Two-Dimensional Separations and Behaviour of Rauwolfia, Corynanthe and Pseudocinchona Alkaloids on Unmodified Silica Gel*

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Key Words

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TLC mild conditions
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Summary

Very efficient procedures for separation by TLC in mild conditions of fifteen alkaloids of the Rauwolfia, Corynanthe, Pseudocinchona group are described. Questions relating to the apparent selectivity of separation systems and separation factors in polar or very polar neutral or acidic mobile phases are discussed.

1 Introduction

One of the major problems in separation science is the search for ever more efficient systems. In TLC [1–3], a high level of efficiency has already been reached for the members of families of general interest after extensive studies of separation systems, for example: amino acids [4], dinitrophenylamino acids [5–8], dansylamino acids [8–10], phenylthiohydantoines [11], nucleotides [12,13] and lipids [14]. It is not the same, however, with other families of compounds – particularly alkaloids (reviews: [15–17]), particularly on account of the lack of knowledge of mobile phase properties and of the origins of apparent selectivities of separation systems.

The above considerations have already lead us to study the separation factors and the chromatographic behaviour of isomeric arylamines [18] and of alkaloids of cocaine and morphine families [19] on unmodified silica gel in neutral or acidic mobile phases. The results obtained [18–20] in these mild conditions have encouraged us to investigate similarly the therapeutically and (or) pharmacologically important family of Rauwolfia, Corynanthe and Pseudocinchona alkaloids. Some interesting information on behaviour in one-dimensional chromatography of some of these bases and other bases of similar structure is already known [16, 21, 22] but not extensive study has been made in connection with the variation of apparent selectivity of mobile phases related to the nature and proportions of solvent components.

The purpose of this preliminary report is to provide some general information on apparent selectivity of separation systems based upon unmodified silica gel with typical mobile phases and to show the possibilities of using this information as a practical guide to devise highly efficient systems of two-dimensional chromatography in mild conditions for the Rauwolfia, Corynanthe and Pseudocinchona alkaloids.

2 Experimental

2.1 Materials

The separations reported were performed on precoated thin-layer plates of unmodified silica gel 60 (200 x 200 x 0.25 mm; 4.58 g adsorbent; n° 5721, Merck, Darmstadt, FRG). The mobile phases were prepared just before use from pure reagent grade chemicals: acetic acid (R.P., Prolabo, Paris), acetone (Merck; water max. 0.2%), dichloro-1,2-ethane (pure; Prolabo; water max. 0.03%), diisopropylether (Merck; inhibited with 10 ppm. 2,6-di-tert-butyl-4-methylphenol; water max. 0.1%), di-ethylamine (Eastman, New York), ethanol (Merck, water max. 0.2%), ethyl acetate (Merck, water max. 0.1%, ethanol max. 0.1%), methanol (Merck; water max. 0.1%), methylethylketone (pure, Fluka, Buchs, Switzerland, water max 0.2%), propanol (pure, Prolabo, water max. 0.2%). Either neutral or acidic solvents are used for two-dimensional development: di-isopropylether/methanol (85:15), dichloro-1,2-ethane/methanol (90:10), acetone/acetic acid (90:10). The alkaloids were obtained from commercial sources. Formulae are drawn from data compiled from reviews [23, 24] or in original works in special cases: Ac [25], Cei and H_2Cei [26, 27], Ceid [28, 29], Ci [30], R [31], αY and Y [30].

2.2 Procedures

The application of samples to the thin-layers and subsequent chromatographic developments were as previously detailed [19, 20]. Development and drying (ventilated

* Proportions in solvents mixtures are v/v except where otherwise indicated. Abbreviations: TLC = thin-layer chromatography, RCP = Rauwolfia, Corynanthe and Pseudocinchona; other abbreviations: see formulaes and Fig. 1.

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hood, 22 °C) of chromatograms are performed in darkness. Because of the danger of oxidation damage of reserpiline and reserpinine during air drying of silica gel after the first run, we recommand quick drying in an air current (30 min) or better the use of a vacuum dryer between the first and second development. For detection of alkaloid spots, a modified Dragendorff reagent [20] has been used.

