High Oxidizing Activity of 3,10-Dimethyl-8-azaisoalloxazine (8-Azaflavin) and its Synthetic Application as a Turnover Redox Catalyst under Aerobic Conditions[1]

Yumihiko Yano,* Masashi Ohshima, Isao Yatsu, and Susumu Sutoh
Department of Chemistry, Gunma University, Kiryu, Gunma 376, Japan
Rafael E. Vasquez, Akira Kitani, and Kazuo Sasaki
Department of Applied Chemistry, Hiroshima University, Saijo, Higashi-Hiroshima 724, Japan

8-Azaflavin (**1**) has been synthesized, and its oxidizing activity has been examined kinetically for the oxidation of *N*-benzyl-1,4-dihydronicotinamide (BNAH), thiols, and nitroalkanes in aqueous solution under anaerobic conditions, by means of a comparison with 3,10-dimethylisoalloxazine (**2**). It was found that (**1**) is much more reactive than (**2**), 41-fold for BNAH, and 10^4—10^6-fold for thiols; (**1**) can be used as a turnover oxidation–reduction catalyst for disulphide and aldehyde syntheses in aqueous solution under aerobic conditions. The hydrolysis of (**1**) and the reducing activity of the reduced (**1**) have been briefly examined.

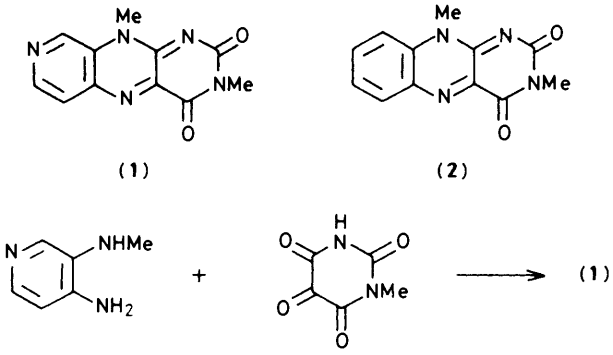
Flavin coenzymes are known to oxidize a variety of substrates such as amino acids, lactic acid, succinic acid, thiols, nitroethane, and so on in biological systems.[2] In model systems, however, the substrates which are oxidized by flavin model compounds are very limited. This may be simply because the oxidizing power of conventional flavin models is quite weak in aqueous solution. Therefore, it is of primary importance to improve the oxidizing power of flavin model compounds not only for mechanistic studies of flavin oxidation, but also for application to organic syntheses as turnover oxidation catalysts under aerobic conditions.

Recently, we have shown that the Hammett ρ values at 8-substituted flavins are relatively large for the oxidation of butane-1,4-dithiol (ρ +5.2) and phenylhydrazine (ρ +4.9) in EtOH, and the substituent at the 6-position of the isoalloxazine ring shows steric hindrance in some reactions.[3] This indicates that the introduction of an electron-withdrawing group at the 8-position increases the oxidizing activity. In fact, 3,10-dimethyl-8-cyanoisoalloxazine (8-cyanoflavin) has been known to oxidize thiophenol and nitroalkanes in aqueous solution, whereas conventional flavins such as (**2**) are unable to oxidize these substrates.[4] However, there is a limitation in the methodology for improving the oxidizing power by employing electron-withdrawing groups in the isoalloxazine ring, since nitro[5] and carbonyl groups[6] are known to be reduced by the reduced flavin, and a halogen at the 8-position is known to undergo nucleophilic substitution.[7] Keeping this in mind, we have employed a ring nitrogen at the 8-position, since it is known to be fairly electron withdrawing.[8]

In this paper, we describe kinetic studies of the oxidation of BNAH, thiols, and nitroalkanes by (**1**), and syntheses of disulphides from thiols and aldehydes from primary nitroalkanes by employing (**1**) as an oxidation–reduction catalyst under aqueous and aerobic conditions, in comparison with (**2**), and also briefly hydrolysis of (**1**) and the reducing activity of the reduced (**1**).

Results and Discussion

Synthesis of Compound (**1**).—It is rather surprising that (**1**) has not been synthesized previously, whereas 6- and 9-azaflavins have been synthesized, although their oxidizing activities have not been examined.[9] Compound (**1**) was prepared by condensation of 4-amino-3-methylaminopyridine and *N*-methylalloxan.



Redox Potentials and Absorption Spectra.—The redox potentials were determined to be −194 mV for (**1**) and −439 mV for (**2**) by cyclic voltammetry by employing an electrochemically preheated glassy carbon working electrode with an Ag–AgCl–0.1M-KCl reference electrode (pH 7.0, N_2, 25 °C). Reversibility of the redox couples was confirmed at scanning speeds of 50 and 100 mV s^{-1}. The redox potential of (**1**) is more positive than that of (**2**) by 245 mV, implying that (**1**) is more oxidation-active.

