

[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY AT CORNELL UNIVERSITY]

DERIVATIVES OF PHENYLBORIC ACID, THEIR PREPARATION  
AND ACTION UPON BACTERIA. II.  
HYDROXYPHENYLBORIC ACIDS<sup>1</sup>

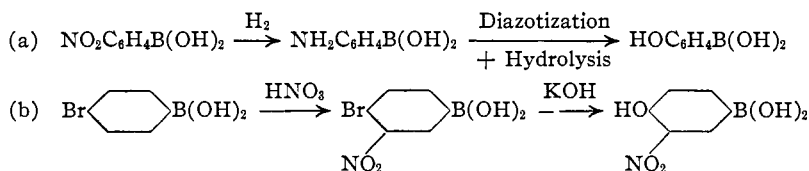
BY FREDERIC R. BEAN AND JOHN R. JOHNSON

RECEIVED JULY 5, 1932

PUBLISHED NOVEMBER 5, 1932

In an earlier investigation<sup>2</sup> various nitro and amino derivatives of phenylboric acid were prepared and their action on certain bacteria was tested. The present study was undertaken with the object of extending the work to other substituted aryl boric acids, particularly those containing a phenolic hydroxyl group.

Since the general methods for the preparation of aryl boric acids do not lend themselves to a direct synthesis of hydroxyphenylboric acids, it was necessary to introduce the phenolic hydroxyl by indirect methods. Of these, the following appeared to be the most promising.



In addition to phenylboric acid, the following substituted boric acids were used as starting materials: *p*-tolyl-, *p*-anisyl- and *p*-bromophenylboric acids. These were prepared by the action of the corresponding organo-magnesium bromides on *n*-butyl borate, at low temperatures. By substituting *n*-butyl borate<sup>3</sup> for methyl borate and effecting the reactions at  $-60^\circ$  (instead of  $-15^\circ$ ),<sup>2</sup> the yields of these acids were improved markedly.

3-Hydroxyphenylboric acid was prepared through the reactions indicated above (a), from *m*-nitrophenylboric acid. The reduction of this and other nitroaryl boric acids was simplified by effecting a catalytic reduction with hydrogen in the presence of platinum<sup>5</sup> instead of using ferrous hydroxide.<sup>6</sup> *m*-Aminophenylboric acid was diazotized in the

<sup>1</sup> This article is an abstract of a thesis submitted by Frederic R. Bean to the Faculty of the Graduate School of Cornell University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, in July, 1931.

<sup>2</sup> Seaman and Johnson, *THIS JOURNAL*, **53**, 711 (1931).

<sup>3</sup> König and Scharrnbeck<sup>4</sup> have recently described the preparation of these aryl boric acids using isobutyl borate, but give no information concerning the yields obtained. They did not succeed in obtaining any nitro or amino derivatives of these acids.

<sup>4</sup> König and Scharrnbeck, *J. prakt. Chem.*, [2] **128**, 157 (1930).

<sup>5</sup> Adams and Shriner, "Organic Syntheses," John Wiley and Sons, Inc., New York, 1928, Vol. VIII, p. 92.

<sup>6</sup> This modification enabled us to isolate *o*-aminophenylboric acid, which previously had been obtained only in solution.<sup>2</sup>

presence of sulfuric acid and the resulting diazonium salt was hydrolyzed in a current of steam. 3-Hydroxyphenylboric acid is a white crystalline solid, readily soluble in water and in ether. It gives a bluish-violet color with ferric chloride solution, and produces 3-hydroxyphenylmercuric chloride in practically quantitative yield on treatment with warm aqueous mercuric chloride solution.

The homolog, 3-hydroxy-4-methylphenylboric acid, was obtained by a similar series of reactions from 3-nitro-4-methylphenylboric acid. The latter was obtained readily by nitrating *p*-tolylboric acid with fuming nitric acid at  $-30^{\circ}$ . The position of the nitro group was established by conversion into 4-bromo-2-nitrotoluene, and into 4-methyl-3-nitrophenylmercuric chloride. Catalytic reduction gave 3-amino-4-methylphenylboric acid, from which 3-hydroxy-4-methylphenylboric acid was obtained by diazotization and subsequent hydrolysis. The constitution of the hydroxy acid was established by conversion into *o*-cresol and into 4-methyl-3-hydroxyphenylmercuric chloride.

In the hope of obtaining a boric acid derivative of guaiacol, *p*-methoxyphenylboric acid was subjected to nitration. Fuming nitric acid, even at low temperatures, produced 2,4-dinitroanisole; likewise, in acetic anhydride, elimination of the boric acid residue occurred and 4-nitroanisole resulted. Difficulties were also encountered in the efforts to obtain derivatives of 4-hydroxyphenylboric acid. *p*-Bromophenylboric acid was used as a starting material in an attempt to use the second series of reactions indicated above (b). Nitration with fuming nitric acid at  $-15^{\circ}$  gave 4-bromo-3-nitrophenylboric acid. The position of the nitro group was established through elimination of the boric acid group, and through replacement by bromine. The products were, respectively, 1,2-bromonitrobenzene and 1,4-dibromo-2-nitrobenzene. Various attempts to replace the bromine atom in the nitrated acid by hydroxyl were unsuccessful.

