EXPERIMENTAL

A. Source of Plant Material

(i) The first batch of Datura

Fresh whole plants of Datura metel Linne'. were obtained from Rajburi, Thailand during December 1970 and were separated as follows:-

(a) Leaves

(b) Flowers

(c) Roots

(d) Seeds

(e) Stems and branches

(ii) The second batch of Datura

Leaves of Datura metel Linne'. were obtained from Kanchanaburi and Rajburi, Thailand during December 1971 to January 1972.

(iii) The first batch of Dioscorea

Tubers of Dioscorea hispida Dennst. were obtained from Saraburi, Thailand during July 1971.

(iv) The second batch of Dioscorea

Tubers of Dioscorea hispida Dennst. were obtained from Chantaburi, Thailand, in August 1972.

All plant materials were identified by the authors.

B. General

1. Thin layer Chromatography (TLC)

The experimental details are summarized as follows:-

a. Analytical

Technique	:	one way, ascending
Adsorbents	:	Aluminium oxide G. (E. Merck.), (Calcium sulphate binding 10%), 70 g./80 ml. distilled water.
Plate size	:	20 cm. x 20 cm.; 10 cm. x 20 cm.
Layer thickness	:	250 μ
Activation	:	Air dried 10 minutes and then heated 1 hour at 105°C.
Solvent systems	:	See details in text.
Distance	:	15 cm.
Temperature	:	20-30°C.
Examination	:	(a) Ultra-violet Light. (b) Dragendorff's spray reagent.

b. Preparative thin layer chromatography

Technique	:	One way, ascending, twice developing
Adsorbents	:	Silica gel G. (E. Merck), $CaSO_4$ binding 13%, 120 g./240 ml. distilled water
Plate size	:	20 cm. x 20 cm.
Layer thickness	:	1 mm.
Activation	:	Air dried for at least 1 hour frequently overnight and then heated 1 hour at 105°C.

Solvent systems : 5% methanol in ether.

Distance : 18 cm.

Temperature : 20-30°C.

Examination : (a) Ultra-violet Light.

(b) Edge of chromatogram with:-
Dragendorff's spray

2. Column Chromatography

Packing of column

(a) Adsorbents packed dry into the column.

(b) Adsorbents poured as a suspension into the column.

Addition of alkaloidal material to column

(a) Solution in small volume of solvent carefully pipetted onto the top of a column holding the same solvent.

(b) Solution in small volume of volatile solvent mixed with small quantity of adsorbent, dried and added to the top of a dry column.

Adsorbents

(a) Kieselguhr (E. Merck)

(b) Hyflo super cel (Celite)

(c) Silica gel (E. Merck) 0.05-0.2 mm.

(d) Alumina neutral (E. Merck)

Solvent

(a) Diethyl ether (Carlo-Erba) Lab. grade.

(b) Chloroform B.P. (I.C.I.)

(c) Methanol (E. Merck)

(d) 95% Ethyl alcohol (The Government Pharmaceutical Organization)

(e) Strong ammonia solution (May and Baker)

(f) Anhydrous sodium sulphate (May and Baker)

(g) Absolute alcohol (E. Merck)

Authentic samples

(a) Aposcopolamine picrate, Meteloidine, Norscopolamine picrate supplied by Dr. W.C. Evans, Pharmacy Department, University of Nottingham, England.

(b) Hyoscyamine hydrobromide, Scopolamine hydrobromide, and Atropine sulphate obtained from E. Merck.

Collection of eluate

Fractions of 25 ml. or more were collected manually.

Examination of eluate

Those fractions giving an orange-red colour with Dragendorff's spray reagent were examined by thin layer chromatography and likely fractions were combined and concentrated to dryness under reduced pressure.

3. Melting point

(a) Determined with Reichert heating stage microscope

(b) Determined with Büchi melting point appratus

4 Optical rotation

Optical rotation were determined with a Billingham and Standley polarimeter using sodium light, solvent, concentration, length of the tube and temperature as state in the text.

5 Ultra-violet absorption spectra

Ultra violet absorption spectra were obtained with a Pye-Unicam S.P. 500 spectrophotometer,

6. Infra-red absorption spectra

Infra-red absorption spectra were determined in the Department of Science, Ministry of Industry with a Perkin-Elmer 421 Grating Spectrophotometer.

