$$R_3P + I_2 \rightleftharpoons R_3PI_2 \quad (1)$$

$$R_3PI_2 + H_2O \longrightarrow R_3PO + 2HI \quad (2)$$

The tertiary phosphine in any solvent can be titrated directly with a solution of iodine in 95% ethanol. The end-point is very sharp and is observed by the appearance of a bright yellow color. The alcohol and water in the titration solutions accelerate the sulfur–phosphine reaction in the aliquots during the direct titration with iodine. This caused an error which was most noticeable in the early part of a run. Such errors were eventually eliminated by quenching each aliquot in an excess of standard iodine solution and then back-titrating with standard triphenylphosphine; however, the rate constants so obtained were identical with those from the latter part of each direct-titration run, and such runs are included in Table III. The solutions of iodine and phosphine for titration were made in benzene containing 20–25% ethanol (95%). The concentration of the phosphine solution must be checked from time to time in order to avoid errors due to the oxidation of the phosphine. When tri-*p*-chlorophenylphosphine is used, oxidation does not occur at room temperatures; on the other hand, trialkyl phosphines cannot be used since they oxidize extremely rapidly in the presence of air. To minimize errors due to oxidation of the liberated HI, about 5 g. of pyridine was added to one liter of iodine solution. Using this method a sample of triphenylphosphine crystallized three times from 95% ethanol analyzed as 99.4% Ph_3P.

The presence of sulfur, phosphine sulfides, phosphine oxides or tertiary amines (in not very large amounts) does not introduce any error nor affect the end-point.

CAMBRIDGE 38, MASSACHUSETTS

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT OF WESTVACO CHLOR-ALKALI DIVISION OF FOOD MACHINERY AND CHEMICAL CORPORATION]

Structure of the Reaction Products of Fully Esterified Phosphorus(III) Acids and α-Halo Carbonyl Compounds[1]

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RECEIVED DECEMBER 14, 1955

The products obtained by the reaction of α-halo carbonyl compounds and completely esterified acids of trivalent phosphorus were shown to be vinyl esters of pentavalent phosphorus acids by the independent synthesis of diethyl vinyl phosphate by the action of sodium hydride on diethyl 2-bromoethyl phosphate, by the addition of hydrogen, chlorine and bromine and by infrared absorption characteristic of the ethylenic double bond.

In a recent paper[2] the reaction products of α-halogenated carbonyl compounds and completely esterified acids of trivalent phosphorus were represented as vinyl esters of the corresponding pentavalent phosphorus acids, and the reasons for the structure assignment were stated briefly. We now wish to present in detail some of the experimental evidence for the vinyl ester structure. In the meantime other investigators[3] have published data supporting the vinyl ester structure and eliminating certain other structures originally thought possible, making a detailed description of some of our work unnecessary.

The vinyl ester structure is supported by a considerable body of evidence, the most important being (a) the independent synthesis of diethyl vinyl phosphate by the dehydrobromination of diethyl 2-bromoethyl phosphate and by the debromination by zinc of the bromine adduct of the product obtained from chloral and triethyl phosphite; (b) the addition of hydrogen, chlorine and bromine to certain members of the series to form saturated products; (c) the hydrolysis of the reaction product of chloral and triethyl phosphite to dichloroacetaldehyde; and (d) infrared absorption at 6.1 μ.

Upson[4] has described the preparation of diethyl vinyl phosphate and divinyl phenylphosphonate by the dehydrochlorination of the corresponding 2-chloroethyl esters. Upson[4b] reported the formation of a viscous polymeric diethyl vinyl phosphate when diethyl chloroethyl phosphate and triethylamine were refluxed for 84 hours, and although we obtained a similar product by the same procedure, neither of the two liquid phases obtained gave a positive test for chloride ion, indicating that triethylammonium chloride was not obtained. Furthermore, when triethylamine was refluxed for 84 hours with the product obtained from triethyl phosphite and chloroacetaldehyde a crystalline solid was obtained which appears to be an addition product of the two reactants. These results suggest that the Upson reaction does not give diethyl vinyl phosphate as an intermediate but that both reactions may involve alkylation (or vinylation in the case of the vinyl phosphate) of the amine. A 5% excess of triethylamine reacted with diethyl 2-bromoethyl phosphate in refluxing isopropyl ether to give a 46% yield of bromide ion and a small liquid fraction distilling at 81–110° (3 mm.) which decolorized bromine and presumably contained diethyl vinyl phosphate. Our efforts to repeat Upson's dehydrochlorination with ethanolic potassium hydroxide and other basic reagents resulted primarily in ethanolysis and degradation and only traces of unsaturated products were obtained.

