[CONTRIBUTION FROM THE COLLEGE OF PHARMACY OF THE UNIVERSITY OF CALIFORNIA]

The Acid Strength of Mono and Diesters of Phosphoric Acid. The *n*-Alkyl Esters from Methyl to Butyl, the Esters of Biological Importance, and the Natural Guanidine Phosphoric Acids

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The esters of phosphoric acid are important compounds in the series of reactions concerned in the metabolism of carbohydrates.[1,2] The fact that the glycerol and sugar esters of phosphoric acid, as well as the naturally occurring guanidine phosphoric acids, have larger dissociation constants[3,4,5] than phosphoric acid is of much theoretical as well as biological interest. The significance for the metabolism of muscle of the alkalinization resulting from the hydrolysis of creatine phosphoric acid has been noted.[5,6] Lipmann[2] has pointed out that "at *p*H 7 with one mole of phosphate combining with hexose or creatine, respectively, 0.25 or 0.4 equivalent of 'acid' appear."

The seemingly anomalous situation wherein the replacement of a phosphoric acid hydrogen by a non-acidic group leads to an increase in the acid strength of the compound has given rise to some speculation.[2,3,4]

This investigation was undertaken to seek an explanation for this phenomenon. Before the behavior of the biologically active compounds can be accounted for, the behavior of the simple alkyl esters must be known. To that end we have measured the normal mono and diesters of phosphoric acid from methyl to butyl. The results have been discussed in terms of the factors known to affect acid strength. The knowledge thus obtained is applied to the compounds of biological interest.

Experimental

The *p*H meter and glass electrode chamber of Goyan, Barnes and Hind[7] was used. The barium salts were converted to the acid by addition of the exact amount of sulfuric acid. Stirring was effected with nitrogen. Carbonate-free sodium hydroxide was used. At least two titration curves were obtained for each compound. Accurate equivalent weights were obtained on the monoalkyl compounds. The dialkyl compounds were considered pure when further recrystallization of the barium salts caused no change in the titration curves. The presence of the barium sulfate had no adverse effect since the pKa' values obtained for phosphoric acid, $pKa'_1 = 1.97$ and $pKa'_2 = 6.82$, are in good agreement with values in the literature, $pKa'_1 = 1.97$ and 1.99 and $pKa'_2 = 6.85$ and 6.81.[4,8]

The dissociation constants were calculated as before.[9] Several points near the middle of each curve were used and the variation of the pKa' values gives an estimate of their accuracy. The over-all error is perhaps greater than the differences indicate, but it is probably less than 0.05 *p*H unit.

Materials

The barium salts of the monoalkyl phosphoric acids were obtained from commercial samples of the corresponding alkyl phosphoric acids supplied by the Monsanto Chemical Company. Purification was effected by recrystallizations from alcohol–water solutions.[10,11] The following equivalent weights were found by titration:

	Found	Theoretical
Barium monomethyl phosphate	248	247
Barium monoethyl phosphate	263	261
Barium mono-*n*-propyl phosphate	275	275
Barium mono-*n*-butyl phosphate	293	289

Barium dimethyl phosphate and barium di-*n*-butyl phosphate were obtained from the filtrates from which the corresponding monoalkyl compounds had been crystallized. The filtrates were evaporated to dryness and extracted with alcohol. Barium diethyl phosphate and barium di-*n*-propyl phosphate were obtained from the corresponding trialkyl phosphates.

Results

The results are presented in Tables I and II. The dissociation constants for the first hydrogen of the mono alkyl phosphoric acids are larger, *i. e.*, the pKa' values are smaller, than the constant for the dissociation of the first hydrogen of phosphoric acid, and the second dissociation constants are larger than the second constant of phosphoric acid, except in the case of monobutyl phosphoric acid which has a second constant about equal to that of phosphoric acid. The constants for both the first and second dissociation become smaller in ascending the series of the monosubstituted acids.

Each of the dialkyl phosphoric acids is stronger than the corresponding monosubstituted acid. As in the case of the monosubstituted acids, the dissociation constants for the disubstituted compounds become smaller in going from dimethyl to dibutyl.

Discussion

The factors affecting acid strength have been discussed by Branch and Calvin.[12] When alkyl groups replace hydrogen atoms in these compounds, the known factors of consequence are as follows: the inductive, statistical, steric and solvation effects. The resonance effect for this change is very small and has little importance.

