[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA]

Synthesis of 2-Thio Analogs of Thiamine[1]

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The synthesis of "methioprim" analogs of thiamine is discussed. The pyrimidylmethyl halides used as intermediates were found to undergo solvolysis readily in warm alcoholic solvents. Physical properties and biological activity data tend to indicate previously reported preparations were frequently the alcoholysis products or mixtures of the alcoholysis product and the desired analog.

INTRODUCTION

Because of our interest in "methioprim" (2-methylthio-4-amino-5-hydroxymethylpyrimidine) (I) as an antimetabolite,[2] we have undertaken a restudy of the synthesis and biological activity of certain previously reported methioprim analogs of thiamine. This paper is concerned with the synthesis of the previously reported 4-methyl-5-(β-hydroxyethyl)-*N*-(2-ethylthio-4-amino-5-pyrimidylmethyl)thiazolium bromide (II)[3] and its hydrobromide (III)[4] and of 4-methyl-5-(β-hydroxyethyl)-*N*-(2-methylthio-4-amino-5-pyrimidylmethyl)thiazolium chloride hydrochloride (IV).[4,5]

The synthetic route to these compounds involved the condensation of the halides of the pyrimidine moiety with the thiazole moiety of thiamine in alcoholic media, in analogy with the routine methods of synthesis for thiamine.[6] We wish to report in this paper that our studies indicate that alcoholysis of the halides of these thiopyrimidines may occur and that some of the thiamine analogy prepared earlier were in fact alcoholysis products.

DISCUSSION

We have found that 2-methylthio-4-amino-5-bromomethylpyrimidine hydrobromide (V) readily undergoes alcoholysis in hot isopropyl alcohol to give 2-methylthio-4-amino-5-isopropoxymethylpyrimidine hydrobromide (VI), analogously to previous observations in methanol and ethanol.[2a] This alcoholysis occurs even when the solution contains an equivalent amount of the thiazole moiety of thiamine. Alcoholysis is also observed with 2-ethylthio-4-amino-5-bromomethylpyrimidine hydrobromide (VII) and 2-methylthio-4-amino-5-chloromethylpyrimidine hydrochloride (VIII), 2-ethylthio-4-amino-5-isopropoxymethylpyrimidine hydrobromide (IX) and 2-methylthio-4-amino-5-isopropoxymethylpyrimidine hydrochloride (X) being obtained as the products.

In order to avoid alcoholysis of the halide intermediates, we have carried out the synthesis of these thio analogs using nonhydroxylic solvents, and the products are indeed found to differ from the compounds obtained using alcoholic solvents.

It is to be noted that with some compounds in this series, the differences in the elementary composition are not large enough to differentiate effectively between the desired thiamine analogs and the corresponding alcoholysis products. Thus, the differences in the calculated percentage analysis for carbon, hydrogen, and nitrogen for the free base of 2-ethylthiothiamine (II) and the isopropoxy compound from alcoholysis (IX) are surprisingly small (Table I). Since analyses for only these three elements were reported by Dornow and Petsch, their analyses are not sufficient to identify their product conclusively. Unlike other thiamine analogs prepared by the same methods, which are obtained as salts, II was reported by Dornow and Petsch to correspond to the free base having only

TABLE I

CALCULATED PERCENTAGE COMPOSITION

Compound	C	H	Br	N	S
2-Ethylthiothiamine, as HBr salt $C_{13}H_{20}Br_3N_4OS$	33.06	4.27	33.84	11.86	13.58
2-Ethylthiothiamine, as free base $C_{13}H_{19}BrN_4OS$	39.89	4.89	20.42	14.32	16.39
2-Ethylthio-4-amino-5-isopropoxymethylpyrimidine $C_{10}H_{18}BrN_3OS$	38.96	5.88	25.92	13.63	10.40

(1) Supported in part by U. S. Public Health Service Grant Cy-2714.

(2) (a) T. L. V. Ulbricht and C. C. Price, *J. Org. Chem.*, **21**, 567 (1956); *Chem. & Ind. (London)*, 1221 (1955); T. Okuda and C. C. Price, *J. Org. Chem.*, **22**, 1719 (1957); T. Okuda and C. C. Price, *J. Org. Chem.*, in press. (b) D. B. McNair-Scott, T. L. V. Ulbricht, M. L. Rogers, E. Chu, and C. Rose, *Cancer Research*, in press; D. F. Dunning, T. L. V. Ulbricht, C. C. Price, and R. Jones, Jr., unpublished results; R. Guthrie, M. E. Loebeck, and M. J. Hillman, *Proc. Soc. Exptl. Biol. Med.*, **94**, 792 (1957); R. Guthrie *et al.*, *Proc. Am. Assoc. Cancer Research*, **2**, 113 (1956); I. J. Slotnick *et al.*, *Proc. Am. Assoc. Cancer Research*, **3**, 251 (1957); F. Rosen, J. F. Holland, and C. A. Nichol, *Proc. Am. Assoc. Cancer Research*, **3**, 243 (1957).

