Journal of Chromatography, 150 (1978) 477–483

CHROM. 10,481

ANALYSIS OF ERGOT ALKALOIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

I. CLAVINES AND SIMPLE DERIVATIVES OF LYSERGIC ACID

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(First received April 26th, 1977; revised manuscript received July 6th, 1977)

SUMMARY

A method of high-performance liquid chromatography has been developed for the separation and quantitative analysis of a mixture of ergot alkaloids on Micro-Pak NH_2 columns using isocratic and gradient elution. The mobile phase is diethyl ether–ethanol. Ultraviolet detection is employed at various wavelengths, and the ergot alkaloids are determined using the method of internal normalization.

INTRODUCTION

Ergot alkaloids (EA) are produced predominantly by the fungus *Claviceps*. More than forty different EA have already been isolated from this pyromycete[1–9]. For practical utilization of EA, a series of methods have been developed for their separation and determination, *e.g.*, thin-layer chromatography[10,11], spectrophotometric determination on a thin layer[12–15] and fluorescence measurement for individual compounds eluted from chromatograms[16]. Gas chromatography has also been used in certain cases, either alone[17–19] or in combination with mass spectrometry[20]. Only preliminary studies of the use of high-performance liquid chromatography (HPLC) for the separation and quantitative analysis of EA have been carried out so far[21,22]. It was the aim of the present paper to develop a rapid and sensitive method for the separation and determination of a mixture of clavine alkaloids and simple derivatives of lysergic acid.

EXPERIMENTAL

The following solvents were used for the separation of mixtures of EA: chloroform, diethyl ether, isopropanol and ethanol (for UV spectroscopy). All of the compounds were of analytical grade (Lachema, Brno, Czechoslovakia). Reference samples of EA were prepared by fraction crystallization of mixtures of EA extracted from cultivation media of *Claviceps purpurea* 129 (CCIM)[23], or were obtained from the laboratories of Professor M. Abe (Tokyo University of Education), Professor

H. G. Floss (Purdue University, Lafayette) and Professor C. Spalla (Farmitalia, Milan).

Analyses were performed on a Varian 8500 liquid chromatograph, in which gradient elution is possible, equipped with a Variscan LC UV detector, A 25 line recorder and 485 integrator (Varian Aerograph, Walnut Creek, Calif., U.S.A.). The chromatographic column (25 cm × 2 mm I.D.) was packed with Lichrosorb Si-60 (particle size, 10 μm) modified by alkylamine (MicroPak NH_2). The column temperature was 25°, and the pressure was 3.0–8.5 MPa (varied according to the type of column, system and composition of the mobile phase).

Three solvent systems served as mobile phases: (a) chloroform–isopropanol (90:10, 80:20); (b) diethyl ether–isopropanol (70:30, 60:40); (c) diethyl ether–ethanol (84:16, 80:20). The flow-rate of the mobile phase during isocratic elution was 1 ml/min. Gradient elution was performed in the system diethyl ether–ethanol (79:21). The separation proceeded first for 15 min in the above system; the concentration of ethanol was then increased at a rate of 4%/min for 2 min and the remaining separation continued at the final composition of the solvents (71:29). The flow-rate was 0.2 ml/min and the pressure on the column was 0.5 MPa.

Individual components in the EA mixture were identified by comparing their elution volumes with the elution volumes of reference samples of EA. The method of internal normalization was used for the quantitative analysis. The integrated area of a compound was corrected by a factor chosen as the ratio of the specific decadic absorption coefficient of the compound to the specific decadic absorption coefficient of the reference compound, agroclavine. This procedure was always applied when the individual peaks were sufficiently separated, so that the integrated areas did not influence each other. When two peaks were poorly separated during the analysis the sample was examined at two or more wavelengths. The relative concentrations of the individual components were calculated by means of a system of linear equations. Correction factors were determined by means of UV spectra measured on a Cary 118 C spectrophotometer (Varian Aerograph). Parameters of the instrument were as follows: wavelength range, 210–350 nm; spectral band width, 0.1 nm; photometric accuracy at 1.0 absorbance, less than 0.001 absorbance; width of cuvettes, 1 cm. All of the spectra were measured in the system diethyl ether–ethanol (80:20) at 25°, and the concentration of the measured solutions was $1 \cdot 10^{-6}$–$2 \cdot 10^{-5}$ g/ml. Within the examined range, a linear relation was found between absorbance and EA concentration in all cases.

