492. *Cinnolines and Other Heterocyclic Types in Relation to the Chemotherapy of Trypanosomiasis. Part III.* Synthesis of* 4 : 4′-*Diamino*-6 : 6′-*azoquinoline Metho-salts.*

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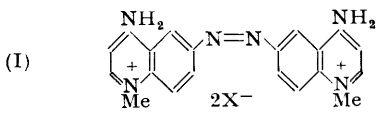
In exploration of the hypothesis that trypanocidal activity might be shown by structures containing two linked quaternised amino-heterocyclic units, 4 : 4′-diamino-6 : 6′-azoquinoline methochloride and methiodide have been synthesised from 4 : 4′-diaminoazobenzene. When tested against *T. congolense* infections in mice, these compounds were not curative, but effected temporary clearance of the blood from trypanosomes.

For reasons recorded in Part I * of this series, it was desired to synthesise 4 : 4′-diamino-6 : 6′-azocinnoline metho-salts. As a preliminary to this, and also because it was not intended to confine later work to the cinnoline series, the preparation of 4 : 4′-diamino-6 : 6′-azoquinoline metho-salts was first investigated.

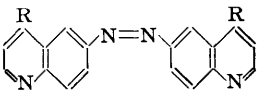
Few azoquinolines are mentioned in the literature. Their preparation from diazonium salts and aminoquinolines, and from diazotised 4 : 6-diaminoquinaldine and phenols or amines, is described in G.P. 622,596. Kneuppel (*Annalen*, 1900, **310**, 75) has stated that 6 : 6′-azoquinoline (II; R = H) is a by-product in the preparation of 6-aminoquinoline from the nitro-compound by reduction with iron and aqueous alcohol in the presence of calcium chloride. In view of the possibility of converting (II; R = H) into (I), we repeated this but found that Knueppel's compound gave no diazotisable product on treatment with stannous chloride in hydrochloric acid; its representation as (II; R = H) is thus open to doubt. Reduction of 6-nitroquinoline with sodium arsenite or sodium stannite in aqueous alcohol gave difficultly separable mixtures of high-melting, faintly coloured products which were not investigated, and preliminary attempts to form (II; R = H) from 4 : 4′-diaminoazobenzene by the Skraup method were not encouraging. Simple aromatic nitro-compounds can be satisfactorily reduced to azo-compounds by

* Parts I and II, preceding paper.

lithium aluminium hydride in ether at −80° (Nystrom and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 3738), but application of the method to nitroquinolines is made unattractive by their low solubility in ether.



(I)



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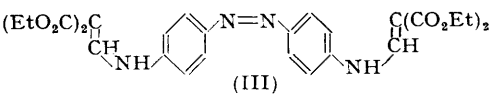

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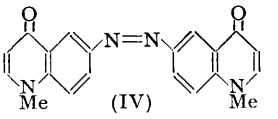

(IV)

The possibility was then investigated of applying the well-known synthesis of 4-hydroxyquinolines from arylamines and ethyl ethoxymethylenemalonate. Condensation of 4 : 4′-diaminoazobenzene with ethyl ethoxymethylenemalonate and cyclisation of the resultant ester (III) in " Dowtherm " under closely defined conditions gave diethyl 4 : 4′-dihydroxy-6 : 6′-azoquinoline-3 : 3′-dicarboxylate; by hydrolysis and decarboxylation this yielded 4 : 4′-dihydroxy-6 : 6′-azoquinoline (II; R = OH). None of the last three compounds melted at 320°; each was amorphous and insoluble in organic solvents, and separated in more or less gelatinous form on acidification of alkaline solutions. Treatment of (II; R = OH) with a mixture of phosphorus pentachloride and phosphoryl chloride gave the crystalline dichloro-compound (II; R = Cl), m. p. 272—274°, and the remaining transformations into the bismetho-salts (I; X = Cl and I) all proceeded by way of crystalline intermediates.

Treatment of pure (II; R = Cl) with potassium hydroxide and phenol, and with ammonia and phenol at 180°, gave the phenoxy- and amino-derivatives (II; R = OPh and NH_2) respectively. The pure phenoxy-compound was also readily prepared from crude chloro-compound, purification of which was difficult and wasteful; the preparation of the amine from the phenoxy-compound, rather than directly from the chloro-compound, would therefore be an advantage. This possibility was suggested to us by the observation of our colleague Mr. J. McIntyre that interaction of 4-chloro-6-nitroquinoline, phenol, and ammonia affords excellent yields of either 6-nitro-4-phenoxy- or 4-amino-6-nitro-quinoline, according to whether a bath temperature of 130° or 180° is used. When ammonia was passed into a solution of (II; R = OPh) at 180°, no reaction occurred, but the addition of 2 mols. of ammonium chloride (with conditions otherwise unchanged) afforded a good yield of the amine. A similar observation, that the presence of amine salt is essential to induce reaction between an alkylamine and 7-chloro-4-phenoxyquinoline, has recently been made by Surrey and Cutler (*J. Amer. Chem. Soc.*, 1951, **73**, 2623).

