Clavicipitic Acid: Its Structure, Biosynthesis, and Role in Ergot Alkaloid Formation

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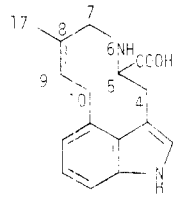
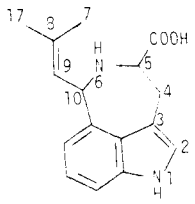
Clavicipitic acid, a metabolite of *Claviceps* strain SD 58, is a mixture of isomers, the major one of which was shown by a single-crystal X-ray analysis to give structure IIa. It is formed from tryptophan and mevalonic acid but is not further converted into the tetracyclic ergot alkaloid elymoclavine. Its formation represents a derailment of ergoline biosynthesis after the first pathway-specific step, the isoprenylation of tryptophan.

In the course of our studies on the biosynthesis of ergot alkaloids[1] we isolated a new indolic amino acid from ergot cultures which we named clavicipitic acid.[2] The compound was first obtained from cultures treated with ethionine to inhibit the N-methylation step in ergoline biosynthesis, as part of a project to identify biosynthetic intermediates by inhibiting specific reactions in the biosynthetic pathway; it was subsequently shown to be also present in smaller quantities in normal cultures of *Claviceps* strain SD 58. On the basis of limited spectral data and some biosynthetic results we[2] proposed structure I for



I



II

clavicipitic acid. King et al.[3] independently isolated an amphoteric indole derivative from cultures of *C. fusiformis* and showed it to be identical with clavicipitic acid. They further resolved the compound into two components, which appeared to be diastereomers, and on the basis of mass spectral data and a proton NMR analysis of the *N*-acetyl methyl ester proposed the revised structure II for clavicipitic acid.[3,4]

In this paper we report results which unequivocally confirm structure II for clavicipitic acid and define its stereochemistry. In addition biosynthetic data pertaining to the formation of clavicipitic acid and its role in ergoline biosynthesis are presented and discussed.

Results

The high-resolution mass spectrum indicated a molecular formula of $C_{16}H_{18}N_2O_2$ (M_r = 270) for clavicipitic acid. A violet-blue color with Ehrlich's reagent and UV absorption maxima at 288 (log ϵ 3.81) and 225 nm (log ϵ 4.58) suggested an unconjugated indole structure. Retention on anion-exchange resin, a strong IR absorption band at 1630 cm^{-1}, and loss of m/e 44 from the M - 1 ion in the mass spectrum support the presence of a carboxyl group in the molecule. Biosynthetic incorporation of tryptophan and mevalonic acid suggested an isoprenylated tryptophan structure, and the presence of characteristic fragment ions[5] at m/e 154, 167, and 169 in the mass spectrum of clavicipitic acid further narrowed down the choices to a 4-substituted indole closely related to the ergolines. Incorporation of tryptophan with retention of the carboxyl group and the α hydrogen and nonincorporation of the methyl group of methionine (see below) indicated that the intact alanine side chain of tryptophan was present, that it was not substituted at the α carbon, and that the amino nitrogen was not methylated. Observation of a single vinyl proton signal at δ 5.7 in the proton NMR spectrum of a methyl derivative of clavicipitic acid suggested the presence of a trisubstituted double bond, which in view of the UV data must be unconjugated to the indole system. Finally, the facts that clavicipitic acid exchanges no more than three hydrogens in D_2O and that it does not react readily with ninhydrin suggest that the nonindolic nitrogen is secondary.

Of the few structures which are compatible with these observations, I was originally proposed because an experiment with [2-^{14}C,5-^{3}H]mevalonic acid had indicated a high degree (~90%) of retention of the tritium, suggesting that clavicipitic acid still contained two hydrogens at C-10.[6] However, more detailed examination of the fate of the hydrogens from C-5 of mevalonate (see below) cast doubt on the proposed structure, showing full retention of the pro-5*S* hydrogen but retention of only half of the pro-5*R* hydrogen. Taken at face value, these unusual figures would indicate that the compound must contain two isoprene residues, both of which still contain the original pro-5*S* hydrogen of mevalonate, but one of which has lost the hydrogen originating from the pro-5*R* position of mevalonate. The thought was entertained that clavicipitic acid might contain an additional isoprene residue, e.g., in the form of a benzylic–allylic isoprenyloxy group, which would be readily lost upon mass spectrometry or derivatization. However, two independent determinations, by field-desorption mass spectrometry[7] and by crystallography,[8] indicated that 270 is the true molecular weight of clavicipitic acid and thus ruled out any such possibility.

