Peroxidic Intermediates in Photosensitized Oxygenation of Tryptophan Derivatives

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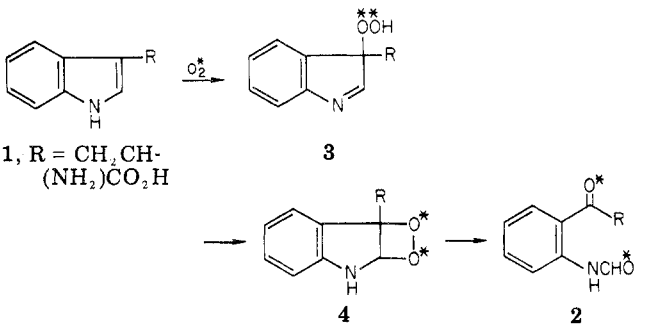
The damaging effects of dyes, light, and oxygen on biological systems have been known since the last century, and are usually termed photodynamic action.[1,2] The biological effects of photodynamic action include membrane damage, mutagenesis, interference with metabolism, and many other life processes. The sites of biological lesion in photodynamic action have been extensively studied.[1,2] Photooxidation of certain amino acids, nucleotides, lipids, and certain cell constituents appears to be the cause of the lesions, although the detailed chemistry is not well understood in most cases.[1-3] Destruction of key active sites of amino acids, particularly tryptophan, methionine, and histidine, causes inactivation of many enzymes.[3] Photooxidation of tryptophyl residues is also responsible for discoloration of irradiated silk and wool fabrics.[4] In order to understand the mechanism of the photodynamic damage caused by tryptophan photooxidation, one must examine the chemistry of the dye-sensitized photooxygenation of free tryptophan or its model compounds. Before we initiated an investigation of the photooxygenation of tryptophan, there had been a number of reports concerned with the photoproducts as well as with the mechanisms of unsensitized irradiation with UV light and dye-sensitized photooxygenation of tryptophan.[3,5]

The reaction of tryptophan (1) with molecular oxygen is also of special interest in view of the mechanism for the metabolic transformation of 1 into formylkynurenine (2) catalyzed by tryptophan 2,3-dioxygenase.[6] When the enzymic reaction is carried out in the presence of $^{18}O_2$, the label is incorporated both into the formyl and ketone moieties of 2.[7] Recently, many other indoles such as 5-hydroxytryptophan, serotonin, tryptamine, and melatonin have been shown to be converted to the corresponding formylkynurenamine-type product by the action of indolamine 2,3-dioxygenase.[8,9] This enzyme utilizes superoxide anion as an oxygen source and is suggested to play an important role in the metabolism of these indolamines.[9] Whatever active oxygen species is involved in the enzymic reactions, it seems very likely that a peroxide bridge is formed at a certain stage of the reaction. Since Witkop's pioneering work in the early 1950s on the chemical behavior of the 3-hydroperoxyindolenines available from the oxidation of 2,3-disubstituted indoles, it has been assumed that the enzymic reaction proceeds by way of 3-hydroperoxyindolenine (3), followed by cyclization to a dioxetane (4; Scheme I).[10-12] Although this mechanism has been widely accepted,[5,11,12] evidence in support of the intermediacy of 3 and/or 4 has not been obtained either in the chemical or in the enzymic oxygenations. In fact, other mechanisms compatible with the experimental results of the enzymic reaction are also possible (vide infra).

Scheme I



Thus, insight into the mechanism of the reaction of tryptophan with molecular oxygen has been seriously hampered by the insufficiency of knowledge concerning

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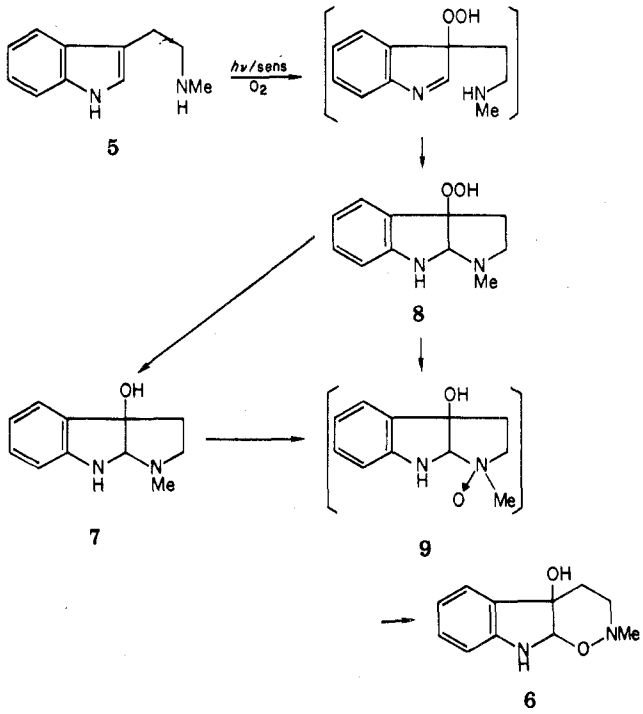
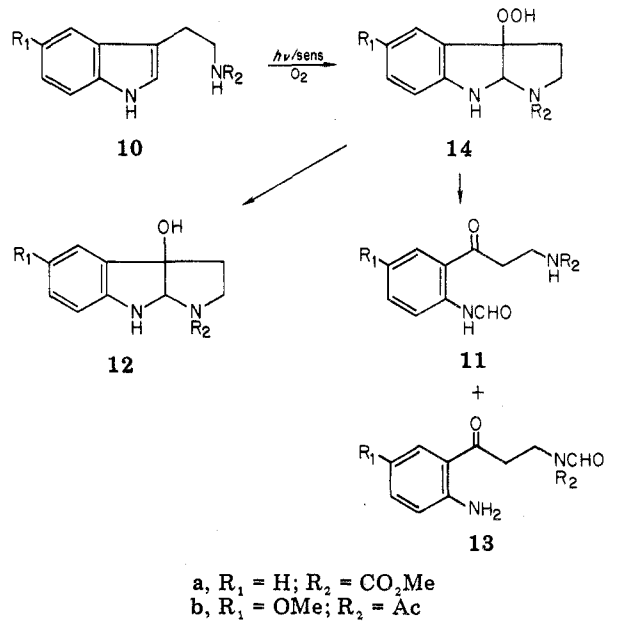
(1) U. Gallo and L. Santamaria, Ed., "Research Progress in Organic, Biological, and Medicinal Chemistry", Vol. II, American Elsevier Publishing Co., New York, N.Y., 1972.
(2) J. D. Spikes and R. Livigton, *Adv. Radiation Biol.*, **3**, 29 (1969).
(3) J. D. Spikes and M. L. MacKnight, *Ann. N.Y. Acad. Sci.*, **171**, 149 (1970).
(4) I. H. Leaver and G. Caird Ramsay, *Photochem. Photobiol.*, **9**, 531 (1969).
(5) A. Fontana and C. Toniolo, *Prog. Chem. Org. Nat. Prod.*, **33**, 309 (1976).
(6) P. Feigelson and F. O. Brady, "Molecular Mechanisms of Oxygen Activation", O. Hayaishi, Ed., Academic Press, New York, N.Y., 1974, p 87.
(7) O. Hayaishi, R. Rothberg, A. H. Mehler, and Y. Saito, *J. Biol. Chem.*, **229**, 880 (1957).
(8) F. Hirata, O. Hayaishi, T. Tokuyama, and S. Senoh, *J. Biol. Chem.*, **249**, 1311 (1974).
(9) F. Hirata and O. Hayaishi, *J. Biol. Chem.*, **250**, 5960 (1975), and references therein.
(10) A. Ek. H. Kissman, J. B. Patrick, and B. Witkop, *Experientia*, **8**, 36 (1952).
(11) R. J. Sundberg, "Chemistry of Indoles", Academic Press, New York, N.Y., 1970, p 282.
(12) G. A. Hamilton, ref 6, p 444.

