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DYE-SENSITIZED PHOTO-OXYGENATION OF TRYPTOPHAN†

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Abstract—Dye-sensitized photo-oxygenation of L-tryptophan (**1**) has been studied at various pH and in various buffers. Disappearance of **1** in both acetate and phosphate buffers was rapid at higher pH. The tricyclic hydroperoxide (**8**) was the sole product in the oxidation of **1** over the range pH 3.6–7.1 in acetate and phosphate buffers. However, the oxidation of **1** in alkaline phosphate and borate buffers (pH 7.7–8.4) gave 5-hydroxyformylkynurenine (**10**) as the major product. The intermediate, 3a,5-dihydroxypyrroloindole (**16**), which may be formed from the tricyclic hydroperoxide (**8**) via the quinoneimine (**15**), was obtained in good yield by the immediate reduction of the reaction mixture with $NaBH_4$. Molecular oxygen (ground state) oxidation of **16** in the alkaline media provided **10**. The similar oxidation and reduction of tryptamine (**17a**) gave 3a,5-dihydroxypyrroloindole (**18b**) which was not further oxidized to 5-hydroxyformylkynureamine. On the other hand, dye-sensitized photo-oxygenation of **1** in Na_2CO_3–AcOH (pH 7) gave formylkynurenine (**3**) as the major product.

Nearly 60 years have passed since Kotake discovered kynurenine (**2**) as a metabolite of L-tryptophan (**1**).[1] During much of this time, a large amount of work has been done to elucidate the nature of the enzyme system[2] as well as the method for the chemical conversion of **1** to **2**.[3] It is now well established that kynurenine (**2**) is formed from **1** via formylkynurenine (**3**) in biological systems.[4]

Despite intense interest in the mechanism for the oxidation of tryptophan (**1**) to formylkynurenine (**3**), there still remains much to be resolved. Hydroperoxylindolenine (**4**) has been suggested as the primary intermediate.[5] Three pathways have been proposed for the transformation of **1** to formylkynurenine (**3**),[4d,6] as shown in Scheme 1. Since 1950, a large number of reports have appeared describing the dye-sensitized photo-oxygenation of **1** but none of the peroxides which have been obtained correspond to the postulated intermediates.

As an extension of our studies on the dye-sensitized photo-oxygenation of tryptamine and tryptophan derivatives,[7] we have isolated the hydroperoxide (**8**) by the dye-sensitized photo-oxygenation of tryptophan, the cyclic tautomer of the primary intermediate, 3-hydroperoxyindolenine (**4**), and also found that **8** rearranged to **3** under various conditions.[3a,8]

In a recent preliminary communication, we reported the dye-sensitized photo-oxygenation of tryptophan to 5-hydroxyformylkynurenine.[9] In this paper we wish to report full details of the effect of pH and the reaction media on the dye-sensitized photo-oxygenation of tryptophan.

Despite intensive work on the photo-oxygenation of tryptophan (**1**) in the presence of a dye as a function of pH, no report of the nature of the oxidation products has been given, but O_2 uptake has been measured.[10] Therefore, we re-examined the oxygenation of **1** over the pH range 1–9.

We first investigated the relative rate of photo-oxidation of tryptophan in acetate buffers in the range pH 3.6–6.2 and in phosphate buffers in the range pH 5.9–8.4 using methylene blue as the sensitizer at all pHs. The results are shown in Fig. 1. The pH of the reaction mixture had a profound influence on the reaction rate. In accord with previous reports,[10] tryptophan (**1**) was oxidized more rapidly with increasing pH.

We next carried out a product analysis of the reaction mixture over the range pH 3.6–8.4. The oxygenation of a buffer solution of L-tryptophan (**1**) was carried out by irradiation ($h\nu > 550$ nm) at 0–5° in the presence of methylene blue and O_2 was bubbled through the solution. The reaction mixture was then treated with Me_2S and was left overnight prior to ion exchange column chromatography (Amberlite CG-50). Lyophilization of the elution with water provided the products summarized in Table 1. In acidic or neutral conditions, the hydroxide, **9**[3] (a 1 : 1 mixture of *cis* and *trans* isomers) was the sole product isolated. In contrast, an alkaline phosphate buffer solution (pH 7.7), **9** became the minor product and a new product, 5-hydroxyformylkynurenine (**10**) was obtained. A similar result was obtained at pH 8.4. The structure of **10** was assigned from its spectral and chemical properties. Hydrolysis of **10** with trifluoroacetic acid, basification and acylation with methyl chloroformate and then esterification with diazomethane followed by acetylation provided the crystalline derivative **11d**.

The formation of **10** was surprising in that the benzene ring was hydroxylated by the dye-sensitized

