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

Aromatic Boronic Acids.
Synthesis of o-Boronophenylalanine.[1]

J. R. KUSZEWSKI, W. J. LENNARZ AND H. R. SNYDER

Syntheses of N-acetyl-o-boronophenylalanine, α-amino-o-boronobenzylmalonic anhydride and o-boronophenylalanine anhydride are described. Alkylation of diethyl acetamidomalonate with o-(bromomethyl)-benzeneboronic anhydride yields o-(2-carbethoxy-2-acetamidoethyl)-benzeneboronic acid rather than the expected o-(2,2-dicarbethoxy-2-acetamidoethyl)-benzeneboronic acid. Decarbethoxylation is explained by postulating that the boronic acid function participates in ester hydrolysis. Decarboxylation of α-amino-o-boronobenzylmalonic anhydride requires an unusually high temperature; this observation is interpreted in terms of a bridged, polycyclic structure. The decarboxylation product, o-boronophenylalanine anhydride, does not possess a zwitterionic structure; this fact indicates that interaction between the amino group and the boron atom occurs.

The synthesis of p-boronophenylalanine (IV) was reported recently.[2] p-(Bromomethyl)-benzeneboronic acid was condensed with sodio diethyl acetamidomalonate and the product was saponified, decarboxylated and hydrolyzed according

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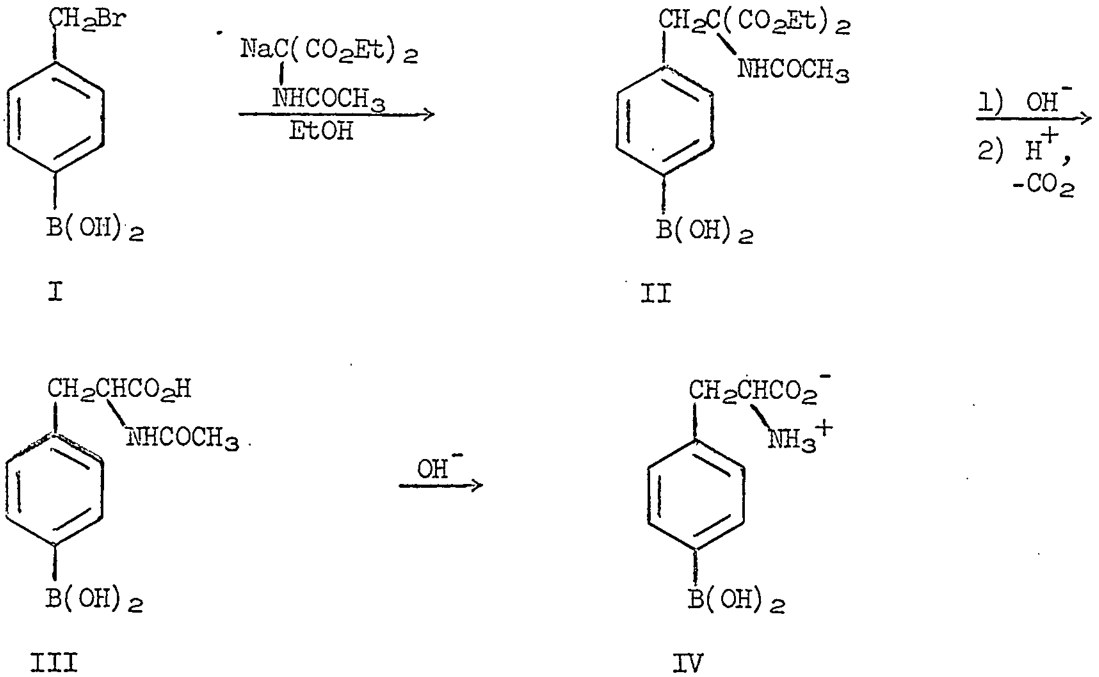
(1) This work was supported in part by a grant [AT(11-1)-314] from the Atomic Energy Commission.

(2) H. R. Snyder, A. J. Reedy and W. J. Lennarz, J. Am. Chem. Soc., 80, 835 (1958).

DISCLAIMER

to the general method of Snyder, Shekleton and Lewis.[3] The infrared spectrum of IV indicated ~~a~~ the zwitterionic structure, ~~in~~ common ~~with almost all other~~ to amino acids.



The above procedure has now been applied in an attempt to prepare o-boronophenylalanine. Condensation of o-(bromomethyl)-benzeneboronic anhydride with sodio diethyl acetamidomalonate does not yield the expected o-(2,2-dicarbethoxy-2-acetamidoethyl)-benzeneboronic acid, but rather o-(2-carbethoxy-2-acetamidoethyl)-benzeneboronic acid (IX). Evolution of carbon dioxide occurs when the alkylation mixture is acidified and warmed to 50°. Decarbethoxylation, with concomitant formation of diethyl carbonate, is known to occur

