TABLE V

CONVERSION OF $[(CH_3)_2CHPCl_3][AlCl_4]$ (XVIII) TO $(CH_3)_2CHP(O)(OR')_2$[a]

Run	R'	B.p. °C.	B.p. Mm.	$n^{25}D$	Yield, calcd. on PCl_3, %
10	CH_3[b]	71	5.5	1.4179	40
11	*i*-C_3H_7 and C_2H_5[c]	47	0.7	1.4138	77
12	*i*-C_3H_7[d]	38.5	0.25	1.4129	21
13	*i*-C_3H_7[e]	44	0.35	1.4148	62

[a] XVIII (0.5 mole) was prepared in all runs in the usual manner from isopropyl chloride in methylene chloride solution. [b] To XVIII (0.5 mole) in 150 cc. of solvent was added dropwise 64 g. (2 moles) of absolute methanol. After the addition of about 0.5 mole of methanol, a white solid began to precipitate. The alcoholysis mixture was refluxed for 4 hr. after the addition of the methanol was completed. The addition of 300 cc. of water to the mixture resulted in the formation of a gelatinous emulsion which was broken by centrifuging. [c] Described in detail in the Experimental part. [d] XVIII in 200 cc. of solvent was refluxed for 6 hr. with 2 moles of isopropyl alcohol. The white solid precipitate, formed upon the addition of 300 cc. of water, was dissolved by adding 100 cc. of concentrated sulfuric acid. In addition to the product, 33 g. of liquid distillation residue was obtained; on attempted distillation of the residue, decomposition occurred. [e] XVIII in 600 cc. of solvent was first treated with 18.4 g. (0.4 mole) of absolute ethanol in 50 cc. of solvent, refluxed for 1 hr., treated with 80 g. (1.33 moles) of isopropyl alcohol, refluxed for an additional 5 hr. and hydrolyzed at −10° with 110 cc. of water by method B.

overnight. About 200 cc. of the solvent was then removed by distillation at atmospheric pressure; the remainder was evaporated under reduced pressure, leaving a pale yellow, crystalline mass which weighed 93.5 g. The product which did not fume in air was pulverized to a light, crystalline powder. Recrystallization from methylene chloride after decolorizing with Norit yielded the complex VIII as a white, microcrystalline powder, m.p. 140–150° (not sharp). The complex VIII could be distilled without decomposition at a pressure of 5 mm.; at 242–245° a liquid distilled which solidified immediately in an attached air condenser.

Anal. Calcd. for CH_3AlCl_5OP: C, 4.5; H, 1.1; Al, 10.1; Cl, 66.6; P, 11.6. Found: C, 5.0; H, 1.6; Al, 9.6; Cl, 63.0; P, 11.6.

Reaction of Methylphosphonyldichloride with Aluminum Chloride.—To a solution of 25 g. of methylphosphonyldichloride in 150 cc. of methylene chloride was added 25 g. of aluminum chloride in portions. A vigorous exothermic reaction occurred keeping the solvent refluxing. After the initial reaction had subsided, the mixture was refluxed for an additional 30 minutes. The light brown solution was filtered to remove a small amount of solid and the filtrate was refrigerated overnight. The resulting deposit of fine, colorless crystals was filtered off, washed on the filter with a small amount of cold methylene chloride and dried in a vacuum desiccator; 93.9% yield.

Anal. Calcd. for CH_3AlCl_5OP: Al, 10.1; Cl, 66.6; P, 11.6. Found: Al, 10.3; Cl, 66.6; P, 12.8.

Reaction of $[CH_3PCl_3][AlCl_4]$ with Two Moles of Methanol.—To a suspension of 107 g. (0.33 mole) of VII in 250 cc. of methylene chloride was added dropwise in the course of 3 hr. a mixture of 21.0 g. (0.66 mole) of absolute methanol and 50 cc. of methylene chloride. After standing overnight, the solvent was removed from the clear, pale yellow solution under reduced pressure leaving 89.5 g. of a pale yellow, crystalline residue. The solid material was kept in a vacuum desiccator for 65 hr.; the loss of weight during this time was 9.5 g. The remaining 80 g. (91.9%) of product was recrystallized from methylene chloride to yield the complex $[CH_3P(O)OCH_3][AlCl_4]$ as colorless crystals.

