Ergogaline, a New Ergot Alkaloid, Produced by *Claviceps purpurea*: Isolation, Identification, Crystal Structure and Molecular Conformation

Ladislav Cvak,[a] Alexandr Jegorov,*[,b] Petr Sedmera,[c] Vladimír Havlíček,[c] Jan Ondráček,[d] Michal Hušák,[d] Svetlana Pakhomova,[d] Bohumil Kratochvíl[d] and Joachim Granzin[e]

[a] *Galena Co., R. & D., 747 70 Opava-Komárov, Czech Republic*
[b] *Galena Co., Research Unit, Branišovská 31, 370 05 České Budějovice, Czech Republic*
[c] *Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic*
[d] *Department of Solid State Chemistry, Prague Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic*
[e] *Institute of Crystallography, Takustrasse 6, Free University, D-1000 Berlin 33, Germany*

A new peptide ergot alkaloid containing L-homoisoleucine has been isolated from ergot sclerotia of the field-growing parasitic fungus *Claviceps purpurea*. The molecular and crystal structures have been studied by X-ray analysis [ergogaline monohydrate, monoclinic space group $P2_1$ with two molecules per unit cell of the dimensions $a = 9.972(1)$, $b = 10.902(1)$ and $c = 14.761(1)$ Å, $\beta = 94.05(1)°$] and compared with the structures in solution as determined by 2D NMR spectroscopy.

There is only a limited number of amino acids incorporated into both groups of natural peptide ergot alkaloids, ergopeptines and ergopeptames.[1] The first amino acid of the peptide moiety in ergopeptines and ergopeptames is either alanine, 2-aminobutyric acid or valine, the second amino acid is either phenylalanine, leucine, isoleucine or valine and the third amino acid is proline. Recently, a new alkaloid, ergobalansine, containing alanine instead of proline was isolated.[2] Some other modified alkaloids were isolated from saprophytic cultures supplemented with other amino acids.[3] Systematically, ergopeptines can be divided into three natural groups according to the first amino acid, ergotamine, ergoxine and ergotoxine. Of the ergotoxine group containing valine as the first amino acid, four natural alkaloids have been isolated: ergocristine, α-ergokryptine, β-ergokryptine and ergocornine. Another member of the ergotoxine group is ergobutyrine having 2-aminobutyric acid as the second amino acid. Bianchi *et al.* isolated it from the submerged cultures of *C. purpurea*.[4] Ohmono and Abe reported the isolation of L-homoleucyl-D-proline lactam, a proposed building block of ergoheptine, an additional member of the ergotoxine family.[5] However, the corresponding cyclol alkaloid has not been isolated to date.

In this paper, we report the isolation, identification and the crystal structure determination of ergogaline, a new natural alkaloid of the ergotoxine family found in two strains of *C. purpurea* grown on rye.

Experimental

Physical Measurements.—M.p.s were determined between cover plates in air and are uncorrected. IR spectra were recorded with a Nicolet 205 FT-IR spectrometer; UV spectra were measured with a Varian DMS 300 spectrometer. NMR spectra were measured on a VXR-400 Varian spectrometer (400 MHz observing frequency for ^1H and 100 MHz for ^{13}C). The chemical shifts are reported in ppm (δ) units downfield from tetramethylsilane which was used as an internal standard. The ^1H and ^{13}C 1D NMR, APT, DEPTGL, COSY, delay-COSY, HOM2DJ, NOESY, ROESY, two-step RELAY and HETCOR experiments were performed with the standard pulse sequences and programming as supplied by Varian.

Positive-ion electron impact (EI) mass spectra were recorded on a Finnigan MAT 90 double-focusing instrument (Finnigan MAT, Bremen, Germany) of BE geometry (magnetic sector preceding the electrostatic one) [ionizing energy 70 eV (*ca.* 1.12×10^{-17} J), source temperature 250 °C, emission current 1 mA, accelerating voltage 5 kV, direct inlet, sample dosed in microgram amount for evaporation]. High resolution measurements were carried out by the peak-matching method using the Ultramark 1600F (PCR Inc., Gainesville, USA) as an internal standard. The resolution was adjusted to 8000 (10% valley definition). The products of metastable collisionally activated decompositions in the first field-free region of the instrument (helium as the collision gas, an applied amount of He attenuated the primary ion beam by 50%) were analysed by daughter B/E constant scan using the manufacturer's software.

