# THE CORRELATION BETWEEN THE STEREOCHEMISTRY OF SOME INDOLE AND OXINDOLE ALKALOIDS AND THEIR BEHAVIOUR ON THIN LAYER CHROMATOGRAMS*

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## INTRODUCTION

SHELLARD AND PHILLIPSON[1] have reported the use of thin layer chromatography as an aid to distinguish and identify 12 indole and oxindole alkaloids isolated from species of *Mitragyna*. These alkaloids were first separated on thin layer chromatograms using a scheme described by WALDI, SCHNACKERZ AND MUNTER[2] and then by a series of systems based on the Waldi scheme. Further alkaloids have been isolated from *Mitragyna speciosa* Korth[3,4] and the behaviour of all these alkaloids on thin layer chromatograms has been compared with some indole and oxindole alkaloids of known stereochemistry.

## METHODS

Adsorbents: Aluminium oxide G (Merck, 15% calcium sulphate), 35 g/60 ml distilled water; Silica gel G (Merck), 30 g/60 ml distilled water.

Plate size: 20 × 20 cm.

Layer thickness: 250 $\mu$.

Activation: Air dried for 15 min, heated at 105° for 60 min, stored in a desiccator over silica gel and reheated for 15 min at 105° immediately prior to use.

Solvents: See below; freshly distilled.

Running distance: 10 cm.

Temperature: 22–25°.

Load: Approx. 1 $\mu$g of alkaloid.

Detection: Dragendorff's spray reagent.

The thin-layer systems used in the modified Waldi scheme were as follows:

*Series A*: *untreated plates, neutral solvents*

1. Cyclohexane, silica gel
2. Cyclohexane–chloroform (5:4), silica gel
3. Chloroform, silica gel.
4. Benzene–ethyl acetate (7:2), silica gel

* This work forms part of a thesis submitted by J. D. PHILLIPSON for the degree of Ph.D of the University of London (July 1965).

5. Chloroform–benzene (1:1), alumina
6. Cyclohexane–chloroform (3:7), alumina
7. Ether, silica gel
8. Chloroform, alumina
9. Chloroform–acetone (5:4), silica gel
10. Methanol, silica gel

*Series B*: *untreated plates, alkaline solvents*

1. Cyclohexane–diethylamine (9:1), silica gel
2. Cyclohexane–chloroform–diethylamine (5:4:1), silica gel
3. Chloroform–diethylamine (9:1), silica gel
4. Benzene–ethyl acetate–diethylamine (7:2:1), silica gel
5. Chloroform–benzene–diethylamine (1:1:0.001), alumina
6. Cyclohexane–chloroform–diethylamine (3:7:0.005), alumina
7. Ether–diethylamine (9:1), silica gel
8. Chloroform–diethylamine (9:1), alumina
9. Chloroform–acetone–diethylamine (5:4:1), silica gel
10. Methanol–diethylamine (9:1), silica gel

*Series C*: *alkaline plates, neutral solvents*

The solvent systems 1–10 were the same as in Series A. Plates were prepared with $N/10$ sodium hydroxide solution instead of distilled water.

## RESULTS

System 2, Series B, silica gel/cyclohexane–chloroform–diethylamine (5:4:1) can be used to distinguish between the known indole and oxindole alkaloids isolated from *Mitragyna* species, indole alkaloids having $hR_F$* values greater than 35 and oxindole alkaloids below 35. System 8, Series A, alumina/chloroform was the best general system for mixtures of indole and oxindole alkaloids. For the separation of the indole alkaloids three systems were found to be most useful:

System 5, Series A, alumina/chloroform–benzene (1:1)
System 7, Series A, silica gel/ether
System 9, Series A, chloroform–acetone (5:4).

The results of the separations obtained are illustrated in Figs. 1–4.

## DISCUSSION

Ajmalicine, isolated from *Mitragyna speciosa* Korth (ref. 4), has been compared with similar alkaloids by means of thin layer chromatography. Fig. 1 illustrates the separation of four ajmalicinoid alkaloids using the three solvent systems which were found to be most useful for the separation of the indole alkaloids of *Mitragyna* species. From these chromatograms it can be observed that these alkaloids behaved as two distinct pairs, those with a methoxy substituent in position 11 having a slightly lower $hR_F$ value than the non-aromatic-substituted alkaloid with the same stereo-

* $hR_F = 100 \times R_F$.

chemistry. Thus it would appear that the effect of a methoxy substituent in the indole ring of these alkaloids is to slightly lower the $hR_F$ value on thin layer chromatograms.

