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## Chromatographic separation of lysergic acid amide and isolysergic acid amide in Morning Glory seeds

Lysergic acid amide (LAA), isolysergic acid amide (isoLAA), elymoclavine and lysergol have been identified by thin-layer chromatography[1–5], as the alkaloidal components in seeds of some kinds of convolvulus plants.

In the present communication, a column chromatographic method for the quantitative separation of LAA and isoLAA, which are known to have psychotomimetic properties, is described.

*Experimental*

Seeds of Heavenly Blue, a horticultural variety of Morning Glory in U.S.A., were used for the experiment. The extraction of the alkaloids in Morning Glory seeds was carried out according to the method described by GENEST[5]. 2 ml of 0.1 *N* sulfuric acid and 4 ml of Van Urk reagent[5] were added to the extract. After 30 min, the absorbance at 550 mμ was measured. The amount of total alkaloids was expressed as LAA equivalents.

*Thin-layer chromatography for identification of the alkaloids.* Silica Gel G was used for the chromatographic plates. Solvent systems for development used were: (A) acetone–piperidine (9:1), (B) chloroform–methanol (4:1), and (C) acetone–ethyl acetate–dimethylformamide (5:5:1). For detection the grey blue fluorescence under 3650 Å UV light was observed and then the plate was sprayed with Van Urk reagent.

*Column chromatography for separation of the alkaloidal components.* An aliquot of the chloroform extract containing 100 μg of total alkaloids was transferred to the first chromatographic column (1 × 22 cm), packed with 4 g of Celite 545 mixed with 3 ml of 0.5 % citric acid aqueous solution. Water-washed chloroform was used as eluant throughout. The mobility of the alkaloids was easily observed by their blue fluorescence when irradiated with UV light. The fluorescent eluate was collected and concentrated in a stream of nitrogen (fraction A). The non-mobile fluorescent band retained in the column was extruded into a tartaric acid solution, rendered alkaline with $NaHCO_3$, and extracted with chloroform. The organic layer was separated and concentrated (fraction B). Fraction B was applied to the second column (1 × 21 cm), which was packed with a mixture of 3.8 g of Celite 545 treated with 2.85 ml of 0.5 % citric acid solution and 0.2 g of silica gel. The initial eluate was combined with fraction A and the next strong fluorescent eluate was collected separately (fraction C). Fluorescent material remaining in the column was extracted as mentioned above (fraction D). Fraction A was then passed through another column (1 × 20 cm), packed with a mixture of 3.6 g of Celite 545 treated with 2.7 ml of 0.5 % citric acid solution and 0.4 g of silica gel. The first fluorescent eluate was collected (fraction E), and the fluorescent band left on the column was extracted and combined with fraction C.

*Results*

The alkaloidal component in each fraction obtained by column chromatography was identified by thin-layer chromatography (Fig. 1).

The major component in fraction A was isoLAA and the minor was LAA.

Clavine alkaloids and LAA remained in fraction B. The first eluate from the second column contained a small amount of isoLAA, and LAA was found in fraction C. Fraction D, obtained by extraction of the material which remained on the second column, contained clavine alkaloids. Fraction E, obtained from the eluate of the third column, contained isoLAA and the material remaining on the third column was combined with fraction C, since it contained LAA. Fractions C and E, obtained in a large scale experiment, were found to be identical with LAA and isoLAA, respectively, by IR spectroscopy using the micro-KBr pellet method.

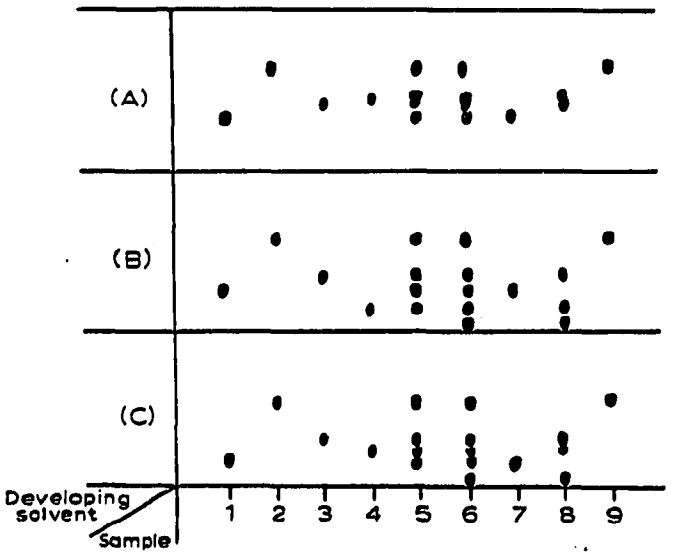


Fig. 1. Thin-layer chromatograms of clavine and lysergic acid alkaloids on Silica Gel G. Developing solvents: (A) acetone–piperidine (9:1); (B) chloroform–methanol (4:1); (C) acetone–ethyl acetate–dimethylformamide (5:5:1). Detection: Observation under UV light and spraying with Van Urk reagent. Samples: 1 = lysergic acid amide; 2 = isolysergic acid amide; 3 = lysergol; 4 = elymoclavine; 5 = 1 + 2 + 3 + 4; 6 = extract from Heavenly Blue seeds; 7 = fraction C obtained by column chromatography; 8 = fraction D obtained by column chromatography; 9 = fraction E obtained by column chromatography.

In the above experiment, LAA and isoLAA were quantitatively recovered from a mixture of authentic compounds by this method. The amounts of alkaloids in fractions C and E were determined by colorimetry. The amount of total alkaloids in fresh seeds of Heavenly Blue was 0.0206 %, and the alkaloids consisted of 44.8 % of LAA, 34.2 % of isoLAA and clavine alkaloids.

The column chromatography technique described here has been used for the determination of LAA and isoLAA in the seeds of horticultural varieties of Morning Glory in our laboratory. This method has some advantages because LAA and isoLAA can be separated quantitatively in a pure state and determined more exactly by colorimetry.

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## DEAE Sephadex LH-20, a new chromatographic medium for the fractionation of acidic lipids

Brain phosphoinositides have been separated on DEAE cellulose with a salt gradient in a mixture of chloroform and methanol[1]. Because cellulose columns are difficult to pack with organic solvents, an exchange medium more compatible with lipid solvents was sought to be used for the application of this technique to the general fractionation of acidic lipids. DEAE Sephadex LH-20 has been prepared, and its use in the separation of the phosphoglycerides of *Escherichia coli* is described here. Conditions for the fractionation of more complex lipid mixtures with this exchanger are currently being investigated.

*Experimental*

Diethylaminoethoxypropylated Sephadex (DEAE Sephadex LH-20) was prepared from Sephadex LH-20 (Pharmacia Fine Chemicals) by the method described by PETERSON AND SOBER[2] for the preparation of DEAE cellulose. Titration of samples of the product in the $OH^-$form in methanol with HCl required 1.3 mequiv./g. The exchanger was stored in the formate form.

*E. coli* lipids were extracted as described elsewhere[3] and were separated into neutral and complex lipid fractions by chromatography on silicic acid[4]. All organic solvents and other chemicals were reagent grade and were used without further treatment.

Columns were packed with a slurry of DEAE Sephadex LH-20 in chloroform–methanol (1 : 1) which had been made 1.0 *M* with formic acid and 0.5 *M* with ammonium hydroxide by the addition of 90% formic acid and 30% ammonium hydroxide solutions, respectively. A 18 × 0.8 (I.D.) cm column was used for 2 g of exchange