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Thiamine Derivatives of Disulfide Type. XI.[1] Oxidation of Dipropyl Disulfide with Hydrogen Peroxide and Properties of the Products[2]

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Investigation was made on the oxidation of dipropyl disulfide with hydrogen peroxide in acetic acid, as a basis for kinetic studies in the synthesis of thiamine propyl disulfide (TPD). Separation and detection methods of the oxidation products by thin-layer chromatography were established. Among these products, thiolsulfinate (PrSSOPr), thiolsulfonate ($PrSSO_2Pr$), sulfinic acid and sulfonic acid were identified. The stability, solubility, IR and UV-spectrum of PrSSOPr and $PrSSO_2Pr$, which react with thiamine to produce TPD, were examined.

Thiamine propyl disulfide (TPD) is one of the disulfide type thiamine derivatives having excellent properties such as being stable against aneurinase and good in absorption through intestinal tracts to sustain high level in blood.[4] As one of the method for TPD synthesis, the reaction between thiamine and propyl propanethiolsulfinate (PrSSOPr) or propyl propanethiolsulfonate ($PrSSO_2Pr$) has been reported by Matsukawa, *et al.*[5] The mechanism of these reactions, however, has not been made clear though it is important in the chemistry and biochemistry of thiamine and thiamine derivatives. The authors have planned a kinetic study on these reactions for TPD production as one of the series of the studies on the basic properties of the disulfide type thiamines. Matsukawa and co-workers[5] prepared PrSSOPr and $PrSSO_2Pr$ used for the TPD production according to the method of Stoll and Seebeck[6] (oxidation of disulfide by hydrogen peroxide), taking only the adjustment of molar ratio of hydrogen peroxide to the disulfide into consideration. Oxidation of the disulfide, however, has been found to be very complicated and the properties of PrSSOPr and $PrSSO_2Pr$ remained unclarified. We have, therefore, attempted to make clear these points. The present paper deals with a basic research on the mechanism of the reaction between thiamine and the oxides of dipropyl disulfide.

Experimental

Materials——Dipropyl Disulfide: It was supplied by Takeda Chemical Industries, Ltd. bp 192.5°[7] Sodium Propylsulfonate: It was prepared according to the method of Houlton and Tartar.[8] IR ν_{max}^{KBr} cm^{-1}: 1180, 1060, 790.[9]

1) Part X: H. Nogami, N. Ikari, K. Andō, and K. Takeuchi, *Yakugaku Zasshi*, **90**, 418 (1970).
2) This work was presented to the Meeting of Kanto Branch Pharmaceutical Society of Japan, Tokyo February 1967.
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4) T. Matsukawa, S. Yurugi, H. Kawasaki, Y. Aramaki, and Z. Suzuoki, *Takeda Kenkyusho Nenpo*, **12**, 1 (1952).
5) T. Matsukawa and S. Yurugi, *Yakugaku Zasshi*, **72**, 1616 (1952); T. Matsukawa and H. Kawasaki, *ibid.*, **73**, 216 (1953).
6) A. Stoll and E. Seebeck, *Experimentia*, **3**, 114 (1947).
7) K. Kawahara, *Yakugaku Zasshi*, **77**, 964 (1957).
8) H.G. Houlton and H.V. Tartar, *J. Am. Chem. Soc.*, **60**, 544 (1938).
9) A.D. Cross, "An Introduction to Practical Infra-Red Spectroscopy," Butterworths Scientific Publications, London, 1960, p. 94.

Sodium Propylsulfinate: It was prepared by the reaction of thiolsulfonate and thiol.[10] A mixture of $PrSSO_2Pr$ and propyl mercaptan in MeOH, was stand at room temperature for five hours. MeOH and excess of propyl mercaptan were removed under reduced pressure. The residue was taken up in ether and the solution was extracted with 2% Na_2CO_3. The Na_2CO_3 solution was washed with ether and was evaporated to dryness. The residue was extracted with absolute EtOH and EtOH was evaporated. Strongly hydroscopic white powder. IR ν_{max}^{KBr} cm^{-1}: 1010, 980.[11]

TPD: It was presented by Takeda Chemical Industries, Ltd. mp 128° (decomp.).

