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Reactions of 10-Thiaisoalloxazines with Alkyl Amines and Benzyl Alcohol

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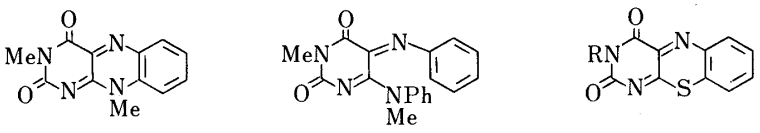
Upon treatment with benzylamine and *n*-propylamine, 10-thiaisoalloxazines **3** undergo a smooth ring-contraction initiated by nucleophilic addition of alkyl amines to the conjugated diimine bond in the molecule to give the corresponding 6′-substituted amino-spiro[benzothiazoline-2,5′-pyrimidine-2′,4′(3′*H*,5′*H*)-diones] **4**, whereas thermal reaction of **3** with benzyl alcohol results in the preferential formation of the redox products, 1,5-dihydro-10-thiaisoalloxazines (*cf.* **6**) and benzaldehyde, proceeding *via* an initial one-electron transfer process.

Keywords——10-thiaisoalloxazine; alkyl amine; ring-contraction; 6′-substituted amino-spiro-[benzothiazoline-2,5′-pyrimidine-2′,4′(3′*H*,5′*H*)-dione]; spiro[benzothiazoline-2,5′-pyrimidine-2′,4′,6′(1′*H*,3′*H*,5′*H*)-trione]; benzyl alcohol; redox reaction; 1,5-dihydro-10-thiaisoalloxazine; benzaldehyde; one-electron transfer process

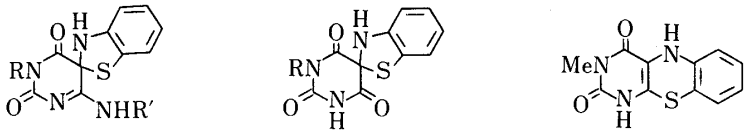
The conjugated diimine moiety (–N=C–C=N–) in the isoalloxazine ring plays a significant role in various biological oxidations catalyzed by flavin.[1] The inherent chemical properties of this moiety, however, are not clear as yet. Our attention has been directed to investigating the changes in the reactivity of the conjugated diimine moiety in the molecule caused by partial chemical modification of the isoalloxazine ring. Recently, we have demonstrated[2] that 6-(*N*-methylanilino)-5-(*N*-phenylimino)pyrimidine-2,4(3*H*,5*H*)-dione (**2**), a ring-opened analogue of 10-methylisoalloxazine **1**, possesses increased susceptibility to nucleophilic addition and an enhanced oxidation capacity compared with the parent isoalloxazine **1**. For example, treatment of **2** with benzyl mercaptan or benzylamine without any base catalyst under mild conditions resulted in the concurrent occurrence of nucleophilic substitution at the C(6)-position of **2** and a redox reaction. In this paper, we describe the reactions of 10-thiaisoalloxazines **3**,[3] in which the nitrogen atom at the 10-position of **1** is replaced by a sulfur atom, with alkyl amines and benzyl alcohol.[4]

A solution of 10-thiaisoalloxazine (**3a**) and a slight excess of benzylamine in acetonitrile was refluxed for 1 h under an argon atmosphere. Evaporation of the solvent followed by trituration of the obtained residue with diethyl ether yielded 6′-benzylamino-spiro-[benzothiazoline-2,5′-pyrimidine-2′,4′(3′*H*,5′*H*)-dione] (**4a**) in a pure state. No formation of other products in this reaction was detected by thin layer chromatographic (TLC) analysis of the reaction mixture. The reaction took place even under mild conditions, though a prolonged reaction time was required for the completion of the reaction (1 d at room temperature). The structure of the product **4a** was confirmed by the microanalytical results and spectral data (see the experimental section) and by the result of acid hydrolysis under mild conditions, leading to spiro[benzothiazoline-2,5′-pyrimidine-2′,4′,6′(1′*H*,3′*H*,5′*H*)-trione] (**5a**), which was prepared independently by the thermal condensation of alloxane with 2-aminothiophenol. Analogous ring-contraction was also observed on treatment of **3a** with *n*-



