CHAPTER III

EXPERIMENTAL

1. Source and Authentication of Plant Material

The leaves of *Gelsemium elegans* Benth. were collected from Phuu-Luang, Loei, Thailand in October, 1985. The plant was identified by Professor Tem Smitinand, the former Deputy Director-General, Royal Forest Department of Thailand.

2. General Technique

2.1 Thin-layer chromatography

2.1.1 Analytical

Technique : one way, ascending

Adsorbents : silica gel GF_{254} (E. Merck), 30 g/60 ml distilled water

: aluminium oxide G (E. merck), 70 g/85 ml distilled water

Plate size : 10 cm x 20 cm and 20 cm x 20 cm

Layer thickness : 250 μ

Activation : air dried for 15 minutes and then at 110°C for 1 hour.

Solvent system : a) silice gel GF_{254}/acetone

b) silice gel GF_{254}/acetone : methanol (8:2)

c) silica gel GF_{254}/acetone : n-hexane (4:6)

d) silica gel GF_{254}/chloroform : acetone (8:2)

e) silica gel GF_{254}/chloroform : ethyl acetate (6:4)

e) silica gel GF_{254}/chloroform : ethyl acetate (6:4)

f) silica gel GF_{254}/chloroform : methanol (8:2)

g) silica gel GF_{254}/chloroform : methanol (7:3)

h) silica gel GF_{254}/chloroform : methanol (6:4)

i) silica gel GF_{254}/diethyl ether GR

j) silica gel GF_{254}/ethyl acetate : ethanol (8:2)

k) silica gel GF_{254}/ethyl acetate : methanol (9:1)

l) silica gel GF_{254}/ethyl acetate : methanol (8:2)

m) silica gel GF_{254}/ethyl acetate : methanol (1:1)

n) silica gel GF_{254}/ethyl acetate : n-hexane (1:1)

o) aluminium oxide G/benzene : ethyl acetate (1:1)

p) aluminium oxide G/ethyl acetate : n-hexane (6:4)

q) aluminium oxide G/ethyl acetate : n-hexane (1:1)

Distance : 15 cm

Temperature : laboratory temperature ($20-30^{\circ}C$)

Detection : a) ultraviolet light at wavelength 254 nm

b) Dragendorff's spray reagent

Solution A : bismuth subnitrate (850 mg), distilled water (40 ml) and acetic acid (10 ml)

Solution B : potassium iodide (8 g) and distilled water (20 ml)

Solutions A and B, each of 5 ml, were mixed. Then 20 ml of glacial acetic acid and 70 ml of distilled water were added and used as spray reagent. The alkaloids give orange spots as positive test.

c) 0.2 M anhydrous ferric chloride in 35 % w/v perchloric acid spray reagent. Plate, after spraying, was warmed gently with hot air stream from a hair dryer for 15 minutes. The indole and oxindole alkaloids give olive green to grey or yellowish brown and pink to purple spots as positive test, respectively.

2.1.2 Preparative

Technique : one way, ascending

Adsorbents : silica gel G + silica gel GF_{254} (E. Merck) (30 + 30 g)/120 ml distilled water

Plate size : 20 cm x 20 cm

Layer thickness: 500 µ

Activation : air dried for 15 minutes and then at 110°C for 1 hour.

Solvent systems: a) ethyl acetate : methanol (45:55)

b) acetone : n-hexane (4:6)

c) diethyl ether

d) chloroform : ethyl acetate (6:4)

d) chloroform : ethyl acetate (95:5)

Distance : 15 cm

Temperature : laboratory temperature (24°-30°C)

Detection : ultraviolet light at wavelength 254 nm

2.2 Column chromatography

Adsorbents : silica gel 0.040-0.063 mm (E. Merck)

: aluminium oxide active, neutral 0.063-0.200 mm (E. Merck)

Packing : adsorbent packed dry into the column

Addition of alkaloidal material

: alkaloidal material was dissolved in small volume of volatile solvent, mixed with small quantity of adsorbent, air dried and added onto the top of a dry column.

