374. *The Constitution of Neoarsphenamine.*

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In a search for a neutral soluble derivative of salvarsan (3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene dihydrochloride) Ehrlich (*Chem. Ztg.*, 1912, 637; Schreiber, *Münch. med. Woch.*, 1912, **59**, 905; compare *Chem. Ztg.*, 1912, 424) prepared neosalvarsan by condensation of salvarsan with sodium formaldehydesulphoxylate. This treatment was supposed also to inhibit oxidation of the reactive arseno- and *o*-aminophenol groups. The manufacture was entrusted to Farbwerke vorm. Meister Lucius and Brüning, who stated (D.R.-P. 245,736) that mono- or di-formaldehydesulphoxylate derivatives of diaminodihydroxyarsenobenzene may be obtained by conducting the condensation reaction at different temperatures, the former being obtained at room temperature and the latter at 60—70°.

Arising out of conditions precipitated by the Great War, products in imitation of neosalvarsan have been manufactured in many countries. The term neoarsphenamine was introduced in the U.S.A. for American products and since 1932 has been adopted by the British Pharmacopœia. In the appropriate monograph neoarsphenamine is defined as a product which may be prepared by treating salvarsan base with sodium formaldehydesulphoxylate.

Methods have been proposed for the examination of neoarsphenamine by Raiziss and Falkov (*J. Biol. Chem.*, 1921, **48**, 209), Macallum (*J. Amer. Chem. Soc.*, 1921, **43**, 643; 1922, **44**, 2578), and Elvove (*U.S. Pub. Health Rep.*, 1925, **40**, 1235) which depend on the use of iodine as an oxidising agent. Certain assumptions as to the iodine requirements of the groups supposed to be present are features of these methods which were severely criticised by Freedman (*J. Lab. Clin. Med.*, 1926, **11**, 6). It was pointed out that, although tervalent arsenic, as in salvarsan, can be converted by iodine almost quantitatively into quinquevalent arsenic as claimed by Ehrlich, Goebel and others, less and less iodine is consumed in presence of acids as the acidity of the solution is increased. In other words, there is an equilibrium in aqueous solution in the following sense:

$$R{\cdot}AsO + I_2 + 2H_2O \underset{\text{acid}}{\overset{\text{neutral}}{\rightleftharpoons}} R{\cdot}AsO_3H_2 + 2HI$$

In the case of neoarsphenamine Freedman found that more and more iodine was consumed as the acidity increased, and this was conclusively traced to progressive hydrolysis of the *N*-methylenesulphoxylate groups. Contrary to previous observers, Freedman concluded that *N*-methylenesulphoxylate groups would theoretically be converted into *N*-methylenesulphites on oxidation with iodine, but that owing to the development of acidity hydrolysis will take place and the results will be *slightly* high.

The full significance of the valuable criticisms of Freedman was apparently not appreciated by Jurist and Christiansen (*J. Amer. Chem. Soc.*, 1928, **50**, 191). From data obtained by the use of iodine as an oxidising agent these authors calculated the percentage of combined and uncombined formaldehydesulphoxylate groups. They were surprised to find that the combined *N*-methylenesulphoxylate content calculated in this way was always low, and to account for this they postulated a type of combination between salvarsan base and sodium formaldehydesulphoxylate differing from their standard type in that it reacted with iodine to give sulphate instead of methylenesulphite. Alternatively a kind of double salt additive compound between salvarsan base and formaldehydesulphoxylate was considered possible. The faith of these authors in their methods of analysis was such that they claimed to have found a new type of sulphur hitherto unrecorded in neoarsphenamine amounting to 0·19—1·27%.

Salkin's (*J. Lab. Clin. Med.*, 1928, **14**, 342) formula for neoarsphenamine is too improbable for serious consideration.

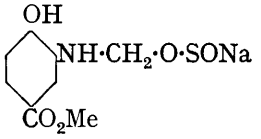
Our elucidation of the constitution of sulpharsphenamine (J., 1933, 1003) depended on a method for the quantitative assay of methylenesulphite radicals. In the present instance we therefore directed our attention to methods for the conversion of methylenesulphoxylate groups into methylenesulphite groups. The use of indigotindisulphonic acid has been suggested by Elvove (*loc. cit.*) for such a purpose, a suggestion based, no doubt, on the well-known use of this dye in the standardisation of sodium hydrosulphite. We found that pure potassium indigotindisulphonate, of oxidation–reduction potential indicator quality, is not reduced by disodium diaminodihydroxyarsenobenzene-*NN'*-dimethylenesulphite at 100° in a current of oxygen-free nitrogen, but that neoarsphenamine can be rapidly titrated to a sharp end-point. Under these conditions the reduction intensity of the arseno-linkage is insufficient to reduce the dye, whereas the methylenesulphoxylate groups have this power. This dye is, however, useless for our purpose, since our standard substance of reference, sodium formaldehydesulphoxylate, under somewhat similar conditions (but in a buffered medium of p_H 3 so as to increase its reactivity) always gave high readings by about 5%. Solutions of indigotin-tri- and -tetra-sulphonates deteriorate like the disulphonate on keeping, but when freshly standardised also gave high values with sodium formaldehydesulphoxylate.

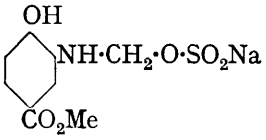
The evidence available for the choice of other dyes is very limited, since, although through the work of Mansfield Clark and others a good range of dyes is at hand for measuring reduction–potential intensities, there is very little known as to their oxidising capacities in a quantitative sense apart from the case of methylene-blue (Atack, *J. Soc. Dyers Col.*, 1915, **31**, 183, 203). Since our work commenced, 2 : 6-dichlorophenolindophenol has been introduced for estimating ascorbic acid; but it is inapplicable to neoarsphenamine.

