83. *New Syntheses of Heterocyclic Compounds. Part III. Azaphenoxazines.*

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The aim of the present investigation was the preparation of analogues of methylene-blue containing nuclear nitrogen in one of the benzenoid rings. Representatives have been obtained in the azaphenoxazine series as exemplified by 7 : 9-*dinitro*-4-, 5-*nitro*-3-, 5 : 9-*dinitro*-3- and 5 : 8-*dinitro*-3-*azaphenoxazines*, and reduced to the corresponding *amino*-compounds. The method was a modification of Turpin's reaction (J., 1891, **59**, 722) and its extension by Misslin and Bau (*Helv. Chim. Acta*, 1919, **2**, 295).

The value of methylene-blue (I) in the treatment of malaria was first demonstrated by Guttmann and Ehrlich (*Berl. Klin. Woch.*, 1891, **28**, 593) and this compound formed the starting point for extensive antimalarial studies which culminated in the preparation of pamaquin, 8-diethylamino*iso*pentylamino-6-methoxyquinoline. Among the possible alternative variations of the methylene-blue molecule, the one involving the replacement of one of the benzenoid rings by one containing nitrogen (II) seemed from the chemotherapeutic standpoint worthy of exploration. Azaphenthiazines have not, however, hitherto been described in the literature. We have carried out model experiments on the synthesis of the related azaphenoxazines, for which the starting materials are relatively more accessible. The application of our results to the preparation of azaphenthiazines will be reported in a subsequent communication.

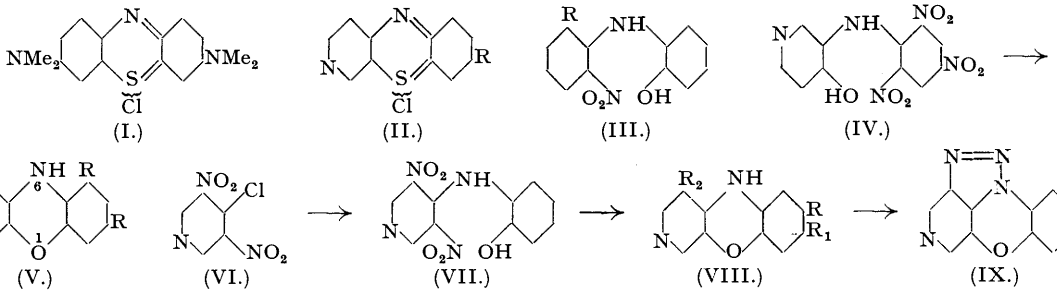
The facile synthesis of dinitrophenoxazine from picryl chloride and *o*-aminophenol in the presence of alkali was discovered by Turpin (J., 1891, **59**, 722) (cf. Kehrmann, *Ber.*, 1899, **32**, 2603). Subsequently the reaction was found to proceed equally readily with 2 : 6-dinitrochlorobenzene (Ullmann, *Annalen*, 1909, **366**, 110) and was ultimately extended to embrace 6-substituted 2-nitrochlorobenzenes *via* the intermediate compounds (III) (Ullmann, *ibid.*, p. 79; G.P. 200,736). The mechanism of the reaction was studied by Brady and Waller (J., 1930, 1218), who found that ring closure of (III) proceeded equally readily when R was either an electropositive or an electronegative substituent. They concluded that the effect of R (formula III) was largely stereochemical, forcing the *o*-nitro-group into close proximity to the hydroxyl group. Scale models confirm this hypothesis.