An unexpected development occurred in an attempt to dehydrobrominate diethyl 2-bromoethyl phosphate with sodium *t*-butoxide in *t*-butyl alcohol. The product closely resembled the product obtained from triethyl phosphite and chloroacetaldehyde in physical properties, including infrared absorption, but the specific gravity and phosphorus content were significantly low, suggesting that the vinyl compound was contaminated with

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[1] Presented at the 128th Meeting of the American Chemical Society, Minneapolis, Minnesota, September 11–16, 1955.

[2] J. F. Allen and O. H. Johnson, THIS JOURNAL, **77**, 2871 (1955).

[3] (a) W. F. Barthel, B. H. Alexander, P. A. Giang and S. A. Hall, *ibid.*, **77**, 2424 (1955); (b) A. M. Mattson, J. T. Spillane and G. W. Pearce, *J. Agr. Food Chem.*, **3**, 319 (1955); (c) W. Perkow, *Chem. Ber.*, **87**, 755 (1954); (d) W. Perkow, W. Krockow and K. Knoevenagel, *ibid.*, **88**, 662 (1955); (e) W. Lorenz, A. Henglein and G. Schraeder, THIS JOURNAL, **77**, 2554 (1955); (f) M. S. Kharasch and I. S. Bengelsdorf, *J. Org. Chem.*, **20**, 1356 (1955).

[4] (a) R. W. Upson, THIS JOURNAL, **75**, 1763 (1953); (b) R. W. Upson, U. S. Patent 2,557,805 (1951).

TABLE I

HALOGEN ADDITION PRODUCTS OF VINYL PHOSPHATES

Compound	Yield,[a] %	Halogen, % or meq./g.[b] Calcd.	Halogen, % or meq./g.[b] Found	Phosphorus, % Calcd.	Phosphorus, % Found	B.p. °C.	B.p. Mm.	n^{25}_{D}
$\mathrm{(EtO)_2P(O)OCHClCHCl_2}$	43	37.3	36.8	10.9	10.7	111–142	1	1.4512
$\mathrm{(EtO)_2P(O)OCHClCCl_3}$	74	44.3	44.4	9.7	9.0	125–145	1.5	1.4600
$\mathrm{(EtO)_2P(O)OCHBrCBrMe_2}$	100	43.4	43.5	8.4	8.6	.....	..	....
$\mathrm{(EtO)_2P(O)O\underline{CBrCHBrCH_2CH_2CH_2CH_2}}$	100	40.6	40.9	7.9	7.9	.....	..	....
$\mathrm{(EtO)_2P(O)OCHClCH_2Br}$[c]	48	6.8	6.7	10.4	10.0	106–112	1	1.4579
$\mathrm{(EtO)_2P(O)OC(CH_2Cl)BrCH_2Br}$	98	7.7	7.9	8.0	8.4	.....	..	....
$\mathrm{(EtO)_2P(O)OCHBrCHBrCl}$	38	8.0	7.4	8.3	8.5	120–122	1	1.4660
$\mathrm{(ClCH_2CH_2O)_2P(O)OCHBrCHBrCl}$	98	11.0	10.9	6.8	7.1	.....	..	....
$\mathrm{(EtO)_2P(O)OCHBrCBrCl_2}$	71	9.8	9.6	7.6	8.0	114–121	0.2	1.4867
$\mathrm{(\mathit{n}\text{-}PrO)_2P(O)OCHBrCBrCl_2}$	47	9.2	8.9	7.1	7.3	152–160	1	1.4828
$\mathrm{(CH_2)_3O_2P(O)OCHBrCBrCl_2}$	100	10.2	10.1	7.9	8.6	.....	..	...
$\mathrm{\underline{OCHEtCHMeCH_2O}P(O)OCHBrCBrCl_2}$	100	9.2	8.6	7.1	7.3	.....	..	....
$\mathrm{(CCl_3CH_2O)_2P(O)OCHBrCBrCl_2}$	100	16.2	17.2	5.0	5.3	.....	..	....

