and a reflux condenser. In order to remove remaining water from the apparatus, the solution was boiled, with no cooling water going through the condenser, until a few ml. had distilled out the top. A calcium chloride drying tube was then fitted to the top of the condenser, the source of heat removed and the cooling water turned on. The bottle containing the silver salt was attached to the third neck of the reaction flask by means of a flexible rubber coupling. The reaction vessel was brought to the desired temperature and silver salt (silver salts of bromo acids were maintained at 0°) was introduced in small portions with good stirring; exit gases were passed through a bubble counter.

The reactions carried out at 0° (ice–salt-bath for cooling) proceeded very slowly after an induction period (up to one hour). Carbon dioxide evolution became more rapid as the reaction progressed; 5–10 bubbles per second constituted a satisfactory rate.

At the end of the addition the reaction mixture was allowed to stir further and was finally brought to room temperature. Cold sodium bisulfite solution was added dropwise to destroy excess bromine. Silver bromide was then filtered off and washed with carbon tetrachloride and the combined organic layers were washed with dilute sodium carbonate solution, dried and distilled.

Identification of Products.—1,1-Dibromobutane (3 g.) was refluxed with 5 g. of silver acetate and 20 ml. of glacial acetic acid for six hours and, after silver salts were filtered off, the diacetate thus formed was decomposed by boiling the mixture for a few minutes with 10 ml. of dilute hydrochloric acid. Distillation gave *ca.* 0.5 g. of buteraldehyde–water binary, b.p. 68°, 2,4-dinitrophenylhydrazone, m.p. 118–120°. 1,1-Dibromopropane was similarly converted to propionaldehyde, 2,4-dinitrophenylhydrazone, m.p. 154°.

1,1,1-Tribromopropane (0.75 g.) was refluxed with 20% aqueous potassium hydroxide for 24 hours. The resulting solution, after extraction with ether, was neutralized with hydrochloric acid and evaporated to dryness. The residue was extracted with 70% ethanol and the extract refluxed together with 0.15 g. of *p*-phenylphenacyl bromide[14] for two hours; recrystallization of the product from ethanol gave *p*-phenylphenacyl propionate, m.p. 102–103°.

1,2,4-Tribromobutane was identified by comparison[15] of its infrared spectrum with that of an authentic sample, b.p. 56–59° (1 mm.), $n^{25}\text{D}$ 1.5588, prepared[16] by the action of hydrogen bromide on 3,4-dibromobutanol-1.

1,2,4-Butanetricarboxylic Acid.—One mole (108 g.) of 1-vinylcyclohexene-3[17] was added, during three hours, dropwise with good stirring and external cooling to 1.3 l. (20 moles) of concentrated nitric acid[18] at 50–60°, using 2 g. of ammonium metavanadate as catalyst. The mixture was cooled, siphoned off and again added dropwise to the flask maintained, this time, at 100°. Further reaction took place and the vessel was heated for one hour at 100°. On cooling, a precipitate was noted which was filtered off and identified as *p*-nitrobenzoic acid, yield 2–3 g. Addition of formic acid at 50° conveniently destroyed excess nitric acid. Volatile materials were then removed *in vacuo* and the residual oil was taken up in acetone and treated with Norit. Removal of solvent gave a solid crystalline mass, crude yield 84%. Triacid of high purity was obtained through the ester. The crude acid after treatment with methanol–hydrogen chloride gave (after accidental loss) 111.5 g. (48%) of trimethyl ester, b.p. 116–117° at 1 mm. This was saponified with sodium hydroxide in 50% ethanol and the reaction mixture, after removal of ethanol by distillation, was acidified and the solution taken to dryness *in vacuo*. Extraction of the residue with acetone gave a nearly quantitative yield of acid, m.p. 118–120°.

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[14] It was found convenient, when making phenacyl esters, to use an excess of the phenacyl halide. The excess of reagent could readily be removed by conversion (warming with a benzene solution of trimethylamine) to a water-soluble quaternary salt.

[15] These spectra are reproduced in the Ph.D. thesis of the author, California Institute of Technology, 1950.

[16] *Cf.* H. Pariselle, *Ann. chim.*, [8] **24**, 317 (1911).

[17] Generously supplied by the Koppers Co.

