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IDENTIFICATION OF ERGOT-PEPTIDE ALKALOIDS, BASED ON GAS–LIQUID CHROMATOGRAPHY OF THE PEPTIDE MOIETY

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SUMMARY

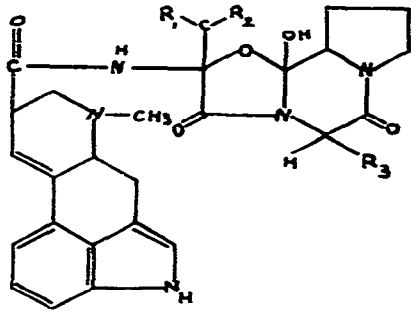
A simple and rapid method for the identification of ergot-peptide alkaloids is described. At temperatures around 300° instantaneous degradation of the free alkaloids occurs in the injection port of the gas chromatograph, each alkaloid yielding a specific set of peptide degradation products, which are subsequently separated on a SE-30 column. Since the lysergic acid moiety cannot be seen in the gas chromatogram, the separation of alkaloids which differ in that part of the molecule is not possible and should be done by thin-layer chromatography or high-performance liquid chromatography. However, combination of these two techniques with the present method provides an excellent identification of all of the possible ergot-peptide alkaloids, including stereoisomers.

INTRODUCTION

Despite numerous attempts, the identification of the ergot-peptide alkaloids has remained cumbersome due to the relatively small differences in the molecular structure of the peptide moiety (Fig. 1). The identification is further complicated by the potential occurrence of isomerization and/or hydrolysis breakdown products[1]. These products result from changes in the lysergic acid moiety, and the hydrolysis breakdown products can be separated relatively easily[1–4].

For the parent alkaloids, ergotamine, ergosine, ergostine, ergocristine, ergokryptine and ergocornine, a large number of thin-layer chromatography (TLC) systems has been described[2,5–7], but, with one exception[7], none has provided an adequate separation of all of these six components. The advantages of high-performance liquid chromatography (HPLC) have not yet been fully investigated[4,8–11] and it remains to be seen whether this technique is able to provide adequate separation efficiency, since the small differences in the peptide moiety hardly seem to affect the chromatographic properties of the total molecule.

Because of the thermal instability and low vapour pressure of the ergot-peptide alkaloids, gas–liquid chromatography (GLC) had been found to be unsuitable for the analysis of this class of compounds[12]. Yet, some observations in a case of death



	R_3			
	$CH_2C_6H_5$	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	
$R_1=R_2=H$	ERGOTAMINE	ERGOSINE	-	ERGOTAMINE-GROUP
$R_1=R_2=CH_3$	ERGOCRISTINE	ERGOKRYPTINE	ERGOCORNINE	ERGOTOXINE-GROUP
$R_1=H;R_2=CH_3$	ERGOSTINE	-	-	-

Fig. 1. Structures of the ergot-peptide alkaloids.

involving ergotamine[13], as well as recent work by Szepesi and Gazdag[14], indicated that GLC may still have some potential. The objective of the present study was to test the applicability of GLC to the identification of the six ergot-peptide alkaloids.

EXPERIMENTAL

Materials

All of the chemicals and solvents were obtained from E. Merck, Darmstadt, G.F.R. and were of analytical grade.

Reference samples of ergotamine, ergosine, ergostine, ergocristine, ergokryptine and ergocornine were kindly supplied as hydrogen maleate or ditartrate salts by Sandoz, Basle, Switzerland. The salts of the respective alkaloids were each dissolved in demineralized water, and the solution was adjusted to pH 10 with 4 *N* ammonia and extracted with chloroform. The chloroform extracts were evaporated to dryness under a gentle stream of nitrogen in a water-bath at 50°, and the residues were taken up in ethanol to give solutions *ca.* 10^{-3} *M* in free base. The free bases were found to be at least 99% pure using the TLC system of Phillips and Gardiner[15]. Volumes of 5 μl of the solutions of the free bases were used in the GLC experiments.

Reference samples of L-phenylalanine-L-proline lactam (L-Phe-L-Pro lactam), L-phenylalanine-D-proline lactam (L-Phe-D-Pro lactam) and pyroergotamine were also gifts from Sandoz. They were used as received and dissolved in ethanol to yield *ca.* 10^{-3} *M* solutions, 5 μl of which were used in the GLC experiments.

