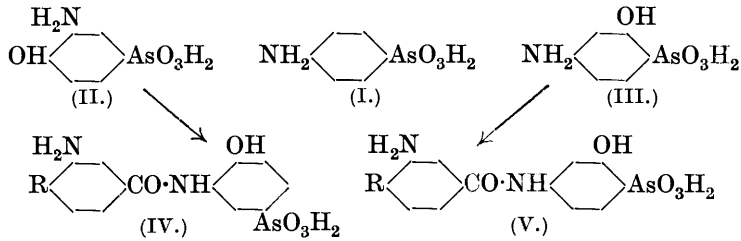
CXII.—*Trypanocidal Action and Chemical Constitution. Part IV. Arylamides of Aminohydroxyphenylarsinic Acids.*

By Leslie Frank Hewitt and Harold King.

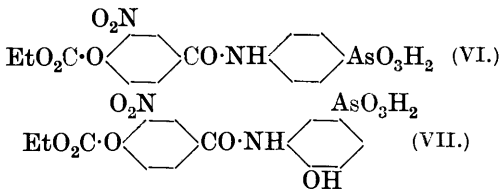
In Parts I and II (King and Murch, J., 1924, **125**, 2595; 1925, **127**, 2632) the preparation and properties of a number of arylamides of 4-aminophenylarsinic acid (I) were described. Inasmuch as Fourneau and his colleagues (*Ann. Inst. Pasteur*, 1923, **37**, 551) have shown that on *Trypanosoma brucei* in mice, 3-amino-4-hydroxyphenylarsinic acid (II) and 4-amino-2-hydroxyphenylarsinic acid (III) with chemo-therapeutic indices (curative dose/tolerated dose) of 1/5 and 1/8 respectively are superior to 4-aminophenylarsinic acid with an index of 1/1, it was thought of interest to introduce into these hydroxy-acids certain substituted aminobenzoyl radicals

which were shown in Part II to have a favourable influence on the trypanocidal activity of 4-aminophenylarsinic acid.



The types (IV) and (V), where R is H, OMe, or OH, were obtained by reduction of the corresponding nitro-acids by ferrous chloride and alkali, the preparation of the nitrohydroxybenzoyl derivatives (R = OH) necessitating the use of ethylcarbonatonitrobenzoyl chloride, where the hydroxyl group is protected by the carbethoxyl group.

Although the ethylcarbonato-group in 3′-nitro-4′-ethylcarbonatobenzoyl-4-aminophenylarsinic acid (VI) is readily hydrolysed with negligible fission at the amide link by brief boiling with *N*-alkali (King and Murch, Part II), in the case of *3′-nitro-4′-ethylcarbonatobenzoyl-3-amino-4-hydroxyphenylarsinic acid* (VII) hydrolysis of this group by cold *N*-alkali leads to appreciable fission of the amide link and much greater fission occurs on rise of temperature.



The maximum dose tolerated by mice, expressed in milligrams per gram of mouse, and the minimum curative dose, where determined, on an experimental infection of *T. equiperdum* in mice, of this group of twelve related compounds is shown below, *R* signifying the para-substituent to the amide group and *r* the number of days before relapse occurred.

Type IV. Derivatives of 3-$NH_2$4-OHC_6H_3·AsO_3H_2.

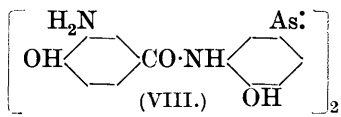
	Nitro-acids.			Amino-acids.		
R	H.	OMe.	OH.	H.	OMe.	OH.
Dosis tolerata	0·075	0·2	0·4	0·3	0·2	0·3
Dosis curativa ...	—	—	—	0·3	0·15	0·3
				(*r* = 13)	(*r* = 4)	(*r* = 8)

Type V. Derivatives of 4-$NH_2$2-OHC_6H_3·AsO_3H_2.

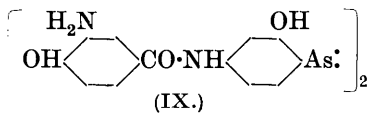
Dosis tolerata	0·2	0·2	0·3	0·1	0·6	0·2
Dosis curativa ...	—	—	—	0·1	0·3	0·2
				(*r* = 12)	(*r* > 30)	(*r* = 12)

On comparing these results with those obtained for the corresponding derivatives of 4-aminophenylarsinic acid (Part II) it will be observed that there is here no enhanced trypanocidal activity such as might be expected from the relative activities of the parent mono-nuclear arsinic acids (I, II, and III). There is, however, a rough parallelism between the activities of the amides of 4-aminophenylarsinic acid and those of 4-amino-2-hydroxyphenylarsinic acid; in each case, the aminoanisoyl derivative is the only one of the three amides with permanent curative properties.* Expressed otherwise, one might say that the introduction of the 2-hydroxyl group into derivatives of 4-aminophenylarsinic acid has not radically altered the distribution of the main and residual affinities of the molecule on which trypanocidal activity as measured by the two determinants, curative dose and tolerated dose, ultimately depends.