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(2) J. E. Robbers and H. G. Floss, *Tetrahedron Lett.*, 1857 (1969).
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(6) For convenience in making biosynthetic comparisons, the ergoline numbering is used for clavicipitic acid.
(7) Carried out at the Mass Spectrometry Biomedical Research Resource at the Department of Biochemistry, Michigan State University. We thank Professor C. C. Sweeley for this measurement.
(8) Measurements of the dimensions of the unit cell of crystals of barium clavicipitate gave a volume of 692.44 Å^3. The density of the crystals was 1.328 g/mL. Assuming two molecules per unit cell, this gives a molecular weight of 276.9 for the free acid. We thank Dr. S. R. Byrn, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, for this analysis.

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Table I. Incorporation of Labeled Precursors into Clavicipitic Acid

expt no.	precursor	$^3H/^{14}C$	product $^3H/^{14}C$	product % T retention	product % ^{14}C incorpn
1	(*R,S*)-[*alanine*-1-^{14}C,2-^{3}H]tryptophan	3.7	1.9	51	4.5
2	(*R,S*)-[*alanine*-3-^{14}C,2-^{3}H]tryptophan	5.8	3.8	66	4.3
3	(*S*)-[*methyl*-^{14}C]methionine				0.1
4	(*R,S*)-[2-^{14}C,5-^{3}H]mevalonic acid	5.5	4.1	75	7.7
5	(3*R*,5*R*)-[5-^{3}H]mevalonic acid + (3*R,S*)-[2-^{14}C]mevalonic acid	4.15	4.37[a]	105	2.6
6	(3*R*,5*S*)-[5-^{3}H]mevalonic acid + (3*R,S*)-[2-^{14}C]mevalonic acid	3.39	6.49	191	43.0

[a] Due to the relatively low incorporation, this value is based on counting a sample containing only 180 dpm of ^{14}C.

Since our efforts at further spectroscopic characterization of clavicipitic acid were frustrated by the small quantities available, the poor solubility of the material, and the difficulties encountered in trying to prepare suitable derivatives, we turned to X-ray crystallography for an unequivocal determination of the structure. Initial work on crystals of the isomeric mixture of clavicipitic acid was unsuccessful.[9] Following the work of King et al.,[3] we further subjected the compound to TLC on silica gel with 75:25:1 chloroform–methanol–concentrated NH_4OH as solvent, which resolved it into two components (R_f 0.29, R_f 0.31). The component of lower R_f value, which in our samples was the major constituent, was purified by preparative TLC and recrystallization and gave crystals suitable for diffraction analysis.

A computer-generated perspective drawing of the final X-ray model of clavicipitic acid less the hydrogens is shown in Figure 1. The X-ray study defined only the relative configuration, and the enantiomer shown assumes that the chiral center at C(5) has the configuration corresponding to L-tryptophan. This assumption seems justified since it has been shown that the enzymatic isoprenylation in *Claviceps* is specific for L-tryptophan and produces only the L isomer of 4-(γ,γ-dimethylallyl)tryptophan.[10] There are two independent molecules of clavicipitic acid in the unit cell but both have the same configuration and essentially the same conformation, so only one is shown. There is an approximate inversion center in the unit cell that relates large parts of the two independent molecules. The two indole fragments are related by this approximate inversion center with a mean error of 0.13 Å. This approximate relationship breaks down entirely in the region of the chiral centers. The molecule crystallizes as the zwitterion and there are a series of hydrogen bonds in the crystal structure. Appropriate contacts are found: O(18)–N(6) ($x - 1$) = 2.741 Å, N(6′)–O(19′) ($x - 1$) = 2.824 Å, O(19)–N(1) ($x - 1, y - 1$) = 2.846 Å, N(1′)–O(18′) ($x - 1, y - 1$) = 2.725 Å. The indole fragment is planar and the seven-membered ring adopts a conformation where five atoms form a plane (C(10), C(11), C(16), C(3), C(4)) and the other two atoms are both on the same side of this plane (N(6), C(5)). Bond distances and angles agree well with generally accepted values.