RESULTS AND DISCUSSION

The separation of a mixture of EA was performed satisfactorily on a MicroPak NH_2 column. The elution volumes of EA are presented in Table I. The best separation was obtained in the system diethyl ether–ethanol (84:16). A decrease in the concentration of the polar component of the mobile phase influenced only polar compounds. The gradient elution was found to be more useful than the isocratic procedure. Under the conditions of gradient elution it was possible to analyze simultaneously groups of polar and non-polar compounds. It follows from Fig. 1 that a suitable combination of the concentration of the polar compound ethanol in the mobile phase with the flow-rate leads to a good separation of the individual EA.

TABLE I

RELATIVE RETENTIONS OF CLAVINE ALKALOIDS AND SIMPLE DERIVATIVES OF LYSERGIC ACID

Column: MicroPak NH_2; particle size, 10 μm; 25 cm × 2.0 mm I.D. Detection: Variscan LC UV detector; wavelength, 225 (240) nm. Flow-rate: 1 ml/min. Pressure: 3.0–8.5 MPa.

Alkaloid	*Solvent system*					
	Diethyl ether–ethanol		*Diethyl ether–isopropanol*		*Chloroform–isopropanol*	
	(84:16)	*(80:20)*	*(70:30)*	*(60:40)*	*(90:10)*	*(80:20)*
Paspaclavine	0.11	0.11	0.11	0.14	0.12	0.12
Isosetoclavine	0.25	0.26	0.27	0.26	0.70	0.70
Lysergene	0.38	0.39	0.46	0.45	0.58	0.63
Setoclavine	0.55	0.61	0.67	0.67	0.98	1.05
Erginine	0.83	0.80	0.69	0.64	0.58	0.55
Lysergine	0.88	0.87	1.20	1.14	1.24	1.30
Agroclavine	1.00	1.00	1.00	1.00	1.00	1.00
Pyroclavine	1.45	1.49	1.80	1.63	1.65	1.71
Festuclavine	2.08	1.98	2.37	2.01	1.46	1.47
Penniclavine	2.80	2.65	2.97	2.72	5.05	3.85
Ergine	2.90	2.51	2.29	1.85	4.00	2.95
Paliclavine	3.12	2.25	1.66	1.26	3.85	2.99
Elymoclavine	3.22	2.90	3.17	2.72	4.48	3.05
Lysergol	3.50	2.99	3.48	2.83	5.80	4.30
Chanoclavine	8.00	6.03	8.56	6.33	9.83	6.03
Retention volume of agroclavine (ml)	4.67	3.00	4.90	3.50	2.67	1.90

The detection of samples with a UV detector at several wavelengths was found to be advantageous for qualitative analysis. This is demonstrated in Fig. 1 which illustrates the analysis of a mixture of EA at 225, 240 and 254 nm. In the analysis of a mixture containing mainly agroclavine (8), the peaks of lysergine (6) and erginine (7) overlapped at 225 nm [in certain cases, also with pyroclavine (9)] with the peak of agroclavine, which made it impossible to detect minor components. In this case it was useful to use a wavelength of 310 nm, at which agroclavine does not absorb (see Table II). The native fluorescence[22] of certain EA was not utilized for detection, since no suitable fluorescence detector was available and a number of EA do not exhibit fluorescence (see Table II).