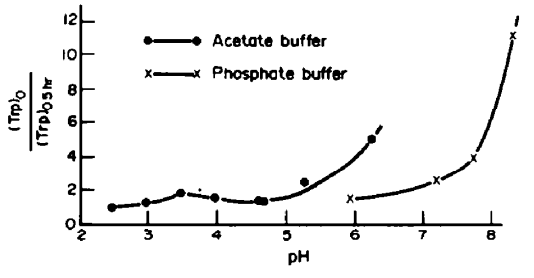
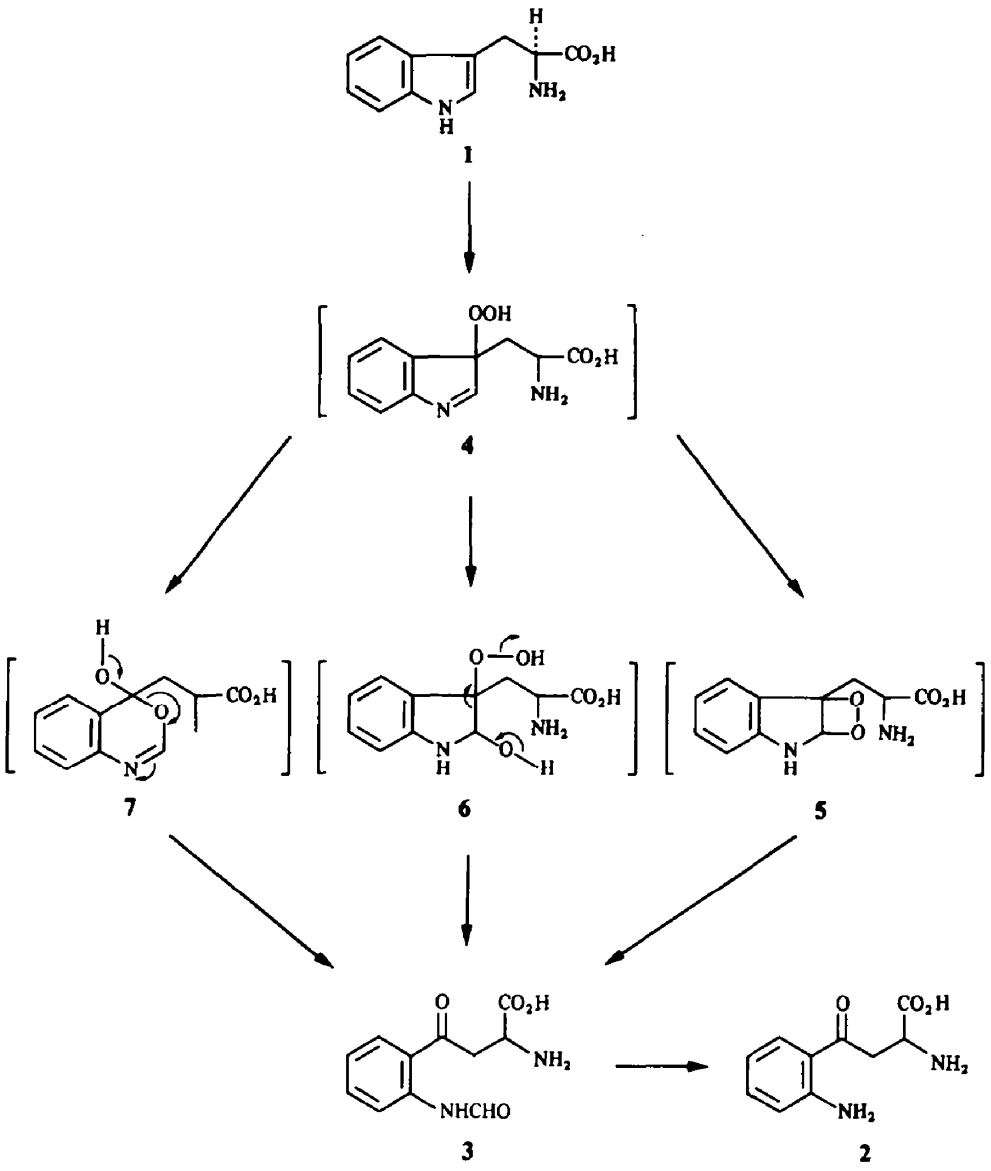


Fig. 1. Relative rate of disappearance of tryptophan.

† Dedicated to Dr. Arnold Brossi on the occasion of his sixtieth birthday.

