Summary

The oxidation of halogenated pyrogallol-2,6-dimethyl ethers leads to the formation of colorless products. The same products are likewise obtained by the further oxidation of phenoxy quinones, derived from the halogenated phenols by oxidation. These colorless compounds differ from previously known quinone oxidation products by virtue of the fact that in their formation by rupture of the quinone ring, only one carbon atom of the ring is lost.

Structures are proposed for these compounds, and the mechanism by which they are formed is discussed.

MINNEAPOLIS, MINNESOTA

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

DERIVATIVES OF PHENYLBORIC ACID, THEIR PREPARATION AND ACTION UPON BACTERIA[1]

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The fact that boric acid acts as a very mild antiseptic agent suggested that, in seeking new types of organic antiseptics, an investigation of the organic boric acids, R-B(OH)$_2$, would be of interest. Michaelis and Becker,[2] who first prepared phenylboric acid, observed that this substance was toxic toward microörganisms and relatively harmless toward higher animals. Notwithstanding the promising indications of this early work, no further investigations have been made upon the antiseptic action of the substituted boric acids. The present study was undertaken with the object of preparing a number of derivatives of phenylboric acid containing nitro and amino groups, and testing their action on microörganisms.

Since none of the usual methods for the preparation of aryl boric acids could be adapted readily to the direct synthesis of the nitrophenylboric acids,[3] it seemed desirable to introduce the nitro group by the nitration of phenylboric acid. This compound is prepared conveniently by the interaction of boron trifluoride[4] or methyl borate[5] and phenylmagnesium bromide.

[1] This article is an abstract of a thesis submitted by William Seaman to the Faculty of the Graduate School of Cornell University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, in March, 1929.

[2] (a) Michaelis and Becker, *Ber.*, **13**, 58 (1880); (b) *ibid.*, **15**, 180 (1882).

[3] An adaptation of the method of preparation of aryl boric acids used by Michaelis and Becker would involve the interaction of boron trichloride or boron tribromide with a bis-(nitrophenyl)-mercury. Although it has been possible to prepare bis-(*m*-nitrophenyl)-mercury (unpublished work of R. S. Shelton in this Laboratory), the relative inaccessibility of such compounds led us to seek other methods.

[4] Krause and Nitsche, *Ber.*, **54**, 2784 (1921); **55**, 1261 (1922); German patent 371,467 [*C. A.*, **18**, 992 (1924)].

[5] (a) Khotinsky and Melamed, *Ber.*, **42**, 3090 (1909); (b) Gilman and Vernon, THIS JOURNAL, **48**, 1064 (1926).

After a preliminary study of the nitration of phenylboric acid, two satisfactory methods of procedure were developed, whereby it was possible to produce the three isomeric nitro compounds. The ortho and meta compounds could be prepared in relatively large amounts but the para isomer was only obtained in very small amounts. Nitration at a low temperature, in the presence of acetic anhydride, with a moderate excess of fuming nitric acid gave a mixture containing about 95% of *o*-nitrophenylboric acid and 5% of *p*-nitrophenylboric acid. The combined products represented yields of about 65% of the theoretical quantities. Nitration with fuming nitric acid alone, at low temperature, led to the production of a mixture of approximately 85% of *m*-nitrophenylboric acid and 15% of the ortho isomer.[6] The combined products in this case represented about 80% of the theoretical yield. The separation of the isomeric nitrophenylboric acids was accomplished by fractional crystallizations from aqueous solutions.

In order to establish the structure of the three isomeric nitrophenylboric acids, the boric acid residue was replaced by the chloromercuri- group,[7] and this in turn by bromine

$$NO_2C_6H_4B(OH)_2 + HgCl_2 + H_2O \longrightarrow NO_2C_6H_4HgCl + HCl + H_3BO_3$$
$$2NO_2C_6H_4HgCl + 2Br_2 \longrightarrow 2NO_2C_6H_4Br + HgCl_2 + HgBr_2$$

Either the nitrophenylmercuric chlorides or the corresponding bromonitrobenzenes were identified by comparison with authentic specimens.

The *o*- and *m*-aminophenylboric acids were obtained by reduction of the nitro compounds by means of freshly precipitated ferrous hydroxide.[8] On account of the small amount of *p*-nitrophenylboric acid at our disposal, the para isomer was not reduced. *m*-Aminophenylboric acid was isolated as a crystalline solid and was converted to the acetyl and benzoyl derivatives. *o*-Aminophenylboric acid was not isolated as such, but was converted to the corresponding benzoyl derivative.

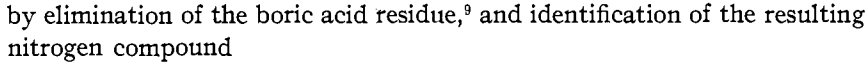
Since the positions of the nitro group in the nitrophenylboric acids had been established, further proof of the orientation of the groups in the

[6] Since the preparation of this manuscript, Ainley and Challenger [*J. Chem. Soc.*, 2176 (1930)] have described the nitration of phenylboric acid to produce *m*-nitrophenylboric acid. These investigators did not isolate the corresponding ortho and para isomers which are described in the present article, but determined the relative proportions of the isomers produced, by examination of the mixed bromonitrobenzenes obtained from them. Their method undoubtedly gives more accurate data concerning this point, but they have dealt only with the nitration technique in which the meta isomer predominates. Their methods of establishing the position of the nitro group were very similar to those used in this investigation.

