mercaptan in pyridine overnight and after pouring on ice, material separated that slowly crystallized; yield 57%. The filtered material was recrystallized twice from ether and petroleum ether; m. p. 87–87.5°, $[\alpha]^{27}D$ + 17° (*c* 5, chloroform).

*Anal.* Calcd. for $C_{15}H_{22}O_9S$: C, 47.62; H, 5.86; S, 8.46. Found: C, 47.64; H, 5.54; S, 8.59.

**Hydrogenolysis of Ethyl Thiol-D-ribonate Tetraacetate.**—One gram of the thiol ester was refluxed for six hours with 15 g. of Raney nickel in 100 cc. of 80% ethanol and after removal of the catalyst and solvent the residue was dissolved in acetone and methanol and then petroleum ether was added to incipient turbidity. *aldehydo*-D-Ribose tetraacetate crystallized on nucleation; yield 0.19 g. (22%), m. p. 101–102°, $[\alpha]^{23}D$ −16.2° (*c* 3.3, acetone); $[\alpha]^{25}D$ −10° (*c* 5, absolute chloroform). Pasternack and Brown[3] cite the constants: m. p. 98–99°, $[\alpha]^{20}D$ −16.7° (*c* 5, acetone).

**Acknowledgment.**—We express our thanks to Dr. Alva Thompson for the optical rotations recorded in this publication.

## Summary

Reduction of the carboxyl group to the aldehyde stage is effected through the reductive desulfurization by Raney nickel of the thiol ester. Application of this reaction is made to the synthesis of benzaldehyde, propionaldehyde and *aldehydo*-D-ribose tetraacetate.

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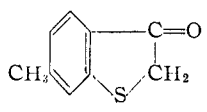
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ROCHESTER]

# Syntheses in the Thionaphthene Series

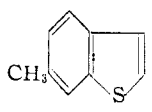
BY D. S. TARBELL AND D. K. FUKUSHIMA

It was shown recently[1] that 5-methyl-4,7-thionaphthenequinone, an isostere of 2-methyl-1,4-naphthoquinone, could be prepared readily by oxidation of 5-methylthionaphthene with chromic acid. The present paper describes experiments designed to prepare 6-methyl-4,7-thionaphthenequinone (III), the isomer of the compound previously reported, by the same general method. It has been possible to obtain a very low yield of this substance.

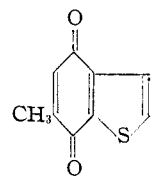
The necessary intermediates for the synthesis are 6-methyl-3-keto-2,3-dihydrothionaphthene (I) and 6-methylthionaphthene (II), obtained from I by reduction. The preparation of I caused



I

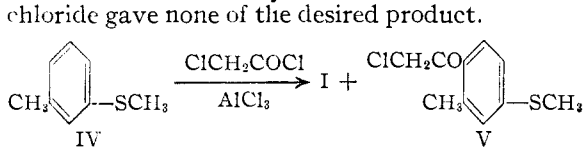


II



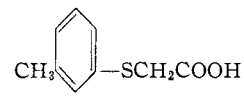
III

much more difficulty than was anticipated from our previous experience with its isomer; the action of chloroacetyl chloride and aluminum chloride on methyl *m*-tolyl sulfide[2] (IV) gave poor and inconsistent yields of impure I, along with a side-product V, which was proved to be 2-methyl-4-methylmercapto-ω-chloroacetophenone as will be shown later. The use of anhydrous stannic chloride as the catalyst instead of aluminum chloride gave none of the desired product.



IV V

In view of these unsatisfactory results, attention was turned to the cyclization of *m*-thiocresoxyacetic acid VI.



VI

Cyclization of the acid chloride with aluminum chloride gave as poor results as in the previous case, and stannic chloride gave no ring closure. Treatment of the acid VI with acetic anhydride, acetic acid and anhydrous zinc chloride,[3] which it was hoped would produce the enol acetate of I, gave only starting material, and cyclization with 80% sulfuric acid[4] was equally unsuccessful. The best method of preparing I was the cyclization of VI with anhydrous hydrogen fluoride, which gave a fair yield of I, containing some impurities. Attempts at purifying the product were unsatisfactory, since I is very easily oxidized by air, so that the crude product was reduced directly to the thionaphthene II with zinc and acetic acid.[5]

Oxidation of the thionaphthene II with chromic acid in acetic acid gave less than 0.1% yield of the desired quinone III; this was identified by analysis, by a positive Craven[5a] test and by its absorption spectrum, which as shown in Fig. 1, is almost identical with that of the 5-methyl isomer.[1] In the oxidation, a colorless neutral substance was obtained in 10–15% yield, which is believed to be the sulfone VII derived from 6-methyl-2,3-dihydrothionaphthene; this struc-

(1) Tarbell, Fukushima and Dam, THIS JOURNAL, **67**, 1643 (1945).

