491. *Cinnolines and Other Heterocyclic Types in Relation to the Chemotherapy of Trypanosomiasis. Part I. General Introduction. Part II. Quaternary Salts of Amino-cinnolines, -quinazolines, and -quinolines.*

By J. R. KENEFORD, E. M. LOURIE, J. S. MORLEY, (the late) J. C. E. SIMPSON, J. WILLIAMSON, and P. H. WRIGHT.

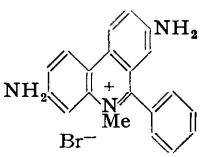
Part I. The general theme and results recorded in the five following papers are summarised.

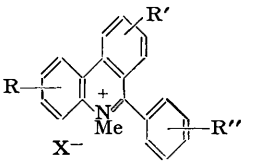
In the hope of simplifying the phenanthridinium quaternary salts while at the same time retaining their trypanocidal properties, various quaternary salts of amino-cinnolines, -quinazolines, and -quinolines were prepared and tested against trypanosome (and spirochæte) infections in mice. Although most of the compounds were inactive, crude preparations of two diamino-cinnoline salts showed definite trypanocidal action. This was attributed to the possible presence in the crude salts of the bis-cinnolinium azo-compounds (IX) and led to the hypothesis that other structures embodying two linked quaternised amino-heterocyclic units (X) might show trypanocidal properties. Di-(4-amino-6-cinnolyl)guanidine dimethiodide was found to have a chemotherapeutic index of the same order as that of " Antrycide."

Part II. The preparation of quaternary salts of amino-cinnolines, -quinazolines, and -quinolines is recorded.

PART I. GENERAL INTRODUCTION

THE form of trypanosomiasis which we have had chiefly in mind in the present work is the bovine infection, caused by *Trypanosoma congolense*, since this is relatively resistant to the arsenicals, and to suramin and pentamidine, which are so useful against human trypanosomiasis. When our work was begun, in 1945, the most effective drug in use against *T. congolense* was dimidium bromide (I). This is one of the most powerful of

(I) 

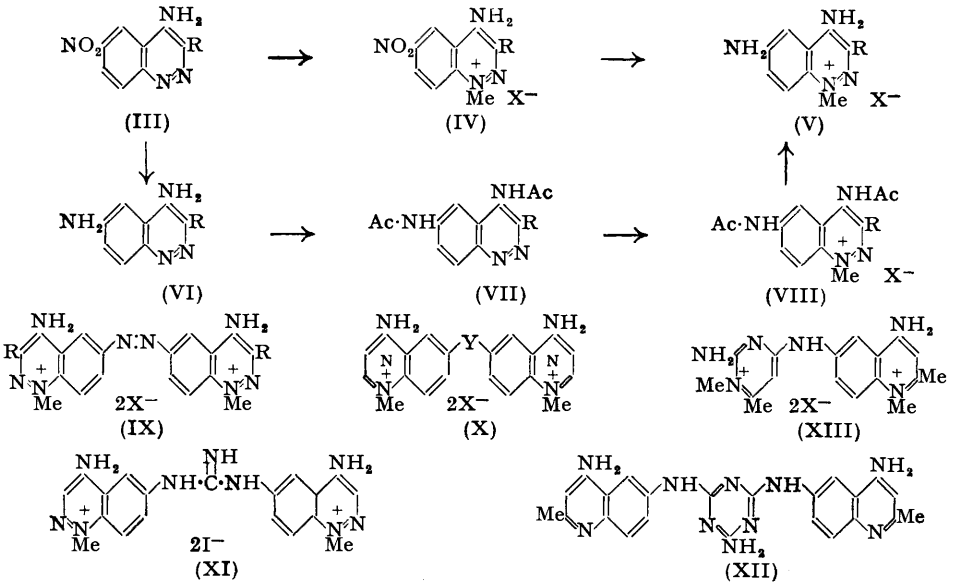
 (II)

several trypanocidal quaternary phenanthridinium salts, the chemistry and chemotherapy of which have recently been reviewed by Walls (*J. Soc. Chem. Ind.*, 1947, **66**, 182; see also *J.*, 1950, 41, 311). The compounds have the general structure (II), in which two of the substituents are primary amino-groups and the third is a hydrogen atom. Although they achieved some success in veterinary practice, a real need remained for other compounds of greater efficacy and lower toxicity.

The work to be described had as its initial objective the synthesis and testing of quaternary salts of 4-amino-quinolines, -quinazolines, and -cinnolines (for reasons discussed in Part II). These had little or no trypanocidal activity. A possible explanation of the ineffectiveness of bicyclic compounds is that the molecular weights of their cations are below a necessary threshold value. Of various devices for increasing the molecular size, that which appeared to offer most promise was suggested by the following observations.

