mol of water from the analysis.

Anal. Calcd for $C_9H_{13}O_2P \cdot 0.5H_2O$: C, 55.95; H, 7.31; P, 16.03. Found: C, 56.12; H, 7.37; P, 16.21.

6-Hydroxy-1-methyl-$\Delta^{3a(7a)}$-2,3,4,5,6,7-hexahydro-1*H*-phosphindole 1-Oxide (17). The isomer mixture of 14 (10 g, 0.036 mol) was dissolved in 150 mL of glacial acetic acid and hydrogenated over 3.0 g of 10% Pd on charcoal at 50 psi for 19 h. The oil that remained from concentration of the solution was converted to crystalline form (2.4 g, 35.6%) by mixing with a small amount of acetone. The residual material was Kugelrohr distilled to give an additional 4.2 g (62.3%, total yield of 17 87.9%) of oil that later solidified. A sample vacuum sublimed for analysis had mp 145 °C. Spectral data are in Table I.

Anal. Calcd for $C_9H_{13}O_2P$: C, 58.06; H, 8.12; P 16.64. Found: C, 58.16; H, 8.33; P, 16.96.

O-Methylation of Hydroxyphosphindole 17. A solution of 1 g (0.0054 mol) of alcohol 17 in 1 mL of water was mixed with a solution of 1 g (0.025 mol) of NaOH in 1.5 mL of water. It was slowly treated with 4 g (0.03 mol) of dimethyl sulfate (caution), and the mixture stirred for 3 h at room temperature. The solution was determined to be acidic and was made basic with NaOH. During the next 20 h, the mixture was treated with two 1-mL portions of dimethyl sulfate and NaOH to keep the solution basic. The solution was then made slightly acidic and continuously extracted with $CHCl_3$. Evaporation of the extract and then distillation (Kugelrohr) at 100 °C (0.1 mm) gave 0.8 g (75%) of 15 as an oil. NMR properties were identical with those of the sample of 15 prepared by the cycloaddition of diene 11.

Anal. Calcd for $C_{10}H_{17}O_2P$: C, 59.99; H, 8.56; P, 15.47. Found: C, 59.89; H, 8.59; P, 15.67.

Registry No. 3, 4746-97-8; 4, 2987-06-6; 5, 13482-23-0; 6, 57707-01-4; 7, 75802-42-5; 8, 75802-43-6; 9, 57707-02-5; 10, 75802-44-7; 11, 75802-45-8; 12 (isomer 1), 75802-46-9; 12 (isomer 2), 75802-47-0; 13, 75802-48-1; 14 (isomer 1), 75802-49-2; 14 (isomer 2), 75802-50-5; 15 (isomer 1), 75802-51-6; 15 (isomer 2), 75802-52-7; 16a, 75802-53-8; 16b, 75802-54-9; 17, 75802-55-0; vinyl bromide, 593-60-2.

Mechanism of Amino Acid α-Hydroxylation and Formation of the Lysergyl Moiety in Ergotamine Biosynthesis

Francoise R. Quigley and Heinz G. Floss*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

Received September 10, 1980

The formation of the modified peptide portion of peptide ergot alkaloids, e.g., ergotamine (I), involves the conversion of an α-amino acid, e.g., alanine in the case of I, into the corresponding α-hydroxy α-amino acid moiety. This transformation is thought to occur after the assembly of the entire lysergyl-tripeptide, possibly at the stage of an N^1-(lysergylalanyl)prolylphenylalanyl lactam (II).[1] In a previous communication[2] we were able to rule out one of three possible mechanisms for this conversion, dehydrogenation to the 2,3-dehydro amino acid followed by addition of water, by showing that deuterium at the 3-position of this amino acid is completely retained during the conversion to the α-hydroxy α-amino acid moiety. We now report results which allow us to distinguish between the remaining two mechanisms, (a) dehydrogenation to the imine followed by addition of water and (b) direct hydroxylation at the α-position, in favor of the latter. The data also shed some light on the mode of formation of the lysergyl moiety from its precursor, elymoclavine (III).

