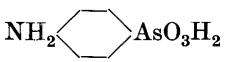
# CLXXVI.—*Trypanocidal Action and Chemical Constitution. Part V. Arylsulphonamides of some Phenylarsinic Acids.*

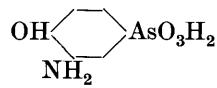
By LESLIE FRANK HEWITT, HAROLD KING, and WILLIAM OWEN MURCH.

PARTS I to IV in this series were almost solely devoted to the preparation, properties, and trypanocidal activity of arylamides of phenylarsinic acids, the common feature of the compounds described being the amide link, –CO·NH–. The present communication is designed to fill the gap in our knowledge of some of the corresponding sulphonamides, with the common link, $-SO_2\cdot NH-$, a group of which only two representatives, both non-amphoteric, have hitherto been described.

*m*-Nitrobenzenesulphonyl and *m*-nitro-*p*-toluenesulphonyl radicals have now been introduced into the amino-group of 4-aminophenylarsinic acid (I), and the former radical into the amino-groups of 3-amino-4-hydroxyphenylarsinic acid (II) and 4-amino-2-hydroxyphenylarsinic acid (III).



(I.)



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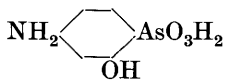
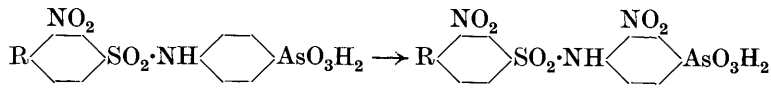


(III.)

For this purpose, the most convenient and by far the most effective method is a modification of the Schotten-Baumann method devised by Fischer and Bergell (*Ber.*, 1902, **35**, 3779) for the preparation of the β-naphthalenesulphonyl derivatives of amino-acids. The reaction with 3-amino-4-hydroxyphenylarsinic acid (II), an acid which is sensitive to caustic alkali, necessitates the use of sodium carbonate, and under these conditions a complex mixture results containing in all probability *O*-sulphonic esters and disulphonamides.

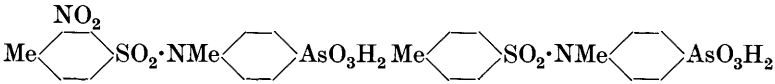
*3′-Nitrobenzenesulphonyl-* (IV; R = H) and *3′-nitro-4′-toluenesulphonyl-4-aminophenylarsinic acid* (IV; R = Me) on further nitration yield exclusively one dinitro-acid (V), the constitution following from the products of hydrolysis.



(IV.) (V.)

*3′-Nitro-4′-toluenesulphonyl-4-methylaminophenylarsinic acid* (VI), which is the methylation product of (IV; R = Me), was obtained by

the action of methyl sulphate and alkali at 100°. It was unobtainable by methylation at room temperature, although *4′-toluenesulphonyl-4-methylaminophenylarsinic acid* (VII) was readily formed under these conditions. The latter acid on solution in sulphuric acid at room temperature was completely hydrolysed and gave an




(VI.) (VII.)

80% yield of 4-methylaminophenylarsinic acid, an acid which is not new. This is, however, the best method for its preparation.

The seven nitro- and dinitro-arsinic acids aforementioned on reduction with ferrous chloride and alkali gave the corresponding amino-compounds quite smoothly and in good yield. When tested for trypanocidal activity on mice infected with *Trypanosoma equiperdum*, they were found to be without exception completely inactive on any dose, including the maximum tolerated, although in structure they differ from the active amides of previous communications only in the replacement of –CO·NH– by –$SO_2$·NH–. This is a surprising result. It might be ascribed to the presence of the strongly acidic hydrogen atom of the –$SO_2$·NH– group in comparison with that contained in the –CO·NH– group, but this was negatived by the inactivity of *3′-amino-4′-toluenesulphonyl-4-methylaminophenylarsinic acid* derived from (VI) by reduction. It is, however, possible to proceed a step further in the analysis of this anomaly.

Ehrlich first advanced the theory that the real active trypanocidal agent in arsenicals is the tervalent arsenic stage, basing it on the two groups of observations, that cures of small animals artificially infected with trypanosomes could be effected by primary quinquevalent arsinic acids and by the corresponding tervalent arseno-compounds and arsenious oxides, but *in vitro*, *i.e.*, outside the animal body, arsinic acids and arseno-compounds had negligible action on trypanosomes, whereas the oxides were very highly active. The view of the importance of the tervalent arsenious oxide has received further support from the experiments of Voegtlin and his associates in Washington, who show that animal tissues are able to reduce the arsinic acids and oxidise the arseno-compounds to the arsenious oxide stage :

$$R{\cdot}AsO_3H_2 \longrightarrow R{\cdot}AsO \longleftarrow R{\cdot}As{=}As{\cdot}R.$$

It was therefore of importance to test whether the oxides corresponding to the above-described inactive acids containing the sulphonamide group were trypanocidally active. *3′-Amino-4′-*