In a series of biosynthetic feeding experiments, the origin and mode of formation of clavicipitic acid were probed. These are summarized in Table I. Experiments 1 and 2 show that tryptophan is incorporated and retains the carboxyl group in the process. The values for retention of tritium from the 2-position of the side chain in these

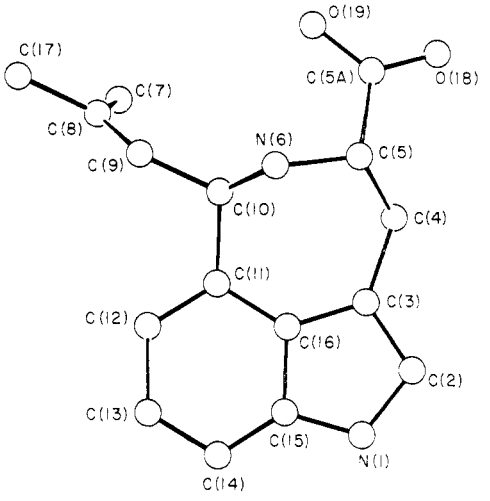

Figure 1. Computer-generated perspective drawing of clavicipitic acid. Hydrogens are omitted for clarity and the absolute configuration is assumed.

two experiments compare to a value of 43% tritium retention in the conversion of (*R,S*)-[*alanine*-3-^{14}C,2-^{3}H]-tryptophan into elymoclavine in the same strain.[11] The partial loss of tritium is due to (a) some conversion of D-tryptophan to the L isomer with loss of the tritium and (b) some reversible transamination of L-tryptophan with loss of tritium.[11] The results support the conclusion that the majority of the L-tryptophan converted to clavicipitic acid retains the α hydrogen of the side chain. In agreement with the structure established for clavicipitic acid, there is no significant incorporation of the methyl group of methionine (experiment 3) but good incorporation of mevalonic acid (experiments 4–6). The doubling of the $^3H/^{14}C$ ratio in experiment 6, in which the 3*R* isomer labeled with tritium and the racemate labeled with ^{14}C were fed, shows that, as in the biosynthesis of the ergot alkaloids, only the 3*R* isomer of mevalonic acid is biologically active.[12,13] The retention of 75% of the tritium from mevalonate tritiated nonstereospecifically at C-5 (experiment 4) is rather unusual; however, the same value was obtained in two independent experiments. Experiment 6 shows complete retention of the pro-5*S* hydrogen of mevalonate. This finding parallels the situation in the formation of the tri- and tetracyclic clavine alkaloids, where the pro-5*S* hydrogen of mevalonate is retained and the pro-5*R* hydrogen is lost.[12,13] However, experiment 5 suggests that clavicipitic acid still contains half of the tritium

(9) A data set was collected on a crystal of the isomeric mixture of clavicipitic acid, but the structure could not be solved. We thank Dr. S. R. Byrn, Purdue University, for this analysis.

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from the pro-5*R* position of mevalonate. Although this figure complements the result of experiment 4, it should be noted that due to low incorporation and the paucity of material the final determination of the $^3H/^{14}C$ ratio involved a sample of relatively low total activity, and its accuracy may thus be questionable. It should also be mentioned that all these measurements were done on the unresolved isomeric mixture of clavicipitic acid.

Finally, clavicipitic acid labeled biosynthetically from [2-^{14}C]mevalonic acid was fed to normal cultures of *Claviceps* strain SD 58 and the resulting elymoclavine was isolated and purified by column chromatography and repeated crystallization. The specific radioactivity of the sample at the last stage of purification corresponded to a maximum incorporation of 1.4%. Lack of material prevented any further purification. Under the conditions of this experiment genuine precursors of the ergoline ring system consistently gave incorporations in excess of 10% and usually in the range of 20–60%. The above result thus indicates that clavicipitic acid is not an intermediate in ergoline biosynthesis.