[18] Oxidation with permanganate [S. Lebedew and N. Skawronskaja, *Chem. Zentr.*, **83I**, 1440 (1912)] is not recommended; the use of nitric acid was suggested by Dr. H. L. Herzog, *cf.* I. G. Farbenindustrie A.-G., British Patent 510,638, *C. A.*, **34**, 5093 (1940).

PASADENA, CALIFORNIA

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

Effect of Structure on Reactivity. VII. The Energies of Activation and the Entropies of Activation for the Ammonolysis of Esters[1]

BY FRANK H. WETZEL, JOHN G. MILLER AND ALLAN R. DAY

RECEIVED AUGUST 12, 1952

The energies of activation and the entropies of activation have been determined for the ammonolysis of methyl, ethyl, *n*-propyl and isopropyl acetate in the presence of 5-*M* ethylene glycol. The same quantities have also been evaluated for the ammonolysis of methyl acetate when catalyzed by 10 *M* water and 10 *M* anhydrous methanol and for the ammonolysis, both catalyzed and uncatalyzed, of methyl lactate at various concentrations. The results show that the differences in the energies of activation and the velocity constants may be interpreted in terms of structural changes in the acetates. In the case of ethyl and *n*-propyl acetate, however, entropy changes must also be considered. It is shown that ethylene glycol is the best catalyst thus far considered for the ammonolysis reaction at temperatures between 25 and 45°. Catalytic activity is less for water and the least in the case of methanol. The reaction as catalyzed by water, however, has the greatest temperature coefficient and water might well be the best catalyst at higher temperatures. In the ammonolysis of methyl lactate a great difference in the entropy change has been found between the uncatalyzed reaction and that catalyzed by ethylene glycol. It has also been shown that the differences in velocity constants of the ammonolysis of methyl acetate and methyl lactate may be interpreted in terms of potential energy changes, *e.g.*, structural variations. The mechanism proposed earlier for either ammonolysis or aminolysis of simple aliphatic esters has been supported by these studies. Attention has been called to heretofore unknown effects of entropy change differences between reactions compared previously in the light of purely potential energy change differences.

Previous investigations[2] in this series were concerned with the determination of the velocity constants for the ammonolysis and aminolysis of a series of esters. The velocity constants for the ammonolysis reactions were interpreted in terms of the structural variations in the ester molecules and the constants for the aminolysis reaction in terms of variations in the amines. The previous work also considered the effect of various hydroxylated solvents on the reaction.

The present paper deals with the determination of the energies of activation and the entropies of activation for the ammonolysis of a representative

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[1] Taken from the thesis of F. H. Wetzel, presented to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1952.

[2] (a) M. Gordon, J. G. Miller and A. R. Day, THIS JOURNAL, **70**, 1946 (1948); (b) *ibid.*, **71**, 1245 (1949); (c) E. McC. Arnett, J. G. Miller and A. R. Day, *ibid.*, **72**, 5635 (1950); (d) *ibid.*, **73**, 5393 (1951).

series of esters. The effects of three hydroxylated catalysts, present at a hydroxyl concentration of 10 moles per liter, have been considered. Such a study seemed warranted in order to test the validity of interpreting the velocity constants for the ammonolysis process in terms of structural changes within the reactant molecules, *i.e.*, in the light of purely potential energy changes.

The data in general show a small energy of activation and a large entropy of activation for the ammonolysis of the esters. The relative activity of the catalysts is clarified by the results.

Experimental

The experimental procedure for ammonolysis of the acetates was essentially that used previously. The rate of ammonolysis was followed by chemical means. Ten-ml. ampoules containing the reaction systems were broken in 4% boric acid and the residual ammonia titrated with 0.5 *N* hydrochloric acid, using a Beckman model G *p*H meter.

Modifications of the original procedure were almost all concerned with decreasing the experimental error caused by ammonia escape. These involved keeping the reactants at 0° from the time they were mixed until they were thermostated at the reaction temperature, design of a special delivery apparatus, and identical treatment of all reaction ampoules, *i.e.*, sealing both blanks and samples in ampoules, breaking in 4% boric acid and titrating.

The procedure used for the lactates was necessarily modified. Because of the absence, or small concentration, of hydroxylated catalyst and the low solubility of ammonia in the diluent, dioxane, the lactate ester was present during the addition of ammonia to the reactant mixture.

Previous work[2] has established the fact that no appreciable hydrolysis of the amide takes place during titration of the residual ammonia. Moreover, it has already been shown that no side reactions, reactions with the catalyst or reversibility of the ammonolysis process, need be considered.

