SYNTHESIS OF 4-KETO-SUGAR PHOSPHATES*

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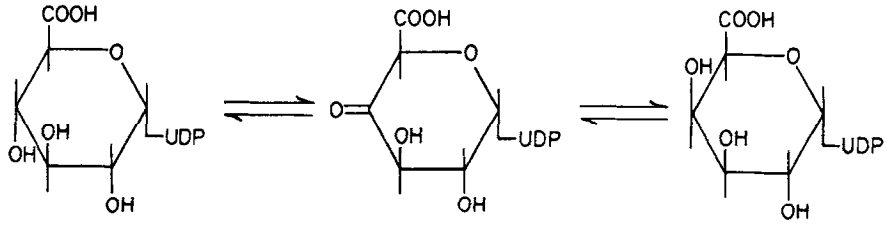
It has been postulated that the conversion of UDP-D-glucuronic acid to UDP-D-galacturonic acid and the conversion of UDP-D-xylose to UDP-L-arabinose by plant enzymes occurs in both cases by an oxidation and subsequent reduction of the C-4 position. To test this hypothesis, the authors decided to attempt the chemical synthesis of UDP-4-keto-D-galacturonic acid and UDP-4-keto-D-xylose. If the enzymes were capable of converting these chemically synthesized intermediates into the normal reduced products, this would be strong evidence for the existence of an oxidation-reduction mechanism of epimerization.



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Two methods of preparation seemed most feasible, (1) the synthesis of the relevant sugar phosphate and oxidation thereof to produce the 4-keto-sugar phosphate, from which the uridine diphosphate derivative might be formed enzymatically, or (2) the direct oxidation of the C-4 position of the monosaccharide sugar nucleotide.

β-L-Arabinose 1-phosphate was synthesized by the method of Cori (1); α-D-galactose 1-phosphate was prepared by the methods of Cori and of McDonald (2).

Oxidation of the secondary hydroxyl groups of suitably substituted glycosides has been achieved using platinum and gaseous oxygen (3) and chromium trioxide dissolved in acetone or pyridine (4). In these instances, the glycosidic carbon was substituted by inert groups such as methyl or benzyl. No instances have been reported of the oxidation of the secondary hydroxyl groups of sugar phosphates. In both L-arabinose and D-galacturonic acid phosphates, only the 4-C carries an axially situated hydroxyl, and it has been shown (5) that axial hydroxyls are oxidized preferentially with platinum and oxygen.

Oxidation of an aqueous solution of arabinose phosphate (0.5 g in 25 ml) with oxygen over freshly reduced Adams catalyst at 50° for 1 to 2 hours gave a compound in about 5% yield (determined colorimetrically by its reaction with o-phenylenediamine (6) using keto sucrose (7) as a standard) which could be separated from the parent sugar phosphate and other products by ionophoresis in sodium bisulfite, pH 5 (8). This new compound gave a positive reaction not only with a phospho-molybdate reagent (9) but also with alkaline silver nitrate and o-phenylenediamine dihydrochloride, thus indicating that a keto-sugar phosphate was present. The compound (20 mg) was reduced at room temperature with sodium borohydride, buffered with boric acid to pH 7.5. After removal of salts by ionophoresis on paper in 0.2 M ammonium formate, pH 3.7, the solution was treated with a crude seminal phosphatase preparation at 37° for 2 hours. The mixture was chromatographed on paper (butanol-acetic acid-water,

52:13:35; phenol-water, 80:20), and the products were detected by spraying with $AgNO_3$ and aniline hydrogen phthalate (10). The chromatographic movements of the compounds were indistinguishable from those of standard D-xylose and L-arabinose in the same solvent systems. The xylose produced by borohydride reduction was isolated by 2-dimensional chromatography and characterized by the preparation of its 2:4 dinitrophenyl hydrazone (7 mg, recrystallized), M.P. 162°; 2:4 dinitrophenyl hydrazone of authentic D-xylose, M.P. 163°. The formation of xylose under these conditions is only possible if the keto-group was located on C-4. Hence, the new compound must be the 4-keto derivative of β-L-arabinose 1-phosphate.

The new compound (0-0.5 mg per 20 µl water) was incubated with 10 µl 0.1 M UTP, 5 µl 0.05 M $MgCl_2$, 5 µl of 1% inorganic pyrophosphatase (11), and 20 µl mung bean pyrophosphorylase (12) for 0-3 hours at temperatures from 20-37°. The mixtures were subjected to electrophoresis on paper in ammonium formate at pH 3.7, and the papers were examined under UV light. In no case was there evidence for the formation of UDP-4-keto-arabinose. However, L-arabinose 1-phosphate when incubated in the above system, was almost completely converted to UDP-L-arabinose. Oxidation of UDP-L-arabinose with Pt and O_2 and with CrO_3 failed to produce any UDP-4-keto-arabinose.

D-Galactose 1-phosphate is commonly oxidized by Pt and O_2 to D-galacturonic acid 1-phosphate (13), and it seemed possible that using suitable conditions, oxidation could be achieved of both the C-4 and C-6 positions simultaneously.

