Oxidation-active Flavin Mimics. Chemical and Redox Properties of 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*)-Tetraone (Benzo-Dipteridine)[1]

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The chemical and redox properties of benzo-dipteridine (BDP), an oxidation-active flavin mimic, were investigated. It was found that (i) BDP undergoes 2e-redox reaction under conventional conditions, (ii) pK_a-values of 2e-reduced BDP (BDP_{red}) are 3.6 and 8.5, respectively, (iii) a mixture of BDP_{ox} and BDP_{red} forms a charge-transfer complex in aqueous solution, (iv) BDP_{ox} exhibits a remarkably high oxidation activity (*ca.* 10^7-fold) toward reactions involving nucleophilic attack at the C(4a)-position compared with 3,10-dimethylisoalloxazine (DMI), and (v) the oxidation of an NADH model by BDP_{ox} is acid-catalysed. As a new model reaction, BDP_{ox} was found to oxidize sulphite ion *via* a BDP–SO_3^- adduct, which decomposes unimolecularly to give $BDPH^-$ and $[SO_3]$ in aqueous solution. This is the first example of an APS (adenosine 5'-phosphosulphate) reductase model.

Flavin coenzymes exhibit diverse functions such as electron transfer, dehydrogenation, and activation of molecular oxygen in biological systems.[2] For instance, flavoenzymes are able to oxidize various substrates such as amines, α-amino acids, α-hydroxy acids, alcohols, dithiols, nitroalkanes, *etc.*[3] In model systems, however, the substrates oxidized by the conventional flavin models so far synthesized are very limited probably due to their lack of oxidizing power. Thus, for construction of artificial flavoenzymes, we thought that a first step would be exploitation of oxidation-active flavin mimics. Such flavin mimics would be quite useful not only for mechanistic studies of the oxidations mediated by flavin but also for exploitation of new model systems. From this point of view, we have continued to search for oxidation-active flavin mimics.

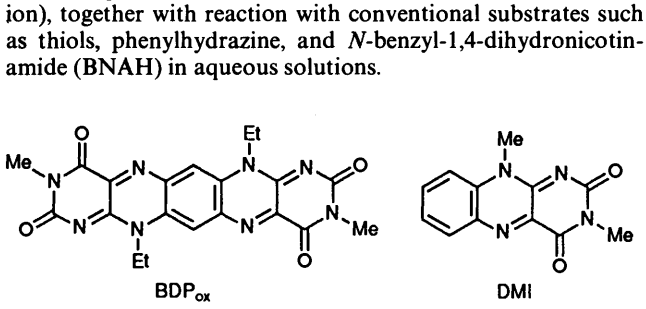
Electron-deficient flavins such as 8-cyano-[4] and 7,8-dichloroflavins[5] are known to show high oxidizing activity. However, the substituent at the 6-position of the isoalloxazine ring, which is a *peri*-position from N(5), occasionally reduces the reactivity owing to steric hindrance.[6] To avoid this disadvantage, we employed the electron-withdrawing nature of ring nitrogen atoms,[7] and found that 8-aza-,[8] 6,8-diaza-,[9] and 6,8,9-triazaflavins[10] show remarkably high oxidizing activity. For example, the oxidation of thiols is facilitated by factors of *ca.* 10^6 for 8-azaflavin, *ca.* 10^8 for 6,8-diazaflavin, and *ca.* 10^{10} for 6,8,9-triazaflavin compared with the corresponding non-azaflavin. However, the reaction conditions for the 6,8-diaza- and 6,8,9-triazaflavins are considerably restricted. The former undergoes reversible covalent hydration in acidic and basic aqueous solutions to lose its oxidizing capability and the latter cannot be used even in a neutral solution due to hydration.[10] Thus we decided to exploit stable oxidation-active flavin model compounds. Meanwhile we had shown that the oxidizing reactivity of 5-deazaflavin is considerably increased by connecting two 5-deazaflavin molecules or a 5-deazaflavin and a flavin, by fusion to a common benzo moiety due to the long conjugative system.[11] Thus we have synthesized benzo-dipteridine (BDP_{ox}) possessing two isoalloxazine rings fused to a common benzo moiety, in which all π-electrons are able to conjugate together, and we have reported hydrolysis products of BDP_{ox} under strongly alkaline conditions.[12]

In this paper, we report chemical and redox properties of BDP_{ox} in comparison with those of DMI and also describe the first example of an APS reductase model (oxidation of sulphite ion), together with reaction with conventional substrates such as thiols, phenylhydrazine, and *N*-benzyl-1,4-dihydronicotinamide (BNAH) in aqueous solutions.

Table 1. Absorption maxima and molar extinction coefficients of BDP_{ox} and DMI at 25 °C

Flavin	Solvent	λ_{max}/nm (log ε, $dm^3\ mol^{-1}\ cm^{-1}$)
BDP_{ox}	Water[a]	390 (4.30), 548 (4.17)
	EtOH	372 (4.29), 545 (4.20)
	MeCN	369 (4.30), 548 (4.23)
DMI	Water[a]	343 (3.88), 434 (3.97)
	EtOH	333 (3.90), 437 (3.98)
	MeCN	329 (3.87), 437 (3.87)

[a] pH 7.1 (0.1 mol dm^{-3} phosphate, μ 0.3 with KCl).