Anal. Calcd. for $C_2H_6AlCl_4O_2P$: C, 9.2; H, 2.3; Al, 10.3; Cl, 54.2; P, 11.8. Found: C, 9.0; H, 2.4; Al, 10.4; Cl, 52.3; P, 11.9.

Preparation of Samples for NMR Spectra.—Solid V (0.3 mole) was dissolved with stirring at room temperature in 150 cc. of methylene chloride and a 2-cc. aliquot removed for determination of the NMR spectrum. The bulk of the solution was then treated with 13.8 g. (0.3 mole) of absolute ethanol in the usual fashion and refluxed for a period of 6 hr. The mixture, containing complex X, was cooled and another 2-cc. sample of the solution removed for NMR analysis. The alcoholysis was continued in two steps by adding each time a 0.3-mole portion of absolute ethanol and withdrawing at each step a sample for spectral analysis.

The solution used for the determination of the NMR spectrum of the complex $[C_2H_5P(O)(OC_2H_5)_2 \cdot AlCl_3]$ was prepared in the following manner. To a solution of diethyl ethylphosphonate (37 g., 0.22 mole) in 100 cc. of methylene chloride was added, after cooling to −78°, 30 g. (0.22 mole) of anhydrous aluminum chloride in two portions. An exothermic reaction took place upon each addition, but the temperature of the reaction mixture was maintained below 0° by cooling. The mixture was stirred below 0° until the aluminum chloride was dissolved and the stirring continued for an additional 2 hr. at room temperature. The trace amounts of insoluble material were allowed to settle by standing undisturbed overnight and an aliquot of the clear supernatant solution of the desired complex was removed for NMR analysis.

The spectra of III, VI, IX and the liquid complex XVI were determined without solvent.

Table I contains the chemical shifts exhibited by the various ethyl complexes and their phosphorus-containing components.

ARMY CHEMICAL CENTER, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF VIRGINIA]

Monoesters and Ester-amidates of Aromatic Phosphonic Acids

BY ALFRED BURGER AND JAMES J. ANDERSON[1]

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Reaction of phosphonic acids with dicyclohexylcarbodiimide yields pyrophosphonic acids, $[RPO(OH)]_2O$, which with alcohols or phenol furnish the respective alkyl or phenyl hydrogen phosphonate esters. These in turn with dicyclohexylcarbodiimide give dialkyl pyrophosphonates, $[RPO(OR)]_2O$, which react with amines to render alkyl N-substituted phosphonamidates. The monoesters and ester-amidates of *p*-aminophenylphosphonic acid exhibited only negligible bacteriostatic activity. The biological observations are discussed in the light of ionization theories of analogous bacteriostatically active sulfanilamide derivatives.

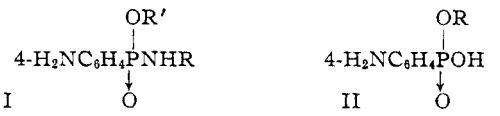
In the course of their studies about the dependence of bacteriostatic activity of sulfanilamide derivatives on the relative negative character of the SO_2 group in these compounds, Bell and Roblin[2] expressed the view that the principles outlined by them should apply to any substance of the type *p*-$H_2NC_6H_4XO_2R$, provided its activity is inhibited by *p*-aminobenzoic acid. If the properties of the aromatic amino group are constant, activity in such compounds should be expressed by the negativity

(1) Virginia-Carolina Chemical Corporation Fellow, 1955–1956.

(2) P. H. Bell and R. O. Roblin, Jr., THIS JOURNAL, 64, 2905 (1942).

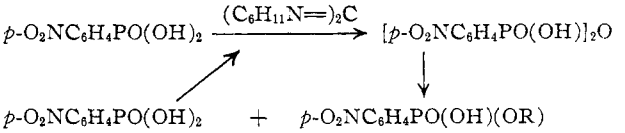
of the XO_2 group. Among compounds predicted to conform with this hypothesis were substances where X is phosphorus.

Since phosphanilic[3,4] and *p*-aminobenzenephosphonous acid[5] as well as phosphanilamide[4] show bacteriostatic activity and the effect of the first two compounds is antagonized by *p*-aminobenzoic acid,[3,5,6] Bell and Roblin's prediction seems to be borne out for these compounds. Moreover, several diamides of phosphanilic acid [$H_2NC_6H_4PO(NHR)_2$] are more highly bacteriostatic than the parent acid,[7] and here the inductive effect of the PO should be equally divided between both NHR groups. It appeared promising to synthesize alkyl N^1-substituted P-(4-aminophenyl)-phosphonamidates (I)[8] because the ionization of the hydrogen of the NHR group could be altered by a change in the electronegativity of one or both R radicals, and this would permit the design of variants with a



range of pK_a values and activities as predicted by Bell and Roblin. In the related, more strongly acidic alkyl hydrogen 4-aminophenylphosphonates (II), acid strength might be manipulated to some extent by varying the structure of the ester group.

