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STUDIES ON THE INDOLE ALKALOIDS OF *GELSEMIUM ELEGANS* (THAILAND): STRUCTURE ELUCIDATION AND PROPOSAL OF BIOGENETIC ROUTE

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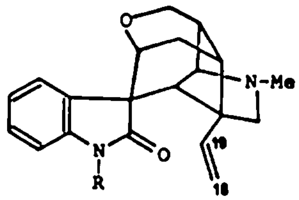
Abstract: Chemical investigation of the roots of Gelsemium elegans collected in Thailand resulted in the isolation of the new indole alkaloids, 16-epi-voacarpine, 19-hydroxydihydrogelsevirine, and 19-(Z)-taberpsychine, along with eight known bases. Among them, the structure of "akuammidine" and koumidine were revised to have 19-(Z) ethylidene configuration. From the seeds of G. elegans 14-hydroxygelsedine was obtained. Furthermore, three new alkaloids, koumine N-oxide, gelsemine N-oxide, and 19-oxo-gelsenicine, were found from the leaves of G. elegans.
A new and plausible biogenetic route of gelsemium alkaloids is proposed.

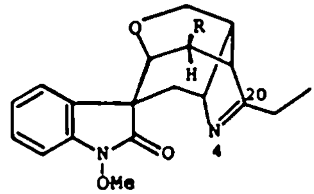
The genus Gelsemium, belonging to the family Loganiaceae, have been used as medicinal plants in the treatment of neuralgia and migrain, and are also used in traditional Chinese medicine. These plants are well known to contain indole and oxindole alkaloids of novel polycyclic systems.[2)] As a part of our program on the chemical studies on the Gelsemium alkaloids,[3)] we report herein our results on the investigation of the alkaloidal constituents of the roots, seeds, and leaves of Gelsemium elegans collected in Thailand. Furthermore, we wish to propose the biogenetic route of Gelsemium alkaloids on the basis of the structures of the alkaloids isolated so far.

1. Structure determination of the alkaloids from the roots, seeds, and leaves of Gelsemium elegans Benth. native to Thailand.

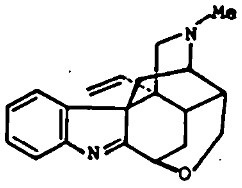
From the methanol extracts of the roots of G. elegans, collected in Thailand, three new alkaloids, 16-epi-voacarpine (14), 19-hydroxydihydrogelsevirine (17), and 19-(Z)-taberpsychine (20), along with eight known bases, i.e. gelsemine (1),[4)5)] gelsevirine (2),[6)7)] koumine (3),[7)8)] gelsenicine (4),[9)] 14-hydroxygelsenicine (5),[10)] humantenine (7),[11)] "akuammidine", and koumidine(10)



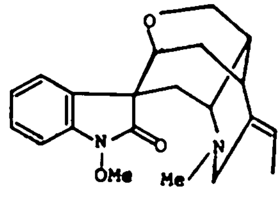
R=H : Gelsemine (1)
R=OMe : Gelsevirine (2)



R=H : Gelsenicine (4)
R=OH : 14-Hydroxygelsenicine (5)
R=OH (4,20-dihydro) : 14-Hydroxygelsedine (6)



Koumine (3)

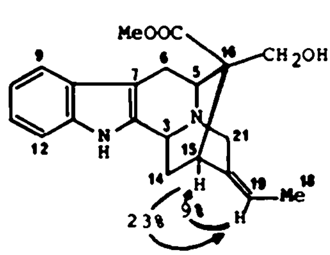


Humantenine (7)

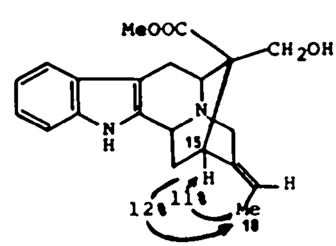
were isolated. The structures of the last two compounds of them, "akuammidine" and koumidine, previously isolated from G. elegans, were revised. Investigation of the seeds of G. elegans resulted in the isolation of 14-hydroxygelsedine (6).[12] Interestingly, highly oxidized new alkaloids, koumine N-oxide (23), gelsemine N-oxide (25), and 19-oxo-gelsenicine (27), were found from the leaves of G. elegans.

19-(Z)-Akuammidine (8)

This indole alkaloid, mp.240-242°C, was previously isolated from the same plants by Chinese group[4] and was assigned to be akuammidine (9), having 19-(E) ethylidene side chain. The mass spectral fission pattern of the isolated alkaloid parallels that for authentic akuammidine (9).[13][14] However, ^{1}H-NMR and ^{13}C-NMR spectra of the alkaloid exhibited similar but not completely identical to (9). ^{13}C-NMR chemical shifts (Table I) 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" (8) compared with these of authentic akuammidine (9) can be reasonably interpreted in terms of γ-gauche effect due to C-18 on the double bond having Z-configuration. Difference NOE experiment also supported the configuration of the ethylidene side chain of both compounds. Thus, irradiation of C-15H (δ3.24) in (9) led to enhancement (12%) of C(18)-H_3 (δ1.68), indicating 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 (8). We finally determined the structure of (8) by X-ray analysis. (see experimental section) The ORTEP drawing is shown in Fig. 1. The CD spectra of both akuammidines (8),(9) exhibited exactly similar curves (Fig. 2), and therefore 19-(Z)-akuammidine (8) has the same absolute configuration as the common indole alkaloids.



19-(Z)-Akuammidine (8)

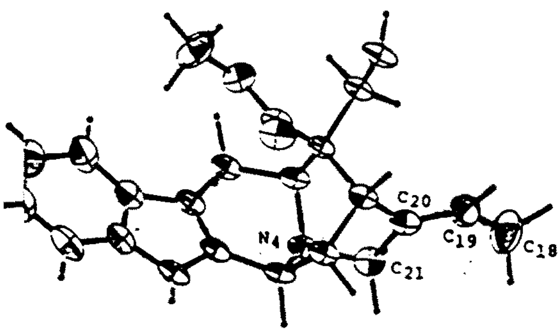


Akuammidine (9)

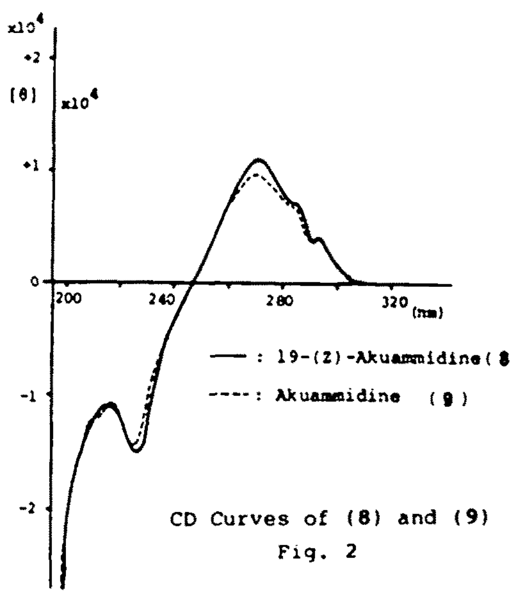
Table I. ^{13}C-NMR Spectral Data of (8), (9), (10), (13), and (14)

No.	(8)	(9)	(10)	(13)	(14)
2	139.1(s)*	139.4(s)*	138.4(s)*	139.0(s)*	137.1(s)*
3	51.5(d)	52.7(d)	51.0(d)**	51.2(d)**	80.5(s)
5	59.5(d)	59.8(d)	54.0(d)**	53.7(d)**	57.5(d)
6	25.1(t)	25.9(t)	23.4(t)	23.5(t)	21.3(t)
7	106.0(s)	106.9(s)	106.0(s)	106.1(s)	107.0(s)
8	128.1(s)	128.8(s)	127.5(s)	122.0(s)	125.7(s)
9	119.8(d)**	120.5(d)**	119.8(d)***	114.8(d)	119.5(d)**
10	118.6(d)**	119.4(d)**	115.3(d)***	109.5(d)	115.7(d)**
11	122.1(d)	122.8(d)	122.1(d)	157.4(s)	122.0(d)
12	112.0(d)	112.8(d)	112.0(d)	96.0(d)	110.9(d)
13	138.9(s)*	139.3(s)*	138.2(s)*	136.9(s)*	136.3(s)*
14	31.4(t)	29.7(t)	29.3(t)	27.8(t)	36.5(t)
15	37.4(d)	31.1(d)	35.1(d)	27.5(d)	33.7(d)
16	53.2(s)	53.5(s)	44.3(d)	43.7(d)	53.2(s)
17	69.1(t)	69.8(t)	61.2(t)	61.1(t)	63.3(t)
18	12.5(q)	14.1(q)	12.6(q)	12.9(q)	12.7(q)
19	118.2(d)**	118.1(d)**	118.7(d)***	119.3(d)	118.5(d)**
20	138.6(s)*	138.8(s)*	142.7(s)*	141.0(s)	135.4(s)*
21	54.0(t)	56.9(t)	54.6(t)	57.0(t)	48.1(t)
<u>C</u>OOMe	174.9(s)	175.6(s)	-	-	175.8(s)
CO<u>OMe</u>	51.5(q)	52.4(q)	-	-	52.1(q)
-OMe	-	-	-	56.1(q)	-

Chemical shifts in ppm downfield from TMS.
Solvent; (8), (9), (10), (13) in CD_3OD. (14) in $CDCl_3$.
*,**,*** Signals may be interchanged within vertical column.