Discussion

The most important features to emerge from this study are the correctness of the structure proposed for clavicipitic acid by King et al. and the relative stereochemistry at C(5) and C(10) of the major isomer (IIa). The substituents at these two atoms are trans with respect to the seven-membered ring; i.e., C(5) has the *S* designation and C(10) the *R* designation. It seems plausible that the component of higher R_f ($\Delta\epsilon_{289}$ −2.95)[3,4] is a diastereomer of the above compound, presumably of 5*S*,10*S* configuration (IIb) as suggested by King et al.[3,4] and supported by their data, but we have no independent evidence relating to this point.

As shown both in this study and by Anderson's laboratory,[14] clavicipitic acid is not a precursor of the clavine alkaloid elymoclavine. Its formation seems to represent a derailment of the metabolism leading to the tetracyclic ergolines between the first and the second pathway-specific step, the isoprenylation of tryptophan[10] and the N-methylation of 4-(γ,γ-dimethylallyl)tryptophan (DMAT).[15,16] Anderson and co-workers have demonstrated the conversion of DMAT into clavicipitic acid in cell-free extracts of *Claviceps*[14] as well as in a thioglycolate–iron(II) model hydroxylation system.[17] The particulate enzyme from *Claviceps*, termed DMAT oxidase, was solubilized and further characterized.[18] It requires oxygen, but no added reducing agent, and catalyzes the following reaction with the stoichiometry shown:

$$\text{DMAT} + O_2 \rightarrow \text{clavicipitic acid} + H_2O_2$$

The reaction may be a direct oxidative cyclization or, alternatively, the primary reaction catalyzed by DMAT oxidase may be the 10-hydroxylation of DMAT; the product, 10-hydroxy-4-(γ,γ-dimethylallyl)tryptophan, could then undergo internal displacement to clavicipitic acid, possibly without further involvement of the enzyme. Our in vivo biosynthetic results are in accord with the formation of clavicipitic acid via DMAT. The peculiar results on the tritium retention from C-5 of mevalonate

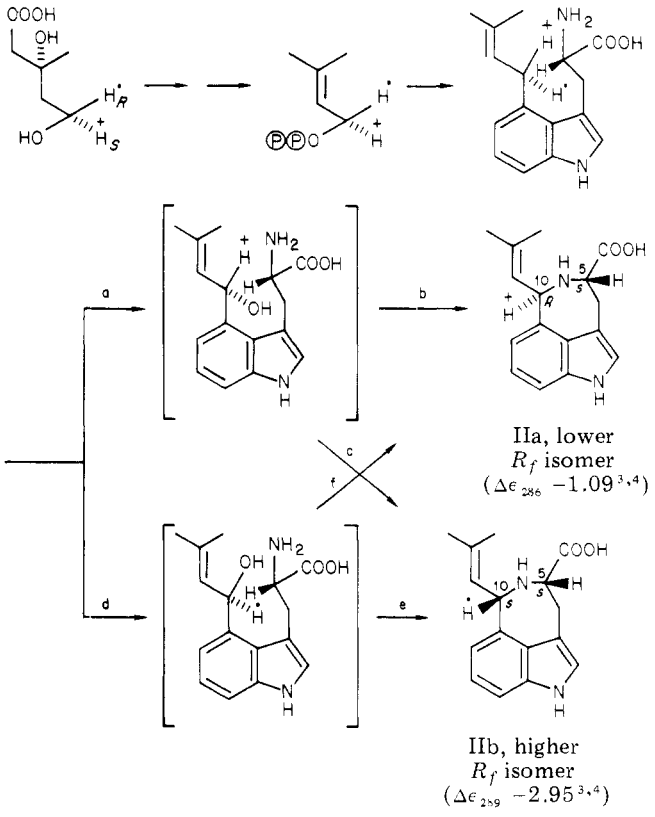

Figure 2. Possible pathways of clavicipitic acid formation.