The possession by the six complex amino-arsinic acids above described of a free hydroxyl group emphasised the desirability of the preparation of the corresponding arseno-derivatives, which like salvarsan should be administrable by solution in alkali. The comparison of six arseno-bases with the parent amino-acids was all the more a desideratum because Fourneau has expressed the opinion that on the evidence available the arsinic acids are at least as active as the arseno-derivatives. Unfortunately this object was only realisable in part because, unexpectedly, the possession of a single hydroxyl group in the same nucleus as the arseno-group did not confer alkaline solubility, the sodium salts of the aminobenzoyl and aminoanisoyl derivatives being quite insoluble in water. In addition, the hydroxyl group ortho to the arseno-group in derivatives of 4-amino-2-hydroxyphenylarsinic acid weakens the attachment of the arsenic atom to the nucleus, thus leading under very mild conditions of reduction by hypophosphorous acid to formation of products containing polyarsenides. This is perhaps not surprising, because the parent acid, 4-amino-2-hydroxyphenylarsinic acid, when reduced by the same reagent gives no arseno-base but only free arsenic. The hydroxyaminobenzoyl derivatives (VIII) and (IX) were, however, alkali-soluble, the former substance being of



(VIII.)



(IX.)

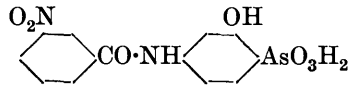
* In this series of communications, the term "permanent curative property" indicates no relapse of some of the animals within 30 days, the blood being examined almost daily over that period. As a rule, each dose is tried on five mice.

special interest because of the duplication of the *o*-aminophenol grouping contained in salvarsan. The maximum tolerated and the minimum curative dose of these two arseno-bases in comparison with their parent arsinic acids are shown below.

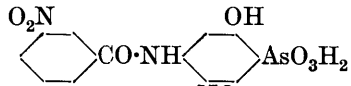
	Acid.	Arseno (VIII).	Acid.	Arseno (IX).
Dosis tolerata	0·3	0·075	0·2	0·075
Dosis curativa	0·3	0·03	0·2	0·02
	($r = 8$)	($r = 5$)	($r = 12$)	($r > 30$)

Whilst there is little difference in activity between the arseno-derivative (VIII) and its parent acid, the arseno-derivative (IX) is permanently curative on one-fourth of its tolerated dose and is thus far superior to its parent acid. It should, however, be borne in mind that the arseno-derivative (IX) contains a certain proportion of polyarsenides, as has been indicated above.

3′-*Nitrobenzoyl*-4-*amino*-2-*hydroxyphenylarsinic acid* (X) on further nitration yields exclusively 3′ : 5-*dinitrobenzoyl*-4-*amino*-2-*hydroxyphenylarsinic acid* (XI), which on reduction yields the corresponding *diaminobenzoylaminohydroxyphenylarsinic acid.*



(X.)



(XI.)

In Part II it was shown that the diamino-arsinic acids were in general permanently curative, but this substance, either on its maximum tolerated dose or on lower doses, was completely devoid of action. Various reasons might be advanced for this, but it is not proposed to discuss them here.

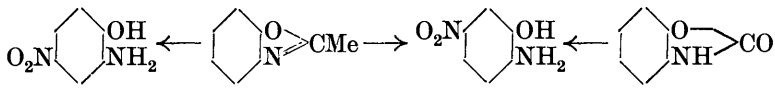
On hydrolysis of (XI) by alkali 5-*nitro*-4-*amino*-2-*hydroxyphenylarsinic acid* was obtained and this on reduction gave a new 4 : 5-*diamino*-2-*hydroxyphenylarsinic acid* of which only one isomeride is known. The activity of this diamino-acid in comparison with the closely related 4-amino-2-hydroxyphenylarsinic acid (III) and 3-amino-4-hydroxyphenylarsinic acid (II) is shown in the following table :

	Diamino-acid.	III.	II.
Dosis tolerata	0·3	0·5	1·5
Dosis curativa	0·05 ($r = 16$)	0·2	0·3

The diamino-acid was very erratic in its curative action and far inferior to (II) or (III). It will be noticed that the indices for (II) and (III) are not as favourable on *T. equiperdum* as those obtained by Fourneau (see opening paragraph) on *T. brucei.*

4-Amino-2-hydroxyphenylarsinic acid (III), required in quantity in this investigation, was prepared by the Bart-Schmidt reaction from 5-nitro-2-aminophenol (Bauer, *Ber.*, 1915, **48**, 1582). The

preparation of the latter from 2-aminophenol presents some points of interest to the student of orientation. Meldola and Wechsler (P., 1900, 180) showed that *ON*-diacetyl-2-aminophenol on attempted mononitration gave exclusively 4 : 6-dinitro-2-aminophenol (picramic acid) after hydrolysis, and we find in support that both mono- and tri-acetyl-2-aminophenol on mononitration yield after hydrolysis almost exclusively picramic acid, although in the case of triacetyl-2-aminophenol traces of both 5-*nitro*- and 4-*nitro*-2-*acetylaminophenol* have been isolated. In D.R.-P. 165650, however, 5-nitro-2-aminophenol is said to be prepared by the nitration of ethenyl-2-aminophenol (XII). An examination of this reaction shows that 5-nitro-2-aminophenol (XIII) is indeed formed together with 4-nitro-2-aminophenol (XIV) in the ratio 4 : 1, the main bulk of the 5-nitro-compound after hydrolysis being separable owing to its lesser solubility in water. The remaining mixture can be separated by fractionally crystallising the acetates from benzene, which obviates both the tendency of the two isomerides to separate from water as brilliant red mixed crystals and the formation of the low-melting hydrate of the 4-nitro-derivative.



