**L-2-Keto-4,5-dihydroxyvaleric Acid: an Intermediate in the Oxidation of L-Arabinose by Pseudomonas saccharophila**

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Crude cell-free preparations of *Pseudomonas saccharophila* grown on L-arabinose oxidize L-arabinose to α-ketoglutaric acid (1). L-Arabonic acid has been identified as an intermediate in this reaction. The investigations described in this communication are concerned with the mechanism of the conversion of L-arabonate to α-ketoglutarate. Evidence is presented for the intermediate formation of L-2-keto-4,5-dihydroxyvaleric acid, a 2-keto-3-deoxy sugar acid, in the transformation. In this respect the metabolism of L-arabonate is similar to that of the D-isomer which is dehydrated to D-2-keto-4,5-dihydroxyvaleric acid (2). The metabolism of D- and L-arabinose differs in that the D-form of the keto acid intermediate is oxidatively cleaved to pyruvic and glycolic acids while the L-isomer is converted to α-ketoglutaric acid. The overall reaction sequence, as it is now known, in the metabolism of L-arabinose by *P. saccharophila* can be represented as follows:

\[
\begin{align*}
\text{L-arabinose} & \rightarrow L-2\text{-keto-4,5-dihydroxyvalerate} \\
& \rightarrow \alpha\text{-ketoglutarate}
\end{align*}
\]

**EXPERIMENTAL.**

Cultures of *P. saccharophila* were grown on L-arabinose as the sole carbon source, harvested, and stored as previously described (1). Cells have been stored at −20° for 2 years without loss of enzymatic activity. Cell-free preparations were obtained by grinding with alumina. The frozen cells were placed in a mortar chilled to approximately 5°, ground for 10 minutes with three parts of alumina (Alcoa A-301), and then extracted with 3 volumes of cold, 0.004 M phosphoric acid; Tris, tris(hydroxymethyl)aminomethane. The extracts were used the same day they were prepared.

L-Arabonic acid was assayed as the lactone (3) which was produced by heating the solution of the acid in 0.1 N HCl in a boiling water bath for 5 minutes. Formaldehyde was measured by its reaction with chromotropic acid (4). 3-Formylpyruvic acid was determined by the colorimetric test for 2-keto-5-deoxy sugar acids described by Weisbach and Hurwitz except that the time for oxidation by periodic acid was not permitted to exceed 5 minutes. Longer oxidative periods resulted in low yields.

The intermediate, L-2-keto-4,5-dihydroxyvaleric acid, was estimated enzymatically by measuring DPN reduction. The intermediate, 0.5 μmole or less, in 0.1 ml. was mixed with 0.05 ml. of crude enzyme extract, 0.1 ml. of 0.01 M DPN, 0.1 ml. of 0.02 M EDTA* (or 0.005 M Na₃S₂O₃), and 2.65 ml. of 0.1 M Tris buffer, pH 8.0. Reduction of DPN was determined spectrophotometrically at 340 μm. Mg**+** stimulated the rate at which DPN was reduced by L-arabonate but had no effect on the rate when the intermediate was the substrate. Thus, interference by L-arabonate in the assay was minimized by the omission of Mg**++.**

For certain purposes, 2-keto-4,5-dihydroxyvaleric acid was qualitatively detected by mixing 1 drop of a solution with 2 drops of a saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl. After 5 minutes the solution was made alkaline. The appearance of a reddish-brown color indicated the presence of the compound. α-Ketoglutaric acid was measured by the method of Friedemann and Haugen (5). 2-Keto-4,5-dihydroxyvaleric acid, when present in equimolar amounts with α-ketoglutaric acid, did not cause more than a 10 per cent error in the measurement.

For paper chromatographic procedures, the solvents used were: Solvent 1, ether:benzene:formic acid:water (70:30:14:10); Solvent 2, sec-butanol (washed with FeSO₄ to remove peroxides and then with water):formic acid (95:5); Solvent 3, n-propanol:formic acid:water (6:3:1); Solvent 4, methyl cellosolve:water:concentrated NH₄OH (80:15:5); Solvent 5, n-butanol:pyridine:water (6:4:3). Acids were detected by spraying with bromothymol blue (6); α-keto acids were detected with a semicarbazide spray (7); and lactones were located with a reagent described by Abdel-Akher and Smith (8). The 2,4-dinitrophenylhydrazones were prepared for chromatography as described by Cavallini (9). The ultraquantumaline derivatives were prepared as described by Heckenhull and Foodgate (10).