*toluenesulphonyl-* and 3 : 3′-*diamino*-4′-*toluenesulphonyl*-4-*aminophenylarsenious oxides* were prepared by reduction of the corresponding acids with sulphur dioxide and hydriodic acid, and the former oxide was examined in detail. It had a maximum tolerated dose of 0·01 mg. per g. of mouse, was thus twenty times as toxic as its parent acid and was completely devoid of trypanocidal activity on the infected animal, but at a dilution of 1 in 10,000 *in vitro* and 30 minutes' exposure, it rendered trypanosomes non-infective to the normal animal. As it can be calculated that this is just one-half the concentration which should obtain in the blood-stream of infected mice when injected intravenously with the maximum tolerated dose, it seems clear that the injected oxide must be de-activated by some body mechanism. Whatever be the ultimate chemical processes involved in the tissues which determine the distribution and fate of these acids or oxides before final excretion, it seems also certain that the sulphonamide linking must confer a general character on these molecules, independent of its orientation with respect to the arsinic acid group, which prevents the oxides from ever attaining a concentration inimical to the continued reproduction of the trypanosomes. Since the toxiphoric grouping is undoubtedly the group containing arsenic, the presence of the $-SO_2\cdot NH-$ group is reflected in an altered distribution of affinities around the arsenic atom. Some light might be thrown on this by precision measurements of the relative reduction potentials of arsinic acids containing the amide link and their analogues with the sulphonamide link.

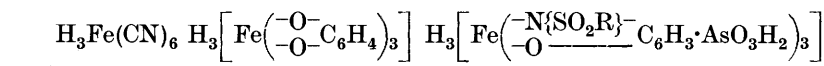
The *arseno*-compounds of 3′-*aminobenzenesulphonyl*-4-*aminophenylarsinic acid* and 3′-*amino*-4′-*toluenesulphonyl*-4-*aminophenylarsinic acid* are white, whereas the arseno-compounds of their benzamide analogues are orange and almost all previously recorded arseno-compounds are yellow or orange. The colour of arseno-compounds is usually ascribed to the presence of the chromophoric grouping –As=As– analogous to the azo-chromophore, –N=N–. The view might therefore be advanced that the loss of colour in the white arsenobenzenes is due to formation of a bimolecular complex.

$$\begin{matrix} R\cdot As{=}As\cdot R \\ R\cdot As{=}As\cdot R \end{matrix} \longrightarrow \begin{matrix} R\cdot As{-}As\cdot R \\ R\cdot As{-}As\cdot R \end{matrix}$$

Michaelis and Schafer (*Ber.*, 1913, **46**, 1742) had found that the molecular weight of crystalline arsenobenzene and arsenotoluene in phenol as cryoscopic solvent agreed with the simple molecular weight. In the present instances, however, the experimental difficulties due to the peculiar properties of the substances have proved insurmountable.

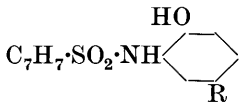
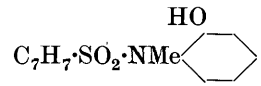
In connexion with some of the compounds described in this communication, the observation has been made that compounds containing a hydroxyl group ortho to a sulphonamide group give a striking series of colour reactions with iron, nickel, cobalt, and copper salts in sodium hydrogen carbonate solution, which are destroyed by excess of caustic alkali or free mineral acid. The cobalt solutions show an absorption band in the yellow-green parts of the spectrum. If a purple alkaline solution of the iron complex of *3′-aminobenzenesulphonyl-3-amino-4-hydroxyphenylarsinic acid*, for instance, be made neutral or weakly acid to litmus, a grey-black precipitate is formed, free from the anion of the precipitating acid, but with an iron–sulphur atomic ratio of approximately 1 : 1 and re-soluble in sodium hydrogen carbonate solution at least in part, with production of the original purple colour. The most reasonable view of the nature of the constituents of the coloured alkaline solutions is that a series of complex anions is formed analogous to the catechol complexes described by Weinland and Binder (*Ber.*, 1912, **45**, 148) and based, at least in the case of iron, on the ferricyanide or ferrioxalate model; but whereas the iron–catechol complexes are of a stability to alkali comparable with that of potassium ferricyanide, the iron–*o*-hydroxysulphonamide complexes seem to be of a stability probably inferior to that of the ferrioxalates.

On this view, the acidic hydrogen atoms of the $-SO_2\cdot NH-$ and $-OH$ groups enter into the co-ordination complex.



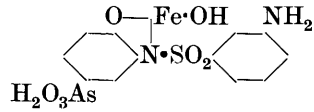
Ferricyanide. Catechol complex. *o*-OH sulphonamides.

In support of this it is found that the benzamide analogues described in Part IV do not give these colour reactions, that *N-p*-toluenesulphonyl-*o*-aminophenol (VIII; R = H) and *toluenesulphonyl-3-amino-4-hydroxybenzoic acid* (VIII; R = $CO_2H$) give the colour reactions, but that N-p-*toluenesulphonyl*-o-*methylaminophenol* (IX) does not.

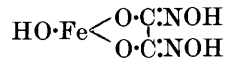
(VIII.)   (IX.)

Incidentally Reverdin's so-called *O*-toluenesulphonyl-*N-p*-methylaminophenol (*Ber.*, 1909, **42**, 1523) has been shown to be *N*-toluenesulphonyl-*N-p*-methylaminophenol. The greyish-black precipitate previously mentioned as being obtained on acidification of the purple iron solutions possibly has the constitution (X) analogous to the ferric oxalohydroxamate (XI) of Hantzsch and Desch (*Annalen*,

1902, **323**, 24), but development of the purple colour by solution of (X) in alkali cannot be mere formation of a soluble salt of the arsinic



(X.)

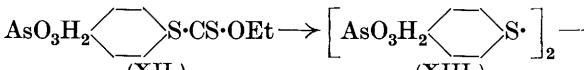


(XI.)

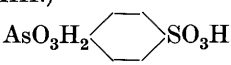
acid grouping, because the same colour is obtained from (VIII; R = H), where the salt-forming group is absent.

