For the preparation of the benzidine dihydrochloride the suspension of the benzidine was stored in a refrigerator for two days before it was collected on a filter. The solid was dried to constant weight in a vacuum desiccator and was then extracted with small portions (about 20 ml.) of boiling absolute ethanol until the amount of undissolved solid appeared to undergo no further decrease. The combined ethanol extracts were diluted with twice the volume of anhydrous ether and dry hydrogen chloride was passed into the solution until precipitation of the hydrochloride was complete. The solid was collected and the mother liquor was tested by further dilution with ether; if more solid separated it was combined with the first crop and the second mother liquor was tested in the same way. The total yield of the dihydrochloride so obtained was 3.1 g. (19.3% based on the nitro compound I). The salt had no definite melting point, but decomposed slowly at temperatures above 300°.

Anal. Calcd. for $C_{12}H_{16}O_4B_2N_2Cl_2$: N, 8.15; B, 6.28. Found: N, 8.27; B, 5.85.

For the isolation of the benzidine directly from the rearrangement mixture the crude diamine was filtered immediately after the precipitation with ammonium hydroxide and the filtrate was stored for two days in a stoppered flask. The solid collected in the filtration was extracted with 100 ml. of 30% ethanol and the extract was allowed to cool. Both the dilute ethanol extract and the ammoniacal mother liquor deposited light brown crystals; the two crops were collected and decolorized in 150 ml. of hot 30% ethanol. This solution on cooling deposited 1.6 g. (13%) of the benzidine as short flesh-colored needles melting at 232–234°. The substance was insoluble in water, aqueous ammonia and ether, but soluble in dilute sodium hydroxide dilute acids, ethanol and glacial acetic acid. A sample dried in the air had the composition of a hemihydrate.

Anal. Calcd. for $C_{12}H_{15}O_{4.5}N_2B_2$: C, 51.25; H, 5.38; B, 7.70. Found: C, 51.01; H, 5.64; B, 7.75.

The combustion analyses on samples dried *in vacuo* over phosphorus pentoxide were erratic with respect to carbon and the combustion residues obviously contained carbon; the hydrogen and nitrogen values on such samples indicated that they consisted of the di-anhydride.

Anal. Calcd. for $C_{12}H_{10}O_2N_2B_2$: H, 4.27; N, 11.88. Found: H, 4.45; N, 11.51.

Removal of the Boronic Acid Groups from Derivatives of the Benzidine (III). (a) **Diacetylbenzidine.**—A solution of 1.36 g. of the diamine in 25 ml. of water containing 0.83 ml. of concentrated hydrochloric acid was treated with 1.16 ml. of acetic anhydride and then warmed to 50°. A solution of 0.82 g. of sodium acetate in 5 ml. of water was added, and the mixture was cooled in an ice-bath. A solid weighing 1.13 g. was collected which was soluble in dilute sodium hydroxide but insoluble in water and acids. A portion of the material was recrystallized from a large volume of 50% ethanol. The analytical data indicated that part of the material had lost boronic acid groups during the reaction.

Anal. Calcd. for $C_{16}H_{18}N_2O_6B_2$: C, 54.0; H, 5.1; B, 6.1. Found: C, 57.4; H, 5.4; B, 4.5.

A sample of about 0.5 g. of the crude acetyl derivative was suspended in 30 ml. of 50% ethanol and treated with 10 ml. of 10% aqueous silver nitrate and 6 drops of concentrated aqueous ammonia. The resulting mixture, which contained a brown precipitate, was refluxed for fifteen minutes and filtered hot. The filtrate deposited a white powder which melted at 329–331°, alone or mixed with authentic diacetylbenzidine.

(b) **Dibenzalbenzidine.**—To a warm solution of 0.5 g. of the benzidine in 70 ml. of 95% ethanol was added 1 ml. of benzaldehyde. The solution soon began to deposit fine crystals, which were collected after four hours. The yellow product melted at 141–143° and weighed 0.8 g. (97%). The substance could be recrystallized from benzene, but the product so obtained had no definite melting point; the material probably suffered the loss of water from the boronic acid groups during the heating with benzene. About 0.2 g. of the freshly prepared benzal derivative was suspended in 50 ml. of water and treated with six drops of aqueous ammonia; dilute aqueous silver nitrate was added until no further precipitation occurred and the brown solid was separated. The solid was added to 25 ml. of water and the mixture was boiled gently for fifteen minutes. The cooled mixture was filtered and the solid was extracted with 20 ml. of hot benzene. The yellow plates which separated when the benzene solution was cooled melted at 232–234°; the substance did not contain boron, and the melting point was not changed by mixture with authentic dibenzalbenzidine.