The inactive methiodides of 4 : 6-diamino- and 4 : 6-diamino-3-methyl-cinnoline were prepared by the route (III) ⟶ (VI) ⟶ (VII) ⟶ (VIII) ⟶ (V). When, however, the alternative route (III) - - ⟶ (IV) ⟶ (V) was investigated, it was found that the crude salt (V; R = H, X = I) obtained by reducing the nitro-salt (IV; R = H, X = I) with iron and water had marked trypanocidal activity which was largely lost during recrystallisation—the final product was indistinguishable from the salt derived by

hydrolysis of (VIII). Similar reduction * of (IV; R = Me, X = I) led to even more marked trypanocidal activity; none of the pure diamino-salt (VIII; R = Me, X = I) was isolated, but the reduced solution showed a chemotherapeutic index of about 2 against *T. congolense* in mice (intraperitoneal injection) as compared with indices of about 15 and 3 for dimidium bromide and phenidium chloride, respectively.



The nitro-amine salt (IV; R = H, X = I) also showed trypanocidal properties, the activity slightly exceeding that of crude preparations of the derived diamine; however, when R = Me the nitro-amine salt is substantially inactive, so that it was unreasonable to ascribe the activity of the crude preparations merely to contamination by unchanged nitro-salt. It followed that the active substances must be impurities formed by side-reactions accompanying the conversion of the nitro- into the amino-salts; and since they were formed from nitro-compounds during reduction in approximately neutral solution, it seemed probable that they were bimolecular, *i.e.*, that they contained two linked cinnoline nuclei, and might well be the azo-salts (IX; R = H and Me).

These considerations, coupled with the fact that several trypanocidal compounds (surfen C, trypan red, suramin, Bayer 7602 Ac, diamidines) are symmetrical (or nearly so), led us to suggest (*Nature*, 1948, **161**, 603) that structure (IX) might be an example of a hitherto undiscovered trypanocidal type (X).

The special hypothesis—that concerning azo-compounds—was first investigated, starting with the more accessible quinoline series. After initial difficulties a series of 6 : 6′-azoquinoline bisquaternary salts were prepared (Macey and Simpson, Part III), but these were biologically disappointing. In the 6 : 6′-azocinnoline series (McIntyre and Simpson, Parts IV and V) one member was encountered which gave two isomeric salts; one of these salts is probably (IX; R = H) and one of them has appreciable biological activity. Proceeding meanwhile to test the general hypothesis, compounds of type (X) in which Y was a urea, thiourea, or guanidine residue were prepared (Morley and Simpson, Part VI). This disclosed that N^1N^3-di-(4-amino-6-cinnolyl)guanidine dimethiodide (XI) has activity of a high order against *T. congolense* infections in mice (Lourie, Morley, Simpson, and Walker, *Brit. J. Pharmacol.*, 1951, **6**, 643). When the synthesis of this compound, which we designate briefly as " 528," was almost complete, the discovery of the now well-known

* The results obtained were somewhat variable, but the best activity was secured by stirring an aqueous solution of the salt, containing a little ammonium chloride, with iron powder for ¾ hour at 10°; the filtered solution was used directly for testing.

" Antrycide " salts was announced (Curd and Davey, *Nature*, 1949, **163**, 89). Despite the fact that these salts were developed from surfen C (XII) (Curd and Davey, *Brit. J. Pharmacol.*, 1950, **5**, 25) and thus arose from a conception differing considerably from our own hypothesis, it is noteworthy that the structure (XIII) of these compounds is similar in many respects to that of " 528 " (XI), and a direct biological comparison of " 528 " with antrycide methyl sulphate showed that the two compounds are indeed very similar in activity (Lourie, Morley, Simpson, and Walker, *loc. cit.*).

These results undoubtedly lend substance to the suggestion that (X) may symbolise a new type of trypanocidal agent, but further work is necessary before the scope or even the general validity of the hypothesis can be regarded as firmly established.

Part II. Quaternary Salts of Amino-cinnolines, -quinazolines, and -quinolines

Our choice of the salts named in the title, for examination as trypanocides, was determined by the following considerations. (i) They could be regarded as simplified versions of the phenanthridinium salts. (ii) Their molecules would still conform to the conditions required for cationic resonance, the general chemotherapeutic significance of which has been stressed in recent years (cf. Keneford, Morley, Simpson, and Wright, *J.*, 1950, 1104). (iii) In the absence of biochemical data on which further synthetic work could be planned, it seemed important to establish the minimum molecular size with which trypanocidal activity may be associated, and also to look for possible relations between activity and structure: the simple bicyclic structures selected appeared to offer a suitable starting point.

