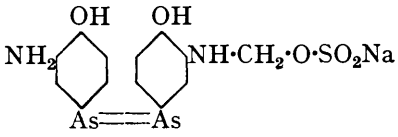
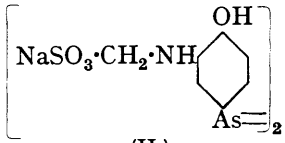
237. *The Constitution of Sulpharsphenamine.*

By W. J. C. Dyke and Harold King.

Sulpharsphenamine is defined by the British Pharmacopœia 1932 as a product which may be prepared by the action of formaldehyde and sodium hydrogen sulphite on 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene dihydrochloride (salvarsan). The first reference to a substance of this type is to be found in D.R.P. 249726 (Farbwerke vorm. Meister Lucius and Brüning, 1911), according to which 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene (salvarsan base) is warmed with formalin (40%) and sodium hydrogen sulphite solution (40%) until all the base is in solution. On addition of hydrochloric acid an acid is liberated containing only one methylenesulphite radical. In 1919 Lévy-Bing, Lehnhoff-Wyld, and Gerbay (*Annales Maladies Vénériennes*, **14**, 520) described a substance called sulfarsenol, and gave it the constitution of a mono-*N*-methylenesulphite of salvarsan base (I), but in the literature accompanying the ampoules later than 1923 it is shown as an *NN*′-dimethylenesulphite of salvarsan base (II).



(I.)



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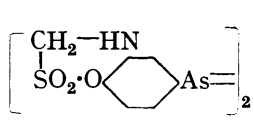


(III.)

A substance to which the same structure (II) was given, and which was called sulpharsphenamine, was described in detail by Voegtlin, Johnson, and Dyer (*U.S. Pub. Health Rep.*, 1922, **37**, 2783), and by Voegtlin and Johnson (*J. Amer. Chem. Soc.*, 1922, **44**, 2573). According to these authors, salvarsan was treated in aqueous solution with formaldehyde (2 mols.; 40%) for about 60 secs., and then sodium hydrogen sulphite solution (4 mols.; 40%) was added in two portions, and the mixture stirred until solution had been effected. The product was then poured into alcohol. The formaldehyde was supposed to form an intermediate formaldehyde-imide, which then formed an ester salt with the sodium hydrogen sulphite analogous to the well-known methylenesulphurous acid derivatives of aniline, R·NH·CH_2·O·SO_2Na (Abelin and Perelstein, *Annalen*, 1916, **411**, 216). Analysis of a large number of batches made with slight variations in the procedure showed that the atomic ratio As : S varied between 1 : 1 and 1 : 1·45. On addition of glacial acetic acid to a concentrated aqueous solution of sulpharsphenamine, a free acid was said to have been obtained with a ratio As : S = 1 : 1·02. According to Newbery and Phillips (J., 1928, 116), this product is probably not the free acid but a sodium salt. Christiansen (*J. Amer. Chem. Soc.*, 1923, **45**, 2184) gave improved practical details for the manufacture of sulpharsphenamine, and found that, using 2 molecular proportions of sodium hydrogen sulphite in its preparation, a product with an atomic ratio As : S = 1 : 0·87 was obtained, but with 4 molecular proportions the ratio became 1 : 1·26. In 1925 Elvove (*U.S. Pub. Health Rep.*, **40**, 1235) introduced a valuable means for differentiating between sulpharsphenamine and neoarsphenamine (neosalvarsan), for he found that iodine in alkaline solution oxidised nearly all the sulphur of neoarsphenamine to sulphate, whereas in sulpharsphenamine less than one-half was so oxidised. To account for the latter results, he supposed that organically bound sulphur in sulpharsphenamine was not oxidised by

iodine in alkaline solution, but that any uncombined sodium formaldehydebisulphite, or any unknown combination which is unstable under the conditions of the assay and behaves like sodium formaldehydebisulphite, was oxidised to sulphate. On this basis, sulpharsphenamine was regarded as a mixture of Voegtlin and Johnson's 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene-*NN*′-dimethylenesulphite and varying proportions of free sodium formaldehydebisulphite.

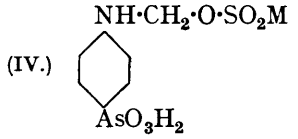
In 1928 an important contribution to the subject was made by Newbery and Phillips (*loc. cit.*), who made the surprising discovery that, when salvarsan base was condensed at 60° with preformed sodium formaldehydebisulphite, the products, described by them as mono- or di-*N*-methylenesulphurous acid substitution products of salvarsan base, contained a type of sulphur linkage which was completely oxidised to sulphate by Elvove's procedure. On the other hand, it was found that when the hydrochlorides of various anilines and aminophenols were condensed with formaldehyde and sodium hydrogen sulphite in succession at room temperature, in aqueous solution, it was only in the case of *o*-aminophenol and its derivatives that a partial differential oxidation of sulphur by Elvove's method was observed. In these cases the significant fact emerged that the atomic ratio of arsenic to non-oxidised sulphur was approximately unity. Furthermore, mild acid hydrolysis at 60—70° of sulpharsphenamine, which is an *o*-aminophenol derivative, gave a product completely oxidisable under Elvove's conditions, and apparently identical with the substance obtained by the action of preformed sodium formaldehydebisulphite on salvarsan base. In the case of *o*-aminophenol and its derivatives, Newbery and Phillips conclusively proved that two types of sulphur linkage were possible, but they were unable to give a completely satisfactory explanation of their novel observations.

