

XLVI.—*The Composition of Salvarsan.*

By ROBERT GEORGE FARGHER and FRANK LEE PYMAN.

COMMERCIAL salvarsan is prepared by the reduction of 3-nitro-4-hydroxyphenylarsinic acid by means of sodium hyposulphite, solution of the base in methyl alcohol containing hydrogen chloride, and precipitation of the salt by means of ether (Ehrlich and Bertheim, *Ber.*, 1912, **45**, 756).

It is a yellow, amorphous compound slightly variable in chemical composition and more so in physical properties; chemically, it is generally regarded as 3:3'-diamino-4:4'-dihydroxyarsenobenzene dihydrochloride containing combined solvent, but published statements on the details of its composition contain many discrepancies.

When salvarsan was first introduced into general use, the makers, Farbwerke vorm. Meister, Lucius & Brüning, stated that it contained about 34 per cent. of arsenic. In 1911, however, Gaebel (*Apoth. Zeit.*, 1911, **26**, 215) found that it lost 7.6 per cent. of its weight on heating, and contained only 31.2 or 31.8 per cent. of arsenic. He communicated these results to Ehrlich, with the result that the later circulars of the issuing firm bore the corrected statement: "the arsenic content of the preparation corresponds to the formula  $C_{12}H_{12}O_2N_2As_2 \cdot 2HCl, 2H_2O$ ."

*The Solvent Content of Salvarsan.*

Ehrlich and Bertheim (*loc. cit.*) have given the results of analysis of a specimen of salvarsan prepared, not by the technical method, but by reducing 3-amino-4-hydroxyphenylarsenious oxide with sodium amalgam and acetic acid, as follows:

Found: C=32.63; H=4.06; N=6.06; As=31.99; Cl=15.41.  
 $C_{12}H_{12}O_2N_2As_2 \cdot 2HCl, MeOH$  requires C=33.1; H=3.8; N=6.0;  
 As=31.85; Cl=15.07.  
 $C_{12}H_{12}O_2N_2As_2 \cdot 2HCl, 2H_2O$  requires C=30.3; H=3.8; N=6.0;  
 As=31.6; Cl=14.9 per cent.

On determining methyl alcohol in 2 grams of this sample, they obtained a "distinctly positive" reaction, and this fact, in conjunction with the percentage of carbon in the compound, led them to put forward the first of the above formulæ for 3:3'-diamino-4:4'-dihydroxyarsenobenzene dihydrochloride prepared in this way.

Recently, Kober (*J. Amer. Chem. Soc.*, 1919, **41**, 442) has claimed that there is no justification for Gaebel's assumption of  $2H_2O$  in salvarsan, and is clearly of opinion that the combined

solvent in salvarsan, precipitated from methyl alcohol by means of ether, is methyl alcohol.

Our experiments show that this view is erroneous. On analysis of two samples of salvarsan precipitated from methyl-alcoholic solution by means of ether, we obtained the following figures:

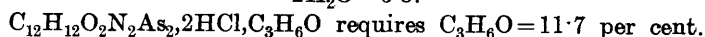
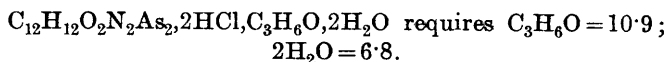
- (A) C=29.5; H=4.0; Cl=12.3; S=2.89; As=30.9; ash=0.43; loss in a vacuum=7.6.  
 (B) C=29.2; H=4.1; Cl=12.0; S=2.76; As=31.0; ash=0.79; loss in a vacuum=7.7 per cent.

Determination of the ratio C to As gives the results 5.97 to 1 and 5.89 to 1 respectively, which show that the total carbon content of the product is accounted for by the six carbon atoms of the benzene nucleus attached to each atom of arsenic.