Crystallographic Studies.—*Crystal data for ergogaline monohydrate* $C_{33}H_{45}N_5O_6$, $M = 607.75$, monoclinic, space group $P2_1$ (No. 4), $a = 9.972(1)$, $b = 10.902(1)$, $c = 14.761(1)$ Å, $\beta = 94.05(1)°$, $V = 1600.7(3)$ Å^3 (determined by least-squares refinement on diffractometer angles of 25 independent reflections, $\lambda = 1.5418$ Å), $D_c = 1.261$ g cm^{-3}, $Z = 2$, $F(000) = 568$, colourless needles, crystal fragment of dimensions $0.15 \times 0.15 \times 0.1$ mm, μ(Cu-Kα) = 6.74 cm^{-1}.

Delft Instruments TurboCAD-4 diffractometer, ω–2θ scan mode, using Ni-filtered Cu-Kα radiation from a rotating-anode X-ray generator (40 kV, 80 mA, 3×0.3 mm^2 focus), temperature 293 K; 2466 reflections measured (h 0 ⟶ 10, k 0 ⟶ 12, l −16 ⟶ 16, $2\theta_{max} = 120°$). In reducing the data, Lorentz and polarization factors were applied, 1856 reflections 'observed and unique' with $I_o \geqslant 1.96\sigma(I_o)$ were considered observed and included in the following analysis. Direct methods were followed by standard Fourier series and block-matrix least-squares calculations, hydrogen atoms were introduced partly in calculated positions and were partly isotropically refined, OH and water hydrogens were constrained in positions found from a difference Fourier synthesis ($\Delta\rho$ map). The presence of a water molecule was revealed from the $\Delta\rho$ map. Function minimized $\Sigma w(|F_o| - |F_c|)^2$, $w = [\sigma(F_o)^2 + 0.0009\ F^2]^{-1}$, 549 parameters refined, ratio of max. least-squares shift to esd (Δ/esd) = 0.005. The refinement converged to $R = 0.046$ {$R = [\Sigma(|F_o| - |F_c|)^2]/(\Sigma|F_o|)^2$}, $R_w = 0.046$ {$R_w = [\Sigma w(|F_o| - |F_c|)^2]/(\Sigma w|F_o|^2)$}, $S = 1.17$ for all parameters ($S = \{\sqrt{[\Sigma w(|F_o| - |F_c|)^2]/(N_{ref.} - N_{par})}\}$ with the largest residual

Table 1 NMR parameters of **1**[a] ($CDCl_3$, 25 °C, 400 and 100 MHz)

Atom	δ_C	Mult.	δ_H	n_H	Mult.	J/Hz
2	119.05	d	6.942	1	dd	1.8, 1.8
3	110.83	s	—			
4	21.59	t	2.843	1	ddd	14.0, 12.1, 1.8
			3.325	1	dd	14.0, 4.9
5	59.20	d	3.884	1	ddddd	12.1, 4.9, 1.9, 1.1, 0.5
7	48.12	t	2.908	1	ddd	12.0, 2.1, 0.8
			2.965	1	dddd	12.0, 3.6, 0.5, 0.5
8	44.33	d	3.145	1	m	
9	118.92	d	6.392	1	ddd	6.2, 1.9, 0.8
10	139.10	s	—			
11	129.71	s	—			
12	111.03	d	7.146	1	dd	7.0, 1.1
13	123.40	d	7.193	1	dd	7.9, 7.0
14	110.03	d	7.241	1	dd	7.9, 1.1
15	133.81	s	—			
16	126.23	s	—			
17	176.22	s	—			
N-CH_3	40.93	q	2.667	3	s	
$N_{(1)}H$	—		8.030	1	d	1.8
CONH	—		9.785	1	s	
1′	165.26	s	—			
1′α	89.75	s	—			
1′β	34.26	d	2.097	1	m	
1′γ_1	16.88	q	0.904	3	d	6.7
1′γ_2	18.79	q	1.020	3	d	6.9
2′	166.56	s	—			
2′α	52.87	d	4.522	1	dd	7.1, 6.2
2′β	41.32	t	1.751	1	ddd	13.7, 7.4, 6.2
			2.107	1	ddd	13.7, 7.1, 6.9
2′γ	31.42	d	1.932	1	ddddq	7.4, 7.1, 6.9, 6.6, 4.6
2′γ-CH_3	15.34	q	1.011	3	d	6.6
2′δ	29.03	t	1.208	1	ddq	14.1, 7.1, 7.4
			1.572	1	ddq	14.1, 4.6, 7.4
2′ε	11.28	q	0.917	3	t	7.4
3′	103.54	s	—			
3′α	64.41	d	3.661	1	ddd	10.0, 6.1, 1.8
3′β	26.47	t	2.166	1	m	
			2.192	1	m	
3′γ	22.14	t	1.800	1	m	
			2.071	1	m	
3′δ	45.93	t	3.545	1	ddd	12.4, 9.2, 2.7
			3.627	1	ddd	12.4, 9.6, 7.3
3′-OH	—		7.424	1	d	1.8

peaks of −0.19 and 0.22 e Å^{-3}. Programs used were: SDP, SHELX76, SHELXS86 and PARST.[6]

