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Norleucine, a natural occurrence in a novel ergot alkaloid γ-ergokryptinine

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Summary. A novel natural peptide ergot alkaloid γ-ergokryptinine containing norleucine has been isolated from ergot sclerotia of the field-growing parasitic fungus *Claviceps purpurea* CCM 8059. Its structure was deduced from the NMR and mass spectral data. The final structural proof was provided by the crystal structure determination, which is the first X-ray structure of a natural Nle-containing secondary metabolite. The conformations of three ergopeptinines: γ-ergokryptinine, ergoladinine, and α-ergokryptinine were compared.

Keywords: Norleucine – Ergot alkaloids – γ-Ergokryptinine – Crystal structure determination

1 Introduction

It seemed until recently that the number of ergot alkaloids of peptidic type is limited to 12 by a combination of 3 amino acids (Ala, α-Abu, and Val) at the first and 4 amino acids (Val, Phe, Leu, Ile) at the second position of the tripeptide moiety of ergopeptines (Stadler, 1982; Buchta and Cvak, 1999). From the historical point of view, the forgotten ergoheptine, which was described based on the paper chromatography only (Abe et al., 1970; Pöhm, 1954), was probably the later identified β-ergokryptine (Schlientz et al., 1967). During the last two decades of 20th century several new alkaloids have been prepared by directed biosynthesis (Beacco et al., 1978; Baumert, 1982; Flieger et al., 1984; Crespi-Perellino et al., 1992), but only a few of new ergopeptines were found in nature with the genus *Claviceps* (Bianchi et al., 1982; Crespi-Perellino et al., 1993; Powell et al., 1990; Cvak et al., 1994; Szántay et al., 1994; Cvak et al., 1996; Halada et al., 1998), other fungal genera, e.g., *Acremonium* (Yates et al., 1985), *Sphacelia* (Atwell and Mantle, 1981), *Dicyma* (Vázquez et al., 2003), and in higher plants (Jenett-Siems et al., 1994). Analyses of amino acids in hydrolysates of sclerotia of the parasitic fungus *Claviceps purpurea* and of crude alkaloid mixtures have revealed the presence of some additional aliphatic amino acids and led to the prediction of the existence of yet unknown natural ergot alkaloids (Jegorov et al., 1997). Here we report the identification and crystal structure determination of a novel alkaloid named γ-ergokryptinine (Fig. 1).

2 Material and methods

2.1 Instruments and methods

NMR spectra were measured on a Varian Inova-400 (399.88 MHz for ^{1}H, 100.55 MHz for ^{13}C, $CDCl_3$, 30°C). Following experiments have been performed: H, C, HOM2DJ, gCOSY, TOCSY, HMQC, gHMQC, 1D-TOCSY. Chemical shifts are reported in δ-scale. Positive-ion ESI spectra were recorded on a Finnigan LCQ ion trap instrument (Finnigan MAT, Bremen, Germany). IR spectrum was measured on Nicolet Nexus in KBr pellet and UV spectrum was recorded on Varian DMS 300 in acetonitrile.

2.2 Isolation of γ-ergokryptinine

Crude α-ergokryptine (70.0 kg) was dissolved in the mixture of toluene (270 l) and of methanol (70 l) and the solution was concentrated to the volume of about 150 l in vacuum and diluted by toluene (150 l). The crystalline α-ergokryptine was filtered off and washed by toluene. The mother liquors were concentrated to dryness and the residue was dissolved in methanol (8 l) and the solution was refluxed for 4 hours. The obtained crystalline product was filtered off and washed with methanol, giving a

