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4-*O*-β-D-Galactopyranosyl-D-xylose: A new synthesis and application to the evaluation of intestinal lactase*

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ABSTRACT

4-*O*-β-D-Galactopyranosyl-D-xylose (**2**) was prepared from benzyl 2,3-*O*-isopropylidene-β-D-xylopyranoside by glycosylation with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide and subsequent deprotection. Compound **2** was hydrolyzed *in vitro* by intestinal lactase; the V_{max} was 25% of that with lactose and the K_m was 370mM (*cf.* 27mM for lactose). Oral administration of **2** to suckling rats led to urinary excretion of D-xylose which could be estimated colorimetrically.

INTRODUCTION

We have reported syntheses of 4-*O*-β-D-galactopyranosyl-3-*O*-methyl-D-glucose (**1**, 3-*O*-methyllactose) and the use of this substance in the evaluation *in vivo* of intestinal lactase[1–3]. This enzyme is involved in adult-type alactasia[4–6] and its diagnostic evaluation is important both in pediatrics and gastroenterology. It can be carried out by a number of procedures[7–16], most of them of difficult application to very young children. The procedure[1–3] using 3-*O*-methyllactose (**1**) is a non-invasive evaluation method based on oral administration of **1**, followed by estimation of 3-*O*-methyl-D-glucose in the urine. 3-*O*-Methyllactose proved to be an acceptable substrate for intestinal lactase and, when administered orally to suckling rats, led to the urinary excretion of 3-*O*-methyl-D-glucose, which could be determined by g.l.c. or h.p.l.c. In spite of its simplicity, the application of this evaluation method is restricted to hospitals having chromatographic analytical facilities, which may be a serious drawback for widespread diagnostic application. We have, therefore, developed a new evaluation method based on 4-*O*-β-D-galactopyranosyl-D-xylose[17] (**2**). This disaccharide has been found to be a substrate of the enzyme, yielding D-galactose and D-xylose[17]. The latter is passively absorbed from

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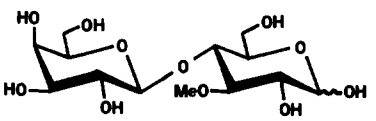

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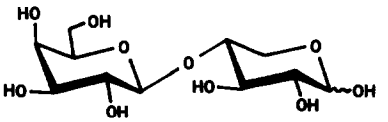

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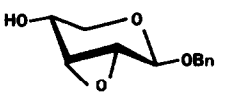

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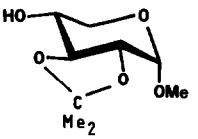

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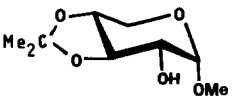

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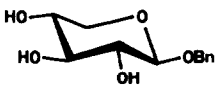

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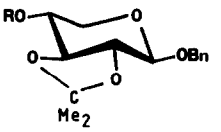

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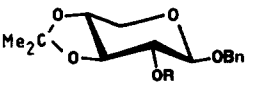
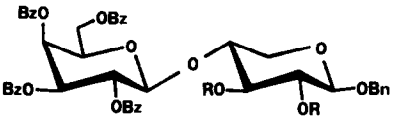
8 , R = H

9 , R = Ac



10 , R = H

11 , R = Ac



12 , R = H

13 , R = Ac

the small intestine[18], it is not phosphorylated[19,20], and it is eliminated in the urine where it can be estimated by a simple colorimetric procedure[21].

Disaccharide **2** is known as a fragment of the linkage region between the protein and the polysaccharide chain of heparin and other animal proteoglycans[22], and syntheses have been reported[23,24]. These syntheses involve glycosylation of benzyl 2,3-anhydro-*β*-D-ribopyranoside (**3**) with a 2,3,4,6-tetra-*O*-acyl-D-galactopyranosyl bromide. The reported synthesis of **2** involves a multistep route starting from benzyl *β*-D-arabinopyranoside[25]. We now report a new synthesis of **2** and its application to the evaluation of intestinal lactase. A communication of the diagnostic evaluation procedure has been submitted for publication[17].