Scheme II

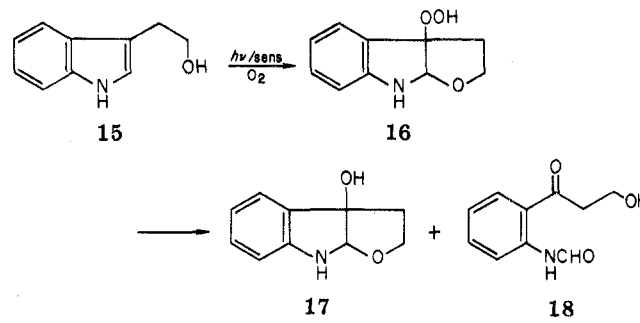


Scheme III



a, R_1 = H; R_2 = CO_2Me
b, R_1 = OMe; R_2 = Ac

Scheme IV



the chemistry of the initially formed peroxide intermediates, although the chemistry of 3-hydroperoxyindolenines is well understood. The transformation of **1** into **2** has been accomplished by several chemical oxidations, e.g., ozonolysis,[5,13,14] photooxidation,[5,15] and transition metal catalyzed oxidation;[16] in none of the cases has any peroxidic product been obtained. We have found that dye-sensitized photooxygenation under carefully controlled conditions (with filtered light at low temperature) makes it feasible to isolate peroxide intermediates from a number of indoles, including tryptophan derivatives. In this Account we present our recent results on the nature of the initially formed peroxide intermediates in singlet-oxygen reactions with tryptophan and related compounds. We also discuss the multiplicity of paths for the decompositions of the peroxidic intermediates.

Photooxygenation of Tryptamine Derivatives

2,3-Disubstituted indoles are known to be susceptible to air oxidation to yield 3-hydroperoxyindolenines as primary products,[11] whereas the hydroperoxides from 3-substituted indoles have not been obtained. Dye-sensitized photooxygenation, however, produces hydroperoxides from 3-substituted indoles like tryptamine and tryptophol. Rose bengal sensitized photooxygenation of N^b-methyltryptamine (**5**) in benzene–methanol at room temperature gave **6** as the major product, together with a small amount of **7**.[17] When the reaction was carried out in methanol at 0 °C, followed by rapid workup, 3a-hydroperoxypyrroloindole **8** was isolated. The hydroperoxide **8**, on standing in pyridine, methanol, or benzene at room temperature, was converted to **6**, suggesting that **6** is formed from **8** by way of **9**. Actually, oxidation of **7** with *m*-chloroperbenzoic acid or 3-hydroperoxy-3-methyl-2-phenylindolenine gave **6** (Scheme II).[18] The product derived from cleavage of the enamine double bond was not obtained from **5**. On the other hand, rose bengal sensitized photooxygenation of N^b-methoxycarbonyltryptamine (**10a**) in a polar solvent like acetone, *tert*-butyl alcohol, or pyridine–methanol, followed by chromatographic separation, provided three products, **11a**, **12a**, and a novel transformylated product, **13a**.[19] The same photooxygenation at low temperature with light filtered through an aqueous $CuCl_2$–$CaCl_2$ solution, followed by alumina chromatography, gave the hydroperoxide **14a**. An important, characteristic reaction of **14a** is its facile transformation into **11a**, **12a**, and **13a** when treated with silica gel or refluxed in benzene.[20] Due to the lack of a basic nitrogen, **14a** undergoes rearrangement to **11a** and **13a**, in contrast to **8**, which undergoes rapid intramolecular oxidation to give **9**, followed by spontaneous rearrangement to **6** (Scheme III). The hydroperoxides (**8**, **14a**) are readily reduced to 3a-hydroxypyrroloindoles (**7**, **12a**), providing a convenient synthetic route for the biogenetic-like transformation of indoles into the 3a-hydroxypyrroloindole ring system frequently found in natural