Work is now in progress on the preparation of substituted naphthylboric acids and diphenylboric acids.

### Experimental Part

*n*-Butyl Borate.—This ester was prepared by the interaction of boric anhydride and *n*-butyl alcohol, or merely by heating together boric acid and *n*-butyl alcohol under conditions such that the water produced in the reaction is eliminated continuously from the reaction mixture.<sup>7</sup> From 666 g. (9 moles) of *n*-butyl alcohol and 124 g. (2 moles) of boric acid there was obtained 410–435 g. (90–95% yield) of pure *n*-butyl borate; b. p. 103–105° (8 mm.), 114–115° (15 mm.), 234–238° (745 mm.),  $d_{27}^{27}$ : $\frac{5}{4}$  0.856. Since *n*-butyl borate is hydrolyzed by atmospheric moisture, the product must be manipulated with a minimum exposure to the air. *n*-Butyl borate is more satisfactory than methyl borate for the preparation of organic boric acids since it is difficult to obtain methyl borate entirely free from methyl alcohol.

<sup>7</sup> W. J. Bannister, U. S. Patent 1,668,797, May 8, 1928; *Chem. Abstracts*, 22, 2172 (1928).

**Phenylboric Acid.**—During the course of an hour, 0.25 mole of phenylmagnesium bromide (in approximately 1 molar solution) was added to a solution of 58 g. (0.25 mole) of *n*-butyl borate in 100 cc. of pure ether. During the reaction the solution was stirred mechanically and cooled in a slush of solid carbon dioxide and acetone, so that the internal temperature was maintained between  $-70$  and  $-75^{\circ}$ . As the Grignard reagent dropped into the solution, there formed immediately a white precipitate which slowly dissolved. After all of the Grignard reagent had been added, the reaction mixture was stirred (at  $-75^{\circ}$ ) until all of the precipitate had disappeared. The resulting yellow-orange solution was allowed to warm slowly to  $0^{\circ}$  in the cooling bath (preferably overnight). The reaction mixture was added slowly, while stirring vigorously, to 150 cc. of cold 10% sulfuric acid. The ether layer was separated and the aqueous layer was extracted twice with 100-cc. portions of ether. The original ether layer and the extracts were combined and the ether was removed by distillation from a steam-bath. The residual solution of phenylboric acid in *n*-butyl alcohol was made distinctly alkaline with potassium hydroxide solution and enough water was added to form a layer of about 150 cc. beneath the alcohol. The *n*-butyl alcohol was removed by steam distillation in vacuum by gentle heating (not above  $45^{\circ}$ ); warm water was added slowly from time to time as needed and the distillation was continued until a mere trace of butyl alcohol remained. Toward the end a small amount of diphenyl distilled off, and a small amount of gummy solid separated in the aqueous solution in the distilling flask.

The residual solution was rendered acid to Congo paper by the addition of sulfuric acid and was diluted, if necessary, to a volume of 150-200 cc. Without separating the precipitate, the mixture was heated to the boiling point, with continuous stirring. After a short while the crystalline solid dissolved completely, leaving a heavy insoluble brown oil.<sup>8</sup> The hot aqueous solution was decanted through a fluted filter. The oil was extracted several times with 20-cc. portions of boiling water; the extracts were filtered while hot and added to the main solution. Phenylboric acid crystallized in white needles upon cooling the combined solutions. The product, after drying, weighed 14-17 g. (50-60% yield) and without further purification melted at  $214-216^{\circ}$  (corr.), in a capillary tube. An additional quantity of 1-2 g. of impure acid can be recovered from the mother liquors. When larger quantities were used in the preparation and more concentrated solutions of the Grignard reagent were employed, the yields were somewhat lower (42-47%).

***p*-Tolylboric Acid.**—*p*-Bromotoluene was converted into *p*-tolylmagnesium bromide in the conventional manner, and the method described for the preparation of phenylboric acid was used for the reaction with *n*-butyl borate. The yields of *p*-tolylboric acid were 43-48% of the theoretical (based upon the original *p*-bromotoluene).

