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CORRELATIONS BETWEEN  $hR_F$  VALUES ON THIN-LAYER CHROMATOGRAMS AND CHEMICAL STRUCTURE OF SOME TERTIARY *STRYCHNOS* ALKALOIDS

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## SUMMARY

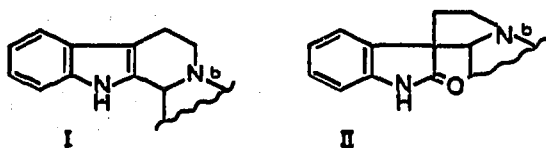
The thin-layer chromatographic behaviour of some thirty compounds of the tertiary alkaloid type found in the genus *Strychnos* has been examined. Seven solvent systems have been used to compare behaviour on alumina and silica gel plates and further comparisons have been made by addition of diethylamine to these same solvent systems. It has been demonstrated that  $R_F$  values cannot be explained solely in terms of  $pK_a$  values and that for *Strychnos* alkaloids the factors affecting adsorption are more complicated than for the previously examined heteroyohimbine and oxindole alkaloids. The adsorption processes, although not fully explained for all compounds, can be understood for the majority by considering: (a) The type of aromatic substituent – methoxyl, amino, hydroxyl; (b) The position of the substituent, its inductive effect on the C(10) carbonyl or hydrogen bond formation to an adjacent hydroxyl; (c) The C(10) carbonyl most certainly takes part in adsorption, its effect being more marked on alumina than on silica gel; (d) N<sub>b</sub> most certainly takes part in adsorption and adsorption is markedly affected by adjacent oxygen atoms at C(16) or C(18); (e) N-oxides are very strongly adsorbed when compared to the parent bases; (f) Reduction of the C(21)–C(22) double bond has a more marked effect on adsorption of pseudo bases than does epoxidation; (g) A 14-hydroxyl group also has a marked effect on adsorption.

## INTRODUCTION

Paper (PC)<sup>1-3</sup>, thin-layer (TLC)<sup>4</sup>, and gas-liquid chromatography (GLC)<sup>5</sup> have been used for the separation of tertiary alkaloids from *Strychnos* species. In the case of GLC, some progress has been made toward correlating certain structural features of these alkaloids with their relative retention times<sup>5</sup>. A connection has been noted between the PC and TLC  $R_F$  values and the  $pK_a$  values of these alkaloids, enabling them to be divided into two groups<sup>6</sup>. However, no serious attempt has been made to correlate TLC adsorption with chemical structure.

The TLC behaviour on alumina and silica gel of some 27 heteroyohimbine

alkaloids (partial, I) has previously been related to aromatic substitution pattern and to  $N_b$  availability. The main factor in adsorption has been explained in terms of hydrogen bond formation between adsorbent hydroxyl groups and nitrogen ( $N_b$ ) lone-pair electrons<sup>7</sup>. Some 30 pentacyclic oxindole alkaloids (partial, II) have also been examined by TLC; it has been concluded that adsorption takes place at the oxindole carbonyl group and at the tertiary nitrogen ( $N_b$ ) if sterically possible<sup>8,9</sup>. Tertiary *Strychnos* alkaloids are similar in structure to the oxindole alkaloids in that many of them possess an aromatic amide carbonyl and a tertiary nitrogen. This study was undertaken to determine whether or not the TLC adsorption of tertiary *Strychnos* alkaloids could also be explained in terms of hydrogen bond formation between adsorbent hydroxyl groups and the alkaloid at the amide carbonyl and/or tertiary nitrogen.



#### METHODS

The details of plate preparation and development of the chromatogram have previously been described<sup>10</sup>. The alkaloids were detected either by Dragendorff's reagent or iodoplatinate reagent. The following solvent systems were used for silica gel and alumina layers:

A — (1) Ether; (2) benzene-ethyl acetate (7:2); (3) cyclohexane-chloroform (3:7); (4) chloroform; (5) chloroform-acetone (5:4); (6) ether-ethanol (95:5); (7) chloroform-ethanol (95:5).

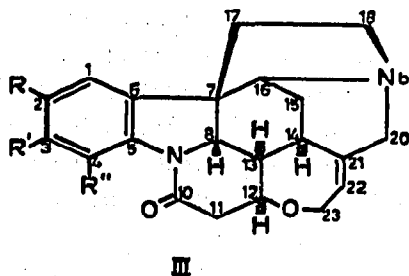
B — (1) Ether-diethylamine (9:1); (2) benzene-ethyl acetate-diethylamine (7:2:1); (3) cyclohexane-chloroform-diethylamine (3:7:1); (4) chloroform-diethylamine (9:1); (5) chloroform-acetone-diethylamine (5:4:1); (6) ether-ethanol-diethylamine (95:5:10); (7) chloroform-ethanol-diethylamine (95:5:10).

#### RESULTS

The results of the separations obtained are shown in Figs. 1-9. The  $hR_F$  values given are the average of at least three separate determinations.

#### DISCUSSION

*The effect of aromatic substitution on the TLC behaviour of bases of the "normal" series*



No.	Alkaloid	R	R'	R''
(1)	Strychnine	H	H	H
(2)	$\beta$ -Colubrine	OMe	H	H
(3)	$\alpha$ -Colubrine	H	OMe	H
(4)	Brucine	OMe	OMe	H
(5)	2-Aminostrychnine	NH <sub>2</sub>	H	H
(6)	3-Aminostrychnine	H	NH <sub>2</sub>	H
(7)	2-Hydroxystrychnine	OH	H	H
(8)	3-Hydroxystrychnine	H	OH	H
(9)	4-Hydroxystrychnine	H	H	OH

The TLC behaviour of strychnine (III, 1), the colubrines (III, 2 and 3), and brucine (III, 4) is shown in Fig. 1. These four alkaloids are more strongly adsorbed on silica gel than on alumina but when diethylamine is added to the solvent system their adsorption is markedly decreased. In all systems used these four compounds have very similar behaviour patterns and their  $R_F$  values are usually very close. There is in fact a definite sequence: strychnine being the least adsorbed, then  $\alpha$ -colubrine (III, 3),  $\beta$ -colubrine (III, 2), and brucine being most adsorbed. It has been shown previously<sup>8</sup> that on TLC oxindole (IV, R = H) is more strongly adsorbed than indole, indicating that the amide group takes part in the adsorption. In line with the explanation for the TLC behaviour of oxindole alkaloids (see INTRODUCTION)<sup>8,9</sup>, it is