Only few monoesters of phosphonic acids have been reported. The methods for their preparation[9-15] include such procedures as the partial alcoholysis of phosphonochloridates or the partial alkaline hydrolysis of dialkyl phosphonate esters and are not generally applicable.[12] The reaction of pyrophosphonic acids [$RPO(OH)]_2O$ with alcohols or phenols should lead to monoesters, but little is known about such anhydrides.[13] It has now been found that the method of Khorana and Todd[16] for the preparation of esters of diphosphoric acids can be extended to the synthesis of alkyl- and arylpyrophosphonic acids. Dehydration of phosphonic acids with one-half molar equivalent of dicyclohexylcarbodiimide in tetrahydrofuran solution quickly furnishes a precipitate of insoluble dicyclohexylurea, and the filtrate contains the desired anhydride. Several phosphonic acids were treated with dicyclohexylcarbodiimide; *n*-hexylphosphonic acid yielded a crystalline pyrophosphonic acid, $[C_6H_{13}PO(OH)]_2O$, while other anhydrides were obtained as viscous oils which we were unable to crystallize. Nevertheless, the tetrahydrofuran solution of the pyrophosphonic acids reacted smoothly with primary or secondary alcohols, or with phenol, to furnish the corresponding monoesters, RPO(OH)OR'. Inevitably, one-half of the phosphonic acid used was recovered from this step, and its separation from the acidic monoester proved difficult. In the case of 4-nitrophenylphosphonic acid, which is obtained by a relatively laborious process,[17] the unavoidable recovery of valuable starting material posed the need of recycling in order to increase the yield of monoesters. These obstacles were overcome by dissolving the phosphonic acid and the alcohol or phenol in tetrahydrofuran and slowly adding *one molar* equivalent of dicyclohexylcarbodiimide. The phosphonic acid recovered from the alcoholysis of the resulting pyrophosphonic acid was thus reconverted to the anhydride *in situ*, and the latter was recycled to more monoester. Excellent yields of monoesters were realized by this procedure (Table I).



When dicyclohexylcarbodiimide was added slowly to a solution of an alkyl hydrogen *p*-nitrophenylphosphonate and an amine, the latter was converted to the corresponding N,N'-dicyclohexyl-N''-alkylguanidine derivative [$C_6H_{11}NHC(NH)=NC_6H_{11}$]. Although the addition of amines to carbodiimides had been known,[18,19] the failure of phosphonate monoesters to form anhydrides which in turn could react with amines was surprising, since in an analogous case the addition of dicyclohexylcarbodiimide to a cold solution of two amino acids yielded peptides.[20] The synthesis of alkyl P-(*p*-nitrophenyl)-N-substituted phosphonamidates did succeed when the alkyl hydrogen *p*-nitrophenylphosphonates were converted to the corresponding dialkyl pyrophosphonates, $[p\text{-}O_2NC_6H_4PO(OR)]_2O$, dicyclohexylurea was filtered, and the filtrate containing the anhydride was heated with an amine The resulting ester-amidates (Table II) could be separated readily from the salts of the monoesters by extraction into ether.

The alkyl or aryl N-substituted P-(*p*-nitrophenyl)-phosphonamidates as well as the alkyl hydrogen *p*-nitrophenylphosphonates were hydrogenated over Raney nickel catalyst to the corresponding *p*-amino derivatives in good yields.

Experimental[21]

Materials.—4-Nitrophenylphosphonic acid was prepared according to Doak and Freedman.[17] Dicyclohexylcarbodi-

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(4) G. O. Doak and L. D. Freedman, THIS JOURNAL, **55**, 4825 (1955); *cf.* U. K. Kanitkar and B. V. Bhide, *Current Sci. (India)*, **16** 223 (1947).

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(8) In analogy to the nomenclature used for sulfanilamide derivatives, the nitrogen atoms of the phosphonamide group are designated N^1, the aromatic *p*-amino-nitrogen, N^4.

(9) J. B. Conant and E. L. Jackson, THIS JOURNAL, **46**, 1003 (1924).