1 2 3

a : R=H
b : R=Me



4 5 6

a: R=H, R′=–CH_2Ph
b: R=H, R′=–*n*-Pr
c: R=Me, R′=–CH_2Ph
d: R=Me, R′=–*n*-Pr

a: R=H
b: R=Me

Chart 1

propylamine, and the employment of 3-methyl-10-thiaisoalloxazine (**3b**) instead of **3a** in this reaction gave the corresponding spiro[benzothiazoline-2,5′-pyrimidine-2′,4′(3′*H*,5′*H*)-diones] (**4b—d**).

We suggest that **4** is formed from **3** by the mechanism depicted in Chart 2,[5] in which the ring-contraction step is a benzylic acid type rearrangement similar to that proposed for the conversion of benzo[1,4]thiazine-2,3(4*H*)-diones to 2-carbamoylbenzothiazoles[6] and 3-chlorobenzo[1,4]oxazin-2-ones to 2-carbamoylbenzoxazoles[7] by amines.

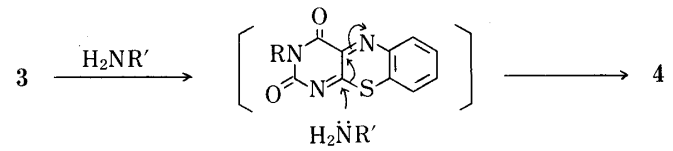


Chart 2

Thus, the treatment of **3** with alkyl amines under mild conditions resulted in the exclusive formation of the corresponding 6′-substituted amino-spiro[benzothiazoline-2,5′-pyrimidine-2′,4′(3′*H*,5′*H*)-diones] **4**, which could proceed by the nucleophilic attack of alkyl amines on the conjugated diimine bond of **3**, followed by ring-contraction.

On the other hand, heating of a mixture of **3b** and a large excess of benzyl alcohol without any solvent under an argon atmosphere with shielding from light at 120 °C for 2 h afforded 1,5-dihydro-3-methyl-10-thiaisoalloxazine (**6**) and benzaldehyde in moderate yields, together with the unchanged starting material **3b**. When the reaction was carried out in the absence of **3b** or under mild conditions (reflux in acetonitrile for 5 h), these products were not obtained. The reduced product **6** was identical with an authentic sample. No formation of intermediates in this reaction was detected by TLC analysis during the reaction. The present reaction was significantly suppressed by the addition of a trace amount of *p*-dinitrobenzene, a one-electron trapping reagent,[8] and was accelerated by exposure to daylight.[9] These observations suggest that a one-electron transfer process could be involved in the thermal reaction of **3b** with benzyl alcohol.

Taking into consideration the above results, the formation of benzaldehyde in this reaction could be explained by a redox mechanism, involving a one-electron transfer from benzyl alcohol to **3** in the initial stage, analogous to that firmly established for the oxidation of

alcohols by 2,3-dichloro-5,6-dicyano-*p*-benzoquinone[10] and *o*-chloranil.[11] The unique feature of the present reaction is that the oxidation of benzyl alcohol takes place without any base catalyst in the dark.[12]

It is generally accepted that the initial step in the oxidation of α-oxy acids, α-ketols, and α-amino acids by flavin is the dissociation of the C–H bond adjacent to the carbonyl group in the substrates by the base catalyst to generate the corresponding carbanion intermediates.[1] Oxidation of the intermediary carbanion, however, is not the sole mechanism available for the flavin-catalyzed oxidation. In particular, substrates which have no structural elements for stabilization of the carbanionic species, as in the case of glucose oxidase, could be oxidized *via* other processes without the initial carbanion formation. Our observation in the oxidation of benzyl alcohol by **3** is suggestive of the presence of an alternate process in the biological oxidation catalyzed by flavin.

