19. *The Search for Chemotherapeutic Amidines. Part V. Phenanthridines.*

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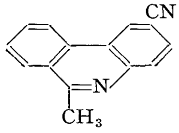
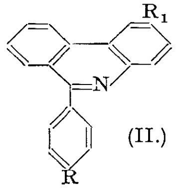
3-*Amidino*-9-*methyl*-, 3-*amidino*-9-p-*amidinophenyl*-, *and* 7-*amidino*-9-p-*amidinophenylphenanthridine*, and the *methochloride* of the last base have been prepared and found to have little trypanocidal activity.

The marked trypanocidal activity found in the phenanthridine series, especially among nuclear quaternised diamines (Walls, *J.*, 1938, 389; 1945, 294), has led us to examine some analogous amidines to see if the therapeutically active amidine group in association with the phenanthridine nucleus gave products having an enhanced trypanocidal action.

As an exploratory experiment, we attempted the preparation of the mono-amidine 3-*amidino*-9-*methylphenanthridine* in order, primarily, to judge the effect of the amidine group on the general toxicity of the nucleus and to see whether any trypanocidal activity could be expected from the mono-amidines of this series.



(I.)



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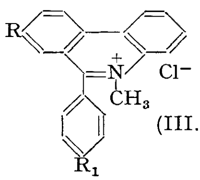

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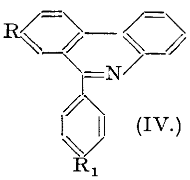

(IV.)

2-Acetamidodiphenyl was brominated in glacial acetic acid to produce 5-bromo-2-acetamidodiphenyl in excellent yield (Scarborough and Waters, *J.*, 1927, 89). Ring closure of this compound with phosphorus oxychloride gave 3-*bromo*-9-*methylphenanthridine*, but smooth cyanation of this product proved difficult. Prior cyanation of the diphenyl was successful, and ring closure by heating with phosphorus oxychloride gave the required 3-*cyano*-9-*methylphenanthridine* (I) in moderate yield. The cyanide was converted *via* the iminoether into the corresponding *amidine dihydrochloride* in the usual way. This compound exhibited little or no trypanocidal activity, and our attention then turned to diamidines of the phenanthridine series.

2-p-*Bromobenzamidodiphenyl* was obtained in satisfactory yield by the interaction of *p*-bromobenzoyl chloride and 2-aminodiphenyl in chlorobenzene. Direct bromination in acetic acid gave 5-*bromo*-2-p-*bromobenzamidodiphenyl*. The longer route involving the stages 2-amino- → 2-acetamido- → 5-bromo-2-acetamido- → 5-bromo-2-amino- → 5-bromo-2-*p*-bromobenzamido-diphenyl gave slightly better yields.

Ring closure of the dibromo-compound was effected in good yield with phosphorus oxychloride in nitrobenzene, the 3-*bromo*-9-p-*bromophenylphenanthridine* (II; $R = R_1 = Br$) so obtained being converted smoothly into the required *dicyanide* (II; $R = R_1 = CN$) by treatment with cuprous cyanide in quinoline.

The dicyanide was converted into the *diamidine dihydrochloride* [II; $R = R_1 = C(:NH)\cdot NH_2,HCl$] through the corresponding iminoether base by treatment with ammonium chloride. The dihydrochloride was very sparingly soluble in water (1% at 23°), and even at this concentration tended to form gels. The amidine exhibited no activity against *T. equiperdum.*

7-Nitro-9-*p*-nitrophenylphenanthridine, prepared by Walls's method (*loc. cit.*), was the starting point for the preparation of the amidines [III and IV; $R = R_1 = C(:NH)\cdot NH_2$], which are analogues of one of the most active phenanthridinediamines (III; $R = R_1 = NH_2$). Reduction to 7-amino-9-*p*-aminophenylphenanthridine (IV; $R = R_1 = NH_2$) was effected by treatment with stannous chloride in glacial acetic acid (cf. Walls, *loc. cit.*), and the required dicyanide (IV; $R = R_1 = CN$) was obtained from the diamine by means of the Sandmeyer reaction.

