CHAPTER III

EXPERIMENTAL

A study of chemical constituents in plant must proceed stepwise from selection and authentication of plant material, through collection, extraction, isolation of the compounds, and structure elucidation of isolated compounds.

1. Source and Authentication of Plant Material

The fresh leaves of *Mitragyna speciosa* (Korth.) Havil. were collected in May, 1989 from a flowering tree growing on the campus of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok Thailand. The material was identified by Professor Tem Smitinand, the former Deputy Director-General, Royal Forest Department of Thailand.

2. General Technique

2.1 Thin-Layer Chromatography (TLC)

2.1.1 Analytical

Adsorbents : The TLC plate for routine work were Pre-Coated TLC Plates of Silica gel 60 F-254 (E.Merck) or Pre-Coated TLC Plates of

Aluminium oxide F-254 (type E, E.Merck), accordingly.

Layer thickness : 250 µm

Technique : one way, ascending, 6.5 cm

Solvent system : (1) silica gel 60 F-254/
n-hexane:ethyl acetate (2:1)

(2) silica gel 60 F-254/
diethyl ether:ethyl acetate (1:1)

(3) silica gel 60 F-254/ ethyl acetate

(4) silica gel 60 F-254/
ethyl acetate:methanol (19:1)

(5) silica gel 60 F-254/ n-hexane:
ethyl acetate:methanol (8:4:1)

(6) silica gel 60 F-254/
chloroform:acetone (5:4)

(7) silica gel 60 F-254/
ammonia saturated-chloroform

(8) silica gel 60 F-254/
chloroform:methanol (9:1)

(9) aluminium oxide F-254 (type E)/
n-hexane:ethyl acetate (5:2)

Temperature : laboratory temperature (20°-30°C)

Detection : (1) ultraviolet light at wavelength 254 nm

(2) 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. This reagent is used as a general alkaloid-detecting reagent, the alkaloids give orange spots as positive test.

(3) 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

Adsorbent : Pre-Coated for preparative layer chromatography plates silica gel 60

F-254 (E.Merck) were used.

Layer thickness : 1 mm

Technique : one way, ascending, 15 cm (double development)

Application : as a continuous streak using a capillary tube

Solvent system : Chloroform:ethanol (4:1)

Temperature : laboratory temperature (20°-30°C)

Detection : The bands were visualized in ultraviolet light (254 nm), scraped off and the alkaloids eluted from the silica gel by shaking with ethanol which was filtered through sintered glass and evaporated to dryness.

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 : (1) adsorbent poured as a suspension into the column

(2) adsorbent packed dry into the column

Addition of alkaloidal material

: alkaloidal material was dissolved in small volume of volatile solvent and gently placed on top of the column.

Technique : Open column chromatography

: Flash column chromatography

Solvents : n-hexane, ethyl acetate, chloroform, ethanol, methanol

Examination of eluate

: fractions were examined by TLC using ultraviolet light at wavelength 254 nm and followed with Dragendorff's spray reagent

2.3 Physical Constant

All melting points were measured on the Buchi 520 melting point apparatus. The values recorded are uncorrected.

2.4 Spectroscopy

2.4.1 Ultraviolet absorption spectra were obtained with a Hitachi U3400 spectrophotometer.

2.4.2 Infrared absorption spectra were performed on 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 GSX-500 (500 MHz) spectrometer. Chemical shifts were reported in ppm scale, using tetramethylsilane (T.M.S.) as internal standard, and deuterochloroform as operating solvent.

2.4.4 ^{13}C-nuclear magnetic resonance (^{13}C-NMR) were obtained with a JEOL FX-270 (67.8 MHz) or a JEOL

GSX-400 (100 MHz) spectrometers, accordingly. Chemical shifts were reported in ppm scale, using tetramethylsilane (T.M.S.) as internal standard, and deuterochloroform as operating solvent.

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

2.5 Solvents

Throughout the work, all organic solvents were redistilled before use.

2.6 Authentic Alkaloids

All authentic alkaloids are kindly supplied by Dr. Dhavadee Ponglux.

