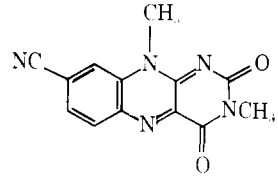
## Oxidation of Thiophenol and Nitroalkanes by an Electron Deficient Isoalloxazine

*Sir:*

Porter, Voet, and Bright[1] have shown that D-amino acid oxidase may employ nitroalkane anions as substrates to yield aldehyde, nitrite ion, and reduced flavoenzyme as products. In nonenzymatic reactions, flavins are not able to catalyze this reaction. Although flavins are reduced by aliphatic mercaptans[2] to yield disulfides, they are not reduced by thiophenols. To date, the mechanism of thiol oxidation by flavins has not appeared in the literature. We report, herein, a kinetic study of an electron deficient isoalloxazine, 3,10-dimethyl-8-cyanoisoalloxazine (I), that does react with thiophenol and nonenzymatically with nitroalkanes.



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The reaction of I with nitromethane and nitropropane was found[3] to be first order in I and strictly first order in nitroalkane anion (eq 1) and found to yield quantitatively

$$\frac{d[I]}{dt} = k_n[R\bar{C}HNO_2][I] \quad (1)$$

dihydroisoalloxazine ($F_{red}$) and RCHO. When nitroalkane was present in great excess over I and the acid–base equilibria of nitroalkane and its anion were preestablished prior to addition of I[3] (pH 7.5–9.4 with phosphate, carbonate, and borate), buffer catalysis could not be detected ($k_n$ (nitromethane anion) = 0.30 $M^{-1}$ $min^{-1}$, $k_n$(2-nitropropane anion) = 0.17 $M^{-1}$ $min^{-1}$). The reaction of I with excess thiophenol was found to follow first-order kinetics at all pH values investigated (pH 4.8–9.9) and to provide $F_{red}$ and $(C_6H_5)_2S_2$ in quantitative yields.[4] At constant pH plots of the pseudo-first-order rate constants ($k_{obsd}$) *vs.* [total RSH + $RS^-$][2] were found to be linear, establishing the reaction to be second order in total thiol. From buffer dilution studies (acetate, phosphate, hydrazine, morpholine, carbonate, and trimethylamine) it could be concluded that thiol oxidation by I is not subject to general catalysis. When the concentration of thiol was kept constant and the pH varied, it was found that a plot of log $k_{obsd}$ *vs.* pH described a "bell-shaped" curve (Figure 1) which could be fit by eq 2. The

$$k_{obsd} = \frac{9a_H K_{app}}{K_{app}^2 + K_{app}a_H + a_H^2} \quad (2)$$

value of $pK_{app}$ = 7.3 may be compared to the $pK_a'$ of thiophenol (6.7) determined by spectral titration under the conditions of the kinetic study. These combined results dictate the kinetic expression of eq 3.

$$\frac{d[RSSR]}{dt} = k_n[RSH][RS^-][I] \quad (3)$$

It has previously been established that 4a-addition of a nucleophile to the isoalloxazine nucleus is general acid catalyzed whereas 5-addition is not.[5] It follows that 4a-addition of nitroalkane anion (eq 4) would be dependent on the ex-

$$I + R\bar{C}HNO_2 \underset{k_{gb}[B]}{\overset{k_{ga}[BH]}{\rightleftharpoons}} 4a\text{-add} \xrightarrow{k_3} \text{product} \quad (4)$$

pression of eq 5 if $k_{ga}$ is rate determining and eq 6 if collapse of adduct to product is rate limiting (where $K_{NH}$ = acid dissociation constant of nitroalkane and $K_{BH}$ = buffer

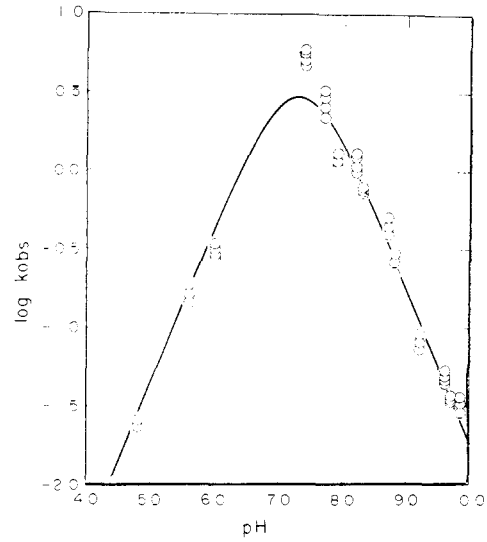
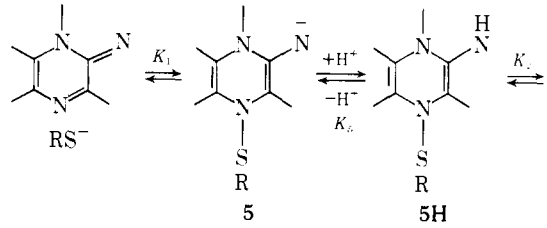


