Biosynthesis of Uridine Diphosphate d-Xylose

IV. MECHANISM OF ACTION OF URIDINE DIPHOSPHOGLUCURONATE CARBOXY-LYASE*

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SUMMARY

UDP-d-glucuronic acid labeled with tritium at either C-3, C-4, or C-5 was converted to UDP-d-xylose by UDP-glucuronate carboxy-lyase (EC 4.1.1.35) from wheat germ and Cryptococcus laurentii. A kinetic isotope effect \( (V_7/V_9) \) of 0.32 and 0.42 for the C. laurentii and wheat germ enzymes, respectively, was observed with the C-4-labeled substrate but not with the C-3- or C-5-labeled substrate. Labeled UDP-d-xylose obtained from UDP-d-glucuronic acid-5T was converted with phosphodiesterase to labeled \( \alpha \)-d-xylosyl phosphate. The latter was oxidized with periodic acid to yield \( \alpha \)-d-phosphoglucosylglycine derived from C-1 and C-2 and from C-4 and C-5; hypobromite oxidation of the dialdehyde yielded \( \alpha \)-d-glucuronic acid in which the intermediate, UDP-4-keto-GlcUA', would be a \( \alpha \)-keto acid. Acid hydrolysis of this compound released unlabeled glycolic acid (which originated from C-1 and C-2) and labeled glycolic acid (from C-4 and C-5). The latter was converted to unlabeled glycolic acid upon oxidation with glycolic oxidase from spinach, which is specific for that hydrogen atom in glycolic acid sterically equivalent to the \( \alpha \)-hydrogen of L-lactic acid. These results show that the configuration at C-5 changed from S to R during the decarboxylation of UDP-d-glucuronic acid. Since UDP-glucuronic acid carboxy-lyase requires NAD for activity, these data suggest that the reaction involves oxidation at C-4 to yield UDP-4-keto-d-glucuronic acid, followed by decarboxylation and formation of UDP-4-keto-d-xylose, which is then stereo-specifically protonated at C-5 and reduced at C-4 to yield product. The presence of an isotope effect with UDP-d-glucuronic acid-4T means that either the initial oxidation or the final reduction is the rate-limiting step of the reaction; it is not possible to differentiate between these two possibilities on the basis of the available data.

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The abbreviations used are: UDP-4-keto-GluA, uridine 5'-\( (\alpha \)-d-xyloxyranosyluronic acid pyrophosphate); UDP-GlcUA, uridine 5'-\( (\alpha \)-d-glucopyranosyluronic acid pyrophosphate); UDP-Xyl, uridine 5'-\( (\alpha \)-d-xylopyranosylpyrophosphate); UDP-4-keto-Xyl, uridine 5'-\( (\alpha \)-d-three-pentopyranosyl-4-ulosyl pyrophosphate). UDP-Glc4T, glucose-6-P4T, glucose-3T, and glucose-5T refer to tritiated compounds labeled specifically at C-3, C-4, and C-5, respectively, of the hexose. UDP-GluUA3T, UDP-GluUA4T, and UDP-GluUA5T refer to tritiated compounds labeled specifically at C-3, C-4, or C-5 of the \( \alpha \)-xylose moiety. UDP-Xyl-3T, UDP-Xyl-4T, UDP-Xyl-5T, and xylose-4T refer to tritiated compounds containing label originally derived from UDP-GluUA5T. UDP-Xyl-5T and glycolic acid T \(_{\text{nuc}} \) refer to tritiated compounds containing label originally derived from the medium (tritiated water).
**EXPERIMENTAL PROCEDURE**

**Materials**

UDP-glucuronate carboxy-lyase was purified from wheat germ and from *C. laurentii* according to the procedures of Ankell and Feingold (3, 4). The specific activities of the wheat germ and the *C. laurentii* enzymes were 0.34 and 0.18 unit per mg, respectively; where 1 unit is defined as the amount of enzyme which catalyzes the formation of 1 μmole of UDP-Xyl per min at 37°.