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(11) J. B. Conant, V. H. Wallingford and S. S. Gandheker, *ibid.*, **45**, 762 (1923).

(12) B. S. Griffin and A. Burger, *ibid.*, **78**, 2336 (1956).

(13) H. H. Hatt, *J. Chem. Soc.*, 776 (1933); 2412 (1929); for a recent preparation of phenylpyrophosphonic acid see L. Anschütz and H. Wirth, *Chem. Ber.*, **89**, 688 (1956).

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(17) G. O. Doak and L. D. Freedman, THIS JOURNAL, **74**, 753 (1952).

(18) A. Huhn, *Ber.*, **19**, 2404 (1886).

(19) W. Weith, *ibid.*, **7**, 1303 (1874).

(20) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955)

(21) Melting points are corrected, boiling points uncorrected. Microanalyses by Mrs. J. Jensen and Miss B. J. Williamson.

TABLE I

MONOESTERS OF PHOSPHONIC ACIDS

R	R′	Yield, %[a]	M.p., °C. (cor.)	Formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
C_6H_5	C_6H_5[b]	82	70–72	$C_{12}H_{11}O_3P$	61.54	4.74	61.69	4.54
n-C_4H_9	C_2H_5	60	B.p. 147–149° (1 mm.)	$C_6H_{15}O_3P$	43.47	9.10	43.28	9.02
p-$O_2NC_6H_4$	C_6H_5	82	139–141	$C_{12}H_{10}NO_5P$	51.62	3.61	51.57	3.48
	C_2H_5	83	111–112.5	$C_8H_{10}NO_5P$	41.57	4.36	41.21	4.40
					N, 6.10		N, 5.98	
	CH_3	60	129–131	$C_7H_8NO_5P$	38.72	3.71	38.63	4.06
	CF_3CH_2[c]	77	115–117	$C_8H_7F_3NO_5P$	33.71	2.48	33.54	2.70
	cyclo-C_6H_{11}	91	123–125	$C_{12}H_{16}NO_5P$	50.53	5.65	50.83	5.49

[a] These yields were also realized if batches up to 0.15 mole were run. [b] Previously prepared by A. Michaelis[14] who reported m.p. 57°. [c] Calcd.: neut. equiv., 285.03. Found: neut. equiv., 283.7.

TABLE II

ESTER-AMIDATES OF p-NITROPHENYLPHOSPHONIC ACID

R	R′	Yield, %	M.p., °C. corr.	Formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
$C_6H_5CH_2$–	C_2H_5[a]	80	81.5–82.5	$C_{15}H_{17}N_2O_4P$	56.25	5.35	56.20	5.37
2-Thiazolyl-	CH_3[b,c]	77	135–136.5	$C_{10}H_{10}N_3O_4PS$	40.14	3.37	40.02	2.87
	C_2H_5[b,d]	85	138–139	$C_{11}H_{12}N_3O_4PS$	42.17	3.86	42.17	4.11
					N, 13.42		N, 13.02	
	C_6H_5[d,e]	67	172–174	$C_{15}H_{12}N_3O_4PS$	49.86	3.35	50.20	3.83
	CF_3CH_2[b,d]	81	143–145	$C_{11}H_9F_3N_3O_4PS$	35.97	2.47	36.06	2.42
2-Pyridyl-	CH_3[e]	55	113–115	$C_{12}H_{12}N_3O_4P$	49.15	4.13	48.97	4.03
	C_2H_5[e]	90	92–94	$C_{13}H_{14}N_3O_4P$	50.82	4.59	50.46	4.59
2-Pyrimidyl-	C_2H_5[b]	50	192–193	$C_{12}H_{13}N_4O_4P$	46.75	4.25	47.25	4.17
2-(5-Methylpyridyl)-	C_2H_5[b,f]	75	142–144	$C_{14}H_{16}N_3O_4P$	52.33	5.02	52.50	5.02

[a] Recrystallized from isopropyl ether. [b] Recrystallized from ethanol–isopropyl ether. [c] Bright yellow. [d] Pale yellow. [e] Recrystallized from butanone–isopropyl ether. [f] Picrate, m.p. 179–180°. Calcd. for $C_{20}H_{19}N_6O_{11}P$: C, 43.64; H, 3.48. Found: C, 43.53; H, 3.48.

imide was obtained by the directions of Schmidt, *et al.*,[22] except that commercial 5.25% sodium hypochlorite solution was used. The compound distilled at 117–120° (0.5 mm.) and melted at 34–35°.