Conversion of (II; R = NH_2) into the quaternary salt (I; X = I) was effected with methyl iodide in phenol. Alternatively, the diacetamido-compound (II; R = NHAc) was heated with a large excess of methyl toluene-*p*-sulphonate and the product hydrolysed with hydrochloric acid. The product (I; X = Cl) so obtained appeared to be identical with material prepared from the above methiodide and silver chloride, but owing to the impossibility of making mixed melting point determinations (the compounds did not melt below 330°) additional evidence was required. This was obtained by boiling the metho-salts with aqueous sodium hydroxide and collecting the gas evolved; it was found that ammonia was liberated in 75% yield, and there was no evidence of the presence of methylamine. The non-volatile product appeared to be the expected 1 : 1′-dimethyl-6 : 6′-azo-4 : 4′-quinolone (IV).

The biological activity of the salts (I; X = Cl and I) has been examined in the Department of Pharmacology, Oxford, by Dr. E. M. Lourie and Dr. J. M. Walker (cf. Lourie, Morley, Simpson, and Walker, *Brit. J. Pharmacol.*, 1951, **6**, 643); when they are injected subcutaneously at about half the maximum tolerated dose into mice infected with *T. congolense*, the blood is temporarily cleared of parasites, but neither compound is curative.

Experimental

M. p.s are uncorrected.

pp'-(2 : 2'-*Dicarbethoxyvinylamino*)*azobenzene* (III).—*pp'*-Diaminoazobenzene (15 g.), ethyl ethoxymethylenemalonate (30 g.), and " Dowtherm " (diphenyl ether 75% + diphenyl 25% by wt.) (150 c.c.) were kept at 95° for ¾ hour at atmospheric pressure and then for 1¼ hours at 20—30 mm. The solid which had separated was collected, freed from " Dowtherm " by being washed with ligroin, and crystallised from alcohol, yielding pp'-(2 : 2'-*dicarbethoxyvinylamino*)*azobenzene* (94%) as orange needles, m. p. 192—193° (Found : C, 60·8; H, 5·7; N, 10·3. $C_{28}H_{32}O_8N_4$ requires C, 60·9; H, 5·8; N, 10·1%).

4 : 4-*Dichloro*-6 : 6'-*azoquinoline*.—A suspension of the foregoing compound (15 g.) in " Dowtherm " (300 c.c.) (vigorously stirred throughout the experiment) was rapidly heated in a flask fitted with a short (8") air-condenser. At 90° a clear red solution was formed, at 245° alcohol was rapidly evolved, and at 253° a solid separated (13 minutes from the start of the heating). The suspension was kept in vigorous ebullition (255—258°), so that the refluxing solvent reached the top of the condenser, for ¼ hour, after which it was cooled and the solid collected and well washed with ligroin (yield, theoretical).

The crude diethyl 4 : 4'-dihydroxy-6 : 6'-azoquinoline-3 : 3'-dicarboxylate from 7 such experiments was boiled with 5% aqueous sodium hydroxide (4200 c.c.) under reflux for 1 hour with mechanical stirring. Most of the solid dissolved, and excessive foaming was reduced by a little amyl alcohol. The liquid was filtered when cold from a residue (6 g.); this was digested with boiling water and the extract added to the main filtrate. The solution was acidified whilst hot with glacial acetic acid, and the crude acid, which was invariably somewhat gelatinous, was collected, washed, and dried at 140°/10—20 mm. over phosphoric anhydride (yield, 92%).

Decarboxylation of this material (10 g.) was effected in " Dowtherm " (400 c.c.). The mechanically-stirred suspension was heated, with gentle refluxing, in a slow stream of nitrogen which was led through barium hydroxide solution; carbon dioxide was steadily evolved, the reaction virtually ceasing after 3 hours. The suspended solid was then collected and washed with ligroin until free from " Dowtherm." In spite of the vigorous drying to which the acid was subjected, water was invariably eliminated during the decarboxylation, indicating either that other pyrolytic reactions occurred or that the acid formed an unusually stable hydrate. On the assumption that the acid was anhydrous, the yield of crude 4 : 4'-hydroxy-6 : 6'-azoquinoline was 105%, and that of barium carbonate was 82%.