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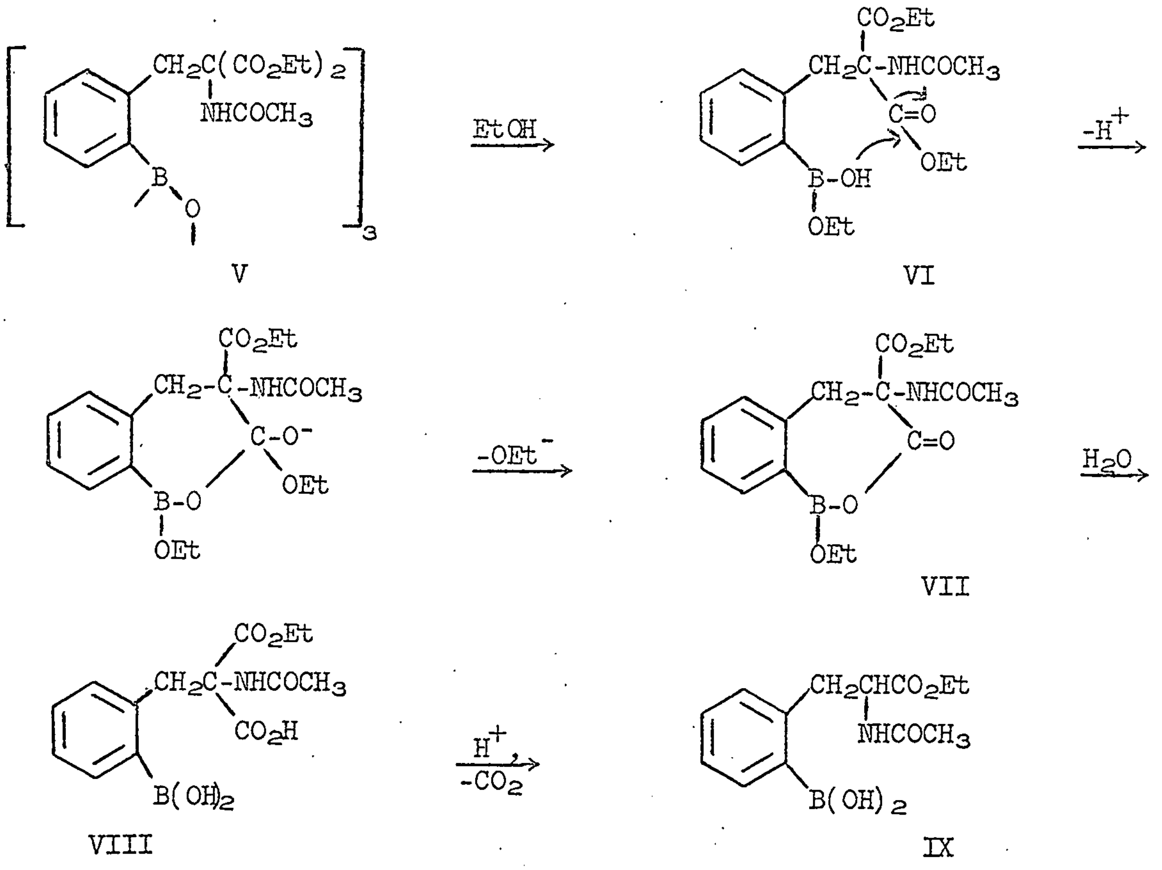
(3) H. R. Snyder, J. F. Shekleton and C. D. Lewis, J. Am. Chem. Soc., 67, 310 (1945).

sometimes as a side reaction in the alkylation of malonic esters;[4] however, decarbethoxylation via diethyl carbonate formation is considered unlikely, since alkylation of the p-bromomethyl analog proceeded normally, and also since, under the conditions employed, diethyl carbonate has been shown not to give rise to rapid generation of carbon dioxide. An alternative explanation that obviates the need of postulating the formation of diethyl carbonate is based on participation of the boronic acid group in the ester hydrolysis reaction. In ethanol, the initially formed boronic anhydride (V) could give the half-acid ester VI, which, after cyclization to the boronic-carboxylic acid anhydride VII, followed by hydrolysis and decarboxylation, would yield



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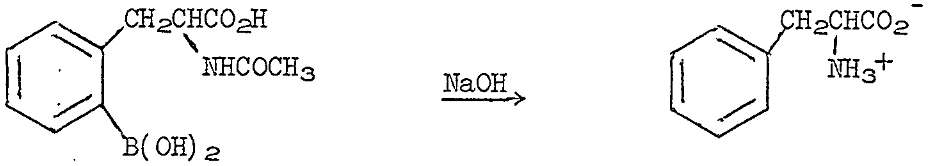
(4) A. C. Cope, H. L. Holmes and H. ~~K.~~ O. House, Org. Reactions, 9, 107 (1957).

the observed product IX. Boronic half-acid esters analogous to VI have not been isolated, but it is probable that they are intermediates in the preparation of boronic esters by the alcoholysis of boronic anhydrides.[5] A~~r~~cyl borates are known to hydrolyze readily,[5] and it might be expected that an a~~r~~cyl boronate such as VII would exhibit similar properties. A somewhat comparable example of intramolecular participation in ester hydrolysis is the facile hydrolysis of acylsalicylic acids due to participation of the *ortho* carboxyl group.[6]

The carbethoxy group in IX was saponified to give the carboxylic acid, X. The N-acetyl group in X proved unexpectedly resistant to alkaline hydrolysis. When refluxed for 11 hours with approximately 1*N* sodium hydroxide, X was recovered in 30% yield; the only other product isolated was phenylalanine.