The earlier work of Morgan and Walls (*J.*, 1938, 389) on the phenanthridinium compounds did not reveal whether quaternisation of the heterocyclic bases was essential for the development of activity against *T. congolense*. Our decision to retain this feature was originally governed by the facts (i) that 7-amino-9-*p*-aminophenyl- and 2 : 7-diamino-9-phenyl-phenanthridine proved to be inactive (these compounds are the parent bases of two of the most active quaternary phenanthridinium salts, and were kindly supplied to us by Messrs. May and Baker, Ltd.), and (ii) that, in contrast to other arsenical drugs, some activity against *T. congolense* is shown by quaternary *iso*arsindolinium hydroxides (Lyon and Mann, *J.*, 1945, 30; Lyon, Mann, and Cookson, *J.*, 1947, 662). Since our work began, however, Walls (*loc. cit.*) has reported that little parasiticidal activity has been found in any phenanthridine compound not possessing a quaternary function, and the same seems to apply to the compounds synthesised by ourselves.

The salts shown in Table 1 were tested against *T. congolense* in mice, and were all completely inactive. With the exception of the methiodides of 4 : 6-diamino-3-methyl-, 4 : 6-diacetamido-3-methyl-, and 4 : 6-diacetamido-cinnoline, which were tested against *T. congolense* only, all the compounds were also inactive against *T. cruzi*, *T. rhodesiense*, *Spirochæta minus*, and *S. recurrentis* infections in mice.

Slight activity against *T. congolense* (amounting merely to prolongation of the infection without even temporary clearance of parasites from the blood) was shown by the methiodides of 5-amino- and 4 : 6-diamino-quinoline and those of 4 : 8-diamino-7-methyl-cinnoline and 4-amino-3-methyl-6- and -8-nitrocinnoline. With the exception of the last compound, these salts were also tested against the other four organisms named above, and were inactive. Slight activity against *T. congolense* was also observed with 4-amino-quinoline and the hydrochloride of 4 : 6-diamino-quinoline and -cinnoline.

The range of quaternary salts tested is far from exhaustive, the number of quinazoline compounds being particularly small because quaternary salts in this series containing a potential 4-amino-group are unstable (Morley and Simpson, *J.*, 1948, 360; 1949, 1354). However, the uniformly negative, or virtually negative, biological results obtained (by intraperitoneal injection of maximum tolerated doses) throughout the series certainly suggested that trypanocidal activity is unlikely to be found among bicyclic amino-quaternary salts as a class; and this conclusion is strengthened by the absence of note-

worthy activity among several diamino-1-phenyl*iso*quinoline metho-salts (McCoubrey and Mathieson, *J.*, 1949, 696), which likewise may be regarded as simplified phenanthridinium salts.

TABLE 1. *Metho-salts tested.*

Substituent	Anion	Ref.	Substituent	Anion	Ref.
Quinoline series.			*Cinnoline series.*		
4-NH_2	I	1	4-NH_2	I	2
6-NH_2	Cl	2	4-NH_2-6-Cl	I	4
6-NH_2	I	2	4-NH_2-7-Cl	I	2
6-NHAc	I	2	4-NHPh-6-NO_2	I	2
4-NH_2-6-NO_2	I	1	6-NH_2-4-NHPh	I	2
3 : 5-$(NH_2)_2$-6-Me	I	2	4 : 6-$(NHAc)_2$	I	2
2-CH:N·NHPh	Cl	2	4 : 6-$(NH_2)_2$	I	2
Quinazoline series.			4 : 6-$(NHAc)_2$-3-Me	I	2
4-NHPh-6-NO_2	I	3	4 : 6-$(NH_2)_2$-3-Me	I	2
6-NH_2-4-NHPh	I	3	4-NH_2-7-Me-8-NO_2	I	2
7-NH_2-4-NHPh	I	3			

Refs.: 1, Simpson and Wright, *J.*, 1948, 1707. 2, Experimental section of this paper. 3, Morley and Simpson, *J.*, 1948, 360. 4, Simpson, *J.*, 1947, 1653.

EXPERIMENTAL

Melting points are uncorrected.

1-*Methyl*-2-*phenylhydrazonomethylquinolinium Chloride.*—This compound was prepared from quinaldine methosulphate and benzenediazonium chloride essentially as described by Koenigs and Bueren (*J. pr. Chem.*, 1936, **146**, 119), and crystallised from 80% acetic acid in red needles, m. p. 241—242° (lit., 241°).

6-*Amino*-1-*methylquinolinium Chloride.*—A solution of 6-acetamidoquinoline methiodide [m. p. 300—302° (decomp.) (Hamer, *J.*, 1921, 1432, gives m. p. 317°); prepared by warming the base and methyl iodide in alcohol (yield, 90%)] (5 g.) in 2N-hydrochloric acid (20 c.c.) was heated under reflux for 1 hour and then evaporated in a vacuum. The residue was refluxed with water (40 c.c.) and silver chloride (5 g.) for 1 hour, and the filtered solution was neutralised to pH 5—7 and concentrated, whereupon 6-amino-1-methylquinolinium chloride separated. The salt formed rectangular prisms, m. p. 242—243°, from aqueous acetone (Decker and Kaufmann, *J. pr. Chem.*, 1911, **84**, 425, give m. p. 242—243°) (Found: C, 56·4; H, 6·3; N, 13·9. Calc. for $C_{10}H_{11}N_2Cl,H_2O$: C, 56·4; H, 6·15; N, 13·2%).

