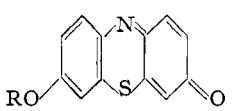
[CONTRIBUTION FROM WESTERN REGIONAL RESEARCH LABORATORY[1]]

Phenothiazine Derivatives: Di-oxygenated Compounds

BY DAVID F. HOUSTON, E. B. KESTER AND FLOYD DEEDS

The synthesis of phenothiazine derivatives with possible tuberculostatic action[2] has been extended to the preparation of a series of ethers of 7-hydroxyphenothiazone-3[3] or "thionol" (I, R = H), which—from structural considerations—might have improved lipid solubility over the parent compound, and yet retain its oxidation-reduction characteristics. The investigation has been closed with the achievement of a minimum objective and the results are presented in this



I

report, although some experimental procedures are incompletely developed.

Bernthsen[4] prepared 7-hydroxyphenothiazone-3 as early as 1885 by digestion of phenothiazine with sulfuric acid. Oxidation of phenothiazine with hydrogen peroxide and hydrochloric acid[5] gave 7-hydroxyphenothiazone-3 which was found to contain difficullty removable chlorine compounds. In 1940, Granick, Michaelis and Schubert[6] called attention to the difficulty of consistently obtaining this product in suitable yields with satisfactory purity. The original Bernthsen method is exceedingly tedious, and the yields are low and variable. A recent modification[7] in which the desired product is isolated as the sparingly soluble lithium salt (I, R = Li) makes the process much more convenient.

The synthesis of 7-hydroxyphenothiazone-3 by fusion of hydroquinone, *p*-aminophenol and sulfur was patented by Vidal[8] as a dye intermediate, but practically no data on properties were recorded. A product obtained[9] by fusing sulfur with *p,p'*-dihydroxydiphenylamine at 180° did not have the properties of 7-hydroxyphenothiazone-3. The recovery of thionol from the urine of animals dosed with phenothiazine has been summarized by Collier[10] and co-workers.

In general, the high melting point (>360°) and the low solubility of thionol have hindered its purification and characterization.

Published information on ethers of 7-hydroxyphenothiazone-3 appears to be limited to the reported formation[11] of the methyl and ethyl leuco compounds by the fusion of 1:1 molar mixtures of hydroquinone and the appropriate *p*-alkoxyaniline with an excess of sulfur. The compounds were converted directly to sulfur dyestuffs.

In the present investigation, 7-hydroxyphenothiazone-3 was prepared by two methods. The first was a modification of Schneider's[9] process, in which iodine was used as a catalyst. The *p,p'*-dihydroxydiphenylamine was prepared according to Knoevenagel and Berlin[12] by condensing two molecules of *p*-aminophenol in the presence of iodine. The purified amine was then fused with sulfur, using an iodine catalyst, to form 3,7-dihydroxyphenothiazine (leucothionol). Solution of the reaction product in dilute alkali afforded the oxidized form, which was precipitated as the lithium salt and subjected to recrystallization. This material represented a 36% yield, and gave chloroform extracts having the visible absorption spectrum characteristic of 7-hydroxyphenothiazone-3. There was still present, however, a congener that was obstinately retained with the desired product. Further investigation may provide a way of capitalizing on this apparently good yield.

The second method of preparation was the lithium salt procedure of Granick and Michaelis.[7] Application of the process in this Laboratory gave somewhat lower yields of the lithium salt than the reported 8%, likely because of differences in experimental details. Further investigation showed that the yield increased as the reaction time at 160–165° was shortened, until a six-hour sulfuric acid digestion of phenothiazine gave 15% of the lithium salt. Shorter digestion periods gave very low yields. Sulfuric acid digestions at 155° with acid of various concentrations gave low yields and poor separations. At temperatures of 172–175° yields were again lower than at 160–165°, and decreased as acid concentration increased. Acid concentrations of 75 to 90% were most favorable at 160–165°, and 80% acid was generally used. The use of commercial phenothiazine melting at 174–178° gave apparently as good yields as did purified phenothiazine melting at 182.5–183.5°. However, lower yields of purified alkyl ethers resulted from the lower-melting phenothiazine. Recrystallizations of the

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted. Presented at the A. C. S. Meeting, San Francisco, Calif., March 27–April 1, 1949.