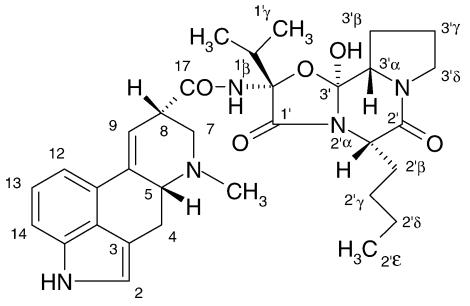

Fig. 1. Numbering scheme for NMR spectrum of γ-ergokryptinine

mixture of ergopeptinines (1620 g). According to the HPLC analysis, the mixture consisted of α-ergokryptinine (85.4%), ergogalinine (3.7%), ergocristinine (2.1%), ergocorninine (2.4%), ergoladinine (1.3%), an unknown alkaloid (1.5%), identified later as γ-ergokryptinine, and some other unknown alkaloids. The mixture of ergopeptinines (500 g) was chromatographed on 10 weight parts of silica gel (Merck, 60 A, 63–200 μm) using dichloromethane with 0.5% methanol as eluent. The fraction containing the unknown alkaloid was chromatographed twice more using the same chromatographic system, giving a concentrate (0.80 g) which was crystallised from methanol, affording the mixture (0.55 g) of γ-ergokryptinine (59.4%) and α-ergokryptinine (35.0%).

The γ-ergokryptinine concentrate (0.50 g) was purified by preparative HPLC on modified silica containing bonded aminopropyl groups (Column Separon SGX NH2, 250 × 25 mm, I.D., 5 μm, from Watrex,

Table 1. ^{1}H and ^{13}C NMR data of γ-ergokryptinine (399.88 and 100.55 MHz, $CDCl_3$, 30°C)

Atom	δ_C	m.	δ_H	n_H	m.	J [Hz]	HMBC (C to H)
2	118.37	D	6.908	1	dd	1.9, 1.8	NH, 4e, 4a
3	110.02	S	–	0			NH, 2, 4e, 4a
4	27.56	T	3.598	1	dd	14.3, 5.4	5
			2.676	1	ddd	14.3, 11.5, 1.8	
5	62.84	D	3.239	1	dddd	11.5, 5.4, 2.2, 2.1	4e, 4a, 7e, 7a, 9, NMe
7	54.73	T	3.161	1	ddd	11.9, 1.3, 1.2	9, NMe
			2.764	1	dd	11.9, 3.7	
8	44.01	D	3.087	1	m		7e, 9
9	117.61	D	6.521	1	ddd	6.4, 2.1, 1.2	5, 7e, 8
10	137.29	S	–	0			4e, 4a, 5, 8, 12
11	127.41	S	–	0			9a, 13
12	112.84	D	7.114	1	dd	7.2, 1.1	14
13	123.40	D	7.155	1	dd	7.7, 7.1	
14	110.16	D	7.220	1	dd	7.7, 1.1	12
15	133.95	S	–	0			NH, 2, 13
16	126.25	S	–	0			NH, 2, 4e, 4a, 12, 14
17	176.26	S	–	0			7a, 7e, 8, 9, CONH
NH	–	–	8.149	1	d	1.9	
NMe	43.36	Q	2.629	3	s		5, 7e
CONH	–	–	9.949	1	s		
1′	165.37	S	–	0			CONH
1′α	89.94	S	–	0			1′β, 1′γ_d, 1′γ_u, CONH
1′β	34.18	D	2.080	1	qq	6.9, 6.8	1′γ_d, 1′γ_u,CONH
1′γ_d	15.53	Q	1.147	3	d	6.9	1′β, 1′γ_u
1′γ_u	17.00	Q	0.920	3	d	6.8	1′β, 1′γ_d
2′	165.86	S	–	0			2′α
2′α	55.13	D	4.380	1	dd	7.0, 5.8	2′β_d, 2′β_u, 2′γ_u
2′β	33.38	T	2.139	1	m		2′α, 2′γ_d, 2′γ_u, 2′δ
			1.950	1	m		
2′γ	28.66	T	1.576	1	m		2′α, 2′β_d, 2′β_u, 2′δ, 2′ε
			1.489	1	m		
2′δ	22.50	T	1.349	2	m		2′β_d, 2′β_u, 2′γ_d, 2′γ_u, 2′ε
2′ε	13.95	Q	0.880	3	t	7.3	2′δ
3′	103.48	S	–	0			2′α, 3′α, 3′–OH
3′α	64.35	D	3.654	1	ddd	10.0, 6.2, 1.8	3′β_d, 3′β_u, 3′–OH
3′β	26.47	T	2.191	1	m		3′α, 3′γ_d, 3′γ_u, 3′δ_u
			2.166	1	m		
3′γ	22.18	T	2.053	1	m		3′β_d, 3′β_u, 3′δ_d, 3′δ_u
			1.797	1	m		
3′δ	45.95	T	3.618	1	m		3′β_d, 3′β_u
			3.540	1	m		
3′–OH	–	–	7.329	1	d	1.8	

