Reactivity of 10-Thiaisoalloxazine. Ring-Contraction by Water (Hydroxide Ion) followed by Redox Reaction

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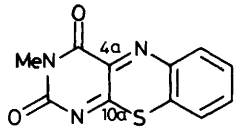
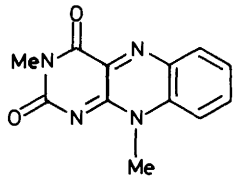
Heating of 3-methyl-10-thiaisoalloxazine (**1**) in aqueous acetonitrile results in the formation of 1,5-dihydro-3-methyl-10-thiaisoalloxazine(**3**),1′-methylspiro[2,3-dihydrobenzothiazole-2,5′-pyrimidine]-2′,4′,6′-trione (**4**), and 1-(benzothiazol-2-yl)carbonyl-3-methylurea (**5**). This type of reaction is significantly accelerated by the addition of a base such as sodium bicarbonate. In sharp contrast, 3,10-dimethylisoalloxazine (**2**) is very stable under these conditions. Our studies suggest that the formation of (**3**) and (**5**) arises from a redox reaction between the ring-contracted product (**4**), initially produced by hydrolysis of (**1**), and unreacted (**1**). These reactions can be attributed to the increased susceptibility of (**1**) to nucleophilic addition and its higher oxidation capacity compared to the isoalloxazine (**2**).

Previous articles from our laboratory[1,2] have described syntheses and chemical reactivities of 10-thiaisoalloxazines (**1**), a thia-analogue of isoalloxazines (**2**). An intriguing observation is that substitution with sulphur at the 10-position of the isoalloxazine ring system increases the susceptibility of the conjugated di-imine moiety (N_1=C_{10a}–C_{4a}=N_5) to nucleophilic addition; it also enhances its oxidation capacity, *i.e.* markedly improves the stability of the corresponding reduced form (**3**) to autoxidation.† Also of interest is that the initial nucleophilic addition to (**1**) occurs at the 10a-position,[2d] rather than at the 4a-position which would be expected to be the most reactive site in (**2**) for nucleophiles.[3]



(**1**)



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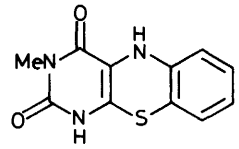

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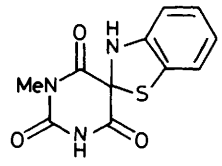
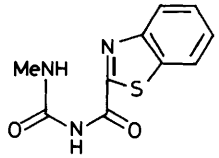
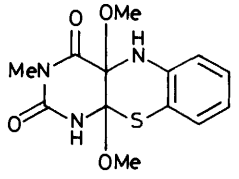


(**4**)



(**5**)



(**6**)

Scheme 1.

This paper describes a novel type of redox reaction, which occurs together with ring-contraction of (**1**) upon treatment with water (hydroxide ion). The present results reflect characteristics of the chemical reactivities of (**1**) and demonstrate a novel reaction of isoalloxazine analogues.

When a solution of 3-methyl-10-thiaisoalloxazine (**1**) in aqueous acetonitrile was heated at 80 °C for 2 h under argon and in the dark, 1,5-dihydro-3-methyl-10-thiaisoalloxazine (**3**), 1′-methylspiro[2,3-dihydrobenzothiazole-2,5′-pyrimidine]-2′,4′,6′-trione (**4**), and 1-(benzothiazol-2-yl)carbonyl-3-methylurea (**5**) were obtained in a ratio of 20:42:21. The structures of these products were confirmed by microanalytical results and spectral data or by spectral comparison with authentic samples available in our own laboratory.[1,2c]

The Table and the Figure show the variation in product distribution as a function of the temperature and reaction time. These results clearly demonstrate that the reaction takes place on slight warming and proceeds *via* initial formation of the ring-contracted product (**4**), which then leads to the concurrent formation of the reduced 10-thiaisoalloxazine (**3**) and the benzothiazole derivative (**5**). These observations can be reasonably explained by ring-contraction of (**1**) with addition of water (hydroxide ion) to give (**4**), followed by redox reaction between (**4**) and the remaining (**1**) to give compounds (**3**) and (**5**).

In fact, treatment of (**4**) with (**1**) under analogous conditions resulted in the smooth production of (**3**) and (**5**). With dry acetonitrile as a solvent, however, there was no appreciable reaction between (**1**) and (**4**), indicating the necessity of water for the redox reaction. No conversion of (**4**) into (**5**) was observed in the absence of (**1**), though (**4**) underwent the ring-cleavage to give (**5**) upon warming in aqueous acetonitrile in the presence of an electron acceptor such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone or 1,4-benzoquinone. It is obvious that (**1**) acts as an electron acceptor in this oxidative ring-cleavage.

