

seeds are not only rich in protein but also contain a large amount of starch.

### Summary.

From the analyses of navy beans, Alaska garden and Canada field peas, it was found that the largest portion of the nitrogen-free extract is starch. Of the 58.97% of nitrogen-free extract in the 1917 sample of navy beans 35.20% was starch, and 15% was distributed among pentosans, dextrans, cellulose, galactans, and sugars, in the order named. Of the 61.80% of nitrogen-free extract of the 1919 sample, 50.54% was total reducing substances, calculated as starch. In the case of the Alaska garden peas, the nitrogen-free extract was 60.26%, and the starch was 51.21%. The Canada field peas had a somewhat lower content of starch, 45.11%, but the nitrogen-free extract was correspondingly low, 54.47%. The starch content of beans varies from year to year, but in general, the legume seeds investigated were found to contain a large amount of starch.

The completeness of digestion of legumes by malt diastase was greatly enhanced by fine grinding. The increase in digestible starch amounted to from 10 to 12% in the finely ground material. Interfering substances, such as cellulose or protein, are broken up and the starch is exposed to the action of the enzyme. The iodine test for the presence of starch is not a satisfactory means of determining when to stop the digestion. After 10 hours' digestion with several additions of malt extract, the residues of legumes still show a blue coloration. The amount of starch obtained by digesting for 10 hours was but little greater than that found with 4 hours' digestion. The iodine test is sensitive to extremely small quantities of starch, and shows that a trace of this may remain undigested even after 10 hours. It is also possible that some substance other than starch gives the blue coloration.

MADISON, WISCONSIN.

[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY OF THE UNIVERSITY OF CHICAGO.]

## PREPARATION OF 5,5'-MERCURI-BIS-3-NITRO-4-HYDROXY-PHENYL-ARSONIC ACID.<sup>1</sup>

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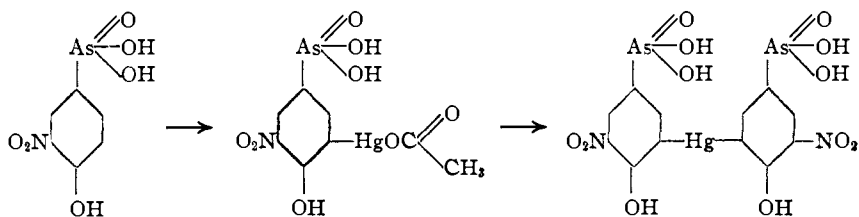
Although arsphenamine and neo-arsphenamine have proved very efficacious in the war on spirochaetes, it has been found most effective to

<sup>1</sup> This investigation was undertaken by Mr. Hanke, Swift Fellow of the University of Chicago, at my suggestion as Special Adviser to the Hygienic Laboratory of the U. S. P. H. S. The coöperation of Dr. Kharasch, National Research Fellow in Organic Chemistry, was invited because of his interest in the theories of the substitution and stability of mercury in aromatic nuclei. As outlined in the text, interesting questions of broader theoretical moment in this field were actually developed.—*J. S.*

<sup>2</sup> The material presented here is used by Martin Hanke in his dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Chicago.

alternate the administration of the arsenicals with mercury preparations. It was with this in mind that the preparation of an arsphenamine containing mercury was undertaken, a compound which would have the effects of both the mercury and the arsenic in the same molecule, and which should have both metals attached directly to carbon. Interesting questions of theory developed in the course of the work, *e. g.*, the replacement of an arsonic acid group by hydrogen in aromatic compounds by means of sodium thiosulfate, and the instability of certain substituted mercury diphenyl derivatives in alkaline solution. The theoretical considerations involved in these reactions will be discussed by one of us (Kh.) in a subsequent paper.

