A STUDY ON THE CONSTITUENTS AND STRUCTURE ELUCIDATION OF INDOLE ALKALOIDS FROM *GELSEMIUM ELEGANS* BENTH. IN THAILAND

THESIS

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SUMPHAN WONGSERIPIPATANA

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ABSTRACT

The roots, stems and branches, leaves and seeds of *Gelsemium elegans* Benth. have been investigated for alkaloids. Sixteen alkaloids have been isolated and characterized. There are seven new alkaloids, three of them have been identified as indole alkaloids 16-epi-voacarpine, 19-(Z)-taberpsychine, and koumine N-oxide;the other being oxindole alkaloids identified as 19-hydroxydihydrogelsevirine, elegansamine, gelsemine N-oxide and 19-oxogelsenicine. Included are three known indole alkaloids koumine, 19-(Z)-akuammidine and koumidine, and six known oxindole alkaloids gelsemine, gelsevirine, gelsenicine (humantenmine), 14-hydroxygelsenicine (humantenidine), humantenine and 14-hydroxygelsedine. The structures of two known alkaloids, koumidine and 19-(Z)-akuammidine have been revised to (19Z)-form.

Among seven new alkaloids, three bases 19-(Z)-taberpsychine, 19-hydroxydihydrogelsevirine and 16-epi-voacarpine were found from the roots; two alkaloids 16-epi-voacarpine and elegansamine were isolated from the stems and branches without leaves; four alkaloids koumine N-oxide, gelsemine N-oxide, 19-oxogelsenicine, and 16-epi-voacarpine were obtained from the leaves. The seeds of this plant contain only known alkaloid, 14-hydroxygelsedine.

Furthermore, partial synthesis and absolute configuration determination of koumidine and 19-(Z)-taberpsychine have been carried out.

The formal synthesis of koumine and the biogenetic route of *Gelsemium* alkaloids have been proposed and discussed in this thesis.

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PART I

INTRODUCTION

INTRODUCTION

Among the natural products, ALKALOID is one of the useful and interesting group of compounds. No other class of natural compounds possesses such an enormous variety of structures. In 1983, over 5000 alkaloids of all structural types were known. One of the big structural types of alkaloids is indole alkaloids which displays a rich variety of structural types, many of which have been established and their syntheses achieved. The number of indole alkaloids of known structure amounts to approximately 1400 (Pelletier, 1983). Some indole alkaloids exert considerable pharmacological activity, three groups are notable for clinically useful alkaloids: (a) the Ergot alkaloids, ergometrine, with its direct action on the contraction of uterine muscle; ergotamine for migraine relief and modified alkaloid, bromocriptine, which suppresses lactation and has some application for the treatment of mammary carcinoma, (b) the *Rauvolfia* alkaloids and specifically reserpine which was the forerunner of the tranquilizers and hypotensive, (c) the dimeric anti-leukemic alkaloids of *Catharanthus*, vinblastine and vincristine which are in current clinical use. It might be thought that interest in indole alkaloids had waned and that they had passed their peak as far as new discoveries were concerned. In fact it is logical to assume that after such intensive research efforts, there would be little novelty left in this area (Phillipson and Zenk, 1980).

The LOGANIACEOUS genus *GELSEMIUM* consists of only three species, all of them are the major sources of indole alkaloids and several alkaloids are toxic. The first *Gelsemium* species, *Gelsemium elegans* Benth. (*Gelsemium sumatranum* Boerl., *Leptopteris sumatrana* Blume and *Medicia elegans* Gardn. & Champ., Hook.) has been used in China, Viet Nam and Borneo as a suicidal poison which is either

ingested or smoked. The flowers of this plant are poisonus to smell, kills butterflies on the flowers. In Burma, it is used as a fish poison. This plant is used in Chinese folk medicine as an analgesic, antispasmodic and as a remedy for certain kinds of skin ulcers (Ornduff, 1970; Lin *et al.*, 1989b). The second *Gelsemium* species, *Gelsemium sempervirens* (L.) Jaume Saint-Hilaire (*Gelsemium lucidum* Poir, *G. nitidum* Michx., *Bignonia sempervirens* L. and *Lisianthus sempervirens* Mill.) causes death and abortion in livestock which feed upon its leaves. Ingestion of nectar and honey produced from *Gelsemium* flowers reportedly has caused death in humans and bees in the southeastern United States (Hardin, 1961; Kingsbery, 1964). However, this plant has been used in the treatment of neuralgia and migraine (Saxton, 1965). Its antispasmodic properties is useful in the treatment of spasmodic disorders such as asthma and whooping cough (Grieve, 1975). Information on the biological properties of last species, *Gelsemium rankinii* Small (*Gelsemium sempervirens* (var.) *inodorum* Nutt.) has not yet been reported.

The genus *Gelsemium* is twining woody vines; leaves opposite, simple, entire, petiolate; stipules represented by stipular lines; flowers pentamerous, distylous or homostylous, one to many, in axillary or terminal inflorescences; corollas funnelform, the lobes imbricated in bud, bright yellow or orange-yellow; stamens five, epipetalous; style quadrifid at apex; seeds flattened, usually winged;n=8. This genus exhibits a pattern of distribution that it is represented by the first species in the southeastern Asia, the second species in the southeastern United States and the highlands of Mexico and Guatemala, and the last species in the southeastern United States (Ornduff, 1970).

GELSEMIUM ELEGANS Benth. is a climbing glabrous evergreen shrub to 3.5m. tall, bark corky, wood porous, vessels numerous. Leaves ovate to ovate-lanceolate, the blades 6-13cm. long, sometimes cuspidate, the petioles 0.5-1.2cm. long; flowers numerous, inflorescence terminal or axillary; corolla 1.2-1.7cm. long including the lobes 0.3-0.8cm. long, bright to orange-yellow, odorless; sepals lanceolate, acuminate 3-4mm. long; pedicels 0.3-1.0cm. long, ebracteolate or with a single subtending bracteole; capsules ovate-elliptic in outline, 0.8-1.5cm. long, inflated; seeds brownish, 3-4mm. in diameter, including an inciso-dentate wing 1-2mm. wide; n=8. Flowering in September to December and occasionally at other times. Fruiting in March and April. This plant is different from the other two species on the points that the latter having borne in inflorescences of 1-8 flowers, capsules not inflated, seeds wingless or with a strongly asymmetrical entire wing.

Gelsemium elegans Benth. is distributed in Assam, northern Burma, northern Thailand, Laos, Viet Nam, southern and southeastern China, Sumatra and northern Borneo. Sea level to 6000 feet (Ornduff, 1970; Brandis, 1971). We found this plant in Phuu Luang National Park, Loei Province, Thailand, known as Mali Saikai Doklueang but in Udon Thani Province as Gok Muan and Nan Province as Ma Khet (Smitinand, 1980).

This thesis was under taken in an effort to provide some observations on alkaloidal constituents in certain plant in the tribe Gelsemieae of the family Loganiaceae. The specific interest was focused on indole alkaloid contents and *Gelsemium elegans* Benth. was the subject of study. This plant was first studied by T.Q. Chou in 1931 and several groups of researchers have continued the study. The

author wished to investigate some other possibly remaining interesting indole alkaloids in this plant.

Gelsemium elegans Benth.

a, flowering stem, $^{x}0.4$; b, mature capsules, $^{x}1.7$; c, seed, $^{x}4$; d, short-homostyled flower, $^{x}1.7$; e, long-styled flowered, $^{x}1.7$; f, short-styled flower, $^{x}1.7$

PART II

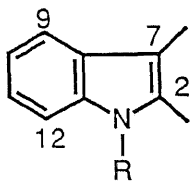
HISTORICAL

HISTORICAL

Alkaloids and their occurrence

The number of known alkaloids has risen dramatically,a review to the middle of 1973 counted 4959 alkaloids, of which 3293 had known structures. By late 1978, the number stood at nearly 4000, structurally defined alkaloids. In 1983, the number of alkaloids was over 5000 (Cordell, 1981; Pelletier, 1983). In recent years, there have been increasingly numerous examples of the occurrence of alkaloids in animals, insects, marine organisms, microorganisms and the lower plants. As the major source of alkaloids still has been the flowering plants, the angiosperms.

Indole alkaloids are defined as the natural organic products containing either the indole nucleus (1) or an oxidized, reduced, substituted equivalent of it, e.g. oxindole (2), pseudoindoxyl or γ-indoxyl (3), indoline or dihydroindole (4), indolenine (5), hydroxyindolenine (6), methyleneindoline (7), N-acylindole (8), 2-acylindole (8.1) and N-acylindolene (8.2). The number of indole alkaloids of known structures in 1983 about to approximately 1400 (Kisakurek *et al.*, 1983; Verpoorte, 1986).



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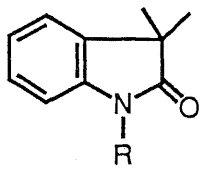

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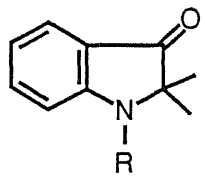

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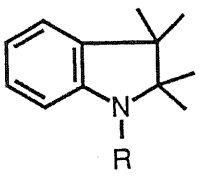

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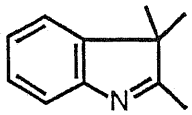

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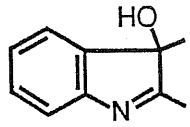

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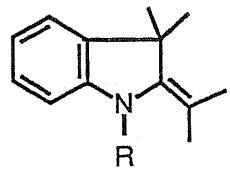

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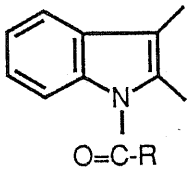

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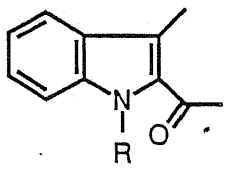

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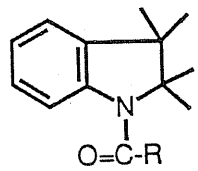


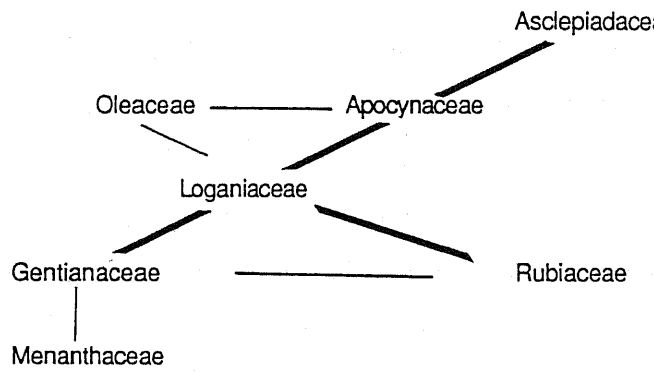
(8.2)

The distribution of indole alkaloids is broad, certain plant groups are noted for containing them. Among the seed plants, the families Apocynaceae, Loganiaceae and Rubiaceae have been a very rich source of indole alkaloids. Another important source is the fungal genus *Claviceps*, which is known to contain more than two dozen different indole alkaloids (Robinson, 1968). Some of them are found in animal, e.g. bufotenine and dehydrobufotenine (Tayler, 1966; Swan, 1967).

Indole alkaloids can be divided into two main classes. The first comprises the simple indole alkaloids. Their structures are not uniform, having only the indole nucleus or a direct derivative of it as a common feature. Depending on the constitution of the rest of the molecule, they occur in many plant families (e.g., harman, obtained from the families Apocynaceae, Chenopodiaceae, Elaeagnaceae, Leguminosae, Loganiaceae, Passifloraceae, Polygonaceae, Rubiaceae, Symplocaceae and Zygophyllaceae) or restricted to very few or only one family (e.g. koenigine obtained only from Rutaceae). The indole alkaloids of the second class contain two structure-elements: tryptamine with the indole nucleus and a C_9- or C_{10}-

monoterpene moiety, derived from secologanin. Probably because they are constructed from two common components and because they are biogenetically interrelated, indole alkaloids of this second class have a more specific distribution and are therefore more suitable as a vehicle for a comparative chemotaxonomic investigation. More than 99.8% of the isolations of this second class are entirely distributed among three plant families: Loganiaceae, Apocynaceae, and Rubiaceae, belonging to the order Gentianales (Kisakurek and Hesse, 1980).

The order Gentianales comprises seven plant families. The three mentioned families, having remarkable morphological similarities, have been classified botanically in close relationship, as shown in the following diagram, the thick lines indicate a close degree of relationship (Leeuwenberg, 1980).



The occurrence of indole alkaloids in the families Apocynaceae, Loganiaceae, and Rubiaceae supports the idea given in the above diagram concerning their chemotaxonomy.

These three families can be recognized and identified easily, as their leaves mostly opposite, simple, pinnately veined, with or without inter-or intrapetiolar stipules. Their flowers mostly 4- or 5-merous, usually actinomorphic, but

sometimes zygomorphic and exceptionally irregular. Corolla segments always united, and stamens inserted on the corolla. Style one. Ovary, except in most Rubiaceae, superior and mostly 2-locular. The Apocynaceae can be differentiated from the Loganiaceae by the presence of milky sap.

The genera of the Loganiaceae, Apocynaceae and Rubiaceae which have species containing indole alkaloids are listed below (Leeuwenberg, 1980).

Family Loganiaceae	
Tribe Gelsemieae	*Gelsemium*
	Mostuea
Tribe Strychneae	*Strychnos*
	Gardneria
Family Apocynaceae	
Subfamily Plumerioideae	
Tribe Carisseae	
Subtribe Carissinae	*Melodinus*
	Leuconotis
Subtribe Landolphiinae	*Landolphia (Carpodinus)*
Subtribe Pleiocarpinae	*Picralima*
	Hunteria (Polyadoa)
	Pleiocarpa
Tribe Plumerieae (Alstonieae)	
Subtribe Craspidosperminae	*Craspidospermum*
Subtribe Plectaneiinae	*Gonioma*
Subtribe Alstoniinae	*Alstonia*
	Tonduzia
Subtribe Aspidospermatinae	*Diplorhynchus*

Aspidosperma

Geissosperum

Subtribe Catharanthinae *Rhazya*

Amsonia

Catharanthus

Vinca

Haplophyton

Tribe Rauvolfieae

Subtribe Rauvolfiinae *Cabucala*

Rauvolfia

Subtribe Ochrosiinae *Ochrosia (Excavatia)*

Subtribe Vallesiinae *Vallesia*

Kopsia

Subtribe Condylocarpinae *Condylocarpon*

Tribe Tabernaemontaneae *Crioceras*

Callichilia (Hedranthera)

Stemmadenia

Capuronetta

Tabernaemontana(Pagiantha, Rejoua, Ervatamia, Hazunta, Peschiera, Conopharyngia, Pandaca, Gabunia)

Tabernanthe

Voacanga

Scizozygia

Family Rubiaceae

Subfamily Rubioideae

Tribe Chiococceae *Hodgkinsonia*

Tribe Psychotrieae *Psychotria*

	Palicourea
	Cephaelis
Tribe Urophylleae	*Pauridiantha*
Tribe Ophiorrhizeae	*Ophiorrhiza*
Tribe Hamelieae	*Hamelia*
Tribe Spermacoceae	*Spermacoce (Borreria)*
	Richardia (Richardsonia)?
Tribe Hedyotideae	*Hedyotis* ?
	Manettia ?
Subfamily Cinchonoideae	
Tribe Naucleeae	*Nauclea (Sarcocephalus)*
	Cephalanthus
	Neonauclea
	Mitragyna
	Uncaria
	Anthocephalus
	Adina
Tribe Cinchoneae	*Cinchona*
	Ladenbergia
	Remijia
	Corynanthe (Pseudocinchona)
	Pausinystalia
	Capirona ?
	Exostema ?
	Coutarea
	Hymenodictyon ?
	Crossopteryx ?

	Ferdinandusa ?
Tribe Rondeletieae	*Pogonopus* ?
	Simira (Sickingia, Arariba)
Tribe Mussaendeae	*Isertia*
Tribe Gardenieae	*Leptactina*
	Tocoyena ?
Tribe Coffeeae	*Tarenna*
Subfamily Guettardoideae	
Tribe Guettardeae	*Antirhea*
	Timonius
Subfamily Hillioideae	
Tribe Hillieae	*Hillia* ?

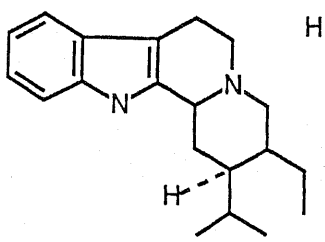
(a. Names in brackets represent synonyms; b. question marks indicate that the alkaloids have not definitely been characterized as indole alkaloids)

The family Loganiaceae comprises about 30 genera with approximately 600 species. These species are distributed in the tropical, subtropical and temperate zones (Heywood, 1978). As mentioned before, only four genera of the Loganiaceae have representatives which contain indole alkaloids. They belong to two different but related tribes, i.e. Gelsemieae and Strychneae. *Gelsemium* with 3 species, is closely allied to *Mostuea*, with 8 species, through their similar leaves, flowers with infundibuliform corolla, and doubly branched stigma. *Mostuea* has seeds which are very different from those of *Gelsemium* but which because of their bony endosperm are very like those of *Strychnos*, a genus comprising about 200 species. On the other hand, the flowers of some *Strychnos* species strikingly resemble those of *Gardneria*, a small genus with only 5 species. The distinct difference is that the ovule in each loculus of

Gardneria is one, while those of *Strychnos* are numerous and the leaves of *Strychnos* are prominently 3-7 nerves starting from near the base (Bor, 1953; Leeuwenberg, 1980).

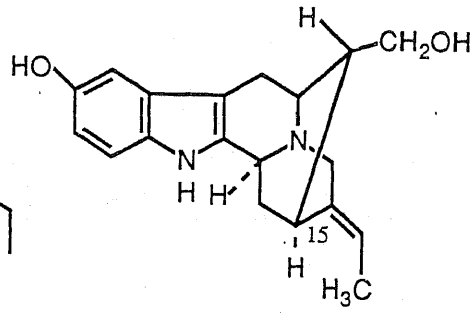
The indole alkaloids derived from tryptamine and secologanin can be classified into eight types, according to the structural characteristics of their skeletons. They are corynanthean- or C-type, e.g. sarpagine (9), ajmalicine, koumidine; vincosan- or D-type, e.g vincoside (10), talbotine; vallesiachotaman- or V-type, e.g. vallesiachotamine (11); strychnan- or S-type, e.g. vomicine (12), akuammicine; aspidospermatan- or A-type, e.g. aspidospermatine, condylocarpine (13); eburnan- or E-type, e.g. vincamine (14), dichotine; plumeran- or P-type, e.g. kopsine (15), aspidospermidine; and ibogan- or J-type, e.g. voaluteine (16), ibogaine.

Alkaloid-types

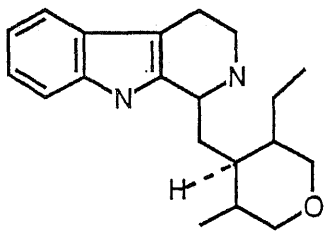


Corynanthean
(C-type)

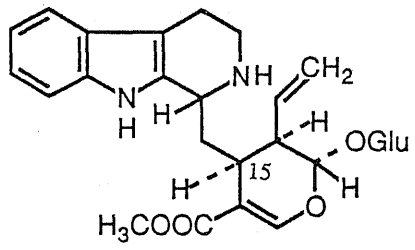
Alkaloids



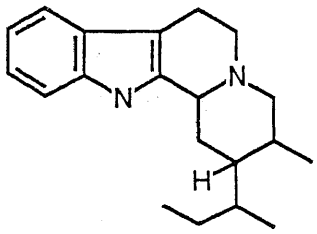
Sarpagine (9)



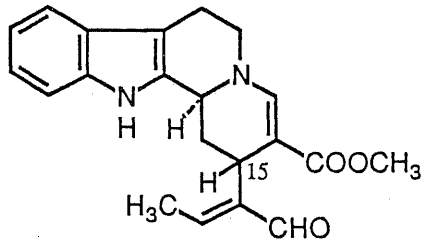
Vincosan
(D-type)



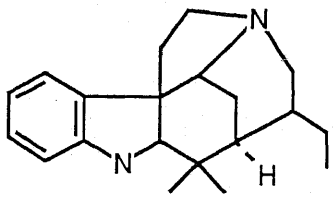
Vincoside (10)



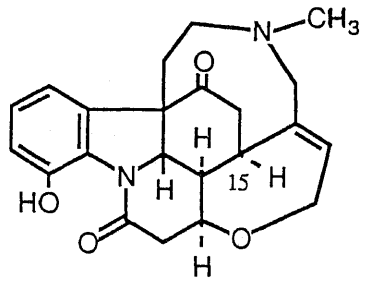
Vallesiachotaman
(V-type)



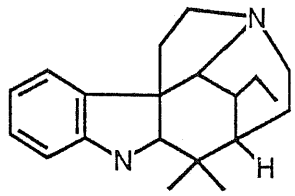
Vallesiachotamine (11)



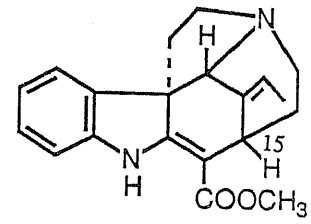
Strychnan
(S-type)



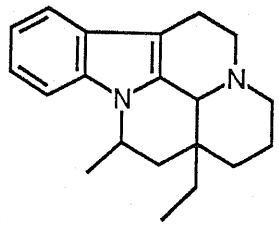
Vomicine (12)



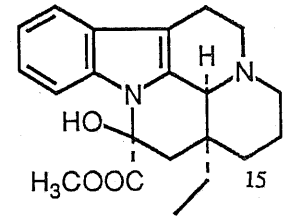
Aspidospermatan
(A-type)



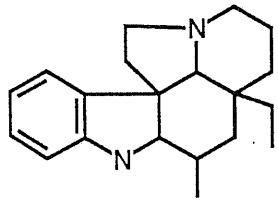
Condylocarpine (13)



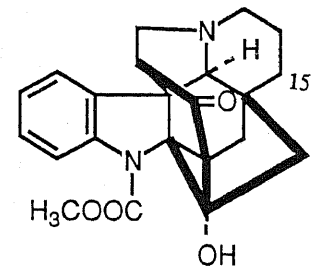
Eburnan
(E-type)



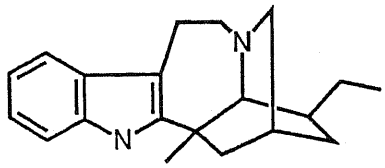
Vincamine (14)



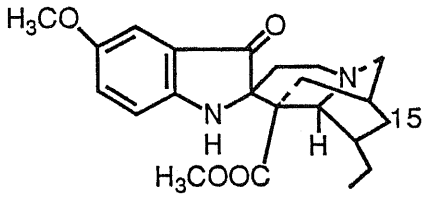
Plumeran
(P-type)



Kopsine (15)

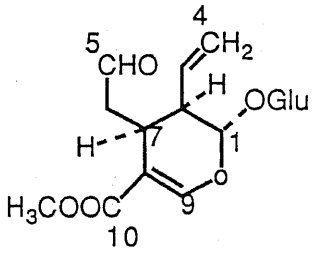


Ibogan
(J-type)



Voaluteine (16)

The eight skeletal types of indole alkaloids can be divided biogenetically into two main groups : the C-, D-, V-, S- and A-types containing a skeleton with a nonrearranged secologanin moiety and the E-, P- and J-types with a rearranged secologanin moiety. This classification is confirmed in addition to the common structural features, by the fact that all of the C-, D-, V-, S- and A-type alkaloids- with known absolute configuration show the same absolute configuration at C(15) as secologanin (17) at C(7). On the other hand, alkaloids with a rearranged secologanin component (E-, P-, J-) can occur with either absolute configuration.



secologanin (17)

The skeletal types with a rearranged secologanin moiety (E-, P- and J-types) occur exclusively in the subfamily Plumerioideae of the Apocynaceae. The occurrence of alkaloids of A- and S- types is restricted to the Loganiaceae and

Apocynaceae. On the other hand, alkaloids of C-, D- and V-types have been detected in all of the three plant families. The Loganiaceae, only C-type alkaloids have been isolated from *Gelsemium* and *Mostuea* of the Gelsemieae. Of the other tribe, Strychneae, *Gardneria* species contain only C-type alkaloids, whereas alkaloids of C-, D-, V-, S- and A-types have been isolated from species of *Strychnos.* The most abundant alkaloids in the Loganiaceae are of the S-type. The occurrence of indole alkaloids in the Loganiaceae is shown below (Kisakurek *et al.*, 1983).

	Number of investigated species	C-	D-	V-	S-	A-	Total
Gelsemieae	4	4					4
Gelsemium	2	2					
Mostuea	2	2					
Strychneae	71	100	2	49	356	1	508
Gardneria	3	24	-	-	-	-	
Strychnos	68	76	2	49	356	1	
Total	75	104	2	49	356	1	512

Chemical Studies on the Alkaloids of the Loganiaceae

As mentioned before, only four genera of the Loganiaceae are known definitely to be alkaloid bearing, the first two genera are *Gelsemium*, with 3 species, and *Mostuea*, with 8 species, belonging to the tribe Gelsemieae. The other two genera, *Strychnos*, a genus comprising about 200 species and *Gardneria*, with only 5 species belonging to the tribe Strychneae.

1. Chemistry of *Gelsemium* Alkaloids

The genus *Gelsemium* comprises of three species : *Gelsemium elegans* Benth. in Southeastern Asia; *Gelsemium sempervirens* (L.) Jaume St.-Hilaire and *Gelsemium rankinii* Small in the United States. About 20 alkaloids have been isolated from *Gelsemium* species and new alkaloids are continually being encountered.

1.1 Alkaloids Isolated from Species of *Gelsemium*

The alkaloids reported to be present in the species of *Gelsemium* are summarized as follows:

1.1.1 *Gelsemium elegans* Benth.

Roots : humantendine (Yang and Chen, 1982)

: humantenmine (gelsenicine), humantendine (14-hydroxy-gelsenicine), gelsevirine, koumine, gelsemine, humantenine, humantenirine (Yang and Chen, 1983)

: 19-(Z)-akuammidine, 16-epi-voacarpine, 19-hydroxy-dihydrogelsevirine, koumidine, gelsemine, koumine, gelsevirine, gelsenicine, 14-hydroxygelsenicine, humantenine (Sakai *et al.*, 1987)

: 19-(Z)-taberpsychine (Ponglux *et al.*, 1988)

Roots : (19*R*)-kouminol and (19*S*)-kouminol (Sun, Xing and Liang, 1989)

Stems and branches : koumine (Janot *et al.*, 1953)

: elegansamine, gelsemine, gelsevirine, koumine, gelsenicine, 14-hydroxygelsenicine, humantenine, 19-(Z)-akuammidine koumidine, 16-epi-voacarpine (Ponglux *et al.*, 1988)

Leaves : gelsemine (Janot *et al.*, 1953)

: koumine N-oxide, gelsemine N-oxide and 19-oxogelsenicine (Ponglux *et al.*, 1988)

Seeds : 14-hydroxygelsedine (Ponglux *et al.*, 1988)

Whole plants : koumine, gelsemine, kouminine, kouminicine and koumini-dine (Chi, Kao and Huang, 1938)

: sempervirine (Janot *et al.*, 1953)

: koumidine, gelsemine, koumine, gelsedine and akuammidine (Jin and Xu, 1982)

: gelsemamide and 11-methoxygelsemamide (Lin *et al.*,1989a)

: N-desmethoxyrankinidine, 11-hydroxyrankinidine, 11-hydroxyhumantenine and 11-methoxyhumantenine (Lin *et al.*, 1989b)

Not mentioned : koumicine and koumidine (Liu *et al.*, 1961)

: humantenmine, humantenine, humantendine, humantenirine, koumine, gelsemine and gelsevirine (Yang and Chen, 1982a; 1984)

: gelsenicine, gelsenidine (Du *et al.*, 1982)

1.1.2 *Gelsemium sempervirens* (L.) Jaume St.-Hilaire

Roots : gelsemine, sempervirine, gelsemicine, gelsedine and gelsevirine (Schwarz and Marion,1953)

Roots and rhizome : gelsemine (Moore, 1910)

: gelsemine, sempervirine and gelsemicine (Forsyth *et al.*, 1945; Ferreiro, 1945)

Stems : 14 β-hydroxygelsedine (Schun and Cordell, 1985)

Not mentioned : sempervirine, gelsemine, gelsemidine and gelsemoidine (Sayre, 1919)

: gelsevirine (1-methoxygelsemine) (Wichtl *et al.*, 1973)

: 21-oxogelsemine (Nikiforov *et al.*, 1974)

1.1.3 *Gelsemium rankinii* Small

Stems : 21-oxogelsevirine, gelsemine and gelsevirine (Schun,Cordell and Garland, 1986)

: rankinidine,humantenirine and humantenine(Schun and Cordell, 1986)

1.2 Structures of *Gelsemium* Alkaloids

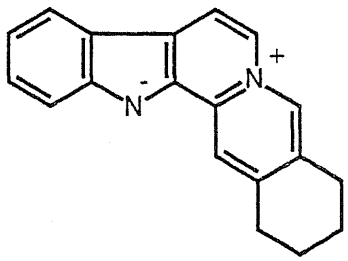
The alkaloids obtained from the species of *Gelsemium*, can be divided into two main groups, indole alkaloids and oxindole alkaloids.

1.2.1 Indole alkaloids

The alkaloids in this group have been classified into three different skeletal types : sempervirine-type, e.g. sempervirine (18); koumine-type, e.g. koumine (19), koumine N-oxide (20); and sarpagine-type (21), e.g. koumidine (22), 19-(Z)-akuammidine (23), 16-epi-voacarpine (24). 19-(Z)-Taberpsychine (25) is another type of indole alkaloids isolated

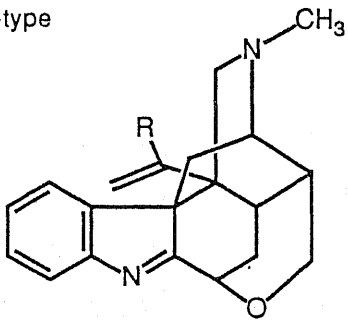
from *G. elegans* Benth. (Ohashi *et al.*, 1963; Denayer-Tournay *et al.*, 1965; Sakai *et al.*, 1987; Liu and Lu, 1988; Ponglux *et al.*, 1989).

Sempervirine-type

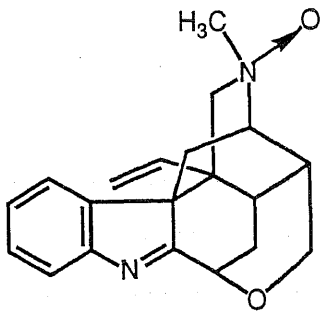


sempervirine (18)

Koumine-type



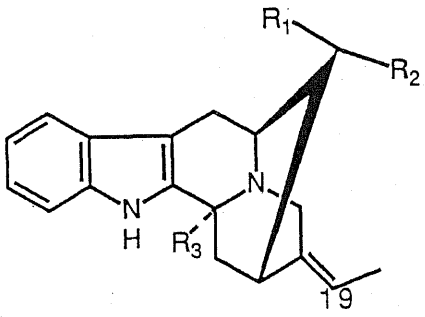
koumine (19) : R = H



koumine N-oxide (20)

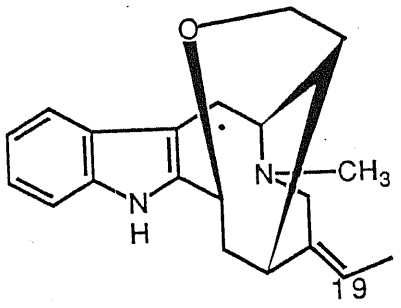
(19*R*)-kouminol and (19*S*)-kouminol : R = OH

Sarpagine-type



(21)

Alkaloids	R_1	R_2	R_3	C(19)
koumidine (22)	CH_2OH	H	H	*Z*
19-(Z)-akuammidine (23)	$COOCH_3$	CH_2OH	H	*Z*
16-epi-voacarpine (24)	CH_2OH	$COOCH_3$	OH	*E*



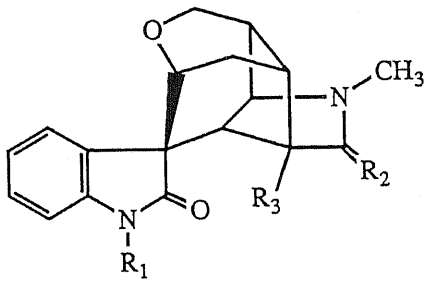
19-(Z)-taberpsychine (25)

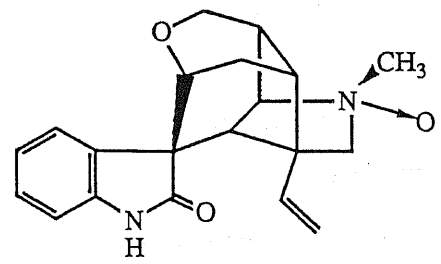
1.2.2 Oxindole alkaloids

The oxindole alkaloids isolated from the species of *Gelsemium* have been classified into three different skeletal types : gelsemine-type, e.g. gelsemine (26), 21-oxogelsemine (27), gelsevirine (28), 21-oxogelsevirine (29), 19-hydroxydihydrogelsevirine (30), gelsemine N-oxide (31); humantenine -type, e.g. N-desmethoxyrankinidine (32), rankinidine (33), 11-hydroxyrankinidine (34), humantenine (35), 11-hydroxyhumantenine (36), humantenirine (37), 11-methoxyhumantenine (38); gelsedine-type, e.g. gelsedine (39), 14-hydroxygelsedine (40), gelsemicine (41), 14-hydroxygelsemicine (42), gelsenicine (43), 14-hydroxygelsenicine (44), 19-oxogelsenicine (45), elegansamine (46) (Lovell, Pepinsky and Wilson, 1959; Wenkert *et al.*, 1972; Nikiforov *et al.*, 1974; Yang and Chen 1984; Schun and Cordell, 1985; Schun and Cordell, 1986; Sakai *et al.*, 1987; Ponglux *et al.*, 1988., Ponglux *et al.*, 1988a; Lin *et al.*, 1989b; Lin *et al.*, 1989a). Furthermore, other two alkaloids, gelsemamide (47), and 11-methoxygelsemamide (48), might be derived from the

humantenine-type, especially, rankinidine (33) and humantenirine (37), by rearrangement of the N_1-C_2 bond to C_2-N_4 (Lin *et al.*, 1989a).

Gelsemine-type

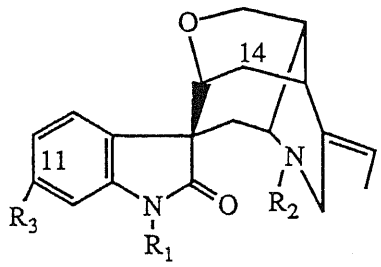




gelsemine N-oxide (31)

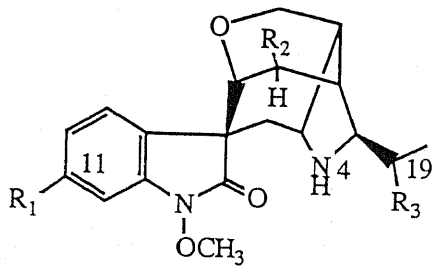
Alkaloids	R_1	R_2	R_3
gelsemine (26)	H	H_2	$HC{=}CH_2$
21-oxogelsemine (27)	H	O	$HC{=}CH_2$
gelsevirine (28)	OCH_3	H_2	$HC{=}CH_2$
21-oxogelsevirine (29)	OCH_3	O	$HC{=}CH_2$
19-hydroxydihydro-gelsevirine (30)	OCH_3	H_2	$HO\text{-}CH\text{-}CH_3$

Humantenine-type

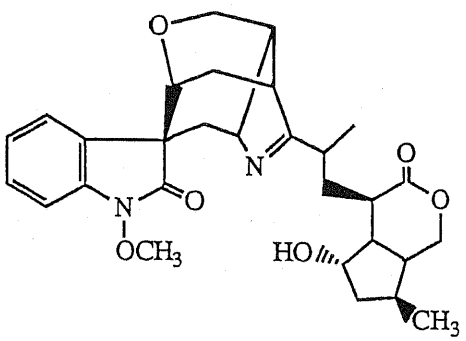


Alkaloids	R_1	R_2	R_3
N-desmethoxyrankinidine (32)	H	H	H
rankinidine (33)	OCH_3	H	H
11-hydroxyrankinidine(34)	OCH_3	H	OH
humantenine (35)	OCH_3	CH_3	H
11-hydroxyhumantenine (36)	OCH_3	CH_3	OH
humantenirine (37)	OCH_3	H	OCH_3
11-methoxyhumantenine (38)	OCH_3	CH_3	OCH_3

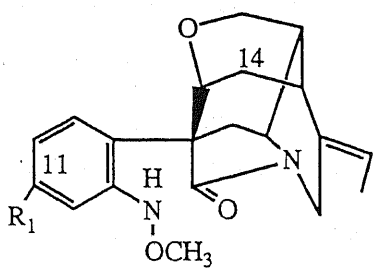
Gelsedine-type



Alkaloids	R_1	R_2	R_3
gelsedine (39)	H	H	H_2
14-hydroxygelsedine (40)	H	OH	H_2
gelsemicine (41)	OCH_3	H	H_2
14-hydroxygelsemicine (42)	OCH_3	OH	H_2
gelsenicine (43)	H	H	H_2
(or 20-N4-didehydrogelsedine)			
14-hydroxygelsenicine (44)	H	OH	H_2
19-oxogelsenicine (45)	H	OH	O



elegansamine (46)



gelsemamide (47) : R=H

11-methoxygelsemamide (48) : R= OCH_3

2. Chemistry of *Mostuea* Alkaloids

The genus *Mostuea* consists of 8 species : one in the northern South America and the rest in tropical Africa. The alkaloids of *Mostuea* and *Gelsemium* are very similar.

2.1 Alkaloids Isolated from Species of *Mostuea* .

The alkaloids reported to be present in the species of *Mostuea* are summarized as follows:

2.1.1 *Mostuea brunonis* Didr. var. *brunonis* f. *augustifolia*

Roots	:	sempervirine (Onanga and Khuong-Huu, 1980)
Stems	:	14-hydroxygelsemicine (Onanga and Khuong-Huu, 1980)
Stems and Leaves	:	mostueine, 20-(N_4)-dehydrogelsemicine, gelsemicine (Onanga and Khuong-Huu, 1980)

2.1.2 *Mostuea buchholzii* Engl.

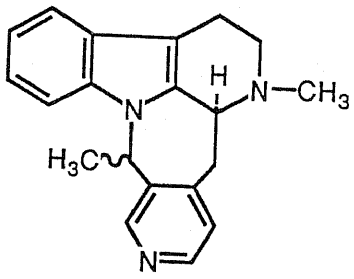
Branches and Leaves	:	sempervirine (Gellert and Schwarz, 1951)

2.1.3 *Mostuea stimulans* A. Chev.

Not mentioned	:	sempervirine, gelsemine (Paris and Moyse-Mignon, 1949)

2.2 Structures of *Mostuea* Alkaloids

Only very few studies have been made on the alkaloids of *Mostuea* and much more certain informations are undoubtedly needed. So far reported almost all of the *Mostuea* alkaloids are those found also in the genus *Gelsemium* and their structures have already been shown. Furthermore, mostueine (49) isolated from *Mostuea brunonis* Didr. var. *brunonis* f. *augustifolia* is of the indole group, but the structure is not similar to those types of *Gelsemium* indole alkaloids.



mostueine (49)

3. Chemistry of *Gardneria* Alkaloids

Japanese representatives of the small genus *Gardneria*, consisting of 5-6 species occurring from India to Central Japan, have been examined in some detail by Sakai and his coworkers, and more than 18 alkaloids have been isolated.

3.1 Alkaloids isolated from Species of *Gardneria*

The alkaloids reported to be found in the species of *Gardneria* are summarized as follows :

3.1.1 *Gardneria insularis* Nakai

Roots and stems : gardneramine, gardnerine, gardnutine, 18-hydroxy-gardnutine, 18-demethoxygardneramine (Haginiwa *et al.*, 1970; Bisset and Phillipson, 1976)

3.1.2 *Gardneria multiflora* Makino

Roots and stems : gardneramine, gardfloramine, 19-(E)-18-demethoxy-gardneramine, 18-desmethoxygardfloramine, chitosenine (alkaloid F)(Sakai *et al.*, 1975)

: gardneramine N-oxide, exomethylene compound,

18-demethylgardneramine (alkaloid G), gardmultine (alkaloid E), alkaloid I, alkaloid J, alkaloid N, alkaloid M, alkaloid L (Sakai, 1976; Sakai *et al.*, 1977)

: 18-demethoxygardmultine (Sakai *et al.*, 1982)

3.1.3 *Gardneria nutans* Sieb. et Zucc.

Roots and stems : gardneramine, gardnerine, gardnutine, hydroxygardnutine (Haginiwa *et al.*, 1967; Sakai, Kubo and Haginiwa, 1969; Sakai *et al.*, 1969; Sakai *et al.*, 1971)

: 19-(E)-18-desmethoxygardneramine (Sakai, 1976)

: 18-hydroxygardnerine (Aimi *et al.*, 1978)

3.1.4 *Gardneria shimadai* Hayata

Roots and stems : gardneramine, 18-demethylgardneramine, gardmultine, chitosenine (Haginiwa *et al.*, 1970; Bisset and Phillipson, 1976)

3.1.5 *Gardneria liukiuensis* Hatsushima

: Alkaloids of this species were proved to be quite similar to that of *Gardneria multiflora* Makino (Sakai *et al.*, 1977)

3.1.6 *Gardneria angustifolia* Wall.

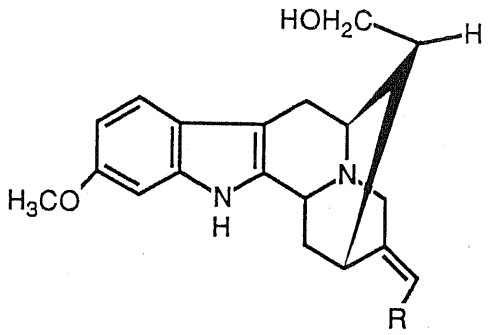
: The leaves of this plant collected from Nepal in 1954, gave an extract which afforded a+++ test; tlc indicated the presence of three major and three minor alkaloids (Bisset and Phillipson, 1976)

3.2 Structures of *Gardneria* Alkaloids

The alkaloids isolated from the species of *Gardneria*, can be divided into three groups, indole, oxindole and imino-ether.

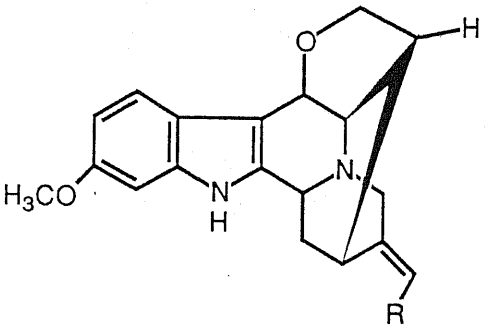
3.2.1 Indole alkaloids

The indole alkaloids isolated from the species of *Gardneria* are gardnerine (50), 18-hydroxygardnerine (51), gardnutine (52), 18-hydroxygardnutine (53) (Sakai *et al.*, 1969; Aimi *et al.*, 1978).



gardnerine (50) : R=CH_3

18-hydroxygardnerine (51) : R=CH_2OH

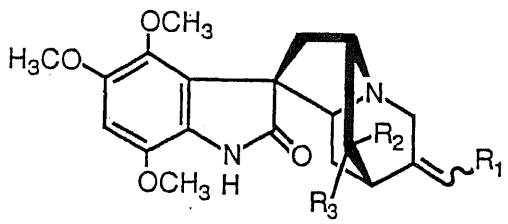


gardnutine (52) : R=CH_3

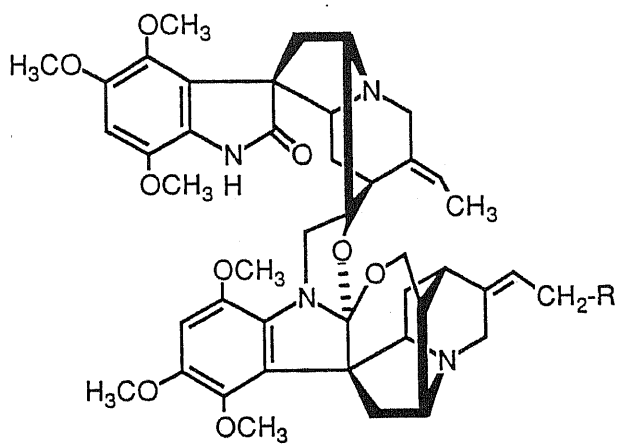
18-hydroxygardnutine (53) : R=CH_2OH

3.2.2 Oxindole alkaloids

The oxindole alkaloids isolated from the species of *Gardneria* are alkaloid M (54), chitosenine (alkaloid F)(55), alkaloid L (56), alkaloid I (57), alkaloid N (58), alkaloid J (59), exomethylene compound (60), and dimeric alkaloids, gardmultine (61), demethoxygardmultine (62) (Sakai, 1976; Sakai *et al.*, 1977; Aimi *et al.*, 1978; Sakai *et al.*, 1982).



Alkaloids	R_1	R_2	R_3	C_{19}
alkaloid M (54)	CH_2OH	H	CH_2OH	*Z*
chitosenine (55)	CH_3	OH	CH_2OH	*E*
alkaloid L (56)	CH_2OH	CH_2OH	H	*Z*
alkaloid I (57)	CH_2OCH_3	H	CH_2OH	*Z*
alkaloid N (58)	CH_2OCH_3	OH	CH_2OH	*Z*
alkaloid J (59)	CH_2OCH_3	CH_2OH	H	*Z*
exomethylene compound (60)	CH_2OCH_3	-	CH_2	*Z*

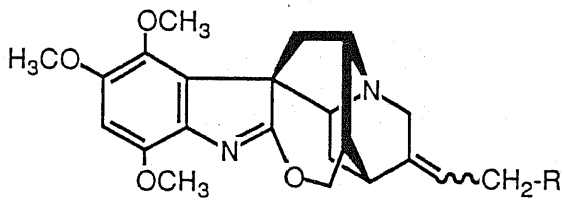


gardmultine (61) : R=OCH_3

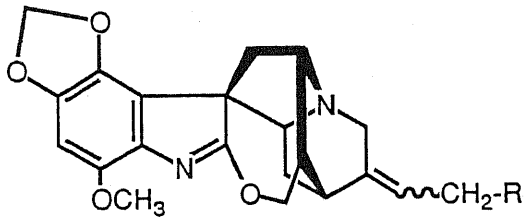
demethoxygardmultine (62) : R=H

3.2.3 Imino-ether alkaloids

The imino-ether alkaloids isolated from the species of *Gardneria* are gardneramine (63), gardneramine N-oxide (64), 18-demethyl-gardneramine (65), 18-demethoxygardneramine (66), gardfloramine (67), demethoxygardfloramine (68) (Bisset and Phillipson, 1976; Sakai, 1976; Sakai *et al.*, 1977).



Alkaloids	R	C_{19}	N(b)
gardneramine (63)	OCH_3	*Z*	
gardneramine N-oxide (64)	OCH_3	*Z*	
18-demethylgardneramine (65)	OH	*Z*	
18-demethoxygardneramine (66)	H	*E*	



Alkaloids	R	C_{19}
gardfloramine (67)	OCH_3	*Z* or *E*
demethoxygardfloramine (68)	H	*Z* or *E*

4. Chemistry of *Strychnos* Alkaloids

The genus *Strychnos* comprises about 200 species distributed through out the tropics and subtropics of the world. There are about 71 species in Central and South America; 75 species in Africa and about 44 species in Asia (Balgooy, 1966; Leeuwenberg, 1980). About 200 alkaloids have been isolated from various *Strychnos* species and new types of alkaloid are continually being encountered (Bisset, 1980).

4.1 Alkaloids isolated from Asian species of *Strychnos*

The alkaloids reported to be present in the Asian species of *Strychnos* are summarized as follows :

Strychnos angustiflora Benth.

: angustine, angustoline, angustidine (Au, Cheung and Sternhell, 1973)

S. axillaris Colebr. (*S. psilosperma* F.v. Muell.)

: strychnospermine (deacetylstrychnospermine), spermostrychnine (Shaw and De la Lande,1948; Anet, Hughes and Rstchie, 1953; Anet

and Sir Robinson, 1955)

S. ignatii Berg.(*S. ovalifolia* Wall. ex G. Don, *S. tieute* Lesch., *S. cuspidata* A.W. Hill)

: strychnine, brucine, pseudostrychnine,pseudobrucine,12-hydroxy-11-methoxy-N-methyl *sec.*-pseudostrychnine, N-methyl *sec.*-pseudo-β-colubrine, diaboline (Casinovi, Marini-Bettolo and Bisset, 1962; Casinovi *et al.*, 1964; Bisset *et al.*, 1965; Bisset and Woods, 1966; Bisset, Choudhury and Walker, 1974; Bisset and Walker, 1974)

: diaboline, icajine, novacine, vomicine (Bisset and Phillipson, 1976)

: longicaudatine (Massiot *et al.*,1983)

S. lucida R. Br. (*S. ligustrina* Bl.)

: strychnine, β-colubrine, brucine (Anet, Hughes and Rstchie, 1953; Mathis and Duquenois, 1963)

: strychnine, brucine, brucine N-oxide, pseudobrucine, β-colubrine, normacusine B, pseudostrychnine, diaboline, α-colubrine, β-colubrine, akuammidine, longicaudatine (Bavovada, 1983)

S. nux-vomica L.

: strychnine, α-colubrine, β-colubrine, 12-hydroxystrychnine, brucine, strychnine N-oxide, brucine N-oxide, pseudostrychnine, pseudo-α-colubrine, pseudo-β-colubrine, pseudobrucine, icajine, vomicine, novacine, isostrychnine, (+)-C-mavacurine, cantleyine (Warnat, 1931; Martin *et al.*, 1952; Chatterjee and Basu, 1967; Bisset and Phillipson, 1971; Bisset and Phillipson, 1973; Heimberger and Scott, 1973; Bisset and Choudhury, 1974a; Galeffi, Delle Manache and Marini-Bettolo, 1974)

: longicaudatine (Massiot *et al.*, 1983)

S. Wallichiana Steud. ex DC. (*S. colubrina* L., *S. gauthierana* Pierre ex Dop)

: strychnine, brucine, 12-hydroxy-11-methoxy-strychnine, strychnine N-oxide, brucine N-oxide, pseudostrychnine, pseudobrucine, icajine, vomicine, N-methyl-*sec.*-pseudo-β-colubrine, novacine, 15-hydroxy-icajine, 15-hydroxynovacine, icajine N-oxide, N-cyano-*sec.*-pseudostrychnine, N-cyano-*sec.*-pseudobrucine (Mathis and Duquenois, 1963; Bisset and Phillipson, 1971; Bisset and Phillipson,1973a; Choudhury,1972; Bisset, Choudhury and Walker, 1974; Bisset and Choudhury, 1974b).

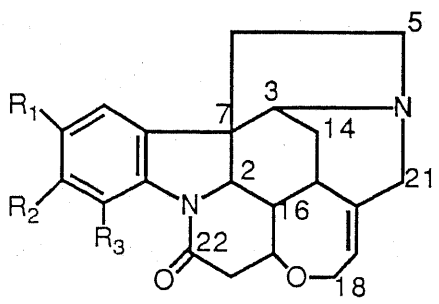
4.2 Structures of Asian *Strychnos* Alkaloids

The alkaloids isolated from the Asian species of *Strychnos*, can be divided into three series, *Normal* series, *Pseudo* series and N-Methyl-*sec.-pseudo* series.

4.2.1 *Normal* Series

This series has strychnine as a model structure and includes aromatic substituted compounds, these alkaloids are strychnine (69), 12-hydroxystrychnine (70), β-colubrine (71), α-colubrine (72), brucine (73), 12-hydroxy-11-methoxystrychnine (74).

Normal Series

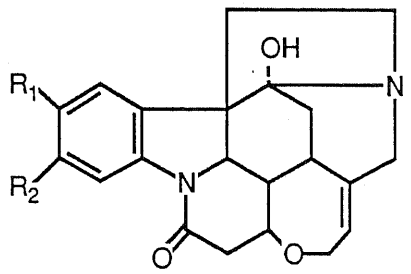


Alkaloids	R_1	R_2	R_3
strychnine (69)	H	H	H
12-hydroxystrychnine (70)	H	H	OH
β-colubrine (71)	OCH_3	H	H
α-colubrine (72)	H	OCH_3	H
brucine (73)	OCH_3	OCH_3	H
12-hydroxy-11-methoxystrychnine (74)	H	OCH_3	OH

4.2.2 *Pseudo* Series

The alkaloids of this series are oxidation products of the *normal* series at the C_3 position to form a carbinol amine. This series may be called the 3-hydroxy series, and the alkaloids of this series isolated from Asian species of *Strychnos* are pseudostrychnine (75), pseudo-β-colubrine (76), pseudo-α-colubrine (77), pseudobrucine (78).

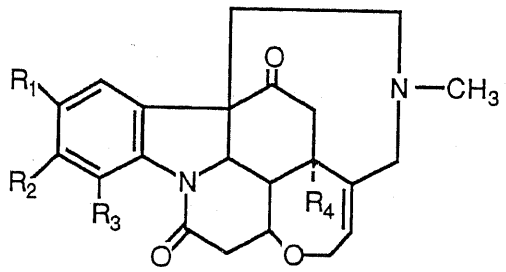
Pseudo Series



Alkaloids	R_1	R_2
pseudostrychnine (75)	H	H
pseudo-β-colubrine (76)	OCH_3	H
pseudo-α-colubrine (77)	H	OCH_3
pseudobrucine (78)	OCH_3	OCH_3

4.2.3 N-Methyl-*sec.-pseudo* series

The alkaloids of this series have a carbonyl function at C_3 and a methyl group attached to the basic nitrogen. The alkaloids of this series are icajine (79), vomicine (80), novacine (81), 15-hydroxyicajine (82), 15-hydroxynovacine (83), 12-hydroxy-11-methoxy-N-methyl-sec.-pseudo-strychnine (84), N-methyl-sec.-pseudo-β-colubrine (85).

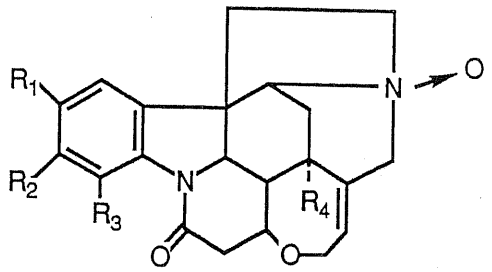


Alkaloids	R_1	R_2	R_3	R_4
icajine (79)	H	H	H	H
vomicine (80)	H	H	OH	H
novacine (81)	OCH_3	OCH_3	H	H
15-hydroxyicajine (82)	H	H	H	OH
15-hydroxynovacine (83)	OCH_3	OCH_3	H	OH
12-hydroxy-11-methoxy-N-methyl-sec.-pseudostrychnine (84)	H	OCH_3	OH	H
N-methyl-sec.-pseudo-β-colubrine (85)	OCH_3	H	H	H

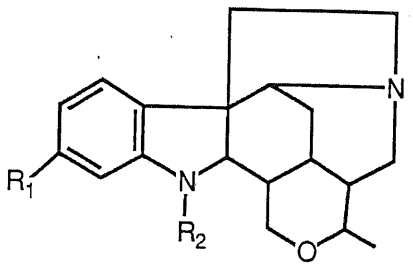
4.2.4 Other Asian *Strychnos* alkaloids

Other indole alkaloids isolated from Asian species of *Strychnos* are strychnine N-oxide (86), brucine N-oxide (87), spermostrychnine (88), strychnospermine (89), deacetylstrychnospermine (90), diaboline (91),

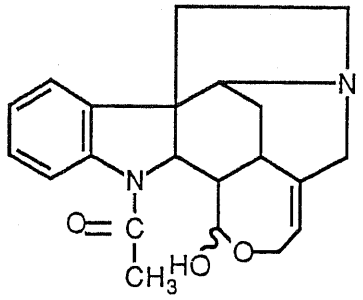
isostrychnine (92), angustine (93), angustoline (94), angustidine (95), C-mavacurine (96), normacusine B(97), akuammidine (98) and longicaudatine (99).