(1) Kalckar, *Chem. Rev.*, **28**, 71 (1941).
(2) Lipmann, *Advances in Enzymol.*, **1**, 99 (1941).
(3) Meyerhof and Lohmann, *Biochem. Z.*, **185**, 113 (1927).
(4) Meyerhof and Suranyi, *ibid.*, **178**, 427 (1926).
(5) Meyerhof and Lohmann, *ibid.*, **196**, 49 (1928).
(6) Fiske and Subbarow, *J. Biol. Chem.*, **81**, 629 (1929).
(7) Goyan, Barnes and Hind, *Ind. Eng. Chem., Anal. Ed.*, **12**, 485 (1940).
(8) Van Slyke, *J. Biol. Chem.*, **52**, 525 (1922).
(9) Kumler, THIS JOURNAL, **60**, 859 (1938); Kumler and Halverstadt, *J. Biol. Chem.*, **137**, 765 (1941).
(10) Lossen and Kohler, *Ann.*, **262**, 209 (1890).
(11) Plimmer and Burch, *J. Chem. Soc.*, 292 (1929).
(12) Branch and Calvin, "The Theory of Organic Chemistry," Prentice-Hall, New York, N. Y., 1941.

TABLE I

All measured at 25°

Molality	(Na^+)	pH	pK'_{a_1}	Average pK'_{a_1}	pK'_{a_2}	Average pK'_{a_2}
		Monomethyl phosphoric acid				
0.0454	0.0156	1.83	1.53			
.0438	.0226	2.00	1.54	1.54		
.0423	.0291	2.24	1.57			
.0367	.0182	6.30			6.31	
.0363	.0205	6.42			6.31	6.31
.0360	.0228	6.55			6.31	
		Monoethyl phosphoric acid				
.0380	.0157	1.97	1.60			
.0374	.0186	2.05	1.60	1.60		
.0369	.0213	2.13	1.59			
.0337	.0143	6.51			6.64	
.0334	.0164	6.60			6.61	6.62
.0330	.0183	6.72			6.62	
		Mono-*n*-propyl phosphoric acid				
.0430	.0207	2.15	1.89			
.0424	.0234	2.23	1.87	1.88		
.0418	.0259	2.32	1.88			
.0362	.0131	6.40			6.65	
.0355	.0165	6.62			6.68	6.67
.0352	.0187	6.74			6.69	
		Mono-*n*-butyl phosphoric acid				
.0427	.0181	2.08	1.88			
.0422	.0208	2.16	1.89	1.89		
.0415	.0235	2.25	1.89			
.0356	.0149	6.70			6.84	
.0350	.0172	6.82			6.84	6.84
.0346	.0194	6.96			6.85	
		Dimethyl phosphoric acid				
.0410	.0174	1.84	1.29			
.0406	.0207	1.92	1.30	1.29		
.0400	.0237	2.00	1.27			
		Diethyl phosphoric acid				
.0336	.0179	2.06	1.33			
.0331	.0210	2.17	1.46	1.39		
.0325	.0242	2.31	1.38			
		Di-*n*-propyl phosphoric acid				
.0276	.0109	2.03	1.59			
.0274	.0126	2.09	1.60	1.59		
.0271	.0143	2.15	1.58			
		Di-*n*-butyl phosphoric acid				
.0228	.0094	2.15	1.73			
.0227	.0113	2.22	1.71	1.72		
.0226	.0129	2.29	1.72			

TABLE II

Acid	pK'_{a_1}	pK'_{a_2}
Phosphoric	1.97	6.82
Monomethyl phosphoric	1.54	6.31
Monoethyl phosphoric	1.60	6.62
Mono-*n*-propyl phosphoric	1.88	6.67
Mono-*n*-butyl phosphoric	1.89	6.84
Dimethyl phosphoric	1.29	
Diethyl phosphoric	1.39	
Di-*n*-propyl phosphoric	1.59	
Di-*n*-butyl phosphoric	1.72	

A negative inductive effect (acid weakening) is associated with the replacement of a hydrogen by an alkyl group and this effect is greater as one goes up the series from methyl to butyl. This accounts for the observation that the dissociation constants of the alkyl phosphoric acids decrease in going up the series from methyl to butyl and from dimethyl to dibutyl.

Since the difference in acid strength between methyl and butyl phosphoric acid is about 0.3 *pKa* unit and the difference between acetic and *n*-valeric is about 0.1 *pKa* unit, it is to be inferred that the two oxygen and one phosphorus atoms in these acids transmit the effect better than the one oxygen and two carbon atoms in the substituted acetic acids. This appears reasonable since the former three atoms are expected to be more polarizable than the latter three atoms.