a axial; *e* equatorial; *d* downfield; *u* upfield

Czech Republic, isocratic elution with the dichloromethane/methanol 99:1 v/v mixture, flow 7 ml/min, detector set at 345 nm). Fractions were pooled according the analytical results of individual fractions (Column Separon SGX NH2, 250 × 4 mm, I.D., 5 μm, from Tessek, Czech Republic, isocratic elution with the dichloromethane/methanol 99:1 v/v mixture, flow 2.5 ml/min, detector set at 310 nm, relative retention time of γ-ergokryptinine was 1.22 with respect to α-ergokryptinine). Finally, about 100 mg of γ-ergokryptinine of purity >99% was isolated. Single crystals were prepared by dissolution of γ-ergokryptinine (23 mg) in methanol (3.5 ml) at 64°C, subsequent addition of water (100 μl), and standing overnight. Mp. 238.8–239.6°C (microscope, between cover plates). IR (KBr pellet): O–H stretching 3309 m, C=O stretching 1665 s, 1645 s, and 1729 s, C=C stretching 1564 w, 1604 w [cm^{-1}]. UV (acetonitrile): 311 nm ($\varepsilon_M = 6.3 \cdot 10^3\ dm^3\ mol^{-1}\ cm^{-1}$), 241 nm ($\varepsilon_M = 1.6 \cdot 10^4\ dm^3\ mol^{-1}\ cm^{-1}$).

2.3 Crystallographic study

γ-Ergokryptinine $C_{32}H_{41}N_5O_5$, $M_r = 575.71$, orthorhombic, space group $P2_12_12_1$, $a = 6.5600(1)$, $b = 17.8830(3)$, $c = 25.4550(4)$ Å, $V = 2986.19(8)$ Å^3, $Z = 4$, $D_c = 1.280\ g\ cm^{-3}$, Nonius Kappa CCD area-detector diffractometer, φ and ω scans technique, MoK_α radiation, $\lambda = 0.71073$ Å, $T = 150(2)$ K. A total of 51419 reflections were measured ($h\ -8 \rightarrow 8$, $k\ -23 \rightarrow 23$, $l\ -32 \rightarrow 33$, $\theta_{max} = 27.48°$). Merging equivalents gave 5401 unique reflections ($R_{int} = 0.07$), of which 3034 were unique and observed ($I > 1.96\ \sigma(I)$) and used for the refinement. The structure was solved by direct methods and anisotropically refined. The H-atoms were placed in their calculated positions and allowed to ride on their attached C-atoms in distances of 1.0 Å. The atoms H511, H531 and H551 were located in difference Fourier maps and refined fixed. The minimized function was $\Sigma w(F_o - F_c)^2$, where $w = [\text{weight}][1 - (\delta F/6\sigma F)^2]^2$, (Prince, 1982; Watkin, 1994), $(\Delta/\delta)_{max} = 0.0003$, $R = 0.042$, $S = 1.075$ with the largest residual peaks of −0.23 and $0.19\ e \cdot Å^{-3}$. Data collection: COLLECT (Nonius, 1997); cell refinement: DENZO/SCALEPACK (Otwinowski and Minor, 1997); data reduction: DENZO/SCALEPACK; program used to solve structure: SHELXS86 (Sheldrick, 1986); program used to refine structure: CRYSTALS (Watkin et al., 2001); molecular graphics: ORTEP-3 (Farrugia, 1997).

Important backbone conformation angles [°]: Val: $\varphi_2 = -63.6(3)$, $\psi_2 = 112.1(2)$, $\omega_2 = -178.3(2)$, $\chi_2{}^{1,1} = 176.2(2)$, $\chi_2{}^{1,2} = -59.5(3)$; Nle: $\varphi_3 = -147.3(2)$, $\psi_3 = 2.6(3)$, $\omega_3 = -5.1(4)$, $\chi_3{}^1 = -49.4(3)$, $\chi_3{}^2 = -171.3(2)$, $\chi_3{}^3 = -58.6(3)$; Pro: $\varphi_4 = -22.6(3)$, $\psi_4 = 49.5(2)$. Full data are deposited with the Cambridge Crystallographic Data Centre.