Solvents : a) ethyl acetate : n-hexane (1:1)

b) ethyl acetate : methanol (9:1)

c) ethyl acetate : methanol (8:2)

d) chloroform : methanol (7:3)

e) methanol

f) ethyl acetate : n-hexane (6:4)

g) chloroform : n-hexane (7:3)

h) ethyl acetate : methanol (95:5)

i) ethyl acetate : n-hexane (9:1)

Collection of eluate

: fractions of 50 ml were collected

Examination of eluate

: fractions were examined by thin layer chromatography using Dragendorff's spray reagent.

2.3 Physical constant

Melting points were determineed by Büchi 520 melting point apparatus. The values recorded are uncorrected.

2.4 Spectroscopy

2.4.1 Ultraviolet absorption spectra were obtained with a Shimadzu Double-beam Spectrophotometer.

2.4.2 Infrared absorption spectra were obtained with a Hitachi 260 Spectrophotometer. The materials were examined in potassium bromide disc.

2.4.3 Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a JEOL FX-270 (270 MHz) Spectrometer in deuterochloroform using tetramethylsilane (T.M.S.) as internal reference.

2.4.4 ^{13}C-nuclear magnetic resonance (^{13}C-NMR) were obtained with a JEOL FX-270 (67.80 MHz) Spectrometer in deuterochloroform using tetramethylsilane (T.M.S.) as internal reference.

2.4.5 Mass spectra were determined on a Hitachi RMU-60 mass spectrometry for EIMS. Operating at 70 eV with inlet temperature 150-240°C.

2.5 Authentic alkaloids

a. gelsemine

b. gelsenicine

c. 14β-hydroxygelsenicine

d. koumine

e. gelsevirine

f. koumidine

g. humantenine

All authentic alkaloids were obtained from the stems of *Gelsemium elegans* Benth. (S. Sakai, E. Yamanaka, M. Kitajima, M. Yokota, N. Aimi, S. Wongseripipatana and D. Ponglux, in press).

3. The Extraction and Isolation of Alkaloids from the Leaves of *Gelsemium elegans* Benth.

3.1 The extraction of alkaloids

The dried coarsely powdered leaves (4.7 kg) 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 distilled 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 alkaline (pH 9) with strong solution of ammonium hydroxide and extracted with chloroform (10 x 300 ml). The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield dry crude ethyl acetate alkaloidal extract (11.38 g).

The marc after macerated with ethyl acetate, had been macerated with portions of methanol (3 x 15 L) and filtered. The combined methanolic filtrate was treated in the same manner as those of crude ethyl acetate alkaloidal extract to yield dry crude methanolic alkaloidal extract (10.03 g).

Thin layer chromatograms of these crude alkaloidal extracts, shown in Figures 23-24, pp. 117-118, indicated that at least 9 alkaloids were present with the addition of base-line alkaloid(s).

3.2 The isolation of alkaloids

Crude ethyl acetate alkaloidal extract (Ce), 5.0 g, was dissolved in chloroform (10ml) and mixed with small amount of silica gel. The content was air dried and packed onto the top of dry silica gel column (8 cm x 44 cm). The column was eluted with ethyl acetate : n-hexane (1:1), ethyl acetate : methanol (9:1), ethyl acetate : methanol (8:2), chloroform : methanol (7:3) and then washed with methanol until no traces of alkaloid could be detected. Fractions of 50 ml were collected and compared by TLC. The eluting solvents were altered to more polar solvent systems when the difference on alkaloidal patterns on TLC were observed. The mentioned solvent systems afforded 10, 40, 80 and 35 fractions, respectively. Those fractions of similar pattern were combined and evaporated to dryness under reduced pressure. By this procedure:

a. fractions 1-11 were combined and designated as Fraction D_1 (0.274 g)

b. fractions 12-20 were combined and designated as Fraction D_2 (0.949 g)

c. fractions 21-34 were combined and designated as Fraction D_3 (1.533 g)

d. fractions 35-40 were combined and designated as Fraction D_4 (0.275 g)

e. fractions 41-68 were combined and designated as Fraction D_5 (0.955 g)

f. fractions 69-77 were combined and designated as Fraction D_6 (0.235 g)

g. fractions 78-115 were combined and designated as Fraction D_7 (0.453 g)

h. fractions 116-143 containing no alkaloid

i. fractions 144-165 were combined and designated as Fraction D_8 (0.131 g)

j. methanolic fractions were combined and designated as Fraction D_9 (0.389 g)

TLC of Fractions D_1-D_9 are shown in Figures 25-26 pp. 119-120.