The following reagents were used: methyl acetate, b.p. 57.2° at 759 mm., $n^{20}D$ 1.3613; ethyl acetate, 76.6–76.9° at 758 mm., $n^{20}D$ 1.3728; *n*-propyl acetate, 100.9–101.8° at 760 mm., $n^{20}D$ 1.3846; isopropyl acetate, 87.9–88.9° at 755 mm., $n^{20}D$ 1.3771; methyl lactate, 47.2–47.9° at 16 mm., $n^{20}D$ 1.4140; 1,4-dioxane, 101° at 758 mm., $n^{20}D$ 1.4221; ethylene glycol, 76.8–77.1° at 2 mm., $n^{20}D$ 1.4316; methanol, 64.6° at 759 mm., $n^{20}D$ 1.3289; ethanol, 78.5° at 760 mm., $n^{20}D$ 1.3610; propanol-1, 96.6–97.1° at 754 mm., $n^{20}D$ 1.3854; propanol-2, 81.9–82.5° at 756 mm., $n^{20}D$ 1.3776.

The acetate esters used were available commercially. The methyl lactate was obtained through the courtesy of the Eastern Regional Research Laboratory, United States Department of Agriculture.

The 1,4-dioxane, obtained commercially, was purified by refluxing with sodium metal for 10 hours and was then distilled from the sodium, using a packed column. Ethylene glycol was dried over magnesium sulfate and distilled *in vacuo*. Methanol was dried and purified, using the method of Lund and Bjerrum.[3] The method involves formation of the magnesium alkoxide and subsequent distillation from this salt. Commercial absolute ethanol was found to require no further purification. Propanol-1 was refluxed over metallic calcium and subsequently distilled. Propanol-2 was refluxed over aluminum isopropoxide for six hours and then distilled off the alkoxide. Anhydrous ammonia was obtained in tanks and added to the reaction mixture through a flow meter and a gas dispersion tube with a sintered glass tip.

Results

The reaction involved in the ammonolysis of esters has been shown to be pseudo-second order. As will be noted in the discussion, a regular though slight increase of the reaction rate with progress of the reaction was found for those reactions catalyzed by ethylene glycol. The value of *k* at 20% ammonolysis was judged to be the most reliable in these cases and this value was obtained by a least squares treatment of the data. These values were then adjusted graphically to a 2 *N* ammonia concentration. The activation energy, E_a, was calculated using the integrated form of the Arrhenius equation and checked by a plot of ln *k* *vs.* 1/*T*. The standard state entropy of activation, ΔS^*, was calculated by means of the equation

$$k = e\,\mathrm{k}T/\mathrm{h}\; e^{-E_a/RT} e^{\Delta S^*/R}$$

The rate constants obtained at the temperatures used are shown in Tables I and II. The values of E_a and ΔS^* are given in Tables III and IV. The plots of ln *k* *vs.* 1/*T* are shown in Fig. 1. Each ΔS^* value is the mean value for the temperature range over which the rate constants were determined. The errors quoted in the tables represent maximum estimates of the errors to be expected in the values affected.

TABLE I

VELOCITY CONSTANTS AT 20% AMMONOLYSIS OF VARIOUS ACETATES (*k* is in liters mole^{-1} hour^{-1})

Ester, acetate	25°		35°		45°	
	$k \times 10^3$	Error ($\times 10^6$)	$k \times 10^3$	Error ($\times 10^6$)	$k \times 10^3$	Error ($\times 10^6$)
Methyl[a]	1.197	±9	1.714	±9	2.443	±0
Methyl[b]	1.024	6	1.575	16	2.320	4
Methyl[c]	0.132	2	0.197	5	0.280	6
Ethyl[a]	.657	12	.920	11	1.232	40
n-Propyl[a]	.568	3	.824	5	1.154	43
Isopropyl[a]	.171	1	.254	1	0.402	10

[a] Catalyzed by ethylene glycol, concn. of 5 mole/l. [b] Catalyzed by water, concn. of 10 mole/l. [c] Catalyzed by methanol, concn. of 10 mole/l.