(13) B. Witkop, *Justus Liebigs Ann. Chem.*, **558**, 103 (1947).
(14) F. Sakiyama and N. Masuda, *Chem. Lett.*, 949 (1973).
(15) A. Pirie and K. J. Dilley, *Photochem. Photobiol.*, **19**, 115 (1974), and references therein.
(16) A. Nishinaga, *Chem. Lett.*, 273 (1975).
(17) M. Nakagawa, T. Kaneko, K. Yoshikawa, and T. Hino, *J. Am. Chem. Soc.*, **96**, 624 (1974).
(18) M. Nakagawa, K. Yoshikawa, and T. Hino, *J. Am. Chem. Soc.*, **97**, 6496 (1975).
(19) M. Nakagawa, H. Okajima, and T. Hino, *J. Am. Chem. Soc.*, **98**, 635 (1976).
(20) M. Nakagawa, H. Okajima, and T. Hino, *J. Am. Chem. Soc.*, in press.

Table I
Variation of the Product Distributions (20, 21) with Temperature[a]

Compound[a]	Solvent	Temp, °C	Products, %[b] 20	21
19a	Methanol	20	20a (95)	
	Methanol	−35	20a (17)	21a (78)
	Methanol	−70	20a (3)	21a (95)
	Acetonitrile	20	20a (85)	
	Acetonitrile	−30	20a (35)	21a (50)
19b	Methanol	20	20b (60)	21b (28)
	Methanol	−70		21b (95)
	Acetonitrile	20	20b (55)	21b (36)
	Acetonitrile	−30	20b (22)	21b (68)

[a] Initial concentration (2 mM); rose bengal was used as a sensitizer ($CuCl_2$-$CaCl_2$ filter). [b] Determined by NMR analysis of the reaction mixture.

products such as sporidesmine,[21] brevianamide E,[22] and hunteracine bromide.[23]

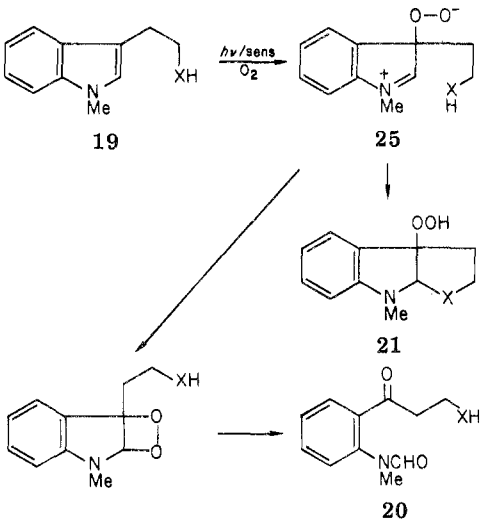
Under similar conditions, photooxygenation of melatonin (**10b**) at −70 °C, followed by reduction with dimethyl sulfide, produced **12b**, whereas at room temperature **10b** gave **11b**, suggesting that the hydroperoxide **14b** is again involved as an intermediate; attempts to isolate **14b**, however, were unsuccessful.[24] Photooxygenation of tryptophol (**15**) at −70 °C gave a quantitative yield of the hydroperoxide **16**,[25] which readily decomposed to give **17** and **18** under a variety of conditions (vide infra) (Scheme IV).

Peroxidic Intermediates of N-Methylindoles

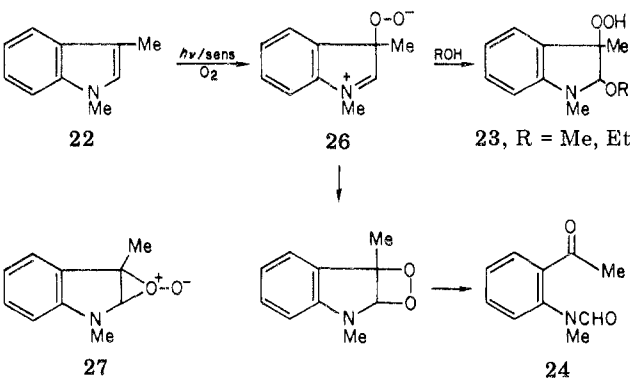
Enamines are well known to react with singlet oxygen to give dioxetanes which can subsequently cleave to carbonyl and amide fragments.[26-28] The 1,2-cycloaddition of singlet oxygen with electron-rich olefins like enol ethers and enamines had been assumed to be a concerted ($_\pi2 + _\pi2$) process.[29,30] However, a nonconcerted process involving an ionic peroxide such as a zwitterion or a perepoxide is also possible.[31] Recent theoretical calculations favor a nonconcerted process involving a zwitterion as the initial intermediate.[32]

N-Methylindoles appear to behave like simple enamines on sensitized oxygenation.[31c,33] Thus, photooxygenation of *N*-methyltryptophol (**19a**) in methanol at room temperature gave a normal C_2–C_3 double bond cleavage product, **20a**. However, when the photooxygenation was carried out at −70 °C, hydroperoxide **21a** was obtained in high yield; no dioxetane was detected at −70 °C.[34] A similar type of reaction has been