L-Arabonic acid was prepared by the hypoiodite oxidation of n-arabinose.

1 A. Weissbach and J. Hurwitz, personal communication.

2 The abbreviations used are: EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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L-arabinose (11). 2,4,5-Trihydroxyvaleric acid, a reduced product of 2-keto-4,5-dihydroxyvaleric acid, was synthesized as described by Nef (12).

The methods of synthesizing 3,4-dihydroxybutyric acid (the expected product of oxidative decarboxylation of 2-keto-4,5-dihydroxyvaleric acid) described by Glattfeld et al. (13) and Glattfeld and Rietz (14) were abandoned because of unsatisfactory yields. Yields approaching 100 per cent were obtained in the following procedure: 25 gm. (0.37 mole) of 3-butenenitrile are dissolved in 250 ml. of 90 per cent formic acid to which are added 45 ml. of 30 per cent H₂O₂ (0.4 mole). The reaction mixture is allowed to stand in a 37° water bath for 24 hours and finally heated on a steam bath for 2 hours to hydrolyze the nitrile completely to the acid. Water and formic acid are removed by distillation under reduced pressure and the residue is redissolved in 100 ml. of water and passed through a Dowex 50 (H⁺) column. The eluate is distilled in a vacuum and the fraction boiling from 155° to 168° at 5 mm. is collected. The yield of lactone is about 35 gm. or 92.5 per cent.

**RESULTS**

**Formation of 2-Keto-4,5-dihydroxyvaleric Acid**

Crude enzyme preparations from *P. saccharophila* reduce DPN with L-arabonate as substrate only after a definite lag period (1). If Na₂S and MgCl₂ are included in the reaction mixture, the time required for the quantitative reduction of DPN is significantly reduced and the lag period is shortened but not entirely abolished (Fig. 1). Other substances combining with ions of heavy metals, such as 2,3-dimercaptopropanol, cysteine, glutathione, and EDTA, can replace sulfide.

It has also been reported that an unstable intermediate is formed by a nonoxidative process when L-arabonate is incubated with crude extract in the absence of DPN (1). The presence of this substance was detected by the immediate and rapid reduction of DPN when DPN was added to the reaction mixture. In preliminary experiments, a method of assay was developed based on the enzymatic reduction of DPN in the presence of the intermediate. Evidence that this was a valid measure of the intermediate was obtained in subsequent experiments with the purified intermediate where the amount of DPN reduced corresponded to the amount of α-ketoglutaric acid produced in the oxidation (Table III). Using this assay, the formation and stability of the intermediate was investigated. The compound apparently is inactivated in some manner in Tris buffer (Table I). In NH₄Cl-NH₄OH buffer, the intermediate is more stable and there is a better correlation between the amount of L-arabonate consumed and the amount of intermediate formed. The optimum pH for formation of the intermediate in the ammonium buffer is 9; however, at higher pH's the compound is unstable in this buffer, also.

The addition of most deproteinizing agents to the reaction mixture resulted in a loss in enzymatic activity of the intermediate. The most satisfactory method of deproteinization was by acidification of the reaction mixture with Dowex 50 (H⁺). The compound may be stored for several months under neutral or acid conditions if kept at −20°. At room temperature there was a slow loss of activity over a period of several days. Alkaline solutions of the intermediate became inactive within a few hours at room temperature. When heated, these alkaline solutions turned a bright reddish-orange color.

The first indication that the intermediate formed from L-arabonate is a keto compound was obtained when it was observed that there was a reaction between the compound and 2,4-dinitrophenylhydrazine. The intermediate also reacted with semicarbazide (15) and α-phenylenediamine (16) to produce addition products which have absorption maxima at 250 μm and 325 μm, respectively. The latter two tests are considered to be specific for α-keto acids.