(1) mitragynine

(2) speciogynine

(3) mitraphylline

(4) isomitraphylline

(5) isopteropodine

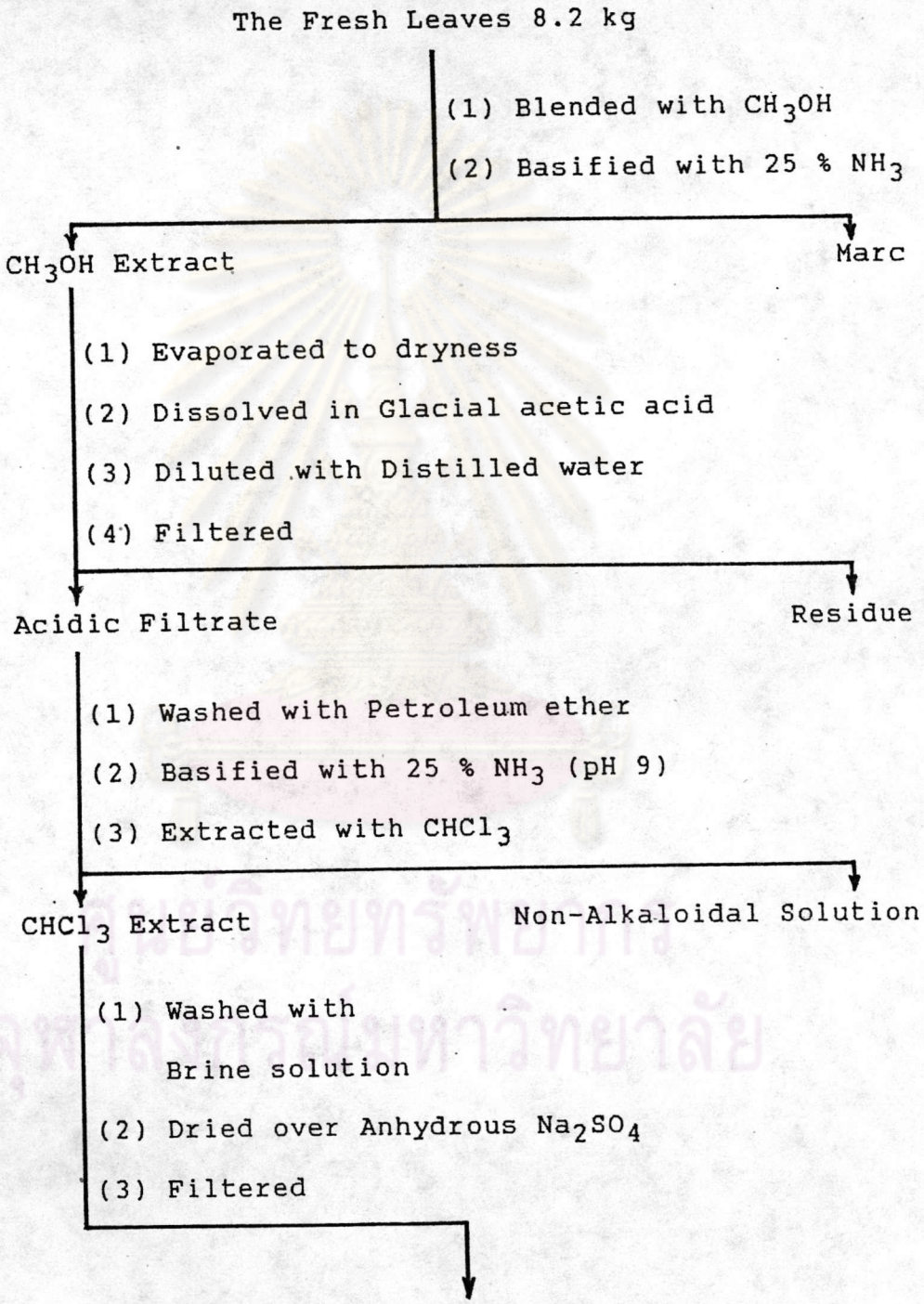
3. The Extraction and Isolation of Alkalods from the Fresh Leaves of *Mitragyna speciosa* (Korth.) Havil.

3.1 The Extraction of Alkaloids

The fresh leaves (8.2 kg) were blended with methanol (20 L) and 200 ml of 25 % ammonia solution was

added. It was then allowed to macerate for two days and filtered. The marc was remacerated for two days with four sucessive portions of methanol (10 L-portion). Testing for complete extraction was carried out with Dragendorff's spray reagent. The combined filtrate was concentrated to syrupy mass under reduced pressure, mixed with glacial acetic acid (400 ml) then distilled water was added to give about 5 % acetic acid solution (8 L), well shaken and left to stand overnight. The acidic filtrate was washed with portions of petroleum ether, then made alkali (pH 9) with 25 % ammonia solution and extracted with chloroform (15 x 300 ml). Testing for complete extraction was carried out with Dragendorff's spray reagent. The combined chloroform extract was washed with Brine solution (saturated sodium chloride in distilled water), dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield dry crude alkaloidal extract 20.17 g (0.25 % based on fresh leaves weight). The procedure was shown diagrammatically in Figure 15. TLC analysis of crude alkaloidal extract showed at least eight alkaloids with the addition of base-line alkaloid(s).

Figure 15 The alkaloidal extraction procedure of *Mitragyna speciosa* (Korth.) Havil.



3.2 The Isolation of Alkaloids

Crude alkaloidal extract (12.0 g) was dissolved in chloroform (20 ml) and gently placed on top of silica gel column (8 x 45 cm) holding n-hexane. The column was eluted with n-hexane:chloroform (4:1), (1:1); chloroform; chloroform:methanol (97:3), (9:1), (4:1), (1:1) 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 20, 30, 30, 60, 30, 20, and 30 fractions, respectively. Those fractions of similar alkaloidal pattern were combined and evaporated to dryness under reduced pressure to give the following fractions:-

(1) fractions 1-23 containing no alkaloid.

(2) fractions 24-82 were combined and assigned as Fraction F-1 (2.535 g).

(3) fractions 83-142 were combined and assigned as Fraction F-2 (6.983 g).

(4) fractions 143-185 were combined and assigned as Fraction F-3 (1.164 g).

(5) fractions 186-220 were combined and assigned as Fraction F-4 (0.860 g).

(6) methanolic fractions were combined and assigned as Fraction F-5 (0.120 g), shown by

TLC to contain traces of alkaloidal mixture and the base-line alkaloid(s). No further study has been made.

3.2.1 Isolation of alkaloids from the Fraction F-1

The Fraction F-1 (2.535 g) was shown by TLC to contain at least 3 alkaloids. It was dissolved in chloroform (5 ml) and gently placed on top of silica gel column (3.5 x 45 cm) holding n-hexane. The column was eluted with n-hexane:ethyl acetate (19:1), (9:1), and (4:1). Twenty ml fractions being collected. The volumes of eluting solvents used were 500, 900, and 900, respectively. By TLC analysis the liked fractions were combined to give the following portions:-

F-1a. fractions 1-32 containing no alkaloid.

F-1b. fractions 33-37 containing one indole alkaloid. It was assigned as DS-1 (23 mg) and subsequently identified as tetrahydroalstonine.