4-Amino-2-hydroxyphenylarsinic acid (III), its 3′-*nitrobenzenesulphonyl*- and 3′-*aminobenzenesulphonyl*-derivatives also give colour reactions with ferric and cupric salts in sodium hydrogen carbonate solution. It is possible that in these cases also co-ordination compounds, involving the arsinic acid and adjacent hydroxyl groups, are formed.

In this communication it is convenient to incorporate some observations on one of the simplest aromatic arsinic acids containing the sulpho-group, namely, p-*sulphophenylarsinic acid*. In 1919, one of the authors (K.) made an unsuccessful attempt to prepare the thiol analogue of salvarsan, and the experiments which follow arose out of that attempt. *p*-Aminophenylarsinic acid was converted into p-*xanthylphenylarsinic acid* (XII) by Leuckardt's method (*J. pr. Chem.*, 1890, **41**, 179) and this on oxidation on the boiling water-bath with 3*N*-nitric acid gave a mixture of the sparingly soluble *diphenyl disulphide* pp′-*diarsinic acid* (XIII) and the extremely soluble p-*sulphophenylarsinic acid* (XIV).



(XII.) (XIII.)



(XIV.)

The latter acid is, however, more readily obtained by oxidation of the disulphide by boiling 6*N*-nitric acid. The disulphide and the sulpho-acid proved to be devoid of trypanocidal action. The sulpho-acid was converted into the corresponding *arseno-acid* by reduction with hypophosphorous acid, but owing to its very ready solubility in water this could not be isolated sufficiently pure for physiological testing. Dr. Voegtlin has also prepared this sulpho-acid and its arseno-derivative (*Physiological Reviews*, 1925, **5**, 91), but he informed us verbally that his method for the preparation was different from ours.

We are indebted to Miss F. M. Durham and Miss J. Marchal of this department for the painstaking care with which they have determined the toxicities and trypanocidal action of the compounds described in this paper.

## EXPERIMENTAL.

*4'-Toluenesulphonyl-4-aminophenylarsinic Acid.*—This acid, briefly described by Little, Cahen, and Morgan (J., 1909, **95**, 1482) as being obtained by the Schotten–Baumann reaction, may be obtained in 74% yield by the Fischer–Bergell method of arylsulphonylation. Sodium *p*-aminophenylarsinate pentahydrate (1 mol.) dissolved in 5 parts of water was shaken with finely-powdered *p*-toluenesulphonyl chloride (2 mols.) and a few drops of ether for 30 minutes. *N*-Sodium hydroxide (1 mol.) was then added, and the mixture again shaken for 30 minutes. This sequence was repeated until 3 molecular proportions of *N*-alkali had been added. The arsinic acid obtained on acidification was sparingly soluble in boiling water, crystallising in microscopic rectangular plates, but readily soluble in boiling 90% formic acid, crystallising therefrom in needles and diamond-shaped plates.

*4'-Toluenesulphonyl-4-methylaminophenylarsinic Acid* (VII).—4'-Toluenesulphonyl-4-aminophenylarsinic acid (1 mol.) was dissolved in 2*N*-sodium hydroxide (2 mols.) and heated in the boiling water-bath with vigorous stirring while methyl sulphate (16 mols.) and an equivalent quantity of 2*N*-sodium hydroxide were run in simultaneously, the reaction of the fluid being faintly alkaline throughout. On acidification a 90% yield of the methylated acid was obtained. It is readily soluble in boiling water and crystallises in needles which are unstable and pass readily into large, hexagonal plates. From alcohol it crystallises in needles and from 90% formic acid, in which it is extremely readily soluble, in microscopic, rectangular plates (Found: S, 8·0. $C_{14}H_{16}O_5NSAs$ requires S, 8·3%). This acid can also be obtained by methylation at room temperature.

*4-Methylaminophenylarsinic Acid.*—*p'*-Toluenesulphonyl-4-methylaminophenylarsinic acid was dissolved in 3½ volumes of concentrated sulphuric acid at room temperature, kept for 30 minutes, and then poured on to ice. The mixture having been made neutral to Congo paper, an 80% yield of *N*-methylaminophenylarsinic acid was obtained (Found: As, 32·6. Calc.: As, 32·4%). It is very soluble in *N*-hydrochloric acid and with nitrite gives an instant precipitate of fine needles of the nitroso-compound. On slight dilution, these rapidly transform into six-sided plates which give the Liebermann nitroso-reaction. This acid had a tolerated dose of 0·05 mg. per g. of mouse, and on this dose mice infected with *T. equiperdum* relapsed in the most favourable cases within 7 days.

*Sodium 3-Nitro-4-toluenesulphonate.*—The following process is an improvement on that of Otto and Gruber (*Annalen*, 1868, **145**, 23)

and of Fichter and Bernouilli (*Ber.*, 1909, **42**, 4309). *p*-Toluenesulphonic acid (100 g.) was heated with 380 c.c. of nitric acid (*d* 1·42) in the boiling water-bath for 30 minutes. The solution was poured into a litre of water and rapidly evaporated to dryness under reduced pressure. The residue, which crystallised in envelope-shaped plates, was re-evaporated with small quantities of water to remove all nitric acid, and the solution then neutralised with sodium carbonate. On concentration, a 72% yield of the *monohydrated sodium* salt was obtained (Found: Loss at 100°, 7·3. $C_7H_6O_5NSNa,H_2O$ requires $H_2O$, 7·0%. Found on dried salt: Na, 9·9. $C_7H_6O_5NSNa$ requires Na, 9·6%). The *barium* salt, which is less soluble than the sodium salt and crystallises in creamy, glistening leaflets, was prepared by addition of barium chloride to a solution of the sodium salt (Found: Loss at 100°, 6·2. $C_{14}H_{12}O_{10}N_2S_2Ba,2H_2O$ requires $H_2O$, 6·0%. Found on dried salt: Ba, 24·3. $C_{14}H_{12}O_{10}N_2S_2Ba$ requires Ba, 24·1%).