C. The isolation of alkaloids from *Datura metel* Linné

1. Comparison of alkaloid contents occuring in different plant parts of Datura metel Linné

Twenty grams of dried powdered materials (leaves, flowers, roots, seeds or stem) were separately macerated with 150 ml. 95% ethanol for 48 hours, and filtered. The filtrate was concentrated under reduced pressure to syrupy mass. The syrupy mass was extracted with (5x5 ml.) 10% hydrochloric acid. The combined acid extract was shaken with (5x5 ml.) petroleum ether to remove pigments or other impurities. The acid layer was made alkaline to pH9 with strong ammonia solution and then extracted with (3x5 ml.) chloroform. The combined chloroform extract was concentrated to dryness under reduced pressure and then reextracted with (5x5 ml.) 10% hydrochloric acid. The combined acid solution was made alkaline to pH 9 with strong ammonia solution and then extracted with (5x5 ml.) chloroform. The combined chloroform extract was evaporated under reduced pressure, weighed and redissolved in 15 ml. chloroform.

This solution was examined by thin layer chromatography technique.

The alkaloid contents are tabulated (see Fig. 1 page 103 and table 3 page 71)

Table 3

Plant Parts	alkaloid content (%) approximately
Flowers	0.18
Leaves	0.17
Roots	0.03
Seeds (extract once with petroleum ether.)	0.01
Stems	0.01

2. The isolation of alkaloids from the flowers of *Datura metel* Linné at various pH values.

The comparison of alkaloid contents from different parts of Datura metal Linné showed that alkaloid contents of flowers and leaves were higher than those of the other parts, and thin layer chromatogram indicated that at least 3 alkaloids were present. (see Fig. 1 page 103)

Dried powdered flowers (2.4 kg.) were macerated for 7 days in 8 l. of ethanol with occational agitation and filtered. The marc was further macerated with (3x4 l.) of 95% ethanol and filtered. The combined filtrate was concentrated under reduced pressure to syrupy mass.

The syrupy mass residue was extracted with 1% hydrochloric acid until the last extraction gave a negative test to Dragendorff's reagent. The combined acid extract was shaken with light petroleum ether to remove some impurities and pigments and then was adjusted to pH 5 with strong ammonia solution and extracted with chloroform until exhaustion, dried with anhydrous sodium sulphate, filtered and evaporated under reduced pressure to dryness yielding residue 3 g.

The aqueous (pH 5) acid solution after extracted with chloroform was made alkaline with strong ammonia solution up to pH range 9-11 and extracted with chloroform until exhaustion.

The chloroform extracts were combined, dried with anhydrous sodium sulphate, filtered and evaporated under

reduced pressure to dryness yielding residue 8.5 g.

Crude extracts at various pH were examined by thin layer chromatography, and the results were shown as follows.

Crude extracts obtained from pH 5 and from pH 9 solutions showed the presence of at least 3 and 5 alkaloids respectively, and both groups contained scopolamine as the main alkaloid and hyoscyamine as the minor alkaloid (see Fig. 2 page 104 and Fig. 3 page 105), crude substances (3.0 g.) obtained from pH 5 solution extraction were divided into two equal parts, each part (1.5 g.) was dissolved in small amount of warm diethyl ether previously saturated with 0.5 M. phosphate buffer (pH 6.4) and applied onto the top of kieselguhr column (30 x 2.5 cm.) loaded with 10 ml. 0.5 M. phosphate buffer (pH 6.4).

Elution began with 350 ml. of diethyl ether (saturated with 0.5 M. phosphate buffer pH 6.4) followed by 325 ml. of chloroform and 750 ml. of 10% methanol in chloroform and 25 ml. fractions were collected manually.

Thin layer chromatogram of these fractions indicated that it could not be possible to separate the mixture of alkaloids by this method (see Fig. 4 page 106).

2.1 The separation of alkaloids from the flowers of Datura metel Linne at pH 9-11

The crude alkaloids extracted at pH 9-11 (8.5 g.) were divided into 4 equal parts, each part (2.1 g.) was dissolved with small amount of warm diethyl ether previously saturated with 0.5 M. phosphate buffer pH 6.4 and applied onto the top of hyflo super cel column (30 x 2.5 cm.) holding diethyl ether (saturated with 0.5 M. phosphate buffer pH 6.4).

Elution of crude alkaloids from the column was begun with diethyl ether (saturated with 0.5 M. phosphate buffer pH 6.4) followed by chloroform; and 25 ml. fractions were collected manually.