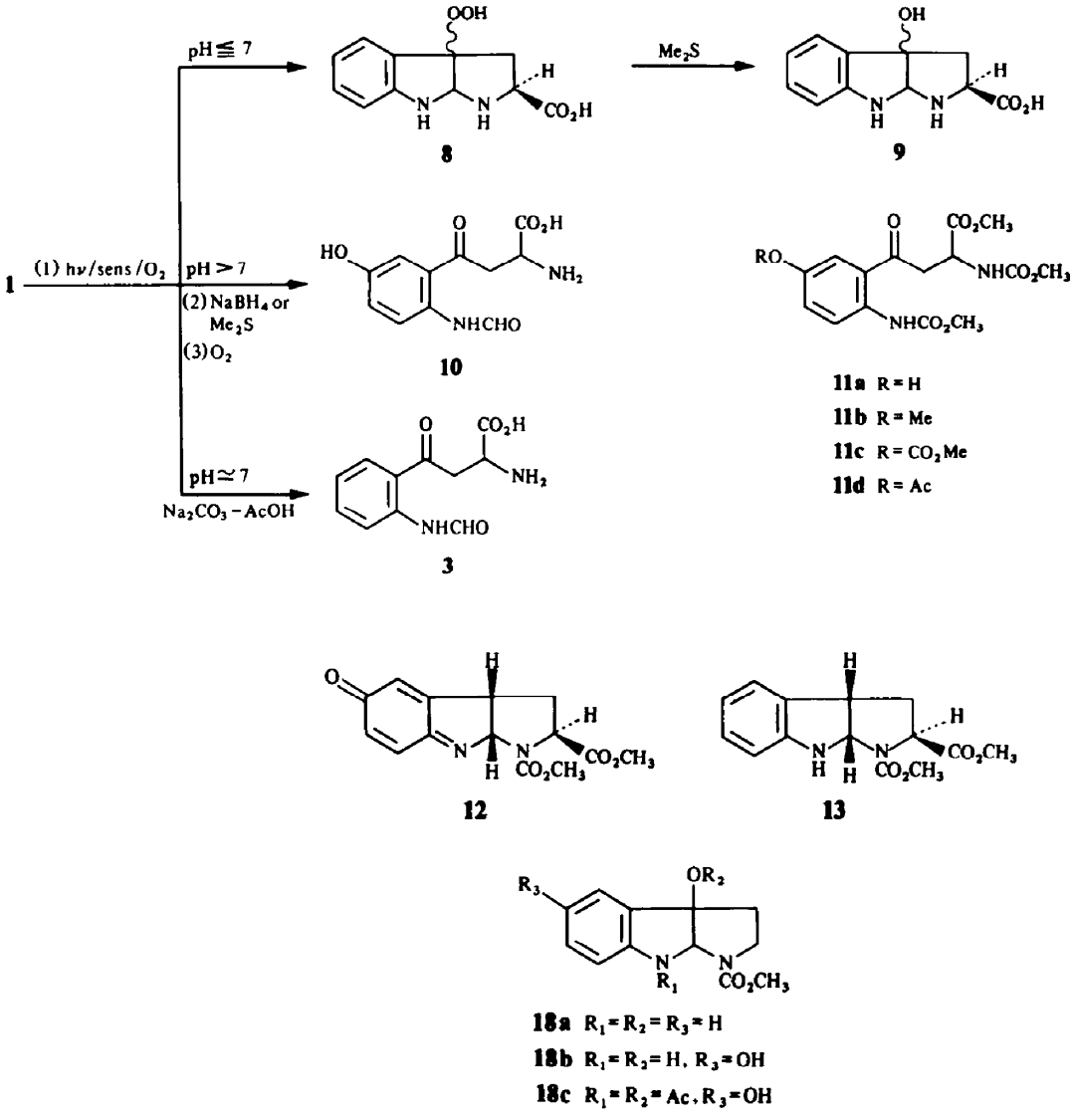


Scheme 1.

Table 1. Photo-oxygenation of tryptophan at various pH

Buffer	pH	Reaction time (hr)	9 (%) UV	9 (%) HPLC	10 (%)
NaOAc–HOAc	3.6	3.5	58	66	—
	4.0	3.5	54	63	—
	4.6	4.0	62	69	—
	5.3	1.5	68	69	—
	6.2	1.0	47	46	—
Na_2HPO_4–KH_2PO_4	5.9	2.0	56	74	—
	7.1	1.0	58	59	Trace
	7.7	1.0	18	15	21
	8.4	1.0	14	15	17

The reaction mixture was reduced with Me_2S and left overnight.



Scheme 2.

photo-oxygenation and, therefore, the mechanism for the formation of **10** was examined. When **1** in phosphate buffer, pH 7.8, was irradiated as above for 1.5 hr, the UV spectrum of the reaction mixture changed from indole chromophore to that having a maximum at 269 nm, which was similar to that of a quinoneimine (**12**) obtained by the $Pb(OAc)_4$ or Fremy's salt oxidation of **13**.[11] The reaction was also followed by high performance liquid chromatography (HPLC), showing the exclusive transformation of **1** (t_R = 24.5 min) to the more polar substance (t_R = 5.8 min), probably **15**. Accordingly, when the reaction mixture was reduced with $NaBH_4$ immediately under N_2 followed by immediate neutralization with dilute HCl and work-up, 3a,5-dihydroxypyrroloindole (**16**) was obtained in 95% yield as a mixture of *cis* and *trans* isomers and **10** was not obtained. In contrast to **9**, **16** was unstable under basic conditions and the surprisingly facile autoxidation of **16** to **10** occurred.

Consequently, the sequence to obtain **10** from **1** was best performed in 47% yield, without isolating **16**, by treating the $NaBH_4$ reduction mixture with oxygen for 3 hr at room temperature. Under similar conditions **3** did not convert to **10** and was recovered unchanged, implying that **3** is not an intermediate for formation of **10**. On the other hand, **8** and **9** were converted to **16** in 42% and 16% yields, respectively, by methylene blue sensitized photo-oxygenation under these conditions whereas, in the absence of methylene blue, **8** and **9** were not oxidized to **10**. Furthermore, the similar photo-oxygenation of **9** followed by immediate reduction with $NaBH_4$ gave **16** in 53% yield but **9** was recovered unchanged when treated with O_2 in alkaline phosphate buffer. These results demonstrated the intermediacy of **16** for **10**.

We turned our attention to the similar oxygenation of **1** in other conditions, as shown in Table 2. When Rose Bengal was used, the reaction proceeded more slowly and **9** and **16** were isolated together with **10**. Reaction in borate buffer also gave a similar result. The

Table 2. Photo-oxygenation of tryptophan in various conditions

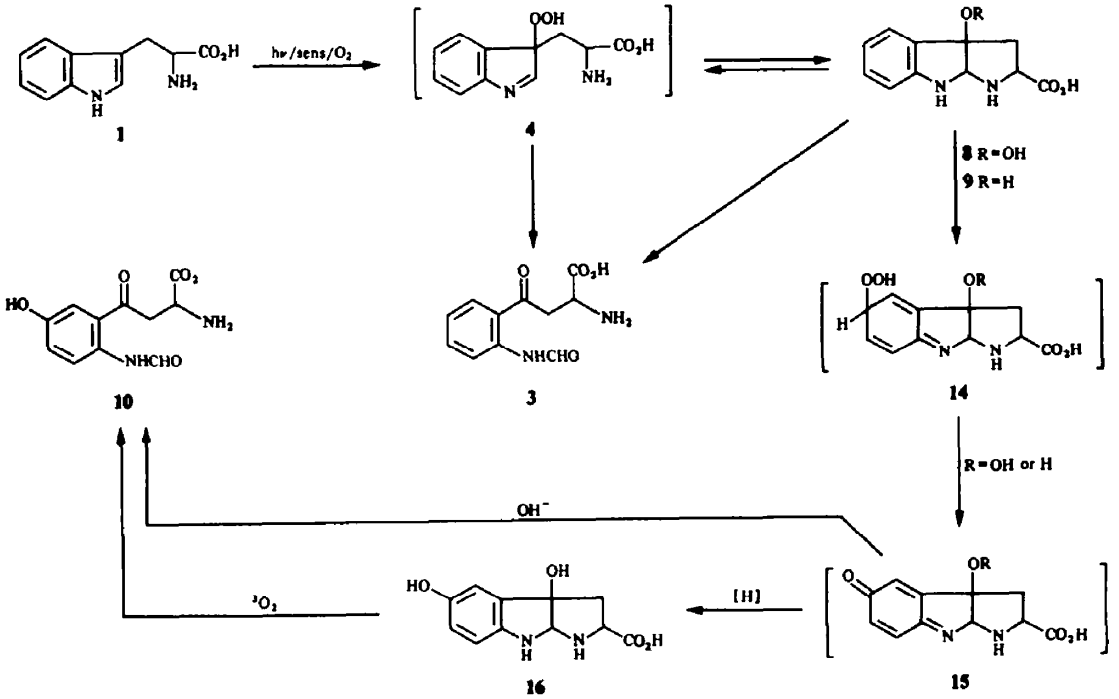
Sensitizer	Buffer (pH 7.7)	Reaction time (hr)	10 (%)	9 (%)	16 (%)
Methylene Blue[a]	KH_2PO_4–Na_2HPO_4	1.5	24	16	—
Rose Bengal[a]	KH_2PO_4–Na_2HPO_4	3.5	18	34	27
Rose Bengal[b]	KH_2PO_4–Na_2HPO_4	3.5	56	33	—
Rose Bengal[a]	H_3BO_3–KCl–Na_2CO_3	4.0	17	34	35
Rose Bengal[b]	H_3BO_3–KCl–Na_2CO_3	3.5	38	38	—
Methylene Blue[b]	H_3BO_3–KCl–Na_2CO_3	1.5	47	—	—

