

A Flavin Mimic possessing Strong Oxidizing Power due to Electron Delocalization: a Benzo-dipteridine

Yumihiko Yano,^{a*} Michiaki Nakazato,^a and Rafael E. Vasquez^b

^a Department of Chemistry, Gunma University, Kiryu, Gunma 376, Japan

^b Department of Applied Chemistry, Hiroshima University, Saijo, Higashi-Hiroshima 724, Japan

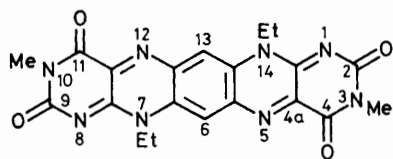
7,14-Diethyl-3,10-dimethylbenzo[1,2-*g*,4,5-*g'*]dipteridine-2,4,9,11(3*H*,7*H*,10*H*,14*H*)-tetrone [benzo-dipteridine (1)] shows remarkably high oxidizing activity towards thiols and phenylhydrazine; acid-catalysis is observed in the oxidation of an NADH model by (1).

A flavin mimic possessing strong oxidizing power would play an important role in model investigations of flavin coenzymes. Recently we have shown that an 8-azaflavin¹ and a 6,8-diazaflavin² exhibit unusually high oxidizing activity. The latter, however, could not be used under alkaline conditions owing to covalent hydration.²

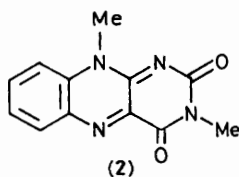
We report here another example of an oxidation-active flavin mimic which has an extended conjugated system consisting of two isoalloxazine skeletons with a benzo unit in common, and also report the detection of acid catalysis in the oxidation of *N*-benzyl-1,4-dihyronicotinamide (BNAH) by this flavin mimic. Recently Yoneda *et al.* have reported a similar class of 5-deazaflavins which exhibit strong oxidizing ability.³ Compound (1)[†] was prepared from *N,N'*-diethyl-*p*-phenylenediamine and 6-chloro-3-methyluracil.⁴

Spectroscopic examination showed that (1) is reduced by NaBH₄, BNAH, thiols, and ethylenediaminetetra-acetic acid-*hν*, and the product has an absorption maximum at 620 nm (ϵ 2.7×10^4 dm³ mol⁻¹ cm⁻¹) at pH 7.0 under anaerobic conditions. Reduction with Na₂S₂O₄, however, led to an absorption maximum at 502 nm (ϵ 3×10^4), which gradually changed to 620 nm. Similar spectral changes were also observed in the reaction of (1) with K₂SO₃, implying that the intermediate with λ_{max} 520 nm is the 4a-sulphite adduct.⁵ The final absorption spectra (λ_{max} 620 nm) changed to the starting spectra in the presence of O₂.

The redox potentials were determined by cyclic voltammetry [(1): -120 and -770 mV vs. Ag/AgCl/0.1 M KCl electrode; pH 7.0; 0.1 M Na₂SO₄; 25 °C; N₂]. The first 2e-redox potential of (1) is about 300 mV more positive than that of the tricyclic analogue (2) (-439 mV), suggesting that (1) should have strong oxidizing ability and the second redox potential (-770 mV) shows that it is difficult for the



(1)

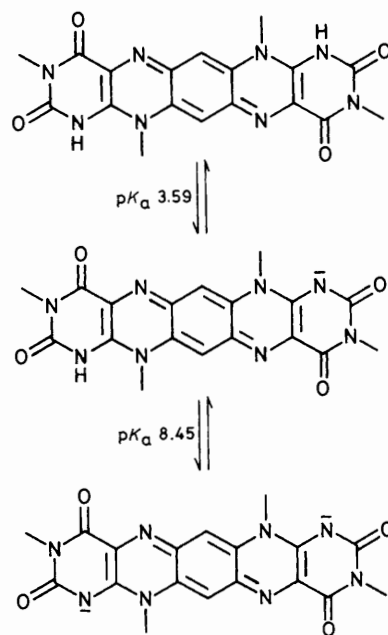


(2)

2e-reduced form of (1) to accept an additional 2e to afford 4e-reduced (1) under conventional conditions.

The activity was examined kinetically for the oxidation of thiols [dithiothreitol (DTT) and HS(CH₂)₂OH (ME)], PhNHNH₂, and BNAH in aqueous solution under anaerobic conditions. The rate constants were determined by following the absorption increase at 620 nm or decrease at 390 nm. Reactions followed first-order kinetics up to more than 3 half-lives for the thiols and PhNHNH₂. The rates were first-order with respect to [DTT] or second-order with respect to [ME], respectively, indicating that the reduced (1) (λ_{max} 620 nm) is not the 4e- but the 2e-reduced form. The p*K*_a values of the 2e-reduced (1) were determined spectrophotometrically (Scheme 1).[‡] The lower p*K*_a compared with that of (2) (~6.7)⁶ may be reasonably explained by stabilization of the conjugated base owing to electron delocalization. Furthermore the much lower p*K*_a value and the absorption at longer wavelengths of reduced (1) compared with reduced (2) strongly suggest that the 2e-reduced form of (1) adopts a 1,8-dihydro structure rather than the 1,5-dihydro form, as shown in Scheme 1.

