Determination of the Kinetic pK_a of a Flavin 4a-Pseudobase from a Study of the Hydrolysis of 4a-Hydroxy-5-ethyl-3-methyl-lumiflavin in H$_2$O. The Oxygen Donation Potential of 4a-Hydroperoxyflavins

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Below pH *ca.* 8.5, 4a-hydroxy-5-ethyl-3-methyl-lumiflavin (**2**) is converted into the spiro(imidazo-4,2-quinoxaline) (**3**), but between pH 8.5—10.5, the product is 1,6,7-trimethyl-4-ethylquinoxaline-2,3-dione (**4**) accompanied by a small amount of (**3**); under strongly basic conditions the product (**4**) is accompanied by a trace of a substance tentatively identified as an imidazo[4,5-*b*]quinoxalinone (**6**); from the pH dependence of the hydrolysis of (**2**) the kinetic pK_{app} = 9.9 for the dissociation of the 4a-hydroxy proton.

In a previous communication[1] it was shown that a free energy correlation exists between the log of the second order rate constant for oxygen transfer from percarboxylic acids or hydroperoxides (YOOH) and the pK_a of YOH. Since the rate of mono-oxygen transfer from the hydroperoxy group of 4a-hydroperoxy-5-ethyl-3-methyl-lumiflavin (**1**) fits this cor-
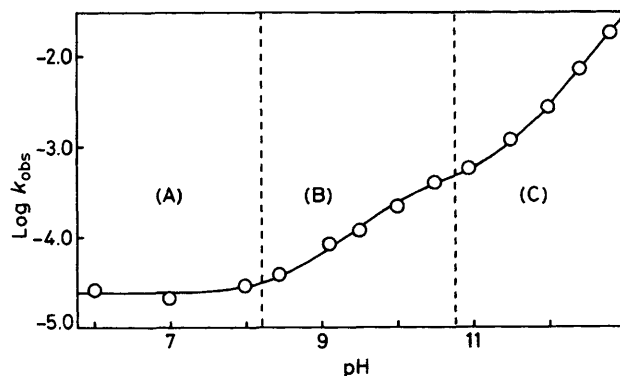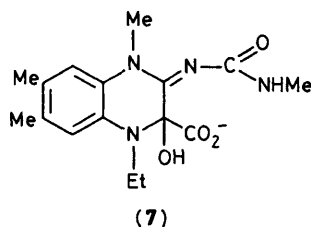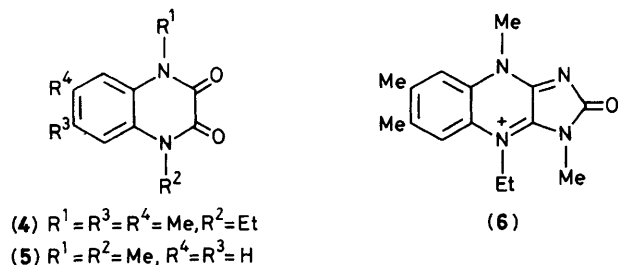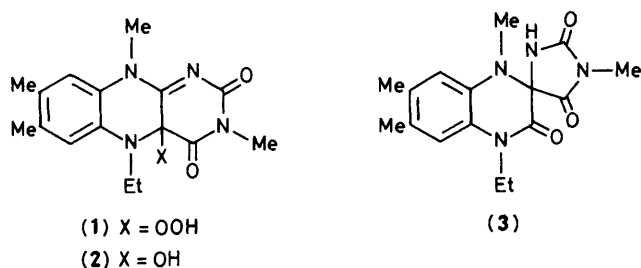

(**1**) X = OOH
(**2**) X = OH

(**3**)

(**4**) R^1 = R^3 = R^4 = Me, R^2 = Et
(**5**) R^1 = R^2 = Me, R^4 = R^3 = H

(**6**)

(**7**)

Figure 1. Log k_{obs} *vs.* pH profile for solvolysis of (**2**) in H$_2$O at 30 °C (μ = 1.0 M).

relation, a rationale was provided for the appreciable mono-oxygen donation potentials for the biochemically important 4a-hydroperoxyflavins. The pK_a values of YOH used in the previous correlation were either experimentally determined (*e.g.* HOH and *m*-ClC$_6$H$_4$CO$_2$H) or calculated [*e.g.* Me$_3$COH, Ph$_2$C(CO$_2$Me)OH, and Ph$_2$C(CN)OH] employing a $\rho_I\sigma_I$ method.[1] However, in the case of (**2**), calculation of its pK_a value is not straightforward and since it decomposes in water, its pK_a cannot be determined experimentally.

Nevertheless, by two separate approaches to the $\rho_I\sigma_I$ method of calculation, the pK_a of the –OH function of (**2**) was determined to be 9.2.† We report here a kinetic study of the pH dependence of the hydrolysis of (**2**) from which an experimental pK_{app} value can be obtained.

