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

Arylboronic Acids. IV. Reactions of Boronophthalide[1]

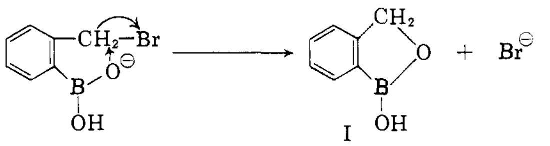
BY W. J. LENNARZ[2] AND H. R. SNYDER

RECEIVED AUGUST 10, 1959

The preparation of derivatives of boronophthalide (I) is reported. The product of nitration of boronophthalide, 5-nitroboronophthalide, is reduced to 5-aminoboronophthalide. 5-Aminoboronophthalide is converted to N-succinyl-5-aminoboronophthalide and to boronophthalide-5-diazonium chloride. The diazonium compound is coupled with 3-carboxy-2-naphthol. Several attempted transformations of the boronolactone ring of boronophthalide are discussed.

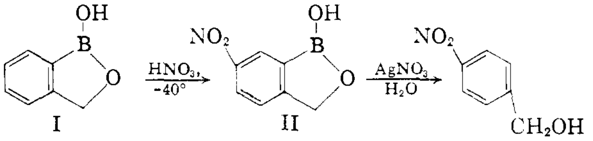
The preparation of a unique boronic acid derivative, boronophthalide (I), has been reported,[3,4] but aside from the resistance of this compound to dehydration and to hydrolytic deboronation,[4] little is known about its chemical reactivity. Therefore, a study of the chemical reactions of boronophthalide was undertaken. Apart from the purely chemical considerations, it was of interest to prepare hydrolytically-stable boronophthalide derivatives that might be useful in boron-disintegration cancer therapy.[4]

Boronophthalide was prepared by alkaline hydrolysis of *o*-(bromomethyl)-benzeneboronic anhydride.[4] The cyclization of the bromide to boronophthalide is very facile, and it seems more likely that the reaction occurs directly by an internal displacement of the bromide ion by the boronate anion, rather than by internal dehydration of an intermediate *o*-(hydroxymethyl)-benzeneboronic acid as suggested by Torssell.[3] The structure of boronophthalide was deduced from the method of synthesis and from the results of combustion analyses.[3,4] In order to substantiate the structural assignment, molecular weight determinations and nuclear magnetic resonance studies[5] were carried out on I. The results of these studies were consistent with the cyclic, monomeric structure assigned to boronophthalide.



It was found that boronophthalide could be nitrated without deboronation or oxidation of the methylene group. The reaction was carried out with fuming nitric acid at −35 to −40°,[6] and 5-nitroboronophthalide (II) was isolated in 84% yield. The infrared spectrum of II contained strong bands at 1345 and 1520 cm.$^{-1}$ which may be attributed to nitro group absorption.[7]

Deboronation with aqueous silver nitrate was used to verify the position of the nitro group in 5-nitroboronophthalide.[8] In a test experiment it was found that boronophthalide was converted to benzyl alcohol. When the reaction was carried out under the same conditions with 5-nitroboronophthalide, the product that was isolated in high yield was found to have an infrared spectrum identical with that of *p*-nitrobenzyl alcohol. The melting point of a mixture of recrystallized deboronation product and *p*-nitrobenzyl alcohol was not depressed. Oxidation of the deboronation product with permanganate afforded *p*-nitrobenzoic acid.



Reduction of 5-nitroboronophthalide to 5-aminoboronophthalide (III) was accomplished readily with hydrogen and Raney nickel catalyst. 5-Aminoboronophthalide very slowly darkened in air and decomposed rapidly when heated. In subsequent experiments that required the use of the amine hydrochloride, it was found expedient to convert the amine directly to its more stable hydrochloride without isolating the free amine.

