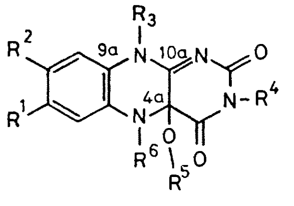
Products of the Decomposition of the Anion of a 4a-Hydroperoxyisoalloxazine Hindered in the 9a and 10a Positions

By Thomas C. Bruice* and Audrey Miller
(*Department of Chemistry, University of California, Santa Barbara, CA* 93106)

Summary Products formed from the decomposition of the 4a-peroxy-anion of the flavin (**1d**) are only accountable through reactions involving the 4a-hydroperoxide anion; kinetic and product studies both argue against the involvement of a 4a → 10a peroxy-anion migration.

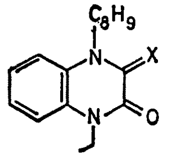
The reaction of molecular oxygen with reduced flavomono-oxygenase provides an enzyme-bound 4a-hydroperoxide of the flavin cofactor.[1] We have found, in biomimetic reactions, that the flavin 4a-peroxide (**1a**) is a most efficient reagent for the mono-oxygenation of organic sulphides[2] and amines[3] and in the chemiluminescent oxidation of aldehydes,[2,4,5] and that the 4a-peroxy-flavin anions, (**1b**) and (**1d**), transfer dioxygen to ambident anions.[6,7,8,9] It has been proposed[10] that the enzyme-bound 4a-hydroperoxy-flavin serves as a 'storage form' and that 4a → 10a migration of the hydroperoxy-group occurs prior to the oxygen transfer events. To test this concept, we synthesized (**1c**) and determined the rate constants for mono-oxygen and dioxygen transfer.[9] The lack of a steric effect[11] upon exchanging methyl for 2′,6′-dimethylphenyl at the 10-position does not support a 4a → 10a migration of the hydroperoxy- and peroxy-anions prior to mono- and dioxygen transfer. The objective of the present investigation has been to ascertain if the products of decomposition of (**1d**) indicate a 4a → 10a peroxy-anion migration *via* a 4a,10a-dioxetan.



(**1**)

a; $R^1 = R^2 = R^3 = R^4 = Me, R^5 = OH, R^6 = Et$
b; $R^1 = R^2 = R^3 = R^4 = Me, R^5 = O^-, R^6 = Et$
c; $R^1 = R^2 = H, R^3 = 2,6\text{-}Me_2C_6H_3, R^4 = Me, R^5 = OH, R_6 = Et$
d; $R^1 = R^2 = H, R^3 = 2,6\text{-}Me_2C_6H_3, R^4 = Me, R^5 = O^-, R^6 = Et$
e; $R^1 = R^2 = R^5 = H, R^3 = 2,6\text{-}Me_2C_6H_3, R^4 = Me, R^6 = Et$

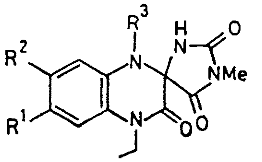
A solution of (**1c**)[9] [$1{\cdot}62 \times 10^{-4}$ mol (94% pure) in 100 ml of dry Bu^tOH] was stirred (N_2) with a solution of Bu^tOK ($2{\cdot}05 \times 10^{-4}$ mol in 100 ml of Bu^tOH) (12 min); mixture was then acidified with oxygen-free acetic acid, the solvent removed, and the residue triturated with CH_2Cl_2. From thick layer chromatography on silica gel (EtOAc eluent) the following were obtained:† 9% (**2a**) (from cyclohexane) (m.p. 155—158 °C), 3—7% (**1e**), and 57% (**3a**) (benzene–cyclohexane) (m.p. 217—218·5 °C). Compound (**2a**) was hydrolysed (3N HCl) to (**2b**), an authentic sample of which was synthesized by thermal condensation of 2-*N*-(2,6-dimethylphenyl)aniline[11b] with diethyl oxalate, and ethylation of the resultant 1,2,3,4-tetrahydro-1-(2,6-dimethylphenyl)-2,3-dioxoquinoxaline (m.p. 297—297·5 °C from methanol)† with diethyl sulphate and NaH in toluene.



