Table I—Nitroglycerin Dissolution from Three 0.4-mg Sublingual Tablet Lots

Lot	Average Percent Dissolved at 0.25 min	0.5 min	0.75 min	1.0 min	t_{90} Average	t_{100} Average	Average Micrograms per Tablet
1	31 (16–37)[a]	78 (66–84)	93 (88–95)	97 (95–98)	0.70	1.6 (1.2–2.4)[b]	433 (418–452)[b]
2	24 (19–30)	72 (69–76)	89 (86–94)	96 (94–98)	0.78	1.5 (1.2–1.8)	443 (424–459)
3	33 (28–39)	82 (71–90)	94 (92–97)	98 (95–100)	0.57	1.2 (1.0–1.5)	394 (360–415)

[a] Range for 10-tablet sample. [b] Range.

Perform a blank determination with the electrolyte to compensate for the small amount of oxygen that diffuses into the cell and take the difference in recorder chart reading between the sample and the blank at each time interval as the sample reading. With no change in experimental conditions, the blank remains constant during the day and only one blank determination need be obtained each day.

Calculate the quantity of nitroglycerin, in milligrams per tablet, from the formula $5C(U/S)$, where C is the concentration, in milligrams per milliliter, of the standard preparation used, whichever is closer to the sample reading; and U and S are the chart readings for the tablet and the standard preparation, respectively.

Note as t_{100} the time required for 100% of the drug to dissolve; this value is determined from the chart as that point where no further rise in reduction current occurs. Calculate the percent of drug released at any time, t, in seconds from the formula (milligrams measured at time t/total milligrams released) × 100.

RESULTS AND DISCUSSION

The proposed dissolution test was performed using 10-tablet samples of each of three lots of a commercial 0.4-mg sublingual tablet formulation[8]. Table I shows the average percentage of drug dissolved from the tablets at 15-sec intervals during the 1st min and the average time required for 90 and 100% of the drug content of the tablets to dissolve. Use of percentage affords direct comparison among individual tablets of differing drug content, thus normalizing the data. Since prompt onset of action is an important criterion for a sublingual nitroglycerin tablet, these data may have clinical significance.

Another advantage of the method is that it provides a measure of the nitroglycerin in each tablet. The data shown in Table I (last column) are consistent with those obtained for these tablets by Dorsch and Shangraw (4), who used an automated assay method. Their method is less time consuming than the dissolution procedure and probably would be preferable if content uniformity information is the only consideration.

[8] NITROPRN Tablets, Warner-Chilcott Laboratories, Morris Plains, NJ. Lot 1 was control number 490254B, Lot 2 was control number 4917124B, and Lot 3 was control number 5964124A.

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[x] To whom inquiries should be directed.

Synthesis and Preliminary Antimicrobial Screening of Two Thiosulfonates

LaVERNE D. SMALL

Abstract □ Tetramethylene bis(methanethiosulfonate), the *S*-ester analog of busulfan, was prepared by reacting 1,4-dibromobutane with potassium methanethiosulfonate. 2,4-Dichlorophenyl methanethiosulfonate was prepared by reacting sodium methanesulfinate with 2,4-dichlorobenzenesulfenyl chloride. Neither compound showed antifungal activity against *Microsporum audouini* or *Trichophyton mentagrophytes*. Although tetramethylene bis(methanethiosulfonate) was more active against *Staphylococcus aureus* than was 2,4-dichlorophenyl methanethiosulfonate, neither compound was as active as the streptomycin control.

Keyphrases □ Thiosulfonates—synthesis of tetramethylene bis(methanethiosulfonate) and 2,4-dichlorophenyl methanethiosulfonate, antimicrobial screening □ Sulfur analog of busulfan—synthesized, antimicrobial screening □ Structure–activity relationships—thiosulfonates synthesized, antimicrobial screening □ Antimicrobial activity—thiosulfonates synthesized and screened

Although thiosulfonates have been known for more than 100 years, the demonstration of antimicrobial activity of some members of this chemical class has been relatively recent (1–3). Two different types of thiosulfonates were made for antimicrobial screening. Busulfan, a bis(sulfonate) ester, is used for treatment of chronic myelocytic leukemia (4). The bis(*S*-ester) analog of this compound was prepared for testing of its antibacterial and antifungal activities and for future screening for antileukemic activity. In addition, the *S*-ester analog of 2,4-dichlorophenyl methanesulfonate, a long acting soil nematocide (5), was prepared. These new thiosulfonates were tested for antibacterial activity against *Staphylococcus aureus* and for antifungal ac-

tivity against *Microsporum audouini* and *Trichophyton mentagrophytes*.

