ANTITUBERCULAR COMPOUNDS

Part II. * 3 : 5-Diiodo-4-aminosalicylic Acid, 4 -Amino-*O*-acetylsalicylic Acid and Other Derivatives of 4-Aminosalicylic Acid

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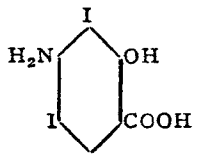
BECAUSE of its low toxicity and usefulness in the treatment of tuberculosis, 4-aminosalicylic acid (PAS) has attracted wide interest, directed towards its synthesis by different methods,[1,2] estimation,[3] pharmacology,[4] preparation of derivatives[5] and the study of compounds analogous to it.[6] With a view to obtain drugs more potent than PAS, numerous derivatives of PAS have been prepared, but none has been found to surpass PAS in *in vivo* activity. A method for the synthesis of PAS from 4-nitro-*o*-toluidine has been described by us[1] and it was shown that this route has the advantage of the accessibility of certain derivatives of PAS. Thus, 3 : 3′-dihydroxyazobenzene-4 : 4′-dicarboxylic acid was synthesized,[1] while the preparation of 4-amino-*O*-acetylsalicylic acid (aminoaspirin) is now reported.

Among the few nuclear substituted derivatives of PAS so far recorded, 3 : 5-dibromo-PAS has been described by Drain *et al.*[5b] and Hirst and Hurni.[5d] The latter workers have determined its inhibition concentration (12·5–6·25), which is much higher than that of PAS (0·0487–0·0293). Diiodosalicylic acid is more stable[7] *in vivo* and *in vitro* and is a more potent antibacterial agent than the corresponding dibromosalicylic acid[8]; Küster and Wagner-Jauregg[9] have shown that sodium 5-iodosalicylate and 3 : 5-diiodosalicylate are bactericidal to acid-fast bacteria, being 1·25 and 4·5 times as active as chaulmoogric acid against tubercle bacilli. Izzo and Cicardo[10] have also found that the injection of thyroxine into guinea pigs infected with tubercle bacilli produces a considerable prolongation of life, whereas thyroid-ectomized guinea pigs have a very much lower resistance to tuberculosis. For these reasons, 3 : 5-diiodo-4-aminosalicylic acid (I) has been prepared by us by the action of iodine monochloride on PAS in glacial acetic acid. The sodium salt is moderately soluble in water. When boiled with dilute hydrochloric acid, (I) decomposes to *m*-aminophenol with the liberation of iodine

* The paper on "3 : 3′-Dihydroxyazobenzene-4 : 4 -dicarboxylic acid and 4-aminosalicylic acid" (*Proc. Ind. Acad. Sci.*, 1949, 29 A, 196) is to be regarded as Part I of the series.

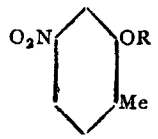
and carbon dioxide, but is unaffected by boiling with water. Because of the iodine atoms adjacent to the amino group, (I) can only be diazotized



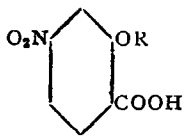
(I)

under special conditions such as the addition of solid sodium nitrite to a solution in concentrated sulphuric acid. *In vitro* tests by Prof. B. V. Bhide, to whom our thanks are due, have shown that (I) inhibits the growth of tubercle bacilli up to a concentration of 1 : 5000 only. In view of the low toxicity of PAS and the readiness with which iodine is liberated from (I), tests on (I) in intestinal infections have been instituted.

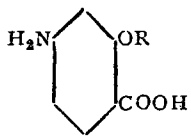
Although many functional derivatives of PAS have been reported by various workers, little attention seems to have been paid to the synthesis of its ethers and *O*-acyl derivatives. 4-Amino-*O*-acetylsalicylic acid has now been obtained by catalytic reduction of *O*-acetyl-4-nitrosalicylic acid.[1] The compound is stable under anhydrous conditions and can be dried at 120° without decomposition. It is deacetylated on warming with 5% aqueous sodium hydroxide. Ethers (IV, R = Me, Et and *n*-C_4H_9) have been prepared by catalytic reduction of *O*-alkyl derivatives (III) of 4-nitrosalicylic acid, obtained by the oxidation of the corresponding ethers (II) of 4-nitro-*o*-cresol. The yield of the *O*-alkyl derivatives obtained by the oxidation of



(II)



(III)

