

CHAPTER III

EXPERIMENTAL

I. Source of Plant Materials

The tubers of *Stephania glabra* (Roxb.) Miers collected from Samoeng, Chiang Mai, Thailand, were purchased from the market in Bangkok in July 1984. The plant materials after cultivation were identified by Mrs. Leena Phuphathanaphong and the voucher specimen is deposited at the Royal Forestry Department, Phahonyothin Road, Bangkok, Thailand.

II. General Techniques

A. Thin Layer Chromatography

- Technique : One way, ascending, tank saturated
- Adsorbent : Silica gel G (E. Merck) 30 g./60 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 45 minutes
- Solvent systems : a) Ether : Hexane (8:2)
- b) Benzene : Ether (2:1)
- c) Chloroform : Methanol (95:5)
- d) Ethyl acetate : Methanol (95:5)
- e) Chloroform : Acetone (5:4)

Distance : 15 cm
Temperature : 25-30°C
Detection : Modified Dragendorff's spray reagent, according to Munier and Macheboenfs.

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

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

Solution A and B, 5 ml each, were mixed, 20 ml of glacial acetic acid and 70 ml of distilled water were added and used as spray reagent. The alkaloids give orange or yellowish orange spots with the reagent.

B. Column Chromatography

Column size : 2.5 cm x 40 cm, 5 cm x 50 cm
Adsorbent : Silica gel 0.040-0.063 mm (E. Merck)
Packing : Adsorbent packed wet into the column
Addition of alkaloidal material to column :

Crude alkaloid was dissolved in small amount of chloroform, then, mixed with small quantity of adsorbent. After the solvent was evaporated, triturated and added to the top of the column.

Solvents : Ether (anh.) J.T. Baker
: Hexane AR. J.T. Baker
: Chloroform GR. Merck
: Methanol GR. Merck



Collection of eluate

: Fractions of 20 ml were collected.

Examination of eluate

: Those fraction giving positive test to the Modified Dragendorff's reagent were examined by thin-layer chromatography.

C. Physical constant

1) Melting points were determined by Gallenkamp MFB 595 Melting Point Apparatus. The values recorded were uncorrected.

2) Optical rotations were determined by Perkin-Elmer 241 Polarimeter.

3) Elemental analysis were determined by Perkin-Elmer 240 C Elemental Analyzer.

D. Spectroscopy

1) Ultraviolet absorption spectra were determined by Hewlett-Packard 8450 A UV/VIS spectrophotometer.

2) Infrared absorption spectra were determined by Perkin-Elmer 281 B Infrared spectrophotometer.

3) Nuclear magnetic resonance spectra were determined by Bruker WP 200 MHz Supercon (FT) Spectrometer.

4) Mass spectra were determined by Kratos MS 9/50 instrument.

III. Extraction and isolation of alkaloids from the tubers of *Stephania glabra* (Roxb.) Miers.

A. Extraction of crude alkaloid

The dried coarsely powdered tubers (700 g) of *Stephania glabra* (Roxb.) Miers were extracted with 95% ethanol (10 L) by percolation. The filtrate was concentrated under reduced pressure. The glacial acetic acid (50 ml) was added to the residue, and then poured into warm distilled water in order to give about 5% acetic acid solution. The mixture was filtered through Kieselguhr pad. The acidic filtrate was extracted with petroleum ether (10 x 500 ml). The combined petroleum ether extracts were washed with water and dried over anhydrous sodium sulfate, then concentrated under reduced pressure to give dried crude alkaloid A (4 g). The aqueous acidic solution was made alkaline (about pH 8-9) with strong ammonia water. The alkaline solution was extracted exhaustively with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to give dried yellowish-brown crude alkaloid B (14.5g). The aqueous layer was further extracted with chloroform : ethanol 2:1 (4 x 200 ml). The combined extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to give brown crude alkaloid C (2.0 g). From TLC examinations on Silica gel plates showed that the crude alkaloid A contains at least 3 major and 2 minor alkaloids, the crude alkaloid B at least 3 major and 2 minor alkaloids and the crude alkaloid C only at the base line. These TLC patterns are shown in Figure I and Figure II, pages 106-107.

B. Isolation of alkaloids

The crude alkaloid B (14.5 g) was divided into six equal portions, each (2.4 g) was treated in the same manner. Dissolved each portion of crude alkaloid B in chloroform (5 ml), mixed with small

amount of silica gel. After the solvent was evaporated, triturated and added to the top of the silica gel column (2.5 cm x 30 cm) which was wet packed before by using ether : hexane (1:1) as solvent.

