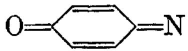
the position *para* to the —OH group is unoccupied. No exceptions are known to this rule.

Phenols containing activating groups stronger than alkyls, *e.g.*, —OH, $—NH_2$, $—OCH_3$ will *generally* not give the test even though the para position may be unoccupied.

An exception to this rule is *m*-methoxy phenol.

Phenols containing deactivating groups stronger than halogens, *e.g.*, $—NO_2$, —CHO, —COOH, $—COCH_3$ will *not* give the test even though the *para* position is unoccupied. An exception to this rule is orthomercurichloride phenol.

The above results are all explicable on the basis of the reactivity of the intermediate  presumed formed in the formation of indophenol.

The nitrosyl ion is also produced in concentrated sulfuric acid by nitrosamines and esters of nitrous acid which therefore give a Liebermann reaction in the presence of phenol.

EXPERIMENTAL

The reaction as described was carried out on the following phenols. *Class A.* Phenols with the *para* position unoccupied which gave a *positive* reaction (*i.e.*, blue or green in concentrated sulfuric acid, red in dilute sulfuric acid, blue or green in alkali): phenol, *o*-cresol, *m*-cresol, *o*-chlorophenol, *o*-bromo phenol, carvacrol, thymol, *o*-hydroxy diphenyl, *m*-methoxy phenol, *o*-mercurichloride phenol, 2,5-xylenol.

Class B. Phenols with the *para* position unoccupied which did not give a *positive* reaction: 3,5-xylenol, saliginen, *o*-methoxy phenol, catechol, resorcinol, pyrogallol, phloroglucinol, *o*-amino phenol, *m*-amino phenol (activating groups), *o*-nitro phenol, *m*-nitro phenol, *o*-hydroxy benzaldehyde, *m*-hydroxy benzaldehyde, *o*-hydroxy benzoic acid, *m*-hydroxy benzoic acid, methyl salicylate, ethyl salicylate, phenyl salicylate, 2,4-dihydroxy benzoic acid, 2,3-cresotic acid, (deactivating groups).

Class C. Phenols with the para position occupied not giving a *positive* test: *p*-amino phenol, *p*-cresol, 2,4-dichlorophenol, *p*-methoxy phenol, *p*-hydroxy benzoic acid, *p*-chloro phenol, *p*-hydroxy phenol, *p*-cresol, 2,4,6-trichlorophenol, 2,4-dinitro phenol, pentachlorophenol, *p*-nitro phenol, *p*-chloro-*m*-cresol.

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2-Nitro-, 3-Nitro-, 2,4-Dinitro-, and 2,5-Dinitrothiophene[1]

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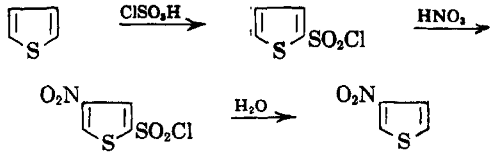
Some years ago we prepared sizeable quantities of 2-nitrothiophene and 2,4-dinitrothiophene and found that there was some confusion in the literature about the properties and/or behavior of these compounds and the related 3-nitrothiophene and 2,5-dinitrothiophene.[2] Since that time we have accumulated enough information about these compounds to clarify the situation for ourselves. Because the confusion in the literature does not appear to be generally recognized, we present in this note what we believe is an accurate description of the compounds in question.

The nitration of thiophene, best done by the nitric acid–acetic anhydride procedure described in Organic Syntheses,[3] leads to 2-nitrothiophene containing small amounts (up to a maximum of about 5%) of 3-nitrothiophene. The presence of 3-nitrothiophene in the mononitration product from thiophene was established by Steinkopf,[4] but there has been no description of a procedure for obtaining pure 2-nitrothiophene from the crude nitration product and for establishing the homogeneity of the 2-nitrothiophene thus obtained. We prepared pure 2-nitrothiophene from the crude mononitration product by crystallization to constant, sharp melting point from purified petroleum ether (b.p. 40–60°) and from ethanol–water. The melting point of this material in a Hershberg apparatus with calibrated thermometer is 43–44°; on a Fisher melting point block, the same material melts over less than a half degree. From the melting point ranges of mixtures of purified 2-nitrothiophene and purified 3-nitrothiophene (whose preparation and purification are described below) it may be concluded that 2-nitrothiophene which melts over less than half a degree range on the Fisher block is pure.

Melting ranges of mixtures of 2-nitrothiophene and 3-nitrothiophene:

% 2-Nitrothiophene	Melting range
95	39–45°
90	39.5–47
75	39–47
50	40–57
25	40–64
10	63–74
5	68–74

The only feasible preparation of 3-nitrothiophene is that described by Steinkopf and Höpner.[4a] We have modified and simplified the procedure, as described in the experimental section, so that 3-nitrothiophene can be prepared in about a 20% over-all



(together with some 5-nitroderivative)

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(1) This note is based on undergraduate research done at Queens College by Mrs. Bach and Mr. Kresch.

