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Formation of Uronic Acid by Galactose Oxidase

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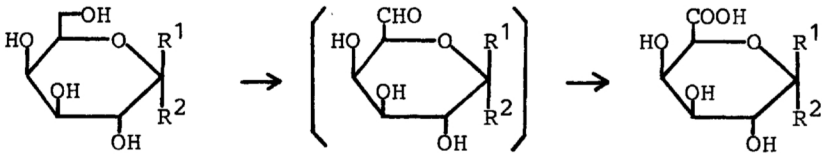
The C-6 hydroxymethyl group of methyl D-galactopyranoside and oligosaccharides containing D-galactopyranosyl residues were found to be oxidized with oxygen by galactose oxidase to the corresponding carboxyl group via an aldehyde intermediate.

Galactose oxidase (D-galactose : oxygen 6-oxidoreductase, EC 1.1.3.9) is an enzyme which catalyzes the oxidation of the C-6 hydroxymethyl of D-galactose in the presence of molecular oxygen to an aldehyde group and produces hydrogen peroxide. This enzyme is used mainly as an analytical tool for determining the presence of D-galactose and D-galactopyranosides.[1] Also, the enzyme is known to be relatively nonspecific and many studies on substrate specificity have been done,[2-3] but the exact oxidation products were not always clear. Recently, Kelleher et al. reported evidence for the conversion of raffinose to 6''-carboxyraffinose in the oxidation products.[4] In this communication, we report the unprecedented oxidation by galactose oxidase in high yield of methyl D-galactopyranoside (1) to methyl D-galactopyranosiduronic acid (3) via an aldehyde (2), and also discuss the oxidation of C-6 hydroxymethyl group of D-galactopyranosyl residues of oligosaccharides in comparison with those of sugar alcohols.

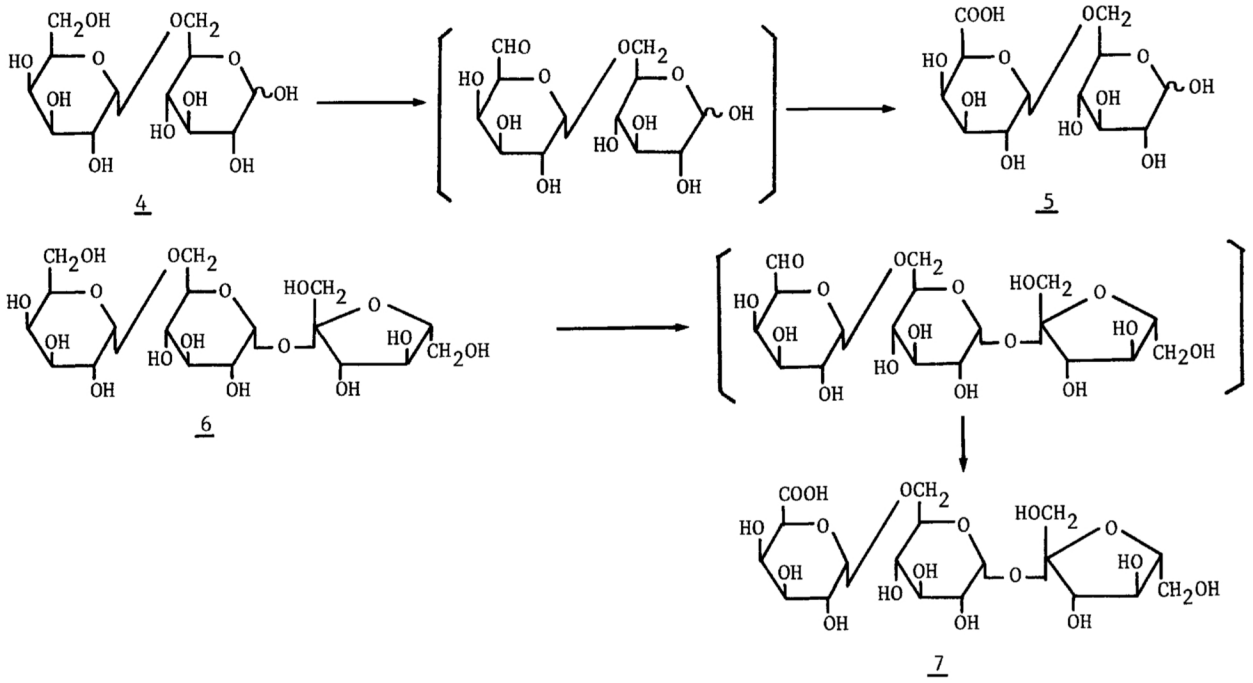


1 2 3

(1a : R^1=H, R^2=OCH_3, α
1b : R^1=OCH_3, R^2=H, β)

Galactose oxidase (EC 1.1.3.9) of Dactylium dendroides (specific activity of 900 units/mg protein) and bovine liver catalase (specific activity of 4000 units/mg protein) were purchased from Sigma. Authentic methyl α-D-galactopyranosiduronic acid was obtained by the oxidation of methyl α-D-galactopyranoside using nitrogen dioxide according to the method of Hardegger et al.[5] Analysis of the enzymatic reaction products was performed by the HPLC method[6] calibrated with the authentic standards.