Compounds containing hydroxyl groups in the side chain of the tetra- or tricyclic nucleus of EA (lysergol, elymoclavine, penniclavine, chanoclavine and paliclavine) had much larger elution volumes. This effect was most pronounced in the case of compounds containing a primary hydroxyl group (lysergol, elymoclavine and chanoclavine) and was most obvious in the system of lowest polarity (chloroform–isopropanol) due to a greater interaction between the stationary phase and the compound. In the case of a secondary hydroxyl group (paliclavine) the increase in the elution volume was less pronounced. Compounds containing tertiary hydroxyl groups (setoclavine and isosetoclavine) had lower elution volumes. This was most obvious in the most polar system (diethyl ether–ethanol). Intramolecular interactions between the primary and tertiary hydroxyl groups were most pronounced in the most

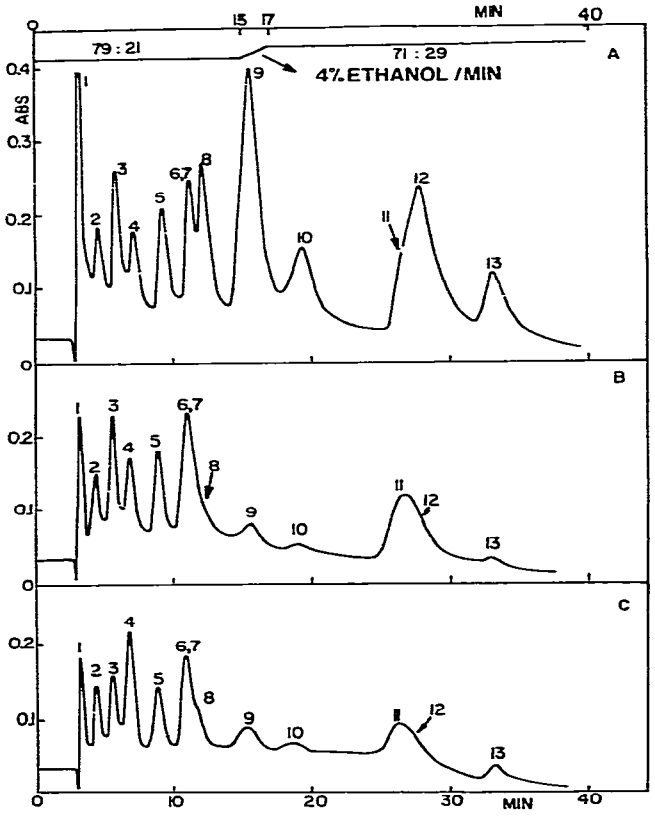

Fig. 1. Chromatogram of a mixture of clavine alkaloids and simple derivatives of lysergic acid. Column, MicroPak NH_2. Mobile phase, diethyl ether–ethanol. Gradient elution: 15 min with diethyl ether–ethanol (79:21), 2 min with increasing ethanol concentration at 4%/min, 30 min with diethyl ether–ethanol (71:29). Flow-rate of the mobile phase, 0.2 ml/min. Pressure, 0.5 MPa. Detector, Variscan UV at 225 (A), 240 (B) and 254 (C) nm. Peaks: 1, 2 = solvent; 3 = isosetoclavine; 4 = lysergene; 5 = setoclavine; 6 = lysergine; 7 = erginine; 8 = agroclavine; 9 = pyroclavine; 10 = festuclavine; 11 = ergine; 12 = elymoclavine; 13 = chanoclavine.

polar system. Therefore, the elution volume of penniclavine was always smaller than the elution volumes of EA containing a single hydroxyl group (*e.g.*, elymoclavine). The elution volume of penniclavine increased markedly with decreasing polarity of the systems, almost by a factor of two in the least polar system. However, even in this case, binding with hydrogen bridges was involved.

Steric isomers (setoclavine and isosetoclavine) were separated similarly in all of the three systems. The elution volume of isosetoclavine was always smaller than that of setoclavine. In the least polar system the elution volumes of both isomers were close to that of agroclavine, and were always greater in more polar systems, in agreement with the more pronounced effect of the stationary phase on the hydroxyl groups of both isomers. The separation of pyroclavine and festuclavine was slightly different. In more polar systems, pyroclavine was eluted before festuclavine. The contrary

TABLE II

SPECIFIC DECADIC ABSORPTION COEFFICIENTS OF CLAVINE ALKALOIDS AND SIMPLE DERIVATIVES OF LYSERGIC ACID

The UV spectra of pyroclavine could not be measured since the amount of the pure reference compound was sufficient only for comparative analysis.

