tion to the bromonitrocamphane. No bromonitrocamphane hydrate is formed, as was supposed by Forster.

3. Potassium hypobromite may also act as an oxidizing agent on camphoroxime to produce hydroxy-nitrosocamphane.

4. Bromonitrocamphane anhydride has been prepared by an improved method and the structure of the anhydride elucidated by its behavior with the Grignard reagent, methyl magnesium iodide. Since the structure of the anhydride is now an established fact, a logical explanation is provided by the unexpected transition from bromonitrocamphane to infra-campholenenitrile.

5. In general, this work fits in with and substantiates the work of Forster on these compounds.

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[CONTRIBUTION FROM THE DIVISION OF PHARMACOLOGY, HYGIENIC LABORATORY, UNITED STATES PUBLIC HEALTH SERVICE]

THE PREPARATION OF SULFARSPHENAMINE[1]

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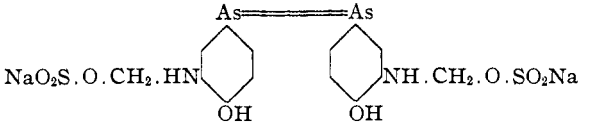
Received June 12, 1922

We have designated as sulfarsphenamine an organic arsenical prepared from arsphenamine (hydrochloride of 3,3′-diamino-4,4′-dihydroxy-arsenobenzene), formaldehyde and sodium bisulfite. This compound is not a pure chemical substance, but when prepared according to the following directions, it is fairly uniform in arsenic, sulfur content and biological behavior. From the method of preparation of sulfarsphenamine and the elementary analysis of its free acid the drug would appear to consist principally of an arsenobenzene derivative of the following constitution.

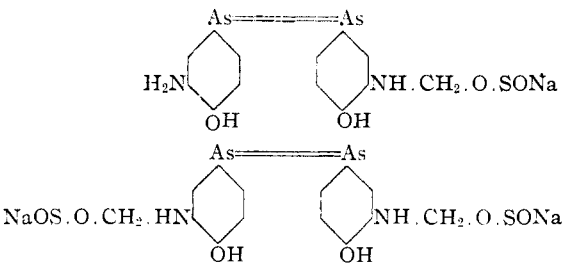


According to Bertheim[2] and Raiziss and Falkov[3] and others, neoarsphenamine may be considered as a mono- or disubstitution product of arsphenamine, or as a mixture of the mono- and disubstitution products, depending on the conditions of manufacture. The probable constitution of the mono- and disubstitution products follow.

[1] Approved for publication by the Surgeon General.

[2] Bertheim, "Handbuch der organischen Arsenverbindungen," Stuttgart, **1913**.

[3] Raiziss and Falkov, *J. Biol. Chem.*, **46**, 210 (1921).



Sulfarsphenamine, therefore, very closely resembles neoarsphenamine, and differs from the latter especially in that the substituting radical in the amino group contains 3 atoms of oxygen instead of 2, being an ester of sulfurous acid.

The preparation is carried out in two principal stages: (1) formaldehyde is allowed to react upon arsphenamine with the formation of a condensation product having the formaldehyde attached to both amino groups (formaldehyde imide derivative), and (2) the formation of a sulfurous acid ester salt by the addition of sodium bisulfite to the formaldehyde imide compound.

The formaldehyde imide derivative of arsphenamine was not isolated, but the formation of formaldehyde imides (Schiff's bases) obtained by the interaction of aromatic primary amines and formaldehyde is so well known that there is little reason to doubt the formation of such an intermediate product, especially as Abelin and Perelstein[4] and others have shown that similar aromatic aldehyde imides react with solution bisulfite to form compounds of the type, $R.NH.CH_2.O.SO_2H$.

Reinking, Dehnel and Labhardt[5] have described a simple test to distinguish compounds containing the group, $\equiv C.O.SO_2Na$, from compounds possessing the group, $\equiv C.O.SONa$. Aqueous solutions of the latter type of compounds reduce indigo carmin whereas the former require treatment with zinc and acetic acid before they give the test. We have found that the indigo carmin test can be used to distinguish sulfarsphenamine from neoarsphenamine. An aqueous solution of neoarsphenamine[6] will decolorize indigo carmin in a few minutes if gently heated, yielding a yellow solution, whereas sulfarsphenamine does not reduce the dye under the same conditions. If, however, an aqueous solution of sulfarsphenamine is first treated with zinc and acetic acid, the filtrate will reduce indigo carmin upon short heating to a yellow solution. Arsphenamine does not reduce indigo carmin. This test, therefore, yields additional

[4] Abelin and Perelstein, *Ann. Chem.*, **41**, 216 (1916).

[5] Reinking, Dehnel and Labhardt, *Ber.*, **38**, 1069 (1905).

