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## A STUDY OF ARSENICAL COMPOUNDS RELATED TO ARSPHENAMINE.

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### Introduction.

Experiences of the last decade have shown to the leading syphilologists that arspfenamine, which is the official American name for 3,3'-diamino-4,4'-dihydroxy-arsenobenzene dihydrochloride,<sup>1</sup> is one of the most efficient remedies in the treatment of syphilis. It has been observed by many that intravenous injections of this drug sometimes produce alarming symptoms. While these have been attributed to various causes, some relating to the condition of the patient, and others relating to the technic of administration, it seemed very probable to us that chemical impurities in arspfenamine may produce the disturbing effects. Therefore, we deemed it advisable to prepare a number of compounds, which are closely allied to arspfenamine and which might appear as impurities in the drug in the course of manufacture.

Arsphenamine is produced by the reduction of 3-nitro-4-hydroxy-phenylarsinic acid which sometimes contains the corresponding dinitro derivative as an impurity. It is possible, therefore, that arspfenamine may be contaminated with 3,5,3',5'-tetra-amino-4,4'-dihydroxy-arsenobenzene tetrahydrochloride. In view of this fact, it was of interest to prepare the latter compound and study its chemical and biological properties.<sup>2</sup> We observed also that it exerts a curative influence upon trypanosome infections, hence the preparation and study of derivatives of the above tetra-amino compound suggested themselves.

In the course of this work it was observed that an increase in the number of amino groups decreases the stability of both the phenylarsinic acids and the arsenobenzenes. On the other hand, the solubilities of the corresponding hydrochlorides in water are increased.

**3,5-Diamino-4-hydroxy-phenylarsinic acid.**—This compound has been very briefly mentioned in a German patent,<sup>3</sup> in which the method of preparation consists of the reduction of 3,5-dinitro-4-hydroxy-phenylarsinic acid by means of sodium hydrosulfite. The same compound has been prepared by us according to the following procedure.

Twenty g. of 3,5-dinitro-4-hydroxy-phenylarsinic acid is suspended in 50 cc. of water and dissolved by adding 13 cc. of 40% sodium hydroxide (2 mols.). To this solution is added a solution of 40 g. of magnesium chloride (crystals) in 400 cc. of water,

<sup>1</sup> P. Ehrlich and A. Bertheim, *Ber.*, **45**, 756 (1912).

<sup>2</sup> J. F. Schamberg, G. W. Raiziss, J. A. Kolmer, and J. L. Gavron. The Chemotherapy of Arsenical Compounds Related to Arspfenamine (to be published later).

<sup>3</sup> *D. R. P.*, 224,953. 1910.

the whole cooled down to 0° and reduction effected by gradually introducing 130 g. of sodium hydrosulfite (commercial powder) with continuous mechanical stirring. During this process, the temperature should not be permitted to rise above 2°, since otherwise further reduction to the arseno compound will occur. When all the hydrosulfite has been introduced, the solution becomes decolorized, and upon continued stirring for 1.5 hours at 0° a white crystalline precipitate of the amino compound appears. This is filtered off and the mother liquor expressed as completely as possible. To purify, the crude product is dissolved by triturating with an excess of diluted hydrochloric acid in a mortar. The solution is filtered, and the filtrate neutralized at low temperature with conc. sodium hydroxide solution until it is but slightly acid to congo red paper. The precipitate of the pure amino compound is filtered off and washed with cold water until it is free from sulfate and chloride, then with methyl alcohol, and finally with ether. It is dried *in vacuo* over sulfuric acid. The yield (after one purification) is about 8.0 g. or 50%.

*Properties.*—3,5-diamino-4-hydroxy-phenylarsinic acid is a white crystalline product which darkens during the process of purification. It is soluble in alkalis, also hydrochloric acid; insoluble in methyl alcohol, ethyl alcohol, ether, acetone, chloroform, benzene. Its solution in alkali darkens very rapidly on standing exposed to the air.

Calc. for  $C_6H_7O_4N_2As$ : N, 11.29; As, 30.24. Found: N, 10.70; As, 29.52.

**3-Amino-4-hydroxy-phenylarsinic Acid.**—This compound was first prepared by Ehrlich and Bertheim by the reduction of 3-nitro-4-hydroxy-phenylarsinic acid with sodium amalgam.<sup>1</sup> Another method in which sodium hydrosulfite is used as the reducing agent has been briefly described in a German patent.<sup>1</sup> Jacobs and Heidelberger<sup>2</sup> prepared this same arsenic acid by the use of ferrous sulfate and sodium hydroxide. The authors in preparing this compound used sodium hydrosulfite as the reducing agent.