(3) A. Dornow and G. Petsch, *Ann.*, **588**, 45 (1954).

(4) T. Sakuragi, *Arch. Biochem. Biophys.*, **74**, 362 (1958).

(5) T. L. V. Ulbricht and J. S. Gots, *Nature*, **178**, 913 (1956).

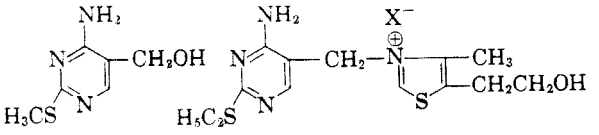
(6) R. R. Williams and J. K. Cline, *J. Am. Chem. Soc.*, **58**, 1504 (1936); H. Andersag and K. Wesphal, *Ber.*, **70B**, 2035 (1937).

one halogen atom, in accordance with their analysis. An additional analysis for bromide and for sulfur would enable one to differentiate between the free base and the alcoholysis product.

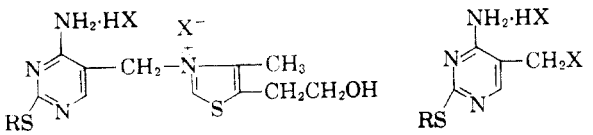
However, the melting point of our alcoholysis product (IX), 171–173° (dec.), is practically the same as that which Dornow and Petsch report for their "2-ethylthiothiamine" (172–174°, dec. at 175°), in contrast to the melting point of our 2-ethylthiothiamine hydrobromide prepared in nonalcoholic solvent (228–230°, dec.).

While this report was being prepared, a paper by T. Sakuragi[4] on an extensive series of 2-alkylthio analogs of thiamine appeared. Sakuragi prepared his 2-alkythio analogs by condensation of the halide intermediates in methanol at room temperature. The melting point reported for his 2-ethylthiothiamine hydrobromide (212–213°, dec.) is lower than ours. This lower melting point is suggestive of some competing alcoholysis even in the cold. His elementary analysis for carbon, hydrogen, and sulfur agreed well with that calculated for 2-ethylthiothiamine hydrobromide.

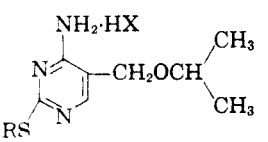
Dornow and Petsch reported their compound to have no thiamine activity when tested on Amoeba. Our alcoholysis product is also inactive. Sakuragi reports some antithiamine effectiveness for his compound. Our studies on 2-ethylthiothiamine hydrobromide are incomplete. A complete report of the biological activity of our series of methioprim analogs will be submitted for publication elsewhere by Dr. Joseph S. Gots.



I II



III. R = C_2H_5, X = Br
IV. R = CH_3, X = Cl
XI. R = CH_3, X = Br
V. R = CH_3, X = Br
VII. R = C_2H_5, X = Br
VIII. R = CH_3, X = Cl



VI. R = CH_3, X = Br
IX. R = C_2H_5, X = Br
X. R = CH_3, X = Cl

In the case of the 2-methylthiothiamine compounds, a sample sent by Ulbricht to the Roswell Park Memorial Institute as 2-methylthiothiamine hydrochloride[6a] was found to be identical to our 2-methylthio-4-amino-5-isopropoxymethylpyrimidine hydrochloride in infrared spectra and to differ from the spectra of our 2-methylthiothiamine hydrochloride prepared in nonalcoholic media. In preliminary tests with *Bacillus subtilis* our 2-methylthiothiamine hydrobromide (XI) has shown a strong inhibition of growth, whereas Ulbricht's sample has shown only a partial inhibitory action.[7]

Sakuragi also reports strong biological activity, using *Kloekera brevis* and *Lactobacillus fermenti*, for his 2-methylthiothiamine hydrobromide. His melting point is again lower than ours (202.5–204.5°, dec., as *cf.* 210–212°, dec.).[8]

EXPERIMENTAL[9]