X

These results indicate that the rate of hydrolytic deboronation [illegible] is comparable to the rate of amide hydrolysis.

Alkylation of diethyl formamidomalonate with *o*-(bromomethyl)-benzeneboronic anhydride, using a smaller excess of base than had been employed in the alkylation of diethyl acetamidomalonate, proceeded in normal fashion to

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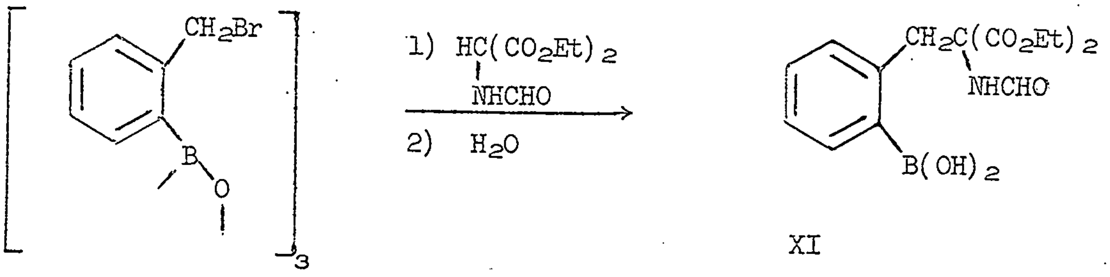
(5) M. F. Lappert, Chem. Revs., 56, 959 (1956).

(6) F. Kagan and R. D. Birkenmeyer, J. Am. Chem. Soc., 81, 1986 (1959).



XI

give the dicarbethoxy compound, XI. It was hoped that the N-formyl derivative could be hydrolyzed readily; Hellman[7] accomplished the one-step hydrolysis and decarboxylation of the analogous tryptophan derivative under mild conditions. When XI was treated under the same conditions, a boron-containing mixture was obtained which gave a positive ninhydrin test; however, this mixture resisted all attempts at purification.

An alternative route to *o*-boronophenylalamine which would not require hydrolysis steps is one based on hydrogenolysis and decarboxylation of an appropriate benzyl ester. Kissman and Witkop[8] synthesized tryptophan by condensation of dibenzyl carbobenzyloxyaminomalonate XII with a substituted Mannich base, followed by hydrogenolysis and decarboxylation. When *o*-(bromomethyl)-benzeneboronic anhydride was treated with XII in the presence of sodium *t*-butoxide, the benzyl ester XIII was produced. Hydrogenolysis of XIII in the presence of palladium-charcoal catalyst proceeded with evolution of one mole of carbon dioxide; recrystallization of the product by addition

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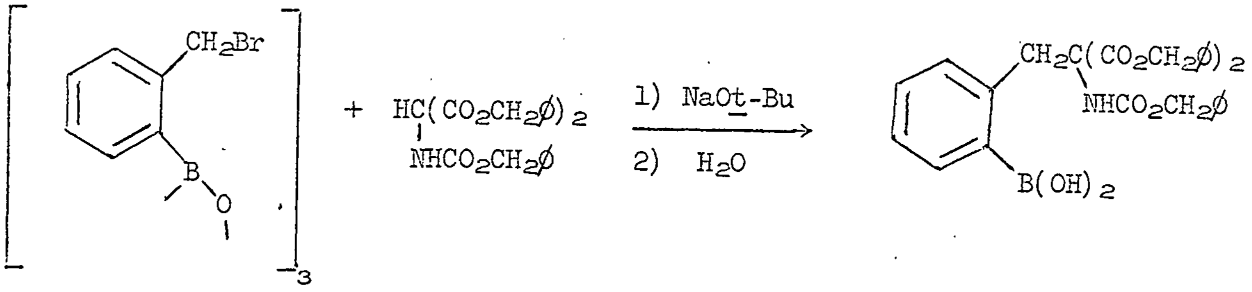
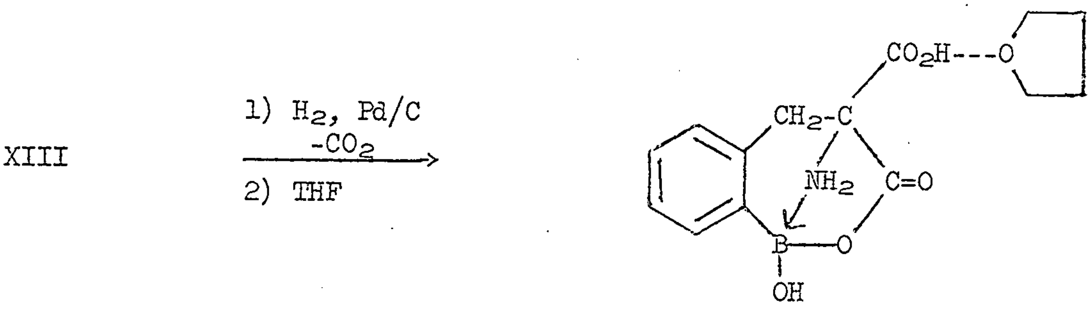
(7) H. Hellman, Z. physiol. Chem., **284**, 163 (1949).

