

CHAPTER III

EXPERIMENTAL

1. Source of Plant Materials

The leaves of *Evodia lepta* Merr. were obtained in April, 1982 and of *E. gracilis* Kurz. in May, 1983 from Doi Suthep, Chiangmai province, Thailand. The plant materials were authenticated by comparison with the herbarium 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 : kieselgel G (E. Merck) Calcium sulphate binder
13%, 30 g/60 ml distilled water.
- Plate size : 10 cm x 20 cm and 20 cm x 20 cm
- Layer thickness : 0.25 mm
- Activation : air dried for 15 minutes and then at 110°C
for 1 hour.
- Solvent system :
1. 5% acetone in chloroform
 2. 5% methanol in ethyl acetate
 3. ethyl acetate : acetone (1:1)
 4. 10% ethanol in chloroform

5. benzene : ethyl acetate (1:1)

6. 5% ethanol in benzene

Distance : 15 cm

Temperature : 25-30°C

Detection : Dragendorff 's spray reagent

solution A : bismuth subnitrate (8.5 g),
distilled water (40 ml) and
glacial acetic acid (10 ml)

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

solution A and solution B, 5 ml each, were
mixed, 20 ml of glacial acetic acid and
80 ml of distilled water were added and
used as spray reagent. The alkaloids give
orange or orange yellow spots with
Dragendorff 's reagent.

2.2 Column Chromatography

Adsorbent : kieselgel 60, 0.040-0.063 mm (E. Merck)
aluminium oxide neutral, 0.063-0.200 mm
(E. Merck)

Packing : dry packing

Alkaloids loading : crude alkaloid was dissolved in small
amount of organic solvent, mixed with
small quantity of adsorbent, air dried,
trituated and added on the top of a dry
column.

2.3 Melting Points

Melting points were determined by means of a Gallenkamp Melting Point Apparatus. The values recorded are uncorrected.

2.4 Ultraviolet Absorption Spectra

Ultraviolet absorption spectra were obtained with a Shimadzu Double-Beam Spectrophotometer Model UV-180.

2.5 Infrared Absorption Spectra

Infrared absorption spectra were determined with a Shimadzu Model IR 440 Spectrophotometer, absorption bands were reported in wave numbers (cm^{-1}).

2.6 Nuclear Magnetic Resonance (NMR) Spectra

Proton NMR spectra were recorded with a Varian T-60A instrument operating at 60 MHz. Tetramethylsilane was used as an internal standard and chemical shifts were reported on the ppm scale.

2.7 Mass Spectra

Mass spectra were obtained with a Varian MAT-112 S double focusing spectrometer operating at 70 eV.

3. Extraction

3.1 Extraction of Alkaloids from *Evodia leptota* Merr. Leaves.

The dried and powdered plant material (600 g) was macerated twice for 3 day-periods with 95% ethyl alcohol (5 L and 4 L). After combination, the alcoholic extracts were evaporated

under reduced pressure until no trace of ethyl alcohol left. The resultant syrupy mass was mixed with glacial acetic acid (500 ml), stirred thoroughly and poured into a large volume of warm water to give about 10% acetic acid solution and filtered. The acidic filtrate was basified with strong solution of ammonium-hydroxide and then extracted exhaustively with chloroform (10 L). The combined chloroform extract was dried over anhydrous sodium sulphate and concentrated under reduced pressure to give a dark brown syrupy mass (18.5 g).

Separation of EL-1, EL-2 and EL-3

The crude extract (18.5 g) was divided into 18 equal portions and each one was treated in the same manner. Each portion (approx. 1 g) was dissolved in chloroform (5 ml), adsorbed onto silica gel (5 g), dried, then placed on the top of a dry silica gel column (2.5 x 15 cm), and eluted with 10% ethyl alcohol in chloroform. Fractions of 5 ml each were collected and examined by thin layer chromatography (TLC). Those fractions of similar pattern were combined.