*p*-Tolylboric acid crystallizes from water in slender needles, which melt at  $258-259^{\circ}$  (corr.) in a capillary tube heated from  $20^{\circ}$ . During the heating a fogginess appears in the capillary tube at  $100-110^{\circ}$ , apparently due to anhydride formation. On the Maquenne block<sup>9</sup> the substance melted without resolidification at  $259-260^{\circ}$  (decomp.); at lower temperatures it melts and instantly resolidifies, which indicates that the observed melting point is that of the anhydride (reported m. p.  $259^{\circ}$ ).<sup>4</sup>

***p*-Methoxyphenylboric Acid.**—*p*-Anisylmagnesium bromide was prepared from *p*-bromoanisole in the customary manner, in yields of 90-95%. The general procedure described above gave the corresponding boric acid in yields of 30-35%. *p*-Methoxyphenylboric acid crystallizes from water in transparent plates which become opaque

<sup>8</sup> This is stated to contain the bis-arylboric acid,  $(C_6H_5)_2B-OH$ , formed by the action of a second molecule of  $C_6H_5MgBr$  upon  $C_6H_5B(OC_4H_9)_2$ .<sup>4</sup>

<sup>9</sup> Cf. Ref. 2, p. 714, footnote.

on drying in the air. The air-dried acid melted at 208.5–209.5° (corr.) in a capillary tube heated from 20°; on the Maquenne block it melted at 208° (decomp.). König and Scharrnbeck<sup>4</sup> reported the melting point 207° for this acid.

***p*-Bromophenylboric Acid.**—*p*-Bromophenylmagnesium bromide was prepared by the action of a dilute ethereal solution of one mole of *p*-dibromobenzene upon one gram-atom of magnesium. The corresponding boric acid was obtained in the customary manner, in yields of 27–33% of the theoretical (based upon the original *p*-dibromobenzene). Since this acid is much less soluble in water than phenylboric acid, the quantity of water used in the last stage of the preparation was increased from 150 cc. to a volume of 600 cc., and the residual insoluble material was extracted with correspondingly larger quantities of hot water to remove the occluded *p*-bromophenylboric acid. From these solutions the acid crystallized in rough needles which melted at 276–281° (corr.) in a capillary tube heated from 20°. After recrystallization from 20% ethyl alcohol, with the addition of decolorizing charcoal, the substance melted with decomposition at 286–289° (corr.); a cloudiness appeared in the tube at about 100°. On the Maquenne block the compound melted without resolidification at 301–302°, which is probably the melting point of the anhydride since the material melts and resolidifies below this temperature. König and Scharrnbeck<sup>4</sup> reported the melting point 266° for this acid and Krause<sup>10</sup> reported 191°.

A sample of 0.5 g. of *p*-bromophenylboric acid was dissolved in hot water and treated with a concd. aqueous solution of 0.62 g. of mercuric chloride. There was precipitated 0.9 g. (95% yield) of *p*-bromophenylmercuric chloride, which after crystallization from 50% alcohol melted at 250°. This value agrees with the melting point of *p*-bromophenylmercuric chloride obtained from the corresponding sulfinic acid.<sup>11</sup> König and Scharrnbeck<sup>4</sup> reported the melting point 235° for the mercuric chloride obtained from their specimen of *p*-bromophenylboric acid.

**3-Nitro-4-methylphenylboric Acid.**—In a 150-cc. cylindrical Pyrex tube was placed 75 cc. of fuming nitric acid (1.50), to which a little urea had been added. The tube was cooled in a bath of liquid ammonia and 10 g. of finely pulverized, air-dried *p*-tolylboric acid was added slowly, with good mechanical stirring, during the course of one and one-half to two hours. The temperature was maintained at –28 to –30° during this addition and the solution was stirred at this temperature for thirty to forty-five minutes after all of the *p*-tolylboric acid had been added. The reaction mixture was carefully poured onto 150–200 g. of ice and the aqueous suspension was stirred thoroughly. The precipitated nitrotolylboric acid was filtered with suction, washed thoroughly with small portions of iced water, and recrystallized from a small volume of water (with the addition of decolorizing charcoal). The yield of the purified acid was 10 g. (75% of the theoretical). This acid proved to be pure 3-nitro-4-methylphenylboric acid; no isomeric nitro acid was isolated from the reaction products.

3-Nitro-4-methylphenylboric acid crystallizes from water in pale yellow needles; it is sparingly soluble in cold water, soluble in aqueous alkalis, alcohol and ether. In a capillary tube, heated from 20°, the acid darkened at 250° and melted at 260–264° (corr.); on the Maquenne block it melted sharply at 264°. For analysis an air-dried sample was crystallized twice from ethylene chloride. The analytical data indicate that this specimen was the anhydride,  $\text{NO}_2\text{C}_7\text{H}_6\text{B}=\text{O}$ .

*Anal.* Calcd. for  $\text{NO}_2\text{C}_7\text{H}_6\text{B}(\text{OH})_2$ : N, 7.74. Calcd. for  $\text{NO}_2\text{C}_7\text{H}_6\text{BO}$ : N, 8.59. Found: N (Dumas), 8.48, 8.43.