RESULTS AND DISCUSSION

It has been reported[26] that isopropylidenation of methyl α-D-xylopyranoside with 2,2-dimethoxypropane in *N,N*-dimethylformamide containing 4-toluenesulfonic acid gives a mixture of 2,3- and 3,4-*O*-isopropylidene derivatives (**4** and **5**) with the former predominating. A considerable increase in the yield could be obtained when the isopropylidenation of methyl β-D-xylopyranoside was performed with 2-methoxypropene in the presence of hydrochloric acid in dry methanol[27]. Treatment of benzyl β-D-xylopyranoside (**6**) under the latter conditions[27] gave a mixture of a mixed acetal (probably **7**, 25%), benzyl 3,4-*O*-isopropylidene-β-D-xylopyranoside (**10**, 8%), and benzyl 2,3-*O*-isopropylidene-β-D-xylopyranoside (**8**, 40%). Conventional acetylation of **8** and **10** gave the corresponding acetylated derivatives **9** and **11**. The spectroscopic and microanalytical data for **8**–**11** were in agreement with the proposed structures. Treatement of **7** with pyridinium 4-toluenesulfonate gave **8** quantitatively (t.l.c.)[28]. Condensation[29] of **8** with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide in nitromethane in the presence of 2,4,6-trimethylpyridine, followed by treatment of the reaction product with 4-toluenesulfonic acid gave **12** (45%), acetylation of which afforded the corresponding diacetate **13**. Debenzoylation and subsequent hydrogenolysis of **12** gave the desired disaccharide **2** (70%).

Compound **2** was hydrolyzed *in vitro* by intestinal lactase, partially purified from sheep. The V_{max} was 25% of that with lactose and the K_m was 370mM (*cf.* 27mM for lactose). For the *in vivo* experiments, 15-day-old suckling rats and two-month-old adult rats were used. All rats were of a single litter. To the suckling rats, fastened in metabolic cages for 4 h at 30°, compound **2** (18.2 mg/0.5 mL of water) was administered orally by intragastric intubation, and urine was collected during 5 h. The adult rats were given 36.4 mg of **2** in 0.5 mL of water, and urine was collected similarly. Both groups of rats were also administered 9.0 mg/0.5 mL (suckling animals) or 18.0 mg/0.5 mL (adult animals) of D-xylose. The urines collected before and after administration of the indicated compounds were analyzed for xylose spectrophotometrically[21] and chromatographically. In parallel experiments, compound **1** and 3-*O*-methyl-D-glucose were

TABLE I

Urinary recovery (%) of D-xylose and 3-*O*-methyl-D-glucose 5 h after oral administration of D-xylose, 3-*O*-methyl-D-glucose, and compounds **1** and **2** to suckling and adult rats[a]

Rats	*Recovered* D-*xylose (%)*				*Recovered 3-*O*-methyl-*D*-glucose (%)*	
	After administration of D-*xylose*		*After administration of* **2**		*After administration of 3-*O*-methyl-*D*-glucose*	*After administration of* **1**
Suckling	40[b]	31[c]	21[b]	13[c]	75[c]	19[c]
Adult	44[b]	29[c]	8[b]	4[c]	90[c]	3[c]

[a] Average values from three experiments. [b] Colorimetric determination. [c] Chromatographic determination.

administered to both suckling and adult animals, the urines were collected, and the excreted 3-*O*-methyl-D-glucose was determined by g.l.c. The results are summarized in Table I. The greater elimination of D-xylose by the suckling rats is evident and, although there are some discrepancies in the percentage depending on the analytical method, a semiquantitative evaluation can confidently and simply be carried out at the present stage. The percentage of both 3-*O*-methyl-D-glucose and D-xylose [derived from 3-*O*-methyllactose (**1**) and 4-*O*-β-D-galactopyranosyl-D-xylose (**2**), respectively] eliminated in the urine was in the same range. Nevertheless, 3-*O*-methyl-D-glucose was more rapidly eliminated in the controls than D-xylose, suggesting that the hydrolysis in the intestine of **2** was faster than that of **1**. This is in agreement with the higher specificity that lactase exhibits for **2**.