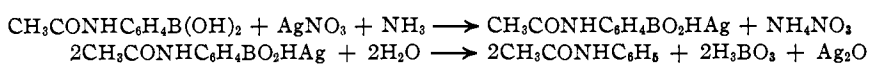
[7] Michaelis and Becker, *Ber.*, 15, 182 (1882).

[8] Subsequently, F. R. Bean, in this Laboratory, has shown that catalytic reduction with the platinum catalyst of Adams and Shriner ("Organic Syntheses," John Wiley and Sons, Inc., New York, Vol. VIII, 1928, p. 92) is a simpler method of effecting this reduction.

amino derivatives was unnecessary. However, in order to confirm the structures of the amino derivatives, it seemed desirable to establish the state of combination of the nitrogen atom. This was accomplished readily by elimination of the boric acid residue,[9] and identification of the resulting nitrogen compound



$$CH_3CONHC_6H_4B(OH)_2 + AgNO_3 + NH_3 \longrightarrow CH_3CONHC_6H_4BO_2HAg + NH_4NO_3$$



$$2CH_3CONHC_6H_4BO_2HAg + 2H_2O \longrightarrow 2CH_3CONHC_6H_5 + 2H_3BO_3 + Ag_2O$$

In the course of this work several attempts were made to devise new methods for the preparation of aromatic boric acids. One of these was an attempt to introduce the boric acid residue directly, by heating aniline with boric acid, in a manner analogous to that used for the preparation of arsanilic acid. It has been shown by Chaudhuri[10] that an anilide of boric acid, $C_6H_5NHB{=}O$, can be obtained by heating aniline with boric acid. None of our efforts to effect a rearrangement of the boric acid group from the side chain into the ring was successful, either with boric anilide or with its acetyl and benzoyl derivatives. Experiments were also made upon the replacement of the sulfinic acid group by the boric acid residue. Sodium benzenesulfinate and *p*-toluenesulfinate were heated with boric acid and with borax, but no trace of an organic boric acid could be observed in the products of these reactions.

Work is now in progress on the preparation of hydroxyphenylboric acids, and of other substituted nitro and amino compounds.

Experimental

Methyl Borate.—For use in the preparation of phenylboric acid, methyl borate should be quite free from methyl alcohol, since the latter caused a marked diminution of the yield, apparently greater than could be accounted for on the basis of the Grignard reagent actually destroyed by the alcohol. Diverse boiling points for methyl borate (from 55 to 72°) are reported in the literature,[11] probably due to the fact that methyl borate forms an azeotropic mixture with methyl alcohol[11d] from which it is relatively difficult to remove the last traces of methyl alcohol. To accomplish this, we employed the method of Schiff,[11b] which consists in washing with cold concd. sulfuric acid. The purified methyl borate obtained thus had the following properties: b. p. 67–67.8° at 750 mm.; d_4^{20} 0.932; n_D^{24} 1.3558.

Phenylboric Acid, C_6H_5-B(OH)$_2$.—Preliminary tests showed that the yields of phenylboric acid were improved by adding the phenylmagnesium bromide to an ethereal solution of methyl borate, instead of the reverse procedure used by Khotinsky and

[9] Michaelis and Becker, *Ber.*, **15**, 181 (1882).

[10] Chaudhuri, *J. Chem. Soc.*, **117**, 1081 (1920).

[11] (a) Gasselin, *Ann. chim. phys.*, [7] **3**, 21 (1894), recorded 55–56°; (b) Schiff, *Ann. Suppl.*, **5**, 160, 184 (1867), reported b. p. 65°, sp. gr. 0.940; (c) Ebelmen, *Ann.*, **60**, 252 (1846), reported b. p. 72°, sp. gr. 0.955; (d) Lecat, *Ann. soc. sci. Bruxelles*, **47B**, I, 63–71 (1927); [*C. A.*, **22**, 4296 (1928)]. At the time when this work was started we were not aware of the work of Lecat on the azeotropic mixture, methyl borate + methyl alcohol.

Melamed.[5] Even this modification did not allow us to attain the yields of 50 and 86% which are claimed by previous investigators.[12]

During the course of forty-five minutes, 0.25 mole of phenylmagnesium bromide (in 1 molar solution) was added to a solution of 26 g. (0.25 mole) of methyl borate in 80 cc. of pure ether. The methyl borate solution was maintained at about −12° during this addition, and the reaction mixture was shaken constantly by hand. After all of the Grignard reagent had been added, the reaction mixture was refluxed gently for fifteen minutes. It was then thoroughly chilled in a freezing mixture, and 125 cc. of a cold 1% solution of sulfuric acid was added cautiously. After shaking thoroughly, the ethereal layer was decanted and the aqueous layer was extracted with four 100-cc. portions of ether. The combined ethereal solutions were dried and the ether distilled off. The solid residue was crystallized from about 70 cc. of water, with the addition of decolorizing carbon. The average yield of a number of runs was 9 g., which is 30% of the theoretical amount. The product obtained in this way was sufficiently pure to be used for nitration.

