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Synthesis of Hypothiaminic Esters[1]

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In a previous paper we reported that hypothiaminic acids (I, VIII) would undergo disproportionation in methanolic hydrogen chloride to the corresponding thiolsulfonates (VII) on the basis of the formation of product (A), which was positive toward thiochrome reaction after treatment with cysteine and isolation of thiaminic acid (VI).[2]

We have made an attempt to isolate the product (A) and confirm its structure.

A solution of I in methanolic hydrogen chloride gave crystals (A), mp 110—112°C. Analogously, the same treatment in ethanolic hydrogen chloride solution of I afforded crystals (B), mp 118—120°C, which were oxidized with hydrogen peroxide to VI.

The structures of these compounds (A, B) were confirmed to be not thiolsulfonate-type compound (VII) but sulfinic esters (V) by means of NMR spectra.

The preparation of sulfinic ester by the reaction of I with diazomethane and the action of ethyl chloroformate on II in methanol gave also Va.[3]

However, sodium hypothiaminate (IX) was treated with ethyl chloroformate in chloroform to yield colorless prisms (mp 133—135°C). On the basis of spectral and analytical data, the structure of the compound was confirmed to be 1,2-oxathiolane-2-oxide derivatives (XII, hereinafter called sulfinothiamine). So far as we know, there is no report on 5-membered ring sulfinic ester.

Compound VIII was allowed to react with thionyl chloride to give sulfinyl chloride (XI) which upon the action of base cyclized to XII. XI could be prepared simply by treating X or XIV with chlorine. Similarly, the disulfide III was converted to open-chain sulfinic ester Vb by chlorine oxidation.

It was noteworthy that the bridge methylene of XII between the pyrimidine and *N*-formyl moiety showed spectrum of AB system (J=15 cps). The magnetic non-equivalence of the methylene seems to be due to a rotational restriction caused by the bonding between the 4-amino group and $^{+}S{=}O^{-}$ group. This might be supported by the fact that no splitting of the methylene was observed in 1,2-oxathiolane XIII, synthesized recently by Nakano and Nishimura.[4] Compound XIII was oxidized with perbenzoic acid to give XII.

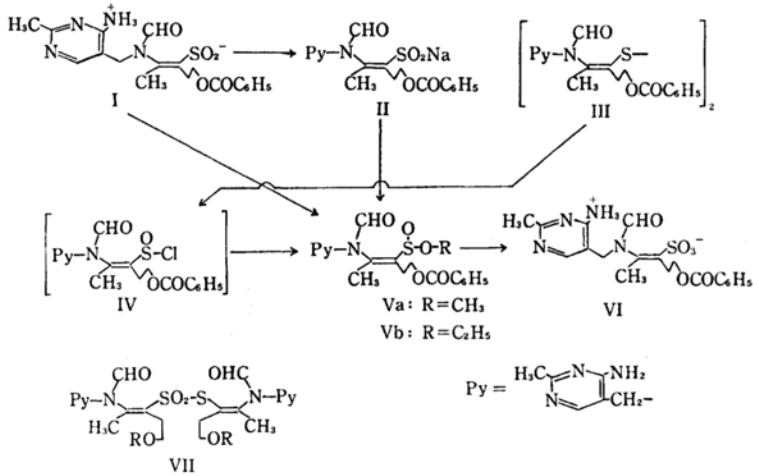


Fig. 1.

1) Part XXXIII in a series on "Studies on Thiamine Disulfide."

2) I. Utsumi, T. Watanabe, K. Harada and G. Tsukamoto, *Chem. Pharm. Bull.* (*Tokyo*), **15**, 1485 (1965).

3) Houben-Weyl, "Methoden der Organischen Chemie" 4 Auflage, Georg Tieme Verlag, **9**, 338 (1955).

4) J. Nakano and O. Nishimura, Abstracts of the 88th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1968, p. 196.