(2) Auwers and Arndt, *Ber.*, **42**, 537 (1909).

(3) Fieser and Hershberg, THIS JOURNAL, **59**, 1028 (1937).

(4) Haworth, *J. Chem. Soc.*, 1125 (1932).

(5) Alternative methods for the preparation of ketodihydrothionaphthenes, such as the ring closure of *o*-carboxythiophenoxyacetic acids, followed by decarboxylation (*e. g.*, Auwers and Thies, *Ber.*, **53**, 2291 (1920), and numerous patents) or alkaline fusion of *o*-methylmercaptobenzoic acids (Auwers and Thies, *loc. cit.*; Lucius and Bruning, German Patent 204,763), were not investigated for the preparation of I, because of the inaccessibility of the necessary starting materials.

(5a) Craven, *ibid.*, 1605 (1931).

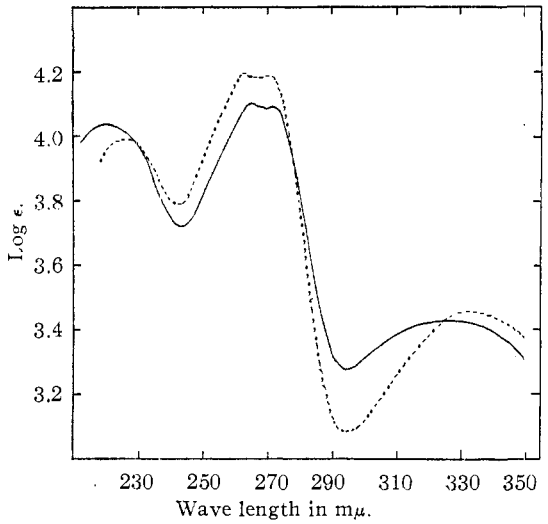
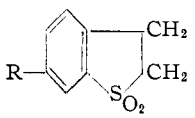



Fig. 1.—Absorption spectra of 6-methyl-4,7-thionaphthenequinone (—) and 5-methyl-4,7-thionaphthenequinone (----).

ture is in best agreement with carbon, hydrogen and sulfur values, and accords with the fact that the compound is neutral and is unaffected by boiling with aqueous alkali. Its occurrence must



VII, R = $CH_3$
VIII, R = H

result from the presence in the thionaphthene II of appreciable amounts of 6-methyl-2,3-dihydrothionaphthene,[6] formed by reduction of the carbonyl group of I to a methylene group during the preparation of II.

In view of the marked difference in the yield of quinone on oxidation of 5-methylthionaphthene[1] compared to the 6-methyl compound, we investigated the oxidation of thionaphthene itself. This yielded with chromic acid only traces of material which gave a Craven's test, but the quinone itself, which is a known compound,[7] could not be isolated. There was formed in about 20% yield in this case also a compound which is presumably the sulfone VIII of dihydrothionaphthene; the two sulfones VII and VIII have very similar absorption curves, as shown in Fig. 2, and hence are not the thionaphthene sulfones, since thionaphthene sulfone is known and is different from VIII.[8] The absorption curve of thionaphthene sulfone, which was taken for comparison, is markedly different from those of the other two compounds as is seen from Fig. 2. The melting point of the product (90.5–91.5°) isolated from the oxidation of thionaphthene is close to that reported for 2,3-di-

(6) Fricke and Spilker, *Ber.*, **58**, 1589 (1925), showed that 2,3-dihydrothionaphthene was one of the products obtained by reduction of thionaphthene with sodium and alcohol.

(7) Fieser and Kennelly, THIS JOURNAL, **57**, 1611 (1935).

(8) Thionaphthene sulfone (Lanfry, *Compt. rend.*, **154**, 519 (1912)) melts at 142-143°.