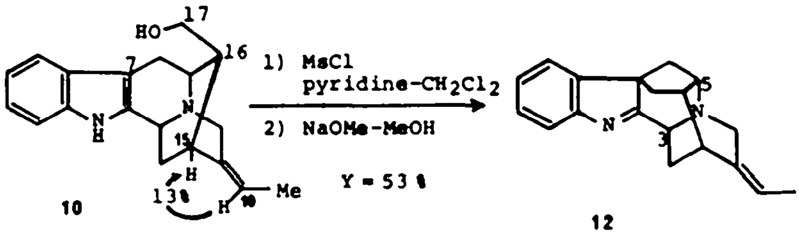
ORTEP Drawing of 19-(Z)-Akuammidine (8)
Fig. 1



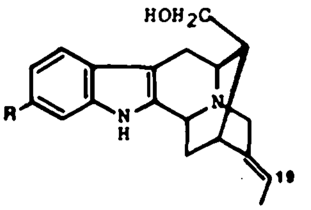
CD Curves of (8) and (9)
Fig. 2

<u>Koumidine (10)</u>

Koumidine, mp. 200-201°C, has also been isolated from <u>G. elegans</u> and the structure was formulated as (11).[4] All the physico- and spectral data of the isolated alkaloid agreed well with those given in literature. In the ^{13}C-NMR spectra (see Table I), the signal due to C-15 of koumidine was observed at downfield (7.6 ppm) and, on the contrary, that of C-21 was observed at upfield (2.4 ppm) than the corresponding signals of gardnerine (13), having (E)-ethylidene side chain. Irradiation of C(19)-H (δ5.36) enhanced C(15)-H (δ2.44) with 13% NOE. From these data we revised the configuration of ethylidene side chain in koumidine to be (Z)-form.[15] Koumidine (10) gave a ring-closed indolenine derivative (12) by mesylation of the hydroxy group at C(17) and subsequent treatment with NaOMe, that made sure the configuration at C(16).[16]



10 12

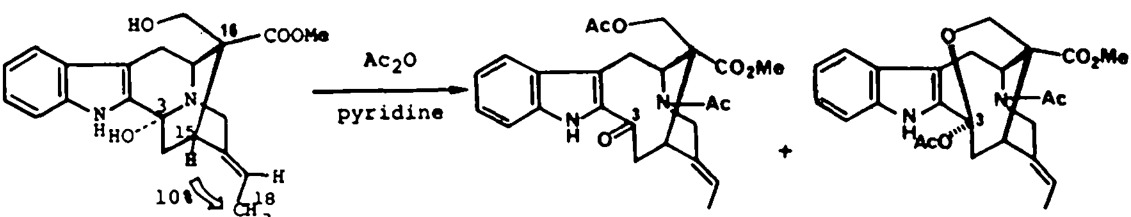


R=H : Published Koumidine (11)

R=OMe : Gardnerine (13)

16-epi-Voacarpine (14)

The new base, 16-epi-voacarpine (14), was obtained as colorless prisms, mp. 162-165°C, $C_{21}H_{24}N_2O_4$. The mass spectrum of (14) presents molecular ion m/z 368 which is 16 a.m.u. higher than the corresponding peak in the spectrum of akuammidine (9). And an exactly similar cleavage patter is observed with voacarpine[14]. On acetylation, (14) gave rise to two products, (15) and (16). The formation of (15), which exhibited a typical 2-acyl indole UV absorption at 314 nm, demonstrated the presence of a hydroxy group at C-3 in (14). The configuration at C-16 was determined by the following two facts: i) The formation of intramolecular hemiacetal-acetate (16) on acetylation of (14). ii) In the NMR spectrum of (15), the signal of acetoxy group was shielded (δ1.45, 3H, s.) by indole nucleus. 10% enhancement observed in difference NOE experiment between C-15H (δ3.12) and C-18 methyl group (δ1.55) indicated the (E)-form ethylidene side chain in (14).



14 15 16

19-Hydroxydihydrogelsevirine (17)

The new alkaloid (17) was isolated as an amorphous solid, whose 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)-OMe oxindole nucleus (λ_{max}; 281(sh), 255, 209 nm). ^{1}H-NMR spectrum showed the characteristic signals due to N(a)-OMe (δ3.99, s.), -O-$C_{(3)}$-H (δ3.84, br-s.), -OCH_2- (δ4.09, dd, J=11.2, 2.3Hz and δ3.91, dd, J=11.2, 2.0Hz), and N(b)-Me (δ2.28, s). Furthermore, the presence of a secondary hydroxy group was deduced by the signals at δ5.13 (q, J=6.6Hz) and δ1.09 (3H, d, J=6.6Hz) in place of a vinyl group in gelsevirine (2). In the ^{13}C-NMR spectrum (Table II), which was very similar to that of gelsevirine (2), the appearance of a new doublet at δ64.3 ppm and a new quartet at δ19.4 ppm, the absence of vinyl carbons (C_{18}, C_{19}) in gelsevirine (2), 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 (17) proposed by spectroscopic analysis, we attempted the preparation of (17) from gelsevirine (2). Gelsevirine N-oxide, prepared by the MCPBA oxidation of (2), was subjected to Wacker oxidation ($PdCl_2$, O_2, DMF-H_2O)[17] to produce 19-keto derivative, which was further converted to compound (18) by the reduction of N-oxide with $NaHSO_3$. Ketone (18) was also obtained from the new alkaloid (17) by means of Swern oxidation. Reduction of ketone (18) with $NaBH_4$ gave a diastereomeric alcohol (19) as the major product, accompanied with trace amounts of (17). This stereospecific reduction enables us to assume the stereochemistry of the isomeric alcohol (19). Thus, in the transition state, ketone derivative (18) may take a conformation A, (more stable than the other conformer B, C and D) as depicted in Fig. 3, 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 (19). Therefore. the secondary hydroxy group on C-19 in (17) takes (R) configuration.

Table II. ^{13}C-NMR Spectral Data

No.	(17)	(2)
2	174.2(s)	173.0(s)
3	69.1(d)*	69.4(d)
5	71.8(d)*	72.3(d)
6	47.5(d)	51.1(d)*
7	53.3(s)	52.3(s)*
8	127.9(s)**	128.1(s)
9	128.4(d)**	128.2(d)
10	123.0(d)**	122.7(d)
11	128.5(d)**	128.2(d)
12	107.2(d)	107.1(d)
13	139.2(s)	139.5(s)
14	22.6(t)	23.1(t)
15	35.7(d)	36.0(d)
16	38.6(d)	38.0(d)
17	61.6(t)	61.6(t)
18	19.4(q)	112.9(t)
19	64.3(d)	138.3(d)*
20	56.8(s)	54.1(s)*
21	58.8(t)	66.3(t)
N-Me	40.7(q)	40.6(q)
-OMe	63.3(q)	63.1(q)

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

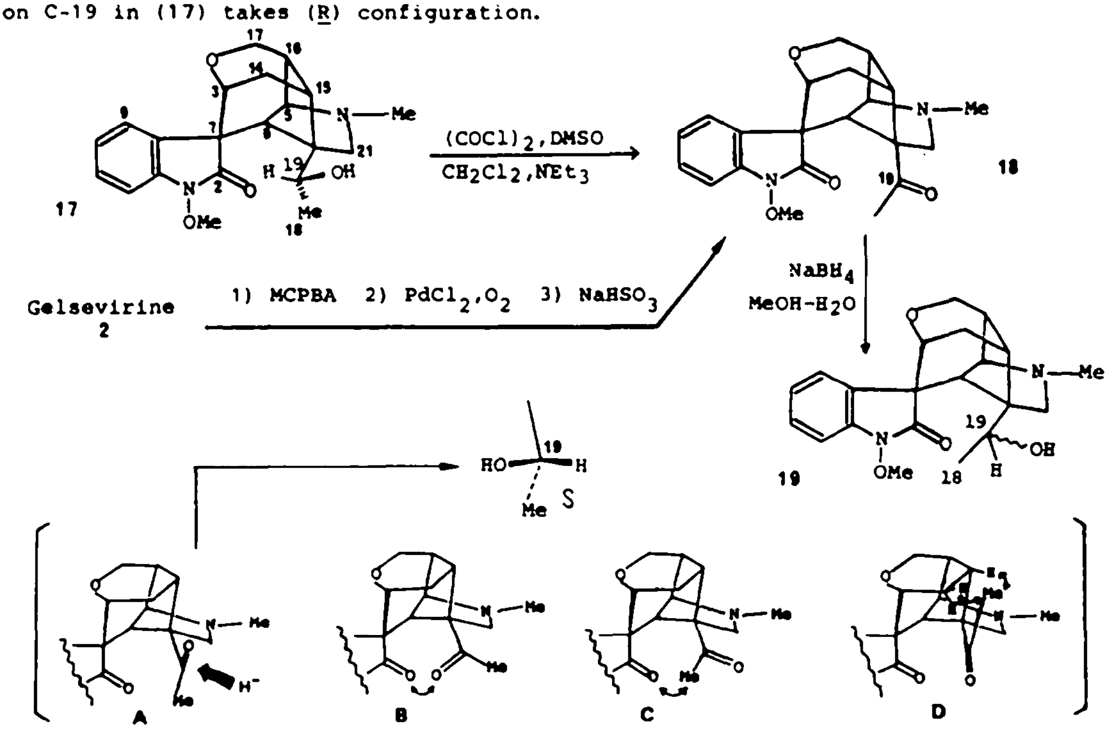
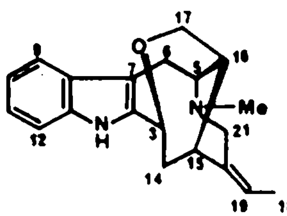