BDP_{ox} DMI

Results and Discussion

BDP_{ox} was synthesized as reported previously.[12]

Absorption Spectra.—A conventional isoalloxazine derivative such as DMI is known to have two characteristic absorptions at *ca.* 340 and 440 nm for the oxidized form. The shorter-wavelength absorption band is known to be sensitive to solvent polarity; it shifts to shorter wavelength in a hydrophobic environment.[13]

The absorption maxima and the molar absorption co-

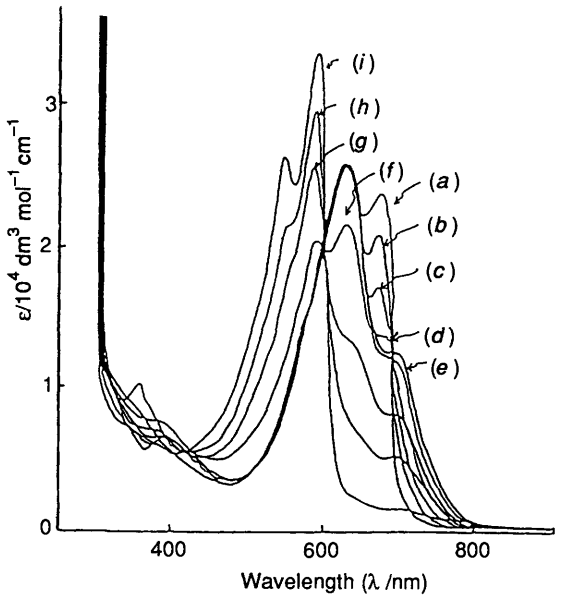


Fig. 1. Absorption spectra of BDP_{red} produced by DTT reduction, N_2, 25 °C; [BDP_{ox}] 1.0 × 10^{-5} mol dm^{-3}, [DTT] 1.00 × 10^{-3}. pH (*a*) 1.84; (*b*) 2.57; (*c*) 3.21; (*d*) 3.92; (*e*) 6.90; (*f*) 7.92; (*g*) 8.54; (*h*) 9.01; (*i*) 9.80

efficients of BDP_{ox} in water, EtOH, and MeCN are shown in Table 1, together with those of DMI. The Table shows that the absorption maxima of BDP_{ox} appear at much longer wavelengths than those of DMI, due to the long conjugative system, and the shorter-wavelength absorption maximum (390 nm) shifts to even shorter wavelength in less polar solvents such as EtOH and MeCN, as observed for DMI.

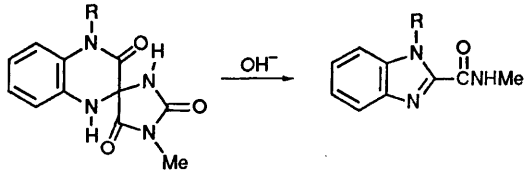
We have previously shown that BDP_{ox} undergoes two electrochemical 2e-redox reactions ($E^{o\prime}$ −120 and −770 mV),[14] indicating that the second 2e-redox reaction would not occur on using conventional reducing reagents. Spectroscopic examination showed that BDP_{ox} reacts with various reducing reagents such as thiols, phenylhydrazine, and BNAH to give a spectrum with λ_{max} 620 nm at pH 7.0 under anaerobic conditions. Ethylenediaminetetracetic acid (EDTA) photoreduction of BDP_{ox} also gave the same spectrum. Introduction of O_2 into the solution regenerated (quantitatively) the starting absorption spectrum. Thus the absorption of λ_{max} 620 nm is that of 2e-reduced BDP ($BDPH^-$). For reduction by $Na_2S_2O_4$, $BDPH^-$ was found to be formed *via* an intermediate, since the absorption spectrum of BDP_{ox} changed immediately to one with λ_{max} 502 nm on addition of $Na_2S_2O_4$, and this absorption peak gradually changed to one with λ_{max} 620 nm. Similar spectral changes were also observed for the reaction of BDP_{ox} with K_2SO_3, suggesting the oxidation of sulphite ion. This will be described in detail later.

pK_a-Value of $BDPH_2$.—The pK_a-values of N(1)–H of reduced flavins are one criterion of their oxidizing power.[14] BDP_{ox} was found to be rapidly reduced by dithiothreitol (DTT) to give BDP_{red} ($BDPH_2$) in aqueous solutions over a wide pH range (1.84–9.80) under anaerobic conditions, indicating that the pK_a-values of $BDPH_2$ are best determined spectrophotometrically. The absorption spectra at various pH-values indicate that there are three species in solution depending on the pH (Fig. 1). Plots of A_{720} *vs.* pH (1.84–6.46) and A_{620} *vs.* pH (6.46–9.80) gave typical titration curves of proton ionization, which allowed us to calculate pK_a-values of 3.6 and 8.5, respectively. The much lower pK_a-value (3.6) of $BDPH_2$ compared with that of the reduced DMI (pK_a 6.7)[3] is accounted for by stabilization of the conjugated base ($BDPH^-$) due to electron delocalization through the long conjugative system. Furthermore, the λ_{max} of BDP_{red} at the longer wavelength compared with that of BDP_{ox} implies that BDP_{red} ($BDPH_2$ + $BDPH^-$) takes a longer conjugative 1,8-dihydro form rather than a 1,5-dihydro one (Scheme 1).