3.2.1 Isolation of alkaloids from the Fraction D_1

The Fraction D_1 was shown by TLC to contain at least three alkaloids (Figures 27-32, pp. 121-126). It was dissolved in chloroform (3 ml), mixed with small amount of silica gel, air dried, and packed onto the top of dry silica gel column (2.5 cm x 44 cm). The column was eluted with ethyl acetate : n-hexane (1:1) and ethyl acetate : n-hexane (9:1). Ten ml fractions were collected, until no traces of alkaloids could be detected. The volumes of eluting solvents used were 200 ml and 150 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions:-

a. fractions 1-3 containing mixture of alkaloids (63 mg)

b. fractions 4-6 containing one indole alkaloid and traces of other alkaloids. The indole alkaloid was separated out by preparative TLC

plates using chloroform : ethyl acetate (6:4) as developing solvent (double development). It was designated as GE-1 (26 mg, 0.52 % of Ce) and subsequently characterized as 16-epi-voacarpine.

c. fraction 7 (21 mg) containing one oxindole alkaloid and traces of other alkaloids. The oxindole alkaloid was obtained by using preparative TLC plates with chloroform : ethyl acetate (95:5) as developing solvent (double development). It was designated as GE-2 (6 mg, 0.12 % of Ce) and subsequently characterized as 19-oxogelsenicine.

d. fractions 8-35 containing traces of alkaloidal mixture.

3.2.2 Isolation of alkaloids from the Fraction D_2

The Fraction D_2 was shown by TLC to contain mixture of at least four alkaloids (Figures 27-32, pp. 121-126). It was dissolved in chloroform (5 ml), mixed with small amount of silica gel, air dried, and packed onto the top of dry silica gel column (3.5 cm x 44 cm). The column was eluted with ethyl acetate : mathanol (95:5) and ethyl acetate : methanol (9:1). Twenty ml fractions were collected, until no traces of alkaloids could be detected. The volumes of eluting solvents were 600 ml and 200 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions:-

a. fractions 2-5 containing traces of alkaloidal mixture

b. fractions 6-11 (30 mg) containing one oxindole alkaloid and traces of other alkaloids. The oxindole alkaloid was separated out by preparative TLC plates using acetone : n-hexane (4:6) as developing solvent (triple development). It was designated as GE-3 (8 mg, 0.16 % of Ce).

c. fractions 12-14 containing traces of alkaloidal mixture

d. fractions 15-22 (119 mg) containing one oxindole alkaloid and traces of other alkaloids. The oxindole alkaloid was separated out by preparative TLC plates using diethyl ether as developing solvent (double development). It was designated as GE-4 (37 mg, 0.74 % of Ce).

e. fractions 23-28 containing one indole alkaloid. White needle crystals (11 mg, 0.22 % of Ce) were crystallized out upon the addition of small volume of methanol. It was designated as GE-5 and subsequently characterized as 19-(Z)-akuammidine.

f. fractions 29-40 containing traces of alkaloidal mixture.

3.2.3 Isolation of alkaloid from the Fraction D_5

The Fraction D_5 contains one oxindole alkaloid and traces of other alkaloids (Figures 33-34, pp. 127-128). It was dissolved in chloroform (5 ml), mixed with small amount of aluminium oxide, air dried,

and packed onto the top of dry aluminium oxide column (3.5 cm x 44 cm). The column was eluted with ethyl acetate : n-hexane (6:4) and chloroform : n-hexane (7:3). Twenty ml fractions were collected and compared by TLC. The volumes of eluting solvent systems were 600 ml and 300 ml, respectively. Those fractions of similar pattern were combined and evaporated to dryness under reduced pressure yielding:-

a. fractions 1-28 containing one oxindole alkaloid. White prism crystals (159 mg, 3.18 % of Ce) were crystallized out upon the addition of small volume of acetone, designated as GE-6 which was subsequently identified as gelsemine.

b. fractions 29-43 containing traces of alkaloidal mixture.