A sample of 0.1 g. of the nitrotolylboric acid was refluxed for one to two hours with an excess of bromine dissolved in aqueous potassium bromide solution. The

<sup>10</sup> Krause, German Patent 371,467; *Chem. Abstracts*, **18**, 992 (1924).

<sup>11</sup> Hanke, *THIS JOURNAL*, **45**, 1321 (1923).

excess bromine was removed by adding a solution of sodium sulfite, and the bromonitrotoluene was purified by steam distillation. The purified product melted at 44.4–45°, and showed no depression of the melting point when mixed with an authentic specimen of 4-bromo-3-nitrotoluene, m. p. 45°.

On treatment with 0.3 g. of mercuric chloride in hot aqueous solution, 0.2 g. of the nitrotolylboric acid gave 0.35 g. of a nitrotolylmercuric chloride. After crystallization from 50% alcohol the product melted at 220–221°, in agreement with the m. p. 220.5–221° reported for 4-methyl-3-nitrophenylmercuric chloride.<sup>12</sup>

**4-Bromo-3-nitrophenylboric Acid.**—Ten grams of pulverized air-dried *p*-bromophenylboric acid was nitrated at –15° according to the procedure described above. The yield of purified product was 5.5–6.0 g. (45–50% of the theoretical). This acid proved to be pure 4-bromo-3-nitrophenylboric acid; no isomeric nitro acid was isolated from the reaction products.

4-Bromo-3-nitrophenylboric acid crystallizes from water in pale yellow needles. It is sparingly soluble in cold water, moderately soluble in hot water; it is soluble in aqueous alkalis and in alcohol and ether. In a capillary tube, heated from 20°, the acid melted at 300–301° (corr.); on the Maquenne block it melted at 320°. Bromine determinations were made by the method of Drogin and Rosanoff,<sup>13</sup> on samples that had been dried in air for ten to twelve hours and then in a desiccator over sulfuric acid for three to four hours. The composition corresponded to that of the acid, RB(OH)<sub>2</sub>, and not the anhydride.

*Anal.* Calcd. for C<sub>6</sub>H<sub>6</sub>O<sub>4</sub>NBrB: Br, 31.24. Found: Br, 31.23, 31.34.

A sample of 0.5 g. of the acid upon treatment with an excess of bromine, in the customary manner, gave 0.3 g. of 1,4-dibromo-2-nitrobenzene. After purification this product melted at 84° and was identified by comparison with an authentic sample of 1,4-dibromo-2-nitrobenzene.

Upon boiling 0.3 g. of the acid with an excess of aqueous silver nitrate solution and steam distilling the mixture, there was obtained 0.2 g. of *o*-bromonitrobenzene. After further purification the product melted at 41.5–42.5°, and showed no depression of the melting point when mixed with an authentic specimen of *o*-bromonitrobenzene, m. p. 42–43°.

***m*-Aminophenylboric Acid.**—A solution of 16.7 g. (0.1 mole) of *m*-nitrophenylboric acid<sup>2</sup> in 40 cc. of methyl alcohol was diluted with 100 cc. of distilled water, and after transferring to a reduction bottle was treated with 0.2 g. of platinum oxide.<sup>5</sup> On shaking with hydrogen under 2–3 atmospheres pressure, in the usual way,<sup>14</sup> the theoretical quantity of hydrogen was taken up in thirty to forty-five minutes. After allowing the catalyst to settle, the solution was decanted through a filter. For the preparation of *m*-hydroxyphenylboric acid the amino acid was not isolated.

A solution of the sulfate of *m*-aminophenylboric acid was prepared by acidifying the filtrate with sulfuric acid and distilling off the methyl alcohol under reduced pressure. Toward the end of the distillation, white crystals of the sulfate separated. To ensure complete removal of methyl alcohol, 100 cc. of water was added and the solution was again evaporated, under reduced pressure, to a small volume. The amount of sodium nitrite solution required for diazotization indicated a yield of about 85–90% of the amino acid.

A small portion of the resulting solution was converted to *m*-benzoylaminophenylboric acid by treatment with benzoyl chloride<sup>2</sup> in the presence of sodium bicarbonate. The product melted at 289° (corr.) in a capillary tube heated from 20°, in good agree-

<sup>12</sup> Coffey, *J. Chem. Soc.*, 129, 639 (1926).

<sup>13</sup> Drogin and Rosanoff, *THIS JOURNAL*, 38, 711 (1916).

<sup>14</sup> "Organic Syntheses," 1928, Vol. VIII, pp. 10, 66.

ment with the reported value (290°). The quantity of the benzoylated product varied in several reductions from 50–53% of the theoretical, based upon the nitro acid.