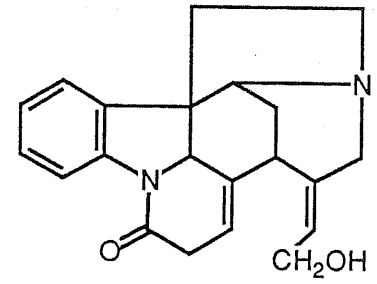
strychnine N-oxide (86) : $R_1=R_2=R_3=H$
brucine N-oxide (87) : $R_1=R_2=OCH_3$, $R_3=H$



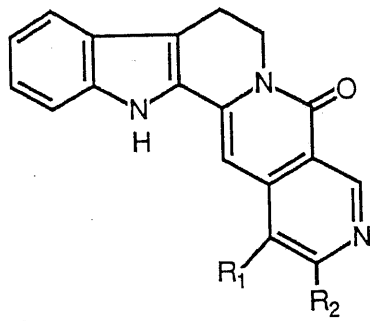
Alkaloids	R_1	R_2
spermostrychnine (88)	H	$COCH_3$
strychnospermine (89)	OCH_3	$COCH_3$
deacetylstrychnospermine (90)	OCH_3	OH



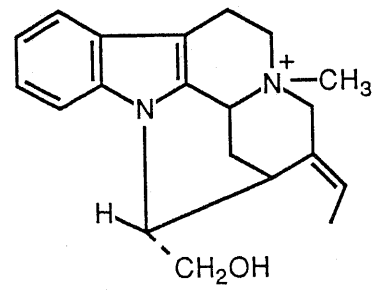
diaboline (91)



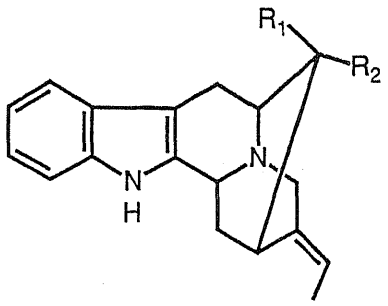
isostrychnine (92)



Alkaloids	R_1	R_2
angustine (93)	$CH{=}CH_2$	H
angustoline (94)	$CH(OH)\text{-}CH_3$	H
angustidine (95)	H	CH_3

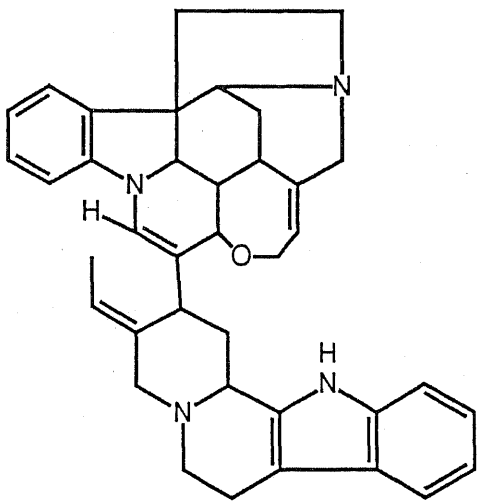


C-mavacurine (96)



normacusine (97) : R_1=H R_2=CH_2OH

akuammidine (98) : R_1=$COOCH_3$ R_2=CH_2OH



longicaudatine (99)

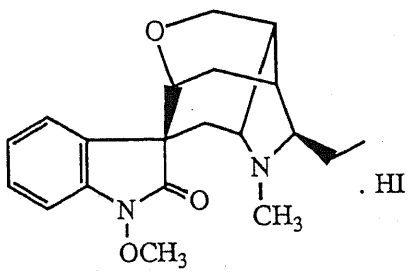
5. Reactions of Alkaloids from Loganiaceae

5.1 Reactions of *Gelsemium* alkaloids

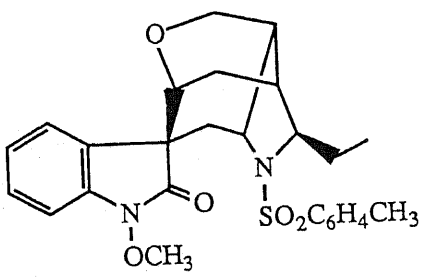
Gelsemine affords acetylgelsemine when boils for one hour with acetic anhydride in the presence of a trace of pyridine (Moore, 1911). On catalytic hydrogenation over palladium, gelsemine gives rise to dihydrogelsemine (Chu and Chou, 1940). Over Adams' platinum catalyst, the dihydro-derivative and then more slowly hexahydrogelsemine are produced. On the other hand, gelsemine is recovered unchanged after reduction with sodium and cyclohexanol

(Forsyth, Marrian and Stevens, 1945). When treated gelsemine in toluene with dispersion of sodium hydride in mineral oil and methyl iodide, N(a)-methyl-gelsemine methiodide is produced (Roe and Gates, 1960).

The degradation of gelsedine (39) to demethoxygelsedine (100), prepared by treatment of solution of gelsedine in ether and tetrahydrofuran, and solution of lithium in liquid ammonia for 30 min. This can also be done by refluxing the solution of gelsedine in benzene and t-butyl alcohol, while sodium is added slowly over a 2.5 hr. period. On treatment with acetic anhydride and pyridine for 18 hr. at room temperature, demethoxygelsedine yields N_b-acetyldemethoxygelsedine (101). On the other hand, a mixture of gelsedine, sodium bicarbonate, methyl iodide and absolute ethanol is refluxed for 24 hr. N_b-methylgelsedine hydroiodide (102) is performed. Gelsedine also gives N_b-*p*-toluenesulfonylgelsedine (103) by treatment of the solution of gelsedine and *p*-toluenesulfonyl chloride in pyridine for 1 hr. on a steam bath and at room temperature for 12 hr. (Wenkert *et al.*, 1963). These reactions are shown as follows:

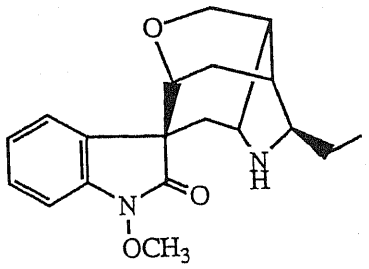


N_b-methylgelsedine hydroiodide (102)

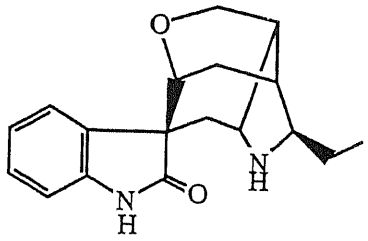


N_b-*p*-toluenesulfonylgelsedine (103)

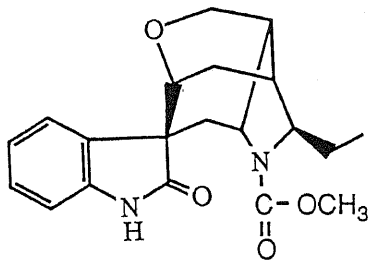
(39)



gelsedine (39)



demethoxygelsedine (100)

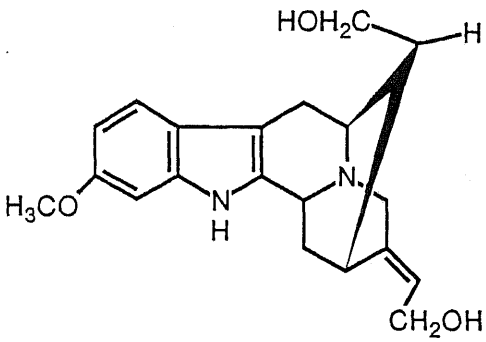


N-acetyldemethoxygelsedine (101)

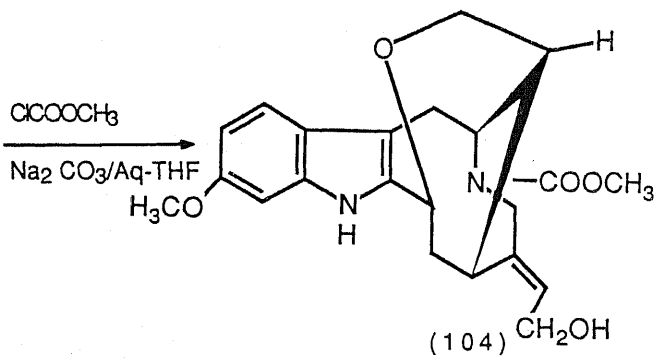
5.2 Reactions of *Gardneria* alkaloids

Sakai and his coworkers reported the biomimetic transformation of 18-hydroxygardnerine (51) to 11-methoxykoumine (107). Upon treatment of 18-hydroxygardnerine with methyl chlorocarbonate and an excess

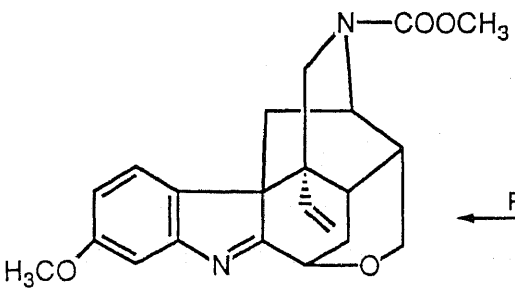
of Na_2CO_3 in aq-THF solution gave N_b-carbomethoxy-apo-18-hydroxygardnerine (104). Allyl alcohol was converted to acetate and methoxy carbonate. The acetate was brought to indole anion with NaH in DMF solution and in the presence of triphenylphosphine, $Pd(OAc)_2$ was added to the DMF solution under Ar and the compound (105) was obtained. Upon reduction with $LiAlH_4$ at rt., (105) was transformed solely into N_b-methylindoline derivative (106). Oxidation of (116) with $Pb(OAc)_4$ gave rise to 11-methoxykoumine (107). These transformations are shown as follows (Sakai *et al.*, 1986):



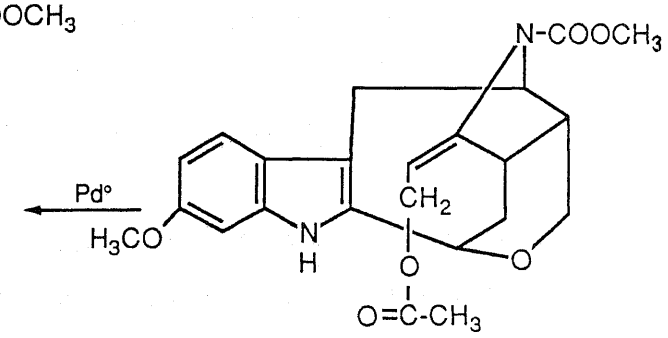
18-hydroxygardnerine (51)



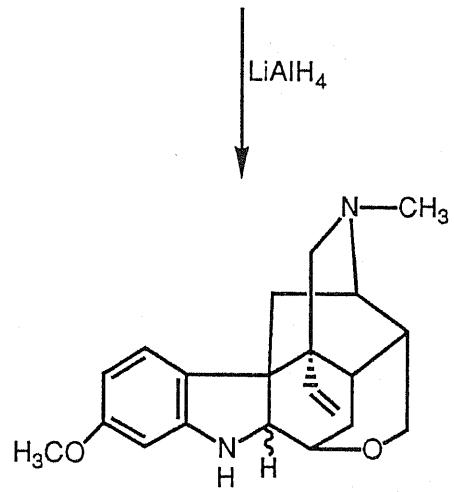
(104)



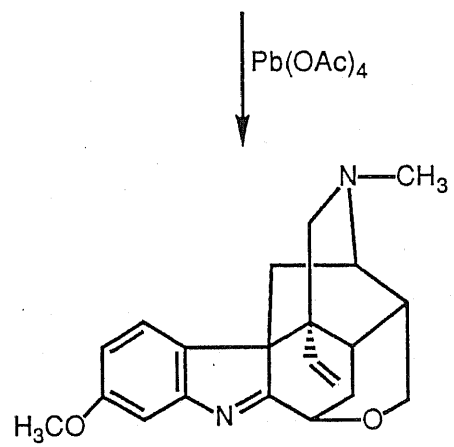
(105)



(105)



(106)



(107)

Biogenesis

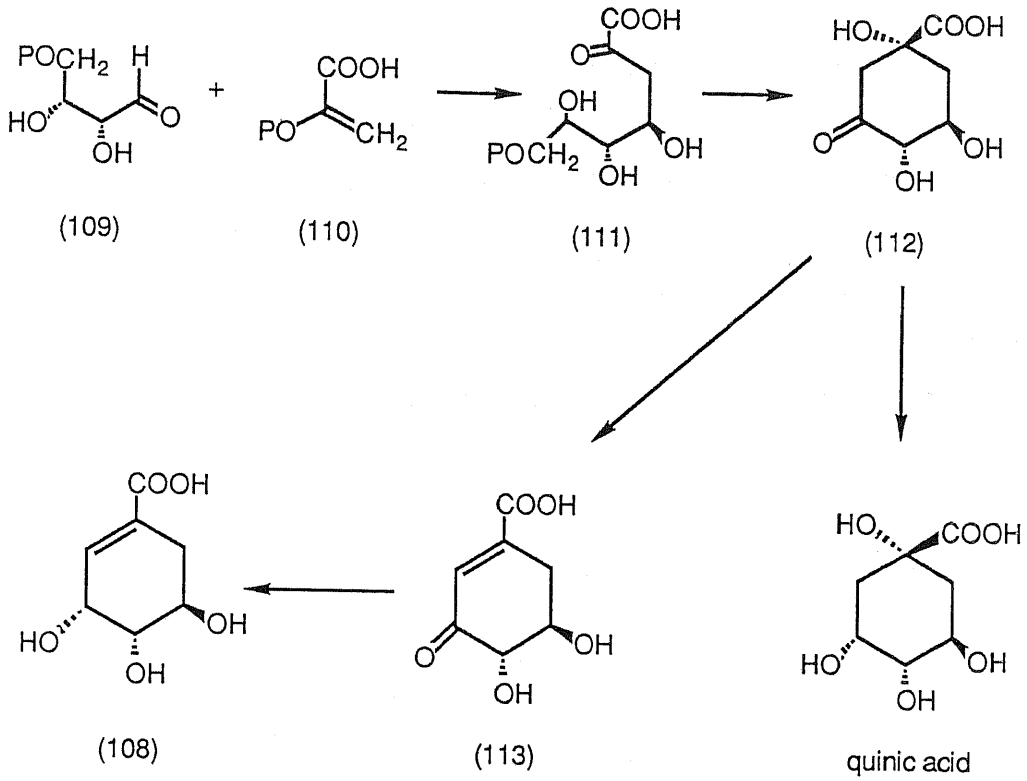
1. Biogenesis of Indole Alkaloids

The biogenesis of indole alkaloids has excited the interest of organic chemists for many years and early speculations were reviewed by Robinson in 1955. Since then radioactive tracer studies have shown that tryptophan is the precursor of the indole portion of the majority of indole alkaloids. Tryptophan itself is derived from shikimic acid. The other portion of indole alkaloids is C_9 or C_{10}-monoterpene moiety, loganin and secologanin (17), which are derived from mevalonate. Loganin and secologanin are also fulfil the conditions for being true precursor of the various types of indole alkaloids (Jackson and Smith, 1968; Kompis, Hesse and Schmid, 1971).

1.1 Formation of Shikimic Acid and Tryptamine

1.1.1 Formation of Shikimic Acid

Tracer studies have confirmed that the formation of shikimic acid (108) in plants follows the same route as that in microorganisms, namely a condensation of D-erythrose-4-phosphate (109) and phosphoenolpyruvate (110) from which 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (111) is obtained. Elimination of phosphoric acid gives the ketone, formally in its enol form, that cyclizes to 3-dehydroquinic acid (112). Shikimic acid is formed by elimination of water and reduction of 3-dehydroshikimic acid (113). Several of the enzymes involved in these transformations have been obtained from plants (Cordell, 1981; Torssell, 1983). The biogenetic pathways is shown as follows :



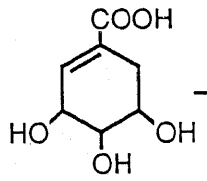
1.1.2 Formation of Tryptamine

Robinson had originally suggested that the two nitrogens and the aromatic portion of all of the then-known indole alkaloids originate from tryptophan via its decarboxylation product, tryptamine. This was later experimentally proved (Kompis, Hesse and Schmid, 1971).

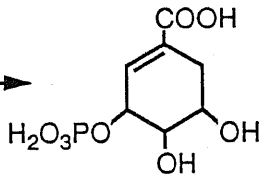
The amino acid tryptophan (120) is derived from shikimic acid (108). By means of a kinase reaction, shikimic acid is formed to be shikimic acid 5-phosphate (114). A reduction involving DPNH or TPNH and a transfer of an amino group from glutamine to the ring are involved in the formation of anthranilic acid (115). In the next phase of the sequence, the formation of the pyrrole ring, phosphoribosyl pyrophosphate (PRPP) provided the two necessary carbon atoms while the carbonyl carbon of anthranilic acid is lost. The

immediate product of the interaction of PRPP and anthranilate is anthranilic ribonucleotide (116), which appears to form anthranilic -l-deoxyribulonucleotide (117). Ring closure, with accompanying production of CO_2 and H_2O gives rise to indole-3-glycerol phosphate (118). Many enzymes catalyse the reversible formation of free indole (119) and triose phosphate or condensation of serine and indole to form tryptophan (120). Tryptamine (130) is formed by decarboxylation of tryptophan (Kompis, Hesse and Schmid, 1971; Lucker, 1972). The reaction is illustrated as follows:

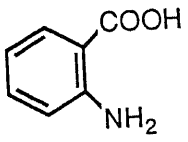
Formation of Tryptamine



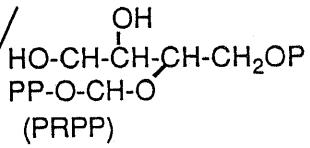
shikimic acid (108)



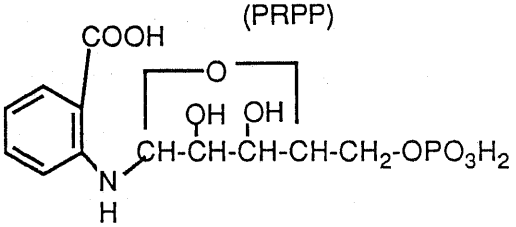
shikimic acid 5-phosphate (114)



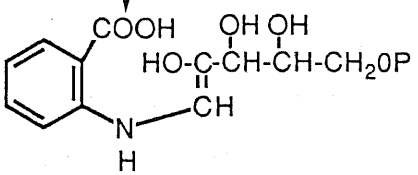
anthranilic acid (115)



(PRPP)

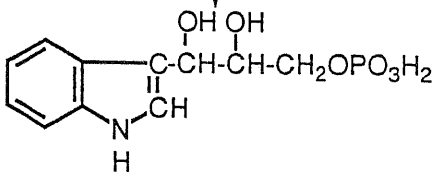


anthranilic ribonucleotide (116)

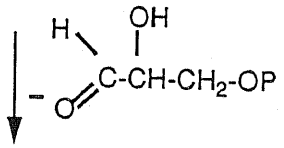


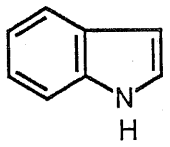
anthranilic-l-deoxyribulonucleotide (117)

(117)

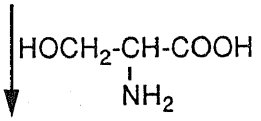


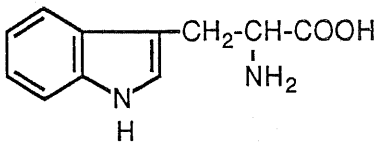
indole-3-glycerol phosphate (118)



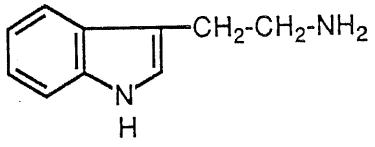


indole (119)





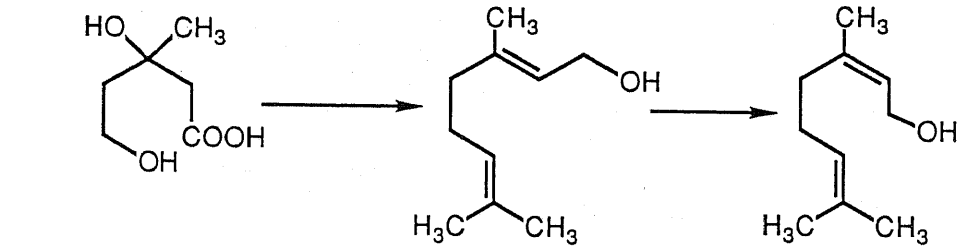
tryptophan(120)



tryptamine(121)

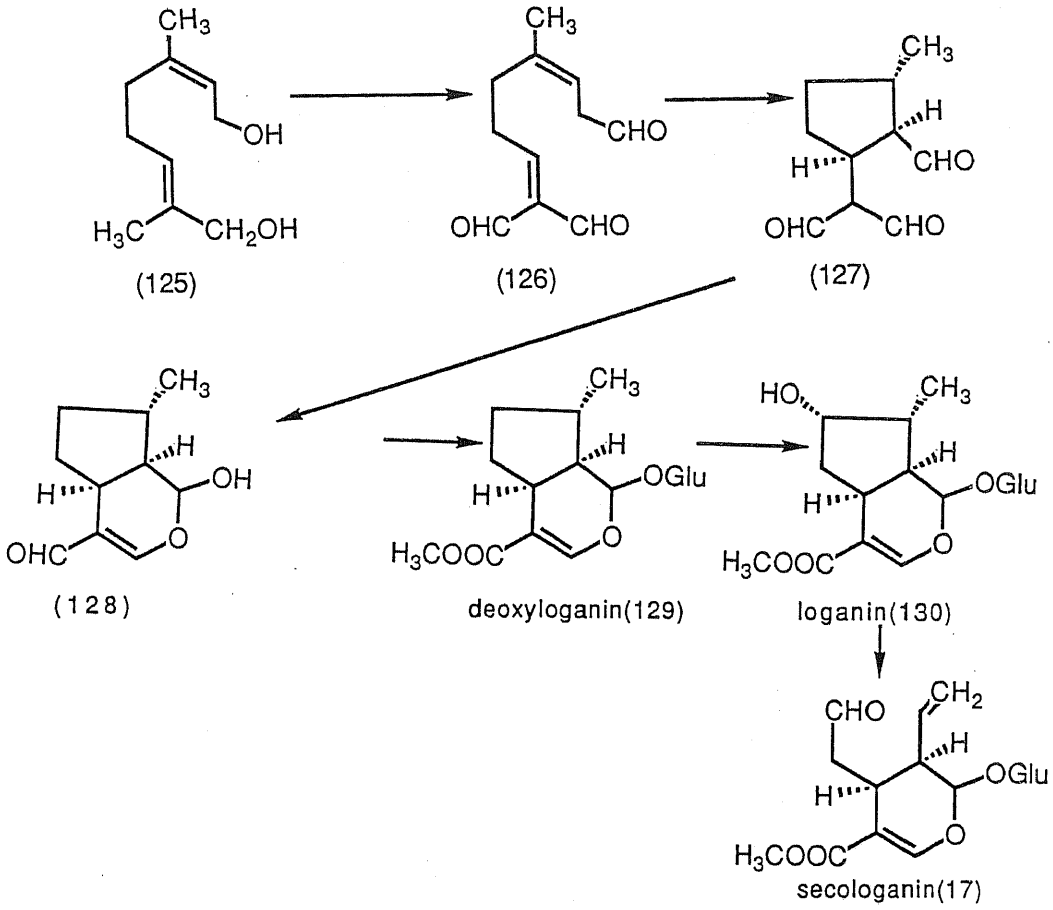
1.2 Formation of Loganin and Secologanin

Mevalonic acid (122) was proved to be the precursor of geraniol through the use of liver and yeast systems. There is an evidence that geraniol (123) and nerol (124) are biosynthetic precursors of loganin (130). It appears that the early steps in the sequence involve (a) a *cis-trans* isomerization of the 2,3-double bond of geraniol (123) to give nerol (124), in which the hydrogen at C-2 of geraniol is retained in nerol, and (b) hydroxylation of nerol at C-10 to give 10-hydroxynerol (125). There is evidence to suggest that at this point further oxidation of C-8 and C-10 occurs to give a trialdehyde such as (126) in which C-8 and C-10 have become equivalent by tautomerization. Probably ring closure occurs at this point to give the monocyclic trialdehyde (127), which exists as the cyclized hemiacetal (128). The next known intermediate is deoxyloganin (129), and it is not difficult to imagine the steps from (128) to (129). Glycosylation possibly of the hemiacetal (128), undoubtedly aids transport. Hydroxylation at C-7 of deoxyloganin (129) occurs stereospecifically to give loganin (130), and the ring cleavage of loganin gives rise to the formation of secologanin (17). The transformations are shown as follows (Battersby, Burnett and Parsons, 1968; Cordell, 1974; Cordell, 1981):



mevalonic acid(122) geraniol(123) nerol(124)

(125)

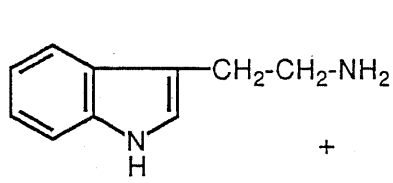


1.3 Formation of Strictosidine and Its Key Role in Alkaloid Biosynthesis.

Strictosidine (131) was derived from the condensation of tryptamine (121) and secologanin (17) by the aid of enzyme strictosidine synthase (Treimer and Zenk, 1979). The role of strictosidine (131) as the sole biosynthetic precursor of a large variety of alkaloids was additionally demonstrated by feeding of (131) to alkaloid producing plants, e.g. *Rhazya stricta, Rhazya orientalis, Amsonia tabernaemontana, Vallesia glabra, Cinchona pubescens* and *Uncaria gambir.* No incorporation of vincoside (132) into the alkaloid fraction of these plants was observed, whereas feeding of strictosidine (131) resulted in the formation of heavily labelled alkaloids (Nagakura *et al.*, 1979). Therefore it can be stated that,

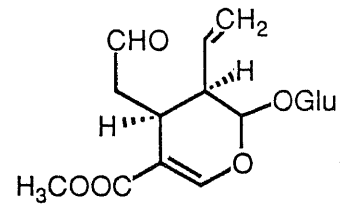
up to now strictosidine (131) is the central precursor for elaboration of the monoterpenoid indole alkaloids derived from the condensation of tryptamine and secologanin in the four plant families Apocynaceae, Loganiaceae, Rubiaceae and Nyssaceae. This key role of strictosidine (131) in alkaloid biosynthesis is summarized as follows (Nagakura, Ruffer and Zenk, 1979; Stockigt, 1980).

Formation of Strictosidine



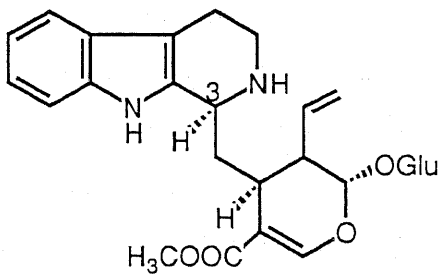
tryptamine (121)

+

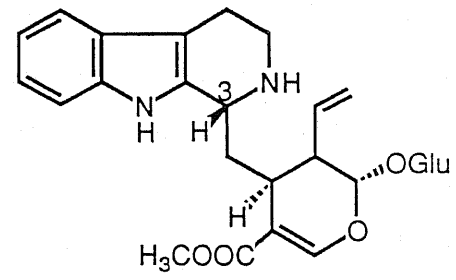


secologanin (17)

strictosidinesynthase

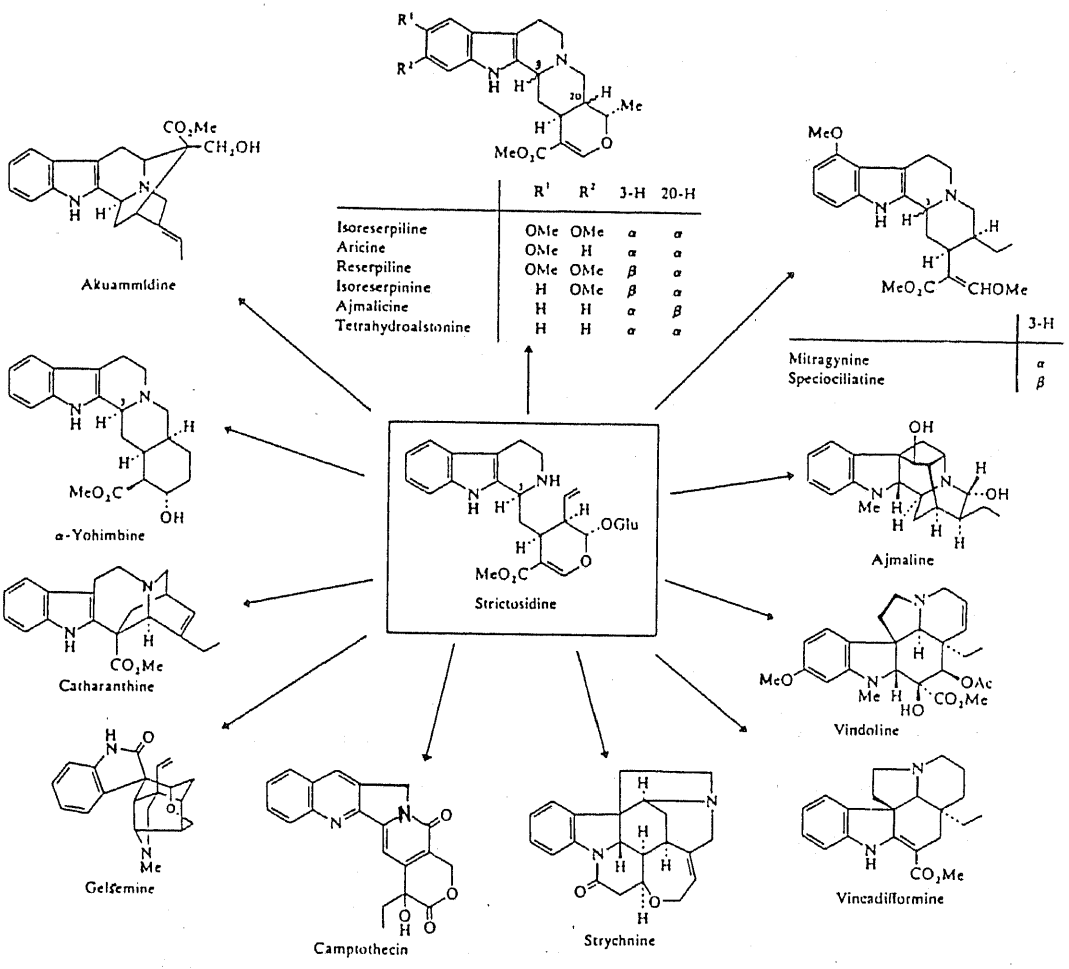


strictosidine (131)



vincoside (132)

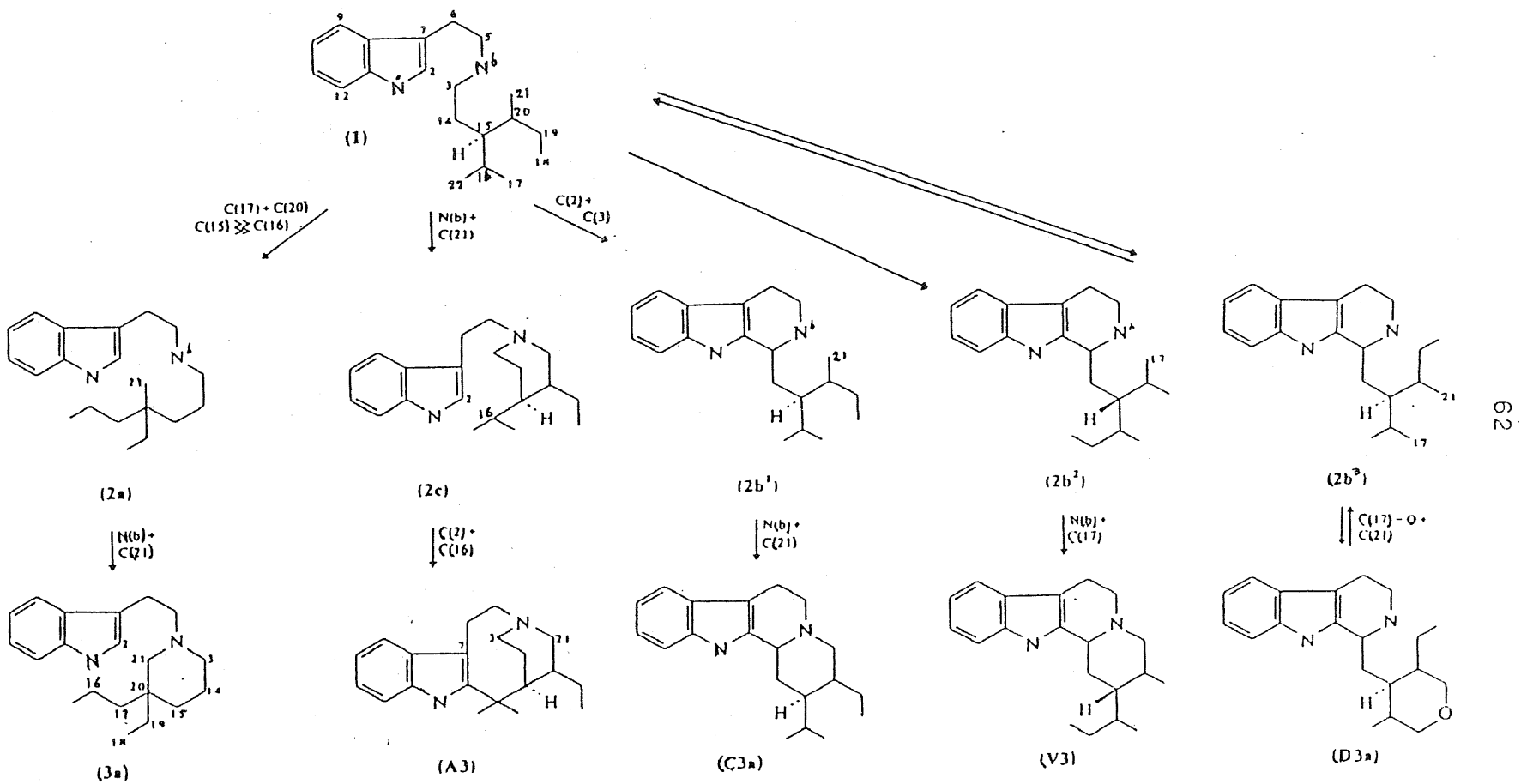
The Key Role of Strictosidine in Alkaloid Biosynthesis

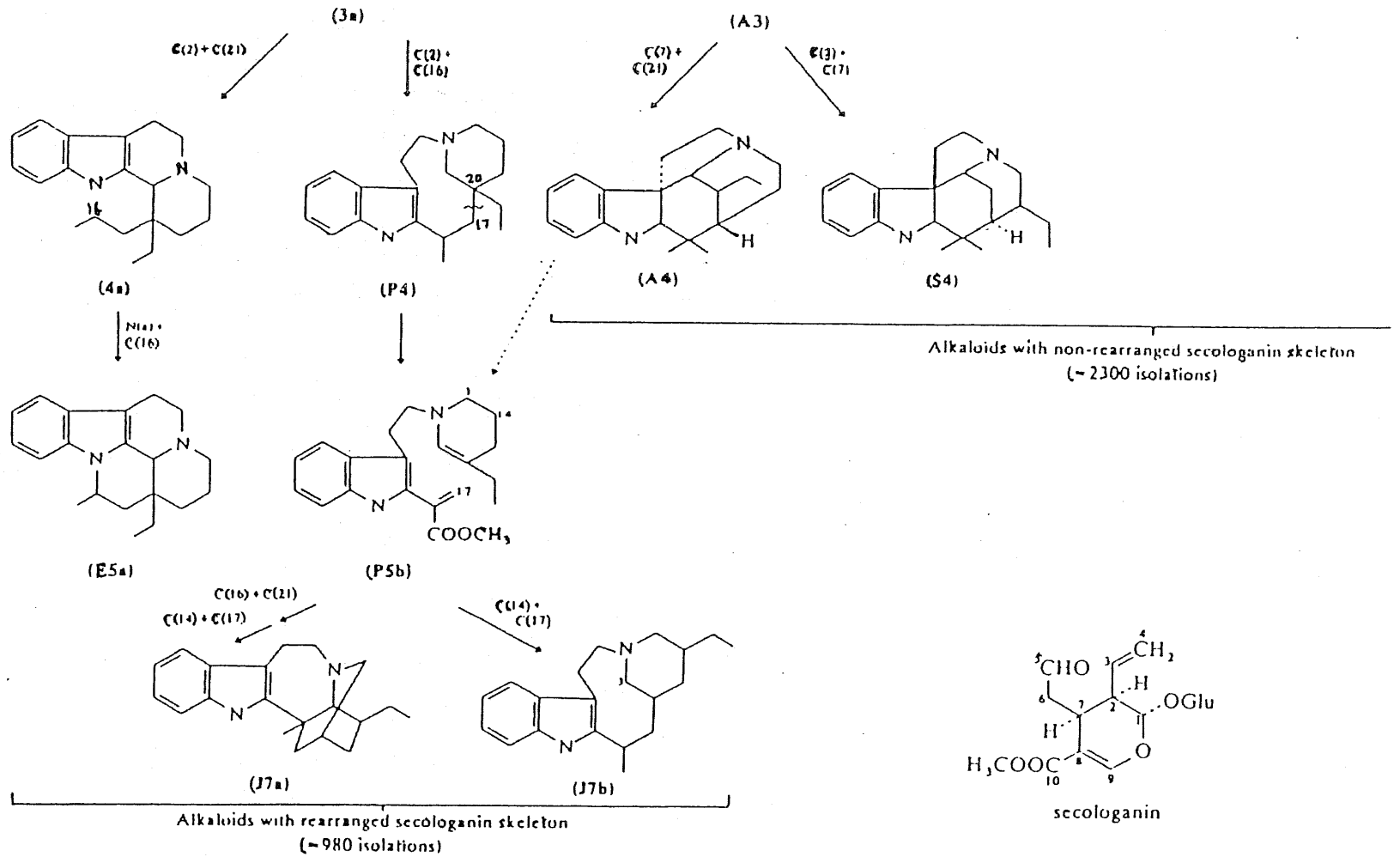


1.4 Biogenetic Relationships of Indole Alkaloids with a C_9- or C_{10}-Monoterpene Moiety.

As mentioned before, indole alkaloids with a C_9- or C_{10}-monoterpene moiety are classified into eight types : corynanthean (C), vincosan (D), vallesiachotaman (V), strychnan (S), aspidospermatan (A), eburnan (E), plumeran

(P) and ibogan (J) types. In a simplified manner, the biogenetic relationships of these main skeletal types are shown in Figure (page 62-63). As an established fact compound D3a is obtained from the condensation of tryptamine (121), or in some other cases tryptophan (120) with secologanin (17). All of the main skeletal types can be derived from D3a. Skeletal D3a can be converted into compound 1 by opening of the C(17)-O-C(21) bond *via* $2b^3$. From compound 1, compounds $2b^1$, $2b^2$, $2b^3$ and 2C can be obtained without rearrangement, or structure 2a by rearrangement of the secologanin portion of the molecule. Ring formation between C(2) and C(3) leads to compound 2b. Intermediates $2b^1$, $2b^2$ and $2b^3$ differ from each other only through rotation about the C(14)-C(15) and C(15)-C(16) bonds respectively. Ring closures between C(21) and N(b) in $2b^1$, and between C(17) and N(b) in $2b^2$ give rise to the main corynanthean-type skeleton C3a and the main vallesiachotaman-type V3, respectively. A new additional bond between C(17)-OH and C(21) in $2b^3$ yields the basic skeleton of vincosan group D3a. Intermediate 2C is obtained by ring closure between C(21) and N(b) in 1. An additional ring closure between C(16) and C(2) in 2C yields A3, the fundamental skeleton of the aspidospermatan group. Starting with A3, S4 is obtained by another ring formation between C(3) and C(7). On the other hand, ring closure between C(21) and C(7) yields A4. Intermediate 2a is derived from 1 by cleavage of the C(15)-C(16) bond followed by the formation of a new bond at C(17)-C(20). Ring closure between C(21) and N(b) leads to 3a, from which 4a and the main skeleton of plumeran group P4 can be derived by additional ring closures [C(2)-C(21) and C(2)-C(16), respectively]. Ring closure [N(a)-C(16)] in 4a yields E5a, the main skeleton of the eburnan group. Cleavage of the C(17)-C(20) bond in P4 forms P3. By further reactions, the main skeletons of ibogan group J7a and J7b can be derived from P3. Further reaction are necessary, starting from C3a, D3, V3, S4, A4, E5a, P4 and J7a, to form derivatives of various other skeletal types (Kisakurek *et al.*, 1983).

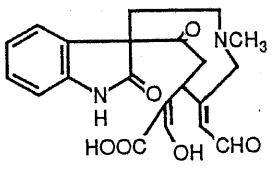

(1)
C(17) + C(20)
C(15) ⋈ C(16)
N(b) +
C(21)
C(2) +
C(3)
(2a)
(2c)
(2b¹)
(2b²)
(2b³)
N(b) +
C(21)
C(2) +
C(16)
N(b) +
C(21)
N(b) +
C(17)
C(17) - O +
C(21)
(3a)
(A3)
(C3a)
(V3)
(D3a)


(3a)
C(2) + C(21)
C(2) + C(16)
(A3)
C(7) + C(21)
C(3) + C(7)
(4a)
(P4)
(A4)
(S4)
Alkaloids with non-rearranged secologanin skeleton
(~ 2300 isolations)
Nb(1) + C(16)
(E5a)
(P5b)
COOCH3
C(16) + C(21)
C(14) + C(17)
C(14) + C(17)
(J7a)
(J7b)
Alkaloids with rearranged secologanin skeleton
(~ 980 isolations)
CHO
CH2
OGlu
H3COOC
secologanin

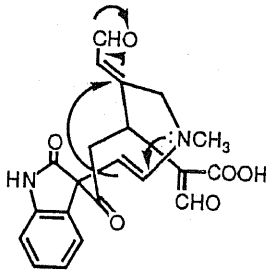
2. Biogenesis of *Gelsemium* Alkaloids

2.1 Biogenesis of Gelsemine

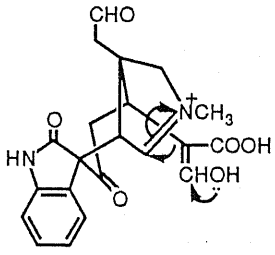
The biogenesis of gelsemine, one of the main alkaloids in *Gelsemium elegans* Benth. and *G. sempervirens* (L.) Jaume Saint-Hilaire has been proposed by Conroy and Chakrabarti (1959). They suggested the proposed precursor (133) derived from equivalents of tryptamine and 3,4-dioxyphenylalanine according to accepted principles. Further dehydrogenation at N_b gave (134); Michael addition of the enamine to the conjugated system establishes the quaternary carbon and formed the five membered ring enclosing N_b. The intermediate (135) was disposed to internal Mannich condensation, to give (136), whence decarboxylation, completion of the oxide ring and adjustment of oxidation state resulted in gelsemine (26). The biogenesis of gelsemine is shown as follows (Conroy and Chakrabarti, 1959):



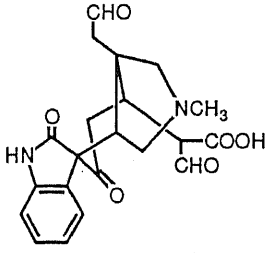
(133)



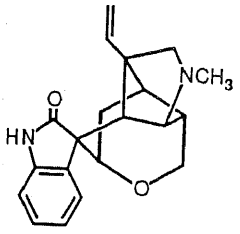
(134)



(135)



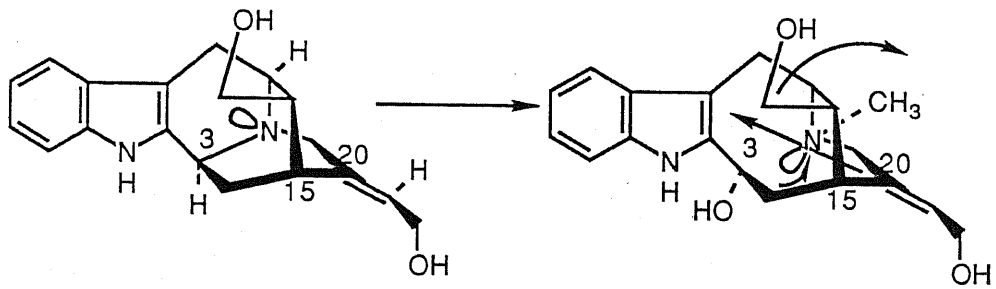
(136)



(26)

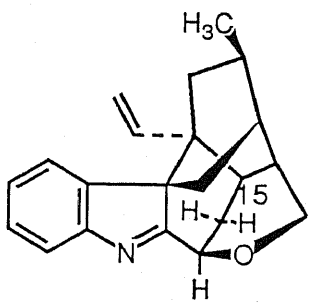
2.2 Biogenesis of Koumine

Lounasmaa and Koshinen proposed a biogenetic route of koumine that it was formed from an 18-hydroxy-deoxysarpagine (137), a close relative of which, hydroxygardnutine (53), has been isolated from *Gardneria nutans* Sieb. et Zucc. The formation of (137) started with oxidative bond rupture between C-3 and N-4 giving rise to the compound (138). Repulsive forces between the nitrogen lone pair electrons and the newly introduced hydroxy function forced the intermediate to capture the conformation of (139) which was further stabilized by hydrogen bonding of the 18-hydroxyl group with the indole N-hydrogen. Expulsion of water and electron pair migrations as depicted would then give rise to the alkaloid koumine (19) (Lounasmaa and Koshinen, 1982). The biogenetic route is shown as follows:

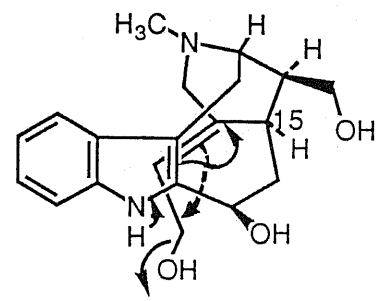


(137)

(138)



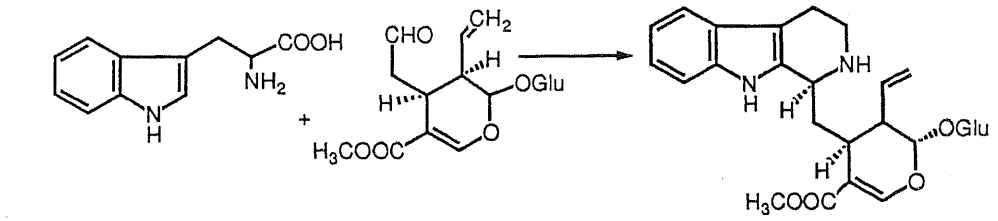
koumine (19)



(139)

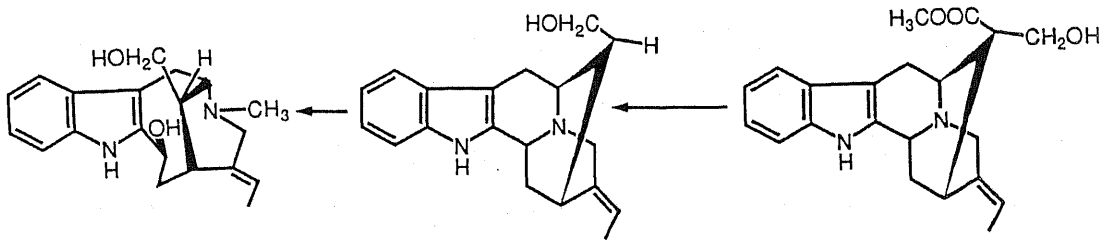
2.3 Biogenesis of Koumine and Gelsemine

Recently, a biomimetic transformation of vobasine to koumine was described and a probable biogenetic pathway was proposed for gelsemine (26) and koumine (17) by Liu and his coworkers. They suggested strictosidine (131), akuammidine (140), (141), vobasindiol (142) and anhydrovobasinediol (143) as intermediates (Liu and Yu, 1987; Liu and Lu, 1988). The biogenetic route is shown as follows:

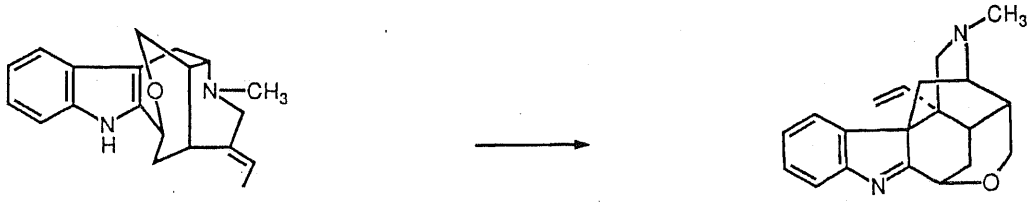


L-Tryptophan (120) Secologanin (17)

Strictosidine (131)

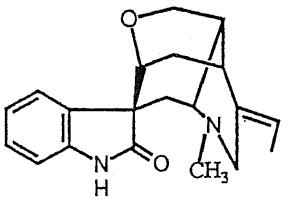


Vobasindiol (142) (141) (140)

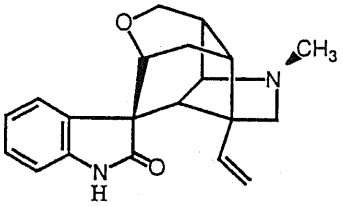


Anhydrovobasinediol (143) Koumine (19)

Anhydrovobasinediol (143)



Humantenine type



Gelsemine (26)

Biological Activity

1. Pharmacology of Gelsemicine

According to its remarkably high toxicity (MLD 0.05-0.06mg/Kg in rabbits, intravenous injection) in comparison to gelsemine (MLD 180mg/Kg), gelsemicine has been considered to be the active principle of *Gelsemium sempervirens* (L.) Jaume St.-Hilaire and attracted the attention of pharmacologists. The main symptoms of toxicity of gelsemicine in mammals are depressed respiration, tremors, incoordination of movement, paralysis of extremities, convulsions, urination, defecation, retchings and salivation. Death apparently results from respiratory failure. The minimum lethal dose in mg per g is for frogs (injection into anterior lymph sac) 0.02 to 0.03, for rats (subcutaneous or intraperitoneal injection) 0.0001 to 0.00012, for rabbits (intravenous injection) 0.0005 to 0.001 (Hou, 1931). In the perfusion on the frog, toad or turtle heart gelsemicine HCl in concentrations of 1-2 mg% produced a primary stimulation followed by a depression of the rate and amplitude of the contractions. A much higher concentration,(about 4mg%) was required to cause this action when the vagal endings were previously paralyzed with atropine. The drug had no action on the spleen or aorta or on the peripheral vessels on the nose, intestine, kidney or leg (Hou, 1932a). Action on intestine, uterus and urinary bladder; gelsemicine HCl in small concentrations caused a slight increase of tone and slight inhibition on pendulum movements of both the isolated intestine and uterus. Larger concentrations lowered the tone and decreased the movements of the intestine but the tone of the uterus was increased. Neither large nor small concentrations had any effect on the urinary bladder muscles. There was a mutual antagonism between gelsemicine and pilocarpine, physostigmine or barium, but none between it and atropine or adrenaline. Neither ergotoxine nor

atropine altered the action of gelsemicine. Similar but less marked results were obtained with the intact intestines and uteri of anesthetized dogs (Hou 1932b). Gelsemicine also increased the hypotensive action of the adrenaline, very small doses of it stimulated respiration but larger doses paralyzed the respiratory centers (Hamet, 1937b). Chen and his coworkers reported that gelsemicine apparently depressed the motor neurons of the brain and spinal cord, this results in generalized muscular weakness. The respiratory failure after the administration of fatal doses was not due to paralysis of the center, but was attributable to that of the spinal motor neurons innervating the respiratory muscles. It had no action on the vagus. The mydriasis, intestinal relaxation and uterine contractions suggested an action upon the sympathetic system (Chen and Chou, 1939).

2. Pharmacology of Gelsemine

Gelsemine inhibited cardiac vagus center and caused contraction of the rabbit uterus, it also stimulated cardiac muscle and acted like atropine (Tamba, 1921). Injection into a dog of 0.2 mg gelsemine-HCl per Kg provoked a fall in blood pressure and a rised in respiratory movements. Gelsemine reinforced the blood pressure activity of adrenaline and suppressed almost completely its apnoeic action (Hamet, 1937a). In the chloralosed dog the single intravenous injection of gelsemine in doses of 0.2 mg/Kg or more produced a marked and prolonged decrease in blood pressure. But when 0.1 mg/Kg was first injected and then, at 5 min. intervals, successively larger doses, a total of 66.25mg/Kg(last dose was 25mg/Kg) was injected in 1.5hrs. without any significant effect on blood pressure. Gelsemine produced a slight vasoconstriction in the kidneys but not in the spleen (Moisset de Espanes, 1938a).

Influence on the effects of adrenaline and excitability of the pneumogastric and the carotid sinus; gelsemine decreased the hypertension action of adrenaline or occlusion of the carotid sinus for the first few min. after its injection, later it may augment the action of adrenaline. Section of the vagi weakened its effects. Eight min. after the injection of 4-10mg/Kg the electrical excitability of the vagus and the sensitivity of the carotid sinus to mechanical stimuli were greatly decreased (Moisset de Espanes, 1938b).

Effect on the electrocardiogram (of the dog); gelsemine of doses larger than 4mg/Kg produced bradycardia, smaller doses decreased vagal tone and produced tachycardia, 60mg/Kg produced clonic convulsions (Moisset de Espanes, 1938c).

Gelsemine injected into the ventral lymph sac of toads and frogs paralyzed the skeletal muscles. The effect was of medullary origin and not due to heterochronism (Moisset de Espanes, 1938d). Gelsemine given intraperitoneally or orally had marked analgesic activity in doses far below the toxic range (Eichler, Hertle and Staib, 1957). Societe Boulonnaise de Recherches et de Diffusion Pharmaceutique (1964) reported that aspirin and gelsemine were combined to give an analgesic preparation and concluded that gelsemine does not have a curarelike action. It is neither ganglioplegic nor a central nervous system sedative. It has a very weak serotonin action and strengthens the hypotensive action of adrenaline. It is a hypotensive in large doses, dose not act on the heart and is not potentiated by barbiturates.