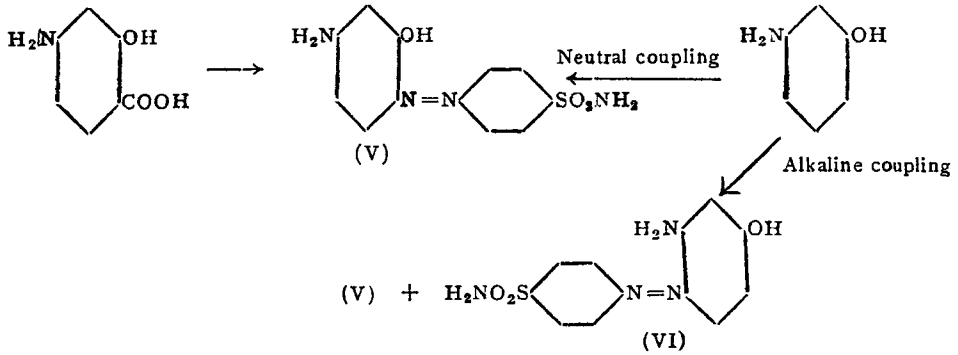


(IV)

(II) considerably diminishes with increase in the length of the alkyl chain. The methyl ester of (IV; R = Me) has also been prepared. The methyl, ethyl and *n*-propyl esters of PAS were obtained from the corresponding esters of 4-nitrosalicylic acid by catalytic reduction.[5a] The synthesis of other esters of PAS was not undertaken, since Drain *et al.*[5b] and Rosdahl *et al.*[5c] have reported the preparation of a series of esters by the same method. Shaefer and Doub[5f] have esterified PAS directly in 70% yield by using boron trifluoride as catalyst. Most of these esters of PAS have about the same bacteriostatic effect as the free acid.[5c]

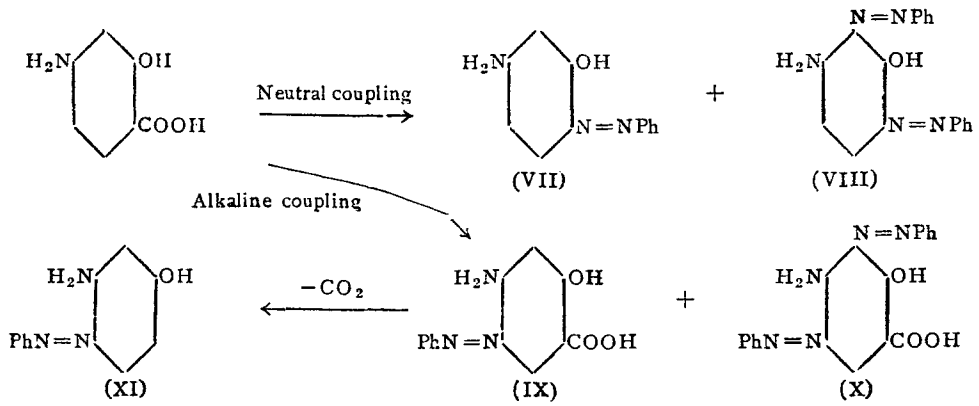
The coupling behaviour of diazotized PAS towards phenolic components has already been reported by us.[1] A study of its coupling be a iour towards

diazonium salts was considered to be of interest since the procedure is employed in its estimation in body fluids.[3] When diazotized sulphanilamide was coupled with PAS in alcoholic sodium acetate solution, a dye (A), m.p. 236°, insoluble in sodium bicarbonate solution, was obtained.[5a] The properties of the dye indicated decarboxylation, and it was identical with the dye obtained by coupling *m*-aminophenol with diazotized sulphanilamide under similar conditions. The alkaline coupling of *m*-aminophenol with one mol. of diazotized sulphanilamide gave a mixture of dyes which were separated from acetone solution by chromatographic adsorption on alumina into two fractions: one melting at 236° and the other (B) at 224°. The dye, m.p. 236°, was weakly adsorbed on the alumina column and was identical



with the dye (A) obtained by neutral coupling of PAS. The formation of the dye (A) under neutral conditions, its chromotographic behaviour and other properties indicated it to be 4-amino-2-hydroxyazobenzene-4′-sulphonamide (V); whereas the dye (B), strongly adsorbed on alumina, must be 2-amino-4-hydroxyazobenzene-4′-sulphonamide (VI). The dyes (V) and (VI) have been described in an I.G. Patent,[11] but the melting points quoted for them are 228° and 106° respectively.

