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DIRECTED BIOSYNTHESIS OF UNNATURAL ERGOT PEPTIDE ALKALOIDS

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ABSTRACT.—Three unnatural lysergic derivatives belonging to the class of ergopeptines have been obtained by feeding L-norvaline to a strain of *Claviceps purpurea*. Ergorine [**1**] corresponds to an analogue of ergovaline, and ergonorine [**2**] is an analogue of ergocornine; both have at C-5′ an *n*-propyl substituent instead of an isopropyl group. Ergonornorine [**3**] has the *n*-propyl group at C-2′ and C-5′ as well. This alkaloid represents the first example of an ergopeptine having an unnatural amino acid in the first position of the cyclol moiety. Additional feeding experiments with natural and unnatural amino acids indicated the maximum possible size of the substitutent in the first position of the peptide moiety.

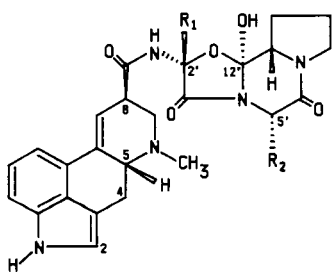
All natural peptide ergot alkaloids are divided into two groups differing only in the extent of cyclization of their peptide moiety. The class of ergopeptine alkaloids is characterized by the presence of the cyclol, a tricyclic structure which is absent in the analogous ergopeptam alkaloids (Figure 1).

It has been shown that unnatural amino acids can also be incorporated in the side chain of ergopeptine alkaloids. Bianchi *et al.* (1) have demonstrated that only lipophilic amino acids can enter position 2. Baumert *et al.* (2) succeeded in substituting 4-thioproline for proline. Finally, Flieger *et al.* (3) have obtained the incorporation of isoleucine in position 1 but only in an ergopeptam structure.

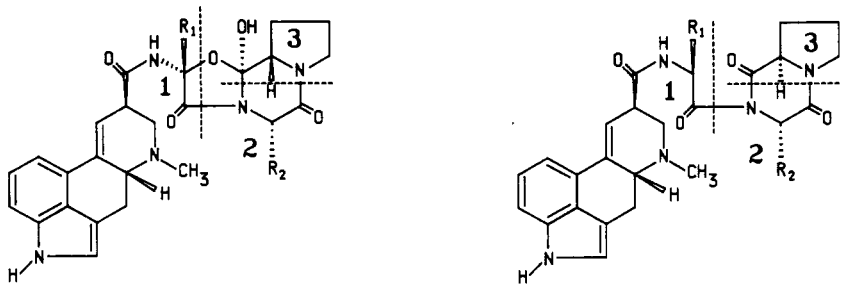
To our knowledge no report is present in the literature concerning the incorporation in position 1 of amino acids, either natural or unnatural, other than alanine, aminobutyric acid, and valine, which are present in the groups of ergotamine, ergoxine, and ergotoxine, respectively. This report is the first example of incorporation in position 1 of the unnatural amino acid norvaline in an ergopeptine structure.

RESULTS AND DISCUSSION

The experiments performed by feeding norvaline to the strain of *Claviceps purpurea* 231 FI led to the isolation of three unnatural ergopeptine alkaloids, for which the names



1 R_1=Me, R_2=*n*-Pr
2 R_1=iPr, R_2=*n*-Pr
3 R_1=R_2=*n*-Pr



A B

FIGURE 1. General structure of ergopeptine (A) and ergopeptam (B) alkaloids. Amino acid positions 1, 2, and 3 are illustrated.

of ergorine [**1**], ergonorine [**2**], and ergonornorine [**3**] are proposed. These alkaloids form a new unnatural series having an *n*-propyl substituent at C-5′ of the cyclol moiety.

Ergorine [**1**] gave norvaline and proline in equal amounts by hydrolysis in acidic conditions, and pyruvic acid and lysergic acid by alkaline hydrolysis. This assigned **1** to the group of ergotamine. Its eims showed the molecular ion at *m/z* 533 and a fragmentation pattern similar to that previously observed for other ergot alkaloids (4). Most significant were the peaks at *m/z* 196 and 337, both deriving from the cleavage of C-12′–O-1′ and C-3′–N-4′ bonds, with charge retention on the peptide fragment and on the lysergic portion of the molecule, respectively. The fragment at *m/z* 196 substantiated the presence of a propyl group at C-5′, while the ion at *m/z* 337 was indicative of the presence of a methyl group at C-2′. The ^{1}H-nmr spectrum of **1** exhibited, among all other signals consistent with the assigned structure, a singlet at δ 1.52 for the methyl group at C-2′ of the cyclol moiety and a triplet at δ 0.94 for the terminal methyl group of the side chain at C-5′.

