dull red heat, a weight loss of 12.5% was found. It was assumed that the only impurity present in significant amount after dehydration was the 1% of carbonate. To estimate the effect of the carbonate, freezing points were determined with known amounts of additional potassium carbonate.

About 50 g. of the hydroxide was melted in a nickel container brought gradually to 475–500°, and kept at this temperature ten to twelve hours to remove water. Further heating did not change the freezing point. The freezing points were obtained from cooling curves. Temperatures were measured with a chromel–alumel thermocouple which was calibrated at the freezing points of tin, lead and zinc, using Bureau of Standards samples. The same freezing point was found with and without a monel metal protecting tube around the couple. When a stream of dry nitrogen was passed over the hydroxide during dehydration and cooling, the freezing point did not differ from the freezing point of the hydroxide in contact with air. The observations are summarized in the accompanying table. The carbonate concentrations are not known with great precision because of the tendency of the molten hydroxide to creep up the walls of the container.

FREEZING POINTS OF $KOH–K_2CO_3$ MIXTURES

K_2CO_3, wt. %	1.0	4.9	7.0	12.2	16.2
F. p., °C.	408.0	400.0	394.5	384.2	373.5
Eutectic, °C.	358.7	365.0	365.0	366.9	366.5

By extrapolation, the freezing point of potassium hydroxide with no carbonate present was estimated to be 410°. A transition which Hevesy found at 248° and Scarpa at 360° was found at 249°. The heat of fusion calculated from the effect of carbonate on the freezing point was 1830 cal. per mole. Kelley[3] has calculated a value of 1980 cal. per mole from the data of Scarpa on the potassium hydroxide–potassium iodide system.

(3) Kelley, U. S. Bureau of Mines, Bulletin 393 (1936).

STATE COLLEGE, PENNSYLVANIA RECEIVED JUNE 3, 1949

Comparison of Some Properties of Thiolsulfonates and Thiolsulfinates

BY LAVERNE D. SMALL,[1] JOHN HAYS BAILEY AND C. J. CAVALLITO

The thiolsulfinates, R–SO–SR, have been shown to be active antibacterial and antifungal agents.[2] Although these oxides were previously unknown, the dioxides frequently have been described. These are now generally believed to have the thiolsulfonate structure, $R–SO_2–SR$. No record could be found of antibacterial tests with these compounds and it was of interest to compare the effect of the thiolsulfonate with that of the thiolsulfinate group in this respect.

The compounds used in this comparison were those where R was ethyl in the preceding structures. The thiolsulfonate was only 1.2% soluble in water compared with approximately 11% for the thiolsulfinate. This reduced interest in the higher alkyl thiolsulfonates. Both compounds react quickly with dilute alkalies.

Tests conducted by methods previously described[2] showed that the two thiol esters are of comparable antimicrobial activity, the thiolsulfonate being more effective against *S. aureus* and *K. pneumoniae*.

The antibiotic activity of thiolsulfinates has been attributed to the ability of the compounds to react with biologically essential –SH groups as exemplified by the reaction with cysteine to yield derivatives of type I, $R–S–S–CH_2–CH(NH_2)–COOH$.[3] With the thiolsulfinates one mole of compound yielded, nearly quantitatively, two moles of I. Under similar conditions the thiolsulfonate gave only one equivalent of I and an acid, presumably ethanesulfinic acid. This in harmony with the observations of Smiles and coworkers[4] who found that thiolsulfonates reacted with mercaptans to yield one mole of disulfide and one of the sulfinic acid. The ability of thiolsulfinates to react rapidly with cysteine (thiols) to yield two moles of I is further support of Smiles' evidence that "disulfoxides" have a thiolsulfonate structure. A disulfoxide presumably would yield two moles of sulfenic acid as an intermediate (plus one cystine) and reaction with more cysteine would give two moles of I. As it is, only one mole of I and one of the sulfinic acid are formed under the conditions.

$$R–SO_2–SR + HS–CH_2–CH(NH_2)–COOH \longrightarrow I + RSO_2H$$

$$R–SO–SR + HS–CH_2–CH(NH_2)–COOH \longrightarrow I + RSOH$$

$$RSOH + HS–CH_2–CH(NH_2)–COOH \longrightarrow I + H_2O$$

The observation of Toennies and Lavine[5] that cystine "disulfoxide" reacts with cysteine to yield a mole of cystine and one of cysteine sulfinic acid favors the thiolsulfonate structure for this compound. Tests in our Laboratories show that cystine dioxide also is inhibitory to bacterial growth. Such compounds may have an *in vivo* biological growth-control function if oxidation of