F-1c. fractions 38-42 containing traces of 2 alkaloids.

F-1d. fractions 43-67 containing one indole alkaloid. It was assigned as DS-2 (1.648 g) and subsequently identified as mitragynine.

F-1e. fractions 68-76 (0.398 g) containing mixture of 2 alkaloids

F-1f. fractions 77-113 containing one indole alkaloid. It was assigned as DS-3 (0.165 g) and subsequently

identified as ajmalicine.

3.2.2 Isolation of alkaloids from the Fraction F-2

The Fraction F-2 (6.983 g) was shown by TLC to contain mixture of at least 5 alkaloids. It was dissolved in chloroform (10 ml) and gently placed on top of silica gel column (5 x 45 cm) holding n-hexane. The column was eluted with n-hexane:ethyl acetate (4:1), (1:1); ethyl acetate; and ethyl acetate:methanol (98:2). Thirty ml fractions being collected. The volumes of eluting solvents used were 700, 1000, 1000, and 1000, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions:-

F-2a. fractions 1-17 containing no alkaloid.

F-2b. fractions 18-44 (1.260 g) containing mixture of 2 alkaloids. This portion was further treated as the Fraction F-1 yielding mitragynine (0.286 g), ajmalicine (0.738 g), and 0.125g of mixture of these two alkaloids.

F-2c. fractions 45-52 (0.186 g) containing mixture of 3 alkaloids.

F-2d. fractions 53-86 (3.618 g) containing at least 3 alkaloids. It was dissolved in chloroform (10 ml) and gently placed on top of aluminium oxide column (4 x 45 cm) holding n-hexane. The column was eluted with n-hexane:ethyl acetate (5:1), (5:2),

(1:1) and ethyl acetate. Thirty ml fractions were collected. The volumes of eluting solvents used were 600, 1200, 600, and 800 ml, respectively. By TLC analysis the liked fractions were combined to give the following portions:-

F-2d-1. fractions 1-23 containing no alkaloid.

F-2d-2. fractions 24-48 (0.702 g) containing at least 3 alkaloids. It was further treated on silica gel-column chromatography using n-hexane:ethyl acetate (3:1) as eluting solvent (1.5 L). This portion yielded ajmalicine (40 mg), two indole alkaloids which were assigned as DS-4 (60 mg) and DS-5 (0.290 g), and 0.138 g of alkaloidal mixture. Compounds DS-4 and DS-5 were subsequently identified as paynantheine and speciogynine, respectively.

F-2d-3. fractions 49-56 (0.240 g) containing alkaloidal mixture.

F-2d-4. fractions 57-108 (2.454 g) containing at least 3 alkaloids. It was further treated on silica gel-column chromatography using n-hexane:ethyl acetate (1:2) as eluting solvent (2 L). This portion yielded DS-6 (0.526 g), DS-7 (0.927 g), DS-8 (0.210 g), and 0.460 g of DS-6 and DS-7 mixture.

These 3 oxindoles, DS-6, DS-7, and DS-8 were subsequently identified as isopteropodine, isomitraphylline, and mitraphylline, respectively.

F-2e. fractions 87-122 (1.561 g) containing at least 3 alkaloids. It was redissolved in ethyl acetate and left to stand from which white amorphous solid of mitraphylline (0.820 g) were deposited.

3.2.3 Isolation of alkaloids from the Fraction F-3

The Fraction F-3 (1.164 g) was shown by TLC to contain at least 3 alkaloids. It was dissolved in chloroform (5 ml) and gently placed on top of silica gel column (3.5 x 45 cm) holding chloroform. The column was eluted with ammonia saturated-chloroform (1 L). Twenty ml fractions were combined to give the following portions:-

F-3a. fractions 1-12 containing traces of alkaloidal mixture.

F-3b. fractions 13-36 (0.712 g) containing at least two alkaloids. It was further treated on silica gel-column chromatography using ethyl acetate:methanol (19:1) as eluting solvent (600 ml). This portion yielded one indole alkaloid together with traces of alkaloidal mixture. The purified indole alkaloid was assigned as DS-9 (0.335 g) and subsequently identified as mitraciliatine.

F-3c. fractions 37-50 (0.430 g) was shown by TLC to contain at least 5 alkaloids including mitraphylline and mitraciliatine.

3.2.4 Isolation of alkaloids from the Fraction F-4

The Fraction F-4 (0.860 g) was shown by TLC to contain at least 4 alkaloids including the base-line alkaloid(s). It was dissolved in chloroform (5 ml) and gently placed on top of silica gel column (2.5 x 45 cm) holding ethyl acetate. The column was eluted with ethyl acetate:methanol (9:1) and (4:1). Twenty ml fractions being collected. The volumes of eluting solvents used were 1200 and 800 ml, respectively, and yielding 4 portions. Each of them containing alkaloidal mixture. The most polar portion (0.140 g) was subjected to aluminium oxide column using ethyl acetate:methanol (9:1) as eluting solvent (500 ml), and yielding 3 portions of alkaloidal mixtures. The most polar portion (33 mg) containing one indole alkaloid and traces of other alkaloids. The indole alkaloid was separated by preparative TLC plates using chloroform:ethanol (4:1) as developing solvent. It was assigned as DS-10 (16 mg) whose structure determination has not been completed yet.