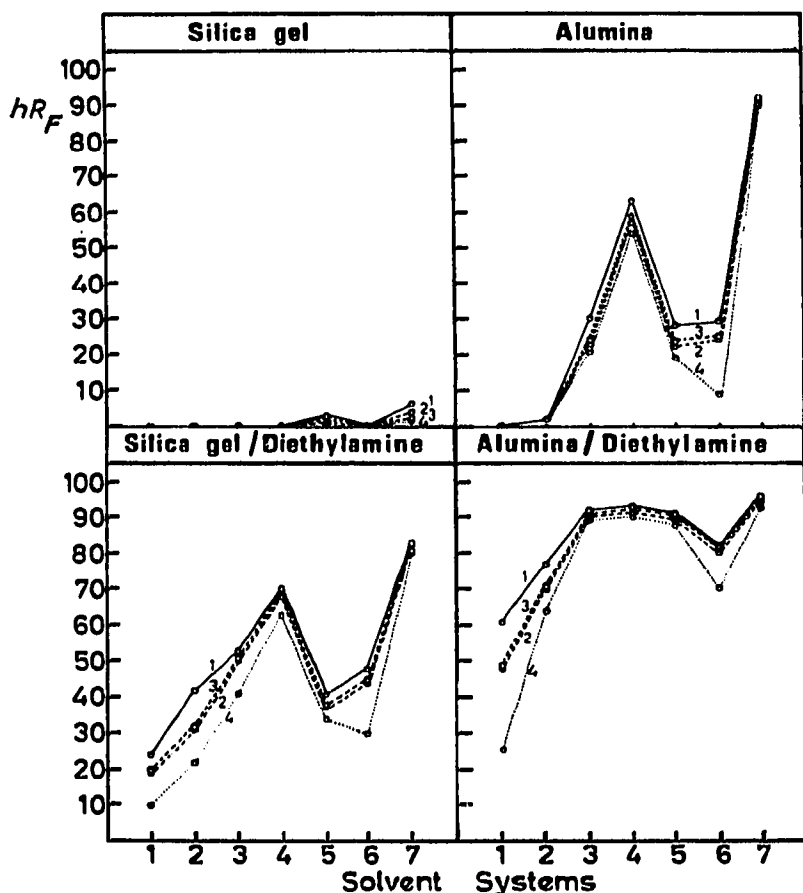
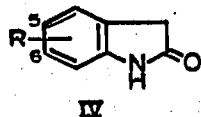


Fig. 1.  $hR_F$  values of: (1) strychnine, ———; (2)  $\beta$ -colubrine, - - - -; (3)  $\alpha$ -colubrine, ·····; and (4) brucine, ·····.

reasonable to assume that in the similar strychnine-type bases (III) the two major sites of adsorption will be the C(10) carbonyl and the basic nitrogen N<sub>b</sub>.

The presence of a methoxy substituent in the aromatic ring offers a further site for adsorption but does not explain the difference in behaviour of  $\alpha$ - and  $\beta$ -colubrine. A study of Dreiding models indicates that a methoxy substituent at C(3) would not sterically hinder availability of the C(10) carbonyl to any greater extent than would a C(2) methoxy substituent.



A study of the methoxy-substituted oxindoles<sup>8</sup> (IV, R = OMe) using the same solvent systems for silica gel and alumina plates, has shown that 5- and 6-methoxyoxindole are both slightly more adsorbed than oxindole and that 5-methoxyoxindole is the most strongly adsorbed. This behaviour is analogous to that of the  $\alpha$ - and  $\beta$ -colubrines and it may be rationalised in terms of the electron-donating properties of the substituent. The inductive effect will be more pronounced when the substituent is *para* to the amide carbonyl, as in  $\beta$ -colubrine (III, 2) and 5-methoxyoxindole, than

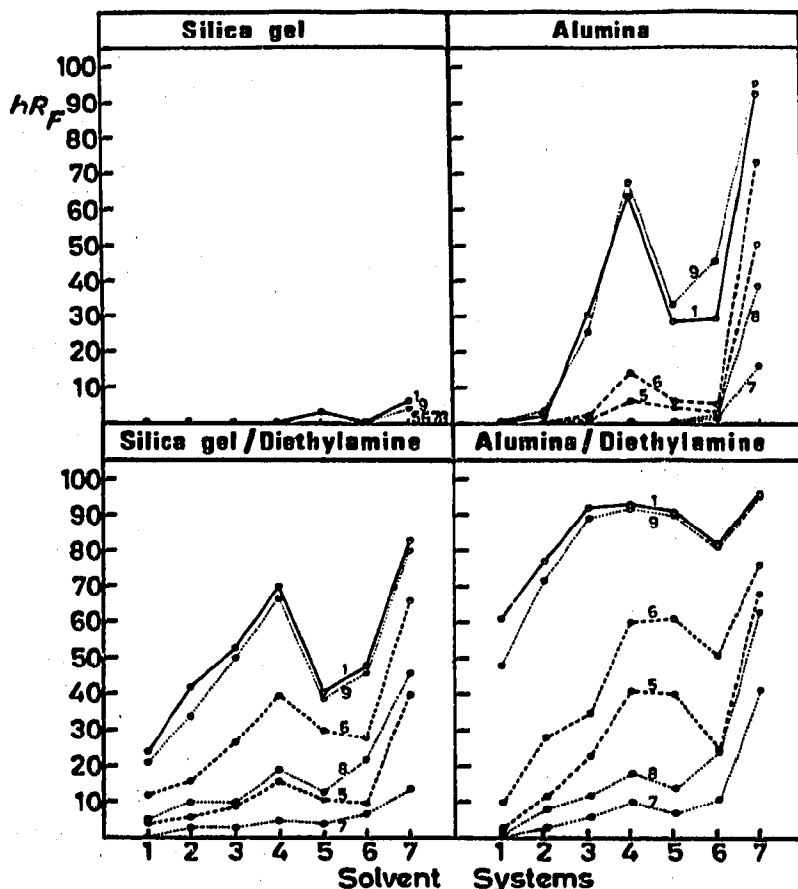
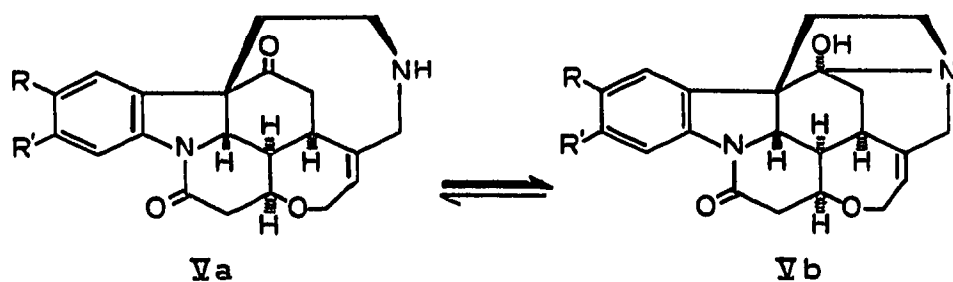


Fig. 2.  $hR_F$  values of: (1) strychnine, ———; (5) 2-aminostrychnine, - - - -; (6) 3-aminostrychnine, - - - -; (7) 2-hydroxystrychnine, ······; (8) 3-hydroxystrychnine, ······; and (9) 4-hydroxystrychnine, ······.

when the substituent is *meta* to the amide carbonyl, as in  $\alpha$ -colubrine (III, 3) and 6-methoxyoxindole. The effect of two methoxy substituents (brucine, III, 4) in the aromatic ring will be to increase further the ring electronegativity and hence the electron availability at the amide carbonyl.