In sharp contrast to the case of **3**, the isoalloxazine **1** did not react with alkyl amines and benzyl alcohol under the conditions employed above, resulting in the recovery of **1**. These findings indicate that the sulfur atom at the 10-position of the isoalloxazine ring results in an increase of susceptibility to nucleophilic addition and an enhancement of the oxidation capacity of **3** compared with the parent isoalloxazine **1**. The remarkable difference observed in the chemical reactivities of the conjugated diimine bond between **1** and **3** appears to reflect the difference of mesomeric effects of nitrogen and sulfur atoms at the 10-position in these compounds.

Experimental

All melting points were determined on a Yanagimoto micro hot stage apparatus and are uncorrected. Elemental analyses were performed at the Analytical Center in our university. Infrared (IR) spectra were recorded on a Hitachi 215 spectrometer in potassium bromide discs. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Hitachi R-24 B (60 MHz) spectrometer in deuteriodimethylsulfoxide or deuteriochloroform containing tetramethylsilane as an internal standard. Mass spectra (MS) were measured at 75 eV with a JEOL JMS-D300 spectrometer and ultraviolet (UV) spectra with a Shimadzu UV-260 spectrophotometer. Gas chromatographic (GC) analyses were carried out with a Hitachi 023 instrument. Column chromatography was performed on silica gel (Wako gel C-300) using chloroform–methanol as the eluent. TLC analyses were carried out by using silica gel plates (Silica gel 70 F_{254} Plate-Wako) and mixed solvents (chloroform–methanol and benzene–ethyl acetate).

Reaction of 10-Thiaisoalloxazines 3 with Benzylamine——A mixture of 10-thiaisoalloxazine (**3a**) (231 mg, 1.0 mmol) and benzylamine (0.15 ml, 1.4 mmol) in dry acetonitrile (20 ml) was refluxed for 1 h under an argon atmosphere. After removal of the solvent *in vacuo*, the residue was triturated with diethyl ether, collected by filtration, and recrystallized from ethanol to yield pure 6′-benzylamino-spiro[benzothiazoline-2,5′-pyrimidine-2′,4′(3′*H*,5′*H*)-dione] (**4a**) (257 mg, 76%); mp 244 °C. *Anal.* Calcd for $C_{17}H_{14}N_4O_2S$: C, 60.35; H, 4.17; N, 16.56. Found: C, 60.10; H, 4.21; N, 16.46. IR ν_{max}^{KBr} cm^{-1}: 3330 (NH), 1720 (C=O), 1695 (C=O). UV λ_{max}^{MeCN} nm (ε): 266 (8.9×10^3), 221 (2.2×10^4). ^{1}H-NMR (in DMSO-d_6) δ: 4.60 (2H, br d, J=6 Hz, –CH_2Ph, the signal coalesced to a singlet upon addition of deuterium oxide), 6.65—7.18 (4H, m, phenyl ring protons), 7.08 (1H, br, deuterium-exchangeable NH), 7.32 (5H, s, phenyl ring protons), 9.48 (1H, br t, J=6 Hz, deuterium-exchangeable benzylamino proton), 10.60 (1H, br, deuterium-exchangeable NH). MS *m/z*: 338 (M^+), 294 ($M^+ - 44$).

Under conditions analogous to those employed in the case of **3a**, the reaction of 3-methyl-10-thiaisoalloxazine (**3b**) (245 mg, 1.0 mmol) with benzylamine was carried out. Chromatographic separation (chloroform : methanol = 20 : 1) of the residue obtained after removal of the solvent yielded the corresponding spiro compound **4c** (250 mg, 71%) as an oily product. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1725 (C=O), 1670 (C=O). UV λ_{max}^{MeCN} nm (ε): 361 (9.1×10), 275 (7.7×10^3), 221 (2.4×10^4). ^{1}H-NMR (in DMSO-d_6) δ: 3.09 (3H, s, N_3-Me), 4.63 (2H, br s, –CH_2Ph), 6.68—7.23 (4H, m, phenyl ring protons), 7.15 (1H, br, deuterium-exchangeable NH), 7.35 (5H, s, phenyl ring protons), 9.56 (1H, br, deuterium-exchangeable NH). MS *m/z*: 352 (M^+), 294 ($M^+ - 58$).