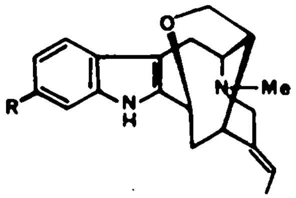
Fig. 3

19-(Z)-Taberpsychine (20)

The new minor alkaloid (20) was obtained as 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 (21),[18] but ^{1}H-NMR spectrum of (20) is not completely identical to that of (21), probably owing to the difference of the configuration of the ethylidene side chain. In the ^{1}H-NMR spectrum, NOE was observed between C_{18}-H and C_{15}-H, suggesting that the configuration of the side chain was (Z)-form. Furthermore, as in the case of akuammidine (9) and 19-(E)-akuammidine (8), we compared ^{13}C-NMR spectra of (20) and appropriate model compound (22),[19] prepared from gardnerine (13).(Table III) The signal due to C-15 of (20) was observed at downfield (6.9ppm) and, on the contrary, that of C-21 was observed at upfield (6.9ppm) than the corresponding signal of (22). From these data, we concluded the structure of this new base as 19-(Z)-taberpsychine.



(20) 19-(Z)-Taberpsychine



R=H : Taberpsychine (21)
R=OMe : (22)

Table III. ^{13}C-NMR Spectral Data

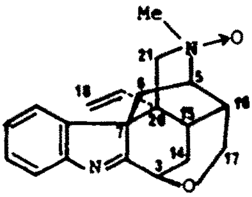
No.	(20)	(22)
2	136.2(s)*	136.5(s)*
3	67.6(d)	67.5(d)
5	60.5(d)	60.6(d)
6	18.0(t)	17.9(t)
7	110.9(s)	111.0(s)
8	128.3(s)	122.8(s)
9	119.8(d)**	119.7(d)**
10	119.3(d)**	109.7(d)
11	122.3(d)**	156.7(s)
12	110.9(d)	94.6(d)
13	135.3(s)*	136.1(s)*
14	29.7(t)	28.0(t)
15	33.5(d)	26.6(d)
16	37.5(d)	37.0(d)
17	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 (23)

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 (0.96ppm) compared with that of koumine (3). MCPBA oxidation of koumine (3) afforded two diastereomeric N(b)-oxides, one of which was identical with natural N-oxide (23). The configuration on N(b) atom was initially deduced by the analysis of ^{1}H-NMR spectra. (See Table V) Thus, in natural N-oxide (23) the signals of C_{15}-H and C_{16}-H are observed at downfield (0.38ppm and 1.30ppm, respectively) than those of koumine (3). While C_6-α-H in the diastereomeric N-oxide (24) is deshielded to downfield compared with that of koumine (3). These phenomena may be attributable to anisotropy of N→O function. Therefore, the configuration on N(b) should be (S) in (23) and (R) in (24) respectively, as depicted in Fig. 5 . Unnatural N-oxide (24) gave the crystal (mp.

214-216°C) suitable for X-ray analysis. The results obtained from X-ray analysis (Fig. 4) (see also experimental section) agreed with the conclusion obtained from ^{1}H-NMR analysis.

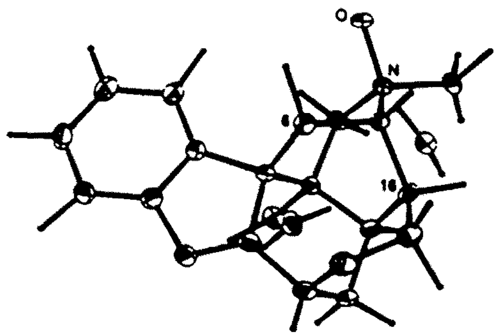


23

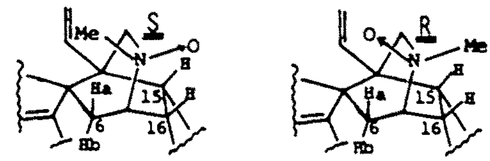
Koumine (3) $\xrightarrow{\text{MCPBA}}$ (23)+(24)

Table V. (δ:ppm)

	Koumine N-oxides (23)	Koumine N-oxides (24)	Koumine (3)
C_{15}-H	2.72	2.23	2.34
C_{16}-H	4.10	2.96	2.8
C_6 -Ha	2.94	3.62	2.34 or 2.41



ORTEP Drawing of 24 Fig.



23 24

Fig. 5

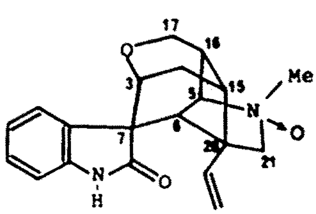
Gelsemine N-oxide (25)

Spectral data indicated that this new alkaloid obtained from the leaves was closely related to gelsemine (1). 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, (25) was obtained by the MCPBA oxidation of gelsemine (1) together with the diastereomeric isomer (26). The configuration on N(b) atom was determined applying the procedure used in the structure elucidation of Koumine N-oxide (23). In the ^{1}H-NMR spectra, C_6-H in the natural N-oxide (25) and C_{16}-H in its diastereomeric isomer (26) are remarkably shifted to downfield (1.43ppm and 1.96ppm), respectively. (See Table VI) Having the steric structure as shown in

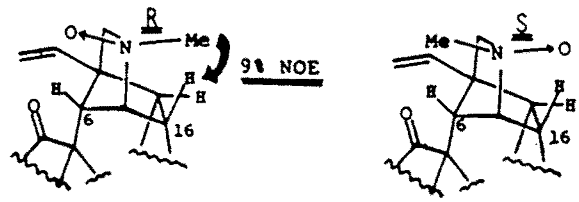
Gelsemine (1) $\xrightarrow{\text{MCPBA}}$ (25)+(26)

Table VI. , (:ppm)

	Gelsemine N-oxides (25)	Gelsemine N-oxides (26)	Gelsemine (1)
C_6 -H	3.41	2.28	1.98
C_{16}-H	2.59	4.26	2.30



Gelsemine N-oxide 25



25 26

Fig. 6

Fig. 6, 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)-Me group enhanced C_{16}-H (δ2.59) with 9% NOE. Therefore, the configuration of N(b) should be (R) (Fig. 6).

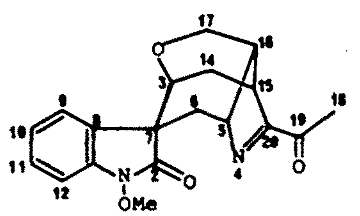
<u>19-Oxo-gelsenicine (27)</u>

The new alkaloid (27) 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 δ2.66 due to methyl group adjacent to carbonyl function. The ^{13}C-NMR spectrum (Table IV) was similar to that of gelsenicine (4) except for the signals of C-18 and C-19, which were shifted downfield to δ26.1 (16.1ppm) andδ197.6 (172ppm), suggesting the presence of ketonic group on C-19 in gelsenicine (4). We finally determined the structure of (27) by X-ray analysis, (see experimental section), as shown in Fig. 7. The CD spectra of (27) and (4) gave the similar curves (See Fig. 8) and therefore 19-oxo-gelsenicine has the same absolute configuration with (4).