TABLE II

VELOCITY CONSTANTS FOR THE AMMONOLYSIS OF METHYL LACTATE, WITH AND WITHOUT ETHYLENE GLYCOL CATALYST

Ester concn. (mole/l.)	Cat. concn. (mole/l.)	25°		35°	
		$k \times 10^3$, l./mole hr.	Error $\times 10^6$	$k \times 10^3$, l./mole hr.	Error $\times 10^6$
3	0.0	1.576	±22	2.259	±71
2	.5	1.879	20	2.503	23
5	.0	3.767	75	5.460	44
4	.5	4.033	118	5.417	94

TABLE III

ENERGIES OF ACTIVATION AND ENTROPIES OF ACTIVATION AT 20% AMMONOLYSIS OF VARIOUS ACETATES

Ester, acetate	E_a, cal.	Error	ΔS^*, e.u.	Error
Methyl[a]	6735	±85	−67.7	±0.20
Methyl[b]	7710	120	−64.7	.28
Methyl[c]	7090	380	−70.8	.84
Ethyl[a]	5925	350	−71.5	.80
n-Propyl[a]	6685	340	−69.3	.78
Isopropyl[a]	8085	220	−67.1	.52

[a,b,c] See Table I.

TABLE IV

ENERGIES OF ACTIVATION AND ENTROPIES OF ACTIVATION FOR THE AMMONOLYSIS OF METHYL LACTATE WITH AND WITHOUT ETHYLENE GLYCOL CATALYST

Ester concn., mole/l.	Cat. concn., mole/l.	E_a, cal.	Error	ΔS^*, e.u.	Error
3	0.0	6576	±630	−67.6	±2.10
2	.5	5240	255	−71.7	0.84
5	.0	6785	420	−65.2	1.40
4	.5	5390	622	−69.7	2.06

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[3] H. Lund and J. Bjerrum, *Ber.*, **64**, 210 (1931).

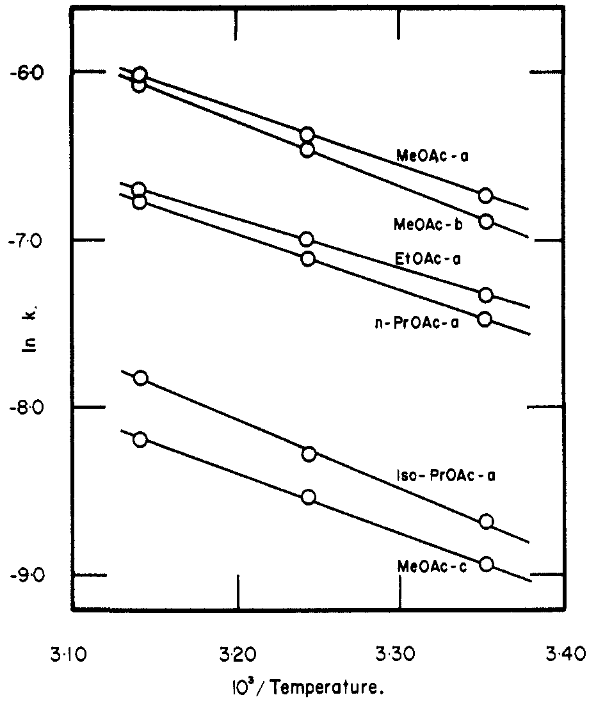
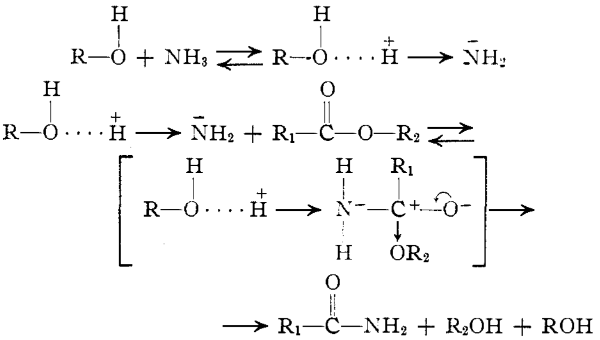


Fig. 1.—Natural logarithm of the velocity constant *vs*. 10^3/temperature (degrees absolute): a, catalyzed by 5 moles/liter ethylene glycol; b, catalyzed by 10 moles/liter water; c, catalyzed by 10 moles/liter methanol.