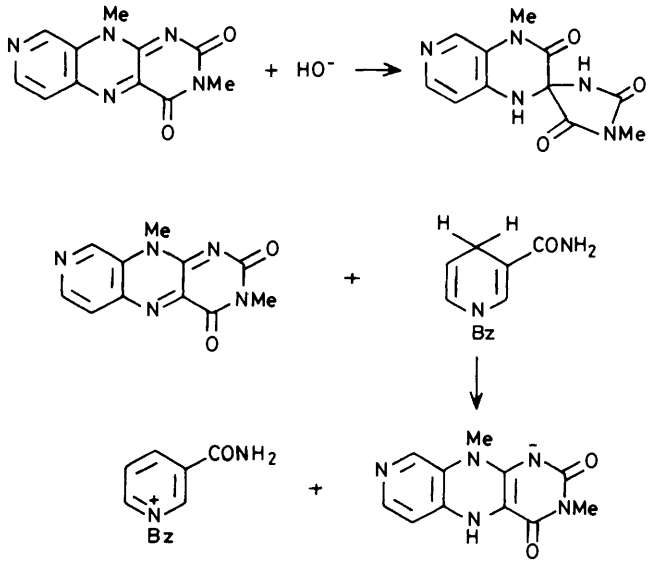
The absorption spectrum of (**1**) shows $\lambda_{max.}$ (pH 7) 442 (ε 6 800 dm^3 mol^{-1} cm^{-1}) and 317 nm (5 600). Reduced (**1**) was obtained by EDTA-photoreduction under anaerobic conditions [$\lambda_{max.}$ (pH 7) 470 (ε 2 000 dm^3 mol^{-1} cm^{-1}) and 359 nm (8 300)]. The absorption spectra of reduced (**1**) at various pH values allowed us to calculate the pK_a of the N(1)–H group to be 4.9. Plots of optical density at 495 nm as a function of the pH gave a titration curve for single proton ionization. This indicates that the 8-aza moiety is more electron withdrawing than the 8-cyano group, since the pK_a of N(1)–H of the 8-cyanoflavin is reported to be 5.9.[4b]

Hydrolysis.—Prior to examination of the oxidizing activity of (**1**), decomposition due to hydrolysis was examined kinetically, since electron-deficient flavins are considered to be susceptible to nucleophilic attack by hydroxide ion. The rate constants were determined by following the absorption decrease of (**1**) at 440 nm. The results are shown in Table 1. The rates are first-order with respect to [HO^-]. The second-order rate constant of (**1**) (k_{HO^-} 2 × 10^2 dm^3 mol^{-1} min^{-1}) is larger than that of (**2**) by two orders of magnitude, and is several times larger than that of 8-cyanoflavin.[4b] Hydrolysis of (**1**) is considered to proceed

Table 1. Pseudo-first-order rate constants of hydrolyses of (1) and (2) at 25 °C[a]

pH	k_{obs}/min^{-1} (1)	(2)
8.18	2.83×10^{-4}	*b*
9.24	3.13×10^{-3}	*b*
10.00	1.94×10^{-2}	*b*
10.33	2.84×10^{-2}	1.8×10^{-4}
10.70	1.82×10^{-1}	*b*

[a] [Flavins] 5×10^{-5}M and [buffer] 0.1M. [b] Not determined.



through HO^- attack at the 10a-position of the isoalloxazine nucleus to give a spirohydantoin as in the hydrolysis of 8-cyanoflavin.[4b]

Oxidation.—The oxidizing activity of (1) was examined for the oxidation of BNAH, thiols, and nitroalkanes in aqueous solution under anaerobic conditions. The rate constants for all the oxidations were determined by following the absorption decrease of (1) or (2) at 440 nm.

BNAH. Although oxidation of *N*-substituted 1,4-dihydronicotinamides by flavins is a biochemically important model reaction, it has no interest from the viewpoint of synthetic chemistry. It is, however, quite a convenient reaction for estimating the oxidizing activity of flavin models in aqueous solution. The oxidation mechanism seems to proceed *via* hydride-ion transfer passing through pre-equilibrium complexing of dihydropyridine and isoalloxazine rings.[10] The kinetic results revealed that (1) is 41 times more reactive than (2) (Table 2).

Thiols. Oxidation of thiols by flavins has been extensively investigated in model systems, since the oxidation is a model reaction of glutathione reductase[11] or lipoamide dehydrogenase.[12] The oxidation mechanism involves nucleophilic attack of a thiol at C(4a) of the isoalloxazine ring to form a 4a-adduct followed by nucleophilic attack upon the adduct by a second thiol anion, giving the corresponding disulphide and 1,5-dihydroflavin.[4,13]

We first examined kinetically whether (1) oxidizes thiols according to the mechanism described above, by employing mercaptoethanol as a substrate. The reaction followed good

Table 2. Rate constants for the oxidation of BNAH[a]

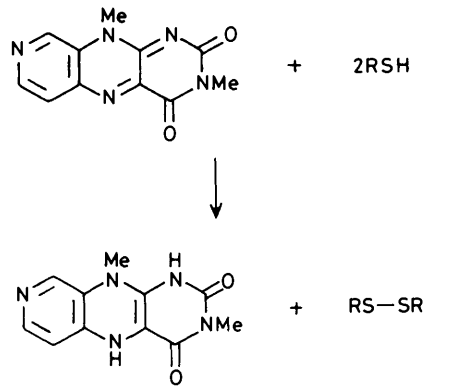
Flavins	k_{obs}/s^{-1}	Relative rate
(1)	1.80×10^{-1}	41
(2)	4.40×10^{-3}	1

[a] [(1)] 1×10^{-4}M, [(2)] 5×10^{-5}M, [BNAH] 5×10^{-4}M, pH 9.27 (0.1M-borate, μ 0.3), 25 °C.

