# PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON THE MEDICINAL PLANTS IN THAILAND

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**THESIS**

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# ABSTRACT

The research work was emphasized on phytochemical and biological studies of selected Thai medicinal plants. A total of 21 samples of plant material represent 11 families have been collected from 10 provinces of Thailand, based on their ethnomedical uses. Phytochemical evaluation of these plants revealed the presence of 47 new compounds among 98 isolates, 5 of which possessed novel skeleta. The bioactivity-directed fractionation of the extract has led to assess some of the isolates. Where evidence was available, either from literature or the medical practitioners, that particular compound marked certain specific types of activity, the following special studies were resorted to : i) cytotoxicity test, ii) antimalarial test, iii) antimicrobial test, iv) anti-inflammatory test and v) spasmolytic test. Results from these evaluations, it was found that most of the tested isolates possessing marked biologically active.

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# LIST OF ISOLATED COMPOUNDS

1. Frullanolide
2. (-)-7α-hydroxylfrullanolide *
3. (+)-11α,13-dihydro-3α,7α-dihydroxyfrullanolide [(+)-grangolide] *
4. eupatorenone **
5. 9-oxoageraphorone
6. 9-oxoageraphorone epimer
7. (-)-dihydroparthenolide
8. (-)-parthenolide
9. (-)-bisparthenolidine *
10. (-)-9α-hydroxydihydroparthenolide *
11. 8α-acetoxy-2α-hydroxydihydroparthenolide (paramicholide) *
12. 2α-hydroxydihydroparthenolide *
13. (+)-N-acetylparthenolide *
14. (+)-N-acetyl-8a-hydroxyparthenolidine *
15. 3-(2'-3'-diacetoxy-2'-methyl butyryl)-cuauhtemone *
16. cuauhtemone
17. entadamide A *
18. entadamide B *
19. 1-(3,4-methylenedioxyphenyl)-1E-tetradecene *
20. N-(3-phenylpropanoyl) pyrrole *
21. N-isobutyl-2E,4E-decadienamide (pellitorine)
22. N-(2E,4E-decadienoyl) pyrrolidine (sarmentine) *
23. N-[7-(3,4-methylenedioxyphenyl)-2E,6E-heptadienoyl] pyrrolidine *(sarmentosine)
24. (+)-tetrandrine
25. (-)-limacine
26. (+)-thalrugosine
27. (+)-homoaromoline
28. (-)-cycleapeltine
29. (-)-2'-norlimacine *
30. (+)-cycleabarbatine *
31. (+)-tetrandrine 2'-β-N-oxide *
32. (+)-protoquercitol
33. (-)-O-methylthaicanine *
34. (-)-thaicanine *
35. (-)-tetrahydropalmatine

36. ancistrocladidine
37. ancistrotectorine *
38. kopsijasminilam **
39. 20-deoxykopsijasminilam **
40. $\Delta^{14}$-kopsijasmine **
41. kopsijasmine *
42. jasminiflorine *
43. fruticosine
44. fruticosamine
45. 21-deoxykopsidasine
46. kopsidasine
47. kopsidasine-N-oxide
48. 10-demethoxykopsidasinine *
49. kopsidasinine
50. heptaphylline
51. 7-methoxy heptaphylline
52. 7-methoxy mukonal
53. 3-formyl-2, 7-dimethoxycarbazole *
54. 7-methoxymurrayacine *
55. 3-formayl-2-methoxycarbazole (0-methylmukonal) *
56. mukonal
57. (-)-12-cytisineacetamide *
58. (-)-cytisine
59. (-)-12-hydroxycytisine
60. (-)-N-methylcytisine
61. (-)-N-formylcytisine
62. (-)-lupanine
63. (+)-5, 6-dehydrolupanine
64. (-)-anagyrine
65. (-)-baptifoline
66. (-)-12-cytisineacetic acid
67. (2R, 3R)-dihydroquercetin-4'-methyl ether *
68. (2R, 3R)-dihydroquercetin-4', 7'-dimethyl ether *
69. (2R, 3R)-dihydroquercetin-3'-methyl ether
70. (2R, 3R)-dihydroquercetin-3', 7'-dimethyl ether
71. tamarixetin
72. (2R, 3R)-quercetin-4', 7'-dimethyl ether
73. isorhamnetin

**74.** rhamnazin
**75.** exiguaflavanone A *
**76.** exiguaflavanone B *
**77.** sophoraflavanone G
**78.** (R)-(-)-claussequinone
**79.** 5-hydroxybowdichione *
**80.** wrightiadione **
**81.** 6-deoxyclitoriacetal *
**82.** rotenone
**83.** micromelin
**84.** microminutin *
**85.** flindersine
**86.** coumarin
**87.** 7-methoxy-coumarin
**88.** microminutin (second possibility)
**89.** 3-hydroxy coumarin glycoside *
**90.** 2-methoxy of **89** *
**91.** xyridin A *
**92.** xyridin B *
**93.** gambogic acid
**94.** isogambogic acid *
**95.** isomorellinol *
**96.** chrysazin
**97.** 3-methoxy chrysazin
**98.** 3-hydroxychrysazin *

* new structure
** structure reported novel skeleton

# ABBREVIATIONS

| | | |
|---|---|---|
| APT | = | Attached Proton Test |
| br s | = | broad singlet (for NMR spectra) |
| °C | = | degree celsius |
| cm | = | centimeter |
| COLOC | = | Correlation by Long-range Coupling |
| COSY | = | Correlation Spectroscopy |
| $^{13}$C NMR | = | carbon-13 nuclear magnetic resonance |
| δ | = | chemical shift (ppm) |
| d | = | doublet |
| dd | = | doublet of doublet |
| DEPT | = | Distortionless Enhancement by Polarization Transfer |
| ε | = | molar absorptivity |
| EIMS | = | Electron Impact Mass Spectrum |
| ev | = | electron volt |
| FABMS | = | Fast Atom Bombardment Mass Spectrum |
| FLOCK | = | Long-range HETCOR with insertion of 3 BIRD pulses |
| g | = | gram |
| $^{1}$H NMR | = | proton nuclear magnetic resonance |
| HETCOR | = | Heteronuclear Chemical Shift Correlation |
| HMBC | = | Heteronuclear Multiple Bond Connectivity |
| HMQC | = | Heteronuclear Multiple Quantum Coherence |
| HREIMS | = | High Resolution Electron Impact Mass Spectrum |
| HR-FAB-MS | = | High Resolution Fast Atom Bombardment Mass Spectrum |
| hRf | = | rate of flow in chromatography multiple by 100 |
| INEPT | = | Insensitive Nuclei Enhanced by Polarization Transfer |
| IR | = | Infrared |
| *J* | = | coupling constant |

| | | |
|---|---|---|
| kg | = | kilogram |
| L | = | litre |
| $\lambda_{max}$ | = | wave length at maximum absorption |
| m | = | multiplet |
| $M^+$ | = | molecular ion |
| *m/z* | = | mass to charge ratio |
| µg | = | microgram |
| MHz | = | mega hertz |
| ml | = | millitre |
| mm | = | millimeter |
| $\upsilon_{max}$ | = | wave number at maximum absorption |
| No. | = | Number |
| ng | = | nanogram |
| nm | = | nanometer |
| NOESY | = | Nuclear Overhauser Enhancement Spectroscopy |
| ppm | = | part per million |
| q | = | quartet |
| ROESY | = | Rotating-frame Overhauser Enhancement Spectroscopy |
| s | = | singlet |
| SINEPT | = | Selective INEPT |
| t | = | triplet |
| TLC | = | Thin Layer Chromatography |
| UV | = | ultraviolet |

# PART I

# INTRODUCTION TO MEDICINAL PLANTS OF THAILAND

Under the law of nature, all human beings are subjected to the same fundamental needs which consist of food, clothing, shelter and medicine. All forms of folklore medicine evolved out of man's desperate attempt to rid himself and his fellowmen of physical suffering, coupled with his overwhelming desire for eternal life. The countless numbers of recipes for longevity in folk medicines bear testimony to man's quest for immortality. Through trial and error, man painstakingly accumulated his knowledge of the curative properties of the indigenous flora abounding all around him. This knowledge was handed down from generation to generation, firstly as word of mouth and later on as written documents.

It is not known exactly when the art of self-healing using medicinal plants began in Thailand. The earliest evidence unearthed was the remnant of a large stone metate and stone roller used in drug compounding: this relic is believed to have originated during the Dvaravati period (circa 6th century). Furthermore, well preserved documents recording the use of herbal medicine have been found etched, engraved of hand-written on various materials ranging from palm leaves to "khoi" paper to marble tablets.

Thai indigenous medicine is a unique blend of knowledge attained through centuries of practice together with the knowledge `adopted' from other systems of medicine, mainly those of Indian and Chinese origins. Thai people in each region of the country have developed their own unique style of indigenous medicine ranging from the simple use of medicinal plants as ingredients in foods and drinks to sophisticated compound drugs formulated as specific remedies for certain ailments.

The art of herbal remedy is a complex one and involves many disciplines of science. It is remarkable that Thai people in the ancient times were able to acquire the knowledge on the medicinal properties as well as the toxicity of so many drug ingredients. With this knowledge, they were able to formulate specific cures for each ailment as well as to devise appropriate techniques for the detoxification of certain drug ingredients. The methods used to detoxify drug ingredients in traditional Thai medicine include sun-drying, roasting, exsiccating, acidifying, fermenting, grilling, and mixing with drugs of opposite attributes.

In the past century, the use of herbal medicine in Thailand has been on the decline, due largely to the introduction of modern medicine which is more effective and easier to use. However, there is a worldwide trend toward the use of drugs of natural origin since they are believed to possess less harmful side-effects than synthetic drugs. This results in an increased public awareness of our rich and valuable heritage in traditional thai medicine. There has also been a concerted effort by both the government and the private sector to develop Thai medicinal plants into safe and effective druge. Examples of these endeavours are the development of an anti-asthmatic drug from `plai' (*Zingiber cassumunar* Roxb), an antipeptic ulcer drug from `plaunoi' (*Croton sublyratus* Kurz) and a drug from goat's foot creeper (*Ipomoea pes-caprae* (Linn.) Sweet) for jelly-fish poison

Thailand,with its prestigious geographical location and advantageous terrain ranging from the cool mountainous areas of the North to the dense tropical rain forests and the vast expanses of coastlines of the South, boasts a large number of indigenous flora, of which over one thousand species are belived to possess medicinal properties. It is pertinent that this vast and untapped natural resource be developed into useful drugs.

This chapter is a brief introduction to traditional Thai medicine and Thai medicinal plants, underlining their history, unique features and vast potential. As history has repeatedly proven, the fact remains that no human invention can ever rival nature's own creation. Based on this logic, natural drugs must be safer and more beneficial to man than synthetic drugs. Thai people are justly proud of the wealth of knowledge in traditional Thai medicine and their rich natural resources. Every endeavour will be undertaken to effect appropriate development of Thai medicinal plants for the benefit of mankind

**The herbal medicine :- a historical account**

Before Suwannaphum "the Land of Gold" came under the rule the "Thai" or "Tai" people, most of the Indochinese Penninsular was under the influence of the ancient Khom Empire. The first recorded evidence of the use of medicinal plants by the Khoms was in the form of stone tablets inscribed in Sanskrit dating back to the reign of King Chaiworaman VII, who ordered the construction of the famous Anger Wat together with 102 arokayasala (hospitals) throughout the kingdom in the 12th century. Remnants of drug inventories inscribed in stone at Prasart Taprom in Cambodia showed a list of 29 items including drugs of botanical origin, such as the infested heartwood of `chan-daeng' (*Dracaena loureiri* Gagnep.),

animal-derived drugs, e.g. beeswax, clarified butter and minerals, e.g. alkaline salt. The inscription also showed a number of drug formulae, one of which was a remedy for haemorrhoids consisting of gum asafetida, banana oil and other oils, `ma-khuea-khuen' (*Solanum aculeatissimum* Jacq.),ginger,`khod-saw' (*Angelica anomala* Pall.), shallots and garlic.

The art of drug compounding in the ancient Khom civilization was largely influenced by the Mahayana Sect of Buddhism which was led by Phra Bhaisajkuru.

**Sukhothai Period**
(1238-1377 A.D.)

Very little is known regarding the form of medicine practised during this period as no concrete evidence in the form of `Khumpee bai Laan' (Khumpee bai Laan, ancient Thai scriptures hand-writtern onto dried young talipot palm (*Corypha lecomtei* Becc.) leaves; such writings may last for several centuries without spoilage.)`Samud Khoi' (Samud Khoi, another form of documentation commissioned on a type of paper made from the bark of Siamese Rough Bush (*Streblus asper* Lour.)) or stone inscription containing drug recipes from this era could be traced. The most important relic left over from the Sukhothai period is the `Ramkamhaeng Stone Inscription', which vividly described the way of life of the period. Unfortunately, there was no mention as to the use of medicinal plants in this important document. However,it is believed that there was much trading between the kingdom and its neighbouring countries, such as Burma, India, Persia, China, Japan, Sumatra and Sri Lanka. There may have been some importation of herbal medicine as well as the transfer of drug compounding technology from these countries. It is most likely that the form of medicine practised during the Sukhothai period was a combination of the knowledge acquired from the ancient Khoms, the Indians, the Chinese as well as indigenous Thai medicine.

It should also be mentioned that during this period the kingdom adopted the Hinayana Sect of Buddhism headed by Chewaka Kamarapaj as its official religion.

**Ayutthaya Period**
(1350-1767 A.D.)

Much of the documentation pertaining to the history of the period was destroyed during the 'Great War'with Burma in 1767. However,there is evidence pointing to the fact that there was much trading between Ayutthaya and Europe in the middle part of the Ayutthaya period, particularly during the reign of King

Ramathibodhi II who had contact with Portugal in 1512. Other countries such as Spain, Holland and Britain also sent trade missions to the capital in the later part of the Ayutthaya period. With the trade envoys from Europe came the missionaries who brought with them not only the Bible but also a myriad of drugs which they distributed to the people while attempting to spread the words of Chritianity.

A number of drug preparations employed during the Ayutthaya period survived the test of time and is still in use in certain districts in the Central Plains today. One of these is `Tamrub Phra Osod Phra Narai' or `King Narai's Drug Recipes' which originated from the period between 1659-1661. It includes an ointment used for boils and open wounds as well as a diuretic preparation. Ingredients used in these recipes include materials derived from plants, animals and minerals.

**Rattanakosin Period**
(1782-present)

Some 15 years elapsed between the fall of Ayutthaya and the beginning of the Rattanakosin period. During this time a new capital was established in Thonburi and the task of rebuilding of the nation began.

In 1782, King Rama I moved the nation's capital to Bangkok, the site of the present metropolis and the nation, once again, began to prosper. The revival of both its economy and culture took place in earnest.

Only 23 herbals, (Old writings on drug matters), originating during the Autthaya period, survived the Great Fire in 1767. Two other herbals were appended to these during the reign of King Rama I : the first one was `Phra Khumpee Suppakun', by HRH Prince Wongsathirajsanit and the second `Phra Khumpee Chantasart' by Phraya Chantaburi (Klom). The latter was a compilation of knowledge from all the important texts on herbal drugs available at the time.

Thai herbal medicine reached its peak in the reign of King Rama III who in 1821, ordered the renovation of Wat Raj Oros in Thonburi. He also gave instructions for the inscription of herbal drug formulae onto marble tablets for use as temple wall decorations. A total of 180 herbal drug formulae was inscribed onto 55 marble tablets which were used to adorn the walls of Phra Ubhosod (principal building) as well as the corridors of the building which housed the reclining Buddha and other corridors in Wat Raj Oros. In 1832, another important temple was constructed under royal

patronage: this temple was named Wat Phra Chetuphon Wimonmangkhalaram (Wat Po) where 317 marble slabs bearing 1,100 drug formulae were used to decorate the inner walls. Similar inscriptions of herbal drug formulae originating from the same period are also to be found at Wat Machimawas in Songkhla Province. Systematic compilation of traditional drug recipes from various sources was also instigated during this period.

During the reign of King Rama V (1868-1910), Krommamuen Phubodirajaharuethai (Chief of the Royal Medical Department), received a Royal Decree to review and revise the state of the art of Thai herbal remedy in order to assemble the information into a Thai medical textbook. This resulted in `Paetsart Sonkhrau Chabub Luang', a two-volume text commissioned in 1870 : this could be considered to be the first comprehensive manuscript on Thai herbal medicine.. In addition to `Paetsart Sonkhrau', there have been nummerous books written on the subject of Thai medicinal plants; these include `Vajasuksa' by Phraya Phisanuprasartvej, the second edition of which appeared in 1913 and `Mai Ted Muang Thai' by S. Pongboonrod, the first edition of which appeared in 1961.

In the early part of the Rattanakosin era, royal herbalists were conferred with `Krabong Daeng' (Baton Rouge), meaning red sceptre, accompanied with `Yaam Daeng' (Scarlet Bag). The holders of these sacred articles had absolute authority to collect drug ingredients from anywhere within the kingdom.

## The Decline of Thai Herbal Medicine

The establishment of Siriraj Hospital in 1888 marked the beginning of the decline in the use of herbal medicine in Thailand. At first, most of the drugs used in this hospital were those derived from medicinal plants. As the number of western-educated physicians began to increase, the use of modern drugs began to rise. This trend was to continue until the use of herbal drugs was entirely replaced by that of modern drugs, particularly instate-run hospitals and health service centres around the country.

## The Revival of Thai Herbal Medicine

With the realization that Thailand has been importing vast quantities of modern drugs from western countries at a cost in excess of 20,000 million baht per annum, the Royal Thai Government, a decade ago, began laying down strategies for research into Thai medicinal plants as well as the reintroduction of scientifically proven herbal drugs as substitutes for modern medicine. Thai scientists as well as

their counterparts, on the other hand, have taken great interest in Thai medicinal plants and have conducted research on a large number of plants samples collected from Thailand for over the past 30 years. One such endeavour has resulted in the discovery of a new `miracle drug' which is one of the most potent antipeptic ulcer drug known to date. This drug was isolated from a plant considered as a troublesome weed in Thailand but whose appearance in at least 30 herbal recipes in four different herbals attested to its curative property.

The search for new drugs from Thai medicinal plants will continue through the concerted effort from all parties concerned and hopefully such endeavours may result in new cures for the seemingly incurable diseases such as cancer and AIDS.

**Preparation of Traditional Thai medicine**

The word `yaa' or drug in traditional Thai medicine usually refers of the use of at least two different ingredients compounded together to yield a drug. Inevitably, traditional Thai drugs consist of numerous ingredients which may be classified into the following categories:

- Major ingredient(s) - those which exert the main action
- Auxiliary drug(s) - those with complimentary or synergistic action
- Flavouring agents - to make the drug more palatable.

Traditional herbalists must have full knowledge of the types, parts used, colours, odours and tastes of all natural drug ingredients. When formulating a preparation, the herbalists must take into consideration the following factors :

- The parts of the ingredients to be prescribed -these may be the bark, roots, flowers or other parts of a plant.
- The form(s) in which they are used - some ingredient may depend on the age and health profile of the patients.
- Other pertinent information - for instance, certain drugs may contain toxic substances which require detoxification before use.

In the formulation of traditional Thai medicine, the old system of measurements is utilized. The most common denominations for weight are the `baht' and `salueng'. An example of the traditional system of measurements for weight, volume and length is given below.

Weight Measurement

| | | | |
|---|---|---|---|
| 1 chang (1,200 g) | = | 20 | tum-lueng |
| 1 tum-lueng (60 g) | = | 4 | baht |
| 1 baht (15 g) | = | 4 | salueng |
| 1 salueng (3.75 g) | = | 2 | feung |
| 1 feung (1.875 g) | = | 4 | pai |
| 1 pai (0.468 g) | = | 2 | utt |

Volume/Capacity Measurement

| | | | |
|---|---|---|---|
| 1 kwian (2,000 lt) | = | 2 | bun |
| 1 bun (1,000 lt) | = | 50 | sud |
| 1 sud (20 lt) | = | 20 | tanan |
| 1 tanan (1 lt) | = | 4 | kob-mue |

Length Measurement

| | | | |
|---|---|---|---|
| 1 ong-ku-lee (2.5 cm) | = | 4 | paddy grains |
| 1 paddy grain | = | 2 | sesame seeds |

Nowadays the acquisition of drug ingredients is made easy by the existence of retail traditional drug stores where a vast array of crude drugs is in stock. In rural areas, traditional herbalists may grow a number of the more common medicinal plants while others may be collected from trees growing in the forests. Once all the ingredients have been acquired, they may be further processed by pulverization if the drug is to be made into pills or tablets. Grinders made of stone or metal alloy may be utilized in the pulverization process and the powdered drug is then passed through a sieve before being shaped into the desired form.

There are several dosage forms in which traditional drugs may be prescribed. These include solid dosage forms, such as pills, tablets, snuff, suppository and liquid dosage forms such as fluid extracts, alcoholic extracts, teas and expressed juices. These drugs may be taken internally, applied externally, used as a snuff, an inhalant or as a poultice. Of the dosage forms previously mentioned, only 5 or 6 are commonly in use and these are described below.

## Fluid Extracts

To prepare an extract, a handful of the ingredients is placed in a clay pot shaped like a gourd. The crude drugs may be coarsely chopped or tied into a sheaf the size of one's palm prior to being transferred to the pot. Water is then a added to

the ingredients until just covers the drugs. The mixture is boiled for 10-30 minutes, after which the supernatant is decanted and drunk when lukewarm.

### Infusions and Herb Teas

The ingredients used in herb teas are usually cut into fine strips and then air-dried. Occasionally, the ingredients may be roasted to give an agreeable aroma. One part of the drug mixture is placed in a container with a lid, into which ten parts of boiling water are added. This is then put aside to brew for 15-20 minutes.

### Alcoholic Macerates

In most cases, air-dried ingredients are roughly pounded and wrapped in a piece of muslin before being placed in a glass jar containing rice whiskey for a period of 7-10 days. The resultant macerate is then decanted and taken as directed.

### Pills

Fresh herbs are sliced and left to dry in the sun. After the drying process, the herbs are pulverized while they are still warm. Two parts of this powdered drug are thoroughly mixed with one part of honey or syrup and allowed to stand for 2 hours. The mixture is then shaped into round balls with a diameter of approximately 0.2-1 centimetre. The finished pills are laid out in the sun to dry and the heat treatment process repeated after a fortnight to prevent fungal infestation.

### Expressed Juices

To prepare the above dosage form, fresh herbs are pounded to a pulp, to which a small amount of water may be added. The juice is then squeezed out and taken as directed. Certain drugs, such as `ka-thue' (*Zingiber zerumbet* (Linn.) Smith) and `kra-chaai' (*Boesenbergia rotunda* (Linn.) Mansfield), must be grilled before use.

### Poultices

Fresh herbs are used for this form of treatment. The pounded herbs, to which a small amount of water or spirit may be added, are made into a thick paste and the paste applied to the affected areas. The drug is kept moist and the dressing changed 2-3 times a day.

The above mentioned forms of drugs are the most commonly prescribed forms in traditional Thai medicine, though other dosage forms are still in use. In the preparation of these drugs a number of vehicles may be used; these include

water, spirit, syrup, honey and lime water. In general, they serve to make the drugs soluble and in some cases they may enhance the action of the major drugs.

As a safety precaution, finite periods of shelf life are recommended for various drug products. Thus, a powdered drug consisting entirely of leaves has a shelf life of 3 to 6 months while a drug containing heartwoods may be stored for as long as 6 to 8 months. In general, pills and tablets store better than powders, and pills consisting of roots or rhizomes are considered to be safe for use for up to one and a half years. Therefore, knowledge of the drug ingredients, appropriate methods of preparation as well as safety aspects is essential in the formulation of traditional Thai drugs.

**Traditional and Modern Usage of Thai Medicinal Plants**

The use of herbal medicine in the Indochinese Peninsular can be traced back to the early days of the khom civilization and beyond. The early form of Thai herbal medicine was greatly influenced by the khoms who were in turn much influenced by the Ayurvedic System of medicine of India. In Thai herbal medicine as practised by traditional healers, the drugs used were almost invariably in a compounded form where a multitude of herbs was mixed together to form a preparation. Each preparation consisted of 2 to 20-30 ingredients or more, derived entirely from plants, animals and/or minerals. Besides the principal drugs, these preparations quite often contained drugs which acted as laxatives and tonics as well as some inert ingredients and flavouring agents. Cetain herbal drug recipes were well guarded secrets in some quarters. Herbal preparations were usually prescribed on an individual basis where the age, sex, history and health profile of each patient were taken into consideration.

While this somewhat sophisticated form of remedy is advocated by traditional healers who inherit their trace from their fore-fathers, a simples form of household remedy also exists. This usually involves the use of single plant drugs which are readily available. These household remedies include the use of aromatic plants such as ginger, sacred basil as carminatives, the use of `phyaa-yaw' (*Clinacanthus nutans* Lindau) for insect bites and the use of betel leaves for skin rashes, for instance. It is probably true to say that most Thai people have an inbred knowledge of the use of medicinal plants from observing their elders. People in rural areas, in particular, still resort to using medicinal plants, mostly for common ailments and in cases of emergency. With the recent revival of interest in herbal medicine and the reintroduction of the use of medicinal plants into the primary health care system, a number of medicinal plants has been carefully selected

for their efficacy and safety and recommendation was made for the use of these plants in primary health care in cases of common ailments.

In contrast to the use of medicinal plants in the form of crude drugs in traditional medicine, modern drugs employ mostly pure substances, although a number of plant extracts are still in use. In this category, medicinal plants represent sources of several important drugs such as digoxin from foxglove, morphine and codeine from opium, quinine and quinidine from cinchona bark, to name but a few. A number of Thai medicinal plants are currently employed for the isolation of active principles for use in modern drug production; these include rauvolfia root (*Rauvolfia serpentina* Benth. ex. Kurz), `plaunoi' (*Croton sublyratus* Kurz) and Asiatic pennywort (*Centella asiatica* (Linn.) Urban).

## Medicinal Plants in Traditional Thai Medicine

Compound Drugs : A large number of herbal recipes has been recorded in various herbals (or khumpee). A total of 25 herbals on Thai medicine is currently in use. Of these numerous recipes, 16 have been selected as common household herbal medicines for use in common ailments. As part of the UNICEF-sponsored project, eight of these recipes were selected for large scale production by the Department of Medical Science for distribution to drug-cooperatives in rural areas. These preparations include antidysenteric and antipyretic drugs as well as simple and stimulating fragrant remedies. Two examples of these are Yaa Lueang Pid Samut, an antidysenteric preparation and Yaa Chantaleela, which is used for fevers.

### Yaa Lueang Pid Samut

This recipe consists of nut grass (*Cyperus rotundus* Linn.); zedoary (*Curcuma zedoaria* Rosc.); the bark of `phay-kaa' (*Oroxylum indicum* Vent.); the roots of`kluai-teep' (*Musa sapientum* Linn.), roasted cloves of garlic (*Allium sativum* Linn.); `chan-yoi' (resin from *Shorea* spp.); stick lacs (*Laccifera chinensis* Mardihassan); black catechu (*Acacia catechu* Willd); pale catechu (*Uncaria gambir* Roxb.); henna leaves (*Lawsonia inermis* Linn.); the leaves of pomegranate (*Punica granatum* Linn.). One part by weight of each of the above ingredients is mixed with six parts of turmeric (*Curcuma longa* Linn.) and the mixture pulverized to a powder. The drug is used as an antidysenteric and antidiarrhoeal in children. When used as an antidysenteric, a vehicle consisting of roasted rhizomes of `kra-chaai' rubbed against the lid of an earthen pot together with lime water is thoroughly mixed with the powder. The mixture is shaped into round balls of

approximately 0.1 gram in weight. As an antidiarrhoeal agent, the decoction, resulting from boiling the pericarp of pomegranate or the bark of agasta (*Sesbania grandiflora* (Linn.) Poiret) with lime water, is used. The dose specified for children is 3-7 pills.

**Yaa Chantaleela**

One part each of `khod-saw' (*Angelica anomala* Linn.); `khod-chulalamphaa' (*Artemisia* sp.); sandalwood (*Santalum album* Linn.); `chan-daeng'; kadom fruits (*Gymnopetalum cochinchinense* Kurz); the stems of `borapet' (*Tinospora crispa* Miers. ex Hook.f. & Thoms.); the roots of `plaalaipueak' (*Eurycoma longifolia* Jack) is mixed together and pulverized. One half to one gram of this powder is mixed with boild or jasmine scented water and taken for various kinds of fevers.

Single Ingredient Drugs : As opposed to the somewhat sophisticated compound drugs precribed by traditional healers, household remedies for common ailments usually consist of sigle ingredient drugs with well proven properties. The use of the fruits of long pepper (*Piper* spp.) as a carminative, the pulp of tamarind (*Tamarindus indica* Linn.) as a laxative and the fruits of ebony tree (*Diospyros mollis* Griff.) for intestinal worms typify this category of herbal medication.

**Medicinal Plants in Modern Medicine**

It is a grossly misguided notion that modern drugs are entirely man-made. In fact, between 20-25 percent of all modern drugs in use today originate from plants, animals, microorganisms and minerals. Important drugs such as the opiate analgesics, morphine and codeine; the anticancer drugs, vincristine and vinblastine; the antimalarial drug, quinine and the cardiotonic glycosides, digoxin and lanatosides A and B are all extracted from plant sources. Steroid drugs,including oral contraceptives, are mostly modified from plant steroids. Microorganisms furnish all the antibiotics in use today. Drugs from animals include hormones,vaccines and a number of enzymes.

A number of Thai medicinal plants are currently employed in the manufacturing of modern drugs. The most recent example of such plants is `plaunoi' which is the source of a novel antipeptic ulcer drug. Rauvolfia root, senna and Asiatic pennywort are all sought after as the sources of antihypertensive,laxative and antikeloid drugs, respectively.

## Spices and Condiments

In addition to their use as medicinal agents, a number of Thai medicinal plants also feature in everyday life in the form of spices and condiments. Thai culinary style requires the use of a large array of spices and condiments to give a special blend of taste and aroma characteristic to Thai food. The most commonly used spices and condiments are chilli peppers, garlic, shallots, sacred basil, lemongrass, pepper, ginger, leech lime, galanga, clove, anise and cumin. Drinks made from certain medicinal plants are also popular in Thailand. One of the most familiar sights in most markets is a push-cart stall selling `nam-bua-bok', a green-coloured drink made from Asiatic pennywort. Herb teas, such as ginger tea, roselle tea and a tea made from dried bael fruits are also common drinks among the Thai people.

## Natural Cosmetics

For centuries, Thai ladies have adorned themselves with natural cosmetics ranging from Thai style fragrant water, scented powder, to lip gloss and rouge. Certain medicinal plants such as aloe gel, gameng (*Eclipta prostrata* Linn.) and leech lime are reputed to keep the hair black and shiny. Thailand is endowed with numerous fragrant flowers, many of which have been used to make aromatic waters, sachets and other odouriferous products.

A form of beautification by cleansing the face and the body with steam saturated with aromatic oils from certain medicinal plants was popular in the kingdom in the old days. This Thai-style sauna was also employed in traditional Thai medicine, particularly for women after childbirth.

## Thai-style Hot Vapour Bath

A form of sauna employing steam saturated with aromatic oils from various medicinal plants has been practised in Thailand for centuries. The practice was recommended in traditional Thai medicine as a mean of cleansing the body,particularly for women after childbirth. It was also used as a cure for certain ailments such as aches and pains as well as certain skin conditions such as rashes and itching. More recently, Thai-style sauna has been employed for cosmetic purposes which include the improvement of skin conditions and weight reduction.

Medicinal plants used in Thai-style sauna can be divided into four main groups.

Group 1 consists of volatile oil-containing plants such as tumeric rhizomes, the leaves of lime (*Citrus aurantifolia* Swing.), the fruits of leech lime and citronella (*Cymbopogon nardus* Rendle).

Group 2 consists of acid-containing plants, including tamarind leaves, the leaves and pods of`som-poi'(*Acacia rugata* Merr.) and the fruits of leech lime cut into halves

Group 3 consists of sublimable substances such as camphor and Borneo-camphor.

Group 4 constitutes medicinal plants for specific ailments, for instance `phak-bung-ruam' (*Enydra fluctuans* Lour.) as an anti-inflammatory, shallots and sweet flag (*Acorus calamus* Linn.) for colds and garden quinine (*Clerodendrum inerme* Gaerth) and sea holly (*Acanthus ebracteatus* Vahl or *A. ilicifolius* Linn.) for skin rashes.

Steps involved in the preparation of Thai-style sauna are as follows:-

- Fresh herbs are placed in a large, closed container and plenty of water is added.
- The mixture is heated to boiling.
- The resulting steam is then routed into a smallen closure ready for use.

Several health clubs and beauty salons in Bangkok offer Thai-style sauna as part of their health or beauty programmes.

**Natural Colouring Agents and Dyes**

Before the advent of synthetic dyes, plants and minerals were the main sources of colouring agents and dyestuff. The oldest plant recorded as being used for this purpose was probably henna, a hair dye and nail polish dating back to the time of the ancient Egyptians. Henna leaves and extracts are still in use to day mainly for cosmetic purposes. Food colouring is another skill acquired since early civilizations. In Thai culinary practice, extracts from a number of plants have been used to impart attractive colours to Thai desserts and drinks for centuries. Examples of these are the use of the blue flowers of butterfly pea (*Clitoria ternatea* Linn.)to give a violet colour to a Thai dessert called `Khanom chaw muang' and the use of the leaves of oyster plant (*Rhoeo spathacea* (Sw.)Stearn) for flavouring and imparting a wine red colour to a drink. Natural dyes derived from barks, leaves and other parts of cirtain plants have been employed to dye hand-woven cloths which are characteristic to that particular part of the country such as `teen-chok' cloth

from Phrae Province and `mud-mee' silk from the Northeast of Thailand. Natural dyes were also used to produce the vivid colours of the famous Thai silk in the olden days. The heartwood of jack fruit tree and the root bark of of wild Indian mulbery yield a yellow dye containing morin while the leaves of Indian almond (*Terminalia catappa* Linn.) give a green dye. Black dyes may be obtained from the friuts of ebony tree and whole plant of gameng. The seed of lipstick tree and indigo leaves yield red and blue dyes, respectively.

## Medicinal Plants for Export

Certain medicinal plants such as bastard cardamom, Siam cardamom, ginger and black peper constitute important export commodities for Thailand, earning valuable foreign currencies. Other Thai medicinal plants sought for export include rauvolfia root, Madagascar periwinkle, valerian root,senna pods and malva nut. West Germany, the United States of America, China and the United Kingdom are the main importers of Thai medicinal plants. According to the Department of Business Economics, Ministry of Commerce, in1986,Thailand exported some 7,000 tons of medicinal plants spices and related products, valued at over 400 million baht. Among the leading export items were betel leaves (2,500 tons), pepper(2,000 tons), ginger(1,250 tons), bastard cardamom (185 tons), rauvolfia root(9.7 tons), and Siam cardamom (4.6 tons)

With its rapid economic growth, Thailand continues to export a relatively large quantity of medicinal plant products each year. The volume of export of Thai medicinal plants is likely to increase since the government has been promoting the cultivation of certain medicinal plants for export in its 6th National Social and Economic Development Plan.

## Miscellany

Apart from the many applications of Thai medicinal previously mentioned,certain medicinal plants such as areca nut and betel leaf found use as a masticatory. The practice of chewing betel quid became popular among the Thai people since ancient times and survived well into the Rattanakosin period when people from all walks of life were seen indulging in this favourite pastime. However, during World War II the practice was banned by Field Marshal P. Pibulsongkhram, the Prime Minister of Thailand, as it was deemed unsociable for a country which had just started to adopt western culture.

With the abundance of fragrant and colourful flowers all year round, Thai ladies, especially courtiers, have busied themselves with the arts of Thai-style arrangement, perfume and sachet making for centuries. These crafts have been passed on from generation to generation and still exist today.

**Thai-style Sachets**

Many Thai flowers are delicately scented and lend themselves well as ingredients in sachet-making. Thai ladies, since the old days, have perfected the arts of making fragrant sachets from these dried flowers. The flowers used for this purpose are usually small in size and possess long lasting fragrance. Flowers from bullet wood, rose petals, jasmine, henna flowers, bread flowers (*Vallaris glabra* Kize.) and *Coffea bengalensis* Roxb. are commonly used. In addition, the leaves of fragrant screw pine and bachelor's button (*Gomphrena globosa* Linn.)are usually included in the pot-pourri. In making the sachets, petals from selected flowers are air-dried while the leaves of fragrant screw pine are finely shredded and air-dried. The dried ingredients are then mixed together and placed in a tightly closed container. The mixture of flowers is sprinkled with the concentrated Thai-style fragrant water and the container sealed for 1-2days. After the impregnation process, the flower mixture can be packed into suitable containers such as small bags of various shapes made of finely netted material, small porcelain of clay pots. These sachets are usually used as souvenirs on special occasions such as wedding and birthday celebrations.

**Thai-style Fragrant Water**

This very popular form of fragrant water is made from freshly gathered flowers and contains no alcohol. The flowers used are selected for their sweet and lingering fragrance, some of the more common ingredients being the flowers of damask rose (*Rosa damascena* Mill.), jasmine, `lam-chiak'(*Pandanus tectorius* Bl.) `kradang-ngaa-thai' (*Canaga odorata* Hook.F.&Th.), orange champaka (*Michelia champaca* Linn.), `chammanaad' (*Vallaris glabra* Ktze.),bullet wood, `lamduan' (*Melodorum fruticosum* Lour.) and`chan ka-pho'(*Vatica diospyroides* Syming.), Other ingredients used include the leaves of fragrant screw pine, the inner bark of 'chaluut' (*Alyxia reinwardtii* Bl. var. *lucida* Markgr.), sandalwood, Siam benzoin, jasmine and hyacinth (*Hyacinthus orientalis* Linn.) oils.

The initial step involves the blanching of coarsely chopped fragrant screw pine leaves, `chaluut' bark and sandalwood with boiling water. After filtration, the extract is allowed to cool and then divided among several tightly-closed containers

made of glass or enamel. This is followed by the absorption process which is usually carried out in the evening when jasmine flowers begin to bloom. The petals of jasmine, shredded petals of `kradang-gaa-thai', orange champaka and the aforementioned flowers are carefully floated on the surface of the prepared water and the containers sealed overnight. After the removal of the flowers, the content of each vessel is transferred into a large porcelain jar, typically a blue and white urn. Fumigation of the water with a mixutre of Siam benzoin, shredded peels of leech lime and sandalwood oil follows. The concluding step involves the mixing of Borneo camphor together with jasmine and hyacinth oils with scented marl in a mortar. A small amount of the scented water is then added to this mixture, at a time, with stirring. The resultant slurry is filtered through muslin cloth into a glass bottle fitted with a tight lid. The procedure is repeated until all the scented water has been processed.

Thai-style fragrant water is applied liberally onto the face, chest and arms during the hot climate. It is also recommended for use in cases of fainting. A thick paste made by mixing scented marl with fragrant water is used as anointment in various ceremonies.

**Thai Medicinal Plants:-Today**

Thailand, being an agricultural country, is richly endowed with a wide variety of indigenous flora, some of which have long been employed for their medicinal properties. The fact that traditional Thai medicine has been in practice long before the introduction of modern medicine into Thailand, is manifested by the existence of `Khampee Bai Laan'or old herbals written on palm leaves and in `Samud khoi' as well as other writings concerning the use of medicinal plants within each region.

After modern medicine had taken root in Thailand with the inauguration of Siriraj Hospital, the first modern-style hospital in Thailand, in 1888, the use of herbal medicine began to dwindle, particularly in state-run hospitals and health centres. In great contrast to the medical professionals whose education in modern medicine dictated their preference for the use of modern drugs, a portion of the thai population still adhered to the old ways of life, in which herbal medicine played a vital role. This is especially true in rural areas where traditional healers and herbalists are still held in reverence by the communities. This being the case, Somdej Phra Wanarat (Poon Poonasiri) the abbot of Wat PhraChetuphon Wimonmang Khallaram(Wat Po) at the time, orderedthe establishment of a school of Thai traditional medicine within the temple in 1957. A succession of schools of Thaitraditional medicine both in Bangkok and in other provinces followed. A number of

temples in rural areas also established their own medicinal plant gardens which served as a source of herbs for the treatment of patients as well as an educational venue. The fact that there are no modern drugs available for the treatment of certain diseases coupled with the rapid increase in the costs of modern drugs has given new impetus to the search for new drugs from age-old medicinal plants. Research work is now in progress both in government institutions and universities in order to isolate and identify the bioactive constituents of plants which may potentially lead to the discovery of more potent drugs with little or no side effects.

The development of Thai herbal medicine may be carried out in three difference directions. Firstly, the traditional drug preparations could be presented in new dosage forms so as to render them more palatable and more convenient to use. Secondly, the active constituents could be isolated and identified; such studies could be carried out on each individual plant or they could be performed on a given drug preparation. More commonly, individual plants are selected for studies which are normally carried out in educational institutions and related government agencies. Research into medicinal plants may follow one of two routes, the first of which is preceded by the isolation and identification of the chemical constituents from selected drug plants and these compounds subsequently tested for their biological activities. On the other hand, crude extracts from selected plants may be primarily screened for their pharmacological activities. Bioassay-directed fractionation of the active principles then follows. The latter practice is preferred by some researchers as the success rate is somewhat higher than the former route. In cases where the active principles are present in the plant (s) in minute quantities, they may have to be synthesized chemically before further studies can be carried out. Once sufficient quantity of the active compound is obtained, toxicological and comprehensive pharmacological studies are performed in test animals. If the drug passes the safety tests and shows promising results in animal tests, clinical studies may be initiated to ensure the efficacy and safety of the drug in human subjects. An integrated study of a drug plant such as the one described above is both time-consuming and costly. Nevertheless, such endeavour has, in recent years,resulted in the discovery of a novel antipeptic ulcer drug from `plaunoi' (*Croton sublyratus* Kurz), a medicinal plant indigenous to Thailand.

The third strategy for the promotion of Thai medicinal plants would be the careful selection and development of medicinal plants as economic crops to supply both the export markets and the local drug industry. At present, Thailand is exporting a number of plant drugs into international markets, for example, `plaunoi', Madagasecar periwinkle and snakeroot.

It is clearly evident that medicinal plants still play an important part in Thai cultural system to day as they have done in the past. The conservation and development of Thai herbal medicine have been made possible through the effort of interested individuals, the private sector and government agencies alike.

**Thai Traditional Medicine :- A basic concept**

Thai traditional medicine is a very ancient system of medicine. It was based on knowledge transmitted orally from generation to generation until such time as it was committed to writing in the Pali language and Khmer script on palm leaf manuscripts. These manuscripts were later translated into Thai, and preserved as sacred texts to be handed down in their original form to future generations.

It is a simple system of medicine based primarily on the philosophy of Ayurveda, the Science of Life of ancient India, which had its beginnings during the same few centuries as the ancient medical systems of Greece and China, about two and a half thousand years ago. Ayurveda attempts to relate the human body and its elements to the macrocosm of the universe, and so it does not adopt a scientific approach to the diagnosis and treatment of disease. It views the body as a co-ordinated whole, classified, not according to function,but according to its constituent components. The material components of the body are classified with reference to the predominant element of the four protoelements of which they are composed.

Consequently, in Thai traditional medicine, disease is seen as a disturbance of the balance of these elements, manifested by disorders of the various component substances of the body. Such disorders may arise from endogenous causes, or from exogenous causes, such as changes in climate or environment, and factors of age and time of day. Immoderate behaviour of the individual is also taken into account as a causal factor of disease.Whatever may be the primary cause, the result is thought to produce changes in the function or structure of one or more of three vital constituents of the body-wind, bile and mucous. These disorders of the tri-dosa are considered to be the ultimate causes of disease.

Disease is diagnosed after a thorough investigationof the history of the patient and his family, the past and present history of the disease, examination of body and mind, and investigation of the patient's symptoms. From the information thus obtained, the primary cause of the disease, and the treatment to be given.

Treatment may be given by means of medicine, surgery, massage, or by magic spells and incantations. Only treatment by medicine has been described here. Crude drugs of plant, animal, and mineral origin are used, usually in standard combinations and quantities. The medicinal properties of drugs are determined by their taste.

This system of medicine is used in Thailand to a much greater extent than modern medicine, which is quite well established in the cities and urban areas, but does not reach the majority of the people, who live in the rural areas. Even where modern medicine is available, many people, including some trained in the modern health professions, patronize the traditional doctors. As the most practical means of providing elementary health care for the thirty five million people of rural Thailand, the World Health Organization has decided to train `village communicators' in Thai traditional medicine.

It may be asked why this ancient system of medicine has survived, why it still retains its popularity even when modern medicine is available, and why the World Health Organization has chosen to encourage its practice ?

How can such an unscientific system be effective-which it must be to have persisted for centuries, and to have commanded the adherence of so many people ? Let us look at the actual practice of Thai traditional medicine,and see what would happen in an imaginary case.

A patient of a certain age, resident in a particular place, with known habits and behaviour pattern, who became ill at a known time of day or night and time of year, presents himself with certain symptoms. The causal factors due to the patient's symptoms, place of birth, age, the time of the onset of the disease, and the primary cause are determined. A conclusion is reached on what is abnormal, and the disease is named. But the cause and the name, although useful psychologically, are irrelevant medically. The medicine prescribed for treatment is selected according to the symptoms.

In actual treatment, experience has shown that a combination of certain drugs is effective in the relief of certain symptoms, and these are then given. If it is a minor complaint, easily treated, the patient recovers in a reasonable time, and is fully satisfied by the treatment. As most illnesses fit into this category, the results,on the whole, are very good.

In the case of serious or potentially fatal illnesses,some of the drugs must surely be effective. Only many years of study of the action of traditional medicines will reveal which these are. At the present time, research is being conducted at hospitals and research institutes in Thailand into these druge, particularly to determine the safety of the use of some which may be dangerous.

Ayurvedic medicine takes a quite practical view of incurable diseases. Traditional practitioner are advised not to treat such cases. But it seems that, although the Thai healer does not attempt to treat incurable diseases, he does offer to try to suppress or alleviate the symptoms, in order to make his patients more comfortable. It is also apparent in the actions of people who consult the traditional doctors when modern medicine has failed them.

It can thus be seen why Thai traditional medicine has survived, and why the World Health Organization has chosen to adopt it for use in the promotion of health care in Thailand. For most purposes it is adequate. In addition it is inexpensive. The drugs are cheap, little equipment is needed, and practitioners can be trained quickly and at little cost.

A total 1,500 species of Thai medicinal plants have been used in 2,449 formularies which registered at the Ministry of Public Health. Almost of formularies are allowed to used for the treatment of mild illnesses such as:

1. Carminative
2. Appetite
3. Blood tonic
4. Stomachica
5. Laxative
6. After child birth remedy
7. Antitussive, expectorant
8. Anodyne
9. Antipyretic
10. Anti-intermittent fever
11. Anthelmintic
12. Antihistaminic
13. Antiashmatic
14. Nerve tonic

The first 50 medicinal plant species mostly used in ancient Thai formularies are listed below :

1. *Zingiber officinale*
2. *Eugenia aromatica*
3. *Piper retrofractum*
4. *Piper nigrum*
5. *Nigella sativa*
6. *Angelica sylvestris*
26. *Curcuma zedoaria*
27. *Aquillaria agallocha*
28. *Terminalia gall*
29. *Diospyros decandra*
30. *Caesalpinia sappan*
31. *Allium stivum*

7. *Amomum krervanh*
8. *Myristica fragrans* (nutmeg)
9. *Conioselinum univittatum*
10. *Cuminum cyminum*
11. *Levisticum officinale*
12. *Atractylodes lyrata*
13. *Myristica fragrans* (mace)
14. *Glycyrrhiza glabra*
15. *Lepidium sativum*
16. Camphor
17. *Plumbago indica*
18. *Foeniculum vulgare*
19. *Dracaena loureiri*
20. *Anethum graveolens*
21 *Artemisia indica*
22. *Piper ribesoides*
23. *Terminalia chebula*
24. *Podocarpus neriifolius*
25. *Zingiber cassumunar*
32. *Mesua ferrea*
33. Borneol
34. *Acorus calamus*
35. *Piper sarmentosum*
36. *Saussurea lappa*
37. *Cinnamonun zeylanicum*
38. *Citrus hystrix*
39. *Tinospora crispa*
40. *Kaempfera galanga*
41. *Aloe vera*
42. Alum
43. *Nelumbo nucifera*
44. Borax
45. *Rauvolfia serpentina*
46. *Cinnamomum burmanii*
47. *Phyllanthus emblica*
48. *Mimusops elengi*
49. *Carthamus tinctorius*
50. *Cyperus rotundus*

**Thai Medicinal Plants :- A Research Trend**

For detection of biological activity one can choose between general screening methods, which will pick up many different types of pharmacological activities, and specific methods which are directed at finding some effects against specific diseases. Both approaches have advantages and disadvantages. A broad test which can pick up many different effects is probably most useful if one is randomly screening the chosen compounds for any kind of pharmacological activity. One disadvantages of using a broad test for screening and for following the isolation of active compounds is that one does not know, until the active compounds has been isolated, if the work was worth doing. It can very well result in the isolation of a trivial compound of no medical use.

Much of the newer work with medicinal plants and/or traditional drugs involves the rediscovery of effects known for a long time. Modern pharmacological research may thus have contributed less that is really new than we like to believe. In fact modern pharmacologists sometimes appear ignorant of the fact that the majority of people in the world rely predominantly on medicinal plants as

drugs. In realization of this, WHO has emphasized the importance of scientific investigations into indigenous herbal medicines. In many countries of the world, native medicinal plants are thus looked upon as possible additions to the WHO list of "Essential Drugs" once their value has been clinically proven. On the other hand, it must also be remembered that the advanced techniques of modern chemistry have made the isolation and stucture determination of new compounds much easier during recent years.

In working with plants used in traditional medicine, one should take advantage of the information about the use of the plant that has been obtained from the traditional healers. Thus, a specific test should preferably be used. As such tests usually are time-consuming and cumbersome, most research groups will have to limit themselves to studying a small number of effects for which they have set up suitable test systems with which they have become thoroughly familiar.

Most work on plants used in traditional medicine has been based on results of in vivo or in vitro tests, performed in the laboratory. In choosing the plants for investigation, a clinical approach might give better results. In Thailand, remedies prepared by a traditional healer from plants of the local flora are the only drugs available for a great many people. An attractive approach to the investigation of such plants would therefore be for a trained physician or pharmacologist to cooperate with the healer, first acting as an observer by establishing proper diagnoses and evaluating whether the treatment given by the healer is likely to have been effective. In this way a number of plants might be selected, extracts of which could then be subjected to more detailed clinical trials, provided that the preliminary observations and a reasonably extensive toxicological evaluation have shown no adverse effects. The further investigation of these plants could then be performed in the laboratory. The advantage of this approach is of course that the chances of finding valuable compounds must be much better when investigating a plant extract for which clinical efficacy has already been proven.

The role of natural products in drug development is obviously not restricted to providing new drugs. Natural sources make available an abundance of compounds, often with unique chemical structures. These compounds, being a part of living systems from which they were isolated and in which they must have played some function, exhibit a diversity of biological activities. Even though most of them are of no immediate interest as drugs, they are indispensable as tools in pharmacological and biochemical investigations. During the last decade the research has developed towards biologically active compounds involving biologists,

chemists and pharmacologists who are engaged in multidisciplinary programs. Natural products will thus continue to strongly influence the development of drugs in an indirect fashion, through an impact on basic sciences upon which drug research depends. Secondly, even if a natural substance is too toxic or not active enough or if it exhibits an undesirable combination of activities, structural modifications of the molecule to acquire a useful activity pattern is a challenging problem for a chemist.

In spite of the hidden promises of the many thousands of medicinal plants that have not yet been evaluated pharmacologically, "new" drugs can also come from "old" natural products, if the necessary research input is made, e.g., with a new drug design.

Thus, the study of natural products has resulted in many valuable therapeutic agents becoming available and it is probable that many potentially useful drugs remain to be discovered. A very large proportion of the Wold's population use herbal remedies but despite this it has been estimated that of the 250,000-500,000 species of higher plants on earth, only a small fraction has been investigated pharmacologically and usually for only one specific type of activity. Therefore, there is no doubt that there are considerable chances if finding new natural compounds with pharmacological activities, useful for the development of new drugs.

# PART II

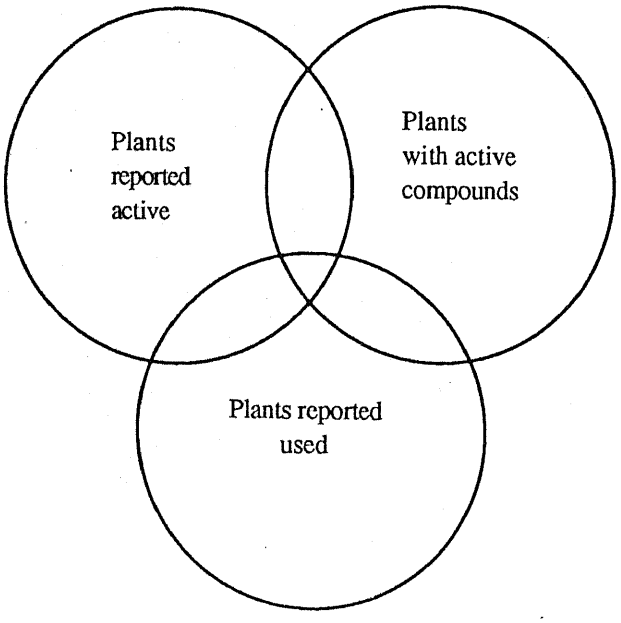
## STRUCTURE ELUCIDATION OF ISOLATED COMPOUNDS

Thailand is rich in natural and are usually available in abundance. Crude drugs are served in every town. After standardization, these materials may provide safe and effective galenical products or may lead to the discovery of new biologically active compounds. Moreover, some of known and novel compounds may reveal as models to organic chemists for synthesis of new drugs. Now we realize that in the realm of science, drug research belongs to the interdisciplinary group of sciences, since it involves a number of heterogenous technical fields which are united in the applications to a common object in order to seek for new drugs.

It has also been our belief that research on natural products, particularly on those derived from Thai medicinal plants, should be performed in a multidisciplinary manner, drawing specific expertise from botanists, chemists, pharmacologists and possibly clinicians. The activities in this area started over the last decade by venturing into the field and selecting plants for chemical study on the basis of simple spot tests.

The selection of plant materials for these researchs is based on i) Random, ii) Phytochemical, iii) Taxonomic, and iv) Ethnomedical approaches.

Logical information of plant to be explored is drawn as below :




**Plant selection**
- Random
- Phytochemical
- Taxonomic
- Ethnomedical

The arrangement of this research compilation will be by phytochemical category, with cross referenced notations certain of the plant species, where appropriate.

## 2.1 Structure elucidation of isolated compounds from Thai medicinal plants

In the past few years, the technology available for the determination of these parameters needed to deduce the stuctures of new natural products has advanced substantially. The most important single development in nmr spectroscopy as far as practical application is concerned is the ability to conduct correlation spectroscopy. Of the many variants of this technique that are available, three in particular have been of importance to the natural product chemist. These are i) $^{1}H$-$^{1}H$ COSY in which either two bond or long range couplings may be emphasized, ii) nOe COSY (NOESY) spectra in which proximate proton-proton relationships are displayed, and iii) $^{1}H$-$^{13}C$ COSY (hetcor) spectra in which the correlation between a carbon and its attached proton (s) is displayed. With these techniques, let's begin the journey through time and the strategies used for structure determination.

### 2.1.1 Sesquiterpenoids

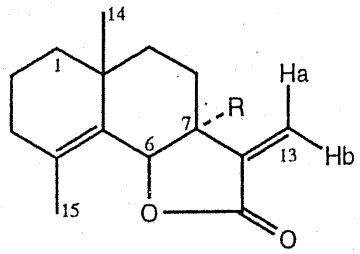
This group of compound is active principal of some traditional medicinal plants of Thailand. The distribution of sesquiterpenoids occuring in the forms of alcohol or lactone is available abundantly in the families Compositae, Magnoliaceae and Aristolochiaceae.

#### i. *GRANGEA MADERASPATANA* (1)

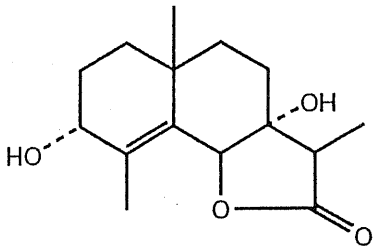
*Grangea* Adanson, in the subtribe Grangeinae of tribe Astereae (Compositae), is a genus of suberect or prostate annual herbs (2). Fourteen species of *Grangea* are found in tropical and subtropical Asia and Africa (3,4). *Grangea maderaspatana* Poir., known locally as "Phayaa-mutti," is the only species found widely and used medicinally throughout Thailand (4). This species is utilized in many countries for medicinal purposes. The leaves are used as a stomachic, a sedative, a carminative, an emmenagogue, and an antiflatulent (5). The plant is claimed to facilitate the return of menses after parturition if the delay is accompanied by abdominal and kidney pain (6). In Thailand, the whole plant has been used in folkloric medicine as a bitter tonic or a carminative and for treatment of flatulence and diarrhea (7). Previous reports have indicated the presence of the following compounds in this species : the diterpenes (-)-hardwickiic acid, *ent*-2β-hydroxy-15, 16-epoxy-3, 13(16), 14-clerodatriene-18-oic acid, and strictic acid, the steroids chondrillaterol and

8-acetoxypentadeca-1,9,14-trien-4,6-diyne (8-10). This is to report the structure elucidation of three sesquiterpene lactones including a new eudesmanolide isolated from *G. maderaspatana.*

The extraction and isolation of three components from the whole plant of *G. maderaspatana* are described in the Experimental, and the structure determination of these compounds will be discussed in the order in which they were eluted from a Si gel column. Compound 1 was a colorless solid which exhibited in its eims a parent ion at *m/z* 232 ($C_{15}H_{20}O_2$) and a base peak at 217, $[M\text{-}Me]^+$. An ir absorption at 1767 $cm^{-1}$ suggested the presence of an α-methylene-γ-lactone moiety, and this was




**1** R = H
**2** R = OH




**3**

confirmed by the $^1$H-nmr spectrum of the substance. The latter spectrum was the same as that reported previously for (-) -frullanolide (11) and established that **1** is this known eudesmanolide. In Table 1 is the report of the 400 MHz $^1$H-nmr spectrum of **1** [previous spectra were recorded at 60 MHz (11)] for comparison with the related components **2** and **3**. The $^{13}$C spectrum of **1** has not been reported previously and is recorded in Table 2. Frullanolide is the allergenic component of a liverwort (12), and its levorotatory enantiomer has been isolated previously from *Frullania tamarisci* while its dextrorotatory form has been reported to be present in *Frullania dilatata* (11). The structure of **1** has been confirmed by synthesis (13).

The second component was a solid whose elemental analysis and eims (parent peak at *m/z* 248) were consistent with the molecular formula $C_{15}H_{20}O_3$. The ir spectrum suggested the presence of an α-methylene-γ-lactone (1772, 1655) and a hydroxyl group (3620 $cm^{-1}$). The $^1$H-nmr spectrum of **2** (Table 1) was similar to that of **1** and suggested the compound possessed a eudesmanolide carbon skeleton. A singlet for the H-6 resonance established that the hydroxyl group must be attached to C-7 and suggested that **2** was (-) -7α-hydroxyl-frullanolide. The base peak at *m/z* 233 in the eims of **2** indicated loss of a methyl group with formation of the stabilized tertiary allylic fragment shown in Figure 1. The same type of fragment also was produced from **1** (*m/z* 217) and appears to be

diagnostic of the $\Delta^{4,5}$-unsaturated eudesmanes. After the investigation of **2** has completed, a paper at a conference described the isolation of the same compound from *Sphaeranthus indicus* (14). A recent publication by another group also reported the isolation of **2** from *S. indicus* and confirmed the structure by an X-ray crystallographic study of a derivative (15). These latter investigators reported **2** was an oil, while we have isolated it in crystalline form and our optical rotation is somewhat higher no doubt that compounds are identical, but as our $^{13}C$ assignments differ substantially from those reported previously (15), our assignments are presented in Table 2. Comparisons with the $^{13}C$ spectra of **1** and **3** were of considerable assistance in making these assignments.

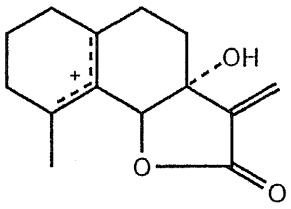



**Figure 1**. Structure of the base peak at *m/z* 233 from eims of **2**.

The third and most polar component was a higher melting solid whose accurate mass was consistent with the molecular formula $C_{15}H_{22}O_4$. In the eims of **3**, a fragment at *m/z* 251, $[M\text{-}Me]^+$, suggested this component was also a $\Delta^{4,5}$-eudesmane (see discussion of **2**) and the *m/z* 248 fragment, $[M\text{-}H_2O]^+$, indicated the compound underwent a facile dehydration reaction. In the ir spectrum of **3**, the absorption at 1771 $cm^{-1}$ indicated a $\gamma$-lactone function, but the absence of any absorption between 3000-3100 $cm^{-1}$ suggested an $\alpha$-methyl rather than an $\alpha$-methylene group was present. Also, the stronger absorption above 3600 $cm^{-1}$ as compared with **2** indicated the additional oxygen atom in **3** was present as a second hydroxyl group. The absorption at 1007 $cm^{-1}$ suggested this hydroxyl group was secondary and allylic (calculated value about 1010 $cm^{-1}$) (16); thus, it was placed on C-3. The $^1H$- and $^{13}C$-spectra of **3** (Tables 1 and 2, respectively) confirmed the suggestions above. In particular, the 3-proton a double at 1.21 in the $^1H$ spectrum established the presence of the C-13 methyl groups, and the triplet at 4.00 ppm was assigned to the carbinyl proton at C-3. With regard to the $^{13}C$ spectrum, it was established previously (17) that a hydroxyl group produced a pronounced downfield shift (about 40 ppm) of the carbon to which it is attached ($\alpha$ effect), a small downfield shift (5-10 ppm) of the $\beta$ carbon, and an upfield shift (a few ppm) of the $\gamma$ carbon. In the $^{13}C$ spectrum of **3** (Table 2) the C-3 resonance is shifted downfield by 36.2 ppm relative to C-3 in 2, C-2 (the $\gamma$ carbon) is shifted downfield by 7.0 ppm, and C-1 (the $\gamma$ carbon) is shifted upfield by 3.2 ppm. Thus, these

assignments confirm the placement of the second hydroxyl group in **3** at C-3 and also indicate the $^{13}C$ assignments for **1,2** and **3** are internally consistent. Finally, it was necessary to establish the stereochemistry of the 13-Me groups and the 3-OH function (assuming that the stereochemistry of all other substituents is the same as in **2**). A detailed $^{13}C$-nmr study of eudesmanolides showed that a β-methyl group at C-13 in a *cis*-fused γ lactone resonated at 9.6 while an α-methyl group in the same moiety appeared at 14.9 ppm (18). In **3**, the 13-Me appears at 7.3 ppm, which strongly suggests it is in a β configuration, and the additional upfield shift of 2.3 ppm (7.3 vs. 9.6) is caused by the γ effect of the 7-hydroxy substituent. The 3-OH group was assigned the α configuration and the carbinyl proton the β position (pseudo-equatorial) because of the equal couplings (tripler, $J$ = 3 Hz) of the latter with the H-2 protons. The chemical shift of H-3 (4.00 ppm) and its coupling constants are very similar to those reported for two eudesmanolides in which the 3-OH function was also assigned the α configuration (19,20). Thus, component **3** is a previously unreported eudesmanolide that we have given the name (+)-grangolide.

(-)-FRULLANOLIDE [**1**]-Mp 70-72° [lit. (12) mp 77°];$[\alpha]^{24}_D$-110° ($CHCl_3$) [lit. (12) $[\alpha]_D$-113° ($CHCl_3$)]; ir $\upsilon_{max}$ ($CCl_4$) 1767, 1264, 1142, 940 $cm^{-1}$; $^1H$ nmr see Table 1; $^{13}C$nmr see Table 2 ; eims *m/z* (rel. int.) $[M]^+$ 232 (16), 217 (100), 171 (21).

(-)-7α-HYDROXYFRULLANOLIDE [**2**] - MP 69-71° [lit. (14) 59-60°, (15) oil]; $[\alpha]^{24}_D$-76° (c = 1.09, $CHCl_3$) [lit.(15) $[\alpha]^{24}_D$-57° (c = 0.43, $CHCl_3$)]; ir $\upsilon_{max}$ ($CCl_4$) 3620,3010, 2933, 1772, 1655, 1142, 956 $cm^{-1}$; $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 248 (19), 233 (100), 230 (8), 178 (18). Calcd for $C_{15}H_{20}O_3$, C 72.55, H 8.12; found C 72.33, H 8.30.

(+)-11α, 13-DIHYDRO-3α, 7α-DIHYDROXYFRULLANOLIDE[(+)-GRANGOLIDE] [**3**] - MP 135-139° $[\alpha]^{24}_D$ + 12° (c = 2.76,$CHCl_3$); ir $\upsilon_{max}$ ($CHCl_3$) 3620, 2940, 1771, 1007, 970 $cm^{-1}$ ; $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 266 (58), 251 (27), 248 (53), 164 (76), 123 (100); hrms *m/z* $[M]^+$ 266.1516 (calcd for $C_{15}H_{22}O_4$, 266.1518).

**Table 1** $^{1}$H-Nmr Spectra of **1**, **2** and **3**[a]

| Proton | Compound | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| 1α | 1.35 td (13.2,3.3) | 1.40 m | 1.69 t (14.0) |
| 1β | 1.43 td (13.2, 3.5) | 1.45 m | 1.77 br d (14.0) |
| 2α | 1.62 m | 1.65 m | 2.0 m |
| 2β | 1.82 m | 1.83 m | 2.0 m |
| 3α | 2.09 br d | 2.12 m | - |
| 3β | 2.11 m | 2.12 m | 4.00 t (3.0) |
| 6 | 5.27 d (5.9) | 5.00 s | 4.89 s |
| 7 | 2.95 dt (10.0, 5.9) | - | - |
| 8α | 1.70 m | 1.68 m | 1.36 br d (13.0) |
| 8β | 1.65 m | 1.99 td (13.2, 3.6) | 1.54 t (13.0) |
| 9α | 1.27 dd (13.2, 12.3, 4.4) | 1.49 td (13.2,3.1) | 1.52 m |
| 9β | 1.48 dt (13.2, 3.9) | 1.68 m | 1.72 m |
| 11 | - | - | 2.79 q (7.2) |
| 13α | 5.58 d (1.0) | 5.81 s | 1.21 3H d (7.2) |
| 13β | 6.16 d (1.0 ) | 6.27 s | - |
| 14 | 1.08 s | 1.09 s | 1.04 s |
| 15 | 1.76 s | 1.78 s | 1.96 s |
| OH | - | 2.30 br s | 2.65 br s |

Coupling constants are in paretheses in Hertz.

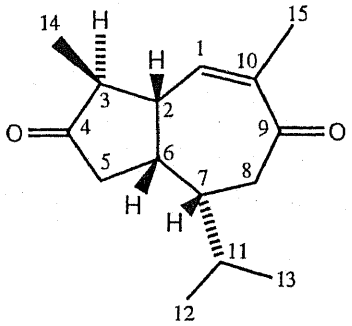
**Table 2** $^{13}$C-Nmr Spectra of **1**, **2** and **3**[a]

| Carbon | Compound | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| 1 | 39.1 (+) | 38.8 (+) | 35.6 (+) |
| 2 | 18.2 (+) | 18.2 (+) | 25.2 (+) |
| 3 | 33.1 (+) | 33.1 (+) | 69.3 (+) |
| 4 | 138.5 (+) | 140.5 (+) | 140.5 (+) |
| 5 | 128.5 (+) | 126.8 (+) | 131.5 (+) |
| 6 | 75.9 (-) | 81.4 (-) | 80.5 (-) |
| 7 | 41.2 (-) | 76.0 (+) | 77.3 (+) |
| 8 | 25.0 (+) | 31.5 (+) | 28.5 (+) |
| 9 | 37.9 (+) | 34.9 (+) | 34.9 (+) |
| 10 | 32.6 (+) | 32.7 (+) | 31.6 (+) |
| 11 | 142.3 (+) | 144.7 (+) | 48.6 (+) |
| 12 | 170.9 (+) | 169.1 (+) | 177.0 (+) |
| 13 | 120.1 (+) | 121.0 (+) | 7.3 (-) |
| 14 | 25.8 (-) | 26.1 (-) | 24.2 (-) |
| 15 | 19.3 (-) | 19.4 (-) | 17.7 (-) |

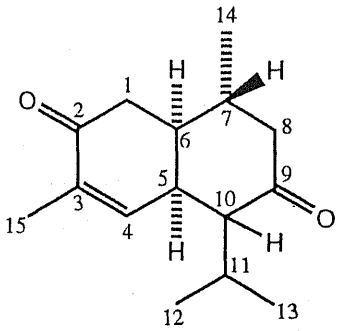
Chemical shifts are in ppm from TMS; solvent for **1** and **2** was $CDCL_3$; solvent for **3** was $Me_2CO$-$d_6$

## ii *EUPATORIUM ADENOPHORUM* (21)

*Eupatorium adenophorum* Spreng.(Compositae), Saap maa, is native to the Chiang Mai Province of Thailand, and is claimed to be useful in traditional Thai medicine. Recently, the binominal name for this plant has been changed to *Ageratina adenophora* (Spreng.) R. King and H. Robinson (22). According to A Geographical Atlas of World Weeds, the *Ageratina adenophora* (Spreng.) R. King and H. Robinson is synonymous with *Eupatorium adenophorum* Spreng (23). Previous phytochemical study on this plant reported the isolation (24) of a cadinane-type sesquiterpene 9-oxoageraphorone (**5**), which was also obtained together with its epimer **6** from *Eupatorium trapezoideum* Kunth (25), subsequently renamed as *Ageratina trapezoidea* (Kunth) R. King and H. Robinson.