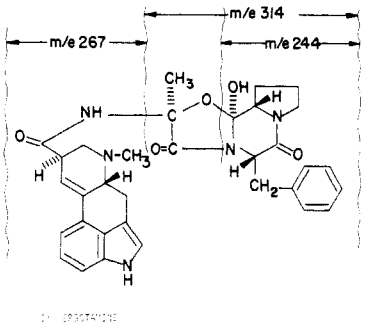
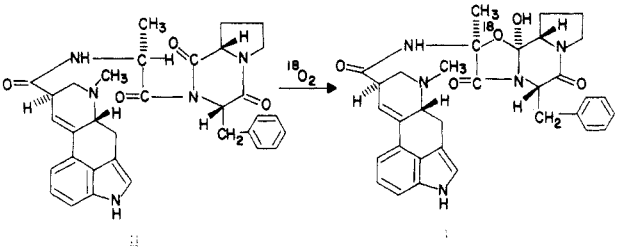


Figure 1.

Scheme I



Results and Discussion

A distinction between the two remaining mechanisms a and b for the amino acid α-oxidation should be possible by determining whether the oxygen in the cyclol ring of I is derived from water (mechanism a) or from molecular oxygen (mechanism b). Consequently, we carried out fermentations of the ergotamine-producing *Claviceps purpurea* strain PCCE1[3] in an atmosphere of 91.7% ^{18}O-enriched oxygen. The ergotamine formed was extracted, purified by preparative thin-layer chromatography, and subjected to EI and CI mass spectrometry. Three mass spectral fragments were analyzed for their isotopic composition, the ions at m/e 244 or 245 and 314 or 315 and, in the case of the EI spectra, also the one at m/e 267. In the CI spectra this ion was too weak for quantitative evaluation. The origin of these fragments,[4] which is supported by high-resolution data, is shown in Figure 1.

The results of two independent experiments are listed in Table I. The fragment of mass 244 clearly shows no significant isotopic enrichment. Thus, as one would expect, ^{18}O from molecular oxygen is not incorporated into the carboxyl groups of amino acids. The fragment of mass 314 contains ^{18}O at an average enrichment of about 43.8 atom %. On the basis of the enrichment of 91.7 atom % of the $^{18}O_2$ used, this indicates the incorporation of 0.48 atoms of oxygen from O_2 into this fragment. This figure reflects the fact that the cultures were only exposed to $^{18}O_2$ starting on day 8, at which time some I was already present. In view of the nonincorporation of ^{18}O into the carboxyl groups of amino acids, as indicated by the absence of labeling in fragment m/e 244, it seems justified to conclude that the ^{18}O in fragment m/e 314 is located in the cyclol oxygen. The results thus support mechanism b, a direct oxygenation, for the conversion of the α-amino to the α-hydroxy α-amino acid moiety (Scheme I). The stereochemistry of this process, replacement of H by OH in a retention mode, as deduced from the configurations of

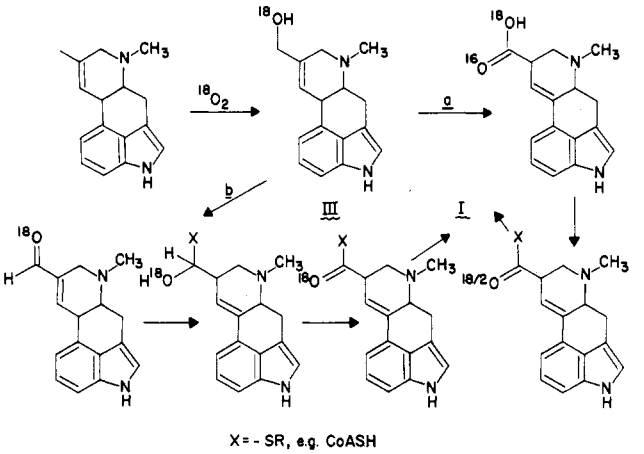
(1) For reviews see: (a) Gröger, D. *Planta Med.* **1975**, *28*, 37; (b) Floss, H. G.; *Tetrahedron* **1976**, *32*, 873.
(2) Belzecki, C. M.; Quigley, F. R.; Floss, H. G.; Crespi-Perellino, N.; Guicciardi, A. *J. Org. Chem.* **1980**, *45*, 2215.
(3) Anderson, J. A.; Kim, I.-S.; Lehtonen, P.; Floss, H. G. *J. Nat. Prod.* **1979**, *42*, 271.
(4) Voigt, D.; Johne, S.; Gröger, D. *Pharmazie* **1974**, *29*, 697.

