603. *The Search for Chemotherapeutic Amidines. Part XIV.* Congeners of 4 : 4′-Diamidinoazobenzene.*

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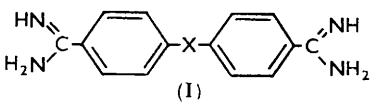
4 : 4′-Diamidino-azoxybenzene, -hydrazobenzene, and -diazo-*N*-methylaminobenzene have been prepared. Only the last diamidine is active against *Trypanosoma congolense* but it is less active than the demethyl analogue 4 : 4′-diamidinodiazoaminobenzene (" Berenil," " Babesin ").

Although 4 : 4′-diamidinodiphenylamine (I*a*) and 4 : 4′-diamidinoazobenzene (I*b*) were described by Ashley *et al.*[1] in 1942, no other diamidines of type (I) (where X is a substituted or unsubstituted imino-group, or a chain of two or more nitrogen atoms, or a cyclic nitrogenous system) have been reported except 4 : 4′-diamidinodiazoaminobenzene[2] (I*c*) which is active against *Trypanosoma congolense* and other species of trypanosome as well as against Babesia. During our investigations, extending over several years, we have examined the effect on trypanocidal activity of varying the linkage X within the types mentioned above. In the present paper we describe the diamidines (I*d*, *e*, and *f*).



(I)

(*a*) X = ·NH·
(*b*) X = ·N:N·
(*c*) X = ·N:N·NH·

(*d*) X = ·N:N·(O)
(*e*) X = ·NH·NH·
(*f*) X = ·N:N·NMe·

4 : 4′-Dicyanoazobenzene, first prepared [1] from the diamine by the Sandmeyer reaction, is better obtained by condensation of *p*-aminobenzonitrile with *p*-nitrosobenzonitrile. Oxidation of *p*-aminobenzonitrile with Caro's acid [3] by a method based on that for the oxidation of *p*-nitroaniline [4] gave a mixture of *p*-nitrosobenzonitrile and 4 : 4′-dicyanoazoxybenzene which were separated by taking advantage of the volatility of the nitroso-compound in steam.

4 : 4′-Dicyanoazoxybenzene was readily converted into the diamidine by Pinner's method and the hydrazino-analogue was obtained by catalytic reduction of 4 : 4′-diamidinoazobenzene dihydrochloride in aqueous solution. The azoxy- and the hydrazino-analogue, like the azo-compound, were inactive against *T. congolense* infections in mice.

An attempt to convert *p*-nitrosobenzonitrile into the amidine gave an unidentified non-basic product.

* Part XIII, *J.*, 1957, 1668.

[1] Ashley, Barber, Ewins, Newbery, and Self, *J.*, 1942, 103.
[2] Milne, Robson, and Lwebandiza, *Vet. Rec.*, 1955, **67**, 280; Jensch, *Arzneimittel Forsch.*, 1955, **5**, 634; B.P. 728,457/1955.
[3] Vallance, *J. Soc. Chem. Ind.*, 1926, **45**, 66.
[4] McIntyre and Simpson, *J.*, 1952, 2609.