The pair of alkaloids with *cis* D/E ring junctions, C-15 H $\alpha$ and C-20 H $\alpha$, reserpinine (I, R = —$OCH_3$) and tetrahydroalstonine (I, R = —H) have higher

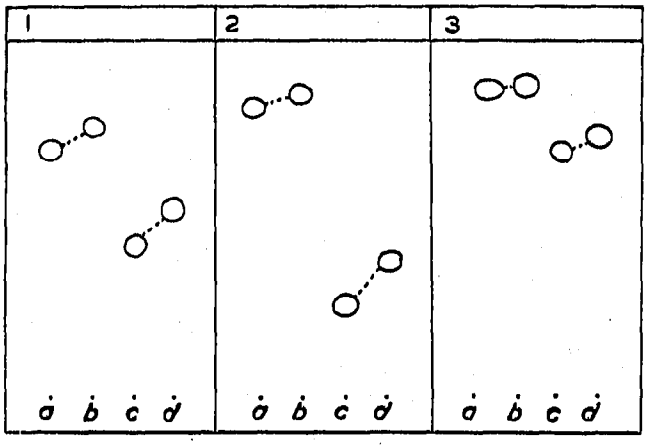
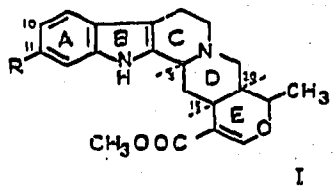


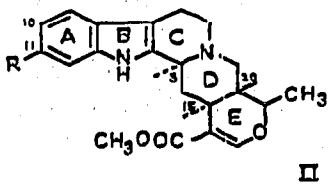

Fig. 1. Thin layer chromatography of some ajmalicinoid alkaloids. a = Reserpinine (I, R = —$OCH_3$); b = tetrahydroalstonine (I, R = —H); c = tetraphylline (II, R = —$OCH_3$); d = ajmalicine (II, R = —H); 1 = chloroform–benzene (1:1), alumina; 2 = ether, silica gel; 3 = chloroform–acetone (5:4), silica gel.

$hR_F$ values than the pair of alkaloids with *trans* D/E ring junctions, C-15 H $\alpha$ and C-20 H $\beta$, tetraphylline (II, R = —$OCH_3$) and ajmalicine (II, R = —H). When aricine was run on the same three thin layer systems it always corresponded in $hR_F$ value with reserpinine and it is interesting to note that the stereochemical structure of reserpinine and aricine is identical; aricine differs from reserpinine in that the methoxy substituent is in position 10 instead of position 11. Thus it appears that the $hR_F$ value is little affected by substitution in either position 10 or 11.




I

(I) Reserpinine (R = —$OCH_3$)
Tetrahydroalstonine (R = —H)



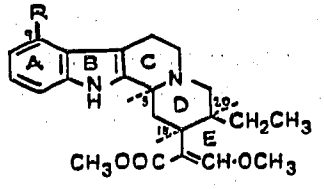

II

(II) Tetraphylline (R = —$OCH_3$)
Ajmalicine (R = —H)

Because of this correlation between $hR_F$ value and the stereochemistry of the D/E ring junction in these ajmalicinoid alkaloids it was decided to investigate the behaviour of some new *Mitragyna* alkaloids in these three systems using mitragynine and corynantheidine (isolated from *Mitragyna speciosa*[4]), corynantheine and dihydrocorynantheine, four alkaloids with a known stereochemistry at C-3, C-15 and C-20[5–10].

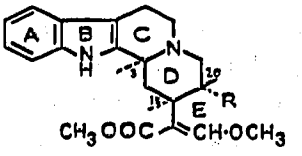
By analyses, equivalent weights, U.V., I.R. and N.M.R. spectra, two new alkaloids from *Mitragyna speciosa* Korth, speciogynine and speciociliatine, have been shown to be isomers of mitragynine (III, R = —$OCH_3$) and a third new alkaloid

paynantheine to be of the mitragynine type with a 9-methoxy substituent but with a vinyl group at C-20, similar to corynantheine (IV, R = —CH=$CH_2$)[4].



