Isolation of ergovaline, ergoptine, and ergonine, new alkaloids of the peptide type, from ergot sclerotia[1,2]

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The isolation of three new ergot alkaloids of the peptide type from sclerotia of *Claviceps purpurea* and from mother liquors of rye ergot alkaloid extraction processes is described. The constitution of the new alkaloids ergovaline, ergoptine, and ergonine has been established by comparison with compounds previously obtained by total synthesis.

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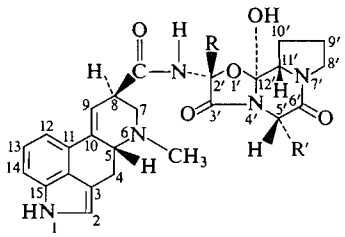
Trois nouveaux alcaloides de l'ergot de type peptidique ont pu être isolés à partir de sclérotes de *Claviceps purpurea* et à partir d'eaux mères accumulées après l'extraction d'autres alcaloides de l'ergot. La structure de ces nouveaux alcaloides ergovaline, ergoptine et ergonine a été etablie par comparaison à des produits obtenus auparavant par synthèse totale.

Genuine ergot alkaloids of the peptide type are found as pairs of diastereomers in nature. Their relative amounts depend strongly on the method of isolation, owing to an easy and reversible isomerization in position 8 of the lysergic acid moiety (1). Up to now seven pairs of alkaloids of the peptide type have been isolated from ergot sclerotia and also partly from mycelia of *Claviceps purpurea* fermentations (2–7). Systematically, ergot peptide alkaloids can be divided into three natural groups by their differing substituents (R) at position 2′ of the peptide moiety (Table 1) (see general structure **1**).



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Of the ergotoxine group, with an isopropyl group at C-2′, four natural alkaloid pairs have been isolated from natural sources: ergocristine–ergocristinine (4), α-ergokryptine–α-ergokryptinine (5), β-ergokryptine–β-ergokryptinine (7), and ergocornine–ergocorninine (5).

Hitherto, in the ergotamine group only two alkaloid pairs have been isolated, ergotamine–ergotaminine (2) and ergosine–ergosinine (3). The ergoxine group has as yet been represented only by the pair ergostine–ergostinine (6).

The total synthesis of ergotamine, achieved some 15 years ago (8), may be regarded as a breakthrough for the total synthesis of new ergot alkaloids of the peptide type. All 12 alkaloid pairs listed in Table 1 have been synthesized following more or less the successful approach employed in the ergotamine total synthesis (6, 9–12).

The availability of synthetic samples of ergot peptide alkaloids also expected to occur in nature somewhat simplified the hunt for the missing links. In a systematic search crude alkaloid extracts of field harvests of new *Claviceps* strains, on the one hand, and mother liquors of the current peptide alkaloid extraction processes, on the other, were tested by paper chromatography for new alkaloids.

Ergovaline **2**

In 1967, an experimental field harvest of our *Claviceps purpurea* strain 235, grown on Swiss rye, produced a mixture of α- and β-ergokryptine, ergocornine, ergosine, and ergometrine as the main alkaloids; the presence of ergovaline in an amount of about 4% of the total alkaloid content was verified by chromatography.

Following the usual procedure, a combined crude alkaloid yield of 0.85% was obtained by extraction of the sclerotia. The main alkaloids mentioned above were separated by chromatography on alumina; some middle fractions probably contained ergovaline: analysis by paper chromatography revealed the presence of about 70% of ergosine and about 20%

[1]Dedicated to the memory of R. H. F. Manske.

[2]88th communication on ergot alkaloids. For part 87, see ref. 14.

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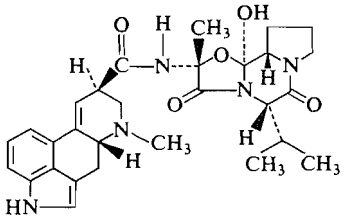
TABLE 1. Natural groups of ergot peptide alkaloids

R′	Ergotamine group R = CH_3	Ergoxine group R = C_2H_5	Ergotoxine group R = $CH(CH_3)_2$
$CH_2—C_6H_5$	Ergotamine	Ergostine	Ergocristine
$CH_2—CH(CH_3)_2$	Ergosine	Ergoptine†	α-Ergokryptine
$CHCH_3—C_2H_5$	β-Ergosine*	β-Ergoptine*	β-Ergokryptine
$CH(CH_3)_2$	Ergovaline†	Ergonine†	Ergocornine

NOTE: All names listed above correspond to those isomers with an equatorial carboxylic acid amide function at C-8 of the lysergic acid moiety; the 8-epimeric isomers derived from isolysergic acid, characterized by the ending -inine, are omitted here for the sake of simplicity.
*Not yet found in nature.
†Isolation reported in this paper.

of a new alkaloid with the same R_f values as synthetic ergovaline.