For more thorough purification, to obtain specimens for determination of physical constants and for bacteriological tests, the substance was recrystallized from petroleum benzene (b. p. 110–120°). The purified material melted at 215.5–219° by the capillary tube method, in a preheated bath; m. p. 221° (corr.) on the Maquenne block.[13]

Michaelis and Becker[2] reported a melting point of 204° for the sample of phenylboric acid that they tested for antiseptic properties; in a later communication the melting point was recorded as 216°.[15] This discrepancy, together with the fact that they reported that phenylboric acid has a pleasant taste and a phenolic odor, leads us to believe that they did not work with perfectly pure material. Our purified specimens were odorless and tasteless, and showed much less antiseptic action.

The residues from the mother liquors which remained after as much as possible of the phenylboric acid had been recovered, contained a yellow solid which melted incompletely near 300°. This material gave a qualitative test for boron[16] and responded to the usual tests for aromatic boric acids with silver nitrate and with mercuric chloride. This material was not examined further; it may consist in part of diphenylboric acid.[15]

***m*-Nitrophenylboric Acid.**—In an ice–salt freezing mixture, 75 g. of colorless fuming

[12] More recent work of F. R. Bean, in this Laboratory, using *n*-butyl borate and effecting the reaction at a temperature of −50 to −60°, gave yields of 50–60% of the theoretical.

[13] The capillary tube melting points of the aromatic boric acids were found to be greatly influenced by the manner of heating, since the substances are capable of undergoing dehydration and other decomposition in the course of heating. The melting points of these substances are much more characteristic and more constant if the Maquenne block [Houben–Weyl, "Die Methoden der organischen Chemie," Georg Thieme, Leipzig, 1921, Vol. I, pp. 734–735] is used, so that preliminary heating is avoided. Since the melting points observed on the Maquenne block frequently differ from those obtained by the usual methods, the values are not directly comparable. In Table I, at the end of the experimental section, are tabulated the various melting points of the principal compounds described in this article. A very convenient and elegant modification of the Maquenne block has been devised in this Laboratory.[14]

[14] Dennis and Shelton, THIS JOURNAL, **52**, 3128 (1930).

[15] Michaelis and Behrens, *Ber.*, **27**, 244 (1894).

[16] Two qualitative tests for boron were used in connection with this work: (1) observation of the green color imparted to a Bunsen flame when the compounds were burned upon a foil, and (2) fusion with metallic sodium, treating with slightly acidulated water and testing the solution with turmeric paper.

nitric acid (sp. gr. 1.50), to which a little urea had been added, was cooled to −15°. During one and one-half hours, 10 g. of finely powdered, dry phenylboric acid was added, with good mechanical stirring, so that the temperature did not rise above −9°. The mixture was stirred for ten minutes after all the phenylboric acid had been added, and was then poured onto 50 g. of ice.[17] The precipitated nitrophenylboric acid (I) was filtered with suction, and recrystallized from a small volume of water, with the addition of decolorizing carbon.

The original filtrate was cooled in an ice-bath, neutralized to congo red with strong sodium hydroxide solution, and then slightly acidulated with nitric acid. The solution was extracted thoroughly with ether, and the solvent evaporated off at room temperature. The residue was fractionally crystallized from a small volume of water, with the addition of decolorizing carbon. The crystals (II) which separated before the solution had cooled completely (30°) consisted chiefly of *m*-nitrophenylboric acid, and were combined with the original precipitate (I) and subjected to further purification. The substance which crystallized upon cooling the filtrate from II, in an ice-bath, consisted largely of *o*-nitrophenylboric acid. The latter was purified by crystallization from a very small volume of water. The average yield of *m*-nitrophenylboric was 10 g. (70% of the theoretical); that of *o*-nitrophenylboric acid was 1.5 g. (10% of the theoretical).

m-**Nitrophenylboric Acid.**—This substance forms yellow prismatic crystals. It is slightly soluble in cold water, moderately soluble in hot water, soluble in alcohol and ether. It dissolves in aqueous sodium hydroxide, sodium carbonate and ammonia. It gives the characteristic tests for aromatic boric acids with silver nitrate and with mercuric chloride, and the usual qualitative tests for boron. The air-dried purified *m*-nitrophenylboric acid melted with charring at 275–276.5° (corr.) in a capillary tube placed in a bath preheated to about 250°, and at 273° (corr.) in a bath which was not preheated.[18] On the Maquenne block the melting point was 319° (corr.).

After drying to constant weight in a desiccator, the substance melted with charring at 285–286° (corr.) in a bath not preheated. This is evidently the melting point of the anhydride, $NO_2C_6H_4B{=}O$, since the analytical data on this sample agreed with the theoretical values for the anhydride. All samples for analysis were dried *in vacuo* over concd. sulfuric acid.[19]

Anal. Calcd. for $NO_2C_6H_4BO$: C, 48.37; N, 9.41. Calcd. for $NO_2C_6H_4B(OH)_2$: C, 43.14; N, 8.39. Found: C, 48.05, 48.17; N, 9.90, 9.75.

Determination of the Position of the Nitro Group.—A hot aqueous solution of 1 g. of the nitrophenylboric acid was treated with a concd. aqueous solution of 1.8 g. of mercuric chloride. The resulting precipitate of nitrophenylmercuric chloride was washed with water, and treated with an excess of a solution of bromine in aqueous potassium bromide. The mixture was shaken for two and one-half hours at room temperature and extracted with ether. The residue, after removal of the solvent, was washed free from bromine and recrystallized from 50% alcohol; m. p. 53.7–54.2° (corr.). An authentic specimen of *m*-bromonitrobenzene (Kahlbaum), used for comparison,

[17] A small amount of nitrobenzene is always formed in these nitrations; if the reaction is carried out at higher temperatures, nitrobenzene and boric acid are produced as the principal products.

