The Synthesis and the Estrogenic Activity of Bis-2, 4-dihydroxyphenyl Disulfide and the Related Thiophenol

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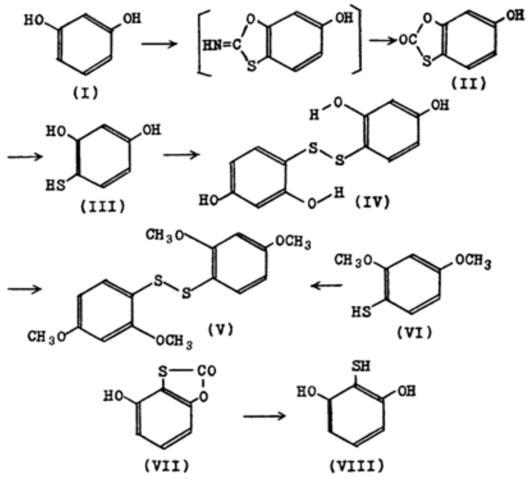
One of the authors (Y. U.) and T. Takahashi[1] reported the synthesis and the estrogenic activity of 2, 4, 2′, 4′-tetrahydroxyazobenzene. It produced full estrus in 100% animals (mice) at a dosage of 20γ injected subcutaneously in two portions. The estrogenic activity of 4, 4′-dihydroxyazobenzene being very small (active in 80% at 1 mg.), the 2, 2′-hydroxyl groups of 2, 4, 2′, 4′-tetrahydroxyazobenzene were considered to form a double chelation, thus effecting a rigid, chrysene-shaped structure favorable for the development of a strong estrogenic activity. The infrared absorption spectrum of the crystals in Nujol showed the presence of hydrogen bonding between the azo nitrogen atom and the phenolic hydroxyl group. The insolubility of the tetrahydroxyazobenzene defied the measurement of its infrared absorption in solution, but 2-hydroxyazobenzene, a reference compound, in carbon tetrachloride solution showed a broad absorption at 3.4–3.5 μ due to an intramolecular hydrogen bonding[2], and thus gave an indirect evidence for the presence of chelation in the tetrahydroxyazobenzene at least in dilute state as when administered to the animal. If the supposed double chelation exists in the tetrahydroxyazobenzene, it is necessarily connected with a coplanar structure of the molecule, which was supposed to be one of the reasons why the substance is less active than the most potent synthetic estrogens[3].

The present authors expected bis-2, 4-dihydroxyphenyl disulfide (IV) might be a compound with a structure similar to that of 2, 4, 2′, 4′-tetrahydroxyazobenzene but not coplanar and thus a stronger estrogen, because of the presence of two unshared pairs of electrons in the valence shell of the covalent-bonded sulfur atom.

C. Lefevre and Ch. Desgrez[4] reported in 1934 that they prepared phenol disulfides by heating several phenols with sulfur and sodium carbonate in glycerol solution at 120°, and that from the ordinary phenol they obtained a disulfide melting at 116°, and from resorcinol a disulfide decomposing without fusion. They assigned the constitutions of bis-4-hydroxyphenyl disulfide and bis-2, 4-dihydroxyphenyl disulfide, respectively, without stating any detailed investigation of their structures. In 1890 R. Leuckart[5] already described bis-4-hydroxyphenyl disulfide melting at 150–151°, and in 1947 S. Magnusson, J. E. Christian, and G. L. Jenkins[6] prepared the same disulfide melting at 151–152°. Hence, the structures of the disulfides of Lefevre and Desgrez were probably different from the supposed. The present authors synthesized bis-2, 4-dihydroxyphenyl disulfide in the following way:



6-Hydroxy-1, 3-benzoxathiolone-(2) (II), m. p. 154.5–155°, was prepared from resorcinol (I), copper sulfate, and ammonium thiocyanate, by a method similar to that described by M. Pantlitschko and H. Benger[7] but without using sodium carbonate. It was hydrolyzed to 2, 4-dihydroxythiophenol (III), m. p. 110–111° by treatment with caustic soda. Oxidation of the thiophenol with hydrogen peroxide gave bis-2, 4-dihydroxyphenyl disulfide (IV), m. p. 170–171°, in white crystalline powder.

