[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY]

A Thiophene Analog of Sulfanilamide

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In view of the well-known fact that the benzene nucleus of physiologically active aromatic compounds can be replaced by the thiophene nucleus without changing the character of the activity, it is of interest to synthesize and determine the activity against bacteria of thiophene analogs of sulfanilamide. Of the six possible isomers, 5-amino-2-thiophenesulfonamide may be considered to be related most closely to sulfanilamide in that the 5-position in thiophenesulfonamide most closely resembles the *p*-position in benzenesulfonamide. However, since nothing is known concerning the effect of the relative positions of the amino group and the sulfonamide group in the thiophene series, all of the isomers are of interest.

At the time this work was begun, none of the thiophene analogs of sulfanilamide had been reported in the literature. In 1948 Burton and Davy[1] reported the synthesis of pure crystalline 4-amino-2-thiophenesulfonamide and an amorphous product presumably 5-amino-2-thiophenesulfonamide. The present paper records the preparation of 5-amino-3-thiophenesulfonamide and the results of attempts to prepare 5-amino-2-thiophenesulfonamide by methods other than that used by Burton and Davy.

The initial attempts to synthesize 5-amino-2-thiophenesulfonamide paralleled the usual procedure for the preparation of sulfanilamide. In the chlorosulfonation of 2-acetamidothiophene, however, the only product that could be isolated was the disulfonyl chloride, regardless of variations in the ratio of reactants, time, temperature and solvent. The product presumably was 5-acetamido-2,4-thiophenedisulfonyl chloride and was converted to the disulfonamide and disulfonanilide.

Hurd and Priestley[2] have reported the preparation of the barium salt of 5-acetamido-2-thiophenesulfonic acid. Attempts to convert the barium salt and the sodium salt to the sulfonyl chloride by reaction with phosphorus pentachloride or thionyl chloride gave only traces of impure oils from which no crystalline amides could be obtained.

Substituted benzenesulfonyl chlorides have been prepared by the action of chlorine on the aryl disulfides in the presence of concentrated nitric and hydrochloric acids.[3] The only satisfactory method reported for the preparation of thienyl disulfides, namely, the reduction of sulfonyl chlorides[4] is of no value for our purpose. The reaction of 5-nitro-2-iodothiophene with sodium disulfide and with sodium polysulfide gave only the sulfide in excellent yield. It was identical with the product obtained from sodium sulfide, and the melting point indicated identity with the 5-nitro-2-thienyl sulfide prepared by Dann and Möller[5] from 5-nitro-2-iodothiophene and thiourea. During the course of this work an improved method for the preparation of iodothiophene was developed.

A small amount of the sulfide also was obtained when 5-nitro-2-iodothiophene was allowed to react with aqueous sodium thiosulfate solution. There was no indication of the formation of the thiosulfate, and hence this compound was not available as an intermediate for the production of the disulfide or the sulfuryl chloride.

When chlorine was passed into a solution of the sulfide in glacial acetic acid containing a small amount of water, the sulfoxide was formed. Dann and Möller[5] have found that the oxidation of the sulfide is difficult and obtained only the sulfone when potassium permanganate in glacial acetic acid was used as the oxidizing agent.

The final attempts to prepare 5-amino-2-thiophenesulfonamide were the treatment of 5-chloro-, 5-bromo- and 5-iodo-2-thiophenesulfonamide with aqueous ammonia, aqueous ammonia in the presence of cuprous chloride or cupric oxide, sodium amide in benzene and sodium amide in liquid ammonia. Only black intractable solids and unchanged starting materials were obtained.