require some comments. A possible explanation could be advanced if one assumes that the DMAT oxidase reaction discriminates only partially between the two heterotopic hydrogens at C-10, replacing one at a rate which differs from that of the other, and that the reaction involves a hydrogen isotope effect. Under these circumstances, the two C-10 epimers of II or of 10-hydroxy-4-(γ,γ-dimethylallyl)tryptophan would be formed in an unequal ratio (excess of the isomer arising by replacement of the original pro-5*R* hydrogen of mevalonate) and each tritiated product species would be enriched in tritium relative to the starting material and the ^{14}C reference label. These relationships are summarized in Figure 2, making additional reasonable assumptions about the steric course of the isoprenylation reaction (inversion[19,20]) and the hydroxylation step (retention).[21] Whether the formation of both C-10 epimers of clavicipitic acid is due to nonstereospecific displacement of the 10-hydroxyl group (e.g., path a and path b plus c, Figure 2), as suggested by the occurrence of IIa and IIb in a 1:1 ratio reported by King et al.[4] and Saini et al.,[18] reflects the stereochemical preference of DMAT oxidase (e.g., path a → [b] → IIa and d → [e] → IIb, Figure 2), as would be compatible with the above interpretation of our tritium data, or is due to a combination of both cannot be decided on the basis of available data.

Experimental Section

General Methods and Materials. *Claviceps*, strain SD 58,[22] which normally produces mainly elymoclavine, was used throughout this study. It was grown in a shake culture at 24 °C by using per 500-mL Erlenmeyer flask 100 mL of medium NL

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406. The latter consists of 50 g of mannitol, 50 g of sucrose, 5.4 g of succinic acid, 3.0 g of Difco yeast extract, 0.1 g of KH_2PO_4, 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, and 0.0044 g of $ZnSO_4 \cdot 4H_2O$, made up to 1 L with boiled tap water. The final pH was adjusted to 5.4 with ammonium hydroxide. For the production of clavicipitic acid, 40 μmol of D,L-ethionine was added to each culture flask on the sixth day of growth and the cultures were then incubated for another 8 days.

The radioactive D,L-[2-^{14}C]mevalonic acid (1.55 mCi/mmol), D,L-[5-^{3}H]mevalonic acid (221 mCi/mmol) (both as the dibenzylethylenediammonium salts), and D,L-[*carboxyl*-^{14}C]tryptophan (10 mCi/mmol) were from New England Nuclear Corp. and D,L-[*alanine*-3-^{14}C]tryptophan (22.4 mCi/mmol) and L-[*methyl*-^{14}C]methionine (56.8 mCi/mmol) were from Amersham-Searle. D,L-[*alanine*-2-^{3}H]Tryptophan (0.67 mCi/mmol),[11] (3*R*,5*R*)-[5-^{3}H]mevalonolactone (10 mCi/mmol), and (3*R*,5*S*)-[5-^{3}H]mevalonolactone (4 mCi/mmol)[13] had been synthesized previously.

Radioactivity measurements were carried out in a Beckman LS 100 or 250 liquid scintillation counter, using PPO and dimethyl POPOP in toluene as the scintillator solution and, if necessary, Biosolv (Beckman) as solubilizer. $^{3}H/^{14}C$ ratios, counting efficiencies, and, in the case of double-labeled samples, the ^{14}C spillover into the tritium channel were determined for each sample by internal standardization. Radioactivity on thin-layer chromatograms was located by scanning the plates in a Packard Model 7201 radiochromatogram scanner. Ultraviolet spectra were recorded on a Bausch and Lomb Spectronic 505 spectrophotometer and IR spectra were measured in KBr on a Perkin-Elmer 21 spectrophotometer. The NMR spectrum of a methyl derivative of clavicipitic acid was determined by averaging 150 runs in deuteriochloroform solution with trimethylsilane as an internal standard on a Varian HA 60 spectrometer equipped with a time-averaging computer. Low-resolution mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6D instrument and the high-resolution spectrum was obtained on a CEC 21-110 spectrometer by the direct inlet system and electron impact ionization.