Discussion

The data are best discussed with reference to the mechanism of the reaction



$$R-\overset{H}{O} + NH_3 \rightleftarrows R-\overset{H}{O}\cdots\overset{+}{H} \longrightarrow \overset{-}{N}H_2$$

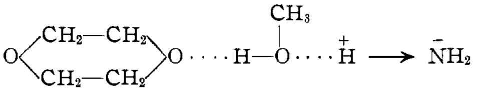
$$R-\overset{H}{O}\cdots\overset{+}{H} \longrightarrow \overset{-}{N}H_2 + R_1-\overset{O}{\overset{\|}{C}}-O-R_2 \rightleftarrows$$

$$\left[R-\overset{H}{O}\cdots\overset{+}{H} \longrightarrow \underset{H}{\overset{H}{N^-}}-\underset{OR_2}{\overset{R_1}{C^+}}-O^-\right] \longrightarrow$$

$$\longrightarrow R_1-\overset{O}{\overset{\|}{C}}-NH_2 + R_2OH + ROH$$

This diagram is an extension of earlier schemes[2b,c] and shows the solvated ammonia molecule. ROH represents the hydroxylated catalyst. R_1 may be aromatic, alkyl or even hydrogen, though it has been found that formates are too readily hydrolyzed in titration of the residual ammonia and cannot be followed by this method. R_2 may be aromatic or alkyl, though esters with sterically hindered ester groupings are extremely slow to react. Either primary or secondary amines may be used instead of ammonia. Although it might appear that ammonolysis or aminolysis would occur at an appreciable rate without the hydroxylated catalyst, it has been shown[2a] that this is not the case.

The regular increase in velocity constants for the ammonolysis process where ethylene glycol is the catalyst has not been noted in this Laboratory heretofore. This increase may be attributed to the catalytic properties of the alcohol, formed in the ammonolysis reaction, since such an alcohol fulfills the requirements for a hydroxylated catalyst. The results of experiments to substantiate this explanation were inconclusive. An alcohol which corresponded to the ester grouping in question was added to the reaction mixture to see if the presence of this alcohol, along with the regular amount of ethylene glycol, would cause an effect comparable to the increase noted in the reactions without added alcohol. A definite increase in initial rate was noted but the rate was much lower than expected. However, in the runs with added alcohol, a certain amount of the diluent, dioxane, was displaced by the alcohol. Although previous and current work in this Laboratory has indicated that dioxane by itself presents no appreciable catalytic function for the ammonolysis reaction, the function of dioxane as inferred from the above may be pictured as a secondary catalytic effect. This may be illustrated, in the case of methanol, as



This catalytic effect is, of course, small with regard to the over-all effect of ethylene glycol. This picture is somewhat substantiated by other workers[4] who have studied hydrogen bonding of cycle ethers and the so-called "wing back effect." Such a secondary catalytic effect has also been observed by Jung.[5]

Consideration of the values for the energies and entropies of activation shows that though critical differences exist when the reactions of the acetates are compared, even with the application of the errors noted, the differences are not sufficiently great to change the mechanism proposed. The same may be said for the effects of the three catalysts on the ammonolysis of methyl acetate. The inferences taken from comparison of velocity constants are therefore generally well founded and no serious change of the mechanism is indicated.

The values of the energies of activation are rather low and the entropies of activation possess surprisingly large magnitudes. Consideration of the free energy of activation shows clearly in any of these reactions that the total energy barrier, composed of both the activation energy and the entropy change, is composed largely of the entropy term.

The entropies of activation of methyl and isopropyl acetates, in the presence of ethylene glycol as a catalyst, are the same within experimental error and the differences in velocity constants here are due to potential energy factors, such as differences in electron release effects of the ester groupings, inductive effects, and the like. In the case of the ethyl and *n*-propyl acetates in the presence of ethylene glycol, entropy changes must be considered. Since the entropies of activation for the reaction between the ester and ammonia are lower than for the methyl and isopropyl esters, these reactions cannot validly be compared simply on the basis of velocity constants.

The greater decrease in the entropy of activation

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[4] S. Searles and M. Tamres, This Journal, **73**, 3704 (1951).

[5] S. L. Jung, Dissertation, University of Pennsylvania, 1951.

for the reaction of ethyl acetate may be caused by a restricted configuration in which there is hydrogen bonding between the β-hydrogens in the ester grouping and the carbonyl oxygen in the activated state. The same picture applies to the *n*-propyl ester, but to a lesser degree since only two such β-hydrogen atoms are available here. This type of H-bonding is, in all probability, found in the isopropyl ester in both reactant and activated state and would therefore not be reflected in the entropy term.

