CHROM. 11,805

ANALYSIS OF ERGOT ALKALOIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

II. CYCLOL ALKALOIDS (ERGOPEPTINES)

M. WURST, M. FLIEGER and Z. ŘEHÁČEK

Institute of Microbiology, Czechoslovak Academy of Sciences, Prague 4 (Czechoslovakia)

SUMMARY

A high-performance liquid chromatographic method has been developed for the separation and quantitative analysis of a mixture of cyclol ergot alkaloids. The method utilizes silica gel treated with alkylamine (LiChrosorb NH_2) as the stationary phase, and isocratic and gradient elution with diethyl ether–ethanol as the mobile phase. Individual alkaloids are detected with a UV detector and determined using the method of internal normalization.

INTRODUCTION

Cyclol ergot alkaloids (CEA) are produced by the pyromycete *Claviceps purpurea*. The attention of pharmacologists is concentrated on cyclol derivatives of L-lysergic acid (designated by the suffix -ine), as derivatives of isolysergic acid (designated by the suffix -inine) are biologically almost inactive. Problems of the separation, isolation, identification and determination of these fungal metabolites have become more urgent with their extended clinical use.

For the analysis of ergot alkaloids (EA), chromatographic methods, mainly paper and thin-layer chromatography[1–14], were applied, utilizing either a spectrophotometric determination (*in situ*) on a thin layer[4,7–9] or measurement of the fluorescence of solutions obtained after extraction from chromatograms[5] and fluorodensitometric measurements[13]. The detection sensitivity can be increased by, *e.g.*, π-acceptors. Gas chromatography, either alone[15–19] or in combination with mass spectrometry[20–22], is less frequently applied in ergot studies, mainly because CEA already have a high molar mass, low vapour pressure and are not stable to heat. In addition, gas chromatography does not give satisfactory results when stereoisomers of lysergic acid are separated[20]. However, in combination with mass spectrometry it appears to be promising for the determination of degradation products of the peptide moiety of CEA[21,22].

Recently, high-performance liquid chromatography (HPLC) has been applied in the analysis of EA[23–33], followed by UV or fluorescence detection. In the HPLC

method, silica gel[23,24,32] and silica gel modified with alkylamine[31] have been used as the stationary phase and reversed phase[25–27,30,32,33], using both isocratic and gradient elution[28,29,31].

In this paper, which extends our previous work[31], an HPLC method is described for the separation and determination of a CEA mixture.

EXPERIMENTAL

Chloroform, isopropanol, diethyl ether and ethanol for UV spectroscopy (Lachema, Brno, Czechoslovakia, all of analytical-reagent grade) served as mobile phases for the separation of the CEA mixture. Reference CEA samples were obtained from Prof. H. G. Floss (Purdue University, Lafayette, Ind., U.S.A.), Prof. C. Spalla (Farmitalia, Milan, Italy) and Dr. E. Udvardy (Gedeon Richter Ltd., Budapest, Hungary).

Analyses of the CEA mixture were performed on a Varian 8500 liquid chromatograph, in which the concentration gradient of the mobile phase can be programmed. The instrument is equipped with a Variscan LC UV detector, A25 line recorder and 485 integrator (Varian Aerograph, Walnut Creek, Calif., U.S.A.). Chromatographic columns were 25 cm long with an I.D. of 2 mm. Silica gel (LiChrosorb Si 60, pore size 10 μm), modified with alkylamine served as the stationary phase. One column (A) was obtained from Varian (MicroPak NH$_2$; number of theoretical plates for agroclavine $N = 280$); the other (B) was packed in this laboratory (LiChrosorb NH$_2$, $N = 1070$; Applied Science Labs., State College, Pa., U.S.A.).

The temperature of the columns was 25°, the pressure on column A was 3.0–8.5 MPa (according to the system used and ratio of the components of the mobile phase), and that on column B was 1.5 MPa. Three solvent systems served as mobile phases: chloroform–isopropanol (90:10), diethyl ether–isopropanol (60:40) and diethyl ether–ethanol (84:16, 88:12 and 93:7). The flow-rates of the mobile phase during isocratic elution were 1 and 0.67 ml·min^{-1} on column A and B, respectively.

Gradient elution was performed with the system diethyl ether–ethanol. The separation was first run for 7 min with a constant ratio of the two components of the mobile phase (97.5:2.5), then the concentration of ethanol was increased at a rate of 0.3%·min^{-1} and the final stage of the separation proceeded at the final composition of the solvents, i.e. diethyl ether–ethanol (95:5). The flow-rate and pressure were the same as during isocratic elution on column B.