Table IV. ^{13}C-NMR Spectral Data

No.	(4)	(27)
2	172.1(s)	171.1(s)
3	74.9(d)*	75.2(d)*
5	72.5(d)*	74.5(d)*
6	37.7(t)	38.0(t)
7	55.8(s)	56.3(s)
8	132.3(s)	131.8(s)
9	124.6(d)	124.5(d)
10	123.3(d)	123.5(d)
11	128.0(d)	128.3(d)
12	106.5(d)	106.7(d)
13	138.0(s)	138.0(s)
14	25.6(t)	27.5(t)
15	39.8(d)**	38.0(d)**
16	42.5(d)**	39.4(d)**
17	62.1(t)	61.7(t)
18	10.0(q)	26.1(q)
19	25.6(t)	197.6(s)
20	184.1(s)	178.1(s)
-OMe	63.3(q)	63.4(q)

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


27

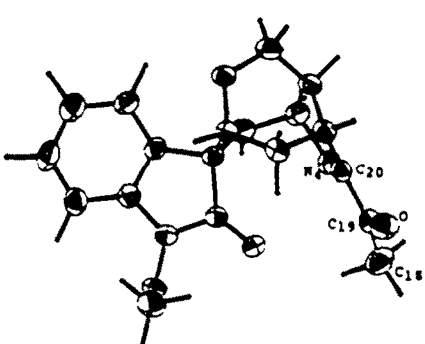
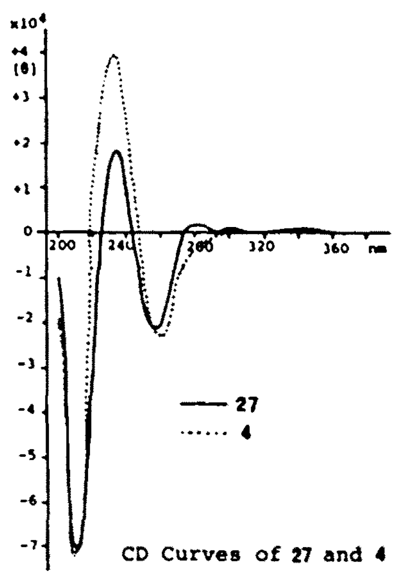


Fig. 7 ORTEP Drawing of 27



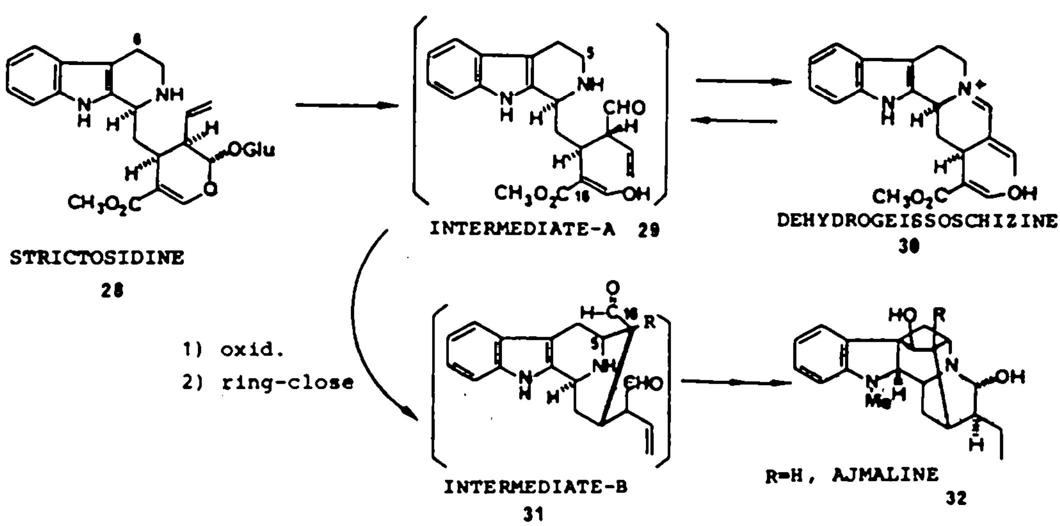
CD Curves of 27 and 4
Fig. 8

2. Proposal of biogenetic route of Gelsemium alkaloids

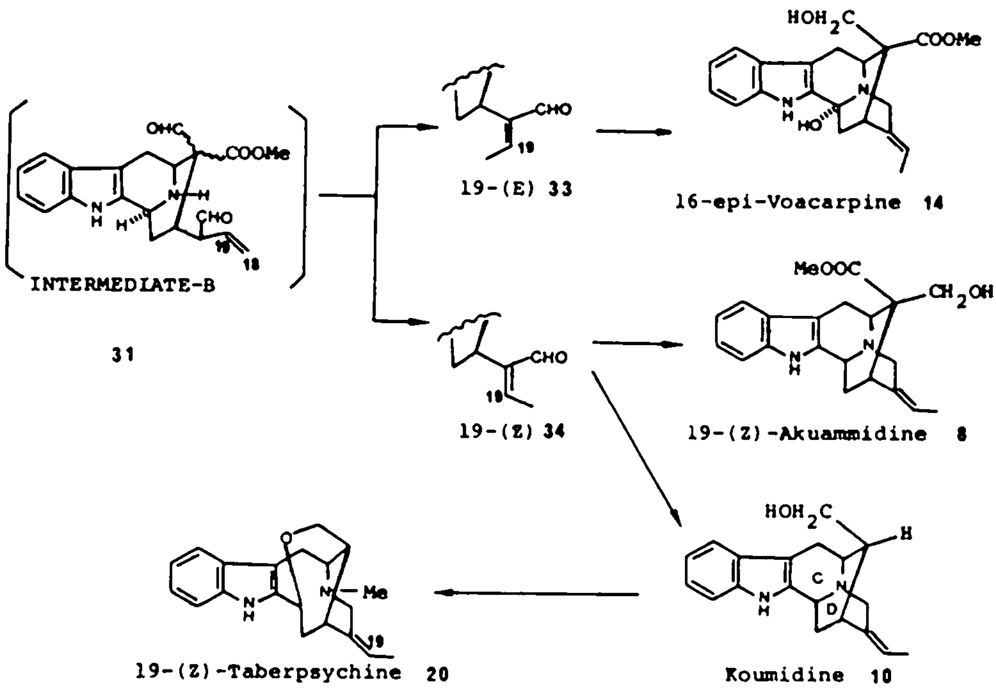
It is well recognized that monoterpenoid indole alkaloids are biosynthesized through the biological transformations of strictosidine (28) which was derived from the condensation of tryptamine with secologanin. M. H. Zenk et al. demonstrated the incorporation of [6-^{14}C]strictosidine (28') into gelsemine (1) in

<u>Gelsemium</u> <u>sempervirens</u>.[20] However, there is no report on the consideration about biosynthesis of gelsemium alkaloids throughout. We would like to propose a new and plausible biosynthetic route of gelsemium alkaloids based on the structure of the compounds isolated so far, including a new alkaloid, 19-(Z)-taberpsychine (20), which is considered to be a key intermediate in the map of biogenetic speculation.[21]

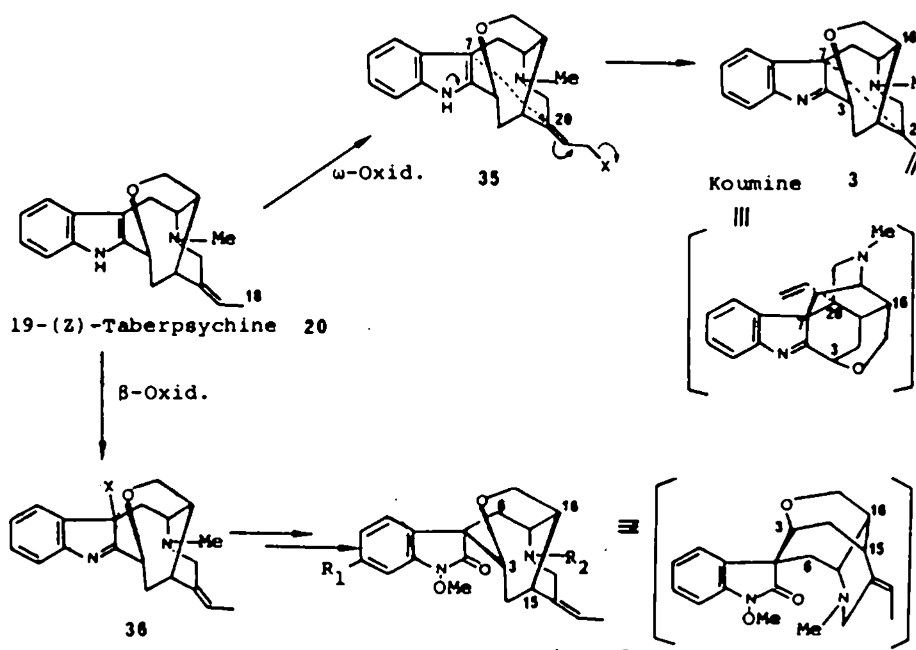
Common intermediate (31) formed from strictosidine (28) by the intramolecular C-C bond formation between C-6 and C-16 will serve as a precursor of sarpagine type indole alkaloids such as koumidine (10), 19-(Z)-akuammidine (8), and 16-epi-voacarpine (14). Koumidine (10) will be metabolized to a C/D ring-opening compound, 19-(Z)-taberpsychine (20). Oxidation on C-18 in (20) and subsequent intramolecular C-C bond formation between C-7 and C-20 will form koumine (3).[22] Very recently, we[3a] and Chinese group[23] independently succeeded the partial synthesis of 18-methoxykoumine and koumine (3) along this biogenetic proposal. β-Oxidation of indole part in (20) will generate indolenine (36), which will further transform into humantenine-type alkaloids, humantenine (7), humantenirine (37),[24] and rankinidine (38),[24] by the rearrangement to oxindole and subsequent N(a)-methoxylation process. After the elimination of HX at C_6-C_7 position in indolenine (36), ene type reaction between C-20 and C-6 will take place to afford indole (40). Through the β-oxidation and successive rearrangement, gelsemine (1) will generate from (40). Further oxidative process will afford gelsevirine (2) and 19-hydroxydihydrogelsevirine (17), in order. It seems that biosynthesis of gelsedine group will branch from the intermediate (31). Thus, (31) will be metabolized to norsarpagine type compound (41), having five membered D-ring, through the release of C_{21}-aldehyde carbon and subsequent ring-closure between N(b) and C-20. (41) will be converted to the C/D ring-opening compound (42), such as 19-(Z)-taberpsychine (20), and then transformed into gelsedine series, (44), (45), (6), (46), (4), (5) and (27), via successive bioconversions. (β-oxidation, rearrangement to oxindole, and oxidation on N(a), N(b), C-14, and/or C-19.)