[18] Ainley and Challenger, Ref. 6, report m. p. 273° for *m*-nitrophenylboric acid, but do not state the conditions of the melting point determination.

[19] Carbon determinations were made by the Parr total carbon method, which did not prove entirely satisfactory. Ainley and Challenger (Ref. 6) reported that the usual combustion method gave very low results for carbon. The nitrogen determinations were made by the Dumas method.

melted at 54.0–54.2° (corr.); a mixture of the two substances melted at 53.7–54.2° (corr.). The formation of *m*-bromonitrobenzene establishes the structure of the above acid as *m*-nitrophenylboric acid.

o-Nitrophenylboric Acid.—Six grams of colorless fuming nitric acid (sp. gr. 1.50), to which a few crystals of urea had been added, was allowed to drop slowly into a suspension of 10 g. of phenylboric acid in 90 cc. of acetic anhydride, cooled to −15°. Mechanical stirring was used throughout the addition, which required about forty-five minutes, and the mixture was stirred for about one and one-half hours after all of the nitric acid had been added. During this period the temperature was allowed to rise slowly to −7°; the freezing-bath was then removed and the stirring was continued until all of the acid had dissolved (about one hour). Occasional cooling was used to keep the temperature below +20°. The reaction mixture was poured slowly into 200 cc. of ice water and the whole was agitated until a clear homogeneous solution was obtained. The solution was concentrated to a small volume at 50°, under reduced pressure; 100 cc. of water was added and the concentration repeated.[20] After cooling, and standing for ten to twelve hours, about 0.3 g. of a crystalline nitrophenylboric acid (III) separated. This was removed by filtration and reserved for examination (see below, *p*-nitrophenylboric acid). The quantity of this product was about 2% of the theoretical yield.

The filtrate was concentrated further, at 50° under reduced pressure, with repeated additions of water, until most of the acetic acid was removed and crystals had separated in the solution. These were separated by filtration, and the mother liquor was concentrated further, until it was reduced to a very small volume After separating the crystals, the final filtrate was made alkaline to congo red with sodium carbonate, slightly acidified with hydrochloric acid, and extracted with ether. Evaporation of the ether extract gave a residue which was combined with the main fractions of *o*-nitrophenylboric acid and recrystallized from a very small amount of water. Approximately 8 g. of crude *o*-nitrophenylboric acid was obtained (60% of the calculated quantity). This product was sufficiently pure for subsequent syntheses; for further purification the acid was washed with hot carbon tetrachloride and recrystallized from water.

o-Nitrophenylboric acid crystallizes from water in yellow needles. It is moderately soluble in water; soluble in alcohol and ether. *o*-Nitrophenylboric acid dissolves in aqueous solutions of sodium hydroxide, sodium carbonate and ammonia; it gives the usual qualitative tests for boron. The air-dried substance melted at 139.2–140.8° (corr.), with darkening, in a bath heated from 20°; m. p. 138.7–139.2° (corr.), in a bath preheated to 120°; m. p. 147.5° on the Maquenne block. After drying to constant weight in a vacuum desiccator, over sulfuric acid, the substance melted at 143.5–147.7° (corr.), in a preheated bath. The vacuum dried substance, evidently the anhydride $NO_2C_6H_4B{=}O$, was analyzed.

Anal. Calcd. for $NO_2C_6H_4BO$: C, 48.37; N, 9.41. Calcd. for $NO_2C_6H_4B(OH)_2$: C, 43.14; N, 8.39. Found: C (Parr bomb),[21] 46.14, 45.97; N (Dumas), 9.26, 9.62.

Determination of the Position of the Nitro Group.—A hot aqueous solution of 0.38 g. of the above acid was treated with a concd. solution of 0.62 g. of mercuric chloride and heated for five minutes. The resulting nitrophenylmercuric chloride was removed by filtration and crystallized three times from alcohol; m. p. 183.4–184.5° (corr.). An

[20] During this process a small quantity of a green oil, possibly a nitroso compound, distilled over. The quantity of the material was too small for identification.

[21] The results for carbon are undoubtedly low, since it seemed impossible to achieve a complete combustion in the Parr peroxide bomb. There were always a few specks of unoxidized carbonaceous matter in the fused mass.

authentic specimen of *o*-nitrophenylmercuric chloride, prepared according to the method of Dimroth,[22] melted at 181.3–183.4° (corr.); a mixture of the two substances melted at 183.4–184.5° (corr.).

p-Nitrophenylboric Acid.—The less soluble fractions (III) of the acid obtained from the nitrations in acetic anhydride were combined and recrystallized from water, with the addition of decolorizing carbon. As a very small amount of this compound was obtained, no analyses were made as it seemed more essential to establish the structure of the compound by conversion to a known compound, and to reserve the remainder for a bacteriological test. The substance gives the usual qualitative tests for boron and for nitrogen.

p-Nitrophenylboric acid crystallizes from water in long, slender, yellow needles. It is slightly soluble in cold water, and is the least soluble of the three isomers; it is soluble in hot water, alcohol and ether. Like the other acids, it dissolves in aqueous solutions of sodium hydroxide, sodium carbonate and ammonia. *p*-Nitrophenylboric acid melts, with decomposition and charring, at 305° (corr.) in a capillary tube in a bath heated from 20°; on the Maquenne block the substance did not melt up to 360°.

