

SCIENTIFIC SECTION

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(To be revised.)

SULPHIDE ANALOGUES OF AZO DYES HAVING BACTERICIDAL PROPERTIES.*

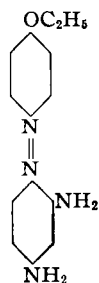
BY W. BRAKER AND W. G. CHRISTIANSEN.¹

The bactericidal properties of a number of diaryl sulphides have been reported by Hilbert and Johnson (1) and by Moness, Braker and Christiansen (2). Since this activity is ascribed to the presence of the sulphide linkage, we have investigated the combination of this grouping with other active groups present in certain azo dyes known to be therapeutically valuable.

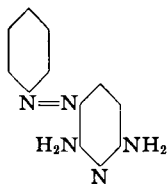
The compounds chosen for preparation contain the diaryl sulphide linkage combined with

- (1) The *p*-ethoxy benzene-azo residue of serenium (I),
- (2) The diamino pyridine grouping of pyridium (II).

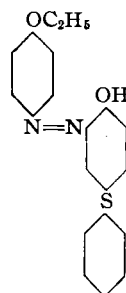
Of the first type we prepared one example: 3-*p*-ethoxy benzene-azo, 4-hydroxy diphenyl sulphide (III). Of the second type two compounds were made: 4,4'-bis-(α,α -diaminopyridine-azo) diphenyl sulphide (IV), and 3,5-bis(4'-amino diphenyl sulphide-azo) 2,6-diamino pyridine (V).



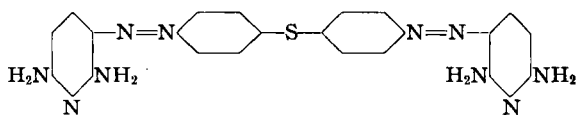
(I)



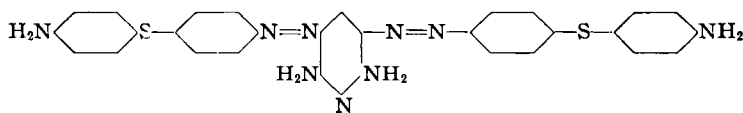
(II)



(III)



(IV)



(V)

* Scientific Section, A. PH. A., Washington meeting, 1934.

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These compounds were readily prepared, but were found to be only slightly soluble in any of the media in which they might be used. Compound III could not be dissolved in mixtures of alcohol, water and glycerin or in water containing an equivalent of NaOH. IV and V were practically insoluble in water containing an equivalent of HCl. They were, however, tested for antiseptic activity by means of the "cup test." In this attempt at evaluation, the powdered compounds were placed in depressions in the center of an inoculated (*Staphylococcus aureus*) area of nutrient agar, and the area of inhibition measured after 48 hours (3). Serenium and pyridium, used as controls, showed clear zones of 4-5 mm. III and IV, under these conditions, were quite inactive, and V was only slightly active.

EXPERIMENTAL.

Preparation of 4,4'-Diamino Diphenyl Sulphide.—The method of Merz and Weith (4) was used, yielding yellowish white needles, m. p. 104-108° C. The authors state 108° C. as the m. p.

Preparation of 2,6-Diamino Pyridine.—The method of Tchitchibabin (5) yielded this substance in the form of silvery leaflets, m. p. 119° C., stated in the literature to be 119-120° C.

Preparation of p-Hydroxy Diphenyl Sulphide.—The preparation of this compound is described in a previous paper (1).

Preparation of 3-p-Ethoxy Benzene-Azo, 4-Hydroxy Diphenyl Sulphide.—1.3 Gm. *p*-phenetidine was dissolved in a mixture of 21.5 cc. of *N*-hydrochloric acid and 25 cc. of water. The substance was diazotized at 0° C. with a solution of 0.69 Gm. NaNO₂ in 10 cc. water. 2.02 Gm. *p*-hydroxy diphenyl sulphide dissolved in 25 cc. 2*N*-NaOH was added with vigorous stirring. The reaction mixture was allowed to stand at 0° C. for 2 hours, then at 30° C. for 4 hours longer. The red dye which had separated out was then filtered off, washed with water and dried *in vacuo*.

Yield—1.54 Gm. of a red powder.

Analysis: Found—S, 9.40%; calculated for C₂₀H₁₈O₂N₂S—S, 9.14%.

Preparation of 4,4'-Bis (α,α-Diamino Pyridine-Azo) Diphenyl Sulphide.—4.2 Gm. 4,4'-diamino diphenyl sulphide was dissolved in a mixture of 20 cc. conc. hydrochloric acid and 50 cc. water. Diazotization was carried out at 0° C. with a solution of 2.7 Gm. NaNO₂ in 20 cc. water. 4.4 Gm. of 2,6-diamino pyridine dissolved in dilute hydrochloric acid (one equivalent of HCl) was then vigorously stirred in. A red precipitate began to separate. After standing at 0° C. for one hour, then at 30° C. for 3 hours, the mixture was made ammoniacal. The precipitate was filtered off, washed with water and dried *in vacuo*.