- a. F₁ containing mixture of alkaloids (4.24 g)
- b. F₂ containing one alkaloid (2.671 g) designated as EL-2
- c. F₃ containing mixture of two alkaloids
- d. F₄ containing one alkaloid (1.529 g) designated as EL-3

(Fig. 1-3 pages 94-96)

Residue from F₁ (4.24 g) was rechromatographed in the same manner as described above, using 10% acetone in chloroform as the eluent. By this procedure, one alkaloid (420 mg) was obtained and designated as EL-1 (Fig. 1-3 pages 94-96).

3.2 Extraction of Alkaloids from *Evodia gracilis* Kurz. Leaves.

The dried coarsely powdered leaves (800 g) were extracted with chloroform (3 L) by soxhlet extractor for 6 hours, and then filtered. The filtrate was concentrated under reduced pressure to give light brown syrupy mass (58 g). The residue (58 g) was treated by the usual procedure (as described in the extraction of alkaloids from *E. lepta* Merr. leaves) to yield dark brown syrupy mass (10 g). Extraction of alkaloids by maceration with 95% ethyl alcohol or by refluxing with chloroform in soxhlet apparatus showed identical patterns of alkaloids on TLC.

Separation of EG-1 and EG-2

Crude extract (10 g) was divided into 5 portions. Each portion was chromatographed by using alumina column (5 x 7 cm) and chloroform was used as an eluent. Fractions of 20 ml being collected and resulting of fractions 2-8 yielded 627 mg of pure alkaloid designated as EG-1, fractions 9-21 yielded mixture of two alkaloids (EG-1 and EG-2) and fractions 21-30 yielded 180 mg of pure alkaloid designated as EG-2. (Fig. 4-6 pages 97-99)

4. Characterisation

EL-1 was obtained as colourless rosette, it is soluble in

chloroform, ether, acetone and ethanol

hRf values

- a) 80 (Fig. 1 page 94)
- b) 57 (Fig. 2 page 95)
- c) 77 (Fig. 3 page 96)

Melting point

110-115°C

Ultraviolet absorption spectrum

$\lambda_{\text{max}}^{\text{EtOH}}$ 288, 325, 336 nm (Fig. 7 page 100)

Infrared absorption spectrum (KBr disc)

ν_{max} 3425, 3220, 1640, 1612, 1584 cm^{-1} (Fig. 8 page 101)

NMR spectrum in deuterochloroform at 60 MHz in δ values (ppm)

form tetramethylsilane (T.M.S) (Fig. 9 page 102)

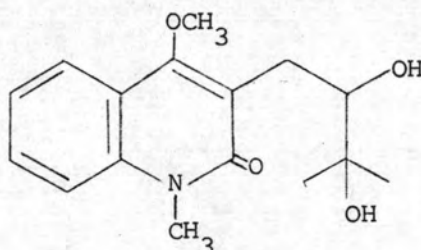
Chemical Shift (δ)	Proton and Multiplicity	Coupling Constants
1.29	6H, s, gem. dimethyl	
2.69	2H, dd, 4'-CH ₂	$J = 14, 10$ Hz
3.13	1H, dd, 3'-OH	$J = 14, 2.3$ Hz
3.40	1H, dd, 3'-H	$J = 10, 2.3$ Hz
3.64	3H, s, N-CH ₃	
3.94	3H, s, O-CH ₃	
5.00	1H, br, 2'-OH	
7.22-7.83	4H, m, aromatic	

Mass spectrum

m/e (% , rel. int.) (Fig. 10 page 103)

291 (M^+ , trace), 276 (1), 273 (3), 258 (3), 233 (16), 232 (100),
203 (21), 202 (21), 188 (35), 172 (13), 160 (5), 144 (11)

These data are in agreement with published values of edulinine (Toube *et al.*, 1967; Higa and Scheuer, 1974). It is therefore concluded that EL-1 is edulinine, the structure of which is shown below.