Table 3. Rate constants for oxidation of thiols at 25 °C[a]

Thiols	k_{obs}/s^{-1} (1)	(2)	Relative rate
PhSH[b]	1.36×10^{-1}	$<10^{-7}$	$>10^6$
$HSCH_2CH_2OH$[c]	3.10×10^{-2}	6×10^{-7}	5×10^4
$HS[CH_2]_4SH$[d]	3.30×10^{-5}	3.0×10^{-5}	1.1×10^4

[a] [(1)] = [(2)] 5×10^{-5}M, [Thiols] 5×10^{-3}M, [Buffer] 0.1M (μ 0.3). [b] pH 8.11(borate, 20% MeCN). [c] pH 8.19(phosphate). [d] pH 7.14(phosphate).



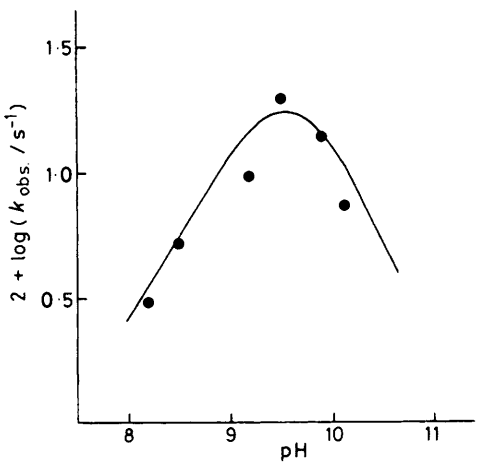
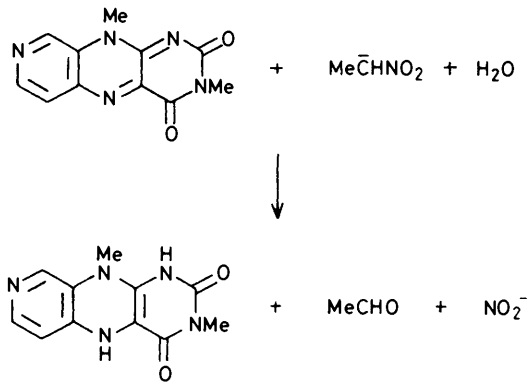


Figure 1. pH–Rate profile for the oxidation by (1): [(1)] 1.5×10^{-4}M, [$HSCH_2CH_2OH$] 5×10^{-3}M, [buffer] 0.1M (μ 0.3), N_2, 25 °C

first-order kinetics up to more than two half-lives. The rates were second-order in [$HSCH_2CH_2OH$] and the pH–log k_{obs} profile showed a bell-shaped curve (Figure 1). A maximum rate was observed at *ca.* pH 9.5, which corresponds to the pK_a value of $HSCH_2CH_2OH$.[14] Thus, the rate is expressed by equation (1), where k_3, K_a, and $[RSH]_o$ represent the third-order rate constant, the dissociation constant of $HSCH_2CH_2OH$, and



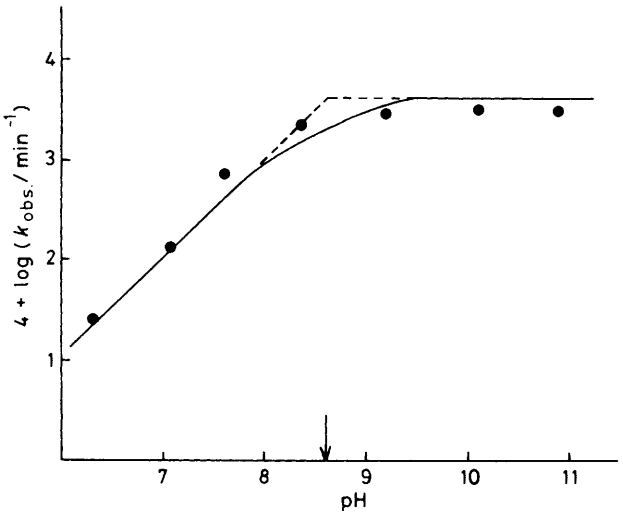


Figure 2. pH–Rate profile for the oxidation of $EtOH_2$ by (**1**): [(**1**)] 5×10^{-5}M, [$EtNO_2$] 1×10^{-2}M, [buffer] 0.1M (μ 0.3), N_2, 25 °C

$$\text{Rate} = k_{obs}[(1)] = k_3[(1)][RSH][RS^-] = \frac{k_3K_a[H^+][(1)][RSH]_o^2}{(K_a + [H^+])^2} \quad (1)$$