*o*-Aminophenylboric Acid.—Five grams of *o*-nitrophenylboric acid<sup>2</sup> was reduced catalytically as described above and the theoretical quantity of hydrogen was taken up. After filtering from the catalyst, the solution of the amino acid was evaporated in vacuum (below 30°) in a slow current of hydrogen. The sirupy residue was treated with 150 cc. of pure acetone and the solution was again evaporated, under the same conditions, until a sirup remained. This operation was repeated with two 100-cc. portions of anhydrous ether. The viscous, sirupy mass obtained finally, when allowed to stand overnight, yielded 1.0 g. of almost colorless crystals of the desired amino acid.

Upon recrystallization from anhydrous ether containing a small amount of acetone, *o*-aminophenylboric acid separated in colorless crystals which melted at 179–180° (corr.), in a capillary tube heated from 20°. The acid gives the usual qualitative tests for boron and nitrogen. It is quite soluble in acetone and in methyl alcohol, sparingly soluble in anhydrous ether. The observation that this substance dissolves slowly in water and in aqueous alkalies, indicates that it is an anhydride. By analogy with *m*-aminophenylboric acid, it would be expected to form an anhydride of the formula  $(\text{NH}_2\text{C}_6\text{H}_4\text{BO})_2\text{O}$ .

Solutions of the amino acid decompose on warming or on long standing in the air. The mother liquors from which the acid had crystallized gradually turned brown on being exposed to the air, and after standing for some time had a distinct odor of aniline. On benzoylation there were obtained 1.5 g. of benzanilide, and 0.5 g. of an unidentified substance melting at approximately 270–275°.

For identification, *o*-aminophenylboric acid was benzoylated. The product melted at 283.6–285.6° (corr.), in good agreement with the value, 283–284° (corr.), reported for *o*-benzoylamino phenylboric acid.<sup>2</sup>

**4-Methyl-3-aminophenylboric Acid.**—A solution of 10 g. of 4-methyl-3-nitrophenylboric acid in 40 cc. of methyl alcohol and 100 cc. of water was reduced catalytically as described above, and took up the theoretical quantity of hydrogen. After removal of the catalyst the solution was evaporated to a small volume, under reduced pressure, in a current of hydrogen. The residue was treated with 100 cc. of water and warmed gently until all the crystalline material had dissolved. The solution was heated with decolorizing charcoal for several minutes, at 60–70°, and filtered. The filtrate was concentrated, under reduced pressure, to a volume of about 25 cc.; during this period a considerable quantity of white crystals of the amino acid had separated. These were filtered with suction and weighed 7 g. (85% of the theoretical). The mother liquor upon further concentration gave a small additional quantity (0.2–0.4 g.). The amino acid was purified by recrystallization from hot water; it crystallizes in small white cubes, which soften at 200° and melt at 209–212° (corr.) without apparent decomposition, in a capillary tube heated from 20°. On the Maquenne block it melts slowly at 267–270°, and instantly at 285°.

4-Methyl-3-aminophenylboric acid is soluble in dilute aqueous alkalies, alcohol, and hot water, but is only sparingly soluble in ether. It gives the usual qualitative tests for boron and nitrogen. A sample for analysis was dried for two days over concd. sulfuric acid; the analyses indicate that anhydride formation did not occur.

*Anal.* Calcd. for  $\text{C}_7\text{H}_{10}\text{O}_2\text{BN}$ : N, 9.26. Found: N (Kjeldahl), 9.04, 9.37.

Treatment of the amino acid with acetic anhydride produced an acetyl derivative; after crystallization from hot water this melted at 244–246°, in a capillary tube. On warming the acetyl derivative with silver nitrate solution, *o*-acetotoluidide was obtained. Upon benzoylation the amino acid gave 4-methyl-3-benzoylamino phenylboric acid. The latter melts and resolidifies at 95–100° and remelts at 315° (corr.). The benzoyl

derivative upon boiling with aqueous silver nitrate solution gave *o*-benzotoluide. The latter after purification melted at 143–144° (corr.); a mixed melting point determination with a known sample of benzoyl-*o*-toluidine (m. p. 142.6–143.6°) gave the value 142.6–143.5° (corr.).

***m*-Hydroxyphenylboric Acid.**—An aqueous solution of the sulfate of *m*-aminophenylboric acid was prepared from the product of reduction of 16.7 g. (0.1 mole) of *m*-nitrophenylboric acid, and freed of methyl alcohol as described under *m*-aminophenylboric acid. The solution was diluted with water to a volume of 200–250 cc. and an additional 0.1 mole of 50% sulfuric acid was added. The strongly acid solution was cooled to –2° in an ice-salt mixture and a solution of 0.1 mole of sodium nitrite in 50 cc. of water was introduced slowly beneath the surface, with mechanical stirring. Throughout the addition of the nitrite the temperature was maintained between 0 and –2°, and the rate of inflow was controlled so that no large excess of free nitrous acid was present. If oxides of nitrogen appeared, the addition of the nitrite solution was stopped until a test with starch-iodide paper was negative. Addition of the nitrite solution was continued until the starch-iodide test was positive after standing for five to ten minutes. This usually required nearly all of the nitrite solution. At this point the solution had acquired a reddish-brown color and a small amount of tarry material was present. After the diazotization was completed the slight excess of nitrous acid was destroyed by adding a 20% solution of urea, until the starch-iodide test was negative. The solution of the diazonium salt was immediately hydrolyzed in an apparatus previously prepared for this purpose.