3. Clinical Applications

The *Gelsemium* alkaloids in crude form have been used as analgesic and antispasm agents for a long time. It was also applied in traditional Chinese medicine as a remedy for dangerous skin ulcers, such as miliary vesicles under the nose. The pure alkaloid gelsemine has been used in an analgesic composition (0.5-2mg gelsemine in 300-500mg aspirin), and it was claimed that this preparation has an onset of action about 15min. and lasts about 8hrs. The action of the combination is greater than either drug used alone. In this analgesic doses, gelsemine does not have any observable side effects. More recently, a preparation of the total alkaloids, which consists of seven individual *Gelsemium* alkaloids (as shown by TLC) and which has an LD_{50} in mice of 0.275mg/Kg (intravenous injection), has been used as an analgesic for the palliation of various acute cancer pains, including hepatic cancer. The normal dosage used was 2-3.5mg/day (intravenous injection). It was claimed that good analgesic activity usually lasted 4-6hrs. and the rate of remarkably effective was 66%, effective 24%, and not effective 10%, thus confirming the analgesic activity of *Gelsemium* alkaloids. Furthermore, the preparation does not show any side effect of addiction and therefore has been recommended as a substitute for morphine or dolantin.

Preliminary observation on 16 cancer patients who have been treated with the above-mentioned total alkaloid preparation indicated that symptoms are improved. Thus hepatic cancer patients have claimed disappearance of pain, improvement of appetite, and reduction of ascites, patients suffering esophageal cancer claimed to have the self-feeling of relaxation of pain and disappearance of vomiting and upset stomach as well as the improvement of appetite. These preliminary results are quite encouraging, but certainly

more extensive investigations are needed before the antitumor action of the *Gelsemium* alkaloids can be established (Societe Boulonnaise de Recherches, et de Diffusion Pharmaceutique, 1964; Liu and Lu, 1988).

4. Toxicity of *Gelsemium* Alkaloids

Okanishi (1933) reported that the toxic components of *Gelsemium elegans* Benth. and of *Gelsemium sempervirens* (L.) Jaume St.-Hilaire are nearly the same. Symptoms of intoxication in humans caused by accidental ingestion of *Gelsemium elegans* Benth. has been described as follows. The effect on the digestive system starts with loss of appetite and turn of the stomach, and continues to severe abdominal pain and intestinal bleeding. The effect on the respiratory system presents as breathing difficulties which finally lead to death by respiratory failure. The effect on muscle innervation usually results in generalized muscular weakness and paralysis of the limbs. The effect on the circulatory system starts with heartbeat disorders and a drop in blood pressure, but heart failure is not a common cause of death. In addition to dilation of pupils, a drop in body temperature and proliferation of white blood cells have also been observed. It is interesting to note that the toxicity of *Gelsemium* species depends not only on the individual alkaloids present but also on the route of administration as well as on the animal used. For example, the LD_{50} values of gelsemine in mice are 1240, 405 and 133mg/Kg, respectively, depending on whether the drug is administered orally, intraperitoneally, or intravenously. (Liu and Lu, 1988). Gelsenicine, the toxic alkaloid from *Gelsemium elegans* Benth. proves to be the most toxic of *G. elegans* Benth. alkaloids, the LD_{50} being 185μg/Kg (mice, intraperitoneal injection) (Du. *et al.*, 1982).

PART III

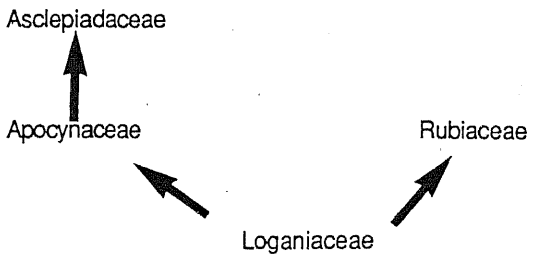
DISCUSSION AND CONCLUSION

DISCUSSION AND CONCLUSION

I. General Discussion

Indole alkaloids can be divided into two main classes. The first class is that of the simple indole alkaloids. They do not present a structural uniformity, having only the indole nucleus or a direct derivative of it as a common feature, e.g. harman. The indole bases of the second class comprise two structure-elements, tryptamine (121) or tryptophan (120) with an indole nucleus and a C_9- or C_{10}-monoterpene moiety, derived form secologanin (17). Very probably, because of both of the common components and the biogenetic relationships, the occurrence of this second class of indole alkaloids is more specific and thereby suitable for comparative chemotaxonomic considerations.

The second class of indole bases can be classified into 8 types, according to the structural characteristics of their skeletons. They are corynanthean (C-type), vincosan (D-type), vallesiachotaman (V-type), strychnan (S-type), aspidospermatan (A-type), eburnan (E-type), plumeran (P-type) and ibogan (J-type). The total number of these alkaloid isolations adding up to 3302, and more than 99.8% of the isolations are distributed among three plant families; Loganiaceae, Apocynaceae and Rubiaceae. Having remarkable morphological similarities, these three plant families have been classified botanically in close relationship which can be shown as follows (Kisakurek and Hesse, 1980):



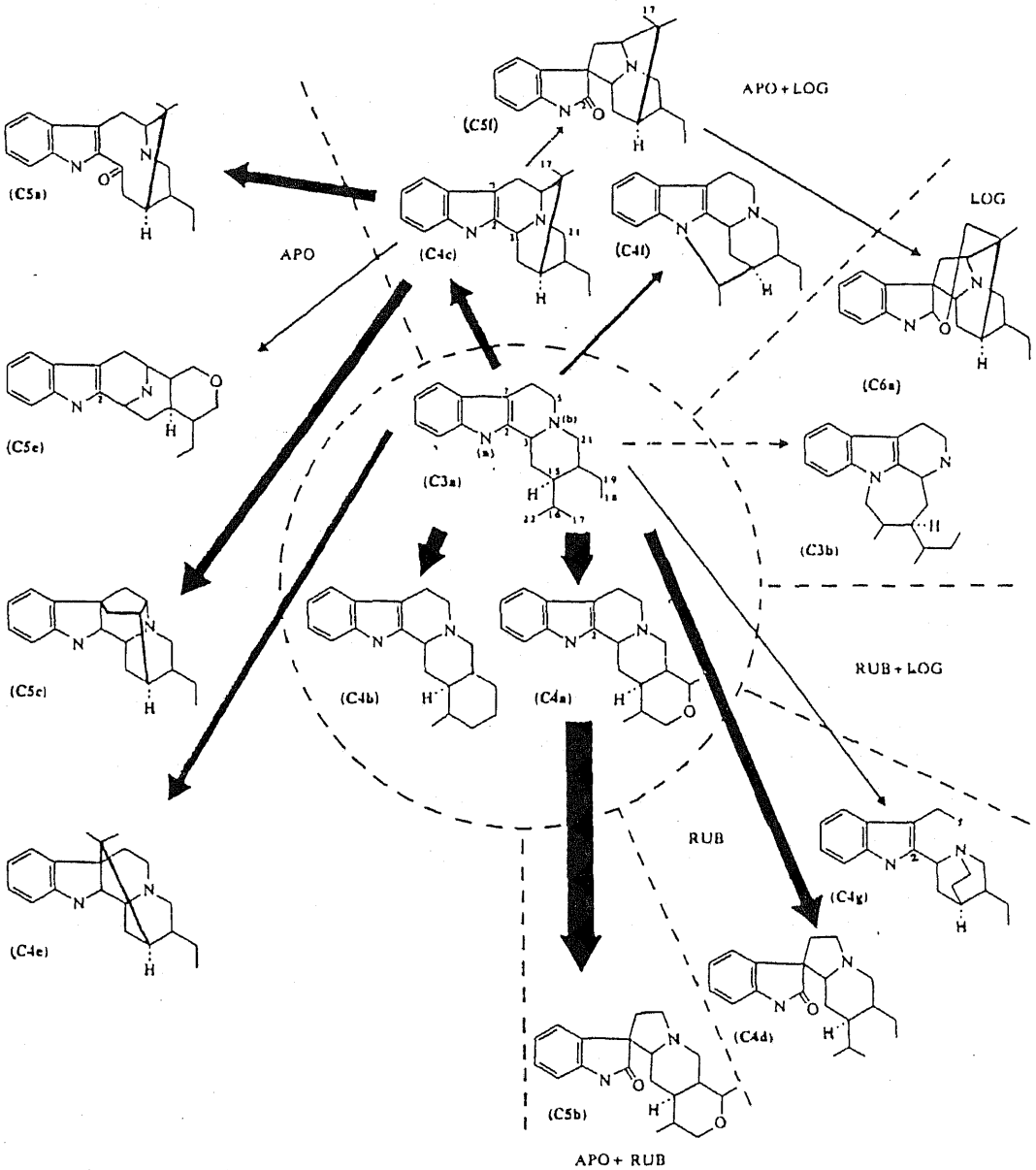
The 8 types of the second class of indole alkaloids can be divided biogenetically into two main groups; the C-, D-, V-, S- and A-types containing a skeleton with a nonrearranged secologanin moiety and E-, P- and J-types with a rearranged secologanin moiety. This argument is confirmed, in addition to the similar skeletal constitutions, also by the fact that all of the C-, D-, V-, S- and A-type alkaloids with known absolute configuration show the same configuration at C(15) as secologanin at C(7). The alkaloids with a rearranged secologanin moiety (E-, P- and J-types) do not present a uniform characteristic in common with respect to their configurations.

The numbers of alkaloid isolations of specific skeletal types in the three plant families are shown as follows (Kisakurek, Leewenberg and Hesse, 1983):

	Skeletal Type							
	With Nonrearranged Secologanin Part					With Rearranged Secologanin Part		
Plant families	C-	D-	V-	S-	A-	E-	P-	J-
Apocynaceae	1078	19	15	51	58	83	316	311
Absolute configuration	α	α	α	α	α	α+β	α+β	α+β
Loganiaceae	104	2	49	356	1			
Absolute configuration	α	α	α	α	α			
Rubiaceae	608	36	23					
Absolute configuration	α	α	α					

The occurrence of alkaloids of A- and S-types is restricted to the Loganiaceae and Apocynaceae but alkaloids of C-, D- and V-types have been detected in all of the three plant families. The Loganiaceae, only alkaloids of C-type have been isolated from *Gelsemium* and *Mostuea* of the Gelsemieae. Of the other tribe, Strychneae, *Gardneria* species contain only alkaloids of C-type, where as alkaloids of C-, D-, V-, S- and A-types have been isolated from species of *Strychnos*.

The main skeletal type (C3a) occurs in all of the three plant families (circle) and so do the skeletal types (C4a) and (C4b). It becomes also evident that the increasing structural complexity brings about a more specific occurrence. For example, (C4c), derived from (C3a) by a new bond formation between C(5) and C(16), leading to an additional ring, is the skeletal type of alkaloids that only occur in Loganiaceae and Apocynaceae but not in Rubiaceae. This tendency to more specific occurrences grows as additional operations are undertaken on (C4a), e.g. oxidation at C(3) leads to (C5a) and by formation of a new bond between C(7) and C(17), (C5c) is obtained. Alkaloids with these last two skeletons only occur in the plants of Apocynaceae. The distribution of alkaloids of corynanthean-type in plant families is shown as follows (Kisakurek and Hesse, 1980):



Distribution of alkaloids of corynanthean-type (C) in plant families.

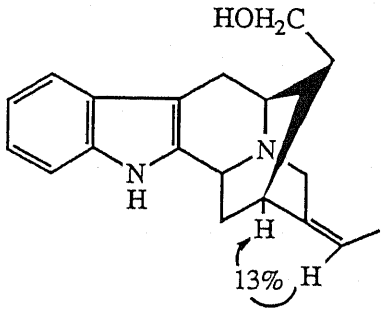
All of the alkaloids obtained from the species of *Gelsemium* can be divided into two main groups, indole and oxindole alkaloids. The indole alkaloids have been classified into three different skeletal types; sempervirine-, koumine- and sarpagine-types which are of indole, indolenine and indole nucleus, respectively. On the other hand, oxindole alkaloids are divided into three different skeletal types; gelsemine-, humantenine and gelsedine-types.

The alkaloids from *Gelsemium elegans* Benth. possess double bond between C(19) and C(20) to afford two configurations *E* and *Z*, e.g. 16-epi-voacarpine (19*E*-form) and 19-(Z)-akuammidine, koumidine, 19-(Z)-taberpsychine (19*Z*-form) respectively. Substitutions at C(14) have been found to be only β-oriented and the substituting group being hydroxy group only, e.g. 14-hydroxygelsenicine (44), 14-hydroxygelsedine (40). On the other hand, hydroxy substitutions have been presented at C(3) as OHα, e.g. 16-epi-voacarpine. For substitutions at N(a), only methoxy group has been found in oxindole alkaloids only. Methyl substitutions at N(b) have been found in both types of alkaloids. Only in oxindole alkaloids, methoxy and hydroxy substitutions in aromatic ring at C(11) have been found, e.g. humantenirine (37) gelsemicine (41), 11-methoxyhumantenine (38) and 11-hydroxyrankinidine (34). 11-hydroxyhumantenine (36) respectively. All of the oxindole alkaloids possess ether bond between C(17) and C(3).

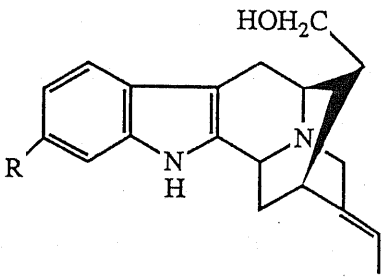
2. Structure Revision of Koumidine and 19-(Z)-Akuammidine

Koumidine (22) isolated from the roots and stems of *Gelsemium elegans* Benth. has mp 200-201° C (dec.) $[\alpha]_D^{21}$ = -9° (c=0.10, MeOH) and all the other spectral data agreed well with those given in literature (Jin and Xu, 1982). The

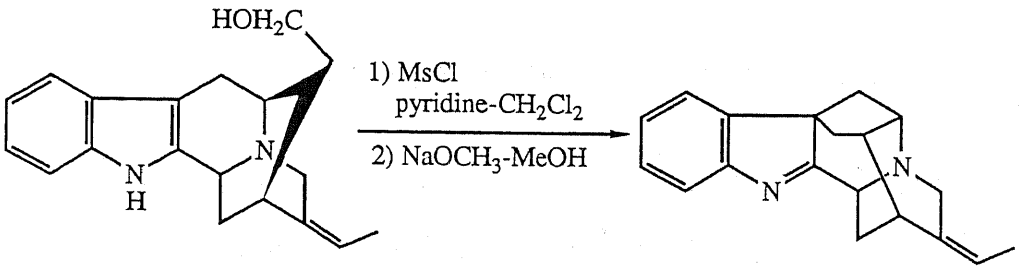
^{13}C-NMR spectra of a koumidine (Table page 81) was compared with that of the known base, gardnerine (50) the signal due to C(15) of koumidine was observed at 7.6 ppm lower shift but that C(21) was observed at 2.4 ppm higher field than the corresponding signals of gardnerine, having (*E*)-ethylidine side chain. Irradiation of C(19)-H(δ 5.36) enhanced C(15)-H(δ 2.44) with 13% NOE. From these data, it is indicated that the configuration of ethylidene side chain in koumidine (22) is (*Z*)-form. Koumidine (22) gave a ring-closed indolenine derivative (144) by mesylation of the hydroxy group at C(17) and subsequent treatment with $NaOCH_3$, that confirmed the configuration at C(16) (Sakai *et al.*, 1973 and 1987; Schun and Cordell, 1987 and Ponglux *et al.*, 1988).



Koumidine (22)



Published Koumidine : R = H
Gardnerine (50) : R = OCH_3



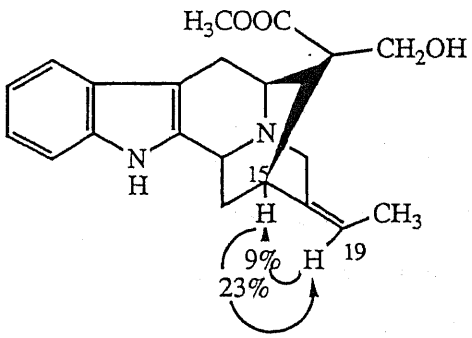
(22) (144)

19-(Z)-Akuammidine (23), mp. 240-242 °C was previously isolated from the same plant by Chinese group (Jin and Xu, 1982) and was assigned to be akuammidine, having 19-(*E*) ethylidene side chain. The mass spectral fission pattern of the isolated alkaloid parallels that for authentic akuammidine 19(*E*) form. However, ^{1}H-NMR and ^{13}C-NMR spectral of the alkaloid exhibited similar but not completely identical to the spectra of authentic akuammidine. The ^{13}C-NMR chemical shifts of C(15) [6.3 ppm lower field than (*E*) form] and C(21) [2.9 ppm upper field than (*E*) form] of the isolated akuammidine (23) compared with the authentic akuammidine can be reasonably interpreted in terms of the γ-gauche effect due to C(18) on the double bond of (*Z*)-configuration. A difference NOE experiment also supported the configuration of the ethylidene side chain of both compounds. Thus, irradiation of C(15)-H(δ 3.24) in akuammidine led to enhancement (12%) of C(18)-H_3(δ 1.68), indicated that the methyl group on the double bond lies *syn* to C(15)-H. On the other hand, 23% enhancement was observed between C(15)-H and C(19)-H in the isolated alkaloid. Finally, the structure of (23) was determined by X-ray analysis [The crystal of (23) has the following data : orthorhombic, $P2_12_12_1$, a=13.962(5), b=20.498(8) c=6.668(2)Å, z=4, Cell volume=1908.38 $Å^3$, Dc=1 227 gcm^{-3}. A total of 2189 unique independent intensities were measured within the range of $3 \leq 2\theta \leq 120^\circ$, 155° on a 4-circle diffractometer (Rigaku AFC-5) using CuKα radiation (λ=1.54Å). The structure was solved by the direct method using MULTAN 80 (UNICS III system) and refined anisotropically (isotropically for H) by the last-squares method to an R value of 0.048, using the 1886 reflections for which F(0)>3σ(Fo)]. The CD spectra of both akuammidines exhibited exactly the same CD curves (Ponglux *et al.*, 1988). And therefore 19-(Z)-akuammidine has the same absolute configuration as the common indole alkaloid.

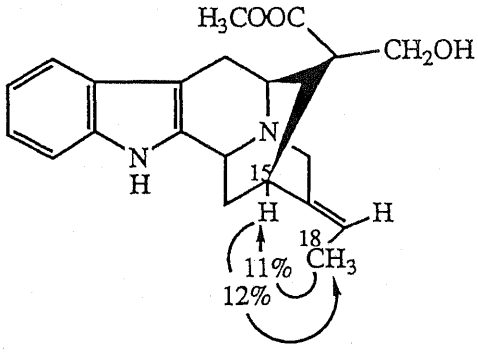
Table ^{13}C-NMR Spectral Data

No.	(23)	(Akuammidine)	(22)	(50)
2	139.1(s)*	139.4(s)*	138.4(s)*	139.0(s)*
3	51.5(d)	52.7(d)	51.0(d)**	51.2(d)**
5	59.5(d)	59.8(d)	54.0(d)**	53.7(d)**
6	25.1(t)	25.9(t)	23.4(t)	23.5(t)
7	106.0(s)	106.9(s)	106.0(s)	106.1(s)
8	128.1(s)	128.8(s)	127.5(s)	122.0(s)
9	119.8(d)**	120.5(d)**	119.8(d)***	114.8(d)
10	118.6(d)**	119.4(d)**	115.3(d)***	109.5(d)
11	122.1(d)	122.8(d)	122.1(d)	157.4(s)
12	112.0(d)*	112.8(d)*	112.0(d)*	96.0(d)*
13	138.9(s)*	139.3(s)*	138.2(s)*	136.9(s)*
14	31.4(t)	29.7(t)	29.3(t)	27.8(t)
15	37.4(d)	31.1(d)	35.1(d)	27.5(d)
16	53.2(s)	53.5(s)	44.3(d)	43.7(d)
17	69.1(t)	69.8(t)	61.2(t)	61.1(t)
18	12.5(q)**	14.1(q)**	12.6(q)***	12.9(q)
19	118.2(d)**	118.1(d)**	118.7(d)***	119.3(d)
20	138.6(s)*	138.8(s)*	142.7(s)*	141.0(s)
21	54.0(t)	56.9(t)	54.6(t)	57.0(t)
COOMe	174.9(s)	175.6(s)	-	-
COOMe	51.5(q)	52.4(q)	-	-
-OMe	-	-	-	56.1(q)

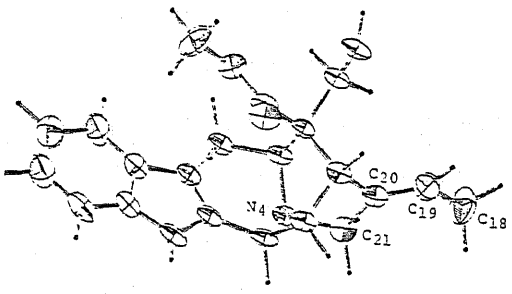
Chemical shifts in ppm downfield from TMS.
Solvent; CD_3OD.
*,**,*** Signals may be interchanged within vertical column.



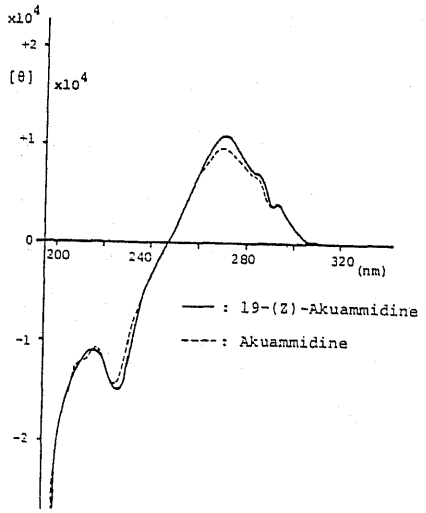
19-(Z)-Akaummidine (23)



Akuammidine



ORTEP Drawing of 19-(Z)-Akuammidine (23)



CD Curves of 19-(Z)-Akuammidine (—) and Akuammidine (----)

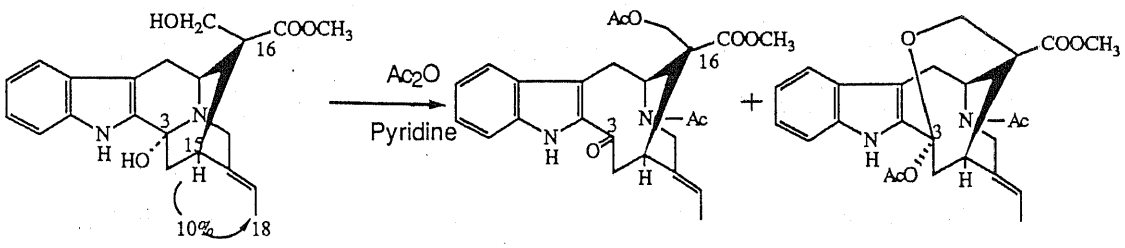
3. Structure Elucidation of New Alkaloids

16-epi-voacarpine (24) showed mp 162-165°C, $[\alpha]_D^{22}$=+42.3° (c=0.20, $CHCl_3$). The mass spectrum of (24) presents molecular ion *m/z* 368 which is 16 a.m.u. higher than the corresponding peak in the spectrum of akuammidine. And the exactly similar cleavage pattern is observed with voacarpine. On acetylation, (24) gave rise to two products (145) and (146). The formation of (145), which exhibited a typical 2-acyl indole UV absorption at 314 nm, demonstrated the presence of a hydroxy group at C(3) in (24). The configuration at C(16) was determined by the following two facts:

1) The formation of intramolecular hemiacetal-acetate (146) on acetylation of (24).

2) In the ^{1}H-NMR spectrum of (145), the signal of acetoxy group was shielded (δ :1.45, 3H, s.) by indole nucleus.

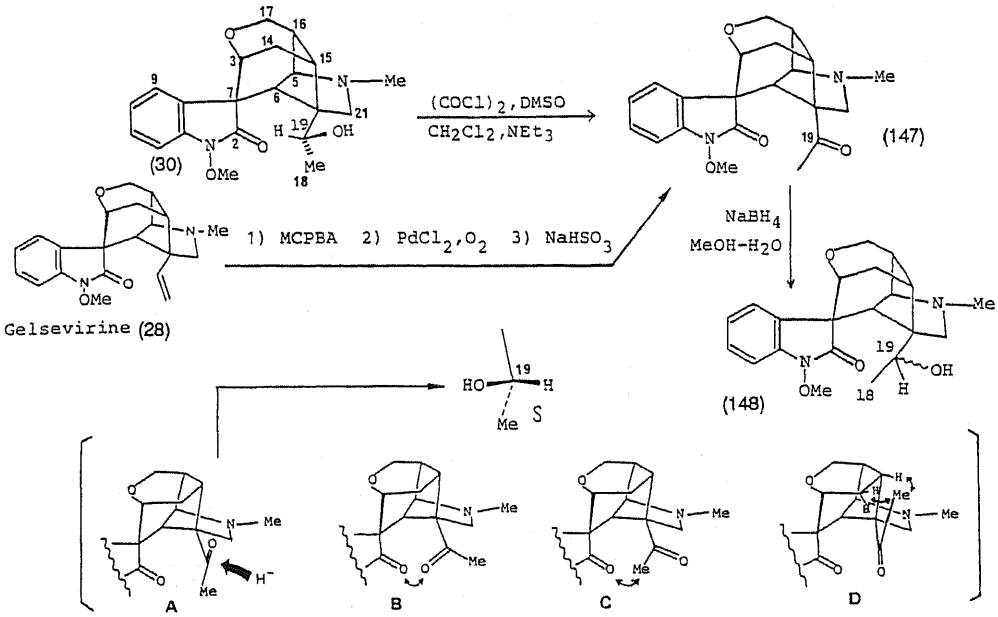
10% Enhancement observed in difference NOE experiment between C(15)-H(δ 3.12) and C(18)-CH_3 (δ 1.55) indicated the (*E*)-form ethylidene side chain in 16-epi-voacarpine (24) (Ponglux *et al.*, 1988).



16-epi-voacarpine(24) (145) (146)

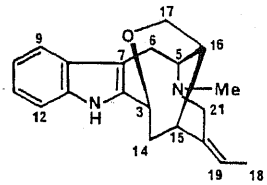
19-Hydroxydihydrogelsevirine (30): This new alkaloid was isolated as an amorphous solid and showed $[\alpha]_D^{21} = +1^\circ$(c=0.11, MeOH). Its high resolution mass spectrum showed the M^+ 370.1889, corresponding to the formula $C_{21}H_{26}N_2O_4$. The UV spectrum indicated N(a)-OCH_3 oxindole nucleus [λ_{max}; 281 (sh), 255, 209 nm]. ^{1}H-NMR spectrum showed the characteristic signals due to N(a)-OCH_3 (δ 3.99, s), -O-C(3)-H (δ 3.84, br-s.), -OCH_2-(δ 4.09, dd, J=11.2, 2.3 Hz and δ 3.91, dd, J=11.2, 2.0Hz), and N(b)-CH_3(δ 2.28,s.). Furthermore the presence of a secondary hydroxy group was deduced by the signals at δ 5.13 (q, J=6.6Hz) and δ 1.09 (3H, d, J=6.6Hz) in place of a vinyl group in gelsevirine (28). The ^{13}C-NMR spectrum (Table3, p.146), with was very similar to that of gelsevirine (28), the appearance of a new doublet at δ 64.3 ppm and a new quartet at δ 19.4 ppm, the absence of vinyl carbons (C_{18}, C_{19}) in gelsevirine (28) and the upfield shift at C(6) (3.6 ppm) and at C(21) (7.5 ppm) also revealed the presence of a secondary hydroxy group on C(19). To confirm the structure (30) proposed by spectroscopic analysis, (30) was prepared from gelsevirine (28). Gelsevirine N-oxide, prepared by the MCPBA oxidation of (28), was subjected to Wacker oxidation ($PdCl_2$, O_2, DMF-H_2O) to produce 19-keto derivative, which was further converted to compound (147) by the reduction of N-oxide with $NaHSO_3$. Ketone (147) was also obtained from the new alkaloid (30) by means of Swern oxidation. Reduction of ketone (147) with $NaBH_4$ gave a diastereomeric alcohol (148) as the major product, accompanied with trace amounts of (30). This stereospecific reduction enables us to assume the stereochemistry of the isomeric alcohol (148). Thus, in the transition state, ketone derivative (147) may take a conformation A, (more stable than the other conformers B, C and D) as depicted in the Fig. p.84, due to the dipole-dipole repulsion and/or steric hindrance. Hydride should approach from less hindered side (*anti* to oxindole nucleus),resulting in the predominant formation of the (*S*)-alcohol

(148). Therefore, the secondary hydroxy group on C(19) in (30) takes (*R*) configuration (Ponglux *et al.*, 1988).

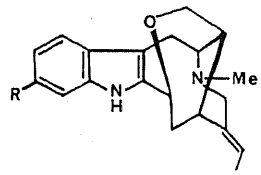


19-(Z)-Taberpsychine (25): This alkaloid was obtained as a colorless oil and its formula, $C_{20}H_{24}N_2O$, was confirmed by HR-MS. The mass spectral fission pattern parallels that for taberpsychine (149), but ^{1}H-NMR spectrum of (25) is not completely identical to that of (149), probably owing to the difference of the configuration of the ethylidene side chain. In the ^{1}H-NMR spectrum, NOE was observed between C(19)-H and C(15)-H, suggesting that the configuration of the side chain was (*Z*)-form. Furthermore, as in the case of akuammidine and 19-(Z)-

akuammidine (23), the ^{13}C-NMR spectra of (25) and appropriate model compound (150), prepared from gardnerine (50) were compared. The signal due to C(15) of (25) was observed at downfield (6.9 ppm) and on the contrary, that of C(21) was observed at upfield (6.9 ppm) than the corresponding signal of (150). From these data, the structure of this new base was concluded as 19-(Z)-taberpsychine (Ponglux *et al.*, 1988).



19-(Z)-Taberpsychine (25)



R=H : Taberpsychine (149)

R=OMe : (150)

^{13}C-NMR Spectral Data

No.	(25)	(150)
2	136.2(s)*	136.5(s)*
3	67.6(d)	67.5(d)
5	60.5(d)	60.6(d)
6	18.0(t)	17.9(t)
7	110.9(s)	111.0(s)
8	128.3(s)**	122.8(s)**
9	119.8(d)**	119.7(d)**
10	119.3(d)**	109.7(d)
11	122.3(d)**	156.7(s)
12	110.9(d)	94.6(d)
13	135.3(s)*	136.1(s)*
14	29.7(t)	28.0(t)
15	33.5(d)	26.6(d)
16	37.5(d)	37.0(d)
17	61.9(t)	61.9(t)
18	12.8(q)	12.4(q)
19	118.2(d)	118.8(d)**
20	131.9(s)	130.6(s)*
21	45.9(t)	52.8(t)
N-Me	43.0(q)	42.7(q)
-OMe	---	55.7(q)

Chemical shifts in ppm downfield from TMS. Solvent; $CDCl_3$
*,** Signals may be interchanged within vertical column.

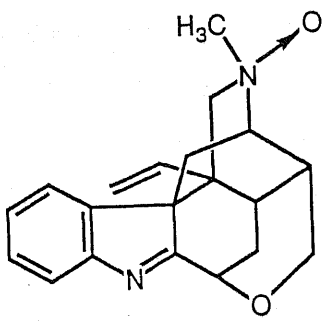
Koumine N-oxide (20) : The molecular formula ($C_{20}H_{22}N_2O_2$) obtained by elemental analysis, as well as UV and ^{1}H-NMR spectral data, indicated that this new indole alkaloid (mp 111-113°C) isolated from the leaves was to be koumine N(b)-oxide. In particular, the signal of N(b)-methyl group is shifted to downfield (0.96 ppm) compared with that of koumine (19). MCPBA oxidation of (19) afforded two diastereomeric N(b)-oxides, one of which was identical with natural N-oxide (20). The configuration on N(b) atom was initially deduced by the analysis of ^{1}H-NMR spectra. Thus, in natural N-oxide (20) the signals of C(15)-H and C(16)-H are observed at downfield (0.38 ppm and 1.30 ppm; respectively) than those of koumine (19). While C(6)-Hα in the diastereomeric N-oxide (151) is deshielded to

downfield compared with that of koumine (19). These phenomena may be attributable to anisotropy of N-->O function. Therefore, the configuration on N(b) should be (*S*) in (20) and (*R*) in (151) respectively, as depicted in Fig. below. Unnatural N-oxide (151) gave the crystal (mp 214-216°C) suitable for X-ray analysis. The results obtained from X-ray analysis agreed with the conclusion obtained from ^{1}H-NMR analysis.

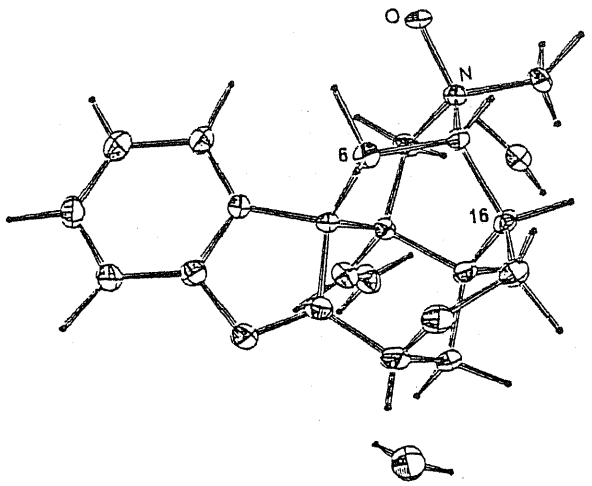
Koumine (19) $\underset{NaHSO_3}{\overset{MCPBA}{\rightleftharpoons}}$ (20) + (151)

(δ: ppm)

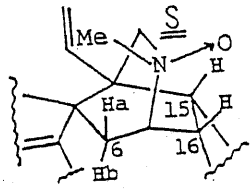
	Koumine N-oxides		Koumine (19)
	(20)	(151)	
C (15)-H	2.76	2.23	2.34
C (16)-H	4.10	2.96	2.80
C (6)-Ha	2.94	3.62	2.34 or 2.41



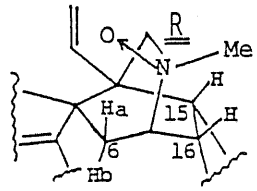
(20)



ORTEP Drawing of (151)



(20)



(151)

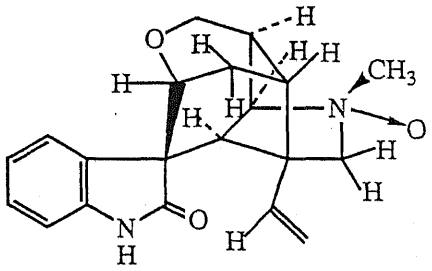
Gelsemine N-oxide (31) : Spectral data indicated that this new alkaloid obtained from the leaves was closely related to gelsemine (26). The characteristic deshielding of N(b)-methyl group (δ 3.41) in ^{1}H-NMR spectrum can be explained by the influence of N(b) oxide. As expected, (31) was obtained by the MCPBA oxidation of gelsemine (26) together with the diastereomeric isomer (152). The configuration on N(b) atom was determined applying the procedure used in the structure elucidation of koumine N-oxide (20). In the ^{1}H-NMR spectra, C(6)-H in natural N-oxide (31) and C(16)-H in its diastereomeric isomer (152) are remarkably shifted to downfield (1.43 ppm and 1.96 ppm), respectively. Having

the steric structure as shown in Figure below. These observation can be reasonably interpreted by the anisotropic effect of oxygen on N(b). To support this conclusion, difference NOE experiment was made. Irradiation of N(b)-methyl group enhanced C(16)-H (δ 2.59) with 9% NOE. Therefore, the configuration of N(b) should be (*R*).

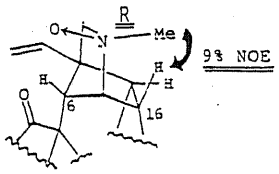
Gelsemine (26) $\underset{NaHSO_3}{\overset{MCPBA}{\rightleftharpoons}}$ (31) + (152)

(δ: ppm)

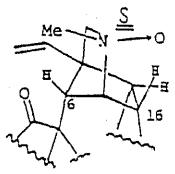
	Gelsemine N-oxides		Gelsemine (26)
	(31)	(152)	
C(6)-H	3.41	2.28	1.98
C(16)-H	2.59	4.26	2.30



Gelsemine N-oxide (31)

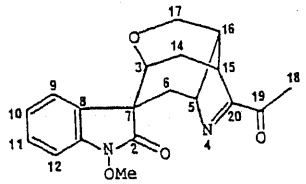


(31)

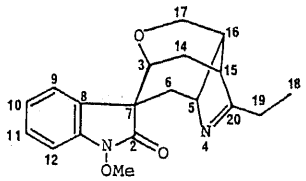


(152)

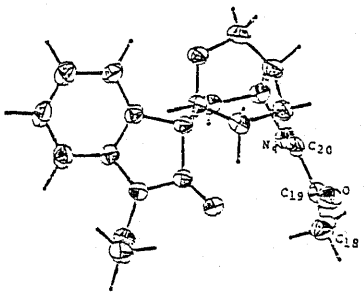
19-Oxogelsenicine (45) : The new alkaloid (45) was obtained as colorless plates, mp 226-227° C, and its formula, $C_{19}H_{20}N_2O_4$, was confirmed by HR-MS spectroscopy. The IR spectrum displayed characteristic absorption for two carbonyl function at 1715 and 1695 cm^{-1}. The ^{1}H-NMR spectrum showed the unusual signal at δ 2.66 due to methyl group adjacent to carbonyl function. The ^{13}C-NMR spectrum (Table 3 and 1,page 146 and 144) was similar to that of gelsenicine (43) except for the signals of C(18) and C(19), which were shifted downfield to δ 26.1 (16.1 ppm) and δ 197.6 (172 ppm), suggesting the presence of ketonic group on C(19) in gelsenicine (43). Finally, the structure of (45) was determined by X-ray analysis, as shown in Figure below. The CD spectra of (45) and (43) gave the similar curves and therefore 19-oxogelsenicine has the same absolute configuration with gelsenicine (Ponglux *et al.*, 1988).



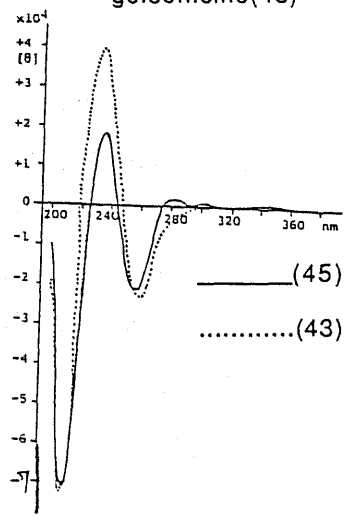
19-oxogelsenicine(45)



gelsenicine(43)



ORTEP Drawing of (45)



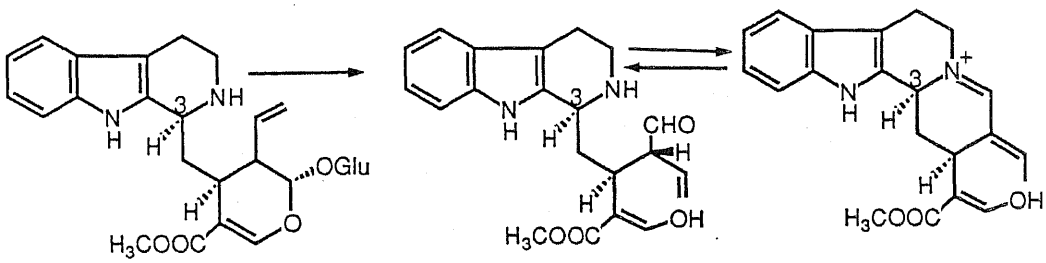
CD Curves of (45) and (43)

Elegansamine (46) : This new alkaloid was obtained from the stems and branches as colorless prisms, mp 172-173° C (MeOH). It showed the UV spectrum characteristic to N(a)-methoxy oxindole nucleus. HR-MS showed the M^+ 508.2572, corresponding to the formula $C_{29}H_{36}N_2O_6$ (caled. 508.2571), and gave the base peak *m/z* 326, corresponding to the molecular weight of gelsenicine (43) $C_{19}H_{22}N_2O_3$, indicated that elegansamine (46) was constructed from gelsenicine (43) or its isomer and a monoterpene unit containing three oxygen atom. In the ^{1}H-NMR spectrum ($CDCl_3$), in addition to some readily assignable signals due to gelsenicine moiety such as four aromatic protons [δ 7.50 C(9)-H, δ 7.26 C(11)-H, δ 7.07 C(10)-H, δ 6.89 C(12)-H], N-OCH_3 (δ 3.95 3H, s), C(3)-H (δ 3.70 1H, dd, J=4.9 and 2.2 Hz, C(15)-H (δ 2.91 1H, t-like, J=9Hz), and C(16)-H (δ 2.54 1H,m), characteristic signals of a doublet on C(18) protons (δ 1.47) 3 H, J=7.3 Hz) and a multiplet due to C(19) proton (δ 2.66) were observed in place of the ethyl group in gelsenicine (43), suggesting that monoterpene unit might be connected at C(19) position. From the ^{13}C-NMR spectrum of (46) (Table 4, page 147), the composing indole alkaloid part and the monoterpene unit were respectively demonstrated to be gelsenicine and an iridoid skeleton, which possessed a lactone function, a C-methyl group, and a secondary hydroxy group. At this stage X-ray structural analysis was carried out. The ORTEP drawing is shown in page143. The CD spectrum of (46)[(1) (c=0.95x10^{-2}, MeOH, 23°C)$[\theta]_{312}0$,$[\theta]_{260}$-18200, $[\theta]_{245.5}0$, $[\theta]_{234}$+18200, $[\theta]_{222}0$, $[\theta]_{209}$-59000. (2) (c=1.0x10^{-2}, MeOH, 23°C) $[\theta]_{314}0$, $[\theta]_{262}$-24200, $[\theta]_{248.5}0$, $[\theta]_{234}$+39300, $[\theta]_{221}0$, $[\theta]_{211}$-73300.] closely resembles that of gelsenicine (43) and therefore gelsenicine part and the iridoid residue in (46) have the same absolute configuration as the conventional indole alkaloids and irridoid monoterpenes, respectively (Ponglux *et al.*, 1988a). And this new type of indole alkaloid has been investicated for the first time.

4. Proposal of Biogenetic Route of *Gelsemium* Alkaloids

It is well recognized that monoterpenoid indole alkaloids are biosynthesized through the biological transformations of strictosidine (131) which was derived from the condensation of tryptamine and secologanin. In this thesis, we proposed a tentative biogenetic route of *Gelsemium* alkaloids as follows. Common intermediate (154) formed from strictosidine (131) by the intramolecular C-C bond formation between C-6 and C-16 will serve as a precursor of sarpagine type indole alkaloids such as koumidine (22), 19-(Z)-akuammidine (23), and 16-epi-voacarpine (24). Koumidine (22) will be metabolized to a C/D ring-opening compound, 19-(Z)-taberpsychine (25) (Tentative biosynthetic route of *Gelsemium* alkaloids-1). Oxidation on C-18 in (25) and subsequent intramolecular C-C bond formation between C-7 and C-20 will form koumine (19). Very recently, we and Chinese group independently succeeded the partial synthesis of 11-methoxykoumine (Sakai *et al.*,1986) and koumine (19) (Liu *et al.*,1987) along this biogenetic proposal. β-Oxidation of indole part in (25) will generate indolenine (159), which will further transform into humantenine-type alkaloids, humantenine (35), humantenirine (37), and rankinidine (33) by the rearrangement to oxindole and subsequent N(a)-methoxylation process (Tentative biosynthetic route of *Gelsemium* alkaloids-2). After the elimination of HX at C_6-C_7 position in indolenine (159), ene type reaction between C-20 and C-6 will take place to afford indole (161). Through the β-oxidation and successive rearrangement, gelsemine (26) will be generated from (161). Further oxidative process will afford gelsevirine (28) and 19-hydroxydihydrogelsevirine (30), in order (Tentative biosynthetic route of *Gelsemium* alkaloid-3). It seems that biosynthesis of gelsedine group will branch from the intermediate (154). Thus, (154) will be metabolized to norsarpagine-type compound (162), having five membered D-ring, through the release of C_{21}-aldehyde carbon and subsequent ring-closure between N(b) and C-20. (162) will

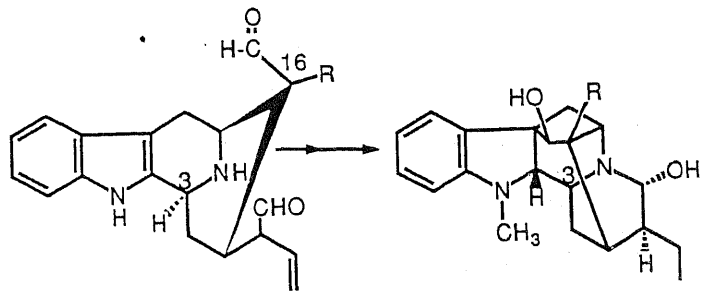
be converted to the C/D ring-opening compound (163), such as 19-(Z)-taberpsychine (25), and then transformed into gelsedine series, gelsedine (39), gelsemicine (41), 14-hydroxygelsedine (40), 14-hydroxygelsemicine (42), gelsenicine (43), 14-hydroxygelsenicine (44) and 19-oxogelsenicine (45), *via* successive bioconversions (Tentative biosynthetic route of *Gelsemium* alkaloids-4) (Ponglux *et al.*, 1988).



Strictosidine (131) Intermediate-A (153) Dehydrogeissoschizine

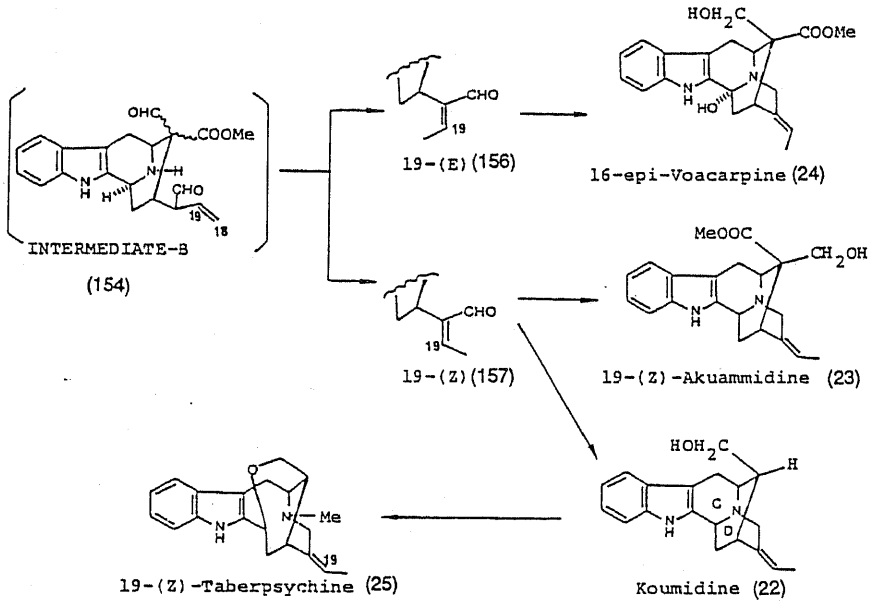
1) oxid.

2) ring-close

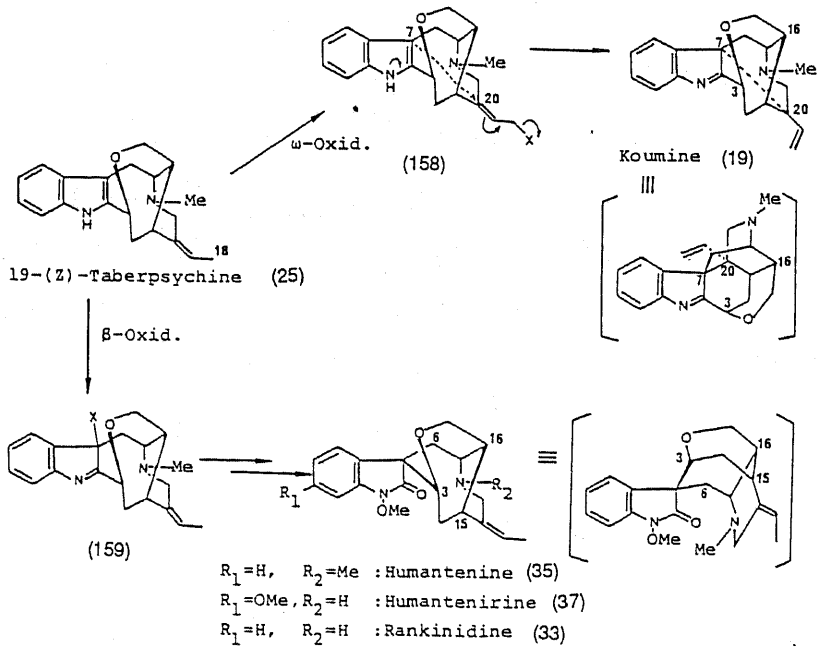


Intermediate-B (154) R=H, Ajmaline (155)

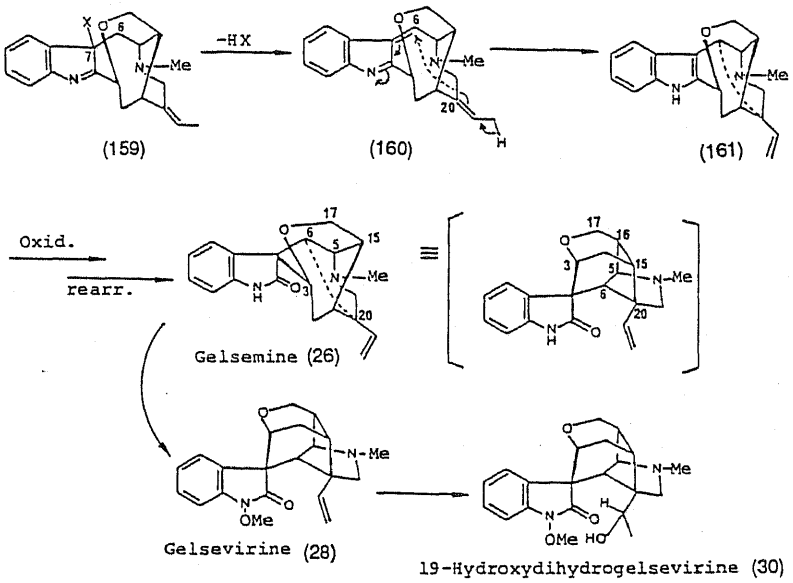
Tentative Biosynthetic Route of *Gelsemium* Alkaloids -1



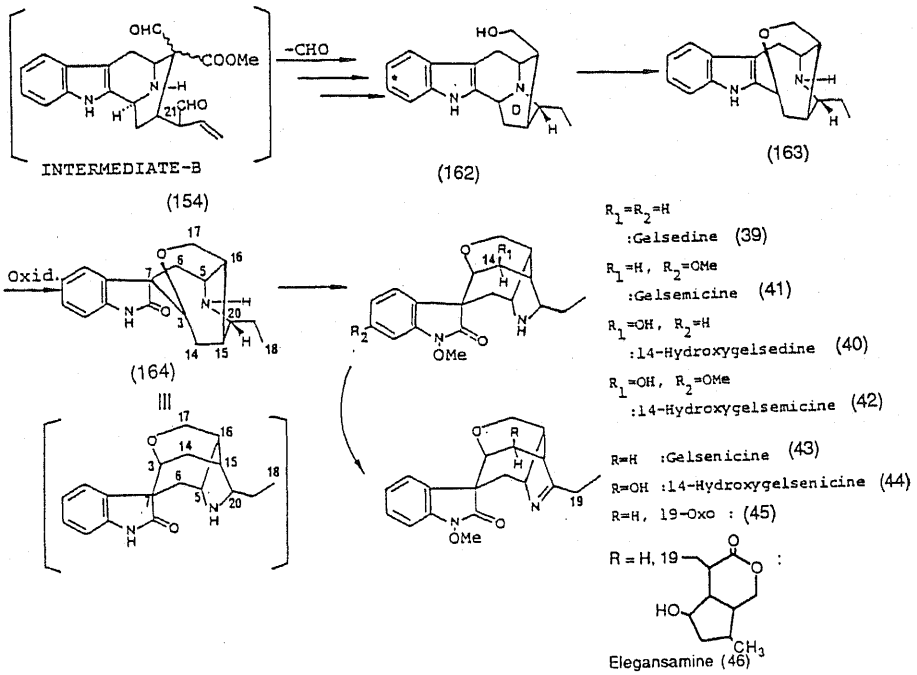
Tentative Biosynthetic Route of *Gelsemium* Alkaloids -2



Tentative Biosynthetic Route of *Gelsemium* Alkaloids -3



Tentative Biosynthetic Route of *Gelsemium* Alkaloids -4



5 Chemical Transformation of Ajamaline to *Gelsemium* Alkaloids

5.1 Partial Synthesis of Koumidine (22) and 19-(Z)-Taberpsychine (25)

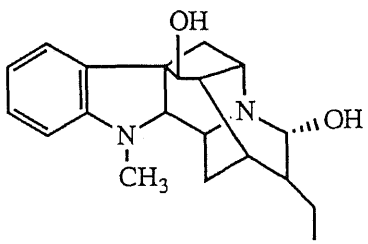
The starting material of this partial synthesis was ajmaline (165) which already reported its absolute configulation. The transformation involves mainly two structural changes of (165), the stereoselective introduction of a double bond into C_{19}-C_{20} position and conversion of indoline moiety into the indolic compound without the epimerization of the C_{16} configuration.

In order to liberate the masked aldehyde (C_{21}) from the amino acetal function and to protect the N(b) group as carbamate, ajmaline (165) was successively treated with N, N-dimethyl hydrazine and catalytic amount of H_2SO_4, methyl chloroformate in 1N-NaOH/CH_2Cl_2, and then $CuCl_2$ in THF-H_2O pH7 to afford the aldehyde (168). The direct conversion of (165) into (168) by the reaction with chloroformates gave the carbonate ($C_{21}OCOOR$) derivatives. After the protection of the C_{17} hydroxy group by methoxyethoxymethyl (MEM) ether, bromine atom was introduced onto the C_{20} position *via* the *t*-butyldimethylsilyl (TBS) enol ether. Treatment of (171) with 1, 8-diazabicyclo [5.4.0]undec-7-ene (DBU) in N, N-dimethylformamide (DMF) gave the desired 19-(Z) olefine (173) in 60% yield, selectively (173):(172) = 5:1. The geometry of the olefines (173) and (172) were unambiguously determined by the NOE experiments [Irradiation of C_{18} methyl protons (δ 2.14) in (173) led to enchancement (17%) of C_{21} aldehyde proton (δ 10.2), while 25% enchancement was observed between C_{19} olefinic proton (δ 6.50) and C_{21} aldehyde proton (δ 9.33) in (172)]. The major α, β-unsaturated aldehyde (173) was reduced with $NaBH_4$ and then ring closure between C_{21} and N(b) was performed by the successive treatment of the resulting alcohol with NaOH in

aqueous ethylene glycol and mesyl chloride in pyridine to afford deoxyajmaline derivative (176).

