Oxidations by a 4a-Hydroperoxyisoalloxazine hindered in the 9a and 10a Positions

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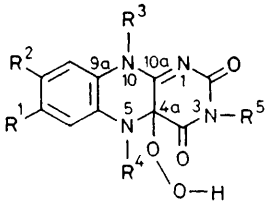
Summary The rates and products of oxidation of thioxan, *NN*-dimethylaniline, and 2,6-di-t-butyl-4-methylphenolate effected by the hindered N^{10}-(2′,6′-dimethylphenyl)-N^5-ethyl-4a-hydroperoxy-3-methyl-4a,5-dihydroisoalloxazine are comparable to the same reactions effected by the unhindered 4a-hydroperoxy-5-ethyl-3-methylumiflavin; this makes it highly unlikely that rearrangement of the 4a-hydroperoxyflavin to a 9a- or 10a-substituted isoalloxazine is required as an explanation for the oxygen transfer potential of the former.

FLAVIN MONO-OXYGENASES can exist in both oxidized and reduced states. When reduced, these enzymes react with molecular oxygen to provide hydrogen peroxide and oxidized flavoenzyme. In the presence of a suitable substrate oxidized enzyme, water, and a mono-oxygenated substrate are formed. For some time the initial reaction of reduced flavoenzyme with oxygen has been considered to provide an enzyme bound peroxyflavin.[1,2] The most viable structures for the hydroperoxide have included the 4a (structure **1a**),[1b] 9a,[1c] and 10a adducts.[1a] There has



1a; $R^1 = R^2 = Me$, R^3 = ribitol phosphate (for FMN) $R^4 = R^5 = H$
b; $R^1 = R^2 = H$, R^3 = 2′, 6′-dimethylphenyl, $R^4 = Et$, $R^5 = Me$
c; $R^1 = R^2 = R^3 = Me$, $R^4 = Et$, $R^5 = Me$

been speculation that the position where the hydroperoxide group resides may depend upon the function of the enzyme.[1b] Oxaziridines,[3] perepoxides,[4] and carbonyl oxides[5a] (or the cyclized trioxide tautomer)[5b,c] have been postulated as ultimate 'oxene' mono-oxidizing species which can be derived from a flavin hydroperoxide. The synthesis of N^5-alkyl-4a-hydroperoxyflavins in this laboratory and the establishment of their spectral identity to the oxygen adducts of a flavin mono-oxygenase has established that the 4a-hydroperoxyflavin species is either the ultimate oxidant or a precursor to the ultimate oxidant.[6] We have also shown that the various oxene mechanisms are not required to explain either the chemiluminescent oxidation of aldehydes[6] or the N- and S-oxidations of tertiary amines and organic sulphides by N^5-alkyl-4a-hydroperoxyflavins in model systems unless a migration of the peroxy group to the 9a or 10a position is envisaged.[7]

Substitution of the isoalloxazine ring at the N^{10}-position by a 2′,6′-dimethylphenyl group (as in **2**) has been shown to prevent nucleophilic addition at the 9a and 10a positions.[8] It may be concluded, from examination of molecular models (Figure), that steric hindrance is not only to the approach

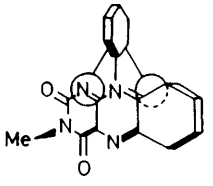


FIGURE. Stereochemical representation of (**2**) showing steric crowding of the 10a position by the methyl groups.

