Journal of Chromatography, 207 (1981) 139–144
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,489

ISOLATION AND SEPARATION OF NEW NATURAL LACTAM ALKALOIDS OF ERGOT BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

Separation of lactam derivatives of ergotoxine alkaloids, of which two were newly isolated from natural material, by high-performance liquid chromatography is described. The separation method utilizes LiChrosorb NH_2 as a stationary phase eluted with diethyl ether–ethanol (96:4) as a mobile phase. Alkaloids are detected with a UV detector. The proposed names for the studied lactam derivatives are ergocristam, ergocristinam, ergocornam, ergocorninam, ergocryptam and ergocryptinam.

INTRODUCTION

Natural ergot alkaloids of the peptide type with a cyclol structure were isolated in diastereomeric pairs either from ergot sclerotia of the parasitic fungus *Claviceps purpurea*, or from the mycelium of saprophytic strains of this fungus. One of the groups of cyclol ergot alkaloids (CEA) is composed of ergotoxine-type alkaloids with an isopropyl group in a C-2′ position. The compounds isolated from natural material are ergocristine[1], ergocornine[2] and ergocryptine[3,4], and their dextrorotatory enantiomers. In 1973 Stütz *et al.*[5] described the isolation of a new alkaloid from a lyophilized mycelium of the strain *C. purpurea* which, owing to the altered structure of its peptide moiety, did not belong to any of the above mentioned groups. It was the first representative of the non-cyclol (lactam) group of peptide ergot alkaloids (LEA) with a D-configuration of proline. The structure of this alkaloid corresponded to the cyclol alkaloid ergocristine (Fig. 1).

Isolation of an identical alkaloid from sclerotia of the ergocristine strain *C. purpurea* of a Czechoslovak production and preparation of its semisynthetic

* Presented at the *6th International Symposium "Advances and Application of Chromatography in Industry", Bratislava, September 16–19, 1980.*

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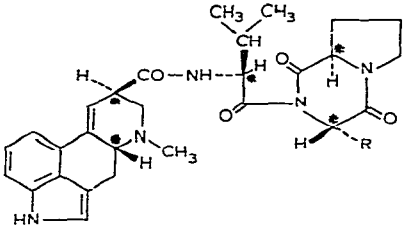

Fig. 1. Structural formula of non-cyclol lactam ergot alkaloids of the ergotoxine group. R = isopropyl (ergocornam), isobutyl (ergocryptam), benzyl (ergocristam).

derivatives was described by Černý *et al.*[6]. The new non-cyclol structure led Floss *et al.*[7] to conclusions concerning the biosynthesis of the peptide moiety of ergot alkaloids. They assumed an effect of a competitive reaction which brings about a reversal of configuration on the optically active carbon of L-proline to the D-configuration. The resulting compound cannot enter the biochemical reactions which lead to the formation of a cyclol structure. The sequence of the presumed biochemical reactions participating in the synthesis of ergocornine, ergocryptine and the corresponding non-cyclol alkaloids was dealt with by Floss *et al.*[7].

The non-cyclol derivatives ergocornine and ergocryptine were obtained during isolation of ergotoxine alkaloids from ergot of Czechoslovak production. Thin-layer chromatography of raw bases of the alkaloids yielded two new spots with properties different from those of the ergotoxine group alkaloids. The alkaloids exhibited properties similar to those of the previously described non-cyclol alkaloid, the structure of which corresponds to ergocristine. The structure of these alkaloids was confirmed by a nuclear magnetic resonance method[8].

High-performance liquid chromatography (HPLC) of ergotoxine ergot alkaloids has been performed by several researchers[9–11]. This study is based on results obtained in an analysis of CEA published earlier[12].

EXPERIMENTAL

Isolation of LEA

Processing of 500 kg of ergocornine–ergocryptine ergot of Czechoslovak production yielded 200 g of bases in which LEA were concentrated. Boiling in ethanol in the presence of phosphoric acid transformed alkaloids into dihydrophosphates of levorotatory forms. After a release of the bases by an aqueous solution of potassium hydrogen carbonate and an extraction into diethyl ether, the alkaloids were concentrated into a foam-like evaporation residue (135 g). Chromatography of this residue on a silica gel column (8.0 kg) using chloroform as the elution agent yielded 35 g of a concentrate containing *ca.* 20% of LEA. Repeated chromatography on a silica gel column (3.5 kg) using an elution mixture of chloroform with 1% methanol yielded, after fraction pooling, oily evaporation residues of N-[N(*d*-lysergyl)-L-valyl]-L-valyl-D-proline-lactam and N-[N(*d*-lysergyl)-L-valyl]-L-leucyl-D-proline-lactam. The proposed names for these compounds are ergocornam and ergocryptam. Precipitation of alkaloids by diethyl ether yielded 0.5 g of raw ergocryptam and 0.9 g of ergocornam. Both alkaloids were obtained in a pure form by repeated chromatography on a silica gel column (200 mg of ergocryptam, 400 mg of ergocornam).