With the object of orienting the nitration product of 3-hydroxypyridine, to which he assigned the 2-nitro-structure, Plazek (*Rocz. Chem.*, 1936, **16**, 504) applied Turpin's reaction (*loc. cit.*) to the corresponding amino-compound, obtaining a product corresponding on analysis to a 7 : 9-dinitro-5-azaphenoxazine, but no evidence in support of this structure was given. Although this result is of interest, 2-amino-3-hydroxypyridines are so difficultly accessible as to preclude work along these lines. We have attempted to extend the reaction, however, to the readily accessible 3-amino-4-hydroxypyridine (Crowe, J., 1925, **127**, 2029). Numerous experiments were carried out under various conditions, but the products, which were all highly coloured compounds containing halogen, exploded on warming and dissolved in alkalis with the production of intense purple colours. This behaviour is paralleled by the reactions of dinitrophenylpyridinium chloride, the addition compound of chlorodinitrobenzene and pyridine, which undergoes conversion into coloured derivatives of glutaconaldehyde on treatment with alkali (Zincke, *Annalen*, 1904, **330**, 361). Similar results were obtained with the analogous 3-amino-4-hydroxyquinaldine of Conrad and Limpach (*Ber.*, 1887, **20**, 950). We were thus forced to conclude that the picryl chloride reacted preferentially with the ring nitrogen and not with the 3-amino-group. It may be mentioned in this connection that Lawson, Perkin, and Robinson (J., 1924, **125**, 628) were unable to condense substituted 3-aminoquinolines with chloronitrobenzenes.

We succeeded ultimately in preparing an azaphenoxazine from 3-amino-4-hydroxypyridine by employing the modification of Turpin's reaction (*loc. cit.*) described by Misslin and Bau (*Helv. Chim. Acta*, 1919, **2**, 295), who replaced picryl chloride by 2 : 4 : 6-trinitroanisole. When approximately equimolecular quantities of the components were heated in methyl-alcoholic solution, the sparingly soluble 3-*picrylamino*-4-*hydroxypyridine* (IV) separated. This compound passed smoothly on warming with one molecule of potassium hydroxide into 7 : 9-*dinitro*-4-*azaphenoxazine* (V; R = NO_2) with simultaneous production of nitrite. Reduction with stannous chloride gave an unstable diamino-compound (V; R = NH_2), isolated as the *trihydrochloride*.

We next turned our attention to the reverse procedure, *viz.*, the condensation of a pyridine analogue of 2 : 6-dinitrochlorobenzene with *o*-aminophenol (cf. Ullmann, *loc. cit.*). 3 : 5-Dinitro-4-hydroxypyridine (Crowe, *loc. cit.*) on treatment with phosphorus pentachloride at 170° was converted into 4-*chloro*-3 : 5-*dinitropyridine* (VI), a compound so susceptible to hydrolysis that it was found desirable to employ it *in situ*. On treating (VI) with *o*-aminophenol in the presence of sodium acetate, *dinitropyridyl*-o-*aminophenol* (VII) was

obtained, smoothly cyclised to 5-*nitro*-3-*azaphenoxazine* (VIII; R = R_1 = H, R_2 = NO_2) by alkalis or aqueous ammonia, with simultaneous formation of nitrite. Ring closure also took place when the intermediate com-



pound (VII) was heated in alcoholic solution with a few drops of piperidine or other secondary base, oxides of nitrogen being evolved. Reduction gave 5-*amino*-3-*azaphenoxazine* (VIII; R = R_1 = H, R_2 = NH_2), characterised by a *monoacetyl* derivative. Treatment of this amino-base with nitrous acid gave the *diazole* (IX). This behaviour is characteristic of an *o*-aminodiphenylamine. It is shown by such structurally analogous compounds as 9-amino-5 : 10-dihydroacridine (Clemo, Perkin, and Robinson, J., 1924, **125**, 1754) and 1 : 3-diaminophenthiazonium chloride (Kehrmann and Steinberg, *Ber.*, 1911, **44**, 3015) and is strong evidence in support of the formulations assigned to our products.

Nitration of 5-nitro-3-azaphenoxazine in glacial acetic acid gave a *dinitro*-derivative. By analogy with the nitration of phenoxazine (Kehrmann and Saager, *Ber.*, 1903, **36**, 478) it was expected that the entrant nitro-group would be introduced in position R_1 (formula VIII) or less probably R. Its formulation as 5 : 9-*dinitro*-3-*azaphenoxazine* (VIII; R_1 = R_2 = NO_2, R = H) followed from the synthesis of a compound of this structure from 4-chloro-3 : 5-dinitropyridine and 5-nitro-*o*-aminophenol, identical in every respect with the product of direct nitration. 5 : 8-*Dinitro*-3-*azaphenoxazine* (VIII; R = R_2 = NO_2, R_1 = H) was similarly prepared from 4-nitro-*o*-aminophenol.