total concentration of $HSCH_2CH_2OH$, respectively. The experimental data in Figure 1 were in accord with the theoretical rate constants obtained from equation (1) by assuming $K_a = 3.16 \times 10^{-10}$ l mol^{-1} and $k_3 = 2.9 \times 10^4$ l^2 mol^{-2} s^{-1}. All the kinetic results obtained here were completely consistent with the previous results,[4,13] indicating the oxidation of $HSCH_2CH_2OH$ by (**1**) proceeds *via* an established mechanism. The rate constants of (**1**) and (**2**) for thiol oxidation are shown in Table 3 which shows that (**1**) is much more reactive than (**2**) (10^4—10^6-fold), whereas the relative rate is only 41 for BNAH oxidation. This may be explained by the electron-withdrawing 8-aza moiety stabilizing a negative charge on N(5), which is generated by nucleophilic attack of a thiol at C(4a) of the isoalloxazine ring.

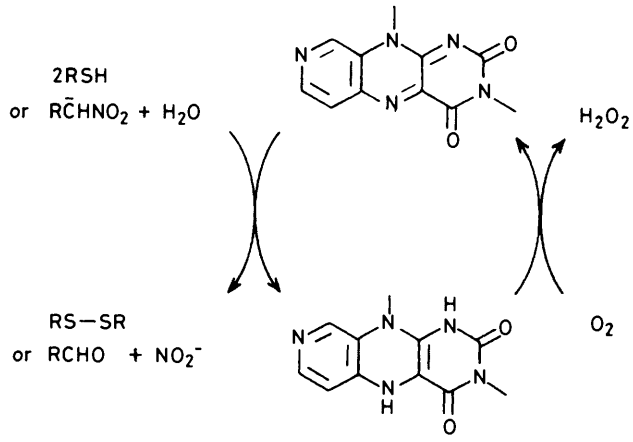
Nitroalkanes. It is known that D-amino acid oxidase oxidizes nitroethane anion to acetaldehyde and nitrite ion coupled with reduction of the flavin coenzyme.[15] In non-enzymatic systems, however, only two examples are known: electron-deficient 8-cyanoflavin[4] and micelle- or polymer-bound flavins.[16] Conversion of nitroalkanes into carbonyl compounds is of interest from the viewpoint of organic syntheses.

The 8-azaflavin (**1**) was found to oxidize various nitroalkanes

Table 4. Rate constants and relative rates for various nitroalkanes at 25 °C under anaerobic conditions[a]

Nitroalkanes	k_{obs}/min^{-1}	Relative rate
$EtNO_2$	8.57×10^{-2}	21
Pr^nNO_2	7.00×10^{-2}	17
Bu^nNO_2	4.57×10^{-2}	11
$n\text{-}C_8H_{17}NO_2$	3.17×10^{-2}	8
Pr^iNO_2	4.04×10^{-3}	1
$CH_2[CH_2]_3CHNO_2$	*b*	
Hydrolysis of (**1**)	6.56×10^{-4}	0.16

[a] [(**1**)] 5×10^{-5}M, [RNO_2] 1×10^{-2}M, pH 9.5(0.1M-borate containing 50% MeCN). [b] Not determined due to hydrolysis of (**1**).



Scheme 1.

to the corresponding carbonyl compounds concomitantly with formation of reduced (**1**) in aqueous solution, whereas (**2**) was unable to oxidize them. The formation of carbonyl compounds was detected as their 2,4-dinitrophenylhydrazone derivatives. The reaction followed first-order kinetics up to more than two half-lives. Further kinetic studies were performed by employing nitroethane. The rates were first-order with respect to nitroethane concentration at constant pH. The pH–log k_{obs} profile showed rate saturation above pH 8.6, which corresponds to the pK_a values of nitroethane,[17] indicating the reactive species to be nitroethane anion (Figure 2). Thus, the rate is expressed by equation (2), where k_2, K_a, and $[EtNO_2]_o$ represent the second-

$$\text{Rate} = k_{obs}[(1)] = k_2[(1)][MeCHNO_2^-] = \frac{k_2K_a[(1)][EtNO_2]_o^2}{(K_a + [H^+])^2} \quad (2)$$

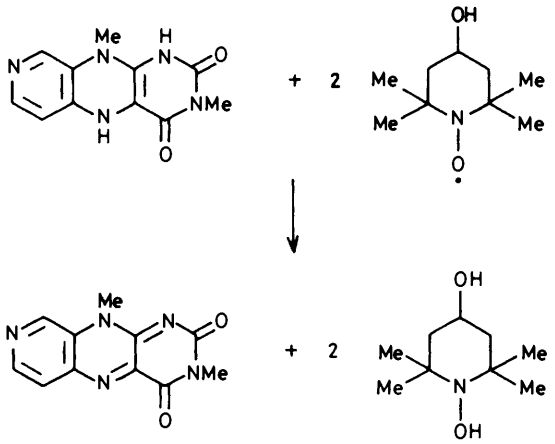
order rate constant of the reaction [(**1**) + $MeCHNO_2^-$], the dissociation constant of nitroethane, and the total concentration of $EtNO_2$, respectively. The second-order rate constant k_2 was 39.8 dm^3 mol^{-1} min^{-1}, which is larger than that of 8-cyanoflavin by two orders of magnitude,[4a] whereas the hydrolysis rate of (**1**) is only several times faster than 8-cyanoflavin. The higher reactivity of (**1**) for oxidation than for hydrolysis compared with 8-cyanoflavin indicates that the reaction site for oxidation may be N(5) which is much more affected by electronic effects from the 8-position, compared with the 10a-position of the isoalloxazine ring. In fact, enzymatic oxidation of nitroethane is known to occur at N(5).[15]

