Cinnolines and Other Heterocyclic Types in Relation to the Chemotherapy of Trypanosomiasis. Part VIII. Attempts to Synthesise* N^1N^3*-Di-(4-amino-7-cinnolyl)guanidine Dimethiodide.*

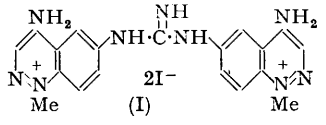
By C. M. Atkinson, (the late) J. C. E. Simpson, and A. Taylor.

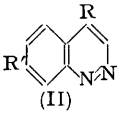
[Reprint Order No. 4837.]

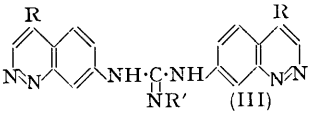
Attempted preparation of the compound named in the title by a method analogous to that used by Morley and Simpson (*J.*, 1952, 2617) for the synthesis of " 528 " (I) gave only a complex mixture of quaternary salts. The difficulties encountered in developing an unambiguous route to these compounds are briefly reported.

4-Hydroxy-7-nitrocinnoline (II; R = OH, R′ = NO_2) was prepared by diazotisation and cyclisation of 2-amino-4-nitroacetophenone in formic acid (McIntyre and Simpson, *J.*, 1952, 2606). Schofield and Theobald (*J.*, 1949, 2404) obtained a similar yield on a much smaller scale using acetic–sulphuric acid and a ten-fold excess of sodium nitrite. Chlorination of the hydroxy-compound and subsequent phenoxylation and amination proceeded satisfactorily on a larger scale, but a single attempt to aminate the chloro-compound (II; R = Cl, R′ = NO_2) directly with ammonia in phenol at 140° gave only a small yield of the nitro-amine (II; R = NH_2, R′ = NO_2).

Schofield and Theobald (*loc. cit.*) reported that the reduction of this compound " offered some difficulty " and were only able to isolate the diacetamido-compound (II; R = R′ = NHAc), in very low yield, after reductive acetylation. We have confirmed these results; four experiments with stannous chloride under different conditions gave unidentified high-melting material; catalytic hydrogenation proceeded beyond the reduction of the nitro-group; and the required diamine (II; R = R′ = NH_2) was finally synthesised by amination of 7-amino-4-phenoxycinnoline (II; R = OPh, R′ = NH_2). The latter derivative was prepared by the reduction of the corresponding nitro-compound with Albert and Linnell's stannous chloride–acetic anhydride reagent (*J.*, 1936, 1617). In a similar way 5- and 8-amino-4-phenoxycinnoline, required for the preparation of other isomers of " 528," were obtained.







4 : 7-Diaminocinnoline reacted with thiocarbonyl chloride, to give a sulphur-containing compound, which, on treatment with methanolic ammonia and mercuric oxide, yielded a base isomeric with that prepared by Morley and Simpson (*loc. cit.*). Quaternisation of the pure base with methyl iodide gave an amorphous salt which was shown to be a mixture of four components by paper chromatography of its aqueous solution. The mixture was inactive when tested against *T. congolense* infections in mice.

This result emphasised the importance of finding an unambiguous route to compounds of the " 528 " type. The possibility of acylation at $N_{(1)}$ and the 4-amino-group was apparent in the recovery of only 60% of 4-aminocinnoline after treatment with thiocarbonyl chloride under the conditions described by Morley and Simpson (*loc. cit.*) and in the isolation of two isomeric acetyl derivatives of the nitro-amine (II; R = NH_2, R′ = NO_2). In order to eliminate this possibility, 7-amino-4-phenoxycinnoline was treated with thiocarbonyl chloride (the phenoxy-compound was shown to be stable to the acidity which developed in the reaction under the usual conditions), but only unchanged material was isolated.

An alternative method of forming the guanidine linkage utilised dichloromethylene-benzamide Ph·CO·N:CCl_2 (Johnson and Chernoff, *J. Amer. Chem. Soc.*, 1912, **34**, 169),

* Part VII, *J.*, 1954, 165.

which with aniline or 4 : 6-diaminocinnoline gave the respective N^2-benzoyl-N^1N^3-disubstituted guanidines. However, the product from 4 : 7-diaminocinnoline methiodide and this reagent was shown by paper chromatography to be a mixture of two compounds, neither of which was the starting material.