0022-3263/81/1946-0464$01.00/0

Table I. Isotopic Composition (%) of Ergotamine Labeled from $^{18}O_2$ As Determined by Mass Spectral Analysis

peak analyzed, m/e:	314		315		267		244		245	
ionization mode:	EI		CI		EI		EI		CI	
sample no.:	1	2	1	2	1	2	1	2	1	2
P	53.0	55.8	50.2	57.6	51.0	50.5	100	95.3	100	98.0
P + 1	0.3	0.2	5.1	2.5	7.9	5.2	0	0	0	2.0
P + 2	46.7	44.0	44.7	39.9	41.2	44.3	0	4.7	0	0
P + 3	0	0	0	0	0	0	0	0	0	0

Scheme II



starting material and product, is in agreement with that generally observed for direct hydroxylations at sp^3 carbons.[5]

Table I indicates that the fragment m/e 267, representing the lysergic acid amide moiety, also contains ^{18}O. This fragment contains only one oxygen which is derived from the carboxyl group of lysergic acid. The average enrichment of 42.8 atom % corresponds to an incorporation of 0.47 atoms of oxygen from O_2 into this position, a value virtually identical with the ^{18}O incorporation into the cyclol oxygen. The precursor of the lysergic acid moiety is elymoclavine (III),[1] which derives its oxygen from O_2, not water,[6] by a mixed-function oxygenase-catalyzed hydroxylation.[7] Two pathways can be envisioned for the conversion of III into the lysergyl moiety of peptide ergot alkaloids, as shown in Scheme II. The conventional pathway (a) would involve stepwise oxidation of III via the aldehyde and double bond isomerization to give free lysergic acid, which would then have to be activated for amide bond formation. Gröger and co-workers[8] have, in fact, shown that cell-free extracts of *Claviceps* can activate lysergic acid to the coenzyme A ester, but it is not clear whether this reaction is involved in alkaloid formation since there is little if any correlation between the activity of the lysergyl-CoA-forming enzyme and alkaloid synthesis. As an alternative, we[9] proposed the pathway (b) in which an activated form of lysergic acid, e.g., the CoA ester, is generated during the oxidation from the aldehyde to the acid oxidation stage, thus preserving some of the free energy of this step. This has analogies in the HMG-CoA reductase reaction[10] and in the phosphoglyceraldehyde dehydrogenase reaction.[11]

As illustrated in Scheme II, the two routes differ in the fate of the ^{18}O introduced into III from atmospheric oxygen. Barring any exchange at the free aldehyde stage, this ^{18}O should be fully carried over into I on the second pathway (b). However, in pathway a the carboxyl group of lysergic acid would contain two oxygen atoms, one ^{18}O from III and one ^{16}O from water. Since these two oxygen atoms are equivalent, the subsequent activation of lysergic acid would remove half of the ^{18}O, leading to I which contains half as much ^{18}O in the carboxamide group of the lysergyl moiety as in the cyclol oxygen. The experimental finding (Table I) that the lysergyl fragment shows the same ^{18}O enrichment as the cyclol oxygen clearly favors the pathway involving direct formation of an activated derivative of lysergic acid from an aldehyde intermediate without further dilution of the ^{18}O of elymoclavine.

Experimental Section

General. $^{18}O_2$ gas (isotopic composition 91.7% ^{18}O, 4% ^{17}O, 4.3% ^{16}O) was purchased from Monsanto Research Corporation. Mass spectra were recorded on a Varian MAT 311A mass spectrometer operating in the EI (70 eV) or CI (isobutane) mode. Multiple scans were recorded for the relevant peak regions, and the isotopic compositions were calculated by using Biemann's formula.[12]

Culture Conditions and ^{18}O Incorporation Experiments. Selected colonies of *Claviceps purpurea* strain PCCEI[3] were grown for 10 days in shake cultures of medium T_2[3] at 24 °C. The cultures were used as inoculum (1 mL per 10 mL of medium) for two stationary cultures in 10 mL of medium NL 833[3] contained in 50-mL suction flasks. The flasks also contained a small upright sample tube. The cultures were grown for 8 days, at which time the alkaloid concentrations were found[13] to be 230 μg/mL and 280 μg/mL, respectively.

On the 8th day, 2–3 mL of 4 N KOH was introduced into the sample tubes inside the flasks and the cotton plug on top of each flask was replaced by a rubber septum. The cultures were flushed with nitrogen by repeated evacuation and introduction of N_2 through the septum via a needle, without disturbing the mycelial mats. The cultures were then evacuated and filled with $^{18}O_2$ gas from a rubber balloon attached to a needle. The cultures were incubated for another 7 days and on the 3rd day fresh $^{18}O_2$ was introduced in the same way.

