Dye-sensitized Photo-oxygenation of Tryptophan to give *N'*-Formylkynurenine

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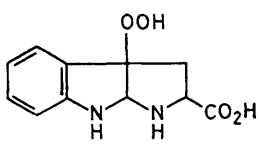
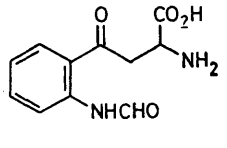
Summary Exclusive conversion of the tricyclic hydroperoxide (**1**) into *N'*-formylkynurenine (**2**) has been found to occur in Na_2CO_3–AcOH (pH 7) and the methylene blue-sensitized photo-oxygenation of tryptophan in the same system has given *N'*-formylkynurenine as the sole product, in contrast with the reaction in water, which gave compound (**1**).

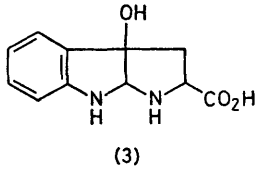
WE have reported the exclusive formation of the tricyclic hydroperoxide (**1**) by the dye-sensitized photo-oxygenation of tryptophan (α-aminoindole-3-propionic acid) in an aqueous solution and compound (**1**) was found to give *N'*-formylkynurenine [3-(*N*-formylanthraniloyl)alanine] (**2**) when heated.[1] We report herein various conditions which effect the conversion of compound (**1**) into (**2**) and also a facile direct conversion of tryptophan into *N'*-formylkynurenine (**2**). We also report the effect of pH on the dye-sensitized photo-oxygenation of tryptophan.



(1)



(2)



(3)

When dissolved in phosphate buffer (pH 7·2), compound (**1**) was converted into (**2**) in 43% yield after 22 h at ambient temperature, together with a mixture of the *trans*- and *cis*-hydroxides (**3**)[1c] in 43% yield. No significant change in the yield and the reaction time for the conversion of (**1**) into (**2**) was observed in the same buffer system at pH 8. On the other hand, compound (**1**) was mainly converted into (**3**) (77%) [compound (**2**), 9%] when dissolved in Hepes buffer (pH 7·3, 5·5 h). At higher pH in the buffer systems H_3BO_3–KCl–NaOH (pH 9·4, 22 h) or Na_2HPO_4–NaOH (pH 11·6, 5·5 h), the yield of compound (**2**) decreased to 23—25%.† A moderate increase in the yield (33% or 48%) of compound (**2**) was observed when compound (**1**) was treated with Menzel buffer solution (Na_2CO_3–$NaHCO_3$, pH 10·3 or Na_2CO_3, pH 11·2) and the conversion was completed within 110 min. An exclusive conversion of the hydroperoxide (**1**) into (**2**) was achieved by dissolving (**1**) in aqueous Na_2CO_3–HCl or Na_2CO_3–AcOH adjusted to pH 9·3–9·7, which selectively formed compound (**2**) within 10 min in 62–72% yields. In this system, even at neutral pH,‡ compound (**1**) was converted into (**2**) within 10 min in 57% yield. Although we have no clear explanation of this solvent effect, the method is simple, rapid, and more efficient both in terms of yield and reaction time than when other conditions were used.

In light of these results, it was conjectured that the dye-sensitized photo-oxygenation of tryptophan performed in aqueous Na_2CO_3–AcOH (pH 7) would produce compound (**2**) directly instead of compound (**1**). In fact, we obtained *N'*-formylkynurenine (**2**) in 54% yield by irradiation ($\lambda > 550$ nm)§ of L-tryptophan (510 mg, 2·5 mM) and methylene blue (20 mg, 0·02 mol. equiv.) in aqueous Na_2CO_3–AcOH (initial pH 7)‡ for 30 min at 0–5 °C under a stream of oxygen,

† The yield of compound (**2**) decreased probably because of the instability of (**2**) under basic conditions.

‡ The initial pH had changed to *ca.* pH 8 after the reaction was completed.

§ Aqueous $K_2Cr_2O_7$ was used as a liquid filter.

which shows that this reaction is a model for the tryptophan 2,3-dioxygenase catalysed reaction.

Despite intensive work on the photo-oxygenation of tryptophan in the presence of a dye as the function of pH, no report of the nature of the oxidation products has been given, but O_2-uptake has been measured.[2] Therefore, we carried out a product analysis over the pH range 2—9. Thus, tryptophan (1 g, 5 mM) was oxygenated in the presence of methylene blue (18 mg, 0·01 mol. equiv.), in a similar manner, at 0 °C in acetate buffer (300 ml) for 4 h. The reaction mixture was treated with dimethyl sulphide and panned through an ion-exchange column (Amberlite CG-50) to give 1,2,3,3a,8,8a-hexahydro-3a-hydroxypyrrolo[2,3-*b*]-indole-2-carboxylic acid (**3**) in 47% (pH 2·7), 64% (pH 3·5), 26% (pH 4·7), and 48% (pH 5·5) yields,¶ and tryptophan was recovered (in 48% yield) only at pH 4·7. The reaction in aqueous acetic acid (pH 3·9) for 4 h gave compound (**3**) (26%) with a 21% recovery of tryptophan. Similar reaction of tryptophan in more acidic solutions, such as 5% HCl, did not proceed, whereas in 0·01 M HCl (pH 2) compound (**3**) was obtained in 86·5% yield after reduction with dimethyl sulphide. Likewise, the oxygenation of tryptophan (1 g, 5 mM) in phosphate buffer was carried out under similar conditions and *N'*-formylkynurenine, together with compound (**3**), was obtained (the results are given in the Table). In accord with previous reports[5a,5d], the reaction proceeded more rapidly under basic rather than acidic or neutral conditions.[1]

TABLE. Oxygenation of tryptophan in phosphate buffer.

pH	*t*/h	Recovered tryptophan/%	Yield of (**1**)/%	Yield of (**2**)/%	Yield of (**3**)/%
4·9	4	5	42		
5·7	4	4			57
6·5	2				63
7·4	2			5	52
8·2	2			17	26
8·9[a]	2			13	38

[a] The pH was adjusted to 8·9 with 0·1 M NaOH.

In the pH range examined, the hydroperoxide (**1**) was the main product and *N'*-formylkynurenine (**2**) was isolated under basic conditions as the minor product, which shows that, even in acidic conditions, the participation of the ethylamino-side chain of the primary intermediate[1] to form compound (**1**) takes precedence over the participation of the 3-hydroperoxy-group (which would give the dioxetan[1]); compound (**1**) then decomposes to *N'*-formylkynurenine (**2**).

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¶ 3-Formylindole was obtained as a minor product at pH 2·7 and 3·5 and other minor products with the 4-quinolone chromophore have been detected in all regions (pH 2·7—5·5).

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