

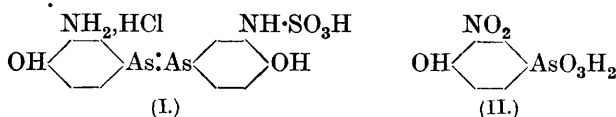
CXXI.—*Derivatives of Sulphur in Commercial
Salvarsan. Part I.*

By HAROLD KING.

THE biological testing of commercial samples of salvarsan has revealed the great difficulty which the manufacturers have to face in the variable toxicity of their preparations. The two main factors which contribute to this variation in toxicity, and therefore

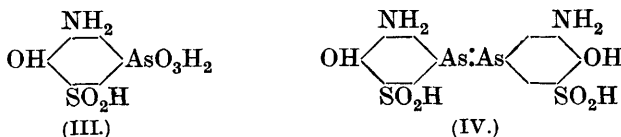
in homogeneity, are, in the first place, the amorphous or doubtfully crystalline nature of the substance, rendering purification difficult, and, secondly, the use of sodium hyposulphite in the reduction process.

Fargher and Pyman (T., 1920, 117, 373) made the highly interesting and important observation that methyl-alcoholic solutions of commercial salvarsan deposit a substance which they regarded as the monohydrochloride of the monosulphamic acid of 3:3'-diamino-4:4'-dihydroxyarsenobenzene (I).



They also adduced evidence that the sulphur enters the molecule during the first stage of the reduction process, namely, the reduction of the nitro-group of 3-nitro-4-hydroxyphenylarsinic acid (II) by the hyposulphite, but they state that they were unable to isolate the substance containing sulphur as an arsenic acid.

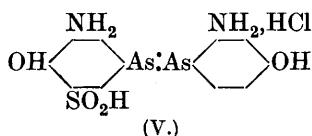
During the examination of some samples of commercial salvarsan for the Salvarsan Committee of the Medical Research Council the present author came across an abnormal commercial sample which, when dissolved in methyl alcohol, gave a 24 per cent. yield of a substance agreeing analytically with Fargher and Pyman's findings. This seemed to indicate that, under suitable conditions, one ought to find a 10 per cent. yield of the sulphur-substituted phenylarsinic acid in the reduction of the nitrohydroxy-acid. A careful examination was accordingly made of the reduction of 3-nitro-4-hydroxyphenylarsinic acid (II) to the corresponding amino-acid by sodium hyposulphite. The chief reduction products which have so far been found and identified are 3-amino-4-hydroxyphenylarsinic acid as the main product, 3-amino-4-hydroxy-5-sulphinophenylarsinic acid (III) and its corresponding arseno-derivative, 3:3'-diamino-4:4'-dihydroxy-5:5'-disulphinoarsenobenzene (IV).



This sulphinic acid * arises constantly under the conditions of the

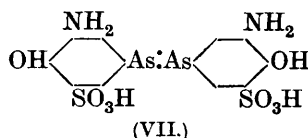
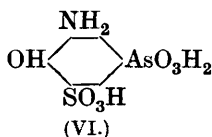
* The possibility that this substance is 3-amino-4-hydroxy-5-sulphophenylarsenous acid seems to be negated by the analyses of the corresponding arsenobenzenes (IV) and (VII), by the different toxicities of these arsenobenzenes and by the parallelism between the solubility in water of this

reduction as described in the experimental part, to the extent of 10 grams from 106 grams of the original nitrohydroxy-acid. This in itself would be sufficient to account for the whole of the sulphur content of many specimens of salvarsan. The corresponding disulphinoarseno-derivative arises in much smaller proportions, the amount increasing at the expense of the sulphinoarsenic acid with rise of temperature. There is some evidence of the formation of the mixed arseno-derivative at the same time. The pure sulphinoarsenic acid can be reduced to its corresponding disulphinoarsenobenzene (IV) by sodium hyposulphite, but better by hypophosphorous acid at 100°. The product agrees in its properties with the disulphinoarsenobenzene referred to above and produced in the reduction of nitrohydroxyphenylarsinic acid. The mixed arsenobenzene, 3:3'-*diamino-4:4'-dihydroxy-5-sulphinoarsenobenzene monohydrochloride* (V), has been prepared by reduction of an equimolecular



mixture of the parent aminohydroxyphenylarsinic acid and its 5-substituted sulphinic acid, both by sodium hyposulphite and by hypophosphorous acid. This is the substance which is the main impurity in commercial salvarsan, and represents in a pure form one of the constituents of the substance which Fargher and Pyman first isolated by reason of its sparing solubility in methyl alcohol.

