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PREPARATIVE SEPARATION OF THE PYRROLIZIDINE ALKALOIDS, INTERMEDINE AND LYCOPSAMINE, AS THEIR BORATE COMPLEXES

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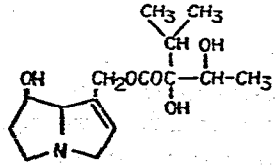
SUMMARY

Intermedine and lycopsamine are diastereoisomers containing vicinal glycol groups of different configuration. The difference in the degree to which they complex with borate is the basis of two procedures for their separation from mixtures on a preparative scale. In the first, the mixture dissolved in chloroform is passed through a column of glass powder moistened with a solution of borax. Intermedine elutes first with chloroform and is cleanly separated from lycopsamine, the more strongly complexing of the pair.

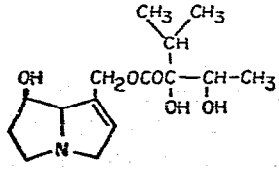
In the other procedure, in which lycopsamine elutes first, a mixture is dissolved in 0.1 *M* borax and the solution passed through a cation-exchange resin (Bio-Rad AG 50W-X2) impregnated with 0.1 *M* borax.

INTRODUCTION

The diastereoisomeric alkaloids, intermedine (1) and lycopsamine (2), occur together in a number of genera of the Boraginaceae, *e.g. Amsinckia*[1] and *Echium*[2] and in the genus *Parsonsia*[3] of the Apocynaceae. They are of interest both for their toxicity and for their conversion into pheromones by certain species of insects[4]. The physical properties of these two alkaloids are closely similar and their complete separation from mixtures 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 of the mixed alkaloids as their



(1)



(2)

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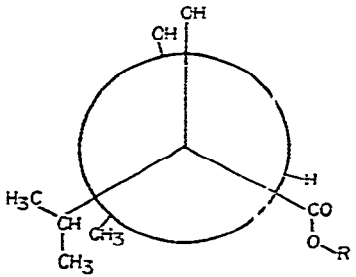
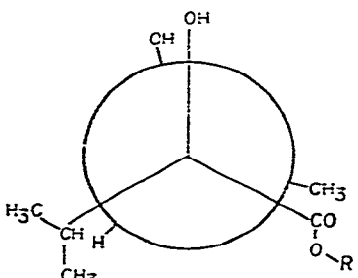
trimethylsilyl[5] or alkylboronate[3] derivatives. When first described from *Amsinckia* species, each alkaloid was isolated in small amounts by counter-current distribution of extracts, but the preparation of the pure alkaloids on a larger scale has remained one of the most difficult separation tasks in the pyrrolizidine alkaloid field.

In an earlier study of the paper electrophoresis of pyrrolizidine alkaloids[6] it was shown that these compounds migrate as cations in non-complexing electrolytes, but that, in sodium borate buffer, compounds containing complexable pairs of hydroxyl groups form anionic complexes to an extent determined by their stereochemistry, and the cationic migration is thereby reduced or changed into anionic migration. In a sodium carbonate electrolyte (pH 9.2), intermedine and lycopsamine were found to have identical cationic mobilities (1.54 cm/h·kV of total potential applied) but, in borate buffer of the same pH, they migrated toward the anode with mobilities of 0.29 and 2.35 cm/h·kV, respectively, and this large difference allowed rapid separation of the isomers, albeit on an analytical scale.



(1a)



(2a)

The greater anionic mobility of lycopsamine is a result of its forming the more stable borate complex, the reason for which is indicated by comparison of the Newman projection formulae (1a) and (2a). Formula (1a) represents a projection along the C_2–C_3 bond of the trachelanthate moiety of intermedine. It is seen that with the hydroxyl groups eclipsed (a condition ideal for the formation of a 5-membered cyclic borate complex) a destabilising non-bonded interaction is set up by the eclipsing of the C_2-isopropyl and C_3-methyl groups. The corresponding projection formula (2a) for the viridiflorate moiety of lycopsamine shows how eclipsing of its *erythro* hydroxyls leads to a conformationally more stable arrangement in which the C_2-isopropyl group interacts only with an eclipsed C_3-H.

The difference in degree to which intermedine and lycopsamine complex with borate has now been used in two different procedures for their separation on a preparative scale.