(**2**)

a; X = NH

b; X = O

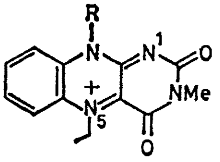


(**3**)

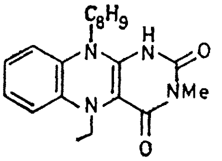
a; $R^1 = R^2 = H$, $R^3 = 2,6\text{-}Me_2C_6H_3$

b; $R^1 = H$, $R^2 = Br$, $R^3 = m\text{-}HOC_6H_4CH_2CH_2$

c; $R^1 = R^2 = R^3 = Me$



(**4**) $R = 2,6\text{-}Me_2C_6H_3$



(**5**)

This authentic (**2b**) (m.p. 133·5—136·5 °C) was identical (m.p., i.r., n.m.r) with the product obtained from the hydrolysis of (**2a**). The yield (%) of (**1e**) was established by its conversion into (**4**) (550 nm, ϵ 8000 l mol^{-1} cm^{-1}) by acidification with $HClO_4$. Assignment of a 10a-spiro structure to (**3a**) is based upon the chemical shift (δ 4·26) of the methylene protons of the ethyl substituent at N-5. These methylene protons, for the known 10a-spirohydantoins, resonate at δ 4·07, [(**3b**); structure by *X*-ray crystallography][12] and 4·01 [for (**3c**)][13] In contrast, the methylene protons of the N^5-ethyl substituent of the 4a-spirohydantoin isomer of the 10a-spirohydantoin (**3c**) fall at higher field (δ 3·05).[13] There is also formed (u.v–vis. spectra) about 5% of the isoalloxazine (Fl_{ox}).

Isoalloxazine-4a-hydroperoxy-anions decompose to yield, in part, 1,5-dihydroisoalloxazine (FlEtH).[6] An aliquot of the acidified reaction solution was treated with excess of 4-hydroxy-2,2,6,6-tetramethylpiperidinone *N*-oxyl[14] and the concentration of N^5-ethylisoalloxazine radical determined spectrally [λ_{max} 636 (ϵ 2800) and 590 nm (ϵ 2700)]. The yield of FlEtH, based on this assay, was 45%. In another experiment, the product analysis for products from (**1d**) was repeated, starting with FlEtH. Compounds (**3a**) (28%) and (**1c**) (9%) were formed. Combination of the results of these experiments leads to equation (1).

$$\begin{array}{l} \ulcorner\!\!\rightarrow \underset{ca.\ 3\%}{(\mathbf{1e})} + \underset{44\%}{(\mathbf{3a})} + \underset{ca.\ 9\%}{(\mathbf{2a})} + \underset{ca.\ 5\%}{Fl_{ox}} \qquad (1) \\ (\mathbf{1d}) \xrightarrow{-O_2} \underset{45\%}{(\mathbf{5})} \xrightarrow{O_2} \underset{ca.\ 4\%}{(\mathbf{1c})} + \underset{13\%}{(\mathbf{3a})} \end{array}$$

It is obvious that the various products of decomposition must arise from (**1d**) without recourse to a 4a → 10a migration of the peroxy-anion.

This study was supported by a grant from the National Institutes of Health (U.S.A.).

(*Received*, 31*st March* 1980; *Com.* 340.)

---

† All new compounds gave satisfactory analytical and spectroscopic data.

[1] V. Massey and P. Hemmerich in 'The Enzymes,' ed. P. D. Boyer, Academic Press, New York, 1976, Vol. XII, p. 191.

[2] C. Kemal, T. W. Chan, and T. C. Bruice, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 405.

[3] S. Ball and T. C. Bruice, *J. Am. Chem. Soc.*, 1979, **101**, 4017.

[4] C. Kemal and T. C. Bruice, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 995.

[5] C. Kemal and T. C. Bruice, *J. Am. Chem. Soc.*, 1977, **99**, 7064.

[6] C. Kemal and T. C. Bruice, *J. Am. Chem. Soc.*, 1979, **101**, 1635.

[7] S. Muto and T. C. Bruice, *J. Am. Chem. Soc.*, 1980, **102**, in the press.

[8] S. Muto and T. C. Bruice, unpublished results.

[9] A. Miller and T. C. Bruice, *J. Chem. Soc., Chem. Commun.*, 1979, 896.

[10] P. Hemmerich and A. Wessiak in 'Flavins and Flavoproteins,' ed. T. P. Singer, Elsevier, Amsterdam, The Netherlands, 1976, p. 1.

[11] (a) S. B. Smith and T. C. Bruice, *J. Am. Chem. Soc.*, 1975, **97**, 2875; (b) L. Main, G. J. Kasperek, and T. C. Bruice, *Biochemistry*, 1972, **11**, 3991.

[12] T. C. Bruice, I. Masaki, H. L. Carrell, and J. P. Glusker, *J. Am. Chem. Soc.*, 1980, **102**, in the press.

[13] H. I. X. Mager, *Tetrahedron Lett.*, 1979, 3549.

[14] T. W. Chan and T. C. Bruice, *J. Am. Chem. Soc.*, 1977, **99**, 7287.