The transformation of indoline moiety into the indolic compound could be accomplished by the deprotection of the C_{17} hydroxy group of the indolenine derivative (179). The epimerization at C_{16} could be prevented by using of trimethylsilyl (TMS) group. Thus, indolenine (179), which was not so stable toward usual work up manner and column chromatography, was treated with AcOH-THF-H_2O (at room temperature) and then reduced with $NaBH_4$ in MeOH to yield koumidine (22), $[\alpha]_D^{23}$-23.8°(c0.6, MeOH), in 70% overall yield from (179), which exhibited ^{1}H-NMR, IR, mass spectra and mp (202-204°C) identical with those of natural koumidine (22), $[\alpha]_D^{20}$-20.8°(c 1.8, MeOH).

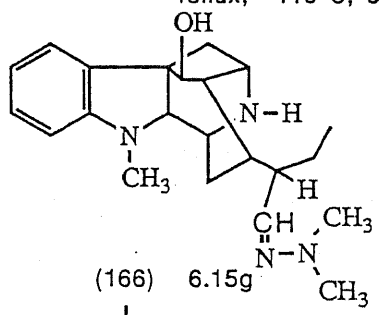
Koumidine (22) was treated with methyl chloroformate in THF-H_2O in the presence of MgO and the resulting carbamate was reduced with lithium aluminium hydride ($LiAlH_4$) to furnish 19-(Z)-taberpsychine (25), $[\alpha]_D^{23}$-251°(c 0.3, $CHCl_3$), in 30% overall yield from (22). The synthetic substance exhibited spectral properties (^{1}H-NMR, IR, UV and MS) in accord with those of an authentic sample, $[\alpha]_D^{23}$-180°(c 0.4, $CHCl_3$) (Takayama *et al.*, 1989). These transformations are summarized as follows:



(165) Ajmaline

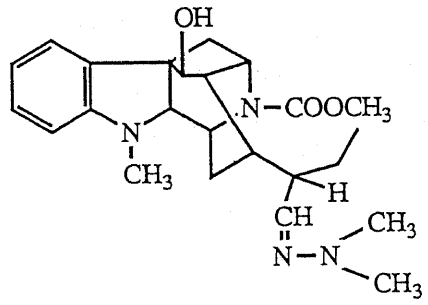
(165)

(165) 5.02g(15.3 m mol)
H_2N-N $(CH_3)_2$ (4.7 ml = 4eq)
conc. H_2SO_4(0.6ml), M-S 3A(3g)
dry EtOH(100ml)
reflux, 110°C, 5 hrs.



(166) 6.15g

$ClCOOCH_3$(1.67ml = 1.4eq)
1N-NaOH aq.(50ml), CH_2Cl_2(200ml)
0°C, 40 min.



Spectral data of (167)

UV λ_{max}^{EtOH}nm : 290, 245, 206.

IR ($CHCl_3$) : 3400, 1690, 1460.

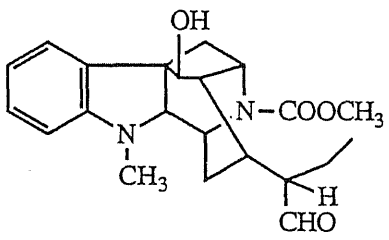
EI-MS *m/z* (%): 426 (M^+, 17), 356(4), 282(61), 144(98), 113(100).

^{1}H-NMR($CDCl_3$) δ : 6.44, 6.40 (1H, each d, J=7.3Hz, C(21)-H)
: 3.69 (3H, s, $COOCH_3$)
: 2.75, 2.74 (each 3H,s,N-CH_3).

(167) 5.166g : 79% yield from (165)

(167) 6.186g (14.5 m mol)
$CuCl_2$ (6.867g =3.5eq)
pH7 phosphate buffer(87ml)
THF(218ml), H_2O(29ml)
rt., 48 hrs.

(168)



Spectral data of (168)

UV λ_{max}^{EtOH}nm : 290, 247, 206.

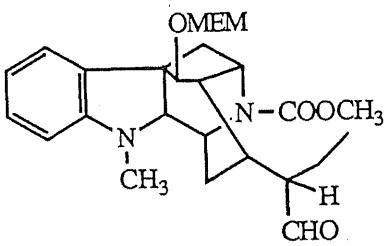
IR ($CHCl_3$) : 3450, 1720, 1690, 1460.

EI-MS *m/z* (%) : 384(M^+,65), 240(32), 173(80), 144(100).

^{1}H-NMR($CDCl_3$) δ : 9.62 (d,J=3.4Hz), 9.59 (d,J=4.3Hz), CHO.

(168) 4.175g : 75% yield from (167)

(168) 100mg (0.260m mol)
MEMCl(90 μl = 3eq)
N, N-diisopropylethylamine(158 μl = 3.5eq)
dry CH_2Cl_2(2ml)
reflux, 70°C, 5 hrs.



Spectral data of (169)

UV λ_{max}^{EtOH}nm : 292, 248, 205.

IR($CHCl_3$) : 1720, 1690, 1460, 1120, 1040.

EI-MS *m/z*(%) : 472(M^+,75), 383(15), 252(33), 182(52), 144(54), 89(98).

^{1}H-NMR($CDCl_3$)δ :9.60(d,J=3.4Hz), 9.57(d, J=4.6Hz), 1H, CHO, 3.73, 3.71 (3H, each s, $COOCH_3$)

(169) 100mg : 81% yield from (168). 3.384, 3.382 (3H, each s, OCH_3).

(169)

(169) 100mg(0.212 m mol)
t-butyldimethylsilyl-
trifluoromethanesulfonate(146μl =3eq)

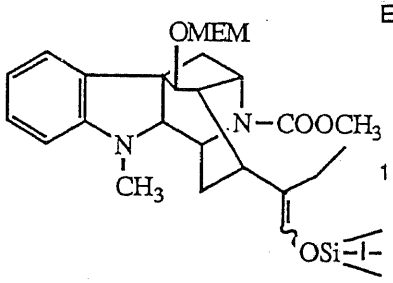
dry Et_3N(117μl =4eq)
dry CH_2Cl_2(1ml)

0°C, 2.5 hrs. Spectral data of (170)

UV λ_{max}^{EtOH}nm : 292, 248, 205.

IR($CHCl_3$) : 1690, 1460, 840.

EI-MS *m/z*(%) : 586(M^+,82), 336(97), 241 (33), 182(67), 144(61), 89 (100), 59(98).



^{1}H-NMR($CDCl_3$)δ: 6.14(1H,s,C(21)-H),
: 0.92(9H,s,t-Bu-Si),
: 0.111(each H,s,CH_3-Si).

(170) 87mg : 71% yield from (169)

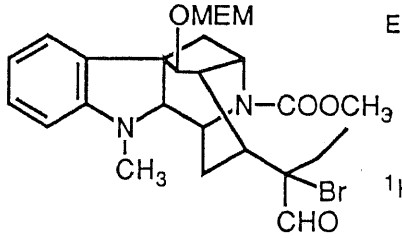
(170) 80mg (0.136 m mol)
N-bromosuccinimide (NBS) (27mg = 1.1eq)
dry THF (4ml)
-20°C, 30 min.

Spectral data of (171)

UVλ_{max}^{EtOH}nm : 291, 244, 205.

IR ($CHCl_3$) : 1710, 1690, 1470, 1110.

EI-MS *m/z* (%): 552(10), 550(M^+,12), 472(14), 182(31), 144(46), 89(98), 59(100).

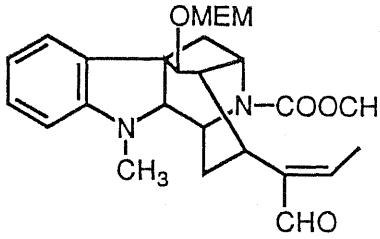


^{1}H-NMR($CDCl_3$)δ :9.39, 9.35(1H,each s,CHO), 3.72, 3.70(3H,each s,$COOCH_3$),3.38(3H,s,OCH_3).

(171) 57mg : 76% yield from (170)

(171) 57mg (0.103 m mol)

DBU(20μl = 1.3eq)
dry DMF(1.0 ml)
rt., 14 hrs.



(172) 6mg : 12% yield from (171)

+

(173) 29mg : 60% yield from (171)

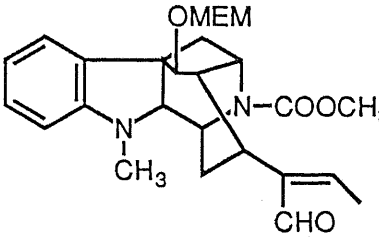
Spectral data of (172)

UV λ_{max}^{EtOH}nm: 293, 252, 224, 207.

IR($CHCl_3$) : 1690, 1455, 1110.

EI-MS *m/z*(%): 470(M^+,79), 381(19), 250(50), 182(61),144(63), 89(66)59(100).

^{1}H-NMR($CDCl_3$) δ : 9.34, 9.32 (1H, each s, CHO), 6.56(1H,m,C(19)-H) 2.07, (dd, J=7.6, 0.6Hz) 2.03(3H,dd,J=7.3,0.6Hz) C(18)-H_3).



Spectral data of (173)

UV λ_{max}^{EtOH}nm : 192, 251(sh), 230, 206.

IR($CHCl_3$) : 1690, 1670, 1460, 1110.

EI-MS *m/z*(%) : 470(M^+,100), 381(26), 250 (74),182(91),144(90),89(90), 59(73).

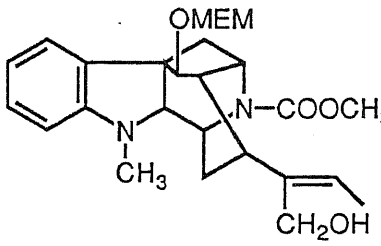
(173)25mg(0.053m mol). ^{1}H-NMR($CDCl_3$)δ :10.20, 10.19(1H,each d, J=1.5Hz,CHO), 6.65(1H, m,C(19)-H), 2.13(3H,d, J=6.7Hz, C(18)-H_3).

$NaBH_4$(2.1mg = 1.1eq)

MeOH(0.5ml)

rt., 30 min.

(174) 22mg : 88% yield from (173)



Spectral data of (174)

UV λ_{max}^{EtOH}nm : 291, 248, 202.

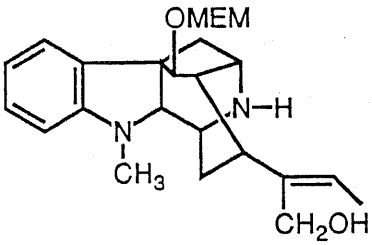
IR($CHCl_3$) : 3450, 1690, 1455, 1100.

El-MS *m/z*(%) : 472(M^+,68), 366(14), 252 (22), 182(55),144(68),89(53),59(100).

(174)22mg : 88% yield from (173)

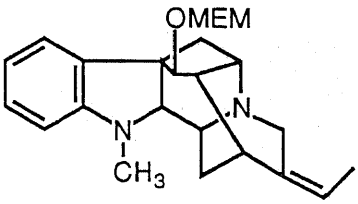
(174) 100mg (0.212 m mol)
NaOH(240mg)
Ethylene glycol(4ml), H_2O(0.8ml)
reflux, 210°C, 6 hrs.

^{1}H-NMR($CDCl_3$)δ:5.47(1H,m,C(19)-H),
4.23(2H,s,C(21)-H_2),1.70,
1.69(3H,each d, J=7.0Hz,
C(18)-H_3).



(175) 77mg : 87% yield from (174)

(175) 1556mg (3.75m mol)
Mesyl chloride(0.28 ml)
dry pyridine(60ml)
rt., 30 min.



Spectral data of (176)

UV λ_{max}^{EtOH}nm : 292, 249, 205.

IR($CHCl_3$) : 1475, 1465, 1300, 1100, 1040.

El-MS *m/z*(%): 396(M^+,100), 307(33), 291 (35),183(37),144(24), 89(18), 59(66).

(176) 925mg : 62% yield from (175).

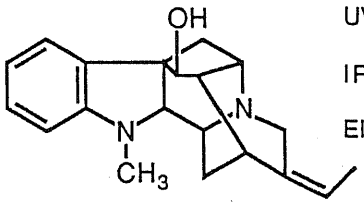
(176)50mg(0.126m mol). ^{1}H-NMR($CDCl_3$) δ : 5.30(1H,qt,J=6.7,2.3Hz,

conc. HCl(1 drop)

MeOH (1 ml)

reflux, 90°C, 5 hrs.

C(19-H),3.60,3.29(each 1H, dt,J=16.5Hz, C(21)-H_2),1.56 (3H,d,J=6.7Hz,C(18)-H_3).



Spectral data of (177)

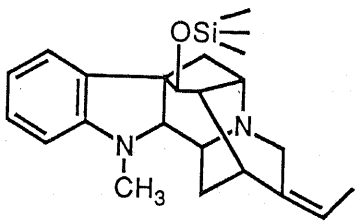
UV λ_{max}^{EtOH}nm : 292, 248, 205.

IR(KBr) : 3050, 1605.

EI-MS *m/z*(%) :308(M^+,100), 291(6), 277(9), 183(40), 182(17), 157(13), 144 (16), 131(6).

(177)25mg:95% yield from(176) ^{1}H-NMR($CDCl_3$)δ:5.31(1H,qt,J=6.7, 2.2Hz,C(19)-H),4.4(1H,s,C (17)-H), 3.62(1H,d,J=17.1Hz,C(21)-H), 3.29(1H,d,J=16.5Hz,C (21)-H),4.44(1H,s,C(17)-H), 3.46 (1H,d,J=9.8Hz,C(3)-H), 2.78(3H,s,N-CH_3), 1.57(3H, d,J=6.7Hz,C(18)-H_3).

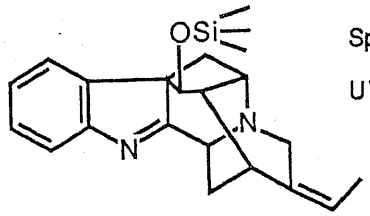
(177)30mg(0.097m mol).
TMS-trifluoromethanesulfonate(40μl).
Et_3N(20μl), dry CH_2Cl_2(3ml)
rt., 30 min.



(178) 33mg : 80% yield from (177)

(178) 123.3mg (0.324 m mol)
$Pb(OAc)_4$(480 mg = 3eq)
dry CH_2Cl_2(2 ml)
-70~-10°C, 5 hrs.

(179) 57 mg : 48% yield from (178)



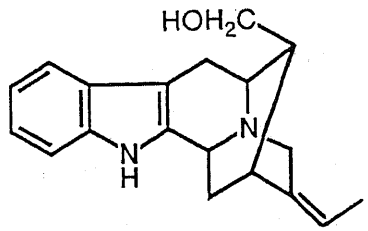
Spectral data of (179)

UVλ_{max}^{EtOH}nm : 261, 226(sh), 221, 215(sh)

(179) 44mg(0.121m mol)

(1) AcOH-THF-H_2O(3:1:1) (1.5ml)
rt. 15 min.

(2) SM(42mg), $NaBH_4$(9mg=2eq),MeOH(1ml)
rt. 15min



Spectral data of (22)

mp : 202-204 °c(acetone).

UV λ_{max}^{EtOH} : 289(sh), 282, 227.

IR(KBr) : 3200, 1450, 1035.

EI-MS*m/z*(%):295(20),294(M^+,100), 293 (91), 277(14), 263(44), 249(11), 182 (11), 170(18), 169(99), 168(67), 167 (11), 156(10), 115(10).

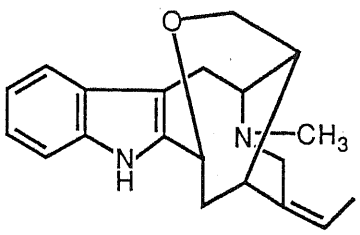
(22) Koumidine 25mg : 70% yield from (179)

1) (22)33mg(0.112mmol), ClCOOCH3(26μl=3eq), MgO(22mg=5eq), THF,H_2O(2.2,0.55ml) rt., 1 hr.

2) SM (20mg),$LiAlH_4$ (22mg),dry THF(1ml) rt., 2 hrs.

^{1}H-NMR(CD_3OD) δ : 5.37(1H,qt,J=6.7Hz,C (19)-H), 4.12(1H,dd,J=9.8,3.7Hz,C (3)-H), 3.76 and 3.60 (each 1H, br-d, J=17.1Hz, C(21)-H_2), 3.52 (1H, dd, J=10.7,6.4Hz,C(17)-H), 3.15(1H,dd, J=10.8,9.0Hz,C(17)-H), 3.01 and 2.9 (each 1H, dd,J=16.2,1.5Hz,C(6)-H_2), 2.44(1H,br-dd,J=5.6,2.9Hz,C (15)-H), 2.24(1H,m,C(16)-H), 1.61 (3H,dt, J=6.7,1.5Hz,C(19)-H_3).

(25) 19-(Z)-Taberpsychine 6.2mg : 29% yield from (22)



(25) 19-(Z)-Taberpsychine.

Spectral data of (25)

UVλ_{max}^{EtOH}nm : 292, 285, 280(sh), 224.

IR($CHCl_3$) : 3460, 1460, 1340, 1075.

EI-MS*m/z*(%):309(23),308(M^+,100, 293 (25), 279(12), 154(54), 123(12), 122 90), 121(59), 120(26).

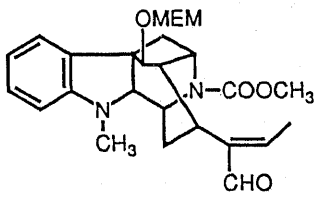
^{1}H-NMR($CDCl_3$) δ : 7.92(1H,s,NH), 7.63(1H,d, J=7.4Hz,C(9)-H), 7.14(1H,t,J=7.4 Hz,C(10)-H), 7.19(1H,t,J=7.4Hz, C(11)-H), 7.32(1H,d,J=7.4Hz,C(12) -H), 5.43(1H,m,C(19)-H), 5.12(1H, d,J=9.9Hz,C(3)-H), 3.84(1H,dd,J= 10.9Hz,C(17)-H), 3.26(1H,d,J=10.9 Hz,C(17)-H), 3.12(1H,m,C(5)-H), 2.82(1H,m,C(15)-H), 2.60(3H,s, NCH_3), 2.44(1H,dt,J=14.2,9.7Hz, C(14)-H), 2.11(1H,dd,J=14.0,10.7 Hz,C(14)-H), 1.60(3H,d,J=6.9Hz, C(18)-H_3).

Both synthetic compounds, koumidine and 19-(Z)-taberpsychine were proved to be the same as alkaloids isolated from *Gelsemiun elegans* Benth. Therefore, the absolute configulation of both natural alkaloids has been confirmed.

5.2 Formal Synthesis of Koumine (19)

Compound (172) as 19-(*E*)-form was reduced with $NaBH_4$ and ring closure between C_{21} and N(b) was performed by treatment of the resulting alcohol with NaOH in aqueous ethylene glycol and mesyl chloride in pyridine to give deoxyajmaline derivative (182). The conversion of indoline moiety into the indolic compound could be accomplished by the protection of the C_{17} hydroxy group of the indolenine derivative (185). The compound (185) was treated with AcOH-THF-H_2O (3:1:1) at room temperature and then reduced with $NaBH_4$ in MeOH to afford 19-(E)-koumidine (186). 19-(E)-koumidine (186) was treated with methyl chloroformate in THF-H_2O in the presence of MgO and reduced with lithium aluminium hydride to furnish anhydrovobasinediol (187).

Liu and Yu (1987) reported that anhydrovobasinediol (187) was treated with SeO_2, H_2O_2 and H_2SO_4 to afford koumine (19). Therefore, the formal synthesis of koumine (19) had been carried out. These conversions are shown as follows:



(172)

(172) 819mg (1.74 m mol)
$NaBH_4$ 65.8mg (1eq)
MeOH 16.5 ml
rt., 30 min.

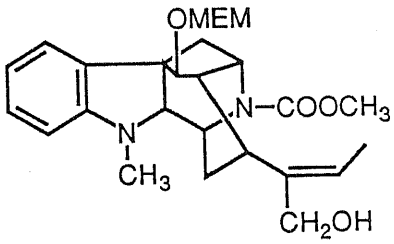
(180) 719 mg : 87.4% yield from (172)

Spectral data of (180)

UV λ_{max}^{EtOH} nm : 291, 248, 202

IR ($CHCl_3$) : 3450, 1690, 1460, 1120

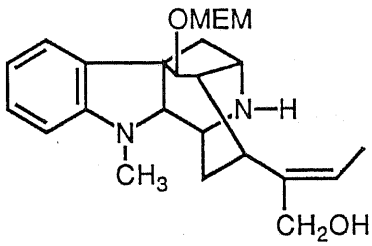
EI-MS *m/z* (%) : 472(M^+,60), 366(18), 252(13), 182(59),144(70), 89(58), 59(100)



(180)

^{1}H-NMR ($CDCl_3$) δ : 5.60(1H, m, C(19)-H)
4.06(2H, s, C(21)-H_2)
1.70(3H,d,J = 6.7Hz, C(18)-H_3

(180) 190mg (0.402 m mol)
NaOH 480mg
ethylene glycol,H_2O(8,1.6ml)
reflux 205°C, 1 hr.



(181) 141mg : 85% yield from (180)

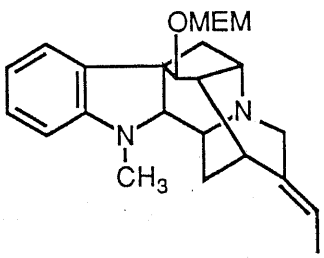
(181) 140mg (0.338m mol)
MsCl (29μl),dry pyridine (3ml)
rt., 1hr.

Spectral data of (182)

UV λ_{max}^{EtOH}nm : 291, 248, 205

IR ($CHCl_3$) : 1605, 1040

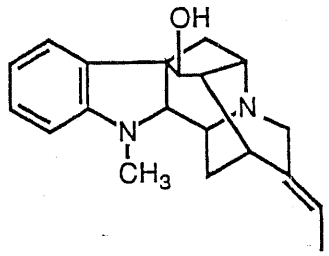
EI-MS *m/z* (%) : 396(M+,100),307(32), 291(31), 183(36), 277(12), 144(20), 89(15), 59(45)



(182) 98mg : 73% yield from (181).

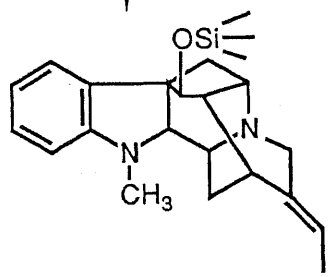
(182)

(182) 47mg(0.119 m mol)
conc. HCl (1 drop)
MeOH (1.5 ml)



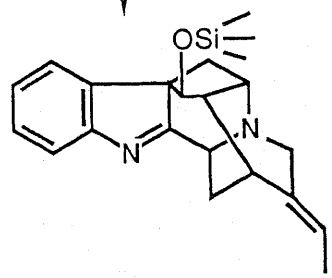
(183) 28mg : 77% yield from (182)

(183) 50mg (0.162 m mol)
TMS-trifluoromethane sulfonate 47μl (1.5eq)
dry Et_3N (34μl =1.5eq), dry CH_2Cl_2 (5ml)



(184) 61mg : 98% yield from (183).

(184) 61mg (0.16 m mol)
$Pb(OAc)_4$ (344mg =4.4eq)
dry CH_2Cl_2 (2ml)



(185) 25mg : 42.8% yield from (184)

^{1}H-NMR ($CDCl_3$) δ :
5.26 (1H, m,C(19)-H), 4.36(1H, s,C(17)-H) 3.55(1H,d,J=12.2Hz C(21)-H_2) 1.65 (3H, dt, J = 6.7, 1.9Hz, C(18)-H_3)

Spectral data of (183)

mp. : 294-296°C
UV λ_{max}^{EtOH}nm : 291, 249, 205
IR (KBr) : 3050, 1605
EI-MS *m/z* (%) : 308 (M^+, 100),291(5), 277(8), 183(44), 182(44), 157(16)
^{1}H-NMR ($CDCl_3$+CD_3OD) δ : 6.64(1H, m, C(19)-H) 4.38(1H, d, J = 0.9Hz, C(17)-H) 3.48(1H,d,J=9.2Hz,C(21)-H 2.76(3H,s, N-CH_3) 1.68 (3H, dq, J = 7.8, 1.1Hz, C(18)-H_3

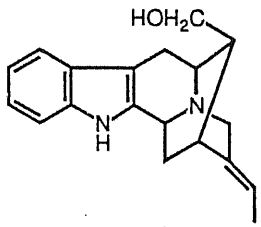
Spectral data of (185)

UV λ_{max}^{EtOH}nm :.261,226(sh) 220, 215(sh)

(185)

1) (185) 25mg (0.069 m mol)
AcOH-THF-H2O = 3:1:1 (0.83ml)

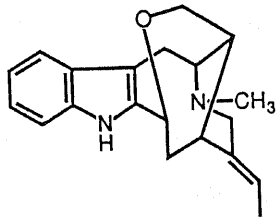
2) SM. 23mg (0.079 m mol)
$NaBH_4$(5mg=2eq), MeOH(1ml)



(186) 7mg : 35% yield from (185)

1) (186) 100mg (0.340 m mol)
$ClCOOCH_3$ (39μl),MgO(69mg),
THF(6.8ml), H_2O(1.7ml)
rt., 30 min.

2) (SM) 92mg
$LiAlH_4$(130mg), dry THF(5ml)
rt., 4.5hrs.

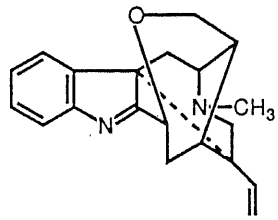


Spectral data of (187)
mp. : 196-200°C
$[\alpha]_D^{22}$: -280° (c =0.4, MeOH)
UV λ_{max}^{MeOH}nm : 292, 283, 222
IR (KBr) : 3289, 2790, 1462, 1340

(187) 58mg : 55% yield from (186). EI-MS *m/z* (%) : 308(M^+,95), 293 (25), 279(10), 122(100)

SeO_2, H_2SO_4
H_2O_2

^{1}H-NMR ($CDCl_3$) δ : 8.34 (1H,br-s, NH) 5.39 (1H, q, J =7Hz,C(19)-H)
5.15 (1H,d, J=9.5Hz, C(3)-H)
3.83 (2H, t, J=10.5Hz,C(17)-H_2)
3.6,2.9(each H,d,J=14.5Hz,C(21)-H_2)
1.69(3H,d, J=7hz,C(18)-H_3)



koumine (19)

6. Conclusion

This investigation also revealed the percentage of crude base from different parts of *Gelsemium elegans* Benth. The roots of this plant contained the highest quantity of crude base, comparing with its stems and branches, leaves and seeds. The yields of crude alkaloids which based on dry roots, stems and branches, leaves and seeds were 1.1, 0.23, 0.24 and 0.6 percentages, respectively. Among isolated alkaloids, gelsemine is the main alkaloid from any mentioned parts of this plant except from the seeds which contains only 14-hydroxygelsedine and the roots, koumine is the major component.

Sixteen alkaloids have been isolated and characterized. They are gelsemine, gelsevirine, koumine, gelsenicine, 14-hydroxygelsenicine, humantenine, 14-hydroxygelsedine, koumidine, 19-(*Z*)-akuammidine, 16-epi-voacarpine, 19-hydroxydihydrogelsevirine, 19-(*Z*)-taberpsychine, koumine N-oxide, gelsemine N-oxide, 19-oxogelsenicine and elegansamine, the last seven isolated bases are new alkaloids. The structures of koumidine and 19-(Z)-akaummidine have been revised from (19*E*)-form to(19*Z*)-form. Furthermore, the synthesis and absolute configuration determination of *Gelsemium* alkaloids koumidine, 19-(Z)-taberpsychine and koumine have been carried out, including the proposal of biogenetic route of *Gelsemium* alkaloids.

PART IV

EXPERIMENTAL

EXPERIMENTAL

Source and Authentication of Plant Material

The roots, stems and branches, leaves and seeds of *Gelsemium elegans* Benth. were collected from Phuu Luang National Park, Loei Province, Thailand in October, 1985. The plant was identified by Dr. Tem Smitinand, the former Deputy Director-General, Royal Forest Department of Thailand. A herbarium specimen is kept in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

General Techniques

1. Chromatography

1.1 Analytical thin-layer chromatography

The TLC plates for routine work were Pre-Coated TLC Plates of Silica gel 60 F-254 and Pre-Coated TLC Plates of Aluminum oxide F-254 (type E)(Merck).

Technique : one way, ascending, 5cm.

Temperature : laboratory temperature (15-35°C)

Solvent systems : the following solvent systems were used depending on the mobility of the alkaloids being examined.

chloroform

acetone

ether

ethyl acetate

ethyl acetate : benzene (80:20)

ethyl acetate : n-hexane (80:20)

ethyl acetate : n-hexane (50:50)

ethyl acetate : methanol (90:10)

ethyl acetate : methanol (80:20)

ethyl acetate : methanol (50:50)

chloroform : benzene (50:50)

chloroform : ethyl acetate (60:40)

chloroform : acetone (50:40)

chloroform : methanol (90:10)

chloroform : methanol (80:20)

chloroform : methanol (60:40)

chloroform : methanol (95:5)

methanol : dichloromethane (0.5:95.5)

Detection : a) ultraviolet light at wavelength 254nm

b) Dragendorff's spray reagent (This reagent was kept as a stock solution consisting of a mixture of bismuth subnitrate 850mg, glacial acetic acid 10ml, distilled water 40ml, and potassium iodide 8gm, distilled water 20ml. The working solution is made by mixing 10ml of the stock solution with 20ml of glacial acetic acid and 70ml of distilled water. Dragendorff's reagent is used as a general alkaloid-detecting reagent.)

c) 0.2M anhydrous ferric chloride in 35% W/V perchloric acid spray reagent. Plates heated at 90°C for 10 minutes. (The indole and oxindole alkaloids give olive green to grey or brown and pink to purple spots as positive test, respectively.)

1.2 Preparative thin-layer chromatography

Pre-Coated for preparative thin-layer chromatography plates silica gel 60 F_{254} (Merck), layer thickness 1 mm were used.

Technique : one way, ascending, 15cm

Temperature : laboratory temperature (15-35°C)

Solvent systems : ether

chloroform : ethyl acetate (6:4)

chloroform : ethyl acetate (95:5)

acetone : n-hexane (4:6)

ethyl acetate : methanol (45:55)

Detection : ultraviolet light at wavelength 254nm

1.3 Column chromatography

Technique : open column chromatography, flash column chromatography and medium pressure column chromatography

Adsorbents : silica gel 60 (Merck) 70-230mesh; silica gel 60 (Merck) 230-400mesh; aluminium oxide 90 active, neutral 70-230mesh (Merck); Merck Aluminium Oxide (activity II-III) and Merck Lober Si 60 (for medium pressure column chromatography)

Temperature : laboratory temperature (15-35°C)

Packing : a) adsorbents packed dry into the column.

b) adsorbents poured slowly into the column containing solvents.

Addition of alkaloidal material to column

: crude alkaloid was dissolved in small amount of organic solvent and added onto the top of column.

Solvent systems : n-hexane

n-hexane : ethyl acetate (10:90)

n-hexane : ethyl acetate (20:80)

n-hexane : ethyl acetate (40:60)

ethyl acetate

ethyl acetate : methanol (95:5)

ethyl acctate : methanol (90:10)

ethyl acetate : methanol (80:20)

ethyl acetate : methanol (70:30)

ethyl acetate : methanol (50:50)

chloroform

chloroform : methanol (95:5)

chloroform : methanol (90:10)

chloroform : methanol (80:20)

chloroform : methanol (50:50)

ether

Detection of eluate : by thin-layer chromatography and ultraviolet light at wavelength 254 nm.

2. Physical Constant

All melting points were measured on a Yamato MP-21 apparatus and are uncorrected.

3. Spectroscopy

3.1 Ultraviolet absorption spectra were measured in MeOH with a Hitachi 340 or Hitachi U3400 spectrometers.

3.2 Infrared absorption spectra were measured with a Hitachi 260 spectrometer. The materials were examined in potassium bromide disc or in chloroform solutions.

3.3 Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM FX-270 and JNM GX-270 (270MHz) spectrometers with tetramethylsilane (T.M.S.) as an internal standard in deuterochloroform ($CDCl_3$) unless otherwise stated.

3.4 ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra were measured with JEOL JNM FX-270 and JNM GX-270 (67.8 MHz) spectrometers with tetramethylsilane as an internal standard.

3.5 Mass spectra were taken with Hitachi RMU-60 and RMU-7M spectrometers

3.6 CD spectra were measured with JASCO J-500A and J-20 in MeOH

4. Solvents

Throughout the work all organic solvents were redistilled before use.

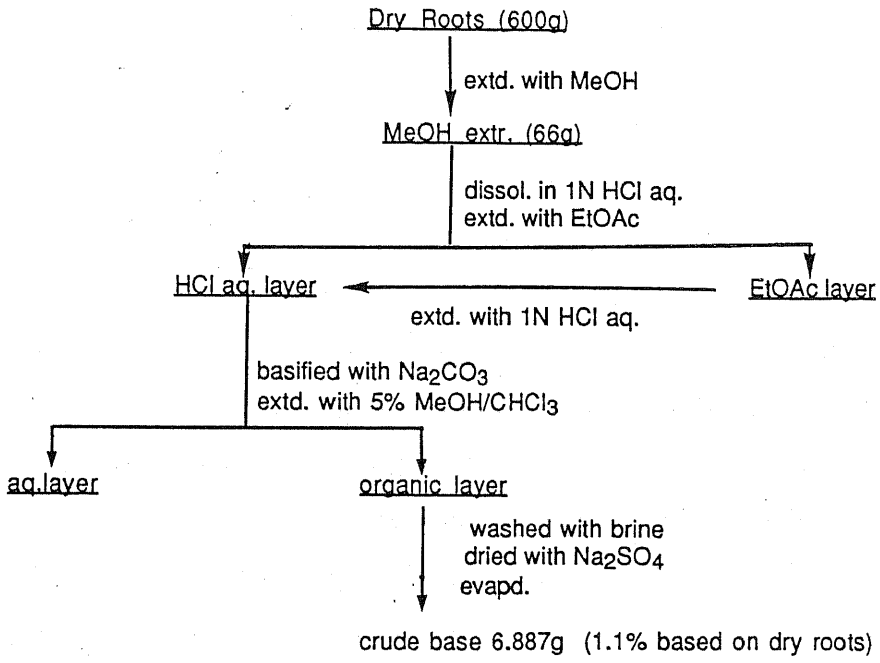
Extraction and Isolation of Alkaloids

1. Extraction and Isolation of Alkaloids from the Roots

1.1 Extraction of alkaloids from the roots

The dried coarsely powdered roots (600g) were extracted with MeOH at room temperature for three times (for 3, 5 and 7 days) and filtered. The combined methanol filtrate was concentrated in vacuo to afford syrupy crude extract (66g), which was dissolved in 1N HCl solution and partitioned to ethyl acetate. After

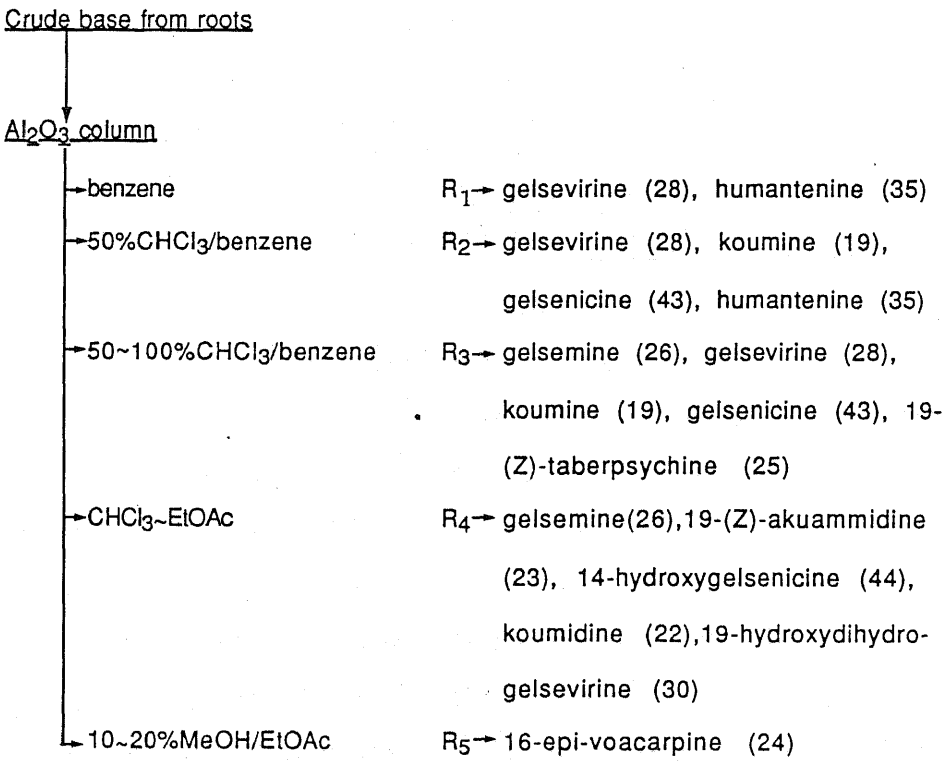
the back-extraction of ethyl acetate with 1N HCl, the combined acidic layer was basified to pH 10 with solid Na_2CO_3 at 0°C and then extracted with 5% MeOH/$CHCl_3$ three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure to give crude base 6.88g (1.1%). The diagram of extraction is shown as follows:



1.2 Isolation of alkaloids from the roots

The portion of alkaloidal fraction (6.8g) was roughly separated with Al_2O_3 column chromatography. The column was eluted with benzene, 50% $CHCl_3$/benzene, 50~100% $CHCl_3$/benzene, $CHCl_3$~EtOAc, and 10~20% MeOH/EtOAc until no traces of alkaloid could be detected. The mentioned solvent systems afforded fractions R_1,R_2,R_3,R_4 and R_5,respectively. These fractions were

purified with SiO_2 column chromatography, flash column chromatography, medium pressure column chromatography and/or preparative TLC where appropriate. The alkaloids gelsevirine (28), and humantenine (35); gelsevirine (28), koumine (19), gelsenicine (43), and humantenine (35); gelsemine (26), gelsevirine (28), koumine (19), gelsenicine (43), and 19-(Z)-taberpsychine (25); gelsemine (26), 19-(Z)-akuammidine (23), 14-hydroxygelsenicine (44), koumidine (22) and 19-hydroxydihydrogelsevirine (30); and 16-epi-voacarpine (24) were obtained respectively. The details are shown as follows:



Gelsemine (26)

The fraction R_3-R_4 eluent from Al_2O_3 column chromatography was subjected to repeat flash column chromatography using 1% MeOH/$CHCl_3$ sat. with aq. NH_3 as a solvent to afford colorless needles of gelsemine (26) (369mg).

Gelsevirine (28)

The benzene-50% $CHCl_3$/benzene eluent from Al_2O_3 column chromatography was subjected to medium pressure column chromatography using 10% MeOH/$CHCl_3$ as a solvent system to give an amorphous solid of gelsevirine (28) (495mg), which was obtained as HCl salt.

Koumine (19)

The 50% benzene/$CHCl_3$-$CHCl_3$ eluent from Al_2O_3 column chromatography was purified by flash column chromatography using 5-10%MeOH/$CHCl_3$ to afford colorless plates or columnar crystals of koumine (574mg).

Gelsenicine (43)

The 50% benzene/$CHCl_3$-$CHCl_3$ eluent from Al_2O_3 column chromatography was subjected to medium pressure column chromatography using 10% MeOH/$CHCl_3$ as a solvent system to give colorless plates or needles of glesenicine (331mg).

14-Hydroxygelsenicine (44)

The fraction R_4 eluent from Al_2O_3 chromatography was isolated by medium pressure column chromatography using 10% MeOH/$CHCl_3$ to afford an amorphous solid of 14-hydroxygelsenicine (169mg).

Humantenine (45)

The benzene-50% $CHCl_3$/benzene eluent from Al_2O_3 column chromatography was subjected to medium pressure column chromatography using

10% MeOH/$CHCl_3$ as a solvent to give an amorphous solid of humantenine (354mg), which was obtained as HCl salt.

19-(Z)-Akuammidine (23)

The fraction R_4 eluent from Al_2O_3 column chromatography was isolated by flash column chromatography using 5% MeOH/$CHCl_3$ as a solvent, the eluent was purified by SiO_2 column chromatography using 1% MeOH/$CHCl_3$ sat. with aq. NH_3 to give colorless needles of 19-(Z)-akuammidine (35mg).

Koumidine (22)

The fraction R_4 eluent from Al_2O_3 column chromatography was isolated by flash column chromatography using 10% MeOH/$CHCl_3$ as a solvent. After that SiO_2 column chromatography was eluted with 1% MeOH/$CHCl_3$ sat. with aq. NH_3 to yield colorless needles of koumidine (47mg).

16-epi-Voacarpine (24)

The fraction R_5 eluent from Al_2O_3 column chromatography was subjected to repeat SiO_2 column chromatography using 5% MeOH/$CHCl_3$ and 1% MeOH/$CHCl_3$ sat. with aq. NH_3 to afford colorless prisms or plates of 16-epi-voacarpine (78mg).

19-Hydroxydihydrogelsevirine (30)

The fraction R_5 eluent from Al_2O_3 column chromatography was isolated by flash column chromatography, eluting with 20% MeOH/$CHCl_3$; purified by SiO_2 chromatography using 2% MeOH/$CHCl_3$ sat. with aq.NH_3; and medium pressure column chromatography using 10% MeOH/$CHCl_3$ to afford colorless amorphous of 19-hydroxydihydrogelsevirine (11mg).

19-(Z)-taberpsychine (25)

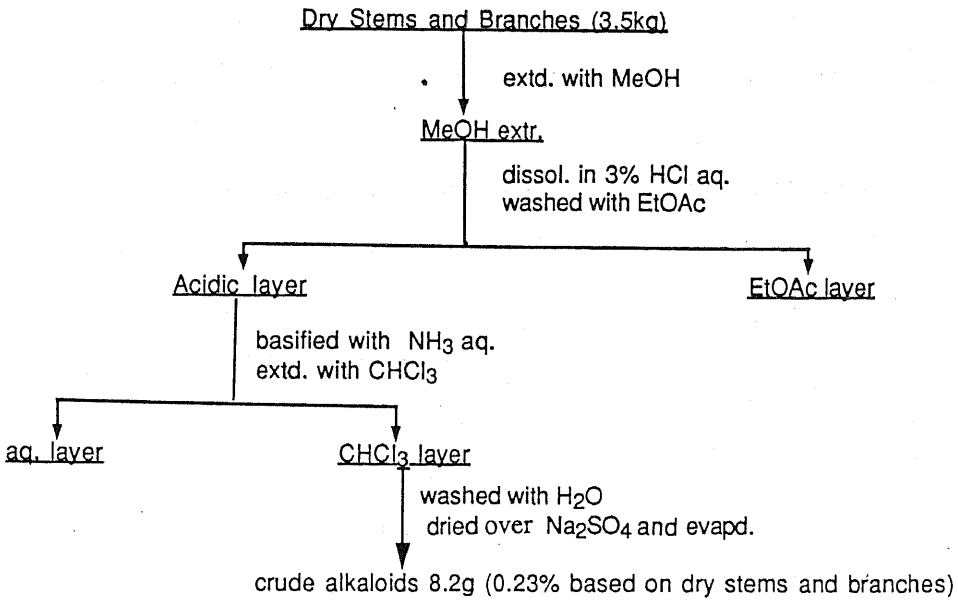
The 50% benzene/$CHCl_3$-$CHCl_3$ eluent from Al_2O_3 column chromatography was isolated by flash column chromatography using 5% MeOH/$CHCl_3$ as a solvent. After that was subjected to repeat medium pressure

column chromatography (10%MeOH/$CHCl_3$), Al_2O_3 column chromatography (EtOAc) and preparative TLC using 40% MeOH/EtOAc as a solvent to give 19-(Z)-taberpsychine (3mg).

2. Extraction and Isolation of Alkaloids from the Stems and Branches

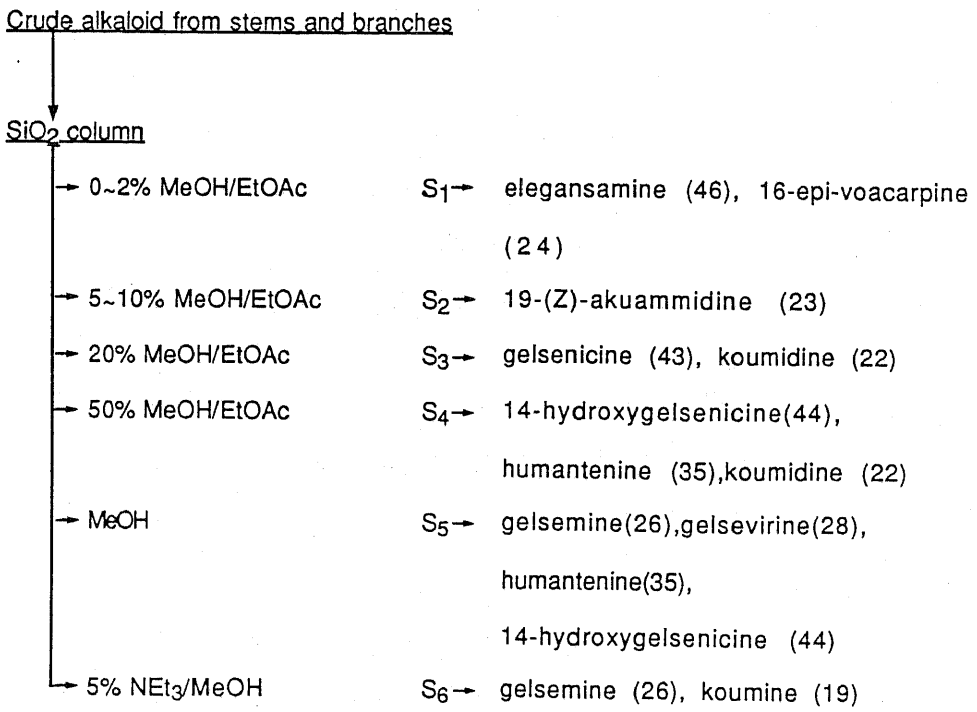
2.1 Extraction of alkaloids from the stems and branches

The dried coarsely powdered stems and branches (3.5kg) were extracted with MeOH at room temperature for three times (for 3, 5 and 7 days) and filtered. The combined methanol filtrate was concentrated to syrupy mass under reduced pressure, and dissolved in 3% HCl solution with well shaken. The acidic filtrate was washed with portions of EtOAc, then made basic (pH10) with strong solution of ammonium hydroxide and extracted with $CHCl_3$ six times. The combined $CHCl_3$ extract was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield crude alkaloids 8.2g (0.23%), as diagram shown as follows :



2.2 Isolation of alkaloids from stems and branches

The portion of alkaloidal extract (7.1g) was roughly separated with SiO_2 column chromatography. The column was eluted with 0~2% MeOH/EtOAc, 5~10% MeOH/EtOAc, 50% MeOH/EtOAc, MeOH, and 5% NEt_3/MeOH until no traces of alkaloid could be detected. According to the solvent systems, they afforded fractions S_1, S_2, S_3, S_4, S_5 and S_6, respectively. These fractions were then purified with SiO_2 column chromatography, flash column chromatography, medium pressure column chromatography and/or preparative TLC where appropriate. The alkaloids elegansamine (46) and 16-epi-voacarpine (24); 19-(Z)-akuammidine (23); gelsenicine (43) and koumidine (22); 14-hydroxygelsenicine (44), humantenine (35) and koumidine (22); gelsemine (26), gelsevirine (28), humantenine (35) and 14-hydroxygelsenicine (44); gelsemine (26) and koumine (19); were obtained respectively. The details are shown as follows:



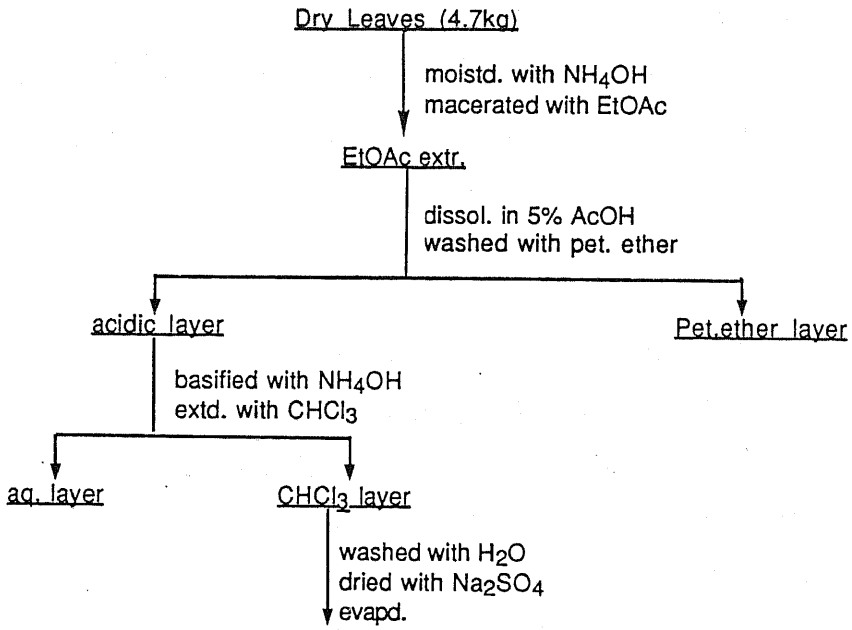
Elegansamine (46)

The 0~2% MeOH/EtOH eluent from the SiO_2 column chromatography was subjected to repeat Al_2O_3 column chromatography using $CHCl_3$ as a solvent to afford colorless prisms of elegansamine (8mg).

3. Extraction and Isolation of Alkaloids from the Leaves

3.1 Extraction of alkaloids from the leaves

The dried coarsely powdered leaves (4.7kg) were moistened with strong ammonium hydroxide solution and allowed to stand overnight. It was then macerated with ethyl acetate for three days and filtered. The marc was remacerated with ethyl acetate for three days and filtered. The combined filtrate was concentrated to syrupy mass under reduced pressure, mixed with glacial acetic acid then poured into a large volume of warm water to give about 5% acetic acid solution, well shaken and left to stand over night. The acidic filtrate was washed with portions of petroleum ether three times, then basified to pH10 with strong solution of ammonium hydroxide and extracted with chloroform. The combined chloroform extract was washed with water, dried over anhydrous sodium sulfate and evaporated to yield crude alkaloids (11.38g).

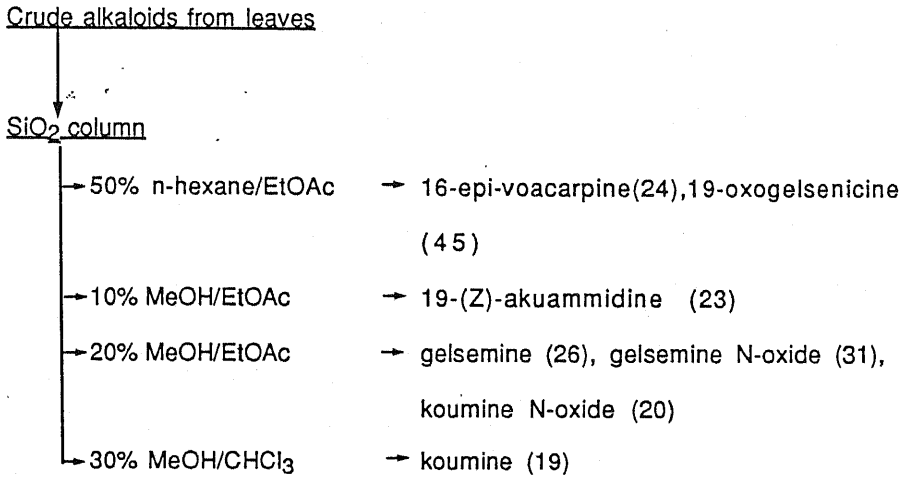


crude alkaloids 11.38g (0.24% based on dry leaves)

3.2 Isolation of alkaloids from the leaves

The portion of alkaloidal extract (2.5g) was dissolved in chloroform (10ml) and mixed with small amount of silica gel. The content was dried and packed onto the top of dry silica gel column. The column was eluted successively with 50% n-hexane/EtOAc, 10% MeOH/EtOAc, 20% MeOH/EtOAc, 30% MeOH/$CHCl_3$ and then with MeOH. The 50% n-hexane/EtOAc eluent was further purified by SiO_2 column chromatography using 50% n-hexane/EtOAc and then by preparative TLC (60%$CHCl_3$/EtOAc) to give 26mg of 16-epi-voacarpine (24) and 6mg of 19-oxogelsenicine (45). The 10% MeOH/EtOAc eluent was subjected to SiO_2 column chromatography and 11mg of 19-(Z)-akuammidine (23) was obtained from the fractions of 5% MeOH/EtOAc eluent. The 20% MeOH/EtOAc eluent was subjected to Al_2O_3 column chromatography. From 40% n-hexane/EtOAc eluent, 159mg of

gelsemine (26) was obtained. The 30% n-hexane/$CHCl_3$ eluent from Al_2O_3 column chromatography was further purified by preparative TLC (45% EtOAc/MeOH, triple development) to yield 18mg of gelsemine N-oxide (31) and 13mg of koumine N-oxide (20). The 30% MeOH/$CHCl_3$ eluent from the first SiO_2 column chromatography was further purified by Al_2O_3 column chromatography using 40% n-hexane/EtOAc to yield 20mg of koumine (19).

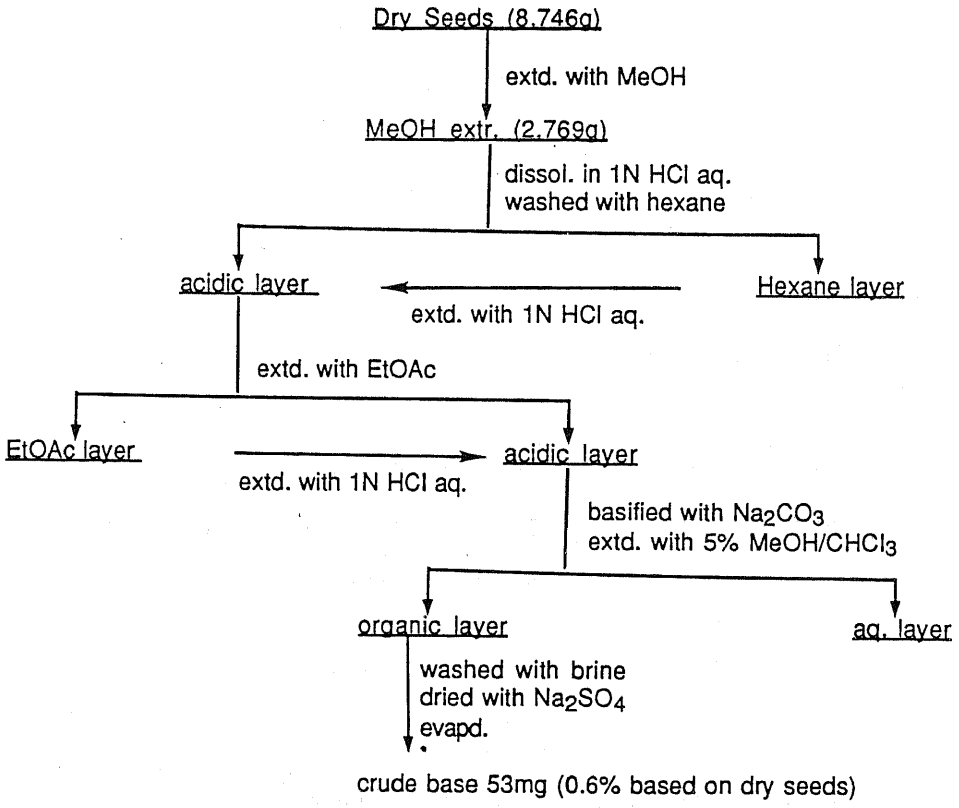


4. Extraction and Isolation of Alkaloid from the Seeds

4.1 Extraction of alkaloid from the seeds

The dried powdered seeds (8.746g) were extracted with MeOH at room temperature three times. The combined methanol extract was concentrated in vacuo to afford crude extract (2.769g) which were dissolved in 1N HCl solution and washed with hexane. After the back-extraction of hexane layer with 1N HCl, the combined acidic layer was extracted with ethyl acetate which was further extracted with 1N HCl. The combined acidic layer was basified to pH10 with solid Na_2CO_3 at 0°C and then extracted with 5% MeOH/$CHCl_3$ three times. The organic layer was

washed with brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give crude base 53mg.



