THE CONVERSION OF GLUCOSE-6-C\textsuperscript{14} TO ASCORBIC ACID
BY THE ALBINO RAT*

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The conversion of glucose-1-C\textsuperscript{14} to ascorbic acid-6-C\textsuperscript{14} and uniformly labeled glucose-C\textsuperscript{14} to uniformly labeled ascorbic acid-C\textsuperscript{14} in the Chloretone-stimulated albino rat has been demonstrated (1, 2). To provide further evidence for a direct conversion of glucose to ascorbic acid and to eliminate pathways similar to that suggested by Bidder (3) for glucuronic acid formation, glucose 6 C\textsuperscript{14} has been used in a similar experiment, and a total degradation of the ascorbic acid thus formed has been carried out.

EXPERIMENTAL

Glucose-6-C\textsuperscript{14}—We are indebted to Dr. John Sowden of Washington University for the sample of glucose-6-C\textsuperscript{14} used. Its activity was found by combustion to be $2.55 \pm 0.02 \times 10^5$ c.p.m. per mg. on our counter\textsuperscript{i} (0.5 µc. per mg. absolute). To test radioactive purity a small amount was diluted with inactive glucose and recrystallized from acetic acid-water. Its calculated activity was $2.56 \pm 0.01 \times 10^5$ c.p.m. per mg.

Isolation of Biosynthetic Ascorbic Acid—Two male albino rats of the Wistar strain were treated with Chloretone for at least 1 week and then injected intraperitoneally with 28.3 and 28.7 mg., respectively, of glucose-6-C\textsuperscript{14} in two doses, each given in 1 ml. of water, on two successive mornings following Chloretone treatment. Ascorbic acid was isolated from urine as described previously (1, 2, 4) and part of it was converted to the osazone.

Degradation of Ascorbic Acid—Carbon 1 was obtained by decarboxylation, carbons 1 and 2 as oxalate, and carbon 6 as dimedon-formaldehyde after periodate cleavage of the osazone as described previously.

It was found that the osazone of ascorbic acid, when dissolved in 0.1 N NaOH, consumed 2 moles of periodate per mole, owing presumably to the opening of the lactone ring in alkali (5) and formic acid formation. The latter, representing carbon 5, was separated as follows from the above reaction mixture.

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\textsuperscript{i} Nuclear Instrument and Chemical Corporation, scaling unit model 103.
After removal of the insoluble nitrogenous material by acidification and filtration and precipitation of formaldehyde as its dimedon derivative, the yellow filtrate was shaken with 1 to 2 gm. of absorbent charcoal for several minutes and filtered. The clear filtrate was acidified with 2 ml. of H₂SO₄ and distilled until the volume had decreased to 25 ml. It was then steam-distilled until the total distillate was 350 to 400 ml. The distillate was treated with 6 gm. of sodium acetate trihydrate and 10 ml. of acetic acid (6) and refluxed under an N₂ sweep to remove any dissolved CO₂ without removing formic acid. 10 gm. of HgCl₂ were added and the mixture was again gently refluxed under an N₂ sweep, the CO₂ being collected in Ba(OH)₂. The BaCO₃ was counted directly.

Since the cleavage was performed in 0.1 N NaOH under conditions in which formaldehyde may be polymerized to aldoses, etc. (7, 8), cleavable by periodate to formate, it was suspected that carbon 5 would be contaminated by radioactivity present in carbon 6. To determine the extent of this contamination, diluted glucose-6-C¹⁴ was cleaved under the same conditions, followed by isolation of both the formaldehyde and formic acid. Total counts in dimedon-formaldehyde, 37,200; in BaCO₃, 562. Contamination therefore occurred to the extent of only 1.5 per cent.

Cleavage of ascorbic acid with NaOI is said to yield oxalic and threonic acids quantitatively (9). This contention is supported by the fact that no more than 4 m.eq. of iodine was consumed after standing 1 hour. The iodine absorbed per mM of ascorbic acid after 5, 10, 23, and 65 minutes, was 3.94, 3.95, 3.96, and 3.98 m.eq., respectively.

The filtrate remaining after oxalate removal, after purification, was treated with periodic acid. Under various conditions of pH, concentration, and temperature, it was found that no glyoxylic acid could be isolated as expected for an α-hydroxy acid (10, 11). Instead, CO₂ was formed.

\[
\begin{align*}
\text{COOH} & \xrightarrow{\text{HCOH}} 1\text{CO}_2 \\
\text{HCOH} & \xrightarrow{\text{HIO}_4} 2\text{HCOOH} + \\
\text{HCOH} & \xrightarrow{0^\circ} + 1\text{H}_2\text{CO} \\
\text{CH}_2\text{OH} &
\end{align*}
\]

At room temperature the yield of CO₂ was over 100 per cent of theoretical and that of formic acid was correspondingly low, but no such difficulty was encountered at 0°. The final procedure used was as follows.

