SYNTHESIS OF THE \textit{d}(+)-\textit{\textalpha}-GLYCEROPHOSPHORIC ACID AND THE ACTION OF PHOSPHATASES ON SYNTHETIC \textit{d}(+)-, \textit{l}(-)-, AND \textit{dl}-\textit{\textalpha}-GLYCEROPHOSPHORIC ACIDS* \\

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The synthesis of the racemic \textit{\textalpha}-glycerophosphoric acid (1) and of the \textit{l}(-)-\textit{\textalpha}-glycerophosphoric acid (2) has been reported. The \textit{l}(-)-\textit{\textalpha}-glycerophosphoric acid has been isolated from various natural sources, such as phosphatides, and as an intermediate product of fermentation or glycolysis. For this reason, in a previous paper we called the \textit{l}(-)-\textit{\textalpha}-glycerophosphoric acid the "biological" acid. The \textit{d}(+)-\textit{\textalpha}-glycerophosphoric acid has not yet been found in nature. We have carried out its synthesis in the same way as that of its optical antipode, and describe in this paper its preparation in the form of the barium salt and silver salt. The diethyl ether diethyl ester possessed in homogeneous substance the rotation $[\alpha]_{D}^{20} = +5.94^\circ$; in absolute ethyl alcohol $[\alpha]_{D} = +6.69^\circ$. The principle of the synthesis, in which \textit{l}(-)-acetone glycerol (3) is used as starting material is given in the accompanying formula which also explains the configurational relationship between \textit{d}(+)-\textit{\textalpha}-glycerophosphoric acid and \textit{l}-glyceraldehyde.

This method of synthesis of the \textit{\textalpha}-glycerophosphoric acids not only gives products of known configuration, but also produces the enantiomorphs in optically pure form, thus having an obvious advantage over previous methods (5, 6) which relied on resolution of the racemic acid.

Since in many cases the deciding factor for the biological utilization of substances with an asymmetrical carbon atom, such as

*This paper is Communication VIII of the series, "Studies on acetone-glyceraldehyde, and optically active glycerides."
sugars and amino acids, seems to be their configuration, we are inclined to believe that also in the field of naturally occurring glycerol derivatives the asymmetry of the \( \beta \)-carbon atom of the glycerol should play a similar rôle.

In an attempt to establish the significance of that asymmetry, we began the investigation of the action of enzymes on the optical isomers of various glycerol derivatives, such as mono-, di-, and triglycerides, glycerol ethers, glycerol acetals with fatty acid residues, and glycerophosphates. A study of the action of phosphatases on glycerophosphates was undertaken first because the glycerophosphates are less liable to acyl migration than glycerides with fatty acid residues and because it was already known that the \( \alpha \)-glycerophosphates react differently with the ferments of muscle press-juice. Meyerhof and Kiessling (7) found, using the racemic compound as substrate, that only the \( l(-)\)-\( \alpha \)-glycerophosphoric acid was used up completely, the \( d(+)\)-\( \alpha \)-glycerophosphoric acid remaining unchanged.

Experiments repeated recently by Professor O. Meyerhof\(^2\)

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\(^1\) In regard to the great number of naturally occurring mixed acid triglycerides, this asymmetry of the \( \beta \)-carbon atom of the glycerol part of the molecule may in the future be found to have biological significance.

\(^2\) Meyerhof, O., private communication.
in Paris with samples of pure \( l(-)- \) and \( d(+) \)-\( \alpha \)-glycerophosphoric acid provided by us have confirmed the previous result obtained.