3.2.4 Isolation of alkaloid from the Fraction D_7

The Fraction D_7 was shown by TLC to contain one indole alkaloid and traces of other alkaloids (Figures 33-34, pp. 127-128). It was dissolved in chloroform (3 ml), mixed with small amount of aluminium oxide, air dried, and packed onto the top of dry aluminium oxide column (2.5 cm x 44 cm). The column was eluted with ethyl acetate : n-hexane (6:4), 600 ml. Twenty ml fractions were collected, until no traces of alkaloid could be detected. The fractions were examined by TLC and the liked fractions were combined to give the following portions:-

a. fractions 2-20 containing one indole alkaloid. It was evaporated under reduced pressure to dryness. White prismatic crystals (20 mg, 0.40 % of Ce) were crystallized out upon the addition of small volume of acetone, designated as GE-7 which was subsequently identified as koumine .

b. fractions 21-30 containing traces of alkaloidal mixture.

4. Identification and Characterisation of the Isolated Alkaloids

The isolated alkaloids were identified by comparison of the hRf values, melting points, ultraviolet, infrared, nuclear magnetic resonance and mass spectra with authentic samples.

The characterization of the isolated alkaloids were performed from the informations of ultraviolet, infrared, nuclear magnetic resonance, mass spectra and colors produced with ferric chloride in perchloric acid spray reagent. X-ray crystallography was used to confirm the structure of GE-2.

The hRf values given are those obtained with the following solvent systems:-

a. silica gel GF_{254}/ethyl acetate : n-hexane (1:1)

b. silica gel GF_{254}/acetone : n-hexane (4:6)

c. silica gel GF_{254}/diethyl ether GR

d. silica gel GF_{254}/chloroform : ethyl acetate (6:4)

e. silica gel GF_{254}/ethyl acetate : methanol (9:1)

f. silica gel GF_{254}/chloroform : acetone (8:2)

g. silica gel GF_{254}/ethyl acetate : ethanol (8:2)

h. silica gel GF_{254}/ethyl acetate : methanol (8:2)

i. silica gel GF_{254}/chloroform : methanol (8:2)

j. silica gel GF_{254}/acetone : methanol (8:2)

k. aluminium oxide G/ethyl acetate : n-hexane (6:4)

4.1 Characterization of GE-1 as 16-epi-voacarpine

GE-1 was obtained as white amorphous solid. It is soluble in ethyl acetate, chloroform, acetone, ethanol and methanol but insoluble in petroleum ether and n-hexane.

hRf values

a. 26 b. 39 c. 40 d. 14 e. 68 f. 24

Molecular weight

368 (mass spectrometry)

Ultraviolet absorption spectrum (ethanol) (Figure 37, p. 131)

λ_{max} 202, 226, 277, 282 and 290 nm

λ_{min} 246 nm

Infrared absorption spectrum (potassium bromide) (Figure 38, p. 132)

ν_{max} (cm^{-1})	
3380 broad	(O-H) and (N-H)
2950	(C-H)
1725	(C=O)
1455	(CH_2)
1440	(CH_3)
1100 and 1025	(C-O) of ester
1060	(C-O) of alcohol

Proton NMR spectrum (in $CDCl_3$, 270 MHz) (Figure 39, p. 133)

Chemical shift (δ) ppm	Proton	Multiplicity
1.57	C(18)-H_3	d
1.77	C(14)-Hα	dd
2.05	C(14)-Hβ	dd
2.70	C(6)-Hβ	d
2.82	C(6)-Hα	dd
3.14	C(15)-H	br-s
3.28	C(17)-H	d
3.40	C(21)-H_2	s
3.69	OCH_3	s
4.18	C(17)-H	br-d
4.44	C(5)-H	d
5.26	C(19)-H	q
6.86	C(10)-H	ddd
7.00	C(11)-H	ddd
7.04	C(12)-H	d
7.04	C(9)-H	d
8.22	N(a)-H	s

^{13}C-NMR spectrum (in $CDCl_3$, 67.8 MHz) (Figure 40, p. 134)

