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THE PREPARATION AND PROPERTIES OF 3,3'BIS(AZOMETA-PHENYLENEDIAMINE)-4,4'-DIHYDROXYARSENOBENZENE AND 3,3'-BIS-(AZO-2,6-DIAMINOPYRIDINE)-4,4'-DIHYDROXYARSENOBENZENE.*

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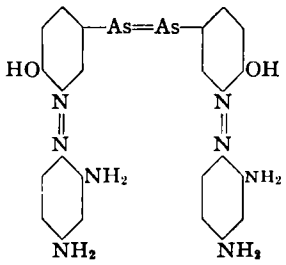
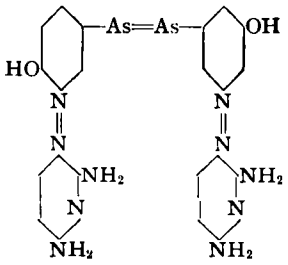
It is well known that certain azo dyes penetrate tissue very readily, thereby being disseminated widely throughout the body. Further, some azo dyes, such as trypan blue and trypan red, are absorbed by trypanosomes and have a definite trypanocidal action. Consequently it was decided to prepare some azo dyes from arsphenamine base by diazotizing the latter and coupling with diamines. A number of arsono and arseno azo compounds have been described by Andreyev (1), Karrer (2), Barrowcliff, Pyman and Remfry (3), Benda (4), Jacobs and Heidelberger (5), and Ehrlich and Bertheim (6). Also some patents have been issued covering such compounds. However, none of them are of the type which we prepared by diazotizing arsphenamine and coupling with metaphenylenediamine and with 2,6-diaminopyridine.

The two substances prepared in this investigation were 3,3'-bis(azometaphenylenediamine)-4,4'-dihydroxyarsenobenzene (I) and 3,3'-bis(azo-2,6-diaminopyridine)4,4'-dihydroxyarsenobenzene (II).



(I)



(II)

When aqueous sodium hydroxide solutions of these compounds were injected intravenously into albino rats, the anticipated tissue staining characteristics resulted. Thus, within a short time after injection the conjunctiva, ears and abdominal cavities of the animals were stained. However, both of these compounds

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were very toxic when compared to other arsphenamines. The sodium salt of 3,3′-bis(azo-2,6-diaminopyridine)-4,4′-dihydroxyarsenobenzene was found to be bacteriostatic to both *B. Typhoid* and *B. Staphylococcus* in concentrations of 1–20,000. Further, although the free base was only slightly soluble in water, a saturated aqueous solution was bacteriostatic to both *B. Typhoid* and *B. Staphylococcus*. However, both compounds appear to be too toxic for therapeutic use.

EXPERIMENTAL.

3,3′-Bis(Azometa-phenylenediamino)-4,4′-Dihydroxyarsenobenzene.—A solution of 8.8 Gm. of arsphenamine in 880 cc. of water was cooled in ice and 3.5 cc. of concentrated hydrochloric acid was added. This was then diazotized by adding 3.0 Gm. of sodium nitrite dissolved in 10 cc. of water. After stirring for ½ hour, 4.3 Gm. of metaphenylenediamine dissolved in an excess of dilute hydrochloric acid was added. After stirring for one hour a slight excess of sodium bicarbonate was added. A finely divided red-brown precipitate formed which was separated by centrifuging. It was resuspended in water, collected on a Buchner funnel and washed with water.

Assay: As found, 28.11%; calculated for $C_{24}H_{22}O_2N_8As_2$, 24.83%.

The dark brown solid was soluble in concentrated aqueous hydrochloric acid and aqueous sodium hydroxide, giving a deeply colored solution. It was also slightly soluble in water, ethyl alcohol, methyl alcohol, acetone and ether. 0.2 Gm. dissolved completely in 20 cc. of water and 1.5 cc. of normal sodium hydroxide.

