THE QUANTITATIVE DETERMINATION OF SOME MITRAGYNA OXINDOLE ALKALOIDS AFTER SEPARATION BY THIN-LAYER CHROMATOGRAPHY*

PART II. COLORIMETRY, USING THE VITALI–MORIN REACTION

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INTRODUCTION

The use of colorimetry in association with TLC for the quantitative determination of alkaloids has been reported by several workers[1–4]. In each case the alkaloids have been eluted from the adsorbent prior to the formation of the colour complex. Since methods of separation and identification of the Mitragyna oxindole alkaloids by TLC have already been described[5–7] and their quantitative determination by

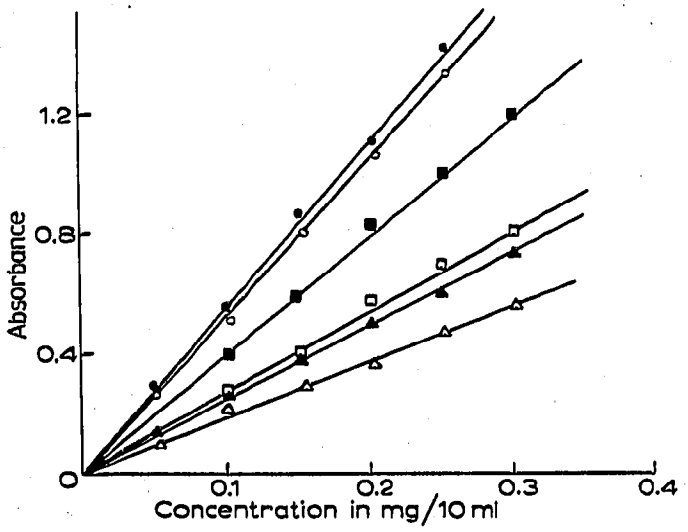

Fig. 1. Calibration curves of the alkaloidal colour complexes (non chromatographed alkaloids). Rotundifoline, (—■—■—■—) at 375 mμ, (—□—□—□—) at 422 mμ; isorotundifoline, (—▲—▲—▲—) at 375 mμ, (—△—△—△—) at 422 mμ; rhynchophylline, (—●—●—●—) at 402 mμ; isorhynchophylline, (—○—○—○—) at 402 mμ; mitraphylline, (—●—●—●—) at 402 mμ; isomitraphylline, (—○—○—○—) at 402 mμ.

means of the Vitali–Morin colour reaction has recently been reported[8] it should be possible to determine these alkaloids in admixture after separation by TLC.

Details of the structure of the Mitragyna oxindole alkaloids were given in

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Part I[9] of this series and the characteristics of the colour complexes have been given by SHELLARD AND ALAM[8]. They are such that the alkaloids can be divided into two groups and the wavelengths 402 mμ and 422 mμ are suitable for measuring the absorbances of Group I and Group II alkaloids, respectively. Calibration curves for the six alkaloids representative of these groups, previously determined by SHELLARD AND ALAM[8], are given in Fig. 1.

This report gives some account of the problems relating to the quantitative determinations of the oxindole alkaloids in admixture by colorimetry in association with TLC.

EXPERIMENTAL

Thin-layer chromatography

The experimental details concerning the preparation of the plates and application of the samples are as described in Part I[9].

Detection of alkaloids. By conc. HNO_3 and heating at 130° for 1 h*.

Removal of adsorbent from the plate. A square of definite size was marked around the nitrated alkaloid and the material from this area was then transferred by means of an aluminium foil scraper to a centrifuge tube.

Elution of nitrated alkaloid. With 5 or 10 ml dimethylformamide.

Production of colour. By the addition of tetraethylammonium hydroxide (TEAH) in dimethylformamide as given in the text.

Preparation of blank solutions. As described above but using a similar area of adsorbent not containing alkaloid.

Spectrophotometry

The spectrophotometers used were:

(a) Beckman DK-2 for automatic recording.

(b) Hilger Uvispek for manual recording.

Dimethylformamide was used as the solvent.