The trypanocidal activity of Stilbamidine (4 : 4′-diamidinostilbene; I; X = ·CH:CH·) [1] against *T. congolense* is increased when the hydrogen atoms in the chain are replaced by methyl groups to give (I; X = ·CMe:CMe·),[5] and, although 4 : 4′-diamidinohydrazobenzene is inactive, it was considered worth trying to make its dimethyl derivative (I; X = ·NMe·NMe·). The corresponding dinitrile was obtained by methylation of 4 : 4′-dicyanohydrazobenzene, but could not be converted into the diamidine. The first attempt to prepare 4 : 4′-dicyano-*NN*′-dimethylhydrazobenzene started from *p*-cyano-*N*-methyl-*N*-nitrosoaniline [6] which was obtained both by nitrosation of *p*-cyano-*N*-methylaniline and by nitrosation (with concomitant loss of a methyl group) of *p*-cyanodimethylaniline using a modification of the method of Sachs and Steinert.[6] Reduction of this *N*-nitroso-compound gave *N*-*p*-cyanophenyl-*N*-methylhydrazine but attempts to oxidise this to 1 : 4-di-*p*-cyanophenyl-1 : 4-dimethyltetrazen by yellow mercuric oxide in ether as used by Fischer [7] for *N*-methyl-*N*-phenylhydrazine caused fission with formation of *p*-cyano-*N*-methylaniline. Oxidation with ferric chloride in hydrochloric acid at 35° decomposed the compound, whilst oxidation at 0—2° gave an unidentified product. *p*-Cyanophenyl-*N*-methyl-*N*-nitrosoaniline did not condense with *N*-*p*-cyanophenyl-*N*-methylhydrazine, and these approaches to 4 : 4′-dicyano-*NN*′-dimethylhydrazobenzene, which might have been obtained by loss of nitrogen from the linear tetrazen,[8] were abandoned. The *p*-cyanodimethylaniline required for these reactions was prepared from *p*-dimethylaminobenzaldoxime, and the *p*-cyano-*N*-methylaniline was obtained by two processes: (*a*) Bromination of *N*-methylacetanilide in acetic acid furnished the *p*-bromo-derivative. This reaction was briefly described by Chowdhury *et al.*[9] without reference to the intermediate formation of a perbromo-derivative. The *p*-bromo- was converted into the *p*-cyano-derivative by treatment with cuprous cyanide and pyridine, and subsequent removal of the acetyl group gave *p*-cyanomonomethylaniline. (*b*) *N*-Methyl-*p*-nitroacetanilide was catalytically reduced to the amine whence the Sandmeyer reaction with cuprous cyanide and subsequent hydrolysis gave *p*-cyanomonomethylaniline. Both 4 : 4′-dicyanoazobenzene and 4 : 4′-dicyanoazoxybenzene were readily reduced in presence of palladised charcoal to 4 : 4′-dicyanohydrazobenzene and this furnished 4 : 4′-dicyano-*NN*′-dimethylhydrazobenzene when boiled with methyl toluene-*p*-sulphonate and potassium carbonate in anisole. An attempt to methylate the hydrazino-linkage with methyl-lithium and methyl sulphate [10] gave a trace of the required dimethyl derivative and some 4 : 4′-dicyanoazobenzene. Attempted conversion of 4 : 4′-dicyano-*NN*′-dimethylhydrazobenzene into the diamidine by Pinner's method caused demethylation with formation of 4 : 4′-diamidinoazobenzene and an unidentified base.

4 : 4′-Diamidinodiazo-*N*-methylaminobenzene was readily prepared by the condensation of *p*-methylaminobenzamidine with *p*-amidinobenzenediazonium chloride. Although curative against *T. congolense* infection in mice, the presence of the methyl group in the nitrogen chain had a dystherapeutic effect and the chemotherapeutic ratio (LD_{50}/CD_{50}) was approximately a quarter of that of " Berenil."

Experimental

Oxidation of p-*Aminobenzonitrile with Caro's Acid.*—Powdered potassium persulphate (500 g.) was added in one portion to concentrated sulphuric acid (558 c.c.) and after being stirred for 1 hr. the mixture was added to ice (9 kg.) and adjusted to pH 5·0—5·5 with anhydrous potassium carbonate (*ca.* 1·5 kg.). The mixture was filtered, and the cold filtrate (*ca.* 9·6 l., containing

[5] Wien, *Brit. J. Pharmacol.*, 1946, **1**, 65; Barber, Slack, and Woolman, *J.*, 1943, 99.
[6] Sachs and Steinert, *Ber.*, 1904, **37**, 1740.
[7] Fischer, *Annalen*, 1878, **190**, 167.
[8] Wieland and Fressel, *ibid.*, 1912, **392**, 133.
[9] Chowdhury, Desai, and Hunter, *J. Indian Chem. Soc.*, 1933, **10**, 637.
[10] Wittig, " Neuere Methoden der preparativen organischen Chemie," 3rd Edn, Weinheim, 1949, Vol. I, 469.

