TWO UNUSUAL ERGOPEPTINES PRODUCED BY A SAPROPHYTIC CULTURE OF *CLAVICEPS PURPUREA*

N. CRESPI-PERELLINO,*

Farmitalia Carlo Erba, Ricerca e Sviluppo Biotecnologico

M.BALLABIO, B. GIOIA,

Farmitalia Carlo Erba, Ricerca e Sviluppo Chimica Analitica, via dei Gracchi 35, 20146 Milano, Italy

and A. MINGHETTI

*Dipartimento di Scienze Farmaceutiche, Universitá di Bologna, via Belmeloro 6, 40100 Bologna, Italy*

ABSTRACT.—Two new ergot alkaloids belonging to the ergopeptine class have been found in saprophytic cultures of strain 231 F.I. of *Claviceps purpurea* that produces mainly ergotoxine. They have an additional methyl group in the peptide side chain and correspond to $O(12')$-methylergocornine [1] and O-12'-methyl-α-ergokryptine [2]. Their structures have been assigned on the basis of spectral data, amino acid composition, and degradation procedures. The involvement of these alkaloids in the biosynthesis of ergopeptines, as suggested by their fragmentation pattern in eims, is discussed, and a possible biosynthetic scheme is proposed.

Ergot alkaloids seem to be a never-ending story, with new alkaloids continuously being identified during the past two decades. All the new alkaloids found recently may be ascribed either to those normally produced by the different strains of *Claviceps purpurea* or to those formed by directed biosynthesis. The former belong to the two known groups of ergopeptines and ergopeptams (1). The latter are synthesized by incorporation of different amino acids, often unnatural, in the cyclol system with formation of anomalous alkaloids (2-5).

The present report deals with two new natural ergot alkaloids **1** and **2**, belonging to the ergopeptine class. They are analogues of ergocornine [**3**] and α-ergokryptine [**4**], respectively, and have an additional methyl group in position 12'. This position is generally considered the starting point of the biosynthesis of the cyclol structure. Modifications at this site could possibly be of great importance and may shed some light onto the still poorly known biosynthetic pathway of ergot alkaloids.

RESULTS AND DISCUSSION

During a study in our laboratories aimed at identifying minor alkaloids produced
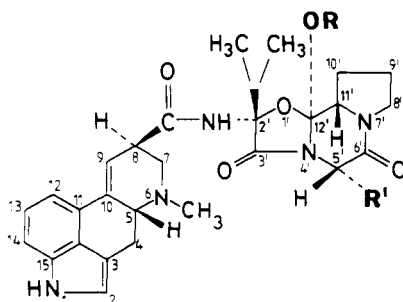
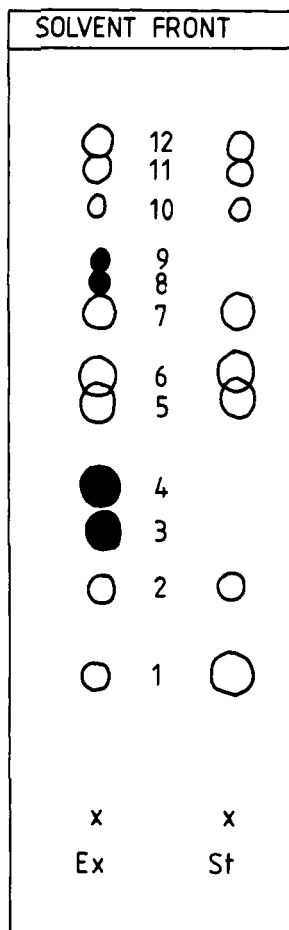|	R	R'	
1	-Me	-CH(Me)$_2$	0-12'-methylergocornine
2	-Me	-CH$_2$-CH(Me)$_2$	0-12'-methyl-α-ergokryptine
3	-H	-CH(Me)$_2$	ergocornine
4	-H	-CH$_2$-CH(Me)$_2$	α-ergokryptine

FIGURE 1. Tlc behavior of the main alkaloids produced by *Claviceps purpurea* strain 231 F.I. on Si gel 60 F 254. Development solvent: two runs in solvent A. Locating reagent: PDAB (see text). St=reference standards; Ex=crude extract. Ergotamine group: 1=ergosine; ergoxine group: 2=ergobutine; 3=O-12'-methyl-ergocornine [1], 4=O-12'-methyl-α-ergokryptine [2]; ergotoxine group: 5=ergocornine, 6=(α+β)-ergokryptine; ergotaminine group: 7=ergosinine; 8=O-12'-methylergocorninine, 9=O-12'-methyl-α-ergocryptinine; ergoxinine group: 10=ergobutinine; ergotoxinine group: 11=ergocorninine, 12=(α+β)-ergokryptinine.

by a strain of *C. purpurea* isolated from strain 231 F.I. (6), two minor alkaloids [1 and 2], evident in analytical tlc, were investigated.