5-Nitro-3-thiophenesulfonamide was prepared by the chlorosulfonation of 2-nitrothiophene and conversion to the amide with ammonia in acetone. These compounds apparently are identical with those prepared by Stadler[6] by sulfonating nitrothiophene, converting to the sulfonyl chloride by heating the potassium salt with phosphorus pentachloride, and forming the amide by treating the sulfonyl chloride with ammonium carbonate. The product probably is 5-nitro-3-thiophenesulfonamide, because it is different from 5-nitro-2-thiophenesulfonamide.[1] The only other possible isomer, 2-nitro-3-thiophenesulfonamide, would not be expected, since its formation would require sulfonation or chlorosulfonation ortho to the nitro group. Reduction of 5-nitro-3-thiophenesulfonamide in alcoholic solution at room temperature and atmospheric pressure with hydrogen and Raney nickel catalyst gave 5-amino-3-thiophenesulfonamide.

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(1) Burton and Davy, *J. Chem. Soc.*, 525 (1948).

(2) Hurd and Priestley, THIS JOURNAL, **69**, 859 (1947).

(3) Fierz, Schlittler and Waldmann, *Helv. Chim. Acta*, **12**, 663 (1929).

(4) Challenger, Miller and Gibson, *J. Chem. Soc.*, 769 (1948).

(5) Dann and Möller, *Chem. Ber.*, **80**, 23 (1947).

(6) Stadler, *Ber.*, **18**, 534 (1885).

amino-3-thiophenesulfonamide. In agar diffusion tests 5-nitro-3-thiophenesulfonamide at a concentration of 0.02 *M* inhibited the growth of *α-Streptococci*, *β-Streptococci* (Rantz), *Neisseria catarrhalis*, *Bacillus subtilis*, *Escherichia coli* and *Pasteurella pestis*, but 5-amino-3-thiophenesulfonamide at the same concentration did not show any inhibition. In broth dilution tests 5-nitro-3-thiophenesulfonamide was four to eight times more active than sulfathiazole against *Bacillus subtilis* and *Pasteurella pestis*. This activity is very high since sulfathiazole is 500 times more active than sulfanilamide. On the other hand 5-amino-3-thiophenesulfanilamide is about as active as sulfanilamide. Limited tests indicated that these compounds are five to ten times as toxic to mice as sulfathiazole.

Experimental

Chlorosulfonation of 2-Acetamidothiophene.—2-Nitrothiophene[7] was reduced to the amine chlorostannate,[8] which in turn was converted to 2-acetamidothiophene.[9] The last step consists of adding sodium hydroxide solution to a stirred mixture of the chlorostannate and water covered with a solution of acetic anhydride in ether. Yields as high as 89% of once crystallized product melting at 157–158° were obtained, if the mixture was not stirred after all the sodium hydroxide had been added. Long stirring decreased the yield and increased the difficulty of isolating a pure product. Steinkopf[9] reported a yield of 65% of material melting at 161–162°.

Eight grams (0.057 mole) of 2-acetamidothiophene was added during 25 minutes to 52 g. (0.45 mole) of chlorosulfonic acid with stirring at 25°. The reaction mixture was protected from moisture. After the addition the mixture was heated at 80–90° for 20 minutes and poured into 75 g. of ice. The gray precipitate was dissolved in benzene, and the solution decolorized with Norit and concentrated at reduced pressure. Cooling gave 6.0 g. (31%) of light crystals melting at 111–113.5°. Two crystallizations from cyclohexane–benzene (2:1) gave white crystals melting at 114–114.5°.

Anal.[10] Calcd. for $C_6H_5Cl_2NO_5S_2$: C, 21.31; H, 1.48; Cl, 20.97. Found: C, 21.52; H, 1.62; Cl, 20.7. This product is assumed to be **5-acetamido-2,4-thiophenedisulfonyl chloride**. All variations from the above procedure gave lower yields of the disulfonyl chloride. When the reaction was run at 0–5°, the disulfonic acid was obtained and isolated as the barium salt.

A solution of 1.8 g. (0.005 mole) of the disulfonyl chloride in 60 cc. of benzene was saturated with ammonia. The precipitated solid was washed with water to give 0.25 g. of gray crystals. Several crystallizations from aqueous alcohol gave white needles of **5-acetamido-2,4-thiophenedisulfonamide**, m. p. 246–247° (dec.).

