

## CHAPTER VII

### EXPERIMENTAL 2



#### 1. Source and Authentication of Plant Materials

The leaves of *Cassia spectabilis* DC. were collected from Singburi, 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%, 50 g/60 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 10cm.
Layer thickness :	0.25 mm.

- Activation : Air dried for 15 minutes and then at 105°C for 1 hour.
- Solvent systems :
- a) Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution, 9+1+0.5 (lower layer)
  - b) Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution, 9+1+0.2
  - c) Aluminium oxide G/Acetone, chloroform, 8+2.
  - d) Aluminium oxide G/Acetone, chloroform, 4+5.
- Distance : 15 cm
- Laboratory temperature : 24°-30°C
- Detection : The spot gave an orange-red colour with Dragendorff's spraying reagent.

## 2.2 Preparative Thin Layer Chromatography

- Technique : One way, ascending.
- Adsorbent : Silica gel G + Silica gel GF<sub>254</sub> (E. Merck) 13% calcium sulphate binder, 60 g/120 ml 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 : Lower layer of Chloroform, 95% ethyl alcohol,  
25% ammonium hydroxide solution, 9+1+0.5.

Distance : 18 cm.

Laboratory temperature : 24°-30°C

Detection : a) Ultraviolet light at wave length 254 nm.  
b) Dragendorff's spray reagent.

#### Solvents and Chemicals

- a) Strong ammonium hydroxide solution
- b) Benzene
- c) Chloroform
- d) 95% Ethyl alcohol
- e) Ethyl alcohol, absolute
- f) Ethyl acetate
- g) Glacial acetic acid
- h) n-Hexane
- i) Hydrochloric acid
- j) Sodium hydroxide, pellet
- k) Sodium sulphate, anhydrous
- l) Sulphuric acid
- m) Tartaric acid

#### 2.3 Melting Point Determination

All melting point were determined by means of Büchi melting point apparatus, and were uncorrected.

## 2.4 Spectroscopy

### Ultraviolet (UV) absorption spectra

UV absorption spectra were recorded by Perkin Elmer 402 Sp in the School of Pharmacy, Brunswick Square, University of London, and by Pye Unicam S.P. 1800 ultraviolet spectrophotometer in Department of Food Chemistry, Chulalongkorn University Faculty of Pharmaceutical Sciences.

### Infrared (IR) absorption spectra

IR absorption spectra were recorded by Unicam S.P. 200 Sp in the School of Pharmacy, Brunswick Square, University of London, and by Beckman IR 4240 in Department of Chemistry, University of Malaya Faculty of Science.

### Nuclear magnetic resonance (NMR) spectra

The NMR spectra were obtained on a Varian A-60D, 60 MHz instrument in Department of Chemistry, Mahidol University Faculty of Science, and using tetramethylsilane (TMS) as the internal standard. The signal multiplicities are denoted as singlet (s), doublet (d), triplet (t), and multiplet (m).

### Mass spectra

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

### 3. The Isolation of Alkaloids from the Leaves of Cassia Spectabilis DC.

#### 3.1 Isolation of Alkaloids

The dried coarsely powdered leaves (4.8 kg) were macerated with 1% ethanolic tartaric acid (20 l) for three days and filtered. The marc was reextracted with 1% ethanolic tartaric acid (20 l) at 50°-60°C. The combined filtrate was concentrated under reduced pressure to 3 l, diluted with 6 l of water, and treated with 300 ml of 2 N sulphuric acid. The acid solution was filtered, washed twice with benzene (5 l), and made alkaline with the strong ammonium hydroxide solution. This solution was extracted with chloroform. The combined chloroform extract (20 l) was dried over anhydrous sodium sulphate and evaporated under reduced pressure yielding a dark brown syrupy mass 79.3 g, (1.6%). It was dissolved in ethyl alcohol and diluted to 150 ml. Each of 50 ml aliquot of this solution, corresponding to 26.4 g of the crude extract, was diluted to 300 ml with 1 N hydrochloric acid and extracted twice with benzene (2 x 300 ml). The aqueous solution was made alkaline with 25 ml of 40% sodium hydroxide solution and extracted with chloroform (3 x 200 ml); the chloroform extracts were washed with water, dried over anhydrous sodium sulphate and concentrated to yield a dark brown syrupy mass 24.6 g. This was digested repeatedly with boiling n-hexane, leaving an insoluble residue of 5.6 g. The n-hexane solution was filtered and concentrated to yield 19.0 g of clear yellow oil. This material was dissolved in 140 ml of ethyl alcohol and made acid with concentrated hydrochloric acid, diluted with 140 ml ethyl acetate, chilled and scratched until crystalline precipitates formed. Further crops could be