50 mg. of ascorbic acid were dissolved in 0.1 N acetic acid and rapidly treated with 15 ml. of 0.1 N iodine in 0.2 N KI solution, followed by 5 ml. of N NaOH. After 5 minutes the solution was brought to pH 5 by adding 0.5 ml. of acetic acid. 1 ml. of M calcium acetate was added to precipitate
calcium oxalate, which was separated by centrifuging. The iodine-colored filtrate was decolorized exactly with 0.1 N sodium arsenite solution (2.6 ml.). Silver nitrate (800 mg.) was added with shaking to precipitate AgI. Precipitation was complete when a drop of AgNO₃ solution produced a brown silver arsenate precipitate. Lead acetate (100 mg. of trihydrate) was added to remove arsenate (pH below 6.5, to avoid coprecipitation of lead hydroxide and threonate). The combined precipitates were removed by gravity filtration and the filtrate was treated with H₂S to remove excess lead. The clear supernatant was divided into two equal parts on which duplicate cleavages were run. The solution was brought to pH 3 to 4 with acetic acid and then boiled under an N₂ sweep to remove H₂S and CO₂. It was then cooled to 0° in an ice bath and treated with 200 mg. (over 100

**Table I**

*Activity in Each Carbon of Ascorbic Acid after Injection of Glucose-C¹⁴*

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>Glucose-1-C¹⁴</th>
<th>Glucose-6-C¹⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>1</td>
<td>11.0</td>
<td>59.5</td>
</tr>
<tr>
<td>2</td>
<td>5.4*</td>
<td>5.5*</td>
</tr>
<tr>
<td>3</td>
<td>6.8</td>
<td>6.4</td>
</tr>
<tr>
<td>4</td>
<td>9.9*</td>
<td>7.0*</td>
</tr>
<tr>
<td>5</td>
<td>8.6</td>
<td>5.3</td>
</tr>
<tr>
<td>6</td>
<td>55.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Total</td>
<td>97.5</td>
<td>98.9</td>
</tr>
</tbody>
</table>

* These values were obtained by difference, subtraction of the value for a single carbon from that of a product representing 2 carbons.

per cent excess) of paraperiodic acid, H₅IO₅, in water. The reaction mixture was swept with N₂, the CO₂ being collected in Ba(OH)₂ solution. After 1 hour, excess arsenious oxide suspended in acid was added to convert the remaining periodate to iodide, and the solution was allowed to warm to room temperature to allow most of the dissolved CO₂ to be swept over by the N₂ in 1 more hour (carbon 3 of ascorbic acid).

The remaining liquid was filtered if necessary, neutralized, and treated with dimeron solution to precipitate the formaldehyde, representing carbon 6.

The filtrate was then acidified and steam-distilled exactly as described above to isolate the formic acid, which was again converted to CO₂ and BaCO₃ for counting purposes (carbons 4 and 5). The yields obtained in a trial run with 49.7 mg. of ascorbic acid in this procedure were as follows: carbon 3, BaCO₃, 19.9 mg., 72 per cent; carbons 4 and 5, BaCO₃, 46.7 mg., 84 per cent; carbon 6, dimeron-formaldehyde, 34.7 mg., 84 per cent.
RESULTS AND DISCUSSION

The two animals converted glucose-6-C\textsuperscript{14} to ascorbic acid in radioactive yields of 0.47 and 0.53 per cent. For comparison the conversion yields from glucose-1-C\textsuperscript{14} were 0.36, 0.79, and 0.51 per cent of the injected dose.

The results of cleavages of ascorbic acid isolated from two animals are given in Table I, along with data obtained for an animal given glucose-1-C\textsuperscript{14}. The results show that glucose-6-C\textsuperscript{14} is converted to ascorbic acid-1-C\textsuperscript{14}, just as glucose-1-C\textsuperscript{14} was converted to ascorbic acid-6-C\textsuperscript{14}. The distribution of activity among the less labeled carbons is similar in both cases and explainable as before on the basis of a partial redistribution of C\textsuperscript{14} among the carbons of glucose before ascorbic acid formation. The similarity of the radioactive yields lends further strong support to the view that the 6th carbon of glucose enters into ascorbic acid via the same molecular pathway that is followed by carbon 1. The conversion is direct, with maintenance of the steric configuration around carbons 2 and 3 of glucose (carbons 5 and 4, respectively, of ascorbic acid).

SUMMARY

1. A method is presented for the stepwise degradation of ascorbic acid.
2. It is shown that glucose-6-C\textsuperscript{14} is converted by the Chloretone-stimulated albino rat to ascorbic acid labeled chiefly in the 1 position. The radioactive yields were the same as those found for glucose-1-C\textsuperscript{14}. Thus further evidence is furnished for direct conversion of \textit{d}-glucose to \textit{l}-ascorbic acid.

BIBLIOGRAPHY

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