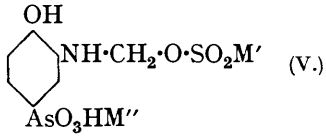
Christiansen (*J. Amer. Pharm. Assoc.*, 1930, **19**, 951), from a study of the p_H of various sulpharsphenamines and of the product precipitated by glacial acetic acid which, following Voegtlin and Johnson, he regarded as the free acid, and of the stability of these in solution, concluded that the sodium salt of sulpharsphenamine is represented by (II) but the free acid by (III).

The present investigation, which we believe affords a satisfactory solution of the problem of the constitution of sulpharsphenamine, was undertaken as a stage in the elucidation of a much more difficult problem, *viz.*, the structure of neoarsphenamine (neosalvarsan) with its highly unsaturated sulphoxylic acid side chains. It was soon realised that in the chemical investigation of products which have been prepared by using a reactive substance like formaldehyde, which might attack various points in the substrate, some analytical method was required which would give a quantitative estimate of the formaldehyde recoverable by hydrolysis. Such a one has been found, and it depends on Signer's (*Helv. Chim. Acta*, 1930, **13**, 43) and Wood's (*J. Soc. Chem. Ind.*, 1933, **52**, 33T) modification of Romijn's iodometric method. When sodium formaldehydebisulphite or *N*-methylenesulphurous acid derivatives of anilines of known constitution are distilled to a small volume from *N*-sulphuric acid solution, and the formaldehyde and sulphur dioxide trapped in *N*-sodium hydroxide, they may both be estimated by the addition of the appropriate amount of *N*/10-iodine. Acidification and back titration with thiosulphate of an aliquot portion gives a combined measure of the formaldehyde and sulphur dioxide, whilst removal of the free iodine by sodium arsenite from the remainder and precipitation of the sulphate as barium sulphate gives a measure of the sulphur dioxide, whence, by difference, the formaldehyde is determined. This method has proved invaluable for the investigation of sulpharsphenamine and allied products.

When *p*-aminophenylarsonic acid is condensed by warming with preformed sodium formaldehydebisulphite in neutral solution (Abelin, *Biochem. Z.*, 1916, **78**, 191) or with formaldehyde and sodium hydrogen sulphite added separately, *p*-aminophenylarsonic acid-*N*-methylenesulphurous acid (IV; M = H) is obtained on making the concentrated solution strongly acid to Congo-red paper, but when the reaction is less acid, but still definitely acid to this indicator, *sodium* p-*aminophenylarsono*-N-*methylenesulphite* (IV; M = Na) separates, thus indicating the strongly acid nature of the *N*-methylenesulphite radical. This crystalline substance gives up all its sulphur as sulphate when oxidised by iodine in alkaline solution under Elvove's conditions, and by our method of estimating the methyl-

enesulphite radical shows formaldehyde and sulphurous acid in almost quantitative amount and in the ratio unity. When 3-amino-4-hydroxyphenylarsonic acid, the parent





substance of sulpharsphenamine, is condensed at 80° in approximately neutral solution with preformed sodium formaldehydebisulphite, *disodium 3-amino-4-hydroxyphenylarsono-N-methylenesulphite hexahydrate* (V; M′,M″ = Na) is obtained in almost quantitative yield on concentration, but if the reaction be adjusted to neutrality to Congo-red paper the *monosodium salt tetrahydrate* (V; M′ = Na, M″ = H) separates as a very soluble product. This important substance is devoid of trypanocidal action, as would be expected (Gough and King, J., 1930, 677; Cohen, King, and Strangeways, J., 1931, 3241); it contains no free amino-group, contains a free phenolic group, as shown by titration, and it gives striking colour reactions under certain conditions with nitrous acid and also with iodine. It is oxidised quantitatively by Elvove's method, and reacts quantitatively in our method of estimating the methylenesulphite radical. When, however, the hydrochloride of 3-amino-4-hydroxyphenylarsonic acid is condensed with formaldehyde (40%) followed by sodium hydrogen sulphite (40%) in aqueous solution and then warmed in neutral solution with sodium formaldehydebisulphite, only a small yield of disodium 3-amino-4-hydroxyphenylarsono-*N*-methylenesulphite (V) can be isolated, but the mother-liquor, on quantitative examination of an aliquot part by Elvove's method and by our method for the methylenesulphite present, free or combined, shows the presence of a methylenesulphite group which cannot be estimated by Elvove's method. Attempts to isolate this new key substance in a complete state of purity as a crystalline solid have so far failed.

Armed with the information afforded by these crystalline methylenesulphurous acid derivatives, we turned our attention to the amorphous products obtained from arseno-compounds.

When commercial sulpharsphenamine, conforming to B.P. 1932 and containing about 20% of arsenic, was examined by Elvove's method for oxidised sulphur, which would include any sulphite or sulphate originally present, the results in Table I were obtained.

TABLE I.

	British.			German.	French.	American.
	A.	B.	C.	D.	E.	F.
S, total	13·0	13·2	12·8	12·0	12·7	12·1
S, Elvove	3·7	3·5	7·4	3·8	4·7	4·1
S,* unoxidised	9·3	9·7	5·4	8·2	8·0	8·0

* By difference.

