Pergamon

PII: S0031-9422(96)00505-5

Phytochemistry, Vol. 44, No. 2, pp. 365–369, 1997
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0031-9422/97 $17.00 + 0.00

8α-HYDROXY-α-ERGOKRYPTINE, AN ERGOT ALKALOID

LADISLAV CVAK, ALEXANDR JEGOROV,*† SVETLANA PAKHOMOVA,‡ BOHUMIL KRATOCHVÍL,‡ PETR SEDMERA,§ VLADIMÍR HAVLÍČEK§ and JOSEF MINÁŘ

Galena Co., R. & D., 747 70 Opava-Komárov, Czech Republic; *Galena Co., Research Unit, Branišovská 31, 370 05 České Budějovice, Czech Republic; ‡Department of Solid State Chemistry, Prague Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic; §Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1983, 142 20 Prague 4, Czech Republic

(*Received* 11 *April* 1996)

Key Word Index—*Claviceps purpurea*; fungus; sclerotia; ergot alkaloids; hydroxylation; ergokryptine; bromokryptine.

Abstract—A new natural ergopeptine alkaloid hydroxylated at C-8 of the lysergic acid moiety has been isolated from sclerotia of the field-growing parasitic fungus *Claviceps purpurea*. Its structure was established by spectroscopic and X-ray diffraction analyses. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Ergopeptines (Fig. 1) are usually considered to be the final step in the synthesis of ergot alkaloids of the peptidic type. Because of the poor specifity of the multienzyme systems involved in their biosynthesis, a number of analogues differing in the amino acid composition of the peptide moiety have been isolated [1–5]. However, only two examples of the natural occurrence of ergopeptines modified at the lysergic acid moiety have been reported, namely 9,10-dihydroergosine produced by *Sphacelia sorghi* [6] and 8-hydroxyergotamine [7, 8]. In the present paper, we describe an additional example of a new natural ergopeptine alkaloid hydroxylated at the C-8 atom of the lysergic acid moiety.

RESULTS AND DISCUSSION

An alkaloid concentrate was obtained by extraction of a field ergot strain producing α-ergokryptine (*Claviceps purpurea* CCM 8059). Crude α-ergokryptine was recrystallized from toluene, and the mother liquors further separated by chromatography on silica gel. To identify potential trace impurities of an important drug, bromokryptine mesylate, produced from this natural source, a fraction rich in minor ergot alkaloids was brominated. An unknown polar compound was detected by HPLC, besides a number of already known 2-bromoergopeptines. The pure compound (**1**) was obtained by preparative HPLC on a reverse-phase column with acetonitrile–water–concentrated ammonia mixture as eluent. Based on mass spectral and NMR data (see Experimental), **1** was identified as 2-bromo-8-hydroxy-α-ergokryptine. The absence of **1** among the side-products obtained by bromination of pure α-ergokryptine indicated that the parent natural compound (**2**) should be contained already in mother liquors.

The presence of **2** among polar compounds in some fractions obtained by the chromatography of mother liquors remaining after α-ergokryptine recrystallization was suspected upon inspection of HPLC chromatograms. Compound **2** was preconcentrated by flash chromatography on silica gel with a dichloromethane–methanol (1–3%) step gradient yielding a 31.3% concentrate of the new alkaloid. Finally, **2** was obtained by preparative HPLC on a reverse-phase column with the methanol–water–concentrated ammonia mixture as eluent. Single crystals were obtained simply by cooling of chromatographic fractions. Owing to their instability in air, crystals were transferred into glass capillaries, and the structure of this solvated form was resolved by X-ray diffraction. Crystal structure determination revealed that the crystalline phase is the methanol solvate-monohydrate of 8α-hydroxy-α-ergokryptine (Fig. 2).