Scheme V



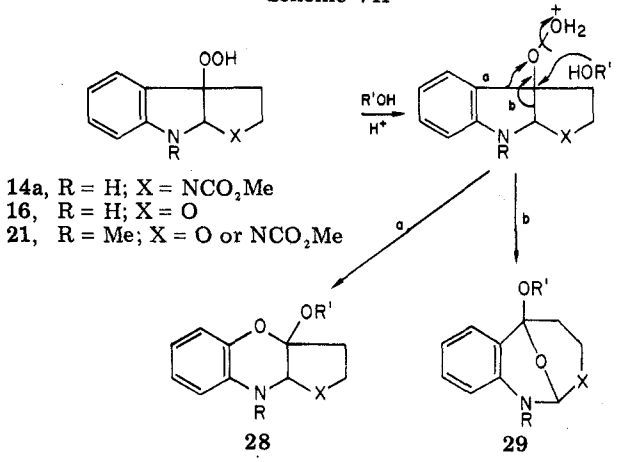
Scheme VI



observed with N^b-methoxycarbonyl-N^a-methyltryptamine (**19b**).[20,35] In both cases, the formation of hydroperoxides **21** occurs preferentially at low temperatures (Table I; Scheme V).[35] Under the reaction conditions the hydroperoxide **21** was confirmed not to be converted to **20**. Photooxygenation of 1,3-dimethylindole (**22**) at −70 °C in alcohols (ROH) produced the hydroperoxide **23**, whereas at room temperature **22** gave **24** exclusively (Scheme VI.)[35] These results indicate that the initial intermediate is *a polar peroxide* capable of undergoing an efficient addition reaction with alcohols or secondary amines even at low temperature. Since dioxetanes,[36] including enamine dioxetane,[26,27] are not known to react with nucleophiles such as alcohols or amines, it seems most likely that the initial peroxide is a zwitterion (**25**, **26**), which presumably corresponds to 3-hydroperoxyindolenines in the case of N-unsubstituted indoles.[37]

(21) The latest report: S. Safe and A. Taylor, *J. Chem. Soc., Perkin Trans.* 1, 472 (1972).
(22) A. J. Birch and J. J. Wright, *Tetrahedron*, **26**, 2329 (1970).
(23) R. H. Burnell, A. Chapelle, and M. F. Khalil, *Can. J. Chem.*, **52**, 2327 (1974).
(24) M. Nakagawa, H. Okajima, J. Chiba, H. Watanabe, and T. Hino, unpublished observations.
(25) I. Saito, M. Imuta, A. Nakada, S. Matsugo, and T. Matsuura, unpublished observations.
(26) C. S. Foote, A. A. Dzakpasu, and J. W. -P. Lin, *Tetrahedron Lett.*, 1247 (1975).
(27) H. H. Wasserman and S. Terao, *Tetrahedron Lett.*, 1735 (1975).
(28) T. Matsuura and I. Saito, *Tetrahedron Lett.*, 3273 (1968).
(29) P. D. Bartlett and A. P. Schaap, *J. Am. Chem. Soc.*, **92**, 3223 (1970).
(30) D. R. Kearns, *Chem. Rev.*, **71**, 395 (1971).
(31) There are several examples of photooxygenations which have been explained in terms of zwitterions; see: (a) T. Matsuura and I. Saito, *Tetrahedron*, **25**, 549 (1969); (b) H. H. Wasserman, *Ann. N.Y. Acad. Sci.*, **171**, 108 (1970); (c) I. Saito, M. Imuta, and T. Matsuura, *Chem. Lett.*, 1173, 1197 (1972).
(32) M. J. S. Dewar and W. Thiel, *J. Am. Chem. Soc.*, **97**, 3978 (1975).
(33) I. Saito, M. Imuta, S. Matsugo, H. Yamamoto, T. Matsuura, *Synthesis*, 255 (1976).
(34) I. Saito, M. Imuta, S. Matsugo, and T. Matsuura, *J. Am. Chem. Soc.*, **97**, 7191 (1975).
(35) I. Saito, M. Imuta, Y. Takahashi, S. Matsugo, and T. Matsuura, *J. Am. Chem. Soc.*, **99**, 2005 (1977).
(36) K. R. Kopecky, J. E. Filby, C. Mumford, P. A. Loockwood, and J-Y. Ding, *Can. J. Chem.*, **53**, 1104 (1975), and references therein.
(37) Perepoxide such as **27** might also be proposed to explain the formation of **21** or **23**. While perepoxides have been proposed to rearrange to ene products and/or dioxetanes,[32] there is no precedent in which perepoxides have been considered to react with alcohols or amines (see also L. M. Stephenson, D. E. McClure, and P. K. Sysak, *J. Am. Chem. Soc.*, **95**, 7888 (1973), and references therein). Note that the compounds (**19**, **22**) having allylic hydrogens do not yield the ene products.

Scheme VII



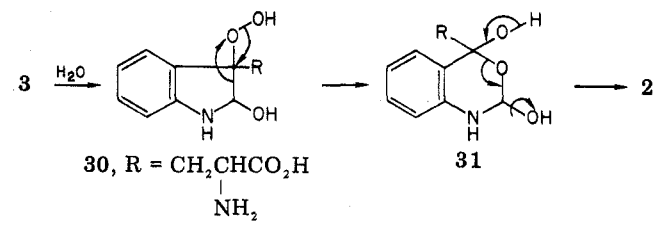
According to the MINDO/3 calculation, the zwitterion, an initial intermediate in enamine–singlet oxygen reaction, is predicted to undergo rearrangement to a dioxetane with a relatively high activation energy compared to that for other processes such as rearrangement to a perepoxide.[32] In such a case it seems very likely that the lifetime of the zwitterion (**25**, **26**) will be longer at lower temperature, permitting the trapping reaction leading to **21** or **23** to be more efficient.