3-Amino-4-hydroxy-5-sulphinophenylarsinic acid, on oxidation with a 3 per cent. solution of hydrogen peroxide in the calculated amount, passes smoothly and without concomitant indophenol formation into 3-amino-4-hydroxy-5-sulphophenylarsinic acid (VI), an acid which has also been isolated by the oxidation of the constituent of salvarsan which is insoluble in methyl alcohol.



sulphinic acid and the corresponding sulpho-acid (VI), and the solubility in water of aromatic sulphinic and sulpho-acids in general. The solubility of the oxide, on the other hand, might be expected to be greater than that of the sulpho-acid (VI), as its nearest analogue, 3-amino-4-hydroxyphenylarsenious oxide, is more soluble than its corresponding arsinic acid. Experiments on the preparation of 3-amino-4-hydroxy-5-sulphophenylarsenious acid for direct comparison are in progress.

This acid, on reduction with hypophosphorous acid at 55°, gives 3 : 3'-diamino-4 : 4'-dihydroxy-5 : 5'-disulphoarsenobenzene (VII). Reduction of an equimolecular mixture of the sulphonic acid and 3-amino-4-hydroxyphenylarsinic acid with hypophosphorous acid at 60° gave the pure mixed arseno-derivative, 3 : 3'-diamino-4 : 4'-dihydroxy-5-sulphoarsenobenzene monohydrochloride, which may also be a constituent of the substance from salvarsan insoluble in methyl alcohol, whilst a mixture of the sulphonic and sulphinic acids in molecular proportions gave 3 : 3'-diamino-4 : 4'-dihydroxy-5-sulpho-5'-sulphinoarsenobenzene on reduction with the same reagent at 100°.

The position of the sulphinic or sulphonic acid group follows from the conversion of 3 : 3'-diamino-4 : 4'-dihydroxy-5 : 5'-disulphoarsenobenzene, by hydrolysis by Karrer's method (*Ber.*, 1915, 48, 1058), into an *o*-aminophenolsulphonic acid, which differs from the two known *o*-aminophenolsulphonic acids, which contain the sulpho-groups *para* to the amino- or hydroxyl-groups. Experiments designed to fix the position with complete certainty are in progress.

The exclusive formation of a mixed arsenobenzene by the reduction of an equimolecular mixture of phenylarsinic acids, when a mixture of the three arsenobenzenes might be expected, has never been satisfactorily explained. The experiment herein described of the reduction of an equimolecular mixture of 3-amino-4-hydroxy-5-sulphinophenylarsinic acid and 3-amino-4-hydroxyphenylarsinic acid furnishes a clue. When the latter acid is reduced by hypophosphorous acid at 60°, the reagents are in solution throughout and the resulting salvarsan base is isolated by addition of sodium hydrogen carbonate. When the former acid, which is insoluble in water, is reduced by hypophosphorous acid at 100°, reduction to the arsenobenzene, which is also insoluble in this medium, takes place through the medium of the solvent by virtue of the small solubility of the arsinic acid. When the equimolecular mixture of the two acids, one of which is soluble, the other insoluble, is reduced, there is a quantitative formation and separation from the solution of the pure, insoluble, mixed arseno-derivative. It is evident that the controlling factor is the smaller solubility of the mixed arsenobenzene. It is probable that all three arsenobenzenes are formed early in the reduction through combination of the free arseno-radicals * $\text{PhAs}<$, which probably exist to a minute amount in the solution in presence of the powerful reducing agent, but the

* Fargher's interpretation (*T.*, 1920, 117, 867) of how a mixed arsenobenzene is formed at all seems unnecessarily complicated; the major difficulty, as to why it is formed exclusively, is left unexplained.

mixed arsenobenzene, being the least soluble, final product of combination of these radicles, is removed from the sphere of action, and is continually formed at the expense of the other arseno-derivatives or radicles, again by means of the minute true solubility of these in the solvent.

