TABLE IV
BIOLOGICAL ASSAYS

Amino acid added	Reaction conditions	Amino acid residues added	Histidine residues in product	Mouse assay, units/mg	Immuno assay, units/mg
Control		0	1.83	21.4 ± 4.3	23.8
Alanine	60 hr (triethylamine)	2.5	1.02	3.6 ± 0.7	10.38
Alanine	4 hr (imidazole)	2.2	2.06	11.8 ± 2.1	15.6
Phenylalanine	5 hr (imidazole)	1.8	2.00	10.2 ± 1.7	15.4

The above results indicate that about 90 ± 5% of the two added alanines are on the α-amino groups of the insulin and about 10 ± 5% on the ε-amino group of the insulin. Also little or no substitution took place on tyrosine or histidine which were lost on dniitrophenylation. If they had been substituted with alanine, they would have been recovered upon hydrolysis of the DNP derivative.

The biological activity of the dialanyl-insulin was 11.8 ± 2.1 units/mg by the mouse convulsion assay. This value may be compared with 10 ± 0.8 units/mg reported by Levy and Carpenter for the trialanyl-insulin.[8]

In the trialanyl-insulin, amino acid residues were added to the ε-amino group as well as to the N-terminal α-amino groups. It was impossible to ascertain whether the decreased biological activity exhibited by this derivative was due to covering the N-terminal groups or of the ε-amino group of lysine at position 29 or perhaps to a combination of both. The fact that the dialanyl derivatives prepared here, which involves primarily the substitution of the N-terminal amino groups with very little reaction on the ε-amino group, have approximately the same biological activity as the trialanyl-insulin indicates that the N-terminal groups are relatively more important for biological activity than the ε-amino group. However, as the dialanyl-insulin was prepared by the phenyldiimide method and the trialanyl-insulin was prepared *via* *p*-nitrophenyl ester, one or the other reaction could have caused a change in the insulin which would be reflected in the assay but not in the amino acid analyses. Diphenylalanyl-insulin prepared by the same method assayed 10.2 ± 1.7 units/mg.

Acknowledgment.—The authors are grateful to Dr. B. Halpern for the glpc determination of the extent of racemization in the reaction of *t*-BOC-amino acid phenyldiimides. We also wish to thank Anna Lisa Valentine for aid with the amino acid analyses.

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

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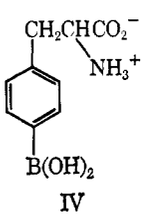
Syntheses of N-acetyl-*o*-boronophenylalanine, α-amino-*o*-boronobenzylmalonic anhydride, and *o*-boronophenylalanine anhydride are described. Alkylation of diethyl acetamidomalonate with *o*-(bromomethyl)benzeneboronic anhydride yields *o*-(2-carbethoxy-2-acetamidoethyl)benzeneboronic acid rather than the expected *o*-(2,2-dicarbethoxy-2-acetamidoethyl)benzeneboronic acid. It is postulated that decarbethoxylation occurs through participation of the boronic acid function 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, the boronic anhydride related to *o*-boronophenylalanine, gives no indication of the zwitterionic structure, presumably because of interaction between the nitrogen and boron atoms.

The synthesis of *p*-boronophenylalanine (IV) has been reported.[2] *p*-(Bromomethyl)benzeneboronic acid



IV

(I) was condensed with sodio diethyl acetamidomalonate, and the product (II) was saponified and decarboxylated to give the acetyl derivative (III) which was hydrolyzed. The general method was that of Snyder, Shekleton, and Lewis.[3] The infrared spectrum of IV indicated the zwitterionic structure common 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 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

(1) This work was supported in part by a grant from the Atomic Energy Commission, Report No. COO-314-11.