A 3-liter round-bottomed Pyrex flask was fitted with a water-cooled condenser, supported in a vertical position, and a jacketed air-cooled condenser bent at an angle of 30° from the vertical. The upper enlarged end of the inclined condenser was bent back to the vertical, and was fitted with a cork bearing a 150-cc. separatory funnel and a 6-mm. steam inlet tube drawn out to a 2-mm. capillary, which extended 2–3 cm. below the stem of the separatory funnel.

Hydrolysis of the diazonium salt was accomplished by allowing the solution to drop at a fairly rapid rate into a strong current of dry steam which entered through the capillary tube. The diazonium salt was decomposed immediately (with frothing) upon striking the steam and the products of decomposition were blown through the air-cooled condenser into the flask. The time required for adding the diazonium solution was thirty to forty minutes and at the end about 500 cc. of a red solution was present in the flask.

The hot solution was treated with decolorizing charcoal and the warm filtrate was saturated with sodium chloride. This caused the separation of a small amount of tarry material, which was filtered off in a fluted filter. The filtrate was cooled to 20° and extracted with three 150-cc. portions and two 75-cc. portions of ether. The ethereal extracts were evaporated over a water-bath and left a viscous, reddish-brown mass. On standing for several hours this solidified and was broken up into a coarse powder. The weight of the crude product was 4.3 g., which is a 30% yield based upon the original *m*-nitrophenylboric acid.

The crude acid was recrystallized from ethylene chloride containing a small amount of acetone. From the concentrated solution the acid separated in faintly pink crystals. After two or three recrystallizations the pink color was removed entirely and 2.5–3.0 g. of the pure acid was obtained. In a capillary tube heated from 20°, the substance softened at 219° (corr.) and melted with decomposition at 225°; on the Maquenne block it did not melt up to 380°.

*m*-Hydroxyphenylboric acid is very soluble in water, alcohol, acetone and ether; sparingly soluble in ethylene chloride and chloroform. It gives the usual qualitative

test for boron, and gives a bluish-violet color on treatment with ferric chloride solution. An aqueous solution of the acid shows the characteristic reactions with silver nitrate and with mercuric chloride. Analyses indicated that the product obtained by crystallization from ethylene dichloride is a mixture of about equal amounts of the acid and the anhydride  $R-B=O$  or double anhydride of the composition  $(HO-C_6H_4-B-OH)_2O$ .

*Anal.*<sup>15</sup> Calcd. for  $HOC_6H_4B(OH)_2$ : C, 52.21; H, 5.89. Calcd. for  $HOC_6H_4B=O$ : C, 60.07; H, 5.89. Calcd. for  $(HOC_6H_4BOH)_2O$ : C, 55.87; H, 4.69. Found: C, 56.50, 56.63; H, 4.62, 4.82.

A few tenths of a gram of the acid was boiled with an excess of aqueous silver nitrate solution and the mixture was steam distilled. The distillate upon treatment with bromine gave instantly a milky precipitate, which after purification melted at 91–92°. The melting point was not lowered by mixture with known 2,4,6-tribromophenol (m. p. 91.5–92°).

***m*-Hydroxyphenylmercuric Chloride.**—A solution of 0.3 g. of *m*-hydroxyphenylboric acid in 100 cc. of water, at 50°, was treated dropwise with a saturated solution of mercuric chloride, until no further precipitation occurred. After ten minutes the precipitate was filtered with suction and washed with hot water to remove excess mercuric chloride. The yield of *m*-hydroxyphenylmercuric chloride was practically quantitative. The substance was recrystallized from 30% alcohol, from which it separated as a white powder, m. p. 240.5–241.5° (corr.). The isomeric *o*- and *p*-hydroxyphenylmercuric chlorides melt, respectively, at 152.5 and 224–225°.<sup>16</sup>

*Anal.* Calcd. for  $C_6H_5OCIHg$ : Cl, 10.77. Found: Cl, 10.61, 10.66.