[a] The yields given are for individual experiments but in several cases the same reactants gave little or no adduct. [b] When two halogens are present the halogen content is expressed as milliequivalents per gram. [c] The positions of the halogen atoms are not known but are assumed on theoretical grounds to be as shown.

diethyl *t*-butyl phosphate, a compound not previously described. This compound was synthesized from diethyl phosphorochloridate and sodium *t*-butoxide and was found to react vigorously with bromine. The reaction with bromine gave isobutene dibromide and other unidentified products. Diethyl *t*-butyl phosphate, as would be expected, did not show infrared absorption at 6.1 μ.

Our only preparation of pure diethyl vinyl phosphate by the dehydrohalogenation reaction was by the action of sodium hydride on diethyl 2-bromoethyl phosphate in ether solution and the yield was only 10%. A careful comparison of physical properties, including infrared absorption, of this product and the product obtained from triethyl phosphite and chloroacetaldehyde indicated them to be identical. When refluxed with triethylamine both products gave similar crystalline derivatives.

The hydrogenation of the product obtained from triethyl phosphite and chloroacetaldehyde to triethyl phosphate was best accomplished in cyclohexane with palladium chloride on carbon as catalyst. The infrared absorption and other physical properties were essentially identical with those determined on a purified sample of commercial triethyl phosphate. Incomplete hydrogenation of the unsaturated phosphate was accomplished by Raney nickel in ether and by palladium chloride on carbon in dioxane, but a modification of reaction conditions would probably have given satisfactory results.

The addition of halogen by vinyl phosphates has been noted by others[3a,b,c,d] but no one has described an anomalous reaction which frequently occurs with either chlorine or bromine. Perkow[3c] described certain tetrabromoethyl esters which he found to be undistillable and which were destroyed by warming with excess bromine, and Mattson and co-workers[3b] noted that although dimethyl dichlorovinyl phosphate readily absorbed bromine the derivative could not be isolated. We have observed that good yields of distillable addition products can frequently be obtained (Table I) from either chlorine or bromine. In other experiments, however, for no apparent reason, the same reactants give rise to very little of the addition product but produce (a) alkyl halide, (b) a product which upon heating decomposes to a carbonyl compound containing one more halogen atom than the original phosphate, and (c) a viscous, non-volatile liquid of an unknown nature.

This anomalous reaction is well illustrated by the addition of bromine at 60° and in the presence of ultraviolet light to an equimolar quantity of diethyl 2-chlorovinyl phosphate dissolved in carbon tetrachloride. The yield of diethyl 1,2-dibromo-2-chloroethyl phosphate was only 5% but considerable quantities of ethyl bromide and bromochloroacetaldehyde, not previously described, were produced.

The normal halogen addition and the anomalous reaction appear to be entirely independent of each other and the relative amounts of the two reactions seem to be independent of temperature, solvent, order of mixing, molar ratios and the presence or absence of ultraviolet light. We have found that 1,2-dibromo-2,2-dichloroethyl diethyl phosphate is stable to excess bromine at 30° but the anomalous reaction has been observed below 0°.

An interesting difference between the halogen adducts and the products of the anomalous reaction is that the former are powerful cholinesterase inhibitors and are highly toxic to certain insects while the latter possess little or no biological activity.