4-Methyl-5-(β-hydroxyethyl)-N-(2-ethylthio-4-amino-5-pyrimidylmethyl)thiazolium bromide hydrobromide (III). 2-Ethylthio-4-amino-5-bromomethylpyrimidine hydrobromide[2a] (10 g., 0.0304 mole) and 5 g. (0.035 mole) of 4-methyl-5-(β-hydroxyethyl)thiazole[10] were refluxed with stirring in 100 ml. of dioxane for 3 hr. After cooling, the solvent was removed by decantation from the precipitate, and the precipitate was dissolved in 100 ml. of boiling absolute ethanol. The solution was concentrated to 50 ml. and cooled, yielding crystals which were fairly hygroscopic. The product was recrystallized from 80 ml. of absolute ethanol to give 4.3 g. of colorless crystals. Recrystallization four times more from absolute ethanol gave a product with m.p. 228–230° (dec.).

Anal. Calcd. for $C_{13}H_{20}Br_2N_4OS$: C, 33.06; H, 4.27; Br, 33.84; N, 11.86; S, 13.58. Found: C, 32.78; H, 4.43; Br, 33.53; N, 11.91; S, 13.58.

Infrared spectrum (in potassium bromide, wave length, and % absorption): 3.00 (52), 3.23 (54), 3.33 (56), 3.65 (54), 6.06 (77), 6.34 (68), 6.55 (56), 6.72 (47), 6.92 (37), 7.23 (48), 7.43 (43), 8.05 (56), 8.54 (42), 9.34 (36), 10.07 (23), 11.40 (19), 12.33 (18), 12.63 (20), 13.03 (32), 14.27 (25).

2-Ethylthio-4-amino-5-isopropoxymethylpyrimidine. 2-Ethylthio-4-amino-5-bromomethylpyrimidine hydrobromide (2 g., 0.0061 mole) was dissolved in 50 ml. of boiling isopropyl alcohol. The solution was refluxed for 30 min., and then concentrated to 4 ml. After cooling, a white, crystalline precipitate was collected and washed with a small amount of isopropyl alcohol; yield, 1.3 g., m.p. 171–173° (dec.).

Anal. Calcd. for $C_{10}H_{18}BrN_3OS$: Br, 25.92. Found: Br, 26.93, 27.09.

The crystals (1 g.) were dissolved in 10 ml. of water, and the solution was made weakly alkaline with sodium hydroxide. The white, crystalline precipitate which appeared on neutralization was collected; yield, 0.6 g. The material was recrystallized from a mixture of benzene and petroleum ether (1:5) to give colorless crystals, m.p. 72–74°.

Anal. Calcd. for $C_{10}H_{17}N_3OS$: C, 52.84; H, 7.54; N, 18.48; S, 14.10. Found: C, 53.15; H, 7.50; N, 18.71; S, 14.30.

4-Methyl-5-(β-hydroxyethyl)-N-(2-methylthio-4-amino-5-pyrimidylmethyl)thiazolium bromide hydrobromide (XI). 2-Methylthio-4-amino-5-bromomethylpyrimidine hydrobromide[2a] (20 g., 0.063 mole) and 9 g. (0.063 mole) of

(6a) This was *not* the same sample studied for biological activity at the University of Pennsylvania.[5]

(7) Private communication from M. E. Loebeck, Roswell Park Memorial Institute, to C. C. Price.

(8) In a private communication, Ulbricht reports that his compound decomposed over 160°.

(9) Melting points are uncorrected. Analyses are by Midwest Microlab., Inc., Indianapolis, Ind.

(10) We are indebted to Dr. Max Tishler, Merck & Co., for samples of this compound.

4-methyl-5-(β-hydroxyethyl)thiazole were refluxed with stirring in 100 ml. of tetrahydrofuran for 30 min. until the solid turned into a viscous mass. After cooling, the solvent was removed by decantation and the viscous material was dissolved in 100 ml. of boiling ethanol. The solution was filtered while hot and 40 ml. of acetone was added until a precipitate appeared. After cooling, a pale yellow, crystalline precipitate which was slightly hygroscopic was collected; yield, 18 g. This product was recrystallized four times from absolute ethanol to give colorless crystals which melted at 210–212° (dec.) [lit.,[4] 202.5–204.5 (dec.)].

Anal. Calcd. for $C_{12}H_{10}Br_2N_4OS$: C, 31.45; H, 3.96; Br, 34.88; N, 12.22; S, 13.99. Found: C, 31.62; H, 4.20; Br, 33.98; N, 12.67; S, 13.82.