6-*Amino*-1-*methylquinolinium Iodide.*—(*a*) A solution of the corresponding nitro-salt [m. p. 245—246° (effervescence) (Hamer, *loc. cit.*, gives m. p. *ca.* 245° with previous decomposition); prepared in theoretical yield from the base and methyl iodide in warm nitrobenzene] (3 g.) in water (15 c.c.) and acetic acid (3 drops) was stirred with iron filings (4 g.) for ½ hour at 90°. 6-Amino-1-methylquinolinium iodide (1·8 g.) separated from the filtered solution in orange needles, m. p. 199—200° (Found: C, 40·4; H, 4·6; N, 9·9. Calc. for $C_{10}H_{11}N_2I,0·5H_2O$: C, 40·7; H, 4·1; N, 9·5%). (*b*) A specimen prepared from the methochloride and aqueous potassium iodide had m. p. 199—200° alone and mixed with the above material.

5-*Amino*-1-*methylquinolinium Iodide.*—This *salt*, prepared from the nitro-compound [m. p. 212—213° (decomp.); Decker (*Ber.*, 1905, **38**, 1144) gives m. p. 215°] similarly to the 6-amino-isomeride, separated from water in soft brown-red needles, m. p. 213—214° (Found: C, 39·9; H, 4·5; N, 9·5. $C_{10}H_{11}N_2I,H_2O$ requires C, 39·5; H, 4·3; N, 9·2%).

3 : 5-*Diamino*-1 : 6-*dimethylquinolinium Iodide.*—An intimate mixture of 6-methyl-3 : 5-dinitroquinoline (1 g.) (Morley and Simpson, *J.*, 1948, 2024) and methyl toluene-*p*-sulphonate (0·9 g.) was heated at 140° (bath-temp.) for 1½ hours. When cold, the melt was pulverised under hot alcohol (20 c.c.), and the solid collected and recrystallised from water (20 c.c.), from which 1 : 6-*dimethyl*-3 : 5-*dinitroquinolinium toluene*-p-*sulphonate* separated in clusters of yellow needles, m. p. 232—233° (decomp.) (Found: C, 52·0; H, 4·4; N, 7·8. $C_{18}H_{17}O_7N_3S$ requires C, 51·6; H, 4·1; N, 7·65%). A solution of this salt (0·5 g.) in hot water (10 c.c.) was treated at 90° with iron powder (1·3 g.) added in portions during ½ hour with vigorous stirring. After a further ½ hour at 90°, the solution was filtered and treated with potassium iodide, yielding almost pure 3 : 5-*diamino*-1 : 6-*dimethylquinolinium iodide* (0·18 g.), m. p. 249—250°. The pure salt separated from water in orange-red prismatic needles, m. p. 254—255° (decomp.) (Found: C, 42·2; H, 4·7. $C_{11}H_{14}N_3I$ requires C, 41·9; H, 4·5%).

4-*Amino*-6-*nitrocinnoline*.—For the large-scale preparation of this and the two following bases, the procedure here described is superior to those previously used (*J.*, 1948, 358, 1707). Dry ammonia was passed into a mixture of recrystallised 4-chloro-6-nitrocinnoline (32 g.) and phenol (320 g.). The mass soon liquefied, and was shaken occasionally until its temperature reached 50—55°; by then a dark, almost clear solution had been formed. The flask was then heated for ½ hour at an internal temp. of 135—145° (bath at 180°) with continuous passage of a brisk stream of ammonia. The reaction-mass was diluted while still slightly warm with ether (*ca.* 1 l.), and the crude amine filtered off and washed with ether. The base was then dissolved in warm 10% aqueous acetic acid (*ca.* 800 c.c.) and the filtered (charcoal) solution was poured into almost boiling N-aqueous ammonia (about 2 l.), yielding the base in a highly granular form. The suspension was diluted somewhat with water and filtered hot, and the solid washed with water; a small second crop was obtained from the cold filtrate. The yield was 25·1 g. (86%), and the m. p. 288—290° (decomp.) on rapid heating.

4-*Amino*-3-*methyl*-6-*nitrocinnoline*.—Pure 4-chloro-3-methyl-6-nitrocinnoline (13 g.) in phenol (130 g.) was treated with a stream of dry ammonia for ¼ hour at 130—140° (internal temp.). The crude product was a mixture of the required amine and the hydroxy-compound, and was digested with warm 30% aqueous acetic acid. 4-Amino-3-methyl-6-nitrocinnoline (60—63%) separated from the filtered and basified solution as an orange-yellow solid, decomp. at 320°. The yield was not appreciably affected by heating for 1 hour, or by lowering the temperature to 100—105°.