The di-iminoether [IV; $R = R_1 = C(:NH)\cdot OC_2H_5$] was not formed on treating the cyanide in chloroform with absolute alcohol and dry hydrogen chloride, but a reasonable yield was obtained in about ten days when the chloroform was omitted. The final product was isolated as the soluble *amidine trihydrochloride*, the isethionate being deliquescent and the corresponding base gel-like.

This amidine exhibited slight trypanocidal activity against *T. equiperdum* infection in mice, and the corresponding *methochloride* was prepared, the quaternary group being an apparently essential feature for marked activity in the diamine series. As it was not possible directly to quaternise the amidine without affecting the amidine group, the dicyanide (IV; $R = R_1 = CN$) in nitrobenzene solution was treated with methyl sulphate to give the corresponding *methyl methosulphate.* 7-*Cyano*-9-p-*cyanophenyl*-10-*methylphenanthridinium chloride* was obtained either by direct treatment of an aqueous solution of the methyl methosulphate with ammonium chloride or by the action of silver chloride on the corresponding *bromide.* Both methods gave products with slightly high halogen values, but the excess halogen was loosely combined; the same phenomenon has been noted independently in the quaternised dinitrophenanthridines (Walls, private communication).

Treatment of an aqueous solution of the chloride with picric acid gave the *picrate*, which analysed correctly and contained no halogen. This disposed of any suggestion of nuclear halogenation by migration from the quaternary nitrogen atom.

The cyanide was smoothly converted through the iminoether hydrochloride into 7-*amidino*-9-p-*amidinophenyl*-10-*methylphenanthridinium chloride dihydrochloride* which was heavily hydrated and quite soluble in water. It exhibited no activity against either *T. equiperdum* or *T. congolense* infections.

EXPERIMENTAL.

3-*Bromo*-9-*methylphenanthridine.*—5-Bromo-2-acetamidodiphenyl (5 g.) was boiled gently under reflux with freshly distilled phosphorus oxychloride (10 g.) for 30 mins. After removal of excess oxychloride under reduced pressure, the residue was treated with warm N-hydrochloric acid (100 c.c.), the solution filtered (charcoal), and excess of concentrated hydrochloric acid added. The phenanthridine hydrochloride was collected after cooling the solution, washed with 2N-hydrochloric acid, and treated with ammonia to give the *base* (2·45 g.), m. p. 128—129° (Found: N, 5·2. $C_{14}H_{10}NBr$ requires N, 5·15%).

2-*Acetamido*-5-*cyanodiphenyl.*—5-Bromo-2-acetamidodiphenyl (20 g.) was heated with cuprous cyanide (8·0 g.) and pyridine (8·0 c.c.) at 170—200° for 3 hours. The melt was poured into a solution of potassium cyanide (20 g.) in water (100 c.c.), and the mixture warmed on the steam-bath and allowed to stand for 1 hour. The solid was filtered off and crystallised from alcohol (30 c.c.; charcoal) to give a crude product (10·85 g.) which still contained copper. Further treatment with warm 20% potassium cyanide solution followed by recrystallisation from alcohol gave the pure *cyano*-compound (9·12 g.), m. p. 129—130° (mixed m. p. with bromo-compound 102—104°) (Found: N, 11·8. $C_{15}H_{12}ON_2$ requires N, 11·9%).

3-*Cyano*-9-*methylphenanthridine.*—The acetamidocyano-compound (3·0 g.) and phosphorus oxychloride (7·5 g.) were boiled gently under reflux for 30 mins. Removal of the oxychloride gave a gum which was kept in contact with water overnight. Solution in pyridine (50 c.c.) followed by precipitation with 0·5N-sodium hydroxide solution (300 c.c.) gave a granular precipitate which was filtered off, washed, dried, and sublimed at 0·5 mm. to give the crude *phenanthridine* (0·85 g., 31%). Recrystallisation from alcohol (75 c.c.) gave the pure compound (0·62 g.), m. p. 202—203·5° (Found: N, 12·8. $C_{15}H_{10}N_2$ requires N, 12·85%). The yield of crude material could probably be improved by the use of nitrobenzene in the reaction mixture as in the later experiments of this series (cf. Walls, *loc. cit.*, and B.P. 520,273).