The plan of the work was as follows. 3-Nitro-4-hydroxy-phenyl-arsonic acid was mercurized, forming 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid. This was converted into the corresponding mercury-diphenyl derivative or "bridged" mercury compound, 5,5'-mercuri-bis-3-nitro-4-hydroxy-phenyl-arsonic acid. The reduction of this



last compound to the corresponding arsphenamine derivative, and the preparation of a soluble compound for therapeutic use are still under investigation.

The method of mercurization of the 3-nitro-4-hydroxy-phenyl-arsonic acid was that of Raiziss, Kolmer and Gavron,<sup>1</sup> in which a solution of the acid in 4 mols of sodium hydroxide solution is treated with an equivalent amount of mercuric acetate and a little acetic acid. The amount of acetic acid added has an important relation to the speed of reaction. With the optimum amount of acetic acid, namely one-half equivalent, the reaction is complete in 3.5 hours. Two or three times as much acid has no effect, but the use of smaller amounts of acid greatly prolongs the time of reaction. In one case, where only a few drops of 6 *N* acid was used, the reaction was not complete in over 15 hours.

The importance of a fine adjustment of hydrogen-ion concentration finds justification in the following considerations. In the first place, mercuric oxide is precipitated from mercuric salt solution by alkali, thus reducing tremendously the concentration of mercuric ion, and since, other

<sup>1</sup> Raiziss, Kolmer and Gavron, *J. Biol. Chem.*, **40**, 533-53 (1919).

factors being constant, the rate of mercurization is presumably directly proportional to the concentration of mercuric ion, the presence of too much alkali greatly interferes with the rate of the reaction. On the other hand, mercurization of nitrophenols proceeds much more rapidly in an alkaline than in an acid or neutral medium, due to the formation of the more reactive quinoid form.<sup>1</sup>

Obviously then it is necessary to adjust the hydrogen-ion concentration so that, on the one hand, there is sufficient acid present to hold the mercuric salt in solution, and on the other hand sufficient alkali to form the reactive quinoid nitrophenate. This condition is attained by the use of the quantities given above. The buffer action of the sodium acetate prevents the slight excess of acetic acid from having any deleterious effect.

The next step in this investigation was the preparation of the 5,5'-mercuri-bis-3-nitro-4-hydroxy-phenyl-arsonic acid. This was attempted with the ordinary reagents used for such purposes, namely, sodium thiosulfate and sodium sulfide,<sup>2</sup> *i. e.*, alkaline reducing agents. The reaction itself is of course not a reduction, and the fact that the reagents are reducing agents seems to be incidental. Their fundamental qualification is that they form with mercuric ion very slightly ionized products. The isolation of intermediate products in the cases of sodium thiosulfate and sodium sulfide indicates that the reaction depends upon the formation of some unstable complex intermediate, which breaks down into the mercury-diphenyl derivative.<sup>3</sup>

Our first attempt at connecting the two phenyl groups by mercury—which we will call “bridging” in future in this article—as in “bridging a gap”—was made with sodium thiosulfate. Although the compound sought was not isolated in pure condition, some very interesting observations were made which suggest further investigation. When 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid was boiled with 3 equivalents of sodium thiosulfate for 15 minutes, until a portion of the solution gave a positive test for inorganic mercury, 10% of a non-arsenized<sup>4</sup> mercury compound was isolated, which proved to be 6,6'-mercuri-bis-2-nitrophenol. The main product was a compound, which, when treated with sodium hydroxide, broke down into mercuric sulfide, sodium sulfate,

<sup>1</sup> Hantzsch and Auld, *Ber.*, **39**, 1105 (1906).

<sup>2</sup> Potassium iodide and sodium stannite were avoided since the use of the former resulted in the removal of the arsenic from the molecule, while the latter would have led to the reduction of the nitro group.

<sup>3</sup> See Pesci, *Gazz. chim. ital.*, **29**, I, 394 (1899). Also Fourneau and Vila, *J. pharm. chem.*, **6**, 433 (1912).