**4**



**5** 10-H α
**6** 10-H β

We report here on the isolation and structure elucidation of eupatorenone (**4**), the first representative of a new bicyclic sesquiterpene skeleton, which was obtained by chromatographic separation of the petroleum ether soluble part of the ethanolic extract of the whole plant of *Eupatorium adenophorum*. Mass spectrometric analysis of eupatorenone (4),mp 66-67°C, $[\alpha]_D$+72.2° (MeOH,c 1.3),indicated a molecular ion at *m/z* 234, corresponding to an elemental composition $C_{15}H_{22}O_2$. Intense absorptions in the UV (MeOH) spectrum at $\lambda_{max}$ 242 nm (log ε 3.86) and in the IR (KBr) spectrum at $\upsilon_{max}$ 1725, 1710, and 1605 $cm^{-1}$ suggested the presence of both saturated and α,β-unsaturated ketonic groups. The $^1H$ NMR spectrum of eupatorenone, obtained in $CDCl_3$ (Table 3), indicated the presence of an olefinic hydrogen ( δ 6.35, d, $J$ = 1.5 Hz), both allylic ( δ 1.73, d, $J$ = 1.5 Hz) and aliphatic ( δ 1.02, d, $J$ = 6.4 Hz) methyl groups, and an isopropyl group ( δ 0.88, d, $J$ = 6.1 Hz; 1.08, d, $J$ = 6.1 Hz; 2.00, m). Two geminally coupled methylene groups ( δ 2.08, 2.20, $J$gem = 8.8 Hz; and 2.54, 2.81, $J$gem = 16.6 Hz) were also observed in the molecule.

From the structural elements found, two types of bicyclic sesquiterpene structures could be proposed for eupatorenone; either a condensed cyclopentanone-cyloheptenone structure (**4**) or a cadinene skeleton comprised of a cyclohexanone and a cyclohexenone unit. Two isomeric sesquiterpenes with the latter skeleton, cadinanes **5** and **6**, have already been isolated from *Eupatorium trapezoidum* Kunth (syn. *Adenophora trapezoidea* (Kunth) R. King and H. Robinson), and their structures have been established by a combination of spectroscopic and chemical correlation studies, together with X-ray analysis of a derivative. The IR and $^{1}H$ and $^{13}C$ NMR spectral data of eupatorenone differ in numerous ways from the corresponding reported values for cadinanes **5** and **6**. The highest wavenumber for carbonyl absorption in the IR spectrum of **5** and **6** is 1700-1705 $cm^{-1}$ versus 1275 $cm^{-1}$ for eupatorenone. The $^{1}H$ NMR spectrum in $CDCl_3$ of **6** exhibits three overlapping methyl groups at 0.90 and a fourth methyl group at 1.60, whereas the corresponding values of eupatorenone are 0.88, 1.02, 1.08, and 1.73. In the $^{13}C$ NMR spectrum of **5**, two doublets and two triplets were reported at 45.2, 50.3 and 23.3, 33.3, respectively. The $^{13}C$ NMR spectrum of eupatorenone,however,shows two doublets at 28.1 and 39.24 and two triplets at 41.04 and 45.80. The reported $\alpha D$ values for cadinanes **5** and **6** are +156° and +72.2°, respectively, whereas the $\alpha D$ value of eupatorenone is +72.2°.

On the basis of the above listed spectroscopic differences, we perceived that eupatorenone could not be characterized by a cadinane structure such as **5** or **6**, where structure elucidation had been performed by reliable chemical derivatization and X-ray crystallography (25). Therefore, from the structural elements present and the coupling pattern of the homonuclear COSY spectrum (measured either in $CDCl_3$ or in pyridine-$d_5$), and unsaturated azulene skeleton was suggested for eupatorenone. The presence of a five-membered ring ketone explains the higher wavenumber carbonyl absorption of eupatorenone than that of cadinanes **5** and **6**. Optimum resolution of the $^{1}H$ NMR signals was achieved in pyridine-$d_5$ and in $CDCl_3$, with $C_6D_6$ yielding minimal signal dispersion (Table 3). The $^{1}H$-$^{1}H$ COSY spectrum indicated a long-range coupling between the allylic methyl protons ( $\delta$ 1.73) and the vinyl hydrogen 1-H (6.35), which itself was coupled to the anellated methine, 2-H ( $\delta$ 3.22). From the relatively small (> 4 Hz) coupling between 2-H and 6-H ( $\delta$ 2.28) a *cis* junction between the five-and seven-membered rings was indicated. An additional small coupling (>1 Hz), observed only in the COSY spectrum for the 2-H signal with 3-H at the $\delta$ 2.05, indicated the near 90° torsion angle between these two hydrogens. Consequently, the orientation of the methyl group at C-3 was deduced to be the same as that of the hydrogens at the anellation positions (2-H and 6-H). The 8-$H_2$ methylene protons showed a characteristic coupling pattern; the signal at $\delta$ 2.03 was a doublet of doublets, while the geminally coupled signal at $\delta$ 2.20 was a doublet,since only one of these two signals

shows coupling with 7-H (δ 2.05). The dd pair of 5-$H_2$ at δ 2.54 and 2.81, however, shows strong geminal coupling, and both signals are coupled with the resonance for 6-H at δ 2.28.

The relative stereochemistry of the substituents as well as certain conformational features of **4** were further established by NOE experiments. Irradiation of the allylic 2-H at δ 3.22 enhanced the C-3 methyl and 6-H methine signals, thereby supporting the *cis* orientation of these three substituents. No NOE enhancement could be expected between the allylic hydrogen and the methyl group in the case of cadinanes **5** and **6** due to the equatorial position of the methyl group. Irradiation of 1-H at δ 6.35 resulted in area increases at δ 2.05 (3-H) and δ 1.73 (10-$CH_3$),but no NOE effect was observed between 1-H and 3-$CH_3$. Separate irradiations of the two dd signals of 5-$H_2$ established the stereotopical nature of the two nonequivalent hydrogens. In the alternative cadinane struture no NOE interaction could be expected between the isopropyl methyl group and any of the methylene protons, assuming that in the thermodynamically preferred conformation the isopropyl group is equatorial. Irradiation of the signal at δ 2.81 resulted in an area increase for the isopropyl methyl groups, but irradiation of the signal at δ 2.54 did not result in any NOE effects being observed.

Further evidence for the unusual carbon framework and substitution pattern of eupatorenone (**4**) came from selective INEPT (26) experiments, which also permitted the unambiguous assignment of the $^{13}C$ NMR spectrum. The APT spectrum of **4** showed four methyl ( δ 15.45, 19.97, 20.28, and 20.93), two methylene ( δ 41.04 and 42.18, six methine ( δ 28.10, 31.78, 39.24, 42.18, 63.90, and 146.89), and three quaternary carbons ( δ 136.00, 198.10, and 212.87), of which the latter two could be assigned as carbonyl carbons. Irradiation of 10-$CH_3$ resulted in enhancements of δ 198.10 and 146.89, which could be assigned as C-9 and C-1, respectively, and irradiation of the isopropyl methyl group enhanced the aliphatic methine (C-7) at δ 28.10. Magnetization transfer *via* irradiation of 2-H with $^3J$ = 6 Hz confirmed the position of the carbonyl function in the five-membered ring leading to enhancements at δ 212.87 (C-4), 136.00 (C-10), 41.04 (C-5), and 28.10 (C-7). Finally, irradiation of 1-H enhanced C-9 at δ 198.10, thereby, confirming the carbonyl placement in the seven-membered ring, C-3 at δ 63.90, C-6 at δ 39.24, and C-15 at δ 15.45 (Figure 2). The assignments for the protonated carbons obtained from the selective INEPT experiments were fully in agreement with the HETCOR spectrum of eupatorenone (**4**), and the complete assignments are shown in Table 3.

Unlike saturated ketones or isolated olefins, α,β-unsaturated ketones are regarded as a class of inherently dissymmetric chromophores (27,28), consequently, no sector rules were applicable for the determination of the absolute configuration of the chiral centers of eupatorenone. The signs of then n-τ* (R band, 320-350 nm) and τ-τ* (K band, 220-260 nm) transitions of trans-enones, however, have been correlated with the sense of helicity for the dissymmetric type chromophore. Thus, the R band is positive and the K band is negative in the case where the helicity of the chromophore is skewed in a left-handed helix, they are opposite when the chromophore helicity is right handed (29,30). Since eupatorenone (**4**) exhibited a CD spectrum characteristic for the presence of a right-handed helicity of the trans-enone chromophore, the absolute configuration of the attached C-2 chirality center was established as S. Experimentally, the CD values in methanol were [θ] + 2150 and -840 at 248 and 300 nm, respectively. On this basis, and according to the prior determination of the relative steric positions of 2-H, 6-H, 3-$CH_3$, and 7-$CH_3$, the absolute configurations of the stereo centers of **4** are proposed as 2S, 3S, 6S, and 7R.

It should be noted that the reported $^1H$ NMR data of one of the cadinanes isolated previously (24) closely resembled those of eupatorenone (**4**). Unfortunately, no $^1H$-$^1H$ COSY measurements or $^{13}C$ NMR data are available for this compound, which might establish the relationship between this sesquiterpene and eupatorenone (**4**) or the possible identity of the two compounds.

Eupatorenone (**4**) having the following physical and spectroscopic properties:mp 66-67°C; $[\alpha]_D$+72.2° (MeOH,c 1.3);UV (MeOH) $\lambda_{max}$ (log ε) 242 (3.86) nm; IR (KBr) $\upsilon_{max}$ 1725, 1710, and 1605 cm-1; $^1H$ NMR, see Table 3; $^{13}C$ NMR, see Table 3; mass spectrum, *m/z* (rel intensity) 234 ($M^+$, 22), 232 (6), 216 (10), 192 (41), 117 (6), 164 (9), 150 (45), 136 (84), 121 (28), 109 (24), 69 (100), 55 (26); CD (MeOH) $[\theta]_{248}$+2150; $[\theta]_{300}$-840.

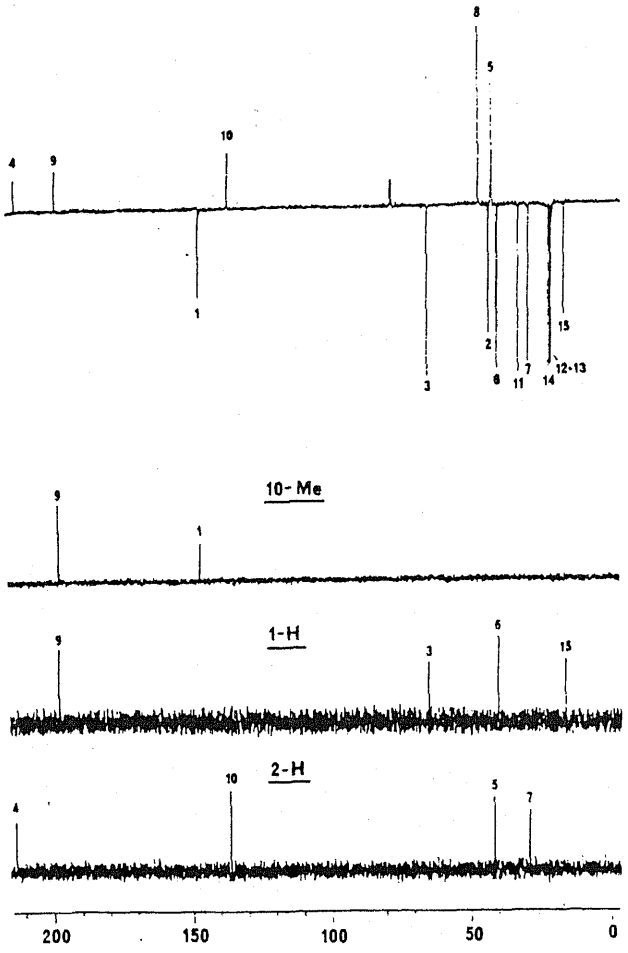



**Figure 2** APT and selective INEPT spectra of eupatorenone (**4**).

Table 3 $^{1}H$ and $^{13}C$ NMR Assignments of Eupatorenone (4)

| | $^{1}H^{a}$ | $^{1}H^{b}$ | $^{1}H^{c}$ | $^{13}C^{a}$ |
|---|---|---|---|---|
| 1 | 6.35 (d, 1.5) | 6.27 (d, 1.5) | 6.24 (d, 1.5) | 146.89 |
| 2 | 3.22 (ddd, 4.6, 4.0,2.2) | 3.15 (ddd, 4.8, 4.0, 2.3) | 2.60 (m) | 41.18 |
| 3 | 2.05 (m) | 2.15 (m) | 1.70 (m) | 63.90 |
| 4 | | | | 212.87 |
| 5a | 2.54 (dd, 16.6, 3.4) | 2.53 (dd, 16.4, 4.4) | 2.01 (dd, 16.6, 4.6) | 41.04 |
| 5b | 2.81 (dd, 8.8, 4.2) | 2.83 (dd, 16.4, 3.7) | 2.53 (dd, 16.6, 3.8) | |
| 6 | 2.28 (m) | 2.17 (m) | 1.65 (m) | 39.24 |
| 7 | 2.05 (m) | 1.94 (m) | 1.87 (m) | 28.10 |
| 8a | 2.08 (dd, 8.8, 4.2) | 2.14 (dd, 9.2, 4.2) | 1.83$^{+}$ (br d, 9.0) | 45.80 |
| 8b | 2.20 (d,8.8) | 2.19 (d, 9.2) | 1.93$^{+}$ (br d, 9.0) | |
| 9 | | | | 198.10 |
| 10 | | | | 136.00 |
| 11 | 2.00 (m) | 1.98 (m) | 1.58 (m) | 31.78 |
| 12 | 0.88$^{*}$ (d, 6.1) | 0.89$^{*}$ (d, 6.3) | 0.75$^{*}$ (d, 6.4) | 19.97$^{*}$ |
| 13 | 1.08$^{*}$ (d, 6.1) | 1.03$^{*}$ (d, 6.3) | 0.77$^{*}$ (d, 6.4) | 20.28$^{*}$ |
| 14 | 1.02 (d, 6.4) | 0.87 (d, 6.5) | 0.57 (d, 6.2) | 20.93 |
| 15 | 1.73 (d, 1.5) | 1.77 (dd, 2.0, 1.4 | 1.69 (d, 1.5) | 15.45 |

[a]Recorded in $CDCl_3$. [b]Recorded in pyridine-$d_6$. [c]Recorded in $C_6D_6$. $^{*,+}$Assignments may be interchanged; proton chemical shifts are reported at values (ppm) from internal TMS at 300 MHZ. Carbon chemical shifts are reported as values (ppm) at 90.8 MHz.

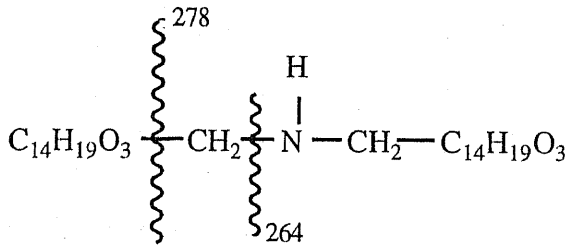
### iii *PARAMICHELIA BAILLONII* (31,52)

The genus *Paramichelia* Hu in the family Magnoliaceae has three species distributed in south and southeast Asia (32). This genus is very similar to that of *Michelia* L. in both external features and ethnomedical properties. *Paramichelia baillonii* (Pierre) Hu [synonymous with *Magnolia baillonii* Pierre, *Michelia baillonii* (Pierre) Fin. and Gagnep., *Aromadendron spongocarpum* (King)Craib., and *A. baillonii* (Pierrer) Craib.] is the only species found in northern Thailand and is known as "champipa" (33,34). The bitter bark of this plant has been used by the natives as a stimulant, febrifuge (35), and as substitute for champaca bark (*Michelia champaca* L.). A decoction of the bark of *M. champaca* has been used as a febrifuge, as a protective medicine for mothers after childbirth (35,36), and in India for the treatment of abdominal tumors (37). There have been no previous reports of

phytochemical studies on any part of *P. baillonii*,and in this article, the structural elucidation of six consituents of the bark of this plant has been reported.

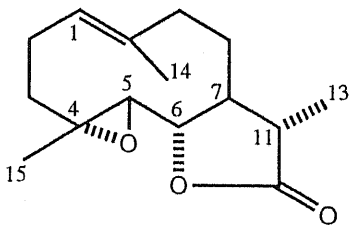
Chromatographic purification of the alcoholic extract of *P. baillonii*, as described in the Experimental section, provided the six components to be discussed. Examination of the spectroscopic data and the optical rotation of of **7** established clearly that this least polar component was (-)-dihydroparthenolide.The 400 MHz $^1$H-nmr spectrum of **7** is reported in Table 4 as previously only low resolution spectra with few proton assignments reported (38,39). The $^{13}$C spectrum of **7** is reported for the first time in Table 5. (-)-Dihydroparthenolide has been isolated previously from *Michelia lanuginosa* (40), *Michelia compressa* (39), and *Ambrosia artemisiifolia* L.(41). Similarly, component **8** was shown to be (-)-parthenolide on the basis of spectroscopic comparisons with literature data. The 200 MHz $^1$H assignments (42) for **8** are included in Table 4 for comparison with **7** and **9** and in a footnote we report additional assignments obtained from our 400 MHz spectrum and an $^1$H-$^1$H 2D-COSY experiment. The previously reported $^{13}$C spectrum of **8** (43) is included in Table 5 for comparison with the spectra of **7** and **9**, also. The structure and conformation of **8** were previously established unambiguously by single crystal X-ray analysis (44), and the optical rotation of this component from *P. baillonii* showed that it possessed the (6S) absolute configuration as depicted in **8** (45).

The third component, **9** was a crystalline solid and its eims (parent peak *m/z* 513, base peak 278)was consistent with a compound containing two sesquiterpenoid units and one nitrogen atom. Accurate mass determinations of the parent peak and two fragments (*m/z* 278 and 264, see Experimental section ) further supported the presence of a nitrogen atom and fragmentations as indicated below:



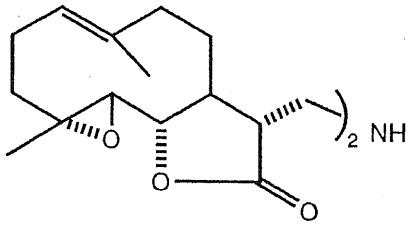

The ir spectrum displayed a strong absorption at 1770 (γ-lactone) and a weak band at 3365 cm$^{-1}$ (N-H). Both the $^1$H-and the $^{13}$C-nmr spectra (Tables 4 and 5, respectively) showed many similarities to the spectra of dihydroparthenolide [**7**] with the only significant differences being in the region of C(13). In particular, the two protons on C(13) in **9** appeared as an AB pattern ($J$ = 13.1 Hz) at about 3 ppm

with additional splitting ($J$ = 2.8 Hz), and in the $^{13}C$ spectrum the chemical shift of C(13) (46.2 ppm) and the attached proton test (APT) were consistent with the presence of a nitrogen atom on a methylene carbon. Assignments for the protons of **9** given in Table 4 were aided by a 2D-COSY experiment. On the basis of this spectroscopic information, we propose that this component is the sesquiterpenoid alkaloid **9**, formed by Michael addition of ammonia to two molecules of parthenolide. The aminomethyl group at C(11) is tentatively assigned the α-configuration because in the $^1H$-nmr spectrum of **9** in $C_6H_6$ solvent, H(7) appears as a well-resolved quartet ($J$ = 8.8 Hz) as a result of trans couplings with H(6), H(8β), and H(11). Presumably **9** is derived from (-)-parthenolide, which has a (6S)-configuration (45), so the same absolute configuration is assigned to this new alkaloid, which we have chosen to call bisparthenolidine.



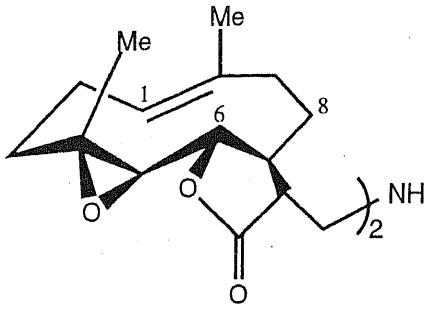

**7**

**8** = 11,13-dehydro **7**




**9**

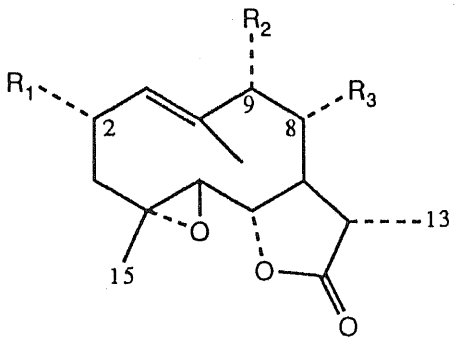
As component **9** is a new germacranolide derivative, we were interested in determining its conformation in solution using $^1H$-nOe experiments. Low-intensity irradiation of a degassed $CDCl_3$ solution of **9** at 1.67 ppm [the resonance for the C(14) methyl group] caused an increase in the intensity of the signals for H(2β), H(8β), and H(9β) of 43, 43, and 10%, respectively. In addition, irradiation of the H(6) resonance at 3.86 ppm resulted in a 47 and 9% enhancement of the H(8β) and C(15) methyl signals, respectively. Because there was interaction between the C(15) methyl group and H(6) but none between H(5) and H(6), the *trans* configuration of the C(4)-C(5) epoxide was confirmed. A 2D-nOe experiment (NOESY) (46) confirmed the interactions mentioned above but also revealed as weak cross-peak correlation between the C(14) and C(15) methyl signals, thus indicating the *syn* relationship between these two groups. The nOe results clearly indicate a conformation for this parthenolide derivative as shown in **9**a. A similar conformation has been reported for parthenolide [**8**] (44).




**9a**

As component **9** is a new and unusual natural product it deserves some comment. At no point in the isolation procedure was $NH_3$ used, and, thus, we are confident that **9** is not an artifact. The $^{13}C$-nmr spectrumof **9** shows only fifteen lines as would be expected for one pure diastereomer; a mixture of diastereomers would be expected if the component was formed chemically rather than in the plant. To our knowledge, **9** is the first reported example of a naturally-occurring germacranolide alkaloid, although a piperidine adduct of a pseudoguaianolide (47) and a tertiary amine derived from $NH_3$ and three molecules of α-methylenebutyrolactone (48) have been isolated previously from natural sources. A secondary amine related to **9** has been synthesized from $NH_3$ and two molecules of the eudesmanolide, alantolactone (49), and there have been numerous reports of reactions of secondary amines with the α-methylene group of germacranolides (50-51).

$^1H$-nmr analysis of the fraction that eluted after bisparthenolidine showed it was a 2:1:1 mixture of components **10**, **11** and **12** respectively. The three components were separated by flash chromatography, and the structural elucidation of each is described herein. Compound **10** exhibited ir absorptions at 3472 (broad) and 1777 $cm^{-1}$, indicating the presence of hydroxyl and γ-lactone functionalities, respectively. Its mass spectrum showed a weak parent ion at




**10** $R_1 = R_3 = H, R_2 = OH$
**11** $R_1 = OH, R_2 = H, R_3 = OAc$
**12** $R_1 = OH, R_2 = R_3 = H$

*m/z* 266 (the exact mass was consistent with the molecular formula $C_{15}H_{22}O_4$) and also showed an $[M-18]^+$ fragment, presumably from dehydration of the alcohol. Examination of the $^1$H-nmr spectrum of **10** revealed many similarities to the spectrum of dihydroparthenolide [7] (see Table 6 for comparisons). The $^1$H- and $^{13}$C-nmr spectra were most instructive in determining the position and stereochemistry of the hydroxyl group that had been indicated by the ir and ms spectra. A comparison of the$^{13}$C-nmr spectra of **10** and the model compound **7** (Table 5,7) indicated the major differences were in the region of C-9. It was established previously (53) that a hydroxyl group produced a pronounced downfield shift (about 40 ppm) of the carbon to which it is attached (αeffect), a smaller downfield shift (5-10 ppm) of the β-carbon, and an upfield shift (a few ppm) of the γ-carbon. In the $^{13}$C spectrum of **10**, the C-9 resonance is shifted downfield by 38.5 ppm relative to **7**, C-8 is shifted downfield by 8.1ppm, and C-7 (the γ-carbon) is shifted upfield by 3.4 ppm.These shifts establish that the hydroxyl group is on C-9. To determine the configuration of the hydroxyl group, a comparison of the $^1$H-nmr spectrum of 9α-hydroxyparthenolide (54) with **10** (a dihydroparthenolide derivative) is helpful. The H-9β resonance at spectrum showed a weak parent ion at *m/z* 266 (the exact mass was consistent with the molecular formula $C_{15}H_{22}O_4$) and also showed an $[M-18]^+$ fragment, presumably from dehydration of the alcohol. Examination of the $^1$H-nmr spectrum of **10** revealed many similarities to the spectrum of dihydroparthenolide [7] (see Table 6 for comparisons). The $^1$H- and $^{13}$C-nmr spectra were most instructive in determining the position and stereochemistry of the hydroxyl group that had been indicated by the ir and ms spectra. A comparison of the $^{13}$C-nmr spectra of **10** and the model compound **7** (Table 5,7) indicated the major differences were in the region of C-9. It was established previously (53) that a hydroxyl group produced a pronounced downfield shift (about 40 ppm) of the carbon to which it is attached (α effect), a smaller downfield

shift (5-10 ppm) of the β-carbon, and an upfield shift (a few ppm) of the γ-carbon. In the $^{13}C$ spectrum of **10**, the C-9 resonance is shifted downfield by 38.5 ppm relative to **7**, C-8 is shifted downfield by 8.1ppm, and C-7 (the γ-carbon) is shifted upfield by 3.4 ppm.These shifts establish that the hydroxyl group is on C-9. To determine the configuration of the hydroxyl group, a comparison of the $^{1}H$-nmr spectrum of 9α-hydroxyparthenolide (54) with **10** (a dihydroparthenolide derivative) is helpful. The H-9β resonance at δ 4.34 ppm in the former compound is in the same region as the proposed H-9β in **10** (4.11 ppm), and the coupling constants are also appropriate for a proton at the β position [i.e., a pseudo-equatorial position and, thus, no large coupling with the protons on C-8 (55) assuming the usual conformation in which the C-14 and C-15 methyls are *cis*]. Thus,we propose that component **10** is (-)-9α-hydroxydihydroparthenolide, a new germacranolide.

The ir spectrum of component **11**, exhibited absorptions at 3527 (hydroxyl), 1778 (γ-lactone),and 1735 $cm^{-1}$ (ester). The parent peak at *m/z* 324 was appropriate for the molecular formular $C_{17}H_{24}O_6$, and the fragment at *m/z* 264, $[M-HOAc]^+$, suggested the presence of an acetoxyl group. The $^{1}H$-and $^{13}C$-nmr spectra (Tables 6 and 7), along with the information above, suggested that **11** was a dihydroparthenolide containing hydroxyl and acetoxyl substituents, In the $^{1}H$-nmr spectrum, H-1 appears as a sharp doublet because of *trans* coupling with H-2β ($J$ = 10.2 Hz), while in **10** and **7** H-1 is a broad doublet because of coupling with both H-2 protons. Of the two unassigned resonances at 4.92 and 4.70 ppm, the more deshielded proton should be attached to the carbon bearing the acetoxyl group, and, consequently, we propose that the hydroxyl group is at the 2α position,and the H-2β resonance is at 4.70 ppm. Placement of the acetoxyl group at C-8 is consistent with the $^{1}H$-nmr spectrum, but, more conclusively, this location results in the anticipated shifts in the $^{13}C$-nmr spectrum relative to that of **7**. The C-8 resonance is downfield by 42.4 ppm (α effect; see related discussion for **10**), the C-7 and C-9 resonances are downfield by 4-8 ppm (β effect), and the C-6 resonance is shifted upfield by 3.5 ppm (γ effect). The 8-acetoxyl group is placed in the α position by analogy to the $^{1}H$-nmr spectrum to the closely related 8β-acetoxydihydroparthenolide (56). In the latter compound the H-8β resonance appears at 4.90, while in **11** it is found at 4.92 ppm. In 8-acetoxygermacranolides in which H-8 is α, this resonance appears much further downfield at about 5.7 ppm (57). The very significant deshielding of the C-13 methyl group in **11** relative to its position in **7** (18.3 vs. 13.2, respectively) suggests a *syn*-orientation of this methyl and the acetoxyl group (i.e., both groups α). Thus, we propose component **11** is 8α-acetoxy-2α-hydroxydihydroparthenolide, for which we suggest the name

paramicholide. For a related discussion of these assignments, see Jakupovic *et al.* (58).

In previous reports on the $^{13}C$-nmr spectra of germacranolides, the resonances assigned to C-2, C-3, C-8,and C-9 are usually indicated as being interchangeable (59). Comparison of the spectrum of dihydroparthenolide [7] with those of the oxygenated derivatives **10** and **11** enabled us to assign each of these resonances for this family of compounds. Clearly, the resonances at 24.0 and 36.6 ppm in **7** must be assigned to C-2 and C-3 because introduction of a hydroxyl group at C-9 in component **10** has little effect on the chemical shifts of these two carbons.The resonance at 36.6 ppm is assigned to C-3 because of the deshielding effect of the neighboring epoxide function. The resonance at 41.1 ppm in **7** is attributed to C-9 because a methylene carbon adjacent to a methyl group on a trisubstituted *trans* double bond, *trans*-[-CH=C($CH_3$)$CH_2$-],has been shown to be significantly deshielded relative to the other allylic carbon (60). Also, the chemical shifts predicted for these four carbons in **10** and **11** based on the $\alpha$, $\beta$, and $\gamma$ effects of oxygen substituents on the model system **7** are in excellent agreement with the actual values.

The ir spectrum of component **12**, suggested the presence of a $\gamma$-lactone and a hydroxyl group, and the $^1H$-nmr spectrum again indicated the compound was a substituted dihydropathenolide (Table 6). The H-1 resonance at 5.25 ppm was a sharp doublet ($J$=10.4 Hz) indicating (as discussed previously for **11**) that the hydroxyl group was attached to C-2 and was in the $\alpha$ position. The chemical shift for H-2$\beta$ is at essentially the same position in both **11** and **12**, suggesting the similarity of structures in this region of the molecules. A $^{13}C$ spectrum of **12** was not obtained because of the limited amount of sample available and because the sample contained a small amount of **11** as an impurity (relative Rf 0.20 and 0.22). Thus, we conclude that **12** is 2$\alpha$-hydroxyldihydroparthenolide.

To our knowledge, none of these three substituted dihydroparthenolides **10-12** has been isolated previously from natural sources. It is of interest from a biosynthetic perspective that, although the oxygenation of the parent dihydroparthenolide has taken place at three different positions in these constituents, in all cases the substituents were introduced on the $\alpha$ face of the molecule.

(-)-DIHYDROPARTHENOLIDE [7]. -Tlc (2% MeOH/$C_6H_6$)Rf 0.50, $[\alpha]^{19}_D$-57° (c 1.4, $CHCl_3$), lit. (38) $[\alpha]]^{26}_D$-62°($CHCl_3$); ir $\upsilon_{max}$ ($CCl_4$) 1775, 1650, 1450, 980 $cm^{-1}$; $^1H$ and$^{13}C$ nmr, see Tables 4 and 5, respectively; eims *m/z* (rel. int.) 250 ($M^+$, 2), 232 (3), 207 (4), 192 (13), 133 (19), 119 (32).

(-)-PARTHENOLIDE [**8**].-Tlc (2% MeOH/$C_6H_6$) Rf 0.42, (MeOH-EtOAc-petrol, 1:3:6) Rf 0.57; $[\alpha]^{20}_D$-78° ($CHCl_3$), lit. (61) $[\alpha]^{20}_D$-81.4° ($CHCl_3$); ir $\upsilon_{max}$ ($CCl_4$) 3020, 2920,1770, 1650, 1281, 1260, 1130, 940 $cm^{-1}$; $^1H$ nmr ($CDCl_3$), see Table 4; eims *m/z* (rel. int.) 248 ($M^+$, 2), 230 (9), 191 (25), 190 (61), 119 (100).

(-)-BISPARTHENOLIDINE [**9**]. -Mp 100-103° ($CHCl_3$);tlc (MeOH-EtOAc-petrol, 1:3:6) Rf 0.39; $[\alpha]^{20}_D$-112° ($CHCl_3$); ir $\upsilon_{max}$ ($CCl_4$) 3365, 3020, 2920, 1770, 1480, 1215,1175, 1000, 940 $cm^{-1}$; $^1H$ and $^{13}C$ nmr, see Tables 4 and 5, respectively; eims *m/z* (rel. int.) 513 ($M^+$, 8) 278 (100),264 (14); hrms (composition interpret., cald. millimass) 513.3077 ($C_{30}H_{43}NO_6$, $M^+$. 513.3090), 278.1752 ($C_{16}H_{24}NO_3$,M-$C_{14}H_{19}O_3$, 278.1756), 264.1600 ($C_{15}H_{22}NO_3$, M-$C_{15}H_{21}O_3$, 264.1594).

(-)-9α-HYDROXYDIHYDROPARTHENOLIDE [**10**].-Tlc MeOH-EtOAc-petroleum ether (10:20:70) Rf 0.32; $[\alpha]^{22}_D$-60° ($CHCl_3$); ir $\upsilon_{max}$ ($CHCl_3$) 3606, 3472 (br), 3550-3400, 2937,1777, 992 $cm^{-1}$; $^1H$ and $^{13}C$ nmr see Table 6 and 7 respecti-vely; eims *m/z* (rel. int.) $[M]^+$ 266 (5), 249 (9), 248 (9), 208 (28), 190 (29), 135 (64); hrms *m/z* 266.1527 $[M]^+$ (calcd for $C_{15}H_{22}O_4$, 266.1518).

8α-ACETOXY-2α-HYDROXYDIHYDROPARTHENOLIDE (PARAMICHOLIDE)[**11**].-Tlc MeOH-EtOAc-petroleum ether (10:20:70)Rf 0.22; ir $\upsilon_{max}$ ($CHCl_3$) 3526, 3400,3200, 2935, 1778, 1735,1645, 1235, 1020 $cm^{-1}$; $_1H$ and $^{13}C$ nmr see Tables 6 and 7,respectively; eims *m/z* (rel. int.) $[M]^+$ 324 (2), $[M-HOAc]^+$ 264 (3), 249 (7), 155 (45).

2α-HYDROXYDIHYDROPARTHENOLIDE [**12**]. -Tlc MeOH-EtOAc-petroleum ether (10:20:70) Rf 0.20; ir $\upsilon_{max}$ ($CHCl_3$) 3600, 3400-3200, 2931, 1774, 1008, 909 $cm^{-1}$; $^1H$ nmr see Table 6; eims *m/z* (rel. int.) $[M]^+$ 266 (1).

**Table 4** $^1$H-nmr Spectra of **7**, **8**, and **9**[a]

| Proton | Compound | | |
|---|---|---|---|
| | **7** | **8**[b] | **9** |
| 1 | 5.15 (dd, 2.3, 11.9) | 5.21 (d, br, 4.0, 12.2) | 5.27 (dd, 2.2,9.8) |
| 2a | 12.11(dddd.2.3,6.0,13.013.0) | 2.09-2.24 (m)[c] | 2.26 (d, 6.0, 12.1) |
| 2b | 2.37 (dddd, 5.0, 11.0, 13.3,13.0 | 2.46 (ddd, 13.8, 12.2, 12.5) | 2.40 (m) |
| 3a | 1.21 (ddd, 6.0, 13.0, 13.0) | 1.25 (m) | 1.23 (dt, 5.9, 13.9) |
| 3b | 2.16 (m) | 2.09-2.24 (m)[c] | 1.88 (dd, 5.9, 14.6) |
| 5 | 2.69 (d, 9.0) | 2.79 (d, 8.9) | 2.74 (d, 8.8) |
| 6 | 3.80 (d, 8.4, 9.0) | 3.86 (dd, 8.9, 8.3) | 3.86 (t, 8.8) |
| 7 | 2.28 (m) | 2.78 (m) | 2.40 (m) |
| 8a | 2.28 (m) | 2.09-2.24 (m)[c] | 2.18 (m) |
| 8b | 1.80 (m) | 1.73 (m) | 2.40 (m) |
| 9a | 1.80 (m) | 2.09-2.24 (m) | 2.10-2.18 |
| 9b | 2.25 (m) | 2.38 (m) | |
| 11b | 2.27 (dq, 6.8, 10.3) | - | 2.40 (m) |
| 13a | 1.25 (d, 6.8, $CH_3$)[d] | 6.34 (d, 3.6) | 3.15 (dd, 2.8, 13.1) |
| 13b | 1.25 (d, 6.8, $CH_3$)[d] | 5.62 (d, 3.1) | 2.92 (dd, 2.8, 13.1) |
| 14 | 1.68 (s) | 1.72 (s) | 1.67 (s) |
| 15 | 1.27 (s)[d] | 1.31 (s) | 1.30(s) |

[a]Chemical shifts are in ppm form TMS, coupling constants are in parentheses in Hertz, and the samples, were dissolved in $CDCl_3$.

[b]Previously assigned 200 MHz spectrum from Badesinsky et al. (42)

[c]Specific assignments possible at 400 MHz with 2D-COSY and decoupling experiments; H-2a, 2.38 (dd, 5.1, 13.1); H-3b 2.17 (m); H-8a, 1.72 (m).

[d]In $C_6D_6$ solvent the C-13 and C-15 methyls were clearly resolved into a doublet at 1.05 and a singlet at 0.98, respectively.

**Table 5** $^{13}$C-nmr Spectra of **7**,**8**, and **9**[a]

| Carbon | Compounds | | |
|---|---|---|---|
| | **7** | **8**[b] | **9** |
| 1 | 125.1 (-) | 125.3 | 125.3 (-) |
| 2 | 24.0 (+)[c] | 24.2[c] | 24.2 (+)[c] |
| 3 | 36.6 (+) | 36.2[c] | 36.5 (+)[c] |
| 4 | 61.4 (+) | 61.5 | 61.6 (+) |
| 5 | 66.3 (-) | 66.4 | 66.1 (-) |
| 6 | 82.1 (-) | 82.5 | 82.3 (-) |
| 7 | 51.9 (-) | 47.7 | 49.0 (-) |
| 8 | 29.7 (+)[c] | 41.2[c] | 30.2 (+)[c] |
| 9 | 41.4 (+) | 30.2[c,d] | 40.9 (+) |
| 10 | 134.4 (+) | 134.7 | 134.3 (+) |
| 11 | 42.4 (-) | 139.5 | 45.5 (-) |
| 12 | 179.6 (+) | 169.3 | 176.7 (+) |
| 13 | 13.2 (-) | 121.0 | 46.2 (+) |
| 14 | 17.1 (-) | 17.3 | 17.2 (-) |
| 15 | 16.8 (-) | 17.0 | 16.8 (-) |

[a]Chemical shifts are in ppm from TMS, solvent was $CDCl_3$, (+) and (-) are signs from the attached proton test.

[b]Data taken from El-Feraly *et al.* (43)

[c]Assignments may be interchanged.

[d]We believe the 41.2 ppm resonance chould be assigned to C-9.

**Table 6** $^1$H-nmr Spectra of **10**, **11** and **12**[a]

| Proton | Compound | | |
|---|---|---|---|
| | **10** | **11** | **12** |
| 1 | 5.32 (dd, 10.2) | 5.30 (d, 10.2) | 5.25 (d, 10.4) |
| 2a | 2.09 (m) | - | - |
| 2b | 2.41 (dddd, 12.5, 12.3, 11.9, 4.9 ) | 4.70 (ddd, 10.3, 10.2, 6.1) | 4.67 (m)[b] |
| 3a | 1.14 (dt, 12.5, 5.5) | 1.23 (dd, 11.4, 10.3) | 1.22 (dd, 10.9, 10.9) |
| 3b | 2.15 (br t, 12.3) | 2.58 (dd, 11.4, 6.1) | 2.56 (dd, 12.2, 5.9) |
| 5 | 2.55 (d, 8.8) | 2.73 (d, 8.8) | 2.77 (d, 9.0) |
| 6 | 3.75 (t, 8.7) | 3.95 (dd, 8.8, 8.8) | 3.79 (dd, 9.0, 9.0) |
| 7 | 1.9 (m) | 2.38 (ddd, 12.1, 8.8, 8.6) | 2.37 (m)[c] |
| 8a | 1.9 (m) | - | 2.37 (m)[c] |
| 8b | 1.9 (m) | 4.92 (ddd, 12.1, 8.2, 3.6) | 1.60 (m)[c] |
| 9a | - | 2.44 (m) | 1.63 (m)[c] |
| 9b | 4.11 (dd, 7.5, 3.9) | 2.44 (m) | 2.11 (m)[c] |
| 11b | 2.26 (dq, 11.4, 7.2) | 2.58 (dq, 8.6, 6.6) | 2.30 (dq, 6.9)[b] |
| 13 | 1.24 (d, 7.2) | 1.45 (d, 6.6) | 1.30 (d, 6.9)[b] |
| 14 | 1.68 (s) | 1.87 (d, 1.1) | 1.78 (s) |
| 15 | 1.26 (s) | 1.30 (s) | 1.30 (s) |
| | | 2.12 (s, OAc) | 1.57 (s, OH) |

[a]Chemical shifts are in ppm from TMS, coupling constants are in Hz, and the samples were dissolved in $CDCl_3$.

[b]These signals were irradiated during decoupling experiments.

[c]Assignments may be interchanged.

**Table 7** $^{13}$C-nmr Spectra of **10** and **11**[a]

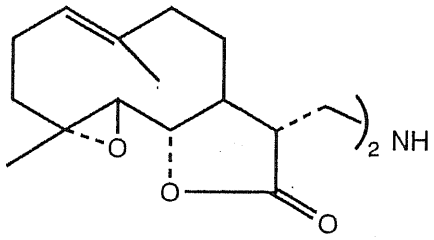
| Carbon | Compound | |
|---|---|---|
| | 10 | 11 |
| 1 | 216.3 (-) | 131.0 (-) |
| 2 | 23.8 (+) | 66.6 (-) |
| 3 | 36.9 (+) | 45.1 (+) |
| 4 | 61.3 (+) | 60.6 (+) |
| 5 | 66.1 (-) | 66.6 (-) |
| 6 | 81.3 (-) | 78.6 (-) |
| 7 | 48.5 (-) | 55.4 (-) |
| 8 | 37.8 (+) | 72.1 (-) |
| 9 | 79.6 (-) | 49.3 (+) |
| 10 | 136.5 (+) | 131.0 (+) |
| 11 | 42.1 (-) | 39.6 (-) |
| 12 | 176.9 (+) | 176.8 (+) |
| 13 | 13.2 (-) | 18.3 (-)[c] |
| 14 | 10.9 (-) | 18.2 (-)[c] |
| 15 | 17.3 (-) | 17.0 (-) |

[a]Chemical shifts are in ppm from TMS; solvent was $CDCl_3$.

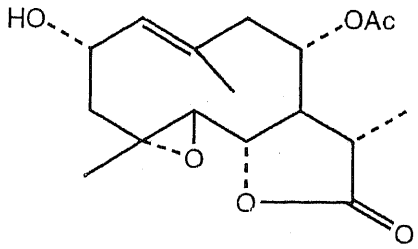
[c]Assignments may be interchanged.

## iv *MICHELIA RAJANIANA* (62)

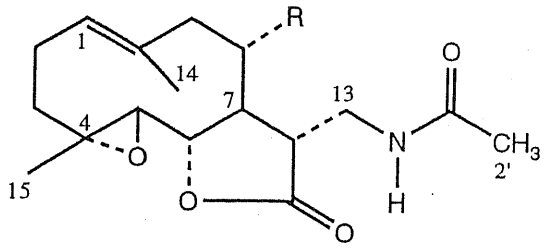
The species *Michelia rajaniana* Craib of the Magnoliaceae family is endemic to northern Thailand and is known to the natives as "Champi luang"(63,64). The timber of *M. rajaniana* is used extensively in the plywood and furniture industries (65), and the bark has been used medicinally as a substitute for *Michelia champaca* L. A decoction prepared from the bark of the latter species has been used as a febrifuge, as a protective medicine for mothers after childbirth (66,67), and in India for the treatment of abdominal tumors (68). There have been no previous phytochemical studies on any part of *M. rajaniana*, and in this report we describe the structural elucidation of five components isolated from the bark of the plant.




**9**




**11**




**13** R = H
**14** R = OH

Chromatographic purification of the alcoholic extract of the bark as outlined in the Experimental section gave five components, which will be discussed in the order they were eluted from the column. The least polar component was found to be identical to (-)-parthenolide, an epoxy germacranolide we identified previously as a constituent of *Paramichelia baillonii* (31). The second component was shown to be identical to bisparthenolidine[**9**], a compound first isolated by us from *P. baillonii* (31). The previously reported $^{1}$H-and $^{13}$C-nmr spectra of **9** are provided (Tables 8 and 9, respectively) for comparison with the new compounds **13** and **14**. The third component was found to be (-)-paramicholide [**11**], a substituted dihydroparthenolide which was isolated first from *P. baillonii* (52). The optical rotation of **11**, which was not reported in our previous study, is given here in detail.

The ir spectrum of the fourth component, **13**, exhibited absorptions at 1770 (s) (γ-lactone), 1670 (s), and 3442 cm$^{-1}$ (w) (secondary amide). The accurate mass of the parent peak at *m/z* 307 in the eims of **13** was consistent with the molecular formula $C_{17}H_{25}NO_4$, and the *m/z* 248 fragment $[M-H_2NAc]^+$ suggested the presence of an NH-acetyl moiety which was expelled *via* a McLafferty-type rearrangement. A comparison of the $^{1}$H- and $^{13}$C-nmr spectra of **9** and **13** (Tables **8** and **9**, respectively)suggested that these compounds were closely related, with the major differences being in the region of C-13 and the presence of acetyl resonances in **13**. Thus, we believe this component possesses the structure dipicted in **13** and suggest the name (+)-N-acetylparthenolidine for this new germacranolide alkaloid. (the names for **9**, **13**,and **14** are based on the

hypothetical compound parthenolidine,which is 13-aminodihydroparthenolide.) The nmr spectral data for **13** indicate that the N-acetyl group adopts the conformation shown in Figure 3 with the amide proton hydrogen bonding to the carbonyl oxygen of the lactone. In the $^{1}H$-nmr spectrum of **13** this amide proton appears as a downfield double of doublets because of different couplings with H-13α and H-13β, which are diastereotopic. Decoupling experiments were performed to confirm these assignments. In the $^{13}C$-nmr spectra, the C-13 resonance in **13** is shielded relative to the corresponding signal in **9** (36.6 vs 46.2 ppm, respectively) as would be expected for a syn orientation of the amide carbonyl group and C-13 (Figure 3) (69,70). Interestingly, the H-13 protons in **13** are deshielded relative to those in **9**. A similar reversal of shifts for the carbon and hydrogen resonances (i.e., carbon shielded while the attached protons were deshielded) in amides has been reported previously (71). To our knowledge, components **13** is the first reported example of a naturally occuring germacranolide amide.

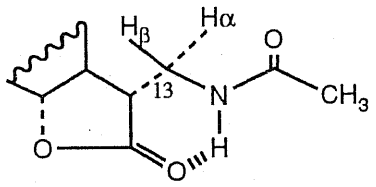



**Figure 3** Partial structure of **13** showing the conformation of the N-acetyl group.

The fifth component exhibited ir absorptions at 1768 (γ-lactone) and 1656 (amide) as well as a broad peak at 3300 $cm^{-1}$ (OH and NH). The mass spectrum and the elemental analysis both were consistent with the molecular formula $C_{17}H_{25}NO_5$. The $^{1}H$- and $^{13}C$-nmr spectra (Tables 8 and 9, respectively), along with the above information, suggested that **14** was a parthenolidine amide derivative containing an hydroxyl substituent. Most of the proton signals in the 400 MHz $^{1}H$-nmr spectrum of **14** were clearly resolved and rich in detail. A $^{1}H$-$^{1}H$ 2D COSY experiment and individual decoupling experiments were exployed to confirm the assignments reported in Table 8, and a few of these results will now be described. Irradiation of the signal for N-H at 6.81 ppm resulted in collapse of the multiple for H-13α at 4.10 to a doublet of doublets and collapse of the broadened doublet of doublets for H-13β at 3.58 ppm to a broadened doublet. Irradiation of H-13α caused sharpening of the N-H signal and simplification of the H-13β resonance but, more importantly, resulted in collapse of the doublet of doublets for H-11 at 2.99 ppm to a doublet ($J$ = 11.2 Hz).This large coupling between H-7

and H-11 clearly established the *trans* stereochemistry of these two protons and confirmed the α orientation of C-13 and the attached amide function. Irradiation of the doublet for the hydroxyl proton at 4.95 ppm changed the broadened quartet (actually a ddd) for H-8 at 3.76 to a broadened triplet (actually a dd). Also, when the $^1$H-nmr spectrum of **14** was obtained in $CDCl_3$ containing a trace of acid, the hydroxyl proton signal was shifted upfield to about 2.3 ppm (disappeared on addition of $D_2O$) because of proton exchange, and the H-8 resonance for *trans* couplings of H-8β with H-9α and H-7 and, thus, confirms the placement of the OH group in the α position. In other germacranolides with an 8α-OH, H-8β also appears at about 3.8 (72), while in related compounds with an 8β-OH, the H-8α resonance appears much further downfield at about 4.7 ppm (73). All of these decoupling experiments, plus additional ones not discussed here, support the structure proposed for **14**. Comparison of the $^{13}$C-nmr spectra of **13** and **14** (Table 9) was very instructive also. It was reported previously (74) that a hydroxy group produces a pronounced downfield shift (about 40 ppm) of the carbon to which it is attached (α effect), a smaller downfield shift (5-10 ppm) of the β carbon, and an upfield shift (a few ppm) of the γ carbon. The resonance for C-8 in **14** is shifted downfield by 42 ppm (α effect) as compared to the same carbon in **13** because of the hydroxyl group in the former. Likewise, C-7 and C-9 in **14** are shifed downfield by 5 and 11 ppm (β effect), respectively, relative to the same carbons in **13**. On the other hand, C-6, C-10, and C-11 in **14** are all shifted upfield by about 4, 4, and 2 ppm (γ effect), respectively, relative to **13**. Thus, the $^{13}$C-nmr results are completely consistent with the structure proposed for **14**, which is another previously unreported germacranolide amide for which we propose the name (+)-N-acetyl-8α-hydroxyparthenolidine.

(-)-PARTHENOLIDE [**8**].-Rotation,ir, $^1$H and $^{13}$C nmr, and eims agree with previously reported values (31).

(-)-BISPARTHENOLIDINE [**9**]. - Mp, rotation, ir, and eims agree with previously reported (31); values $^1$H and $^{13}$C nmr see Tables 8 and 9, respectively.

(-)-8α-ACETOXY-2α-HYDROXYDIHYDROPARTHENOLIDE (PARAMICHOLIDE) [**11**].-Ir, eims, $^1$H and $^{13}$C nmr agree with previously reported values (52); $[\alpha]^{24}_D$-47° (c= 0.76, $CHCl_3$).

(+)-N-ACETYLPARTHENOLIDINE [**13**].-Tlc [MeOH-EtOAc-petroleum ether (1:3:6)] Rf 0.37; $[\alpha]^{24}_D$ + 23° (c = 0.67, $CHCl_3$); ir $\upsilon_{max}$ ($CHCl_3$) 3442, 3010, 2928, 1770(s), 1670,909 cm$^{-1}$; $^1$H nmr see Table 7; $^{13}$C nmr see Table 8; eims *m/z* rel.

int.) $[M]^+$ 307 (25), 289 (11), 279 (10), 264 (11), 248 (14), 131 (100) hrms *m/z* 307.1788 $[M]^+$ (calcd for $C_{17}H_{25}NO_4$, 307.1783).

(+)-N-ACETYL-8α-HYDROXYPARTHENOLIDINE [**14**]. - MP188-190°; tlc [MeOH-EtOAc-petroleum ether (1:3:6)] Rf0.31;$[\alpha]^{20}_D$ + 51° (c = 0.75, $CHCl_3$; ir $\upsilon_{max}$ ($CHCl_3$) 3300 br, 2932, 1768, 1656, 1073, 937 $cm^{-1}$; $^1H$ nmr see Table 7; $^{13}C$ nmr see Table 8; eims *m/z* (rel. int.) $[M-H_2O]^+$ 307 (4), 256 (100), 214 (39), 156 (64). Calcd for $C_{17}H_{25}NO_5$; C 63.14, H 7.79, N 4.33; found C 62.57, H 7.96, N 4.19.

**Table 8** $^1H$-nmr Spectra of **9**, **13** and **14**[a]

| Proton | Compound | | |
|---|---|---|---|
| | **9** | **13** | **14** |
| 1 | 5.27 (dd, 9.8, 2.2) | 5.17 (br, d, 9.7) | 5.17 (br d, 9.1) |
| 2a | 2.26 (dd, 12.1, 6.0) | 2.17 (m) | 2.10 (m) |
| 2b | 2.40 (m) | 2.40 (m) | 2.35 (m) |
| 3a | 1.23 (td, 13.9, 5.9) | 1.21 (ddd, 13.0, 13.0, 6.0) | 1.15 (ddd, 12.9, 12.9, 6.6) |
| 3b | 1.88 (dd, 14.6, 5.9) | 2.10 (m) | 2.45 (ddd, 12.9, 12.9, 7.8) |
| 5 | 2.74 (d, 8.8) | 2.70 (d, 8.9) | 2.69 (d, 8.7) |
| 6 | 3.86 (dd, 8.8, 8.8) | 3.87 (dd, 8.9, 8.9) | 3.94 (dd, 8.7, 8.7) |
| 7 | 2.40 (m) | 2.40 (m) | 2.38 (m) |
| 8a | 2.18 (m) | 2.20 (m) | - |
| 8b | 1.70 (m) | 1.25 (m) | 3.76 (ddd, 11.2, 9.3, 9.3) |
| 9a | 2.10-2.18 (m) | 2.20 (m) | 2.35 (m) |
| 9b | 2.10-2.18 (m) | 2.20 (m) | 2.65 (br d, 12.7) |
| 11 | 2.40 (m) | 2.40 (m) | 2.99 (dd, 11.2, 5.2) |
| 13a | 3.15 (dd, 13.1, 2.8) | 3.66 (ddd, 14.1, 6.5, 3.5) | 4.10 (ddd, 12.9, 6.5, 5.2) |
| 13b | 2.92 (dd, 13.1, 2.8) | 3.53 (ddd, 14.1, 6.5, 6.3) | 3.58 (dd, 12.9, 6.5) |
| 14 | 1.67 (s) | 1.69 (s) | 1.72 (s) |
| 15 | 1.30 (s) | 1.30 (s) | 1.28 (s) |
| N-H | - | 6.31 (br dd, 6.5, 6.5) | 6.81 (dd, 6.5, 6.5) |
| Ac | - | 2.02 (s) | 2.08 (s) |
| OH | - | - | 4.95 (d, 11.2) |

[a]Chemical shifts are in ppm from TMS, coupling constants are in parentheses in Hertz, and the samples were dissolved in $CDCl_3$.

**Table 9** $^{13}$C-nmr Spectra of **9**, **13**, and **14**[a]

| Carbon | Compound | | |
|---|---|---|---|
| | **9** | **13** | **14** |
| 1 | 125.3 (-) | 125.1 (-) | 127.3 (-) |
| 2 | 24.2 (+) | 24.1 (+) | 24.4 (+) |
| 3 | 36.5 (+) | 36.3 (+)[b] | 35.8 (+) |
| 4 | 61.6 (+) | 61.7 (+) | 61.9 (+) |
| 5 | 66.1 (-) | 66.2 (-) | 66.0 (-) |
| 6 | 82.3 (-) | 82.9 (-) | 78.9 (-) |
| 7 | 49.0 (-) | 46.6 (-) | 51.5 (-) |
| 8 | 30.2 (+) | 29.8 (+) | 72.3 (-) |
| 9 | 40.9 (+) | 40.9 (+) | 52.4 (+) |
| 10 | 134.3 (+) | 134.6 (+) | 130.3 (+) |
| 11 | 45.5 (-) | 48.6 (-) | 46.8 (-) |
| 12 | 176.7 (+) | 176.6 (+) | 177.0 (+) |
| 13 | 46.2 (+) | 36.6 (+)[b] | 39.6 (+) |
| 14 | 17.2 (-)[b] | 17.2 (-)[c] | 17.5 (-)[b] |
| 15 | 16.8 (-)[b] | 16.9 (-)[c] | 17.3 (-)[b] |
| 1' | - | 170.7 (+) | 173.2 (+) |
| 2' | - | 23.2 (-) | 22.9 (-) |

[a]Chemical shifts are in ppm from TMS, solvent was $CDCl_3$, and (+) and (-) are signs from the attached proton test.

[b,c] Assignments the same column with the same superscripts may be interchanged.

## v *PLUCHEA INDICA* (97)

The genus *Pluchea* in the Compositae, tribe Inuleae, is composed of 50 species distributed in the New World and Far East. A number of *Pluchea* species are noted for their ethnomedical properties, of which the reputed emmenagogue (98-100) and abortifacient (101,102) activities of *Pluchea odorata* in the region of Central America and Caribbean are probably the best known. Extracts of *Pluchea lanceolata* have shown uterine relaxation activity at low doses (103) and possibly both antiimplantation and abortifacient effects (104).

*Pluchea indica* Less. (syn.*P.foliosa* D.C., *Coryza corymbosa* Roxb., *C. indica* Miq., *Baccharis indica* L.), also known as kukronda, has no established *in vitro* or *in vivo* activities, although in Thailand and Java the leaves and root have

been reported to possess astringent and antipyretic properties and are used as a diaphoretic in fevers. Fresh leaves are used in the form of poultices against atonic and gangrenous ulcers (105). Cigarettes prepared from the chopped stem bark are smoked to relieve the pain of sinusitis (106), and in Indo-China, the leaves and young shoots are crushed, mixed with alcohol, and applied to the back in cases of lumbago and also are used for rheumatic pains and in baths to treat scabies (107).

A number of compounds have been isolated from *Pluchea* species, the most characteristic of which are the eudesmane derivatives in the cuauhtemone series from *P. chingoyo* (108), *P. foetida* (109), *P. odorata* (110,111), *P. rosea* (112), and *P. suaveolens* (113). There have been no previous reports of any phytochemical studies on *P. indica.*

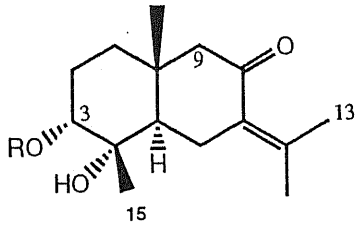
In this report we describe the isolation of a new cuauhtemone derivative **15** from *P.indica* and elucidate its structure through interpretation of the high-field proton nmr spectrum and hydrolysis to cuauhtemone (**16**).

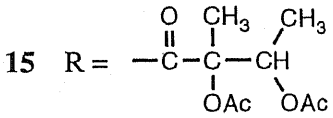
Mass measurement of the molecular ion of the isolate established a molecular formula $C_{24}H_{36}O_8$, and of particular interest in the mass spectrum were the ions at *m/z* 217($C_{15}H_{21}O$) and *m/z* 131($C_6H_{11}O_3$). Whereas the latter suggested a polyfunctional ester unit, the former indicated the nucleus to be a sesquiterpene. Based on prior phytochemical work with *Pluchea* species it was considered that the nucleus might be of the cuauhtemone type. From the ir spectrum, hydroxy, saturared ester, and α,β-unsaturated ketone functionalities were evident. From the $\lambda_{max}$ at 258 nm it was apparent that the ketone was α,β,β-trisubstituted (calcd 254 nm), and this was confirmed by the absence of any olefinic proton below 5.4 ppm.

The structure of the isolate was established by the complete assignment of the 400 MHz proton nmr spectrum through use of double resonance and INDOR techniques. Preliminary examination of the spectrum in comparison with published nmr spectral data for compounds in this series(109,110,113), indicated the presence of two acetates at 2.09, quaternary methyl groups at C-10 (0.98 ppm) and C-4(1.28 ppm), and two olefinic methyl groups at δ 1.86 (13-H3) and δ 2.10(12-H3). Two other three-proton singlets in the spectrum ( δ 1.26 and 1.66) were assigned to groups in an ester side chain. These data suggested a cuauhtemone derivative esterified with a side chain having the molecular formula $C_9H_{13}O_5$. The presence of a downfield quartet at δ 5.24 coupled to the three-proton doublet ($J$ =6 Hz) at δ 1.26 was confirmed through irradiation and indicated that this must be a 2,3-diacetoxy-2-methyl butyrate unit. It, therefore, remained for us to establish the location of the acylating unit.

Mild hydrolysis of the isolate afforded cuauhtemone (**16**) thereby establishing the skeleton and the location and stereochemistry of the hydro groups. This stereochemical array was confirmed through irradiation of the triplet ($J$ = 3 Hz) at δ 5.02, which considerably simplified through irradiation of the complex multiplet in the region δ 1.84-1.76. Irradiation at δ 1.81 confirmed the coupling to an equatorial 3β-H and also permitted assignment of the 1α-H to a slightly broadened doublet ($J$ = 15 Hz) at δ 1.49 and the 1β-H to a doublet at δ 1.33 in the decoupled spectrum.

Two aliphatic doublets ($J$ = 15 Hz) at δ 2.17 and 2.25 were assigned to the 9-$H_2$. Irradiation at δ 2.17 collapsed the signal at δ 2.25 to a singlet, and this coupling was further substantiated through INDOR experiments monitoring each proton successively. Individual assignment of the 9α-H and 9β-H resonances was based initially in prior data (109,110,113) for compounds in this series.




**16** R = H

The slightly broadened doublet of doublets ($J$ =4, 13 Hz) at δ 1.92 was assigned to the 5α-H, and irradiation collapsed the signal at δ 3.01 to a doublet ($J$ = 15 Hz), which must therefore be the 6α-H. Although significant changes were also observed in the region δ 2.15-2.27,it was only through irradiation at δ 2.17 that the signals at 3.01 and 1.92 were collapsed to doublets ($J$ = 4 Hz) permitting the assignment of the 6β-H. Only the methyleneprotons at C-2, both of which must be in the region δ 1.76-1.86 remained to be assigned. Because irradation at δ 1.49 (1α-H) simplified the region around δ 1.84 more than irradiation at δ 1.33, the 2β-H must be in this region and the 2α-H at about δ 1.81. Because of the non-first-order nature of this part of the spectrum, these values must be considered approximate.

That the ester group is a C-3 and the hydroxy groups is at C-14α (rather than in the side chain) is apparent from the 3β-H at δ 5.02 and the 4-$CH_3$ at δ 1.28. These data are in agreement with those for a 4-hydroxy derivative and not for the corresponding 4-acetyl derivative, where these signals are observed at δ 5.86 and

1.60, respectively (110). The isolate, therefore, has the structure 3-(2',3'-diacetoxy-2'-methyl butyryl)-cuauhtemone (**15**).

One further interesting observation was made; namely, that the 1α-,5α-and 9α-resonances were all slightly broadened. This was particularly clear for the 1- and 9-methylene protons where direct comparison could be made with the corresponding β-protons, which were invariably sharp. We suggest this broadening to be caused by weak coupling with the 10-methyl group. Indeed, the resolution enhanced, 400-MHz spectrum clearly shows this methyl group to be broadened significantly in comparison with the 5'-H3.

**15** was obtained as colorless prisms on standing, mp 165°,ir, $\upsilon_{max}$ (KBr) 3420, 2960, 2940, 2890, 1740, 1660,1585, 1445, 1390, 1370, 1240, 1200, 1120, 1070, 1020, 940, 870, 750.and 620 $cm^{-1}$; uv, $\lambda_{max}$ (EtOH) 258 nm (log ε 4.15); $^1$H-nmr, (400 MHz, $CDCl_3$) δ 0.98 (3H, bd, s, 14-H3), 1.26 (3H, d, *J* = 6 Hz, 4'-H3), 1.28 (3H, s, 15-H3), 1.33 (1H,dt, *J* = 3, 3, 15 Hz, 1β-H), 1.49 (1H, bd, ddd, *J* = 3, 12, 15 Hz, 1α-H), 1.66 (3H, s, 5'-H3), 1.81 (1H, m, 2α-H), 1.84 (1H, m, 2β-H), 1.86 (3H, s, 13-H3), 1.92 (1H, dd, *J* = 4, 13 Hz, 5α-H), 2.09 (6H, s, 2 x OAc), 2.10 (3H, s, 12-H3), 2.17 (1H,d, *J* = 15, Hz, 9α-H), 2.17 (1H, dd, *J* = 13,15 Hz, 6β-H), 2.25 (1H, d, *J* = 15 Hz, 9β-H), 3.01 (1H, dd, *J* = 4, 15 Hz, 6α-H), 5.02 (1H, t, *J* = 3 Hz,3β-H) and 5.24 (1H, q, *J* = 6 Hz, 3'-H); ms, *m/z* (rel. int., %) 452 ($M^+$,1), 434 (2), 218 (8), 217(47), 216 (26), 201(11),193 (5), 173 (5), 159 (8), 149 (6), 131 (22), 123 (9), 121 (8), 109 (8), 97(10), 95(15), 83 (12), 81 (13), 71 (16), 69 (19), 67 (12), 56 (27) and 55 (25). Mass measurements, Obsvd. 452.2412, $C_{24}H_{36}O_8$ requires 452.2410; 217.1594, $C_{15}H_{21}O$ requires 217.1591; 131.0704, $C_6H_{11}O_3$ requires 131.0707.

HYDROLYSIS OF **15**.-To a solution of **15** (11.4 mg) in anhydrous MeOH (10 ml) was added a small quantity of $Na_2CO_3$. After 2 h at room temperature, the solvent was removed *in vacuo*, cold $H_2O$ (10 ml) was added and the mixture extracted with $CHCl_3$ (5 x 10 ml), and with $H_2O$ until neutral, dried over $Na_2SO_4$ and evaporated *in vacuo* to afford a gummy mass (**16**, 4.4 mg), uv, $\lambda_{max}$ (EtOH) 258 nm (4.03); $^1$H-nmr (60 MHz, $CDCl_3$), δ 0.93 (3H, s, 15-H3), 1.21 (3H, s, 14-H3), 1.81 (3H, s, 13-H3), 2.01 (3H, s, 12-H3), 2.19 (2H, m, 9-H2), 2.67 (1H, br s, exchangeable with $D_2O$, 3-OH), 2.88 (1H, br s, exchangeable with $D_2O$, 4-OH), 3.01 (1H, m, 6α-H) and 3.65 (1H, br s, 3-H); ms, *m/z* (rel. int., %) 252 ($M^+$ ,6), 201 (11), 194 (20), 178 (6), 177 (4), 175 (4), 166 (6), 165 (6), 152 (17), 151 (11), 149 (9), 137 (9), 125 (21), 124 (15), 123 (11), 121 (11),110 (13), 109 (14), 95 (11), 84 (14), 83 (14), 81 (12) and 73 (64). These physical data are in agreement with

those obtained previously for cuauhtemone (**16**)(110),and identity was confirmed by comparison (tlc, ms) with an authentic sample.