Although the  $R_F$  differences are small for methoxy substituents they are larger for the more strongly adsorbed amino-substituted strychnines and for the most strongly adsorbed hydroxy-substituted strychnines. Fig. 2 shows that 2-aminostrychnine (III, 5) is more strongly adsorbed in all systems than 3-aminostrychnine (III, 6). This behaviour can likewise be explained in terms of the greater inductive effect on the amide carbonyl of the 2-amino substituent (*para*) in comparison to the 3-amino substituent (*meta*). Fig. 2 also shows that 2-hydroxystrychnine (III, 7) and 3-hydroxystrychnine (III, 8) have the same behaviour pattern as the 2- and 3-amino- and -methoxy-substituted strychnines but that 4-hydroxystrychnine (III, 9) has a behaviour pattern similar to that of strychnine. It is known that the 4-hydroxy substituent is hydrogen bonded to the C(10) amide carbonyl<sup>11</sup>, thus reducing the electron availability of the hydroxyl group and of the amide carbonyl group for adsorption. The combined effect of the hydrogen bonded hydroxyl/carbonyl in 4-hydroxystrychnine is such as to give adsorptive properties similar to those of strychnine. On alumina without diethylamine in the solvent 4-hydroxystrychnine is nearly always slightly less adsorbed than strychnine, but this effect is reversed for silica gel and for alumina when diethylamine is present. This effect, although slight, is distinct, but as the system is so complex it is difficult to explain. It is probable, for example, that diethylamine will hydrogen bond to differing extents with 4-hydroxystrychnine and strychnine. It was thought that this might affect the strength of the phenolic hydroxyl-amide carbonyl bond. However, there was no change in the carbonyl region of the infrared spectrum of strychnine ( $1670\text{ cm}^{-1}$ ) or 4-hydroxystrychnine ( $1642\text{ cm}^{-1}$ ) when dissolved in chloroform and also in chloroform containing 10% diethylamine.

*TLC behaviour of bases of the pseudo series*



No.	Alkaloid	R	R'
(10)	Pseudostrychnine	H	H
(11)	Pseudobrucine	OMe	OMe

Studies of the infrared spectra of pseudostrychnine (V, 10) and pseudobrucine (V, 11) in solution and in solid phase have indicated that these bases exist almost entirely in the carbinolamine form (Vb)<sup>12</sup>. The keto-amine form (Va) almost certainly exists in equilibrium as deduced by chemical reactions, *e.g.* methanol at room temperature yields the O-methyl ether. However, sufficient keto-amine form is not present to be detected by physical means<sup>12, 13</sup>.

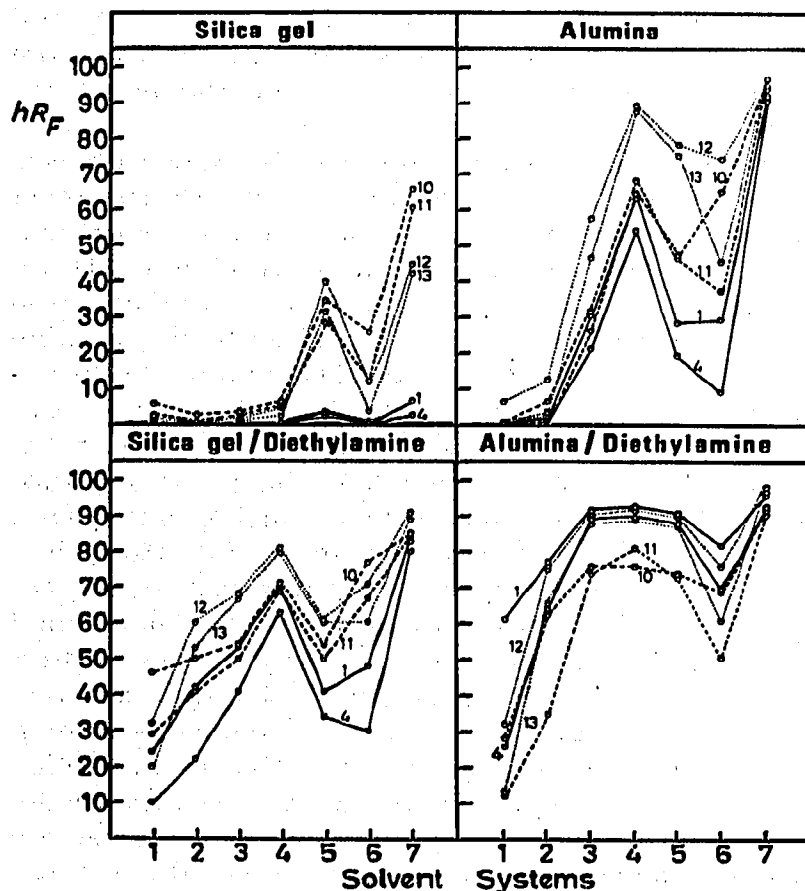


Fig. 3.  $hR_F$  values of: (1) strychnine, ———; (4) brucine, ———; (10) pseudostrychnine, - - - -; (11) pseudobrucine, - · - · -; (12) icajine, ·····; and (13) novacine, ·····.

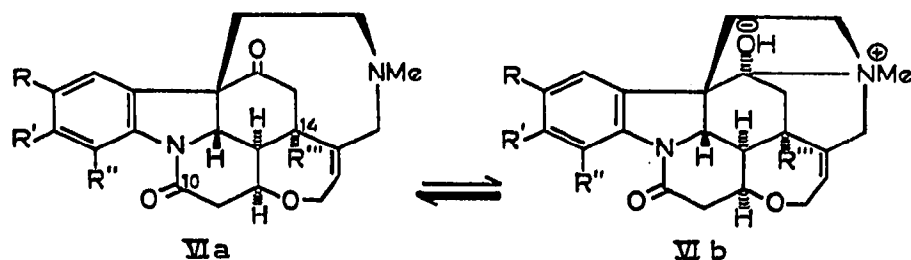
Fig. 3 shows that pseudostrychnine (V, 10) is less adsorbed than strychnine (III, 1) on alumina and silica gel plates with neutral solvents and on silica gel plates with added diethylamine. This shows that the weaker base, pseudostrychnine ( $pK_a$  5.60)<sup>14</sup>, is less adsorbed than strychnine ( $pK_a$  7.37) (ref. 14). However, on alumina plates and solvent systems containing diethylamine this behaviour is reversed so that pseudostrychnine is more strongly adsorbed than strychnine. Pseudobrucine (V, 11) and brucine (III, 4) show exactly the same behaviour pattern (Fig. 3), both being slightly more adsorbed than the corresponding unsubstituted alkaloids. It is apparent therefore that the  $R_F$  values of these alkaloids cannot be explained solely in terms of  $pK_a$  values.

