Synthesis of Vinyl Phosphate, a Possible Intermediate in Carbohydrate Metabolism and Photosynthesis

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(Received for publication, June 23, 1958)

It is now well established that phosphate esters are essential intermediates in a wide range of biochemical conversions. Unexpected instability of some of these esters has, on previous occasions, delayed their positive identification in biological materials. For example, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, which had long been suspected to be intermediates in fermentation and glycolysis, were identified only after a synthesis of D-glyceraldehyde-3-phosphate (1) had revealed its chemical properties, and had suggested methods for the isolation of the highly labile triose phosphates. It seems probable that our knowledge of the intermediates in carbohydrate metabolism, and particularly of those involved in photosynthesis, is by no means complete. The possibility that vinyl phosphate is one of the unknown intermediates in anabolism and catabolism of carbohydrates has been considered by various workers. Involvement of vinyl phosphate in both biochemical degradation and synthesis of phosphate esters of the 3-carbon acids can readily be envisaged. Scheme I suggests tentatively the relationship of vinyl phosphate to such esters by carboxylation and decarboxylation reactions, and its decomposition to the penultimate product of fermentation, acetaldehyde.

\[
\begin{align*}
\text{COOH} \\
\text{C-OPO}_3\text{H}_2 \rightleftharpoons \text{H-C-OPO}_3\text{H}_2 \rightleftharpoons \\
\text{CH}_3 \quad \text{CH}_3 \\
\text{H-C-OH} \rightleftharpoons \text{HC=O} \\
\text{CH}_2 \quad \text{CH}_2
\end{align*}
\]

Scheme I

As expected, vinyl phosphate was found to be hydrolyzed fairly rapidly at room temperature with phosphoric acid and acetaldehyde being formed. Hence it might be argued that the substance could not possibly serve as an intermediate in aqueous systems except during degradation. However, its stability towards hydrolysis increases considerably on esterification. To liberate acetaldehyde from diesters of vinyl phosphate, either a strong acid at high temperature or a catalyst (a mercuric salt) is required. It is possible that biologically formed vinyl phosphate may be linked via its phosphoric acid to some material that confers greater stability on it.

The synthesis of vinyl phosphate has not yet, to our knowledge, been satisfactorily accomplished. A method yielding an aqueous solution containing a compound with the properties expected for vinyl phosphate has been reported by Fager (2). The synthesis of esters of vinyl phosphoric acid has been carried out by several methods (3-9), but none of these seemed suitable for the synthesis of vinyl phosphoric acid itself. On the other hand, some of the procedures that had been developed for the synthesis of vinyl esters of organic acids (10-16) seemed applicable also to the synthesis of vinyl phosphoric acid. Of these, the method of Toussaint and MacDowell (13) for the preparation of vinyl esters of higher aliphatic acids by a mercuric ion-catalyzed exchange reaction between vinyl acetate and the aliphatic acid appeared to be the most promising one for the preparation of vinyl phosphoric acid.

In accordance with the procedure of the above authors, a mixture of vinyl acetate, crystalline orthophosphoric acid, and mercuric acetate was heated to boiling under reflux (Equation 1, R = CH₃).

\[
\text{CH}_2=\text{HC-OCONO}_3\text{H}_2 + \text{H}_3\text{PO}_4 \xrightarrow{\text{mercuric acetate}} \text{CH}_2=\text{HC-OPO}_3\text{H}_2 + \text{R} \cdot \text{COOH}
\]

The mixture, however, turned dark almost immediately with the formation of tarry condensation products. There was some indication that an exchange had taken place, but evidently the vinyl phosphate had undergone further reactions at the temperature of the boiling vinyl acetate. Subsequently, numerous experiments were carried out in which the reaction time and temperature, as well as the molar ratios of the reactants, were varied in order to establish more suitable conditions for the transesterification process. As a result, it was established that if a solution of approximately 1 gm. of crystalline orthophosphoric acid and 20 mg. of mercuric acetate in 20 ml. of vinyl acetate is kept, with stirring, for 1 hour at 30° ± 1°, a reaction mixture is obtained that contains considerable amounts of vinyl phosphate, but that is, nevertheless, almost colorless.