By analytical procedures 1 and 2 were shown to be lysergic acid derivatives, containing valine and proline [1] and leucine and proline [2] in equimolar amounts. This assigned them to the class of ergopeptines, but their position in tlc (Figure 1) excluded all known groups of ergopeptines; they were more polar than ergotoxine and less polar than the ergoxine and ergotamine groups. Their corresponding isomers at the C-8 center of lysergic acid had an even more surprising tlc behavior, falling between the ergotaminine and ergoxinine groups instead of ergoxinine and ergotoxinine as expected. By alkaline hydrolysis, 1 and 2 were shown to contain 2-oxo-valeric acid, correspond-

ing to valine as the first amino acid. These facts led us to regard them as a new group in the general class of ergotoxines.

Eims of **1** and **2** are shown in Figures 2 and 3, respectively. Their molecular ions are clearly recognizable, and molecular weights of 575 for **1** and 589 for **2** can be determined. These data were confirmed by fdms, which showed only molecular ions at the same m/z values. The molecular weights indicate the presence of an additional methyl group in comparison with the structures of ergocornine [**3**] and α-ergokryptine [**4**], probably at the oxygen atom in C-12' (see also nmr results). The presence of the methyl group is confirmed by exact mass measurements of the molecular ion of both compounds, which are in agreement with a molecular formula of $C_{32}H_{41}N_5O_5$ for **1** and $C_{33}H_{43}N_5O_5$ for **2**.

However, the fragmentation pattern under eims conditions differed from that previously described for this class of compounds (7,8) and was elucidated with the aid of high resolution mass measurements on major ions and analysis of metastable ions using linked scan (B/E=const. and B^2/E=const.) and defocusing techniques (scan of accelerating voltage with constant magnetic field and constant electric sector voltage). The results of these studies are reported in Scheme 1 (structures represented are hypothetical). At first glance, the reported fragmentation pattern seems to be in agreement with an open chain structure; the losses of proline methylester (m/z 446 for **1** and 460 for **2**) and of the last two amino acids (m/z 347 for both **1** and **2**) from the molecular ion would be particularly in favor of this preliminary hypothesis. However, nmr data (see later) confirm that the peptidic moiety has the usual cyclic structure.

The mass spectral data can be explained considering an equilibrium between the cyclic and the open structures, as depicted in the upper part of Scheme 1, which probably takes place very easily for the molecular ion in the gas phase. The open form subsequently undergoes the indicated fragmentation. The loss of 32 mass units (MeOH)
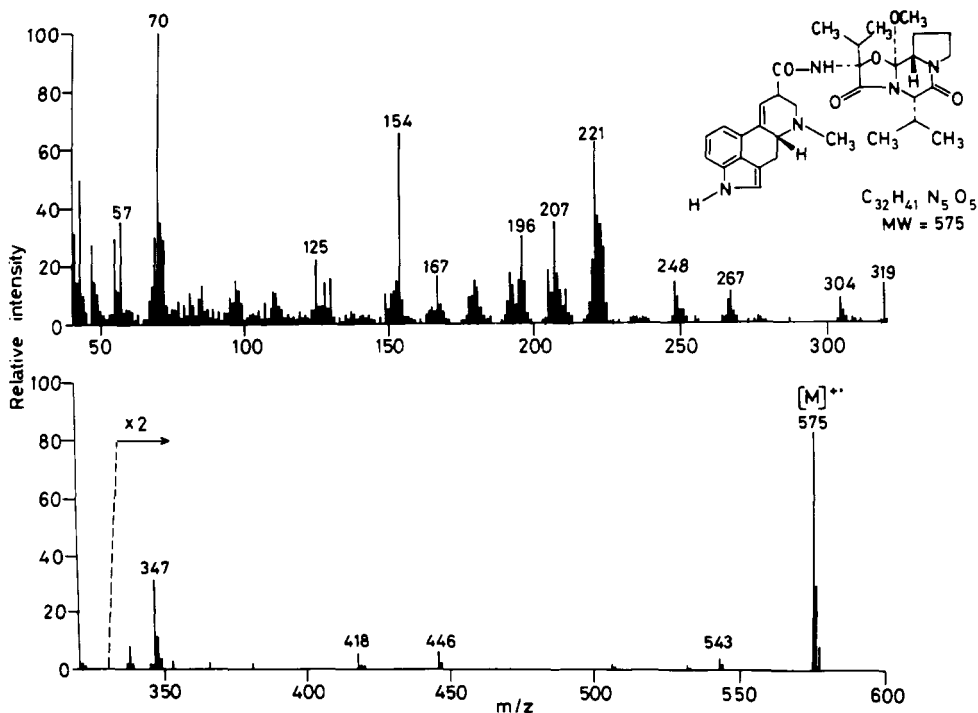