Thin layer chromatography of these fractions indicated that it could not be possible to separate the mixture of alkaloids by this adsorbent (see Fig. 5 page 107).

3. The isolation of alkaloids from the flowers of *Datura metel* Linné

Dried powdered flowers (5 kg.) were moistened with distilled water, mixed with calcium hydroxide (1 kg.) and then macerated with diethyl ether (20 l.) for two days.

The marc was remacerated with another portions of diethyl ether (3 x 20 l.). The combined filtrate was concentrated under reduced pressure and extracted with 0.25 N. sulphuric acid to exhaustion. The combined acid solution was made alkaline with strong ammonia solution and then extracted with chloroform. The chloroform extracts were combined and concentrated under reduced pressure to 500 ml. and extracted with 0.25 N. sulphuric acid (5 x 400 ml.); the combined acid extract was made alkaline with strong ammonia solution, extracted with chloroform (4 x 300 ml.), dried with anhydrous sodium sulphate and concentrated under reduced pressure to yield a crude base (4.5 g.).

Thin layer chromatography indicated that this crude base contained at least five alkaloids and two of them corresponded to scopolamine and hyoscyamine (see Fig. 6,7 pages 108,109).

Crude alkaloids (4.5 g.) were divided into 2 equal parts each part (2.2 g.) was dissolved in a small amount of warm diethyl ether and applied onto the top of a silica gel column holding diethyl ether.

The fractions (30 ml.) were collected manually. Elution

of the column began with diethyl ether (210 ml.) followed by diethyl ether : methanol (8 : 2) (240 ml.) and eluted with methanol (180 ml.) until exhaustion.

The fractions were examined by thin layer chromatography and likely fractions were combined and evaporated under reduced pressure to dryness.

The combined fractions were treated as follows:-

The diethyl ether eluates fraction 1 to fraction 7 (210 ml.) were combined and evaporated under reduced pressure to dryness. Examination of the combined concen trated fractions by thin layer chromatography did not give intense colour with Dragendorff's reagent, it showed the presence of a trace of alkaloid with R_F value = 0.87 (on silica gel G / diethyl ether : ethanol (1 : 1) (see Fig. 8 page 110).

The remaining 4 fractions of diethyl ether eluates (8-11) and 2 fractions of diethyl ether : methanol (8 : 2) eluates were combined and concentrated under reduced pressure to small volume.

The thin layer chromatography of the combined fractions indicated that at least 2 alkaloids were present.

Fractions 3-5 from the elution of diethyl ether:methanol (8 : 2) were combined and concentrated under reduced pressure to small volume, yielding very small amount of crystals.

R_F value of this crystal on silica gel G thin layer

chromatography / diethyl ether : ethanol (1 : 1) = 0.18 (see Fig. 8 page 110).

The remaining diethyl ether : methanol (8 : 2) eluates (120 ml.) and methanol eluates (180 ml.) were combined and after evaporation under reduced pressure to dryness yielded very small amount of alkaloid.

Comparision with the authentic materials (E. Merck) by thin layer chromatography, it corresponds to hyoscyamine (see Fig. 8 page 110).

4. The isolation of alkaloids from the leaves of *Datura metel* Linné

Dried powdered leaves (4.5 kg.) were moistened with distilled water, mixed with calcium hydroxide (500 g.) and allowed to stand for overnight. The powdered mixture was macerated with diethyl ether (16 l.) and filtered. The marc was remacerated with another portion of diethyl ether (3 x 15 l.).

The combined filtrate was concentrated under reduced pressure to 1 l., and then extracted with 0.25 N. sulphuric acid (5 x 400 ml.). The acid solutions were combined, made alkaline with strong ammonia solution and extracted with chloroform (5 x 400 ml.). The combined chloroform extracts were dried with anhydrous sodium sulphate and concentrated under reduced pressure to get a crude base (4.8 g.).

Crude base (4.8 g.) was dissolved in chloroform, mixed with small amount of alumina, let the content be air-dried and packed onto the top of dry alumina column.

The column was eluted with chloroform, the eluate was collected and concentrated under reduced pressure to yield a crude base (3.2 g.).

Thin layer chromatograms of the crude base obtained by the above method as shown in (Fig. 9,10 pages 111,112) indicated that at least six alkaloids were present.

Crude alkaloids (3.22 g.) were dissolved in small amount of diethyl ether, and poured onto the top of silica gel column (2.5 cm. x 30 cm.) holding diethyl ether.