Qualitative analysis of CEA mixtures was performed by comparing the elution volumes of the components with those of reference CEA samples. The method of internal normalization was used for quantitative analysis. Peak areas were evaluated by using the integrator. Correction factors were calculated from values of the absorption coefficients (absorption coefficient of the analysed sample/absorption coefficient of the reference sample) obtained from UV spectra (Table I) measured in a Cary 118 C spectrometer (Varian Aerograph) with a wavelength range of 210–350 nm and a spectral bandwidth of 0.1 nm; the photometric accuracy at 1.0 absorbance was less than 0.001 absorbance. The cuvettes were 1 cm wide. All spectra were measured in the system diethyl ether–ethanol (80:20) at 25°. The concentration of the measured solutions varied within the range $1 \cdot 10^{-6}$–$2 \cdot 10^{-5}$ g·ml^{-1}. In this range, a linear relationship was found between absorbance and CEA concentration in all instances.

TABLE I

SPECIFIC DECADIC ABSORPTION COEFFICIENTS OF CYCLOL ERGOT ALKALOIDS

Alkaloid	Absorption coefficient				
	225 nm	240 nm	254 nm	282 nm	310 nm
Ergocryptine	343	320	165	66	143
Ergocryptinine	332	342	225	57	151
Ergocornine	325	304	153	59	134
Ergocorninine	409	420	277	73	188
Ergocristine	365	318	161	61	139
Ergocristinine	301	295	197	51	134
Ergosine	335	345	228	59	154
Ergosinine	286	288	184	55	126
Ergostine	426	361	186	73	159
Ergostinine	343	323	208	56	140
Ergotamine	394	329	172	67	139
Ergotaminine	303	292	187	52	130
Ergobasine	507	502	258	103	232
Ergobasinine	508	569	352	108	257
Ergine	786	786	385	157	363
Erginine	673	730	449	138	329
Agroclavine*	1175	130	102	302	3.1

* Reference sample.

RESULTS AND DISCUSSION

Satisfactory results were obtained when analysing clavines and simple derivatives of lysergic acid on silica gel modified with alkylamine as the stationary phase. Therefore, separations were carried out on both columns with the same stationary phase: MicroPak NH_2 and LiChrosorb NH_2. According to our previous experience with the mobile phases used, the best separation of the CEA mixture was obtained with the system diethyl ether–ethanol (Table II). This solvent mixture makes it possible to make measurements within the whole range of absorption maxima of all EA, particularly clavines, gives relatively stable solutions and is hence suitable for use in quantitative analysis. The isocratic elution of CEA with diethyl ether–ethanol as the mobile phase yielded good results. The optimal separation of the CEA mixture was obtained with ratios of these components of 88:12 and 93:7 on columns A and B, respectively. Column B (packed in this laboratory) was three times more effective for agroclavine than column A (obtained commercially). A composition of the mobile phase of 84:16 is given in Table II for comparison of the elution data of CEA with the elution values of clavines and simple derivatives of lysergic acid.

The chromatographic separation of CEA stereoisomers is made possible probably owing to the steric arrangement of the amide group and hydrogen at position C-8 of the ergolene ring. A change of configuration at position C-8 influences most pronouncedly their elution volumes (Fig. 1). The elution volumes of "-ines" are substantially higher than those of "-inines". It follows that the amide group of "-ines" is in the equatorial position with respect to the D-ring of the ergolene nucleus, whereas in the "-inines" its position is axial and is stabilized by an intramolecular hydrogen

TABLE II

RELATIVE RETENTIONS OF CYCLOL ERGOT ALKALOIDS

Column: (A) MicroPak NH$_2$; (B) LiChrosorb NH$_2$. Detection: UV Variscan LC detector; wavelength 310 nm. Flow-rate: (A) 1 ml $\cdot$ min^{-1}; (B) 0.67 ml $\cdot$ min^{-1}. Pressure: (A) 3.0–8.5 MPa; (B) 1.5 MPa.