Summary

m-Hydrazobenzeneboronic acid can be prepared in good yield by the reduction of *m*-nitrobenzeneboronic acid with zinc in the presence of alkali. Rearrangement of the hydrazo compound by acids leads to complex mixtures, but under certain conditions benzidine-2,2'-diboronic acid is produced and can be isolated in yields of about 20%.

URBANA, ILLINOIS RECEIVED AUGUST 18, 1947

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Synthesis of Azo Boronic Acids. II. Dyes from Tetrazotized Benzidine-2,2'-diboronic Acid

BY H. R. SNYDER AND SEYMOUR L. MEISEL[1]

The speculations of Kruger[2] and Zahl and others[3-6] on the possible development of an irradiation therapy based on the nuclear disintegration process (equation 1) occurring when the boron isotope of atomic weight 10 captures a slow neutron have provoked an interest in the synthesis of boron-containing dyes having structures similar to those of Trypan Blue and Evans Blue. Such substances might be expected to concentrate in certain tissues, which might then be irradiated with slow neutrons with the result that the energetic nuclear disintegration would be localized. Recent studies[7] on the localization of derivatives of Trypan Blue and Evans Blue containing radioactive bromine indicate that the relative concentra-

$$ {}_5B^{10} + {}_0N^1_s \longrightarrow {}_3Li^7 + {}_2He^4 \qquad (1) $$

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[1] Present address: Socony-Vacuum Oil Co., Inc., Paulsboro, N. J.

[2] Kruger, *Proc. Nat. Acad. Sci.*, **26**, 181 (1940).

[3] Zahl, Cooper and Dunning, *ibid.*, **26**, 589 (1940).

[4] Zahl and Cooper, *Science*, **93**, 64 (1941).

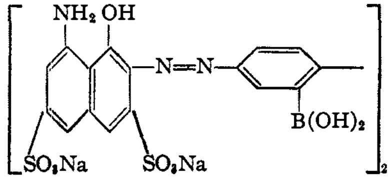
[5] Zahl and Waters, *Proc. Soc. Exper. Biol. Med.*, **48**, 304 (1941).

[6] Zahl and Cooper, *Radiology*, **37**, 673 (1941).

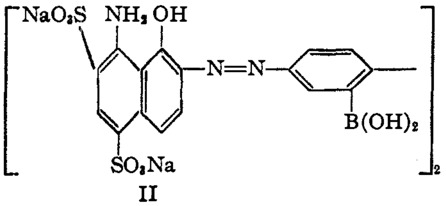
[7] Moore, Tobin and Aub, *J. Clin. Invest.*, **22**, 161 (1943).

tion of the dyes in tumors is by no means as favorable as would be required for therapeutic application. Nevertheless, the possibility remains that a more selective action might be characteristic of the proposed boron-containing dyes, and in any event they should be of value in the study of the general effects of the nuclear disintegration of boron occurring within living tissues.

The dye (I) obtained by coupling benzidine-2,2'-diboronic acid[8] with 1-amino-8-naphthol-3,6-disulfonic acid (H acid) presumably has a structure differing from that of Trypan Blue only in the presence of the two boronic acid groups in the 2 and 2' positions of the benzidine unit instead of methyl groups in the 3 and 3' positions. The structure of the dye was not proved, but the linkage of the azo groups at the 7 positions of the 8-naphthol residues may be assumed by analogy to other dyes prepared by coupling with H-acid under comparable conditions.[9] No displacement of boron occurred during the coupling and purification of the product (I). Since the localization of Trypan Blue depends on the fact that it forms colloidal dispersions rather than true aqueous solutions, a qualitative dialysis experiment was carried out with the dye (I); it was found to diffuse only very slowly through a membrane which was readily permeable to phenolphthalein.



I



II

The analog (II) of Evans Blue was prepared by coupling the same benzidine with 1-amino-8-naphthol-2,4-disulfonic acid (Chicago acid). This substance also contained nearly the theoretical amount of boron and it also diffused very slowly through a collodion membrane. Dyes of the expected composition likewise resulted from the coupling of the benzidinediboronic acid with β-naphthol and with acetoacetanilide. Dyes were obtained by coupling of the benzidine with 2-naphthol-3,6-disulfonic acid (R acid), 1-naphthol-4-sulfonic acid (NW acid) and β-naphthylamine, but no satisfactory analytical samples of the products have been obtained.

Dyes of higher boron content and of higher molecular weight should be available by coupling diazotized aminobenzeneboronic acids into the 2 position of I. This possibility is being tested.