The effect of diethylamine will be to hydrogen bond to the hydroxyl groups of the adsorbents thus lessening the adsorptive capacity of alumina and silica gel. In agreement with this, the  $R_F$  values of each of these four alkaloids is increased when diethylamine is added to the solvent system.

This increase in  $R_F$  values is most marked for the "normal" bases and is rather less for the two pseudo bases, being much less marked on alumina. Most probably competition takes place between adsorbent/diethylamine, adsorbent/alkaloid, and alkaloid/diethylamine. In spite of the complexity of the systems, the fact remains that when diethylamine is present in the solvent system the pseudo bases are less

adsorbed than the "normal" bases on silica gel and more adsorbed on alumina. It may be possible that the equilibrium  $Va \rightleftharpoons Vb$  is displaced by differing amounts when adsorbed on alumina and on silica gel or that the C(16) oxygen function, whether hydroxyl or keto, plays a role in adsorption.

*TLC behaviour of alkaloids of the N-methyl-sec.-pseudo series*



No.	Alkaloid	R	R'	R''	R'''
(12)	Icajine	H	H	H	H
(13)	Novacine	OMe	OMe	H	H
(14)	Icajidine (C(10) deoxy)	H	H	H	H
(15)	Vomicine	H	H	OH	H
(16)	Dihydroicajine (C(20)-C(21) double bond saturated)	H	H	H	H
(27)	14-Hydroxyicajine	H	H	H	OH

It is known that icajine (VI, 12) exhibits trans-annular amide-type neutralisation between the C(16) carbonyl and the N<sub>b</sub> methyl functions<sup>12</sup>. This leads to reduction in the availability of the unshared electrons on N<sub>b</sub> which is reflected in the lower *pK<sub>a</sub>* value of 6.07 (ref. 14) as compared with that of strychnine (7.37) and in the lowering of the carbonyl stretching frequency in the infrared spectrum (chloroform) to 1668 cm<sup>-1</sup> (combined absorption of C(10) amide carbonyl and C(16) carbonyl; in icajidine (VI, 14) the C(16) carbonyl absorbs at 1655 cm<sup>-1</sup>) (ref. 15). However, it is difficult to define the degree of interaction between the C(16) carbonyl and the N<sub>b</sub> methyl functions. The situation has been described as a case of mesomerism involving VIa and VIb as canonical forms. In view of the stereochemical differences at C(16) in VIa (trigonal) and VIb (tetrahedral), HENDRICKSON has proposed a situation resembling tautomerism where the molecule can exist in two forms, one being a slightly polarised form of VIa and the other approximating VIb (ref. 13). However, it has not yet proved possible to isolate two forms of icajine. The C(16) carbonyl band does show in the infrared spectrum of the solid, which must approximate VIa, but it is claimed that the C(16) carbonyl band of the solution spectrum disappears rapidly, presumably due to solvation, and that form VIb becomes the more stable one.

Fig. 3 shows a clear behaviour pattern on alumina plates, with and without diethylamine, icajine (VI, 12) being less adsorbed than pseudostrychnine (V, 10). The corresponding dimethoxy compounds are slightly more adsorbed and show the same behaviour pattern, novacine (VI, 13) being less adsorbed than pseudobrucine (V, 11). When the *R<sub>F</sub>* values on alumina, without diethylamine in the solvent system, of the N-methyl-sec.-pseudo bases icajine (VI, 12) and novacine (VI, 13) are compared with those of the corresponding "normal" alkaloids, strychnine (III, 1) and brucine (III, 4), and of the pseudo bases pseudostrychnine (V, 10) and pseudobrucine (V, 11), it can be seen that the pseudo bases tend to be in an intermediate position. If the most

important factor in adsorption is considered to be electron availability at  $N_b$  then the order of adsorption would be reflected by  $pK_a$  values

strychnine (7.37) > icajine (6.07) > pseudostrychnine (5.60)

It is obvious when comparing the TLC behaviour of these three alkaloids, as shown in Fig. 3, that this is not the case. It is interesting to note that on alumina plates, in the presence of diethylamine, strychnine and icajine have very similar  $R_F$  values in six out of seven solvent systems and that brucine and novacine also have a very similar behaviour pattern. This may indicate that on alumina the C(16) oxygen function plays little part in adsorption when diethylamine is present.

However, on silica gel plates the behaviour is not so straightforward, although the alkaloids do tend to behave in pairs, strychnine/brucine, pseudostrychnine/pseudobrucine and icajine/novacine having similar behaviour patterns. The effect of the two aromatic methoxyl groups in the "normal", pseudo, and N-methyl-*sec.*-pseudo series is always to increase adsorption. In six out of seven solvent systems without diethylamine, on silica gel, icajine (VI, 12) is more strongly adsorbed than pseudostrychnine (V, 10) and novacine (VI, 13) is more strongly adsorbed than pseudobrucine (V, 11). This is the behaviour which would be expected if  $N_b$  were the most important factor (as reflected by  $pK_a$  values). When diethylamine is added to the solvent system this behaviour is reversed and icajine is less adsorbed than pseudostrychnine and novacine is less adsorbed than pseudobrucine in five out of the seven solvent systems.

Fig. 4 shows that vomicine (VI, 15) behaves very similarly to the corresponding unsubstituted alkaloid icajine (VI, 12). On silica gel plates without diethylamine, vomicine is consistently less adsorbed than icajine and this may probably be due to less electron availability at the C(10) carbonyl (*cf.* discussion of 4-hydroxystrychnine/strychnine), a further indication that the amide carbonyl at C(10) takes part in adsorption. However, when diethylamine is added to the solvent and also on alumina plates with and without diethylamine, this effect tends to be reversed, although in many systems these two alkaloids have virtually identical  $R_F$  values.