[a] The reaction mixture was reduced with Me_2S and left overnight.
[b] The reaction mixture was reduced with $NaBH_4$ followed by immediate oxidation with O_2.

yield of **10** was increased when the reaction mixture was reduced with $NaBH_4$ followed by immediate oxidation with O_2.

A possible rationale for the oxidation leading from tryptophan (**1**) to 5-hydroxyformylkynurenine **10** is outlined in Scheme 3. We conclude that the initial hydroperoxidation at the *para*-position of the primary product, **8**, probably by singlet oxygen in alkaline phosphate buffers, gave the quinoneimine **15** via **14**, which was converted to **16** on treatment with $NaBH_4$. When Me_2S was used, we conclude that disproportionation between intermediates must have occurred to form **16** in accord with the observation for the low yield of **10**. Supporting evidence for the transformation of **9** to **16** via a quinoneimine was demonstrated by the Fremy's salt oxidation of **9** to **16** in a phosphate buffer followed by $NaBH_4$ reduction. However, the mechanism for oxidation of **16** to **10** is not yet clear but may well involve the initial oxidation of the phenolate anion of **16** with triplet oxygen, since **16**, is stable in neutral media in the presence of O_2 as well as in alkaline phosphate buffer in Ar.

This oxidation of **1** in alkaline phosphate buffer constrasted to the similar reaction carried out in water where **1** was converted to **8** almost quantitatively.[3a,8] In accord with the suggested mechanism, the benzene ring of Nb-methoxycarbonyltryptamine (**17a**) was similarly oxidized by methylene blue sensitized photo-oxy-



Scheme 3.

Table 3. Conversion of **8** to **3** and **9** in various conditions

Buffer soln	pH	Reaction time[b]	3 (%)	9 (%)
Hepes[a]	7.3	5.5 hr	9	68
1/3 M KH_2PO_4–1/30 M Na_2HPO_4	7.2	23 hr	43	38
1/15 M KH_2PO_4–1/15 M Na_2HPO_4	8.1	22 hr	43	30
0.1 M Na_2HPO_4–0.1 M NaOH	11.6	5.5 hr	23	20
0.2 M H_3BO_3–0.2 M KCl–0.2 M NaOH	9.4	22.0 hr	25	Trace
1/20 M Na_2CO_3–0.1 M $NaHCO_3$	8.8	80 min	57	7
1/20 M Na_2CO_3–0.1 M $NaHCO_3$	10.3	110 min	48	9
0.2 M Na_2CO_3	11.2	110 min	33	+
10% Na_2CO_3–30% HCl	9.7	10 min	62	Trace
10% Na_2CO_3–CH_3COOH	9.3	10 min	72	6
10% Na_2CO_3–CH_3COOH	10.3	10 min	56	20
10% Na_2CO_3–CH_3COOH	7.0	10 min	57	—
H_2CO_3	3.9	60 min	—	101

[a] $HOCH_2CH_2N$⟨⟩$NCH_2CH_2SO_3H$.

[b] Required time for negative KI–starch test.

genation in an alkaline phosphate buffer to give 3a,5-dihydroxy-1-methyoxycarbonylpyrroloindole (**18b**), which was identified as its 3a,8-diacetate, **18c**, in 54% yield. However, the 1-methoxycarbonyl derivative (**18b**) was stable to air oxidation in an alkaline phosphate buffer and was not converted to the corresponding 5-hydroxy-N′-formylkynurenine derivative.

Furthermore, deuterium retention upon the oxidation of 5-deutero-Nb-methoxycarbonyltryptamine (**17b**) was examined in an identical condition with that of **17a**. The NMR, mass, and IR spectra of the diacetate, **18c**, obtained from **17b**, were identical with those of **18c** obtained from **17a** and confirmed its complete loss of the deuterium, indicating that the NIH shift was not involved during oxidation of the benzene ring.

In the above conditions, neither formylkynurenine (**3**) nor kynurenine (**2**) were formed in significant amounts from **1**. Therefore, we next examined the reaction conditions for the direct oxidation of **1** to **3**.[12] We found that the hydroperoxide, **8**, converted to **3** when dissolved in various buffers as shown in Table 3.

By dissolving in Hepes buffer, **8** was mainly reduced to **9**[13] accompanied with a small amount of **3** whereas, in alkaline phosphate and borate buffers, **8** converted gradually to an appreciable amount of **3**. However, by dissolving **8** in aq Na_2CO_3–HCl or Na_2CO_3–HOAc adjusted to pH 9.3–9.7, **3** was formed within 10 min in 62–72% yields. In this system, even at neutral pH, **8** was converted to **3** within 10 min in 57% yield.

Formation of **3** strongly catalysed by a buffer containing Na_2CO_3 solution suggested that Na_2CO_3 may not only act as a base to promote rapid cyclic–chain tautomerism between **8** and **4** but also act as a good trapping agent for **4** by forming a carbamic acid derivative with the ethylamino group under the reaction conditions. Although such an intermediate could not be isolated, the formation of it might prevent **4** from reverting to **8** and the reaction proceeding exclusively to give **3**.