2-Aminopyridine, 2-aminothiazole and 2-aminopyrimidine were supplied generously by Dr. Gilmer T. Fitchett, American Cyanamid Co. 3,4-Dimethyl-5-aminoisoxazole was donated by Hoffmann–LaRoche Co.

General Directions. (a) **Monoesters of Phosphonic Acids.**—A solution of 0.01 mole of the phosphonic acid and 0.01 mole of phenol, or the respective alcohol, in 25 ml. of dry tetrahydrofuran was heated to boiling, and a solution of 0.011 mole of dicyclohexylcarbodiimide in 10 ml. of the same solvent was added over a period of 2.5–3 hr. N,N′-Dicyclohexylurea separated out and was filtered after the mixture had been heated for another 8–10 hr. and allowed to stand at 37° for several hours. The solvent was evaporated under reduced pressure, the resulting pale-brown viscous oil was dissolved in 25 ml. of warm 1 *N* ammonium hydroxide, the solution was filtered and acidified with 6 *N* hydrochloric acid. The viscous oily esters crystallized on scratching to colorless solids which were recrystallized from mixtures of butanone and isopropyl ether, except phenyl hydrogen phenylphosphonate which crystallized from dilute ethanol.

t-Butyl alcohol did not react by this procedure. Data on the monoesters are collected in Table I.

(b) **Ester-amidates of 4-Nitrophenylphosphonic Acid.**—A solution of 22 mmoles of dicyclohexylcarbodiimide in 10 ml. of dry tetrahydrofuran was added slowly with stirring to a solution of 20 mmoles of the respective monoester of 4-nitrophenylphosphonic acid in 40 ml. of dry tetrahydrofuran at 25°. N,N′-Dicyclohexylurea precipitated out immediately. After the mixture was allowed to stand for 30 minutes, the N,N′-dicyclohexylurea was filtered, the filtrate heated to boiling and 22 mmoles of the respective amine was added. Refluxing was continued for 10 hr., and the solvent then evaporated under reduced pressure. The residual light brown tar was extracted with 75 ml. of boiling ether. The undissolved crystalline amine salt of the starting ester was filtered and the ether removed. When the remaining oil was taken up in the minimum amount of ethanol and the solution was diluted with a little water, a viscous oil precipitated which crystallized on scratching. The crude ester-amidates crystallized from isopropyl ether, or from mixtures of isopropyl ether and ethanol or butanone. Data on ester-amidates are listed in Table II. 3,4-Dimethyl-5-aminoisoxazole did not react by this procedure.

(c) **Monoesters and Ester-amidates of 4-Aminophenylphosphonic Acid.**—The derivatives of p-nitrophenylphosphonic acid (0.01 mole) were dissolved in 100 ml. of absolute methanol and hydrogenated with the aid of Raney nickel catalyst at 3 atm. and 25° in 2–3 hr. The catalyst was filtered, the solvent evaporated under reduced pressure, the crystalline or viscous residue was taken up in butanone and treated with isopropyl ether. In some cases a sparingly soluble amino compound precipitated during the hydrogenation; it had to be dissolved by addition of a few drops of ammonium hydroxide before the catalyst could be filtered, and the amine was reprecipitated by adjustment of the filtrate to *p*H 5–6. The colorless amino compounds obtained by this procedure are recorded in Table III.

P,P′-(Di-*n*-hexyl) Dihydrogen Pyrophosphonic Acid.—A solution of 1.13 g. (0.055 mole) of dicyclohexylcarbodiimide in 5 ml. of dry ether was added slowly with stirring to a solution of 1.66 g. (0.01 mole) of *n*-hexylphosphonic acid in 50 ml. of dry ether. N,N′-Dicyclohexylurea precipitated immediately and quantitatively. It was filtered and the filtrate concentrated to 5 ml. On cooling, 1 g. (65%) of colorless crystals precipitated which after recrystallization from ligroin melted at 64–66°.

Anal. Calcd. for $C_{12}H_{28}O_5P_2$: C, 45.85; H, 8.98. Found: C, 45.57; H, 9.11.

Determination of pK_a Values.—The apparent pK_a values for the compounds listed in Table IV were determined by measuring the *p*H of a solution containing equimolar concentrations of the acidic material and its salt, prepared by adding 0.2–0.7 mmole of the compound to 50–100 ml. of water followed by addition of the exact amount of standard base to neutralize one-half of the acidic substance.