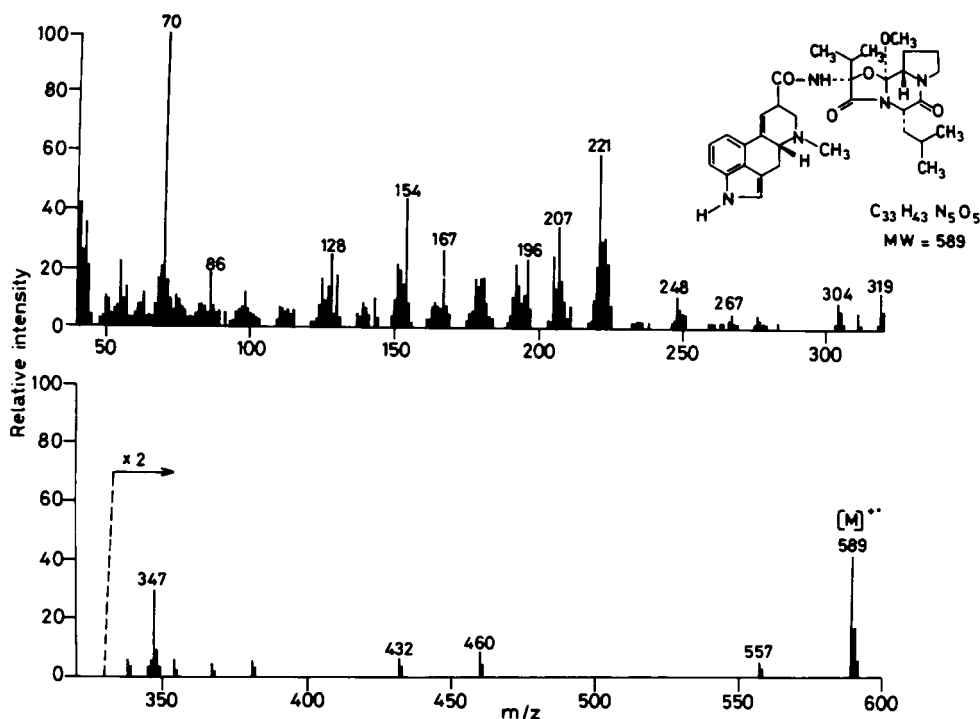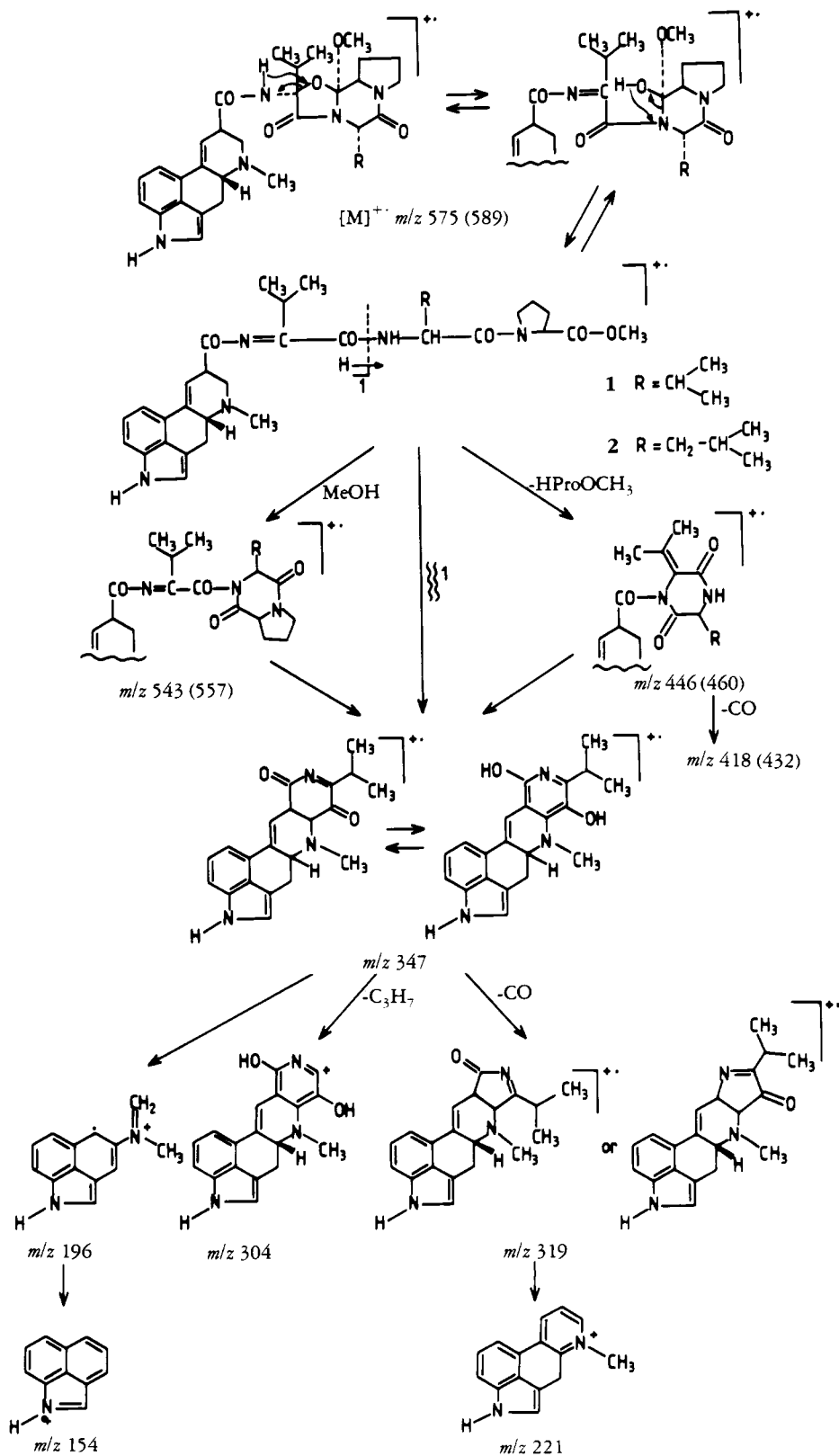FIGURE 2.   Electron impact mass spectrum of alkaloid **1**