Several unsuccessful attempts were made to perform other reactions with diethyl vinyl phosphate and other products which normally add to an ethylenic double bond. These included epoxidation in acetic acid, hydrogen chloride with anhydrous aluminum chloride, 2,4-dinitrobenzenesulfenyl chloride in acetic acid, and carbon tetrachloride with benzoyl peroxide. The last of these may have been partly successful since a product was isolated in 31% yield which contained 71% of the theoretical chlorine content of the expected adduct.

The hydrolysis of the product obtained from the reaction of chloral with triethyl phosphite to dichloroacetaldehyde is consistent with the vinyl structure.

Experimental

Dehydrobromination of Diethyl 2-Bromoethyl Phosphate.—This reaction was carried out with a 100% excess of phosphate in order to minimize side reactions. To a solution of 636.5 g. (2.44 moles) of diethyl 2-bromoethyl phosphate in 300 ml. of dry ether was gradually added 29.3 g. of sodium hydride over a period of 4 hours. The solution was refluxed (43°) for 85 hours, filtered by gravity and the ether removed *in vacuo*. Distillation of the product through a 10-inch Vigreux column gave 42.5 g. distilling at 52–78° and fractionation of the distillate through a 1-foot column packed with glass helices gave 21.5 g. (10% yield) of diethyl vinyl phosphate, b.p. 60–64° (1 mm.), n^{35}_{D} 1.4070, and d^{35}_{4} 1.0687 (literature[2] values for the product obtained from chloroacetaldehyde and triethyl phosphite are b.p. 79° (6 mm.), n^{35}_{D} 1.4100, and d^{35}_{4} 1.0724). Infrared spectrograms prepared from this product and the one obtained from chloroacetaldehyde and triethyl phosphite were essentially identical.

Anal. Calcd. for $C_6H_{13}O_4P$: P, 17.2. Found: P, 17.5.

The diethyl 2-bromoethyl phosphate used in this preparation was made by mixing equimolar quantities of pyridine, bromoethanol and diethyl phosphorochloridate in the order given at 0–10°, stirring the mixture at room temperature for 2 hours, and removing of pyridinium chloride by filtration. Distillation of the product gave a 61% yield, b.p. 100–102° (1 mm.), n^{35}_{D} 1.4388, and d_4^{35} 1.3898.

Anal. Calcd. for $C_6H_{14}BrO_4P$: Br, 30.6; P, 11.9. Found: Br, 29.6; P, 12.3.

Hydrogenation of Diethyl Vinyl Phosphate.—The catalyst for this reaction was prepared by hydrogenating palladium chloride on carbon in cyclohexane at 50 pounds pressure for 2 hours in a Parr low-pressure apparatus. To 50 g. of highly purified product obtained from chloroacetaldehyde and triethyl phosphite was added 8.1 g. of wet catalyst (equivalent to 4 g. of dry catalyst) and 150 g. of pure cyclohexane. After 1 hour at 60 pounds pressure a sample of product was isolated for an infrared spectrogram and found to contain an estimated 10% of diethyl vinyl phosphate. Six grams of wet catalyst was then added to the reaction mixture and hydrogenation contained at 50 pounds pressure over the week end. Distillation gave a 73% yield of triethyl phosphate, b.p. 75° (5 mm.), n^{35}_{D} 1.3975, d^{35}_{4} 1.0549, and no absorption at 6.1 μ. In order to obtain a direct comparison under the same conditions with an authentic sample of triethyl phosphate, a sample of commercial product was fractionated at 10% take off through a 6-foot column packed with glass helices. The fraction chosen for comparison had b.p. 79° (5 mm.), n^{35}_{D} 1.3990, and d^{35}_{4} 1.0544. Infrared spectrograms of the two samples were essentially identical.

Anal. Calcd. for $C_6H_{15}O_4P$: P, 17.0. Found: for hydrogenation product, 16.4 and 16.6; for the purified commercial product, 16.5 and 16.7.