Some indication of the role played by the C(10) carbonyl in adsorption is obtained from comparing the behaviour of icajidine (VI, 14), *i.e.* icajine in which the C(10) carbonyl is replaced by methylene. Fig. 4 shows that on alumina plates without diethylamine in the solvent system and on both silica gel and alumina plates with diethylamine present, icajidine is always less adsorbed than icajine. It may be inferred that the C(10) carbonyl takes part during adsorption in these systems and this behaviour is analogous to that of the oxindole alkaloids<sup>8,9</sup>. However, it is very surprising to note that on silica gel without added diethylamine, icajidine is more adsorbed than icajine. The lack of an oxygen function at C(10) does mean that  $N_b$  is more basic and this is reflected in the  $pK_a$  values of icajine (6.07) and icajidine (8.29)<sup>14</sup>. Nevertheless, studies of Dreiding models indicate that sterically the indoline nitrogen ( $N_b$ ) is not available for adsorption. It may be that the increased adsorption of icajidine on silica gel without diethylamine in the solvent system can be attributed to increased electro-negativity of the benzenoid system.

Probably the most unusual behaviour of these *Strychnos* alkaloids on TLC is that of dihydroicajine (VI, 16). Fig. 4 shows that when the behaviour of icajine and dihydroicajine (*i.e.* C(21)-C(22) double bond saturated) is compared, dihydroicajine shows a dramatic increase in adsorption on silica gel and alumina without added



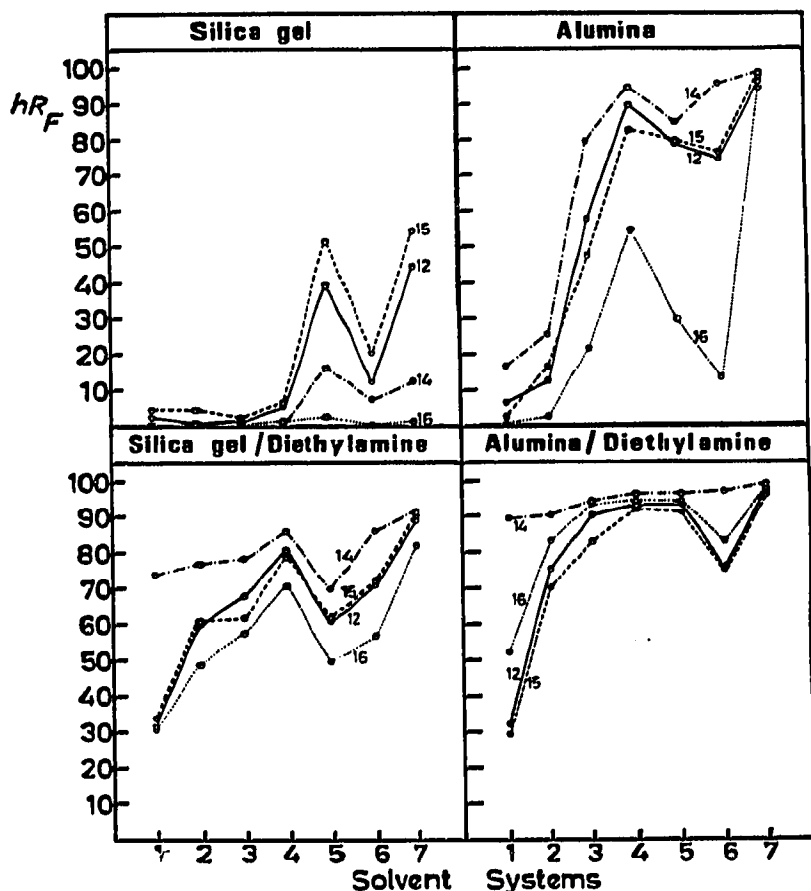
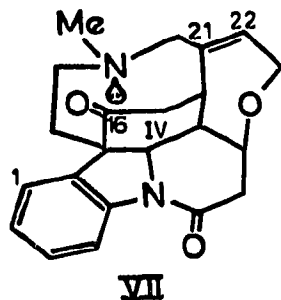


Fig. 4.  $hR_F$  values of: (12) icajine, —; (14) icajidine, -·-·-; (15) vomicine, - - -; and (16) dihydroicajine, ·····.

diethylamine and on silica gel with added diethylamine. In fact, the behaviour in all systems is far more like strychnine than a *N*-methyl-*sec.*-pseudo alkaloid. This indicates that possibly  $N_b$  is more available for binding. The  $pK_a$  value of dihydroicajine does not appear to be in the literature, but reduction of the C(21)–C(22) double bond in strychnine does lead to a slight increase in  $pK_a$  value, *viz.* strychnine 7.37 and dihydrostrychnine 7.45 (ref. 14).

Saturation of the C(21)–C(22) double bond of icajine (VII) most probably involves a change in conformation in ring IV, affecting the proximity of the C(16) carbonyl and  $N_b$ .

This is supported by a study of Dreiding models and the fact that in the NMR spectrum of icajine the C(1) proton occurs at  $\delta$  7.60–8.0 p.p.m. whilst in dihydro-



icajine it appears at  $\delta$  6.8–7.55 p.p.m., indicating that the C(1) proton is more shielded by the C(16) oxygen function in dihydroicajine<sup>15</sup>.

Weakening of the trans-annular amide-type bond could result in inversion at N<sub>b</sub> during chromatography on alumina and silica gel. There has been previous evidence for conformational changes of some indole alkaloids during chromatography<sup>7</sup>. Such a conformational change would also result in increased availability of the C(16) oxygen for binding to the adsorbents.

*TLC behaviour of 18-oxostrychnine, strychnine N-oxide, and brucine N-oxide*

Fig. 5 shows the effect of the introduction of a further oxygen in "normal" alkaloids at C(18) and N<sub>b</sub>. When an oxygen is present at C(18), N<sub>b</sub> is no longer basic as it forms part of an amide function. 18-Oxostrychnine (III, 19, oxygen at C(18)) is less adsorbed on silica gel and alumina without diethylamine present in the solvent system and on silica gel with diethylamine present. This can be explained in terms of less electron availability at N<sub>b</sub>. On alumina plates with diethylamine in the solvent system 18-oxostrychnine is slightly more adsorbed than strychnine. This behaviour pattern is exactly analogous to that of pseudostrychnine when compared to strychnine and is a further indication that N<sub>b</sub> electron availability is an important factor in adsorption on alumina and silica gel.