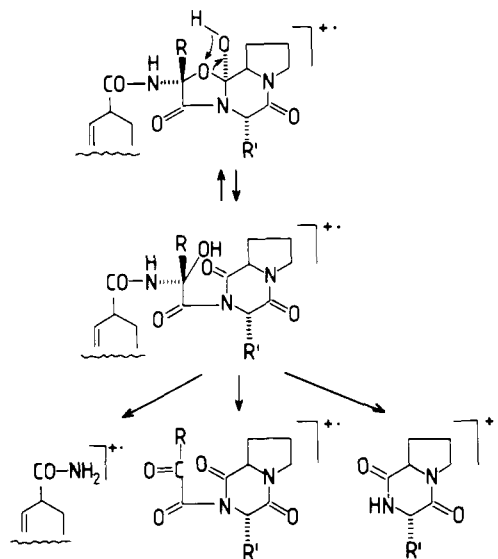FIGURE 3. Electron impact mass spectrum of alkaloid **2**.

from the molecular ion (*m/z* 543 for **1** and 557 for **2**) is of particular diagnostic value because it confirms the presence of a methoxy group in the molecule. The base peak at *m/z* 70, whose origin is not indicated in Scheme 1, is due to the five-membered ring of proline as already observed in normal ergot alkaloids (7,8).

Even though the reason for the striking differences between compounds **1** and **2** and the other ergot alkaloids has not been fully investigated, one explanation can be offered: The starting point of the fragmentation pathway of usual alkaloids is probably the opening of the oxygenated five-membered ring of the cyclol moiety (see Scheme 2). In fact, since the cyclol ring does not differ from a cyclic sugar, it can achieve equilibrium between the acyclic and cyclic hemiacetalic forms. In the alkaloid class, this equilibrium is not evident in solution as for carbohydrates, but very likely can take place in the high-energy conditions of the gas phase ions generated in eims. All the ions previously described for ergot alkaloids (7) can be derived from the open intermediate by simple cleavages accompanied by hydrogen rearrangements (Scheme 2). However, in compounds **1** and **2** it is clear that this fragmentation pathway is prevented by the methyl group on the oxygen atom at C-12'. An alternative mechanism, therefore, becomes operative, as described in Scheme 1. There are great similarities between the two ring-opening mechanisms, the only difference being the role played by a nitrogen instead of an oxygen atom.

Prompted by the finding of an additional 14 mass units in the ms spectra of **1** and **2**, the new alkaloids were subjected to nmr analysis to pinpoint the site of substitution. ^{13}C nmr (Table 1) gave evidence of an additional methoxy group (49.2 δ in **1**, 48.8 δ in **2**) at the 12' position. The neighboring sites in both **1** and **2** presented chemical shifts suggesting such a substitution when compared to the values found for the parent alkaloids ergocornine [**3**] and α-ergokryptine [**4**] (Δδ = +3 ppm Cα, Δδ = −2.5 ppm Cβ). All the chemical shifts pertinent to the lysergic skeleton were similar to those of

$[M]^{+\cdot}$ m/z 575 (589)

1    $R = CH\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}$

2    $R = CH_2-CH\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}$

MeOH

-HProOCH$_3$

m/z 543 (557)

m/z 446 (460)

-CO

m/z 418 (432)

m/z 347

-C$_3$H$_7$

-CO

m/z 196

m/z 304

m/z 319

or

m/z 154

m/z 221

SCHEME 1.   Fragmentation pattern of alkaloids **1** and **2** under eims conditions