The isolation of ergovaline **2** on a preparative scale from this fraction proved to be a tedious task owing to its structural similarity to ergosine. Comparison of all chemical and physical properties of synthetic ergovaline with ergosine revealed practically no differences in solubilities, pK values, partition coefficients, and chromatographic behavior on silica gel thin-layer plates.



Ergovaline, **2**

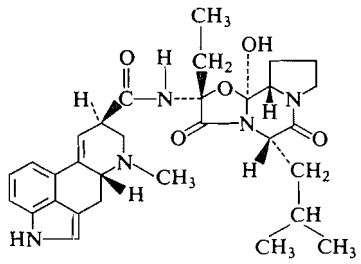
After testing several chromatographic systems on alumina thin-layer plates, it was found that the two alkaloids showed a slight R_f difference of 0.06 in a solvent mixture of ethyl acetate – n-butanol 9:1. Column chromatography on alumina using this mixture permitted sufficient enrichment of the new alkaloid for further purification to be effected by crystallization.

Natural ergovaline **2** melted at 207–208°C (dec.) in contrast to the reported (9) melting point of synthetic ergovaline (177–178°C, dec.). Yet after a few recrystallizations of an original sample of synthetic ergovaline from ethyl acetate, its melting point could be raised to a constant 209–210°C (dec.). This phenomenon can be attributed to dimorphism, often observed with ergot alkaloids. Otherwise the specimen of ergovaline isolated from *Claviceps purpurea* sclerotia was completely identical with a sample originating from total synthesis.

Ergoptine 3, Ergonine 4

Ergoptine **3** and ergonine **4** are members of the ergoxine group of ergot peptide alkaloids, hitherto represented only by ergostine, sometimes accompanying ergotamine as a minor constituent ($\leqq 0.5\%$) in nature (6). As a working hypothesis it was therefore assumed that these alkaloids might occur in ergokryptine–ergocornine-producing strains. Indeed, after thorough analysis of all types of mother liquors derived from technical ergokryptine–ergocornine extraction plants, a few revealed the presence of ergoptine and ergonine, previously obtained by synthesis (11), in the alkaloid mixture.

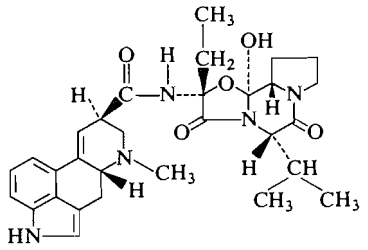
For example, in the isolation of ergoptine **3** a



Ergoptine, **3**

mother liquor with an estimated content of about 10% served as starting material. Its main constituent, about 40%, was ergocornine which could be partially separated by two consecutive chromatographic steps. Synthetic ergoptine gives a well crystallizing and more sparingly soluble salt with di-*p*-toluyl-L-tartaric acid than ergocornine. Thus, a fraction containing about 80% ergoptine, could be further purified as the above mentioned salt. Subsequent chromatography of the base on a silica gel column followed by crystallization from acetone–water gave pure natural ergoptine **3**, identical in all respects with a synthetic specimen.

For the isolation of ergonine **4** a mother liquor consisting of almost 90% ergosine and about 10% ergonine was at hand. Again, the different solubilities of the di-*p*-toluyl-L-tartrates of ergosine and ergonine in methanol could be exploited for their separation,



Ergonine, **4**

the salt of the latter being more soluble. Therefore, most of the ergosine present could be removed in pure form. The mother liquors, enriched in ergonine, were subjected to further purification as free base by column chromatography on alumina. Crystallization from ethanol – diisopropyl ether finally led, with great losses, to pure, natural ergonine **4**, identical with a synthetic specimen.

The separatory procedures described above do not, of course, provide a basis for accurate estimation of the relative amounts of **3** and **4** occurring in raw alkaloid extracts obtained from ergokryptine–ergocornine-producing strains. Very approximately, the concentration of ergoptine may be assumed not to exceed 0.5% of the total alkaloids present, whereas the ergonine concentration is even lower, probably in the region of 0.1%. The occurrence of ergovaline **2** in certain strains of *Claviceps purpurea* appears to be only sporadic.

Experimental

Owing to decomposition, melting points of ergot alkaloids were determined on a Tottoli apparatus in high vacuum and are uncorrected. Ultraviolet (uv) spectra were recorded on a Beckman spectrophotometer, model DK 2, in dichloromethane solution. The wavelengths of absorption maxima are reported in nanometers (nm) with log ε values in parentheses. Infrared (ir) spectra were measured on a Perkin Elmer model 21 spectrophotometer. The absorption maxima are reported in wavenumbers (cm^{-1}). Proton magnetic resonance (^{1}Hmr) spectra were measured on a Varian high resolution spectrometer. Chemical shift values are given in the δ (ppm) scale relative to tetramethylsilane (TMS) used as internal standard. The integrated peak areas, signal multiplicities, and eventual proton assignments are given in parentheses. Low resolution mass spectra (ms) were determined on an AEI-MS-30 spectrometer, high resolution ms on a CEC-21-110B apparatus.

