653. *Some Potential Trypanocidal and Antibacterial Compounds in the Heterocyclic Series. Part II.*

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Diamidine salts in the 2-phenyl-benzoxazole, -benziminazole, and -quinoline series and a monoamidino-2 : 3-diphenylquinoxaline have been prepared. The trypanocidal activities of these compounds are described.

The only compound showing significant trypanocidal action among those described in Part I (preceding paper) was 6-amidino-2-*p*-amidinophenylbenzthiazole and we considered it of interest to prepare, among other compounds, the corresponding benzoxazole and benziminazole derivatives. It was not expected *a priori* that the diamidino-2-phenylbenzoxazoles would show markedly different action from the equivalent benzthiazoles, but in the case of the benziminazoles it seemed possible that the existence of the mesohydric tautomeric system (Hunter, *J.*, 1945, 806) might considerably increase the activity (cf. Schönhöfer, *Z. physiol. Chem.*, 1942, **274**, 1, regarding antimalarial drugs).

Synthetical methods for the extension of the work reported in Part I into the benzoxazole and benziminazole series have already been reported (Stephens and Bower, *J.*, 1950, 1722), and 5-cyano-2-*p*-cyanophenylbenzoxazole (*idem*, *ibid.*) was converted into the corresponding diamidine by the usual Pinner method. On reaction with cuprous cyanide, 6-bromo-2-*p*-cyanophenylbenzoxazole (*idem*, *bid.*) was easily transformed into 6-cyano-2-*p*-cyanophenylbenzoxazole, and the diamidine prepared therefrom. Aqueous solutions of salts prepared from these two diamidines were unstable, being decomposed with precipitation of solid on boiling, and the preparation of analytically pure salts was difficult.

5(6)-Cyano-2-*p*-cyanophenylbenziminazole (*idem*, *ibid.*) was easily converted into the diamidine, and this, as the dihydrochloride, was found to be one of the most stable substances of the series (aqueous solutions could be boiled apparently indefinitely without decomposition and the compound was easily recrystallisable from water). The corresponding diamidrazone was prepared as described in the benzthiazole series (Part I), and so was the 2-*m*-amidinophenyl isomer. For the latter purpose *m*-cyanobenzaldehyde was required, and attempts to prepare this compound by Slotta and Kethur's method (*Ber.*, 1938, **71**, 59) gave a product contaminated with 3-chlorobenzaldehyde (indeed, this is to be expected; cf. *Org. Synth.*, Coll. Vol. II, p. 132). The aldehyde was prepared in poor yield by Reinglass's method (*Ber.*, 1891, **24**, 2416), and it reacted with 4-cyano-*o*-phenylenediamine to give a Schiff's base (compare the behaviour of *p*-cyanobenzaldehyde; Stephens and Bower, *loc. cit.*), which was oxidised by lead tetra-acetate to produce 5(6)-cyano-2-*m*-cyanophenylbenziminazole.

5(6)-Amidino-2-*p*-amidinophenylbenziminazole dihydrochloride was considerably more active as a trypanocide than any of the diamidines in the benzthiazole or benzoxazole series and it appeared of interest to attempt to decide if this was due to the tautomeric iminazole system. For this purpose the nuclear imino- was replaced by the methylimino-group, 5-cyano- and 6-cyano-1-methyl-2-*p*-cyanophenylbenziminazole being prepared and converted into diamidines. Direct methylation of 5(6)-cyano-2-*p*-cyanophenylbenziminazole, which would probably give rise to a mixture of the isomers, was attempted by means of (i) methyl sulphate, (ii) formic acid and formaldehyde, and (iii) methyl iodide with sodamide in liquid ammonia. As these methods failed, the two dinitriles were synthesized separately. Catalytic reduction of 4-methylamino-3-nitrobenzonitrile (Mattaar, *Rec. Trav. chim.*, 1922, **41**, 24) to 3-amino-4-methylaminobenzonitrile, followed by condensation with *p*-cyanobenzaldehyde, produced 5-cyano-2-*p*-cyanophenyl-1-methylbenziminazole. Attempts to prepare 3-methylamino-4-nitrobenzonitrile from 3-bromo-4-nitrobenzonitrile by reaction with methylamine in a sealed tube, as in the case of the isomer (Mattaar, *loc. cit.*), gave only very poor yields of the desired compound. The bromo-compound was recovered unchanged after it had been heated with methylamine hydrochloride, a method used by Phillips (*J.*, 1930, 2400) for the preparation of 3-methylamino-4-nitrophenylarsonic acid. This replacement was effected in moderate yield by heating the bromo-compound with methylamine hydrochloride and sodium hydrogen carbonate in pyridine (Campbell, *J. Amer. Chem. Soc.*, 1949, **71**, 740). Catalytic reduction of 3-methylamino-4-nitrobenzonitrile gave 4-amino-3-methylaminobenzonitrile, which was condensed with *p*-cyanobenzaldehyde to produce 6-cyano-2-*p*-cyanophenyl-1-methylbenziminazole.

