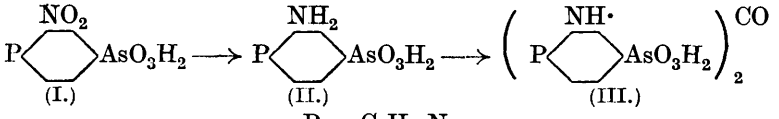
CXLII.—*Trypanocidal Action and Chemical Constitution. Part VI. Amphoteric* s-*Carbamidoarylarsinic Acids.*

By Harold King.

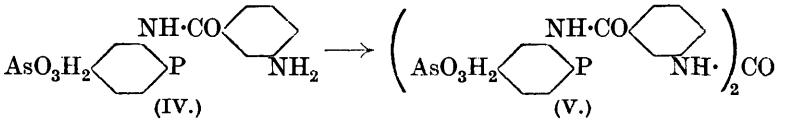
The physiological action of a drug is primarily a matter of its distribution in the tissues, and among the trypanocidally active aromatic arsinic acids experience has shown that those of an amphoteric nature are the more frequent. This may be attributed to the favourable distribution conferred on substances built somewhat analogously to the simple protein units, the amino-acids. In an earlier communication (Part I; J., 1924, **125**, 2595), it was shown that the *s*-carbamides of some complex aromatic arsinic acids were devoid of trypanocidal action and it was conceivable that this was associated with their non-amphoteric nature. It was therefore deemed of sufficient interest to examine some *s*-carbamidoarsinic acids which still retained a free basic centre. These could be prepared by phosgenation of aminoarsinic acids containing a second basic centre unattacked by phosgene (carbonyl chloride) or by nitration and reduction of *s*-carbamidoarsinic acids. Instances of both classes are recorded below.

As an example of the first type, 4-chloro-3-nitrophenylarsinic acid on treatment with piperidine gave 3-*nitro*-4-*piperidinophenylarsinic acid* (I), which can be reduced to the amino-acid (II). This gives an *acetyl* derivative and also, on phosgenation, a s-*carbamide* (III).



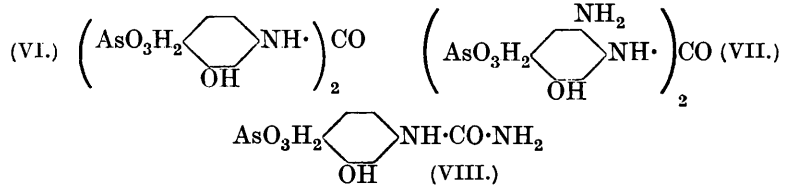
$P = C_5H_{10}N$.

On *m*-nitrobenzoylation, (II) gave 3-m-*nitrobenzamido*-4-*piperidinophenylarsinic acid*, which on reduction gave the 3-m-*aminobenzamido*-derivative (IV); and this on phosgenation gave the



s-*carbamide* (V). These acids were relatively toxic to mice and had no influence on an experimental infection of *Trypanosoma equiperdum* in mice.

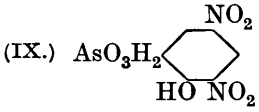
As an example of the second type, 4-amino-2-hydroxyphenylarsinic acid on phosgenation gave the s-*carbamido*-derivative (VI), which could be dinitrated to the s-*carbamide* of 5-*nitro*-4-*amino*-2-*hydroxyphenylarsinic acid*. The latter acid on reduction with sodium hyposulphite gave the 4-s-*carbamide* of 4 : 5-*diamino*-2-*hydroxyphenylarsinic acid* (VII). For comparison with the

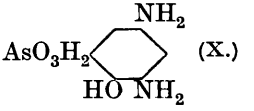


symmetrical carbamides (VI) and (VII), 2-*hydroxy*-4-*carbamido*-*phenylarsinic acid* (VIII) was prepared by the action of potassium cyanate on 4-amino-2-hydroxyphenylarsinic acid. The maximum dose tolerated by mice expressed in milligrams per gram of mouse, and the minimum curative dose on an experimental infection of *Trypanosoma equiperdum* in mice, of these three carbamides are shown below, *r* signifying the number of days before relapse occurred.