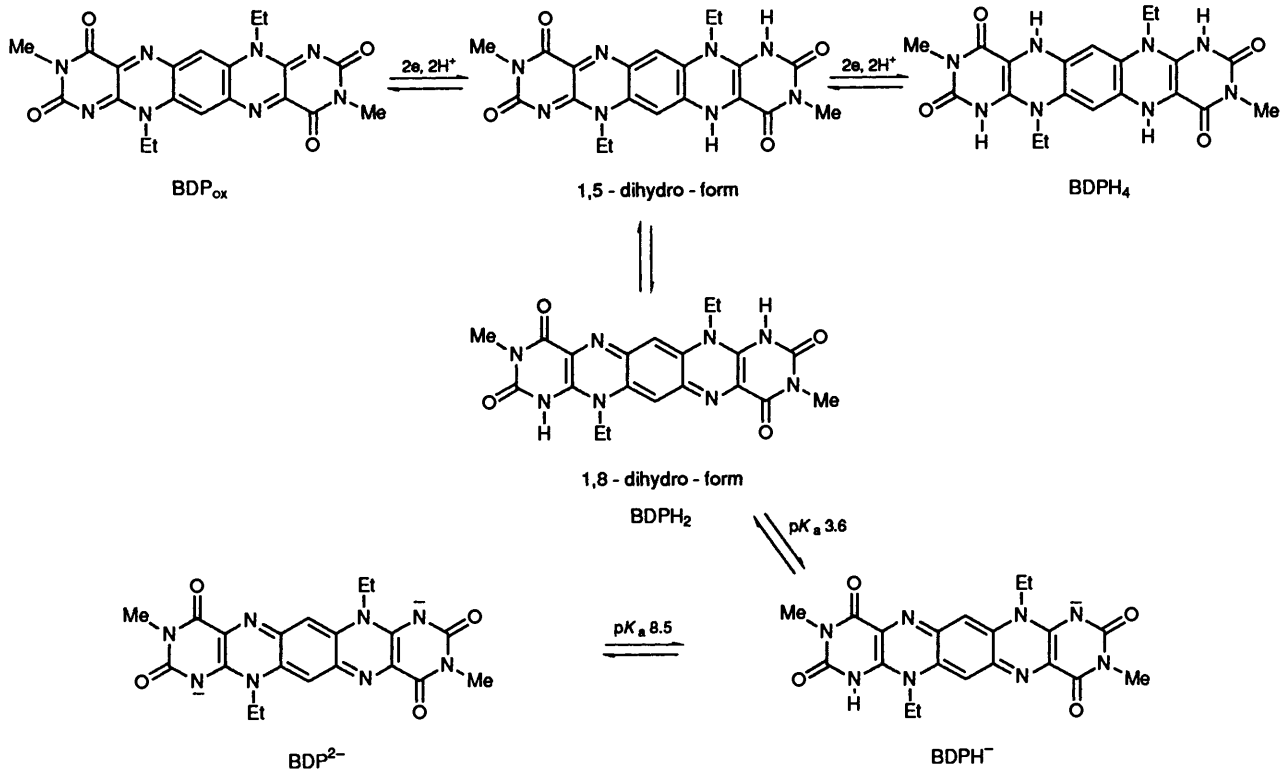
Charge-transfer(CT) Complex between BDP_{ox} *and* BDP_{red}.—A mixture of flavin mononucleotide (FMN) and $FMNH_2$ is known to form a CT complex (700–900 nm) at relatively high concentration (4.0 × 10^{-3} mol dm^{-3}) in aqueous solution.[15] We have also shown that a half-reduced bis-flavin linked by a trimethylene group at the 10,10′-positions of the isoalloxazine rings forms an intramolecular CT complex at relatively low concentration (1.7 × 10^{-5} mol dm^{-3}) in aqueous solution, and that the oxidation activity decreases due to CT-complex formation.[16] Thus formation of a CT-complex between BDP_{ox} and BDP_{red} was examined prior to examination of the oxidizing activity. In particular, since both BDP_{ox} and BDP_{red} are expected to be planar molecules due to the long conjugative systems, the CT-complex should be formed in lower concentrations.

Detection of the CT complex was performed spectrophotometrically by employing EDTA-photoreduction,[15] since progress of the reduction of BDP_{ox} can be controlled by switching on and off of the light. Time-courses of the spectral changes of BDP_{ox} (1.0 × 10^{-5} mol dm^{-3}) by EDTA-photoreduction at pH 8.0 are shown in Fig. 2, which shows clearly that a new absorption band at 860 nm appears, develops with progress of the reduction to reach a maximum at *ca.* 50% reduction, and disappears with further reduction. Thus the absorption band at 860 nm (ε 3000 dm^3 mol^{-1} cm^{-1}) could be ascribed to the CT complex between BDP_{ox} and BDP_{red}. A plot of the maximum absorption at 860 nm at various pH-values gave a sigmoid curve which reached maximum absorption above pH *ca.* 8.5, indicating that the CT-complex formation is most favourable between BDP_{ox} and the dianion form of BDP_{red} (BDP^{2-}) (Fig. 3).

Hydrolysis.—It is established that hydrolysis of an electron-deficient flavin such as 7- or 8-cyanoflavin proceeds *via* OH^- attack at the 10a-position of the isoalloxazine ring to give the corresponding 4a-spirohydantoin.[4b] We have already reported hydrolysis products of BDP_{ox} in the reaction with Triton B (40% in MeOH) in dimethylformamide (DMF) at 120–130 °C for 50 h.[12] The isolated products are the corresponding 4a-spirohydantoin and benzimidazole-2-carboxamide derivatives. We have also reported that a 4a-spirohydantoin derivative gives the corresponding benzimidazole-2-carboxamide after one hour at 125 °C as shown below.[17]



Prior to examination of the oxidizing activity, the stability of BDP_{ox} was kinetically studied. Pseudo-first-order rate constants were determined by following the absorption decrease of BDP_{ox} at 390 nm under anaerobic conditions at 25 °C. A plot of log k_{obs} *vs.* pH gave a straight line of unit slope as shown in Fig. 4. The second-order rate constant (k_{OH^-}) is 8.9 × 10^2 dm^3 mol^{-1} min^{-1} for BDP_{ox}, 10^3-times larger than that of DMI (k_{OH^-} 8.4 × 10^{-1} dm^3 mol^{-1} min^{-1}). The hydrolysis product (λ_{max} 505 nm) was found still to possess some oxidizing activity toward BNAH under anaerobic conditions, and introduction of O_2 into the solution regenerated quantitatively the spectrum



Scheme 1.