Ergovaline 2

Sclerotia (1.4 kg) of *Claviceps purpurea* (strain No. 235, grown in the summer of 1967 on rye in an experimental field in the outskirts of Basle were ground and consequently defatted by extraction with petrol ether (2 × 5 L). The dark violet residue was extracted with a mixture of 70% acetone and 30% water containing 5% tartaric acid (4 × 5 L). The extracts were concentrated *in vacuo* at 50°C to a volume of 5 L, removing practically all acetone. The acidic solution was rendered alkaline by addition of Na_2CO_3, extracted with ethyl acetate (3 × 5 L), washed with water, dried (Na_2SO_4), and evaporated at 50°C *in vacuo*. The raw residue (12 g) showed a total alkaloid content of 40% on colorimetric determination (reagent of Van Urk (15)); semiquantitative determination of the alkaloids by paper chromatography gave ergometrine (7%), ergometrinine (1%), ergosine (14%), ergosinine (3%), ergocristine (5%), α- and β-ergokryptine (26%), ergocornine (26%), mixture of ergotoxinine (12%), and a new alkaloid (4%) with the same R_f values as synthetic ergovaline.

Chromatography of the raw extract (12 g) on alumina (600 g, activity II) afforded fraction 1 (chloroform – 0.5% methanol, 5 g, ergotoxine alkaloids, discarded), fraction 2 (chloroform – 1.5% methanol, 1.5 g, ergosine and new alkaloid), and fraction 3 (chloroform – 3% methanol, 0.6 g, ergometrine, discarded). Chromatography of fraction 2 (1.5 g) on alumina (100 g) gave a purified fraction 2 (chloroform – 1.5% methanol, 0.7 g, colorimetric alkaloid content 80%, paper chromatography: ergosine, 70%, and the new alkaloid, 30%). Chromatography of purified fraction 2 (0.7 g) on alumina (420 g, DS-O Camag, activity I, elution with ethyl acetate – *n*-butanol 9:1, fractions of 20 mL) gave the following fractions. Fraction 6 (15 mg), rest of ergokryptine and ergocornine, discarded. Fractions 7–10 (382 mg) primarily ergosine, discarded. Fractions 11–13 (199 mg), containing the new alkaloid, were combined and purified by crystallization: natural ergovaline **2** (60 mg); mp 207–208°C (ethyl acetate), mixture mp 207–208°C (synthetic ergovaline mp 207–208°C (ethyl acetate)); $[\alpha]_D^{20}$ −172° (*c* 0.5, chloroform); uv λ_{max}: 237 (4.3), 307 (3.95); ir (dichloromethane) ν_{max}: 3466, 1727, 1666 (sh), 1650; ^{1}Hmr (DMSO) δ: 10.8 (1H, s, N1-H), 9.37 (1H, s, CONH), 7.0–7.3 (4H, m, aromatic H), 6.75 (1H, d, J = 2 Hz, OH), 6.33 (1H, s, C9-H), 4.31 (1H, d, J = 5 Hz, C5′-H), 2.53 (3H, s, N6-CH_3), 1.7–3.9 (~14H, m), 1.58 (3H, s, C2′-CH_3), 1.08 (6H, d, J = 7 Hz, $CH(CH_3)_2$). *Exact Mass* calcd. for $C_{29}H_{35}N_5O_5$: 533.2638; found (ms): 533.2602.