obtained by concentrating the filtrate and adding ethyl acetate. Thus a total of 11.3 g of crystalline hydrochloride was obtained. The free base, obtained by dissolving the hydrochloride in a small amount of ethyl alcohol, diluting with chloroform adding ammonia, washing the organic phase with water, dried over anhydrous sodium sulphate; concentrated under reduced pressure, and crystallised in n-hexane as white needle crystals (2 g), designated as Sp I. The mother liquor was concentrated to yield a yellow oil, 5.6 g. One gramme of this yellow oil was separated by preparative thin layer chromatography. It yielded 0.336 g of Sp I and 0.281 g of Sp II.

### 3.2 Alkaloidal Salts

Either Sp I or Sp II (160 mg) was dissolved in absolute ethyl alcohol (5 ml) and made acid with concentrated hydrochloric acid, diluted with ethyl acetate (15 ml). After standing in a cool place (5 hours) the crystals were formed, filtered, washed and recrystallised in ethyl alcohol. The silky white needle crystals of Sp I HCl obtained, weighed 200 mg, and by mean of this process obtained the white needle crystals of Sp II HCl (190 mg).

### 3.3 Preparation of Dihydro-alkaloids<sup>(7)</sup>

Either Sp I or Sp II (100 mg) was dissolved in 3 ml of methyl alcohol and treated with sodium borohydride (4 x 40 mg), and let it stand for half an hour. It was acidified with diluted hydrochloric acid, extracted twice with ether, made alkaline with 10% sodium hydroxide, and extracted twice with chloroform and twice with a mixture of chloroform and ethyl alcohol (4+1). The combined organic layer was evaporated

obtained by concentrating the filtrate and adding ethyl acetate. Thus a total of 11.3 g of crystalline hydrochloride was obtained. The free base, obtained by dissolving the hydrochloride in a small amount of ethyl alcohol, diluting with chloroform adding ammonia, washing the organic phase with water, dried over anhydrous sodium sulphate; concentrated under reduced pressure, and crystallised in n-hexane as white needle crystals (2 g), designated as Sp I. The mother liquor was concentrated to yield a yellow oil, 5.6 g. One gramme of this yellow oil was separated by preparative thin layer chromatography. It yielded 0.336 g of Sp I and 0.281 g of Sp II.

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until free from solvents. The residue was dissolved in diluted hydrochloric acid and dried under reduced pressure. The dried amorphous powder was dissolved in a small amount of ethyl alcohol and ethyl acetate was added dropwise, to yield the white crystals of dihydro Sp I HCl (105 mg) or dihydro Sp II HCl (103 mg).

#### 4. Identification

##### 4.1 Identification of Sp I as Cassine

Sp I is obtained as white needle crystals from n-hexane (m.p.  $53^{\circ}$ - $54^{\circ}$ C). It is soluble in chloroform, slightly soluble in n-hexane.

Sp I HCl is obtained as silky white needle crystals from ethyl alcohol (m.p.  $164^{\circ}$ - $165^{\circ}$ C).

Dihydro Sp I HCl is obtained as white crystals from ethyl alcohol-ethyl acetate (m.p.  $166^{\circ}$ - $167^{\circ}$ C).

#### hR<sub>f</sub> value

Sp I on Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution,  $9+1+0.5$  (lower layer) = 70 (Fig. XI, p. 143).

Sp I on Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution,  $9+1+0.2$  = 17 (Fig. XII, p. 144).

Sp I on Aluminium oxide G/Acetone, chloroform,  $8+2$  = 26 (Fig. XIII, p. 145).

Sp I on Aluminium oxide G/Acetone, chloroform,  $4+5$  = 71 (Fig. XIV, p. 146).

Dihydro Sp I on Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution,  $9+1+0.5$  (lower layer) = 54 (Fig. XI, p.143).



Cassine on Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution, 9+1+0.2 = 17 (Fig. XII, p. 144).

Cassine on Aluminium oxide G/Acetone, chloroform, 4+5 = 71 (Fig. XIV, p. 146).