(8) H. M. Kissman and B. Witkop, J. Am. Chem. Soc., **75**, 1967 (1953).


XII

XIII

of tetrahydrofuran (THF) to a concentrated aqueous solution gave a material whose analysis corresponded ~~with~~ to that calculated for a 1:1 complex of THF with a dehydrated form of the aminomalonic acid. Infrared and n.m.r. spectra further indicated the presence of THF. Structure XIV is favored for this


XIV

complex, ~~due to~~ because of the similarity of its infrared spectrum with that of the non-complexed anhydride (XV), whose preparation and structure determination are described below.

When, in place of THF, t-butanol was used in the recrystallization of the hydrogenolysis product, a similar complex with t-butanol formed. However, upon drying this complex overnight in vacuo, some of the t-butanol was lost, as indicated by the slightly low percentage of carbon in the sample. The n.m.r. spectrum of this complex possessed a strong signal at $\tau = 8.78$ ~~p.p.m.~~, providing further evidence for the presence of t-butanol.

The THF proved unexpectedly difficult to remove from the complex, XIV. After ~~heating~~ the complex had been heated in vacuum overnight at 100°, the elemental analysis indicated that some THF was still present. Heating at 180° for 30 minutes in vacuum proved sufficient to remove the THF; surprisingly, no decarboxylation took place during this treatment.

For larger-scale syntheses, the hydrogenolysis product could be recrystallized from a minimum amount of water. After drying at 100°, the product was the same aminomalonic anhydride (XV) that had been obtained by heating the THF complex to 180°.

Several possible structures were considered for this anhydride. The nuclear magnetic resonance spectrum of XV, obtained in deuterated dimethyl sulfoxide, was of value in ruling out most of the proposed structures. Multiplets at $\tau = 2.95$ ~~p.p.m.~~ (relative area 3) and at $\tau = 2.45$ ~~p.p.m.~~ (relative area 1) were assigned to the aromatic protons. A singlet at $\tau = 6.75$ ~~p.p.m.~~ (relative area 2) was attributed to the aliphatic $-CH_2-$ group. Only two other peaks were present in the spectrum, each having a relative area of 2, the first at $\tau = 1.30$ ~~p.p.m.~~ (broad) and the second at $\tau = 6.18$ ~~p.p.m.~~ (broad). One of these two peaks represents protons of XV which are either of the carboxylic acid or hydroxyl variety (or both, since the hydrogen atoms of carboxylic acids and hydroxyl compounds are known to undergo rapid exchange[9]). The other peak must, therefore, represent the

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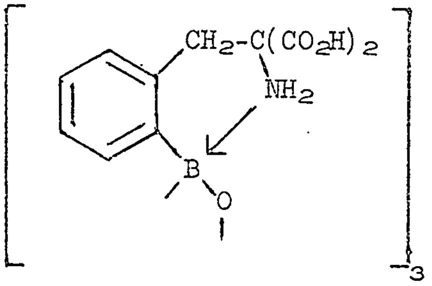
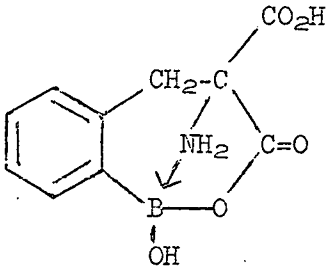
(9) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon, New York, 1959.

two protons of an $-NH_2$ group. Amines do not undergo rapid exchange unless a base is present to promote it. The broadness of the two peaks at $\tau = 1.30$ and 6.18 may be ascribed to slow exchange between the amine hydrogen atoms and the carboxylic-hydroxylic hydrogen atoms.

Since two hydrogen atoms are present on the nitrogen atom, only structures XVa and XVb need be considered for the aminomalonic anhydride. A molecular weight measurement in 95% ethanol was in agreement with that calculated for a monomeric species; however, the boroxine XVa cannot be ruled out on this basis, since in 95% ethanol a boroxine could be converted to a boronate ester, which process would furnish as many particles as a monomer and hence the apparent molecular weight would correspond to a monomeric anhydride.


XVa


XVb

The infrared spectrum of XV had absorption peaks at 645 cm.$^{-1}$ (medium, broadened), 734 cm.$^{-1}$ (strong, sharp) and 764 cm.$^{-1}$ (medium, sharp). Snyder, Konecky and Lennarz[10] found that boroxines possess a characteristic peak,

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(10) H. R. Snyder, M. S. Konecky and W. J. Lennarz, J. Am. Chem. Soc., 80, 3611 (1958).

invariably strong and sharp, in the region 680-705 cm.$^{-1}$. Hawkins[11] found this band as low as 672 cm.$^{-1}$ for two o-dialkylaminomethylbenzeneboronic anhydrides. Serafinowa and Makosza[12] have suggested that the region of absorption characteristic of boroxines be extended to 736-688 cm.$^{-1}$. The strong, sharp peak at 734 cm.$^{-1}$ in XV might be ascribed to a boroxine, but it is more likely due to ortho-disubstitution of the aromatic ring.[13]