The demonstration that 7-amino-4-phenoxycinnoline (II; R = OPh, R′ = NH_2) condensed smoothly with dichloromethylenebenzamide under the same conditions offered a means of avoiding these difficulties. The identity of the product obtained by amination and quaternisation of (III; R = OPh, R′ = Bz) with one of the products obtained from the condensation of 4 : 7-diaminocinnoline methiodide and dichloromethylenebenzamide would have fixed the position of quaternisation, and, further, there would have been no ambiguity about the position of the guanidine bridge. However, amination of the compound (III; R = OPh, R′ = Bz) gave a crystalline compound, m. p. 206—207°, the analytical figures for which precluded its representation as (III; R = NH_2, R′ = Bz).

Experimental

M. p.s were determined on a Kofler block and are corrected.

4-Hydroxy-7-nitrocinnoline.—An ice-cold solution of 2-amino-4-nitroacetophenone (Part VII, *J.*, 1954, 165; 20 g.) in formic acid (200 c.c.) was diazotised with an aqueous solution of sodium nitrite (10 g. in 50 c.c.) with stirring and external cooling. The mixture was left for 4 days at room temperature and the cinnoline, m. p. 300° (15 g.), was then collected. In experiments where gentle warming was used to assist the initial solution of the amine, the only product was *2-formamido-4-nitroacetophenone*, m. p. 155° (Found: C, 51·5; H, 3·4; N, 13·3. $C_9H_8O_4N_2$ requires C, 51·9; H, 3·8; N, 13·4%), readily hydrolysable to the amine, m. p. and mixed m. p. 166°.

7-Amino-4-phenoxycinnoline.—A stirred suspension of the nitro-compound (5 g.) (Schofield and Theobald, *loc. cit.*) in glacial acetic acid (25 c.c.) was treated with the reducing agent [125 c.c.; prepared according to Albert and Linnell (*loc. cit.*)], added during *ca.* 10 min. with external cooling to maintain the temperature at 20—25°. The complex which separated from the clear red solution towards the end of the addition was stirred for 20 min. and poured on ice (1 kg.) and aqueous sodium hydroxide (800 c.c. of 40%). The product, m. p. 178° (3·8 g., 86%), was isolated by evaporation to dryness of a washed and dried ($MgSO_4$) chloroform extract; pure *7-amino-4-phenoxycinnoline*, m. p. 179—180°, formed greenish-yellow elongated plates from benzene (Found: C, 70·5; H, 4·5; N, 17·5. $C_{14}H_9ON_3$ requires C, 70·9; H, 4·6; N, 17·75%). Attempted catalytic reduction gave no useful product although the mixture was worked up after the theoretical uptake of hydrogen (Adams catalyst in acetic acid).

4 : 7-Diaminocinnoline.—The above phenoxy-compound (0·5 g.) was added to stirred, molten ammonium acetate (5 g.) at 137° and kept at this temperature for 30 min. The clear orange solution was cooled, treated with aqueous sodium hydroxide (100 c.c.; 10%), and set aside for 4 days; the pure *diamine* (0·23 g., 76%) separated as long very pale yellow needles, m. p. 250° (decomp.) unchanged by recrystallisation from water (Found: C, 59·7; H, 5·0; N, 35·45. $C_8H_8N_4$ requires C, 60·0; H, 5·0; N, 35·0%).

N^1N^3-Di-(4-amino-7-cinnolyl)guanidine (?).—4 : 7-Diaminocinnoline (0·4 g.) was dissolved in aqueous acetone (20 c.c.; 50%) and to the hot solution thiocarbonyl chloride (0·13 c.c.) was added. The solution was stirred and refluxed for 30 min., set aside overnight, and diluted with acetone (150 c.c.), to precipitate yellow needles, m. p. 256—257° (decomp.) (0·3 g.). This material gave a positive qualitative test for sulphur and was used directly for the next stage. The above product (0·2 g.), yellow mercuric oxide (0·2 g.), and methanolic ammonia (25 c.c.; 12% w/v) were stirred together at 43—45° for 4 hr. The cooled mixture was then set aside overnight and was evaporated to dryness at room temperature. The resulting solid was broken up and extracted with hydrochloric acid (2N; 6 × 10 c.c.). The inorganic material was removed as sulphide, and the resulting mixture filtered hot. Concentration of the filtrate yielded a colourless salt which crystallised unchanged from dilute hydrochloric acid, to give *N^1N^3-di-(4-amino-7-cinnolyl)guanidine trihydrochloride* (?), m. p. 330° (decomp.) (Found: C, 41·85; H, 5·0. $C_{17}H_{15}N_9,3HCl,2H_2O$ requires C, 41·6; H, 4·5%). On dissolution in warm dilute ammonia (0·2 g. in 10 c.c.) and addition of aqueous sodium hydroxide (2 c.c.; 10%) the free base was obtained, m. p. 238—240° (0·15 g.).