**4-Methyl-3-hydroxyphenylboric Acid.**—This acid was obtained from 4-methyl-3-nitrophenylboric acid, by catalytic reduction, diazotization and hydrolysis of the diazonium salt, following the general procedure used for the preparation of *m*-hydroxyphenylboric acid. From 10 g. (0.55 mole) of the nitro acid there was obtained 4 g. (45% yield) of the crude hydroxy acid. After two crystallizations from ethylene chloride + acetone, 3.3 g. of pure material was obtained; m. p. 266–268° (corr.) with decomposition, in a capillary tube. On the Maquenne block the substance does not melt up to 380°.

4-Methyl-3-hydroxyphenylboric acid is moderately soluble in water, from which it crystallizes in small cubes. It is soluble in alcohol, acetone and ether. The acid gives a boron flame test, and gives a violet color with ferric chloride solution. It gives the characteristic reactions with silver nitrate and mercuric chloride. Analyses indicate that the product obtained by crystallization from ethylene chloride is the anhydride,  $R-B=O$ .

*Anal.* Calcd. for  $C_7H_7O_2B$ : C, 62.74; H, 5.27. Found: C, 62.75, 62.64; H, 5.53, 5.46.

On boiling this acid with silver nitrate solution, and following the procedure outlined under *m*-hydroxyphenylboric acid, there was obtained a dibromocresol, m. p. 53–54°. A mixture of this product with authentic 3,5-dibromo-2-hydroxytoluene (m. p. 55°) melted at 54–55°.

**4-Methyl-3-hydroxyphenylmercuric Chloride.**—This substance was prepared in almost quantitative yield from the corresponding boric acid by the method described under *m*-hydroxyphenylmercuric chloride. After recrystallization from 40% alcohol, the pure substance melted at 143.6–144.6° (corr.), with darkening.

*Anal.* Calcd. for  $C_7H_7OCIHg$ : Cl, 10.30. Found: Cl, 10.34, 10.44

<sup>15</sup> Microanalyses by Schoeller.

<sup>16</sup> Dimroth, *Ber.*, 32, 761 (1899).



Bacteriological Tests<sup>17</sup>

**Bactericidal Tests by the Reddish Method.**<sup>18</sup>—Two series of experiments were made with *Staphylococcus aureus*, at 37°, using 5 cc. of the "antiseptic" in the dilutions noted and 0.5 cc. of a twenty-four hour bouillon culture of the organism. In one series the test solution was allowed to act for five minutes, and in a second series for fifteen minutes. The results of these tests are given in Table I.

**Bacteriostatic Tests.**<sup>19</sup>—To each mixture of organisms and "antiseptic" solutions used in the bactericidal tests, an equal volume of standard plain broth was added and the mixture was incubated for forty-eight hours at 37°. The results of these tests are given in Table I.

TABLE I

S <sup>a</sup>	Bactericidal tests <sup>b</sup>										Bacteriostatic tests <sup>b</sup>				
	Five minutes					Fifteen minutes					200	400	1000	2000	4000
	100 <sup>c</sup>	200	500	1000	2000	100	200	500	1000	2000					
I	—	+	+	+	+	—	+	+	+	+	—	—	+	+	+
II		+	+	+	+		+	+	+	+		—	+	+	+
III			—	+	+			—	—	+			—	—	—
IV			+	+	+			+	+	+			—	—	+
V		—	—	+	+		—	—	+	+	—	—	—	—	+
VI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VII	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+
VIII	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+

<sup>a</sup> (I) *p*-Tolylboric acid; (II), *p*-methoxyphenylboric acid; (III), *p*-bromophenylboric acid; (IV), 4-methyl-3-nitrophenylboric acid; (V), 4-bromo-3-nitrophenylboric acid; (VI), 4-methyl-3-aminophenylboric acid; (VII), *m*-hydroxyphenylboric acid; (VIII), 4-methyl-3-hydroxyphenylboric acid.

<sup>b</sup> A minus sign indicates a positive effect (killing or inhibition of growth); a plus sign indicates continued growth.

<sup>c</sup> Dilution of the test solution; *i. e.*, 1/100.

**Discussion of Bacteriological Tests.**—While the chemistry of disinfection—that is, the mechanism of the process—may be explained by a general theory,<sup>20</sup> a consideration of many observations shows that there is little, if any, obvious relation between the antiseptic potency of a sub-

<sup>17</sup> The authors wish to express their thanks to Dr. E. H. Volwiler, Chemical Director, and to Mr. E. B. Carter, Director of Biologic Laboratories, of the Abbott Laboratories, North Chicago, Illinois, for their kind coöperation in carrying out the tests described in this communication.

<sup>18</sup> An antiseptic solution in the dilution recommended must kill a *Staphylococcus aureus* culture, under the conditions of the Reddish test, in five minutes. The culture of *Staphylococcus aureus* when tested against phenol should be killed by a 1:70 dilution in ten minutes, and may or may not be killed by a 1:60 dilution in five minutes.

<sup>19</sup> The term bacteriostatic action denotes the property of hindering the growth of organisms without killing them. This is commonly called an antiseptic effect but does not conform to the official ruling of the Bureau of Chemistry.