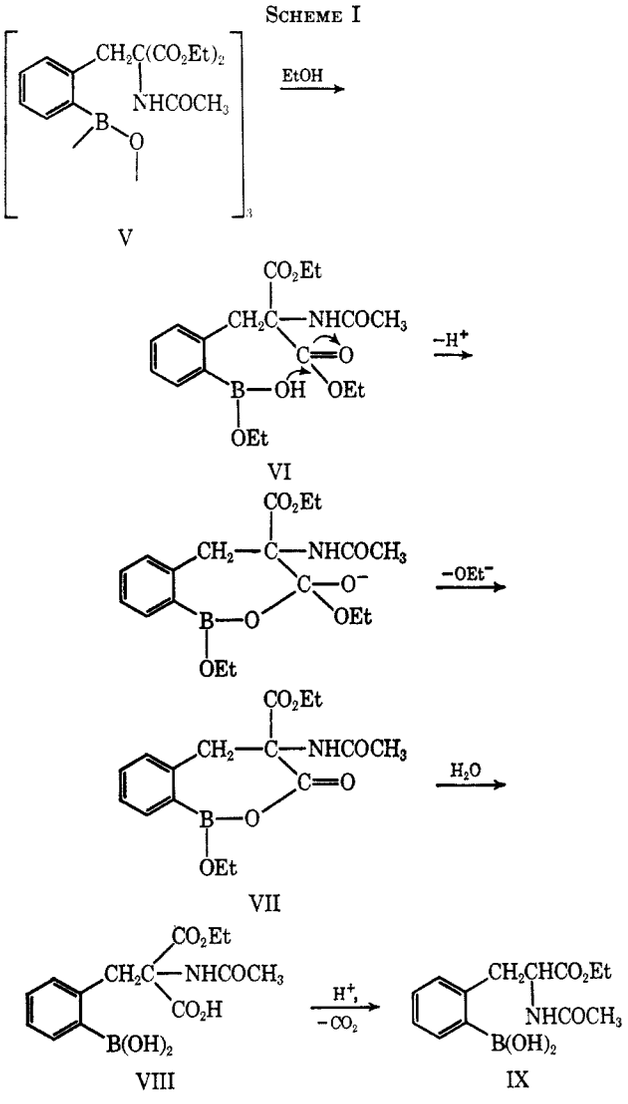
(2) H. R. Snyder, A. J. Reedy, and W. J. Lennarz, *J. Amer. Chem. Soc.*, **80**, 835 (1958).

(3) H. R. Snyder, J. F. Shekleton, and C. D. Lewis, *ibid.*, **67**, 310 (1945).

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

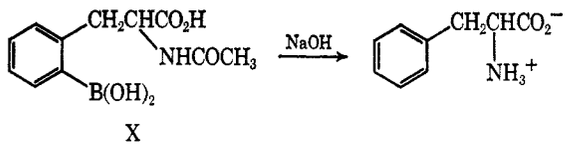
conditions employed, diethyl carbonate has been shown not to give rise to rapid generation of carbon dioxide. An alternative explanation is based on participation of the boronic acid group in the ester hydrolysis reaction. In ethanol, the initially formed boronic anhydride V could give the half-acid ester VI, which, after cyclization to the boronic carboxylic acid anhydride VII, followed by hydrolysis and decarboxylation, would yield the observed product IX (Scheme I). Boronic

SCHEME I



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] Acyl borates are known to hydrolyze readily,[5] and it might be expected that an acyl boronate such as VII would exhibit similar properties. A comparable example of intramolecular participation in ester hydrolysis is the facile hydrolysis of acylsalicylic acids due to participation of the *ortho* carboxyl group.[6]

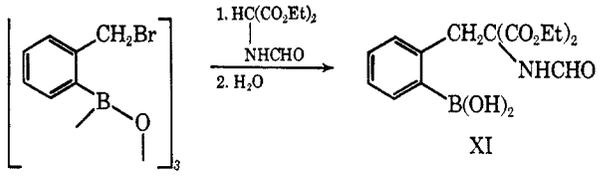
The carbethoxy group in IX was saponified to give the carboxylic acid X. The N-acetyl group in X proved unexpectedly resistant to alkaline hydrolysis. When refluxed for 11 hr with approximately 1 *N* sodium



X

hydroxide, X was recovered in 30% yield; the only other product isolated was phenylalanine. These results indicate that the rate of hydrolytic deboronation is comparable to the rate of amide hydrolysis.