Glucose-3T and glucose-5T were gifts from Dr. J. Katz, Institute for Medical Research, Cedars-Sinai Hospital, Los Angeles, California. Glucose-4T was a gift from Dr. O. Gabriel, Department of Biochemistry, Georgetown University School of Medicine and Dentistry, Washington, D.C. UDP-GlcUA uniformly labeled with 14C (125 mCi per mmole) and tritiated water (100 mCi per μg) were purchased from New England Nuclear. Snake venom phosphodiesterase (EC 3.1.4.1) and crystalline catalase (EC 1.11.1.6) were purchased from Worthington, and purine bovine liver UDP-glucose dehydrogenase (EC 1.11.1.22) was prepared in our laboratory (8). Glycylate oxidase (EC 1.1.1.31) was partially purified from spinach according to the procedure of Zelitch and Ochoa (9). All other chemicals used in these experiments were of commercial reagent grade quality.

**Methods**

**Chromatography and Electrophoresis**—Ascending paper chromatography was carried out on Whatman No. 1 or 3MM filter paper in the following solvent systems (v/v): A, 1 M ammonium acetate (pH 7.3)-95% ethanol (3:7); B, 1-propanol-ethyl acetate-water (7:1:2); C, ethyl acetate-acetic acid-water (2:1:1); D, diethyl ether-acetic acid-water (13:3:1).

Electrophoresis was carried out on Whatman No. 1 or 3MM filter paper in the solvent-cooled Gilson Medical Electronics model D high voltage electrophonstor. The buffer systems employed were 0.1 M ammonium formate, pH 3.6, or 0.1 M ammonium acetate, pH 6.8 (10). Compounds were detected on paper with the use of the following spray reagents: p-anisidine phosphate (10) or ammoniacal silver nitrate (11) for free sugars; molybdate acid (12) for organic phosphates; and a saturated aqueous solution of ammonium vanadate or the potassium ferrocyanide reagent of Stern et al. (13) for organic acids. Nucleotides and nucleotide sugars were detected by visual inspection under short wave (254 μm) ultraviolet light. Radioactive compounds were located with the use of a Tracerlab 4-π paper strip scanner or by autoradiography with Eastman Kodak No-screen or Blue Brand x-ray film.

**Analytical Procedures**—Xylose was determined by a modification of the methods of Roe and Rice (14) as previously described (15), glycolic acid with 2,7-dihydroxyphenylalanine in concentrated sulfuric acid (16), and glyoxalic acid with phenylhydrxyl hydrochloride by the procedure of McFadden and Howes (16), except that the total volume was reduced to 2.4 ml. Acid-labile organic phosphate (released by hydrolysis in 0.01 M tritiated sulfuric acid (16), and glyoxalic acid with phenylhydrazine (17)) were located with the use of a Tracerlab 4-π paper strip scanner. Radioactivity measurements were carried out in a Packard Tri-Carb model 3375 scintillation spectrometer in Solvent A. Those areas which cochromatographed with authentic unlabeled UDP-Xyl or UDP-GlcUA were excised and counted in the toluene base scintillation mixture. The H:14C ratio was determined both in the product and the remaining substrate during the course of the reaction. This was done to determine the rate of decarboxylation of 14C-labeled and 3H substrate independently and simultaneously in the same reaction mixture. Reaction rates were expressed as percentage of conversion of UDP-GlcUA to UDP-Xyl (UDP-Xyl uniformly labeled with 14C counts were corrected for loss of 14C02).

**Preparation of Specifically Tritiated UDP-GlcUA—**Specifically tritiated g-glucose was converted to UDP-Glc with an enzyme preparation from Bakers' yeast according to the procedure of Wright and Robbins (19). UDP-GlcUA-3T, UDP-GlcUA-4T, and UDP-GlcUA-5T were prepared from the correspondingly tritiated UDP-Glc with UDP-Glc dehydrogenase in the presence of a large excess of NAD in 0.1 M Tris-HCl buffer, pH 8.0. Tritiated UDP-GlcUA was purified by chromatography in Solvent A, followed by high voltage electrophoresis at pH 5.8.