These products, each of which originates from a different firm of manufacturers, show a very close similarity in total sulphur content, and 5 out of 6 show a similar distribution of oxidised and unoxidised sulphur. The problem thus resolved itself into finding, with the new analytical methods available, the nature of the linkage by which the sulphur unoxidised by Elvove's method was attached to the molecular structure.

A number of ampoules of these products (E) being available, a more detailed analysis was made of the combined product, with the results shown in Table II.

TABLE II.

	As.	Total S.	Volatile S.	Oxidised S.	Unoxidised S.	CH_2O.
Per cent.	19·1	11·9	11·5	3·5	8·4	10·9
Atomic ratios	2·0	2·9	2·8	0·9	2·1	2·9

The product contained 1·35% of sulphur as free sulphate, and this has been deducted from the values for total sulphur and oxidised sulphur (Elvove). The results for volatile sulphur or

sulphur dioxide and formaldehyde, determined by our method for methylenesulphite groups, prove conclusively that the sulphur which is not oxidised to sulphate by iodine in alkaline solution is still present as combined sulphur dioxide in association with an equimolecular proportion of formaldehyde. Other oxidising agents, such as bromine or hydrogen peroxide in boiling ammoniacal solution, do also, in fact, convert this unoxidised sulphur into sulphate. In this sample of sulpharsphenamine, therefore, on the simplest assumption there are 2·8 methylenesulphite radicals for every 2 atoms of arsenic, and of these radicals, 1·9, being the difference between the volatile sulphur and the oxidised sulphur, are unattacked by iodine in alkaline solution. They cannot be present as free sodium formaldehydebisulphite, since this substance gives up its sulphur quantitatively as sulphate under the Elvove conditions.

Newbery and Phillips (*loc. cit.*) showed that sulpharsphenamine on mild acid hydrolysis at 70° gives a product which does not contain any appreciable amount of sulphur which resists oxidation by Elvove's method. It is familiar to workers in this field, that, when sulpharsphenamine is dissolved in water and the solution acidified, there is no precipitate formed until a large excess of mineral acid has been added, whereupon a precipitate suddenly appears. On a preparative scale, this delay in the appearance of any precipitate until the requisite acidity is reached is very striking and indicates the probable operation of hydrolytic processes. The composition of the acid precipitated from the solution of sulpharsphenamine (combined sample E) is shown in Table III.

TABLE III.

	As.	Total S.	Volatile S.	Oxidised S.	Unoxidised S.	CH_2O.
Per cent.	31·4	7·7	6·6	5·8	1·9	6·5
Atomic ratios	2·0	1·1	1·0	0·9	0·3	1·0

By comparison with the previous table, it will be observed that this acid now contains only one methylenesulphite radical, and almost all of it is of the type which is oxidised by iodine in alkaline solution. By analogy with the similar behaviour on oxidation of Abelin's acid and of crystalline 3-amino-4-hydroxyphenylarsono-*N*-methylenesulphite, this methylenesulphite radical is attached to nitrogen. It follows that treatment of sulpharsphenamine in solution with mineral acid at room temperature effects the same hydrolysis as was observed by Newbery and Phillips at 70°. During this mild process, 1·9 methylenesulphite radicals which are unattacked by Elvove's method have been detached from their union with the arsenobenzene molecule. That these groups are really attached to the main molecular structure and are not free in solution in some unreactive form, follows from the results of precipitation of sulpharsphenamine by glacial acetic acid. A sample of sulpharsphenamine was prepared by Christiansen's method (*loc. cit.*) and analysed. A portion was precipitated by glacial acetic acid following Voegtlin and Johnson's procedure, and another portion precipitated with excess of hydrochloric acid. Analysis of these products, as summarised in the atomic ratios (Table IV), demonstrates that after acetic acid treatment the product is still a sodium salt, as was suggested by Newbery and Phillips, and the major portion of the methylenesulphite radical which is unattacked by iodine in alkaline solution is still present. Incidentally, the proof that the acetic acid product is a sodium salt disposes of Christiansen's formula (III).

TABLE IV.

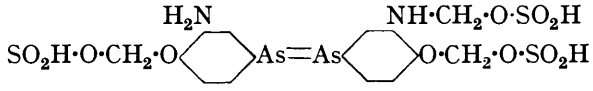
Atomic ratios.

	As.	Total S.	Volatile S.	Oxidised S.	Unoxidised S.	CH_2O.	Na.
Sulpharsphenamine	2	2·4	2·1	0·7	1·7	2·2	1·7
After $CH_3{\cdot}CO_2H$	2	—	1·8	0·7	—	2·2	1·3
After HCl.....	2	1·2	1·0	0·7	0·5	1·0	—

Furthermore, it will be noticed that the composition of the free acid precipitated by mineral acid agrees very well with the composition of the corresponding acid made from a commercial sulpharsphenamine (Table III). The fact that the ratio of atoms of arsenic

to atoms of oxidised sulphur is unaltered by hydrochloric acid treatment either in the commercial product (Tables II and III) or in our own preparation (Table IV), shows that there is no appreciable amount of free sodium hydrogen sulphite or sodium formaldehydebisulphite in these preparations.

