

134. Attempts to find New Chemotherapeutic Amidines. Part VII.

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The preparation of 4:4'-diamidino-2-methylstilbene and 4:4'-diamidino-2:2'-dimethylstilbene as potential trypanocidal compounds is described.

THE high activity of 4:4'-diamidino- $\alpha\beta$ -dimethylstilbene against *T. cruzi* (Barber, Slack, and Woolman, *J.*, 1943, 99) as compared with 4:4'-diamidinostilbene, together with the observation that certain nuclear substituents in the latter molecule enhanced its trypanocidal effect (Ashley and Harris, *J.*, 1946, 567), made the synthesis of 4:4'-diamidino-2-methylstilbene and 4:4'-diamidino-2:2'-dimethylstilbene desirable. The preparation of these compounds required the hitherto undescribed 5-bromo-*o*-tolualdehyde and 5-bromo-*o*-tolylacetic acid. Both these have been prepared from 5-bromo-*o*-toluic acid, the latter by the Arndt-Eistert reaction (*Ber.*, 1935, 68, 200) and the former by the method of McFadyen and Stevens (*J.*, 1936, 584).

5-Bromo-*o*-tolylacetic acid was condensed with 4-cyanobenzaldehyde under the conditions of the Perkin reaction. The resulting *cis*-4-bromo-4'-cyano-2-methylstilbene- α -carboxylic acid was decarboxylated to yield *cis*-4-bromo-4'-cyano-2-methylstilbene, which with cuprous cyanide in pyridine gave *cis*-4:4'-dicyano-2-methylstilbene. It was observed that whilst the latter compound readily isomerised to the *trans*-isomer with iodine in nitrobenzene, the former under similar conditions remained unchanged. Conversion of either the *cis*- or the *trans*-dicyanide into the amidine by the method of Pinner ("Die Imido Äther") gave *trans*-4:4'-diamidino-2-methylstilbene.

An attempt to obtain *cis*-4-bromo-4'-cyano-2-methylstilbene by the method of Meerwein (*J. pr. Chem.*, 1939, 132, 237) from *p*-cyanocinnamic acid and diazotised 5-bromo-*o*-toluidine gave a product from which the required substance could be isolated only with considerable difficulty and in too small a yield for preparative purposes.

The Perkin condensation between 5-bromo-*o*-tolylacetic acid and 5-bromo-*o*-tolualdehyde yielded in addition to *cis*-4:4'-dibromo-2:2'-dimethylstilbene- α -carboxylic acid small amounts of *trans*-4:4'-dibromo-2:2'-dimethylstilbene and 4-bromo-2-methylcinnamic acid.

cis-4:4'-Dibromo-2:2'-dimethylstilbene- α -carboxylic acid did not undergo decarboxylation on being heated in a vacuum or with copper chromite in quinoline. However, as anticipated, replacement of bromine with the more electron seeking -CN group facilitated decarboxylation, since treatment of the above acid with cuprous cyanide in pyridine followed by high vacuum sublimation yielded *cis*-4:4'-dicyano-2:2'-dimethylstilbene. This was converted into *trans*-4:4'-diamidino-2:2'-dimethylstilbene which was prepared in better yield from *trans*-4:4'-dicyano-2:2'-dimethylstilbene obtained by inversion of the *cis*-isomer.

These amidines have been tested for trypanocidal activity; the results will be discussed elsewhere.

EXPERIMENTAL.

5-Bromo-*o*-toluoyl Chloride.—5-Bromo-*o*-toluic acid (34 g.) was refluxed on the steam-bath with thionyl chloride (75 c.c.) for 30 minutes. Excess of thionyl chloride was removed under reduced pressure, and the residue distilled. 5-Bromo-*o*-toluoyl chloride, b. p. 136–138°/16 mm., was collected; it solidified to a mass of colourless plates (34 g.), m. p. 43° (Found: Cl, 30.1. C₈H₆OClBr requires Cl, 30.4%).