(XIV.) (XII.) (XIII.) (XV.)

The sulphonation product of carbonyl-2-aminophenol (XV) is known to yield 2-aminophenol-5-sulphonic acid on hydrolysis (D.R.-P. 197496; King, J., 1921, **119**, 1117), but although 5-nitrocarbonyl-2-aminophenol is prepared by the action of nitric acid on carbonylaminophenol and yields nitrocatechol on alkaline hydrolysis (Chelmicki, *J. pr. Chem.*, 1890, **42**, 441), there is no record of its hydrolysis by acids. This should also lead to the required 5-nitro-2-aminophenol. This has now been effected, but only with difficulty, as 5-nitrocarbonyl-2-aminophenol is very resistant to acid hydrolysis. Of the two possible routes to the required nitroaminophenol, the one *via* the ethenyl derivative proved the more convenient.

Thus, whilst the nitration of any of the acetyl derivatives of 2-aminophenol yields as main product picramic acid in which the orienting power of the hydroxyl may be said to overwhelm that of the acetylated amino-group in agreement with the behaviour of 4-aminophenol derivatives, in ethenyl- and carbonyl-aminophenol the reverse is true. The latter cases are also in agreement with the nitration of another cyclic derivative of 2-aminophenol, acetylphenoxazine, which yields 3 : 9-dinitrophenoxazine, the nitro-

groups entering the para-position to the nitrogen atom (Kehrmann and Saager, *Ber.*, 1903, **36**, 477).

To Miss F. M. Durham and Miss J. Marchal of this department we again tender our thanks for the efficient and painstaking care bestowed on the biological side of our subject.

Experimental.

o-*Aminophenol.*—The large quantities of *o*-aminophenol required were prepared in part by an improvement on Grandmougin's process (*Ber.*, 1906, **39**, 3561). *o*-Nitrophenol dissolved in 2*N*-sodium hydroxide (1 mol.) was treated with solid sodium hyposulphite (1·6 mols.) and sufficient sodium hydroxide to keep the reaction mixture faintly alkaline, the solution being well stirred and the temperature kept below 30°. The yield of *o*-aminophenol, including ether-extracted material, was 67%.

*Nitration of Ethenyl-*o*-aminophenol* (XII).—Ethenyl-*o*-aminophenol was obtained in 90% yield by a simplification of Ladenburg's method (*Ber.*, 1876, **9**, 1524). *o*-Aminophenol (carefully freed from inorganic salts) was distilled with 3 parts of acetic anhydride, and the distillate refractionated through a short column. The fraction, b. p. 200—210°, consisted of ethenyl-*o*-aminophenol. Of this, 53·9 g. were dissolved in sulphuric acid (200 c.c.) below 0° and treated with nitric acid (*d* 1·42; 1·1 mols.) dissolved in an equal volume of sulphuric acid. On pouring on to ice, a bulky yellow compound was obtained, but attempts to isolate a pure nitroethenyl-*o*-aminophenol from this were unsuccessful. (When dried in a vacuum, it lost 90% of its weight and liquefied, and repeated crystallisation from alcohol gave eventually pure 5-*nitro*-2-*acetylaminophenol.*) The wet, bulky precipitate was heated at 100° with 325 c.c. of hydrochloric acid (*d* 1·16) until dissolved, and the solution almost neutralised. Orange needles of 5-nitro-2-aminophenol (37·7 g.) separated immediately. On adjusting the reaction of the filtrate to neutrality, a red, crystalline solid (9·4 g.) separated which was almost pure 4-nitro-2-aminophenol. On one crystallisation from benzene with addition of glacial acetic acid, it yielded the pure *acetate*, yellow sheaves, which on drying at 95° gave 8·5 g. of the pure base. Ether extraction of the mother-liquors gave a semi-solid product which, fractionally crystallised as acetate from benzene and a little glacial acetic acid, gave further small quantities of 5-nitro-2-aminophenol (1·8 g.) and 4-nitro-2-aminophenol (3·0 g.). The total yield of pure nitroaminophenols was 83%.

5-Nitro-2-aminophenol (XIII) when quite pure melts at 203—204° and crystallises from water in orange or dark brown needles (Friedlaender and Zeitlin, *Ber.*, 1894, **27**, 192, give m. p. 201—202°).

