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# OBSERVATIONS ON THE INCORPORATION OF $C^{14}$ INTO TARTARIC ACID AND THE LABELING PATTERN OF D-GLUCOSE FROM AN EXCISED GRAPE LEAF ADMINISTERED L-ASCORBIC ACID-6-$C^{14}$ [1,2]

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Vickery and Palmer (12) have speculated on the possible precursor relationship of D-glucose to (+)-tartaric acid in plants. C-2 and C-3 of D-glucose have the same configuration as C-2 and C-3 of (+)-tartaric acid. They proposed a metabolic path in which C-5 and C-6 were lost, presumably via an intermediate such as 5-keto-gluconic acid.

Hough and Jones (9) have stated that hexuronic acids, L-ascorbic acid, and (+)-tartaric acid commonly occur together in nature. Conceivably, L-ascorbic acid might be the precursor of (+)-tartaric acid through loss of C-1 and C-2 of the former and subsequent oxidations of the terminal carbons of the four carbon fragment to carboxyl groups. C-4 and C-5 of L-ascorbic acid have the same configuration as (+)-tartaric acid.

We have attempted to test the latter possibility. L-Ascorbic acid-6-$C^{14}$ [4] (3 mg containing 4.7 $\mu$c of $C^{14}$) was fed through the cut stem to a single grape leaf (Mission variety, second leaf from the tip of an actively growing vine) in 0.3 ml of distilled water. The leaf was illuminated by a pair of 10-watt daylight fluorescent bulbs at a distance of 12 cm. Practically all of the radioactive solution was taken up in five hours. After 8 hours, the leaf was placed in a closed 350-ml container in the dark for an additional 17 hours. Finally, the accumulated respiratory $CO_2$ was aspirated into a gas trap of $N$ NaOH. Approximately 5 % of the administered label was lost as $CO_2$ during the dark period.

The soluble constituents of the leaf were separated and recovered as described in another paper (11). Most of the activity remained in the particle-free extract. Very little activity was removed during passage through a cationic exchange resin (Dowex 50, $H^+$). About one half of the activity remained on the anionic exchange column (Dowex 1, formate). Most of this activity was eluted with a 3 $N$ formic acid gradient (10) in four peaks. The 1st peak coincided with the first traces of acid through the column and might be due to inadequate washing of the column after loading. The 2nd, a very narrow sharp peak, came in the region characteristic of ascorbic acid. Two peaks of lesser activity followed, the last corresponding to malic acid on a paper chromatogram (10). The tartaric acid peak, located by its acid titration curve and by its ammonium metavanadate reaction, had such low activity as to be undetectable by the solid sample counting procedure employed. After three recrystallizations, the potassium acid tartrate from this peak had a specific activity of 24 cpm per mg of carbon (gas-phase counter, 80 % efficient). This amount of $C^{14}$ was too low to be of any significance as concerns the possible metabolic conversion of ascorbic acid to tartaric acid in the grape leaf. The fact that some activity did enter the tartrate molecule from C-6 of L-ascorbic acid suggested that this label was derived, indirectly perhaps, from the sugar pool of the leaf.

In order to explore the latter possibility, the neutral effluent of the Dowex 1 column was concentrated in vacuo to a thick sirup and chromatographed on paper using a solvent composed of ethyl acetate, pyridine, and water (8 : 2 : 1) (13). About 50 % of the $C^{14}$ remained very close to the origin after development. Another 30 % was found in the sucrose. About 5 % each was found in the glucose, fructose, and xylose bands. The glucose region, which was free of other reducing sugars and showed a single darkened area in the corresponding radioautogram, was cut out and eluted. About 0.7 mg of glucose was recovered. It had a specific activity of 100,000 cpm per mg of carbon as determined by wet combustion of an aliquot after dilution with unlabeled D-glucose.

[1] Received December 9, 1957.

[2] Work done at the Western Regional Research Laboratory, Albany, California.

[3] Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[4] Kindly supplied by Dr. H. S. Isbell, National Bureau of Standards, Washington, D. C.

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN THE D-GLUCOSE RECOVERED FROM A GRAPE LEAF LABELED WITH L-ASCORBIC ACID-6-$C^{14}$

| CARBON ATOM OF GLUCOSE | PERCENT OF TOTAL ACTIVITY |
|---|---|
| 1 | 21.5 |
| 2 | 6.0 |
| 3 | 23.0 |
| 4 | 21.0 |
| 5 | 2.5 |
| 6 | 26.0 |

This activity was about one tenth of the specific activity of the original L-ascorbic acid-6-$C^{14}$. The distribution of this activity as determined by degradation with *Leuconostoc mesenteroides* (7) is given in table I. Carbons 1, 3, 4, and 6 were almost equally labeled and together accounted for over 90 % of the total activity of the glucose. A significantly greater amount of label appeared in C-2 than in C-5.

The results indicate that L-ascorbic acid-6-$C^{14}$ was extensively metabolized in the grape leaf and that C-6 readily entered the triose phosphate pool in such a manner that label in the terminal carbons of the triose became equilibrated. It has recently been reported that in the guinea pig (3) ascorbic acid is metabolized by oxidation to diketogulonic acid and subsequent decarboxylation. The L-xylose formed in this reaction could enter into the pentose metabolism of the animal via a conversion to D-xylulose (8) which could then be metabolized by the pentose phosphate pathway (2) to hexose. If such processes of conversion exist in the plant, then it would be quite easy to visualize a pathway leading to the observed labeling pattern. Label from L-ascorbic acid-6-$C^{14}$ would first be converted to L-pentose-5-$C^{14}$, hence to D-pentose-1-$C^{14}$ (an inversion of the carbon chain would occur with the intermediate polyol formation), and then by pentose phosphate metabolism to hexose phosphate-1,3-$C^{14}$ (1, 6). The latter, upon equilibration with the triose phosphate metabolism of the leaf would give rise to the observed D-glucose labeled primarily in carbons 1, 3, 4, and 6 (5). The slightly greater amount of label in C-2 than in C-5 would be a consequence of pentose phosphate metabolism (1, 4).

The facile incorporation of label from L-ascorbic acid-6-$C^{14}$ into the carbohydrate metabolism of the grape leaf probably accounts for the small amount of label observed in (+)-tartaric acid (11). There does not appear to be a direct pathway for the formation of (+)-tartaric acid from L-ascorbic acid in the grape leaf.

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