Freedman (*loc. cit.*) stated, on the basis of unpublished evidence, that methylene-blue oxidised *N*-methylenesulphoxylates quantitatively to sulphites. We found that diamino-dihydroxyarsenobenzene-*NN'*-dimethylenesulphite had no action on methylene-blue at room temperature but reduced the dye readily on warming. Since sulphites cannot reduce methylene-blue, this action must be ascribed to the arseno-group. This is not surprising, since in the oxidation–reduction potential scale methylene-blue is removed from the region of the indigotinsulphonates to the region of lower reducing potentials. Under more drastic conditions at 100° methylene-blue oxidises sodium formaldehydesulphoxylate quantitatively to sulphite, the conditions necessary approximating to an acidity of p_H 3 and a temperature of 100°. The reducing property of the arseno-linkage on methylene-blue in warm solution naturally restricts the use of the latter in the case of neoarsphenamine to solutions at room temperature.

When various commercial samples of neoarsphenamine were titrated with methylene-blue under the rigid conditions prescribed in the experimental section, a great variation was observed between the consumption of the different makes, and the products of one manufacturer often showed a significant variation between different batches of material. Since sodium formaldehydesulphoxylate under parallel conditions showed only a small and almost negligible consumption of methylene-blue (a reaction catalysed by light), the reduction of methylene-blue by neoarsphenamine must be ascribed to its content of combined *N*-methylenesulphoxylate groups.

To throw light on the quantitative side of the methylene-blue reaction it was necessary to synthesise some substituted anilino-*N*-methylenesulphoxylates of undoubted purity. The condensation products with aniline and *o*-toluidine of Reinking, Labhardt, and Dehnel (*Ber.*, 1905, **38**, 1069) and with *o*- and *p*-aminobenzoic acids of Binz and Holzapfel (*ibid.*, 1920, **53**, 2022) did not, on repetition, meet our requirements. A satisfactory substance was found in the methyl ester of 3-amino-4-hydroxybenzoic acid, having a similar orientation of substituents to neoarsphenamine. This ester reacted readily with sodium formaldehydesulphoxylate to form *sodium 2-hydroxy-5-carbomethoxyanilino*-N-*methylenesulphoxylate* (I), which crystallised with facility in large hexagonal plates with all the

(I.) 



(II.)

attributes of homogeneity. For comparison the analogous N-*methylenesulphite* (II) was prepared by means of sodium formaldehydebisulphite. With these substances it was possible to investigate the action of methylene-blue and of iodine under a variety of conditions unhampered by an arseno-group. It was found that neither the *N*-methylenesulphoxylate (I) nor the *N*-methylenesulphoxylates of aniline, *o*-toluidine, and anthranilic acid reduced methylene-blue to any extent in neutral sodium acetate-buffered solution but reduction was markedly catalysed by acids. The *N*-methylenesulphoxylate (I) reduced methylene-blue to the extent of 71% of the theoretical quantity in an aqueous alcoholic solution containing sodium acetate (the medium necessary in neoarsphenamine titrations) at 70—75°, but in *N*/10-acetic acid solution (inapplicable in the case of neoarsphenamine) about 90% reduction took place finally in almost boiling solution, the major reduction having been effected at room temperature.

In the titration of *N*-methylenesulphoxylates by methylene-blue we seem to be driven to the conclusion that an equilibrium

$$R{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SONa + M\text{-}B \rightleftharpoons R{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SO_2Na + \textit{leuco}\ M\text{-}B$$

is attained depending on the conditions (compare Fruton, *J. Biol. Chem.*, 1934, **105**, 80). In the example just mentioned, in *N*/10-acetic acid solution equilibrium is apparently attained in boiling solution when 90% of the *N*-methylenesulphoxylate has been converted into *N*-methylenesulphite and a corresponding amount of *leuco*-methylene-blue has been formed. Unlike the above-mentioned crystalline *N*-methylenesulphoxylates, neoarsphen-

amine reduces methylene-blue in neutral solution at room temperature, an effect which must be attributed to activation by the highly unsaturated arseno-linkage, which does not, however, take part in the reaction. That an equilibrium with its consequential incomplete oxidation of *N*-methylenesulphoxylate groups by methylene-blue is also concerned in the case of neoarsphenamine is very probable from the results to be recorded later.

When 0·1*N*-iodine solution was added to sodium 2-hydroxy-5-carbomethoxyanilino-*N*-methylenesulphoxylate (I), absorption took place until 90% of the sulphur had appeared in the solution as sulphate. Although the starch–iodide reaction was reasonably permanent, the solution still contained sulphite, probably as combined *N*-methylenesulphite, since sulphur dioxide was liberated on boiling with mineral acid. On the other hand, the corresponding *N*-methylenesulphite (II) was not acted upon by iodine until 3*N*-hydrochloric acid had been added. It follows that in the reaction between iodine and the substituted anilino-*N*-methylenesulphoxylate (I) a small proportion of *N*-methylenesulphite is formed at first and the acidity developed by liberation of hydriodic acid rapidly causes hydrolysis of the remainder of the combined *N*-methylenesulphoxylate to free formaldehydesulphoxylate, which then undergoes oxidation to formaldehyde and sulphate. The reactions may be represented thus :

$$R{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SONa + I_2 \longrightarrow R{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SO_2Na + HI \quad \text{(subsidiary reaction)}$$

$$\left.\begin{aligned} R{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SONa + HI &\longrightarrow R{\cdot}NH_2 + HO{\cdot}CH_2{\cdot}O{\cdot}SONa \\ HO{\cdot}CH_2{\cdot}O{\cdot}SONa + I_2 &\longrightarrow CH_2O + Na_2SO_4 \end{aligned}\right\} \text{(main reaction)}$$

The analytical figures given without comment by Binz and Holzapfel (*loc. cit.*) for sodium anthranilino-*N*-methylenesulphoxylate show that they must have encountered the same reactions.

These results on a pure crystalline *N*-methylenesulphoxylate completely invalidate the conclusions of all previous workers, with the possible exception of those of Freedman, on the quantitative action of iodine on neoarsphenamine. The possibility of error is further enhanced when arseno-groups are present, as is shown by the direct titration with iodine of disodium diaminodihydroxyarsenobenzene-*NN'*-dimethylenesulphite. Although the consumption of iodine corresponded to 7·2 atoms (the arseno-group requires 8), the reaction liquor contained amounts of sulphate which increased with the degree of acidification. It is evident that, although apparently the consumption of iodine corresponds approximately to the requirements of the arseno-group, actually a considerable proportion of iodine has been consumed in forming sulphate at the expense of the arsonic acid, which reverts to arsenoxide as shown in the equation p. 1708. In the case of neoarsphenamine the amount of acid generated by oxidation with iodine is still larger owing to its greater degree of unsaturation; in addition the *N*-methylenesulphoxylate groups are more susceptible to hydrolysis than *N*-methylenesulphite groups, so that the formation of sulphate is more pronounced.