Rearrangement of the Hydroperoxides to 2,3-Dihydro-1,4-benzoxazines

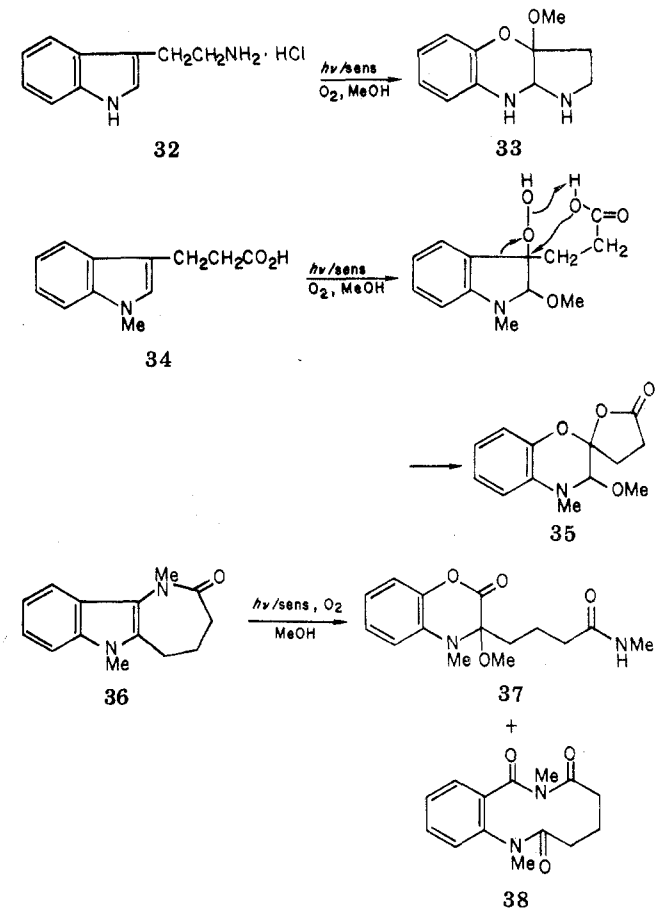
The 3-hydroperoxyindolines thus obtained readily undergo an acid-catalyzed rearrangement to 2,3-dihydro-1,4-benzoxazines; this rearrangement provides a synthetically useful method for the oxidative transformation of indoles into the 1,4-benzoxazine system.[34] For example, treatment of **14a**, **16**, and **21** with methanol containing catalytic amounts of HCl gave the corresponding 1,4-benzoxazine **28** in high yield (Scheme VII).[19,20,34,35] The first step of the rearrangement probably involves a protonation on the hydroperoxy group, followed by O–O heterolysis and aryl migration (path a). An analogous acid-catalyzed rearrangement of hydroperoxides is well established, where relative migratory aptitudes are aryl >> hydrogen >> *tert*-alkyl > *sec*-alkyl > *n*-alkyl.[38] It might also be expected that the product resulting from alkyl migration (path b), **29**, is formed during the reaction. This type of rearrangement is of special interest with regard to the mechanistic understanding of the enzymic transformation of **1** into **2**. For the tryptophan-2,3-dioxygenase-catalyzed reaction, Hamilton has recently proposed a mechanism involving O–O heterolysis of the hydroperoxide **30**, followed by alkyl migration to yield **31**, which subsequently breaks down to **2** (Scheme VIII).[12,39] In the present case, however, aryl migration occurs preferentially.

It should be noted that the rearrangement occurs under very mild conditions, in contrast to that of other peroxides, which usually require a strong acid and/or high temperature. It is also possible to prepare 1,4-benzoxazines from indoles without isolating the hydroperoxides. Methylene blue sensitized photo-

Scheme VIII



Scheme IX



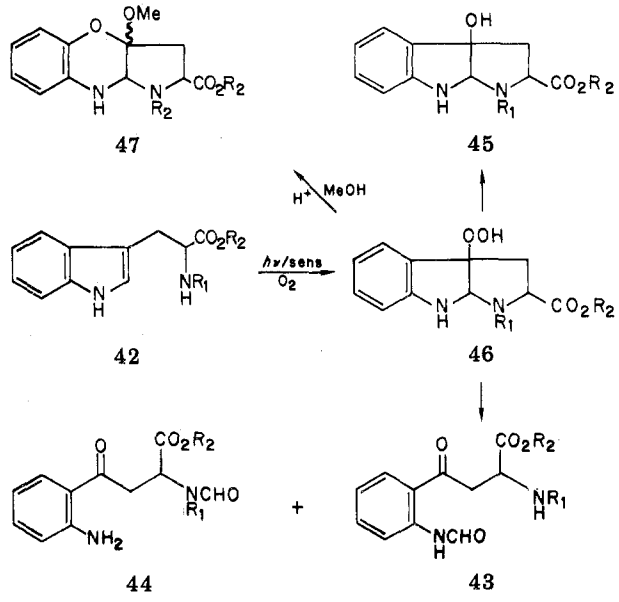
oxygenation of tryptamine hydrochloride (**32**) in methanol gave **33**. In the case of **34**, where the addition of HCl is not necessary, photooxygenation of **34** in methanol led to the clean formation of **35**.[34] Likewise, photooxygenation of **36** in methanol, followed by silica gel chromatography, gave **37** in addition to **38**[40] (Scheme IX).