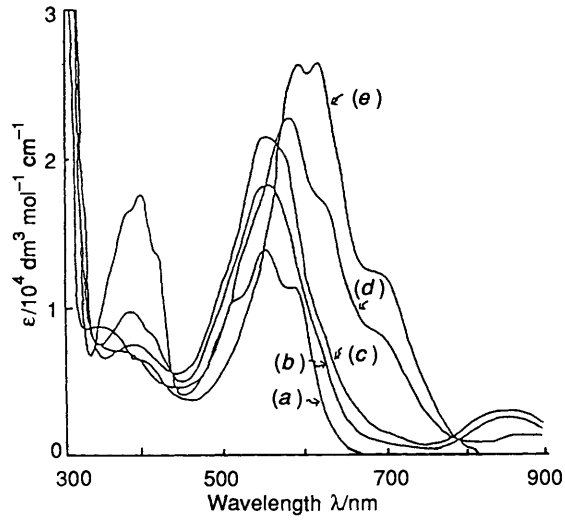


Fig. 2. Time-course of spectral changes of BDP_{ox} by EDTA-photoreduction (60 W tungsten lamp, 15 cm) at pH 8.0; [BDP_{ox}] 1.0×10^{-5} mol dm^{-3}, [EDTA] 1.00×10^{-4} mol dm^{-3}, N_2, 25 °C; irradiation interval: (*a*) 0 min; (*b*) 0.17 min; (*c*) 0.5 min; (*d*) 6 min; (*e*) 30 min

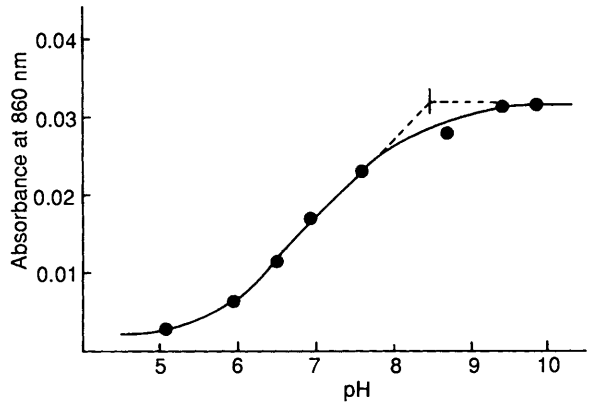
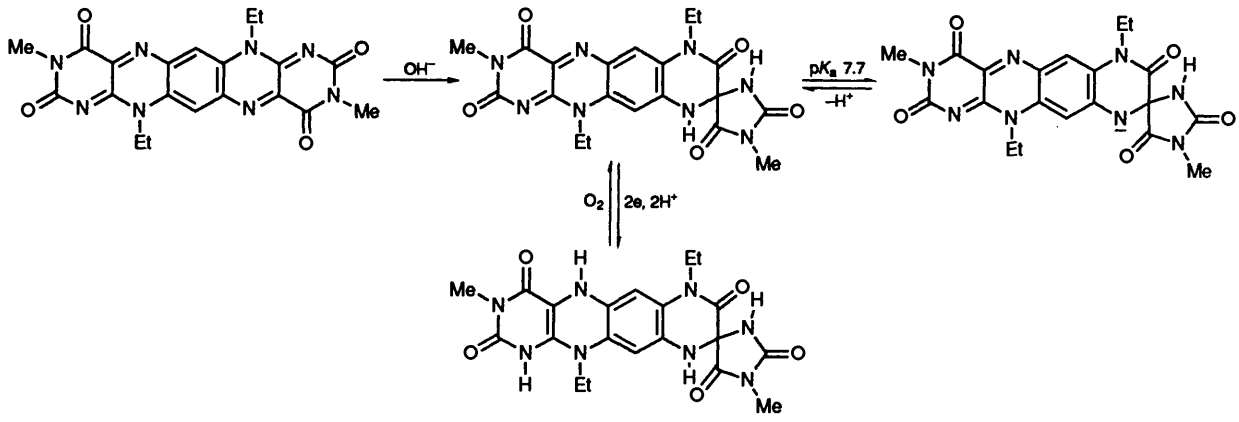


Fig. 3. Maximum formation of CT complex (860 nm) between BDP_{ox} and BDP_{red} during EDTA-photoreduction at various pH-values; [BDP_{ox}] 1.0×10^{-5} mol dm^{-3}, [EDTA] 1.00×10^{-4} mol dm^{-3}, N_2, 25 °C

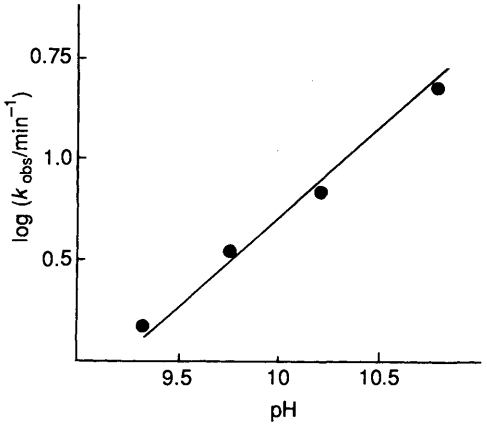
with λ_{max} 505 nm, indicating that an isoalloxazine skeleton is still present in the hydrolysis product. Furthermore it was found that the absorption spectrum (λ_{max} 505 nm; ε 2.7×10^4 dm^3 mol^{-1} cm^{-1}) shifts to one with λ_{max} 474 nm (ε 1.6×10^4 dm^3 mol^{-1} cm^{-1}), showing an isosbestic point, upon lowering of the pH (6.12–10.0), and that the spectral change is reversible upon change of pH. A plot of A_{500} *vs.* pH gave a titration curve to provide pK_a (7.7), indicating that an ionizable hydrogen is involved in the hydrolysis product. These spectral changes were consistent with those of the authentic 4a-spirohydantoin derivative as shown in Scheme 2. Meanwhile spectroscopic examination showed that BDP_{red} is stable at pH 10.0 for 48 h (25 °C).