All other chemicals and solvents were reagent grade.

Oxidation of Dipropyl Disulfide——To a solution of disulfide dissolved in thirty times of AcOH, a certain portion of H_2O_2 (30%) was added. This reactions were conducted at 25° for a fixed period (usually 24 hr) and at 100° for 0.5 hr.

Thin-Layer Chromatography (TLC)——Samples were withdrawn at different time intervals from the oxidation solutions, spotted on the silica gel (Wakogel B-5, Wako Pure Chem. Ind., Ltd.) thin-layer plates of 0.25 mm thickness and the spots were developed with heptane: dioxane (9:1 v/v) or benzene. Ultraviolet (UV)-lamp and the following reagents were used for the detection of spots developed.

$KMnO_4$-Reagent: Approximately 5% portion of 2N H_2SO_4 was added to 2% $KMnO_4$ (yellow spots on a purple background).

I_2-Vapour: I_2 vapour was fulfilled in a desiccator. (Brown spots on a white background).

BPB Reagent: 0.1% Brom phenol blue solution adjusted to pH 7. (yellow spots on a blue background).

Cysteine-BPB Reagent: According to the method of Barnard and Cole,[12] 2% cysteine and 0.1% brom phenol blue solution adjusted to pH 7 was freshly prepared (yellow spots on a blue background.).

HCl-KI-Starch Reagent: Modified the method of Barnard and Cole,[12] and Thompson, *et al.*,[13] the plates developed were exposed to hydrochloric acid fume about 1 min. and after removal of fume, sprayed by KI-starch solution (0.5% KI and 5% water soluble starch) (Blue-violet spots on a white background.).

As illustrated in Fig. 1, eight spots (A-H) were detected in all. (H shows dipropyl disulfide produced by the decomposition of oxidation products. A and B are on starting point.)

All spots except A were detected by $KMnO_4$ or I_2-vapour reagent. B, D, E, and F(E and F: weak) were detected by HCl-KI-starch reagent. A and B were detected by BPB reagent and D,E and F (D and F:weak) were detected by cysteine-BPB reagent.

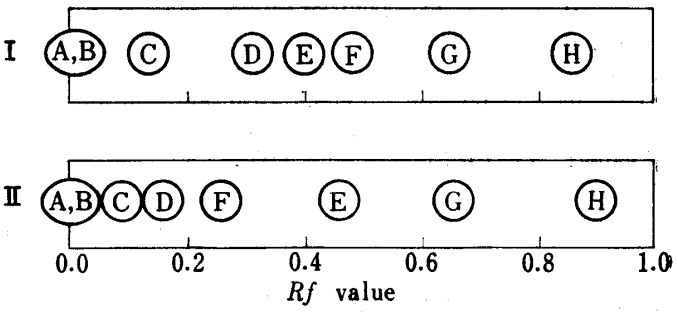

Fig. 1. Thin-Layer Chromatographic Detection of the Compounds obtained from the Oxidation of Dipropyl Disulfide by Hydrogen Peroxide in Acetic Acid

plate: silica gel
developing solution I: heptane: dioxane (9: 1) II: benzene
reagent (positive) HCl-KI-starch: B,D,E, and F; BPB: A, and B; cysteine-BPB: D,E, and F; $KMnO_4$ or I_2: B,C,D,E,F, G, snd H

Isolation of Oxidation Products——Fraction C, D, E, G, and H were isolated by the following method. The reaction solution oxidised by one molar portion of H_2O_2 at 100° for 0.5 hr was poured into water. $NaHCO_3$ was added to the solution in order to neutralize AcOH and the solution was extracted by ether. Ether layer was separated, washed with water, dried with Na_2SO_4 and concentrated by evaporation. The concentrated ether solution was spotted linearly on the silica gel plates of 1 mm thickness and developed using benzene or heptane: dioxane (9: 1 v/v). Each fraction marked with UV-lamp *etc.* and raked together with spatulla. Each powder separated was packed into the column for chromatography and eluted with ether. These procedures were repeated, until one spot on chromatogram was obtained. Although the developing solvent was adequately chosen according to fractions, both solvent system benzene and heptane-dioxane were used alternatively on usual separation.