Paper chromatographic examination indicated that only one intermediate accumulates in the reaction mixture. The reac-
tion mixture, after being treated with Dowex 50 (H\(^+\)), was applied to the chromatogram sheet and developed by ascending chromatography in one of the solvents listed in the section on "Methods." Location of the compound was detected by spraying the sheet with either an acid or an \(\alpha\)-keton acid indicator. All chromatograms have shown the presence of only one compound in the reaction mixture other than the substrate, L-arabonate.

In each of the five solvents the intermediate has an \(R_F\) different from either L-arabonate or \(\alpha\)-ketoglutarate (Table II).

### Isolation of 2-Keto-4,5-dihydroxyvaleric Acid

Isolation of the intermediate was accomplished by chromatography on Dowex 1 columns. A reaction mixture composed of 4.0 ml. of crude enzyme preparation, 2.0 ml. of 0.5 m potassium L-arabonate, 2.0 ml. of 0.02 m EDTA, 1.0 ml. of 0.05 m MgSO\(_4\), and 1.0 ml. of 0.5 M \(\text{NH}_4\text{OH}-(\text{NH}_4)_2\text{SO}_4\) buffer, pH 9.0, was incubated at 30° for 3 hours. The pH of the mixture, which decreased as the reaction proceeded, was readjusted to pH 9 with 1.0 M KOH at frequent intervals. Assay of the incubation mixture by the DPN reduction method indicated that 920 \(\mu\)moles of 2-keto-4,5-dihydroxyvalerate had been formed. The reaction mixture, which contained a flocculent precipitate, was deproteinized by passing it through a column of Dowex 50 (H\(^+\)), and the column was washed with water until the effluent gave a negative test for \(\alpha\)-keto acid (as determined by reaction with 2,4-dinitrophenylhydrazine). The effluent was then passed through a Dowex 1 (formate) column. Next, the column was washed thoroughly with 0.25 m formic acid which removed residual L-arabonate. The intermediate was then eluted from the column with 0.275 m formic acid. Fractions giving a positive \(\alpha\)-keto acid test were pooled and concentrated by lyophilization. The residue was dissolved in water and assayed. At this point recovery of the enzymatically active intermediate was 90 per cent. Solvent could not be removed by distillation under reduced pressure without complete loss of activity.

The lyophilized material was a gummy syrup which showed no tendency to crystallize. It was soluble in water, ethanol, and acetone but insoluble in ether. Various salts were prepared by neutralization and lyophilization. These were obtained as syrups which showed the same solubility properties as the unneutralized intermediate.

Aqueous solutions of the lyophilized intermediate gave a positive ferric chloride test for the enol group. By chemical means to be discussed later, it was found that 80 to 90 per cent of this preparation existed in the lactone form and the remainder as the free acid. At pH 7, the lactone was completely hydrolyzed within 3 hours while only 5 minutes were required at pH 8. Chromatograms of the lactone showed the presence of a single spot characterized by an \(R_F\) which is different from that of L-arabonate-\(\gamma\)-lactone (Table II). Chromatograms of the free acid, obtained by neutralizing and hydrolyzing the lactone and then passing the solution through a Dowex 50 (H\(^+\)) column, also revealed a single spot when sprayed with either an acid or \(\alpha\)-keton acid indicator.

The intermediate was identified chromatographically by comparison with DL-2-keto-4,5-dihydroxyvaleric acid which has been chemically synthesized. The \(R_F\) values of the free acid and lactone form of the intermediate are identical with those of the corresponding form of the synthetic compound. In addition, both the intermediate and synthetic DL-2-keto-4,5-dihydroxyvaleric acid will be presented in a subsequent report.