4-*Amino*-6-*nitroquinoline*.—Dry ammonia was bubbled slowly for 2 hours into a solution of 4-chloro-6-nitroquinoline (30 g.) in phenol (300 g.) at 170—180°. Basification in the cold with excess of 10% aqueous sodium hydroxide gave 4-amino-6-nitroquinoline, purified by dissolution in 10% aqueous acetic acid (500 c.c.) and reprecipitation with sodium hydroxide solution. The yield was 26·5 g. (97%), and the m. p. 310—313° (decomp.).

4 : 6-*Diaminoquinoline*.—A solution of the foregoing nitro-amine (26 g.) in acetic acid (180 c.c.) was added during 5 minutes at room temperature to one of stannous chloride (96 g.; " AnalaR ") in concentrated hydrochloric acid (192 c.c.) and water (48 c.c.) (mechanical stirring). After 30 minutes at 60—65° (reduction did not proceed at a lower temperature) the suspension was treated with excess of 20% sodium hydroxide, and the crystalline precipitate was filtered off rapidly, washed with a little ice-cold water, and dried *in vacuo*. A solution of the base in acetone (charcoal) was treated with concentrated hydrochloric acid; the dihydrochloride (24 g., 75%), m. p. >330°, so obtained was dissolved in water and treated with sodium hydroxide, yielding almost colourless, brittle needles, m. p. 212—213° (decomp.), of 4 : 6-diaminoquinoline [Simpson and Wright, *J.*, 1948, 1707, give m. p. 208° (decomp.)].

4 : 6-*Diaminocinnoline*.—The following conditions are superior to those previously described (*J.*, 1950, 1104). The almost clear solution formed by dissolving 4-amino-6-nitrocinnoline (10 g.) in hot 0·5N-hydrochloric acid (200 c.c.) was added while still slightly warm, during ¼ hour, to a stirred solution of stannous chloride dihydrate (" AnalaR "; 38 g.) in 10N-hydrochloric acid (100 c.c.). The temperature, initially 20°, was allowed to rise to 45° and kept at 40—45° by occasional cooling. Copious crystallisation occurred soon after the addition of the nitro-compound was complete. After being set aside for 1 hour, the mixture was treated with 40% aqueous sodium hydroxide (300 c.c.), the temperature being allowed to rise freely to 50—55°, and the crude diamine was filtered off cold and washed with a little water. The crude base from 30 g. of nitro-compound was dissolved in water (450 c.c.) containing acetic acid (18 c.c.), and the filtered (charcoal) solution was treated with 40% sodium hydroxide (90 c.c.), whereupon 4 : 6-diaminocinnoline (22·7 g., 90%) separated in almost colourless soft needles, m. p. 262—264° (decomp.).

4 : 6-*Diamino*-3-*methylcinnoline*.—A solution of stannous chloride (" AnalaR "; 18·8 g.) in 10N-hydrochloric acid (25 c.c.) and water (25 c.c.) was added dropwise during ¼ hour at 25—30° to a stirred solution of 4-amino-3-methyl-6-nitrocinnoline (5 g.) in acetic acid (25 c.c.). After a further ¼ hour's stirring at 25—30°, the yellow suspension was heated quickly to 60°, kept at this temperature for 5 minutes, and then treated with ice and 20% sodium hydroxide solution (250 c.c.). The crude 4 : 6-diamino-3-methylcinnoline which separated as a colourless solid on scratching was immediately dissolved in hot very dilute hydrochloric acid (100 c.c.). The solution was treated with acetone (100 c.c.) and concentrated hydrochloric acid (100 c.c.), whereupon the diamine hydrochloride (4·9—5·2 g.) separated in almost colourless prismatic needles, m. p. 325° (decomp.). Basification of a concentrated aqueous solution of the salt with potassium hydroxide gave the free diamine, m. p. 270° (decomp.), which was freely soluble in warm water. The *diacetyl* derivative, obtained in theoretical yield by refluxing acetic anhydride

(10 parts by volume; 5 minutes) and washing of the precipitated solid with ether, crystallised from acetone in colourless needles, m. p. 297—298° (decomp.) after sintering at 290° (Found: C, 60·45; H, 5·4; N, 20·8. $C_{13}H_{14}O_2N_4$ requires C, 60·4; H, 5·45; N, 21·7%).

6-*Amino*-4-*anilinocinnoline Hydrochloride*.—A solution of crude 4-anilino-6-nitrocinnoline (Schofield and Simpson, *J.*, 1945, 512) (5 g.) in acetic acid (30 c.c.) was treated with a solution of stannous chloride (14·5 g.) in concentrated hydrochloric acid (25 c.c.), added during ¼ hour at 60—65°. After 1 hour at 95°, the solid obtained by basification with 2N-sodium hydroxide was collected and digested with small quantities of hot 0·25N-hydrochloric acid until no crystals separated from the extracts on cooling. The 6-*amino*-4-*anilinocinnoline hydrochloride* so obtained (3·5 g.; m. p. 298—300°) separated in glittering, rust-coloured needles, m. p. 301—302° (decomp.), from very dilute hydrochloric acid (Found: C, 58·3; H, 5·25; N, 19·1. $C_{14}H_{13}N_4Cl,H_2O$ requires C, 57·85; H, 5·2; N, 19·3%); the free base separated from water as a yellow gelatinous mass, m. p. *ca.* 259—261° (decomp.). Treatment of the hydrochloride (1·62 g.) with anhydrous sodium acetate (0·99 g.) and acetic anhydride (6 c.c.) at 90° for 1½ hours, followed by addition of water (25 c.c.), gave light-brown needles, m. p. 289—290° (decomp.), of 6-acetamido-4-anilinocinnoline (1·15 g.).

