Oxidation of 3-Hydroxykynurenine. An EPR investigation

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A weak alkaline solution of 3-hydroxykynurenine was oxidized using potassium ferricyanide. A stable epr signal was recorded, probably arising from a phenoxazine intermediate. A plausible radical mechanism for this reaction is presented.

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Introduction.

Ommochromes are a class of natural pigments which occur mainly in arthropods and cephalopods. Chemical and biochemical evidence [1] indicates that these pigments are products of the metabolism of tryptophan and, in particular, are believed to arise from the oxidative condensation of two or more molecules of 3-hydroxykynurenine (**1**), a final product of tryptophan metabolism.

The yellow pigment xanthommatin (**2**), the most investigated of the ommochrome class, has been obtained *in vitro* by Butenandt and Schäfer [2] following oxidation of 3-hydroxykynurenine by potassium ferricyanide. This suggests that xanthommatin formation occurs in chromatophore cells as well as *in vitro* and the phenoxazin-3-one formation is a key step in the pathway leading to the pigment (Figure 1).

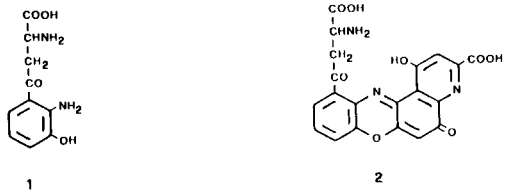
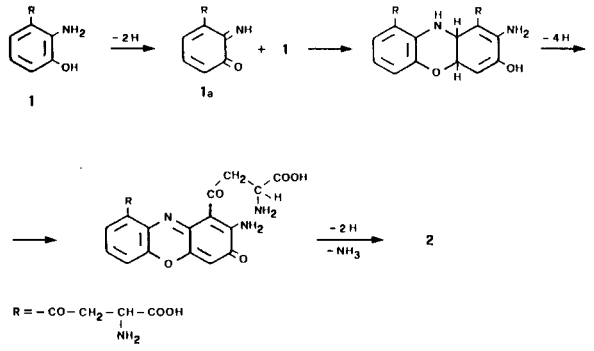


Figure 1

According to the above mentioned authors, the phenoxazinone system would arise, under oxidizing conditions, from a nucleophilic attack of the aromatic amino group of **1** on the *o*-quinonimine **1a** arising *in situ* from **1** (Scheme 1). This mechanism was believed to operate in the synthesis of phenoxazinones from *o*-aminophenols, under an oxidizing medium [3]. Xanthommatin is formed by the cyclization of the terminal α-amino acid moiety on the amino group of the phenoxazinone ring [4]. Strangely enough, under the oxidizing conditions, 3-hydroxykynurenine (**1**) does not undergo an intramolecular cyclization like that of DOPA to 5,6-dihydroxyindol-2-carboxylic acid.

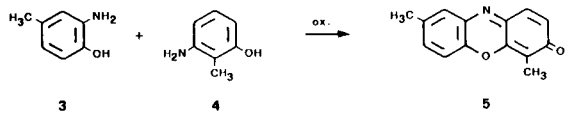
Successive investigations carried out by Corbett [5] on the oxidation of *p*-phenylendiamine and *p*-aminophenol, showed that, instead of the expected attack of the aromatic amino group on the quinonediimine and quinonimine systems, an electrophilic attack of the quinonimine group occurs on the electron-rich aromatic substrate. This is more evident when *para*- and *meta*-aminophenols are oxidized together [6].

Scheme 1



In agreement with these results, we recently reported [7] the formation of 4,8-dimethylphenoxazin-3-one (**5**) by ferricyanide oxidation of 2-amino-4-methylphenol (**3**) in the presence of 2-methyl-3-aminophenol (**4**) (Scheme 2). In this case, the first step in the formation of the phenoxazinone system should involve, according to Corbett, an electrophilic attack of the *o*-quinonimine species formed by the oxidation of **3**, on the electron-rich *m*-aminophenol **4**. Once again, these results strongly suggest that in *o*-aminophenol oxidation, the phenoxazinone system arises from an electrophilic attack on the aminophenolic species. On the other hand, it has already been shown that a radical mechanism is involved in the oxidation of catechols [8] as well as of aromatic diamines [9,10]; therefore, this additional possibility cannot be ruled out. The presence of radical intermediates, under conditions where xanthom-

Scheme 2



matin is generally formed [2], is investigated in the present paper.

