158. *New Syntheses of Heterocyclic Compounds. Part IV. 3-Azaphenthiazines.*

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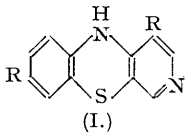
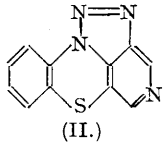
The synthesis of azaphenoxazines described in Part III (Petrow and Rewald, J., 1945, 313) has now been extended to the corresponding *azaphenthiazines*. 1-*Nitro*-3-*azaphenthiazine* (I; R = NO_2; R′ = H) has been prepared and its reduction and nitration studied. Biological examination of these new azaphenthiazines and of the related azaphenoxazines described in Part III (*loc. cit.*) did not reveal any outstanding activity.

The synthesis of 3-azaphenoxazines described in Part III (*loc. cit.*) from 3 : 5-dinitro-4-chloropyridine and *o*-aminophenol has now been extended to the preparation of the hitherto unknown 3-azaphenthiazines. These substances formed the ultimate object of our investigation, and, from their formal analogy to methylene blue, appeared to us of interest from the chemotherapeutic standpoint.

Condensation of 4-chloro-3 : 5-dinitropyridine (Part III, *loc. cit.*) with *o*-aminothiophenol or its readily accessible zinc double salt (Bogert and Snell, *J. Amer. Chem. Soc.*, 1924, **46**, 1309) led to the formation of 1-*nitro*-3-*azaphenthiazine* (I; R = NO_2; R′ = H) with evolution of oxides of nitrogen. In contrast to the corresponding oxazine analogue (Part III, *loc. cit.*) we were unable to isolate the intermediate dinitropyridyl-*o*-aminothiophenol, which appeared to undergo spontaneous ring closure on formation. The reactions of (I; R = NO_2; R′ = H) were analogous to those of 1-nitro-3-azaphenoxazine. The compound gave a deep purple *methiodide*, and on reduction passed into 1-*amino*-3-*azaphenthiazine* (I; R = NH_2; R′ = H) characterised by a *monoacetyl* derivative and by a *monohydrochloride*. Treatment with nitrous acid led to the formation of 3-*azaphenthiazine*-1 : 10-*diazole* (II), a reaction typical of an *o*-aminodiphenylamine residue. Nitration of 1-nitro-3-azaphenthiazine in concentrated sulphuric acid gave a *dinitrosulphoxide*. We have


(I.)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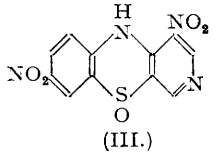
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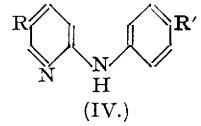
(IV.)

assigned the constitution of a 1 : 7-*dinitro*-3-*azaphenthiazine sulphoxide* (III) to this compound from analogy with (a) the nitration of the corresponding 1-nitro-3-azaphenoxazine (Petrow and Rewald, *loc. cit.*) and (b) the nitration of phenthiazine which leads to a 3 : 7-dinitrophenthiazine sulphoxide (Bernthsen, *Ber.*, 1884, **17**, 611). Reduction of (III) gave 1 : 7-*diamino*-3-*azaphenthiazine* (I; R = R′ = NH_2), isolated as the *dihydrochloride*, but all attempts to obtain a quinonoidal oxidation product from this diamino-compound were unsuccessful.

Finally, we have examined the possibility of extending the Bernthsen synthesis of phenthiazine from diphenylamine by sulphur fusion (*Annalen*, 1884, **230**, 77; cf. G.P. 25,150) to the following suitably constituted pyridine analogues: 4-anilinopyridine (Kermack and Weatherhead, *J.*, 1942, 726), 4-anilinoquinaldine (Fischer, Diepolder and Wölfel, *J. prak. Chem.*, 1925, **109**, 61), 4-*p*-acetamidophenylamino-2-methylquinoline (Glen, Sutherland, and Wilson, *J.*, 1939, 491), and 5-amino-2-(4′-aminophenylamino)pyridine (IV; R = R′ = NH_2).