I am indebted to my colleagues, Dr. J. H. Burn, Miss F. M. Durham, and Miss J. Marchal for a comparison of the relative efficacy of some of the arsenobenzenes described in this communication, with a pure sulphur-free salvarsan prepared by hypophosphorous acid. The maximum tolerated and the minimum lethal dose were determined by intravenous injection into mice, and the minimum curative dose by intravenous injection into mice infected with *Trypanosoma equiperdum*. The results obtained are tabulated below and are expressed in milligrams per gram of mouse.

	Dosis Letalis.	Dosis Tolerata.	Dosis Curativa.	C/T.
Disulphino-derivative (IV.)	0.1	0.09	>0.06	—
Disulpho-derivative (VII.)	0.07	0.06	>0.05	—
Monosulphino-derivative* (V.)	0.1	0.09	0.02	1/4.5
Salvarsan (hypophosphorous)	0.2	0.175	0.013	1/13.5

* Base.

A reduction of the basic property of the salvarsan molecule by the introduction of a sulphinic or sulphonic acid group has a dystherapeutic effect, the effect on the toxicity of the sulphonic acid group being somewhat greater than that of the sulphinic. The effect is not, however, so marked as that of the carboxyl group in 5 : 5'-diamino-4 : 4'-dihydroxyarsenobenzene-2 : 2'-dicarboxylic acid (Karrer, *Ber.*, 1915, **48**, 1058), which has a toxic dose of 0.03 mg. per gram of mouse.

The evidence, so far as it goes, shows that the presence of the monosulphinoarsenobenzene in commercial salvarsan does not contribute to the efficacy of this preparation.

EXPERIMENTAL.

Reduction of 3-Nitro-4-hydroxyphenylarsinic Acid with Sodium Hyposulphite : Isolation of 3-Amino-4-hydroxy-5-sulphinophenyl-arsinic Acid, and 3 : 3'-Diamino-4 : 4'-dihydroxy-5 : 5'-disulphinoarsenobenzene.

3-Nitro-4-hydroxyphenylarsinic acid (106 grams) was dissolved in 800 c.c. of *N*-sodium hydroxide solution in a thin-walled vessel and the solution was mechanically stirred and cooled to -2° . A preliminary experiment on a small-scale batch having determined

the minimum quantity of sodium hyposulphite required to decolorise the nitrohydroxy-acid, the calculated quantity was added in four equal portions. For a good commercial sample of sodium hyposulphite 216 grams were required. The temperature before each addition of hyposulphite was lowered to -2° , the successive additions of hyposulphite generally raising the temperature to between 5° and 10° , depending on the efficiency of the stirring and cooling arrangements. On continuing the stirring for an hour or more after the last addition of hyposulphite, 3-amino-4-hydroxyphenylarsinic acid separated in quantity, and to complete the separation the solution was kept in the ice-chamber overnight. The crystals were collected and amounted when dry to 48 grams.

The filtrate was made slightly acid to Congo-paper by the addition of 120 c.c. of concentrated hydrochloric acid and kept at 0° or below for about a week, when a mixture of three substances separated, sodium sulphate, 3-amino-4-hydroxy-5-sulphinophenylarsinic acid, and its corresponding arseno-derivative. These were separated as follows. The solids were collected by filtration, suspended in 100 c.c. of water and warmed with stirring to 40° , when all the sodium sulphate dissolved. The undissolved solids, when collected and dried, amounted to 13.6 grams. A perfect separation of the two components of this mixture was effected by suspending in 80 c.c. of water, and adding 2*N*-ammonia (25 c.c.) until all passed into solution. On addition of 10 c.c. of 4 per cent. calcium chloride solution and thorough mixing, the calcium salt of the arseno-acid was quantitatively precipitated as a gelatinous, yellow mass, which carried down with it a certain amount of the soluble calcium salt of the arsenic acid. The solution was centrifuged and the slightly tinted, water-bright, supernatant fluid syphoned off and acidified to Congo-paper with hydrochloric acid, when pure 3-amino-4-hydroxy-5-sulphinophenylarsinic acid separated. The gelatinous calcium coagulum was resuspended in recently boiled water, well stirred, and again centrifuged. The supernatant fluid, on separation and acidification, gave a second crop of the pure acid. The treatment was repeated so long as any acid separated on acidification, usually three extractions being required in all. The crops of acid were combined and, when air-dried, amounted to 9.3 grams. The gelatinous calcium salt of the arseno-benzene was suspended in air-free water and acidified with hydrochloric acid. The gelatinous acid was obtained by centrifuging and resuspending in fresh water so long as there was any chloride left in the supernatant fluid. When all precipitating ions are removed, the free acid tends to pass into colloidal solution. The gelatinous solid was dried in a vacuum over sulphuric acid and amounted to 2.75 grams.