### Table II

Comparison of \(R_F\) values of synthetic compounds with enzymatically produced intermediate and its derivatives.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arabonic acid</td>
<td>0.00</td>
<td>0.33</td>
<td>0.34</td>
<td>0.56</td>
<td>0.16</td>
</tr>
<tr>
<td>L-Arabono-(\gamma)-lactone</td>
<td>0.30</td>
<td>0.34</td>
<td>1.00</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>(\alpha)-Ketoglutaric acid</td>
<td>0.40</td>
<td>0.71</td>
<td>0.86</td>
<td>0.50</td>
<td>0.21</td>
</tr>
<tr>
<td>2-Keto-4,5-dihydroxyvaleric acid</td>
<td>0.15</td>
<td>0.59</td>
<td>0.64</td>
<td>0.74</td>
<td>0.29</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.16</td>
<td>0.59</td>
<td>0.63</td>
<td>0.77</td>
<td>0.29</td>
</tr>
<tr>
<td>Lactone of 2-keto-4,5-dihydroxyvaleric acid</td>
<td>0.71</td>
<td>0.75</td>
<td>1.00</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Lactone of intermediate</td>
<td>0.71</td>
<td>0.75</td>
<td>1.00</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>3,4-Dihydroxybutyric acid</td>
<td>0.11</td>
<td>0.64</td>
<td>0.62</td>
<td>0.64</td>
<td>0.34</td>
</tr>
<tr>
<td>Oxidized intermediate</td>
<td>0.11</td>
<td>0.64</td>
<td>0.60</td>
<td>0.60</td>
<td>0.30</td>
</tr>
<tr>
<td>Lactone of 3,4-dihydroxybutyric acid</td>
<td>0.77</td>
<td>0.88</td>
<td>1.00</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Lactone of oxidized intermediate</td>
<td>0.78</td>
<td>0.83</td>
<td>1.00</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>2,4,5-Trihydroxyvaleric acid</td>
<td>0.03</td>
<td>0.54</td>
<td>0.32</td>
<td>0.68</td>
<td>0.21</td>
</tr>
<tr>
<td>Reduced intermediate</td>
<td>0.03</td>
<td>0.57</td>
<td>0.30</td>
<td>0.66</td>
<td>0.19</td>
</tr>
<tr>
<td>Lactone of 2,4,5-trihydroxyvaleric acid</td>
<td>0.61</td>
<td>0.65</td>
<td>1.00</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Lactone of reduced intermediate</td>
<td>0.67</td>
<td>0.65</td>
<td>1.00</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

* See "Methods" for composition of the solvents.
† Lactones were prepared by heating a 0.1 m solution of the compound to 0.1 N HCl for 5 minutes in a boiling water bath.
‡ Prepared by treating the intermediate with \(\text{H}_2\text{O}_2\).
§ Prepared by treating the intermediate with sodium borohydride.

### Table III

Comparison of various methods of assay for DL-2-keto-4,5-dihydroxyvaleric acid and enzymatically produced intermediate in aqueous solution.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration of substrate* as determined by:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPN reduction†</td>
<td>(\alpha)-Ketoglu- Decarboxyl- taric acid tion with determina- hydrogen tion: peroxides</td>
<td>(\mu)moles/ml</td>
<td>(\mu)moles/ml</td>
<td>(\mu)moles/ml</td>
<td>(\mu)moles/ml</td>
<td>(\mu)moles/ml</td>
</tr>
<tr>
<td>DL-2-Keto-4,5-dihydroxyvaleric acid</td>
<td>18.7</td>
<td>19.9</td>
<td>36.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>55.5</td>
<td>56.6</td>
<td>55.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Lyophilized preparations of the substrates were dissolved in arbitrary amounts of water and assayed by the methods listed.
† Dilutions of the aqueous solution were made so that a 0.1-ml aliquot of the diluted sample, when added to a cuvette containing the assay mixture, would result in the reduction of 0.5 \(\mu\)mole or less of DPN.
‡ Reaction mixtures, used to measure DPN reduction, were pooled, and 2,4-dinitrophenylhydrazine added. The derivative was extracted and measured colorimetrically.
§ A volume of solution containing 2 to 5 \(\mu\)moles of substrate (as determined by DPN reduction) was treated with \(\text{H}_2\text{O}_2\) and \(\text{CO}_2\) production measured as described in text.

valeric acid are enzymatically oxidized to \(\alpha\)-ketoglutaric acid. While the intermediate is quantitatively oxidized in a reaction mixture of crude enzyme, DPN, EDTA, and Tris buffer, pH 8.0, 50 per cent of the synthetic keto acid is transformed which is the expected result for the DL-compound (Table III).
Attempts to isolate the intermediate from deproteinized reaction mixtures as the calcium salt following the procedure described by Palleroni and Doudoroff (17) for the precipitation of the calcium salt of n-2-keto-4,5-dihydroxyvaleric acid were unsuccessful.