The same product was obtained by treatment of 3-bromo-9-methylphenanthridine with cuprous cyanide in pyridine, but it could not be freed from halogen.

3-*Amidino*-9-*methylphenanthridine*.—3-Cyano-9-methylphenanthridine (2·5 g.) was dissolved in dry chloroform (75 c.c.) and dry alcohol (1·8 c.c.). After saturation with dry hydrogen chloride at 0°, the mixture was kept at 15° for 16 days. The solid was filtered off, washed with chloroform and ether, and dried in a vacuum to give the crude iminoether hydrochloride (3·57 g.). Treatment with concentrated alcoholic ammonia (75 c.c.), and crystallisation of the residue from hot water (175 c.c.) by addition of excess concentrated hydrochloric acid, gave the *amidine dihydrochloride* (3·05 g.) as fine needles. Drying of this heavily hydrated form was effected by boiling with dry alcohol, the dihydrate then being formed (Found: N, 12·2; Cl, 19·9. $C_{15}H_{13}N_3,2HCl,2H_2O$ requires N, 12·2; Cl, 20·7%).

2-p-*Bromobenzamidodiphenyl*.—2-Aminodiphenyl (17·0 g.) was dissolved with *p*-bromobenzoyl chloride (24·0 g.) in dry chlorobenzene (150 c.c.) and heated under reflux for 1 hour. A clear solution rapidly formed and evolution of hydrogen chloride was complete after about 45 mins. The solution was filtered while still hot (charcoal) and 50 c.c. of chlorobenzene were removed by distillation. On slow cooling (rapid cooling produced gels) stellate clusters of the crude product separated (21·3 g.; 61%). Addition of light petroleum to the filtrate gave a further crop (9·2 g.; 26%). Recrystallisation from light petroleum (b. p. 80—100°) gave the pure *amide*, m. p. 128—129°, in colourless needles (Found: N, 4·0; Br, 22·6. $C_{19}H_{14}ONBr$ requires N, 3·98; Br, 22·7%).

5-*Bromo*-2-p-*bromobenzamidodiphenyl*.—5-Bromo-2-aminodiphenyl (12·5 g.) (Scarborough and Waters, *loc. cit.*) in pyridine (20 c.c.) was treated with *p*-bromobenzoyl chloride (11 g.) and warmed to complete solution. After standing overnight the whole was poured into excess of ice-cold dilute hydrochloric acid, and the solid filtered off, washed, and recrystallised from alcohol to give the *dibromo*-compound, m. p. 161° (16·5 g.). The same product was obtained in 80% yield (pure) by aroylation in boiling chlorobenzene solution and in 71% yield by direct bromination of *p*-bromobenzamidodiphenyl under conditions similar to those used for bromination of the acetamido-compound (Found: N, 3·3. $C_{19}H_{13}ONBr_2$ requires N, 3·25%).

3-*Bromo*-9-p-*bromophenylphenanthridine*.—The above amide (15 g.) in nitrobenzene (50 c.c.) and phosphorus oxychloride (15 c.c.) were heated under reflux for 2 hours. Treatment with ice and water, followed by removal of the nitrobenzene in steam, gave a quantitative yield of the *phenanthridine*, m. p., after crystallisation from alcohol, 234—235°. Omission of the nitrobenzene led to a decreased yield of material (Found: N, 3·6; Br, 38·2. $C_{19}H_{11}NBr_2$ requires N, 3·4; Br, 38·7%).

3-*Cyano*-9-p-*cyanophenylphenanthridine*.—The foregoing dibromo-compound (10 g.) and cuprous cyanide (4·35 g.) were intimately mixed and fed slowly (15 mins.) into boiling quinoline (40 c.c.). Refluxing was then continued for 30 mins., and the mixture was then cooled, treated with dilute hydrochloric acid, and the solid filtered off, washed, and dried. Sublimation at 280—320°/2 mm. gave a crude product (5·8 g.) which recrystallised from pyridine or nitrobenzene to give the pure *cyanide* (3·5 g.), m. p. 340° (Found: N, 13·4. $C_{21}H_{11}N_3$ requires N, 13·75%).