Experimental

Tetrazotization of Benzidine-2,2'-diboronic Acid.—A solution of 1 g. of benzidine-2,2'-diboronic acid[8] (or 1.27 g. of the dihydrochloride) in 30 ml. of 3*N* hydrochloric acid was cooled in an ice-bath. About 8 ml. of a solution of 0.52 g. of sodium nitrite in 10 ml. of water was added in small portions with good stirring, and the solution then was tested with starch-iodide paper; if the test was negative further small portions of the nitrite solution were added until a positive test resulted. The mixture was kept cold for one-half hour during which time the positive reaction to starch-iodide was maintained by further additions of small portions of the nitrite solution, if necessary. The excess of nitrous acid then was removed by the addition of very small amounts of the benzidinediboronic acid, and the resulting solution was used immediately in a coupling reaction.

Coupling with H Acid.—The technical H acid was purified as follows: A solution of 100 g. of the technical material in 500 ml. of hot water was filtered, decolorized with 15 g. of charcoal and salted out by the addition of about 70 g. of sodium chloride to the filtrate. The material so obtained was recrystallized from the minimum volume of boiling water, and the purified material was dried in a vacuum oven at 110°.

The cold tetrazonium solution from 1 g. of the benzidinediboronic acid was added over a period of one-half hour to a cold, stirred solution of 2.36 g. of purified H acid and 5 g. of sodium carbonate in 35 ml. of 5% aqueous sodium hydroxide. The resulting mixture was cooled and stirred for two hours, after which time it was warmed to 85° and filtered. The filtrate was stirred at 85° for fifteen minutes and then treated with 35 g. of hydrated sodium acetate, added in five portions. After fifteen minutes the heating bath was removed and the stirred mixture was allowed to come to room temperature. The precipitated dye was collected, dissolved in 50 ml. of hot water and again salted out as before with 30 g. of the hydrated sodium acetate. The product so obtained was extracted ten times with portions of about 15 ml. of boiling ethanol, each extract being removed by filtration. After four to seven extractions the residual dye gave a negative test for sodium acetate.[10] The purified dye weighed 1.5 g. It was a very dark purple powder which did not melt or decompose at temperatures up to 340°. Analytical samples, dried over phosphorus pentoxide at about 95° and at 5 mm. pressure, had the composition of the trihydrate. The boron content was determined by the sodium peroxide fusion method.[11]

Anal. Calcd. for $C_{32}H_{28}O_{21}N_6B_2S_4Na_4$: C, 35.79; H, 2.61; B, 2.02. Found: C, 35.64; H, 2.89; B, 1.88.

For the dialysis test enough of the dye was added to 75 ml. of water to produce an intense purple solution and a few drops of phenolphthalein indicator solution was added. The solution was placed in a collodion bag suspended in 200 ml. of water. After one day the water remained entirely colorless, and portions of it gave the phenolphthalein color when made alkaline. Even after a week the water in the outer compartment had assumed only a very faint color.

Coupling with Chicago Acid.—Technical Chicago acid was purified in the same way as the H acid. In an experiment in which the tetrazotization, coupling and isolation of the product were carried out exactly as described above for the coupling with H acid the yield of the dye II was only 0.6 g. It appeared that the lower yield resulted from lower efficiency in the salting-out process. Other experiments in which ammonium bromide was used for the

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(8) Snyder, Weaver and Parmerter, THIS JOURNAL, **70**, 773 (1948).

(9) Saunders, "The Aromatic Diazo Compounds," Edward Arnold and Co., London, 1936, p. 108.

(10) Hartwell and Fieser, "Org. Syn.," Coll. Vol. II, 1943, p. 145.

(11) Snyder, Kuck and Johnson, THIS JOURNAL, **60**, 105 (1938).

salting-out (with subsequent extraction of the product with methanol for the removal of ammonium bromide) gave about the same yield. The dye II was a very dark purple powder which did not melt or decompose at temperatures up to 340°. It rapidly imparted a purple color to water, but was insoluble in the organic solvents that were tested. Analytical samples, dried at about 95° at 5 mm., had the composition of the trihydrate. In a dialysis experiment it behaved exactly as did I.

Anal. Calcd. for $C_{32}H_{28}O_{21}N_6B_2S_4Na_4$: C, 35.79; H, 2.61; B, 2.02: Found: C, 35.76; H, 2.89; B, 1.77.

Coupling with β-Naphthol.[12]—The tetrazonium solution prepared, as described above, from 0.5 g. of benzidine-2,2′-diboronic acid was added with stirring to a cold solution of 0.54 g. of β-naphthol and 5 g. of sodium carbonate in 18 ml. of 5% aqueous sodium hydroxide. The mixture was stirred in the ice-bath for two hours and then added with stirring to 30 ml. of 5% hydrochloric acid. The mixture was diluted with 25 ml. of ethanol and filtered hot. The red solid was dissolved in the minimum amount of Cellosolve (less than 5 ml.) and the solution was diluted with 5 ml. of ethanol and filtered hot. Water was added to the filtrate until the precipitation of the dye was complete, and the mixture was boiled to coagulate the solid. The solid was collected and dried at 95° at 5 mm. About 0.2 g. of a bright red powder, easily soluble in Cellosolve, less soluble in ethanol and insoluble in water, was obtained; it decomposed at about 240°.