### 2.1.2 **Amides**

Natural products possessing amide group are usually marked biological interests. For instance, the pungent substances capsaicin and piperine and the antigout colchicine are play as important natural drug products up to now. The distribution of naturally occuring amides is indefinite for chemotaxonomic consideration.

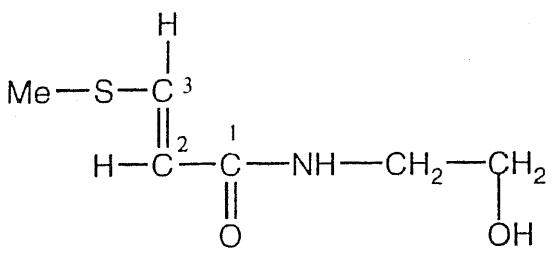
#### i. *ENTADA PHASEOLOIDES* (75,76)

*Entada phaseoloides* Merr. (Leguminosae) is a woody climber growing thoughout south-east asia and known commonly in Thai as "Sabaa mon". The seeds of this plants are utilizing as a folk medicine to treat skin diseases and as a soap plant in Thailand and other tropics. Barua *et al.* have shown that the seeds contain oleanolic acid and entagenic acid (77). This present investigation had led to the isolation of two new sulfur-containing compouds **17** and **18**, (entadamide A and entadamide B)

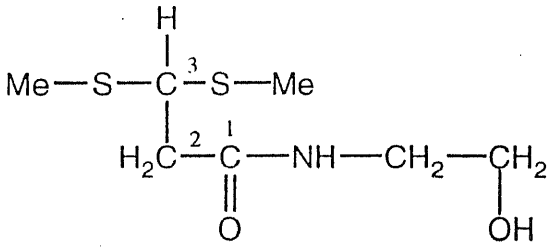
The molecular formula of **17** was determined to be $C_6H_{11}NO_2S$ ($M^+$, *m/z* 161.0554, calcd 161.0511) by high resolution MS(EI) measurement. The IR spectrum of **17** in $CHCl_3$ revealed bands at 3200-3500 $cm^{-1}$ (br,NH and OH), 1640 $cm^{-1}$ (C=O) and 1580 $cm^{-1}$ (C=C). The $^{13}$C-NMR spectrum in $CDCl_3$ indicated that the molecule was made up by one α,β-disubstituted olefine conjugated with a carbonyl group, one methyl, two methylene and one amide function, as shown in Table 10. Considering the $^{13}$C-NMR spectral data, all signals of the $^1$H-NMR spectrum of **17** in $CDCl_3$ were assigned as follows: δ7.64 ppm (1H, d, $J$ = 14.5 Hz, *trans*-C<u>H</u>=CHCO-), δ 5.68 ppm (1H, d, $J$ = 14.5 Hz, *trans*-C<u>H</u>=CHCO-), δ 6.20 ppm(1H, br, CONH, disappears on addition of $D_2O$), δ 3.69 ppm (2H, t, $J$ = 5 Hz, -OC<u>H</u>$_2$CH$_2$N), 3.20-3.55 ppm (3H, m, OH +-OCH$_2$CH$_2$N, becomes a 2H triplet ($J$ = 5 Hz) centered at δ 3.44 ppm on addition of $D_2O$), δ 2.32 ppm (3H, s, S-$CH_3$). From the aboue results, the structure of Entadamide A was elucidated to be *trans*-N-(2-hydroxyethyl)-3-methylthiopropenamide.

Entadamide B, **18**, $C_7H_{15}NO_2S_2$ ([M]$^+$ *m/z* 209.0553,calcd 209.0545), differed in composition by the increment $CH_4S$ from entadamide A. The IR spectrum ($CHCl_3$) of 18 showed bands at 3250-3550 $cm^{-1}$ (br, NH and OH) and 1660 $cm^{-1}$ (amide CO) like the spectrum of **17**. The $^1$H NMR and $^{13}$C NMR spectra ($CDCl_3$) of **18** also exhibited similar signals to those due to the -CONHCH$_2$CH$_2$OH moiety of **17**, as shown in Tables 10 and 11. The other signals of both spectra showed the presence of an

isolated =CH-CH2- moiety and two equivalent S-methyl groups in the molecule (**18**). From the above results, the structure of entadamide B was shown to be N-(2-hydroxyethyl)-3,3-bis (methylthio) propanamide.



Entadamide A



Entadamide B

**Table 10** $^{13}$C-NMR spectral data of entadamide A and B

| Carbon | Entadamide A* | Entadamide B* |
|---|---|---|
| C-1 | 165.9 (s) | 170.4 (s) |
| C-2 | 115.7 (d) | 42.9 (t)[a] |
| C-3 | 143.4 (d) | 50.4 (d) |
| NH-$CH_2$ | 42.6 (t) | 42.5 (t)[a] |
| $CH_2$-OH | 62.2 (t) | 61.8 (t) |
| S-$CH_3$ | 14.7 (q) | 13.4 (q) |

* (100 MHz, $CDCl_3$)

a Assignments may be interchange

**Table 11** $^1$H NMR spectral data of entadamide B in $CDCl_3$ ( δ ppm)

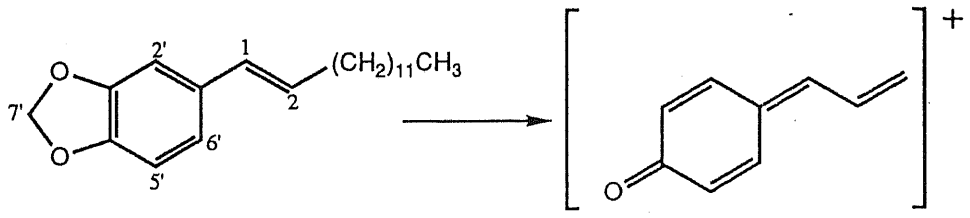
| δ | |
|---|---|
| 6.20 | (1H, *br*, conH-, disappears on addition of $D_2O$) |
| 4.15 | (1H, *t*, *J* = 7 Hz, C<u>H</u>-$CH_2$CO-) |
| 3.75 | (2H, *t*, *J* = 5 Hz, -OC<u>H</u>$_2$, $CH_2$N-) |
| 3.45 | (2H, *m*, -OCH$_2$C<u>H</u>$_2$, N-, becomes a 2H triplet (*J* = Hz) on addition of $D_2O$) |
| 2.68 | (2H, *d*, *J* = 7 Hz, CH-C<u>H</u>$_2$CO-) |
| 2.4 | (1H, *br*, -OH, disappears on addition of $D_2O$ |
| 2.16 | (6H, *s*, S-Me |

## ii. *PIPER SARMENTOSUM* (78)

The genus *Piper* in the Piperaceae family is composed of approximately 2000 species distributed primarily in tropical regions (79). A number of *Piper* species are noted for their ethnomedical properties, of which the reputed stimulant, carminative, diuretic and diaphoretic activities of *P. nigrum* are probably the best known(80,81).The species of interest in the present study, *Piper sarmentosum* Roxb., also known as "Cha-plu", has exhibited *in vitro* activity in the reduction of blood sugar in alloxan diabetic rabbits (82) and in Thailand the plant and fruit are used as an expectorant (80). In the Malay and Indonesian Archipelago, the leaves and roots of this species have been reported to provide an effective remedy for toothache, fungoid dermatitis on the feet, coughing, asthma and pleurisy (81). A previous investigation of *P. sarmentosum* yielded β-sitosterol and dihydrocinnamic acid as two constituents of the leaves (83). Herein, is the report of the isolation and structural elucidation of five components isolated from the dried fruit of *P. sarmentosum*. Four of these components are previously unreported natural products and two of these have been synthesized to confirm their structures.

Five of the compounds extracted from the dried fruit of *P. sarmentosum* were isolated as described in the Experimental section. The structural elucidation of these compounds will be described in the order in which they were eluted from the chromatography column.

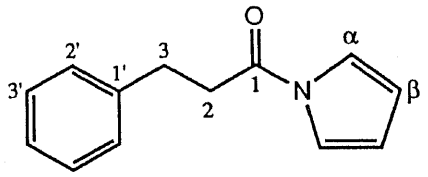
Compound **19**, the least polar component, was a low-melting solid which exhibited a parent peak in its electron impact mass spectrum(eims) at *m/z* 316 and an accurate mass consistent with the molecular formula $C_{21}H_{32}O_2$. The mass spectral fragment at *m/z* 131 (84) (see proposed structure below), the ultraviolet (85) and $^1H$ NMR spectra (85) all suggested the presence of a 3,4-methylenedioxy-phenyl moiety conjugated to an alkenyl side chain. The $^1H$ NMR also indicated that the configuration of the 1,2-double bond is *trans* ($J$ = 15.6 Hz) and both the $^1H$ and the




**19** *m/z* 131

$^{13}C$ NMR spectra showed that the side chain is linear. The structure proposed for this first component, **19**, has not been reported previously in the literature although related natural products with C11 (isolated from *P. longum* (86)) and C12 *trans*-alkenyl side chains (pipataline, isolated from *P. peepuloides* (85)) have been reported.

The second compound eluted from the column was a crystalline solid which exhibited an eims parent ion at *m/z* 199 and an accurate mass consistent with the formula $C_{13}H_{13}NO$. The infrared absorption at 1725 $cm^{-1}$ (unusual position for an amide) (87) and the strong ultraviolet absorption at 240 nm (88) both were consistent with the presence of an N-acylpyrrole moiety. Detailed analysis ofthe $^{1}H$ and $^{13}C$ NMR spectra indicated this component possesses structure **20** Because of the lower barrier to rotation about the carbonyl-nitrogen bond in acylpyrroles (87), the protons and carbons at the two α-positions of the pyrrole ring appear at the same position in the $^{1}H$ and $^{13}C$ NMR spectra, respectively, unlike the situation with saturated amides (to be discussed later). To our knowledge, **20** has neither been isolated from natural sources nor synthesized previously but it is worth noting that 3-phenylpropanoic acid, the parent acid of amide **20**, was isolated previously from the leaves of *P. sarmentosum* (83).



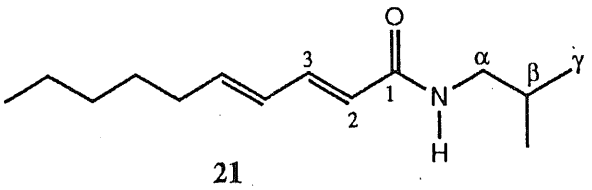

**20**

The eims ($M^+$, 223), infrared spectrum (C=O, 1673$cm^{-1}$), melting point, and nmr spectra all supported the conclusion that the third component is pellitorine, **21**. A detailed analysis of the 400 MHz $^{1}H$ NMR spectrum of **21** (89) is presented in Table 11a for comparison with **22** and **23** and in Table 11b the 100 MHz $^{13}C$ NMR data for **21** is reported for comparison with **22** and **23** also because the chemical shifts for several carbons differ from those reported previously (90). Pellitorine has been found in a number of *Piper* species as well as in the Compositae and the Rutaceae families (91). **21** has also been the target of numerous synthetic investigations (92) presumably because of its significant biological activity.

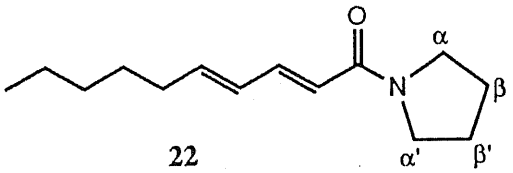
The fourth component, **22**, was an oil which exhibited a parent iont at *m/z* 221 in its eims and an accurate mass consistent with the formula $C_{14}H_{23}NO$. The infrared (C=O, 1650 $cm^{-1}$) and ultraviolet spectra ( $\lambda_{max}$ 274 nm) suggested the presence of a dienamide moiety and the $^{1}H$ NMR spectrum indicated that both double bonds in the

diene system were of the *trans*-configuration $J_{2,3}$ = 15.2, $J_{4,5}$ =14.1 Hz). The $^1H$ spectrum (Table 11a) also suggested that the nitrogen atom was present in a pyrrolidine ring and because of the higher barrier to rotation about this carbonyl-nitrogen bond (93) (as compared with the barrier in **20** (87), the α- or syn-protons resonate upfield from the $\alpha^1$-or anti-protons (93) (anti to the carbonyl group). Conversely, it was discovered by a $^1H$-$^1H$ 2D-COSY experiment that the β syn protons resonate downfield from the β' antiprotons. The $^{13}C$ NMR spectrum of component **22**, which is recorded in Table 11b, shows that the carbon chain beyond the diene moiety is linear and also exhibits four resolved resonances for the carbons of the pyrrolidine ring, with the α- or syn-carbon being assigned the more upfield resonance at 46.0 ppm (93). The evidence described above indicates that this fourth component is the previously unreported N-acylpyrrolidine, **22**, for which we propose the name sarmentine.

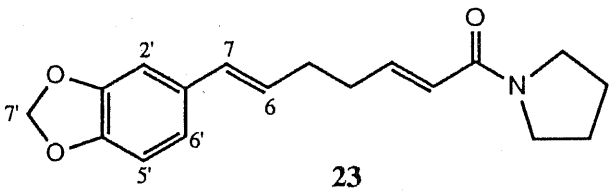
The fifth and final component in this study, **23**, exhibited in its eims a parent ion at *m/z* 299 and an accurate mass appropriate for a molecular formula of $C_{18}H_{21}NO_3$. The IR and UV spectra suggested the presence of an unsaturated amide function and the base peak in the eims at *m/z* 131 indicated a partial structure consisting of a double bond conjugated to a 3,4-methylenedioxyphenyl substituents (i.e. aryl-CH=CH-$CH_2$-, see discussion of eims of **19**). Analysis of the $^1H$ (Table 11a) and $^{13}C$ NMR spectra (Table 11b) for this compound indicated that the two double bonds present in **23** were of the *trans*-configuration ($J_{2,3}$ = 15.2, $J_{6,7}$ = 15.6 Hz) and that the nitrogen atom was again present in a pyrrolidine ring. All of this spectroscopic data supports the conclusion that this compound is another unsaturated N-acylpyrrolidine, **23**, for which we propose the name sarmentosine.




21




22




23

**1-(3,4-methylenedioxyphenyl)-1E-tetradecene, 19**

TLC (1% EtOAc/petrol) Rf 0.70; MP 33.5-36.5°C; UV (EtOH) $\lambda_{max}$ 216 nm (20,000), 260 (12,000), 268 (11,500), 312 (5,300); IR ($CCl_4$) 3010, 2920, 1500, 1480, 1245, 1040 $cm^{-1}$; $^1H$ NMR, 400 MHz ($CDCl_3$) δ 6.91 (1,s,H-2'),6.75 (2,m,H-5' and 6'), 6.29 (1,d, $J$ = 15.6 Hz, H-1), 6.06 (1,dt, $J$ = 15.6, 6.5 Hz, H-2), 5.94 (2,s,H-7'), 2.17 (2,dt, $J$ = 7.2, 7.0 Hz, H-3),1.45 (2,m,H-4), 1.28 (18, m, H-5 to H-13), 0.89 (3,t, $J$ = 6.4 Hz), assignments confirmed by 2D-COSY experiment; $^{13}C$ NMR, 100 MHz ($CDCl_3$) 147.9 (+,C-3'),146.5 (+,C-4'),132.5 (+,C-1'), 129.5(-,C-1), 129.2(-,C-2),120.1(+,C-6'), 108.2(-,C-5'), 105.3(-,C-2'), 100.9(+,C-7'),32.9(+), 31.9(+), 29.6(+), 29.5(+), 29.3(+), 29.2(+), 22.6(+), 14.1(-,C-14); MS (rel. int.) *m/z* 316 ($M^+$,4),288(82),161(69), 131(100);exact masses calcd. for $C_{21}H_{32}O_2$ 316.2402 and $C_9H_7O$ fragment 131.0494, found 316.2400 and 131.0496, respectively.

**N-(3-phenylpropanolyl)pyrrole, 20**

TLC (5% EtOAc/petrol) Rf 0.75;MP 48.5-50.0°C; UV(EtOH) $\lambda_{max}$ 205 nm (2500), 240 (8000) ; IR ($CCl_4$) 3060, 3030, 2940, 1725, 1470, 1370, 1325, 700 $cm^{-1}$; $^1H$ NMR, ($CDCl_3$) δ 7.27 (7,m,H-2',-3',-4' and -α) 6.27 (2,t, $J$ = 2.5 Hz, H-β), 3.12 (2,t), 3.11 (2,t,H-2 or -3); $^1H$ NMR ($C_6D_6$) δ 7.09 (5,m,H-3',-4', and -α), 6.94 (2,d, $J$ = 7.3 Hz, H-2');6.07 (2,t, $J$ = 2.3 Hz, H-β), 2.78 (2,t, $J$ = 7.6 Hz), 2.37(2,t, $J$ = 7.6, Hz,H-2 or -3); $^{13}C$ NMR ($CDCl_3$) 164.6 (C-1),140.2 (C-1'), 128.7 (C-2'), 128.4 (C-3'), 126.5 (C-4'), 118.9 (2 x C-α), 113.2 (2 x C-β); 36.4, 30.4 (C-2 or

-3)MS (rel. int.) *m/z* 199 ($M^+$, 58), 105 (55), 91(100), 77(21), 65(17); exact masses calcd. for $C_{13}H_{13}NO$ 199.0997, found 199.1005.

**N-isobutyl-2E,4E-decadienamide (pellitorine), 21**

TLC (5% EtOAc/petrol) Rf 0.10; MP 66-68°C (lit. (91) 69°C); IR and MS the same as those reported previously (94); $^1H$ and $^{13}C$ NMR, see Tables 11a and 11b, respectively.

**N-(2E,4E-decadienoyl)pyrolidine (sarmentine), 22**

TLC (30% EtOAc/petrol) Rf 0.42; a yellow oil; UV (EtOH) $\lambda_{max}$ 274 nm (16,900); IR ($CCl_4$) 2940, 2920, 2860,1650, 1635, 1610, 1420 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Tables 11a and 11b, respectively; MS (rel. int.) 221 ($M^+$,39), 192(6), 178(14),150(100), 81(74) ; exact masses calcd. for $C_{14}H_{23}NO$ 221.1780 and $C_9H_{12}NO$ fragment 150.0920, found 221.1778 and 150.0917, respectively.

**N-[7-(3,4-methylenedioxyphenyl)-2E,6E-heptadienoyl]pyrrolidine (sarmentosine), 23**

TLC (30% EtOAc/petrol) Rf 0.32; MP 77.5-79.5°C;UV (EtOH) $\lambda_{max}$ 217 nm ( ε 36,700), 267(18,700), 309(8,900);IR($CCl_4$) 2865, 1660, 1615, 1480, 1245, 1040 $cm^{-1}$, $^1H$ and $^{13}C$ NMR, see Tables 11a and 11b, respectively; MS (rel. int.) 299 ($M^+$,16), 201(18), 131(100), 100(57); exact mass calcd.for $C_{18}H_{21}NO_3$ 299.1521, found 299.1526.

**Table 11a** $^1H$ NMR Data for **21**, **22**, and **23**[a]

| H | 21 | 22 | 23 |
|---|---|---|---|
| 2 | 5.76 (d, 15,0) | 6.01 (d, 15.2) | 6.13 (d, 15.2) |
| 3 | 7.19 (dd, 15.0, 10.0) | 7.21 (dd, 15.2, 9.5) | 6.92 (dt, 15.2, 6.2) |
| 4 | 6.10 (dd, 13.1, 10.0) | 6.08 (dd, 14.1, 9.5) | 2.36 (br s) |
| 5 | 6.12 (dt, 13.1, 7.0) | 6.10 (dt, 14.1, 7.0) | 2.36 (br s) |
| 6 | 2.14 (dd, 6.8, 7.3) | 2.08 (dd, 6.8, 7.0) | 6.02 (dt, 15.6, 6.2) |
| 7 | 1.42 (quint, 7.1) | 1.35 (quint, 6.9) | 6.32 (d, 15.6) |
| 8 | 1.30 (m) | 1.23 (m) | b |
| 9 | 1.30 (m) | 1.23 (m) | - |
| 10 | 0.89 (t, 6.9) | 0.81 (t, 4.3) | - |
| α | 3.16 (t, 6.5) | 3.44 (t, 6.9) | 3.50 (t, 7.0) |
| β | 1.80 (m) | 1.89 (quint, 7.0) | 1.95 (quint, 6.4) |
| β' | - | 1.79 (quint, 7.0) | 1.86 (quint, 6.4) |
| α' | - | 3.47 (t, 6.9) | 3.53 (t, 7.0) |
| γ | 0.92 (d, 6.7) | - | - |
| N-H | 5.60 (br, s) | - | - |

[a]Chemical shifts are in ppm from TMS, multiplicities and coupling constants in Hertz are in parentheses and the samples were dissolved in $CDCl_3$.

[b]Assignments of aryl protons : δ6.88 (br s, H-2'), 6.74 (m, H-5' and 6'), 5.94 (s, H-7')

**Table 11b** $^{13}C$ NMR Data for **21**, **22**, and **23**[a]

| Carbon | 21 | 22 | 23 |
|---|---|---|---|
| 1 | 166.4 (+) | 165.2 (+) | 164.7 (+) |
| 2 | 121.8 (-) | 119.8 (-) | 120.3 (-) |
| 3 | 143.2 (-) | 143.1 (-) | 144.4 (-) |
| 4 | 128.2 (-) | 128.6 (-) | 32.2 (+) |
| 5 | 141.3 (-) | 142.2 (-) | 31.6 (+) |
| 6 | 32.9 (+) | 32.9 (+) | 130.2 (-) |
| 7 | 28.5 (+) | 28.5 (+) | 127.5 (-) |
| 8 | 31.4 (+) | 31.4 (+) | b |
| 9 | 22.5 (+) | 22.5 (+) | - |
| 10 | 14.0 (-) | 14.0 (-) | - |
| α | 46.9 (+) | 45.9 (+) | 45.7 (+) |
| β | 28.6 (-) | 26.1 (+) | 26.0 (+) |
| β' | - | 24.3 (+) | 24.2 (+) |
| α' | - | 46.5 (+) | 46.4 (+) |
| γ | 20.1 (-) | - | - |

[a]Chemical shifts are in ppm from TMS with $CDCl_3$ as solvent.

[b]Chemical shifts for carbons 1' to 7' are : 132.0 (+), 105.3 (-), 147.9 (+), 108.1 (-), 122.2 (-), 100.9 (+), respectively. See reference (26) for assignments of related aromatic compounds.

### 2.1.3 **Alkaloids**

Alkaloids have played an important part in the development of the chemical and biological sciences. They were originally defined as pharmacologically active, nitrogen heterocyclic bases of plant origin. A definition which was a natural consequence of the early experiences of alkaloid isolation, and which focussed on plants which had been used as sources of drugs for centuries, or well known to be extremely toxic.

The alkaloid groups presented here is roughly devided based on the major amino acid from which they are derived.

#### 2.1.3.1 **Isoquinolines**

##### a. **Bisbenzylisoquinolines**

###### i. *CYCLEA BARBATA* (114,115)

*Cyclea barbata* (Wall.) Miers (Menispermaceae), a plant growing in East Asia and used in Thailand for the treatment of fevers associated with malaria. This plant has been studied previously and found to afford a number of alkaloids (116,117). In this investigation, five alkaloids, (+)-tetrandrine [**24**], (-)-limacine [**25**], (+)-thalrugosine [**26**], (+)-homoaromoline [**27**] and (-)-cycleapeltine [**28**],were found from the first alkaloidal fraction. Although these alkaloids are diversely distributed (116-

119), thus far, their complete and unambiguous $^{1}H$- and $^{13}C$-nmr analyses have not appeared due to the severe overlap of the proton and carbon signals from the corresponding positions of the dimeric units. As a result, some of the previously reported $^{13}C$ assignments of tetrandrine [**24**], limacine [**25**] and homoaromoline [**27**] require definition and revision. High field nmr, and particularly the use of various two-dimensional NMR techniques (COSY, ROESY, HETCOR and FLOCK), permitted these determinations.

Tetrandrine [**24**] was previously isolated from various plants, including *C. barbata*, and NMR parameters have been partially assigned (116-119). The $^{1}H$ NMR spectrum showed coupling signals for the four protons of the C'-ring, and according to convention(118), the most downfield signal ( $\delta$ 7.30, dd, $J$ = 8.2, 2.2 Hz) should be assigned to H-14'. A decoupling study on the $^{1}H$ NMR spectrum at 300MHz showed that H-14' coupled to H-13' ( $\delta$ 7.10, dd, $J$ = 8.2, 2.3 Hz) and H-10' ( $\delta$ 6.27, dd, $J$ = 8.2, 2.3 Hz), H-11'( $\delta$ 6.76, dd, $J$ = 8.2, 2.3 Hz) coupled to H-13' and H-10', and H-14 ( $\delta$ 6.86, dd, $J$ = 8.2, 2.2 Hz) coupled to H-13 ( $\delta$ 6.82, d, $J$ = 8.2 Hz) and H-10 ( $\delta$ 6.52, d, $J$ = 2.2 Hz). These relationships were confirmed by the COSY spectrum that also showed that H-1 ( $\delta$ 3.72) coupled to two H-$\alpha$ protons at $\delta$ 2.48 and 2.67, and that H-1' ( 3.84) coupled to the two H-$\alpha$' protons at $\delta$ 2.75 and 3.22.

The ROESY spectrum (Fig. 4) (120,121) showed the presence of NOE correlation contours between H-5 ( $\delta$ 6.26) and 6-OMe ( $\delta$ 3.70), H-13 ( $\delta$ 6.82) and 12-OMe ( $\delta$ 3.88), H-5'( $\delta$6.48) and 6'-OMe ( $\delta$ 3.33), and H-8' ( $\delta$ 5.96) and H-14'. Thus, H-14' is spatially closer to H-8' than H-10', which is not apparent from the planar diagram (118). The $^{1}H$ NMR data for the methyl and methine signals were consistent with the reported data (118), but there were no previous reports on the assignment of the six methylene groups, which are assigned here. A sample (70 mg) of **24** in about 0.4 ml $CDCl_3$, was used for the $^{1}H$ and also for $^{13}C$ NMR assignment. The $^{13}C$ spectrum of **24** originally showed only 22 separated aromatic carbon signals. However, after zerofilling to 32 K, two signals near $\delta$ 127.7 (C-4a and C-4a'), and two signals near $\delta$121.6 (C-11' and C-13'), were disclosed, permitting the emergence of all 24 aromatic carbon signals, consistent with the structure. The HETCOR spectrum (122) displayed each of the protonated carbons, and the FLOCK spectrum (Fig. 5) (123) indicated the long-range correlations between $^{1}H$ and $^{13}C$ through three bonds and two bonds.

Analysis of the corresponding cross-correlations revealed that the N-Me ( $\delta$ 2.30) coupled to C-1 ( $\delta$ 61.19) and C-3 ( $\delta$ 43.91), the N'-Me ( $\delta$ 2.58) coupled to C-

1' ( δ63.64) and C-3' ( δ 45.00), the H-1 coupled to C-8a ( δ 122.64), C-4a ( δ 127.72), C-α ( δ 41.70), C-8 ( δ 148.19) and C-9 ( δ 134.68), the H-1' coupled to C-α' ( 37.93), theH-5 ( δ 6.26) coupled to C-4 ( δ 21.81),C-8a,C-7 ( δ 137.63), C-6 ( δ151.18) and C-4a, the H-5' ( δ 6.48) coupled to C-7'( δ 143.57), C-6' ( δ 148.39), C-4' ( δ 24.92) and C-8a' ( δ 127.84), the H-8' (δ 5.96) coupled to C-6', C-7', C-1' and C-4a', the H-13 coupled to C-11 (δ 149.12), C-9 and C-10, the H-13' coupled to C-12' (δ 153.58), C-9' and C-11', the 6-OMe ( δ 3.70) coupled to C-6, the 7-OMe ( δ 3.15) coupled to C-7, the 12-OMe ( δ 3.88) coupled to C-12 and the 6'-OMe ( δ 3.33) coupled to C-6'. Therefore, this experiment permitted the complete assignment of the $^{13}C$ nmr data. Retrospective analysis of the HETCOR spectrum then disclosed the assignments of the partly overlapped signals of the six methylene groups as the H-3 signals at δ 2.87 and 3.47, the H-3' signals at δ 2.83 and 3.39, the H-4 signal at δ 2.39 and 2.89, the H-4' signals at 2.69 and 2.91, the H-α signals at δ 2.48 and 2.67, and the H-α' signals at δ 2.75 and 3.22. The unambiguous $^{1}H$ and $^{13}C$ nmr data 25 are shown in Tables 12 and 13, respectively. Comparison with the reported $^{13}C$ data for **24** (119) indicates that the data for C-8a, C-11, and C-7' should be revised. In addition, the parameters for the N-Me and N'-Me,and for C-α and C-α' now have been clearly assigned, and the assignments for the OMe signals have been defined.

Limacine [**25**] also was previously isolated from this and other plants, and partial assignment of the $^{1}H$ and $^{13}C$ nmr data has been reported (116-119). Like tetrandrine [**24**], a sample (70 mg) of **25** in about 0.4 ml $CDCl_3$ was used for this study, and a corresponding one-and two-dimensional NMR study led to the complete assignment of the $^{1}H$ and $^{13}C$ nmr data. Comparison with the reported $^{13}C$ data (Table 13), indicated that C-11 and C-7'should be revised. The N-Me, N'-Me, C-3, C-3', C-α and C-α' have now been clearly assigned, and assignments for the three OMe signals have been made.

Thalrugosine [**26**], a stereoisomer of limacine [**25**], was previously isolated from this and other plants, and partial assignments of the $^{1}H$ NMR data have been made(116-118). A sample (80 mg) of **26** in about 0.4 ml of $CDCl_3$ was used in this study, and the $^{1}H$ and $^{13}C$ nmr data have now been completely assigned by the use of COSY, ROESY, HETCOR and FLOCK techniques, and some of the signals differ from those of **25** (Tables 12 and 13). The major difference in the proton data between **25** and **26** is that the 6'-OMe signal of **26** is 0.5 ppm farther downfield than that of **25**, and the H-1 and H-5' resonances of **26** are about 0.2 ppm farther downfield than those of **25**, respectively, and the H-10, H-14 and H-1' signals of **26** are 0.2-0.3

ppm farther downfield than those of **25** respectively. Furthermore, the C-5, C-8, C-4a' and C-8a' signals of **26** are 2-3 ppm farther up- or downfield than those of **25**.

Homoaromaline [**27**] has been isolated previously from about ten plants, but this is the first reported isolation from *C. barbata* (116,117). In our study, it was found that its proton nmr spectra showed remarkable difference between dilute and concentrated solutions in $CDCl_3$, especially, one of the N-methyl signals moved upfield by more than 0.1 ppm. In this study, a sample (40 mg) of **27** in about 0.4 ml of $CDCl_3$ was used, and its complete $^1H$ and $^{13}C$ nmr data were assigned by a combination of COSY, ROESY, HETCOR and FLOCK experiments(Tables 12 and 13). Comparison with the reported $^1H$ nmr data (118) indicated that the assignments for N-Me and N'-Me should be revised. Comparison with the reported $^{13}C$ nmr data (117,124) indicated that the assignments of C-9, C-9', C-3, C-3', C-11, C-12, C-4a'. C-8a', 6-OMe and 6'-OMe also should be revised, and that C-4a, C-9', C-5, C-13, N-Me and N'-Me have been definitively assigned.

Cycleapeltine [**28**], a stereoisomer of homoaromoline **27**, was isolated previously from *C. peltata* (125), and this is the first isolation from *C. barbata* (116,117). A sample (13 mg) of **28** about 0.4 ml of $CDCl_3$ was used forthe nmr studies, and the $^1H$ and $^{13}C$ nmr data of **28** were assigned by the use of the COSY, ROESY, HETCOR and FLOCK tecnigues (Tables 12 and 13). The major differences between the proton data of **27** and **28** are that the H-10 signal of **28** is about 1 ppm farther downfield than that of **27**, and the signals for H-1, 6-OMe, and H-8 are about 0.2 ppm farther downfield than those of **27**. The major differences in the carbon data between **27** and **28** is that the C-a' signal of 29 is about 29 ppm farther downfield than that of **27**, but the C-3 signal of **28** is 5 ppm farther upfield than that of **27**. Futhermore, the signals for C-8,C-8a, $\alpha$, 9, 10, 12, 13 and 13' of **28** are 2-3 ppm farther upfield than those of **27**, and the signals for C-4, C-4a,C-4' and C-9' of **28** are 2-3 ppm farther downfield than those of **27**, respectively.

Alkaloids **24**, **25** and **26** belong to a groups of bisbenzylisoquinoline alkaloids with two ether linkages between 8-7' and 11-12', and their H-8' signal is farther upfield than that of H-5'. In contrast, in alkaloids **27** and **28**, which have ether linkages between 7-8' and 11-12', the H-8 signal is farther downfield than that of H-5. Futhermore, in contrast to the alkaloids **24**, **25** and **26**, and also in contrast to the normal bisbenzylisoquinolines with two or three ether linkages (with certain exceptions, such as candicusine, 12-O-desmethyllaberine, osornine) (116-118), alkaloids **27** and **28** showed the proton resonance of the N'-Me farther upfield than the signal for

the corresponding N-Me. A study to re-examine the spectra of other alkaloids with these structural characteristics is currently being undertaken.

Continuing studies of the alkaloids fraction afforded two new bisbenzylisoquinoline alkaloids, namely, (-)-2'-norlimacine [**29**], and (+)-cycleabarbatine [**30**]. (+)-Tetrandrine 2'-β-N-oxide [**31**] was also found in the extract. This alkaloid was isolated previously from this plant as the first bisbenzylisoquinoline N-oxide, but its structure was not fully determined (127). The known alkaloids (+)-berbamine, (-)-repandine, (+)-cycleanorine, (+)-daphnandrine, (-)-curine, (+)-coclaurine, and (-)-N-methylcoclaurine were also identified.

The mass spectrum of (-)-2'-norlimacine [**29**] is characteristic of a doubly bridged, tail-to-tail dimer with a molecular peak at *m/z* 594 (100%), corresponding to $C_{36}H_{38}N_2O_6$. A strong fragment ion at *m/z* 367 corresponds to the upper part of the molecule. These two fragments are 14 daltons less than the corresponding ions in the mass spectrum of limacine.

The $^1H$ nmr spectrum presents only one N-methyl group signal at δ 2.32 ppm which should be assigned to the left hand side isoquinoline unit (128). Compared to the $^1H$ nmr spectrum of (-)-limacine, the absence of a three proton singlet around 2.55 ppm indicates the presence of a secondary amine on the right hand side of the molecule. As expected, signals due to the protons situated near N-2' are shifted further downfield : H-1' at δ 4.17 ppm, instead of 3.87 ppm for limacine (126), the methylene protons at C-3' are at 3.20 ppm and 3.51 ppm instead of 2.83 and 3.49 ppm, and the protons at C-α' appear at 3.20 ppm and 2.98 ppm instead of 2.76 ppm and 3.22 ppm (128). The other resonances in the spectrum are similar to those observed for (-)-limacine (126,128).

A ROESY experiment showed a spatial relationship between the 2-N-methyl signal (2.32 ppm) and H-1 (3.74 ppm), as well as between the 2-NMe and H-4 (3.51 ppm). An effect between H-1' (4.17 ppm) and H-8' (6.07 ppm) was also visible. These results confirmed the presence of a N-2' secondary amine. Homonuclear COSY, HMQC and HMBC experiments permitted the complete assignment of the $^1H$ and $^{13}C$ spectra of (-)-2'-norlimacine [**29**] as presented around structures **29**a and **29**b.

The second new bisbenzylisoquinoline, (+)-cycleabarbatine [**30**], afforded a mass spectrum showing a molecular ion peak at *m/z* 608 ($C_{37}H_{40}N_2O_6$) accompanied by a base peak at *m/z* 381. The mass of the latter fragment corresponds to

$C_{22}H_{26}N_2O_4$, and indicates that the upper part of the molecule is substituted by two methoxyl groups and one hydroxyl group, with two methylated tertiary amines, or by three methoxyl groups and a N-methyl group and a secondary amine. The presence in the $^1H$ nmr spectrum of two three-proton singlets at $\delta$ 2.25 ppm and 2.58 ppm due to two N-methyl groups indicated the former structural hypothesis to be correct. Except for the absence of a three proton singlet near 3.75 ppm, the signals observed in the spectrum are similar to those observed in the spectrum of (-)-isotetrandrine (128). This similarity suggested that compound **30** was identical to C-O-demethyl-isotetrandrine.

The small absolute value of the positive specific rotation of **30** confirmed that (+)-cycleabarbatine belong to the tetrandrine subgroup and incorporates the 1R,1'S configuration (129).

A ROESY experiment confirmed these assignments. In particular, effects were observed between H-5' (6.55 ppm) and OMe-6' (3.55 ppm), H-1' (3.98 ppm) and H-8' (6.08ppm), 2'NMe (2.58 ppm) and H-1' (3.98 ppm), and 2-NMe (2.25 ppm) and H-1 (3.95 ppm). The $^1H$ nmr data are summerized on structure **30**.

The mass spectrum of (+)-tetrandrine 2'-β-N-oxide [**31**] displays a weak molecular ion at *m/z* 638 (16%) which corresponds to $C_{38}H_{42}N_2O_7$. The base peak at *m/z* 622 is due to the loss of an oxygen atom and suggests the presence of a N-oxide function. The other fragment ions are similar to those observed in the mass spectrum of(+)-tetrandrine, with a strong peak at *m/z* 395 (45%) due to the bistetrahydroisoquinoline fragment following facile clavage of the benzylic bonds (128).

The $^1H$ nmr spectrum (in $CDCl_3$, 500 MHz), presented around structure **31a**, indicated a three proton singlet due to a 2-N-Me at 2.34 ppm while the 2'-N-methyl group resonates further downfield than usual at 3.36 ppm. The doublet of doublets due to H-1' at 4.44 ppm, as well as the two multiplets corresponding to the C-3' methylene group at 3.76 and 4.13 ppm, are also more downfield than in the spectrum of (+)-tetrandrine (respectively at 3.84, 2.83,and 3.39 ppm). These features confirmed the presence of a N-oxide function at N-2'. Other resonances, especially those of the aromatic protons, were very similar to those observed for (+)-tetrandrine (126).

The downfield shifts of 0.8 ppm for the 2'-N-methyl singlet and of 0.6 ppm for the H-1' doublet of doublets suggested that H-1' is on the side opposite to the N-

oxide(127). The positive optical rotation indicates that **31** possesses the 1S,1'S configuration (127). Thereforethe N-oxide function occupies the β orientation.

This configuration of the N-oxide function was confirmed by a ROESY experiment; a significant correlation was observed between H-1' (4.44 ppm) and the 2'-N Me (3.36 ppm), while no effects were detected between H-3' and the 2'-N Me. Therefore, the NMe group is on the same side of the molecule as H-1', and the oxygen atom on the opposite side.

The complete assignments of $^{1}H$ and $^{13}C$ spectra have been confirmed by homonuclear COSY, HMBC and HMQC experiments and are presented around structures **31a** and **31b**.

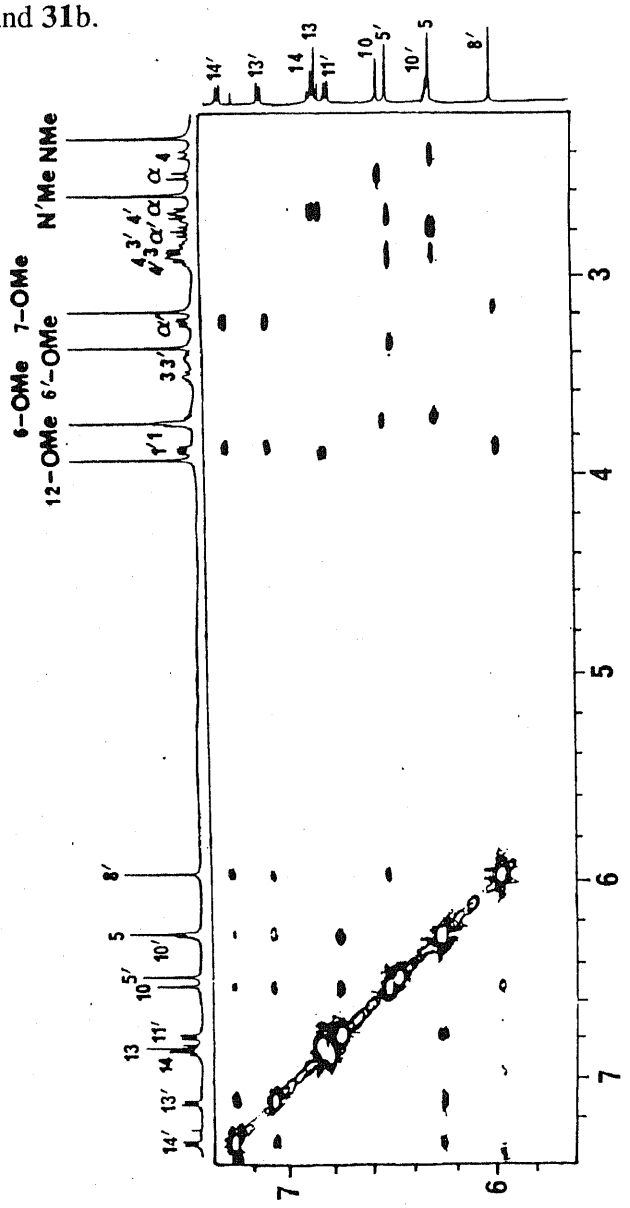



**Figure 4** ROESY Spectrum of **24**

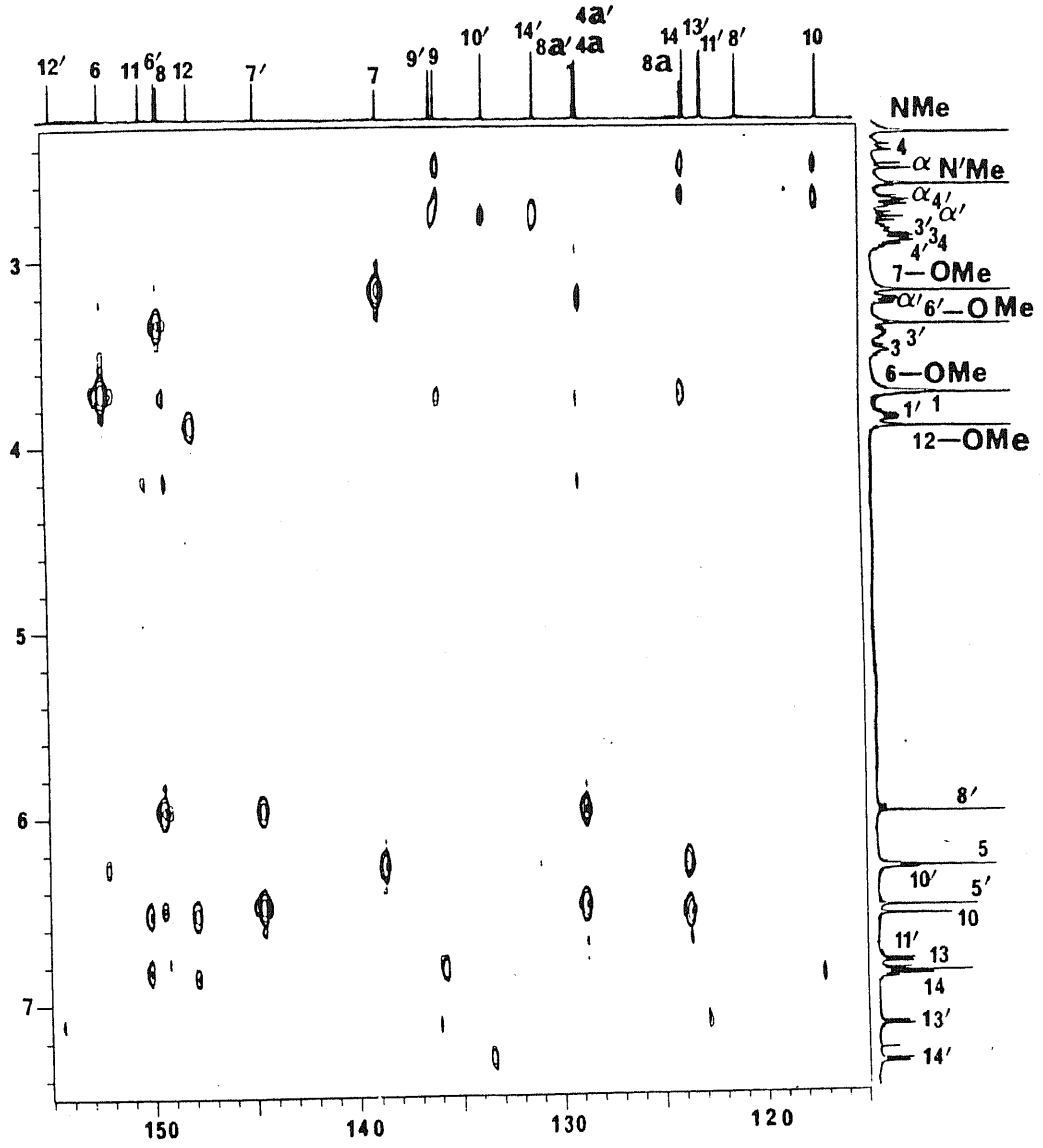



**Figure 5** FLOCK Spectrum of **24**

**Table 12** $^1$H-NMR Spectra Data of the Bisbenzylisoquinoline Alkaloids **24-28** from *C. barbata* [a]

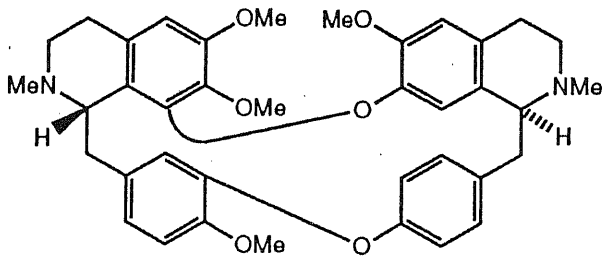
| proton | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|
| 1 | 3.72(d,9.5) | 3.75(dd,10.9,2.5 | 3.98(dd,10.9,2.5) | 3.63(dd,4,2.6) | 3.45(d,7.7) |
| NMe | 2.30 (s) | 2.32(s) | 2.28(s) | 2.53(s) | 2.57(s) |
| 3 | 2.87 (m) | 2.85(m) | 2.74(m) | 3.02(m) | 2.66(m) |
| 3 | 3.47 (m) | 3.49(m) | 3.21(m) | 2.73(m) | 2.97(m) |
| 4 | 2.39 (m) | 2.39(m) | 2.32(dd,15,4.7) | 2.40(m) | 2.60(m) |
| 4 | 2.89 (m) | 2.90(m) | 2.79(m) | 2.40(m) | 2.78(m) |
| 5 | 6.26 (s) | 6.27(s) | 6.32(s) | 6.33(s) | 6.41(s) |
| 6-OMe | 3.70 (s) | 3.70(s) | 3.27(s) | 3.60(s) | 3.34(s) |
| 7-OMe | 3.15 (s) | ..... | ..... | ..... | ..... |
| 8 | ..... | ..... | ..... | 6.66(s) | 6.44(s) |
| α | 2.45(dd,14,1.8) | 2.57(d,13.9) | 2.61(dd,14.8,10.9) | 2.68(m) | 2.50(d,13.4,7.8) |
| α | 2.67(dd,14,10) | 2.69(dd,13.9,10) | 2.90(dd,14.8,2.5) | 2.94(dd,14.1,4) | 3.05(d,13.4) |
| 10 | 6.52 (d,2.2) | 6.57(d,2.2) | 6.26(d,2.2) | 5.54(br.s) | 6.60(d,2.2) |
| 11 | ..... | ..... | ..... | ..... | ..... |
| 12-OMe | 3.88(s) | 3.91(s) | 3.88(s) | 3.88(s) | 3.94(s) |
| 13 | 6.82(d,8.2) | 6.83(d,8.2) | 6.76(d,8.3) | 6.74(d,8.4) | 6.94(d,8.4) |
| 14 | 6.86(dd,8.2,2.2) | 6.85(dd,8.2,2.2) | 6.60(dd,8.3,2.2) | 6.70(dd,8.4,2.3) | 6.88(dd,8.4,2.2) |
| 1' | 3.84(dd,11,5.9) | 3.87(dd,10.9,5.6) | 3.58(dd,11.5,5.0) | 4.13(d,6.5) | 4.19(br.d,9.8) |
| N'Me | 2.58(s) | 2.59(s) | 2.45(s) | 2.43(s) | 2.48(s) |
| 3' | 2.83(m) | 2.83(m) | 2.74(m) | 2.55(m) | 2.84(m) |
| 3' | 3.39(m) | 3.49(m) | 3.31(m) | 2.86(m) | 3.37(m) |
| 4' | 2.69(m) | 2.72(m) | 2.88(m) | 2.73(m) | 2.63(m) |
| 4' | 2.91(m) | 2.94(m) | 2.90(m) | 3.08(m) | 2.93(m) |
| 5' | 6.48(s) | 6.51(s) | 6.70(s) | 6.20(m) | 6.38(s) |
| 6'-OMe | 3.33(s) | 3.33(s) | 3.86(s) | 3.76(s) | 3.75(s) |
| 8' | 5.96(s) | 6.05(s) | 6.04(s) | ..... | ..... |
| α' | 2.75(dd,11.0,12.3) | 2.75(dd,12.5,10.9) | 2.76(m) | 2.61(m) | 2.77(dd,12.7,9.8) |
| α' | 3.22*dd,12.3,5.9) | 3.22(dd,12.5,5.6) | 3.18(m) | 3.22(d,13.8) | 3.13(dd,12.7,1.6) |
| 10' | 6.27(dd,8.2,2.3) | 6.30(dd,8.2,2.3) | 6.41(dd,8.3,2.2) | 6.95(dd,8.4,2.2) | 6.84(dd,8.4,2.2) |
| 11' | 6.76(dd,8.2,2.3) | 6.79(dd,8.2,2.3) | 6.80(dd,8.3,2.2) | 6.40(dd,8.4,2.2) | 6.84(dd,8.4,2.2) |
| 13' | 7.10(dd,8.2,2.3) | 7.12(dd,8.2,2.3) | 7.02(dd,8.3,2.2) | 6.91(dd,8.4,2.2) | 7.06(br.d,2.2) |
| 14' | 7.30(dd,8.2,2.3) | 7.32(dd,8.2,2.3) | 7.28(dd,8.3,2.2) | 7.33(dd,8.4,2.2) | 7.30(br.d,2.2) |

[a] Recorded in $CDCl_3$, chemical shift values are reported as values (ppm) from TMS at 500 MHz ; signal multiplicity and coupling constants (Hz) are shown in parentheses. In order to follow the convention (117), the most downfield signal is assigned for H-14', one of the four protons of ring C'.

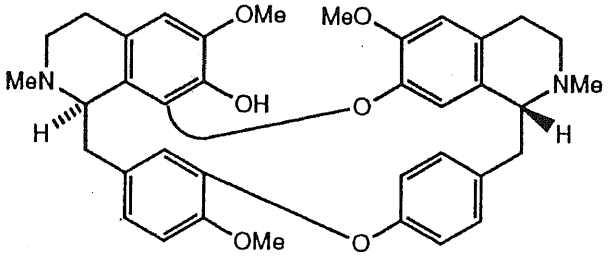
**Table 13** $^{13}$C-NMR Spectra Data of Bisbenzylisoquinoline Alkaloids **24-28** from *C. barbara* [a]

| Carbon | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|
| 1 | 61.19 | 61.36 | 60.09 | 64.26 | 65.31 |
| NMe | 42.05 | 42.25 | 42.13 | 43.72 | 42.36 |
| 3 | 43.91 | 44.14 | 43.72 | 51.10 | 46.78 |
| 4 | 21.81 | 21.76 | 22.34 | 28.45 | 26.59 |
| 4a | 127.72 | 123.21 | 122.14 | 130.56 | 127.92 |
| 5 | 105.55 | 104.80 | 107.46 | 111.10 | 112.38 |
| 6 | 151.18 | 145.75 | 146.84 | 148.50 | 149.12 |
| 7 | 137.63 | 134.57 | 136.32 | 143.50 | 144.15 |
| 8 | 148.19 | 141.86 | 144.20 | 116.93 | 120.73 |
| 8a | 122.64 | 123.41 | 124.22 | 128.02 | 131.31 |
| a | 41.70 | 41.85 | 39.06 | 38.32 | 40.38 |
| 9 | 134.68 | 134.96 | 133.17 | 130.95 | 133.90 |
| 10 | 115.99 | 116.14 | 144.77 | 117.00 | 120.46 |
| 11 | 149.12 | 149.29 | 150.10 | 148.70 | 148.58 |
| 12 | 146.83 | 146.93 | 146.51 | 146.64 | 148.50 |
| 13 | 111.33 | 111.45 | 111.35 | 110.71 | 112.77 |
| 14 | 122.56 | 122.67 | 121.78 | 123.65 | 123.45 |
| 1' | 63.64 | 63.65 | 64.92 | 60.46 | 60.22 |
| N'Me | 42.32 | 42.52 | 42.89 | 41.50 | 41.54 |
| 3' | 45.00 | 45.21 | 45.80 | 44.96 | 44.26 |
| 4' | 24.92 | 25.35 | 25.39 | 24.96 | 22.70 |
| 4a' | 127.72 | 128.03 | 130.57 | 122.99 | 122.95 |
| 5' | 112.50 | 112.99 | 112.18 | 104.50 | 105.82 |
| 6' | 148.39 | 148.71 | 148.96 | 147.61 | 146.42 |
| 7' | 143.57 | 143.50 | 143.17 | 133.39 | 134.91 |
| 8' | 119.96 | 120.57 | 121.15 | 142.47 | 143.06 |
| 8a' | 127.84 | 128.64 | 130.77 | 122.91 | 122.95 |
| a' | 37.93 | 37.86 | 37.93 | 38.20 | 43.99 |
| 9' | 134.91 | 135.10 | 135.17 | 138.17 | 136.46 |
| 10' | 132.42 | 132.49 | 131.85 | 131.49 | 131.69 |
| 11' | 121.63 | 121.86 | 122.79 | 121.12 | 120.38 |
| 12' | 153.58 | 153.67 | 154.36 | 152.74 | 155.42 |
| 13' | 121.63 | 121.82 | 122.49 | 121.90 | 121.55 |
| 14' | 129.90 | 130.07 | 129.93 | 128.34 | 129.78 |
| 6-OMe | 55.55 | 56.00 | 55.84 | 55.21 | 55.21 |
| 7-OMe | 59.99 | .... | .... | .... | .... |
| 12-OMe | 55.87 | 56.04 | 56.07 | 55.79 | 56.22 |
| 6'-OMe | 55.56 | 56.19 | 55.89 | 55.68 | 55.82 |

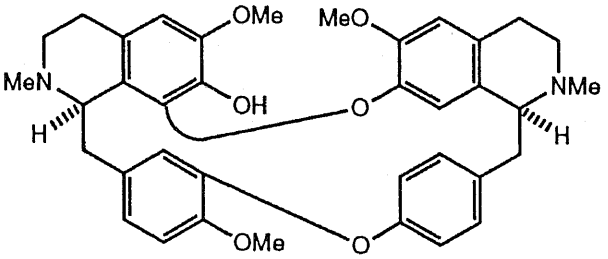
[a]Recorded in $CDCl_3$, chemical shift values are reported as values (ppm) at 125.8 MHz.


OMe
MeO
MeN
NMe
OMe
O
H
H
OMe
O

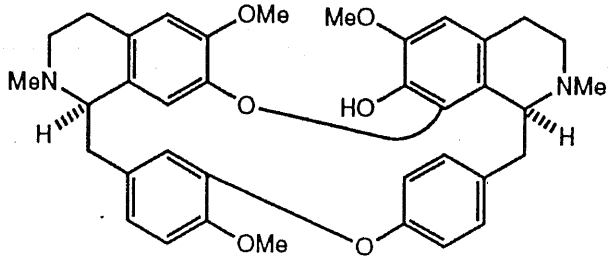
24


OMe
MeO
MeN
NMe
OH
O
H
H
OMe
O

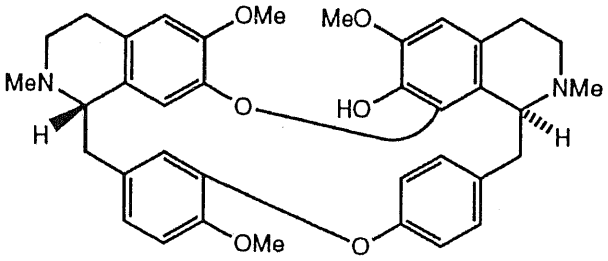
25


OMe
MeO
MeN
NMe
OH
O
H
H
OMe
O

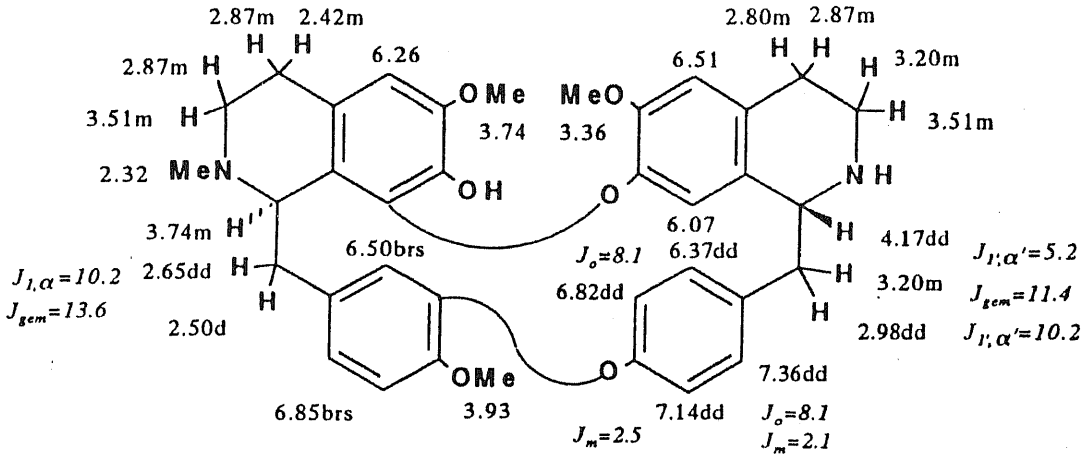
26


OMe
MeO
MeN
NMe
O
HO
H
H
OMe
O

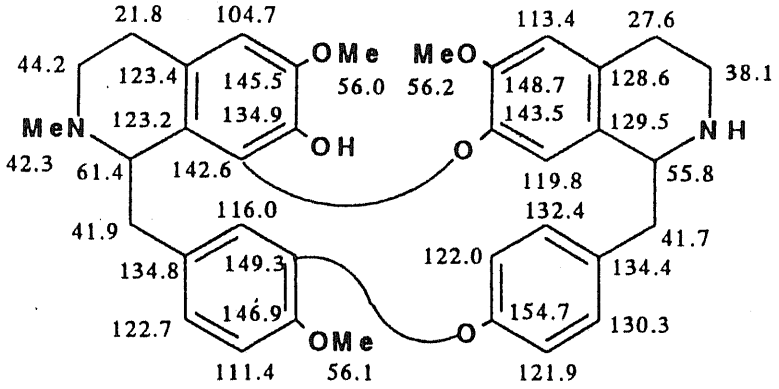
27


OMe
MeO
MeN
NMe
O
HO
H
H
OMe
O

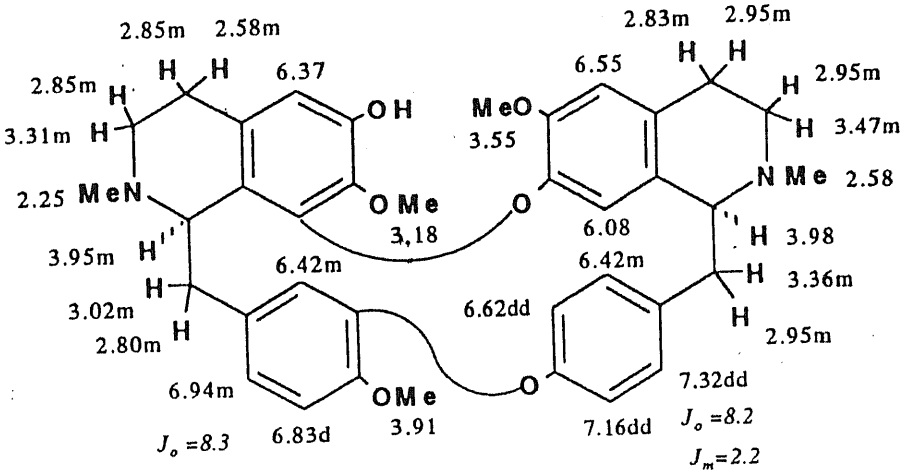
28


2.87m 2.42m
2.87m
3.51m
2.32 MeN
3.74m
2.65dd
2.50d
6.26
OMe
3.74
OH
6.50brs
OMe
6.85brs
3.93
2.80m 2.87m
6.51
3.20m
3.51m
MeO
3.36
NH
6.07
6.37dd
4.17dd
3.20m
6.82dd
2.98dd
7.36dd
7.14dd
O
$J_{1,\alpha}=10.2$
$J_{gem}=13.6$
$J_o=8.1$
$J_m=2.5$
$J_{1',\alpha'}=5.2$
$J_{gem}=11.4$
$J_{1',\alpha'}=10.2$
$J_o=8.1$
$J_m=2.1$

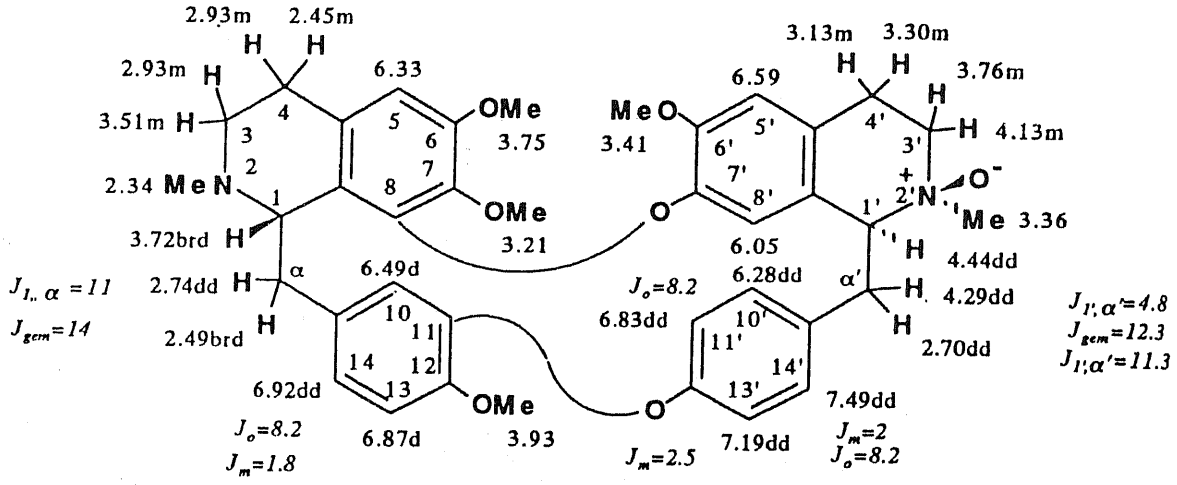
29a


21.8 104.7
44.2
123.4 145.5
OMe
56.0
MeN
123.2 134.9
OH
42.3
61.4
142.6
116.0
41.9
134.8 149.3
122.7 146.9
OMe
111.4 56.1
113.4 27.6
MeO
56.2
148.7 128.6
38.1
143.5 129.5
NH
119.8
55.8
132.4
41.7
122.0 134.4
154.7 130.3
121.9
O

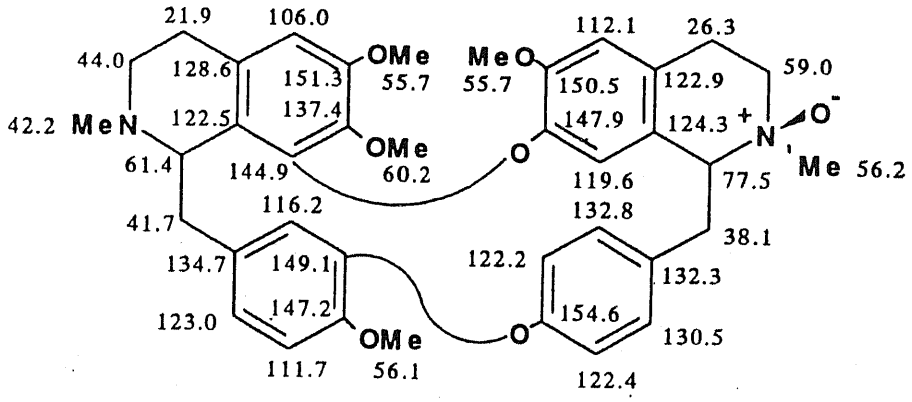
29b


2.85m 2.58m
2.85m
3.31m
2.25 MeN
3.95m
3.02m
2.80m
6.37
OH
OMe
3,18
6.42m
6.94m
OMe
6.83d
3.91
2.83m 2.95m
6.55
2.95m
MeO
3.55
3.47m
NMe 2.58
6.08
3.98
6.42m
3.36m
6.62dd
2.95m
7.32dd
7.16dd
O
$J_o=8.3$
$J_o=8.2$
$J_m=2.2$

30


2.93m
2.45m
2.93m
3.51m
2.34 MeN
3.72brd
2.74dd
2.49brd
6.33
OMe
3.75
OMe
3.21
6.49d
6.92dd
6.87d
OMe
3.93
3.13m
3.30m
3.76m
4.13m
MeO
3.41
6.59
6.05
6.28dd
6.83dd
4.44dd
4.29dd
2.70dd
Me 3.36
7.49dd
7.19dd
$J_{1,\alpha}=11$
$J_{gem}=14$
$J_o=8.2$
$J_m=1.8$
$J_m=2.5$
$J_m=2$
$J_o=8.2$
$J_{1';\alpha'}=4.8$
$J_{gem}=12.3$
$J_{1';\alpha'}=11.3$

**31a**


21.9
106.0
44.0
128.6
151.3
55.7
42.2 MeN
122.5
137.4
OMe
61.4
144.9
60.2
116.2
41.7
134.7
149.1
147.2
123.0
OMe
111.7
56.1
112.1
26.3
MeO
55.7
150.5
122.9
59.0
147.9
124.3
Me 56.2
119.6
77.5
132.8
38.1
122.2
132.3
154.6
130.5
122.4

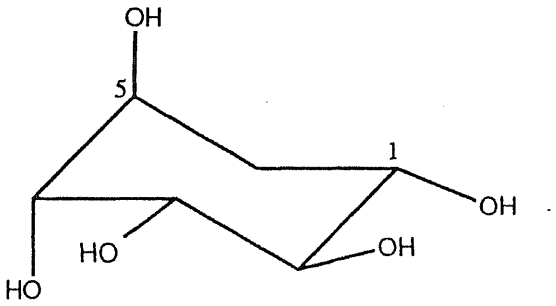
**31b**

## b. Tetrahydroprotoberberines

### i. *PARABAENA SAGITTATA* (130)

*Parabaena sagittata* Miers (Menispermaceae) is a lofty climber indigenous to the northern part of Thailand and has been used by the hill tribes of this region for medicinal purposes (131). A decoction of stems and leaves affords a treatment for jaundice, indigestion, and painful intestinal disturbances. All parts of the plant may be used as a febrifuge and tonic. There have been no previous reports of any phytochemical or pharmacological studies on this species, but a recent tlc screening of *P. sagittata* Mooney suggested the presence of magnoflorine in the petiole (132). In this report, we describe the structural elucidation of four components isolated from the leaves of *P. sagittata* Miers. Two of these components are alkaloids not previously reported.