The rate constants for various nitroalkanes (listed in Table 4) indicate that secondary nitroalkanes are less reactive than

Table 5. Rate constants for the reaction of the nitroxide radical with the reduced flavins[a]

Reduced flavins	k_{obs}/min^{-1}	Relative rate
(1)	0.250	1/35
(2)	8.73	1

[a] Reduced flavins (5×10^{-5}M) were prepared by EDTA-photoreduction, [>N–O·] 3×10^{-3}M, pH 6.65 (0.1M-phosphate, μ 0.3) at 25 °C under N_2.



primary ones owing to the size of the secondary alkyl groups, and the decomposition of (1) through hydrolysis is negligible for the reaction with primary nitroalkanes.

Synthetic Application of (1) *as Turnover Oxidation Catalyst under Aerobic Conditions.*—From the kinetic studies, (1) was found to show remarkably high oxidizing activities for the oxidations of thiols and nitroalkanes in aqueous solution. Thus, it is of interest to examine whether (1) can act as turnover oxidizing catalyst in syntheses of disulphides and aldehydes under aerobic conditions as shown in Scheme 1.

Yoneda *et al.* have employed coenzyme models such as 5-deazaflavin as a recycling oxidation catalyst, and since then they have extensively investigated oxidations of alcohols and amines by employing various synthetic catalysts possessing the 5-deaza moiety.[18] However, the reactions are limited to the oxidations of alcohols and amines.

To achieve an efficient catalytic cycle in Scheme 1, it is of primary importance that (i) the flavin possesses a strong oxidizing power and the reduced flavin is rapidly reoxidized by molecular oxygen, and (ii) the catalyst and products are stable under the reaction conditions including hydrogen peroxide produced with the progress of the reactions.

A flavin possessing a stronger oxidizing power is expected to show a weaker reducing power. The turnover catalytic activity is controlled by both rates of substrates oxidation and O_2 oxidation of the reduced flavin. Thus, the reducing activity was estimated kinetically for the reaction of 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (>N–O·) with the reduced flavins in aqueous solution under anaerobic conditions.[19] The rate constants were determined by following the absorption increase at 440 nm (Table 5). As expected, the flavin showing a higher oxidizing reactivity revealed a lower reducing activity.

(a) *Disulphides syntheses.* There seems to be no factor to obstruct the catalytic cycle for the oxidation of thiols in Scheme 1, since thiols are known to be oxidized to disulphides by molecular oxygen[20] and hydrogen peroxide[21] in alkaline solution.

Table 6. Yields of disulphides RS–SR[a]

	RS–SR (%)		
Starting thiol	(1)	(2)	None
PhSH[b]	4 700(95)	1 300(25)	(6)
$PhCH_2SH$[c]	4 100(82)	800(16)	
p-MeC_6H_4SH[d]	4 000(80)	2 600(52)	(24)
p-$MeOC_6H_4SH$[e]	4 600(91)		
Bu^nSH	3 600(72)	650(13)	(3)
n-$C_6H_{13}SH$	3 500(70)	450(9)	

[a] Isolated yields after 1 h stirring at room temperature. Yields are based on (1), and the numbers in parentheses are yields based on thiols. [b] pH 7.8. [c] pH 9.0. [d] pH 8.6(MeCN:buffer = 2:1). [e] pH 9.0(MeCN:buffer = 2:1).

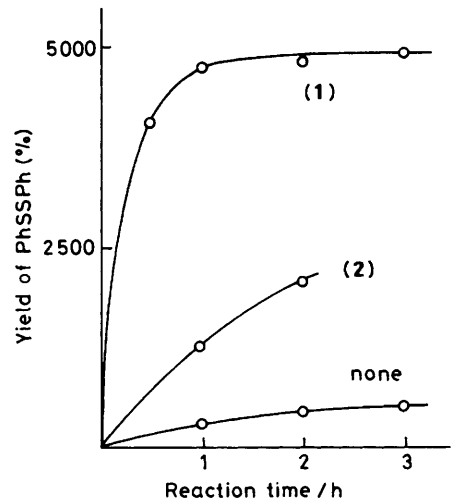


Figure 3. Time course of PhSSPh yields

The catalytic activity of (1) was examined by stirring a mixture of PhSH (0.5 mmol) and a small amount of (1) (1/100 that of PhSH) in aqueous buffer solution (3 ml) in air at room temperature. The yield PhSSPh with time is shown in Figure 3. As can be seen in Figure 3, PhSSPh was obtained quantitatively with 1 h stirring in the case of (1). During the reaction, the solution turned pale pink due to the presence of reduced (1). This suggests that regeneration of oxidized (1) is rate limiting for disulphide synthesis. The results of the oxidation of various thiols are summarized in Table 6. Disulphides were obtained in fairly good yields. The absorption spectra of the reaction mixtures showed no hydrolysis of (1) during the reactions. To the best of our knowledge, this is the first example to employ a flavin model as a turnover oxidation catalyst for disulphide syntheses.