3 : 3'-Diamino-4 : 4'-dihydroxy-5 : 5'-disulphinoarsenobenzene, prepared in this way, is obtained in dark brown, brittle masses still retaining several units per cent. of water, which is almost completely removed by powdering and further drying. The finely ground product is a fawn-coloured substance insoluble in acids, but readily soluble in strong or weak alkalis (Found : N=5.7 *; S=11.9; As=29.6.† $C_{12}H_{12}O_6N_2S_2As_2$ requires N=5.7; S=13.0; As=30.3 per cent.). The somewhat low sulphur content which was consistently observed in several preparations is probably due to the presence of the mixed arseno-derivative. In its properties it agrees substantially with the synthetic product prepared by the reduction of the pure 3-amino-4-hydroxy-5-sulphinophenylarsinic acid.

Properties of 3-Amino-4-hydroxy-5-sulphinophenylarsinic Acid (III).—This acid is almost insoluble in water and crystallises on acidification of solutions of its salts in minute, elongated plates with pointed ends. It remains white and apparently unchanged at 280°. It is insoluble in acids, but dissolves with the production of a bright yellow colour on addition of sodium nitrite, and then couples with alkaline β -naphthol with the production of a deep reddish-brown, soluble dye. The sulphur is not removed by heating with alkaline lead acetate solution, and the acid is remarkably stable to boiling dilute sodium hydroxide solution. The acid, when dissolved in dilute ammonia, gives no precipitate with lithium chloride, calcium chloride, or magnesia mixture in cold or hot solution, but gives an immediate heavy precipitate of sphærocrystals on addition of barium chloride solution at room temperature. Under comparable conditions, the parent acid, 3-amino-4-hydroxyphenylarsinic acid, shows an identical behaviour to nitrous acid and alkaline β -naphthol, gives, however, a beautifully crystalline lithium salt in boiling ammoniacal solution, a crystalline calcium salt under the same conditions, and a microcrystalline magnesium salt. With barium chloride there is slight precipitation. Both acids reduce ammoniacal silver nitrate solution in the cold instantaneously to metallic silver, but only the sulphinic acid decolorises iodine solution, two atoms being consumed. Thus, 0.2971 gram of the dried acid decolorised 20.25 c.c. of N/10-iodine, whereas 20 c.c. are required by theory (Found : loss in a vacuum = 2.5, 2.2. $C_6H_5O_6NSAs, \frac{1}{2}H_2O$ requires $H_2O=2.9$. Found : in dried material, N=4.7; As=25.2; S=10.9. $C_6H_5O_6NSAs$ requires N=4.7; As=25.2; S=10.7 per cent.).

* All nitrogen estimations were made by the Kjeldahl-Gunning process.

† All arsenic estimations were made by Lehmann's method except where otherwise stated.

Oxidation of 3-Amino-4-hydroxy-5-sulphinophenylarsinic Acid to 3-Amino-4-hydroxy-5-sulphophenylarsinic Acid (VI).

The choice of suitable oxidising agents is limited owing to the reactive *o*-aminophenol group, thus permanganate and sodium *N*-chloro-*p*-toluenesulphonamide proved inapplicable. The calculated amount of iodine oxidises the sulphinic acid quantitatively to the sulpho-acid, but the product is difficult to isolate completely. By use, however, of the calculated amount of 3 per cent. hydrogen peroxide solution, the reaction proceeds quantitatively.

Ten grams of the sulphinic acid were treated with 36 c.c. of 3 per cent. hydrogen peroxide solution. The temperature of the suspension was raised to 40°, when the oxidation proceeded rapidly and exothermally, the temperature rising to 78° with dissolution of the sulphinic acid as the readily soluble sulphonic acid, which crystallised out on keeping in large, well-formed crystals. The yield was almost quantitative.