α-Ergokryptinine (IVAX Pharmaceuticals, crystallised from methanol/water) $C_{32}H_{41}N_5O_5$, $M_r = 575.71$, orthorhombic, space group $P2_12_12_1$, $a = 6.571(1)$, $b = 18.337(4)$, $c = 26.234(5)$ Å, $V = 3161(1)$ Å^3, $Z = 4$, $D_c = 1.210\ g\ cm^{-3}$, Nonius CAD4 diffractometer, $2\theta/\omega$ scans technique, CuK_α radiation, $\lambda = 1.54184$ Å, $T = 293$ K. A total of 6283 reflections were measured ($h\ 0 \rightarrow 8$, $k\ 0 \rightarrow 22$, $l\ -31 \rightarrow 31$, $\theta_{max} = 69.93°$), 5130 of them were unique and observed ($I > 2\ \sigma(I)$) and included in the structure analysis. The structure was solved by direct methods and anisotropically refined. C28 in the valine residue is disordered in two positions labelled as C28 of occupancy 0.68(1) – conformation denoted as **A**, and C28′, occupancy 0.32(1) – **B**. The most of the leucine residue is disordered in two branches – C30, C31 and C32 of occupancy 0.29(2) – **A**, and C30′, C31′ and C32′ of occupancy 0.71(2) – **B**. The H-atoms were located in difference Fourier maps and isotropicaly refined, except of H1, H4, H52, H232, which were refined riding in distance of 0.95(1) Å and those H-atoms in disordered residues, that were refined riding in their calculated positions with their respective occupancy values. The minimized function was $\Sigma w(Fo^2 - Fc^2)^2$, where $w = 1/[\sigma^2(Fo^2) + (0.1236P)^2 + 1.0004P]$ *and* $P = (Fo^2 + 2Fc^2)/3$, $(\Delta/\delta)_{max} = -0.005$, $R = 0.0670$, $S = 1.028$ with the largest residual peaks of −0.18 and $0.74\ e \cdot Å^{-3}$. Data collection, cell refinement and data reduction: SDP (Frenz, 1985); program used to solve structure: SHELXS86 (Sheldrick, 1986); program used to refine structure: SHELXL (Sheldrick, 1993).

Important backbone conformation angles [°]: conformation of Val: $\varphi_2 = -47.2(5)$, $\psi_2 = 112.1(2)$, $\omega_2 = -177.8(3)$, $\chi_2{}^{1,2} = -63.0(5)$, conformation of Val **A**: $\chi_2{}^{1,1} = 166.6(5)$, conformation of Val **B**: $\chi_2{}^{1,1} = 65(1)$; Leu: $\varphi_3 = -149.5(3)$, $\psi_3 = -7.9(5)$, $\omega_3 = 0.9(6)$, conformation of Leu **A**: $\chi_3{}^1 = -63(1)$, $\chi_3{}^{2,1} = -58(2)$, $\chi_3{}^{2,2} = 179(2)$; conformation of Leu **B**: $\chi_3{}^1 = 157(1)$, $\chi_3{}^{2,1} = -87(1)$, $\chi_3{}^{2,2} = 179(2)$; Pro: $\varphi_4 = -19.8(5)$, $\psi_4 = 44.5(4)$. Full data are deposited with the Cambridge Crystallographic Data Centre.

CCDC contains the supplementary crystallographic data for this paper: γ-ergokryptinine CCDC 227875 and α-ergokryptinine CCDC 227876. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk)

3 Results and discussion

In our program of searching for potential impurities of ergotoxine, we identified an unusual ergopeptine containing norleucine. A crude alkaloid concentrate was obtained by the extraction of an ergot strain producing α-ergokryptine (*Claviceps purpurea* CCM 8059), but was also found in other ergot strains producing ergotoxine. The concentrate of α-ergokryptine was crystallised from toluene and the mother liquors were concentrated and converted to a mixture of ergopeptinines by crystallisation from methanol. The mixture of ergopeptinines was then separated by chromatography on a silica gel. Finally, γ-ergokryptinine fraction was purified by preparative HPLC and crystallised from methanol/water.