These results contradict Kober's assumption that the combined solvent in salvarsan consists of methyl alcohol, and are confirmed by direct determinations of the methyl alcohol by the method described by Simmonds (*Analyst*, 1912, **37**, 16), and later modified by Jones (*ibid.*, 1915, **40**, 218), when percentages of methyl alcohol varying from nil to 1.4 per cent. were found.\*

It may be noted here that when salvarsan is precipitated from methyl-alcoholic solution by means of acetone, the product contains one molecular proportion of acetone, which cannot be removed in a vacuum at the ordinary temperature, and, beyond this, the usual amount of water which can be removed in a vacuum. The acetone was estimated by distilling the compound with water and determining the volume of N/10-iodine required to convert the acetone in the distillate into iodoform.†

Mean values found: In air-dried substance, acetone=10.9; loss in a vacuum=6.1; in dried substance, acetone=11.2.



\* The method consisted in distilling from an aqueous solution of salvarsan (1 in 20 to 1 in 40), half its volume of liquid and determining methyl alcohol in the distillate. Experiments on the delicacy of the reaction confirmed the figures given by Jones (*loc. cit.*), and indicated that in our experiments as little as 0.2 per cent. of methyl alcohol, expressed in terms of the salvarsan employed, should have been readily detected.

† This method of estimation is the subject of a recent communication by L. F. Goodwin (*J. Amer. Chem. Soc.*, 1920, **42**, 39), who includes a summary of previous literature. The results confirm our experiments on the accuracy and delicacy of the estimation.

*The Sulphur Content of Salvarsan.*

The presence of a considerable proportion of sulphur in commercial salvarsan—not disclosed in the reputed formula—calls for comment. Ehrlich and Bertheim (*Ber.*, 1911, **44**, 1264; 1912, **45**, 762) mention that crude salvarsan base, prepared by means of hyposulphite, contains inorganic matter and slight quantities of a substance containing arsenic united with sulphur, but indicate that these impurities are removed by converting the base into the hydrochloride. Myers and Du Mez (*U.S.A. Public Health Reports*, 1918, **33**, 1003), and Kober (*loc. cit.*), have recently recorded the bare fact that commercial salvarsan contains sulphur.

Several years ago the present authors (compare Medical Research Committee, *Special Report Series*, No. 44, "Reports of the Special Committee upon the Manufacture, Biological Testing, and Clinical Administration of Salvarsan and its Substitutes," No. 1, p. 8) found that commercial salvarsan of both British and German origin invariably contained sulphur, of which the amount varied from 1 to 2 per cent., and, exceptionally, up to 3 per cent. In our opinion, the sulphur occurs at least in part in the form of the sulphamo-group,  $\cdot\text{NH}\cdot\text{SO}_3\text{H}$ . Our reasons for this view are as follows:

(1) It is well known that the reduction of nitro-compounds with hyposulphite in alkaline solution leads, sometimes in part and sometimes wholly, to the formation of sulphamic acids of the type  $\text{R}\cdot\text{NH}\cdot\text{SO}_3\text{H}$ . Karrer (*Ber.*, 1915, **48**, 1061) states that "nitro-carboxyphenylarsinic acid does not give the normal arseno-derivative when reduced with hyposulphite, but gives an easily soluble substance, probably a sulphamic acid, as has frequently been observed in other cases." Moreover, in the somewhat analogous case of 3:5-dinitro-4-aminophenylarsinic acid, an alkali-soluble reduction product containing sulphur has been obtained which, on treatment with warm acids, yields free sulphur dioxide and salts of hexa-aminoarsenobenzene (E.P. 8041 of 1913). Although no constitution is assigned in the patent, Giemsa (*Deut. med. Woch.*, 1919, **45**, 95) describes the product as a monosulphamic acid of hexa-aminoarsenobenzene,  $\text{HSO}_3\cdot\text{NH}\cdot\text{C}_6\text{H}_2(\text{NH}_2)_2\cdot\text{As}_2\cdot\text{C}_6\text{H}_2(\text{NH}_2)_3$ .