Oxidation Activity.—The oxidizing reactivity of BDP_{ox} was examined kinetically by employing conventional substrates such as thiols, phenylhydrazine, and BNAH in aqueous solutions under anaerobic conditions, and comparing the results with those for DMI. Pseudo-first-order rate constants were determined by following the absorption increase at 620 nm for BDP_{ox} and the absorption decrease at 440 nm for DMI unless otherwise stated. For all the substrates, the rates followed first-order kinetics up to >2 half-lives below pH *ca.* 7. Above pH 7, however, the first-order plots showed downward curvature after 50% reaction, due to the CT-complex. Thus the rate constants were determined from the initial slopes of the first-order plots.

Owing to the large rate difference of BDP_{ox} and DMI, the



Scheme 2.



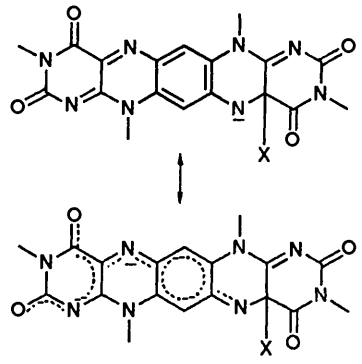


Fig. 4. Plot of k_{obs} *vs.* pH for hydrolysis of BDP_{ox}; [BDP_{ox}] 1.00 × 10^{-5} mol dm^{-3}, 0.1 mol dm^{-3} buffer (μ = 0.3), N_2, 25 °C

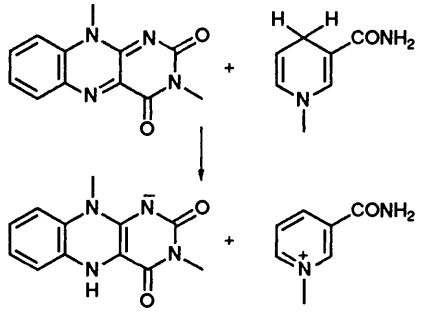
Table 2. Pseudo-first-order rate constants and relative rates for the oxidation of ME, DTT, and $PhNHNH_2$[a]

Substrate	pH[b]	k_{obs}/min^{-1} BDP$_{ox}$	DMI	Rel rate[c]
ME	6.73	0.735[d]	3.79 × 10^{-2}[e]	1.9 × 10^7
DTT	4.96	6.14[f]	4.4 × 10^{-4}[g]	1.4 × 10^6
$PhNHNH_2$	8.59	2.11[h]	2.3 × 10^{-4}[i]	1.0 × 10^7

[a] [BDP_{ox}] 1.0 × 10^{-5} mol dm^{-3}, [DMI] 5.0 × 10^{-5} mol dm^{-3}, N_2, 25 °C. [b] [Buffer] 0.1 mol dm^{-3} (μ 0.3). [c] Relative rates were obtained from k_{obs}/[ME]^2, k_{obs}/[DTT] and k_{obs}/[$PhNHNH_2$]. [d] [ME] 1.00 × 10^{-2} mol dm^{-3}. [e] [ME] 1.00 mol dm^{-3}. [f] [DTT] 1.00 × 10^{-4} mol dm^{-3}. [g] [DTT] 1.00 × 10^{-2} mol dm^{-3}. [h] [$PhNHNH_2$] 1.00 × 10^{-4} mol dm^{-3}. [i] [$PhNHNH_2$] 1.00 × 10^{-1} mol dm^{-3}.

concentrations of the substrates were changed for estimation of the relative rates at the same pH-value. For this, it was confirmed that the rates were second order with respect to [2-mercaptoethanol (ME)], first order with respect to [DTT], and first order with respect to [$PhNHNH_2$], respectively. As shown in Table 2, BDP_{ox} is much more reactive than DMI by a factor of more than 10^7 for oxidation of ME and of $PhNHNH_2$. In addition the oxidations of thiols[4–6] and $PhNHNH_2$[6] by flavin models have been well investigated, and these oxidations are considered to proceed *via* nucleophilic attack at the C(4a)-position of the isoalloxazine rings. Thus the large rate enhancement could be accounted for by stabilization of the transition states, in which a negative charge developed on the N(5)-position by nucleophilic attack of the substrates at the C(4a)-position is delocalized as shown below.

The oxidation of BNAH by flavin is known to proceed *via* a hydride (or its equivalent) transfer to the N(5)-position of an isoalloxazine ring.[18] The mechanism is different from those involved in the oxidation of thiols or $PhNHNH_2$. The rates of the oxidation of BNAH by BDP_{ox} were followed by monitoring of the absorption increase at 580 nm at pH > 8.5. During estimation of relative rates, the rates were found to be pH-dependent. Since a negative charge is produced on the N(1)-position as shown below, the reaction should be facilitated by



acid. Although the oxidation of NADH models by flavins has been extensively investigated,[18] the effect of acid has not been examined because of the instability of NADH models toward acid.[19] The rate constants for acid decomposition of BNAH were determined by following the absorption decrease of BNAH at 350 nm under anaerobic conditions; 5.2 × 10^{-2} min^{-1} at pH 5.93, and 3.6 × 10^{-2} min^{-1} at pH 6.48. These rate constants are much smaller than those for the oxidation by BDP_{ox}. Thus the rate constants for the oxidation by BDP_{ox} at pH *ca.* 6 can be

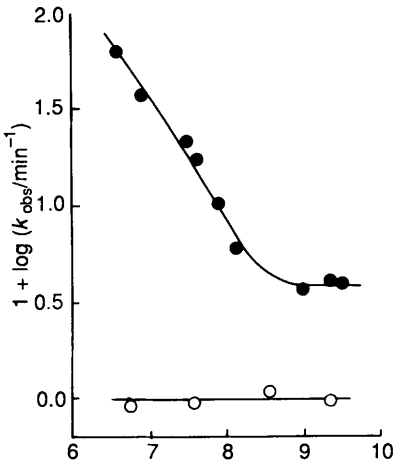


Fig. 5. pH–Rate profiles for the oxidations of BNAH by BDP_{ox} and DMI; $[BDP_{ox}]$ $[DMI] = 1.0 \times 10^{-5}$ mol dm^{-3}, [BNAH] 1.00×10^{-4} mol dm^{-3}, N_2, 25 °C; ●, BDP_{ox}; ○, DMI