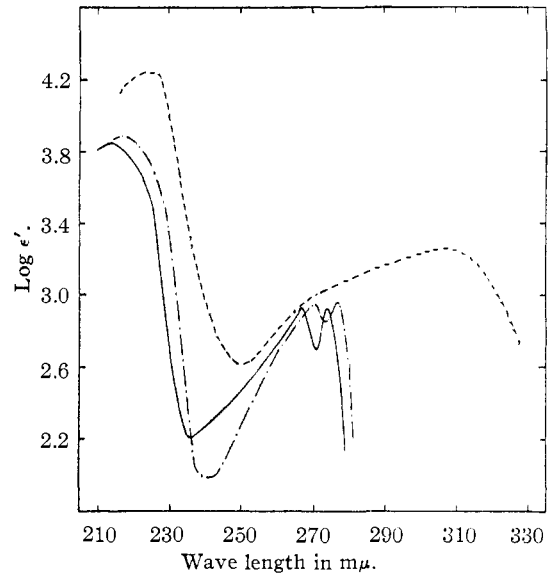
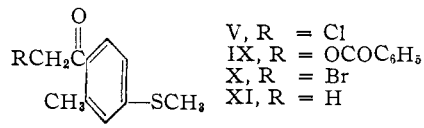


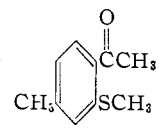

Fig. 2.—Absorption spectra of 2,3-dihydrothionaphthene-sulfone (—), 6-methyl-2,3-dihydrothionaphthene-sulfone (·-·-) and thionaphthene sulfone (---).

hydrothionaphthene sulfone,[9] which supports the structures assigned to these products VII and VIII.

The structure of the by-product V was proved as follows.



V, R = Cl
IX, R = $OCOC_6H_5$
X, R = Br
XI, R = H



XII

Methyl *m*-tolyl sulfide yielded a mixture of the two acetophenones XI and XII, when treated with acetyl chloride and aluminum chloride. The mixture of isomeric ketones was desulfurated with Raney nickel,[10] yielding a mixture of *o*- and *p*-methylacetophenones; the 2,4-dinitrophenylhydrazones of the mixture were prepared and separated, and identified by mixed melting points with authentic samples. The *o*-methylacetophenone 2,4-dinitrophenylhydrazone was five times as abundant as the *p*-isomer in the mixture. This means that more XI than XII was present in the original mixture. Another portion of the mixture of XI and XII was now converted to the semicarbazones, and the latter were separated by fractional crystallization. The more abundant isomer, which must have been the semicarbazone of XI, was hydrolyzed to pure XI. This was brominated, giving X, and when the bromine was replaced by the benzoate group, the compound obtained (IX) was identical with the benzoate formed from the by-product V.

(9) This compound is reported to melt at 91.5-92° (Fricke and Spilker[6]) and at 98° (v. Braun, *Ber.*, **58**, 2165 (1925); Bennett and Hafez, *J. Chem. Soc.*, 287 (1941)).

(10) Mozingo, Wolf, Harris and Folkers, THIS JOURNAL, **65**, 1013 (1943).

## Experimental[11]

*m*-Thiocresol.[12]—*m*-Toluidine (80 g.), dissolved in 150 g. of crushed ice and 150 cc. of concentrated hydrochloric acid, was diazotized at 0–4° with 55 g. of sodium nitrite in 125 cc. of water. The cold diazonium solution was added dropwise to a solution of 140 g. of potassium ethyl xanthate in 180 cc. of water maintained at 40–45°. The diazonium solution, on contact with the xanthate solution, formed a yellow intermediate compound, which rapidly decomposed with evolution of nitrogen, leaving a red oil. After addition of the diazonium solution, the mixture was maintained at 40–45° for one-half hour. The red oil was separated, the aqueous layer extracted with ether, and the combined oil and ether extract washed with 10% sodium hydroxide solution, then with water until the washing was neutral to litmus, dried over anhydrous calcium chloride and the ether evaporated.