In another experiment using 15 g. of acid and 50 c.c. of nitric acid, the solution obtained on pouring the product into water was extracted thrice with ether. The ethereal extract was freed from acids by extraction with alkali and on evaporation gave 0·75 g., a 6·3% yield, of *p*-nitrotoluene. The main bulk melted at 54°, as did a mixture with pure *p*-nitrotoluene, but a trace melted 10° higher, suggesting the presence of 2 : 4-dinitrotoluene.

3′-*Nitro*-4′-*toluenesulphonyl*-4-*aminophenylarsinic Acid* (IV; R = Me).—Sodium *p*-aminophenylarsinate was submitted to the action of *m*-nitro-*p*-toluenesulphonyl chloride (2 mols.) by the Fischer–Bergell process described above. The yield of required acid was 77%. It is sparingly soluble in boiling water and crystallises therefrom in minute, rectangular plates (Found: S, 7·8. $C_{13}H_{13}O_7N_2AsS$ requires S, 7·7%). The maximum tolerated dose for mice is 0·3 mg. per g. of mouse and subsequent toxicities are expressed on the same basis.

3′-*Nitro*-4′-*toluenesulphonyl*-4-*methylaminophenylarsinic Acid* (VI).—This acid was obtained in 93% yield by methylation of the preceding acid by the process described for its analogue without the nitro-group. It is not formed in recognisable quantity by methylation at room temperature, using a large excess of methyl sulphate and a variety of concentrations of potassium or sodium hydroxide. It is soluble in 200 parts of boiling water and crystallises in feathery needles, but from alcohol in large, hexagonal plates and from 90% formic acid in diamond-shapedplates (Found: S, 7·3. $C_{14}H_{15}O_7N_2SAs$ requires S, 7·4%). The maximum tolerated dose is 0·03 mg.

3′-*Amino*-4′-*toluenesulphonyl*-4-*aminophenylarsinic Acid*.—The nitro-acid (8·3 g.) was reduced with ferrous chloride and alkali as

described in previous papers of this series. The alkaline extracts (0·2*N*-NaOH) of the ferric hydroxide precipitate were neutralised to Congo-paper, and the crystalline amino-acid which separated was kept over-night. It was collected, dissolved in 140 c.c. of *N*-hydrochloric acid at 50°, and when precipitated by careful addition of saturated sodium acetate separated in small, prismatic needles. The first precipitation mother-liquors treated at 100° with ammonia and magnesium chloride gave a small quantity of *magnesium* salt which gave a further small proportion of the required amino-acid. The total yield was 73% (Found : S, 8·3. $C_{13}H_{15}O_5N_2SAs$ requires S, 8·3%). This *amino-acid* is readily soluble in 2*N*- or 3*N*-mineral acids, the *hydrochloride* being precipitated in small, oblique rhombs by concentrated hydrochloric acid. The maximum tolerated dose is 0·2 mg.

3′-*Amino*-4′-*toluenesulphonyl*-4-*aminophenylarsenious Oxide*.—The foregoing acid (1·8 g.) was dissolved in 10·8 c.c. of water with the aid of 8·2 c.c. of concentrated hydrochloric acid. On addition of 0·45 g. of potassium iodide in a few drops of water there was rapid separation of iodine and a brown gum, but on passing sulphur dioxide for successive ½-hour intervals with intermediate kneading of the now pale yellow gum, it eventually crystallised completely in almost quantitative yield. It was titrated by Ehrlich and Bertheim's method for *p*-aminophenylarsenious oxide (*Ber.*, 1910, **43**, 917) (Found : 0·2185 g., dry, required 9·02 c.c. of *N*/10-iodine. Calc. for $C_{13}H_{13}O_3N_2SAs$ : 9·06 c.c.). The agreement is fortuitous, as the product contained hydrochloride. It was dissolved in water, and the free *oxide* precipitated as a white, amorphous powder by addition of sodium hydrogen carbonate (Found : 0·2132 g. equiv. to 11·53 c.c. of *N*/10-iodine. Calc. : 12·04 c.c. Whence the purity was 96%). This arsenious oxide reduces ammoniacal silver solution only on warming. It is readily soluble in *N*-hydrochloric acid and diazotises and couples in the usual way. It is also readily soluble in 0·5*N*-sodium carbonate and 2*N*-ammonia but not in sodium hydrogen carbonate.

3′-*Aminotoluenesulphonyl*-4-*aminoarsenobenzene*.—The foregoing arsenious oxide (1·25 g.) was dissolved in water (6 c.c.) with addition of 6 c.c. of hypophosphorous acid (*d* 1·137) and a crystal of potassium iodide. The solution was stirred and the temperature slowly raised to 80°. The arsenobenzene separated in microscopic needles which were centrifuged off, washed first with water and then with sodium hydrogen carbonate solution, and finally with several changes of water. The crystalline character was retained throughout. The yield was 1·1 g. The same compound was also obtained in the crystalline state very readily by reduction of the corresponding acid

at 50—55° (Found : 0·1158 g. required 13·9 c.c. of *N*/10-iodine in the presence of sodium acetate. Calc. : 13·7 c.c. Found : As, as $Mg_2As_2O_7$, 22·6. $C_{26}H_{26}O_4N_4As_2S_2$ requires As, 22·3%). This arseno-compound is white or of a pale cream colour. It is readily soluble in molten phenol, but insoluble in acetic acid. It is insoluble in mineral acids, but dissolves on addition of nitrite and then couples in the usual way. It dissolves in an equivalent amount of sodium hydroxide, but is precipitated as the sodium salt by a slight excess. It is also slowly soluble in sodium carbonate solution. The dried powder is very easily electrified and consequently very difficult to handle. The maximum tolerated dose is 0·1 mg.