Determination of the Position of the Nitro Group.—By the method given for *m*-nitrophenylboric acid, 0.2 g. of the nitrophenylboric acid described above was converted to a bromonitrobenzene. The latter, after crystallization from 50% alcohol melted at 122.8–124.4° (corr.). An authentic specimen of *p*-bromonitrobenzene (Kahlbaum) melted at 126.4–127.4° (corr.); a mixture of the two substances melted at 125.9–126.9° (corr.).

m-Aminophenylboric Acid.—Reduction of the nitro acids was effected by the use of freshly precipitated ferrous hydroxide, according to a modification of the procedure of Claisen and Thompson, and Jacobs, Heidelberger and Rolf.[23]

In a 1-liter flask (or bottle), 71 g. of ferrous sulfate crystals (95% $FeSO_4 \cdot 7H_2O$ as determined by titration) was dissolved in 250 cc. of air-free water; a suspension of 81 g. of hydrated barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$) in 150 cc. of water was added in several portions, the mixture was shaken thoroughly until it reacted alkaline to litmus, and a uniform suspension of ferrous hydroxide was obtained. A uniform suspension of 12 g. of hydrated barium hydroxide and 6 g. of *m*-nitrophenylboric acid in 125 cc. of air-free water was added in one portion to the ferrous hydroxide, and the mixture was shaken vigorously for ten minutes, during which the green color of the ferrous hydroxide changed to the brown of ferric hydroxide. The insoluble material was filtered off with suction, suspended in water and washed thoroughly. The filtrate and washings were united and the barium ions were removed by exact precipitation with sulfuric acid, so that a test portion of the filtered solution gave no turbidity with either sulfuric acid or barium hydroxide solution. The filtered solution was concentrated at 50°, under reduced pressure, in an atmosphere of nitrogen. The resulting yellow crystals of the crude *m*-aminophenylboric acid were manipulated, as far as practicable, in an inert atmosphere. The filtrate from the main crop of crystals could be concentrated further to obtain a small additional quantity of the amino acid; it proved more satisfactory, however, to make use of this solution for the preparation of the benzoyl derivative. The yield of crude *m*-aminophenylboric acid was 2.2–2.4 g. (44–48%), and 0.3–0.5 g. of the benzoyl derivative was obtained from the mother liquors. The crude amino acid was recrystallized from a very small volume of water (in an atmosphere of nitrogen), with the addition of decolorizing carbon. The purified product formed pale yellow hexagonal plates; the slight tinge of color could not be removed by several recrystallizations.

[22] Dimroth, *Ber.*, **35**, 2036 (1902).

[23] (a) Claisen and Thompson, *ibid.*, **12**, 1946 (1879); (b) Jacobs, Heidelberger and Rolf, This Journal, **40**, 1580 (1918).

Undoubtedly the pure compound is colorless; the color of our product was probably due to the merest traces of impurity, since the substance had a fairly sharp melting point. In a capillary tube, either from a bath at 20° or a preheated bath, the substance melted at 92.8–93.8° (corr.), and resolidified if kept just above this temperature; upon further heating, the compound melted indefinitely, with darkening, near 200°. On the Maquenne block the material melted sharply at 176° (corr.). *m*-Aminophenylboric acid is soluble in water and alcohol, sparingly soluble in ether.

For analysis the substance was dried to constant weight in a vacuum desiccator (ten days) over sulfuric acid. After drying the melting point was 193° (corr.) on the Maquenne block; the dried substance melted indefinitely at about 168° (with darkening), in a capillary tube in a bath heated from 20°.

Anal. Calcd. for $NH_2C_6H_4B(OH)_2$: N, 10.23. Calcd. for $NH_2C_6H_4BO$: N, 11.78. Calcd. for $(NH_2C_6H_4BOH)_2O$: N, 10.95. Found: (Kjeldahl) N, 11.11, 11.12.

The nitrogen determinations indicated that the completely dried sample was an anhydride formed by the elimination of one molecule of water from two molecules of the acid.[24] The air-dried substance was undoubtedly the acid and not an anhydride, since it dissolved immediately in water and gave the typical tests for aromatic boric acids. Experience with the anhydrides of various boric acids indicates that the anhydrides do not react at once with cold water to regenerate the acids.

Since the position of the amino group was established by the relationship to *m*-nitrophenylboric acid, *m*-aminophenylboric acid was characterized merely by conversion to the acetyl and benzoyl derivatives, and by the identification of aniline after removal of the boric acid residue.

Elimination of the Boric Acid Residue.—To a slightly ammoniacal aqueous solution of 0.2 g. of the substance, an excess of 5% aqueous silver nitrate solution was added; the resulting precipitate of the silver salt was filtered and washed with water. This was suspended in water and boiled gently for fifteen minutes to remove completely the boric acid group, and the aniline was recovered by steam distillation. Aniline was identified by the following tests: (1) development of a transient violet color with calcium hypochlorite solution; (2) formation of a colorless precipitate with bromine water; (3) conversion to an alkali-soluble benzene sulfonamide, m. p. 107.3–110.3° (corr.), which showed no depression of the melting point when mixed with an authentic specimen of benzenesulfonanilide, m. p. 109.3–111.3° (corr.).