In order to examine more closely the decarboxylation of PAS during diazonium coupling, PAS was coupled with one mol. of diazotized aniline under neutral conditions. In this case also decarboxylation occurred, and a monoazo dye, m.p. 124°, accompanied by a small amount of a *dis*azo dye, m.p. 179-80°, was obtained. The monoazo dye was sparingly soluble in 2% caustic soda solution and largely retained in the ether layer when an ether solution was shaken with aqueous caustic soda, indicating the presence of a hydroxyl group *ortho* to the azo group. Alkaline coupling of PAS with one mol. of diazotized aniline, however, yielded 4-amino-5-benzeneazosalicylic acid (IX), m.p. 223°, together with a small amount of 4-amino-3: 5-*bis*benzeneazosalicylic acid (X), m.p. 256°. The dye (IX) could be smoothly



decarboxylated to 2-amino-4-hydroxyazobenzene (XI), m.p. 187°; the constitution of the monoazo dye, m.p. 124°, obtained by neutral coupling of PAS, was therefore 4-amino-2-hydroxyazobenzene (VII). For final confirmation of the constitutions of the monoazo dyes (VII and XI), their preparation from *m*-aminophenol was necessary. *m*-Aminophenol was coupled with one mol. of diazotized aniline in neutral as well as alkaline conditions. Under either condition a mixture of dyes was formed. They were separated from their benzene solution by chromatographic adsorption on alumina and elution with the same solvent. 4-Amino-2-hydroxyazobenzene (VII) was weakly adsorbed owing to the chelation of the hydroxyl and azo groups, whereas 2-amino-4-hydroxyazobenzene (XI) was retained at the top of the column as expected. In neutral coupling, the dye (VII) was formed in major yield, whereas in alkaline coupling both were formed in almost equal quantities. The visual colour, mode of formation, chromatographic behaviour, alkali-solubility and coloration with concentrated sulphuric acid of the dyes (VII) and (XI) were very similar to the properties of the dyes (V) and (VI) respectively, obtained from PAS and sulphanilamide.

The lower m.p. of the *dis*azo dye obtained in the neutral coupling of PAS with diazotized aniline in comparison with (XI) indicated its constitution as 3-amino-2: 6-*bis*benzeneazo-phenol (VIII).

Thus, the coupling of PAS with diazotized sulphanilamide and aniline under neutral conditions takes place with displacement of the carboxyl group; but coupling takes place normally under alkaline conditions. On treatment with only one mol. of diazotized aniline PAS forms *dis*azo dyes simultaneously with monoazo dyes.

Experimental

3: 5-*Diiodo*-4-*aminosalicylic acid* (I).—A solution of 4-aminosalicylic acid (5·0 g., 0·0326 mole) in glacial acetic acid (250 c.c.) was treated with

iodine monochloride (9·72 g., 0·0598 mole) in glacial acetic acid (30 c.c.) under mechanical stirring. During addition of iodine monochloride a precipitate appeared; water (150 c.c.) was then added when further precipitation occurred. The reaction mixture was gradually heated to 55–60° on the water-bath during 20 minutes and maintained at this temperature for further 20 minutes. After cooling to room temperature the reaction mixture was filtered. The product was washed with water, extracted with 1% sodium bicarbonate solution and the solution acidified with acetic acid. The precipitate was collected and dried (11·0 g.). 3:5-Diiodo-4-aminosalicylic acid crystallizes from alcohol in colourless flat needles, which decomposed at 174° with liberation of iodine vapour (Found: C, 20·9; H, 1·4; N, 3·3; I, 62·3; Equiv. wt., 397. $C_7H_5I_2NO_3$ requires, C, 20·7; H, 1·2; N, 3·4; I, 62·4%; Equiv. wt., 405). The compound is insoluble in water, but moderately soluble in alcohol and acetic acid, and sparingly soluble in acetone and benzene. It decomposes with liberation of iodine, when boiled with 8% hydrochloric acid. With alcoholic ferric chloride it gives a violet colouration. With concentrated sodium bicarbonate solution it forms an insoluble sodium salt, which dissolves on dilution. The sodium salt crystallizes from water, in which it has a solubility of about one in 60 at room temperature.

O-Acetyl-4-aminosalicylic acid.—A solution of the freshly prepared acetate of 4-nitrosalicylic acid, m.p. 156° (1·5 g.) in absolute alcohol (5 c.c.) was shaken with hydrogen under 40 lb. pressure and Raney nickel for half an hour. The residue obtained after filtration of the catalyst and distillation of alcohol under reduced pressure at 60° from the filtrate, crystallized from a mixture of ether and *n*-hexane in colourless plates, m. p. 225° (decom.) (Found; C, 55·4; H, 4·8; N, 7·4. $C_9H_9NO_4$ requires C, 55·4; H, 4·6; N, 7·2%). The substance is very soluble in alcohol, ether and acetone but sparingly soluble in water, benzene and petroleum-ether. The alcoholic solution gives a very faint brown colouration with alcoholic ferric chloride. The compound is deacetylated by treatment with 5% caustic soda solution at room temperature for 60 hours.