(2) Whilst Ehrlich and Bertheim's analysis of pure, as distinct from commercial, salvarsan shows a chlorine content of 15.4 per cent., we have found considerably less chlorine in commercial salvarsan of both British and German origin, the content of samples taken direct from sealed ampoules varying generally from 12.5 to 14.5 per cent. It is thus always lower than that calculated for  $\text{C}_{12}\text{H}_{12}\text{O}_2\text{N}_2\text{As}_2\cdot 2\text{HCl}\cdot 2\text{H}_2\text{O}$ , which requires  $\text{Cl}=14.9$  per cent.,

and, moreover, is usually lower than that necessitated by the neutralisation value (that is, the number of parts of sodium hydroxide required to neutralise 100 parts of salvarsan to phenolphthalein). The difference between the chlorine found on estimation and the quantity corresponding with the acidity of salvarsan must be due to some other acid. The fact that salvarsan forms a sparingly soluble sulphate led us to test for the presence of sulphate in the product, but only negative results, or, at most, indications of traces, were obtained. In view of the evidence of the formation of sulphamic acids in analogous cases, we believe that that part of the acidity of salvarsan which is not due to the hydrochloric acid is due to the presence of the sulphamo-group.

Determinations of sulphur, chlorine, and neutralisation value in ten consecutive preparations, with the results deduced from them, are given below. They are typical of many others.

S.	Cl.	Neut. val.	Cl calculated from neut. val.	Difference in chlorine.	Whence S as NH·SO <sub>3</sub> H.	Other S.
1·7	14·0	16·45	14·57	0·57	0·51	1·19
1·3	14·1	16·25	14·40	0·30	0·27	1·03
1·26	14·06	16·35	14·50	0·44	0·40	0·86
1·48	13·5	16·36	14·51	1·01	0·91	0·57
1·36	13·8	16·34	14·49	0·69	0·62	0·74
1·44	13·7	16·42	14·54	0·84	0·76	0·68
1·47	13·06	16·45	14·57	1·52	1·37	0·10
1·51	13·66	16·47	14·59	0·93	0·84	0·67
1·77	13·23	16·21	14·35	1·12	1·01	0·76
1·74	13·51	16·35	15·50	0·99	0·89	0·85

The possibility, in the case of salvarsan, of the transformation of the sulphamic acid into a true sulphonic acid would seem to be precluded by the conditions of the experiment, which, from a study of the literature, do not appear to favour the change.

The formation of an internal imide,  $R \cdot NH \cdot SO_2 \cdot NH \cdot R$ , receives, at first sight, support from the low neutralisation values sometimes encountered, but these are more probably due to partial hydrolysis of the dihydrochloride. We have found that reprecipitation of the hydrochloride from methyl-alcoholic solution leads to a product containing rather less chlorine, whilst the chlorine content, when low, is raised by reprecipitation in presence of a further quantity of hydrogen chloride.

Thus, 8·9 grams of 3-nitro-4-hydroxyphenylarsinic acid were reduced in the usual manner, and the resulting base was dissolved in 100 c.c. of methyl alcohol containing 1·13 grams of hydrogen chloride. From a part of the solution, salvarsan was precipitated immediately by the addition of ether (fraction A). To 25 c.c. of the remainder a further quantity of 0·2 gram of hydrogen chloride

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was added, and the liquor kept overnight before precipitation (fraction *B*). Both fractions were dried under the same conditions and analysed. Found, in *A*, Cl=13.6; in *B*, Cl=14.9 per cent.

Another instance is supplied by a commercial sample of high sulphur content. It contained Cl=12.3; S=3.1 per cent. 1.2 Grams were kept overnight in 10 c.c. of methyl alcohol containing 0.2 gram of hydrogen chloride, and then precipitated by ether. The product contained 13.5 per cent. of chlorine and 2.8 per cent. of sulphur.