Tetramethylene bis(methanethiosulfonate) (I), the analog of busulfan, was prepared by reacting 1,4-dibromobutane with 2 equivalents of potassium methanethiosulfonate, using a modification of the procedure of Spring (6). The 2,4-dichlorophenyl methanethiosulfonate (II) was prepared by reacting sodium methanesulfinate with 2,4-dichlorobenzenesulfenyl chloride.

Both I and II were purified with the aid of TLC, using aluminum sheets coated with silica gel F-254. The sulfur-containing compound, as well as compounds containing aromatic groups, was visualized as fluorescent quenching spots under short wavelength UV light. This technique was used to determine the best solvents for separating various components from the final reaction mixture and to check the purity of the final product.

EXPERIMENTAL[1]

TLC sheets, precoated with 0.25-mm silica gel F-254[2] on an aluminum backing, were used for the TLC separations. The NMR spectra[3] of I and II were obtained using deuterated chloroform and deuterated dimethyl sulfoxide as the solvents, respectively. Tetramethylsilane was used as the internal standard for all NMR measurements. The IR spectra[4] were obtained using chloroform as the solvent for I and carbon tetrachloride for II.

Potassium Methanethiosulfonate—An alcoholic solution of potassium sulfide was prepared by dissolving 12 g (0.3 mole) of bright metallic potassium in 600 ml of anhydrous ethanol under an atmosphere of dry nitrogen. One-half of this solution was saturated with hydrogen sulfide according to the procedure described by Brauer (7) for the preparation of potassium trisulfide. Water, 60 ml, was added to this alcohol solution containing 0.15 mole of potassium sulfide, and the solution was cooled in an ice–water bath to less than 10°.

Methanesulfonyl chloride, 11.6 g (0.15 mole), was slowly dropped into the cold sulfide solution while the mixture was stirred rapidly. Stirring was continued for 3 hr while the mixture was allowed to warm to room temperature. The potassium methanethiosulfonate was not isolated from the reaction mixture.

Tetramethylene Bis(methanethiosulfonate) (I)—To the alcoholic suspension of potassium methanethiosulfonate was added 1,4-dibromobutane, 8.1 ml (0.068 mole). The mixture was heated under gentle reflux for 36 hr. The reaction mixture was allowed to cool to room temperature and then was stirred for an additional 12 hr. The white precipitate was removed by filtration, and the alcoholic filtrate of the reaction mixture was placed in a freezer for 24 hr to obtain additional material.

The precipitated material was washed with cold water, dried under reduced pressure at 40°, and crystallized by dissolving in boiling acetone and allowing the solution to cool. The substance was recrystallized by dissolving in boiling benzene. After the addition of hexane to the warm benzene solution and cooling, I, 12.4 g (66% yield), mp 90.0–90.5°, was obtained. The NMR spectrum and the melting point were the same as those of the product previously obtained from commercial potassium sulfide by TLC.

The NMR spectrum showed a strong, sharp absorption peak at δ 3.40, a smaller peak at δ 3.30, and a third broad peak centered at δ 1.93. If the integration of the peak at δ 1.93 represents four protons, the integration of the two peaks at δ 3.30 and 3.40 represents 10 protons. The NMR spectrum of 1,4-dibromobutane showed two broad band peaks at δ 2.03 and 3.50.

The IR spectrum for I showed absorption at 1130 (1) (strongest absorption), 1321 (2), 949 (3), and 1182 (4) cm^{-1}. Literature values (8) for ethyl methanethiosulfonate indicate absorption at 1136 (SO_2), 1321, 962, and 751 cm^{-1}. The mass spectrum for I indicated 278 (M^+, $C_6H_{14}O_4S_4$).

Anal.—Calc. for $C_6H_{14}O_4S_4$: C, 25.88; H, 5.07; S, 46.06. Found: C, 26.00; H, 4.96; S, 46.22.