Pantlitschko and Benger observed that the product (m. p. 160°) from the reaction of

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[1] This Bulletin, **26**, 162 (1953).

[2] T. Takahashi, *J. Chem. Soc. Japan*, **74**, 673 (1953).

[3] Cf. M. Ōki and Y. Urushibara, This Bulletin, **25**, 109 (1952).

[4] *Compt. rend.*, **198**, 1791 (1934).

[5] *J. prakt. Chem.*, **41**, 179 (1890).

[6] *J. Am. Pharm. Assoc. Sci. Ed.*, **36**, 257 (1947).

[7] *Monatsh.*, **81**, 293 (1950); *Chem. Abstr.*, **45**, 2471 (1951).

resorcinol, copper sulfate, and ammonium thiocyanate, was 4-hydroxy-1, 3-benzoxathiolone-(2) (VII), from which they obtained 2, 6-dihydroxythiophenol (VIII), m. p. 83–84°. G. Werner[8] claimed a preparation of the same or isomeric compound melting at 158°, and, although the Chemical Abstracts, Vol. 38, gives the name 6-hydroxy-1, 3-benzoxathiolone-(2) for the substance in the Index, it is not shown in either the Chemical Abstracts or the original patent comment how its constitution was determined. It is also unknown whether the substance of G. Werner is identical with the substance melting at 154.5-155° obtained by the present authors or not. The following facts, however, support the structures assigned to the substances described in this paper.

Namely, the methylation of the disulfide, m. p. 170–171°, gave a product identical with the bis-2, 4-dimethoxyphenyl disulfide (V) prepared from the known 2, 4-dimethoxythiophenol (VI). The latter compound (VI) was prepared, and the constitution was determined, by C. M. Suter and H. L. Hansen[9]. The estrogenic activity of the disulfide (IV) and the thiophenol (III) is also a strong evidence for their structures.

Bis-2, 4-dihydroxyphenyl disulfide (IV) produced full estrus in 100% animals (mice) at a dosage of 10 γ by a single subcutaneous injection in oil solution, while 2, 4-dihydroxythiophenol (III) was fully active in 60–70% animals at the same dosage and in 100% at 20 γ. 2, 4-Dihydroxythiophenol appears to be an estrogen of the least molecular weight ever known, but it must be considered that it is oxidized in vivo to bis-2, 4-dihydroxyphenyl disulfide and acts as such.

Bis-4-hydroxyphenyl disulfide, 4-$HOC_6H_4 \cdot SSC_6H_4OH$-4, prepared by the method described by Magnusson et al., showed much less activity. Namely, it was totally inactive at 50 γ, but produced estrus in 20–40% animals at 100 γ and in 80% at 500–1000 γ. The structure of this compound may be noncoplanar contrary to those of 4, 4′-dihydroxyazobenzene and 4, 4′-dihydroxystilbene, considering the possible repulsion of the unshared pairs of electrons of the two sulfur atoms, and considering certain results from the investigations on the molecular structure of sulfur monochloride S_2Cl_2 by means of electron diffraction[10] and on the dipole moments of diphenyl disulfides[11]. Bis-4-hydroxyphenyl disulfide and bis-2, 4-dihydroxyphenyl disulfide may possibly possess the same or very similar twisted conformation of the hydrogen peroxide type, and then it may facilitate a chelation in the latter compound because one of the two unshared pairs of electrons of each sulfur atom is oriented towards the ortho hydroxyl group of the farther ring, but the distance between the two para hydroxyl groups may be shorter than the optimum for estrogenic potency[3].

At any rate, it has been demonstrated that the introduction of an ortho hydroxyl group into each ring of bis-4-hydroxyphenyl disulfide to form bis-2, 4-dihydroxyphenyl disulfide (IV) increases the estrogenic potency to a great extent, but it has not yet been determined in any other way whether a chelation really exists.

