Reactivity Studies of Roseoflavin Analogues: A Correlation between Reactivity and Absorption Maxima

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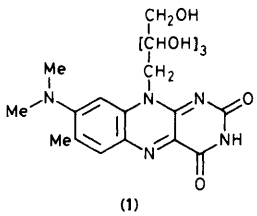
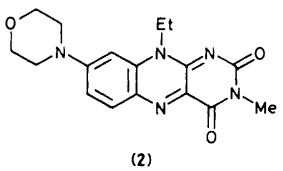
The absorption spectra of roseoflavin analogues are very sensitive to solvent effect and the oxidising ability (in the photo-oxidation of 1-benzyl-1,4-dihydronicotinamide) correlates well with the shift in the absorption maxima.

Roseoflavin (**1**), isolated from a culture medium of Streptomyces strain No. 768,[1] has a dimethylamino group at the 8-position instead of a methyl group as in conventional flavin coenzymes.[2,3] Roseoflavin and its 8-*N*-alkyl analogues exhibit an inhibitory effect on growth of Gram-positive bacteria.[4] This anti-riboflavin activity is thought to occur because the isoalloxazine ring loses its oxidising ability owing to intramolecular charge transfer from the 8-dimethylamino group to the pteridine moiety.[5] Roseoflavin and its analogues are red in aqueous solution, the colour being attributable to the charge transfer band, and they invariably have redox potentials more negative than conventional flavin coenzymes.[4] To investigate the relationship between intramolecular charge transfer and reactivity, we synthesised a roseoflavin analogue (**2**) and a monoazacrown ether flavin mimic (**3**).† We here report that, in contrast to the redox properties of conventional flavins, the absorption spectra of these roseoflavin analogues are very



(1)



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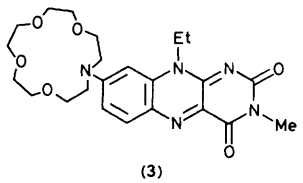

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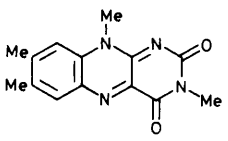

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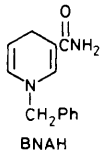


BNAH

† (**2**) (m.p. >300 °C) and (**3**) (m.p. 215—217 °C) were synthesised by the reaction of 3-methyl-8-chloro-10-ethylisoalloxazine with the corresponding amines and identified by i.r. and n.m.r. spectroscopy and elemental analysis.

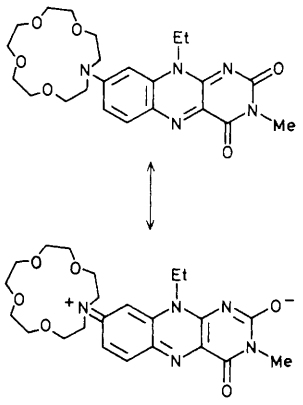
Table 1. Absorption maxima and pseudo-first-order rate constants for the photo-oxidation of BNAH.[a]

	$\lambda_{max.}$/nm		$10^3 \cdot k_1'$/min^{-1}	
Solvent	(2)	(3)	(2)	(3)
MeOH	485	495	*ca.* 0.1	*ca.* 0.1
MeCN	480	487	1.0	0.7
THF[b]	478	486	96	60
Dioxane	473	483	220	140
Benzene	479	486	170	90
MeCN ([$NaClO_4$] = 21.3 mM)			1.0	13

[a] [flavin] = 1.82×10^{-5} M, [BNAH] = 5.00×10^{-3} M, 30 °C, under N_2. The distance between a 17-W fluorescent lamp and a Thunberg cuvette was maintained at 14 cm. [b] THF = tetrahydrofuran.

sensitive to solvent effects and the oxidising ability correlates well with the shifts in the absorption maxima.

In polar solvents such as water and methanol (**2**) and (**3**) give a red colour. This colour, due to the first absorption band at *ca.* 490 nm, is related to the intramolecular charge transfer. However, in non-polar solvents such as dioxane and benzene (**2**) and (**3**) produce a yellow to orange solution as well as a strong green fluorescence (Table 1). The hypsochromic shift in nonpolar solvents is rationalised in terms of suppression of the intramolecular charge transfer [equation (1)]. The situation is very similar to the effect of solvent on the absorption spectra of indophenol derivatives.[6] Also Dimroth's transition energy values, E_T, correlate well with the $h\nu$ values of the maximum absorption frequencies of (**2**) and (**3**).



(1)

The thermal reaction of the flavins with 1-benzyl-1,4-dihydronicotinamide (BNAH) in aqueous solution was first order in flavin and BNAH. The second-order rate constants, k_2, for (**2**) and (**3**) (k_2 = 2.58 and 2.10 dm^3 mol^{-1} min^{-1}, respectively; 30 °C, pH 8.18, water–methanol = 3 : 7 v/v) were 17—21-fold smaller than that for 3-methyl-lumiflavin (**4**) (k_2 = 44.9 dm^3 mol^{-1} min^{-1}), indicating that the electron donating 8-amino group significantly weakens the oxidising ability of the isoalloxazine ring. The k_2 value for (**3**) was enhanced in the presence of 0.20 M NaCl (4.38 dm^3 mol^{-1} min^{-1}). This rate enhancement is attributed to specific Na^+–crown interaction in (**3**), because the addition of 0.20 M CsCl was quite ineffective for the oxidation of BNAH by (**3**) and such a salt effect was not observed for (**2**).

The anaerobic photo-oxidation of BNAH was carried out in a thermostated water bath (30 °C).‡ The thermal reaction was negligible in the solvents listed in Table 1. The photo-oxidation rate, which was followed by monitoring the disappearance of the absorption maxima of the flavin, could be approximated by the first-order rate equation for up to two half-lives in the presence of excess BNAH. The pseudo-first-order rate constants, k_1', thus obtained are summarised in Table 1. The photo-oxidation is very slow in polar solvents such as methanol and acetonitrile while the reaction is relatively fast in nonpolar solvents such as dioxane and benzene, the rate difference being more than 10^3-fold. In other words, the 'red' flavins are redox-inactive while the 'yellow' flavins are capable of oxidising BNAH. A plot of log k_1' (in min^{-1}) *vs.* E_T showed a linear relationship (r = 0.97) as expressed by equation (2). This indicates that the photo-oxidising ability of (**2**) and (**3**) is directly related to their transition energies.

$$\log k_1' = -0.17E_T + 5.15 \quad (2)$$

Generally flavin-dependent reactions are accelerated in polar media, because they have polar transition states. The contrasting results obtained for the roseoflavin analogues imply that their oxidising ability is governed by the extent of the intramolecular charge transfer. Nonpolar solvents which suppress the charge separation on the isoalloxazine ring can activate the roseoflavin analogues as oxidising agents. Another method of suppression of the charge transfer band would be the co-ordination of the 8-amino group to metal ions. As recorded in Table 1, the addition of $NaClO_4$ (21.3 mM) enhanced the k_1' value for (**3**) 19-fold but such a salt effect was not observed for (**2**). The result establishes that the rate increase is caused by Na^+–crown interaction.

In conclusion, the present study established that the roseoflavin analogues, which are inactive in polar media, are activated in nonpolar media because of the suppression of the intramolecular charge transfer from the 8-amino group to the pteridine moiety. The finding suggests that roseoflavin may be activated when it is bound to hydrophobic pockets of enzymes.

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‡ The product analysis showed that BNAH is quantitatively oxidised to the 1-benzylnicotinamide salt.