As the hydroxyl has the 8α-orientation, the alkaloid is derived from lysergic acid. All cyclol amino acids belong to the L-series. The D ring of ergine adopts the energetically favoured flap-up conformation. [9]. Various authors describe this conformation also as E [10], D_1 [11] or $^2H_3$ [12]. This conformation is typical for ergopeptine alkaloids recrystallized from protic solvents or for the ergot alkaloid salts, whereas ergopeptine bases having a flap-down configuration of the D (CB [10]; D_2 [11] or $^3H_2$ [12]) upon recrystallization from

†Author to whom correspondence should be addressed.

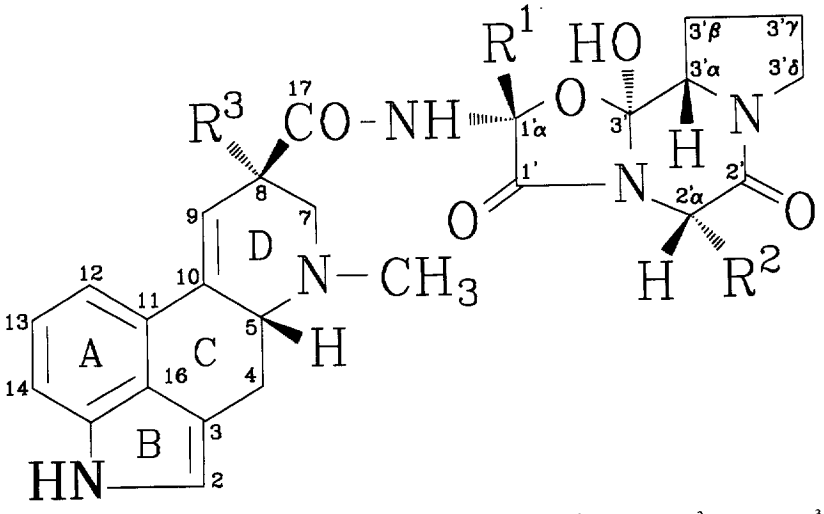

Fig. 1. General structure of ergot alkaloids. 8β (8*R*)-series—ergopeptines; for R^1 = methyl: R^2 = benzyl, R^3 = H-ergotamine; R^1 = isopropyl, R^2 = isobutyl, R^3 = H-α-ergokryptine; for R^1 = methyl: R^2 = benzyl, R^3 = OH-8α-hydroxyergotamine; R^1 = isopropyl, R^2 = isobutyl, R^3 = OH-8α-hydroxy-α-ergokryptine (**2**).

non-protic solvents. A ubiquitious hydrogen bond between the cyclol OH and lysergic acid carbonyl [1] is also present in this structure.

The amorphous form obtained by the evaporation of dichloromethane solutions was studied by mass and NMR spectroscopy. The protonated form of **2** was observed in the positive ion electrospray mass spectrum at *m/z* 592. Diagnostic fragment ions were generated by electron impact ionization. The hydroxylated ergine and peptide parts were characterized by ions *m/z* 283 and 308, respectively. The nature of the R^1 and R^2 substituents in the peptide moiety (R^1 = *i*-Pr, R^2 = *i*-Bu, see Fig. 1) was deduced from the occurrence of the corresponding acylium (*m/z* 71) and immonium (*m/z* 86) ions [13]. The ^{1}H NMR spectrum measured in $CDCl_3$ contained singlets of CONH (9.48 ppm), tertiary OH (4.14 ppm) and *N*-methyl (2.65 ppm), and doublets of indole NH (8.06 ppm, J = 1.7 Hz) and 3'-OH (7.28 ppm, J = 1.9 Hz). Besides the features characteristic for a peptide alkaloid (three carbonyls, –OCO– and –OCN– carbons), the presence of an additional quaternary carbon attached to oxygen (71.5 *s*) was noticeable. Spin systems found by COSY comprise three vicinal aromatic protons, giving the partial struc-