3-Amino-4-hydroxy-5-sulphophenylarsinic acid differs from the sulphinic acid and the parent 3-amino-4-hydroxyphenylarsinic acid in many respects. The reactions of the latter acids, for comparison, are given above. The sulphonic acid decomposes at 258° (corr.). It is soluble in four times its weight of boiling water. With nitrous acid it gives a deep yellow colour and couples with alkaline β -naphthol with production of a deep reddish-brown, soluble dye. It reduces ammoniacal silver nitrate solution instantaneously. Its ammoniacal solution, on heating, gives a crystalline calcium salt on addition of calcium chloride, and a white precipitate with magnesia mixture. The reaction with barium chloride is characteristic. Addition of the reagent in excess precipitates an oil which dissolves on heating, and on boiling gently a barium salt separates in rosettes of crystals. With lithium chloride there is no precipitation. On boiling the acid with baryta solution, there is no separation of barium sulphate, but, instead, a crystalline barium salt is deposited (Found: N=4.5; S=10.2; As=23.5. $C_6H_8O_7NSAs$ requires N=4.5; S=10.2; As=23.9 per cent.).

3 : 3'-Diamino-4 : 4'-dihydroxy-5 : 5'-disulphinoarsenobenzene (IV).

(a) *Reduction with Sodium Hyposulphite*.—Two grams of 3-amino-4-hydroxy-5-sulphinophenylarsinic acid, dissolved in 40 c.c. of water containing 6 c.c. of 2*N*-sodium hydroxide, were reduced at 50° for forty-five minutes, by addition of 12 grams of sodium hyposulphite and 3 grams of magnesium chloride. The insoluble arsenobenzene was collected by centrifuging, suspended in recently

boiled water, and again centrifuged. The solid was dissolved in 2.7 c.c. of 2*N*-ammonia and centrifuged to remove insoluble impurities. The liquor was acidified to Congo-paper with hydrochloric acid, diluted, and the solid collected by centrifuging. It was resuspended in air-free water and again centrifuged several times until most of the hydrochloric acid had been removed. When dried in a vacuum, it amounted to 1.05 grams (Found: N=5.4; S=13.7; As=27.9. $C_{12}H_{12}O_6N_2S_2As_2$ requires N=5.7; S=13.0; As=30.3 per cent.). It was not found possible to obtain a purer substance by the use of sodium hyposulphite. At 60°, a temperature often reached during the commercial preparation of salvarsan, there is in the present instance fission of some arsenic from the nucleus and a deepening of the colour of the product, the atomic ratios found being N : S : As=1 : 1.56 : 1.36. In this connexion, the author has observed that boiling 3-nitro-4-hydroxyphenylarsinic acid with a concentrated solution of sodium hydrogen sulphite gives *o*-nitrophenol in excellent yield. The extension of this reaction may prove of service in determining the constitution of phenylarsinic acids.

(b) *Reduction with Hypophosphorous Acid*.—One gram of the sulphinic acid, suspended in a mixture of 5 c.c. of water and 5 c.c. of glacial acetic acid, was reduced with 5 c.c. of hypophosphorous acid (D 1.14) at 100° (at 60° or 80° only a partial reduction takes place) for one hour. The addition of 0.05 gram of potassium iodide is advantageous. The insoluble, primrose-yellow arsenobenzene was collected by centrifuging, suspended in air-free water, and again centrifuged three or four times until the supernatant liquor was no longer acid to Congo-paper. The product was dried in a vacuum, and the yield was quantitative (Found: N=5.6; S=12.7; As=30.2 per cent.). The qualitative reactions are indistinguishable from those of the corresponding disulphoarsenobenzene described below.

3 : 3'-Diamino-4 : 4'-dihydroxy-5-sulphinoarsenobenzene Hydrochloride (V).