(22) E. Schmidt, M. Seefelder, R. G. Jennen, W. Striewsky and H. v. Martius, *Ann.*, **571**, 83 (1951).

TABLE III

MONOESTERS AND ESTER-AMIDATES OF PHOSPHANILIC ACID

p-$H_2NC_6H_4PO(Y)(OR)$ Y	R	Yield, %	M.p., °C. corr.	Formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
OH	CH_3[a]	90	196.5–197	$C_7H_{10}NO_3P$	44.92	5.39	44.48	5.67
OH	C_2H_5[a]	80	208–210	$C_8H_{12}NO_3P$	47.76	6.01	47.70	6.09
OH	C_6H_5[b]	74	245.5–246	$C_{12}H_{12}NO_3P$	57.83	4.86	57.70	5.14
OH	cyclo-C_6H_{11}	77	236–238	$C_{12}H_{18}NO_3P$	56.46	7.11	56.44	7.43
2-Pyridylamino-	CH_3[c]	80	162–164	$C_{12}H_{14}N_3O_2P$	54.75	5.36	54.69	5.75
	C_2H_5[c]	75	167–169	$C_{13}H_{16}N_3O_2P$	56.31	5.82	56.82	5.60
2-Thiazolylamino-	CH_3[c]	79	162–163	$C_{10}H_{12}N_3O_2PS$	44.60	4.49	44.34	4.68
	C_2H_5[c]	80	163–165	$C_{11}H_{14}N_3O_2PS$	46.64	4.98	46.59	4.76
	CF_3CH_2[d]	76	145–147	$C_{11}H_{11}F_3N_3O_2PS \cdot {}^1/_2C_6H_{14}O$	43.30	4.67	42.94	4.91
							43.42	4.76
	C_6H_5[c]	91	209–211 d.	$C_{15}H_{14}N_3O_2SP$	54.37	4.26	53.67	4.29
2-Pyrimidylamino-	C_2H_5[c]	75	171–173	$C_{12}H_{15}N_4O_2P$	51.80	5.43	52.14	5.42
$C_6H_5CH_2NH$–	C_2H_5[a]	75	91– 93	$C_{15}H_{19}N_2O_2P$	62.06	6.60	62.43	6.47

[a] Recrystallized from methanol. [b] Purified by reprecipitation from dilute hydrochloric acid. [c] Recrystallized from butanone. [d] Recrystallized from butanone–isopropyl ether, dried at 110° (1 mm.). The presence of 0.5 mole of isopropyl ether of crystallization is based on the analytical values only, since infrared absorption shows all major peaks for ether oxygen where NH_2 may be expected to absorb. The compound contained nitrogen, phosphorus, sulfur and fluorine (small amounts in qualitative test) and exhibited a strong absorption band at 3 μ in the infrared.

A Beckman model G *p*H meter equipped with a glass electrode in conjunction with a saturated calomel electrode was used. From control experiments with known acids, the accuracy was judged to be ± 0.1 for pK_a values of 4 to 10, while for higher values a drift of 0.1–0.3 was observed. For values below 3.8, see Table IV.

TABLE IV

pK_a VALUES OF SOME DERIVATIVES OF 4-NITRO- AND 4-AMINOPHENYLPHOSPHONIC ACID

R	*p*-$RC_6H_4PO(R')(OR'')$ R′	R″	pK_a[a]	pK_a of sulfonamide analog $RC_6H_4SO_2R'$[2]
NO_2	OH	C_2H_5	2.5	
NO_2	OH	C_6H_5	2.5	
NO_2	OH	CF_3CH_2	2.5	
NO_2	OH	cyclo-C_6H_{11}	2.6	
NH_2	OH	CH_3	3.8	
NH_2	OH	C_2H_5	3.9	
NH_2	OH	C_6H_5	4.0	
NH_2	OH	cyclo-C_6H_{11}	3.9	
NO_2	2-Thiazolylamido-	CF_3CH_2	8.3	
NO_2	2-Thiazolylamido-	CH_3	8.6	
NO_2	2-Thiazolylamido-	C_2H_5	8.7	
NO_2	2-Thiazolylamido-	C_6H_5	8.9	
NO_2	2-Pyridylamido-	CH_3	10.2	
NO_2	2-Pyridylamido-	C_2H_5	10.4	
NH_2	2-Thiazolylamido-	CF_3CH_2	8.7	7.2
NH_2	2-Thiazolylamido-	CH_3	9.2	7.2
NH_2	2-Thiazolylamido-	C_2H_5	9.3	7.2
NH_2	2-Thiazolylamido-	C_6H_5	9.7	7.2
NH_2	2-Pyridylamido-	CH_3	10.4	8.43
NH_2	2-Pyridylamido-	C_2H_5	10.5	8.43
NH_2	2-Pyrimidylamido-	C_2H_5	10.4	7.86
NH_2	$C_6H_5CH_2$–	C_2H_5	10.7	..