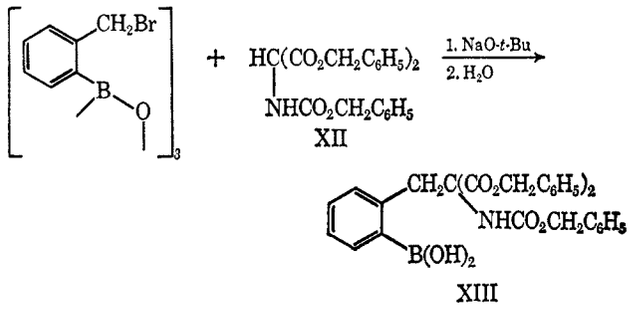
Alkylation of diethyl formamidomalonate with *o*-(bromomethyl)benzeneboronic anhydride, with a smaller excess of base than had been employed in the alkylation of diethyl acetamidomalonate, proceeded in normal fashion to give the dicarbethoxy compound



XI

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*-boronophenylalanine 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



XII

XIII

of XIII in the presence of palladium–charcoal catalyst proceeded with evolution of 1 mol of carbon dioxide; recrystallization of the product by addition of tetrahydrofuran (THF) to a concentrated aqueous solution gave a material whose analysis corresponded to that calculated for a 1:1 complex of THF with a dehydrated form of the aminomalonic acid. Infrared and nmr spectra further indicated the presence of THF. Struc-

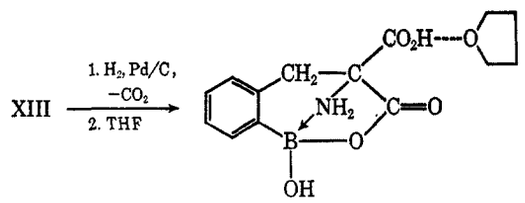
(5) M. F. Lappert, *Chem. Rev.*, **56**, 959 (1956).

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

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

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

ture XIV is favored for this complex, because of the similarity of its infrared spectrum with that of the non-complexed anhydride XV, whose preparation and structure determination are described below.



XIV

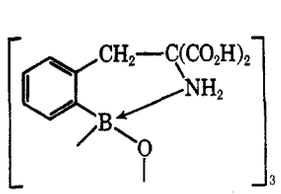
When *t*-butyl alcohol was used in the recrystallization of the hydrogenolysis product, a similar complex formed. However, upon drying overnight *in vacuo*, some of the *t*-butyl alcohol was lost from this complex, as indicated by the slightly low percentage of carbon in the sample. The nmr spectrum of this complex possessed a strong signal at τ 8.78, providing further evidence for the presence of *t*-butyl alcohol.

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

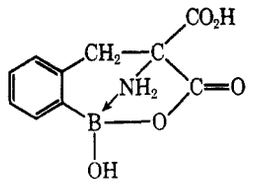
For larger scale syntheses, the hydrogenolysis product could be recrystallized from a minimum amount of water. After drying at 100°, the product was the same aminomalonic anhydride XV that had been obtained by heating the THF complex to 180°.

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 τ 2.95 (relative area 3) and at τ 2.45 (relative area 1) were assigned to the aromatic protons. A singlet at τ 6.75 (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 τ 1.30 (broad) and the second at τ 6.18 (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 two protons of the NH_2 group. Amines do not undergo rapid exchange unless a base is present to promote it. The broadness of the two peaks at τ 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 into a boronate ester, which process would furnish as many



XVa



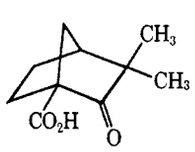
XVb

particles as a monomer and hence the apparent molecular weight would correspond to a monomeric anhydride.