We prepared the latter compound by condensing 5-nitro-2-chloropyridine with *p*-aminoacetanilide in glacial acetic acid solution in the presence of potassium acetate to give *5-nitro-2-(4'-acetamidophenylamino)-pyridine* (IV; R = NO_2, R' = NHAc). Reduction gave *5-amino-2-(4'-acetamidophenylamino)pyridine* (IV; R = NH_2, R' = NHAc), hydrolysed with hydrochloric acid to the diamino-compound, isolated as the *dihydrochloride*. We were not able to convert any of these diphenylamine analogues into the azaphenthiazines by the known standard methods. For example, fusion with sulphur in *o*-dichlorobenzene (cf. G.P. 237,771) gave in all instances black, microcrystalline substances containing carbon, hydrogen, nitrogen and sulphur, resembling in general properties the so-called "sulphur dyes" to which they are probably analogous structurally.

Biological examination of some of the above compounds was very kindly undertaken by the Therapeutic Research Corporation of Great Britain Ltd. (*see* Experimental).

Experimental.

M. p.'s are corrected. Most of the analyses are by Drs. Weiler and Strauss, Oxford.

1-*Nitro*-3-*azaphenthiazine* (I; R = NO_2, R' = H).—(a) 4-Chloro-3 : 5-dinitropyridine (3·7 g.) (Petrow and Rewald, *loc. cit.*), *o*-aminothiophenol hydrochloride (3 g.) (Claasz, *Ber.*, 1912, **45**, 1029) and anhydrous sodium acetate (7 g.) were heated for 20 minutes under reflux in absolute alcohol (30 ml.). Aqueous sodium hydroxide (33%) was gradually added to the boiling blood-red solution until a permanent purple colouration was obtained, and the mixture diluted with an equal volume of water and left to stand at 0°. The black crystalline product on crystallisation from alcohol (charcoal) gave 1-*nitro*-3-*azaphenthiazine* in purple-brown platelets, m. p. 141—142° (Found: C, 53·6; H, 2·9; N, 16·9; S, 13·0. $C_{11}H_7O_2N_3S$ requires C, 53·9; H, 2·9; N, 17·1; S, 13·1%). (b) 4-Hydroxy-3 : 5-dinitropyridine (20 g.) was treated under reflux with phosphorus pentachloride (36 g.) and a little phosphorus oxychloride for one hour at 150—160°, and the phosphorus halides removed under reduced pressure (cf. Part III). The crude 4-chloro-3 : 5-dinitropyridine, dissolved in boiling benzene, was treated with *o*-aminothiophenol zinc double salt (15 g.; Bogert and Snell, *J. Amer. Chem. Soc.*, 1924, **46**, 1309) followed immediately by excess anhydrous sodium acetate. The mixture was heated under reflux until evolution of oxides of nitrogen had ceased (*ca.* 2 hours), the benzene removed on the water bath under reduced pressure and the dark residue extracted with boiling alcohol (500 ml.). The filtrate (charcoal) was concentrated to 100—150 ml. and poured into water (500 ml.). The precipitated material in alcohol (500 ml.) was treated at the boiling point with 33% aqueous sodium hydroxide until the appearance of a permanent purple colouration. The solution was then concentrated and poured into water as above. Crystallisation from alcohol gave 1-nitro-3-azaphenthiazine (7 g., 30%) identical with the material described under (a). The *methiodide*, purple plates from water, m. p. 268° (decomp.) (Found: I, 33·2. $C_{12}H_{10}O_2N_3IS$ requires I, 32·8%), was prepared *via* the methosulphate, obtained in nearly quantitative yield when dimethyl sulphate (1 g.) was added to a solution of (I; R = NO_2, R' = H) (2 g.) in nitrobenzene (20 ml.) at about 150°.

1-*Amino*-3-*azaphenthiazine* (I; R = NH_2, R' = H).—1-Nitro-3-azaphenthiazine (1·5 g.), reduced iron (8 g.), and a little calcium chloride were heated under reflux in 70% alcohol (40 ml.) for one hour. The filtrate and washings were concentrated until crystallisation commenced. 1-*Amino*-3-*azaphenthiazine* formed yellow crystals from spirit, m. p. 310—311° (sinters 280°) (Found: C, 61·3; H, 4·2; N, 19·4; S, 14·9. $C_{11}H_9N_3S$ requires C, 61·4; H, 4·2; N, 19·5; S, 14·9%). The *hydrochloride* separated from dilute hydrochloric acid in glittering golden needles, m. p. >300° (Found: Cl, 14·1. $C_{11}H_{10}N_3ClS$ requires Cl, 14·1%). The *acetyl* derivative formed white crystals from 50% alcohol, m. p. 230—231° (Found: C, 60·6; H, 4·3; N, 16·4; S, 12·4. $C_{13}H_{11}ON_3S$ requires C, 60·7; H, 4·3; N, 16·3; S, 12·5%).