TABLE I

ANTIMICROBIAL ACTION OF THE THIOLSULFINATE AND THIOLSULFONATE

Compound	Inhibitory concentration,[a] mg. per cc. *Clostridium perfringens*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Escherichia coli*	*Mycobacterium tuberculosis* H37Rv	*Trichophyton mentagrophytes*
$C_2H_5SOSC_2H_5$[b]	0.05	0.05	0.05	0.015	0.005	0.005
$C_2H_5SO_2SC_2H_5$	.05	.015	.005	.01	.005	.005

[a] Concentration producing complete inhibition of growth in serial dilution tests. [b] For preparation see [2].

(1) Present address: University of Nebraska, Lincoln, Nebraska.

(2) Small, Bailey and Cavallito, THIS JOURNAL, **69**, 1710 (1947).

(3) Cavallito, Buck and Suter, *ibid.*, **66**, 1952 (1944).

(4) Smiles and Gibson, *J. Chem. Soc.*, **125**, 176 (1924); Miller and Smiles, *ibid.*, **127**, 224 (1925).

(5) Toennies and Lavine, *J. Biol. Chem.*, **113**, 593 (1936).

–S–S– groups to the mono- or dioxide stage is demonstrated to occur in an organism.

Experimental

Ethyl Ethanethiolsulfonate. Method A.—One liter of ethyl acetate and 24.5 g. (0.2 mole) of ethyl disulfide were placed in a three-liter, three-neck flask. The solution was cooled with an ice-bath and while stirring there was added, during fifteen minutes, a solution of 5 moles of 40% peracetic acid in 500 cc. of ethyl acetate. After stirring and cooling for an additional hour the solution was allowed to stand at room temperature overnight. The reaction mixture was stirred with a solution of 30 g. of ferrous sulfate heptahydrate in 150 cc. of water to decompose excess per-acid. The ethyl acetate layer was separated and shaken with sufficient saturated aqueous sodium bicarbonate solution to remove acids. The ethyl acetate extract was dried over anhydrous sodium sulfate and then evaporated under reduced pressure to remove solvent. The liquid residue was distilled, yielding the thiolsulfonate, b. p. 56° at 0.2 mm.; $n^{25}D$ 1.4972; yield 3.7 g. or 12%.

Anal. Calcd. for $C_4H_{10}O_2S_2$: C, 31.15; H, 6.54. Found: C, 31.42; H, 6.41.

Method B.—Ethyl ethanethiolsulfonate was first prepared by Otto by reaction of an ethyl halide with sodium ethanethiolsulfonate, but few details were given and the compound was poorly characterized.[6,7]

An alcoholic solution of potassium sulfide was prepared by dissolving 0.5 mole of potassium hydroxide in 250 cc. of absolute ethanol, saturating the solution with hydrogen sulfide and adding a second 0.5 mole of alkali to the solution.

Ethanesulfonyl chloride ($n^{25}D$ 1.4515) was prepared by the method of Lee and Dougherty.[8] To the potassium sulfide solution was added dropwise, during four hours of stirring under anhydrous conditions, a solution of 0.5 mole of ethanesulfonyl chloride in 250 cc. of ethanol. The mixture was cooled with an ice-bath during this period. The mixture was then made alkaline to litmus with alcoholic potassium hydroxide and allowed to stand for fifteen hours at 25° and then heated to 50° for ten minutes. The solution was filtered and the filtrate refluxed for four hours with 0.75 mole of ethyl bromide. After cooling, the solution was filtered, the filtrate concentrated under reduced pressure and the residual thiolsulfonate distilled: yield 23.2 g. or 30%.

Anal. Found: C, 31.07; H, 6.23; $n^{25}D$ 1.4977.

Reaction with Cysteine.—Unbuffered aqueous solutions of cysteine hydrochloride and the thiolester adjusted to *p*H 6.5 with sodium hydroxide solution were mixed[3] and within a few seconds there appeared a white crystalline precipitate of I where R is ethyl, m. p. 196° dec. The thiosulfinate yielded nearly two moles of I (compare where R is allyl[3]) with no change in *p*H; the thiolsulfonate gave 90% yield for one mole of I and the *p*H dropped to 3.0.

