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

SOME DERIVATIVES OF ARSPHENAMINE[1]

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The Preparation of Arsphenamine Polyarsenide

In a previous communication from this Laboratory,[2] the preparation of arsphenamine polyarsenides by reduction of a mixture of 3-amino-4-hydroxyphenylarsenious oxide and sodium arsenite with hypophosphorous acid is described. The arsenic content of the product varies from 47.3 to 53.7% depending upon the ratio of the organic arsenical to the sodium arsenite prior to the reduction. In spite of the fact that these specimens contain $^2/_3$ as much arsenic again as arsphenamine, they are tolerated in doses as large as 140 mg./kg.; many samples of arsphenamine are not tolerated at this dose.

It was shown[3] that the toxicity of aromatic amino arseno compounds in general, when prepared from nitro aryl arsonic acids by reduction with sodium hydrosulfite, can be made to vary considerably depending upon the conditions under which the nitro group is reduced, and two sets of conditions for reduction of the nitro group have been developed so that, while using the same quantities of all reagents in both cases, the arsphenamine will be tolerated (a) in fairly large doses and (b) in only rather small doses.[4] An investigation of the sulfur compounds in arsphenamine evinced that the more toxic samples prepared by improper reduction of the nitro group contain approximately 2% of sulfur, whereas when the most favorable conditions are employed the sulfur content is only 0.8%.[5] A more recent study[6] proved that when a certain amount of acetic acid is present while 3-nitro-4-hydroxyphenylarsonic acid is being reduced under the most favorable conditions by commercial hydrosulfite to arsphenamine base, the yield is increased without any alteration in the toxicity, but that when too much acid is employed the toxicity of the product suddenly increases.

A study of the production of arsphenamine polyarsenide by reduction of 3-nitro-4-hydroxyphenylarsonic acid and sodium arsenite with sodium

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[1] This is the 13th of a series of studies on the properties contributing to the toxicity of arsphenamine being made under a grant from the United States Interdepartmental Social Hygiene Board to the Harvard Medical School; the work is under the general direction of Doctor Reid Hunt.

[2] Christiansen, THIS JOURNAL, **43**, 373 (1921).

[3] Christiansen, *ibid.*, **43**, 2202 (1921).

[4] Ref. 3, p. 2207.

[5] Christiansen, THIS JOURNAL, **44**, 848 (1922).

[6] Christiansen, *ibid.*, **45**, 1319 (1923).

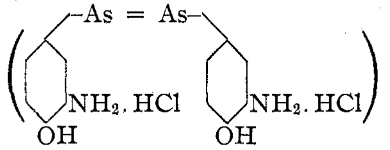
hydrosulfite shows clearly that the relations found between the mode of synthesis and the toxicity of arsphenamine as summarized above hold true in this case also. Tabulation of the results brings out the differences very distinctly.

TABLE I

THE RELATION BETWEEN THE MODE OF SYNTHESIS AND THE TOXICITY OF ARSPHENAMINE POLYARSENIDE

Substances reduced	Reducing agent	Expt.	Tol. dose Mg./kg.	As content %	S content %
3-Amino-4-hydroxy-phenylarsenious oxide and sodium arsenite[2]	H_3PO_2	3	140	50.81	
			140	47.31	
			140	53.72	
3-Nitro-4-hydroxy-phenylarsonic acid and sodium arsenite.........	$Na_2S_2O_4$ (*a*)	2	120	44.76	
			120	43.76	1.78
	(*b*)	1	80	41.87	2.38
	(*c*)	1	<80	45.55	

(*a*) The nitro group was reduced under the most favorable conditions.
(*b*) The nitro group was reduced under the least favorable conditions.
(*c*) An excess of acetic acid was present during the reduction.

The arsenic determinations show that the composition of arsphenamine polyarsenide is very variable, and the substance may be best represented by the formula  As_n. When $n = 2$ the calculated arsenic content is 50.9%. One of the factors controlling n is the ratio between the quantities of arsonic acid and the sodium arsenite used.

Experimental Part

A. To a vigorously stirred, cold (8°) solution of 10.6 g. of magnesium chloride hexahydrate in 260 cc. of water, 50 g. of sodium hydrosulfite is added, and after about 30 seconds a cold (8°) solution of 2 g. of 3-nitro-4-hydroxyphenylarsonic acid in 46 cc. of water containing 0.66 g. of sodium hydroxide is added gradually during 1 to 1.5 minutes. Two g. of decolorizing carbon is added, and the material, after being heated rapidly to 30°, is filtered. A solution of 0.9 g. of arsenious oxide in 45 cc. of water containing 0.37 g. of sodium hydroxide is added to the clear filtrate, and the solution is heated rapidly with stirring to 55° and maintained at 55–60° for 90 minutes. The arseno compound separates as a finely divided, brownish-yellow solid. The base is removed by filtration and, after it is washed and dried in a vacuum over sodium hydroxide, is converted into the dihydrochloride by dissolving it in 10.5 cc. of absolute methyl alcohol containing a slight excess over 2 moles of dry hydrogen chloride. Filtration will not remove some of the very finely divided insoluble matter, but centrifuging for 5 or 10 minutes produces a clear, bright red alcoholic solution which is poured into 110 cc. of ether previously cooled to 0–5°. After this is filtered, washed with ether and dried in a vacuum over sodium hydroxide, 1.4 g. of a reddish-orange arsphenamine polyarsenide is obtained which dissolves very readily in water forming a clear, deep red solution. The product is tolerated in doses of 120 mg./kg. and contains 44.2% of arsenic (the average of 2 experiments) and 1.78% of sulfur.