(b) *Aldehydes syntheses from primary nitroalkanes.* The kinetic study demonstrated that the oxidation of primary nitroalkanes by (1) proceeds much faster than the hydrolysis of (1). Thus, primary nitroalkanes were employed as the substrates. In contrast to thiol oxidation, however, the oxidation mechanism of nitroalkanes by flavins has not been established in model systems. Thus, spectroscopic examination was firstly performed for the reaction of nitroethane by (1): the absorbance changes at 440 nm by O_2 introduction are shown in Figure 4. Figure 4 suggests that (1) is able to act as turnover catalyst. It was found, however, that (1) slowly changes to an unidentified product ($\lambda_{max.}$ 380 nm), which is not due to the hydrolysis

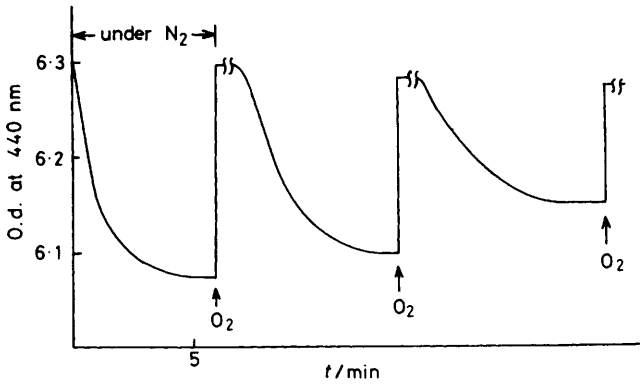


Figure 4. Absorbance changes of (1) at 440 nm by O_2 introduction in the reaction of $EtNO_2$ in aqueous MeCN [0.1 M buffer (pH 8.13): MeCN = 1:1], [(1)] 5×10^{-5} M, [$EtNO_2$] 1×10^{-2} M, 25 °C

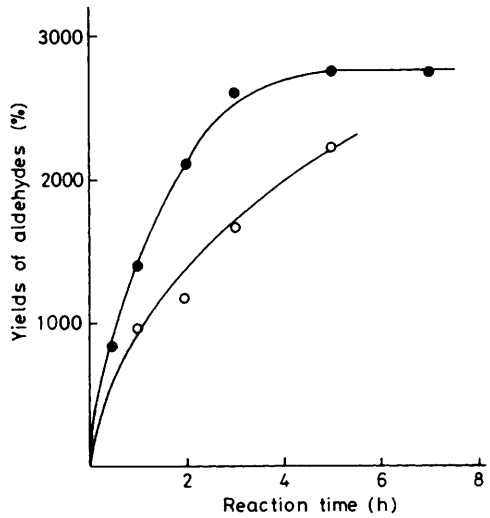
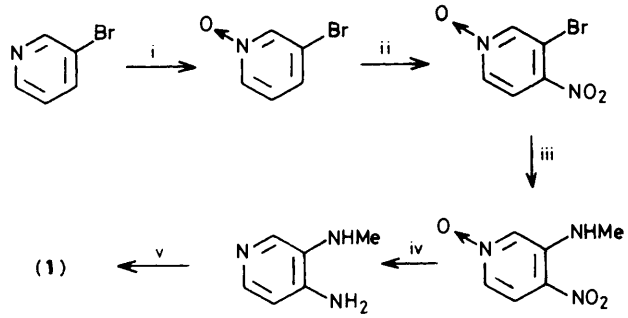


Figure 5. Time course of yields of aldehydes: ○, propanal, pH 8.0—8.1, 40 °C; ●, butanal, pH 8.0—8.2, 50 °C

product of (1). Furthermore, the absorption at $\lambda_{max.}$ 380 nm was found to appear only when the reaction [(1) + $EtNO_2$] was carried out under aerobic conditions, or anaerobic conditions in the presence of hydrogen peroxide. This observation suggests that the reaction intermediate reacts with H_2O_2 to form the product ($\lambda_{max.}$ 380 nm), which clearly limits the usefulness of (1) as the oxidation catalyst. However, we did not study the product further because of the failure of isolation of the product.