Anal. Calcd. for I, $C_5H_{11}O_2NS_2$: N, 7.75. Found: N, 7.79.

(6) Otto, *Ber.*, **15**, 122 (1882).

(7) Hilditch, *J. Chem. Soc.*, **97**, 1098 (1910).

(8) Lee and Dougherty, *J. Org. Chem.*, **5**, 83 (1940).

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Further Studies on Oxythiamine

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The preparation of oxythiamine from thiamine by deamination with gaseous nitrogen oxides has been previously reported from this Laboratory.[2] In the present communication, we wish to report additional information on this compound.

During the course of this work the thiochrome method was used in following the fate of thiamine, and the Prebluda–McCollum reagent as modified by Melnick and Field[3] served in tracing both the thiamine and oxythiamine, since both compounds give a positive reaction with equal color production. We have found that Melnick and Field[3] and also Rosenberg[4] are incorrect in assuming that the amino group in position 4 of the pyrimidine moiety is necessary for the production of color. Todd and Bergel,[5] and Prebluda and McCollum[6] had already shown that the essential features required for the production of color are the β-hydroxyethyl group on position 5 and a free hydrogen on position 2 of the thiazole moiety. In fact, Bergel and Todd[7] were able to synthesize oxythiamine and showed that it gave a positive formaldehyde–azo reaction. Thus, whereas thiamine gives both positive thiochrome and Prebluda–McCollum reactions, oxythiamine reacts only with the Prebluda–McCollum reagent, and the chloroxy- and bromoxythiamine give neither reaction. Our findings, therefore, confirm those of Todd and Bergel.

Oxythiamine is very similar to thiamine in its chemical properties. Both substances form chloride–hydrochloride salts, picrates and picrolonates, and they can be adsorbed on and eluted from Decalso under similar conditions.[8] Neither compound is attacked by sodium in the presence of glacial acetic acid[9] and both are split by the sulfite treatment of Williams.[10] Thus, by treatment of oxythiamine with sulfite, we have obtained 2-methyl-4-oxypyrimidine-5-methylsulfonic acid.

The ultraviolet absorption spectrum of oxythiamine chloride–hydrochloride was determined for aqueous solutions at neutral and acid reactions, by means of a Model DU Beckman spectrophotometer. Oxythiamine shows two maxima at 223 and 266 mμ, respectively, at *p*H 7.2 (phosphate buffer, 0.02 *M*), and at 221.5 and 265 mμ in 0.02 *M* phosphoric acid. The spectrum is very similar to that of oxychlorothiamine.[11]

Experimental[12]

Picrate and Picrolonate of Oxythiamine.—These compounds were prepared in the usual manner. They were recrystallized several times from an ethanol–water (1:1) mixture.

Oxythiamine picrate, m. p. 102–108°. *Anal.* Calcd. for $C_{12}H_{16}O_2N_3S[C_6H_2OH(NO_2)_3]_2$: C, 39.78; H, 3.00. Found: C, 39.46; H, 2.88.

(1) Present address: Biochemical Research Laboratory, Massachusetts General Hospital, Boston, Mass.

(2) Soodak and Cerecedo, THIS JOURNAL, **66**, 1988 (1944).

(3) Melnick and Field, *J. Biol. Chem.*, **127**, 505 (1939).

(4) H. R. Rosenberg, "Chemistry and Physiology of the Vitamins," Interscience Publishers, Inc., New York, N. Y., 1942, p. 129.

(5) Todd and Bergel, *J. Chem. Soc.*, 1559 (1936).

(6) Prebluda and McCollum, *J. Biol. Chem.*, **127**, 495 (1939).

(7) Bergel and Todd, *J. Chem. Soc.*, 1504 (1937).

(8) Cerecedo and Hennessy, THIS JOURNAL, **59**, 1617 (1937).

(9) Tolpin, Foy and Cerecedo, *ibid.*, **63**, 2848 (1941).

(10) Williams, Waterman, Keresztesy and Buchman, *ibid.*, **57**, 536 (1935).

(11) Buchman and Williams, *ibid.*, **57**, 1751 (1935).

(12) All melting points are uncorrected. The analyses were performed by Mr. M. Bier.