Isolation of Ergogaline, **1**.—Crude α-ergokryptine (30 kg) was isolated from sclerotia (12 000 kg) of the ergot strain CCM 8059 growing on rye in Northern Moravia (Czech Republic). This crude α-ergokryptine was recrystallized from toluene and alkaloid mixture (5.1 kg) was isolated from the mother liquor. This material was chromatographed on silica gel (18.0 kg SiO_2, CH_2Cl_2). Fractions containing α-ergokryptine were recrystallized from toluene again and a concentrate containing 9.2% of ergogaline was isolated from the mother liquor. Two preparative HPLC methods were used for the final purification. As the first step ergocristine was removed by reversed-phase chromatography on a phenyl column (material SGX Phenyl, 7 μm, column 500 × 7.1 mm i.d., from Tessek, Czech Republic). Isocratic elution was carried out with the methanol–water–conc. ammonia solution (725:275:0.2, v/v/v) mixture. Finally, the α-ergokryptine–ergogaline mixture was separated on a octadecyl reversed phase column (SGX RPS, 7 μm, 250 × 8 mm i.d., from Tessek, Czech Republic). Isocratic elution was carried out with acetonitrile–water–conc. ammonia solution (500:500:0.2 v/v/v). Crystals of **1** were obtained directly by the moderate cooling of appropriate fractions.

The **1**·monohydrate was isolated as colourless crystals, m.p. 182 °C (Found: C, 65.1; H, 7.6%; $C_{33}H_{43}N_5O_5 \cdot H_2O$ requires C, 65.22; H, 7.46%); positive ion EI MS, m/z 589 (M^+, 0.5%), 322.1895 ($C_{17}H_{26}N_2O_4$, calc. 322.1893, 10%), 268 (18), 267.1372 ($C_{16}H_{17}N_3O$, calc. 267.1372, 89%), 252.1105 ($C_{12}H_{16}N_2O_4$, calc. 252.1110), 252.1460 ($C_{13}H_{20}N_2O_3$, calc. 252.1474, the sum of both isobaric ions 42%), 225 (16), 224 (78), 221 (46), 207 (46), 206 (20), 196 (24), 195.0780 ($C_9H_{11}N_2O_3$, calc. 195.0770, 49), 182 (15), 181 (16), 180 (29), 179 (15), 167 (29), 154 (99), 153 (15), 100.1127 ($C_6H_{14}N$, calc. 100.1126, 5), 71.0500 (C_4H_7O, calc. 71.0497, 70), 70 (100), 43 (69) and 41 (25); ν/cm^{-1} 1727s, 1674m, 1632vs and 1535m (KBr); λ_{max}(10^{-4} mol dm^{-3} solution in methanol)/nm 312 (ε 8.7 × 10^3 dm^3 mol^{-1} cm^{-1}) and 238 (2.0 × 10^4) ^{1}H and ^{13}C NMR data are listed in Table 1, for numbering see Fig. 1 (the partial fragmentation scheme of ion m/z 589). All reported transitions were verified by linked scan techniques.

Results and Discussion

A new alkaloid **1** was found in two strains of *C. purpurea* Fr. (Tul.), growing on rye—the strain CCM 8057 producing a mixture of ergocornine, α-ergokryptine and β-ergokryptine and the strain CCM 8059 producing α-ergokryptine.

The structure of **1** was primarily solved by X-ray methods. Ordinary ergot alkaloids of the peptide type are described as

