### Experimental

The lactones were prepared according to literature procedures and purified by recrystallization from appropriate solvents. All spectra were run in Nujol mulls. The instrument was a Perkin–Elmer model 13 recording spectrometer operated at slow chart speeds and at speed–response ratios which gave maximum resolution (estimated at 10 cm.$^{-1}$). A wave length calibration preceded each set of runs. This was achieved by operating the spectrometer on "direct mode" and recording the water vapor spectrum.

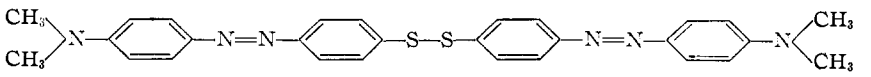
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF SOUTHERN CALIFORNIA
LOS ANGELES 7, CALIFORNIA

## The Preparation and Biological Properties of 4,4′-Bis-(*p*-dimethylaminophenylazo)-phenyl Disulfide[1,2]

BY R. K. BURKHARD, D. E. SETTER[3] AND R. M. GROSSMAN

RECEIVED APRIL 23, 1954

4,4′-Bis-(*p*-dimethylaminophenylazo)-phenyl disulfide (DS) was prepared for two reasons. DS



might serve as a suitable intermediate in the synthesis of azo dyes related to methyl orange but which would contain lower oxidation states of the sulfur atom than that which is found in methyl orange. The disulfide linkage in this molecule might enable it to react with mercaptan groups in a protein and hence form azo dye–protein derivatives which would contain the carcinogenic moiety found in 4-dimethylaminoazobenzene (DAB). It has been found that when carcinogenic azo dyes are fed to rats these dyes are incorporated into the liver proteins of the rat through a linkage which probably involves the dimethylamino group of the dye.[4] It might be that the feeding of DS would result in the formation of azo dye–protein derivatives in which the carcinogenic moiety found in DAB would be attached to liver proteins through a disulfide linkage. It might be that an azo dye bound to liver proteins in such a manner would also induce tumor formation.

### Experimental

**Preparation of 4,4′-Bis-(*p*-dimethylaminophenylazo)-phenyl Disulfide (DS).**—The starting material for this synthesis was acetanilide which was converted into *p*-acetaminobenzene sulfonyl chloride according to the procedure of Smiles and Stewart.[5] The crude acid chloride was dried with an acetone–benzene mixture according to the procedure of Adams and Johnson[6] if it was to be stored for any length of time. This acid chloride was reduced to bis-(*p*-acetamino)-phenyl disulfide and hydrolyzed to 4,4′-diaminophenyl disulfide dihydrochloride according to the method of Bauer and Cymerman.[7] The tetrazotization of this diamine dihydrochloride and its subsequent coupling to dimethylaniline were accomplished in a manner similar to that used by Burawoy and Turner.[8] A suspension of 4,4′-diaminophenyl disulfide dihydrochloride (1.61 g.) in concentrated sulfuric acid (7 ml.) was prepared and cooled to 0°. This suspension was then slowly added to a cold solution of nitrosyl sulfuric acid. After tetrazotization the cold solution of the tetrazonium salt was slowly added to a cold solution of 1.21 g. of dimethylaniline in 200 ml. of 50% alcohol containing 60 g. of sodium acetate. The *p*H of the solution was intermittently measured and not allowed to go below 5.0 by the addition of more sodium acetate. The reaction mixture became very viscous and alcohol and chipped ice were added occasionally to maintain an easily stirred mixture. After the addition of the tetrazonium salt was completed the resulting mixture was then stirred for an additional 30 minutes followed by the addition of a small amount of urea. The crude product was then collected and washed several times with water to dissolve the large quantities of inorganic salts present. This procedure gave 2.1 g. (82% yield) of crude DS. The crude DS was first crystallized from a pyridine–water mixture, then from pyridine and finally from ethylene chloride to give red crystals melting at 198–199.5°. Analysis of DS for nitrogen and sulfur contents gave 15.8 and 12.0%, respectively. These data yield upon calculation a nitrogen/sulfur ratio of 1.32/1.00. The values calculated for $C_{28}H_{28}N_6S_2$ are 16.4% nitrogen, 12.5% sulfur and a nitrogen/sulfur ratio of 1.31/1.00. The absorption spectrum of DS in ethylene chloride shows a maximum at 4300 Å. with a molar extinction coefficient of $3.04 \times 10^4$.

