OXIDATION OF DITHIOLS BY 10-THIAISOALLOXAZINE

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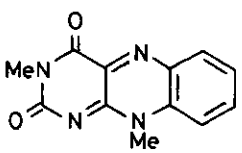
Abstract ——— Reactions of 3-methyl-10-thiaisoalloxazine (2) with dihydrolipoamide (4a) and 1,4-butanedithiol (4b) gave almost exclusively redox products, whereas reactions of 2 with 1,3-propanedithiol (4c) and 1,2-ethanedithiol (4d) resulted in the concurrent occurrence of 4a,10a-cyclic addition and the redox reactions.

Our previous report[1] has demonstrated that the sulfur displacement at the 10-position of the isoalloxazine ring (cf. 2) increases susceptibility for nucleophilic addition to the conjugated diimine moiety ($-N_1=C_{10a}-C_{4a}=N_5-$) and enhances the oxidation capacity comparing with the parent isoalloxazine (1), e.g., 3-methyl-10-thiaisoalloxazine (2)[2] reacted with the lower primary alcohols under mild conditions to give 4a,10a-diadducts (3), whereas 2 oxidized secondary alcohols in the neutral medium on exposure to daylight. These observations prompted us to investigate the reaction of 2 with dithiols in connection with the flavin redox chemistry.

In this paper, we describe oxidation of lower aliphatic dithiols (4) by 2 which occurs with ease under mild conditions. The oxidation, however, largely depends upon the structure of the dithiols employed and competes with the 4a,10a-cyclic adduct formation. The present results suggest that the oxidation of dithiols by 2 proceeds via a covalent intermediate which is formed in the initial stage of the reaction.



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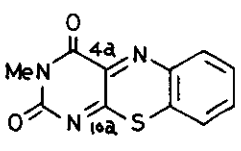

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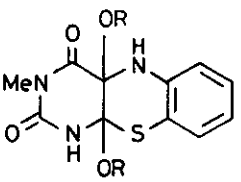

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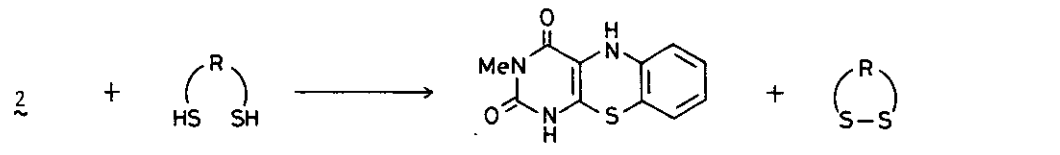
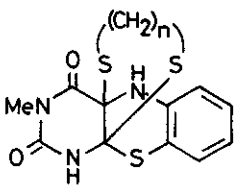


4: a): R=-$(CH_2)_2$-CH-($(CH_2)_4CONH_2$); b): R=-$(CH_2)_4$-; c): R=-$(CH_2)_3$-; d): R=-$(CH_2)_2$-

6: a): R=-$(CH_2)_2$-CH-($(CH_2)_4CONH_2$); b): R=-$(CH_2)_4$-; c): R=-$(CH_2)_3$-; d): R=-$(CH_2)_2$-



7 a): n=3 b): n=2

A mixture of the 10-thiaisoalloxazine (2) and an equimolar amount of dihydrolipoamide (4a) in acetonitrile was stirred at ambient temperature under an argon atmosphere. After disappearence of 2 (about 1 day, monitored by tlc), column chromatography of the reaction mixture allowed isolation of 1,5-dihydro-3-methyl-10-thiaisoalloxazine (5)[2,3] and lipoamide (6a) in almost quantitative yields, respectively. No formation of other products in this reaction was shown by tlc and hplc analyses of the reaction mixture. Upon shielding from daylight, analogous results were obtained. The redox products (5 and 6a) were identical in every respect with authentic samples.

Analogously, the reaction of 2 with 1,4-butanedithiol (4b) proceeded smoothly to give 5 and 1,2-dithiane (6b)[4] (detected by gc-mass).