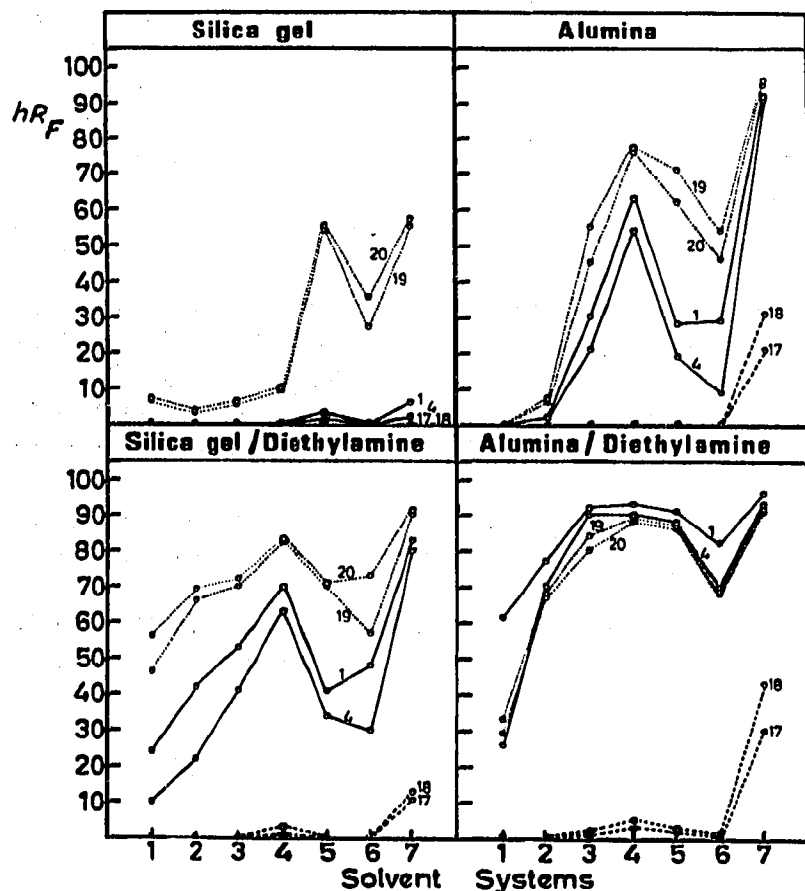
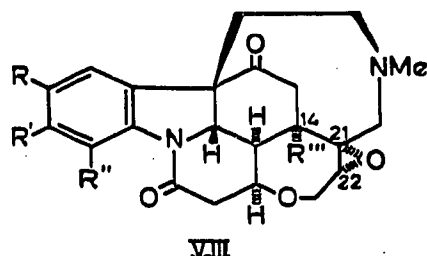


Fig. 5.  $hR_F$  values of: (1) strychnine, ———; (4) brucine, ———; (17) strychnine N-oxide, - - - -; (18) brucine N-oxide, - - - -; (19) 18-oxostrychnine, ·····; and (20) 18-oxostrychnine epoxide, ·····.

When the oxygen function is present at N<sub>b</sub>, forming N-oxides, then a markedly different behaviour pattern occurs (Fig. 5). In all systems used the N-oxides of strychnine and brucine are much more strongly adsorbed than the parent bases. This clearly cannot be explained solely by differences in basicity, as the  $pK_a$  for strychnine N-oxide is 5.17 compared with a  $pK_a$  for strychnine of 7.37 (ref. 14). If basicity alone were the major factor in adsorption, the N-oxides would be less adsorbed than the corresponding bases. The N<sub>b</sub> oxygen of the N-oxides projects from the molecule and it is most likely that adsorption takes place via the electron pairs of the oxide oxygen atom. In the relatively few systems where strychnine and brucine N-oxides move off the base line, brucine N-oxide is less adsorbed than strychnine-oxide, a reversal of the behaviour of the parent compounds. Perhaps the presence of the two methoxy substituents in brucine N-oxide hinders the orientation of the molecule at the surface of the adsorbent and thus slightly reduces the otherwise very strong adsorption at the N-oxide oxygen.

*TLC behaviour of N-methyl-sec.-pseudo alkaloids containing a 21,22- $\alpha$ -epoxide and/or a 14-hydroxyl group*



No.	Alkaloid	R	R'	R''	R'''
(21)	Epoxy novacine	OMe	OMe	H	H
(22)	Epoxy vomicine	H	H	OH	H
(23)	2-Methoxyepoxyvicajine	OMe	H	H	H
(24)	3-Methoxyepoxyvomicine	H	OMe	OH	H
(25)	14-Hydroxyepoxy novacine	OMe	OMe	H	OH
(26)	14-Hydroxyepoxyvomicine	H	H	OH	OH

The effect of an  $\alpha$ -epoxide function at C(21)–C(22) is shown in Fig. 5 where the  $R_F$  values of 18-oxostrychnine (III, 19) are compared with those of 18-oxostrychnine 21,22- $\alpha$ -epoxide (20). The behaviour of these two alkaloids is very similar in all systems. On alumina the epoxide alkaloid is very slightly more adsorbed and this is reversed on silica gel. The epoxide function in 18-oxostrychnine 21,22- $\alpha$ -epoxide, therefore, has very little effect on adsorption.

The TLC behaviour of novacine (VI, 13) and epoxy novacine (VIII, 21) is compared in Fig. 6. The effect of the epoxide is to increase adsorption on alumina without added diethylamine and on alumina and silica gel with added diethylamine. Although the  $R_F$  values are extremely close on silica gel without diethylamine in the solvent system, epoxy novacine is very slightly less adsorbed than novacine. This fact is difficult to explain and we are unable to give any satisfactory reason for it. However, it is clear that, in general, alumina is a much more satisfactory adsorbent for separating corresponding alkaloids with and without epoxide functions because the epoxide alkaloids tend to be more adsorbed. This is to be expected because Dreiding models show that the oxygen of the  $\alpha$ -epoxide is in a good position for binding to the adsor-

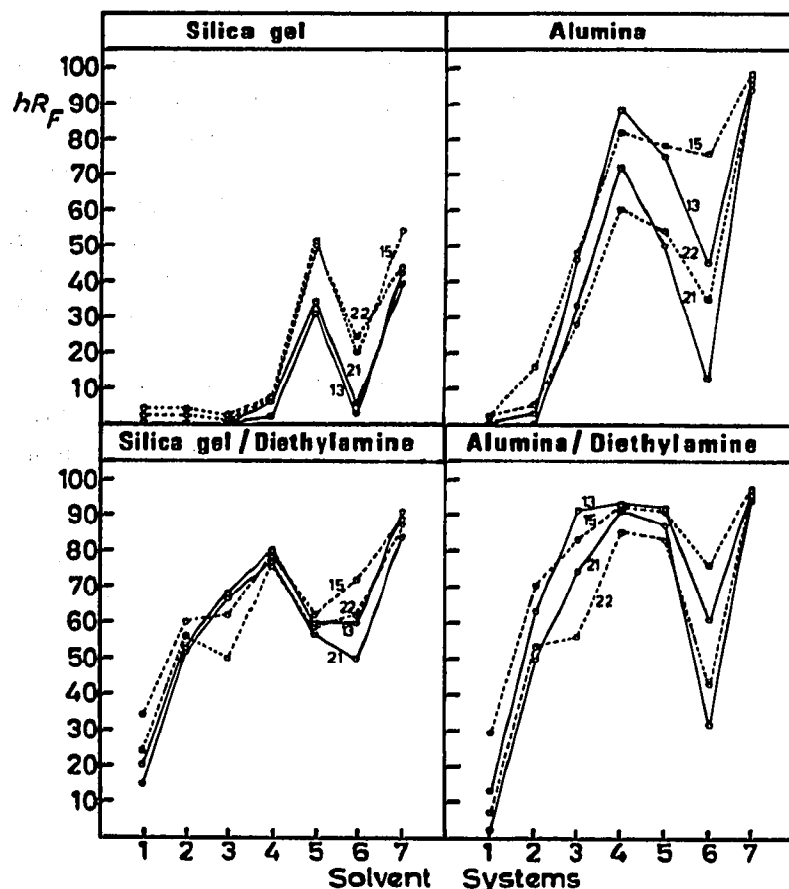


Fig. 6.  $hR_F$  values of: (13) novacine, ———; (15) vomicine, - - - -; (21) epoxynovacine, ———; and (22) epoxyvomicine, - - - -.