RESULTS AND DISCUSSION

There are three possible methods of applying the Vitali–Morin colour reaction to alkaloids separated by TLC: (i) Elution of the alkaloids from the adsorbent using the techniques described in Part I[9] and then following the procedure given by SHELLARD AND ALAM[8]. This necessitates prior location of the alkaloids. (ii) Production of the Vitali–Morin colour complex on the thin layer followed by eluting the complex and measuring its absorption. This necessitates nitration of the alkaloid on the layer by spraying the chromatographed plate with concentrated nitric acid and keeping the plate at a given temperature for a given time. The layer must then be sprayed with TEAH in dimethylformamide, by which means the position of the alkaloid is also located. This method is not entirely suitable because in order to obtain a colour which does not fade quickly it is necessary to spray with excessive amounts of reagent and this frequently leads to damage of the surface of the layer and interference with the boundaries of adjacent spots so that they may merge. Furthermore, elution of the colour complex is not always complete even with 10 ml dimethylformamide. (iii) Nitration of the alkaloid directly on the layer, which also locates the alkaloids,

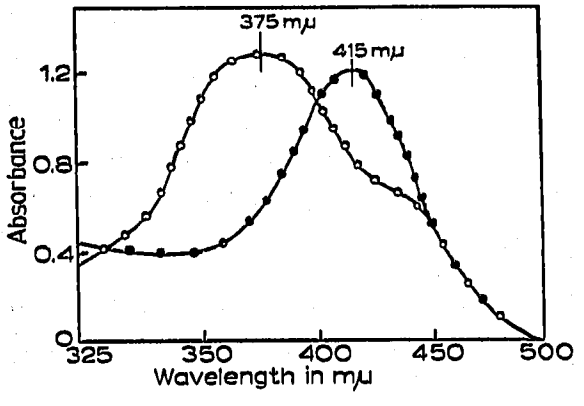
followed by elution of the nitrated alkaloid and subsequent treatment with TEAH in dimethylformamide. This method proved very satisfactory, giving very consistent and reproducible results and an account of this method is given below.

It is essential that the alkaloids are completely nitrated, the extent of nitration depending upon the temperature of the oven and the time for which the nitric acid treated layer is maintained at this temperature. Although rotundifoline and isorotundifoline are more readily nitrated than the other alkaloids, when silica gel is used as the adsorbent all six alkaloids are fully nitrated after heating for 1 h at 130°. With alumina layers, rotundifoline and isorotundifoline are fully nitrated after 1 h at 110° but the other alkaloids require 1 h at 130–140°. Nitration on silica gel is certainly more satisfactory than on alumina; further the nitrated alkaloids on alumina are very pale yellow in colour and are difficult to locate satisfactorily so that it is necessary to spray lightly with TEAH in dimethylformamide after nitration in order to be certain of their location.

There is some evidence that while the nitrated alkaloid remains stable on the adsorbent for 24 h some change may occur after this, the absorbance of the nitrated alkaloid increasing slightly with length of time on the layer up to 72 h so that it is desirable to elute the nitrated alkaloid within 24 h of nitration.

The first noticeable feature of this method is that while the spectra of the colour complexes can again be used to divide the alkaloids into the same two groups, the spectra are quite different from those obtained following direct nitration of the alkaloids (*i.e.*, in a flask when thin-layer chromatography is not involved (SHELLARD AND ALAM[8]); Group I alkaloids having a maximum absorbance at 415 mμ and Group II alkaloids a maximum at 440 mμ. The spectra are remarkably stable.

Figs. 2 and 3 show the two spectra of rotundifoline and mitraphylline colour complexes, respectively: (a) when the alkaloid is nitrated directly and (b) when the alkaloid is nitrated on the thin layer. This difference is not influenced by the TEAH since the spectrum of the nitrated alkaloid itself in dimethylformamide differs according to the method of nitration, that nitrated directly having a maximum at 400 mμ and that nitrated on the thin layer having a maximum at 338 mμ (Fig. 4). A similar



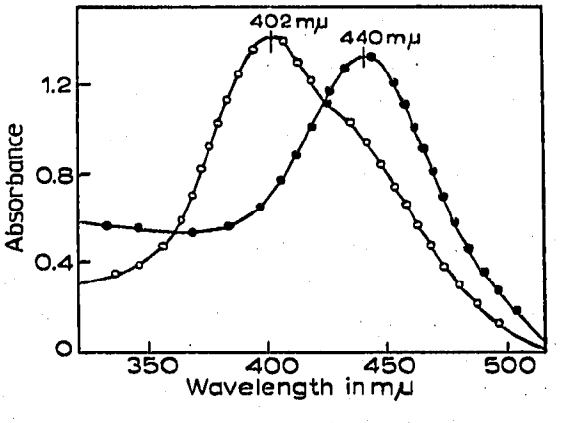
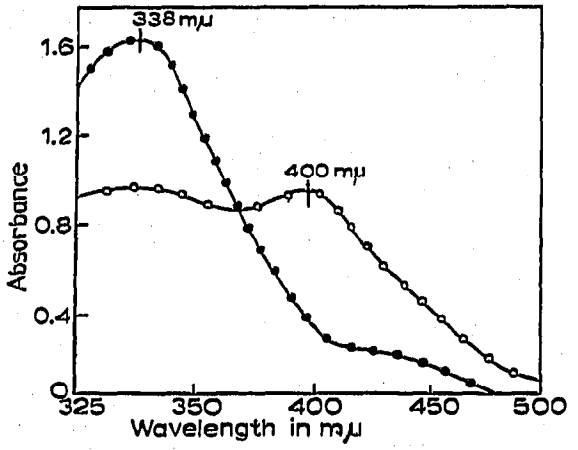