4. Identification of 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, as indicated.

The hRf values given in Table 4 are those obtained with the following solvent systems:-

(1) silica gel 60 F-254/ diethyl ether:ethyl acetate (1:1)
(2) silica gel 60 F-254/ n-hexane:ethyl acetate:methanol (8:4:1)
(3) silica gel 60 F-254/ chloroform:acetone (5:4)
(4) silica gel 60 F-254/ chloroform:methanol (9:1)
(5) aluminium oxide F-254 (type E)/ n-hexane:ethyl acetate (5:2)

4.1 Identification of DS-1 as Tetrahydroalstonine

DS-1 was obtained as colorless feather crystals from ethyl acetate - n-hexane. It was soluble in ethyl acetate, chloroform, and methanol.

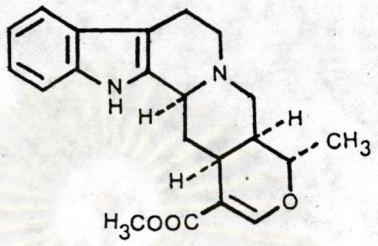
Melting point : 216°-217°C
Molecular weight : 352
hRf values : see Table 4 (page 136)
UV λ_{max} (nm) : 226.2, 281.4 (page 182)

^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 183)

Chemical shift (ppm)	Proton	Multiplicity
7.76	-NH	1H, br-s
7.56	C(17)-H	1H, d (J= 0.5 Hz)
7.45	C(9)-H	1H, dd (J= 7.5,1.1 Hz)
7.28	C(12)-H	1H, dd (J= 7.5,1.1 Hz)
7.12 *	C(11)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
7.08 *	C(10)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
4.49	C(19)-H	1H, dq (J= 12.0,6.0 Hz)
3.75	C(23)-OCH_3	3H, s
3.36	C(3)-H	1H, dd (J= 12.0,3.0 Hz)
3.10	C(21)-Hβ	1H, dd (J= 12.0,3.0 Hz)
2.94	C(5)-Hβ	1H, ddd (J= 12.0,6.0,0.5 Hz)
2.90	C(6)-Hβ	1H, ddd (J= 12.0,11.0,6.0 Hz)
2.77	C(15)-H	1H, dt (J= 12.0,3.0 Hz)
2.72	C(21)-Hα	1H, dd (J= 12.0,4.0 Hz)
2.68	C(6)-Hα	1H, br-d (J= 12.0 Hz)
2.56	C(5)-Hα	1H, ddd (J= 12.0,11.0,4.0 Hz)
2.49	C(14)-Hα	1H, dt (J= 12.0,3.0 Hz)
1.70	C(20)-H	1H, br-d (J= 4.0 Hz)
1.55	C(14)-Hβ	1H, q (J= 12.0 Hz)
1.40	C(18)-CH_3	3H, d (J= 6.0 Hz)

* Assignments may be interchanged

These data are in agreement with the published values of tetrahydroalstonine (Lounasmaa and Kan, 1980). It is therefore concluded that DS-1 is tetrahydroalstonine.



Tetrahydroalstonine

4.2 Identification of DS-2 as Mitragynine

DS-2 was obtained as pale yellow amorphous solid. All attempts on crystallization were unsuccessful. It was soluble in diethyl ether, ethyl acetate, acetone, chloroform, and methanol.

Melting point : 93°-95°C

Molecular weight : 398

hRf values : see Table 4 (page 136)

UV λ_{max} (nm) : 224.2, 291.5 (page 184)

IR absorption spectrum (potassium bromide) : (page 185)

$\bar{\nu}_{max}$ (cm^{-1})	
3460	N-H (imino)
3000-2905	C-H
1690	C=O (ester)
1625	C=C

^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 186)

Chemical shift (ppm)	Proton	Multiplicity
7.70	-NH	1H, br-s
7.42	C(17)-H	1H, s
6.98	C(11)-H	1H, t (J= 8.0 Hz)
6.88	C(12)-H	1H, d (J= 8.0 Hz)
6.45	C(10)-H	1H, d (J= 7.7 Hz)
3.86	C(9)-OCH_3	3H, s
3.72	C(17)-OCH_3	3H, s
3.69	C(23)-OCH_3	3H, s
3.15	C(3)-H	1H, dd (J= 12.0,1.5 Hz)
3.11	C(5)-Hα	1H, td (J= 12.0,4.0 Hz)
3.04	C(5)-Hβ	1H, dt (J= 12.0,6.0 Hz)
2.98	C(21)-Hα	1H, dd (J= 12.0,3.0 Hz)
2.92	C(6)-Hβ C(21)-Hβ	2H, m
2.53	C(6)-Hα C(15)-H	2H, m
2.46	C(14)-Hβ	1H, td (J= 12.0,6.0 Hz)
1.78	C(19)-H	2H, m
1.61	C(14)-Hα	1H, br-d (J= 10.2 Hz)
1.19	C(20)-H	1H, m
0.87	C(18)-CH_3	3H, t (J= 7.2 Hz)

^{13}C-NMR spectrum : see Table 5 (in $CDCl_3$, 100 MHz)(page 137)

Mass spectrum : *m/e* (%, relative abundance)

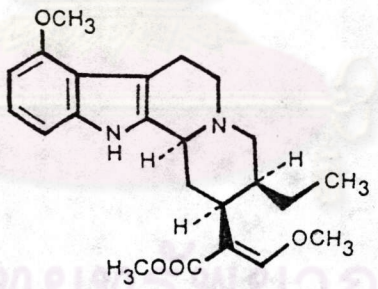
(EIMS) 398(M^+, 85.88), 397(79.07),

(page 188) 269(23.24), 214(100.00), 200(27.47),

199(21.30), 186(26.29), 170(5.42),

75(8.28), 28(5.00)

DS-2 is identical in hRf values, melting point, UV, IR, and ^{1}H-NMR spectra with authentic sample of mitragynine obtained from *Mitragyna speciosa* Korth. (Shellard, Houghton and Resha, 1978b) and also confirmed by ^{13}C-NMR spectrum. It is therefore concluded that DS-2 is mitragynine.