4.2 Isolation of alkaloid from the seeds

The crude base 53mg was subjected to flash column chromatography. The 20% MeOH/$CHCl_3$ eluent was further purified by SiO_2 column chromatography using 3% MeOH/$CHCl_3$ sat. with aq. NH_3 as a solvent system to give colorless needles of 14-hydroxygelsedine (40) 6mg.

5. Isolated Alkaloids and Their Yields

The isolated alkaloids and their yields from the roots, stems and branches, leaves and seeds of *Gelsemium elegans* Benth. are shown as follows.

Alkaloids	Roots		Stems and branches		Leaves		Seeds	
	mg	%	mg	%	mg	%	mg	%
Gelsemine (26)	369	5.4	1094	15.4	159	62.8	-	-
Gelsevirine (28)	495	7.3	95	1.3	-	-	-	-
Koumine (19)	574	8.4	251	3.5	20	7.9	-	-
Gelsenicine (43)	331	4.9	600	8.5	-	-	-	-
14-Hydroxygelsenicine (44)	169	2.5	501	7.1	-	-	-	-
Humantenine (35)	354	5.1	79	1.1	-	-	-	-
14-Hydroxygelsedine (40)	-	-	-	-	-	-	6	11.3
19-(Z)-Akuammidine (23)	35	0.5	55	0.8	11	4.3	-	-
Koumidine (22)	47	0.7	22	0.3	-	-	-	-
16-epi-Voacarpine (24)	78	1.1	94	1.3	26	10.3	-	-
19-Hydroxydihydro-gelsevirine (30)	11	0.2	-	-	-	-	-	-
19-(Z)-Taberpsychine (25)	3	0.04	-	-	-	-	-	-
Elegansamine (46)	-	-	8	0.1	-	-	-	-
19-Oxogelsenicine (45)	-	-	-	-	6	2.4	-	-
Gelsemine N-oxide (31)	-	-	-	-	18	7.1	-	-
Koumine N-oxide (20)	-	-	-	-	13	5.1	-	-
Total	2457	36.1	2799	39.5	253	10.2	6	11.3

% : Based on crude base

Characterization and Identification of Isolated Alkaloids

1. Gelsemine (26)

Gelsemine (26) was obtained as colorless needle crystals from acetone.

Melting point : 176-178°C

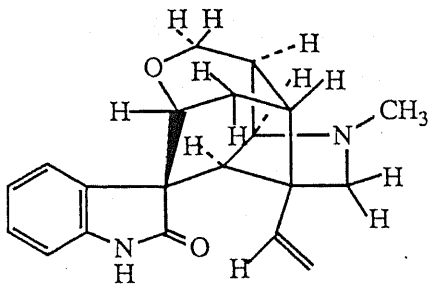
UV λ_{max}nm : 293 (sh), 280, 251, 208.

IR (KBr) : 1715, 1475, 1225, 1095.

MS *m/z* (%) : 322 (M^+, 47), 249 (55), 108 (100).

^{1}H-NMR δ : 7.86 (1H, br-s, NH), 7.24 (1H, d-like, J=7.6 Hz, C(9)-H), 7.00 (1H, t-like, J=7.6 Hz, C(10)-H), 7.19 (1H, t-like, J=7.6 Hz, C(11)-H), 6.77 (1H, d-like, J=7.6 Hz, C(12)-H), 6.26 (1H, dd, J=17.8, 11.0 Hz, C(19)-H), 5.10 (1H, dd, J=11.0, 1.3 Hz, C(18)-H), 4.94 (1H, dd, J=17.8, 1.3 Hz, C(18)-H), 4.11 (1H, dd, J=11.1, 2.3 Hz, C(17)-H), 3.91 (1H, dd, J=11.1, 2.0 Hz, C(17)-H), 3.82 (1H, br-s, C(3)-H), 3.44 (1H, br-s, C(5)-H), 2.83 (1H, dd, J=14.4, 3.0 Hz, C(14)-H), 2.00 (1H, ddd, J=14.2, 6.0, 3.0 Hz, C(14)-H), 2.42 (1H, br-d, J=8.9 Hz, C(16)-H), 2.25 (3H, s, N-CH_3), 2.78 and 2.31 (each 1H, d, J=10.2 Hz, C(21)-H_2).

^{13}C-NMR : See Table 1, page 144



2. Gelsevirine (28)

This base (28) was obtained as amorphous solid from acetone, which afforded as HCl salt.

Melting point : 255-260°C (dec.)

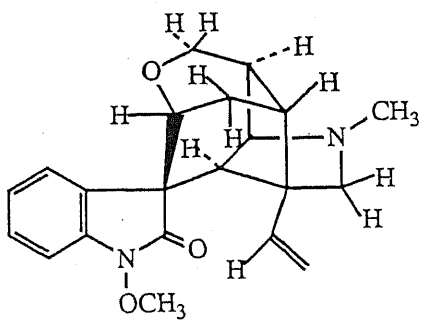
UV λ_{max}nm : 280 (sh), 254, 208.

IR (HCl salt, KBr) : 2440, 1735, 1463, 1078.

MS *m/z* (%) : 352 (M^+, 26), 321 (63), 309 (28), 108 (100).

^{1}H-NMR δ : 7.46 (1H, d-like, J=7.6, C(9)-H), 7.05 (1H, t-like, J=7.6 Hz, C(10)-H), 7.29 (1H, t-like, J=7.6 Hz, C(11)-H), 6.95 (1H, d-like, J=7.6 Hz, C(12)-H), 6.24 (1H, dd, J=17.8, 11.0 Hz, C(19)-H), 5.13 (1H, dd, J=11.0, 1.3 Hz, C(18)-H), 4.97 (1H, dd, J=17.8, 1.3 Hz, C(18)-H), 4.10 (1H, dd, J=10.9, 2.3 Hz, C(17)-H), 3.89 (1H, dd, J=10.9, 2.0 Hz, C(10)-H), 3.96 (3H, s, OCH_3), 3.81 (1H, br-s, C(3)-H), 3.38 (1H, d, J=1.3 Hz, C(5)-H), 2.84 (1H, dd, J=14.5, 3.0 Hz, C(14)-H), 2.00 (1H, ddd, J=14.5, 5.7, 2.6 Hz, C(14)-H), 2.42 (1H, br-d, J=8.6 Hz, C(5)-H),. 2.28 (1H, br-d, J=9 Hz, C(15)-H), 2.24 (3H, s, N-CH_3), 2.78 and 2.31 (each 1H, d, J=10.3 Hz, C(21)-H_2), 1.94 (1H, br-s, C(6)-H).

^{13}C-NMR : See Table 1, page 144



3. Koumine (19)

Koumine (19) crystallized from acetone as colorless plates or columnar crystals.

Melting point : 168-169°C

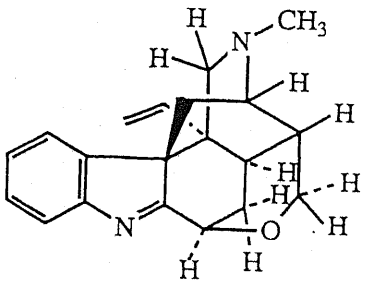
UV λ_{max}nm : 296 (sh), 262, 230 (sh), 221, 215 (sh).

IR (KBr) : 1450, 1215, 1085, 1070.

MS *m/z* (%) : 306 (M^+, 100), 224 (28), 71 (51).

^{1}H-NMR δ : 7.61 (1H, d-like, J=7.3 Hz, C(9)-H), 7.24 (1H, t-like, J=7.3 Hz, C(10-H), 7.35 (1H, t-like, J=7.55 Hz, C(11)-H), 7.55 (1H, d-like, J=7.55 Hz, C(12)-H), 5.01 (1H, br-s, C(3)-H), 4.83 (1H, dd, J=16.8, 2.0 Hz, C(18)-H), 4.78 (1H, dd, J=10.9, 2.0 Hz, C(18)-H), 4.67 (1H, dd, J=16.8, 10.9 Hz, C(19)-H), 4.25 (1H, dd, J=11.9, 4.3 Hz, C(17)-H), 3.61 (1H, d, J=11.9 Hz, C(17)-H), 3.08 and 3.18 (each 1H, each d, J=11.4 Hz, C(21)-H_2), 2.8 (1H, br-d, J=10 Hz, C(16)-H), 2.78 (1H, br-s, C(5)-H), 2.61 (3H, s, N-CH_3), 2.41 (H, dd, J=14.2, 1.7 Hz, C(6)-H), 2.34 (1H, dd, J=14.2, 2.0 Hz, C(6)-H), 2.61 (1H, dt, J=14.5, 4.0 Hz, C(14)-H), 1.88 (1H, dt, J=14.5, 2.3 Hz, C(14)-H), 2.34 (1H, br-d, J=~10 Hz, C(15)-H).

^{13}C-NMR : See Table 1, page 144



4. Gelsenicine (43)

This base (43) crystallized from Et_2O as colorless plates or needle crystals.

Melting point : 168-170°C

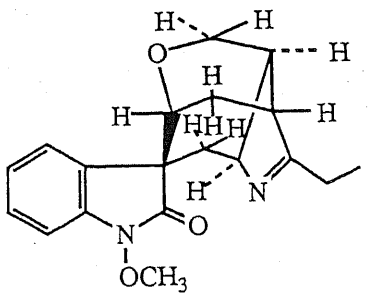
UV λ_{max}nm : 281 (sh), 257, 208.

IR (KBr) : 1730, 1465, 1220, 1110, 1018.

MS *m/z* (%) : 326 (M^+, 53), 294 (100), 150 (74).

1H-NMR δ : 7.53 (1H, d-like, J=7.6 Hz, C(9)-H), 7.07 (1H, t-like, J=7.6 Hz, C(10)-H), 7.25 (1H, t-like, J=7.6 Hz, C(11)-H), 6.88 (1H, d-like, J=7.6 Hz, C(12)-H), 4.41 (1H, m C(5)-H), 4.31 (1H, dd, J=11.2, 3.0 Hz, C(17)-H), 4.26 (1H, dd, J=11.2, 1.0 Hz, C(17)-H), 3.95 (1H, s, OCH_3), 3.72 (1H, dd, J=4.5, 5.3 Hz, C(3)-H), 2.86 (1H, dd, J=9.9, 9.2 Hz, C(15)-H), 2.56 (1H, m, C(16)-H), 2.37 (1H, dd, J=14.8, 2.3 Hz, C(14)-H), 2.10 (1H, ddd, J=14.8, 10.1, 4.7 Hz, C(14)-H), 2.72 and 2.41 (each 1H, dq, J=17.1, 7.6 Hz, C(19)-H_2), 2.40 (1H, dd, J=15.4, 4.6 Hz, C(6)-H), 2.28 (1H, dd, J=15.4, 2.4 Hz, C(6)-H), 1.29 (3H, t, J=7.3 Hz, C(18)-H_3).

^{13}C-NMR : See Table 1, page 144



5. 14-Hydroxygelsenicine (44)

This base (44) was obtained as amorphous solid.

Melting point : 158-162°C

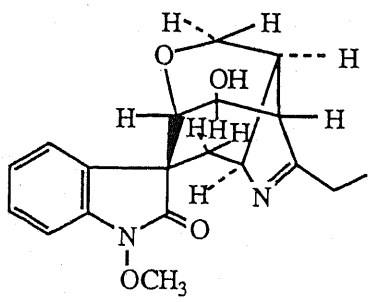
UV λ_{max}nm : 281 (sh), 265 (sh), 258, 209.

IR (KBr) : 3400, 1715, 1470, 1045, 1015.

MS *m/z* (%) : 342 (M^+, 83), 312 (45), 311 (100), 108 (34).

^{1}H-NMR δ : 7.51 (1H, d-like, J=7.6 Hz, C(9)-H), 7.07 (1H, t-like, J=7.6 Hz, C(10)-H), 7.26 (1H, t-like, J=7.6 Hz, C(11)-H), 6.89 (1H, d-like, J=7.6 Hz, C(12)-H), 4.44 (1H, d, J=3.0 Hz, C(14)-H), 4.44 (1H, dd, J=10.9, 3.6 Hz, C(17)-H), 4.31 (1H, br-d, J=10.9 Hz, C(17)-H), 4.41 (1H, m, C(5)-H), 3.93 (3H, s, OCH_3), 3.76 (1H, br-s, C(3)-H), 2.88 (1H, br-d, J=7.2 Hz, C(15)-H), 2.58 (1H, m, C(16)-H), 2.73 and 2.79 (each 1H, each-q, J=7.3 Hz, C(19)-H_2), 1.28 (3H, t, J=7.3 Hz, C(18)-H_3).

^{13}C-NMR : See Table 2, page 145



6. Humantenine (35)

Humantenine was obtained as amorphous solid from acetone which afforded as HCl salt.

Melting point : 202-205°C (dec.)

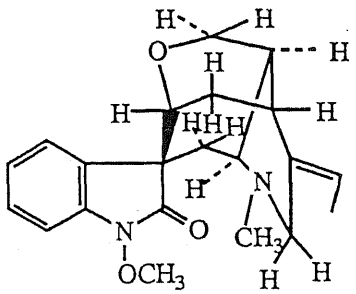
UV λ_{max}nm : 282 (sh), 253, 207.

IR (HCl salt, KBr) : 1715, 1620, 1470, 1430, 1218, 1210, 765.

MS *m/z* (%) : 344 (M^+, 65), 323 (100), 122 (86).

1H-NMR δ : 7.40 (1H, d-like, 7.6 Hz, C(9)-H), 7.11 (1H, t-like, J=7.6 Hz, C(10)-H), 7.32 (1H, t-like, J=7.6 Hz, C(11)-H), 7.00 (1H, d-like, J=7.6 Hz, C(12)-H), 5.38 (1H, br-q, J=6.7 Hz, C(19)-H), 4.20 (1H, d, J=10.9 Hz, C(17)-H), 4.06 (1H, dd, J=10.9, 4.9 Hz, C(17)-H), 4.07 (1H, s, OCH_3), 3.63 (1H, br-d, J=6.9 Hz, C(3)-H), 3.41 and 3.3 (each 1H, d, J=15.0 Hz, C(21)-H_2), 3.35 (1H, m, C(5)-H), 2.25 (3H, s, N-CH_3), 2.3 (1H, m, C(16)-H), 2.51 (1H, dd, J=15.5, 8.6 Hz, C(6)-H), 1.69 (1H, dd, J=15.5, 8.6 Hz, C(6)-H), 1.65 (3H, br-d, J=6.7 Hz, C(18)-H_3).

^{13}C-NMR : See Table 2, page 145



7. 19-(Z)-Akuammidine (23)

This base (23) crystallized from acetone as colorless needle crystals.

Melting point : 240-242°C

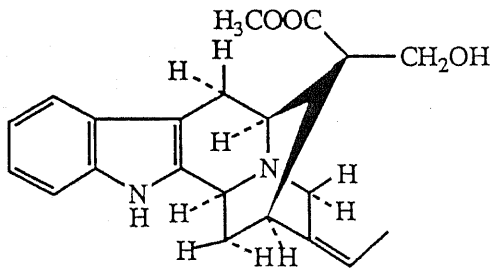
UV λ_{max}nm : 290, 280, 274 (sh), 226.

IR (KBr) : 3275, 1720, 1460, 1225.

MS *m/z* (%) : 352 (M^+, 100), 351 (53), 321 (48), 293 (22), 249 (60, 169 (66), 168 (41).

^{1}H-NMR (CD_3OD) δ : 7.36 (1H, d-like, J=7.3 Hz, C(9)-H), 6.98 (1H, t-like, J=7.3 Hz, C(10)-H), 7.03 (1H, t-like, J=7.3 Hz, C(11)-H), 7.26 (1H, d-like, J=7.3 Hz, C(12)-H), 5.44 (1H, m, C(19)-H), 4.2 (1H, br-d, J=8.3 Hz, C(3)-H), 3.74 and 3.69 (each 1H, each-d, J=10.2 Hz, C(17)-H_2), 3.57 and 3.63 (each 1H, br-d, J=13.5 Hz, C(21)-H_2), 2.96 (3H, s, $COOCH_3$), 2.78 (1H, br-d, J=~5 Hz, C(5)-H), 2.75 (1H, dd, J=4.1, 1.3 Hz, C(15)-H), 2.84 (1H, dd, J=15.2, 4.3 Hz, C(6)-H), 3.42 (1H, dd, J=15.2, 1.3 Hz, C(6)-H), 2.66 (1H, br-d, J=10.9 Hz, C(14)-H), 1.93 (1H, ddd, J=10.7, 8.5, ~2Hz, C(14)-H), 1.62 (3H, br-d, J=6.9 Hz, C(18)-H_3).

^{13}C-NMR : See Table 2, page 145



8. Koumidine (22)

Koumidine (22) was obtained as colorless needle crystals from acetone.

Melting point : 200-201°C

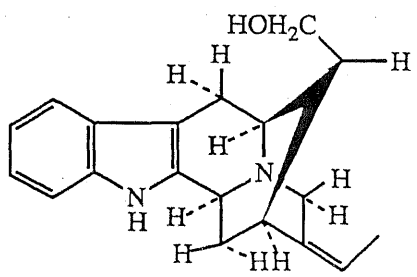
UV λ_{max}nm : 289, 279, 273 (sh), 225.

IR (KBr) : 3220, 1450, 1035.

MS *m/z* (%) : 294 (M^+, 100), 293 (87), 263 (41), 249 (9), 182 (13), 169 (88), 168 (57).

^{1}H-NMR (CD_3OD) δ : 7.59 (1H, d-like, J=7.6 Hz, C(9)-H), 6.97 (1H, t-like, J=7.6 Hz, C(10)-H), 7.05 (1H, t-like, J=7.6 Hz, C(11)-H), 7.40 (1H, d-like, J=7.6 Hz, C(12)-H), 5.36 (1H, m, C(19)-H), 4.11 (1H, dd, J=9.6, 4.0 Hz, C(3)-H), 3.75 and 3.62 (each 1H, br-d, J=15.5 Hz, C(21)-H_2), 3.60 (1H, br-dd, J=12.0, 4.3 Hz, C(5)-H), 3.51 (1H, dd, J=10.9, 6.6 Hz, C(17)-H), 3.15 (1H, dd, J=10.6, 8.9 Hz, C(17)-H), 3.00 (1H, dd, J=15.8, 1.7 Hz, C(6)-H), 2.91 (1H, dd, J=16.3, 5.5 Hz, C(6)-H), 2.44 (1H, br-dd, J=5.9, 3.0 Hz, C(15)-H), 2.24 (1H, m, C(16)-H), 1.91 (1H, ddd, J=13.2, 10.0,~2Hz, C(14)-OH), 1.83 (1H, dt, J=13.2,~4 Hz, C(14)-H), 1.60 (3H, br-d, J=6.9 Hz, C(18)-H_3).

^{13}C-NMR : See Table 2, page 145



9. 16-epi-Voacarpine (24)

This base (24) crystallized from CH_2Cl_2 as colorless prisms or plates.

Melting point : 162-165°C

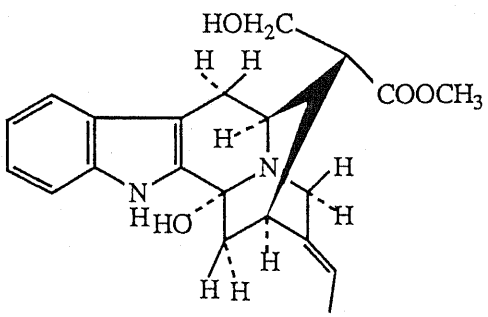
UV λ_{max}nm : 290, 281, 275 (sh), 226.

IR (KBr) : 3380, 1725, 1455, 1100, 1060.

MS *m/z* (%) : 368 (M^+, 62), 351 (29), 337 (28), 265 (65), 184 (100).

^{1}H-NMR (CD_3OD) δ : 7.39 (1H, d-like, J=7.9 Hz, C(9)-H), 6.97 (1H, t-like, J=7.9 Hz, C(10)-H), 7.07 (1H, t-like, J=7.9 Hz, C(11)-H), 7.33 (1H, d-like, J=7.9 Hz, C(12)-H), 5.26 (1H, br-q, J=6.9 Hz, C(19)-H), 4.38 (1H, br-d, J=5.5 Hz, C(5)-H), 4.16 (1H, br-d, J=16.8 Hz, C(21)-H), 3.3 (1H, br-d, J=17 Hz, C(21)-H), 3.68 (3H, s, $COOCH_3$), 3.52 (2H, s, C(17)-H_2), 3.2 (1H, br-s, C(15)-H), 3.18 (1H, dd, J=16.5, 1.7 Hz, C(6)-H), 3.09 (1H, dd, J=16.5, 5.5 Hz, C(6)-H), 2.25 (1H, dd, J=14.3, 3.8 Hz, C(14)-H), 1.79 (1H, dd, J=14.2, 2.3 Hz, C(14)-H), 1.63 (3H, dt, J=6.9, 1.0 Hz, C(18)-H_3).

^{13}C-NMR : See Table 3, page 146



10. 19-Hydroxydihydrogelsevirine (30)

This base (30) was obtained as colorless amorphous solid.

$[\alpha]_D^{21}$ + 1°(c=0.11, MeOH).

UV λ_{max}nm : 282 (sh), 255, 209.

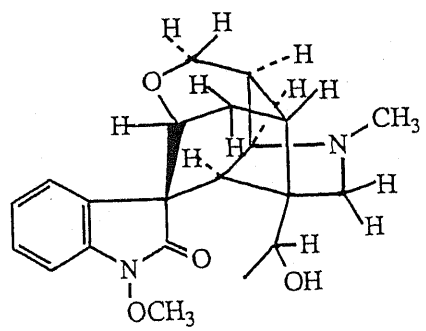
IR ($CHCl_3$) : 3400, 1715, 1620, 1470, 1085.

MS *m/z* (%) : 370 (M^+, 23), 339 (100), 325 (46), 295 (33), 275 (28).

HR-MS : Calcd. for $C_{21}H_{26}N_2O_4$ 370.1890; Found, 370.1889 .

^{1}H-NMR δ : 7.45 (1H, d-like, J=7.9 Hz, C(9)-H), 7.04 (1H, t-like, J=7.9 Hz, C(10)-H), 7.30 (1H, t-like, J=7.9 Hz, C(11)-H), 6.97 (1H, d-like, J=7.9 Hz, C(12)-H), 5.13 (1H, q, J=6.6 Hz, C(19)-H), 4.09 (1H, dd, J=11.2, 2.3 Hz, C(17)-H), 3.91 (1H, dd, J=11.2, 2.0 Hz, C(17)-H), 3.99 (3H, s, OCH_3), 3.84 (1H, br-s, C(3)-H), 3.43 (1H, br-s, C(5)-H), 3.07 and 2.33 (each 1H, d, J=8.9 Hz, C(21)-H_2), 2.28 (3H, s, N-CH_3), 2.56 (1H, br-dd, J=14.2, 2.6 Hz, C(14)-H), 2.04 (1H, ddd, J=14.2, 6.0, 2.3 Hz, C(14)-H), 2.05 (1H, m, C(15)-H), 2.16 (1H, br-s, C(6)-H), 1.09 (3H, d, J=6.6 Hz, C(18)-H_3).

^{13}C-NMR : See Table 3, page 146



11. 19-(Z)-Taberpsychine (25)

This base (25) was obtained as colorless oil.

$[\alpha]_D^{23}$ -180°(c=0.4, $CHCl_3$).

UV λ_{max}nm : 292, 284, 277 (sh), 222.

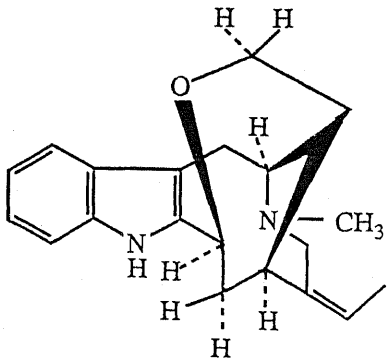
IR ($CHCl_3$) : 3460, 2930, 1460, 1340.

MS *m/z* (%) : 308 (M^+, 86), 293 (27), 279(35), 154 (16), 130 (15), 122 (100), 121 (62), 108 (27), 107 (20).

HR-MS : Calcd. for $C_{20}H_{24}N_2O$, 308.1886; Found, 308.1881.

^{1}H-NMR δ : 7.98 (1H, s, NH), 7.62 (1H, d-like, J=7.9 Hz, C(9)-H), 7.13 (1H, t-like, J=7.9 Hz, C(10)-H), 7.19 (1H, t-like, J=7.9 Hz, C(11)-H), 7.31 (1H, d-like, C(12)-H), 5.43 (1H, br-q, J=6.7 Hz, C(19)-H), 5.12 (1H, dd, J=9.8, 1.2 Hz, C(3)-H), 3.84 (1H, dd, J=11.6, 10.1 Hz, C(17)-H), 3.26 (1H, d, J=11.6 Hz, C(17)-H), 3.15 (1H, m, C(5)-H), 2.82 (1H, br-td, J=10, 6 Hz, C(15)-H), 2.59 (3H, s, N-CH_3), 2.43 (1H, dt, J=14.3, 9.8 Hz, C(14)-H), 2.12 (1H, ddd, J=14.2, 10.7, 1.2 Hz, C(14)-H), 1.61 (3H, br-d, J=6.7 Hz, C(18)-H_3).

^{13}C-NMR : See Table 3, page 146



12. 14-Hydroxygelsedine (40)

14-Hydroxygelsedine (40) crystallized from acetone as colorless needle crystals.

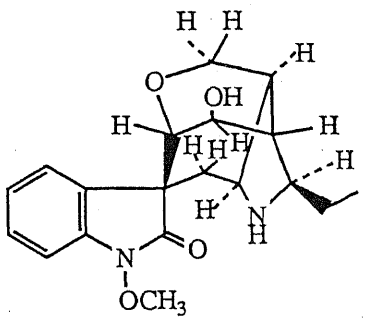
Melting point : 214 -216°C

UV λ_{max}nm : 281 (sh), 265 (sh), 258, 209.

IR (KBr) : 3260, 1695, 1620, 1470, 1340.

MS *m/z* (%) : 344 (M^+, 44), 313 (51), 168 (60), 97 (44), 84 (100).

^{1}H-NMR δ : 7.44 (1H, d-like, J=7.5 Hz, C(9)-H), 7.13 (1H, t-like, J=7.5 Hz, C(10)-H), 7.30 (1H, t-like, J=7.5 Hz, C(11)-H), 6.95 (1H, d-like, J=7.5 Hz, C(12)-H), 4.45 (1H, dd, J=11.2, 4.3 Hz, C(17)-H), 4.23 (1H, br-d, J=10.9 Hz, C(17)-H), 4.01 (3H, s, OCH_3), 3.64 (1H, dt, J=9.2, 3.3 Hz, C(5)-H), 3.43 (1H, br-s, C(3)-H), 3.01 (1H, dt-like, J=7.1, 3.9 Hz, C(20)-H), 2.51 (1H,ddd, J=9.2, 4.7, 4.3 Hz, C(16)-H), 2,18 (1H, dd, J=15.8, 3.6 Hz, C(6)-H), 2.02 (1H, dd, J=16.2, 2.3 Hz, C(6)-H), 2.07 (1H, t-like, J=4 Hz, C(15)-H), 1.91 (2H, m, C(19)-H2), 1.10 (3H, t, J=7.3 Hz, C(18)-3H).



13. 19-Oxogelsenicine (45)

This base (45) was obtained as amorphous solid from acetone.

Melting point : 226-227°C

UV λ_{max}nm : 281, 256, 209.

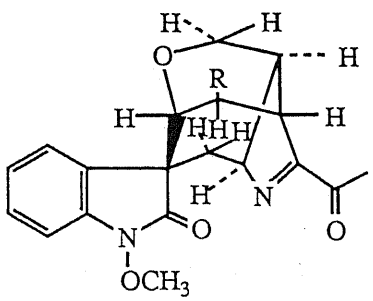
IR (KBr) : 1715, 1695, 1618, 1462, 1088.

MS *m/z* (%) : 340 (M^+, 100), 309 (40), 165 (55), 144 (38), 136 (39), 122 (65).

HR-MS : Calcd. for $C_{19}H_{20}N_2O_4$, 340.1421. Found, 340.1412.

^{1}H-NMR δ : 7.54 (1H, d-like, J=7.6 Hz, C(9)-H), 7.09 (1H, t-like, J=7.6 Hz, C(10)-H), 7.27 (1H, t-like, J=7.6 Hz, C(11)-H), 6.89 (1H, d-like, J=7.6 Hz, C(12)-H), 4.73 (1H, ddd, J=7.6, 4.9, 2.1 Hz, C(5)-H), 4.34 (1H, dd, J=11.3, 2.8 Hz, C(17)-H), 4.28 (1H, dd, J=11.3, 1.5 Hz, C(17)-H), 3.99 (3H, s, OCH_3), 3.75 (1H, br-dd, J=4.2, 2.3 Hz, C(3)-H), 3.43 (1H, br-t, J=8.8 Hz, C(15)-H), 2.69 (1H, m, C(16)-H), 2.68 (1H, dd, J=15.3, 2.3 Hz, C(14)-H), 2.22 (1H, ddd, J=15.3, 8.8, 4.2 Hz, C(14)-H), 2.56 (1H, dd, J=15.6, 4.9 Hz, C(6)-H), 2.23 (1H, dd, J=15.6, 2.1 Hz, C(6)-H), 2.66 (3H, s, C(18)-H_3).

^{13}C-NMR : See Table 3, page 146



14. Koumine N-oxide (20)

Koumine N-oxide (20) was obtained as amorphous solid from acetone.

Melting point : 111-113°C $[\alpha]_D^{19}$-237°(c=0.14, MeOH).

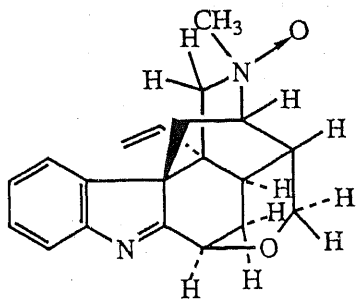
UV λ_{max}nm : 260, 226 (sh), 220, 215.

IR (KBr) : 1640, 1587, 1445, 1080.

MS *m/z* (%) : 306 (M^+-16,100), 223 (25), 120 (29), 70 (52).

^{1}H-NMR δ : 7.66 (1H, d-like, J=7.6 Hz, C(9)-H), 7.29 (1H, t-like, J=7.6 Hz, C(10)-H), 7.42 (1H, t-like, J=7.6 Hz, C(11)-H), 7.23 (1H, d-like, J=7.6 Hz, C(12)-H), 5.06 (1H, br-s, C(3)-H), 4.93 (1H, d, J=17.8 Hz, C(18)-H), 4.93 (1H, J=11.2 Hz, C(18)-H), 4.59 (1H, dd, J=17.8, 11.2 Hz, C(19)-H), 4.38 (1H, dd, J=12.5, 5.3 Hz, C(17)-H), 3.68 (1H, d, J=12.5 Hz, C(17)-H), 4.10 (1H, m, C(16)-H), 3.84 and 4.04 (each 1H, d, J=13.5 Hz, C(21)-H_2), 3.57 (3H, s, N-CH_3), 3.52 (1H, br-s, C-(5)-H), 2.94 (1H, dd, J=16.2, 4.0 Hz, C(6)-H), 2.43 (1H, br-d, J=16.2 Hz, C(6)-H), 2.72 (1H, br-d, J=10 Hz, C(15)-H), 2.67 (1H, dt, J=14.5, 4 Hz, C(14)-H), 1.90 (1H, br-d, J=14.5 Hz, C(14)-H).

^{13}C-NMR : See Table 4, page 147



15. Gelsemine N-oxide (31)

Gelsemine N-oxide (31) was obtained as amorphous solid.

$[\alpha]_D^{25}$ -16.9°(c=0.9, MeOH).

UV λ_{max}nm : 284, 252, 208.

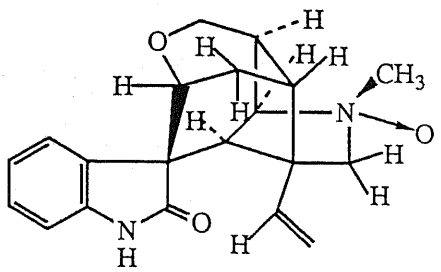
IR ($CHCl_3$) : 1715, 1620, 1475, 1105.

MS *m/z* (%) : 322 (M^+-16, 40), 279 (64), 108 (100).

RH-MS (in beem) : Calcd. for $C_{20}H_{22}N_2O_3$, 338.1629; Found, 338.1640.

^{1}H-NMR δ : 10.13 (1H, br-s, NH), 7.24 (1H, d-like, J=7.6 Hz, C(9)-H), 6.87 (1H, t-like, J=7.6 Hz, C(10)-H), 6.95 (1H, t-like, J=7.6 Hz, C(11)-H), 6.45 (1H, d-like, C(12)-H), 6.32 (1H, dd, J=17.7, 11.0 Hz, C(19)-H), 5.21 (1H, d, J=11.0 Hz, C(18)-H), 5.02 (1H, d, J=17.7 Hz, C(18)-H), 4.17 (1H, dd, J=11.3, 2.4 Hz, C(17)-H), 4.03 (1H, dd, J=11.3, 1.8 Hz, C(17)-H), 4.15 (1H, br-s, C(5)-H), 3.86 (1H, br-s, C(3)-H), 3.41 (3H, s, N-CH_3), 3.75 and 3.25 (each 1H, d, J=12.0 Hz, C(21)-H_2), 3.41 (1H, br-s, C(6)-H), 2.94 (1H, dd, J=14.6, 2.7 Hz, C(14)-H), 2.07 (1H, ddd, J=14.6, 6.0, 2.9 Hz, C(14)-H), 2.59 (1H, br-d, J=8.2 Hz, C(16)-H), 2.48 (1H, br-dd, J=8.3, 6.0 Hz, C(15)-H).

^{13}C-NMR : See Table 4, page 147



16. Elegansamine (46)

Elegansamine (46) crystallized from MeOH as colorless prisms.

Melting point : 172-173°C

UV λ_{max}nm : 281 (sh), 257, 208.

IR (KBr) : 2930, 1730, 1470, 1230, 1040.

MS *m/z* (%) : 508 (M^+, 58), 477 (36), 339 (22), 326 (100), 295 (58), 150 (30).

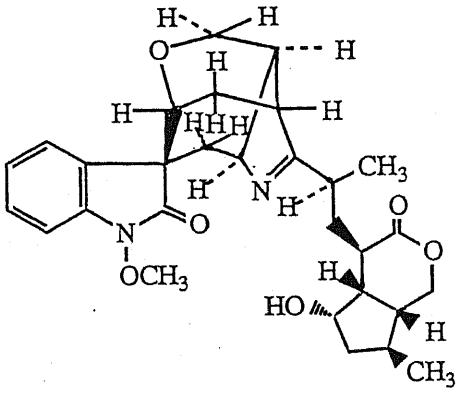
HR-MS : Calcd. for $C_{29}H_{36}N_2O_6$ 508.2571; Found, 508.2572.

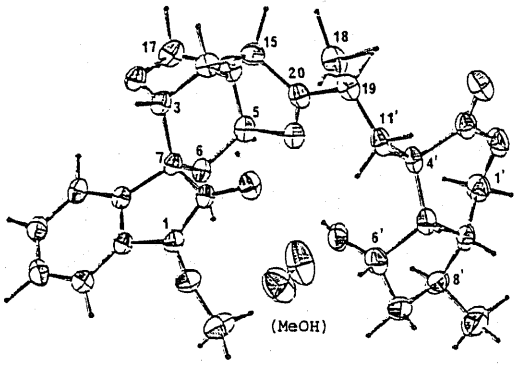
^{1}H-NMR δ : 7.50 (1H, C(9)-H), 7.07 (1H, C(10)-H), 7.26 (1H, C(11)-H), 6.89 (1H, C(12)-H), 3.95 (3H, s, O-CH_3), 3.70 (1H, dd, J=4.9, 2.2 Hz, C(3)-H), 2.91 (1H, t-like, J=9 Hz, C(15)-H), 2.66 (1H, m, C(19)-H), 2.54 (1H, m, C(16)-H), 1.47 (3H, d, J=7.3 Hz, C(18)-H_3),

^{13}C-NMR : See Table 4, page 147

Crystal Data and Data Collection Parameters : monoclinic, $P2_1$, a=10.701(3), b=7.940(2), c=17.039(4)Å, Z=2, Cell volume=1415Å^3, Dc=1.26 gcm^{-3}. A total of 3115 unique independent intensities were measured with the range of $3 \leq 2\Theta \leq 120°,150°$ on a four-circle diffractometer (Rigaku AFC-5) using CuKα radiation (λ =1.54 Å). The structure was solved by the direct method using MULTAN80 and refined anisotropically (isotropically for H) by the least-squares method to an R value of 0.0496, using the 2834 reflections for which F(0)>3σ(Fo). The structure and ORTEP drawing is shown in the next page.

Structure of elegansamine (46)





ORTEP Drawing of elegansamine (46)

TABLE 1

^{13}C-NMR chemical shifts and assignments for gelsemine (26), gelsevirine (28), koumine (19) and gelsenicine (43).

No.	(26)	(28)	(19)	(43)
2	179.5(s)	173.0(s)	185.4(s)	172.1(s)
3	69.5(d)	69.4(d)	70.8(d)	74.9(d)*
5	72.1(d)	72.3(d)	56.8(d)	72.5(d)*
6	50.7(d)	51.1(d)	28.5(t)	37.7(t)
7	54.2(s)*	52.3(s)*	59.7(s)	55.8(s)
8	132.0(s)	128.1(s)	143.6(s)	132.3(s)
9	128.2(d)	128.2(d)	122.9(d)	124.6(d)
10	121.7(d)	122.7(d)	125.8(d)	123.3(d)
11	127.9(d)	128.2(d)	128.0(d)	128.0(d)
12	109.0(d)	107.1(d)	121.0(d)	106.5(d)
13	140.7(s)	139.5(s)	154.8(s)	138.0(s)
14	22.9(t)	23.1(t)	25.2(t)	25.6(t)
15	35.8(d)	36.0(d)	33.0(d)	39.8(d)**
16	38.2(d)	38.0(d)	38.8(d)	42.5(d)**
17	61.6(t)	61.6(t)	61.2(t)	62.1(t)
18	112.2(t)	112.9(t)	115.7(t)	10.0(q)
19	138.8(d)	138.3(d)	137.3(d)	25.6(t)
20	54.0(s)*	54.1(s)*	45.2(s)	184.1(s)
21	66.2(t)	66.3(t)	57.7(t)	-
N-CH_3	40.6(q)	40.6(q)	42.6(q)	-
N-OCH_3	-	63.1(q)	-	63.3(q)

Chemical shifts in ppm downfield from TMS.

Solvent : $CDCl_3$

*, ** : signals may be interchanged within vertical column.

TABLE 2

^{13}C-NMR chemical shifts and assignments for 14-hydroxygelsenicine (44), humantenine (35), 19-(Z)-akuammidine (23) and koumidine (22).

No.	(44)	(35)	(23)	(22)
2	171.0(s)	174.4(s)	139.1(s)*	138.4(s)*
3	79.2(d)	72.2(d)*	51.5(d)	51.0(d)**
5	71.1(d)	67.0(d)*	59.5(d)	54.0(d)**
6	37.5(t)	28.3(t)**	25.1(t)	23.4(t)
7	53.8(s)	55.3(s)	106.0(s)	106.0(s)
8	131.7(s)	129.2(s)	128.1(s)	127.5(s)
9	124.6(d)	125.9(d)	119.8(d)**	119.8(d)***
10	123.5(d)	123.0(d)	118.6(d)**	115.3(d)***
11	128.2(d)	128.1(d)	122.1(d)	122.1(d)
12	106.7(d)	107.3(d)	112.0(d)	112.0(d)
13	137.9(s)	139.0(s)***	138.9(s)*	138.2(s)*
14	66.0(d)	25.3(t)**	31.4(t)	29.3(t)
15	52.1(d)	34.6(d)	37.4(d)	35.1(d)
16	38.4(d)	38.4(d)	53.2(s)	44.3(d)
17	61.1(t)	61.6(t)	69.1(t)	62.2(t)
18	10.0(q)	12.8(q)	12.5(q)	12.6(q)
19	26.0(t)	119.5(d)	118.2(d)**	118.7(d)***
20	181.5(s)	137.2(s)***	138.6(s)*	142.7(s)*
21	-	45.7(t)	54.0(t)	54.6(t)
N-CH_3	-	42.6(q)	-	-
N-$COCH_3$	63.3(q)	63.4(q)	-	-
$\underline{C}OOCH_3$	-	-	175.6(s)	-
$COO\underline{C}H_3$	-	-	52.4(q)	-
Solvent	$CDCl_3$	$CDCl_3$	CD_3OD	CD_3OD

Chemical shifts in ppm downfield from TMS

*, **, *** : signals may be interchanged within vertical column.

TABLE 3

^{13}C-NMR chemical shifts and assignments for 16-epi-voacarpine (24), 19-hydroxydihydrogelsevirine (30), 19-(Z)-taberpsychine (25) and 19-oxogelsenicine (45).

No.	(24)	(30)	(25)	(45)
2	137.1(s)*	174.2(s)	136.2(s)*	171.1(s)
3	80.5(s)	69.1(d)*	67.6(d)	75.2(d)*
5	57.5(d)	71.8(d)*	60.5(d)	74.5(d)*
6	21.3(t)	47.5(d)	18.0(t)	38.0(t)
7	107.0(s)	53.3(s)	110.9(s)	56.3(s)
8	125.7(s)	127.9(s)	128.3(s)	131.8(s)
9	119.5(d)**	128.4(d)**	119.8(d)**	124.5(d)
10	115.7(d)**	123.0(d)	119.3(d)**	123.5(d)
11	122.0(d)	128.5(d)**	122.3(d)**	128.3(d)
12	110.9(d)	107.2(d)	110.9(d)	106.7(d)
13	136.3(s)*	139.2(s)	135.3(s)*	138.0(s)
14	36.5(t)	22.6(t)	29.7(t)	27.5(t)
15	33.7(d)	35.7(d)	33.5(d)	38.0(d)**
16	53.2(s)	38.6(d)	37.5(d)	39.4(d)**
17	63.3(t)	61.6(t)	61.9(t)	61.7(t)
18	12.7(q)	19.4(q)	12.8(q)	26.1(q)
19	118.5(d)**	64.3(d)	118.2(d)	197.6(s)
20	135.4(s)*	56.8(s)	131.9(s)	178.1(s)
21	48.1(t)	58.8(t)	45.9(t)	-
$\underline{C}OOCH_3$	175.8(s)	-	-	-
$COO\underline{C}H_3$	52.1(q)	-	-	-
N-CH_3	-	40.7(q)	43.0(q)	-
N-OCH_3	-	63.3(q)	-	63.4(q)

Chemical shifts in ppm downfield from TMS

Sovent : $CDCl_3$

*, ** : signals may be interchanged within vertical column.

TABLE 4

^{13}C-NMR chemical shifts and assignments for koumine N-oxide (20), gelsemine N-oxide (31), and elegansamine (46).

No.	(20)	(31)	(46)	(46) continued
2	183.2(s)	179.8(s)	171.5(s)	1'= 70.4(t)
3	70.5(d)*	70.5(d)	75.0(d)	3'= 177.9(s)
5	72.7(d)*	87.3(d)	72.5(d)	4'= 48.7(d)
6	26.0(t)	51.1(d)	37.4(t)	5'= 37.7(d)**
7	55.5(s)	55.0(s)*	56.1(s)	6'= 71.2(d)
8	142.1(s)	132.2(s)	131.9(s)	7'= 43.7(t)
9	124.0(d)	129.6(d)**	124.7(d)	8'= 35.1(d)**
10	126.8(d)	122.9(d)	123.4(d)	9'= 45.1(d)
11	128.8(d)	129.7(d)**	128.1(d	10'= 18.7(q)*
12	121.3(d)	110.5(d)	106.6(d)	11'= 33.1(t)
13	154.6(s)	142.7(s)	138.0(s)	
14	24.6(t)	23.4(t)	27.7(t)	
15	31.1(d)	36.9(d)	40.1(d)	
16	35.1(d)	38.9(d)	42.5(d)	
17	60.2(t)	61.6(t)	61.9(t)	
18	117.8(t)	115.1(t)	19.3(q)*	
19	134.3(d)	137.5(d)	37.7(d)**	
20	45.9(s)	53.6(s)*	186.2(s)	
21	75.6(t)	83.3(t)	-	
N-CH_3	60.2(q)	53.9(q)	-	
N-OCH_3	-	-	63.2(q)	
Solvent	$CDCl_3$	CD_3OD	$CDCl_3$	

Chemical shifts in ppm downfield from TMS

*, **, : signals may be interchanged within vertical column.

APPENDIX

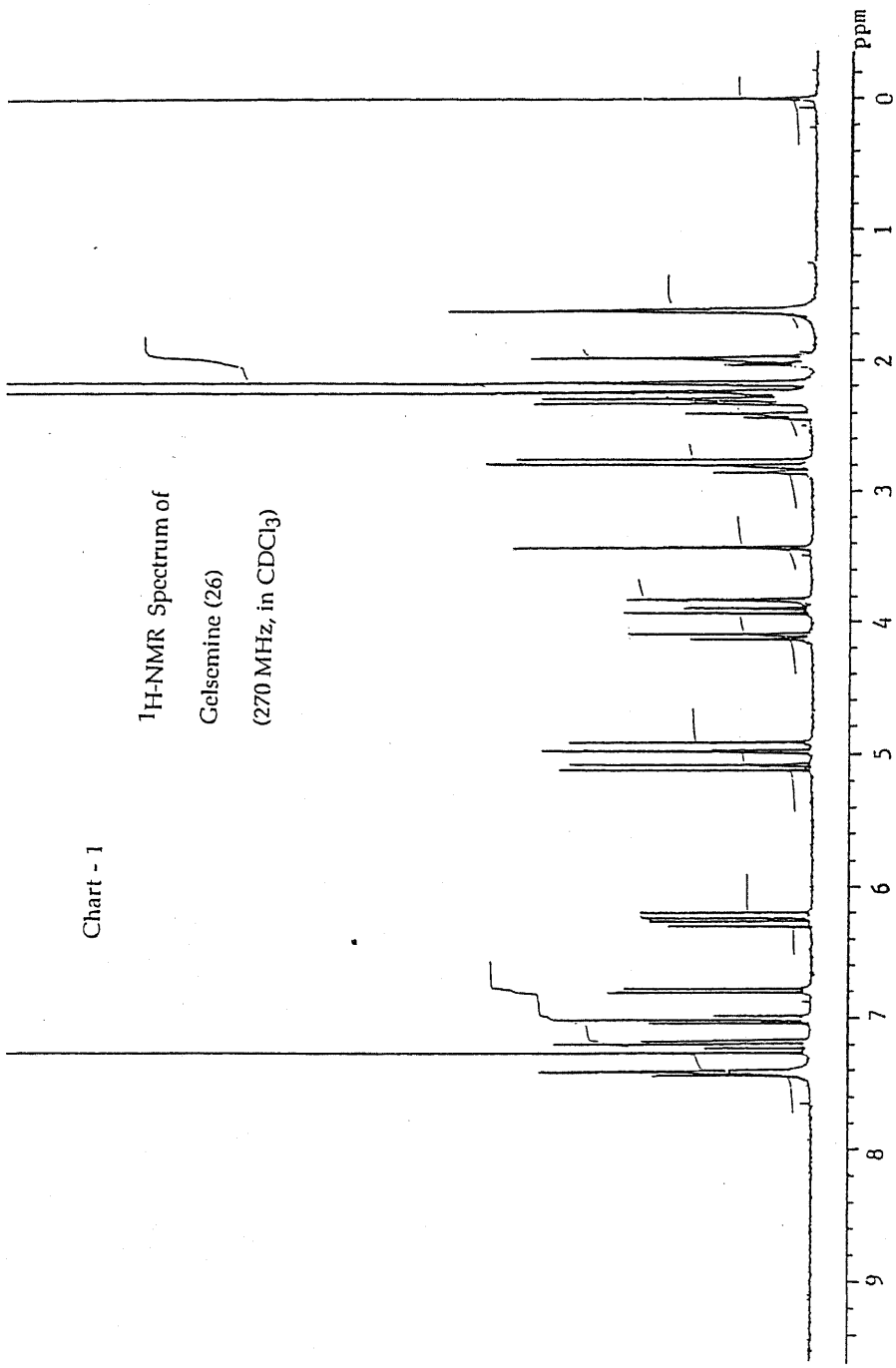


Chart - 1
^{1}H-NMR Spectrum of
Gelsemine (26)
(270 MHz, in $CDCl_3$)
ppm
0
1
2
3
4
5
6
7
8
9

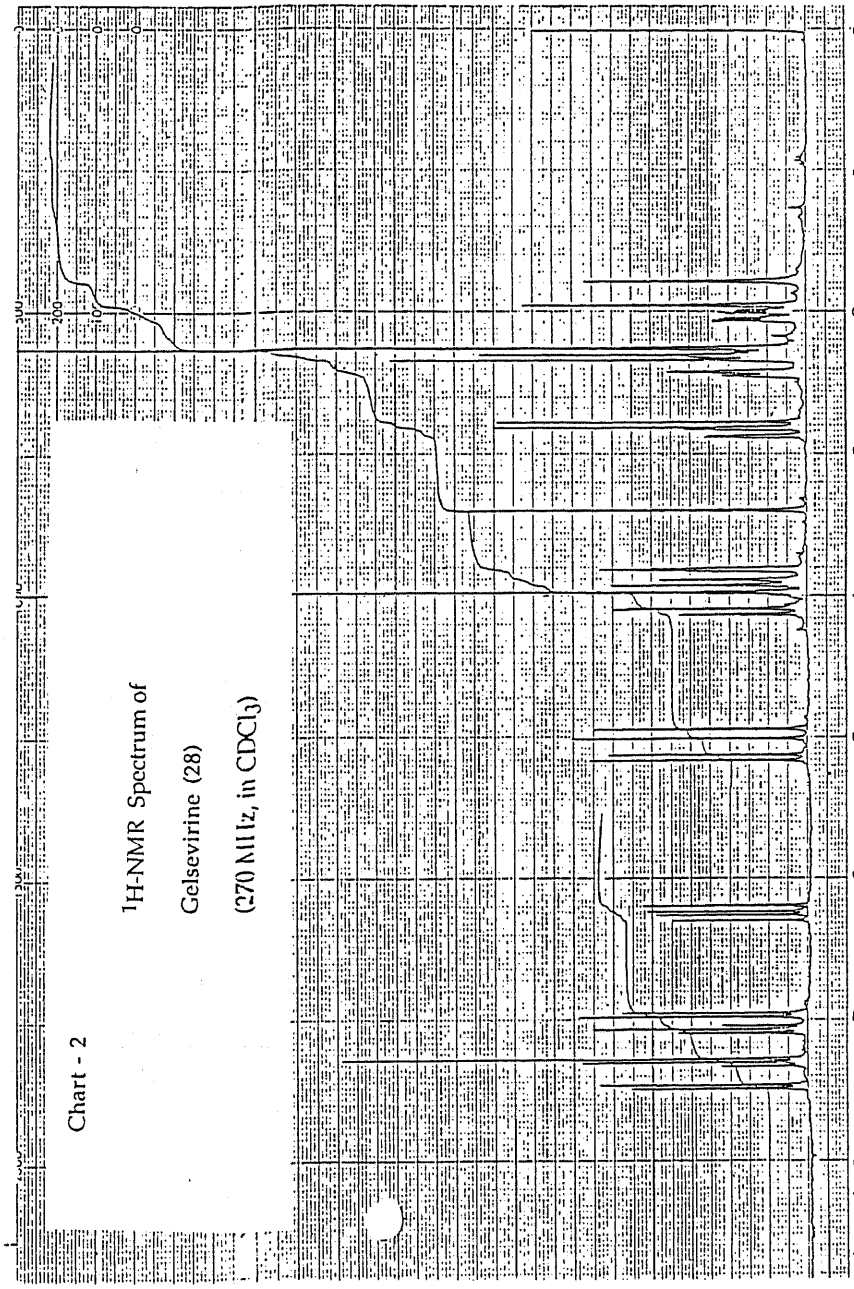


Chart - 2
^{1}H-NMR Spectrum of
Gelsevirine (28)
(270 MHz, in $CDCl_3$)
(ppm)
8
7
6
5
4
3
2
1
0

Chart - 3

^{1}H-NMR Spectrum of

Koumine (19)

(270 MHz, in $CDCl_3$)