of the nucleophile but also destabilizes the adduct through contact between the 2′-methyl group and the 9a or 10a substituent. To test whether the 4a or the 9a, 10a positions are involved in the reactions of hydroperoxyflavins, we have synthesized N^{10}-(2′,6′-dimethylphenyl)-N^5-ethyl-4a-hydroperoxy-3-methyl-4a,5-dihydroisoalloxazine (**1b**), and measured the rate constants for its reactions with a number of substrates. Synthesis of (**1b**) from the known N^{10}-(2′,6′-dimethylphenyl)-3-methylisoalloxazine (**2**)[8b] was accomplished in three steps. Compound (**2**) was ethylated at the 5-position using acetaldehyde, H_2, Pd/C, and a trace of perchloric acid. Oxidation of the crude product with sodium nitrite and perchloric acid gave an excellent yield of N^{10}-(2′,6′-dimethylphenyl)-N^5-ethyl-3-methylisoalloxazinium perchlorate which gave satisfactory elemental analyses. The very low pK_a (0·95) for pseudo-base formation (spectral titration) from the N^5-ethylflavinium salt accounts for the rapid formation of (**1b**) on treatment of a solution of the cation in acetonitrile with 30% hydrogen peroxide.

TABLE. Comparison of rate constants for oxidations by (**1b**) and (**1c**)

Substrate	Rate constants (**1b**)	(**1c**)
Thioxan (MeOH, anaerobic)	0·27 l mol^{-1} s^{-1}	0·66 l mol^{-1} s^{-1} [a]
NN-Dimethylaniline (Bu^{t}OH, anaerobic)	$7{\cdot}4 \times 10^{-4}$ l mol^{-1} s^{-1}	$9{\cdot}6 \times 10^{-4}$ l mol^{-1} s^{-1} [b]
2,6-Di-t-butyl-4-methylphenolate (Bu^{t}OH, anaerobic)	0·26 s^{-1}	0·33 s^{-1} [c]

[a] See C. Kemal, T. W. Chan, and T. C. Bruice, *Proc. Nat. Acad. Sci. U.S.A.*, **1977**, **74**, 405. [b] See S. Ball and T. C. Bruice, *J. Amer. Chem. Soc.*, 1979, **101**, 4017. [c] See C. Kemal and T. C. Bruice, *ibid.*, **1979**, **101**, 1635. The rate constant reported was determined in the presence of an excess of base.

The rate constants for the reaction of (**1b**) and (**1c**) to give S-oxidation of thioxan, N-oxidation of *NN*-dimethylaniline (equation 1), and dioxygen transfer to the 4-position of 2,6-di-t-butyl-4-methylphenolate (equation 2) are given in the Table. The Table (FlEt = N^5-ethylflavin) shows

$$\text{4a-FlEt-OOH} + :\text{N}\lesssim \text{ (or } :\text{S}\lesssim\text{)} \longrightarrow \text{4a-FlEt-OH} + {}^{-}\text{ON}^{+}\!- \text{ (or OS}\lesssim\text{)} \quad (1)$$

$$\text{4a-FlEt-OO}^{-} + \text{C}_{15}\text{H}_{23}\text{O}^{-} \longrightarrow \text{FlEt}^{-} + (\text{C}_{15}\text{H}_{23}\text{O})\text{OO}^{-} \quad (2)$$

that in all cases the reactivity of the hindered hydroperoxyflavin (**1b**) is comparable to that of the unhindered hydroperoxyflavin (**1c**). There appears to be little or no steric effect in the reactions of (**1b**). Furthermore, the oxidation of *NN*-dimethylaniline by (**1b**) gave a 100% yield of the *N*-oxide (as determined by the method of Ziegler and Pettit),[9] while the oxidation of 2,6-di-t-butyl-4-methylphenolate gave a substantial amount of 2,6-di-t-butyl-4-methyl-4-hydroperoxycyclohexa-2,5-dienone mixed with the corresponding 4-hydroxy compound. In the course of the reaction (**1b**) was reduced to the corresponding N^5-ethyl-1,6-dihydroflavin (equation 2) in 60% yield.†

This work shows that the oxygenase activity of flavins can be explained on the basis of formation of a 4a-hydroperoxyflavin adduct without subsequent formation of a 9a or 10a derivative.

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† Analysed by the method of Kemal and Bruice, see Table footnote c.

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