**Chemical Properties**

**Oxidation by Cerio Sulfate and Hydrogen Peroxide**—Cerio sulfate is an oxidizing agent that is usually specific for α-keto acids if the reaction is measured by CO₂ production. However, it could not be used for the decarboxylation of 2-keto-4,5-dihydroxyvaleric acid since it caused extensive degradation of the compound resulting in the production of large amounts of CO₂.

Hydrogen peroxide will also decarboxylate α-keto acids, and has the advantage over ceric sulfate that strongly acidic conditions are not required. It was found that hydrogen peroxide produced exactly 1 mole of CO₂ per mole of intermediate under the following conditions: 5 μmoles of the intermediate were placed in a Warburg flask with two side arms. An excess (0.2 ml.) of 0.1 n KOH was added and the volume was brought to 1.0 ml. with water. In one side arm was placed 0.1 ml. of 0.5 N acetic acid and in the other 0.1 ml. of 3 per cent H₂O₂. After temperature equilibration, the acetic acid was tipped into the flask. After another 10 minutes, peroxide was added. Decarboxylation was usually complete within 5 to 10 minutes.

Since peroxide will not react with the lactone, it is necessary to hydrolyze the lactone ring by the addition of excess base. Acetic acid was then used to acidify the reaction mixture since acetate buffer is most efficient at pH 4. At this pH, (a) the intermediate does not lactonize, (b) the solubility of CO₂ is negligible, and (c) hydrogen peroxide does not spontaneously decompose to oxygen and water.

In another experiment, 100 μmoles of the intermediate were decarboxylated with hydrogen peroxide. When the oxidation was complete, the reaction mixture was passed through a Dowex 1 (formate) column. The column was washed with water and the decarboxylated compound then eluted with 0.1 N HCOOH. It was detected in the eluate by the lactone test. However, the compound did not react with 2,4-dinitrophenylhydrazine. Fractions giving a positive lactone test were pooled and lyophilized. The residue was dissolved in water, a known excess of NaOH added, and the solution back-titrated to the phenolphthalein end point. The lyophilized eluate contained 92 μeq. of acid. Chromatography of the eluted compound on paper as the free acid and as the lactone revealed the presence of a single compound possessing an Rₚ indistinguishable from that of the corresponding forms of 3,4-dihydroxybutyric acid (Table I). It was also found that both 3,4-dihydroxybutyrate and the compound formed by the decarboxylation of the intermediate could be oxidized by periodate with the production of 1 μmole of formaldehyde per μmole of substrate.

These results demonstrate that the intermediate is oxidized by hydrogen peroxide to CO₂ and 3,4-dihydroxybutyric acid. Comparable results were obtained with a known sample of DL-2-keto-4,5-dihydroxyvaleric acid.

This reaction furnishes a convenient method for measuring 2-keto-4,5-dihydroxyvaleric acid. By omitting the hydrolysis step in the assay described above, it is possible to measure the amount of free acid in the presence of the lactone.

**Periodic Acid Oxidation**—The course of oxidation of 2-keto-4,5-dihydroxyvaleric acid, of either enzymatic or chemical origin, by periodate was followed by assaying for formaldehyde and β-formylpyruvate. Since periodate will not oxidize the lactone form of the intermediate, the lactone was hydrolyzed with base and then the required amount of periodate was added along with enough H₂SO₄ to give a final concentration of 0.1 N acid. A μmole of formaldehyde was produced per μmole of substrate within 5 minutes, and the amount did not significantly change in the next 120 minutes (Table IV).

The maximal quantity of β-formylpyruvate was obtained 5 minutes after mixing the intermediate with periodate (Table IV). Its concentration then decreased until it had almost disappeared at the end of 60 minutes. The spectrum of the color produced had an absorption maximum at 550 μm. The results are given in optical density units since an authentic sample of formylpyruvate was not available for molar absorption determinations.