Mitragynine

4.3 Identification of DS-3 as Ajmalicine

DS-3 was obtained as colorless prismatic crystals from methanol. It is soluble in ethyl acetate, acetone, chloroform, and methanol.

Melting point : 250°-251°C

Molecular weight : 352

hRf values : see Table 4 (page 136)

^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 189)

Chemical shift (ppm)	Proton	Multiplicity
7.79	-NH	1H, br-s
7.53	C(17)-H	1H, d (J= 1.5 Hz)
7.46	C(9)-H	1H, dd (J= 7.5,1.1 Hz)
7.29	C(12)-H	1H, dd (J= 7.5,1.1 Hz)
7.13 *	C(11)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
7.08 *	C(10)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
4.43	C(19)-H	1H, qd (J= 6.0,3.0 Hz)
3.74	C(23)-OCH_3	3H, s
3.40	C(3)-H	1H, dd (J= 12.0,3.0 Hz)
3.20	C(14)-Hα	1H, dt (J= 12.0,3.0 Hz)
3.10	C(5)-Hβ	1H, br-dd (J= 12.0,6.0 Hz)
3.03	C(6)-Hβ	1H, m
2.96	C(21)-Hβ	1H, dd (J= 12.0,3.0 Hz)
2.72	C(5)-Hα C(6)-Hα	2H, m
2.42	C(15)-H	1H, tdd (J= 12.0,3.0,1.5 Hz)

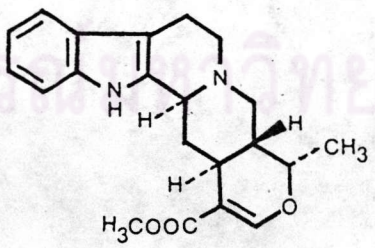
Chemical shift (ppm)	Proton	Multiplicity
2.26	C(21)-Hα	1H, t (J= 12.0 Hz)
2.17	C(20)-H	1H, tt (J= 12.0,3.0 Hz)
1.31	C(14)-Hβ	1H, q (J= 12.0 Hz)
1.19	C(18)-CH_3	1H, d (J= 6.0 Hz)

* Assignments may be interchanged

^{13}C-NMR spectrum : see Table 5 (in $CDCl_3$, 100 MHz)(page 137)

Mass spectrum (EIMS) (page 191) : *m/e* (%, relative abundance)
352(M^+, 100.00), 351(67.45), 337(5.36), 265(5.17), 209(11.23), 184(43.34), 169(16.17), 156(59.92), 115(4.70), 55(9.25)

These data are in agreement with the published values of ajmalicine (Lounasmaa and Kan, 1980). It is therefore concluded that DS-3 is ajmalicine.



Ajmalicine

4.4 Identification of DS-4 as Paynantheine

DS-4 was obtained as pale yellow amorphous solid. It was soluble in ethyl acetate, chloroform, and methanol.

Melting point : 97°C

Molecular weight : 396

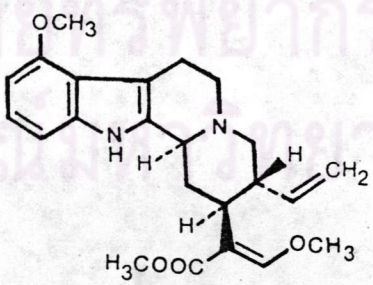
hRf values : see Table 4 (page 136)

^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 192)

Chemical shift (ppm)	Proton	Multiplicity
7.74	-NH	1H, br-s
7.33	C(17)-H	1H, s
6.99	C(11)-H	1H, t (J= 8.0 Hz)
6.88	C(12)-H	1H, d (J= 8.0 Hz)
6.45	C(10)-H	1H, d (J= 7.7 Hz)
5.58	C(19)-H	1H, ddd (J= 17.6,10.2,3.0 Hz)
5.00	C(18)-H (*trans*)	1H, dd (J= 17.3,2.0 Hz)
4.95	C(18)-H (*cis*)	1H, dd (J= 10.2,2.0 Hz)
3.87	C(9)-OCH_3	3H, s
3.77	C(17)-OCH_3	3H, s
3.68	C(23)-OCH_3	3H, s
3.27	C(3)-H	1H, br-d (J= 10.7 Hz)
3.17	C(5)-Hβ	1H, m

Chemical shift (ppm)	Proton	Multiplicity
3.03	C(6)-Hβ C(6)-Hα C(20)-H C(21)-Hβ	4H, m
2.76	C(15)-H	1H, td (J= 11.8,3.6 Hz)
2.58	C(5)-Hα	1H, td (J= 11.6,4.4 Hz)
2.28	C(21)-Hα	1H, t (J= 10.7 Hz)
2.09	C(14)-Hβ	1H, br-q (J= 12.1 Hz)
1.95	C(14)-Hα	1H, br-d (J= 12.4 Hz)

These physical data are in agreement with the published values of paynantheine (Beckett *et al.*, 1966b) and also confirmed by 500 MHz ^{1}H-NMR spectrum. It is therefore concluded that DS-4 is paynantheine.



Paynantheine

4.5 Identification of DS-5 as Speciogynine

DS-5 was obtained as colorless prismatic crystals from absolute ethanol. It was soluble in ethyl acetate chloroform, ethanol, and methanol.

