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Note

Ion-pair adsorption chromatography of pyrrolizidine alkaloids

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In the United States, comfrey (*Symphytum officinale*) is used commercially to make a common herbal tea. The water-soluble pyrrolizidine alkaloids in this plant, however, are known to be highly hepatotoxic and caused liver cancer in rodents when fed at a level of 50 ppm[1].

Various methods have been described for the chromatographic separation of these pyrrolizidine alkaloids. Most attention has been paid to systems using methanol as a solvent for sodium hydroxide-impregnated silica gel plates[2–5] and to straight-phase systems using more or less complex mixtures of organic solvents and ammonia[3,6–8]. In the first system the hR_F values of separated alkaloids depend on the nature of the amino alcohol and the type of esterification of the ester alkaloid. The effects of molecular weight and number of hydroxyl groups proved to be of minor importance[5].

The straight-phase system of Sharma *et al.*[6] gave a wider spread of hR_F values for distinct alkaloids. Reproducibility, however, was difficult to achieve[2] and the separation was highly influenced by factors such as temperature and humidity. The separation of echimidine and symphytine, both having retronecine as the amino alcohol and being main alkaloids in *S.x uplandicum* and obviously in *S. officinale*[7–9], was highly unsatisfactory when using sodium hydroxide-impregnated plates on comparison of the hR_F values with data published by Pedersen[3]. With the solvent system of Sharma *et al.* we were able to discriminate between several pyrrolizidine alkaloids in extracts from *S. taxa*, but for reliable qualitative comparison of extracts the system proved to be much less useful because of frequent overlap of detected spots.

De Zeeuw *et al.*[10] described the use of ion-pair adsorption chromatography of basic drugs on silica gel plates using inorganic counter ions and organic solvents: basic compounds migrated as uncharged ion pairs under neutral conditions. The method was relatively insensitive to small changes in atmospheric conditions. Its major advantage is the combination of separational selectivity of the silica gel with the differences in the physico-chemical properties of the compounds in the ion-pair system.

In this paper we describe a method for the ion-pair thin-layer chromatographic (TLC) separation of mono- and diester pyrrolizidine alkaloids, by which is shown that the alkaloid patterns of different cytotypes of *S. officinale* are very similar.

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EXPERIMENTAL

Isolation of pyrrolizidine alkaloids

Pyrrolizidine alkaloids from *Symphytum officinale* L. ($2n = 24$ and 48) and *S.x uplandicum* Wym. ($2n = 40$) were isolated as described previously[11].

Pyrrolizidine alkaloids from *Cynoglossum nervosum* were derived according to Pedersen[3].

Thin-layer chromatography

Silica gel 60 F_{254} thin-layer plates (10 × 5 and 20 × 20 cm) from Merck (Darmstadt, G.F.R.) and G1500 F_{254} plates (20 × 20 cm) from Schleicher & Schüll (Dassel, G.F.R.) were dipped for 5–25 sec into methanol saturated in potassium chloride or methanol containing 0.1, 0.15 or 0.2 *M* sodium iodide or lithium chloride.

For comparison of the influence of the nature of the counter ion on the separation patterns, the dipped plates were blotted and dried in a stream of air at ambient temperature. Further drying conditions tested after lithium chloride impregnation of the plates were drying for 3 h at ambient temperature and drying at 130°C for 15 and 30 min. The mobile phases used were chloroform–methanol (90:10, 80:20, 75:25 and 70:30). Occasionally 0.15 *M* lithium chloride was added to the eluent. The straight (basic) eluting system used was chloroform–methanol–25% ammonia solution (85:14:1)[6].

Extracts in chloroform were spotted by means of Modulohm 5-μl microcapillary pipettes (Herlev, Denmark). Spots were revealed with chloranil[12]. For the preparative separation of alkaloids, extracts were applied to plates with a Camag Chromatocharger 2 cm from the bottom in an amount such that the brown colour of the extract just became visible on the back of the plate. After development of a plate over 10 cm and drying at ambient temperature, the complete plate was sprayed with Dragendorff reagent. The coloured bands were scraped off and powdered and the powders were washed with an aqueous saturated solution of sodium carbonate in a fluted filter-paper in a filter-funnel until the yellow colour had disappeared completely. The washings were collected in a separating funnel and extracted three times with chloroform. The chloroform was distilled off *in vacuo* and from the remaining clear glassy products mass spectra were recorded on a Finnigan quadrupole mass spectrometer with a 6110 data system at an electron energy of 70 eV and an ionizing current of 100 μA.