Reaction of 3 with *n*-Propylamine——The reaction of **3a** (231 mg, 1.0 mmol) and *n*-propylamine (0.13 ml, 1.6 mmol) under conditions (reflux for 1 h) similar to those used in the reaction of **3a** with benzylamine, followed by recrystallization of the residue obtained after evaporation of the solvent, gave 6′-*n*-propylamino-spiro-[benzothiazoline-2,5′-pyrimidine-2′,4′(3′*H*,5′*H*)-dione] (**4b**) (203 mg, 70%); mp 249 °C (from ethanol). *Anal.* Calcd for $C_{13}H_{14}N_4O_2S$: C, 53.79; H, 4.86; N, 19.30. Found: C, 53.65; H, 4.98; N, 19.02. IR ν_{max}^{KBr} cm^{-1}: 3320 (NH), 3200 (NH), 1725 (C=O), 1695 (C=O). UV λ_{max}^{MeCN} nm (ε): 266 (1.3×10^4), 223 (2.2×10^4). ^{1}H-NMR (in DMSO-d_6) δ: 0.88

(3H, br t, $-CH_2-CH_2-CH_3$), 1.25—1.85 (2H, m, $-CH_2-CH_2-CH_3$), 3.10—3.55 (2H, m, $-CH_2-CH_2-CH_3$), 6.58—7.21 (4H, m, phenyl ring protons), 7.08 (1H, br, deuterium-exchangeable NH), 8.98 (1H, br, deuterium-exchangeable NH), 10.52 (1H, br, deuterium-exchangeable NH). MS *m/z*: 290 (M^+), 246 (M^+-44).

The reaction of **3b** (245 mg, 1.0 mmol) and *n*-propylamine under conditions employed above and the purification of the reaction mixture by column chromatography (chloroform : methanol = 20 : 1) gave the corresponding spiro compound **4d** (252 mg, 83%) as an oily product. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1725 (C=O), 1670 (C=O). UV λ_{max}^{MeCN} nm (ε): 361 (1.3×10^2), 276 (7.7×10^3), 221 (2.5×10^4). ^{1}H-NMR (in DMSO-d_6) δ: 0.89 (3H, br t, $-CH_2-CH_2-CH_3$), 1.35—1.83 (2H, m, $-CH_2-CH_2-CH_3$), 3.08 (3H, s, N_3–Me), 3.17—3.55 (2H, m, $-CH_2-CH_2-CH_3$), 6.69—7.22 (4H, m, phenyl ring protons), 7.07 (1H, br, deuterium-exchangeable NH), 9.06 (1H, br, deuterium-exchangeable NH). MS *m/z*: 304 (M^+), 246 (M^+-58).

Acid Hydrolysis of 4——A solution of **4a** (170 mg, 0.5 mmol) in acetonitrile (10 ml) containing a small amount of 6 N HCl (0.05 ml) was stirred at room temperature for 3 h. The precipitated solid was collected by filtration and recrystallized from ethanol to yield spiro[benzothiazoline-2,5′-pyrimidine-2′,4′,6′(1′*H*,3′*H*,5′*H*)-trione] (**5a**) (117 mg, 94%); mp 250 °C. *Anal.* Calcd for $C_{10}H_7N_3O_3S$: C, 48.20; H, 2.83; N, 16.86. Found: C, 47.92; H, 2.89; N, 16.81. IR ν_{max}^{KBr} cm^{-1}: 3370 (NH), 3270 (NH), 3220 (NH), 1770 (C=O), 1750 (C=O), 1715 (C=O). UV λ_{max}^{MeCN} nm (ε): 302 (2.8×10^3), 222 (2.0×10^4). ^{1}H-NMR (in DMSO-d_6) δ: 6.46 (1H, br, deuterium-exchangeable NH), 6.57—7.12 (4H, m, phenyl ring protons), 11.25 (2H, br, deuterium-exchangeable NH). MS *m/z*: 249 (M^+), 221 (M^+-28), 204 (M^+-45).