<sup>20</sup> Bancroft and Richter, *J. Phys. Chem.*, 35, 511 (1931).

stance and its molecular structure and physical properties. Certain groups introduced into a structure of recognized antiseptic potency (such as phenol) diminish its effectiveness and others augment it. Prediction of the antiseptic value of a particular substance depends upon deductions drawn from analogy and upon empirical rules derived from a large number of observations.

In the present study, *m*-hydroxyphenylboric acid and 4-methyl-3-hydroxyphenylboric acid were of particular interest. They can be regarded from the two aspects: as derivatives of phenol and *o*-cresol, whence the effects of the boric acid group can be compared with that of other substituents, or as derivatives of phenyl and *p*-tolylboric acids, whence the effect of introducing the phenolic hydroxyl can be observed.

Both of these substances were found to have practically no bactericidal action (toward *Staphylococcus aureus*) and to exert very little bacteriostatic effect. From the first point of view, this marked decrease in antiseptic action with the introduction of the boric acid group into the phenolic structure in the *m*-position appears to parallel that produced by a carboxyl, or a second phenolic hydroxyl, in the *m*-position.<sup>21</sup> The relative potency of these hydroxyarylboric acids and other substituted phenylboric acids can be deduced approximately by comparing the maximum dilutions at which the substances exhibit bacteriostatic action. Using phenylboric acid as standard, the effect of the substituents can be expressed simply by means of a "phenylboric acid index,"<sup>23</sup> which is the ratio of the maximum bacteriostatic dilution of the test substance to that of phenylboric acid.

TABLE II  
COMPARISON OF SUBSTITUTED PHENYLBORIC ACIDS

Substituents	Maximum bacteriostatic dilution	Index	Substituents	Maximum bacteriostatic dilution	Index
3-NH <sub>2</sub>	>1:200	<0.5	2-NO <sub>2</sub>	1:2000	5.0
3-NH <sub>2</sub> , 4-CH <sub>3</sub>	>1:200	<0.5	3-NO <sub>2</sub>	1:2000	5.0
3-OH	1:200	0.5	4-NO <sub>2</sub>	1:2000	5.0
3-OH, 4-CH <sub>3</sub>	1:200	0.5	3-NO <sub>2</sub> , 4-CH <sub>3</sub>	1:2000	5.0
4-CH <sub>3</sub>	1:400	1.0	3-NO <sub>2</sub> , 4-Br	1:2000	5.0
4-OCH <sub>3</sub>	1:400	1.0	4-Br	1:4000	10.0
None	1:400	1.00			

<sup>21</sup> *m*-Hydroxybenzoic acid has practically no antiseptic action; resorcinol has a good phenol coefficient (*B. typhosus*) of 0.29.<sup>22</sup>

<sup>22</sup> Morgan and Cooper, *Orig. Com. 8th Intern. Congr. Appl. Chem.*, 19, 243 (1912); *Chem. Abstracts*, 6, 2944 (1912).

<sup>23</sup> This notation is used rather than the conventional phenol coefficient because we were concerned primarily with the effect of introducing substituents into the parent structure, phenylboric acid. The phenol coefficient of phenylboric acid is approximately 0.5 against *B. typhosus* (20°) and *Staph. aureus* (37°).

The relatively high bacteriostatic action of the nitrophenylboric acids and of *p*-bromophenylboric acid indicates that the effect of introducing these substituents into phenylboric acid parallels the effect produced in phenol, since the nitrophenols and *p*-bromophenol have a stronger antiseptic action than phenol itself. The effects are not quantitatively the same since *p*-tolylboric acid has an index of approximately one and *p*-cresol has a phenol coefficient of two. In view of the fact that the indices used in the present work are based upon bacteriostatic effects and phenol coefficients are based upon bactericidal effects, this discrepancy is not surprising.

It is unwise to draw any generalization from the limited numbers of compounds tested but it does not seem unreasonable to assume, as a guide for the selection of other substituted aryl boric acids for study, that the effect of introducing a substituent into phenylboric acid will be of the same general nature as that produced in phenol.

### Summary

3-Hydroxyphenylboric acid and 3-hydroxy-4-methylphenylboric acid were prepared from phenyl- and *p*-tolylboric acids. Several substituted aryl boric acids used in their preparation have been described and characterized.

The preparation of aminoaryl boric acids from the corresponding nitro acids has been improved by the use of catalytic reduction in the presence of platinum. This modification made possible the isolation of *o*-aminophenylboric acid, which had been obtained previously only in solution.

The bacteriostatic effects of a number of substituted phenylboric acids upon *Staphylococcus aureus* were determined. The effect of introducing various substituents appears to be analogous to that produced by the same substituents in phenol.

ITHACA, NEW YORK