The infrared spectrum of XV had absorption peaks at 654 (medium, broadened), 734 (strong, sharp) and 764 cm^{-1} (medium, sharp). Snyder, Konecky, and Lennarz[10] found that boroxines possess a characteristic peak, 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, 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 hr. Decarboxylation of XV, on the other hand, took place at 180–220°. An explanation for this extraordinary difficulty of decarboxylation can be derived from structure XVb. The polycyclic skeleton present in XVb is extremely rigid, 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. On the basis of chemical and spectral characteristics, XVb is therefore considered the most likely structure for the aminomalonic anhydride.



XVI

On standing for a few days in an atmosphere saturated with water vapor, the anhydride XV absorbed 2 mol of water. The infrared spectrum of the resulting acid did not differ greatly from that of its anhydride in the regions attributed to boroxine absorption and carbonyl absorption.

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

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

(11) R. T. Hawkins and H. R. Snyder, *ibid.*, **82**, 3863 (1960).

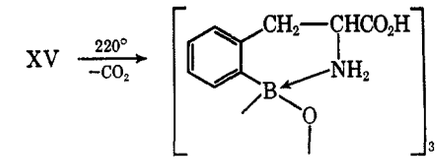
(12) B. Serafinowa and M. Makosza, *Rocz. Chem.*, **35**, 937 (1961).

(13) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley & Sons, Inc., New York, N. Y., 1959.

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

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

The product of decarboxylation of XV was the amino acid anhydride. The boroxine structure XVII is suggested by the strong infrared absorption at 660



XVII

cm^{-1}; other structures are conceivable. A strong, broad band at 1710 cm^{-1}, characteristic of an unionized carboxylic acid, was present in the infrared spectrum. The *para* isomer of XVII, *p*-boronophenylalanine,[2] existed as the normal zwitterionic form, IV. The boron atom in close proximity to the nitrogen atom of XVII probably reduces the basicity of the amine function sufficiently to prevent protonation by carboxylic acid. Boronic acids are much weaker acids than carboxylic acids, and it is surprising that the boronic anhydride function of XVII 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 XVII more than compensates for the normal difference in acidity.

In contrast to the aminomalonic anhydride XV, the amino acid anhydride XVII 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 would form.

The amino acid derivatives IX, XV, and XVII 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 XVII, which contain polar functional groups, may offer possibilities for meeting some of the requirements of the proposed therapy.

Experimental Section[18]

***o*-(2-Carbethoxy-2-acetamidoethyl)benzeneboronic Acid.**—To a solution of sodium ethoxide prepared from 0.92 g (0.040 g-atom) of sodium and 75 ml of absolute ethanol was added 12.96 g (0.045 mol) of diethyl acetamidomalonate. After the malonate had dissolved, 5.92 g (0.010 mol) of *o*-(bromomethyl)benzeneboronic anhydride[19] was added. A white precipitate began to form almost immediately. The mixture was stirred and heated to reflux for 6 hr, cooled to *ca.* 50°, and acidified with 4 ml of 3 *N* hydrochloric acid. The solution was then maintained at 50–60° for 30 min, during which time carbon dioxide evolution was followed by bubbling the evolved gases into calcium hydroxide solution. At the end of this period, gas evolution was very slow. The pH was adjusted to *ca.* 4–5 with 10% sodium hydroxide and the solution was concentrated *in vacuo*. The resulting semisolid residue was recrystallized from 95 ml of boiling water. The crystals (4.31 g) melted at 135–145°.

Concentration of the mother liquors afforded a second crop (4.26 g, mp 65–175°), which was combined with the first crop 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, mp 147–150°, was prepared by twofold recrystallization from water and subsequent drying *in vacuo* over calcium chloride for 10 hr.

