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Note

Separation of diastereomeric pyrrolizidine alkaloids by chromatography on alkalised silica gel

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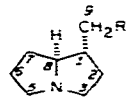
The chemistry of the pyrrolizidine alkaloids is of considerable importance due to their wide distribution in the plant kingdom, their hepatotoxicity and carcinogenicity and their more recent discovery in specialised organs of Lepidoptera[1,2]. Efficient separation techniques are vital because of the frequent co-occurrence among pyrrolizidine alkaloids of several structurally related and diastereomeric bases. Separation of pure diastereomers from mixtures has remained one of the most difficult tasks in this field. For example, the complete separation of the diastereomeric alkaloids intermedine (8) and lycopsamine (9) by column, thin-layer or paper chromatographic methods has not yet been achieved, even on an analytical scale. Resolution satisfactory for analytical purposes is possible by gas chromatography (GC) of the mixed alkaloids as their trimethylsilyl[3] or alkyl boronate[4] derivatives. When first described from *Amsinckia intermedia*, *A. hispida* and *A. lycopsoides*, intermedine and lycopsamine were separated in small amounts by a series of counter-current separations[5].



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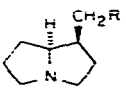

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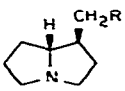

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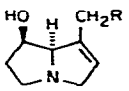

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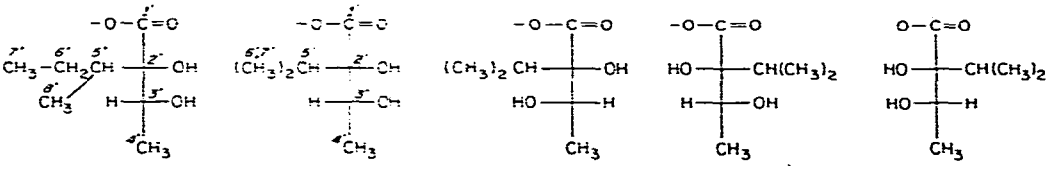
9 R=E



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During our work on pyrrolizidine alkaloids of *Heliotropium curassavicum*[6] we developed a new chromatographic method to separate the diastereomeric alkaloids curassavine (1) and heliocurassavinine (4), coromandaline (2), heliocoromandaline (5), heliovicine (3), heliocurassavicine (6) and heliocurassavine (7), which is described here. This method involves column chromatography on alkalised silica gel with chloroform–methanol–25% ammonia (17:3.8:0.25) as eluent and is simple and effective.

When our work was completed, a paper by Frahn *et al.*[9] appeared describing the separation of intermedine (8) and lycopsamine (9) as their borate complexes. Their procedures are based on the difference in the degree to which the vicinal glycol groups of different configurations (*erythro* and *threo*) complex with borate and is limited to separation of diastereomers containing such groups. Difficulty was experienced in recovering the free alkaloids in high yields from the borate solutions. Our method is superior in that it involves separation of the alkaloids as such and can be used for separating any diastereomeric pyrrolizidine alkaloids, not necessarily only those alkaloids with vicinal glycol groups of different configurations.

EXPERIMENTAL

Silica gel for column chromatography

Silica gel C or G (TLC grade; ACME, Bombay, India) was shaken well with twice the amount of 0.1 *N* sodium hydroxide solution, poured into an evaporating basin, set aside for 4 h, heated at 120°C for 9 h, powdered well and kept for at least 2 days before use.

Alkaloid mixtures M, P and Q

Extraction and fractionation of the alkaloids of *Heliotropium curassavicum* were as described previously[7,8]. Chromatography of alkaloid fraction A (ether extract before reduction) on a column of neutral alumina gave base M as a mixture of curassavine (1) and coromandaline (2). Alkaloid fraction D (ether extract after reduction) on chromatography over neutral alumina with a chloroform–methanol gradient as eluent gave the alkaloid mixtures P (230 mg) and Q (160 mg). P is a mixture of curassavine (1), coromandaline (2), heliovicine (3), heliocurassavine (4), heliocurassavicine (6) and heliocurassavinine (7). Q is a mixture of curassavine (1), coromandaline (2), heliovicine (3) and heliocoromandaline (5).

Thin-layer chromatography (TLC) was performed on silica gel C or impregnated with *N*/10 sodium hydroxide (20 cm × 5 cm × 0.25 mm) using chloroform–methanol–25% ammonia (17:3.8:0.25) as the solvent system. Alkalised silica gel plates were kept for at least 2 days before use. Spots were visualized with iodine and/or Dragendorff's reagent.

Nuclear magnetic resonance (NMR) monitoring was done on a Varian T-60 instrument and confirmed by using a Bruker HX-270 spectrometer[10].

Column chromatography

Alkaloid mixture P or Q was dissolved in chloroform and applied to a column (2.2 cm I.D.) of alkalised silica gel (50–70 g) set up in chloroform. The column was initially eluted with chloroform and then continuously with chloroform–methanol–

25% ammonia (68:15.2:1) at the rate of 5 drops per minute. 0.5-ml fractions were collected and monitored by TLC followed by NMR.