For fraction D (or E), the column chromatography was also available. The residue of ether extraction prepared by the above method from D (or E) rich solution, was introduced over a silica gel column. Elution was conducted with hexane at first then with benzene. Benzene eluate was collected in every five ml. Each

10) R. Otto and A. Rössing, *Chem. Ber.*, **20**, 2079 (1887); S. Smiles and D.T. Gibson, *J. Chem. Soc.*, **125**, 176 (1924).
11) J.L. Bellamy, "Organic Sulfur Compounds," Vol. 1, ed. by N. Kharasch, Pergamon, Press, New York, N. Y., 1961, p. 47.
12) D. Barnard and E.R. Cole, *Arch. Chim. Acta*, **20**, 540 (1963).
13) J.F. Thompson, W.N. Arnord and C.J. Morris, *Nature*, **197**, 380 (1963).

fraction was checked by TLC, gathered, and evaporated with *vacuo* to remove benzene completely. Physical chemical properties of these fractions are listed as follows:

C: Sticky liquid, IR ν_{max}^{cap} cm^{-1}: 3420, 1740, 1055.

D: UV $\lambda_{max}^{2\%EtOH} m\mu$ (ε): 240 (2100), IR cm^{-1}: ν_{SO} 1085. *Anal.* Calcd. for $C_6H_{14}OS_2$: C, 43.4; H, 8.5. Found: C, 43.6; H, 8.6. Solubility in H_2O *ca.* 1% (dipropyl disulfide *ca.* 0.01%). It is unstable even at room temperature and decomposed to fraction C and H, then it should be stored in refregerator.

E: It has no specific UV absorption over 200 mμ. IR cm^{-1}: ν_{SO_2} 1131 (sym.), 1342 (asym.). *Anal.* Calcd. for $C_6H_{14}O_2S_2$: C, 39.6; H, 7.9. Found: C, 39.5; H, 7.6. Solubility in H_2O. 0.01—0.1%.

F: It is unable to isolate by the above method, because of the decomposition to fraction D, H, E, G *etc.* during the isolation procedure.

G: It has an offensive odor and IR ν_{max} cm^{-1}: 1755.

H(dipropyl disulfide produced by the decomposition of oxidation products): It is identified by infrared (IR) spectrophotometry.[14]

Fraction A was isolated by the following method.

From the oxidation solution by six molar portions of H_2O_2 for 24 hours, AcOH was evaporated under reduced pressure at 50°. The residue was dissolved with 1N NaOH until neutralized (the volume of 1N NaOH required approximately agreed to that estimated as sulfonic acid). The solution was dried and the residue was crystallized from EtOH (white amorphous powder).

Fraction B was isolated by the following method.

From the solution oxidized by four molar portions of H_2O_2 for 24 hours, AcOH was removed under reduced pressure at room temperature. The residue was dissolved in ether and the solution was extracted with 2% Na_2CO_3. Na_2CO_3 layer was thoroughly washed with ether and dried under reduced pressure. The residue was extracted with absolute EtOH and EtOH was evaporated (white amorphous powder).

Stability of Oxidation Products——Each fraction isolated by the above method (except F), was treated in the following conditions and examined by TLC.

a) 25°, 2 weeks.

b) 100°, 0.5 hour in AcOH.

c) 60°, 2 hours in pH 8 phosphate buffer containing 10% EtOH (each fraction dissolved in EtOH was added to the buffer, the condition was the same as in the reaction with thiamine).

Reaction with Thiamine——According to the method of Matsukawa, *et al.*,[5] EtOH solution of each fraction (except F) was added to aqueous solution of thiamine (the volume of EtOH was 10% finally) and kept at pH 8 at 60° for 2 hours. Reaction solutions were examined by TLC; silica gel plate of 0.25 mm thickness, MeOH as developing medium, dragendorff and cysteine–ferricyan reagents for the identification (*Rf* value: TPD 0.75, thiamine 0.05).