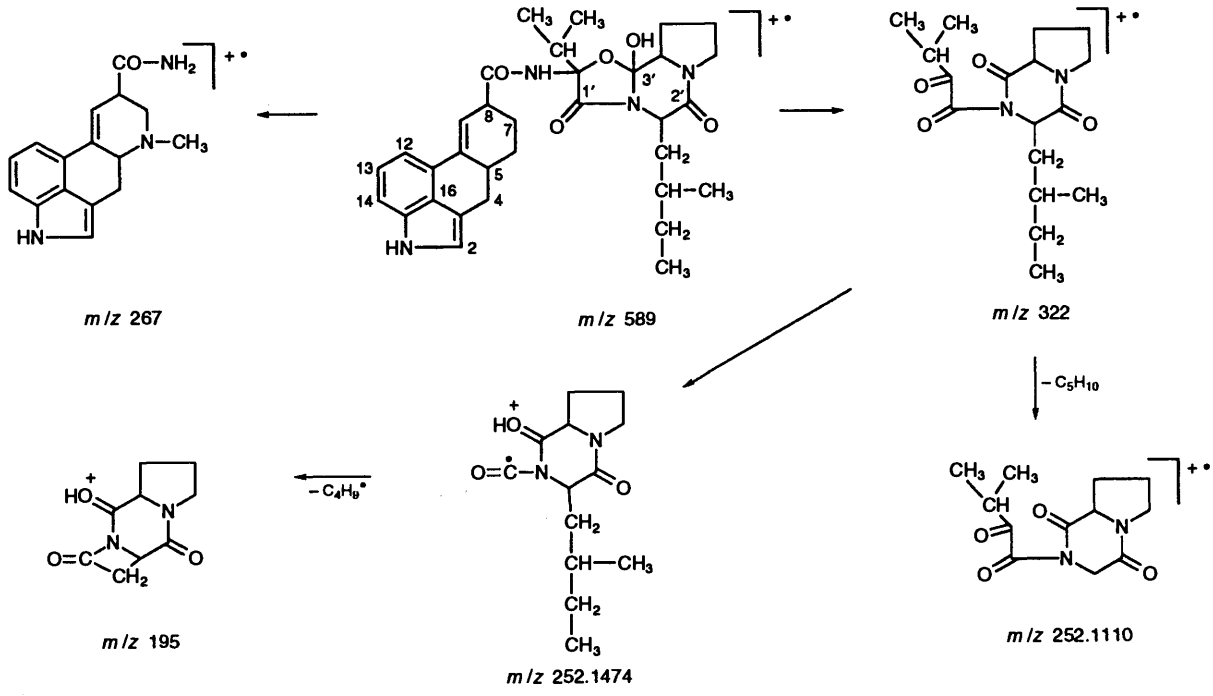

Fig. 1 Dominating ergogaline fragmentation pathways under positive ion EI conditions

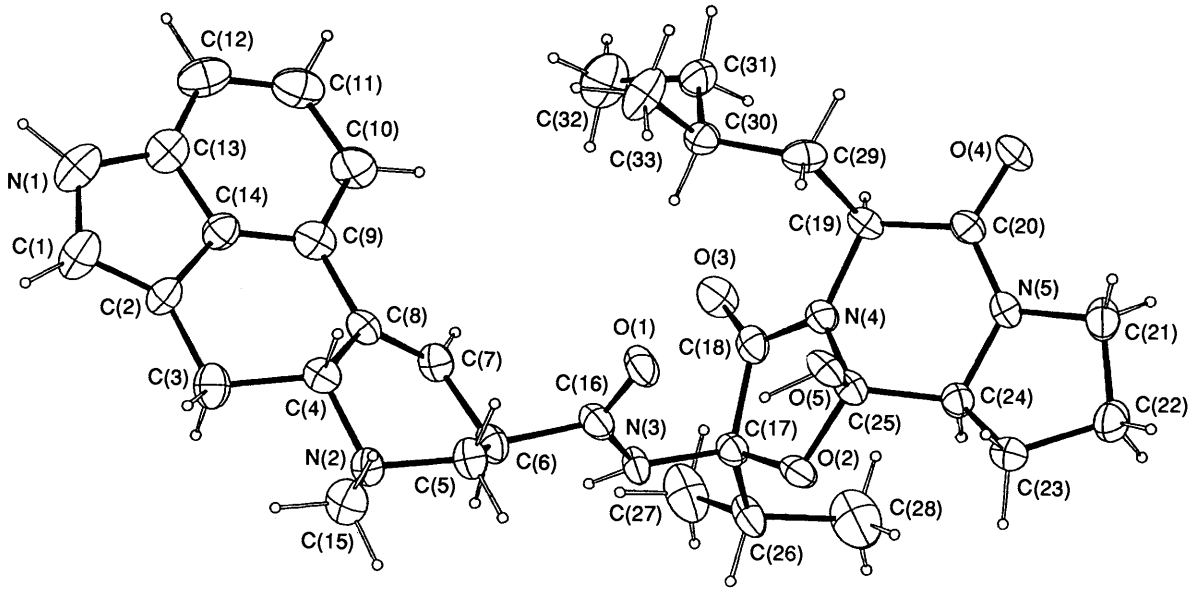

Fig. 2 Structure of ergogaline **1** showing the numbering scheme. Thermal ellipsoids are drawn at 50% probability.