Acetyl-*m*-aminophenylboric Acid.—A solution of the amino acid obtained by the reduction of 6.8 g. of *m*-nitrophenylboric acid was evaporated almost to dryness and treated with 25 g. of acetic anhydride. The mixture became warm and the solid particles dissolved. The acetic acid was removed by distillation at 50°, in an atmosphere of nitrogen, by addition of small portions of water and repeated evaporations. The acetyl derivative separated from the solution as the acetic acid was removed. The first crop of crystals, amounting to 2.2 g., was colorless; an additional amount, 1.5 g., of a slightly colored product was recovered from the mother liquors. Both fractions were purified by recrystallization from a small volume of hot water, with the addition of decolorizing carbon. The total yield of the crude product was 3.7 g., which is 56% of the theoretical amount calculated from the *m*-nitrophenylboric acid.

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[24] An explanation of the formation of this type of anhydride is that the amino acid may exist as a salt (as do glycine and sulfanilic acid) formed by intramolecular neutralization; thus, the elimination of a molecule of water can only occur between two molecules:

$$2\overset{+}{N}H_3—C_6H_4—B\overset{-}{O}—OH \xrightarrow{-H_2O} \overset{+}{N}H_3—C_6H_4—B\overset{-}{O}—O—B\overset{-}{O}—C_6H_4—\overset{+}{N}H_3$$

Acetyl-*m*-aminophenylboric acid crystallizes from water in colorless prismatic plates; it is moderately soluble in water, alcohol and ethyl acetate, but almost insoluble in ether. It dissolves in aqueous alkalies. In a capillary tube the substance melted, with darkening, at 274–275° (corr.) when heated from 20°. On the Maquenne block the compound melted and immediately resolidified, without decomposition, at about 160°. The transition temperature could not be determined accurately, as only the smallest particles (viewed through a magnifying glass) could be seen to melt completely and the resolidification was almost instantaneous. The air-dried product lost no appreciable weight in a vacuum desiccator over sulfuric acid.

Anal. Calcd. for $C_8H_{10}O_3NB$: N, 7.83. Found: (Kjeldahl) N, 7.72, 7.64.

The acid gives the usual qualitative tests for boron, and the characteristic precipitates with aqueous solutions of mercuric chloride and of silver nitrate. Elimination of the boric acid group, effected by heating the silver salt in water, gave acetanilide, m. p. 112.3–114.3 (corr.), identified by comparison with an authentic specimen.

Benzoyl-*m*-aminophenylboric Acid.—A concd. solution of the amino acid obtained by the reduction of 2.0 g. of *m*-nitrophenylboric acid was treated with 2 g. of sodium bicarbonate and benzoylated with 2 g. of benzoyl chloride. The crude pale yellow benzoyl derivative weighed 1.2 g. (42% of the theoretical yield, based on the nitro acid). A preliminary purification was effected by dissolving the crude acid in dilute alkali, filtering and reprecipitating by pouring into dilute hydrochloric acid. A final purification was made by crystallization from 30% alcohol, with the addition of decolorizing carbon.

Benzoyl-*m*-aminophenylboric acid crystallizes from dilute alcohol in colorless prisms; it is almost insoluble in cold water and in ether, soluble in hot water and in alcohol. It is completely soluble in dilute sodium hydroxide solutions but only slightly soluble in sodium bicarbonate and sodium carbonate solutions. The air-dried substance melted in a capillary tube, with darkening, at 290° (corr.) in a bath heated from 20°; on the Maquenne block the substance melted with immediate resolidification, at about 200° (corr.). As the behavior of this compound was very similar to that of the acetyl derivative, it was not possible to determine accurately the temperature of the transition point. An air-dried sample did not lose weight in a vacuum desiccator over sulfuric acid.

Anal. Calcd. for $C_{13}H_{12}O_3NB$: N, 5.82. Found: (Kjeldahl) N, 5.59, 5.80.

This acid gives the usual qualitative tests for boron, and the characteristic precipitates with aqueous solutions of mercuric chloride and of silver nitrate. Elimination of the boric acid group, effected by heating the silver salt in water, gave benzanilide, m. p. 163.3–164.4° (corr.), which was identified by comparison with an authentic sample. Hydrolysis with hot concd. aqueous sodium hydroxide (one and one-half hours) gave benzoic acid, m. p. 121.3–122.3° (corr.). An attempt to hydrolyze the benzoyl derivative by boiling for one-half hour with strong potassium hydroxide in 90% alcohol was unsuccessful.

Benzoyl-*o*-aminophenylboric Acid.—Although several attempts to isolate *o*-aminophenylboric acid and its acetyl derivative were unsuccessful,[25] it was possible to obtain benzoyl-*o*-aminophenylboric acid. *o*-Nitrophenylboric acid was reduced by the same general procedure as the meta isomer, except that the reduction was effected at 95–

[25] Our experiments indicate that *o*-aminophenylboric acid must be present in the solution obtained by reducing *o*-nitrophenylboric acid. Attempts to isolate the *o*-amino compound yielded phenylboric acid, due evidently to a reductive deamination; phenylboric acid was isolated likewise when the crude reduction product was treated with acetic anhydride in an effort to obtain the acetyl derivative. In one experiment, after treatment with acetic anhydride, a small amount of boric acid was isolated.