An aqueous solution of the polyarsenide is slightly acid to litmus, gives an orange-colored precipitate of the sulfate when treated with sodium sulfate, and an orange-colored precipitate of the free base when sodium acetate or carbonate is added; these precipitates redissolve in sodium hydroxide forming clear, red solutions. When the aqueous solution is added to concd. hydrochloric acid or to acetone, the dihydrochloride is reprecipitated. When iodine is added to the red aqueous solution, the material is oxidized and the red color is destroyed. In alkaline solution, the compound is oxidized by hydrogen peroxide and the red color disappears.

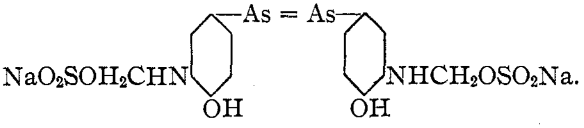
B. All of the reagents are used in the same quantities as in A. The dilute, warm (30°) aqueous magnesium chloride solution is stirred very slowly, and immediately after the hydrosulfite has been added, the warm (30°) nitro solution is added very rapidly (5 seconds). The solution is stirred rapidly for about 1 minute to dissolve the remainder of the hydrosulfite completely and then is filtered. The arsenite solution is added, and after the mixture is heated slowly to 55° and stirred vigorously the experiment is continued as in A; yield, 1.4 g. of reddish-orange polyarsenide which dissolves very readily in water to give a clear, red solution. The product is tolerated in doses of only 80 mg./kg. and contains 41.87% of arsenic and 2.38% of sulfur.

C. This experiment is conducted as described in A, but after the arsenite solution has been added 4 cc. of glacial acetic acid is added; yield, 1.5 g. of reddish-orange polyarsenide which is very readily soluble in water, forming a clear, red solution and which contains 45.55% of arsenic. It is not tolerated in doses of 80 mg./kg.

The toxicity determinations were made by injecting 2% alkalinized solutions intravenously into albino rats. The solutions were prepared by adding 4 molecular equivalents of *N* sodium hydroxide solution to an aqueous solution of the dihydrochloride.

Sulfarsphenamine

Recently the production of sulfarsphenamine, a compound similar to neo-arsphenamine, has been described and, owing to its low toxicity, therapeutic efficiency, stability in aqueous solution and the fact that it can be injected subcutaneously, it may become quite important in the treatment of syphilis.[7] Voegtlin and Johnson prepared this substance by condensing arsphenamine with 2 molecular equivalents of formaldehyde and adding 4 equivalents of sodium bisulfite to the condensation product; the following formula has been assigned to it.



In a few cases 3 and 6 molecular equivalents of the aldehyde and bisulfite, respectively, were used.

When an attempt was made to prepare this substance by following Voegtlin and Johnson's directions, using 2 moles of formaldehyde and 4 moles of bisulfite per mole of arsphenamine, the precipitate formed by the addition of bisulfite redissolved only partially; consequently, the yield

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[7] Voegtlin and Johnson, This Journal, **44**, 2573 (1922).

was very low. This was found to be due to incomplete condensation between the arsphenamine and the formaldehyde. However, when 3 moles, that is 50% excess of the aldehyde are used, the initial precipitate redissolves completely when only 2 moles of bisulfite have been added. The reaction product which is isolated when 3 moles of aldehyde and 2 moles of bisulfite are employed is not tolerated in doses above 250 mg./kg. and contains less than 1 sulfur atom per atom of arsenic; in this case complete reaction has not been obtained between the bisulfite and the formaldehyde condensation product. By adding another mole of bisulfite after the initial precipitate has redissolved complete reaction is obtained, and the product is tolerated in doses of 350–400 mg./kg. It seems that 3 moles of formaldehyde and 3 moles of bisulfite are much more favorable than 2 and 4 moles, respectively, and the use of bisulfite in excess of the necessary amount, namely, 3 moles, increases the opportunity for contamination of the product with bisulfite and does not improve the product in any noticeable way.