<sup>4</sup> This loss of arsenic by thiosulfate treatment is of great theoretical interest, and it also affords a convenient method of replacing an arsonic acid group by a hydrogen atom. The reaction will be further investigated.

and a mercury-diphenyl derivative,<sup>1</sup> from which we inferred that a mercury thiosulfate complex had been formed. Analyses of this product indicate that some of the arsenic had also been lost here. Finally, the fact that the thiosulfate compound contained more than the theoretical quantity of sulfur, and that the mercury diphenyl derivative isolated in the alkaline decomposition always contained sulfur, indicates that some sulfur may have attached itself to the ring as a result of the thiosulfate treatment.

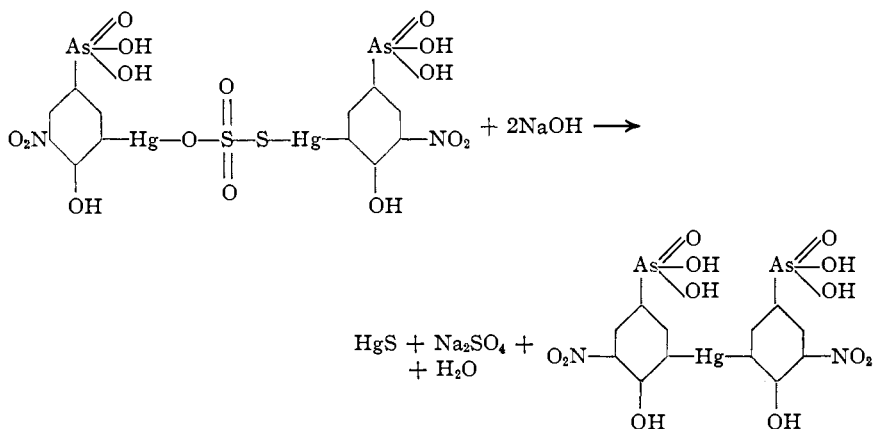
The involved character of the reaction, with its loss of arsenic, introduction of sulfur, and small yield, led us to look for some other method for carrying out the bridging reaction.

We finally decided upon sodium sulfide as bridging agent, being influenced by the researches of Fourneau and Vila,<sup>2</sup> who bridged the mercury derivative of *p*-nitrophenol in this way. The sodium salt of 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid was treated with sodium sulfide. An orange colored precipitate was formed immediately which was not investigated by us, but which in the light of the investigations of Fourneau and Vila is "di-2-hydroxy-3-nitro-5-arsonophenyl-mercuric sulfide." When heated on the water-bath it gradually decomposes into mercuric sulfide and 5,5'-mercuri-bis-3-nitro-4-hydroxy-phenyl-arsonic acid.

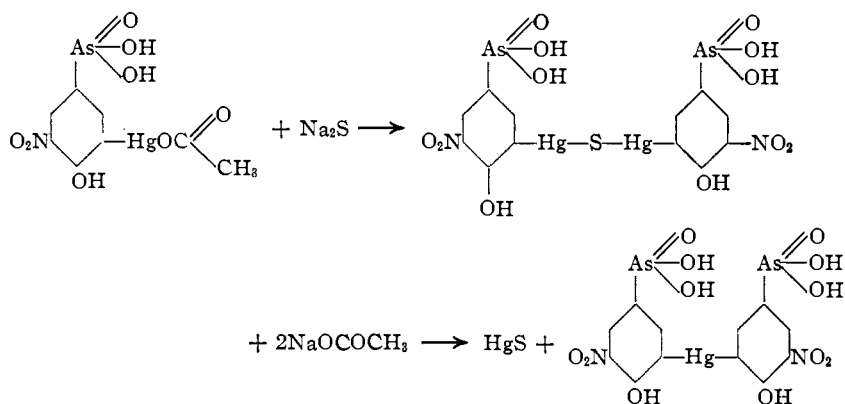
The mercuric sulfide, when dried and weighed, was found to have separated in equivalent amount, but when the product was isolated from the alkaline solution and analyzed, it was found to contain still a large percentage of the original unbridged mercury compound.