EXPERIMENTAL

Methods — Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer 141 polarimeter. T.l.c. was performed on Silica gel GF_{254} (Merck) with detection by charring with H_2SO_4. Column chromatography was performed on Silica gel Merck (70–230). ^{1}H-N.m.r. spectra were recorded with a Varian XL-300 (300 MHz) or a Bruker AM-200 (200 MHz) spectrometer, and ^{13}C-n.m.r. spectra with a Bruker AM-200 (50 MHz) or a Bruker WP-80 (20 MHz) spectrometer.

Protein was determined by the method of Bradford[30], bovine serum albumin being used as a standard. D-Xylose in urine was determined with a Lambda-4 Perkin–Elmer spectrophotometer. D-Xylose, D-galactose, and 3-*O*-methyl-D-glucose in urine were determined with a Hewlett–Packard 5890 gas chromatograph. The urine was lyophilized after addition of mannitol (4 mg), and a solution of the dry residue in pyridine (1 mL) was heated to 70° for 3 min, filtered, and treated with chlorotrimethylsilane (0.5 mL) and hexamethyldisilazane (0.5 mL).

Preparation of lactase. — Intestinal lactase was purified from young sheep essentially as described by Schlegel-Hauter *et al.*[31]. Intestinal mucosa was scraped off and homogenized in M NaCl (4 mL). The pellet was digested with papain and the supernatant, obtained after centrifugation at 105 000*g* for 1.5 h, was eluted from a column of Sepharose 2B. The fractions containing lactase activity were obtained and fractionated by gel filtration on Sephadex G-200. Fractions containing lactase activity were combined, concentrated overnight by ultracentrifugation, and fractionated again on DEAE-cellulose. Fractions containing lactase activity were combined for use in the assays. The purification factor was 25 from the papain digest. A standard reaction mixture contained, in a final volume of 40 μL, 67mM maleate (pH 6.0), 0.5M lactose or **2** (20 μL), and the enzyme preparation (20 μL). After incubation for 45 min at 37°, the reaction was stopped by addition of 180mM Tris buffer (pH 8.4, 140 μL) and boiling for 2 min. D-Galactose was determined with D-galactose dehydrogenase[32]. One unit of enzyme activity is defined as the amount of enzyme that can produce 1 μmol of D-galactose · min^{-1} at 37°.

Benzyl β-D-xylopyranoside (**6**). — A mixture of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (3.6 g), HgO (1.3 g), $HgBr_2$ (0.1 g), benzyl alcohol (15 mL), and molecular sieves 4A (1.5 g) was stirred for 20 h at room temperature. The solids were filtered and thoroughly washed with chloroform. The combined filtrate and washings were collected and concentrated. To the residue was added ethanol (75 mL), and from the resulting solution cristals of benzyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (2.1 g) were removed and added to a 0.1M solution of sodium methoxide in methanol (8 mL), and stirred for 3 h at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, and the solution was evaporated to give pure **6** as a solid (1.3 g, 51%), which cristallized from acetone–hexane, m.p. 112°, $[\alpha]_D^{20}$ −67° (*c* 0.9, water); ^{1}H-N.m.r. (D_2O): δ 7.50–7.30 (m, 5 H, Ph), 4.87, 4.70 (2d, 2 H, *J* 11.6 Hz, PhCH_2), 4.47 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.95 (dd, 1 H, $J_{4,5e}$ 5.4, $J_{5a,5e}$ 11.5 Hz, H-5*e*), 3.62 (m, 1 H, H-4), 3.40 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3), and 3.30 (m, 2 H, H-2, 5*a*).

Anal. Calc. for $C_{12}H_{16}O_5$: C, 59.99; H, 6.71. Found: C, 59.74; H, 7.00.

