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SHORT COMMUNICATION

THE SEPARATION OF ERGOT AND CLAVINE ALKALOIDS BY GEL FILTRATION*

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Abstract—Gel filtration on Sephadex LH-20 and Sephadex G-25 with 96% ethanol, acetone, *N,N*-dimethylformamide and water as solvents has been applied for the separation of clavine and ergot alkaloids from seed extract of *Ipomoea violacea* L. and some authentic commercial samples.

INTRODUCTION

THE TECHNIQUES of separation, purification and identification of clavine alkaloids are of great interest. Chromatographic methods such as TLC and column chromatography have been used for the separation of different clavine and ergot alkaloids. For example, Niwaguchi *et al.*[1] described a column chromatographic method for the quantitative separation of lysergic acid amide and isolysergic acid amide on Celite 545 from seed extract of Heavenly Blue, a horticultural variety of *Ipomoea violacea* L.

Since the discovery of the dextran gel, Sephadex, by Porath and Flodin[2] gel filtration has been used increasingly in the separation of naturally occuring compounds differing in molecular size. Low molecular weight aromatic and heterocyclic compounds, however, can produce adsorption affects which do not conform exactly to theory. These adsorption affects can be used to separate substances where the difference in molecular weights is very small.

RESULTS

Using Sephadex LH-20 and Sephadex G-25 we have applied gel filtration for the separation of clavine and ergot alkaloids. A typical results is shown in Fig. 1, which demonstrates the separation of 6 ergot and clavine alkaloids on Sephadex LH-20 with 96% ethanol as an eluent.

We have also separated the alkaloids using *N,N*-dimethylformamide on Sephadex LH-20 but in this case ergocristine and ergotaminine were present in the same fraction as was also the case using acetone. Lysergic acid and ergometrine were well separated using water as eluent on Sephadex G-25 and the mixture of lysergic and isolysergic acid amide present in extracts of seeds of *I. violacea*, Heavenly Blue, were separated from other alkaloids by 96% ethanol on Sephadex LH-20.

DISCUSSION

Gel filtration on Sephadex LH-20 with 96% ethanol, acetone or *N,N*-dimethylformamide as eluents show that the clavine alkaloids are eluted according to molecular size.

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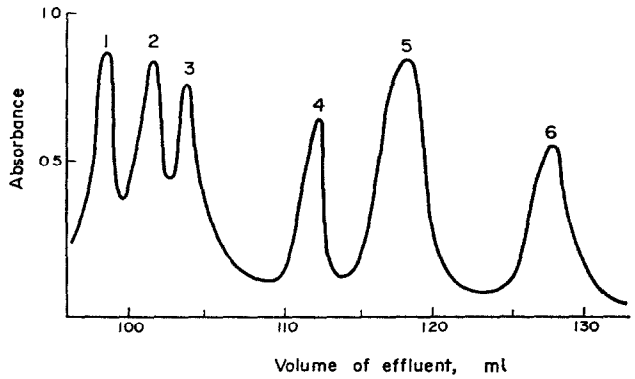

FIG. 1. SEPARATION OF ERGOT AND CLAVINE ALKALOIDS ON SEPHADEX LH-20 WITH 96% ETHANOL. Bed dimensions: 1 × 130 cm. Sample 3 ml containing 0·7–2·0 mg of each alkaloid. Flow rate 1 ml/10 min. 1—ergometrine; 2—ergocristine; 3—ergotaminine; 4—penniclavine; 5—elymoclavine; 6—agroclavine.

In contrast, ergot alkaloids separated on Sephadex LH-20 with 96% ethanol as eluent show slight retention and are not separated strictly according to molecular size. Apparently ergot alkaloids having aromatic rings interact with Sephadex LH-20, resulting in delayed elution. Thus, ergometrine (MW 325) with one ring elutes before ergotaminine (MW 581) and ergocristine (MW 609) which have two rings. None of the alkaloids tested were irreversibly adsorbed on the columns.

Ergot alkaloids on Sephadex LH-20 column with acetone or *N,N*-dimethylformamide do not show this adsorption effect and are separated according to molecular size.

EXPERIMENTAL

Seeds of Heavenly Blue a cultivar of *Ipomoea violacea* L. (25 g) were extracted according to the modified Hofmann method described by Marderosian.[3] Evaporated extracts were dissolved in 3–4 ml of 96% EtOH and applied directly to the Sephadex LH-20 column. Filtration experiments were carried also using Sephadex G-25.

Most of the experiments were performed on columns of about 1 × 100 cm. The dry gel was allowed to swell in appropriate solvents used as an eluent. The substances to be tested were dissolved in an appropriate eluent and put on the column in a volume of 2–3 ml. The concentration of the substances tested varied between 0·7–3·0 mg. All experiments were carried out in 7–21 hr at room temp. The flow rate was approximately 6–12 ml/hr. Fractions were analysed quantitatively using van-Urk reagent.

Fractionated alkaloids were identified by means of TLC on Silica Gel-G in a system $CHCl_3$–MeOH 17:3[4] except for ergotaminine and ergocristine which were determined in system $CHCl_3$–EtOH 9:1[5]. Elymoclavine and agroclavine after intensive UV irradiation give a yellow fluorescence. The plates were then sprayed with van-Urk reagent, and all substances gave a blue color except penniclavine which gave a green.

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Key Word Index—Ipomoea violacea; Convolvulaceae; ergot; alkaloids; lysergic acid; gel filtration.