Melting point : 213°-214°C

Molecular weight : 398

hRf values : see Table 4 (page 136)

UV λ_{max} (nm) : 225.9, 291.7 (page 193)

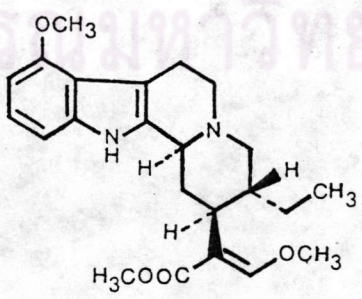
^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 194)

Chemical shift (ppm)	Proton	Multiplicity
7.67	-NH	1H, br-s
7.35	C(17)-H	1H, br-s
6.99	C(11)-H	1H, t (J= 8.0 Hz)
6.88	C(12)-H	1H, d (J= 8.0 Hz)
6.45	C(10)-H	1H, d (J= 7.7 Hz)
3.87	C(9)-OCH_3	3H, s
3.72	C(17)-OCH_3	3H, s
3.70	C(23)-OCH_3	3H, s
3.23	C(3)-H	1H, dd (J= 12.0,1.5 Hz)
3.20	C(5)-Hα	1H, td (J= 12.0,1.5 Hz)
3.15	C(21)-Hβ	1H, dd (J= 12.5,7.0 Hz)
3.07	C(5)-Hβ	1H, ddd (J= 12.0,6.5,1.5 Hz)
2.99	C(21)-Hα	1H, br-d (J= 12.4 Hz)
2.59	C(6)-Hα C(6)-Hβ	2H, m

Chemical shift (ppm)	Proton	Multiplicity
2.28	C(15)-H	1H, br-d (J= 11.0 Hz)
2.05	C(14)-Hβ	1H, td (J= 12.0,1.5 Hz)
1.96	C(19)-H	2H, m (deformed)
1.43	C(14)-Hα	1H, br-d (deformed)
1.05	C(20)-H	1H, m (deformed)
0.86	C(18)-CH_3	3H, t (J= 7.2 Hz)

Mass spectrum (EIMS) (page 195) : *m/e* (%, relative abundance)
398(M^+, 100.00), 397(82.02), 383(51.48), 269(13.30), 225(21.40), 214(81.45), 200(42.74), 186(33.62), 170(7.71), 75(11.44), 42(4.77)

These physical data are identical with authentic sample of speciogynine obtained from *Mitragyna speciosa* Korth. (Shellard, Houghton and Resha, 1978c) and also confirmed by 500 MHz ^{1}H-NMR spectrum. It is therefore concluded that DS-5 is speciogynine.



Speciogynine

4.6 Identification of DS-6 as Isopteropodine

DS-6 was obtained as colorless needle crystals from chloroform-ethyl acetate. It was soluble in diethyl ether, chloroform, and methanol.

Melting point : 204°-205°C

Molecular weight : 368

hRf values : see Table 4 (page 136)

^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 196)

Chemical shift (ppm)	Proton	Multiplicity
7.71	-NH	1H, br-s
7.41	C(17)-H	1H, s
7.27	C(9)-H	1H, dd (J= 7.5,1.1 Hz)
7.18 *	C(11)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
7.02 *	C(10)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
6.85	C(12)-H	1H, dd (J= 7.5,1.1 Hz)
4.34	C(19)-H	1H, dq (J= 12.0,6.0 Hz)
3.60	C(23)-OCH_3	3H, s
3.28	C(21)-Hβ	1H, dd (J= 10.7,1.6 Hz)
3.22	C(5)-Hβ	1H, td (J= 8.0,1.5 Hz)
2.54	C(3)-H	1H, dd (J= 12.0,2.7 Hz)
2.44	C(5)-Hα, C(6)-Hβ, C(14)-Hα, C(21)-Hα	4H, m
1.99	C(6)-Hα	1H, m

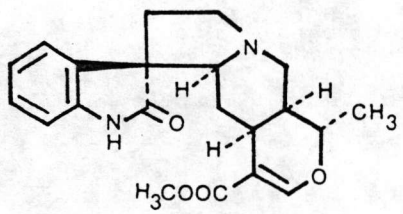
Chemical shift (ppm)	Proton	Multiplicity
1.59	C(15)-H C(20)-H	2H, m
1.41	C(18)-CH_3	3H, d (J= 6.0 Hz)
0.86	C(14)-Hβ	1H, q (J= 12.0 Hz)

* Assignments may be interchanged

^{13}C-NMR spectrum : see Table 6 (in $CDCl_3$, 67.8 MHz)(page 138)

Mass spectrum (EIMS) (page 198) : *m/e* (%, relative abundance)
368(M^+, 100.00), 337(7.37), 267(5.28), 224(13.83), 223(95.53), 208(22.34), 180(21.64), 130(16.32), 69(48.88), 42(16.59)

DS-6 is identical in melting point and hRf values with authentic sample of isopteropodine obtained from *Uncaria homomalla* (Ponglux *et al*, 1977 : Planta Med, 31: 26-30, 1977). The spectral data are in agreement with the published values of isopteropodine (Martin, Sanduja and Alam, 1986). It is therefore concluded that DS-6 is isopteropodine.



Isopteropodine

4.7 Identification of DS-7 as isomitraphylline

DS-7 was obtained as amorphous cream colored solid. It was soluble in ethyl acetate, acetone, chloroform, and methanol.