The crude *m*-tolyl ethyl xanthate which remained was dissolved in 500 cc. of ethanol, the solution brought to boiling and the source of heat removed. To the refluxing solution was slowly added 175 g. of potassium hydroxide pellets at a rate sufficient to continue the refluxing, and the solution refluxed eight hours longer. About 400 cc. of ethanol was removed by distillation on the steam-bath, and the residue taken up in the minimum amount of water. The aqueous solution was extracted with ether and then strongly acidified to congo red with 6 *N* sulfuric acid. To prevent oxidation to the disulfide, 2 g. of zinc dust was added, and the mixture steam distilled. The distillate was extracted with ether, the ether dried, removed, and from the residue 65–69 g. (70–75%) of colorless *m*-thiocresol obtained, b. p. 103–108° (50 mm.). The reported[12] b. p. is 107.5° (50 mm.).

**Methyl *m*-Tolyl Sulfide (IV).**—To a warm solution of 40 g. of *m*-thiocresol in 125 cc. of 10% sodium hydroxide solution was added dropwise 25 cc. of dimethyl sulfate. After the addition, the reaction mixture was warmed on the steam-bath for one-half hour, made distinctly alkaline and warmed for another half hour. The mixture was cooled, extracted with ether, the ether extract washed with 10% sodium hydroxide solution, dried, evaporated, and the residue distilled, yielding 40.5 g. (90%) of methyl *m*-tolyl sulfide, b. p. 110–112.5° (31 mm.). The analytical sample had b. p. 110° (31 mm.), $n^{24}D$ 1.5736.

*Anal.* Calcd. for $C_8H_{10}S$: C, 69.49; H, 7.30. Found: C, 69.66; H, 7.28.

***m*-Thiocresoxyacetic Acid (VI).**[13]—To a warm solution of 10 g. of *m*-thiocresol in 50 cc. of 33% sodium hydroxide solution was added slowly 15 g. of chloroacetic acid. The mixture was warmed on the steam-bath for one hour, strongly acidified with dilute sulfuric acid and extracted with ether. The ether solution was extracted with 5% sodium carbonate solution, and the alkaline layer strongly acidified with sulfuric acid; the precipitated *m*-thiocresoxyacetic acid (13.3 g., 90%) was collected by filtration, and after several recrystallizations from benzene-petroleum ether (b. p. 40–60°) or from water, was obtained as white needles, m. p. 67.5–68°. This melting point differs from that (103–104°) reported by Behaghel[14]; he recrystallized the product from water.

*Anal.* Calcd. for $C_8H_8O_2S$: C, 59.29; H, 5.53; neut. equiv., 182.1. Found: C, 59.15; H, 5.69; neut. equiv., 182.

**A. 6-Methyl-3-keto-2,3-dihydrothionaphthene (I).**[15] **From Methyl *m*-Tolyl Sulfide.**—To a cold solution of 10 g. of methyl *m*-tolyl sulfide and 8.5 g. of chloroacetyl chloride in 100 g. of dry carbon disulfide was added 12 g. of anhydrous aluminum chloride in small portions. The mixture was slowly brought to room temperature and then refluxed for five hours on the steam-bath, which caused darkening. The solvent was evaporated, the aluminum chloride complex decomposed with ice and concentrated hydrochloric acid, and steam distilled. The distillate contained 2 g. (17%) of crude I, m. p. 70–85°. Several recrystallizations from methanol in a carbon dioxide atmosphere using charcoal, yielded soft, slightly pink needles, m. p. 87.5–88.5°.[16] The product is sensitive to oxygen and rapidly turns red on exposure to air; it is soluble in 10% sodium hydroxide, forming a yellow solution which turns red to blue on heating.

The yield reported in this run was the best obtained; in some other runs almost none of the desired product was obtained, and this was also the case when stannic chloride was used instead of aluminum chloride.

From the residue of the steam distillation was obtained 2.5 g. of product, m. p. 100–104°, which, after recrystallization from acetone and from methanol with charcoal, yielded light yellow prisms, m. p. 103.5–105°, raised to 104.5–105.5° by vacuum sublimation. The compound was proved to be **2-methyl-4-methylmercapto-ω-chloroacetophenone V,** as described below.

*Anal.* Calcd. for $C_{10}H_{11}ClOS$: C, 55.91; H, 5.16. Found: C, 55.96; H, 5.22.

The **2,4-dinitrophenylhydrazone** was obtained in the usual manner and, after recrystallization from glacial acetic acid, melted at 183.5–184.5°.