[a] For pK_a values below 3.8 the accuracy is 0.2. Calculated by equation (252), S. Glasstone, "Textbook of Physical Chemistry," 2nd Ed., Van Nostrand Co., Inc., New York, N. Y., 1946. The other values were calculated by the Henderson equation.

Infrared Absorption Spectra.—Major assigned characteristic absorptions of *p*-nitro- and *p*-aminophenylphosphonic acid monoesters and ester-amidates were observed as follows: CF_3 at 9.2 μ[23]; NH_2 at 2.9 and 2.98 μ[23]; *p*-disubstituted phenyl at 12.1 μ[23]; NO_2 at 7.4 μ[23]; thiazole at 6.12 and 6.6 μ[24]; P–O–C aliphatic at 9.7–9.9 μ[25]; P=O at 7.8 μ.[25] In addition, all these compounds absorbed in the region of 10.2–10.4 μ which may be considered characteristic for many phosphorus compounds including phosphonates.[25]

The infrared data indicate that the ester-amidate derivatives of phosphanilic acid contain a free amino group and most probably do not exist as zwitterions. Thus the pK_a values should express the dissociation of the phosphonamidate group. In the case of monoalkyl hydrogen phosphanilates, no bond appears in the region of 3 μ. This implies that the amino group is associated and the pK_a values of these compounds represent probably the dissociation of the inner salt, *p*-$H_3N^+C_6H_4P(O)(OR)O^-$.

Discussion of Bacteriostatic Activity

The *in vitro* bacteriostatic activities of ethyl N^1-(2-pyridyl)-P-(*p*-aminophenyl)-phosphonamidate, the corresponding N^1-(2-pyrimidyl) and N^1-(2-thiazolyl) derivatives and the lower methyl N^1-(2-thiazolyl) homolog, as well as those of methyl and ethyl N^1-(2-pyridyl)- and N^1-(2-thiazolyl)-P-(*p*-nitrophenyl)-phosphonamidates were measured by the agar diffusion method in the Microbiology Section of Smith, Kline and French Laboratories. Also included in this series were methyl and ethyl hydrogen *p*-nitrophenylphosphonate and methyl hydrogen *p*-aminophenylphosphonate. The organisms employed were *Pseudomonas sp.*, *Escherichia coli*, *Micrococcus pyogenes* var. *aureus*, *Mycobacterium smegmadis*, and *Diplococcus pneumoniae* Type I. Only *D. pneumoniae* was inhibited slightly by all but two of the compounds tested in a 2×10^{-4} % solution, and *M. aureus* responded equally slightly to the four *p*-nitrophenylphosphonamidates. The relatively most active derivative, ethyl N^1-(2-pyridyl)-P-(*p*-aminophenyl)-phosphonamidate, did not protect mice against pneumococcus infection at 100 and 800 mg./Kg.

These disappointing findings are not easily fitted into the framework of the Bell and Roblin theory. The lack of correlation between bacteriostatic values and acid dissociation data points to

(23) H. Gilman, "Organic Chemistry," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1953, Chapter 2.

(24) H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determinations of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949.

(25) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, Chapter 18.

the influence of other factors which are not so pronounced in the sulfonamide series. Apparently the SO_2 and PO groups cannot compete equally for the negative charge left by the dissociation of the proton of the amide. In the bacteriostatically ineffective alkyl hydrogen *p*-aminophenylphosphonates the PO_2^- carries a formal negative charge as does the CO_2^- in the *p*-aminobenzoate ion, and the pK_a (3.8 to 4.0) of these esters approaches that of PABA (4.68). The effects of solubility, steric influences, etc., may outweigh those of acid dissociation; alkyl N^1-heterocyclically substituted P-(*p*-aminophenyl)-phosphonamidates are uniformly more soluble in base than the corresponding sulfonamide drugs. The valence requirements of the phosphonamidates place an alkyl group on one of the oxygens whereas this is absent in the sulfonamides. While it is conceivable that the extra group could interfere with the fit at an essential receptor site, the bacteriostatic activity of *p*-aminophenylphosphonamidates containing two relatively bulky substituents in the amidate group[7] cannot readily be reconciled with this view.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Synthesis of Peptides of Arginine Related to Arginine-vasopressin[1]