One cannot arrive easily at the magnitude of the inductive effect of the methyl group when it replaces hydrogen in these acids. The change from formic acid, *pKa'* 3.77, to acetic acid, *pKa'* 4.76, is not a good analogy because in formic acid resonance effects complicate the situation by making the acid abnormally strong. The decrease in acidity due to the inductive effect resulting from replacing hydrogen by methyl in the phosphoric acids is thus probably less than 1 *pKa* unit (4.76−3.77), but greater than 0.3 *pKa* unit which is the difference in inductive effects between methyl and butyl (1.89−1.54). For purposes of calculation we will assume a value of 0.5 *pKa* unit.

The statistical effect concerns the number of equivalent hydrogen atoms that can ionize compared with the number of negatively charged oxygen atoms to which they can return. This effect causes the first constant of the monoalkyl acids to be abnormally weak by 0.18 *pKa* unit (log 3/2 − log 2/2) compared with the first of phosphoric, the second to be weak by 0.30 *pKa* unit (log 2/3 − log 1/3) compared with the second of phosphoric; and the dialkyl acids to be weak by 0.48 *pKa* unit (log 3/2 − log 1/2) compared with the first of phosphoric.

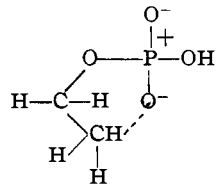
The solvation effect results from the solvation of the acidic hydrogen atoms by the base water. Since the extent of solvation is greater for the acid than for the ion, the effect is acid weakening. When an acidic hydrogen in an acid containing two or more equivalent acid hydrogen atoms is replaced by an alkyl group the number of hydrogen atoms that can be solvated is less. The decrease in solvation energy results in an increase in the strength of the acid. This effect is of major importance in the alkyl phosphoric acids. It is sufficiently large to overcome the inductive and statistical effects which are both acid weakening.[13]

In order to ascertain the true magnitude of the solvation effect, one must correct the value for the decrease in acid strength that results from these other effects. By using the above values for the inductive and statistical effects, we can estimate

(13) Another mechanism whereby the solvation of an acid with two or more acid groups can be acid weakening results from the reduction of the acid strengthening effect of one group on the other through dipole interaction and hydrogen bonding with the solvent. From this viewpoint, replacement of an acid hydrogen with an alkyl group would be acid strengthening due to prevention of dipole interaction and hydrogen bonding.

the value of the solvation effect. The substitution of one alkyl group for hydrogen in phosphoric acid results in an increase in acid strength, due to solvation, of about 1 *pKa* unit [1.97 −(1.54 − 0.5 − 0.18)]. The solvation effect for the substitution of two alkyl groups results in an increase of acid strength of about 2 *pKa* units [1.97 − (1.29 − 0.5 − 0.18)].

There is evidence that the same type of steric effect is present in the alkyl phosphoric acids as has been observed in the case of the fatty acids.[14] In comparison to the other members of the series, the first dissociation constant of monoethyl phosphoric acid is abnormally strong. This we attribute to the formation of a weak hydrogen bond in the ion



which reduces the tendency of the anion to recombine with the proton. This effect, although weak, has been used to explain the anomaly in the fatty acid series.[14] In the latter series, butyric acid is abnormally strong. Ethyl phosphoric acid, in its series, is analogous to butyric in the fatty acid series because each compound has the correct number of atoms to form the six-membered chelate ring. In the dialkyl series the diethyl compound likewise appears to be abnormally strong.

Esters of Biological Interest.—The same factors that are observed above to affect the acid strength of the alkyl phosphoric acids are also operative in the compounds of biological interest, such as glycerol phosphoric acid, the sugar phosphoric acids, creatine phosphoric acid, etc. (Table III).

TABLE III

Acid	pK'_{a_1}	pK'_{a_2}	pK'_{a_3}
α-Glycerol phosphoric[a]	1.40	6.44	
β-Glycerol phosphoric[a]	1.37	6.34	
Glyceraldehyde phosphoric[a]	2.10	6.75	
Dihydroxyacetone phosphoric[a]	1.77	6.45	
Fructose-6-phosphoric (Neuberg ester)[b]	0.97	6.11	
Glucose-6-phosphoric (Robison ester)[b]	0.94	6.11	
Glucose-3 (or 4)-phosphoric[b]	0.84	5.67	
Glucose-1-phosphoric[d]	1.10	6.13	
Fructose-1,6-diphosphoric[c]	1.48	6.32	
3-Glyceric phosphoric[a]	1.42	3.42	5.98
Enol-pyruvic phosphoric[c]		3.5	6.38
2,3-Diphosphoglyceric[c]	7.46 (pK'_{a_4})	7.99 (pK'_{a_5})	

[a] Kiessling, *Biochem. Z.*, **273**, 103 (1934). [b] Meyerhof and Lohmann, *ibid.*, **185**, 113 (1927). [c] Meyerhof and Suranyi, *ibid.*, **178**, 427 (1926). [d] Cori, Colowick and Cori, *J. Biol. Chem.*, **121**, 465 (1937).