	VI.	VII.	VIII.
Dosis tolerata	0·3	0·8	0·4
Dosis curativa	—	0·8	0·4
	r = 11 days.		$r > 30$ days.

The facile way in which the amino-group in 5-nitro-2-aminophenol can be replaced by the arsinic acid group by the Bart–Schmidt reaction (Bauer, *Ber.*, 1915, **48**, 1582; Hewitt and King, J., 1926, 817) suggested its application to picramic acid, which likewise forms an intermediate sparingly soluble quinone-diazide. Here again a good yield of the acid, 3 : 5-dinitro-2-hydroxyphenylarsinic acid (IX), was produced, probably identical with the acid obtained by Benda (*Ber.*, 1911, **44**, 3296) by the nitration of 5-nitro-2-hydroxyphenylarsinic acid.

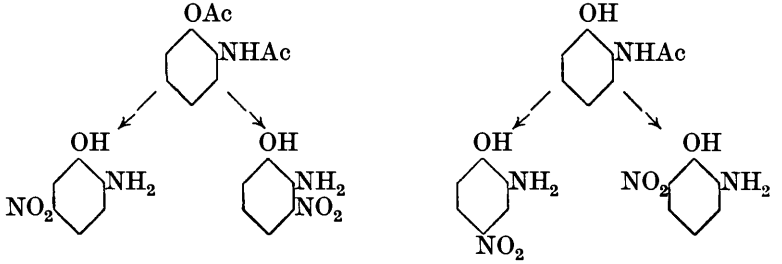




On reduction with sodium hyposulphite it gave a new *diamino-hydroxyphenylarsinic acid* (X), of which only two isomerides are known. This proved to be very toxic to mice and was devoid of trypanocidal action.

Nitration of 2- and 4-Aminophenols.

In Part IV of this series (Hewitt and King, *loc. cit.*) it was shown that *N*-acetyl-2-aminophenol or *ONN*-triacetyl-2-aminophenol, when nitrated in sulphuric acid solution with one equivalent of nitric acid, gave mainly 4 : 6-dinitro-2-aminophenol (picramic acid), but that ethenyl-2-aminophenol under similar conditions gave 5- and 4-nitro-2-aminophenols, approximately in the proportion 4 : 1. It has now been shown that *ON*-diacetyl-2-aminophenol in sulphuric acid also nitrates with production of a large proportion of picramic acid and a small proportion of 4-nitro-2-aminophenol. Meldola and Wechsler (P., 1900, **16**, 180) showed that nitration of *ON*-diacetyl-2-aminophenol by fuming nitric acid gave solely 4 : 6-dinitro-2-aminophenol, which they oriented by conversion into 2-chloro-4 : 6-dinitrophenol. This constitution has been confirmed by other means in the present communication, such confirmation being necessary since Ingold and Ingold (J., 1926, 1321), in repeating the earlier work of Meldola, Woolcott, and Wray (J., 1896, **69**, 1330), ascribed the constitution 3 : 5-dinitro-2-aminophenol to this substance, as it was deemed to be in agreement with their results on the mononitration of *ON*-diacetyl-2-aminophenol in acetic anhydride solution. The latter nitration, which leads to the formation of 3- and 5-nitro-2-aminophenols, is of considerable interest, because it has now been shown that under exactly parallel conditions *N*-acetyl-2-aminophenol gives good yields of the other two isomerides —4- and 6-nitro-2-aminophenols—and a small proportion of picramic acid.



These results, at first sight somewhat involved, bear a simple interpretation, since it has been found that *O*-acetyl is hydrolysed to hydroxyl when *ON*-diacetyl-2-aminophenol is dissolved in cold concentrated sulphuric acid and that, although *ON*-diacetyl-2-aminophenol is moderately stable in concentrated nitric acid at — 5°, when nitration has taken place the products have the hydroxyl group free, pointing to immediate nitration after hydrolysis. In either case, solution in sulphuric acid or nitric acid, if hydrolysis precedes nitration the results are consistent.