EL-2 was obtained as colourless plates, it is soluble in chloroform, ether, acetone and ethanol.

hRf values

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

- a) 57 (Fig. 1 page 94)
- b) 21 (Fig. 2 page 95)
- c) 20 (Fig. 3 page 96)

Melting point

215-216°C

Ultraviolet absorption spectrum

$\lambda_{\text{max}}^{\text{EtOH}}$ 213, 238, 316, 328 nm (Fig. 11 page 104)

Infrared absorption spectrum (KBr disc)

ν_{max} 3200, 2990, 1622, 1602, 1578, 1540, 1510 cm^{-1}

(Fig. 12 page 105)

NMR spectrum in deuteriochloroform at 60 MHz in δ values (ppm)

from tetramethylsilane (T.M.S) (Fig. 13 page 106)

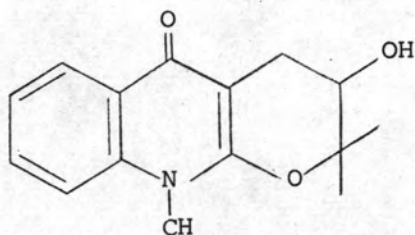
Chemical Shift	Proton and Multiplicity	Coupling Constants
(δ)		
1.31	3H, s, 2'-CH ₃	
1.52	3H, s, 2'-CH ₃	
2.93	2H, d, 4'-CH ₂ -	$J = 4.2$ Hz
3.44	3H, s, N-CH ₃	
3.91	1H, t, 3'-OH	$J = 4.2$ Hz
4.94	1H, br, 3'-OH	
7.10-7.80	3H, m, aromatic 6,7,8-H	
8.19-8.30	1H, m, aromatic 5-H	

Mass spectrum

m/e (% rel. int.) (Fig. 14 page 107)

259 (M^+ , 30), 242 (4), 230 (5), 229 (5), 226 (5), 216 (5),
200 (6), 188 (100), 187 (11), 186 (6), 181 (5)

These data are in agreement with published values of ribalinine (Corral *et al.*, 1973; Torres and Cassels, 1978; Vaquette *et al.*, 1976). It is therefore concluded that EL-2 is ribalinine, the structure of which is shown below :



EL-3 was obtained as colourless prisms, it is soluble in chloroform, ether, acetone and ethanol.

hRf values

The hRf values given are those obtained with the following systems.

- a) 48 (Fig. 1 page 94)
- b) 14 (Fig. 2 page 95)
- c) 13 (Fig. 3 page 96)

Melting point

179-182°C

Ultraviolet absorption spectrum

$\lambda_{\text{max}}^{\text{EtOH}}$ 238, 252, 308, 318 nm (Fig. 15 page 108)

Infrared absorption spectrum (KBr disc)

ν_{max} 3430, 2980, 1620, 1580, 1518, 1458 cm^{-1}
(Fig. 16 page 109)

NMR spectrum in deuteriochloroform at 69 MHz in δ values (ppm)

from tetramethylsilane (T.M.S) (Fig. 17 page 110)

Chemical Shift	Proton and Multiplicity	Coupling Constants
(δ)		
1.13	3H, s, 2'-CH ₃	
1.34	3H, s, 2'-CH ₃	
2.76	1H, br, 4'-OH	
3.22	2H, d, 3'-CH ₂ -	$J = 9$ Hz
3.57	3H, s, N-CH ₃	
4.82	1H, t, 4'-H	$J = 9$ Hz
7.12-7.62	3H, m, aromatic, 6,7,8-H	
8.22-8.37	1H, m, 5-H	

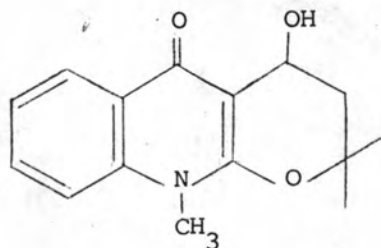
Mass spectrum

m/e (% rel. int) (Fig. 18 page 111)

259 (M^+ , 54), 244 (7), 226 (18), 216 (35), 200 (33), 189 (40)

188 (100), 134 (23), 187 (9), 144 (12), 134 (23)

All of these data are suggested the possibility of EL-3 as an isomer of EL-2. This skeleton has never been found elsewhere either naturally or synthetically. The tentative structure of unusual 4'-OH pyroquinolone (pyranoquinolone) is shown below.