RESULTS AND DISCUSSION

The use of chloride as the counter ion for the ion-pair TLC separation of pyrrolizidine alkaloids using chloroform–methanol mixtures as eluent gave a wide range of hR_F values for distinct pyrrolizidine alkaloids from *Symphytum x uplandicum* extracts. Comparing Figs. 1 and 2, it could be concluded that higher methanol concentrations in the eluent gave higher hR_F values, whereas higher chloride ion concentrations in the impregnants mainly gave lower hR_F values.

The disadvantage of potassium chloride as an impregnant was its poor solubility in methanol. Molar solutions of lithium chloride in methanol can be prepared, hence making the introduction of this salt directly into eluents consisting of chloro-

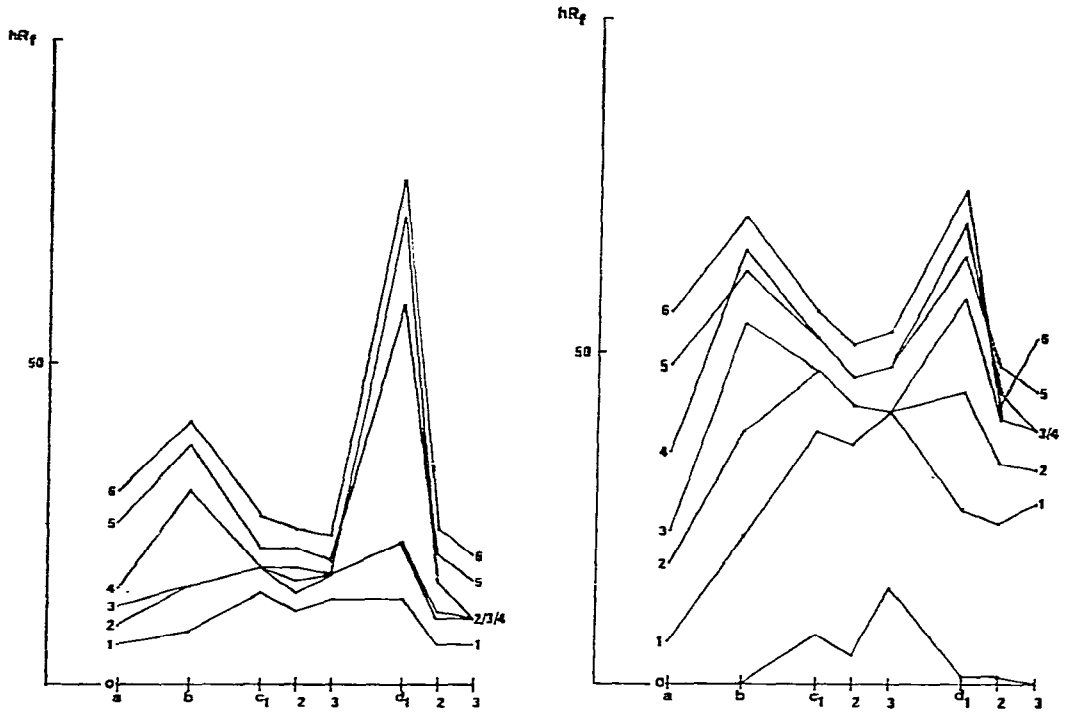


Fig. 1. hR_F values of pyrrolizidine alkaloids from *Symphytum x uplandicum* from ion-pair separation using chloroform–methanol (90:10) as the eluent. Impregnation parameters: impregnation for 15 sec by dipping into: (a) no treatment; (b) saturated KCl in methanol; (c_1) 0.10 *M* NaI in methanol; (c_2) 0.15 *M* NaI in methanol; (c_3) 0.20 *M* NaI in methanol; (d_1) 0.10 *M* LiCl in methanol; (d_2) 0.15 *M* LiCl in methanol; (d_3) 0.20 *M* LiCl in methanol. 0, 1, 2 and 3 = unknowns; 4 = acetyllycopsamine or diastereoisomer; 5 = echimidine; 6 = symphytine.

Fig. 2. hR_F values of pyrrolizidine alkaloids from *S. x uplandicum* from ion-pair separation using chloroform–methanol (80:20) as the eluent. Impregnation parameters as in Fig. 1.

form and methanol feasible. With separation using chloroform–methanol (80:20) with added lithium chloride (0.15 *M*) and omitting impregnation of the plates, a sub-front appeared, leading to distortion of the separation pattern. The use of this eluent with impregnated plates, however, gave very good separations of pyrrolizidine alkaloids with no sub-front.

The ease with which the impregnant and eluent can be varied is of great convenience when fitting the separation system to special separation requirements.