The apparent nitration of mono-, di-, or tri-acetyl-2-aminophenol in sulphuric acid or of *ON*-diacetyl-2-aminophenol in fuming nitric acid is therefore really the nitration of *N*-acetyl-2-aminophenol and in each case, whether the solvent be sulphuric acid, nitric acid, or acetic anhydride, the nitro-groups enter positions ortho or para, or ortho and para, to the hydroxyl group. When the hydrolysis of *O*-acetyl is prevented by use of acetic anhydride, the nitro-groups enter the ortho- or the para-position to the *N*-acetyl group. These results are in complete agreement with the observations of Reverdin and his associates on the nitration of *N*-acetyl-4-aminophenol (Reverdin and Dresel, *Ber.*, 1905, **38**, 1593), *ON*-diacetyl-4-aminophenol (R. and D., *Ber.*, 1904, **37**, 4453; 1905, **38**, 1593), *N*-benzoyl-4-aminophenol (Reverdin and Delétra, *Ber.*, 1906, **39**, 125), and *ON*-dibenzoyl-4-aminophenol (Reverdin and Dresel, *Ber.*, 1904, **37**, 4453; Reverdin and Delétra, *Ber.*, 1906, **39**, 125), where, however, *O*-acetyl and *O*-benzoyl are not hydrolysed prior to nitration by solution in nitric acid, but *O*-benzoyl is hydrolysed prior to nitration by solution in sulphuric acid. The relative orienting powers for nitro-groups as determined for these aminophenols may be conveniently summarised as follows :

2-Aminophenol.	*4-Aminophenol.*
OH > NHAc	OH > NHAc *or* NHBz
NHAc > OAc	NHAc > OAc; NHBz > OBz

The fact that, with the same solvent, acetic anhydride, and the same nitrating agent, nitric acid or acetylnitric acid, *N*-acetyl-2-aminophenol nitrates ortho and para to the hydroxyl group whilst *ON*-diacetyl-2-aminophenol nitrates ortho and para to the *N*-acetyl group is strong evidence in favour of the view that the induced polarity of the benzene molecule is independent of the solvent or nitrating agent as a major influence, and is probably an electromeric phase of the molecule induced by the substitution of the hydroxyl and amino-groups. From this point of view it is not difficult to understand the peculiar nitration of ethenyl-2-aminophenol which gives 5- and 4-nitro-2-aminophenols, there being two induced electromeric phases. That the solvent or nitrating agent is not without influence is shown by the great tendency for N-acetyl-2-aminophenol to become dinitrated in sulphuric acid solution, although but one equivalent of nitric acid be added, whereas in acetic anhydride solution this is a subsidiary phenomenon.

The author is deeply indebted to Miss F. M. Durham and Miss J. Marchal of this Department for the determination of the trypanocidal activities of the substances herein recorded, to Dr. J. Marshall of the Research Laboratories of Messrs. Boots Pure Drug Co. for a

gift of 4-chloro-3-nitrophenylarsinic acid, and to Dr. J. T. Conroy of the United Alkali Co. for a liberal and free supply of phosgene.

EXPERIMENTAL.

3-Nitro-4-piperidinophenylarsinic Acid (I).—4-Chloro-3-nitrophenylarsinic acid (2·81 g.) and excess of piperidine (5·4 c.c.) were boiled together in 20 c.c. of alcohol for 3 hours. The orange-coloured solution was diluted with water, evaporated to a small volume under reduced pressure, and made neutral to Congo-paper. The gum that separated in quantitative yield, and soon crystallised on being rubbed, was dissolved in 60 c.c. of 0·4*N*-ammonia, and excess of calcium chloride solution added; the finely divided *calcium* salt thus obtained was washed, suspended in water, and treated with hydrochloric acid (Congo-paper). The required *acid* (3·0 g.) separated in pale yellow leaflets (Found : As, 22·3. $C_{11}H_{15}O_5N_2As$ requires As, 22·7%). It crystallises from boiling water in laminated leaflets, is readily soluble in hot acetic or warm 90% formic acid, and dissolves in concentrated hydrochloric acid, forming an almost colourless solution. The *barium* salt separates in filmy leaflets when an ammoniacal solution of the acid is boiled with barium chloride.