Intravenous injections were made in rats using a solution of 0.5 Gm. of compound in 5 cc. of normal sodium hydroxide and 45 cc. of water. A dose of 100 mg./Kg. was lethal and stained the conjunctiva, ears and abdominal cavity a pale yellow color. Also a blue-greenish color of the skin of the back and shoulders was noted immediately after injection which disappeared in 30 minutes. Owing to the toxicity of this compound it was not further tested.

3,3′-Bis(Azo-2,6-diaminopyridine)-4,4′-Dihydroxyarsenobenzene.—A solution of 5 Gm. of arsphenamine base in 250 cc. of water and 9 cc. of concentrated hydrochloric acid was cooled to 0° C. by the addition of ice and diazotized by adding 3.0 Gm. of sodium nitrite dissolved in 20 cc. of water. Then 3.0 Gm. of 2,6-diaminopyridine dissolved in 12 cc. of hydrochloric acid and 60 cc. of water was added. The mixture was stirred for ¾ of an hour and then neutralized with sodium bicarbonate. The precipitate so obtained was collected on a Buchner funnel and washed with water. The brown solid was insoluble in water, slightly soluble in dilute hydrochloric acid and readily soluble in aqueous alkali.

Assay: Found, As 24.79%, N 22.94%; calculated for $C_{22}H_{20}O_2N_{10}As_2$, As 24.74%, N 23.10%.

This compound was tested for toxicity by intravenous injection. The solution was prepared by dissolving 1.0 Gm. in 4 cc. of normal sodium hydroxide solution and sufficient water to make solutions of 0.5% to 3.0% concentration. The results of these tests are given in the following table.

These results show that this compound is extremely toxic when injected intravenously since no rats survived even at doses of 5 mg./Kg. This compares unfavorably with a tolerated dose of 200 mg./Kg. for arsphenamine.

The oral toxicity was found to be between 24 and 52 mg./Kg. of body weight.

This compound was also subjected to germicidal and bacteriostatic tests on *B. Typhoid* and *B. Staphylococcus*. The results of these tests are given in Table II.

TABLE I.

Dosage, Mg./Kg.	Concentration of Solution Used, Per Cent.	Number of Rats Injected.	Number of Rats Died.	Survival Time.
5	0.5	2	2	4 to 21 hours
10	0.5	2	2	½ to 3 hours
10	1.0	2	2	2 to 6 hours
20	1.0	3	3	2 to 6 hours
40	1.0	3	3	2 to 5 hours
50	2.0	1	1	14 minutes
100	2.0	1	1	6 minutes
200	3.0	1	1	2 minutes
277	3.0	1	1	30 seconds

TABLE II.

Germicidal Tests.		Bacteriostatic Tests.	
Concentration.	Result.	Concentration.	Result.
Alkaline solution 1–100	Inactive	Alkaline solution 1– 1,000	Active
Alkaline solution 1–500	Inactive	Alkaline solution 1– 5,000	Active
Alkaline solution 1–1000	Inactive	Alkaline solution 1–10,000	Active
Saturated aqueous solution	Inactive	Alkaline solution 1–20,000	Active
		Alkaline solution 1–50,000	Inactive
		Saturated aqueous solution	Active

NOTE: These results apply equally to *B. Typhoid* and *B. Staphylococcus.*

SUMMARY.

1. Two azo derivatives of arsphenamine were prepared by coupling diazotized arsphenamine with metaphenylenediamine and 2,6-diaminopyridine, respectively.

2. These two substances were found to penetrate the tissue of test animals very rapidly, but were too toxic for therapeutic purposes.

3. One of these compounds, 3,3′-bis(azo-2,6-diaminopyridine)-4,4′-dihydroxy-arsenobenzene was found to be bacteriostatic but not germicidal to *B. Typhoid* and *B. Staphylococcus.*

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

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Harvard University has created a new Doctor of Philosophy degree in the "History of Science and Learning." Dr. Conant in explanation of this degree stated that "the history of science, the history of ideas, the history of scholarship and the history of universities should now be occupying the attention of many instead of a few."

Winners of the degree will be required to have a knowledge of six major fields and to master the technique of historical and scientific investigation. Harvard's Graduate School of Arts and Sciences now offers more than a score of courses in various phases of the history of knowledge.