Anal. Calcd for $C_{13}H_{18}BNO_5$: 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.**—A solution prepared from 8.39 g (0.03 mol) of crude *o*-(2-carbethoxy-2-acetamidoethyl)benzeneboronic acid and 70 ml of 5% sodium hydroxide was refluxed for 4 hr and then concentrated *in vacuo* to *ca.* 50 ml and the solution acidified with 25 ml of 4 *N* hydrochloric acid. A white precipitate formed. The mixture was stirred and heated to *ca.* 50–70° for 30 min, 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 chilled for several hours to give 3.00 g (43% yield) of product, mp 192–193°.

Recrystallization from 350 ml of 50% ethanol afforded 1.95 g of crystals which melted at 196–197°.

An analytical sample was prepared by twofold recrystallization from 50% ethanol and drying *in vacuo* over calcium chloride (mp 203–204°).

Anal. Calcd for $C_{11}H_{14}BNO_5$: 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.—A solution prepared from 500 mg (0.0020 mol) of *o*-(2-carboxy-2-acetamidoethyl)benzeneboronic acid and 560 mg (0.0136 mol) of sodium hydroxide in 11 ml of water was refluxed for 11 hr and then cooled and acidified to pH 2–3 by dropwise addition of concentrated hydrochloric acid. The resultant white precipitate (*ca.* 150 mg) was identified as impure *o*-(2-carboxy-2-acetamidoethyl)benzeneboronic acid from its infrared spectrum.

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 g-atom) of sodium and 40 ml of absolute ethanol. Next, 3.78 g (0.0186 mol) of diethyl formamidomalonate was added. After the malonate dissolved, 2.96 g (0.0050 mol) of *o*-(bromomethyl)-benzeneboronic anhydride[19] was added. A white precipitate began to form almost immediately. The mixture was stirred and heated to reflux for 5 hr, then cooled to *ca.* 40°, and acidified with 2 ml of 4 *N* hydrochloric acid. After 20 min, the inorganic salt was removed by filtration and the filtrate concentrated *in vacuo*. Recrystallization of the residue from 70 ml of boiling water afforded 1.68 g (33.4%) of crystals, mp 115–124° dec.

An analytical sample prepared by twofold recrystallization from water melted at 125–131° dec.

Anal. Calcd for $C_{15}H_{20}BNO_7$: 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*-butyl alcohol was prepared by dissolving 4.06 g (0.176 mol) of sodium in 310 ml of dry *t*-butyl alcohol. The solution was stirred under nitrogen and heated nearly to boiling; then 78.00 g (0.180 mol) of dibenzyl carbobenzyloxyaminomalonate was added. To the clear, yellow solution was added 37.20 g (0.063 mol) of *o*-bromomethylbenzeneboronic anhydride (prepared according to the method of Kurz[19]). After refluxing for 1 hr, the reaction mixture was no longer alkaline. 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 water, dried, and evaporated *in vacuo* to give 108.4 g of

(16) P. G. Kruger, *Proc. Natl. Acad. Sci. U. S.*, **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.
(19) R. K. Kurz, Ph.D. Thesis, University of Illinois, 1961.

white crystals. These were taken up in a mixture of benzene and petroleum ether (bp 60–70°), applied to a column of 2 lb 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-butyl alcohol. An analytical sample was prepared by threefold recrystallization from 1,2-dichloroethane–petroleum ether, mp 110–111°.

Anal. Calcd for $C_{32}H_{30}BNO_8$: 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.—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. To a solution of 15.00 g (0.026 mol) of o-2,2-dicarbobenzyloxy-2-carbobenzyloxyaminoethyl)benzeneboronic acid in 200 ml of ethyl acetate under nitrogen, 3 g of 30% palladium-on-charcoal catalyst was added. The mixture was stirred while hydrogen was passed through rapidly for 7.5 hr. During this time, a precipitate of barium carbonate formed in a barium hydroxide trap connected to the apparatus; after being washed and dried *in vacuo*, this precipitate weighed 5.22 g (0.026 mol).

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 which could be recrystallized from water, mp 249–262°.

Anal. Calcd for $C_{10}H_{10}BNO_5$: 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*, mp 248–264°.