Yield—8.2 Gm. of an orange-colored powder.

Analysis: Found—S, 6.80%; calculated for C₂₂H₂₀N₁₀S—S, 7.02%.

Preparation of 3,5-Bis (4'-Amino Diphenyl Sulphide-Azo) 2,6-Diamino Pyridine.—5.0 Gm. 4,4'-diamino diphenyl sulphide in 3 equivalents of dilute hydrochloric acid was diazotized at 0° C. with a solution of 1.53 Gm. NaNO₂ in 20 cc. water. A slight residue, present after diazotization, was removed by filtration. To the clear filtrate was added, with vigorous stirring, and at 0° C., 1.17 Gm. 2,6-diamino pyridine dissolved in dilute hydrochloric acid. Immediate precipitation occurred. After standing at 30° C. for 4 hours, the reaction mixture was made alkaline. The precipitate was filtered off, washed with water, sucked dry, washed with several portions of warm benzene and dried *in vacuo*.

Yield—4.5 Gm. of a red powder.

Analysis: Found—S, 11.29%; calculated for C₂₉H₂₄N₈S₂—S, 11.37%.

The biological tests on the compounds here reported were made in the Biological Research Laboratories of E. R. Squibb and Sons and we gratefully acknowledge their assistance.

SUMMARY.

1. Three new dyes, containing the diaryl sulphide linkage, together with residues present in serenium and pyridium, have been prepared.
2. These substances have been tested for antiseptic activity and have been found to be inactive.

BIBLIOGRAPHY.

- (1) Hilbert and Johnson, *J. A. C. S.*, 51, 1526 (1929).
- (2) Moness, Braker and Christiansen, *JOUR. A. PH. A.*, 21, 558 (1932).
- (3) U. S. Department of Agriculture, *Circular No. 198*; "United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants."
- (4) Merz and Weith, *Ber.*, 4, 384 (1871).
- (5) Tchitchibabin and Seide, *J. Russ. Phys. Chem. Soc.*, 46, 1216 (1914).

PHYTOCHEMICAL NOTES.*¹

No. 108. A PHYTOCHEMICAL STUDY OF THE SEED OF THE DIGGER PINE.

BY JOSEPH SEMB.

In the early part of November 1931, forty pounds of Digger pine seed were received from Mr. J. W. Preston, Chico, Calif. One hundred of these seeds were cracked and separated into seed coats and endosperms. The former weighed 53.20 Gm. (= 76.9 per cent), the latter 15.95 Gm. (= 23.1 per cent). Inasmuch as the 100 seeds weighed 69.15 Gm., the average weight of the individual seed is a trifle less than 0.7 Gm. That of the endosperm is about 0.16 Gm.

Samples of ground seed, of seed coats and of endosperm were extracted with selective solvents. The data are recorded below:

PERCENTAGE EXTRACTED.

| Solvent. | From Seed. | | From Seed Coat. | | From Endosperm. | |
|-------------|------------|--|---------------------|---------------------|---------------------|---------------------|
| | Per Cent. | | A (2). Per Cent. | B (3). Per Cent. | C (4). Per Cent. | D (5). Per Cent. |
| Pet. ether | 11.47 (1) | | 0.18 | 0.13 | 53.00 | 12.20 |
| Ethyl ether | 0.27 | | 0.30 | 0.23 | 1.00 | 0.23 |
| Chloroform | 0.11 | | 0.00 | 0.00 | 0.74 | 0.17 |
| Alcohol | 0.90 | | 0.76 | 0.59 | 2.90 | 0.66 |
| Water | 1.98 | | 2.00 | 1.44 | 8.20 | 1.89 |
| 1% HCl | 15.00 | | 18.20 | 14.00 | 27.18 | 6.26 |
| 1% NaOH | 2.60 | | 5.00 | 3.70 | .. | .. |

Of the 40 pounds of seed, 37.5 pounds or 17 Kg. were ground in the horizontal disk mill. The coarsely comminuted material was extracted in the cold with petroleum ether for 24 hours. After the percolate had been drawn off, the dregs were reground in a ball mill. This reground residue was subsequently extracted twice with petroleum ether in the cold. A test sample of the last extraction indicated that the extraction was almost complete. Most of the petroleum ether was recovered. The last traces were removed by heating on a water-bath under somewhat reduced pressure for several hours. During part of this time a current of CO₂ was passed through the liquid to assist in the complete removal of the volatile solvent. The petroleum ether extract thus obtained weighed 1950 Gm. = 11.47% of the seed or 49.66% of the endosperm.

* Scientific Section, A. PH. A., Miami meeting, 1931.

¹ From the laboratory of Edward Kremers.