The above hydroxy-compound (25 g.), phosphorus pentachloride (62·5 g.), and phosphorus oxychloride (125 g.) were boiled under reflux for 2 hours. The suspension was poured on crushed ice, set aside for 1 hour, and then made just alkaline to phenolphthalein. The product was collected, washed, dried thoroughly, and extracted with boiling benzene (2400 c.c.). Concentration of the extracts gave fairly pure crystalline material (11·7 g., 42%) which melted within the range 255—265°. Further purification was achieved only by crystallisation from hot 2N-sulphuric or 2N-hydrochloric acid (150 c.c./g.); a final crystallisation from benzene then yielded 4 : 4'-*dichloro*-6 : 6'-*azoquinoline* as rosettes of orange needles, m. p. 273—274° (Found : N, 15·6; Cl, 19·9. $C_{18}H_{10}N_4Cl_2$ requires N, 15·9; Cl, 20·1%).

4 : 4'-*Diphenoxy*-6 : 6'-*azoquinoline*.—The foregoing crude dichloroazo-compound (8 g.), m. p. 264—266° with previous softening, was heated with a solution of potassium hydroxide (3·2 g.) in phenol (40 g.) at 140—147° for 1 hour. The melt was then cooled and stirred with excess of 5% aqueous sodium hydroxide, and the solid was collected, digested with alcohol, and crystallised from ethyl methyl ketone, from which almost pure 4 : 4'-*diphenoxy*-6 : 6'-*azoquinoline* (57%) separated in orange blades, m. p. 236—238°. An analytically pure specimen, prepared from pure 4 : 4'-dichloro-6 : 6'-azoquinoline, had m. p. 238·5—239·5° (Found : C, 76·5; H, 4·4; N, 12·4. $C_{30}H_{20}O_2N_4$ requires C, 76·9; H, 4·3; N, 12·0%).

4 : 4'-*Diamino*-6 : 6'-*azoquinoline*.—(*a*) A solution of the pure dichloroazoquinoline (5 g.) in phenol (50 g.) was heated at 170—180° under a reflux condenser for 2 hours, a brisk stream of dry ammonia being passed through the mixture. The cold melt was digested with excess of 5% sodium hydroxide solution, the solid collected and dissolved in 5% aqueous acetic acid, and the base precipitated from the hot filtered solution by sodium hydroxide. The dried solid was dissolved in warm glacial acetic acid (100 c.c.); on addition of ethyl acetate (130 c.c.) 4 : 4'-*diamino*-6 : 6'-*azoquinoline triacetate dihydrate* (5·1 g.) separated in yellow needles which did not melt at 330° (Found, for material dried at room temp. : C, 54·0; H, 5·6; N, 15·2. $C_{18}H_{14}N_6,3CH_3{\cdot}CO_2H,2H_2O$ requires C, 54·3; H, 5·7; N, 15·8%). This salt formed a red solution in water; addition of aqueous ammonia precipitated 4 : 4'-*diamino*-6 : 6'-*azoquinoline*,

which crystallised in orange needles from phenol containing a little alcohol (Found: C, 67·5; H, 4·6; N, 25·6. $C_{18}H_{14}N_6,0{\cdot}25H_2O$ requires C, 67·5; H, 4·6; N, 26·3%). The base was insoluble in the common solvents and did not melt at 330°; salts with mineral acids could not be obtained crystalline.

(*b*) 4 : 4′-Diphenoxy-6 : 6′-azoquinoline (6 g.), phenol (60 g.), and ammonium chloride (1·4 g.) were heated in a stream of dry ammonia at 175—180° for 1¼ hours. The product was worked up as above and converted into the acetate (5·3 g.) which, after being dried at 100° in a vacuum, appeared to be the *monoacetate monohydrate* (Found: C, 61·3; H, 5·05; N, 21·4. $C_{18}H_{14}N_6,CH_3{\cdot}CO_2H,H_2O$ requires C, 61·2; H, 5·1; N, 21·4%).

4 : 4′-*Diacetamido*-6 : 6′-*azoquinoline*.—The above mentioned amine triacetate (1·5 g.), dissolved in acetic acid (10 c.c.), was heated under reflux for 1 hour with acetic anhydride (10 c.c.), and the product collected after dilution with ether. The *compound* was insoluble in common organic solvents, but dissolved in slightly aqueous acetic acid. It formed yellow needles, which did not melt at 330°, from acetic acid (97%)–ethyl acetate (Found: C, 64·2; H, 4·8; N, 19·7. $C_{22}H_{18}O_2N_6,H_2O$ requires C, 63·45; H, 4·85; N, 20·2%).

