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A Total Synthesis of the Diazaphenanthrene Alkaloid Perlolidine

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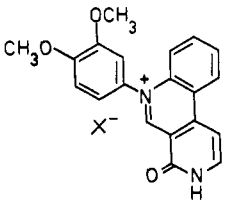
Abstract: A total synthesis of the diazaphenanthrene alkaloid perlolidine is reported. A key intermediate, 3-cyano-4-phenylpyridone, was synthesized by two methods. This intermediate was then converted to 2-aza-1-keto-fluorenone by a polyphosphoric acid cyclization. Perlolidine was prepared from the corresponding fluorenol by a Schmidt rearrangement. Alkylation of perlolidine with 4-bromoveratrole gave an O-alkylated product instead of the alkaloid perloline. A synthetic route patterned after a proposed biosynthesis of perlolidine led to several compounds containing elements of the diazaphenanthrene skeleton.

Several alkaloids containing a diazaphenanthrene skeleton have been discovered in various species of grass. Perloline, the most abundant member of this family of alkaloids, was the target for most of the structural investigations. Chemical studies and an X-ray crystallographic investigation led to the establishment of the correct structure (**1**).[2] Perlolidine, one of the minor alkaloids, was first isolated from the New Zealand perennial rye grass *Lolium perenne* L.,[3] and has since been found in other species of grasses along with perloline.[4] Perloline is degraded into perlolidine by oxidizing agents or by storage of its hydrochloride. Since these transformations involve loss of the dimethoxyphenyl substituent,[3,5] the structure of perlolidine (**2**) is defined.[2] In the present paper we describe a total synthesis of this novel diazaphenanthrene alkaloid.[6]



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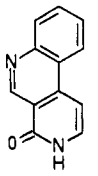

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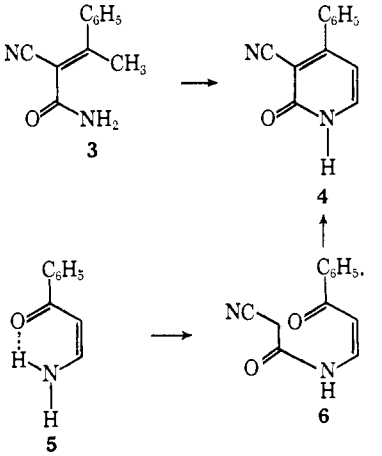
The pyridone **4** was an obvious intermediate for the synthesis of perlolidine. Once prepared, we planned to utilize the cyano group of **4** in the formation of the tricyclic diazaphenanthrene skeleton. Since the obvious route to **4**, condensation of cyanoacetamide with formylacetophenone or its equivalent, could be expected to give predominately the isomeric 6-substituted 2-pyridone,[7] our first synthesis of 3-cyano-4-phenyl-2-pyridone (**4**) utilized a new method for the formation of pyridone rings. A Knoevenagel condensation of cyanoacetamide with actophenone yielded 1-cyano-2-methylcinnamide which was predominately the *trans* isomer **3**.[8] Condensation of the product with ethyl formate using sodium hydride as a base gave a small yield of the desired pyridone **4**. The reasons for the low yield were soon apparent. The cinnamide **3** reacts readily with strong bases to yield a dimer, 3-cyano-3,4-dihydro-4,6-diphenyl-4-methyl-2-pyridone. And a reaction of ethyl formate with strong bases was causing the destruction of both the base and the formylating agent.[9]



3 **4** **5** **6**

A practical route to **4** was found in the reaction of ethyl cyanoacetate with the enamino ketone **5**. This synthesis was based on a report by Hauser and Basu[10]

(1) Author to whom inquiries should be addressed, at the Department of Biochemistry, University of Washington, Seattle, Wash. 98105.

(2) J. A. D. Jeffreys, G. A. Sim, R. H. Burnell, W. I. Taylor, R. E. Corbett, J. Murray, and B. J. Sweetman, *Proc. Chem. Soc.*, 171 (1963); J. A. D. Jeffreys, *J. Chem. Soc.*, 4504 (1964); G. Ferguson, J. A. D. Jeffreys, and G. A. Sim, *J. Chem. Soc., B*, 454 (1966).

(3) I. Reifer and E. P. White, *New Zealand J. Sci. Technol.*, **27B**, 242 (1945), and references therein.

(4) S. G. Yates, *J. Chromatog.*, **12**, 423 (1963).

(5) W. S. Metcalf, *New Zealand J. Sci. Technol*, **29B**, 98 (1947); **35B**, 473 (1954).