(3) Many attempts, both synthetic and analytic, were made to isolate the compound containing sulphur in acid form. Analysis of the commercial hyposulphite employed in the reduction revealed the presence of sulphate, sulphite, thiosulphate, and sulphide as impurities containing sulphur, these, with the exception of sulphide, having been recognised previously by Binz and Sondag (*Ber.*, 1905, **38**, 3830). Increase of the proportion of each in the reduction mixture was without material effect on the sulphur content of the product, and since it was found that the reduction of 3-amino-4-hydroxyphenylarsinic acid with hyposulphite led to a substance containing relatively little sulphur (0.34 per cent.), it was concluded that the acidic sulphur originates during the reduction of the nitro-group by the hyposulphite, a conclusion in harmony with our view of its mode of combination.

Eventually, however, we found that commercial salvarsan, and indeed all specimens prepared by the reduction of 3-nitro-4-hydroxyphenylarsinic acid with hyposulphite, gradually separated to some extent from concentrated methyl-alcoholic solution, this property being much less marked in the preparation obtained by reduction of the amino-acid by hyposulphite and absent in that prepared by the reduction of the amino-acid by hypophosphorous acid. Preliminary examination of the precipitates showed that they contained a greater proportion of sulphur than the parent substance, and were practically insoluble even in excess of methyl alcohol. The isolation of this sparingly soluble portion in a state of comparative purity was accomplished with some difficulty. Ten grams of a specimen of commercial salvarsan (containing Cl=13.7; S=1.0; As=33.0 per cent.) were dissolved in 50 c.c. of methyl alcohol and kept overnight in an inert atmosphere. The deposit was separated centrifugally, very thoroughly washed with methyl alcohol, and dried in a vacuum. It amounted to 0.4 gram of a bright yellow powder, somewhat sparingly soluble in water and insoluble in methyl alcohol. On analysis, it gave the following results:

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Found: Cl=6.84; S=5.1; As=30.8\*; neutralisation value=15.5.\*

The combination of a low chlorine content, a high sulphur content, and a normal neutralisation value prove the presence of acidic sulphur. It may be pointed out, further, that the product approximates in composition to the monohydrochloride of the mono-sulphamic acid of 3:3'-diamino-4:4'-dihydroxyarsenobenzene, the formation of which would be expected in view of Bertheim's statement (*Chem. Zeit.*, 1914, **38**, 756) that a mixture of two arsenic acids gives on reduction only the unsymmetrical arsenobenzene.

$C_{12}H_{12}O_5N_2SAs_2.HCl$  requires Cl=7.3; S=6.6; As=31.1 per cent.; neut. val., 16.6.

The remainder of the sulphur is probably attached to arsenic, this mode of combination being indicated by the fact that the distillate from an acid solution of salvarsan often contains traces of hydrogen sulphide, which can be detected by lead acetate and other reagents, whilst 3-amino-4-hydroxyphenylarsenic trisulphide (D.R.-P. 253157) appears to give up at least part of its sulphur as hydrogen sulphide on boiling with dilute hydrochloric acid.

It should be remembered, however, that salvarsan has been shown to possess some of the properties of a colloid (compare Karrer, *Ber.*, 1919, **52**, [B], 2319), and it is therefore possible that a portion of the sulphur unaccounted for by the sulphamo-group may be present in physical association with the compound.

*The Preparation of 3:3'-Diamino-4:4'-dihydroxyarsenobenzene Dihydrochloride.*

During the progress of the foregoing experiments, it became necessary to prepare pure, as distinct from commercial, salvarsan.

The reduction of 3-amino-4-hydroxyphenylarsenious oxide to the arsenobenzene by means of sodium amalgam in acetic acid had already been described by Ehrlich and Bertheim (*loc. cit.*), and this and several other modifications involving the reduction of 3-nitro-4-hydroxyphenylarsinic acid step by step were investigated.

The first series of experiments involved the preliminary reduction of the nitro-group without affecting the arsenic acid group, leading to 3-amino-4-hydroxyphenylarsinic acid. This had been accomplished by Ehrlich and Bertheim (*loc. cit.*) by means of sodium amalgam, and we employed this process until it became

\* Owing to the small amount of substance available these results are only approximate.