Anal. Calcd. for $C_{32}H_{24}O_6N_4B_2$: C, 66.01; H, 4.13; N, 9.63. Found: C, 65.99; H, 4.21; N, 9.75.

Coupling with Acetoacetanilide.—The coupling procedure was a modification of that described by Linstead and Wang.[13] The tetrazonium solution prepared from 0.64 g. of the benzidine dihydrochloride was added over a period of fifteen minutes to a stirred mixture of 0.64 g. of acetoacetanilide, 1 g. of sodium acetate and 50 ml. of 1% aqueous sodium hydroxide. The resulting mixture was stirred in an ice-bath overnight. The solid was separated by filtration and recrystallized three times from dilute ethanol. The bright yellow solid contained boron. It was insoluble in water or aqueous sodium carbonate, but soluble in 5% aqueous sodium hydroxide. It decomposed sharply at 174–175° to give a black residue which melted at 270–280°.

Anal. Calcd. for $C_{32}H_{30}N_6O_8B_2$: C, 59.29; H, 4.63; N, 12.97. Found: C, 58.87; H, 4.70; N, 12.96.

Summary

Analogs of Trypan Blue and Evans Blue have been prepared by the coupling of tetrazotized benzidine-2,2′-diboronic acid with 1-amino-8-naphthol-3,6-disulfonic acid (H acid) and with 1-amino-naphthol-5,7-disulfonic acid (Chicago acid), respectively. The dyes so obtained have the expected boron contents. Boron-containing dyes also have been prepared by coupling the benzidinediboronic acid with β-naphthol and with acetoacetanilide.

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[12] The authors are indebted to Dr. Stanley M. Parmerter for the first experiments on this reaction.

[13] Linstead and Wang, *J. Chem. Soc.*, 807 (1937).

[CONTRIBUTION FROM THE DEPARTMENT OF INDUSTRIAL CHEMISTRY, FACULTY OF ENGINEERING, KYÔTO UNIVERSITY]

The Preparation of Synthetic Estrogens. I. The Synthesis of Diethylstilbestrol through the Pinacol–Pinacolone Compounds

BY KEIITI SISIDO AND HITOSI NOZAKI

Wessely[1] and others[2] have described synthetic approaches to the estrogenic diethylstilbestrol involving the use of the pinacol–pinacolone and retropinacolone rearrangements. We have carried out further investigations on this approach and have been able to improve several of the steps involved. The synthesis is outlined in the flow sheet.

Model experiments on the reduction of acetophenone and *p*-methoxyacetophenone to the pinacols using the method of Newman[3] gave the pinacols as a stereoisomeric mixture in 73 and 80% yields, respectively. With *p*-methoxypropiophenone (I) the yield of crystalline pinacol (II) was considerably lower, but after rearrangement of the crude pinacol the pinacolone (III) was obtained in 54% yield from the propiophenone (I). After reduction to carbinol (IV) and rearrangement with dilute sulfuric acid, only a small amount of crystalline product was obtained. However, heating the oily mixture with iodine in chloroform[1] effected isomerization and permitted the isolation of the dimethyl ether of diethylstilbestrol (V) in about 14% over-all yield from *p*-methoxypropiophenone (I). By further development of these procedures the yields can probably be improved.

By similar procedures starting with acetophenone we have prepared 2,3-diphenyl-2-butene in 30% over-all yield and 2,3-dianisyl-2-butene from *p*-methoxyacetophenone in 32% over-all yield.

According to a Swedish patent,[4] not accessible to us yet, diethylstilbestrol is said to be obtained from *p*-hydroxypropiophenone in an almost identical way. We have noticed in our model experiments with *p*-hydroxyacetophenone as well as with *p*-acetoxyacetophenone that the existence of free hydroxyl or acetoxyl groups hinders the smooth reaction of the pinacolic reduction.

Experimental[5]

(I) Pinacolic Reduction

2,3-Diphenyl-2,3-butanediol.—To a solution of 30 g. of acetophenone in 300 cc. of absolute alcohol and 200 cc. of dry, thiophene-free benzene were added 12 g. of alu-

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[1] Wessely, Kerschbaum, Kleedorfer, Prillinger and Zajic, *Monatsh.*, **73**, 127 (1940).

[2] For a review of these and other syntheses of diethylstilbestrol see Solmssen, *Chem. Rev.*, **37**, 481 (1945).

[3] Newman, THIS JOURNAL, **62**, 1683 (1940).

[4] Adler, Gie and von Euler, Swedish Patent No. 115,816, Feb. 12, 1946; *C. A.*, **41**, 486 (1947).

[5] Microanalyses by Miss Yasuko Meizyô of our Laboratory.