(6) Perlolidine has been synthesized independently by two different routes: M. A. Akhtar, W. G. Brouwer, J. A. D. Jeffreys, C. W. Gemenden, W. I. Taylor, R. N. Seelye, and D. W. Stanton, *J. Chem. Soc.*, 859 (1967).

(7) H. Meislich in "Pyridine and Its Derivatives," Part III, E. Klingsberg, Ed., Interscience Publishers, New York, N. Y., 1962, pp 525–535.

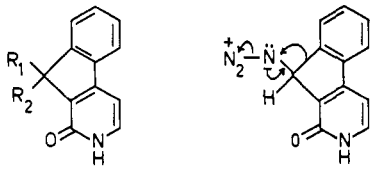
(8) A. Foucand, H. Person, and A. Robert, *Bull. Soc. Chim. France*, 1873 (1964).

(9) J. C. Powers, R. Seidner, T. G. Parsons, and H. J. Berwin, *J. Org. Chem.*, **31**, 2623 (1966).

(10) C. R. Hauser and C. J. Ray, *J. Am. Chem. Soc.*, **79**, 728 (1957); U. Basu, *J. Indian Chem. Soc.*, **12**, 299 (1935).

that condensation of ethyl cyanoacetate with the enamino ketone derived from benzoylacetone yielded 6-methyl-4-phenyl-2-pyridone, while the 2-pyridone possessing the reverse order of substituents was obtained upon reaction with malonamide. Thus the ethyl cyanoacetate reaction probably proceeded by way of **6** which subsequently cyclized to the desired pyridone **4**. Hydrolysis and decarboxylation to 4-phenyl-2-pyridone confirmed the structure of the cyanopyridone **4**.

The conversion of the 3-cyano-4-phenyl-2-pyridone (**4**) to perlolidine proceeded quite smoothly. Cyclization of **4** to the bright yellow ketone **7** was effected with polyphosphoric acid. A by-product of this reaction was the amide 3-carbamoyl-4-phenyl-2-pyridone which was produced exclusively at a lower reaction temperatures. Reduction of the ketone **7** with sodium borohydride in methanol was easily followed by the disappearance of the yellow color. Work-up of the reaction produced an excellent yield of the alcohol **8**. This was easily converted to perlolidine (**2**) upon reaction with hydrazoic acid in sulfuric acid–chloroform. This Schmidt rearrangements proceeds by way of the azide **9**. Since the phenyl ring has a greater migratory aptitude than the pyridone ring, it migrates preferentially to the positive nitrogen in the transition state and the rearrangement proceeds in the sense shown.[11] The identity of synthetic perlolidine with natural material was established by comparison of infrared and ultraviolet spectra, by tlc behavior and by mixture melting point.



7, R_1R_2 = O

8, R_1=H; R_2=OH

9

Consideration of the structures of the diazaphenanthrene alkaloids led to the realization that these alkaloids could be derived from tryptophan (**10**).[1] A logical intermediate would be kynurenine (**13**). This is synthesized in bacterial and mammalian systems by the action of two enzymes, tryptophan pyrolase, which oxidizes tryptophan to N-formylkynurenine, and formylkynurenineforamidase, which removes the formyl group.[12] Introduction of a three-carbon fragment, probably as malonyl CoA as in the case in fatty acid biosynthesis would yield perlolidine (**2**) after a series of secondary transformations.[13] Concurrently with our other synthetic work we therefore undertook an approach to perlolidine modeled after this proposed biosynthesis.[14]

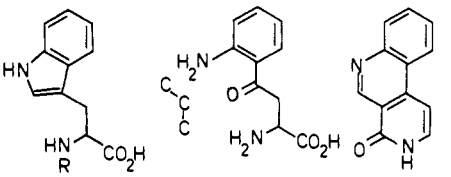
(11) Treatment of 2-nitrofluorenol with hydrazoic acid gave nitrophenanthrene containing 97% of the 7-nitro isomer formed as phenyl rather than nitrophenyl migrated preferentially: C. L. Arcus and M. M. Coombs, *J. Chem. Soc.*, 4319 (1954).

(12) A. Meister, "Biochemistry of the Amino Acids," Academic Press, New York, N. Y., 1965, pp 850–853.

(13) The three-carbon fragment could be introduced prior to oxidation of the indole ring. Malonyltryptophan is formed in spinach leaves, tomatoes, and peas: N. E. Good and W. A. Andreae, *Plant Physiol.*, **32**, 561 (1957).