TENTATIVE BIOSYNTHETIC ROUTE OF GELSEMIUM ALKALOIDS -1-



TENTATIVE BIOSYNTHETIC ROUTE OF GELSEMIUM ALKALOIDS -2-

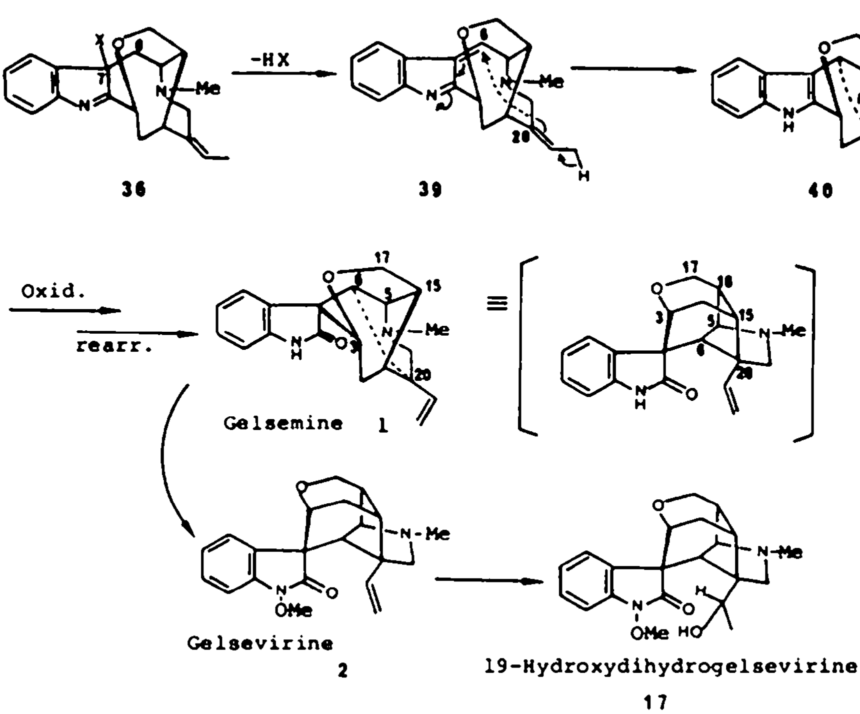


R_1=H, R_2=Me :Humantenine 6

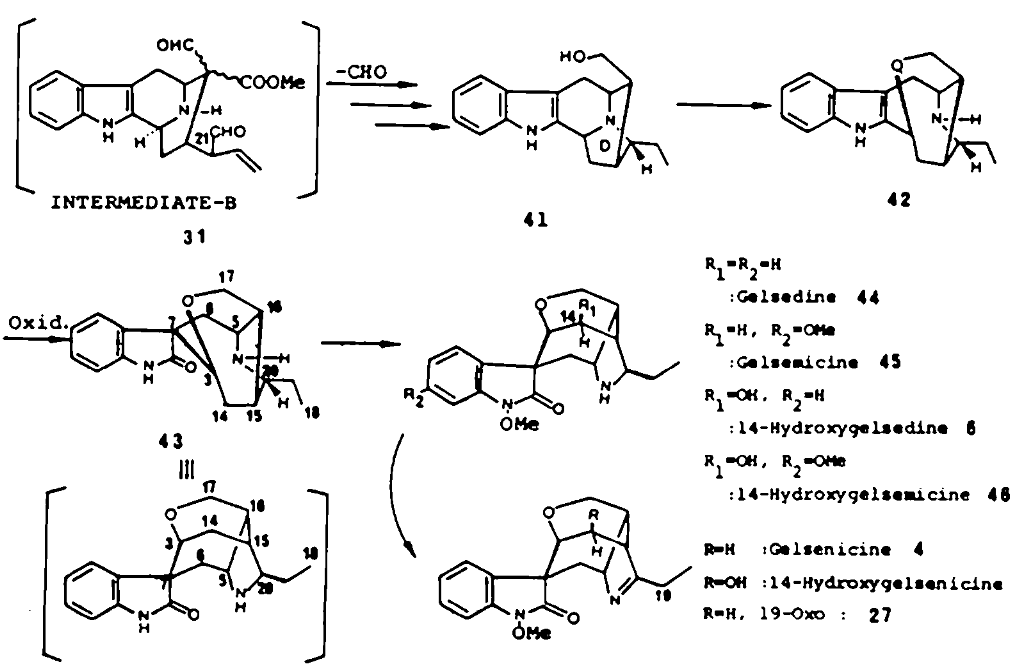
R_1=OMe, R_2=H :Humantenirine 37

R_1=H, R_2=H :Rankinidine 38

TENTATIVE BIOSYNTHETIC ROUTE OF GELSEMIUM ALKALOIDS -3-



TENTATIVE BIOSYNTHETIC ROUTE OF GELSEMIUM ALKALOIDS -4-



Experimental

All melting points were measured on a Yamato MP-21 apparatus and are uncorrected. IR spectra were measured with a Hitachi 260 spectrometer, and UV spectra were measured in MeOH with a Hitachi 340 or Hitachi U3400 spectrometers. ^{1}H-NMR spectra were recorded on JEOL JNM FX-270 and JNM GX-270 (270 MHz) spectrometers with tetramethylsilane as an internal standard in $CDCl_3$ unless otherwise stated. ^{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. Mass spectra were taken with Hitachi RMU-6E and RMU-7M spectrometers. CD spectra were measured with JASCO J-500A and J-20 in MeOH. Thin layer chromatography was performed on Merck precoated Silica gel 60F-254 and Aluminium oxide 60F-254 plates. Column chromatography utilized Merck Silica gel 60 [70-230 and 230-400 mesh (for flash chromatography)], Merck Lober Si60 (for medium pressure column chromatography), and Merck Al_2O_3(activity II-III). Organic solution were dried with anhydrous Na_2SO_4. Abbreviations used are: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), shoulder (sh).

Extraction and Isolation of Alkaloids from the roots of G. elegans

The dried powdered roots (600g) of G. elegans, collected at Phu Laung, Loei Province, Thailand, in January 1985, were extracted with MeOH at room temperature three times (for 4 days, 5 days, and 7 days). The combined MeOH extracts were concentrated in vacuo to afford crude extracts (66g), which were dissolved in 1N-HCl solution and partitioned to ethyl acetate. After the back-extraction of ethyl acetate layer 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 and then evaporated under reduced pressure to give crude base 6.887g. The portion of alkaloidal fraction (6.800g) was roughly separated with Al_2O_3 column chromatography and then purified with silica gel column chromatography, flash column chromatography, medium pressure column chromatography and/or preparative TLC as described below.

Gelsemine (1)

The 50%$CHCl_3$/benzene-$CHCl_3$ eluent from the Al_2O_3 column chromatography was subjected to repeated flash column chromatography using 1%MeOH/$CHCl_3$ sat. with aq.NH_3 as a solvent to afford colorless needles of (1)(369 mg). mp. 176-178°C (acetone). IR(KBr):1715, 1475, 1225, 1095. MS m/z(%):322(M^+, 47), 249(55), 108(100). UV λ_{max}nm:293(sh), 280, 251, 208. ^{1}H-NMR δ:7.86(1H, br-s, NH), 6.26(1H, dd, J=17.8, 11.0Hz, C(19)-H), 5.10(1H, dd, J=11.0, 1.3Hz, C(18)-H), 4.94(1H, dd, J=17.8, 1.3Hz, C(18)-H), 4.11(1H, dd, J=11.1, 2.3Hz, C(17)-H), 3.91(1H, dd, J=11.1, 2.0Hz, C(17)-H), 3.82(1H, br-s, C(3)-H), 2.25(3H, s, N-Me), 2.78 and 2.31(each 1H, d, J=10.2Hz, C(21)-H_2).

Gelsevirine (2)

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 (2)(495 mg), which was obtained as HCl salt, mp. 255-260°C(dec.)(acetone). IR(HCl salt)(KBr):2440, 1735, 1463, 1078. MS m/z(%):352(M^+, 26), 321(63), 309(28), 108(100). UVλ_{max}nm:280(sh), 254, 208. ^{1}H-NMR δ:6.24(1H, dd, J=17.8, 11.0Hz, C(19)-H), 5.13(1H, dd, J=11.0, 1.3Hz, C(18)-

H), 4.97(1H, dd, J=17.8, 1.3Hz, C(18)-H), 4.10(1H, dd, J=10.9, 2.3Hz, C(17)-H), 3.89(1H, dd, J=10.9, 2.0Hz, C(17)-H), 3.96(3H, s, OMe), 3.81(1H, br-s, C(3)-H), 2.24(3H, s, N-Me), 2.78 and 2.31(each 1H, d, J=10.3Hz, C(21)-H_2).