Bis(2,4-dichlorophenyl) Disulfide—Sodium 2,4-dichlorothiophenol was prepared by a slight modification of the method of Baddeley and Bennett (9), starting with 20 g (0.123 mole) of 2,4-dichloroaniline. About 200 ml of 50% (w/w) sulfuric acid was necessary to dissolve and maintain the dichloroaniline in solution. This amount was more than twice the concentration of acid used by Baddeley and Bennett (9).

After preparing the diazonium sulfate, about 85% of the acid was neutralized by the addition of sodium hydroxide solution while the temperature of the mixture was maintained below 10°. Sodium borate solution was then used to adjust the reaction mixture to about pH 4. The dichlorophenyl xanthogenate was prepared and then hydrolyzed as described by Baddeley and Bennett (9). The resulting sodium 2,4-dichlorothiophenol was oxidized in the aqueous alcoholic solution by addition of excess iodine and sodium iodide solution.

After the mixture was stirred for 20 min, sodium thiosulfate solution was added until the color of the iodine was discharged. The disulfide was extracted from the mixture by the addition of chloroform. The chloroform extract was dried, using anhydrous sodium sulfate, and the chloroform was evaporated. The residue, crystallized from a mixture of acetone and methanol, provided 7.6 g (17% yield) of bis(2,4-dichlorophenyl) disulfide, mp 82–84° [lit. (10) mp 82–83°].

2,4-Dichlorobenzenesulfenyl Chloride—A solution containing 7.6 g (0.02 mole) of bis(2,4-dichlorophenyl) disulfide in 100 ml of dry carbon tetrachloride was treated with chlorine according to the method of Hubacher (11) for the preparation of *o*-nitrophenylsulfur chloride, except for the reaction time. The flow of chlorine through the warm reaction mixture was maintained for 20 hr. The solvent was then evaporated, and the residual dark oil was distilled at about 5 mm of pressure. The fractions distilling at 100–102° and showing a constant refractive index, n_D^{25} 1.6330 ± 0.0002, were combined. A fuming red oil, 4.5 g (98% yield), was obtained [lit. (10) bp 94–96°/4 mm].

2,4-Dichlorophenyl Methanethiosulfonate (II)—Methanesulfinyl chloride, 1.4 g (0.014 mole), was prepared by the reaction of chlorine on methyl disulfide in acetic anhydride (12) and was then added dropwise, with stirring, to 25 ml of a cold (5°) aqueous solution containing 1.13 g (0.028 mole) of sodium hydroxide to form sodium methanesulfinate. A solution containing 3.0 g (0.014 mole) of 2,4-dichlorobenzenesulfenyl chloride in 5 ml of dichloromethane was slowly added, with stirring, to the cold aqueous solution of the sodium methanesulfinate. The cold mixture was stirred for 1 hr and then allowed to warm to room temperature.

The reaction mixture was extracted with carbon tetrachloride, and the extract was washed with 5% sodium bicarbonate solution and dried with anhydrous sodium sulfate. A thin-layer chromatogram of a small portion of the carbon tetrachloride extract was developed as indicated below. The carbon tetrachloride was removed in a flash evaporator, and the remaining white solid was washed with hexane until a thin-layer chromatogram showed a single spot. The remaining compound, II, 2.1 g (58% yield), was crystallized from benzene–hexane, mp 88–88.5°.

The NMR spectrum, using deuterated dimethyl sulfoxide as the solvent, showed a sharp absorption peak at δ 3.6 that integrated for three protons. A multiplet, integrating for three protons, occurred between δ 7.6 and 8.1.

The IR spectrum for II showed strong absorption at 1347 (1), 1146 (2), and 956 (3) cm^{-1} and a broad band of absorption between 710 and 840 cm^{-1}. The mass spectrum showed M^+ 256, M + 2 at 258, and a lower peak M + 4 at 260.

Anal.—Calc. for $C_7H_6Cl_2O_2S_2$: C, 32.69; H, 2.35; S, 24.94. Found: C, 32.59; H, 2.26; S, 24.83.