Preparation of Quaternary Salts from Cinnoline Bases.—The following compounds were prepared by heating the base (1 part by wt.) with a large excess (*ca.* 5 parts by vol.) of methyl iodide in alcohol (10—15 parts by vol.); if necessary, the solutions were concentrated for isolation of the products. Unless otherwise stated, clear solutions were obtained at some period during the reactions. The references in parentheses are to the preparation of the cinnoline bases, and the figures refer to times of reflux.

4-*Amino*-1-*methylcinnolinium iodide* (*J.*, 1948, 358; ½ hour), greenish-yellow prismatic needles from alcohol (yield, crude 70%, pure 35%), had m. p. 252—253° (decomp.) (Found: C, 38·05; H, 3·6; N, 14·65. $C_9H_{10}N_3I$ requires C, 37·65; H, 3·5; N, 14·65%).

4-*Amino*-7-*chloro*-1-*methylcinnolinium iodide* (*loc. cit.*; ¼ hour) formed orange prismatic needles (crude 87%, pure 45%), m. p. 282—283° (decomp.), from water (Found: C, 34·1; H, 2·6; N, 13·2. $C_9H_9N_3ClI$ requires C, 33·6; H, 2·85; N, 13·05%); unidentified colourless needles, m. p. 258—260° (decomp.), were obtained from the first filtrate.

4-*Amino*-1-*methyl*-8-*nitrocinnolinium iodide* (*J.*, 1948, 1702; 3 hours) separated from water as a mixture of small bronze prisms and soft leafy aggregates of needles; each form had m. p. and mixed m. p. 237—238° (decomp.) (crude 75%, pure 50%) (Found: C, 32·65; H, 2·3; N, 16·6. $C_9H_9O_2N_4I$ requires C, 32·55; H, 2·75; N, 16·85%).

4-*Amino*-1 : 3-*dimethyl*-6-*nitrocinnolinium iodide* (*J.*, 1948, 358; 3—5 hours) had m. p. 280—281° (decomp.), and crystallised from water in deep red prisms or plates (Found: C, 35·35; H, 3·6; N, 16·7. $C_{10}H_{11}O_2N_4I$ requires C, 34·7; H, 3·2; N, 16·2%). The m. p. varied somewhat with different specimens; the yield of easily purified material was *ca.* 50%, a further 20% of poorly crystalline material, m. p. 245—250° (decomp.), being obtainable from the alcoholic filtrate.

4-*Amino*-1 : 3-*dimethyl*-8-*nitrocinnolinium iodide* (*loc. cit.*; 6 hours; at no time during the reaction was a clear solution obtained), m. p. 263—264° (decomp.), crystallised from water in dark bronze irregular plates (crude 61%, pure *ca.* 40%) (Found: C, 35·3; H, 3·45; N, 16·6. $C_{10}H_{11}O_2N_4I$ requires C, 34·7; H, 3·2; N, 16·2%).

4-*Amino*-1 : 7-*dimethyl*-8-*nitrocinnolinium iodide* (*J.*, 1948, 1702; 2 hours; some material was in suspension throughout the reaction) crystallised from water in long reddish-orange prismatic needles, m. p. 287° (decomp.) (yield of almost pure salt, 84%) (Found: C, 35·25; H, 3·35; N, 15·65. $C_{10}H_{11}O_2N_4I$ requires C, 34·7; H, 3·2; N, 16·2%).

4-*Anilino*-1-*methyl*-6-*nitrocinnolinium iodide* (*J.*, 1945, 512; ½ hour) crystallised from water in glittering scarlet needles, m. p. 233—234° (decomp.) (Found: C, 44·0; H, 3·5; N, 13·4. $C_{15}H_{13}O_2N_4I$ requires C, 44·1; H, 3·2; N, 13·7%); the salt was obtained in the same yield (7 g.), but more conveniently, by heating a solution of the base (5 g.) in nitrobenzene (50 c.c.) with methyl iodide (10 c.c.) for ½ hour on the steam-bath under reflux.