The catalytic activity of (1) was examined as follows: a mixture of (1) (23 mg, 0.1 mmol) and nitroalkanes (10 mmol) in aqueous MeCN (50 ml) (MeCN:0.1 M buffer = 1:1) was stirred under air at 40—50 °C. The pH of the solution was maintained at *ca.* 8 during the reaction by adding alkaline solution (2M-NaOH). Yields of aldehydes were determined by isolation of their 2,4-dinitrophenylhydrazone derivatives. The time course of aldehyde formation (propanal and butanal) is shown in Figure 5. Spectroscopic examinations of the reaction mixture showed that considerable amounts of (1) (50% for Pr^nNO_2 and 80—85% for Bu^nNO_2 oxidations) change to the product of $\lambda_{max.}$ 380 nm. This is a reason for saturation of aldehyde formation in Figure 5. Propanal, butanal, and octanal were produced in 2 200, 2 700, and 2 500% based on (1) after 5 h stirring (the oxidation of nitro-octane was performed under the same conditions as that of



Scheme 2. *Reagents:* i, H_2O_2; ii, HNO_3; iii, $MeNH_2$; iv, Fe–AcOH; v, *N*-methylalloxan

Bu^nNO_2). Although accompanying the side reaction, it is noteworthy that primary nitroalkanes can be converted into the corresponding aldehydes in fair yields by using a flavin model under aqueous and aerobic conditions.

Conclusions.—It has been shown that the 8-azaflavin reveals a remarkably high oxidizing activity, and it can be used as a turnover oxidation catalyst for syntheses of disulphides from thiols and of aldehydes from primary nitroalkanes in aqueous and aerobic conditions, although a side reaction occurs for the oxidation of nitroalkanes.

Experimental

Absorption spectra were recorded with a Hitachi model 200-10 spectrophotometer. pH Values were measured with a Hitachi–Horiba 7-7DE pH meter.

Materials.—3,10-Dimethylisoalloxazine (2), BNAH, and 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl were available from our previous study.[22] 3,10-Dimethyl-8-azaisoalloxazine (1) was synthesized according to Scheme 2. 4-Amino-3-methylaminopyridine was prepared from 3-bromopyridine according to literature procedures,[23] m.p. 113 °C (lit.,[23] 114 °C). Condensation of 4-amino-3-methylaminopyridine and *N*-methylalloxan was performed according to literature procedures.[24] To a stirred solution of 4-amino-3-methylaminopyridine (0.12 g, 1 mmol) in AcOH (5 ml), *N*-methylalloxan (0.43 g, 2.8 mmol) and H_3BO_3 (0.075 g) were added, and stirring was continued for 20 h at room temperature. The crystals formed were collected by filtration, washed with H_2O, and recrystallized from EtOH (yield 0.071 g, 29%), m.p. >320 °C; M^+ 243 (Found: C, 54.5; H, 3.8; N, 28.6. $C_{11}H_9N_5O_2$ requires C, 54.3; H, 3.7; N, 28.8%). Commercial thiols and nitroalkanes were purified by distillation under N_2. Nitro-butane, -cyclopentane, and -octane were prepared from the corresponding bromides and $NaNO_2$ in dimethylfomamide (DMF).[25] The following buffers were employed to maintain pH; K_2HPO_4–Na_2HPO_4 for pH 6.5—8.2, $Na_2B_4O_7$–KH_2PO_4 for pH 8.2—9.2, and Na_2CO_3–$Na_2B_4O_7$ (or $NaHCO_3$) for pH >9.2. Ionic strength was adjusted to be 0.3 with KCl. Deionized water was distilled and used for the kinetics.

Determination of Redox Potentials.—The redox potentials were determined by cyclic voltammetry with a polarographic analyser (Yanagimoto model P-1100) equipped with an *X–Y* recorder (Rika Electronics RY-101A). Glassy carbon (Tokai Electrode GC-20, diam. 3 mm), which was electrochemically preheated by holding its potential at 1.4 V *versus* Ag–AgCl–0.1M-KCl at pH 7.0 (0.1M-McIlvaine buffer, 0.1M-Na_2SO_4), was employed as a working electrode. The $E^{0\prime}$ values were

calculated from cathodic and anodic peaks of the cyclic voltammograms obtained at a fixed scanning rate (50 mV s^{-1}).

Rate Measurements.—The measurement of the kinetics of BNAH oxidation was performed as described previously.[22] The concentrations of stock solutions were: flavins (5×10^{-3}M in DMF), thiols (3×10^{-1}M in EtOH), $EtNO_2$ [1M in aqueous KOH (1M)], other nitroalkanes (1M in MeCN), and nitroxide radical (3×10^{-1}M in MeCN). In a Thunberg cuvette, substrate (30 μl) was placed in the cell section with buffer solution (2.94 ml), and flavin solution (30 μl) was placed in the upper section. Both the solutions were degassed by bubbling vanadous-ion-scrubbed N_2 for 20 min. After equilibrium at 25 °C, the reaction was initiated by mixing. For the reduction of the nitroxide radical, the flavins were placed in the cell with buffer containing EDTA (1×10^{-3}M), and the nitroxide solution (30 μl) was placed in the upper section. The flavin in the cell part was reduced by irradiation with a 60 W tungsten lamp, and the reaction was initiated.

Determination of Yields of Disulphides and Aldehydes, and Recovery of (1).—The reaction mixture described in the text was extracted with diethyl ether (10 ml, twice), and the ether layer was washed with 1M-NaOH (10 ml) to remove unchanged thiol, washed with water, and dried (Na_2SO_4). After evaporating off ether, the disulphide remaining was weighed without purification. Compound (1) was much more soluble in the aqueous layer than in ether. Thus, compound (1) was recovered from the aqueous solution by $CHCl_3$ extraction (10 ml, twice) in almost quantitative yield.