[6] We have also found that the free acid obtained from neoarsphenamine by treatment with hydrochloric acid (according to Raiziss and Falkov) reduces indigo carmin, thus showing that the reduction of the dye obtained with neoarsphenamine cannot be attributed altogether to the admixture of free sodium sulfoxylate.

evidence to the effect that sulfarsphenamine contains a side chain, —NH.-CH_2.O.SO_2Na, whereas neoarsphenamine contains the side chain, —NH.-CH_2.O.SONa.

A German patent (No. 249,726) describes a product which, it is claimed, represents the monosubstitution product, only one of the amino groups of arsphenamine being substituted by the formaldehyde and bisulfite. This product is described as a red-brown mass and is prepared by treating arsphenamine-base suspended in water with formaldehyde and sodium bisulfite on a steam-bath. Numerous attempts to prepare this substance have failed in our hands and we believe that the substance described in the patent is not identical with sulfarsphenamine.[7]

The principal reason for reporting the preparation of sulfarsphenamine is the fact that extensive animal experimentation carried out in this laboratory and which will be published elsewhere has demonstrated that this drug is suitable for subcutaneous injection and that it is very effective in the treatment of protozoal diseases; furthermore, its aqueous solutions are remarkably stable and do not increase in toxicity on standing in contact with air for 24 hours. These properties of sulfarsphenamine make it more than probable that this drug will play an important part in the treatment of syphilis. The drug is now being tested clinically.

Preparation.—Lot 1420. To 50 g. of arsphenamine (1 mol.) is added 75 cc. of 95% alcohol, and the mixture is stirred thoroughly. The drug is then dissolved at about 20° by the addition of 675 cc. of water and the use of a mechanical stirring device. After complete solution is obtained, 18.15 cc. of 33.8% formaldehyde (2 mol.) is added rapidly with vigorous stirring and 69 seconds later 65 cc. of 32.64% sodium bisulfite (2 mol.) at once. The light yellow precipitate, which forms immediately with the simultaneous liberation of sulfur dioxide, gradually redissolves as the stirring is continued. After 7 minutes another 65 cc. of the same bisulfite solution (2 mol.) is added and stirring is continued for 17 minutes. A very slight amount of undissolved material is separated and the dark orange solution poured in a fine stream under vigorous stirring into 4040 cc. of 95% alcohol. The light yellow precipitate is filtered off, washed with 95% alcohol, followed by absolute alcohol, and the product is dried in a vacuum desiccator over sodium hydroxide; yield, 64 g.

Several conditions must be observed for the successful preparation of sulfarsphenamine: (1) the solution of arsphenamine must be complete before the formaldehyde is added; alcohol facilitates the rapidity of solution, and also has a tendency to prevent the formation of a jelly after the addition of formaldehyde; (2) sufficient time should elapse to allow the formaldehyde to react with arsphenamine, usually 60 seconds, but not enough time to cause the formation of a viscous mass which will not

[7] Under the name of sulfarsenol, a product of French manufacture has been put on the market. The manufacturers of sulfarsenol have not published anything concerning the method of its preparation or composition, but claim that it is the monosubstitution product, having only one of the amino groups of arsphenamine substituted by formaldehyde bisulfite.

readily dissolve upon the addition of the bisulfite; (3) the bisulfite should be added in 2 portions, each portion being added rapidly and with an interval of 7 minutes between the additions; (4) the formaldehyde and bisulfite solutions should be analyzed for their strength; (5) the sodium bisulfite must be prepared freshly from sodium carbonate and sulfur dioxide; (6) 2 mol. of formaldehyde and 4 mol. of sodium bisulfite for each mol. of arsphenamine are usually satisfactory, although 3 mol. and 6 mol., respectively, seem to be more favorable proportions; (7) best results in the precipitation of sulfarsphenamine are obtained by adding the final solution in a fine stream to 5 volumes of 95% alcohol; (8) the drug is best dried by treatment with absolute alcohol on the filter, ether being unsatisfactory for drying.

For the calculation of the molecular proportions of arsphenamine, formaldehyde and bisulfite, the actual arsenic content of arsphenamine was taken as a basis and the strength of formaldehyde and bisulfite was determined quantitatively. The arsenic estimation was carried out according to Lehmann's method.[8] The sulfur estimation of sulfarsphenamine was made by ashing the sample with a mixture of 7 parts of sodium carbonate to 1 part of potassium nitrate in an electric furnace at approximately 350°, dissolving the ash in hydrochloric acid by means of prolonged slight boiling. The sulfate was precipitated as barium sulfate and weighed. Nitrogen was determined by the Kjeldahl method. The hydrogen-ion concentration was estimated by means of bromophenol blue and methyl red; the colors of these indicators are not destroyed by sulfarsphenamine, thus making the results fairly reliable. The osmotic pressure was estimated by the freezing-point method.