Isolation of Products I and II—The desired compound was initially separated from the reaction mixture by TLC. Solvents and other volatile substances were removed from the reaction mixture by means of a flash vacuum evaporator and heating at 50°. Several drops or a few milligrams of the reaction residue was dissolved in about 0.3 ml of acetone or carbon tetrachloride. About 5 μl of this solution was applied to a 5 × 20-cm sheet coated with silica gel F-254. A diluted solution of methyl disulfide or 2,4-dichlorophenyl disulfide was applied to the chromatographic sheet as a control. The chromatogram of the reaction product of I was developed with benzene–hexane–ether (8:8:1), and the chromatogram of II was developed with benzene–0.5% acetic acid.

Four or five spots were visible on the developed chromatograms when viewed under short wavelength UV light. The solubility of each

[1] Element analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.
[2] Brinkmann Instruments, Des Plaines, Ill.
[3] Varian model EM-360 spectrometer.
[4] Beckman IR-18A spectrophotometer.

spot on the developed chromatogram was determined by the dropwise addition of hexane to each spot and reexamination of the chromatogram under UV light. The areas apparently not influenced by the hexane were then treated with benzene. The areas containing the more polar substances had either little or no apparent solubility in benzene. Most impurities in the reaction products of both I and II were removed by washing the reaction residues with hexane. The washed residues, representing nearly pure I and II, were recrystallized from mixtures of benzene and hexane until a chromatogram indicated a pure substance.

Antibacterial and Antifungal Testing—Both I and II were tested for antifungal activity against *M. audouini*[5] and *T. mentagrophytes*[5] in Sabouraud liquid medium by the serial dilution method (13). Griseofulvin, used as the control agent, prevented growth of the microsporum at 1.25 μg/ml, as indicated by the absence of turbidity in the culture. Griseofulvin prevented growth of the trichophyton at 2.5 μg/ml. Neither thiosulfonate showed antifungal activity at the highest concentration tested, 125 μg/ml.

The antibacterial activity was tested by the serial dilution method using a culture of *S. aureus* (subcultured from FDA 209) in nutrient broth[6] (14). Streptomycin, used as the control agent, prevented growth of the *S. aureus* at 5 μg/ml but not at 2.5 μg/ml.

Compound I prevented growth of the *S. aureus* at 16 μg/ml but not at 8 μg/ml, while II prevented growth at 62.5 μg/ml but not at 31.3 μg/ml.

DISCUSSION

Compound I was first prepared by reacting methanesulfonyl chloride with a solution of commercial potassium sulfide to form potassium methanethiosulfonate. After combining the product of this reaction with 1,4-dibromobutane and purifying the products as described, 150 mg (representing a 2% yield) of a white crystalline compound was obtained. IR, NMR, and mass spectra were obtained for this material, Compound I.

The small yield of I obtained by this procedure was probably due to the fact that much of the commercial potassium sulfide is actually sulfurated potash, a mixture of potassium sulfides. Knowledge of the properties of I obtained from this initial preparation facilitated the separation of I in a much better yield from the reaction mixture as described. Previous attempts to synthesize I using commercial sodium sulfide had proven unsuccessful.

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Effect of Compression Force and Corn Starch on Tablet Disintegration Time

PHILIP M. HILL

Abstract □ An unusual occurrence of decreasing tablet disintegration time with increasing tablet hardness was explored. Tablets were made with varying ratios of starch disintegrant to starch paste and compressed with eight different forces. This unusual disintegration pattern is modified by changes in the starch ratios. The reason for this phenomenon is ascribed to grain swelling as the mechanism by which starch acts as a disintegrant.

Keyphrases □ Tablets—disintegration time, hardness, effect of various ratios of starch disintegrant to starch paste and various compression forces □ Disintegration time, tablet—effect of various ratios of starch disintegrant to starch paste and various compression forces □ Hardness, tablet—effect of various ratios of starch disintegrant to starch paste and various compression forces □ Dosage forms—tablets, disintegration time, hardness, effect of various ratios of starch disintegrant to starch paste and various compression forces □ Starch—effect of various ratios of disintegrant to paste on tablet disintegration time and hardness

Most articles dealing with tablet compressing report that increased compression forces result in tablets with longer disintegration times. Lowenthal (1) cited 31 references supporting this conclusion in a review of tablet disintegration. This cause–effect relationship is logical and not surprising. Higher compression forces