(14) Dr. Taylor's synthetic approach uses similar reasoning. A protected kynurenamine is used as a starting material and the three carbon fragment is introduced by a reaction with dimethylacetylene dicarboxylate. Dr. J. A. D. Jeffreys has proposed an alternate route to the diazaphenanthrine alkaloids from tryptophan.

Dolby and Booth have recently reported that sodium periodate oxidatitively cleaves the pyrrole ring of indoles under very mild conditions;[15] for example, skatole (**14**) is cleaved to *o*-formaminoacetophenone (**15**) in over 80% yield. Periodate thus imitates the action of tryptophan pyrrolase on tryptophan. Sodium ethoxide catalyzed condensation of diethyl malonate with the oxidation product **15** gave a small yield of the 2-quinolone **16**. This reaction sequence imitates the proposed mode of diazaphenanthrine alkaloid synthesis in the sense that a three-carbon unit was allowed to react with an oxidized indole to give a product which contained several of the elements of the perlolidine structure.



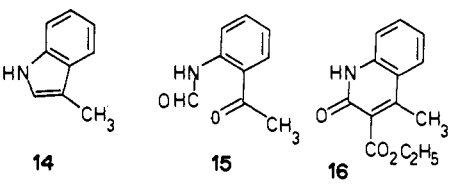
10, R=H

11, R=COCH$_3$

12, R=COCH$_2$CO$_2$C$_2$H$_5$

13

2



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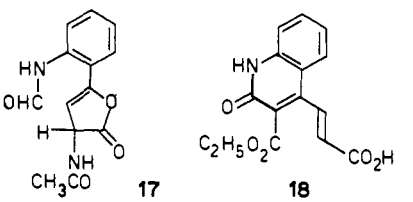
15

16



17

18

Oxidation of N-acetyltryptophan (**11**) or N-malonyltryptophan (**12**) led to a complex mixture of products; tryptophan itself was inert under the reaction conditions. The only product that could be isolated was assigned the oxazolone structure **17**. This was evidently formed by participation of the carboxyl group in the periodate oxidation of N-acetyltryptophan (**11**). The oxazolone structure was assigned on the basis of the oxidation product's spectral properties and its hydrolysis under acidic conditions to kynurenine.

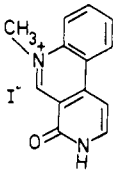
The base-catalyzed condensation of diethyl malonate with kynurenine (**13**) was investigated next. Under the influence of sodium ethoxide the reaction produced in low yield the 2-quinolone acid **18** which contains most of the structural features of the perlolidine skeleton. Unfortunately, the amino group that we had hoped would form the third ring of the diazaphenanthrene skeleton was eliminated under the reaction conditions to form the double bond of **18**. Kynurenine (**13**) itself eliminates ammonia under the influence of sodium bicarbonate to yield a cyclized product called kynurenine yellow, 2-carboxy-2,3-dihydroquinolone-4.[16] Thus **18**

(15) L. J. Dolby and D. L. Booth, *J. Am. Chem. Soc.*, **88**, 1049 (1966).

(16) T. Tokuyama, S. Senoh, T. Sakan, K. S. Brown, Jr., and B. Witkop, *ibid.*, **89**, 1017 (1967).

may in reality be a condensation product of diethyl malonate with kynurenine yellow.

Perloline is potentially available by alkylation of perlolidine with a suitable alkylating agent. Reaction of perlolidine with methyl iodide gave a nearly quantitative yield of the methiodide **19**. The structure assignment of **19** is based mainly on consideration of the most probable alkylation site of perlolidine in neutral solution. The ultraviolet of the methiodide is consistent with this assignment, but lacking appropriate models for all three possible monoalkylated products, the structure assignment is considered tentative. Attempted alkylation of perlolidine, on the other hand, in neutral solution with 4-bromoveratrole and several related alkylating agents under a variety of conditions led either to recovery of starting materials or to complete destruction of the reactants. Perloline, if formed, probably would not have withstood the reaction conditions since it has been reported to be thermally unstable. Attempted alkylation of dihydroperlolidine (**22**), produced by reduction of perlolidine with lithium aluminum hydride, gave no better results. Dihydroperlolidine (**22**) underwent rapid dehydrogenation to perlolidine either upon standing in solution or upon heating.[17]



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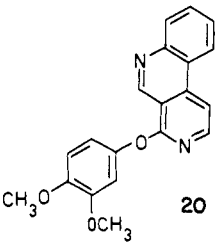