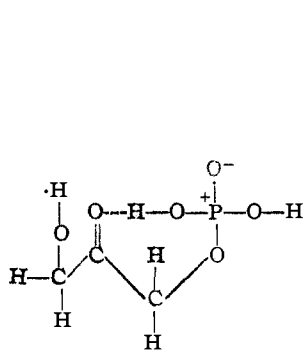
(14) Dippy, *Chem. Rev.*, **25**, 189 (1939).

The statistical effect will, of course, be identical in both sets of compounds. The positive inductive effect of the additional oxygen and nitrogen atoms in the compounds (Fig. 1) of biological interest will be responsible for an increase in acid strength. The amount of this increase can be calculated through the use of the inductive constants of Branch and Calvin. The inductive effect of an oxygen atom four atoms removed from the dissociating OH group strengthens the acid by 0.064 *pKa* unit, an oxygen 5 atoms removed by 0.024 *pKa* unit, etc. Since all oxygen atoms in the alcohol part of these esters, with the single exception of glucose-1-phosphoric acid, are four or more atoms away from the acid hydroxyl, it is evident that this effect can account for an increased acid strength of only about 0.1 *pKa* unit. Such an increase is not nearly sufficient to account for the greater strength of about 1 *pKa* unit shown by some sugar esters over the alkyl esters.

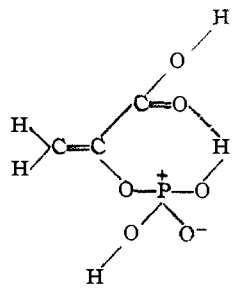
The steric effect is doubtless greater in the sugar esters than in the alkyl esters because of the stronger hydrogen bonds formed by the hydroxyl groups. Chelate rings may be formed in two ways in these compounds: type one, where an alcoholic hydroxyl bonds to a negatively charged phosphoric acid oxygen, and type two, where a phosphoric acid hydroxyl bonds to an alcoholic oxygen. Type one bonding is acid strengthening while type two is acid weakening. The type one bonding could account for the added strength of α-glycerol phosphoric acid over monopropyl phosphoric acid but it cannot account for the greater acidity of the glucose-6-phosphoric acid, and fructose-6-phosphoric acid over that of α-glycerolphosphoric acid. These sugar esters do not have hydroxyl groups situated so that 6 or 7 membered chelate rings can be formed by type one bonding. These can form chelate rings only by the second type of bonding which is acid weakening.

That type two bonding is important in some of these compounds is indicated by the fact that glucose-1-phosphoric acid is weaker than glucose-6-phosphoric acid in spite of the fact that the 1 compound has an oxygen one atom nearer the phosphoric acid group. The inductive effect of an oxygen 3 atoms removed, over one 4 atoms removed, will make the 1 compound stronger by 0.12 *pKa* unit $[(1/2.8)^3 \times 4 - (1/2.8)^4 \times 4]$. The glucose-1-phosphoric acid has the acetal oxygen in the most suitable position for type two bonding. A six-membered ring can be formed in this case while with the other sugar esters under consideration only seven-membered rings are possible. The acid weakening effect of type two bonding here amounts to about 0.3 *pKa* unit (1.10 + 0.12 − 0.94).

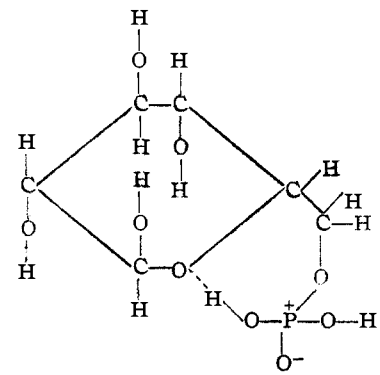
Since the statistical, inductive and steric effects do not account adequately for the greater strength of the sugar esters over the glycerol esters it appears that a solvation effect is involved here