(a) *Reduction with Sodium Hyposulphite*.—An equimolecular proportion of 3-amino-4-hydroxy-5-sulphinophenylarsinic acid and 3-amino-4-hydroxyphenylarsinic acid, when reduced with this reagent at 50–55° in the usual manner, gave the mixed arsenobenzene. The product and yield are not, however, satisfactory (Found: N=6.1; Cl=8.5; S=6.8; As=32.1. $C_{12}H_{12}O_4N_2SAs_2 \cdot HCl$ requires N=6.0; Cl=7.6; S=6.9; As=32.1 per cent.).

(b) *Reduction with Hypophosphorous Acid*.—0.88 Gram of 3-amino-

4-hydroxyphenylarsinic acid and 1.12 grams of its 5-sulphino-derivative were reduced in a solution made up from 10 c.c. of hypophosphorous acid (D 1.14), 5 c.c. of glacial acetic acid, 5 c.c. of water, and a crystal of potassium iodide, at 100° for one hour. The insoluble yellow solid was collected by centrifuging, suspended in recently boiled water, and again centrifuged, then treated twice with excess of 2*N*-hydrochloric acid and centrifuged each time. Finally, the hydrochloride was suspended twice in air-free water, centrifuged, and dried in a vacuum. The yield of the initial base was quantitative, the yield of the hydrochloride was smaller (1 gram), owing to losses in washing (Found: N=6.0; Cl=7.7; S=6.9; As=31.7 per cent.). The qualitative reactions are indistinguishable from those of the corresponding monosulpho-derivative described below.

3 : 3'-Diamino-4 : 4'-dihydroxy-5 : 5'-disulphoarsenobenzene (VII).

Reduction with Hypophosphorous Acid.—One gram of 3-amino-4-hydroxy-5-sulphophenylarsinic acid was suspended in 5 c.c. of water, 5 c.c. of glacial acetic acid, and 5 c.c. of hypophosphorous acid (D 1.14). A crystal of potassium iodide was added and the temperature raised to 55°. On stirring, the sulphonic acid dissolved and reduction to the arsenobenzene was rapid. After one hour, the arsenobenzene was collected by centrifuging, suspended in recently boiled water, and centrifuged four times. The final supernatant liquor was barely acid to litmus. The yield was quantitative (Found: N=5.4; S=12.6; As=28.5. $C_{12}H_{12}O_8N_2S_2As_2$ requires N=5.3; S=12.2; As=28.5 per cent.).

3 : 3'-Diamino-4 : 4'-dihydroxy-5 : 5'-disulphoarsenobenzene is insoluble in water and does not form salts with acids. It diazotises, forming a deep yellow solution and couples then with β -naphthol. It is soluble in 2*N*-sodium hydroxide solution, but is precipitated by a slight excess. Its solution in sodium carbonate is precipitated by excess of concentrated sodium carbonate solution, and likewise its solution in dilute ammonia by concentrated ammonia solution. This precipitation is apparently a coagulating or flocculating property of the hydroxyl ion. The compound dissolves readily in sodium hydrogen carbonate solution and is not precipitable by a saturated solution of this reagent. It is not appreciably soluble in saturated sodium acetate solution, but dissolves on dilution. The ammoniacal solution gives an immediate and copious gelatinous precipitate with calcium and barium chlorides, with magnesia mixture, and with lanthanum or thorium nitrate. With lithium chloride the precipitate is slight.

Hydrolysis of 3 : 3'-Diamino-4 : 4'-dihydroxy-5 : 5'-disulphoarsenobenzene.—One gram of the sulphoarsenic acid was reduced as described above to the arsenobenzene. The still damp solid was dissolved in 20 c.c. of hot water by addition of 2 c.c. of saturated sodium acetate solution and heated in a sealed tube for ten hours at 100°. The red solution was made acid to Congo-paper with hydrochloric acid and filtered. The colourless filtrate was extracted with ether, which only removed acetic acid. The aqueous solution was reduced by saturation with sulphur dioxide, the excess removed from the warmed solution in a vacuum, and the arsenic completely removed as arsenious sulphide by passing hydrogen sulphide. The filtered solution on concentration gave 0.3 gram of pure 6-aminophenol-*o*-sulphonic acid, crystallising in long, silky needles (Found : in air-dried solid, $H_2O=4.2$; in anhydrous material, $N=7.5$. $C_6H_7O_4NS, \frac{1}{2}H_2O$ requires $H_2O=4.5$; $C_6H_7O_4NS$ requires $N=7.4$ per cent.). It differs from 6-aminophenol-*m*-sulphonic acid, with which a direct comparison was made, in forming a very sparingly soluble barium salt, in its slighter stability in alkaline solution, in forming a hydrate, and in not forming a sparingly soluble diazo-oxide.