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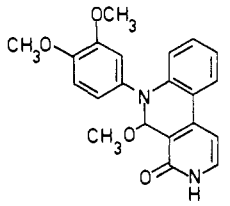

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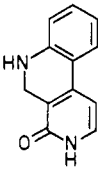

22

Perlolidine reacted with 4-bromoveratrole in the presence of potassium carbonate and copper powder to give the O-alkylated product **20**. The ultraviolet spectrum was misleading since it was almost identical with that of perlodidine in dilute acid and was very similar to that of perlodidine in dilute base. The mass spectrum, however, supplied conclusive evidence for the assigned structure. The second most intense peak in the spectrum (*m/e* 197) corresponds to cleavage of the ether linkage with loss of the veratrole group and the oxygen atom. The mass spectrum of perloline methyl ether (**21**), on the other hand, had an intense peak at *m/e* 196 which corresponds to loss of methoxy and the veratrole group to give the perlolidine ion. We were unable to detect any perloline-related substances in other alkylation attempts.

Experimental Section

Microanalyses were performed by Miss Heather King, University of California, Los Angeles, Calif. Melting points were determined with a Buchi melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer recording spectrometer, Model 21, with sodium chloride prisms, or an a Perkin-Elmer Model 137 Infracord; in general the listing of infrared bands include only those relevant to the structural argument. Ultraviolet spectra were measured on a Cary recording spectrophotometer, Model 11. A Varian Associates A-60 instrument was used for recording nmr spectra. Peak positions are given in parts per million (ppm) from an internal tetramethylsilane standard. Complete spectra are quoted when deemed appropriate and when adequately resolved; otherwise the pertinent and salient features only are given. The mass spectra were measured with an AEI MS-9 instrument using a direct inlet system, with ionization energy 70 eV; the strongest peaks only are listed. Thin layer chromatograms (tlc) were made with Merck (Darmstadt) Silica Gel G. Matheson activated alumina was used in preparing column chromatograms unless otherwise indicated. Anhydrous magnesium sulfate was used as a drying agent in working up reactions.

3-Cyano-4-phenyl-2-pyridone (4) from 1-Cyano-2-methylcinnamide (3). Sodium hydride (0.1 mol) was added slowly to a stirred warm solution of 5 g (0.025 mol) of 1-cyano-2-methylcinnamide[8] in 50 ml of ethyl formate. This reaction mixture was stirred overnight and then poured into water. The pyridone separated from the mixture, was filtered and recrystallized from methanol–benzene to yield 0.5 g of material with mp 228.5–231.5°; ir (KBr) 2215, 1615, and 1660 cm^{-1}; uv_{max} (95% C_2H_5OH) 241 mμ (ε 15,000), 283 (8300), and 340 (7800); nmr (DMSO-d_6) δ 7.81 (d, 1, J = 7 Hz) and 6.43 (d, 1, J = 7 Hz) characteristic of an AB system for the two hydrogens on the pyridone ring, 7.60 (m, 5, phenyl hydrogens); mass spectrum *m/e* (relative intensity) 196 (100) molecular ion, 168 (33) M − CO.

Anal. Calcd for $C_{12}H_8N_2O$: C, 73.46; H, 4.11; N, 14.28. Found: C, 73.49; H, 3.90; N, 13.94.

3-Cyano-4-phenyl-2-pyridone (4) from Sodium Formylacetophenone and Ethyl Cyanoacetate. A solution of 64.8 g (1.2 mol) of ammonium chloride in 200 ml of water was added to a solution of 204 g (1.2 mol) of sodium formylacetophenone[18] in 500 ml of water. After this mixture was allowed to stand overnight, a red oil formed. Extraction with ether, drying, and evaporation gave crude 1-amino-2-benzoylethylene (40–60%);[19] ir (film) 3400 cm^{-1} (N–H). The crude product (**5**) was used without further purification.

To a solution of sodium ethoxide (0.48 mol) in 400 ml of absolute ethanol was added a solution of 53.6 g (0.48 mol) of ethyl cyanoacetate and 68.8 g (0.48 mol) of crude 1-amino-2-benzoylethylene in 200 ml of alcohol. The mixture was refluxed for 24 hr and allowed to cool to room temperature. A solution of 40 ml of concentrated hydrochloric acid in 500 ml of water was added and the mixture was placed in a refrigerator for 24 hr. Collection of the precipitate and recrystallization from a 1:1 mixture of methanol–benzene gave 9.2 g of 3-cyano-4-phenyl-2-pyridone with mp 233.5–234°.