Diethyl 1,2,2,2-Tetrachloroethyl Phosphate.—One-half mole (124.5 g.) of diethyl 2,2-dichlorovinyl phosphate and 185 ml. of carbon tetrachloride were placed in a 500-ml. 3-neck flask equipped with a stirrer, an adapter bearing a thermometer and a Dry Ice condenser, and a 100-ml. graduated addition funnel surmounted by a Dry Ice condenser. About 0.75 mole of chlorine, liquefied in the condenser and collected in the addition funnel, was added dropwise to the reactor at about 60°. The reaction proceeded readily under the influence of a mercury vapor lamp. When the reaction was complete the solvent and excess chlorine were removed *in vacuo* and the product distilled. The yield of diethyl 1,2,2,2-tetrachloroethyl phosphate was 118 g. (74%), b.p. 125–145° (1.5 mm.), and n^{35}_{D} 1.4600.

Anal. Calcd. for $C_6H_{11}Cl_4O_4P$: Cl, 44.3; P, 9.7. Found: Cl, 44.4; P, 9.0.

Diethyl 1,2-Dibromo-2,2-dichloroethyl Phosphate.—One-half mole (80 g.) of bromine was added to 0.5 mole (124.5 g.) of diethyl 2,2-dichlorovinyl phosphate dissolved in 300 ml. of carbon tetrachloride. The temperature rose spontaneously to about 60° and the reaction was allowed to continue in a dark cabinet for 3 days. The solvent and a small amount of bromine were removed *in vacuo* and the product distilled. The yield of diethyl 1,2-dibromo-2,2-dichloroethyl phosphate was 145 g. (71%); b.p. 114–121° (0.2 mm.), n^{35}_{D} 1.4867 and d^{35}_{4} 1.741.

Anal. Calcd. for $C_6H_{11}Br_2Cl_2O_4P$: halogen, 9.8 meq./g.; P, 7.6. Found: halogen, 9.6; P, 8.0.

Diethyl Bromochloroethyl Phosphate.—One-tenth mole each of bromine and chlorine were dissolved in 75 ml. of carbon tetrachloride at 0° and 0.2 mole of diethyl vinyl phosphate added gradually with cooling to about 20°. Removal of the solvent *in vacuo* and distillation gave 28 g. (48%) of diethyl bromochloroethyl phosphate, b.p. 106–112° (1 mm.), n^{35}_{D} 1.4518 and d^{35}_{4} 1.4579.

Anal. Calcd. for $C_6H_{13}BrClO_4P$: halogen, 6.8 meq./g.; P, 10.4. Found: halogen, 6.7; P, 10.0.

Anomalous Reaction of Bromine with Diethyl 2-Chlorovinyl Phosphate.—Two moles (429 g.) of diethyl 2-chlorovinyl phosphate was dissolved in 1 liter of carbon tetrachloride and 2 moles of bromine added gradually with cooling at about 60°. A mercury vapor lamp was used to activate the halogen. The carbon tetrachloride, which was removed first at atmospheric pressure and then *in vacuo*, contained a considerable quantity of lower boiling liquid, presumably ethyl bromide, but this was not isolated and identified. An attempt to distil the product at reduced pressure resulted largely in decomposition and only 40 g. (5%) of diethyl 1,2-dibromo-2-chloroethyl phosphate was obtained, b.p. 148–150° (1.5 mm.).

Anal. Calcd. for $C_6H_{12}Br_2ClO_4P$: halogen, 8.0 meq./g.; P, 8.3. Found: halogen, 7.8; P, 8.6.

A smaller (28 g.) and more volatile fraction of product collected at 70–95° (1 mm.) had n^{35}_{D} 1.4025 and was probably triethyl phosphate.

About 40 g. of liquid collected in a cold trap placed between the vacuum source and the condenser and an additional quantity was probably lost. Redistillation of this material at atmospheric pressure gave 27 g., b.p. 111.5–112°, n^{35}_{D} 1.4864 and d^{35}_{4} 1.8576.

Anal. Calcd. for C_2H_2BrClO: halogen, 12.1 meq./g. Found: halogen, 12.7.

