**Table I.** Chemical Shifts and Coupling Constants for the Methyl Diazonium and Methylene Diazenium Ions

| | species[a] | | | | |
|---|---|---|---|---|---|
| | $CH_2LN_2^+$ | $CH_2N_2^+L$ | $CH_2LOSO_2F$ | $CH_3OH_2^+$ | $CH_2N_2$ |
| $\delta(^1H)$[b] | 4.75 | 6.09 | 4.21 | 4.7, 9.4[c] | 3.28[d] |
| $\delta(^{13}C)$ | 43.78 | 73.28 | 63.20 | 62.9[e] | 23.1[f] |
| $^2J_{HD}$[g] | 2.2 | | 1.6 | | |
| $^1J_{HC}$ | 163.3 | 176.0 | 155.5 | 157.5[e] | |
| $^1J_{DC}$ | 24.7 | | 23.5 | | |

[a] L signifies H or D. [b] Chemical shifts were measured in parts per million relative to $Me_4Si$ = 0. [c] Reference 6. [d] Reference 7. [e] Reference 8. [f] Reference 9. [g] Coupling constants were measured in hertz.

formed on heating this sample was monodeuterated, as witnessed by triplet resonances in its $^1H$ and $^{13}C$ spectra; therefore H-D exchange and, consequently, reversible protonation on carbon is insignificant in the intermediates.

The most reasonable structures compatible with these observations are **1** for the major intermediate and **2** for the minor one; their chemical shifts and coupling constants are collected in Table I along with reference data for diazomethane, methyl fluorosulfate, and the methyl oxonium ion.

It is possible that the methyl diazonium ion be not free in solution, but rather associated with, or bonded to, the fluorosulfate counterion.[10] This is considered unlikely, however, as incorporation of antimony pentafluoride (up to 50% the acid concentration) to the solution did not change the $^1H$ or $^{13}C$ shifts. Furthermore, the $^{19}F$ spectrum of the reaction mixture[11] at −100 °C showed only the peaks of $SO_2ClF$ at −99.1 ppm and $HFSO_3$ at −41.7 ppm (relative to $CFCl_3$). After the mixture was heated to −80 °C, the resonance of methyl fluorosulfate appeared at −31.4 ppm (lit.[12] −31.2 ppm).

Protonated diazirene could be considered as an alternative structure to **2**.[10] It can be excluded, however, as introduction of diazirene[13,11] into the reaction medium at −120 °C did not produce any previously observed species, but only one with $^{13}C$ shift of 33.8 ppm ($\delta(^{13}C)$ of diazirene 10.9 ppm), which was unaffected by heating the mixture to −80 °C.

In accordance with the proposed structures **1** and **2**, both the $^1H$ and $^{13}C$ chemical shifts of **2** are considerably greater than those of **1**. Surprisingly, although the proton chemical shift of **1** is very similar to those of methyl fluorosulfate and the methyl oxonium ion, indicating similar electron distributions in the three methyl groups, the $^{13}C$ shift of **1** is almost 20 ppm upfield from the other two, though nonetheless 20 ppm downfield from the diazomethane value. As the one-bond coupling constants $^1J_{CH}$ and $^1J_{CD}$ in Table I indicate that the diazonium group is slightly more electron withdrawing than the fluorosulfate or oxonium groups, this must be taken as another example of the unreliability of the $^{13}C$ shift as a measure of electron density, at least for diazo and diazonium compounds.[14]

The broadening (15 Hz) of the $^{13}C$ resonance of **2** was observed not only for the singlet in the proton decoupled spectrum, but also for the triplet in the proton coupled spectrum, and so is unlikely to derive from poor resolution of fortuitously close peaks. An alternative explanation—rapid exchange of the N-H proton—is acceptable only if confined to an intimately bound complex, as otherwise the diazomethane formed would also be reprotonated on carbon, leading to the eventual demise of the N-protonated species. A probable source of broadening for the resonance of **2** but not of **1** is a greater degree of $^{13}C$-$^{14}N$ coupling in the former. It has previously been shown that, whereas in diazomethane this coupling can be observed at room temperature,[15] less symmetrically substituted diazoalkanes have merely broadened lines for the diazo carbons. The electrical field gradient around the central nitrogen in **1** is less symmetrical than that in **2**, entailing a more rapid $^{14}N$ quadrupolar relaxation. This in turn removes the $^{13}C$-$^{14}N$ coupling and gives a narrower line for the diazonium ion resonance.