(2) D. F. Houston, E. B. Kester and Floyd DeEds, THIS JOURNAL, **71**, 3816 (1949).

(3) A discussion of nomenclature occurs in ref. 2.

(4) A. Bernthsen, *Ann.*, **230**, 187 (1885).

(5) F. DeEds and C. W. Eddy, THIS JOURNAL, **60**, 1446 (1938).

(6) S. Granick, L. Michaelis and M. Schubert, *ibid.*, **62**, 1802 (1940).

(7) S. Granick and L. Michaelis, *ibid.*, **69**, 2982 (1947), and personal communications.

(8) H. R. Vidal, German Patent 103,301; March, 1897.

(9) F. Schneider, *Ber.*, **32**, 689 (1899).

(10) H. B. Collier, D. E. Allen and W. E. Swales, *Can. J. Research*, Sect. D., **21**, 151 (1943).

(11) Gesellschaft für Chemische Industrie in Basel, Swiss Patents 209,501 (methyl) and 209,502 (ethyl); July, 1940.

(12) E. Knoevenagel and H. J. Berlin, *J. prakt. Chem.*, **197**, 24 (1914).

product from boiling glacial acetic acid yielded crystalline 7-hydroxyphenothiazone-3 which had optical and crystallographic properties in agreement with those of Granick and Michaelis' product.[13] This method was used to provide the needed amounts of 7-hydroxyphenothiazone-3 and its lithium salt.

The ethyl, amyl, octyl, dodecyl and hexadecyl ethers have been prepared by the reaction between the silver salt of thionol and the proper *n*-alkyl iodides in refluxing benzene. Bromides proved unsatisfactory. In most cases, the silver salt was made directly from the precipitated lithium salt by treatment with silver nitrate solution. Purification was achieved by chromatographing the benzene solution of the ether on alumina, eluting with benzene–chloroform mixtures, removing all volatile material (finally in vacuum), and crystallizing from hexane–benzene or acetone.

Absorption spectra of solutions of the ethers were nearly identical, though the orange-to-rust-red colors of the crystals changed appreciably with size and shape. Some evidence points toward polymorphism, although this may be induced by residual impurities. For example, crystallization of the octyl ether in one case yielded both chunky garnet-red crystals and spherulitic clusters of orange needles. Microscopical examination showed the two forms were very similar optically and crystallized from melts in apparently identical forms. The orange needles seemed to change to the darker form below the melting point.

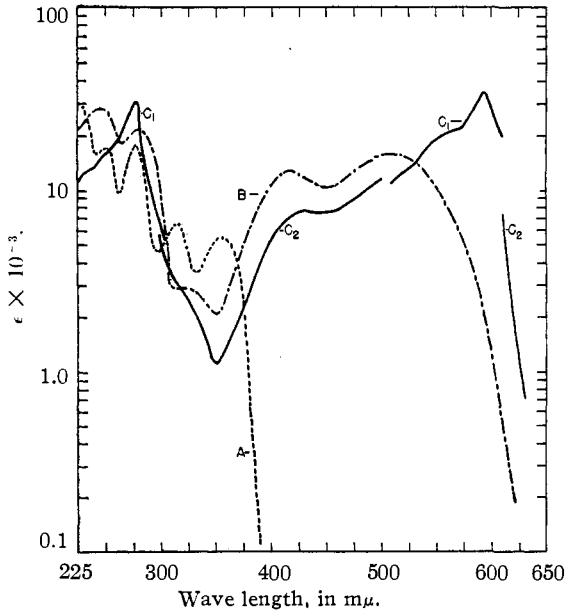


Fig. 1.—Molar extinction curves in absolute methanol: (A) 3-*n*-octoxyphenothiazine-5-oxide (2.33γ/ml.); (B) 7-*n*-octoxyphenothiazone-3 (4.33γ/ml.); (C_1 – C_2) 7-hydroxyphenothiazone-3 (C_1 = 0.588γ/ml.; C_2 = 2.94γ/ml.).