Reaction between the Foregoing Guanidine and Methyl Iodide.—The base (0·1 g.) was refluxed in dry methanol (15 c.c.) for 2 hr. with methyl iodide (1 c.c.). The mixture yielded on con-

centration to dryness under reduced pressure a product, m. p. 178°, which after recrystallisation from water had m. p. 234°, with previous softening at 178° and 210° (Found: C, 34·6; H, 3·7; N, 17·45; I, 41·2. Calc. for $C_{19}H_{21}N_9I_2,2H_2O$: C, 34·3; H, 3·5; N, 18·9; I, 38·2%). Paper chromatography with a descending mixture of *tert.*-butanol (70 c.c.), 6N-hydrochloric acid (1·65 c.c.), and water (to 100 c.c.) (Atkinson and Taylor, unpublished work) showed this to be a mixture of four components, and the mother-liquors, which gave a solid, m. p. 176—178°, on evaporation to dryness, were even more complex.

Stability of 7-Nitro- and 7-Amino-4-phenoxycinnoline to Acid Hydrolysis.—Weighed samples of each compound were refluxed for 45 min. in acetone which contained dilute hydrochloric acid to make the concentration shown. In the case of the nitro-compound the percentage of unchanged material was calculated from the yield of hydroxy-compound.

Unchanged material (%).

Concentration of acid used	pH 5·91 *	N/100	N/10	N/5	N/2	N
7-Nitro-4-phenoxycinnoline	—	26·9	13·5	0	—	—
7-Amino-4-phenoxycinnoline	100	90·5	—	—	75	22

* Phosphate buffer.

Acetylation of 4-Amino-7-nitrocinnoline.—The nitro-amine (1 g.) was heated on the steam-bath with acetic anhydride (20 c.c.) for 35 min. and the hot mixture poured into water and left overnight. The crude product (0·6 g.; m. p. *ca.* 197°) gave a mixture of pale yellow needles, m. p. 234—238°, and yellow prisms, m. p. 194—196°, from alcohol. Fractional recrystallisation provided *4-acetamido-7-nitrocinnoline*, m. p. 235° (Found: C, 51·95; H, 4·3; N, 23·9. $C_{10}H_8O_3N_4$ requires C, 51·7; H, 3·45; N, 24·1%), and from the mother-liquors an isomeric *compound*, m. p. 194—195° (Found: C, 51·8; H, 4·1%).

4-Amino-7-nitrocinnoline Methiodide.—The nitro-amine (0·3 g.) (Schofield and Theobald, *loc. cit.*) was refluxed with methyl iodide (2·2 c.c.) in ethanol (7·5 c.c.) for 1½ hr.; the crude salt separated overnight. Recrystallisation from water gave orange needles of the *methiodide*, m. p. 236—238° (decomp.) (Found: C, 32·65; H, 3·0; N, 16·1; I, 37·6. $C_9H_9O_2N_4I$ requires C, 32·6; H, 2·7; N, 16·9; I, 38·2%).

4-Acetamido-7-nitrocinnoline Methiodide.—A solution of the acetamido-compound (0·35 g.; m. p. 235°) in alcohol (15 c.c.) was refluxed with methyl iodide (0·5 c.c.) for 2 hr. and the mixture was evaporated to dryness. Recrystallisation from ethanol (carbon) yielded dark red needles of *4-acetamido-7-nitrocinnoline methiodide*, m. p. 188—189° (Found: C, 35·55; H, 4·4; N, 14·25; I, 32·85. $C_{11}H_{11}O_3N_4I$ requires C, 35·3; H, 2·9; N, 14·3; I, 33·9%). Hydrolysis in boiling N-hydrochloric acid gave 4-amino-7-nitrocinnoline methiodide, m. p. 236—238° (decomp.).