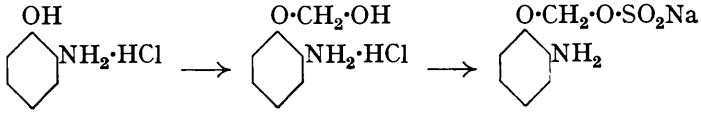
We believe that these results admit of only one interpretation: the methylenesulphite groups which are shown by Elvove's method are attached to amino-groups, whilst those which are not shown are attached to phenolic groups. On this view, commercial sulpharsphenamine (F), examined above, would correspond approximately to a sodium salt of 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene-*OO′N*-trimethylenesulphurous acid (VI), which on treatment with excess of mineral acid at room temperature gives 3 : 3′-diamino-



(VI.)

4 : 4′-dihydroxyarsenobenzene-*N*-methylenesulphurous acid (as in I), with loss of the radicals attached to the phenolic groups. The instability of formaldehyde attached to oxygen groups towards strong mineral acids and stability towards alkali are well known. We have, however, been unable to find any example in the literature of the attachment of methylenesulphite groups to phenolic groups, although there are a few cases known of formaldehyde attaching itself to isolated phenolic groups (D.R.P. 89979). The possibility that there is some ring formation involving the amino- and hydroxyl groups, as was suggested by Newbery and Phillips to account for the fact that *m*- or *p*-aminophenols do not show the Elvove phenomenon, seems to be excluded by our results.

On our view, when formaldehyde and sodium hydrogen sulphite in succession are added to the hydrochlorides of *o*-aminophenol derivatives at room temperature, the formaldehyde attacks the phenolic group in preference to the amino-group, which is protected by salt formation, but some substitution on the latter does take place:



This has been amply exemplified by the cases of salvarsan and of the hydrochloride of its parent amino-acid. When, however, preformed sodium formaldehydebisulphite reacts with *o*-aminophenol derivatives in neutral or weakly alkaline solution at 60—70°, condensation takes place on the amino-groups. This is shown by the fact that salvarsan base condensed in this way gives a product in which the methylenesulphite radicals are all oxidised to sulphate under Elvove's conditions as the following results demonstrate:

TABLE V.

	As.	Total S.	Volatile S.	Oxidised S.	Unoxidised S.	CH_2O.
Atomic ratio	2	2·1	1·8	1·8	0·3	1·7
Free acid	2	1·2	0·8	0·8	0·3	0·8

We also find that when sodium 3-amino-4-hydroxyphenylarsonate is similarly condensed with preformed sodium formaldehydebisulphite, the mother-liquors after removal of the 3-amino-4-hydroxyarsono-*N*-methylenesulphite show only a quite small proportion of methylenesulphite radicals unoxidised by iodine in alkaline solution. Moreover, when a product which approximates to a disodium 3 : 3′-diamino-4 : 4′-dihydroxyphenylarseno-*NN′*-dimethylenesulphite is treated in aqueous solution at 0° with hydrochloric acid, an acid is immediately precipitated which corresponds approximately to a 3 : 3′-diamino-4 : 4′-dihydroxyphenylarseno-*N*-methylenesulphurous acid (Table V). The free acid of the disubstituted product is therefore unstable, and this accounts for the results obtained in the earliest patent on this subject. It also explains why Newbery and Phillips, by mild acid hydrolysis of sulpharsphenamine at 60—70°, obtained a product apparently identical with the product of mild acid hydrolysis in hot solution of the condensation

product of salvarsan base with preformed sodium formaldehydebisulphite, since both products are essentially the same mono-*N*-methylenesulphite.

In further confirmation of our interpretation of the results, it has been found that the monosubstituted acid obtained, *e.g.*, from commercial sulpharsphenamine (E) by the action of excess of mineral acid (Table III), on analysis for carbon content showed a ratio of arsenic to carbon of 2 : 12·7, in agreement with the distillation results. This indicates that none of the formaldehyde used in the condensation in weakly acid solutions has entered the nucleus. It should therefore be possible to isolate 3-amino-4-hydroxyphenylarsonic acid by the oxidation of the hydrolytic products of sulpharsphenamine, and this we have been able to do both by iodine and by hydrogen peroxide.

It was shown by Voegtlin and Johnson that sulpharsphenamine made according to their procedure had a p_H between 3·2 and 4·6, and Christiansen (*J. Amer. Pharm. Assoc.*, 1930, **19**, 951) found values between 2·44 and 4·36 for commercial products. Christiansen has also shown that the number of atoms of sulphur usually exceeds the number of atoms of sodium present. On a sample of sulpharsphenamine made by ourselves by Christiansen's procedure, we find (Table IV) that for every 2·1 methylenesulphite radicals there are only 1·7 atoms of sodium. This ratio is also very little changed by precipitation of the product with acetic acid. The origin of the acidity is due to the use of salvarsan (dihydrochloride) in the preparation of sulpharsphenamine. The hydrochloric acid groups create an initial acidity in the reaction mixture which is only partially neutralised by loss of sulphur dioxide on addition of the sodium hydrogen sulphite and thus persists in the final product. Since there is a deficiency of sodium for all the acidic groups present, one of the strongly acid methylenesulphurous acid radicals of sulpharsphenamine is partly neutralised by a free amino-group in the solid product, and this internal salt is hydrolysed to an acid reaction on solution in water. In our own preparation of sulpharsphenamine (Table IV), out of 2·1 methylenesulphite radicals only 1·7 are neutralised by sodium, or if we take the total number of sulphur atoms, 2·4, which is made up of 2·1 methylenesulphite groups and 0·3 atom of nuclear sulphur probably present as sulphonic acid groups (King, J., 1921, **119**, 1415), there is an excess of 0·7 acidic group over the sodium atoms present. This should be ample to account for the observed acidity. As certain commercial samples of sulpharsphenamine are neutral in reaction it would appear that the reaction mixture in these cases is neutralised before precipitation by alcohol.