*Anal.* Calcd. for $C_{16}H_{15}ClN_4O_4S$: C, 48.65; H, 3.83. Found: C, 48.73; H, 3.97.

The **benzoate (IX)** was prepared by heating with sodium benzoate in aqueous alcohol, and was obtained as white crystals from ethanol, m. p. 109.5–110°.

*Anal.* Calcd. for $C_{17}H_{16}O_3S$: C, 67.95; H, 5.37. Found: C, 68.01; H, 5.25.

**B. Cyclization of *m*-Thiocresoxyacetyl Chloride with Aluminum Chloride.**—A solution of 9 g. of *m*-thiocresoxyacetic acid and 7 cc. of purified thionyl chloride was warmed on the steam-bath until the reaction ceased, excess thionyl chloride was removed under diminished pressure, and anhydrous benzene was added and removed in the same way. To the acid chloride, 100 cc. of dry carbon disulfide was added, the solution cooled to 10° and 8 g. of anhydrous aluminum chloride added in small portions. The reaction mixture was slowly brought to room temperature, and then refluxed for ten minutes. Longer periods of refluxing resulted in much smaller yields. The solvent was removed under reduced pressure, the complex decomposed with ice and concentrated hydrochloric acid and steam distilled. From the distillate was obtained 1.2 g. (14%) of crude I; ether extraction of the filtrate yielded 0.6 g. additional product, which was reduced directly to 6-methylthionaphthene, since much was lost on attempted purification. This is the best yield obtained in several runs.

**C. Cyclization of *m*-Thiocresoxyacetic Acid with Anhydrous Hydrogen Fluoride.**—In a copper flask to which was attached a reflux condenser with a copper inner tube, was placed 20 g. of *m*-thiocresoxyacetic acid and 400–450 g. of anhydrous hydrogen fluoride. The solution was allowed to stand at room temperature for seven hours, was poured into a copper beaker and the hydrogen fluoride allowed to evaporate (about two hours) at room temperature. A shorter time of standing lowers the yield. Water was added to the residue and the mixture steam distilled, while a nitrogen stream was passed through the apparatus to prevent air oxidation of the product. The distillate, after standing in the refrigerator overnight, yielded 10–20 g. of crude I, contaminated with insoluble silicates. It was used in the next step without further purification. This method was much more reliable than the others.

**6-Methylthionaphthene (II).**[1]—To a refluxing solution of 1.8 g. of crude I (from methods A or B) in 30 cc. of glacial

(11) All melting points corrected; analyses by Dr. Carl Tiedcke, Mr. Carl Claus and the Micro-Tech Laboratories. Absorption curves were taken with a Beckmann spectrophotometer, using alcohol as solvent.

(12) Bourgeois, *Rec. trav. chim.*, **18**, 447 (1899).

(13) *Cf.* Koelsch, THIS JOURNAL, **53**, 304 (1937).

(14) Behaghel, *J. prakt. Chem.*, (2) **114**, 287 (1926).

(15) *Cf.* Auwers and Arndt, ref. 2.

(16) Auwers and Thies, *Ber.*, **53**, 2285 (1920), report the m. p. as 86–87°.

acetic acid was added in small portions 2 g. of zinc dust and 1 cc. of concentrated hydrochloric acid. The mixture was refluxed for six hours, cooled, saturated with solid sodium hydroxide and steam distilled. The distillate was extracted with ether, the solution dried, the solvent evaporated and the residue distilled, yielding 0.45 g. (27%) of crude 6-methylthionaphthene, b. p. 110–115° (13 mm.), which solidified in an ice-bath. The **picrate** melted at 114.5–115.5°. On decomposition of the picrate and sublimation of the product, pure 6-methylthionaphthene, m. p. 42–42.5°, was obtained.

*Anal.* Calcd. for $C_9H_8S$: C, 72.91; H, 5.44. Found: C, 72.79; H, 5.40.

**Picrate.** *Anal.* Calcd. for $C_{15}H_{11}N_3O_7S$: C, 47.73; H, 2.94; neut. equiv., 377.2. Found: C, 47.24; H, 2.91; neut. equiv., 357.5.

By reduction of the crude 6-methyl-3-keto-2,3-dihydrothionaphthene from the anhydrous hydrogen fluoride method, 3.5 g. (11%, based on 40 g. of *m*-thiocresoxyacetic acid) of crude 6-methylthionaphthene, b. p. 110–115° (12 mm.), was obtained.