Mass spectrometry revealed that the compound is isobaric with α- or β-ergokryptines ($C_{32}H_{41}N_5O_5$). Also the ms^2 of the $[M + H]^+$ ion m/z 576 provided identical fragment ion $[M + H - H_2O]^+$ m/z 558 and ion m/z 348, representing the splitting of the diketopiperazine moiety. The later ion, analogous to the fragmentation observed under EI conditions (Crespi-Perellino et al., 1987), makes it possible to calculate the summary composition of the side chains of the first and second amino acid of the tripeptide moiety as C_3H_7 and C_4H_9, respectively, but lacks the information about the structure and chirality of these side chains.

The observation of three carbonyls and two sp^3-hybridized carbons attached to two heteroatoms each shows that the investigated compound belongs to ergot peptide alkaloids. There are thirty-two signals in the ^{13}C NMR spectrum: four methyls, eight methylenes, ten methines (four =CH among them), and ten quaternary carbons. Thirty-eight hydrogens are bonded to carbons; three to heteroatoms (two N–H's and one OH, see below). These facts verify the summary formula $C_{32}H_{41}N_5O_5$. ^1H NMR spectrum contains, besides the singlets of N-methyl and CONH, a three-spin system made of vicinal aromatic protons, and partial structures $(CH_3)_2CH$–, $NCH(CH_2)_3CH_3$, –NHCH=CCH_2CHC=CHCH(X–)CH_2–, $C(OH)CHN(CH_2)_3$. Indole

NH, CONH and 3′-OH were easily differentiated according to their coupling pattern in HMBC (Fig. 2). This experiment also determines the attachment of CONH to C-8 (X above) and the location of N-methyl between C5 and C7. Therefore, the molecule contains a 9-ergolene moiety. To determine the configuration at C8, the conformation of the ring D has to be solved first (Pierri et al., 1982; Cvak et al., 1994a). Coupling $J_{8,9} = 6.4$ Hz means a pseudoequatorial H8. Chemical shift of CONH proton corresponds to its participation in hydrogen bonding; that of H5 (3.239 ppm) is diagnostic for a *trans* arrangement of H5 and the nitrogen lone electron pair (Bailey and Grey, 1972). Therefore, the D-ring exists in so-called flap-up conformation and the cyclol is 8α- attached (i.e., indeed an ''-inine''). Isolated isopropyl group is attached to C1′α. The *n*-butyl side chain is attached to C2′α and the proline is located in its usual position. Long-range coupling between H2′α and H3′α suggest that both these atoms are located on the same side of the (modified) diketopiperazine ring.

The final structural proof was provided by the crystal structure determination, Fig. 3. The X-ray study has confirmed the elemental composition, presence of norleucine in γ-ergokryptinine and provided relative configuration of all its chiral centres. Up to now, only few X-ray structures of norleucine (Harding et al., 1995) or its synthetic derivatives (Nigovic et al., 1992; Fenude and Casalone, 1996) have been reported. Structure of γ-ergokryptinine is apparently the first X-ray structure of natural norleucine-containing secondary metabolite. The conformation of γ-ergokryptinine is very close to that found in ergoladinine structure (Cvak et al., 1996). The almost isostructural molecular packing explains this surprisingly high similarity. The analogous force fields of the neighbours allow molecules to settle in similar conformations and vice versa the chemical and sterical demands of the molecules make the compounds crystallise in the same structural type. For comparison, the crystal structure of α-ergokryptinine was also determined. Despite some obvious differences in the conformation of the side chain of leucine (see Experimental), this third structure of ergopeptinines determined so far indicates that these compounds tend to prefer the same structural arrangement.