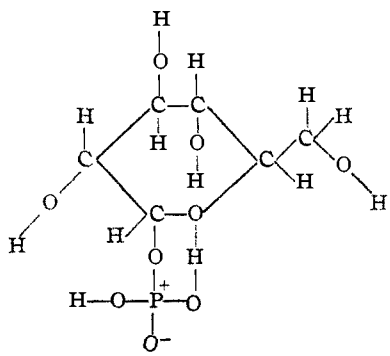
Dihydroxyacetone phosphoric acid



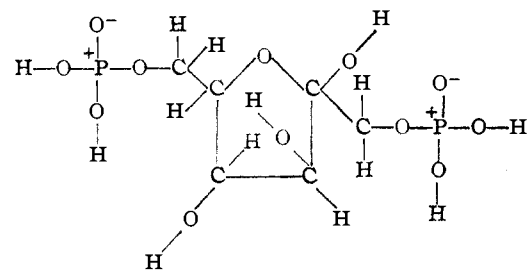
Enol pyruvic phosphoric acid



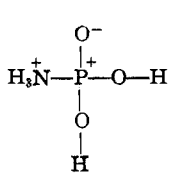
Glucose-6-phosphoric acid



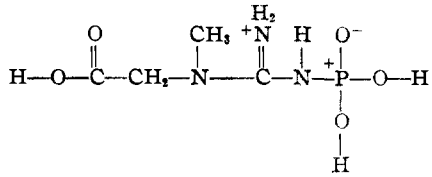
Glucose-1-phosphoric acid



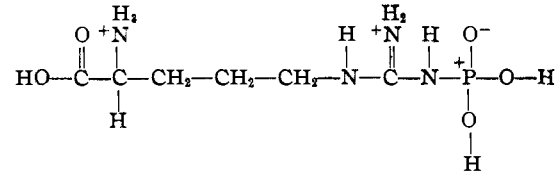
Fructose-1,6-diphosphoric acid



Aminophosphoric acid



Creatine phosphoric acid



Arginine phosphoric acid

Fig. 1.—The most acid structure is written in each case. In accordance with the assignment of the various dissociation constants, the neutral molecule of aminophosphoric acid will be a zwitterion, that of creatine phosphoric acid will be a zwitterion with a plus charge on a guanidino nitrogen and a negative charge on a phosphoric acid oxygen while that of arginine phosphoric acid will be double zwitterion with plus charges on both the guanidino and α amino nitrogen atoms and negative charges on phosphoric and carboxylic acid oxygens.

also. The solvation of the strongly acid hydroxyl groups by the base water is acid weakening and, as is shown above, accounts for the fact that the alkyl esters of phosphoric acid are stronger than phosphoric acid. This factor will also be operative in the glycerol and sugar esters but since these compounds also contain alcoholic hydroxyl groups which are more basic than water and since these alcoholic hydroxyl groups will be solvated by the relatively stronger acid water, an additional solvation effect will function in these esters. This effect will be greater in the ion than in the undissociated molecule due partly to the assistance given this solvation by the presence of the negative charge. As a result, this additional solvation effect will be acid strengthening. Since there are twice as many alcoholic hydroxyl groups in the sugar esters as in the glycerol esters the greater acid strength of the sugar esters is accounted for.

The greater strength of glucose-3-phosphoric acid over that of glucose-1-phosphoric acid is to be attributed to the greater possibility of hydrogen bonding of type one plus the positive inductive effect of the oxygen atoms, which being closer in the 3 compound will strengthen the acid by about 0.04 pKa unit.

β-Glycerol phosphoric acid is stronger than α-glycerol phosphoric acid for the same reasons.

The constants given for fructose diphosphoric acid are really hybrid constants. The first pKa' of 1.52 is a combination constant for the first and second hydrogen ions coming from both phosphoric acid residues and the second pKa' of 6.31 is a combination constant for the third and fourth dissociating hydrogen ions. These constants are weaker than those for the glucose-6- and fructose-6-phosphoric acids because of the weakening effect of the negative charge. This effect is relatively small (1.52–0.94) because of the large dis-

tance between the two phosphoric acid residues and because the effect of the charge is masked by the positive inductive effect of the other phosphoric acid residue.

The weakness of the fourth and fifth constants of 2,3-diphosphoglyceric acid is also due to the effect of the negative charges. The case is analogous to that of fructose diphosphoric acid. It has been pointed out[15] that the shape of the titration curve for ribonucleic acid can be accounted for on the basis of a depression of the ionization of the individual nucleotides due to the presence of the negative charges on the phosphoric acids groups.

That dihydroxyacetone phosphoric acid is weaker than α-glycerol phosphoric can be explained by a large amount of type two bonding in dihydroxyacetone phosphoric acid. This is reasonable since the carbonyl oxygen favors this type of bonding. However, the constants of the much weaker 3-glyceraldehyde phosphoric acid are not explicable on such a basis and it appears possible that the published constants for this unstable compound may be too small.