A comparison of several experiments using both straight-phase and ion-pair systems (for an example, see Fig. 3) indicated that the latter system is much less sensitive to small atmospheric changes, as was also found by De Zeeuw *et al.*[10], and that it gives more information.

On examination of the influence of different methods of drying the dipped plates, it was found that methanol still adhering to plates dried at ambient temperature gave rise to an increase in hR_F values, because the resulting eluent was more polar than that when the plates were thoroughly dried. Hence drying at 130°C resulted in

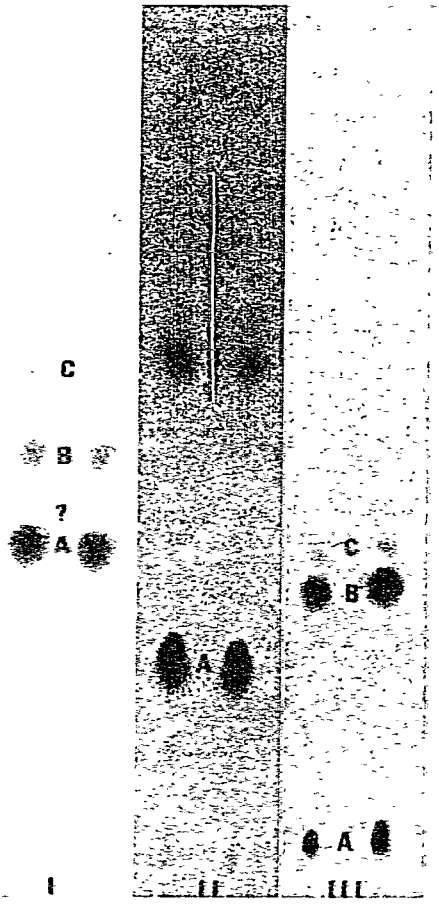
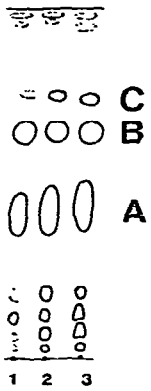


Fig. 3. Chromatography of pyrrolizidine alkaloids from *S. officinale*. I = Ion-pair system; impregnant 0.15 *M* LiCl; eluent, chloroform–methanol (75:25) with 0.15 *M* LiCl added. II, III = Straight-phase system (separation in August 1980 and December 1979, respectively). Extracts I and II are identical. A = lycopsamine and/or diastereoisomer; B = acetyllycopsamine and/or diastereoisomer; C = symphytine; ? = unknown.

a poorer separation, which could nevertheless be restored completely be eluting with more polar eluents.

Pyrrolizidine alkaloids from white and purple flowering tetraploid ($2n = 48$) and white flowering diploid ($2n = 24$) *S. officinale* plants could be separated effectively by the use of ion-pair TLC, as shown in Fig. 4. The alkaloid patterns from these *S. officinale* cytotypes showed a striking resemblance. The molecular weights of the alkaloids, determined by mass spectrometry after preparative isolation, were 299 ($hR_F = 42$; lycopsamine and/or diastereoisomer), 341 ($hR_F = 64$; acetyllycopsamine and/or diastereoisomer) and 381 ($hR_F = 75$; symphytine). From *Cynoglossum nervosum* an alkaloid was isolated having a molecular weight of 299 (ref. 13). Using the straight-phase system of Sharma *et al.*[6], this alkaloid migrated faster than did the



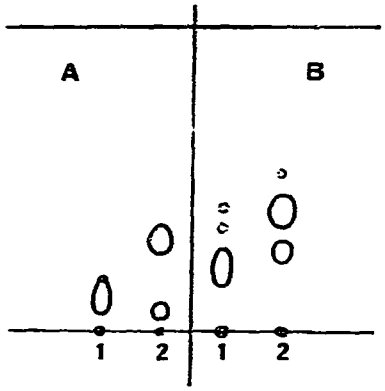


Fig. 4. Ion-pair separation of pyrrolizidine alkaloids from *S. officinale*. Impregnant, 0.15 *M* LiCl; eluent, chloroform–methanol (80:20). Cytotypes: 1 = white flowering, $2n = 48$; 2 = purple flowering, $2n = 48$; 3 = white flowering, $2n = 24$. See also Fig. 3.

Fig. 5. Comparison of migration of alkaloids from *Cynoglossum nervosum* (1) and *Symphytum officinale* (2). Separation conditions: A, straight phase system; B, ion-pair system as described in Fig. 4.

alkaloid with the same molecular weight from *S. officinale* (Fig. 5). As an ion pair however, the alkaloid from *S. officinale* migrated faster. A combination of the two systems could be of great value for comparative investigations.

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