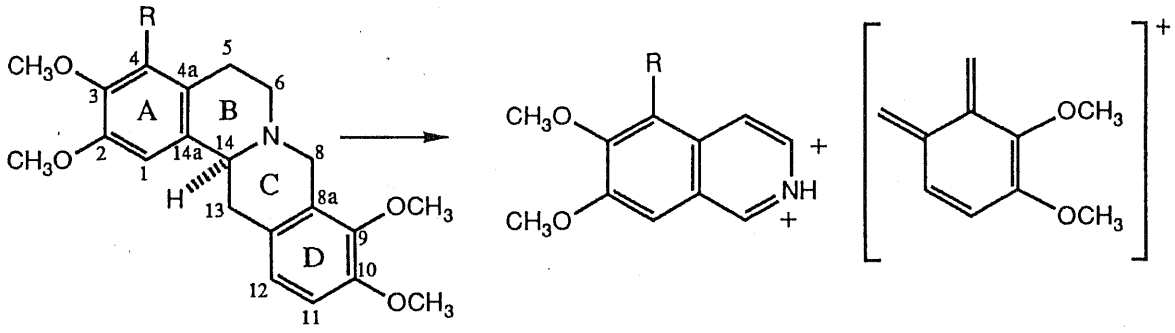
The colorless crystalline solid, **32**, was isolated as derscribed in the Experimental section and was found to be the cyclohexanepentol (+)-protoquercitol (**32**) on the basis of the data reported below. Our 400 MHz $^{1}$H-nmr spectrum of this cyclitol was essentially the same as that previously reported at 220 MHz (133). The $^{13}$C-nmr spectrum of **32** was reported recently (134), but two of the six carbon resonances could not be assigned unambiguously. We have found that $^{1}$H-$^{13}$C shift-correlated 2-D nmr spectroscopy (135,136) showed clearly that the multiplet at δ 3.59 ppm assigned to H-1 (133) was associated with the $^{13}$C resonance at δ 68.9 ppm and that the doublet of doublets at δ 3.85 for H-5 was attached to the carbon resonating at δ 68.6. Thus, we have been able to assign all six resonances, and these are reported in this section. The isolation of (+)-protoquercitol from the family Menispermaceae has been reported previously (137-138).




**32**

The three components, **33**,**34**, and **35** which were isolated from the basic fraction of the leaf extracts, gave positive Dragendorff and Mayer's tests, suggesting they were alkaloids. Mass spectral analysis of the compounds indicated a close

structural relationship as all three exhibited the charcteristic retro Diels-Alder fragments associated with a tetrahydroprotoberberine skeleton possessing two methoxy groups in the D ring (i.e., fragment *m/z* 164, Figure 6 and at least two methoxyl groups in the A ring (139). These analyses also suggested that **33** contained an additional methoxyl group and **34** a hydroxyl group in ring A which was absent in **35** (*m/z* 220 vs. 206 vs. 190, respectively, Fig. 6)




| | | *m/z* | *m/z* |
|---|---|---|---|
| **33** | R = OC $H_3$ | 220 | 164 |
| **34** | R = OH | 206 | 164 |
| **35** | R = H | 190 | 164 |

**Figure 6.** Mass Spectral Fragmentations of **33, 34** and **35**

A variety of spectroscopic techniques was employed to determine the positions of the substituents in rings A and D. The presence of a C-9 methoxyl group in all three alkaloids was indicated by significant relative intensity of the (M-$OCH_3$) fragment in the mass spectra of **33,34**, and **35** (18, 21, and 12%, respectively) (140).Further evidence for this substitution was obtained from the $^1$H-nmr signal for the C-8 methylene group. In compounds with no C-9 oxygen substituent, this methylene appears as a broad singlet, but when a methoxyl group is present on C-9, the C-8 pseudo axial proton is shifted upfield to become a doublet ($J$ = 16 Hz) at about 3.65 ppm, and the pseudo equatorial proton is deshielded to about 4.35 ppm

(141,142). Examination of the $^1H$ spectra of these alkaloids (Table 14) shows clearly that the latter situation exists for the protons at C-8 [e.g.,in **33** $\delta$ 3.55(d, $J$ = 15.7 Hz), 4.25 (d, $J$ = 15.7 Hz)]. Placement of the second methoxyl group in ring D at C-10 was indicated by the ortho coupling ($J$ = 8.1 Hz) between H-11 and H-12. Thus, we can conclude that the two methoxyl groups in ring D of these compounds are located at C-9 and C-10.

Both $^1H$- and $^{13}C$-nmr were employed to determine the substitution patterns in ring A of the compounds. A 400 MHz $^1H$-nmr spectrum of **35** showed singlets for the two aromatic protons in ring A, which suggested that they were para to each other (i.e., at the 1- and 4-positions) and thus that the two methoxyl groups were at C-2 and C-3. Comparison of the $^{13}C$-nmr spectrum of **35** with that of tetrahydropalmatine (143,144), a known tetrahydroprotoberberine with methoxy groups at the 2,3,9, and 10-position, established that the compounds were the same. Further comparison of other physical properties confirmed that the isolate was indeed **35**. Although portions of the $^1H$-nmr spectrum of **35** have been reported (145), in Table 14 we have indicated the assignment for each resonace in the 400 MHz spectrum. Determination of the optical rotation of **35** established that it was (-)-tetrahydropalmatine with the (S) absolute configuration as depicted in Fig. 6 (146,147).

Mass spectral analyses established that **33** was a pentamethoxyl derivative which contained an additional $OCH_3$ groups in ring A as compared with **35** and that of **34** had an additional OH group in this ring. Reaction of **34** with MeI and $K_2CO_3$ in DMF yielded **33** and, thus, showed that **33** was the methyl ether of **34**. On biosynthetic grounds, the three methoxyl groups in ring A of **33** would be expected at the 1-, 2- and 3-positions, but comparison of the $^{13}C$ spectrum of **33** (Table 15) with that of O-methylcapaurine (144,148), the known tetrahydroprotoberberine with methoxyls at the 1,2,3,9, and 10-positions, showed that they were not the same.

**Table 14** $^{1}H$ 400 MHz NMR spectra of **33**,**34**, and **35**[a]

| Hydrogen | Compound | | |
|---|---|---|---|
| | 33 | 34 | 35[b] |
| 1 | 6.56 s | 6.37 s | 6.75 s |
| 4 | - | 5.84 br. s (*OH*) | 6.64 s |
| 5 ax | 2.87 m | 2.84 m | 3.17 m |
| 5 eq | 2.56 m | 2.58 m | 2.67 m |
| 6 ax | 2.87 m | 2.84 m | 2.67 m |
| 6 eq | 3.22 m | 3.22 m | 3.21 m |
| 8 ax | 3.55 d (15.7) | 3.55 d (15.9) | 3.58 d (15.8) |
| 8 eq | 4.25 d (15.7) | 4.25 d (15.9) | 4.26 d (15.8) |
| 11 | 6.79 d (8.1) | 6.80 d (8.1) | 6.81 d (8.1) |
| 12 | 6.89 d (8.1) | 6.87 d (8.1) | 6.88 d (8.1) |
| 13 ax | 2.87 m | 2.84 m | 2.84 dd (16.0, 12.0) |
| 13 eq | 3.24 dd (10.9, 4.0) | 3.26 dd (10.5, 4.4) | 3.28 dd (16.0, 4.0) |
| 14 | 3.52 m | 3.51 m | 3.55 dd (12.0, 4.0) |
| $OCH_3$'s | 3.850 | 3.849 | 3.850 |
| | 3.853 | 3.852 | 3.856 |
| | 3.871 | 3.874 | 3.870 |
| | 3.877 | 3.892 | 3.892 |
| | 3.883 | | |

[a]Chemical shifts are in ppm from TMS and coupling constants are in parentheses in Hertz.
[b]See Tourwe *et al.* (145) for a discussion of some of these assignments.

The following evidence unambiguously established that the third methoxy group in ring A of **33** was at the 4-position and that no substituent was on C-1. Tetrahydroprotoberberines such as **35**, which contain no methoxyl group on C-1, have been shown to exist in a *trans*-conformation with respect to the B/C ring fusion (148,149). The observation of a high field (3.5-4.0 ppm) H-14 resonance is one indication of this *trans*-conformation (e.g., 3.55 ppm in **35**) (145,148). In **33** and **34**, then H-14 resonance was found in this region (Table 14), indicating that both compounds have no substituent on C-1 and that they exist in the *trans*-conformation. Further, in similar compounds with no substituent on C-1, the C-6 resonance in the $^{13}C$-nmr spectrum appears at about 51 ppm, while in compounds with an oxygen substituent at this position, the C-6 signal appears in the region 47-49 ppm (148,150). In **33** and **34**, this signal appeared at 51.2 and 50.9 ppm, (Table 15) respectively, as expected for compounds with no C-1 substituent.

It could be assumed that because there is no substituent at C-1 in either of these alkaloids, the three substituents in ring A must be at the 2,3, and 4-positions. But because substitution at the 4-position in protoberberines is without precedent,

Table 15 $^{13}$C-nmr spectra of **33**, **34** and **35**[a]

| Carbon | Compounds | | |
|---|---|---|---|
| | **33** | **34** | **35**[b] |
| 1 | 104.6 | 100.6 | 108.9 |
| 2 | 150.8 | 150.4 | 147.6 |
| 3 | 140.5 | 133.5 | 147.6 |
| 4 | 151.8 | 146.3 | 111.5 |
| 4a | 121.4 | 114.8 | 127.8 |
| 5 | 23.6 | 23.1 | 29.1 |
| 6 | 51.2 | 50.9 | 51.5 |
| 8 | 54.0 | 54.1 | 54.0 |
| 8a | 127.7 | 127.7 | 126.9 |
| 9 | 150.3 | 150.2 | 150.3 |
| 10 | 145.1 | 145.0 | 145.2 |
| 11 | 111.0 | 111.0 | 111.1 |
| 12 | 123.8 | 123.7 | 123.7 |
| 12a | 128.7 | 128.6 | 128.7 |
| 13 | 36.4 | 36.2 | 36.4 |
| 14 | 59.5 | 59.5 | 59.3 |
| 14a | 133.6 | 133.7 | 129.9 |
| 2-$OCH_3$ | 55.9 | 55.9 | 55.8 |
| 3-$OCH_3$ | 60.6[c] | 61.0 | 55.8 |
| 4-$OCH_3$ | 60.9[c] | - | - |
| 9-$OCH_3$ | 60.2 | 60.2 | 60.1 |
| 10-$OCH_3$ | 56.2 | 55.9 | 56.1 |

[a]Chemical shifts are in ppm from TMS.
[b]Data taken from Hyghes and MacLean (144)
[c]Assignments may be interchanged.

evidence will be presented to establish clearly this substitution pattern. In compounds without an oxygen substituent at the 4-position, the $^{13}$C resonance for C-5 appears at about 30 ppm (e,g., 29.1 ppm in **35**). In both **33** and **34**, this carbon is strongly shielded (23.6 and 23.1 ppm, respectively) because of the presence of a C-4 oxygen substituent. A similar shielding of C-5 has been reported for simple tetrahydroisoquinolines (151,152) and for more complex systems (153,154) possessing a methoxy group at the 4-position. Also, the $^{13}$C resonance for all carbons in the A ring of **34** (Table 15) showed excellent agreement with a similarly substituted tetrahydroisoquinoline system (153). $^{1}$H 2-D nOe spectroscopy (NOESY)(155) was also employed to confirm the substitution pattern in ring A. For **33**, this technique showed that one of the methoxyl groups was in close proximity to H-5ax ( $\delta$ 2.87), a result that is only possible if a methoxyl group is on C-4. The NOESY technique was even more informative with **34**, as it showed that the hydroxyl proton at $\delta$ 5.84 was in close proximity to H-5eq ( $\delta$ 2.58). This result established that the hydroxyl group in ring A must be at the 4-position. In making the $^{1}$H-nmr assignments for all protons in **33** and **34** (Table 14), we were assisted by a $^{1}$H homonuclear shift-correlated 2-D experiment (COSY) (156) performed with **33**.

On the basis of the above evidence, we propose the illustrated structures for **33** and **34**. As neither of these compounds has been reported previously, we suggest the names (-)-O-methylthaicanine for **33** and (-)-thaicanine for **34**. Closely related levorotatory substances are of the (S) configuration (146,147), so we propose the absolute configurations for **33** and **34** are as depicted in Fig 6. An unusual biosynthetic feature of the two new alkaloids is the presence of oxygen substituents at C-4. Of the more than 70 known protoberberines (149), to our knowledge these are the first which have been reported with substituents at the 4-position.

(+)-PROTOQUERCITOL (**32**).-Mp 228-230°, lit. (137) mp 235-237°; $[\alpha]^{20}_D$ + 23° ($H_2O$), lit. (137)$[\alpha]^{20}_D$ + 25.6° ($H_2O$); ir $\upsilon_{max}$ (KBr) 3320 (st.,br.), 1070, 1050 $cm^{-1}$; $^1H$ nmr, as reported previously (133); $^{13}C$ nmr ($D_2O$) 33.3 (C-6), 68.6 (C-5), 68.9(C-1), 71.0(C-3), 72.3(C-4), 74.6(C-2); eims *m/z* (rel.int.) 165 ($M^+$ + 1,1), 128(10), 102(18), 99(23), 86(24), 74(53), 73(100).

(-)-O-METHYLTHAICANINE (**33**).-Mp 119-120° (MeOH/$CHCl_3$); $[\alpha]^{20}_D$- 259° ($CHCl_3$); ir $\upsilon_{max}$ ($CHCl_3$) 3040, 2940,2850, 1495, 1120, 910 $cm^{-1}$; uv $\lambda_{max}$ (EtOH) 208 nm ( ε 26,000), 229 sh ( ε 15,000), 278 ( ε 1,700); $^1H$ and $^{13}C$ nmr,see Tables 14 and 15; eims *m/z* (rel. int.) 385 ($M^+$, 69), 384(39), 354(18), 220(26), 165(24), 164(100), 149(61); tlc (50% EtOAc/30-60° petroleum ether) Rf 0.44. Calcd for $C_{22}H_{27}NO_5$: C, 68.55; H, 7.06; N, 3.63. Found : C, 68.41; H, 7.40; N, 3.87.

(-)-THAICANINE (**34**).-Mp 144-146° (MeOH/$CHCl_3$);$[\alpha]^{20}_D$ - 243° ($CHCl_3$); ir $\upsilon_{max}$ ($CHCl_3$) 3520, 3020, 2830,2750, 1495, 1120, 910 $cm^{-1}$; uv $\lambda_{max}$ (EtOH) 208 nm ( ε 22,000), 221 sh ( ε 8100), 280 ( ε 1300); with added OH- 216 ( ε 32,000), 280 (ε 3200); $^1H$ and $^{13}C$ nmr, see Tables 14 and 15; eims *m/z* (rel. int.) 371 ($M^+$, 60), 370(29), 340(12), 206(16), 165(23), 164(100), 149(57); tlc (50% EtOAc/30-60° petroleum ether) Rf 0.35. Calcd for $C_{21}H_{25}NO_5$: C, 67.91; H, 6.78; N, 3.77. Found: C, 67.75; H, 6.30; N, 3.80.

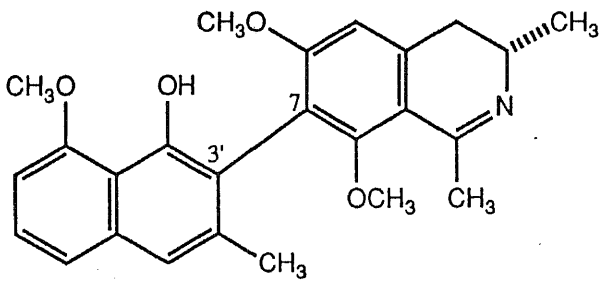
(-)-TETRAHYDROPALMATINE (**35**)-Mp 139-141°, lit.(157) mp 141°; $[\alpha]^{20}_D$ - 203° ($CHCl_3$), -236° (EtOH), lit.(157) $[\alpha]^{28}_D$ - 258°, -271° (EtOH); ir $\upsilon_{max}$ ($CHCl_3$) 3020,2830, 2750, 1595, 1255, 910 $cm^{-1}$; uv $\lambda_{max}$ (EtOH) 205 nm,225 sh, 281; $^1H$ and $^{13}C$ nmr spectra, see Table 14 and 15 and references (145) and (144), respectively; eims *m/z* (rel. int.) 335 (M+, 67), 354(41), 324(12), 190(24), 165(23), 164(100), 149(162).

CONVERSION OF **34** TO **33**. - To a solution of **34** (24.7 mg) in DMF (1.5 ml) was added 24 mg finely powdered $K_2CO_3$. To this suspension was introduced 8.23 µl of MeI, and the reaction mixture was stirred at room temperature under an atmosphere of $N_2$ for 2.5 h. $H_2O$ (10 ml) was added to the reaction mixture, and the product was extracted with $CHCl_3$ (3 times). The combined organic extracts were washed once with brine, dried (anhydrous $MgSO_4$), and the solvent removed at reduced pressure to give a yellow oil. Purification of the crude product by preparative tlc (50% EtOAc/30-60° petroleum ether,Rf 0.44) gave 16.4 mg (64%) of a compound indentical to **33**.

## c. Naphthalene-isoquinolines

### i. *ANCISTROCLADUS TECTORIUS* (158,184)

*Ancistrocladus* is the only genus of the plant family Ancistrocladaceae and is composed of nearly 20 species distributed in tropical Asia, Malaysia, and West Africa (159). The genus is a source, together with two genera in the Dionchophyllaceae, of the naphthalene-isoquinoline group of alkaloids (160,161). Eighteen alkaloids in this series have been isolated to date, and the compounds may be grouped according to the location of the δ-bond joining the two units. Most of the alkaloids (ten) are linked 5-1' (162-169), whilst seven others are linked 7-1' (166,170-172). The remaining alkaloid, ancistrocladidine (**36**) from *Ancistrocladus heyneanus* Wall. (173), is linked 7-3', and the crystallographic analysis of this alkaloid was recently presented (174). We report here the structure determination of a second alkaloid having a 7-3' linkage, ancistrotectorine (**37**) from the leaves of *Ancistrocladus tectorius* (Lour.) Merr.




**36**

Previous work on the stem and twigs of *A. tectorius* has yielded ancistrocladeine (167), ancistrocladine (169), hamatine (169), and ancistrocline

(169), subsequent to a demonstration that the species contained alkaloids (175). The roots of *A. tectorius* have been used to treat dysentery and malaria.

After partition of the EtOH extract, the $CHCl_3$ fraction was chromatographed on alumina to afford ancistrotectorine (0.016% yield). From its uv spectrum, which displayed $\lambda_{max}$ 234, 286, 306, 323, and 337 nm, ancistrotectorine was a member of the naphthalene-isoquinoline series of alkaloids containing a phenolic hydroxy group ($\upsilon_{max}$ 3375 $cm^{-1}$), even though no positive $FeCl_3$ test or shift in base (2N NaOH solution) in the uv spectrum was observed. No molecular ion was observed in the electron impact (ei) mass spectrum, but a clear ($M + H^+$) was found at *m/z* 422 in the chemical ionization mass spectrum using methane as the reagent gas. Important fragments in the ei mass spectrum were observed at *m/z* 406 and 203.

The low-field $^1$H-nmr spectrum of ancistrotetorine firmly established the naphthalene-isoquinoline nature of the compound and indicated the presence of two doublet methyl group, an aromatic methyl group, an N-methyl group,and three aromatic methoxy groups. The aromatic region was complex, although two singlets were apparent.

In order to evaluate the structure more explicitly, a detailed examination of the high-field $^1$H-nmr spectrum of ancistrotectorine was undertaken. Two doublet methyl groups were observed at $\delta$ 1.268 and $\delta$ 1.466 from the 3-$CH_3$ and the 1-$CH_3$, respectively, and their methine protons were observed at $\delta$ 2.544 and $\delta$ 3.731. Irradiation at $\delta$ 1.27 collapsed the signal at $\delta$ 2.544 to a doublet of doublets ($J$ =3.0, 10.3 Hz), clearly establishing the 3-$CH_3$ to be equatorial. The aromatic region was now clearly resolved with singlets observed at $\delta$ 6.518 and $\delta$ 7.232, doublets at $\delta$ 6.689 and $\delta$ 7.343, and a triplet at $\delta$ 7.266. The latter three signals were assigned to adjacent protons on the naphthalene nucleus, and on biogenetic grounds a methyl group ( $\delta$ 2.169) was placed at C-2' and oxygenation at C-4' and C-5'. Irradiation of the methoxyl group singlet at $\delta$ 3.691 caused an 8.1% nOe effect in the singlet at $\delta$ 6.518, and the reverse irradiation produced an nOe (4%) only in the $\delta$ 3.691 singlet. A similar spatial relationship was established between the aromatic methoxy group at 3.985 and the doublet at $\delta$ 6.689, which could therefore be assigned to a 5'-$OCH_3$ and its adjacent 6'-H. There were a number of alternatives to explain the former nOe experiments: (a)a 7-1' linkage, with the OH at C-4' and the third $OCH_3$ at C-8, (b) a 7-3' linkage with the OH/$OCH_3$ at C-8/C-4' or reverse, and (c) a 5-3' linkage with OH at C-8 and $OCH_3$ at C-4'. Potentially, these structures could be distinguished by the base-induced shift of a proton ortho or para to the hydroxy group and whether or not

the proton shifted is ortho to a methoxy group. However, the failure of ancistrotectorine to undergo a bathochromic shift in the uv spectrum on the addition of 2N NaOH did not augur well for the success of this experiment, and consequently, the single crystal X-ray crystallographic analysis was carrried out.

Ancistrotectorine crystallized in the monoclinic $P2_1$ space group with unit cell dimensions of a = 11.858(14), b = 7.043(7), C = 14.693(28) A° and $\beta$ = 114.43(12). The crystal structure was elucidated, using the program MULTAN (176), and refined, using the program SHELX (177). The hydrogen bond lengths were restricted to 1.0 A° during refinement, and the largest peak on a final electron density difference map was 0.12 eA°.$^{-3}$ The final R = 8.0% over 1087 independent reflexions using a unit weighting scheme.

Final position parameters, bond lengths, and valency angles are listed in Tables 16-18, respectively. The crystallographic structure deduced for ancistrotectorine (Figure 7) indicates that it is indeed a 7-3' linked naphthalene-isoquinoline alkaloid having the molecular array shown in 70 in which the C-1 and C-3 methyl groups are *cis* and the N-containing ring to the isoquinoline moiety adopts sofa conformation with C-3 the out-of-plane atom. Steric hindrance about the diaryl linkage results in a dihedral angle of 56.4° beween the two aromatic rings.

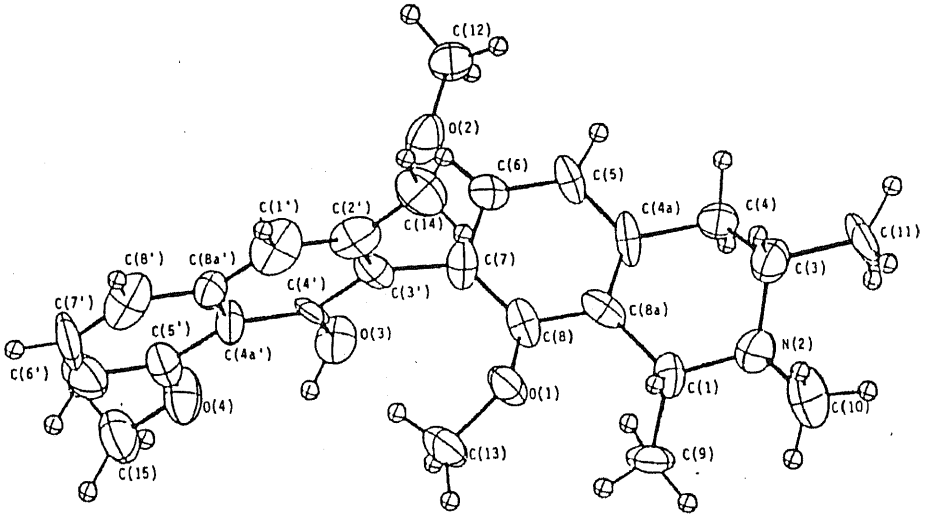



**Figure 7** ORTEP display of the crystallographic structure of ancistrotectorine (**37**)

It now became possible to assign the $^1$H spectrum completely. From the nOe experiments the singlets at $\delta$ 3.691 and $\delta$ 3.985 could be assigned to the 6- and 5'-

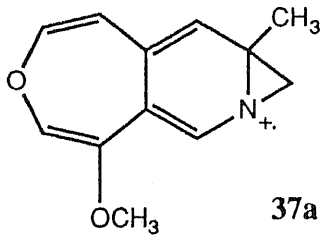
$OCH_3$, repectively, leaving the signlet at δ 3.323 to be a somewhat shielded methoxy group at C-8. Similarly, the aromatic singlet at δ 6.518 could be assigned to H-5 and at δ 7.232 to H-1'. Examination of the coupling constants permitted the doublets of doublets at δ 2.655 and δ 2.787 to be assigned to H-4β and H-4α respectively. The complete assignments are shown on structure **37**. Ancistrotectorine (**37**) is therefore a member of the rare 7-3'-linked naphthalene-isoquinoline alkaloids of which ancistrocladidine (**36**) (173,174), is the only other example.

**Table 16** Fractional atomic Co-ordinates (x $10^4$) with e.s.d's (in parenthesis)

| Atom | x | y | z |
|---|---|---|---|
| O (1) | 1240 (8) | 9594 (20) | 0433 (6) |
| O (2) | 4670 (7) | 6549 (22) | 2800 (6) |
| O (3) | 3753 (9) | 11387 (23) | 2826 (7) |
| O (4) | 3645 (10) | 13613 (23) | 4207 (6) |
| C (1) | 1943 (12) | 8794 (27) | -1116 (9) |
| N (2) | 2331 (11) | 7931 (24) | -1864 (8) |
| C (3) | 2868 (13) | 6042 (27) | -1609 (10) |
| C (4) | 3963 (11) | 6199 (29) | -0718 (10) |
| C (4A) | 3682 (12) | 7021 (28) | 0111 (9) |
| C (5) | 4374 (11) | 6427 (28) | 1072 (10) |
| C (6) | 4056 (11) | 7043 (25) | 1837 (9) |
| C (7) | 3025 (12) | 8244 (29) | 1655 (9) |
| C (8) | 2347 (12) | 8766 (27) | 0679 (9) |
| C (8A) | 2689 (11) | 8158 (25) | -0056 (9) |
| C (1') | 1312 (11) | 7786 (30) | 3363 (11) |
| C (2') | 1769 (1) | 7380 (27) | 2658 (10) |
| C (3') | 2589 (10) | 8651 (25) | 2481 (9) |
| C (4') | 2962 (11) | 10278 (23) | 3052 (9) |
| C (4A') | 2490 (13) | 10752 (27) | 3776 (9) |
| C (5') | 2789 (12) | 12383 (27) | 4364 (10) |
| C (6') | 2339 (18) | 12833 (30) | 5069 (13) |
| C (7') | 1546 (16) | 11543 (32) | 5221 (11) |
| C (8') | 1207 (11) | 9955 (35) | 4686 (10) |
| C (8A') | 1656 (13) | 9490 (26) | 3945 (9) |
| C (9) | 2074 (14) | 10965 (24) | -1185 (11) |
| C (10) | 1288 (16) | 7917 (32) | -2780 (12) |
| C (11) | 3135 (14) | 5103 (3) | -2472 (10) |
| C (12) | 5591 (12) | 5143 (29) | 2970 (9) |
| C (13) | 1163 (16) | 11377 (31) | 0861 (11) |
| C (14) | 1417 (14) | 5579 (28) | 2077 (12) |
| C (15) | 4087 (14) | 15272 (29) | 4814 (11) |
| OH (3) | 3799 (102) | 12787 (37) | 2937 (80) |
| H (1) | 1102 (41) | 8521 (175) | -1144 (71) |
| H (3) | 2306 (81) | 5109 (127) | -1480 (79) |
| H (4A) | 443 (81) | 5016 (86) | -0463 (68) |
| H (4B) | 4559 (77) | 7121 (122) | -0795 (77) |
| H (5) | 5014 (69) | 5531 (121) | 1053 (74) |
| H (1') | 0662 (71) | 6984 (134) | 3440 (73) |
| H (6') | 2342 (100) | 14212 (56) | 5234 (79) |
| H (7') | 1116 (75) | 11783 (176) | 5685 (55) |
| H (8') | 0583 (72) | 9069 (124) | 4760 (74) |
| H (9A) | 1546 (77) | 11586 (160) | -0873 (64) |
| H (9B) | 2922 (39) | 10879 (185) | -0680 (52) |
| H (9C) | 2105 (94) | 11794 (152) | -1731 (60) |
| H (10A) | 0896 (106) | 9195 (78) | -2902 (88) |
| H (10B) | 0712 (97) | 6960 (116) | -2689 (90) |
| H (10C) | 1516 (101) | 7534 (144) | -3343 (62) |
| H (11A) | 3642 (84) | 3971 (140) | -2491 (95) |
| H (11B) | 2300 (38) | 4926 (158) | -2963 (65) |
| H (11C) | 3510 (89) | 6260 (106) | -1646 (91) |
| H (12A) | 5926 (88) | 4500 (150) | 3619 (40) |
| H (12B) | 5199 (96) | 4183 (126) | 2448 (56) |
| H (12C) | 6272 (70) | 5770 (154) | 2829 (70) |
| H (13A) | 0396 (52) | 12051 (147) | 0480 (69) |
| H (13B) | 1102 (88) | 10847 (161) | 1476 (52) |
| H (13C) | 1854 (67) | 12306 (135) | 1050 (79) |
| H (14A) | 2230 (49) | 5140 (165) | 2110 (79) |
| H (14B) | 0997 (82) | 4533 (126) | 2288 (81) |
| H (14C) | 0919 (74) | 5983 (177) | 1399 (43) |
| H (15A) | 4807 (59) | 15680 (158) | 4660 (75) |
| H (15B) | 3619 (94) | 16424 (107) | 4869 (83) |
| H (15C) | 4383 (87) | 14600 (147) | 5464 (49) |

The mass spectrum of ancistrotectorine also merits some additional comment. A facile loss of 15 amu was apparent to give the stable species at *m/z* 406. The most interesting ion though is *m/z* 203 since this does not correspond to either half of the

molecule as might be expected, althought it clearly is derived from the isoquinoline unit. In our estimation this ion may have the structure **37a** in which one of the aliphatic methyl groups has been lost together with one of the methyls from an aromatic methoxy group. Ancistrocladidine (**36**) shows aloss of 15 amu from the molecular ion at *m/z* 405, but no stable species are observed below this mass.




**37a** *m/z* 203

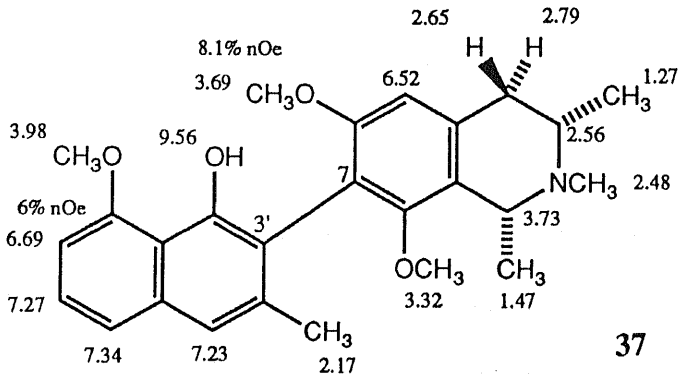
The absolute configuration of naphthalene-isoquinoline alkaloids has been studied previously (165,178). The cd spectrum of ancistrotectorine in MeOH showed a negative first Cotton effect ($[\theta]_{234}$ - 34, 179) and a positive second Cotton effect ($[\theta]_{218}$ + 70,167) which clearly demonstrates the absolute configuration of ancistrotectorine to be that shown in **37**.

**Table 17** Bond lenghts (Å ) with eds's (in parentheses)

| | | | |
|---|---|---|---|
| 0(1)-C(18) | 1.356 (18) | C(5)-C(6) | 1.380 (22) |
| 0(1)-C(13) | 1.422 (25) | C(6)-C(7) | 1.429 (22) |
| 0(2)-C(6) | 1.379 (14) | C(7)-C(8) | 1.413 (17) |
| 0(2)-C(12) | 1.427 (21) | C(7)-C(3') | 1.510 (21) |
| 0(3)-C(4') | 1.352 (18) | C(8)-C(8A) | 1.354 (22) |
| 0(4)-C(5') | 1.416 (22) | C(1')-C(2') | 1.363 (24) |
| 0(4)-C(15) | 1.447 (23) | C(1')-C(8A') | 1.443 (25) |
| 0(10)-N(2) | 1.469 (22) | C(2')-C(3') | 1.414 (23) |
| 0(1)-C(8A) | 1.550 (17) | C(2')-C(14) | 1.501 (25) |
| 0(1)-C(9) | 1.554 (25) | C(3')-C(4') | 1.393 (21) |
| N(2)-C(3) | 1.463 (24) | C(4')-C(4A') | 1.411 (22) |
| N(2)-C(10) | 1.458 (17) | C(4A')-C(5') | 1.403 (25) |
| C(3)-C(4) | 1.473 (16) | C(4A')-C(8A') | 1.417 (25) |
| C(3)-C(11) | 1.562 (25) | C(5')-C(6') | 1.365 (28) |
| C(4)-C(4A) | 1.492 (23) | C(6')-C(7') | 1.383 (31) |
| C(4A)-C(5) | 1.413 (18) | C(7')-C(8') | 1.341 (30) |
| C(4A)-C(8A) | 1.370 (21) | C(8')-C(8A') | 1.414 (23) |

**Table 18** Valency Angles (Å) with eds's (in parentheses)

| | | | |
|---|---|---|---|
| C(13)-O(1)-C(8) | 118.2 (13) | C(7)-C(8)-O(1) | 121.2 (12) |
| C(8A)-C(8)-O(1) | 117.7 (12) | C(12)-C(2)-C(6) | 116.0 (11) |
| C(5)-C(6)-O(2) | 123.9 (14) | C(7)-C(6)-O(2) | 115.9 (11) |
| C(3')-C(4')-O(3) | 115.0 (11) | C(4A')-C(4')-O(3) | 123.4 (14) |
| C(15)-C(4)-C(5') | 120.9 (12) | C(4A')-C(5')-O(4) | 115.8 (13) |
| C(6')-C(5')-C(4) | 118.9 (16) | C(8A)-C(1)-N(2) | 114.4 (13) |
| C(9)-C(1)-N(2) | 107.1 (13) | C(3)-N(2)-C(1) | 114.2 (12) |
| C(10)-N(2)-C(1) | 107.5 (13) | C(9)-C(1)-C(8A) | 108.5 (12) |
| C(4A)-C(8A)-C(1) | 119.1 (12) | C(8)-C(8A)-C(1) | 118.9 (13) |
| C(10)-N(2)-C(3) | 111.9 (14) | C(4)-C(3)-N(2) | 108.6 (15) |
| C(11)-C(3)-N(2) | 112.1 (12) | C(11)-C(3)-C(4) | 112.3 (14) |
| C(4A)-C(4)-C(3) | 111.6 (13) | C(5)-C(4A)-C(4) | 118.7 (15) |
| C(8A)-C(4A)-C(4) | 120.9 (12) | C(8A)-C(4A)-C(5) | 120.0 (13) |
| C(6)-C(5)-C(4A) | 119.3 (14) | C(8)-C(8A)-C(4A) | 122.0 (13) |
| C(7)-C(6)-C(5) | 120.2 (12) | C(8)-C(7)-C(6) | 118.3 (13) |
| C(3')-C(7)-C(6) | 118.9 (12) | C(3')-C(7)-C(8) | 122.1 (14) |
| C(8A)-C(8)-C(7) | 120.1 (15) | C(2')-C(3')-C(7) | 119.2 (14) |
| C(4')-C(3')-C(7) | 120.9 (14) | C(8A')-C(1')-C(2') | 121.3 (16) |
| C(3')-C(2')-C(1') | 119.9 (16) | C(14)-C(2')-C(1') | 120.3 (16) |
| C(4A')-C(8A')-C(1') | 118.8 (13) | C(8')-C(8A')-C(1') | 123.0 (16) |
| C(14)-C(2')-C(3') | 119.8 (13) | C(4')-C(3')-C(2') | 119.9 (12) |
| C(4A)-C(4')-C(3') | 121.5 (14) | C(5')-C(4A')-C(4') | 125.3 (15) |
| C(8A')-C(4A')-C(4') | 118.6 (15) | C(8A')-C(4A')-C(5') | 116.2 (13) |
| C(6')-C(5')-C(4A') | 125.2 (16) | C(8')-C(8A')-C(4A") | 118.2 (16) |
| C(7')-C(6')-C(5') | 116.6 (18) | C(8')-C(7')-C(6') | 121.9 (16) |
| C(8A'-C(8')-C(7') | 121.9 (17) | | |




**37**

PHYSICAL PROPERTIES OF ANCISTROTECTORINE. -Ancistrotectorine (**37**) crystallized from $Me_2CO$ as pale yellow needles, mp 134-140°; ir $\upsilon_{max}$ (KBr) 3375, 2960, 2925,2840, 2770, 1640, 1600, 1555, 1458, 1400, 1362, 1320, 1115, 1095, 1085, 825, and 755 $cm^{-1}$; uv $\lambda_{max}$ (EtOH) 234 (log ε 4.73),286(3.64),306(3.61), 323(3.66), and 337 nm(3.69); ms *m/z* (ei, 70eV, 180°) no $M^+$ observed, 420 (1%),407(28), 406(100), 360(17), 203(72), 202(5), 195(6), 189(4), 188(9), 187(5), 181(8), 180(36), 172(28), 167(9), 165(28), 158(31), 151(33), 145(8), 144(8), 143(8), 137(7), 136(8), 131(7), 129(10), 128(8), 115(12), and 107(7); ms, *m/z* (ci, $CH_4$ gas, 70eV) 422($M + H^+$, 100%) and 406(9); $^1$H-nmr (360 MHz, $CDCl_3$) δ 1.268 (d, 3H, *J* = 6.1 Hz, 3-$CH_3$), 1.466 (d, 3H, *J* = 6.4 Hz, 1-$CH_3$), 2.169 (s, 3H, 2'-$CH_3$), 2.484 (s, 3H, N-$CH_3$), 2.544 (ddq, 1H, *J* = 3.0, 6.1, 10.3Hz, 3-H), 2.655 (dd, 1H, *J* = 3.0, 15.3 Hz, 4-Hβ), 2.787 (dd, 1H, *J* = 10.3,

15.4 Hz, 4-Hα), 3.323 (s, 3H, 8-$OCH_3$),3.691 (s, 3H, 6-$OCH_3$), 3.731 (q, 1H, *J* = 6.4 Hz, 1-H), 3.985 (s, 3H, 5'-$OCH_3$), 6.518 (s, 1H, 5-H), 6.689 (d, 1H, *J* = 7.5 Hz, 6'-H), 7.232 (s, 1H, 1'-H), 7.266 (t, 1H, *J* =7.8 Hz, 7'-H), 7.343 (d, 1H, *J* = 8.0 Hz, 8'-H), and 9.565 (s, 1H, 4'-OH); $[\alpha]^{26}_D$ O° ($CHCl_3$); cd (MeOH) $[\theta]_{218}$ + 70,167, $[\theta]_{234}$ - 34,179, $[\theta]_{283}$ = -10,291, $[\theta]_{308}$ - 5,146, $[\theta]_{321}$ - 6,549 and $[\theta]_{336}$ - 8,420.

CRYSTALLOGRAPHIC ANALYSIS. - Crystal data,$C_{26}H_{31}NO_4$, M = 421.5, monoclinic, a = 11.858(14), b = 7.043(7), c = 14.693(28) A°, β = 111.43(12)°, U = 1142.6 $A°^3$, Z= 2 Dc = 1.22 g. cm-3, space group $P2_1$, μ (MO-Kα) = 0.46 $cm^{-1}$.

The crystal structure was elucidated using MULTAN(176) and refined using SHELX (177). In the final cycles of least-squares refinement, the positional parameters for all atoms, anisotropic thermal parameters for the C and O atoms and common isotropic thermal parameters for the methyl and non-methyl hydrogens were varied. Structure amplitudes and thermal parameters are listed in material available from the authors.

Previous work on the chloroform fraction of the ethanol extract of the leaves of *Ancistrocladus tectorius* (Lour.) Merr.(Ancistrocladaceae) yielded a new 7,3'-linked naphthalene-isoquinoline alkaloid, whose structure was deduced through a combination of spectroscopic and X-ray crystallographic analysis (158). Subsequent crystallization of a related fraction from acetone afforded ancistrocladidine (**36**), whose identity was established by means of mixed mp,co-tlc, uv, ir, mas and $^1$H-nmr in comparison with an authentic sample (173).

Attention then focussed on the nmr spectroscopic properties of this compound, given the presence of three methyl, one methylene, one aliphatic methine,three methoxy, five aromatic protonated carbons and twelve quaternary aromatic carbons in the attached proton test (APT) spectra. Analysis of the $^1$H-nmr and homonuclear COSY spectra indicated that a phenolic proton appeared at δ 9.63 ppm, and that three of the protons on the naphthalene nucleus appeared as an ABX pattern at δ 7.36 (d, *J* = 7.3 Hz), 7.28(t, *J* = 7.3 Hz), and 6.71 ppm (d, *J* = 7.3 Hz) assigned to H-8', H-7' and H-6', respectively. Two other aromatic singlets appeared at δ 7.25 and 6.63 ppm and were assigned to H-1'and H-5, respectively.

Further evidence to assign H-5 and H-6' was apparent from the NOESY spectrum, which revealed the presence of a nOe effect between a methoxy group

singlet at 3.99 ppm and H-6', and between a singlet at δ 3.75 ppm and H-5 (6.63 ppm). Consequently, these two methoxy group singlets were assigned to C-5' and C-6, respectively. The remaining methoxy singlet,which does not show any nOe with an adjacent aromatic proton, could therefore be attributed to position 8.

The most high-field methyl group resonance at δ 1.43 ppm (d, $J$ = 6.7 Hz) and assigned to position 3, was coupled to a multiplet at δ 3.41 ppm, which was in turn coupled to a pair of geminally-coupled methylene protons at δ 2.69 (dd, $J$ = 15.5,4.5 Hz) and 2.42 (dd, $J$ = 15.5, 1.8 Hz), as well as being long-range coupled to a methyl doublet at 2.49 ppm ($J$ = 1.8 Hz). The signals were assigned to H-3, H-4α, H-4β and 1-$CH_3$, respectively. Attribution of the chemical shifts of the protons α and β at H-4 was confirmed by the observation of nOe cross peaks in the NOESY spectrum between H-3 (3.41 ppm) and H-4β (2.42 ppm). Additionally, as noted from the X-ray crystal structure data of ancistrotectorine (**37**), the isoquinoline moiety adopts a sofa conformation with C-3 out-of-plane (158). Under these conditions, and, given the coupling constant data, the assignments of H-4α and H-4β were confirmed. The remaining methyl singlet at 2.16 ppm was assigned to H-2'.

Examination of the broad-band decoupled and the APT carbon-13 nmr spectra did not permit complete and unambiguous resonance identification for ancistrocladidine (**36**). This could only be achieved through the application of the CSCM 1D (179) and selective INEPT (180) techniques. Initially, the protonated carbons were assigned using the former technique (Figure 8). Magnetization transferred from the down field satellite of H-5 afforded two positive resonances for C-5 (105.87 ppm) and C-6'(103.01 ppm) and were distinguished as described below. Magnetization transferred from the upfield carbon-13 satellite of H-7' showed three negative resonances for C-1', C-8' and C-7' at δ 118.70, 121.07 and 125.58 ppm because of the proximity of the proton chemical shifts of H-8' and H-1'. A similar situation was encountered with H-8'. Irradiation of the downfield satellite of H-8' enhanced the resonance at δ 121.07 ppm maximally, suggesting that this might be C-8', and to a lesser extent the signal at δ 125.58 ppm, which was tentatively assigned to C-7'. A positive distinction between these resonances was made with the selective INEPT technique, as illustrated subsequently. Irradiation of the downfield carbon-13 satellite of the 5'-$OCH_3$ protons positively enhanced the 5'-$OCH_3$ carbon at δ 55.80 ppm. Similarly, irradiation of the downfield satellite of 8-$OCH_3$ yielded two positive resonances for the 8-$OCH_3$ carbon (60.82 ppm) and for C-3 (51.40 ppm), and a negative resonance for the 6-$OCH_3$ carbon (55.93 ppm). These data supported the presence of H-3 partially hidden below the 8-$OCH_3$ protons. Irradiation of the downfield carbon-13 satellite of

1-$CH_3$ positively enhanced the 1-$CH_3$ (26.85 ppm) and to a lesser extent C-4 (35.20 ppm). When the downfield satellite of 3-$CH_3$ was irradiated, only a positive resonance for 3-$CH_3$ (21.93 ppm) was observed.

The oxygenated carbons on the naphthalene and isoquinoline rings were assigned using the selective INEPT technique employing a delay time corresponding to $J$ = 4 Hz (Figure 9 and 10). Selective INEPT irradiation of the methoxy singlets attached to C-5', C-6 and C-8 resulted in the enhancement of these carbons at 156.01, 159.28 and 157.75 ppm, respectively. Irradiation of H-4$\alpha$ with $J$ = 5 Hz yielded the three-bond coupled carbons C-5(105.87 ppm), 3-$CH_3$ (21.93 ppm) and C-8a (116.93 ppm), as well as the two-bond couplings of C-4a(141.25 ppm) and C-3(51.40 ppm).

Assignment of the remaining naphthalene ring carbons was achieved with $J$ = 8 Hz. Irradiation of H-1' led to the enhancement of the carbon resonances at $\delta$ 136.08, $\delta$ 121.07, 116.79 and $\delta$ 113.25 ppm. Irradiation of H-6' also enhanced the resonance at 113.25 ppm, indicating that it should be C-4'a and also the resonance at 121.07 ppm, indicating it to be C-8'. Irradiation of the 2'-$CH_3$ enhanced the signals at $\delta$ 118.70, 116.79 and 137.71 ppm, and consequently the signal at $\delta$ 116.79 ppm could be ascribed to C-3', the signal at $\delta$ 137.71 ppm to C-2' and the signal at $\delta$ 118.70 ppm to C-1'. The remaining resonance at $\delta$ 136.08 ppm was attributed to C-8'a, thereby completing the unambiguous assignment of the carbon-13 nmr spectrum of ancistrocladidine (**36**).

*Physical Properties of Ancistrocladidine* - Ancistrocladidine (**36**) crystallized from acetone as pale yellow needles, mp 255-258° dec., $[\alpha]_D$ = 129.7° ($CHCl_3$; c 0.064); ir $\upsilon_{max}$ (KBr): 3367, 3013, 2934, 1609, 1090 $cm^{-1}$; uv $\lambda_{max}$ (MeOH):238 (log $\varepsilon$ 4.22), 255 sh(3.89), 285(3.63), 306 sh (3.41), 320(3.34), 335 nm (3.42); $^1$H-nmr (360 MHz, $CDCl_3$) $\delta$ : 9.63 (1H, s, 4'-OH), 7.36 (1H, d, $J$ = 7.3 Hz, H-8'), 7.28 (1H, t, $J$ = 7.3 Hz, H-7'), 7.25 (1H, s, H-1'), 6.71 (1H, d, $J$ = 7.3 Hz, H-6'), 6.63 (1H, s, H-5), 3.99 (3H, s, 5'-$OCH_3$), 3.75 (3H, s, 6-$OCH_3$), 3.41 (1H, m, H-3), 3.37 (3H, s, 8-$OCH_3$), 2.69 (1H, dd, $J$ = 15.5, 4.5 Hz,. H-4$\alpha$), 2.49 (3H, d, $J$ = 1.8 Hz, 1-$CH_3$), 2.42 (1H, dd, $J$ = 15.5, 1.8 Hz, H-4$\beta$), 2.16 (3H, s, 2'-$CH_3$), 1.43 (3H, d, $J$ = 6.7 Hz, 3-$CH_3$); $^{13}$C nmr ($CDCl_3$) : 163.26 (C-1), 159.28 (C-6), 157.75 (C-8), 156.01 (C-5'), 151.26 (C-4'), 141.25 (C-4a), 137.71 (C-2'), 136.08 (C-8'a), 125.58 (C-7'), 121.07 (C-8'), 118.70 (C-1'), 118.63 (C-7), 116.93 (C-8a), 116.79 (C-3'), 113.25(C-4'a), 105.87 (C-5), 103.01 (C-6'), 60.82 (8-$OCH_3$), 55.93 (6-$OCH_3$), 55.80 (5'-$OCH_3$), 51.40 (C-3), 35.20 (C-4), 26.85 (1-$CH_3$); 21.93 (3-

$CH_3$), 20.46 (2'-$CH_3$); eims, *m/z* (rel. int.) 405 ($M^+$, 100%), 388(37), 344(2); cims, *m/z* (rel. int.) 405 ($M^+$, 100%).

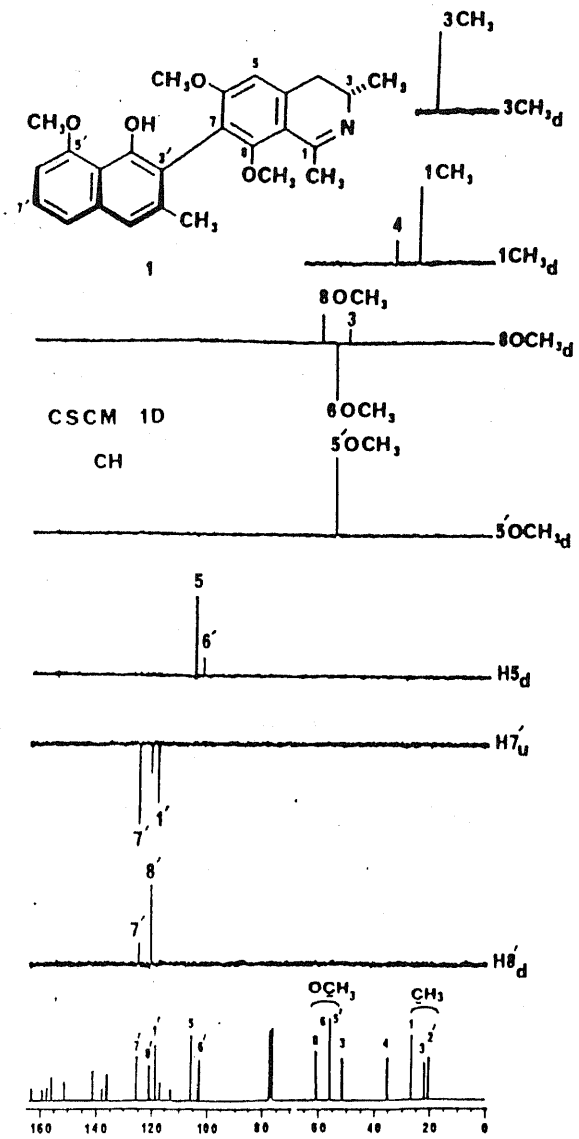



**Fig. 8** Representative CSCM 1 D spectra of ancistrocladidine (**36**)

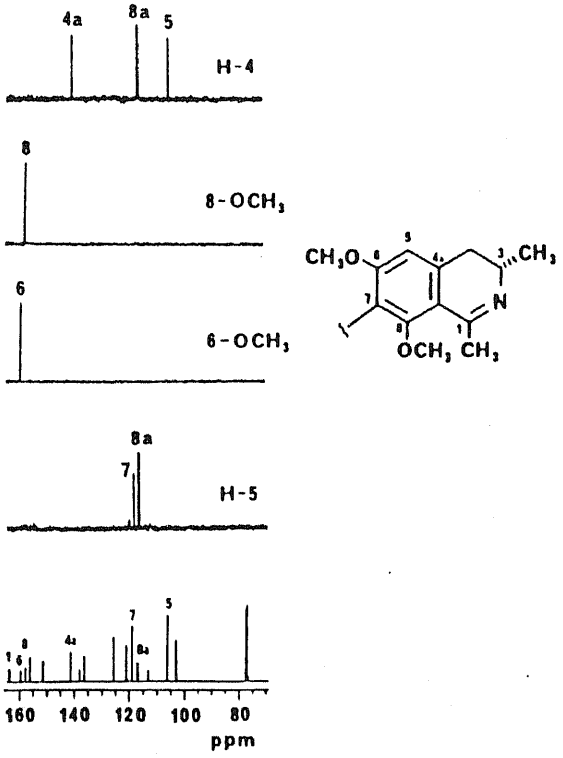



**Fig. 9** Selective INEPT irradiations of isoquinoline ring of **36**

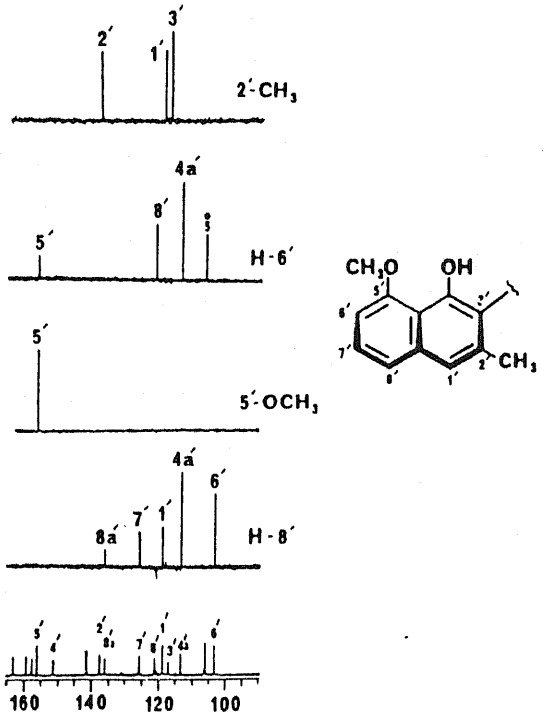



**Fig. 10** Selective INEPT irradiations of naphthalene ring of **36**

### 2.1.3.2 **Indoles**

#### i. ***KOPSIA JASMINIFLORA*** (181,185)

A study of the indole alkaloid constituents was made on *Kopsia jasminiflora* Pitard (Family Apocynaceae, subfamily Plumerioideae, tribe Rauvolfieae), a species on which no chemical report has been published. From the methanol extract of the leaves collected at Chiang Mai province of Thailand two known and five new alkaloids were obtained. Among the new alklaoids three were found to be novel class of *Kopsia* alkaloids.

Compound **38** showed the UV spectrum characteristic to N-acylated indoline chromophore. The $^1$H-NMR spectrum, though revealed some readily assignable functional groups such as N(a)-methoxycarbonyl ( $\delta$ 3.79 & 3.81, rotamers), aromatic ring protons ( $\delta$ 7.12 (H-9), $\delta$ 7.06 (H-10), $\delta$ 7.23 (H-11, and $\delta$ 7.50 & 7.94 (H-12,rotamers)), and a conjugated methyl ester ( $\delta$ 3.77 ($CO_2Me$) and 7.04(H-17)), strongly suggested a hitherto unencountered type basic skeleton. At this stage X-ray structural analysis was carried out.

The crystal of compound **38** had the following crystal data : triclinic, $P_1$, a = 10.108(2), b = 15.118(2), c = 7.221(1) A°, $\alpha$ = 99.84 (1), $\beta$ = 108.81(2), $\gamma$ = 80.09(1). Intensity data of 4039 reflections, F(o) > 3 $\delta$ F(o), were measured on a four circles diffractometer. The structure was solved by the direct method and the result was refined by block-diagonal least squares calculations to an R value of 0.062. The ORTEP drawing is shown below. Compond **38**, now named kopsijasminilam (**38**), turned out to be the first example of D/E seco *Kopsia* alkaloids.

In the $^1$H-NMR spectrum of kopsijaminilam (**38**) a highly deshielded signal is observed at $\delta$ 4.10, which is ascribable to H-3$\alpha$ suffering from deshielding anisotropic effect caused by C-21 amide carbonyl group. Other protons adjacent to nitrogen were observed at $\delta$ 3.00 (H-3$\beta$), 2.88 (H-5$\alpha$), and 3.50(H-5$\beta$). The $^{13}$C-NMR data are shown in Table 19. Based on these and other spectroscopic data, structures of two additional closely related alkaloids, compound **39** and compound **40**, were elucidated as described below.

Compound **39** showed the $^1$H-NMR signals due to the methylenes on C-3( 3.02(H-3$\beta$) and $\delta$ 4.15(3$\alpha$)) and C-5 ( $\delta$ 2.87(H-5$\alpha$) and $\delta$ 3.56(H-5$\beta$)) at almost the

same positions as kopsijasminilam (**38**). The $^{13}$C-NMR (Table 19) indicated this compound to be 20-deoxykopsijasminilam (**39**). The C-20 signal was found at δ 33.0 as a doublet. The signal due to C-18 was observed at δ 30.4, at a position shifted downfield by 4.1 ppm from **38** owing to the relief from the γ-gauche effect of the hydroxyl group at C-20 of **38**.

Compound **40** possessed one double bond in the molecule of kopsijasminilam (**38**). The $^{1}$H-NMR spectrum of **40** showed two olefinic protons, H-14 and H-15, at δ 5.44 and δ 5.80, respectively. The characteristic H-3α signal moved further downward to δ 4.94 in this compound. These findings, together with $^{13}$C-NMR spectral data (Table 19), indicated the structure to be $\Delta^{14}$-kopsijasminilam (**40**). Catalytic reduction of **40** ($H_2$/Pd-C, atmospheric pressure) offorded kopsijasminilam (**38**) as expected.

Two additional new alkaloids, compound **41** and compound **42**, were isolated and the structures were deduced as follows.

Compound **41** showed the UV spectrum superimposable to that of (**38**), thus indicating the chromophores of N(a)- methoxycarbonyl indoline and acrylic ester. The $^{1}$H-NMR spectral data of the non-aromatic moiety and the mass spectral fragments were remarkably analogous to those of a reported compound **45** derived from natural kopsidasine **46** or kopsidasine N-oxide **47** by Homberger and Hesse (182). The $^{13}$C-NMR of **41** (Table 19) strongly supported the elucidated structure. The name of kopsijasmine was given to compound **41**.

Compound **42** (jasminiflorine) was found to have a 12-methoxy indoline chromophore by the UV spectrum and the $^{1}$H-NMR spectrum ( δ 3.85(OMe), δ 6.76(H-11), δ 6.85(H- 10), and δ 6.97(H-9). The IR absorption at 1725 cm$^{-1}$ and $^{13}$C-NMR signal at δ 214.8(s) indicated the ketonic group of the type of fruticosine-fruticosamine. These and other spectral data demostrated the structure shown above. The orientation of the hydroxyl group at C-16 was deduced to be β on the basis of the coupling constant ($J$ = 6.6 Hz) between 16-H and 17-H (183).

Along with the above new alkaloids two more bases were isolated and were shown to be fruticosine **43** and fruticosamine **44** through comparison of the spectral data with those given in the literature (183).

Compound **38** (kopsijasminilam) : mp 245-246° C, $[\alpha]_D$ -220° ($CHCl_3$), $C_{23}H_{26}N_2O_6$. MS *m/z* : 426 ($M^+$, 70), 408(43), 394(32), 339(100), 42(45). IR(KBr) 3450, 1720, 1700, 1690 $cm^{-1}$. UV(MeOH) 208, 247, 280, 290 nm. $^1$H-NMR (270 MHz, $CDCl_3$) δ : 1.61-2.35(8H), 2.48 (1H, ddd, *J* = 15.3, 15.3, 5.6 Hz), 2.88 (1H, dd, *J* = 9.6, 9.6 Hz), 3.00 (1H, dd, *J* = 14.2, 4.6 Hz), 3.50 (1H, ddd, *J* = 11.4, 11.4, 11.4 Hz), 3.67 (1H, br.s), 3.79 & 3.81 (each s, 6H altogether), 4.10(1H, dd, *J* = 13.8, 11.9 Hz), 7.04 (1H,s), 7.06 (1H, dd, *J* = 7.3, 7.3 Hz), 7.12 (1H, d, *J* = 6.3 Hz), 7.23 (1H, t-like), 7.50 (d-like) & 7.94 (br) (1H).

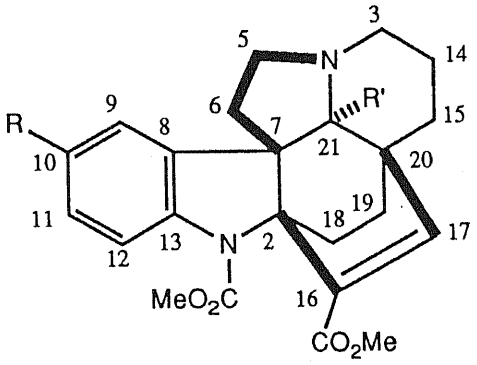
Compound **39** (deoxykopsijasminilam) : mp 192- 195°C, $C_{23}H_{26}N_2O_5$, $[\alpha]_D$ -210° ($CHCl_3$). MS *m/z* 410($M^+$,81), 378(78), 323(100), 59(54). IR(KBr) 1720, 1710, 1690 $cm^{-1}$. UV(MeOH) 210(4.62), 243(4.25), 282(3.47), 289(3.43) nm. $^1$H-NMR (270 MHz, $CDCl_3$) δ : 1.5-2.3 (1 OH), 2.87 (1H, dd, *J* = 7.1, 7.1 Hz), 2.90 (1 H,m), 3.02 (1H, dd, *J* = 13.2, 4.6 Hz), 3.56 (1H, br.), 3.64(s) & 3.83(s) (3H), 3.76(3H, s), 4.15 (1H, dd, *J* = 12.8, 12.8 Hz), 7.06 (1H, dd, *J* = 7.6, 7.6 Hz), 7.14 (1H, d, *J* = 7.1 Hz), 7.23 (1H, t- like), 7.26(1H, s, overlapped with $CHCl_3$ signal), 7.51 (d, *J* = 7.9 Hz) & 7.95 (d, *J* = 7.9 Hz) (1H).

Compound **40** ($\Delta^{14}$-kopsijasminilam) : amorphous powder. High Resolution MS : 424.163 ($M^+$) (Calcd. for $C_{23}H_{26}N_2O_5$; 424.1632). MS *m/z* 424 ($M^+$,68), 406(7), 365(7), 338(24), 337(100), 259(7), 167(9), 115(8), 59(15). UV(MeOH) 209, 242, 281, 290 nm. $^1$H-NMR (270 MHz, $CDCl_3$) δ : 1.92 (1H, ddd, *J* = 14.4, 7, 9.7 Hz), 1.90-2.15 (4H,m), 2.32(1H,m), 2.40(1H,m), 2.65 (1H, ddd, *J* = 16.6, 14.0, 6.4 Hz), 2.90 (1H,m), 2.96 (1H, dd, *J* = 9.5, 9.5 Hz), 3.37 (1H, dd, *J* = 17.2, 6.8 Hz), 3.40 (1H,m), 3.72 & 3.80 (each s, 6H altogether), 4.94 (1H, ddd, *J* = 17.4, 2.7, 2.7 Hz), 5.44 (1H, dd, *J* = 12.5, 6.1 Hz), 5.80 (1H, dd, *J* = 12.5, 2.7 Hz), 6.82 (1H, s), 7.06(2H), 7.24(1H), 7.52(d, *J* = 7.9 Hz) & 7.98 (d, *J* = 8.6 Hz) (1H).

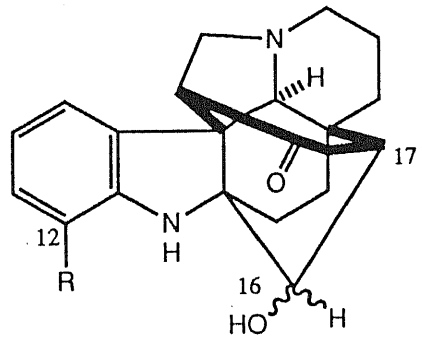
Compound **41** (kopsijasmine) : mp 199-202° C, $[\alpha]_D$ - 202° ($CHCl_3$. High Resolution MS : 394.1885 ($M^+$) (Calcd. for $C_{23}H_{26}N_2O_4$; 394.1890). MS *m/z* : 394 ($M^+$,100), 393(20), 379(31), 363(12), 336(20), 335(66), 307(19), 282 (19), 275(19), 226(21), 59(12). $^1$H-NMR (270 MHz, $CDCl_3$) : δ 1.2-1.4 (3H), 1.46(1H, ddd, *J* = 13.3, 13.3, 3.5 Hz), 1.60 (1H, ddd, *J* = 13.3, 9.1, 9.1 Hz), 1.45-1.65(1H, m), 1.70-1.90 (2H, m), 2.05 (1H, br.d, *J* = 13.1 Hz), 2.47 (1H, dd, *J* = 13.7, 6.8 Hz), 2.58 (1H, ddd, *J* = 8.6, 8.6, 6.8 Hz), Hz), 2.68 (1H, dd, *J* = 2.7, 2.7 Hz), 3.05 (2H, br, d, *J* = 6.7 Hz), 3.33 (1H,s), 3.74 & 3.79 (br.s and s, 6H altogether), 6.85 (1H,

br.s), 7.03 (1H, dd, $J$ = 7.0, 7.3 Hz), 7.20 (1H, dd, $J$ = 7.0, 7.0 Hz), 7.29 (1H, d, $J$ = 7.2 Hz), 7.50 (br) & 7.91 (br) (1H).

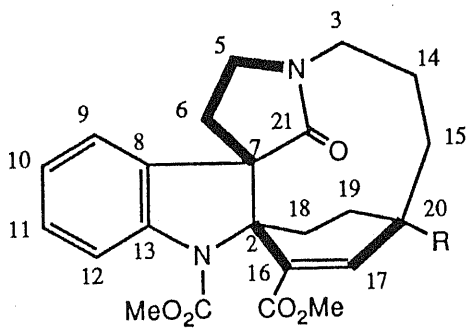
Compound **42** (jasminiflorine) : mp 230-233° C, $[\alpha]_D$ -55.3° ($CHCl_3$). High Resolution MS : 353.1784 (Calcd. for $C_{21}H_{24}N_2O_3$ : 352.1785). UV(EtOH) 210(4.50), 245(3.84), 291(3.37) nm. MS $m/z$ 352 ($M^+$, 100), 232(90), 254(17), 253(10), 210(9), 124(60). IR(KBr) 3500-3300, 1730 $cm^{-1}$. 1H-NMR (270 MHz, $CDCl_3$) δ : 1.1-1.5 (5H,m), 1.78 (1H, dd, $J$ = 11.5, 3.3 Hz), 1.87 (1H, ddd, $J$ = 12.8, 9.6, 9.6 Hz), 2.20 (1H, ddd, $J$ = 11.5, 9.9, 9.9 Hz), 2.41(1H, d, $J$ = 4.6 Hz), 2.58(1H, d, $J$ = 6.6 Hz), 2.84 (1H, d, $J$ = 10.6 Hz), 2.85-2.90 (2H, br), 3.22 (1H, s), 3.59 (1H, dd, $J$ = 11.6, 4.6 Hz), 3.85 (3H, s), 4.20(1H, d, $J$ = 6.6 Hz), 6.76 (1H, dd, $J$ = 8.0, 1.0 Hz), 6.86 (1H, dd, $J$ = 8.0, 8.0 Hz), 6.96 (1H, dd, $J$ = 7.3, 1.0 Hz).