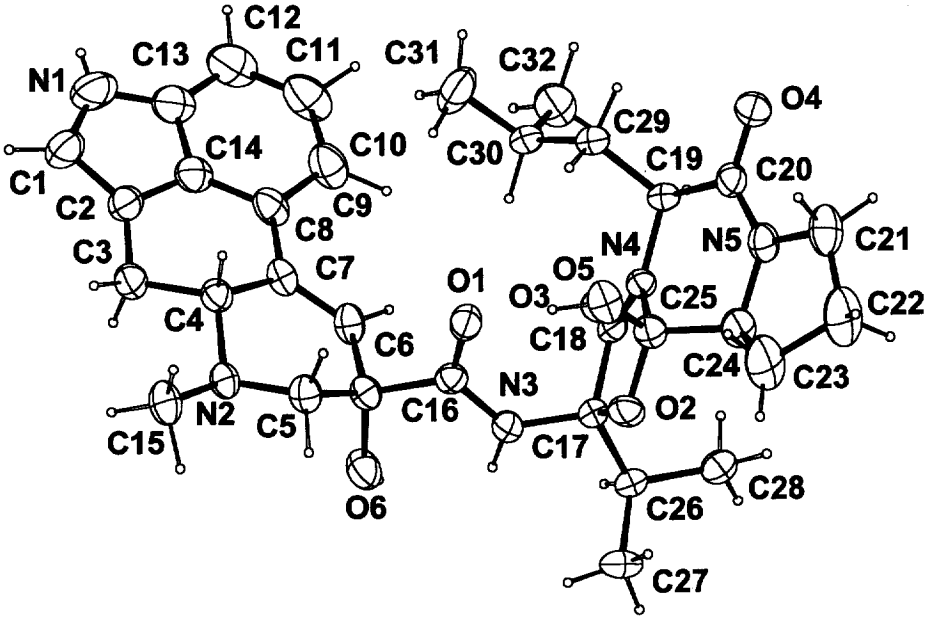

Fig. 2. Crystal structure of 8α-hydroxy-α-ergokryptine methanol solvate-monohydrate with numbering system used for X-ray data.

ture $-NHCH=CCH_2CHC=CH-$, $-CH_2-$, $(CH_3)_2CH-$, $-CHNCH_2CH(CH_3)_2$, and $CHN(CH_2)_3$ (proline). Unfortunately, the C-7 protons forming an AB system in this solvent were indistinguishable; moreover, some carbon signals (C-4, C-5 and C-7) were extensively broadened. For these reasons and to facilitate comparison with similar compounds, the experiments were repeated in CD_3OD (Table 1). The H-7e proton was identified using its long-range coupling (W-type) to H-9. NOE observed between the *N*-methyl and H-4e and H-7e indicated its pseudoequatorial orientation; NOE between H-5 and H-7a suggested that the D ring exists in the same conformation (flap-up [9]), as in the solid state. Therefore, the amorphous form was identified as unsolvated **2**.

Six 8-hydroxylated clavines (setoclavine, isosetoclavine, norsetoclavine, 9,10-dihydrosetoclavine, penniclavine and isopenniclavine) [1], two lysergamide derivatives (8α-hydroxyergine and 8β-hydroxyerginine) [14] and one peptide alkaloid (8α-hydroxyergotamine) [7, 8] have been described to date. 8α-Hydroxy-α-ergokryptine reported in this paper is the second example in the latter series. Hydroxylation at C-8 might be due to post-synthetic modifications. [15] As peroxidases occur frequently in various *Claviceps* species, other 8-hydroxylated ergopeptines might be found in nature. However, the additional hydroxyl group increases the polarity of these compounds; they are strongly adsorbed on silica gel columns and eluted in polar fractions together with many interfering substances. Probably for this reason most of them have escaped the attention of researchers to date. Contrary to peroxidases attacking the indole moiety of ergot alkaloids, oxidation by microsomal cytochromes P450 takes place at the proline ring in the cyclol part [16]. Nevertheless, 2-bromo-8-hydroxy-α-ergokryptine might be also expected among metabolites of bromokryptine in humans, because of the presence of peroxidases in their bodies (see also [17]).