5-Bromo-*o*-tolyl Diazomethyl Ketone.—5-Bromo-*o*-toluoyl chloride (6.0 g.) in absolute ether was added to excess of diazomethane in absolute ether and left overnight. The solvent was removed under reduced

pressure and the residue crystallised from light petroleum (b. p. 40—60°). The *diazoketone* separated as yellow needles (3.7 g.), m. p. 83° (Found: N, 11.3. $C_8H_5ON_2Br$ requires N, 11.6%).

5-Bromo-*o*-tolylacetic Acid.—5-Bromo-*o*-tolyl diazomethyl ketone (34 g.) in dioxan (200 c.c.) was added with stirring to a suspension of silver oxide (4.0 g.) in a solution of sodium carbonate (9.8 g.) and sodium thiosulphate (6.4 g.) in water (400 c.c.) at 60°. The reaction was maintained at this temperature for 1 hour and then slowly raised to 90—100° and maintained at this temperature for a further 3 hours. The mixture was cooled, filtered, and extracted with ether. The aqueous layer was freed from ether, acidified with dilute nitric acid, the precipitate removed and crystallised from 20% aqueous alcohol, and the *acid* separated as colourless fluffy needles (21.0 g.), m. p. 116° (Found: Br, 34.2. $C_8H_5O_2Br$ requires Br, 35.0%).

***cis*-4-Bromo-4'-cyano-2-methylstilbene-*a*-carboxylic Acid.**—Potassium 5-bromo-*o*-tolylacetate (11.2 g.), *p*-cyanobenzaldehyde (5 g.), pyridine (1 c.c.), and acetic anhydride (80 c.c.) were heated under reflux at 140—160° for 4 hours. The mixture was poured into water to decompose the excess of acetic anhydride, and the liquid extracted with ether (100 c.c.). The ether layer was separated, water (30 c.c.) added, and the liquid made alkaline with solid sodium carbonate; an oil then separated. The oil and aqueous layer were run off and cooled in ice. The sodium salt, when solidified, was collected, dissolved in hot water, and *cis*-4-bromo-4'-cyano-2-methylstilbene-*a*-carboxylic acid (9.9 g.) (50%) precipitated by acidification with hydrochloric acid. The acid crystallised from benzene-light petroleum as colourless prisms (6.2 g.), m. p. 178° (decomp.) (Found: N, 4.1; Br, 23.2. $C_{17}H_{12}O_2NBr$ requires N, 4.1; Br, 23.4%). The aqueous carbonate mother liquor from the above separation yielded on acidification a solid which crystallised from alcohol as colourless needles (0.7 g.), m. p. 260° alone and mixed with *p*-cyanocinnamic acid.

***cis*-4-Bromo-4'-cyano-2-methylstilbene.**—(1) *cis*-4-Bromo-4'-cyano-2-methylstilbene-*a*-carboxylic acid (10.4 g.) was heated at 200—230° for 20 minutes and the residue distilled in a high vacuum over a free flame. The distillate set to a glass which was crystallised from ethyl alcohol; the *stilbene* separated as clusters of colourless needles (4.1 g.), m. p. 152° (Found: N, 3.9. $C_{16}H_{12}NBr, C_2H_5OH$ requires N, 4.0%).

Attempts to convert this into the *trans*-compound with iodine in nitrobenzene were unsuccessful, the original substance being obtained unchanged after 1½ hours under reflux.