Isolation and Characterization of Clavicipitic Acid. Ethionine-treated cultures of *Claviceps*, strain SD 58, were harvested by homogenizing the mycelia with the culture medium in a blender and then removing the mycelia by filtration. The filtrate was passed through a column of Dowex 50 (H^+) (3 × 30 cm for a batch of 20 cultures), and the alkaloids and amino acids were eluted from the resin with 5% ammonium hydroxide. The alkaloids were removed from the column eluate by partitioning with chloroform. The remaining fraction containing the water-soluble compounds was evaporated to near dryness under reduced pressure and completely dried at 70 °C on Merck silica gel (particle size 0.05–0.2 mm). This dried mixture was placed on top of a column (3 × 40 cm) of the same silica gel and chromatographed (30% ethanol in chloroform as the solvent system). The elution was followed by TLC and clavicipitic acid was obtained by pooling the appropriate fractions and evaporating them to dryness. The crude residue was washed with ether and recrystallized from ethanol, producing colorless plates which decomposed at 262 °C. From 20 cultures about 16 mg of pure clavicipitic acid was obtained: M_r = 270.1361 (calcd for $C_{16}H_{18}N_2O_2$, 270.1368); UV (ethanol) λ (log ε) 288 (3.81), 225 (4.58) nm; IR (KBr) 3090 (indole), 1630 (indole, amino acid), 1375 (CCH_3) cm^{-1}; mass spectrum (EI, 70 eV), *m/e* (relative intensity, elemental composition) 270 (100, $C_{16}H_{18}N_2O_2$), 269 (77, $C_{16}H_{17}N_2O_2$), 255 (18), 225 (48, $C_{15}H_{17}N_2$), 215 (66, $C_{12}H_{11}N_2O_2$), 196 (51, $C_{14}H_{14}N$), 183 (65, $C_{12}H_{11}N_2$), 182 (81, $C_{13}H_{12}N$), 169 (92, $C_{11}H_9N_2$), 167 (63, $C_{12}H_9N$), 154 (98, $C_{11}H_8N$).

For the determination of the number of exchangeable hydrogens, about 1 mg of clavicipitic acid was boiled with 5 mL of deuterium water (99.7% D) for 1 min, the mixture was filtered, and the clear solution was lyophilized to dryness. The residue was analyzed in the mass spectrometer (57% D_0, 27% D_1, 11% D_2, 5% D_3, 0% D_4). There was considerable reexchange of deuterium in the instrument, but the presence of a peak at *m/e* 273 and the absence of one at *m/e* 274 indicated the exchange of not more than three hydrogens in the molecule.

Attempts to obtain an NMR spectrum of clavicipitic acid failed because the compound was too insoluble in most solvents (pyridine, Me_2SO) or decomposed instantaneously in others (trifluoroacetic acid). A soluble derivative was finally prepared by treating 5 mg of clavicipitic acid dissolved in 5 mL of ethanol with an ether solution of diazomethane (prepared from 0.5 g of *N*-nitroso-*N*-methylurea). Three products giving a positive test with Ehrlich's reagent were obtained from this reaction, which were separated by TLC on silica gel G with 10% acetone in benzene as the solvent. The major reaction product had the highest R_f value and the mass spectrum indicated that it was a methyl derivative of clavicipitic acid. For NMR spectroscopy it was eluted from the silica gel with chloroform, the eluate was evaporated to dryness, and the residue was redissolved in deuteriochloroform.

Analysis of several samples of clavicipitic acid (purified as described) by TLC (silica GF_{254}, solvent 75:25:1 $CHCl_3$–CH_3OH–concentrated NH_4OH, 1 day old) showed the presence of a major (R_f 0.28) and a minor component (R_f 0.31). From several combined samples, the lower R_f component was isolated by preparative TLC (silica gel, solvent 80:20:1 $CHCl_3$–CH_3OH–concentrated NH_4OH developed twice), elution with 100:1 CH_3OH–concentrated NH_4OH, crystallization from ethanol, and rechromatography of the mother liquor, to give two samples of 0.9 and 2.0 mg which were submitted for X-ray crystallography.