3 : 3'-Diamino-4 : 4'-dihydroxy-5-sulphoarsenobenzene Hydrochloride.

Reduction with Hypophosphorous Acid.—1.15 Grams of 3-amino-4-hydroxy-5-sulphophenylarsinic acid and 0.85 gram of 3-amino-4-hydroxyphenylarsinic acid were reduced with a mixture of 10 c.c. of hypophosphorous acid (D 1.14), 5 c.c. of water, and 5 c.c. of glacial acetic acid at 60°. A crystal of potassium iodide was added as a catalyst. Both acids dissolved at this temperature and the quantitative separation of the mixed arsenobenzene was complete at the end of one hour. The working up of the product as hydrochloride for analysis was exactly as described above for the corresponding mixed sulphino-derivative. The yield was 1.8 grams (Found : $N=5.7$; $Cl=7.1$; $S=6.9$; $As=30.8$. * $C_{12}H_{12}O_5N_2SAs_2, HCl$ requires $N=5.8$; $Cl=7.3$; $S=6.6$; $As=31.1$ per cent.).

3 : 3'-Diamino-4 : 4'-dihydroxy-5-sulphoarsenobenzene hydrochloride is sparingly or slowly soluble in water. It is soluble in 2*N*-sodium hydroxide solution and is precipitated by excess. It is, however, soluble in dilute or concentrated ammonia. It only dissolves slowly in sodium carbonate solution and is not precipitated by excess of the saturated solution. It is insoluble in sodium hydrogen carbonate solution. The ammoniacal solution forms gelatinous

* Estimated gravimetrically by the Carius method as $Mg_2As_2O_7$. Lehmann's method gave low results.

precipitates with calcium, barium, or magnesium chloride, but with lithium chloride only on adding excess.

3 : 3'-Diamino-4 : 4'-dihydroxy-5-sulpho-5'-sulphinoarsenobenzene.

Reduction of an equimolecular mixture of the sulpho- and sulphinoarsinic acids with hypophosphorous acid at 100° proceeded normally. The yield was quantitative. The product is indistinguishable in its qualitative reactions from either the disulphoarsenobenzene or the disulphinoarsenobenzene (Found : N=5.4; S=12.1; As=29.5. $C_{12}H_{12}O_7N_2S_2As_2$ requires N=5.5; S=12.6; As=29.4 per cent.).

The Constituents of Salvarsan insoluble in Methyl Alcohol.

9.5 Grams of a commercial salvarsan A containing 2.7 per cent. of sulphur were dissolved in 29 c.c. of dry methyl alcohol (compare Fargher and Pyman, *loc. cit.*) and kept overnight in absence of air and moisture. The solidified contents were broken up and centrifuged in stoppered tubes, the supernatant liquor syphoned off, and the yellow solid mixed with 30 c.c. of dry methyl alcohol. The contents were again centrifuged and submitted to fresh quantities of methyl alcohol five times in all. The final product was dried in a vacuum and then weighed 2.3 grams or 24 per cent. of the weight of the starting material. The finely powdered solid had a bright yellow colour (Found : N=5.3; Cl=7.25; S=5.7. A second preparation from the same original batch of material gave N=5.3; Cl=7.4; S=5.8; As=30.0*; whence the ratio of the atoms is N : As : S : Cl=2 : 2.1 : 0.94 : 1.1. Fargher and Pyman, for the small quantity of material isolated by them, found Cl=6.8; S=5.1; As=30.8. The mixed sulphinoarsenobenzene hydrochloride, $C_{12}H_{12}O_4N_2SAs_2.HCl$, requires N=6.0; Cl=7.6; S=6.9; As=32.1 per cent., whilst the mixed sulphoarsenobenzene hydrochloride, $C_{12}H_{12}O_5N_2SAs_2.HCl$, requires N=5.8; Cl=7.3; S=6.6; As=31.1 per cent.). The approximate agreement with these monohydrochlorides is fortuitous, for the product contained a portion of its sulphur in the form of sulphate. Examination of several samples of the constituent insoluble in methyl alcohol from other batches of commercial salvarsan showed that in general this insoluble constituent is a mixture, and consists of 3 : 3'-diamino-4 : 4'-dihydroxy-5-sulphinoarsenobenzene monohydrochloride (the possibility of the presence of the corresponding sulpho-derivative is not excluded, since there is no means of detecting one in the presence of the other, the iodine absorption figures proving valueless) and

* By Carius as $Mg_2As_2O_7$.