Chemical Shift (δ) ppm	Carbon No.	Multiplicity
12.67	C(18)	q
21.25	C(6)	t
33.64	C(15)	d
36.54	C(14)	t

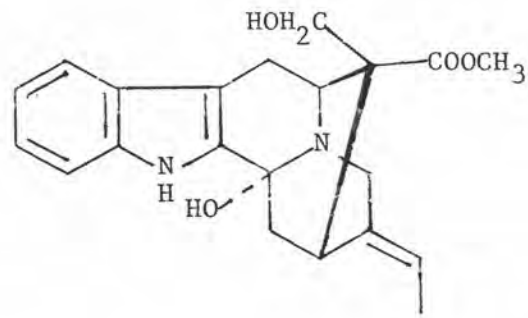
Chemical Shift (δ) ppm	Carbon No.	Multiplicity
47.92	C(21)	t
52.24	OCH_3	q
53.22	C(16)	s
57.62	C(5)	d
63.32	CH_2OH	t
80.10	C(3)	s
107.83	C(7)	s
111.03	C(12)	d
116.22	C(9)	d
118.55	C(19)	d
119.62	C(10)	d
122.18	C(11)	d
125.72	C(8)	s
134.53	C(20)	s
136.46	C(13)	s
136.69	C(2)	s
175.50	C=O	s

Mass spectrum (EIMS) (Figure 41, p. 135)

m/e (% , relative abundance)

368 (M^+, 62), 353 (5.2), 351 (29.5), 350 (8.6), 338 (27.5)
309 (19), 266 (22), 265 (76), 264 (5.5), 251 (10.5),
237 (17), 194(15), 185 (82), 184 (100), 180 (35.5), 169 (9),
168 (15.5), 167 (27), 166 (17.5), 158 (17), 130 (28),
129 (24), 91 (9) and 28 (51.5)

From the molecular weight, ultraviolet, infrared, ^{1}H-nuclear magnetic resonance, ^{13}C-nuclear magnetic resonance, mass spectra and by comparison with the spectra of similar compounds, i.e. voacarpine (Denayer-Tournay, Pecher, Martin, Friedmann-Spiteller and Spiteller, 1965), voachalotine (Clayton, Reed and Wilson, 1962; Lounasmaa, Jokela, Tolvanen and Kan, 1985), koumidine (Jin and Xu, 1982), and 19-(*E*)-akuammidine (Clayton *et al.*, 1962; Ohashi, Budzikiewicz, Wilson, Djerassi, Levy, Gosset, Lemen and Janot, 1963; Bieman , 1966; Lounasmaa *et al.*, 1985), it is therefore concluded that GE-1 is 16-epi-voacarpine and the structure is shown below.



4.2 Characterization of GE-2 as 19-oxogelsenicine

GE-2 was obtained as white amorphous solid. It is soluble in ethyl acetate, chloroform, acetone, ethanol and methanol but insoluble in petroleum ether and n-hexane.

hRf values

a. 28 b. 63 c. 45 d. 45 e. 65 f. 73

Molecular weight

340 (mass spectrometry)

Ultraviolet absorption spectrum (ethanol) (Figure 42, p. 136)

λ_{max} 208.6 and 255.2 nm

λ_{min} 242.4 nm

Infrared absorption spectrum (potassium bromide)(Figure 43, p. 137)

ν_{max} (cm^{-1})	
2970-2780	(C-H)
1725 and 1695	(C=O)
1460	(CH_2)
1085	(C-O) of ether

Proton NMR spectrum (in $CDCl_3$, 270 MHz) (Figure 44, p. 138)

Chemical Shift (δ) ppm	Proton	Multiplicity
2.21	C(14)-H_2	m
2.33	C(6)-Hβ	dd
2.56	C(6)-Hα	dd
2.61	C(16)-H	s
2.66	C(18)-H_3	s
3.44	C(15)-H	t
3.75	C(5)-H	q
3.93	N(a)-OCH_3	s
4.30	C(17)-H_2	dd,dd
4.73	C(3)-H	m
6.89	C(12)-H	dd
7.09	C(11)-H	ddd

Chemical Shift (δ) ppm	Proton	Multiplicity
7.27	C(10)-H	ddd
7.54	C(9)-H	dd

^{13}C-NMR spectrum (in $CDCl_3$, 67.8 MHz) (Figure 45, p. 139)