**Enzyme Assays—**UDP-GlcUA carboxy-lyase was routinely assayed during purification as previously described (4); however, in order to measure the effect of isotope on the rate of decarboxylation, a different type of assay was used. Ninety microliters of 0.1 M sodium-potassium phosphate buffer, pH 7.0, containing 0.5 g of EDTA per liter (Buffer A) and tritiated UDP-GlcUA and UDP-GlcUA uniformly labeled with 14C at a final concentration of 1 to 5 × 104 m was incubated at 30°. Reaction mixtures for the *C. laurentii* enzyme contained, in addition, NAD at a final concentration of 1 mM. Either before or immediately after the reaction was started by addition of 20 μl of suitably diluted enzyme, 10 μl of the reaction mixture were sampled. At appropriate time intervals, 10-μl portions of the reaction mixture were removed in capillary tubes which then were sealed and placed into boiling water for 1 min. The contents of the capillaries were spotted on filter paper and the reaction product was separated from remaining substrate by chromatography in Solvent A. Those areas which cochromatographed with authentic unlabeled UDP-Xyl or UDP-GlcUA were excised and counted in the toluene base scintillation mixture. The H:14C ratio was determined both in the product and the remaining substrate during the course of the reaction. This was done to determine the rate of decarboxylation of 14C-labeled and 2H substrate independently and simultaneously in the same reaction mixture.

**Preparation of Tritiated UDP-Xyl—**UDP-Xyl-3T, UDP-Xyl-4T, and UDP-Xyl-5T were prepared from the correspondingly tritiated UDP-GlcUA with UDP glucuronate carboxy-lyase prepared from wheat germ and from *C. laurentii*. Reaction mixtures containing UDP-GlcUA (5 to 10 × 104 cpm) (1 μmole of NAD when the *C. laurentii* enzyme was used) and enzyme (0.1 unit) in 1 ml of Buffer A were incubated for 4 hours. The reaction was stopped by heating at 100° for 5 min. Denatured protein was removed by centrifugation and tritiated UDP-Xyl was isolated by chromatography in Solvent A.

UDP-Xyl-5T was prepared by carrying out the decarboxylation of unlabeled UDP-GlcUA in tritiated water (100 mCi per g) with the wheat germ enzyme. A reaction mixture containing UDP-GlcUA (4 μmoles), in 0.5 ml of Buffer A was lyophilized; the water was replaced with tritiated water and wheat germ enzyme (0.4 unit in 25 μl of Buffer A) was added to start the reaction. After 2 hours at 25°, the reaction mixture was lyophilized and UDP-Xyl-5T was isolated by chromatography in Solvent A. Identical reaction mixtures were set up in which UDP-GlcUA was replaced by UDP-Xyl.

**Degradation of UDP-Xyl and Isolation of Carbon-bound Hydrogen at C2 and C5—**UDP-Xyl (Fig. 1) was hydrolysed with snake venom phosphodiesterase and the xylose-1-P released (II)
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yield formic acid from C-3 and n-phosphodiglycolic aldehyde. The hydrogen at C-2 is lost during formation of osazone and oxidation of the osazone to yield crystalline mesoxalaldehyde 1,2-bis(phenylhydrazone).

The tritiated xylosazones were subsequently oxidized with periodic acid to yield mesoxalaldehyde 1,2-bis(phenylhydrazone) (III) from C-1, C-2, and C-3 of n-xylose (21). Periodic acid (24 μmoles) was added to the xylosazone (8 μmoles) in a total volume of 1.2 ml of 66% ethanol. The orange precipitate which immediately formed was recovered by filtration, washed with 66% ethanol, and dissolved in absolute ethanol. The spectrum of the isolated product was compared with authentic mesoxaldehyde 1,2-bis(phenylhydrazone) (22) and the concentration was determined from the optical density at 420 nm based on the molar absorptivity of the authentic compound. Aliquots of the osazone solution were counted in Bray's solution; the counts were corrected for the quenching caused by the yellow color of the osazone.

The tritated xylosazones were subsequently oxidized with periodic acid to yield mesoxalaldehyde 1,2-bis(phenylhydrazone) (III) from C-1, C-2, and C-3 of n-xylose (21). Periodic acid (24 μmoles) was added to the xylosazone (8 μmoles) in a total volume of 1.2 ml of 66% ethanol. The orange precipitate which immediately formed was recovered by filtration, washed with 66% ethanol, and dissolved in absolute ethanol. The spectrum of the isolated product was compared with authentic mesoxaldehyde 1,2-bis(phenylhydrazone) (22) and the concentration was determined from the optical density at 420 nm. Aliquots of the orange solution were counted in Bray's solution and the counts were corrected for quenching.