In the light of these results, it was expected that the dye-sensitized photo-oxygenation of **1** performed in aq Na_2CO_3–HOAc (pH 7) would produce **3** directly instead of **8**. In fact, we obtained **3** as the sole product in 54% yield by irradiation of **1** and methylene blue in aq Na_2CO_3–HOAc (initial pH 7) for 30 min at 0–5° under a stream of O_2. In the absence of methylene blue, the reaction did not take place and **1** was recovered in 85%

Table 4. Stability of L-formylkynurenine in various solvents

Run	Condition	pH	Reaction time	Recovered 3 (%)
1	H_2O (room temp)	6.6	72 hr	84
2	H_2O (reflux)	6.6	1 hr	42
3	1/100 M NaOH	12.0	24 hr	17
4	2 M NaOH	12.0	10 min	Trace
5	1/20 M Na_2CO_3–1/10 M $NaHCO_3$	8.8	60 min	54
6	1/20 M Na_2CO_3–1/10 M $NaHCO_3$	10.2	60 min	53
7	0.1 M Na_2CO_3	11.3	60 min	47
8	10% Na_2CO_3–CH_3CO_2H	9.3	10 min	67
9	15% Na_2CO_3–CH_3CO_2H	7.2	10 min	81

L-Formylkynurenine, **3** (33 mg, 0.14 mM) was dissolved in the solvent (50 ml) and its recovery determined by UV analysis after ion-exchange resin column separation.

yield. The yield of **3** was not increased due to its instability. Table 4 shows the recovery of **3** when dissolved in various solvents, indicating that **3** was particularly unstable in basic media. Although the yield of **3** was poor, it does satisfactorily demonstrate the tryptophan 2,3-dioxygenase catalysed reaction.

We would hope that these chemical transformations may assist in the understanding of the oxidation of tryptophan in biological systems and of the mechanism of photodynamic action.

EXPERIMENTAL

^{1}H-NMR spectra were recorded with a JEOL MH-270 instrument in $CDCl_3$ using Me_4Si as an internal standard, except where otherwise indicated; chemical shifts are expressed in δ (ppm). IR spectra were run on Hitachi EPI-G 3, IR-215 and IR-295 instruments. Mass spectra were recorded on Hitachi RMU-60 and 7M instruments. Microanalyses were performed on a Perkin–Elmer 240 C, H and N analyser. UV–visible absorption spectra were obtained on Hitachi 323 and 340 spectrophotometers. Optical rotations were taken with a JASCO DIP-140 digital polarimeter. All m.ps (Yamato m.p. apparatus and Yanagimoto micro hot-stage apparatus) reported are uncorrected. All photo-oxygenations were carried out in a Pyrex immersion apparatus using an Ushio tungsten–halogen JCV 500W lamp with a Pyrex cooling, vacuum jacket and an aq $K_2Cr_2O_7$ filter soln. HPLC was performed on a Hitachi model 655 liquid chromatograph (Hitachi Seisakusho, Japan) equipped with a variable wavelength UV monitor and a 833-data processor. The column was μBondapak C_{18} (3.9 × 300 mm) (part No. 27324, Waters Associates). The ion-exchange chromatography was conducted on Amberlite CG-50 (CO_2H form).

General procedure for the methylene blue-sensitized photo-oxygenation of tryptophan in various conditions. (1) In acetate buffers. L-Tryptophan (500 mg, 2.5 mM) was dissolved in a pH adjusted acetate buffer (100 ml) by brief heating and then cooled to room temp. This soln was transferred into a reaction vessel and methylene blue (9 mg, 0.025 mM), the acetate buffer (185 ml) and EtOH (15 ml) were added. The mixture was cooled to 0–5° using an ice bath and irradiated with a 500 W halogen lamp through a liquid filter while O_2 was bubbled through. The reaction was monitored by HPLC and observing the UV spectrum. Me_2S (2 ml) was added and then the mixture was stirred for 1 hr until the starch–KI test was negative. Excess Me_2S and EtOH were removed under reduced pressure at *ca* 10°. The resulting aq soln was washed with CH_2Cl_2 and filtered to remove insoluble material. (The CH_2Cl_2 extracts of the mixture showed the presence of 3-formylindole.) The aq soln was concentrated to *ca* 10 ml by lyophilization and then chromatographed on an ion exchange column (4.2 × 17 cm).

Elution (500 ml) was with water containing a mixture (roughly 1 : 1) of *cis*- and *trans*-**9** and the affluent was collected. The yield of **9** was determined by UV spectrometry (at 294 nm, ε = 2070) and also by HPLC (t_R = 10.9 min) using **1** (t_R = 24.5 min) as the internal standard. A portion of the eluent was lyophilized to give **9** as a powder which was identified (UV, HPLC, NMR) by direct comparison with an authentic specimen.

(2) In aq Na_2CO_3–HOAc soln. (a) A soln of **1** (510 mg, 2.5 mM) was dissolved, as above, in 5% Na_2CO_3–HOAc (285 ml, pH 7), prepared from 5% Na_2CO_3 (300 ml) and HOAc (10.5 ml). To this soln were added methylene blue (21 mg, 0.125 mM) and EtOH (15 ml). The mixture was irradiated followed by work-up as described above in (1). Eluent (1 l.) with water containing **3** was collected. The yield of **3** was determined by quantitative UV analysis (at 320 nm, ε = 3400). A portion of the effluent was lyophilized to give **3** as a colourless powder which was identified by direct comparison (IR, UV, TLC, HPLC) with an authentic specimen of formylkynurenine (**3**).

(b) Without methylene blue. A soln of **1** (510 mg, 2.5 mM) in 5% $NaHCO_3$–HOAc (285 ml, pH7.0) and EtOH (15 ml) was irradiated for 3.5 hr as above in (2a) and after similar work-up **1** was recovered in 85% yield.