Gas chromatography

Analyses were done on a Hewlett-Packard Model 5830 instrument, equipped with a hydrogen flame detector. Glass columns (1.8 m × 2 mm I.D.) coated with

HMDS were packed with 3% SE-30 on Chromosorb G HP (80–100 mesh). The injection port had a glass inlet liner (2 mm I.D.) and was kept at 300°. The oven temperature was 225°, and the detector temperature was 300°. Nitrogen was used as carrier gas at a flow-rate of 30 ml/min. The flow-rates of hydrogen and air in the detector were 30 ml/min and 300 ml/min, respectively.

Thin-layer chromatography

The system of Phillips and Gardiner[15] was used, with chloroform–methanol (9:1) on NaOH-impregnated precoated silica gel plates (silica gel F_{254}; E. Merck). Development was carried out in unsaturated chambers, whereas detection was done in UV light of wavelength 254 nm and 365 nm, followed by spraying with a 5% (w/v) solution of 4-dimethylaminobenzaldehyde in methanol–hydrochloric acid (1:1) (DMBA).

Gas chromatography–mass spectrometry (GC–MS)

Analyses were done on a Finnigan 3300 GC/MS/COM instrument. For chemical ionization (CI) spectra, methane was used as carrier gas and as reactant gas, with the gas chromatograph coupled to the mass spectrometer by a 2 mm I.D. stainless steel tube and a venting valve. The methane flow-rate was 20 ml/min, resulting in an ion-chamber pressure of *ca.* 0.5 Torr. The ion-source temperature was 250°, the electron energy 70 eV and the ion-repeller voltage was 3 V. For electron impact spectra, helium was used as carrier gas, with the gas chromatograph coupled to the mass spectrometer by an all-glass jet separator. The ion-source pressure was kept at *ca.* 10^{-5} Torr, the ion-source temperature was 250° and the electron energy 70 eV.

High-resolution mass spectrometry

Exact mass determinations were obtained on an AEI Model MS-9 instrument via a direct inlet system with a probe temperature of 180°. The electron energy was 70 eV and the acceleration voltage 8 kV. Calculations were carried out on an Atlas–Ferranti computer system.

RESULTS AND DISCUSSION

During a recent investigation into the cause of death of a drug addict we applied hydrolysis in strongly acid medium[16] to the post-mortem blood samples so as to liberate any bound drug from blood cells or blood proteins. An alkaline chloroform extract of the hydrolyzate then revealed the presence of L-Phe-D-Pro lactam, L-Phe-L-Pro lactam and a pyruvoyl precursor of L-Phe-D-Pro lactam[13]. Since these products, in particular L-Phe-D-Pro lactam, are characteristic of the decomposition of ergotamine under acidic conditions[1], we concluded that the latter drug had been present in the blood of the deceased.

Fig. 2 shows the decomposition pathways for ergotamine. However, further investigations into the quantitative aspects of the lactam formation showed that this degradation took place to only a limited extent during the acid hydrolysis, but that reproducible and instantaneous degradation was achieved in the injection port of the gas chromatograph. Fig. 3 represents a gas chromatogram obtained by injecting a