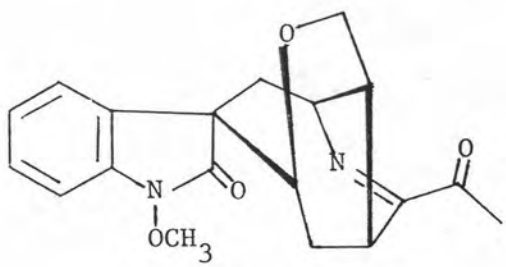
Chemical Shift (δ) ppm	Carbon No.	Multiplicity
26.09	C(18)	q
27.53	C(14)	t
38.01	C(6)	t
38.93	$-OCH_3$	q
39.39	C(15)	d
56.27	C(7)	s
61.74	C(17)	t
63.35	C(16)	d
74.53	C(5)	d
75.19	C(3)	d
106.72	C(12)	d
123.51	C(10)	d
124.52	C(9)	d
128.29	C(11)	d
131.77	C(8)	s
138.02	C(13)	s
171.11	C(20)	s
178.08	C(2)	s
197.60	C(19)	s

Mass spectrum (EIMS) (Figure 46, p. 140)

m/e (% , relative abundance)

340 (M^+, 100), 310 (42), 267 (8), 176 (10), 164 (54), 154 (10), 144(38), 136 (42), 130 (31), 122 (72), 116 (37), 81 (38), and 43 (92).

From the molecular weight, ultraviolet, infrared, ^{1}H-NMR, ^{13}C-NMR, mass spectra and by comparison with the spectra of gelsenicine (humantenmine) (Du *et al.*, 1982; Yang and Chen, 1983), it is therefore concluded that GE-2 is 19-oxogelsenicine. The structure was confirmed by X-ray crystallography (S. Sakai, E. Yamanaka, M. Kitajima, M. Yokota, N. Aimi, S, Wongseripipatana and D. Ponglux, in press) and shown as follows:-



4.3 Characterization of GE-5 as 19-(Z)-akuammidine

GE-5 was obtained as white needle crystals from methanol. It is soluble in ethanol and methanol, slightly soluble in chloroform and insoluble in benzene.

hRf values

a. 7 b. 27 c. 10 d. 12 e. 53 f. 22

Melting point

249° C

Molecular weight

352 (mass spectrometry)

Ultraviolet absorption spectrum (ethanol) (Figure 47, p. 141)

λ_{max} 225, 274, 282 and 290 nm

λ_{min} 250 nm

Infrared absorption spectrum (potassium bromide) (Figure 48, p. 142)

ν_{max} (cm^{-1})	
3300	(O-H)
3050	(N-H)
2850-2800	(C-H)
1720	(C=O)
1460	(CH_2)
1440	(CH_3)
1220 and 1100	(C-O) of ester

Proton NMR spectrum (in CD_3OD, 270 MHz) (Figure 49, p. 143)

Chemical Shift (δ) ppm	Proton	Multiplicity
1.63	C(18)-H_3	d
1.94	C(14)-Hα	ddd
2.67	C(14)-Hβ	dd
2.75	C(15)-H	dd
2.78	C(6)-Hα	dd
2.87	C(5)-H	dd
2.96	-OCH_3	s
3.42	C(6)-Hβ	dd
3.61	C(21)-H_2	br-s
3.66	C(17)-H	d
3.74	C(17)-H	d
4.21	C(3)-H	d
5.44	C(19)-H	br-q
6.95	C(10)-H	ddd
7.03	C(11)-H	ddd
7.26	C(12)-H	dd
7.36	C(9)-H	dd

^{13}C-NMR spectrum (in CD_3OD, 67.8 MHz) (Figure 50, p. 144)

Chemical Shift (δ) ppm	Carbon No.	Multiplicity
12.5	C(18)	q
25.1	C(6)	t
31.4	C(14)	t
37.2	C(15)	d