Upon the addition of petroleum ether, an oil precipitated which consisted of a mixture of vinyl phosphate, small amounts of inorganic phosphoric acid, pyrophosphoric acid (identified by its white, water-insoluble, silver salt), and possibly vinyl pyrophosphate, divinyl phosphate, and phosphate esters of acetaldehyde condensation products. Somewhat purer vinyl phosphate preparations were obtained by dissolving the oily precipitate immediately in an inert solvent, such as anhydrous ether or dioxane, and fractionally precipitating the reaction

1 We have observed that polyphosphoric acids are formed from phosphoric acid by the action of mercuric acetate on its ethereal solution.
product with petroleum ether of low boiling points. At room temperature, especially when the attempt was made to remove adhering solvents, the mixture decomposed with the liberation of acetaldehyde and the formation of a black residue. For analysis, the fractions were dissolved immediately in anhydrous ether or dioxane, and the molar ratios of phosphoric acid and acetaldehyde were estimated. Our best fractions were found to contain phosphoric acid and acetaldehyde in almost theoretical ratios (1:1), but still they were contaminated with considerable amounts of pyrophosphate (15 to 20 per cent) and condensation products of acetaldehyde. Attempts to remove these by other methods were unsuccessful. When we replaced the vinyl acetate with vinyl formate* (Equation 1, R = H) and carried out the transesterification at higher dilution (1 gm. of phosphoric acid per 40 ml. of vinyl formate), we obtained phosphorus-containing transesterification products, which upon separation by fractional precipitation, gave vinyl phosphoric acid preparations containing usually not more than 5 per cent of pyrophosphates or acetaldehyde condensation products. Vinyl formate, however, is not readily obtainable commercially. Therefore, when it was found that addition of ethylenediamine-tetracetate tetrasodium salt to the reaction mixture also reduces the formation of both pyrophosphates and condensation products of acetaldehyde, we returned to the use of the more readily available vinyl acetate. Attempts to obtain material for elementary analyses by freeing the vinyl phosphoric acid from adhering solvents in a vacuum led to the decomposition of the vinyl ester. It was observed, however, that the vinyl phosphoric acid can be kept for several days with apparently very little decomposition if it is dissolved immediately in anhydrous ether or dioxane, provided the concentration of the ester does not exceed 0.5 per cent, and the solution is kept in the cold. The analysis for phosphoric acid, pyrophosphoric acid, acetaldehyde, and condensation products of acetaldehyde disclosed that the dissolved material contained phosphoric acid and acetaldehyde in a molar ratio of 1.00:0.95, and that pyrophosphoric acid and acetaldehyde condensation products amount to 3.2 per cent and 2.3 per cent of the total phosphoric acid and acetaldehyde, respectively.

The transesterification product gave reactions which were consistent with those expected of vinyl phosphoric acid. In dilute hydrochloric acid, the material was hydrolyzed readily and formed approximately equimolecular amounts of phosphoric acid and acetaldehyde. This acid hydrolysis proceeds so rapidly that no distinction between bound and free phosphoric acid was possible by the customary methods for the determination of phosphoric acid. When added to a lukewarm solution of 2,4-dinitrophenylhydrazine in 2.5 N hydrochloric acid, the product rapidly formed acetaldehyde 2,4-dinitrophenylhydrazone. The rate of formation of this precipitate is similar to the rate of that from another water soluble vinyl ceter, vinyl formate. Upon cautious addition of diazomethane to a cold ethereal solution of the vinyl phosphoric acid, with care to avoid an excess of diazomethane, and fractional distillation of the reaction product in a vacuum, a fraction was obtained that contained considerable amounts of dimethylvinyl phosphate. The methylation product, however, contained somewhat less bound acetaldehyde per mole of phosphoric acid than did the original material, perhaps as the result of a partial loss of the vinyl group during the preparation of the ester.

Since, on removal of solvents, the vinyl phosphoric acid decomposes at too rapid a rate to permit its isolation in a pure state, various salts were prepared in the hope that these would prove more stable and facilitate further purification. The salts examined were those of the cyclohexyloxammonium, dimethylanilinium, pyridinium, brucinium, ammonium, lithium, and magnesium cations. Of these, only the pyridinium and lithium salts were obtained in a reasonably satisfactory degree of purity. However, even these salts were not stable enough to allow further purification after the initial precipitation. The preparation of the pyridinium and lithium salts of vinyl phosphoric acid is described in "Experimental."

The composition of the monopyridinium and dilithium vinyl phosphates, the latter obtained via the pyridinium salt and containing 1 mole of ethanol, varied slightly from experiment to experiment even with the methods of preparation finally adopted (cf. Tables I and II below).