**Test for Carcinogenicity.**—The procedure for testing of DS was similar to that used by the Wisconsin group wherein the dye is fed to albino rats by its incorporation into a special basal ration.[9]

Three groups of Sprague-Dawley male albino rats of weights averaging 200 g. were arranged. Each group was fed *ad libitum* for a fourteen week period one of the following rations: group I, basal ration; group II, DS ration; and group III, 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) ration. The DS ration and the 3′-Me-DAB ration both incorporated these dyes at 0.06% concentration by weight rather than at equal molar concentrations because it was thought that DS could be cleaved *in vivo* to yield two (*p*-dimethylaminophenylazo)-phenyl moieties. At the end of the fourteen week period the animals were anesthetized and an examination of the internal organs made.

**Preparation of Blood-free Liver Homogenates.**—The liver of each animal was perfused *in situ* with 0.89% saline, excised, quick frozen and then stored for a week in the frozen state. After this time the livers were homogenized and dialyzed at 0° to remove soluble colored materials and then lyophilized.

### Results and Discussion

Examination of the three groups of rats showed that the livers from the rats fed the basal ration and the rats fed the DS ration could not be differentiated one from another by gross examination. The livers from the rats fed the 3′-Me-DAB ration were cirrhotic and small tumors were noted. Histological examination of the livers revealed that the livers from the DS fed rats were normal.[10] From these data it was concluded that DS is not a hepatic carcinogen for the male albino rat under the conditions of the test.

These findings brought up the question as to the

(1) Presented in part before the Biological Section of the 125th National Meeting of the American Chemical Society, Kansas City, Missouri, March 25, 1954.

(2) Supported by the National Cancer Institute, U. S. Public Health Service, Bethesda 14, Maryland.

(3) U. S. Public Health Service Predoctoral Research Fellow, 1952–1953.

(4) E. C. Miller, J. A. Miller, R. W. Sapp and G. M. Weber, *Can. Res.*, **9**, 336 (1949).

(5) S. Smiles and J. Stewart, "Organic Syntheses," Coll. Vol. I, H. Gilman and A. H. Blatt, Editors, 2nd Edition, John Wiley and Sons, Inc., New York, N. Y., 1946, pp. 8–10.

(6) R. Adams and J. R. Johnson, "Elem. Lab. Exp. in Org. Chem.," 3rd Edition, the Macmillan Co., New York, N. Y., 1945, p. 361.

(7) L. Bauer and J. Cymerman, *J. Chem. Soc.*, 3434 (1948).

(8) A. Burawoy and C. Turner, *ibid.*, 469 (1950).

(9) H. P. Rusch, C. A. Bauman, J. A. Miller and B. E. Kline, "Experimental Liver Tumors," in A. A. A. S. Res. Conf. on Cancer, F. R. Moulton, Editor, A. A. A. S., Washington, D. C., 1945, pp. 267–283.

(10) The authors are indebted to Mr. Ralph Pyke of the Chemistry Department and Dr. Melvin J. Swenson of the School of Veterinary Medicine, Kansas State College, for the histological examination.

ability of the rat to incorporate DS into the liver tissue as was originally hoped might be true. Accordingly, another group of rats was fed DS for a period of six weeks and after this time interval blood-free liver homogenates were prepared for analysis of the dye. This time interval (six weeks) was chosen since the Wisconsin group has shown that both carcinogenic and non-carcinogenic azo dyes appear in the liver tissue in about equal amounts after this length of time.[4] Thus whether DS is or is not a carcinogen should have little effect on the amount of dye found in the liver after six weeks of feeding. Analysis of the liver preparations according to the method of Miller and Miller[11] failed to show the presence of dye. From this it was concluded that DS was either not incorporated into the rat liver, or that if it was then it was destroyed too rapidly to accumulate.

It was thought desirable to determine the intestinal absorption of DS since this factor has contributed to the low carcinogenicity of certain azo dyes.[12] Analysis of the feces from rats fed DS showed that approximately 15–25% of the dye ingested is excreted in the feces within a 48-hour period after ingestion.

Lastly, it was noted that the rats fed the DS ration gained weight much more rapidly than either of the other two groups of rats. This observation may not have any significance, however, since the rats were not limited in their intake and also since it was noted that the rats fed the DS ration consistently ate more than the other rats. In fact, at the end of 1.5 months of feeding the rats which were fed the DS ration had eaten all of the ration which had originally been calculated to be adequate for three or four months of feeding. The factor responsible for this observation was not determined.