6-*Acetamido*-4-*anilino*-1-*methylcinnolinium iodide* (this paper; 1¼ hours) formed yellow prismatic needles (0·39 g. from 0·9 g. of base), m. p. 267—268° (decomp.), from water (Found: C, 46·3; H, 4·35; N, 12·5. $C_{17}H_{17}ON_4I,H_2O$ requires C, 46·6; H, 4·35; N, 12·8%). The corresponding chloride, obtained by recrystallising the iodide from 2N-hydrochloric acid, had m. p. 240—241° (decomp.) and did not depress the m. p. of the analytical sample described below. Concentration of the alcoholic filtrate from the iodide (reduced pressure) and crystallisation of the product from water gave a *substance* (0·5 g.), m. p. 189—191°, which could not again be satisfactorily crystallised from water, but gave deep yellow needles, m. p. 278—280°

(decomp.), when crystallised from dilute hydrochloric acid (Found : C, 65·0; H, 5·05; N, 19·05%).

4 : 6-*Diacetamido*-1-*methylcinnolinium iodide* (*J.*, 1950, 1104; ½ hour) crystallised from water in yellow prismatic needles, m. p. 265° (decomp.) (yield of pure salt, 75%) (Found : C, 39·2; H, 4·3; N, 13·8. $C_{13}H_{15}O_2N_4I,0·5H_2O$ requires C, 39·5; H, 4·1; N, 14·2%). A solution of this salt (6 g.) in N-hydrochloric acid (120 c.c.) was heated under reflux for 1 hour. Potassium iodide (20 g.) in water (20 c.c.) was added to the solution, from which almost pure 4 : 6-*diamino*-1-*methylcinnolinium iodide* (4·5 g., 95%) slowly separated. The pure salt crystallised from water in yellow needles, m. p. 273—275° (decomp.) (Found : C, 35·8; H, 3·8; N, 21·8; I, 42·7. $C_9H_{11}N_4I$ requires C, 35·75; H, 3·7; N, 18·5; I, 42·05%). 4 : 6-*Diamino*-1-*methylcinnolinium bromide* (6·6 g.) was prepared by refluxing a solution of the iodide (8 g.) in water (200 c.c.) with silver bromide (20 g.) for 1 hour, and then adding sodium bromide (40 g.) to the filtered solution; the salt was easily soluble in water, and crystallised in golden-yellow needles, m. p. 286—287° (decomp.) (Found : C, 42·5; H, 4·5; N, 19·3; Br, 31·35. $C_9H_{11}N_4Br$ requires C, 42·4; H, 4·35; N, 21·9; Br, 31·3%). The *chloride*, similarly prepared, crystallised from water or aqueous acetone in yellow needles, m. p. about 305° (decomp., depending somewhat on the rate of heating) (Found : C, 51·8; H, 5·55; N, 24·9; Cl, 16·45. $C_9H_{11}N_4Cl$ requires C, 51·3; H, 5·25; N, 26·6; Cl, 16·8%). The *thiocyanate* crystallised when aqueous solutions of the methobromide and of ammonium thiocyanate were mixed; it formed golden-yellow needles which melted at 187—188° (without decomposition but somewhat dependent on the rate of heating) (Found : C, 50·4; H, 4·65; N, 31·3; S, 12·75. $C_{10}H_{11}N_5S$ requires C, 51·45; H, 4·75; N, 30·0; S, 13·7%).

4 : 6-*Diacetamido*-1 : 3-*dimethylcinnolinium iodide* (this paper; 1 hour) separated from alcohol in stout, orange-red, prismatic needles, m. p. 243—244° (decomp.) (crude, 91%; pure, 53%) (Found : C, 42·5; H, 4·55; N, 14·0. $C_{14}H_{17}O_2N_4I$ requires C, 42·0; H, 4·3; N, 14·0%). The salt (4·35 g.) was heated under reflux for 1 hour with N-hydrochloric acid (100 c.c.). The product (2·65 g.) which separated on cooling had m. p. 294—295°, and was probably a mixture of chloride and iodide. Treatment of its aqueous solution with potassium iodide gave long golden needles of 4 : 6-*diamino*-1 : 3-*dimethylcinnolinium iodide*, m. p. 282—283° (decomp.), and more was obtained by treating the original acid filtrate with potassium iodide (total yield 87%, based on acetamido-compound) (Found : C, 35·7; H, 4·7; N, 16·6. $C_{10}H_{13}N_4I,H_2O$ requires C, 35·9; H, 4·5; N, 16·75%). 4 : 6-*Diamino*-1 : 3-*dimethylcinnolinium chloride* (2·35 g.), prepared by treating the iodide (3·0 g.) with freshly precipitated silver chloride (6 g.) in boiling water (100 c.c.) for ¾ hour and then adding sodium chloride (6 g.) to the filtered solution, separated from dilute aqueous sodium chloride in long, golden-yellow needles, m. p. 314—315° (decomp.), and was appreciably soluble in cold water (Found : C, 52·9; H, 5·55; N, 24·4. $C_{10}H_{13}N_4Cl$ requires C, 53·4; H, 5·8; N, 24·9%).