3′-*Amino*-4′-*toluenesulphonyl*-4-*methylaminophenylarsinic Acid*.—This acid was obtained in 75·3% yield by reduction of the corresponding nitro-acid with ferrous chloride and alkali. It was purified by solution in *N*-hydrochloric acid and precipitation by sodium acetate (Found : S, 8·1. $C_{14}H_{17}O_5N_2SAs$ requires S, 8·0%). This acid crystallises readily from boiling water in clusters of thin plates. It is readily soluble in warm *N*-hydrochloric acid, the *hydrochloride* crystallising in prisms. The *sulphate* and *nitrate* are sparingly soluble in their respective *N*-acids and both crystallise in fine needles. The maximum tolerated dose is 0·03 mg.

3 : 3′-*Dinitro*-4′-*toluenesulphonyl*-4-*aminophenylarsinic Acid* (V; R = Me).—This acid was prepared in 73% yield from 8·3 g. of 3′-nitrotoluenesulphonyl-4-aminophenylarsinic acid, dissolved in 25 c.c. of sulphuric acid and nitrated with 2 g. of nitric acid (*d* 1·42) in 2 g. of sulphuric acid. It crystallises from boiling water, in which it is sparingly soluble, in very small needles, and from glacial acetic acid, in which it is moderately soluble, in bunches of small, prismatic needles (Found : S, 6·9. $C_{13}H_{12}O_9N_3SAs$ requires S, 6·9%). The maximum tolerated dose is 0·1 mg.

3 : 3′-*Diamino*-4′-*toluenesulphonyl*-4-*aminophenylarsinic Acid*.—This acid was prepared by reduction of the preceding dinitro-acid (9·2 g.) with ferrous chloride and alkali. The alkaline extracts of the ferric hydroxide gave 2·7 g. of acid by direct precipitation and 2·0 g. by way of the *magnesium* salt in hot ammoniacal solution. The total yield was 60%. This *diamino-acid*, precipitated from acid solution by sodium acetate, separates in small, rhomb-shaped plates. It is very soluble in dilute mineral acids and diazotises and couples with alkaline β-naphthol with a blood-red colour (Found : As, 18·9. $C_{13}H_{16}O_5N_3SAs$ requires As, 18·7%). The maximum tolerated dose is 0·5 mg.

3 : 3′-*Diamino*-4′-*toluenesulphonyl*-4-*aminophenylarsenious Oxide*.—One g. of the preceding acid was dissolved in 4·5 c.c. of con-

centrated hydrochloric acid and 6 c.c. of water. Potassium iodide (0·25 g.) was added and sulphur dioxide passed for an hour. A crystalline salt separated in long, yellow needles (Found for anhydrous material : 0·1138 g. equiv. to 3·57 c.c. of $N$/10-iodine. Calc. for the pure dihydriodide : 3·97 c.c.). The remainder was dissolved in water, and the oxide precipitated with sodium hydrogen carbonate. The yield was 0·4 g. (Found for anhydrous oxide : 0·1623 g. equiv. to 8·41 c.c. of $N$/10-iodine. $C_{13}H_{14}O_3N_3SAs$ requires 8·84 c.c. Hence the purity was 95%).

3′-*Nitrobenzenesulphonyl*-4-*aminophenylarsinic Acid* (IV; R = H).—This acid was prepared from sodium *p*-aminophenylarsinate (1 mol.) and finely powdered nitrobenzenesulphonyl chloride (2 mols.) by the Fischer–Bergell method. The yield was 69% (Found : S, 7·7. $C_{12}H_{11}O_7N_2SAs$ requires S, 8·0%). It is soluble in boiling 90% formic acid, and crystallises in short prisms, less readily in boiling acetic acid and very sparingly in boiling water. The maximum tolerated dose is 0·4 mg.

3′-*Aminobenzenesulphonyl*-4-*aminophenylarsinic Acid*.—The corresponding nitro-acid was reduced by ferrous chloride and alkali in the usual way at 0°, and the amino-acid isolated through its *magnesium* salt in hot ammoniacal solution. The yield was 70%. It is best purified as its *hydrochloride*, which is only moderately soluble in cold water and separates readily on addition of stronger acid in rhomb-shaped plates (Found : Cl, 8·5. $C_{12}H_{13}O_5N_2SAs,HCl$ requires Cl, 8·7%). On addition of saturated sodium acetate solution to a warm concentrated solution of the hydrochloride, the *aminoarsinic acid* separates as a gum which rapidly crystallises, on rubbing, in long, narrow plates with sloping ends. It is moderately soluble in cold water. The maximum tolerated dose is 0·5 mg.

3′-*Aminobenzenesulphonyl*-4-*aminoarsenobenzene*.—This was prepared from the corresponding acid by means of hypophosphorous acid at 45—50° in the usual way (Found : As, 23·6. $C_{24}H_{22}O_4N_4S_2As_2$ requires As, 23·3%). It is a white or creamy-white, amorphous powder when dry, soluble in sodium hydroxide, and slowly soluble in sodium carbonate solution. It dissolves in molten phenol, but the solution soon becomes turbid and finally fills with a finely-divided precipitate.