Melting point : 119°-120°C

Molecular weight : 368

hRf values : see Table 4 (page 136)

^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 199)

Chemical shift (ppm)	Proton	Multiplicity
7.65	-NH	1H, br-s
7.38	C(17)-H	1H, d (J= 1.5 Hz)
7.35	C(9)-H	1H, dd (J= 7.5,1.1 Hz)
7.18 *	C(11)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
7.00 *	C(10)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
6.84	C(12)-H	1H, dd (J= 7.5,1.1 Hz)
4.36	C(19)-H	1H, qd (J= 6.0,3.0 Hz)
3.57	C(23)-OCH_3	3H, s
3.31	C(5)-Hβ	1H, m
3.12	C(3)-H	1H, dd (J= 12.0,0.5 Hz)
2.59	C(21)-Hβ	1H, dd (J= 12.0,6.0 Hz)
2.53	C(21)-Hα	1H, t-like (J= 12.0 Hz)
2.41	C(5)-Hα	1H, m
2.19	C(6)-Hβ C(14)-Hα	2H, m
2.04	C(6)-Hα	1H, m

Chemical shift (ppm)	Proton	Multiplicity
1.93	C(15)-H C(20)-H	2H, m
1.12	C(18)-CH_3	3H, d (J= 6.0 Hz)
0.61	C(14)-Hβ	1H, q (J= 12.0 Hz)

* Assignments may be interchanged

^{13}C-NMR spectrum : see Table 6 (in $CDCl_3$, 67.8 MHz)(page 138)

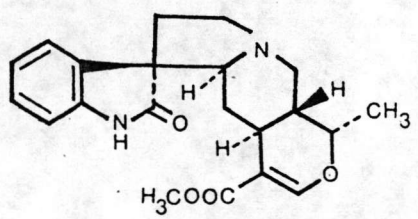
Mass spectrum : *m/e* (%, relative abundance)

(EIMS) 368(M^+, 51.09), 337(4.86),

(page 201) 224(14.27), 223(100.00), 208(9.89),

130(8.38), 69(27.91), 42(9.57)

DS-7 is identical in melting point and hRf values with authentic sample of isomitraphylline obtained from *Mitragyna tubulosa* Havil. (Shellard and Rungsiyakul, 1973). The result is fully supported by spectral data of ^{1}H- and ^{13}C-NMR. It is therefore concluded that DS-7 is isomitraphylline.



Isomitraphylline

4.8 Identification of DS-8 as Mitraphylline

DS-8 was obtained as white needle crystals from absolute ethanol. It was soluble in ethyl acetate and methanol.

Melting point : 273°-274°C

Molecular weight : 368

hRf values : see Table 4 (page 136)

^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 202)

Chemical shift (ppm)	Proton	Multiplicity
7.88	-NH	1H, br-s
7.43	C(17)-H	1H, d (J= 1.5 Hz)
7.19	C(9)-H	1H, dd (J= 7.5,1.1 Hz)
7.18 *	C(11)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
7.03 *	C(10)-H	1H, ddd (J= 7.5,7.6,1.1 Hz)
6.85	C(12)-H	1H, dd (J= 7.5,1.1 Hz)
4.37	C(19)-H	1H, qd (J= 6.0,3.0 Hz)
3.59	C(23)-OCH_3	3H, s
3.38	C(5)-Hβ	1H, m
3.21	C(21)-Hβ	1H, dd (J= 12.0,3.0 Hz)
2.49	C(3)-H C(5)-Hα	2H, m
2.38	C(6)-Hβ C(21)-Hα	2H, m

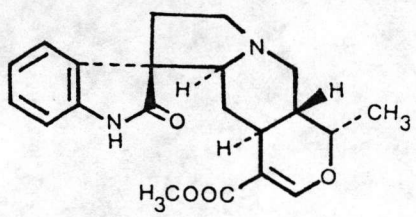
Chemical shift (ppm)	Proton	Multiplicity
2.08	C(6)-Hα C(14)-Hα C(15)-H	3H, m
1.84	C(20)-H	1H, t-like (J= 12.0 Hz)
1.20	C(14)-Hβ	1H, q-like (J= 12.0 Hz)
1.11	C(18)-CH_3	3H, d (J= 6.0 Hz)

* Assignments may be interchanged

^{13}C-NMR spectrum : see Table 6 (in $CDCl_3$, 100 MHz)(page 138)

Mass spectrum (EIMS) (page 204) : *m/e* (%, relative abundance)
368(M^+, 51.30), 337(4.54), 224(14.25), 223(100.00), 208(9.13), 130(8.23), 69(24.66), 42(8.12)

DS-8 is identical in melting point and hRf values with authentic sample of mitraphylline obtained from *Mitragyna tubulosa* Havil. (Shellard and Rungsiyakul, 1973). The result is fully supported by spectral data of ^{1}H- and ^{13}C-NMR. It is therefore concluded that DS-8 is mitraphylline.



Mitraphylline

4.9 Identification of DS-9 as Mitraciliatine

DS-9 was obtained as colorless fine needle crystals from ethyl acetate - n-hexane. It was soluble in ethyl acetate, chloroform, and methanol.

Melting point : 139°-140°C

Molecular weight : 398

hRf values : see Table 4 (page 136)

UV λ_{max} (nm) : 225.4, 292.3 (page 205)

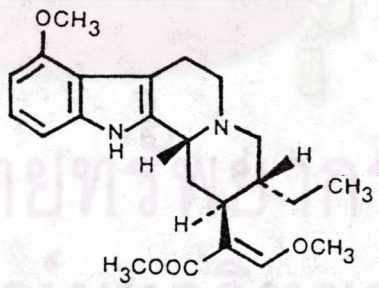
^{1}H-NMR spectrum : in $CDCl_3$, 500 MHz (page 206)