3-Amino-4-piperidinophenylarsinic Acid (II).—The above-described nitro-acid (14·8 g.) was reduced with ferrous chloride and alkali in the usual manner. The filtrate from the ferric hydroxide and the weakly alkaline extracts thereof were neutralised (Congo-paper), and concentrated under reduced pressure at 50°. The crystalline *amino-acid* (10·3 g.) thus obtained was purified by solution in 50 c.c. of *N*-hydrochloric acid and careful addition of saturated sodium acetate solution till acidity to Congo-paper was removed (yield 75%) (Found : loss at 100°, 6·0. $C_{11}H_{17}O_3N_2As,H_2O$ requires H_2O, 5·7. Found in anhydrous material : As, 25·2. $C_{11}H_{17}O_3N_2As$ requires As, 25·0%). This amino-acid is instantly soluble in excess of *N*-hydrochloric acid, and yields, with nitrite, an orange-brown solution which couples intensely with alkaline β-naphthol. The pure acid readily forms, when the excess of mineral acid in *N*-hydrochloric acid solution is removed by sodium acetate, a *monohydrochloride*, which crystallises in well-formed, clear prisms (Found : Cl, 10·2. $C_{11}H_{17}O_3N_2As,HCl$ requires Cl, 10·5%). In ammoniacal solution, the acid yields a crystalline *calcium* salt, but the *magnesium* and *barium* salts are precipitated only on boiling. The maximum tolerated dose is 0·01 mg. per g. of mouse.

3-Acetamido-4-piperidinophenylarsinic acid was prepared from the foregoing amino-acid by shaking its alkaline solution with excess of acetic anhydride, added in portions. On neutralisation to Congo-

paper, it separated in small prisms. The yield was quantitative (Found : As, 22·4. $C_{13}H_{19}O_4N_2As$ requires As, 21·9%). This acid is sparingly soluble in boiling water, readily soluble in boiling acetic acid, and crystallises therefrom in diamond-shaped plates. It is instantly soluble in 90% formic acid and in *N*-mineral acids. The maximum tolerated dose is 0·03 mg.

3-m-*Nitrobenzamido*-4-*piperidinophenylarsinic Acid.*—Amino-piperidinophenylarsinic acid monohydrate (6·0 g.), dissolved in 90 c.c. of water containing 2 mols. of sodium hydroxide, was shaken for ½ hour with 7·4 g. of *m*-nitrobenzoyl chloride, and at half-hourly intervals two further quantities of 20 c.c. of *N*-sodium hydroxide were added. On the solution being made definitely acid (Congo-paper) the required *acid* separated, as a gum which rapidly crystallised, mixed with nitrobenzoic acid. The latter was removed by ether extraction. The yield was 92%. For analysis, the acid was purified by solution in dilute aqueous alkali and very gradual precipitation of the warm solution with acid (Found : loss at 100°, 7·3. $C_{18}H_{20}O_6N_3As,2H_2O$ requires H_2O, 7·4. Found in anhydrous material : As, 17·1. $C_{18}H_{20}O_6N_3As$ requires As, 16·7%). This acid is readily soluble in boiling acetic acid and crystallises well in spiked rods. It is soluble in concentrated hydrochloric acid, but is precipitated in well-formed, flattened prisms on dilution. In ammoniacal solution, it yields crystalline precipitates of the *calcium* and *barium* salts on addition of the corresponding chlorides.

3-m-*Aminobenzamido*-4-*piperidinophenylarsinic Acid* (IV).—The foregoing nitro-acid (7·3 g.) was reduced with ferrous chloride and alkali, and the ferric hydroxide precipitate extracted with dilute alkali solution. The combined filtrates were neutralised (Congo-paper), a copious, amorphous precipitate separating; from the filtered solution, concentrated under reduced pressure, a crystalline acid separated. This was dissolved in *N*-hydrochloric acid and fractionally precipitated from the hot solution by saturated sodium acetate solution. After removal of much weakly basic, amorphous material, the required *amino-acid* was obtained in poor yield (2·25 g.) (Found : As, 18·2. $C_{18}H_{22}O_4N_3As$ requires As, 17·9%). This amino-acid is extremely readily soluble in *N*-hydrochloric acid and diazotises and couples with alkaline β-naphthol with formation of a red precipitate insoluble in excess of alkali. In hot ammoniacal solution, it yields a *magnesium* salt, narrow plates, and a *barium* salt, clusters of leaflets, on addition of the corresponding chlorides. The maximum tolerated dose is 0·1 mg.