**Oxidation of 6-Methylthionaphthene.**—To an ice-cold solution of 14 g. of chromic oxide in 35 cc. of 80% acetic acid was added dropwise a solution of 3.1 g. of crude 6-methylthionaphthene in 40 cc. of glacial acetic acid. The reaction mixture was kept below 10° during the addition, then slowly brought to room temperature and allowed to stand for ten hours. The mixture was diluted with water and extracted with ether; the extract was washed with water, with sodium bicarbonate solution and with water, then dried. Concentration of the ether solution by evaporation of solvent yielded 0.5 g. of white crystals, m. p. 150–155°, which was raised to 160.5–161.5° after several crystallizations from methanol and ethanol.

The carbon–hydrogen analysis of this product is fairly close to that of a hydrated acetoxyl derivative of 6-methylthionaphthene, as well as of 6-methyl-2,3-dihydrothionaphthene sulfone. The former structure was ruled out by the sulfur analysis of the compound, and by the fact that it was recovered unchanged after refluxing for four hours with 10% sodium hydroxide solution.

*Anal.* Calcd. for $C_9H_{10}O_2S$ (6-methyl-2,3-dihydrothionaphthene sulfone): C, 59.32; H, 5.53; S, 17.6. Found: C, 59.18, 59.07: H, 5.29, 5.42: S, 18.4, 18.6.

**6-Methyl-4,7-thionaphthenequinone (III).**—The yellow ethereal filtrate from the sulfone described above was concentrated further and more of the white crystals were separated. The ether solution was then evaporated to dryness and the residue vacuum sublimed. The sublimate, after recrystallization from methanol and resublimation, yielded yellow crystals of the quinone, m. p. 130.5–131.5°, which gave a positive Craven test.

*Anal.* Calcd. for $C_9H_6O_2S$: C, 60.64; H, 3.4. Found: C, 60.55; H, 3.58.

An unsuccessful attempt was made to oxidize 6-methylthionaphthene to an acetoxyl derivative with red lead and glacial acetic acid. The starting material was recovered in 60% yield.

**2-Methyl-4-methylmercaptoacetophenone (XI).**—To a solution of 5 g. of methyl *m*-tolyl sulfide and 4.5 g. of acetyl chloride in 50 g. of carbon disulfide was added slowly 7 g. of anhydrous aluminum chloride. The mixture was gradually brought to refluxing, which was continued for four hours. The carbon disulfide was evaporated, the residue decomposed with ice and concentrated hydrochloric acid and steam distilled. The distillate was extracted with ether, and distillation of the product remaining after evaporation of the ether gave 4.82 g. (74%) of the mixture of 2-methyl-4-methylmercapto- and 4-methyl-2-methylmercaptoacetophenone (XI and XII), b. p. 118–124° (1–1.5 mm.). The isomeric ketones were separated by formation of the semicarbazones and fractional crystallization of the derivatives from ethanol and benzene. The ketones could be regenerated by hydrolysis of the purified semicarbazone with dilute sulfuric acid. From 4.5 g. of the mixture, 3 g. of pure 2-methyl-4-methylmercaptoacetophenone (XI) was obtained; the analytical sample had b. p. 125–126° (2 mm.), $n^{25}D$ 1.6090.

*Anal.* Calcd. for $C_{10}H_{12}OS$: C, 66.61; H, 6.71. Found: C, 66.50; H, 6.72.

The **semicarbazone,** recrystallized three times from ethanol and twice from benzene, was obtained as white crystals, m. p. 177–178°.

*Anal.* Calcd. for $C_{11}H_{15}N_3OS$: C, 55.66; H, 6.37. Found: C, 55.76; H, 6.58.

**2-Methyl-4-methylmercapto-ω-bromoacetophenone (X).**[17]—To a solution of 2 g. of pure 2-methyl-4-methylmercaptoacetophenone (XI) in 15 cc. of carbon disulfide was added a solution of 1.8 g. of bromine in 8 cc. of carbon disulfide. The solution was refluxed on the steam-bath until the evolution of hydrogen bromide had ceased, the solvent was evaporated and the residue steam distilled. Filtration of the distillate yielded 0.5 g. of solid, m. p. 70–80°, which, after recrystallization from methanol, melted at 82–85°; the mixture of this substance with 6-methyl-3-keto-2,3-dihydrothionaphthene (I, m. p. 87–88.5°) melted at 65–80°. Further recrystallization of X raised the m. p. to 84–86°. The **benzoate IX** prepared from this compound was identical with that from compound V, as shown by mixed m. p.

