

CHAPTER III

EXPERIMENTAL

Source of Latex

The latex of *Papaver somniferum* L. was obtained from Ban Phui Nuea, Maecham District, Chiangmai Province, Thailand in December 1980 and January 1982. Opium Poppies of white flowers and red flowers were used. Capsules of the plant were incised and the exuded latex were collected in the next morning.

General Technique

Thin Layer Chromatography

Technique	: one way, ascending
Adsorbent	: silica gel G (E. Merck), calcium sulphate binding 13 %, 30 g/60 ml distilled water
Plate Size	: 20 cm x 20 cm
Layer Thickness	: 250 nm
Activation	: air dried for 15 minutes and then at 110°C for 1 hr
Solvent System	: ethyl acetate + methanol + strong ammonium hydroxide solution (85 + 10 + 5)
Distance	: 15 cm
Temperature	: 20°-30°C

Detection : Modified Dragendorff's spray reagent

Solution A : bismuth subnitrate (850 mg), distilled water (40 ml) and glacial acetic acid (10 ml).

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

Solution C : glacial acetic acid (20 ml) and distilled water (80 ml).

Solution A and B, 5 ml each, were mixed with 100 ml of solution C and used as spray reagent. The alkaloids give orange-red color spots with modified Dragendorff's reagent.

Collection of Latex

During September to October of the late rainy season in 1980 and 1981, the poppy seeds were sowed by the villagers. They grew naturally without scientific help. The plants flowered in December and the petal fall in 24-72 hours after the opening of the buds. The capsules took another 5-10 days to become fully swollen when they are ready for lancing.

In December 1980, the latex was drawn from two fields, Ban Phui and Mae La Thung Phei. One capsule was chosen from each plant. About 60 capsules of each white and red flowered plants were grouped into 6 groups (10 capsules each). They were coded as follows:

TP/W 1, 2, 3, ... for white flowered capsules from Mae La Thung Phei (แม่ลาทุ่งผี).

TP/R 1, 2, 3, ... for red flowered capsules from Mae La Thung
Phei (แม่ลาทุ่งผี).

P/W 1, 2, 3, ... for white flowered capsules from Ban Phui
(บ้านพุย).

P/R 1, 2, 3, ... for red flowered capsules from Ban Phui
(บ้านพุย).

The first lancements of all capsules were made at the same morning. The capsules were incised longitudinally with a three-blade knife and left them over night. Then the latex from each capsule was scraped with a blade and kept in a tight container. In the second lancements, each group was incised in different periods, i.e. 1, 2, 3, ... and 6 days respectively after the first lancements. Time of lancements is shown in Table 6.

In January 1982, the collections were made at Ban Phui and Hauy Sai Neau. One hundred capsules of each white flowers and red flowers were chosen and grouped into 10 groups (10 capsules each). They were coded as follows:

BP/W 1, 2, 3, ... for white flowered capsules from Ban Phui
(บ้านพุย).

BP/R 1, 2, 3, ... for red flowered capsules from Ban Phui
(บ้านพุย).

HS/W 1, 2, 3, ... for white flowered capsules from Hauy Sai Neau
(หัวทรายเหนือ).

HS/R 1, 2, 3, ... for red flowered capsules from Hauy Sai Neau
(หัวทรายเหนือ).

The lancements of these are shown in Table 7.

Table 6

Lancings of Opium Poppy capsules in December 1980

Date	Capsule numbers (TP & P)					
	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 60
22 Dec.	← incised →					
23 Dec.	← collected →					
	← incised →					
24 Dec.	← collected →	← incised →				
25 Dec.		← collected →	← incised →			
26 Dec.			← collected →	← incised →		
27 Dec.				← collected →	← incised →	
28 Dec.					← collected →	← incised →
29 Dec.						← collected →

Table 7

Lancings of Opium Poppy capsules in January 1982

Date	Capsule numbers (BP & HS)									
	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 60	61 - 70	71 - 80	81 - 90	91 - 100
15 Jan.	←————— incised —————→									
16 Jan.	←————— collected —————→									
17 Jan.	← collected →									
18 Jan.		← incised →								
19 Jan.		← collected →								
20 Jan.			← incised →							
21 Jan.			← collected →	← incised →						
22 Jan.				← collected →	← incised →					
23 Jan.					← collected →	← incised →				
24 Jan.						← collected →	← incised →			
25 Jan.							← collected →	← incised →		
26 Jan.								← collected →	← incised →	
27 Jan.									← collected →	← incised →
28 Jan.										← collected →



Extraction and Examination of Alkaloids from the Latex

1. Extraction of Alkaloids

The latex from each capsule was weighed and each group of ten was macerated in 10-20 ml of 95 % ethanol for one week. It was then filtered and the filtrate be adjusted to 25 ml. Then the solution was diluted to 0.5 % W/V in 95 % ethanol. This solution was ready for quantitative examination.

2. Quantitative Examination of Alkaloids

2.1. Standard Solution of Reference Alkaloids

Standard solution of morphine, codeine, thebaine and narcotine in 95 % ethanol were prepared in different concentration depending on their sensitivities to the spray reagent as shown in Table 8. Accurate volumes of these standard solutions were applied to TLC plates via a Hamilton syringe. The volumes of 1 μ l to 12 μ l were applied to each plate, without damaging the surface of the layer. The solvent system used for TLC plate was:

ethyl acetate + methanol + strong ammonium hydroxide solution
(85 + 10 + 5)

The chromatogram was dried by means of gentle stream of air. The least μ l of alkaloid spots which gave positive results with modified Dragendorff's spray reagent were recorded and sensitivity in μ g were calculated. The results are presented in Table 8.

Table 8
Sensitivities of Standard Alkaloids

Standard alkaloid	Conc. mg/ml	First performance (μ l)	Second performance (μ l)	Third performance (μ l)	Mean μ l	Mean sensitivity (μ g)
Morphine	0.5	2	2	2	2	1.00
	0.2	5	5	5	5	
Codeine	0.4	2	2	2	2	0.77
	0.2	3	4	4	3.67	
Thebaine	0.1	2	3	3	2.67	0.25
	0.05	4	5	5	4.67	
Narcotine	0.2	2	2	2	2	0.40
	0.04	10	10	10	10	

2.2. Opium Alkaloids Solutions

The opium latex in strength of 0.5 % W/V in 95 % ethanol was prepared. The volumes of 1 μ l to 12 μ l of each group were applied to TLC plates via Hamilton syringe. Ethyl acetate + methanol + strong ammonium hydroxide solution (85 + 10 + 5) was used as developing solvent and modified Dragendorff's spray reagent was used for detection.

Percentages of the alkaloids presented were calculated by the following formula:

$$\text{Percentage of alkaloid} = \frac{\text{S.Q.} \times 10}{C \times \text{S.V.}}$$

S.Q. = sensitivity in μg

S.V. = sensitivity volume of alkaloids solution in μl

C = concentration of latex in g/100 ml solution



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