This product was further identified as bromochloroacetaldehyde by reaction with an equimolar quantity of triethyl phosphite to form ethyl bromide (80%, b.p. 33–40°) and diethyl chlorovinyl phosphate (63%), b.p. 88–94° (1 mm.), and n^{35}_{D} 1.4269 (literature[2] values are b.p. 92–105° at 1–2 mm., and n^{35}_{D} 1.4276).

The non-volatile material from this reaction (424 g.) was a viscous liquid, soluble in ether and methanol but insoluble in water, and was found to contain 12.9% phosphorus and 6.7 milliequivalents of halogen per gram.

Debromination of Diethyl 1,2-Dibromo-2,2-dichloroethyl Phosphate.—Fifty grams (0.122 mole) of diethyl 1,2-dibromo-2,2-dichloroethyl phosphate dissolved in 100 ml. of ether was treated gradually with an equivalent quantity of zinc dust (8 g.). The initial reaction was very vigorous and some of the product was lost by foaming. After refluxing for 3 hours the solution was extracted with water, the organic layer dried and the ether removed *in vacuo*. Rectification of the product gave a fraction with b.p. 60° (0.1 mm.) and n^{35}_{D} 1.4423 (literature[2] value for diethyl 2,2-dichlorovinyl phosphate, n^{35}_{D} 1.4428). Spectral traces from 1.8 to 15 μ prepared from this sample and a sample of diethyl 2,2-dichlorovinyl phosphate were essentially identical.

Hydrolysis of Diethyl 2,2-Dichlorovinyl Phosphate.—Sixty-five grams (0.262 mole) of diethyl 2,2-dichlorovinyl phosphate, 126 ml. of 37% hydrochloric acid and 100 ml. of water were mixed and refluxed for 5 hours. The vent gases were dried and passed into a cold trap which condensed 34 g. (79%) of ethyl chloride, b.p. 12.5°. The reaction mixture was extracted 5 times with ether, the combined ether extracts dried over sodium sulfate, and the ether removed *in vacuo*. Fractionation of the residue gave 15.6 g. (53%) of dichloroacetaldehyde, b.p. 95.5–96.4°. This product and an authentic sample of dichloroacetaldehyde were converted to 1:1 condensation products with urea by dissolving 5 g. of the aldehyde and 5 g. of urea in 6 ml. of water and separating the resulting white solid by filtration. The melting point and mixed melting point were 125–126°.

Reaction of Diethyl Vinyl Phosphate with Triethylamine.—One-tenth mole (18 g.) of diethyl vinyl phosphate and 0.11 mole (11 g.) of triethylamine were refluxed for 84 hours. Upon cooling a crystalline solid separated and this was washed with ether and dried.

Anal. Calcd. for $C_{12}H_{28}NO_4P$: P, 11.0. Found: P, 11.1.

Diethyl *t*-Butyl Phosphate.—Sodium *t*-butoxide was prepared by refluxing 125 ml. (1.32 moles) of *t*-butyl alcohol containing 3.45 g. (0.15 mole) of finely cut sodium until reaction was complete. To this mixture was added gradually with stirring and cooling 25.9 g. (0.15 mole) of diethyl phosphorochloridate at 25–30°. After stirring for 2 hours at room temperature the excess *t*-butyl alcohol was removed *in vacuo*, 25 ml. of water added to dissolve the sodium chloride, and the resulting solution extracted with ether. Removal of the ether and distillation gave 9.8 g. (31%) of diethyl *t*-butyl phosphate, b.p. 63–66° (1 mm.), $n^{35}D$ 1.4042 and $d^{35}{}_4$ 1.0105.

Anal. Calcd. for $C_8H_{19}O_4P$: P, 14.8. Found: P, 15.2.

To 21 g. (0.1 mole) of diethyl *t*-butyl phosphate was gradually added 16 g. (0.1 mole) of bromine at 10–15°. Distillation gave a high boiling product and 18 g. (83%) of 1,2-dibromo-2-methylpropane, b.p. 140–154°, and $n^{35}D$ 1.4992 (literature[5] b.p. 145–148°, $n^{25}D$ 1.5050).

