[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL.]

HYPOPHOSPHOROUS ACID PREPARATION OF ARSPHENAMINE. (3,3[1]-DIAMINO-4,4[1]-DIHYDROXY-ARSENOBENZENE DIHYDROCHLORIDE.)[1]

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In the course of our work it was found that the reduction of 3-amino-4-hydroxy-phenyl-arsonic acid by hypophosphorous acid consistently gives arsphenamine of low toxicity. The amino acid was secured most readily by reducing 3-nitro-4-hydroxy-phenyl-arsonic acid with sodium hydrosulfite[2] and then purified by precipitation from a hydrochloric acid solution with sodium acetate. Instead of isolating the arsphenamine base after the reduction of the amino acid, as done by Fargher and Pyman, the reduced solution was poured into hydrochloric acid, thereby precipitating the arsephenamine as the dihydrochloride, which, although difficultly soluble in cold water, conforms to the qualitative tests for arsphenamine outlined in the Public Health Reports.[3]

It has been found that by oxidizing toxic arsphenamine to the 3-amino-4-hydroxy-phenyl-arsonic acid and then reducing this intermediate with hypophosphorous acid arsphenamine of low toxicity was obtained.

Fargher's[4] modification of Lehmann's method was used in the arsenic determinations, and the toxicity of the products was determined by intravenous injection into albino rats according to the standard method adopted by the U. S. Public Health Service.

The amino acid was synthesized by 4 different methods, and the arsphenamine secured from it was, in each case, of low toxicity.

Experimental.

Oxidation of Arsphenamine.[5]—Thirty-five g. of arsphenamine is dissolved in 360 cc. of water, 40 cc. of 10 *N* sodium hydroxide solution is added, and then hydrogen peroxide (3%) slowly. The reaction is quite exothermic, and the solution becomes dark brown. After a while, a precipitate forms which is redissolved by adding a little 10 *N* sodium hy-

[1] This is the first of a series of studies on the properties contributing to the toxicity of arsphenamine being made under a grant from the Interdepartmental Social Hygiene Board to the Harvard Medical School; the work is under the general direction of Dr. Reid Hunt, who is also responsible for the biological tests reported in this paper.

The experimental work reported here was largely completed before the publication of the paper by Fargher and Pyman, *J. Chem. Soc.*, 117, 370 (1920).

[2] Fargher, *ibid.*, 115, 982 (1919).

[3] Myers and Du Mez, *Public Health Reports*, 33, 1004 (1918).

[4] *Loc. cit.*

[5] This method is suggested by Ehrlich and Bertheim, *Ber.*, 45, 756 (1912), but details were not given.

droxide solution. More hydrogen peroxide is now added until the precipitate reappears. This alternate addition of the sodium hydroxide and hydrogen peroxide is continued until a precipitate no longer appears when the oxidizing agent is added. The total quantity of hydrogen peroxide used is about 310 cc., and 14 cc. of 10 *N* sodium hydroxide was added to redissolve the precipitates. The solution is acidified with glacial acetic acid (39 cc.); if a mineral acid is used care must be taken to reach the point when the solution is acid to litmus, but not to congo red, as the amino-hydroxy-arsonic acid is soluble in an excess of mineral acid. Yields, 60% of brown solid.

Purification of Crude 3-Amino-4-hydroxy-phenyl-arsonic Acid.—Ten g. of the crude acid is dissolved in 100 cc. of water plus 8 cc. of hydrochloric acid (1.19), super-filtchar[1] is added and the solution agitated for 15 minutes at room temperature. Heating was found to hinder the decolorization. After filtering, 20% sodium acetate solution (about 90 cc.) is added to the colorless or light yellow filtrate until the latter is no longer acid to congo red. White or slightly pink, minute plates crystallize, and after thorough cooling, they are filtered off and air-dried. The yield is 78%.

Subs., 0.1999: 34.91 cc. of 0.04889 *N* $Na_2S_2O_3$ solution.
Calc. for $C_6H_8O_4NAs$: As, 32.17. Found: 31.99.

Preparation of Arsphenamine.—Twenty-three g. of purified 3-amino-4-hydroxy-phenyl-arsonic acid is dissolved in 736 cc. of water plus 138 cc. of 50% hypophosphorous acid plus 11.5 cc. of 3% potassium iodide solution. The mixture is heated gradually to 55° and maintained at 55–60° for 90 minutes. The deep yellow solution is cooled to 10° and poured with vigorous stirring into 1640 cc. of 1 : 1 hydrochloric acid which has been previously cooled to 2°. The precipitate is filtered and washed with 100 cc. of cold 1 : 1 hydrochloric acid and dried in thin layers *in vacuo* over flake sodium hydroxide. Yield, 98.3% of light yellow powder of high specific gravity.

Subs., 0.2007: 13.38 cc. of 0.1280 *N* $Na_2S_2O_3$.
Calc. for $C_{12}H_{14}O_2N_2Cl_2As_2.H_2O$: As, 32.82. Calc. for $2H_2O$: As, 31.58. Found: 31.99.

The product is yellow, dissolves readily in warm water, and its aqueous solution is clear bright yellow. With cold water it forms a jelly. The material was found to be free from inorganic arsenic and sulfur and conformed to the qualitative tests for arsphenamine.

Arsenic Content.—The average of the analyses of 25 samples prepared by hydrosulfite reduction[2] and Kober's[3] conversion of the base into

[1] A high grade of decolorizing carbon prepared by the Industrial Chemical Co., N. Y., who kindly placed a supply at our disposal.