Because of the large rate difference between (1) and (2), the substrate concentration was changed for estimation of the relative rates at the same pH. Table 1 shows that (1) exhibits a markedly higher oxidizing activity compared with (2) towards



Scheme 1

[†] M.p. >300 °C, *M*⁺, *m/z* 434, λ_{max} (pH 7.0) 390 (ϵ 1.75×10^4 dm³ mol⁻¹ cm⁻¹) and 548 nm (ϵ 1.4×10^4). Satisfactory elemental analyses were obtained.

[‡] From the absorption spectra after the reaction with DTT in the pH range 1.84–10.10.

Table 1. Rate constants and relative rates at 25 °C.^a

Substrate	pH	$k_{\text{obs.}}/\text{min}^{-1}$		Rel. rate ^b [(1)/(2)]
		(1)	(2)	
ME	6.75	0.735 ^c	3.79×10^{-2} ^d	2×10^7
DTT	4.96	6.14 ^c	4.4×10^{-4} ^f	1×10^6
PhNHNH ₂	8.59	2.11 ^g	$<2.3 \times 10^{-4}$ ^h	$>1 \times 10^7$

^a [(1)] = 1×10^{-5} M, [(2)] = 5×10^{-5} M, 0.1 M buffer (μ 0.3 M).

^b The relative rates were calculated from $k_{\text{obs.}}/[\text{ME}]^2$, $k_{\text{obs.}}/[\text{DTT}]$, and $k_{\text{obs.}}/[\text{PhNHNH}_2]$. ^c [ME] = 1×10^{-3} M. ^d [ME] = 1 M. ^e [DTT] = 1×10^{-4} M. ^f [DTT] = 1×10^{-2} M. ^g [PhNHNH₂] = 1×10^{-4} M. ^h [PhNHNH₂] = 0.1 M.

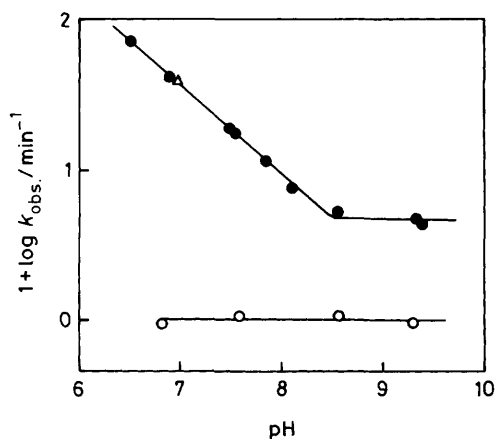


Figure 1. pH-rate profiles for the oxidation of BNAH, [(1)] = [(2)] = 1×10^{-5} M, [BNAH] = 1×10^{-4} M, [buffer] = 0.1 M (μ 0.3 M with KCl); 25 °C: ●, (1); ○, (2); pH < 8.5; phosphate (Δ , imidazole), pH > 8.5; borate.

the thiols and PhNHNH₂ (10^6 – 10^7 fold). It seems to be well established that the mechanism for the oxidation of thiols and PhNHNH₂ by flavin models involves 4a-adduct formation; the former gives the disulphide by nucleophilic attack of a second

thiol anion⁷ and the latter affords phenyldiazene by base-catalysed elimination.⁸ Thus the large rate enhancement may be best accounted for by stabilization of the 4a-adducts due to delocalization of negative charge generated on the N(5) atom. The oxidation of BNAH was found to be acid-catalysed for (1), although no buffer catalysis [imidazole buffer (0.025–0.2 M), pH 7.0] was observed. In contrast, no acid-catalysis was observed for (2) (Figure 1). As shown in Figure 1, the acid catalysis begins to appear below the pH corresponding to the pK_a value of the product [2e-reduced (1)]. Although the oxidation of NADH models by flavin models has been extensively investigated,⁹ acid catalysis in these systems has not been examined because of instability of the NADH model towards acids. Thus the higher pK_a (8.45) of the 2e-reduced (1) made it possible to detect acid catalysis. The small rate enhancement of (1) above pH ca. 8.5 may be attributed to the formation of unstable dianion of the reduced (1).

We thank Professor E. Ohta (Gunma University) for mass spectral measurements, and one of the referees for helpful comments.

Received, 23rd July 1984; Com. 1071

References

- 1 Y. Yano, I. Yatsu, E. Ohya, and M. Ohshima, *Chem. Lett.*, 1983, 775; Y. Yano, M. Ohshima, and S. Sutoh, *J. Chem. Soc., Chem. Commun.*, 1984, 695.
- 2 Y. Yano, M. Ohshima, S. Sutoh, and M. Nakazato, *J. Chem. Soc., Chem. Commun.*, 1984, 695.
- 3 F. Yoneda, K. Kuroda, M. Koga, and T. Ibuka, *J. Chem. Soc., Chem. Commun.*, 1984, 872.
- 4 F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shiomura, *J. Am. Chem. Soc.*, 1976, **98**, 830.
- 5 S. Shinkai, N. Honda, Y. Ishikawa, and O. Manabe, *Chem. Lett.*, 1984, 327.
- 6 T. C. Bruice, *Prog. Bio-org. Chem.*, 1976, **4**, 1.
- 7 I. Yokoe and T. C. Bruice, *J. Am. Chem. Soc.*, 1975, **97**, 450; E. L. Loechler and T. C. Hollocher, *ibid.*, 1980, **102**, 7312, 7322, 7328.
- 8 Y. Yano, N. Nakazato, and E. Ohya, *J. Chem. Soc., Perkin Trans. 2*, in the press.
- 9 M. F. Powell, W. H. Wong, and T. C. Bruice, *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 4604, and references cited therein.