Figure 1 shows a plot of the log of the pseudo first-order rate constants *vs.* pH for disappearance of (**2**) (H$_2$O, 30 °C, μ = 1 M with KCl). The sole product in region (A) is the spiro compound (**3**) (identified by comparison of h.p.l.c. retention time and u.v.–visible spectrum with an authentic[2] sample). In region (B) the product is 1,6,7-trimethyl-4-ethylquinoxaline-2,3-dione (**4**) accompanied by a small amount of (**3**). In region (C), the product is (**4**) accompanied by a trace of what is presumed to be the pseudo-base of (**6**). Compound (**4**) was isolated by preparative h.p.l.c. and identified by high resolution mass spectroscopy and by the similarity of its u.v.–visible

$$k_{obs} = k_1' + k_2' \frac{K_{app}}{K_{app} + a_H} + k_3[HO^-] \frac{K_{app}}{K_{app} + a_H} \quad (1)$$
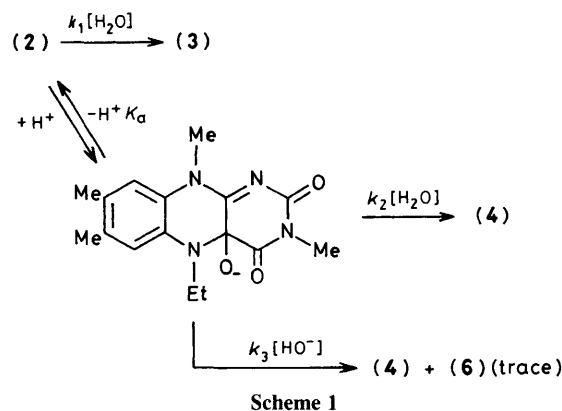
(A)   (B)   (C)

(**6**) pK_a 4.4 −H$^+$ / +H$^+$ (**2**)

(**2**) pK_a 4.0 −H$^+$ / +H$^+$ (**2**) (**3**)

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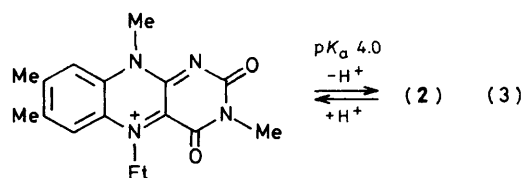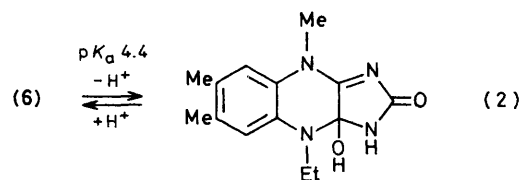
† These procedures are based on the general method of J. P. Fox and W. P. Jencks (*J. Am. Chem. Soc.,* 1974, **96**, 1436) and involve division of the ring structure of (**2**) into domains with assignable σ_I values so that the C(4)–OH moiety may be considered as a substituted methanol. The first procedure is described in ref. 1. Professor Marvin Charton employed an alternate choice of domains.

spectrum with that of authentic (5).[3] The formation of (4) is dependent upon the presence of O_2, while the rate of disappearance of (2) [equation (1)] is not. Therefore, (4) must arise by O_2 oxidation of a hydrolysis product of (2). By analogy with previous work[4] this hydrolysis product is (7).

The structure of (6) is presumed on the basis that a structure identical to (6), with the exception of the absence of the N(5)–ethyl substituent, is formed on base catalysed aerobic hydrolysis of 3-methyl-lumiflavin[4] and the finding that (6) exists as both 'acid' (λ_{max} 410, 342 nm) and 'base' (λ_{max} ca. 320 nm) species with pK_a of 4.4 (by spectrophotometric titration) [equation (2)]. The pK_a of 4.4 is similar to that associated with pseudo-base formation of (2) [equation (3)].[5]

The points on Figure 1 are experimental and the line joining the points has been generated using equation (1) which can be derived from Scheme 1.‡ The constants employed to fit equation (1) to the experimental points of Figure 1 are: $k_1' = 2.43 \times 10^{-5}$ s^{-1}, $k_2' = 3.75 \times 10^{-4}$ s^{-1}, $k_3 = 1.0 \times 10^{-3}$ mol^{-1} dm^3 s^{-1} and $pK_{app} = 9.9$. The value of pK_{app} differs from that calculated by the use of the $\rho_I\sigma_I$ relationship (9.2) by 0.7 pH units. Since kinetic and thermodynamic pK_a values often differ by up to 0.5 pH units, the calculated and kinetically determined constants are in reasonable agreement. In any event, a pK_a of 9.9 results in only a five-fold positive deviation of the rate constant from the plot of log k_{rate} vs. pK_a of YOH for the reaction of the 4a-hydroperoxyflavin. This is insignifi-

‡ It is interesting to note that in MeOH–MeO$^-$, compound (1) yields (2) (cf. M. Iwata, T. C. Bruice, H. L. Carrell, and J. P. Glusker, J. Am. Chem. Soc., 1980, **102**, 5036). Presumably, in H_2O, the –O$^-$ function of the anion of (1) behaves as a neighbouring general base to promote attack of H_2O upon the C(4) position to yield (6). Attack at C(4) is apparently disfavoured in MeOH.

Scheme 1

cant since the linear free energy plot holds for a range of 10^{13} in K_a and 10^8 in k_{rate}.

References

1 T. C. Bruice, J. Chem. Soc., Chem. Commun., 1983, 14; T. C. Bruice, J. B. Noar, S. S. Ball, and U. V. Venkataram, J. Am. Chem. Soc., 1983, **105**, 2452.
2 M. Iwata, T. C. Bruice, H. L. Carrell, and J. P. Glusker, J. Am. Chem. Soc., 1980, **102**, 5036.
3 G. W. H. Cheeseman, J. Chem. Soc., 1958, 108.
4 B. S. Smith and T. C. Bruice, J. Am. Chem. Soc., 1975, **97**, 2875.
5 S. Ghisla, U. Hartmann, P. Hemmerich, and F. Muller, Liebigs Ann. Chem., 1973, 388; C. Kemal and T. C. Bruice, J. Am. Chem. Soc., 1976, **96**, 3955.