CHAPTER III

EXPERIMENTAL

1. Source of plant materials

The leaves of *Micromelum minutum* Seem. were obtained from Erawan Waterfall, Kanchanaburi Province, Thailand in June 25, 1979. The plant materials were authenticated by comparison with the specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives.

2. General techniques

2.1 Analytical thin layer chromatography

Technique:	one way, ascending
Adsorbent:	silica gel G (E. Merck), calcium sulphate binder 13%; 30 g/60 ml of distilled water.
Plate size:	10 cm x 20 cm, 20 cm x 20 cm.
Layer thickness:	0.25 mm.
Activation:	air dried for 15 minutes and then at 105^{o}C for 1 hour.
Solvent system:	a) ethyl acetate : chloroform = 9 : 1
	b) chloroform : ethyl alcohol = 9 : 1
	c) chloroform : acetone = 5 : 4
	d) diethyl ether : chloroform = 1 : 1
	e) diethyl ether

Distance : 15 cm.

Laboratory temperature: 24 - 30°C

Detection: Dragendorff's spray reagent, giving an orange colour after spraying.

2.2 Preparative thin layer chromatography

Technique: one way ascending, double development.

Adsorbent: silica gel G (E. Merck) : silica gel GF_{254} (E. Merck) = 2 : 1, 60 g/120 ml of distilled water.

Plate size: 20 cm x 20 cm.

Layer thickness: 0.5 mm.

Activation: air dried for 30 minutes and then at 105°C for 1 hour.

Solvent system: ethyl acetate : chloroform = 9 : 1.

Distance: 18 cm.

Laboratory temperature: 24 - 30°C

Detection: under ultraviolet light (wavelength 254 nm)

2.3 Solvents and chemical used

a) Ammonium hydroxide, strong solution

b) Acetic acid, glacial

c) Sodium sulphate, anhydrous

d) Ethyl alcohol, 95%

e) Ethyl acetate

f) Chloroform

g) Acetone

h) Diethyl ether, anaesthetic

i) Diethyl ether, anhydrous

2.4 Melting point determination

Melting point was determined by heating stage microscope (Reichert).

2.5 Ultraviolet absorption spectrum

Ultraviolet absorption spectrum was recorded in ethanol using a Unicam SP 1800 ultraviolet spectrophotometer.

2.6 Infrared absorption spectrum

Infrared absorption spectrum was obtained in a potassium bromide disc using a Perkin-Elmer 283 grating spectrometer.

2.7 Nuclear Magnetic Resonance (NMR) spectrum

NMR spectrum was determined in deuterochloroform using a Varian A-60D instrument. Tetramethylsilane (TMS) was used as the internal standard.

2.8 Mass spectrum

Mass spectrum was recorded in a Dupont 21-490B mass spectrometer.

3. The isolation of alkaloid(s) from the leaves of *Micromelum minutum* Seem.

The dried coarsely powdered leaves (1.5 kg) were macerated with 95% ethyl alcohol (4 l) for five days and filtered. The marc was remacerated with another portion of 95% ethyl alcohol (2 x 4 l). The combined filtrate was concentrated under reduced pressure to syrupy mass till no traces of ethyl alcohol left, mixed with glacial acetic acid (300 ml) then poured into a large number of warm water to give about 5% acetic acid solution, well shaken and left to stand overnight. The filtrated acid extract was made alkaline (pH 9) with strong solution of ammonium hydroxide and extracted with chloroform (10 x 400 ml). The combined chloroform extract was washed with distilled water (5 x 400 ml), dried over anhydrous sodium sulphate and concentrated under reduced pressure to yield a syrupy crude base (5.35 g).

The crude base (0.2 g each) was dissolved in chloroform (1.5 ml) and applied onto the preparative thin layer chromatographic plates by streaking.

The chromatogram was developed twice in ethyl acetate: chloroform = 9 : 1 and then observed under UV light as a guide to the alkaloidal bands. Two bands were scraped off and kept separately. Each scraped band was packed into a column (diameter 2.5 cm), eluted with ethyl acetate : 95% ethyl alcohol = 1 : 1 till no traces of alkaloid could be detected. These combined eluates were evaporated to dryness under reduced pressure. The treatment of remaining crude base was done similarly.

The eluate from band 1 gave light brown syrupy mass (309 mg) which was examined by thin layer chromatography showing that it was

a mixture of two alkaloids (Fig. 15, p. 64). However, the mass was present in too small the quantity to be separated and identified. No further study of this band has been made.

The base eluate from band 2 after evaporated to dryness yielded pale yellow needle crystals (324 mg). Recrystallisation of the crystals in anhydrous diethyl ether gave white needle crystals (139 mg) designated as V-1 and was subsequently identified as flindersine.

4. Identification of V-1 as flindersine

V-1 was obtained as white needle crystals from anhydrous diethyl ether. It was soluble in methyl alcohol, ethyl alcohol, chloroform, diethyl ether and petroleum ether, and insoluble in water.

hR_f values

a) 37, on silica gel G / ethyl acetate : chloroform = 9 : 1.

b) 57, on silica gel G / chloroform : ethyl alcohol = 9 : 1.

c) 45, on silica gel G / chloroform : acetone = 5 : 4

d) 25, on silica gel G / diethyl ether.

Melting point

182-183°C

Molecular weight

227 (mass spectrometry)

Ultraviolet absorption spectrum

λ_{max}^{EtOH} 236, 332, 349, 366 nm

Infrared absorption spectrum

ν_{max}^{KBr} 1660 (C = O), 1625 (C = C)

NMR spectrum in deuterochloroform

δ values	Proton	Multiplicity
1.57	6H	s (gem-dimethyl)
5.58	1H	d, J = 10 Hz (olefinic, C-3')
6.88	1H	d, J = 10 Hz (olefinic, C-4')
7.40-7.71	3H	m (aromatic, C-6, C-7, C-8)
8.02	1H	d, J = 7.5 Hz (aromatic, C-5)

Mass spectrum

m/e (%) 227 (M^+, 48.0), 212 (M^+-CH_3,100), 198 (4.0), 186 (2.3
184 (3.1), 183 (3.5), 166 (4.0), 154 (2.7),
146 (3.1), 140 (2.3), 130 (1.9), 129 (2.3),
128 (2.3), 120 (4.0), 115 (2.7), 106 (5.5)

V-1 is identical in melting point, ultraviolet, infrared and mass spectra with flindersine isolated from *Flindersia australis* R.Br. (Brown *et al.*, 1954), *Haplophyllum tuberculatum* (Lavie *et al.*, 1968) and *Atalantia roxburghiana* Hook f. (Bowen and Lewis, 1978), also with synthetic flindersine (Brown *et al.*, 1956).