#### Ultraviolet absorption spectrum

Sp I HBr (Fig. XV, p. 147).

$$\lambda_{\text{max}}^{\text{MeOH}} = 277 \text{ nm}$$

Cassine HCl (7)

$$\lambda_{\text{max}}^{\text{EtOH}} = 276 \text{ nm}$$

#### Infrared absorption spectra

Sp I (Fig. XVI, p. 147).

$$\nu_{\text{max}}^{\text{CCl}_4} = 3630, 3520, 2930, 2860, 2810, 1720, 1360, 690 \text{ cm}^{-1}.$$

Cassine (7)

$$\nu_{\text{max}}^{\text{CCl}_4} = 3530, 2930, 2860, 2810, 1720, 1360, 690 \text{ cm}^{-1}.$$

Sp I HCl (Fig. XVII, p. 148).

$$\nu_{\text{max}}^{\text{KBr}} = 3300, 2930, 2850, 1715, 1530, 1170, 1160, 1012, 995 \text{ cm}^{-1}$$

Cassine HCl (7)

$$\nu_{\text{max}}^{\text{Nujol}} = 3290, 1715, 1530, 1170, 1160, 1010, 990 \text{ cm}^{-1}$$

Dihydro Sp I HCl (Fig. XXVI, p. 153).

$$\nu_{\text{max}}^{\text{KBr}} = 3350, 2920, 2840, 1570, 1460, 1420, 1360, 1120, 980, 710 \text{ cm}^{-1}.$$

NMR spectrum of Sp I in  $\text{CDCl}_3$  (Fig. XVIII, p. 148).

<u><math>\delta</math> values</u> (in ppm)	<u>Proton</u>	<u>Multiplicity</u>
1.10	3 H	d, $J = 6$ Hz (methyl at C-2)
1.28	18 H	s (methylene of side chain)
2.00	1 H	s, broad (hydroxyl at C-3)
2.14	3 H	s (methyl adjacent to carbonyl)
2.40	2 H	t, $J = 6.5$ Hz (methylene adjacent carbonyl)
2.40-2.90	2 H	m (hydrogens at C-2, C-6)
3.55	1 H	s, broad ( $\omega_{1/2} = 6$ Hz) (equatorial hydrogen at C-3)

Mass spectra

Sp I HCl (Fig. XIX, p. 149).

$m/e$  (%) = 297( $M^+$ , 4.0); 282( $M^+ - \text{CH}_3$ , 7.5); 279( $M^+ - \text{H}_2\text{O}$ , 1.5);  
 254( $M^+ - \text{CH}_3 - \text{CO}-$ , 3.0); 240( $M^+ - \text{CH}_3 - \text{CO} - \text{CH}_2-$ , 31.5);  
 238( $M^+ - \text{C}_3\text{H}_6\text{OH}$ , 7.5); 236( $M^+ - \text{CH}_3 - \text{CO}$ ,  $-\text{H}_2\text{O}$ , 1.0);  
 226(2.0); 222(1.5); 213(4.0); 184(2.0); 170(2.8);  
 156(3.2); 140(2.5); 138(2.0); 114(100); 96(42.0).

Cassine HCl (Fig. XX, p. 149).

$m/e$  (%) = 297( $M^+$ , 1.1); 282(2.2); 240(7.6); 213(1.2); 114(100); 96(8.7).

Dihydro Sp I (Fig. XXVII, p. 153).

$\underline{m/e}$  (%) = 299(M<sup>+</sup>, 14.4); 284(52.7); 282(16.4); 266(13.3); 254(10.5);  
 242(14.4); 240(61.2); 238(12.5); 236(3.6); 226(5.8);  
 222(9.8); 212(12.6); 198(6.9); 196(6.6); 184(8.4); 170(9.4);  
 156(14.8); 145(10.2); 142(8.6); 140(9.4); 138(4.5);  
 128(7.5); 127(32.0); 124(6.2); 120(23.8); 118(26.6);  
 114(100); 96(72.5).

The UV, IR, NMR, and Mass spectra of Sp I are identical with the authentic cassine isolated from Cassia excelsa Shrad.<sup>(7)</sup> Then Sp I is conclude as cassine.

#### 4.2 Identification of Sp II as Iso-6-cassine

Sp II is obtained as colourless oil.