s-*Carbamide* of 3-*Amino*-4-*piperidinophenylarsinic Acid* (III).—Into a solution of the amino-compound (3 g.) in 200 c.c. of water containing 21·2 g. of anhydrous sodium carbonate (20 mols.),

carbonyl chloride was passed until an acid reaction was obtained. The crude arsinic acid was precipitated by adjusting the reaction to faint acidity to Congo-paper. It was recrystallised by solution in 30 c.c. of *N*-ammonia and addition of concentrated hydrochloric acid, separating as a microscopic, white powder (yield 2·9 g.) (Found : loss at 100°, 2·9. $C_{23}H_{32}O_7N_4As_2,H_2O$ requires H_2O, 2·8. Found in the anhydrous acid : As, 23·8. $C_{23}H_{32}O_7N_4As_2$ requires As, 23·9%). This acid is almost insoluble in boiling glacial acetic acid, but dissolves readily on addition of a small proportion of water and then crystallises, on cooling, in microscopic rods. It is readily soluble in 3*N*-hydrochloric acid. The *magnesium* salt crystallises in elongated leaflets from ammoniacal solution containing ammonium chloride. The maximum tolerated dose is 0·075 mg.

s-*Carbamide of* 3-m-*Aminobenzamido*-4-*piperidinophenylarsinic Acid* (V).—Prepared similarly to the preceding one, this *carbamide* was obtained in quantitative yield. It was purified for analysis and physiological testing by dissolving it in 5 volumes of 90% formic acid and adding the solution to 50 volumes of boiling water. On continuing the boiling, the carbamide gradually separated (Found : loss at 100°, 5·8. $C_{37}H_{42}O_9N_6As_2,3H_2O$ requires $3H_2O$, 5·9. Found in the anhydrous acid : As, 17·8. $C_{37}H_{42}O_9N_6As_2$ requires As, 17·4%). This carbamide is soluble in concentrated hydrochloric or formic acid and is precipitated on dilution. It is practically insoluble in boiling glacial acetic acid or 50% formic acid, but its crystalline form may be seen by dissolving it in cold 90% formic acid, adding water until a turbidity is produced, boiling the solution, and continuing the addition of water until the solution is again turbid; on cooling, the carbamide crystallises in microscopic, woolly needles. The amorphous *magnesium*, *calcium*, and *barium* salts are immediately precipitated from dilute ammoniacal solution, and the *sodium* salt is precipitated in amorphous particles by addition of excess of 2*N*-sodium hydroxide to a solution of the sodium salt. The maximum tolerated dose is 0·2 mg.

s-*Carbamide of* 4-*Amino*-2-*hydroxyphenylarsinic Acid* (VI).—The amino-acid (4·7 g. of monohydrate) was phosgenated in the presence of sodium carbonate (10 mols. in 200 c.c. of water) until the reaction was acid. The crystalline precipitate (3·5 g.) was dissolved in dilute ammonia and reprecipitated by acid (yield 3·3 g.) (Found : loss at 100°, 7·6. $C_{13}H_{14}O_9N_2As_2,2H_2O$ requires H_2O, 6·8. Found in anhydrous solid : As, 30·5. $C_{13}H_{14}O_9N_2As_2$ requires As, 30·5%). This carbamide is practically insoluble in boiling acetic or formic acid; it is sparingly soluble in boiling water, and crystallises therefrom in rods. Dilute ammoniacal solutions gave immediate amorphous precipitates with calcium, magnesium, and barium chlorides.