comprising lysergic acid and the 'tripeptide moiety'. It should be noted, however, that from a biosynthetic point of view,[1] all these parts originate from amino acids but in the structure of the alkaloid only one of them retains a bonding environment characteristic of peptides and all others are considerably 'modified', Fig. 2. To describe sufficiently the bonding environment of four peptidic bonds, the peptide nomenclature[7] corresponding to tetrapeptides can be, with some obvious restrictions, applied to describe individual torsion angles ω_i, φ_i, ψ_i and χ_i in **1** as well as in all other ergopeptines. The final positional and thermal parameters of the non-hydrogen atoms, bond distances and angles are deposited as supplementary data.* The packing of molecules in the structure is shown in Fig. 3. The molecules form ribbons extending in the *a* direction. [OW···O(5) = 3.03(1) Å, OW–H(2)W···O(5) = 154(7)°; OW···N(2)($x-1$, y, z) = 2.98(1) Å, OW–H(1)W···N(2) = 154(7)°; and N(3)···O(4)(x, $y-1$, z) = 3.12(1) Å, N(3)–H(1)N(3)···O(4) = 164(4)°].

The indole moiety comprising the *A* and *B* rings of lysergic acid is only slightly puckered. The dihedral angle between the benzene and pyrrole mean planes (*A* and *B* rings) is 1.8(2)°. Ring *C* [C(8), C(9), C(14), C(2), C(3) and C(4)] possesses an E_6 envelope conformation with C(4) atom displaced 0.601(5) Å above the mean plane of remaining five atoms. Puckering parameters[8] are Q = 0.429(5) Å, φ = −60.1(9)° and θ = 56.8(7)°. Ring *D* [N(2), C(5), C(6), C(7), C(8) and C(4)] has a $^1H_2$ half chair conformation with Q = 0.555(5) Å, φ = 39.5(6)° and θ = 55.1(6)°. Torsion angles about the *C*/*D* ring junction C(3)–C(4)–C(8)–C(7) and N(2)–C(4)–C(8)–C(9) are −132.1(5) and 173.8(4)°, respectively. The sp^3 hybridized N(2) atom deviates by 0.485(4) Å from the plane of atoms C(4), C(5)

* This data has been deposited with the Cambridge Crystallographic Data Centre. See 'Instructions for Authors (1994),' *J. Chem Soc., Perkin Trans. 2*, 1994, Issue 1.

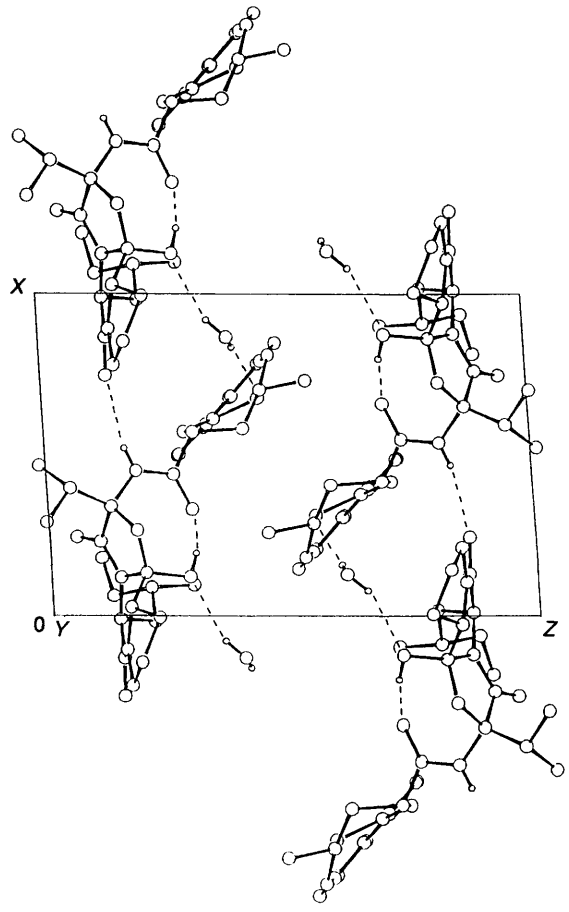

Fig. 3 Packing scheme of ergogaline **1**; dashed lines indicate hydrogen bonds

and C(15). *N*-Methyl and C(8) substituents are in equatorial positions.