| Alkaloid	Molar mass	Mobile phase				
		Diethyl ether–ethanol			Diethyl ether–isopropanol, 60:40(A)	Chloroform–isopropanol, 90:10(A)
		93:7(B)	88:12(A)	84:16(A)		
Ergocryptine	575.7	2.82	0.50	0.47	0.31	0.13
Ergocryptinine	575.7	1.59	0.25	0.21	0.14	0.03
Ergocornine	561.7	3.06	0.64	0.56	0.32	0.12
Ergocorninine	561.7	2.00	0.34	0.30	0.18	0.05
Ergocristine	609.7	3.76	0.89	0.78	0.45	0.15
Ergocristinine	609.7	2.35	0.45	0.38	0.26	0.04
Ergostine	595.7	7.38	1.85	1.30	0.82	0.33
Ergostinine	595.7	3.15	0.75	0.57	0.37	0.05
Ergosine	547.6	8.47	1.83	1.21	0.71	0.47
Ergosinine	547.6	3.09	0.63	0.46	0.28	0.07
Ergotamine	581.7	12.44	2.25	1.94	1.08	0.53
Ergotaminine	581.7	4.56	0.94	0.88	0.52	0.07
Ergobasine	325.4	12.82	5.12	4.16	2.55	4.93
Ergobasinine	325.4	3.82	1.05	1.00	0.57	0.98
Ergine	267.3	—	—	2.90	1.85	4.00
Erginine	267.3	—	—	0.83	0.64	0.58
8-Hydroxy-ergotamine	597.7	8.88	—	—	—	—
Agroclavine	238.3	1.00	1.00	1.00	1.00	1.00
Retention volume of agroclavine (ml)	—	1.10	6.00	4.67	3.50	2.67
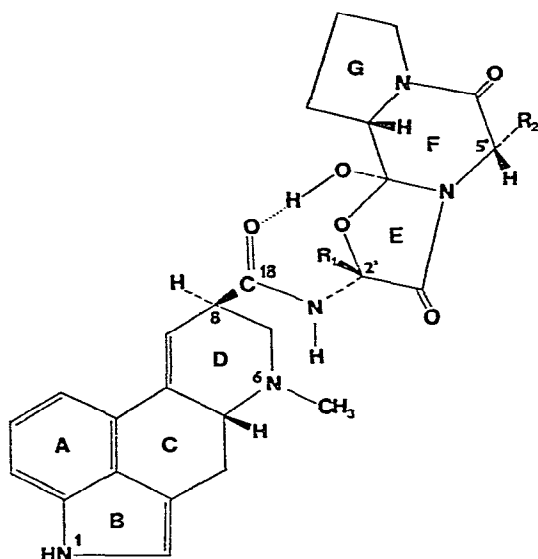
Fig. 1. Structure of cyclol ergot alkaloids.

bond between the basic nitrogen in position N-6 and the hydrogen of the amide group in position C-18.

Aromatic or aliphatic groups (benzyl, isobutyl and isopropyl) at position C-5', together with an alkyl group (methyl, ethyl and isopropyl) at position C-2', bound to the cyclic peptide of CEA influence considerably the elution volumes (Fig. 1). The elution volumes of CEA with a benzyl group at position C-5', together with an alkyl group at position C-2', are higher than those of CEA with alkyl groups in both positions. Free π-electrons of the benzene nucleus play a role in the interaction between the analysed compound and the stationary phase. The smaller the alkyl group in position C-2', the higher are the elution volumes. In the series ergocristine, ergostine ergotamine, the elution volume increases with decreasing molar mass (Table II).

CEA with aliphatic groups at positions C-2' and C-5' have elution volumes lower than those of CEA with a single aromatic group at position C-5' and it also holds here that the larger the alkyl groups in both positions the lower are the elution volumes. Substitution of the aromatic group by a branched alkyl group at position C-5' and of the methyl group at position C-2' by ethyl and especially isopropyl produces greater steric hindrance on the peptide moiety of the alkaloids for interaction with the stationary phase. In the series ergosine, ergocornine, ergocryptine, the elution volume decreases with increasing molar mass (Table II).

The elution volume of 8-hydroxyergotamine, which has a tertiary hydroxyl group at position C-8, is lower than that of ergotamine, which has only hydrogen at position C-8 (Table II, Fig. 1). The effect of the hydrogen bond between the
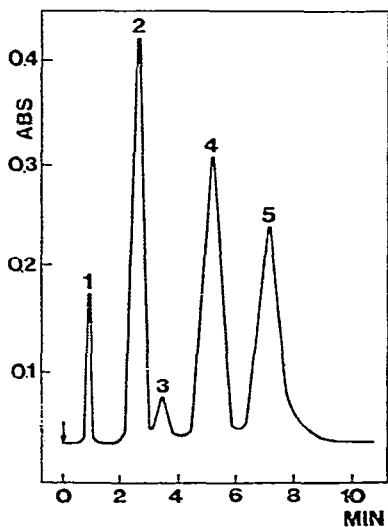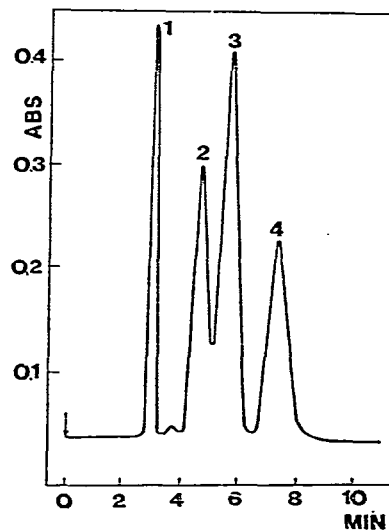