Result and Discussion

Detection of Oxidation Products by TLC

On the oxidation of disulfide, different oxidation products have been assumed. Yoshimura[15] has reported the method of detection of dipropyl disulfide and PrSSOPr by paper partition chromatography. As the method of the detection for oxides of disulfide, Barnard, *et al.*,[12] and Thompson, *et al.*[13] have reported on paper partition chromatography, and Calam, *et al.*[16] have investigated on paper electrophoresis. We investigated on TLC analysis which is more convenient and more advantageous. As the result it was found that the developing medium of heptane: dioxane (9:1) or benzene on silica gel plate was suited in this case. As the method of detection, such a reagent as HCl–KI–starch[12,13] or cysteine-BPB[11] used in paper partition chromatography, could be also suitably applied on thin-layer chromatography. But hydroxylamine reagent[15] was unsuitable in the case of TLC.

Oxidation Conditions and Products

Fig. 2 shows the chromatograms of solution oxidized by different molar portions of hydrogen peroxide at 25°. When hydrogen peroxide was one molar, D was major product and E and F was mixed. The production of E was also seen in the case of one half molar hydrogen peroxide. When hydrogen peroxide were increased in the portions of two molar,

14) J.V. Jacobsen, R.A. Barnhard, L.K. Mann and A.R. Saghir, *Arch. Biochem. Biophys.*, **104**, 473 (1964).
15) M. Yoshimura, *Vitamin*, **14**, 627 (1958).
16) D.H. Calam and S.G. Waley, *Biochem. J.*, **85**, 410 (1962).

D was rich at initial stage and E, G and B were increased with the time elapsed. In four molar portions, at first D, E, F and B were found and after 24 hours D was little observed. In six molar portions, the similar course was revealed, but after 24 hours A became predominant product.

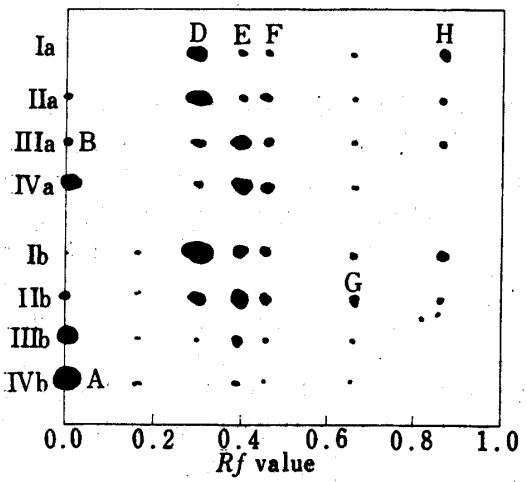

Fig. 2. Thin-Layer Chromatograms Illustrating the Oxidation of Dipropyl Disulfide by Hydrogen Peroxide in Acetic Acid at 25°

I: 1 molar H_2O_2 for dipropyl disulfide, II: 2 molar, III: 4 molar, IV: 6 molar
a: after 2 hr b: after 24 hr
developing solution: heptane: dioxane (9: 1)

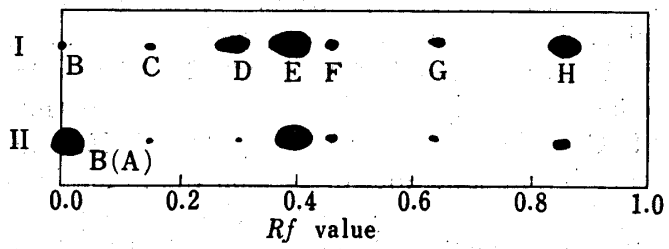

Fig. 3. Thin-Layer Chromatograms Illustrating the Oxidation of Dipropyl Disulfide by Hydrogen Peroxide in Acetic Acid at 100° for 0.5 hr.