This compound was also obtained as green plates, which melted at 170°, but crystallised almost instantaneously, and then melted at 203—204°. The velocity of transformation in contact with aqueous solutions is, however, slow. The N-*monoacetyl* derivative, obtained by the action of acetic anhydride without the application of heat, is sparingly soluble in boiling alcohol and crystallises in stout, pale yellow prisms, m. p. 271—272° (Found : C, 49·0; H, 4·1. $C_8H_8O_4N_2$ requires C, 49·0; H, 4·1%). The ON-*diacetyl* derivative is formed very readily by boiling the aminonitrophenol with acetic anhydride. It is readily soluble in boiling alcohol and crystallises as a felt of fine needles, m. p. 193—194° (Found : C, 50·5; H, 4·3. $C_{10}H_{10}O_5N_2$ requires C, 50·4; H, 4·2%). The *triacetyl* derivative was isolated from the acetylation mother-liquors of the preceding. It crystallises from absolute alcohol, in which it is readily soluble, in fern-like clusters of stout crystals, m. p. 138—139° (Found : C, 51·6; H, 4·5. $C_{12}H_{12}O_6N_2$ requires C, 51·4; H, 4·3%).

4-Nitro-2-aminophenol (XIV) forms an N-*monoacetyl* derivative, m. p. 279—280°, by the action of acetic anhydride at room temperature. It forms clusters of needles from absolute alcohol, in which it is very sparingly soluble at the boiling point (Found : C, 48·9; H, 4·2. $C_8H_8O_4N_2$ requires C, 49·0; H, 4·1%). The ON-*diacetyl* derivative, formed with difficulty by long boiling with excess of acetic anhydride, crystallises from boiling absolute alcohol, in which it is readily soluble, in needles or prisms, m. p. 187—188° (Found : C, 50·2; H, 4·3. $C_{10}H_{10}O_5N_2$ requires C, 50·4; H, 4·2%).

Nitration of Carbonyl-o-aminophenol (XV).—Carbonyl-*o*-aminophenol (0·5 g.) was warmed with 2 c.c. of nitric acid (*d* 1·42); a violent reaction then occurred accompanied by frothing. The mononitro-compound crystallised out and was isolated by pouring into water. The yield was 0·5 g., m .p. 255° (Bender, *Ber.*, 1886, **19**, 2271, gives m. p. 256°). When boiled for 6 hours with 16% hydrochloric acid, it was for the most part undissolved and unchanged, but neutralisation of the filtrate and ether extraction gave 0·15 g. of 5-nitro-2-aminophenol, m. p. 201°. The unchanged residue was boiled with hydrochloric acid (32%) and alcohol for 8 hours and gave a portion of unchanged compound and 0·2 g. of 5-nitro-2-aminophenol.

Nitration of N-*Acetyl-2-aminophenol.*—On nitration with nitric acid (1 mol.) as described for ethenyl-2-aminophenol, the sole product obtained was 4 : 6-dinitro-2-acetylaminophenol (acetylpicramic acid).

Nitration of Triacetyl-2-aminophenol.—When nitrated with nitric acid (1 mol.) as for the ethenyl derivative, this phenol yields small quantities of the monoacetyl derivatives of 4- and 5-nitro-2-amino-

phenol, readily isolated by reason of their sparing solubility in alcohol. If, however, the nitration product be hydrolysed, 4 : 6-dinitro-2-aminophenol (picramic acid) is readily isolated as the main product of the nitration.

4-Amino-2-hydroxyphenylarsinic Acid (III).—4-Nitro-2-hydroxyphenylarsinic acid was obtained in 78% yield from 5-nitro-2-aminophenol as described by Bauer (*loc. cit.*) except that the main bulk of arsinic acid separated on neutralisation to Congo-paper, without proceeding *via* the magnesium salt. On reduction with ferrous chloride by the method of Jacobs, Heidelberger, and Rolf (*J. Amer. Chem. Soc.*, 1918, **40**, 1580) it gives an 84% yield of 4-amino-2-hydroxyphenylarsinic acid. The *hydrochloride*, *nitrate*, and *sulphate* all crystallise from the corresponding *N*-acids in acicular crystals, and with the exception of the sulphate are readily soluble on warming. On addition of nitrite, a yellow colour is developed, and on adding to alkaline β-naphthol, a red colour is produced. The same colour is produced by pouring into alkali, probably through self-coupling. Addition of excess of nitrite destroys the coupling property almost instantaneously.

3'-Nitrobenzoyl-4-amino-2-hydroxyphenylarsinic Acid (X).—The *m*-nitrobenzoylation was carried out exactly as described for the isomeric 3-amino-4-hydroxyphenylarsinic acid, an 89% yield of the amide being obtained. It is almost insoluble in hot glacial acetic acid, but slightly soluble in hot 90% formic acid, from which it crystallises in bunches of needles (Found : As, 19·5. $C_{13}H_{11}O_7N_2As$ requires As, 19·6%). The *sodium* salt crystallises in fine needles.