RESULTS

Effect of Isotope on Rate of Decarboxylation—When the rate of decarboxylation of each of the tritiated substrates was measured, only UDP-GlcUA-4T was found to show an isotope effect. In Tables I and II are presented the results of one experiment with UDP-glucuronate carboxyl-lyase from C. laurentii and wheat germ, respectively. A summary of the results of a number of such experiments is presented in Table III. Ankel and Feingold (3, 4) have shown previously that label is retained upon the wheat germ enzyme. In contrast, the decarboxylation of UDP-GlcUA-4T also occurs with complete retention of label (Table IV). UDP-GlcUA-4T reacts at 32% of the rate (Vf/VH = 0.32) of nonlabeled UDP-GlcUA (measured as the rate of decarboxylation of UDP-GlcUA uniformly labeled with 14C) when decarboxylation is carried out with the C. laurentii enzyme and at 42% of the rate (Vf/VH = 0.42) when the reaction is carried out with the wheat germ enzyme. In contrast, the decarboxylation of UDP GlcUA 3T and UDP GlcUA 5T occurs without an isotope effect (Vf/VH = 1.0) (Table III).
The assay was carried out as described under "Methods." The reaction mixture contained UDP-GlcUA-4T, UDP-GlcUA-14C (0.02 µmole), NAD (0.1 µmole), and UDP-GlcUA carboxylase (C. laurentii, 0.02 unit) in a total volume of 100 µl of Buffer A. Approximately 10 µl aliquots were withdrawn for assay at the times indicated.

**TABLE I**

| Time (min) | UDP GlcUA | UDP Xyl | Rate<sup>b</sup> | VT/VI<sup>b</sup> | %C | %H | %C | %H |
|------------|------------|---------|------------------|-----------------|----|----|----|----|
| 0          | 1060       | 341     | 711              | 19              | 0  | 0  | 0  | 0  |
| 1          | 1549       | 388     | 456              | 37              | 3.4| 3.4| 0.29| 0.29|
| 2          | 1480       | 357     | 512              | 30              | 22.4| 2.9| 0.22| 0.22|
| 5          | 1155       | 335     | 1200             | 70              | 43.1| 12.0| 0.28| 0.28|
| 15         | 552        | 327     | 1610             | 115             | 57.4| 20.7| 0.36| 0.36|
| 30         | 288        | 170     | 1250             | 138             | 73.0| 39.4| 0.55| 0.55|
| 60         | 127        | 160     | 2070             | 234             | 86.2| 54.0| 0.65| 0.65|

<sup>a</sup> Rate of decarboxylation for each isotope-labeled species. Expressed as percentage of conversion of UDP-GlcUA to UDP-Xyl for each isotope species. 
<sup>b</sup> VT = observed rate of decarboxylation of UDP-GlcUA-T at 2 min; VI = observed rate of decarboxylation of UDP-GlcUA-T at 5 min (measured as the rate of decarboxylation of UDP-GlcUA-14C).

### TABLE II

| Time (min) | UDP GlcUA | UDP Xyl | Rate<sup>b</sup> | VT/VI<sup>b</sup> | %C | %H | %C | %H |
|------------|------------|---------|------------------|-----------------|----|----|----|----|
| 0          | 1180       | 625     | 190              | 48              | 0  | 0  | 0  | 0  |
| 5          | 675        | 437     | 232              | 48              | 11.2| 2.9| 0.26| 0.26|
| 15         | 574        | 385     | 511              | 60              | 20.7| 8.0| 0.39| 0.39|
| 30         | 642        | 439     | 494              | 103             | 29.1| 12.0| 0.41| 0.41|
| 60         | 450        | 486     | 607              | 204             | 55.4| 22.3| 0.42| 0.42|
| 15         | 270        | 433     | 1330             | 330             | 86.8| 35.4| 0.51| 0.51|

<sup>a</sup> Rate of decarboxylation for each isotope species. Expressed as percentage of conversion of UDP-GlcUA to UDP-Xyl. 
<sup>b</sup> VT = observed rate of decarboxylation of UDP-GlcUA-4<sup>T</sup> at 5 min; VI = observed rate of decarboxylation of UDP-GlcUA at 5 min (measured as the rate of decarboxylation of UDP-GlcUA-14<sup>C</sup>.