2-Aza-1-ketofluorenone (7). Solid 3-cyano-4-phenyl-2-pyridone (11.9 g) was added to 200 ml of polyphosphoric acid. This mixture was stirred and heated at 160–164° under nitrogen for 4 hr. Heating at temperatures greater than 165° resulted in charring of the organic materials. The reaction mixture was poured into water and the resulting solution refluxed overnight. After cooling and adjusting the pH to 5–6, the product separated. Recrystallization from ethanol yielded 5.3 g of the yellow 2-aza-1-ketofluorenone, mp 331–333° (decomposition, sealed tube); ir (KBr) 3440 (N–H), 1705 (ketone), and 1630 cm^{-1} (amide carbonyl); nmr δ 6.86 (d, 1, J = 6 Hz) and 7.90 (d, 1, J = 6 Hz), characteristic for the two protons on the pyridone ring, and 7.60 (m, 4, phenyl hydrogens).

Anal. Calcd for $C_{12}H_7NO_2$: C, 73.09; H, 3.58. Found: C, 73.23; H, 3.80.

3-Carbamoyl-4-phenyl-2-pyridone. If the aqueous mother liquors from the polyphosphoric acid reaction were allowed to stand overnight after removal of 2-aza-1-ketofluorenone, 3-carbamoyl-4-phenyl-2-pyridone crystallized from solution. This could be isolated efficiently by concentration of the aqueous mother liquors followed by continuous ether extraction. The yield from the above reaction was 0.9 g with mp 285–287°; ir (KBr) 3415 (N—H) and 1660–1600 cm^{-1} (amide and pyridone C=O); uv_{max} (95% C_2H_5OH) 231 mμ (ε 19,400), 260 (6500), and 317 (5800); mass spectrum *m/e* 196 (M − H_2O, base peak, corresponds to **4**), 214 (molecular ion), 198 (M − NH_2), 170 (M − $CONH_2$), 168 (196 − CO), and 140 (168 − H_2CN). This product was also formed from the

(17) Dihydroperlolidine (**22**) is probably an intermediate in Dr. J. A. D. Jeffreys' synthesis of perlolidine.

(18) W. S. Johnson, E. Woroch, and F. J. Mathews, *J. Am. Chem. Soc.*, **69**, 566 (1947).

(19) F. Asinger, L. Schroder, and S. Hoffman, *Ann.*, **648**, 83 (1961).

polyphosphoric acid reaction carried out at 145–155° and from hydrolysis of the pyridone **4** with 10% aqueous sodium hydroxide.

Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 67.28; H, 4.71; N, 13.08. Found: C, 66.37; H, 4.74; N, 12.59.

4-Phenyl-2-pyridone. A mixture of 250 mg of 3-carbamoyl-4-phenyl-2-pyridone and 15 ml of constant-boiling HCl was refluxed for 14 hr. The solution was cooled and 4-phenyl-2-pyridone crystallized from solution. Recrystallization from methanol–benzene gave 100 mg of product with mp 233.5–234.5° (lit.[20] 227–228°); ir (KBr) 1650 and 1610 cm^{-1} (pyridone C=O); uv_{max} (95% C_2H_5OH) 233 mμ (ε 18,000), 261 (11,500), and 315 (4300); mass spectrum 171 (molecular ion), 143 (M − CO), 115 (143 − 28).

2-Aza-1-ketofluorenol (8). A solution of 100 mg of sodium borohydride in methanol was added to 148 mg of 2-aza-1-ketofluorenone in 20 ml of methanol. The yellow color of the fluorenone was discharged by the borohydride to yield a clear colorless solution. After stirring for 15–30 min, the solution was poured into water and the pH adjusted to 5–6 with hydrochloric acid. A white solid precipitated, and this was recrystallized from ethanol to give an 85% yield of 2-aza-1-ketofluorenol with mp 250–251°; ir (KBr) 3260 (OH) and 1640 cm^{-1} (C=O); nmr (DMSO-d_6) δ 6.68 (d, 1, J = 7 Hz) and 7.60 (d, 1, J = 7 Hz) characteristic of the pyridone hydrogens, 7.50 (m, 4, aromatic hydrogens), and 5.45 (s, 1, *H*C-OH).

Anal. Calcd for $C_{12}H_9NO_2$: C, 72.35; H, 4.55. Found: C, 72.73; H, 4.71.