EXPERIMENTAL

General. NMR spectra were measured at 400 MHz for ^1H and 100 MHz for ^{13}C, in $CDCl_3$ and CD_3OD at 25°. Chemical shifts are reported in ppm (δ) units downfield from TMS as int. standard. Proton-coupled ^{13}C NMR, APT, DEPTGL, COSY, delay-COSY, ROESY, HOM2DJ, HETCOR and long-range HETCOR experiments were performed with standard manufacturer's software. All positive-ion MS were recorded on a double-focusing instrument of BE geometry. Experimental conditions for EI were, ionizing energy 70 eV, source temp. 250°, emission current 1 mA, accelerating voltage 5 kV, direct inlet, sample dosed in μg amounts for evapn. For electrospray ionization, the sample was dissolved in H_2O–MeOH (1:1) and introduced into the electrospray source via a linear syringe pump (10 μl min^{-1}).

2-Bromo-8-hydroxy-α-ergokryptine (**1**). A part of the fr. containing 1.9% of **2** (68 g dry wt) was brominated [18]. The crude mixt. of brominated alkaloids was sepd on a reverse-phase column (SGX RPS, 5 μm, 250 × 12.5 mm, i.d., from Tessek, Czech Republic) using MeCN–H_2O–conc. NH_4OH (1200:800:1). Amorphous pure **1** was obtained by evapn of appropriate chromatographic frs. The presence of bromine in the molecule was inferred from the isotopic pattern of ergine ions *m/z* 316/318 and 361/363, cyclol-size

Table 1. ^1H and ^{13}C NMR data for compound **2** (400 and 100 MHz, CD_3OD, TMS, 25°)

Atom		δ_C	δ_H	Mult.	*J* (Hz)	Atom		δ_C	δ_H	Mult.	*J* (Hz)
C-1*	2†	120.8	7.01	*dd*	1.8, 0.6	C-18	1′	167.9	–		
C-2	3	110.6	—			C-17	1′α	91.7	–		
C-3	4	26.1	2.80	*ddd*	14.2, 11.5, 1.8	C-26	1′β	35.5	2.24	*qq*	6.9, 6.8
			3.55	*ddd*	14.2, 5.6, 0.6	C-27	1′γ_d	17.4	1.13	*d*	6.9
C-4	5	63.5	3.41	*ddd*	11.5, 5.6, 2.0	C-28	1′γ_u	16.1	1.0‡	*d*	6.8
C-5	7	61.0	2.95	*dd*	11.7, 0.9	C-20	2′	168.2	—		
			3.03	*d*	11.7	C-19	2′α	54.9	4.55	*dd*	7.5, 6.1
C-6	8	73.9	–			C-29	2′β	45.1	1.86	*m*	
C-7	9	121.6	62.6	*dd*	2.0, 0.9				1.90	*m*	
C-8	10	141.5	–			C-30	2′γ	26.3	2.05	*m*	
C-9	11	128.5	–			C-31	2′δ_d	22.6	1.08§	*d*	6.5
C-10	12	113.1	7.09	*m*	strongly coupled	C-32	2′δ_u	23.8	0.94	*d*	6.6
C-11	13	123.9	7.12	*m*	strongly coupled	C-25	3′	105.4	–		
C-12	14	112.0	7.25	*m*	strongly coupled	C-24	3′α	65.9	3.83	*dd*	9.4, 6.5
C-13	15	135.9	–			C-23	3′β	27.6	2.14	*m*	
C-14	16	128.2	–						2.16	*m*	
C-16	17	178.5	–			C-22	3′γ	23.2	1.88	*m*	
C-15	*N*-Me	43.2	2.64	*s*					2.06	*m*	
						C-21	3′δ	47.6	3.55	*m*	

*Crystallographic numbering (Fig. 2).

†Chemical numbering (Fig. 1).