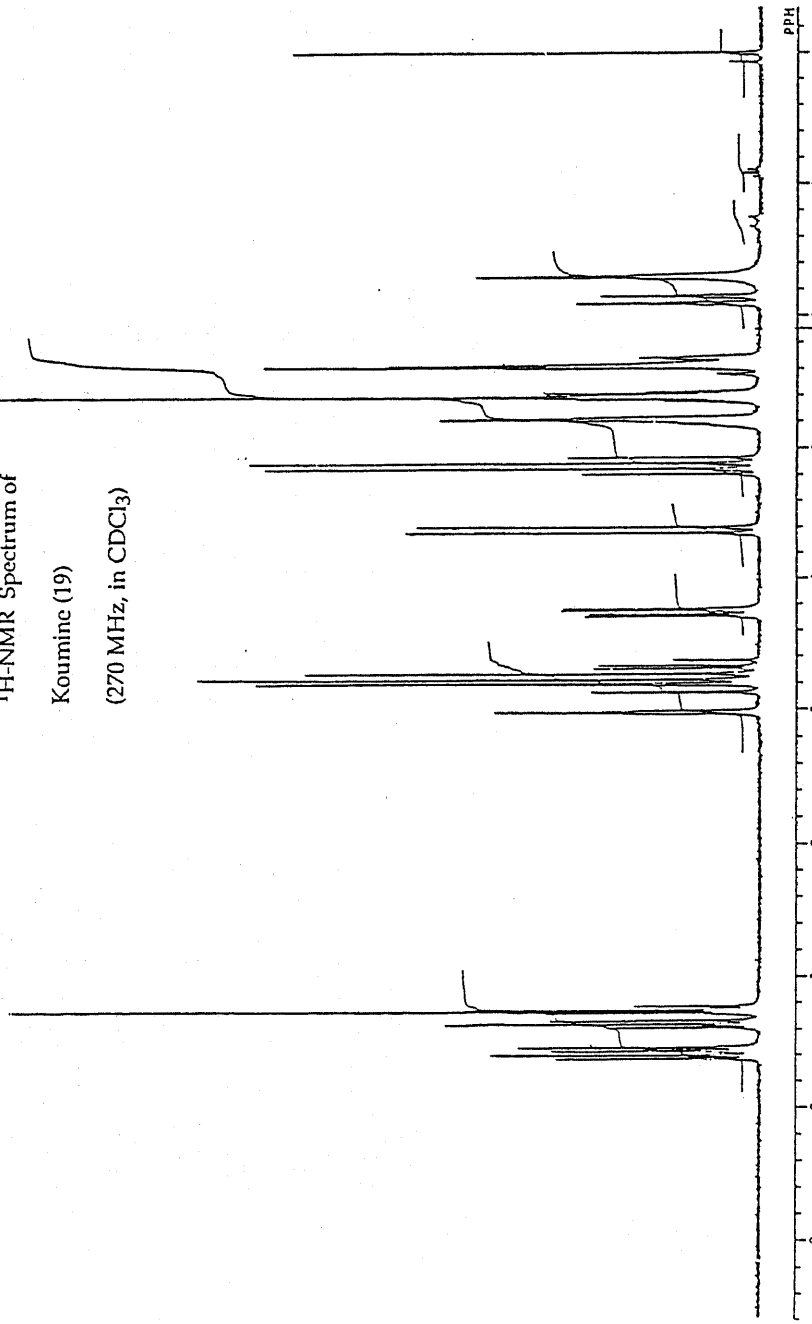


Chart - 4

^{1}H-NMR Spectrum of

Gelsenicine (43)

(270 MHz, in $CDCl_3$)

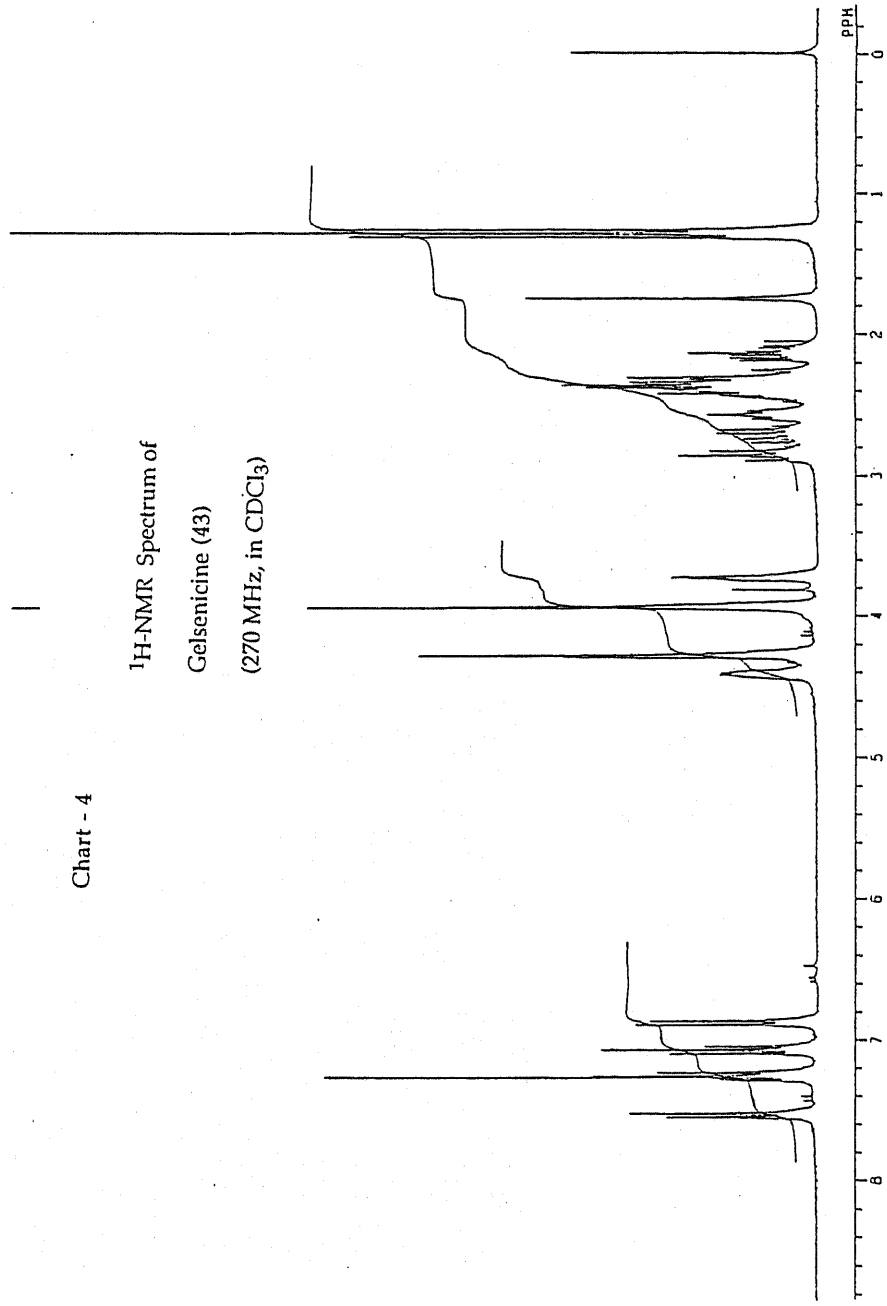


Chart - 5

^{1}H-NMR Spectrum of

14-Hydroxygelsenicine (44)

(270 MHz, in $CDCl_3$)

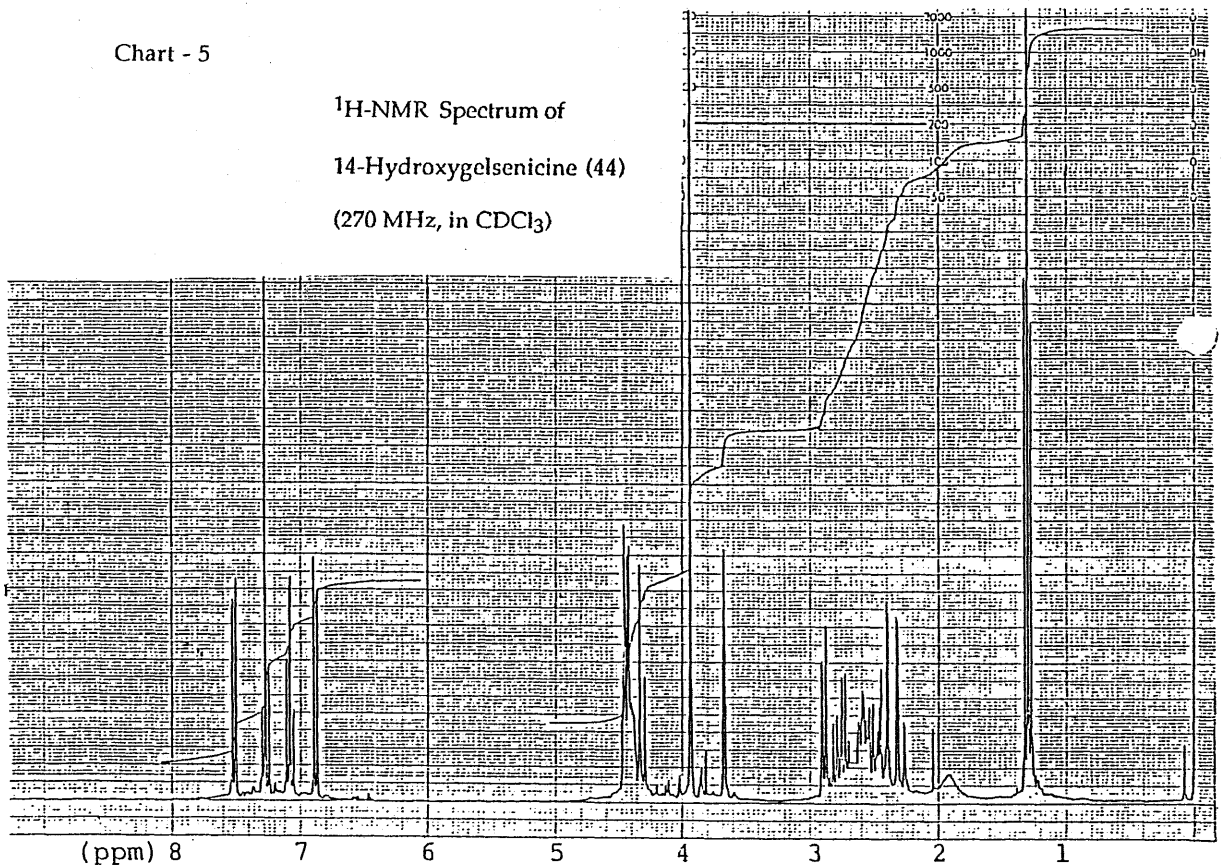


Chart - 6

^{1}H-NMR Spectrum of

Humantenine (35)

(270 MHz, in $CDCl_3$)

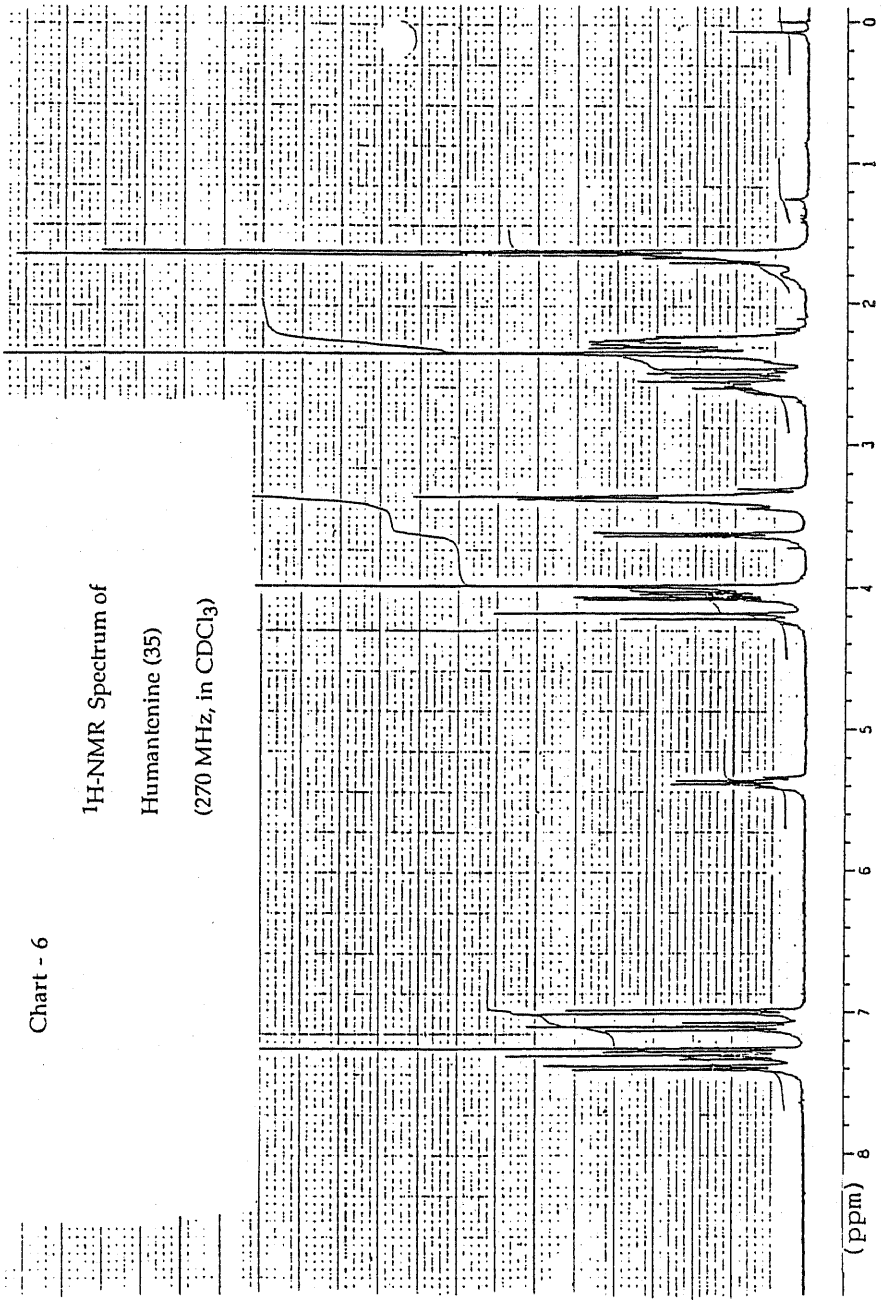
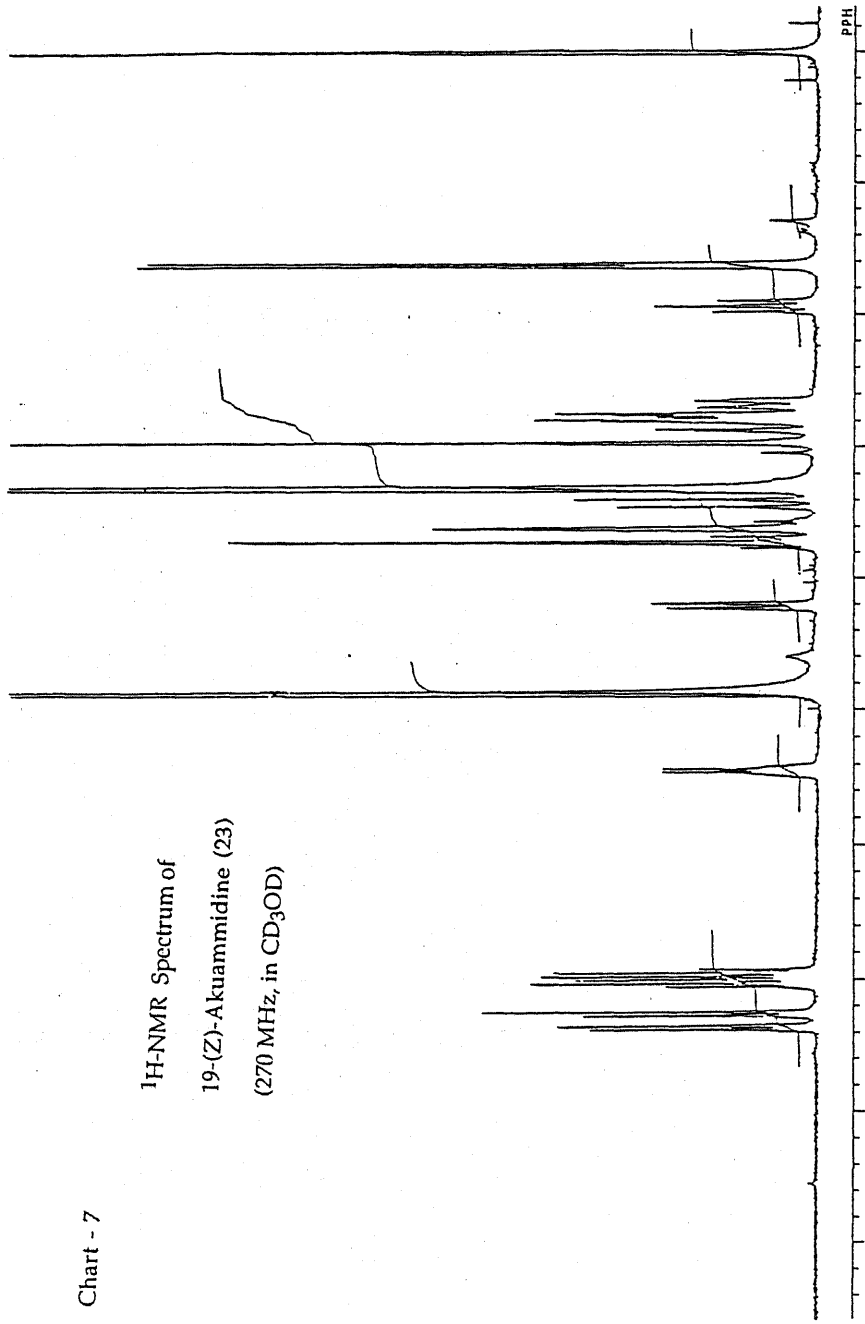


Chart - 7

^{1}H-NMR Spectrum of

19-(Z)-Akuammidine (23)

(270 MHz, in CD_3OD)



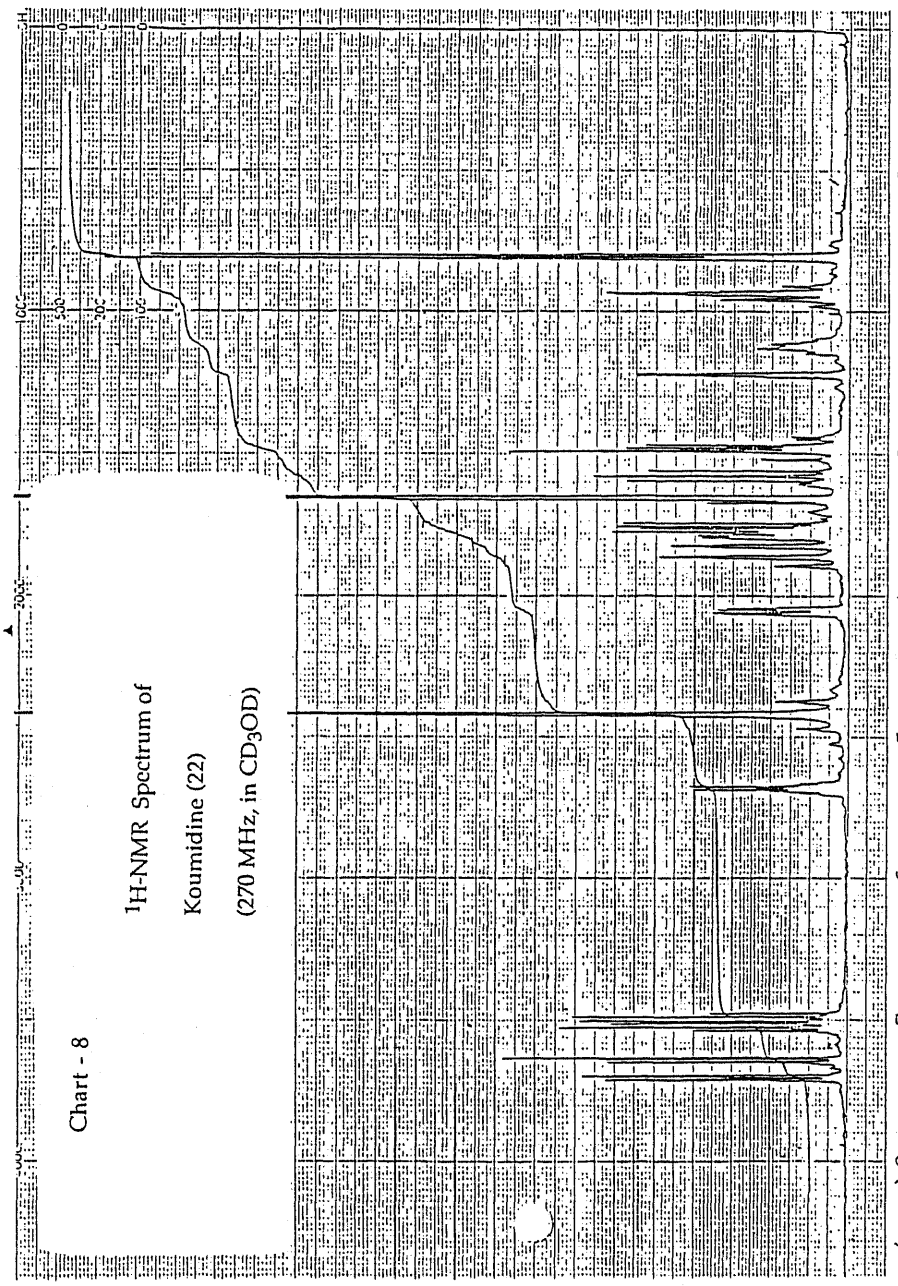


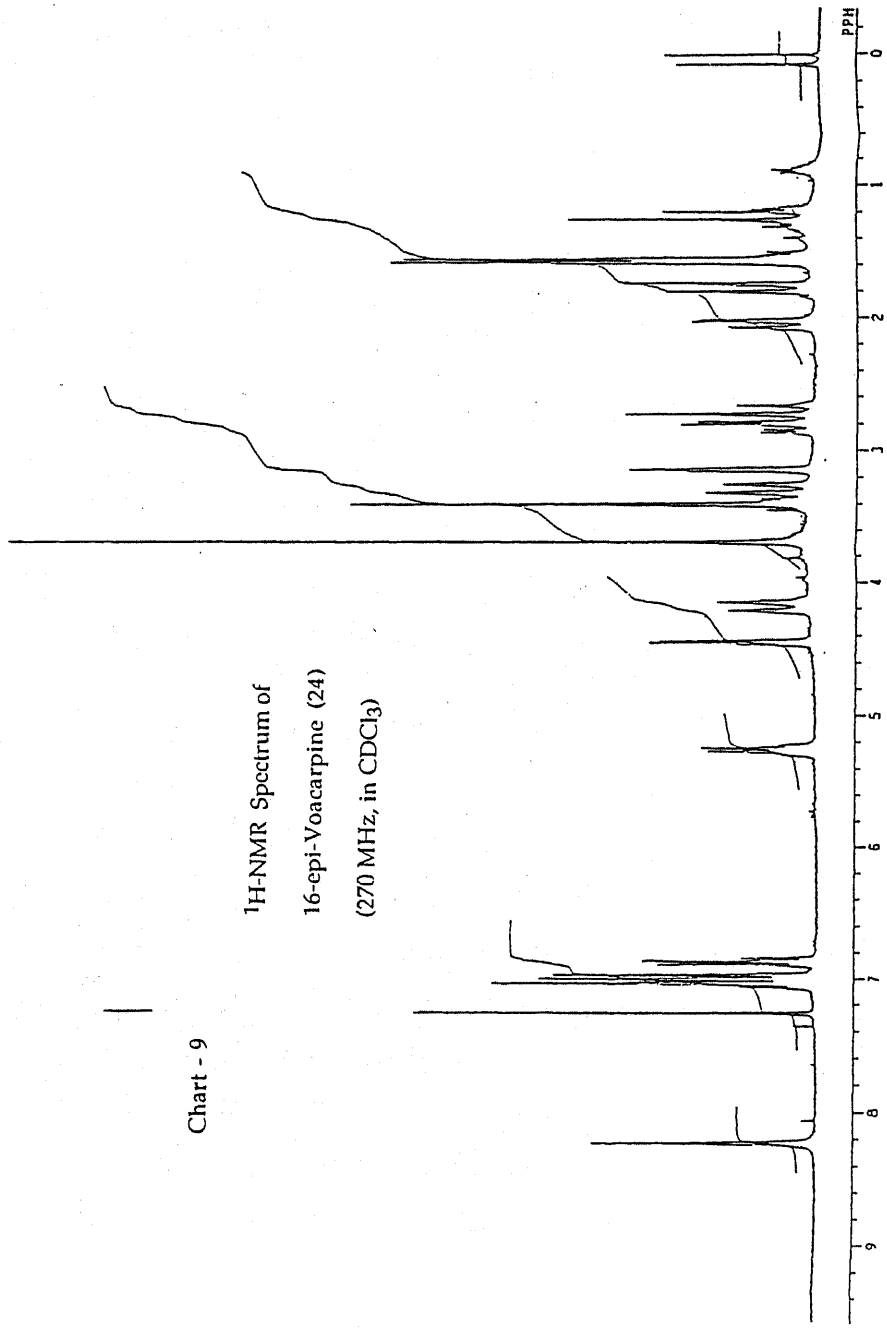
Chart - 8
^{1}H-NMR Spectrum of
Koumidine (22)
(270 MHz, in CD_3OD)
(ppm) 8
7
6
5
4
3
2
1
0

Chart - 9

^{1}H-NMR Spectrum of

16-epi-Voacarpine (24)

(270 MHz, in $CDCl_3$)



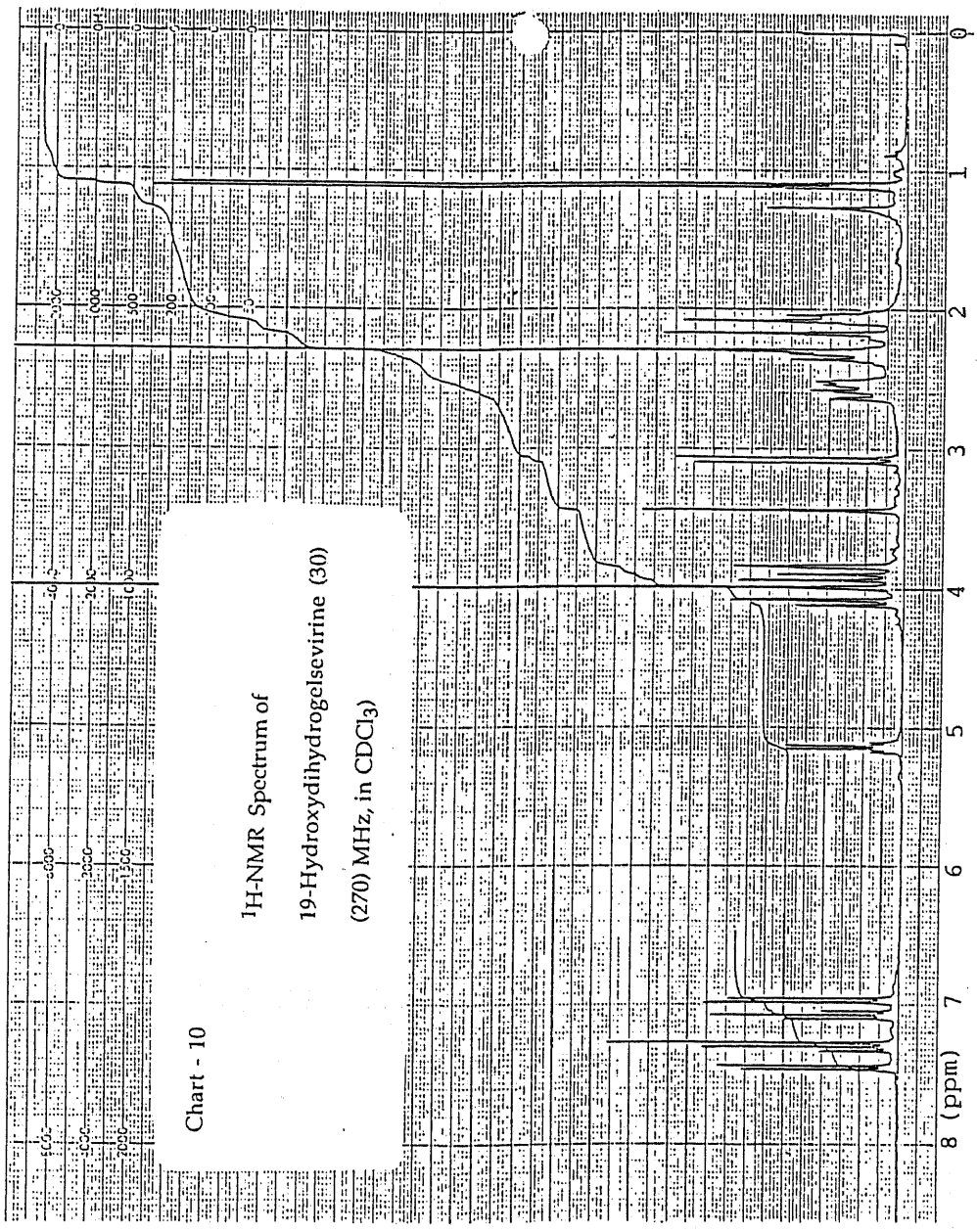


Chart - 10
^{1}H-NMR Spectrum of
19-Hydroxydihydrogelsevirine (30)
(270) MHz, in $CDCl_3$)
δ (ppm)

Chart - 11

^{1}H-NMR Spectrum of

19-(Z)-Taberpsychine (25)

(270 MHz, in $CDCl_3$)

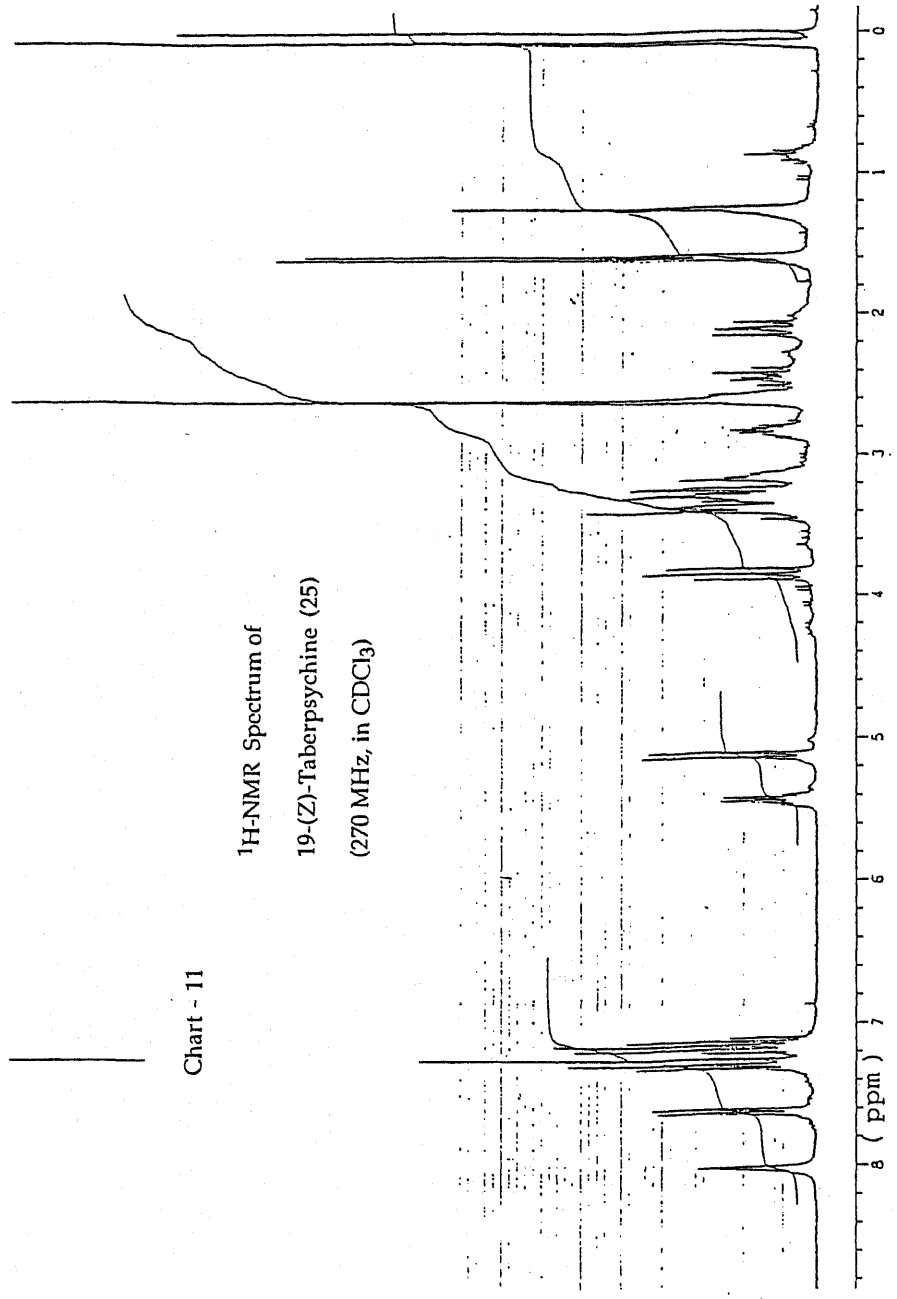


Chart - 12

^{1}H-NMR Spectrum of

14-Hydroxygelsedine (40)

(270 MHz, in $CDCl_3$)

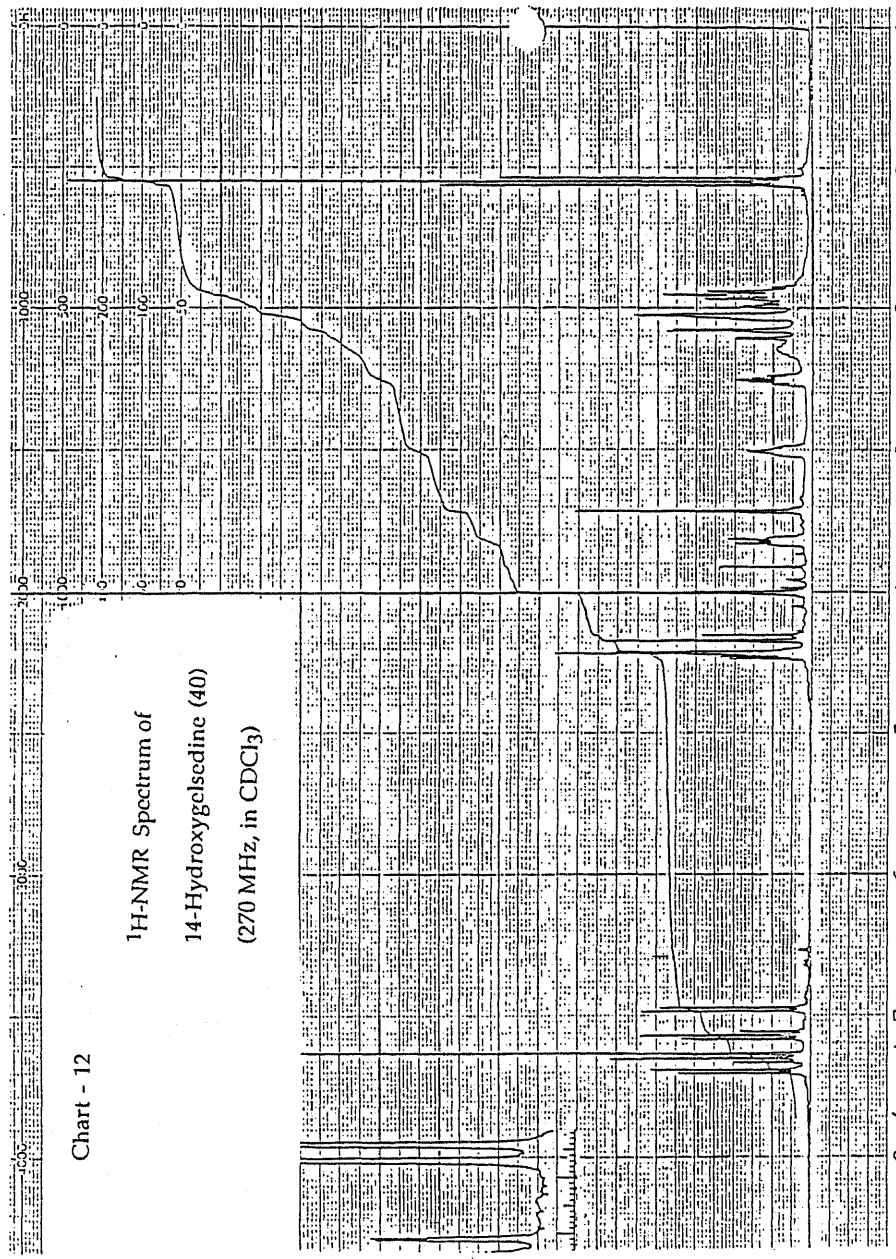
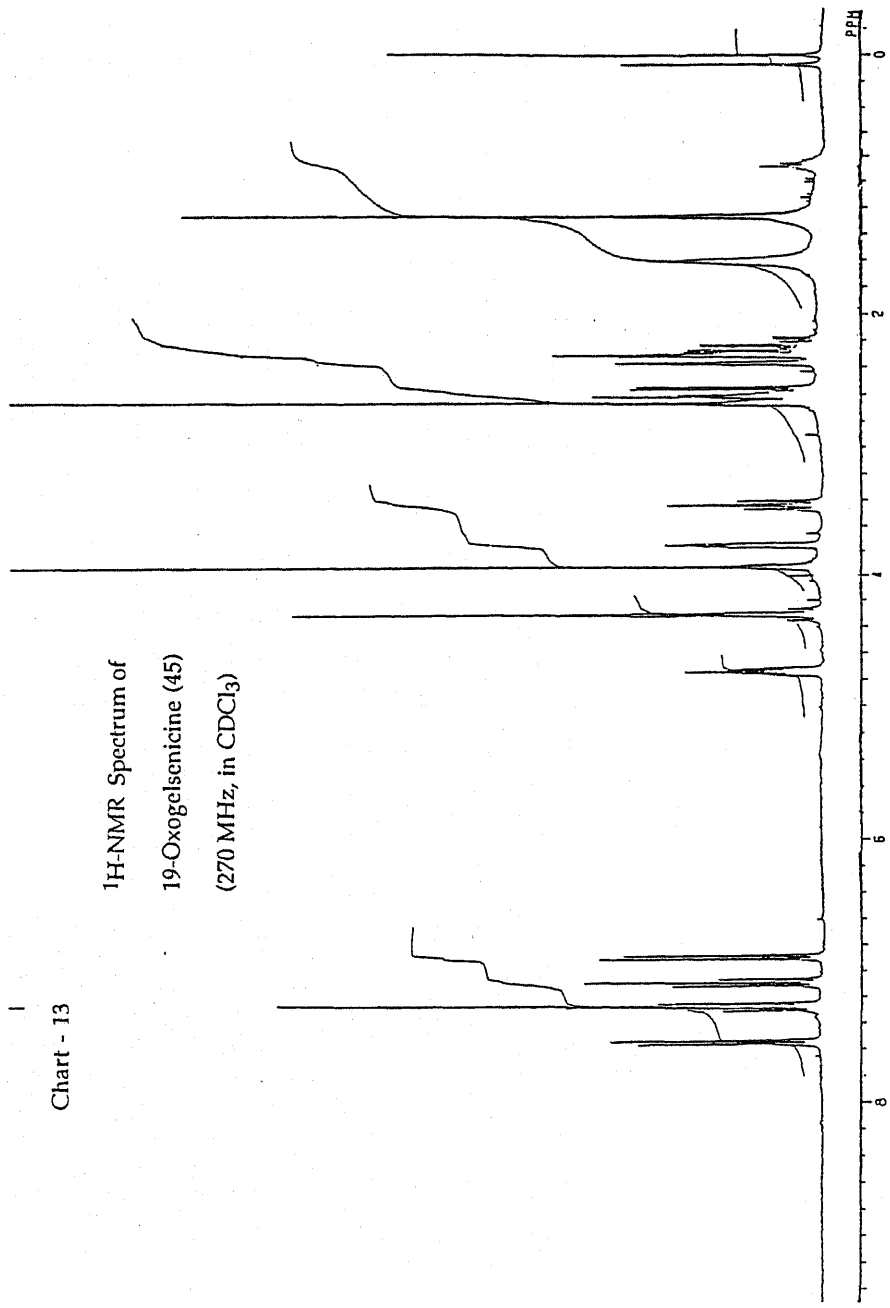


Chart - 13

^{1}H-NMR Spectrum of

19-Oxogelsenicine (45)

(270 MHz, in $CDCl_3$)



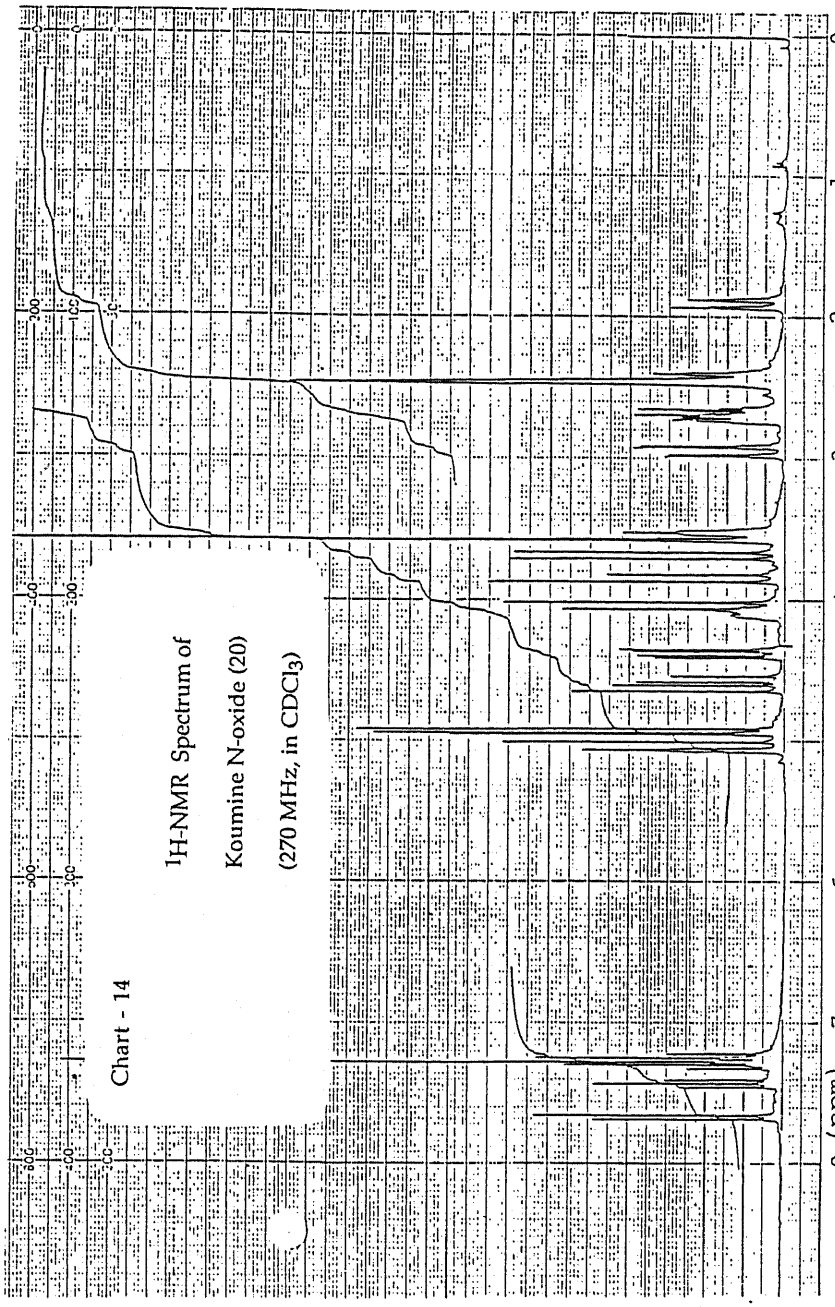


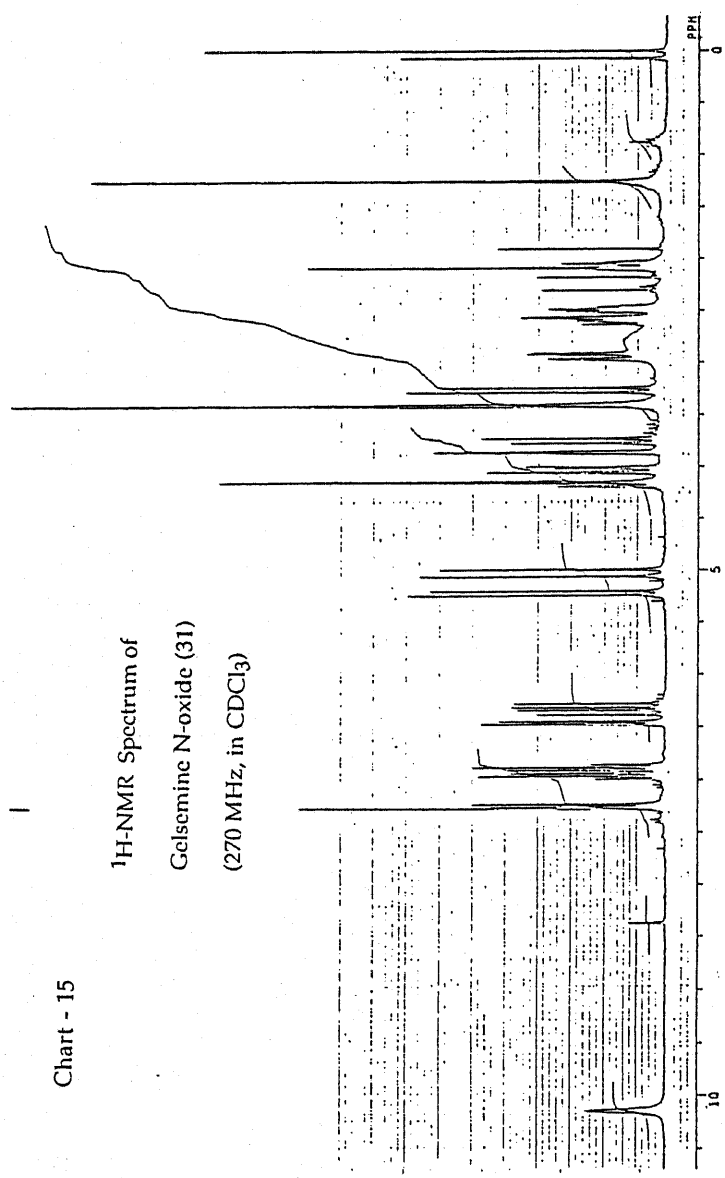
Chart - 14
^{1}H-NMR Spectrum of
Koumine N-oxide (20)
(270 MHz, in $CDCl_3$)
8 (ppm) 7 6 5 4 3 2 1 0

Chart - 15

^{1}H-NMR Spectrum of

Gelsemine *N*-oxide (31)

(270 MHz, in $CDCl_3$)



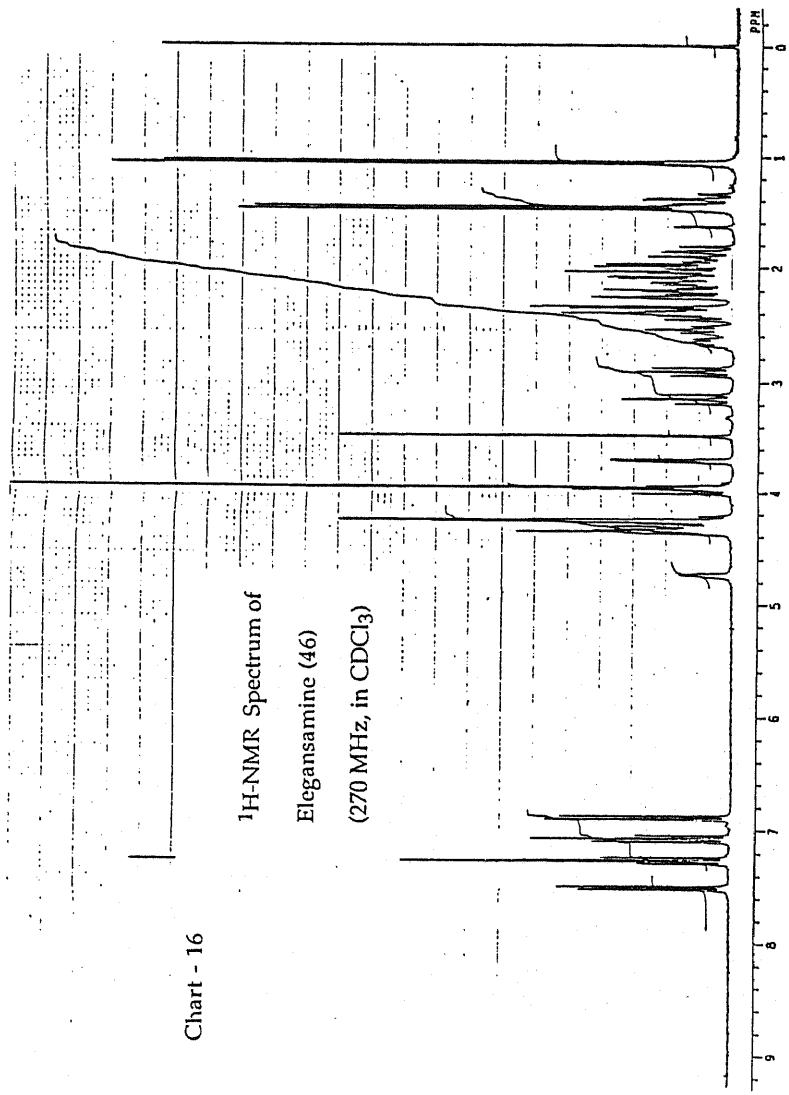


Chart - 16

^{1}H-NMR Spectrum of

Elegansamine (46)

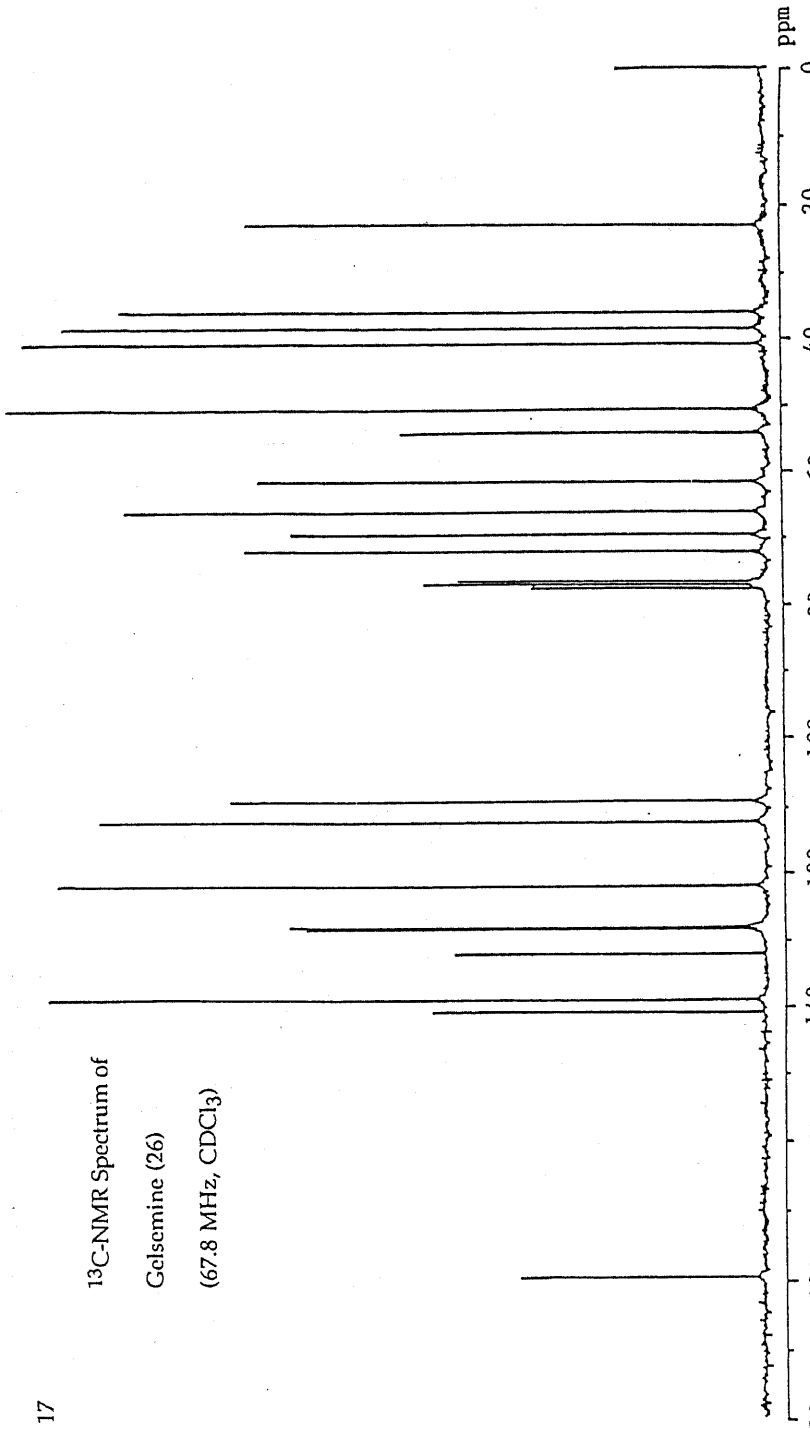
(270 MHz, in $CDCl_3$)

Chart - 17

^{13}C-NMR Spectrum of

Gelsemine (26)

(67.8 MHz, $CDCl_3$)



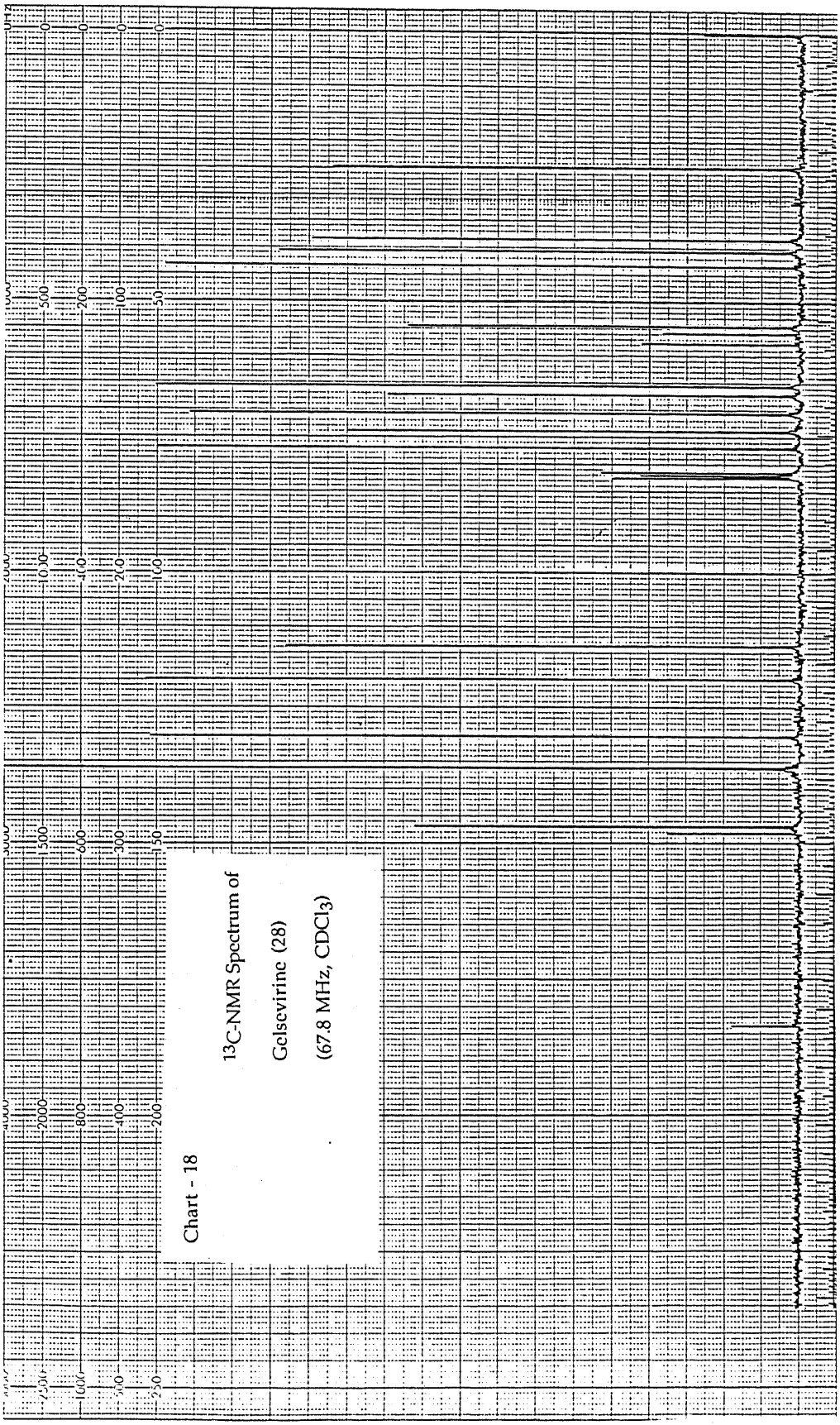


Chart - 18

^{13}C-NMR Spectrum of

Gelsevirine (28)