EXPERIMENTAL

Materials

The mixture of intermedine (approx. 30%) and lycopsamine (approx. 70%) contained a small amount of echimidine and was the product of an earlier investigation of the alkaloids of *Amsinckia* species[1]. The long-stored mixture was cleaned up by dissolving it in dilute sulphuric acid, filtering the solution from a black precipitate,

washing the filtrate with diethyl ether and with chloroform, adding ammonium hydroxide to pH 9.5, saturating the basic solution with sodium chloride and extracting it with at least five lots of chloroform.

Borate partition column. Powdered soda glass (passing 150 mesh; 48 g) was moistened with a 5% aqueous solution of disodium tetraborate (borax) (8 ml) and packed in light petroleum in a 55 × 1.1 cm column with the aid of a perforated plunger. The alkaloid mixture (47.7 mg) was dissolved in a small volume of chloroform which was absorbed into glass powder and evaporated under vacuum. The glass powder was then packed on top of the column. Chloroform was applied as eluent and fractions of approximately 5 ml collected and titrated with toluene-4-sulphonic acid (0.01 *M*) in chloroform using dimethyl yellow as indicator. The titres indicated two peaks (Fig. 1), comprised of fractions 13–24 and 51–80, which were combined appropriately for recovery of alkaloid. Each bulk fraction was evaporated and the residue taken up in dilute sulphuric acid. The solutions were made alkaline by adding ammonium hydroxide and then washed several times with light petroleum to remove dimethyl yellow, saturated with sodium chloride and extracted with five lots of chloroform. Fractions 13–24 gave 14 mg and fractions 51–80 gave 23.5 mg colourless crystalline alkaloid with the electrophoretic properties in borate buffer of pure intermedine and lycopsamine, respectively.

In a larger scale run, 1.7 g mixed alkaloid was applied to a 70 × 2.2 cm column packed with 340 g glass powder bearing 55 ml borate solution. Fractions of 40 ml were collected and examined by spotting on filter paper and spraying with manganese sulphate–potassium permanganate–sulphuric acid reagent[7]. Fractions were grouped appropriately and worked up as before. Fractions 5–15 gave 0.58 g intermedine and fractions 23–60 gave 0.83 g lycopsamine, both electrophoretically pure.

Intermedine. After recrystallisation from acetone, intermedine formed thick prisms, m.p. 141–142°, $[\alpha]_D^{20}$ + 9.8° (c, 1.49 in ethanol). ^{1}H NMR, δ(100 Mhz, $CDCl_3$): 0.93, d, 6H, $(CH_3)_2CH$; 1.20, d, 3H, CH_3CHOH; 4.80, AB nearly *s**, 2H, H9; 5.94, m, 1H, H2.

Lycopsamine. After recrystallisation from acetone, lycopsamine formed thick prisms, m.p.. 132–134°, $[\alpha]_D^{20}$ + 5.7° (c, 0.89 in ethanol). ^{1}H NMR, δ(100 Mhz, $CDCl_3$): 0.89, d, 3H, CH_3CH; 0.93, d, 3H, CH_3CH; 1.25, d, 3H, CH_3CHOH; 4.76, 4.81, ABq*, 2H, H9; 5.91, m, 1H, H2. The m.p. of a mixture of lycopsamine and intermedine was depressed to 119–125°.

Ion-exchange separation of borate complexes of intermedine and lycopsamine

A solution of mixed alkaloids (0.52 g) in 0.1 *M* di-sodium tetraborate (1 ml) was applied to the top of a 100 × 3 cm column of Bio-Rad AG 50W-X2 resin, (200–400 mesh; sodium form in 0.1 *M* borate). Elution was continued with 0.1 *M* borate at a flow-rate of 2 ml/min. Fractions of 10 ml were collected and examined for alkaloid by spotting on filter paper and spraying with manganese sulphate–potassium permanganate–sulphuric acid reagent. Visual assessment of the spots indicated that the alkaloids had eluted as in Fig. 2. Fractions were grouped appropriately and worked up by adding glucose (20 g/100 ml), potassium chloride (30 g/100 ml) and 10 *M* sodium hydroxide (3–4 ml/100 ml, until pH 11 was indicated by test papers).

* AB nearly *s*: AB quartet which has the appearance of a broadened singlet; ABq: AB quartet.

Immediate extraction with five lots of chloroform gave products as follows: fractions 1–30, 10 mg echimidine; fractions 31–70, 200 mg lycopsamine; fractions 71–110, 121 mg intermedine. Electrophoresis confirmed that the peaks shown in Fig. 2 were pure single components.