Anal. Calcd. for $C_6H_9N_3O_6S_2$: C, 24.07; H, 3.03. Found: C, 24.42; H, 2.99.

Heat was evolved when the disulfonyl chloride was mixed with aniline. The product was extracted with acetone, the acetone evaporated and the residue extracted with dilute sodium hydroxide. The solution was treated with Norit and the filtrate slowly neutralized with hydrochloric acid. The white amorphous precipitate of **5-acetamido-2,4-thiophenedisulfonanilide** was crystallized twice from aqueous alcohol (1:1) when it melted at 170–171°.

Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$: C, 47.90; H, 3.80. Found: C, 48.21; H, 3.82.

2-Iodothiophene.—To a solution of 38 g. (0.15 mole) of iodine in 42 g. (0.50 mole) of thiophene was added dropwise with stirring 28 cc. (0.43 mole) of nitric acid (sp. gr. 1.42) diluted with an equal volume of water. The mixture was warmed to start the reaction but then required cooling with an ice-bath to control the reaction. After addition of the nitric acid the mixture was refluxed for 30 minutes. The oily layer that settled to the bottom was separated, mixed with 40 cc. of 10% sodium hydroxide solution, and steam distilled. The yellow oil was separated, dried over anhydrous calcium chloride and distilled at reduced pressure from a Claisen flask having a fractionating side arm. The yield of 2-iodothiophene distilling at 89–93° (36 mm.) was 45–47 g. (72–75%). The yield is the same as that obtained by the usual procedure[11] and avoids the use of mercuric oxide.

Reaction of Sodium Disulfide with 5-Nitro-2-iodothiophene.—To a solution of 5 g. (0.02 mole) of 5-nitro-2-iodothiophene[12] in 50 cc. of 95% ethyl alcohol was added dropwise a solution of 1.65 g. (0.015 mole) of sodium disulfide[13] in 20 cc. of water. On contact of the sulfide solution with the orange alcoholic solution of nitroiodothiophene, a whitish-yellow precipitate formed with slight evolution of heat. After the addition of the aqueous sodium disulfide solution a large amount of precipitate had formed, and the mixture was stirred for another hour at room temperature.

The reaction mixture was poured into 150 cc. of ice and water and allowed to stand for 15 minutes, filtered through a sintered-glass funnel, and the precipitate washed with ice-water and dried in air. The crude solid, weighing 3.2 g. and melting at 65–90° was dissolved in 70 cc. of hot methyl alcohol, and the solution was treated with Norit and filtered. The filtrate on cooling gave yellow needles which sintered at 99° and melted at 101–103°. More yellow needles were obtained by concentration of the methyl alcohol solution to give a total of 2.9 g. of product. After recrystallization from methyl alcohol the yellow needles sintered at 100° and melted at 100.5–103.5° to a yellow liquid. A similar product melting at 104–105° was obtained from a solution of sodium disulfide prepared from aqueous sodium sulfide and sulfur. The product of the reaction of 5-nitro-2-iodothiophene with aqueous alcoholic sodium sulfide solution melted at 103–105° and did not depress the melting point of the material obtained from sodium disulfide. The same compound also was obtained when 5-nitro-2-iodothiophene was refluxed with an aqueous alcoholic solution of sodium thiosulfate. The recorded melting point of 5-nitro-2-thienyl sulfide prepared from thiourea[5] is 104–106°.

Chlorine was passed into a solution of 0.34 g. of the sulfide in 14 cc. of glacial acetic acid and 1 cc. of water for one half hour at 50–60°. Pouring onto ice gave a fine yellow precipitate which after crystallization from benzene weighed 0.23 g. and melted at 135–136°. Analysis indicated that the compound is **5-nitro-2-thienyl sulfoxide**. It is insoluble in water, ether, and petroleum ether, and slightly soluble in ethyl alcohol and benzene.

Anal. Calcd. for $C_8H_4O_5N_2S_3$: C, 31.57; H, 1.32; S, 31.62. Found: C, 31.86; H, 1.35; S, 31.69.