Koumine (3)

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 (3)(574 mg). mp. 168-169°C (acetone). IR(KBr):1450, 1215, 1085, 1070. MS m/z(%):304(M^+, 100), 224(28), 71(51). UV λ_{max}nm:296(sh), 262, 230(sh), 221, 215(sh). ^{1}H-NMR δ:5.01(1H, br-s, C(3)-H), 4.83(1H, dd, J=16.8, 2.0Hz, C(18)-H), 4.78(1H, dd, J=10.9, 2.0Hz, C(18)-H), 4.67(1H, dd, J=16.8, 10.9Hz, C(19)-H), 4.25(1H, dd, J=11.9, 4.3Hz, C(17)-H), 3.61(1H, d, J=11.9Hz, C(17)-H), 3.08 and 3.18(each 1H, d, J=11.4Hz, C(21)-H_2), 2.61(3H, s, N-Me).

Gelsenicine (4)

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 afford colorless plates or needles of (4)(331 mg). mp. 168-170°C (Et_2O). IR(KBr):1730, 1465, 1220, 1110, 1018. MS m/z(%):326(M^+, 53), 294(100), 150(74). UV λ_{max}nm:281, 257, 208. ^{1}H-NMR δ:4.41(1H, m, C(5)-H), 4.31(1H, dd, J=11.2, 3.0Hz, C(17)-H), 4.26(1H, dd, J=11.2, 1.0Hz, C(17)-H), 3.95(3H, s, OMe), 3.72(1H, dd, J=4.5, 2.3Hz, C(3)-H), 2.86(1H, dd, J=9.9, 9.2Hz, C(15)-H), 2.37(1H, dd, J=14.8, 2.3Hz, C(14)-H), 2.10(1H, ddd, J=14.8, 10.1, 4.7Hz, C(14)-H), 2.72 and 2.41(each 1H, dq, J=17.1, 7.6Hz, C(19)-H_2), 1.29(3H, t, J=7.3Hz, C(18)-H_3). CD(c=1.0x10^{-2}, MeOH, 23°C): $[\theta]_{314}$0, $[\theta]_{262}$-24200, $[\theta]_{248.5}$0, $[\theta]_{234}$+39300, $[\theta]_{221}$0, $[\theta]_{205}$-45800.

14-Hydroxygelsenicine (5)

The 50%benzene/$CHCl_3$-$CHCl_3$ eluent from Al_2O_3 chromatography was purified by medium pressure column chromatography using 10%MeOH/$CHCl_3$ to yield an amorphous solid of (5)(169 mg). IR(KBr):3400, 1715, 1470, 1045, 1015. MS m/z(%): 342(M^+, 83), 312(45), 311(100), 108(34). UV λ_{max}nm:281(sh), 265(sh), 258, 209. ^{1}H-NMR δ:4.44(1H, d, J=3Hz, C(14)-H), 4.44(1H, dd, J=10.9, 3.6Hz, C(17)-H), 4.31(1H, br-d, J=10.9Hz, C(17)-H), 4.41(1H, m, C(5)-H), 3.93(3H, s, OMe), 3.76(1H, br-s, C(3)-H), 2.73 and 2.79(each 1H, q, J=7.3HZ, C(19)-H_2), 1.28(1H, t, J=7.3Hz, C(18)-H_3).

Humantenine (7)

The benzene-50%$CHCl_3$/benzene eluent from Al_2O_3 chromatography was subjected to medium pressure column chromatography using 10%MeOH/$CHCl_3$ as a solvent to afford an amorphous solid of (7)(354 mg). HCl salt: mp. 202-205°C (dec.)(acetone). IR(HCl salt)(KBr):1715, 1620, 1470, 1430, 1218, 1210, 765. MS m/z(%): 344(M^+, 65), 323(100), 122(86). ^{1}H-NMR δ:5.38(1H, br-q, J=6.7Hz, C(19)-H), 4.20(1H, d, J=10.9Hz, C(17)-H), 4.06(1H, dd, J=10.9, 4.9Hz, C(17)-H), 4.07(3H, s, OMe), 3.63(1H, br-d, J=6.9Hz, C(3)-H), 3.41 and 3.33(each 1H, d, J=15Hz, C(21)-H_2), 2.25(3H, s, N-Me), 1.65(3H, br-d, J=6.7Hz, C(18)-H_3).

19-(Z)-Akuammidine (8)

The $CHCl_3$-AcOEt eluent from Al_2O_3 column chromatography was purified with flash column chromatography (5%MeOH/$CHCl_3$) and then with SiO_2 chromatography (1%MeOH/$CHCl_3$ sat. with aq. NH_3) to give colorless needles of (8)(35 mg). mp. 240-242°C (acetone). IR(KBr):3275, 1720, 1460, 1225. MS m/z(%): 352(M^+, 100),351(53), 321(48), 293(22), 249(60), 169(66), 168(41). UV λ_{max}nm: 290, 280, 274(sh), 226. $[\alpha]_D^{16}$ +9°(c=0.16, MeOH). CD(c=1.0x10^{-2}, MeOH, 22°C):$[\theta]_{310}$0, $[\theta]_{293}$+4100, $[\theta]_{291}$+3700, $[\theta]_{285}$+7000, $[\theta]_{271}$+11000, $[\theta]_{247}$0, $[\theta]_{226}$-15000, $[\theta]_{215}$-10900. ^{1}H-NMR(CD_3OD) δ:5.44(1H, m, C(19)-H), 4.20(1H, br-d, J=8.3Hz, C(3)-H), 3.74 and 3.69(each 1H, d, J=10.2Hz, C(17)-H_2), 3.63 and 3.57(each 1H, br-d, J=13.5Hz, C(21)-H_2), 2.96(3H, s, COOMe), 2.75(1H, dd, J=4.1, 1.3Hz, C(15)-H), 1.62(3H, br-d, J=6.9Hz, C(18)-H_3).

The crystal of (8) 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 $3 \leq 2\theta \leq 120°$, 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 least-squares method[25] to an R value of 0.048, using the 1886 reflections for which F(0)>3σ(Fo).[26]

Koumidine (10)

The $CHCl_3$-AcOEt eluent from Al_2O_3 column chromatography was purified successively by flash column chromatography (10%MeOH/$CHCl_3$) and by SiO_2 column chromatography (1%MeOH/$CHCl_3$ sat. with aq. NH_3) to yield colorless needles of (10)(47 mg). mp. 200-201°C (acetone). IR(KBr):3220, 1450, 1035. MS m/z(%):294(M^+, 100), 293(87), 263(41), 249(9), 182(13), 169(88), 168(57). UV λ_{max}nm:289, 279, 273(sh), 225. ^{1}H-NMR (CD_3OD) δ: 5.36(1H, m, C(19)-H), 4.11(1H, dd, J=9.6, 4.0Hz, C(3)-H), 3.75 and 3.26(each 1H, br-d, J=15.5Hz, C(21)-H_2), 3.51(1H, dd, J=10.9, 6.6Hz, C(17)-H), 3.15(1H, dd, J=10.6, 8.9Hz, C(17)-H), 2.44(1H, br-dd, J=5.9, 3.0Hz, C(15)-H), 1.60(3H, br-d, J=6.9Hz, C(18)-H_3).

Preparation of indolenine (12) from koumidine (10)

To a solution of koumidine (8 mg) in dry CH_2Cl_2 (0.3 ml) and dry pyridine (0.3 ml) MsCl (4.2 μl) was introduced at 0°C under Ar atmosphere. After 30 min, MsCl (3.2 μl) was added and the mixture was stirred for 1h at room temperature. The solvent was removed and the crude residue was treated with NaOMe/MeOH at room temperature for 1h. The reaction mixture was concentrated under reduced pressure and was extracted with $CHCl_3$. The organic extract was washed with brine, dried and then concentrated. The crude product was purified with medium pressure column chromatography using 3%MeOH/$CHCl_3$ as solvent to afford 4mg (53%) of (12). mp. 158-159 °C (AcOEt/Et_2O). IR(KBr):1587, 1455, 1085, 750. MS m/z(%):276(M^+, 100), 181(24), 168(58). UV λ_{max}nm:259, 226(sh), 219, 213, 208(sh). ^{1}H-NMR δ:5.35(1H, qt, J=6.7, 2.4Hz, C(19)-H), 4.15(1H, br-d, J=9.2Hz, C(3)-H), 3.77(1H, br-d, J=16.8Hz, C(21)-H), 3.46(1H, dt-like, J=16.8, 2Hz, C(21)-H), 2.23(1H, dd, J=12.8, 9.5Hz, C(17)-H), 1.82(1H, ddd, J=12.8, 2.4, 1Hz, C(17)-H).