Alkaloid	*Absorption coefficient at indicated wavelength (nm)*				
	225	*240*	*254*	*282*	*310*
Paspaclavine*	993	71	64	227	1.8
Isosetoclavine	767	810	381	169	370
Lysergene	1290	1523	1566	846	867
Setoclavine	845	806	393	138	350
Erginine	673	730	449	138	329
Lysergine	1065	980	503	220	452
Agroclavine*	1175	130	102	302	3.1
Pyroclavine*	—	—	—	—	—
Festuclavine*	1290	92	92	279	2.1
Panniclavine	795	777	425	141	344
Ergine	786	786	385	157	363
Paliclavine*	1157	107	88	268	4.6
Elymoclavine*	983	110	83	267	12
Lysergol	845	826	433	177	385
Chanoclavine*	1560	148	140	382	18

* This compound does not exhibit a natural fluorescence.

could be observed in the least polar phase. The elution volumes of ergine and erginine differed considerably. The difference was most pronounced in the least polar system due to the presence of the highly polar amidic group.

In the case of the mixture of agroclavine and lysergine (isomers differing in the position of the double bond on the ergoline ring), lysergine was eluted before agroclavine when separation took place in the most polar system. In less polar systems the elution volume of agroclavine was smaller than that of lysergine. The separation of the pair elymoclavine–lysergol was roughly similar in both polar systems. The elution volume of lysergol was always larger than that of elymoclavine. The difference between the elution volumes of the isomers was most pronounced in the least polar system (effect of the stationary phase on the hydroxyl groups of both isomers).

The determination of a mixture of EA was demonstrated on an artificially prepared mixture. During the analysis, the peaks of erginine, agroclavine, elymoclavine and ergine were separated only partially. Therefore, ergine and erginine were detected at 310 nm, the absorption of agroclavine and elymoclavine being negligible at this wavelength. Corrected areas for all the four compounds were calculated from integrated areas measured at 282 nm, together with the values for other EA, and were verified at 225 and 240 nm. The values obtained in this way were used to calculate the arithmetic means for the individual components of the mixture and the results obtained gave an indication of their relative occurrence (Table III). Slightly higher errors of the determined values of some clavines were caused by manual control of the integrator (human error), since automatic integration could not be used in this case. More satisfactory results could be obtained by using a more advanced integrator of a new type.

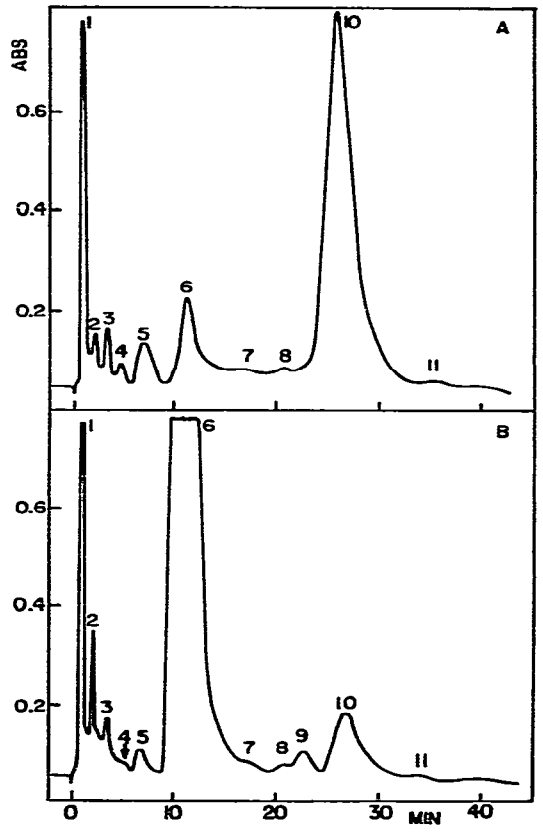

Fig. 2. Chromatogram of a mixture of clavine alkaloids produced by the submersed culture of *Claviceps purpurae* 129: (A) cultivation in the 10-l volume of the laboratory fermentation tank; (B) cultivation in a 300 ml flask. Detector, Variscan UV at 225 nm. Other details as in Fig. 1. Peaks: 1, 2 = solvent; 3 = isosetoclavine; 4 = lysergene; 5 = setoclavine; 6 = agroclavine; 7 = pyroclavine; 8 = festuclavine; 9 = ergine; 10 = elymoclavine; 11 = chanoclavine.