Fig. 2. Spectra of rotundifoline Vitali–Morin colour complexes. (—O—O—O—) When nitrated directly; (—●—●—●—) when nitrated on adsorbent.

Fig. 3. Spectra of mitraphylline Vitali–Morin colour complexes. (—O—O—O—) When nitrated directly; (—●—●—●—) when nitrated on adsorbent.

difference is shown by the spectra of the colour complexes of the other alkaloids when the nitration is carried out directly and when the nitration is carried out on the thin layer.

It is clear that the product of nitration is affected by the presence of the adsorbent but the same result is obtained whether the adsorbent is silica gel, alumina or magnesium silicate (Fig. 5). This phenomenon applies equally to all six alkaloids and is not, therefore, a function of the hydroxyl group at C (9). If the alkaloids are



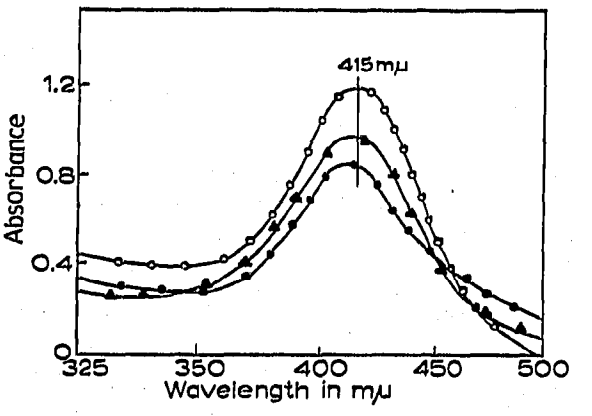


Fig. 4. Spectra of nitrated mitraphylline. (—○—○—○—) When nitrated directly; (—●—●—●—) when nitrated on adsorbent.

Fig. 5. Spectra of rotundifoline Vitali–Morin colour complexes (120 μg/5 ml). (—○—○—○—) When nitrated on silica gel layer; (—●—●—●—) when nitrated on alumina layer; (—▲—▲—▲—) when nitrated on magnesium silicate layer.

nitrated in a flask in the presence of added adsorbent, the spectra are the same as those obtained when the alkaloids are nitrated on the adsorbent, either still adhering to the plate or in a flask after scraping the adsorbent containing the alkaloid from the plate. When the alkaloid is nitrated directly in a flask and the adsorbent is added afterwards the addition of TEAH in dimethylformamide produces a colour complex having a maximum at 375 mμ (Group I) or 402 mμ (Group II) and not at 415 mμ (Group I) or 440 mμ (Group II). Thus the new nitrated compound is obtained only when the alkaloid is nitrated in the presence of adsorbent.

The colour complex obtained when silica gel is the adsorbent has a higher extinction coefficient than that obtained on alumina which further confirms that alumina is not a very satisfactory adsorbent to use. For the separation of certain mixtures of alkaloids, however, alumina may be necessary.

The second noticeable feature of this method is that, whereas with directly nitrated alkaloids, the full colour intensity develops immediately, with alkaloids nitrated on the adsorbent the development of the colour takes place more slowly and 30–40 min are required for the full colour intensity to develop.

A third feature is that, in contrast to the directly nitrated alkaloid which only requires 0.12 ml of 2.5 % TEAH in dimethylformamide to produce full colour intensity (though 1 ml is necessary to prevent the colour from fading), the adsorbent nitrated alkaloid requires 2.5 ml of 2.5 % TEAH in dimethylformamide.