**Desulfuration of XI and XII.**—A suspension of 2 g. of the mixture of the methylmethylmercaptoacetophenones XI and XII, 25 g. of Raney nickel and 135 cc. of 70% ethanol was refluxed for five hours.[10] The mixture was diluted with 400 cc. of water and distilled until the temperature rose to 100°. The distillate was refractionated until the temperature rose to 85°, water was added to the distillate and the solution extracted with ether. The distilland of the refractionation was extracted with ether and the two ether solutions combined, dried and the solvent evaporated. The residual oil gave 1.15 g. (77%) of a mixture of *o*- and *p*-methylacetophenone, b. p. 103–108° (30 mm.).

The mixed **2,4-dinitrophenylhydrazones** of the ketonic mixture were prepared and fractionally crystallized from ethanol, giving 0.44 g. of crude *o*-methylacetophenone 2,4-dinitrophenylhydrazone and 0.08 g. of the *p*-derivative; the latter is not very soluble in hot ethanol. The pure *o*- and *p*-methylacetophenone 2,4-dinitrophenylhydrazones thus obtained melted at 162–163° and 259–261°, respectively, and neither showed a depression on mixed m. p. with an authentic sample.[18]

**Experiments on Thionaphthene.**—Following the procedure employed in the preparation of 5-methylthionaphthene,[1,19] 5 g. of 3-keto-2,3-dihydrothionaphthene yielded 2 g. of crude thionaphthene, b. p. 102–107° (15 mm.). Purification through the picrate, m. p. 149–149.5° (reported value 149°)[20] yielded crystalline thionaphthene, m. p. 26–28° (reported value 30–31°[20]).

Thionaphthene (1 g., crude) was oxidized with chromic acid and glacial acetic acid by the method described for the 6-methyl compound. The oxidation mixture was extracted with ether instead of being steam distilled. Although the ether extract gave a positive Craven test, no crystalline 4,7-thionaphthenequinone could be obtained from it. The ether solution did yield, however, white crystals (0.2 g.) which were insoluble in base and gave a negative Craven test. After several recrystallizations from ethanol and ether, the product melted at 90.5–91.5°.

*Anal.* Calcd. for $C_8H_8SO_2$: C, 57.12; H, 4.79. Found: C, 57.41; H, 4.78.

(17) *Cf.* Krollpfeiffer and Schneider, *Ber.*, **61**, 1284 (1928).

(18) *o*-Methylacetophenone for comparison was prepared by the action of di-*o*-tolylcadmium on acetyl chloride (*cf.* Gilman and Nelson, *Rec. trav. chim.*, **55**, 518 (1936)). The dinitrophenylhydrazone melted at 164–165°; the reported value is 161° (Borsche and Wagner-Roemmich, *Ann.*, **546**, 273 (1941). *p*-Methylacetophenone dinitrophenylhydrazone is reported to melt at 260° (Ferrante and Bloom, *Am. J. Pharm.*, **105**, 383 (1933)).

(19) *Cf.* a recent paper by Hansch and Lindwall, *J. Org. Chem.*, **10**, 381 (1945).

(20) Gattermann and Lockhart, *Ber.*, **26**, 2808 (1893).

## Summary

6-Methyl-3-keto-2,3-dihydrothionaphthene has been prepared by several methods, and has been reduced to 6-methylthionaphthene, which has been oxidized in very low yield to 6-methyl-4,7-thionaphthenequinone. A by-product in the oxidation has been shown to be probably the 2,3-dihydro-6-methylthionaphthene sulfone. The action of chloroacetyl chloride and aluminum chloride on methyl *m*-tolyl sulfide leads to 3-keto-2,3-dihydro-6-methylthionaphthene and to a chloroacetyl compound, which has been proved to be 2-methyl-4-methylmercapto-ω-chloroacetophenone.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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

# Streptomyces Antibiotics. VIII. Isolation of Streptomycin

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Some chemical and biological properties of streptomycin helianthate, hydrochloride, sulfate and *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonate have been described.[1] The preliminary steps in the purification of the streptomycin concentrates and the preparation of these salts of streptomycin are described herein.