4 : 7-Diaminocinnoline Methiodide.—A solution of 4-amino-7-nitrocinnoline methiodide (0·2 g.) in water (5 c.c.) was treated at 50° with a solution of stannous chloride (0·5 g.; dihydrate) in dilute hydrochloric acid (2 c.c. of water and 8 c.c. of concentrated acid). The pale yellow crystalline precipitate which separated on cooling redissolved at 65° and after 20 min. at this temperature the mixture was cooled, giving a colourless precipitate. After 1 hr. at room temperature the mixture was diluted with water and freed from tin salts with hydrogen sulphide, and the filtrate was evaporated (desiccator) to a black dry solid. This was dissolved in boiling water (charcoal), and the methiodide precipitated with saturated aqueous potassium iodide. *4 : 7-Diaminocinnoline methiodide* formed long yellow needles, m. p. 262—263° (0·1 g.), from ethanol (Found: C, 36·25; H, 4·2; N, 18·4. $C_9H_{11}N_4I$ requires C, 35·85; H, 3·6; N, 18·5%).

Dichloromethylenebenzamide (Johnson and Chernoff, *loc. cit.*).—Potassium thiocyanate (25 g.) in water (50 c.c.) was treated with a filtered solution of lead acetate (55 g.) in water (200 c.c.). The heavy white precipitate was filtered off, washed twice with water, then with ethanol, and dried in a desiccator over concentrated sulphuric acid (yield, 27 g.). Lead thiocyanate (54 g.) and benzoyl chloride (45 g.) in dry benzene were stirred at reflux temperature (88°) for 1 hr., the solvent was removed after filtration, and benzoyl thiocyanate (34·3 g.; 62%) distilled off (b. p. 104°/0·5 mm.). Dry chlorine was passed through a solution of benzoyl thiocyanate (34 g.) in anhydrous carbon tetrachloride (110 c.c.) for 4 hr. The mixture was filtered after 66 hr., the sulphur chloride and carbon tetrachloride were removed at slightly reduced pressure, and the residue was distilled (b. p. 119—120°/15 mm.). The pale yellow product (36·2 g.) was redistilled, giving dichloromethylenebenzamide, a stable colourless liquid, b. p. 79°/0·5 mm., n_{18}^{D} 1·5675 (27·15 g., 61%).

N^1N^3-*Di*-(4-*amino*-6-*cinnolyl*)*guanidine*.—A stirred suspension of 4 : 6-diaminocinnoline (0·3 g.) (*J.*, 1952, 2597) in nitromethane (25 c.c.) was treated at *ca.* 5° with a solution of dichloromethylenebenzamide (0·3 c.c.) in nitromethane (5 c.c.). After 3½ hr. the red precipitate (0·35 g.) was filtered off and dried, and the finely divided solid was stirred with dilute aqueous ammonia, to give a yellow base. This (0·1 g.) was refluxed for 2 hr. with alcoholic potassium hydroxide (0·5 g. in 30 c.c.), then concentrated under reduced pressure, and the residue was washed with water and dried at 70°. The product (0·06 g.), m. p. 250—255°, was dissolved in dilute hydrochloric acid (charcoal), filtered, and further acidified with concentrated hydrochloric acid. Colourless fibrous needles were obtained on seeding with the authentic guanidine (Morley and Simpson, *loc. cit.*), which showed the same solubility and m. p. characteristics.

N^2-*Benzoyl*-N^1N^3-*di*-(4-*phenoxy*-7-*cinnolyl*)*guanidine*.—To 7-amino-4-phenoxycinnoline (0·1 g.) in nitromethane (5 c.c.), dichloromethylenebenzamide (0·1 g.) in nitromethane (2 c.c.) was added. An orange solid, m. p. >350° (0·15 g., average for 6 experiments), which was slowly precipitated, was collected after 1½ hr. at room temperature. The guanidine, m. p. >350°, crystallised from nitromethane in very low yield as the *trihydrochloride* (Found : C, 61·1; H, 4·5. $C_{36}H_{25}O_3N_7,3HCl$ requires C, 61·0; H, 4·0%). Passage of dry ammonia through a suspension of the salt in nitromethane gave a white base, m. p. 240—242°. This crystallised (with poor recovery) from Cellosolve (2-ethoxyethanol) as colourless needles, m. p. 244—245°.