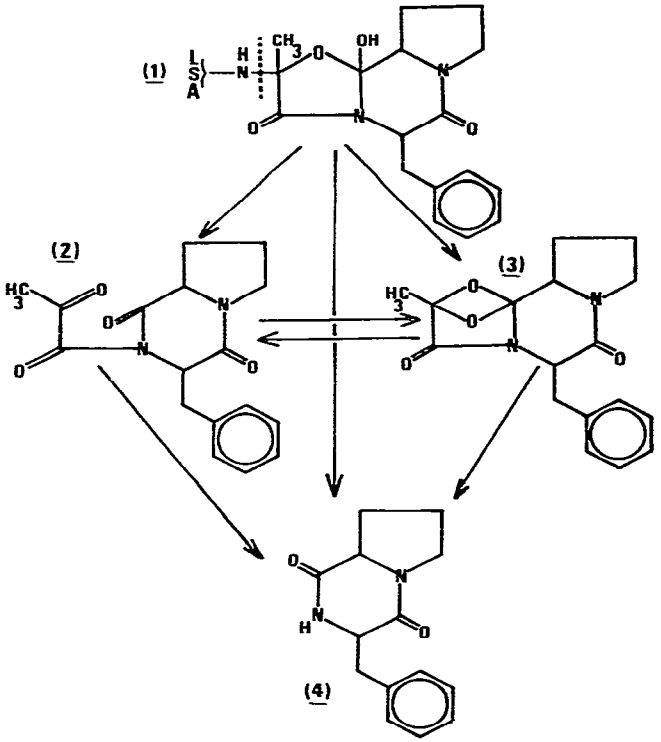


Fig. 2. Decomposition pathways of ergotamine. 1 = Ergotamine. LSA indicates the lysergic acid moiety. The dashed line indicates that the cleavage takes place between the α-nitrogen atom and the α-carbon atom of the amino acid involved, namely α-hydroxyalanine. This results in a pyruvoyl precursor of phenylalanine-proline lactam which can have structure 2 or 3 (ref. 17). Structure 3 is that of pyroergotamine, a reference sample of which showed the same GC and MS behaviour as the above pyruvoyl precursor. However, this does not preclude structure 2 (which is more stable) for this precursor, but for which no reference sample was available. Structure 4 is the phenylalanine-proline lactam, which is obtained in two forms, namely L-Phe-D-Pro lactam and L-Phe-L-Pro lactam.

freshly prepared solution of ergotamine base in ethanol. The identity of the decomposition products was further confirmed by GC–MS and high-resolution MS, and by comparison with authentic reference samples[17].

The optimal injection-port temperature was 300°. Lower temperatures, down to 180°, decreased the amount but not the number of decomposition products. Other solvents, such as benzene, dichloromethane, chloroform, dichloroethane and acetonitrile, gave exactly the same degradation patterns, but diethyl ether, dimethyl sulphoxide and carbon disulphide were less satisfactory, probably due to the poor solubility of ergotamine in these solvents.

The other ergot-peptide alkaloids, ergosine, ergostine, ergocristine, ergokryptine and ergocornine, showed similar decomposition patterns in the injection port, in that they all gave cyclic lactams containing two amino acids and a precursor of these lactams which, besides these two amino acids, also contained a deaminated third hydroxy-amino acid. This was confirmed by GC–MS. Table I summarizes the

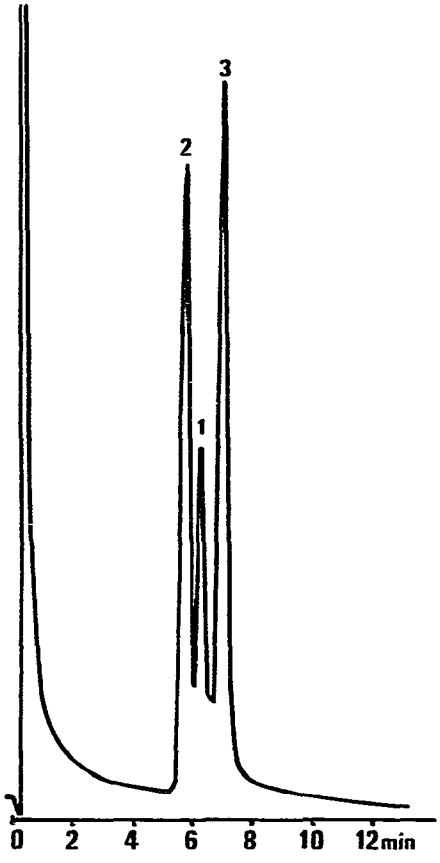


Fig. 3. Gas chromatogram of ergotamine base on SE-30. Peaks: 1 = L-Phe-L-Pro lactam; 2 = L-Phe-D-Pro lactam; 3 = pyruvoyl precursor of L-Phe-D-Pro lactam.

various degradation products, their retention times and retention indices[18] on SE-30, and their quasi-molecular ions in CI–MS. Fig. 4 shows a gas chromatogram of a mixture of the six ergot-peptide alkaloids, injected as bases in ethanol. It can be seen that each individual alkaloid can be identified by the presence of two or three characteristic degradation products.