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evident that, on repeated use, without elaborate purification, the mercury became contaminated with arsenic and the yield of the amino-acid showed progressive diminution. Several other processes, such as cautious reduction with zinc in acid solution, reduction with ammonium sulphide, and reduction with ferrous hydroxide in alkaline solution, were investigated, and eventually we drew the conclusion that the most satisfactory method was to treat the nitro-acid in cold aqueous alkaline solution with just sufficient hyposulphite to reduce the nitro-group (D.R.-P. 224953; Fargher, T., 1919, 115, 982). The crude product was contaminated with sulphur, but purification as described by Ehrlich and Bertheim (*loc. cit.*) effected the complete removal of this impurity, an important factor in view of our further experiments.

The reduction of 3-amino-4-hydroxyphenylarsinic acid to the corresponding arsenobenzene was readily accomplished by means of hypophosphorous acid, as follows: Five grams of the acid were dissolved in 25 c.c. of hypophosphorous acid (D 1.15) and 25 c.c. of water, 0.1 gram of potassium iodide being added as a catalyst. The resulting solution was heated to 60° for two hours in an atmosphere of carbon dioxide, cooled, made slightly alkaline with 10 per cent. aqueous sodium carbonate, the precipitated base filtered, washed very completely with water, converted into the hydrochloride by solution in methyl alcohol containing hydrogen chloride, precipitated by dry ether, collected, and dried for several hours in a vacuum. The yield amounted to 3.7 grams. The following figures were obtained on analysis:

Found: As=31.8, 31.84; Cl=14.15; loss in a vacuum=6.38.

In the substance dried at 60° in a vacuum:

Found: C=33.3, 33.0; H=3.6, 3.4; N=6.5.

$C_{12}H_{12}O_2N_2As_2, 2HCl$  (439.0) requires C=32.9; H=2.8; N=6.4 per cent.

The base dissolved less readily in methyl alcohol containing hydrochloric acid than did that obtained by the reduction of 3-nitro-4-hydroxyphenylarsinic acid with sodium hyposulphite. The dihydrochloride, grey or very pale yellow in colour, was less readily soluble in water or methyl alcohol than the commercial product. Its solution in three parts of methyl alcohol gave no precipitate on keeping out of contact with air. It decomposed at 187° (corr.).

The reduction of the amino-acid by sodium hyposulphite was also undertaken, the product, in most of its properties, resembling commercial salvarsan rather than the pure substance. It differed, however, considerably in the readiness with which it precipitated

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from concentrated methyl-alcoholic solution, and contained only 0.34 per cent. of sulphur. Other figures obtained on analysis indicated a normal composition.

The second series of experiments, conducted by the converse process, namely, reduction of 3-nitro-4-hydroxyphenylarsinic acid to 3:3'-dinitro-4:4'-dihydroxyarsenobenzene and subsequent reduction of the nitro-groups, was less successful.

The first stage was readily accomplished by means of hypophosphorous acid essentially as described in D.R.-P. 269886, although the somewhat elaborate precautions taken in isolating the product to ensure freedom from the corresponding diamine were found to be superfluous, since the reduction of the nitro-group by the addition of potassium iodide to the reduction mixture (D.R.-P. 271894) could not be confirmed.

Owing to its insolubility, dinitrodihydroxyarsenobenzene could not be reduced with sodium amalgam in acetic acid solution, and although in alkaline solution reduction took place readily, the product was unsatisfactory. The base obtained by reducing dinitrodihydroxyarsenobenzene in alkaline solution with sodium hyposulphite proved somewhat difficult to filter and wash. The hydrochloride prepared from it differed but little from commercial salvarsan.

In conclusion, we are glad to have the opportunity of recording that this work was carried out in collaboration with the technical experts of Messrs. Burroughs Wellcome & Co., and to thank especially Dr. H. A. D. Jowett and Mr. F. H. Lees, F.I.C., for their valuable help and advice in the course of the work.

THE WELLCOME CHEMICAL RESEARCH LABORATORIES,

LONDON, E.C. 1.

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