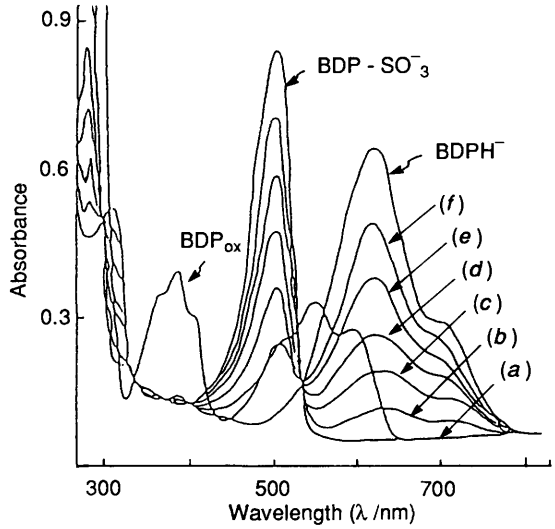


Fig. 6. Time-course of the spectral changes of the reactions of BDP_{ox} with HSO_3^-; $[BDP_{ox}]$ 2.0×10^{-5} mol dm^{-3}, $[NaHSO_3]$ 2.00×10^{-3} mol dm^{-3} pH 5.8, N_2, 25 °C; (*a*) 0; (*b*) 30; (*c*) 90; (*d*) 210; (*e*) 360; (*f*) 720 min.

determined without disturbance by the acid decomposition of BNAH. The pH–rate profiles for BDP_{ox} and DMI are shown in Fig. 5 which indicates that acid catalysis for BDP_{ox} begins to appear below the pH corresponding to the pK_a-value of the product ($BDPH^- \rightleftharpoons BDP^{2-} + H^+$, p$K_a$ 8.5), whereas no acid catalysis was observed for DMI. The pK_a-value of N(1)–H of the conventional reduced flavin models is usually <7.[3,4b] Thus, we presume that the higher pK_a-value (8.5) of $BDPH^-$ made it possible to detect any acid catalysis. The pK_a-value of the product is an important factor in the operation of acid catalysis.[20] It has been reported that acid catalysis is detected for the reduction of carbonyl[21] and nitroso[22] groups by acid-stable NADH models. This is, to our knowledge, the first example of the demonstration of acid catalysis in the oxidation of NADH models by flavins. It should be noted that the rate enhancement of the oxidation of BNAH by BDP_{ox} is rather small compared with those of thiols and $PhNHNH_2$. This indicates that BDP_{ox} could be regarded as a flavin model which accelerates specifically those oxidations proceeding *via* C(4a)-attack by stabilizing a negative charge generated on the N(5)-position due to the long conjugative system.

Since we have a remarkably highly oxidation-active flavin mimic in hand, the next subject for study is exploitation of new flavin model systems. As one new model system, we have already reported the oxidative dealkylation of *N*-nitrosamine derivatives by BDP_{ox} in aqueous acetonitrile,[23] which is a model for metabolic activation of *N*-nitrosamines.

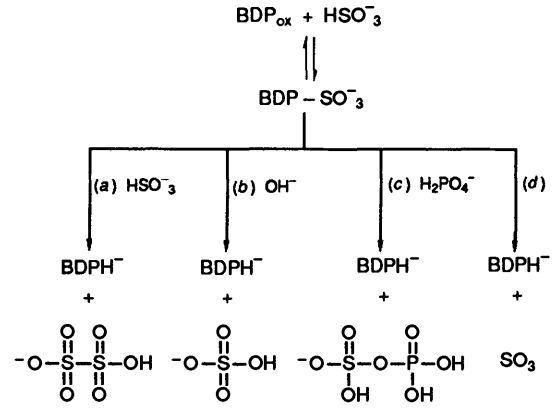
Oxidation of Sulphite Ion.—An APS reductase is a flavoprotein containing FAD and non-haeme iron as prosthetic groups, and catalyses the reaction of adenosine monophosphate (AMP) + $SO_3^{2-} \rightleftharpoons$ APS. The mechanism of formation of APS (adenosine 5′-phosphosulphate), which is a key intermediate in microbial sulphur metabolism, has been said to involve a flavin adenine dinucleotide (FAD)–SO_3^- adduct followed by sulphite transfer to AMP to yield APS and reduced FAD,[24] in which oxidation of sulphite ion by FAD is involved. Thus, for model studies of this enzyme, it would be of primary importance to find a system with flavin mimic which is able to oxidize sulphite ion. However, there has been no precedent in the literature that a reduced flavin is produced in the reaction of sulphite ion with a flavin model compound, although formation of flavin–sulphite adducts is reported in flavin models[25] and flavoprotein oxidases.[26]