The disappearance of formylpyruvate very likely was due to its decarboxylation by periodate. In a separate experiment in which the oxidation was performed in a Warburg respirometer at 30°, it was found that 1 μmole of CO₂ was produced in 60 minutes from 1 μmole of 2-keto-4,5-dihydroxyvaleric acid. No steam volatile acids could be detected in the experiment described in Table IV or in similar experiments performed on a larger scale.

The amount of periodate consumed could not be quantitatively measured by iodine titration because the oxidized products reacted slowly with the iodine resulting in false and fading end points. Also, it was not possible to measure periodate reduction spectrophotometrically since the products of the reaction had larger extinction coefficients than the periodate.

These results are consistent with the identification of the intermediate of L-arabinose metabolism by P. saccharophila as 2-keto-4,5-dihydroxyvaleric acid. The fact that the compound gives a positive reaction with 2-thiobarbituric acid after being oxidized by periodate establishes it as a 2-keto-3-deoxy sugar acid. The simultaneous formation of formaldehyde and β-formylpyruvate without the concomitant production of formic acid from this oxidation can be obtained with only one 2-keto-3-deoxy sugar acid, 2-keto-4,5-dihydroxyvaleric acid.
Lactone contained a carbonyl group adjacent to the carboxyl, it
enediamine — Although it was demonstrated that the intermedi-
as the acid or as the lactone, the reduced intermediate possessed
be dissolved in ethyl acetate and recrystallized from ethanol.
leroni and Doudoroff (17) were able to obtain a solid 2,4-dinitro-
tives by extraction from acid solution into ethyl acetate followed
mixtures were made alkaline. However, the derivatives did not
4-nitro-o-phenylenediamine reacted with the intermediate as
could be shown by the intense color produced when the reaction
was not possible to obtain a solid 2,4-dinitrophenylhydrazone of the n-isomer of the a-keto acid which could be
dissolved in ethyl acetate and recrystallized from ethanol.
Reduction with Sodium Borohydride — An aqueous solution, 5
ml. of 0.04 M intermediate was neutralized with NaOH until the
pH remained at 8 and 100 mg. of solid sodium borohydride were
added. Reduction was complete within 5 minutes as determined
by the absence of a reaction with 2, 4-dinitrophenylhydrazine.
After 15 minutes the excess borohydride was destroyed by acid-
fying with HCl to approximately pH 1 and then the reaction
mixture was diluted to 10 ml. with water. A volume of 0.1 ml.
was removed and oxidized by periodate; 1.8 μmol of formalde-
yde were produced in the oxidation but no CO₂. Also, no CO₂
was produced when a 1-ml sample was treated with hydrogen
peroxide (with the use of the procedure described for decarboxyl-
ating the intermediate).
To separate the reduced derivative from HCl and borate, it
was converted into the lactone by heating for 5 minutes in a boi-
ing water bath. After cooling to room temperature, the solution
was passed through a Dowex 1 (formate) column. The lactone
is not adsorbed by the resin. The eluate containing the reduced
intermediate was neutralized to pH 8 to 9 in order to hydrolyze
the lactone and the solution was again passed through a Dowex 1
(formate) column. The reduced intermediate, as a salt, is ad-
sorbed by the column. After washing the column with 0.025 M
formic acid, the compound was eluted with 0.03 M formic acid.
The presence of the derivative in fractions of the eluate was de-
tected by the lactone test. Fractions giving a positive test were
pooled, lyophilized, and redissolved in water. The recovery of
lactonizable material was 80 percent. When chromatographed
as the acid or as the lactone, the reduced intermediate possessed
R₇ values corresponding to those of a known sample of 2, 4, 5-
trihydroxyvaleric acid or its lactone (Table II).