**41** R=H R'=H
**45** R=OMe R'=H
**46** R=OMe R'=OH
**47** R=OMe R'=OH
N-Oxide




**42** R=OMe
16β-OH
**43** R=H
16β-OH
**44** R=H
16α-OH




**38** R=OH
**39** R=H
**40** R=OH
$\Delta^{14}$

**Table 19** $^{13}$C-NMR (67.5 MHz, $CDCl_3$) of compounds **38-42**

| Carbon | **38** | **39** | **40** | **41** | **42** |
|---|---|---|---|---|---|
| 2 | 69.2[a)] | 69.5 | 68.9[a)] | 71.1 | 65.5 |
| 3 | 42.3 | 42.9 | 40.8 | 47.3 | 48.5 |
| 5 | 43.9 | 44.0 | 45.2 | 50.2 | 54.7 |
| 6 | 32.9[b)] | 32.4 | 31.6 | 39.0[a)] | 55.4 |
| 7 | 60.3 | 61.3 | 65.0 | 62.5 | 60.6 |
| 8 | 130.5 | 130.4 | 129.5[b)] | 135.2 | 130.6 |
| 9 | 124.9 | 124.4, 124.9 | 124.3[c)] | 122.1 | 110.4 |
| 10 | 123.6 | 123.3 | 123.6 | 123.6 | 117.3 |
| 11 | 128.7 | 128.5, 128.6 | 128.8 | 124.7 | 121.5 |
| 12 | 115.4 | 115.3 | 115.6 | 115.2 | 146.4 |
| 13 | 140.6 | 140.9 | 140.8 | 142.0 | 138.8 |
| 14 | 22.5 | 22.6 | 124.9[c)] | 16.1 | 17.8 |
| 15 | 41.3 | 31.4 | 138.8 | 26.0 | 35.7 |
| 16 | 130.1 | - | 130.1[b)] | - | 70.9 |
| 17 | 145.7 | 148.0, 148.6 | 142.9 | 143.4 | 59.1 |
| 18 | 26.3 | 29.3, 30.4 | 26.6 | 38.2[a)] | 31.6 |
| 19 | 32.2[b)] | 23.9 | 29.7 | 32.3 | 29.5 |
| 20 | 69.4[a)] | 33.0 | 68.2[a)] | 34.0 | 35.4 |
| 21 | 172.0[a)] | 172.0 | 172.3 | 69.0 | 67.0 |
| C-C=O | 166.5 | 166.9 | 166.5 | 166.1 | 214.9 |
| N-C=O | 153.4 | 153.2 | 153.3 | - | |
| O-Me | 52.3 | 51.8 | 52.2 | 51.8 | 53.7 |
| O-Me | 52.8 | 52.0 | 52.8 | 52.2 | |

a), b), c) : Assignments may be interchanged

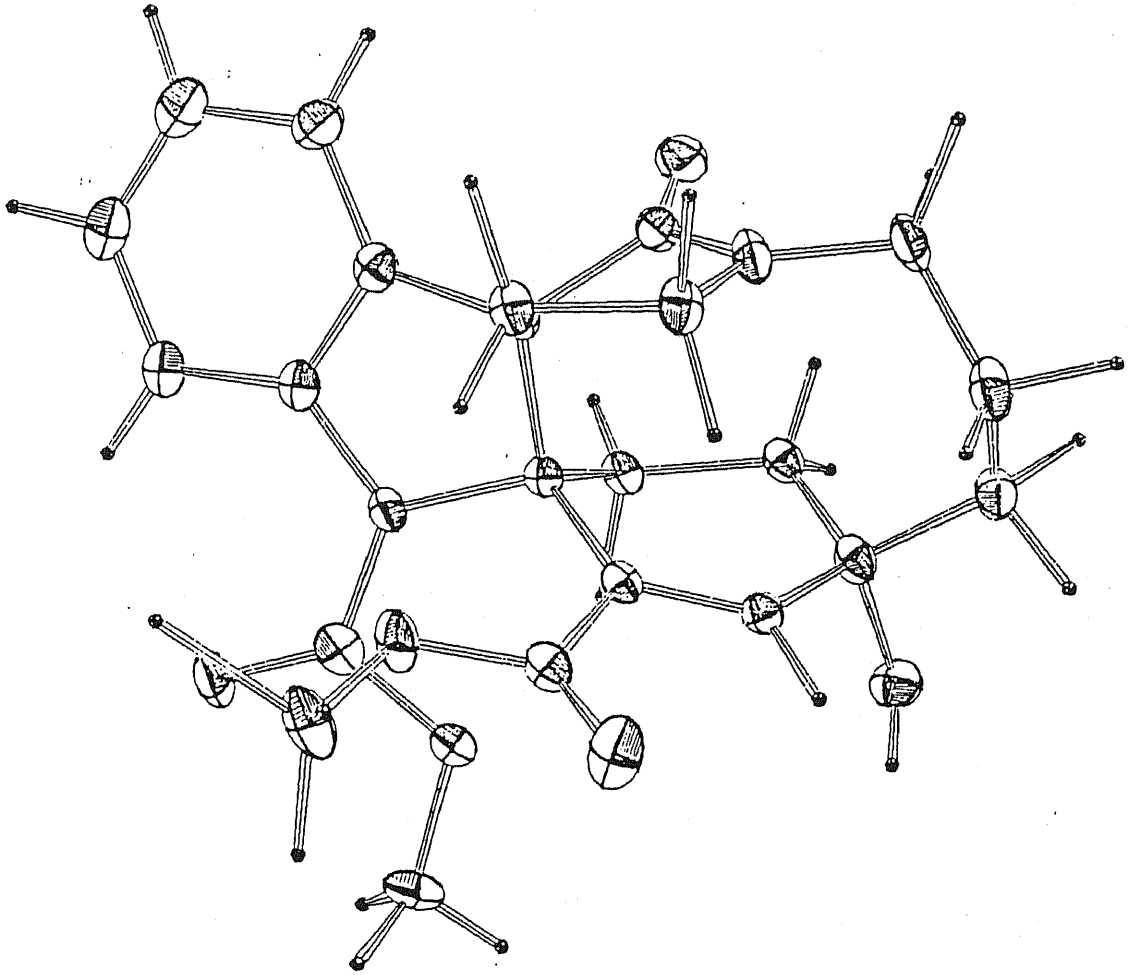
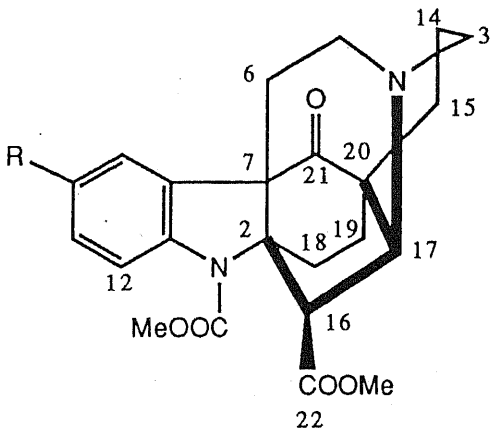


**Fig 11** ORTEP drawing of kopsijasminilam

Further extraction with ethanol, followed by the usual work-up procedures and repeated chromatography over silica gel yielded alkaloid **48**, whose molecular formula $C_{23}H_{26}N_2O_5$ was established by high resolution mass measurement. In the region 1690-1750 $cm^{-1}$, the IR spectrum displayed three carbonyl bands, one of them indicative for an ester (1745 $cm^{-1}$). The second band at 1727 $cm^{-1}$ could be assigned with the aid of the $^{13}C$ NMR spectrum to a keto function (214 ppm); while the absence of amine proton in the $^1H$ NMR spectrum and the observation of signals at




**48** R=H
**49** R=OMe

153 and 52 ppm in the $^{13}C$ NMR spectrum allowed the assignment of the absorption band at 1697 $cm^{-1}$ to a urethane moiety. The UV spectrum displayed a similarity to a N-methoxy carbonyl-2,3,-dihydroindole chromophore.

The $^1H$ NMR spectrum recorded at 22° integrated for 26 protons, but displated three pairs of identical signals (7.91 and 7.48, 4.10 and 3.89, and 1.95 and 1.78 ppm) integrating for one half proton each. Increasing temperature led to a gradual broadening of those signals and to a collapse of the broad singlet buried under the methyl ester signals at 3.74 ppm. This observation suggested that 86 was a 1:1 mixture of two conformers at room temperature in which the N-carbomethoxy group was in two energetically available planar conformations. Splitting of all but three of the signals in the $^{13}C$-NMR spectrum supported that notion. The equilibrium of energetically favorable conformers for indole alkaloids bearing a urethane group has been reported previously(186,187).

The $^1H$-NMR data were further analysed with the aid of resolution enhanced COSY spectra at 22° and 55° (see Fig 12a and b, Table 20). In addition to the N-carbomethoxy and methyl ester groups, five isolated spin systems were observed. Four aromatic protons with a coupling pattern typical for an o-disubstitutes aromatic ring were observed in the region 7.0-7.9 ppm, and a two- proton spin system was

comprised of a split doublet at 4.10 and 3.89 ppm, coupled to a doublet at 3.52 ppm ($J$ = 9.4 Hz). Two pairs of methylene protons, one pair of which, because of their chemical shifts (2.97 and 2.75 ppm) had to be vicinal to a heteroatom, and a second pair were observed at 1.53 and 3.54 ppm. The large chemical shift difference ($\Delta\delta$ 2.01) between these two protons indicated a strong anisotropic effect of a spacially close carbonyl. Another spin system consisted of three pairs of methylene protons (2.92 and 2.79, 2.38 and 2.49 and 1.21 ans 1.33 ppm), the first of which was also adjacent to nitrogen. Finally, the signals for another two pairs of methylene protons were observed at 1.95 (1.78) and 1.43, and 1.35 and 1.58 ppm, respectively.

The APT spectrum of **48** displayed resonances for four aromatic CH and two aromatic quaternary carbons, three non-aromatic quaternary, two methine and seven methylene carbons together with signals attributable to the N-carbomethoxy, methyl ester and keto functionalities. The latter signal, due to its unusually high chemical shift (214 ppm) was reminiscent of kopsidasinine **49** (186), and a tentative assignments of the other carbon resonances showed good agreement with a kopsidasinine type skeleton.

Positive proof of the nature of the nucleus was obtained through extensive NOE difference experiments at 22° and 55°, which linked all of the discrete spin systems together and completely established the relative sterochemistry and the preferred conformation. Furthermore, the spectral simplification permitted substantially overlapping signals in the region 1.2-1.7 ppm.

The results of the NOE difference experiments are schematically represented in Fig. 13, which also shows the preferred conformation of **48** in solution. On irradiation of the N-carbomethoxy resonance, a weak enhancement of the H-12 signals was observed. The fact that the H-12 resonances of both conformers were enhanced can be explained by consideration of an equilibrium states where conformational exchange is much faster than the rather slow NOE build-up and decay. NOE's from H-16 to H- 18a, from H-17 to H-19a, H-15a and H-3a, and between H-14b and H-5b defined the relative stereochemistry around the quaternary carbons C-2, C-7 and C-20. Irradiation of H-6b led to an enhancement of H-9, thereby establishing a link to the indole nucleus. The stereochemistry around the urethane moiety was defined by the anisotropic effect of the N-carbomethoxy carbonyl group on H-12, H-16 and H-18b, and their spatial proximity was confirmed by a molecular model. The stereochemistry of the C-16 ester group was established from the coupling constant $J_{16,17}$ = 9.4 Hz, indicating a small dihedral angle for the H-C-16 to H-C-17 bond. The ester

function had therefore to be endo relative to the C-18-C-19 bridge. Molecular models revealeld a very close spatial relationship between the ester carbonyl and H-6a, thereby explaining the unusually strong deshielding observed for that proton. Additional NOE's that could be observed are also indicated in Fig 13.

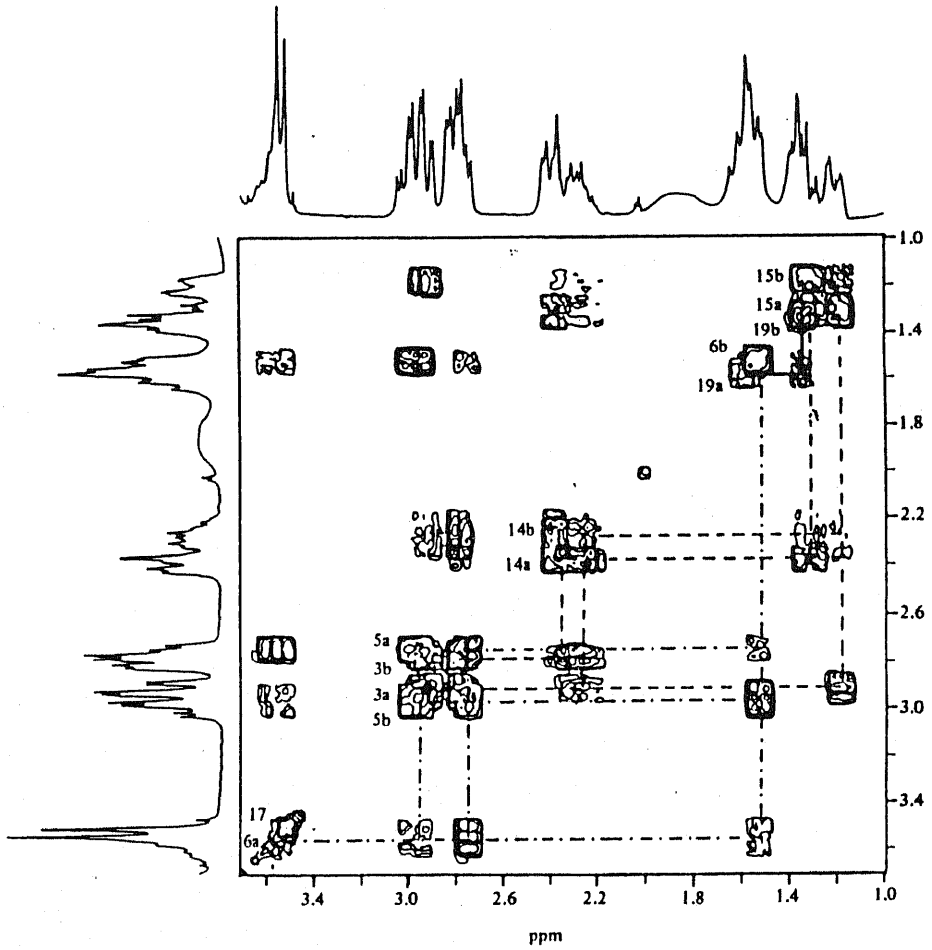



**Fig 12** Upfield region of the COSY spectra of 10-demethoxykopsidasinine (**48**);
(a) recorded at 22°, (b) recorded at 55°

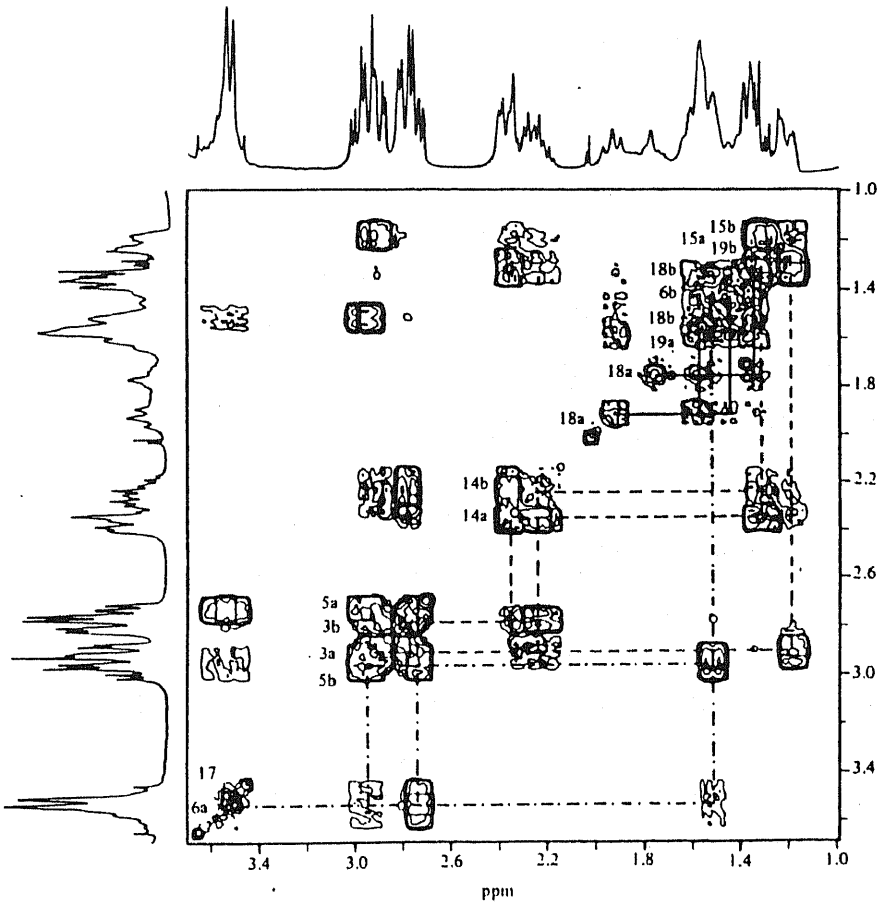

15a
15b
19b
18b
6b
18b
19a
18a
18a
14b
14a
5a
3b
3a
5b
17
6a
1.0
1.4
1.8
2.2
2.6
3.0
3.4
3.4
3.0
2.6
2.2
1.8
1.4
1.0
ppm

**Fig 12b**

The $^{13}C$-NMR spectral data were assigned through 1D heteronuclear correlation using the CSCM 1D pulse sequence (188). Selective population transfer from both the upfield and downfield $^{13}C$ satellites in the $^{1}H$ NMR spectrum allowed for the unambiguous assignment of all of the protonated carbons (Table 20). Compared to the previously reported assignments of the closely related kopsidasinine(186), all of the interchangeable assignments have been clarified. The assignment of the C-3 and C-5 resonances had to be inverted, and the resonances of the aromatic carbons C-9, C-10 and C-11, all of them close in chemical shift, could be distinguished based on the well resolved $^{1}H$-NMR spectrum. In a similar fashion, the four signals in the region 28-35 ppm were attributed to the aliphatic methylene carbons C-6, C-14, C-18 and C-19.

Based on this spectral evidence, compound **48** was identified as 10-demethoxykopsidasinine. To our knowledge it is only the second indole alkaloid known with this particular skeleton, and since the structure of kopsidasinine **49** was established essentially through chemical correlation with kopsidasine and pleicarpine, the present report represents the first detailed NMR spectral analysis of a compound in this series.

10-Demethoxykopsidasinine (**48**). Colourless prisms, 60 mg, 0.0086%, mp 194-196°, $[\alpha]^{20}_{D}$ - 159° (MeOH, c 1.00); UV $\lambda_{max}$ (MeOH) nm (ε) : 208 (26,800), 243 (13,600), 280 (2,200), 287 (2,000); IR $\upsilon_{max}$ KBr $cm^{-1}$: 2800, 2720, 1747, 1727, 1697, 1585, 1570, 1481, 1463, 1444, 1365, 1359, 1197, 1169, 740; EIMS *m/z* (rel. int.) 410(47, $M^+$), 379(37), 378(100), 351, 350(16), 323(8), 322(7), 215(19), 201(10), 182(8), 180(8), 168(9), 167(8), 156(9), 154(9), 122(15), 109(14); HR EIMS *m/z* found 410.1842 ($M^+$), calcd $C_{23}H_{26}N_2O_5$ 410.1841; $^{1}H$ NMR : see Table 20; $^{13}C$ NMR : see Table 21.

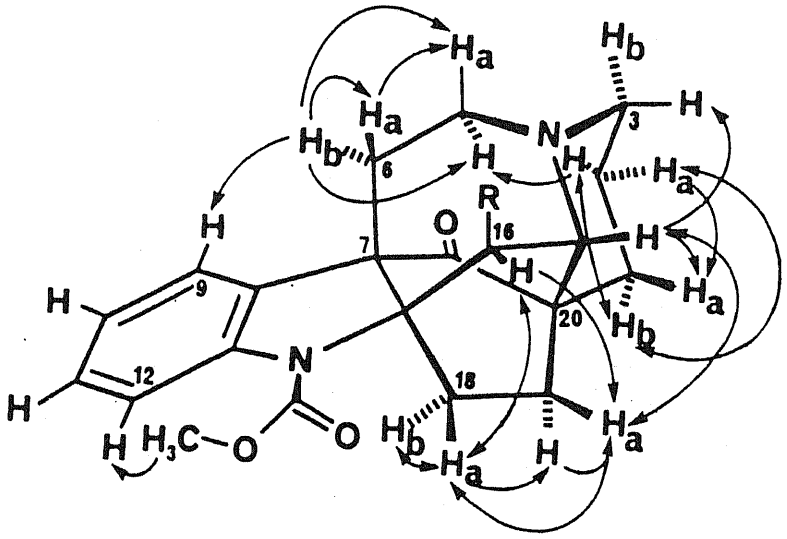



**Fig 13** NOE's observed for 10-demethoxykopsidasinine (48)

**Table 20** $^1H$ NMR spectral data of 10-demethoxykopsidasinine (**48**)*

| H | Chemical Shift | | | | Coupling constant (Hz) | |
|---|---|---|---|---|---|---|
| | 22°C | | 55°C | | | |
| 3a | 2.92 | *ddd* | 2.94 | *ddd* | 5a, 6b | 1.4 |
| 3b | 2.79 | *dt* | 2.78 | *dt* | 5a, 5b | 13.0 |
| 5a | 2.75 | *ddd* | 2.72 | *ddd* | 5a, 6a | 6.1 |
| 5b | 2.79 | *ddd* | 2.96 | *ddd* | 6a, 6b | 13.7 |
| 6a | 3.54 | *ddd* | 3.56 | *ddd* | 6a, 5b | 13.0 |
| 6b | 1.53 | *dt* | 1.53 | *dt* | 5b, 6b | 5.4 |
| 9 | 7.73 | *br d* | 7.73 | *d* | 3a, 3b | 13.0 |
| 10 | 7.05 | *dt* | 7.03 | *dt* | 3a, 14a | 5.2 |
| | 7.06 | *dt* | | | 3a, 14b | 12.9 |
| 11 | 7.20 | *dt* | 7.21 | *dt* | 3b, 14a | 1.5 |
| | 7.24 | *dt* | | | 3b, 14b | 5.0 |
| 12 | 7.91 | *br d* | - + | | 3b, 15b | 1.5 |
| | 7.48 | *br d* | - + | | 14a, 15a | 5.2 |
| 14a | 2.39 | *dddd* | 2.39 | *dddd* | 14a, 15b | 2.0 |
| 14b | 2.28 | *ddddd* | 2.28 | *ddddd* | 14b, 15a | 12.0 |
| 15a | 1.33 | *ddd* | 1.33 | *ddd* | 14b, 15b | 5.0 |
| 15b | 1.21 | *dddd* | 1.19 | *dddd* | 14a, 14b | 12.9 |
| 16 | 4.10 | *d* | - + | | 15a, 15b | 13.2 |
| | 3.89 | *d* | - + | | 9, 10 | 7.6 |
| 17 | 3.52 | *d* | 3.52 | *d* | 10, 11 | 7.5 |
| 18a | 1.95 | *ddd* | - + | | 11, 12 | 7.5 |
| | 1.78 | *ddd* | - + | | 16, 17 | 9.4 |
| 18b | 1.43 | *dt* | - + | | 18a, 18b | 10.0 |
| 19a | 1.58 | *br dd* | 1.61 | *dt* | 18a, 19a | 8.8 |
| 19b | 1.35 | *dt* | 1.35 | *dt* | 18b, 19b | 3.0 |
| 16-$CO_2Me$ | 3.74 | *s* | 3.74 | *s* | 19a, 19b | 10.0 |
| $NCO_2Me$ | 3.74 | *br s* | 3.81 | *br s* | | |
| | 3.85 | *br s* | | | | |

* Obtained at 300 MHz, in $CDCl_3$
\+ Extremely broadened signals

**Table 21** $^{13}C$ NMR spectral asignments of 10-demethoxykopsidasinine (**48**)*

| C | Chemical shift | | C | Chemical shift | |
|---|---|---|---|---|---|
| 2 | 9.95 | *s* | 14 | 29.10 | *t* |
| | 69.76 | *s* | | | |
| 3 | 54.40 | *t* | 15 | 14.83 | *t* |
| 5 | 44.41 | *t* | 16 | 49.98 | *d* |
| | | | | 49.09 | *d* |
| 6 | 34.18 | *t* | 17 | 66.90 | *d* |
| | | | | 66.81 | *d* |
| 7 | 62.11 | *s* | 18 | 29.36 | *t* |
| | 61.58 | *s* | | 28.15 | *t* |
| 8 | 131.50 | *s* | 19 | 29.04 | *t* |
| | 131.26 | *s* | | | |
| 9 | 125.29 | *d* | 20 | 47.85 | *s* |
| | 124.88 | *d* | | 47.77 | *s* |
| 10 | 123.49 | *d* | 21 | 213.53 | *s* |
| | 123.32 | *d* | | 213.95 | *s* |
| 11 | 128.26 | *d* | 22 | 170.95 | *s* |
| | 128.07 | *d* | | 170.74 | *s* |
| 12 | 115.30 | *d* | $N\underline{C}O_2Me$ | 153.84 | *s* |
| | 115.27 | *d* | | 152.69 | *s* |
| 13 | 141.38 | *s* | $NCO_2\underline{Me}$ | 52.70 | *q* |
| | 140.36 | *s* | | 52.05 | *q* |
| | | | $CO_2\underline{Me}$ | 51.76 | *q* |

* Obtained at 90.8 MHz, in $CDCl_3$

### 2.1.3.3 Carbazoles

#### i. *MURRAYA SIAMENSIS* (189)

*Murraya* L., in the tribe Clauseneae, subfamily Aurantioideae, family Rutaceae, is a genus of shrubs or small trees distributed from Southeast Asia to Australia (190). *Murraya siamensis* Craib is one of the three species found throughout Thailand and is known locally as "Prong faa" (191). The powdered root of this species mixed with $H_2O$ is claimed to be taken externally and internally for eye sores, for snakebite, and for tuberculosis (192). There have been no previous phytochemical reports on any part of *M. siamensis*, so in this study we describe the isolation and structural elucidation of eight constituents, including three new carbazole alkaloids **53**, **54**, and **55**. The eight components extracted from the roots were isolated by a combination of chromatographic techniques as described in the Experimental section. The structural elucidation of these compounds will be described in the order in which they were eluted from the chromatography column.

The first and least polar compound was shown to be the carbazole heptaphylline [**50**] by comparison with the mp and spectral data reported in the literature (193). In Table 23 we include the previously unreported $^{13}C$-nmr spectrum of **50**. After we had determined independently the structures of the second and third components, these new carbazoles, **51** and **52**, were reported to be present in *Clausena harmandiana*, another Rutaceae species (194). We believe the assignments in the previous report (194) for C-2 and C-7 in **52** should be reversed (Table 23).

The fourth component was a pale yellow solid that exhibited a parent ion in its eims at *m/z* 255 and an accurate mass consistent with the molecular formula $C_{15}H_{13}NO_3$. The uv spectrum of the compound indicated a 3- formylcarbazole chromophore (195,196), and, as addition of base did not alter the spectrum, it was concluded that no phenolic hydroxyl groups were present. The $^1H$- and $^{13}C$-nmr spectra (Table 22 and 23) indicated the presence of two methoxy groups and a formyl substituent. The $^1H$ resonance for H-5, H-6, and H-8 were very similar to those in **51** and **52** and indicated that one of the methoxyl groups was attached to C-7. The other two aromatic resonances as singlets, were assigned to H-1 and H-4 and showed that the second methoxyl group must be at C-2. Thus, this component was assigned the structure 3-formyl- 2,7-dimethoxycarbazole [**53**], a new alkaloid and the methylated derivative of **52**. The related 3-formyl-2,6-dimethoxycarbazole (glycozolide)

has been isolated from *Glycosmis pentaphylla* (197) and prepared by oxidation of the corresponding 3-methyl derivative (198).

The fifth component was shown to be the known coumarin xanthoxyletin (199) by comparison of its mp and $^1$H-nmr spectrum with an authentic sample. This compound was also isolated from *C. harmandiana* (200) along with **51** and **52**, which are mentioned above (194).

The next component was found to be mukonal [**56**], a carbazole previously isolated from *Murraya koenigii* (201). The $^{13}$C-nmr spectrum of **56** is given in Table 23, as a number of our assignments, particularly in the unsubstituted benzene ring, differ from those reported previously for mukonal (201). Our assignments for this ring are similar to those reported for carbazole itself (202,203).

The seventh component was a yellow crystalline solid that showed a weak parent ion in its eims at *m/z* 307 and an accurate mass consistent with the molecular formula $C_{19}H_{17}NO_3$. The uv spectrum suggested the presence of a 3- formylcarbazole partial structure (195,196), and, as addition of base did not shift the uv maxima, it was assumed that the other oxygens are not present as phenolic hydroxyl groups. The $^1$H-nmr spectra in the three solvents used in this study (Table 22) indicated the presence of a 7-methoxy substituents as the familiar pattern seen in the spectra of **51, 52**, and **53** for H-5, H-6, and H-8 was observed here also. The only other aromatic proton, a significantly deshielded singlet, was assigned to H-4. Two vinyl protons, which were coupled to each other, and a 6- proton singlet were readily accounted for by a 2,2- dimethyl-$\Delta^3$-pyran system fused to C-1, and C-2 of the carbazole nucleus, and, thus, this component is a new alkaloid possessing structure **54**. A compound without the methoxyl substituent, murrayacine, has been isolated previously from *M. koenigii* (204) and from *Clausena heptaphylla* (205), and we therefore suggest that **54** be called 7-methoxymurrayacine. A comparison of the $^1$H-nmr spectrum of murrayacine (204) with that of **54** (Table 22) fully supports the structure proposed for the latter, as does the $^{13}$C-nmr spectrum reported in Table 23. The weaker parent ion in the eims of **54** is explained by the facile loss of one of the methyl groups at C-11 to give a carbazolopyrilium ion (203), the base peak in the spectrum.

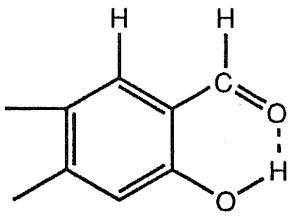
The final component isolated was a cream- colored solid that gave a parent peak in its eims (*m/z* 225) and an accurate mass corresponding to the molecular formula $C_{14}H_{11}NO_2$. The uv spectrum again suggested a 3-formylcarbazole skeleton, and as it was unchanged upon addition of base, the remaining oxygen is not present as

a phenolic hydroxyl group. The $^1H$ nmr was quite simple (Table 22, compound **55**); other than the aromatic, amine, and formyl protons it showed only one methoxyl group, and the aromatic protons H-5 to H-8 displayed a pattern similar to that found in **50** and **56**. In addition, two aromatic singlets, with chemical shifts similar to the corresponding protons in **56**, were assigned to H-1 and H-4. Thus, component **55** is 3-formyl-2-methoxycarbazole or O-methylmukonal. This compound has been systhesized (198), but to our knowledge this is the first time it has been reported in *M.koenigii* (206) and *C.heptaphylla* (207). $^1H$- or $^{13}C$-nmr spectra of **55** were not previously reported; these are included in Tables 22 and 23, respectively.

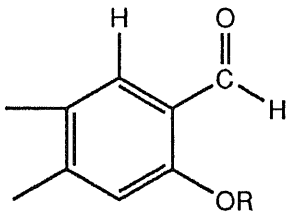
The isolation of seven carbazole components from the species under investigation provides an unusual opportunity for a detailed comparison, so the 400 MHz $^1H$- and 100 MHz $^{13}C$-nmr spectra of all these compounds are recorded in Tables 22 and 23, respectively. A compilation of the $^1H$-nmr spectra of known carbazole alkaloids was reported in 1977 (196). In our study we recorded the $^1H$-nmr spectra of the seven carbazoles in three different solvents: the polar solvent $Me_2CO$-$d_6$, the common nmr solvent $CDCl_3$, and the aromatic medium $C_6D_6$. The solvent $Me_2CO$-$d_6$ was found to be particularly effective in resolving the aromatic protons H-5 to H-8. For all compounds, the NH proton in $C_6D_6$ is shielded, while in $Me_2CO$-$d_6$ it is deshielded relative to its position in $CDCl_3$. The aromatic solvent-induced shift (ASIS) is explained by assuming that $C_6D_6$ is aligned perpendicular to the polar N-H bond at the positive end of the dipole, resulting in shielding of the amine proton by the $\tau$ cloud (208). It should be noted that with an alkyl groups at the 1 position (e.g., in **50** and **51**), this shielding effect is not as pronounced. However, in $Me_2CO$-$d_6$ this proton is deshielded because of the interaction between the amine as proton-donor and the $Me_2CO$-$d_6$ oxygen as electron pair donor (209). Interestingly, in compounds **50-52** and **56**, the 2-OH, which is H-bonded to the 3-formyl oxygen, is deshielded in $C_6D_6$ compared with the other two solvents.

A comparison of the chemical shift of H-4 in compounds containing a 2-OH substituent **50-52, 56** with those containing a 2-OR substituent (**53, 54, 55**) is instructive. In the latter compounds in $C_6D_6$,H-4 resonates considerably further downfield than in the former compounds. We suggest that in compounds **50-52** and **56**, the 3-formyl group exists in conformation A because of intramolecular H-bonding with the 2-OH substituent, while compounds **53, 54** and **55** conformation B predominates. Application of the so-called carbonyl plane rule (209,210) would predict that in $C_6D_6$, H-4 in conformation B would be deshielded because it is in front of the carbonyl plane, whereas in conformation A it would be shielded relative to its

position in $CDCl_3$. It should also be noted that in the A-type compounds **50-52, 56** the formyl proton is more shielded in all solvents compared with the B-type compounds(**53, 54, 55**). On the other hand, the formyl carbon (Table 23) is deshielded in the A-type group (~ 196 ppm) because of intramolecular H-bonding, as compared with the other group (~ 188.5 ppm). A comparison of the chemical shifts of the two methoxyl groups in **53** with the single methoxyl groups in **51, 52, 54,** and **55** establishes unambiguously that in **53** the downfield resonance in $Me_2CO$-$d_6$ and $CDCl_3$ (3.99 ppm),but the upfield resonance in $C_6D_6$ (3.32) is the 2-methoxyl group. It is hoped that knowledge of these solvent effects may find application in the structural elucidation of new carbazole natural products.




A

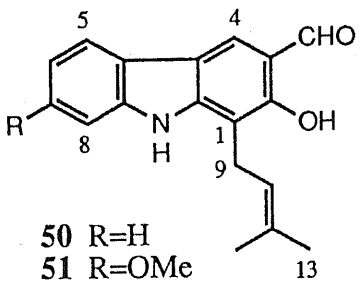



B

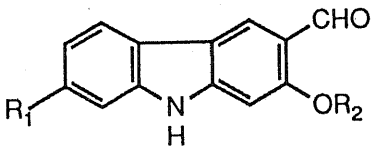
3-FORMYL-2,7-DIMETHOXYCARBAZOLE [**53**]. - Mp 219- 220°; uv (EtOH) $\lambda_{max}$ (log ε ) 299(4.69), 344(4.14); ir ($CCl_4$) 3340, 2964, 1687, 1662, 1632, 1148, 1063 $cm^{-1}$, $^1H$ nmr see Table 22; $^{13}C$ nmr see Table 23; eims (rel. int.) *m/z* [$M^+$] 255(81), 240(33); exact calcd for $C_{15}H_{13}NO_3$, 255.0895, found 255.0875.

7-METHOXYMURRAYACINE [**54**].-Mp 211-213°; uv (EtOH) $\lambda_{max}$ (log ε ) 306(4.58), 354(4.09); ir ($CHCl_3$) 3460, 1666, 1628,1603,1156 $cm^{-1}$ ; $^1H$ nmr see Table 22; $^{13}C$ nmr see Table 23 ; eims (rel.int.) *m/z* [$M^+$] 307(44), 292(100), 255(58), 210(65) ; exact mass calcd for $C_{19}H_{17}NO_3$, 307.1208, found 307.1208.

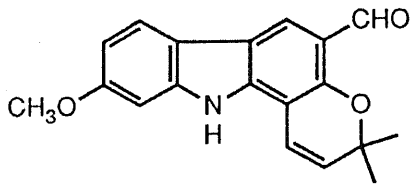
3-FORMYL-2-METHOXYCARBAZOLE(O-METHYLMUKONAL)[**55**]. -Mp 189.0-189.5° ; uv (EtOH) $\lambda_{max}$ (log ε ) 296(4.64), 350 (4.19); ir ($CHCl_3$) 1668, 1628, 1606, 1153 $cm^{-1}$; $^1H$ nmr see Table 22; $^{13}C$ nmr see Table 23; eims (rel.int.) *m/z* [$M^+$] 225(100), 208(12), 179(18), 154(14); exact mass calcd for $C_{14}H_{11}NO_2$, 225.0790, found 225.0796.




**50** R=H
**51** R=OMe




**52** $R_1$=OMe, $R_2$=H
**53** $R_1$=OMe, $R_2$=Me
**56** $R_1$=H, $R_2$=H
**55** $R_1$=H, $R_2$=Me




**54**

**Table 22** 400 MHz $^1$H-nmr spectra of compounds **50-56** in three solvents[a]

| Proton | Compound | | | |
|---|---|---|---|---|
| | **50** | **51** | **52** | **53** |
| H-1 | - | - | 6.85 (s) | 7.11 (s) |
| | | | [6.84, 6.61] | [96.85, 6.13] |
| H-4 | 8.22 (S) | 8.12 (s) | 8.29 (s) | 8.37 (s) |
| | [8.04, 7.40] | [7.94, 7.34] | [8.05, 7.36] | [8.45, 8.82] |
| H-5 | 8.01 (d, 7.6) | 7.88 (d, 8.7) | 7.92 (d, 8.8) | 7.97 (d, 8.3) |
| | [7.97, 7.80] | [7.84, 7.66] | [7.85, 7.61] | [7.87, 7.56] |
| H-6 | 7.20 (t, 7.6) | 6.82 (dd, 8.7, 2.2) | 6.83 (dd, 8:8, 2.2) | 6.88 (dd, 8.3, 2.2) |
| | [7.27, 7.21] | [6.87, 6.93] | [6.88, 6.86] | [6.88, 6.74] |
| H-7 | 7.35 (t, 7.6) | - | - | - |
| | [7.40, 7.27] | | | |
| H-8 | 7.48 (d, 7.6) | 7.01 (d, 2.2) | 7.02 (d, 2.2) | 7.02 (d, 2.2) |
| | [7.40,6.90] | [6.91, 6.44] | [6.89, 6.50] | [6.90, 6.62] |
| H-9 | 3.61 (d, 6.8) | 3.59 (d, 6.9) | - | - |
| | [3.64, 3.53] | [3.63, 3.57] | | |
| H-10 | 5.34 (br t, 6.8) | 5.32 (br t, 6.9) | - | - |
| | [5.32, 5.22] | [5.32, 5.26] | | |
| H-12 | 1.64 (d, 10) | 1.64 (d, 1.0) | - | - |
| | [1.77, 1.57] | [1.78, 1.58] | | |
| H-13 | 1.81 (s) | 1.81 (s) | - | - |
| | [1.90, 1.69] | [1.90, 1.71] | | |
| CHO | 9.91 (s) | 9.90 (s) | 9.95 (s) | 10.43 (s) |
| | [9.91, 9.47] | [9.90, 9.52] | [9.93, 9.49] | [10.50, 10.93] |
| NH | 10.62 (br s) | 10.51 (br s) | 10.72 (br s) | 10.64 (br s) |
| | [8.20, 7.48] | [8.10, 7.39] | [8.12, 6.31] | [8.11, 6.58] |
| OH | 11.75 (s) | 11.72 (s) | 11.42 (s) | - |
| | [11.70, 12.35] | [11.60, 12.38] | [11.43, 12.18] | |
| OMe | - | 3.84 (s) | 3.85 (s) | 3.85, 3.99 (s)[b] |
| | | [3.90, 3.46] | [3.90, 3.47] | [3.90, 3.99] ; |
| | | | | 3.46, 3.32] |

[a]For each proton the first number is the chemical shift in the solvent $Me_2CO$-$d_6$ while the two numbers in square brackets are values obtained in $CDCl_3$ and $C_6D_6$, respectively. In parentheses are given the multiplicity for the proton and the coupling constant (s) in Hertz.
[b]In each pair, the first value is for the 7-OMe and the second for the 2-OMe

**Table 22** *Continued*

| Proton | Compound | | |
|---|---|---|---|
| | **54** | **55** | **56** |
| H-1 | - | 7.12 (s) | 6.89 (s) |
| | | [6.88, 6.10] | [6.88, 6.59] |
| H-4 | 8.23 (s) | 8.50 (s) | 8.43 (s) |
| | [8.30, 8.69] | [8.56, 8.88] | [8.18, 7.42] |
| H-5 | 7.97 (d, 8.4) | 8.10 (d, 8.0) | 8.07 (d, 7.6) |
| | [7.84, 7.53] | [8.00, 7.70] | [7.99, 7.75] |
| H-6 | 6.83 (dd, 8.4, 2.2) | 7.15 (t, 8.0) | 7.22 (t, 7.6) |
| | [6.86, 6.73] | [7.25, 7.09] | [7.28, 7.18] |
| H-7 | - | 7.35 (t, 8.0) | 7.38 (t, 7.6) |
| | | [7.38, 7.25] | [7.40, 7.26] |
| H-8 | 6.79 (d, 2.2) | 7.47 (d, 8.0) | 7.48 (d, 7.6) |
| | [6.90, 6.55] | [7.40, 6.96] | [7.40, 6.88] |
| H-9 | 6.91 (d, 9.8) | - | - |
| | [6.61, 6.03] | | |
| H-10 | 5.90 (d, 9.8) | - | - |
| | [5.80, 5.32] | | |
| H-12 | 1.54 (s) | - | - |
| | [1.55, 1.26] | | |
| H-13 | 1.54 (s) | - | - |
| | [1.55, 1.26] | | |
| CHO | 10.45 (s) | 10.45 (s) | 9.98 (s) |
| | [10.49, 10.92] | [10.49, 10.89] | [9.95, 9.45] |
| NH | 10.68 ( br s) | 10.61 (s) | 10.72 (bt s) |
| | [8.14, 6.55] | [8.88, 6.65] | [8.22, 6.31] |
| OH | - | - | 11.46 (s) |
| | | | [11.46, 12.16] |
| OMe | 3.85 (s) | 4.00 (s) | - |
| | [3.89, 3.46] | [3.99, 3.30] | |

**Table 23** $^{13}$C-nmr spectra of compounds **50-56**[a]

| Carbon | Compounds | | | | | | |
|---|---|---|---|---|---|---|---|
| | **50** | **51** | **52** | **53** | **54** | **55** | **56** |
| C-1a | 145.3 (+) | 145.5 (+) | 147.0 (+) | 146.6 (+) | 141.6 (+) | 146.2 (+) | 146.8 (+) |
| C-1 | 109.7 (+) | 109.6 (+) | 97.0 (-) | 93.9 (-) | 105.2 (+) | 93.5 (-) | 97.1 (-) |
| C-2 | 158.0 (+) | 157.5 (+) | 160.9 (+) | 161.6 (+) | 154.4 (+) | 162.2 (+) | 161.7 (+) |
| C-3 | 115.7 (+) | 115.6 (+) | 115.8 (+) | 116.0 (+) | 117.2 (+) | 117.7 (+) | 116.1 (+) |
| C-4 | 126.0 (-) | 124.5 (-) | 126.7 (-) | 121.5 (-) | 121.5 (-) | 121.2 (-) | 128.3 (-) |
| C-4a | 117.7 (+) | 117.9 (+)[b] | 118.4 (+) | 119.4 (+) | 119.1 (+) | 119.3 (+) | 118.6 (-) |
| C-5a | 124.1 (+) | 117.5 (+)[b] | 117.3.(+) | 118.2 (+) | 118.0 (+) | 124.2 (+) | 124.0 (+) |
| C-5 | 120.7 (-)[b] | 120.7 (-) | 121.0 (-) | 120.2 (-) | 118.4 (-) | 120.7 (-)[b] | 121.0 (+) |
| C-6 | 120.0 (-)[b] | 109.0 (-) | 109.2 (-) | 109.5 (-) | 109.3 (-) | 120.5 (-)[b] | 120.4 (-) |
| C-7 | 125.9 (-) | 159.5 (+) | 159.8 (+) | 159.7 (+) | 160.1 (+) | 126.2 (-) | 126.5 (-) |
| C-8 | 111.6 (-) | 95.9 (-) | 96.1 (-) | 96.2 (-) | 96.2 (-) | 111.7 (-) | 111.8 (-) |
| C-8a | 141.5 (+) | 142.9 (+) | 143.0 (+) | 143.0 (+) | 143.2 (+) | 141.4 (+) | 141.7 (+) |
| 2-OMe | - | - | - | 56.3 (-) | - | 56.1 (-) | - |
| 3-CHO | 196.3 (-) | 196.3 (-) | 196.2 (-) | 118.6 (-) | 118.3 (-) | 188.4 (-) | 196.1 (1) |
| 7-OMe | - | 55.3 (-) | 55.5 (-) | 55.8 (-) | 55.7 (-) | - | - |
| C-9 | 23.0 (+) | 22.9 (+) | - | - | 130.8 (-) | - | - |
| C-10 | 121.7 (-) | 121.8 (-) | - | - | 117.4 (-) | - | - |
| C-11 | 132.7 (+) | 132.6 (+) | - | - | 77.7 (+) | - | - |
| C-12 | 17.6 (-) | 17.6 (-) | - | - | 27.6 (-) | - | - |
| C-13 | 25.3 (-) | 25.3 (-) | - | - | 27.6 (-) | - | - |

[a]Chemical shifts are in ppm with the solvent $Me_2$-CO-$d_6$ as internal reference.
[b]Assignments may be interchanged.

### 2.1.3.4 Lupine Alkaloids

#### i. *SOPHORA EXIGUA* (211)

*Sophora exigua* Craib. has been used as a folk medicinal plant in Thailand for antipyretic and respiratory diseases (212). In this reports, a new lupin alkaloid, (-)-cytisineacetamide isolated from the roots of *S. exigua* is presented.

Separations by silica gel chromatography, preparative TLC and preparative HPLC of a 75% ethanol extract of the dry roots of *S.exigua* gave a new alkaloid, (-)-12- cytisineacetamide (**57**), together with eight known bases, (-)-cytisine (**58**), (-)-12-hydroxycytisine (**59**), (-)-N-methylcytisine (**60**), (-)-N-formylcytisine (**61**), (-)-lupanine (**62**), (+)-5,6-dehydrolupanine (**63**), (-)-anagyrine (**64**), and (-)-baptifoline (**65**) (Table 24).

The new alkaloid (**57**) (0.005% dry wt), was an oil, $[\alpha]^{25}_{D}$ - 204°, which gave the molecular formula $C_{13}H_{17}N_3O_2$ by high resolution mass spectrometry. The presence of an α-pyridone ring was suggested by absorptions in the UV spectrum at 309 nm(log ε 3.88) and 233(3.84) and in the IR spectrum at 1650 $cm^{-1}$. The fragment ions at *m/z* 189, 160 and 146 in the mass spectrum of **57** also showed the presence of an α-pyridone ring system [213]. The IR spectrum of **57** showed the absorption of an amide groups at 1690 $cm^{-1}$. Furthermore, two signals at δ 61.7 (t, C-14) and at δ 175.5 (s, C-15) were observed compared to those of (-)-cytisine (**58**) in the $^{13}C$ NMR DEPT spectrum (Table 25). The $^{1}H$ NMR spectrum of **57**, except for the two isolated signals at δ 2.97 (1H, d, $J$ = 16.2 Hz, H-14) and at δ 2.87 (1H, d, $J$ = 16.2 Hz, H-14'), showed essentially similar signals to that of (-)-cytisine (**58**). In the $^{13}C$ nmr spectrum of **57** (Table 25), the signals of C-11 and C-13 were shifted downfield in the range of 7-8, compared to those of **58**. From these results, the structure of **57** was suggested to be an acetamide of (-)-cytisine (**58**) at the N-12 position, namely (-)-12-cytisineacetamide (**57**). Further confirmation of the identify of the new alkaloid as **57** was obtained by comparing the natural product directly with a synthetic sample, prepared from **58** with α-chloroacetamide.

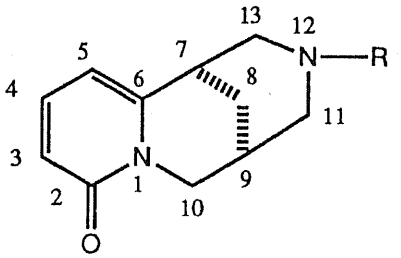
It was possible that **57** might be an artifact of (-)-12-cytisineacetic acid (**66**) as extraction of the compound was performed using 28% $NH_4OH$ [214,215]. Therefore, the presence of **66** in the original plants was examined by two methods. The first method used extraction without 28% $NH_4OH$ [216]. The second was preparation of the amino acid fraction using a cationic exchange resin. Aqueous EtOH extracts from the dry roots of *S. exigua* were treated with Dowex-50W X4 ($H^+$ form) and the fraction containing the zwitter-ion compounds obtained. All alkaloidal fractions

obtained using these two methods were analysed by HPLC. However, analysis of both fractions did not reveal the presence of **66** but demonstrated the presence of **57**. Consequently, it was confirmed that **57** is not an artificial product of **66**.

The absolute configuration of **57** was determined by comparison of the CD spectrum with that of **58**. The CD spectrum of natural and synthetic **57** gave a negative Cotton effect at 307 nm ($[\theta]_{307}$ - 26100) and a positive one at 232 nm ($[\theta]_{232}$ + 28600). These are the same as those of **58** ($[\theta]_{310}$ - 21000, $[\theta]_{233}$ + 43000), reported previously [217]. Therefore, the absolute configuration of **57** was determined to be 7R,9S, the same as that of (-)- cytisine (**58**) (7R,9S) [218].

Compound **59**, (-)-12-hydroxycytisine has already been isolated from *Spartium junceum* (Leguminosae) with the name of cytisine N-oxide, which was considered to be in equilibrium with 12-N-hydroxycytisine [219]. However, no detailed characterization of **59** has been a vailable so far. Therefore, we report a full characterization of **59** in this investigation.

So far, we have isolated N-12-cytisine derivatives such as **60**, **61**, **66**, (-)-rhombifoline, (-)-N-ethylcytisine, (-)-N-acetylcytisine, and (-)-N-(3-oxobutyl) cytisine from several leguminous plants and demonstrated a few enzymatic activities for biotransformation of N-12-cytisine derivatives [214, 220-226]. Recently, Greinwald *et al.* reported the isolation and structural determination of cytisine 12-carboxy-ethylester as a new compound from suspension culture of *Laburnum watereri* (Leguminosae) [227]. It is of interest that the occurrence of these N-12 derivatives reveals their possible role as key intermediates in the biosynthetic pathway between tricyclic lupin alkaloids and tetracyclic alkaloids.




| | R (14 15) |
|---|---|
| **57** | $CH_2CONH_2$ |
| **58** | H |
| **59** | OH |
| **66** | $CH_2CO_2H$ |

**Table 24** Physical constants and yield of lupin alkaloids from roots of *S. exigua*

| Akaloids<br>Total base | Mp | $[\alpha]_D$ (% EtoH) | | Yield:mg<br>5.4 g | (% dry wt)<br>(1.5) |
|---|---|---|---|---|---|
| (-)-12-Cytisineacetamide (**57**) | oil | -204 | (0.17) | 17 | (0.005) |
| (-)-Cytisine (**58**) | 155 | -112 | (0.54) | 1640 | (0.456) |
| (-)-12-Hydroxycytisine (**59**) | 238 + | -209 | (0.11) | 11 | (0.003) |
| (-)-N-Methylcytisine (**60**) | 137 | -219 | (0.48) | 1640 | (0.456) |
| (-)-N-Formylcytisine (**61**) | 170 | -230 | (0.15) | 15 | (0.004) |
| (-)-Lupanine (**62**)* | oil | - | | trace | - |
| (+)-5,6-Dehydrolupanine (**63**)* | oil | - | | trace | - |
| (-)-Anagyrine (**64**) | oil | -153 | (0.03) | 3 | (0.001) |
| (-)-Baptifoline (**65**) | 207 | -137 | (0.20) | 219 | (0.061) |

*CD spectra were measured instead of $[\alpha]_D$
+Decomposition

**Table 25** $^{13}C$ NMR spectral data of compounds **57** and **58**

| C | | **57** | **58** | **57-58** |
|---|---|---|---|---|
| 2 | s | 165.5 | 166.6 | -1.1 |
| 3 | d | 116.8 | 117.8 | -1.0 |
| 4 | d | 141.4 | 142.1 | -0.7 |
| 5 | d | 107.9 | 108.9 | -1.0 |
| 6 | s | 153.2 | 153.4 | -0.2 |
| 7 | d | 36.7 | 36.9 | -0.2 |
| 8 | t | 26.0 | 27.3 | -1.3 |
| 9 | d | 29.5 | 29.5 | 0 |
| 10 | t | 51.5 | 51.8 | -0.3 |
| 11 | t | 61.2 | 53.6 | +7.6 |
| 13[a] | t | 61.8 | 54.6 | +7.2 |
| 14[a] | t | 61.7 | - | - |
| 15 | s | 175.5 | - | - |

[a]Assignments may be reversed

(-)-12-Cytisineacetamide (**57**). Oil. $[\alpha]^{25}_D$ - 204° (EtOH; c 0.17). CD (MeOH): $[\theta]_{307}$ - 26100, $[\theta]_{232}$ + 28600. UV $\lambda_{max}$ MeOH nm (log ε ) : 309(3.88), 233(3.84),

IR $\upsilon_{max}$ $CHCl_3$ $cm^{-1}$; 3500, 3370, 1140 (NH), 2990, 2940, 2800 (C-H), 1690 (amide) 1650, 1550 (α-pyridone). EI-MS *m/z* (rel. %): 247.1321 $[M]^+$ (20) ($C_{13}H_{17}N_3O_2$, calc. 247.1321), 230(5), 203(100),189(8), 160(19), 146(12), 117(7), 101(9), 58(62), 44(18). $^{13}C$ NMR (67.8 MHz, $CD_3OD$): δ 175.5 (s,C-15), 165.5 (s, C-2), 153.2 (s, C-6), 141.4 (d, C-4), 116.8 (d, C-3), 107.9 (d, C-5), 61.8 (t, C-13)*, 61.7 (t, C-14)*, 61.2 (t, C-11), 51.5 (t, C-10), 36.7 (d, C-7), 29.5 (d, C-9), 26.0 (t, C-8); *assignments may be reversed. $^1H$ NMR (500 MHz, $CD_3OD$): δ7.48 (1H, dd, *J* = 8.8 and 6.9 Hz, H-4), 6.43 (1H, dd, *J* = 8.8 and 1.4 Hz, H-3), 6.32 (1H, dd, *J* = 6.9 and 1.1 Hz, H-5), 4.12 (1H, d, *J* = 15.5 Hz, H-10eq), 3.91 (1H, dd, *J* = 15.5 and 6.0 Hz, H-10ax), 3.14 (1H, d, *J* = 2.2 Hz, H-7), 3.00 (1H, d, *J* = 12.0 Hz, H-11eq), 2.97 (1H, d, *J* = 16.2 Hz, H-14), 2.90 (1H, dm, *J* = 11.1 Hz, H-13eq), 2.87 (1H, d, *J* = 16.2 Hz, H-14'), 2.60 (1H, d, *J* = 12.0 Hz, H-11ax), 2.57 (1H, dd, *J* = 11.1 and 2.1 Hz, H-13ax), 2.50 (1H, d, *J* = 2.5 Hz, H-9), 1.96 (1H, dt, *J* = 12.9 and 1.5 Hz, H-8eq), 1.87 (1H, d, *J* = 12.9 Hz, H-8ax).

(-)-12-Hydroxycytisine (**59**). Pale yellow needles. Mp 238o (dec.). $[\alpha]^{25}_D$ - 209° (EtOH; c 0.11), CD (MeOH) : $[\theta]_{308}$ - 19100, $[\theta]_{232}$ + 17400, UV $\lambda_{max}$ MeOH nm (log ε ) : 308(3.83), 232(3.78). IR $\upsilon_{max}$ KBr $cm^{-1}$; 3200, 1150 (N-H), 2940, 2900, 2820 (C-H), 1640, 1540 (α-pyridone). EI-MS *m/z* (rel. %): 206.1053 $[M]^+$ (62) ($C_{11}H_{14}N_2O_2$, calc. 206.1054), 190(29), 189(40), 160(43), 148(28), 147(54), 146(100), 134 (26), 117(16), 93(8), 44(40), 41(30). $^{13}C$ NMR ($CD_3OD$), 125.65 MHz): δ 165.5 (s, C-2), 153.0 (s, C-6), 141.3 (d, C- 4), 116.8 (d, C-3), 107.8 (d, C-5), 66.0 (t, C-13), 65.9 (t, C-11), 51.4 (t, C-10), 37.5 (d, C-7), 30.6 (d, C-9), 25.9 (t, C-8). $^1H$ NMR ($CD_3OD$, 500 MHz): δ 7.45 (1H, dd, *J* = 8.8 and 6.9 Hz, H-4), 6.39 (1H, dd, *J* = 8.8 and 1.1 Hz, H-3), 6.29 (1H, dd, *J* = 6.9 and 0.8 Hz, H-5), 4.05 (1H, d, *J* = 15.4 Hz, H-10eq), 3.87 (1H, dd, *J* = 15.4 and 6.7 Hz,H- 10ax), 3.32 (1H, dm, *J* = 12.1 Hz, H-11eq), 3.26 (1H, dt, *J* = 9.6 and 1.8 Hz, H-13eq), 3.18 (1H, d, *J* = 2.5 Hz, H-7), 2.72 (2H, br d, *J* = 9.6 Hz, H-11ax, 13ax), 2.60 (1H, dd, *J* = 6.1 and 3.0 Hz, H-9), 1.83 (2H, dd, *J* = 13.9 and 3.0 Hz, H-8).

### 2.1.4 Flavonoids

The flavonoids are a very large class of naturally occurring compounds that can be suitably subclassified according to the oxygenation patterns of the shikimate-derived aromatic ring (C). They are common in the higher plants and have been extensively used as chemotaxomonic markers. The flavonoids presented here are included dihydroflavonol, prenylated flavonoids, isoflavonoids, homoisoflavonoids and rotenoids.

#### i. *BLUMEA BALSAMIFERA* (228)

*Blumea balsamifera* DC is a member of the tribe Inuleae (Compositae) which has been used in the traditional medicine of some Oriental cultures. The Chinese have used preparations of this plant as a carminative, a mild stimulant, a vermifuge, as a topical application for septic ulcers, and as a preventive medicament in times of epidemics. The ancient Chinese medical literature has recorded its use as an abortifacient (229).

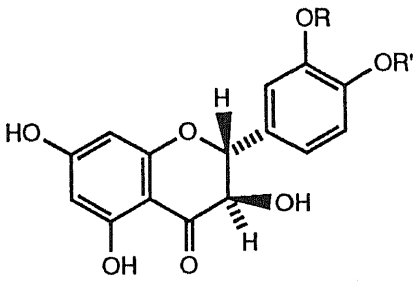
Preparations of *Blumea* are presently finding use in the traditional medicine of Thailand, where they are available at local herbal drug shops. Cigarettes are prepared from the chopped, dried leaves of *B. balsamifera* and smoked to relieve the pain of sinusitis, An infusion prepared from leaf material is used as a stomachic, carminative, diaphoretic, expectorant and emmenagogue. A decoction of fresh leaves is used, alone or in combination with other plant preparations, as a bath for women following childbirth.

The chemistry of *Blumea* constituents has been of some interest for at least 65 years. The essential oil was the first product to be studied (230). In this initial study, d-carvotanacetone, l-tetrahydrocarvone, a mixture of butyric, isobutyric and n-octanoic acids, and an unidentified phenol were isolated. Subsequently, l-borneol (229), fenchone (231), 1,8-cineol (231), two carvotanacetone derivatives (232) a diester of coniferyl alcohol (233), some polyacetylenes and thiophene derivatives (233,234), campesterol (235), stigmasterol (236), sitosterol (237), xanthoxylin (229), erianthin and 5,3',4'-trihydroxy-3,6,7-trimethoxyflavone (236,238-240), other unidentified flavonoids (229, 238), coumarins and triterpenes (229), and myristic acid (231) have been isolated from *Blumea* species.

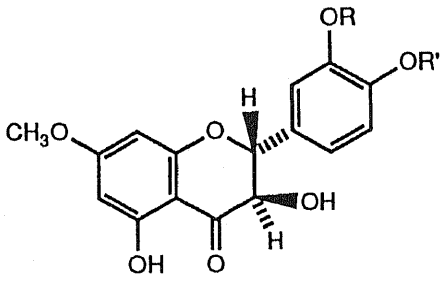
The present study was undertaken in order to expand our knowledge of the constituents of this medicinally interesting genus, and describes the isolation of two flavonoid derivatives, (2R,3R)-dihydroquercetin-4'-methyl ether (**67**) and (2R,3R)-dihydroquercetin-4',7-dimethyl ether (**68**), both of which are new natural products.

The proton nmr data of isolate **67** strongly suggested that it was a dihydroflavonol derivative, and the characteristic 11 Hz doublets at 4.48 and 5.03 ppm indicated a *trans*-diaxial arrangement of 2-H and 3-H in such a system. That the signal for 3-H was further split and, on addition of $D_2O$,sharpened to a doublet indicated that the hydroxy at C-3 was not methylated (241a). The AB system centered at 5.87 ppm, showing *meta* coupling of approximately 1 Hz, suggested that they were protons at C-6 and C-8 in a dihydroflavonol oxygenated at C-5 and C-7 (241b).

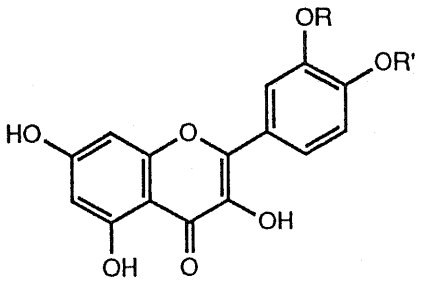
The pronounced bathochromic shift of the band II absorption in the uv spectrum of **67** on addition of sodium acetate indicated the presence of a free hydroxyl at C-7 (241c). Addition of aluminum chloride reagent produced bathochromic shifts of band I (54 nm) and band II (25 nm) characteristic of a second free hydroxyl at C-5 (241d). Mass spectral peaks at *m/z* 166 and 137 substantiated that the remaining hydroxyl and the methoxyl group were indeed on the B-ring (242, 243).



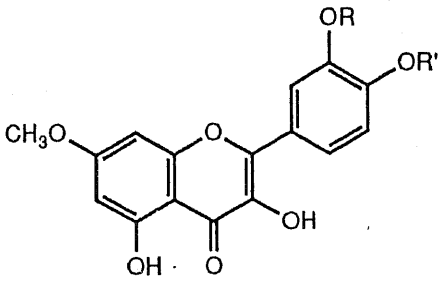

**67** R = H, R' = $CH_3$
**69** R = $CH_3$, R' = H




**68** R = H, R' = $CH_3$
**70** R = $CH_3$, R' = H




**71** R = H, R' = $CH_3$
**73** R = $CH_3$, R' = H




**72** R = H, R' = $CH_3$
**74** R = $CH_3$, R' = H

The multiplet at 6.91 (DMSO) or 7.00-7.10 (acetone) in the nmr spectrum of **67** are suggestive of oxygenation of C-3 and C-4 (241e). However, definitive assignment of

the substitution pattern could not be made on the basis of these spectra. A portion of the isolate was oxidized (2N $H_2SO_4$ /air (244) to the corresponding flavonol, which should be either tamarixetin (**71**) or isorhamnetin (**73**). Comparison of the nmr spectum of the TMS ether of this unknown flavonol with published spectra of the two reference compounds (241f) showed that the unknown flavonol corresponded to tamarixetin (**71**). This was further substantiated by the stability of the uv spectrum of the oxidation product in the presence of sodium methoxide. Flavonols having a free hydroxyl at C-4 are unstable in sodium methoxide solution, and their uv spectra degenerate in a few minutes (241g). The flavonol produced by the oxidation of **67** was seen to be stable in the presence of sodium methoxide for at least 24 hours. Also, tlc comparison of the oxidation product with isorhamnetin (**73**) showed them to be different in several systems. A sample of tamarixetin was, unfortunately, not available.

The absolute stereochemistry at C-2 and C-3 was determined by means of circular dichroism. Comparison of the cd curves of **67** with those of dihydroflavonols of known configuration (245) established the absolute configuration as 2R,3R, the signs of the Cotton effects from 400 to 200 nm being respectively +, -, +, +. Thus **67** is assigned the structure (2R,3R,)-dihydroquercetin-4'-methyl ether or (2R,3R)-dihydrotamarixetin.

The nmr spectrum of the isolate **68** was identical to that of **67** except that it showed the loss of one exchangeable proton and the addition of a second aromatic methoxyl group. Since the uv spectrum was unchanged on addition of sodium acetate, this methoxyl group is located at C-7 (241c). Mass spectal evidence confirmed that the additional 14 mass units were indeed in the A-ring. Sulfuric acid oxidation of **68** was used to establish the B-ring substitution pattern as in **68** rather than **70**. Thus, the uv spectrum of the oxidation product **72** was found to be quite stable in sodium methoxide solution, and tlc comparison with a reference sample of rhamnazin (**74**) showed **72** to different in several systems. Circular dichroism again establised the absoluted configuration to be 2R,3R. Thus, **68** is assigned the structure (2R,3R)-dihydroquercetin-4',7-dimethyl ether.

In 1972, Herz *et al.* (246) reported the isolation of a dihydroflavonol from a *Eupatorium* hybrid (Compositae) having either **68** or **70** as its sturcture. They were unable to completely define their isolate, having lost the remaining sample in an unsuccessful oxidation reaction.

Comparison of the aromatic region of the nmr spectrum (in DMSO) of their isolate with that of **68** showed them clearly to be different. Therefore, from the data presented in ref. 246 and comparison with our isolate, **68**, we can now propose the

structure of the *Eupatorium* isolate as (2R,3R)-dihydroquercetin-3',7-dimethyl ether (**70**), which is also a previously unreported natural product.

**Table 26** Ultraviolet spectra data of **67** and **68**[a]

| | **67** | **68** |
|---|---|---|
| MeOH | 327 sh (log ε = 3.89) | 327 sh (3.76) |
| | 290 (4.48) | 287 (4.49) |
| | 235 (4.54) | 230 sh (4.57) |
| | 206 (4.82) | 216 sh (4.68) |
| | | 205 (4.81) |
| $NaOCH_3$ | 327 (4.62) | 327 sh (3.76) |
| | 290 sh (4.03) | 290 (4.47) |
| | 250 sh (4.09) | 230 sh (4.57) |
| | 206 (4.82) | 217 sh (4.70) |
| | | 207 (4.79) |
| NaOAc | 327 (4.58) | 327 sh (3.76) |
| | 294 sh (4.19) | 287 (4.49) |
| NaOAc + $H_3BO_3$ | 327 sh (3.89) | 327 sh (3.76) |
| | 290 (4.48) | 287 (4.49) |
| $AlCl_3$ | 381 (3.92) | 384 (3.92) |
| | 315 (4.55) | 315 (4.57) |
| | 282 (4.11) | 287 sh (4.11) |
| | 223 (4.64) | 225 (4.70) |
| | 206 (4.76) | 206 (4.92) |
| $AlCl_3$ + HCl | 377 (4.01) | 384 (3.89) |
| | 315 (4.55) | 312 (4.50) |
| | 282 (4.25) | 287 sh (4.22) |
| | 223 (4.64) | 225 (4.68) |
| | 206 (4.77) | 206 (4.91) |

[a]All UV spectra were recorded using the standard procedures given in reference 241

(2R,3R)-DIHYDROQUERCETIN-4'-METYL ETHER (**67**)-Fraction **67**, when evaporated to dryness *in vacuo*, yielded 766 mg ($1.7x10^{-4}$%) of a light yellow, amorphous powder, mp 173-4°; $[\alpha]^{24}_D$+14.9°,ir, $\upsilon_{max}$ (KBr), 3400, 1645, 1600, 1280, and 1160 $cm^{-1}$: uv, data summarized in table 31; cd (MeOH),$[\theta]_{328}$=+3.35x$10^5$,$[\theta]_{293.5}$=-1.31x$10^6$,$[\theta]_{253.5}$=+1.95x$10^5$, $[\theta]_{220}$=+1.44 x $10^6$ deg.$cm^2$ dmole; $^1$H-nmr (DMSO-$d_6$), δ 3.78 (3H,s,$ArOCH_3$)24.48(1H,bd, *J* =11 Hz, 3-H),3.81 (3H,m,3xOH),5.03 (1H,d, *J*=11Hz,2-H),5.87(2H,AB system,6-H and 8-H and 8-H),6.91(3H,bm 2'H,5'H and 6'H),and 11.88(1H, bs,OH);$^1$H-nmr(acetone-$d_6$) ,3.88(3H,s,$ArCH_3$),4.61(1H,d, *J* =11.5 Hz,3-H),5.08(1H,d, *J* =11.5 Hz,2-H),5.98(2H,AB system,6-H and 8-H),7.00-7.10(3H,m,2'-H,5'-H and 6'-H), and 11.68 (1H,bs,OH); ms,*m/z* 318 ($M^+$, 37%, $C_{16}H_{14}O_7$), 289(44), 166(50), 165(26), 164(36), 153(100), and 137(39).

(2R,3R)-DIHYDROQUERCETIN-4',7-DIMETHYL ETHER (**68**).-Fraction **68**,when evaporated to dryness *in vacuo*, yielded 94 mg ($2.1x10^{-5}$%)of a light yellow, amorphous powder, mp 164-7°;$[\alpha]^D_{24}$+14.8°; ir, $\upsilon_{max}$(KBr), 3480m, 1630, 1610, 1273, 1150 and 1130 $cm^{-1}$;uv, data summarized in table 1;cd (MeOH),

$[\theta]_{331.5}$=+3.59x$10^5$,$[\alpha]_{224}$=1.26x$10^6$,$[\theta]_{253.5}$=+1.71x$10^5$,$[\theta]_{221.5}$= +1.28x$10^6$ deg $cm^2$/dmole; $^1$H-nmr (DMSO-$d_6$) , δ 3.79(6H, bs, 2x$ArOCH_3$), 4.55 (1H,m,3-H), 5.09 (1H,d, *J* =11 Hz,2-H),6.10(2H,AB system, *J* =2 Hz,6-H and 8-H), and 6.95 (3H,m,2'-H,5'-H and 6'-H):$^1$H-nmr (acetone-$d_6$) , δ 2.78 (3H,bm, 3xOH), 3.86(3H, s, $ArOCH_3$),3.87(3H,s,$ArOCH_3$), 4.62(1H,d, *J* =11.6 Hz,3-H), 5.11(1H,d, *J* =11.6 Hz, 2-H),6.07(2H,AB system, 6-H and 8-), and 7.00-7.10(3H, m, 2'-H,5'-H and 6'-H) ; ms, *m/z* 332 ($M^+$, 25%, $C_{17}H_{16}O_7$), 303 (36), 179 (16), 167 (100), 166 (29), 164 (35), 151 (20), and 137 (23).