TABLE III

DETERMINATION OF A MIXTURE OF CLAVINE ALKALOIDS AND SIMPLE DERIVATIVES OF LYSERGIC ACID

Alkaloid	*wt. %*		*Standard deviation*	
	weighed	*determined**	*absolute*	*relative*
Paspaclavine	13.00	12.2	0.23	1.9
Setoclavine	10.87	9.9	0.50	5.0
Erginine	14.73	14.9	0.79	5.3
Agroclavine	12.74	13.9	0.60	4.3
Festuclavine	16.27	17.2	0.66	3.8
Ergine	14.97	14.1	0.57	4.0
Elymoclavine	17.42	17.8	1.42	7.9

* A total of 8 determinations was performed.

CONCLUSION

Conditions for application of the HPLC method to the separation and determination of a mixture of EA have been described. The results obtained indicate that the method can be applied to the routine determination of EA in natural material and in chemically synthesized preparations (Fig. 2), and to the determination of EA in pharmaceutical drugs.

REFERENCES

1 E. Ramstad, *Lloydia*, 31 (1968) 327.
2 A. J. Birch, B. J. McLoughlin and H. Smith, *Tetrahedron Lett.*, (1960) 1.
3 D. Gröger, K. Mothes, H. Simon, H. G. Floss and F. Weygand, *Z. Naturforsch.*, 15b (1960) 141.
4 E. M. Taylor and E. Ramstad, *Nature (London)*, 188 (1960) 494.
5 V. E. Tyler, Jr., *J. Pharm. Sci.*, 50 (1961) 629.
6 Z. Řeháček, *Biologické listy*, 38 (1973) 213.
7 R. B. Woodward, *Angew. Chem.*, 68 (1956) 13.
8 E. Wenkert, *Experientia*, 15 (1959) 165.
9 L. D. BuLock and J. G. Barr, *Lloydia*, 31 (1968) 342.
10 S. Agurell, *Acta Pharm. Suecica*, 2 (1965) 357.
11 A. Kornhauser, M. Perpar and L. Gasperut, *Arch. Pharm. (Weinheim)*, 303 (1970) 882.
12 K. Genest, *J. Chromatogr.*, 19 (1965) 531.
13 T. Niwaguchi and T. Inoue, *J. Chromatogr.*, 59 (1971) 127.
14 S. Keipert and R. Voigt, *J. Chromatogr.*, 64 (1972) 327.
15 A. Cavallaro, G. Rossi and G. Elli, *Boll. Lab. Chim. Prov.*, 21 (1970) 303.
16 L. A. Dal Cortivo, J. R. Broich, A. Dihrberg and B. Newman, *Anal. Chem.*, 38 (1966) 1959.
17 C. A. Radecka and J. C. Nigam, *J. Pharm. Sci.*, 55 (1966) 861.
18 M. Lerner, *Bull. Narcotics*, 19 (1967) 39.
19 K. D. Barrow and F. R. Quigley, *J. Chromatogr.*, 105 (1975) 393.
20 S. Agurell and A. Ohlsson, *J. Chromatogr.*, 61 (1971) 339.
21 J. Wittwer and J. H. Kluckhohn, *ISA Analysis Instrumentation Symposium, Cleveland, 1972*, Paper No. 192.
22 R. A. Heacock, K. R. Langille, J. D. MacNeil and R. W. Frei, *J. Chromatogr.*, 77 (1973) 425.
23 Z. Řeháček, J. D. Desai, P. Sajdl and S. Pažoutová, *Can. J. Microbiol.*, 23 (1977) 596.