strength[7] is usually reflected in decreasing bond length, the C—C bond will be longer and the N=N bond shorter in **2** than in **1**; this makes **2** geometrically closer to the transition state (principle of least motion)[8] and consequently more reactive.

It is already known that copper complexes of the azo compounds as well as the N-oxides of the azo compounds are thermally much more stable.[9] Such would be predicted for the reasons given above.

(7) The increased bond strength of the N=N bond in **2** compared to **1** is also reflected in the corresponding vibrational stretching frequency, i.e., for 2 = 1502 cm⁻¹ (R. C. Cookson, S. S. H. Gilani, and I. D. R. Stevens, *J. Chem. Soc. C*, 1905 (1967)) and for 1 = 1493 cm⁻¹ (B. M. Trost and R. M. Cory, *J. Amer. Chem. Soc.*, **93**, 5572 (1971)).

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(10) Camille and Henry Dreyfus Teacher–Scholar Grant Recipient.

Harmut Schmidt, Armin Schweig*

Fachbereich Physikalische Chemie der Universität Marburg/Lahn
Marburg/Lahn, Germany

Barry M. Trost,*[10] Hans B. Neubold, Paul H. Scudder

Department of Chemistry, University of Wisconsin
Madison, Wisconsin 53706

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Photosensitized Oxygenation of Tryptophan Methyl Ester and N_b-Methyltryptamine. Isolation and Identification of 3a-Hydroxypyrroloindole and 4a-Hydroxy-1,2-oxazinoindole

Sir:

Oxidation mechanisms of aromatic substrates catalyzed by oxygenases have received much attention in recent years.[1] Not long ago we reported[2] a model reaction for the oxidation of tryptophan by monooxygenases, viz., the conversion of N_a,N_b-dimethyltryptamine to 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrroloindole (**1**) by photolysis with pyridine 1-oxide (path A).

In the metabolic transformation of tryptophan to kynurenine by tryptophan 2,3-dioxygenase[3] the hydroperoxyindolenine (**3**) has been suggested as a primary intermediate.[4] The photosensitized oxygenation of tryptophan to N-formylkynurenine (**5**) provides a model reaction for the enzymatic oxidation which is believed to proceed via a dioxetane intermediate[5] derived from **3**. In a third pathway (C) the ethylamino side chain[4] in **3** participates with the formation of 3a-
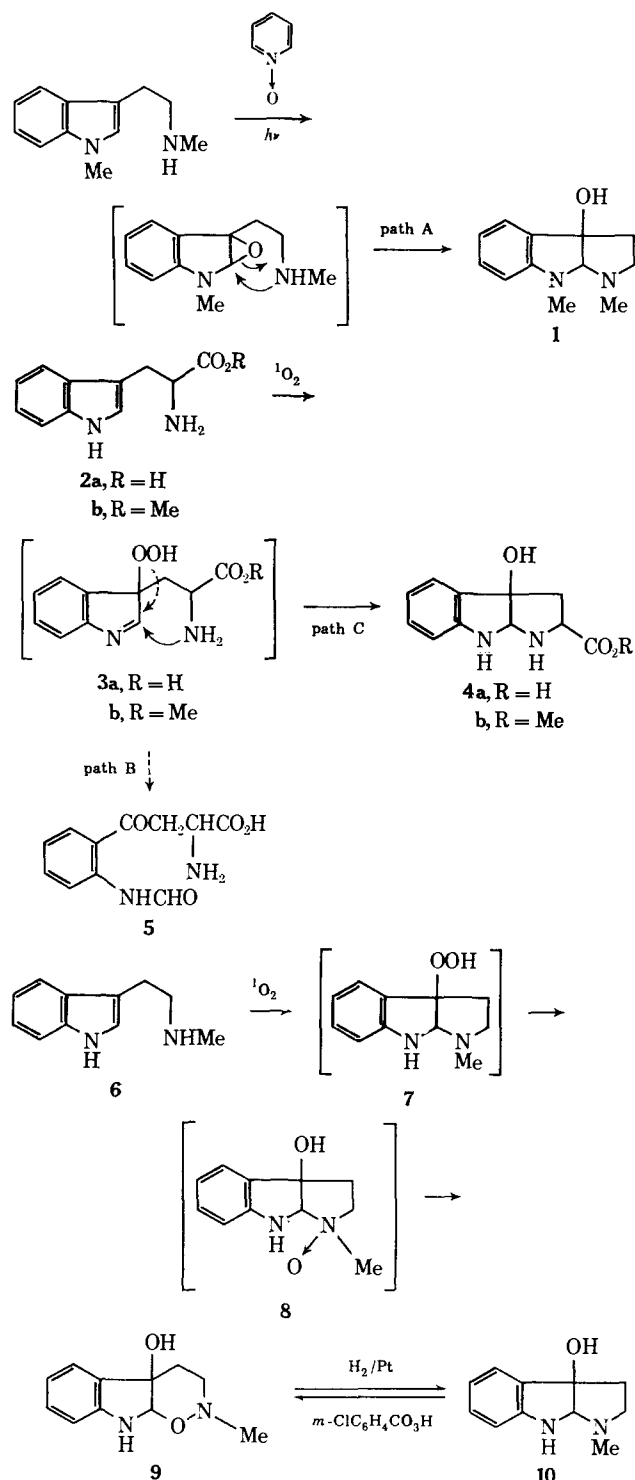
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hydroxy-1,2,3,3a,8,8a-hexahydropyrroloindoles (**4**), possessing the novel ring system of the sporidesmins[6] and brevianamide E.[7]