Application of the above quaternisation conditions (3 hours) to 4-amino-6-nitrocinnoline gave a product which was later found (Atkinson, forthcoming publication) to be substantially the expected salt contaminated with some isomeric material. As thus prepared, the *salt* crystallised from water in rosettes of orange-red needles, m. p. 209—210° (decomp.) (yield, crude 60%, pure 40%) (Found : C, 33·05; H, 2·85. $C_9H_9O_2N_4I$ requires C, 32·5; H, 2·7%).

Preparation of Bz-*Aminocinnolinium Salts*.—The following compounds were prepared by reducing the appropriate nitro-salt in water with iron powder; figures in parentheses are, respectively, weight of nitro-compound, volume of water, weight of iron, temperature, time taken for addition of iron, and total time of reduction. The filtered solutions were concentrated if necessary in an evacuated desiccator until crystallisation set in, and the salt were purified by recrystallisation from water. Yields given are for pure or nearly pure material.

4 : 6-Diamino-1-methylcinnolinium iodide (4·1 g., 75 c.c., 9 g., 90°, ¼ hour, 1 hour) formed brown prismatic needles (1·35 g.), m. p. 273—274° to a dark liquid after shrinking at 265°; the m. p. was not depressed by admixture with the authentic sample described above (Found : C, 35·6; H, 3·7; N, 18·5; I, 42·4. Calc. for $C_9H_{11}N_4I$: C, 35·75; H, 3·7; N, 18·5; I, 42·05%); unidentified products were also formed. 4 : 6-Diamino-1 : 3-dimethylcinnolinium iodide [1 g. (crude), 40 c.c., 2 g. (added all at once), 88°, 1 hour, mechanical stirring] crystallised in long bronze-coloured needles (0·25 g.), m. p. 283—284° (decomp.) alone and when mixed with the authentic material described above (Found : C, 36·0; H, 4·55; N, 16·8. Calc. for $C_{10}H_{13}N_4I,H_2O$: C, 35·9; H, 4·5; N, 16·75%). Other products were also present in the filtrate from this compound. 4 : 8-*Diamino*-1 : 7-*dimethylcinnolinium iodide* (1·5 g., 150 c.c., 2·7 g. added all at once, 90°, 1 hour) separated in small red needles (1 g.), m. p. 338° (decomp.) (Found : C, 37·3; H, 4·2; N, 17·4. $C_{10}H_{13}N_4I,\frac{1}{4}H_2O$ requires C, 37·45; H, 4·25; N, 17·5%).

6-*Amino*-4-*anilino*-1-*methylcinnolinium iodide* (1 g., 80 c.c., 1·25 g., 92—94°, ½ hour, 1¾ hours, mechanical stirring) (0·39 g.) crystallised from alcohol in orange needles, m. p. 240—241° (decomp.) (Found: C, 48·0; H, 4·8; N, 13·8. $C_{15}H_{15}N_4I,C_2H_5{\cdot}OH$ requires C, 48·0; H, 5·0; N, 13·2%). 6-*Acetamido*-4-*anilino*-1-*methylcinnolinium chloride*, formed by heating a solution of the foregoing salt (100 mg.) in water (2 drops) and acetic acid (1 c.c.) with acetic anhydride (1 c.c.) at 95° for 1½ hours and then adding 2N-hydrochloric acid to the cold solution, separated in orange-yellow needles (80 mg.), m. p. 237—238° (decomp.) (238—239° when mixed with the sample described above) (Found: C, 58·6; H, 5·4; N, 15·5; Cl, 10·4. $C_{17}H_{17}ON_4Cl,H_2O$ requires C, 58·8; H, 5·5; N, 16·1; Cl, 10·2%).

4 : 6-*Diamino*-1-*methylquinolinium Iodide*.—4 : 6-Diacetamido-1-methylquinolinium iodide (Simpson and Wright, *J.*, 1948, 1707) (5 g.) was heated under reflux for 1 hour in N-hydrochloric acid (50 c.c.); potassium iodide (25 g.) was then added, and the solution was cautiously neutralised in the cold with aqueous ammonia to pH 6—7. The crude 4 : 6-*diamino*-1-*methylquinolinium iodide* (4·75 g.; m. p. 245—250°) which separated was recrystallised from water, from which the pure salt, m. p. 257—258°, was obtained in small, yellow prisms followed by long, yellow blades (Found: C, 40·0; H, 4·1; N, 13·5. $C_{10}H_{12}N_3I$ requires C, 39·9; H, 4·0; N, 13·95%). Dilute solutions exhibited a beautiful blue fluorescence, destroyed by increase or decrease in pH. Treatment with nitrous acid gave a solution which coupled immediately with alkaline β-naphthol.

WARRINGTON YORKE DEPARTMENT OF CHEMOTHERAPY,
LIVERPOOL SCHOOL OF TROPICAL MEDICINE.
MEDICAL RESEARCH COUNCIL, GROUP FOR RESEARCH IN CHEMOTHERAPY,
THE UNIVERSITY, MANCHESTER.
[*Received, December 11th*, 1951.]