Methyl ether of 4-aminosalicylic acid.—4-Nitro-2-methoxybenzoic acid, m.p. 147° (3·0 g.) obtained by the method of Simonsen and Rau,[12] was reduced by shaking with hydrogen at 40 lb. pressure in the presence of Raney nickel (about 0·2 g.) for 2 hours. The catalyst was filtered off and alcohol removed under reduced pressure, when the ether separated in colourless needles. It was crystallized from methanol in plates, m.p. 153° (Found: C, 57·8; H, 5·5; N 8·6. $C_8H_9NO_3$ requires C, 57·5; H, 5·8; N, 8·4%). Froeliches and Cohen[16] quote m.p. 217–8°.

A similar catalytic hydrogenation of methyl-4-nitro-2-methoxybenzoate, m.p. 89° (Simonsen and Rau[12] record the same m.p.) gave *methyl-4-amino-2-methoxybenzoate*, which crystallized from alcohol in plates, m.p. 157–58° (Found: N, 7·8. $C_9H_{11}NO_3$ requires N, 7·7%).

4-Nitro-2-ethoxytoluene.—To sodium ethoxide (24 c.c. alcohol and 0·9 g. sodium) was added 4-nitro-*o*-cresol (6·0 g.) and the mixture warmed on the water bath. An excess of ethyl bromide (12·0 cc.) was then added and the mixture refluxed until it gave no test for alkalinity (litmus). Alcohol and unreacted ethyl bromide were removed under reduced pressure and the residue was extracted with 5% sodium hydroxide solution to remove any unreacted cresol. 4-Nitro-2-ethoxytoluene crystallized from 70% alcohol (Norit) (6·2 g; 93% yield), in colourless needles, m.p. 56–58° (Spiegel *et al.*[13] who prepared the compound by using ethyl iodide quote m.p. 61°) (Found: N, 7·8. $C_9H_{11}NO_3$ requires N, 7·7%). 4-Nitro-2-ethoxytoluene has also been obtained in 85–90% yield by direct ethylation of 4-nitro-*o*-cresol with ethyl sulphate in alkaline medium at 50–60° temperature.

4-Nitro-2-n-butoxytoluene obtained by the alkyl bromide method crystallised from 70% alcohol in long needles, m.p. 48° (Found: N, 7·2. $C_{11}H_{15}NO_3$ requires N, 6·7%).

4-Nitro-2-ethoxybenzoic acid.—To 4-nitro-2-ethoxytoluene (4·0 g.) in boiling water (50 cc.) containing magnesium sulphate (12·0 g.) was added under mechanical agitation, potassium permanganate solution (250 c.c., 4% solution) in small quantities during 2 hours. Boiling was continued for a further 1½ hours. Excess of potassium permanganate was destroyed by adding a few drops of alcohol and the reaction mixture filtered while hot. The precipitate was washed with hot water and the filtrate and washings acidified with hydrochloric acid. The precipitated acid was filtered and purified by treatment with sodium bicarbonate solution. It crystallized from water in colourless elongated plates (0·6 g.) m.p. 148° (Found: N, 6·6. $C_9H_9NO_5$ requires N, 6·6%).

4-Nitro-2n-butoxybenzoic acid similarly prepared, crystallized from 80% alcohol in plates, m.p. 125° (Found: N, 6·3. $C_{11}H_{13}NO_5$ requires N, 5·9%).

The ethyl and n-butyl ethers of 4-aminosalicylic acid were obtained from the corresponding nitro compounds by reduction, similar to that of 4-nitro-2-methoxybenzoic acid described earlier. The former crystallized from methanol in colourless plates, m.p. 151° (Found: N, 7·9. $C_9H_{11}NO_3$ requires N, 7·7%); and the latter from methyl alcohol in plates, m.p. 167° (decomp.) (Found: N, 6·7. $C_{11}H_{15}NO_3$ requires N, 6·7%).