I: 1 molar H_2O_2 for dipropyl disulfide, II: 2 molar
developing solution: heptane: dioxane (9: 1)

When the molar ratios of peroxide were 0.5 to 1 at 100°, various spots (B-H) were detected as illustrated in Fig. 3. However, in more than two molar portions, the spots observed were nearly equal to those after 24 hours at 25° and D was little observed. As the result of these experiment, it was revealed that a single oxidation product could not be prepared only by the control of molar ratio of hydrogen peroxide, but at least a mixture of several products was obtained. On this view point, PrSSOPr (regarded to be product by one molar hydrogen peroxide) or $PrSSO_2Pr$ (two molar hydrogen peroxide) used in the earlier report[5] was suggested to be contaminated with other products.

Isolation and Identification of Oxidation Products

From the observation for the progress of the oxidation on chromatogram and for the coloration by reagents, it has been suggested that A was sulfonic acid, B sulfinic acid, D PrSSOPr and E $PrSSO_2Pr$. The suggestion was identified by the following results. A and B, acidic substances obtained from the reaction solution oxidized by six and four molar portions of hydrogen peroxide, were essentially identical with the authentic samples in IR spectra. D and E separated from the oxidation solution were also identified by elementary analyses, IR and UV spectra.

Evidence was obtained to indicate that the oxidation pathway was at least shown as follows

$$PrSSPr \longrightarrow PrSSOPr \longrightarrow PrSSO_2Pr \longrightarrow PrSO_2H \longrightarrow PrSO_3H$$

where PrSSPr is dipropyl disulfide, $PrSO_2H$ propylsulfinic acid and $PrSO_3H$ propylsulfonic acid. C, F, and G have not been identified. Among these products (except F), PrSSOPr and $PrSSO_2Pr$ had the reactivity with thiamine, which become a main purpose of this series of study, then the separation of PrSSOPr and $PrSSO_2Pr$ by column chromatography and the investigation on their properties were conducted. It has been reported that methionine

sulfoxide could be separated by gas chromatography[17] and the purification of aliphatic sulfoxides contaminated with further oxidation products could be accomplished by ion-exchange chromatography.[18] In this case, it was found that silica gel column was suited for the separation of PrSSOPr especially. On alumina column, an interesting phenomenon was observed; $PrSSO_2Pr$ converted into PrSSOPr. The reaction is similar with the hydrolytic breakdown of $PrSSO_2Pr$ by alkali described later.

Properties of PrSSOPr and $PrSSO_2Pr$

PrSSOPr (D)——A strong absorption maximum characteristic of SO stetching vibration at 1085 cm^{-1} in IR spectrum. Although ν_{SO} is generally shown at nearly 1050 cm^{-1}[19] a slight shift is observed in disulfide bond (such as cystine[19] and benzoyl thiamine disulfide.[20] The λ_{max} at 240 mμ (ε 2100) in 2% EtOH in ultraviolet (UV) spectrum is closely related to the fact that simple sulfoxides have λ_{max} at 210 mμ (ε 1500)[21] and agrees well with the fact that MeSSOMe shows λ_{max} at 246 mμ (ε 2080) in EtOH.[22] It has been explained that such a strong absorption in sulfoxides derives on the formation of hydrogen bond[21] with solvent. In this case, the existance of resemble sulfoxide group in SSO may be confirmed. Further the change in the concentration of PrSSOPr (10^{-4}—10^{-2} M) and in pH (2—10) had not effect on its spectrum. It can be explained by the formation of hydrogen bond that the water solubility of PrSSOPr (*ca.* 1%) becomes higher than that of dipropyl disulfide (*ca.* 0.01%).

$PrSSO_2Pr$ (E)——Strong absorption maximums are shown at 1130, 1324 cm^{-1} in IR spectrum. Such maximums are shown in sulfones and the former absorption belongs to symmetric stretching vibration and the latter asymmetric.[9,11,23] In respect of the UV spectrum, $PrSSO_2Pr$ has no specific absorption maximum in the wave length longer than 200 mμ. The observation may be considered similarly with the fact that sulfones are transparent to radiations of wave lengths longer than 180 mμ.[21] Sulfoxides form strong hydrogen bond as described above, on the contrary sulfones weak.[24] In disulfide bond, a similar tendency may be confirmed, *viz.* SSO>SSO_2. The water solubility of $PrSSO_2Pr$ (0.01—0.1%) is lower than that of PrSSOPr.