Ergonorine [**2**] gave norvaline and proline in equal amounts by hydrolysis in HCl, and dimethylpyruvic acid by alkaline hydrolysis: this assigned **2** to the group of ergotoxine. Its field desorption mass spectrum (fdms) confirmed the mol wt with the molecular ion at *m/z* 561. Among all other signals consistent with the assigned structure, the ^{1}H-nmr spectrum of **2** showed a triplet at δ 0.96 for the terminal methyl group of the side chain at C-5′ and two doublets at δ 0.88 and δ 1.00, characteristic of the two diastereotopic methyl groups of an isopropyl chain, such as the one at C-2′ of the cyclol moiety.

Ergonornorine [**3**] gave norvaline and proline in equal amounts by acidic hydrolysis, and α-keto-valeric acid by alkaline hydrolysis. Compound **3** represents the first member of a new unnatural group having an *n*-propyl substituent at C-2′. The fdms confirmed its mol wt with a peak at *m/z* 561. In the ^{1}H-nmr spectrum of **3**, two triplets were found at δ 0.91 and δ 0.96 for the terminal methyl groups of the side chains at C-2′ and C-5′. All the other signals in the spectrum were in agreement with the proposed structures.

Experiments on strains of *C. purpurea* B21, 275 FI, and S40, producers mainly of ergocornine, ergotamine, and ergocristine, respectively, showed that all strains were able to incorporate norvaline in position 1, although in very small amounts. These strains could not incorporate leucine, isoleucine, or norleucine.

These findings suggest that incorporation of amino acids in position 1 of ergopeptines is possible only when their side chains, in addition to a lipophilic character, have the proper dimension. This may range from 1-carbon (as for alanine in ergotamine group) to 3-carbon units, either in branched (as for valine in ergotoxine group) or in linear form (as for norvaline in the newly characterized ergonornorine group).

Under the experimental conditions used, none of the above-mentioned strains were able to produce ergopeptam alkaloids even in trace amounts. Leucine, isoleucine, and norleucine were not incorporated, probably because their dimensions exceeded the size of the active site of the multienzymatic complex involved in the ergopeptine biosynthesis. In ergopeptam biosynthesis, the incorporation of at least one of these amino acids, isoleucine, has been shown to occur (3), probably because the size requirements are not so strict: in fact the lack of the third ring in the cyclol should allow a larger variety of side chains to be accommodated in position 1, without incurring severe crowding of the structure at the moment of the concerted formation of the ergopeptide alkaloid.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hplc analyses were performed on a Beckman Model 332 solvent delivery system equipped with two 110 A pumps and a Photo Diode Array detector, model 168. Two RP_{18} Hibar (Merck) columns in series were utilized in isocratic conditions with the following solvent system: phosphate buffer (0.15 M pH 8)- MeCN (40:60); detection at 310 nm. Uv spectra were recorded on a Bausch & Lomb Spectronic 2000 in EtOH. Extractions were performed on Chemelut (Analytichem International) preparative columns of Kieselguhr, and cc on Si gel Si 60 (E. Merck). Kieselgel 60 F 254 (E. Merck) tlc plates were used for analytical and preparative separations. Final purification was carried out on Si gel sheets Empore (Analytichem International). Fdms and eims were recorded on a Varian Mat 311 A mass spectrometer equipped with a FI/FD/EI combined ion source. ^{1}H-nmr spectra were recorded at 200 MHz on an XL-200 Varian instrument, in $CDCl_3$ solution. Chemical shifts are given in ppm (δ) from TMS.

STRAINS.—Submerged cultures of *C. purpurea* strain 231 and its mutant B21 (ATCC 20106), producers mainly of ergocornine, and strains 275 FI (ATCC 15383) and S40 (ATCC 20103), producers mainly of ergotamine and ergocristine, respectively, were cultivated as described elsewhere (5). To all strains, natural (L-valine, L-leucine, and L-isoleucine) and unnatural (L-norvaline and L-norleucine) amino acids were administered. All the reported amino acids (Fluka) were fed to 7-day-old cultures at the dose of 4 mg/ml. After 7 additional days of fermentation, cultures were harvested and processed.

ALKALOID EXTRACTION AND PURIFICATION.—In all experiments 50 Erlenmeyer flasks, each containing 50 ml of medium, were used. On the fourteenth day of fermentation, cultures were harvested, pooled, and filtered, and the mycelium was homogenized in Me_2CO- 2% aqueous tartaric acid (1:1). The suspension was filtered and the mycelium re-extracted twice. Pooled extracts were evaporated under reduced pressure, made alkaline by dilute NH_4OH, and extracted with a preparative Chemelut column. Elution was performed with CH_2Cl_2 (2 liters). The organic extract was concentrated under reduced pressure to a minimum volume. The oily residue was precipitated with 10 volumes of petroleum ether. The crude alkaloids were submitted to cc on Si gel Si 60 (Merck) and eluted with CH_2Cl_2 and EtOH in increasing amounts. Fractions were analyzed by hplc and pooled according to their alkaloid content.

