

CHAPTER III

EXPERIMENTAL 1



1. Source and Authentication of Plant Materials

The young leaves of Cassia siamea Lamk. were obtained from a market in Bangkok, Thailand in May 1976. The plant materials were identified by comparison with the herbarium specimen in the Botany section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

2. General Techniques

2.1 Thin Layer Chromatography (TLC)

Analytical

- Technique : One way, ascending
- Adsorbents : a) Aluminium oxide G (E. Merck), calcium sulphate binder 10%; 70 g/80 ml of distilled water.
b) Silica gel G (E. Merck), calcium sulphate binder 13%; 30 g/60 ml of distilled water.
- Plate size : 20 cm x 20 cm, 20 cm x 10 cm.
- Layer thickness : 0.025 mm.
- Activation : Air dried for 15 minutes and then at 105°C for 1 hour.
- Solvent systems : a) Silica gel G/Chloroform, methyl alcohol 9+1.

- b) Silica gel G/Butyl alcohol, concentrated hydrochloric acid, water 4+1+5 (Upper layer).
- c) Aluminium oxide G/Chloroform, 95% ethyl alcohol 95+5.
- d) Aluminium oxide G/Acetone, chloroform 5+4.

Distance : 15 cm.

Laboratory temperature : 25° - 30°C.

- Detection :
- a) The spot gave yellow fluorescence in ultraviolet light at 365 nm.
 - b) The spot gave an orange-red colour with Dragendorff's spraying reagent.

Solvents and Chemicals

- a) Acetone
- b) Strong ammonium hydroxide solution
- c) Anhydrous sodium sulphate
- d) Butyl alcohol
- e) Chloroform
- f) Ethyl alcohol
- g) Glacial acetic acid
- h) Methyl alcohol

2.2 Melting Point Determination

Melting point was determined by means of Büchi melting point apparatus and was uncorrected.

2.3 Spectroscopy

Ultraviolet (UV) absorption spectrum

UV absorption spectrum was recorded by Perkin Elmer 402 Sp in the School of Pharmacy, Brunswick Square, University of London.

Infrared (IR) absorption spectrum

IR absorption spectrum was recorded by Unicam S.P. 200 Sp in the School of Pharmacy, Brunswick Square, University of London.

Nuclear magnetic resonance (NMR) spectrum

NMR spectrum was obtained on a Perkin Elmer R12 60 MHz instrument in the Department of Medical Science, Ministry of Public Health, and using tetramethylsilane (TMS) as the internal standard. The signal multiplicities are denoted as singlet (s), doublet (d).

Mass spectrum

Mass spectrum was run in an AEI MS 902 mass spectrometer in the School of Pharmacy, Brunswick Square, University of London.

3. Isolation of Chemical Substance from the Young Leaves of

Cassia Siamea Lamk.

The shredded fresh young leaves of Cassia siamea Lamk. (3 kg) were boiled with 2% acetic acid (3 X 7 l) and filtered. The combined acid filtrate was made alkaline with strong ammonium hydroxide solution and extracted with chloroform. The combined chloroform extract (20 l) was dried over anhydrous sodium sulphate and concentrated under reduced pressure to about 500 ml and extracted with 5% acetic acid (3 X 300 ml). Each acid extract was neutralised with strong ammonium hydroxide solution

to pH 6.5 - 7.0, the lemon yellow needle crystals precipitated out (3 g). The crystals were collected and recrystallised several times by dissolving in 2% acetic acid and neutralised with strong ammonium hydroxide solution. The yellow needle crystals were designated as S.I (1 g).

4. Identification

4.1 Identification of S.I as Barakol

S.I was obtained as yellow needle crystals, turned green in vacuum desiccator. It decomposed at 164°C to give brownish black substance. It was soluble in methyl alcohol, ethyl alcohol, and moderately soluble in water. The aqueous and alcoholic solutions of S.I gave yellowish fluorescence in ultraviolet light at wavelength of 365 nm.

R_f values of S.I

on silica gel G/chloroform, methyl alcohol 9+1 = 28

(Fig. V, p. 140).

on silica gel G/butyl alcohol, conc. hydrochloric acid, water

4+1+5 (Upper layer) = 24 (Fig. V, p. 140).

on aluminium oxide G/chloroform, 95% ethyl alcohol, 95+5 = 50

(Fig. V, p. 140).

on aluminium oxide G/acetone, chloroform 5+4 = 33

(Fig. V, p. 140).

Ultraviolet absorption spectrum

S.I (Fig. VI, p. 141).

$\lambda_{\text{max}}^{\text{MeOH}}$ = 241, 247 (major absorption), 271, 333, 390 nm.

Barakol⁽¹⁷⁵⁾

$$\lambda_{\text{max}}^{\text{EtOH}} = 241, 246, 384, \text{nm.}$$

Infrared absorption spectrum

S.I (Fig. VII, p. 141).

$$\nu_{\text{max}}^{\text{Nujol}} = 3430 \text{ cm}^{-1} \text{ (broad, hydroxyl group), } 1675 \text{ cm}^{-1} \text{ (strong, either to a hydrogen-bonded carbonyl group or an enol ether), } 1635 \text{ cm}^{-1}.$$

Barakol⁽¹⁷⁵⁾

$$\nu_{\text{max}}^{\text{Nujol}} = 3450 \text{ cm}^{-1} \text{ (strong), } 1670 \text{ cm}^{-1} \text{ (strong), } 1630 \text{ cm}^{-1} \text{ (strong).}$$

NMR spectrum of S.I in deuteriochloroform (CDCl_3) (Fig. VIII, p. 142).

<u>δ values</u> (in ppm)	<u>Proton</u>	<u>Multiplicities</u>
2.21	3H	s (methyl group)
2.34	3H	s (methyl group)
6.35, 6.23	2 X 1H	d, J_{meta} (2 meta coupled aromatic protons)
6.22, 5.98	2 X 1H	s, slightly broad (2 olefinic protons)

Mass spectrum

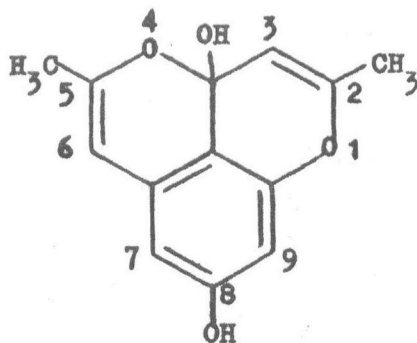
S.I (Fig. IX, p. 142).

$$\underline{m/e} (\%) = 232(\text{M}^+, 2), 214(100), 186(78), 158(23), 143(6), 110(17), 105(15), 93(10), 89(10), 51(13).$$

Barakol⁽¹⁷⁵⁾

$$\underline{m/e} (\%) = 232(\text{M}^+, 1.5), 214(100), 186(59), 158(23), 143(6), 110(17), 105(15), 93(10), 89(8), 51(13).$$

The ultraviolet, infrared, and mass spectra of S.I were identical with barakol as described by Bycroft et al. (175) It is therefore concluded that S.I is barakol (3a,4-Dihydro-3a,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene).



Barakol