3 Results and Discussions

3.1 One-Dimensional Chromatography

One-dimensional chromatography on unmodified silica gel makes it possible to know the position of the bases studied in the polarity scale (Fig. 1), from the approximate relation between mobility of bases and dielectric constant of pure neutral solvent [18]. These investigations have shown that these bases can be classified in two groups according to their polarities. The alkaloids of the more polar group (ajmaline, serpentine, tetraphyllicine) are only eluted by very polar pure solvents ($\epsilon > 22$; for example: methanol, ethanol) and have only a weak mobility if the concentration of the more polar solvent in the mixture of weakly and strongly polar solvents reaches 30% (e.g., di-isopropylether/methanol, Table II). All the bases from the least polar group (reserpinine to reserpiline, Table I) have appreciable mobility in pure neutral solvent having dielectric constants of 20 (e.g., propanol, Table I).

Favorable R_F-values are obtained for these alkaloids if the concentration of the more polar component in the mixture of weakly and strongly polar solvent is about 10–15% (e.g., di-isopropylether/methanol – Table II and Fig. 1, first dimension – or 1,2-dichloroethane/methanol – Fig. 2, first dimension). The least polar bases of this second group (reserpinine, corynantheidine, corynanthine) can be eluted by a weakly polar solvent (e.g., di-isopropylether, $\epsilon = 4$, Table I). They are not eluted by carbon tetrachloride ($\epsilon = 2.23$).

Comparison of the data obtained with various pure neutral solvents (Table I) also provides some interesting information on solvent selectivity. The relative mobilities of bases change slightly when passing from one solvent to another if they are of the same chemical type. The order of migration of particular bases changes if the chemical type of solvent is modified. For example, if we compare the results (Table I) obtained with ethyl acetate and with methanol, we find that the relative order of migration for four bases (R_F-values) are Ceid > H_2Cei > D > Ra and D > Ra > Ceid = H_2Cei respectively. Comparison of results obtained with propanol and with acetone shows that the migration order for two bases is H_2Cei > αY and αY > H_2Cei respectively. This second type of variation in selectivity of a pure neutral solvent is particularly interesting in practice. Numerous modifications in the migration order of spots appear when the pure solvent is acidified. Two examples of large variation in the apparent selectivity of systems are given in

Table I. R_F-values. Systems: silica gel and one-component solvents or acidic (or alkalin) solvents (a)

Solvents	DIPE	EA	M	E	P	P/DEA	A	A/A	MEK	MEK/A
Reserpinine	0.31	0.82	0.78	0.80	0.75	0.84	0.91	0.72	0.85	0.37
Deserpidine	0	0.32	0.68	0.69	0.58	0.79	0.84	0.85	0.61	0.55
Ajmalicine	0.05	0.59	0.68	0.73	0.61	0.83	0.86	0.56	0.73	0.20
Corynantheine	0.09	0.68	0.66	0.77	0.70	0.85	0.83	0.64	0.75	0.29
Reserpine	0	0.22	0.64	0.62	0.48	0.77	0.81	0.84	0.53	0.49
Rescinnamine	0	0.22	0.64	0.62	0.47	0.77	0.83	0.86	0.53	0.54
α-Yohimbine	0.02	0.23	0.62	0.66	0.51	0.79	0.79	0.19	0.53	0.03
Corynantheidine	0.22	0.76	0.58	0.73	0.65	0.85	0.86	0.54	0.79	0.22
Dihydrocorynantheine	0.03	0.46	0.58	0.69	0.63	0.85	0.69	0.59	0.54	0.26
Corynanthine	0	0.09	0.57	0.58	0.43	0.81	0.61	0.27	0.29	0.08
Yohimbine	0	0.09	0.55	0.50	0.40	0.74	0.54	0.27	0.23	0.06
Reserpiline	0	0.09	0.54	0.49	0.29	0.66	0.59	0.45	0.31	0.16
Tetraphyllicine	0	0.01	0.28	0.17	0.11	0.76	0.04	0.31	0.02	0.13
Ajmaline	0	0.01	0.24	0.12	0.11	0.68	0.04	0.82	0.02	0.56
Serpentine	0	0.01	0.03	0.01	0.01	0.16	0.01	0.19	0	0.06
Conditions	b	b	b	b	b	b	c	c	c	c