“salvarsan sulphate” and “hydrochloride.” The latter are probably chiefly present as the mixed salts analogous to the well-known mixed salts of dibasic quinine, such as $2Q, H_2SO_4, 2HBr$. There is no evidence of the presence of a monosulphamic acid, as suggested by Fargher and Pyman. These statements are supported by the following evidence.

Twenty-one grams made up from thirty-five samples, each 0.6 gram, of successive batches of a well-known make of salvarsan, were dissolved or suspended in 63 c.c. of dry methyl alcohol for twenty-four hours and then worked up for the insoluble constituent exactly as described above. There were obtained 1.8 grams of dry solid, which on analysis gave N=5.6; total S=3.3; S as sulphate=1.9; Cl=8.4; As=31.5 per cent., whence the atomic ratios are

N.	As.	Cl.	Total S.	Free S as SO_4 .	Combined S.
2	2.09	1.18	0.51	0.29	0.22

On the assumption that the constituents are as stated above, it is evident that out of 200 NH_2 -groups 118 are combined with hydrochloric acid, 58 with sulphuric acid, and 22 neutralised internally by the sulphinic acid group, that is to say, the acid groups present are equivalent within 1 per cent. to the NH_2 -groups.

Reactions of the Substance insoluble in Methyl Alcohol.—It dissolves in water completely, first passing through a gelatinous state. On addition of neutral barium chloride solution there is immediate separation of barium sulphate, and, after centrifuging, the clear supernatant liquor gives no further separation of barium sulphate on heating, either alone or with acids or alkalis. This proves the absence of the sulphamo-group, $\cdot NH \cdot SO_3H$ (Traube, *Ber.*, 1890, **23**, 1653). The presence of “salvarsan sulphate” in a soluble state is at first surprising, as the pure substance is insoluble in cold water. Experiment has, however, shown that a preparation of the pure sulphate dissolves readily on the addition of dilute hydrochloric acid or sodium chloride solution and is readily soluble up to 33 per cent. in “salvarsan hydrochloride” solution.

When dissolved in thirty parts of water and treated with 3*N*-hydrochloric acid, a flocculent precipitate was obtained. On collecting this by centrifuging and washing in the centrifuge, first with *N*-hydrochloric acid and then with water, and drying the product, a substance was obtained which contained S=5.7; N=5.9 per cent. There was no free sulphate present. This evidently consists of the mixed arseno-derivative, 3:3'-diamino-4:4'-dihydroxy-5-sulphinoarsenobenzene hydrochloride, in a crude state. Unlike the starting material, it was no longer soluble or only very

Q Q*

slowly so in water, in this respect showing a close resemblance to the synthetic product.

Oxidation of the Constituent insoluble in Methyl Alcohol.—2.35 Grams of the insoluble constituent from salvarsan *A* were oxidised in alkaline solution by hydrogen peroxide, following Ehrlich and Berthelm's process for salvarsan (*Ber.*, 1912, 45, 756). By acidifying to Congo-paper with hydrochloric acid and concentrating, 0.8 gram of 3-amino-4-hydroxyphenylarsinic acid was obtained. The mother-liquors were evaporated to dryness after neutralising with sodium carbonate, the residue was dissolved in a small volume of water, the solution filtered from a small quantity of insoluble material, then made alkaline with ammonia, and treated with barium chloride solution. On heating on the water-bath, a pigmented crystalline barium salt separated, which on acidification gave eventually 0.1 gram of pure 3-amino-4-hydroxy-5-sulphophenylarsinic acid. Its identity was proved by comparison with the pure material obtained by oxidation of the sulphinic acid. Both melted at the same temperature and showed an identical behaviour in the calcium salt, and also when diazotised and coupled, and in other reactions.

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