(13) A sample for comparison was kindly supplied by Dr. Granick.

An attempted formation of 7-*n*-octoxyphenothiazone-3 by ferric chloride oxidation of 3-*n*-octoxyphenothiazine[2] according to the method of preparing phenothiazone-3 from phenothiazine[14] caused hydrolysis of the ether linkage and yielded chiefly phenothiazone-3. Traces of 7-hydroxyphenothiazone-3 were noted, but no octoxy compound. Reaction of 3-octoxyphenothiazine with alkaline solutions of hydrogen peroxide effectively oxidized the sulfur atom, yielding 3-octoxyphenothiazine-5-oxide. This type of di-oxygenated derivative shows sensitivity to light and air similar to that of 3-alkoxyphenothiazines.

Spectral Absorptions.[15]—Granick and Michaelis[7] have recently published data on the spectral absorption of 7-hydroxyphenothiazone-3 and its semiquinone form. Lipson[16] has recorded absorption peaks at 552 and 500–510 mμ for an ethanol solution of 7-hydroxyphenothiazone-3, recovered from the urine of sheep treated with phenothiazine. Collier and co-workers,[10] discussing the spectrometry of phenothiazine derivatives in animal experimentation, pointed out that the peak at 552 mμ was due to the semiquinone form of phenothiazone-3.

The spectral absorption of thionol prepared in this work is in agreement with the results of Granick and Michaelis. This was conclusively shown by the identity of absorption curves obtained on methanol solutions of the two preparations. It is of further interest to note that the sharp peak at 590 mμ for these preparations in 0.01 *N* alkali is concordant with the predicted peak at 587 ± 3 mμ calculated by the rules of Lewis.[17] The visible absorption maxima of 7-hydroxyphenothiazone-3 shift with solvent (411, 502 mμ in chloroform and 424, 518 in methanol), and the absorption shows some deviation from Beer's law. This deviation is consistent with the similar characteristics of methylene blue and thionine,[18,19] amino analogs of thionol. Absorption curves for representative di-oxygenated compounds are shown in Fig. 1.

Tuberculostatic properties are being investigated by other workers and will be reported in a separate publication.

Experimental[20]

Materials.—Phenothiazine was purified by crystallization from toluene, followed by washing with hexane.

(14) R. Pummerer and S. Gassner, *Ber.*, **46**, 2324 (1913).

(15) Visible absorption was usually measured on a Hardy-General Electric Recording Spectrophotometer; ultraviolet (and some visible) absorption was determined with a Beckman Model DU quartz spectrophotometer.

(16) M. Lipson, *Australian J. Exptl. Biol. Med. Sci.*, **18**, 269 (1940).

(17) G. N. Lewis, THIS JOURNAL, **67**, 770 (1945).

(18) L. Michaelis, M. P. Schubert and S. Granick, *ibid.*, **62**, footnote p. 210 (1940).

(19) L. F. Epstein, F. Karush and E. Rabinowitch, *J. Opt. Soc. Am.*, **31**, 80 (1941).

(20) All melting points are corrected unless otherwise indicated. Nitrogen analyses were made by the Kjeldahl procedure of White and Secor, *Ind. Eng. Chem., Anal. Ed.*, **18**, 457 (1946).

TABLE I

PROPERTIES OF ETHERS OF 7-HYDROXYPHENOTHIAZONE-3

Alkyl group of ether	Color	Yield (crude), %	Empirical formula	Melting point, °C.	Analytical data, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
Ethyl	Orange	58, 71	$C_{14}H_{11}NO_2S$	208–209	65.35	65.4	4.31	4.27	5.44	5.45
n-Amyl (1)	Deep rust	38, 41	$C_{17}H_{17}NO_2S$	124.5–126	68.20	68.2	5.73	5.63	4.68	4.66
n-Amyl (2)	Deep rust		$C_{17}H_{17}NO_2S$	131–132	68.20	68.3	5.73	5.69	4.68	4.68
n-Octyl	Garnet-red	53	$C_{20}H_{23}NO_2S$	115.5–116	70.35	70.8	6.79	6.88	4.10	4.02
n-Dodecyl	Garnet-red	52	$C_{24}H_{31}NO_2S$	121–122.5	72.50	72.9	7.86	7.88	3.52	3.47
n-Hexadecyl	Orange-red	44	$C_{28}H_{39}NO_2S$	120.5–121.5	74.13	74.0	8.66	8.65	3.09	3.04