Perlolidine. A suspension of 1.3 g of 2-aza-4-ketofluorenol in 57 ml of chloroform was added to 780 mg of sodium azide in 31 ml of chloroform and 15 ml of concentrated sulfuric acid. This mixture was stirred for 1 hr at 25° and for 1 hr at 50°. Ice was added and this mixture was allowed to stand overnight. Filtration yielded a yellow solid which was dissolved in hot ethanol by careful addition of dilute sodium hydroxide solution. After filtration, the clear white solution was neutralized by bubbling CO_2 through it. A white solid separated and this was crystallized from hot ethanol to yield 520 mg of perlolidine, mp 337–341°; ir (KBr) 3400 (N—H) and 1645 cm^{-1} (C=O); uv_{max} (95% C_2H_5OH) 239 mμ (ε 50,300), 243 (49,300), 252 (45,000), 322 (11,600), 337 (13,800); uv_{max} (0.01 *N* NaOH) 237, 284, 325, 355; uv_{max} (0.01 *N* HCl) 240 (19,400), 249 (23,500), 256 (28,000), 272 (10,700), 283 (8800), 377 (12,200); uv_{max} (pH 8 buffer) 238 (59,000), 251 (41,000), 321 (9350), 337 (10,500), 353 (11,000); mass spectrum *m/e* 196 (M^+, base peak), 168 (M − CO), 140 (168 − H_2CN). The ir and uv spectra of synthetic perlolidine were identical with those of the natural material isolated by Dr. J. A. D. Jeffreys. A mixture melting point of synthetic perlolidine with natural material supplied by Dr. W. I. Taylor showed no depression.

3-Carbethoxy-4-methyl-2-quinolone (16). Diethyl malonate (5 g) and *o*-formaminoacetophenone[15] (5 g) were added to a cooled ethanol solution of sodium ethoxide (from 0.7 g of Na^0). This mixture was heated at reflux for 14 hr, cooled, and poured into water. Extraction with chloroform and evaporation of the organic solvents yielded 320 mg of crystals, mp 256–258° (recrystallized from hot ethanol); ir (Nujol) 1745 (ester) and 1640 cm^{-1} (amide C=O); mass spectrum *m/e* 231 (M^+), 186 (M − OC_2H_5), 185 (M − HOC_2H_5), 159 [base peak, M − (CO_2, C_2H_4)], 141 (159 − H_2O), 130 (159 − CO), and 103 (130 − HCN).

Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67. Found: C, 67.77; H, 5.92.

Periodate Oxidation Product (17) of N-Acetyltryphtophan. N-Acetyltryphtophan (2 g) was oxidized with sodium periodate in methanol–water under nitrogen using the procedure of Dolby and Booth.[15] After stirring overnight, the cooled reaction mixture was filtered and the filtrate evaporated. Chromatography of the residue on alumina (elution with 20% methanol in $CHCl_3$) gave 200 mg of a white solid. This was recrystallized from ethanol, mp 305–310° dec; ir (Nujol) 3450 and 3250 cm^{-1} (N—H), 1810 and 1750 (oxazolone),[21] 1680 and 1645 (amide C=O); uv_{max} (95% C_2H_5OH) 212 mμ (ε 26,000), 254 (4800), 298 (1600), essentially no change when the spectrum is run in 0.01 *N* NaOH; in 1 *N* NaOH solution the lactone ring of **17** probably opens and the uv_{max} became 223 mμ (ε 12,000), 248 (4800), 270 (5250); nmr (DMSO-d_6) δ 11.0 (broad, 1, N—H), 8.9 (d, 1, C*H*O), 7.2 (m, 4, aromatic hydrogens), 4.9 (q, 1, C=C—*H*), a multiplet at *ca.* 2.6–2.7 on the side of DMSO peak (tertiary H), 1.95 (s, 3, CH_3CO); mass spectrum *m/e* 160 (M^+), 218 (base peak, M − H_2C=C=O), 174 (218 − CO_2), 146 (174 − CO), 145 (174 − HCO), 144 (174 − HCN), 130 (174 − OHCNH, probably a quinolinium ion). N-Acetylaminoacetophenone, a model for the chromophor of opened **17**, has uv_{max} 242 mμ (log ε 4.1) and 280 (3.0) and *o*-aminostyrene, a model for **17** itself, has uv_{max} 221 (4.3) and 250 (3.9).

The oxidation product **17** upon heating with 10% NaOH was converted to a mixture of three compounds. The major component of this mixture was identified as kynurenine by its tlc behavior in three independent solvent system and by its characteristic color upon reaction with Ehrlich's reagent.