The fourth difference between the colour complex of the directly nitrated alkaloid and that of the adsorbent nitrated alkaloid is that whereas the former is obtained in the most stable form by using a reagent more than 6 h old (or after a time of 60–90 min, using freshly prepared reagent) no colour at all is obtained by using the old reagent with the adsorbent nitrated alkaloids. A colour is only obtained by using freshly prepared 2.5 % TEAH in dimethylformamide or 25 % aqueous TEAH. No changes occur in the spectra of any of the alkaloids and all the colours are extremely stable.

The intensity of the colour for any given amount of alkaloid is the same irrespective of the solvent system used for the development of the chromatogram. Table I shows the results obtained with rotundifoline using four different solvent systems on silica gel and two on alumina.

TABLE I

EFFECT OF USING DIFFERENT CHROMATOGRAPHIC SOLVENT SYSTEMS ON THE ABSORBANCE OF COLOUR COMPLEX

Alkaloid	*Chromatographic system*	*Theoretical amount (μg)*	*Absorbance of the colour complex*	*Average*
Rotundifoline	Silica gel/ethyl acetate–benzene (7:2)	60.0	0.602, 0.605, 0.605, 0.601, 0.595	0.601
	Silica gel/ether		0.602, 0.605, 0.607, 0.607, 0.597	0.603
	Silica gel/benzene–acetone (1:1)		0.605, 0.605, 0.607, 0.591, 0.595	0.600
	Silica gel/methanol		0.607, 0.605, 0.598, 0.595, 0.602	0.601
	Alumina/chloroform–cyclohexane (7:3)		0.325, 0.351, 0.341, 0.348, 0.325	0.338
	Alumina/chloroform		0.335, 0.341, 0.319, 0.329, 0.339	0.332

Dimethylformamide, as the solvent, recovers approximately 99 % of the alkaloid colour complex using 5 ml reagent unless the load is greater than 80.0 μg when at least 2 × 5 ml extractions are necessary.

Since the colour complexes have maxima at 415 mμ and 440 mμ (Group I and Group II alkaloids respectively) it is unlikely that the impurities extracted from the adsorbent by the dimethylformamide will interfere to any great extent with the adsorbance readings. There is, however, some absorbance from the eluate of the blank adsorbent and therefore such an eluate must be used as the reference solution. The investigation shows that there is no difference in the absorbance figures whether the reference solution is prepared from adsorbent taken from different portions of the same plate or from identical portions on different plates (Tables II and III) but it is not possible to measure the absorbance of an eluate of any blank adsorbent and use this as a standard reference since the actual absorbance from the adsorbent eluate will vary according to the solvent system used for the chromatography (Table IV).

TABLE II

EFFECT OF OBTAINING REFERENCE SOLUTIONS FROM DIFFERENT POSITIONS (R_F VALUES) OF THE SAME PLATE

Alkaloid	*Chromatographic system*	*R_F value*	*Blank solution extracted from R_F values of*	*Absorbance of the colour complex*	*Mean*
Rotundifoline (60.0 μg)	Silica gel/chloroform–acetone (5:4)	0.66–0.74	0.00	0.605, 0.592, 0.601, 0.592, 0.599	0.597
			0.40–0.50	0.602, 0.592, 0.609, 0.597, 0.605	0.601
(at 415 mμ)			0.66–0.74	0.610, 0.592, 0.591, 0.595, 0.601	0.597
Isomitraphylline 80.0 μg		0.60–0.67	0.00	0.585, 0.690, 0.599, 0.592, 0.601	0.593
			0.40–0.50	0.595, 0.592, 0.594, 0.585, 0.601	0.593
(at 440 mμ)			0.60–0.67	0.585, 0.592, 0.602, 0.601, 0.601	0.596
Mitraphylline 56.0 μg		0.38–0.45	0.00	0.425, 0.430, 0.431, 0.412, 0.418	0.423
			0.38–0.45	0.432, 0.419, 0.418, 0.423, 0.421	0.422
(at 440 mμ)			0.60–0.70	0.418, 0.428, 0.431, 0.432, 0.428	0.427