Preparation and Analysis of the Free Acid of Sulfarsphenamine

Pure arsphenamine was prepared from pure 3-amino-4-hydroxy-phenylarsonic acid by the method of Christiansen.[9] Analysis of the product showed that it had an arsenic content of 29.87%, and contained no sulfur.

Five g. of this arsphenamine was converted in the manner described in this article into sulfarsphenamine. After the precipitation by alcohol and filtration on a Büchner funnel, the product was dissolved in a small amount of water and precipitated with a large excess of glacial acetic acid. It was centrifuged, washed with glacial acetic acid and water, and drained each time in the centrifuge. The free acid so formed was dried in a vacuum desiccator over soda lime until all traces of acetic acid were gone; yield, 5.8 g.

Analyses. Subs., 0.1850, 0.222: H_2O, 0.0700, 0.0780; CO_2, 0.1860, 0.2220. Subs. 0.2000, 0.2000: 5.99, 5.79 cc. of 0.1 *N* acid (Kjeldahl). Subs., 0.2000, 0.2000: $BaSO_4$, 0.1420, 0.1430. Subs., 0.2000, 0.2000: 11.99, 12.04 cc. of 0.1 *N* $Na_2S_2O_3$ (Lehmann).

Found: C, 27.43, 27.28; H, 4.23, 3.93; N, 4.20, 4.13; S, 9.75, 9.82; As, 22.47, 22.57.

Ratio: As : S = 1 : 1.02; As : N = 1 : 0.98; As : 7 C = 1 : 1.09; As : 8 H = 1 : 1.69.

[8] Meyers and Du Mez, *U. S. Pub. Health Reports*, 33, 1012 (1918).

[9] Christiansen, THIS JOURNAL, 42, 2042 (1920).

The compound, therefore, appears to have the constitution given in the introduction (free acid instead of the sodium salt), with 3 or 4 molecules of water of crystallization.

The accompanying table includes the data of 18 different lots of sulfarsphenamine prepared. Lot 1413 was prepared from an arsphenamine of French origin, all the other lots being made from arsphenamine of four of the principal American manufacturers of this drug.

TABLE I

DATA ON SULFARSPHENAMINE

Lot	Arsphenamine used	HCHO	$NaHSO_2$	Yield	N	As	S	At. ratio	P_H 1% soln.	Atm. of osmotic pressure	
	G.	Mol.	Mol.	G.	%	%	%	As : S		5% sol.	10% sol.
1357[a,b]	5	2	4	7.6	2.78	16.49	13.53	1 : 1.92	4.4		
1364[b]	5	2	4	5.9	3.93	21.67	12.13	1 : 1.31	4.0		
1366[b]	5	2	4	5.5	4.12	22.45	12.14	1 : 1.26	3.8		
1389[b]	50	2	4	61.0	4.00	21.80	11.68	1 : 1.25	3.6	2.59	4.75
1390[b]	10	2	4	5.4	3.85	23.81	11.47	1 : 1.12	4.4		
1391[b]	50	2	4	63.0	4.22	22.24	11.04	1 : 1.16	3.2	2.47	4.21
1393[c]	5	2	4	6.0	4.09	23.47	10.85	1 : 1.08	4.4		
1395[b]	5	2	4	4.0	4.04	23.74	10.22	1 : 1.01	4.4		
1398[b]	5	2	4	5.0	3.90	23.16	10.82	1 : 1.09	4.4		
1399[c]	5	2	4	6.0	4.11	23.60	10.95	1 : 1.08	4.4		
1400[b]	30	2	2	36.0	4.25	22.32	8.72	1 : 0.91	3.2	2.41	4.64
1413[b]	10	2	4	11.9	3.92	21.31	11.21	1 : 1.23	4.2		
1414[b]	7	2	4	7.2	4.13	23.05	10.89	1 : 1.10	4.6		
1415[b]	4.8	2	4	3.8	4.00	22.06	10.85	1 : 1.15	4.6		
1420[b]	50	2	4	64.0	3.95	21.68	10.48	1 : 1.13	3.8	2.87	5.54
1426[b]	10	3	4	14.5	3.53	19.40	11.99	1 : 1.44	3.8		
1428[b]	10	3	6	11.4	3.89	21.68	10.48	1 : 1.22	4.6		
1451[b]	10	3	6	13.0	3.63	20.77	12.88	1 : 1.45	4.6		

[a] For the precipitation of this lot 10 volumes of alcohol were used, instead of 5 volumes. This accounts for the high sulfur content, which is probably in part in the form of $NaHSO_3$.

[b] 15 H_2O solvent.

[c] 1.5 alcohol + 13.5 H_2O solvent.

Summary

The preparation, chemical compostion and physical properties of a derivative of 3,3′-diamino-4,4′-dihydroxyarsenobenzene made by treatment of this substance with formaldehyde and sodium bisulfite is described. This substance will be known under the name sulfarsphenamine.

WASHINGTON, D. C.