Reduction of these dinitro-compounds gave the corresponding 5 : 9-*diamino*-3-*azaphenoxazine* (VIII; R_1 = R_2 = NH_2, R = H) and 5 : 8-diamino-3-azaphenoxazine (VIII; R = R_2 = NH_2, R_1 = H), isolated as the *dihydrochlorides*. The free *base* from the 5 : 9-isomeride proved very susceptible to oxidation, rapidly darkening on exposure to air or on liberation from its salts with sodium hydroxide. All attempts to oxidise it to a well-defined quinonoidal form, however, proved unsuccessful.

The results of the pharmacological tests on some of the above compounds will be published elsewhere.

Experimental.

M. p.'s are corrected. Microanalyses are by Mr. R. Maxim, University Chemical Laboratory, Cambridge, and The Wellcome Chemical Works, Dartford, Kent.

3-*Picrylamino*-4-*hydroxypyridine* (IV).—3-Nitro-4-hydroxypyridine (5 g.) (Crowe, *loc. cit.*), reduced iron (15 g.), and anhydrous calcium chloride (1 g.) were heated under reflux in 80% methanol (125 ml.) for 90 minutes, the iron residues collected and extracted with a further quantity of boiling methanol (50 ml.), and the combined filtrates treated with trinitroanisole (5 g.; 0·6 mol.) (Brady and Horton, J., 1925, **127**, 2232). After 30 minutes' refluxing, 3-*picrylamino*-4-*hydroxypyridine* separated; it formed golden-yellow platelets from aqueous acetic acid, m. p. 201° (decomp.) (Found : C, 41·3; H, 2·5; N, 22·0. $C_{11}H_7O_7N_5$ requires C, 41·1; H, 2·2; N, 21·8%). Yield, 5 g. (80%).

7 : 9-*Dinitro*-4-*azaphenoxazine* (V; R = NO_2).—To 3-picrylamino-4-hydroxypyridine (10 g.), suspended in spirit (200 ml.), potassium hydroxide (2 g.; 1·1 mols.) was added, the mixture refluxed for 2 hours, and the product collected after cooling. 7 : 9-*Dinitro*-4-*azaphenoxazine* formed red needles from alcoholic acetic acid, m. p. 229—230° (Found : C, 47·9; H, 2·3; N, 20·1. $C_{11}H_6O_5N_4$ requires C, 48·2; H, 2·2; N, 20·4%). Yield, 6 g. (70%). The filtrate was evaporated to dryness; an aqueous extract of the residue gave positive nitrite tests with the Griess–Ilosvay reagent, dilute acid, etc.

7 : 9-*Diamino*-4-*azaphenoxazine Trihydrochloride* (V; R = NH_2,HCl).—The corresponding dinitro-compound (2 g.), suspended in concentrated hydrochloric acid (15 ml.), was treated with stannous chloride (10 g.) in concentrated hydrochloric acid (15 ml.). After being heated for 1 hour on the water-bath, the mixture was diluted with water (1 l.), and the tin removed as sulphide. The filtrate, on concentration under reduced pressure on the water-bath to *ca.* 10 ml., deposited pale yellow needles of 7 : 9-*diamino*-4-*azaphenoxazine trihydrochloride*, m. p. above 300° (Found : Cl, 33·4. $C_{11}H_{10}ON_4$,3HCl requires Cl, 32·9%). Yield, 20—25%. The compound was very susceptible to oxidation, undergoing partial decomposition when attempts were made to recrystallise it from a variety of solvents. Attempts to isolate the free base were unsuccessful, rapid oxidation taking place.

4-*Chloro*-3 : 5-*dinitropyridine* (VI).—3 : 5-Dinitro-4-hydroxypyridine (10 g.) (Crowe, *loc. cit.*) was treated under reflux with phosphorus pentachloride (18 g.; 1·2 mols.) and a little phosphorus oxychloride at 160—170° for 1 hour. After removal of the phosphorus halides under reduced pressure on the water-bath the residue was extracted with boiling light petroleum (b. p. 80—100°). 4-*Chloro*-3 : 5-*dinitropyridine*, which separated on cooling, formed colourless needles from light petroleum, m. p. *ca.* 240° (decomp.) (with preliminary softening at *ca.* 60°) (Found : Cl, 17·1. $C_5H_2O_4N_3Cl$ requires Cl, 17·4%). The compound rapidly decomposes on keeping, reverting to the dinitrohydroxypyridine.