The rate of hydrolysis of \( l(-)- \)\( \alpha \)-glycerophosphoric acid and \( d(+) \)-\( \alpha \)-glycerophosphoric acid by unrefined kidney phosphatase, taka-phosphatase, rat bone phosphatase, and a purified phosphatase from dog feces has been investigated by us. We were able to show with all four preparations that, under the conditions specified, the \( d(+) \)-\( \alpha \)-glycerophosphoric acid was hydrolyzed with greater velocity than the \( l(-)- \)\( \alpha \)-glycerophosphoric acid. This fact has probably some physiological significance. We were interested primarily in the synthesis of the \( \alpha \)-glycerophosphoric acids with definite configuration. The behavior of the optical isomers toward ferments was investigated to prove the biological significance of the asymmetry of the \( \beta \)-carbon atom in glycerol derivatives. Having established this point, we do not propose to extend the enzyme work on glycerophosphates any further.

In the experimental part of this paper details are given for the hydrolysis with two enzymes only; namely, kidney phosphatase with ammonium buffer (pH 9.6) and phosphatase from dog feces with carbonate-veronal buffer (pH 8.62). The method used to follow the rate of hydrolysis was the same as that described by King and Armstrong (8), and the determination of free phosphoric acid was carried out as applied by King (9). Data showing the rate of hydrolysis of the two optical isomers are given in Tables I and II and Figs. 1 and 2, and in Fig. 2 a curve for the racemic compound is included. The racemic compound is hydrolyzed, as was to be expected, at a rate approximately midway between those of its two components. Taka-phosphatase with phthalate buffer (pH 3.8) and rat bone enzyme with carbonate-veronal buffer (pH 8.62) gave essentially similar results.

Conclusive proof, however, that the configuration of asymmetrically substituted glycerols has an important bearing on their physiological activity will require extension of the present research to other types of glycerol derivatives. In this connection we are at present studying the enzymatic hydrolysis of the optically active glycerides with fatty acid residues and hope to present our results in the near future.
We would like to thank Dr. E. J. King, British Post-Graduate Medical School, London, England, for having kindly put at our disposal his veronal-CO$_2$ buffer (10) before publication. We also wish to thank him and Dr. C. C. Lucas of the Department of Medical Research, Banting Institute, University of Toronto, for their kind advice and assistance during our enzyme experiments.
Our thanks are also due to Miss Shirley Platt for the care with which she conducted the enzyme experiments.

**Table I**

*Hydrolysis of l(-)- and d(+)-α-Glycerophosphate by Pig Kidney Phosphatase*

8 cc. of ammonium buffer (pH 9.6), 8 cc. of substrate (containing 8.0 mg. of organic P), and 4 cc. of enzyme solution were used.

<table>
<thead>
<tr>
<th>Time</th>
<th>Phosphorus hydrolyzed (corrected for blank)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>l(-)-</td>
</tr>
<tr>
<td></td>
<td>mg.</td>
</tr>
<tr>
<td>0 min.</td>
<td>0.0</td>
</tr>
<tr>
<td>30 &quot;</td>
<td>0.840</td>
</tr>
<tr>
<td>60 &quot;</td>
<td>1.480</td>
</tr>
<tr>
<td>90 &quot;</td>
<td>1.918</td>
</tr>
<tr>
<td>120 &quot;</td>
<td>2.264</td>
</tr>
<tr>
<td>6 hrs.</td>
<td>3.751</td>
</tr>
</tbody>
</table>

**Table II**

*Hydrolysis of Inactive, l(-)-, and d(+)-α-Glycerophosphate by Phosphatase Prepared from Dog Feces*

25 cc. of carbonate-veronal buffer (pH 8.62), 20 cc. of substrate (containing 6.2 mg. of organic P), and 5 cc. of enzyme solution were used.