On day 15 after inoculation the cultures were harvested. The medium, 7 mL and 8 mL,[14] respectively, with alkaloid concentrations of 470 μg/mL and 370 μg/mL, was made alkaline with concentrated NH_4OH and extracted 3 times with $CHCl_3$, and the extracts from each medium and mycelium were combined and subjected to preparative layer chromatography (0.5-mm Merck silica gel G with indicator, $CHCl_3$/MeOH, 95:5). The band of ergotamine was eluted with $CHCl_3$/MeOH (80:10). After evaporation, the extracts were purified further by redissolving in 2% succinic acid and partitioning with ether. The aqueous phase was

(5) See: Hanson, K. R.; Rose, I. A. *Acc. Chem. Res.* **1975**, *8*, 1.
(6) Floss, H. G.; Günther, H.; Gröger, D.; Erge, D. *J. Pharm. Sci.* **1967**, *56*, 1675.
(7) Hsu, J. C.; Anderson, J. A. *Biochim. Biophys. Acta* **1971**, *230*, 518.
(8) Maier, W.; Erge, D.; Gröger, D. *Biochem. Physiol. Pflanzen* **1972**, *163*, 432.
(9) For a more detailed discussion of the available data pertaining to this conversion see ref 1b.
(10) Retey, J.; Stetten, E. V.; Coy, U.; Lynen, F. *Eur. J. Biochem.* **1970**, *15*, 72.
(11) Cf.: Walsh, C. "Enzymatic Reaction Mechanisms"; W. H. Freeman and Co.: San Francisco, 1979; p 322–330.
(12) Biemann, K. "Mass Spectrometry. Organic Chemical Applications"; McGraw-Hill: New York, 1962; pp 223–227.
(13) Smith, M. J. *Publ. Health. Rep.* **1950**, *45*, 1466.
(14) It is not possible to calculate from these data the amount of alkaloid formed de novo during the incubation under $^{18}O_2$, because alkaloid is also degraded during this time period. However, we know from analyses of such fermentations that the pool sizes of intermediates are extremely small; hence, any alkaloid formed during this time period closely represents de novo synthesis of the entirely molecule of I.

made basic with concentrated ammonium hydroxide and extracted with chloroform. The chloroform extracts were evaporated and the ergotamine obtained was submitted to mass spectrometry for isotopic analysis.

Acknowledgment. We are indebted to Professor K. L. Rinehart and Dr. C. E. Snipes, Roger Adams Laboratory, University of Illinois, Urbana, for the mass spectral analyses and to Professor J. C. Vederas, Department of Chemistry, University of Alberta, Edmonton, for advice on the ^{18}O feeding technique. Support of this work by the U.S. Public Health Service through NIH Research Grant CA 17482 is gratefully acknowledged.

Registry No. Ergotamine, 113-15-5.

Reductive Formylation of *N,N*-Dimethyl-*p*-nitrosoaniline by Glyoxylic Acid. Evidence for a Hydroxamic Acid Intermediate[1]

Michael D. Corbett* and Bernadette R. Corbett

Rosenstiel School of Marine and Atmospheric Science, The University of Miami, Miami, Florida 33149

Received August 28, 1980

We recently reported that nitroso aromatics react rapidly with glyoxylic acid in aqueous solution to produce *N*-hydroxyformanilides.[2] During an attempt to apply this reaction for the synthesis of a previously unknown and highly reactive hydroxamic acid **2**, we found that the final product was actually the corresponding formanilide **3**. The direct condensation of glyoxylic acid and *N,N*-dimethyl-*p*-nitrosoaniline (**1**) to give **3** is not possible, so it was of interest to determine how **3** is produced. We now provide evidence which indicates that production of the formanilide **3** follows the initial production of the hydroxamic acid **2** (Scheme I). Since a similar reductive reaction was not observed for the *N*-hydroxyformanilides previously reported,[2] the *p*-dimethylamino substituent in **1** represents a special case of the nitroso–glyoxylate reaction.