Methyl 4-*aminosalicylate.*—A solution of methyl 4-nitro-salicylate, m.p. 97°[5a] (1·5 g.) in benzene (15 c.c.) was shaken with hydrogen under 40 lb. pressure and Raney nickel for one hour. The residue obtained after filtration of the catalyst and removal of benzene from the filtrate crystallized from a mixture of benzene and *n*-hexane in colourless prisms, m.p. 120–1° (118°, 120–1° and 121–2° in references 5*c*, 5*b* and 5*f* respectively) (Found: N, 8·4. $C_8H_9NO_3$ requires N, 8·3%).

Ethyl-4 *aminosalicylate* crystallized from benzene-hexane in colourless needles, m.p. 114–15° (115°, 111·5–113° and 114–5° in references 5*c*, 5*b* and 5*f* respectively) (Found: N, 7·6. $C_9H_{11}NO_3$ requires, N, 7·7%). n-*Propyl*-4-*aminosalicylate* crystallized from benzene-hexane in colourless needles, m.p. 102–4° (102–3° and 102–3° in references 5*c* and 5*b* respectively) Found: N, 7·0. $C_{10}H_{13}NO_3$ requires N, 7·2%).

Coupling of 4-*aminosalicylic acid with diazotized sulphanilamide under neutral conditions and formation of* 4-*amino*-2-*hydroxyazobenzene*-4′-*sulphonamide* (*V*).—A solution of 4-aminosalicylic acid (0·77 g.; 0·005 mole.) in alcohol (20 c.c.) and sodium acetate solution (6·0 g. in 20 c.c. water) was treated with the diazonium solution obtained from sulphanilamide (0·7 g.; 0·005 mole.). After stirring for half an hour, the dye was filtered and crystallized from 60% alcohol. The long orange-red needles had m.p. 236° (Found: N, 18·4. $C_{12}H_{12}N_4O_3S$ requires N, 18·4%). The dye is insoluble in sodium bicarbonate solution, but soluble in caustic soda and sodium carbonate solutions, and when mixed with the dye obtained by coupling *m*-aminophenol with diazotized sulphanilamide in alcoholic sodium acetate solution (see below) showed no depression in m.p. It gives a yellow colouration with concentrated sulphuric acid.

Coupling of m-*aminophenol with diazotized sulphanilamide under neutral conditions.*—On coupling in alcoholic sodium acetate solution with diazotized sulphanilamide, *m*-aminophenol gave a dye which crystallized from 60% alcohol in orange-red needles, m.p. 236° (Found: N, 18·4. $C_{12}H_{12}N_4O_3S$ requires N, 18·4%).

Coupling of m-*aminophenol with diazotized sulphanilamide under alkaline condition and formation of* 2-*amino*-4-*hydroxyazobenzene*-4′-*sulphonamide* (*VI*).—To a mechanically stirred solution of *m*-aminophenol (2·18 g.) in 2% caustic soda solution (200 c.c.) maintained at 0° was added the diazonium solution prepared from sulphanilamide (3·44 g.). The reaction mixture was stirred for half an hour and acidified with acetic acid. The precipitate was collected and dried at about 80°, when the red dye became much darker in colour. The dye was insoluble in benzene, *n*-hexane, carbon tetrachloride,

toluene, ether and chloroform, slightly soluble in boiling water and moderately soluble in acetone, alcohol and acetic acid. An acetone solution of the dye was chromatographed on a column of alumina. The chromatogram was developed with alcohol-acetone (1 : 4) when two bands appeared: an upper orange band and a lower yellow band. The lower band was eluted with alcohol-acetone, and the upper was extracted with boiling alcohol. On working the alcohol-acetone solution, a dye, m.p. 236°, was obtained; while the residue obtained on distillation of the alcohol extract of the upper band yielded after crystallization from water, yellow plates, m.p. 224° (Found: N, 18·7. $C_{12}H_{16}N_4O_3S$ requires N, 18·4%). The dye gives an orange colouration with concentrated sulphuric acid.

Coupling of 4-aminosalicylic acid with diazotized aniline under neutral conditions and formation of (VII).—To a mechanically stirred solution of 4-aminosalicylic acid (1·53 g., 0·01 mole.) in alcohol (40 c.c.), water (30 c.c.) and sodium acetate solution (6·0 g. in 20 c.c. water) at 0° was added a diazonium solution prepared from aniline (0·93 g., 0·01 mole.). The reaction mixture was stirred for half an hour and diluted with water (100 c.c.). The reddish orange dye was filtered, washed with water and taken up in ether. The ethereal solution was extracted successively with 5% sodium bicarbonate solution and 5% sodium hydroxide solution. On acidifying the latter extract with acetic acid, a dye separated, which crystallized from dilute alcohol in reddish orange needles, m.p. 124° (Found: N, 19·4. $C_{12}H_{11}N_3O$ requires N, 19·7%). It forms a yellow solution in concentrated sulphuric acid.