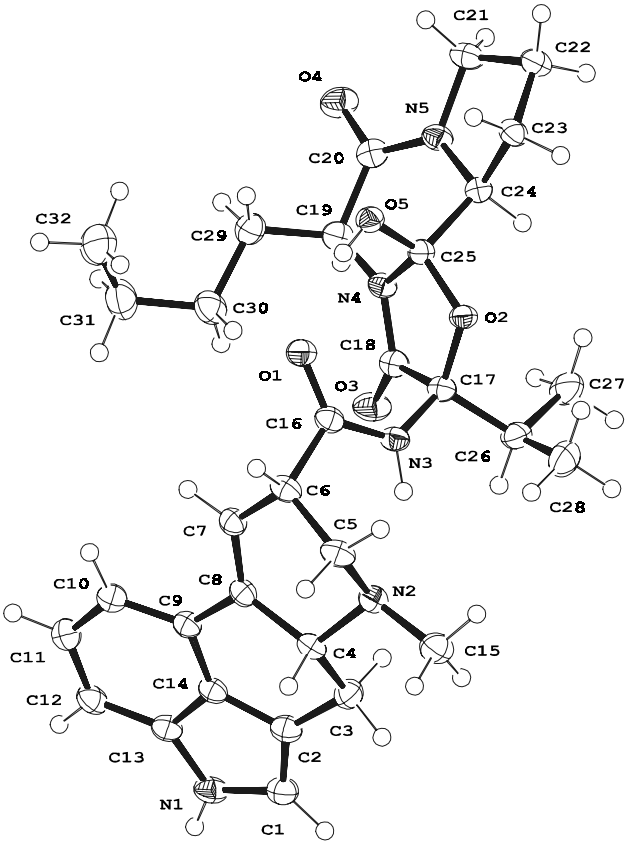

Fig. 3. ORTEP drawing of γ-ergokryptinine, thermal elipsoids are drawn at 50% probability

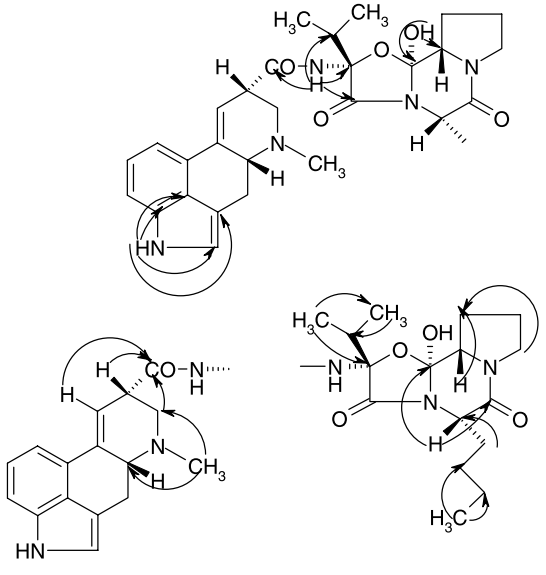

Fig. 2. Long range couplings in NMR spectrum of γ-ergokryptinine

There are two intramolecular hydrogen bonds in the structure of γ-ergokryptinine: the obligatory O5-H551 . . . O1 typical for ergopeptine alkaloids and N3-H531 . . . N2, which stabilises the 'flap-up' conformation present in bases only. The only intermolecular bond is provided by N1-H511 . . . O4 $(-x + 1/2, -y, +z - 1/2)$, which forms infinite chains along the 2_1 screw axis in Z direction. This interaction appears to be responsible for the needle-like shape of the crystals.

γ-Ergokryptinine belongs to the *iso*-lysergic acid series (-inines) with the 8α configuration at the C-8 atom. Similarly as with other series of secondary metabolites synthesised extraribosomally by multienzyme system (Keller, 1999), the possibility that norleucine can be incorporated instead of aliphatic amino acids is not unexpected and has already been detected by *in vitro* experiments (Beacco et al., 1978). Moreover, also the misincorporation of norleucine (amino acid side chain $-(CH_2)_3-CH_3$) instead of methionine ($-(CH_2)_2-S-CH_3$) is well known even with ordinary protein synthesis (Jakubowski and Goldman, 1992). It is worth mentioning that just methionine containing ergot alkaloid was described recently (Cvak et al., 1996). Thus, γ-ergokryptinine is the 8th member of ergotoxine (-inine) series (Bianchi et al., 1982; Cvak et al., 1994b, 1996).