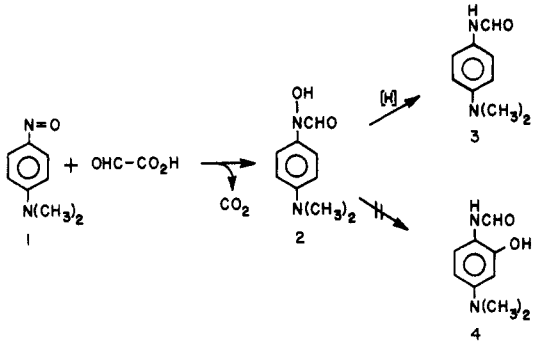
After discovering that the product isolated from the reaction of **1** with glyoxylic acid in aqueous solution was not the hydroxamic acid **2**, we suspected that it might be the isomeric rearrangement product **4**. However, all spectral data indicated that the product was identical with an authentic sample of the formanilide **3**. Typical yields of the formanilide **3** from synthetic-scale reactions were in the 50–60% range after column chromatography and recrystallization. Reactions carried out in more dilute solutions under suitable conditions were found to give **3** in quantitative yields as shown by high-pressure LC analysis. We do not have any evidence for the production of **4** as a secondary reaction product.

The use of TLC and a special high-pressure LC analytical technique[3] enabled us to detect an intermediate in the reaction of the nitroso compound **1** with glyoxylic acid. That this intermediate is the hydroxamic acid **2** is supported by all available evidence and is consistent with our previous report.[2]

The effect of pH upon the rate of formation of this hydroxamic acid intermediate (probable structure **2**) and its subsequent conversion to the final product **3** were studied by high-pressure LC. It was observed that at pH 2.0 the conversion of the intermediate **2** to **3** was quite slow and that a high concentration of the intermediate **2** was produced. Analysis by TLC of similar reactions conducted at pH 2.0, but at higher substrate concentrations, indicated the presence of a compound with a slightly lower R_f than was observed for **3**. Spraying of the TLC plate with 1% $FeCl_3$ resulted in an immediate violet color reaction for this compound. The formation of violet-colored complexes with Fe^{3+} is highly characteristic of hydroxamic acids.[4] Attempts to isolate this hydroxamic acid **2** from pH 2.0 reactions were unsuccessful due to its highly unstable nature.

At pH 3.0 the intermediate hydroxamic acid **2** was still detectable by TLC analysis of reactions. As the pH was increased further, the intermediacy of **2** could be detected only by high-pressure LC analysis since the rate of conversion of **2** to **3** increases greatly with increasing pH. The

Scheme I. Pathway for Reductive Formylation of *N,N*-Dimethyl-*p*-nitrosoaniline (1) by Glyoxylic Acid



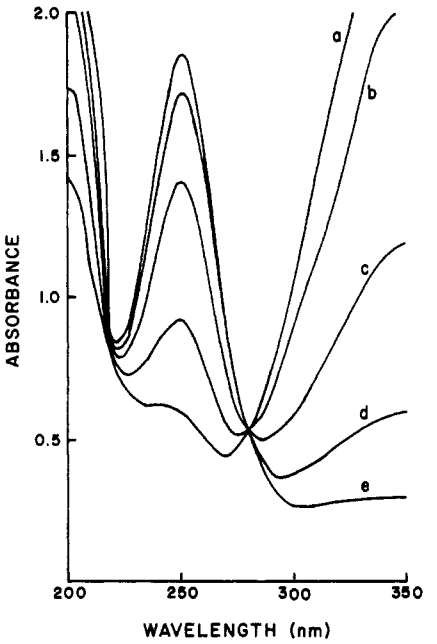


Figure 1. Change in UV spectrum during the reaction of *N,N*-dimethyl-*p*-nitrosoaniline (**1**) with glyoxylic acid at pH 2.0. An initial reaction mixture containing 0.125 mM **1** and 5.0 mM glyoxylic acid in 2.0 mL of 0.05 M, pH 2.0, KH_2PO_4 buffer was placed in a 1.0-cm path length quartz cell and scanned at the indicated times: a, 1.0 min; b, 5.0 min; c, 15 min; d, 30 min; e, 60 min.

(1) This investigation was supported by Grants CA-21992 and CA-23492 from the National Cancer Institute and by Research Career Development Award ES-00038 to M.D.C. from the National Institute of Environmental Health Sciences, DHEW.

(2) M. D. Corbett and B. R. Corbett, *J. Org. Chem.*, **45**, 2834 (1980).

(3) M. D. Corbett and B. R. Chipko, *Anal. Biochem.*, **98**, 169 (1979).

(4) (a) J. B. Neilands, *Science*, **156**, 1443 (1967); (b) L. Bauer and O. Exner, *Angew. Chem., Int. Ed. Engl.*, **13**, 376 (1974); (c) B. Monzyk and A. L. Crumbliss, *J. Am. Chem. Soc.*, **101**, 6203 (1979).

0022-3263/81/1946-0466$01.00/0