‡*pro(R)* according to NOE with H-3′α.

§*pro(S)* according to NOE with H-2′α.

from the ion m/z 308. The ergopeptine character of this compound was supported by signals for three carbonyls and two sp^2-type carbons attached to two heteroatoms. Bromination at C-2 followed from the absence of a typical H-2 signal, as well as of its couplings to indole NH and H-4*a*. Amino acid side-chains were deduced using COSY spectra. Broadened and downfield-shifted signals of *N*-Me, H-4e, H-5 and H-7 indicated protonation at $N_{(6)}$. EI MS (rel. int.) m/z: 363 (9), 361 (9), 320 (8), 318 (8), 308 (7), 252 (6), 238 (16), 210 (15), 209 (38), 195 (23), 154 (45), 129 (12), 123 (16), 71 (65), 70 (80), 57 (25), 43 (100), 41 (55), 29 (16), 27 (15), 18 (23). ^{1}H NMR (CD_3OD): δ 0.89 (3H, *d*, $J = 6.6$ Hz, H-2′δ_u), 1.05 (3H, *d*, $J = 6.8$ Hz, H-1′γ_u), 1.07 (3H, *d*, $J = 6.5$ Hz, H-2′δ_d), 1.18 (3H, *d*, $J = 6.8$ Hz, H-1′γ_d), 1.86 (1H, *m*, H-2′β), 1.91 (1H, *m*, H-3′γ_u), 2.01 (1H, *m*, H-2′γ), 2.07 (1H, *m*, H-3′γ_d), 2.15 (2H, *m*, 2 × H-2′β), 2.24 (1H, *qq*, $J = 6.8$, 6.8 Hz, H-1′β), 2.92 (1H, *dd*, $J = 14.4$, 12.0 Hz, H-4a), 3.15 (3H, *s*, *N*-Me), 3.53–3.59 (2H, *m*, 2 × H-3′δ), 3.67 (1H, *dd*, $J = 14.4$, 6.2 Hz, H-4e), 3.86 (1H, *dd*, $J = 9.6$, 6.1 Hz, H-3′α), 4.27 (1H, *m*, H-5), 4.57 (1H, *dd*, $J = 6.8$, 6.8 Hz, H-2′α), 6.54 (1H, *bs*, H-9), 7.19 (1H, *dd*, $J = 7.6$, 7.5 Hz, H-13), 7.23 (1H, *dd*, $J = 7.5$, 1.1 Hz, H-12), 7.29 (1H, *dd*, $J = 7.6$, 1.1 Hz, H-14). ^{13}C NMR (CD_3OD): δ 16.2 (C-1′γ_u), 17.5 (C-1′γ_d), 22.5 (C-2δ'_d), 23.2 (C-3′β), 23.78 (C-2′δ_u), 25.3 (C-4), 26.3 (C-2′γ), 27.5 (C-3′γ), 35.3 (C-1′β), 43.2 (*N*-Me), 45.1 (C-2′β), 47.7 (C-3′δ), 55.1 (C-2′α), 61.2 (C-7), 63.9 (C-5), 65.9 (C-3′α), 72.7 (C-8), 92.0 (C-1′α), 105.7 (C-3′), 106.8 (C-2), 107.8 (C-3), 112.8 (C-14), 115.0 (C-12), 120.7 (C-9), 124.7 (C-13), 127.9 (C-16), 128.2 (C-11), 136.3 (C-15), 136.7 (C-10), 167.5 (C-1′), 168.0 (C-2′), 176.3 (C-17).