Single-Crystal X-ray Diffraction Study of Clavicipitic Acid. Suitable crystals of clavicipitic acid in the form of very long needles could be grown by slow evaporation of aqueous ethanol solutions. Preliminary X-ray photographs showed only triclinic symmetry, and accurate lattice constants, obtained from a least-squares fitting of 15 diffractometer measured 2θ values, were a = 5.669 (1) Å, b = 8.282 (2) Å, c = 15.183 (3) Å, α = 99.18 (2)°, β = 100.47 (1)°, and γ = 86.45 (2)°. Assuming two molecules of composition $C_{16}H_{18}N_2O_2$ per unit cell gave a plausible calculated density of 1.30 g/cm^3 but the limited quantity of acid available precluded an accurate density measurement. Since clavicipitic acid is known to be chiral, the observations are uniquely accommodated by the space group *P*1 with two independent molecules of clavicipitic acid in the asymmetric unit.

All unique diffraction maxima with $2\theta \leq 48°$ were recorded on a four-circle diffractometer using graphite-monochromated Mo Kα (0.71069 Å) radiation and a variable θ–2θ scan technique. A total of 2200 reflections were examined in this fashion and after correction for Lorentz, polarization, and background effects, 1999 (91%) were judged observed ($F_o \geq 3\sigma(F_o)$).

The angular dependence of the scattering was removed and the reflection intensities were converted to normalized structure factors.[23] As anticipated, the intensity statistics of the normalized structure factors were indicative of a centric[24] arrangement but no explicit use was made of this. An initial phasing model was achieved by a multisolution weighted tangent formula approach and a weighted *E* synthesis of the most probable solution showed a plausible 18-atom fragment. This fragment proved to be all of the atoms in one of the independent molecules of clavicipitic acid minus the two isopropylidene methyls. The remaining atoms were located in subsequent *F* syntheses with no difficulty. After block-diagonal least-squares refinement all 36 hydrogen positions were located on Δ*F*-syntheses. Least-squares refinement with anisotropic nonhydrogen atoms and isotropic (fixed) hydrogens resulted in convergence to a residual of 0.042 for the observed reflections. Additional crystallographic details are available as supplementary material.

Incorporation of Labeled Precursors into Clavicipitic Acid. Mycelia from 4-day-old shake cultures of *Claviceps* strain SD 58 grown in 100 mL of NL406 without ethionine were filtered

(23) All crystallographic calculations were done on a Prime 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principal programs used were REDUCE and UNIQUE (data reduction programs: M. E. Leonowicz, Cornell University, 1978), BLS (block-diagonal least-squares refinement: K. Hirotsu, Cornell University, 1978), ORFLS (modified) (full-matrix least squares: W. R. Busing, K. O. Martin, and H. S. Levy, ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, TN), ORTEP (crystallographic illustration program: C. K. Johnson, ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, TN), BOND (structural parameters and errors: K. Hirotsu, Cornell University, 1978), and MULTAN-76 (direct methods and fast Fourier transform: G. Germain, P. Main, and M. Woolfson, University of York).

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off aseptically, washed with sterile water, and divided in half; each half was then placed into a flask containing 100 mL of fresh medium NL 406. Both flasks received 40 μmol of D,L-ethionine each, along with the respective radioactively labeled precursor which was added as a filtration-sterilized aqueous solution. The cultures were harvested 6 days later and the amino acid fraction was isolated by adsorption on Dowex 50 (H^+), elution with NH_4OH, and removal of the alkaloids with chloroform. Purification of the clavicipitic acid was achieved by TLC of an aliquot of the amino acid fraction (silica gel H, solvent 40:9:1 2-propanol–water–concentrated NH_4OH). The clavicipitic acid was eluted with 50% ethanol and further purified by repeated rechromatography on silica gel G plates until scanning of the plates in a radiochromatogram scanner indicated absence of radioactive contaminants or, in the case of double-labeled samples, until the $^3H/^{14}C$ ratio was constant. It was estimated that there was an 80% loss of clavicipitic acid during each separate chromatography and elution. This value was obtained by averaging the loss of radioactivity encountered in subjecting radiochemically pure clavicipitic acid to this purification procedure. The amount of radioactive clavicipitic acid in the original extract could thus be estimated by back calculation, and this figure was employed in determining the approximate percentage of incorporation.