### TABLE III

| Substrate | VT/VI<sup>b</sup> | Wheat germ enzyme | C. laurentii enzyme |
|-----------|-------------------|-------------------|--------------------|
| UDP-GlcUA | 1.0               | 1.0               | 0.42               |
| UDP-GlcUA-4<sup>T</sup> | 0.63            | 0.63              | 0.22               |
| UDP-GlcUA-5<sup>T</sup> | 0.36            | 0.36              | 0.53               |

<sup>a</sup> Average isotope effect calculated from observed rates at 2 min, four trials: 0.40, 0.40, 0.42, 0.47.
<sup>b</sup> Average isotope effect calculated from observed rates at 5 min, three trials: 0.26, 0.36, 0.35.

### TABLE IV

| Enzyme source | UDP-GlcUA-3<sup>T</sup> | UDP-GlcUA-4<sup>T</sup> | UDP-GlcUA-5<sup>T</sup> | Percentage of control |
|---------------|-------------------------|-------------------------|-------------------------|-----------------------|
| Control (boiled enzyme) | 2580 | 124 | 2639 | 100 |
| C. laurentii (boiled enzyme) | 2639 | 124 | 2639 | 100 |
| Wheat germ | 2639 | 124 | 2639 | 100 |

### Location of Label in UDP-Xyl

In order to determine the location of the label in the reaction product, UDP-Xyl was degraded by two different procedures. The first procedure involved periodate oxidation followed by bromine oxidation of the tritiated xylose-1-P derived from UDP-Xyl. This procedure permitted the isolation and identification of 14C bound at either C-1 or C-5 of the D-xylosyl moiety. Glyoxalic acid, containing that hydrogen originally bound at C-1, is derived from C-1 and C-2, and glycolic acid, containing those hydrogen atoms originally present at C-5 is derived from C-4 and C-5 of the D-xylosyl moiety.

The second degradation procedure involved acid hydrolysis of UDP-Xyl to yield the free sugar, formation of the osazone of xylose, and periodate oxidation of the osazone (Fig. 2). That hydrogen atom originally present at C-2 is lost during formation of the osazone, and after periodate oxidation, those hydrogen atoms originally bound at C-1 and C-3 are isolated as the phenylhydrazone of mesoxaldehyde.

The specific activity of xylose-1-P<sup>T</sup> derived from the decarboxylation product of UDP-GlcUA-4<sup>T</sup> (wheat germ enzyme) was 1400 cpm per µmole; however, glyoxalic acid and glycolic acid obtained from it contained less than 1% of the label originally present. On the other hand, the specific activity of xylose-1-P<sup>T</sup> derived from the decarboxylation product of UDP-GlcUA-5<sup>T</sup> (wheat germ enzyme) was 2300 cpm per µmole, that of the glycolic acid derived from it was 2000 cpm per µmole, while the glyoxalic acid contained less than 2% of the original activity.
Specific activities of xylose-1-P, glyoxalic acid, and glycolic acid obtained from UDP-Xyl-5T

Xylose-1-P-T, glyoxalic acid-T and glycolic acid-T were prepared from UDP-Xyl-5T as described under "Methods" and their specific activities were determined. Values in parentheses represent a second series of experiments.

| Compound                  | Enzyme          | Specific activity | Product, glycolic acid |
|---------------------------|-----------------|-------------------|------------------------|
|                           | C. laurentii    | Wheat germ        |                        |
|                           | cpm/µmole       | cpm/µmole         |                        |
| Xylose-1-P-T<sub>sub</sub> | 2600 (1900)     | 2300 (1600)       |                        |
| Glyoxalic acid            | 20              | 50                |                        |
| Glyoxalic acid-T<sub>sub</sub> | 2800 (2000)    | 2600 (1700)       |                        |
| Xylose-1-P-T<sub>med</sub> |                 |                   |                        |
| Glyoxalic acid            |                 |                   |                        |
| Glyoxalic acid-T<sub>med</sub> |                 |                   |                        |

These data show that there is no migration of label from C-4 to either C-1 or C-5 during the decarboxylation.

| Substrate                  | Specific activity | Product, glycolic acid |
|----------------------------|-------------------|------------------------|
|                            | cpm/µmole         | cpm/µmole              |
| C. laurentii               |                   |                        |
| Glycolic acid-T<sub>sub</sub> | 2900             | 66                     |
|                            | (310)             | (8)                    |
| Wheat germ                 |                   |                        |
| Glycolic acid-T<sub>sub</sub> | 2700             | 66                     |
|                            | (800)             | (27)                   |
| Glycolic acid-T<sub>med</sub> | 1700             | 1700                   |
|                            | (1700)            | (1700)                 |

These results show that there is no migration of label from C-4 to either C-1 or C-5 during the decarboxylation.