TABLE III

EFFECT OF OBTAINING REFERENCE SOLUTIONS FROM DIFFERENT PLATES BUT AT THE SAME R_F VALUE AS THAT OF THE ALKALOID

Alkaloid	*Chromatographic system*	*R_F value*	*Blank solution extracted from*	*Absorbance of the colour complex (at 415 or 440 mμ)*	*Mean*
Rotundifoline 60.0 μg (at 415 mμ)	Silica gel/chloroform–acetone (5:4)	0.66–0.74	Same plate	0.612, 0.592, 0.590, 0.601, 0.611	0.601
			2nd plate	0.591, 0.592, 0.596, 0.601, 0.612	0.598
			3rd plate	0.602, 0.605, 0.592, 0.595, 0.597	0.598
Isomitraphylline 80.0 μg (at 440 mμ)		0.60–0.67	Same plate	0.591, 0.592, 0.612, 0.585, 0.607	0.597
			2nd plate	0.590, 0.612, 0.602, 0.612, 0.585	0.600
			3rd plate	0.591, 0.591, 0.604, 0.612, 0.581	0.595
Mitraphylline 36.0 μg (at 440 mμ)		0.38–0.45	Same plate	0.431, 0.435, 0.412, 0.418, 0.421	0.423
			2nd plate	0.418, 0.421, 0.429, 0.409, 0.431	0.419

TABLE IV

ABSORBANCE OF THE REFERENCE SOLUTIONS ELUTED FROM 1.0 SQUARE IN. AREA OF SILICA GEL PLATES, SPRAYED WITH CONC. NITRIC ACID AND HEATED AT 130° FOR 1 HOUR, AFTER DEVELOPMENT WITH DIFFERENT SOLVENT SYSTEMS

Elution solvent: dimethylformamide (5 ml). The nitrated thin layer extracted from a position of R_F value = 0.5.

Solvent system	*Absorbance between 350–500 mμ each reading is an average of 5*
Chloroform	0.032
Ether	0.029
Ether–diethylamine (99:1)	0.042
Chloroform–acetone (5:4)	0.035
Benzene–acetone	0.043
Methanol	0.012

Thus the recommended procedure is:

(1) Spray the developed plates with concentrated nitric acid.

(2) Silica gel plates: maintain at 130° for 1 h.
Alumina plates: maintain at 140–150° for 1 h.

(3) Silica gel plates: scrape the adsorbent containing the nitrated alkaloid from the plate (1 sq. in.) and transfer to a centrifuge tube.

Alumina plates: spray with *freshly prepared* 2.5 % TEAH in dimethylformamide and wait for the yellow colour to develop in order to locate the alkaloids. Then scrape the adsorbent containing the alkaloid colour complex from the plate (1 sq. in.) and transfer to a centrifuge tube.

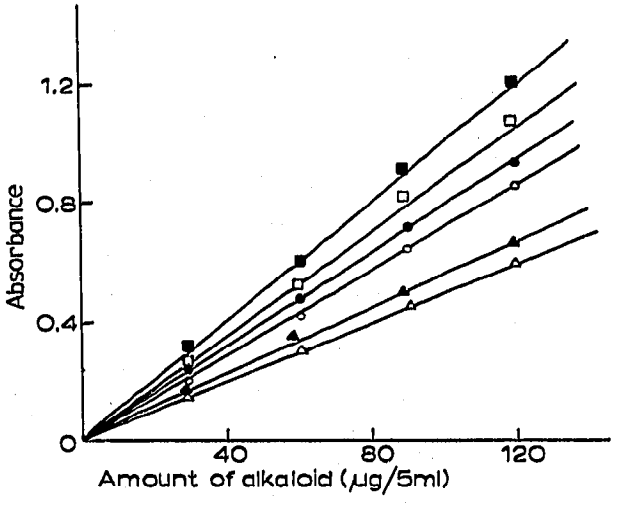


Fig. 6. Calibration curves of the alkaloidal colour complexes (after chromatographic separation and nitration on the plate). Rotundifoline: from silica gel, at 415 mμ (—■—■—■—); from alumina, at 415 mμ (—▲—▲—▲—). Isorotundifoline: from silica gel, at 415 mμ (—□—□—□). Rhynchophylline: from silica gel, at 440 mμ (—●—●—●—). Isorhynchophylline: from silica gel, at 440 mμ (—○—○—○—); from alumina, at 440 mμ (—△—△—△—). Mitraphylline: from silica gel, at 440 mμ (—●—●—●). Isomitraphylline: from silica gel, at 440 mμ (—○ —○—○—); from alumina, at 440 mμ (—△—△—△—).

TABLE V

APPARENT SPECIFIC EXTINCTION COEFFICIENTS OF THE OXINDOLE ALKALOIDS

Wavelength mμ	*Rotundifoline*	*Isorotundifoline*	*Rhynchophylline*	*Isorhynchophylline*	*Mitraphylline*	*Isomitraphylline*
390	29.95	28.38	15.69	14.22	15.22	14.72
440	35.06	30.20	39.24	34.94	38.94	35.22

(4) Scrape 1 sq. in. of adsorbent from one of the plates used in the determination and transfer to a centrifuge tube.