In experiment 4, Table I, approximately 25 μCi of D,L-[2-^{14}C]mevalonic acid and 100 μCi of D,L-[5-^{3}H]mevalonic acid were added to 20 cultures. In this experiment, the workup followed the methods used for the production of unlabeled clavicipitic acid, and the radioactive material was isolated in crystalline form.

Test for Incorporation of Clavicipitic Acid into Elymoclavine. A solution of 0.18 mg of crystalline radioactive clavicipitic acid (8.4 μCi of ^{14}C/mmol) from experiment 4, Table I, in 2% succinic acid was added aseptically through a Millipore filter to a 6-day-old shake culture of *Claviceps* strain SD 58 which had not been treated with ethionine. After another 5 days the cultures were harvested by filtering off the mycelia, and the alkaloid titer in the culture filtrate was determined colorimetrically with van Urk's reagent.[25,26] Elymoclavine was isolated as described previously[27] by extraction from the alkaline culture filtrate into methylene chloride and chromatography on alumina (Brockmann) using methylene chloride with increasing amounts of methanol (0.5%, 2%) as the eluant. It was recrystallized repeatedly from methanol until the material was used up, and at each crystallization stage the specific radioactivity was determined.

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Supplementary Material Available: Fractional coordinates and temperature factors (Table I), bond distances (Table II), bond angles (Table III), and observed and calculated structure factors for clavicipitic acid (15 pages). Ordering information is given on any current masthead page.

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Synthesis of 15-Methyl-*cis*-Δ^4-prostaglandins[1]

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(15*S*)-15-Methyl-*cis*-Δ^4-prostaglandin $F_{1\alpha}$ methyl ester (**4**) and (15*R*)-15-Me-*cis*-Δ^4-$PGF_{1\alpha}$ methyl ester (**5**) were prepared in three steps from keto acetal **1**. First **1** was allowed to react with methylmagnesium bromide, and the product was then hydrolyzed to remove the methoxyl group and, finally, reacted with sodium 4-(triphenylphosphoranylidene)butyrate and diazomethane to give a mixture of **4** and **5**. After separation, **4** was converted to the free acid **6** by saponification, to (15*S*)-15-Me-*cis*-Δ^4-PGE_1 methyl ester (**7**) by selective silylation, oxidation, and deprotection, and to (15*S*)-15-Me-*cis*-Δ^4-PGD_1 methyl ester (**10**) by selective oxidation with Jones reagent at −40 °C. (15*S*)-15-Me-*cis*-Δ^4-PGA_1 methyl ester (**9**) was prepared from **7**. (15*R*)-15-Me-*cis*-Δ^4-PGE_1 methyl ester (**8**) was prepared from **5**. The lactol **3** was oxidized with silver oxide to give lactones **11** and **12**. Spectral properties were used to assign configurations at C-15 in various of the new products.

The prostaglandins found occurring naturally in most mammalian cells are capable of eliciting various powerful biological responses. The exceedingly rapid metabolism of these compounds renders their biological activity of short duration. The two most rapid modes of metabolic attack upon the prostaglandins are, first, the oxidation of the allylic C-15 alcohol accompanied by reduction of the 13,14 double bond, and, second, the oxidative degradation of the carboxylic acid side chain by the processes of β oxidation.[2]

An early objective for the chemical modification of the prostaglandins was the inhibition of these metabolic processes while maintaining the potent biological activities of the molecules. To this end, the preparation of prostaglandins having, for example, a methyl substituent at C-15,[3] dimethyl substitution at C-16,[4] or a phenyl substituent at C-17 (replacing the C_{18-20} carbons)[5] has had the result of effectively blocking or slowing the metabolic attack at the C_{13}–C_{15} allylic alcohol system.[6] Likewise,

(1) A preliminary account of these results was presented at the International Conference on Prostaglandins, Florence, Italy, May 1975. See: Samuelsson, B.; Paoletti, R. *Adv. Prostaglandin Thromboxane Res.* **1976**, *2*, 873.
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