Elution of the column began with diethyl ether, followed by diethyl ether : ethanol (8 : 2), and methanol respectively. Thirty ml. fractions were collected manually.

Twenty six, 14, and 6 fractions of diethyl ether, diethyl ether : methanol (8 : 2) and methanol eluates respectively were collected.

Thin layer chromatogram of those fractions indicated that the separation and isolation of crude alkaloids by this method was unfavourable.

5. The isolation of alkaloids from the leaves of Datura metel Linné using preparative layer chromatography.

Dried powdered leaves (23.3 kg.) were moistened with distilled water, mixed with calcium hydroxide (2.5 kg.) and allowed to stand overnight. The mixture was macerated with diethyl ether (62 l.) for a week and then filtered. The marc was remacerated with diethyl ether (4 x 40 ml.). The filtrates were combined and concentrated under reduced pressure to 2.5 l. and then extracted with 0.25 N. sulphuric acid (5 x 2 l.). The combined acid extract was made alkaline with strong ammonia solution and extracted with chloroform (5 x 2 l.). The combined chloroform extract was evaporated under reduced pressure to yield a crude base (48.6 g.)

The crude base was divided into six equal parts (8.1 g.) and every part was treated as follows:-

Crude base was dissolved in a small volume of chloroform and mixed with alumina, air dried and packed onto the top of alumina column the column was eluted with chloroform until no trace of alkaloid could be detected in the last fraction. Chlorophyll and some impurities were removed by this treatment.

The combined eluates were evaporated under reduced pressure to dryness yielding crude base (42 g.)

Crude alkaloid (0.25 g. each) was dissolved in chloroform (1 ml.) and applied onto the silica gel plate by streaking.

The chromatogram developed twice with 5% methanol in diethyl ether.

The developed chromatogram was located with Dragendorff's spray reagent at the edge of the chromatogram, and the band appeared after spraying was numbered 1,2,3, and 4 respectively(see Fig. 11 page 113). These bands were used as a guide for scraping off. Each scraped band was kept separately and packed into the column, eluted with chloroform and followed by methanol. The combined eluates evaporated to dryness under reduced pressure to yield a brown residue. The treatment of remaining crude alkaloids were done similarly.

The eluates from bands of various plates were combined and evaporated and the result are as follows:-

Band 1 yielding 11.8 g. of crude base.

Band 2 yielding 4.42 g. of crude base.

Band 3 yielding 12.61 g. of crude base.

Band 4 yielding 10.4 g. of crude base.

Band 1 gave brown syrupy mass (11.8 g.) which was crystallized in the mixture of absolute ethanol and dry diethyl ether yielding cream colour needle crystals (172 mg.). Recrystallization of the crystals in absolute ethanol gave white needle crystals (20 mg.) designated as (Ra 1). Ra 1 was subsequently identified as hyoscyamine (m.p. 106°C.).

Band 2, crude base (4.4 g.) gave (50 mg.) of a cream colour needle crystals from a mixture of absolute ethanol and dry diethyl ether. Thin layer chromatography of eluted alkaloids from band 2 indicated that at least 3 alkaloids were present (see Fig. 12 page 114).

The base eluted from Band 3 (12.6 g.) was examined by thin layer chromatography and then crystallised in absolute ethanol and dry diethyl ether, yielding white needle crystals (120 mg.), designated as Ra 3. The mother liquor from which Ra 3 was crystallised out contained scopolamine as the main alkaloid and traces of Ra 3, when examined by thin layer chromatography (see Fig. 12 page 114). This mixture was made picrate salt yielding yellow needle crystals and was designated as Ra 4. Recrystallisation from absolute ethanol gave yellow needle crystals of Ra 4 (1.5 g.). Ra 4 was subsequently identified as scopolamine picrate.

Crude base (10.4 g.) from Band 4 was crystallised in absolute ethanol yielded white needle crystals (117 mg.) and was examined by thin layer chromatography showing that it is a mixture of two alkaloids. The R_F values of these two alkaloids are very close to each other and cannot be able to separate. They do not correspond to aposcopolamine, meteloidine and norscopolamine kindly supplied by Dr. W.C. Evans, Department of Pharmacy, University of Nottingham, England. According to Evans the R_F values of the two alkaloids neither correspond to 3-tigloyl-6-acetoxytropane, 3-tigloyltropane nor tigloidine.[55] (see Fig. 12 page 114).