The conclusions of Jurist and Christiansen (*loc. cit.*) are thus seen to be unjustified and their iodine method appears to be valueless. The new labile type of methylenesulphoxylate grouping of these authors is mainly *N*-methylenesulphoxylate which has become hydrolysed both during treatment with iodine and on subsequent acidification.

In the following pages an account is given of processes which will allow a manufacturer to control his final product and will reveal the approximate composition of a commercial neoarsphenamine. On the marketed material the analyses necessary are determination of arsenic, free sulphate, total sulphur exclusive of nuclear sulphur (by Elvove's alkaline iodine method), and a methylene-blue titration. The total sulphur obtained by fusion methods is only slightly higher than the sulphur obtained by Elvove's method, the difference corresponding to nuclear sulphur, as has been amply recorded by other observers.

Table I shows the results which have been obtained on a series of typical commercial neoarsphenamines (C_1 to C_6) made by six different firms, three British and three American, during the last year or two in comparison with a preparation of neosalvarsan of German origin.

A pure disubstituted methylenesulphoxylate of diaminodihydroxyarsenobenzene requires As, 26·5% and a monosubstituted product requires As, 32·2%. The British

TABLE I.

	a. As, %.	*a.* Total S (Elvove), %.	*b.* M-B S,* %.	*c.* Sulphate S, %.	$100b/(a-c)$.	Atomic ratios. As : S† : M-B S
C1	20·9	9·3	1·8	0·5	20·3	2 : 2·0 : 0·4
C2	17·8	9·9	3·5	1·0	39·1	2 : 2·35 : 0·9
C3	19·0	8·3	3·3	0·5	42·9	2 : 1·9 : 0·8
C4	20·9	10·3	5·9	0·3	59·5	2 : 2·2 : 1·3
C5	21·6	9·0	3·2	0·7	38·9	2 : 1·8 : 0·7
C6	20·5	7·2	3·5	0·5	52·6	2 : 1·5 : 0·8
German	20·4	9·5	5·3	0·4	55·8	2 : 2·1 : 1·2

* M-B S means sulphur found by methylene-blue.
† Sulphur calculated using the value $a-c$.

Pharmacopœia (1932) demands that neoarsphenamine should contain about 20% of arsenic and it is probable that sodium chloride is added to commercial samples so as to meet the requirements of the various official control organisations.

To gain a deeper insight into the composition and structure of commercial neoarsphenamine preparations, further experiments are necessary. When the commercial neoarsphenamines recorded in Table I were dissolved in water and precipitated with glacial acetic acid and the precipitates were collected and washed with alcohol—all operations being carried out in an apparatus designed to exclude all traces of oxygen (Fig. 2)—a series of products was obtained free from inorganic salts and uncombined reagents such as sodium formaldehydesulphoxylate. Table II summarises the analytical results.

TABLE II.

	As, %.	S (Elvove), %.	M-B S, %.	Na, %.	Atomic ratios. As : S : Na	100 Na/S.
C1	28·3	6·7	0·5	2·3	2 : 1·1 : 0·5	46·8
C2	26·4	10·0	—	2·9	2 : 1·8 : 0·7	40·1
C3	27·7	9·0	1·7	1·5	2 : 1·5 : 0·35	23·0
C4	27·1	10·2	—	1·6	2 : 1·8 : 0·4	21·6
C5	27·5	8·7	2·7	1·7	2 : 1·5 : 0·4	27·7
C6	30·1	7·7	3·3	0·6	2 : 1·2 : 0·1	10·8
German	27·6	9·4	4·8	1·2	2 : 1·6 : 0·3	18·2

In Table III are given the results which have been obtained on synthetic products prepared as standards by carefully controlled condensation of salvarsan base with sodium formaldehydebisulphite, with sodium formaldehydesulphoxylate and with an equimolecular mixture of the two.

TABLE III.

	Condensation product. As.	S (Elvove).	M-B S.	Acetic acid precipitate. As.	S.	M-B S.	Na.	Atomic ratio 100 Na/S.
Salv. base + $2CH_2(OH)\cdot SO_3Na$	20·8%	9·9%	0%	23·7%	8·8%	0%	5·0%	
Atomic ratios	2	: 2·0 :	0	2	: 1·7	: 0	: 1·4	79
Salv. base + $2CH_2(OH)\cdot SO_2Na$ = neoarsphenamine	24·5%	10·4%	6·2%	27·3%	9·3%	4·6%	1·0%	
Atomic ratios	2	: 2·0 :	1·2	2	: 1·6	: 0·8	: 0·23	14·5
Salv. base + $\begin{cases} CH_2(OH)\cdot SO_3Na \\ CH_2(OH)\cdot SO_2Na \end{cases}$	24·8%	9·9	2·8%	24·9%	8·7%	1·8%	2·0%	
Atomic ratios	2	: 1·9 :	0·5	2	: 1·5	: 0·3	: 0·5	32·4

A study of the columns of atomic ratios in Table I shows that the sulphur content of commercial neoarsphenamine in most cases approximates to that of a disubstituted derivative. If treatment with glacial acetic acid at about 5° is not accompanied by appreciable hydrolysis of *N*-methylenesulphoxylate groups, comparison of Tables I and II shows that 20% or more of the sulphur as found by Elvove's method represents uncombined sulphur derivatives.