DISCUSSION

Ankel and Feingold proposed that UDP-4-keto-GlcUA would be a likely intermediate in the decarboxylation of UDP-GlcUA by the carboxylases. The role of NAD in the reaction would be to accept the hydride ion removed from C-4 of the n-glucuronosyl moiety. This reduced NAD would remain enzyme-bound and the hydride ion would then be stereospecifically introduced into a second intermediate, UDP 4-keto Xyl, to yield UDP-Xyl (4). The proposed mechanism is similar to that invoked to explain the 4-epimerization of UDP-Glc (5) except that the β-keto acid, UDP-4-keto-GlcUA, would be a reaction intermediate. Ankel and Feingold were unable to demonstrate the presence of a 4-keto intermediate, and attempts to trap such an intermediate were unsuccessful. Evidence to support the proposed reaction
The reaction mechanism for the decarboxylation of UDP-GlcUA is presented in Fig. 3. The enzyme would combine with the substrate to form an enzyme-substrate complex, which would then undergo a rate-limiting step involving the participation of NAD. The reaction would proceed through the formation of a 4-keto intermediate, which would then be decarboxylated. The mechanism involves the participation of NAD and NADH, and the over-all reaction is consistent with the known stereochemistry of the reaction. The mechanism has been demonstrated to be consistent with the known stereochemistry of the reaction, and it has been shown that the decarboxylation of UDP-GlcUA is irreversible.

Additional evidence for the presence of a 4-keto intermediate is provided by the results of the studies of the stereochemistry of the reaction. The absolute configuration at C-5 of the UDP-GlcUA moiety is determined by the stereochemistry of the reaction. If a hydride ion is removed upon oxidation at C-4, it must be stereospecifically reintroduced at the same position from which it was removed. All the label originally present at C-4 of the UDP-GlcUA moiety in tritiated water with the wheat germ enzyme results in the incorporation into UDP-Xyl of only 1 proton with an absolute configuration of S at C-5. These results show that the proton is incorporated at C-5 on the side opposite the leaving carboxyl group.

Because of the presence of the pyranose ring, it is unlikely that the decarboxylation of UDP-GlcUA occurs by direct replacement. Such a mechanism would necessitate opening of the pyranose ring, displacement of the carbohydrate group by the proton with inversion of configuration, and re-formation of the pyranose ring structure. In addition, since in a direct displacement reaction there would be no need to facilitate the decarboxylation by formation of a 4-keto intermediate, there should be no isotope effect at C-4.

The difference in the type of isotope effect noted with the carboxy-lyase, which indicates that UDP-Xyl is not activated to the 4-keto intermediate by the enzyme. There is no evidence at this time, however, to establish whether $k_5$ or $k_6$ is the rate-limiting step in the reaction, but this difference would not affect the over-all mechanism.

This reaction mechanism appears to be consistent with the data which have been presented. The small difference between the size of the isotope effects for the decarboxylation of UDP-GlcUA-4T and UDP-GlcUA-5T, however, seems to indicate that the enzymatic steps involved after oxidation are entirely different. Whereas reduction of the 4-keto intermediate of the decarboxylation reaction is stereospecific, reduction of the 4-keto intermediate in the epimerase-catalyzed reaction is dictated only by the equilibrium constant. With the carboxy-lyase, the hydride ion must be held in a fixed orientation in respect to the intermediate, whereas the same may not be true for the epimerase.

Conversion of myo-inositol to D-xylosyl and D-glucuronosyl moieties of polysaccharides has been indicated in higher plants (25). From labeling patterns it is evident that the following metabolic sequence is involved: myo-inositol → D-glucuronic acid → α-D-glucopyranosyluronic acid phosphate → UDP-GlcUA → UDP-Xyl. Loewus demonstrated that myo-inositol can be converted to UDP-GlcUA by an enzyme from higher plants, and it has been shown that UDP-GlcUA is the immediate precursor of UDP-Xyl.
inositol-2\textsuperscript{T} is converted by plant tissues to d-xylose-5\textsuperscript{T} with inversion of configuration at the labeled carbon atom (7). Our findings show that this inversion occurs during the decarboxylation of UDP-GlcUA.

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