100°, and the reaction mixture was boiled gently for five minutes before filtering off the precipitated ferric hydroxide and barium sulfate. The filtrate, containing the excess of barium hydroxide, was divided into three portions for benzoylation. Each portion was treated with 1 g. of solid sodium bicarbonate and 1 g. of benzoyl chloride, and shaken thoroughly until there was no odor of benzoyl chloride. Upon acidifying with hydrochloric acid, the crude benzoyl derivative separated in yellow crystals. The yield was 1.6 g. from 3 g. of the nitro acid (37% of the theoretical). The crude product was purified by crystallization from 30% alcohol, with the addition of decolorizing carbon.

Benzoyl-*o*-aminophenylboric acid crystallizes from dilute alcohol in colorless, transparent platelets. It is very slightly soluble in cold water and ether, soluble in hot water and alcohol. It dissolves completely in dilute sodium hydroxide solution, but not in sodium carbonate or bicarbonate solutions. It gives the usual qualitative tests for nitrogen and boron, and the characteristic tests with mercuric chloride and with silver nitrate solutions. In a capillary tube, immersed in a bath preheated to 200°, the substance melts with darkening at 283–284° (corr.); on the Maquenne block the melting point was 319.5° (corr.), with darkening. The air-dried product did not lose weight in a vacuum desiccator over sulfuric acid.

Anal. Calcd. for $C_{13}H_{12}O_3NB$: N, 5.82. Found: (Kjeldahl) N, 5.92, 5.92.

The compound was characterized by elimination of the boric acid residue in the usual way. The boron-free product, m. p. 161.5–162° (corr.), was identified as benzanilide.

TABLE I

MELTING POINTS OF SUBSTITUTED BORIC ACIDS

Substituted boric acid, $-B(OH)_2$	M. p., °C. (corr.), capillary tube method: From bath at 20°	From preheated bath[a]	M. p., °C. (corr.), Maquenne block
Phenyl	210.6–212.6	215.5–219.4	221
m-Nitrophenyl	273 (dec.)	275–276.5 (dec.)	319 (dec.)
o-Nitrophenyl	139.2–140.8	138.7–139.2	147.5
p-Nitrophenyl	305.5 (dec.)	....	Above 360
m-Aminophenyl	92.8–93.8[b]	92.8–93.8[b]	176
Acetyl-*m*-aminophenyl	274–275	....	*circa* 170[b]
Benzoyl-*m*-aminophenyl	290	....	*circa* 200[b]
Benzoyl-*o*-aminophenyl	....	283–284	319.5

[a] In these determinations the bath was heated to a temperature about 10° below the melting point, before immersion of the capillary tube. [b] Resolidifies.

Bacteriological Tests[26]

For the bacteriological tests the substances to be examined were prepared in a high state of purity, as evidenced by constant melting points after repeated crystallizations and homogeneous appearance under the microscope. The first experiments were made with *Staphylococcus aureus*, using the Reddish test. Five cc. of the antiseptic in the dilution noted, was added to 0.5 cc. of a twenty-four hour bouillon culture of *Staphylococcus aureus*. This *Staphylococcus aureus* when tested against phenol should be

[26] The authors wish to express their thanks to Dr. E. H. Volwiler, Chemical Director, and to Dr. J. F. Biehn, Director of the Department of Clinical Research, of the Abbott Laboratories, North Chicago, Illinois, for their kind coöperation in carrying out the bacteriological tests described in this communication.

killed by a 1:70 dilution in ten minutes, and may or may not be killed by a 1:60 dilution in five minutes.[27]

The following compounds were tested in aqueous solution at a dilution of 1:1000: phenylboric acid, *o*- and *m*-nitrophenylboric acids sodium *m*-aminophenylborate, sodium benzoyl-*m*-aminophenylborate and sodium benzoyl-*o*-aminophenylborate. The results were entirely negative, since the organisms were not killed by any of these solutions, even after fifteen minutes. Similar experiments were made using *B. typhosus* in place of *Staphylococcus*, and the following compounds were tested, at dilutions of 1:1000: boric anilide ($C_6H_5NHB{=}O$), acetyl-*m*-aminophenylboric acid, and *p*-nitrophenylboric acid; phenylboric acid and boric acid itself were tested at concentrations of 1:200. In this series the tests were allowed to run for two hours. The only positive result was obtained with phenylboric acid (1:200), which killed the organisms within one hour.

Since the preliminary tests indicated a much weaker action than had been anticipated, a milder test was used to enable us to determine the effect of various substituents upon the bacteriostatic action of phenylboric acid. For this purpose the following modified technique was used. To 5 cc. of the test solution were added 5 cc. of plain bouillon and 0.1 cc. of a bouillon culture of *Staphylococcus aureus*. These cultures were incubated for forty-eight hours, after which they were examined to determine whether or not a bacteriostatic effect[28] had been produced. The results of these tests, shown in Table II, led us to make a more complete examination of three compounds (phenylboric acid, *m*-nitrophenylboric acid, and *m*-aminophenylboric acid), against *Staphylococcus aureus*. The same modified technique for bacteriostatic action was used, and the following results were obtained: (1) phenylboric acid showed bacteriostatic action in dilutions of 1:400 to 1:450, but not at dilutions from 1:475 to 1:1500; (2) *m*-nitrophenylboric acid showed bacteriostatic action in dilutions of 1:500 to 1:2400, but not at a dilution of 1:3200; (3) *m*-aminophenylboric acid showed no bacteriostatic action in dilutions ranging from 1:400 to 1:3200.