An obvious extension of this work was the preparation of 6-amidino-2-*p*-amidinophenylquinoline. Pfitzinger reaction between 5-bromoisatin and 4-bromoacetophenone (cf.

Lindwall, Bandes, and Weinberg, *J. Amer. Chem. Soc.*, 1931, **53**, 317) produced 6-bromo-2-*p*-bromophenylcinchoninic acid which, by reaction with cuprous cyanide, was converted into 6-cyano-2-*p*-cyanophenylquinoline and thence into the required diamidine.

A monoamidine of the quinoxaline series, 6-amidino-2 : 3-di-*p*-aminophenylquinoxaline, was obtained by condensation of 4-cyano-*o*-phenylenediamine with 4 : 4′-diacetamidobenzil (Gee and Harley-Mason, *J.*, 1947, 251) and thence by conventional reactions. The reason for preparing this compound was that several trypanocidal drugs (*e.g.*, the phenanthridinium type) have for their broad structural plan two aromatic amino-groups disposed about a central quaternary nitrogen atom, carrying a positive charge, and we were interested to see if activity could be obtained when this charge was associated with the amidine group rather than with nitrogen.

2-Guanidino-5(6)-nitrobenziminazole hydrochloride was prepared for antibacterial examination.

Trypanocidal Action.—The activities of some of the compounds described in this and the preceding paper are summarised below. The tests were carried out on mice by Mr. J. Kershaw, to whom we are indebted for the following data.

Compound.	*T. equiperdum.* I.P.	*T. equiperdum.* S.C.	*T. rhodesiense.* I.P.	*T. rhodesiense.* S.C.	*T. congolense.* I.P.	*T. congolense.* S.C.
6-Amidino-2-*p*-amidinophenylbenzthiazole di-isethionate	—	3	—	2	—	2
6-Amidino-2-*m*-amidinophenylbenzthiazole dihydrochloride	—	1	—	1	—	1
6-Amino-2-*m*-aminophenylbenzthiazole methochloride hydrochloride	0	—	0	—	1	—
6-Amidino-2-*p*-amidinophenylbenzoxazole dihydrochloride	1	—	2	—	1	—
5(6)-Amidino-2-*p*-amidinophenylbenziminazole dihydrochloride	—	4	4	5 *	—	2
5(6)-Amidrazono-2-*p*-amidrazonophenylbenziminazole dihydrochloride	1	—	1	—	0	—
5(6)-Amidino-2-*m*-amidinophenylbenziminazole dihydrochloride	1	—	1	—	1	—
5-Amidino-2-*p*-amidinophenyl-1-methylbenziminazole dihydrochloride	1	—	2	—	2	—
6-Amidino-2-*p*-amidinophenyl-1-methylbenziminazole dihydrochloride	1—2	—	2	—	1—2	—
6-Amidino-2-*p*-amidinophenylquinoline di-isethionate	2	—	4	—	1	—
6-Amidino-2 : 3-di-*p*-aminophenylquinoxaline isethionate	0	—	0	—	0	—

I.P. = Intraperitoneal. S.C. = Subcutaneous.