Methyl D-galactopyranoside (1) (0.15 mmol) was incubated with galactose oxidase (25 units) and catalase (6000 units) in 30 mL of 0.05 mol dm^{-3} phosphate buffer at pH 7.0. The incubation tube was flashed with oxygen, stoppered, and shaken at 30 °C in the dark. After 7 h incubation, 1 disappeared in the reaction mixture and the ^{13}C NMR spectra of the isolated products indicated the conversion of the C-6 hydroxymethyl group of 1 to an aldehyde group. ^{13}C NMR analyses suggested that the aldehyde group only exists as the aldehyde hydrate and as a mixture of the internal hemiacetals formed by the cyclization of a hydroxyl group with the C-6 aldehyde group in the aqueous solution. After 7 h incubation, the C-6 aldehyde group was further oxidized to the corresponding carboxylic group by the galactose oxidase. After 20 days incubation, chromatographically isolated product was analyzed by IR, ^{1}H NMR, and ^{13}C NMR. From these results, it was indicated that methyl D-galactopyranosiduronic acid (3) was formed. These spectral data agreed completely with those of the authentic methyl D-galactopyranosiduronic acid.[7] The yield for 3a was 53% and 50% for 3b after 20 days incubation. During the oxidation by the enzyme at various intervals, an aliquot of sample was withdrawn from the reaction tube and analyzed for reaction products by HPLC. Figure 1 shows the oxidation of 1a, and Fig.2 shows the oxidation of 1b. Formation of the intermediate aldehyde 2 was shown by the relative value from the UV response of the HPLC. Compound 1 disappeared within 7 h with the formation of an aldehyde. The concentration of the aldehyde 2 reached its maximum with the disappearance of 1. At this time the C-6 aldehyde was further oxidized to the C-6 carboxylic group by the enzyme. Compound 1a and 1b were oxidized to the corresponding aldehydes with similar rates, but the rate of formation of uronic acid (3b) was slower than that of 3a. From these results, it was indicated that the galactose oxidase oxidizes the C-6 hydroxymethyl of the galactopyranosyl group to a C-6 carboxylic group via an aldehyde by a two step reaction.



Some oligosaccharides containing galactopyranosyl residues in the molecule were also oxidized by the enzyme. The experimental procedures are the same as that of 1. Pure and isolated 6'-carboxymelibiose (5) was obtained with a yield of 41% from melibiose (4) and 6''-carboxyraffinose (7) was obtained with a yield of 14% from raffinose (6) after 20 days incubation.

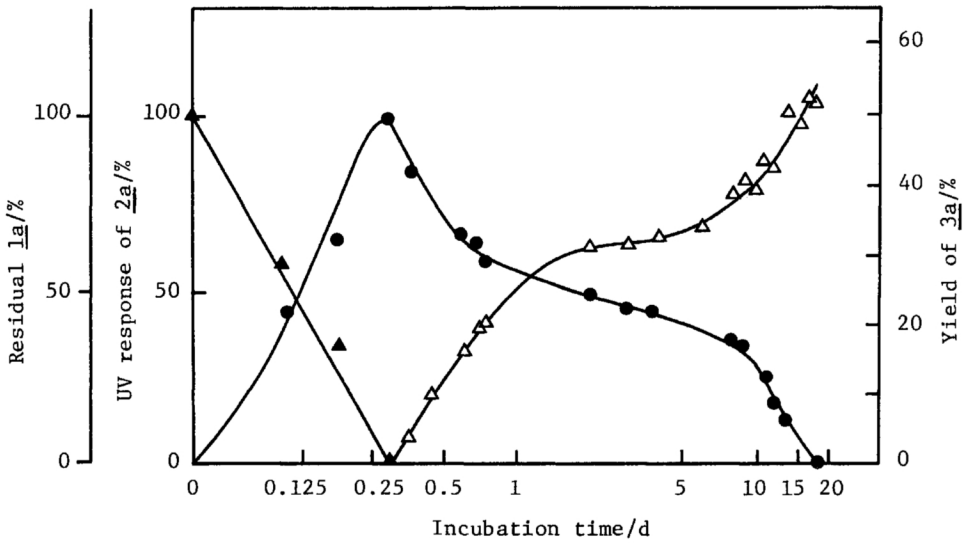


Fig.1. Oxidation of methyl α-D-galactopyranoside (1a) to methyl α-D-galactopyranosiduronic acid (3a) via an aldehyde (2a) by galactose oxidase at 30 °C. See text for the reaction conditions. ▲:1a, ●:2a, △:3a