Lysergic acid and the remaining tripeptide moiety are connected together by three formally single bonds. The free rotation around two single bonds, restricted rotation around the partial double bond CO–NH, and the two possible symmetrical 'twist' conformations of the ergolene *D* ring apparently give the molecule a fairly large number of possible conformations. The degrees of conformational freedom are described by torsion angles: τ [C(4)–N(2)–C(5)–C(6)] = 70.1(5)° (the '*E*' type backbone),[9] φ_1 [C(5)–C(6)–C(16)–N(13)] = 137.6(5)°, ω_1 [C(6)–C(16)–N(3)–C(17)] = −175.9(4)°, and φ_2 [C(16)–N(3)–C(17)–C(18)] = −54.6(6)°. The values obtained indicate that, as in other ergopeptines,[9] the amide possesses a nearly planar *trans* configuration. Also a strong intramolecular hydrogen bond between the cyclol hydroxy and the amide carbonyl [H(105)···O(1) = 1.74(6), O(5)···O(1) = 2.66(1) Å, O(5)–H(105)···O(1) = 167(5)°] is present thus fixing the steric arrangement of the peptide backbone and the central amide.

The hydroxyvaline, homoisoleucine and proline residues are connected together to create three rings denoted *E*, *F* and *G*. The five-membered *E* ring [C(18), C(17), O(2), C(25) and N(4)] is nearly planar [max. deviation is −0.05 Å for the C(25) atom]. The piperazine ring *F* [C(24), N(5), C(20), C(19), N(4) and C(25)] adopts an E_6 conformation [Q = 0.453(4) Å, φ = 69.2(6)°, θ = 58.0(5)°]. The L-proline residue creates the *G* ring [C(23), C(22), C(21), N(5) and C(24)] which possesses an E_1 conformation [Q = 0.407(6), φ = 177.4(7)°]. This can be alternatively described as an *A* conformation with a C^{β} *-endo* pucker {χ_4^1 [N(5)–C(24)–C(23)–C(22)] = −39.5(5)°}. The exceptionally high value of the puckering amplitude, which is not usually found in other proline-containing structures,[10] is caused by the sp^3 hybridization of the C(25) atom, thus influencing 'ψ_4' [C(20)–N(5)–C(24)–C(25)].[11]

The presence of homoisoleucine is probably the most interesting feature of ergogaline. With regard to the Cambridge Data Files[12] the structure of **1** seems to be the first structure containing this unique amino acid. Because there are two asymmetric carbon atoms in homoisoleucine, four stereoisomers are possible (L,L; L,D; D,L and D,D). The present structure of **1** revealed the presence of a L,L-stereoisomer. The conformation of homoisoleucine is described by the torsion angles: φ_3^1 [N(4)–C(19)–C(20)–N(5)], χ_3^1 [N(4)–C(19)–C(29)–C(30)], $\chi_3^{2,1}$ [C(19)–C(29)–C(30)–C(31)], $\chi_3^{2,2}$ [C(19)–C(29)–C(30)–C(33)], χ_3^3 [C(29)–C(30)–C(31)–C(32)]. The value of φ_2^1 = 0.7(6)° corresponds roughly to the ideal geometry which is, however, seldom found in structures containing Leu or Ile.[13] The conformation about the C^{α}–C^{β} bond is described by the torsion angle χ^1 which has three equilibrium values χ^1 = 60, 180 and −60°, in **1** χ_3^1 = −59.7(6)° was found. The homoisoleucine $C^{\delta 1}$ [C(31)] and $C^{\delta 2}$ [C(33)] carbon atoms adopt a distorted *gauche I* conformation [$\chi_3^{2,1}$ = −72.4(6), $\chi_3^{2,2}$ = 164.9(5)°], which is the preferred conformation for all the $C^{\delta 1}$ and $C^{\delta 2}$ atoms in leucine and/or the $C^{\gamma 1}$ and $C^{\gamma 2}$ atoms in isoleucine and/or valine.[13] The *gauche I* conformation is also adopted by the 2-hydroxyvaline residue in the tripeptide moiety of **1** [$\chi_2^{1,1}$ = 63.9(6), $\chi_2^{1,2}$ = −168.3(5)°]. The C^{ε} atom on the homoisoleucine side-chain is expected[14] to be *trans*-oriented to C^{β} [C(29)] [χ_3^3 = 164.0(5)°]. Thus it can be concluded that the homoisoleucine in **1** possesses in all respects the conformation corresponding to the expected energetic minimum.

Although the structure of **1** was primarily determined by the crystal structure analysis, it can be inferred unambiguously also from the MS and NMR data. Molecular cation–radical *m/z* 589 gave ergine and peptide ions *m/z* 267 and 322 (Fig. 1). According to its fragmentation pattern, the compound is a higher homologue of ergokryptine. The determination of isopropyl as R^1 might be derived from the occurrence of characteristic acylium ion *m/z* 71.0500 (elemental composition C_4H_7O), the nature of R^2 might be determined either from the existence of an immonium ion *m/z* 100.1126 (composition $C_6H_{14}N$), or from the fragmentation of *m/z* 252.