In the case of 3-glyceric phosphoric acid the second constant due to the carboxyl group is slightly larger than the constant for glyceric acid (pKa' 3.64) and the third constant which represents the last hydrogen from the phosphoric moiety is larger than the second constant of glycerol phosphoric. Apparently, here the positive inductive effect of the oxygen and phosphorus atoms of the phosphoric acid slightly overcomes the acid weakening effect of the negative charge on the phosphoric acid oxygens and the same holds true for the carboxyl oxygen atoms.

The second constant of enol pyruvic phosphoric acid is weaker than pyruvic, $pKa' = 2.5$,[16] because of two factors; the absence of the carbonyl oxygen in the ester and the presence of the negative charge. Since the acid weakening effect of the negative charge just about equals the positive inductive effect of the phosphoric acid moiety most of the difference must be attributed to the substitution of a doubly bonded carbon atom for a double bonded oxygen.

The weakness of the third constant of enol-pyruvic phosphoric acid compared with the third of 3-glyceric phosphoric acid is due to hydrogen bonding of type two between the last OH on phosphoric and the carboxylate ion. This bonding does not take place in 3-glyceric phosphoric acid because the carboxyl group is too far removed from the phosphoric acid moiety.

Natural Guanidine Phosphoric Acids.—According to the work of Meyerhof and Lohmann,[5] the titration curve for arginine phosphoric acid shows the presence of three buffer regions within the range pH 3–11. The presence of a strongly dissociating fourth acidic group is indicated. The several buffer regions were associated with the various functional groups on the basis of a comparison of the titration curve for the compound with that of an equal molar mixture of arginine and phosphoric acid as well as on the basis of an analogy drawn from their interpretation of the titration curve for aminophosphoric acid.

The approximate values $pKa' = 4.5$, $pKa' = 9.6$ and $pKa' = 11.2$[17] were assigned for the dissociation of the carboxyl group, the secondary hydroxyl of the phosphoric acid moiety, and for the acid dissociation of the α-amino group, respectively. The strongly dissociating acidic group was attributed to the first hydrogen of the phosphoric acid residue.

The titration curve for creatine phosphoric acid shows three equivalents of buffer capacity in the acid range.

Through a comparison of the titration curves for creatine and phosphoric acid, the two more strongly dissociating groups were attributed to the carboxyl group ($pKa' = 2.7$) and to the first hydroxyl of the phosphoric acid portion of the molecule. The clearly defined buffer maximum at pH 4.5 was referred to the secondary hydroxyl of the phosphoric acid group. However, a consideration of the titration curve for aminophosphoric acid led the authors to cast doubt on the validity of these assignments.

In the case of aminophosphoric acid, the buffer regions showing maxima at pH 2.8 and pH 8.2 were associated with first and second hydroxyl hydrogens of the substituted phosphoric acid.

Calculations, involving the use of the constants of Branch and Calvin[12] for the acid strength of the several functional groups in aminophosphoric acid, confirm our opinion that the value 2.8 must represent the dissociation constant of the secondary hydrogen of the phosphoric acid. Accordingly, the first hydrogen must be proportionately stronger; in fact, calculations yield a value $pKa = -0.9$ for this group. The value 8.2 agrees well with that expected for the acid dissociation of the amino group.

The value $pKa'_2 = 2.8$ and $pKa'_3 = 8.2$ for the dissociation of this zwitterion molecule should, then, be associated with the secondary hydrogen and the amino group, respectively. These considerations permit us to re-associate the functional groups of arginine phosphoric acid with the various approximate dissociation constants. The structural formula for arginine phosphoric acid (Fig. 1) shows that this compound will have 5 groups that may be titrated between the extremes of the pH scale. In keeping with the Brönsted definition of acids and bases, all the ionization constants of this molecule may be written as acid constants. The most strongly ionizing group

(15) Allen and Eiler, *J. Biol. Chem.*, **137**, 757 (1941).

(16) Böeseken, Hansen and Bertram, *Rec. trav. chim.*, **35**, 313 (1915).

(17) In the absence of protocols one can assume only that points of maximum buffer activity were obtained by inspection of the curve. Accordingly, only approximate values are reported. All pK_B' reported by Meyerhof and Lohmann have been converted to pKa' (acid dissociation).

will be that of the first dissociation for the phosphoric acid moiety. Due to the solvation effect and the strongly positive inductive effect of the positive charge on the guanidine nitrogen, as well as to the positive inductive effect of the more distant positively charged α-amino nitrogen, this group will be much stronger than the corresponding dissociation in phosphoric acid.