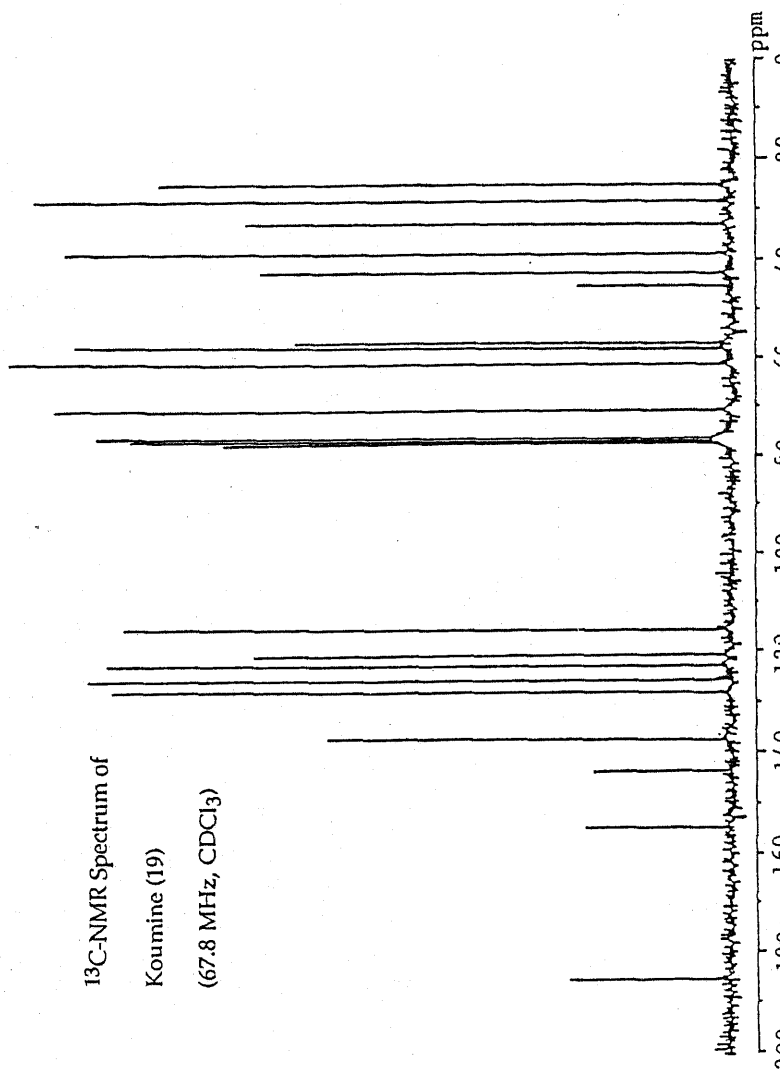
(67.8 MHz, $CDCl_3$)

Chart - 19

^{13}C-NMR Spectrum of

Koumine (19)

(67.8 MHz, $CDCl_3$)



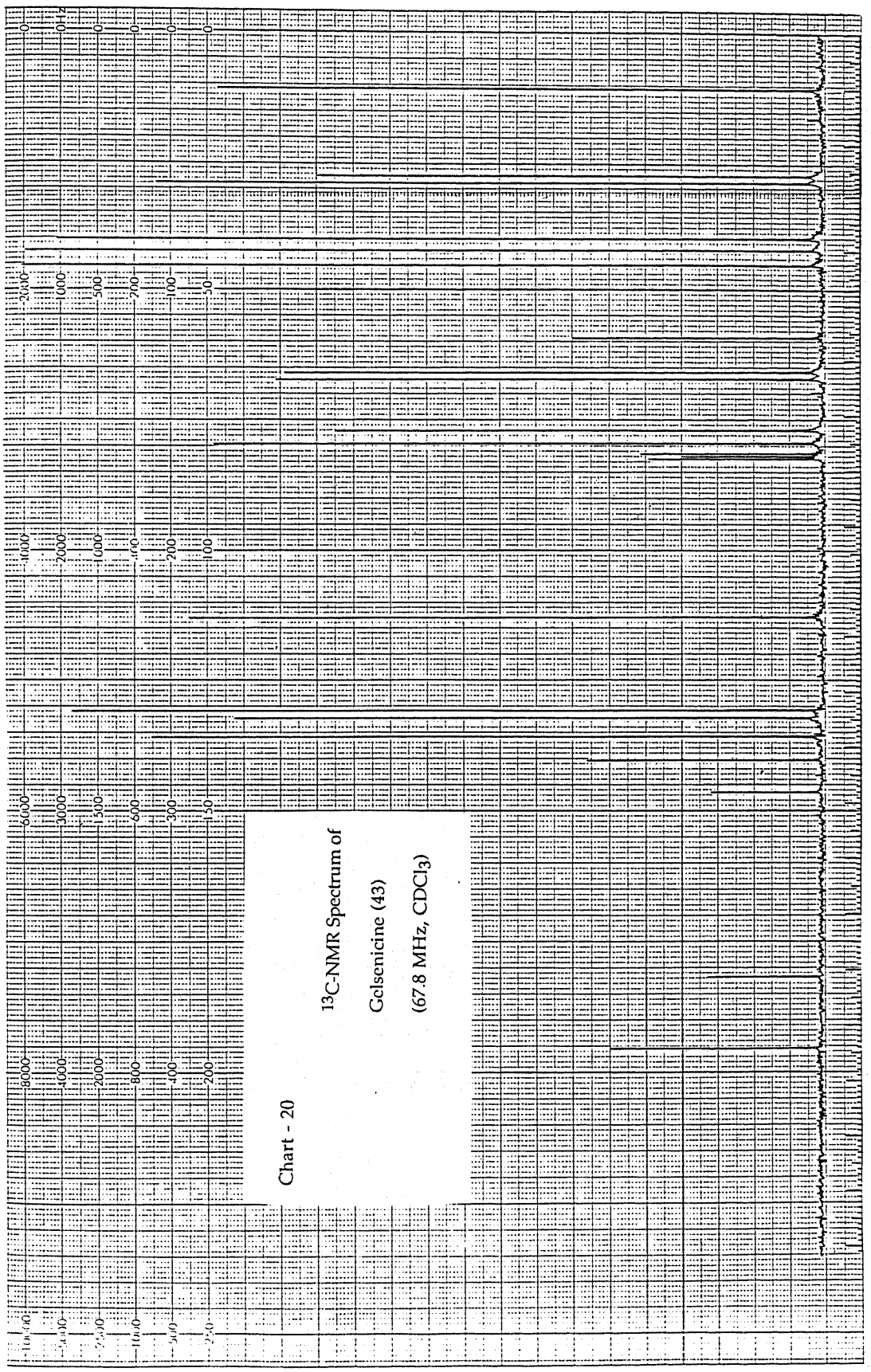


Chart - 20

^{13}C-NMR Spectrum of

Gelsenicine (43)

(67.8 MHz, $CDCl_3$)

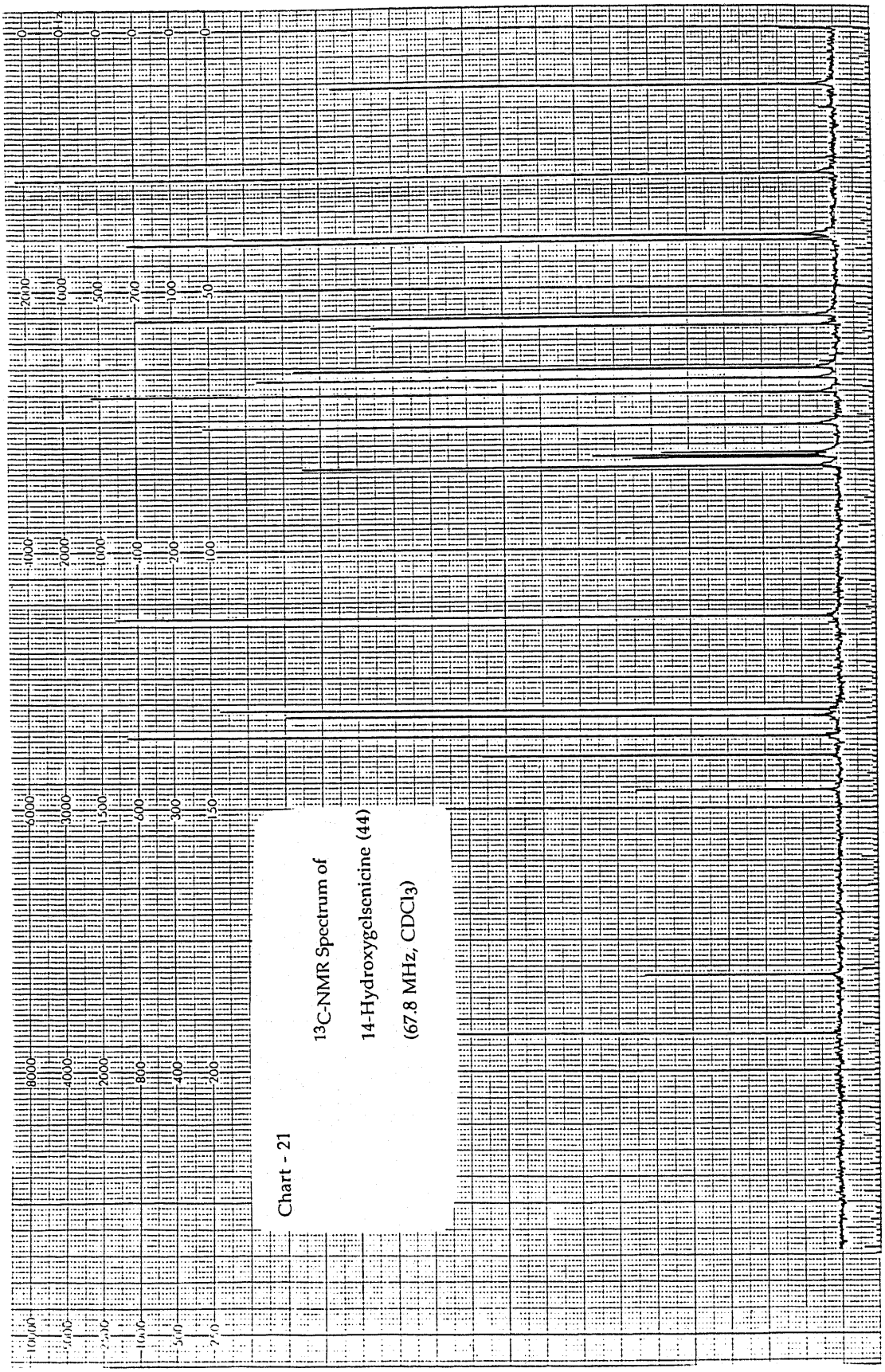


Chart - 21

^{13}C-NMR Spectrum of

14-Hydroxygelsenicine (44)

(67.8 MHz, $CDCl_3$)

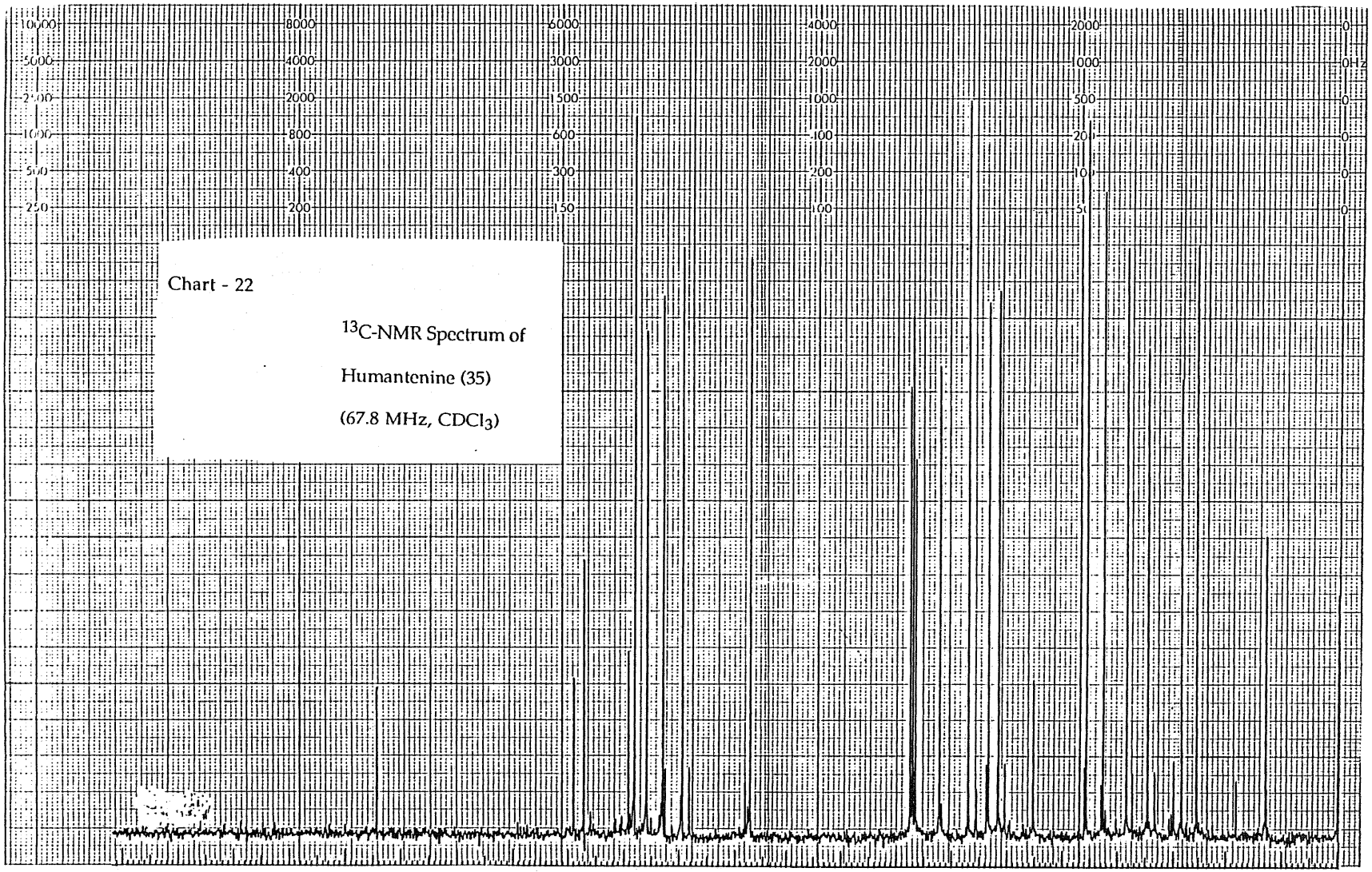


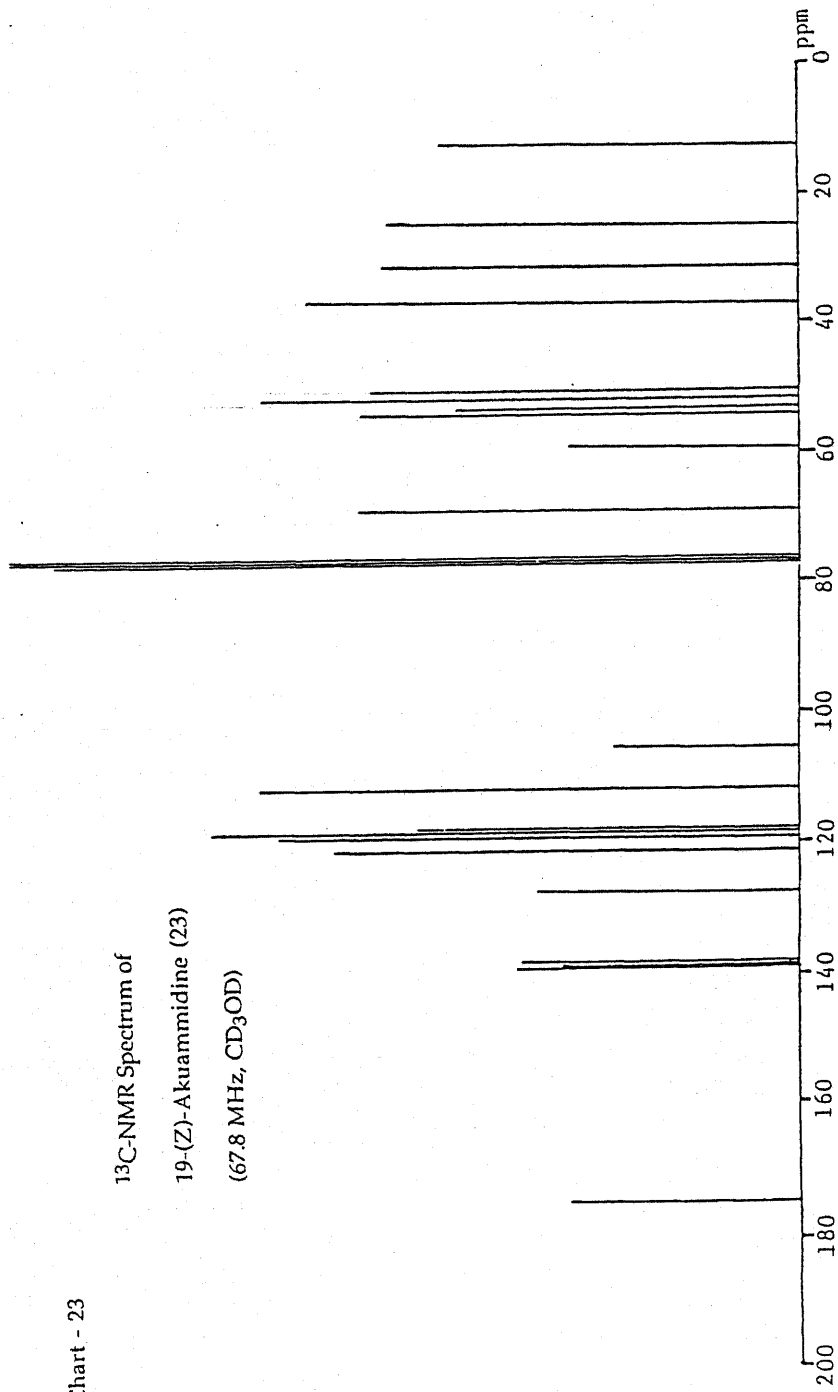
Chart - 22
^{13}C-NMR Spectrum of
Humantenine (35)
(67.8 MHz, $CDCl_3$)

Chart - 23

^{13}C-NMR Spectrum of

19-(Z)-Akuammidine (23)

(67.8 MHz, CD_3OD)



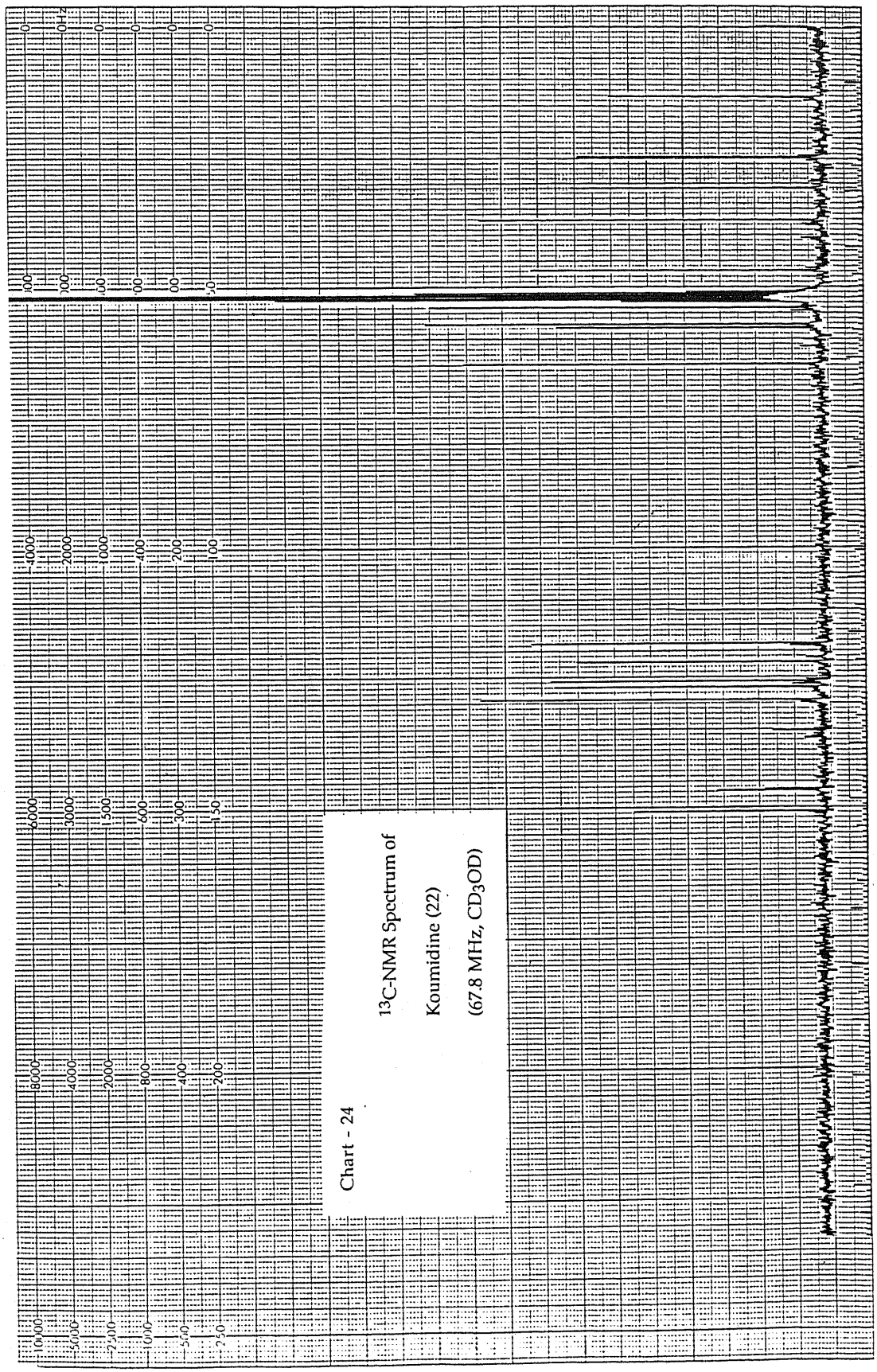


Chart - 24

^{13}C-NMR Spectrum of

Koumidine (22)

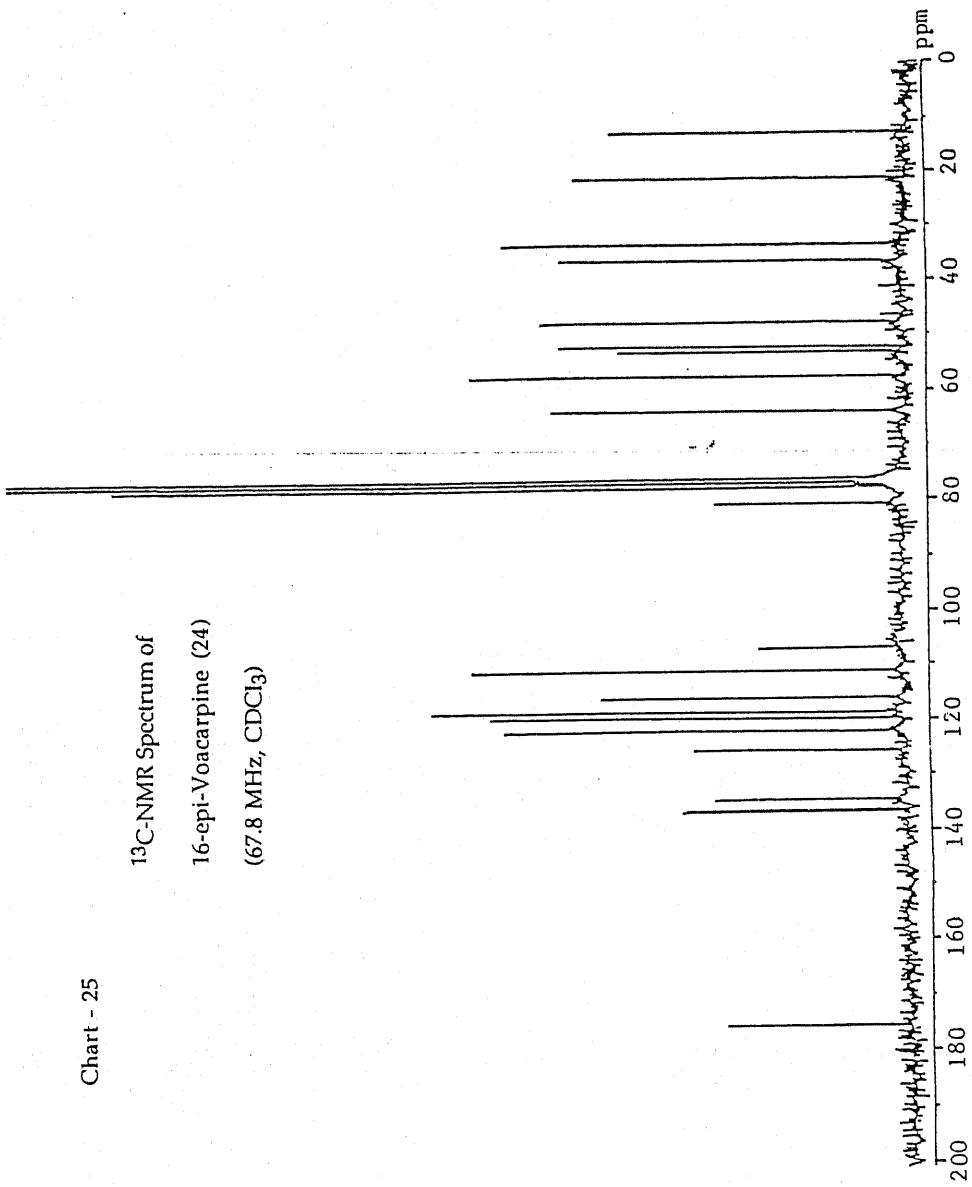
(67.8 MHz, CD_3OD)

Chart - 25

^{13}C-NMR Spectrum of

16-epi-Voacarpine (24)

(67.8 MHz, $CDCl_3$)



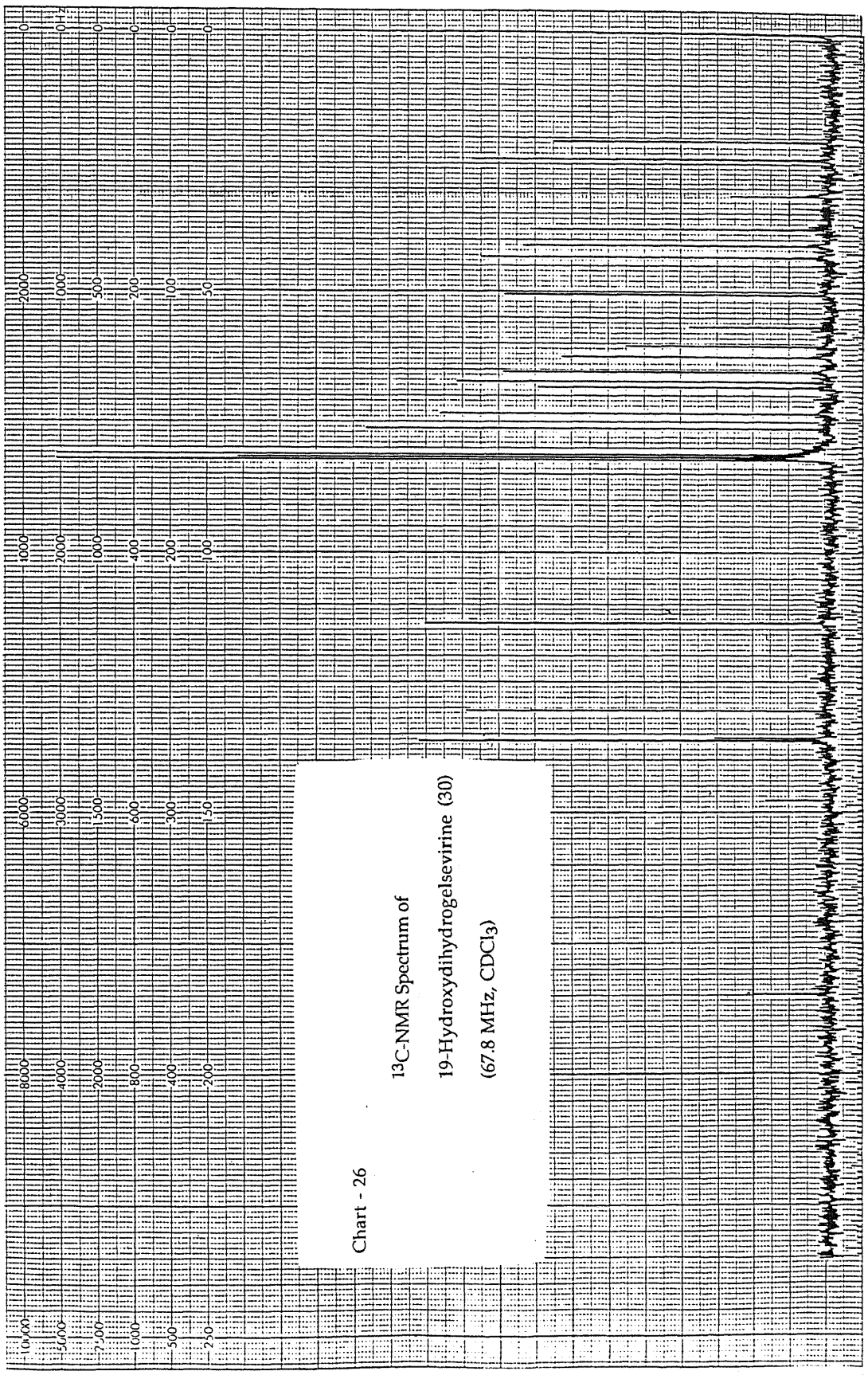


Chart - 26

^{13}C-NMR Spectrum of

19-Hydroxydihydrogelsevirine (30)

(67.8 MHz, $CDCl_3$)

Chart - 27

^{13}C-NMR Spectrum of

19-(Z)-Taberpsychine (25)

(67.8 MHz, $CDCl_3$)

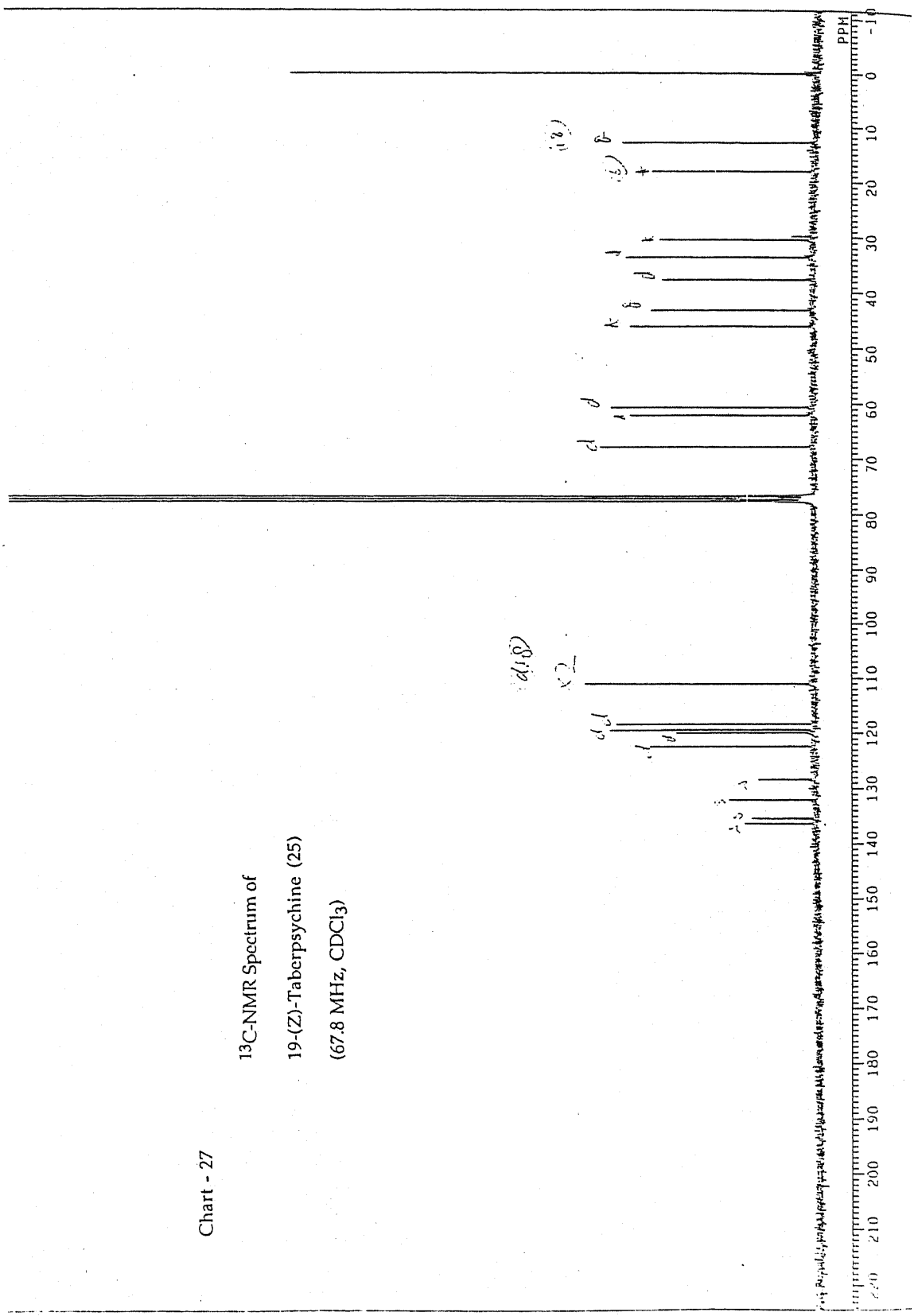
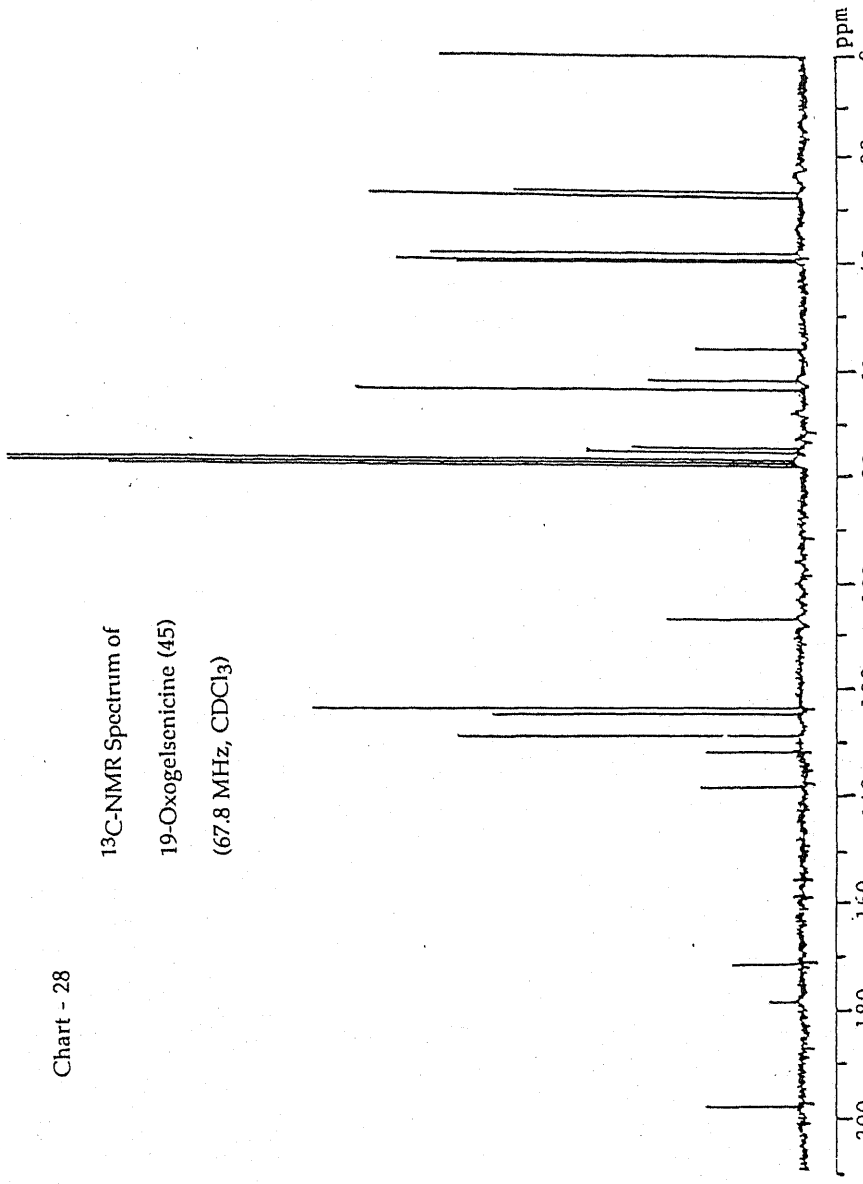


Chart - 28

^{13}C-NMR Spectrum of

19-Oxogelsenicine (45)

(67.8 MHz, $CDCl_3$)



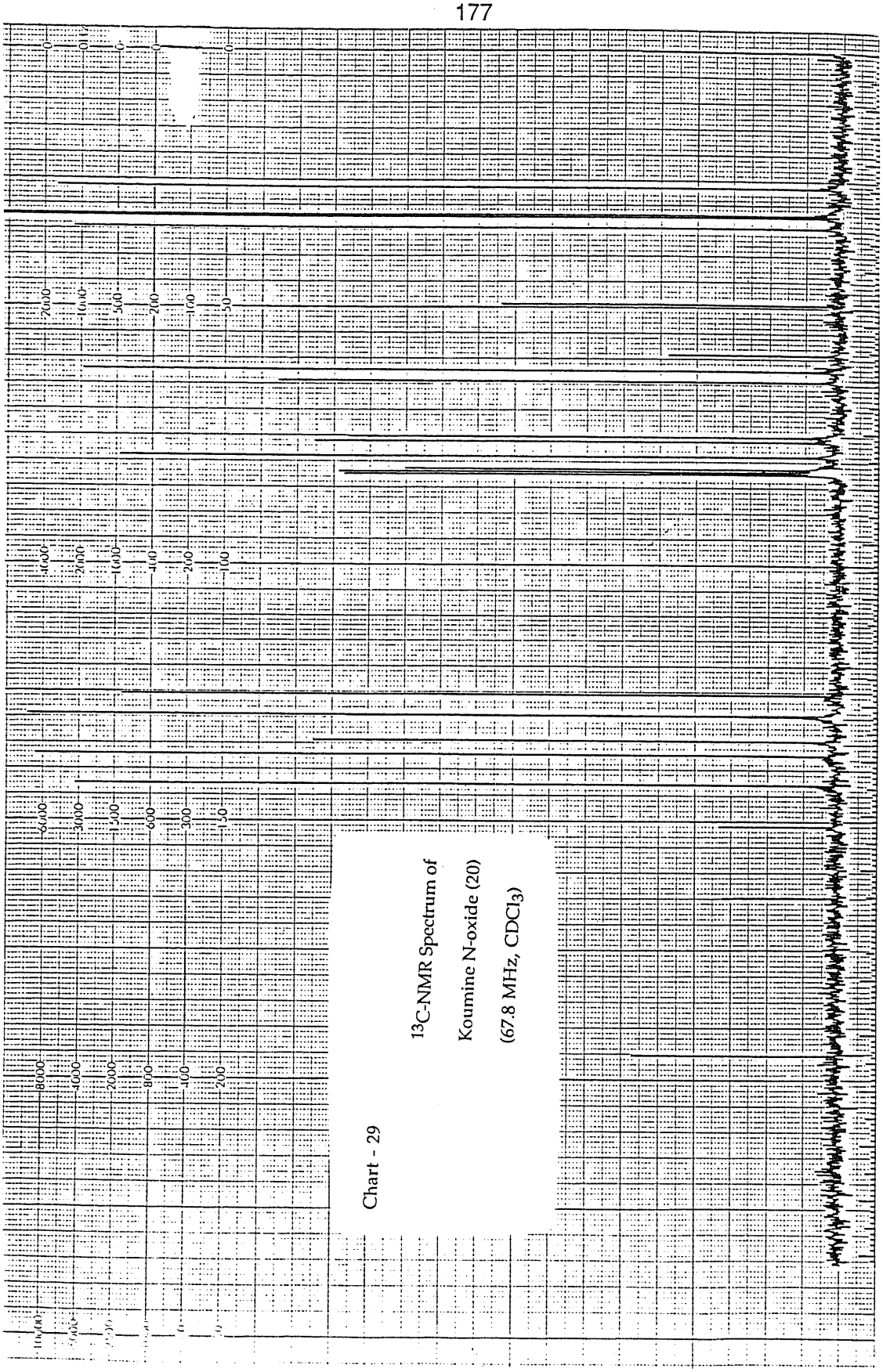


Chart - 29

^{13}C-NMR Spectrum of

Koumine N-oxide (20)

(67.8 MHz, $CDCl_3$)

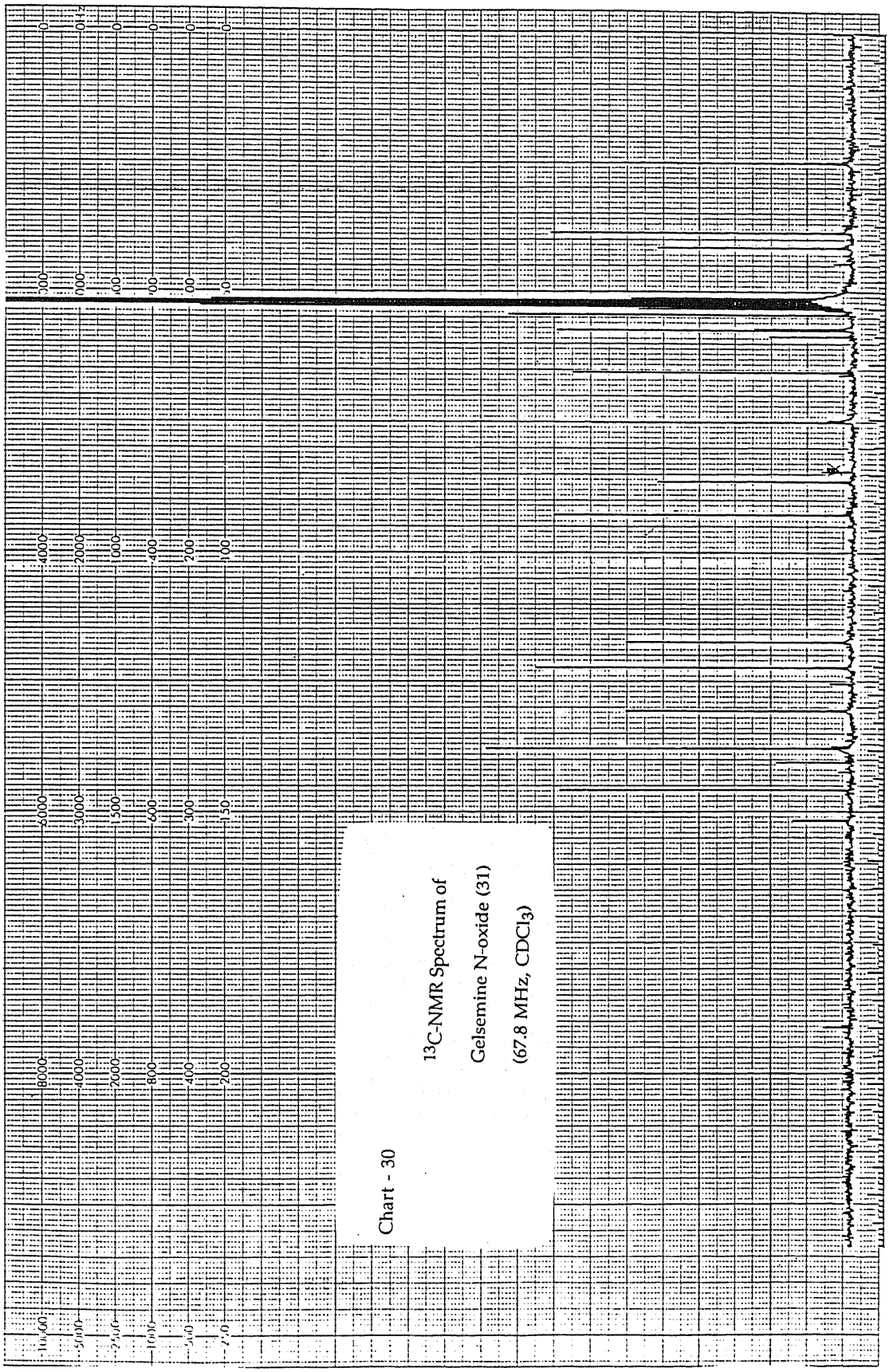


Chart - 30

^{13}C-NMR Spectrum of

Gelsemine N-oxide (31)

(67.8 MHz, $CDCl_3$)

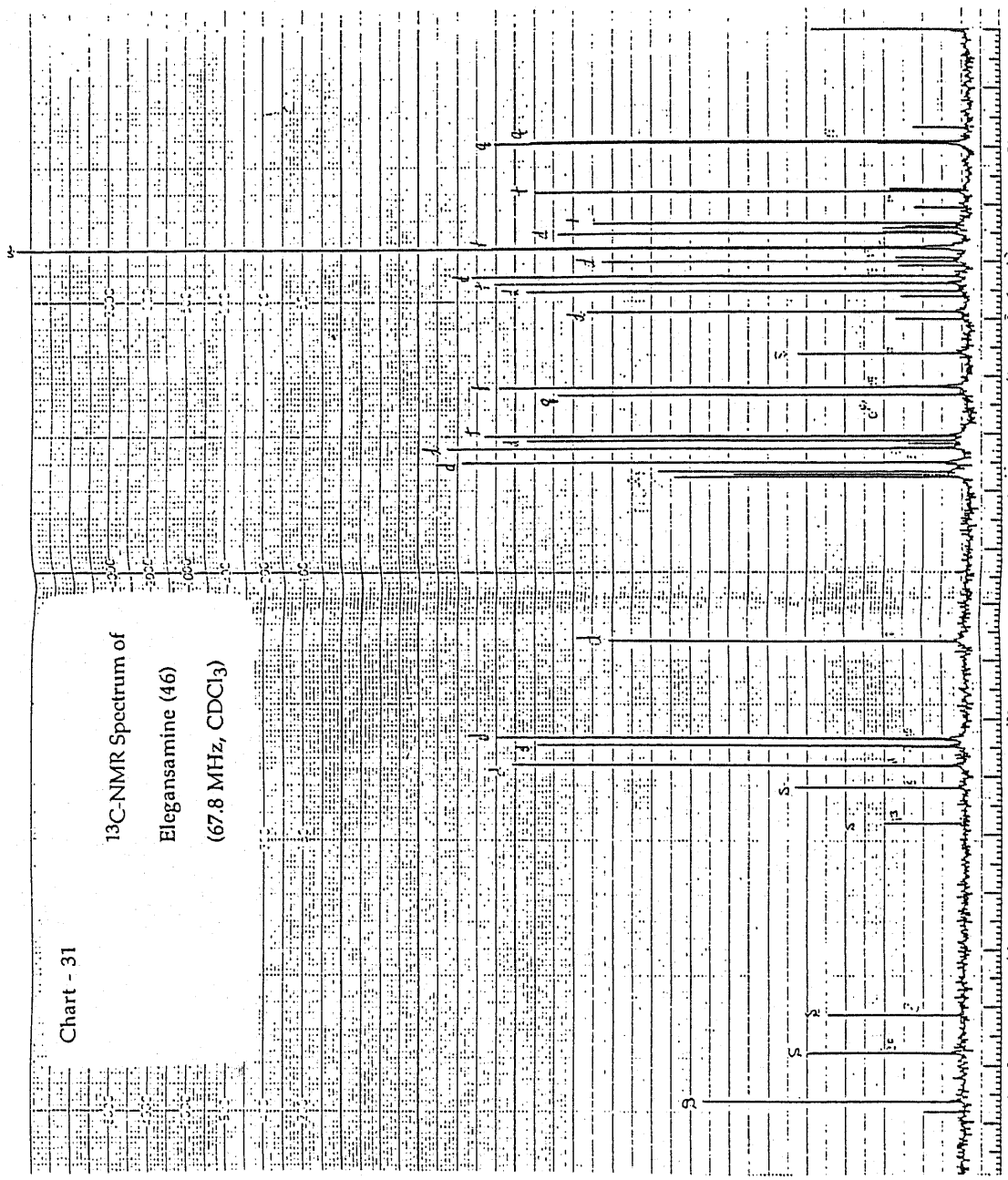


Chart - 31
^{13}C-NMR Spectrum of
Elegansamine (46)
(67.8 MHz, $CDCl_3$)

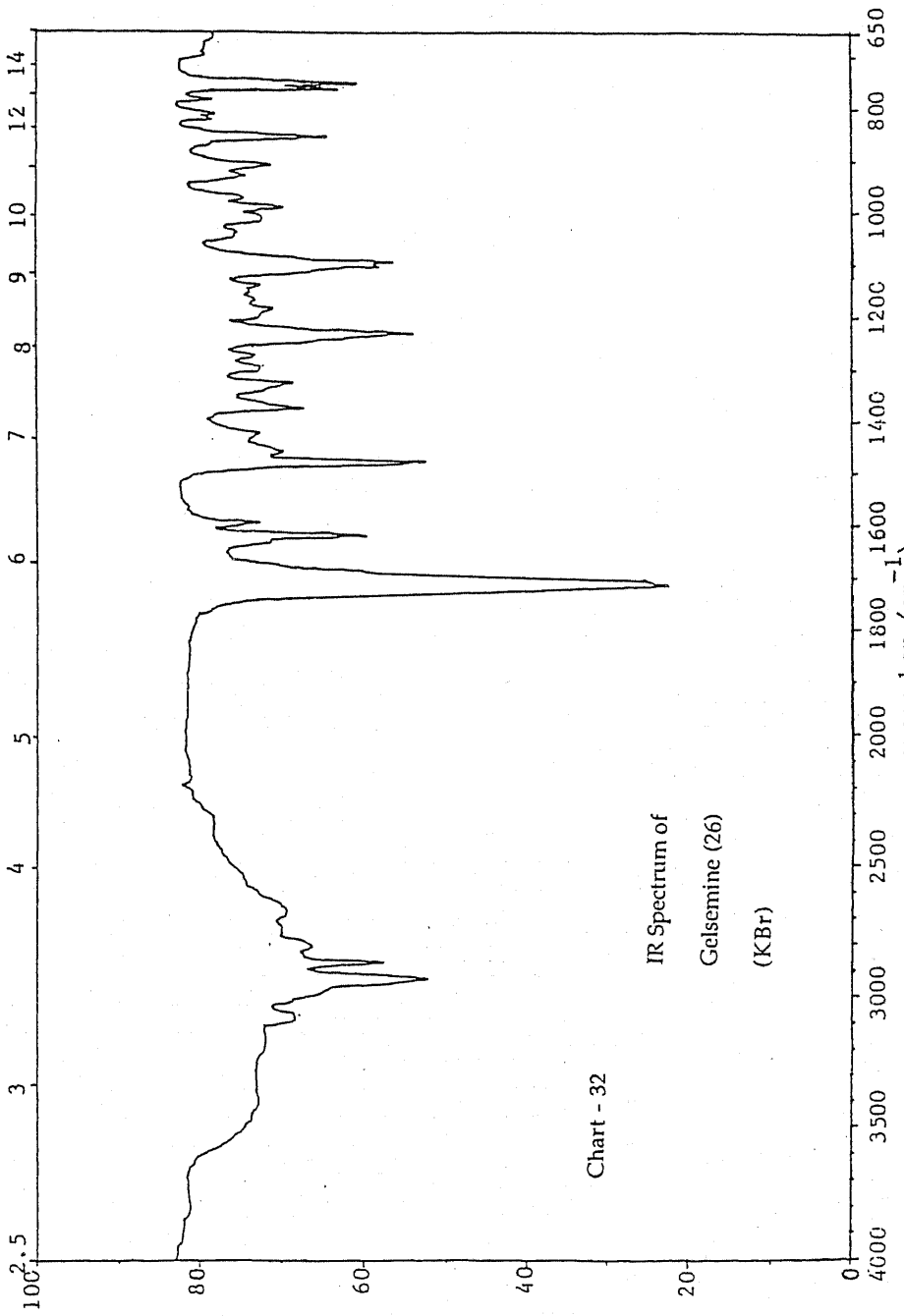


Chart - 32
IR Spectrum of
Gelsemine (26)
(KBr)
wavenumber (cm-1)
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
650
100
80
60
40
20
0
2.5
3
4
5
6
7
8
9
10
12
14