Three vicinal aromatic protons, a system –NHCH=CCH_2CHC=CHCHCH_2–, N-methyl, and CONH–, determined by COSY and delay-COSY, were attributed to the 9-ergolene moiety. The chemical shift of the amido proton (δ 9.785) indicates its participation in hydrogen bonding. The large coupling $J_{8,9}$ = 6.2 Hz defines 8-H as pseudoequatorial, as well as the couplings $J_{7a,8}$ = 3.6 Hz and $J_{7e,8}$ = 2.1 Hz. The N-methyl exhibiting NOE cross-peaks to 4e-H and 7e-H is also pseudoequatorial. That leaves a *gauche*-orientation for the nitrogen lone pair with respect to 5-H, corroborated by its characteristic chemical shift[15] (around 3.8 ppm). Therefore, the *D*-ring of ergolene adopts a flap-down conformation similar to ergotamine.[16] The presence of the cyclol group is demonstrated by characteristic signals of sp^3-hybridized carbons attached to two heteroatoms and carbonyls in the ^{13}C NMR spectrum (Table 1). The remaining spin systems in the ^1H NMR spectrum inferred from COSY, triple-RELAY, and decoupling experiments, –$CH(CH_3)_2$, –$CHCH_2CH(CH_3)CH_2CH_3$, and HO–C–$CH(N)(CH_2)_3N$–, belong to the cyclol part of the molecule. NOE between one isopropyl methyl and the amide NH and between the other Pr^i-methyl and proline α-H indicates that the first amino acid is valine. Whereas several hydrogen atoms in the second amino acid moiety are antiperiplanar in the solid state, the observed vicinal couplings among the corresponding protons (Table 1) show that this is not the case in solution. These values require either a significant contribution of the

gauche rotamers or eclipsed forms. The existence of a preferred conformation of this side chain is supported by NOE cross-peaks between 2'α-H and the downfield resonating 2'β-H, 2'α-H and 2'γ-CH_3. Furthermore, the different couplings of 2'γ-H to the methylene protons of the ethyl group point to the same phenomenon. The W-type coupling of 3'-OH to 3'α-H (1.8 Hz) often observed in other cyclol peptide alkaloids is consistent with a fixed conformation of this hydroxy group (probably due to hydrogen bonding to the amide carbonyl, see also the X-ray part). Large vicinal couplings of 3'α-H to its neighbours (10.0 and 6.1 Hz) reflect a strong puckering of the proline ring (C-β above the plane). Even if our set of vicinal couplings is incomplete, it is evident that the proline ring conformation is not identical with any of the several already described.[17]

The resulting structure represents a new ergot alkaloid of the ergotoxine group containing homoisoleucine. The presence of this acid is very unusual not only judging from the structural features of ergopeptines so far isolated,[1] but also from the point of the biosynthetic origin of this non-protein amino acid. The peptide part of ergot alkaloids is synthetized by a multienzyme complex, as is the case of the biosynthesis of other fungal cyclic peptides and depsipeptides.[18] In general, fungi are able to synthetize amino acids *de novo*[19] independently from external sources. Various feeding experiments have shown that the addition of some natural or unnatural amino acids to the nutrient medium of submerged cultures of *C. purpurea* can alter the proportions of corresponding alkaloids and even some other modified alkaloids were isolated.[3,4] Based on these experiments, it was concluded that the biosynthesis of the peptide moiety of ergot alkaloids is controlled by the relative concentration of their parent amino acids in the internal pool. Since homoleucine may act as a leucine antagonist and thus produce growth inhibition,[20] an intriguing question remains to be solved, whether and why is this acid produced by the fungus or by its host plant. So far, homoisoleucine and their related unsaturated amino acids were found in higher plants but not in fungi.[21] It was proposed, that they may act as nitrogen storage material in seeds.[20] The origin of homoleucine and its possible role in fungal physiology is a subject of further study.

Acknowledgements

This research was in part supported by an EC grant 27ERB40450PL 93-2014 (Commission of the European Communities) and by the Grant Agency of the Czech Republic under Grant 203/94/01504.