Anal. Calcd for $C_{14}H_{18}BNO_6$: 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-Butyl Alcohol.—A concentrated aqueous solution of α-amino-o-boronobenzylmalonic acid was treated with ten times its volume of t-butyl alcohol and allowed to stand at 5° for 3 days; the crystals which separated were dried at room temperature *in vacuo* overnight, mp 250–253° dec, with gas evolution at 170–180°.

Anal. Calcd for $C_{14}H_{20}BNO_6$: 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 (0.2588 g, 0.0011 mol) 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 the addition of 2 mol of water was 0.2992 g.

Anal. Calcd for $C_{10}H_{14}BNO_7$: 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 mol) was heated at 220° (0.1 mm) for 3.75 hr. 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, mp 252–262°.

Anal. Calcd for $C_9H_{10}BNO_3$: C, 56.59; H, 5.28; N, 7.33. Found: C, 56.49; H, 5.22; N, 7.44.

Registry No.—IX, 17604-89-6; X, 5115-46-8; XI, 17604-90-9; XIII, 17604-91-0; XIV, 17659-05-1; XVb, 17604-92-1; α-amino-o-boronobenzylmalonic anhydride complex with t-butyl alcohol, 17604-93-2; α-amino-o-boronobenzylmalonic acid, 77604-94-3; o-boronophenylalanine anhydride, 17604-95-4.

Arylboronic Acids. Imino Derivatives from o-Formylbenzeneboronic Acid[1]

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o-Formylbenzeneboronic anhydride reacts with aniline, p-toluidine, benzylamine, and n-propylamine to give Schiff bases which are isolated as the trimeric boronic anhydrides. These substances react with catechol to give the catechol derivatives of the boronic acids, in which the boron atom interacts with the neighboring nitrogen atom and becomes tetracovalent. When the free acid, o-formylbenzeneboronic acid, reacts with methoxyamine, the expected oxime ether is formed. Attempts to convert the product, N-o-boronobenzalmethoxyamine, into the boronic anhydride are complicated by the occurrence of a transformation of the Beckmann type, by which oximino ether groups are converted into nitrile groups, some of which are hydrolyzed to amide groups, with the result that a complex mixture of simple and mixed trimeric boronic anhydrides is formed. The previously known heterocyclic substance, 4-hydroxy-4,3-boroxaroisoquinoline, formed from the formylboronic acid and hydroxylamine, likewise undergoes a Beckmann transformation on heating and yields a mixture of trimeric simple and mixed anhydrides containing nitrile and amide groups.

Because of the ease of formation and the stability of the lactone ring in boronophthalide, I, one might expect o-formylbenzeneboronic acid, or its anhydride II, to react with primary amines to form substituted aminoboronophthalides rather than simple Schiff bases. The lactone ring of I forms so readily that the hydroxyboronic acid is unknown,[3,4] and the carbon–boron bond of I is stable to strong acids or strong bases[4] under conditions which effect the deboronation of most boronic acids.[5,6] It is surprising to find that the trimeric anhydride of II reacts with primary aromatic and aliphatic amines, such as aniline, p-toluidine, benzylamine, and n-propylamine, to give Schiff bases, readily obtained as the trimeric boronic anhydrides, III. The structures IIIa–d are indicated by the analyses, by the occurrence of –C=N– absorption in the region of 1622–1648 cm^{-1}, and by the occurrence of the boronic anhydride[7] B—O absorption in the 1315–1390-cm^{-1} region, as well as by the absence of absorption due to OH or NH

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(3) K. Torssell, *Arkiv Kemi*, **10**, 509 (1957).

(4) H. R. Snyder, A. J. Reedy, and W. J. Lennarz, *J. Amer. Chem. Soc.*, **80**, 835 (1958).

(5) M. F. Lappert, *Chem. Rev.*, **56**, 994 (1956).

(6) A. D. Ainley and F. Challenger, *J. Chem. Soc.*, 2171 (1930).

(7) See R. L. Letsinger, Advances in Chemistry Series, No. 42, American Chemical Society, Washington, D. C., p 3.