Ergoptine 3

A mother liquor (251 g) of the ergokryptine–ergocornine production with a total alkaloid content of about 80% (determined colorimetrically with the reagent of Van Urk) was chromatographed on alumina (8 kg, activity II). Elution with chloroform and 0.3% methanol led to a first fraction (110 g) which consisted primarily of ergokryptine and ergocornine and was not further considered. A second fraction (95 g) was obtained on elution with chloroform and 0.6% methanol which contained on analysis by paper chromatography about 25% ergoptine. This fraction was chromatographed again on alumina (3 kg, activity II). A first fraction obtained on elution with chloroform and 0.3% methanol again contained ergokryptine and ergocornine and was discarded. Elution with chloroform and 0.6% methanol gave a second fraction (23 g). Its analysis by paper chromatography revealed a content of about 80% ergoptine **3**, 5% ergonine **4**, and 15% ergotoxine alkaloids. For the isolation of ergoptine this fraction was converted into the salt with di-*p*-toluyl-L-tartaric acid. The fraction (23 g, ~40 mmol) was dissolved in methanol (230 mL), a solution of di-*p*-toluyl-L-tartaric acid (17 g, 44 mmol) in methanol (170 mL) was added whereupon the salt (30 g, 31.6 mmol) crystallized. Being too poorly soluble to be recrystallized in a reasonable amount of solvent, the salt (30 g) was cleaved by partition between chloroform – 2 *N* Na_2CO_3 giving an amorphous resin (17.2 g, 30.6 mmol) which was again converted into the salt with di-*p*-toluyl-L-tartaric acid by the same procedure. The salt (27.3 g, 28.8 mmol) thus obtained, mp 177–178°C, was cleaved by partition in chloroform – 2 *N* Na_2CO_3 and afforded 15.2 g (27 mmol) base, purity about 95%. Further purification of that base by chromatography on a column of silica gel (700 g, Merck, 70–230 mesh, ASTM) followed by crystallization from acetone–water 7:3 yielded homogeneous ergoptine **3** (7.8 g); mp 198–199°C, mixture mp 199–200°C (synthetic ergoptine mp 199–200°C);

$[\alpha]_D^{20}$ −188° (*c* 0.8, chloroform); uv λ_{max}: 238 (4.34), 308 (3.98); ir (dichloromethane) ν_{max}: 3470, 3150–3300, 1732, 1671 (sh), 1653; ^{1}Hmr ($CDCl_3$) δ: 9.58 (1H, s, N1-H), 8.17 (1H, s, CONH), 7.1–7.3 (3 + 1H, m aromatic H, OH), 6.99 (1H, s, C2-H), 6.34–6.5 (1H, m, C9-H), 4.58 (1H, t, J = 6 Hz, C5′-H), 2.8–4 (~9H, m, C4-H, C5-H, C7-H, C8-H, C8′-H, C11′-H), 2.68 (3H, s, C6-CH_3), 1.7–2.4 (~9H, m, C2′-CH_2, C5′-CH_2, C9′-CH_2, C10′-CH_2, side-chain CH), 1.9–2.2 (9H, m, side-chain CH_3). *Exact Mass* calcd. for $C_{31}H_{39}N_5O_5$: 561.2951; found (ms): 561.2875.

Ergonine 4

A mother liquor (2.9 g, ~5.3 mmol) of an ergokryptine–ergocornine-producing strain of *Claviceps purpurea* was shown by paper chromatography to consist of almost 90% ergosine and 10% ergonine. For the removal of ergosine the mother liquor was dissolved in a mixture of dichloromethane (15 mL) and methanol (15 mL) and a solution of di-*p*-toluyl-L-tartaric acid (1.87 g, 4.84 mmol) in methanol (20 mL) was added. The resulting salt of di-*p*-toluyl-L-tartaric acid with ergosine crystallized on concentration of the solution *in vacuo*. Recrystallization of the salt from dichloromethane–methanol yielded ergosine di-*p*-toluyl-L-tartrate (2.47 g, 2.65 mmol), mp 184–185°C, chromatographic purity 99%. The mother liquors were recrystallized several times from dichloromethane–methanol giving additional ergosine di-*p*-toluyl-L-tartrate (0.94 g, 1 mmol), mp 181–182°C. The combined mother liquors of the salt were cleaved by partition between dichloromethane and 2 *N* Na_2CO_3 and the resulting base (670 mg) chromatographed on alumina (67 g, activity II). With dichloromethane and 0.3% methanol the ergonine containing fractions were eluted from the column. Further purification was performed by crystallization from a little ethanol and diisopropylether and yielded natural ergonine **4** (20 mg), chromatographic purity 99%; mp 206–207°C, mixture mp 206–207°C (synthetic ergonine mp 207–208°C); uv λ_{max}: 238 (4.31), 307.5 (3.95); ir (dichloromethane) ν_{max}: 3460, 1728, 1668 (sh), 1649; ^{1}Hmr ($CDCl_3$) δ: 9.05 (1H, s, N1-H), 8.2 (1H, s, CONH), 7.0–7.3 (3 + 1H, m, aromatic H, OH), 6.9 (1H, s, C2-H), 6.2–6.5 (1H, m, C9-H), 4.4 (1H, d, J = 5 Hz, C5′-H), 1.5–4.0 (~16H, m), 2.6 (3H, s, N6-CH_3), 1.15 (3 + 3H, d, J = 7 Hz, $CH(CH_3)_2$), 0.91 (3H, t, J = 7 Hz, CH_2—CH_3). *Mol. wt.* calcd. for $C_{30}H_{37}N_5O_5$: 547; found (field desorption): 547.

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