0 = No activity. 1 = Life of animal prolonged for less than one month when highest possible single dose administered. 2 = Therapeutic index <5. 3 = Index 5—10. 4 = Index 10—20. 5 = Index 30—40.

* Toxicity L.D.50 (mg./g.) = 0·16. Curative activity C.D.50 (mg./g.) = 0·004.

It would appear from these results that the superior trypanocidal activity of 6-amidino-2-*p*-amidinophenylbenziminazole is related to the tautomeric iminazole system.

Experimental.

6-*Cyano*-2-p-*cyanophenylbenzoxazole*.—6-Bromo-2-*p*-cyanophenylbenzoxazole (2·5 g.) and cuprous cyanide (0·8 g.) were intimately mixed and added to boiling quinoline (10 ml.). The mixture was boiled under reflux for 35 minutes, then cooled, the quinoline dissolved in 2N-hydrochloric acid, and the crude *dinitrile* filtered off. After being washed and dried, it was vacuum-sublimed, and the sublimate recrystallised from benzonitrile to give white needles (1·62 g.; m. p. 245°) (Found : C, 73·8; H, 3·0; N, 16·8. $C_{15}H_7ON_3$ requires C, 73·5; H, 2·9; N, 17·1%).

5(6)-*Cyano*-2-m-*cyanophenylbenziminazole*.—*m*-Cyanobenzaldehyde (1·5 g.; m. p. 80—81°) and 4-cyano-*o*-phenylenediamine (1·5 g.) were boiled together in glacial acetic acid (15 ml.) for 5 minutes. Addition of water precipitated a bright yellow solid (2·1 g.) which, after being dried, was oxidised with lead tetra-acetate (3·8 g.) in glacial acetic acid (20 ml.). The *dinitrile* was isolated by dilution, and recrystallised from benzonitrile in fawn needles (1 g.; m. p. 255—256°) (Found : N, 22·2. $C_{15}H_8N_4$ requires N, 22·9%).

3-*Amino*-4-*methylaminobenzonitrile*.—4-Methylamino-3-nitrobenzonitrile (5 g.) was suspended in ethanol (100 ml.) and reduced by hydrogen in the presence of a platinum–charcoal catalyst (9% of platinum). Evaporation to dryness of the catalyst-free solution, followed by extraction of the resulting solid with water, gave a very pale fawn solid (3·5 g.; m. p. 140—141°) which recrystallised from water as slender white needles (m. p. 140—141°) (Found : C, 65·7; H, 6·3. $C_8H_9N_3$ requires C, 65·3; H, 6·1%).

5-*Cyano*-2-p-*cyanophenyl*-1-*methylbenziminazole*.—The foregoing *nitrile* (2·0 g.) and *p*-cyanobenzaldehyde (1·8 g.) were added to glacial acetic acid (20 ml.). The solution was boiled for 5 minutes, cooled, and poured into water, and the *dinitrile* (2·2 g.) collected, dried, and recrystallised from nitrobenzene, giving fawn needles (1·3 g.; m. p. 263·5—264·5°) (Found : C, 73·9; H, 4·3. $C_{16}H_{10}N_4$ requires C, 74·4; H, 3·9%).

3-*Bromo*-4-*nitrobenzonitrile*.—3-Bromo-4-nitroaniline (18 g.), sulphuric acid (36 ml.; *d* 1·84), and water (36 ml.) were heated until the solid dissolved, diluted with water (240 ml.), and cooled. The precipitated base was diazotised by addition of sodium nitrite (6 g.) in water (30 ml.) at 0°, after which the cooling bath was removed and the mixture allowed to reach room temperature (with stirring) during 2 hours. The diazo-solution was filtered and added to sodium cyanide (80 g.) and cuprous cyanide

(40 g.) in water (800 ml.) at 10°. The resultant mixture was heated to 90° in a steam-bath and cooled, and the product filtered off. Recrystallisation from aqueous alcohol gave yellow needles (10 g.; m. p. 104°) (Found : N, 12·1. Calc. for $C_7H_3O_2N_2Br$: N, 12·3%).