Soloway has found that while *m*-aminobenzeneboronic acid has a tumor–brain tissue localization factor of only 1.2 in mice, the corresponding N-succinyl derivative has a factor of approximately 8.[9] For this reason it was desirable to prepare N-succinyl-5-aminoboronophthalide. Reaction of equimolar quantities of 5-aminoboronophthalide and succinic anhydride in absolute ethanol afforded N-succinyl-5-aminoboronophthalide (IV) in 89% yield. The infrared spectrum of IV had strong bands at 1665 and 1695 cm.$^{-1}$ which may be attributed to amide carbonyl absorption and carboxyl carbonyl absorption, respectively.[7]

Attention was next directed to the diazotization of 5-aminoboronophthalide and the diazo coupling of the diazonium salt with a phenol. Several azo dyes have shown promise in boron-disintegration therapy,[10] and it was hoped that demonstration of the feasibility of coupling boronophthalide-5-diazonium chloride with a simple phenol would open the way for later studies involving the preparation of more complex dyes containing the boronophthalide moiety.

5-Aminoboronophthalide hydrochloride was diazotized by the addition of a solution of sodium

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(1) Part of this work was supported by a grant [AT(11-1)314] from the Atomic Energy Commission.

(2) Public Health Service Research Fellow of the National Cancer Institute, 1958–1959.

(3) K. Torssell, *Arkiv. Kemi*, **10**, 507 (1957).

(4) H. R. Snyder, A. J. Reedy and W. J. Lennarz, THIS JOURNAL, **80**, 835 (1958).

(5) For details see forthcoming paper V in this series, H. R. Snyder, R. T. Hawkins and W. J. Lennarz.

(6) K. Torssell, *Arkiv. Kemi*, **10**, 497 (1957).

(7) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," ed. 2, Methuen and Co., London, 1956.

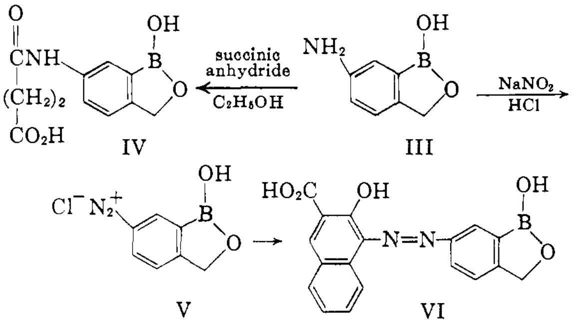
(8) A. Michaelis and P. Becker, *Ber.*, **15**, 180 (1882); A. Michaelis and M. Behrens, *ibid.*, **27**, 244 (1894).

(9) A. H. Soloway, private communication; *cf.* A. H. Soloway, *Science*, **128**, 1572 (1958).

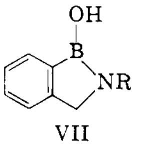
(10) H. R. Snyder and S. L. Meisel, THIS JOURNAL, **70**, 774 (1948); P. G. Kruger, *Radiation Research*, **3**, 1 (1955).

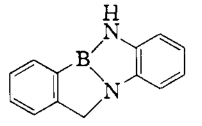
nitrite to a slurry of the amine hydrochloride in hydrochloric acid. Addition of a slurry of the diazonium compound to a basic solution of 3-carboxy-2-naphthol resulted in the copious precipitation of a red solid. The deep red powder that was isolated in 95% yield was insoluble in water and soluble in warm dimethylformamide and in base.

The infrared spectrum of the dye, which was tentatively assigned the structure VI, was very diffuse and hence difficult to interpret. Analytically pure VI was obtained by dissolving the dye in dimethylformamide and precipitating it from the solution by the addition of water.



Aside from the unsuccessful attempts by Reedy to brominate and to oxidize the methylene group of boronophthalide,[11] and the previously mentioned deboronation of boronophthalide with silver nitrate, no reactions involving the five-membered ring of boronophthalide had been attempted. It was of interest to determine whether boronophthalide could be converted to boronophthalimidine (VII, R = H) or one of its derivatives by reaction with amines. Attempts to effect this conversion were made with anhydrous ammonia under pressure at 100°, and with boiling benzylamine. In neither case was any nitrogen-containing product isolated. It was thought it might be possible to convert boronophthalide to the known boradiazole VIII[12] by reaction of boronophthalide with *o*-phenylenediamine. When the reaction was carried out under conditions similar to those developed by Letsinger and Nazy[13] *o*-phenylenediamine and boronophthalide were recovered.