(2) (a) "Thiophene and Its Derivatives," Howard D. Hartough, Interscience, New York, 1952, p. 221; (b) L. S. Levitt and Edgar Howard, Jr., *J. Am. Chem. Soc.*, **76**, 1951 (1954).

(3) V. S. Babasinian, *Org. Syntheses*, **Coll. Vol. II**, 466 (1943).

(4) (a) W. Steinkopf and T. Höpner, *Ann.*, **501**, 174 (1933); (b) W. Steinkopf, *Ann.*, **545**, 38 (1940).

yield from thiophene by the following three reactions.

Apparently 3-nitrothiophene has not hitherto been prepared in sufficient quantity to permit careful purification, for the melting points reported for the compound vary from 68° to 79°. 3-Nitrothiophene is higher melting and less soluble than the 2-nitro derivative and it can be purified easily by crystallization from ethanol. Material purified in this way to constant, sharp melting point melts at 75–76° in a Hershberg apparatus with calibrated thermometer; on the Fisher block the material melts over less than 0.5°-range and can be seen to sublime between the glass slides. From the melting point ranges given earlier for mixtures of 2-nitro- and 3-nitro-thiophene, the 3-nitro derivative can be considered pure when it melts over a half-degree range.

Two dinitrothiophenes have been prepared by nitration of the mononitrothiophenes; 3-nitrothiophene furnishes exclusively 2,4-dinitrothiophene, m.p. 49–50°, and 2-nitrothiophene furnishes predominantly the same product together with smaller amounts (~15%) of the isomeric *2,5-dinitrothiophene, m.p. 78–82°.* There is no satisfactory way of preparing 2,5-dinitrothiophene and only one sample of the material has been reported. That sample was obtained by heating the crude dinitration product from 2-nitrothiophene with nitric and sulfuric acids, which removes the 2,4-dinitrothiophene by oxidation and leaves the 2,5-dinitrothiophene unattacked.[4a] We have done no work with 2,5-dinitrothiophene, but we have prepared and purified 2,4-dinitrothiophene, m.p. 49.8–50.2° (corr.) and have confirmed the structure originally assigned to it by showing that it is identical with the product obtained from 2-iodo-3,5-dinitrothiophene when the iodine is replaced by hydrogen by means of dilute hydriodic acid.

The behavior of 2-nitrothiophene on nitration—formation principally of a 2,4-disubstituted derivative—is characteristic of those 2-monosubstituted thiophenes in which the substituent is strongly *meta* directing. As a consequence, direct substitution is not satisfactory for the preparation of 2,5-disubstituted thiophenes in which both substituents are *meta* directing, and for many such disubstituted thiophenes there is no good method of preparation. The orientation pattern just described was clearly stated by Steinkopf upon whose work it is principally based.[4a] Restatement is necessary because later workers have either overlooked or misread his work and have assigned the 2,5 structure to disubstitution products that are actually 2,4 derivatives.

The infrared spectra of 2-, 3-, and 2,4-dinitrothiophene have been determined by Dr. R. C. Gore of the Research Division of the American Cyanamid Co. and are included in the collection of the American Petroleum Institute. 2-Nitro, 3-nitro, and 2,4-dinitrothiophene give the characteristic colors with sodium iodide.[5] Neither 2- nor 3-nitrothiophene gives a positive isatin reaction.

EXPERIMENTAL

2-Nitrothiophene. Crude 2-nitrothiophene[3] was dissolved in petroleum ether (b.p. 40–60°, freshly distilled over potassium permanganate) by keeping the solvent and excess solute in a glass-stoppered flask in a water bath at 40°. The clear saturated solution was chilled overnight in a refrigerator. About 7 g. of 2-nitrothiophene dissolves in 100 ml. of the solvent and the recovery of crystallized product averages about 75%. Four crystallizations as just described followed by one crystallization from dilute ethanol (1.0 g. of solute, 10 ml. of ethanol, and 8 ml. of water) and a final crystallization from petroleum ether gave a product of m.p. 43–44° (corr.) in a Hershberg apparatus, and 43–43.4° (uncorr.) on a Fisher block. The melting point was not changed by the last three crystallizations.

3-Nitrothiophene. In a 400-ml. beaker, 100 g. of chlorosulfonic acid and 50 ml. of anhydrous chloroform were mixed and stirred mechanically while 25 ml. of thiophene was added during 4 min. The temperature of the reaction mixture was held at 0° by the direct addition of solid carbon dioxide. The reaction mixture was allowed to warm to room temperature and then chilled again with solid carbon dioxide while ice was added to decompose the excess chlorosulfonic acid. The layers were separated and the aqueous layer was extracted with 25 ml. of chloroform. The combined chloroform layers were washed with 50 ml. of water, dried over anhydrous sodium sulfate, and distilled. From six such runs there was obtained 119 g. (37%) of thiophene-2-sulfonyl chloride, b.p. 99–101°/6 mm., which solidified on cooling.

