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ERGOLADININE, AN ERGOT ALKALOID

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Abstract—A new natural peptide ergot alkaloid, ergoladinine, containing methionine, has been isolated from sclerotia of the field-growing parasitic fungus, *Claviceps purpurea*. Its structure was deduced from the X-ray analysis, NMR and mass spectral data.

INTRODUCTION

Some years ago, ergot alkaloids of the peptidic type, ergopeptines, seemed to be a closed group of natural products, containing only a limited number of amino acids [1–3]. (Fig. 1, R^1 = methyl, ethyl, *iso*propyl; R^2 = benzyl, *iso*butyl, *sec*-butyl, *iso*propyl). During the past two decades several new alkaloids have been prepared by directed biosynthesis [4–7], but only a few new ergopeptines were found in nature, [8–12]. Until now, it was generally accepted that only amino acids where R^1 and R^2 are benzyl or aliphatic carbon chains can be incorporated into the tripeptide moiety of ergopeptines [1–3].

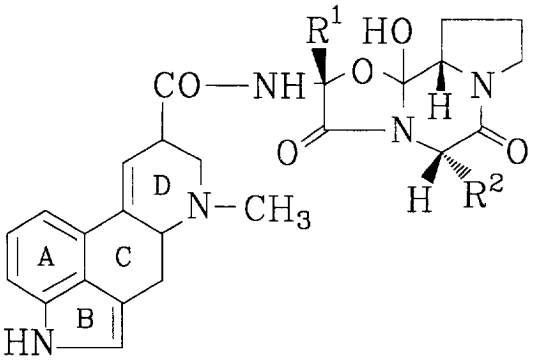

Fig. 1. Structure of ergot alkaloids: 8β(8*R*)-series-ergopeptines; for R^1 = *iso*propyl: R^2 = *iso*propyl-ergocornine; R^2 = *iso*butyl-α-ergokryptine; R^2 = *sec*-butyl-β-ergokryptine; R^2 = benzyl-ergocristine; R^2 = 2-methyl-butyl-ergogaline; R^2 =

In the present paper, we report the isolation of a new natural ergot alkaloid which has an unexpected structure.

RESULTS AND DISCUSSION

In our programme searching for potential impurities in drugs (ergotamine tartarate, ergotoxine mesylate and bromokryptine mesylate), we identified an unusual ergopeptine containing methionine (R^2 = $(CH_2)_2SCH_3$). A crude alkaloid concentrate was obtained by the extraction of an ergot strain producing α-ergokryptine (*Claviceps purpurea* CCM 8059). The concentrate was recrystallized from toluene and the mother liquors were separated by chromatography. Finally, **1** was obtained by recrystallization from methanol.

The molecular formula $C_{31}H_{39}N_5SO_5$ was deduced from elemental compositions of complementary peptide (*m/z* 326, $C_{15}H_{32}N_2SO_4$) and ergine (*m/z* 267, $C_{16}H_{17}N_3O$) ions observed in the EI mass spectrum. Diagnostic ions used for the determination of the cyclol structure were *m/z* 71 (C_4H_7O, acyclium ion of R^1), *m/z* 70 (C_4H_8N, proline-related), *m/z* 104 ($C_4H_{10}NS$, immonium ion of R^2) and *m/z* 61 (C_2H_5S, from Met). Typical cyclol signals were observed in the ^{13}C NMR spectrum (3 × C=O and 2 × sp^3-C). Detailed examination of ^1H NMR parameters of 9-ergolene protons ($J_{7,8}$, $J_{8,9}$, δ(H-5), δ(CONH)) showed that the D-ring exists in the flap-up conformation [13] with an axial 8-substituent. Spin systems due to the cyclol moiety

The coupling of *iso*propyl methyls to C-1′, SCH_3 to C-2′γ and vice versa, and 3′-OH to C-3 and C-3′ indicated that $R^1 = CH(CH_3)_2$ and $R^2 = (CH_2)_2SCH_3$. The final structural proof was provided by the crystal structure determination (see Experimental). The X-ray study confirmed the presence of methionine in **1** and provided the absolute configuration of all its chiral centres.