Infrared spectrum (in potassium bromide, wave length, and % absorption): 3.03 (55); 3.30 (61), 3.67 (54), 6.05 (82), 6.35 (66), 6.56 (60), 6.73 (50), 6.92 (40), 7.22 (48), 7.43 (49), 8.05 (55), 8.51 (45), 9.34 (33), 10.25 (22), 10.60 (22), 13.02 (30).

2-Methylthio-4-amino-5-chloromethylpyrimidine hydrochloride (VIII). To a solution of 10 g. (0.06 mole) of 2-methylthio-4-amino-5-hydroxymethylpyrimidine in 600 ml. of boiling chloroform was slowly added 30 ml. (0.41 mole) of thionyl chloride, and the mixture was refluxed for 1 hr. After cooling, a white, crystalline powder was collected and washed three times with 50-ml. portions of chloroform, followed by drying at 80° for 1 hr.; yield, 13.2 g. (100%). The product did not melt below 300°. It was recrystallized from acetic acid for analysis.

Anal. Calcd. for $C_6H_9Cl_2N_3S\cdot 2H_2O$: Cl, 27.02; N, 16.03; S, 12.23. Found: Cl, 26.61; N, 15.63; S, 12.82.

4-Methyl-5-(β-hydroxyethyl)-N-(2-methylthio-4-amino-5-pyrimidylmethyl)thiazolium chloride hydrochloride (IV). (a) 2-Methylthio-4-amino-5-chloromethylpyrimidine (5 g., 0.02 mole) and 3.2 g. (0.02 mole) of 4-methyl-5-(β-hydroxyethyl)-thiazole were refluxed with stirring in 100 ml. of tetrahydrofuran for 7 hours. After cooling, a white precipitate was collected and dried at 80° for 1.5 hr.; yield, 5.2 g. The product was refluxed with 300 ml. of isopropyl alcohol and filtered. The solid (*ca.* 200 mg.) was recrystallized twice from 40 ml. of absolute ethanol, giving colorless crystals, which were dried at 110° (1 mm. Hg) for 1 hr., m.p. 218–220° (dec.).

Anal. Calcd. for $C_{12}H_{18}Cl_2N_4OS_2\cdot C_2H_6O$: C, 40.48; H, 5.82; Cl, 17.07; N, 13.49. Found: C, 40.11; H, 5.11; Cl, 16.75; N, 13.65.

The filtrate from the above product was concentrated to 30 ml., and acetone was added until a precipitate appeared. After cooling, the hygroscopic precipitate was collected; yield, 1.2 g. It did not melt below 300°.

An aqueous solution of this product, on neutralization with sodium hydroxide, gave a colorless, crystalline precipitate, which, after recrystallization from a mixture of benzene and ligroin, melted at 105–108° and showed no melting point depression on admixture with authentic 2-methylthio-4-amino-5-isopropoxymethylpyrimidine. This evidently was formed from some unreacted chloromethyl compound in the crude precipitate.

(b) 2-Methylthio-4-amino-5-chloromethylpyrimidine hydrochloride (5 g., 0.002 mole) and 3.2 g. (0.02 mole) of 4-methyl-5-(β-hydroxyethyl)thiazole were refluxed with stirring in 100 ml. of dioxane for 3 hr. After cooling, the solvent was removed by decantation and the viscous residue was dissolved in 150 ml. of hot absolute ethanol, filtered while hot, and the filtrate concentrated to 40 ml. After cooling, the crystalline precipitate was filtered and washed with absolute ethanol, and then dried at 80° overnight; yield, 2.6 g. This product was recrystallized from absolute ethanol, giving colorless crystals which melted at 199–201° (dec.).

Anal. Calcd. for $C_{12}H_{18}Cl_2N_4OS_2\cdot H_2O$: C, 37.21; H, 5.20; S, 16.55. Found: C, 37.28; H, 5.35; S, 16.16.

Infrared Spectra (T. Okuda's sample in potassium bromide, wave length, and % absorption): (a) *Alcoholate:* 3.08 (71), 3.30 (75), 3.66 (62), 6.02 (85), 6.10 (85), 6.33 (77), 6.57 (80), 6.74 (57), 6.84 (57), 7.20 (59), 7.47 (67), 8.02 (74), 8.35 (53), 8.50 (54), 9.32 (41), 10.30 (27), 10.60 (29), 11.42 (26), 12.02 (38), 12.55 (33), 13.20 (42).