Sublimed sulfur (U. S. P.) was used in fusions. *p,p'*-Dihydroxydiphenylamine prepared according to Knoevenagel[12] melted at 167.5–168.5° in agreement with 169° reported by him. Ethyl and amyl iodides, amyl, octyl, dodecyl and hexadecyl bromides were reagent grade chemicals boiling within two-degree ranges, and were used as received. Octyl, dodecyl and hexadecyl iodides were prepared by treating the corresponding bromides with sodium iodide in refluxing acetone. The octyl and dodecyl iodides were distilled at reduced pressures, and hexadecyl iodide was recrystallized at 0° from alcohol–benzene to a melting point of 22°.

Oxidation of 3-Alkoxyphenothiazines.—(a) A 0.5-g. portion of 3-isopropoxyphenothiazine[2] was suspended in 100 cc. of 0.5% potassium hydroxide and aerated for thirty minutes. Filtration yielded rust-brown solids. A chloroform extract showed the absorption spectrum of phenothiazone-3, and recrystallization gave brick-red crystals, m. p. 158–161° (uncor.). Phenothiazone-3 melts at 161–162°. The original purple alkali solution suggested 7-hydroxyphenothiazone-3, but none was found.

(b) Oxidation of 0.5 g. of 3-*n*-octoxyphenothiazine[2] by the ferric chloride–hydrogen peroxide procedure of Pummerer and Gassner[14] resulted in a marked odor of *n*-octanol and yielded 0.34 g. of crude phenothiazone-3. Extraction with hexane gave 0.24 g. (75% on original ether) melting at 157.5–160.5° (uncor.). Chromatographing a benzene solution of this material on Alorco F-20 alumina and eluting with benzene–chloroform provided 0.2 g. of phenothiazone-3, m. p. 160–161°. Indications were obtained of a trace of 7-hydroxyphenothiazone-3.

(c) A solution of 0.5 g. of 3-octoxyphenothiazine in 20 cc. of 1% alcoholic potassium hydroxide was treated on the steam-bath with 0.5 cc. of 30% hydrogen peroxide, and with a further 0.5 cc. after one-half hour. During the two-hour heating, the original brown color changed gradually to a pale yellow with a green fluorescence. Crystallization at 0° from the reaction solution yielded 0.42 g. of light yellow solid, m. p. 166° (uncor.). Recrystallization from acetone gave 0.28 g. (53.5%) of platelets, m. p. 168.2–168.7° (dec.). This type of oxidation is common in converting sulfides to sulfoxides; the analysis corresponds to 3-*n*-octoxyphenothiazine-5-oxide.

Anal. Calcd. for $C_{20}H_{25}NO_2S$: C, 69.93; H, 7.34; N, 4.08. Found: C, 69.6; H, 7.25; N, 4.06.

7-Hydroxyphenothiazone-3. (a) Bernthsen Synthesis.[4]—The digestion of 50.0 g. of phenothiazine according to Bernthsen's original procedure provided 1.15 g. of crude 7-hydroxyphenothiazone-3. The leuco compound was extracted with ether in a carbon dioxide atmosphere, reoxidized by air in ammonia solution, and crystallized from acetic acid. The product required intensive drying, because of strong retention of moisture, before analyses approximating the theoretical were obtained.

(b) **Lithium Salt Process.**—Application of the method of Granick and Michaelis[7] to 22.0 g. of phenothiazine gave 0.98 g. of lithium thionol (3.8%) instead of the 2.01 g. reported. Liquid temperatures of 160–165° were maintained for twenty-six hours without stirring in a one-liter flask heated with a glass-cloth electric heater. These conditions may have varied from those used by Granick and Michaelis. Their recommended rapid filtration of the sodium carbonate extracts of the product was not achieved.