3-*Azaphenthiazine*-1 : 10-*diazole* (II).—1-Amino-3-azaphenthiazine hydrochloride (2·5 g.) dissolved in water (30 ml.) and concentrated hydrochloric acid (10 ml.) was treated at 0°, whilst mechanically stirred, with a solution of sodium nitrite (1 g.) added dropwise over 30 minutes. After bringing to the boil and cooling, the white hydrochloride of (II) was collected, dissolved in water and the base liberated with ammonia. 3-*Azaphenthiazine*-1 : 10-*diazole* separated from alcohol in white, felted needles, m. p. 199—200° (Found: C, 58·4; H, 2·8; N, 24·8. $C_{11}H_6N_4S$ requires C, 58·4; H, 2·7; N, 24·8%).

1 : 7-*Dinitro*-3-*azaphenthiazine Sulphoxide* (III).—Finely powdered 1-nitro-3-azaphenthiazine (2 g.) dissolved in conc. sulphuric acid (20 ml.) was mechanically stirred and treated at 0° with concentrated nitric acid (*d*, 1·42; 7 ml.) added at the rate of 1 ml. every 10 minutes. After 90 minutes, the solution was poured into water, ammonia (*d*, 0·880) added in slight excess followed by acetic acid until the orange colour of the mixture changed to bright yellow. The precipitated material was collected and crystallised from alcohol–pyridine. 1 : 7-*Dinitro*-3-*azaphenthiazine sulphoxide* formed pale yellow needles, m. p. 282° (decomp.) (Found: C, 43·6; H, 2·4; N, 18·1; S, 10·7. $C_{11}H_6O_5N_4S$ requires C, 43·1; H, 2·0; N, 18·3; S, 10·5%). Reduction to the dinitroazaphenthiazine could not be achieved either by alcoholic sulphuric or hydrochloric acid (cf. Kehrmann and Nossenko, *Ber.*, 1913, **46**, 2816) or by prolonged treatment with anhydrous formic acid.

1 : 7-*Diamino*-3-*azaphenthiazine Dihydrochloride* (I; R = R' = NH_2,HCl).—Dinitroazaphenthiazine sulphoxide (2 g.), reduced iron (6 g.) and anhydrous calcium chloride (1 g.) were heated under reflux in 80% alcohol (100 ml.) for one hour. The filtrate and washings were concentrated to small bulk and saturated with hydrogen chloride. 1 : 7-*Diamino*-3-*azaphenthiazine dihydrochloride* separated from aqueous alcohol in yellow crystals with a faintly green reflex, m. p. >300° (Found: Cl, 23·7. $C_{11}H_{10}N_4S$,2HCl requires Cl, 23·4%). Oxidation with ferric chloride in aqueous solution gave a black insoluble amorphous product. When the hydrochloride (1·1 g.) and chloranil (1 g.; 1·1 mols.) were heated under reflux in alcohol (200 ml.) for 16 hours and the product collected and thoroughly washed with boiling water and acetic acid, a black microcrystalline substance was obtained, m. p. >300°. It was insoluble in the usual solvents, pyridine and dilute hydrochloric and nitric acids. It dissolved in concentrated sulphuric acid (blue-green colour) and was slightly soluble in concentrated nitric acid (green colour). The compound is probably a condensation product of 2 mols. of base with 1 mol. of chloranil with elimination of 2HCl (Found: Cl, 11·7. $C_{28}H_{18}N_8S_2O_2Cl_2$ requires Cl, 11·2%).