The present reaction was accelerated by the addition of sodium bicarbonate or a pH 8.8 buffer solution, and occurred even at room temperature.

In view of the above facts, we propose a plausible reaction sequence for the present reactions as shown in Scheme 2. Our previous work[2a] has shown that (**1**) produces with ease the

† Reduction and oxidation potentials (in MeCN, V *vs* SCE) are measured to be $E^{red}_{1/2}$ = −0.38 for (**1**) and −0.81 for (**2**), and $E^{ox}_{1/2}$ = 0.59 for (**3**) by cyclic voltammetry.

Table. Temperature dependence of product distributions in the reaction of 3-methyl-10-thiaisoalloxazine (1) with water[a]

Temp.	Yield (%)[b] Recovered (1)	(3)	(4)	(5)
room temp.	100	0	0	0
40 °C	61	0	39	0
80 °C	17	20	42	21

[a] The reactions were carried out under the conditions as described in the Experimental section. [b] The yields were estimated by TLC densitometry.

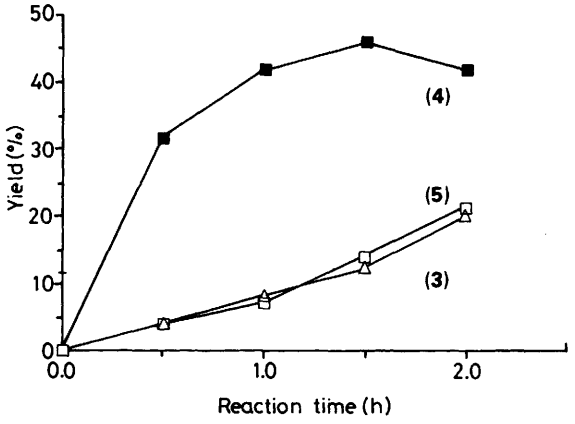
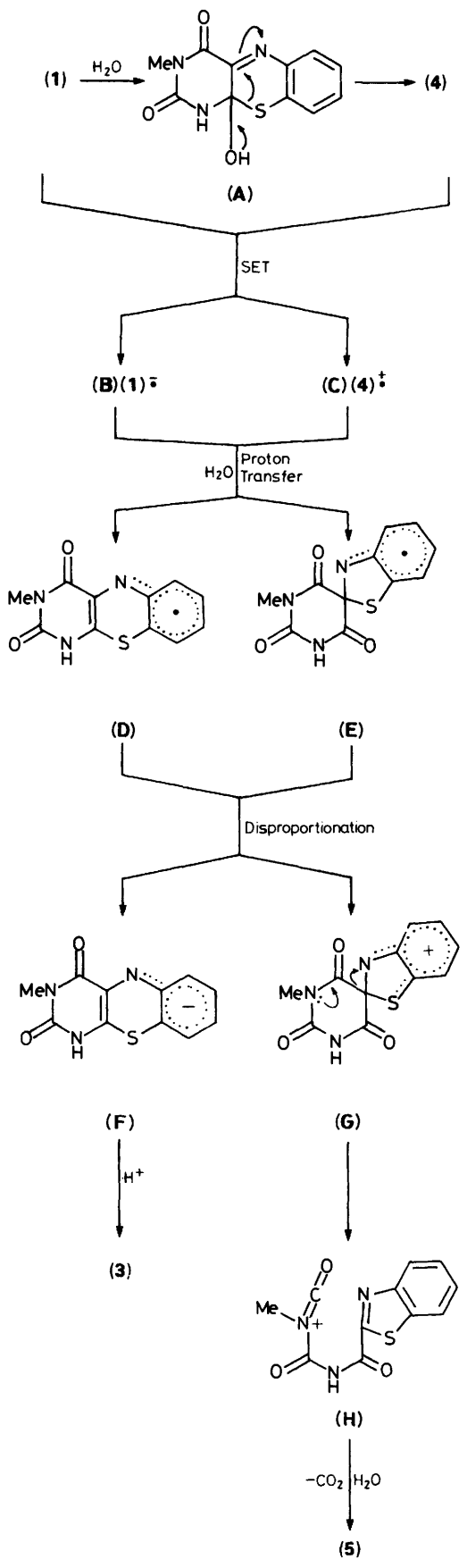


Figure. Changes of product distributions during the reaction of 3-methyl-10-thiaisoalloxazine (1) in aqueous acetonitrile at 80 °C.