(3) In phosphate buffers. (a) Reducing agent—dimethyl sulphide: formation of **10**. A soln of **1** (500 mg, 2.5 mM), methylene blue (9 mg, 0.025 mM), and EtOH (15 ml) in phosphate buffer (285 ml) was irradiated followed by work-up as described above. (The CH_2Cl_2 extracts of the mixture showed the presence of 3-formylindole.) Ion exchange column chromatography of the mixture gave **9** from the first elution (200–250 ml) with water. Further elution (600–700 ml) with water was lyophilized to give **10** as a powder. $[\alpha]_D^{11}$ = −41.3 (c = 1, H_2O). UV $\lambda_{max}^{H_2O}$ 234, 261sh, 347 nm; $\lambda_{max}^{H_2O-OH^-}$ 243, 285sh, 365 nm. IR ν_{max}^{KBr} 3200br (NH_3), 1650br (CO_2^-), 1510, 1400 cm^{-1}. ^{1}H-NMR (5% CF_3CO_2H–D_2O) 7.15 (1H, dd, J = 8.9, 3.0 Hz, H-4), 7.36 (1H, d, J = 2.6 Hz, H-6), 7.84 (1H, d, J = 8.9 Hz, H-3), 8.27 (1H, s, CHO). HPLC t_R = 13.5 (min); eluent H_2O–MeOH (9 : 1) pressure 60 kg/cm^2; detector 254 nm.

(b) The similar reaction of **1** (510 mg, 2.5 mM) in phosphate buffer (285 ml, pH 7.8) and ethanol (15 ml) was carried out in the absence of methylene blue for 2 hr and **1** (466 mg, 91%) was recovered unchanged.

5 - *Acetoxy*- N,N' - *dimethoxycarbonylkynurenine methyl ester* (**11d**). To a soln of **10** (512 mg, 2 mM) in water (80 ml) was added CF_3CO_2H (4 ml). The mixture was stirred for 2.5 hr at room temp until the UV spectrum changed from that (λ_{max} 234, 261sh, 347 nm) of **10** to a new chromophore, 5-hydroxykynurenine (λ_{max} 219, 252, 313, 368 nm). To this mixture were added 10% NaOH, CH_2Cl_2, Cl_2CO_2Me (4 ml) with ice cooling and the mixture was stirred for 30 min and acidified with conc HCl. The CH_2Cl_2 extracts were washed, dried and evaporated to leave a residue (565 mg) which was dissolved in a mixture of MeOH (10 ml) and ether (10 ml), and diazomethane in ether was added. After 2.5 hr stirring, the solvent was evaporated *in vacuo* to give a residue (482 mg) which was chromatographed on silica gel (30 g). Elution with EtOAc–hexane (2 : 1) gave **11c** (TLC showed the presence of a small amount of **11b**). Further elution with the same solvent gave the 5-hydroxy derivative, **11a** (69 mg). UV λ_{max}^{EtOH} 231, 256, 262, 335 nm; $\lambda_{max}^{EtOH-OH^-}$ 248, 282sh, 405 nm. IR ν_{max}^{KBr} 3350br (NH), 2950 (CH), 1770, 1750, 1665 (CO), 1600, 1540 (amide **11**), 1260, 1220 cm^{-1}. ^{1}H-NMR (100 MHz) δ 3.4–4.1 (2H, m, CH_2), 3.68 (3H, s, CO_2CH_3), 3.75 (3H, s, $NHCO_2CH_3$), 3.78 (3H, s, $NHCO_2CH_3$), 3.92 (3H, s, OCO_2CH_3), 4.74 (1H, m, NHC<u>H</u>CO_2CH_3), 5.80 (1H, m, NH, exchangeable), 7.38 (1H, dd, J = 9, 3 Hz, H-4), 7.66 (1H, d, J = 3 Hz, H-6), 8.52 (1H, d, J = 9 Hz, H-3), 10.73 (1H, s, ArNH). MS m/z 412 (11) $[M]^+$, 278 (48), 220 (100), 176 (30), 162 (39), 59 (35).

To a stirred soln of crude **11c** (238 mg) in MeOH (7 ml) was added 10% Na_2CO_3 aq (0.8 ml) with ice cooling. The mixture was stirred for 95 min and 10% Na_2CO_3 aq (0.6 ml) was added. After 45 min stirring, 10% Na_2CO_3 aq (0.6 ml) was again added to the mixture which was further stirred for a further 10 min and poured into a mixture of ice–water (80 ml) and CH_2Cl_2 (100 ml) and acidified with 10% HOAc to pH 5. The CH_2Cl_2 layer was separated and the aq layer extracted with CH_2Cl_2. The extracts were washed, dried and evaporated. The residue (188 mg) was subjected to silica gel chromatography (30 g). Elution with Me_2CO–CH_2Cl_2 (1 : 9) gave **11a** (130 mg, 18% yield from **10**) which was dissolved in pyridine (3 ml) and treated with Ac_2O (1 ml) for 1 hr. The solvent was then evaporated. The residue was treated with water (20 ml) and extracted with benzene. The benzene extracts were washed with 5% HCl and water, dried and evaporated. The residue (118 mg) was chromatographed on silica gel (4 g, CH_2Cl_2) to give **11d** (115 mg, 79% based on **11a**). Crystallization from benzene–hexane gave pale yellow crystals, m.p. 141.5–142.5°. $[\alpha]_D^{19}$ = 15.0 (c = 0.246, EtOH) UV $\lambda_{max}^{95\%EtOH}$ 231 (ε = 34,800), 256 (ε = 11,700), 262sh (ε = 10,600), 338 nm (ε = 4790). IR ν_{max}^{KBr} 3325 (NH), 1774, 1747, 1734, 1700, 1672, 1665 (CO), 1590, 1530 (amide **11**), 1256, 1224, 1200, 1170 cm^{-1} (C–O–C). ^{1}H-NMR 2.32 (3H, s, AcO), 3.50–4.00 (2H, m, CH_2), 3.69 (3H, s, CO_2CH_3), 3.76 (3H, s, $NHCO_2CH_3$), 3.79 (3H, s,