The carbonyl stretching frequencies at 1730 and 1685 cm.$^{-1}$ are consistent with structures XVa and XVb; un-ionized carboxylic acids absorb in the range 1725-1700 cm.$^{-1}$,[13] and acyloxy boron compounds at 1786-1700 cm.$^{-1}$.[14]

The difficulty of decarboxylation of XV provides a basis for favoring structure XVb over XVa. Generally speaking, malonic acids lose carbon dioxide fairly readily; the para-substituted acetamidomalonic ester II, for instance, after saponification with dilute base, was decarboxylated by refluxing in dilute acid for 1 hour. Decarboxylation of XV, on the other hand, took place at 180-220°. Whereas no explanation for this extraordinary difficulty of decarboxylation is apparent from structure XVa, structure XVb does afford such explanation. The polycyclic skeleton present in XVb is extremely rigid,

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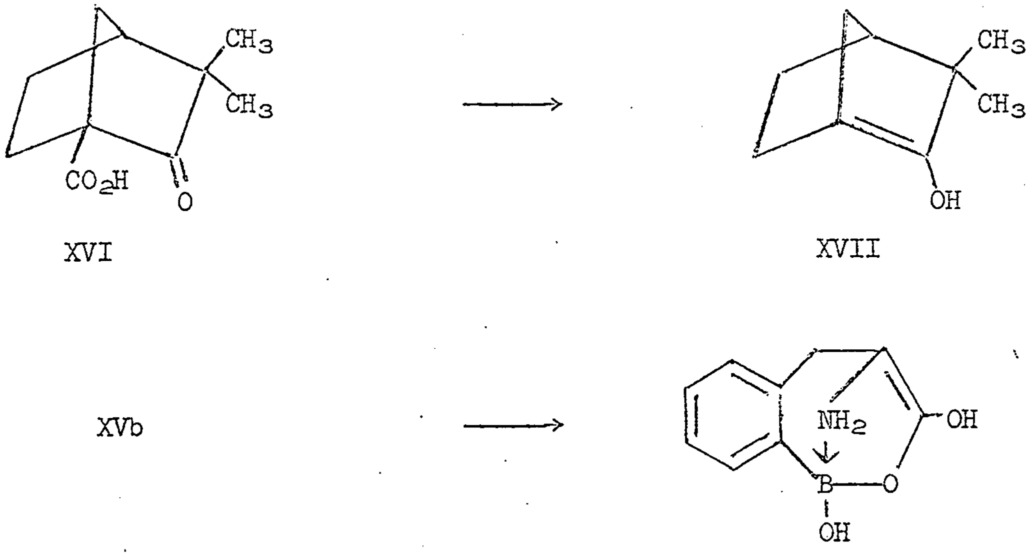
(11) R. T. Hawkins and H. R. Snyder, J. Am. Chem. Soc., 82, 3863 (1960).

(12) B. Serafinowa and M. Makosza, Roczniki Chem., 35, 937 (1961).

(13) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed., Wiley, New York, 1959.

(14) L. A. Duncanson, W. Gerrard, M. F. Lappert, H. Pyszora and R. Shafferman, J. Chem. Soc., 3652 (1958).

and the carboxyl group may be considered as attached to a "bridgehead" carbon atom. Bridgehead carboxylic acids such as XVI[15] may be decarboxylated only with extreme difficulty; the enol XVII that would result from decarboxylation of XVI contains a bridgehead double bond, which is prohibited on steric grounds (Bredt's Rule[15]). Similar considerations would govern the decarboxylation of XVb to the hypothetical enol XVIII. On the basis of chemical and spectral characteristics, XVb is therefore considered the most likely structure for the aminomalonic anhydride.



XVI

XVII

XVb

XVIII

On standing for a few days in an atmosphere saturated with water vapor, the anhydride XV absorbed two moles of water. ~~were taken up per mole of the anhydride XV.~~ The infrared spectrum of the resulting acid did not differ greatly from that of its

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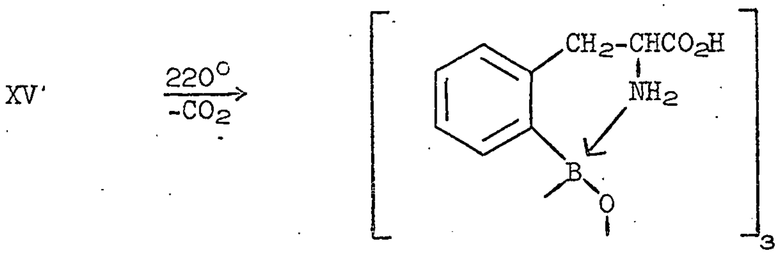
(15) F. S. Fawcett, Chem. Revs., 47, 219 (1950).

anhydride in the regions attributed to boroxine absorption and carbonyl absorption.