Dinitropyridyl-o-*aminophenol* (VII).—3 : 5-Dinitro-4-hydroxypyridine (10 g.) was chlorinated as described above, and the phosphorus halides removed. The residue was treated *in situ* with anhydrous sodium acetate (10 g.), followed immediately by a solution of *o*-aminophenol (6 g.; 1 mol.) in spirit. The mixture was refluxed for 30 minutes, an equal volume of water added, and the product collected after cooling. *Dinitropyridyl*-o-*aminophenol* formed orange platelets from alcohol (charcoal), m. p. 195° (decomp.) (Found : C, 47·9; H, 2·9; N, 20·2. $C_{11}H_6O_5N_4$ requires C, 47·8; H, 2·9; N, 20·3%). Yield, 9·3 g. (60%).

5-Nitro-3-azaphenoxazine (VIII; R = R_1 = H, R_2 = NO_2).—Crude dinitropyridyl-*o*-aminophenol (9·3 g.), suspended in alcohol, was heated with excess of aqueous ammonia (*d* 0·880) for 15 minutes on the water-bath; after cooling, the solid was collected. *5-Nitro-3-azaphenoxazine* separated from aqueous acetic acid in deep red crystals, m. p. 209—210° (Found : C, 57·6; H, 3·0; N, 18·3. $C_{11}H_7O_3N_3$ requires C, 57·6; H, 3·1; N, 18·3%). Yield, 5·5 g. (70%). The mother-liquors gave positive tests for nitrite (see above).

5-Amino-3-azaphenoxazine (VIII; R = R_1 = H, R_2 = NH_2).—Finely powdered 5-nitro-3-azaphenoxazine (2·5 g.), suspended in alcohol (25 ml.), was treated with stannous chloride (10 g.) in concentrated hydrochloric acid (25 ml.) under reflux until the original red substance had been completely replaced by brown needles; after cooling, the product was collected. *5-Amino-3-azaphenoxazine hydrochloride* formed bright yellow needles from water (charcoal), m. p. above 300° (Found : C, 56·1, H, 4·4; N, 18·3; Cl, 15·6. $C_{11}H_9ON_3$,HCl requires C, 56·1; H, 4·3; N, 17·8; Cl, 15·1%). Yield, 2 g. (80%). *5-Amino-3-azaphenoxazine*, precipitated from a solution of the hydrochloride with ammonia, separated from water (charcoal) in white crystals, m. p. 258—259° (Found : C, 66·0; H, 4·6; N, 21·3. $C_{11}H_9ON_3$ requires C, 66·3; H, 4·5; N, 21·1%). Yield, nearly quantitative. The free base could be obtained in 75% yield directly from the nitro-compound by reduction with reduced iron in aqueous alcohol. When 5-amino-3-azaphenoxazine hydrochloride (2·7 g.) was heated under reflux for 30 minutes with acetic anhydride (40 ml.) and sufficient anhydrous sodium acetate to bring the yellow suspension into solution, *5-acetamido-3-azaphenoxazine* was obtained; it separated from spirit (charcoal) in felted white needles, m. p. 288—289° (decomp.) (Found : C, 64·9; H, 4·6; N, 17·4. $C_{13}H_{11}O_2N_3$ requires C, 64·7; H, 4·6; N, 17·4%).

3-Azaphenoxazine-5 : 6-diazole (IX).—A solution of 5-amino-3-azaphenoxazine hydrochloride (5 g.) in water (15 ml.) and concentrated hydrochloric acid (15 ml.) was cooled to 0°, and an aqueous solution of sodium nitrite (1·5 g.) added dropwise with mechanical stirring. After 1 hour the mixture was heated to boiling; a sparingly soluble hydrochloride then separated. The mixture was cooled, the product collected, and the free base liberated with aqueous ammonia. *3-Azaphenoxazine-5 : 6-diazole* formed white needles from spirit, m. p. 171° (decomp.) Found : C, 63·0; H, 2·7; N, 26·7. $C_{11}H_6ON_4$ requires C, 62·9; H, 2·9; N, 26·7%).