[2] Ehrlich and Bertheim, *loc. cit.*

[3] THIS JOURNAL, 41, 442 (1919).

dihydrochloride shows 31.30% of arsenic, while the average of the analyses of 10 samples prepared by hypophosphorous acid reduction followed by hydrochloric acid precipitation of the dihydrochloride showed 31.00% of arsenic. Theoretically, arsphenamine with 2 molecules of water of crystallization should contain 31.58% of aresnic.

Toxicity.—The average tolerated dose of 44 samples prepared by hydrosulfite reduction and methyl alcohol-ether[1] or Kober's method of conversion of the base into the dihydrochloride is 100 mg./kg. bodyweight, and the doses ranged from below 60 to 140 mg./kg. The average tolerated dose of 10 samples prepared by the method outlined above is 140 mg./kg. and ranged from 120 to 160 mg./kg.

Reworking of Toxic Arsphenamine.—Four samples of arsphenamine which would not pass the present U. S. P. H. S.[2] requirements were oxidized to the amino-hydroxyphenyl-arsonic acid which, after purification, was reduced to arsphenamine with hypophosphorous acid and precipitated by pouring into 1 : 1 hydrochloric acid.

Ext. No.	Tolerated dose of original.	Tolerated dose of arsphenamine obtained.
51	Below 100 mg./kg.	150 mg./kg.
53	Below 80 mg./kg.	140 mg./kg.
38	Doubtful at 80 mg./kg.	Above 140 mg./kg.
42	Below 80 mg./kg.	140 mg./kg.

In order to try this procedure on a little larger scale, Mr. Lewis I. Nurenberg, chemist in charge of the arsphenamine laboratory of the Massachusetts Health Department prepared arsphenamine by this method from some amino-hydroxyphenyl-arsonic acid which was secured by oxidation of an old toxic lot of arsphenamine and obtained a product which had a tolerated dose of 130 mg./kg. Another batch gave a product which had a tolerated dose of 130–140 mg./kg.

Summary.

1. A method for the preparation of 3,3[1]-diamino-4,4[1]-dihydroxy-arsenobenzene dihydrochloride directly from the aminohydroxyphenyl-arsonic acid has been developed which, in every case in this laboratory, has given a relatively non-toxic product and which, when carried out in another laboratory by other manipulators, gave equally good results.

2. By application of this method toxic arsphenamine has been converted into relatively non-toxic material.

3. Although the product obtained by this method is less readily soluble in water than that obtained by the methyl alcohol-ether method, the use of warm water in dissolving it has not been found to be injurious.

4. The isolation of arsphenamine base and its possible oxidation during manipulation are avoided.

[1] Ehrlich and Bertheim, *loc. cit.*

[2] U. S. Public Health Service.

5. The qualitative tests and the analyses of the product show that it is chemically the same as that obtained by the older method.

6. The source of the amino-hydroxyphenyl-arsonic acid does not affect the toxicity of the arsphenamine obtained from it.

The writer wishes to express his thanks to Dr. Reid Hunt for his interest in the work, his helpful suggestions and his kindness in determining the toxicity of the preparations.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA.]

A BIVALENT NITROGEN DERIVATIVE OF CARBAZOLE.

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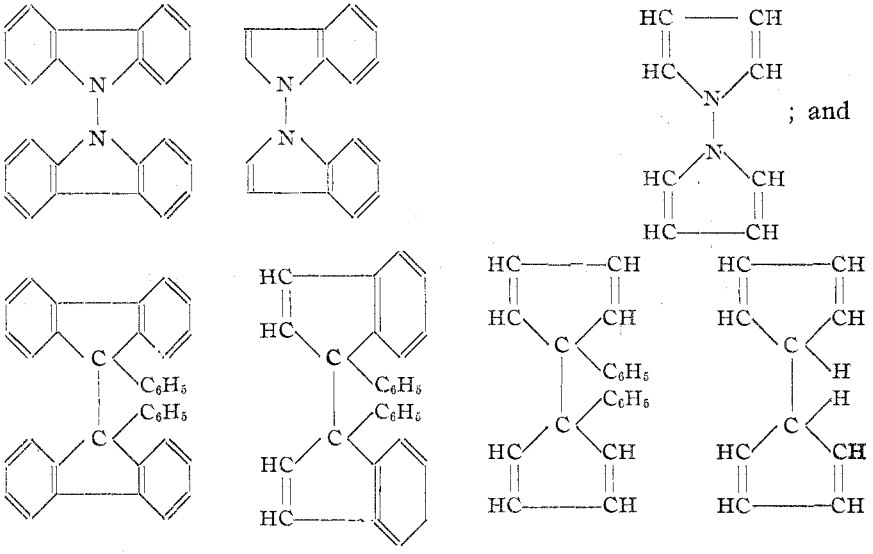
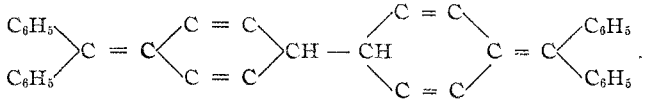
Introduction.

The two series of compounds



are of interest since they vary by graduated steps from compounds closely allied to the tetra-aryl hydrazines and hexa-arylethanes to purely aliphatic types which are similar in form to the quinoid modifications such as



Substances of Type I p. 2406 have been prepared and appear to show some evidence of dissociation, but to a less extent than the corresponding hexa-aryl ethanes. They do not give colored solutions and no dissociation has been shown by molecular weight determinations, but, on the other hand,