The sulphur found by methylene-blue titration expressed as a percentage of the Elvove sulphur, less the free sulphate content, as shown by column 6 in Table I as $100b/(a-c)$ is a very variable quantity in commercial products and in three cases approaches 60%. Our

own product (Table III) from pure formaldehydesulphoxylate gives the same figure of 60%. On this basis the German and the two commercial preparations C4 and C6 approximate to pure sulphoxylate preparations. Again, in our preparation of neoarsphenamine the percentage of Elvove sulphur in the original material in combination with the arsenobenzene structure is 8·3% (9·3 × 34·5/27·3). The methylene-blue figure of 6·2 on this product thus corresponds to 75% of the combined *N*-methylenesulphoxylate, and this we believe to be the position of equilibrium arrived at in the titration of combined *N*-methylenesulphoxylate groups by methylene-blue in neoarsphenamine. It is of interest and significance that the German sample and the commercial sample C4 give exactly the same point of equilibrium at 75% conversion on methylene-blue titration, again, evidence of their being pure sulphoxylate preparations.

In the last column of Tables II and III is recorded the percentage of methylenesulphoxylate (or methylenesulphite) radicals which remain combined with sodium after acetic acid precipitation. We were already familiar with the fact that an *OO'N*-trimethylenesulphite preparation as exemplified by sulpharsphenamine (Dyke and King, *loc. cit.*) on similar treatment furnished a material which was mainly a sodium salt. The results now obtained on our *NN'*-dimethylenesulphite of salvarsan base (Table III) are of the same order, for almost 80% of the groups are combined with sodium. On the other hand, the acetic acid precipitate from a pure sulphoxylate preparation, neoarsphenamine of Table III, has only 14·5% of its *N*-methylenesulphoxylate groups combined with sodium. As might be expected, a product prepared from equimolecular proportions of sodium formaldehydesulphoxylate and sodium formaldehydebisulphite has an intermediate percentage of groups combined with sodium, namely, 32·4%.

On this basis, therefore, the German and the commercial products C4 and C6 again correspond approximately to pure sulphoxylate preparations. The percentage of sulphur-containing radicals combined with sodium in the remaining preparations leads us to believe that C3 is mainly but not entirely a sulphoxylate preparation, C2 contains still less, whilst C1 contains very little sulphoxylate. The methylene-blue titration figures of the original preparations given in Table I supply full confirmation of this view. In commercial preparations of low sulphoxylate content and high percentage of radicals combined with sodium, a comparison of the analytical figures with those of our synthetic products suggests that the deficiency is made up by methylenesulphite radicals, sulphate being excluded. We have refrained from utilising in the discussion the methylene-blue figures for the acetic acid precipitated solids, since they are possibly subject to greater errors owing to certain experimental difficulties in their determination.

In conclusion we may compare our preparation of neoarsphenamine (made under great precautions in a specially designed apparatus, Fig. 1, which enables all the operations to be effected in absence of oxygen) with the International Standard, No. 2204 of 1930 (*League of Nations Rep.*, CH. 734 of 1928, p. 57). Table IV records atomic ratios so as to facilitate comparison on a common basis.

TABLE IV.

	Original preparations.			Acetic acid precipitate.			
	As.	S.	M-B S.	As.	S.	M-B S.	Na.
International standard	2	2·1	1·2	2	1·6	0·8	0·29
Preparation A of D. & K.	2	2·0	1·2	2	1·6	0·8	0·23

As far as the chemical properties and analytical data are concerned, the substances appear to be identical. The International Standard is therefore a pure methylenesulphoxylate preparation substituted only on nitrogen.

In our previous communication we showed that another soluble derivative of salvarsan, namely, sulpharsphenamine, was a derivative of salvarsan base in which both phenolic groups and one amino-group were combined with methylenesulphite groups. In a later communication in this field of work it is hoped to correlate the toxicities and therapeutic activities of many of the theoretically possible *O*- and *N*-methylenesulphites and *N*-methylenesulphoxylates of salvarsan base with their constitution.

Experimental.

Titration of Sulphoxylate Compounds with Methylene-blue.—A solution of methylene-blue (5 g. of the pure material in 1 l. of water) was standardised by titration against titanous chloride solution as described by Knecht and Hibbert ("New Reduction Methods in Volumetric Analysis," London, 1925, p. 101). For convenience in calculation it is advisable to express the strength of the dye solution as grams of sulphoxylate sulphur per c.c. (one molecule of methylene-blue oxidises one atom of sulphoxylate sulphur to sulphite).

A small round-bottomed flask is fitted with a bung with four apertures, through which pass an inlet and an outlet tube for nitrogen, the tip of the burette containing the dye, and a small funnel. The nitrogen is conveniently obtained free from oxygen by passing the commercial product successively through bottles containing strips of copper wire in aqueous ammonia and thence through dilute sulphuric acid to remove ammonia—the method used in the work on Vitamin D in this Institute (Angus *et al.*, *Proc. Roy. Soc.*, 1931, *B*, **108**, 340). The efficacy of the process is shown by the fact that *leuco*-dyes remain colourless indefinitely in an atmosphere of nitrogen purified in this manner. The gas is bubbled through the liquid in the titration flask to facilitate mixing.

Application to neoarsphenamine. Sodium acetate solution (30 c.c. of 0·1*N*) is placed in the flask, and nitrogen passed through for some time to de-aerate the liquid. The neoarsphenamine (0·1 g.) in a small capsule is quickly introduced into the flask, followed by 40 c.c. of alcohol saturated with nitrogen, this quantity being such that the final solution after titration contains about 50% alcohol, which keeps the *leuco*-methylene-blue in solution. The liquid is then titrated against the dye at room temperature. It is advisable to add the dye drop-wise, since otherwise some undesirable flocculation occurs. In the case of neoarsphenamine reduction is usually rapid at first but subsequently proceeds at a slower rate. After much experimentation we find that consistent results are obtained when the end-point is taken at the stage where a shade of blue persists, in the bluish-green colour due to 2 drops of the dye, for 30 seconds. The function of the sodium acetate is to act as a buffer against the acidity produced by reduction of the methylene-blue.

In the case of acidic substances insoluble in water, such, for instance, as the products precipitated from neoarsphenamine by glacial acetic acid, the following modification has been adopted. The weighed substance is placed in the titration flask, which is swept out with nitrogen as before, and then dissolved in saturated sodium bicarbonate solution (2 c.c.). Glacial acetic acid is then added drop by drop until a slight turbidity appears. Sodium acetate solution (25 c.c. of 0·1*N*) and alcohol (40 c.c.), both saturated with nitrogen, are added, and a clear liquid should then result. The titration is thereafter carried out as before.