Photooxygenation of Tryptophan Derivatives

The photooxygenations thus far reported with tryptophan (**1**) were carried out in aqueous media and were reported to yield a complex mixture of products.[5,41] Extensively degraded products such as carbon dioxide,[42] ammonia,[42] kynurenine (**39a**),[43] 3-hydroxykynurenine (**39b**),[43] anthranilic acid,[44] and aspartic acid[45] were

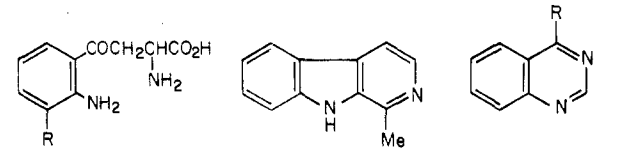
(38) R. Hiatt, "Organic Peroxides", Vol. II, D. Swern, Ed., Wiley-Interscience, New York, N.Y., 1970, p 65.
(39) G. A. Hamilton, private communication.
(40) I. Saito, Y. Takahashi, M. Imuta, S. Matsugo, H. Kaguchi, and T. Matsuura, *Heterocycles*, **5**, 53 (1976).
(41) T. Matsuura and I. Saito, "Photochemistry of Heterocyclic Compounds", O. Buchardt Ed., Wiley, New York, N.Y. 1976, p 456.
(42) L. Weil, *Arch. Biochem. Biophys.*, **33**, 90 (1951).
(43) Z. Yoshida and M. Kato, *J. Am. Chem. Soc.*, **76**, 311 (1954).
(44) S. Hara, *Nogei Kagaku Zashi*, **34**, 493 (1960).
(45) C. A. Benassi, E. Scoffone, and G. Galiazza, *Photochem. Photobiol.*, **6**, 857 (1967).

Scheme X



a, R_1 = Ac; R_2 = Me
b, R_1 = CO_2Me; R_2 = Me
c, R_1 = H; R_2 = H
d, R_1 = H; R_2 = Me

detected. Proflavin-sensitized photooxygenation of **1** in formic acid has been reported to give **39a** in good



39a, R = H
b, R = OH

40

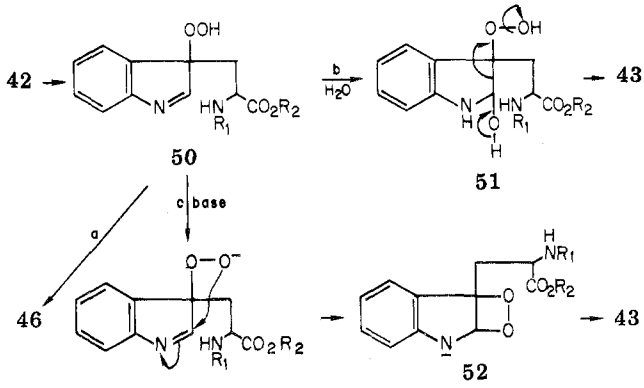
41, R = $CH_2CH(NH_2)CO_2H$

yield,[45] whereas the photooxygenation in acetic acid yielded β-carboline (**40**) into which the solvent was incorporated.[46] Methylene blue sensitized photooxygenation at pH 9.0 gave seven products, one of which was identified as dioxindolylalanine.[47] Savige,[48] however, has reported that the major product is **39a** and that dioxindolylalanine is not formed under these conditions. In the presence of ammonia (pH 8–9), the photooxygenation gave the quinazoline **41** as the main product.[5,48] The complexity of the photoproducts is probably due to secondary reactions of the primary product, formylkynurenine (**2**), under the photooxygenation conditions.

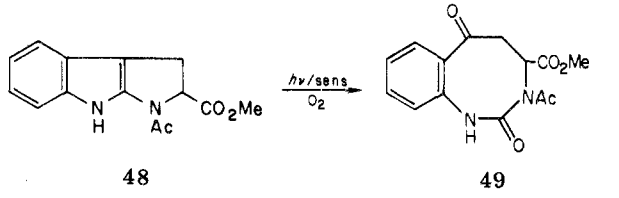
Photooxygenation of tryptophan derivatives in organic solvents gives more clean results. Rose bengal sensitized photooxygenation of **42a** in methanol at room temperature gave **43a** in quantitative yield.[40] Photooxygenation of **42b** in pyridine–methanol, followed by chromatographic separation, gave three products, **43b**, **44b**, and **45b**.[19] Low-temperature photooxygenation followed by rapid workup made it possible to isolate the hydroperoxide **46b**.[24] Treatment of **46b** with silica gel give **43b**, **44b**, and **45b**, in the same manner as **14a** (Scheme X). Photooxygenation of free tryptophan (**42c**) in aqueous ethanol gave an unstable peroxidic

(46) G. Guazzo and G. Jori, *J. Org. Chem.*, **37**, 1429 (1972).
(47) S. Gurnani, M. Arifuddin, and K. T. Augusti, *Photochem. Photobiol.*, **5**, 495 (1966).
(48) W. E. Savige, *Aust. J. Chem.*, **24**, 1285 (1971).

Scheme XI



product which on reduction gave **45c**.[24] Photooxygenation under acidic conditions produced a different type of product; methylene blue sensitized photooxygenation of **42d** hydrochloride in methanol proceeded slowly and gave **47d**.[40] Photooxygenation of the cyclic tryptophan derivative **48** in methanol yielded **49**.[40]



48 **49**

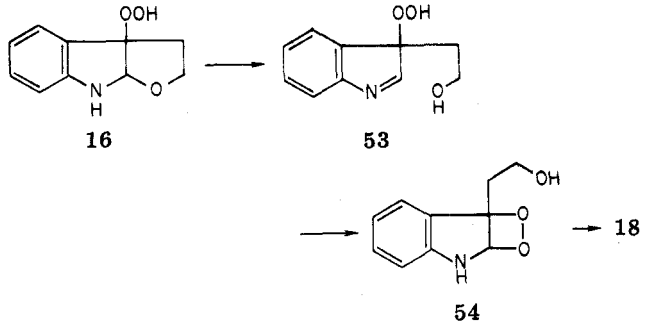
Mechanisms for the 2,3-Bond Cleavage

Heterocyclic compounds bearing —C=C—NH moieties have been suggested to undergo reaction in a manner formally analogous to the "ene" reaction with singlet oxygen, and the hydroperoxidic products are isolable in several systems.[41] A 2,3-disubstituted indole such as 3-methyl-2-phenylindole gives the corresponding 3-hydroperoxyindolenine, either by singlet-oxygen reaction[25] or by radical-induced autoxidation.[11] In an analogous fashion, tryptophan derivative **42** may produce **50** as a primary product which is subsequently intercepted by the functional group of the side chain to yield **46** (path a). In aqueous media, it seems likely that **50** may yield **51** (path b), in competition with cyclization to **46**. The hydroperoxide **51** thus formed may readily decompose to **43** in a manner analogous to that of the known ring opening of 3-hydroperoxyindolenines in aqueous solvents.[11] It is also possible that in strongly alkaline conditions the hydroperoxide **50** undergoes cyclization to dioxetane **52**, as was suggested in the base-catalyzed autoxidation of indoles (path c) (Scheme XI).[49,50]

