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HIGH OXIDIZING ACTIVITY OF 8-AZA-3,10-DIMETHYLISOALLOXAZINE (8-AZAFLAVIN)

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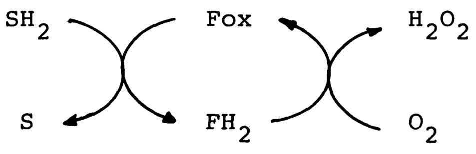
8-Azaflavin was synthesized for the first time, and it was found that 8-azaflavin is much more reactive ($>10^4$) than 3,10-dimethylisoalloxazine for oxidation of thiols in aqueous solution. Catalytic efficiency for disulfides synthesis was also examined under aerobic conditions.

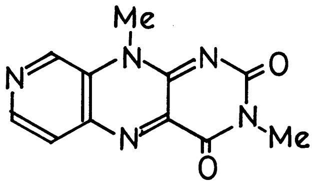
To improve an oxidizing power of flavin model compounds is of special interest not only for the mechanistic study of flavin oxidation, but also for their application to organic syntheses as oxidation-reduction catalyst as described below.



SH_2; substrate
Fox; oxidized flavin
FH_2; reduced flavin

Electron-withdrawing substitutent at 8-position of isoalloxazine nucleus is known to increase the oxidizing reactivity. For example, 8-cyano-3,10-dimethylisoalloxazine(8-cyanoflavin) oxidizes thiophenol and nitroalkane anions in aqueous solutions, whereas conventional flavins do not oxidize these substrates.$^{1)}$ Another way to facilitate flavin oxidation is to employ hydrophobic environment provided by micelle and polymer bound flavins.$^{2)}$

We report herein that 8-azaflavin (**1**) exhibits remarkably high reactivity for the oxidation of thiols in aqueous solution compared to 3,10-dimethylisoalloxazine (**2**).



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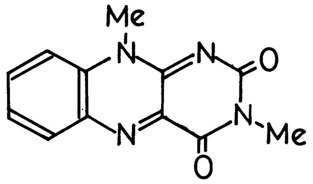


2

8-Azaflavin (**1**) was synthesized by condensation of 4-amino-3-N-methylaminopyridine and N-methylalloxan.$^{3)}$ The absorption spectra of **1** are λmax 442 nm (ε=6,800) and 317 nm (ε=5,600) for oxidized and λmax 470 nm (ε=2,000) and 359 nm (ε=8,300) for reduced forms, respectively. The absorption maxima are very similar to those of 8-cyanoflavin.$^{1)}$ This suggests that 8-aza moiety must be fairly electron-withdrawing.

At the first place, the reactivity of **1** was examined kinetically for oxidation of thiols in aqueous solution. The rate constants were determined by following the decrease of the absorption of flavins (≈440 nm) under anaerobic conditions. Oxidized flavins were regenerated quantitatively by O_2 bubbling into the cuvette after the reactions, and the corresponding disulfides were identified by TLC. The results together with hydrolysis rates$^{4)}$ are listed in Table 1.

Table 1. The pseudo first order rate constants $^{a)}$

Thiols[SH]	k_{obsd} (s^{-1}) **1**	**2**	Rel.rates
PhSH $^{b)}$	1.36×10^{-1}	$\sim10^{-7}$	$\sim10^{6}$
$HSCH_2CH_2OH$ $^{c)}$	3.10×10^{-2}	$\sim6\times10^{-7}$	$\sim5\times10^{4}$
$HS(CH_2)_4SH$ $^{d)}$	3.30×10^{-1}	3.0×10^{-5}	1.1×10^{4}
^{-}OH $^{e)}$	4.73×10^{-4}	3.0×10^{-6}	1.6×10^{2}

a) [**1**]=[**2**]=5×10^{-5}M, [SH]=1×10^{-3}M, [Buffer]=0.1 M (μ=0.3), 25°C

b) pH 8.11 (borate, 20%(v/v) CH_3CN) c) pH 8.19 (phosphate),

d) pH 7.14 (phosphate), e) pH 10.33 (carbonate)

The table indicates that **1** is much more reactive than **2**. The oxidation of $HSCH_2CH_2OH$ revealed; (a) the rate was second-order to $[HSCH_2CH_2OH]$, (b) the pH-rate profile showed a bell-shaped curve, and (c) the maximum rate came out at pH 9.5, which corresponds to pKa of $HSCH_2CH_2OH$.5) These kinetic results were completely consistent with the results reported previously.1,6) Thus, the mechanism involves nucleophilic attack of thiol anion at C(4a) position to form adduct followed by nucleophilic attack upon the adduct by a second thiol anion.1) Thus, larger rate enhancement of **1** could be explained by that the two nucleophilic attacks of thiol anions are facilitated by electron withdrawing 8-aza moiety. In fact, **1** was found to be several times reactive compared with 8-cyanoflavin for thiophenol oxidation.1) It is worthy to note that the rate acceleration of **1** is greater for the thiol oxidation than for its own hydrolysis.