During 1 hr. 69.2 g. of thiophene-2-sulfonyl chloride was added dropwise to 281 ml. of fuming nitric acid (d. 1.59–1.60) that was stirred mechanically and held between 25 and 30° by a cold water bath. After the addition the reaction mixture was held at 40° for 1 hr., then was poured onto 1 kg. of ice and the organic material separated with the aid of 350 ml. of carbon tetrachloride. The carbon tetrachloride solution was washed with water, dried, and distilled. In this way from 119 g. of thiophene-2-sulfonyl chloride there was obtained 105 g. (76%) of 4-nitrothiophene-2-sulfonyl chloride boiling at 145–150°/4 mm. and less than 5 g. of 5-nitrophene-2-sulfonyl chloride boiling at 133–136°/4 mm.

The 105 g. of 4-nitrothiophene-2-sulfonyl chloride and 530 ml. of water were boiled under reflux for 4 hr. during which time all but traces of the acid chloride dissolved. The clear yellow solution was left overnight, then 655 g. of ice and 920 ml. of concentrated sulfuric acid were added and the resulting solution was divided in five portions and distilled with superheated steam. The solution was heated to 140° (thermometer in the liquid) before the superheated steam was introduced and was kept at this temperature during the distillation. Each portion of the reaction mixture required about 90 min. for the distillation and the entire distillate amounted to 1.2 l. The 3-nitrothiophene that precipitated in the distillate weighed 44 g. (74%).

The 3-nitrothiophene is readily purified by crystallization from ethanol. Using 3 ml. of solvent per gram of solute the recovery is between 75 and 80%. Two such crystallizations furnished pure material whose melting point (75–76° corr., in a Hershberg apparatus; 75–75.5°, uncorr., in a Fisher block) was not changed by a third crystallization from 60–90° ligroin.

Two comments on the preparation of 3-nitrothiophene are in order. The least satisfactory step is the reaction with chlorosulfonic acid. This step, which has the advantage of convenience, could probably be improved by the isolation

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(5) A. H. Blatt and Norma Gross, *J. Org. Chem.*, 22, 1046 (1957).

of the sulfonic acid as the sodium salt and its conversion to the sulfonyl chloride,[6] but these operations require equipment not available in the ordinary laboratory. The nitration of the sulfonyl chloride according to the literature[4a] furnishes the 4-nitro and 5-nitro derivative in an 8:3 ratio. In our work the ratio of 4-nitro to 5-nitro was closer to 9:1.

2,4-Dinitrothiophene. Crude 2,4-dinitrothiophene obtained by the nitration of 2-nitrothiophene[4a] was purified by three crystallizations from ethanol and one from petroleum ether (b.p. 40–60°). Solutions were prepared and handled in the same way as with 2-nitrothiophene. About 2 g. of the dinitrothiophene will dissolve in 10 ml. of ethanol or 250 ml. of petroleum ether at 40°. The recovery from ethanol is about 15%, from petroleum ether about 60%. The pure dinitrothiophene melts at 49.8–50.2°, corr., in a Hershberg apparatus.

2,4-Dinitrothiophene from 2-Iodo-3,5-dinitrothiophene. A solution of 4.5 g. of sodium iodide in 15 ml. of acetone was added to 3.0 g. of 2-iodo-3,5-dinitrothiophene dissolved in 15 ml. of acetone and 5 ml. of glacial acetic acid. After two weeks the dark brown reaction mixture was poured into a solution of 5.0 g. of sodium bisulfite in 140 ml. of water and the dark oily precipitate was stirred until it became granular. The yellow-brown solid was dissolved in 25 ml. of hot ethanol and the solution, after it had been decolorized with Norit and filtered, was diluted with 40 ml. of water to yield 1.2 g. (66%) of 2,4-dinitrothiophene, m.p. 51–52°, whose identity was confirmed by a mixed melting point. In another experiment the solid was digested with ligroin; the extract on evaporation furnished 2,4-dinitrothiophene. The residue from the ligroin extraction when crystallized from ethanol gave unreacted 2-iodo-3,5-dinitrothiophene.

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(6) See reference 2a. p. 513.

An Improved Micro Synthesis of Thiamine-S^{35}[1]

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In connection with a study of the metabolism of thiamine which is in progress in this laboratory, it became necessary to prepare thiamine-S^{35} of a high specific activity. Williams and Ronzio[2] have synthesized labeled thiamine from thiourea-S^{35} and thiourea-2-C^{14}. We attempted to duplicate this synthesis, and obtained very low yields when less than 300 mg. of thiourea-S^{35} was used for the initial step of the synthesis. We therefore revised the steps of the synthesis and, starting with 50–100 mg. of carrier-free thiourea-S^{35}, have obtained high yields.