Ergoladinine belongs to the *iso*-lysergic acid series (-inines) with the 8α-configuration at the C-8 atom. This new alkaloid is the first sulphur-containing natural ergot alkaloid. It is interesting to note that methionine is absent also in many other series of fungal secondary metabolites produced extraribosomally, e.g. destruxins, cyclosporins or beauverolides [14]. The rare exception is probably the presence of methionine sulphone in cycloamanides, toxins of the fungus, *Amanita phalloides* [15]. However, the possibility that methionine can be incorporated instead of aliphatic amino acids is not unexpected since the opposite example, the incorporation of norleucine into proteins instead of methionine, is well known [16]. The mischarging of $tRNA^{Met}$ with norleucine by methionyl-tRNA synthetase is the only mischarging error known in which a non-cognate amino acid is used to charge a cognate tRNA. In contrast, due to the fact that ergot alkaloids are synthesized by a quite different mechanism (multienzyme complex [17]) the list of amino acids which can be incorporated either naturally or be a directed biosynthesis into the second position of ergopeptine alkaloids includes at present: Phe [1], Leu [1], Ile [1], Val [1], Nva [4, 7], Nle [4], α-Abu [4, 8, 9], *p*-chloro-Phe [4], *p*-fluoro-Phe [4], 5,5,5-trifluoro-Leu [4], β-hydroxy-Leu [4], homo-Ile [11, 12] and Met.

Based on the studies of directed biosynthesis, it was proposed that incorporation of a particular amino acid depends on its concentration in the free amino acid pool [4–9, 18]. Considering the fact that the concentration of methionine in ergot protoplasts is by one to two orders lower than that of all other amino acids [18], the ratio of Leu/Met, for example, is similar to the ratio of the corresponding ergopeptines (α-ergokryptine/ergoladine). Thus, it can be concluded, that incorporation of methionine into ergopeptine proceeds with similar specificity to that of incorporation of aliphatic amino acids. Therefore, we assume that methionine-containing peptides can be expected also as minor products among other extraribosomally produced fungal secondary metabolites.

EXPERIMENTAL

General. NMR spectra were measured at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm (δ) units downfield from TMS as int. standard. Positive-ion EI-MS were recorded on a double-focusing instrument of BE geometry (ionizing measurements were carried out by peak-matching using the Ultramark 1600F (PCR Inc.) as int. standard. Products of metastable collisionally-activated decompositions in the first field-free region of the instrument were analysed by daughter B/E constant scans using the manufacturer's software (He as collision gas).

Isolation. 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). The concentrate was recrystallized from toluene and the mother liquors sepd by chromatography (silica gel, CH_2Cl_2 with 1–3% of MeOH). A fr. containing 3.8% of an unknown alkaloid was obtained. Further chromatography of this fr. afforded 83.2% of a concentrate; recrystallization from MeOH yielded the new alkaloid, ergoladinine (**1**) (2.1 g).

