CHAPTER III

EXPERIMENTAL

Source of Plant Materials

The bark of *Erythrophleum teysmannii* Craib *var. puberulum* Craib was obtained from Pakthongchai, Nakhon Ratchasima, Thailand in May, 1981. The plant materials were authenticated by comparison with herbarium specimens at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

General Techniques

A. Thin Layer Chromatography

Technique : one way, ascending

Adsorbent : silica gel G + silica gel GF_{254} (E. Merck) (15 + 15) g/ 60 ml distilled water,

aluminium oxide G + aluminium oxide GF_{254} (E. Merck) (35 + 35) g/80 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 : 1) silica gel (G + GF) / cyclohexane + chloroform + diethylamine (5 + 4 + 1)

2) silica gel (G + GF) / cyclohexane + chloroform + methanol (3 + 6 + 1)

3) aluminium oxide (G + GF) / cyclohexane + chloroform + diethylamine (5 + 4 + 1)

4) aluminium oxide (G + GF) / ether + ethanol + diethylamine (98 + 1.5 + 0.3)

5) aluminium oxide (G + GF) / benzene : chloroform (1 : 9)

6) aluminium oxide (G +GF) / chloroform : methanol (98 : 2)

Distance : 15 cm

Temperature : 25°-30°C

Detection : 1) UV light of 254 nm

The alkaloids become visible as dark spots on the yelloish green fluorescent background plate in UV light of 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, 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 orange yellow spots with Dragendorff's reagent.

B. Column Chromatography

Column size : 5 cm x 50 cm

Adsorbent : aluminium oxide neutral, activity III 0.063-0.200 mm (E. Merck).

Packing : adsorbent packed dry into the column.

Adding of alkaloidal material to column : crude alkaloid was dissolved in small amount of organic solvent, mixed with small quantity of adsorbent, air dried, triturated and added onto the top of a dry column.

Solvent : 1) petroleum ether (40°-60°C)

2) benzene

3) chloroform

4) methanol

Collection of eluate : fractions of 20 ml were collected.

Examination of eluate : those fractions giving an orange color with Dragendorff's spray reagent were examined by thin-layer chromatography.

C. Physical Constant

Melting point was determined on a Reichert Melting Point Apparatus. The value recorded is uncorrected.

D. Spectroscopy

1) Ultraviolet absorption spectrum was obtained with a Shimadzu Double-Beam Spectrophotometer.

2) Infrared absorption spectrum was obtained with a Perkin-Elmer 283 Spectrophotometer.

3) Nuclear magnetic resonance spectrum was obtained with a HA-100 Instrument in deuterochloroform, using tetramethylsilane (T.M.S.) as internal reference.

4) Mass spectrum was determined on a Hitachi Hi-Resolution Mass System at 70 eV with inlet temperature 130°C

Extraction and Isolation of Alkaloids from the Bark of *Erythrophleum teysmannii* Craib *var. pubérulum* Craib

A. Extraction of Crude Alkaloid

The dried coarsely powdered bark (8.0 kg) was moistened with 3 liters of 5 % sodium carbonate solution overnight. The moistened powder was then macerated with dichloromethane (20 l) for three days and filtered. The marc was remacerated wiyh dichloromethane (20 l) for three days and filtered. The filtrates were concentrated under reduced pressure and combined to yield the total crude extract. The total crude extract was dissolved in glacial acetic acid (300 ml) and then poured into warm distilled water to give 5 % acetic acid solution (6 l) and the insoluble materials were removed by filtration through kieselguhr. The filtrate was shaken with ether (5 x 500 ml) and the ether layers rejected. The acidic layer was cooled in refrigerator overnight and made alkaline, (pH about 8), with strong ammonia water. The alkaline solution was extracted with chloroform (6 x 500 ml) and the complete extraction was controlled with Dragendorff's reagent. The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulfate and evaporated under reduced pressure to dryness to give a crude alkaloid (3.8 g). The crude alkaloid contained at least ten alkaloids as indicated by thin layer chromatography on silica gel plates (Figure 12-13, pp.64-65).