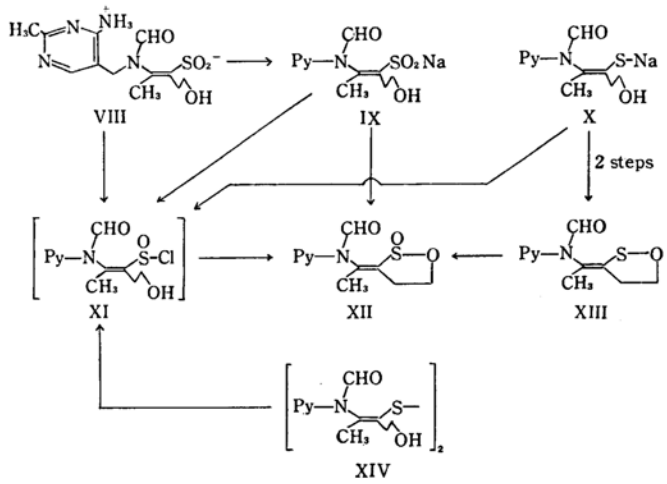
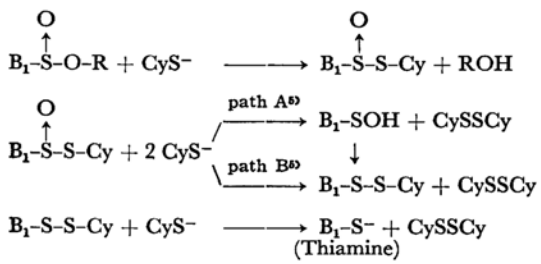
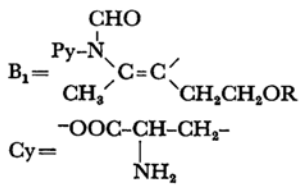


Fig. 2.

It was found that V was reduced quantitatively to thiamine with 1000 mol of cysteine per mol of Vb at pH 8.6 and 50°C for 2 hr. Accordingly, the following mechanism appears applicable:





Reduction of XII to thiamine proceeds with difficulty in comparison with that of open-chain sulfinic esters (V). This might be unusual in view of the fact that the rates of hydrolysis of sultones[6] or lactones[7] are known to be very fast in comparison with the open-chain analogs. Unfortunately, there has been no data for the properties of cyclic sulfinate, even for the synthesis. A molecular model suggests a close proximity between the 4-amino group and $^{+}S{=}O^{-}$ group which would cause a strong interaction between the two groups resulting in a hindered rotation around the bridged methylene bonds as discussed for the unusual splitting of methylene protons in the NMR spectrum of XII. The same interaction might be also the cause of the resistance to the reduction, although further work is necessary in order to elucidate this interesting phenomenon.

Experimental

Methyl *O*-Benzoylhypothiaminate (Va). *i) From I.* A solution of I (840 mg) in absolute methanol (20 m*l*) containing 0.1M hydrogen chloride was warmed in a bath kept at 70° for 1 hr. The solution was concentrated *in vacuo*. After the residue dissolved in water was washed with ether, the aqueous solution, which was made alkaline with sodium bicarbonate, was extracted with chloroform. The extract, which was washed with water and dried over sodium sulfate, was concentrated *in vacuo* and to the residue was added ether to give colorless prisms (500 mg), mp 108—110°C. Recrystallization from ethanol - ether gave Va as colorless prisms, mp 110—112°C.

Found: C, 55.76; H, 5.94; N, 12.72; S, 7.16%. Calcd for $C_{20}H_{24}O_5N_4S$: C, 55.55; H, 5.59; N, 12.96; S, 7.42%.

IR ($CHCl_3$) cm^{-1}: 1713 (C=O), 1665 (NCHO), 1115 (S=O), 980 (S–O). NMR (in $CDCl_3$) τ: 5.55 (s $C-CH_2-N$).

The reaction of I (120 mg) in ethanol with a large quantity of diazomethane afforded Va (100 mg).

ii) From II. To a solution of II (420 mg) in methanol (15 m*l*) was added ethyl chloroformate (110 mg) under cooling with ice water. The mixture was allowed to stand at room temperature over night, concentrated *in vacuo*, and to the residue was added water and chloroform. The chloroform layer was washed with water and dried over sodium sulfate. The chloroform solution was chromatographed over alumina to afford Va (50 mg).

Ethyl *O*-Benzoylhypothiaminate (Vb). *i) From I.* The same procedure and treatment were followed as described above. The reaction between I (840 mg) and absolute ethanol (20 m*l*) containing 0.1M hydrogen chloride afforded colorless prisms (620 mg), mp 118—

5) G. Tsukamoto, T. Watanabe and I. Utsumi, This Bulletin, **42**, 2566 (1969).

6) F. G. Bordwell, C. F. Osborne and R. D. Chapman, *J. Am. Chem. Soc.*, **81**, 2698 (1959).

7) T. C. Bruice and S. J. Benkovie, "Bioorganic Mechanisms," Vol. 1, W. A. Benjamin, New York (1966), p. 21.