Chemical shift (ppm)	Proton	Multiplicity
7.89	-NH	1H, br-s
7.31	C(17)-H	1H, s
7.03	C(11)-H	1H, t (J= 8.0 Hz)
6.98	C(12)-H	1H, d (J= 8.0 Hz)
6.49	C(10)-H	1H, d (J= 7.7 Hz)
4.43	C(3)-H	1H, br-d (J= 3.0 Hz)
3.88	C(9)-OCH_3	3H, s
3.72	C(17)-OCH_3	3H, s
3.65	C(23)-OCH_3	3H, s
3.25	C(5)-Hβ	1H, ddd (J= 12.0,10.3,0.5 Hz)
3.18	C(5)-Hα C(6)-Hβ	2H, m
2.83	C(6)-Hα	1H, m
2.77	C(21)-Hα	1H, dd (J= 12.0,7.0 Hz)

Chemical shift (ppm)	Proton	Multiplicity
2.43	C(20)-H	1H, m
2.38	C(21)-Hβ	1H, t (J= 12.0 Hz)
2.21	C(15)-H	1H, td (J= 12.0,3.2 Hz)
2.15	C(14)-Hβ	1H, td (J= 12.0,6.0 Hz)
1.94	C(14)-Hα	1H, br-d (J= 12.5 Hz)
1.27	C(19)-H	2H, m
0.76	C(18)-CH_3	3H, t (J= 7.4 Hz)

^{13}C-NMR spectrum : see Table 5 (in $CDCl_3$, 67.8 MHz)(page 137)

Based on physical and spectral data, especially ^{1}H- and ^{13}C-NMR, DS-9 is therefore concluded to be mitraciliatine.



Mitraciliatine

Table 4 hRf values of the isolated alkaloids

Alkaloid	Solvent system				
	(1)	(2)	(3)	(4)	(5)
DS-1	88	66	78	81	64
DS-2	82	56	74	77	56
DS-3	74	50	71	79	50
DS-4	76	42	68	70	38
DS-5	66	36	62	64	36
DS-6	68	41	72	71	12
DS-7	62	38	66	67	10
DS-8	28	24	52	66	2
DS-9	6	12	11	38	7
DS-10	0	2	2	26	0

Note :

$$hRf = \frac{\text{distance of spot center from start point} \times 100}{\text{distance of solvent front from start point}}$$

Table 5 ^{13}C-NMR spectra of DS-2, DS-3, and DS-9

Carbon	Chemical shift (ppm)		
	DS-2	DS-3	DS-9
C(2)	133.74 (s)	134.33 (s)	131.31 (s)
C(3)	61.28 (d)	60.37 (d)	54.03 (d)
C(5)	53.80 (t)	53.27 (t)	50.62 (t)
C(6)	23.93 (t)	21.69 (t)	24.29 (t)
C(7)	117.67 (s)	106.54 (s)	118.01 (s)
C(8)	107.84 (s)	127.12 (s)	107.77 (s)
C(9)	154.52 (s)	118.00 (d)	154.32 (s)
C(9)-O$\underline{C}$H$_3$	55.33 (q)	-	55.21 (q)
C(10)	99.74 (d)	119.11 (d)	99.55 (d)
C(11)	121.80 (d)	121.26 (d)	121.68 (d)
C(12)	104.21 (d)	111.08 (d)	104.53 (d)
C(13)	137.27 (s)	136.28 (s)	137.13 (s)
C(14)	29.96 (t)	32.50 (t)	31.92 (t)
C(15)	39.95 (d)	30.56 (d)	34.85 (d)
C(16)	111.54 (s)	107.25 (s)	111.93 (s)
C(17)	160.47 (d)	155.19 (d)	159.62 (d)
C(17)-O$\underline{C}$H$_3$	61.53 (q)	-	61.38 (q)
C(18)	12.87 (q)	14.99 (q)	11.30 (q)
C(19)	19.10 (t)	73.84 (d)	19.12 (t)
C(20)	40.71 (d)	40.71 (d)	39.07 (d)
C(21)	57.78 (t)	56.76 (t)	51.70 (t)
C(22): ester	169.26 (s)	167.98 (s)	168.96 (s)
C(23): O$\underline{C}$H$_3$	51.36 (q)	51.11 (q)	51.18 (q)

Table 6 ^{13}C-NMR spectra of DS-6, DS-7, and DS-8

Carbon	Chemical shift (ppm)		
	DS-6	DS-7	DS-8
C(2): amide	181.16 (s)	181.22 (s)	181.55 (s)
C(3)	71.31 (d)	71.79 (d)	73.85 (d)
C(5)	54.15 (t)	54.31 (t)*	54.38 (t)#
C(6)	34.89 (t)	35.46 (t)	35.20 (t)
C(7)	56.94 (s)	56.41 (s)	55.61 (s)
C(8)	133.79 (s)	133.85 (s)	133.39 (s)
C(9)	124.60 (d)	124.91 (d)	122.89 (d)
C(10)	122.54 (d)	122.36 (d)	122.54 (d)
C(11)	127.70 (d)	127.57 (d)	128.03 (d)
C(12)	109.60 (d)	109.56 (d)	109.86 (d)
C(13)	140.20 (s)	140.23 (s)	140.98 (s)
C(14)	30.21 (t)	29.18 (t)	28.42 (t)
C(15)	30.51 (d)	30.08 (d)	30.49 (d)
C(16)	109.89 (s)	107.40 (s)	106.95 (s)
C(17)	154.98 (d)	153.85 (d)	154.08 (d)
C(18)	18.66 (q)	14.89 (q)	14.87 (q)
C(19)	72.16 (d)	74.04 (d)	74.61 (d)
C(20)	37.94 (d)	40.96 (d)	40.53 (d)
C(21)	53.55 (t)	53.40 (t)*	54.32 (t)#
C(22): ester	167.62 (s)	167.09 (s)	167.13 (s)
C(23): O$\underline{C}H_3$	50.98 (q)	50.93 (q)	50.76 (q)

* Assignments may be interchanged

Assignments may be interchanged