Obviously then, it became necessary to develop methods for removing the last traces of unbridged mercury compound, and for detecting its presence by a qualitative test, so that it could be conveniently ascertained

<sup>1</sup> This might happen according to the following equation:



<sup>2</sup> *Loc. cit.*



whether or not the product was completely bridged. For the latter, sodium stannite served as the reagent. When sodium stannite is added to an alkaline solution of our unbridged mercury compound, metallic mercury is precipitated immediately in the cold. If the mercury is completely bridged the solution remains clear. This difference in the stability of mercury compounds of the type R-Hg-X<sup>1</sup> (where X is inorganic) and mercury compounds, R-Hg-R, toward sodium stannite is the basis of our qualitative differentiation of bridged from unbridged compounds. It was found that as little as 0.1 mg. of 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid in 1 cc. of solution when treated with sodium stannite, threw out a perceptible cloud of metallic mercury, while the corresponding bridged compound, 5,5'-mercuri-bis-3-nitro-4-hydroxy-phenyl-arsonic acid, when similarly treated, remained perfectly clear in any concentration.

Sodium hyposulfite acts similarly toward mercury compounds, and for a while we used it to detect the presence of unbridged mercury. It was soon found, however, that it is not nearly so sensitive a test as sodium stannite. It requires 0.6 mg. of 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid in one cc. of solution to produce the faintest perceptible cloud with sodium hyposulfite. Many of our preparations which were negative to hyposulfite, gave a strong stannite test.<sup>2</sup> In these cases quantitative analyses also indicated that the compounds were incompletely bridged. However, some other bridged compounds which had been prepared in this laboratory, which gave excellent analyses, and which also remained clear with hyposulfite, produced a cloud of metallic mercury when treated with sodium stannite. This then indicates that the stannite test is a much more sensitive criterion of the completeness with which an alkali soluble compound is bridged than a quantitative analysis.

With this delicate method of differentiating between bridged and un-

<sup>1</sup> R stands for an aryl radical, either simple or substituted.

<sup>2</sup> Hereafter we shall use the abbreviation, positive and negative stannite test, respectively, to denote whether or not the compound gives a precipitate of metallic mercury, when treated with a solution of sodium stannite.

bridged compounds at our disposal, we could more easily ascertain the exact conditions for carrying the bridging reaction to completion. The method for removing the last traces of unbridged mercury was simply to allow the alkaline solution to react with small amounts of sodium sulfide until the product gave a negative stannite test. If the bridged compound is now isolated, it gives excellent analyses.

There are several conditions which must be carefully fulfilled if the bridging reaction is to be carried out successfully. Most important, as Fourneau and Vila also pointed out, is that the solutions must be concentrated. In fact we could not obtain a completely bridged product, *i. e.*, one giving a negative stannite test, with any amount of sodium sulfide when working in dilute solutions. Then, at least 3 mols of alkali should be used; any other proportion leads to a material reduction in the yield. The reaction should be carried out with all dispatch, for the mercury bridge is opened on continued standing in hot alkaline solution,<sup>1</sup> as is evidenced by the stannite test. On the other hand, the sodium sulfide must not be added in too large quantities, for this also reduces the yield.

In the final precipitation of the bridged compound from the alkaline solution by acid, the immediate addition of a large excess of acid is very important. If the acid is added gradually, the precipitate darkens and the product gives a strong stannite test. The slower the addition of the acid, the greater the decomposition. The decomposition is more pronounced with acetic or oxalic than with sulfuric acid. Prolonged standing in sulfuric acid, however, also causes decomposition. Thus there seems to be a certain range of hydrogen-ion concentration in which the compound is very unstable, and above or below which it is relatively stable.

Preliminary experiments on the reduction of 5,5'-mercuri-bis-3-nitrohydroxy-phenyl-arsonic acid, have been carried out. The results will be published in the next paper.