OXIDATION OF **67** -A sample (15 mg) of **67** was suspended in 2N $H_2SO_4$ (5 ml) and heated on a steam bath under a gentle stream of air for 24 hours. After the sample was cooledto room temperature and extracted with ethyl acetate (4 x 5 ml), partition was effected against a saturated aqueous solution of sodium bicarbonate to remove residual acid. The organic phase, when dried ($Na_2SO_4$) and evaporated in vacuo, yielded 14.2 mg (95%) of a dark yellow, waxy solid, **71** ; uv, max (MeOH), 369, 292, 270 sh, 255 and 205 nm ; max (MeOH + $NaOCH_3$), 388, 326, 277 and 205 nm, with no change for at least 24 hours.

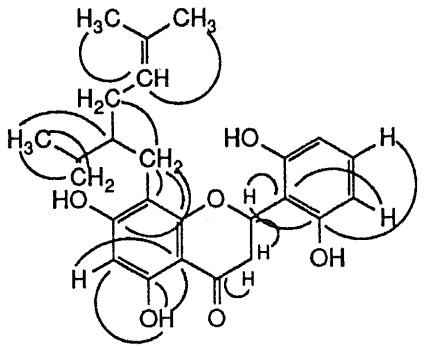
OXIDATION OF **68** -A sample (15 mg) of **68** was suspended in 2N $H_2SO_4$ (5 ml) and heated on a steam bath under a gentle stream of air for 48 hours. After the sample was cooled to room temperature and extracted with ethyl acetate (4 x 5 ml), partition was effected against a saturated aqueous solution of sodium bicarbonate to ramove residual acid. The organic phase, when dried ($Na_2SO_4$) and evaporated *in vacuo*, yielded 12.4 mg (83%) of a yellow waxy solid, **72** ; uv, $\upsilon_{max}$ (MeOH), 364, 288, 260, 213 and 208 nm ; $\upsilon_{max}$ (MeOH + $NaOCH_3$), 413, 333, 290, 230 and 211 nm, with no change for at least 24 hours.

## ii. *SOPHORA EXIGUA* (247)

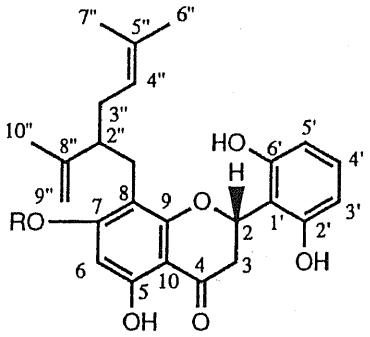
The genus *Sophora* is an abundant source of prenyl flavonoids [248] and some species of the genus have been used as a folk medicine in China (*S. tonkinensis*) and Japan (*S. flavescens*). The genus is divided into two subgenera, *Sophora* and *Styphnolobium* [249,250], Previous studies show that the prenylflavonoids occur exclusively in the subgenus *Sophora*. *Sophora exigua*, in subgenus *Sophora* and series *Rubriflorae* [249,250], is an endemic species in Thailand and the roots of the plant are used for treating respiratory diseases [251]. Previous studies on the chemical constituents of this plant revealed the presence of nine lupine alkaloids which were

studied by Takamatsu *et al.* [211]. We now describe the isolation from the roots and structural determination of two new flavanones with a lavandulyl residue, named exiguaflavanones A and B.

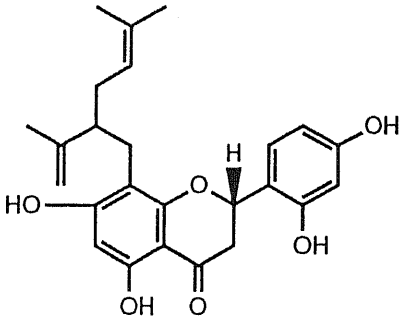
Purification of a chloroform soluble extract of the roots of *Sophora exigua* with silica gel column chromatography resulted in the isolation of three flavanones (**75-77**). Three one-proton double doublets at δ2.54 $J$ = 17, 3Hz), 3.87 ($J$ = 17,14 Hz) and 6.02 ($J$ = 14,3 Hz) assigned to H-3 and H-2 in the $^1$H NMR spectrum showed **75** to be a flavanone derivative. A two-proton doublet at δ6.47 ($J$ = 8Hz) assignable to H-3' and H-5', and a triplet at δ7.05 ($J$ = 8 Hz) assignable to H-4' indicated that **75** is di-oxygenated at C-2' and C-6' on the B ring. In the EI mass spectrum, prominent fragment ions at *m/z* 301 and 165 showed that A ring is also di-oxygenated, and the ring bears a C-alkyl group. The alkyl group was determined to be lavandulyl (5-methyl-2-isopropenyl-hex-4-enyl) by the carbon chemical shifts [253] assigned by $^1$H-$^{13}$C COSY. The position of the lavandulyl residue was concluded to be at C-8, because a one-proton singlet assigned to a chelated hydroxyl group at C-5 appeared at δ12.27 [252,253], and that **75** exhibited a bathochromic shift on addition of $AlCl_3$ (ca 23 nm) in the UV spectrum [254]. The position of the lavandulyl residue was further confirmed by 2D NMR. In the COLOC spectrum, the chelated hydroxyl group caused a cross peak with a carbon at δ96.8 which was correlated with a one-proton singlet at δ6.01 assigned to the A ring proton (H-6) in the $^1$H-$^{13}$C COSY. By means of the CD spectral results , the configuration at C-2 is 2S . Consequently, **75** is (2S) -5,7,2',6'-tetrahydroxy-8-lavandulylflavanone, named exiguaflavanone A.



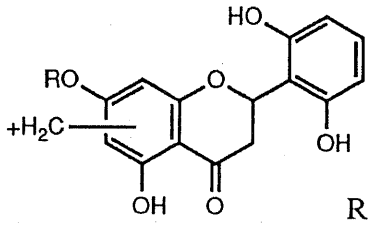

$^{13}$C-$^1$H long range coupling in the COLOC spectrum of **75**



**75** R = H
**76** R = Me

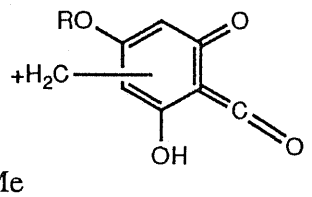


**77**



**75a** *m/z* 301
**76a** *m/z* 315

R = H and Me



**75b** *m/z* 165
**76b** *m/z* 179

EIMS fragments of **75** and **76**

The $^1$H NMR spectrum of **76** was almost superimposable on that of **75** except for the presence of a methoxyl group at δ 3.87. In the EI mass spectrum, prominent fragment ions were observd at *m/z* 315 and 179. These results showed **76** is a monomethyl ether of **75**. It is clear that one of the hydroxyl groups on the A ring of **75** is methylated. In the COLOC spectrum (acetone-$d_6$), a chelated hydroxyl group assigned to C-5 (δ 12.38) caused a cross peak with a carbon signal at C-6(δ 93.2). The results of the COLOC spectrum indicated that the A ring substitution is 5-hydroxy-8-lavandulyl-7-methoxy. The CD spectral data showed that the configuration at C-2 is 2S. Consequently, **76** is (2S)5,2',6'-trihydroxy-8-lavandulyl-7-methoxyflavanone, named exiguaflavanone B. Compound **77**, obtained as needles, was identified as sophoraflavanone G (5,7,2',4'-tetrahydroxy-8-lavandulylflavanone) by comparison with an authentic sample isolated from *S. leachiana* [253].

In the present study, we isolated two 2',6'-dioxygenated flavanones from the roots of *S. exigua*. Such flavanones are only known previously from *Lonchocarpus orotinus* (Leguminosae) (orotonin and orotonin 5-methyl ether) [255], *Scutellaria baicalensis* (Labiatae) (5,7,2',6'-tetrahydroxyflavanone and its 5-methyl ether) (5,7,2',6'-tetrahydroxyflavanone and its 5-methyl ether) [256,257] and *S. discolor* (5,2'-dihydroxy-7,8,6'-trimethoxyflavanone and 5,2'-dihydroxy-6,7,6'-trimethoxyflavanone) [258]. Up to the present, the flavanones from *Sophora* are oxygenated at 2'-,4'-,2',4'-[248] and 2',4',6'- [259,260]. Our present discovery of 2',6'-dioxygenated flavanone in *S. exigua* is therefore of note.

5,7,2',6'-Tetrahydroxy-8-lavandulylflavanone (exiguaflavanone A, **75**). mp 178.5-179° ($C_6H_6$). EIMS *m/z* (rel. int.): 424 (11), 301 (100), 283 (93), 165 (73), 136 (4). HRMS *m/z* : $[M]^+$424.1917 (Calcd. 424.1886 for $C_{25}H_{28}O_6$). CD $\Delta\varepsilon_{273}$ -7.3 (negative maximum), $\Delta\varepsilon_{303}$ + 3.1 (positive max.). UV λ MeOH nm:292.338, + $AlCl_3$:314,392. $^1H$ NMR (acetone-$d_6$) δ :1.48 (3H,br s, Me-7"), 1.54,(3H, br s, Me-6"), 1.61 (3H,br s, Me-6"), 1.61 (3H, br s, Me-10"), 2.08(2H,m,H-3'),2.54(1H,dd, *J* = 17,3 Hz,H-3eq),2.56-2.60(3H,m,H-1",H-2").3.87(1H,dd, *J* = 17,14 Hz,H-3ax),4.55 (2H,bt s, H-9"),4.98 (1H, t like, H-4'), 6.01 (1H,s,H-6), 6.02 (1H,dd, *J* = 14,3 Hz,H-2),6.47(2H,d, *J* = 8 Hz,H-3',H-5'), 7.05 (1H,t, *J* = 8 Hz,H-4'),8.50 (2H, br s, OH-2' and OH-6'), 9.45 (1H, s, OH-5). $^{13}C$ NMR data are shown in Table 27.

5,2',6'-Trihydroxy-8-lavandulyl-7-methoxyflavanone (exiguaflavanone B, **76**). A pale yellow viscous oil. EIMS *m/z* (rel. int.): 438 (7), 369 (7), 315 (100), 297 (93), 133 (11), 179 (69), 136 (3). HRMS *m/z*: $[M]^+$ 438.2010 )Calcd. 438.2042 for $C_{26}H_{30}O_6$). CD:$\Delta\varepsilon_{275}$-6.5 (negative max.),$\Delta\varepsilon_{302}$ +2.2(positive max.). UV MeOH nm:290,343, + $AlCl_3$:315,340. $^1H$ NMR (acetone-$d_6$) δ : 1.47 (3H,br s, Me-6"), 1.60 (3H, br s, Me 10"), 2.00-2.55 (5H,m, H-1", H-3"), 2.56 (1H, dd, *J* = 16,3 Hz, H-3eq), 3.87 (3H, s, OMe), 3.89 (1H, dd, *J* = 16, 14 Hz H3ax), 4.45,4.49 (1H, each br s,H-9"), 4.94 (1H, t like, H-4"), 6.02 (1H,dd, *J* =14,3 Hz,H-2),6.13 (1H,s,H-6),6.48(2H,d, *J* = 8 Hz,H-3', H-5'), 7.03 (1H,t, *J* = 8 Hz, H-4'), 8.55 (2H, br s, OH-2' and OH-6'), 12.38 (1H, s, OH-5). $^{13}C$ NMR spectral data are shown in Table 27

**Table 27** $^{13}C$ NMR spectral data of compounds **75** and **76** (acetone-$d_6$)

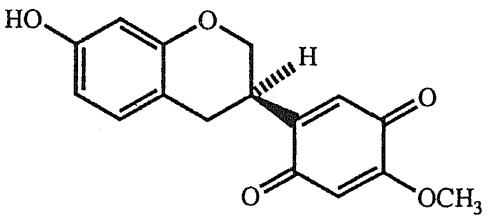
| C | **75** | **76** |
|---|---|---|
| 2 | 74.3 | 74.3 |
| 3 | 41.2 | 41.1 |
| 4 | 199.2 | 199.3 |
| 5 | 163.7 | 163.9 |
| 6 | 96.8 | 93.2 |
| 7 | 165.7 | 166.8 |
| 8 | 108.4 | 108.3 |
| 9 | 162.8 | 161.1 |
| 10 | 103.8 | 103.8 |
| 1' | 112.4 | 111.9 |
| 2',6' | 158.2 | 157.7 |
| 3',5' | 108.9 | 108.7 |
| 4' | 131.3 | 131.0 |
| 1" | 28.3 | 27.6 |
| 2" | 48.4 | 48.3 |
| 3" | 32.5 | 32.2 |
| 4" | 125.0 | 124.6 |
| 5" | 132.1 | 132.0 |
| 6" | 18.3 | 18.2 |
| 7" | 26.3 | 26.2 |
| 8" | 148.7 | 149.1 |
| 9" | 111.6 | 111.9 |
| 10" | 19.6 | 19.2 |
| OMe | | 56.5 |

all carbons were assigned with the aid of $^{13}C$-$^{1}H$ and $^{13}C$-$^{1}H$ long range COSY

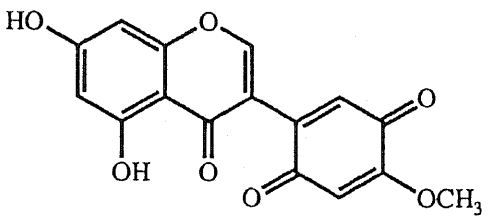
## iii. *DALBERGIA CANDENATENSIS* (261)

Continuing our studies on the traditional medicinal plants of Thailand, we have investigated *Dalbergia candenatensis* Prain (Leguminosae), a tree indigenous to eastern Asia. The heartwood has a deep red color and is used in Thailand as a red dyestuff. There have been no previous phytochemical studies on this species. In a preliminary biological evaluation, the $CHCl_3$ and MeOH extracts were found to display antibacterial and antifungal properties. The same extracts also showed cytotoxic activity aginst the P-388 lymphocytic leukemia test system *in vitro*. (262).

The isolation of the antibacterial and antifungal components was monitored by direct bioautographic assay on tlc (263,264). Four of the isolated compounds were identified as (±)-mucronulatol (265,266), (*R*)-(-)-claussequinone **78** (267), formononetin (265), and (*R*)-(-)-vestitol (267,363). The new isoflavone quinone **79** was characterized in the following manner.




**78**




**79**

The bathochromic shift in band II of the uv spectrum, on the addition of $AlCl_3$/HCl and NaOAc, indicated the presence of a hydroxy group *peri* to a carbonyl group and of a second acidic hydroxy group. In the ir spectrum a complex absorption pattern was observed in the region 1650-1680 $cm^{-1}$ suggesting the presence of more than one α,β-unsaturated ketone moiety. In the mass spectrum obtained at 20 eV, compound **79** showed a fragmentation pattern typical for methoxybenzoquinones (269).

An intense molecular ion was observed at *m/z* 314. Elimination of a methyl group and subsequent loss of CO resulted in ions at *m/z* 299 and 271, respectively. The ion at *m/z* 243 arose from the loss of a methyl group and two molecules of CO, and this ion decomposed by further elimination of CO leading to a $M^+$ -99 species at *m/z* 215. A fragment ion at *m/z* 203 was due to the rupture of the C-1'-C-2' and C-5'-C-6' bonds, and an intense peak, characteristic of methoxybenzoquinones, appeared at *m/z* 69

($C_3HO_2^+$). RDA fragmentation of the molecule and subsequent elimination of CO led to the ions at *m/z* 152 and *m/z* 124.

In the relatively simple $^1$H-nmr spectrum a singlet at 8.36 ppm was assigned to H-2 of an isoflavone with the resonances for the hydroxyl groups at C-5 and C-7 appeearing at 12.46 and 11.06 ppm, respectively. Two one-proton singlets for H-3' and H-6' were observed at 6.26 nd 7.05 ppm, respectively. Assignment of the $^{13}$C-nmr spectrum of **79** was achieved through comparison with literature data (270,271), with the data for claussequinone **78**, and by the attached proton test (APT) spectrum. Based on this spectroscopic information compound **79** has been identified as 5-hydroxybowdichione, the second isoflavone quinone to be described (272).

(*R*)-(-)-Claussequinone [**78**].--Mp 193-196° decomp. [lit. (267) mp 189-194° decomp.]; [α]D, uv, ir, eims: see lit. (362); $^1$H nmr (360 MHz, DMSO-$d_6$) δ 2.72 (1H, dd, *J*=15.5, 9.0 Hz, 4-$H_{ax}$), 2.80 (1H, dd, *J*=15.5, 5.7 Hz, 4-$H_{eq}$), 3.21 (1H, m, 3-$H_{ax}$), 3.78 (1H, s, 4'-$OCH_3$), 3.92 (1H, dd, *J*=10.5, 8.4 Hz, 2-$H_{ax}$), 4.16 (1H, dd, *J*=10.4, 1.9 Hz, 2-$H_{eq}$), 6.17 (1H, s, 3'-H), 6.18 (1H, d, *J*=2.5 Hz, 8-H), 6.30 (1H, dd, *J*=8.3, 2.3 Hz, 6-H), 6.54 (1H, s, 6'-H), 6.86 (1H, d, *J*=8.3 Hz, H-5), 9.25 (1H, bs, 7-OH); $^{13}$C nmr (90.8 MHz, DMSO-$d_6$) δ 28.8 (C-4), 30.6 (C-3), 56.4 (4'-$OCH_3$), 67.9 (C-2), 102.5 (C-8), 107.9 (C-6), 108.4 (C-3'), 111.3 (C-4a), 130.0 (C-5), 130.4 (C-6'), 148.4 (C-1'), 154.2 (C-8a), 156. 6(C-7), 158.3 (C-4'), 181.7 (C-5'), 186.5 (C-2').

5-Hydroxybowdichione [**79**].--Yellow crystals, mp 241-245° decomp. ($CHCl_3$/MeOH); uv λ max (MeOH) 318 sh, 279, 262, and 223 nm sh, (+ NaOMe) 334, 292, 240 nm sh; (+NaOAc) 328, 271 nm; (+$AlCl_3$) 372, 308, 269 nm, no alteration on addition of HCl; ir $\upsilon_{max}$ (KBr) 3480, 3440, 1660, 1647, 1630, 1621, 1598, 1585, 1233, 1205, 1190, 1170, 1154, 830, 825 $cm^{-1}$; $^1$H nmr (300 MHz, DMSO-$d_6$) δ 3.83 (3H, s, 4'-$OCH_3$), 6.26 (1H, s, 3'-H), 6.27 (1H, d, *J*=1.5 Hz, 8-H), 6.44 (1H, d, *J*=1.5 Hz, 6-H), 7.05 (1H, s, 6'-H), 8.36 (1H, s, 2-H), 11.06 (1H, s, 7-OH), 12.46 (1H, s, 5-OH); $^{13}$C nmr (75.44 MHz, DMSO-$d_6$) δ 56.3 (4'-$OCH_3$), 94.1 (C-8), 99.5 (C-6), 104.0 (C-4a), 107.9 (C-3'), 115.5 (C-3), 133.2 (C-6'), 137.9 (C-1'), 157.1 (C-8a), 157.5 (C-2), 158.4 (C-4'), 161.6 (C-5), 164.7 (C-7), 178.8 (C-4), 181.4 (C-5'), 185.0 (C-2'); ms (20 eV) *m/z* (rel. int.) 314 (1000), 299 (17), 271 (99), 255 (3), 243 (3), 229 (6), 215 (7), 203 (14), 153 (7), 152 (6), 124 (6), 69 (37).

## iv. *WRIGHTIA TOMENTOSA* (273)

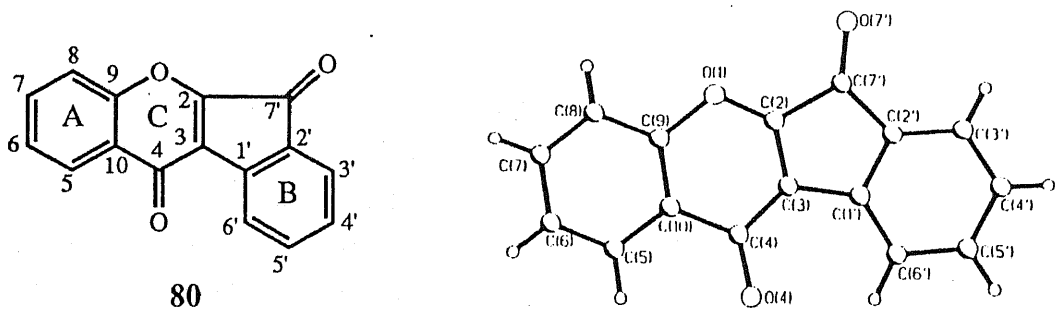
*Wrightia tomentosa* Roem. & Schult. (Apocynaceae) has been used in Ayurvedic medicine for many years for the treatment of menstrual disorders, renal complaints and amoebic dysentery [274]. In Thailand, the dried bark of this species is used as an antipyretic [275]. In the course of an evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase, the methanol extract of the dried leaves and stems of this plant showed weak activity (10% inhibition) against HIV-1 reverse transcriptase [276]. Previous phytochemical studies on this plant have revealed the presence of unsaturated sterols, triterpenes, flavonoids, phenolic acids and alkaloids [277,278]. However, detailed information about these isolates was lacking. In this investigation, we report the isolation and structure determination of an isoflavone with a new skeleton from the bark of *W. tomentosa.*

The $^{1}H$ NMR spectrum of wrightiadione showed eight aromatic proton resonances at δ 8.60, 8.41, 8.01, 7.89, 7.83, 7.77, 7.65 and 7.41. The scalar couplings observed among δ 8.60, 7.77, 7.41 and 7.89 and among δ 8.41, 7.65, 7.83 and 8.01 in the COSY spectrum clearly revealed that they belonged two isolated spin systems. To determine the carbon framework of **80**, a series of $^{13}C$ NMR experiments, including BB, APT, HETCOR and selective INEPT [280,281] was performed. However, in the course of proton broad-band decoupling experiments using an optimum pulse angle (Ernst angle) and a number of different post-acquisition delays (5, 10, 20 and 30 sec) to avoid the saturation of NMR signals, only 15 of the anticipated 16 $^{13}C$ resonances were consistently observed. These data were inconsistent with those obtained from the IR spectrum which showed two distinct carbonyl frequencies ($\upsilon_{max}$ 1726 and 1686 $cm^{-1}$) and the HRMS (obsd 248.0472), which indicated a molecular formula $C_{16}H_8O_3$, and suggested that one of the carbonyl carbon resonances was absent from the $^{13}C$ NMR spectrum. All efforts to locate this carbonyl carbon in the spectrum have thus far failed.

Based on the NMR and mass spectral data, several possible structures were possible. Comparison of the $^{13}C$ chemical shifts of wrightiadione **80** with those of chromone, xanthone, flavone and quinone derivatives [282] immediately ruled out several alternatives and suggested that it might be a flavone or isoflavone. The presence of two absorption maxima at 259 (band II) and 313 nm (band I, shoulder) in the UV spectrum, characteristic of isoflavones, further suggested that this compound is **80**.

The carbonyl carbon resonance of C-7' which was expected to appear in the range δ 190-210 [282] was not observed; consequently, the selective INEPT technique could not be used to place this carbonyl function. However, selective INEPT

experiments did provide very useful information for the unambiguous assignment of the remaining carbon resonances. Irradiation of H-5 at δ 7.89 using a pulse delay corresponding to $J$=8 Hz resulted in the enhancement of C-4 (δ 182.5), C-7 (δ 138.3), and C-9 (δ 146.3) (Fig. 15). However, enhancement of the C-4 resonance was not as significant as that of C-7 and C-9, due to the different dihedral angles existing between H-5 and C-4, and between H-5 and C-7/C-9. When the pulse delay was increased ($J$=5 Hz), the C-4 resonance was significantly enhanced revealing that one of the carbonyl goups was located at C-4. Irradiation of H-6 at δ7.41 significantly enhanced C-8 (δ 117.9) and C-10 (δ 121.9), but C-5 (δ 125.4) was only weakly enhanced indicating three-bond and two-bond coupling with H-6, respectively. Irradiation of H-7 at δ 7.77 enhanced the carbon resonances at δ 125.4 and 146.3, further confirming the assignment of C-5 and C-9. Furthermore, the unambiguous assignment of the A-ring carbons, and some of the C-ring carbons was achieved by irradiating H-8 at δ 8.60 using a pulse delay corresponding to $J$=1 Hz. It was found that the C-4 and C-5 resonances, which were four-bond coupled to H-8, were significantly enhanced. Similarly, irradiation of H-3' at δ 8.01 using the same pulse delay as that for H-8 (i.e. for $J$=1 Hz) resulted in the enhancement of five carbon resonances. Among them, C-2 (δ 144.3), C-3 (δ 158.0), and C-6' (δ 127.5) were four-bond coupled to H-3'. The complete asignment of the C/D rings thus could be made. Respective irradiation of H-4' (δ 7.83) H-5' (δ 7.65) and H-6' (δ 8.41), using a pulse delay corresponding to $J$=8 Hz, resulted in the enhancement of the carbon resonances which were three-bond coupled to the corresponding proton, further permitting the unequivocal assignment of the ring B carbons. To firmly establish its structure, an X-ray analysis was performed, which demonstrated that wrightiadione has the structure **80** (Fig. 14)




**80**

**Fig. 14** X-Ray crystal structure of wrightiadione.

Structurally, wrightiadione may be considered to be an oxidized cyclic 2-methylisoflavone. It has been reported that some 2-methylisoflavones, also lacking any B-ring substitution, have been isolated from *Glycyrrhiza glabra* roots [283]. Whether or not these compounds are true isoflavonoids, i.e. derived from chalcone-flavanone precursors and involving an aryl migration, remains to be established. The presence of wrightiadione may help to answer this question.

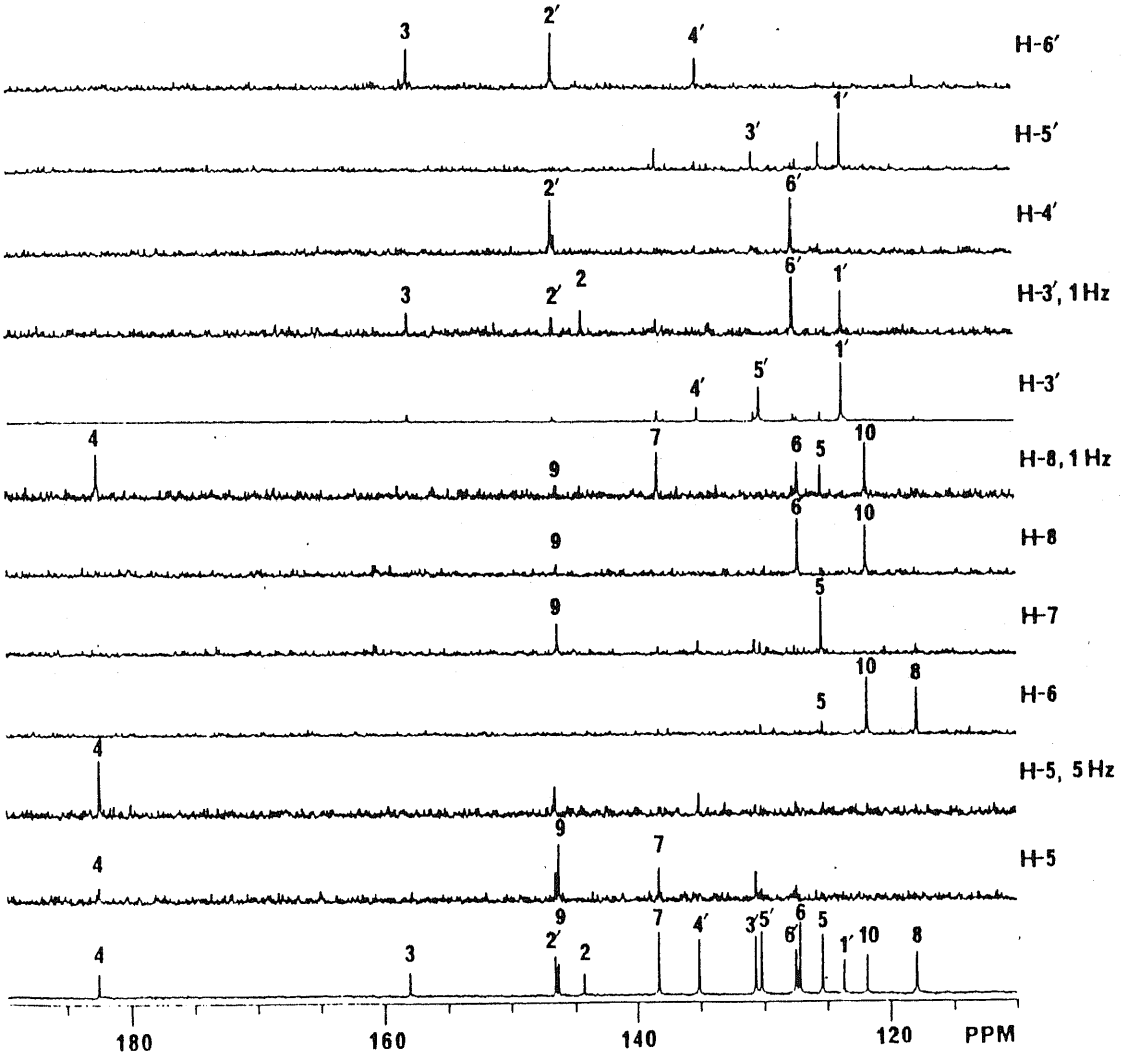



**Fig. 15** Selective INEPT spectra of wrightiadione (**80**) the *J* value used in these experiments was 8 Hz, unless otherwise specified.

Wrigtiadione exhibited the following physical and spectroscopic properties : mp 228-230°; [α]D 0° ($CHCl_3$; 0.1); UV $\lambda_{max}$ ($CHCl_3$) nm (log ε) : 259 (4.68), 272 (1.98), 282 (1.88), 306 (2.02), 313 (2.05), 336 (1.91), 354 (1.68), 398 (1.68); IR $\upsilon_{max}$ $cm^{-1}$ : 2930, 2865, 1726, 1686, 1595, 1460, 1354, 1314, 1190, 1117, 1040, 756; $^1H$ NMR (360.1 MHz, $CDCl_3$) δ 8.60 (1H, *dd*, *J*=8.1, 1.4 Hz, H-8), 8.41 (1H, *dd*, *J*=7.9, 1.6 Hz, H-6'), 8.01 (1H, *dd*, *J*=8.0, 1.2 Hz, H-3'), 7.89 (1H, *dd*, *J*=7.6, 1.4 Hz, H-5), 7.83 (1H; *ddd*, *J*=8.0, 7.7, 1.6 Hz, H-4'), 7.77 (1H, *ddd*, *J*=8.1, 7.9, 1.4 Hz, H-7), 7.65 (1H, *ddd*, *J*=7.9, 7.7, 1.2 Hz, H-5'), 7.41 (1H, *ddd*, *J*=7.9, 7.6, 0.9 Hz, H-6); $^{13}C$ NMR (90.8 MHz, $CDCl_3$) C-7' not observed, 182.5 (s, C-4), 158.0 (s, C-3), 146.5 (s, C-2'), 146.3 (s, C-9), 144.3 (s, C-2), 138.3 (*d*, C-7), 135.1 (*d*, C-4'), 130.7 (*d*, C-3'), 130.2 (*d*, C-5'), 127.5 (*d*, C-6'), 127.2 (*d*, C-6), 125.4 (*d*, C-5), 123.7 (s, C-1'), 121.9 (s, C-10), 117.9 (*d*, C-8); EIMS 70 eV (probe) *m/z* (rel. int.) 248 $[M]^+$ (100), 220 (41), 192 (17), 144 (6), 128 (12), 115 (10), 102 (13), 99 (11), 91 (15), 90 (10), 77 (10), 76 (11), 57 (15), 55 (10), 50 (7); HRMS found : $[M]^+$ 248.0472; $C_{16}H_8O_3$ requires 248.0473.

X-Ray crystallographic data of wrightiadione. Slow evapn of an $Me_2CO$ soln of wrightiadione yielded yellow transparent crystals as prisms of size 1.0 x 0.08 x 0.04 mm. Crystal data : monoclinic space group P2(1)/n, a=7.392(2) A° , *b*=7.592(2) A° , *c*=19.481(4) A° , *Z*=4, *d*=1.509 g $cm^{-3}$; on a Siemens R3m diffractometer, 1364 unique reflections were measured with Nifiltered CuKα radiation, 1109 observed with F>4σ(F). Absorption correction was applied. The structure was solved by direct methods using SHELXL (284). The hydrogen atoms were calculated from the positions of the carbons to which they are bound. Anisotropic refinement cycles converged at wR=6.92% (with unit weights). The atomic coordinates as well as the bond distances and angles are deposited at the Cambridge Crystallographic Data Centre. Atomic coordinates of non-hydrogen atoms are given in Table 28.

**Table 28** Atomic coordinates ($x10^4$) and equivalent isotropic displacement coefficients ($A^2 \times 10^3$)

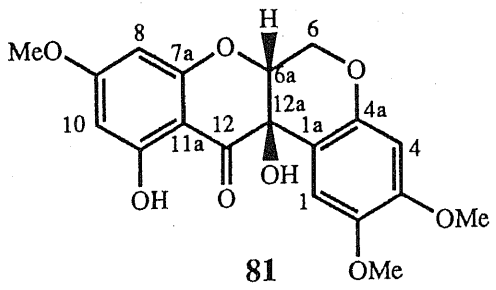
| Atom | x | y | z | $U^*_{eq}$ |
|---|---|---|---|---|
| O(1) | 3492 (6) | 7603 (6) | 5068 (2) | 68 (2) |
| C(2) | 2889 (7) | 6185 (7) | 5372 (3) | 40 (2) |
| C(3) | 2324 (5) | 4681 (5) | 5059 (2) | 17 (1) |
| C(4) | 2285 (7) | 4493 (7) | 4353 (3) | 40 (2) |
| O(4) | 1674 (5) | 3182 (5) | 4080 (2) | 56 (1) |
| C(5) | 3132 (7) | 6030 (7) | 3264 (3) | 45 (2) |
| C(6) | 3766 (7) | 7497 (8) | 2917 (3) | 52 (2) |
| C(7) | 4255 (7) | 8992 (8) | 3276 (3) | 53 (2) |
| C(8) | 4181 (7) | 9034 (7) | 3986 93) | 47 (2) |
| C(9) | 3562 (7) | 7538 (7) | 4341 (2) | 38 (2) |
| C(10) | 3006 (7) | 6030 (7) | 3983 (3) | 38 (2) |
| C(1') | 1812 (6) | 3422 (7) | 5566 (3) | 38 (2) |
| C(2') | 2033 (7) | 4156 (7) | 6222 (3) | 39 (2) |
| C(3') | 1634 (7) | 3187 (8) | 6810 (3) | 47 (2) |
| C(4') | 997 (7) | 1472 (8) | 6736 (3) | 51 (2) |
| C(5') | 787 (7) | 759 (8) | 6086 (3) | 51 (2) |
| C(6') | 1201 (7) | 1708 (7) | 5488 (3) | 44 (2) |
| C(7') | 2733 (7) | 5939 (7) | 6144 (3) | 44 (2) |
| C(7') | 3129 (6) | 7032 (5) | 6570 (2) | 63 (2) |

* Equivalent isotropic U is defined as one-third of the trace of the orthogonalized $U_{ij}$ tensor.

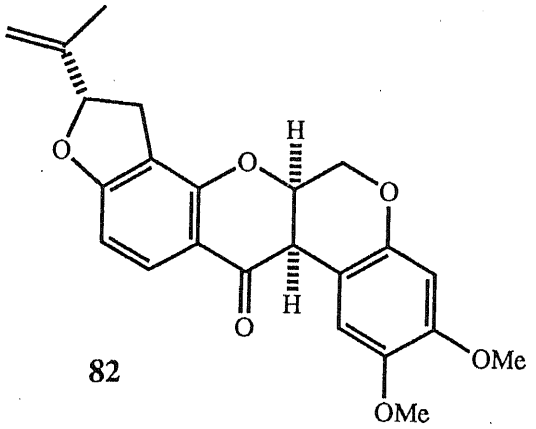
## v. *CLITORIA MACROPHYLLA* (285)

*Clitoria macrophylla* is traditionally used in Thailand for the treatment of skin diseases and for pest control in horticulture and animal husbandry. Earlier phytochemical investigation resulted in the isolation of two rotenoids, clitoriacetal and stemonacetal, from a chloroform extract of this plant (286). It was reported that the major component, clitoriacetal, possessed remarkable antiinflammatory and antipyretic activities (287). In this investigation, we report the isolation of a new rotenoid, 6-deoxyclitoriacetal (**81**), from the roots of *C. macrophylla.*

Compound **81** was obtained as white rosette crystals. The $^1H$ NMR spectrum showed a H-bonded hydroxyl signal at $\delta$ 11.52, two aromatic singlets at $\delta$ 6.65 and $\delta$ 6.41, two meta-coupled proton signals at $\delta$ 5.94 and 5.87, a pair of nonequivalent methylene proton signals at $\delta$ 4.51 and 4.39, a methine proton doublet at $\delta$4.49, and three methoxy singlets at $\delta$ 3.73, 3.66 and 3.65. Comparison of the $^1H$ NMR spectral data of **81** with those of clitoriacetal (286) revealed the close similarity in structure between these two compounds. The mass spectrum of **81** exhibited a $[M]^+$ at *m/z* 374, 16 mu less than that of clitoriacetal, strongly suggesting that **81** is the deoxy derivative of clitoriacetal. Confirmation of this observation was supported by the HR mass spectrum which indicated a molecular formula $C_{19}H_{18}O_8$. The presence of a methylene carbon resonance at $\delta$63.5 in the $^{13}C$ NMR spectrum of **81** demonstrated that **81** is the 6-deoxy derivative of clitoriacetal.




**81**




**82**

Compound **81** was deduced to be a 12a-hydroxyrotenoid on the basis of both the NMR and mass spectral data. Two prominent fragment ion peaks at *m/z* 167 and 208, due to cleavage between the B/C rings, revealed that ring D possessed methoxy and hydroxy groups (*m/z* 167), and rings A and B had two methoxy and one hydroxy groups (*m/z* 208). The observation of an AB quartet at δ 4.51 (*J*=11.0, 2.5 Hz) and 4.39 (*J*=12.0, 2.0 Hz), attributed to H-6, and a small doublet at δ 4.49 (*J*=2.5 Hz), assigned to H-6a, led to location of the hydroxy group at C-12a. Based on the H-1 chemical shift value (δ 6.65) the B/C ring junction in **81** was determined to be *cis* (288). The unambiguous assignments of the quaternary carbons and the location of the methoxy and hydroxy groups were established by COLOC ($^{lr}J_{CH}$=8 Hz) [289] and NOE difference experiments.

In the NOE difference experiments, separate saturation of the methoxy signals at δ 3.73, 3.66 and 3.65 resulted in the enhancements of H-4 (δ 6.41), H-8 (δ 5.87)/H-10 (δ 5.94) and H-1 (δ 6.65), respectively, indicating that these methoxy groups were at C-3, C-9 and C-2, respectively. The correlation peaks between each methoxy proton signal, and its corresponding quaternary carbon resonance in the COLOC spectrum, allowed the carbon resonances at δ 143.8, 151.2 and 168.8 to be assigned to C-2, C-3 and C-9, respectively (Fig. 16). Furthermore, the correlation peaks between H-4 and C-2, H-1 and C-3, and H-8 and C-9 confirmed these assignments. The carbon resonance at δ 108.1, showing two correlation peaks with H-4 and H-6a, could be unambiguously assigned to C-1a, whereas the resonance at δ 148.2, showing correlation with H-1, H-4 and H-6, was assigned to C-4a. Four carbon resonances, at δ 95.3 (C-10), 100.0, 161.4 and 168.8 (C-9), showed correlation with H-8 indicating that they were either two-bond or three-bond coupled to H-8. The carbon resonances at δ 100.0 and 161.4 thus could be tentatively assigned to C-11a and C-7a, respectively. Based on the correlations between the OH-11 proton signal (δ 11.52) and the carbon resonances at δ 95.3, 100.0 and 164.2, it became evident that the carbon resonance at δ 164.2 should be

asigned to C-11 and the one at δ 100.0 attributed to C-11a. Therefore, the carbon resonance at δ 161.4 could be assigned to C-7a. A correlation peak observed between H-6a (δ 4.49) and the carbonyl resonance at δ 195.0 further revealed that the carbonyl function was located at C-12. Comparison of the optical rotation of **81** with those of 12a-hydroxyrotenoids (290) indicated that the absoluted configurations at C-6a and C-12a were *R,R*, respectively. The structure of **81** was thus determined to be (+)-(6a*R*, 12a*R*)-11,12a-dihydroxy-2,3,9-trimethoxyrotenoid (6-deoxyclitoriacetal).

6-Deoxyclitoriacetal (190 mg, 0.063%), mp 130-131°C, $[\alpha]_D$+233° ($CHCl_3$; *c*0.1). UV $\lambda_{max}^{MeOH}$ nm (log ε) : 293 (4.56). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$ : 3460, 3003, 2936, 2851, 1642, 1577, 1513, 1458, 1361, 1271, 1205, 1161, 1108, 1041, 1018, 828, 760. $^1H$ NMR (300 MHz, $CDCl_3$) δ 11.52 (1H, *s*, OH-11), 6.65 (1H, *s*, H-1), 6.41 (1H, *s*, H-4), 5.94 (1H, *d*, *J*=2.3 Hz, H-10), 5.87 (1H, *d*, *J*=2.3 Hz, H-8), 4.51 (1H, *dd*, *J*=11.0, 2.5 Hz, H-6), 4.49 (1H, *d*, *J*=2.5 Hz, H-6a), 4.39 (1H, *dd*, *J*=12.0, 2.0 Hz, H-6), 3.73 (3H, *s*, OMe-3), 3.66 (3H, *s*, OMe-9), 3.65 (3H, *s*, OMe-2) $^{13}C$ NMR (75.4 MHz, $CDCl_3$) δ 195.0 (*s*, C-12), 168.8 (*s*, C-9), 164.2 (*s*, C-11), 161.4 (*s*, C-7a), 151.2 (*s*, C-3), 148.2 (*s*, C-4a), 143.8 (*s*, C-2), 109.5, (*d*, C-1), 108.1 (*s*, C-1a), 101.0 (*d*, C-4), 95.3 (*d*, C-10), 94.3 (*d*, C-8), 75.4 (*d*, C-6a), 66.9 (*s*, C-12a), 63.5 (*t*, C-6), 56.2 (*q*, OMe-2), 55.5 (*q*, OMe-3), 55.5 (*q*, OMe-9), EIMS 70 eV (probe) *m/z* (rel. int.) : 374 $[M]^+$ (13), 356 (2), 208 (100), 207 (41), 193 (10), 167 (16), 109 (13), 97 (13), 85 (19), 79 (17), 69 (50); HRMS found : $[M]^+$ 374.1000; $C_{19}H_{18}O_8$ requires 374.1002.

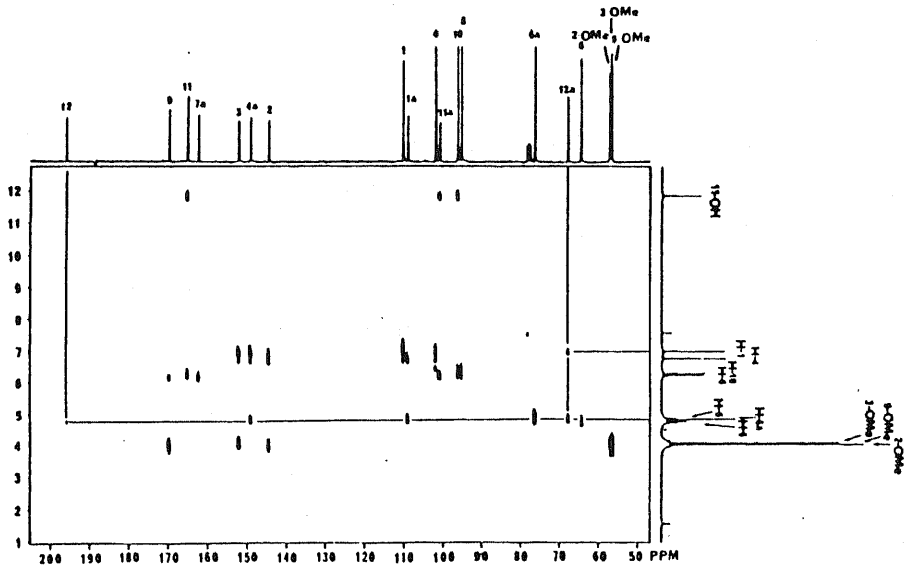


**Fig. 16** COLOC spectrum of 6-deoxyclitoriacetal (**81**)

### 2.1.5 Coumarins

During the past two decades there has been a notable reawakening of interest in natural coumarins. This has led to their discovery in hundreds of plant species and to the elucidation of many novel structures. In part, this progress has stemmed from the development of much improved method for their isolation combined with a variety of powerful modern techniques for their structural identification. However, much interest has also emanated from the wide range of physiological activity many display and from the characteristic fluorescence of most coumarins on ultraviolet (UV) irradiation.

#### i. *MICROMELUM MINUTUM* (291)

Previous studies of *Micromelum* species, family Rutaceae, (292-295) have revealed a number of unique coumarins, e.g., micromelin (**83**, in which an α,β-unsaturated γ-lactone is bound to a coumarin nucleus. Cassady *et al.* (294) have recently reported that **83** displays in vivo activity in the P-388 lymphocytic leukemia test system. (296)

The present study is concerned with the isolation and structure elucidation of microminutin (**84**), a novel cytotoxic coumarin from the leaves of *M. minutum* (Forst. f.) Seem. (syn. *Micromelum pubescens* Blume), collected in Thailand. The pyranoquinoline alkaloid flindersine **85** was also isolated in the course of these studies. Microminutin (**84**) was isolated in almost 1% yield from the plant, and its structure was determined from the following spectroscopic studies.

The molecular formula $C_{15}H_{12}O_6$ indicated for the isolate was established by high-resolution mass spectrometry. The infrared spectrum showed a very strong, wide carbonyl band at 1740 $cm^{-1}$ and suggested the presence of two unsaturated lactones or of one unsaturalted lactone and one saturated ester group, an ambiguity that was resolved by NMR analysis. The IR spectrum did, however, indicate an absence of hydroxyl groups.

From previous studies (292-295) a coumarin nucleus was suspected for microminutin. This and the location and nature of the substituents on the nucleus were deduced by a combination of $^1H$ and $^{13}C$ NMR spectroscopy.

The proton noise decoupled (PND) $^{13}C$ spectrum of microminutin as well as the coupled $^{13}C$ spectrum (Figure 17) exhibit resonances of δ 160.4 as a doublet of doublets ($J$=4.6, 11.4 Hz), δ 113.2 as a doublet ($J$=172.6 Hz), and δ 143.6 as a doublet of

doublets ($J$=4.9, 163.0 Hz). These three resonance patterns are characteristic (297) of C-2, C-3, and C-4, respectively, of the coumarin nucleus.

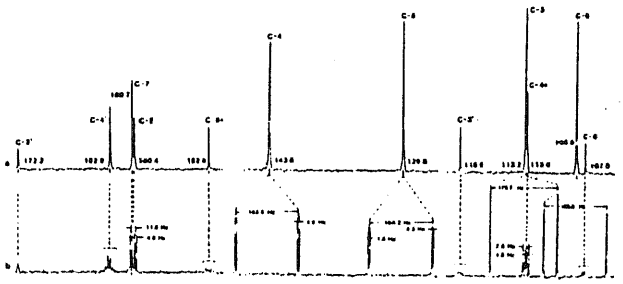


**Fig. 17** Carbon-13 NMR spectrum (25.05 MHz) of microminutin (**84**) : (a) proton noise decoupled (PND); (b) coupled spectrum.

The aromatic region of the $^1H$ NMR spectum of microminutin at 360 MHz contains only four resonance patterns, all of which are doublets. A pair of doublets (9.5 Hz) at δ 6.25 and 7.68 are characteristic of H-3 and H-4 in a coumarin nucleus. (298) Irradiation of the H-4 doublet at δ 7.68 produces a positive nuclear Overhauser effect (NOE) in the doublet ($J$=8.8Hz) at δ 7.50, which can therefore be assigned to H-5, leaving the doublet at δ 6.93 as H-6. The absence of any other $^1H$ signals in the aromatic region clearly indicates that positions C-7 and C-8 are substituted and furthermore that their substituents do not possess any aromatic or olefinic protons.

The three-proton singlet at δ 3.90 may be assigned to either aromatic methoxy (OMe) or carbomethoxy (COOMe) protons, and this ambiguity could also be resolved by performing a double resonance experiment. Irradiation at the frequency of this signal shows a strong, positive (23.4%) NOE in the H-6 doublet at δ 6.93. This experiment suggests that the resonance at δ 3.90 should be the protons of a methoxy group located at C-7. Similar irradiation of a resonance from the protons of either a COOMe group at C-7 or a methoxy group at C-8 would not be expected to produce the above effect.

With the coumarin nucleus and its substitution pattern firmly established, attention was turned to the molecular array attached at C-8 in microminutin. From the high-resolution mass spectrum this fragment should have the molecular formula $C_5H_5O_2$

and the nature of this moiety was deduced through total assignment of the $^{13}C$ and $^{1}H$ NMR spectra. As an initial step, the carbon resonances representing the coumarin nucleus and the C-7 methoxy group were assigned by comparison of the $^{13}C$ spectra of microminutin with those of coumarin (**86**), 7-methoxy-coumarin (**87**), and micromelin (**83**). (294,299,300) Thus the resonances at δ 107.0, 108.0, 113.0, 113.2, 129.8, 143.6, 152.9, 160.4, and 160.7 represent C-8, C-6, C-4a, C-3, C-5, C-4, C-8a, C-2, and C-7, respectively (Figure 17 and Table 29). The five remaining $^{13}C$ resonances in the microminutin spectrum must correspond to the carbon atoms occurring in the C-8 substituent. A correlation of these resonances in the PND and the single-frequency-off-resonance-decoupled (SFORD) spectra provide the following structural assignments : δ 172.2 singlet in PND, remains singlet in SFORD (-OC(O)-); δ 119.2 and 160.9 singlets in PND, remain singlets in SFORD (>C=CHC(O)-); δ73.1 singlet in PND, becomes triplet in SFORD (-$CH_2$OC(O)-); δ 13.7 singlet in PND, becomes quartet in SFORD (-C($CH_3$)=C<). Construction of the above structural fragments into a ring framework suggests a γ-butenolide moiety as the C-8 substituent on the coumarin nucleus. Additional structural information could be deduced from a consideration of the proton NMR spectral data. The broadened 2 H signal (100 or 200 MHz) centered at δ 4.88 is characteristic of the protons of a methylene group flanked by a lactone oxygen and an olefinic carbon atom, and the slightly broadened 3 H singlet (100 or 200 MHz) at δ 2.02 is typical of a vinyl methyl. At 360 MHz, however, the methylene protons (δ 4.87 and 4.95) were each observed as quartets of doublets due to molecular dissymetry showing $^{2}J$=17.4 and $^{4}J$=0.8 Hz (Figure 18). The only remaining ambiguity in the structure of microminutin is therefore the relative location of the vinyl methyl and the carbonyl function in the γ-butenolide moiety. Two alternative structures, **84** and **88** were therefore considered for microminutin.

**Table 29** $^{13}C$-$^{1}H$ Coupling Constants for Microminutin (84)

| C | multiplicity[a] | coupling const, Hz | coupling assignment |
|---|---|---|---|
| 2 | dd | 11.6 | $H_4$ |
| | | 4.9 | $H_3$ |
| 3 | d | 172.7 | $H_3$ |
| 4 | dd | 163.8 | $H_4$ |
| | | 4.9 | $H_5$ |
| 4a | tt | 7.9 | $H_6$, $H_3$ |
| | | 1.8 | $H_5$, $H_4$ |
| 5 | ddd | 164.2 | $H_5$ |
| | | 4.3 | $H_4$ |
| | | 1.8 | $H_6$ |
| 6 | d | 163.6 | $H_6$ |
| 7 | | m | $OCH_3$ |
| 8 | | m | |
| 8a | | m | |
| 2' | | m | |
| 3' | | m | |
| 4' | m | 6.7 | $CH_3$[b] |
| | | 4.3 | $CH_2$[b] |
| 5' | tq | 151.8 | $H_3$, |
| | | 5.1 | $CH_3$ |

[a] dd=doublet of doublets, d=doublet, ddd=doublet of doublets of doublets, tt=triplet of triplets, t=triplet, q=quartet, m=unresolved multiplet. [b] Obtained from low-power single frequency selective decoupling (LPSFSD) experiments.

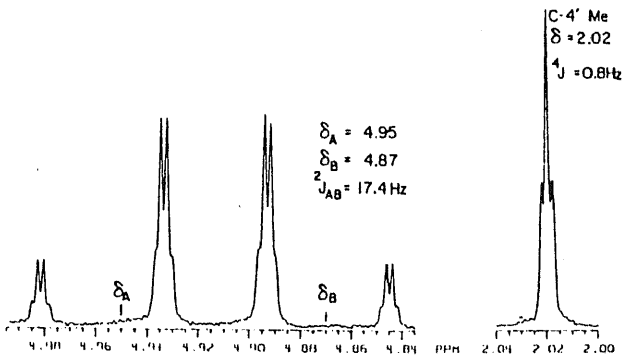



**Fig. 18** Methylene and olefinic methyl regions in the proton NMR spectrum (360 MHz) of microminutin (**84**).

Distinction between structures **84** and **88** was achieved independently by (i) application of intramolecular nuclear Overhauser difference spectroscopy and (ii) single frequency selectively decoupling of the $^{13}C$ NMR spectrum of microminutin. Thus, irradiation of the methyl at δ 2.02 (360 MHz) using the NOE difference technique (301--312) leads to a 2.5% NOE for the pattern due to the methylene protons. No appreciable NOE was observed when the methoxyl protons (δ 3.90) were similarly irradiated. The NOE observed for the methylene protons is much smaller than that observed for the aromatic CH because the dominant relaxation of each methylene proton is through its geminal neighbor. Nevertheless there are some contributions from the neighboring C-$CH_3$ protons that would only occur in structure **84**.

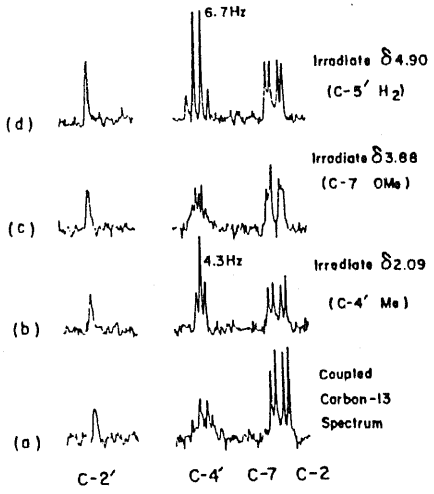
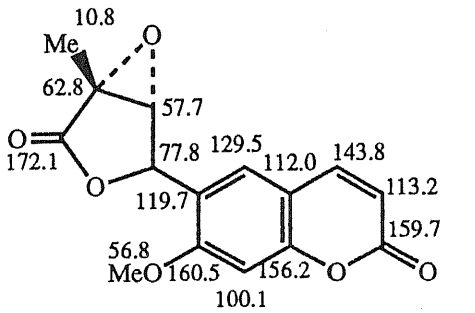


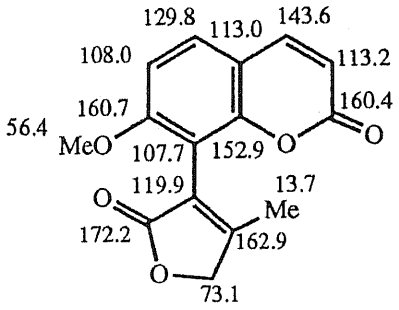

**Fig. 19** Low-power single frequency selective decoupling $^{13}C\{^1H\}$ spectra of microminutin (**84**), observation of low-field spectral region.

Low-power single frequency selective decoupling (LPS-FSD) of the $^{13}C$ spectrum (25.05 MHz, 50°C) gave complimentary results (Figure 19). Thus irradiation of the C-$CH_3$ protons caused the multiplet at δ 162.9 observed in the coupled $^{13}C$ spectrum to collapse to a triplet ($^3J_{CH}$=4.3 Hz). Conversely, irradiation of the methylene proton signal centered at δ 4.90 (100 MHz, $^1H$) caused the signal at δ 162.9 to collapse to a quartet ($^3J_{CH}$=6.7 Hz). The observation that only *one* carbon signal is affected in these LPSFSD experiments argues in favor of both the methyl and the methylene groups being attached to the *same* $sp^2$ carbon atom; i.e., microminutin has the structure **84**.

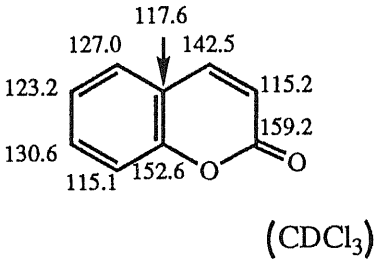
Microminutin (**84**) is the first member of a new series of prenylated coumarins in which neither the "head" nor "tail" of the isoprene unit is attached either to a hetero-atom or the coumarin nucleus, but rather an adjacent carbon forms the crucial bond.




83




84

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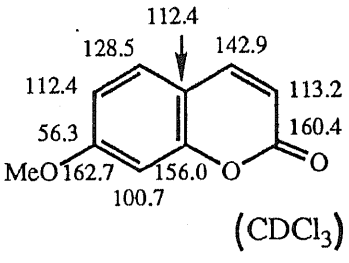
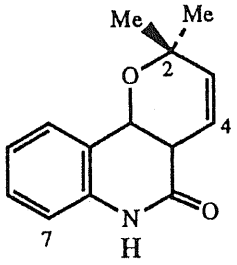



87

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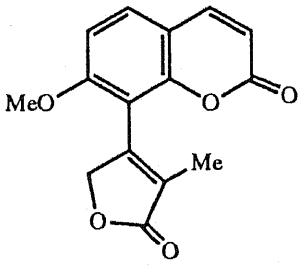



88

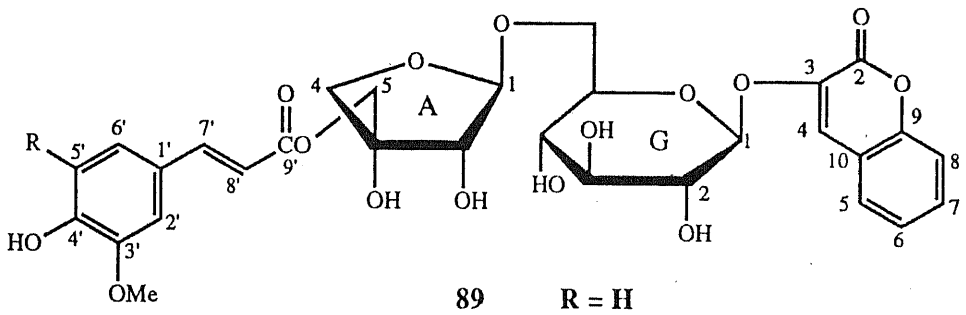
Microminutin (**84**,6.35 g, 0.98%) : mp 154-155°C; IR (KBr) $\upsilon_{max}$ 1740 (vs), 1675 (m), 1600 (s), 1560 (m), 1495 (m), 1440 (m), 1395 (m), 1285 (s), 1247 (s), 1142 (s), 1108 (s), 1090 (s), 1075 (m), 1060 (m), 1033 (s), 1003 (m), 928 (w), 890 (m), 830 (s), 760 (m), 720 (w), 640 (w), 610 (w), 460 (w), 390 (w) $cm^{-1}$; UV (EtOH) $\lambda_{max}$ (log ε) 268 nm (3.59), 321 (4.23); $^1H$ NMR (360 MHz, $CDCl_3$) δ 2.02 (t, *J*=0.8 Hz, 3H, $C_{4'}$-$CH_3$), 3.88 (s, 3H, $C_7$-$OCH_3$), 4.87 (qd, *J*=0.8, 17.4 Hz, 1H, $C_{5'}$-H), 4.95 (qd, *J*=0.8, 17.4 Hz, 1H, $C_{5'}$-H), 6.25 (d, *J*=9.5 Hz, 1H, $C_3$-H), 6.93 (d, *J*=8.8 Hz, 1H, $C_6$-H), 7.50 (d, *J*=8.8 Hz, 1H, $C_5$-H), 7.68 (d, *J*=9.5 Hz, 1H, $C_4$-H); $^{13}C$ NMR (25.05 MHz, $CDCl_3$), see structure **84** ; MS *m/z* 272 ($M^+$, 7), 257 (57), 243 (12), 227 (42), 216 (15), 215 (100), 213 (27), 199 (25), 187 (16), 185 (14), 172 (16), 171 (16), 159 (16), 128 (22). Mass measurement : obsd, 272.0599; calcd for $C_{15}H_{12}O_5$, 272.0685.

## ii. *ALYXIA REINWARDTI* (313)

*Alyxia reinwardti* Bl. var. *lucida* Markjor. (Apocynaceae), a traditional Thai medicinal plant, is widely used in Thailand for making perfume, tobacco products and is a constituent of several therapeutic remedies. Previous investigation of the leaves of this species resulted in the isolation of two iridolactones, alyxialactone and 4-epi-alyxialactone (314). Three monohydroxy coumarins, 3-hydroxycoumarin, 5-hydroxycoumarin and 8-hydroxycoumarin, have been isolated from the stem of *A. lucida*, a closely related species to *A. reinwardti* (315). We report here the isolation of two novel 3-hydroxycoumarin glycosies, **89** and **90**, from the inner bark of *A. reinwardti* var. *lucida*, whose structures were established by spectroscopic methods.

The $^1$H NMR spectrum of **89** (Table 30) showed eight aromatic proton resonances in the region δ 6.76-7.50, two *trans*-coupled olefinic proton resonances at δ 6.28 ($J$=15.8 Hz) and 7.55, one methoxyl singlet at δ 3.82, and 13 sugar proton resonances in the region δ 3.36-5.00, including two anomeric proton resonances at δ 4.95 ($J$=7.4 Hz) and 5.00 ($J$=2.5 Hz). These data suggested that **89** was a phenylpropanoid diglycoside. Investigation of the COSY spectrum (Table 31) revealed an ABX spin system at δ 6.76, 6.97 and 7.06 belonging to one of the two phenylpropanoid moieties and an AMM'X spin system at δ 7.50, 7.23, 7.36 and 7.20 belonging to the other. However, based on these data, it was not possible to assign the remaining olefinic proton resonances at δ 6.28 (*d*), 7.55 (*d*) and 7.39 (*s*) to a specific phenylpropanoid unit. The observation of a base peak at *m/z* 177 in the positive FABMS further revealed the presence of a ferulyl moiety, $C_{10}H_9O_3$, which normally shows a characteristic ABX spin sytem in the $^1$H NMR spectrum (316). Furthermore, the long-range couplings observed between δ 7.06 (H-2') and 7.55 (H-7'), and between δ 6.97 (H-6') and 7.55 in the delayed (250 msec) COSY spectrum confirmed this structural unit. The other phenylpropanoid moiety was deduced to be a 3-hydroxycoumarin based on the literature data, in particular, the $^{13}$C NMR data reported for 3-hydroxycoumarin (315,317). Consistent evidence for this moiety was also present in the FAB mass spectra which showed a prominent fragment ion peak at *m/z* 471 $[M^+-C_9H_5O_3]^+$ in the positive FAB and 470 $[M^+-1-C_9H_5O_3]^+$ in the negative FAB. The high resolution mass indicated a molecular formula $C_{30}H_{32}O_{15}$ suggesting that the two sugar residues were a pentose and a hexose. Comparison of the $^1$H and $^{13}$C NMR data of **89** (Tables 30, 32) with those reported in the literature (318,319) revealed the presence of glucose and apiose. In order to establish the linear connectivities between the different units and to assign the structure unambiguously, a series of NMR experiments including COSY, HOHAHA, ROESY, HMQC, HMBC and selective INEPT was performed.

The observation of an AMM'X spin system in the $^1H$ NMR spectrum of **89** indicated that quite unusually no substitution was present at C-5, C-6, C-7 and C-8 of the coumarin nucleus. A singlet at δ 7.39 suggested that a substituent might be at C-3 or C-4. A long-range coupling observed between δ 7.39 (H-4) and 7.20 (H-8) in the delayed COSY spectrum confirmed that the substituent was at C-3. Irradiation of the anomeric proton at δ 4.95 enhanced a carbon resonance at δ 142.9 (C-3) in the selective INEPT spectra (Fig. 20) revealing the linkage between the coumarin and glucose moieties. A long-range correlation contour between the glucose H-1 (δ 4.95) and C-3 in the HMBC spectrum confirmed this connectivity. Furthermore, the observation of a prominent fragment ion peak at *m/z* 309 $[M^+-C_9H_5O_3-C_6H_{10}O_5]^+$, due to successive losses of coumarin and glucose, in the positive FAB spectrum, provided additional evidence for this connectivity. The selective INEPT experiments showed that irradiation of H-4 enhanced two carbon resonances at δ 159.8 and 142.9 (C-3) allowing the carbon resonance at δ 159.8 to be assigned to C-2. In the HMBC spectrum, H-4 showed correlations with three carbon resonances at δ 159.8, 151.3 and 142.9 not only confirming the assignment of C-2 and C-3, but also allowing the carbon resonance at δ 151.3 to be assigned to C-9. Irradiation of H-5 at δ 7.50 significantly enhanced the carbon resonances at δ 151.3, 130.5 (C-7), 120.8 (C-4) and confirmed the assignment of C-9. Irradiation of H-6 at δ 7.23 resulted in the enhancement of the carbon resonances at δ 151.3 (C-9), 120.9 and 117.0 (C-8) permitting the quaternary carbon resonance at δ 120.9 to be assigned to C-10. Confirmation of this assignment was achieved by irradiating H-8 at δ 7.20 which resulted in the enhancement of C-9, C-6 and C-10 at δ 151.3, 126.2 and 120.9, respectively. The assignment of the coumarin moiety was thus complete.