III

(III) Mitragynine (R = —$OCH_3$)
Corynantheidine (R = —H)



IV

(IV) Dihydrocorynantheine (R = —$CH_2CH_3$)
Corynantheine (R = —CH = $CH_2$)

The results of the separations of speciogynine and paynantheine with mitragynine, corynantheidine, dihydrocorynantheine and corynantheine on thin layer chromatograms are illustrated in Fig. 2. In these three systems mitragynine (III, R = —$OCH_3$) and corynantheidine (III, R = —H) have almost identical $hR_F$ values but the $hR_F$ value of mitragynine is always slightly lower. This behaviour is similar to that of the 11-methoxy ajmalicinoid alkaloids. Speciogynine has similar $hR_F$ values (slightly lower) to dihydrocorynantheine (IV, R = —$CH_2CH_3$) and different $hR_F$ values (much lower) from mitragynine/corynantheidine thus indicating that the D/E ring junction is *trans* and not *cis*. The I.R. spectra of mitragynine and speciogynine indicate *trans* C/D ring junctions and thus if the stereochemistry at C-17 is identical across the double bond then speciogynine can differ from mitragynine in having a *trans* D/E ring junction[4].

Fig. 2 also illustrates that corynantheine (IV, R = —CH=$CH_2$) and dihydrocorynantheine (IV, R = —$CH_2CH_3$) have similar $hR_F$ values in these three thin layer systems but that of corynantheine is always slightly higher, thus the effect of a vinyl group at C-20 instead of an ethyl group is to slightly increase the $hR_F$ value. Paynantheine runs more closely to speciogynine and corynantheine in these three systems than it does to mitragynine so it would appear that paynantheine resembles these two alkaloids in having a *trans* D/E ring junction.

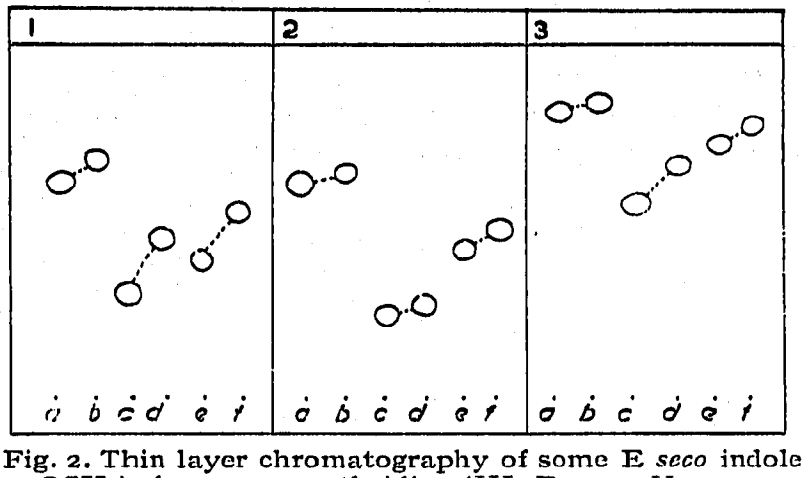
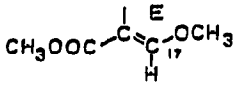


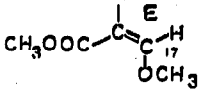

Fig. 2. Thin layer chromatography of some E *seco* indole alkaloids. a = Mitragynine (III, R = —$OCH_3$); b = corynantheidine (III, R = —H); c = speciogynine; d = dihydrocorynantheine (IV, R = —$CH_2CH_3$); e = paynantheine; f = corynantheine (IV, R = —CH = $CH_2$); 1 = chloroform–benzene (1:1), alumina; 2 = ether, silica gel; 3 = chloroform–acetone (5:4), silica gel.

The conclusions drawn from these three thin layer systems (Fig. 2) that mitragynine resembles corynantheidine stereochemically and that paynantheine resembles speciogynine stereochemically were further substantiated when these four alkaloids were run on all the systems of the modified Waldi scheme (Fig. 3). The close $hR_F$ values in all these systems between corynantheidine and mitragynine suggest that these two alkaloids have the same stereochemistry at all centres and thus it would appear that the stereochemistry at C-17 is identical for these two alkaloids and not different as proposed, V (mitragynine) and VI (corynantheidine)[9,10].