3-Methylamino-4-nitrobenzonitrile.—3-Bromo-4-nitrobenzonitrile (10 g.) and sodium hydrogen carbonate (10·6 g.) were added to pyridine (64 ml.), and to the mixture was added methylamine hydrochloride (5·3 g.) dissolved in the minimum quantity of hot water. The mixture was boiled under reflux for 10 hours and filtered hot, and the residue washed with acetone. By cooling the acetone–pyridine filtrate and dilution of it with water, the required *nitrile* (3·75 g.; m. p. 213—214°) was isolated as bright red needles (Found : N, 23·2. $C_8H_7O_2N_3$ requires N, 23·7%).

4-Amino-3-methylaminobenzonitrile.—3-Methylamino-4-nitrobenzonitrile (1·75 g.) was reduced in ethanol (60 ml.) by hydrogen in the presence of a 10% platinum–charcoal catalyst to give white plates (1·35 g.; m. p. 129—130°) from water (Found : N, 29·3. $C_8H_9N_3$ requires N, 28·6%).

Amidine.	Salt analysed.	Crystn. solvent.	M. p.	Analysis. Found, %. N.	Cl.	Required, %. N.	Cl.
5-*Amidino*-2-p-*amidinophenylbenzoxazole*	$C_{15}H_{13}ON_5,2C_7H_7{\cdot}SO_3H(p)$	I.M.S.*	306°	11·1	—	11·2	—
6-*Amidino*-2-p-*amidinophenylbenzoxazole*	$C_{15}H_{13}ON_5,2HCl,3H_2O$	Dil. HCl	328	17·1	—	17·2	—
6-*Amidino*-2-p-*amidinophenylbenzoxazole*	$C_{15}H_{13}ON_5,2C_6H_5{\cdot}SO_3H$	H_2O	272—273	11·6	—	11·8	—
5(6)-*Amidino*-2-p-*amidinophenylbenziminazole* [1]	$C_{15}H_{14}N_6,2HCl,5H_2O$	H_2O	352	18·9	16·6	19·0	16·1
5(6)-*Amidrazono*-2-p-*amidrazonophenylbenziminazole* [2]	$C_{15}H_{16}N_8,2HCl,4H_2O$	H_2O	> 360	25·5	15·8	24·7	15·7
5(6)-*Amidino*-2-m-*amidinophenylbenziminazole* [3]	$C_{15}H_{14}N_6,2HCl,4H_2O$	Dil. HCl	305 (decomp.)	20·2	—	19·9	—
5-*Amidino*-2-p-*amidinophenyl*-1-*methylbenziminazole* [4]	$C_{16}H_{16}N_6,2HCl,4H_2O$	Dil. HCl	305—306	19·1	—	19·2	—
6-*Amidino*-2-p-*amidinophenyl*-1-*methylbenziminazole* [5]	$C_{16}H_{16}N_6,2HCl,2H_2O$	H_2O	315—317	20·6	—	20·9	—
6-*Amidino*-2-p-*amidinophenylquinoline*	$C_{17}H_{15}N_5,2C_7H_7{\cdot}SO_3H(p),2H_2O$	H_2O	304	10·7	—	10·5	—
2 : 3-*Di*-p-*acetamidophenyl*-6-*amidinoquinoxaline*	$C_{25}H_{22}O_2N_6,HCl,3H_2O$	H_2O	255	16·4	6·8	15·9	6·7
	$C_{25}H_{22}O_2N_6,HCl$	—	254	17·7	7·36	17·7	7·48
6-*Amidino*-2 : 3-*di*-p-*aminophenylquinoxaline*	$C_{21}H_{18}N_6,2H_2O$ †	—	190	21·1	—	21·5	—

* Industrial methylated spirit. † Free amidine-base.

Ultra-violet absorption of aqueous solutions of salts showed maxima as follows :
[1] 3130 A. (ε = 33,080); 2580 A. (ε = 29,350). [2] 3150 A. (ε = 34,000). [3] 3080 A. (ε = 27,500).
[4] 3000 A. (ε = 19,800); 2530 A. (ε = 26,660). [5] 3000 A. (ε = 25,260).