The production of decarboxylation of XV was the amino acid anhydride. The boroxine structure XIX is suggested by the strong infrared absorption at



XIX

660 cm.$^{-1}$; other structures are conceivable. A strong, broad band at 1710 cm.$^{-1}$, characteristic of an un-ionized carboxylic acid, was present in the infrared spectrum. The para-isomer of XIX, p-boronophenylalanine,[2] existed as the normal zwitterionic form, IV. The boron atom in close proximity to the nitrogen atom of XIX probably reduces the basicity of the amine function sufficiently to prevent protonation by the carboxylic acid. Boronic acids are much weaker acids than carboxylic acids, and it is surprising that the boronic anhydride function of XIX can compete effectively with the carboxylic group for the basic center, the nitrogen atom. Evidently, the favorable steric orientation of the boron and nitrogen atoms in XIX more than compensates for the normal difference in acidity.

In contrast to the aminomalonic anhydride XV, the amino acid anhydride XIX did not absorb water readily when allowed to stand in an atmosphere saturated with water vapor. Some water was taken up very slowly; it is possible that on prolonged standing a hydrated species will form.

The amino acid derivatives IX, XV and XIX are of interest in connection with a proposed cancer therapy based on nuclear disintegration of the B^{10} atom upon capture of a neutron.[16] Soloway[17] has found that compounds which possess a high water-to-benzene partition coefficient show the greatest tendency to localize selectively in mouse-brain tumors. Organoboron compounds such as IX, XV and XIX, which contain polar functional groups, may offer possibilities for meeting some of the requirements of the proposed therapy.

Experimental[18]

o-(2-Carbethoxy-2-acetamidoethyl)-benzeneboronic Acid. -- A solution of sodium ethoxide was prepared from 0.92 g. (0.040 g. atom) of sodium and 75 ml. of absolute ethanol in an oven-dried flask equipped with a magnetic stirrer and a condenser protected by a drying tube. To this solution was added 12.96 g. (0.045 mole) of diethyl acetamidomalonate. After the malonate had dissolved, 5.92 g. (0.030 mole) of o-(bromomethyl)-benzeneboronic anhydride was added. A white precipitate began to form almost immediately. The mixture was stirred and heated to reflux for 6 hours, cooled to ca. 50°, and acidified with 4 ml. of 3 N hydrochloric acid. The solution was then maintained at 50-60° for 30 minutes, during which time carbon dioxide evolution was followed

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(16) P. G. Kruger, Proc. Natl. Acad. Sci., 26, 181 (1940).

(17) A. H. Soloway, Science, 128, 1572 (1958).

(18) Microanalyses and molecular weight determinations were performed by Mr. Josef Nemeth and associates.

by bubbling the evolved gases into calcium hydroxide solution. At the end of this period, ~~of time~~ gas evolution was very slow. The pH ~~of~~ the solution was adjusted to ca. 4-5 with 10% sodium hydroxide and was concentrated in vacuo. The resulting semi-solid residue was dissolved in 95 ml. of boiling water. The solution was cooled overnight and the crystals which resulted were collected and dried in vacuo. The crystals (4.31 g.) melted at 135-145°.

Concentration of the mother liquors in vacuo to ca. 45 ml. afforded a second crop of crystals (4.26 g., m.p. 65-175°).

no ¶ The first and second crops ~~of crystals~~ were combined (8.57 g., 102% yield) and used without further purification in the preparation of o-(2-carboxy-2-acetamidoethyl)-benzeneboronic acid.

From a small sample of the first crop of crystals an analytical sample was prepared by two-fold recrystallization from water and subsequent drying in vacuo over calcium chloride for 10 hours. The resulting crystals melted at 147-150°.

Anal. Calcd. for $C_{13}H_{18}NO_5B$: C, 55.94; H, 6.50; N, 5.01. Found: C, 56.20; H, 6.47; N, 5.28.

o-(2-Carboxy-2-acetamidoethyl)-benzeneboronic Acid. -- Into a 100-ml. flask equipped with a magnetic stirrer and a reflux condenser were placed 8.39 g. (0.03 mole) of crude o-(2-carbethoxy-2-acetamidoethyl)-benzeneboronic acid and 70 ml. of 5% sodium hydroxide. The solid dissolved when the solution was warmed. After the solution was heated to reflux for 4 hours, the total volume was reduced to ca. 50 ml. by concentration in vacuo. The solution, cooled to ca. 40°, was acidified by the addition of 25 ml. of 4 N hydrochloric acid. A white precipitate formed. The mixture was stirred and heated to ca. 50-70° for 30 minutes, during which time no carbon dioxide evolution was observed. The mixture was cooled, then partially neutralized by the addition of 8 ml. of 10% sodium hydroxide, and stored in the refrigerator for several hours. The crystals, after being collected by filtration and dried in vacuo, weighed 3.00 g. (43% yield) and melted at 192-193°.

Recrystallization from 350 ml. of 50% ethanol afforded 1.95 g. of crystals which melted at 196-197°. The mother liquors were concentrated in vacuo to ca. 150 ml.; an additional quantity of 0.35 g. of product (m.p. 190-193°) was obtained.