4 : 4′-*Diamino*-6 : 6′-*azoquinoline Metho-salts*.—(*a*) The base (1·5 g., prepared from the pure acetate) was heated with phenol (30 g.) and methyl iodide (6 c.c.) on the steam-bath (reflux condenser). After 1½ hours, when a clear red solution had been formed, more methyl iodide (3 c.c.) was added, whereupon crystallisation slowly took place. After a total of 3 hours, the mass was stirred with ether and the crude 4 : 4′-*diamino*-1 : 1′-*dimethyl*-6 : 6′-*azoquinolinium di-iodide* (2·8 g.) collected. The crystalline form was largely lost on recrystallisation from a large volume of water, but on further crystallisation from phenol–alcohol–ether the salt separated in well-formed orange-yellow needles which did not melt at 330° (Found: C, 38·0; H, 3·9; N, 12·8. $C_{20}H_{20}N_6I_2,2H_2O$ requires C, 37·9; H, 3·8; N, 13·3%).

(*b*) The above salt (1·5 g.), silver chloride (from 1·75 g. of silver nitrate), and water (250 c.c.) were boiled under reflux for 1¾ hours. The resultant *dimethochloride* (orange-red plates; 1·05 g.) separated from the filtered solution on addition of 2N-hydrochloric acid (30 c.c.) and was recrystallised from 0·04N-hydrochloric acid (Found: C, 52·25; H, 5·65; N, 18·9; Cl, 15·7. $C_{20}H_{20}N_6Cl_2,2H_2O$ requires C, 53·2; H, 5·4; N, 18·7; Cl, 15·7%). It did not melt at 330°, and separated from water alone in an amorphous state.

(*c*) 4 : 4′-Diacetamido-6 : 6′-azoquinoline (0·7 g.) and methyl toluene-*p*-sulphonate (14 g.) were slowly heated in an oil-bath. At 180°, when the base had largely dissolved, crystallisation suddenly occurred, and after a further ¼ hour at 160—180° the product (1·25 g.) was isolated by dilution with ether and hydrolysed by heating it under reflux with 2·5N-hydrochloric acid (250 c.c.). Orange-red plates rapidly separated; this material, which did not melt at 330°, appeared to be identical with the salt prepared as in (*b*). The solvent was largely removed at 100° in a vacuum, but satisfactory analyses could not be obtained (Found: C, 56·6; H, 4·7; N, 17·2. Calc. for $C_{20}H_{20}N_6Cl_2$: C, 57·8; H, 4·85; N, 20·25%).

Alkaline Decomposition of 4 : 4′-*Diamino*-1 : 1′-*dimethyl*-6 : 6′-*azoquinolinium Dichloride and Di-iodide*.—The methochloride [0·2 g., prepared by method (*b*)] was boiled under reflux with 0·2N-sodium hydroxide (55 c.c.) in a stream of nitrogen, the issuing gases being passed through water which was periodically neutralised with picric acid. After 1½ hours this aqueous solution was concentrated, yielding ammonium picrate (60 mg., 25%), m. p. 280° (decomp.) alone and when mixed with an authentic specimen [m. p. 290° (decomp.)], and 200° when mixed with methylammonium picrate (m. p. 210—212°).

In a second experiment, the methiodide (0·5 g.) and 0·5N-sodium hydroxide (50 c.c.) were boiled under reflux in a stream of nitrogen; the gases were passed through dilute sulphuric acid, which was back-titrated at intervals. The following Table gives the amount of ammonia (as % of that theoretically possible) evolved after the times stated:

Time (hours)	1½	2½	4	5½	7	13½
NH_3, %	33·4	42·7	50·8	57·0	61·1	75·0

The non-volatile product of the reaction was filtered off (0·28 g.), digested with boiling water, and crystallised from phenol–alcohol, yielding yellow rhombs, which did not melt at 330°, of 1 : 4-*dihydro*-4 : 4′-*diketo*-1 : 1′-*dimethyl*-6 : 6′-*azoquinoline* (Found: C, 69·05; H, 5·05; N, 16·7. $C_{20}H_{16}O_2N_4$ requires C, 69·75; H, 4·7; N, 16·3%). The compound was very sparingly soluble in common organic solvents, and easily soluble in dilute acids.

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