**89** R = H

**90** R = OMe

**Table 30** $^1$H-NMR data of **89** and **90** (500.1 MHz, $CD_3OD$)

| Proton | **89** | **90** |
|---|---|---|
| H-4 | 7.39 (*s*) | 7.40 (*s*) |
| H-5 | 7.50 (*dd*, *J*=7.8, 1.5 Hz | 7.50 (*d*, *J*=7.9, 1.5 Hz) |
| H-6 | 7.23 (*ddd*, *J*=7.8, 7.9, 1.2 Hz) | 7.23 (*ddd*, *J*=7.5, 7.7, 1.5 Hz) |
| H-7 | 7.36 (*ddd*, *J*=8.0, 8.0, 1.5 Hz) | 7.36 (*ddd*, *J*=7.8, 7.8, 1.5 Hz) |
| H-8 | 7.20 (*dd*, *J*=7.9, 1.2 Hz) | 7.20 (*dd*, *J*=8.1, 1.5 Hz) |
| H-2' | 7.06 (*d*, *J*=1.7 Hz) | 6.78 (*s*) |
| H-5' | 6.76 (*d*, *J*=8.4 Hz) | - |
| H-6' | 6.97 (*dd*, *J*=8.6, 1.7 Hz) | 6.78 (*s*) |
| H-7' | 7.55 (*d*, *J*=15.8 Hz) | 7.53 (*d*, *J*=6.0 Hz) |
| H-8' | 6.28 (*d*, *J*=16.0 Hz) | 6.29 (*d*, *J*=15.8 Hz) |
| 3'-OMe | 3.82 (*s*) | 3.81 (*s*) |
| 5'-OMe | - | 3.81 (*s*) |
| G: H-1 | 4.95 (*d*, *J*=7.4 Hz) | 4.95 (*d*, *J*=7.4 Hz) |
| G: H-2 | 3.51 (*dd*, *J*=9.1, 7.4 Hz) | 3.51 (*dd*, *J*=9.1, 7.4 Hz) |
| G: H-3 | 3.49 (*dd*, *J*=9.1, 9.1 Hz) | 3.49 (*dd*, *J*=9.1, 9.1 Hz) |
| G: H-4 | 3.36 (*dd*, *J*=9.6, 9.6 Hz) | 3.36 (*dd*, *J*=9.1, 9.1 Hz) |
| G: H-5 | 3.69 (*m*) | 3.70 (*m*) |
| G: H-6 | 4.09 (*dd*, *J*=10.9, 1.7 Hz) | 4.09 (*dd*, *J*=10.9, 1.7 Hz) |
| G: H-6 | 3.62 (*dd*, *J*=11.0, 6.9 Hz) | 3.63 (*dd*, *J*=11.0, 6.8 Hz) |
| A: H-1 | 5.00 (*d*, *J*=2.5 Hz) | 5.00 (*d*, *J*=2.2 Hz) |
| A: H-2 | 3.95 (*d*, *J*=2.2 Hz) | 3.95 (*d*, *J*=2.2 Hz) |
| A: H-4 | 4.04 (*d*, *J*=9.9 Hz) | 4.05 (*d*, *J*=9.6 Hz) |
| A: H-4 | 3.84 (*d*, *J*=9.9 Hz) | 3.84 (*d*, *J*=9.9 Hz) |
| A: H-5 | 4.25 (**AB** quartet, *J*=11.5 Hz) | 4.26 (**AB** quartet, *J*=11.5 Hz) |

**Table 31** Summary of 2D NMR spectral data of **89**.

| Proton | COSY | HOHAHA | ROESY |
|---|---|---|---|
| H-4 | | | G-1 |
| H-5 | H-6, H-7 | H-6, H-7, H-8 | |
| H-6 | H-5, H-7 | H-5, H-7, H-8 | |
| H-7 | H-5, H-6, H-8 | H-5, H-6, H-8 | |
| H-8 | H-6, H-7 | H-5, H-6, H-7 | |
| G-1 | G-2 | G-2, G-3, G-4, G-5 | H-4, G-3, G-5 |
| G-2 | G-2, G-3 | G-1, G-3, G-4, G5 | |
| G-3 | G-2, G-4 | G-1, G-2, G-4, G-5 | G-1, G-5 |
| G-4 | G-3, G-5 | G-1, G-2, G-3, G-5, G-6b | |
| G-5 | G-5, G-6b | G-2, G-3, G-4, G-6a, G-6b | G-1, G-6b |
| G-6a | G-5, G-6b | G-4, G-5, G-6b | G-6b, A-1 |
| G-6b | G-5, G-6a | G-4, G-5, G-6a | G-5, G-6a, A-1 |
| A-1 | A-2 | A-2 | G-6a, G-6b, A-2 |
| A-2 | A-1 | A-1 | A-1, A-5 |
| A-4a | A-4b | A-4b | A-4b |
| A-4b | A-4a | A-4a | A-4a, A-5 |
| A-5 | | | A-2, A-4b |
| H-2' | H-6' | H-5', H-6' | H-7', H-8', 3'-OMe |
| H-5' | H-6' | H-2', H-6' | |
| H-6' | H-2', H-5' | H-2', H-5' | H-8' |
| H-7' | H-8' | H-8' | H-2' |
| H-8' | H-7' | H-7' | H-2', H-6' |
| 3'-OMe | | | H-2' |

**Table 32** $^{13}C$ NMR data of **89** and **90** (125.8 MHz, $CD_3OD$)

| Carbon | **89** | **90** |
|---|---|---|
| C-2 | 159.8 (*s*) | 159.9 (*s*) |
| C-3 | 142.9 (*s*) | 153.1 (*s*) |
| C-4 | 120.8 (*d*) | 120.9 (*d*) |
| C-5 | 128.6 (*d*) | 128.8 (*d*) |
| C-6 | 126.2 (*d*) | 126.4 (*d*) |
| C-7 | 130.5 (*d*) | 130.7 (*d*) |
| C-8 | 117.0 (*d*) | 117.2 (*d*) |
| C-9 | 151.3 (*s*) | 151.5 (*s*) |
| C-10 | 120.9 (*s*) | 121.0 (*s*) |
| G: C-1 | 102.4 (*d*) | 102.6 (*d*) |
| G: C-2 | 74.5 (*d*) | 74.6 (*d*) |
| G: C-3 | 77.4 (*d*) | 77.5 (*d*) |
| G: C-4 | 71.4 (*d*) | 71.6 (*d*) |
| G: C-5 | 77.2 (*d*) | 77.4 (d) |
| G: C-6 | 68.5 (*t*) | 68.6 (*t*) |
| A: C-1 | 110.5 (*d*) | 110.7 (*d*) |
| A: C-2 | 78.3 (*d*) | 78.7 (*d*) |
| A: C-3 | 78.9 (*s*) | 79.1 (*s*) |
| A: C-4 | 75.0 (*t*) | 75.2 (*t*) |
| A: C-5 | 67.3 (*t*) | 67.5 (*t*) |
| C-1' | 127.5 (*s*) | 126.6 (*s*) |
| C-2' | 111.7 (*d*) | 107.1 (*d*) |
| C-3' | 149.3 (*s*) | 149.6 (*s*) |
| C-4' | 150.7 (*s*) | 139.8 (*s*) |
| C-5' | 116.5 (*d*) | 149.6 (*s*) |
| C-6' | 124.2 (*d*) | 107.1 (*d*) |
| C-7' | 147.3 (*d*) | 147.7 (*d*) |
| C-8' | 115.0 (*d*) | 115.6 (*d*) |
| C-9' | 168.8 (*s*) | 168.9 (*s*) |
| 3'-OMe | 56.4 (*q*) | 57.0 (*q*) |
| 5'-OMe | - | 57.0 (*q*) |

Examination of the $^{13}C$ NMR (Table 32) spectrum in the sugar carbon region showed that the glucose C-6 resonated at δ 68.5, which is more down-field than the corresponding free alcohol, suggesting the presence of a substituent at C-6. Irradiation of the anomeric proton of apiose at δ 5.00 enhanced three carbon resonances, at δ 68.5 (G-6), 75.0 (A-4) and 78.9 (A-3) allowing the linkage between the apiose and glucose moieties to be made. The unambiguous assignment of the proton resonances in the glucose moiety was made posible by performing a series of 2D NMR experiments including COSY, HOHAHA and ROESY. The HOHAHA spectrum (Table 31) showed that the glucose H-1 exhibited four-step relayed connectivity to glucose H-5 and that H-4 had connectivities with glucose H-1, H-2, H-3, H-5 and H-6. A significant NOE observed between glucose H-1 and the coumarin H-4 in the ROESY spectrum (Table 42) revealed the close proximity of these two moieties. In addition, the glucose H-1, showing a NOE with the glucose H-3 and H-5, further confirmed the configuration of these OH groups.

A strong correlation was observed between the apiose H-5 ( δ 4.25) and C-9' (δ 168.8) in the HMBC spectrum revealing the connection between the apiose and the feruloyl moieties. However, the selective INEPT experiment on irradiating the apiose H-5 at δ 4.25 resulted in the enhancement of only the apiose C-4 at δ 75.0. This might be due to the different *J* values used in these two experiments. Owing to the introduction of an electron withdrawing group into the apiose C-5, the $^{13}C$ chemical shift of C-5 ( δ 67.3) was shifted down-field and C-3 shifted up-field, as compared to the corresponding free sugar (318). The NOEs observed between apiose H-1 and H-2, and between H-4 and H-5 in the ROESY spectrum confirmed the configuration of the OH groups in apiose. Furthermore, the NOE between the apiose H-1 and the glucose H-6 demonstrated the spatial relationship between these two sugar moieties. Comparison of the chemical shifts and coupling constants of the anomeric protons in apiose and glucose moieties with those reported in the literature (318) led to the conclusion that both sugars were in the β-D-configuration.

In the feruloyl moiety, the observation of a NOE between the 3'-OMe and H-2' (δ 7.06) confirmed that the methoxyl group was at C-3'. A correlation peak observed between the 3'-OMe protons (δ 3.82) and a carbon at δ 149.3 in the HMBC spectrum allowed this carbon resonance to be assigned to C-3'. The correlation of C-3' with two other protons, H-2' and H-5', further supported this assignment. Three proton resonances, H-2', H-5' and H-6', showed correlation with the same carbon resonance at δ 150.7 permitting this carbon resonance to be assigned to C-4'. The carbon resonance at δ 127.5 was attributed to C-1', due to the observation of a correlation of this carbon

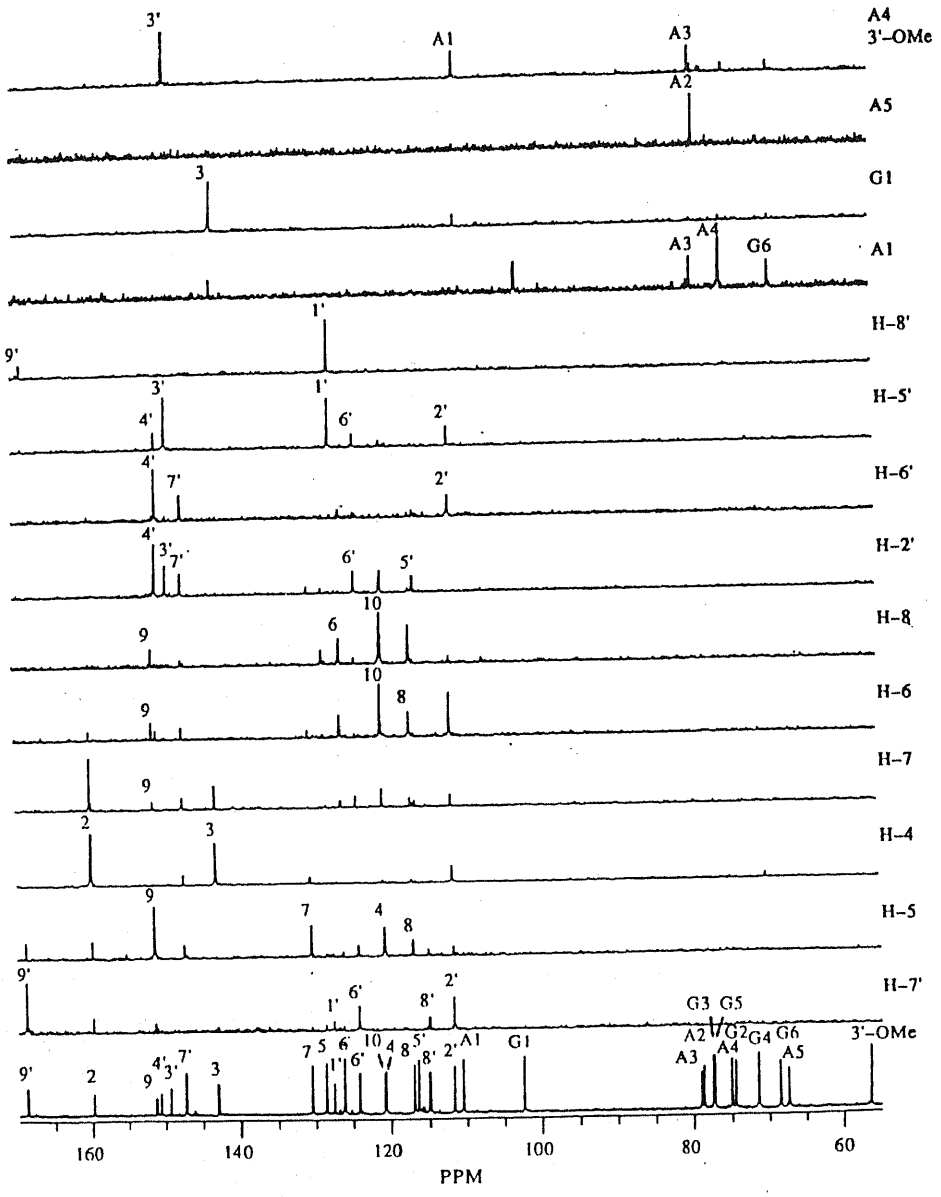



**Fig. 20** Selective INEPT spectra of **89**

with H-5' and H-8'. The carbonyl resonance at δ 168.8, showing correlations with three proton resonances, H-7', H-8' and the apiose H-5, not only revealed the connectivity between the ferulyl moiety and the apiose residue, but also confirmed the assignment of these protons. Comparable results were also obtained from selective INEPT experiments (Fig. 20). The structure of **89** thus could be determined and its structural assignment was established as the first member of a new class of natural products.

**Table 33** Summary of 2D NMR spectral data of **90**

| Proton | COSY | HOHAHA | ROESY |
|---|---|---|---|
| H-4 | | | G-1 |
| H-5 | H-6, H-7 | H-6, H-7, H-8 | |
| H-6 | H-5, H-7 | H-5, H-7, H-8 | |
| H-7 | H-5, H-6, H-8 | H-5, H-6, H-8 | |
| H-8 | H-6, H-7 | H-5, H-6, H-7 | |
| G-1 | G-2 | G-2, G-3, G-4, G-5 | H-4, G-3, G-5 |
| G-2 | G-1, G-3 | G-1, G-3, G-4, G5 | |
| G-3 | G-2, G-4 | G-1, G-2, G-4, G-5 | G-1, G-5 |
| G-4 | G-3, G-5 | G-1, G-2, G-3, G-5, G-6b | |
| G-5 | G-4, G-6a, G-6b | G-2, G-3, G-4, G-6a, G-6b | G-1, G-6b |
| G-6a | G-5, G-6b | G-4, G-5, G-6b | G-6b, A-1 |
| G-6b | G-5, G-6a | G-4, G-5, G-6a | G-5, G-6a, A-1 |
| A-1 | A-2 | A-2 | G-6a, G-6b, A-2 |
| A-2 | A-1 | A-1 | A-1, A-5 |
| A-4a | A-4b | A-4b | A-4b |
| A-4b | A-4a | A-4a | A-4a, A-5 |
| A-5 | | | A-2, A-4b |
| H-2' | | | H-8', 3'-OMe |
| H-6' | | | H-7'-5'-OMe |
| H-7' | H-8' | H-8' | H-6' |
| H-8' | H-7' | H-7' | H-2' |
| 3'-OMe | | | H-2' |
| 5'-OMe | | | H-6' |

Compound **90** displayed a molecular ion peak at *m/z* 663 in the positive FAB and *m/z* 661 in the negative FAB spectra revealing that this isolate contained 30 a.m.u. more than **89**. The HRMS indicated a molecular formula $C_{31}H_{34}O_6$ further suggesting the presence of an additional methoxyl group. However, due to the symmetrical nature of the phenylpropanoid moiety, only one methoxyl resonance was observed in $^1H$ and $^{13}C$ NMR spectra of **90**. Comparison of the $^1H$ NMR spectral data of **90** with those of **89** (Table 30) indicated that the only difference was in the phenylpropanoid moiety which showed a singlet with an integration for two protons at δ 6.78 in **90**. Based on these observations it was deduced that sinapyl moiety was present in **90**. Consistent with this finding was the $^{13}C$ NMR data which showed four, instead of six, aromatic carbon resonances for this group (Table 32). A base peak at *m/z* 207 ($C_{11}H_{11}O_4$) present in the

positive FAB and a prominent fragment ion peak at *m/z* 223 ($C_{11}H_{11}O_5$) observed in the negative FAB further supported this speculation.

The selective INEPT experiments performed on **90** not only confirmed the connectivity between different moieties to be the same as **89**, but also permitted the unambiguous assignment of the sinapyl moiety. Irradiation of H-7' at δ 7.53 resulted in the enhancement of two carbon resonances at δ 168.9 and 107.1 allowing these two resonances to be readily assigned to C-9' and C-2'/C-6', respectively. Two carbon resonances at δ 168.9 and 126.6 were enhanced as a result of the irradiation of H-8' at δ 6.29 revealing that the quaternary carbon resonance at δ 126.6 was C-1'. A significant NOE observed between H-2'/H-6' and the methoxyl proton resonance at δ 3.81 in the ROESY spectrum indicated that the locations of these methoxyl groups were at C-3'/C-5'. Irradiation of the 3'-OMe/5'-OMe protons enhanced the carbon resonance at δ 149.6, permitting this resonance to be assigned to C-3'/C-5'. The enhancement of three carbon resonances at δ 149.6, 147.7 and 139.8 on irradiation of H-2'/H-6' further allowed the carbon resonance at δ 139.8 to be assigned to C-4'. The assignment of the sinapic acid residue thus could be made unambiguously.

The 2D NMR data of **90** (Table 33) showed close resemblance to those of **89**, confirming the identical stereochemistry and connectivities in these two isolates. Both compounds exhibited comparable optical activity, further demonstrating their similarity in stereochemistry.

The isolation of 3-hydroxycoumarin glycosides from *A. reinwardti* var. *lucida* implied that the aglycone, 3-hydroxycoumarin, should also exist in this plant. Further investigation of the less polar fractions of the extract may provide the answer. It is interesting to note that three monohydroxy coumarins, including 3-hydroxycoumarin, have been isolated from *A. lucida* (315). The occurrence of this very unusual coumarin and its glycoside in two different species suggests a close taxonomic relationship. Compounds **89** and **90** were not active against cultured P-388 lymphocytic leukemia cells ($ED_{50}$>4μg $ml^{-1}$).

Compound **89** exhibited the following physical and spectroscopic properties : $[\alpha]_D$-111 (MeOH; *c* 0.1); UV $\lambda_{max}^{(MeOH)}$ nm (log ε) : 232 (4.26) 297 (4.37), 315 (4.40); IR $\upsilon_{max}$ $^{(KBr)}$ $cm^{-1}$ : 3441, 2942, 1728, 1709, 1628, 1605, 1514, 1458, 1431, 1294, 1273, 1161, 1065, 1028; $^1H$ and $^{13}C$ NMR, Table 30 and 32 respectively; positive FABMS, *m/z* (rel. int.) 633 ($[M+1]^+$, 25), 554 (11), 471 (17), 309 (46), 291 (12), 194 (10), 177 (100), 163 (20); negative FABMS, *m/z* (rel. int.) 631 ($[M-1]^+$,

100), 605 (21), 487 (11), 471 (23), 470 (24), 469 (56), 443 (15), 367 (24), 343 (10), 325 (13), 311 (11), 293 (12), 265 (11); HRMS found : $[M+1]^+$ 633.1822; $C_{30}H_{33}O_{15}$ requires 633.1819.

Compound **90** exhibited the following physical and spectroscopic properties : [α]D-101° (MeOH; *c* 0.1); UV $\lambda_{max}^{(MeOH)}$ nm (log ε) : 225 (4.36), 291 (4.34), 313 (4.38); IR $\upsilon_{max}^{(KBr)}$ $cm^{-1}$ : 3435, 2940, 1727, 1717, 1628, 1607, 1516, 1458, 1426, 1294, 1153, 1113, 1067; $^1H$ and $^{13}C$ NMR, Tablet 30 and 32, respectively; positive FABMS, *m/z* (rel. int.) 663 ($[M+1]^+$, 17), 501 (7), 475 (2), 339 (37), 321 (20), 207 (100), 163 (17), 162 (6); negative FABMS, *m/z* (rel. int.) 661 ($[M-1]^+$, 100), 500 (22), 499 (45), 367 (39), 355 (11), 295 (13), 273 (25), 223 (60), 162 (10), 161 (72), 133 (7); HRMS found : $[M+1]^+$ 663.1925; $C_{13}H_{35}O_{16}$ requires 633.1925.

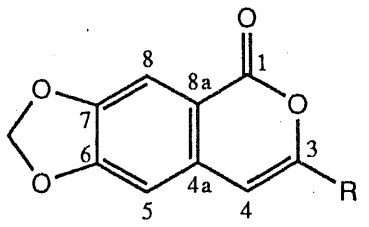
### 2.1.6 **Isocoumarins**

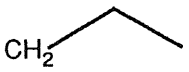
Isocoumarin or 1H-2-benzopyran-1-one is an unsaturated lactone which differs from coumarin in that the olefin linkage is not conjugated with the carbonyl group. It may be regarded as a vinyl ester. Isocoumarin compounds could found not only in higher plants but also in fungi.

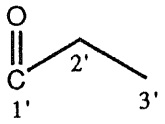
#### i. *XYRIS INDICA* (320)

The genus *Xyris* Gronov. ex L. in the Xyridaceae family is composed of approximately 250 species distributed primarily in tropical and subtropical region (321). *Xyris indica* L. (Tall yellow-eyed grass) is one of the five species found throughout Thailand and is known locally as "Kra thin thung"(322). It is a weed which is widespread in paddy fields of Prachin buri. In Bengal, the plant has been used in folklore medicine as a cure for ringworm, itch and leprosy (323,324). Phytochemical study of the genus *Xyris* has previously led to the isolation of two antraquinones; chrysazin and 3-methoxy chrysazin from *Xyris semifuscata* (325). Herein we report the structure determination of two new isocoumarins isolated from a non-polar chromatographic fraction obtained from the flowering heads of *X. indica.*

Purification of a chloroform soluble extract of the flowering heads of *Xyris indica* with silica gel column chromatography resulted in the isolation of two new isocoumarins (**91** and **92**).




**91** R = 


**92** R = 


Compound **91**, m.p. 67-68°C, was obtained as a pale yellow plate. The molecular formula of **91** was deduced to be $C_{13}H_{12}O_4$ (Calcd. 232.0732 amu) from its molecular ion at *m/z* 232.0731 in the hreims. The benzopyrone structure was evident from its UV absorptions at 334 and 282 nm (log ε 3.65 and 3.77)(326), and the pyrone-carbonyl stretching frequency was found in the region 1725 $cm^{-1}$ from IR spectrum (327,328). The presence of a methylenedioxy group at C-6 and C-7 was indicated from the isolated methylene proton signal at δ 6.03 (s) and the aromatic proton resonances of H-5 at δ 6.66 (s), H-8 at δ 7.51 (s) and H-4 at δ 6.09 (s) in the $^1$H-NMR spectrum. Analysis of the $^{13}$C-nmr and DEPT spectra of **91** revealed the presence of 3 methylene carbons ( δ 20.18, 35.16 and 102.02), 3 aromatic methine carbons (δ102.96, 103.50 and 107.19), one methyl carbon (δ 13.40), and 6 quaternary carbons (δ 114.48, 135.19, 147.72, 153.51, 156.99 and 162.63) including one carbonyl carbon. The n-propyl side chain could be assigned from $^1$H-signals at δ 0.93, 1.66 and 2.42 (t [$J$ = 7.3 Hz], tq[ $J$ = 7.3, 7.3 Hz] and t [ $J$ = 7.3 Hz])). It could be inferred from the above data that there are a number of alternatives structure of **91** to be deduced : (a) a 3-n-propyl or 4-n-propyl-6,7-(methylenedioxy-1H-2-benzopyran-1-one and (b) a 3-n-propyl or 4-n-propyl-6,7-(methylenedioxy)-2H-1-benzopyran-2-one. In order to evaluate the structure more explicitly, a detailed examination of the HMBC of **91** was undertaken. The significant long range $^1$H-$^{13}$C correlations contour are indicated by arrows in Fig 21. The quaternary carbon at δ156.99 (C-3) is correlated with the methylene protons at δ 2.42 (H-1') and δ 1.66 (H-2') and with the olefinic proton at δ 6.09 (H-4). The carbonyl carbon at δ 162.63 (C-1) is correlated with the olefinic proton at δ 7.51 (H-8). The quaternary carbon at δ 114.48 (C-4a) is correlated with the olefinic protons at δ 6.09 (H-4) and δ 6.66 (H-5). The quaternary carbon at δ 135.19 (C-8a) is correlated with the olefinic proton at δ 7.51 (H-8). These results strongly support the proposed structure of **91** as 3-n-propyl-6,7-(methylenedioxy)-1H-2-benzopyran-1-one, a new isocoumarin named Xyridin A.

Compound **92**, m.p. 198-199°C, was obtained as a colorless plate, possessing the molecular formula $C_{13}H_{10}O_5$ as determined by HREIMS spectrum. A variety of spectroscopic techniques was employed to determine the structrue of **92** and indicated a close structural relationship with **91** as all two exhibited the same carbon framework. UV

spectrum of **92** showing absorption maxima (log ε) at 333(4.16) and 262(4.57) nm, suggested the feature of benzopyrone (326). The two carbonyl stretching frequency were found in the regions 1695 and 1733 $cm^{-1}$ in IR spectrum (327,328) . $^{1}H$-NMR spectrum of **92** indicated the presence of three aromatic protons at δ 7.24 (s), 6.92 (s), and 7.62 (s) and two methylene protons at δ 6.11 (s) and 2.92 (q), suggested a close resemblance to **91** based on the 6,7-methylenedioxy-1H-2-benzopyran-1-one skeleton. Further, the triplet at δ1.14 and quartet at δ 2.92 ($J$ =7.3 Hz) indicated the $CH_3$-$CH_2$- side chain. In the $^{13}C$-NMR and DEPT spectra, it was shown that **92** consisted of 2 methylene carbons (δ 31.63 and 102.71), 3 methine carbons (δ 106.32, 108.09 and 108.52), one methyl carbon (δ 7.33) and 7 quaternary carbons (δ 118.39, 132.57, 148.68, 150.34, 153.86, 160.42 and 194.91 (including two carbonyl carbons). It is clearly shown that **92** has an additional carbonyl on the propyl side chain of **91** as an oxopropyl group from the NMR and MS data. Confirmation by C-H long range connectivity of **92** by HMBC spectrum indicated that the carbonyl carbon (C-1') at δ 194.91 of oxopropyl side chain is correlated with the olefinic proton at δ 7.24 (H-4), methylene proton at δ 2.92 (H-2') and methyl proton at δ 1.14 (H-3'). The quaternary carbon at δ 148.68 (C-3) is correlated with the olefinic proton at δ 7.24 (H-4). The carbonyl carbon at δ 160.42 (C-1) is correlated with the olefinic proton at δ 7.62 (H-8). The quaternary carbon at δ 118.39 (C-4a) is correlated with the olefinic protons at δ 7.24 (H-4) and δ 6.92 (H-5). The quaternary carbon at δ 132.57 (C-8a) is correlated with the olefinic proton at δ 7.62 (H-8). The structure of **92** was thus determined to be 3-(1'-oxopropyl)-6,7-(methylenedioxy)-1H-2-benzopyran-1-one unambiguously and named Xyridin B.

From a biogenetic point of view, there seems to be no doubt that xyridin A (**91**) and xyridin B (**92**) arise from the acetate-malonate pathway through cyclization reactions of a C12-polyketide chain. This secondary metabolic route appears to be responsible for the other constituents which have so far been isolated from *Xyris* sp (325).

Compound **91** exhibited the following physical and spectroscopic properties : mp 67-68°C; UV $\lambda_{max}$ (MeOH) nm (log ε) : 203 (4.26), 239 (4.60), 282 (3.77), 334 (3.65); IR $\upsilon_{max}$ $cm^{-1}$ 3090, 2960, 2925, 2865, 1725, 1662, 1595, 1503, 1484, 1412, 1358, 1261, 1110, 1031, 1015, 981, 920, 848; $^{1}H$ and $^{13}C$ NMR, Tables 34and 35, respectively ; EIMS 70 eV (probe) *m/z* (rel. int.) 232 $[M]^+$ (100), 204 (14), 203 (77), 175 (76), 162 (23),161 (12), 133 (37), 75 (12) ; HREIMS found : $[M]^+$ 232.0731; $C_{13}H_{12}O_4$ requires 232.0732

Compound **92** exhibited the following physical and spectroscopic properties : mp 198-199°C ; UV $\lambda_{max}$ (MeOH) nm (log ε) : 227 (4.09), 262 (4.57), 333 (4.16) ; IR

$\upsilon_{max}$ $cm^{-1}$ 3080, 3055, 2980, 2925, 1733, 1695, 1632, 1613, 1505, 1490, 1412, 1346, 1275, 1243, 1192, 1048, 1015, 943, 908, ; $^1H$ and $^{13}C$ NMR Tables 34 and 35 respectively ; ElMS 70 eV (probe) *m/z* (rel. int.) 246 $[M]^+$ (80), 189 (16), 162 (2), 161 (16), 133 (100), 75 (3) ; HREIMS found : $[M]^+$ 246.0534; requires 246.0525

**Table 34** $^1H$ NMR spectral data for compounds **91** and **92** ($CDCl_3$ ; 500 MHz ; TMS)

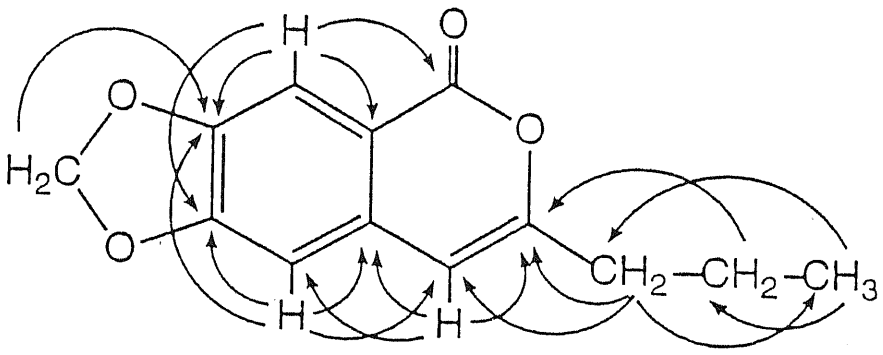
| H | **91** | **92** |
|---|---|---|
| 4 | 6.09 s | 7.24 s |
| 5 | 6.66 s | 6.92 s |
| 8 | 7.51 s | 7.62 s |
| -O-$CH_2$-O- | 6.03 s | 6.11 s |
| 1' | 2.42 t (7.3) | - |
| 2' | 1.66 tq (7.3, 7.3) | 2.92 q (7.3) |
| 3' | 0.93 t (7.3) | 1.14 t (7.3) |

δ (ppm). Coupling constants (*J* in Hz) in parentheses.

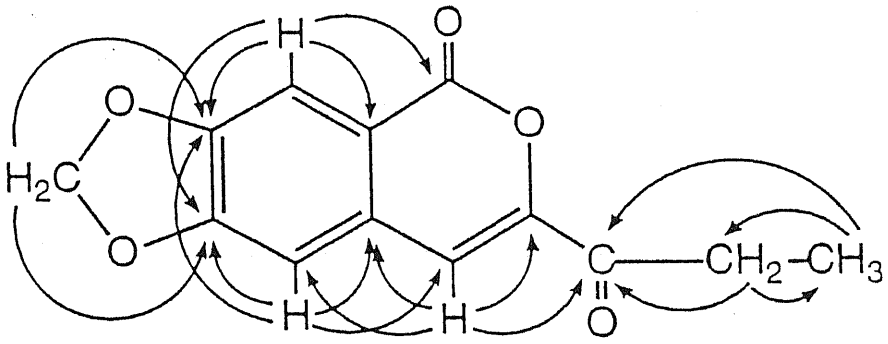
**Table 35** $^{13}C$ NMR spectral data for compounds **91** and **92** ($CDCl_3$ ; 125.65 MHz;TMS)

| C | **91** | **92** |
|---|---|---|
| 1 | 162.63 | 160.42 |
| 3 | 156.99 | 148.68 |
| 4 | 102.96 | 108.52 |
| 4a | 114.48 | 118.39 |
| 5 | 103.50 | 106.32 |
| 6 | 147.72 | 150.34 |
| 7 | 153.51 | 153.86 |
| 8 | 107.19 | 108.09 |
| 8a | 135.19 | 132.57 |
| -O-$CH_2$-O- | 102.02 | 102.71 |
| 1' | 35.16 | 194.91 |
| 2' | 20.18 | 31.63 |
| 3' | 13.40 | 7.33 |

δ (ppm). All carbons were assigned with the aid of $^{13}C$-$^1H$ and $^{13}C$-$^1H$ long range COSY




**91**




**92**

**Fig. 21** C-H Correlation observed in the HMBC spectra of **91** and **92**

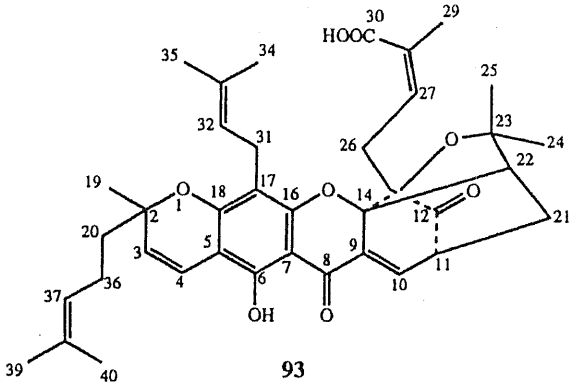
### 2.1.7 Xanthones

Xanthonoid compounds are regarded as dibenzopyrone with or without prenylated functionalities. Distribution of xanthonoids in higher plants is significant in chemotaxonomic consideration. It occurs in the families Guttiferae, Gentianaceae, Moraceae as well as in fungi. Some of which are marked biological interests.

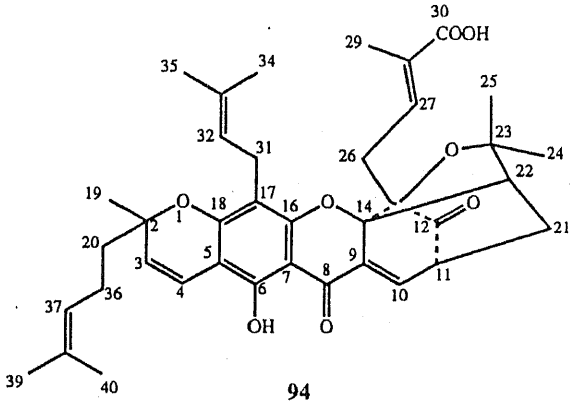
#### i. *GARCINIA HANBURYI* (329)

The pigment gamboge is the latex exuded from various *Garcinia* (Guttiferae) species, including *Garcinia hanburyi* Hook.f. and *G. morella* Desr. In Thailand, *G. hanburyi* is known as 'Rongthong' (gold resin) and is used internally as a drastic purgative, an emetic and a vermifuge to treat tapeworm. For external use, the resin, mixed with coconut milk, is applied for the treatment of chronic dermatitis (330,331). From the resin, gambogic acid has been isolated and claimed to be the major active principle (332). Owing to the complexity of this type of xanthone derivative, chemical studies have been carried out on gambogic acid and a related compound, morellin (333-336). The structure of morellin has been established by X-ray crystallographic study of its p-bromobenzenesulfonyl ester, (335) and the structure of gambogic acid deduced inferentially (334). No $^{13}C$ NMR data have been reported in support of the structures of this class of compounds, and the existing $^{1}H$ NMR assignments were also found to be incomplete. With the availability of high-field NMR instrumentation and the various newly developed NMR techniques, it became possible to reaffirm the proposed sructure and assign completely the spectra of gambogic acid and its related derivatives and to evaluate their biological activity.

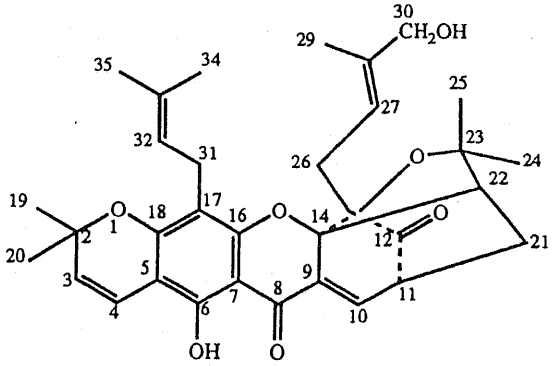
Previous phytochemical investigations of *G. morella* have resulted in the isolation of seven closely related compounds, all of which contain a xanthone moiety ($C_{13}$) with four isoprene units, i.e. morellin, (335) isomorellin, (337) desoxymorellin, dihydroisomorellin, (338) morellic acid, isomorellic acid (339) and morellinol (341). However, only two related derivatives, which consist of one xanthone moiety with five isoprene units, have been reported from *G. hanburyi*, viz. gambogic acid (**93**) (332,334,339) and neogambogic acid (341). In the current investigation, the latex of *G. hanburyi* collected in Thailand yielded two new derivatives, isogambogic acid (**94**) and isomorellinol (**95**).




93




94




95

Electron impact mass spectrometry (EIMS) of gambogic acid (**93**) showed a molecular ion peak at *m/z* 628 suggesting a molecular formula $C_{38}H_{44}O_8$. Investigation of the broadband decoupled $^{13}C$ NMR and APT spectra confirmed the presence of 38 carbons. Comparison of the $^{1}H$ NMR data (Table 36) with those reported (334) for gambogic acid indicated close identity, although some ambiguities in the structure remained to be clarified. In order to elucidate its structure, a series of NMR experiments including COSY, ROESY, (342) selective INEPT, (337) HMQC and HMBC were performed.

In the COSY spectrum of gambogic acid (**93**), a proton resonance at δ 3.46 (H-11) showed scalar couplings with two resonances at δ 7.53 (H-10) and 2.29. The latter resonance showed further couplings to a resonance at δ 2.49 suggesting that the resonance at δ 2.29 was one of two H-21 methylene protons. The carbon resonance at δ 25.09 (Table 37) showed correlations with two proton resonances at δ 1.27 and 2.29, and the carbon resonance at δ 48.92 showed a correlation with the proton resonance at δ 2.49 in the HMQC spectrum, clearly revealing that the proton resonances at δ 2.29 and 2.49 were H-21 and H-22, respectively. The previous assignment (334) for these two protons thus has to be reversed. The cross peaks present in the COSY spectrum also allowed the proton resonances of H-26 (δ 2.98), H-27 (δ 6.11), H-31 (δ 3.13/3.27), H-32 (δ 5.04), H-36 (δ 2.03), and H-37 (δ 5.07) to be unequivocally assigned. Through correlative interpretation of the NMR spectral data obtained from selective INEPT, HMQC and HMBC experiments it became possible to assign these resonances unambiguously.

In the selective INEPT spectra (Fig. 22) it was observed that irradiation of 6-OH at δ 12.74 enhanced three carbon resonances at δ 157.50 (C-6), 102.75/102.57 (C-5) and 100.40/100.33 (C-7). Three carbon resonances, C-5, C-6 and C-7, showed the same correlation with 6-OH in the HMBC spectrum (Fig. 23), thereby confirming this finding. Irradiation of H-3 at δ 5.40 enhanced the carbon resonances at δ 102.75/102.57 and 81.14/80.99 (C-2), confirming the assignment of C-5. In the HMBC spectrum, correlation of both C-2 and C-5 with H-3 was also observed. Irradiation of H-4 at δ 6.55 resulted in the enhancement of four carbon resonances at δ 161.34, 157.50, 102.75/102.57, and 81.14/80.99, allowing the carbon resonance at δ 161.34 to be assigned to C-18. The assignments of C-2, C-5 and C-6 were thus further confirmed by this irradiation. The correlation peaks between H-4 and C-2, and between H-4 and C-6, were observed in this HMBC spectrum. Irradiation of H-10 at δ 7.53 enhanced four carbon resonances at δ203.26, 178.79, 90.98/90.87 and 46.70, which could be assigned to C-12, C-8, C-14 and C-11, respectively. Confirmation of this assignment was based on the observation that these carbon resonances showed the same correlation with H-10 in the HMBC spectrum.

**Table 36** $^1$H NMR data for gambogic acid (**93**), isogambogic acid (**94**) and isomorellinol (**95**)[a]

| H | Gambogic acid (**93**) | Isogambogic acid (**94**) | Isomorellinol (**95**) |
|---|---|---|---|
| H-3 | 5.40 (d, 10.0) | 5.45/5.43 (d, 10.0) | 5.53 (d, 10.0) |
| H-4 | 6.55 (d, 10.0) | 6.67 (d, 10.0) | 6.64 (d, 10.0) |
| H-10 | 7.53 (d, 6.5) | 7.55/7.54 (d, 10.0) | 7.46 (d, 7.0) |
| H-11 | 3.46 (m) | 3.50 (m) | 3.53 (m) |
| H-19 | 1.34/1.31 (s) | 1.39/1.38 (s) | 1.45 (s) |
| H-20 | 1.68 (m) | 1.68 (m) | 1.45 (s) |
| H-20 | 1.78 (m) | 1.84 (m) | - |
| H-21 | 2.29 (d, 13.0) | 2.33 (dd, 4.5, 13.0) | 1.36 (dd, 5.0, 13.5) |
| H-21 | 1.27 (m) | 1.45 | 1.35 (dd, 9.5, 13.5) |
| H-22 | 2.49 (dd, 3.0, 9.0) | 2.53 (m) | 2.51 (d, 9.5) |
| H-24 | 1.27 (s) | 1.29 (s) | 1.30 (s) |
| H-25 | 1.69/1.67 (s) | 1.71 (s) | 1.72 (s) |
| H-26 | 2.98 (d, 7.0) | 2.62 (m) | 2.64 (d, 7.0) |
| H-26 | 2.98 (d, 7.0) | 2.63/2.53 (m) | 2.64 (d, 7.0) |
| H-27 | 6.11 (d, 7.0) | 6.67/6.54 (t, 7.0) | 4.78 (t, 7.0) |
| H-29 | 1.73/1.72 (s) | 1.29 (s) | 1.06 (s) |
| H-30 | - | - | 3.65 (q, 11.0) |
| H-30 | - | - | 3.65 (q, 11.0) |
| H-31 | 3.27 (m) | 3.28 (m) | 3.35 (dd, 6.1, 7.5) |
| H-31 | 3.13 (m) | 3.24 (m) | 3.30 (dd, 6.1, 7.5) |
| H-32 | 5.04 (m) | 5.11 (m) | 5.24 (t, 7.5) |
| H-34 | 1.71/1.70 (s) | 1.74/1.72 (s) | 1.78 (s) |
| H-35 | 1.62/1.60 (s) | 1.64 (s) | 1.68 (s) |
| H-36 | 2.03 (m) | 2.05 (m) | - |
| H-36 | 2.03 (m) | 2.05 (m) | - |
| H-37 | 5.07 (m) | 5.06 (m) | - |
| H-39 | 1.66/1.64 (s) | 1.65 (s) | - |
| H-40 | 1.56/1.52 (s) | 1.54/1.52 (s) | - |
| 6-OH | 12.74 (s) | 12.76/12.75 (s) | 12.72 (s) |

[a] Recorded in $CDCl_3$ ; chemical shifts are reported as $\delta$ values (ppm) from TMS at 500.1 MHz; signal multiplicity and coupling constants (Hz) are given in parentheses.

**Table 37** $^{13}C$ NMR data for gambogic acid (**93**), isogambogic acid (**94**) and isomorellinol (**95**) (500 MHz, $CDCl_3$)

| Carbon | Gambogic acid (**93**) | Isogambogic acid (**94**) | Isomorellinol (**95**) |
|---|---|---|---|
| C-2 | 81.14, 80.99 (s) | 81.29, 81.24 (s) | 78.75, 78.65 (s) |
| C-3 | 124.62, 124.32 (d) | 124.75 (d) | 126.35, 126.25 (d) |
| C-4 | 115.81, 115.32 (d) | 115.94 (d) | 115.44, 115.36 (d) |
| C-5 | 102.75, 102.57 (s) | 102.88, 102.75 (s) | 103.11, 103.05 (s) |
| C-6 | 157.50 (s) | 157.58 (s) | 157.31 (s) |
| C-7 | 100.40, 100.33 (s) | 100.43, 100.33 (s) | 100.66 (s) |
| C-8 | 178.79 (s) | 178.84 (s) | 180.22 (s) |
| C-9 | 133.26, 133.13 (s) | 133.29 (s) | 133.79 (s) |
| C-10 | 135.48, 135.25 (d) | 135.33 (d) | 134.31 (d) |
| C-11 | 46.70 (d) | 46.91, 46.83 (d) | 47.00 (d) |
| C-12 | 203.26 (s) | 203.06, 202.98 (s) | 203.18 (s) |
| C-13 | 83.69, 83.54 (s) | 83.64, 83.57 (s) | 84.48 (s) |
| C-14 | 90.98, 90.87 (s) | 90.67, 90.57 (s) | 90.42 (s) |
| C-16 | 157.45, 157.22 (s) | 157.33 (s) | 157.66 (s) |
| C-17 | 107.63, 107.45 (s) | 107.86 (s) | 108.38 (s) |
| C-18 | 161.34 (s) | 161.41, 161.29 (s) | 160.94 (s) |
| C-19 | 27.56, 26.82 (q) | 27.46, 27.31 (q) | 28.30 (q) |
| C-20 | 41.87, 41.59 (t) | 41.85 (t) | 28.26 (q) |
| C-21 | 25.09 (t) | 25.27 (t) | 25.35 (t) |
| C-22 | 48.92 (d) | 48.98 (d) | 49.09 (d) |
| C-23 | 83.69, 83.54 (s) | 83.72 (s) | 83.40 (s) |
| C-24 | 28.76 (q) | 28.92 (q) | 29.03 (q) |
| C-25 | 29.81 (q) | 29.97, 29.89 (q) | 30.23, 30.14 (q) |
| C-26 | 29.17 (t) | 28.95 (t) | 29.71 (t) |
| C-27 | 138.39 (d) | 137.09, 136.86 (d) | 118.28, 117.92 (d) |
| C-28 | 127.36 (s) | 128.89, 128.53 (s) | 138.28, 137.92 (s) |
| C-29 | 20.61 (q) | 11.32 (q) | 12.58 (q) |
| C-30 | 171.79, 171.58 (s) | 172.25, 172.09 (s) | 67.97 (t) |
| C-31 | 21.51 (t) | 21.59 (t) | 21.62 (t) |
| C-32 | 122.20 (d) | 122.12 (d) | 121.87, 121.75 (d) |
| C-33 | 131.27 (d) | 131.73 (s) | 131.96, 131.83 (s) |
| C-34 | 18.09, 17.96 (q) | 18.11, 18.04 (q) | 18.23 (q) |
| C-35 | 25.62, 25.55 (q) | 25.62, 25.42 (q) | 25.80 (q) |
| C-36 | 22.66 (t) | 22.71 (t) | - |
| C-37 | 123.78 (d) | 123.81, 123.73 (d) | - |
| C-38 | 131.62 (s) | 131.88, 131.78 (s) | - |
| C-39 | 25.62, 25.55 (q) | 25.62, 25.42 (q) | - |
| C-40 | 17.50 (q) | 17.58 (q) | - |

When H-11 (δ 3.46) was irradiated, five carbon resonances at δ 203.26 (C-12), 135.48/135.25 (C-10), 133.26/133.13, 83.69/83.54 and 48.92 (C-22) were enhanced.

The result not only confirmed the assignment of C-12, C-10 and C-22, but also permitted the assignment of the carbon resonances at δ 133.26/133.13 and 83.69/83.54 as C-9 and C-13, respectively. However, in the HMBC spectrum only C-9, C-12 and C-22 showed correlation with H-11; the less intense resonances, C-10 and C-13, enhanced in the selective INEPT spectrum were not observed. Irradiation of H-27 at δ 6.11 resulted in the enhancement of three carbon resonances at δ 171.79/171.58, 83.69/83.54 (C-13) and 20.61 allowing the carbon resonances at δ 20.61 and 171.79/171.58 to be assigned to C-29 and C-30, respectively. Owing to the overlap of H-32 and H-37 resonances, irradiation of these resonances at δ 5.06 resulted in the enhancement of C-35/C-39 (δ 25.62/25.55), C-36 (δ 22.66), C-31 (δ 21.51), C-34 (δ 18.03/17.96) and C-40 (δ 17.50). Irradiation of H-31 at δ 3.26 and 3.11 enhanced the same and other carbon resonances at δ 161.34 (C-18), 157.45/157.22 (C-16), 137.27 (C-33), 122.20 (C-32) and 107.63/107.45 (C-17), albeit with slightly different intensities. Irradiation of H-26 at δ 2.98 enhancement five carbon resonances at δ 203.26 (C-12), 138.39 (C-27), 127.36 (C-28), 90.98/90.87 (C-14) and 83.69/83.54 (C-13). The correlation of these carbons with H-26 was also observed in the HMBC spectrum. By irradiation H-22 at δ2.49, it was found that four carbon resonances at δ 90.98/90.87, 83.69/83.54, 46.70 and 29.81 (C-25) were enhanced, allowing the assignment of C-25 to be made. Identical information was also obtained from the HMBC spectrum, which showed four correlation peaks of these carbons with H-22. Irradiation of H-21 at δ 2.29 enhanced five carbon resonances at 135.48/135.25, 90.98/90.87 (C-14), 83.69/83.54 (C-23), 48.92 and 46.70. In the HMBC spectrum, however, only two carbon resonances, C-14 and C-23, showed correlation with H-21. Irradiation of H-36 at δ 2.03 enhanced three carbon resonances at δ 131.62, 123.78 and 81.14/80.99, allowing these resonances to be assigned to C-38, C-37 and C-2, respectively. The correlation of these carbons with H-36 was also observed in the HMBC spectrum, when the contour level was increased.

In the HMBC spectrum, both C-2 and C-20 showed a correlation with the methyl resonance at δ 1.34/1.31, indicating this methyl group should be H-19. The methyl resonance at δ 1.73/1.72 showed correlation with both C-27 and C-28, revealing that this methyl group was $H_3$-29. The reverse correlation observed between H-27 and C-29 confirmed this assignment. Through two and three-bond $^{13}C$-$^{1}H$ correlations the location of each *gem*-dimethyl group could be determined unambiguously. Both C-22 and C-23 showed correlations with two methyl resonances at δ 1.27 and 1.69/1.67, indicating these two methyl groups were $H_3$-24 and $H_3$-25, respectively. The correlation peaks observed between $H_3$-24 and C-25, and between C-24 and $H_3$-25 provided further

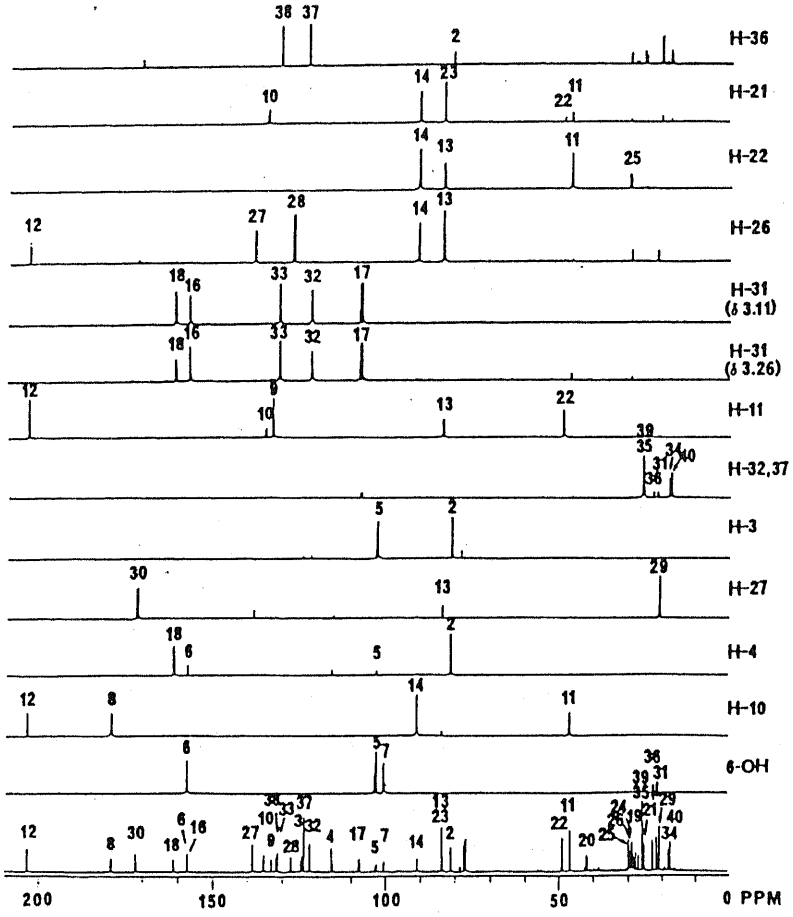



**Fig. 22** Selective INEPT spectra of gambogic acid (**93**).

evidence to support these assignments. Similarly, C-32 and C-33 showed correlations with both $H_3$-34 (δ 1.71/1.70) and $H_3$-35 (δ 1.62/1.60) and C-37 and C-38 had correlations with both $H_3$-39 (δ 1.66/1.64) and $H_3$-40 (δ 1.56/1.52), permitting the assignment of these methyl resonances to be made unequivocally. The reverse correlation between each pair of *gem*-dimethyl groups was also observed.

In a ROESY experiment, the contour observed between H-27 and $H_3$-29 confirmed the *trans*-configuration between H-27 and the carboxyl group (Fig. 24), as reported previously (334). The structure of gambogic acid was thus determined (Fig. 22), its NMR spectral assignment was complete and the groundwork laid for the structure determination of the two new isolates.

Isogambogic acid (**94**) has the same molecular formula $C_{38}H_{44}O_8$ [by high-resolution mass spectrometry (HRMS)] as that of gambogic acid (**93**), and also showed very similar UV, IR, MS, $^1H$ and $^{13}C$ MR data to those of **93**, revealing that **94** should be an isomer of **93**. A significant downfield shift for H-27 (δ 6.67/6.54, Δδ =

0.56/0.43) and upfield shifts for H-26 (δ 2.63/2.53, Δδ = -0.35/-0.45) and $H_3$-29 (δ 1.29, Δδ = -0.44/-0.43), as compared with the corresponding signals of gambogic acid, strongly suggested that the difference was in the side-chain bearing the carboxyl group. The observation of a significant upfield shift of the C-29 resonance (δ 11.32, Δδ = -9.29), due to non-bonding steric interaction (γ effect) (456) with the methylene group at C-26, further delineated a *cis* configuration between H-27 and the C-30 carboxyl group. The presence of a contour between H-26 and $H_3$-29 in the ROESY spectrum confirmed the stereochemistry of the C-27/C-28 double bond as *cis*.

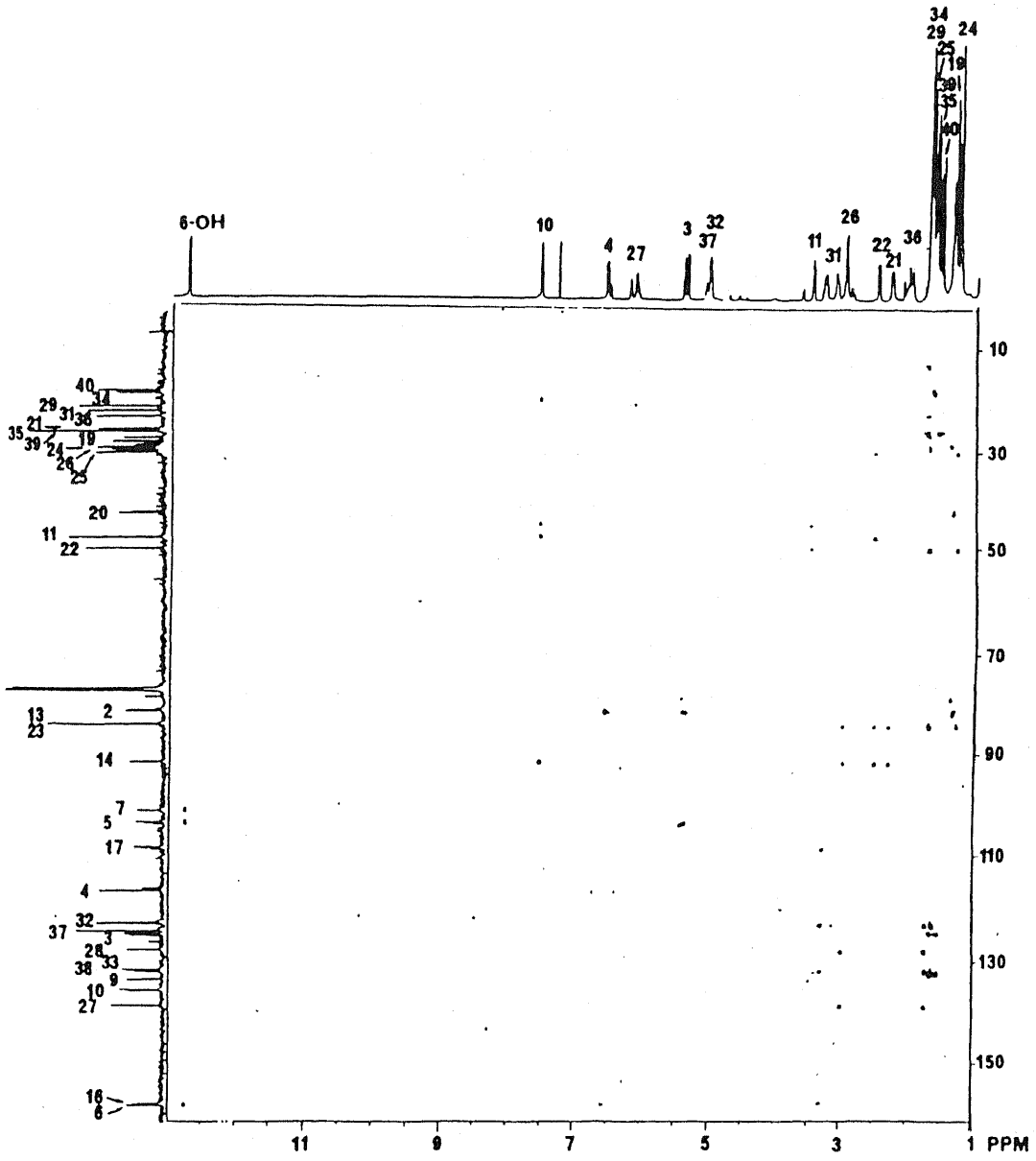



**Fig. 23** HMBC spectrum of gambogic acid (**93**).

The complete $^{1}H$ and $^{13}C$ NMR assignments of isogambogic acid (**94**) were made by COSY, selective INEPT, HMQC and HMBC experiments, and are shown in Tables 36 and 37, respectively. Similarly to the situation with gambogic acid (**93**), more than the eight expected methyl proton resonances were observed in the $^{1}H$ NMR spectrum of **94** owing to the existence of two conformers, or stereoisomers. Further, many of the carbon chemical shifts appeared as two close small peaks instead of one large peak in their $^{13}C$ spectra for the same reason, and some protons showed two very distinct chemical shifts such as H-27 (δ 6.67, 6.54), $H_3$-34 (δ 1.74, 1.72) and $H_3$-40 (δ 1.54, 1.52). The $^{1}H$ NMR spectrum of **94** was taken at different temperatures (25, 35, 45 and 55°C); the spectra were nearly identical and showed two almost equal peak sets, supporting the existence of two stereoisomers in the ratio 1:1. As compared to the NMR spectra **93** and **94**, the $^{1}H$ and $^{13}C$ NMR spectra of isomorellinol (**95**) did not show two peak sets or two close peaks for a proton or carbon signal.

Comparison of the $^{1}H$ NMR spectral data of isomorellinol (**95**) with those of gambogic acid (**93**) and isogambogic (**94**) showed that two characteristic proton resonances, H-36 and H-37, present in the spectra of gambogic acid and its isomer were missing in this isolate. In addition, only seven methyl resonances were observed in the NMR spectra of isomorellinol, suggesting the absence of the isoprene unit attached to C-20. The significant upfield shift of H-27 (δ 4.78), when compared with those of gambogic acid and its isomer, and the presence of an AB pattern at δ 3.65, further implied that a primary alcohol had replaced the carboxy group at C-30. In the $^{13}C$ NMR spectrum only 33 carbon resonances were observed, indicating the compound to have five carbons less than gambogic acid and its isomer. Further, the methylene carbon resonance observed at δ 67.97 suggested the presence of a primary OH group in the molecule. In support of this, the IR spectrum showed an absorption band at 3466 $cm^{-1}$ and the absence of an, α,β-unsaturated carbonyl absorption at 1690 $cm^{-1}$. EIMS gave a molecular ion peak at *m/z* 546 and HREIMS established a molecular formula $C_{33}H_{38}O_8$, supporting the proposed general structure.

In order to assign completely the structure of isomorellinol, a series of 2D NMR experiments, including COSY, ROESY, HMQC and HMBC, was performed. The HMQC spectrum showed that the most upfield methyl proton resonance ($H_3$-29) was correlated with the carbon resonance at δ 12.58 (C-29). The chemical shift of C-29 in isomorellinol is thus comparable to that of isogambogic acid (δ 11.32) and suggested that these two compounds had the same stereochemistry in the C-13 side-chain. The observation of correlation between H-27 and $H_2$-30 and between $H_2$-26 and $H_3$-29 in the ROESY spectrum provided strong evidence for the *cis* configuration between H-27 and

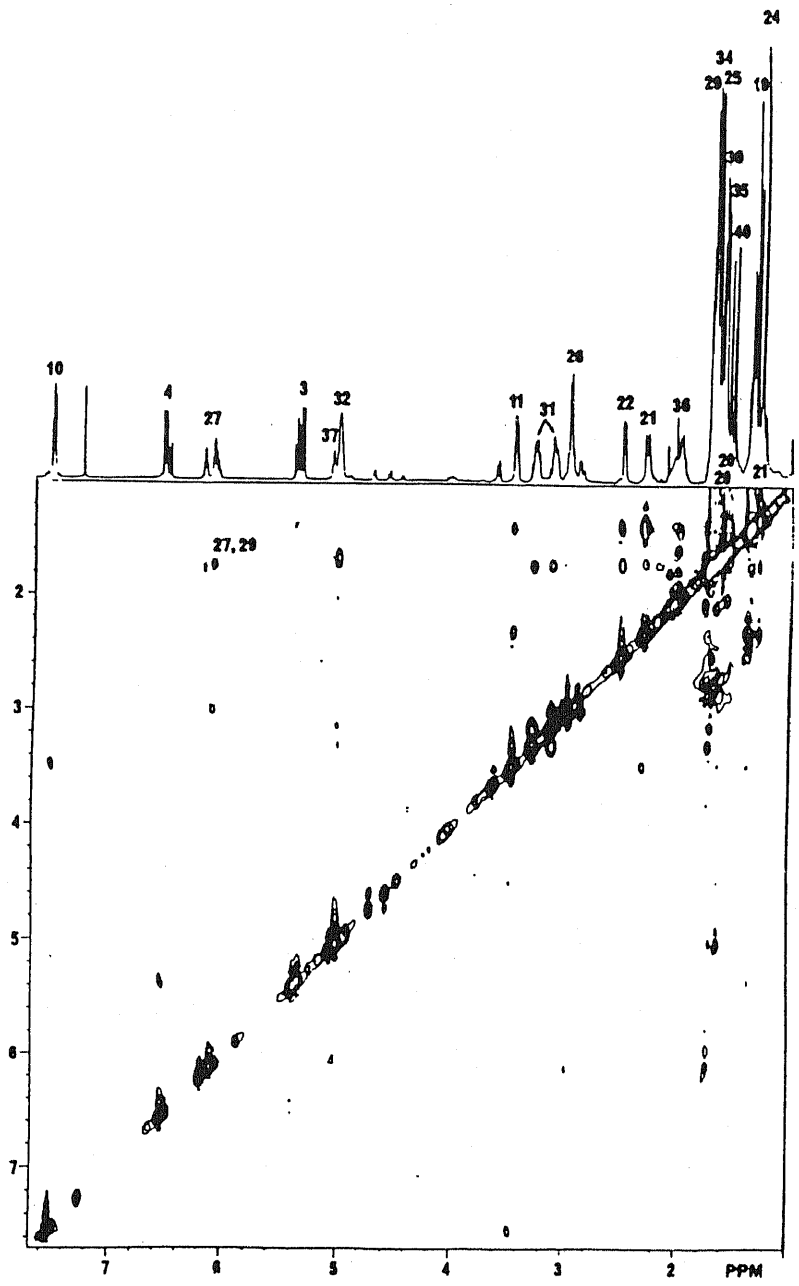



**Fig. 24** ROESY spectrum of gambogic acid (**93**).

$H_2$-30. Based on correlations shown in the HMBC spectrum, all the carbon resonances could be assigned unambiguously. The additional information provided through reverse correlation was of particular importance for the assignment of those methyl proton resonances in congested regions of the spectrum. For example, the correlation between $H_3$-24 and C-25 and between C-24 and $H_3$-25 permitted the recognition of these two *gem*-dimethyl groups, and the correlations of $H_3$-24 and $H_3$-25 with both C-22 and C-23 also allowed the location of these *gem*-dimethyl groups to be determined. In this way, the carbon network of isomorellinol (**95**) could be established and its structure completely assigned.

An interesting finding is that two pairs of *trans-cis*-isomers, morellin and isomerellin (335,337) and morellic acid and isomorellic acid (336,337) have been isolated from *G. morella*. However, only the *trans* isomer, morellinol, was isolated from the species (338). All these biosynthetically related compounds contain four isoprene units. In contrast, the major components found in *G. hanburyi*, such as gambogic acid (332,334,337) and neogambogic acid (339) are structurally more complicated, i.e. they have one more isoprene unit at C-20. The isolation of isogambogic acid from *G. hanburyi* may suggest that a *cis-trans* isomerase is also active in this species. The presence of isomorellinol in *G. handuryi* provided further evidence to demonstrate the closeness of these two species, which may have some sigmificance from the chemotaxonomic point of view.

Gambogic acid (**93**) exhibited the following physical and spectroscopic properties : $[\alpha]_D^{20}$-510° (*c* =0.1, $CHCl_3$); UV $\lambda_{max}$ (MeOH) (log ε) 290 (4.24), 360 (4.18) nm; IR $\upsilon_{max}$ (neat) 2974, 2934, 2874, 1738, 1692, 1645, 1634, 1593, 1456, 1435, 1402, 1383, 1333, 1260, 1177, 1138, 1047, 959, 885, 808, 758 $cm^{-1}$; $^1H$ NMR data, see Table 36; $^{13}C$ NMR data see Table 37; EIMS, *m/z* (relative intensity, %) $[M]^+$ 628 (20), 600 (10), 546 (34) 545 (100), 517 (23), 473 (12), 389 (8), 347 (8), 245 (14), 215 (28), 189 (14), 129 (12), 105 (15).

Isogambogic acid (**94**) exhibited the following physical and spectroscopic properties : $[\alpha]_D^{20}$-408° (*c* =0.1, $CHCl_3$); UV $\lambda_{max}$ (MeOH) (log ε) 290 (4.24), 359 (4.18) nm; IR $\upsilon_{max}$ (neat) 2969, 2926, 2888, 2859, 1738, 1690, 1645, 1634, 1592, 1456, 1435, 1402, 1385, 1333, 1264, 1175, 1138, 1049, 959, 884, 855, 810, 758 $cm^{-1}$; $^1H$ NMR data, see Table 36; $^{13}C$ NMR data, see Table 37; EIMS, *m/z* (relative intensity, %) $[M]^+$ 628 (36), 613 (5), 600 (5), 546 (35), 517 (15), 473 (8), 379 (9), 355 (6), 347 (6), 271 (4), 245 (8), 215 (15), 189 (6); HREIMS, *m/z* $[M]^+$ 628.3042 (calculated for $C_{38}H_{44}O_8$, 628.3036).

Isomorellinol (**95**) exhibited the following physical and spectroscopic properties : $[\alpha]_D^{20}$-332° (c=0.125, $CHCl_3$) ; UV $\lambda_{max}$ (MeOH)(log ε) 232 (4.21), 288 (4.20), 358 (4.09) nm; IR $\upsilon_{max}$ (neat) 3466, 2976, 2928, 2861, 1738, 1645, 1634, 1593, 1462, 1437, 1400, 1385, 1333, 1302, 1250, 1213, 1186, 1165, 1140, 1047, 961, 878, 812; $^1H$ NMR, see Table 36; $^{13}C$ NMR data, see Table 37; EIMS, *m/z* (relative intensity, %) $[M]^+$ 546 (100), 531 (25), 519 (20), 518 (66), 504 (23), 503 (66), 485 (16), 435 (19), 406 (34), 405 (53), 389 (23), 379 (19), 363 (37), 349 (29), 347 (20), 307 (21), 287 (49), 285 (25), 263 (16), 245 (20), 231 (51), 213 (20), 189 (14), 135 (17), 105 (21); HREIMS, *m/z* $[M]^+$ 546.2626 (calculated for $C_{33}H_{38}O_7$,546.2618).

### 2.1.8 Anthraquinones

Anthraquinones is a tricyclic benzene ring structure having diketone at C-9 and C-10 position (9, 10-anthracenedione). The anthraquinones are the largest group of natural quinones, nearly half of which have been found in higher plants and a similar mumber in fungi a well as in lichens.

#### i. *XYRIS INDICA* (343)

The dried flowering heads *X. indica* was extracted with chloroform and the components were separated by column chromatography as described in the Experimental section. The compounds will be discussed in the order in which they were eluted from the column. The first and major component was shown to be 1, 8-dihydroxy anthraquinone (chrysazin) **96**, by comparison of its m.p. and spectral data with the values reported in the literature (325) . We report the 500 MHz $^{1}H$ and $^{13}C$ NMR spectra of **96** in $CDCl_3$. The $^{13}C$-NMR spectrum of **96** exhibited only eight lines as would be expected if the component was symmetry.