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References

Abe M, Fukuhara T, Ohmono S, Hori M, Tabuchi T (1976) Production of alkaloids and related substances by fungi. Part VII. Isolation of prolyldiketopiperazines corresponding to peptide portions of peptide-type alkaloids from sclerotia and saprophytic cultures of Ergot fungi. J Agr Chem Soc Japan 50: 543–579

Atwell SM, Mantle PG (1981) Hydroxydihydroergosine, a new ergot alkaloid analogue from directed biosynthesis by *Sphacelia sorghi*. Experientia 37: 1257–1258

Bailey K, Grey AA (1972) Conformational study of lysergic acid and isolysergic acid dialkylamides by proton magnetic resonance spectroscopy. Can J Chem 50: 3876–3885

Baumert A, Erge D, Gröger D (1982) Incorporation of thiazolidine-4-carboxylic acid into ergosine by *Claviceps purpurea*. Planta Med 44: 122–123

Beacco E, Bianchi ML, Minghetti A, Spalla C (1978) Directed biosynthesis of analogues of ergot peptide alkaloids with *Claviceps purpurea*. Experientia 34: 1291–1293

Bianchi ML, Crespi Perellino N, Gioia B, Minghetti A (1982) Production by *Claviceps purpurea* of two new peptide ergot alkaloids belonging to a new series containing α-aminobutyric acid. J Nat Prod 45: 191–196

Buchta M, Cvak L (1999) Ergot alkaloids and other metabolites of the genus *Claviceps*. In: Kŕen V, Cvak L (eds) Ergot. The genus *Claviceps*. and aromatic plants – Industrial profiles, vol. 6. Harwood Academic Publishers, Amsterdam, pp 173–200

Crespi-Perellino N, Ballabio M, Gioia B, Minghetti A (1987) Two unusual ergopeptines produced by a saprophytic culture of *Claviceps purpurea*. J Nat Prod 50: 1065–1074

Crespi-Perellino N, Malyszko J, Ballabio M, Gioia B, Minghetti A (1992) Directed biosynthesis of unnatural ergot peptide alkaloids. J Nat Prod 55: 424–427

Crespi-Perellino N, Malyszko J, Ballabio M, Gioia B, Minghetti A (1993) Identification of ergobine, a new natural peptide ergot alkaloid. J Nat Prod 56: 489–493

Cvak L, Stuchlík J, Schreiberová M, Sedmera P, Havlíček V, Flieger M (1994a) 2,3-Dihydro-2-oxoergolene derivatives, Collect. Czech Chem Commun 59: 929–942

Cvak L, Jegorov A, Sedmera P, Havlíček V, Ondráček J, Hušák M, Pakhomova S, Kratochvíl B, Granzin J (1994b) Ergogaline, a new ergot alkaloid, produced by *Claviceps purpurea*: Isolation, identification, crystal structure and molecular conformation. J Chem Soc, Perkin Trans 2: 1861–1865

Cvak L, Minář J, Pakhomova S, Ondráček J, Kratochvíl B, Sedmera P, Havlíček V, Jegorov A (1996) Ergoladinine, an ergot alkaloid. Phytochemistry 42: 231–233

Farrugia LJ (1997) ORTEP-3 for Windows – a version of ORTEP-III with a Graphical User Interface (GUI) by J. Farrugia. J Appl Cryst 30: 565–565

Fenude E, Casalone G (1996) Three protected tetrapeptides. Acta Crystallographica C52: 973–978

Flieger M, Sedmera P, Vokoun J, Řeháček Z, Stuchlík J, Malinka Z, Cvak L, Harazim P (1984) New alkaloids from a saprophytic culture of *Claviceps purpurea*. J Nat Prod 47: 970–976

Frenz BA, Associates Inc. (1985) SDP. Structure determination package. Enraf-Nonius, Delft, The Netherlands

Halada P, Sedmera P, Havlíček V, Jegorov A, Cvak L, Ryska M (1998) Mass spectrometric amino acid structure determination in ergopeptines. Eur. Mass Spectrom 4: 385–392