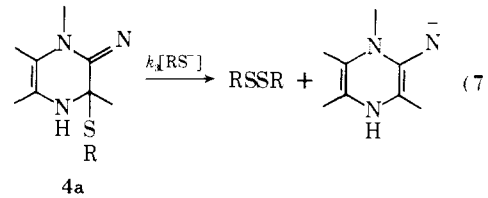

**Figure 1.** pH dependence for the oxidation of thiolphenol ($1 \times 10^{-3}$ $M$) by I ($1 \times 10^{-5}$ $M$) in 20% acetonitrile (30°; $\mu$ = 1.0).

$$k_{obsd} = k_{ga}[BH][R\bar{C}HNO_2] \quad (5)$$

$$k_{obsd} = \frac{K_{NH}k_{ga}k_3[RCH_2NO_2]}{K_{BH}k_{gb}} \quad (6)$$

acid dissociation constant). Since the reaction of I with nitromethane and 2-nitropropane is dependent upon $[RC^-HNO_2]$ and independent of [B] (or [BH]), either the formation of a 5-adduct or a free-radical reaction must be favored—the two, of course, not being mutually exclusive.[6] Rather strong evidence has been presented[1] (trapping, isolation, and partial characterization) for a kinetically competent 5-adduct in the case of D-amino acid oxidase reacting with nitroalkane. For the thiophenol oxidation the lack of buffer catalysis may be ascribed to either 5-addition followed by 5 → 4a migration of $RS^+$ as in eq 7 or general-



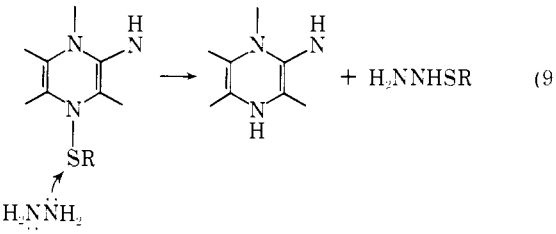




(7)

acid catalyzed 4a-addition as in eq 8 with rate limiting nu-

$$RS^- + I \underset{k_{gb}[B]}{\overset{k_{ga}[BH]}{\rightleftharpoons}} \mathbf{4a} \xrightarrow{k_3[RS^-]} \text{product} \quad (8)$$

cleophilic attack of $RS^-$ upon the 4a-adduct.[7] Though precedence exists for 5H → 4a cation migrations,[8-10] the mechanism of eq 8 is less encumbered and probably is to be preferred.[11] In addition, a 5-adduct would be a sulfenamide which would be anticipated[12] to be susceptible to transamination by the strong $\alpha$-effector hydrazine (eq 9) at a rate



(9)

comparable[10] to the 5H → 4a shift. Hydrazine at concentrations 0.1–0.4 *M* at pH 7.90 had no effect on the rate of reaction. The free radical mechanisms of eq 10 and 11 may

$$RS^- + F_{ox} \overset{K_1}{\rightleftharpoons} RS\cdot + F_{rad}$$

$$F_{rad} + RSH \overset{K_2}{\rightleftharpoons} F_{red} + RS\cdot \qquad (10)$$

$$2RS\cdot \xrightarrow{k_3} RSSR$$

$$RSH + F_{ox} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RS\cdot + F_{rad} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} RS^+ + F_{red} \qquad (11)$$

$$RS^+ + RS^- \xrightarrow{k_3} RSSR$$

be dismissed since the values of $k_{obsd}$ on both the alkaline and basic side (pH 5.6 and 9.8) of the bell-shaped pH–log $k_{obsd}$ profile were found to be independent of the ratio of oxidized to reduced I at the time of initiation of the reaction. Kinetics indicative of autocatalytic processes were not observed.

The results of a previous study[5] established that a given nucleophile could add to either the 4a- or 5-position of an isoalloxazine ring. The present results point out that both positions may be implicated in flavin catalysis depending on the substrate and, of course, the directional influence of the enzyme.

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## References and Notes


(1) D. J. T. Porter, J. G. Voet, and H. J. Bright, *J. Biol. Chem.*, **248**, 4400 (1973).

(2) I. M. Gascoigne and G. K. Radda, *Biochim. Biophys. Acta*, **131**, 498 (1967).

(3) The disappearance of I was followed spectrophotometrically at 443 nm under an argon atmosphere. In practice, a solution of nitroalkane (0.1–1.0 *M*) in 0.1–1.0 *M* aqueous KOH was allowed to sit for 45 min to allow completion of formation of nitroalkane anion; 0.1 ml of this solution was added to 4.8 ml of the appropriate buffer. The resulting solution was degassed and saturated with argon for 30 min, and the reaction was initiated by mixing in a Thunberg cuvet under argon with 0.1 ml of a $2.5 \times 10^{-3}$ *M* solution of I in $CH_3CN$. The resulting reaction mixture being $5 \times 10^{-5}$ *M* in I and $2 \times 10^{-2}$ *M* in nitroalkane (2% aqueous acetonitrile, v/v, $\mu$ = 1.0 with KCl, 30°). At completion of the reaction, admittance of $O_2$ regenerated I quantitatively. Acetaldehyde was found *via* polarography to be produced quantitatively.