5-Nitro-2-(4'-acetamidophenylamino)pyridine (IV; R = NO_2, R' = NHAc).—5-Nitro-2-chloropyridine (10 g.), *p*-aminoacetanilide (10 g.) and anhydrous potassium acetate (7 g.) in glacial acetic acid (50 ml.) were heated under reflux for 6 hours. The mixture was concentrated to small bulk and precipitated with water. The product was crystallised first from alcohol and finally from 50% acetic acid. *5-Nitro-2-(4'-acetamidophenylamino)pyridine* (13 g., 75%) formed red crystals, m. p. 239—240° (Found: C, 56·9; H, 4·4; N, 20·6. $C_{13}H_{12}O_3N_4$ requires C, 57·4; H, 4·4; N, 20·6%). Hydrolysis of the acetamido-compound (5 g.) with concentrated hydrochloric acid (50 ml.) for 3 hours under reflux followed by concentration and precipitation with ammonia gave *5-nitro-2-(4'-aminophenylamino)pyridine*, separating from alcohol–benzene in pale red crystals (3·5 g., 80%), m. p. 176—177° (Found: C, 57·8; H, 4·2; N, 23·9. $C_{11}H_{10}O_2N_4$ requires C, 57·4; H, 4·3; N, 24·4%).

5-Amino-2-(4′-acetamidophenylamino)pyridine (IV; R = NH_2, R′ = NHAc).—5-Nitro-2-(4′-acetamidophenylamino)-pyridine (5 g.), reduced iron (20 g.), anhydrous calcium chloride (0·5 g.) and 70% alcohol (200 ml.) were heated under reflux for one hour and the filtrate and washings (charcoal) concentrated to small bulk. *5-Amino-2-(4′-acetamido-phenylamino)pyridine* formed silvery white platelets from alcohol, m. p. 242—243° (Found: C, 64·0; H, 5·9; N, 23·5%. $C_{13}H_{14}ON_4$ requires C, 64·4; H, 5·8; N, 23·2%). The foregoing acetamido-compound was hydrolysed by heating for 2 hours under reflux with concentrated hydrochloric acid (25 ml.) and the mixture taken to dryness. The residue was washed with ether and crystallised from aqueous alcohol and alcohol–ligroin. *5-Amino-2-(4′-aminophenylamino)-pyridine dihydrochloride* formed yellowish-green crystals, m. p. *ca.* 292° (decomp.) (Found: Cl, 25·8. $C_{11}H_{12}N_4$,2HCl requires Cl, 26·0%).

Sulphur fusions. The compounds were heated with 2 mols. of sulphur at 280—300°, or in *o*-dichlorobenzene under reflux with traces of iodine as catalyst until evolution of hydrogen sulphide ceased. The products were isolated by extraction with hot spirit, and formed in all instances black, microcrystalline powders, m. p. >300° containing C, H, N and S.

Biological Examination.—Dr. R. Wien and Dr. J. Harrison (Biological Division, May and Baker Ltd.), on behalf of the Therapeutic Research Corporation of Great Britain Ltd., have very kindly tested the following substances for therapeutic activity. Antibacterial activity was tested by the serial dilutional method in nutrient broth against *Staphylococcus aureus* (a), *B. coli* (b), and *Pseudomonas pyocyanea* (c). The bacteriostatic activity (minimal effective concentration) was assessed by the turbidity of the broth after 18 hours' incubation at 37°. The toxicity ($L.D._{50}$) was determined by both intravenous and subcutaneous administration. Some of the compounds were described in Part III (Petrow and Rewald, *loc. cit.*).

TABLE.

Compound (hydrochloride).	$L.D._{50}$ (mg./kg.). i/v.	s/c.	Antibacterial activity (min. effective concn.). (a).	(b).	(c).
1-Amino-3-azaphenoxazine	100	—	1 : 8000	1 : 8000	1 : 2000
1 : 7-Diamino-3-azaphenoxazine	120	70	1 : 16000	1 : 4000	1 : 8000
1 : 8-Diamino-3-azaphenoxazine	200	600	1 : 2000	1 : 4000	1 : 2000
7 : 9-Diamino-3-azaphenoxazine 1-Amino-3-azaphenthiazine 1 : 7-Diamino-3-azaphenthiazine	Too insoluble to give results of value.				

The above did not show activity against either *T. equiperdum* infections in mice or *Pl. gallinaceum* in chicks.

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QUEEN MARY COLLEGE (UNIVERSITY OF LONDON). [*Received, June 14th,* 1945.]