Isolation of N-[N(*d*-lysergyl)-L-valyl]-L-phenylalanyl-D-proline-lactam (proposed name ergocristam) was performed according to Černý *et al.*[6].

Isomerization of LEA

N-[N(*d*-Isolysergyl)-L-valyl]-L-valyl-D-proline-lactam (ergocorninam), N-[N(*d*-isolysergyl)-L-valyl]-L-leucyl-D-proline-lactam (ergocryptinam) and N-[N(*d*-isolysergyl)-L-valyl]-phenylanyl-D-proline-lactam (ergocristinam) were prepared by diluting individual LEA in ethyl acetate acidified by 60% acetic acid at room temperature. After equilibration (*ca.* 5 h) individual solutions were extracted by water, concentrated and chromatographed on a silica gel column using chloroform as an elution agent.

High-performance liquid chromatography

Separation of the mixture of CEA and LEA was performed with diethyl ether (p.a.) and ethanol (UV-spectroscopic purity) from Lachema, Brno, Czechoslovakia, as mobile phases. Standards of all used compounds were prepared in Galena, Opava, Czechoslovakia.

Separation of a mixture of CEA and LEA was performed on a Varian 8500 high-performance liquid chromatograph. Detection was performed by a Variscan LC UV detector, and integration of peaks by a C 111 integrator (Varian Aerograph, Walnut Creek, CA, U.S.A.). The chromatographic column was 25 cm × 2 mm I.D. The stationary phase was silica gel modified by alkylamine, particle size 10 μm. The column was packed in the laboratory[13] with LiChrosorb NH_2 ($N_{agroclavine}$ = 1070). The column temperature was 20°C and the pressure 2.5 MPa. Elution was performed with diethyl ether–ethanol (96:4). The flow-rate of the mobile phase was 40 ml/h. The qualitative analysis of substances in the mixture was performed by comparing their elution volumes with those of pure reference substances.

RESULTS AND DISCUSSION

The separation of a mixture of LEA was performed using the results of an analysis of CEA by the HPLC method[12]. Differences in mutual interactions of the screened substances with a stationary and a mobile phase were sufficient for a satisfactory separation of individual components of the mixture of CEA and LEA during isocratic elution. Retention data of diastereomeric pairs of LEA and CEA are given in Table I.

The chromatographic behaviour of the studied substances is markedly influenced by the steric arrangement of the C-8 carbon of the ergolene nucleus. An intramolecular hydrogen bond, N-6···H—N-20, is formed in an axial position of the amide group of derivatives of isolysergic acid (-inines), bringing about a decrease in polarity of these derivatives. Interaction between the imine group of alkaloid and the amine group of the stationary phase decreases, -inines are retained and their elution volumes are smaller than those of -ines, in which the amide group is in an equatorial position.

This fact can be used for separation of biologically effective cyclol derivatives from isolysergic acid derivatives with a negligible biological activity. The same holds for the separation of the LEA diastereomeric pairs. The difference between retention volumes of the LEA isomers is more marked than that between the volumes of the

TABLE I

ELUTION DATA FOR LACTAM ERGOT ALKALOIDS AND CYCLOL ERGOT ALKALOIDS

$r_{t,s}$ = relative retention; k' = capacity factor.

CEA and LEA	*Molecular weight*	*Mobile phase diethyl ether–ethanol (96:4)*	
		$r_{t,s}$	k'
Ergocorninine	561.7	0.88	6.80
Ergocorninam	545.8	0.88	6.80
Ergocornine	561.7	1.36	11.00
Ergocornam	545.8	2.02	16.80
Ergocryptinine	575.7	0.77	5.80
Ergocryptinam	559.8	0.68	5.00
Ergocryptine	575.7	1.45	11.80
Ergocryptam	559.8	1.66	13.60
Ergocristinine	609.7	1.00	7.80
Ergocristinam	593.8	0.86	6.60
Ergocristine	609.7	1.61	13.20
Ergocristam	593.8	1.70	14.00
Retention volume of ergocristinine (ml)		3.12	

CEA isomers (Table I). With respect to the ergolene nucleus the axial position of the peptide is affected by the polarity of the hydroxyl group bound to C-12′. The CEA-inines are therefore more polar than the -inams. The elution rate of the CEA-inines is therefore, under the given conditions, considerably lower than that of the lactam derivatives. The elution rate difference is most clearly manifested with ergocristinine

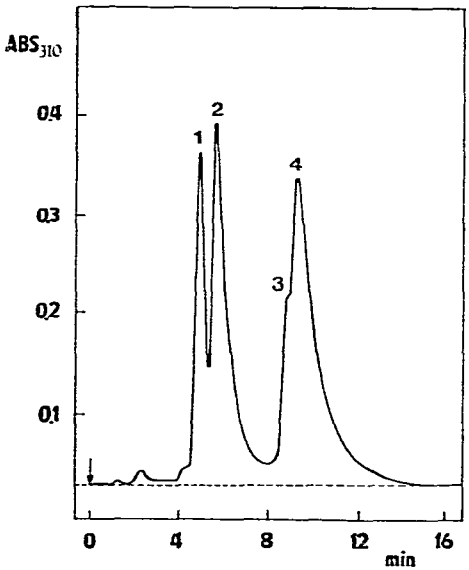

Fig. 2. Chromatogram of a mixture of diastereomeric pairs of ergocristine and ergocristam. Column, LiChrosorb NH_2; mobile phase, diethyl ether–ethanol (96:4); flow-rate of the mobile phase, 40 ml/h; pressure, 2.5 MPa; detector, Variscan UV at 310 nm. Peaks: 1 = ergocristinam; 2 = ergocristinine; 3 = ergocristine; 4 = ergocristam.