SCHEME 2.   Usual fragmentation pattern of the cyclol
moiety of ergopeptines

the parent compounds, except for the amide carbon in position 8, which was found 2 ppm more upfield in **1** and **2** (173.9 δ, 174.0 δ) than in the parent alkaloids **3** and **4** (176.2 δ). There the hydroxyl group in 12′ is strongly hydrogen-bound in CDCl$_3$ solution to the amide group in C-8, hence the lower shielding of the amide carbonyl (Table 1) and of the amide proton (Table 2). In addition, long-range coupling is observed with H-11′ through a W pathway, due to the fixed spatial position of the hydroxyl hydrogen (9,10). In compounds **1** and **2** the absence of this hydrogen bond causes the higher shielding of both the amide carbonyl and the amide proton.

The most interesting feature of the proton spectrum of the new alkaloid **2** was the coupling exhibited by H-5′ in both CDCl$_3$ and DMSO solution. In the parent alkaloid, α-ergokryptine H-5′ has coupling with the neighboring -CH$_2$- of leucine of 7.3 and 5.6 Hz (CDCl$_3$) (6.5, 6.5 Hz in DMSO), indicating a freely rotating isopropyl group. In **2** instead the H-5′ signal shows markedly different coupling constants, 3.8 and 10.8 Hz in CDCl$_3$ (3.5 and 10.7, respectively, in DMSO). This suggested a large change in the conformer distribution around the C5′/CH$_2$-Leu bond, caused by a different hindrance of the methoxy substituent at C-12′, all other features of the cyclol moiety remaining unchanged. As reported in the literature (9), when the substituent at C-5′ is benzyl, the conformer distribution calculated from the coupling of H-5′ with the side chain hydrogens gives 36% (g⁻), 26% (t), and 38% (g⁺) in CDCl$_3$. The first two staggered rotamers, corresponding to torsion angles of 300 and 180, respectively (Figure 4), are those preferred by the molecule in the solid state. In our case, the conformer distribution, calculated according to Bystrov (11), gave 45% (g⁻), 29% (t), and 26% (g⁺) for α-ergokryptine, while for **2** the preferred staggered rotamer was g⁻ (77%), t and g⁺ accounting for 13% and 10% of the population, respectively. When the temperature of the CDCl$_3$ solution was raised to 90° the H-5′/CH$_2$ J values began to converge toward less extreme values, namely 4.9 and 9.8 Hz. From these, the rotamer distribution at 90° was calculated to be 68-23-9 (g⁻, t, g⁺), the conformer g⁺, having the side chain staggered between N-4′ and the C-6′ carbonyl still being the least populated.

Considering the molecular model of the cyclol moiety and keeping the B ring of

TABLE 1. ^{13}C-nmr Chemical Shifts of **1**, **2**, **3** (Ergocornine) and **4** (α-Ergokryptine) in CDCl$_3$

Carbon atoms	Compounds			
	1	**2**	**3**	**4**
C-2	(119.0)[a]	(119.0)	(119.2)	(119.1)
C-3	110.8	110.3	110.6	110.7
C-4	26.6	26.4	26.6	26.5
C-5	59.4	59.5	59.3	59.2
N(6)-CH$_3$	44.1	44.6	44.4	44.3
C-7	49.2	48.4	48.2	48.1
C-8	41.3	41.3	40.9	40.9
C-9	(119.2)	(119.2)	(118.8)	(118.8)
C-10	138.8	138.8	139.1	139.1
C-11	129.8	129.8	129.6	129.6
C-12	112.0	112.1	111.9	111.9
C-13	123.4	123.5	123.3	123.3
C-14	110.0	110.0	110.1	110.1
C-15	133.8	133.8	133.9	133.8
C-16	126.2	126.2	126.2	126.2
(8)-CONH	173.9	174.0	176.2	176.2
C-2′	89.2	89.2	89.6	89.7
C-3′	(164.7)	(165.7)	(164.7)	(165.8)
C-5′	60.7	53.1	60.7	53.3
C-6′	(168.7)	(167.4)	(166.8)	(166.2)
C-8′	45.8	46.1	45.8	46.0
C-9′	(22.1)	(21.9)	(21.7)	(21.6)
C-10′	(22.2)	(22.0)	(22.1)	(22.1)
C-11′	61.4	61.9	64.1	64.5
C-12′	106.8	106.4	103.6	103.5
(12′)-OCH$_3$	49.2	48.8	—	—
(2′)-C	35.7	35.9	34.3	34.3
(2′)-(CH)-(CH$_3$)$_2$	16.0, 16.7	16.0, 16.8	15.3, 16.9	15.4, 16.9
(5′)-C	32.3	42.2	32.8	43.5
(5′)-(C)-C	—	25.0	—	25.1
(5′)-(C)-(C)-(CH$_3$)$_2$	19.5, 19.2	22.1, 21.4	19.0, 20.5	22.2, 22.6