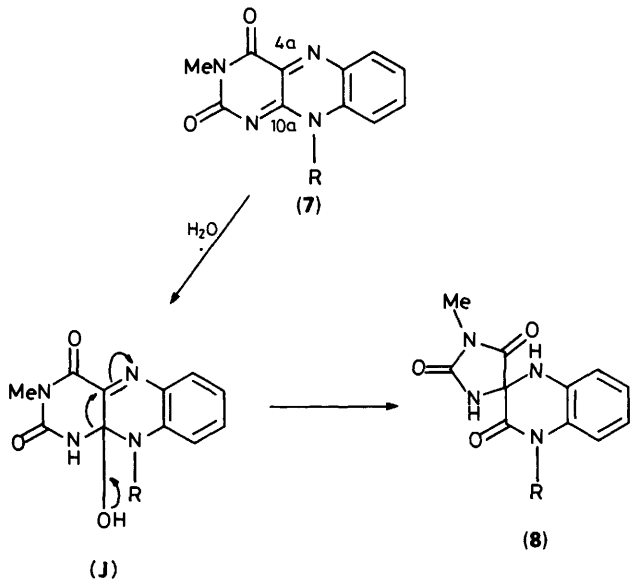
4a,10a-diadduct [*cf.* (6) in Scheme 1] upon treatment with methanol at room temperature, *via* an initial addition of methanol to the 10a-position in the conjugated imine system. In the present case, the ring-contraction of (1) to (4) could occur *via* an intermediate covalent 10a-monoadduct (A), initially produced because of the favourable juxtaposition of the thiohemiacetal and imine (C_{4a}=N_5) groups for intramolecular rearrangement. Single-electron transfer (SET) from (4) to the remaining (1) leads to the formation of a pair of radical ions (B) and (C). The redox chemistry of flavins is often rationalised by involvement of the SET process.[3] Subsequent proton transfer from (C) to (B) generates the radical species (D) and (E); in this stage, the intervention of water may be required to prevent a back electron transfer from (B) to (C).[4] Intermolecular disproportionation between (D) and (E) could give an ion pair (F) and (G). Fragmentation of the cation (G) gives rise to an iminoketenium ion intermediate (H) which can be converted into (5) *via* the addition of water followed by decarboxylation. The anion (F) is protonated to give (3).

There have been some investigations on the reaction of isoalloxazines with hydroxide ion.[5,6] Yoneda and co-workers[6] have demonstrated that 10-substituted 3-methylisoalloxazines (7) afford 1-methylspiro[imidazolidine-4,2′(1′*H*)-3′,4′-dihydroquinoxaline]-2,3′,5-triones (8) as a major product* upon treatment with 40% methanolic Triton B in DMF at room temperature. This indicates that nucleophilic attack of hydroxide ion on the conjugated di-imine bond of the isoalloxazine ring occurs mainly at the 10a-position [see (J) in Scheme 3], which is different from the reactive site (4a-position) for other nucleophiles such as thiols and sulphite ion.[3,6]

* The differences in the ring-contractions of (1) and (7) by hydroxide ion can be explained by considering the bond strength of the C_{10a}–S_{10} and C_{10a}–N_{10} bonds in transient intermediates, (A) and (J).



Scheme 2.



Scheme 3. R = 2',6'-$Me_2C_6H_3$, Ph, CH_2Ph, Et.

The ring-contraction of (7) to (8) on treatment with water, however, has been never observed even under drastic conditions; in fact, the isoalloxazine (2) is inert under the conditions employed in the case of (1). Thus, the present reaction of (1) with water demonstrates its unusual chemical reactivity involving a high susceptibility to nucleophilic addition and enhanced oxidation capacity compared with its parent isoalloxazines.

Experimental

M.p.s (uncorrected) were determined on a Yanagimoto micro-hot stage apparatus. Spectroscopic measurements were performed with the following instruments: IR spectrum with Hitachi Model 215 spectrometer; UV absorption spectrum with Shimadzu-260 spectrophotometer; ^1H NMR spectra with a JEOL JNM-GX270 (270 MHz) FT-NMR spectrometer using tetramethylsilane as an internal standard; mass spectrum with JEOL JMS-D300 machine operating at 70 eV. TLC was performed on Silica gel plates (Wako, Silicagel 70 F_{254} Plate-Wako) with a mixed solvent (methanol–chloroform, 1:10 or acetone–chloroform, 1:10). TLC scanning was performed on a Shimadzu CS-9000 dual-wavelength flying-spot scanner [detector 277 nm for (1)–(5)].