<table>
<thead>
<tr>
<th>Time</th>
<th>Phosphorus hydrolyzed (corrected for blank)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>l(-)</td>
</tr>
<tr>
<td></td>
<td>mg.</td>
</tr>
<tr>
<td>min.</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>0.275</td>
</tr>
<tr>
<td>10</td>
<td>0.500</td>
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<td>15</td>
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</tr>
<tr>
<td>25</td>
<td>0.983</td>
</tr>
<tr>
<td>30</td>
<td>1.100</td>
</tr>
</tbody>
</table>

**Experimental**

*Synthesis of Substrate*

*Barium Salt of d(+)-α-Glycerophosphoric Acid (Prepared from l(-)-Acetone Glycerol)*—The method used for the preparation of d(+)-α-glycerophosphoric acid is the same as that given by us
for the simplified preparation of \(l(-)-\alpha\)-glycerophosphoric acid (2). 4.1 gm. of \(l(-)-acetone\) glycerol (3) yielded 3.7 gm. of barium salt (37 per cent of the theoretical).

\[
\text{C}_3\text{H}_7\text{O}_4\text{PBa} (307.48). \text{ Calculated.} \quad \text{C} 11.7, \text{ H} 2.3, \text{ P} 10.08 \\
\text{Found.} \quad " 11.7, " 2.6, " 10.04
\]

**Diethyl Ether of Ethyl \(d(+)\)-\(\alpha\)-Glycerophosphate—**

\[
\text{H}_{3}\text{C} \cdot \text{O} \cdot \text{P} = \text{O} \\
\text{II} \text{H}_{2}\text{C} \cdot \text{O} \cdot \text{C} \cdot \text{HOC}_2\text{H}_4 \]

The diethyl ether of ethyl \(d(+)\)-\(\alpha\)-glycerophosphate was prepared according to the prescription given for its antipode, diethyl ether of ethyl \(l(-)\)-\(\alpha\)-glycerophosphate.

**Silver \(d(+)\)-\(\alpha\)-Glycerophosphate—** 2.4 gm. of barium \(d(+)\)-\(\alpha\)-glycerophosphate gave 2.42 gm. of silver salt (80 per cent of the theoretical).

\[
\text{C}_3\text{H}_7\text{O}_4\text{PAg} (385.8). \text{ Calculated.} \quad \text{Ag} 56.4; \text{ found, Ag} 56.3
\]

**Ethylation—** 2.1 gm. of silver salt yielded 1.2 gm. of diethyl ether of ethyl \(d(+)\)-\(\alpha\)-glycerophosphate (77.6 per cent of the theoretical); b.p. (0.09 mm.) = 95–97°, b.p. (0.22 mm.) = 104–105°; \(n_\text{D}^\text{II} = 1.4252, n_\text{D}^\text{II} = 1.4238.

\[
\text{C}_{11}\text{H}_{22}\text{O}_6\text{P} (284.2). \text{ Calculated.} \quad \text{C} 46.4, \text{ H} 8.8, \text{ P} 11.00 \\
\text{Found.} \quad " 45.3, " 8.6, " 10.88
\]

**Optical Rotation—** (1) In homogeneous substance in a 1 dm. tube, \(d_1 = 1.090, \alpha_\text{D} = +6.48°, [\alpha]_\text{D} = +5.94°; (2) in absolute ethyl alcohol (distilled over sodium), 0.3403 gm. in 2 cc., \(c = 14.01, 1 \text{ dm. tube,} \alpha_\text{D} = +0.94°, [\alpha]_\text{D} = +6.69°.

* See also Paper VI (2), especially p. 499, for the corresponding deficiency of carbon in the analysis of the isomer.

The respective rotations of the diethyl ether of ethyl \(l(-)\)-\(\alpha\)-glycerophosphate were (1) in homogeneous substance \([\alpha]_\text{D} = -5.31°, (2) in absolute ethyl alcohol \([\alpha]_\text{D} = -5.76°. The optical rotation of the diethyl ethers of diethyl \(d(+)\)- and \(l(-)\)-\(\alpha\)-glycerophosphates is strongly dependent on the water content of the alcohol used as solvent.

**Enzymatic Studies**

**Substrate Solutions**—The action of phosphatases on the \(\alpha\)-glycerophosphoric acids was studied with the sodium salts of
l(-)-, d(+)-, and dl-α-glycerophosphoric acids. These were prepared from the corresponding barium salts (1, 2) by treatment with sodium sulfate. The solutions of the sodium salts were kept in the ice box and never used when more than 1 week old.