Anal. Calcd. for $C_4H_8Br_2$: Br, 74.1. Found: Br, 74.3. The high-boiling product, 10.2 g., b.p. 136–148° (1 mm.), may be a mixture of diethyl hydrogen phosphate and diethyl phosphorobromidate. Analysis indicated 17.9% phosphorus, 10.8% bromine and 4.9 milliequivalents of acid per gram.

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[5] N. A. Milas and C. N. Winnick, THIS JOURNAL, **71**, 748 (1949).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

The Synthesis and Properties of Hydroxypyruvic Acid Phosphate

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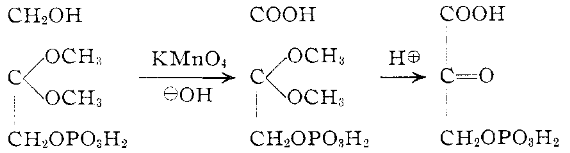
Hydroxypyruvic acid phosphate, a possible intermediate in the biosynthesis of serine phosphate, has now been obtained by synthesis and some of its properties have been determined. This synthetic material has been shown by other workers to be a good precursor of serine in a partially purified enzyme system prepared from rat liver.

Hydroxypyruvic acid phosphate has been postulated as a possible precursor of serine phosphate and of serine *via* the pathway[1]

$$\text{hydroxypyruvic acid phosphate} \xrightarrow{\text{transamination}}$$
$$\text{serine phosphate} \xrightarrow{\text{enzymatic dephosphorylation}} \text{serine}$$

However, since the hydroxypyruvic acid phosphate has not been available either by isolation or synthesis, it has been impossible to test its biological activity in such a role.

We wish to report the synthesis of hydroxypyruvic acid phosphate by a definitive method, using as starting material the cyclohexylammonium salt of dihydroxyacetone phosphate dimethyl or diethyl ketal.[2] The reaction scheme is outlined below.



I II III

The cyclohexylammonium salt of dihydroxyacetone phosphate dimethyl ketal (I) was converted to the potassium salt, which was oxidized to II with potassium permanganate. The salt of II was converted to the free acid by passage through a column of Dowex 50 (H^+). The acid eluate was neutralized with cyclohexylamine to give the salt, which was isolated in crystalline form from water by the addition of acetone.

The titration curves for I and II show the presence of a carboxyl group in the oxidation product, II (one mole per mole of primary or secondary phosphate), and are confirmation of the assigned structure.

The ketal structure of II is more stable at the *p*H of its own free acid than is that of I, being only slowly hydrolyzed to the ketone III, at 40°. However, incubation of the acidic solution at 40° for 4 days does hydrolyze the ketal, with the liberation of very little inorganic phosphate. The ultraviolet absorption spectra of the ketal and of the hydrolyzed compound show a shift in absorption toward the longer wave lengths following the hydrolysis of the ketal structure, which may serve as the basis of a useful spectrophotometric assay. The preparation of III by way of the diethyl ketal is in some respects preferable, since hydrolysis of the diethyl ketal proceeds about three times more readily than the dimethyl ketal.

III has the following properties. The phosphate group was liberated by acid hydrolysis at a rate comparable to that of dihydroxyacetone phosphate (half-time of hydrolysis about 20 minutes in 1 *N* hydrochloric acid at 90°).[2] In contrast, liberation of the phosphate group in alkali was more difficult, requiring 15 hours at room temperature in 1 *N* alkali for the elimination of 50% of the phosphate. Conditions for the quantitative elimination of phosphate in alkali were not found. Dephosphorylation

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[1] H. J. Sallach, in H. B. McElroy and B. Glass, "A Symposium on Amino Acid Metabolism," Johns Hopkins Press, Baltimore, Md., 1955, p. 782.

[2] C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **78**, 1659 (1956).