VII


VIII

Benzyl alcohol has been converted to benzaldehyde by oxidation with selenium dioxide.[14] No oxidation of boronophthalide was observed when it was treated with either one or two molecular equivalents of selenium dioxide in aqueous dioxane.

In a continuation of our studies of the infrared spectra of boronic acids and boronic acid derivatives,[15] the infrared spectra of boronophthalide derivatives have been examined. The absorption band which invariably occurs near 1350 cm.$^{-1}$ appears to be the most reliable criterion for the presence of the boron–oxygen linkage in aryl boronic acids and anhydrides.[15] However, it has been found that this is not the case with boronophthalide and its derivatives. If an absorption band due to the boron–oxygen linkage is present in the 1350 cm.$^{-1}$ region in these compounds, it is very weak and not useful for interpretation. It appears that a boron–oxygen absorption band in boronophthalide derivatives occurs in the 1375–1475 cm.$^{-1}$ region, but due to the complex absorption bands in this region and the limited number of compounds available for study, it has not been possible to determine the location of this boron–oxygen band with certainty.

An infrared absorption band that may be characteristic of boronophthalide derivatives has been found. The source of this absorption band, which occurs in the 1000–970 cm.$^{-1}$ region, is not definitely known. The C–O stretching frequency band of four- and five-membered cyclic ethers[7] falls in the range 1100–970 cm.,$^{-1}$ and it may be that the band in boronophthalide derivatives is due to the stretching absorption of the C–O band in the boronolactone ring.

In Table I is listed the region of absorption of the "C–O" band of each boronophthalide derivative, together with the location of the hydroxyl group absorption band of that compound.

TABLE I

SELECTED INFRARED ABSORPTION BANDS OF BORONOPHTHALIDE AND ITS DERIVATIVES[a]

Compound	Hydroxyl band, cm.$^{-1}$	"C–O" band, cm.$^{-1}$
Boronophthalide	3300	970
5-Nitroboronophthalide	3250	980
5-Aminoboronophthalide	3350	985
5-Aminoboronophthalide hydrochloride	3440	975
N-Succinyl-5-aminoboronophthalide	3400	980
6-Methylboronophthalide[5]	3310	970

[a] All spectra obtained from Nujol mulls.

Experimental[16]

Boronophthalide.—*o*-(Bromomethyl)-benzeneboronic anhydride was prepared as described by Snyder, *et al.*, for the preparation of *p*-(bromomethyl)-benzeneboronic anhydride,[15] and was converted to boronophthalide by the method previously reported.[4] It was found that crude boronophthalide may be purified by sublimation at 50° (0.05 mm.). The compound sublimes readily to give boronophthalide of analytical purity. The results of molecular weight determinations on boronophthalide are: mol. wt. calcd., 134; mol. wt. found, 143 (ebullioscopic, in benzene), 124 (Rast, in camphor).

5-Nitroboronophthalide.—To 10 ml. of fuming nitric acid (sp. gr. 1.49), maintained at −45 to −40° was added 1.60 g. (0.0115 mole) of boronophthalide with stirring. The addition was done portionwise and was complete in 4 min. The mixture was stirred and maintained at −45 to −30° for 20 min.; at the end of this time the mixture was very pasty.

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(11) A. J. Reedy, Thesis, Doctor of Philosophy, University of Illinois, 1957.

(12) See forthcoming paper VI in this series, H. R. Snyder and R. T. Hawkins.

(13) R. L. Letsinger and J. R. Nazy, THIS JOURNAL, **81**, 3013 (1959).

(14) N. Rabjohn, *Org. Reactions*, **5**, 331 (1949).

(15) H. R. Snyder, M. S. Konecky and W. J. Lennarz, THIS JOURNAL, **80**, 3611 (1958).

(16) Microanalyses were carried out by Mr. Josef Nemeth and his associates. Infrared spectra were determined by Mr. Paul McMahon and his associates. Molecular weight determinations were carried out by Clark Microanalytical Laboratories, Urbana, Ill. Capillary melting points are corrected.