(4) Disappearance of I was followed at 443 nm. All reactions were carried out in Thunberg cuvets under an argon atmosphere employing solution presaturated (for 30 min) with argon. Reactions were initiated by mixing an acetonitrile solution of I with thiophenol in aqueous acetonitrile solution. The reaction solution was $10^{-5}$ *M* in I and $10^{-3}$ *M* in thiophenol with buffer concentrations of 0.1–0.5 *M* (20% aqueous acetonitrile, v/v, $\mu$ = 1.0 with KCl, 30°). At completion of reaction, admittance of air regenerated I quantitatively. Carried out on a preparative scale 98% yield of $(C_6H_5)_2S_2$ product could be collected as a precipitate (ir, uv, and melting point).

(5) T. C. Bruice, L. Hevesi, and S. Shinkai, *Biochemistry*, **12**, 2083 (1973).

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(7) A similar mechanism has been proposed for the oxidation of glutathione by 2,6-dichloroindolphenol: D. S. Coffey and L. Hellerman, *Biochemistry*, **3**, 394 (1964).

(8) W. Haas and P. Hemmerich, *Z. Naturforsch. B*, **27**, 1035 (1972).

(9) W. H. Walker, P. Hemmerich, and V. Massey, *Eur. J. Biochem.*, **13**, 258 (1970).

(10) D. Clerin and T. C. Bruice, *J. Amer. Chem. Soc.*, **96**, 5571 (1974).

(11) The observation of general-base catalysis in the oxidation of lipoic acid by oxidized flavin [ref 2] is in accord with the mechanism of eq 8 where $k_{ga}$ is rate determining. E. Loechler and T. Hollocher (private communication) present cogent arguments in support of the mechanism of eq 8. These include a change in rate-determining step from $k_3$ to $k_{ga}$ in going from a monoalkylthiol to dithiothreitol.

(12) C. Steelink, M.S. Thesis in Chemistry, 1949, University of Southern California.

(13) Postdoctoral Fellow, University of California, Santa Barbara, California 93106.


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## A Novel Route to Bicyclo[3.3.1]non-1-ene. Supporting Evidence for Wiseman's Postulate

*Sir:*

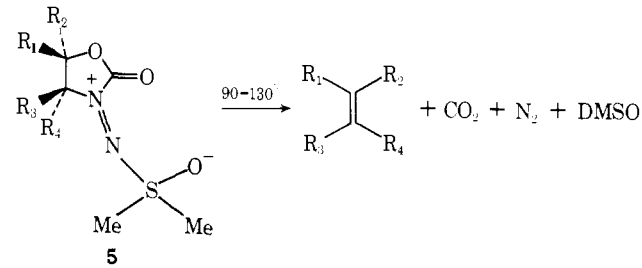
The failure of Bredt's rule, as formulated in the quantitative expression ("S number") of Fawcett, to account for differences in strain between isomeric bridgehead olefins, *e.g.*, **1** and **2**, represents a serious shortcoming of this numerical approach.[1] In contrast, the proposal by Wiseman[2] that the strain in bridgehead alkenes is closely related to the strain of the corresponding trans cycloalkene accounts well for the properties of known bridgehead olefins and leads to the clear-cut prediction that the bridgehead double bond will be more stable when it is oriented trans in the larger ring. Thus, *Z* isomer **2** (*trans*-cyclooctene) should be more stable than the *E* isomer **1** (*trans*-cyclohexene).

**1**, X = $CH_2$ **2**, X = $CH_2$
**3**, X = $SO_2$ **4**, X = $SO_2$

Support for the Wiseman postulate comes from the synthesis of several "anti-Bredt" bridgehead olefins,[3-6] including bicyclo[3.3.1]non-1-ene[7-9] and certain heterocyclic derivatives.[10,11] Except for the sulfones **3** and **4**, where the presence of *E* and *Z* isomers was inferred from the stereochemistry of Diels–Alder adducts,[10] the methods of synthesis provide no information concerning the preferred geometry of these bridgehead olefins. A study of the thermal decomposition of sulfoximines (**5**) derived from *N*-aminooxazolidones has led to the finding that these substances extrude $CO_2$, $N_2$, and DMSO at 90–130° with liberation of the olefin stereospecifically (cis elimination) and in high yield (Scheme I).[12] It was therefore of interest to apply this olefin synthesis to *E* (**1**) and *Z* (**2**) isomers of bicyclo[3.3.1]non-1-ene.

Ketoester **6** was reduced under Meerwein-Ponndorf conditions to a mixture of exo (**7**) and endo (**8**) alcohols, which were separated by gas chromatography.[9] The minor exo al-

**Scheme I**



**5**