The column was eluted with ether : hexane (2:1) and 20 ml fractions were collected until no traces of alkaloid could be detected. Fractions were determined by TLC, combined the like fractions together and obtained in three portions of the eluate. Each portion was concentrated to dryness under reduced pressure.

The first portion yielded an amorphous yellow alkaloid (180 mg), designated as SG₁ (melting point 145°C).

The yellow crystals were obtained from the second portion and recrystallized in methanol yield pale yellow prisms (6.6 g), designated as SG₂ (melting point 138-139°C).

The yellow solid was obtained from the third portion and recrystallized in methanol yield pale yellow prisms (0.6 g), designated as SG₃ (melting point 175°C).

C. Identification of Alkaloid SG₁ as 1-Capaurine

SG₁ was obtained as pale yellow solid. It is soluble in chloroform, in ethanol and in methanol.

1. hRf values

The hRf values were obtained from the solvent systems mentioned on page 65 : a = 64, b = 16, c = 76, d = 78, e = 68.

(hRf = Rate of flow in chromatography multiplied by 100)

The thin layer chromatograms of alkaloid SG₁ are shown in Figures III-VII, pages 108-112.

2. Melting Point

145°C

3. Elemental Analysis

The percentage of carbon, hydrogen, nitrogen and oxygen are shown below :-

C = 67.45

H = 6.71

N = 3.60

O = 22.24

Then molecular formula should be $C_{21}H_{25}NO_5$.

4. Specific Optical Rotation
 $[\alpha]_D^{24} -259.8^\circ$ (C = 0.112 g/100 ml, chloroform)
5. Ultraviolet Absorption Spectrum
 $\lambda_{\max}^{\text{MeOH}}$ (log ϵ) : 213 (4.6567), 223 sh (4.3964),
279 (3.4994) nm

 $\lambda_{\max}^{\text{MeOH} + \text{NaOH}}$ (log ϵ) : 215 (4.7294), 281 (3.6915) nm

 $\lambda_{\max}^{\text{MeOH} + \text{HCl}}$ (log ϵ) : 212 (4.7001), 224 sh (4.3489),
275 (3.6217) nm
6. Infrared Absorption Spectrum
 ν_{\max}^{KBr} 830, 985, 1100, 1270, 1490, 1585, 2820,
2930, 2985, 3520 cm^{-1}
7. Nuclear Magnetic Resonance Spectrum (200 MHz, CDCl_3

and T.M.S. as internal standard)

δ (ppm)	3.52 (2H, d, J = 15.4)	$\text{C}_8\text{-H}$
	3.85 (6H, s)	2 x OCH_3
	3.87 (3H, s)	OCH_3
	3.89 (3H, s)	OCH_3



4.25 (1H, d, J = 16)	C ₈ -H
6.36 (1H, s)	C ₄ -H
6.78 (1H, d, J = 8.4)	C ₁₁ -H
6.88 (1H, d, J = 8.4)	C ₁₂ -H

8. Mass Spectrum

m/e (% rel.int.)	371 (M ⁺ , 73.16), 370 (42.30)
	208 (6.96), 206 (17.92), 164 (100)
	and 149 (68.70)

SG₁ is identical in ultraviolet, infrared, nuclear magnetic resonance and mass spectra with l-capaurine, the known alkaloid from *Corydalis* species (Kametani *et al.*, 1968). It is therefore concluded that SG₁ is l-capaurine.

D. Identification of Alkaloid SG₂ as l-Tetrahydropalmatine

SG₂ was obtained as pale yellow prisms and is soluble in chloroform, in ethanol and in methanol.

1. hRf Value

The hRf value were obtained from the solvent systems as mentioned on page 65 : a = 53, b = 22, c = 93, d = 68, e = 73.

The thin layer chromatograms of alkaloid SG₂ are shown in Figures III-VII, pages 108-112.

2. Melting Point

148-149°C

3. Elemental Analysis

The percentage of carbon, hydrogen, nitrogen and oxygen are shown below :

$$C = 71.23$$

$$H = 7.12$$

$$N = 3.95$$

$$O = 17.70$$

Then molecular formula should be $C_{21}H_{25}NO_4$.