3 : 3′-*Dinitrobenzenesulphonyl*-4-*aminophenylarsinic Acid* (V; R = H).—3′-Nitrobenzenesulphonyl-4-aminophenylarsinic acid (10·0 g.) in 30 c.c. of sulphuric acid was slowly treated at 0° with nitric acid (1·75 c.c.; $d$ 1·42) dissolved in a little sulphuric acid (5 c.c.). After 30 minutes, the solution was poured on to ice. The yield was 93%. This *dinitro-acid* crystallises well from boiling water, in which it is sparingly soluble, in short needles and similarly

from boiling 90% formic acid, in which it is readily soluble (Found : S, 7·2. $C_{12}H_{10}O_9N_3SAs$ requires S, 7·2%). On boiling with 32% hydrochloric acid for 8 hours, it was only partly hydrolysed, giving an 83% yield of 3-nitro-4-aminophenylarsinic acid. No 4-aminophenylarsinic acid could be detected in the mother-liquors. The maximum tolerated dose is 0·1 mg.

3 : 3′ - *Diaminobenzenesulphonyl* - 4 - *aminophenylarsinic Acid.*— Reduction of the corresponding dinitro-acid by ferrous chloride and alkali at 0° gave this *diamino-acid* in 70% yield. It was isolated through the magnesium salt in ammoniacal solution (Found : As, 19·5. $C_{12}H_{14}O_5N_2SAs$ requires As, 19·4%). When liberated from acid solution by addition of sodium acetate, it crystallises in slightly distorted, diamond-shaped plates. The maximum tolerated dose is 1·5 mg.

3′-*Nitrobenzenesulphonyl*-3-*amino*-4-*hydroxyphenylarsinic Acid.*— 3-Amino-4-hydroxyphenylarsinic acid (11·6 g.) was dissolved in water (60 c.c.) with the aid of 3·85 g. of anhydrous sodium carbonate. Nitrobenzenesulphonyl chloride (2 mols.; 22 g.) was added at once and a few drops of ether. The mixture was shaken for 30 minutes, and three successive portions of *N*-sodium carbonate (1 mol.) at 30-minute intervals were then added with intermediate shaking. The solution, on being made definitely acid to Congo-paper, deposited a pale-coloured gum immediately and, after 12 hours in the cold room, a crystalline powder. These were collected and stirred with 75 c.c. of *N*-hydrochloric acid in the boiling water-bath until the non-soluble portion had crystallised. This treatment was essential to remove aminohydroxyphenylarsinic acid carried down from acid solution with the gum. The crystalline, chalky solid obtained on cooling was a complex mixture probably containing *O*- and di-*N*-sulphonylated derivatives (Found : S, 9·0. A disulphonamide requires S, 10·6%). It was dissolved in 20 volumes of 0·5*N*-sodium hydroxide, boiled for 30 minutes, and while still hot treated with concentrated hydrochloric acid so long as an oily turbidity was produced. After brief stirring, the hot solution was freed from oil by filtration through a fluted paper. The filtrate showed no further tendency to deposit oil, but deposited the required nitro-acid in a crystalline state. The original mother-liquors on concentration under reduced pressure gave a succession of crops of the required nitro-acid and unchanged aminohydroxyphenylarsinic acid separable by extraction with *N*-hydrochloric acid. The total yield was 33%. If one molecular proportion of sulphonyl chloride was originally used instead of two, the same difficulties were encountered and the yield was 28%. For analysis, the *acid* was recrystallised from 20 volumes of boiling water, from which it crystallised extremely well

as a *dihydrate* in large, straw-coloured prisms or rhombs (Found : Loss at 95°, 8·4. $C_{12}H_{11}O_8N_2SAs,2H_2O$ requires $H_2O$, 7·9%. Found on anhydrous material : S, 8·0. $C_{12}H_{11}O_8N_2SAs$ requires S, 7·7%). When dissolved in sodium hydrogen carbonate solution and treated with a drop of dilute ferric chloride, it gives an intense purple solution which on addition of caustic alkali becomes redder; the colour is discharged by excess of caustic alkali with separation of ferric hydroxide. On addition of acid, however, to neutrality to litmus, the colour is also discharged with precipitation of a grey, amorphous precipitate which, after separation, redissolves in sodium hydrogen carbonate solution with the same purple colour. In a similar way, cobalt nitrate gives a rose-coloured solution with an absorption band in the yellowish-green part of the spectrum. The reaction is more intense in sodium carbonate solution, but is discharged by excess of acid or caustic alkali. A nickel salt gives a clear yellowish-brown solution, whilst a copper salt gives an intense deep brown solution. The maximum tolerated dose is 0·5 mg.