Discussion

There are certain similarities in the metabolism of α-glucose
(14, 18), α-galactose (19), and α-arabinose (2, 17) by P. saccharo-
phila. The sequence of events is as follows. The sugar is ox-
idized to the aldonic acid which is then dehydrated to form the
2-keto-3-deoxy sugar acid. This latter compound is cleaved
with the formation of pyruvic acid from the first three carbons.
Details of the mechanism are different for different sugars.
For example, glucose and all of its intermediates are phosphorylated.
On the other hand, a phosphorylated compound does not appear
in the pathway of galactose metabolism until just before the
cleavage step. No phosphorylated compound appears to be
formed in α-arabinose oxidation. With α-arabinose, the pattern
also varies in that an oxidation occurs along with the cleavage
of the 2-keto-3-deoxy sugar acid to form pyruvic and glycolic acids.

The oxidation of α-arabinose in P. saccharophila follows the
same general metabolic scheme, except that α-ketoglutaric acid
is formed rather than pyruvic acid (1). It has now been shown
that L-arabonic acid may be dehydrated to a 2-keto-3-deoxy
sugar acid. The identification of this compound is based on a
comparison of its chemical properties with those of a known sam-
ple of 2-keto-4,5-dihydroxyvaleric acid, and by its reduction by
sodium borohydride to a compound chromatographically identi-
ced as 2, 4, 5-trihydroxyvaleric acid.

The configuration of the hydroxyl group on the C₂ of the in-
termediate has not been determined. Since this carbon does not
appear to be involved in the dehydration step, it probably re-
mains in the L-configuration. This assumption is based on the
stereospecificity of the enzymes involved in the dehydration
of 6-phosphogluconic acid and of galactonic acid, and the subse-
quent cleavage of the dehydrated sugar acids (19). In the me-
tabolism of these sugars, the configuration about the C₂ remains
unchanged in the dehydration step. Comparable results have
been obtained in L-arabinose metabolism. The dehydrase in the
crude enzyme preparation is active on L-arabonate but not on
α-arabonate (1). Also, only 50 percent of α-l-keto 4, 5 dihy-
droxyvaleric acid are oxidized to α-ketoglutaric acid by the en-
zyme system.

A minimum of two steps would be required for the enzymatic
oxidation of 1-2-keto-4, 5-dihydroxyvaleric acid to α-ketoglutaric
acid. It could occur either by a dehydrination between carbons 4
and 5 followed by an oxidation, or the oxidation could be the
first step and followed by a rearrangement. There is no direct
evidence available to indicate which of these two schemes, or
perhaps even another, is operating in P. saccharophila. It ap-
ppears likely, however, that the oxidation is the first step. In
reaction mixtures in which L-arabonate is the substrate but which
lack DPN, no new compounds other than 2-keto-4, 5-dihydroxy-
valeric acid have been detected. Also, there is no lag or induc-
tion period in DPN reduction when 2-keto-4, 5-dihydroxyval-
erate is being oxidized to α-ketoglutarate. An induction period
would be expected if the 2-keto-3-deoxy sugar acid first had to be
converted to another compound for the oxidation to take place.

The only other report on 2-keto-4, 5-dihydroxyvaleric acid is
its biological formation from n-arabinose (2). While there are
a few discrepancies, the properties described for the compound
formed by the dehydration of L-arabonate agree well with those
described for the keto acid formed from n-arabinose. It seems
probable, therefore, that these two compounds are stereoisomers
of each other.

Summary

A new intermediate, 1-2-keto-4, 5-dihydroxyvaleric acid, has
been identified in the oxidation of L-arabinose to α-ketoglutaric
acid by Pseudomonas saccharophila. It is formed by a nonoxi-
dative process from L-arabonate and is isolated by elution from a
Dowex 1 column. The intermediate has been characterized by
chemical degradative procedures and by reducing it to 2, 4, 5-
trihydroxyvaleric acid.

In the presence of diphosphopyridine nucleotide the interme-
diate is quantitatively oxidized to α-ketoglutaric acid at a much
greater rate than is L-arabonate. It is suggested that at least
two steps are required for this conversion, an oxidation being the
first step. The enzyme system seems to exhibit a specificity for the L-configuration since in this system only 50 per cent of the DL-2-keto-4,5-dihydroxyvaleric acid are oxidized, and d-arabonate not at all.

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I-2-Keto-4,5-dihydroxyvaleric Acid: an Intermediate in the Oxidation of I-Arabinose by *Pseudomonas saccharophila*
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