It was poured with stirring into water and ice and stored at 0–10° for 2 hr. The pale yellow crystals were collected, washed with *ca.* 25 ml. of water, and dried *in vacuo* over phosphorus pentoxide. The crystals weighed 2.03 g. (98.5% yield). Recrystallization from water afforded 1.73 g. (84% yield) of pale yellow crystals that melted at 186–191°.

An analytical sample was prepared by twofold recrystallization from water and subsequent drying *in vacuo* over phosphorus pentoxide. The resulting yellow needles melted at 203–206°. The crystals, when observed on the Kofler micro-melting point stage, were found to melt partially between 150–190° and then resolidify in the form of cubes.

Anal Calcd. for $C_7H_6NO_4B$: C, 46.92; H, 3.36; N, 7.84. Found: C, 47.07; H, 3.39; N, 7.68.

Test Deboronation[8] of Boronophthalide.—To 500 mg. of boronophthalide was added 12 ml. of 0.4 *N* silver nitrate. The mixture was warmed on the steam-bath for 75 min. during which time all the boronophthalide dissolved. The solution then was cooled and extracted with three 15-ml. portions of ether. The combined ether extract was dried over magnesium sulfate and evaporated under a stream of nitrogen. The resulting oil was distilled through a small column; b.p. 110–115° (35 mm.), n^{25}D 1.5355. Literature values for benzyl alcohol are: b.p. 93° (10 mm.), n^{25}D 1.5395.[17] The infrared spectrum of the deboronation product was identical with that of an authentic sample of benzyl alcohol.

Deboronation of 5-Nitroboronophthalide.—To 600 mg. of 5-nitroboronophthalide was added 15 ml. of 0.4 *N* silver nitrate. The mixture was warmed on the steam-bath. After *ca.* 30 min. solution was complete, and the solution was brown. Heating was continued for another 90 min. and then the solution was allowed to stand at room temperature for 2 hr. The flask was warmed in order to dissolve some crystalline material and the solution was extracted with four 10-ml. portions of ether. The combined ether extract was evaporated under a stream of nitrogen. The solid weighed 500 mg. (92% yield). After recrystallization from carbon tetrachloride and then from water, the sample melted at 88–90°; a mixture of this material and an authentic sample of *p*-nitrobenzyl alcohol melted at 90–92°. The infrared spectra of *p*-nitrobenzyl alcohol and of the sample prepared by deboronation of 5-nitro-boronophthalide were identical.

A sample of the crude deboronation product was oxidized using the procedure outlined by Vogel.[18] After recrystallization, the product melted at 240–242°; the mixed melting point with an authentic sample of *p*-nitrobenzoic acid was 240–242°. The infrared spectra of the oxidized deboronation product and of *p*-nitrobenzoic acid were identical.

5-Aminoboronophthalide.—To a solution of 2.50 g. (0.0140 mole) of 5-nitroboronophthalide in *ca.* 50 ml. of absolute ethanol, contained in a Parr hydrogenation bottle, was added *ca.* 0.5–1.0 g. of Raney nickel catalyst (W-2). The initial pressure during the hydrogenation was 1.7 atm.; after 16 hr. the theoretical amount of hydrogen had been consumed. The nickel was removed by filtration and the ethanol was evaporated from the filtrate *in vacuo* at room temperature.[19] When a drop of water was added to the residual oil, crystallization ensued. The solid was recrystallized from 12 ml. of 25% ethanol. The tan crystals obtained (1.64 g., 79% yield) decomposed at 154° (bath preheated to 100°) sealed in a capillary under nitrogen. 5-Amino-boronophthalide decomposes very slowly in air at room temperature and rapidly at elevated temperatures. Difficulty was encountered in the preparation of an analytically pure sample of 5-aminoboronophthalide. Recrystallization from 95% ethanol afforded pale yellow crystals, m.p. 157° dec.

Anal.[20] Calcd. for $C_7H_8NO_2B$: C, 56.44; H, 5.41; N, 9.39. Found: C, 56.06; H, 5.41; N, 9.47.