The lithium salt was extracted with two 100-cc. portions of boiling glacial acetic acid, which on cooling and filtration gave 0.43 g. of crystalline 7-hydroxyphenothiazone-3. Recrystallization provided rosettes of fine brownish-red needles that did not melt at 360°. Drying *in vacuo* over Anhydrone at 78° for ninety hours gave an analytical sample.

Anal. Calcd. for $C_{12}H_7NO_2S$: C, 62.87; H, 3.08; N, 6.11. Found: C, 62.7; H, 2.99; N, 6.07.

In view of the differences in yield, further exploration of reaction conditions was made. The optimum reaction conditions of digestion for about six hours at 160–165° with 75–90% acid gave 15–16% of lithium salt. Separation of hot sodium carbonate extracts with a basket centrifuge was much more convenient than filtration, though the yields were reduced slightly. Mechanical stirring did not increase the yield. The effect of changing the 4% concentration of phenothiazine in the reaction mixture was not investigated.

(c) **Sulfur Fusion Process.**—Equivalent amounts of sulfur (1.28 g., 0.04 atom) and *p,p'*-dihydroxydiphenylamine (4.06 g., 0.02 mole) ground together were placed in a long-necked flask with 0.1 g. of iodine. The flask was immersed in a metal-bath preheated to 190° and held at 195–200° until evolution of hydrogen sulfide was practically complete. This required forty-five to sixty minutes, during which time the dark reaction mixture solidified. Some ammonia and phenol were generated during the reaction. The coke-like residue, readily broken out of the flask, was digested with successive one-liter portions of 0.7 and 0.6% boiling sodium carbonate solution and filtered. The combined heated filtrates were treated with 20 g. of lithium chloride and chilled to 0°. Filtration left 0.65 g. of dark solids. Treatment of the reheated filtrate with a further 25 g. of lithium chloride gave 1.70 g. (36.3%) of lithium salt of 7-hydroxyphenothiazone-3. Recrystallizations from glacial acetic acid eventually yielded the crystalline product, though removal of persistent congeners was difficult and gave low final yields.

Anal. Calcd. for $C_{12}H_7NO_2S$: C, 62.87; H, 3.08; N, 6.11. Found: C, 62.2; H, 3.01; N, 6.18.

Ethers of 7-Hydroxyphenothiazone-3.—Preparations of the individual ethers were closely similar, and are illustrated by the details of synthesis for the amyl ether. Data on the products are collected in Table I. Excesses of alkyl iodide varied from 2.5 to 5 equivalents, but smaller amounts would likely be suitable. The vacuum treatment can be omitted in larger-scale preparations.

7-*n*-Amyloxyphenothiazone-3.—The silver salt was prepared from 2.35 g. (0.01 mole) of crude lithium salt by solution in one liter of water on the steam-bath, treatment with 2.5 g. (0.015 mole) of silver nitrate in 100 cc. of water, digestion for one hour, filtration, exhaustive washing, and drying at 105°.

The silver salt was dispersed in 500 cc. of refluxing benzene and treated with 9.9 g. of *n*-amyl iodide (5 equivalents). After seven hours reflux the liquid was cooled and filtered. The solids were washed with 200 cc. of hot benzene. Duplicate experiments showed 38 and 41% of

crude product at this point. The combined solutions were chromatographed on Alorco F-20 alumina (3.3 cm. diam., 15 cm. long), then developed and eluted with 250 cc. of benzene, 1000 cc. of 9:1 and 2500 cc. of 4:1 benzene–chloroform. The ether traveled through the column as a deep-red band, and was saved in a separate eluate. Unetherified 7-hydroxyphenothiazone-3 and some dark impurities remained strongly adsorbed at the top of the column. Evaporation of the eluate left the ethers as a partially crystalline solid. Vacuum treatment at 78° removed some volatile material, and recrystallization from hexane–benzene and from acetone provided garnet-red lath-like crystals (1) m. p. 124.5–126°. The substitution of amyl bromide for the iodide resulted in only 7% yield of the desired ether.