An aqueous or, better, alcoholic solution of the acid gives a port-wine colour on addition of ferric chloride. The maximum tolerated dose is 0·3 mg.

s-*Carbamide of* 5-*Nitro*-4-*amino*-2-*hydroxyphenylarsinic Acid.*—The preceding carbamide (7·0 g.) in 28 c.c. of sulphuric acid was nitrated at 0° with 2·8 g. of nitric acid (*d* 1·42) in 3 c.c. of sulphuric acid. The solution was poured into water, and a yellow, crystalline solid (7·8 g.) slowly separated. When stirred with cold water, this carbamide yields a yellow, opalescent solution. It is readily soluble in boiling water; on cooling, the solution becomes acid to Congo-paper, and gradually becomes limpid with deposition either of the anhydrous *acid*, crystallising in yellow plates, or of the *dihydrate* in fine, yellow needles (Found: loss at 100°, 6·0. $C_{13}H_{12}O_{13}N_4As_2,2H_2O$ requires H_2O, 5·8%). The *magnesium*, *barium*, and *calcium* salts are precipitated amorphous from ammoniacal solution. The *sodium* salt crystallises well and is only moderately easily soluble in water. The *tetra-ammonium* salt is sparingly soluble and crystallises in fine, orange-yellow needles. It is partly hydrolysed by washing with water, as is shown by analysis [Found: loss at 100°, 10·1. $C_{13}H_{12}O_{13}N_4As_2,3\frac{1}{2}NH_3,4H_2O$ requires H_2O, 10·1. Found in the anhydrous salt: volatile NH_3, 8·7; As (Lehmann), 23·3. $C_{13}H_{12}O_{13}N_4As_2,3\frac{1}{2}NH_3$ requires NH_3, 9·3; As, 23·3%].

s-4-*Carbamide of* 4 : 5-*Diamino*-2-*hydroxyphenylarsinic Acid* (VII)—A clear solution of the preceding nitro-compound (1·16 g.) in 12 c.c. of *N*-sodium hydroxide at 0° was treated with 5 g. of sodium hyposulphite, and the external cooling agent simultaneously removed. On stirring for about 2 hours, the precipitated yellow nitro-compound was replaced by the required *amino*-compound. This was collected, dissolved in 11 c.c. of *N*-hydrochloric acid, and precipitated, as an almost white, microcrystalline powder, by addition of sodium acetate (yield 85%) (Found: loss at 100°, 6·9. $C_{13}H_{16}O_9N_4As_2,2H_2O$ requires H_2O, 6·5. Found in dried substance: As, 28·4. $C_{13}H_{16}O_9N_4As_2$ requires As, 28·7%). This amino-acid is readily soluble in dilute mineral acids, but its salts are hydrolysed on dilution. It gives a yellow solution on addition of sodium nitrite but does not couple with alkaline β-naphthol, probably owing to the formation of a soluble diazoimide. The maximum tolerated dose is 0·8 mg.

2-*Hydroxy*-4-*carbamidophenylarsinic Acid* (VIII).—The parent amino-acid (1·35 g.) in 2·5 c.c. of water was treated with 1·74 g. of potassium cyanate (4 mols.); all then passed into solution. After addition of 0·8 c.c. of glacial acetic acid the solution was kept for 24 hours and then made acid (Congo-paper), and the precipitate was

collected (yield 1·25 g.). On careful addition of sodium acetate to the filtrate, 0·15 g. of unchanged amino-acid was recovered (Found : loss at 100°, 3·3. $C_7H_9O_5N_2As,\frac{1}{2}H_2O$ requires H_2O, 3·2. Found in dried substance : As, 27·2. $C_7H_9O_5N_2As$ requires As, 27·2%). This acid is soluble in 66 parts of boiling water and crystallises in fine needles. Its ammoniacal solution gives immediate precipitates of the amorphous *magnesium* and *calcium* salts on addition of the respective chlorides, but the *barium* salt, obtained on boiling, is crystalline. The maximum tolerated dose is 0·4 mg.

3 : 5-*Dinitro*-2-*hydroxyphenylarsinic Acid* (IX).—Picramic acid (10·0 g.) was suspended in 60 c.c. of 3*N*-hydrochloric acid at 0° and diazotised by addition of 3·8 g. of sodium nitrite in 38 c.c. of water. Arsenic trioxide (6·2 g.) in 37·2 c.c. of 2*N*-sodium hydroxide was now added rapidly, and into the still acid solution 2*N*-sodium hydroxide (about 150 c.c.) was run slowly until all the brightly coloured quinone-diazide had disappeared and evolution of nitrogen had ceased. The solution was acidified (Congo-paper) and, after removal of amorphous matter, concentrated at 50°. Two crops (10·15 g. in all) of crude dinitro-acid were obtained which, on two crystallisations from 50 c.c. of boiling water, gave yellow leaflets (yield 8·7 g.), m. p. 244—246° (Benda, *loc. cit.*, gives 237°) (Found : As, 24·7. Calc. : As, 24·3%). This acid, treated with 2*N*-ammonia, gives a sparingly soluble *ammonium* salt, which crystallises from water in needles. The *calcium* and *magnesium* salts are precipitated amorphous from ammoniacal solution, but the *barium* salt is crystalline.