Anal. Calcd for $C_{13}H_{12}N_2O_4$: C, 60.00; H, 4.65. Found: C 60.20; H, 4.87.

Condensation Product (18) of Diethyl Malonate with Kynurenine. A mixture of 400 mg of kynurenine, 1 ml of diethyl malonate, and sodium ethoxide (from 125 mg of Na^0) was heated at reflux in ethanol solution for 14 hr. The mixture was then evaporated to dryness under reduced pressure. Water was added to the resulting solid, and the mixture was extracted with chloroform. Evaporation gave an oil which solidified upon standing. Recrystallization from ethanol–benzene gave 50 mg of yellow solid with mp 292–293° dec; ir (Nujol) 1740 (ester), 1685 (acid), and 1650 cm (amide C=O); uv_{max} (95% C_2H_5OH) 223 mμ (ε 23,000), 282 (5300), 339 (4600); mass spectrum *m/e* 287 (M^+), 242 (M − OC_2H_5), 214 (base peak, M − $CO_2C_2H_5$), 198 (242 − CO_2), 196 (242 − CO_2H), 170 (198 − CO), 140 (214 − CO, CO_2H_2).

Anal. Calcd for $C_{15}H_{13}NO_5$: mol wt, 287.07936. Found: mol wt, 287.07937.

Perlolidine Methiodide (19). A mixture of 200 mg of perlolidine and 5 ml of methyl iodide was heated at 90° for 14 hr. The mixture was cooled and evaporated to dryness. Recrystallization of the resulting solid from ethanol gave 280 mg of pure methiodide with mp 279–284° dec; uv_{max} (95% C_2H_5OH) 222 mμ (ε 31,000), 242 (28,000), 249 (20,500), 257 (21,000), 276 (9000), 286 (8400), 400 (10,500); uv_{max} (0.01 *N* NaOH) 226 (44,800), 241 (42,000), 256 (15,000), 290 (9300), strong adsorption (ε 10,000) at 400; uv_{max} (0.1 *N* HCl) 251 (34,600), 256 (43,100), 274 (14,000), 284 (11,900), and 382 (20,300).

Anal. Calcd for $C_{13}H_{11}N_2OI$: C, 46.19; H, 3.28. Found: C, 47.01; H, 3.61.

Alkylation of Perlolidine. A mixture of 100 mg of perlolidine, 2 ml of 4-bromoveratrol, 1 g of anhydrous potassium carbonate powder, and 250 mg of copper powder was heated at 200–210° under nitrogen for 24 hr. The reaction mixture was poured into water and extracted with $CHCl_3$. The chloroform extracts were extracted with 10% sulfuric acid solution and the acid extract made basic with excess potassium carbonate. The basic solution was then extracted with chloroform; this was dried and evaporated. All filtrations were carried out with sintered glass and not paper filters. The solid which was obtained was crystallized from $CHCl_3$–CH_3OH to give *ca.* 25 mg of **20** with mp 266–269°; ir (Nujol) 1670, 1625, and 1610 cm^{-1}; uv_{max} (CH_3OH) 246 mμ (ε 47,000), 253 (46,000), 324 (8000), 344 (11,000), 357 (12,000); uv_{max} (0.1 *N* NaOH in CH_3OH) 246 mμ (ε 36,000), 253 (35,000), 324 (6400), 344 (8900), 357 (10,000); uv_{max} (0.1 *N* HCl in CH_3OH) 236 mμ (ε 26,000), 252 (infl, 35,000), 258 (43,500), 384 (17,000); mass spectrum *m/e* 332 (base peak, M^+) 317 (M − CH_3), 289 (317 − CO), 246 (M − $(CH_3)_2(CO)_2$), 179 (second most intense peak, M − veratrol group and oxygen), and 152 (179 − HCN).

The mass spectrum of perloline methyl ether (**21**) has peaks at *m/e* 364 (very weak molecular ion), 334 (loss of CH_2O), 333 (base peak, M − CH_3O), 332 (M − CH_3OH), 305 (334 − CO), 304 (333 − CO), 168 (second most intense peak, loss of the veratrol group and methoxyl), 168 (196 − CO), and 140 (168 − 28).

Anal. Calcd for $C_{20}H_{16}N_2O_3$: mol wt, 332.11608. Found: mol wt, 332.11631.