Ergoladinine (**1**). Positive-ion EI-MS *m/z* (rel. int.): 593 ($[M]^{+\cdot}$, 0.2), 326.1390 ($C_{15}H_{22}N_2SO_4$, calc. 326.1300, 10), 267.1400 ($C_{16}H_{17}N_3O$, calc. 267.1372, 26), 252.1132 ($C_{12}H_{16}N_2O_4$, 18), 250 (15%), 249 (13), 228.0942 ($C_{10}H_{16}N_2SO_2$, calc. 228.0933, 17), 224 (6), 223 (6), 221 (13), 207 (11), 196 (7), 181 (6), 180 (9), 167 (13), 155 (9), 154 (100), 125 (4), 104.0535 ($C_4H_{10}NS$, calc. 104.0534, 2), 71.0500 (C_4H_7O, calc. 71.0497, 9), 70 (28), 61.0129 (C_2H_5S, calc. 61.0112, 15), 43 (20), 41 (11). ^1H NMR (400 MHz, $CDCl_3$, TMS, 25°): δ 0.92 (3H, *d*, J = 6.7 Hz, H-1′γ_u), 1.14 (3H, *d*, J = 6.8 Hz, H1′γ_d), 1.82 (1H, *m*, H-3′γ_u) 2.06 (1H, *m*, H-3′γ_d), 2.08 (1H, *qq*, J = 6.8, 6.7 Hz, H-1′β), 2.09 (3H, *s*, SMe), 2.14 (1H, *m*, H-3′β_u), 2.18 (1H, *m*, H-3′β_d), 2.27 (1H, *ddt*, J = 14.0, 6.8, 7.8 Hz, H-2′β_u), 2.44 (1H, *ddt*, J = 14.0, 5.9, 7.0 Hz, H-2′β_d), 2.62 (3H, *s*, NMe), 2.64 (1H, *ddd*, J = 14.3, 11.5, 1.8 Hz, H-4a), 2.74 (1H, *dd*, J = 11.9, 3.6 Hz, H-7a), 2.78 (2H, *dd*, J = 9.0, 7.8 Hz, H-2′γ), 3.07 (1H, *m*, H-8), 3.13 (1H, *ddd*, J = 11.9, 1.4, 1.2 Hz, H-7e), 3.22 (1H, *dddd*, J = 11.5, 5.5, 2.2, 2.0 Hz, H-5), 3.54 (1H, *ddd*, J = 12.2, 9.2, 2.4 Hz, H-3′δ_u), 3.59 (1H, *dd*, J = 14.3, 5.5 Hz, H-4a), 3.61 (1H, *ddd*, J = 12.0, 10.0, 7.3 Hz, H-3′δ_d), 3.68 (1H, *ddd*, J = 9.3, 6.6, 1.8 Hz, H-3′α), 4.61 (1H, *dd*, J = 6.8, 5.7 Hz, H-2′α), 6.50 (1H, *ddd*, J = 6.2, 2.0, 1.2 Hz, H-9) 6.89(1H, *dd*, J = 1.8, 1.8 Hz, H-2), 7.10 (1H, *dd*, J = 7.2, 1.3 Hz, H-12), 7.13 (1H, *dd*, J = 7.5, 7.2 Hz, H-13), 7.20 (1H, *dd*, J = 7.5, 1.3 Hz, H-14), 7.22 (1H, *d*, J = 1.8 Hz, 3′-OH), 8.276 (1H, *d*, J = 1.8 Hz, N-H), 10.05 (1H, *s*, CONH). ^{13}C NMR (100 MHz, $CDCl_3$, TMS, 25°): δ 15.0 *q* (SMe), 15.5 *q* (C-1′γ_d), 16.9 *q* (C-1′γ_u), 22.2 *t* (C-3′γ), 26.4 *t* (C-3′β), 27.5 *t* (C-4), 31.0 *t* (C-2′γ), 32.7 *t* (C-2′β), 34.0 *d* (C-1′β), 43.3 *q* (NMe), 43.8 *d* (C-8), 46.0 *t* (C-3′γ), 53.6 *d* (C-2′α), 54.6 *t* (C-7), 62.7 *d* (C-5), 64.2 *d* (C-3′α), 89.9 *s* (C-1′α), 103.3 *s* (C-3′), 109.7 *s* (C-3), 110.2 *d* (C-14), 112.7 *d* (C-12), 117.3 *d* (C-9), 118.4 *d* (C-2), 123.3 *d* (C-13), 126.1 *s* (C-16), 127.2 *s* (C-11), 133.8 *s* (C-15), 137.3 *s* (C-10), 165.2 *s* (C-2′), 165.4 *s* (C-1′), 176 *s* (C-17).

Crystallographic study. Ergoladinine, $C_{31}H_{39}N_5O_4S$.

CAD4 diffractometer, graphite monochromator, ω–2θ scan technique, CuK_α radiation, $\lambda = 1.54056$ Å. A total of 6406 reflections were measured (h 0→8, k 0→22, l −31→31, $\theta_{max} = 69.80°$), 5375 of them were observed ($I > 2\sigma(I)$) and included in the structural analysis. The structure was solved by direct methods and anisotropically refined by block-diagonal least-squares. The positions of H atoms were found from difference synthesis and expected geometry. All H atoms were refined isotropically. Absorption was ignored, extinction correction was included. The minimized function was $\Sigma w(F_0^2 - F_c^2)^2$, where $w = 1/[\sigma^2(F_0) + (0.0758P)^2 + 0.6288P]$, and $P = (F_0^2 + 2F_c^2)/3$, $(\Delta/\delta)_{max} = 0.003$, $R = 0.048$, $S = 1.054$ with the largest residual peaks of −0.49 and 0.32 e. Å^{-3}. Programs used were SDP [19], SHELXS86 [20], SHELXL93 [21] and PARST [22]. Important backbone conformation angles (°): Val: $\varphi_2 = -59.9(3)$, $\psi_2 = 113.0(2)$, $\omega_2 = -179.5(2)$, $\chi_2^{1,1} = 175.5(3)$, $\chi_2^{1,2} = -60.0(3)$; Met: $\varphi_3 = -149.0(2)$, $\psi_3 = 3.6(3)$, $\omega_3 = -6.1(4)$, $\chi_3^1 = -51.3(3)$, $\chi_3^2 = -176.1(2)$, $\chi_3^3 = -60.8(4)$; Pro: $\varphi_4 = 148.1(2)$, $\psi_4 = 48.5(2)$. Full data are deposited at the Cambridge Crystallographic Data Centre, U.K.

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