$NHCO_2CH_3$), 4.73 (1H, m, $NHC\underline{H}CO_2CH_3$), 5.74 (1H, d, J = 8.6 Hz, $N\underline{H}CHCO_2CH_3$, exchangeable), 7.31 (1H, dd, J = 9.2, 2.4 Hz, H-4), 7.58 (1H, d, J = 2.4 Hz, H-6), 8.53 (1H, d, J = 9.2 Hz, H-3), 10.86 (1H, s, ArN$\underline{H}$). ^{13}C-NMR (67.8 MHz) (C-3, C-4 and C-6 are determined by selective decoupling) 20.99 (q, $\underline{C}H_3CO$), 42.04 (t, CH_2), 49.93 (d, $NHCHCO_2CH_3$), 52.41 (q, $NHCO_2CH_3$), 52.84 (q, CO_2CH_3), 120.47 (d, C-4), 120.82 (s, C-1), 123.38 (d, C-3), 129.11 (d, C-6), 139.39 (s, C-2), 144.23 (s, C-5), 154.13, 156.61, 169.40, 171.41, 200.49 (S, C=O). MS *m/z* (%) 396 (6) $[M]^+$, 354 (51) $[M-CH_2{=}C{=}O]^+$ 220 (54), 162 (100), 43 (33), Ac. (Found: C, 51.75; H, 5.07; N, 7.0%. Calc for $C_{17}H_{20}N_2O_9$: C, 51.51; H, 5.09; N, 7.07%.)

(c) Reducing agent—$NaBH_4$—isolation of 3a,5-dihydroxypyrroloindole (**16**). A soln of **1** (510 mg, 2.5 mM) in phosphate buffer (285 ml, pH 7.8), EtOH (15 ml) and methylene blue (9 mg, 0.025 mM) was irradiated as above. After 100 min, the UV spectrum of the mixture showed a maximum at 269 nm and had an intense peak at t_R = 5.8 min (detected at 270 nm). $NaBH_4$ (437 mg) was added with stirring to the mixture while bubbling N_2 gas through the soln. The UV spectrum changed (λ_{max} 238, 312 nm) and HPLC showed a new peak at t_R = 6.0 min and the peak at t_R = 5.8 min disappeared after 1 hr. The mixture was extracted with CH_2Cl_2. A portion (25 ml) of the aq soln (350 ml) was immediately added to an ion exchange column. Elution (200 ml) with water followed by lyophilization gave **16** as a pale brown powder (40.2 mg) and **10** was not detected. The calculated total yield of **16** was 563 mg, 95%. Work-up of the rest of the soln (325 ml), in an identical manner, provided **16** (360 mg, 61%) and also **10** (17.4 mg, 3%) during prolonged work-up. Compound **16**: UV $\lambda_{max}^{H_2O}$ 238, 312 nm; $\lambda_{max}^{H_2O-OH^-}$ 243, 326 nm. IR ν_{max}^{KBr} 3300br (NH_2), 1627 (C=O), 1500, 1477, 1400 cm^{-1}; ^{1}H-NMR (D_2O, determined by WEFT and also Homogate decoupling methods) 2.56 (0.7H, t, J = 1.25 Hz, ABX, *cis* H-3), 2.86 (0.7H, m, AB$\underline{X}$, *cis* H-3 + 0.6H, $\underline{AB}X$, *trans* H-3), 3.91 (0.7H, dd, J = 11.7, 6.6 Hz, AB$\underline{X}$, *cis* H-2), 4.35 (0.3H, t, J = 6.9 Hz, AB$\underline{X}$, *trans* H-2), 5.32 (0.3H, d, J = 5.9 Hz, *trans* H-8a), 5.42 (0.7H, s, *cis* H-8a), 6.77 (1H, d, J = 8.6 Hz, H-7), 6.86 (1H, dd, J = 8.6, 2.6 Hz, H-6), 6.96 (1H, d, J = 2.6 Hz, H-4). Exact mass calc for $C_{11}H_{12}N_2O_4$: 236.0797. Found: 236.0821. HPLC t_R = 6.0 (min) Packing material: Waters μBondapak C_{18}. Eluent: H_2O–MeOH (9:1). Pressure: 60 kg/cm^2. Detector 254 nm.

(d) Reducing agent—$NaBH_4$, air oxidation—synthesis of **10** from **1**. A soln of **1** (510 mg, 2.5 mM) in phosphate buffer (pH 7.8) was oxygenated as described above. After 2.5 hr, $NaBH_4$ was added, the mixture was adjusted to pH 7.8 with dil. HCl and O_2 was bubbled through it for 3 hr. The mixture was washed with CH_2Cl_2 and filtered to remove the insoluble material. The filtrate was concentrated to a volume of 20 ml by lyophilization and chromatographed on an ion exchange column in a similar manner as above to give **9** (68 mg, 12%) and **10** (295 mg, 47%) whose structures were identified (IR, UV, HPLC) by comparisons with the specimen obtained in (3a) above.

Oxidation of **9** *with Fremy's salts—an alternate synthesis of* **16**. To a stirred soln of Fremy's salts $ON(SO_3K)_2$ (1.23 g, 4.6 mM) in phosphate buffer (30 ml, pH 6.9) was added a cooled soln of **9** (500 mg, 2.3 mM) in phosphate buffer (20 ml) under ice cooling. After 70 min stirring, the mixture (λ_{max} 269 nm) was treated with $NaBH_4$ (435 mg), stirred for 20 min and concentrated to a volume of 10 ml by lyophilization. Ion exchange column chromatography (3.8 × 40 cm) of the mixture, as described above, gave **16** (174 mg, 32%) which by UV, NMR and HPLC proved identical with those obtained by methylene blue-sensitized photo-oxygenation of **1** in alkaline phosphate buffer described in (3c) above.