The rather facile hydrolysis of vinyl phosphoric acid in acetic acid, as indicated by the rapid formation of acetaldehyde and 2,4-dinitrophenylhydrazine, aroused some suspicion as to its structure. However, the facts that it yields salts containing bound acetaldehyde, and that, on methylation with diazomethane, it forms a methyl ceter in which the vinyl group is as firmly bound as in authentic esters of vinyl phosphoric acid, effectively dispose of any possibility that it is merely a physical mixture of acetaldehyde and phosphoric acid in the fortuitous molar ratio of 1:1, or else a carbonyl addition compound or complex of acetaldehyde and phosphoric acid. The vinyl ester structure of the transesterification product receives further support by the presence of a strong absorption band in the infrared spectrum at 6.08 μ (dioxane) which is shown by esters of vinyl phosphoric acid (4, 9) and is characteristic of the vinylic double bond.

Vinyl phosphoric acid, as an end ester, may be expected to yield on hydrolysis a standard free energy decrease of the same order of magnitude as phosphoryl-enolpyruvic acid. This would place the vinyl phosphoric acid in a group of compounds that is referred to generally as containing "high energy phosphate bonds" (17-19).

**Experimental**

Materials-Stabilized vinyl acetate (practical grade) was obtained from the Eastman Kodak Company, and the vinyl formate, from The Borden Company. Both preparations, which should be of recent manufacture, were redistilled immediately before use, and a first fraction amounting to approximately 10 per cent of the original volume was discarded. Diethyl ether and petroleum ether (b.p., 35-60°) were distilled and stored over metallic sodium. The dioxane was purified and dried as described by Hess and Frahm (20). Anhydrous ethanol was prepared by the method of Lund and Bjerrum (21). The anhydrous pyridine was obtained by refluxing pyridine (analytical reagent grade) over barium oxide, and distilling the product with careful exclusion of moisture. The mercuric acetate and tetrasodium salt of ethylenediaminetetraacetic acid (practical grade) were dried in a vacuum at room temperature over phosphorus pentoxide. Anhydrous lithium carbonate was prepared by dissolving lithium carbonate in slightly more than the theoretical amount of dilute acetic acid, filtering the solution, and allowing the resulting solution to stand for several days. Anhydrous lithium acetate was prepared by dissolving lithium carbonate in slightly more than the theoretical amount of dilute acetic acid, filtering the solution, and allowing the resulting solution to stand for several days. Anhydrous lithium acetate was prepared by dissolving lithium carbonate in slightly more than the theoretical amount of dilute acetic acid, filtering the solution, and allowing the resulting solution to stand for several days.

*We wish to thank Dr. Donald D. Clarke for recommending to us the use of vinyl formate.*
removing most of the water by distillation in a vacuum, and
driving off the residual water by placing the concentrate in an
oven at 130°. To prepare a 2 per cent ethanolic solution of
lithium acetate, 1.0 gm. of the salt was added to 50 ml. of an-
hydrous ethanol, and the mixture was shaken for 1 hour and
cleared by centrifugation.

Crystalline Anhydrous Orthophosphoric Acid—Concentrated
phosphoric acid (sp. gr. 1.75) was placed in a platinum crucible
and heated in an electric furnace to 105° ± 1° for a period
of 24 hours. The anhydrous phosphoric acid, while still hot,
was transferred to 4-ml. test tubes of Pyrex glass, filling them
half-full. The tubes were closed and set aside at room tem-
perature. Shortly before they were used, crystallization was
induced by immersing the tubes briefly in a cold bath of ace-
tone and solid carbon dioxide. When about two-thirds of
the phosphoric acid had crystallized at room temperature, the
stopper was replaced by a piece of strong filter paper, kept in
place by rubber bands. The test tube was placed in an inverted
position in a centrifuge glass which contained at its bottom a
layer of coarse glass fiber. The centrifuge tube was closed with
a rubber cap, and the mother liquor was removed by centrifuga-
tion. The remaining crystalline orthophosphoric acid usually
contained not more than 3 per cent of pyrophosphoric acid (22)
and melted at 41.7-42.5° (reported m.p. 42.35° (23)).