The C-protonated species **1** has been calculated to have in the gas phase a lesser free enthalpy of formation than that of the N-protonated isomer by 125 kJ $mol^{-1}$ (basis set 4-31G), or 209 kJ $mol^{-1}$ (basis set STO-3G).[16] The solvational energy assigned to an $^+N$-H bond in fluorosulfuric acid is 46 kJ $mol^{-1}$;[17] so from these values **1** would be predicted to be the only observable isomer in $HFSO_3$-$SO_2ClF$. Hence, the isomer ratio of 4:1 observed in our study could indicate the relative rates of irreversible protonation on the two basic centers of diazomethane.

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## 3a-Hydroperoxypyrroloindole from Tryptophan. Isolation and Transformation to Formylkynurenine

*Sir:*

Since the early 1950s, when the first report on Methylene Blue sensitized photooxygenation of tryptophan appeared,[1] this field of study has been developed as a tool for the elucidation of the active site in enzymes, and as a model reaction for the dioxygenase-catalyzed reaction of tryptophan to formylkynurenine.[2] There is, however, considerable variation in the earlier results and very little attention has been given to the reaction mechanism.

Recently we reported that Rose Bengal sensitized photooxygenation of tryptophan[3] and tryptophan derivatives[4] followed by $Me_2S$ reduction gave 3a-hydroxypyrroloindoles,

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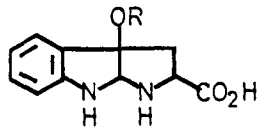
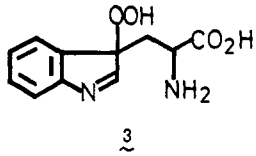
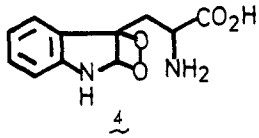


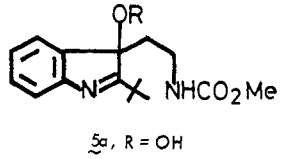
1a, R = OH
b, R = H



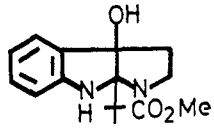
3



4



5a, R = OH
b, R = H



6

whereas formylkynurenine was isolated by heating the oxygenation mixture obtained from tryptophan. In this paper we report the first successful isolation, the nature, and the reactivity of the initially formed peroxidic intermediate in the dye-sensitized photooxygenation of tryptophan.

L-Tryptophan (1 g, 5 mM) was irradiated[5] in 5% EtOH–$H_2O$ containing Rose Bengal (15 mg, $^1/_{300}$ mol equiv) as sensitizer at 0–5 °C for 3.5 h, while oxygen was bubbled through the mixture, which was then acidified with AcOH and extracted with $CH_2Cl_2$ to remove the sensitizer. Lyophilization of the aqueous solution[6] gave the tricyclic hydroperoxide **1a** as a powder in 75–85% yield[7] which was successfully purified on a Sephadex G-10 column to give a colorless powder. **1a:** $\lambda_{max}$ ($H_2O$) nm ($\epsilon$), 235 (6300), 294.5 (2000); $\nu_{max}$ (KBr) 1610 $cm^{-1}$; NMR $\delta$ ($D_2O$) 2.50–3.20 (m, 2 H, $C_3$ H), 4.01 (t, 0.6 H, cis $C_2$ H), 4.39 (t, 0.4 H, trans $C_2$ H), 5.64 (s, 0.4 H, trans NCHN), 5.75 (s, 0.6 H, cis NCHN), 6.70–7.15, 7.15–7.60 (m, 4 H, aromatic H); *m/e* 236 ($M^+$). Chromatographic analysis on silica gel as well as the NMR spectrum revealed **1a** to be a mixture of cis and trans isomers, reduction of which with $Me_2S$ furnished the corresponding hydroxy derivatives **1b**.[8] The hydroperoxide **1a** was partially converted to formylkynurenine (**2**, 21%) along with **1b** (55%) when heated in water at 100 °C for 30 min. The aqueous solution of **1a** was also found to convert to **2** (11%) and **1b** (70%) after 1 week at room temperature. Decomposition of **1a** was catalyzed by silica gel, metal ions such as $Fe^{3+}$ and $Fe^{2+}$, and light (253.7 or >300 nm); however, cupric and cuprous ions did not show any significant effect.[9] On the other hand, the exclusive formation of formylkynurenine (**2**, 64%) from **1a** was observed when an aqueous solution of **1a** was basified with $Na_2CO_3$ to about pH 9.[10] In contrast, by acidification with HCl, **1a** underwent a facile acid-catalyzed rearrangement followed by hydrolysis to give *o*-aminophenol in 40% yield.[4c,e]