D. The isolation of alkaloids from the tubers of *Dioscorea hispida* Dennst.

Fresh wild yam tubers (19.5 kg.) were sliced into thin pieces blended with 95% ethanol (20 l.), filtered, and the filtrates from 4 times of maceration were combined. The combined filtrate was concentrated under reduced pressure to syrupy mass.

The syrupy mass dissolved in 5% acetic acid and filtered. The acid solution was made alkaline with 10% sodium carbonate solution and followed by extraction with chloroform until the base was exhausted.

The combined chloroform extract was concentrated under reduced pressure to give a syrupy crude base (28.6 g.).

Examination of the crude base by thin layer chromatography indicated at least two alkaloids were present (see Fig. 14 page 116).

Crude alkaloid (9 g.) was dissolved in a small amount of absolute ethanol, and saturated picric acid in absolute alcohol was added to form yellow crystalline picrate (3.5 g.). This crystalline picrate was designated as Ra 6.

Ra 6 subsequently identified as dioscorine picrate. Another portion of crude alkaloid (9 g.) was dissolved in acetone and 5% of hydrobromic acid in acetone was added dropwise (using congo red paper as indicator) until the indicator paper changing from red to greenish blue (pH 3-5), the solution become turbid. After standing for a while, it yielded white needle crystals and designated as Ra 7.

After twice recrystallizations with absolute ethanol white needle crystals (500 mg.) were obtained. Ra 7 was subsequently identified as dioscorine hydrobromide.

The hydrochloride derivative from crude alkaloid (9 g.) was prepared by the same procedure shown above by using 5% hydrochloric acid in acetone.

After twice crystallizations white needle crystals (356.5 mg.) designated as Ra 8 were obtained and subsequently identified as dioscorine hydrochloride.

Code numbers of the alkaloids and alkaloidal salts.

Ra 1 = Isolated hyoscyamine.

Ra 2 = --

Ra 3 = Isolated substance(s) from Band 3 of preparative layer chromatography.

Ra 4 = Picrate salt of isolated scopolamine.

Ra 5 = Mixture of two alkaloids from Band 4 of preparative layer chromatography.

Ra 6 = Picrate salt of isolated dioscorine.

Ra 7 = Hydrobromide salt of isolated dioscorine.

Ra 8 = Hydrochloride salt of isolated dioscorine.

E. Characterisation and identification of the isolated alkaloids.

The characterisation of Ra 1 as hyoscyamine

Ra 1 was obtained as white needle crystals from the mixture of ethanol and diethyl ether (m.p. 106°C.) and its picrate (m.p. 165°C.). The alkaloid is soluble in ethanol, chloroform and slightly soluble in water and benzene. (Hyoscyamine m.p. 108.5°C., Hyoscyamine Picrate m.p. 165-166°C.)[4,27]

Optical rotation

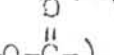
$[\alpha]_D^{23}$ = -18.23 (C = 1 in ethanol, l = 0.25 dm.)

Ultra-violet absorption spectrum

λ max.:- 248 mμ, 255 mμ, 260 mμ, 265 mμ, and 280 mμ. in methanol. (see Fig. 15 page 117).

Infra-red absorption spectrum (KBr disc)

$\bar{\nu}$ max.:- 1425 ($\overset{|}{\underset{|}{N}}-CH_3$) $cm.^{-1}$,

1600 (phenyl ) $cm.^{-1}$,

1720 (ester $-O-\overset{O}{\overset{\|}{C}}-$) $cm.^{-1}$,

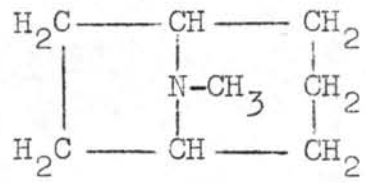
3405 (free -OH) $cm.^{-1}$.

(see Fig. 16 page 118).

Chemical test

Vitali's test

To about 1 mg. of Ra 1 contained in a small porcelain dish, add 1 ml. of nitric acid, and evaporated on a stream-bath just to dryness. Cool the residue, and add to it 2 drops of 1 in 10 solution of potassium hydroxide in ethanol, an intense violet colour is produced. The positive result of this test indicated that tropane group was present.



tropane alkaloid

Thin layer chromatography

Examination of Ra 1 by using thin layer chromatography correspondedtto the reference hyoscyamine (regenerated from hyoscyamine hydrobromide E. Merck.)