Voegtlin and Johnson lay great stress upon the quality of the bisulfite and state that it must be pure and freshly prepared from sodium carbonate and sulfur dioxide. This is entirely unnecessary and the sulfarsphenamine made from the purified sodium bisulfite which is on the market is the equal in every respect of that secured by the use of freshly prepared, pure bisulfite.

Moreover, it is unnecessary to prepare arsphenamine, that is, the dihydrochloride, to start with. When the dried base obtained by the reduction of 3-nitro-4-hydroxyphenylarsonic acid with sodium hydrosulfite is dissolved in water by the addition of a small amount of hydrochloric acid, this solution serves just as well as one prepared from the dihydrochloride itself.

These two changes, namely, the use of commercial bisulfite and of arsphenamine base instead of the purified dihydrochloride, simplify the production of sulfarsphenamine and lower the cost of its preparation considerably.

Also, sulfarsphenamine that will be tolerated in doses of 350–400 mg./kg. can be prepared from toxic arsphenamine, that is, arsphenamine that is not tolerated at 70 mg./kg., and which was prepared by improper reduction[3] of the nitrohydroxy acid.

In Table II, the results of a series of experiments are recorded on which the above conclusions are based. Three molecular equivalents of formaldehyde were used in each experiment.

When the comparatively toxic arsphenamine that has been secured by improper reduction of the nitro acid with hydrosulfite is converted into sulfarsphenamine, the initial precipitate formed by the addition of the bisulfite after the condensation with formaldehyde redissolves in less than 40 seconds, which is very much more rapidly than when relatively non-toxic arsphenamine is used. Since the arsphenamine in this case contains about 2% of sulfur, most of which is present as a sulfonic acid derivative of arsphenamine, it is to be expected that the resulting sulfarsphenamine would have a larger ratio of sulfur to arsenic than when the original material contains little sulfur.

Table II

The Preparation of Sulfarsphenamine

		Sulfarsphenamine			
Arsphenamine Tol. dose about	$NaHSO_4$ Mol.	As %	S %	As:S	Tol. dose Mg./kg.
100	2[a]	24.48	8.89	1:0.85	<250
100	2[b]	25.11	9.34	1:0.87	250
100	2[b]	24.75	9.48	1:0.901	250
100	3[b]	21.83	10.96	1:1.17	400 or above
100	3[b]	21.99	11.10	1:1.18	400
100	4[b]	20.80	11.13	1:1.26	350
140 or above[e]	3[b]	21.55	11.16	1:1.21	400
70[c]	3[b]	19.36	10.97	1:1.33	350–400
Arsphenamine base[d]	3[b]	21.55	11.16	1:1.21	400

[a] Freshly prepared, pure bisulfite.

[b] Commercial, pure bisulfite.

[c] The arsphenamine was prepared by improper reduction of the nitro acid with hydrosulfite.

[d] This base is the same as that from which *e* was prepared.

[e] Compare *d*, above.

Since sulfarsphenamine should contain 25.1% of arsenic, the material obtained by these reactions is only about 87% pure. To determine the effect, if any, that the impurities have on the toxicity, some of the crude sulfarsphenamine which was tolerated at 350 mg./kg. was converted into the free acid[7] by treatment with acetic acid. The dry, purified acid was reconverted into the sodium salt by solution in the calculated quantity of sodium carbonate and precipitation with alcohol. The sodium salt then contained 25.9% of arsenic and was tolerated in doses of 300–350 mg./kg. Therefore, the impurities in crude sulfarsphenamine are comparatively inert and have little bearing on the toxicity of the substance.

Experimental Part[8]

A. Two and one-half g. of arsphenamine is moistened with 4 cc. of alcohol, dissolved in 32 cc. of water with the aid of mechanical stirring, and 1.25 cc. of formalin (about 37% formaldehyde) is added. After 1 minute, 3.3 cc. of a solution of commercial sodium bisulfite (3.7 g. of bisulfite in 10 cc. of solution) is added; the precipitate that forms gradually redissolves, and after 10 minutes 1.6 cc. more of the bisulfite solution is added. The stirring is continued for 20 minutes, and then, after filtration, the clear, orange-yellow solution is poured into 5 volumes of alcohol. The sulfarsphenamine is separated,

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[8] The experimental procedure is, in general, that reported by Voegtlin and Johnson[7] but owing to the changes in quantities and time, which seem advisable as a result of experiments in this Laboratory, the entire process is outlined again for the sake of clarity.

washed with 95% alcohol and absolute alcohol, and dried in a vacuum over sodium hydroxide; yield, 3 g. of sulfarsphenamine that is very readily soluble in water, forming a clear, orange-yellow solution; it contains arsenic and sulfur in the ratio of 1: 1.19. The product is tolerated in doses of 400 mg./kg.