Reaction of the Preceding Guanidine with Ammonium Acetate.—The guanidine (0·7 g.) was added to molten ammonium acetate (20 g.) at 135° and the temperature raised to 160° and kept thereat, with occasional stirring, for ½ hr. The clear solution which was obtained after 10 min. was cooled, poured into aqueous sodium hydroxide (10%; 30 c.c.), and, next morning, the crystalline precipitate (0·13 g.), m. p. 204—205°, was collected. This *compound* formed colourless blades, m. p. 206—207°, from ethanol (Found : C, 61·3; H, 4·5; N, 16·8%). A derivative, $C_{24}H_{19}ON_9,H_2O$, of (III) requires C, 61·6; H, 4·5; N, 27·0%.

8-*Amino*-4-*phenoxycinnoline*.—8-Nitro-4-phenoxycinnoline (2·8 g.) (Schofield and Theobald, *loc. cit.*) was suspended in glacial acetic acid (5 c.c.), and frozen at 15°. This solid was treated with Albert and Linnell's reagent (*loc. cit.*) (100 c.c.) at ⩾15°. An almost colourless solid separated from the deep maroon solution which was formed initially, and the mixture was kept for ½ hr. at 10°, then poured on to ice (500 g.) and aqueous sodium hydroxide (250 c.c.; 40%). The product which separated was extracted with chloroform, and evaporation of the washed and dried ($MgSO_4$) extract yielded a crude product, m. p. 128—129° (2·1 g.). 8-*Amino*-4-*phenoxycinnoline*, b. p. 130°, separated from benzene–light petroleum (b. p. 60—80°; 1 : 1, v/v) in rhombs, m. p. 130° (Found : C, 71·35; H, 4·75; N, 17·6. $C_{14}H_{11}ON_3$ requires C, 70·9; H, 4·6; N, 17·75%).

5-*Amino*-4-*phenoxycinnoline*.—4-Chloro-5-nitrocinnoline (0·5 g.; Schofield and Theobald, *loc. cit.*), ammonium carbonate (2 g.), and phenol (50 g.) were rapidly ground together and then heated on a steam-bath for ½ hr. The mixture was diluted with water and treated with 40% aqueous sodium hydroxide (120 c.c.), and the resulting solution extracted with chloroform. The washed and dried extract was evaporated to dryness (0·6 g.), and the residue was recrystallised from light petroleum (b. p. 40—60; 50 c.c.)–benzene (2 c.c.) (charcoal), to provide long very pale green needles, m. p. 114—115°. Further recrystallisation (as before) gave needles, m. p. 122—123°. 5-*Nitro*-4-*phenoxycinnoline* separated from light petroleum (b. p. 40—60°) in almost colourless needles, m. p. 123° (Found : C, 62·4; H, 3·8. $C_{14}H_9O_3N_3$ requires C, 62·9; H, 3·4%). The product (0·15 g.; m. p. 122—123°) was suspended in glacial acetic acid (1 c.c.) and treated at 10° with the reagent (3·5 c.c.; Albert and Linnell, *loc. cit.*). An almost colourless precipitate separated immediately and the mixture was gently shaken for 20 min. and poured into 40% aqueous sodium hydroxide (100 c.c.) and ice (100 g.). Evaporation of a washed and dried ($MgSO_4$) chloroform extract gave a residue which yielded, on cooling of a filtered benzene digest and further crystallisation from benzene, pure 5-*amino*-4-*phenoxycinnoline* as yellow needles, m. p. 199—200° (decomp.) (0·08 g., 79%) (Found : C, 70·65; H, 4·4; N, 17·6. $C_{14}H_{11}ON_3$ requires C, 70·9; H, 4·6; N, 17·75%).

The authors thank Mr. E. S. Morton and Mr. H. Swift for the microanalyses.

MEDICAL RESEARCH COUNCIL GROUP FOR RESEARCH IN CHEMOTHERAPY,
UNIVERSITY OF MANCHESTER.
DEPARTMENT OF CHEMISTRY, CHELSEA POLYTECHNIC. [*Received, November 26th*, 1953.]