Twenty g. of 3-nitro-4-hydroxy-phenylarsinic acid is suspended in 50 cc. of water and dissolved by adding 15 cc. of 40% sodium hydroxide solution (2 mols.). A solution of 40 g. of magnesium chloride crystals in 300 cc. of water is added, the whole cooled down to 0°, and reduced by adding 85 g. of sodium hydrosulfite and proceeding exactly as in the case of the previous compound. The yield of purified product is about 10.8 g. or 61%.

*Properties.*—3-Amino-4-hydroxy-phenylarsinic acid is a white crystalline substance which during purification turns slightly brown. It is soluble in hydrochloric acid, sodium and ammonium hydroxides; insoluble in acetic acid and the usual organic solvents. Its alkaline solution darkens rapidly on standing in air.

Calculated for  $C_6H_8O_4NAs$ : N, 6.01. Found: 5.74.

**Acetyl-3-amino-4-hydroxy-phenylarsinic Acid.**—Ten g. of 3-amino-4-hydroxy-phenylarsinic acid is suspended in 50 cc. of water, immersed in an ice-bath, and mechanically stirred. Nine cc. of acetic anhydride (one mol equals 4.4 cc.) is gradually introduced and the stirring maintained for 3 hours. The whole is filtered, and the precipitate suspended in dil. hydrochloric acid. This removes the unchanged amino compound, the acetyl derivative remaining undissolved. This is filtered off, washed with water till free from hydrochloric acid, then with methyl alcohol, and finally ether. It is dried *in vacuo* over sulfuric acid. The yield is 3.5 g. of pure substance.

<sup>1</sup> D. R. P., 224,953, 1910.

<sup>2</sup> THIS JOURNAL, 40, 1580 (1918).

*Properties.*—Acetyl-3-amino-4-hydroxy-phenylarsinic acid is a light brown crystalline substance, soluble in dil. alkalis. It is insoluble in hydrochloric acid, acetic acid and the usual organic solvents.

Calc. for  $C_9H_{10}O_6NAs$ : N, 5.09; As, 27.27. Found: N, 5.10; As, 27.61.

**Diacetyl-3,5-Diamino-4-hydroxy-phenylarsinic Acid.**—This compound is prepared from 3,5-diamino-4-hydroxy-phenylarsinic acid in exactly the same way as the previously described compound. The yield is 75%.

*Properties.*—A pale brown-crystalline substance, soluble in sodium and ammonium hydroxides. Insoluble in water, hydrochloric acid, and the usual organic solvents.

Calc. for  $C_{16}H_{18}N_2O_6As$ : N, 8.43; As, 22.60. Found: N, 8.49; As, 22.98%.

**3,5,3',5'-Tetra-amino-4,4'-dihydroxy-arsenobenzene Tetrahydrochloride.**—Although this amino compound has been very briefly described in a German patent, its tetrahydrochloride has not been described. It is interesting to note that it appears to exist in 2 modifications, one easily soluble in methyl alcohol, and the other only sparingly so.

Twenty-five g. of the corresponding dinitro-hydroxyphenylarsinic acid is dissolved in 525 cc. of water to which has been added 16 cc. of 40% sodium hydroxide solution (2 mols.) Reduction is effected by a solution of 575 g. of sodium hydrosulfite and 65 g. of magnesium chloride in 2600 cc. of water. After 1.5 hours' stirring at 55° to 60° the yellow precipitate of the tetra-amino compound is filtered, washed with distilled water until most of the hydrosulfite has been removed, and then dried on porous plates.

To convert the dried base into its tetrahydrochloride, it is introduced into 220 cc. of absolute methyl alcohol containing hydrogen chloride. About  $\frac{1}{2}$  of the tetrahydrochloride dissolves. This is decanted off. The remaining insoluble portion is triturated in a mortar with more methyl alcohol-hydrochloric acid solution in order to remove the soluble fraction completely. The entire methyl alcohol solution of the tetrahydrochloride is mixed with 5 volumes of U. S. P. ether. A light yellow precipitate is immediately produced. This is allowed to settle, the supernatant fluid decanted, the precipitate washed several times by decantation with ether, rapidly filtered and finally dried *in vacuo* over sulfuric acid. The yield is 8.0 g.

The undissolved fraction of the tetrahydrochloride is washed with ether and also dried *in vacuo* over sulfuric acid. The yield is 7.5 g.