5-Aminoboronophthalide Hydrochloride.—The reduction of 1.75 g. (0.00978 mole) of 5-nitroboronophthalide in *ca.* 80 ml. of absolute ethanol was carried out as described above. After the nickel was removed by filtration, the filtrate was cooled in an ice-bath, and anhydrous hydrogen chloride was bubbled into the solution for several minutes. The solution was then concentrated *in vacuo* to *ca.* 30 ml. Addition of 75 ml. of absolute ether effected the precipitation of the hydrochloride. The mixture was cooled in the refrigerator for an hour and then the solid was collected and dried *in vacuo*. The pale yellow crystals obtained weighed 1.58 g. (87% yield), and darkened but did not melt below 350°.

An analytical sample was prepared by recrystallization from 4 *N* hydrochloric acid and subsequent drying *in vacuo*.

Anal. Calcd. for $C_7H_9NO_2BCl$: C, 45.34; H, 4.88; N, 7.54; B, 5.85; Cl, 19.00. Found: C, 44.61; H, 4.91; N, 7.69; B, 5.30; Cl, 18.94.

N-Succinyl-5-aminoboronophthalide.—A mixture of 1.645 g. (0.01105 mole) of 5-aminoboronophthalide, 1.105 g. (0.01105 mole) of resublimed succinic anhydride and 18.0 ml. of absolute ethanol was stirred and heated to 60° under nitrogen. After *ca.* 10 min. a copious precipitate began to form. The flask was warmed to *ca.* 75° in order to effect solution of the precipitate and then maintained at 65° for 10 hr. The solution was cooled ultimately to −10°. The white crystals were collected by filtration, washed with a small amount of cold ethanol and dried *in vacuo;* 2.22 g., 80% yield, m.p. 205–207°. A second crop of crystals (0.24 g., 9% yield) was obtained by concentration of the filtrate.

The first and second crops were combined and recrystallized from 190 ml. of 25% ethanol (dissolution was very slow). The pale tan plates were collected and dried *in vacuo*. The product weighed 2.07 g. (75% yield) and melted at 210.5–212.5°.

An analytical sample was prepared by recrystallization from 25% ethanol and subsequent drying at 5 mm. over phosphorus pentoxide for 22 hr.

Anal. Calcd. for $C_{11}H_{12}NO_5B$: C, 53.08; H, 4.85; N, 5.62. Found: C, 52.79; H, 4.87; N, 5.48.

Diazotization of 5-Aminoboronophthalide.—A solution of 1.500 g. (0.0081 mole) of 5-aminoboronophthalide hydrochloride in 4.5 ml. of water, was cooled in an ice-bath, treated with 0.97 ml. of concentrated hydrochloric acid, stirred well and cooled to 0–5°. A solution of 0.567 g. (0.0081 mole) of sodium nitrite in 4.5 ml. of water was added at such a rate that the temperature of the amine hydrochloride suspension did not exceed 5°. Near the end of the addition the suspension was tested for excess of nitrite ion with starch–iodide paper. When all but *ca.* 0.3 ml. of the sodium nitrite solution had been added, the test was positive. An additional 10–20 mg. of amine hydrochloride was added; the starch–iodide test was then negative. One drop of sodium nitrite solution was added and the suspension stirred for 3 min.

Coupling of the Diazonium Compound with 3-Carboxy-2-naphthol.—The suspension of diazotized 5-aminoboronophthalide prepared as described above was added to a cool, stirred solution of 1.521 g. (0.0081 mole) of 3-carboxy-2-naphthol in 13.0 ml. of 10% sodium hydroxide, the bright red, viscous mixture was stirred for 15 minutes, and poured into a stirred solution of 15 ml. of 10% hydrochloric acid and 5 ml. of water. A deep red precipitate resulted. The mixture was stored in the refrigerator for 1 hr. and then the precipitate was collected, washed well with water and dried *in vacuo* overnight. The red powder weighed 2.68 g. (95% yield). It darkened gradually when heated but did not melt below 350°. The infrared spectrum of the dye was very diffuse and difficult to interpret.

