*Carbohydrate Research* 54 (1977) 145–147


## Note

# Identification of 3-*O*-α-D-glucopyranosyl-L-sorbose—a product of L-sorbose metabolism in plants

ELIZABETH A. MCCOMB AND VICTOR V. RENDIG

*Department of Land, Air and Water Resources, Soils and Plant Nutrition Section, University of California, Davis, California 95616 (U.S.A.)*

(Received October 21st, 1976; accepted for publication, November 1st, 1976)

The *in vivo* synthesis of L-*galacto*-heptulose[1] and L-threitol[2] in plant leaves from shoots that had imbibed L-sorbose was studied by using L-[$^{14}$C]sorbose. Four radioactive areas were detected on radioautographs and Geiger–Mueller strip-scans of paper chromatograms of leaf juice. Three of the areas corresponded to threitol, sorbose, and *galacto*-heptulose; the fourth had an $R_F$ value near to that of sucrose, but it was not sucrose, as carbon-14 activity was evident in the same area after invertase hydrolysis.

This metabolite of L-sorbose was originally detected in alfalfa (*Medicago sativa*). Subsequently, confirmation was obtained for its biosynthesis in tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*). A quantity (~400 mg) of the saccharide was isolated from alfalfa leaves, and it was identified as 3-*O*-α-D-glucopyranosyl-L-sorbose, owing to the agreement of its characteristics with those of a disaccharide reported[3] to be synthesized by the α-D-glucosidase of brewers' yeast.

## EXPERIMENTAL

*Formation and isolation of the saccharide.* — Leaves were separated from shoots of alfalfa plants that had imbibed 0.1M L-sorbose under illumination for about 24 h, and they were then placed directly in boiling ethyl alcohol. The alcohol extract was concentrated, the suspension filtered through a Celite mat, and the filtrate passed through a column of Amberlite IR-120 ($H^+$) and then through one of Duolite A-4 ($OH^-$) ion-exchange resin. The eluate was then concentrated to ~15% of solids, and the concentrate was treated with a 2% solution of yeast invertase (EC 3.2.1.26). After completion of the reaction, further separation was made by using heavy-paper chromatography as previously described[1].

For chromatographic separation and detection, the papers were irrigated with 8:2:1 ethyl acetate–pyridine–water and then with 5:1 liquefied phenol–water, and developed with an orcinol[4] and with an aniline[5] reagent. In preliminary tests for the presence of the compound, samples of expressed juice[6] were "inverted" directly[7] on

the paper before chromatography β-D-Glucosidase (EC 8 2 1 21) activity was determined by the method outlined in the Worthington manual[8] The phenylosazone was formed as described by Chiba and Shimomura[3], and the reducing values were determined by a modification of the Shaffer–Somogyi procedure[9]

*Identification of the disaccharide* — The saccharide isolated was amorphous and very hygroscopic Chromatographically, it gave a yellow color for ketose with orcinol, and had an $R_{Fru}$ value identical with that of turanose (3-*O*-α-D-glucopyranosyl-D-fructofuranose) The saccharide was not hydrolyzed by invertase or by β-D-glucosidase, and gave a negative Raybin[10] diazouracil test, indicating that the glycosidic linkage was not that found in sucrose It was reducing, and, after hydrolysis (0 1M HCl), the molar ratio of total hexose hexulose was 1 00 0 45 Glucose and sorbose were detected in the hydrolyzate by paper chromatography The components of a hydrolyzed sample were separated on heavy chromatographic paper, and isolated The two crystalline fractions that were obtained gave X-ray powder diffraction patterns identical with those of authentic glucose and sorbose The disaccharide isolated as a product of L-sorbose metabolism in plants was chromatographically similar to the 3-*O*-α-D-glucopyranosyl-L-sorbose of Chiba and Shimomura[3], and its identity as this compound was confirmed by its $[\alpha]_D$ +81° (*c* 2 0, water), and phenylosazone of m p 185–187°, agreeing with their values[3] of $[\alpha]_D$ +81° (*c* 1 0, water), and phenylosazone of m p 183–185° for this disaccharide

## DISCUSSION

The formation of "α-D-glucopyranosido-α-L-sorbofuranoside" by a sucrose glucosyltransferase from the bacterium *Pseudomonas saccharophila* was reported by Hassid *et al*[11] in 1945 Later Bean and Hassid[12] found that an enzyme from pea synthesized a "D-glucosyl-L-sorboside" from a substrate of UDPG and L-sorbose, the structure of the disaccharide was not established, but they implied that it was that reported by Hassid *et al*[11] From several plant-tissues, Leloir and collaborators[13,14] isolated two enzymes that catalyze the reversible formation of sucrose (β-D-fructofuranosyl α-D-glucopyranoside) from UDPG and D-fructose, these investigators did not find a substance reacting like sucrose when D-fructose was replaced by L-sorbose, suggesting that the inference of Bean and Hassid[12] might not be correct

A series of 3-*O*-α-D-linked, L-sorbose-terminated malto-oligosaccharides has been found by Abdullah and Whelan[15] to result from the activity of a transglycosylase from the D enzyme of potato In addition to 3-*O*-α-D-glucopyranosyl-L-sorbose, Chiba and Shimomura[3] identified 1-*O*-α-D-glucopyranosyl-L-sorbose and 4-*O*-α-D-glucopyranosyl-L-sorbose as being synthesized by the α-D-glucosidase of brewers' yeast To the best of the authors' knowledge, the present report is the first on the formation of 3-*O*-α-D-glucopyranosyl-L-sorbose as a product of the *in vivo* metabolism of L-sorbose in plants

## REFERENCES


1 E A McComb and V V Rendig, *Arch Biochem Biophys*, 95 (1961) 316–319
2 E A McComb and V V Rendig, *Arch Biochem Biophys*, 103 (1963) 84–86
3 S Chiba and T Shimomura, *Agric Biol Chem*, 35 (1971) 1363–1370
4 A Bevenue and K T Williams *Arch Biochem Biophys*, 34 (1951) 225–227
5 R M McCready and E A McComb, *Anal Chem*, 26 (1954) 1645–1647
6 E A McComb and V V Rendig *Chemist Analyst*, 49 (1960) 55
7 K T Williams and A Bevenue, *Science*, 113 (1951) 582
8 *Worthington Enzymes Enzyme Reagents*, Worthington Biochemical Corporation Freehold, N J, 1972
9 *Methods of Analysis, A O A C* 12th edn, Association of Official Analytical Chemists, Washington, D C, 1975
10 H W Raybin *J Am Chem Soc*, 55 (1933) 2603–2604
11 W Z Hassid M Doudoroff, H A Barker, and W H Dore, *J Am Chem Soc*, 67 (1945) 1394–1397
12 R C Bean and W Z Hassid *J Am Chem Soc*, 67 (1955) 5737–5738
13 L F Leloir and C E Cardini *J Am Chem Soc* 75 (1953) 6084
14 C E Cardini, L F Leloir, and J Chiriboga *J Biol Chem* 214 (1955) 149–155
15 M Abdullah and W J Whelan *Arch Biochem Biophys*, 112 (1965) 592–598