6-Cyano-2-p-*cyanophenyl*-1-*methylbenziminazole.*—The foregoing *nitrile* (1·35 g.), *p*-cyanobenzaldehyde (1·2 g.), and glacial acetic acid (20 ml.) were boiled together for 5 minutes. Dilution with water gave a gum which on being stirred rapidly changed to a solid and on recrystallisation from ethanol gave the *dinitrile* (2·12 g.; m. p. 241—242°) as white needles (Found : N, 21·9. $C_{16}H_{10}N_4$ requires N, 21·7%).

6-Bromo-2-p-*bromophenylcinchoninic Acid.*—5-Bromoisatin (23·2 g.), *p*-bromoacetophenone (23·2 g.), ethanol (125 ml.), and aqueous potassium hydroxide solution (62 ml., 33%) were boiled together under reflux for 10 hours, after which the mixture was steam-distilled to remove unchanged bromoacetophenone. After cooling, the solid was filtered off, suspended in water, and acidified with hydrochloric acid. The precipitate was collected and washed first with water, then alcohol, and finally acetone (to remove bromoisatin), and dissolved in 2N-sodium hydroxide solution. Filtration and acidification of the filtrate with sulphuric acid gave the pale yellow *acid* (30 g.; m. p. 279—280°). Recrystallisation from acetic acid gave pale yellow needles (m. p. 279—280°) (Found : N, 3·24; Br, 37%; Br : N = 2 : 1. $C_{16}H_9O_2NBr_2,\frac{1}{2}CH_3{\cdot}CO_2H$ requires N, 3·2; Br, 36·6%).

6-Cyano-2-p-*cyanophenylquinoline.*—This *compound* was obtained in poor yield by treating the above dibromo-acid with cuprous cyanide and quinoline (*a*) as described under 6-cyano-2-*p*-cyanophenylbenzoxazole and (*b*) by the method of U.S.P. 2,195,076. Crystallisation from acetic acid and vacuum sublimation gave colourless needles (m. p. 290°) (Found : C, 80·4; H, 3·5. $C_{17}H_9N_3$ requires C, 80·0; H, 3·5%).

2 : 3-*Di*-p-*acetamidophenyl*-6-*cyanoquinoxaline.*—4-Cyano-*o*-phenylenediamine (1·5 g.) and 4 : 4′-diacetamidobenzil (3·6 g.) were heated together in boiling glacial acetic acid (50 ml.) for 15 minutes, and the crude *quinoxaline* (3·8 g.) isolated by dilution with water. Recrystallisation from 50% acetic acid gave pale yellow needles (m. p. 198—199°) (Found : N, 16·3. $C_{25}H_{19}O_2N_5$ requires N, 16·6%). Hydrolysis with 10% sulphuric acid gave 2 : 3-*di*-p-*aminophenyl*-6-*cyanoquinoxaline*, orange needles, m. p. 280—282° (from aqueous dioxan) (Found : N, 20·5. $C_{21}H_{15}N_5$ requires N, 20·8%).

Amidines and Amidrazone.—The salts and the amidine and amidrazone detailed in the table were prepared by the general Pinner method as described in Part I (preceding paper), nitrobenzene, dioxan, or ethanol being used as solvents for the preparation of the imino-ethers. All the amidines gave a positive Fuller reaction (*Nature*, 1944, **154**, 773), and the amidrazone gave a bright yellow precipitate in this test (as did the amidrazone prepared in Part I). The amidine hydrochlorides appeared considerably to reduce the surface tension of water, in which they were very soluble.

2-*Guanidino*-5(6)-*nitrobenziminazole Hydrochloride.*—This *compound* was obtained from 4-nitro-*o*-phenylenediamine by the method described in Part I as yellow-brown needles (m. p. 197—198°) from dilute hydrochloric acid (Found : N, 31·3; Cl, 13·1. $C_8H_9O_2N_6Cl,\frac{1}{2}H_2O$ requires N, 31·64; Cl, 13·4%).

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