(5) Add 4.5 ml dimethylformamide and 0.5 ml *freshly prepared* 2.5 % TEAH in dimethylformamide. Shake occasionally during 45 min and then centrifuge the suspension.

(6) Remove the supernatant solution and read the absorbance at 415 mμ or 440 mμ (according to the alkaloid) using as the reference solution, the eluate of blank adsorbent.

The calibration curves obtained with the six oxindole alkaloids are shown in Fig. 6.

TABLE VI

DETAILS OF REGRESSION ANALYSIS OF THE EXPERIMENTAL DATA OF ROTUNDIFOLINE COLOUR COMPLEX EXTRACTED FROM SILICA GEL PLATES

Source of variation	*Sum of squares*	*F°*	*Mean squares*
Between concentrations	4.2709	5	0.8541
Within concentrations	0.0136	30	0.00045
Total	4.2845	35	—

$$F^{5}_{30} = \frac{0.8541}{0.00045} = 1898;\ p = <<< 0.001,$$

i.e. the greater mean square against lower mean square is significant.

Source of variation	*Sum of squares*	*F°*	*Mean squares*
Between concentration due to regression	4.2676	1	4.2676
Deviation from regression	0.0033	4	0.000845
Within concentrations (residual)	0.0136	30	0.00045
Total	4.2845	35	

$$F^{1}_{30} = \frac{4.2676}{0.00045} = 9483;\ p = <<< 0.001.$$

Therefore the regression line is highly significant,

$$F^{4}_{30} = \frac{0.000845}{0.00045} = 1.833;\ p = <<< 0.001.$$

Departures from linearity are insignificant. The statistical test therefore does not disapprove the linearity of concentration–spectrophotometric readings.

TABLE VII

CONSTANTS OF THE REGRESSION LINES OF THE EXPERIMENTAL DATA OF THE VITALI–MORIN COLOUR COMPLEX OF THE ALKALOIDS EXTRACTED FROM ADSORBENT LAYER BY DIMETHYLFORMAMIDE (5 ml)

Alkaloid	*Adsorbent*	*Slope of the line*	*Variance about the line*
Rotundifoline	Silica gel	0.010080	$9.7 \cdot 10^{-5}$
Isorotundifoline		0.0090	$6.58 \cdot 10^{-4}$
Rhynchophylline		0.007952	$4.21 \cdot 10^{-4}$
Isorhynchophylline		0.007503	$1.48 \cdot 10^{-4}$
Mitraphylline		0.007952	$4.20 \cdot 10^{-4}$
Isomitraphylline		0.00744	$3.51 \cdot 10^{-4}$
Rotundifoline	Alumina	0.005292	$2.14 \cdot 10^{-4}$
Isorotundifoline		0.00502	$0.76 \cdot 10^{-4}$
Rhynchophylline		0.004182	$2.55 \cdot 10^{-4}$
Isorhynchophylline		0.005104	$3.28 \cdot 10^{-4}$
Mitraphylline		0.004227	$5.32 \cdot 10^{-4}$
Isomitraphylline		0.005090	$1.35 \cdot 10^{-4}$

It may be that with some TLC systems, two adjacent alkaloids are not separated sufficiently to permit each alkaloid to be completely removed from the layer. If these two alkaloids are one from each group, then they can be removed from the layer as one spot and after elution and treatment with TEAH, each can be estimated by measuring the absorbance at two suitable wavelengths, *e.g.* 390 mμ and 440 mμ and calculating the results from the simultaneous equations:

$$A_{390} = K_{390}^{I} + K_{390}^{II} C^{II}$$
$$A_{440} = K_{440}^{I} + K_{440}^{II} C^{II}$$

where A, K and C are absorbance, apparent specific extinction coefficient and concentration (μg/5 ml) respectively. I and II refer to the two alkaloids.

The apparent specific extinction coefficient of the alkaloid colour complexes at 390 mμ and 440 mμ are as given in Table V.

The use of the Vitali–Morin colour complex as the basis of the quantitative determination involves a procedure with three stages, two of which are chemical reactions, while the ultraviolet spectrophotometric method only involves one stage—the physical elution of the alkaloid from the layer. There is thus a greater possibility of variation on the resultant product, the absorbance of which is the basis of the determination. However, the regression analysis of the experimental data confirms that a linear relationship exists between the amount of alkaloid and the absorbance of the colour complex (Table VI).