5-Chloro-2-thiophenesulfonamide and 5-Chloro-2-thiophenesulfonanilide.—These compounds were prepared by heating 5-chloro-2-thiophenesulfonyl chloride[14] with ammonium carbonate and with aniline. **The amide** was soluble in methyl alcohol, ethyl alcohol and acetic anhydride but insoluble in water and chloroform. Repeated crystallizations from methyl alcohol gave white crystals melting at 115–116°.

Anal. Calcd. for $C_4H_4ClNO_2S_2$: C, 24.30; H, 2.04. Found: C, 24.60; H, 2.06.

The anilide after crystallization from methyl alcohol was obtained as white crystals melting at 84–85°.

(7) Babasinian, "Organic Syntheses," Coll. Vol. II, p. 466, 1943.

(8) Steinkopf and Lützkendorf, *Ann.*, **403**, 28 (1914).

(9) Steinkopf and Lützkendorf, *ibid.*, **403**, 31 (1914).

(10) All carbon, hydrogen and nitrogen analyses are by Microchemical Specialties Co., Berkeley, Calif.

(11) Minnis, "Organic Syntheses," Coll. Vol. II, p. 357 1943.

(12) Rinkes, *Rec. trav. chim.*, **53**, 643 (1934); Dann, *Ber.*, **76**, 419 (1943).

(13) Rule and Thomas, *J. Chem. Soc.*, **105**, 177 (1914).

(14) Steinkopf and Koehler, *Ann.*, **532**, 250 (1937).

Anal. Calcd. for $C_{10}H_8ClNO_2S_2$: C, 43.87; H, 2.95. Found: C, 44.17; H, 3.08.

Attempts to replace the chlorine atom of the amide by heating with aqueous ammonia and cuprous chloride at 180–190° for 4 hours gave a black product from which no crystalline material could be isolated. Unchanged amide was recovered after refluxing with sodium amide in benzene for 8 hours.

5-Bromo-2-thiophenesulfonamide[15] was recovered unchanged after heating with aqueous ammonia at 150° for 2 hours. When cupric oxide was present a dark product was obtained from which only a small amount of starting material could be isolated. A similar result was obtained when the amide was heated with sodium amide in liquid ammonia for 2 hours at 90°. Likewise only starting material was isolated after 5-iodo-2-thiophenesulfonamide[15] was refluxed with sodium amide in benzene for 6 hours.

5-Nitro-3-thiophenesulfonyl Chloride and 5-Nitro-3-thiophenesulfonamide.—One-half of a solution of 39 g. (0.3 mole) of 2-nitrothiophene in 100 cc. of chloroform was added dropwise with stirring and refluxing to 93 g. (0.8 mole) of chlorosulfonic acid in 100 cc. of chloroform. An additional 93 g. of chlorosulfonic acid then was added in one lot to the flask and the remainder of the 2-nitrothiophene solution was added dropwise. The addition required about 40 minutes, and refluxing on the steam-bath was continued for 10 hours.

After cooling, the mixture was poured into a separatory funnel. On standing two layers formed with the acid layer on the bottom. The two layers were added separately to ice and water mixtures. The aqueous layers were extracted with several portions of chloroform, and the chloroform extracts were combined and washed with water. The chloroform solution was dried over anhydrous sodium sulfate, treated with Norit, filtered and distilled on a steam-bath under reduced pressure to remove the chloroform. The residue was transferred to a modified Claisen flask, and the fraction distilling at 146–151° (5–7 mm.) was collected to give 45 g. (74%) of 5-nitro-3-thiophenesulfonyl chloride as a yellow oil. Even after further purification and cooling, it would not crystallize. Stadler's product[6] was a viscous oil.