90—93 g. of persulphuric acid) was stirred at 20° whilst *p*-aminobenzonitrile (50 g.) dissolved in dioxan (200 c.c.) was added gradually. The mixture was stirred at room temperature for 20 hr., then the light brown solid was filtered off and steam-distilled. The distillate (*ca.* 12 l.) furnished p-*nitrosobenzonitrile* (25 g., 44·5%) which crystallised from ethanol in yellow prismatic needles, m. p. 136—137° (Found: C, 63·6; H, 3·3; N, 21·2. $C_7H_4ON_2$ requires C, 63·6; H, 3·0; N, 21·2%). The non-volatile solid was crystallised from acetic acid and gave 4 : 4′-*dicyanoazoxybenzene* (19 g., 38%), brown needles, m. p. 229—230° (sinters at 218°) (Found: C, 67·4; H, 3·4; N, 22·4. $C_{14}H_8ON_4$ requires C, 67·7; H, 3·2; N, 22·6%).

4 : 4′-*Dicyanoazobenzene*.—*p*-Nitrosobenzonitrile (2·64 g.) and *p*-aminobenzonitrile (2·38 g.) in acetic acid (100 c.c.) were refluxed for 1 hr. The product (2·8 g., 60%) separated from the solution in red needles, m. p. 268—270°, identical with a specimen prepared originally from the diamine.[1]

4 : 4′-*Diamidinoazoxybenzene*.—This was prepared by the general method described by Ashley *et al.*[1] from the dinitrile (15 g.) in chloroform (400 c.c.)–ethanol (75 c.c.). The *diamidine dihydrochloride* (12·3 g.) separated from 2N-hydrochloric acid in brown needles, m. p. 282° (decomp.; sinters at 145°) (Found: N, 22·3; Cl, 19·0; H_2O, 5·0. $C_{14}H_{14}ON_6,2HCl,H_2O$ requires N, 22·5; Cl, 19·0; H_2O, 4·8%).

4 : 4′-*Diamidinohydrazobenzene*.—4 : 4′-Diamidinoazobenzene dihydrochloride [1] (10 g.) in water (200 c.c.) was reduced with a slightly positive pressure of hydrogen in presence of 10% palladised charcoal (2 g.). Reduction was complete in 1·5 hr. (380 c.c.) and after removal of the catalyst the colourless solution was treated with sodium chloride (20 g.). The *dihydrochloride* (10 g., 100%) separated in cream prisms which were filtered off, washed with 90% ethanol, and dried over sulphuric acid *in vacuo*; they had m. p. 294—296° (decomp.) (Found: C, 47·3; H, 5·8; Cl, 19·8; H_2O, 4·95. $C_{14}H_{16}N_6,2HCl,H_2O$ requires C, 46·8; H, 5·6; Cl, 19·8; H_2O, 5·0%). A 4% aqueous solution was stable in air for 2 hr.

Attempted Preparation of p-*Nitrosobenzamidine*.—A suspension of finely powdered *p*-nitrosobenzonitrile (5 g.) in chloroform (30 c.c.) and ethanol (5 c.c.) at 0—10° was saturated with hydrogen chloride and then kept at room temperature for 7 days. The crystalline solid (7 g.) was filtered off and added to saturated ethanolic ammonia (70 c.c.). The red suspension was kept at 55—60° for 6 hr. and, after being cooled, the yellow, non-basic *substance* was filtered off. It crystallised from acetic acid in yellow needles, m. p. 228—230° (decomp.) (Found: C, 61·5; H, 3·2; N, 16·7%).

p-*Cyano*-N-*methyl*-N-*nitrosoaniline*.—*p*-Cyanodimethylaniline [6] (14·6 g.) in warm 4N-sulphuric acid (146 c.c.) was treated dropwise (below the surface of the solution) during 10 min. with sodium nitrite (14·6 g.) in water (40 c.c.). The mixture was shaken in a closed vessel until nearly all the solid had dissolved, and after being kept at 0° for 3 days the yellow nitroso-compound was filtered off. It crystallised from ethanol (75 c.c.) in yellow needles (6·4 g., 40%), m. p. 120—122°. Sachs and Steinert [6] give m. p. 125° (but no yield). A similar yield was obtained by nitrosation of *p*-cyano-*N*-methylaniline.