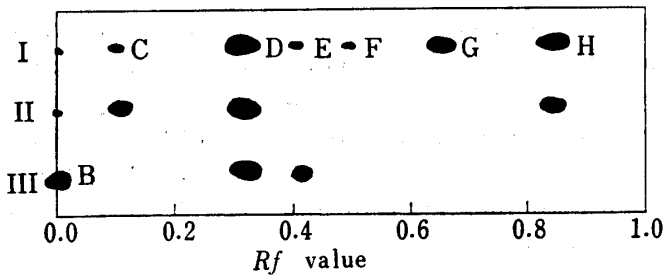

Fig. 4. Thin-Layer Chromatograms Illustrating the Stability of PrSSOPr (D) and $PrSSO_2Pr$ (E)

I: PrSSOPr at 100° for 0.5 hr in acetic acid
II: PrSSOPr at 20—25° for 2 wk
III: $PrSSO_2Pr$ st 37° for 2 hr in pH 8 phosphate buffer
developing solution: heptane: dioxane (9: 1)

Stability and Reactivity of Oxidation Products

PrSSOPr is rather unstable and the heating in acetic acid causes degradation to give various spots as shown in Fig. 4. The chromatogram is resemble with that of oxidation of dipropyl disulfide by one molar portion of hydrogen peroxide at 100° as shown in Fig. 3. Therefore, it can be assumed that some of oxidation products given in Fig. 3 derived from the unstability of PrSSOPr. This phenomenon agrees with that of Barnards' report used ^{35}S labelled thiolsulfinate, in

17) D.E. Johnson, S.J. Scott and A. Meister, *Anal. Chem.*, **33**, 669 (1961).
18) V. Horak and J. Pecka, *J. Chromatog.*, **14**, 97 (1964).
19) W.E. Savige, J. Eagar, J.A. Maclaren and C.M. Roxburgh, *Tetrahedron Letters*, **1964**, 3289.
20) I. Utsumi, K. Harada and G. Tsukamoto, *Vitamin*, **29**, 556 (1964).
21) A.E. Gillam and E.S. Stern, "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry," Edward Arnold Ltd., 1955, p. 59.
22) H.J. Backer and H. Kloosterxiel, *Rec. Trav. Chim.*, **73**, 129 (1954).
23) B.J. Sweetman, *Nature*, **183**, 744 (1959).
24) A. Mangini, *Gazz. Chim. Ital.*, **88**, 1063 (1958).

which thiolsulfinate undergoes complicated disproportionation, breakage, scrambling.[25] It was also reported that such a decomposition proceeds easily in the existence of P_2O_5 under *vacuo*.[26] Similar tendency was also observed in PrSSOPr, thus such a kind of drying method is inapplicable. PrSSOPr is also unstable even at room temperature and degrades mainly into C and dipropyl disulfide. On the other hand, $PrSSO_2Pr$, C, and G was stable in heat-treatment in acetic acid, but sulfinic acid was decomposed. The unstability of sulfinic acid has also been reported in 1-dodecanesulfinic acid.[27] Among these compounds (except F), PrSSOPr and $PrSSO_2Pr$ react with thiamine to give TPD. Further it was found that $PrSSO_2Pr$ undergoes the following hydrolysis by alkali similar with SS dioxide of cystine.[19]

$$PrSSO_2Pr \xrightarrow{H_2O} PrSO_2H + 1/2PrSSOPr$$

As described above, the reactivities of the oxidation products of disulfides are much complicated, then, further investigations are expected.

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25) D. Barnard, J.M. Fabian and H.P. Koch, *J. Chem. Soc.*, 1949, 2442.
26) D. Barnard, *J. Chem. Soc.*, 1957, 4675.
27) C.S. Marvel and R.S. Johnson, *J. Org. Chem.*, 13, 822 (1948).