Reaction of 3-*Methyl*-10-*thiaisoalloxazine* (1) *with Water.*—(*a*) *Reaction at* 80 °C. A solution of (1) (49 mg, 0.2 mmol) in 33% aqueous acetonitrile (10 ml) was heated at 80 °C under argon and in the dark for 2 h. After removal of the solvent under reduced pressure, the residue was purified on a silica gel column (eluant, methanol–chloroform, 1:30) to afford 1,5-dihydro-3-methyl-10-thiaisoalloxazine (3), 1'-methylspiro[2,3-dihydrobenzothiazole-2,5'-pyrimidine]-2',4',6'-trione (4), and 1-(benzothiazol-2-yl)-carbonyl-3-methylurea (5). TLC analysis of the reaction mixture showed that 17% of the starting material (1) remained and that the product ratio of (3), (4), and (5) was 20:42:21. R_F values of (1), (3), (4), and (5) were as follows: 0.57 for (1), 0.41 for (3), 0.29 for (4), and 0.72 for (5) (solvent: methanol–chloroform, 1:10). The products (3) and (4) were identical in every respect with authentic samples.[1,2c] The structure of (5) was confirmed by microanalytical results and spectral data, m.p. 221–223 °C (from ethanol) (Found: C, 50.96; H, 3.99; N, 17.59. $C_{10}H_9N_3O_2S$ requires C, 51.06; H, 3.86; N, 17.87%); *m/z* 235 (M^+), 206 ($M^+ - 29$), 178 ($M^+ - 57$), 135 ($M^+ - 100$, 100%); ν_{max}(KBr) 3 350 (NH), 1 710 (C=O), and 1 680 (C=O) cm^{-1}; λ_{max}(MeCN) 293 (ε, 1.2 × 10^4), 243 (7.9 × 10^3), and 220 (1.4 × 10^4) nm; δ_H[$(CD_3)_2SO$] 2.80 (3 H, d, *J* 6 Hz, NCH_3), 7.64–7.74 (2 H, m, ArH), 8.05 (1 H, d, *J* 6 Hz, deuterium exchangeable, NH), and 8.22–8.33 (2 H, m, ArH).

(*b*) *Changes of product distributions during the reaction.* Compound (1) (in aqueous acetonitrile, 20mM) was heated at 80 °C and sampled every 30 min for 2 h. Analysis of these sampled mixtures was carried out by TLC densitometry, and the results were as follows; yields (reaction time, h): 4% (0.5), 8% (1.0), 12% (1.5), and 20% (2.0) for (3); 32% (0.5), 42% (1.0), 46 (1.5), and 42 (2.0) for (4); 4% (0.5), 7% (1.0), 14% (1.5), and 21% (2.0) for (5).

(*c*) *Temperature dependence of the product distributions.* Compound (1) (in 33% aqueous acetonitrile, 20 mM) was stirred at room temperature, 40 °C, or 80 °C under argon and shielded from daylight for 2 h. TLC analysis of the mixture clearly indicated that the product ratios of these reactions were markedly dependent upon temperature and that (4) was the first-formed product in the reaction. The results are shown in the Table.

Reaction of 3,10-*Dimethylisoalloxazine* (2) *with Water.*—Under analogous conditions to those for compound (1), the compound (2) was reacted with water at 80 °C for 2 h. The isoalloxazine (2), however, was inert under these conditions and most of (2) was recovered after purification by column chromatography (methanol–chloroform, 1:10).

Thermal Reaction of (4) *with* (1).—(*a*) *In the presence of water.* A solution of (1) (4.9 mg, 0.02 mmol) and (4) (5.2 mg, 0.02 mmol) in 33% aqueous acetonitrile (1.0 ml) was heated at 80 °C for 1 h under an argon atmosphere and shielded from daylight. TLC analysis of the reaction mixture showed the smooth formation of (3) and (5) in almost equal quantities. Under analogous conditions, (4) itself was very stable.

(*b*) *In the absence of water.* The above reaction of (4) with (1) was attempted in dry acetonitrile. TLC analysis of the mixture showed no reaction even after 2 h.

Redox Reaction between (4) *and Electron Acceptors.*—A solution of (4) (2.6 mg, 0.01 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (2.4 mg, 96% purity, 0.01 mmol) or 1,4-benzoquinone (BQ) (1.1 mg, 0.01 mmol) in 33% aqueous acetonitrile (1.0 ml) was heated in the dark at 80 °C under argon for 1 h. TLC analysis of the reaction mixtures showed the formation of (5) in 29% (for DDQ) and 9% (for BQ) yields, respectively. The structure of (5) was confirmed by spectral comparison with the authentic sample after purification.

Reaction of (1) *with Water in the Presence of a Base.*—(*a*) *pH* 8 *Borate buffer.* A solution of (1) (20mM) in pH 8 borate buffer–acetonitrile (1:2) was stirred at room temperature, under argon and in the dark. TLC analysis of the mixture after stirring for 5 h showed the consumption of (1) in 57% yield and the formation of (3), (4), and (5) in a product ratio of 14:28:11.

(*b*) *Sodium bicarbonate.* Under conditions analogous to the above case, a mixture of (1) (4.9 mg, 0.02 mmol) and sodium bicarbonate (1.7 mg, 0.02 mmol) in 33% aqueous acetonitrile (1.0 ml) was stirred for 5 h. TLC analysis of the reaction mixture showed 91% consumption of (1) and the formation (3), (4), and (5) in a product ratio of 30:12:49.

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