Evaporation of the ether, from which the alkali-soluble dye had been extracted, gave a crystalline residue, which on recrystallization from 60% alcohol yielded shining brown plates, m.p. 179–80° (Found: N, 22·4. $C_{18}H_{15}N_5O$ requires N, 22·1%). The dye gives a bordeaux-red colouration with concentrated sulphuric acid.

Coupling of 4-aminosalicylic acid with diazotized aniline under alkaline condition and formation of 4-amino-5-benzeneazosalicylic acid (IX).—Aniline (1·86 g.), in a mixture of concentrated hydrochloric acid (6 c.c.) and water (6 c.c.) was diazotized with a solution of sodium nitrite (1·6 g.) in water (10 cc.) at 0°. The solution was then slowly added to a mechanically stirred, cold solution of 4-aminosalicylic acid (3·16 g.) dissolved in 10% sodium hydroxide solution (50 c.c.) and water (40 c.c.). The temperature of the reaction mixture was kept below 5° by external cooling. On addition of the diazonium solution coupling began immediately with the development of a red colour. After the addition of the diazonium solution was over, stirring was continued for one hour. The reaction mixture was then filtered, and the residue washed

with water. Crystallization from 80% acetic acid gave a brown dye, m.p. 256° (decomp.) (Found: N, 19·3. $C_{19}H_{15}N_5O_3$ requires N, 19·3%). The dye gives a pure green colouration with concentrated sulphuric acid.

Acidification of the filtrate with acetic acid gave the dye (IX) which on repeated crystallizations from 50% alcohol (norit) was obtained as long yellow needles, m.p. 223° (decomp.) (Found: N, 16·6. $C_{13}H_{11}N_3O_3$ requires N, 16·3%). The dye forms an insoluble sodium salt in 10% sodium bicarbonate solution, which dissolves on dilution. It gives an orange colouration with concentrated sulphuric acid.

Decarboxylation of (IX).—The acid (IX) (40 mg.) was suspended in glycerol (10 c.c.) and the mixture heated for 20 minutes in a glycerol bath at 180°. The dye slowly dissolved in glycerol and evolution of gas was perceptible during heating. The reaction mixture was then cooled and diluted with water (40 c.c.) when a crystalline dye separated. It was filtered and crystallized from dilute alcohol, m.p. 187°.

Coupling of m-*aminophenol with diazotized aniline under neutral conditions and formation of (VII) and (XI).*—The dye obtained by adding diazotized aniline (0·93 g.) to a solution of *m*-aminophenol (1·09 g.) in alcohol (30 c.c.) and sodium acetate solution (6 g. in 20 c.c. water), was dissolved in benzene and chromatographed on a column of alumina. Using the same solvent for development of the chromatogram, two bands appeared: an upper orange-coloured band and a lower yellow band. The alumina column was taken out and the two zones were separated. Extraction of the upper portion with alcohol, partial evaporation of the solvent and dilution with water yielded golden yellow plates, m.p. 187°. The m.p. of this dye admixed with the dye obtained by decarboxylation of (IX) was not depressed (Found: N, 19·4. $C_{12}H_{11}N_3O$ requires, N, 19·7%). Similar treatment of the lower part of the alumina column gave bright orange needles, m.p. 124° (Found N, 19·4. $C_{12}H_{11}N_3O$ requires N, 19·7%). The m.p. of the latter dye was not depressed by admixture with the monoazo dye obtained by coupling PAS with diazotized aniline in alcoholic-sodium acetate solution, described earlier. This dye gives with concentrated sulphuric acid a yellow colouration, and the dye m.p. 187°, an orange colouration.

Coupling of m-*aminophenol with diazotized aniline under alkaline conditions.*—On coupling in 2% caustic soda solution with diazotized aniline, *m*-aminophenol gave a mixture of dyes, m.p. 124° and m.p. 187°, in nearly equal proportion. The two dyes were separated by chromatographic adsorption as described in the previous experiment.

SUMMARY

3 : 5-Diiodosalicylic acid, 4-amino-*O*-acetylsalicylic acid (aminoaspirin), and a series of ethers of 4-aminosalicylic acid have been prepared.

The behaviour of 4-aminosalicylic acid towards diazonium salts has been investigated.

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