The solution of the problem of the constitution of sulpharsphenamine presented in this paper raises the important question of the relation between the structure of the 9 possible *O*- and (or) *N*-methylenesulphites of salvarsan and their toxicity and therapeutic action. Some progress has already been made in this direction, and we hope to communicate our results shortly.

Experimental.

Analytical Methods.—Estimation of methylenesulphite groups. 0·15—0·2 G. of the substance is dissolved in water (20 c.c.), with the addition of a few drops of dilute sodium hydroxide solution, if necessary, in a flask fitted with a rubber stopper carrying an anti-splash bulb sealed to the inner tube of a water condenser. Approximately *N*-sulphuric acid (150 c.c.) is added to the solution in the flask, which is then gradually heated. Distillation is continued until the volume of the original solution is reduced to 20 or 30 c.c. The sulphur dioxide which comes over first is absorbed beneath the surface of *N*-sodium hydroxide (20 c.c.) in the receiver. Standard 0·1*N*-iodine (50 c.c.) is then pipetted into the distillate, and the mixture allowed to stand for 5 min. before acidification with *N*-hydrochloric acid (21 c.c.). The solution is then made up accurately to 250 c.c. Back titration of 100 c.c. of this solution with 0·1*N*-sodium thiosulphate gives a combined measure of the formaldehyde and sulphur dioxide. The remaining 150 c.c. are transferred to a beaker, and decolorised by the addition of approx. 0·05*M*-sodium arsenite solution. After addition of *N*-hydrochloric acid (5 c.c.), the sulphur is gravimetrically determined as barium sulphate in the usual manner. The amount of iodine required to oxidise the sulphur dioxide to sulphate may then be calculated, and subtraction of this value from the total iodine consumption of the distillate enables the formaldehyde content to be ascertained. The formaldehyde determination, containing accumulated errors, is obviously less accurate than that of sulphur. The method described has, however, been

found quicker and more convenient and reliable than Clowes and Tollens's gravimetric method for *O*-methylene groups (*Ber.*, 1899, **32**, 284), which tends to give high results. The following are typical results for methylenesulphite groups.

	Found.		Calc.	
Substance.	S, %.	CH_2O, %.	S, %.	CH_2O, %.
$CH_2(OH)\cdot SO_3Na,H_2O$	21·1	18·8	21·1	19·7
$AsO_3H_2\cdot C_6H_4\cdot NH\cdot CH_2\cdot SO_3H$	10·4	9·4	10·3	9·6
$AsO_3HNa\cdot C_6H_3(OH)\cdot NH\cdot CH_2\cdot SO_3Na,6H_2O$	6·8	6·1	6·7	6·3

The results for formaldehyde determinations on $AsO_3H_2\cdot C_6H_4\cdot NH\cdot CH_2\cdot SO_3Na$ by Clowes and Tollens's method were: Found: 10·2, 10·2, 10·4. Calc.: 9·0%.

Elvove sulphur and oxidised sulphur. The method of Elvove (*loc. cit.*) was used. About 0·15 g. of the substance is dissolved in water (50 c.c.), treated with 0·1*N*-iodine (50 c.c.), and then *N*-sodium hydroxide (20 c.c.). After 1 hour, the excess iodine is first liberated by acidification of the solution with hydrochloric acid (21 c.c.) and then removed by the addition of sufficient 0·05*M*-sodium arsenite. Another 5 c.c. of *N*-acid are added, and the sulphate in the solution estimated as barium sulphate in the usual way. The amount of sulphur weighed in this form (called Elvove sulphur) includes any originally present as free inorganic sulphate. The true value for the *oxidised sulphur* is obtained by difference.

Inorganic sulphate. To avoid oxidation of any sulphites which might be present in the products examined, the following method was used. 0·3*N*-Hydrochloric acid (200 c.c.) is boiled to remove dissolved air. While the solution is still hot, 0·25 g. of substance is added, and the methylenesulphite sulphur removed as sulphur dioxide by boiling the liquid down to half its volume. The inorganic sulphate left in solution is then precipitated as barium sulphate, which is separated and weighed after 24 hours. A somewhat similar method has been used by Elvove (*J. Ind. Eng. Chem.*, 1922, **14**, 624) for free sulphate in neosalvarsan.

Total sulphur. This was determined by fusing the sample with a mixture of anhydrous sodium carbonate (2 parts) and sodium peroxide (1 part) in a covered nickel crucible. The melt was dissolved in water, filtered, and acidified with hydrochloric acid, the sulphate being then estimated as barium sulphate.

Arsenic. This element was determined by the method of Ewins (J., 1916, **109**, 1356), and the *equivalents* of arsonic acids were determined by the titrimetric method of King and Rutterford (J., 1930, 2138).