The position taken by the mercury in the molecule of 3-nitro-4-hydroxyphenyl-arsonic acid was not established by Raiziss, Kolmer and Gavron. From the known rules of substitution they assumed that the mercury oriented *ortho* to the hydroxyl group. However, since the entrance of mercury into the benzene ring is not always in accord with the ordinary rules of substitution,<sup>2</sup> and especially since the effect of an arsonic acid group on the position of substituting groups has not been exhaustively studied, we undertook to prove the position of the mercury. This was done by replacing the mercury and arsenic successively with iodine, and identifying the known di-iodo-*o*-nitrophenol thus obtained. Our results

<sup>1</sup> This observation was made by the authors in August 1920, before their attention was called to the paper of Whitmore and Middleton, read at the meeting of the American Chemical Society in September 1920, in which they reported that mercury-bis-*o*-phenol decomposes in a similar manner under the influence of alkali.

<sup>2</sup> See Dimroth, *Ber.*, **35**, 2032 (1902).

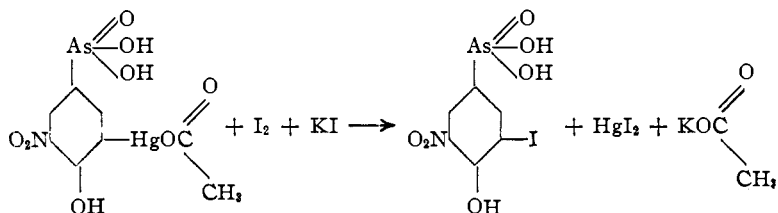
established the correctness of the assumption of Raiziss, Kolmer and Gavron.

### Experimental Part.

**The Preparation of 3-Nitro-4-hydroxy-5-acetoxymercuri-phenyl-arsonic Acid.**—As starting product a preparation of 3-nitro-4-hydroxy-phenyl-arsonic acid<sup>1</sup> was used, which had been employed extensively before in this laboratory in researches on arsphenamine and its derivatives with excellent results, and it was considered suitable for our work without further purification. Analyses of our products bore out the correctness of this assumption.

The method employed was that of Raiziss, Kolmer and Gavron,<sup>2</sup> except that one cc. of glacial acetic acid was used for every 10 g. of the original nitro acid. We also found that the time of reaction was 3.5 hours instead of 1.5 hours, as stated by the authors mentioned.

**Identification of the Position of the Mercury in 3-Nitro-4-hydroxy-5-acetoxymercuri-phenyl-arsonic Acid.**—(1) *Replacement of the Mercury by Iodine and Preparation of 3-Nitro-4-hydroxy-5-iodo-phenyl-arsonic acid.*—



Five g. of 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid was suspended in 120 cc. of water in a 250-cc. glass-stoppered bottle. A solution of 2.6 g. of iodine and 4.0 g. of potassium iodide in 30 cc. of water was added gradually in small portions, with vigorous shaking of the mixture after each addition. After this it was shaken for about 2 hours longer until the color of the iodine was completely discharged. The mixture was filtered, and the nitro-hydroxy-iodo-phenyl-arsonic acid precipitated from the filtrate by the addition of dil. sulfuric acid. The precipitate was collected on a filter and washed with water. For analysis the compound thus obtained was dissolved in sodium hydroxide solution and reprecipitated with sulfuric acid. It was then washed successively with water, alcohol, and ether, and dried *in vacuo* at 100° over phosphorus pentoxide.

*Analyses:* Subs., 0.1658: 16.16 cc. of I<sub>2</sub> soln. (1 cc. of I<sub>2</sub> soln. = 00.001975 g. of As). Subs., 0.2065: AgI, 0.1265.

Calc. for C<sub>6</sub>H<sub>5</sub>O<sub>6</sub>NAs: As, 19.26; I, 32.63. Found: As, 19.29; I, 33.09.