In organic solvents, on the other hand, **46** was found to be the precursor of the formylkynurenine-type product **43**. Hydroperoxides **14a** and **46b** produced **11a** and **43b**, respectively, when treated with silica gel or refluxed in dry benzene (Scheme X).[19,20] Refluxing of the hydroperoxide **16** in toluene gave **18**, together with **17**.[25] When a solution of **16** in toluene–CCl_4 was refluxed in the presence of 9,10-dibromoanthracene as a fluorescer, no chemiluminescence was observed.[25] NMR analyses of the solutions of **16** in benzene-d_6 and ace-

(49) F. McCapra and Y. C. Chang, *Chem. Commun.*, 522 (1966).
(50) An analogous reaction, see G. Rio, A. Ranjon, O. Pouchot, and M-J. Scholl, *Bull. Soc. Chim. Fr.*, 1667 (1969).

Scheme XII



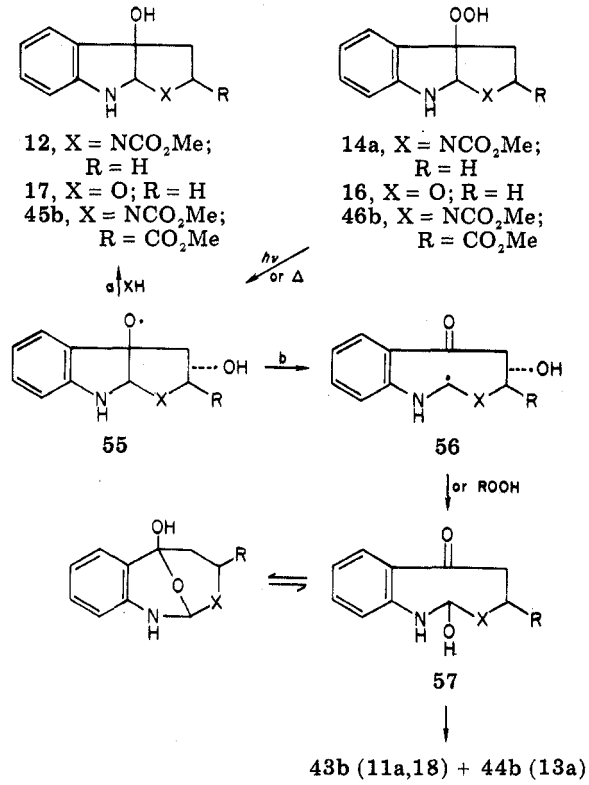
tone-d_6 preclude the possibility that **16** is equilibrated with **53**.[25] These observations seem to eliminate a mechanism involving dioxetane **54** formed via **53** (Scheme XII).

The hydroperoxides (**14a**, **16**, **46b**) are not only thermally unstable but also sensitive to light. For example, irradiation of **16** by visible light without a filter solution under nitrogen atmosphere gave **17** and **18**.[25] Thus, low temperature and irradiation with filtered light are indispensable conditions for isolating the hydroperoxides. Moreover, the redox reaction of **16** with ferrous sulfate or cuprous chloride in aqueous methanol was found to be instantaneous and yielded **17** and **18**.[25] This is particularly interesting in relation to the mechanism of the enzymic oxygenation of indolamines, since an enzyme such as tryptophan or indolamine 2,3-dioxygenase is a heme-containing enzyme.[6] These results, taken together, suggest that the thermal decomposition of the hydroperoxide (**14a**, **16**, **46b**) may proceed via O–O homolysis to give **55** and an hydroxy radical. The *tert*-alkoxy radical **55** may abstract hydrogen (path a) or undergo β scission to give **56** (path b). Both the formylkynurenine-type product **43b** (**11a**, **18**) and the transformylated product **44b** (**13a**) are presumably derived from the common intermediate **57**, which is formed either by recombination of **56** with an hydroxy radical[51] or by radical-chain decomposition of the hydroperoxide (**14a**, **16**, or **46b**) induced by **56** (Scheme XIII).[52] Our results, however, do not necessarily exclude an ionic mechanism involving heterolytic cleavage of the O–O bond of the hydroperoxides (Scheme XIV) or Hamilton's mechanism (Scheme VIII) under one set of conditions. In fact, treatment of the hydroperoxide **14a** with acetic anhydride in pyridine gave only the transformylated product **58**.[24] Further studies are required to clarify the reaction mechanism of the decomposition of the hydroperoxides.

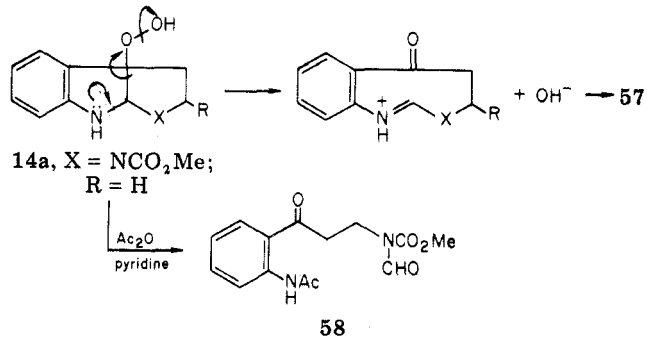
Involvement of Singlet-Oxygen Process

Most products of the indole photooxygenations result from cleavage of the enamine double bond, a reaction which would be expected with singlet oxygen; however, radical reactions are known to give the same type of chemistry with indoles.[11] A variety of experimental tests have been applied to the dye-sensitized photooxygenation of tryptophan to distinguish between a singlet oxygen and a type I[53] (free radical) mechanism.