and ergocristinam. Separation of the mixture of ergocristine (or ergocristam), ergocristinine and ergocristinam poses no problems. On the other hand, ergocristine and ergocristam are practically inseparable (Fig. 2). In an equatorial arrangement of the CEA molecule (-ines) a hydrogen bond, 0-19···H···0-13′ is formed, not N-6···H—N-20. This explains why the CEA-ines are less polar than the LEA-ams, and why their retention volumes are smaller. The most marked difference between retention volumes was found with the ergocornine–ergocornam pair. Similarly, no problems are met in the separation of a mixture of ergocorninine (or ergocorninam), ergocornine and ergocornam. Retention volumes of ergocorninine and ergocorninam are identical (Fig. 3). The best separation of both pairs of alkaloids is obtained with a mixture of ergocryptine, ergocryptam, ergocryptinine and ergocryptinam, in which all four substances are readily separated (Fig. 4).

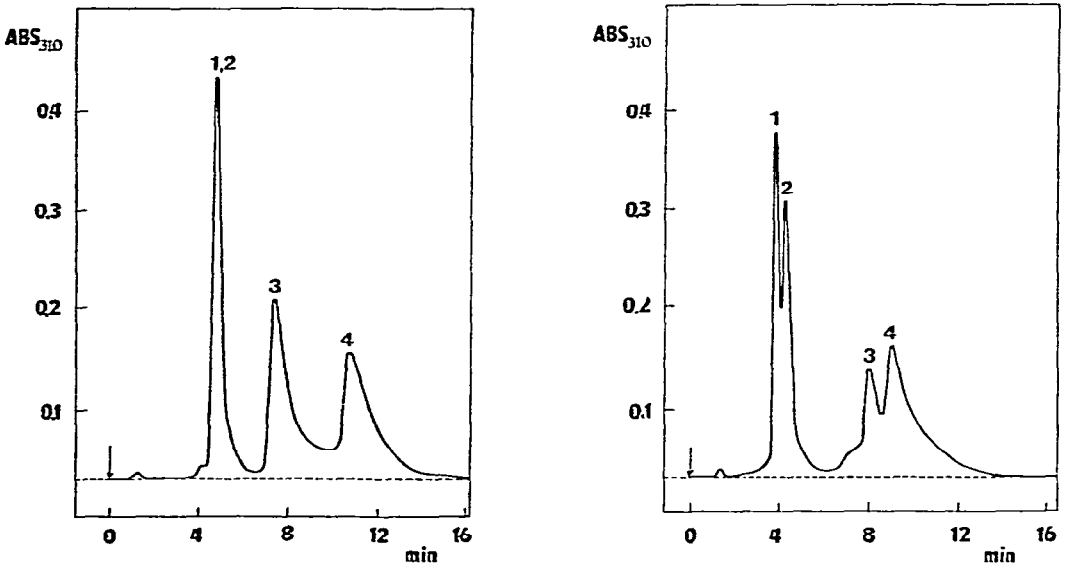

Fig. 3. Chromatogram of mixture of diastereomeric pairs of ergocornine and ergocornam. Column, LiChrosorb NH_2; mobile phase, diethyl ether–ethanol (96:4); flow-rate of the mobile phase, 40 ml/h; pressure, 2.5 MPa; detector, Variscan UV at 310 nm. Peaks: 1 and 2 = ergocorninam and ergocorninine; 3 = ergocornine; 4 = ergocornam.

Fig. 4. Chromatogram of mixture of diastereomeric pairs of ergocryptine and ergocryptam. Column, LiChrosorb NH_2; mobile phase, diethyl ether–ethanol (96:4); flow-rate of the mobile phase, 40 ml/h; pressure, 2.5 MPa; detector, Variscan UV at 310 nm. Peaks: 1 = ergocryptinam; 2 = ergocryptinine; 3 = ergocryptine; 4 = ergocryptam.

The chromatographic study of separation rendered possible the specification of conditions for the application of the HPLC method in the analysis of CEA in a mixture with LEA. The results could be used for a routine determination of the CEA/LEA mixture in natural materials, in the field of biogenesis of alkaloids, and in the control of pharmaceutical preparations.

The authors expect new lactam alkaloids corresponding to the remaining cyclol alkaloids to be found soon. Hence a new terminology is proposed in which the suffixes -ine or -inine for the cyclol alkaloids will be substituted by suffixes -am or -inam for the lactam alkaloids.

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