3-*Amidino*-9-p-*amidinophenylphenanthridine*.—The dicyanide (5 g.) was finely ground (200 mesh) and suspended in dry chloroform (100 c.c.) and dry alcohol (5 c.c.). Saturation with dry hydrogen chloride was carried out at 0°, and after standing for 8 days the crude iminoether base, m. p. 150—153°, was isolated in the usual manner.

The base (4·9 g.) was dissolved in alcohol (130 c.c.), and a solution of ammonium chloride (1·32 g.) in water (5 c.c.) added. The whole was kept at 70° for 4 hours and deposition of the *amidine dihydrochloride*, which began after 1 hour, was completed by addition of cold acetone. The solid (4·0 g.) was filtered off and dried, but purification was rendered difficult by its tendency towards gel formation with solvents. A solution in hot water rapidly set to a loose gel which was reversed only on boiling, and the forms obtained by precipitation with acetone from methanolic or acidic aqueous solution appeared to be amorphous. Purification was finally effected by careful precipitation of an aqueous gel with acetone. The solubility of the salt was approximately 1% at 23° (Found: N, 15·5; Cl, 15·8. $C_{21}H_{17}N_5,2HCl,2H_2O$ requires N, 15·6; Cl, 15·9%). Only one molecule of water was lost by drying in a vacuum at 100° and as the resulting monohydrate combined with atmospheric moisture during weighing, it was impossible to obtain accurate loss in weight figures.

7-*Amino*-9-p-*aminophenylphenanthridine*.—A solution of stannous chloride (360 g.) in hydrochloric acid (360 c.c., *d* 1·16), heated to about 70°, was added all at once to a boiling suspension of 7-nitro-9-*p*-nitrophenylphenanthridine (50 g.) in glacial acetic acid (350 c.c.). When the vigorous reaction had subsided, the mixture was refluxed for 75 mins. and allowed to cool. The stannichloride was filtered off, dissolved in the least quantity of water, and the ice-cooled solution made alkaline to phenolphthalein with 50% sodium hydroxide. The amine thus liberated was dissolved in a small volume of dioxan, the solution filtered, and the amine reprecipitated by adding a large volume of water; it was collected, washed with water, dried at 100°, and crystallised from *n*-butyl alcohol to give yellow needles, m. p. 208—210° (20·5 g., 50%) (Found: N, 14·5. Calc. for $C_{19}H_{15}N_3$: N, 14·7%).

7-*Cyano*-9-p-*cyanophenylphenanthridine*.—A suspension of the foregoing amine (20 g.) in hydrochloric acid (140 c.c., *d* 1·16) and water (200 c.c.) was cooled in ice and mechanically stirred. Sodium nitrite (10·3 g. in 40 c.c. of water) was added at 0—5°, and the solution then neutralised with sodium carbonate. The diazonium solution was added to potassium cuprocyanide solution (15·7 g. CuCN; 35·2 g. KCN; 210 c.c. H_2O) at 60°, and the resulting mixture heated on the steam-bath for 2 hours. The solid was separated, washed well with water, and dried at 100°. It was then sublimed at 300°/0·1 mm., the *cyanide* being obtained as a pale yellow, crystalline sublimate (6·9 g.). It crystallised from pyridine in pale yellow, matted needles, m. p. 322—323° (4·5 g., 21%) (Found: C, 82·2; H, 3·7; N, 13·4. $C_{21}H_{11}N_3$ requires C, 82·5; H, 3·6; N, 13·8%).