For converting the sulfonyl chloride to the amide, the best results were obtained by saturating a solution of 10 g. of the sulfonyl chloride in 50 cc. of acetone with ammonia, removing the ammonium chloride by filtration, diluting with 15 cc. of water, and evaporating until all of the acetone was removed. The residue of 5-nitro-3-thiophenesulfonamide weighed 7.7 g. Crystallization from acetone–water (1:1) gave 5.6 g. (60%) of light yellow crystals melting at 169.5–172°. After three recrystallizations from water the product melted at 171–173°. Stadler[6] reported 172–173° for his product.

Anal. Calcd. for $C_4H_4N_2O_4S_2$: C, 23.07; H, 1.94. Found: C, 23.22; H, 1.82.

When 13.5 g. of the sulfonyl chloride was heated with 10 g. of aniline for 15 minutes on the steam-bath, a solid was obtained which was crystallized from benzene to give 12.5 g. of yellow crystals melting at 129–133°. After three recrystallizations it melted at 135–136°.

Anal. Calcd. for $C_{10}H_8N_2O_4S_2$: C, 42.24; H, 2.84. Found: C, 42.66; H, 2.73.

5-Amino-3-thiophenesulfonamide.—A solution of 2 g. of 5-nitro-3-thiophenesulfonamide in 50 cc. of absolute alcohol was reduced with hydrogen in the presence of Raney nickel using a small-scale apparatus.[16] The calculated amount of hydrogen was absorbed in 6 hours. After removal of the catalyst (dry weight 2.2 g.) the solution was concentrated to 10 cc. Cooling gave 1.0 g. of grayish-yellow crystals. Further concentration of the mother liquor gave a small amount of crystals contaminated with a red oil. Repeated crystallization from ethyl alcohol–isopropyl alcohol (1:1) gave a product melting at 158° with decomposition.

Anal. Calcd. for $C_4H_6N_2O_2S_2$: C, 26.95; H, 3.39; N, 15.72. Found: C, 26.89; H, 3.36; N, 15.59.

Attempts to reduce 5-nitro-3-thiophenesulfonanilide gave a red viscous oil from which only an amorphous brown solid could be obtained.

Summary

5-Amino-3-thiophenesulfonamide has been prepared together with several new compounds obtained as intermediates in its preparation or in attempts to prepare 5-amino-2-thiophenesulfonamide.

(15) Steinkopf, Jacob and Penz, *Ann.*, **512**, 136 (1934).

(16) Noller and Barusch, *Ind. Eng. Chem., Anal. Ed.*, **14**, 907 (1942), **18**, 730 (1946).

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[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Phosphorylation of Starch

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As part of a general program on the reaction of starch with polyfunctional reagents, we have prepared esters by treating several starches with phosphorus oxychloride in pyridine. The literature contains several references[2,3] to esters of amylaceous polysaccharides prepared in this manner. Those derivatives were reported to be soluble in water. Starch,[4] "soluble starch,"[5] and various starch materials[6,7,8,9] have been phosphorylated in aqueous media to give soluble products containing up to 2% phosphorus.

In this paper we describe the preparation of water-insoluble phosphates of a variety of starches by reaction with phosphorus oxychloride in pyridine. We found that the pretreatment given the starches controlled their reactivity and the nature of the products formed.

Starches which had been oven-dried or dried

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) H. Vogel, *Ber.*, **72**, 2052 (1939).

(3) P. Karrer, H. Koenig and E. Usteri, *Helv. Chim. Acta*, **26**, 1296 (1943).

(4) H. K. Barrenscheen and J. Pany, *Biochem. Z.*, **219**, 364 (1930).

(5) J. Kerb, *ibid.*, **100**, 3 (1919).

(6) H. Pringsheim and K. Goldstein, *Ber.*, **56**, 1520 (1923).

(7) P. Koets, *Proc. Acad. Sci. Amsterdam*, **38**, 63 (1935).

(8) P. Koets and H. R. Kruyt, *C. A.*, **32**, 1543 (1938); *Kolloid-Beihefte*, **47**, 100 (1937).

(9) M. Samec, "Kolloidchemie der Stärke," Theodor Steinkopff, Dresden-Blasewitz, 1927, pp. 25–28.