Sodium p-*Aminophenylarsono*-N-*methylenesulphite* (IV; M = Na).—*p*-Aminophenylarsonic acid (4·3 g.) in *N*-sodium hydroxide (20 c.c.) was treated with sodium formaldehydebisulphite (6 g.), complete solution being effected by warming to 70°. After 12 hours at 37° the reaction was made slightly acid to Congo-red paper by addition of 3*N*-hydrochloric acid. The crude *monosodium* salt (5·3 g.) which separated was ground under 75% alcohol to remove chlorides, and then crystallised from water (4 c.c.) [Found: As, 19·6; Na, 6·4; S (Elvove), 8·7; S (volatile), 8·5; CH_2O, 7·7; H_2O, 13·1; equiv., 189·6. $C_7H_9O_6NSAsNa,2\frac{1}{2}H_2O$ requires As, 19·8; Na, 6·1; S, 8·5; CH_2O, 7·9; H_2O, 11·9%; equiv., 189·1].

The same compound was also obtained by treating a solution of *p*-aminophenylarsonic acid (2·2 g.) in *N*-sodium hydroxide (10 c.c.) at room temperature for 5 mins. with formalin (40%, 1·5 c.c.) followed by sodium hydrogen sulphite solution (40%, 5 c.c.). After standing for 12 hours at 37°, the liquid was worked up as above; yield 1·85 g. (Found, on anhydrous product dried in a vacuum: equiv., 168·3. Calc.: equiv., 166·6).

If the reaction of the solution is made strongly acid to Congo-red paper, the free *p*-aminophenylarsono-*N*-methylenesulphurous acid (IV; M = H) of Abelin (*loc. cit.*) separates instead of the sodium salt [Found: S (Elvove), 10·4; S (volatile), 10·4; CH_2O, 9·4; equiv., 103·3. Calc.: S, 10·3; CH_2O, 9·6%; equiv., 103·7].

Disodium 3-*Amino*-4-*hydroxyphenylarsono*-N-*methylenesulphite* (V; M′,M″ = Na).—This substance was obtained in almost quantitative yield by adding sodium formaldehydebisulphite (30 g.) to a solution of 3-amino-4-hydroxyphenylarsonic acid (23·3 g.) in 2*N*-sodium hydroxide (51 c.c.) and heating the solution for 30 mins. at 80°. The pale yellow solution was concentrated to one-half its volume at 50°, and kept at 0° for 24 hours. Two crops, 36·9 g. and 8·5 g., were obtained, and these were combined and crystallised three times from half their weight of water. The *disodium* salt separated as a *hexahydrate* in long acicular crystals [Found: C, 17·3; H, 4·5; As, 15·6; Na, 9·9; S (Elvove), 6·7; S (volatile), 6·8; CH_2O, 6·1; H_2O at 110°, 19·6. $C_7H_8O_7NSAsNa_2,6H_2O$ requires C, 17·5; H, 4·2; As, 15·6; Na, 9·6; S, 6·7; CH_2O, 6·3; $5H_2O$, 18·8%]. 0·1174 G. of the substance required 4·71 c.c. of 0·1*N*-sodium hydroxide for

neutralisation by the method of King and Rutterford, the calculated value for one acidic group being 2·45 c.c. This shows therefore a 92% neutralisation of the phenolic group. No free amino-group could be detected by diazotisation and coupling with sodium β-naphthoxide. A deep red coloration is obtained when an aqueous solution of the compound is treated with iodine, ferric chloride, nitric acid, or bromine; also in acid solution with potassium permanganate or dichromate, sodium nitrite, or chloramine-T.

After removal of the crystalline solid, equal aliquot portions of the mother-liquor from the above preparation were analysed for " Elvove sulphur " and " volatile sulphur." The weights of barium sulphate were in the ratio 17 : 19, thus showing the absence of any appreciable amount of condensation product containing a type of methylenesulphite linkage not oxidised under Elvove's conditions.

Sodium 3-*Amino*-4-*hydroxyphenylarsono*-N-*methylenesulphite Tetrahydrate* (V; M′ = Na, M″ = H).—This salt (2·2 g.) was obtained when the above disodium salt (9·2 g.) was dissolved in *N*-hydrochloric acid (20 c.c.) and the solution concentrated in a desiccator. The substance was recrystallised twice from water, in which it is extremely soluble, and then separated in minute needles (Found : As, 17·4; Elvove S, 7·7. $C_7H_9O_7NSAsNa,4H_2O$ requires As, 17·8; S, 7·6%). 0·1058 G. required 7·05 c.c. of 0·1*N*-sodium hydroxide (Calc., for 2 acidic groups : 5·02 c.c.), whence it follows that 80% of the phenolic group is neutralised during titration. This *monosodium* salt is much less stable than the disodium salt; it chars on drying at 90°. Its aqueous solution is acid to Congo-red and gives a deep red colour with sodium nitrite without addition of acid.