*3′-Aminobenzenesulphonyl-3-amino-4-hydroxyphenylarsinic Acid.*—The reduction of the foregoing nitro-acid was carried out with ferrous chloride and alkali. The isolation of the pure amino-acid was effected as follows. The filtrate from the ferric hydroxide together with the alkaline extracts (0·2*N*-sodium hydroxide) of the ferric hydroxide was of a deep purple colour, and on being made neutral to Congo-paper deposited, after standing for 12 hours, the required amino-acid mixed with an amorphous, grey complex of ferric hydroxide and the amino-acid. These were collected, dissolved in 2*N*-sodium hydroxide, and filtered from the ferric hydroxide, which separated in part. The filtrate, on being made neutral to litmus, deposited the remainder of the bound iron as the complex, which could be removed, and thereafter the pure amino-acid was obtained on making the filtrate neutral to Congo-paper. The iron complex could be re-submitted to the same treatment with caustic alkali, when ferric hydroxide was again precipitated, but not quite completely, and the filtrate gave a further small crop of iron complex and the pure amino-acid on being made neutral to litmus and Congo-paper, respectively. The original mother-liquors, on concentration under reduced pressure, yielded further crops of pure amino-acid. For physiological testing and analysis, the combined crops of amino-acid were dissolved in *N*-hydrochloric acid and precipitated with saturated sodium acetate solution. The yield was 76% (Found : Loss at 95°, 7·5; S, 7·5. $C_{12}H_{13}O_6N_2SAs,2H_2O$ requires $H_2O$, 8·1; S, 7·6%). It crystallises from boiling water, in which it is fairly readily soluble, in glistening prisms. It is readily soluble in *N*-hydro-

chloric, nitric, or sulphuric acid. The *hydrochloride* crystallises in rectangular tablets, the *sulphate* in short needles, and the *nitrate* in prisms. In sodium hydrogen carbonate solution, the amino-acid gives a purple colour with ferric chloride and on neutralisation to litmus a complex containing iron is precipitated as a dove-grey powder. Some preparations, however, are almost black. This is free from chloride and dissolves in sodium hydrogen carbonate with restoration of the purple colour (Found on air-dried material: Fe, 10·4; S, 6·5, whence Fe : S = 1 : 1·08). The rose-pink colour developed on similar addition of cobalt nitrate is very marked. There is an absorption band in the yellowish-green. A soluble nickel complex is similarly formed with a yellowish-brown colour, and with copper salts a very intense brownish-red solution is obtained showing considerable stability to caustic alkali. The maximum tolerated dose is 0·5 mg.

*3′-Nitrobenzenesulphonyl-4-amino-2-hydroxyphenylarsinic Acid.*—4-Amino-2-hydroxyphenylarsinic acid (11·6 g.) was converted into the sulphonamide by the Fischer–Bergell process, nitrobenzenesulphonyl chloride (2 mols.) being used. The solution, on being made acid to Congo-paper, deposited a gum and, on standing some hours, a crop of crystals. The total precipitate was collected and warmed on the water-bath with *N*-hydrochloric acid; it then became wholly crystalline. It was dissolved in 10 volumes of *N*-sodium hydroxide and heated on the water-bath for 30 minutes. The *nitro-acid* was obtained on acidification in colourless needles rapidly transforming to plates; yield 9·1 g. The original mother-liquors contained additional acid, which was isolated by concentration. This acid is a dihydrate (Found: Loss at 95°, 8·4. $C_{12}H_{11}O_8N_2SAs,2H_2O$ requires $H_2O$, 8·2%. Found on anhydrous material: S, 8·0, 7·6. $C_{12}H_{11}O_8N_2SAs$ requires S, 7·7%). It is soluble in 32 parts of boiling water and separates in very pale buff-coloured rhombs or prisms. (For its colour reactions, see next paragraph.) The maximum tolerated dose is 0·2 mg.

*3′-Aminobenzenesulphonyl-4-amino-2-hydroxyphenylarsinic Acid.*—This acid was prepared from the nitro-acid in 63% yield by means of ferrous chloride and alkali. It tends to form an iron complex in the alkaline extracts of the ferric hydroxide precipitate just as does its isomeride described above. It can be isolated, however, in exactly the same way (Found: S, 8·1. $C_{12}H_{13}O_6N_2SAs$ requires S, 8·3%). It is readily soluble in boiling water and crystallises in tufts of fine needles. In *N*-mineral acids it is very readily soluble. When dissolved in sodium hydrogen carbonate solution, it gives a clear deep yellowish-brown colour with ferric chloride and a bright emerald-green colour with copper sulphate. Under comparable

conditions, the parent nitro-acid gives with ferric chloride a clear reddish-brown solution and with copper salts an olive-green solution. The parent acid of this group, 4-amino-2-hydroxyphenylarsinic acid, gives a reddish-brown solution with ferric salts and an emerald-green solution with copper sulphate. The maximum tolerated dose is 0·025 mg.

N-p-*Toluenesulphonyl*-o-*aminophenol* (VIII; R = H).—When prepared by the process described by Reverdin and de Luc (*Ber.*, 1914, **47**, 1538) for the isomeric derivative of *m*-aminophenol, this sulphonamide was obtained in very good yield and agreed in properties with the description given by Tröger and Ullmann (*J. pr. Chem.*, 1895, **51**, 441). It dissolves on warming with dilute aqueous sodium carbonate; the solution becomes turbid on cooling and gives a bright purple colour on addition of ferric chloride. With cobalt nitrate, it gives a pink colour with an absorption band in the yellow-green.

N-p-*Toluenesulphonyl*-o-*methylaminophenol* (IX) was prepared by extending Reverdin and de Luc's method to *o*-methylaminophenol. *o*-Methylaminophenol sulphate (" Ortol," 4·3 g.) and sodium acetate (6·8 g.; hydrated) were dissolved in 50% alcohol (100 c.c.) and heated for 30 minutes on the water-bath, *p*-toluenesulphonyl chloride (4·75 g.) being added in several portions from time to time. On cooling, N-p-*toluenesulphonyl*-o-*methylaminophenol* (4·6 g.) separated in white needles. It was recrystallised from glacial acetic acid and showed m. p. 127—128° (Found: S, 11·3. $C_{14}H_{15}O_3NS$ requires S, 11·6%). It is readily soluble in caustic alkali but insoluble in dilute acids. It is unchanged by nitrous acid. Its solution in boiling dilute aqueous sodium carbonate becomes turbid on cooling, but gives no purple colour with ferric chloride and no pink colour with cobalt nitrate.