(2) 5-Bromo-*o*-toluidine sulphate (4.75 g.) was dissolved in water (5 c.c.) and sulphuric acid (2.0 c.c.), and the whole diazotised with sodium nitrite (1.2 g.) in water (5 c.c.) at 5—10°. The diazonium sulphate solution was added with agitation to a solution prepared from *p*-cyanocinnamic acid (4 g.), sodium acetate (15.0 g.), and cupric chloride (0.10 g.) in acetone (100 c.c.) and water (20 c.c.) at 23—27°. Stirring was continued for 3 hours, and the mixture was then left overnight. The acetone was removed, the mixture shaken with benzene, and the benzene layer extracted with 2*N*-sodium carbonate. The benzene layer was dried and passed through alumina soaked in benzene and the column eluted with more benzene. The benzene eluate was again evaporated, the residue dissolved in light petroleum and adsorbed on alumina and eluted with light petroleum. The residue on removal of the solvent was crystallised from ethyl acetate and separated as colourless needles (0.1 g.), m. p. 152° alone and mixed with *cis*-4-bromo-4'-cyano-2-methylstilbene. The sodium carbonate mother liquor on acidification yielded unchanged *p*-cyanocinnamic acid (3.6 g.).

***cis*-4 : 4'-Dicyano-2-methylstilbene.**—*cis*-4-Bromo-4'-cyano-2-methylstilbene (4 g.), cuprous cyanide (2.0 g.), and pyridine (2.0 c.c.) were heated at 200—210° for 1½ hours. A test attempt to extract the copper with hot concentrated hydrochloric acid resulted in hydrolysis of the cyano-group. Excess of pyridine was removed and the residue was distilled over a free flame in a high vacuum. The distillate was dissolved in benzene, passed through alumina and eluted with more benzene. The solvent was removed and the residue crystallised from ethyl alcohol; *cis*-4 : 4'-dicyano-2-methylstilbene separated in clusters of colourless needles (1.4 g.), m. p. 128° (Found: N, 11.3. $C_{17}H_{12}N_2$ requires N, 11.4%).

***trans*-4 : 4'-Dicyano-2-methylstilbene.**—*cis*-4 : 4'-Dicyano-2-methylstilbene (0.8 g.) was heated in nitrobenzene (2.0 c.c.) with a trace of iodine for 30 minutes; dilution with light petroleum produced a white precipitate. This crystallised from alcohol, and *trans*-4 : 4'-dicyano-2-methylstilbene separated as colourless needles (0.7 g.), m. p. 208° (Found: N, 11.4. $C_{17}H_{12}N_2$ requires N, 11.4%).

***trans*-4 : 4'-Diamidino-2-methylstilbene Dihydrochloride.**—*trans*-4 : 4'-Dicyano-2-methylstilbene (0.7 g.) in dry alcohol (25 c.c.) was saturated with hydrogen chloride at 0° and the mixture kept for 3 days. The voluminous precipitate of iminoether was filtered off and heated with alcoholic ammonia at 40° for 8 hours. The *amidine* crystallised from aqueous hydrochloric acid as light yellow prisms (0.5 g.), m. p. >360° (Found: N, 15.0; Cl, 19.1. $C_{17}H_{20}N_4Cl_2, H_2O$ requires N, 15.2; Cl, 19.1%).

***cis*-4 : 4'-Dicyano-2-methylstilbene** treated in the above manner yielded only *trans*-amidine in poor yield (< 10%).

5-Bromo-*o*-toluoylhydrazide.—Methyl 5-bromo-*o*-toluate (23 g.), 50% aqueous hydrazine hydrate solution (35 c.c.), and ethanol (70 c.c.) were refluxed overnight. The mixture was cooled and 5-bromo-*o*-toluoylhydrazide separated as colourless needles (21 g.), m. p. 160°. A small portion crystallised from ethyl alcohol for analysis had m. p. 163° (Found: N, 10.2. $C_8H_5ON_2Br, C_2H_5OH$ requires N, 10.2%).

Benzenesulphonyl-5-bromo-*o*-toluoylhydrazide.—5-Bromo-*o*-toluoylhydrazide (25 g.) in pyridine (260 c.c.) was cooled to and maintained at 0—5° while benzenesulphonyl chloride (30 c.c.) was added with stirring. Agitation was continued for 2 hours after the final addition of benzenesulphonyl chloride. The mixture was poured into excess of dilute hydrochloric acid and the crude product crystallised from alcohol; the *compound* separated as colourless needles (36 g.), m. p. 188° (Found: N, 7.6. $C_{14}H_{13}O_3N_2BrS$ requires N, 7.6%).