In contrast to the above results, treatment of 2 with 1,3-propanedithiol (4c) or 1,2-ethanedithiol (4d) under analogous conditions gave 5 and 4a,10a-cyclic adduct (7a or 7b). Although hitherto unknown 1,2-dithiethane (6d) was not detected, the formation of 1,2-dithiolane (6c)[4] in the reaction of 2 with 4c was proved by gc-mass spectroscopy of the reaction mixture. The structures of the cyclic adducts (7a,b) were fully supported by microanalytical and spectral data.

The results in the reaction of the 10-thiaisoalloxazine (2) with dithiols (4) are summarized in Table 1.

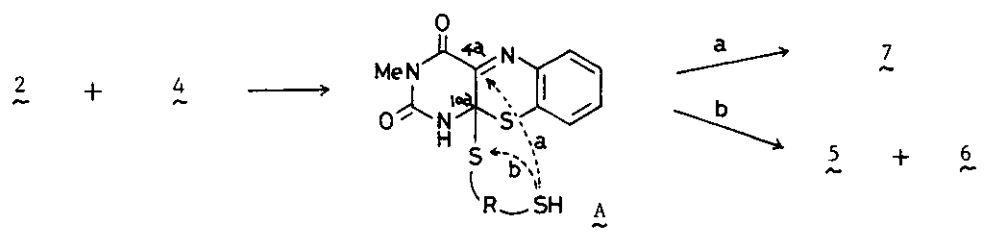
Table 1 Reactions of 3-Methyl-10-thiaisoalloxazine (2) with Dithiols (4)

Dithiol	Dihydro-3-methyl-10-thiaisoalloxazine (5) Yield (%)[a]	Cyclic adduct (7) Yield (%)	mp (°C)
4a	95	-	
4b	93	-	
4c	75	14 (7a)	190 (decomp.)
4d	24	75 (7b)	270 (decomp.)

a : Yields were estimated by hplc.

The cyclic adduct (7a) obtained above was stable upon heating under reflux in acetonitrile. This fact indicates that 7a is not a productive intermediate for the formation of the redox products (5 and 6c).

The cyclic adduct formation can be explained in terms of initial formation of an intermediary 10a-addition product (A),[5] which could give the cyclic adduct (7) via further intramolecular addition of the second thiol group to the azomethine bond ($-C_{4a}=N_5-$)(route a). The redox reaction appears to occur via intramolecular nucleophilic attack of the thiol group at the linked sulfur in the 10a-addition product (A)(route b), which becomes preferable to the addition (route a) with increasing the chain length and bulkiness of dithiols due to the steric reason. Reaction of 2 with ethanethiol resulted in the smooth formation of the 4a,10a-diadduct. In a sharp contrast, reaction of 2 with bulky t-butanethiol did not give the redox products as well as the addition product. These facts also support that the initial formation of the intermediary adduct (A) is a requisite for the redox reaction between 2 and dithiols such as 4a and 4b.



There have been some model reactions[6-11] for an understanding the catalytic reactions of flavin-requiring enzymes such as dihydrolipoamide dehydrogenase. In non-enzymatic oxidations of thiols by flavins and its analogues under basic conditions, strong kinetic evidence exists for an intermediacy of the thiol addition product across the azomethine bond ($-C_{4a}=N_5-$) of the isoalloxazine ring (cf. 1). The present results are of interest in connection with the mechanism of flavin-catalyzed dithiol oxidations.

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3 Contrary to dihydroisoalloxazine, the dihydro-10-thiaisoalloxazine (5) was fairly stable to autoxidation reverting to the 10-thiaisoalloxazine (2) (half life: ca. 6 h). Thus, quantitative analysis and isolation of 5 can be performed with ease.

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5 Previously, we have demonstrated that the initial addition site in the conjugated diimine moiety of 2 is the 10a-position rather than the 4a-position. cf. Y. Maki, M. Tanabe, M. Sako, K. Hirota, and K. Harano, Chem. Lett., 1983, 1093.

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