R_F value on Alumina G / Chloroform = 0.032

R_F value on Silica gel G / methyl ethyl ketone: 7.5% MeOH : 25% ammonia (6 : 3 : 1) = 0.35 (see Fig. 12,13 pages 114, 115).

Characterisation of Ra 3 (unidentified substance(s)

Ra 3 was obtained as colourless needle crystals (m.p.257°C) from the mixture of absolute ethanol and dry diethyl ether. The substance(s) is soluble in methanol, ethanol, [illegible] and very slightly soluble in ether, benzene, and water.

Ultra-violet absorption spectrum

λmax. 222 mμ. in methanol. (see fig. 17 page 119).

Infra red absorption spectrum

$\bar{\nu}$max.:- 1425 ($\overset{|}{\underset{|}{N}}$-$CH_3$) $cm.^{-1}$,

1675 (ester) $cm.^{-1}$,

3400 (free -OH group) $cm.^{-1}$

(see Fig. 18 page 120).

Chemical test

Vitali's test

Ra 3 gave positive result to the Vitali's test and indicated that tropane group was present.

Characterisation of Ra 4 as scopolamine picrate

Ra 4 was obtained as yellow needle crystals from absolute ethanol (m.p. 187°C.) and its base colourless syrupy mass.

Optical rotation

$[\alpha]_D^{23}$ = -5.3 (C = 2.7, H_2O, l = 0.25 dm.)

Ultra-violet absorption spectrum

Ra 4 was used.

λ max. 247 mμ, 252 mμ, 258 mμ, 264 mμ, in water.

(see Fig. 19 page 121)

Infra-red absorption spectrum (KBr disc.)

Base of Ra 4 (isolated scopolamine) was used.

λ max.:- 850 (epoxy) $cm.^{-1}$,

1425 ($\overset{|}{\underset{|}{N}}-CH_3$) $cm.^{-1}$,

1625 (phenyl ) $cm.^{-1}$,

1700 (ester) $cm.^{-1}$,

3029 (epoxy) $cm.^{-1}$,

3058 (epoxy) $cm.^{-1}$,

3400 (free -OH group) $cm.^{-1}$

(see Fig. 20 page 122).

Chemical test

Vitali's test

Base of Ra 4 gave positive result to the Vitali's test, so it indicated that tropane group was present.

Thin layer chromatography

Examination of the base of Ra 4 by using thin layer chromatography showed that the base of Ra 4 corresponded to authentic scopolamine (regenerated from scopolamine hydrobromide E. Merck.)

R_F value of Ra 4 on Alumina G./Chloroform = 0.32

R_F value of Silica gel G./methyl ethyl ketone : 7.5% MeOH : 25% ammonia (6 : 3 : 1) = 0.609 (see Fig. 12,13 pages 114,115).

Characterisation of Ra 6 as dioscorine picrate, Ra 7 as dioscorine hydrobromide and Ra 8 as dioscorine hydrochloride.

Ra 6 was obtained as yellow needle crystals (m.p. 187°C) from absolute ethanol. (Dioscorine picrate m.p. = 187°C)[4,27]

Ra 7 was obtained as white needle crystals (m.p. 207°C) from acetone. (Dioscorine hydrobromide m.p. = 207°C)[4,27]

Ra 8 was obtained as white needle crystals (m.p. 210°C) from acetone. (Dioscorine hydrochloride m,p. =210°C ,207°C)[4,27]

Ultra-violet absorption spectrum of Ra 6 base

λ max. = 215.4 mμ. in methanol.(see Fig. 21 page 123).

Infra red absorption spectrum of Ra 8 (KBr disc.)

$\bar{\nu}$ max. 1425 ($\overset{|}{\underset{|}{N}}-CH_3$) $cm.^{-1}$,

1620-1640 (double bond -C=C-) $cm.^{-1}$,

1700-1733 (lactone or ester) $cm.^{-1}$,

3400-3500 (free -OH group) $cm.^{-1}$

(see Fig. 22page 124)

Chemical test

Colour test (described by Pinder A.R. 1952)[28]

(a) The alkaloid gave a reddish violet colour with alkaline nitroprusside solution.

(b) The alkaloid gave a violet color with potassium iodate and sulphuric acid.

Vitali's test of Ra 7 and Ra 8

Either sample of alkaloid gave positive violet colour with Vitali's test, and showed that they were tropane alkaloids.