EG-1 was obtained as colourless clusters, it is soluble in chloroform, ether, acetone and ethanol.

hRf values

The hRf values given are those obtained with the following systems.

d) 56 (Fig. 4 page 97)

e) 57 (Fig. 5 page 98)

f) 45 (Fig. 6 page 99)

Melting point

165-166°C

Ultraviolet absorption spectrum

$\lambda_{\max}^{\text{EtOH}}$ 221, 245, 251, 309, 321, 335 nm (Fig. 19 page 112)

Infrared absorption spectrum (KBr disc)

ν_{\max} 3130, 3000, 2950, 1625, 1590, 1483, 1363, 1253, 1202 cm^{-1} (Fig. 20 page 113)

NMR spectrum in deuterochloroform at 60 MHz in δ values (ppm)

from tetramethylsilane (T.M.S) (Fig. 21 page 114)

Chemical Shift	Proton and Multiplicity	Coupling Constants
(δ)		
3.98	3H, s,)	6,7-OCH ₃
4.00	3H, s,)	
4.27	3H, s, 4-OCH ₃	
7.27	1H, s, 8-H	
7.33	1H, s, 5-H	

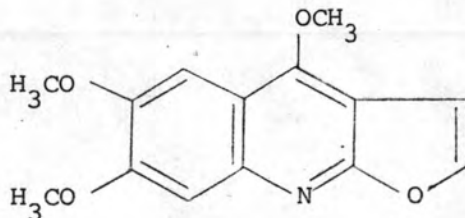
Chemical Shift	Proton and Multiplicity	Coupling Constants
(6)		
6.94	1H, d, β -furan (3'-H)	$J = 2.7$ Hz
7.46	1H, d, α -furan (2'-H)	$J = 2.7$ Hz

Mass spectrum

m/e (% rel. int.) (Fig. 22 page 115)

259 (M^+ , 100), 244 (57), 216 (26), 201 (19), 186 (21), and
173 (13)

These data are in complete agreement with published values of kokusaginine (Higa and Scheuer, 1974; Robertson, 1963; Silva *et al.*, 1979). It is therefore concluded that EG-1 is kokusaginine, the structure of which is shown below.



EG-2 was obtained as colourless monoclinic crystals, it is soluble in chloroform, ether, acetone and ethanol.

hRf values

The hRf values given are those obtained with the following systems.

- d) 48 (Fig. 4 page 97)
- e) 35 (Fig. 5 page 98)
- f) 41 (Fig. 6 page 99)

Melting point

172-174°C

Ultraviolet absorption spectrum

 $\lambda_{\text{max}}^{\text{EtOH}}$ 249, 320 and 333 nm (Fig. 23 page 116)

Infrared absorption spectrum

 ν_{max} 3120, 2860, 1620, 1581, 1510, 1495, 1444, 1392, 1365
and 1269 cm^{-1} (Fig. 24 page 117)

NMR spectrum in deuteriochloroform at 60 MHz in δ values (ppm)
from tetramethylsilane (T.M.S) (Fig. 25 page 118)

Chemical Shift (δ)	Proton and Multiplicity	Coupling Constants
4.00	3H, s, 7-OCH ₃	
4.10	3H, s, 8-OCH ₃	
4.34	3H, s, 4-OCH ₃	
6.97	1H, d, β -furan (3'-H)	$J = 2.7$ Hz
7.52	1H, d, α -furan (2'-H)	$J = 2.7$ Hz
7.17	1H, d, 6-H	$J = 8.4$ Hz
7.97	1H, d, 5-H	$J = 8.4$ Hz

Mass spectrum

 m/e (% rel. int.) (Fig. 26 page 119)

259 (M^+ , 77), 258 (25), 245 (15), 244 (100), 242 (10),
230 (52), 229 (26), 228 (21), 216 (26), 215 (13), 213 (30),
201 (26), 200 (10), 199 (16) and 173 (14)

These data are in agreement with published values of skimmianine (Mitscher *et al.*, 1975; Robertson, 1963; Steck *et al.*, 1971)

It is therefore concluded that EG-2 is skimmianine, the structure of which is shown below.