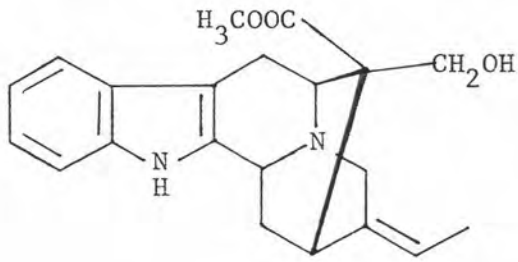
Chemical Shift (δ) ppm	Carbon No.	Multiplicity
51.2	$-OCH_3$	q
51.8	C(5)	d
53.2	C(16)	s
54.0	C(21)	t
59.5	C(3)	d
69.1	C(17)	t
106.0	C(7)	s
112.0	C(12)	d
118.2	C(9)	d
118.6	C(19)	d
119.8	C(10)	d
122.1	C(11)	d
128.1	C(8)	s
138.6	C(20)	s
138.9	C(2)	s
139.1	C(13)	s
174.9	C=O	s

Mass spectrum (EIMS) (Figure 51, p. 145)

m/e (% , relative abundance)

352 (M^+,100), 351 (65), 337 (22), 335 (22), 321(60), 293 (27), 253 (11), 250 (18), 249 (71), 182 (19), 169 (88), 168 (57), 156 (19), and 115 (14).

From the molecular weight, ultraviolet, infrared, ^{1}H-NMR, ^{13}C-NMR, mass spectra and by comparison with the spectra of 19-(*E*)-akuammidine (Clayton *et al.*, 1962; Ohashi *et al.*, 1963; Bieman, 1966; Lounasmaa *et al.*, 1985), GE-5 is therefore characterized as 19-(Z)-akuammidine and the structure is shown below.



4.4 Identification of GE-6 as gelsemine

GE-6 was obtained as white prismatic crystals from acetone. It is soluble in ethyl acetate, chloroform, acetone, ethanol and methanol but insoluble in petroleum ether and n-hexane.

hRf values

g. 25 h. 25 i. 43 j. 41 k. 35

Melting point

178°C

Molecular weight

322 (mass spectrometry)

Ultraviolet absorption spectrum (ethanol) (Figure 52, p. 146)

λ_{max} 208 and 251 nm

λ_{min} 226 and 273 nm

shoulder at 283 nm

Infrared absorption spectrum (potassium bromide) (Figure 53, p. 147)

$\nu_{max}(cm^{-1})$	
3080	(N-H)
2930-2860	(C-H)
1720	(C=O)
1620-1590	(C=C)
1470	(CH_2)
1220	(C-N)
1100	(C-O)

Proton NMR spectrum (in $CDCl_3$, 270 MHz) (Figure 54, p. 148)

Chemical shift (δ) ppm	Proton	Multiplicity
1.98	C(6)-H	t-like
2.00	C(14)-Hβ	ddd
2.25	N(b)-CH_3	s
2.30	C(15)-H	m
2.31	C(21)-H	d
2.42	C(16)-H	br-d
2.78	C(21)-H	d
2.83	C(14)-Hα	dd
3.44	C(5)-H	br-s
3.82	C(3)-H	br-s

Chemical Shift (δ) ppm	Proton	Multiplicity
3.91	C(17)-H	dd
4.11	C(17)-H	dd
4.94	C(18)-H	dd
5.10	C(18)-H	dd
6.26	C(19)-H	dd
6.77	C(12)-H	d
7.00	C(10)-H	ddd
7.19	C(11)-H	ddd
7.42	C(9)-H	d
7.86	N(a)-H	br-s

^{13}C-NMR spectrum (in $CDCl_3$, 67.8 MHz) (Figure 55, p. 149)

Chemical Shift (δ) ppm	Carbon No.	Multiplicity
22.9	C(14)	t
35.8	C(15)	d
38.2	C(16)	d
40.7	N(b)-CH_3	q
50.7	C(6)	d
54.1	C(20)	s
54.2	C(7)	s
61.6	C(17)	t
66.2	C(21)	t
69.5	C(3)	d
72.1	C(5)	d
109.0	C(12)	d

Chemical Shift (δ) ppm	Carbon No.	Multiplicity
112.2	C(18)	t
121.7	C(10)	d
127.9	C(11)	d
128.3	C(9)	d
132.0	C(8)	s
138.8	C(19)	d
140.7	C(13)	s
179.4	C(2)	s