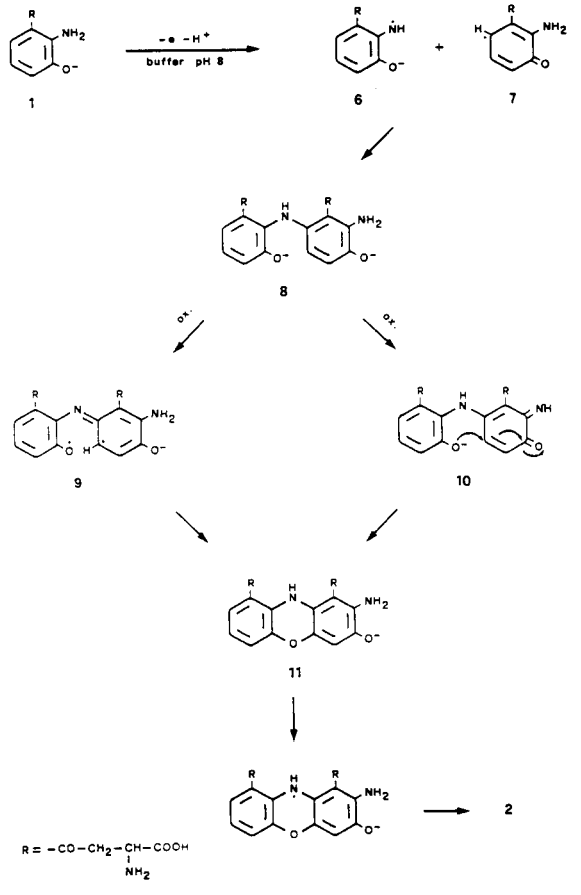
Results and Discussion.

One ml of a solution 10^{-4} *M* 3-hydroxykynurenine (**1**) in 1 *M* phosphate buffer (*p*H 8) was rapidly added to a 1 ml solution of 6 x 10^{-4} *M* potassium ferricyanide and the epr spectrum of this mixture was immediately recorded at room temperature.

A uniformly spaced four-line spectrum, with the line intensity in the ratio 1:2:2:1, was obtained. The measured coupling constant was 4.99 gauss, while the line width and g values were 9.0 gauss and 2.009 respectively. The hyperfine structure was interpreted as a basic three-line splitting by a nitrogen-14 nucleus and a doublet splitting due to a proton, with very similar coupling constants.

The epr spectrum features of this stable radical are quite similar to those reported for radicals arising from some phenoxazines [11] and suggest that the 1,9-diaspartoyl-2-amino-3-hydroxyphenoxazine radical **12** could be the intermediate responsible for the spectrum observed on the oxidation mixture of **1**. The formation of such a radical can be hypothesized as reported in Scheme 3.

Scheme 3



According to Scheme 3, the dimer **8** resulting from the coupling between the initial radical and the semiquinonimine, may undergo ring closure to a phenoxazine from which the detected radical could arise. The radical formation can be induced by ferricyanide, a well known monoelectronic oxidizing agent. Ferricyanide can promote radical formation in acidic, neutral and especially in alkaline solutions with reactive amines and phenols.

Although conversion of **8** to **11** could be hypothesized to occur through in pairing of the intermediate diradical **9**, as reported in the oxidation of anthranilic acid [12], the alternative cyclization of an *o*-quinonimine, involving a fast intramolecular heterolytic reaction, cannot be excluded. However, utilizing the experimental conditions in the present work, no other radical hypothesized in Scheme 3 was detected; nevertheless, the formation of the radical **12** is in good agreement with the occurrence of reduced products which are found in natural sources of ommochrome pigments. Furthermore, a radical pathway to the formation of the phenoxazinone ring which is simulated by the oxidation of *o*-aminophenols by ferricyanide, could justify the dimerization reaction of 3-hydroxykynurenine to **2** instead of the more likely ionic intramolecular cyclization.

EXPERIMENTAL

All the epr measurements were carried out on a Bruker ER 200 D spectrometer equipped with a standard low-temperature apparatus, at room temperature. The magnetic field of each scan was carefully measured by means of an nmr gaussmeter (ER 035 *M*), which leaves markers at preselected values, the klystron frequency being checked by using DPPH as reference (g = 2.0036). The uv reference spectra were recorded on a Perkin-Elmer 550-S spectrophotometer. The 3-Hydroxykynurenine was a commercial sample.

REFERENCES AND NOTES

[1] A. Butenandt and W. Schäfer, Recent Progress in the Chemistry of Natural and Synthetic Colouring Matters and Related Fields, Academic Press, New York, 1962, p 13.

[2] A. Butenandt, U. Schiedt, E. Biekert and R. I. T. Cromartie, *Ann. Chem.*, **590**, 75 (1955).

[3] The Chemistry of Quinonoid Compounds, S. Patai, ed, John Wiley & Sons Ltd., 1974, p 877.

[4] W. Schäfer. *Progr. Org. Chem.*, **6**, 135 (1964).

[5] J. F. Corbett, *J. Chem. Soc. (B)*, 827 (1969).

[6] K. C. Brown, J. F. Corbett and R. Labinson, *J. Chem. Soc., Perkin Trans. II*, 1292 (1978).

[7] A. Bolognese, R. Liberatore and W. Schäfer, *J. Heterocyclic Chem.*, **26**, 1083 (1989).

[8] The Chemistry of the Hydroxyl Group, S. Patai, ed, Interscience Publishers, London, 1971, p 505.

[9] T. J. Stones and W. A. Waterers, *Proc. Chem. Soc.*, 253 (1962).

[10] The Chemistry of Amino, Nitroso and Nitro Compounds, Supplement F, S. Patai, ed, John Wiley & Sons, 1982, p 1151.

[11] Electron Spin Resonance in Chemistry, P. B. Ayscough, Metthuen & Co. Ltd., London, 1967, p 260.

[12] L. R. Morgan and C. C. Aubert, *Proc. Chem. Soc.*, 73 (1962).