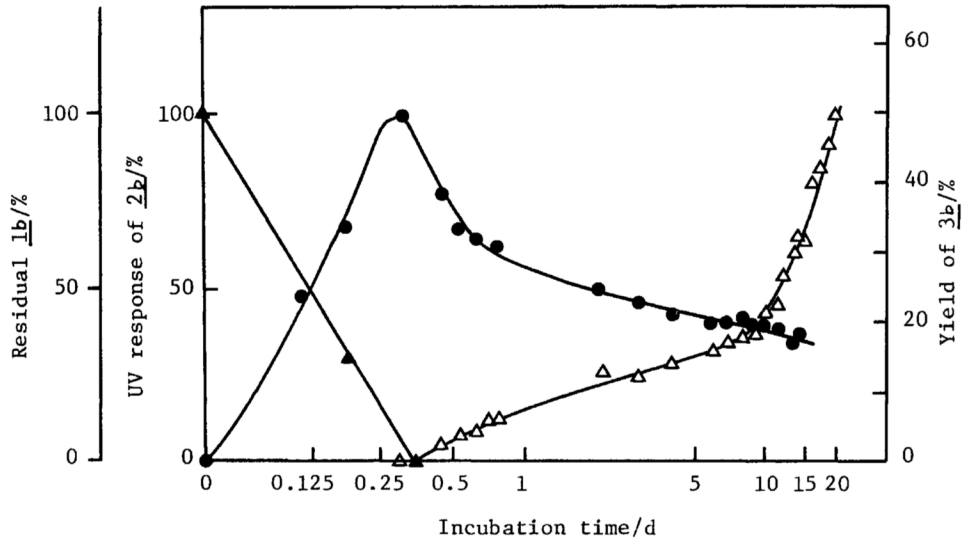
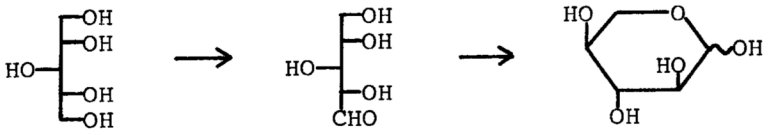


Fig.2. Oxidation of methyl β-D-galactopyranoside (1b) to methyl β-D-galactopyranosiduronic acid (3b) via an aldehyde (2b) by galactose oxidase at 30 °C. See text for the reaction conditions. ▲:1b, ●:2b, △:3b



Xylitol L-xylose

It appears that the C-6 aldehyde of galactopyranosyl group is susceptible to oxidation and is further oxidized to the C-6 carboxyl group in the presence of galactose oxidase. In contrast to the oxidation of galactopyranosides, it is reported that some sugar alcohols, such as galactitol and xylitol, are oxidized by galactose oxidase to L-galactose and L-xylose, respectively.[8] In this case, the L-galactose and L-xylose produced will be stabilized by forming the pyranose ring, and no further oxidation occurs. In fact L-xylose was obtained exclusively after 12 days incubation of xylitol with galactose oxidase under the same experimental conditions as those for the oxidation of 1.

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References

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4) F. M. Kelleher and V. P. Bhavanandan, J. Biol. Chem., 261, 11045 (1986).
5) E. Hardegger and D. Spitz, Helv. Chim. Acta, 32, 2165 (1949).
6) HPLC column : TOSOH Co. Ltd., Cation-exchange chromatographic column, TSK-Gel SCX ; Eluant : 0.05 mol dm^{-3} $HClO_4$; UV detector : JASCO 875-UV (208 nm) ; RI detector : SHOWA DENKO Co. Ltd., Shodex RI SE-51.
7) ^{13}C NMR(D_2O): For enzymatically synthesized 1a δ 173.2(C-6), 100.4(C-1), 70.8(C-5,C-3), 69.7(C-4), 68.5(C-2), 56.4(α-OCH_3); authentic 1a δ 173.2(C-6), 100.4(C-1), 70.9, 70.8(C-5,C-3), 69.7(C-4), 68.5(C-2), 56.4(α-OCH_3).
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