bent. The same behaviour pattern is shown by vomicine (VI, 15) and epoxyvomicine (VIII, 22), as illustrated in Fig. 6.

The effect of the C(21)–C(22) epoxide is also shown in Fig. 7 where the behaviour of icajine and 2-methoxyepoxyicajine (VIII, 23) is illustrated. Although these two alkaloids differ in two ways, *i.e.* epoxide and a 2-methoxy substituent, it is thought that the effect of the 2-methoxy substituent will be to slightly increase adsorption (*cf.* strychnine/colubrines, Fig. 1; epoxyvomicine/3-methoxyepoxyvomicine, Fig. 7). Thus the effect of the 2-methoxyl substituent and the epoxide in 2-methoxyepoxyicajine (VIII, 23) will probably be to increase adsorption in all systems, when compared with icajine (VI, 12). The difference in adsorption is more marked on alumina than on silica gel and this can be attributed mainly to the  $\alpha$ -epoxide.

The introduction of the 14-hydroxyl into icajine (VI, 12) causes a marked increase in adsorption (*cf.* icajine/14-hydroxyicajine, Fig. 8). Dreiding models show that a 14-hydroxyl group in the  $\alpha$ -configuration would be easily accessible for binding to adsorbents. Fig. 8 also shows the same behaviour pattern when the pairs of alkaloids epoxynovacine (VIII, 21)/14-hydroxyepoxynovacine (VIII, 25) and epoxyvomicine (VIII, 22)/14-hydroxyepoxyvomicine (VIII, 26) are compared. However, the  $R_F$  values of these latter two pairs tend to be somewhat closer together than those of icajine/14-hydroxyicajine. It would be expected, particularly on alumina, that a

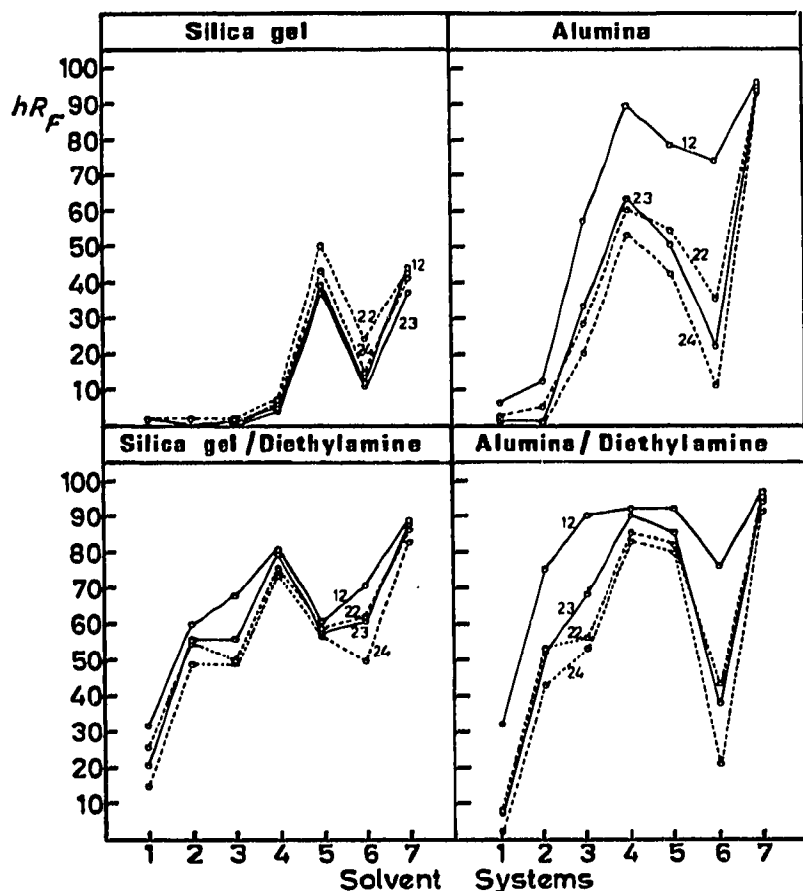
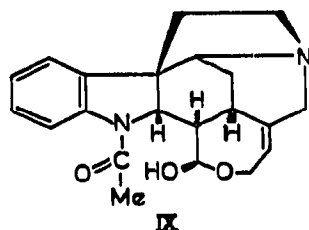


Fig. 7.  $hR_F$  values of: (12) icajine, ———; (22) epoxyvomicine, - - - -; (23) 2-methoxyepoxy-icajine, ·····; and (24) 3-methoxyepoxyvomicine, - · - · -.

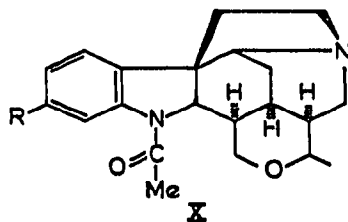
greater increase in adsorption would be noted for a 14-hydroxyl and epoxide group than for just a 14-hydroxyl group. A study of Dreiding models shows that the 14-hydroxyl and epoxide groups are close together and it may be that the 14-hydroxyl group hydrogen bonds weakly to the epoxide, thus explaining why compounds with the combination of 14-hydroxyl and epoxide are somewhat less adsorbed than with 14-hydroxyl alone.

The presence of a 14-hydroxyl group, therefore, is clearly demonstrated by marked adsorption on silica gel and alumina. Parallel behaviour has been noted on GLC, since it has been shown that the effect of a C(21)–C(22) epoxide is to increase only slightly the retention time, whereas the presence of a 14-hydroxyl group causes a greater increase in retention time<sup>5</sup>.