An analytical sample was prepared by dissolving 500 mg. of the dye in 15 ml. of warm dimethylformamide and then precipitating it by the addition of 10 ml. of water acidified with 2 drops of 10% hydrochloric acid. The resulting mixture was cooled in the refrigerator for several hours and the precipitate collected and dried overnight in a vacuum oven at 50°. The resulting dark red powder was dried further at room temperature *in vacuo* over phosphorus pentoxide for 2 days.

Anal. Calcd. for $C_{18}H_{13}N_2O_5$: C, 62.09; H, 3.76; N, 8.05. Found: C, 61.92; H, 4.07; N, 8.16.

Attempted Reaction of Boronophthalide with Amines. With Ammonia.—To 2.0 g. (0.0149 mole) of boronophthalide contained in a steel bomb was added *ca.* 75 ml. of liquid ammonia. The bomb was sealed and heated to 100° for 3 hr. The recorded pressure during this time was 34 atm. The bomb was allowed to cool to room temperature, opened and the ammonia allowed to evaporate in a hood. The

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(17) C. D. Hodgman, "Handbook of Chemistry and Physics," Chemical Rubber Publishing Co., ed. 31, 1954, p. 708.

(18) A. I. Vogel, "Practical Organic Chemistry," Longmans, Green and Co., London, ed. 3, 1956, p. 755.

(19) When the solution was concentrated at higher temperatures, the amine decomposed.

(20) This analytical sample was prepared by Dr. T. Tanaka.

solid residue was dissolved in absolute ethanol. The resulting Burgundy-colored solution was treated with Darco and filtered. The filtrate, which was still colored, was evaporated to dryness *in vacuo*. The resulting mixture of brown oil and crystals was dissolved in 5 ml. of 10% potassium hydroxide, filtered and acidified with 10% hydrochloric acid. The white precipitate that resulted was collected and dried. It was identified as boronophthalide from its infrared spectrum.

With Benzylamine.—A mixture of 1.0 g. (0.0074 mole) of boronophthalide in 15 ml. of benzylamine was heated to reflux for 25 hr. under nitrogen, during which the boronophthalide dissolved. The solution was allowed to cool and 12 ml. of water was added. Two phases formed. The lower, organic phase was separated and washed with three 10-ml. portions of water. The oil was then suspended in 10 ml. of water and this mixture extracted with three 10-ml. portions of ether. The combined ether extract was dried over magnesium sulfate and the ether evaporated under a stream of nitrogen. The remaining oil, which had a slight odor of benzylamine, was dried *in vacuo* overnight and distilled; b.p. 80–110° (*ca.* 5 mm.). The distillate was identified as benzyl alcohol from its infrared spectrum.

With *o*-Phenylenediamine.[13]—A mixture of 803 mg. (0.0060 mole) of boronophthalide, 648 mg. (0.0060 mole) of *o*-phenylenediamine and 20 ml. of absolute ethanol was heated to reflux for 10 min. While the solution continued to boil, toluene was added in 2-ml. portions during a 30-minute period. At the end of this time all the ethanol had boiled away and the temperature of the refluxing solution was 109°. The total volume was *ca.* 25 ml. Boiling was continued for 10 min., and then the solution was filtered through absorbent cotton. The filtrate was allowed to cool to room temperature, whereupon crystallization occurred. The tan crystals (*ca.* 500 mg.) were collected. The crystals were identified as *o*-phenylenediamine from their infrared spectrum.

Attempted Oxidation of the Methylene Group of Boronophthalide.—A mixture of 1.0 g. (0.0075 mole) of boronophthalide, 0.83 g. (0.0075 mole) of selenium dioxide and 25 ml. of 80% aqueous dioxane was heated under reflux for 12 hr. The solution, which had a yellow color and contained a small amount of black, metallic selenium, was filtered. The selenium weighed only 30 mg.

A second experiment was run using the same solvent and reaction conditions except that 1.66 g. (0.0150 mole) of selenium dioxide was used. No appreciable amount of selenium was precipitated.