5-Bromo-*o*-tolualdehyde.—Benzenesulphonyl-5-bromo-*o*-toluoylhydrazide (4.8 g.) in ethylene glycol (30 c.c.) was heated to 160°, anhydrous sodium carbonate (3.6 g.) was added, and heating continued for 75 seconds. The mixture was diluted with boiling water, cooled, and extracted with ether. The solvent was removed, and the residue was shaken with an excess of saturated sodium hydrogen sulphite solution and left overnight. The aldehyde bisulphite was filtered off, washed with a little cold water,

and decomposed by warming on the steam-bath with dilute hydrochloric acid (100 c.c.). The liquor was extracted with benzene, separated, the solvent removed, and the residue distilled under reduced pressure. 5-Bromo-*o*-tolualdehyde distilled as a colourless liquid, b. p. 95°/0.05 mm., n_D^{25} 1.5960; this solidified to a mass of colourless crystals (1.7 g.), m. p. 26° (Found: Br, 38.8. C_8H_7OBr requires Br, 40.0%). The *dinitrophenylhydrazone* crystallised from dioxan in clusters of orange needles, m. p. 256° (Found: N, 14.2. $C_{14}H_{11}O_4N_4Br$ requires N, 14.8%). The *semicarbazone* crystallised from glacial acetic acid as colourless prismatic needles, m. p. 228—230° (Found: N, 15.2. $C_9H_{10}ON_3Br \cdot H_2O$ requires N, 15.3%). Found after drying: N, 16.2. $C_9H_{10}ON_3Br$ requires N, 16.4%). The *azine* obtained from the above aldehyde in 4% aqueous alcoholic hydrazine hydrate solution crystallised from glacial acetic acid as yellow needles, m. p. 176° (Found: N, 7.0. $C_{16}H_{14}N_2Br_2$ requires N, 7.1%). This compound sublimed unchanged in high vacuum.

4-Bromo-2-methylcinnamic Acid.—5-Bromo-*o*-tolualdehyde (2.5 g.), fused potassium acetate (1.7 g.), acetic anhydride (25 c.c.), and pyridine (1.0 c.c.) were heated at 140—150° for 6 hours. The mixture was poured into water, extracted with ether, and separated. The ether layer was extracted with 2*N*-sodium carbonate, separated, and the aqueous layer acidified. The precipitate was removed, dried, and crystallised from absolute alcohol. 4-Bromo-2-methylcinnamic acid separated as colourless plates (0.5 g.), m. p. 208° (Found: Br, 33.1. $C_{10}H_8O_2Br$ requires Br, 33.2%).

cis-4 : 4'-Dibromo-2 : 2'-dimethylstilbene-*α*-carboxylic Acid.—Dry potassium 5-bromo-*o*-tolylacetate (14.0 g.), 5-bromo-*o*-tolualdehyde (10 g.), acetic anhydride (150 c.c.), and pyridine (3.0 c.c.) were heated at 140—160° for 4 hours. The mixture was poured into water and extracted with ether. The residue from evaporation of the ether extract was steam distilled to remove acetic acid, extracted with benzene, separated, and the benzene layer repeatedly distilled until the residue was dry. The gum was triturated with light petroleum (b. p. 80—100°) and the solid which deposited was filtered off. The residue was boiled with 2*N*-sodium carbonate, filtered hot, and the residue (0.25 g.) crystallised from glacial acetic acid. *trans*-4 : 4'-Dibromo-2 : 2'-dimethylstilbene separated as colourless prismatic needles, m. p. 182° (Found: Br, 43.7. $C_{16}H_{14}Br_2$ requires Br, 43.7%). The light petroleum solution was concentrated, and an intractable gum remained which resisted all immediate attempts at crystallisation. The residue was kept for three weeks; some solid had then been deposited. The residue was triturated with light petroleum (b. p. 80—100°) and filtered. This procedure was repeated thrice, and the solid fractions bulked together. This material was boiled with 2*N*-sodium carbonate (charcoal), filtered hot, and cooled. The solid which separated was removed, dissolved in boiling water, and the hot solution acidified with hydrochloric acid. The residue was removed, dried, and crystallised from light petroleum (b. p. 80—100°). *cis*-4 : 4'-Dibromo-2 : 2'-dimethylstilbene-*α*-carboxylic acid separated as colourless prisms (4.6 g.), m. p. 175° (Found: Br, 38.5. $C_{17}H_{14}O_2Br_2$ requires Br, 39.0%). The sodium carbonate mother liquors were acidified and the residue was crystallised from ethyl alcohol; m. p. 208° alone and mixed with 4-bromo-2-methylcinnamic acid (3.1 g.).