16-epi-Voacarpine (14)

The 10-20%MeOH/AcOEt eluent from Al_2O_3 column chromatography was subjected to repeated SiO_2 chromatography (5%MeOH/$CHCl_3$, 1%MeOH/$CHCl_3$ sat. with aq.NH_3) to afford colorless prisms or plates of (14)(78 mg). mp. 162-165°C (dec.)(CH_2Cl_2). $[\alpha]_D^{22}$ +42.3°(c=0.20, $CHCl_3$). Anal. Calcd. for $C_{21}H_{26}N_2O_4 \cdot 1/3H_2O$: C;67.36, H;6.64, N;7.48, Found. C;67.25, H;6.49, N;7.42. MS m/z(%):368(M^+, 62), 351(29), 337(28), 265(65), 184(100). UV λ_{max}nm:290, 281, 275(sh), 226. ^{1}H-NMR(CD_3OD) δ:5.26(1H, br-q, J=6.9Hz, C(19)-H), 4.38(1H, br-d, J=5.5Hz, C(5)-H), 3.68(3H, s, COOMe), 3.52(2H, s, C(17)-H_2), 4.16(1H, br-d, J=16.8Hz, C(21)-H), 3.30(1H, br-d, J=17Hz, C(21)-H), 3.20(1H, br-s, C(15)-H), 3.18(1H, dd, J=16.5, 1.7Hz, C(6)-H), 3.09(1H, dd, J=16.5, 5.6Hz, C(6)-H), 2.25(1H, dd, J=14.3, 3.8Hz, C(14)-H), 1.79(1H, dd, J=14.3, 3.8Hz, C(14)-H), 1.63(1H, dt, J=6.9, 1.0Hz, C(18)-H_3).

Acetylation of 16-epi-voacarpine (14)

A mixture of (14)(10 mg), Ac_2O (0.3 ml), and pyridine (0.5 ml) was stirred at room temperature overnight. The reaction mixture was concentrated and the residue was basified with aq.NH_3, then extracted with $CHCl_3$. The organic layer was washed with brine, dried and evaporated to give the residue, which was purified by p-TLC (5%MeOH/$CHCl_3$) to afford 5mg(41%) of (15) and 4mg(33%) Of (16). (15): mp. 138-140 °C (acetone/Et_2O). IR(KBr):3320, 1742, 1645, 1410, 1235. MS m/z(%):452(M^+, 71), 409(100), 309(34), 280(38), 194(43), 178(74), 172(77). UV λ_{max}nm:314, 238, 203. ^{1}H-NMR δ:3.17(1H, dd, J=14.6, 12.5Hz, C(14)-H), 3.48(1H, br-d, J=14.6Hz, C(14)-H), 3.85(1H, br-d, J=12.5Hz, C(15)-H), 4.66(1H, d, J=11.6Hz, C(17)-H), 4.82(1H, d, J=11.9Hz, C(17)-H), 5.25(1H, br-q, J=7.0Hz, C(19)-H), 3.65(3H, s, COOMe), 1.45(3H, s, OAc), 2.12(3H, s, NAc). (16): mp. 143-145°C (AcOEt/n-Hexane). IR(KBr):3400, 1742, 1442, 1360, 1275, 1220, 1095, 1048, 1020. MS m/z(%):452(M^+, 38), 393(87), 351(43), 333(100), 307(32), 247(43). UV λ_{max}nm:291, 282, 275, 224. ^{1}H-NMR δ :2.79(1H, dd, J=15.3, 4.0Hz, C(14)-H), 2.14(1H, dd, J=15.3, 2.1Hz, C(14)-H), 3.18(1H, br-dd, J=4.0, 2.1Hz, C(15)-H), 5.30(1H, br-q, J=6.9Hz, C(19)-H), 3.65(3H, s, COOMe), 1.91(3H, s, OAc), 2.20(3H, s, NAc).

19-Hydroxydihydrogelsevirine (17)

The $CHCl_3$-AcOEt eluent from Al_2O_3 column chromatography was purified successively by flash column chromatography (20%MeOH/$CHCl_3$), SiO_2 chromatography (2%MeOH/$CHCl_3$ sat. with aq.NH_3), and medium pressure column chromatography (10%MeOH/$CHCl_3$) to afford colorless amorphous of (17)(11 mg). $[\alpha]_D^{21}$ +1° (c=0.11, MeOH). IR($CHCl_3$): 3400, 1715, 1620, 1470, 1085. MS m/z(%):370(M^+, 23), 339(100), 325(46), 295(33), 275(28). UV λ_{max}nm:282(sh), 255, 209. High resolution MS: Calcd. for $C_{21}H_{26}N_2O_4$ 370.1890; Found, 370.1889. ^{1}H-NMR δ:5.13(1H, q, J=6.6Hz, C(19)-H), 4.09(1H, dd, J=11.2, 2.3Hz, C(17)-H), 3.91(1H, dd, J=11.2, 2.0Hz, C(17)-H), 3.99(3H, s, OMe), 3.84(1H, br-s, C(3)-H), 3.07 and 2.33(each 1H, d, J=8.9Hz, C(21)-H_2), 2.28(3H, s, N-Me), 2.56(1H, br-dd, J=14.2, 2.6Hz, C(14)-H), 2.04(1H, ddd, J=14.2, 6.0, 2.3Hz, C(14)-H), 1.09(3H, d, J=6.6Hz, C(18)-H_3).

Preparation of (18) from gelsevirine (2)

To a solution of gelsevirine (2)(108 mg) in dry CH_2Cl_2 (5 ml), MCPBA (84 mg) was added and the mixture was stirred at room temperature for 15 min. After addition of aq.NH_3 (7 ml) the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried and evaporated. The residue was chromatographed on Al_2O_3 using 5%MeOH/$CHCl_3$ to give a diastereomeric mixture of gelsevirine N-oxides, 102 mg (95.4%). Suspension of $PdCl_2$ (60 mg) in DMF (1 ml) and H_2O (0.3 ml) was stirred under oxygen atmosphere for 30 min. A solution of gelsevirine N-oxides in DMF (0.5 ml) was added and the reaction mixture was vigorously stirred at 35-40°C. After 6h 5% aq.NH_3 (10 ml) was added to the reaction mixture and the whole was extracted with 5%MeOH/$CHCl_3$. The extract was washed with brine, dried and evaporated. The crude residue was purified by SiO_2 column chromatography. Elution with 20%MeOH/$CHCl_3$ gave two diastereomeric mixture of 19-oxo-gelsevirine N-oxides (48 mg). The N-oxides were dissolved in CH_2Cl_2 (10 ml) and the solution was vigorously stirred with sat.$NaHSO_3$ solution (1 ml). After 30 min. 5% aq.NH_3 solution (6 ml) was added to the reaction mixture and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried and evaporated to give colorless needles of (18), 45 mg (44% from gelsevirine (2)). mp. 225-227°C (acetone). IR(KBr):1726, 1695, 1462, 1350, 1320, 1088, 765. MS m/z(%):368(M^+, 20), 337(66), 326(40), 325(100), 294(55), 264(42), 263(59). UV λ_{max}nm:282(sh), 255, 208. ^{1}H-NMR δ:3.99(3H, s, OMe), 3.96(1H, br-s, C(3)-H), 2.32(3H, s, N-Me), 2.16(3H, s, C(18)-H_3).

Swern oxidation of 19-hydroxydihydrogelsevirine (17)

A solution of dry CH_2Cl_2 (48 μl) and oxalyl chloride (2 μl) was placed in a 10ml two neck flask equipped with a $CaCl_2$ drying tube and a rubber septum under Ar atmosphere. The DMSO (2.4 μl) in dry CH_2Cl_2 (18 μl) was added to the stirred oxalyl chloride solution at -70°C. The reaction mixture was stirred for 10 min, to which 5 mg of (17) in dry CH_2Cl_2 (20 μl) was added over 1 min. Stirring was continued for an additional 30 min. Triethylamine (10 μl) was added and the reaction mixture was allowed to warm to room temperature over 1h. 5% aq.NH_3 solution (3 ml) was then added and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried and evaporated. The crude products were purified by SiO_2 column chromatography (1%MeOH/$CHCl_3$ sat. with aq.NH_3) to give (18)(1 mg), which was identical with 19-oxo-gelsevirine (18) prepared from gelsevirine (2) by the comparison of mixed mp., ^{1}H-NMR, and MS spectra.

$NaBH_4$ reduction of 19-oxo-gelsevirine (18)

19-Oxo-gelsevirine (18) (9 mg) in MeOH (1 ml) was treated with $NaBH_4$ (16 mg) at room temperature for 3h. After addition of H_2O (0.2 ml) and $NaBH_4$ (16 mg), the reaction mixture was stirred at 35-40°C overnight. Ice and acetic acid was added to decompose the excess $NaBH_4$ and the mixture was concentrated. The residue was basified with 5% aq.NH_3 solution and extracted with $CHCl_3$. The extract was washed with brine, dried and concentrated. The residue was purified by SiO_2 column chromatography (2-5%MeOH/$CHCl_3$ sat. with aq.NH_3) to give trace amounts of (17) detectable on TLC and 2mg of (19).[27] MS m/z(%):370(M^+, 2), 355(26), 354(100), 324(37), 323(32), 296(30), 295(95), 251(75), 132(28), 122(31), 121(31), 108(54). UV λ_{max}nm:282(sh), 255, 209. ^{1}H-NMR δ:3.65(1H, br-s, C(3)-H), 3.40(1H, br-s, C(5)-H), 2.55(1H, br-s, C(6)-H), 2.40(br-d, J=8.2Hz, C(16)-H), 3.71(1H, dd, J=11.3, 2.1Hz, C(17)-H), 3.92(1H, dd, J=11.3, 1.8Hz, C(17)-H), 1.09(3H, d, 6.4Hz, C(18)-H_3), 4.30(1H, q, J=6.7Hz, C(19)-H), 2.07 and 3.18(each 1H, d, J=9.8Hz, C(21)-H_2), 3.87(3H, s, OMe), 2.28(3H, s, N-Me).