Isolation of 8α-*hydroxy*-α-*kryptine* (**2**). Crude α-ergokryptine (30 kg) was isolated from sclerotia (12 000 kg) of the ergot strain CCM 8599 growing on rye in Northern Moravia (Czech Republic). The product was recrystallized from toluene and the mother liquors (3.7 kg dry wt) were sepd by chromatography (silica gel, CH_2Cl_2 with 1–3% MeOH). A fr. containing 1.9% of unknown alkaloid was obtained (210 g dry wt). Further chromatography of a part this fr. (100 g dry wt) yielded a fr. containing 31.3% of the new alkaloid (2.7 g). This concentrate was sepd on a reverse-phase column (SGX RPS, 5 μm, 250 × 12.5 mm, i.d.) with MeOH–H_2O–conc. NH_4OH (3650:1350:1). Single crystals of **2** methanol solvate-monohydrate were obtained directly by moderate cooling of chromatographic frs (4°, overnight). An additional amount of amorphous unsolvated **2** was obtained by partial evapn of pooled chromatographic frs, partitioning between H_2O–CH_2Cl_2 and evapn of the organic layer. EI MS (rel. int.) m/z: 309 (4), 308 (5), 284 (4), 283 (25), 267 (15), 240 (36), 239 (10), 238 (16), 237 (7), 223 (6), 221 (12), 211 (12), 210 (18), 209 (41), 196 (14), 195 (29), 194 (11), 192 (14), 168 (10), 167 (33), 155 (10), 154 (100), 125 (10), 86 (6), 71 (35), 70 (97), 43 (53), 41 (16). For ^{1}H and ^{13}C NMR spectra: see Table 1 and Fig. 1 for numbering system.

Crystallographic study. 8α-Hydroxy-α-ergokryptine methanol solvate-monohydrate, $C_{32}H_{41}N_5O_6 \cdot CH_4O \cdot H_2O$, $M_r = 641.76$, monoclinic space group $P2_1$, $a = 8.795(1)$, $b = 18.970(3)$, $c = 10.842(2)$ Å, $\beta = 111.19(2)°$, $V = 1686.6(5)$ Å^3, $Z = 2$, $D_c = 1.178$ g cm^{-3}, Enraf-Nonius CAD4 diffractometer, graphite monochromator, ω-2θ scan technique, Mo K_α radiation, $\lambda = 0.71073$ Å. A total of 7052 reflections were measured (h $0 \rightarrow 10$, k $-23 \rightarrow 23$, l $-13 \rightarrow 13$, $\theta_{max} = 26.01°$), 2338 of them were observed and unique ($I > 2\sigma(I)$). The structure was solved by direct methods and anisotropically refined by full-matrix least-squares. The presence of solvate molecules was revealed from a difference map. The positions of H atoms were found from a difference map and expected geometry. All H atoms were isotropically refined. The minimized function was $\Sigma w(F_0^2 - F_c^2)^2$, where $w = 1/[\sigma^2(F_0) + (0.0519P)^2]$, and $P = (F_0^2 + 2F_c^2)/3$, $(\Delta/\sigma)_{max} = -0.006$, $R = 0.047$, $R_w(F^2) = 0.093$, $S = 1.165$ with the largest residual peaks of -0.15 and 0.18 e Å^{-3}. Programs used were SDP [19], SHELXS86 [20], SHELXL93 [21] and PARST [22]. Important backbone conformation angles [3, 23] [°]: Val; $\varphi_2 = -51.6(6)$, $\psi_2 = 111.8(4)$, $\omega_2 = -176.4(4)$, $\chi_2^{1,1} = 170.1(4)$, $\chi_2^{1,2} = -67.9(5)$; Leu: $\varphi_3 = -149.8(4)$, $\psi_3 = 4.5(5)$, $\omega_3 = -4.0(6)$, $\chi_3^1 = -65.9$, $\chi_3^{2,1} = 171.7(5)$, $\chi_3^{2,2} = -65.0(6)$; Pro: $\varphi_4 = -26.9(6)$, $\psi_4 = 54.2(5)$. Full data are deposited at the Cambridge Crystallographic Data Centre, U.K.

Acknowledgements—This research was supported by grants 27ERB40450PL 93-2014 and CIPA-CT94-0189 (Commission of the European Communities) and 203/94/0135 (Grant Agency of the Czech Republic).

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