For aldehydes, the reaction mixture (5 ml) described in the text was pipetted out into 2M-HCl solution (10 ml) saturated with 2,4-dinitrophenylhydrazine. The crystals formed were collected and dried in a vacuum desiccator overnight and weighed without further purification.

Acknowledgements

We are grateful to Professor N. Furukawa, University of Tsukuba, for helpful discussions.

References

1 Preliminary communications, 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 C. Walch, *Acc. Chem. Res.*, 1980, **13**, 148; T. C. Bruice, *ibid.*, p. 256.
3 Y. Yano, M. Nakazato, and E. Chya, *J. Chem. Soc., Perkin Trans. 2*, 1985, 77.
4 (*a*) I. Yokoe and T. C. Bruice, *J. Am. Chem. Soc.*, 1975, **97**, 450; (*b*) T. C. Bruice, T. W. Chan, J. P. Taulene, I. Yokoe, D. E. Elliott, R. F. Williams, and M. Novak, *ibid.*, 1977, **99**, 6713.
5 M. J. Gibian and A. L. Baumstark, *J. Org. Chem.*, 1971, **36**, 1389; D. Voshall and D. O. Can, *Biochem. Pharmacol.*, 1973, **22**, 1521; Y. Yano, T. Sakaguchi, and M. Nakazato, *J. Chem. Soc., Perkin Trans. 2*, 1984, 595; W. R. Knappe, *Liebig's Ann. Chem.* 1979, 1067.
6 T. C. Bruice, in 'Progress in Bioorganic Chemistry,' eds. E. T. Kaiser and F. J. Kezdy, Wiley, New York, 1976, vol. 4, p. 1.
7 E. G. More, G. Ghisla, and V. Massey, *J. Biol. Chem.*, 1979, **254**, 8173; F. Yoneda, K. Shinozuka, K. Hiromatsu, R. Matsushita, Y. Sakuma, and M. Hamana, *Chem. Pharm. Bull.*, 1980, **28**, 3576.
8 R. G. Shepherd and J. L. Fedrick, in 'Advances in Heterocyclic Chemistry,' ed. A. R. Katritzky, Academic Press, New York, 1965, vol. 4, p. 145.
9 W. R. Knappe, in 'Flavins and Flavoproteins,' eds. K. Yagi and T. Yamano, University Park Press, Baltimore, 1980, p. 33.
10 G. Blankenhorn, *Eur. J. Biochem.*, 1975, **50**, 351; R. Stewart and D. J. Norris, *J. Chem. Soc., Perkin Trans. 2*, 1978, 246; M. F. Powell, W. H. Wong, and T. C. Bruice, *Proc. Natl. Acad. Sci., U.S.A.*, 1982, **79**, 464.
11 G. E. J. Ataal and C. Veeger, *Biochim. Biophys. Acta.* 1969, **185**, 49.
12 V. Massey and G. Palmer, *J. Biol. Chem.*, 1962, **237**, 2347.
13 E. L. Loechler and T. C. Hollocher, *J. Am. Chem. Soc.*, 1980, **102**, 7312, 7322.
14 M. M. Kreevoy, E. T. Harger, R. E. Duvall, H. S. Wilgus, and L. T. Ditsch, *J. Am. Chem. Soc.*, 1960, **82**, 4899.
15 D. T. Porter, J. G. Voet, and H. J. Bright, *J. Biol. Chem.*, 1972, **247**, 1951; 1973, **248**, 4400.
16 S. Shinkai, in 'Flavins and Flavoproteins,' eds. K. Yagi and T. Yamano, University Park Press, Baltimore, 1980, p. 45.
17 R. G. Pearson and R. L. Dillon, *J. Am. Chem. Soc.*, 1953, **75**, 7287.
18 F. Yoneda, *Yakugaku Zasshi*, 1984, **104**, 97 and references cited therein.
19 T. W. Chan and T. C. Bruice, *J. Am. Chem. Soc.*, 1977, **99**, 7287.
20 G. Gapozzi and G. Modena, in 'The Chemistry of the Thiol Group,' ed. S. Patai, Wiley, New York, 1981, Part II, p. 791.
21 T. McAllan, T. W. Cullum, R. A. Dean, and F. A. Fidler, *J. Am. Chem. Soc.*, 1951, **73**, 3627.
22 Y. Yano and E. Ohya, *J. Chem. Soc., Perkin Trans. 2*, 1984, 1227.
23 W. Clark-Lewis and R. P. Singh, *J. Chem. Soc.*, 1962, 2397.
24 R. Kuhn and F. Weygand, *Ber. Dtsch. Chem. Ges.*, 1934, **67**, 1409.
25 N. Kornblum, H. D. Larson, R. K. Blackwood, D. D. Mooberry, E. P. Oliveto, and G. E. Graham, *J. Am. Chem. Soc.*, 1956, **78**, 1497.

Received 11*th June* 1984; *Paper* 4/979