The second hydrogen, in order of strength, will be that resulting from the ionization of the carboxyl group. On the basis of the titration curve for arginine, Meyerhof and Lohmann assign the value $pKa' = 2.8$ ($pKs' = 2.8$ for arginine) to the carboxyl group in arginine phosphoric acid. More recent values for the dissociation constants for arginine are given by Schmidt, Kirk and Appleman.[18] The values $pK'_1 = 2.02$, $pK_2' = 9.0$, $pK_3' = 12.48$ are attributed to the carboxyl group, the acid dissociation of the α-amino group, and to the acid dissociation of the guanidine group, respectively. Accordingly, the value for the dissociation of the carboxyl group in arginine phosphoric acid should not be far removed from the value $pKa' = 2.02$. The acid strengthening positive inductive effect of the phosphoric acid oxygen and phosphorus would be compensated by the acid weakening effect of the negative charge resulting from the first dissociation.

There is no doubt that the group showing a maximum buffer activity at pH 4.5 must be the secondary hydroxyl of the phosphoric acid. The solvation effect along with the positive inductive effect of the positively charged guanidine nitrogen causes the dissociation of this group to be markedly increased over that for the secondary hydroxyl of phosphoric acid. The increase in strength of this group does not approach that observed for aminophosphoric acid because of the greater distance of the positive charge in arginine phosphoric acid. In the latter compound the resonance distributes the plus charge among the three guanidine nitrogen atoms.

The acid dissociation of the α-amino group must account for the buffer maximum at pH 9.6. It appears that the presence of the two negative charges on the phosphoric acid group not only negates the acid strengthening effect of the phosphoric acid oxygens but reduces the value for the dissociation of this group in arginine phosphoric acid below the level observed for arginine ($pKa_2' = 9.0$).

The fifth group, showing a buffer maximum at pH 11.2 must be attributed to the acid dissociation of the guanidine group.

The structural formula for creatine phosphoric acid (Fig. 1) reveals the presence of four acid groups. In keeping with the assignments made for arginine phosphoric acid, and in the order of decreasing acid strength, the groups may be assigned to the first dissociation of phosphoric acid group, the carboxyl group, the secondary hydroxyl of the phosphoric acid, and to the acid dissociation of the guanidine group.

For reasons similar to those given for arginine phosphoric acid, the primary hydroxyl of the phosphoric acid group in creatine phosphoric acid would be a much stronger acid than the same group in phosphoric acid.

The carboxyl group in creatine phosphoric acid should show an acid strength comparable to that of the carboxyl group in creatine which according to Meyerhof and Lohmann has a pK' of 2.7.

In accordance with the findings for arginine phosphoric acid, the secondary hydroxyl of the phosphoric acid group will be responsible for the buffer maximum at pH 4.5.

The acidic strength of the guanidine group in creatine, and presumably in creatine phosphoric acid, is extremely weak. The greater acid strength of the guanidine group in arginine is to be attributed to the greater distance of the acid weakening effect of the negatively charged carboxyl group as well as to the small positive inductive effect of the uncharged α-amino nitrogen.

The above interpretations of the behavior of the acid dissociating groups in arginine phosphoric acid and in creatine phosphoric acid stand, we believe, in good agreement with the experimental facts. A more accurate estimation of the influence of various factors must await more complete experimental data on the magnitude of the various constants. It is appreciated that the lability of these compounds at high (H^+) makes this task difficult.

Factors Affecting Biological Activity.—As has been pointed out above, the formation of a chelate ring, as well as the positive inductive effect of the phosphoric acid phosphorus and oxygens, especially in the absence of negative charges on the phosphoric acid group, have considerable influence on the strength of several of these acids. It is within the bounds of speculation to assume that these two effects may exert considerable influence on the biological activity of these compounds. For example, it is possible that chelation and the positive inductive effect may contribute, in an important fashion, to the ease of the enzymatic scission of hexose-di-phosphoric acid into dihydroxyacetone phosphoric acid and glyceraldehyde phosphoric acid.[18a] It has been shown[3] that although the enzyme concerned in the above reaction catalyzes the condensation of dihydroxyacetone phosphate with a variety of aldehydes, no reaction is observed when dihydroxyacetone phosphate is replaced by the unphosphorylated molecule.