#### TLC behaviour of diaboline, spermostrychnine, and strychnospermine



(28) Diaboline



(29) Spermostrychnine  
(30) Strychnospermine

R = H  
R = OMe

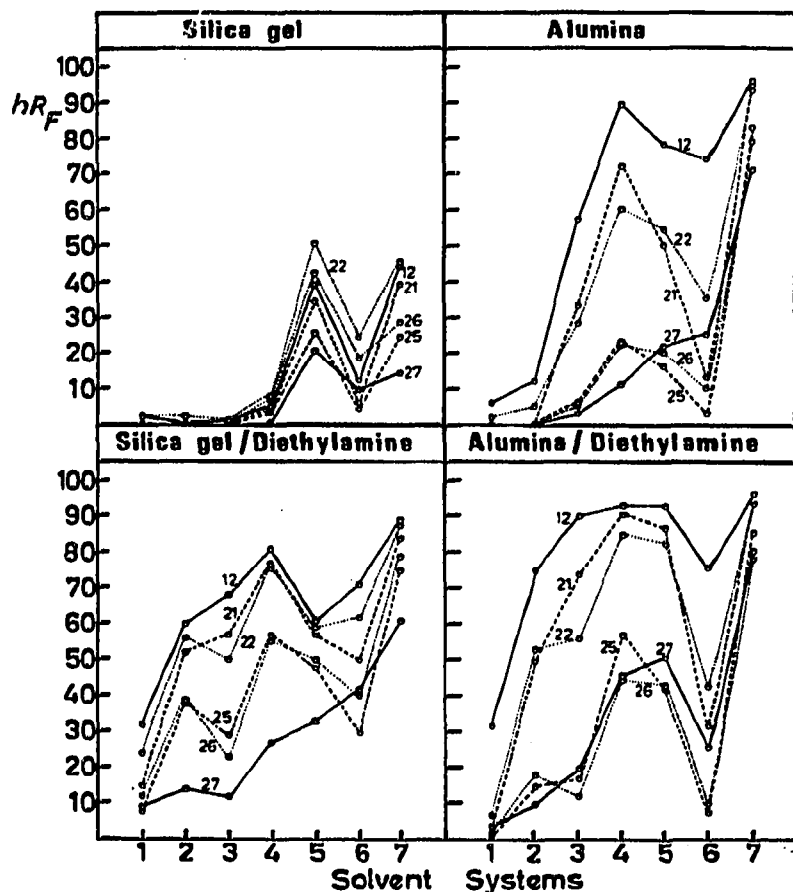


Fig. 8.  $hR_F$  values of: (12) icajine, ———; (21) epoxynovacine, - - - -; (22) epoxyvomicine, ······; (25) 14-hydroxyepoxynovacine, - · - · - ·; (26) 14-hydroxyepoxyvomicine, ······; and (27) 14-hydroxyicajine, ———.

Diaboline (IX, 28) differs from strychnine by having ring III open and a hydroxyl function present at C(12). Fig. 9 shows that diaboline is more adsorbed than strychnine on alumina and silica gel in all systems. This increase in adsorption is most probably due to the C(12) hydroxyl group and the  $N_a$  acetyl group which is now free to take up the most suitable orientation for adsorption. Deacetyldiaboline has previously been shown to be more strongly adsorbed on silica gel than diaboline<sup>16</sup>, indicating that the indoline nitrogen takes part in adsorption.

$R_F$  values have been reported<sup>17</sup> for the isomers jobertine (O-acetyldiaboline B), which has an acetyl group with  $\alpha$ -configuration at C(12), and for henningsamine (O-acetyldiaboline A), whose acetyl group has the corresponding  $\beta$ -configuration. In one silica gel system without diethylamine, jobertine is more adsorbed than henningsamine, whilst in four silica gel systems with added diethylamine this behaviour is reversed. This again illustrates that  $pK_a$  is not the only criterion of adsorption. It would be expected that jobertine with the  $\alpha$ -acetyl group at C(12) would be more adsorbed than henningsamine, if the difference in adsorption is due to the difference in the configuration of the acetyl group at C(12). However, it is difficult to understand why this should be reversed when diethylamine is added.

It is difficult to make meaningful comparisons between spermostrychnine (X,

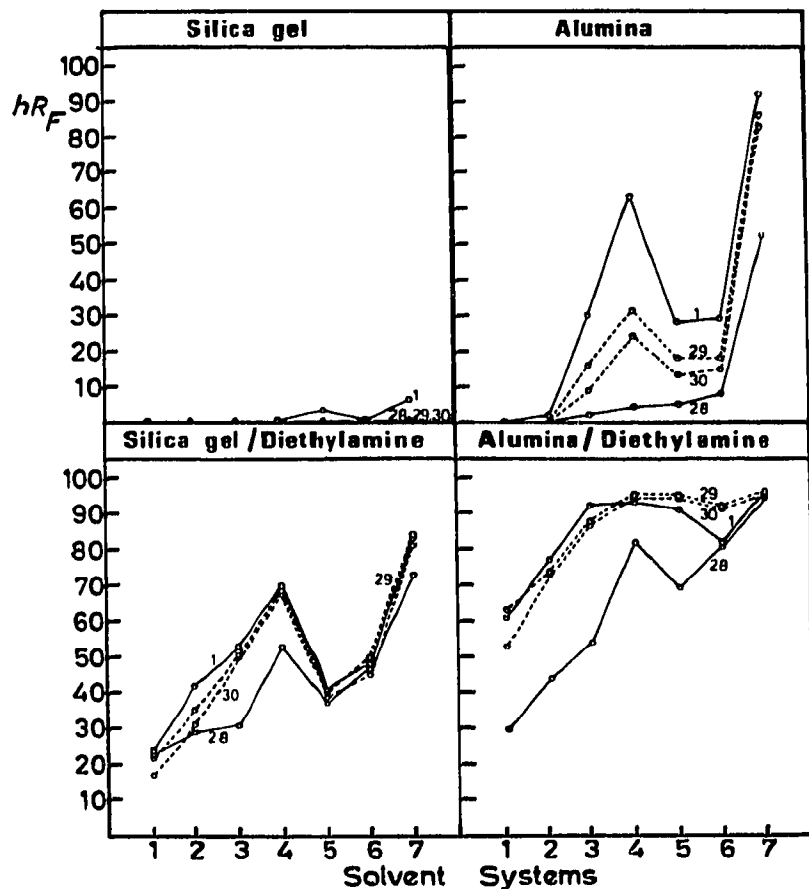


Fig. 9.  $hR_F$  values of (1) strychnine, ———; (28) diaboline, ———; (29) spermostrychnine, - - - -; and (30) strychnospermine, · · · ·.

29)/strychnospermine X, 30) and strychnine because of the difference in skeleton, ring VII being six-membered and not seven-membered. However, Fig. 9 does show that these two alkaloids do have a very similar adsorption pattern to each other and that in common with all heteroyohimbine, oxindole, and *Strychnos* alkaloids so far examined the effect of a methoxy substituent, in the indole ring, is to slightly increase adsorption.

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