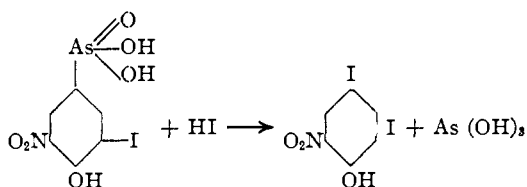
The 3-nitro-4-hydroxy-5-iodo-phenyl-arsonic acid is a very hygroscopic light yellow powder, insoluble in water, alcohol and ether, but soluble in acetone. It is very soluble in dil. sodium hydroxide and sodium carbonate solutions forming deep-red colored solutions.

(2) *Replacement of the Arsonic Acid Group by Iodine<sup>3</sup> and Identification of the 4,6-di-iodo-2-nitro-phenol thus obtained.*—

<sup>1</sup> This product was obtained from the Dermatological Research Laboratories of Philadelphia.

<sup>2</sup> *Loc. cit.*

<sup>3</sup> See Ehrlich and Bertheim, *Ber.*, **40**, 3297 (1907).



One g. of the 3-nitro-4-hydroxy-5-iodo-phenyl-arsonic acid prepared above was dissolved in 0.66 cc. of 15% sodium hydroxide solution (one equivalent) and 3 cc. of water, and a solution of 2 g. of potassium iodide in 2 cc. of dil. sulfuric acid was added. The mixture was heated on the water-bath for 4 hours under a reflux condenser during which time a molten mass appeared at the bottom of the flask. The mixture was cooled and the solid thus formed collected on a filter. It was ground and well mixed with ether, and the ether solution filtered. Any unchanged arsenic compound remained undissolved and was thus removed. The ether solution was allowed to evaporate spontaneously, and the di-iodo-*o*-nitro-phenol thus obtained melted at 95°. One recrystallization from 50% alcohol-ether mixture, raised the melting point to 97°. The melting point was not lowered when a mixture of the compound with some known 4,6-di-iodo-2-nitro-phenol<sup>1</sup> was used.

**Preparation of 6,6'-Mercuri-bis-Nitrophenol.**—To 12 g. of 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid, suspended in 150 cc. of water, a solution of 18 g. of sodium thiosulfate (3 equivalents) was added. The mixture became clear at once. It was then boiled for 15 minutes, at the end of which time a sample portion of the solution gave a positive test for inorganic mercury, when treated with ammonium sulfide. The solution was then allowed to stand overnight. A precipitate separated out, which was collected on a filter, washed successively with water and alcohol, and dried *in vacuo* over sulfuric acid. Yield 0.6 g.

*Analysis:* Subs., 0.2451: 13.26 cc. dry N<sub>2</sub> at 28° and 721.4 mm. Calc. for C<sub>12</sub>H<sub>8</sub>O<sub>6</sub>N<sub>2</sub>Hg: N, 5.88. Found: 5.62.

The compound is of a light yellow color. It is soluble in sodium hydroxide solution, imparting to the latter a red color. It is insoluble in water and most common organic solvents. It gives a negative stannite test. It does not melt when heated to 295°.

The position of the mercury in this compound was established by replacing the mercury by iodine in the usual manner. A compound was thus obtained which melted at 110°. The melting point also was not lowered when a mixture of this compound with known 6-iodo-*o*-nitrophenol (m. p. 110°) was used.

**Preparation of 5,5'-Mercuri-bis-3-nitro-4-hydroxy-phenyl-arsonic Acid.**—Four g. of 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid was suspended in 30 cc. of water, and 6 cc. of 15% sodium hydroxide solution (3 equivalents) was added. The mixture assumed a deep red color but did not become clear entirely until it was warmed. Two cc. of 1 *N* sodium hydrosulfide<sup>2</sup> solution was then added and the resulting mixture allowed to react on the steam-bath for about 1/2 hour. During this time the orange precipitate which had formed as a result of the sulfide addition, gradually decomposed leaving a clear red liquid overlying a black residue of mercuric sulfide. An additional 2 cc. of sodium hydrosulfide was then added, and the mixture allowed to react on the steam-bath for 1/2 hour longer. The alkaline solution, although having separated an

<sup>1</sup> This was prepared by the treatment of *o*-nitro-phenol with iodine. See Körner, *Jahresber.*, 1867, 617.