Preparation of Enzymes. Phosphatase from Kidney—The phosphatase solution from fresh pig kidney was prepared in the usual manner (11) by grinding the tissue with sand and extracting the enzyme with chloroform water. The enzyme solution finally obtained was brought to pH 9.0.

Phosphatase from Dog Feces—According to Armstrong's prescription (12), 0.34 gm. of an active phosphatase preparation (activity about 25,000 units per gm.) was obtained from 1 kilo of dog feces. Standard enzyme solutions were prepared by dissolving approximately 10 mg. of this solid material in 25 cc. of physiological saline. This stock solution was diluted to give enzyme solutions of a suitable activity (ranging from 0.1 to 0.6 unit per cc. (8)).

Buffer Solutions. Ammonia Buffer—An ammonia buffer of pH 9.6 was prepared by adding 1 volume of 0.2 M NH₄Cl to 6 volumes of 0.2 M NH₄OH.

Carbonate-Veronal Buffer—A carbonate-beronal buffer of pH 8.62 (at 37°) was prepared according to King and Delory (10).

Rate of Hydrolysis with Kidney Phosphatase—Ammonia buffer, enzyme, and substrate solutions were separately brought to 37.5°. Then 8 cc. of buffer and 8 cc. of substrate solution (0.033 M), followed by 4 cc. of enzyme solution were thoroughly mixed in a large test-tube and kept at 37.5° in a thermostat with occasional stirring. Aliquots (3 cc.) were removed at zero time, 30 minutes, 60 minutes, 1½ hours, 2 hours, and 6 hours, and immediately run into 2 cc. of trichloroacetic acid to inactivate the enzyme. The phosphoric acid liberated was determined colorimetrically by the method described by King (9). The inorganic phosphate found in the zero time aliquot was taken as representing the sum of the free phosphate in the enzyme and substrate used. The figures obtained are corrected for this "blank" value and reported in Table I and Fig. 1. Similar results were obtained with other concentrations of the substrate solution.

Rate of Hydrolysis with Phosphatase from Dog Feces—The experiments with the enzyme from dog feces were conducted as described above with the following changes: (1) the aforementioned carbonate-beronal buffer (pH 8.62) was used; (2) a weaker
substrate solution was used (0.010 M) and a shorter period of time was allowed for the hydrolysis, as the enzyme was so much more active than the kidney preparation; (3) the use of trichloroacetic acid to precipitate protein was unnecessary because of the purified nature of the enzyme. In this case the hydrolysis was stopped by running the aliquots into the 1 cc. of perchloric acid used for the colorimetric phosphate determination.

The data for these hydrolyses are given in Tables I and II and Figs. 1 and 2.

**SUMMARY**

1. With \( l(-)-acetone \) glycerol as starting material the \( d(+)\)-\( \alpha \)-glycerophosphoric acid has been prepared.

2. In experiments on the action of various phosphatases on \( l(-)-\alpha \)-glycerophosphoric acid and \( d(+)\)-\( \alpha \)-glycerophosphoric acid, the \( d(+)\)-\( \alpha \)-glycerophosphoric acid was hydrolyzed with markedly greater velocity than the \( l(-)\)-\( \alpha \)-glycerophosphoric acid, while the rate of hydrolysis of the racemic compound was about midway between the rates of the other two. In contrast, muscle press-juice has been observed by Meyerhof to utilize \( l(-)\)-\( \alpha \)-glycerophosphoric acid completely, while it does not utilize \( d(+)\)-\( \alpha \)-glycerophosphoric acid at all.

**BIBLIOGRAPHY**

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SYNTHETIC \( d(+)\)-, \( l(-)\)-, AND \( dl\)-\( \alpha \)
-GLYCEROPHOSPHORIC ACIDS
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