N-p-*Cyanophenyl*-N-*methylhydrazine*.—Acetic acid (36 c.c.) was added during 30 min. to a stirred suspension of *p*-cyano-*N*-methyl-*N*-nitrosoaniline (16·1 g.) and zinc dust (100%; 24 g.) in water (120 c.c.). The oil which separated solidified after a further hour. The liquid was decanted and the solid was extracted with ether (3 × 200 c.c.). Extraction into 2N-hydrochloric acid (3 × 100 c.c.) and basification at 0—5° gave the substituted *hydrazine*, which crystallised from *iso*propyl alcohol (60 c.c.) in cream needles (8·8 g., 60%), m. p. 71—72° (Found: C, 64·95; H, 6·2; N, 28·7. $C_8H_9N_3$ requires C, 65·3; H, 6·1; N, 28·6%).

4 : 4′-*Dicyanohydrazobenzene*.—4 : 4′-Dicyanoazoxybenzene (or 4 : 4′-dicyanoazobenzene) (25 g.) in dioxan (250 c.c.) was catalytically reduced at just above 1 atm. in presence of 10% palladised charcoal (2·5 g.). Reduction was complete in 4·5 hr. at 40—50° and gave the *product* (4·0 g., 80%), yellow needles [from acetic acid (250 c.c.)], m. p. 201—202° (Found: N, 23·9. $C_{14}H_{10}N_4$ requires N, 24·0%).

4 : 4′-*Dicyano*-NN′-*dimethylhydrazobenzene*.—4 : 4′-Dicyanohydrazobenzene (11·7 g.), methyl toluene-*p*-sulphonate (29·2 g.), anhydrous potassium carbonate (20·7 g.), and dry anisole (300 c.c.) were refluxed together at 165—170° for 2·5 hr., then cooled. Chloroform (500 c.c.) removed the *product* which crystallised from ethanol (300 c.c.) in yellow needles (9·2 g., 70%), m. p. 197—198° (Found: C, 73·6; H, 5·6; N, 21·2. $C_{16}H_{14}N_4$ requires C, 73·3; H, 5·35; N, 21·35%).

Attempted Preparation of 4 : 4′-*Diamidino*-NN′-*dimethylhydrazobenzene*.—A green solution of

the dinitrile (18 g.) in chloroform (180 c.c.) and ethanol (18 c.c.) at 0—10° was saturated with hydrogen chloride, becoming red. Crystallisation occurred after 36 hr. After being kept at room temperature for 7 days, the pale red solid (10·4 g.) was filtered off, added to saturated ethanolic ammonia (300 c.c.), and kept at 55—60° for 6 hr. This gave 4 : 4′-diamidinoazobenzene dihydrochloride (6·4 g.), m. p. >360° (Found : N, 24·0; Cl, 20·3. Calc. for $C_{14}H_{14}N_6$,2HCl,0·5H_2O : N, 24·1; Cl, 20·4%). The filtrate from the di-imidoate stage was treated with ether (1 l.); the oil which separated soon solidified. When this was treated with ethanolic ammonia it furnished a brown hygroscopic solid and attempts to crystallise it gave only gums.

p-*Bromo*-N-*methylacetanilide*.—*N*-Methylacetanilide (m. p. 100°; b. p. 112—116°/10 mm.) (149 g.) in acetic acid (700 c.c.) was stirred and treated with bromine (240 g.) in acetic acid (250 c.c.) during 2 hr. while illuminated with a tungsten lamp (the internal temperature during the bromination was 32—44°). After 5 hours' stirring hydrogen bromide and excess of bromine were removed by a stream of nitrogen and a *perbromide* was filtered off. This had m. p. 140° (decomp.) (Found : Br, 66·0. $C_9H_{12}ONBr_3$ requires Br, 66·2%). When this was ground with 2N-sodium carbonate bromine was evolved and a yellow oil was formed which soon solidified. This (188 g.) crystallised from light petroleum (b. p. 60—80°) and afforded the bromo-derivative (156 g., 68%), white prisms, m. p. 97—98°. Wurster and Scheibe [11] give m. p. 99°.

p-*Amino*-N-*methylacetanilide*.—*N*-Methyl-*p*-nitroacetanilide [12] (65 g.) in methanol (5·6 l.) was catalytically reduced in presence of Adams catalyst (3·3 g.) to the amine (51·5 g., 93·5%), m. p. 63—65° (Morgan and Grist [10] give m. p. 59—60°), which was used without further purification.