4 : 4'-Dibromo-2 : 2'-dimethylstilbene-*α*-carboxylic acid was heated at 170—200°/1 mm. The sublimate was removed and crystallised from light petroleum (b. p. 80—100°); m. p. 175° alone and mixed with starting material. The stilbenecarboxylic acid (1.0 g.) in quinoline (5 c.c.) with a trace of copper chromite was heated at 220° for 1½ hours. The residue was poured into hydrochloric acid and extracted with ether. No workable material was isolated even after chromatographic purification.

cis-4 : 4'-Dicyano-2 : 2'-dimethylstilbene.—*cis*-4 : 4'-Dibromo-2 : 2'-dimethylstilbene-*α*-carboxylic acid (3.2 g.), cuprous cyanide (2 g.), and pyridine (2.4 c.c.) were heated under reflux at 200—220° for 1½ hours. Excess of pyridine was removed and the residue sublimed at 200—260°/0.05—0.1 mm.; the sublimate was removed and crystallised from alcohol. *cis*-4 : 4'-Dicyano-2 : 2'-dimethylstilbene separated as colourless prismatic needles (0.6 g.), m. p. 166° (Found: N, 10.85. $C_{18}H_{14}N_4$ requires N, 10.85%).

trans-4 : 4'-Dicyano-2 : 2'-dimethylstilbene.—*trans*-4 : 4'-Dibromo-2 : 2'-dimethylstilbene (0.05 g.) from the above Perkin condensation, cuprous cyanide (0.05 g.), and pyridine (0.04 c.c.) were heated under reflux at 200—220° for 1½ hours. The residue was extracted with hot concentrated hydrochloric acid, and the solid recrystallised from nitrobenzene as colourless prisms, m. p. 272° alone and mixed with the product obtained by inversion of the above *cis*-4 : 4'-dicyano-2 : 2'-dimethylstilbene with iodine in nitrobenzene.

trans-4 : 4'-Diamidino-2 : 2'-dimethylstilbene Dihydrochloride.—*trans*-4 : 4'-Dicyano-2 : 2'-dimethylstilbene (0.3 g.) in dry alcohol was saturated with hydrogen chloride at 0° and kept for 10 days. The residue (0.3 g.) was filtered off, treated at 40° with alcoholic ammonia for 8 hours, and the product crystallised from 5% aqueous hydrochloric acid. *trans*-4 : 4'-Diamidino-2 : 2'-dimethylstilbene dihydrochloride separated as colourless needles (0.28 g.) (Found: N, 14.5; Cl, 17.9. $C_{18}H_{22}N_4Cl_2 \cdot H_2O$ requires N, 14.6; Cl, 18.3%).

cis-4 : 4'-Dicyano-2 : 2'-dimethylstilbene treated in the above manner yielded only the *trans*-amidine in poor yield (10%).

The author wishes to express his indebtedness to Mr. S. Bance, B.Sc., for the several semimicro-analyses, to Dr. A. J. Ewins, F.R.S., for his interest, help, and encouragement, and to the Directors of May & Baker, Ltd., for permission to publish the results.

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[Received, August 8th, 1946.]