3'-Aminobenzoyl-4-amino-2-hydroxyphenylarsinic Acid (V; R = H).—This acid was prepared as described for the isomeric derivative of 3-amino-4-hydroxyphenylarsinic acid (*vide infra*) in 72% yield. Liberated from acid solution by sodium acetate, it separates microcrystalline (Found : As, 20·8; 20·7. $C_{13}H_{13}O_5N_2As$ requires As, 21·3%). The *sodium* salt crystallises in platelets, the *ammonium* salt as a felt of needles. The *hydrochloride*, clusters of needles, and *nitrate*, minute needles, are readily soluble in their respective warm *N*-acids, but the *sulphate*, clusters of needles, is rather sparingly soluble in boiling *N*-sulphuric acid.

The *arseno*-derivative prepared by the action of hypophosphorous acid at 60° contained 30% of As as against 24·8% (theoretical) and consisted therefore mainly of polyarsenides. It was insoluble in sodium hydroxide or mineral acids, but dissolved on adding nitrite.

3' : 5-Dinitrobenzoyl-4-amino-2-hydroxyphenylarsinic Acid (XI).—The above-described mononitro-acid (11·3 g.) was dissolved in sulphuric acid (45 c.c.), cooled to 0°, and nitrated with a mixture

of 2·15 c.c. of nitric acid (*d* 1·42) and sulphuric acid (2 c.c.). On pouring on to ice, a yellow, microcrystalline precipitate separated. It was collected, dissolved in sodium carbonate, and reprecipitated by acid. The yield was 95% (Found: As, 17·7. $C_{13}H_{10}O_9N_3As$ requires As, 17·6%). This acid is sparingly soluble in boiling acetic acid and crystallises in microscopic needles, more soluble in formic acid, from which it separates in much longer needles.

On hydrolysis by boiling with 15 volumes of *N*-sodium hydroxide for 40 minutes, it gave pure *m*-nitrobenzoic acid, and *5-nitro-4-amino-2-hydroxyphenylarsinic acid*, short, yellow prisms crystallising readily from hot water, in which it is fairly readily soluble. It is sparingly soluble in 3*N*-hydrochloric acid, but readily soluble in concentrated acid. It diazotises with nitrite and thereafter couples with alkaline β-naphthol. Excess of nitrite, however, destroys the coupling property (Found: As, 27·1. $C_6H_7O_6N_2As$ requires As 27·0%).

3′ : 5-*Diaminobenzoyl-4-amino-2-hydroxyphenylarsinic Acid*.—This was obtained in 71% yield by reduction of the dinitro-acid with ferrous chloride and alkali. When liberated from the alkaline extracts of the ferric hydroxide, it separates in very fine needles, but occasionally as an amorphous, voluminous solid which becomes crystalline on warming the precipitation liquor (Found: As, 20·6. $C_{13}H_{14}O_5N_3As$ requires As, 20·4%). The *hydrochloride* is very soluble in *N*-hydrochloric acid, but separates from stronger acid in narrow leaflets with domed ends; the *sulphate*, minute needles, is only moderately soluble in *N*-sulphuric acid, but the *nitrate* is very readily soluble in *N*-nitric acid and crystallises from more concentrated acid in needles and in lenticular prisms. On addition of nitrite, it diazotises and then gives the same red colour on pouring into alkali, probably owing to self-coupling, as on adding to alkaline β-naphthol. This acid is very resistant to hydrolysis. It is unchanged after boiling with *N*-alkali for an hour, or after 7 hours' boiling with concentrated hydrochloric acid. It was recovered to the extent of more than 60% after 5 hours' heating with concentrated hydrochloric acid under pressure at 150°. *m*-Aminobenzoic acid was identified as a hydrolytic product.

4 : 5-*Diamino-2-hydroxyphenylarsinic Acid*.—5-Nitro-4-amino-2-hydroxyphenylarsinic acid (2·0 g.) was dissolved in 22 c.c. of *N*-sodium hydroxide (3 mols.) at 0° and to the vigorously stirred solution were added all at once 5·0 g. of sodium hyposulphite (1 mol. of 80% = 4·7 g.) with removal of the external bath. After being stirred for 2 hours, the solution was filled with fine needles of the required amino-acid. This was collected and washed with ice-cold water (yield 93%). For analysis and physiological testing, it was

dissolved in 20 c.c. of 0·7*N*-hydrochloric acid and reprecipitated by addition of saturated sodium acetate. On 1·65 g. there was a loss of 0·1 g. The *diamino-acid* crystallises from boiling water, in which it is sparingly soluble, in fine needles. It is very soluble in *N*-hydrochloric acid and gives, on addition of nitrite, a red coloration, which becomes pale after a few seconds. This is followed by separation of the *diazoimine*, in rectangular leaflets. The diamino-acid reduces ammoniacal silver nitrate instantly at room temperature. In acid solution, potassium dichromate gives a port wine colour (Found : As, 29·0. $C_6H_9O_4N_2As,\frac{1}{2}H_2O$ requires As, 29·0%).