Fig. 2. Chromatogram of a mixture of cyclol ergot alkaloids of the ergotamine type. Column, LiChrosorb NH₂. Mobile phase, diethyl ether–ethanol (93:7). Flow-rate of mobile phase, 0.67 ml · min⁻¹. Pressure, 1.5 MPa. Detector, Variscan UV at 310 nm. Peaks: 1 = solvent; 2 = ergosinine; = ergotaminine; 4 = ergosine; 5 = ergotamine.

Fig. 3. Chromatogram of a mixture of cyclol ergot alkaloids of the ergotoxine type. Column, LiChrosorb NH₂. Mobile phase, diethyl ether–ethanol (97.5:2.5). Flow-rate of mobile phase, 0.67 ml · min⁻¹. Pressure, 1.5 MPa. Detector, Variscan UV at 310 nm. Peaks: 1 = ergocryptinine; 2 = ergocristinine; 3 = ergocryptine; 4 = ergocristine.

oxygen atom of the carbonyl group at position C-18 and the hydrogen atom of the hydroxyl group at position C-8 is involved here. Therefore, the sorption of 8-hydroxy-ergotamine is less pronounced than that of ergotamine. The same also holds true for the sorption of setoclavine and lysergine[31]. In simple derivatives of lysergic acid, e.g., ergine, the elution volume is influenced by the primary amino group at position C-18 and in ergobasine by the primary hydroxyl group on isopropyl in the same position (Fig. 1, Table II).

The analysis of CEA at the optimal ratio of the components of the diethyl ether–ethanol mobile phase (93:7) made it possible to separate very well CEA stereo-isomers and mixtures of the ergotamine type (Fig. 2). CEA mixtures of the ergotoxine type were separated best in the system diethyl ether–ethanol (97.5:2.5) (Fig. 3). Elution with a programmed concentration gradient of the mobile phase was more advantageous for a CEA mixture than isocratic elution. Under these conditions it was
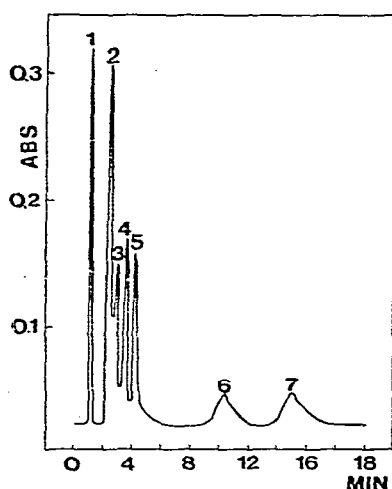

Fig. 4. Chromatogram of mixture of cyclol ergot alkaloids. Column, LiChrosorb NH_2. Gradient elution: 7 min diethyl ether–ethanol (97.5:2.5), 10 min with increasing ethanol concentration at 0.3% · min^{-1}, 5 min with diethyl ether–ethanol (95:5). Flow-rate of mobile phase, 0.67 ml · min^{-1}. Pressure, 1.5 MPa. Detector, Variscan UV at 310 nm. Peaks: 1 = solvent; 2 = ergocorninine; 3 = ergocristinine; 4 = ergocornine; 5 = ergocristine; 6 = ergosine; 7 = ergotamine.

TABLE III

DETERMINATION OF MIXTURES OF CYCLOL ERGOT ALKALOIDS USING THE HPLC METHOD

Alkaloid	Amount (wt.-%)		Standard deviation	
	Weighed	Determined*	Absolute	Relative
Ergocorninine	11.8	10.8	0.21	1.94
Ergocristinine	27.8	27.9	0.32	1.15
Ergocornine	21.1	22.9	0.56	2.45
Ergocristine	13.7	12.8	0.39	3.05
Ergotamine	25.6	25.6	0.52	2.03

* A total of 8 determinations were performed.

possible to analyse simultaneously CEA of both the ergotamine and ergotoxine types. Programmed elution of the mobile phase was performed with the same solvent mixture as was used in isocratic elution. An increase in the ethanol content from 2.5% to 5% gave good results (Fig. 4).