Table VII shows the slope of the best fitting lines and the variance about the regression line. It will be seen that the variation about the regression line is insignificant and therefore the slope of the regression line can be used directly for calculating the amount of alkaloid in 5 ml of dimethylformamide from the equation:

$$x = \frac{y - c}{b}$$

TABLE VIII

ANALYSIS OF ALKALOIDS BY VITALI–MORIN REACTION AFTER SEPARATION BY THIN-LAYER CHROMATOGRAPHY

(*a*) *Rotundifoline and isorotundifoline*

Alkaloid	*Chromatographic system*	R_F *value*	*Theoretical amount* (μg)	*Found experimentally* (μg)	*Average* (μg)	% *Coefficient of variation*
Rotundifoline	Silica gel/chloroform–acetone (5:4)	0.66–0.74	18.0	17.20, 18.90, 19.00, 18.20, 18.50	18.36	3.92
			36.00	35.20, 37.00, 36.20, 37.25, 35.75	36.28	2.35
			72.00	70.90, 72.10, 72.80, 73.60, 73.00	72.48	1.45
Isorotundifoline		0.40–0.44	10.00	11.00, 11.02, 10.01, 11.00, 9.95	10.59	3.83
			20.00	20.05, 20.07, 21.00, 19.03, 19.25	19.88	3.96
			40.00	40.05, 40.00, 40.03, 38.50, 40.03	39.72	1.78
Rotundifoline	Alumina/chloroform–cyclohexane (7:3)	0.30–0.35	18.00	18.90, 16.20, 17.20, 17.19, 16.35	17.16	6.18
			36.00	34.25, 35.28, 35.81, 37.18, 35.20	35.54	3.00
			72.00	71.28, 70.02, 70.69, 74.01, 69.21	71.04	2.57
Isorotundifoline		0.17–0.22	10.00	9.21, 9.19, 10.21, 9.81, 9.12	9.50	5.00
			20.00	18.12, 19.26, 18.21, 19.21, 18.71	18.59	2.97
			40.00	36.28, 36.91, 37.98, 39.21, 40.18	38.11	4.27

(*b*) *Rhynchophylline and isorhynchophylline*

Alkaloid	*Chromatographic system*	R_F *value*	*Theoretical amount* (μg)	*Found experimentally* (μg)	*Average* (μg)	% *Coefficient of variation*
Rhynchophylline	Silica gel/chloroform–acetone (5:4)	0.24–0.33	15.20	14.81, 15.91, 14.92, 15.10, 15.80	15.30	3.32
			30.40	29.49, 29.21, 28.52, 28.91, 30.01	29.22	1.92
			60.80	61.28, 62.10, 61.01, 60.81, 59.18	60.87	1.74
Isorhynchophylline		0.63–0.70	14.00	13.28, 13.00, 13.18, 13.18, 13.91	13.31	2.60
			28	27.91, 26.91, 26.81, 27.11, 29.12	27.57	3.49
			42.00	40.81, 40.81, 41.21, 39.21, 39.10	40.22	2.46

(*continued on p. 499*)

TABLE VIII (*continued*)

Alkaloid	*Chromatographic system*	R_F *value*	*Theoretical amount* (μg)	*Found experimentally* (μg)	*Average* (μg)	% *Coefficient of variation*
Rhynchophylline	Alumina/chloroform	0.10–0.15	15.20	13.20, 14.81, 13.01 13.91, 15.00	13.98	6.46
			30.40	29.81, 32.00, 30.12, 32.91, 32.81	31.37	3.75
			60.80	62.81, 58.28, 59.12, 60.10, 58.18	59.69	3.17
Isorhynchophylline		0.44–0.50	14.0	13.21, 15.21, 13.12, 14.91, 15.18	14.32	7.53
			28.00	26.21, 26.91, 28.12, 26.12, 26.91	26.85	5.97
			42.00	45.09, 40.81, 40.12, 41.81, 41.28	42.20	4.36