(a) DIPE = di-isopropylether (dielectric constant, ϵ, 25 °C, about 4), EA = ethyl acetate (ϵ, 6.02), M = methanol (ϵ, 32.6), E = ethanol (ϵ, 24.3), P = propanol (ϵ, 20.1), P/DEA = propanol/diethylamine (95:5), A = acetone (ϵ, 20.7), A/A = acetone/acetic acid (90:10), MEK = methylethylketone, MEK/A = methylethylketone/acetic acid (90:10).

(b) 10 x 20 cm thin-layer plates in 20.5 x 9 x 21 cm tank (inner lengths), 100 cm^3 solvent; 21 °C

(c) same conditions but with 20 x 20 cm plates.

Table II. R_F-values. System: silica gel, di-isopropylether/methanol (m:n)*

m:n	95:5	90:10	80:20	65:35	50:50	75:25
Reserpinine	0.73	0.79	0.85	0.86	0.86	0.81
Corynantheidine	0.63	0.75	0.82	0.83	0.75	0.66
Corynantheine	0.41	0.66	0.79	0.82	0.80	0.73
Ajmalicine	0.39	0.66	0.79	0.83	0.80	0.75
Dihydrocorynantheine	0.25	0.54	0.73	0.71	0.67	0.62
α-Yohimbine	0.13	0.38	0.65	0.68	0.67	0.64
Deserpidine	0.08	0.36	0.73	0.78	0.80	0.75
Reserpiline	0.06	0.17	0.44	0.53	0.57	0.55
Reserpine	0.05	0.21	0.65	0.74	0.75	0.72
Corynanthine	0.05	0.20	0.52	0.59	0.61	0.59
Yohimbine	0.03	0.13	0.38	0.49	0.53	0.55
Rescinnamine	0.02	0.15	0.61	0.74	0.76	0.72
Tetraphyllicine	0	0.03	0.08	0.14	0.20	0.27
Ajmaline	0.01	0.03	0.06	0.10	0.14	0.22
Serpentine	0	0	0	0.01	0.02	0.04

* 10 x 20 cm thin-layer plate in 20.5 x 9 x 21 cm tank (inner lengths), 100 cm^3 solvent; 21 °C