It is interesting to note that the formation of an L–L dipeptide lactam together with an L–D dipeptide lactam can only be seen with ergotamine, ergostine and ergocristine, the three alkaloids that contain both phenylalanine and proline. The other alkaloids, which do not contain phenylalanine, yield only one dipeptide lactam. Two reasons may account for the latter phenomenon: the degradation occurs in favour of only one configuration, or, if two configurations are formed, the separation of these two is not accomplished under the present GLC conditions. Since the respective L–L and L–D dipeptide lactams derived from ergosine, ergokryptine and ergocornine were not available this question remained unanswered.

Our results do not seem to be in agreement with those of Szepesi and Gazdag[14]. These workers studied the GLC behaviour of dihydroergotoxine alkaloids and

TABLE I

GLC DEGRADATION PRODUCTS, RETENTION TIMES, RETENTION INDICES AND QUASI-MOLECULAR IONS OF THE ERGOT-PEPTIDE ALKALOIDS STUDIED

Alkaloid	*Amino acids in peptide moiety*			*Degradation products detectable by GC*	*Peak number in Fig. 4*	*Retention time (min)*	*Retention index*	*MH^+ (CI–MS)*
Ergotamine	α-hydroxy-Ala	Phe	Pro	L-Phe-L-Pro lactam	1	6.33	2300	245
				L-Phe-D-Pro lactam	2	5.76	2275	245
				pyruvoyl-Phe-Pro lactam*	3	7.00	2340	315
Ergosine	α-hydroxy-Ala	Leu	Pro	Leu-Pro lactam	4	1.93	1900	211
				pyruvoyl-Leu-Pro lactam*	5	2.81	2075	281
Ergostine	α-hydroxy-α--aminobutyric acid	Phe	Pro	L-Phe-L-Pro lactam	1	6.33	2300	245
				L-Phe-D-Pro lactam	2	5.76	2275	245
				α-ketobutyryl-Phe-Pro lactam*	6	9.20	2435	329
Ergocristine	α-hydroxy-Val	Phe	Pro	L-Phe-L-Pro lactam	1	6.33	2300	245
				L-Phe-D-Pro lactam	2	5.76	2275	245
				α-ketoisovaleryl-Phe-Pro-lactam*	7	10.58	2480	343
Ergokryptine	α-hydroxy-Val	Leu	Pro	Leu-Pro lactam	4	1.93	1900	211
				α-ketoisovaleryl-Leu-Pro lactam*	8	4.28	2175	309
Ergocornine	α-hydroxy-Val	Val	Pro	Val-Pro lactam	9	1.51	1810	197
				α-ketoisovaleryl-Val-Pro lactam*	10	3.55	2100	295

* The exact structure of this component is unknown at the present time. As indicated in Fig. 2, pyruvoyl-Phe-Pro lactam may be present as an α,β-diketo structure (2) or as a structure with a dioxane ring (3). This also applies to the other deaminated tripeptide lactam components in this table.

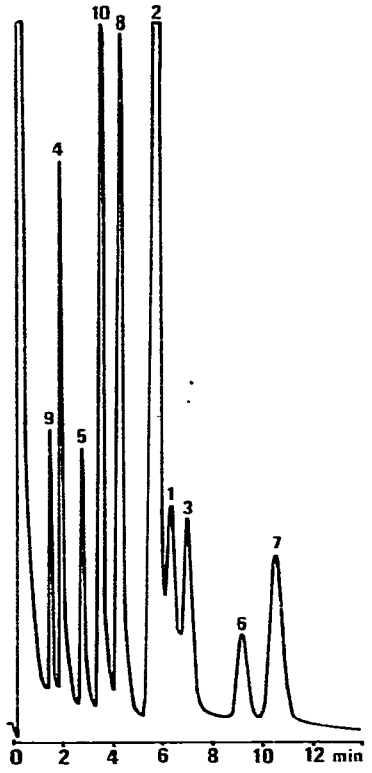


Fig. 4. Gas chromatogram of the six ergot-peptide alkaloids on SE-30. Ergotamine: peaks 1, 2 and 3; ergosine: 4 and 5; ergostine: 1, 2 and 6; ergocristine: 1, 2 and 7; ergokryptine: 4 and 8: ergocornine: 9 and 10.

reported only one GLC peak for the cornine, kryptine and cristine peptide moieties using steel injection ports at temperatures around 235°. Furthermore, they assumed that there was cleavage of the amide bond between the lysergic acid moiety and the peptide moiety, resulting in a tripeptide moiety containing three nitrogen atoms. In our studies, GC–MS and high-resolution MS showed that the initial cleavage takes place between the α-nitrogen atom and the α-carbon atom of the α-hydroxy-amino acid in the peptide moiety, resulting in a deaminated tripeptide structure*. We found no difference between the behaviour of steel liners and glass liners.