Further purification was achieved by analytical Si F_{254} tlc plates (Merck) and Empore (Analytichem International) sheets, in the following solvent systems: (a) CH_2Cl_2-iPrOH (92:8), (b) CH_2Cl_2-EtOH (98:2), (c) Et_2O.

Purified alkaloids were eluted with CH_2Cl_2-EtOH (4:1), concentrated under reduced pressure, and precipitated with petroleum ether.

DEGRADATION PROCEDURES.—Acid and alkaline hydrolyses were performed as described previously (6). Ammonolysis for detection of ergopeptam alkaloids was carried out as reported elsewhere (3).

Ergorine [**1**].—Compound **1** (75 mg) was obtained as an amorphous powder with the procedure described above: ^{1}H nmr ($CDCl_3$) 0.94 (t, J = 7.2 Hz, 3H, 5′-CH_2CH_2*Me*), 1.52 (s, 3H, 2′-Me), 2.63 (s, 3H, N-Me), 4.41 (m, 1H, H-5′), 6.51 (dd, J = 2.0, 6.0 Hz, 1H, H-9), 6.78 (d, J = 1.7 Hz, 1H, OH-12′), 6.92 (s, 1H, H-2), 7.1–7.2 (m, 3H, H-12, H-13, H-14), 7.96 (bs, 1H, NH-1), 9.87 (bs, 1H, CONH).

Ergonorine [**2**].—Compound **2** (87 mg) was obtained as an amorphous powder by the same procedure: ^{1}H nmr ($CDCl_3$) 0.88 [d, J = 6.7 Hz, 3H, *Me*-CH(C-2′)-Me], 0.96 (t, J = 7.3 Hz, 3H, 5′-CH_2CH_2*Me*), 1.00 [d, J = 6.7 Hz, 3H, Me-CH(C-2′)-*Me*], 2.66 (s, 3H, N-Me), 2.86 (dd, J = 12.0, 14.0 Hz, 1H, H_{ax}-4), 3.17 (m, 1H, H-8), 3.34 (dd, J = 5.0, 14.0 Hz, 1H, H_{eq}-4), 3.90 (ddd, J = 2.0, 5.0, 12.0 Hz, 1H, H_{ax}-5), 4.44 (m, 1H, H-5′), 6.38 (dd, J = 2.0, 6.0 Hz, 1H, H-9), 6.94 (s, 1H, H-2), 7.1–7.3 (m, 3H, H-12, H-13, H-14), 7.38 (d, J = 1.7 Hz, 1H, 12′-OH), 7.98 (bs, 1H, 1-NH), 9.84 (bs, 1H, CONH).

Ergonornorine [**3**].—Compound **3** (15 mg) was isolated as an amorphous powder by the same procedure: ^{1}H nmr ($CDCl_3$) 0.91 (t, J = 7.2 Hz, 3H, 2′-CH_2CH_2*Me*), 0.96 (t, J = 7.3 Hz, 3H, 5′-

CH_2CH_2*Me*), 2.65 (s, 3H, *N*-Me), 2.82 (dd, J = 12.0, 14.0 Hz, 1H, H_{ax}-4), 3.14 (m, 1H, H-8), 3.31 (dd, J = 5.0, 14.0 Hz, 1H, H_{eq}-4), 3.84 (ddd, J = 2.0, 5.0, 12.0 Hz, 1H, H_{ax}-5), 4.44 (m, 1H, H-5′), 6.36 (dd, J = 2.0, 6.0 Hz, 1H, H-9), 6.94 (s, 1H, H-2), 7.10 (d, J = 1.7 Hz, 1H, 12′-OH), 7.1–7.2 (m, 3H, H-12, H-13, H-14), 7.96 (bs, 1H, 1-NH), 9.52 (bs, 1H, CONH).

LITERATURE CITED

1. M.L. Bianchi, P.A. Cattaneo, N. Crespi Perellino, A. Guicciardi, A. Minghetti, and C. Spalla, in: "Overproduction of Microbial Metabolites." Ed. by V. Krumphanzl, B. Sikyta, and Z. Vanek, Academic Press, London, 1982, p. 235.
2. A. Baumert, D. Erge, and D. Gröger, *Planta Med.*, **44**, 970 (1982).
3. M. Flieger, P. Sedmera, J. Vokoun, Z. Řeháček, J. Stuchlik, Z. Malinka, L. Cvak, and P. Harazim, *J. Nat. Prod.*, **47**, 970 (1984).
4. S. Vokoun and Z. Řeháček, *Collect. Czech. Chem. Commun.*, **40**, 1731 (1975).
5. M.L. Bianchi, A. Minghetti, and C. Spalla, *Experientia*, **32**, 145 (1966).
6. M.L. Bianchi, N. Crespi Perellino, B. Gioia, and A. Minghetti, *J. Nat. Prod.*, **45**, 191 (1982).

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