Experimental Part[12]

6-Hydroxy-1, 3-benzoxathiolone-(2) (II).—To a solution of 22 g. of resorcinol and 100 g. of crystalline copper sulfate in 500 ml. of water, 62 g. of ammonium thiocyanate dissolved in 100 ml. of water were added with stirring. A black precipitate appeared and turned gray in color. After standing for one hour with occasional stirring, the precipitate was removed, and the orange-red filtrate was heated on a waterbath and left to stand to complete the precipitation of a yellow substance. The yellow precipitate was collected on a filter and recrystallized three times from water-alcohol. Pale yellow needles, m. p. 154.5–155°. Yield, 12 g. (36% of the theory).

Anal. Found: C, 49.83; H, 2.36; S, 18.90. Calcd. for $C_7H_4O_3S$: C, 49.99; H, 2.40; S, 19.07%.

2, 4-Dihydroxythiophenol (III).—In the stream of nitrogen 100 ml. of 2 N caustic soda was added gradually to 10 g. of 6-hydroxy-1, 3-benzoxathiolone-(2) (II) contained in an Erlenmeyer flask. The mixture was gently heated to complete dissolution, left to cool down to the room temperature, acidified to acid reaction to Congo Red with 2 N hydrochloric acid, and extracted with ether. The white residue from the ethereal extract was recrystallized twice from benzene. A white crystalline powder, m. p. 110–111°. Yield, 6.5 g. (79% of the theory).

Anal. Found: C, 50.90; H, 4.37; S, 22.49. Calcd. for $C_6H_6O_2S$: C, 50.68; H, 4.25; S, 22.55%.

Bis-2, 4-dihydroxyphenyl Disulfide (IV).—A solution of 6 g. of the thiophenol (III) in 40–60 ml. (as little as possible) of water was cooled below 10°, about 4 ml. of 35% hydrogen peroxide was added gradually with stirring at a temperature not exceeding 15°, and the mixture was left to stand with cooling. The white crystalline substance was collected on a filter and recrystallized twice from water. A white crystalline powder,

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[8] U.S. Patent, 2,332,418; *Chem. Abstr.*, 38, 1889 (1944).

[9] *J. Am. Chem. Soc.*, 55, 2080 (1933).

[10] E. Hirota and Y. Morino, private communication. Cf. also S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press, New York (1954), p. 93.

[11] E. Bergmann and M. Tschudnowsky, *Z. physik. Chem.*, B, 17, 107 (1932).

[12] Melting points uncorrected.

m. p. 170–171°, Yield, 4 g. (67% of the theory).

Anal. Found: C, 51.14; H, 3.42; S, 22.27. Calcd. for $C_{12}H_{10}O_4S_2$: C, 51.05; H, 3.57; S, 22.72%.

Bis-2, 4-dimethoxyphenyl Disulfide (V).—2, 4-Dimethoxythiophenol, prepared by the method of C. M. Suter and H. L. Hansen⁹⁾, was treated with a slight excess of iodine in alcoholic solution. The precipitate was recrystallized from alcohol. Colorless needles, m. p. 113–115°. Yield, quantitative.

Anal. Found: C, 56.59; H, 5.19; S, 18.79. Calcd. for $C_{16}H_{18}O_4S_2$: C, 56.78; H, 5.36; S, 18.91%.

Bis-2, 4-dihydroxyphenyl disulfide (IV), dissolved in caustic soda, and dimethyl sulfate gave a white crystalline product. Recrystallized from alcohol, it showed a melting point 109.5–112.5° and no depression of the melting point was observed in admixture with the bis-2, 4-dimethoxyphenyl disulfide described above.

Attempted methylation of 2, 4-dihydroxythiophenol (III) to the known methyl 2, 4-dimethoxyphenyl sulfide, $C_6H_3(SCH_3)(OCH_3)_2$ (1, 2, 4), was accompanied by oxidation and resulted in the formation of 2, 4-dimethoxyphenyl disulfide (V).

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