4. Specific Optical Rotation

$$[\alpha]_D^{24} -294.2^\circ \quad (C = 0.121 \text{ g/100 ml, chloroform})$$

5. Ultraviolet Absorption Spectrum

$$\lambda_{\max}^{\text{MeOH}} (\log \epsilon) : 209 (4.6387), 219 \text{ sh } (4.2318), \\ 282 (3.6182) \text{ nm}$$

$$\lambda_{\max}^{\text{MeOH} + \text{NaOH}} (\log \epsilon) : 221 \text{ sh } (4.2926), 282 (3.6718) \text{ nm}$$

$$\lambda_{\max}^{\text{MeOH} + \text{HCl}} (\log \epsilon) : 220 \text{ sh } (4.0913), 230 (4.1668), \\ 282 (3.6937) \text{ nm}$$

6. Infrared Absorption Spectrum

$$\nu_{\max}^{\text{KBr}} \quad 885, 1000, 1120, 1240, 1500, 1605, 2825, \\ 2930, 3000 \text{ cm}^{-1}$$

7. Nuclear Magnetic Resonance Spectrum (200 MHz, CDCl_3

and T.M.S. as internal standard)

δ (ppm)	3.54 (1H, d, $J = 14.76$)	C_8 -H
	3.85 (6H, s)	2 x OCH_3
	3.87 (3H, s)	OCH_3
	3.89 (3H, s)	OCH_3
	4.25 (1H, d, $J = 15.7$)	C_8 -H
	6.62 (1H, s)	C_4 -H
	6.73 (1H, s)	C_1 -H
	6.79 (1H, d, $J = 8.4$)	C_{11} -H
	6.89 (1H, d, $J = 8.4$)	C_{12} -H

8. Mass Spectrum

m/e (% rel.int.) 355 (M^+ , 68.34), 354 (52.78),
192 (8.94), 190 (28.34), 164 (100)
and 149 (71.75)

The UV, IR, NMR and Mass spectra of SG_2 are identical with data from the literature of 1-tetrahydropalmatine isolated from *Stephania glabra* (Roxb.) Miers. in all aspects (Patra *et al.*, 1980; Bhakuni and Gupta, 1982). It is therefore concluded that SG_2 is 1-Tetrahydropalmatine.

E. Identification of Alkaloid SG_3 as 1-Xylopinine

SG_3 was obtained as pale yellow prisms. It is soluble in chloroform, in ethanol and in methanol.

1. hRf Value

The hRf values were obtained from the solvent systems as mentioned on page 65 : a = 20, b = 10, c = 90, d = 50, e = 65.

The thin layer chromatograms of alkaloid SG_3 are shown in Figures III-VII, pages 108-112.

2. Melting Point

175°C

3. Elemental Analysis

The percentage of carbon, hydrogen, nitrogen and oxygen are shown below :

C = 70.58

H = 7.06

N = 3.90

O = 18.46

8. Mass Spectrum

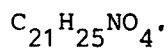
m/e (% rel.int.) 355 (M^+ , 29.13), 354 (11.98),
192 (1.49), 190 (12.99), 164 (100)
and 149 (5.18)

The UV, IR, NMR and Mass spectra of SG_3 are identical with data from the literature of 1-xylopinine isolated from *Stephania suberosa* Forman in all aspects (Patra *et al.*, unpublished at this time). It is therefore concluded that SG_3 is 1-xylopinine.



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Then the molecular formula of SG₃ should be



4. Specific Optical Rotation

$[\alpha]_D^{24} -298^\circ$ (C = 0.100 g/100 ml, chloroform)

5. Ultraviolet Absorption Spectrum

$\lambda_{\max}^{\text{MeOH}}$ (log ϵ) : 220 sh (3.7019),
285 (3.2095) nm

$\lambda_{\max}^{\text{MeOH} + \text{NaOH}}$ (log ϵ) : 211 (4.0711), 220 sh (4.7008),
286 (3.2423) nm

$\lambda_{\max}^{\text{MeOH} + \text{HCl}}$ (log ϵ) : 209 (4.0452), 220 sh (3.4455),
229 (3.5141),
284 (3.1905) nm

6. Infrared Absorption Spectrum

ν_{\max}^{KBr} 835, 1000, 1120, 1250, 1505, 1610, 2820,
2930, 3000 cm^{-1}

7. Nuclear Magnetic Resonance Spectrum (200 MHz, CDCl_3

and T.M.S. as internal standard)

δ (ppm)	3.68 (1H, d, J = 15.7)	C ₈ -H
	3.86 (3H, s)	OCH ₃
	3.86 (3H, s)	OCH ₃
	3.87 (3H, s)	OCH ₃
	3.89 (3H, s)	OCH ₃
	3.95 (2H, d, J = 14.7)	C ₈ -H
	6.58 (1H, s)	C ₉ -H
	6.62 (1H, s)	C ₄ -H
	6.66 (1H, s)	C ₁₂ -H
	6.74 (1H, s)	C ₁ -H