Structures were established[6–8,10] by high-resolution ^{1}H and ^{13}C NMR, mass spectrometry (MS), GC, rotation and paper electrophoresis of the alkaloids and their hydrolysis products.

RESULTS AND DISCUSSION

Base M (a mixture of curassavine and coromandaline) is homogeneous by TLC on alkalised silica gel with (a) methanol, (b) chloroform–methanol–ammonia (17:2.8:0.2)[11], (c) chloroform–acetone–ethanol–ammonia (10:6:2:2)[12] and (d) benzene–ethyl acetate–diethylamine (14:4:2)[13] as solvent systems. In an attempt to select a solvent system for resolving base M on alkalised silica gel plates, the following systems were tried: various proportions of benzene–ethanol–ammonia, benzene–ethanol–ethyl acetate–ammonia, chloroform–methanol–ammonia, chloroform–methanol–ethyl acetate–ammonia, chloroform–acetone–ethanol–ammonia and benzene–ethyl acetate–diethylamine. Base M showed up as a single spot or at best as an elongated one in all these systems. Finally, however, TLC on silica gel impregnated with 0.1 *N* sodium hydroxide with chloroform–methanol–25% ammonia (17:3.8:0.25) proved to be successful for resolution of base M into two spots, R_F 0.88 (curassavine) and 0.85 (coromandaline). With this system, alkaloid mixtures P and Q could be resolved into more than two spots at low concentrations. This system works best only at temperatures 27–31°C.

The next step was to develop an efficient chromatographic method for the separation of the diastereomeric alkaloids. Preparative TLC was unsuccessful. After standardisation of the conditions (see Experimental), separation of the bases could be effected on a column of alkalised silica gel (TLC grade) with chloroform–methanol–25% ammonia (68:15.2:1) as eluent. To provide an illustration of the method, two typical separations are described herein.

Alkaloid mixture P (220 mg) was chromatographed on alkalised silica gel with the above solvent system as described in the Experimental section with the above solvent system; the results are given in Table I. The first six fractions were pure heliocurassavine (4) followed by mixtures of 4 and curassavine (1). Fractions 9–12 were pure 1 and fraction 13 was a mixture of 1 and heliocurassavine (7). Fractions 14 and 15 were pure 7 followed by a mixture of 7 and heliocurassavicine (6). Pure 6 was eluted next in fractions 17–19, followed by a mixture of 6 and heliovicine (3) and then by pure 3 in fractions 21 and 22. Fraction 23 was a mixture of 3 and coromandaline (2). The last fractions 24 and 25 were pure 2. In this separation, 25 fractions were collected out of which 19 fractions were pure diastereomeric alkaloids. Out of the total recovery (90%), 73% comprised six pure diastereomers and 27% partially separated mixtures.

In a similar way, alkaloid mixture Q (150 mg) was chromatographed; the results are shown in Table II. Fractions 1–5 were pure curassavine (1) whereas fractions 6 and 7 were mixtures of 1 and heliovicine (3). Fractions 8–10 were pure 3 followed by a mixture of 3 and coromandaline (2). Pure 2 was eluted next followed by a mixture of 2 and heliocoromandaline (5). The last fraction was pure 5. In this separation 15 fractions were collected of which 11 represented pure alkaloids. Total

TABLE I

SEPARATION OF THE ALKALOIDS 1, 2, 3, 4, 6 AND 7 FROM ALKALOID MIXTURE P

Fraction No.	*Amount (mg)*	*Alkaloids*	*Approximate percentage abundance (by NMR)*
1–6	25	4	100
7	7	4, 1	60:40
8	9	4, 1	30:70
9–12	35	1	100
13	8	1, 7	65:35
14, 15	15	7	100
16	9	7, 6	30:70
17–19	30	6	100
20	11	6, 3	35:65
21, 22	25	3	100
23	9	3, 2	70:30
24, 25	14	2	100

recovery was 85% of which 69% comprised pure alkaloids and 31% mixtures of partially separated bases.

Fractions were best distinguished by NMR monitoring. Column chromatographic separation with this system works best at temperatures between 27 and 31°C. Humidity may also play a rôle but its importance cannot be specified precisely.

Diastereomeric pyrrolizidine alkaloids have not previously been separated by silica gel chromatography. The method should be applicable to other mixtures of diastereomeric alkaloids with little modification.

TABLE II

SEPARATION OF THE ALKALOIDS 1, 2, 3 AND 5 FROM ALKALOID MIXTURE Q

Fraction No.	*Amount (mg)*	*Alkaloids*	*Approximate percentage abundance (by NMR)*
1–5	40	1	100
6	11	1, 3	60:40
7	12	1, 3	30:70
8–10	29	3	100
11	9	3, 2	65:35
12, 13	11	2	100
14	8	2, 5	40:60
15	7	5	100

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