N-4-*Toluenesulphonyl*-3-*amino*-4-*hydroxybenzoic Acid* (VIII; R = $CO_2H$).—Aminohydroxybenzoic acid (1·5 g.) was dissolved in 10 c.c. of pyridine and treated with 2·37 g. of toluenesulphonyl chloride. After 12 hours, the mixture was poured into *N*-hydrochloric acid, the solid collected, and boiled for 30 minutes with *N*-sodium hydroxide. On acidification the sulphonamide separated straightway almost pure in 77% yield. For analysis it (2·3 g.) was recrystallised from the minimum volume (50 c.c.) of 50% (by vol.) acetic acid and separated in glistening, pale straw-coloured, wedge-shaped prisms, m. p. 250° (decomp.) (Found: S, 10·7. $C_{14}H_{13}O_5NS$ requires S, 10·4%). In sodium hydrogen carbonate solution it gives an intense purple colour with ferric chloride and a bright pink colour with cobalt nitrate, the solution showing an absorption band in the yellowish-green part of the spectrum.

N-p-*Toluenesulphonyl*-p-*methylaminophenol* was prepared following Reverdin's directions for his so-called *O*-toluenesulphonyl-*N*-*p*-monomethylaminophenol. It melts at 136—137° (Reverdin gives 135°) and crystallises in needles from dilute alcohol or benzene. It is insoluble in dilute acids and unchanged by nitrous acid, but is readily soluble in sodium hydroxide and in sodium carbonate solution on warming. When dissolved in sodium carbonate solution, it does not give a coloration with ferric chloride.

p-*Xanthylphenylarsinic Acid* (XII).—Sodium *p*-aminophenylarsinate pentahydrate (32·9 g.) was diazotised at 0° in 200 c.c. of water and 30 g. of concentrated hydrochloric acid, and the product run slowly into a rapidly stirred solution of 20 g. of potassium xanthate in 420 c.c. of a 10% solution of hydrated sodium carbonate, kept at 80°. After being stirred for 1 hour, the solution was cooled and acidified. The precipitated resin solidified after a few hours and was submitted to a partial purification by dissolution in sodium carbonate solution, filtration from insoluble by-products, and reprecipitation by acids. The yield of crude xanthate was 62% (Found: S, 18·8. $C_9H_{11}O_4S_2As$ requires S, 19·9%). The acid could not be obtained crystalline nor of a higher sulphur content, but it behaved normally on oxidation.

*Oxidation of* p-*Xanthylphenylarsinic Acid*.—Five g. of crude *p*-xanthylphenylarsinic acid were heated on the water-bath for 2 hours with 100 c.c. of 3*N*-nitric acid and finally to boiling for ½ hour. When cold, the crystalline acid was collected (yield 82%). *Diphenyl disulphide* pp′-*diarsinic acid* (XIII) dissolves in 900 parts of boiling water, from which it crystallises in narrow plates or needles. It crystallises much more readily from 200 parts of 2*N*-nitric acid (Found: As, 32·2; S, 13·9. $C_{12}H_{12}O_6S_2As_2$ requires As, 32·2; S, 13·8%). A 1% solution in 0·2*N*-ammonia treated with 5% barium chloride solution gave, on heating, a *barium* salt crystallising in needles. On cooling, the barium salt rapidly re-dissolved, but was again precipitated on heating. The acid has a maximum tolerated dose of 0·002 mg.

The nitric acid mother-liquors of the above oxidation were evaporated to a syrup with repeated additions of water to remove the excess of nitric acid. They were then diluted and treated with sufficient baryta to precipitate the sulphuric acid present. On evaporation the solution set to a crystalline magma of p-*sulphophenylarsinic acid* (XIV). This was redissolved in water, and the solution evaporated until a crust formed. On keeping, the acid separated in long prisms. These are extremely soluble in water, but can be freed from the mother-liquors by washing with glacial acetic acid. The whole of the *p*-xanthylphenylarsinic acid unaccounted

for as disulphide appears to be present as sulpho-acid. The sulpho-acid was, however, more readily obtained as follows. The disulphide (5 g.) was boiled with 50 c.c. of 6*N*-nitric acid for 4 hours. The clear solution was evaporated dry and left 6·0 g. of crude sulpho-arsinic acid. This was crystallised once from water and washed with glacial acetic acid (Found: Loss at 95°, 5·8. $C_6H_7O_6SAs,H_2O$ requires $H_2O$, 6·0%. Found on anhydrous material: S, 11·6. $C_6H_7O_6SAs$ requires S, 11·4%). This acid also crystallises occasionally in hexagonal plates, possibly an anhydrous form, as it frequently appears as a crust on the surface of hot saturated solutions. The maximum tolerated dose is 0·5 mg.

The disulphide is reduced by hypophosphorous acid in the presence of a trace of iodide to an insoluble, yellow arseno-compound, probably *thiolarsenobenzene*, because it is insoluble in sodium hydrogen carbonate but soluble in caustic alkali. Under similar conditions, at 50—55° *p*-sulphophenylarsinic acid is reduced to p-*sulphophenyl-arsenobenzene*, which is very soluble in water and can be isolated in a crude condition as a yellow, gelatinous product by evaporation of the reduction liquors in a vacuum over sulphuric acid.

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