B. Isolation of Alkaloid KS_1

Neutral aluminium oxide (activity I, 350 g) was deactivated with distilled water (15 ml) to give aluminium oxide activity III. The moistened aluminium oxide was packed in a column (5 cm x 50 cm). The crude alkaloid

(3.5 g) was dissolved in chloroform (10 ml) and adsorbed on small amount of aluminium oxide activity III. It was air dried, triturated and packed onto the top of aluminium oxide activity III column. The eluates were collected in 20 ml fractions. The various fractions were analyzed by using silica gel (G + GF) / cyclohexane + chloroform + diethylamine (5 + 4 + 1) tlc system.

tography of the crude alkaloid.

Table 8

Thin layer chromatography of the crude alkaloid

Tube No.	Solvent system: petroleum ether+ benzene+chloroform	Volume (l)	Fraction No.	Remark
1-80	300 + 75 + 100	1.6	1	no alkaloid
81-150	275 + 75 + 400	1.4	2	no alkaloid
151-210	275 + 75 + 600	1.2	3	trace of alkaloids
211-315	0 + 1 + 3	2.0	4	KS_1 as main alkaloid
316-330	0 + 0 + 1	0.3	5	KS_1 and the mixture of alkaloids
331-390	0 + 0 + 1	1.2	6	mixture of three amorphous alkaloids
391-480	2 % methanol in chloroform	1.8	7	mixture of three amorphous alkaloids

Fraction 4 contained one alkaloid and was concentrated under reduced pressure to dryness, dissolved in chloroform (1 ml), mixed with diethyl ether (15 ml) and allowed to stand in the dark for 24 hours. White prisms were obtained which were washed with diethyl ether. Drying the alkaloid in desiccator overnight yielded 160 mg of alkaloid designated as KS_1.

C. Identification of Alkaloid KS_1 as Norerythrophlamide

KS_1 was obtained as white prisms. It was soluble in chloroform, in ethanol and in ethyl acetate.

1. hR_f Values

The hR_f values given are those obtained with the following systems:

a) silica gel (G +GF) / cyclohexane + chloroform + diethylamine (5 + 4 + 1) = 15

b) silica gel (G + GF) / cyclohexane + chloroform + methanol (3 + 6 + 1) =37

c) aluminium oxide (G + GF) / chloroform : methanol (98 : 2) = 77

d) aluminium oxide (G + GF) / benzene : chloroform (1 : 9) = 9

e) aluminium oxide (G + GF) / ether + ethanol + diethylamine (98 + 1.5 + 0.3) = 6

The thin layer chromatograms of alkaloid KS_1 are shown in Figures 14-18, pp. 66-70.

2. Melting Point

167°-168°C (uncorrected)

3. Molecular Weight

435 (mass spectrometry)

4. Ultraviolet Absorption Spectrum (Ethyl Alcohol)

λ_{max} 214 nm

5. Infrared Absorption Spectrum (Potassium Bromide Disc)

ν_{max} (cm^{-1})	3370, 3190	(hydroxyl group)
	2980-2850	(C-H)
	1735, 1720	(C_7-carbonyl group)
	1703	(carbonyl group of C_4-carbomethoxy)
	1645	(double bond conjugation)
	1600	(α,β unsaturated amide)
	1450,1203, 1160, 1125, 865	

6. N.M.R Spectrum in Deuterochloroform at 100 MHz in δ Values (ppm) from Tetramethylsilane (T.M.S)

δ (ppm)	0.84 (3 H, s)	C_{10}-CH_3
	1.08 (3 H, d, *J* 7 Hz)	C_{14}-CH_3
	1.40 (3 H, s)	C_4-CH_3
	3.08 (3 H, s)	N-CH_3
	3.52-3.62 (4 H, m)	N-CH_2-CH_2-O
	3.71 (3 H, s)	-$COOCH_3$
	5.90 (1 H, s)	olefinic

7. Mass Spectrum

m/e (%) 436 (34), 435 (M^+, 100), 376 (23), 362 (16), 361 (41), 360 (33), 301 (13), 283 (7), 183 (15), 109 (14), 107 (10), 91 (21), 81 (11), 74 (22), 69 (13), 57 (14), 55 (12), 53 (11), 44 (14), 43 (9)

KS_1 is identical in ultraviolet, infrared, nmr and mass spectra with the identified norerythrophlamide spectral data from *Erythrophleum ivorense* A. Chev. and *E. chlorostachys* Baill. (Cronlund and Sandberg, 1971 and Loder *et al.*, 1974). It is therefore concluded that KS_1 is norerythrophlamide.