(11) E. C. Miller and J. A. Miller, *Can. Res.*, **7**, 468 (1947).
(12) J. A. Miller, R. W. Sapp and E. C. Miller, *ibid.*, **9**, 652 (1949)

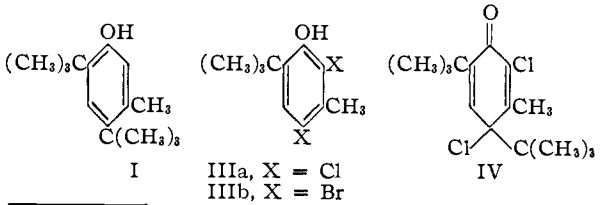
DEPARTMENT OF CHEMISTRY
KANSAS STATE COLLEGE
MANHATTAN, KANSAS

## Reactions of 3,4,6-Trialkylphenols. I. Halogen Derivatives of 3-Methyl-4,6-di-*t*-butylphenol

BY LAWRENCE E. FORMAN AND WILLIAM C. SEARS

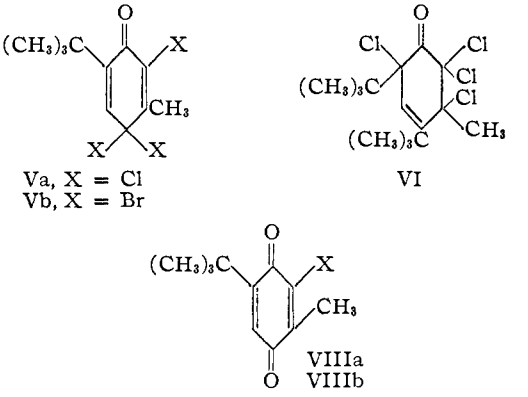
RECEIVED AUGUST 13, 1953

Chlorination of 3-methyl-4,6-di-*t*-butylphenol (I) yields 2-chloro-3-methyl-4,6-di-*t*-butylphenol (II) as the initial product. Further chlorination in carbon tetrachloride solution gives two products—a new compound (IV) containing two atoms of chlorine, and the partially dealkylated dichlorophenol IIIa. Compound IV is insoluble in sodium hydroxide solutions. The infrared spectrum shows that the hydroxyl band (3554 cm.$^{-1}$)[1] of a partially hindered phenol present in II and III has disappeared, while a new band attributable to a carbonyl group has appeared at 1659 cm.$^{-1}$. Similarly, the ultraviolet absorption spectrum, $\lambda_{max}$, has undergone a hypsochromic shift indicating a loss of benzenoid character as shown by a maximum at 284 m$\mu$ displacing to 246 m$\mu$ with a large increase in log $E$. Fisher–Hershberger–Taylor models indicate considerable steric hindrance to *para* substitution by chlorine at position 5, but admit the possibilities of substitution at positions 2 or 4 to give "ketohalogenides" similar to the compounds described by von Auwers[2] and Zincke.[3] This leads us to believe that further chlorination of II proceeds by the absorption of a positive chlorine atom at position 4 and the elimination of a proton from the hydroxyl group. Coppinger and Campbell[4] have indicated that a similar situation exists for the attack of bromine on 2,6-di-*t*-butyl-4-methylphenol.



I IIIa, X = Cl IIIb, X = Br IV

Further chlorination causes elimination of the 4-*t*-butyl group and the formation of a mixture of IIIa and Va.



Va, X = Cl Vb, X = Br VI VIIIa VIIIb

Chlorination of I in acetic acid in the presence of pyridine at 12°, gives rise to a tetrachloro addition product. The infrared spectrum indicates that this product is a non-conjugated cyclohexenone and therefore structure VI has been assigned.

The trichloro derivative Va may be prepared also by the addition of chlorine to 3-methyl-6-*t*-butylphenol (VII) in cold acetic acid.

Bromine reacts with I in carbon tetrachloride solution in the presence of iron to give the dibromo analog of III, 2,4-dibromo-3-methyl-6-*t*-butylphenol (IIIb). This compound may be prepared more readily by the addition of bromine to 3-methyl-6-*t*-butylphenol dissolved in acetic acid. No bromo derivative corresponding to IV was isolated. The bromo analog of V may be prepared by treating VII with sufficient bromine in cold acetic acid followed by refrigeration. This tribromo derivative, 2,4,4-tribromo-3-methyl-6-*t*-butylphenol (Vb) is unstable and loses bromine on standing in air at room temperature.

Oxidation of III or V, either by nitric or chromic acids in acetic acid, gives rise to the corresponding *p*-quinone, 2-halogeno-3-methyl-6-*t*-butylquinone-

(1) L. J. Kitchen and W. C. Sears, THIS JOURNAL, **71**, 4110 (1949).
(2) K. von Auwers, *Ann.*, **301**, 203 (1898); *Ber.*, **32**, 2987 (1899).
(3) Th. Zincke, *Ann.*, **328**, 282 (1903).
(4) G. M. Coppinger and T. W. Campbell, THIS JOURNAL, **75**, 735 (1952).