Scheme XIII



Scheme XIV



In the type I mechanism, the primary reaction is an electron (or a hydrogen) transfer from the substrate to the triplet dye to give the semireduced dye and the semioxidized substrate radical. The latter species subsequently reacts with triplet oxygen to give oxidized products. The rate constants of quenching of the triplet state of eosine,[54] thionine,[54] and methylene blue[55] by tryptophan suggest that the type I mechanism is predominant under anaerobic conditions in aqueous buffered solution. Kepka and Grossweiner[56] have proposed an alternative mechanism involving semioxidized eosine when tryptophan is irradiated at low concentration in the presence of oxygen and eosine. Moreover, the excited singlet states of rose bengal and methylene blue are shown to be quenched by tryptophan and other indoles,[57] suggesting that the excited singlet states of the dyes are involved in the photo-

(51) For example, see G. Rousseau, P. Le Perchec, and J. M. Conia, *Tetrahedron*, **32**, 2533 (1976).
(52) Y. Sawaki and Y. Ogata, *J. Org. Chem.*, **41**, 2340 (1976), and references therein.
(53) K. Gollnick, *Adv. Photochem.*, **6**, 1 (1968).
(54) I. Kraljić and L. Lindqüist, *Photochem. Photobiol.*, **20**, 35 (1974).
(55) A. Knowles and S. Gurnani, *Photochem. Photobiol.*, **16**, 95 (1972).
(56) A. G. Kepka and L. I. Grossweiner, *Photochem. Photobiol.*, **14**, 621 (1971).
(57) R. S. Davidson and K. R. Trethemey, *J. Am. Chem. Soc.*, **98**, 4008 (1976).

oxygenation at high substrate concentrations.

On the contrary, the inhibition by singlet-oxygen quenchers and the D_2O test,[58] a diagnostic test for the participation of singlet oxygen, suggest that the photooxygenation of tryptophan proceeds primarily via a singlet-oxygen mechanism in unbuffered aqueous methanol.[59] Thus, the mechanistic situation is by no means clear in this case, which is probably borderline and particularly sensitive to the reaction conditions; depending on sensitizer, solvent (neutral or basic), and the concentrations of the substrate and oxygen, the mechanism of the photooxygenation may change from type I to singlet-oxygen mechanism.[60]

Singlet oxygen generated by nonphotochemical means was found to react with indoles to give the same type of products as those of the photooxygenations. Singlet oxygen from hypochlorite–hydrogen peroxide systems converts 3-methylindole into *o*-formamidoacetophenone.[61] Solid-phase reaction of 1,3-dimethyl-2-phenylindole adsorbed on silica gel with singlet oxygen generated by microwave discharge method gave the corresponding C_2–C_3 double-bond cleavage product.[62] Oxidation of tryptophan derivative **42a** under the same conditions yielded the formylkynurenine-type product **43a**.[40] This result indicates that singlet oxygen can produce formylkynurenine from tryptophan even in the absence of solvent. The photooxygenation of tryptophan, tryptophol, and tryptamine derivatives in methanol was inhibited by the addition of known singlet-oxygen quenchers.[63] For example, photooxygenation of **19a** in methanol was inhibited by the presence of 1,4-diazabicyclo[2.2.2]-octane[64] or triethylamine[65] at low concentration.[34] All the described results suggest that a major portion of the photooxygenation of tryptophan derivatives, especially in organic solvents at sufficient oxygen concentrations, is a singlet-oxygen-mediated reaction, although the possibility that a radical process involving excited states of dye, operating as a minor competitive process, cannot be ruled out rigorously.

Summary

We have shown that low-temperature photooxygenation of tryptamine, tryptophol, and tryptophan derivatives gives the corresponding 3-hydroperoxyindolines, which result from the interception of the initial peroxides by a nucleophilic functional group of the side chain. One of the important reactions of the hydroperoxides is their facile transformation into formylkynurenine-type products, providing a new pathway for the oxidation of tryptophan to formylkynurenine other than the widely accepted dioxetane pathway. The hydroperoxides are also converted to the 2,3-dihydro-1,4-benzoxazine system. These reactions suggest that such transformations may be involved in the oxidation of various indoles in biological systems. We have also shown that the low-temperature photooxygenation of *N*-methylindoles proceeds via a zwitterion, which is efficiently intercepted inter- and intramolecularly by alcohols and amines. We believe that an understanding of the chemistry of 3-hydroperoxyindolines resulting from tryptophan and its model compounds might be of help in the elucidation of the mechanisms of the enzymic reaction and of photodynamic action.

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(58) P. B. Merkel, R. Nilsson, and D. R. Kearns, *J. Am. Chem. Soc.*, **94**, 1030 (1972).
(59) R. Nilsson, P. B. Merkel, and D. R. Kearns, *Photochem. Photobiol.*, **16**, 117 (1972).
(60) C. S. Foote, *Free Radicals Biol.*, **3**, 85 (1976).
(61) N. A. Evans, *Aust. J. Chem.*, **24**, 1971 (1971).
(62) I. Saito, Y. Takahashi, and T. Matsuura, unpublished observations.
(63) K. Inoue, I. Saito, and T. Matsuura, unpublished observations.
(64) C. Ouannès and T. Wilson, *J. Am. Chem. Soc.*, **90**, 6527 (1968).
(65) R. H. Young and R. L. Martin, *J. Am. Chem. Soc.*, **94**, 5183 (1972).