120°C. Recrystallization from ethanol - ether gave Vb as colorless prisms, mp 118—120°C.

Found: C, 56.26; H, 5.85; N, 12.64; S, 6.95%; mol wt (Rast), 450. Calcd for $C_{21}H_{26}O_5N_4S$: C, 56.49; H, 5.87; N, 12.55; S, 7.18%; mol wt, 446.5.

IR ($CHCl_3$) cm^{-1}; 1710 (C=O), 1665 (NCHO), 1115 (S=O), 1010 (S–O). NMR (in $CDCl_3$) τ: 5.75—6.50 (m $-\overset{O}{\overset{|}{S}}-O-CH_2-$), 5.50 (s $C-CH_2-N$).

ii) From III. A solution of III (4.36 g) dissolved in 7.2 g of acetic acid and ethanol (50 m*l*) was cooled to −10——15°C. To the mixture was added dropwise a chloroform solution (10 m*l*) containing chlorine (0.43 g), and then the mixture was titrated with a chloroform solution of dimethylamine to make it alkaline. The mixture was concentrated *in vacuo* and to the residue was added ethanol to separate crystals, which were identical in IR spectrum with III. The mother liquor was evaporated *in vacuo* and the residue dissolved in chloroform was washed with water and dried over sodium sulfate. The chloroform solution was chromatographed over alumina to yield Vb (0.60 g).

Oxidation of Vb with Hydrogen Peroxide. A solution of Vb (90 mg) dissolved in acetic acid (1 m*l*) and 30% hydrogen peroxide (0.1 m*l*) was allowed to stand at room temperature for 2 weeks. The mixture was concentrated *in vacuo*, and the residue was crystallized from 95% aqueous ethanol to yield colorless prisms (20 mg), which was identical in infrared spectrum with authentic sample (VI).

Sulfinothiamine (XII). *i) From IX.* Compound IX (1.1 g) was suspended in chloroform (10 m*l*) and ethyl chloroformate (0.35 g) was added. After the mixture was stirred for 2 hr at room temperature, it was washed with water and dried over sodium sulfate. The chloroform solution was chromatographed over alumina to afford crystals (0.13 g). Recrystallization from ethyl acetate gave colorless prisms mp 133—135°C.

Found: C, 47.94; H, 5.59; N, 18.77; S, 10.55%. Calcd for $C_{12}H_{16}O_3N_4S$: C, 48.64; H, 5.44; N, 18.91; S, 10.82%.

IR (KBr) cm^{-1}: 1110 (S=O), 990 (S–O). NMR (in $CDCl_3$) τ: 5.02 and 5.90 (q $C-CH_2-N$, $J=15$ cps), 5.00—5.50 (m $-\overset{O}{\overset{|}{S}}-O-CH_2-$).

ii) From VIII. A suspension of VIII (9.5 g) in chloroform (30 m*l*) was cooled to −3——5°C and thionyl chloride (0.38 g) was added. After the solution was stirred for 1 hr below 0°C it was alkalified under cooling by addition of a chloroform solution of triethylamine, and then allowed to stand until room temperature was reached. The mixture was washed with water and dried over magnesium sulfate. To the residue obtained by the evaporation of the solvent was added ethyl acetate to yield XII (30 mg).

iii) From XIV. A solution of XIV (3.4 g) dissolved in acetic acid (7.2 g) and chloroform (60 m*l*) was cooled to −10°C and a chloroform solution (5.5 g) containing chlorine (0.6 g) was added dropwise. The mixture was similarly treated as described above to afford XII (0.38 g).

iv) From X. A suspended solution of X (3.8 g) in chloroform (50 m*l*) was cooled to −10°C. After acetic acid (6.0 g) was added to the solution, a chloroform solution (13.0 g) containing chlorine (1.4 g) was added dropwise under cooling to −10°C. Similar treatment of the mixture to that descibed in *i)* gave XII (0.10 g).

Oxidation of XIII with Perbenzoic Acid. To a solution of XIII (280 mg, 1.0×10^{-3} mol) in chloroform (8 m*l*) was added a chloroform (2.4 m*l*) solution containing perbenzoic acid (1.2×10^{-3} mol). The mixture was allowed to stand for 30 min at room temperature, and was shaken with aqueous sodium bicarbonate solution, washed with water, and then dried over sodium sulfate. The residue obtained by the concentration of the chloroform solution was recrystallized from benzene to give XII (120 mg).

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