It should be noted that at all temperatures at which these studies were made ethylene glycol is definitely the best catalyst of the three considered, when comparison is made on the basis of equivalent hydroxyl concentration. Water is somewhat lower in catalytic activity and methanol is almost a power of ten lower than either of the other two catalysts.[6] It is apparent, as seen in Fig. 1, that, because of the temperature coefficients, at a higher temperature water might very well become a better catalyst than ethylene glycol.

Separating the various factors affecting catalytic activity of the three catalysts studied presents a difficult problem. The fact that the ammonolysis reaction as catalyzed by water gives the smallest decrease in the entropy of activation is not surprising in view of the small and unhindered water molecule. The high activation energy for water catalysis may very well be caused by the many and complex ways in which water and ammonia can be associated. Catalysis by ethylene glycol gives a low activation energy, possibly because of the adjacent hydroxyl groups, both of which may be attached to the same ammonia molecule. The fact that the total energy barrier for the reaction catalyzed by methanol is the same as that for ethylene glycol may possibly indicate that not one but two molecules of methanol bond with one molecule of ammonia to form the amide ion. This would also account for the relative order of decreasing entropies of activation.

Because of the necessary modifications of experimental procedure for ammonolysis of the lactates and the relatively large experimental error, quantitative deduction from the data obtained in these reactions is difficult. The reaction is self-catalyzed *via* the hydroxyl group contained in the lactate ester. The presence of ethylene glycol may be expected to lower the potential energy barrier, because of the increased availability of the hydroxyl groups, but evidently it causes a larger decrease in the total energy barrier by a large decrease in the entropy of activation. The latter may be caused by hydrogen bonding between the second hydroxyl group of the glycol and the hydroxyl group of the lactate. This in turn causes a more rigid complex in the activated state than would be true in the uncatalyzed reaction.

It is to be noted that comparison of the uncatalyzed reaction of methyl lactate with ammonia may be made with the reaction of methyl acetate with ammonia in the presence of ethylene glycol on the basis of either velocity constants or energies of activation, *i.e.*, potential energy factors. This is justified in view of the equality, within experimental error, of the entropies of activation of these two reactions. Such an interpretation has been made in the earlier work.[2a,b]

PHILADELPHIA, PENNA.

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[6] R. Baltzly, I. M. Berger and A. A. Rothstein (THIS JOURNAL, **72**, 4149 (1950)) have described methanol as the best catalyst for the aminolysis of a series of esters. These workers, however, used methanol as both solvent and catalyst for their investigation and a fair comparison of catalysts cannot be made under these circumstances.

[CONTRIBUTION FROM THE BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY]

The Separation of Sugar Phosphates by Ion Exchange with the Use of the Borate Complex[1]

BY JOSEPH X. KHYM AND WALDO E. COHN

RECEIVED OCTOBER 18, 1952

Borate complexing is used to effect separations of the commonly encountered monophosphorylated sugars by anion exchange in an alkaline chloride system. Phosphoglyceric acid, fructose diphosphate and the adenosine polyphosphates are separated from the sugar monophosphates and from each other by simple *p*H and ionic strength adjustment. Using a sequence of eluting solutions of varying borate concentration, chloride concentration and *p*H, each member of both groups of substances can be isolated from all of the others in a single continuous process.

Introduction

Except for the almost complete separation of ribose-5-phosphate from ribulose-5-phosphate as achieved by Horecker and Smyrniotis,[2] a direct separation of monophosphorylated sugars by ion-exchange methods has not yet been reported. The complete separation of fructose-6-phosphate from fructose-1,6-diphosphate by anion exchange has been demonstrated by Benson, *et al.*[3] In this instance the presence of two phosphate groups in fructose-1,6-diphosphate accounts for the high degree of separation obtained. However, the similarity of the structural configurations and the nearly identical dissociation constants[4] of the commonly occurring sugar monophosphates are two factors which make unlikely their complete separation by simple ion exchange.

For purposes of identification of phosphate esters in complex mixtures, the techniques of paper chromatography have been more successful. Bandurski and Axelrod[5] used this technique to identify

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[1] Work performed under contract No. W-7405-eng-26 for the Atomic Energy Commission.

[2] B. L. Horecker and P. Z. Smyrniotis, *Arch. Biochem.*, **29**, 232 (1950).

[3] A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Hass and W. Stepka, THIS JOURNAL, **72**, 1710 (1950).

[4] W. D. Kumler and J. J. Eiler, *ibid.*, **65**, 2355 (1943).

[5] R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).