EXPERIMENTAL

2-Amino-5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} hydrochloride. Thiourea-S^{35} (100 mg.)[3] was coupled with 330 mg. of γ-aceto-γ-chloropropanol (1:1.5 molar ratio, respectively) according to the method of Todd *et al.*[4] The substances were mixed in a 5-ml. beaker on the steam bath, and the mixture stirred continuously until the thiourea-S^{35} went into solution, and then 5 min. longer. The solution was removed from the steam bath, and stirred until the mass solidified. The solid mass was broken up, transferred to a centrifuge tube, extracted with anhydrous ether, and the ether was discarded. The residue was dissolved in the minimum amount of hot anhydrous ethanol (1–1.5 ml.). After the solution was cooled, absolute ether was added dropwise until crystallization began. A fivefold excess of ether was then added and the crystallization allowed to go to completion while standing for about 12 hr. in the refrigerator. The precipitate was filtered, rinsed with anhydrous ether, and dried in a vacuum desiccator to yield 220 mg. (86%) of 2-amino-5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} hydrochloride, m.p. 148–150°.

It was found that when 75–100 mg. of thiourea were coupled with γ-aceto-γ-chloropropanol in a molar ratio of 1:1.5, respectively, an average yield of 82% of the 2-aminothiazole was obtained. When very small amounts (less than 75 mg.) of thiourea were used, increasing this ratio of reactants to 1:2 resulted in an average yield of 78%.

5-Methyl-4-(β-hydroxyethyl)thiazole-S^{35}. 2-Amino-5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} hydrochloride (220 mg.) was dissolved in 5.8 ml. of concentrated hydrochloric acid, with cooling in a methanol–ice bath at −5°. Sodium nitrite (1.8 ml. of a 1*N* solution, precooled to 0°) was added dropwise. The mixture was allowed to stand for 30 min. at 0° to −5°. Then 5.8 ml. of water (also precooled to 0°) was slowly added in order to reduce the concentration of hydrochloric acid to 6*N*. During approximately 10 min., 2.6 ml. of cold 32% hypophosphorous acid was added, with the temperature maintained at −5°. The solution was stirred rapidly during all of the above additions. The reaction mixture was placed in the refrigerator at 0° to 2° for 12–15 hr., during which time nitrogen was evolved. The solution was cooled to −5° and neutralized by the addition of 30% sodium hydroxide. Excess base was then added to bring the *p*H to 11–12, and the solution was washed into a continuous extractor and extracted for 16 hr. with ether.[5] After removal from the extractor, the aqueous phase was extracted with several portions of ether in a separatory funnel. The combined ethereal extracts were dried over anhydrous magnesium sulfate, and the ether was removed by distillation from the steam bath. The yield of 5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} was 70 mg. (50%). The reduced thiazole was obtained in yields ranging from 50–70% when 150–250 mg. of the 2-aminothiazole were used. The crude thiazole-S^{35} was identified by the conversion of a small amount to the picrate, which melted at 159–161° when precipitated from anhydrous ether.

Since the condensation of the crude thiazole with the pyrimidine moiety gave very low yields of thiamine, we found it necessary to purify the crude material by distillation under reduced pressure. The pure thiazole-S^{35} was obtained in recoveries of 85–90%, when 50–100 mg. of the crude material were distilled under reduced pressure (b.p. 126–128° at 2–3 mm. and 128–130° at 3–4 mm. pressure).

Thiamine-S^{35} bromide hydrobromide. The 5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} (25 mg.) and 60 mg. of 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide (1:1 molar ratio) were dissolved in 2 ml. of anhydrous ethanol[6] in a 20-ml. pear-shaped flask fitted with a reflux condenser,

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(1) This investigation was aided, in part, by Contract No. AT (30-1)-1056 between the U.S. Atomic Energy Commission and Fordham University.

(2) D. L. Williams and A. R. Ronzio, *J. Am. Chem. Soc.*, **74**, 2409 (1952).

(3) Obtained from Abbott Laboratories, Division of Radio-Pharmaceuticals, North Chicago, Ill.

(4) A. R. Todd, F. Bergel, H. L. Fraenkel-Conrat, and A. Jacob, *J. Chem. Soc.*, 1601 (1936).

(5) The extraction period can be shortened from 40 hr. without any decrease in the yield of thiazole.[2]

(6) Williams and Ronzio[2] carried out the condensation in butanol at 125°. The side reaction (ether formation between the butanol and the β-hydroxyethyl group of the thiazole moiety) which occurs under those conditions was completely avoided when anhydrous ethanol was used as the solvent.