URBANA, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

Association Phenomena. I. Specific Cation Effects on the Hydrolysis and Glycinolysis of Acetyl Phosphate[1]

BY JOSEPH L. KURZ[2] AND C. DAVID GUTSCHE

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The kinetics of the disappearance of acetyl phosphate in glycine–glycinate buffers has been analyzed in terms of rate constants defined by the equation $-\mathrm{d}[\mathrm{AcPO_4^{=}}]/\mathrm{d}t = (k_\mathrm{w}[\mathrm{H_2O}] + k_\mathrm{OH}a'_\mathrm{OH} + k_\mathrm{HG}[\mathrm{HG}] + k_\mathrm{G}[\mathrm{G^-}])[\mathrm{AcPO_4^{=}}]$, where a'_OH is the *p*H meter-determined hydroxide ion activity and the quantities in brackets are concentrations. The four rate constants have been studied as a function of cation species (tetramethylammonium ion and lithium ion), cation concentration, temperature and solvent. A rationalization of the results is based on the assumption of complex formation between the cation and one or more of the reactants. With the exception of the activation parameters for k_W, the behavior of these specific rates is consistent with these assumptions: (1) in water solution, there is little association between the reactants and the cations, but appreciable association of the cations with the triply charged transition states; (2) in 50% dioxane, there is association of the cations with all the reactant anions, the association constants increasing with the charge and polarizability of the anion; (3) although tetramethylammonium ion associates, it has little ability to polarize the anion with which it associates. Preliminary studies with polymeric quaternary ammonium compounds indicate that these are more effective catalysts than tetramethylammonium ion.

Many reactions occurring in living systems take place in aqueous solution and involve charged reactants. The mode of action of enzymes mediating such processes should involve the same types of forces, electrostatic and otherwise, which are operative in the interactions of simple ions. To gain insight into the nature of these interactions, the reaction of acetyl phosphate with aqueous glycine has been investigated using lithium ion and tetramethylammonium ion as models for the metal ions and quaternary ammonium ion centers which may be involved in comparable enzymatic reactions.

Acetyl phosphate was chosen as a substrate because it is a known component of several biological systems[3] and because it already has received a certain amount of attention from a purely chemical viewpoint. Lynen[4] observed that the hydrolysis of acetyl phosphate is first order at *p*H 7.4; Lipmann and Tuttle[5] studied the hydrolysis rate as a function of *p*H and observed catalysis in acid by molybdate ion and in base by calcium ion; Weil-Malherbe and Green[6] also studied the molybdate-catalyzed hydrolysis; Bentley[7] studied the hydrolysis with H_2O^{18} and also investigated the acetylation of some amines; similarly, Lipmann and Tuttle[8] and Chantrenne[9] studied amine acylation with particular reference to hydroxylamine; and finally, Koshland[10,11] has made an extensive study of both the hydrolysis reaction and the glycinolysis reaction which provided the starting point for the work described in this communication.

Koshland's study of the kinetics of the reaction

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[2] Du Pont Fellow in Chemistry, 1957–1958; Monsanto Research Associate, summer, 1957.

[3] *Cf.*, for instance, L. P. Hager, D. M. Geller and F. Lipmann, *Fed. Proc.*, **13**, 734 (1954); I. A. Rose, M. Grunberg-Manago, S. R. Korey and S. Ochoa, *J. Biol. Chem.*, **211**, 737 (1954); E. R. Stadtman, *ibid.*, **196**, 527 (1952); B. L. Horecker, E. C. Heath, J. Hurwitz and A. Ginsburg, *Fed. Proc.*, **16**, 198 (1957).

[4] F. Lynen, *Ber.*, **73**, 367 (1940).

[5] F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **153**, 571 (1951).

[6] H. Weil-Malherbe and R. H. Green, *Biochem. J.*, **49**, 286 (1951).

[7] R. Bentley, THIS JOURNAL, **70**, 2183 (1948).

[8] F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 286 (1945).

[9] H. Chantrenne, *Compt. rend. trav. lab. Carlsberg, Ser. chim.*, **26**, 231 (1948).

[10] D. E. Koshland, THIS JOURNAL, **73**, 4103 (1951).

[11] D. E. Koshland, *ibid.*, **74**, 2286 (1952).