When D-galactose 1-phosphate was oxidized in aqueous solution (25 mg per ml) with Pt and O_2 at 60° for 1 hour, the solution being maintained at pH 7 by addition of $KHCO_3$, subsequent ionophoresis in bisulfite revealed the formation of a variety of products.

One of these compounds (I) gave positive reactions with $AgNO_3$/NaOH, o-phenylenediamine, phosphomolybdate reagent and with the Dische carbazole test (14) for uronic acids. I was present in about 2-3% yield (o-phenylene-

diamine reaction) most of the remaining material being D-galacturonic acid 1-phosphate. When the oxidation was performed on a larger scale (4 g), I was isolated by ionophoresis in bisulfite buffer and chromatography in propanol-ethyl acetate-water (7:1:2) as an amorphous powder (63 mg), $[\alpha]_D^{20}$ + 30° (c, 0.5 in H_2O). Analysis of I by the method of Fiske and Subbarow (15) gave a total phosphorus content of 7.65% (theory 8.03%). The ultraviolet absorption spectra of I revealed a peak at 335 mμ (extinction coefficient, 0.15) in 0.1 N NaOH, in addition to an absorption maximum at 275 mμ (extinction coefficient, 0.7). This secondary peak which is characteristic of keto sugars (16) was absent in 0.1 N HCl, but could be obtained when the solution was readjusted to an alkaline pH. This peak was not obtained with galacturonic acid 1-phosphate. Optical density measurements (at 275 mμ) indicated that I was stable in acid, but underwent slow decomposition in 0.1 N NaOH (at 335 mμ) as would be expected for a keto-sugar phosphate (17). I (45 mg) was reduced with buffered sodium borohydride, pH 7.5, and salts were removed by ionophoresis in 0.2 M NH_4 formate, pH 3.7. After subsequent hydrolysis with seminal phosphatase, galacturonic acid and glucuronic acid were isolated by ionophoresis in 0.2 M NH_4 formate buffer, pH 3.4. The galacturonic acid was characterized by oxidation with nitric acid to the insoluble mucic acid, M.P. 215° (13 mg) while the glucuronic acid was characterized by preparation of its p-nitrophenyl hydrazone (4 mg), M.P. 222-223° (18). Thus, I was 4-keto-α-D-galacturonic acid 1-phosphate.

Incubation of I (0-0.5 mg per 20 μl) with the mung bean pyrophosphorylase system as described above failed to reveal any UDP-4-keto-D-galacturonic acid on subsequent ionophoresis, although the system was able to convert D-galacturonic acid 1-phosphate almost quantitatively to UDP-D-galacturonic acid.

UDP-D-galactose was synthesized chemically from α-D-galactose 1-phosphate by the method of Khorana (19). UDP-D-galactose could be oxidized with Pt and O_2 to UDP-D-galacturonic acid, but no keto-

derivatives were obtained, either during this oxidation or on re-oxidation of the UDP-D-galacturonic acid. Attempts to oxidize UDP-D-galactose or UDP-D-galacturonic acid with chromium trioxide resulted in considerable degradation.

REFERENCES

(1) Cori, C. F., Colowick, S. P., and Cori, G. T., J. Biol. Chem. 121, 465 (1937).

(2) McDonald, C., J. Org. Chem. 27, 1107 (1962).

(3) Brimacombe, E., Brimacombe, J. S., and Lindberg, B., Acta Chem. Scand. 14, 2236 (1960).
Heyns, K., and Lenz, J., Angewandte Chemie 73, 299 (1961).

(4) Assarsson, A., and Theander, O., Acta Chem. Scand. 1507 (1958).
Walton, E., et al., J.A.C.S. 5168 (1958).

(5) Heyns, K., and Paulsen, H., Adv. Carb. Chem. 17, 169 (1962).

(6) Lanning, M. C., and Cohen, S. S., J. Biol. Chem. 189, 109 (1951).

(7) Feingold, D. S., and Grebner, E. E., Am. Chem. Soc., Abstract, 3D, Chicago, Illinois meeting, September 3-8 (1961).

(8) Theander, O., Acta Chem. Scand. 717 (1957).

(9) Hanes, C. S., and Isherwood, F. A., Nature 164, 1107 (1949).

(10) Partridge, S. M., Nature 164, 443 (1949).

(11) Kunitz, M., J. Gen. Physiol. 35, 423 (1952).

(12) Neufeld, E. F., et al., J. Biol. Chem. 236, 3102 (1961).

(13) Marsh, C. A., J. Chem. Soc. 1578 (1952).

(14) Dische, Z., J. Biol. Chem. 167, 189 (1947).

(15) Fiske, C. H., and Subbarow, Y., J. Biol. Chem. 66, 375 (1952).

(16) Okazaki, R., Okazaki, T., Strominger, J. L., and Michelson, A. M., J. Biol. Chem. 237, 3014 (1962).

(17) Theander, O., Acta Chem. Scand. 1887 (1958).

(18) Kiliani, H., Berichte 58, 2344 (1925).

(19) Roseman, S., Distler, J. J., Moffatt, J. G., and Khorana, H. G., J.A.C.S. 659 (1961).