The method was verified with a mixture of standard CEA and the results are presented in Table III. Deviations of the values determined for individual CEA varied within the range commonly observed in most chromatographic analyses.

The method described is suitable for the determination of ergot alkaloids in fermentation media with emerged and submerged cultures of *Claviceps purpurea*, *C. paspali*, *Aspergillus flavus*, *Penicillium* sp., etc., and for determination of these alkaloids in natural materials and in semi-synthetic and pharmaceutical commercial preparations.

REFERENCES

1 J. Kolsek, *Microchim. Acta*, 10 (1956) 1500.
2 J. L. McLaughlin, J. E. Goyan and A. G. Paul, *J. Pharm. Sci.*, 53 (1964) 306.
3 S. Agurell, *Acta Pharm. Suecica*, 2 (1965) 357.
4 K. Genest, *J. Chromatogr.*, 19 (1965) 531.
5 L. A. Dal Cortivo, J. R. Broich, A. Dihrberg and B. Newman, *Anal. Chem.*, 38 (1966) 1959.
6 A. Kornhauser, M. Perpar and L. Gasperut, *Arch. Pharm. (Weinheim)*, 303 (1970) 882.
7 A. Cavallaro, G. Rossi and G. Elli, *Boll. Lab. Chim. Prov.*, 21 (1970) 303.
8 T. Niwaguchi and T. Inoue, *J. Chromatogr.*, 59 (1971) 127.
9 S. Keipert and R. Voigt, *J. Chromatogr.*, 64 (1972) 327.
10 R. Fowler, P. J. Gomm and D. A. Patterson, *J. Chromatogr.*, 72 (1972) 351.
11 J. Reichelt and S. Kudrnáč, *J. Chromatogr.*, 87 (1973) 433.
12 E. Eckart and S. Walter, *Planta Med.*, 27 (1975) 58.
13 M. Prošek, E. Kučan, M. Katič and M. Bano, *Chromatographia*, 9 (1976) 273 and 325; 10 (1977) 147.
14 G. Rücker and A. Taha, *J. Chromatogr.*, 132 (1977) 165.
15 C. A. Radecka and J. C. Nigam, *J. Pharm. Sci.*, 55 (1966) 861.
16 M. Lerner, *Bull. Narcot.*, 19 (1967) 39.
17 A. R. Sperling, *J. Chromatogr. Sci.*, 12 (1974) 265.
18 K. D. Barrow and F. R. Quigley, *J. Chromatogr.*, 105 (1975) 393.
19 G. Szepesi and M. Gazdag, *J. Chromatogr.*, 122 (1976) 479.
20 S. Agurell and A. Ohlsson, *J. Chromatogr.*, 61 (1971) 339.
21 J. W. F. van Mausvelt, J. E. Greving and R. A. De Zeeuw, *J. Chromatogr.*, 151 (1978) 113.
22 T. A. Plomp, J. G. Leferink and R. A. A. Maes, *J. Chromatogr.*, 151 (1978) 121.
23 J. D. Wittwer Jr. and J. H. Kluckholm, *J. Chromatogr. Sci.*, 11 (1973) 1.
24 R. A. Heacock, K. R. Langille, J. D. MacNeil and R. W. Frei, *J. Chromatogr.*, 77 (1973) 425.
25 I. Jane and B. B. Wheals, *J. Chromatogr.*, 84 (1973) 181.
26 R. V. Vivilecchia, R. L. Cotter, R. J. Limpert, N. Z. Thimot and J. N. Little, *J. Chromatogr.*, 99 (1974) 407.
27 J. Christie, M. W. White and J. M. Wiles, *J. Chromatogr.*, 120 (1976) 496.
28 H. Bethke, B. Delz and K. Stich, *J. Chromatogr.*, 123 (1976) 193.
29 F. Erni, R. W. Frei and W. Lindner, *J. Chromatogr.*, 125 (1976) 265.
30 J. Dolinar, *Chromatographia*, 10 (1977) 364.
31 M. Wurst, M. Flieger and Z. Řeháček, *J. Chromatogr.*, 150 (1978) 477.
32 L. Szepesy, I. Fehér, G. Szepesi and M. Gazdag, *J. Chromatogr.*, 149 (1978) 271.
33 V. Hartmann, M. Rödniger, W. Ableidinger and H. Bethke, *J. Pharm. Sci.*, 67 (1978) 98.