Harding MM, Kariuki BM, Williams L, Anwar J (1995) DL-Norleucine: redetermination of structure and observations with synchrotron radiation Laue diffraction on heating towards transformation. Acta Cryst B51: 1059–1062

Jakubowski H, Goldman E (1992) Editing of errors in amino acids selection for protein synthesis. Microbiol Rev 56: 412–429

Jegorov A, Šimek P, Heydová A, Cvak L, Minář J (1997) Free and bonded homoisoleucine in sclerotia of the parasitic fungus *Claviceps purpurea*. Amino Acids 12: 9–19

Jenett-Siems K, Kaloga M, Eich E (1994) Ergobalansine/ergobalansinine, a proline-free peptide-type alkaloid of the fungal genus *Balansia*, is a constituent of *Ipomea piurensis*. J Nat Prod 57: 1304–1306

Keller U (1999) Biosynthesis of ergot alkaloids. In: Kŕen V, Cvak L (eds) Ergot. The genus *Claviceps*. and aromatic plants – Industrial profiles, vol. 6. Harwood Academic Publishers, Amsterdam, pp 95–163

Nigovic B, Kojic-Prodic B, Puntarec V (1992) Structure of a biologically active conjugate of auxin: *N*-indol-3-ylacetyl-L-norleucine at 297 and 133 K. Acta Cryst C48: 1079–1082

Nonius (1997) COLLECT. Nonius BV Delft, The Netherlands

Otwinowski Z, Minor W (1997) Processing of X-Ray diffraction data collected in oscillation mode. In: Methods in enzymology, vol. 276. Macromolecular crystallography, Part A. In: Carter CW Jr, Sweet RM (eds) Academic Press, New York, pp 307–326

Pierri L, Pitman IH, Rae ID, Winkler DA, Andrews PR (1982) Conformational analysis of the ergot alkaloids ergotamine and ergotaminine. J Med Chem 25: 937–942

Pöhm M (1954) Über zwei neue Peptid-Alkaloide aus Mutterkorn. Monatsh 85: 1010–1012

Powell RG, Plattner RD, Yates SG, Clay K, Leuchtmann J (1990) Ergobalansin, a new ergot-type peptide alkaloid isolated from *Cenchrus echinatus* (Sandbur grass) infected with *Balansia obtecta* and produced in liquid culture of *B. obtecta* and *Balansia cyperi*. J Nat Prod 53: 1272–1279

Prince E (1982) Mathematical techniques in crystallography and materials science. Springer, New York

Sheldrick GM (1986) SHELXS86. Program for crystal structure solution. University of Göttingen, Germany

Sheldrick GM (1993) SHELXL93. Program for the refinement of crystal structures. University of Göttingen, Germany

Schlientz W, Brunner R, Rüegger A, Berde B, Stürmer E, Hofmann A (1967) β-Ergokryptine, a new alkaloid of the ergotoxine group. Experientia 23: 991–992

Stadler PA (1982) Neuere Ergebnisse der Mutterkornalkaloid-Forschung. Planta Med 46: 131–144

Szántay C Jr, Bihari M, Brlik J, Csehi A, Kassai A, Aranyi A (1994) Structural elucidation of two novel ergot alkaloid impurities in α-ergokryptine and bromokryptine. Acta Pharm Hung 64: 105–108

Vázquez MJ, Roa AM, Reyes F, Vega A, Rivera-Sagredo A, Thomas DR, Díez E, Hueso-Rodríguez JA (2003) A novel ergot alkaloid as a 5-HT_{1A} inhibitor by *Dicyma* sp. J Med Chem 46: 5117–5120

Watkin DJ (1994) The control of difficult refinements. Acta Cryst A50: 411–437

Watkin DJ, Prout CK, Carruthers JR, Betteridge PW, Cooper RI (2001) CRYSTALS. Issue 11. Chemical Crystallography Laboratory, Oxford, England

Yates SG, Plattner RD, Garner GB (1985) Detection of ergopeptine alkaloids in endophyte infected, toxic Ky-31 tall fescue by mass spectrometry/mass spectrometry. J Agric Food Chem 33: 719–722

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