Both modifications are yellow substances easily soluble in cold water. From this solution the base is precipitated by the addition of sodium hydroxide and redissolved when sufficient alkali has been added to form the monosodium salt. This solution on standing rapidly becomes turbid due to the action of the carbon dioxide of the air. A solution of disodium salt is less readily precipitated. Both salts in solution when exposed to the air, rapidly oxidize, more so than the corresponding salts of 3,3'-diamino-4,4'-dihydroxy-arsenobenzene dihydrochloride.

*Analysis.*—Neutralization of the hydrochloride and formation of monosodium salt. Soluble modification: subs., 0.100: 9.0 cc. 0.1 N NaOH. Insoluble modification: subs., 0.100: 7.5 cc. 0.1 N NaOH. Calculated for  $C_{12}H_{18}N_4Cl_4O_2As_2 \cdot 1CH_3OH$ : 8.7 cc. 0.1 N NaOH.

Oxidation by iodine.

Soluble modification. Subs., 0.100: 13.50 cc. 0.1 N iodine.

Insoluble modification. Subs., 0.100: 13.6 cc. 0.1 N iodine.

Calc. for  $C_{12}H_{18}N_4Cl_4O_2As_2 \cdot 1CH_3OH$ : 13.95 cc. 0.1 N iodine; 11.16 mg. oxygen. Found: Sol. mod., 10.80 mg. oxygen; insol. mod., 10.88.

In view of the fact that this compound cannot be recrystallized, the only method of purification consisting of redissolving in methyl alcohol-hydrochloric acid solution and reprecipitating from ether, we are reporting these results as the best we have been able to obtain thus far.

Calc. for  $C_{12}H_{15}N_4Cl_4O_2As_2 \cdot 1CH_3OH$ : N, 9.76; As, 26.13. Found: sol. mod., N, 8.80; As, 26.62; insol. mod., N 8.80; As, 25.09%.

**Tetra-acetyl-3,3',5,5'-tetra-amino-4,4'-dihydroxy-arsenobenzene.**—Two g. of diacetyl-3,5-diamino-4-hydroxy-phenylarsinic acid is dissolved in 7.0 cc. of water by the addition of 4 cc. of 15% sodium hydroxide solution. Two g. of magnesium chloride (crystals), and 10 g. of sodium hydrosulfite are dissolved in 45 cc. of water and immediately mixed with the solution of the arsenic acid. The combined solutions are filtered rapidly, and warmed up to 55 or 60°. This temperature is maintained for 30 minutes, during which time the mixture is continually stirred. The precipitate which forms is yellow at first but at the end of the reduction it appears white. It is filtered off, washed thoroughly with water and dried *in vacuo* over sulfuric acid. The yield is 1.2 g.

The compound is a white powder insoluble in water, dil. hydrochloric acid, and the usual organic solvents, soluble in glacial-acetic acid however. It is soluble in dil. alkalis, forming a yellow solution which is characteristic of arseno compounds; also soluble in dil. sodium hydrogen carbonate solution. Like all other arseno compounds it decolorizes iodine, thereby becoming oxidized to its corresponding arsenic acid. This reaction cannot be utilized for its quantitative estimation, thereby differing from other arseno compounds, because of difficulty in distinguishing the exact end-point.

Calc. for  $C_{20}H_{22}O_6N_4As_2$ : N, 9.93; As, 26.60. Found: N, 10.14; As, 25.75.

**Diacetyl-3,3'-diamino-4,4'-dihydroxy-arsenobenzene.**—The method of preparation of this substance from acetyl-3-amino-4-hydroxy-phenylarsinic acid is the same as that of the tetra-acetyl compound described above. The yield is 73.17%.

The substance is a pale yellow powder insoluble in water, methyl alcohol or sodium hydrogen carbonate solution, soluble in sodium hydroxide solution from which it is reprecipitated by hydrochloric acid.

Calc. for  $C_{16}H_{16}N_2O_4As_2$ , N, 6.22; As, 33.33. Found: N, 6.16; As, 33.00.

### Summary.

The preparation and chemical properties of the following compounds have been studied: 3,5-diamino-4-hydroxy-phenylarsinic acid; 3-amino-4-hydroxy-phenylarsinic acid; acetyl-3-amino-4-hydroxy-phenylarsinic acid; diacetyl-3,5-diamino-4-hydroxy-phenylarsinic acid; 3,5,3',5'-tetra-amino-4,4'-dihydroxy-arsenobenzene-tetrahydrochloride, tetra-acetyl-3,3',5,5'-tetra-amino-4,4'-dihydroxy-arsenobenzene, and diacetyl-3,3'-diamino-4,4'-dihydroxy-arsenobenzene.

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