B. A suspension of 2.1 g. of arsphenamine base in 32 cc. of water is stirred mechanically and treated with 0.9 cc. of concd. hydrochloric acid (approximately 2 moles). As soon as the base has dissolved completely, the experiment is continued as in A. In this case the solution commences to become viscous about 30 seconds after the aldehyde has been added, whereas in A this never occurs in less than 1 minute. The bisulfite is added as soon as marked signs of viscousness appear. Three g. of cream-colored sulfarsphenamine is obtained, which dissolves readily in water forming a clear, deep yellow solution and which contains arsenic and sulfur in the ratio of 1:1.21. The product is tolerated in doses of 400 mg./kg.

The toxicity of these preparations was determined by intravenous injection of 6% solutions into albino rats.

During large scale work in the Arsphenamine Laboratory of the Massachusetts State Health Department, the same trouble was encountered as in this Laboratory in obtaining satisfactory results with the quantities recommended by Voegtlin and Johnson. Since the adoption of the changes in quantities herein recommended, these difficulties have been avoided. Also, comparatively large quantities of sulfarsphenamine have been prepared from toxic and non-toxic arsphenamine using commercial bisulfite and the product was tolerated in doses of 400 mg./kg.

A Polyarsenide of Sulfarsphenamine

When arsphenamine polyarsenide is subjected to the procedure used in preparing sulfarsphenamine, a very soluble sodium salt is obtained which may be called sulfarsphenamine polyarsenide. When the bisulfite is added, after the arsphenamine polyarsenide has been condensed with formaldehyde, an orange-colored precipitate forms that redissolves very quickly. The dried sodium salt is an orange-colored powder that dissolves in water forming a deep red solution. Although the toxicity of arsphenamine polyarsenide differs very little from that of arsphenamine, the polyarsenide of sulfarsphenamine is not tolerated in doses even as low as 200 mg./kg., whereas sulfarsphenamine itself is tolerated at 400 mg./kg.

Summary

Arsphenamine polyarsenide may be obtained by reduction of 3-nitro-4-hydroxyphenylarsonic acid and sodium arsenite with sodium hydrosulfite, and the toxicity of the product depends on the mode of reduction just as does that of arsphenamine. In spite of its high arsenic content as compared with arsphenamine, its toxicity differs but little from that of the latter substance.

By modifying the quantities of reagents certain difficulties encountered while preparing sulfarsphenamine by the original method have been overcome. It is unnecessary to use freshly prepared, pure sodium bisulfite

in this reaction; commercial bisulfite suffices. Also, it is unnecessary to start with arsphenamine, that is, the dihydrochloride; equally good results can be secured by starting with dry arsphenamine base.

As the impurities present in the crude sulfarsphenamine have little bearing on the toxicity, it is unnecessary to purify the material.

Arsphenamine polyarsenide can be converted into a polyarsenide of sulfarsphenamine, but this substance is comparatively toxic.

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THE ARSONATION OF PHENOL[1]

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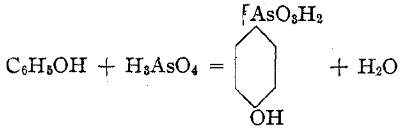
In synthesizing arsphenamine from aniline or phenol by any of the common methods,[2] all of the reactions except that in which the arsenical group is introduced into the benzene nucleus progress smoothly, and excellent yields (75% or over) are easily obtained. Owing to the low yields obtained when aniline or phenol is arsonated with arsenic acid, the cost of production of the final product is greatly influenced by this reaction. Cheetham and Schmidt,[3] by modifying the conditions for arsonating aniline, secured recrystallized arsanilic acid in 26% yields, whereas the yields obtained by previous methods averaged approximately 15%. The arsonation of phenol with arsenic acid has been discussed in two papers; one by Conant[4] and the other by Jacobs and Heidelberger,[5] and in both cases the yield of hydrated sodium *p*-hydroxyphenylarsonate is given as approximately 20%. Conant suggests that by proper adjustment of the experimental conditions, it should be possible to raise the yield to about 30%.

For the production of sodium *p*-hydroxyphenylarsonate in this Laboratory, the method of Jacobs and Heidelberger was adopted but the yield averaged only 18% instead of 20. The main reaction is expressed by the equation



$$C_6H_5OH + H_3AsO_4 = C_6H_4(AsO_3H_2)(OH) + H_2O \quad (1)$$

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[1] This investigation was undertaken in connection with a study of arsphenamine which was made under a grant from the United States Interdepartmental Social Hygiene Board to the Harvard Medical School; the work was under the general direction of Dr. Reid Hunt.

[2] For a brief outline of these methods see Lythgoe, *Chem. Age*, **28**, 390 (1920).

[3] Cheetham and Schmidt, This Journal, **42**, 828 (1920).

[4] Conant, *ibid.*, **41**, 431 (1919).

[5] Jacobs and Heidelberger, *ibid.*, **41**, 1440 (1919).