Dihydroperlolidine (22). Solid perlolidine (530 mg) was added to 1.1 g of lithium aluminum hydride in 100 ml of THF. The perlolidine dissolved with the evolution of gas and the formation of a yellow solution. This mixture was refluxed for 14 hr and the excess hydride destroyed by the sequential addition of ethylacetate, ethanol, and ammonium chloride solution. Extraction with chloroform and evaporation gave 225 mg of dihydroperlolidine (yellow needles from ethanol); ir (Nujol) 3350 (N—H) and 1645 cm^{-1} (C=O); mass spectrum *m/e* 198 (M^+), 197 (M − 1, base peak), 196, the rest of the spectrum was identical with that of perlolidine. Dihydroperlolidine melts at 279–280°, but it rapidly looses its color and solidifies to remelt at 340° dec. This indicates that dihydroperlolidine is thermally dehydrogenating to perlolidine (mp 337–341°); this was confirmed by tlc (8% methanol in $CHCl_3$) of the

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melt. Due to the ease with which dihydroperlolidine underwent air oxidation upon standing in solution, we were never able to obtain this compound completely free of perlolidine.

Anal. Calcd for $C_{12}H_{10}N_2O$: mol wt, 198.07931. Found: mol wt, 198.07923.

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The Structure of Acetone-Oxytocin with Studies on the Reaction of Acetone with Various Peptides[1]

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Abstract: The structure of acetone-oxytocin has been shown to possess a 2,2-dimethyl-4-imidazolidinone ring structure in which the isopropylidene group from acetone forms a bridge between the nitrogen of the free amino group of one of the half-cystine residues of oxytocin and the nitrogen of the peptide bond between this half-cystine residue and the succeeding tyrosine residue. These results were obtained by extensive comparison of the chemical and spectral properties of the acetone derivative of S-benzyl-L-cysteinyl-L-tyrosine, from which acetone-oxytocin can be synthesized, with those of 2,2-dimethyl-4-imidazolidinone and 5-imino-2,2-dimethyloxazolidine. When L-prolyl-L-leucylglycinamide was treated with acetone under anhydrous conditions, a product was isolated which similarly was shown to be a substituted 2,2-dimethyl-4-imidazolidinone. On the other hand, treatment of S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide which can form neither an imidazolidinone nor an oxazolidine with acetone afforded no isolable product. However, the rapid formation of a Schiff base could be demonstrated by a trapping experiment using sodium borohydride which led to the formation of N-isopropyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.

In a recent communication,[3] the inactivation of oxytocin (Figure 1) by acetone was reported, and the new compound which formed, referred to as acetone-oxytocin, was isolated. Analytical and chemical evidence showed acetone-oxytocin to be a monoisopropylidene derivative of oxytocin. Further, the N-terminal amino group of oxytocin was shown to be involved since deamino-oxytocin, a highly potent analog of oxytocin in which the free amino group is replaced by hydrogen,[4,5] is not inactivated by acetone. Parallel experiments with lysine-vasopressin[6] led to similar inactivation, and a monoisopropylidene derivative of lysine-vasopressin, referred to as acetone-lysine-vasopressin, was isolated. As in the case of deamino-oxytocin, 1-deamino-8-lysine-vasopressin[7] was not inactivated by treatment with acetone, no isolable derivative of the deamino analog was formed, and only 1-deamino-lysine-vasopressin was recovered. This indicated that the amino group at position 1 of lysine-vasopressin, as in oxytocin, is necessary for formation of acetone-lysine-vasopressin.

The full biological activities of oxytocin and lysine-vasopressin could be recovered from acetone-oxytocin and acetone-lysine-vasopressin, respectively, by treatment of the derivatives with 0.25% acetic acid at 90° for 30 min. Acetone is liberated from both compounds mole for mole when either is heated at 100° in 0.1 *N* acetic acid for a short period of time.

More recently it was found[8] that S-benzyl-L-cysteinyl-L-tyrosine (I), the N-terminal dipeptide segment of S,S′-dibenzyl-oxytoceine, gave a crystalline monoisopropylidene derivative with acetone from which total synthesis of acetone-oxytocin was accomplished. The synthesis was achieved by first coupling the isopropylidene dipeptide with the heptapeptide L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The resulting isopropylidene derivative of S,S′-dibenzyl-oxytoceine was treated with sodium in liquid ammonia for removal of the S-benzyl groups, the disulfhydryl intermediate was oxidized with potassium ferricyanide, and the product was isolated. Since the synthetic product was found to be identical with acetone-oxytocin obtained by inactivation of oxytocin with acetone, the isopropylidene groups in both acetone-oxytocin and the isopropylidene derivative of S-benzyl-L-cysteinyl-L-tyrosine

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