(*c*) *Mitraphylline and isomitraphylline*

Alkaloid	*Chromatographic system*	R_F *value*	*Theoretical amount* (μg)	*Found experimentally* (μg)	*Average* (μg)	% *Coefficient of variation*
Mitraphylline	Silica gel/chloroform–acetone (5:4)	0.38–0.43	10.00	9.21, 9.81, 10.21, 9.42, 9.18	9.56	4.55
			20.00	18.91, 18.99, 20.93, 19.21, 18.72	19.35	4.65
			30.00	27.17, 28.28, 29.18, 29.81, 28.17	28.52	3.56
Isomitraphylline		0.60–0.67	21.00	19.21, 18.92, 21.08, 20.12, 18.81	19.62	4.89
			42.00	41.21, 39.21, 41.81, 40.81, 41.01	40.81	2.38
			63.00	61.12, 60.21, 64.01, 62.01, 62.18	61.90	0.72
Mitraphylline	Alumina/chloroform	0.15–0.20	10.00	8.91, 10.12, 8.52, 9.28, 9.17	9.20	6.43
			20.00	18.20, 21.81, 19.20, 19.72, 19.58	19.70	6.70
			30.00	27.21, 28.21, 29.18, 31.21, 31.91	29.54	6.71
Isomitraphylline		0.35–0.40	21.00	18.50, 19.71, 19.91, 21.21, 21.19	20.10	5.62
			42.00	41.21, 39.21, 41.18, 42.81, 42.01	41.28	3.25
			63.00	61.21, 60.18, 59.22, 62.81 63.18	61.32	2.75

(*continued on p. 500*)

TABLE VIII (*continued*)

(*d*) *Rotundifoline, isorotundifoline and rhynchophylline*

Alkaloid	*Chromatographic system*	R_F *value*	*Theoretical amount* (μg)	*Found experimentally* (μg)	*Average* (μg)	% *Coefficient of variation*
Rotundifoline	Silica gel/chloroform–acetone (5:4)	0.66–0.74	20.0	19.01, 19.21, 20.10, 21.01, 19.17	19.70	4.22
			40.0	38.22, 38.91, 39.21, 40.81, 41.29	39.68	3.31
Isorotundifoline		0.40–0.44	15.0	14.81, 13.81, 15.28, 14.21, 14.92	14.60	4.05
			30.0	28.29, 29.17, 31.21, 30.18, 28.18	29.40	5.78
Rhynchophylline		0.24–0.33	16.20	15.21, 16.91, 15.91, 14.91, 15.81	15.75	4.81
			32.40	31.81, 30.91, 31.22, 31.89, 32.82	31.73	2.31

TABLE IX

ANALYSIS OF A FOUR COMPONENT MIXTURE BY SIMULTANEOUS EXTRACTION OF TWO ALKALOIDAL SPOTS

(a) Rotundifoline and isorhynchophylline together.
(b) Isorotundifoline and rhynchophylline together.

	Alkaloid	*Chromatographic system*	R_F *value*	*Theoretical amount* (μg)	*Found experimentally* (μg)	*Average* (μg)	% *Coefficient of variation*
(a)	Rotundifoline	Silica gel/chloroform–acetone (5:4)	0.63–0.74	20.00	19.01, 18.23, 17.91, 20.81, 19.81	19.15	6.21
				40.00	39.21, 37.81, 36.91, 47.91, 39.92	38.35	3.125
	Isorhynchophylline			18.00	17.01, 18.21, 16.21, 17.12, 18.92	17.49	6.10
				36.00	34.29, 33.29, 36.92, 37.21, 33.21	34.98	5.54
(b)	Isorotundifoline		0.33–0.45	18.00	17.81, 19.28, 18.21, 17.21, 19.28	18.35	4.99
				36.00	33.29, 34.22, 33.80, 36.29, 36.71	34.36	4.41
	Rhynchophylline			25.40	26.22, 24.28, 25.21, 27.12, 26.91	25.94	4.56
				50.80	48.20, 47.01, 49.12, 47.20, 49.81	48.26	2.49

where:

x = amount of alkaloid (μg/in 5 ml)
y = average of 5 spectrophotometric readings
c = intercept (= O)
b = slope of the regression line.

Results of the analyses of mixtures of alkaloids, after separation by TLC are shown in Table VIIIa, b, c and d. (The fact that alumina is not as satisfactory as silica gel for this technique is evidenced by the high figures for the % coefficient of variation with alumina.)

Table IX shows the results obtained by the use of binary mixtures when the separation of the alkaloid is not sufficiently good for their individual determination.

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SUMMARY

A method for the colorimetric determination of some oxindole alkaloids based on the Vitali–Morin reaction, after their separation by thin-layer chromatography, is described. Problems associated with the nitration and formation of the colour complex with tetraethylammonium hydroxide, TEAH in dimethylformamide are discussed together with the related problems of quantitative recovery from the layers and the effect of interfering substances. A statistical analysis of results obtained by the method is given.

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