Sodium formaldehydesulphoxylate reduces methylene-blue extremely slowly, one drop partially in 3 minutes, under the above conditions, and when added to a neoarsphenamine does not significantly affect the titre. The reaction with sodium formaldehydesulphoxylate is catalysed by strong sunlight, an electric arc-lamp, or a new mercury vapour lamp. For this reason neoarsphenamine titrations should be performed in diffused daylight. When sodium formaldehydesulphoxylate is titrated with methylene-blue in weakly acid media, such as a p_H 3 buffer solution or 0·2*N*-acetic acid at 100°, quantitative results are obtained [Found: S, 20·8. Calc. for $CH_2(OH)\cdot O\cdot SONa,2H_2O$: S, 20·8%]. Thionine gives equally good results, but suffers from the disadvantage of being less soluble.

The strength of the methylene-blue solutions stored in a brown bottle in the dark was checked at intervals, either against titanous chloride or against fresh recrystallised sodium formaldehydesulphoxylate of known iodine titre, without showing any depreciation in titre over several weeks.

In our experience sodium formaldehydesulphoxylate of commerce is strongly alkaline in reaction and keeps well. When recrystallised from 0·8 part of water, the pure neutral salt is obtained. The freshly recrystallised material, crystallised in a nitrogen atmosphere, was used in all our experiments. Its purity was always checked by iodine titration. Such neutral preparations do not, however, keep well.

Crystalline N-*Methylenesulphoxylates and* N-*Methylenesulphites and their Behaviour towards Iodine and Methylene-blue.—Sodium anilino*-N-*methylenesulphoxylate.* A suspension of aniline (3 g.) in a solution of recrystallised sodium formaldehydesulphoxylate (5·1 g.) in water (6 c.c.) was vigorously stirred in a small vessel fitted with a rubber bung provided with a mercury-sealed stirrer and inlet and outlet tubes for oxygen-free nitrogen. After 1·5 hours' stirring at 70—80° in an atmosphere of nitrogen, the aniline suddenly went into solution, the liquid at this stage having an acid reaction. The solution was concentrated in a vacuum over sulphuric acid until

crystallisation set in. The product, silky needles (1 g.), was rapidly dried on porous plate and dehydrated over sulphuric acid in a high vacuum [Found in two preparations: S (Elvove), 17·2, 17·1; S (methylene-blue) at 60°, 13·4. Calc. for $C_6H_5{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SONa$: S, 16·6%]. The two preparations consumed on direct titration 18·1 and 18·3 c.c. of 0·1*N*-iodine per 0·1 g. and the liquor therefrom gave gravimetrically 15·0 and 15·4% respectively of S as sulphate, corresponding to about 90% oxidation of the sulphur to sulphate. The calculated titre for 0·1 g. if oxidised completely to sulphate by iodine is 20·7 c.c. of 0·1*N*. The compound is unstable in air, rapidly reddening with liberation of aniline and hydrogen sulphide. It reduces methylene-blue very slowly at room temperature.

Sodium o-*toluidino*-N-*methylenesulphoxylate*. Prepared similarly to the aniline compound (compare Reinking, Dehnel, and Labhardt, *loc. cit.*) from *o*-toluidine (2·2 g.), sodium formaldehydesulphoxylate (3·3 g.), and water (3 c.c.), this compound crystallised as a *tetrahydrate* in long needles (2·6 g.). After drying on porcelain the product was analysed immediately. During condensation hydrogen sulphide was evolved and the liquor became acid in reaction [Found: S (Elvove), 11·7; Na, 8·6. $C_7H_7{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SONa,4H_2O$ requires S, 11·5; Na, 8·2%]. On direct titration 0·1 g. required 11·8 c.c. of 0·1*N*-iodine and the sulphate found in the liquor corresponded to S, 10·4% or 90% of the total. For complete oxidation to sulphate, 0·1 g. would require 14·3 c.c. of 0·1*N*-iodine. A second preparation was dehydrated in a high vacuum over sulphuric acid [Found: S (Elvove), 15·6; S (methylene-blue, mainly at room temperature, finally at 70°), 11·6. Calc. for $C_7H_7{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SONa$: S, 15·5%]. On direct titration 0·1 g. required 17·1 c.c. of 0·1*N*-iodine and S in liquor as sulphate was 14·1% or 90% of the total. For complete oxidation to sulphate 0·1 g. would require 19·3 c.c. of 0·1*N*-iodine. This substance is very unstable, has an acid reaction in aqueous solution, and owing to this acidity reduces methylene-blue at room temperature.

Sodium 4-*carboxyanilino*-N-*methylenesulphoxylate*. This compound (compare Binz and Holzapfel, *loc. cit.*) was prepared by condensation of *p*-aminobenzoic acid (3 g.) with sodium formaldehydesulphoxylate (4 g.) in water (4 c.c.) at 60° in a nitrogen atmosphere. Condensation was rapid and the required substance crystallised as a *tetrahydrate* on cooling. It was collected in an atmosphere of carbon dioxide and dried on porcelain (yield, 3·5 g.) [Found: S (Elvove), 10·5; S (methylene-blue at 60—100°), 7·9; Na, 7·1. $C_6H_4(CO_2H){\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}SONa,4H_2O$ requires S, 10·4; Na, 7·4%]. On direct titration 0·1 g. required 9·8 c.c. of 0·1*N*-iodine, whereas the calculated value assuming complete oxidation to sulphate is 12·9 c.c. This substance crystallises in small delicate plates which are very unstable: they slowly reduce methylene-blue at room temperature.

Sodium anthranilino-N-*methylenesulphoxylate*. Prepared similarly to the foregoing compound (compare Binz and Holzapfel), this substance formed a *tetrahydrate*, crystallising in plates (yield, 4 g.) [Found: S (Elvove), 10·0; S (methylene-blue), 6·9; Na, 6·9. $C_8H_8O_4NSNa,4H_2O$ requires S, 10·4; Na, 7·4%]. On direct titration 0·1 g. required 9·3 c.c. of 0·1*N*-iodine and 9·8 c.c. in sodium bicarbonate solution. The substance is unstable and evolves hydrogen sulphide.