(b) *Hydrate:* 3.35 (82), 3.85 (68), 6.11 (92), 6.35 (85), 6.60 (83), 6.76 (70), 7.25 (69), 7.44 (73), 8.06 (74), 8.53 (63), 9.40 (51), 10.30 (34), 10.63 (37), 11.60 (31), 12.63 (41), 13.10 (41).

2-Methylthio-4-amino-5-isopropoxymethylpyrimidine hydrochloride (X). 2-Methylthio-4-amino-5-chloromethylpyrimidine hydrochloride (2 g., 0.009 mole) was heated with 50 ml. of isopropyl alcohol, the crystals being completely dissolved in 15 min. The solution was refluxed for 15 min. more and then concentrated to 5 ml. After cooling, a colorless crystalline precipitate was collected; yield, 1.55 g. The product did not melt below 300°.

Anal. Calcd. for $C_9H_{16}ClN_3OS$: Cl, 14.19. Found: Cl, 14.18.

Infrared spectrum: (Hydrochloride, in potassium bromide, wave length, and % absorption): 3.00 (68), 3.17 (79), 3.33 (65), 3.80 (79), 6.02 (94), 6.35 (86), 6.56 (80), 7.16 (67), 7.23 (64), 7.44 (78), 7.98 (79), 8.08 (77), 8.57 (65), 8.78 (65), 8.90 (72), 9.48 (80), 10.27 (42), 10.54 (39), 10.89 (44), 11.46 (46), 11.84 (47), 12.79 (50), 13.03 (44), 13.40 (43), 14.54 (42).

Infrared spectrum of Ulbricht's sample of "2-methylthiothiamine" hydrochloride: 3.00 (64), 3.17 (72), 3.33 (60), 3.80 (72), 6.02 (91), 6.35 (79), 6.56 (73), 7.16 (62), 7.23 (61), 7.44 (71), 7.98 (71), 8.08 (70), 8.57 (60), 8.78 (60), 8.90 (67), 9.48 (74), 10.27 (44), 10.54 (41), 10.89 (44), 11.46 (49), 11.84 (50), 12.79 (50), 13.03 (47), 13.40 (48), 14.54 (44).

A solution of the above obtained hydrochloride (0.5 g., 0.002 mole) in 10 ml. of water was made alkaline with sodium hydroxide. A colorless crystalline precipitate was collected and recrystallized from a mixture of benzene and ligroin (1:3), yielding colorless needles, m.p. 105–108°.

Anal. Calcd. for $C_9H_{15}N_3OS$: C, 50.70; H, 7.04; N, 19.72; S, 15.02. Found: C, 50.64; H, 6.94; N, 20.00, S, 15.33.

2-Methylthio-4-amino-5-isopropoxymethylpyrimidine hydrobromide (VI). 2-Methylthio-4-amino-5-bromomethylpyrimidine hydrobromide (2 g., 0.006 mole) was treated with 50 ml. of boiling isopropyl alcohol in the same way as the previous experiment, yielding 1.1 g. of colorless needles, which did not melt below 300°.

Anal. Calcd. for $C_9H_{16}BrN_3OS$: Br, 27.16. Found: Br, 27.96.

A solution of this hydrobromide (0.5 g., 0.0017 mole) in 10 ml. of water was made alkaline with sodium hydroxide. The resulting colorless crystals were collected and recrystallized from a mixture of benzene and ligroin (1:3); m.p. 105–108°. No melting point depression was observed on admixture with the sample obtained from the hydrochloride.

2-Methylthio-4-amino-5-methoxymethylpyrimidine hydrobromide. 2-Methylthio-4-amino-5-bromomethylpyrimidine (10 g., 0.015 mole) was added to a solution of 4.5 g. (0.03 mole) of 4-methyl-5-(β-hydroxyethyl)thiazole in 100 ml. of methanol, and the mixture was heated at 55–65° for 12 hr., after which the solution was concentrated to 30 ml. After cooling, white crystals were collected and washed with dry ether; yield, 10.2 g. These crystals were reprecipitated by dissolving in 20 ml. of hot methanol, adding 10 ml. of dry ether, and cooling, m.p. 167–168° (dec.).

Anal. Calcd. for $C_7H_{12}BrN_3OS$: Br, 30.02. Found: Br, 30.13.

When 0.5 g. of the product was dissolved in 10 ml. of water and the solution was made weakly alkaline with sodium hydroxide, colorless crystals, m.p. 104–106°, were collected. These were identified as 2-methylthio-4-amino-5-methoxymethylpyrimidine by mixed melting point.

PHILADELPHIA, PA.