The reaction of BDP_{ox} with sulphite ion ($HSO_3^- + SO_3^{2-}$)* was examined spectrophotometrically in aqueous solution under anaerobic conditions. The time-course of the spectral changes of the reaction is shown in Fig. 6, which indicates that BDP_{ox} (390 and 548 nm, reddish) reacts with HSO_3^- immediately to give a BDP–SO_3^- adduct (λ_{max} 502 nm, pale yellow), which decays gradually to $BDPH^-$ (λ_{max} 620 nm, blue) with tight isosbestic points. After the reaction, introduction of O_2 into the solution regenerated the absorption peak at 502 nm quantitatively, indicating that BDP_{ox} regenerated from $BDPH^-$ by O_2 reacts instantly with excess of HSO_3^- to give the adduct. Incidentally, addition of H_2O_2 (30%) to consume the excess of HSO_3^- gave quantitatively the starting spectrum of BDP_{ox}. Further addition of HSO_3^- to this solution showed the same spectral changes. Even after these procedures had been repeated several times, only BDP_{ox} was detected on TLC [silica gel, R_f 0.36 (EtOH) and 0.45 (HCO_2H)]. This clearly indicates that the redox reaction occurs without any side-reaction such as ring substitution. Next, the reaction was kinetically examined to determine the oxidation mechanism. The rate of adduct formation was too fast to be measured by our kinetic method. Instead the dissociation constant (K_d) of the adduct was determined spectrophotometrically by changing $[HSO_3^-]$. The value of K_d found was 1.6×10^{-5} mol dm^{-3} (pH 6.1; 25 °C), which is smaller than that of 7,8-dimethyl-1,10-ethyleneisoalloxazinium ion (K_d 1.3×10^{-4} mol dm^{-3} at pH 7.0).[25a] This readily occurring adduct formation suggests that the reaction site is the C(4a)-position of BDP_{ox}, although the structure of the adduct could not be determined directly owing to our failure to isolate it.

First-order rate constants for formation of $BDPH^-$ from BDP–SO_3^- were determined by following the absorption increase of $BDPH^-$ at 620 nm or the absorption decrease of the adduct at 502 nm under anaerobic conditions. The rates followed first-order kinetics up to more than two half-lives at pH < 6.5. However, the rates did not follow first-order kinetics with increasing pH, and the accompanying spectral changes did not show isosbestic points. Thus, the rate measurements were performed at pH < 6.5.

For the formation of $BDPH^-$ from the adduct, the following four mechanisms are conceivable (Scheme 3). The mechanisms

* $HSO_3^- \rightleftharpoons H^+ + SO_3^{2-}$, p$K_a$ 6.7 [ref. 25(*b*)].



Scheme 3.

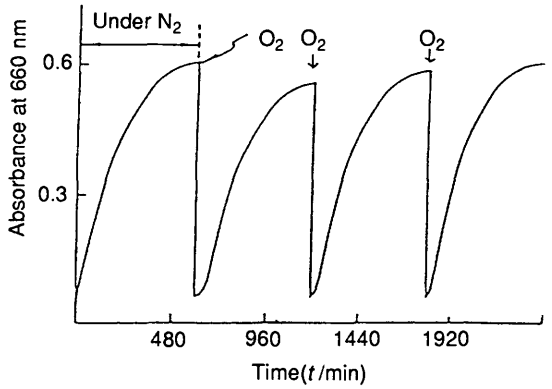


Fig. 7. Absorption changes of $BDPH^-$ (660 nm) by introduction of O_2 in the reaction of $NaHSO_3$ in EtOH; [BDP_{ox}] 2.0 × 10^{-5} mol dm^{-3}, [$NaHSO_3$] 2.00 × 10^{-3} mol dm^{-3}, 25 °C

can be easily distinguished by kinetics. Namely, the rates are dependent on [HSO_3^-] for path (*a*), [OH^-] for path (*b*), [buffer] for path (*c*), and are independent of all of these species for path (*d*). The rates were found to be independent of pH over the range 3.6–6.5 (k_{obs} 4.80 ± 0.32 × 10^{-4} min^{-1}) and [HSO_3^-] over the range 1.0 × 10^{-4}–1.0 × 10^{-3} mol dm^{-3} (k_{obs} 4.82 ± 0.08 × 10^{-4} min^{-1} at pH 5.80). It was also confirmed that the rates were not affected by buffer species ([acetate] 0.05–0.4 mol dm^{-3} at pH 4.9, and [phosphate] 0.05–0.2 mol dm^{-3} at pH 5.8). These kinetic results undoubtedly indicate that $BDP–SO_3^-$ decomposes *via* path (*d*). However, it is unclear whether or not the unimolecular decomposition proceeds *via* free sulphur trioxide.[27]

Detection of the oxidation product of sulphite ion is the next problem to be solved in order to determine the oxidation mechanism. Since detection of sulphate ion formed in water is fairly difficult in the presence of a large excess of sulphite ion, we chose EtOH as the solvent in which the oxidation product of sulphite ion is trapped by EtOH to give $EtOSO_3^-$. Meanwhile the spectral changes in the reaction of BDP_{ox} with $NaHSO_3$ in EtOH were found to be similar to those in aqueous solution (pH < 6.5). It was also confirmed that BDP_{ox} acts as a turnover catalyst in EtOH under aerobic conditions (Fig. 7). Thus product analysis was performed in EtOH under aerobic conditions to accumulate $EtOSO_3^-$. $EtOSO_3Na$ was successfully isolated by column chromatography (Experimental section).* A control experiment without BDP_{ox} showed no formation of $EtOSO_3Na$. From all the data obtained and the specific enhancement of BDP_{ox} for oxidations proceeding *via* C(4a)-adduct formation, we propose the reaction Scheme 4. At any rate, to the best of our knowledge, it is the first example of a flavin mimic oxidizing sulphite ion *via* an adduct to give a reduced flavin.

In conclusion, benzo-dipteridine is considered to be a unique flavin mimic which possesses an extremely high oxidizing activity for reactions proceeding *via* C(4a)-attack. Acid catalysis was observed for the first time in the oxidation of BNAH by BDP_{ox} due to the higher pK_a-value of $BDPH^-$. As a new model reaction, the oxidation of sulphite ion is described. We believe that this unique flavin mimic, BDP_{ox}, opens up new flavin chemistry in model systems.