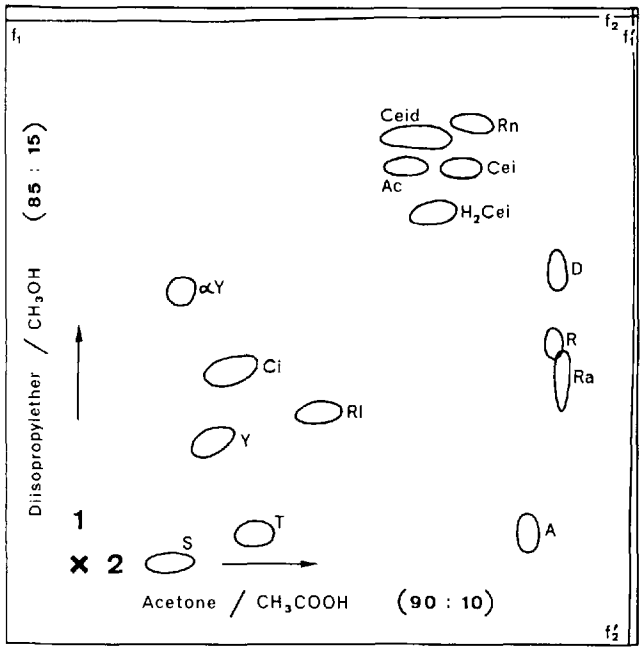
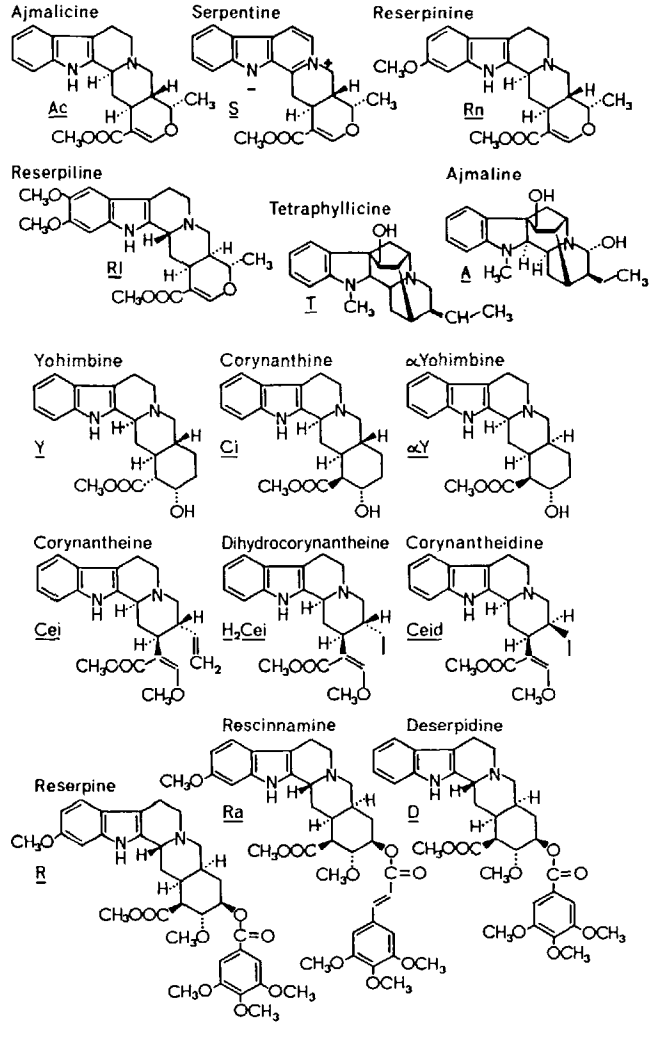


Fig. 1

Two-dimensional chromatography: unmodified silica gel, di-isopropylether/methanol (85:15) then acetone/acetic acid (90:10). First dimension 101 min; second dimension 74 min; sample: 40 μg (ajmaline), 20 μg (all other products); 20 x 20 cm plates, 20.5 x 9 x 21 cm tank (inner lengths), 100 cm^3 solvent; for details see [20].

x = origin; ff' = solvent fronts. Visualisation: Dragendorff's reagent. A = ajmaline, Ac = ajmalicine, Cei = corynantheine, Ceid = corynantheidine, Ci = corynanthine, D = deserpidine, H_2Cei = dihydrocorynantheine, R = reserpine, Ra = rescinnamine, RI = reserpiline, Rn = reserpinine, S = serpentine, T = tetraphyllicine, Y = yohimbine, αY = α-yohimbine.