RESULTS AND DISCUSSION

The separation of intermedine and lycopsamine on the borate partition column depended on differences in the chloroform solubility of the free bases and their respective borate complexes. The free bases are easily soluble in chloroform but the salt-like complex anions may reasonably be expected to have little or no solubility in this solvent. The order of elution of the alkaloids was therefore determined by the proportion in which each existed as the free base in its equilibrium mixture and, as expected, intermedine appeared first and was cleanly separated from lycopsamine (Fig. 1). Each alkaloid crystallised readily from its pure fraction.

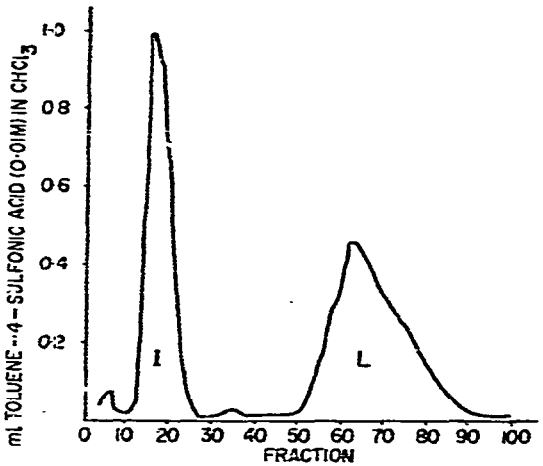


Fig. 1. Elution pattern of intermedine (I) and lycopsamine (L) from borate partition column.

In the other procedure, in which the alkaloid mixture was dissolved in 0.1 *M* sodium borate and the aqueous solution passed through a cation-exchange resin, it was expected that the negative charge induced on the alkaloid molecules by borate complexing would hinder binding to the resin and that the order of elution would, in this case, depend on the equilibrium proportion in which each alkaloid existed as its anionic complex. Lycopsamine, as the more strongly complexed of the pair of alkaloids, was indeed the first eluted, and complete separation from intermedine was again achieved (Fig. 2).

Difficulty was experienced in recovering lycopsamine in high yield from the borate solution, and, to a lesser extent, this was also true for intermedine. In the procedure adopted, glucose was added to decompose the alkaloid complexes by competing for borate, and the freed bases were salted out into chloroform by adding potassium chloride after making the solution strongly alkaline. In a trial experiment with lycopsamine, recovery was 80%.

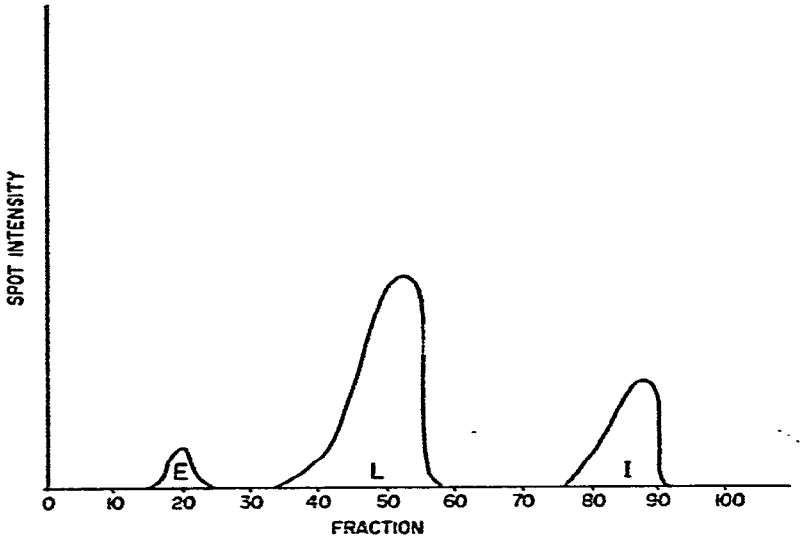


Fig. 2. Elution pattern of echimidine (E), lycopsamine (L) and intermedine (I) from Bio-Rad AG 50W-X2 ion-exchange column.

Both methods are suitable for separating gram quantities of the alkaloids and make intermedine and lycopsamine readily available in a pure state for the first time. The methods should be applicable to other mixtures of diastereoisomeric alkaloids which differ in being viridifloric and trachelanthic esters, for example, those of 7-angelylheliotridine which occur together in *Heliotropium supinum* L[8].

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