Pyridinium Hydrogen Vinyl Phosphate—In a thoroughly dried
100-ml. round flask with ground glass joint, equipped with an
oil-sealed mechanical stirrer, were placed 65 ml. of freshly dist-
tilled vinyl acetate, 125 mg. of mercuric acetate, 500 mg. of
ethylenediaminetetraacetic acid tetrasodium salt, and 2.0 gm.
of crystalline orthophosphoric acid. The flask was immersed
in an oil bath at 30° ± 1°, and the mixture was stirred at this
temperature for 2.5 hours. The colorless solution was decanted
from the solid and divided equally between two 200-ml. centri-
fluence tubes, each containing 90 ml. of petroleum ether (low
boiling). The mixtures were centrifuged, the supernatant sol-
utions were decanted, and the precipitates were washed with
30 ml. of petroleum ether. The remaining materials, colorless
viscous oils, were each extracted with 45 ml. of anhydrous ether,
and both extracts were cleared by centrifugation. The ether
extracts were transferred to 200-ml. centrifuge tubes, and
to each were added 60 ml. of petroleum ether. The mixtures
were separated by centrifugation, the supernatant solutions
were transferred to two 200-ml. centrifuge tubes, and to each
was added a solution of 0.25 ml. of anhydrous pyridine in 30
ml. of ether. The oily precipitates were centrifuged, freed from
supernatant solutions, and each triturated with a solution of
0.25 ml. of pyridine in 2 ml. of ether. The precipitates were
then washed, each with two 10-ml. portions of petroleum ether,
and kept in a vacuum (0.5 mm.) at room temperature over potas-
sium hydroxide pellets for 1 hour to remove most of the solvents.
The pyridinium hydrogen vinyl phosphate, a viscous oil weigh-
ing 1.18 gm. (25.7 per cent of the theory calculated for phosphoric
acid), was analyzed for its contents of phosphoric acid, acet-
aldheyde, and pyridine.

3 It is essential that from this point all operations are carried
out as rapidly as possible, with the exclusion of moisture (rubber
caps on all tubes), and that the ether and petroleum ether are
cooled to 5°.

4 There is probably at least as much again of the vinyl phos-
phoric acid in the other fractions that have been discarded, but
it is accompanied by substances that make it difficult to isolate
the ester in the state of acceptable purity.

The phosphoric acid was determined by the method of King
(24) on (a) aqueous solutions of pyridinium hydrogen vinyl
phosphate that had been kept for 4 hours at room temperature,
giving the phosphoric acid, both free and bound as vinyl
phosphate, and on (b) solutions that had been subjected to
acid hydrolysis at 100° giving the total phosphoric acid. The
difference between these values was considered to represent
pyrophosphoric acid which in general amounted to less than 4
per cent of the total phosphoric acid. Almost all of the o-phos-
phoric acid was bound to acetaldehyde.

The acetaldehyde was determined as 2,4-dinitropheny-
hydrazone either by adding an aqueous solution of the pyridinium
hydrogen vinyl phosphate at room temperature to a saturated
solution of 2,4-dinitrophenylhydrazine hydrochloride in 2.5
n hydrochloric acid, or by bubbling a stream of nitrogen through
an acidified aqueous solution of the pyridinium vinyl phosphate
which was kept in a water bath at 45° + 2°, and absorbing the
acetaldehyde in a saturated solution of 2,4-dinitrophenylhy-
drazine hydrochloride in hydrochloric acid. The first procedure
yields mixtures of the 2,4-dinitrophenylhydrazones of acetalde-
hyde and its condensation products, whereas the second pro-
cedure separates the acetaldehyde from its less volatile con-
densation products and permits their separate determination
as 2,4-dinitrophenylhydrazones. In general, the condensation
products amounted to less than 3 per cent of the acetaldehyde.
The pyridine was determined colorimetrically as reineckate.

The molar ratios of phosphoric acid:acetaldehyde:pyridine of
five independent preparations of pyridinium hydrogen vinyl
phosphate are shown in Table I.

---

**TABLE I**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Total phosphoric acid</th>
<th>Acetaldehyde*</th>
<th>Pyridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>V 119</td>
<td>1.00</td>
<td>0.94</td>
<td>0.99</td>
</tr>
<tr>
<td>V 120</td>
<td>1.00</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>V 121</td>
<td>1.00</td>
<td>1.03</td>
<td>0.92</td>
</tr>
<tr>
<td>V 122</td>
<td>1.00</td>
<td>0.95</td>
<td>0.94</td>
</tr>
<tr>
<td>V 123</td>
<td>1.00</td>
<td>0.99</td>
<td>0.96</td>
</tr>
</tbody>
</table>

* The figures represent minimal values, since determinations
upon pure acetaldehyde gave values of about 3 per cent too low
when determined as 2,4-dinitrophenylhydrazone.