V

(V) Mitragynine



VI

(VII) Corynantheidine

ZACHARIAS, ROSENSTEIN AND JEFFREY[11] have shown by X-ray crystallography that the C(17)-H in mitragynine is *cis* to the carbomethoxy group (V) and PHILLIPSON[12] as a result of thin layer chromatographic and N.M.R. considerations suggested that the arrangement is the same in corynantheidine. This postulation was independently confirmed by WEISBACH *et al.*[13].

There are four known isomers with a mitragynine-like structure, mitragynine, speciogynine, speciociliatine[4] and mitraciliatine[14]. The relationship between the $hR_F$ values of these four isomers is shown in Fig. 3 and it can be seen that in most systems mitragynine has the highest $hR_F$, then speciogynine, speciociliatine and the lowest mitraciliatine. The I.R. spectra of mitragynine and speciogynine indicate *trans* C/D ring junctions whilst the I.R. spectra of speciociliatine and mitraciliatine indicate *cis* C/D ring junctions[4,12]. Thus the two alkaloids with *cis* C/D ring junctions have lower $hR_F$ values than the two alkaloids with *trans* C/D ring junctions. If it is assumed that the stereochemistry at C-17 is identical for these four alkaloids then it is likely that these four alkaloids correspond to the four possible stereochemical skeletons for mitragynine, namely *normal*, *pseudo*, *allo* and *epiallo*. The behaviour on thin layer chromatograms suggests that since mitragynine has a *cis* D/E ring junction then speciogynine has a *trans* D/E ring junction and the same argument would suggest that speciociliatine has a *cis* D/E ring junction and mitraciliatine a *trans* D/E ring junction. These proposals are summarised in Table I:

Insufficient alkaloids are available for comparison of the stereochemistry of the oxindole alkaloids with their behaviour on thin layer chromatograms but it is

TABLE I

| | *Configuration of H atom at* | | |
|---|---|---|---|
| | *C-3* | *C-15* | *C-20* |
| Mitragynine (*allo*) | α | α | α |
| Speciociliatine (*epiallo*) | β | α | α |
| Speciogynine (*normal*) | α | α | β |
| Mitraciliatine (*pseudo*) | β | α | β |