Solutions of these three compounds were also tested in concentrations of 1:200, using 5 cc. of the test solution and 0.1 cc. of a twenty-four hour bouillon culture of *Bacillus typhosus*. *m*-Nitrophenylboric acid killed these organisms within five minutes, phenylboric acid in ninety minutes and *m*-aminophenylboric acid did not kill them within two hours.

[27] To be labeled as an antiseptic solution, under the ruling of the Bureau of Chemistry, the antiseptic solution in the dilution recommended, must kill this *Staphylococcus* culture, under the conditions of the above test, in five minutes.

[28] A bacteriostatic effect is commonly called an antiseptic effect. According to a ruling of the U. S. Department of Agriculture, the terms antiseptic and germicide are synonymous, and the previously stated definition of antiseptic is employed. The term bacteriostatic denotes the property of hindering the growth of organisms without killing them.

TABLE II

TESTS FOR BACTERIOSTATIC ACTION

Substance	Dilution[a]	Result[b]
Phenylboric acid	1:2000	−
m-Nitrophenylboric acid	1:2000	+
o-Nitrophenylboric acid	1:2000	+
p-Nitrophenylboric acid	1:2000	+
Sodium *m*-aminophenylborate	1:2000	−
Sodium benzoyl-*m*-aminophenylborate	1:2000	−
Sodium benzoyl-*o*-aminophenylborate	1:2000	−
Acetyl-*m*-aminophenylboric acid	1:2000	−
Boric anilide	1:2000	−
Phenylboric acid	1:400	+
Boric acid	1:400	−
Boric acid	1:50	+
Control	..	−

[a] Five cc. of the test solution was mixed with 5 cc. of bouillon and 0.1 cc. of a culture of *Staphylococcus aureus*, and incubated for forty-eight hours.

[b] A minus sign indicates continued development of the organisms; a plus sign denotes arrested development (bacteriostatic action).

Discussion of Bacteriological Tests

It appears that the claims of Michaelis and Becker[2] for the antiseptic efficacy of phenylboric acid were exaggerated. Aside from the question of purity of the phenylboric acid used in their work, it should be pointed out that their tests were made on heterogeneous mixtures of bacteria of unknown vitality, whereas our samples were tested on pure cultures by means of a standardized procedure.

Although none of the substances examined was found to be a powerful antiseptic, several of them possessed marked bacteriostatic properties. The introduction of an amino group (in the meta position) into the molecule of phenylboric acid resulted in a practically complete loss of bacteriostatic action, while the introduction of a nitro group markedly increased the bacteriostatic action.[29]

The fact that phenylboric acid and the derivatives here reported do not act as general antiseptics, does not preclude the possibility that they may have a specific action on certain organisms. This point remains to be tested. Since some of these compounds are soluble both in ether and in water, they may be expected to possess good penetrating power. It is possible that the introduction of a more powerfully antiseptic group (such as a phenolic hydroxyl) into the aromatic boric acid may prove fruitful, since the phenylboric acid residue may serve to give the molecule a satisfactory penetrating power.

[29] This effect of the nitro group is to be expected, since the nitrophenols are more powerful antiseptics than phenol [Morgan and Cooper, Orig. Com. 8th Intern. Congr. Appl. Chem., **19**, 243 (1912); *C. A.*, **6**, 2944 (1912)].

Summary

Phenylboric acid was prepared in a pure state, and the three isomeric nitrophenylboric acids were obtained from this acid by nitration under appropriate conditions. *m*-Aminophenylboric acid, its acetyl and benzoyl derivatives, and the benzoyl derivative of *o*-aminophenylboric acid were prepared. The physical characteristics of these substances are reported.

Bacteriological tests showed that phenylboric acid and the three nitrophenylboric acids exert a bacteriostatic effect upon *Staphylococcus aureus;* *m*-aminophenylboric acid and its derivatives do not produce a bacteriostatic effect.

The nitrophenylboric acids are more highly bacteriostatic than phenylboric acid, which in turn is much more effective than boric acid. Although no direct comparison was made, it appears that *m*-nitrophenylboric acid (1:200) approaches or slightly surpasses phenol (1:70) in its bactericidal action toward *B. typhosus*, but phenylboric acid (1:200) is much weaker than phenol (1:70).

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THE EFFECT OF ETHYLENE UPON THE HYDROLYSIS OF SALICIN BY EMULSIN

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The nature of the transformations brought about by the use of ethylene in the preparation of fruits and vegetables for marketing and the mechanism of the action continue to attract attention. It is generally believed that the changes produced are due to acceleration of enzymic activity. In an earlier paper[1] a brief consideration of the subject from this point of view, along with the results of experiments as to the effect of ethylene upon diastase and invertase with pure substrate material, was presented. No significant difference between the ethylene-treated and untreated materials was observed. The enzymes investigated were selected as representative of those concerned with normal ripening changes of carbohydrates and the original plan of experimentation anticipated studies upon other enzymes more definitely associated with color changes. It has been pointed out by Harvey[2] that an overdose of ethylene causes bananas to become brown. This coloration may be similar to leaf darkening and result from an increased hydrolysis of some glucoside followed by oxidase action upon the non-sugar component. It also seems probable that other coloration phenomena might have, as an initial stage, the change of a

[1] Englis and Zannis, THIS JOURNAL, 52, 797 (1930).

[2] Harvey, Bulletin 247, U. of Minn., Agr. Exptl. Sta., October, 1928.