p-*Cyano*-N-*methylacetanilide*.—(*a*) *p*-Bromo-*N*-methylacetanilide (114 g.) was added to the stirred complex prepared by addition of cuprous cyanide (67·1 g.) to dry pyridine (40 c.c.) at 120°. When the bath temperature was then raised to 220—230° a mildly exothermic reaction occurred and the internal temperature remained steady around 200° for a further 2 hr. The mixture was then distilled; most of the product distilled at 220—240°/35 mm. The distillate was added to ice-water, and the crude product (65 g.) was filtered off, dried, and crystallised from *iso*propyl alcohol (150 c.c.) to give p-*cyano*-N-*methylacetanilide* (44 g., 60·5%), white needles, m. p. 144—145° (Found : C, 69·2; H, 6·1; N, 16·1. $C_{10}H_{10}ON_2$ requires C, 69·0; H, 5·75; N, 16·1%).

(*b*) A stirred suspension of *p*-amino-*N*-methylacetanilide (133 g.) in concentrated hydrochloric acid (192·5 c.c.) and water (770 c.c.) was diazotised, at 0—10°, with sodium nitrite (58 g.) in water (190 c.c.). The red solution was stirred for a further 0·5 hr. and, after being neutralised (litmus) with sodium carbonate, it was added during 0·5 hr. to a stirred solution of cuprous cyanide (95 g.) and potassium cyanide (200 g.) in water (385 c.c.) at 5—15° in presence of chloroform (1 l.). After 1 hr. the mixture was kept at 50° for 0·5 hr., then the chloroform layer furnished the anilide (92 g., 65%) identical with the product prepared by method (*a*).

p-*Cyano*-N-*methylaniline*.—Concentrated hydrochloric acid (136 c.c.) was added to *p*-cyano-*N*-methylacetanilide (34·8 g.) partly dissolved in hot water (136 c.c.). The mixture was heated at 95° for 5 min., cooled to 10°, and basified (pH 9) at 10—15° with 50% aqueous sodium hydroxide. The amine crystallised from water (charcoal) in prismatic needles (14·2 g., 54%), m. p. 89—91° (Sachs and Steinert [6] give m. p. 85—86°) (Found : C, 72·6; H, 6·2; N, 20·9. Calc. for $C_8H_8N_2$: C, 72·7; H, 6·1; N, 21·2%).

p-*Methylaminobenzamidine*.—This was prepared by the imidoate method from the nitrile (40 g.) in dry chloroform (400 c.c.) and ethanol (40 c.c.). The *monohydrochloride* crystallised in prisms, m. p. 193—195°, from ethanol–acetone (Found : N, 22·4; Cl, 19·2. $C_8H_{11}N_3$,HCl requires N, 22·6; Cl, 19·1%).

4 : 4′-*Diamidinodiazo*-N-*methylaminobenzene*.—A stirred suspension of *p*-aminobenzamidine monohydrochloride (7·25 g.) in water (30 c.c.) and concentrated hydrochloric acid (5 c.c.) was treated at 0—5° with sodium nitrite (2·5 g.) in water (25 c.c.). To the diazonium solution was added, in one portion, at 5—15°, *p*-methylaminobenzamidine monohydrochloride (7·0 g.) in water (100 c.c.), and the deep red solution was then treated with saturated aqueous sodium acetate (42·5 c.c.) After being stirred at 5—15° for 1 hr. the yellow solid was filtered off, washed with brine, and dried. It was crystallised by dissolution in hot methanol (350 c.c.), filtration, and addition of acetone (600 c.c.) to the hot filtrate. The *dihydrochloride* (9 g., 59%) was

[11] Wurster and Scheibe, *Ber.*, 1879, **12**, 1818.
[12] Morgan and Grist, *J.*, 1918, 688.

deposited as yellow granules, decomp. 249—250° (Found: N, 24·3; Cl, 17·4; H_2O, 9·0. $C_{15}H_{17}N_7,2HCl,2H_2O$ requires N, 24·25; Cl, 17·6; H_2O, 8·9%).

Thanks are offered to Dr. H. J. Barber for his interest, Mrs. R. Stone and Mr. K. N. Brown for the biological tests, Mr. S. Bance for the semimicroanalyses, and the Directors of May & Baker Ltd. for permission to publish this work.

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[*Received, March 6th,* 1957.]