5 : 9-Dinitro-3-azaphenoxazine (VIII; R_1 = R_2 = NO_2, R = H).—(a) Finely powdered 5-nitro-3-azaphenoxazine (2·2 g.) was suspended in glacial acetic acid (16 ml.), and fuming nitric acid (*d* 1·5) (5 ml.) added dropwise with mechanical stirring at 0° during 15 minutes. The bright red product was collected, washed with glacial acetic acid, and crystallised from nitrobenzene (charcoal), yielding bright red crystals of *5 : 9-dinitro-3-azaphenoxazine*, m. p. 312° (decomp.) (Found : C, 48·5; H, 2·3; N, 20·6. $C_{11}H_6O_5N_4$ requires C, 48·2; H, 2·2; N, 20·4%). A further small quantity of material was obtained by partial neutralisation of the acid mother-liquors with aqueous ammonia. Yield, 2·4 g. (90%).

(b) Crude 4-chloro-3 : 5-dinitropyridine (prepared from 10 g. of 3 : 5-dinitro-4-hydroxypyridine) was treated with anhydrous sodium acetate (10 g.), followed immediately by an alcoholic solution of 5-nitro-2-aminophenol (8 g.) (Hewitt and King, J., 1926, 882). The mixture was heated under reflux for 15 minutes, and then cooled after addition of sufficient water to produce a turbidity. The product was collected (12 g.; m. p. 220—230°), suspended in alcohol, and heated under reflux with excess of aqueous ammonia (*d* 0·880) for 15 minutes. The product, crystallised from nitrobenzene (charcoal), gave red crystals of 5 : 9-dinitro-3-azaphenoxazine, m. p. 316° (decomp.) (Found : C, 48·6; H, 2·3; N, 20·6%), not depressed by the compound obtained in (a). Yield, 5 g. (35%).

5 : 8-Dinitro-3-azaphenoxazine (VIII; R = R_2 = NO_2, R_1 = H).—Prepared from 4-nitro-2-aminophenol (Hewitt and King, *loc. cit.*) by the method described in the previous preparation, this formed orange crystals from nitrobenzene, m. p. 318° (Found : C, 48·4; H, 2·2; N, 20·3. $C_{11}H_6O_5N_4$ requires C, 48·2; H, 2·2; N, 20·4%). Yield, 35%. In admixture with the 5 : 9-dinitro-compound a m. p. depression of 40° was obtained

5 : 9-Diamino-3-azaphenoxazine (VIII; R = R_1 = NH_2, R_2 = H).—5 : 9-Dinitro-3-azaphenoxazine (2 g.), reduced iron (8 g.), and anhydrous calcium chloride (0·5 g.) were heated under reflux in 70% alcohol (30 ml.) for 2 hours. After addition of charcoal and a further few minutes' heating the liquid was filtered. White needles of *5 : 9-diamino-3-azaphenoxazine* separated on cooling, m. p. *ca.* 270° (decomp.) (Found : C, 62·4; H, 4·7; N, 26·0. $C_{11}H_{10}ON_4$ requires C, 61·7; H, 4·7; N, 26·2%). Attempts at recrystallisation led to severe discoloration. The *dihydrochloride* formed yellow-green needles from hydrochloric acid, m. p. above 300° (Found : Cl, 24·6. $C_{11}H_{10}ON_4$,2HCl requires Cl, 24·7%). *5 : 9-Diacetamido-3-azaphenoxazine*, prepared by heating the base with 20 vols. of acetic anhydride under reflux for 1 hour, separated from alcohol as the *monohydrate* (Found : loss on drying, 6·2. $C_{15}H_{14}O_3N_4$,H_2O requires H_2O, 5·7%). The anhydrous compound formed lemon-yellow crystals, m. p. above 300° (Found : C, 60·6; H, 4·4; N, 18·6. $C_{15}H_{14}O_3N_4$ requires C, 60·4; H, 4·7; N, 18·8%).

5 : 8-Diamino-3-azaphenoxazine dihydrochloride (VIII; R = R_2 = NH_2,HCl, R_1 = H), prepared as described for the 5 : 9-isomeride, separated from water in yellow needles, m. p. above 300° (Found : Cl, 24·7. $C_{11}H_{10}ON_4$,2HCl requires Cl, 24·7%).

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