We now report one-step syntheses of 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrroloindole (**4b**) and 4a-hydroxy-1,2,3,3a,8,8a-hexahydrooxazinoindole (**9**) which are probably formed (path C) via 3a-hydroperoxytetrahydropyrroloindole(**7**)when tryptophan methyl ester(**2b**) and N_b-methyltryptamine (**6**), respectively, were photooxygenated. A 4.4 mM solution of **2b** in benzene (250 ml) was irradiated (300-W flood lamp) for 15 hr in the presence of Rose Bengal (50 mg in 5 ml of MeOH) while oxygen was bubbled through the reaction vessel. Column chromatography followed by preparative tlc of the crude photolysate gave 3.7% **4b**, mp 166–167°:[8] λ_{max}^{EtOH} nm (ϵ) 244 (7900), 302 (2300); $\lambda_{max}^{EtOH-HCl}$ nm (ϵ) 236.5 (7400), 295 (2300);[9] m/e 234 (M⁺); ν_{max}^{KBr} cm⁻¹ 3417, 3395, 3272, 3240 (OH, NH); δ (CDCl₃) 2.30–2.60 (m, 2, CH₂), 3.07 (broad s, 3, NH, OH), 3.73 (s, 3, CH₃), 3.60–3.90 (m, 1, C₂H), 5.02 (s, 1, NCHN).

Previously, photosensitized oxidations of tryptophan have been conducted in either water or organic acids such as HCOOH or CH₃CO₂H,[10] where participation of the ethylamino side chain is unfavorable and the reaction consequently proceeds via path B. When photooxygenation of **6** was carried out under similar reaction conditions (200-W Halogen lamp) for 7 hr, crystalline 4a-hydroxy-2-methyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-b]indole (**9**) (25–30%) was isolated:[11] mp 197–198°, m/e 206 (M⁺); λ_{max}^{EtOH} nm (ϵ) 242 (7460), 297 (2300); $\lambda_{max}^{EtOH-HCl}$ nm (ϵ) 236 (7590), 293 (2050); δ (C₅D₅N) 2.50 (s, N_b-Me), 2.20–2.90 (m, CH₂CH₂), 4.70 (broad s, OH, NH), 5.37 (s, NCHO), ν_{max}^{KBr} cm⁻¹ 3300, 3150 (OH, NH), 990 (N–O). The formation of **9** may result from the intramolecular oxidation of the intermediate 3a-hydroperoxyindolenine (**7**) to the N-oxide (**8**),[12] which then spontaneously rearranges to **9**.

Catalytic hydrogenation (PtO₂) of **9** in MeOH in the presence of a catalytic amount of HCl gave **10**: mp 151°; m/e 190 (M⁺); λ_{max}^{EtOH} nm (ϵ) 243 (8740), 302 (2470); $\lambda_{max}^{EtOH-HCl}$ nm (ϵ) 236.5 (7980), 294.5 (2280); δ (CDCl₃) 2.10–2.90 (m, CH₂CH₂), 2.35 (s, N_b-Me), 3.45 (broad s, OH or NH), 4.10 (broad s, OH or NH), 4.38 (s, NCHN); δ (C₅D₅N) 2.46 (s, N_b-Me), 4.88 (s, NCHN); ν_{max}^{KBr} cm⁻¹ 3300, 3080 (OH, NH).