The second component, isolated in a fair amount, was the 3-methoxy 1, 8-dihydroxy anthraquinone (3-methoxy chrysazin) **97**, which displayed the expected methoxy group in $^{1}H$-nmr spectrum. We report in the Experimental the 500 MHz $^{1}H$-nmr spectrum of **98** in $CDCl_3$ compared with those reported previously in $C_6D_6$. 3-Methoxychrysazin was first isolated from *X. semifuscata* as a new chrysazin derivative (325).

The third and minor component **98** gave positive Borntrager test, suggesting it was anthraquinone compound. Mass spectral analysis of the compounds **96-98** indicated a close structural relationship as all three exhibited-1, 8-dihydroxy anthraquinone skeleton (Fig. 25).

Preliminary examination of the spectrum in comparison with published proton NMR spectral data of compounds in this series indicated that **98** was a chrysazin derivative (325). The $^{1}H$-NMR spectrum of **98** exhibited two hydroxyl protons appeared as two singlets at $\delta$ 12.03 and 12.07 ppm. The downfield chemical shift of five aromatic methine proton were observed at $\delta$ 7.74, 7.65, 7.33, 7.11 and 6.58 ppm indicated protons at H-6, H-5, H-7, H-4 and H-2 respectively. The equivalent coupling constant indicated that H-6 coupled to H-5 and H-7 (see table 38). Two sets of doublets centered at $\delta$ 6.58 and 7.11 ppm represented the *meta* aromatic protons at the position 2 and 4 of ring C. This also indicated that the remaining aromatic proton at position 3 was substituted with hydroxyl group which corresponded to HREIMS. The moisture of

DMSO-$d_6$ as solvent caused the disappearing of 3-hydroxyl proton signal. The $^{13}C$-NMR of **98** provided 14 lines of carbon signals, also indicated that **98** has no carbon substituent. From the DEPT spectrum, it was shown that **98** consisted of 9 quaternary carbons (δ109.07, 115.60, 113.12, 135.13, 161.24, 164.53, 165.76, 181.29 and 190.07 ppm) and 5 methine carbons (δ107.94, 108.84, 119.29, 124.47, and 136.76 ppm). **98** was previously found to produce from the symbiosis of the bacterium *Xenorhabdus luminescens* and the insect pathogen nematode *Heterorhabdites* sp. by fermentation (344). This is the first reported of **98** occurring in higher plant. The $^{1}H$ and $^{13}C$ spectral data of **98** reported earlier were ambiguously assigned (344). In this present investigation, $^{13}C$-$^{1}H$ and $^{13}C$-$^{1}H$ long range COSY were undertaken to determine the complete assignment of **98**. From HMBC spectrum, the significant long range $^{1}H$-$^{13}C$ correlations contour were indicated by arrows in Fig. 26. The assignment of carbon and proton signals were summarized in Table 38. Thus, it was concluded the structure **98** to be 1, 3, 8-trihydroxy-9, 10-anthracenedione.

1,8-Dihydroxyanthraquinone (chrysazin) **96** obtained as orange plate, m.p.188°C [lit 191°C] ; UV, IR and MS the same as those reported previously (325); $^{1}H$ NMR (500 MHz, $CDCl_3$ ) : δ7.23 (2H, dd, *J* = 1.3, 7.8 Hz, H-2 and H-7), 7.62 (2H, dd, *J* = 1.3, 7.8 Hz, H-3 and H-6), 7.75 (2H, dd, *J* = 1.3, 7.8 Hz, H-4 and H-5) and 11.9 (bs, 2OH), $^{13}C$ NMR (12.65 MHz, $CDCl_3$ ) : δ 162.5 (C-1, C-8), 124.6 (C-2, C-7), 137.23 (C-3, C-6), 133.55 (C-8a, C-9a), 120.01 (C-4, C-5) and 115.81 (C-4a, C-10a)

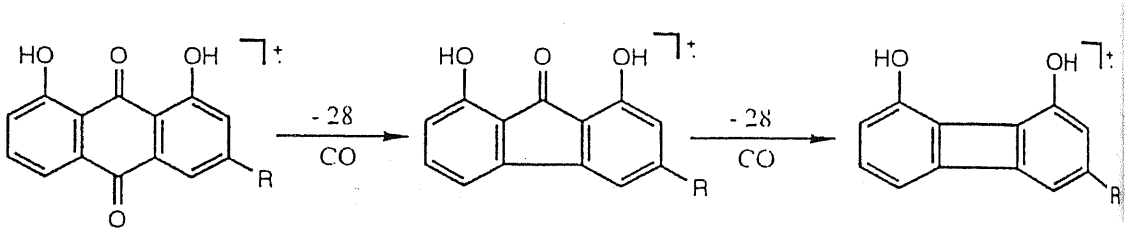
3-Methoxy-1, 8-dihydroxyanthraquinone (3-methoxychrysazin) **97** obtained as orange cluster, mp. 180°C [ lit 180-183°C ] ; UV, IR and MS the same as those reported previously (325) ; $^{1}H$ NMR (500 MHz, $CDCl_3$ ) : δ 3.87 (3H, s, $-OCH_3$), 6.61 (1H, d, *J* = 2.5 Hz, H-2) 7.21 (1H, dd, *J* = 1.2, 7.3 Hz, H-7), 7.30 (1H, d, *J* = 2.5 Hz, H-4), 7.56 (1H, dd, *J* =1.2, 7.3 Hz, H-6), 7.73 (1H, dd, *J* = 1.2, 7.3 Hz, H-5), 12.11 (1H, bs, 1-OH) and 12.19 (1H, bs, 8-OH)

1,3,8-Trihydroxyanthraquinone (3-hydroxy chrysazin) **98** obtained as orange plate ; m.p. 294-295°C ; UV λ max (MeOH) nm (log ε ) : 218.4(4.46), 244.8 (4.21), 265.6 (4.22), 248.5 (4.27) ; IR υ max (KBr) $cm^{-1}$ 3392, 1671, 1611, 1578, 1476, 1363, 1281, 1238, 1163, 758 ; $^{1}H$ and $^{13}C$ : see table 38; MS $[M]^+$ 256 (100%), 255 (2), 239 (4), 229 (3), 228 (20), 200 (14), 171 (7), 155 (2), 154 (4), 126 (4), 115 (60, 114 (4), 69 (6), 63 (4), 57 (4)

**Table 38** $^{1}H$ and $^{13}C$ NMR assignments of **98** (DMSO-$d_6$ ; 500 and 125.65 MHz ; TMS)

| Atom | $^{13}C$ | $^{1}H$ |
|---|---|---|
| 1 | 164.53 | 12.03 bs (OH) |
| 2 | 107.94 | 6.58 d (2.5) |
| 3 | 165.76 | |
| 4 | 108.84 | 7.11 d (2.5) |
| 4a | 109.07 | |
| 5 | 119.29 | 7.65 dd (8.5, 1.2) |
| 6 | 136.76 | 7.74 dd (8.5, 1.2) |
| 7 | 124.47 | 7.33 dd (8.5, 1.2) |
| 8 | 161.24 | 12.07 bs (OH) |
| 8a | 115.60 | |
| 9 | 190.01 | |
| 9a | 135.13 | |
| 10 | 181.29 | |
| 10a | 133.12 | |

δ (ppm) Coupling constants (*J* in Hz) in parentheses. All carbons were assigned with the aid of $^{13}C$-$^{1}H$ and $^{13}C$-$^{1}H$ long range COSY




| | | m.w. | *m/z* | *m/z* |
|---|---|---|---|---|
| **96** | R = H | 240 | 212 | 184 |
| **97** | R = $OCH_3$ | 270 | 242 | 214 |
| **98** | R = OH | 256 | 228 | 200 |

**Fig. 25** Mass fragmentation of **96**, **97** and **98**.

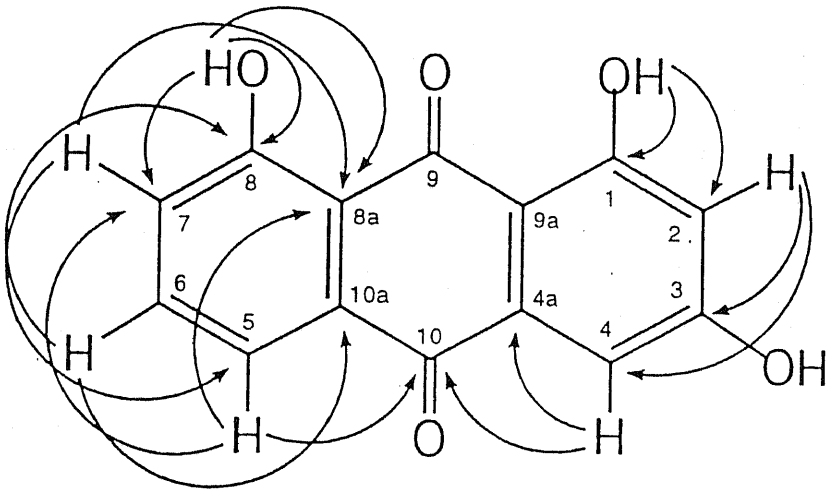



**Fig. 26** The C-H correlations observed in HMBC spectrum of **98**.

# PART III

# BIOLOGICAL ACTIVITIES OF SOME ISOLATED COMPOUNDS

About 30 years ago modern chemical and biological methods were first used to investigate traditional medicine materials in Thailand. With the growth in our knowledge about chemistry, biochemistry, physiology, and phamaceutics and progress made in scientific instrumentation, there have been an increasing number of scientific reports characterizing the biological activities of the chemical constituents of Thai medicinal materials. The present biological evaluation of the isolates from Thai medicinal plants provides information on recent advances and perspectives for future research into thai medicinal materials. This information may be of value for the development of new drugs and may stimulate further investigations.

Some plant isolates were subjected to the following biological investigations.

## BIOLOGICAL ACTIVITY

## 1. CYTOTOXIC ACTIVITY :

**Evaluation of the Cytotoxic Potential of Compounds Isolated from *Clitoria macrophylla* (285)**

| Compound tested | Cell line tested ($ED_{50}$ µg/ml) | | | |
|---|---|---|---|---|
| | | | KB-V1 | |
| | P-388 | KB | -VLB | +VLB |
| 6-Deoxyclitoriacetal (**81**) | 0.031 | 30 | 19 | 6.1 |
| Rotenone (**82**) | 0.011 | 0.9 | 6.9 | 3.8 |

**Evaluation of the Cytotoxic Potential of Compounds Isolated from *Garcinia hanburyi* (329)**

| | Cytotoxic Activity $ED_{50}$ (µg/ml) | |
|---|---|---|
| | KB-3 | KB-VI |
| Gambogic acid (**93**) | 0.7 | 2.3 |
| Isogambogic acid (**94**) | 0.9 | 3.0 |
| Isomorellinol (**95**) | 2.3 | 3.1 |

**Evaluation of the Cytotoxic Potential of Compounds Isolated from *Cyclea barbata.*** (114)

| | Cytotoxic Activity $ED_{50}$ (μg/ml) | | |
|---|---|---|---|
| | P-388 | KB-3 | KB-VI |
| Extract | 1.5 | 3.6 | 12 |
| Tetrandrine (**24**) | 0.40 | 2.1 | 3.7 |
| Limacine (**25**) | 0.25 | 9.8 | 11 |
| Thalrugosine (**26**) | 0.36 | 3.4 | 11 |
| Homoaromaline (**27**) | 0.31 | 3.6 | 15 |
| Cycleapeltine (**28**) | 0.57 | 2.2 | 4.4 |

**Cytotoxic Evaluation of Isolated Compounds 1,2,8,9,80,84** (1,31,273,291)

| Compound tested | Cell line tested ($ED_{50}$, μg/ml) | |
|---|---|---|
| | P-388 | KB |
| Frullanolide (**1**) | - | 0.4 |
| 7α-Hydroxyfrullanolide (**2**) | - | 0.35 |
| Parthenolide (**8**) | - | 0.45 |
| Bisparthenolidine (**9**) | - | 0.6 |
| Wrightiadione (**80**) | 1.1 | - |
| Microminutin (**84**) | 3.7 | - |

## 2. ANTIMALARIAL ACTIVITY

**Evaluation of the Antimalarial Activity of the Alkaloid Extract and Compounds isolated from *Cyclea barbata*** (114)

| Compound | Antimalarial activity, $ED_{50}$ (ng/ml) | |
|---|---|---|
| | Strain D-6 | Strain W-2 |
| Alkaloid Extract | 163 | 224 |
| Tetrandrine [**25**] | 179 | 160 |
| Limacine [**26**] | 52.7 | 164 |
| Thalrugosine [**27**] | 65.1 | 78.0 |
| Homoaromoline [**28**] | 232 | 451 |
| Cycleapeltine [**29**] | 29.0 | 40.6 |
| Quinine | 7.6 | 30.8 |

## 3. ANTIMICROBIAL ACTIVITY :

**Minimal Inhibitory Concentration (MIC μg/ml) of compounds (96, 97 and 98) isolated from *Xyris indica* againt various microorganisms (343)**

| Microorganism | 96 | 97 | 98 | Miconazole |
|---|---|---|---|---|
| *Bacillus subtilis* ATTCC6633 | >100 | >100 | 12.5 | 1.56 |
| *Staphylococcus aureus* FDA209P | >100 | >100 | 6.25 | 1.56 |
| *Escherichia coli* NIHJ | >100 | >100 | >100 | 50 |
| *Pseudomonas aeruginosa* NCTC1490 | >100 | >100 | >100 | 50 |
| *Candida albicans* ATCC48130 | >100 | >100 | >100 | <0.2 |
| *Saccharomyces cerevisiae* ATCC18824 | 3.13 | >100 | 3.13 | <0.2 |
| *Aspergillus niger* F828 | >100 | >100 | >100 | 1.56 |
| *Rhizopus chinensis* IF04747 | >100 | >100 | >100 | 25 |
| *Trichophyton mentagrophytes* TIMM1189 | >100 | >100 | 0.78 | 0.78 |
| *T. rubrum* TIMM2659 | 1.56 | >100 | 0.78 | <0.2 |

**Antimicrobial Activities of Isoflavonoids Isolated from *Dalbergia candenatensis*** (261)

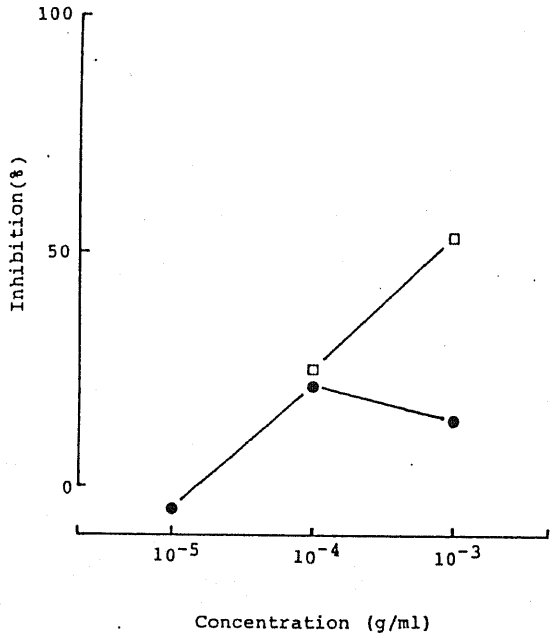
| Microorganism | Compound | | | | | | |
|---|---|---|---|---|---|---|---|
| | mucronulatol | clausse-quinone [78] | 5-hydroxy-bowdichioe [79] | formo-nonetin | vestitol | tetra-cycline | nystatin |
| *Staphylococcus aureus* ATCC 25923 | >100[a] | 6 | >100 | >100 | >100 | 0.6 | |
| *Bacillus subtilis* ATCC 6633 | >100 | 3 | 100 | >100 | 50 | 2.5 | |
| *Escherichia coli* ATCC 25922 | 50 | 6 | 50 | 100 | 50 | 1.2 | |
| *Candida albicans* ATCC 10259 | >100 | 50 | >100 | >100 | >100 | | 1.5 |
| *Penicillium expansum* ATCC 7861 | >100 | >100 | >100 | 50 | >100 | | 6.0 |
| *Aspergillus niger* ATCC 6275 | >100 | 100 | >100 | 100 | >100 | | 6.0 |
| *Trichophyton mentagrophytes* ATCC 9533 | 25 | 25 | 100 | >100 | 25 | | 3.0 |

[a]Minimum inhibitory concentration (μg/ml).

## 4. ANTI-INFLAMMATORY ACTIVITY :

**Biological activity of Entadamide-A (17) and -B (18)** (75,76)

Entadamide-A (**17**) and -B (**18**) showed marked inhibitory effects on the 5-lipoxygenase activity of RBL-1 cells. The inhibition % values were 25.1 and 53.5% at $10^{-4}$ and $10^{-3}$ g/ml of **18** and 21.5 and 14.5% at $10^{-4}$ and $10^{-3}$ g/ml of **17**, respectively. These results indicated that **18** was more effective than **17** as an inhibitor on the 5-lipoxygenase activity. The present findings suggest that Entadamide-A (**17**) or -B (**18**) can be used to treat the inflammatory diseases such as a bronchial asthma.




Effects of Entadamide-A and Entadamide-B on 5-lipoxygenase activities.
-●-●- : Entadamide-A, -❑-❑- : Entadamide-B.

## 5. SPASMOLYTIC ACITIVITY :

### Spasmolytic Activity of Ancistrotectorine (37) Isolated from *Ancistrocladus tectorius.*

The various pharmacological effects in mice, rat, guinea-pig and rabbit were studied both in normal and anesthetized animals and also in isolated organ preparations. Ancistrotectorine $1x10^{-5}$ to $5x10^{-5}$ molar reduced not only spontaneous contraction but also the contraction induced by acetylcholine, histamine, serotonin, barium, potassium and calcium chloride, also by electrical stimulation, in rabbit's jejunum and guinea-pig's ileum. Reduction of both the whole isolated mice stomach and rat-stomach strip induced by acetylcholine, carbachol, serotonin, barium and calcium chloride. Intravenous administration to conscious mice (50 mg/kg) suppressed the propulsive movement of the stomach and small intestine and showed antagonism to spasmogenic activity of carbachol in mice and also in anesthetized rabbits (1-1.5 mg/kg). The reduction of rat and guinea-pig uterine contraction in both spontaneous and contraction induced by oxytocin were also observed. In rat vas deferens, ancistrotectorine reduced, in dose-related manner, both phasic and tonic contraction induced by various agonists and also by electrical stimulation. Ancistrotectorine could relax the isolated rat thoracic aortic strip induced contraction by cumulative dose of 1-phenylephrine and also showed dose-dependent inhibition of the contraction of rabbit aortic strip evoked by calcium chloride in the high potassium chloride solution. Ancistrotectorine showed less inhibition the histamine induced contraction of guinea-pig trachea. The results of the present study clearly indicated that Ancistrotectorine has a spasmolytic effect on a wide variety of smooth muscle preparations from various species. These non-specific spasmolytic activities may suggest a papaverine-or calcium antagonist-like action of Ancistrotectorine.(352)

# PART IV

# EXPERIMENTAL

## SOURCE OF PLANT MATERIAL :

The plant materials were collected from various localities in Thailand and period of times as the following :

| Plant | Part | Province | Duration |
|---|---|---|---|
| *Grangea maderaspatana* | W.P. | Suphan Buri | October, 1986 |
| *Eupatorium adenophorum* | W.P. | Chiang Mai | May, 1987 |
| *Paramichelia baillonii* | S.B. | Chiang Mai | July, 1985 |
| *M. rajaniana* | S.B. | Chiang Mai | August, 1985 |
| *Pluchea indica* | W.P. | Nakhon Pathom | May, 1981 |
| *Entada phaseoloides* | S | Chiang Mai | June, 1983 |
| *Piper sarmentosum* | Fr | Kanchanaburi | February, 1986 |
| *Cyclea barbata* | R | Sukhothai | March, 1989 |
| *Parabaena sagittata* | L | Chiang Mai | June, 1983 |
| *Ancistrocladus tectorius* | L | Nakhon Ratchasima | October, 1982 |
| *Kopsia jasminiflora* | L | Chiang Mai | December, 1985 |
| *Murraya siamensis* | R | Sukhothai | October, 1988 |
| *Sophora exigua* | R | Nakhon Ratchasima | June, 1989 |
| *Blumea balsamifera* | L | Chiang Mai | July, 1980 |
| *Dalbergia candenatensis* | W | Chiang Mai | April, 1984 |
| *Wrightia tomentosa* | S.B. | Nakhon Pathom | September, 1990 |
| *Clitoria macrophylla* | R | Rayong | May, 1991 |
| *Micromelum minutum* | L | Saraburi | May, 1980 |
| *Alyxia reinwartii* | S.B. | Nakhon Pathom | September, 1990 |
| *Xyris indica* | Fl | Prachin Buri | November, 1991 |
| *Garcinia hanburyi* | La | Chanthaburi | March, 1990 |

W.P. = whole plant, S.B. = stem bark, S = seed, Fr = fruit, T = tuber, L = leaf, R = root, W = wood, Fl = flower, La = latex

Authentication was achieved by comparison with the herbariam specimens at Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand. The voucher specimen of plant materials have been

deposited at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

**INSTRUMENTATION :** Melting points were determined on a Kofler hot plate or a Yanaco melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Beckman DU-7 spectrometer or a Hitachi U-3400 spectrometer, and the IR spectra were measured on a Nicolet MX-1 FT-IR (KBr) interferometer or a Hitachi 260-10 IR spectrometer. $^{1}$H-NMR, homonuclear COSY, $^{13}$C-NMR, DEPT, and HETCOR spectra were recorded in $CDCl_3$ with TMS as internal standard, employing a Varian XL-300 instrument or a JEOL alpha FT nmr spectrometer (500 MHz). The MS were obtained with a Varian MAT 112S instrument or a Hitachi M-60 mass spectrometer (using a direct inlet system) operating at 70 eV.

**BIOASSAY :**

**ASSAYS OF ANTIMALARIAL ACTIVITY** :- Cultures of *P. falciparum* (chloroquine-sensitive strain D-6 derived from CDC Sierra Leone, and chloroquine-resistant strain W-2 derived from CDC Indochina III) were maintained in human erythrocytes in vitro according to established methods (345). Parasites were inoculated into type A + human erythrocytes at a hematocrit of 6% in RPMI-1640 culture medium (GIBCO Laboratories, Grand Island, NY) supplemented with 32 mM $NaHCO_3$ (GIBCO), 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, Sigma Chemical Co., St. Louis, MO), and 10% heat-inactivated human plasma type A$^+$. Parasitemia was maintained below 4% under an atmosphere of 5% $O_2$, 5% $CO_2$, and 90% $N_2$ in 25-cm$^2$ culture flasks at 37°.

The antimalarial activity of test compounds was assessed with an in vitro radioisotope-incorporation method (346,347). In brief, a suspension (200 µl) of *P. falciparum*- infected red blood cells (0.5-1.0%) parasitemia, 1.0% cell hematocrit) was added to wells of a standard 96-well tissue culture plate containing 25 µl of drug to be tested. Each test compound was assayed in duplicate over a concentration range of 10,000-14 ng/ml. In addition, the known antimalarial drugs quinine, chloroquine, mefloquine, and artemisinin were tested in each experiment over a range of 250-0.3 ng/ml. Microtiter plates were incubated for 24 h at 37° in a sealed chamber under an atmosphere of 5% $CO_2$, 5% $O_2$, and 90% $N_2$. After this incubation period, 0.5 µCi of [$^3$H(G)] hypoxanthine (New England Nuclear Research Products, Boston, MA) was added to each well (25 µl of 20 µCi/ml), and the microtiter plate was returned to the sealed chamber at 37° for an additional 18 h incubation. The assay was terminated by harvesting the contents of each microtiter plate onto a glass fiber filter using a Skatron

model 11021 semi-automatic cell harvester. Filters were dried, and the radioactivity from individual wells was excised from the filter and placed in 4-ml vials with toluene-based scintillation cocktail. Radioactivity was determined with a Beckman LS 5801 liquid scintillation counter. Concentrations of both test compounds and positive controls that inhibited parasite-specific incorporation of [$^3$H] hypoxanthine by 50% ($IC_{50}$) were determined by non-linear regression analysis. Zero-drug controls defined 100% incorporation.

**CELL LINES FOR CYTOTOXICITY ASSAYS** :- Human breast cancer (UISO-BCA-1), colon cancer (UISO-COL-1), lung cancer (UISO-LUC-1), and melanoma (UISO-MEL-2) cell lines were established from primary human tumors in the Specialized Cancer Center, University of Illinois College of Medicine at Chicago. Each of these cell lines has been shown to grow after as injection of 1-4 x $10^6$ cultured cells in male or female (breast cancer only) athymic mice, and diagnosis of the original patient specimen was consistent with analysis of the cultured cells by electron microscopy and analysis of nude mouse tumors by light microscopy. Each cell type has also been found to contain human isozymes when analyzed using the Corning Authentic Kit electrophoresis system. Fibrosarcoma (HT-1080) cells were purchased from the American Type Culture Collection (Rockville, MD), as were P-388 cells. KB-3 and a multidrug-resistant cell line, KB-Vl, which was established by treating KB-3 cells with a chronic sublethal dose of vinblastine, were supplied by Dr. I.B. Roninson (Department of Genetics, University of Illinois College of Medicine at Chicago, Chicago, IL), and A-431 (human squamous cell carcinoma), LNCaP (human prostatic cancer), and ZR-75-1 (human breast cancer) cell lines were supplied through the courtesy of Dr. R.M. Tait, Glaxo Group Research, Greenford, UK. UISO-BCA-1, and UISO-COL-2 were cultured in Eagle's minimal essential medium with Eagle's salts ( MEME) (GIBCO Laboratories, Grand Island, NY) supplemented with 15% fetal bovine serum (FBS) (Biofluids, Rockville, MD), 100 units/ml penicillin G, 100 µg/ml streptomycin sulfate, 0.25 µg/ml amphotericin B (Fungizone) (PSF) (GIBCO), and 1% nonessential amino acids (NAA) (sigma); HT-1080 and UISO-LUC-1 were maintained in MEME (GIBCO) supplemented with 10% FBS, PSF, and 1% NAA; KB-3 and A-431 were cultured in Dulbecco's modified minimum essential medium (D-MEM) (GIBCO) supplemented with 10% FBS and PSF; KB -V1 was cultured in D-MEM (GIBCO) supplemented with 10% FBS, PSF and 1 µg/ml vinblastine (Sigma); UISO-MEL-2 was grown in minimal essential medium with Hanks' salts (MEMH) (GIBCO) supplemented with 10% FBS and PSF. ZR-75-1 and LNCaP were grown in RPMI 1640 media (phenol red-free) supplemented with 10% FBS (hormone-free) and PSF. The medium for ZR-75-1 cells was further supplemented with 0.1 nM estradiol, and the medium for LNCaP cells was also supplemented with 0.1

nM testosterone. P-388 cells were cultured in Fisher medium containing 10% FBS and PSF. All cell lines were cultured at 37° in 100% humidity with a 5% $CO_2$ atmosphere in air, except the melanoma cell line, which was kept at 37° in closed tissue culture flasks.

EVALUATION OF CYTOTOXIC POTENTIAL :- Cell were typically grown to 60% -70% confluence; the medium was then changed and the cells were used for test procedures one day later. In each case, 96-well tissue culture plates were used. Test samples were initially dissolved in DMSO and the diluted 10-fold with $H_2O$. Serial dilutions were performed using 10% aqueous DMSO as the solvent, and 10 µl were added to the wells. In general, five concentrations were rested (in triplicate), and preliminary studies were conducted to determine test concentrations that were above and below the $ED_{50}$ of the samples. Control groups were also added in which 10 µl of 10% DMSO were added to wells. After the plates were prepared, cells were removed from the tissue culture flasks by treatment with trypsin, enumerated, and diluted with fresh media. Various quantities of cells (in 190 µl of media) were then added to the 96-well plates, and incubations were performed for various periods of time, as follows [cell number ; incubation time (d)] : UISO-BCA-1 ($10 \times 10^4$ ; 3), UISO-COL-1 ($6.5 \times 10^4$ ; 3), UISO-LUC-1 ($5 \times 10^4$ ; 3), UISO-MEL-2 ($10 \times 10^4$ ; 3), HT-1080 ($5 \times 10^4$ ; 3), KB-3 ($5 \times 10^4$ ; 3), KB-VI ($6 \times 10^4$ ; 3), A-431 ($6 \times 10^4$ ; 3), LNCaP ($6.5 \times 10^4$ ; 3), and ZR-75-1 ($7 \times 10^4$ ; 3). All incubations were performed at 37° in a $CO_2$ incubator with the plates capped in the normal fashion, except for UISO-MEL-2, for which the plates were sealed with Parafilm.

After the incubation period, cells were fixed to the plastic substratum by the addition of 50 µl of cold 50% aqueous trichloroacetic acid. The plates were incubated at 4° for 1 h, washed with tap $H_2O$ (4 x), and air-dried. The trichloroacetic-acid-fixed cells were stained by the addition of 0.4% sulforhodamine B (w/v) dissolved in 1% HOAc (30 min). Free sulforhodamine B solution was then removed by washing with 1% aqueous HOAc (4 x). The plates were then air-dried, and the bound dye was solubilized by the addition of 10 mM unbuffered Tris base, pH 10 (200 µl). The plates were placed on a shaker for 5 min, and the absorption was determined at 515 nm using an ELISA plate reader. In each case, a zero-day control was performed by adding an equivalent number of cells to several wells of the 96-well plates and incubating at 37° for a period of 10 min. The cells were then fixed with trichloroacetic acid and processed as described above.

In a procedure similar to that described above for cells capable of attaching to the surface of the culture dishes, P-388 cells were enuerated and diluted to a concentration of $10 \times 10^4$ cells/ml, 190 µl were added to the wells containing the test substances, and the

incubation was performed at 37° in a $CO_2$ incubator for 2 days. After the incubation period, the plates were centrifuged (10 min, 2000 rpm) and the supernatant fractions were carefully removed. The cells were treated with 100 µl of 20% aqueous trichloroacetic acid (4°) and incubate at 4° for 1 h. The cells were rinsed with $H_2O$ (4 x), dried, and treated with sulforhodamine B as described above.

Finally, the absorption values obtained with each of the treatment procedures were averaged, and the average value obtained with the zero day control was subtracted. These values were then expressed as a percentage, relative to the solvent-treated control incubations, and $ED_{50}$ values were calculated using non-linear regression analysis (percent survival versus concentration). These experimental conditions were established in preliminary studies wherein it was shown (a) there is at least a 7-fold increase in cell number relative to the amount of cells added to the plates at time zero, (b) the resulting absorption values were in a range to assure reading accuracy (i.e., <1.4 $A_{515}$ units), and (c) the cell number attained during the incubation period did not reach a plateau region on the growth curve.

**ASSAY OF ANTIINFLAMMATORY ACTIVITY** :- The assays werre carried out based on the methods of Jakschik and Lee (349) and Steinhoff *et al.* (350). RBL-1 cells were grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum and antibiotic-antimycotic mixture. The harvested cells were washed once with phosphate-buffered saline, suspended in 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA, and sonicated 4 sec four times at 5 sec intervals. The resulting homogenate was centrifuged at 105,000 g for 1 hr to yield supernatant fraction (cytosol) for assaying arachidonate 5-lipoxygenase.

The assay mixtures contained (1-$^{14}$C) arachidonic acid (0.1 µ Ci, 16.8 nmol), 1 mM $CaCl_2$ and cytosolic fraction in 0.5 ml of 50 mM Tris-HCl buffer, pH 7.4 with or without test compounds (EtOH concentration : 2%). The mixtures were incubated at 37°C for 10 min with shaking and then terminated by the addition of 1.9 ml of $CHCl_3$/MeOH (1:2, v/v). The substrate and metabolites were extracted by the method of Bligh and Dyer ( ) to avoid lactone formation. Lipid extract was then chromatographed on silica gel $60F_{254}$ aluminum plates along with standards using petroleum ether-$Et_2O$-HOAc (45 : 55 : 1, v/v) as a solvent. Labelled products and substrate were localized by an Aloka radio-chromatoscanner (TLC-101). Radioactive peaks were scraped off and finally counted in a Beckman LS 5800 liquid scintillation counter.

**ASSAY OF ANTIMICROBIAL ACTIVITY** :- MIC of the isolates were determined by micro broth dilution method with 96-well flat bottom microplate (Nunc)

(351). To prepare the inoculum, tested organisms were prepared by suspending in buffered saline with 0.1% Tween 80. Spores of *Aspergillus* and *Rhizopus* were collected from a culture grown at 35°C for a week on Potato Dextrose agar (Difco) slant. For *Trichophyton*, conidia formed after cultivation at 27°C for 2 week on Malt agar (Difco) slant. Yeasts were grown at 35°C for 2 days in Sabouraud Dextrose broth (BBL), other bacteria were cultivated in Mueller Hinton broth (Difco) at 35°C for 20 hours. Test compounds were initially dissolved in dimethyl sulfoxide (DMSO) and prepared serial 2-fold dilution. Unless otherwise stated, each well contained appropriate test substance, Mueller Hinton broth (for bacteria) or Sabouraud Dextrose broth (for fungi) and approximately 2000 cfu in a total volume of 200 µl. MICs were determined by the observing the turbidity of each well with EIA reader (Bio-Rad) at 630 nm. After shaking the plates for a few seconds on a microplate shaker, the absorbance of the wells were measured and after appropriate incubation periods (20 hours for bacteria at 35°C, 7 days for fungi at 25°C) a second absorbance measurement was carried out.

*Trichophyton* and *Saccharomyces* were grown as described above. Twenty ml of Sabouraud Dextrose agar was poured into petri dishes (diameter 9 cm). After the medium had solidified, 100 µl of spores or mycerial fragments suspension ($10^{7-8}$ CFU/ml) were spread on the agar surface and sterile 8-mm disks (Toyo) containing 30 µl of test compounds solution (1000, 100, 10, 1 µl/ml) were added. Plates were incubated at 25°C for 5 days.

**ASSAY OF SPASMOLYTIC ACTIVITY :-** J.R. Vane firstly used the rat fundus strip for testing antiserotonic activity in 1959. We have merrily used this organ strip for testing atropine-like action and papaverine-like action. This strip had a characteristic in a point that an agonist could be affected through the wide range of doses as compared to other organ preparations.

For making organ preparation, a rat is killed by a blow on the head and the abdomen is opened. The fundal part of the somach can easily be identified because it is grey, whereas the pyloric part is pink. It is cut away, opened out longitudinally, placed in a dish containing Kreb's solution and made into a strip about 4 or 5 cm long by suitable transverse cut. A thread is attached at each end and the preparation is mounted in Kreb's solution at 37°, aerated with a mixture of oxygen (95%) and carbon dioxide (5%). The organ bath should be relatively long (8-10 cm) but can be narrow, so its volume can still be quite small (5-10 ml). One end of the strip is attached to fixed pin in the bath and the other end to a transducer. The preparation should be left for 30 min. to stretch and a weight of 0.5 g as resting tension should be given.

The spasmolytic activity of a test material is examined in various isolated organs, for instance : small intestine and uterus.

This assay is a specific and non-specific inhibition test aimed at identifying a material that exert spasmolysis in the smooth muscle.

## EXTRACTION AND ISOLATION OF COMPOUNDS.

### Isolation of compounds 1-3 from *Grangea maderaspatana*

Dried, powdered plant material (2 kg) from *G. maderaspatana* was macerated twice with EtOH (2 x 5 liters) for 3-day periods and then filtered. The combined filtrate was evaporated *in vacuo* to give a syrupy mass (250 g). This residue was suspended in $H_2O$ (150 ml), extracted with $CHCl_3$ (6 x 300 ml), and the combined extract was dried (anhydrous $Na_2SO_4$) and the solvent removed to yield 45 g of a crude $CHCl_3$ extract. A portion of this extract (3 g) was chromatographed on a Si gel column (5 x 5 cm) using $CHCl_3$-$Me_2CO$ (5:1) as eluent and collecting 20-ml fractions. Fractions 3-4 gave 24 mg of **1**, fractions 7-11 afforded 155 mg of **2**, and fractions 21-28 yielded 117 mg of **3**.

### Isolation of compound 4 from *Eupatorium adenophorum*

The powdered dried plant material (2.3 kg) was extracted with 95% EtOH (20 L), and the combined extracts were evaporated *in vacuo*. The residue was distributed between $H_2O$ (5 L) and petrol (3 x 2 L), and the organic layer was dried and evaporated to a residue (40 g), which was chromatographed on Si gel column, eluting first with benzene and later with benzene containing increasing amounts of acetone to 2, 4, 5, 10, and 15%, respectively. The fractions were evaporated, examined by TLC, and purified further through prep. TLC to yield eupatorenone (**4**) (120 mg, 0.003%).

### Isolation of compounds 7-12 from *Paramichelia baillonii*

The fresh bark of *P. baillonii* (3 kg) was blended with 95% EtOH, macerated twice over a period of 3 days (10 and 5 liters), and then filtered. The filtrate was concentrated under reduced pressure to give a residue which was treated with $H_2O$ (5 liters), followed by extraction with $CHCl_3$ (3 times, 2 liters). The combined organic extracts were dried (anhydrous $Na_2SO_4$) and removal of the solvent gave a residue (8.59 g), which was chromatographed on a Si gel column (8 x 15 cm). The products were eluted with a 70% EtOAc/petrol solvent system and 25 ml fractions were collected. Fractions 67-126 afforded a crude mixture (0.52 g), which was further purified using the following sequence of solvents and collecting 25-ml fractions : (a) 50% $CHCl_3$/petrol, 25 fractions, (b) $CHCl_3$, 10 fractions, and (c) $CHCl_3$-$Me_2CO$ (8:2), 15 fractions.

Fractions. Fractions 11-12 gave 85 mg of **8**, fractions 28-36 gave 136 mg of **9**, and fractions 49-50 gave 97 mg of a mixture.

After increasing the polarity of the solvent in the large 8 x 15 cm column to 100% EtOAc, fractions 152-186 afforded a crude yellow residue (0.89 g) which was further purified by chromatography with $CHCl_3$ to give a yellow powder (92 mg). Purification of a portion of this powder (13 mg) by flash chromatography (2% MeOH/$C_6H_6$) gave **7** (3.6 mg) followed by liriodenine (8.6 mg). The large Si gel column was finally eluted with MeOH to give a residue (6.64 g) which was not investigated.

Fractions 49 and 50 from this latter purification gave 97 mg of an oil which was shown by $^1H$ nmr to be a 2:1:1 mixture of components **10**, **11**, and **12**, respectively. This mixture (50 mg) was separated by flash chromatography using the solvent system MeOH-EtOAc-petroleum ether (10:20:70) to yield 20 mg of **10** and 25 mg of a mixture of **11** and **12**. The latter mixture was separated by flash chromatography using the same solvent system to give 5 mg of **11** and 3 mg of **12** plus several fractions that were still mixtures of **11** and **12** ($R_f$ 0.22 and 0.20, respectively).

### Isolation of compounds 9-14 from *Michelia rajaniana*

The fresh bark of *M. rajaniana* (3 kg) was macerated twice with 95% EtOH (10 and 15 liters) over a 3-day period, and the suspensions were filtered. The combined filtrates were evaporated under reduced pressure, $H_2O$ (5 liters) was added to the residue, and the mixture was extracted with $CHCl_3$ (8 x 500 ml). The combined extracts were dried (anhydrous $Na_2SO_4$) and the solvent removed to yield 12.5 g of a syrupy residue. Purification of the residue was effected using Si gel chromatography with $C_6H_6$-EtOAc (1:1) as eluent and collecting 25-ml fractions. Fractions 1-13, 17-39, and 41-53 afforded, after evaporation, residues A, B, and C, respectively. Residue A was rechromatographed on Si gel using $C_6H_6$-$Me_2CO$ (6:1) to provide 138 mg of parthenolide and 79 mg of **9**. Similarly, residue B with $CHCl_3$-$Me_2CO$ (6:2) gave 84 mg of **11**, 80 mg of **13**, and 126 mg of a fraction which was found by $^1H$ nmr to be a mixture of at least three components and is still under investigation. Purification of residue C with $CHCl_3$ solvent yielded 344 mg of **14** and 150 mg of liriodenine.

### Isolation of compound 15 from *Pluchea indica*

The powdered fresh leaves of *P. indica* (50 kg) were macerated twice, for two-day periods, with 95% ethanol (70 and 50 liters). After combination, the extracts were

evaporated *in vacuo*, and the residue was suspended in warm 10% ethanol (10 liters) and filtered. The filtrate was treated with a 5% aqueous lead acetate solution until no further precipitation occurred. The final solution was extracted with chloroform (8 x 500 ml), and the combined chloroform extracts were dried over $Na_2SO_4$, and evaporated *in vacuo* to afford a yellow, syrupy mass (5.5 g).

The chloroform-soluble fraction (5.5 g) was divided into 11 portions, and each portion was dissolved in chlorofrom (2 ml), mixed with a small amount of silica gel, and dried. The residue was placed on a dry, silica gel column (2.5 x 40 cm) and eluted with ether to obtain 15-ml fractions. Fractions 11-14 were homogeneous by TLC and afforded colorless prisms (110 mg) of **15** on standing.

### Isolation of compounds 17-18 from *Entada phaseoloides*

A 75% aqueous ethanol extract of the air-dried powdered seed kernels of *Entada phaseoloides* (750 g) was concentrated *in vacuo*, saturated with anhydrous $K_2CO_3$ and then extracted with $CH_2Cl_2$. The basic fraction (806 mg, 0.12% of dry wt) obtained was subjected to Si-gel CC (Merck, type 60, 70-230 mesh, 3 x 20 cm) using $Et_2O$-MeOH-28% $NH_4OH$ (70:10:1, v/v) and $CH_2Cl_2$-MeOH (8:1, v/v) as eluting solvents and 5 ml fractions were collected monitoring by UV-detection at 254 nm. The **17**-rich fraction was further subjected to preparative TLC on Si-gel $60GF_{254}$ (0.5 mm thick, Merck) and developed with the same solvents to yield a colorless sirup of **17** (186 mg, 0.025% of dry wt). **17** did not react with Dragendorff's reagent, but formed a violet-grey color with Iodoplatinate reagent. **18** was obtained as colorless syrup in a 0.004% yield of the dry plant material and behaved like **17** with chromogenic reagents.

### Isolation of compounds 19-23 from *Piper sarmentosum*

The dried, powdered fruit from *P. sarmentosum* (450 g) was extracted with 2 L of petroleum ether (40-60°C) for 8 h using a soxhlet apparatus. Removal of the solvent *in vacuo* yielded 21 g of a dark brown syrupy mass, of which 3 g was chromatographed on silica gel. The components were eluted using a solvent gradient of chloroform up to 50% MeOH/$CHCl_3$, to afford 19 fractions (25 ml each) which were examined by TLC. Portions containing components of similar polarity were combined as follows : fractions 2-5 were designated as residue A (1.5 g), 9-15 residue B (700 mg), and 16-19 residue C (684 mg).

Residue A was rechromatographed on silica gel with benzene as solvent to provide 50 mg of **19** and 215 mg of **20**. Residue B was eluted with 15%

EtOAc/benzene to afford 10 mg of β-sitosterol and 82 mg of pellitorine, **21**. Residue C was eluted with 30% EtOAc/benzene to give 200 mg of **22** and 80 mg of **23**.

### Isolation of compounds 24-31 from *Cyclea barbata*

The powdered roots of *C. barbata* (3.75 kg) were extracted with MeOH at room temperature three times, and the MeOH solution was evaporated *in vacuo*. The residue was treated with 1% HCl, and the acidic solution was basified with $NH_4OH$ to pH 9, then extracted with $CHCl_3$. After evaporation of the $CHCl_3$ solution, the crude alkaloid extract (45 g, yield 1.2%) was dissolved in $Me_2CO$ and gave crystalline tetrandrine [**24**] (12 g). Chromatography of the remaining extract (30 g) on Si gel, eluting with increasingly polar mixtures of $CHCl_3$/MeOH and followed by preparative TLC, afforded alkaloids **24** (6 g, total 18 g, 0.48%), **25** (0.5 g, 0.013%), **26** (0.7 g, 0.019%), **27** (0.4 g, 0.011%) and **28** (15 mg, 0.0004%) which were identified as tetrandrine [**24**] limacine [**25**], thalrugosine [**26**], homoaromoline [**27**], and cycleapeltine [**28**]. The minor alkaloids were also isolated and identified as 2'-norlimacine [**29**], cycleabarbatine [**30**] and tetrandrine-2'-β-N-oxide [**31**].

### Isolation of compounds 32-35 from *Parabaena sagittata*

The powdered, dried leaves (1.2 kg) were extracted thoroughly by percolation with 95% EtOH (20 liters), and the percolate was concentrated under reduced pressure. During the concentration, **32** precipitated as colorless crystals which were removed by filtration. The filtrate was further evaporated to dryness (580 g). Recrystallization of **32** from 50% aqueous EtOH yield colorless prisms (2.38 g). The residue from above (580 g) was acidified with 5% aqueous HOAc (1.5 liters) and filtered. The filtrate was basified with $NH_4OH$ and extracted with $CHCl_3$ (10 liters). The $CHCl_3$ extract was washed with $H_2O$ (1 liter), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to yield the syrupy crude bases (11.8 g). These were divided into five portions, and each portion was chromatographed on a silica gel colum (70 g, 5 x 7 cm) with $CHCl_3$ as the eluent. Fractions of 20 ml were collected and monitored by TLC. Fractions 2-8 yielded 34 mg of yellow prisms (**33**), fractions 10-14 gave 32 mg of fine off-white needles (**34**), and fractions 19-25 yielded 64 mg of **35** as colorless needles. The polarity of the eluent was increased to 20% EtOH in $CHCl_3$, and fractions 33-39 yielded 54 mg of yellow crystals which were shown to be berberine by comparison with authentic sample.

**Isolation of compound 37 from *Ancistrocladus tectorius***

The dried powdered leaves of *A. tectorius* (800 g) were macerated twice for 3-day periods with 95% EtOH (8 liters and 5 liters) and the combined EtOH extracts pooled and evaporated under reduced pressure. The residue (125 g) was suspended in distilled $H_2O$ (5 liters) and the filtrate extracted with $CHCl_3$ (2 x 5 liters). The combined $CHCl_3$ extracts were dried ($Na_2SO_4$), filtered, and evaporated under reduced pressure to afford a light brown powder (13 g).

The powder was divided into six portions, and each was separately chromatographed as follows. Each portion was dissolved in $CHCl_3$ (10 ml), absorbed onto neutral alumina (5 g), dried, and placed on top of a dry alumina column (6 x 20 cm) and eluted with $Et_2O$. Twenty fractions were collected, examined by tlc and yielded ancistrotectorine (**37**, 125 mg, 0.016% yield).

**Isolation of compound 48 from *Kopsia jasminiflora***

The air-dried and powdered leaves (700 g) of *K. jasminiflora* were exhaustively extracted with 95% EtOH at room temp. The EtOH extracts were pooled, the alcohol removed *in vacuo*. The syrupy residue (120 g) was acidified with 2% tartaric acid to pH 2.7, filtered and partitioned against EtOAc. The aqueous phase was rendered alkaline (pH 9) by the addition of $NaHCO_3$. Partitioning with EtOAc yielded a crude alkaloid fraction (4.6 g) which was chromatographed over silica gel. Elution with $CHCl_3$ and increasing amounts of MeOH yielded 10 fractions. Fraction 10 (629 mg) was further purified over silica gel with $C_6H_6$-EtOAc (3:7) as eluent. Crude **48** was crystallized from EtOH.

**Isolation of compounds 50-56 from *Murraya siamensis***

The dried, chipped roots (1 kg) were macerated twice for 2-day periods with 95% EtOH (6 and 5 liters). The combined extracts were evaporated in vacuo, and the residue was suspended in $H_2O$ (2 liters). The aqueous phase was extracted with $CHCl_3$ (4.5 liters). This organic phase was dried (anhydrous $Na_2SO_4$) and filtered, and the solvent was removed to yield 9.3 g of a gummy residue. The residue was divided into four equal portions, and each was purified by Si gel flash chromatography using $CHCl_3$ as eluent. Fractions of 25 ml each were collected and examined by tlc, and those containing the same material were combined to give the following components : fractions 2-3 gave 230 mg of **50**, fractions 5-7 gave 120 mg of **51**, fraction 9 gave 24 mg of **52**, fractions 11-23 gave residue A and fractions 26-32 gave 16 mg of **53**. Residue A was further purified by Si gel preparative tlc using EtOAc-petroleum ether (30-60°) (8:2) to yield 143 mg of xanthoxyletin, 28 mg of **54**, and residue B. Purification of residue B by Si gel

flash chromatography using EtOAc-petroleum ether (30-60°) (1:1) gave 22 mg of **55** and 21 mg of **56**.

**Isolation of compounds 57-66 from *Sophora exigua***

Air-dried roots (360 g) of *S. exigua* were extracted x 5 with 75% EtOH at room temp. The combined extracts were concd *in vacuo* and acidified with 5% HCl. The acidic aq. soln was extracted x 2 with $CH_2Cl_2$ and made basic with 28% $NH_4OH$. The basic aq. soln was extracted x 3 with $CH_2Cl_2$. The aq. layer was made strongly basic by addition of dry $K_2CO_3$ under ice-cooling and extracted x 3 with $CH_2Cl_2$. All $CH_2Cl_2$ extracts from the alkaline aq. soln were combined, dried ($K_2CO_3$) and concd to dryness *in vacuo*. The total alkaloid (5.4 g) was obtained in a yield of 1.5% of the dry wt. The total base was applied to a silica gel column and eluted with a $CH_2Cl_2$-MeOH-28% $NH_4OH$ gradient. All alkaloids **58-66** were purified by repeated silica gel CC. The **57**-rich fraction was purified by prep. TLC and prep. HPLC to give pure **57** (17 mg).

**Isolation of compounds 67-68 from *Blumea balsamifera***

The air dried and powdered plant material (4.5 kg) was macerated twice for 5 day periods with 95% ethanol (20 and 15 liters). The ethanol extracts were pooled, the alcohol removed *in vacuo*, and the residue suspended in 3 liters of 10% ethanol. After filtration, the filtrate was treated with a 10% aqueous lead acetate solution until no further precipitation occurred. Further filtration then afforded a clear solution which was extracted with chloroform (3 x 7 liters). The combined chloroform extract was dried ($Na_2SO_4$) and then evaporated *in vacuo* to yield 13.5 g of a brown syrupy mass which, when stirred with chloroform (90 ml), produced a pale yellow precipitate. Recovery by filtration and drying yielded a solid (Fraction A, 977 mg) and a filtrate which was evaporated *in vacuo* to a yellow syrup (Fraction B, 12 g).

Thin layer chromatography (tlc) of Fractin A (silica gel G, chloroform : acetone (9:1)) indicated the presence of only two components (Rf = 0.17 and 0.35). The total fraction was divided into four portions. Each portion was dissolved in chloroform (2 ml), adsorbed onto silica gel (5 g), dried, then placed on top of a dry silica gel column (2.5 x 40 cm), and eluted with chloroform-acetone (9:1). Fractions of 20 ml each were collected and compared by tlc. Those fractions of similar composition were combined. This procedure produced 3 major fractions which were evaporated *in vacuo*. Fractions 1-5 yielded no residue on evaporation ; fractions 6-10 were homogeneous by tlc and were designated **67** ; fractions 11-19 were also homogeneous by tlc and were designated **68**.

**Isolation of compounds 75-77 from *Sophora exigua***

The dried and ground roots of *S. exigua* (1 kg) were extracted with $CHCl_3$ (5 L) to yield a syrupy mass (150 g) after evapn. A part of $CHCl_3$ extract (40 g) was subjected to silica gel CC (n-hexane-$Me_2CO$;gradient system). Repeated CC and recrystallization afforded **75** (40 mg), **76** (120 mg) and **77** (25 mg) in pure form.

**Isolation of compounds 78-79 from *Dalbergia cadenatensis***

The powdered plant material (430 g) was extracted at room temperature successively with light petroleum ether, $CHCl_3$, and MeOH. The $CHCl_3$ and MeOH extracts showed and $ED_{50}$ of 2.5 µg/ml and 27 µg/ml, respectively, against the P-388 test system *in vitro*. Neither extract exhibited significant activity against the KB cell culture system. The presence of antifungal and antibacterial in both extracts was detected by bioautography on Si gel ($CHCl_3$-MeOH, 95 : 5 ).

The pigments of the MeOH extract (100 g) were removed by chromatography over Si gel eluting with EtOAc-MeOH-$H_2O$ (100 : 6 : 3). The active fraction was further separated on Si gel eluting with $CHCl_3$-MeOH (95 : 5) into five fractions (A-E). Recrystallization of fraction B yielded (R,S)-(+)-mucronulatol as colorless cubes (300 mg)

Fraction C was rechromatographed over Si gel eluting with $CHCl_3$-MeOH (98 : 2) to afford formononetin (95 mg), (R)-(-)-claussequinone [**78**] (155 mg) and 5-hydroxybowdichione [**79**], (4 mg) as crystalline compounds.

**Isolation of compound 80 from *Wrightia tomentosa***

The air-dried plant material (3 kg) was ground into a powder and extd with MeOH. After concn the MeOH extract (200 g) was chromatographed on silica gel using $CHCl_3$ and $CHCl_3$-MeOH mixtures as eluents. The fractions which contained wrightiadione were combined and purified by prep. TLC using petrol-EtOAc-$Me_2CO$ (100 : 8 : 8) as developing eluant to yield **80** (2 mg, 0.00006%).

**Isolation of compound 81 from *Clitoria macrophylla***

Dried powdered roots (300 g) were macerated twice with 95% EtOH (11 and 0.5 L) for 3-day periods and filtered. The combined filtrates were evapd *in vacuo* and the residue (43 g) chromatographed on silica gel column using $CHCl_3$-EtOH (19 : 1) as eluent. Frs of 25 ml each were collected and monitored by TLC. The homgenous frs (36-45) were combined and evapd to afford a yellow solid (260 mg). After

recrystallization from a mixt. of $CHCl_3$ and EtOH, white rosette crystals of **81** (190 mg, 0.063%) were obtained.

### Isolation of compound 84 from *Micromelum minutum*

Dried powdered leaves (650 g) of *Micromelum minutun* were macerated with 95% EtOH (2 L) for 3 days. After evaporation of the eluent *in vacuo* (to 200 mL), distilled $H_2O$ (300 mL) and saturated Pb $(OAc)_2$ solution (120 mL) were added, the mixture was centrifuged, filtered, extracted with $CHCl_3$ (3 L), and the $CHCl_3$ fraction was dried ($Na_2SO_4$). The residue after evaporation (8.5 g) was chromatographed on Si gel eluting with $CHCl_3$ and the main constituent crystallized from absolute EtOH to afford cream-colored rosettes of microminutin (**84**, 6.35 g, 0.98%).

### Isolation of compounds 89-90 from *Alyxia reinwardti*

The inner bark of *A. reinwardti* (3 kg) was extracted with MeOH at room temp and partitioned with petrol and aq. MeOH. The aq. fr. was concd *in vacuo* and after $H_2O$ addition was partitioned with $H_2O$ satd EtOAc fr. (17.4 g) was subjected to silica gel CC eluting with mixts of $CHCl_3$ and MeOH of increasing polarity. The column fractions which contained **89** and **90** were combined and further purified by prep. TLC.

One third of the combined column frs was repeatedly purified by prep. TLC using the organic phases of EtOAc-MeOH-$H_2O$ (3:1:3) and $CHCl_3$-EtOAc-MeOH-$H_2O$ (4:4:2:1) to afford **89** with higher Rf value (43 mg, 0.0014%) and **90** in amorphous powder (50 mg, 0.001%).

### Isolation of compounds 91-92, 96-98 from *Xyris indica*

The dried flowering heads (1 kg) was extracted with $CHCl_3$ (10 and 5 L) for two 7-day periods and filtered. The combined filtrates were evaporated *in vacuo* until dryness to yield a syrupy mass residue (45 g). The residue (10 g) was chromatographed on silical gel CC (8x12 cm) using Pet. ether-EtOAc, 8 : 1 as eluent and collecting 50-ml fraction. Those fractions containing homogeneous components as judged by TLC were combined and the solvent removed. Fractions 20-41 and 59-176 afforded residues XV and XB respectively. XV was rechromatographed on silica gel CC (2.5x26 cm) using $CHCl_3$ as eluent to furnish **91**, 77 mg (0.035%). XB was rechromatographed on silica gel CC (4.5x20 cm) using Benz-EtOAc, 15 :1 as eluent to yield 112 mg (0.05%) of **92**. Fractions 3-19 and 59-176 afforded residues $XI_1$ and $XI_2$ respectively. $XI_1$, was rechromatographed on silica gel CC (4.5x20 cm) using hexane-EtOAc, 99 : 1 as eluent to yield **96** 0.974 g (0.44%) and **97** 0.1006 g (0.04%). $XI_2$ was rechromatographed on

silica gel CC (4.5x20 cm) using Benz-EtOAc, 15 : 1 as eluent to furnish 74 mg (0.03%) of **98**.

### Isolation of compounds 93-95 from *Garcinia hanburyi*

The dried gum resin (200 g) was ground into a powder and dissolved in $CHCl_3$ (5 L) for 24 h and then filtered. The filtrate was evaporated to dryness *in vacuo* to give a yellow powder (125 g). A portion of dried yellow powder (2 g) was chromatographed on silica gel using $CHCl_3$-EtOH (97.5 : 2.5, v/v) as eluent. The fractions which contained gambogic acid were combined. Repeated purification of these column fractions by preparative TLC using $CHCl_3$-$Me_2CO$-EtOAc (10:1:1, v/v) and $CHCl_3$-MeOH (19 : 1, v/v) as developing eluent yielded three distinct bands. Elution of the first band of highest Rf value with $CHCl_3$ afforded isomorellinol (**95**) (5.5 mg, 0.17%). Elution of the second and third bands with $CHCl_3$ gave gambogic acid (**93**) 150 mg. 4.69%) and isogambogic acid (**94**) (45 mg, 1.41%), respectively.

## SYNTHESIS OF SOME AMIDE

### Synthesis of 19

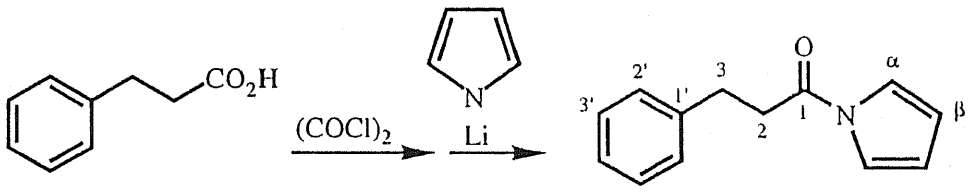
A solution of tridecylmagnesium bromide was prepared from 92 mg (3.8 mol) of Mg turnings and 1.0 g (3.8 mmol) of 1-bromotridecane in 25 mL of anhydrous ether. To this solution at 0°C was added over 15 min. a solution of 570 mg (3.8 mmol) of piperonal in 10 mL of ether. The mixture was heated to reflux for 2 h, then cooled and 20 mL of 20% aqueous $H_2SO_4$ was added cautiously. The layers were separated, the aqueous layer was extracted with ether (2x10 mL) and the combined ether layers were washed with saturated $NaHCO_3$ solution (1x30 mL), with brine (1x20 mL) and then dried (anhydrous $MgSO_4$). Removal of the solvent *in vacuo* gave 868 mg (68%) of crude alcohol as a white solid.

A sample of 452 mg (1.4 mmol) of this crude alcohol and 450 mg of neutral alumina were heated under $N_2$ at 180-200°C for 30 min. The alumina was washed with ether and the solution dried ($MgSO_4$). Removal of the solvent gave, after recystallization from petrol/ethanol, 386 mg (87%) of **19** as a white solid, m.p. 33.5-36.5°C

**Synthesis of 20**

To a solution of 800 mg (5.3 mmol) of 3-phenylpropanoic acid in 5 mL of dry $CH_2Cl_2$ was added dropwise 1.34 g (0.92 mL, 10.6 mmol) of oxalyl chloride and the reaction was stirred under $N_2$ at room temperature for 3 h. The solvent was removed in vacuo and the residue washed four successive times (4x20 mL) to remove unreacted oxalyl chloride. The yellow residue of crude acid chloride was used in the next step.

The pyrrole anion was prepared by reacting 355 mg (0.367 mL, 5.3 mmol) of freshly distilled pyrrole in 10 mL of dry THF under $N_2$ at -78°C with 2.1 mL (5.3 mmol) of 2.5 M n-butyl lithium in hexane and stirring for 15 min. The crude 3-phenylpropanoyl chloride was then added dropwise to the pyrrole anion solution at -78°C and the temperature brought to room temperature gradually. After stirring for one-half hour at room temperature, water was added and the product was extracted with ether. The combined organic phases were dried ($MgSO_4$) and the solvent removed to give one major UV positive spot on TLC at Rf 0.45 (10% EtOAc/petrol). Purification of the crude product by MPLC gave 815 mg (77%) of N-(3-phenylpropanoyl) pyrrole, **20**, which was identical in all respects with the second component isolated from *P. sarmentosum.*




**20**

# PART V

# CONCLUSION

The present investigation has led to the isolation of several bioactive compounds from selected Thai medicinal plants. Various kinds of phytochemical categories have been isolated and identified on the basis of detailed spectroscopic analysis. Modern technique of NMR have been employed to establish the structure of new natural products isolated from a selection of Thai medicinal plants. Results from 21 species of plant materials revealed the presence of 47 new natural products among 98 isolated compounds. Five of isolates were found to be novel skeleta.

Perhaps the most important single development in nmr spectroscopy as far as practical application is concerned is the ability to conduct correlation spectroscopy. Of the many variants of this technique that are available, three in particular have been of importance to the natural product chemist. These are i) $^{1}H$-$^{1}H$ COSY in which either two bond or long range couplings may be emphasized, ii) nOe COSY (NOESY) spectra in which proximate proton-proton relationships are displayed, and iii) $^{1}H$-$^{13}C$ COSY (hetcor) spectra in which the correlation between a carbon and its attached proton (s) is displayed.

Unfortunately, the latter technique has three substantial disadvantages : i) significant amounts of material (at least 30 mg) are normally required, ii) such spectra typically afford no information concerning the assignment of quaternary carbons, iii) the assignment of carbon signals with very close or identical chemical shifts may be difficult.

Variety of models in biological evaluation of plant extracts and/or pure isolates have been established e.g. cytotoxic, antimalarial, antimicrobial, anti-inflammatory and spasmolytic activities, to pursue this research as a discovery of new medicinal agents.

## COMPOUNDS POSSESSING BIOLOGICAL ACTIVITY :

*Cytotoxicity* : The cytotoxic evaluation of the isolates was found that the chemical categories of marked inhibitory of cells growth are sesquiterpene lactones, bisbenzylisoquinoline alkaloids, aporphine alkaloids, amaryllidaceous alkaloids, rotenoid, isoflavonoids, coumarins and xanthones. Plants in families Compositae (*Grangea maderaspatana*) and Magnoliaceae (*Michelia rajaniana* and *Paramichelia baillonii*) are representative of sesquiterpene lactones to be studies. It was found that sesquiterpene lactones containing α-methylene-γ-lactone functionality displaying cytotoxicity. This activity was assumed that the Michael addition reaction between α-

methylene-γ-lactone and sulfhydryl group of amino acid in DNA strand such as methionine or cysteine was occurred. The plant belonging to Menispermaceae; *Cyclea barbata* which yielded bisbenzylisoquinoline alkaloids have been found to possess strong cytotoxic activity. The cytotoxic potential of bisbenzylisoquinoline alkaloids was then measured with a battery of culture mammalian cells. Each of the alkaloids was found to demonstrate a general cytotoxic response with no discernable cell-type selectivity. In order to determine the cytotoxic activity of the three isolated from *Garcinia hanburyi*, these compounds were evaluated using KB cell lines. The $ED_{50}$ values in the KB cell system were 0.7, 0.9 and 2.3 μg/ml for gambogic acid, isogambogic acid and isomorellinol, respectively. Therefore, although the KB-VI cell are slightly more resistant to these compounds compared with cultured KB cells, it is apparent that they are not efficiently removed from the cells due to the P-glycoprotein. One cytotoxic mechanism consistent with these data is nucleophilic attack at position C-10, which results from the presence of the α,β-unsaturated carbonyl moiety. The evaluation of microminutin, a coumarin isolated from *Micromelum minutum*, on P-388 lymphocytic leukemia possesses promising activity. The presence of unsaturated butano-lactone as substituent of coumarin causes nucleophilic attack in the cytotoxic mechanism. Group of isoflavonoids including rotenoids was also exhibited cytotoxic effects. The cytotoxic mechanim was probably due to γ-pyrone moiety.

*Antimalarial activity* : Numbers of alkaloids isolated from *Cyclea barbata* have been subjected to evaluate their potential with *Plasmodium falciparum* strains D-6 and W-2. Results that nearly all were capable of inhibiting the growth of cultured of both strains. Although the intensity of the response was not equivalent to the known antimalarial agents investigated, appreciable activity was demonstrated with some isolates. In general, the concentration of test agent required to inhibit the growth by 50% was roughly equivalent or greater in the drug-resistant strain W-2 as compared with strain D-6.

*Antimicrobial activity* : The *in vitro* antifungal and antibacterial activities of the isolated isoflavonoids was performed. Claussequinone showed good antibacterial activity with MIC values ranging from 3 to 6 μg/ml. 5-Hydroxybowdichione, which had previously shown good activity in the bioautographic assay, was only weakly active in the microdilution test. We attribute this diminished activity to the instability of this compound in solution. The activity mechanism of claussequinone might be due to quinone moiety. The *in vitro* antifungal and antibacterial activities of the isolated anthraquinones from *Xyris indica* was assessed using miconazole as reference compound. Minimum inhibitory concentration (MIC) were determined through a microdilution assay. The three anthraquinones exhibited only marginal or no antibacterial

activity at the concentrations tested. 3-Hydroxychrysazin, however, showed good antifungal activity with MIC value 0.78 μg/ml against *Trichophyton mentagrophytes* and *T. rubrum*, a dermatophytes causing ring worm and athelete's foot. Therefore, it is concluded that 3-hydroxychrysazin is an antifungal principle in this medicinal plant.

*Anti-inflammatory activity* : Entadamide A and B were assayed for anti-inflammatory activity using 5-lipoxygenase activity of RBL-1 cells. It was found that entadamide B was more effective than A based on inhibition % values.

*Spasmolytic activity* : Antispasmodic activity were investigated in various smooth muscle. Ancistrotectorine reduced not only spontaneous contraction but also the contraction induced by acetylcholine, histamine, serotonin, barium, potassium and calcium chloride in rabbit's jejunum and guinea-pig's ileum. Reduction of rat and guinea-pig uterine contraction in both spontaneous and contraction induced by oxytocin were also observed. Ancistrotectorine produced the dose-dependent reduction of phasic contraction of rat vas deferens induced by histamine, serotonin, barium, potassium and calcium chloride and also showed dose-dependent inhibition of the contraction of rabbit aortic strip evoked by calcium chloride in the high potassium chloride solution. These results may suggest a calcium antagonist-like action of Ancistrotectorine.

Most medicinal materials contain a large variety of known of still unknown compounds. Thai traditional medicine prefers modalities characterized by a combination of numerous individual materials, sometimes up to ten or more. It is obvious that the interaction between individual materials might be of considerable relevance for the biological effectiveness of these combination. Studies on the chemical constituents and pharmacological activities of combined preparation have been very scarce up to now and an appropriate discussion of the effects of such combinations is therefore not possible at the present time.

To assess the chemotherapeutic value of the herbal flora of Thailand, it is essential that the plant material selected for investigation should comprise those species that are extensively used in herbal medicine and prominently described in literature. A reference to 25 plants from 12 different families, which form part of this study, shows that a large majority of them satisfy these criteria. Nevertheless, it is quite clear from our study that a certain proportion of Thai medicinal plants do possess marked biological properties to form the basis of further investigations in order to make them interesting from the clinical point of view. The development of a modern therapeutic agent, however, is a long and an arduous process, necessitating the cooperation of botanists, chemists, pharmacologists, toxicologists microbiologists, biochemists and finally

clinicians. However, in a developing country like Thailand, where every effort is being made to utilize our own resources. The availability of plant materials obtainable from the herbal flora of the country is a primary consideration and, as such, must be linked to our scientific investigations for the promotion of research and development in order to attain as much self-sufficiency in this field as possible.

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