<sup>2</sup> For the preparation of this solution H<sub>2</sub>S was passed in excess into 1 *N* NaOH solution.



equivalent amount of mercuric sulfide by this time, still gives a positive stannite test, showing the presence of considerable quantities of unbridged compound. The addition of sodium hydrosulfide was continued in 0.5 cc. portions every 15 minutes, until a portion of the clear supernatant liquid gave a negative stannite test. Three or four such extra additions were found to be necessary.

The alkaline solution was filtered hot and the mercuric sulfide washed with hot water, and finally with hot dil. sodium sulfate<sup>1</sup> solution, the total volume of the filtrate and washings being kept down to about 100 cc. Ammonium sulfate crystals were now added in excess, and as the ammonium sulfate dissolved, the ammonium salt of the bridged compound was salted out as golden-yellow flakes. The mixture must be allowed to stand for several hours, or better, overnight, to insure complete crystallization. Cooling does no good because it lowers the solubility of the ammonium sulfate, while warming must be avoided lest decomposition take place. Repeated stirring is advantageous. The precipitate was collected on a filter and washed repeatedly with saturated ammonium sulfate solution. Polysulfides and an unmercurized arsenic-nitro-phenol passed into the filtrate.

The washed ammonium salt was now dissolved in about 200 cc. of water, and a little ammonium hydroxide, and the resulting solution filtered. The clear liquid was heated to boiling, and a large excess of dil. sulfuric acid added at once with constant stirring. A translucent gel of greenish tinge was formed, which soon changed to a heavy granular yellow powder. The latter was transferred to a filter, washed well with water and alcohol, and dried *in vacuo* at 100° over phosphorus pentoxide. Yield, 78%.

*Analyses:* Subs., 0.3596: 12.54 cc. dry N<sub>2</sub> at 23.7° and 736.9 mm.

Subs., 0.1701: 17.65 cc. I<sub>2</sub> soln. (1 cc. I<sub>2</sub> soln. = 0.00199 g. As).

Subs., 0.4483: HgS, 0.1447.

Calc. for C<sub>12</sub>H<sub>10</sub>O<sub>12</sub>N<sub>2</sub>As<sub>2</sub>Hg: N, 3.87; As, 20.69; Hg, 27.68. Found: N, 3.89; As, 20.66; Hg, 27.82.

The compound is a yellow powder, very hygroscopic, and insoluble in all organic solvents. An alkaline solution remains perfectly clear when treated with sodium stannite. Like other compounds of this type, it has no melting point.

### Summary.

1. The position of the mercury in the compound obtained by mercurizing 3-nitro-4-hydroxy-phenyl-arsonic acid has been established. It is *ortho* to the hydroxyl, *i. e.*, in position 5.

2. The preparations of new compounds, 5,5'-mercury-bis-3-nitro-4-hydroxy-phenyl-arsonic acid and 3-nitro-4-hydroxy-5-iodo-phenyl-arsonic acid, are described.

3. A method of differentiating between alkali-soluble mercury compounds of the type R-Hg-R and R-Hg-X is given. Alkaline sodium stannite does not react with the former but does with the latter, giving a precipitate of metallic mercury.

4. A non-arsenized mercury compound was isolated, as one of the products, when 3-nitro-4-hydroxy-5-acetoxy-mercuri-phenyl-arsonic acid was boiled with sodium thiosulfate. It was proved to be 6,6'-mercuri-bis-2-nitrophenol.

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<sup>1</sup> Hot water and an electrolyte are used to keep the mercuric sulfide from passing into the filtrate as a colloid.