In the region of pH 7 dihydroxyacetone phosphoric acid will possess two negative charges which will minimize the electron attracting influence of the positively inductive phosphorus

(18) Schmidt, Kirk and Appleman, *J. Biol. Chem.*, **88**, 285 (1930).

(18a) The importance of phosphoric acid in contributing to the ease of scission of carbon bonds is indicated by the observation of Rosenfeld, *J. Biol. Chem.*, **150**, 281 (1943), that phosphate catalyzes the non-enzymatic scission of dihydroascorbic acid.

and oxygens. However, these charges are neutralized, to a large extent, through the attachment of the molecule to the enzyme protein. In this fashion the electron mobilizing influence of the phosphorus and oxygen, as well as the influences derived from chelation, may assist the progress of the enzyme catalyzed reaction.

In the course of the energy yielding reactions of carbohydrate metabolism, 3-glyceric phosphoric acid is converted, by way of 2-glyceric phosphoric acid, to enol-pyruvic phosphoric acid. There is little doubt that chelation of type one bonding makes possible the intramolecular migration of the phosphoric acid group from position 3 of glyceric acid to position 2 of glyceric acid. Studies involving the use of radioactive phosphorus[19] make certain that it is the phosphoric acid located in position 3, and not the inorganic phosphate of the reaction mixture, that migrates to position 2.

In the course of the oxido-reduction reaction whereby 3-glyceraldehyde phosphate is oxidized to 3-glyceric acid phosphate, both 1,3-diphospho glyceraldehyde and 1,3-diphospho glyceric acid appear as intermediates.[3] Chelation can well account for the necessary stability of the aldehyde addition product with phosphoric acid. The inductive effects of the two phosphoric acid groups may facilitate the removal of the hydrogen necessary to the oxidation of the hydrogen acceptor.

The concept that the three OH groups of phosphoric acid are different seems to prevail in some quarters. For instance, Lipmann[20] states "On phosphorylation, always the undissociated third OH group enters the organic linkage. Solely from the replacement of a 'homoiopolar' bonded H by an organic radical, no change of the acid-base equilibrium would be expected." There is, of course, no difference between the three OH groups in phosphoric acid, and when esterification takes place the alcohol does not pick out the undissociated third OH group. The reason the second and third dissociation constants of phosphoric are weaker than the first is due entirely to the acid weakening effect of the negative charges on the ions. When one of the hydrogen atoms in phosphoric is replaced by an organic radical, a "'homoiopolar' bonded H," is not thus replaced, but a very acid H is replaced. The acidity of the solutions is, however, increased by this process since the substituted acids are stronger than phosphoric because of the various effects that have been discussed.

The property which the mono and diesters of phosphoric acid have of becoming less acid upon hydrolysis suggests their suitability for industrial applications where the development of acidity during a process is undesirable.

It appears likely that the mono and diesters of other polybasic acids may be found to be stronger acids than the parent compounds.

(19) Meyerhof and Kiessling, *Biochem. Z.*, **276**, 239 (1935).

(20) Lipmann, "Advances in Enzymology," Interscience Publishers, Inc., New York, N. Y., p. 113, 1941.

Summary

1. The dissociation constants of monomethyl, monoethyl, mono-*n*-propyl, mono-*n*-butyl, dimethyl, diethyl, di-*n*-propyl, and di-*n*-butyl phosphoric acids have been measured. The monoalkyl acids are stronger than phosphoric and the dialkyl acids are stronger than the monoalkyl acids.

2. The factors affecting the acid strength of these compounds are the solvation, inductive, statistical and steric effects. Of these the solvation effect is chiefly responsible for the substituted acids being stronger than phosphoric acid.

3. A steric effect similar to that found in the fatty acids is present in the alkyl phosphoric acids. Ethyl phosphoric acid in this series corresponds to butyric acid in the other series, each being abnormally strong.

4. Information obtained from the study of the alkyl compounds has been applied to the glycerol and sugar esters of phosphoric acid. The greater strength of these compounds is due in small part to the positive inductive effect of the negative oxygen atoms, and in larger part to the solvation of the alcoholic hydroxyl groups. Chelation due to hydrogen bonding occurs in these compounds and either increases or decreases the acid strength depending on whether or not the hydrogen in the bond comes from an alcoholic or phosphoric acid hydroxyl group.

5. The groups responsible for the dissociation constants of the natural guanidine phosphoric acids have been assigned on a basis of analogies and theoretical considerations.

6. The factors of chelation through hydrogen bonding and the inductive effect of the phosphoric acid group offer a possible mechanism whereby the presence of the phosphoric acid moiety facilitates some of the reactions of biological importance.

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