Catalytic activities of **1** and **2** for oxidation of thiols were examined under aerobic conditions. Namely, a mixture of PhSH (0.5 mmol) and a small amount of **1** (1/100 mol to PhSH) in 3 ml of aqueous buffer solution (pH 7.8, 0.1 M phosphate) was stirred under air atomosphere. The yield of diphenyl disulfide vs. reaction time are shown in Fig. 1. Diphenyl disulfide was obtained quantitatively within 1 h stirring in the case of **1**. The results of the oxidation of various thiols are summarized in Table 2.

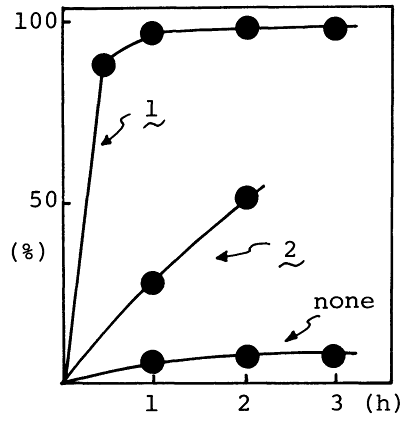


Fig. 1. PhSSPh yields vs. reaction time.

Table 2. R-SS-R (%) after 1 h stirring a)

RSH	R-SS-R(%)		none
	1	**2**	
C_6H_5- b)	95	25	6
$C_6H_5CH_2$- c)	82	16	-
p-Me-C_6H_5- d)	80	52	24
p-MeO-C_6H_5 e)	91	-	-
n-C_4H_9 e)	72	13	3
n-C_6H_{13} e)	70	9	-

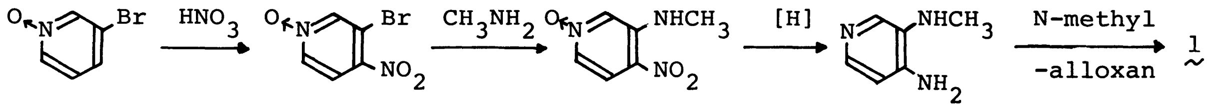
a) At room temp. Isolated yields. b) pH 7.8, c) pH 9.0, d) pH 8.6 (CH_3CN: buffer=2:1), e) pH 9.0 (CH_3CN: buffer=2:1), [RSH]=0.5 mmol, [RSH]/[Fox]=100.

Although it is desireable to adjust the pH of the reaction medium to pKa values of the thiols employed, the pH should not exceed 9 to avoid hydrolysis of **1**.[7)]

The present study demonstrated that 8-azaflavin possesses a high oxidizing reactivity for oxidation of thiols and it can be used as oxidation-reduction catalyst for disulfides syntheses in aqueous solution under aerobic conditions.

References

1) I. Yokoe and T. C. Bruice, J. Am. Chem. Soc., 97, 540 (1974); T. C. Bruice, T. W. Chan, J. P. Taulane, I. Yokoe, D. E. Elliott, R. F. Williams, and M. Novak, ibid., 99, 6713 (1977).
2) S. Shinkai, "Flavin and Flavoproteins," ed by K. Yagi and T. Yamano, University Park Press, Baltimore (1980), p. 45.
3) Synthetic routes;



Mp > 320°C

Found: C; 54.46, H; 3.77, N; 28.59%.

Calcd for $C_{11}H_9N_5O_2$: C; 54.32, H; 3.73, N; 28.79%.

4) The hydrolysis rates were obtanied by following the decrease of the absorption of flavins (≈ 440 nm) under anaerobic conditions: S. B. Smith, and T. C. Bruice, J. Am. Chem. Soc., 97, 2875 (1975).
5) The rate equation is; $k_{obsd} = k[S^{-1}][SH] = \frac{kKa[H^+]}{(Ka+[H^+])^2}[SH]_o^2$.
6) S. Shinkai, R. Ando, and F. Yoneda, Chem. Lett., 1977, 147; E. L. Loechler and T. C. Hollocher, J. Am. Chem. Soc., 102, 7312, 7322 (1980).
7) Hydrolysis of **1** was not detected by the absorption spectra at pH 9 or below.

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