Methylene blue-sensitized photo-oxygenation Nb-methoxycarbonyltryptamine (**17**) *in phosphate buffer*. (a) A soln of **17a** (500 mg, 2.3 mM) in EtOH (30 ml) was added to phosphate buffer (270 ml, pH 7.8) containing methylene blue (9 mg, 0.023 mM). The mixture was irradiated with a halogen lamp for 1 hr while O_2 was bubbled through. Half of the soln (150 ml) was reduced with $NaBH_4$ and stirred for 45 min and Ac_2O (1 ml) added. After a further 2 hr stirring, the mixture was extracted with CH_2Cl_2. The extracts were washed with brine, dried and evaporated to give a residue (235 mg) which was chromatographed on silica gel (20 g). Elution with CH_2Cl_2–Me_2CO (9:1) gave **18c** (148 mg, 39%). Crystallization from MeOH gave colourless needles, m.p. 172–173°; UV λ_{max} 247 (ε = 14,500), 282 nm (ε = 1770); λ_{max} 270, 315 nm. IR λ_{max}^{KBr} 3308 (OH), 1767, 1712, 1655 (C=O), 1373, 1343, 1220, 1178 cm^{-1}. ^{1}H-NMR 2.10 (3H, s, AcO), 2.29 (3H, s, AcN), 2.39 (2H, m, H-3), 2.80–2.95 (1H, m, H-2), 3.67 (3H, s, CO_2CH_3), 3.72–3.84 (1H, m, H-2) 4.80 (1H, rs, OH), 5.65 (1H, s, H-8a), 7.03 (1H, dd, J = 8.9, 2.6 Hz, H-6), 7.15 (1H, d, J = 2.6 Hz, H-4), 7.89 (1H, d, J = 8.9 Hz, H-7). ^{13}C-NMR (67.8 MHz) 21.12, 22.29 (q, CH_3CO), 35.27 (t, C-3), 45.84 (t, C-2), 52.69 (q, CO_2CH_3), 83.07 (d, C-8a), 85.55 (s, C-3a), 116.79 (d, C-4), 120.70 (d, C-6), 123.21 (d, C-7), 134.81 (s, C-3b), 140.14 (s, C-7a), 147.87 (s, C-5), 155.26, 169.48, 172.36 (s, C=O). The assignment of C-4, C-6 and C-7 was confirmed by the selective decoupling method. MS *m/z* (%): 334 (28) $[M]^+$, 292 (65) $[M-CH_2{=}C{=}O]^+$, 250 (100), 232 (55), 162 (40), 43 (34). (Found: C, 57.39; H, 5.45; N, 8.31%. Calc for $C_{16}H_{18}N_2O_6$: C, 57.48; H, 5.43, N, 8.38%.)

(b) The similar reaction of **17a** (500 mg, 2.3 mM) was carried out as described in (a) above, followed by $NaBH_4$ reduction. O_2 was then introduced into the soln for 2 hr.

The mixture was treated with Ac_2O (10 ml), dil. NaOH (50 ml), CH_2Cl_2 (250 ml) at room temp for 3 hr followed by work-up as described above in (a) to give **18c** (411 mg, 54%). A **10** type compound was not detected. Recrystallization from MeOH gave colourless needles of **18c** (355 mg) m.p. 171–172°, identical (m.p. IR, UV, TLC) with the specimen obtained in (a) above.

(c) A soln of **17b**† (147 mg, 0.67 mM) and methylene blue (2.5 mg, 0.0069 mM) in phosphate buffer (270 ml, pH 7.8) and EtOH (30 ml) was oxygenated for 1 hr in similar manner as for the oxidation of **17a**. The mixture (λ_{max} 269 nm) was reduced with $NaBH_4$ (127 mg, 3.4 mM) for 20 min, to which (λ_{max} 238, 308 nm) were added Ac_2O (5 ml) and dil. NaOH (50 ml). The mixture was stirred for a further 2 hr at room temp and extracted with CH_2Cl_2. The extracts were washed, dried and evaporated to leave a residue (196 mg) which was chromatographed on silica gel (20 g). Elution with CH_2Cl_2–Me_2CO (9:1) gave **18a** (78 mg, 35%). Recrystallization from methanol gave colourless needles, m.p. 172–173°, identified as **18a** by direct comparison (m.p., IR, UV, MS, NMR) with the specimen prepared from **17a**.

Methylene blue-sensitized photo-oxygenation of **8**. The hydroperoxide, **8** (556 mg, 2.5 mM) was dissolved in phosphate buffer (100 ml, pH 7.8) by brief heating and then cooled to room temp. To this soln was added phosphate buffer (185 ml), EtOH (15 ml) and methylene blue (9 mg). The mixture was irradiated as above for 1.5 hr and acidified with 5% HCl (5 ml) followed by addition of Me_2S (5 ml). The mixture was stirred overnight. After usual work-up **10** (91 mg, 14%), **3** (71 mg, 13%) and a small amount of **9** were obtained. When $NaBH_4$ was used instead of Me_2S, **10** (192 mg) was obtained in 42% yield.

Methylene blue-sensitized photo-oxygenation of **9**. A soln of **9** (550 mg, 2.5 mM) in phosphate buffer was oxygenated followed by work-up, without reduction of the mixture, to give **10** (106 mg, 16%), whereas the similar oxygenation of **1** followed by $NaBH_4$, neutralization with 5% HCl and work-up provided **16** (313 mg, 53%). The similar reaction of **9** without methylene blue for 2 hr gave **10** (23 mg, 4%), and **9** (475 mg) was recovered in 84% yield.

Attempted oxygenation of **3**. The analogous oxygenation of **3** (563 mg, 2.5 mM) in phosphate buffer (285 ml, pH 7.8) and EtOH (15 ml) was carried out for 2 hr in the presence of methylene blue and neutralized with 5% HCl. It was then stirred overnight after the addition of Me_2S. Usual work-up recovered **3** (474 mg) in 84% yield which was identified (UV, HPLC, m.p.) by direct comparison with an authentic specimen.

† 5-Deutero-Nb-methoxycarbonyltryptamine (**17b**) contained **17a**. The ratio of **17b**–**17a** was 58.5:41.5.

Autoxidation of **16** *to* **10** *in alkaline phosphate buffer.* A soln of **16** (100 mg, 0.4 mM) in phosphate buffer (30 ml, pH 7.8) after bubbling O_2 through was added to an ion exchange column (7.0 × 6.5 cm). Lyophilization of the first eluent (200 ml) with water gave **16** (48 mg, 48%). Further elution (200 ml) with the same solvent gave **10** (31 mg, 29%).

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