Mass spectrum (EIMS) (Figure 56, p. 150)

m/e (% , relative abundance)

322 (M^+, 49), 279 (54), 261 (5), 251 (23), 236 (9), 222 (5), 196 (7), 158 (10), 146 (10), 145 (10), 134 (13), 120 (15), 109 (10), 108 (100), 93 (10), 91 (11), 70 (10), and 42 (21)

These data are in agreement with the published values of gelsemine (Wenkert, Chang, Cochran and Pellicciari, 1972; Bindra, 1973; Schun and Cordell 1985 b). Direct comparison with an authentic sample of gelsemine isolated from the stems of *Gelsemium elegans* Benth. (Wongseripipatana, 1985) on TLC also gave good correspondence. It is therefore concluded that GE-6 is gelsemine.

4.5 Identification of GE-7 as koumine

GE-7 was obtained as white prismatic crystals from acetone. It is soluble in ethyl acetate, chloroform, acetone, ethanol and methanol but insoluble in petroleum ether and n-hexane.

hRf values

g. 17 h. 16 i. 64 j. 31 k. 45

Melting point

169.5°C

Molecular weight

306 (mass spectrometry)

Ultraviolet absorption spectrum (ethanol) (Figure 57, p. 151)

λ_{max} 221 and 262 nm

λ_{min} 237 nm

Infrared absorption spectrum (potassium bromide) (Figure 58, p. 152)

ν_{max} (cm^{-1})	
3060-2750	(C-H)
1620-1580	(C=C)
1480	(CH_2)
1440	(CH_3)
1210	(C-N)
1080, 1070	(C-O)

Proton NMR spectrum (in $CDCl_3$, 270 MHz) (Figure 59, p. 153)

Chemical Shift (δ) ppm	Proton	Multiplicity
1.87	C(14)-Hα	dt
2.36	C(6)-H_2	br-s
2.36	C(15)-H	br-d
2.60	C(14)-Hβ	dt
2.60	N(b)-CH_3	s

Chemical Shift (δ) ppm	Proton	Multiplicity
2.78	C(5)-H	br-s
2.81	C(16)-H	br-d
3.13	C(21)-H_2	d,d
3.61	C(17)-Hα	d
4.25	C(17)-Hβ	dd
4.69	C(19)-H	dd
4.75	C(18)-H	dd
4.84	C(18)-H	dd
5.02	C(3)-H	br-s
7.24	C(10)-H	ddd
7.36	C(11)-H	ddd
7.56	C(9)-H	d
7.62	C(12)-H	d

^{13}C-NMR spectrum (in $CDCl_3$, 67.8 MHz) (Figure 60, p. 154)

Chemical Shift (δ) ppm	Carbon No.	Multiplicity
25.2	C(14)	t*
28.5	C(6)	t*
33.1	C(15)	d**
38.8	C(16)	d**
42.6	N(b)-CH_3	q
45.3	C(20)	s
56.8	C(5)	d
57.7	C(21)	t
58.0	C(7)	s

Chemical Shift (δ) ppm	Carbon No.	Multiplicity
61.3	C(17)	t
70.9	C(3)	d
115.8	C(18)	t
121.1	C(12)	d
123.0	C(10)	d
126.0	C(11)	d
128.1	C(9)	d
137.3	C(19)	d
143.7	C(8)	s
154.8	C(13)	s
185.5	C(2)	s

* Values may be interchanged

** Values may be interchanged

Mass spectrum (EIMS) (Figure 61, p. 155)

m/e (% , relative abundance)

306 (M^+,100), 291 (8), 277 (11), 263 (17), 251 (16), 223 (33), 205 (19), 194 (20), 180 (21), 167 (18), 120 (34), 71 (32), 70 (85), 42 (31)

These data are in agreement with published values of koumine (Khuong-Huu *et al.*, 1981; Sakai, *et al.*,1986). Direct comparison with an authentic sample of koumine isolated from the stems of *Gelsemium elegans* Benth. (Wongseripipatana, 1985) on TLC also gave good correspondence. It is therefore concluded that GE-7 is koumine.