Sodium 2-*hydroxy*-5-*carbomethoxyanilino*-N-*methylenesulphoxylate* (I). Methyl 3-amino-4-hydroxybenzoate (" orthoform "), suspended in water (3·3 g. in 5 c.c.), was condensed with sodium formaldehydesulphoxylate (3·8 g.) in a nitrogen atmosphere. After 30 minutes at 50—55° all the base passed into solution. Water (2 c.c.) was added together with a few drops of dilute alkali solution to remove the slight acidity which had developed. On cooling, the required *compound* crystallised well in hexagonal plates, which were collected in an atmosphere of carbon dioxide and rapidly dried between filter-paper. This salt crystallised with $3\frac{1}{2}$ molecules of water and in the interval between analyses was preserved in a vacuum [Found: S (Elvove), 10·0; 10·0; S (methylene-blue), 8·1 in sodium acetate solution at 70°; 8·9 in 0·1*N*-acetic acid at 95—100°; Na, 7·0, 6·9; Me, 5·0; H_2O, 19·7. $C_9H_{10}O_5NSNa,3\frac{1}{2}H_2O$ requires S, 9·7; Na, 7·0; Me, 4·5; H_2O, 19·1%]. On direct titration 0·1 g. required 10·7 and 10·3 c.c. of 0·1*N*-iodine and these liquors contained 9·1 and 8·9% of sulphur respectively as sulphate as found gravimetrically. The iodine oxidation liquors also contained combined sulphite as shown by hydrolysis. For complete oxidation to sulphate 0·1 g. would require 12·1 c.c. of 0·1*N*-iodine. The sulphur oxidised by iodine and found as sulphate is about 90% of the total possible. On titration with phenolphthalein as indicator 0·1 g. required 2·2 c.c. of 0·1*N*-sodium hydroxide, the calculated value for one acidic group being 3·0 c.c. This is consistent with the presence of a free phenolic group which is neutralised to the extent of 70%. This compound is relatively stable in a vacuum, but rapidly decomposes on exposure to the air.

Sodium 2-*hydroxy*-5-*carbomethoxyanilino*-N-*methylenesulphite* (II). Without special pre-

cautions, " orthoform " (3·3 g.), sodium formaldehydebisulphite (3·1 g.; 2 equivs.), and water (5 c.c.) were warmed together at 70—75°. After 5 minutes a clear solution was obtained which was kept at the same temperature for 30 minutes more. On cooling, the required salt crystallised readily (yield anhydrous, 4·8 g.). A trace of unchanged " orthoform " was removed by ether extraction, and the residual solid (4·7 g.) crystallised from water (7 c.c.). This salt separated as a *dihydrate* in elongated plates which showed no coupling reaction after diazotisation [Found : N, 4·5; S (Elvove), 10·2; Na, 7·0; H_2O, 11·4. $C_9H_{10}O_6NSNa,2H_2O$ requires N, 4·4; S, 10·0; Na, 7·2; H_2O, 11·3%].

Preparation of Neoarsphenamine.—Although it is stated in the patent literature that neoarsphenamine may be prepared by adding sodium formaldehydesulphoxylate to salvarsan (dihydrochloride), it is obvious that, since hydrochloric acid is present, decomposition products of sulphoxylate, which is sensitive to acids, would be expected in the final product. For this reason salvarsan base only was used in the following condensations. The base was prepared from 3-amino-4-hydroxyphenylarsonic acid by sodium hydrosulphite reduction (Christiansen, *J. Amer. Chem. Soc.*, 1921, **43**, 2202), treated with dilute sodium bicarbonate solution, and well washed to remove certain sulphur-containing impurities. The salvarsan base after drying was stored in evacuated ampoules and the same preparation was used throughout our work.

Since neoarsphenamine is extremely susceptible to atmospheric oxidation, the apparatus shown diagrammatically in Fig. 1, in which all operations may be carried out in an inert atmosphere, was designed for use in its preparation. A is a small vessel in which the condensation is carried out and is provided with a bung carrying inlet and outlet tubes for nitrogen and a mercury-sealed stirrer. The stirrer is constructed of hollow glass tubing, open at both ends, so that the liquid in the vessel A may be withdrawn through it, after the condensation, along the bent tube BC. During the condensation this tube is disconnected at B, and the stirrer sealed, full of nitrogen, with a small stopper. D is a sintered glass filter (Jena, 3G3) which is attached to a dropping funnel E by means of a rubber bung. The bungs in D and E are provided with inlet and outlet tubes for nitrogen. The apparatus is first well swept out with nitrogen and after the condensation is completed the liquor is drawn through the stirrer by applying suction at D_1 after the remaining four taps have been closed. To clarify the neoarsphenamine solution it is advisable to have a small kieselguhr bed on the sintered filter D. The clear solution having been collected in E, nitrogen is re-admitted. G is a vessel in which precipitations in vigorously stirred alcohol are carried out. It is fitted with a rubber bung carrying a mercury-sealed stirrer, a dropping funnel H, inlet and outlet tubes for nitrogen, and an adapter F. At the bottom of G is a wide-bore tap fitted into the rubber stopper of the sintered glass filter J (Jena 11G2). This filter is also provided with inlet and outlet tubes for nitrogen and is attached to the filter flask K. Air-free alcohol may be obtained by further de-aeration of nitrogen-saturated alcohol in the reservoir H by further passage of nitrogen. The aqueous liquor in E is added dropwise to the alcohol in G in a nitrogen atmosphere and the precipitated neoarsphenamine is collected on the funnel J, a slight positive pressure of nitrogen being a sufficient aid to filtration. The product is finally washed with alcohol from H, and the vessel J, without its fittings, is quickly transferred to a desiccator for drying in a high vacuum.

Preparation A. A mixture of salvarsan base (1·83 g.), recrystallised sodium formaldehydesulphoxylate (1·54 g.; 2 equivs.), and water (10 c.c.) was stirred at 60° until the base just went into solution (5 minutes). The filtered solution was added dropwise to alcohol (170 c.c.), and the precipitated neoarsphenamine collected, washed with alcohol (40 c.c.), and dried in a high vacuum. The substance was a pale yellow solid, very easily soluble in water, its aqueous solution having a p_H value of 6 (yield, 2·3 g.) [Found : As, 24·5; S (Elvove), 10·4; S (methylene-blue), 6·2; whence As : S (Elvove) : S (methylene-blue) = 2 : 2 : 1·2].