3′-*Nitroanisoyl*-4-*amino*-2-*hydroxyphenylarsinic Acid*.—Prepared in 83% yield in the same way as its isomeride (*vide infra*), this acid is almost insoluble in hot glacial acetic acid, but crystallises from hot 90% formic acid in spear-shaped needles (Found : As, 18·4. $C_{14}H_{13}O_8N_2As$ requires As, 18·2%). The *sodium* salt, silky needles, and *ammonium* salt, diamond-shaped plates, were only moderately soluble in water.

3′-*Aminoanisoyl*-4-*amino*-2-*hydroxyphenylarsinic Acid* (V; R = OMe).—This acid, obtained in 77% yield by reduction of the nitro-acid with ferrous chloride and alkali at 0°, separates as a jelly on acidification of the alkaline extracts of the ferric hydroxide. It soon crystallises, however, in silky needles (Found : As, 19·4. $C_{14}H_{15}O_6N_2As$ requires As, 19·6%). The *sodium* salt crystallises in prisms readily soluble in water. The *hydrochloride*, spiked leaflets, is readily soluble in warm *N*-hydrochloric acid, but the *sulphate*, short, white needles and stout prisms, and *nitrate*, soft, woolly needles, are much less soluble in the warm *N*-acids.

The *arseno*-base could not be obtained sufficiently pure for physiological testing. Reduction with hypophosphorous acid under the mildest conditions gave polyarsenides containing between 26% and 56% of arsenic as against 22·6% required by theory. Reduction with hyposulphite even at 70° gave no trace of arseno-base.

3′-*Nitro*-4′-*ethylcarbonatobenzoyl*-4-*amino*-2-*hydroxyphenylarsinic Acid*.—3-Nitro-4-ethylcarbonatobenzoyl chloride (28·2 g.; 2 mols.) was added in several portions to 4-amino-2-hydroxyphenylarsinic acid (12 g.), dissolved in 2*N*-sodium hydroxide (45 c.c.), saturated sodium acetate solution (50 c.c.), and water (20 c.c.), with vigorous shaking. The reaction mixture was acidified to Congo-paper, and the precipitate collected after being well washed with dilute acid and water. When dry, it was extracted in a Soxhlet apparatus with ether and reprecipitated from sodium carbonate solution by acid. The yield was 73% of the theoretical. This acid is practically insoluble in hot glacial acetic acid, but separates from 90% formic

acid in microscopic, fluffy needles (Found : As, 15·9. $C_{16}H_{15}O_{10}N_2As$ requires As, 15·9%).

3'-Nitro-4'-hydroxybenzoyl-4-amino-2-hydroxyphenylarsinic Acid.—The preceding acid (18·5 g.) was dissolved in 4 equivalents of *N*-sodium hydroxide (158 c.c.) and kept at room temperature for 18 hours. On acidification carbon dioxide and ethyl alcohol were liberated and a white, microcrystalline precipitate was thrown down. This was collected, dried, and extracted with ether in a Soxhlet apparatus (yield 89%). This acid dissolves in alkali with a bright yellow colour, is almost insoluble in warm acetic acid, but crystallises from 90% formic acid in microscopic needles (Found : As, 18·8. $C_{13}H_{11}O_8N_2As$ requires As, 18·8%).

3'-Amino-4'-hydroxybenzoyl-4-amino-2-hydroxyphenylarsinic Acid (V; R = OH).—Reduced as described for its isomeride (*vide infra*), this acid was obtained in 76% yield. Liberated from alkaline solutions by acid, it separates in a gelatinous condition, but rapidly crystallises, on warming, in elongated leaflets (Found : As, 20·0. $C_{13}H_{13}O_6N_2As$ requires As, 20·4%). The *hydrochloride*, small needles, is readily soluble in *N*-hydrochloric acid; the *nitrate*, diamond-shaped plates, is sparingly soluble in hot *N*-nitric acid, and the *sulphate*, needles, is moderately soluble in warm *N*-sulphuric acid. On adding nitrite to a salt with mineral acids, a very sparingly soluble yellow *diazo-oxide* is precipitated, of amorphous appearance, but microscopically anisotropic. It couples intensely with alkaline β-naphthol.

3'-Amino-4'-hydroxybenzoyl-4-amino-2-hydroxyarsenobenzene (IX). —The above-described arsinic acid (3 g.) was suspended in 15 c.c. of hypophosphorous acid (*d* 1·14), diluted with an equal volume of water, and stirred at 45° for 2½ hours after addition of a crystal of potassium iodide. The orange-yellow arsenohypophosphite was worked up as described for aminobenzoyl-3-amino-4-hydroxyarsenobenzene. The yield was 1·0 g. When dried, it formed a brittle, brownish-yellow powder, instantly soluble in sodium hydroxide to a pale yellow solution. It was not soluble in mineral acids, but dissolved on adding nitrite and then coupled with alkaline β-naphthol. On analysis, the product proved to contain polyarsenide (Found : As, 28·7. $C_{26}H_{22}O_6N_4As_2$ requires As, 23·6%). When prepared at 55°, the proportion of polyarsenide was increased, as the arsenic content was 29·8%.