3 : 5-*Diamino*-2-*hydroxyphenylarsinic Acid* (X).—The preceding dinitro-acid (1·0 g.), dissolved in 10 c.c. of *N*-sodium hydroxide at 0°, was treated with 5 g. of sodium hyposulphite all at once, the external bath being simultaneously removed. The temperature rose to about 30° and on continuing the stirring the *diamino*-compound crystallised in long needles (yield 0·5 g.) [Found : As, 30·8 (Carius), 29·7 (Lehmann). $C_6H_9O_4N_2As$ requires As, 30·2%]. This diamino-acid is very soluble in dilute mineral acids and gives a reddish-brown solution on addition of a trace of nitrite. It reduces ammoniacal silver nitrate solution instantaneously and gives a port-wine colour on addition of potassium dichromate solution. The maximum tolerated dose is 0·04 mg.

Nitration of ON-*Diacetyl*-2-*aminophenol*.—(a) *By nitric acid*. Ten g. of the diacetyl compound were nitrated as described by Meldola, Woolcott, and Wray (*loc. cit.*) and Ingold and Ingold (*loc. cit.*). On dilution with ice *N*-acetylpicramic acid was obtained, m. p. 203°, in 63% yield. On crystallisation from much boiling water it separated in soft, yellow needles, m. p. 205—206°. Its identity was

proved by the melting points of its mixture with monoacetylpicramic acid and of mixtures of its acid hydrolytic product with picramic acid and of its diacetylated derivative with *ON*-diacetylpicramic acid. ON-*Diacetylpicramic acid* is readily obtained by boiling picramic or monoacetylpicramic acid with acetic anhydride and a few drops of pyridine. It crystallises from 5 volumes of boiling acetic acid in needles, m. p. 190—191°. *N*-Acetylpicramic acid is obtained by allowing picramic acid to react at room temperature with a small volume of acetic anhydride.

(b) *By nitric acid in sulphuric acid.* Anhydrous *ON*-diacetylaminophenol (9·7 g.) was dissolved at — 5° in 40 c.c. of sulphuric acid and nitrated by the gradual addition of 5 g. of nitric acid (*d* 1·42) mixed with 2·5 c.c. of sulphuric acid. On pouring the solution on ice, 9·0 g. of crude nitration products separated, and ether extraction of the mother-liquor gave 1·1 g. of highly coloured gum. The main crop of solid was hydrolysed by 10% hydrochloric acid and gave, on dilution and partial neutralisation, 3·3 g. of picramic acid. On neutralisation to Congo-paper, the mother-liquor gave 0·9 g. of 4-nitro-2-aminophenol, m. p. 140° (anhydrous) and not depressed by admixture with pure 4-nitro-2-aminophenol. Ether extraction of the final liquors gave 1·05 g., which, on acetylation, gave 1·1 g., m. p. 266°; this, on crystallisation from 45 c.c. of alcohol, gave 0·75 g., melting, alone or mixed with 4-nitro-2-acetamidophenol, at 280°. The aqueous liquors from the acetylation deposited 0·05 g., m. p. 178°, which, on crystallisation from alcohol, gave 4-nitro-*ON*-diacetylaminophenol; this, alone or mixed with a genuine sample of 4-nitrodiacetylaminophenol, melted at 184°. These yields indicate a 66% conversion of the available nitric acid into dinitro-derivative (picramic acid) and a 17·5% conversion into mononitro-derivative.