The acid hydrolysis of **4c** was carried out under conditions similar to those employed in the case of **4a**, to obtain **5b** (96%); mp 198 °C (from ethanol). *Anal.* Calcd for $C_{11}H_9N_3O_3S\cdot H_2O$: C, 46.98; H, 3.94; N, 14.94. Found: C, 47.22; H, 3.94; N, 14.86. IR ν_{max}^{KBr} cm^{-1}: 3390, 3260 (NH), 3200 (NH), 1760 (C=O), 1725 (C=O), 1690 (C=O). UV λ_{max}^{MeCN} nm (ε): 302 (2.0×10^3), 220 (2.0×10^4). ^{1}H-NMR (in $CDCl_3$) δ: 3.29 (3H, s, N_3Me), 5.08 (1H, br, deuterium-exchangeable NH), 6.93—7.15 (4H, m, phenyl ring protons), 8.74 (1H, br, deuterium-exchangeable NH). MS *m/z*: 263 (M^+), 235 (M^+-28), 206 (M^+-57).

Thermal Condensation of Alloxane with 2-Aminothiophenol——A solution of alloxane monohydrate (320 mg, 2.0 mmol) and 2-aminothiophenol (0.32 ml, 3.0 mmol) in acetonitrile (20 ml) was refluxed for 5 h. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography (chloroform : methanol = 10 : 1) and recrystallized from ethanol to give **5a** (282 mg, 57%).

Thermal Reaction of 3b with Benzyl Alcohol——A solution of **3b** (245 mg, 1.0 mmol) in benzyl alcohol (10 ml) without any solvent was heated at 120 °C under an argon atmosphere with shielding from light for 2 h. GC analysis of the reaction mixture proved the presence of benzaldehyde (48% yield based on **3b** employed). After removal of the solvent *in vacuo*, 1,5-dihydro-10-thiaisoalloxazine (**6**) (143 mg, 58%) was isolated by trituration of the residue with chloroform followed by filtration. The starting material **3b** (74 mg, 30%) was recovered by column chromatography of the filtrate.

References and Notes

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4) We have already reported that treatment of **3** with lower primary alcohols or thiols under mild conditions affords the 4a,10a-diadducts or 4a,10a-cyclic adducts across the conjugated diimine bond of **3**, whereas **3** readily oxidizes dihydrolipoamide and 1,4-butanedithiol even under mild conditions with shielding from light. *Cf.* Y. Maki, M. Tanabe, Y. Kojima, M. Sako, and K. Hirota, *Heterocycles*, **22**, 13 (1984); M. Sako, Y. Kojima, K. Hirota, and Y. Maki, *ibid.*, **22**, 1017 (1984).
5) Previously, we have demonstrated that the initial addition site in the conjugated diimine moiety of **3** is the 10a-position. *Cf.* Y. Maki, M. Tanabe, M. Sako, K. Hirota, and K. Harano, *Chem. Lett.*, **1983**, 1093.
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12) Yoneda *et al.* have demonstrated that 2*H*-chromeno[2,3-*d*]pyrimidine-2,4(3*H*)-diones, which are deazaflavin analogues, and 10-substituted pyrimido[5,4-*g*]pteridines oxidize benzyl alcohol and cyclopentyl alcohol under thermal conditions analogous to those employed in the case of **3**, though they did not propose a detailed mechanism for these redox reactions. *Cf.* F. Yoneda, R. Hirayama, and M. Yamashita, *Chem. Lett.*, **1980**, 1157; T. Nagamatsu, E. Matsumoto, and F. Yoneda, *ibid.*, **1982**, 1127. We have also recently confirmed that **2** possesses the capacity to oxidize benzyl alcohol under thermal conditions without any base catalyst. *Cf.* ref. 2. The results obtained in the present paper provide a further example of the flavin-mimicking oxidation of alcohols under neutral conditions.