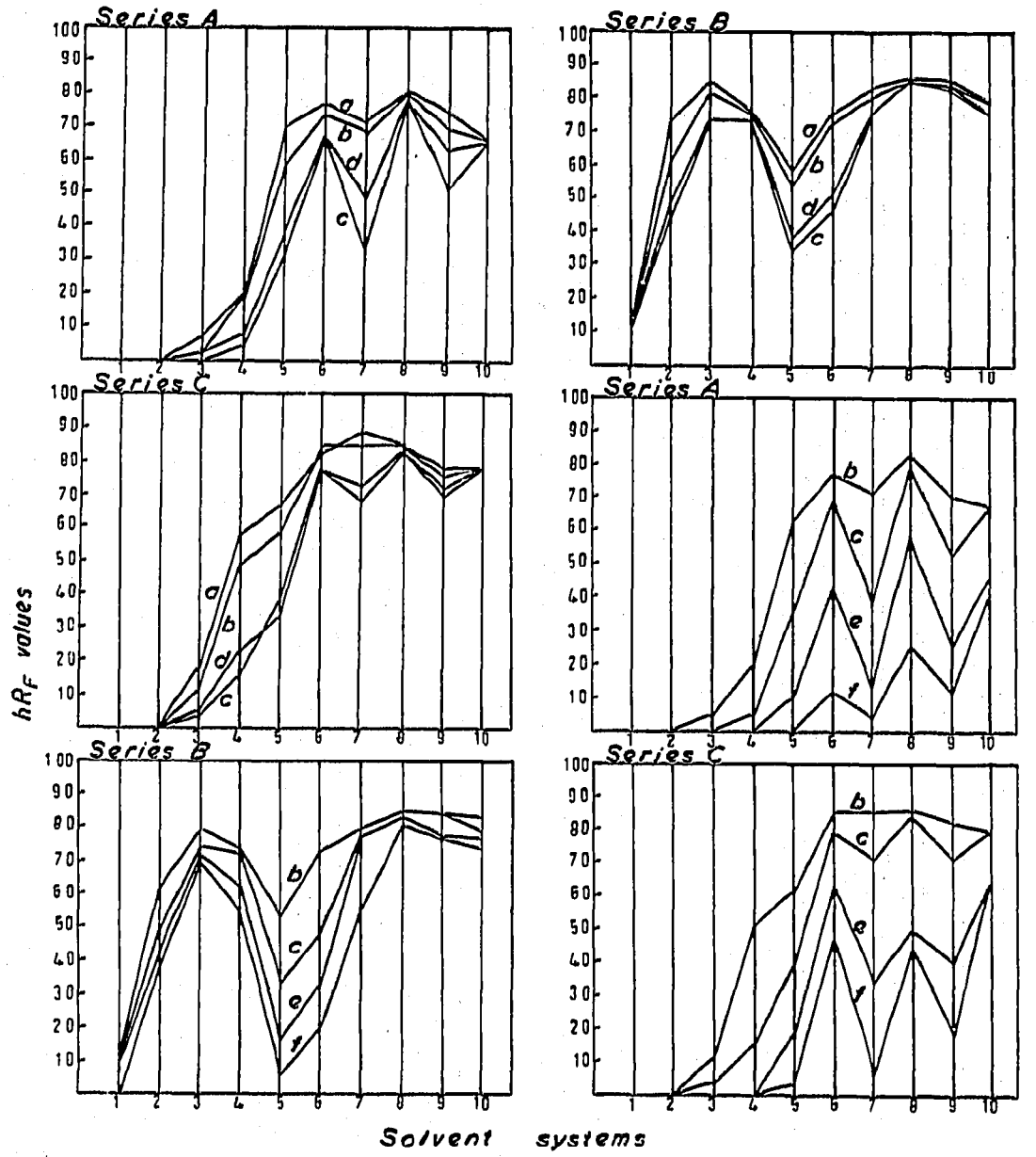



Fig. 3. $hR_F$ values of corynantheidine (a), mitragynine (b), speciogynine (c), paynantheine (d), speciociliatine (e) and mitraciliatine (f) in the modified Waldi scheme.

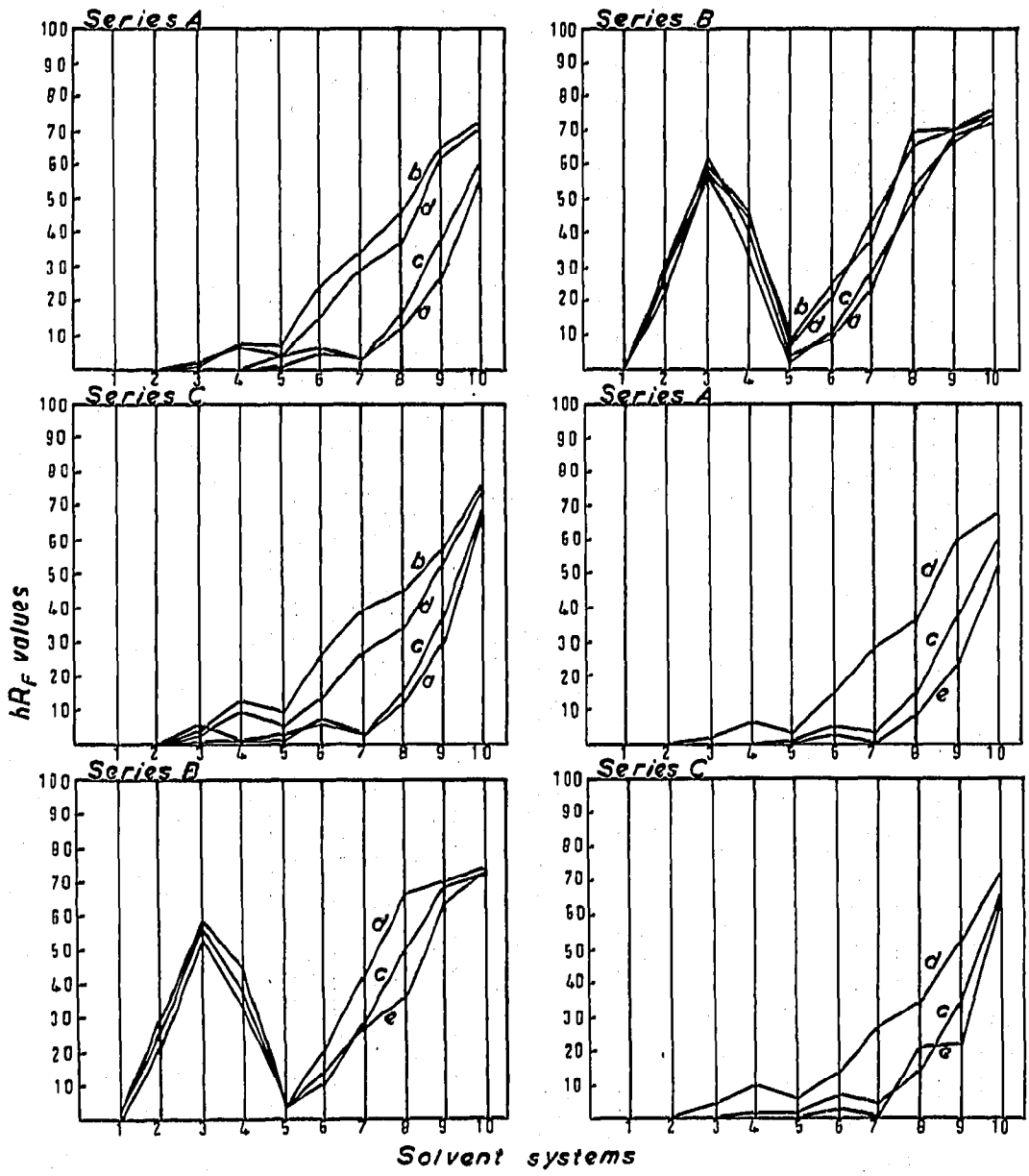
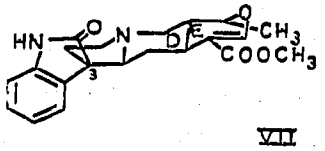