[a]Close values in parentheses may be interchanged.

cyclol in a pseudo-chair conformation as seen in the solid state (12), the closeness in space of the substituent at C-12′ and the side chain at C-5′ is evident. In α-ergokryptine [**4**] the hydroxyl group in 12′ is kept far from the chain at C-5′ by the above men-

TABLE 2.   Main ^{1}H-nmr Signals for **1**, **2**, **3** (Ergocornine) and **4** (α-Ergokryptine) in CDCl$_3$[a]

	Compounds			
	1	**2**	**3**	**4**
N(1)-H	8.07	8.09	7.97	7.96
N(6)-CH$_3$	2.66	2.66	2.66	2.64
H-9	6.40	6.41	6.40	6.38
	(2.0, 5.9)[a]	(1.8, 5.5)	(1.5, 6.1)	(2.0, 6.2)
(8)-CONH	9.17	9.16	9.86	9.84
OH-12′	—	—	7.84 (1.6)	7.38 (1.7)
OCH$_3$-12′	3.31	3.24	—	—
H-5′	4.51	4.62	4.43	4.50
	(4.1)	(3.8, 10.8)	(3.5)	(5.6, 7.3)

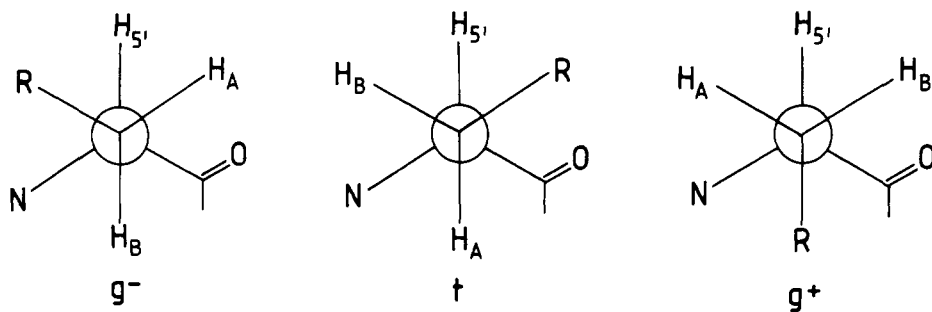[a]Coupling constants in parentheses in Hz.

FIGURE 4. Staggered rotamers about C-5'/CH$_2$-R bond in the cyclol moiety. Torsion angles are 300 (g$^-$), 180 (t), 60 (g$^+$).

tioned hydrogen bond with the amide at C-8, thus allowing a free rotation about the C-5'/CH$_2$-Leu bond. When the methoxy group is substituted for the hydroxyl, as in **2**, the hindrance of the substituent becomes larger not only because of the bulkiness of the substituent itself but also because of the lack of the restraint formerly caused by the hydrogen bond. The motion of the methoxy group around the C-12'/O bond can sweep a large portion of space at the bottom face of the cyclol, strongly interacting with the *syn* side chain at C-5' in the g$^+$ rotamer. The above considerations are valid for any bulky C-5' substituent and have been discussed here for the case of **2**, where the side chain is isobutyl, but must also hold true for a smaller substituent such as isopropyl in **1**. Here the vicinal coupling constant H-5'/CH-Val changes from 3.5 Hz (ergocornine) to 4.1 Hz [**1**] (CDCl$_3$) suggesting again a somewhat different rotamer distribution caused by the presence of the methoxy group in C-12'.

Alkaloids **1** and **2** represent the first natural examples of O-CH$_3$ substitution at 12'. Two 12'-OCH$_3$ derivatives of a cyclol tripeptide generated photochemically and chemically were found to have inverted stereochemistry at the C-12' and C-11' centers (13,14). A chemically methylated derivative of cyclol tripeptide with the same stereochemistry as natural ergopeptines has also been synthesized, but it lacked the lysergic moiety (14).

In order to ascertain if alkaloids **1** and **2** were genuine natural products and not artifacts arising from the MeOH used in the extraction procedures, a separate experiment was performed. A batch of ten Erlenmeyer flasks was cultured and extracted as reported in the Experimental section, but using *n*-BuOH instead of MeOH. The crude extract, analyzed by tlc in system A, gave alkaloids **1** and **2**, confirmed by ms analyses, and in the same percentage as found in the main batch processed with MeOH.

Methylation in our laboratories of the alkaloids **3** and **4** with chemical methods (CH$_2$N$_2$, Me$_2$SO$_4$) was unsuccessful, in agreement with literature data (15).

Taking account of the fact that the oxygen bearing the anomalous methyl group belongs to the carboxylic group of proline (16), feeding experiments with l-proline methylester were made in our laboratories, but no increase in the production of **1** and **2** was achieved.