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**TABLE II**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Total phosphoric acid</th>
<th>Acetaldehyde*</th>
<th>Lithium</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>V 124</td>
<td>1.00</td>
<td>0.85</td>
<td>1.95</td>
<td>0.94</td>
</tr>
<tr>
<td>V 125</td>
<td>1.00</td>
<td>0.84</td>
<td>2.06</td>
<td>0.94</td>
</tr>
<tr>
<td>V 126</td>
<td>1.00</td>
<td>0.89</td>
<td>1.95</td>
<td>0.96</td>
</tr>
<tr>
<td>V 127</td>
<td>1.00</td>
<td>0.91</td>
<td>2.08</td>
<td>0.95</td>
</tr>
</tbody>
</table>

* The figures represent minimal values, since determinations
upon pure acetaldehyde gave values of about 3 per cent too low
when determined as 2,4-dinitrophenylhydrazone.
phate, the pyridinium salt (1.178 gm.), obtained as described above from 2.0 gm. of crystalline phosphoric acid but not dried in a vacuum, was immediately dissolved in 25 ml. of anhydrous ethanol. The solution was placed in a 75-ml centrifuge tube, and 7.5 ml. of a 2 per cent solution of lithium acetate in anhydrous ethanol were added. After the mixture was cleared by centrifugation, the supernatant solution was transferred to another centrifuge tube (75-ml.), and 20 ml. of the 2 per cent ethanolic solution of lithium acetate were added. The mixture was separated by centrifugation, and the supernatant solution was decanted. The precipitate was washed successively with 10 ml. of anhydrous ethanol and two 20-ml portions of anhydrous ether, with separation of the mixture by centrifugation each time. The precipitate was dried in a vacuum (2 mm.) at room temperature over phosphorus pentoxide and potassium hydroxide pellets for 20 hours. The dilithium vinyl phospate, a white, powdery material weighing 0.582 gm., was obtained in a yield of 60.8 per cent of theory calculated from the pyridinium salt. It contained 1 mole of ethanol which was identified qualitatively as its 3,5-dinitrobenzoic acid ester, and determined quantitatively as phosphoric acid by a mercuric ion-catalyzed acid exchange reaction, and its isolation as its pyridinium and lithium salts are described.

A synthesis of vinyl phosphoric acid from vinyl acetate and phosphoric acid by a mercuric ion-catalyzed acid exchange reaction, and its isolation as its pyridinium and lithium salts are described.

Acknowledgments—The authors acknowledge with gratitude their indebtedness to the Kate E. Taylor Fund of the Banting Research Foundation, and the Eli Lilly Company for their financial support of this investigation; to Dr. J. Forrest Allen of the Food Machinery and Chemical Corporation (South Charleston, West Virginia) for gifts of various esters of vinyl phosphoric acid; and to Miss Elizabeth Kirby, B. A., of the Ontario Research Foundation, for the infrared determinations.

REFERENCES

10. Roper, W., German patent 588,352 (1933); U. S. patent 2,006,075 (1936).

4 These values for nitrogen and phosphorus, and those reported in Table II are for the same four preparations of vinyl phospate.

Characterization of Alcohol in Dilithium Vinyl Phosphate—Lithium vinyl phosphate was added at room temperature to a saturated solution of 2,4-dinitrophenylhydrazine in 2.5 N hydrochloric acid. After 1 hour the acetaldehyde 2,4-dinitrophenylhydrazone was removed by filtration, the filtrate was distilled at atmospheric pressure, and the alcohol of the distillate was esterified with 3,5-dinitrobenzoyl chloride as described by Wild (26). The crude 3,5-dinitrobenzoic acid ethyl ester, which was obtained in a yield of about 20 per cent of theory, on recrystallization from petroleum ether melted at 91.5-92.0°; the melting point of authentic 3,5-dinitrobenzoic acid ethyl ester is 93° (26), (the melting point of a mixture was not depressed).

SUMMARY

A synthesis of vinyl phosphoric acid from vinyl acetate and phosphoric acid by a mercuric ion-catalyzed acid exchange reaction, and its isolation as its pyridinium and lithium salts are described.
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