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The preparation of L-prolyl-L-arginylglycinamide dihydrobromide and S-benzyl-L-cysteinyl-L-prolyl-L-arginylglycin amide dihydrobromide, peptides containing sequences that are found in arginine-vasopressin, is described.

As a result of degradation studies on purified preparations of arginine-vasopressin, the principal pressor and antidiuretic hormone of the beef posterior pituitary gland, the sequence of amino acids in arginine-vasopressin was obtained[3] and a structure was postulated.[4,5] For the purpose of investigating synthetic routes to arginine-vasopressin, the peptides L-prolyl-L-arginylglycinamide dihydrobromide (I) and S-benzyl-L-cysteinyl-L-prolyl-L-arginylglycinamide dihydrobromide (II) were desired. The preparation of these peptides and their intermediates are described in this paper.

N^{α}-*p*-Nitrobenzyloxycarbonyl-L-arginylglycinamide hydrochloride (III) was prepared by the method of Gish and Carpenter[6] and the peptide derivative was isolated as the crystalline picrate IV. The picrate IV was cleaved with acetic acid saturated with hydrogen bromide to give L-arginylglycinamide dihydrobromide (V) as an amorphous solid. The dipeptide amide V was characterized as its crystalline dipicrate VI. The dihydrobromide V was converted to the monohydrobromide and the monohydrobromide was condensed with carbobenzoxy-L-proline by the tetraethyl pyrophosphite method[7] to give carbobenzoxy-L-prolyl-L-arginylglycinamide hydrobromide (VII). The tripeptide amide derivative VII was purified by countercurrent distribution and then treated with acetic acid saturated with hydrogen bromide to give L-prolyl-L-arginylglycinamide dihydrobromide (I). The amorphous product was converted to the crystalline diflavianate for characterization.

S-Benzyl-N-*p*-nitrobenzyloxycarbonyl-L-cysteine was converted to its acid chloride and then condensed with proline benzyl ester to give S-benzyl-N-*p*-nitrobenzyloxycarbonyl-L-cysteinyl-L-proline benzyl ester (VIII) and with proline methyl ester to give the corresponding methyl ester IX. The esters VIII and IX were obtained as oils, but could be converted to the crystalline hydrazide in high yield. Saponification of these esters yielded S-benzyl-N-*p*-nitrobenzyloxycarbonyl-L-cysteinyl-L-proline (X). Yields of 50–85% were obtained on various runs of the saponification of the benzyl ester of the *p*-nitrobenzyloxycarbonyl derivative of this dipeptide, whereas a yield of 52% was obtained in the saponification of the methyl ester. Saponification of S-benzyl-N-carbobenzoxy-L-cysteinyl-L-proline methyl ester (XI), prepared in a manner similar to that used for the preparation of IX, gave consistent yields of about 85%. No difficulties had been encountered previously during the saponification of *p*-nitrobenzyloxycarbonyl derivatives of peptides.[6,8] The dipeptide derivative X was condensed with L-arginylglycinamide monohydrobromide by the tetraethyl pyrophosphite method to give the S-benzyl-N-*p*-nitrobenzyloxycarbonyl-L-cysteinyl-L-prolyl-L-arginylglycinamide hydrobromide (XII). The crude product was purified by countercurrent distribution and the tetrapeptide amide derivative was characterized as its crystalline picrate XIII. Treatment of the purified tetrapeptide amide derivative XII with acetic acid saturated with hydrogen bromide gave S-benzyl-L-cysteinyl-L-prolyl-L-arginylglycinamide dihydrobromide (II) as an amorphous solid. Countercurrent distribution of this material in the system 2-butanol–0.1% acetic acid revealed a single peak and the tetrapeptide amide dihydrobromide

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(7) G. W. Anderson, J. Blodinger and A. D. Welcher, *ibid.*, **74**, 5309 (1952).

(8) F. H. Carpenter and D. T. Gish, *ibid.*, **74**, 3818 (1952).