It is generally assumed (1,17) that throughout biosynthesis of the ergopeptine alkaloids the molecule is covalently bound to a multienzymatic complex linked at the carboxyl group of the proline as in the thioester **5** in Scheme 3. This hypothesis, however, still lacks experimental support. The presence of the methoxy group in 12' may confer to the open ergopeptine structure a stability similar to the molecule when bound to the enzyme. In fact, the first step of eims fragmentation (Scheme 1), corresponding to the open chain form of the molecular ion, could represent such a stable form similar to **5** and, moreover, it is not observed in the eims fragmentation of unsubstituted alkaloids such as **3** and **4**.

R'   = isopropyl or isobutyl
R"   = H or methyl
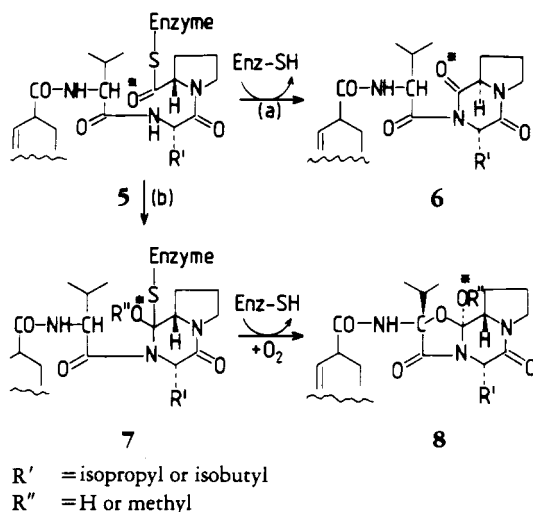
SCHEME 3.   Hypothetical biosynthetic pathways leading to
ergopeptam (a) and ergopeptine (b) alkaloids

Methylation of the hydroxyl group occurs necessarily during cyclol biosynthesis, and the event can be placed in a mechanism as depicted in Scheme 3 (where the oxygen of the thioester is labeled for convenience). The enzyme is linked to the tripeptide in linear form **5** and gives rise to the diketopiperazine structure by two different mechanisms. In one case (Path a), it gives the ergopeptam alkaloid **6** (1) by the formation of a carbonyl group in C-12' and the conversion of the L-proline to its D form through the change of stereochemistry at C-11' (14). In the other case (Path b), a hydroxyl group is formed at C-12' with a bridge between N-4' and C-12' [7]. At this point methylation may occur leading eventually to the formation of the reported ergopeptines **1** and **2**. The final step is the oxygenation of position 1' by O_2 from the air (16), together with splitting of the enzyme from the complete ergopeptine structure [8].

The alternative pathways a and b leading to the formation of either an ergopeptam or an ergopeptine alkaloid depend probably on the availability of molecular oxygen in the medium. In fact, noticeable amounts of ergopeptam alkaloids have been found in surface cultures of *C. purpurea* grown in polyethylene bags (5); in contrast, in well aereated fermenters only ergopeptine alkaloids are produced.

The presence of a methoxy group at C-12' could also explain the anomalous tlc behavior of **1** and **2** (shown in Figure 1). This may be due to the absence of the "normal" hydrogen bond between the amide in C-8 and the OH-12' group (9,10,18).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectra were obtained on a Bausch & Lomb Spectronic 2000 spectrophotometer. Amino acid analyses were performed with a Technicon TMS Sequential Multisample Analyzer for amino acids.

Eims were recorded on a Varian MAT-311 A mass spectrometer at 70 eV using the direct inlet technique at a temperature of 110°-130°; the ion source temperature was 250°. The linked scan spectra were recorded in the same conditions using the Varian Metascan unit. Exact mass measurements were made on the same instrument at a resolution of 10,000 (10% valley) using the peak matching technique and perfluorokerosene as reference compound. Measured masses were within ±2 ppm of the calculated values. Fdms were obtained using benzonitrile-activated emitters. The total potential difference between the field emitter anode and the cathode was about 9 kV; the emitter heating current was in the range 20-28 mA and the ion source temperature was 150°.

Nmr spectra were recorded on a XL-200 Varian spectrometer at 200 MHz for ^{1}H and 50 MHz for ^{13}C. For ^{1}H nmr, data were usually collected on 10K, and FIDs were zero-filled to 32 K prior to Fourier transformation. All chemical shifts are expressed in ppm downfield from zero TMS. When necessary, assignments were checked through suitable decoupling experiments. The variable temperature experiment for **2** was performed with a thick-wall sealed nmr tube containing the CDCl$_3$ solution.

STRAIN.—A strain isolated from *C. purpurea*, strain 231 FI (6), was grown in Erlenmeyer flasks; the medium and conditions were previously reported (8).