Repetition of this preparation with a larger amount of crude material (31.5 g.), eliminating the vacuum heating step, gave material (2) melting at 131–132°.

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Summary

Thionol (7-hydroxyphenothiazone-3) has been prepared by condensation of *p,p'*-dihydroxydiphenylamine with sulfur, and by sulfuric acid oxidation of phenothiazine followed by isolation as the lithium salt. The yield from the previously reported latter process has been increased by shortening the reaction time.

The ethyl, amyl, octyl, dodecyl and hexadecyl ethers of 7-hydroxyphenothiazone-3 have been prepared by treating the silver salt with the appropriate alkyl iodides.

3-Octoxyphenothiazine-5-oxide has been made by alkaline peroxide treatment of 3-octoxyphenothiazine.

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[CONTRIBUTION FROM THE BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

Synthesis of Inositol-5-monophosphoric Acid and Scyllitol Monophosphoric Acid[1]

BY BEAT M. ISELIN[2]

The widespread occurrence of inositol phosphoric acids in plants has long been recognized.[3] For many years inositol hexaphosphoric acid, or phytic acid was the only inositol phosphate known until Anderson,[4] in 1914, succeeded in isolating inositol monophosphoric acid from wheat bran. The same substance has been obtained by the action of the enzyme phytase on phytic acid[5,6] or by partial hydrolysis of phytic acid with dilute sulfuric acid.[7] More recent investigations have shown that inositol monophosphoric acid is a constituent of many phosphatides. Anderson has found this substance as a polysaccharide in the phosphatide fraction of tubercle bacilli.[8,9] Klenk and Sakai have described a preparation of inositol monophosphoric acid from soy bean lipositol[10] in which, as has been demonstrated by Woolley,[11] it is combined with galactose in glycosidic linkage.

The inositol monophosphoric acids isolated from natural sources are optically inactive. Their structure has not been investigated as yet. However, in the light of the present knowledge of the configuration of meso-inositol it is evident that only those mono-substituted derivatives are optically inactive in which the substituent is in position 2 or 5[12]; substitution in other positions is expected to yield products with optical activity. Assuming that the natural inositol monophosphates are not resolvable it may be concluded that the phosphoric acid residue is attached to carbon atom 2 or 5.

The synthesis of an inositol monophosphoric acid carrying the substituent on carbon atom 5 was effected by taking advantage of the known fact that *Acetobacter suboxydans* oxidizes meso-inositol specifically in position 5 yielding scyllo-meso-inosose. This substance, by acetylation and subsequent catalytic hydrogenation of the keto group with platinum oxide in glacial acetic acid, is converted to 1,2,3,4,6-pentaacetyl-meso-inositol as has been described by Posternak.[13] When the directions for the hydrogenation of scyllo-meso-inosose pentaacetate given by this author were closely followed, a product was obtained that had the recorded melting point (161–162°); acetylation to the hexaacetate and the bioassay of the hydrolyzed material with *Saccharomyces cerevisiae* revealed, however, that the product contained approximately 25% of the scyllitol isomer. It was found impossible to achieve a satisfactory separation of the two isomers by fractional crystallization. If the hydrogenation of scyllo-meso-inosose pentaacetate was carried out using methanol in-

(1) Presented at the 115th meeting of the American Chemical Society, San Francisco, March, 1949.

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(3) *E. g.*, E. Winterstein, *Ber.*, **30**, 2299 (1897).

(4) R. J. Anderson, *J. Biol. Chem.*, **18**, 441 (1914).

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(7) R. J. Anderson, Ph.D. Dissertation, Cornell University, 1919.

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(12) Numbering according to H. O. L. Fischer, "Harvey Lectures," Ser. **40**, 156 (1945), and H. G. Fletcher, "Advances in Carbohydrate Chemistry," **3**, 45 (1948).

(13) Th. Posternak, *Helv. Chim. Acta*, **24**, 1045 (1941).