Sp II HCl is obtained as white needle crystals from ethyl alcohol (m.p. 129.5°C).

Dihydro Sp II HCl is obtained as white crystals from ethyl alcohol - ethyl acetate (m.p. 146° - 147°C).

#### hR<sub>f</sub> value

Sp II on Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution, 9+1+0.5 (lower layer) = 44 (Fig. XI, p. 143).

Sp II on Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution, 9+1+0.2 = 8 (Fig. XII, p. 144).

Sp II on Aluminium oxide G/Acetone, chloroform, 8+2 = 6 (Fig. XIII, p. 145).

Dihydro Sp II on Silica gel G/Chloroform, ethyl alcohol, 25% ammonium hydroxide solution, 9+1+0.5 (lower layer) = 28 (Fig. XI, p. 143).

Ultraviolet absorption spectrum

Sp II HCl (Fig. XXI, p. 150).

$$\lambda_{\text{max}}^{\text{EtOH}} = 275 \text{ nm}$$

Infrared absorption spectra

Sp II (Fig. XXII, p. 150).

$$\nu_{\text{max}}^{\text{CCl}_4} = 3620, 3510, 2930, 2850, 1720, 1360, 1160, 980, 675 \text{ cm}^{-1}$$

Iso-6-cassine<sup>(15)</sup>.

$$\nu_{\text{max}}^{\text{CCl}_4} = 3635, 3535 \text{ cm}^{-1}.$$

Sp II HCl (Fig. XXIII, p. 151).

$$\nu_{\text{max}}^{\text{KBr}} = 3350, 2935, 2850, 1715, 1575, 1465, 1370, 1160, 1184, 1015, 960, 720 \text{ cm}^{-1}.$$

Iso-6-cassine HCl<sup>(15)</sup>.

$$\nu_{\text{max}}^{\text{KBr}} = 3350, 2860, 2820, 1715, 1570, 1467, 1370, 720 \text{ cm}^{-1}.$$

Dihydro Sp II HCl (Fig. XXVIII, p. 154).

$$\nu_{\text{max}}^{\text{KBr}} = 3360, 2920, 2850, 1560, 1460, 1370, 1010, 930, 710 \text{ cm}^{-1}.$$

NMR spectrum of Sp II in  $\text{CDCl}_3$  (Fig. XXIV, p. 151).

<u><math>\delta</math> value</u> (in ppm)	<u>Proton</u>	<u>Multiplicity</u>
1.15	3 H	d, $J = 6$ Hz (methyl at C-2)
1.30	18 H	s (methylene of side chain)
1.95	1 H	s, broad (Hydroxyl at C-3)
2.14	3 H	s (methyl adjacent to carbonyl)
2.44	2 H	t, $J = 6.5$ Hz ( $-\text{CH}_2-\text{CO}-$ )
2.90	1 H	m (hydrogen at C-6)
3.22	1 H	m, $J = 4$ Hz (hydrogen at C-2)
3.75	1 H	s, broad ( $\omega_{1/2} = 14$ Hz) (carbinolic hydrogen at C-3)

Mass spectra

Sp II HCl (Fig. XXV, p. 152).

$\underline{m/e}$  (%) = 297( $\text{M}^+$ , 2.2); 282(4.2); 240(14.0); 238(4.0); 212(2.0);  
170(1.0); 156(1.0); 114(100); 96(18).

Iso-6-cassine<sup>(15)</sup>.

$\underline{m/e}$  (%) = 298( $\text{M}^++1$ , 1.8); 282(0.5); 254(0.9); 240(3.0); 238(0.8);  
226(0.5); 212(0.7); 198(0.6); 184(0.5); 170(0.6); 156(0.7);  
142(0.55); 128(0.4); 114(100); 96(8).

Dihydro Sp II (Fig. XXIX, p. 154).

$\underline{m/e}$  (%) = 299( $\text{M}^+$ , 22.4); 284(96.1); 282(18.4); 266(41.7); 254(11.8);  
242(25.0); 240(54.6); 226(8.2); 212(13.8); 198(7.6); 184(8.7);  
170(11.8); 156(15.4); 142(10.4); 140(5.1); 138(5.1);  
127(15.3); 114(100); 96(81.1).

By the evidences of UV, IR, NMR, and Mass spectra Sp II should probably be iso-6-cassine.