19-(Z)-Taberpsychine (20)

The 50%benzene/$CHCl_3$-$CHCl_3$ eluent from Al_2O_3 column chromatography was purified successively by flash chromatography (5%MeOH/$CHCl_3$), medium pressure column chromatography (10%MeOH/$CHCl_3$), Al_2O_3 column chromatography (AcOEt), and p-TLC (40%MeOH/AcOEt) to yield 3mg of (20). $[\alpha]_D^{23}$ -180°(c=0.4, $CHCl_3$). 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). High resolution MS: Calcd. for $C_{20}H_{24}N_2O$, 308.1886; Found. 308.1881. UV λ_{max}nm:292, 284, 277(sh), 222. ^{1}H-NMR δ:7.98(1H, s, NH), 5.43(1H, br-q, J=6.7Hz, C(19)-H), 5.12(1H, dd, J=9.8, 1.2Hz, C(3)-H), 3.84(1H, dd, J=11.6, 10.1Hz, C(17)-H), 3.26(1H, d, J=11.6Hz, C(17)-H), 3.15(1H, m, C(5)-H), 2.82(1H, br-td, J=10, 6Hz, C(15)-H), 2.59(3H, s, N-Me), 2.43(1H, dt, J=14.3, 9.8Hz, C(14)-H), 2.12(1H, ddd, J=14.2, 10.7, 1.2Hz, C(14)-H), 1.61(3H, br-d, J=6.7Hz, C(18)-H_3).

Isolation of 14-hydroxygelsedine (6) from the seeds of G. elegans

From the dried powdered seeds (8.746g), 53 mg of crude base was obtained by the same procedure described in the experimental section of the roots. The crude base was subjected to flash column chromatography. The 20%MeOH/$CHCl_3$ eluent was further purified by SiO_2 chromatography using 3%MeOH/$CHCl_3$ sat. with aq. NH_3 as a solvent system to give colorless needles of (6)(6 mg). mp. 214-216°C (acetone). IR(KBr): 3260, 1695, 1620, 1470, 1340. MS m/z(%):344(M^+, 44), 313(51), 168(60), 97(44), 84(100). UV λ_{max}nm:281(sh), 265(sh), 258, 209. ^{1}H-NMR δ:4.44(1H, br-s, C(14)-H), 4.45(1H, dd, J=11.2, 4.3Hz, C(17)-H), 4.23(1H, br-d, J=10.9Hz, C(17)-H), 4.01(3H, s, OMe), 3.64(1H, dt, J=9.2, 3.3Hz, C(5)-H), 3.43(1H, br-s, C(3)-H), 2.51(1H, ddd, J=9.2, 4.7, 4.3Hz, C(16)-H), 1.10(3H, t, J=7.3Hz, C(18)-H_3).

Extraction and isolation of alkaloids from the leaves of G. elegans

The dried coarsely powdered leaves (4.7 kg) collected in October 1985, were moistened with strong ammonium hydroxide solution and allowed to stand overnight. It was then macerated with ethyl acetate (20 L) for three days and filtered. The marc was remacerated with ethyl acetate (15 L) for three days and filtered. The combined filtrate was concentrated to syrupy mass under reduced pressure, mixed with glacial acetic acid (300 ml) then poured into a large volume of warm water to give about 5% acetic acid solution (6 L), well shaken and left to stand overnight. The acidic filtrate was washed with portions of petroleum ether (3 x 400 ml), then made basic (pH 9) with strong solution of ammonium hydroxide and extracted with chloroform (10 x 300 ml). The combined chloroform extract was washed with water, dried over anhydrous sodium sulfate and evaporated to yield crude alkaloids (11.38 g). The portion of alkaloidal extract (5.0 g) was dissolved in chroloform (10 ml) 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/AcOEt, 10% MeOH/AcOEt, 20% MeOH/AcOEt, 30% MeOH/$CHCl_3$, and then with MeOH. The 50% n-hexane/AcOEt eluent was further purified by SiO_2 column chromatography using 50% n-hexane/AcOEt and then by p-TLC ($CHCl_3$/AcOEt) to give 26 mg of 16-epi-voacarpine (14) and 6 mg of 19-oxo-gelsenicine (27). The 10% MeOH/AcOEt eluent was subjected to SiO_2 column chromatography and 11 mg of 19-(Z)-akuammidine (8) was obtained from the fraction of 5% MeOH/AcOEt eluent. The 20% MeOH/AcOEt eluent was subjected to Al_2O_3 column chromatography. From the 40% n-hexane/AcOEt eluent, 159 mg of gelsemine (1) was obtained. The 30% n-hexane/$CHCl_3$ eluent from Al_2O_3 column chromatography was further purified by p-TLC (45% AcOEt/MeOH, triple development) to yield 18 mg of gelsemine N-oxide (25) and 13 mg of koumine N-oxide (23). 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/AcOEt to yield 20 mg of koumine (3).

Koumine N-oxide (23)

(23): mp. 111-113°C (acetone). IR(KBr): 1640, 1587, 1445, 1080. MS m/z(%): 306(M^+-16, 100), 223(25), 120(29), 70(52). UVλ_{max}nm: 260, 226(sh), 220, 215. Anal. Calcd. for $C_{20}H_{22}N_2O_2\cdot 1/4H_2O$: C;73.84, H;6.94, N;8.57, Found. C;73.60, H;6.90, N;8.54. $[\alpha]_D^{19}$ -237°(c=0.14, MeOH). ^{1}H-NMR δ: 5.06(1H, br-s, C(3)-H), 4.93(1H, d, J=11.2Hz, C(18)-H), 4.93(1H, d, J=17.8Hz, C(18)-H), 4.59(1H, dd, J=11.2, 17.8Hz, C(19)-H), 4.38(1H, dd, J=12.5, 5.3Hz, C(17)-H), 3.68(1H, d, J=12.5Hz, C(17)-H), 4.10(1H, m, C(16)-H), 3.57(3H, s, N-Me), 2.94(1H, dd, J=16.2, 4.0Hz, C(6)-H), 2.43(1H, br-d, J=16.2Hz, C(6)-H), 2.72(1H, br-d, J=10Hz, C(15)-H).

MCPBA oxidation of koumine (3)

To a solution of koumine (3) (100 mg) in CH_2Cl_2 (5 ml), MCPBA (62 mg) was added portionwise and the mixture was stirred at room temperature for 15 min. After the addition of aq.NH_3 (10 ml), the whole was extracted with 5%MeOH/$CHCl_3$. The organic layer was washed with brine, dried and evaporated. The residue was purified by SiO_2 column chromatography (2-5%MeOH/$CHCl_3$ sat. with aq.NH_3) to afford 42mg (40%) of (23) and 35mg (33%) of (24). The former was identical with natural N-oxide (23) by the comparison of TLC and ^{1}H-NMR spectra. (24): mp. 214-216°C (acetone). IR($CHCl_3$): 1585, 1450, 1436, 1073. UVλ_{max}nm: 263, 226(sh), 221, 215(sh). Anal. Calcd. for $C_{20}H_{22}N_2O_2 \cdot 2H_2O$: C;67.02, H;7.31, N;7.82, Found. C;67.08, H;7.06, N;7.76. $[\alpha]_D^{19}$ -157°(c=0.14, MeOH). ^{1}H-NMR δ:5.07(1H, br-s, C(3)-H), 4.91(1H, d, J=11.0Hz, C(18)-H), 4.83(1H, d, J=17.8Hz, C(18)-H), 4.65(1H, dd, J=17.8, 11.0Hz, C(19)-H), 4.34(1H, dd, J=12.5, 4.9Hz, C(17)-H), 3.74((1H, d, J=12.5Hz, C(17)-H), 3.68(1H, ddd, J=15.3, 2.1, 1.8Hz, C(6)-H), 2.51(1H, dd, J=15.3, 4.0Hz, C(6)-H), 3.48(3H, s, N-Me), 2.96(1H, br-d, J=11.9Hz, C(16)-H), 2.23(1H, br-d,J=11.9Hz, C(15)-H). Crystal data for (24): orthorhombic, $P2_12_12_1$, a=16.264(28), b=12.814(13), c=8.440(7)Å, Cell volume=1758.79Å^3, Dc=1.218 gcm^{-3}, z=4. Intensity data were measured on an automated diffractometer with CuKα radiation. In total 2093 reflections were measured, of which 1901 were judged significant (F(0)>3σ(Fo)). The structure was solved by the direct method using MULTAN 80 and refined by the block-diagonal least-squares with anisotropic temperature factors for all non-hydrogen atoms and with isotropic ones for all hydrogen atoms. The final R value was 0.078.[26)]

Gelsemine N-oxide (25)

(25):amorphous, IR($CHCl_3$): 1715, 1620, 1475, 1105. UVλ_{max}nm: 284, 252, 208. MS

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