3'-Nitrobenzoyl-3-amino-4-hydroxyphenylarsinic Acid.—To a solution of 3-amino-4-hydroxyphenylarsinic acid (7·4 g.) in 2*N*-sodium hydroxide (25 c.c.) and half-saturated sodium acetate solution (200 c.c.) was added 3-nitrobenzoyl chloride (11·8 g.) dissolved in ether. The mixture was vigorously shaken, and sodium

hydroxide solution run in from time to time to maintain a slight alkalinity to phenolphthalein. On completion of the reaction, the mixture was made neutral to Congo-paper, the precipitated solid collected, washed with dilute acid, and, after drying, extracted thoroughly with ether. It was dissolved in alkali, reprecipitated by acid, dried, and again extracted with ether. The yield was 90%. This nitro-acid is almost insoluble in hot glacial acetic acid, but crystallises from hot 90% formic acid in silky needles (Found: As, 19·6. $C_{13}H_{11}O_7N_2As$ requires As, 19·6%). The *ammonium* salt is sparingly soluble and crystallises in tufts of needles.

3′-*Aminobenzoyl*-3-*amino*-4-*hydroxyphenylarsinic Acid* (IV; R = H).—The nitro-acid (7·6 g.) was dissolved in 72 c.c. of 2*N*-sodium hydroxide solution at 0°, and ferrous chloride (28 g.) dissolved in 40 c.c. of water run in slowly with vigorous stirring. A further quantity (72 c.c.) of 2*N*-sodium hydroxide was then run in under the same conditions. After stirring for 90 minutes, the ferric hydroxide was filtered off and extracted twice by thorough disintegration with 150 c.c., each time, of 0·5*N*-sodium hydroxide. The combined alkaline filtrates were neutralised to Congo-paper, and the crystalline precipitate was collected. It was dissolved in 150 c.c. of *N*-hydrochloric acid and reprecipitated by addition of saturated sodium acetate solution. The yield was 44%. This amino-arsinic acid, so prepared, separates in microscopic platelets of indefinite shape (Found: loss at 100°, 4·9, 4·7, 5·1. $C_{13}H_{13}O_5N_2As,H_2O$ requires H_2O, 4·9%. Found in anhydrous material: As, 20·8. $C_{13}H_{13}O_5N_2As$ requires As, 21·3%). It is readily soluble in warm *N*-mineral acids, the *hydrochloride* crystallising in microscopic, narrow leaflets, the *sulphate* in rectangular leaflets, and the *nitrate* in microscopic plates.

3′-*Aminobenzoyl*-3-*amino*-4-*hydroxyarsenobenzene*.—The corresponding arsinic acid (1 g.) was suspended in a mixture of 30% hypophosphorous acid (5 c.c.), glacial acetic acid (5 c.c.), and water (5 c.c.), with addition of a crystal of potassium iodide. After stirring for 2 hours at 57°, the orange-yellow solid was centrifuged off, and the deposit washed several times with boiled-out water. It was then made alkaline by addition of aqueous sodium hydrogen carbonate and washed free from alkali by repeated centrifuging with water. The product was dried in a vacuum (yield 0·53 g.). This arsenobenzene is insoluble in aqueous acids or alkalis, but soluble in warm 90% formic acid. In acid solution, it dissolves immediately on addition of nitrite and then couples with alkaline β-naphthol with a deep red colour (Found: As, 24·8. $C_{26}H_{22}O_4N_4As_2$ requires As, 24·8%).

3′-*Nitroanisoyl*-3-*amino*-4-*hydroxyphenylarsinic Acid*.—This acid

was obtained in 80% yield by the same process as is described for the corresponding 3′-nitrobenzoyl derivative. It is practically insoluble in boiling glacial acetic acid, but crystallises from 90% formic acid in square leaflets (Found: As, 17·9. $C_{14}H_{13}O_8N_2As$ requires As, 18·2%). The *sodium* salt, acicular crystals, is readily soluble in water.

3′-*Aminoanisoyl*-3-*amino*-4-*hydroxyphenylarsinic Acid* (IV; R = OMe).—The reduction of the nitro-compound was carried out precisely as described for the preparation of the 3′-aminobenzoyl compound. The yield was 61%. Liberated from acid solution by sodium acetate, this acid crystallises in colourless plates (Found: As, 20·0. $C_{14}H_{15}O_6N_2As$ requires As, 19·6%). The *hydrochloride*, rosettes of needles, is readily soluble in hot *N*-hydrochloric acid, the *sulphate*, microscopic, square leaflets, is less soluble in hot *N*-sulphuric acid, and the *nitrate*, long, silky needles, is sparingly soluble in hot *N*-nitric acid.

3′-*Aminoanisoyl*-3-*amino*-4-*hydroxyarsenobenzene*.—Prepared in the same way as the previously described arsenobenzene in 77% yield, this is a yellow powder insoluble in acids or alkalis, but instantly soluble in acid solution on addition of nitrite. It then couples with alkaline β-naphthol (Found: As, 22·7. $C_{28}H_{26}O_6N_4As_2$ requires As, 22·6%).