Experimental

Absorption spectra were recorded on a Hitachi UV-200 spectrophotometer. The pH-values were measured with a Hitachi-Horiba F-7DE pH meter. Mass spectra were taken on a Shimazu QP-1000 instrument. ^1H NMR spectra (200 MHz) were obtained with a Nippon Electronics FX-200 Gemini-200 instrument. IR spectra were recorded on a Hitachi 270–50 instrument.

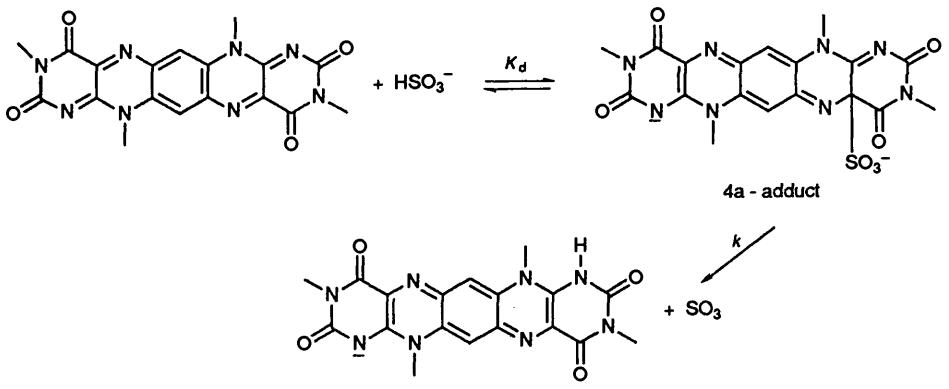
Materials.—Substrates (ME, DTT, and $PhNHNH_2$) were purified by distillation under N_2. K_2SO_3 and $NaHSO_3$ (Wako Chemical Co.) were used without further purification. DMI and BNAH were supplied from our previous study.[15] BDP_{ox} was synthesized as described in our previous paper,[12] except for the final step. Namely, deoxygenation of benzo-dipteridine di-*N*-oxide (0.5 g, 1 mmol) was conducted by stirring of the mixture with $Na_2S_2O_4$ (1.1 g, 6 mmol) in water (2 cm^3) for 3 h at room temperature, and the mixture was stirred overnight after addition of 30% H_2O_2 (0.5 cm^3). The crystals which formed were collected, washed with water, and recrystallized from DMF to give violet crystals (0.2 g, 40%), m.p. > 300 °C; δ_H (200 MHz; $CDCl_3$) 1.50 (t, 6 H, Me), 3.55 (s, 6 H, NMe), 4.75 (q, 4 H, CH_2) and 8.55 (s, 2 H, ArH); *m/z* (20 eV) 434 (M^+) (Found: C, 54.2; H, 4.95; N, 24.6. $C_{20}H_{18}N_8O_4$·DMF requires C, 54.43; H, 4.96; N, 24.84%).

Redox potentials were determined by cyclic voltammetry as described previously.[8c]

CT-Complex between BDP_{ox} *and* BDP_{red}.—A typical run (BDP_{ox}:BDP_{red} 1:1) was as follows. In a Thunberg cuvette, a solution of BDP_{ox} in dimethylacetamide (DMAC) (1.0 × 10^{-3} mol dm^{-3}; 15 mm^3) was placed in the cell part with a solution of EDTA in water (1.0 × 10^{-2} mol dm^{-3}; 20 mm^3) and buffer (3 cm^3), and an aliquot (15 mm^3) of BDP_{ox} solution was placed in the upper counterpart. Both the solutions were bubbled for 20 min with N_2 which had been scrubbed with aq. vanadium(II) ion and prehumidified with water. BDP_{ox} in the cell part was photoreduced by use of a 60 W tungsten lamp. After mixing of both the solutions, the absorption spectra were recorded.

Rate Measurements.—Kinetic measurements were performed similarly to those described previously.[8c] In a Thunberg cuvette, an aliquot (30 mm^3) of flavin stock solution (1.0 × 10^{-3} mol dm^{-3} in DMAC) was mixed with buffer solution (3 cm^3), and a suitable amount of the substrate (usually 30 cm^3 of 0.2 mol dm^{-3} Me in MeCN, 1.0 × 10^{-2} mol dm^{-3} of DDT or BNAH in EtOH) was placed in the upper part of the cuvette. Both the solutions were bubbled for 20 min with vanadium(II) ion-scrubbed N_2. After temperature equilibration at 25 °C for 15 min, the reaction was initiated by mixing of both the solutions, and the absorption increase of BDP_{red} at 620 nm or the absorption decrease of BDP_{ox} at 390 nm was followed. The following buffers (0.1 mol dm^{-3}) (μ 0.3 with KCl) were employed

* It was confirmed beforehand that $EtOSO_3Na$ can be recovered from excess of $NaHSO_3$ by column chromatography (silica gel; EtOH).



Scheme 4.

to maintain constant pH: acetate for pH 5.5–7.5, borate for 7.5–9.0, and carbonate for pH > 9.0.

Product Analysis for the Oxidation of Sulphite Ion by BDP_{ox} *in* EtOH.—A heterogeneous mixture of BDP_{ox} (13 mg, 0.03 mmol) and $NaHSO_3$ (650 mg, 6 mmol) in EtOH (300 cm^3) was stirred for 20 days in the dark at room temperature under aerobic conditions. After filtration of excess of $NaHSO_3$, EtOH was evaporated off and the remaining materials were washed well with $CHCl_3$ to remove BDP_{ox}, and subjected to column chromatography (silica gel; EtOH) to give $EtOSO_3Na$ (150 mg, 3400% based on BDP_{ox}). ^1H NMR and IR spectra of $EtOSO_3Na$ thus obtained were completely consistent with those of an authentic sample: δ_H(90 MHz; D_2O) 1.30 (t, 3 H, Me) and 4.13 (q, 2 H, CH_2); and ν_{max}(KBr)/cm^{-1} 1070 and 1228.

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