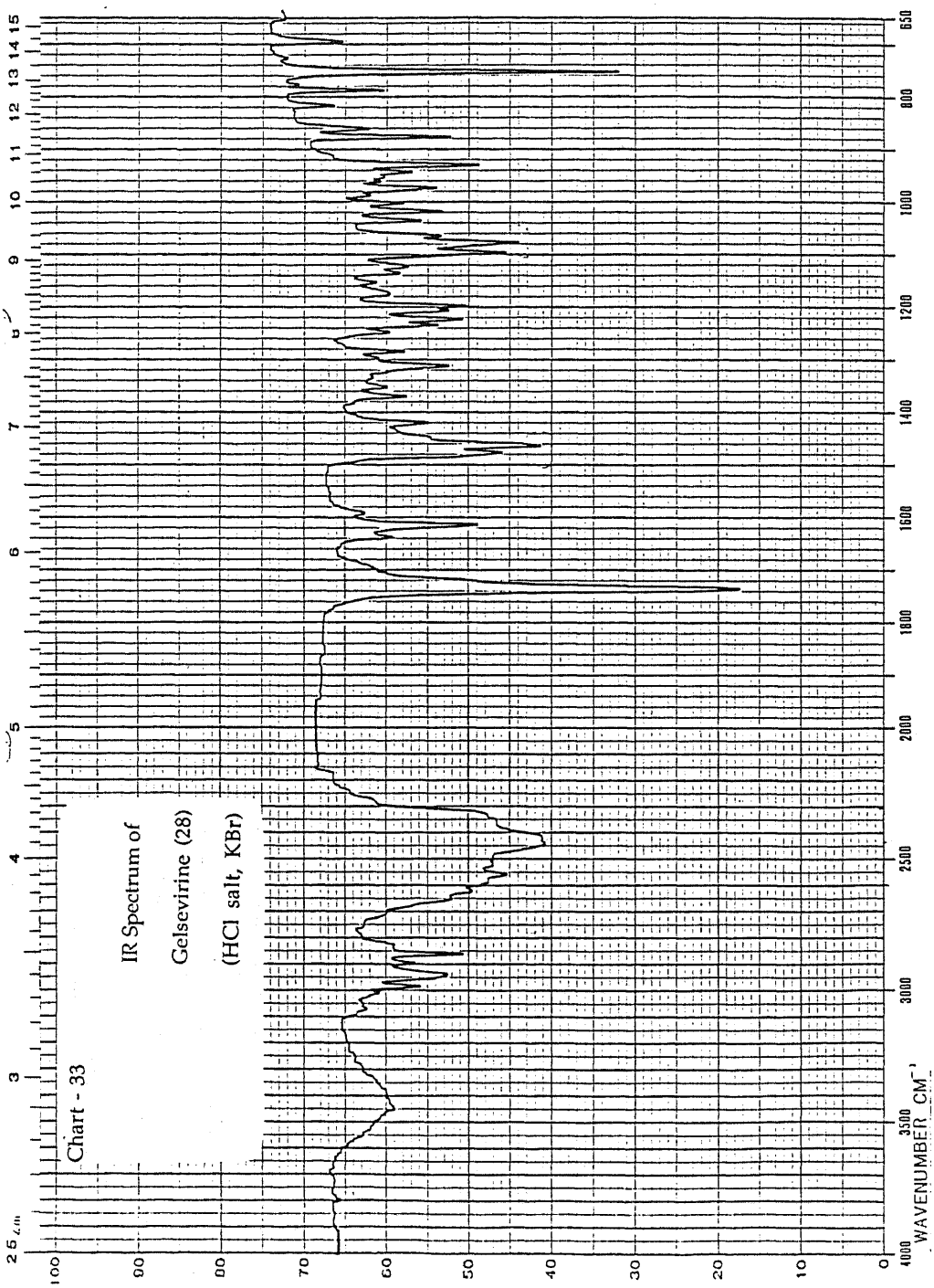


Chart - 33
IR Spectrum of
Gelsevirine (28)
(HCl salt, KBr)
WAVENUMBER CM⁻¹

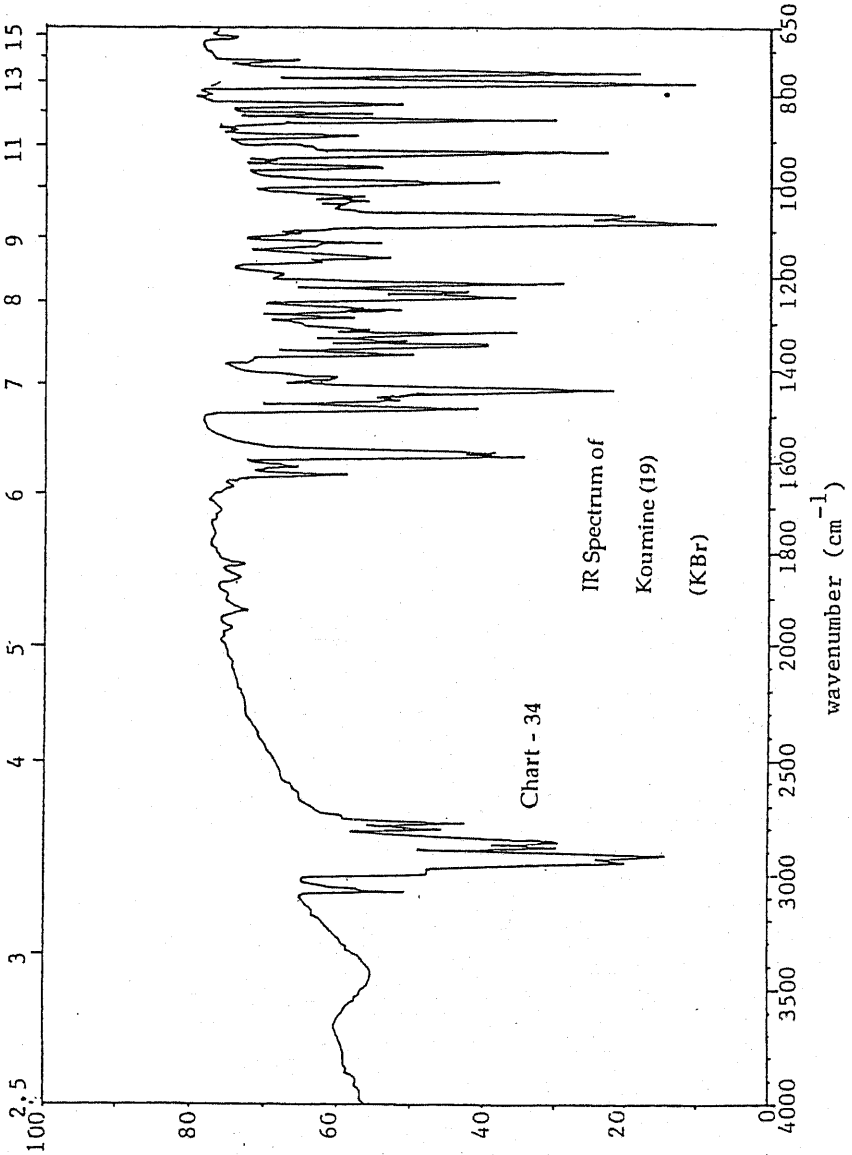


Chart - 34
IR Spectrum of
Koumine (19)
(KBr)
wavenumber (cm⁻¹)
4000 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 650
2.5 3 4 5 6 7 8 9 11 13 15
100 80 60 40 20 0

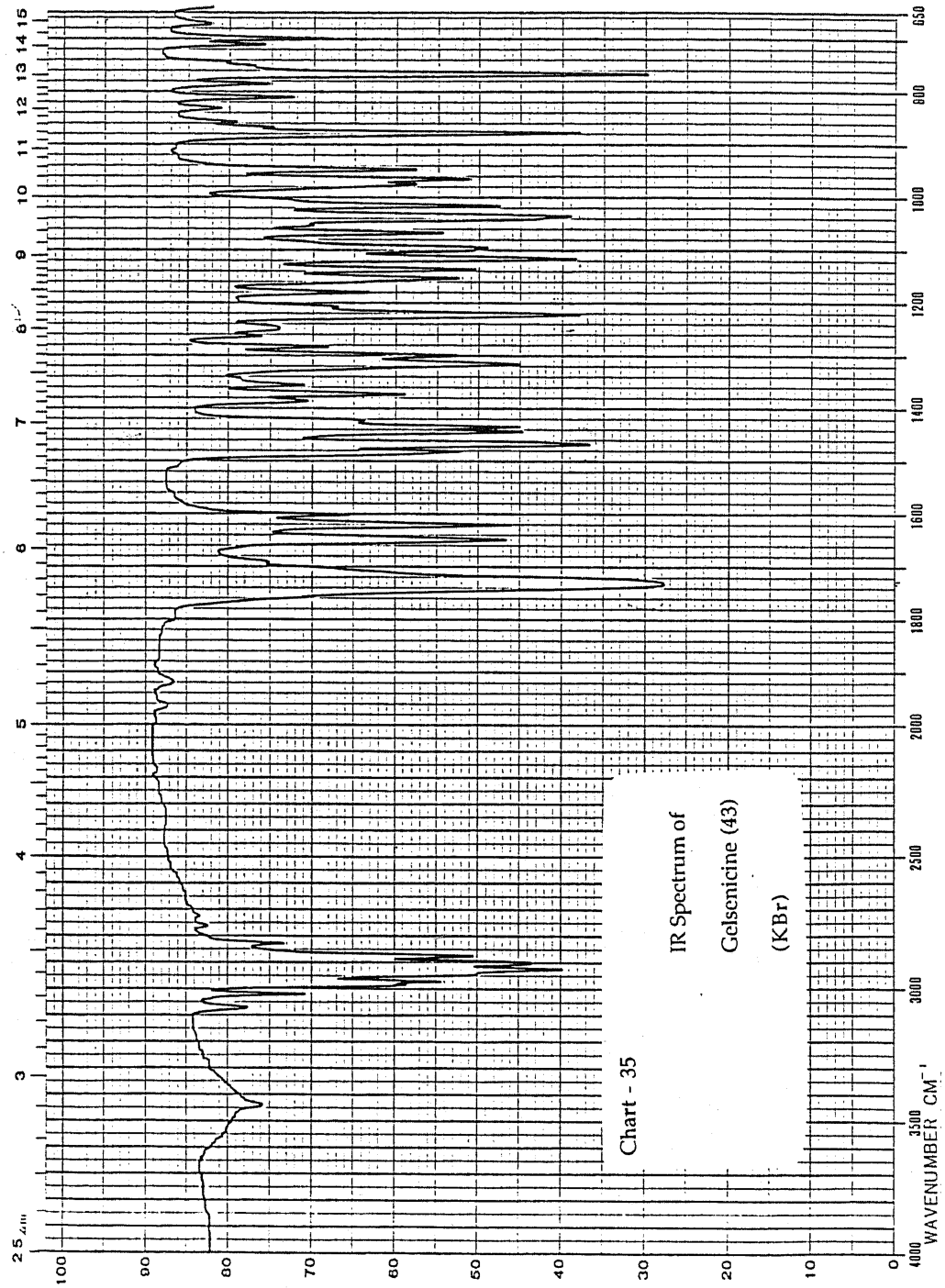


Chart - 35
IR Spectrum of
Gelsenicine (43)
(KBr)
WAVENUMBER CM⁻¹
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
650
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

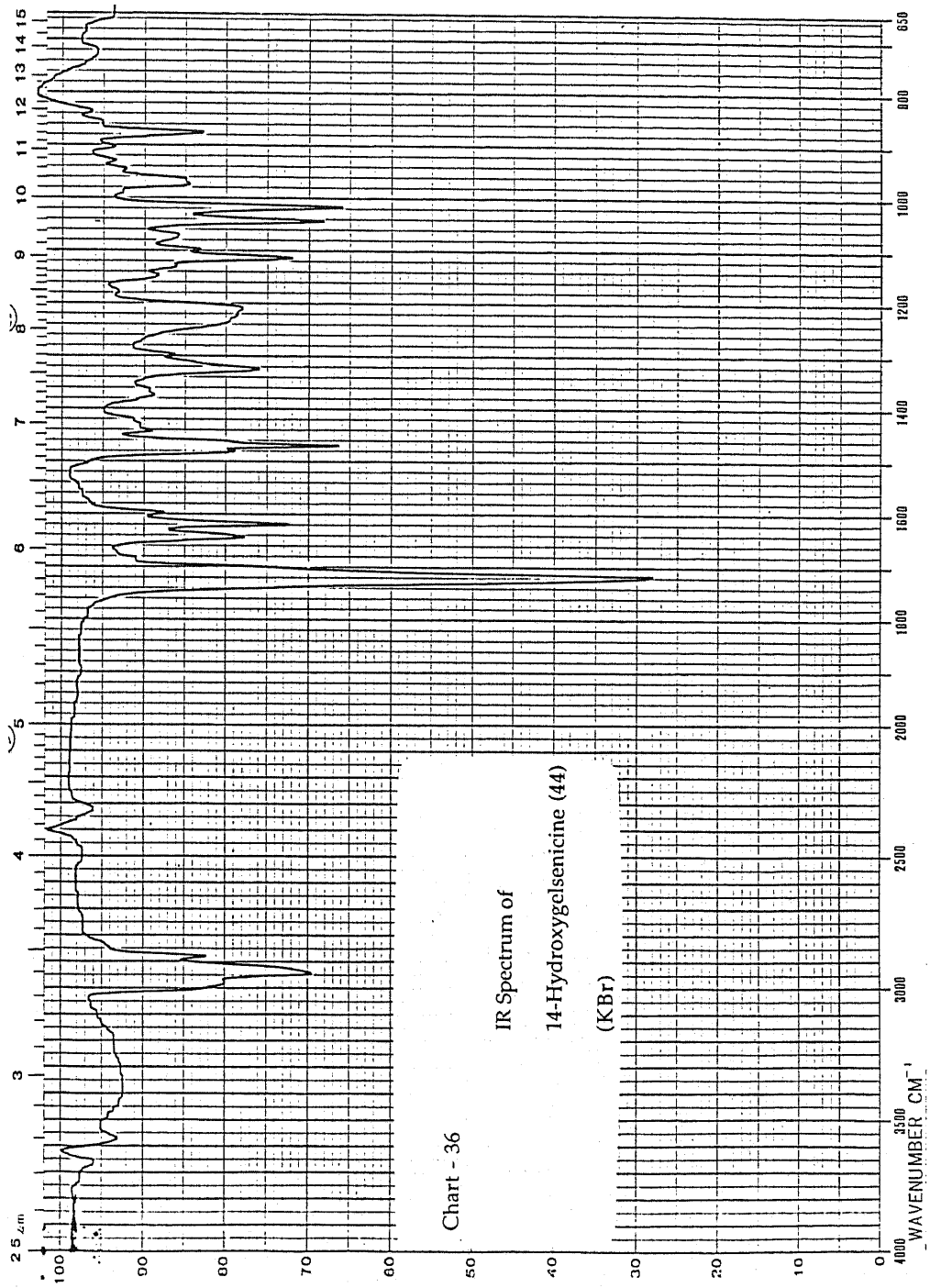


Chart - 36
IR Spectrum of
14-Hydroxygelsenicine (44)
(KBr)
WAVENUMBER CM⁻¹

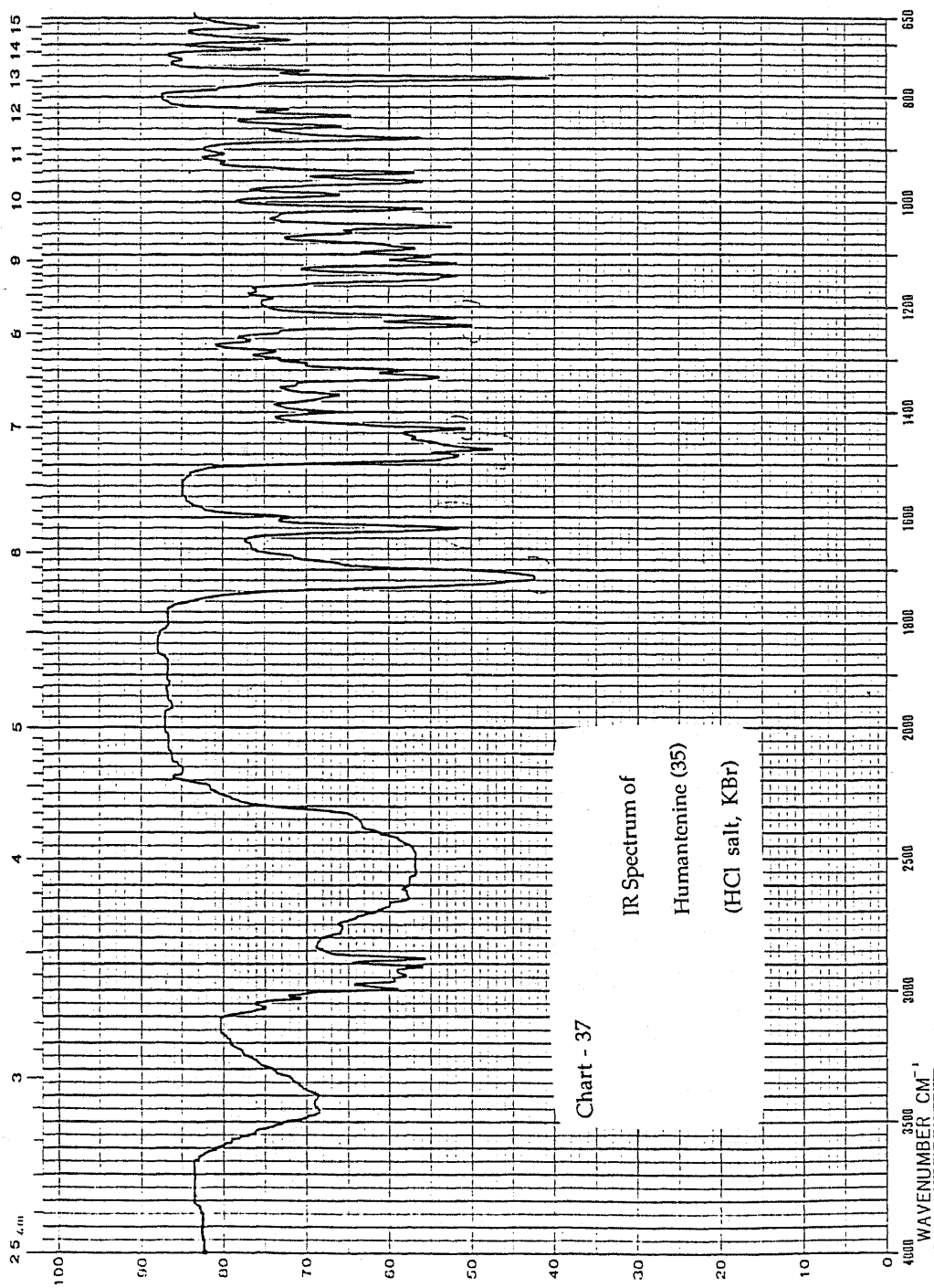


Chart - 37
IR Spectrum of
Humantenine (35)
(HCl salt, KBr)
WAVENUMBER CM⁻¹

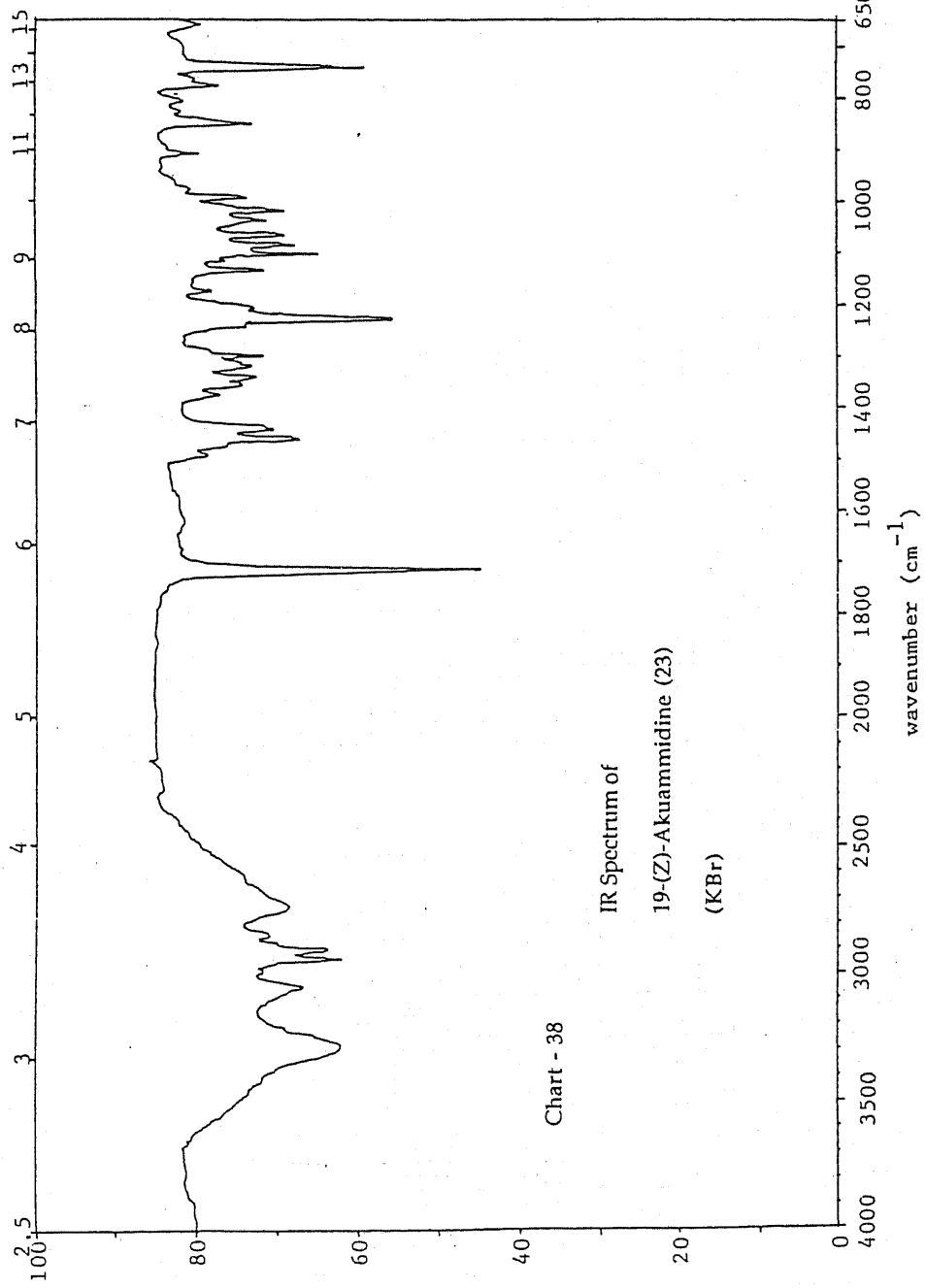


Chart - 38

IR Spectrum of

19-(Z)-Akuammidine (23)

(KBr)

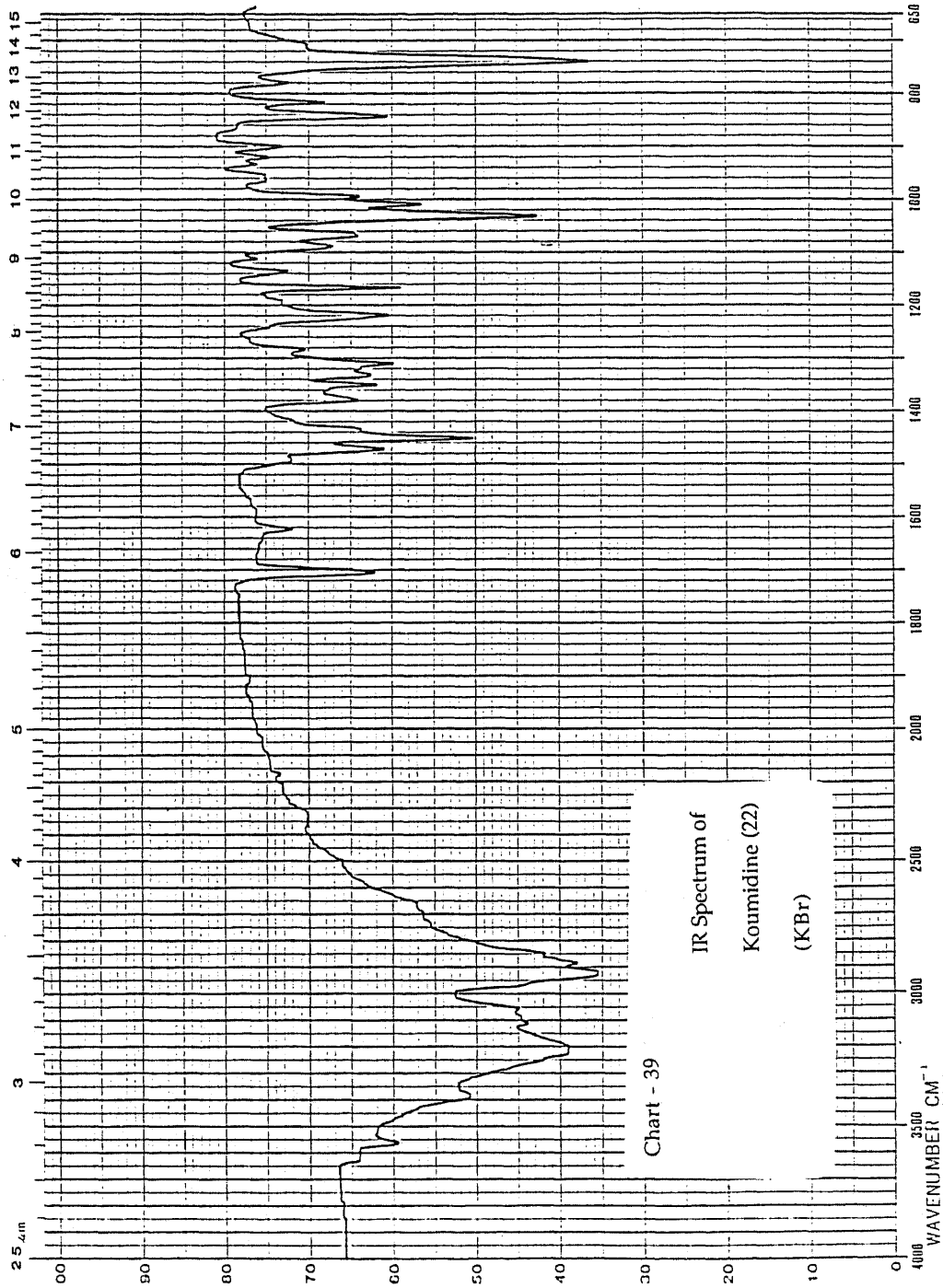


Chart - 39
IR Spectrum of
Koumidine (22)
(KBr)
WAVENUMBER CM⁻¹

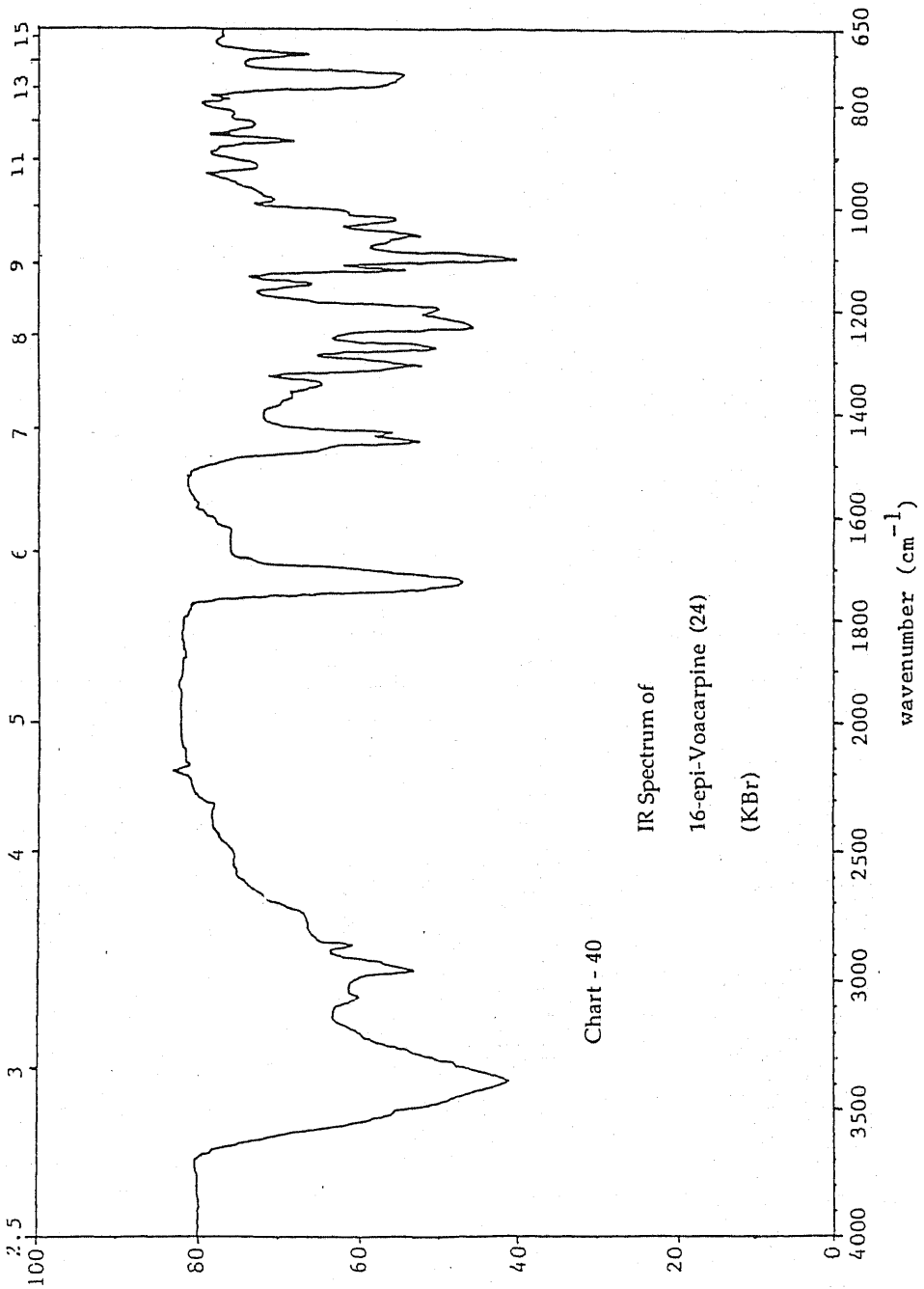


Chart - 40

IR Spectrum of

16-epi-Voacarpine (24)

(KBr)

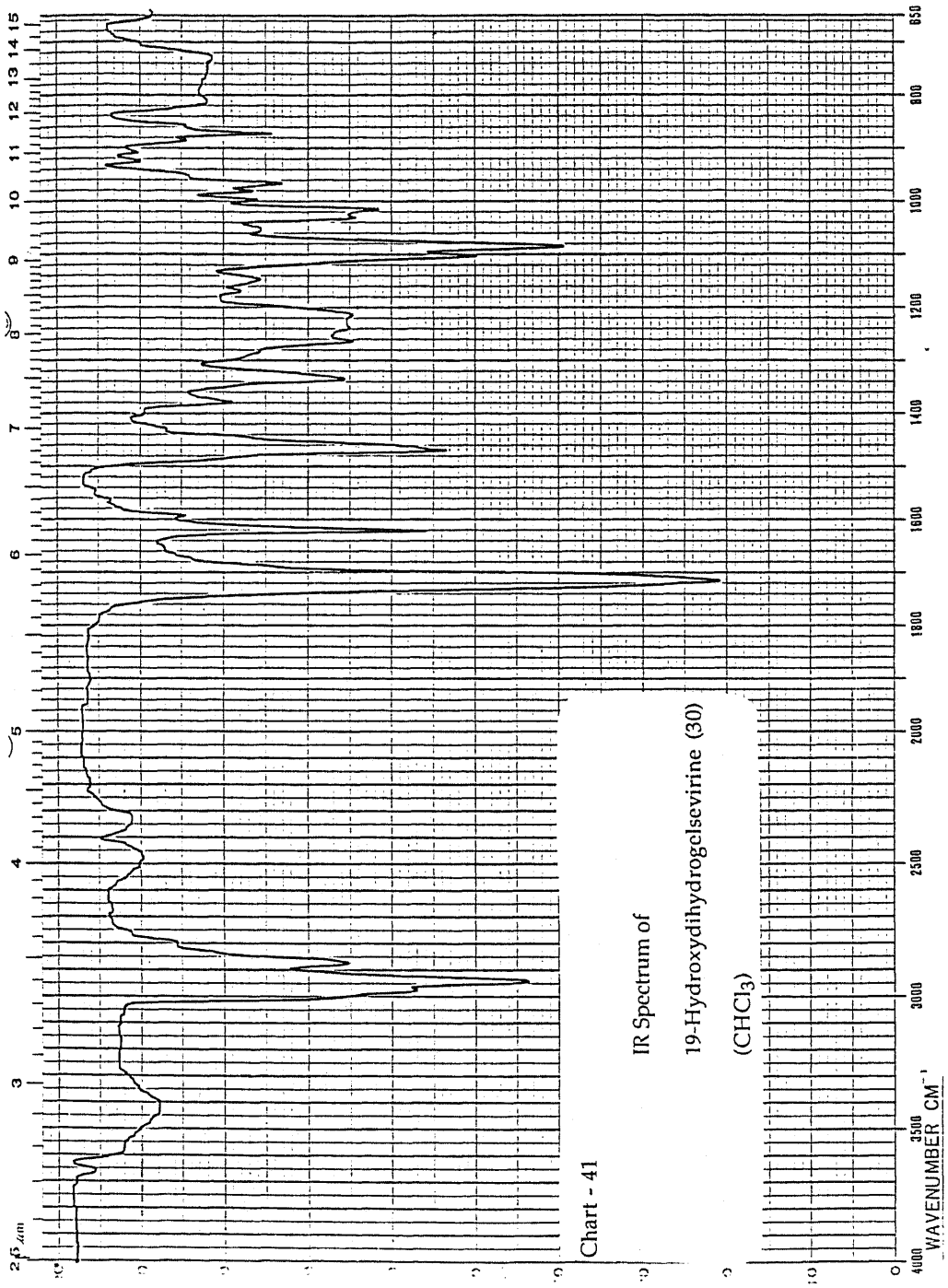


Chart - 41

IR Spectrum of

19-Hydroxydihydrogelsevirine (30)

($CHCl_3$)

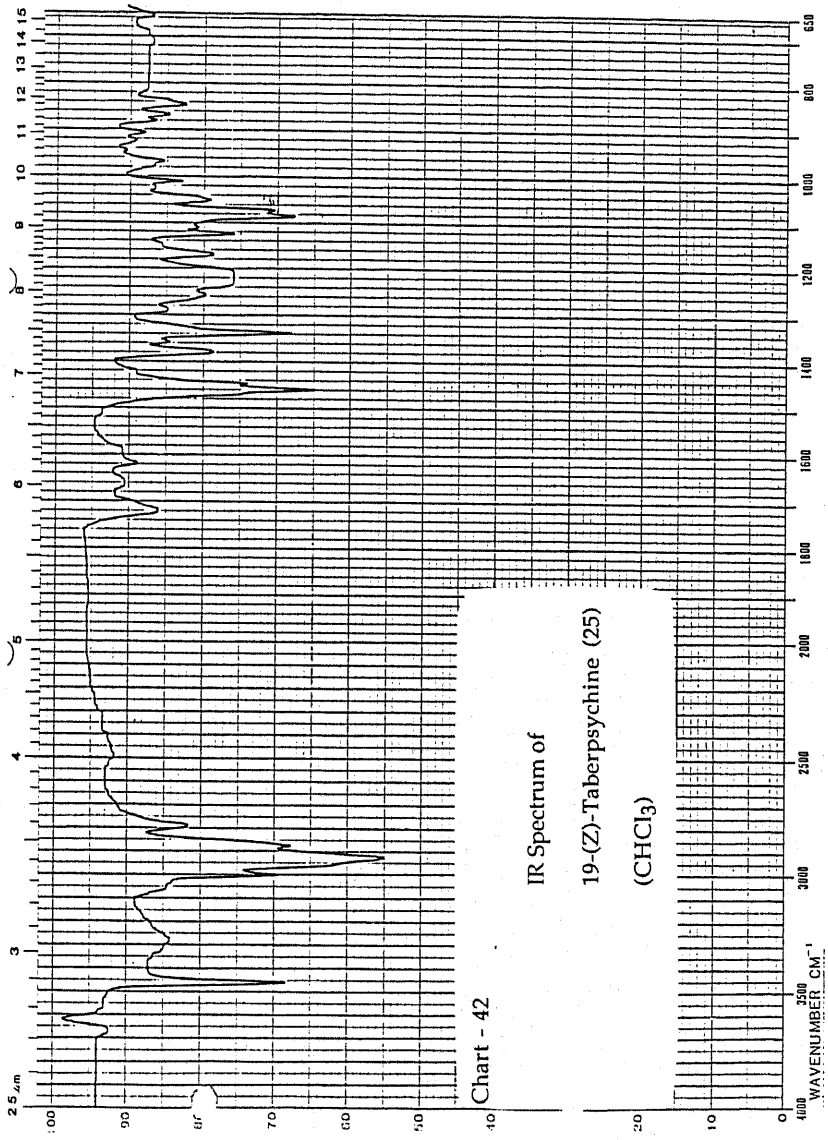


Chart - 42

IR Spectrum of

19-(Z)-Taberpsychine (25)

($CHCl_3$)

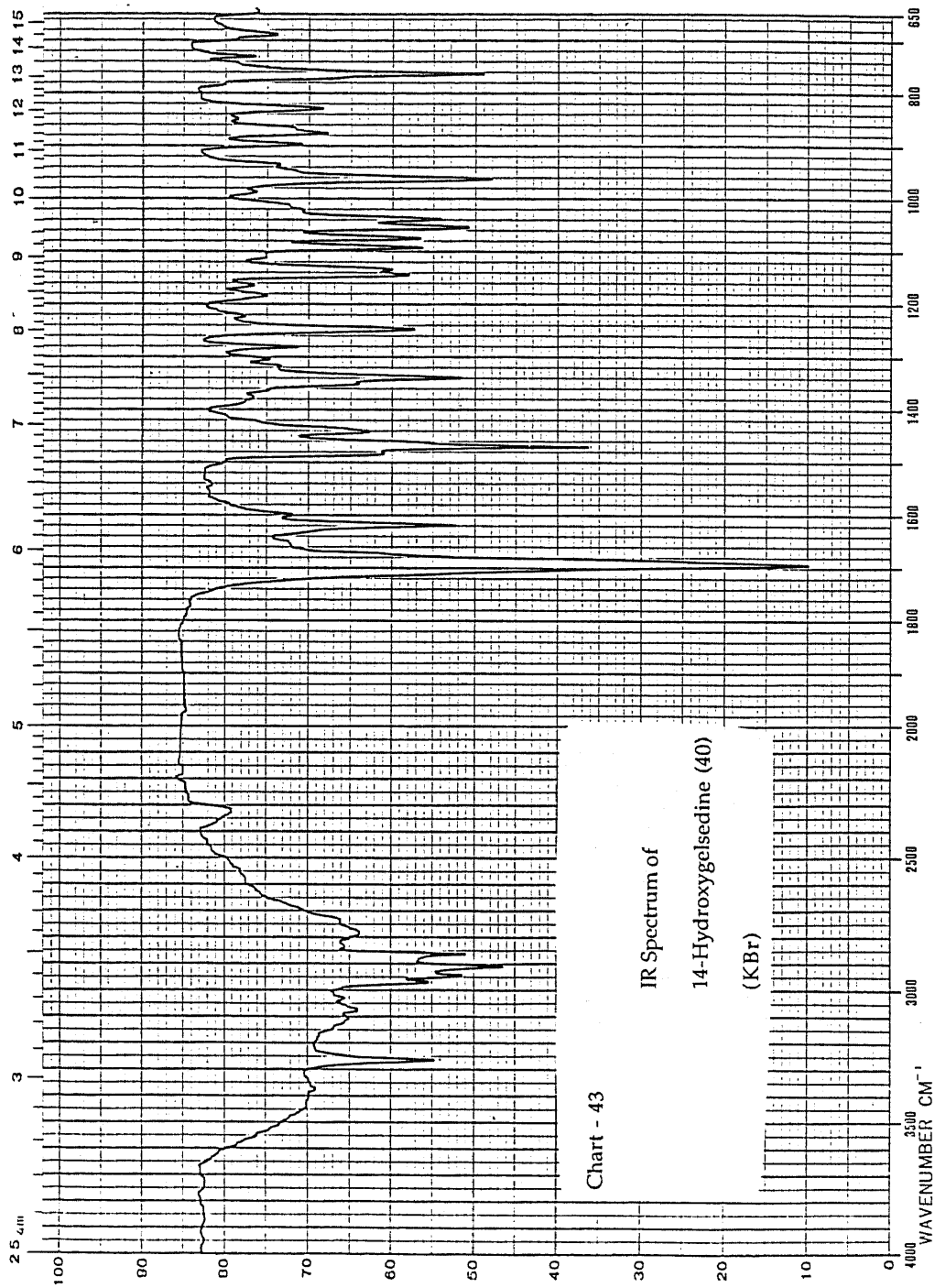


Chart - 43
IR Spectrum of
14-Hydroxygelsedine (40)
(KBr)
WAVENUMBER_CM⁻¹

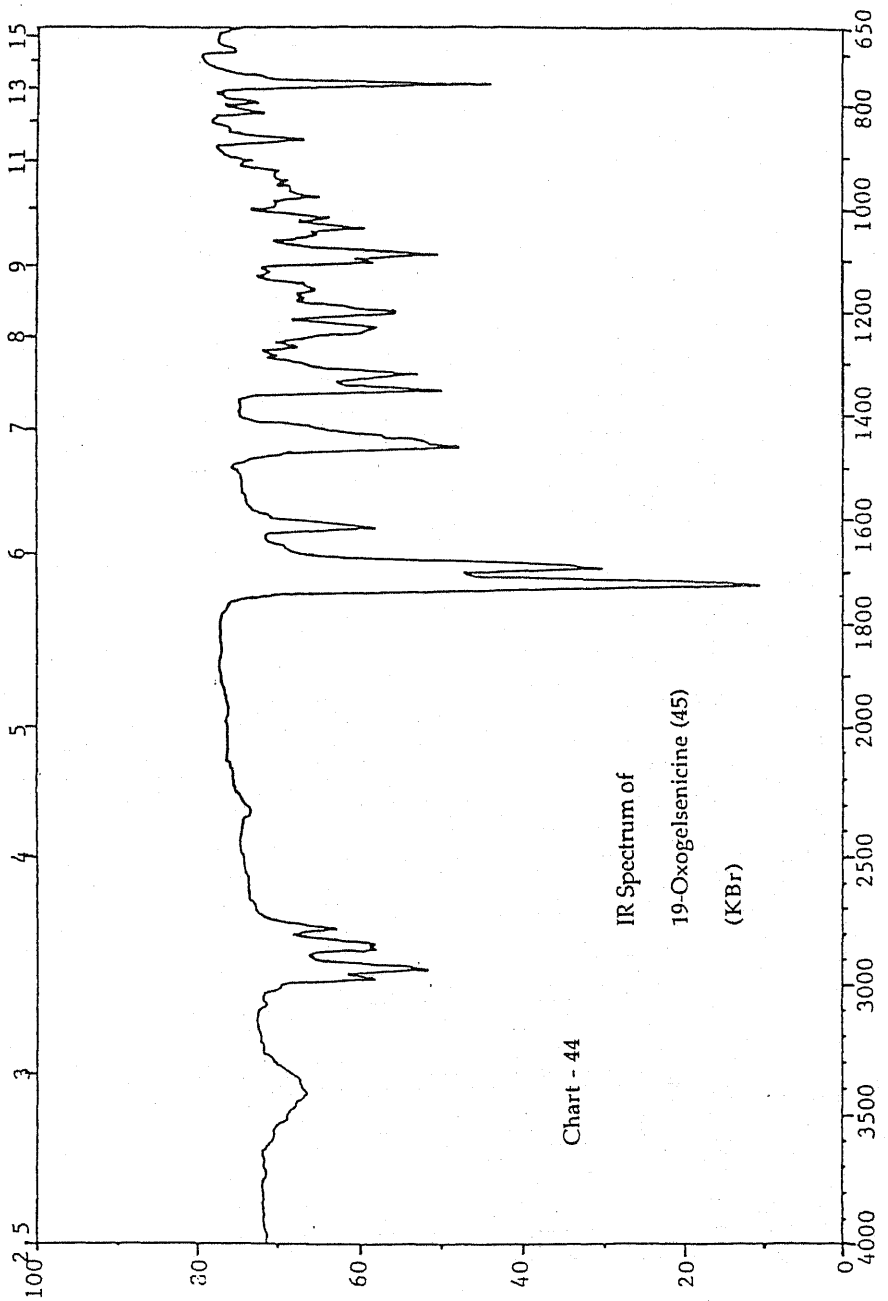


Chart - 44

IR Spectrum of

19-Oxogelsenicine (45)

(KBr)

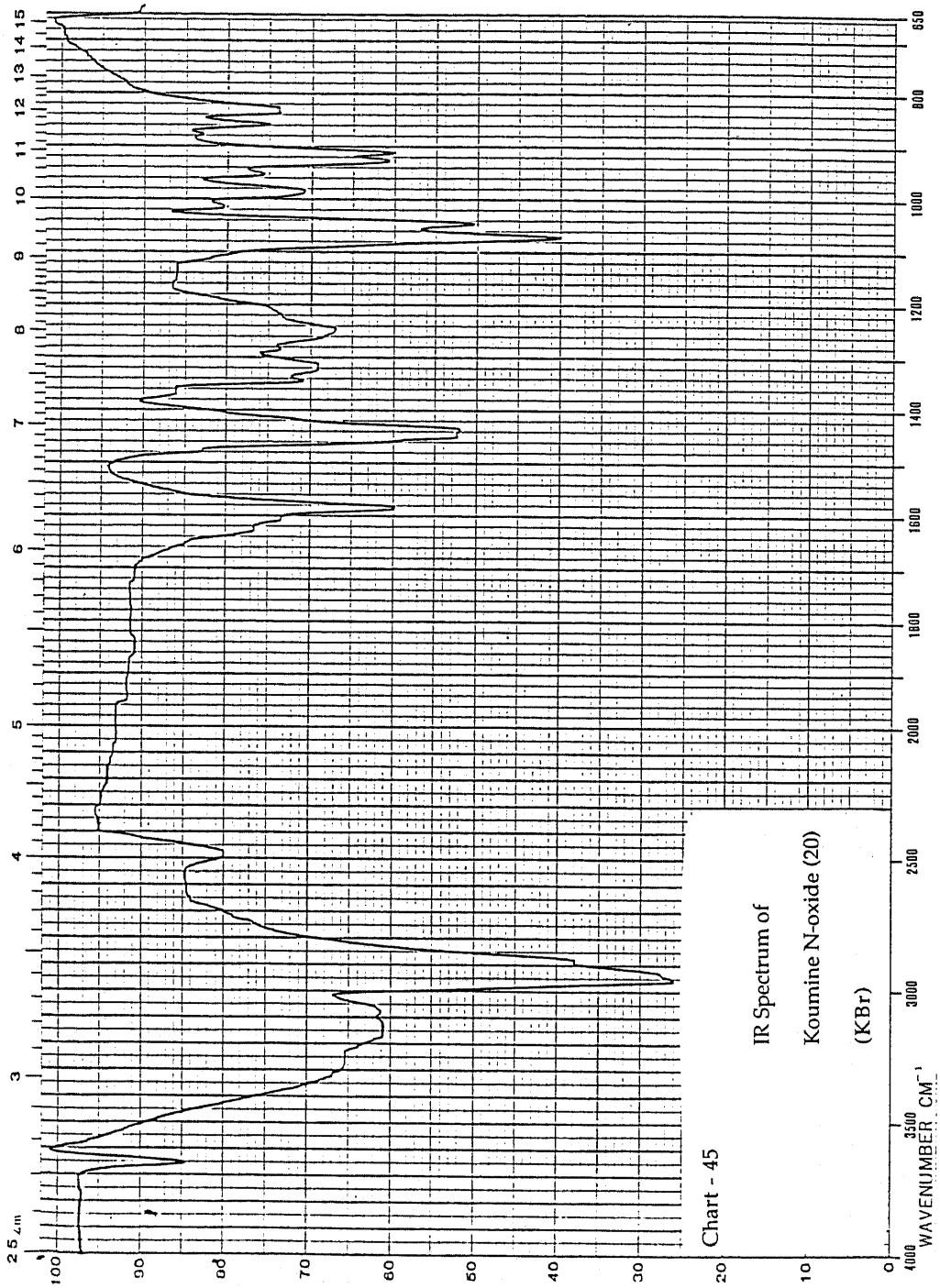


Chart - 45
IR Spectrum of
Koumine N-oxide (20)
(KBr)
WAVENUMBER CM⁻¹

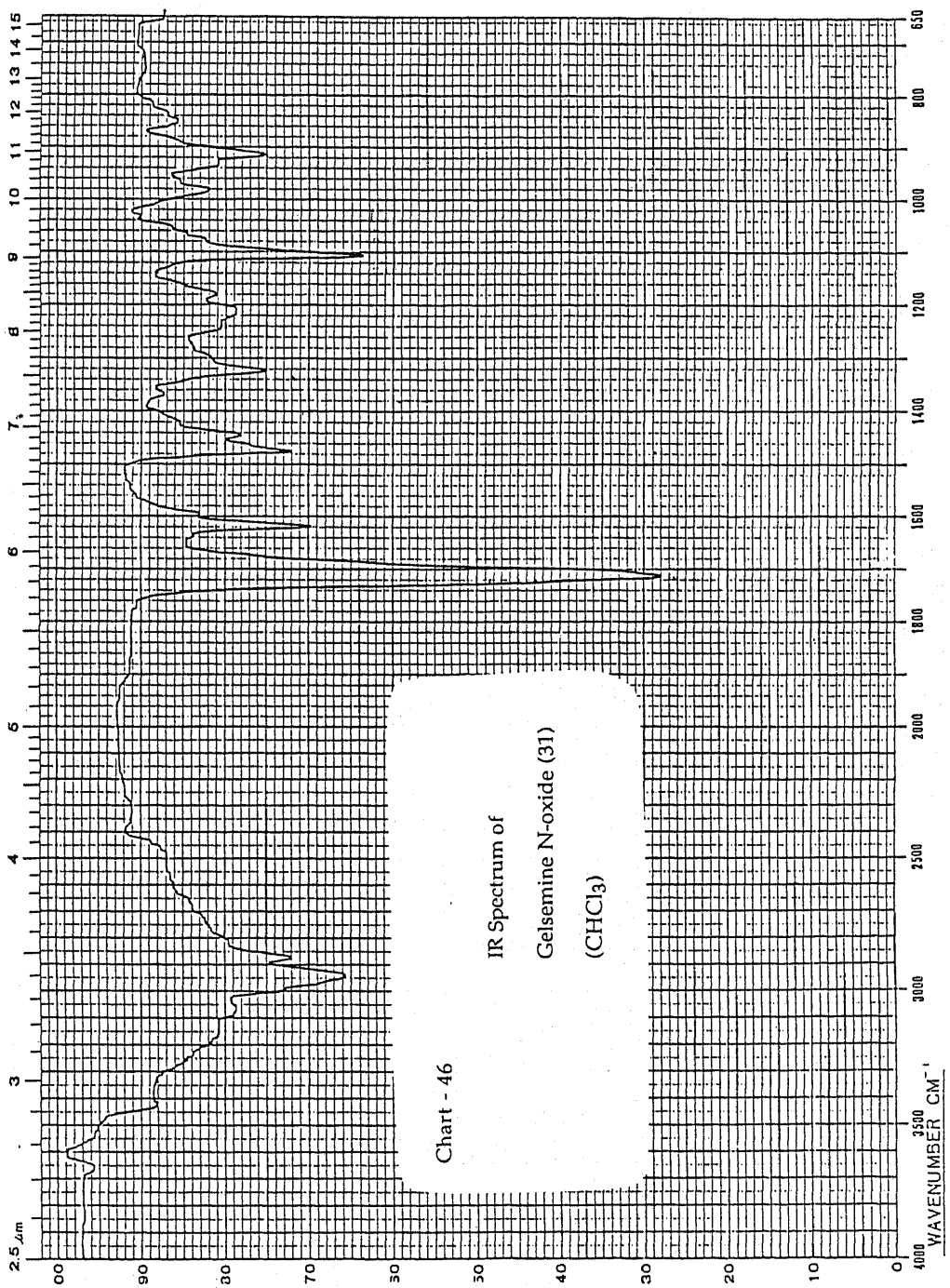


Chart - 46

IR Spectrum of

Gelsemine N-oxide (31)

($CHCl_3$)

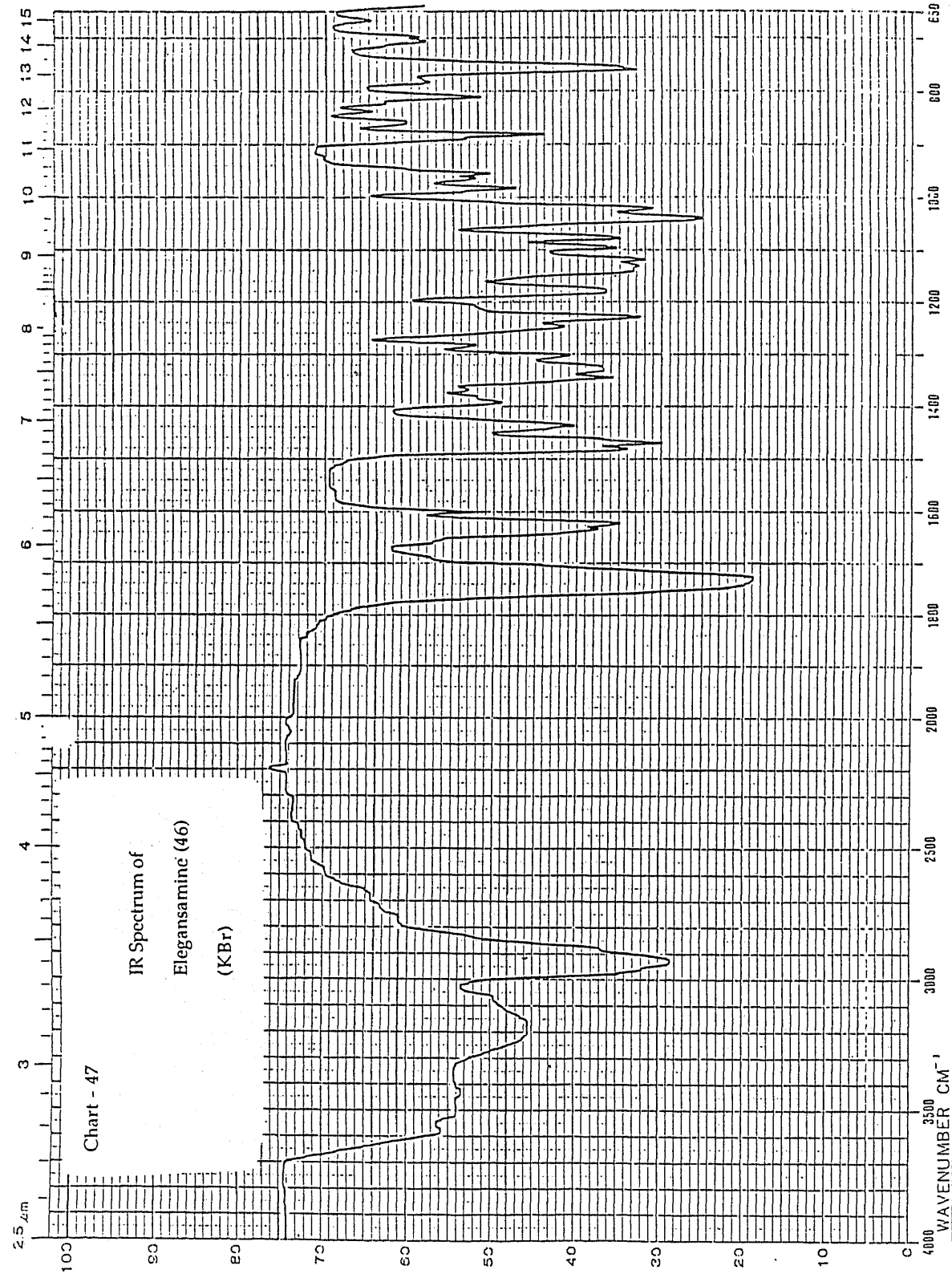


Chart - 47
IR Spectrum of
Elegansamine (46)
(KBr)
WAVENUMBER CM⁻¹

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