The rearrangement of **1a** to formylkynurenine could be explained by assuming an eight-membered unstable cyclic carbinol as an intermediate.[4e] Yet there is another possibility involving **3** derived from **1a** to give **2** via the dioxetane **4**, although evidence for the existence of an equilibrium between **1a** and **3** is lacking. In order to demonstrate tautomerism between **1a** and **3**, we have carried out the analogous photosensitized oxygenation of 2-*tert*-butyl-$N^b$-methoxycarbonyltryptamine which has the bulky substituent at the 2 position. As expected, the hydroperoxyindolenine **5a**,[11] corresponding to the putative primary intermediate **3** of the dye-sensitized photooxygenation of tryptophan and tryptamines, was isolated in 90% yield. Reduction of **5a** with $Me_2S$ gave **5b**, mp 157–159 °C.[12] The NMR spectrum of **5b** showed that **5b** exists as a tautomeric mixture of **5b** and **6** (15:85 in $CDCl_3$, 4:1 in $CD_3OD$). Refluxing **5b** in $CH_2Cl_2$ followed by recrystallization from $CH_2Cl_2$–hexane gave **6**, mp 136–138 °C, which showed an NMR spectrum identical with that obtained from **5b** after equilibrium is reached.

These results, by reasons of analogy, may be regarded as strong evidence for the presence of an equilibrium between hydroperoxyindolenine **3** and the hydroperoxypyrroloindole **1a** and support the mechanism previously proposed for the formation of formylkynurenine (**2**) from **1a** via **3** and **4**.[3,13] These results provide a powerful predictive model for mechanism of biological oxidation of tryptophan.

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(5) A 500-W halogen lamp with $CuCl_2$–$CaCl_2$ filter was used.
(6) Neither formylkynurenine nor $N^b$-formylkynurenine has been detected (TLC, UV spectrum).
(7) The yield was determined by iodometry. The extent of decomposition of **1a** was shown to be <5% when kept at about −70 °C for 2 months, while **1a** decomposes at room temperature within 24 h.
(8) The L-series **1b** showed UV and NMR spectra identical with those of the DL series **1b**. The stereochemistry of the two isomers of 1b has been established.[3] Cf. W. E. Savige, *Aust. J. Chem.*, **28**, 2275 (1975).
(9) Decomposition was monitored by iodometry as well as TLC. For an example, when $FeCl_3$ (0.1 mol equiv) was added, **1a** was decomposed after 24 h. Acid hydrolysis of the reaction mixture with $F_3CCO_2H$ followed by Dowex 50 × 8 column separation afforded kynurenine (2%) and **1b** (59%).
(10) The mechanism of the base-catalyzed conversion is not clear. However, the reverse reaction of **1a** to **3** might be catalyzed by the base. The dioxetane formation or addition of water to the C=N double bond of the indolenine **3** may be favored in alkaline conditions.
(11) **5a:** chromatographically homogeneous oil; $\lambda_{max}$ (EtOH), nm, 222, 264, 292 (sh); $\nu_{max}$ (KBr), $cm^{-1}$, 1700, 1560, 1535; $\delta$ ($CDCl_3$) 1.44 (s, 9 H, $Me_3C$), 2.00–2.44 (m, 2 H, $CH_2$), 2.44–3.30 (m, 2 H, $CH_2N$), 3.56 (s, 3 H, $CO_2CH_3$); *m/e* 306 ($M^+$). **5a** was converted to the formylkynurenine analogue, mp 120.5–122.5 °C, when refluxed in MeOH.
(12) Satisfactory elemental analysis and spectral data were obtained for all new compounds characterized by melting points.
(13) A weak chemiluminescence was observed in a dark room when **1a** was heated in $Me_2SO$ at 170 °C.

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