References

1 H. G. Floss, *Tetrahedron*, 1976, **32**, 873; P. A. Stadler, *Planta Med.*, 1982, **46**, 131; in *The Alkaloids Chemistry and Pharmacology*, ed. A. Brosi, Academic Press, San Diego, 1990, p. 1.
2 R. G. Powell, R. D. Plattner and S. G. Yates, *J. Nat. Prod.*, 1990, **53**, 1272.
3 E. Beacco, M. L. Bianchi, A. Minghetti and C. Spalla, *Experientia*, 1978, **34**, 1291; S. M. Atwell and P. G. Mantle, *Experientia*, 1981, **37**, 1257; A. Baumert, D. Erge and D. Gröger, *Planta Med.*, 1982, **44**, 122; M. Flieger, P. Sedmera, J. Vokoun, Z. Řeháček, J. Stuchlík, Z. Malinka, L. Cvak and P. Harazim, *J. Nat. Prod.*, 1984, **47**, 970; N. Crespi-Perellino, J. Malyszko, M. Ballabio, B. Gioia and A. Minghetti, *J. Nat. Prod.*, 1992, **55**, 424.
4 M. L. Bianchi, N. Crespi Perellino, B. Gioia and A. Minghetti, *J. Nat. Prod.*, 1982, **45**, 191.
5 S. Ohmono and M. Abe, *J. Agric. Chem. Soc. Jpn.*, 1976, **50**, 543.
6 B. A. Frenz and Associates Inc. SDP, structure determination package, Enraf–Nonius, Delft, The Netherlands, 1985; G. M. Sheldrick, SHELX76, *Program for Crystal Structural Determination*, University of Cambridge, 1976; G. M. Sheldrick, SHELXS86, *Program for Crystal Structure Solution*, University of Göttingen, 1986; M. Nardelli, PARST, a system of computer routines for calculating molecular parameters from results of crystal structure analysis, *Comput. Chem.*, 1983, **7**, 95.
7 IUPAC–IUB Commission on Biochemical Nomenclature, *J. Mol. Biol.*, 1970, **52**, 1.
8 D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, 1975, **97**, 1354.
9 H. P. Weber, in *Ergot Compounds and Brain Function*, eds. M. Goldstein, A. Liberman, D. B. Calne and M. O. Thorner, Raven Press, New York, 1980, p. 25.
10 M. E. Druyan, C. L. Coulter, R. Walter, G. Kartha and G. K. Ambady, *J. Am. Chem. Soc.*, 1976, **98**, 5496.
11 F. A. A. M. de Leeuw, C. Altona, H. Kessler, W. Bermel, A. Friedrich, G. Krack and W. E. Hull, *J. Am. Chem. Soc.*, 1983, **105**, 2237.
12 Cambridge Crystallographic Data File, Crystallographic Data Centre, University Chemical Laboratory, Cambridge, 1993.
13 V. Cody, in *Chemistry and Biochemistry of the Amino Acids*, ed. G. C. Barrett, Chapman and Hall, London, 1985, p. 625.
14 P. K. Ponnuswamy and V. Sasisekharan, *Int. J. Protein Res.*, 1971, **III**, 9.
15 K. Bailey and A. A. Grey, *Can. J. Chem.*, 1972, **50**, 3876.
16 L. Pierri, I. H. Pitman, I. D. Rae, D. A. Winkler and P. R. Andrews, *J. Med. Chem.*, 1982, **25**, 937.
17 H. Kessler, *Angew. Chem.*, 1982, **94**, 509; H. Kessler, A. Friedrich and W. E. Hull, *J. Org. Chem.*, 1981, **46**, 3892;. F. A. A. M. de Leeuw, C. Altona, H. Kessler, W. Bermel, A. Friedrich, G. Krack and W. E. Hull, *J. Am. Chem. Soc.*, 1983, **105**, 2237; Z. L. Mádi, C. Griesinger and R. R. Ernst, *J. Am. Chem. Soc.*, 1990, **112**, 2908; R. M. Brunne, W. F. van Gunsteren, R. Brüschweiler and R. R. Ernst, *J. Am. Chem. Soc.*, 1993, **115**, 4764.
18 H. G. Floss, M. Tcheng-Lin, H. Kobel and P. Stadler, *Experientia*, 1974, **30**, 1369; W. Maier, D. Erge, B. Schumann and D. Gröger, *Biochem. Biophys. Res. Commun.*, 1981, **99**, 155.
19 M. O. Garraway and R. C. Evans, in *Fungal Nutrition and Physiology*, Wiley, New York, 1984, p. 96.
20 L. Flowden and A. Smith, *Phytochemistry*, 1968, **7**, 809.
21 S. Hunt, in *Chemistry and Biochemistry of the Amino Acids*, ed. G. C. Barrett, Chapman and Hall, London, 1985.

Paper 4/01156B
Received 25*th February* 1994
Accepted 9*th May* 1994