3′-*Nitro*-4′-*ethylcarbonatobenzoyl*-3-*amino*-4-*hydroxyphenylarsinic Acid* (VII).—3-Amino-4-hydroxyphenylarsinic acid (11·6 g.) was dissolved in 100 c.c. of water with addition of 5·3 g. (2 mols.) of sodium carbonate. After addition of 100 c.c. of saturated sodium acetate solution, 27·3 g. (2 mols.) of 3-nitro-4-ethylcarbonatobenzoyl chloride were added in three portions with a few c.c. of ether, and well shaken. After ½ hour, the solution was made definitely acid to Congo-paper, and the mixture of acids collected, dried, and divided by ether extraction into 17·0 g. of the required arsinic acid, insoluble in ether, and 16·0 g. of recovered nitroethylcarbonatobenzoic acid, soluble in ether. This arsinic acid is readily soluble in cold 90% formic acid and crystallises therefrom in microscopic needles; from glacial acetic acid, in which it is readily soluble when hot, it separates either as needles or rectangular leaflets (Found: As, 16·1. $C_{16}H_{15}O_{10}N_2As$ requires As, 15·9%).

3′-*Nitro*-4′-*hydroxybenzoyl*-3-*amino*-4-*hydroxyphenylarsinic Acid*.—The foregoing ethylcarbonato-acid (10 g.) was dissolved in *N*-sodium hydroxide (4 mols.) and kept for a day. It was diluted with an equal volume of water and precipitated with concentrated hydrochloric acid. On warming the mixture, the precipitated acid became partly crystalline. The dried acid, when extracted with ether to remove hydroxynitrobenzoic acid, was obtained in 84% yield. The

aqueous mother-liquors, extracted with ether, gave nitrohydroxybenzoic acid and also contained 3-amino-4-hydroxyphenylarsinic acid, as was proved by its coupling strongly with alkaline β-naphthol after diazotisation. The required arsinic acid dissolves in alkali with a bright yellow colour. It crystallises in diamond-shaped spicules from either boiling glacial acetic acid, in which it is sparingly soluble, or boiling 90% formic acid, in which it is readily soluble (Found: As, 19·2. $C_{13}H_{12}O_8N_2As$ requires As, 18·8%).

3′-Amino-4′-hydroxybenzoyl-3-amino-4-hydroxyphenylarsinic Acid. (IV; R = OH).—The nitro-acid (8·8 g.) was dissolved in 33 c.c. of water with the aid of two equivalents (23 c.c.) of 2*N*-sodium hydroxide, and ferrous chloride (31 g.) in 42 c.c. of water added slowly with vigorous stirring at −5°. Sufficient sodium hydroxide (167 c.c. of 2*N*) was now slowly added to make the reaction of the fluid distinctly alkaline to litmus. The filtered ferric hydroxide was thoroughly extracted, each time, with 170 c.c. of 0·2*N*-sodium hydroxide. On rendering the combined extracts neutral to Congo-paper, a copious separation of crystalline amino-acid ensued. This was purified by solution in 100 c.c. of *N*-nitric acid at 50° and precipitation with sodium acetate, and gave 4·0 g. The main mother-liquors were rapidly concentrated below 50° to a small volume and gave a mixture of the amino-compound and the corresponding azoxy-compound, readily separable by dilute nitric acid into 0·75 g. of azoxy-compound (9% yield) and 1·0 g. of amino-compound. The total yield of amino-compound was 62% (Found: As, 20·4. $C_{13}H_{13}O_6N_2As$ requires As, 20·4%). The amino-acid diazotises with production of a deep yellow solution, from which an insoluble *diazo-oxide* crystallises in dense clusters of needles. It couples readily with alkaline β-naphthol. The *hydrochloride*, microscopic needles, is very sparingly soluble in boiling *N*-hydrochloric acid; the *sulphate*, leaflets, is sparingly soluble in warm *N*-sulphuric acid; and the *nitrate*, microscopic needles, is readily soluble in warm *N*-nitric acid.

The *azoxy*-compound crystallises very readily from boiling 90% formic acid, in which it is readily soluble, in pale brown, truncate, diamond-shaped plates. It dissolves in ammonia with a pale yellow colour and gives an insoluble magnesium salt on addition of magnesium chloride.

3′-Amino-4′-hydroxybenzoyl-3-amino-4-hydroxyarsenobenzene (VIII was obtained in quantitative yield, allowing for recovered acid, by reduction of the corresponding acid (4·0 g.) with 20 c.c. of hypophosphorous acid (*d* 1·14), 20 c.c. of water, 10 c.c. of acetic acid, and a crystal of potassium iodide at 50—55° for 3 hours. The pure arseno-base was isolated as described for the preceding members.

It is a yellow powder soluble in caustic alkalis, but forming insoluble orange salts with acids. It is instantly soluble to a yellow solution on addition of nitrous acid. From more concentrated solutions, the amorphous *diazo-oxide* separates; this couples with alkaline β-naphthol (Found : As, 23·8. $C_{26}H_{22}O_6N_4As_2$ requires As, 23·6%).

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