The first concentrates of streptomycin,[2] which were prepared for use in microbiological experiments, were obtained from the culture broths of *Streptomyces griseus* by the following steps: adsorption on Norite-A, elution with dilute acid, neutralization and concentration *in vacuo* to a residue.

The crystalline reineckate of streptomycin, which has been described,[3] was obtained by a sequence of steps similar to that used for streptothricin reineckate.[3] These steps were: charcoal adsorption, elution with mineral acid, precipitation with phosphotungstic acid, conversion of regenerated bases to crude picrate, chromatography of picrate and finally preparation of the reineckate.

Another method for the purification and isolation of streptomycin has been described.[4] The steps were as follows: clarification of broth with charcoal, adsorption on charcoal, elution with methanolic hydrogen chloride, and chromatography with aluminum oxide. A three-step charcoal adsorption process for the purification of streptomycin also has been used.[5]

Our sequence of steps for the isolation of streptomycin, which differs in several respects from those described above, is as follows: charcoal adsorption, elution with methanolic formic acid, precipitation with picric acid, conversion to the hydrochloride, chromatography, and finally conversion to the helianthate. The steps consisting of charcoal adsorption, elution with methanolic formic acid, precipitation with picric acid, and direct conversion to the hydrochloride were carried out essentially as described for streptothricin.[6] The concentrates of streptomycin hydrochloride showed an average activity of about 100–200 units/mg. The chromatographic step was carried out either with columns of aluminum oxide or Darco G-60. By these chromatographic procedures, fractions showing activities up to about 750 units/mg. were obtained.

Treatment of samples of streptomycin hydrochloride, which showed an activity of about 400 units/mg., or higher, with the sodium salt of helianthine (methyl orange) or of *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonic acid (orange II), yielded the corresponding crystalline salts of streptomycin. The helianthate was utilized for preparative purposes After recrystallization the helianthate was converted into the hydrochloride or other salts such as the hydrobromide or sulfate. The sulfate was obtained crystalline, but it was difficult to reproduce and it was frequently contaminated with streptidine sulfate.[7] The helianthate also was converted directly into the crystalline streptomycin trihydrochloride–calcium chloride double salt.[8] The desirable precautions in the conversion of the helianthate into other salts have already been noted.[7]

Potentiometric titrations were carried out on three salts of streptomycin and the data obtained were as follows: helianthate, eq. wt. 1600, $pK'a$ 7.50; calcd. for $C_{21}H_{37}N_7O_{12}\cdot 3C_{14}H_{15}N_3O_3S$, 1495.6; hydrochloride, eq. wt. 740, $pK'a''$ 7.66; calcd. for $C_{21}H_{37}N_7O_{12}\cdot 3$ HCl, 689; hydrobromide, eq. wt. 869; calcd. for $C_{21}H_{37}N_7O_{12}\cdot 3$ HBr, 823. As already stated,[8] a cryoscopic molecular weight determination on streptomycin hydrochloride in water gave a value of about 800 for the free base (calcd., 580). A colorimetric determination of the helianthine in streptomycin helianthate gave a value of 205 for the combining weight (calcd. combining weight, 193). The results of these determinations and the microanalytical

(1) Kuehl, Peck, Walti and Folkers, *Science*, **102**, 34 (1945).

(2) Schatz, Bugie and Waksman, *Proc. Soc. Exp. Biol. Med.*, **55**, 66 (1944).

(3) Fried and Wintersteiner, *Science*, **101**, 613 (1945).

(4) Carter, Clark, Dickman, Loo, Skell and Strong, *J. Biol. Chem.*, **160**, 337 (1945).

(5) Le Page and Campbell, *ibid.*, **162**, 163 (1946).

(6) Peck, Walti, Graber, Flynn, Hoffhine, Allfrey and Folkers, THIS JOURNAL, **68**, 772 (1946).

(7) Peck, Graber, Walti, Peel, Hoffhine and Folkers, *ibid.*, **68**, 29 (1946).

(8) Peck, Brink, Kuehl, Flynn, Walti and Folkers, *ibid.*, **67**, 1866 (1945).