An analytical sample was prepared by two-fold recrystallization from 50% ethanol and subsequent drying in vacuo over calcium chloride ~~; the crystals~~ melted ~~at~~ (m.p. 203-204°).

Anal. Calcd. for $C_{11}H_{14}NO_5B$: C, 52.60; H, 5.62; N, 5.59. Found: C, 53.06; H, 5.50; N, 5.54.

Attempted Hydrolysis of o-(2-Carboxy-2-acetamidoethyl)-benzeneboronic Acid. -- Into a 50-ml. flask equipped with a magnetic stirrer and a reflux condenser were placed 500 mg. (0.0020 mole) of o-(2-carboxy-2-acetamidoethyl)-benzeneboronic acid and a solution of 560 mg. (0.0136 mole) of sodium hydroxide in 11 ml. of water. The boronic acid dissolved readily. The solution was heated to reflux for 11 hours, and then cooled and acidified to pH 2-3 by dropwise addition of concentrated hydrochloric acid. The white precipitate that resulted was collected by filtration and dried. This material (ca. 150 mg.) was identified as impure o-(2-carboxy-2-acetamidoethyl)-benzeneboronic acid from its infrared spectrum. ~~The main impurity is thought to be a small amount of silica.~~

The pH of the filtrate from the acidified mixture described above was adjusted to 6.8-7.0 with dilute ammonium hydroxide. A small amount of white, flocculent precipitate formed. The mixture was concentrated in vacuo to ca.

one-half the original volume. The flask was cooled in an ice bath and the precipitate collected and dried. This material (ca. 70 mg.) reacted with ninhydrin and was identified as phenylalanine from its infrared spectrum. Another 30-40 mg. of phenylalanine was obtained by further concentration of the filtrate.

o-(2,2-Dicarbethoxy-2-formamidoethyl)-benzeneboronic Acid. -- A solution of sodium ethoxide was prepared from 0.41 g. (0.0180 mole) of sodium and 40 ml. of absolute ethanol in an oven-dried flask equipped with a magnetic stirrer and a reflux condenser protected by a drying tube. Next, 3.78 g. (0.0186 mole) of diethyl formamidomalonate was added. After the malonate dissolved, 2.96 g. (0.0150 mole) of o-(bromomethyl-benzeneboronic anhydride was added. A white precipitate began to form almost immediately. The mixture was stirred and heated to reflux for 5 hours, then cooled to ca. 40° and acidified with 2 ml. of 4 N hydrochloric acid. The mixture was then maintained at 50-70° for 20 minutes, during which time a small amount of carbon dioxide was evolved. The inorganic salt was removed by filtration, and the filtrate concentrated in vacuo to a semi-solid residue. Recrystallization of this residue from 70 ml. of boiling water afforded 1.68 g. (33.4%) of crystals, m.p. 115-124° (dec.).

An analytical sample was prepared by two-fold recrystallization from water and subsequent drying at 1 mm. over calcium chloride for 24 hours. The crystals melted at 125-131° (dec.).

Anal. Calcd. for $C_{15}H_{20}NO_7B$: C, 53.43; H, 5.96; N, 4.16; B, 3.22. Found: C, 53.47; H, 6.02; N, 4.31; B, 2.93.

Attempts were made to improve the yield in the synthesis of o-(2,2-dicarbethoxy-2-formamidoethyl)-benzeneboronic acid; however, condensations carried out in benzene with sodium hydride, in benzene-ethanol mixture with sodium ethoxide, and in dimethylformamide with sodium hydride led only to oily or gummy materials which resisted attempts at purification.

Preparation of o-(2,2-Dicarbobenzyloxy-2-carbobenzyloxyaminoethyl)-benzeneboronic Acid. -- A solution of sodium *t*-butoxide in *t*-butanol was prepared by dissolving 4.06 g. (0.176 mole) of sodium in 310 ml. of *t*-butanol which had been dried by refluxing over sodium. The reaction vessel was purged with dry nitrogen. The solution was stirred, heated nearly to boiling, and 78.00 g. (0.180 mole) of dibenzyl carbobenzyloxyaminomalonate was added. To the clear, yellow solution was added 37.20 g. (0.189 mole) of *o*-bromomethylbenzeneboronic anhydride (prepared according to the method of Kurz[19]). A white precipitate appeared. After refluxing for 1 hr., the reaction mixture was no longer alkaline toward moist Hydrion paper. The mixture was cooled and poured onto 300 ml. of a mixture of ice and water; the resulting slurry was extracted with one 500-ml. and three 100-ml. portions of chloroform. The combined chloroform extracts were washed with 200 ml. of water in 2 portions, dried over magnesium sulfate, and evaporated *in vacuo* to give 138.4 g. of sticky white crystals. These were taken up in a mixture of benzene and petroleum ether, applied to a column of 2 lb~~s~~. of alumina, and chromatographed. Elution with benzene gave 25 g. of impure dibenzyl carbobenzyloxyaminomalonate. Further elution with ether and absolute ethanol gave 47.2 g. (47%) of *o*-(2,2-dicarbobenzyloxy-2-carbobenzyloxyaminoethyl)-benzeneboronic acid as an oil, which was crystallized from aqueous *t*-butanol. An analytical

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(19) R. K. Kurz, Thesis, Doctor of Philosophy, University of Illinois, 1961.

sample was prepared by threefold recrystallization from 1,2-dichloroethane/ petroleum ether; m.p. 110-111°.