Fig. 4. $hR_F$ values of rhynchophylline (a), isorhynchophylline (b), mitraphylline (c), isomitraphylline (d) and speciophylline (e) in the modified Waldi scheme.

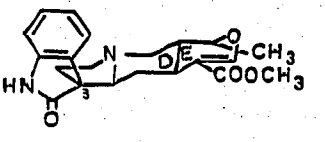
possible to use thin layer chromatography in order to ascertain the stereochemistry at the spiro carbon C-3.

Mitraphylline (VII) and isomitraphylline (VIII) and rhynchophylline (IX) and isorhynchophylline (X) have the same stereochemistry at the D/E ring junction and each pair is isomeric at the spiro carbon C-3[15–17]. Fig. 4 shows that rhynchophylline and mitraphylline have similar $hR_F$ values in most systems of the modified Waldi scheme whilst isorhynchophylline and isomitraphylline have similar $hR_F$ values to each other, the latter pair moving more quickly. Although no explanation can be offered, it is interesting to note that mitraphylline usually has a higher $hR_F$ value than rhynchophylline whilst the reverse is true for the iso alkaloids.



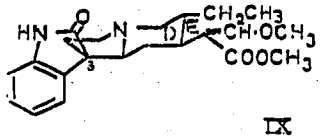
VII

(VII) Mitraphylline



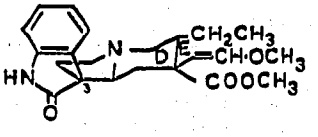
VIII

(VIII) Isomitraphylline



IX

(IX) Rhynchophylline



X

(X) Isorhynchophylline

Speciophylline, a new oxindole alkaloid from *Mitragyna speciosa*, has been characterised by equivalent weight, analysis, U.V., I.R., and N.M.R. spectra as an isomer of mitraphylline. Thin layer chromatography has indicated that speciophylline is different from mitraphylline, isomitraphylline, uncarine A and uncarine B, the four known isomers of mitraphylline. Fig. 4 shows that on thin layer chromatograms speciophylline resembles mitraphylline rather than isomitraphylline; thus it is proposed that the stereochemistry of the spiro carbon at C-3 is the same for speciophylline as for mitraphylline.

ACKNOWLEDGEMENTS

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The dihydrocorynantheine was obtained from S.B. Penick and Co. (as the hydrochloride).

SUMMARY

Thin layer chromatography may be used to distinguish between indole alkaloids having *cis* or *trans* C/D and D/E ring junctions, with or without methoxy substituents,

and with vinyl groups instead of ethyl groups at C-20. The behaviour on thin layer chromatograms of some new indole and oxindole alkaloids from *Mitragyna* species has been examined and proposals made for their stereochemical differences.

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