EXTRACTION OF THE CRUDE ALKALOIDS.—A batch of crude alkaloids was obtained by extracting the pooled content of 200 Erlenmeyer flasks as reported elsewhere (8). After extraction with CH$_2$Cl$_2$-MeOH (80:20) followed by precipitation with petroleum ether, the crude alkaloid extract was loaded on 40 Extrelut (Merck) columns, each eluted with 200 ml of CH$_2$Cl$_2$; 12.4 g of alkaloids calculated as ergotamine was recovered.

CHROMATOGRAPHY.—A crude extract of alkaloids was loaded on a Si gel 60 (Merck) column (250 g) in CH$_2$Cl$_2$-MeOH (98:2). The column was eluted with the same solvent. Each fraction (100 ml) was checked by uv and tlc analyses in system A. Fractions 1-13 contained dextrorotatory isomers of alkaloids (6.3 g). In fractions 14-19 levorotatory isomers of compounds **1**, **2**, **3**, and **4** were present (3.4 g). Fractions 20-27 gave mainly ergosine (300 mg). Only fractions 14-19 were considered, pooled, and further purified by preparative and analytical tlc in a cold room in the dark; 150 mg of pure **1** and 120 mg of pure **2** were obtained.

THIN LAYER CHROMATOGRAPHY.—Tlc was performed on Si gel F 254, 0.25 thick plates (Merck) in the following solvent systems: (A) CH$_2$Cl$_2$-iPrOH (92:8); (B) CH$_2$Cl$_2$-MeOH (90:10). Chromatographic separations of alkaloids in system A were performed with a double run of the solvent.

A typical chromatographic pattern of alkaloid separation in solvent system A is reported in Figure 1. The plates were examined under uv at 254 and 366 nm, and some were sprayed only at the edge with *N,N*-*p*-dimethylaminobenzaldehyde (PDAB) (19). The fluorescent bands corresponding to the blue reaction with PDAB were scraped off and eluted from the Si gel with CH$_2$Cl$_2$-MeOH (1:1).

DEGRADATION PROCEDURES.—Acidic and alkaline hydrolysis were performed as reported elsewhere (8).

LITERATURE CITED

1. P.A. Stadler, *Planta Med.*, **46**, 131 (1982).
2. E. Beacco, M.L. Bianchi, A. Minghetti, and C. Spalla, *Experientia*, **34**, 1291 (1978).
3. M.L. Bianchi, P.A. Cattaneo, N. Crespi-Perellino, A. Guicciardi, A. Minghetti, and C. Spalla, in: "Overproduction of Microbial Metabolites. Mechanisms of Control in the Qualitative Biosynthesis of Lysergic Acid Alkaloids," Ed. by V. Krumphanzl, B. Sikyta, and Z. Vanek, Academic Press, London, 1982, p. 235.
4. A. Baumert, D. Erge, and D. Groger, *Planta Med.*, **44**, 122 (1982).
5. M. Flieger, P. Sedmera, J. Vokoun, Z. Rehacek, J. Stuchlik, Z. Malinka, L. Cvak, and P. Harazim, *J. Nat. Prod.*, **47**, 970 (1984).
6. M. Bianchi, A. Minghetti, and C. Spalla, *Experientia*, **32**, 145 (1976).
7. J. Vokoun and Z. Rehacek, *Collect. Czech. Chem. Commun.*, **40**, 1731 (1975).
8. M.L. Bianchi, N. Crespi-Perellino, B. Gioia, and A. Minghetti, *J. Nat. Prod.*, **45**, 191 (1982).
9. L. Pierri, I.H. Pitman, I.D. Rae, D.A. Winkler, and P.R. Andrews, *J. Med. Chem.*, **25**, 937 (1982).
10. H.P. Weber, H.R. Loosli, and T.J. Petcher, *Proc. Symp. Steric Eff. Biomol.*, Eger, Hungary, 1981, 39.
11. V.F. Bystrov, *Prog. Nucl. Magn. Reson. Spectrosc.*, **10**, 41 (1976).
12. G. Lucente, F. Pinnen, G. Zanotti, S. Cerrini, F. Mazza, A.L. Segre, and W. Fedeli, *J. Chem. Soc., Perkin Trans. 2*, 1169 (1982).
13. M. Przybylska and F.R. Ahmed, *Acta Crystallogr., Sect. B*, **B37**, 168 (1981).
14. H. Ott, A.J. Frey, and A. Hofmann, *Tetrahedron*, **19**, 1675 (1963).
15. H. Ott, A. Hofmann, and A.J. Frey, *J. Am. Chem. Soc.*, **88**, 1251 (1966).
16. F.R. Quigley and H.G. Floss, *J. Org. Chem.*, **46**, 464 (1981).
17. H.G. Floss, *Tetrahedron*, **32**, 873 (1976).
18. Z. Rehacek, *Adv. Biochem. Eng.*, **14**, 33 (1980).
19. E. Stahl, "Thin Layer Chromatography: A Laboratory Handbook," Springer-Verlag, New York, 1969, p. 689.