(c) *By nitric acid in acetic anhydride.* Following Ingold and Ingold (*loc. cit.*), 14·5 g. of diacetyl-2-aminophenol were nitrated in acetic anhydride at 10°. *ON*-Diacetyl-3-nitro-2-aminophenol (1·45 g.) separated in diamond-shaped plates; the product obtained by pouring the mother-liquor into water was fractionally crystallised from alcohol, giving *ON*-diacetyl-5-nitro-2-aminophenol. The 3-nitro-derivative (1·0 g.) was hydrolysed by boiling it with 3*N*-hydrochloric acid and gave, on removal of the acidity to Congo-paper, 0·55 g. of 3-*nitro*-2-*aminophenol*; this was soluble in 100 parts of boiling water and crystallised in red needles, m. p. 216—217° (Found: C, 46·2; H, 3·9. $C_6H_6O_3N_2$ requires C, 46·7; H, 3·9%). It gave an intense red colour on addition of alkali.

A comparison of the relative colour intensities of 0·2% solutions of 3-, 4-, 5-, and 6-nitro-2-aminophenols in 0·5*N*-sodium

hydroxide by means of the Lovibond tintometer gave the following values :

	3-*Nitro.*	4-*Nitro.*	5-*Nitro.*	6-*Nitro.*
Red	36	16	31	15
Yellow	11	18	14	15

Definite maxima of redness which control the general visible intensity are thus found for the two compounds which are *m*-nitrophenols. It thus appears that the chromophoric character in alkaline solution of the *o*- and *p*-nitroaniline groups may be greater than that of the *o*- and *p*-nitrophenolic groups.

Nitration of N-*Acetyl-2-aminophenol.—By nitric acid in acetic anhydride.* A solution of monoacetyl-2-aminophenol (1·5 g.) in 50 c.c. of acetic anhydride at 80° was cooled to 30°, and nitric acid (0·5 c.c.; *d* 1·5) in 2·5 c.c. of acetic anhydride then slowly added. On cooling, there separated 0·5 g. of 4-nitro-2-acetamidophenol, m. p. 279—280°. In admixture with an authentic specimen and with 5-nitro-2-acetamidophenol, this melted at 279—280° and at 245°, respectively. The mother-liquor, on concentration at 70°, gave no further deposit. It was therefore evaporated twice with water at 50°, the residue hydrolysed by boiling for 1 hour with 3*N*-hydrochloric acid, and the solution neutralised (Congo-paper). The brown solid (0·4 g.), m. p. about 100°, thus obtained was ground with *N*-hydrochloric acid, and water gradually added to dissolve the crystalline hydrochloride. The insoluble red solid (0·05 g.) was picramic acid, m. p. 165°, mixed m. p. with pure picramic acid 167°. The soluble fraction, on neutralisation at 80°, gave 0·35 g. of 6-nitro-2-aminophenol; this crystallised in long, maroon-coloured needles, m. p. 112—113°, and its properties agreed with those described for 6-nitro-2-aminophenol by Benda (*Ber.*, 1914, **47**, 1010), except that it gave a red and not a blue solution when diazotised and added to R-salt; with alkaline β-naphthol, it gave a purple precipitate. The original aqueous liquors were neutralised (litmus), and extracted with ether; this removed 0·25 g. of unchanged 2-aminophenol which, alone or mixed with pure 2-aminophenol, melted at 174°.

Action of Sulphuric Acid on ON-*Diacetyl-2-aminophenol.*—A solution of 1 g. of *ON*-diacetyl-2-aminophenol in 2 c.c. of sulphuric acid at 10—15° was, after 1 hour, diluted with ice; extraction with ether than gave *N*-acetyl-2-aminophenol in almost quantitative yield.

Action of Nitric Acid on ON-*Diacetyl-2-aminophenol at* — 5°.— One g. of diacetylaminophenol was dissolved in a mixture of 4 c.c. of nitric acid (*d* 1·42) and 2 c.c. (*d* 1·5) at — 5° and kept at this temperature for ½ hour. When the solution was mixed with ice,

0·5 g. of unchanged diacetyl-2-aminophenol separated; ether extraction of the mother-liquor gave a further 0·15 g. containing traces of nitrated products.

The thanks of the author are due to Mr. W. K. Anslow for help in the preparations and analyses.

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HAMPSTEAD. [*Received. March 18th*, 1927.]