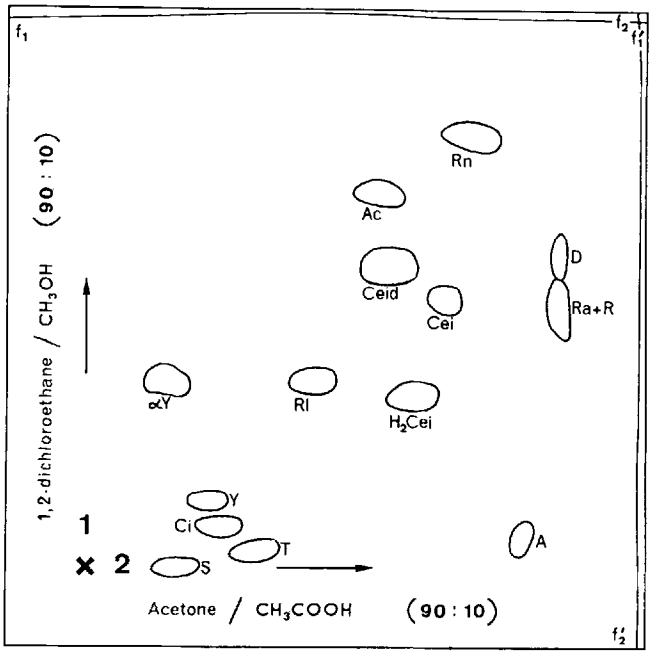


Fig. 2

- Two-dimensional chromatography: unmodified silica gel, 1,2-dichlorethane/methanol (90:10) then acetone/acetic acid (90:10). First dimension 105 min; second dimension 74 min; sample, conditions, visualisation, abbreviations: see Fig. 1. x = origin, ff' = solvent fronts.

Table I when mixtures of acetone/acetic acid (90:10) or methylethylketone/acetic acid (90:10) replace acetone or methylethylketone respectively. If the pure solvent is modified by addition of base (e.g.: di-ethylamine), two parameters are simultaneously changed – activity of adsorbent and ionic properties of alkaloids – and in these conditions pure solvent and basified solvent cannot be suitably compared (Table I). In practice, a less polar solvent such as benzene modified by addition of base such as di-ethylamine is used [16] and this time the selectivity comparison cannot be made because the corresponding pure solvent like benzene cannot elute the alkaloids.

Another way to modify the apparent selectivity of separation systems is to use mixtures of neutral solvents of different polarities. When the proportion of two components of the solvent is changed, the order of migration of particular alkaloids is modified. Examples illustrating this fact can be found in Table II concerning the system: unmodified silica gel, di-isopropylether/methanol (m:n). For example, if we compare the results (Table II) obtained with di-isopropylether/methanol (90:10) and with di-isopropylether/methanol (80:20), we find that the order of migration for six bases based on R_F-values is $H_2Cei > \alpha Y > D > R \neq Ci > RI$ and $D = H_2Cei > R = \alpha Y > Ci > RI$. The dependence of apparent selectivity of the systems upon the proportion of solvent components appear as a general fact. As we recently showed for various types of solutes – phenylthiohydantoins [11, 32], arylamines [18], di-nitrodimethylaminophenyl amino acids or peptides [32] – each solute is characterized by a specific relation between chromatographic mobility (R_F value) and solvent composition and curves for the various solutes intersect if their structures are sufficiently different. Further studies of this type for RCP alkaloids are currently under way.

3.2 Two-Dimensional Chromatography

The first experiments have been devoted to the evaluation of some pure neutral solvents or mixture of neutral solvents and acidic solvents for the separation of the Rauwolfia, Corynanthe and Pseudocinchona alkaloids on unmodified silica gel. In short, through one-dimensional chromatography, not one system alone has sufficient apparent selectivity or even sufficient efficiency adequately to resolve the fifteen alkaloids studied. Nevertheless, optimum separation conditions and chromatographic behaviour of bases have been established.

Thus by combining neutral mobile phases with a compatible acidic mobile phase possessing the most promising apparent selectivities [20], all the alkaloids investigated can be separated on 20 x 20 cm thin-layers of unmodified silica gel in mild condition (Figs. 1 and 2).

Depending on the concentration of methanol, the apparent selectivity of systems with the first run solvent – di-isopropylether/methanol or 1,2-dichlorethane/methanol – may be increased to the optimum value as desired and thus this first run solvent can be favorably associated with a second run solvent having a very different apparent selectivity because of its acidic reaction. Comparison of Figs. 1 and 2 shows clearly that the apparent selectivity of the systems is also depending on the least polar component (di-isopropylether, 1,2-dichlorethane) of the first run neutral solvent.

4 Conclusion

By combining systems with favourable and very different apparent selectivities, two-dimensional thin-layer chromatography on unmodified silica gel in mild conditions provides very efficient separation procedure in the family of the Rauwolfia, Corynanthe and Pseudocinchona alkaloids as in other alkaloid families [20]. Clean, reproducible and predictable separations are obtained.

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