Anal. Calcd. for $C_{32}H_{30}NO_8B$: C, 67.73; H, 5.33; N, 2.47; B, 1.92. Found: C, 67.89; H, 5.33; N, 2.59; B, 1.83.

Preparation of α-Amino-o-boronobenzylmalonic Anhydride. ~~CAUTION~~ The 30% palladium-on-charcoal catalyst used in this experiment was washed with distilled water and absolute ethanol and dried *in vacuo*. Thus treated, the catalyst was highly pyrophoric; it was weighed as rapidly as possible, and added to the reaction mixture only after the reaction flask had been flushed well with nitrogen.

A 300-ml., 3-necked flask was equipped with a gas-delivery tube which passed almost to the bottom of the flask, and with a gas exit tube connected to two bubbler tubes in series. The bubbler tubes were filled with saturated aqueous barium hydroxide. After addition of a solution of 15.00 g. (0.026 mole) of *o*-2,2-dicarbobenzyloxy-2-carbobenzyloxyaminoethyl)-benzeneboronic acid in 200 ml. of ethyl acetate, the system was flushed with nitrogen and 3 g. of 30% palladium-on-charcoal catalyst was added. The mixture was stirred while hydrogen was passed through rapidly for 7.5 hrs. During this time, a precipitate of barium carbonate formed in the bubblers; after washing and drying *in vacuo*, this precipitate weighed 5.22 g. (0.026 mole). Therefore, one mole of carbon dioxide was evolved per mole of *o*-(2,2-dicarbobenzyloxy-2-carbobenzyloxyaminoethyl)-benzeneboronic acid.

The hydrogenolysis mixture was filtered and washed with 100 ml. of ethyl acetate. The residue remaining on the filter was extracted with 250 ml. of water; the resultant aqueous solution was freed of a small amount of insoluble material by filtration through a fine sintered-glass funnel, and evaporated in vacuo, giving 6.668 g. (99.7%) of white crystals. An analytical sample was prepared by twofold recrystallization from a minimum amount of water and drying in vacuo at 100°; m.p. 249-262°.

Anal. Calcd. for $C_{10}H_{10}NO_5B$: C, 51.11; H, 4.29; N, 5.96; B, 4.60. Found: C, 51.09; H, 4.49; N, 5.94; B, 4.27.

Preparation of α-Amino-o-boronobenzylmalonic Anhydride Complex with Tetrahydrofuran. -- A concentrated aqueous solution of α-amino-o-boronobenzylmalonic acid was treated with one-fourth its volume of tetrahydrofuran; the crystals which separated were dried overnight at room temperature in vacuo; m.p. 248-264°.

Anal. Calcd. for $C_{14}H_{18}NO_6B$: C, 54.75; H, 5.91; N, 4.56. Found: C, 54.89; H, 5.96; N, 4.63.

Preparation of α-Amino-o-boronobenzylmalonic Anhydride Complex with t-Butanol. -- A concentrated aqueous solution of α-amino-o-boronobenzylmalonic acid was treated with 10 times its volume of t-butanol and allowed to stand at 5° for 3 days; the crystals which separated were dried at room temperature in vacuo overnight; m.p. 250-253°(d$\overset{ec}{\wedge}$.), with gas evolution at 170-180°.

to read (dec.)

Anal. Calcd. for $C_{14}H_{20}NO_6B$: C, 54.39; H, 6.52; N, 4.53. Found: C, 53.91; H, 6.48; N, 4.42.

Preparation of α-Amino-o-boronobenzylmalonic Acid Hydrate. -- α-Amino-o-boronobenzylmalonic anhydride (0.2588g.; 0.0011 mole) was allowed to stand for 7 days in a desiccator saturated with water vapor. At the end of this time, the sample weighed 0.2997 g. The calculated value for $\overset{\text{the addition}}{\text{take-up}}$ of two moles of water was 0.2992 g.

Anal. Calcd. for $C_{10}H_{14}NO_7B$: C, 44.32; H, 5.21; N, 5.17. Found: C, 44.28; H, 5.16; N, 5.09.

Preparation of o-Boronophenylalanine Anhydride. -- α-Amino-o-boronobenzylmalonic acid hydrate (2.271 g.; 0.008 mole) was heated at 220° and 0.1 mm. pressure for 3.75 hrs. The yellow powder was extracted with 125 ml. of hot water and the insoluble residue removed by filtration. The filtrate was treated with Darco and evaporated *in vacuo* to give 1.178 g. (73.5%) of white crystals. An analytical sample was prepared by twofold recrystallization from a minimum amount of water, followed by drying at 100° *in vacuo* overnight; m.p. 252-262°.

Anal. Calcd. for $C_9H_{10}NO_3B$: C, 56.59; H, 5.28; N, 7.33. Found: C, 56.49; H, 5.22; N, 7.44.

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