Condensation of 3-*Amino*-4-*hydroxyphenylarsonic Acid with Formaldehyde and Sodium Hydrogen Sulphite in Succession in Acid Solution.*—The amino-acid (4·7 g.) in 3*N*-hydrochloric acid (12 c.c.) and water (10 c.c.) was treated with formalin (3 c.c.) for 1 min. at room temperature. Sodium hydrogen sulphite solution (40%, 10 c.c.) was added, and after 5 mins. the solution was neutralised to litmus by the addition of sodium hydroxide. After addition of sodium formaldehydebisulphite (4·5 g.), the solution was heated at 80° for 30 mins. and then concentrated in a vacuum over sulphuric acid. On being kept at 0° for some days, a crop (2·6 g.) of crystals separated, and on further concentration and prolonged keeping further small crops containing sodium formaldehydebisulphite and sodium chloride were removed. The combined crops gave pure disodium 3-amino-4-hydroxyphenylarsono-*N*-methylenesulphite (0·55 g.) on two crystallisations from water [Found : As, 15·3; S (volatile), 6·8; H_2O, 19·3. Calc. : As, 15·6; S, 6·7; H_2O, 18·8%]. The viscous mother-liquor could not be induced to crystallise further at neutrality or at p_H 4—5. On analysis of equal portions for " Elvove sulphur " and " volatile sulphur " weights of barium sulphate were obtained in the ratio 0·21 : 0·31, indicating the presence of sulphite sulphur in some form unoxidised by iodine in alkaline solution. The residual gum was diluted slightly and poured in a thin stream into alcohol (100 c.c.) with vigorous stirring. The precipitated solid was reprecipitated in a similar manner, and thus obtained as a cream-coloured, amorphous, hygroscopic powder (5—6 g.) [Found in vacuum-dried material : As, 10·3; S (Elvove), 7·6; S (volatile), 12·6; S (free SO_4''), 0·6; CH_2O, 10·9%; As : oxidised S : volatile S : CH_2O = 1 : 1·6 : 2·9 : 2·5]. This crude material probably contains the condensation product of 3-amino-4-hydroxyphenylarsonic acid containing two methylenesulphite groups, one of which resists oxidation by the Elvove method.

Commercial Sulpharsphenamine.—The results of the analysis of British, German, French, and American sulpharsphenamine are shown in Table I. As a rule about one-third of the total sulphur is found by oxidation with iodine in alkaline solution (Elvove's method), the major portion of the remainder being found by our method for methylenesulphite groups or by other oxidising agents. Thus the American sulpharsphenamine (F) gave S, 11·3% by oxidation with ammoniacal hydrogen peroxide and S, 10·6% by oxidation with bromine water.

A characteristic colour reaction, given by all sulpharsphenamines examined, is the deep-red coloration obtained when their aqueous solutions are treated with sodium nitrite and then acidified with hydrochloric acid. This may be the same colour reaction as is given by crystalline 3-amino-4-hydroxyphenylarsono-*N*-methylenesulphite. On treatment with " perhydrol," solutions of sulpharsphenamine give a transient purple coloration which rapidly becomes dark red.

A more detailed analysis of French sulpharsphenamine (E) was possible by combining 22 ampoules to yield 13·2 g. of material [Found : As, 19·1; total S, 13·3; S (Elvove), 4·9; unoxidised S (by diff.), 8·4; S (volatile), 11·5; S (free SO_4''), 1·35; CH_2O, 10·9%]. The chief impurity in commercial sulpharsphenamines appears to be a sulphate, probably sodium sulphate. Thus the above value of free sulphate, 1·35%, would correspond to the presence of 6% of sodium

sulphate, and if a correction is made for this, the resulting arsenical would correspond approximately to a condensation product of salvarsan base containing 3 methylenesulphite groups (Found: As, 20·3; volatile S, 12·2; CH_2O, 11·6. $C_{15}H_{15}O_{11}N_2S_3As_2Na_3$ requires As, 21·0; S, 13·5; CH_2O, 12·6%).

Hydrolysis of Commercial Sulpharsphenamine.—The above mixed sample (E, 3 g.) was dissolved in water (10 c.c.), and 3*N*-hydrochloric acid (150 c.c.) added at room temperature. After a few secs., sulphur dioxide was evolved and a yellow solid precipitated. After 30 mins. this solid (1·7 g.) was collected, well washed with water, and dried in a vacuum. It was light brown and dissolved in sodium hydrogen carbonate solution [Found: C, 31·8; H, 3·8; As, 31·4; total S, 7·7; oxidised S, 5·8; unoxidised S (by diff.), 1·9; S (volatile), 6·6; CH_2O, 6·5. A mono-*N*-methylenesulphite, $C_{13}H_{14}O_5N_2SAs_2$, would require C, 33·9; H, 3·1; As, 32·6; S, 7·0; CH_2O, 6·5%].

The above *acid* and two other preparations from commercial sulpharsphenamine, as well as our own preparation shown in Table IV, show a value for volatile sulphur, *i.e.*, sulphur dioxide, higher than the figure for oxidised sulphur obtained by Elvove's method. We interpret this to mean that during the hydrolysis of the poly-substituted methylenebisulphite and precipitation by hydrochloric acid some of the unhydrolysed *O*-substituted derivative is carried down with the precipitate.