7-*Amidino*-9-p-*amidinophenylphenanthridine*.—The foregoing dicyanide (2 g.) in lime-dried alcohol (15 c.c.) was saturated with hydrogen chloride and left sealed at 0° for 12 days. The iminoether base, m. p. 147—148° (1·53 g., 55%), isolated in the usual manner, was used for the preparation of the amidine without further purification. It (0·86 g., 1 mol.) was heated with ammonium chloride (0·26 g., 2·2 mols.)

in aqueous alcohol (1 c.c., 4 c.c.) at 60—65° for 4 hours. Precipitation with acetone and crystallisation (0·72 g.) from ethyl alcohol gave prismatic needles, m. p. 282° (decomp.) (0·3 g.), which were a mixture of di- and tri-hydrochlorides (Found : N, 14·4; Cl, 19·2%; N : Cl = 5 : 2·6). This solid, on treatment with 4N-hydrochloric acid, was converted into the *trihydrochloride*, m. p. 294—295° (decomp.) (Found : N, 13·8; Cl, 20·8; N : Cl = 5 : 3. $C_{12}H_{17}N_5,3HCl,3H_2O$ requires N, 13·9; Cl, 21·2%).

7-*Cyano*-9-p-*cyanophenyl*-10-*methylphenanthridinium Chloride*.—Methyl sulphate (2·8 c.c.) was added to a solution of 7-cyano-9-*p*-cyanophenylphenanthridine (5 g.) in nitrobenzene (35 c.c.) at 180°. The red solution was kept at 200° for 15 mins., and the nitrobenzene removed by steam-distillation. The resultant aqueous solution (200 c.c.) was filtered hot, and ammonium chloride (45 g.) added to the hot filtrate. The solid which separated was filtered off and crystallised from aqueous methanol (7 c.c.; 70 c.c.). The *chloride* crystallised in pale yellow needles, m. p. 314—315° (decomp.) (2·8 g., 48%) (Found : N, 11·4, 11·0, 11·1; Cl, 10·8, 10·3, 10·3. $C_{22}H_{14}N_3Cl$ requires N, 11·8; Cl, 10·0%).

The *picrate* was prepared from the chloride in aqueous solution, and crystallised twice from acetone to give flat needles, m. p. 218—219° (decomp.) (Found : C, 62·0; H, 2·3; N, 15·2, 15·35. $C_{28}H_{16}O_7N_6$ requires C, 61·5; H, 2·9; N, 15·4%).

The *bromide* was prepared exactly as above, ammonium bromide being substituted for the chloride. The solid which separated was filtered off, washed with a little water, and crystallised from aqueous alcohol (150 c.c.; 100 c.c.) to give the bromide, m. p. 304—306° (decomp.) (3·4 g., 60%) (Found : N, 10·3, 10·4; Br, 21·0, 21·6. $C_{22}H_{14}N_3Br$ requires N, 10·5; Br, 20·0%). It was converted into the above chloride as follows. To silver chloride (prepared from 2·15 g. of nitrate, and well washed by decantation with water and methanol) were added the finely ground bromide (2·15 g.) and methanol (40 c.c.), and the mixture was refluxed for 1 hour, filtered hot, and the filtrate evaporated to dryness in a vacuum to give the chloride (1·7 g., 89%). This solid, together with that from a similar experiment (2·44 g. in all), crystallised from 2N-hydrochloric acid (110 c.c.) in almost colourless needles, m. p. 310—311° (1·84 g., 75% return) (Found : N, 11·1; Cl, 10·3%).

7-*Amidino*-9-p-*amidinophenyl*-10-*methylphenanthridinium Chloride*.—A suspension of 7-cyano-9-*p*-cyanophenyl-10-methylphenanthridinium chloride (0·4 g.) in lime-dried alcohol (7 c.c.) was saturated with dry hydrogen chloride below 0°, and then left at 0° for two weeks. The excess alcohol was then removed in a vacuum at 35—40°, leaving the di-iminoether dihydrochloride as a pale yellow solid.

The *diamidine dihydrochloride*, m. p. 270—272° (decomp.), obtained by treatment of the above hydrochloride with alcoholic ammonia, was washed with acetone and dried in a vacuum (0·36 g., 56%) (Found : N, 12·3; Cl, 18·9; N : Cl = 5·0 : 3·0. $C_{22}H_{20}N_5Cl,2HCl,6H_2O$ requires N, 12·3; Cl, 18·7%).

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