Furthermore, instead of **8**, we obtained **9** upon oxidation of **10** with m-chloroperbenzoic acid,[13] indicative

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(11) The compound **9** was not obtained when **10** was treated under the reaction condition. When the reaction was carried out in MeOH under similar reaction conditions, only 6% yield of **9** was obtained. The stereochemistry of **9** has not yet been determined. Both **4b** and **9** were not produced when the reactions were carried out in absence of Rose Bengal, respectively.

(12) 3-Hydroperoxy-3-methyl-2-phenylindolenine oxidized Et₃N to triethylamine oxide in high yield (unpublished data): M. Nakagawa, H. Yamaguchi, and T. Hino, *Tetrahedron Lett.*, 4035 (1970); M. Nakagawa, T. Suzuki, T. Kawashima, and T. Hino, *Chem. Pharm. Bull.*, **20**, 2413 (1972).

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of easy rearrangement of **8** to **9** at room temperature.[14] The oxidation of physostigmine by hydrogen peroxide and rearrangement to geneserine has been reported.[15] The isolation of **4b** and **9** provides new evidence for 3a-hydroperoxyindolenines as intermediates in the reaction of tryptophan derivatives with singlet oxygen.

The fact that both dioxygenase and monooxygenase model reactions produce the 3a-hydroxyhexahydro-pyrrolo[2,3-*b*]indole ring system[16] suggests that the

(14) We obtained geneserine upon oxidation of physostigmine with *m*-ClC₆H₄CO₃H.

(15) C. Hootel, *Tetrahedron Lett.*, 2713 (1969); B. Robinson, *Alkaloids*, 13, 213 (1971).

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hydroxyl group at the **3a** position in the sporidesmins and brevianamides most likely arises biogenetically *via* path A or path C. Kynurenine derivatives were not formed under our reaction conditions.

Masako Nakagawa,* Takao Kaneko
Kensei Yoshikawa, Tohru Hino
*Faculty of Pharmaceutical Sciences, Chiba University
1-33, Yayoi, Chiba, 280, Japan*
Received September 4, 1973

Sulfite Esterase Activity of Pepsin Modified at Active Site Carboxyl Groups

Sir:

Since the discovery was made that pepsin catalyzes the hydrolysis of sulfite esters,[1] the following lines of evidence have been adduced in support of the hypothesis that the active site requirements for the sulfite esterase action of the enzyme are the same as those for its peptidase action. (a) Peptides which bind to the active site of pepsin have been shown to act as competitive inhibitors toward the sulfite esterase activity of the enzyme, and the inhibition constants obtained with these peptides correspond closely with the Michaelis constants calculated from their pepsin-catalyzed hydrolysis.[1,2] (b) The diazocarbonyl reagent *N*-diazoacetyl-D,L-norleucine methyl ester which is known to inactivate pepsin as a peptidase[3] has also been reported to cause the inactivation of the enzyme as a catalyst for the hydrolysis of diphenyl sulfite and methyl phenyl sulfite at pH 2.[1] (c) The pH dependency of the rate parameter k_{cat}/K_m for the pepsin-catalyzed hydrolysis of the reactive sulfite ester substrate bis-*p*-nitrophenyl sulfite[4] corresponds fairly closely to the pH dependency of this parameter for the hydrolysis of the neutral dipeptide *N*-acetyl-L-phenylalanyl-L-phenylalanylamide.[5]

We now wish to report our discovery that pepsin modified by treatment with either the diazoketone α-diazo-*p*-bromoacetophenone (I) in the presence of cupric ion[6] or the epoxide 1,2-epoxy-3-(*p*-nitrophenoxy)-propane (II)[7,8] at pH 5 and 25°, retaining less than 1% activity toward the peptide substrate hemoglobin,[9] remains very active over a range of pH values as a catalyst for the hydrolysis of a variety of symmetrical and unsymmetrical sulfite ester substrates, including bis-*p*-nitrophenyl sulfite (III), phenyl *p*-nitrophenyl sulfite (IV), and methyl *p*-nitrophenyl sulfite (V).

Pseudo-first-order kinetics were obtained, and no evidence for enantiomeric specificity was seen when the rates of hydrolysis of the latter sulfite ester catalyzed by

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