Sulpharsphenamine (Voegtlin and Johnson).—Sulpharsphenamine was prepared by Christiansen's (*loc. cit.*) modified method from salvarsan base (5 g., 1 mol.) in *N*-hydrochloric acid (31 c.c.) and water (40 c.c.) by successive treatment with formalin (3 c.c., 3 mol.) and 40% sodium hydrogen sulphite solution (10·2 c.c., 3 mol.), and subsequently pouring into alcohol (400 c.c.); yield 7·9 g. The salvarsan base was prepared by the sodium hyposulphite (hydrosulphite) process from 3-nitro-4-hydroxyphenylarsonic acid and contained 1·5% of sulphur. The sulpharsphenamine obtained was a cream-coloured powder, and gave similar colour reactions to the commercial products [Found: As, 21·6; total S, 11·7; Elvove S, 3·9; S (free SO_4''), 0·6; volatile S, 9·8; CH_2O, 9·5; Na, 5·6%].

Precipitation with acetic acid. To a solution of the above preparation (1 g.) in water (3 c.c.), acetic acid (20 c.c.) was added. The precipitate was collected, and dried in a vacuum; yield 0·9 g. It was readily soluble in water (Found: As, 22·4; oxidised S, 3·9; volatile S, 8·6; CH_2O, 9·9; Na, 4·5%).

Hydrolysis. A solution of the sulpharsphenamine prepared above (5 g.) in water (20 c.c.) was treated with 3*N*-hydrochloric acid (250 c.c.) at room temperature. After a slight delay, a voluminous yellow solid was suddenly precipitated. This was collected after 30 mins., well washed with water, and dried in a vacuum; yield 3·5 g. The product was light brown and soluble in sodium bicarbonate solution [Found: As, 29·1; total S, 7·5; oxidised S, 4·6; unoxidised S (by diff.), 2·9; volatile S, 6·0; CH_2O, 5·8%].

Disodium 3 : 3′-Diamino-4 : 4′-dihydroxyarsenobenzene-NN′-dimethylenesulphite.—Salvarsan base (1 mol.) obtained from $NO_2{\cdot}C_6H_3(OH){\cdot}AsO_3H_2$ (8·5 g.) by the sodium hydrosulphite process was, without drying, suspended in water (60 c.c.) containing sodium formaldehydebisulphite (6·8 g., 3 mol.), and the mixture stirred at 60° for 3 hours. The solution thus obtained was clarified by filtration through a thin kieselguhr bed, and poured into alcohol (700 c.c.) with vigorous stirring. The lemon-yellow solid (6 g.) was dried in a vacuum [Found: As, 23·6; total S, 11·4; Elvove S, 9·8; S (free SO_4''), 0·8; oxidised S (by diff.), 9·0; volatile S, 8·9; CH_2O, 8·2. An *NN′*-dimethylenesulphite, $C_{14}H_{14}O_8N_2S_2As_2Na_2$, requires As, 25·1; S, 10·7; CH_2O, 10·0%]. This substance is less soluble in water than sulpharsphenamine, and gives a red colour with perhydrol, but unexpectedly does not give the normal colour reaction of sulpharsphenamine with sodium nitrite solution on acidification.

Hydrolysis. The above product (2·5 g.) in water (75 c.c.) was cooled in ice and treated with cold 3*N*-hydrochloric acid (25 c.c.). A yellow solid was immediately formed, and after 30 mins. was collected, washed with water, and dried in a vacuum; yield 1·6 g. The acid was similar in properties to that prepared from sulpharsphenamine (Found: As, 29·2; total S, 7·2; Elvove S, 5·1; volatile S, 4·9; CH_2O, 4·5%).

Oxidation of the Acid Hydrolytic Product from Sulpharsphenamine.—(a) *With iodine.* A mixture of the acid (2 g.), obtained by cold hydrochloric acid treatment of sulpharsphenamine, sodium bicarbonate (6 g.), and water (50 c.c.) was well stirred and cooled to 0°. Solid iodine (4 g.) was added in portions, effervescence occurring as the iodine reacted. The liquid was filtered, neutralised to Congo-red paper, and a succession of amorphous crops and sodium iodide removed after successive concentrations in a desiccator. Finally, crude 3-amino-4-hydroxyphenylarsonic acid (0·4 g.) was obtained, and on purification gave 0·3 g. of analytically pure

material (Found : C, 30·5; H, 3·5; N, 6·0; As, 31·6. Calc. : C, 30·9; H, 3·5; N, 6·0; As, 32·2%).

(b) *With hydrogen peroxide.* In this case the acid (8 g.) obtained by the action of hydrochloric acid on some old collected ampoules of sulpharsphenamine was not completely soluble in sodium bicarbonate (3·6 g.) and water (100 c.c.). Its content of acidic groups was correspondingly low (As : S = 2 : 0·4). Solution was effected on addition of *N*-sodium hydroxide (25 c.c.), the excess alkalinity being removed by carbon dioxide. Perhydrol (6·5 g.) in water (13 c.c.) was added dropwise at 0° with stirring. The dark brown solution was neutralised to Congo-red paper and then concentrated under reduced pressure with removal of successive crops of amorphous material. On keeping at 0°, 3-amino-4-hydroxyphenylarsonic acid was slowly deposited. A further small amount was isolated by boiling the acidified solution and proceeding as before. The combined crops (3·3 g.) were dissolved in *N*-hydrochloric acid (20 c.c.), and any amorphous material separated by fractional precipitation with saturated sodium acetate solution. At neutrality to Congo-red paper pure 3-amino-4-hydroxyphenylarsonic acid (2 g.), completely free from sulphur, was obtained (Found : C, 30·7; H, 3·7; N, 6·0%).

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