The effect of varying some of the factors is summarised in the following table.

Preparation.	Salv. base, equiv.	$CH_2(OH)\cdot O\cdot SONa$, equiv.	Temp.	Time, mins.	As, %.	S (E),† %.	S (M-B), ‡ %.	Atomic ratios. As : S : M-B S
A	1	2	60°	61	24·5	10·4	6·2	2 : 2 : 1·2
B	1	2	50	165	24·2	10·1	5·1	2 : 2 : 1·0
C	1	2·5	60	30*	21·6	11·6	6·4	2 : 2·5 : 1·4
D	1	3	58	45*	22·0	11·9	7·7	2 : 2·5 : 1·6
E	1	4	60	30*	18·5	12·2	5·6	2 : 3·1 : 1·4
F	1	1·5	60—65	180	28·0	8·4	3·2	2 : 1·4 : 0·5

* Heating was continued for a further 30 minutes after complete solution had been effected.
† (E) means Elvove. ‡ (M-B) means methylene-blue.

In all the above experiments 1·83 g. of salvarsan base were used except in C, where double quantities were employed. The volume of water throughout was 10 c.c. and the volume of alcohol for precipitation 170 c.c., except in F, where only 100 c.c. were used. The p_H of the final material was usually 6·0.

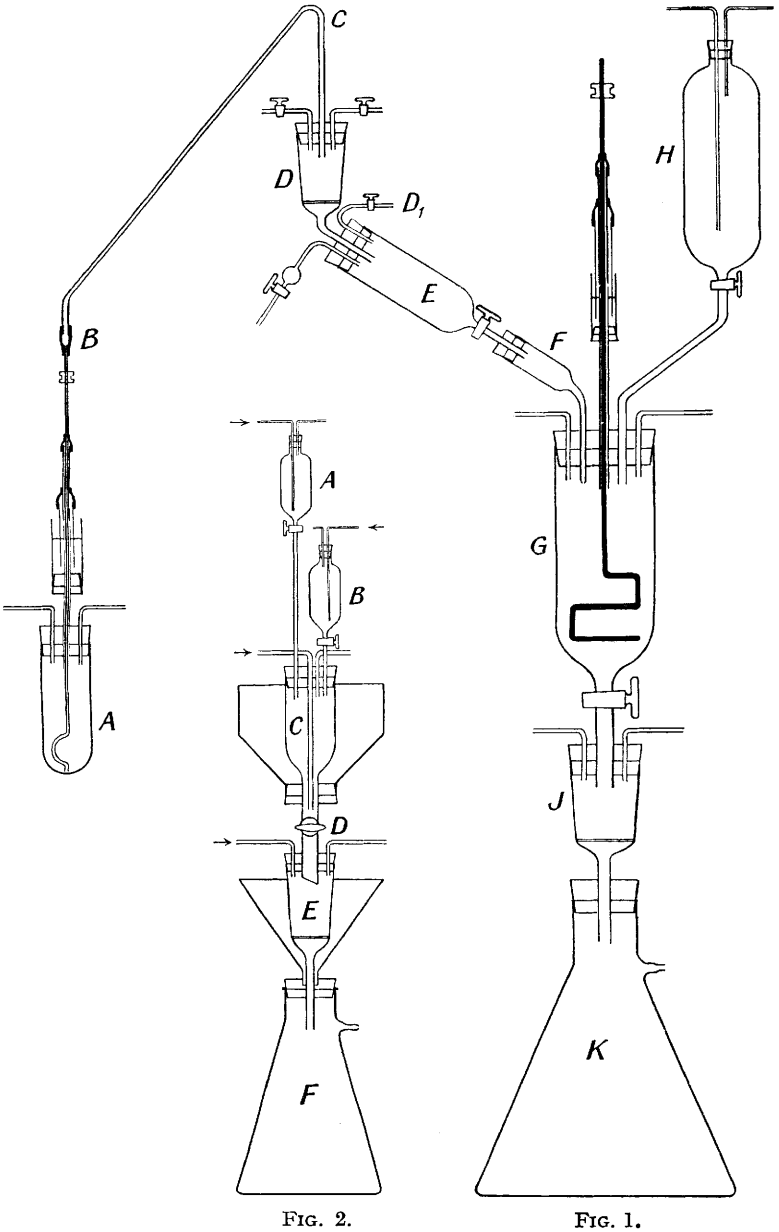


FIG. 2. FIG. 1.

Of preparation C, 4·5 g. were dissolved in water (12·5 c.c.) in the above-described apparatus and reprecipitated by alcohol (170 c.c.) [Found: As, 22·5; S (Elvove), 11·7; S (methylene-blue), 6·3%; whence As : S (Elvove) : S (methylene-blue) = 2 : 2·4 : 1·3]. This preparation could not be precipitated by pouring a concentrated aqueous solution into pure methyl alcohol or into a 50% ethyl alcohol–methyl alcohol mixture.

Of preparation E, 2·1 g. were dissolved in water (10 c.c.) and reprecipitated by alcohol

(170 c.c.) [Found: As, 21·9; S (Elvove), 12·4; S (methylene-blue), 6·2%; whence As : S (Elvove) : S (methylene-blue) = 2 : 2·7 : 1·3]. Of this product, 1·25 g. and sodium formaldehydesulphoxylate (1·25 g.) were dissolved in water (10 c.c.) and poured into alcohol (170 c.c.) [Found: As, 20·4; S (Elvove), 13·4%; whence As : S = 2 : 3·1].