Since the present method only identifies the peptide part of the molecule, differentiation between components that differ in their lysergic acid moiety (stereo-isomers such as -ine and -inine components, *aci*-components and derivatives such as lumi- and dihydro-components) is not possible. Yet, as the latter products can be differentiated by TLC[3,13] or HPLC[4], combination of these techniques with the present GLC method allows unequivocal identification of all of the ergot alkaloids, also in mixtures. On the other hand, differences in the structure of the lysergic acid moiety

* Szepesi and Gazdag have recently obtained GC–MS information on the site of cleavage of the peptide moiety which is in agreement with our findings[19].

do not affect the GLC degradation of the peptide moiety. For example, *aci*-ergotamine, ergotamine and dihydroergotamine were found to give the same gas chromatogram as ergotamine (see Fig. 3).

Combination of the present GLC technique with TLC or HPLC systems will also provide a more reliable analysis of materials, suspected of containing lysergide (LSD). For example, when subjected to TLC, various street drug samples show a multitude of blue fluorescent spots giving a blue colour with DMBA and have R_F values close to that of LSD which may be caused by ergot-peptide alkaloids. By collecting the spots from the plate and subjecting them to GLC, it can easily be established whether these products contain a peptide moiety.

It should be noted that neither lysergide, lysergic acid nor the lysergic acid moiety of ergot-peptide alkaloids can be detected under the present GLC conditions.

The quantitative aspects of the method are still under investigation. With ergotamine, it seems that the ratio in which the three degradation components are formed differs slightly, but that the total area of the three peaks remains fairly constant with a variation coefficient of *ca.* 5%.

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REFERENCES

1 A. Hofmann, *Die Mutterkornalkaloide*, Enke Verlag, Stuttgart, 1964.
2 K. Macek, *Pharmaceutical Applications of Thin-layer and Paper Chromatography*, Elsevier, Amsterdam, 1972.
3 B. Kreilgaard, *Ph.D. Thesis*, College of Pharmacy, Copenhagen, 1973.
4 H. Bethke, B. Delz and K. Stich, *J. Chromatogr.*, 123 (1976) 193.
5 J. L. McLaughlin, J. E. Goyan and A. G. Paul, *J. Pharm. Sci.*, 53 (1964) 306.
6 R. Fowler, P. J. Gomm and D. A. Patterson, *J. Chromatogr.*, 72 (1972) 351.
7 J. Reichelt and S. Kudrnáč, *J. Chromatogr.*, 87 (1973) 433.
8 R. A. Heacock, K. R. Langille, J. D. MacNeil and R. W. Frei, *J. Chromatogr.*, 77 (1973) 425.
9 J. D. Wittwer, Jr. and J. H. Kluckholm, *J. Chromatogr. Sci.*, 11 (1973) 1.
10 I. Jane and B. B. Wheals, *J. Chromatogr.*, 84 (1973) 181.
11 J. Christie, M. W. White and J. M. Wiles, *J. Chromatogr.*, 120 (1976) 496.
12 S. Agurell and A. Ohlsson, *J. Chromatogr.*, 61 (1971) 339.
13 R. A. de Zeeuw, F. J. W. van Mansvelt and J. E. Greving, *J. Forensic Sci.*, 22 (1977) 550.
14 G. Szepesi and M. Gazdag, *J. Chromatogr.*, 122 (1976) 479.
15 G. F. Phillips and J. Gardiner, *J. Pharm. Pharmacol.*, 21 (1969) 793.
16 J. V. Jackson, in I. Sunshine (Editor), *Handbook of Toxicology*, CRC Press, Cleveland, Ohio, 1969, p. 398.
17 R. A. de Zeeuw, F. J. W. van Mansvelt and J. E. Greving, in *Advances in Mass Spectrometry, Vol. 7, Proc. 7th Int. Conf. on Mass Spectrometry, Florence, 1976*, Heyden, London, 1977.
18 E. Kováts, *Helv. Chim. Acta*, 41 (1958) 1915.
19 G. Szepesi, personal communication, 1977.