Precipitation of Neoarsphenamine and Allied Products with Glacial Acetic Acid.—To ensure oxygen-free conditions the apparatus shown in Fig. 2 was used. C is a vessel of about 60 c.c. capacity in which precipitations are carried out. It is fitted with a rubber bung carrying small dropping-funnels A and B with inlets and outlets for pure nitrogen. The vessel C is provided with a large-bore tap D, and fits into the rubber bung in a sintered glass filter E (Jena 3G4), which is also provided with inlet and outlet tubes for nitrogen. The filter E is attached to a filter flask F. The vessels C and E are surrounded by ice-baths. The apparatus is first swept out with nitrogen. A solution of neoarsphenamine (2 g.) in air-free water (5 c.c.) is prepared in C and cooled by means of the external bath of ice. Glacial acetic acid (40 c.c.) in A and alcohol (20 c.c.) in B are de-aerated by passage of nitrogen. The acetic acid is then added drop-wise to the solution in C, nitrogen bubbling vigorously through the liquid to ensure thorough mixing. When all has been added, the precipitate is collected on the filter E by opening D. Ice-cooling and a nitrogen atmosphere are maintained during the filtration. The precipitate is finally washed with alcohol from B and then quickly transferred on the filter E to a high vacuum for drying. In the case of pure sulphoxylate compounds, owing to the extremely finely divided nature of the precipitate, some loss is encountered during filtration, but average yields of 1 g. are obtained. The following table summarises the analytical data for the acetic acid precipitates of neoarsphenamine preparations A, B, and D of the previous table.

Preparation.	As, %.	S (Elvove), %.	S (M-B), %.	Na, %.	Atomic ratios. As : S (E) : S (M-B) : Na
A	27·3	9·3	4·6	1·0	2 : 1·6 : 0·8 : 0·23
B	27·3	9·4	4·9	1·0	2 : 1·6 : 0·8 : 0·24
D	27·5	10·3	—	—	2 : 1·7 : — : —

The results for the neoarsphenamine preparations and the acetic acid precipitations therefrom may be briefly summarised. The amount of combination on the amino-groups increases with the proportion of sodium formaldehydesulphoxylate employed. Condensation is more rapid the more concentrated the sodium formaldehydesulphoxylate solution used. It is impossible to remove excess of sodium formaldehydesulphoxylate by means of alcohol, since sodium formaldehydesulphoxylate is less soluble than its condensation product, but possible by means of acetic acid. At least 1·5 equivalents of sodium formaldehydesulphoxylate must be used to effect condensation; and prolonged condensation times are undesirable as well as high temperatures, since slow decomposition sets in, as is shown by evolution of hydrogen sulphide. There is no evidence for the presence of substituents on the phenolic groups.

Preparations containing Formaldehydebisulphite.—Preparation G. Salvarsan base (1·83 g.) and two equivalents of a 50% mixture of sodium formaldehydesulphoxylate and sodium formaldehydebisulphite formed a clear solution in 30 minutes at 55—60°. The product precipitated in alcohol (200 c.c.) was a light yellow powder (2·3 g.), easily soluble in water, p_H 8 [Found: As, 24·8; S (Elvove), 9·9; S (methylene-blue), 2·8%; whence As : S (Elvove) : S (methylene-blue) = 2 : 1·9 : 0·5].

Preparation H. Using 2·5 equivalents of a 50% mixture of sodium formaldehydesulphoxylate and sodium formaldehydesulphite, solution of salvarsan base (1·83 g.) was effected in 15 minutes at 58°, but this temperature was maintained for a further 15 minutes; yield 2·7 g., p_H 8 [Found: As, 22·0; S (Elvove), 11·2; S (methylene-blue), 3·0%; whence As : S (Elvove) : S (methylene-blue) = 2 : 2·4 : 0·6].

Preparation J. Disodium 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene-*NN*′-dimethylenesulphite (J., 1933, 1011) was prepared by condensing 2·5 equivalents of sodium formaldehydebisulphite with salvarsan base [Found: As, 20·8; S (Elvove), 9·9; free SO_4'' S, 0·7%. As : S (sulphite) = 2 : 2·0].

The effect of glacial acetic acid precipitation on these products is tabulated below.

Preparation.	As, %.	S (Elvove), %.	S (M-B), %.	Na, %.	Atomic ratios. As : S (E) : S (M-B) : Na
G	27·0	8·7	1·8	2·0	2 : 1·5 : 0·3 : 0·5
H	26·3	9·7	1·7	2·5	2 : 1·7 : 0·3 : 0·6
J	23·7	8·8	0·0	5·0	2 : 1·7 : 0·0 : 1·4

Action of Iodine on N-*Methylenesulphoxylates and* N-*Methylenesulphites in the Presence of Arseno-groups.—Disodium* 3 : 3′-*diamino*-4 : 4′-*dihydroxyarsenobenzene*-NN′-*dimethylenesulphite.* On direct titration of 0·1 g. (Preparation J) the consumption of iodine was 10·0 c.c., corresponding to 7·2 atoms of iodine. Similarly, when treated with excess of iodine and back-titrated, 0·1 g. consumed 10·5 c.c. 0·1*N*-iodine. The liquor from the former titration without further acidification gave on gravimetric analysis S, 2·7% free as sulphate. The experiment was repeated with the addition of 5 c.c. of 3*N*-hydrochloric acid to the liquor after exact oxidation with iodine and here the free sulphate corresponded to S, 5·6%. Thus, although the iodine titre corresponds approximately to the theoretical requirements of the arseno-groups alone (compare Wright, *Proc. Soc. Exp. Biol. Med.*, **1933**, **31**, 170), nevertheless combined sulphur in the side chain becomes oxidised to sulphate, the amount depending on the degree of acidity of the solution.

N-*Methylenesulphoxylate*-N-*methylenesulphite preparation.* Preparation H on direct titration with iodine required 15·9 c.c. of 0·1*N*-solution for 0·1 g., and the liquor without acidification gave S, 5·0% in the form of free sulphate. In a parallel experiment hydrochloric acid was added after iodine titration and the sulphur found as free sulphate was 8·2%, whereas the total sulphur found by Elvove's method was 11·2%.

N-*Methylenesulphoxylate preparation.* Neoarsphenamine, preparation A, required 19·7 c.c. of 0·1*N*-iodine for 0·1 g. on direct titration. The liquor was assayed for sulphate without further acidification and gave S, 8·1% in the form of free sulphate. In a parallel experiment hydrochloric acid was added after iodine titration and the sulphur found as free sulphate was S, 9·2%, whereas the total sulphur found by Elvove's method was 10·4%.

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