Isopropylidenation of benzyl β-D-xylopyranoside. — To a solution of **6** (0.45 g, 1.9 mmol) in dry *N,N*-dimethylformamide (1 mL) under Ar was added 4M HCl in dry methanol (14 μL) under stirring at 70°. 2-Methoxypropene (0.9 mL, 9.4 mmol) was added and the reaction was allowed to proceed for 2 h at 70°, and then overnight at room temperature. The acid was neutralized with triethylamine (34 μL) and the solvent removed under vacuum at 30°. The residue was dissolved in chloroform (25 mL) and the solution was washed with water (2 × 25 mL), dried (Na_2SO_4), and concentrated to dryness. The resulting syrup was chromatographed on a column of silica gel, in 3:1→1:1 hexane–ethyl acetate to afford, first probably *benzyl 2,3-isopropylidene-4-O-[1-methoxy-1-methylethyl]-β-D-xylopyranoside* (**7**; 0.16 g, 25%) as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.20 (m, 5 H, Ph), 4.86 (d, 1 H, *J* 11.7 Hz, PhC*H*), 4.72 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 4.66 (d, 1 H, *J* 11.7 Hz, PhC*H*), 4.08 (m, 2 H, H-4,5*e*), 3.56 (dd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 8.4 Hz, H-3), 3.44 (dd, 1 H, H-2), 3.30 (dd, 1 H, $J_{4,5a}$ 9.0, $J_{5a,5e}$ 13.9 Hz, H-5*a*), 3.24 (s, 3 H, OCH_3), 1.43, 1.42, 1.39, and 1.35 (4s, 3 H each, 4 Me).

The second compound eluted was *benzyl 3,4-O-isopropylidene-β-D-xylopyranoside* (**10**; 0.04 g, 8%) as a syrup, $[\alpha]_D^{20}$ −78° (*c* 1.0, chloroform).

Anal. Calc. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.82; H, 7.61.

Conventional treatment of **10** with acetic anhydride–pyridine gave *benyzyl 2-O-acetyl-3,4-O-isopropylidene-β-D-xylopyranoside* (**11**) as a syrup, $[\alpha]_D^{20}$ −89° (*c* 1.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.20 (m, 5 H, Ph), 5.02 (dd, 1 H, $J_{1,2}$ 5.1, $J_{2,3}$ 9.6 Hz, H-2), 4.44 (d, 1 H, H-1), 4.11 (dd, 1 H, $J_{4,5a}$ 5.1, $J_{5a,5e}$ 10 Hz, H-5*a*), 3.73 (dd, 1 H, $J_{4,5e}$ 5.0 Hz, H-5*e*), 3.55 (m, 2 H, H-3,4), 1.99 (s, 3 H, $OCOCH_3$), 1.38 and 1.37 (2 s, 3 H each, 2 Me).

Anal. Calc. $C_{17}H_{22}O_6$: C, 63.38; H, 6.83. Found: C, 63.40; H, 6.70.

The third compound to be eluted as a syrup was *benzyl 2,3-O-isopropylidene-β-D-xylopyranoside* (**8**; 0.21 g, 40%), $[\alpha]_D^{20}$ −47° (*c* 1.1, chloroform).

Anal. Calc. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.11; H, 7.40.

Acetylation of **8** with acetic anhydride in pyridine gave *benzyl 4-O-acetyl-2,3-O-isopropylidene-β-D-xylopyranoside* (**9**), $[\alpha]_D^{20}$ −97° (*c* 1.0, chloroform); ^{1}H-n.m.r.

($CDCl_3$): δ 7.40–7.20 (m, 5 H, Ph), 4.95 (m, 1 H, H-4), 4.78 (d, 1 H, $J_{1,2}$ 6.9 Hz, H-1), 4.15 (dd, 1 H, $J_{4,5e}$ 5.1, $J_{5e,5a}$ 12.7 Hz, H-5*e*), 3.73 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 3.51 (dd, 1 H, H.2), 3.33 (dd, 1 H, $J_{4,5a}$ 5.0 Hz, H-5*a*), 2.02 (s, 3 H, $OCOCH_3$), 1.39 and 1.38 (2s, 3 H, each, 2 Me).

Anal. Calc. for $C_{17}H_{22}O_6$: C, 63.38; H, 6.83. Found: C, 63.43; H, 6.58.

Benzyl O-*(2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-β-*D*-xylopyranoside (***12***).* — To a mixture of **8** (1.39 g, 4.75 mmol), silver triflate (1.56 g, 6 mmol), 2,4,6-trimethylpyridine (0.71 mL, 5.6 mmol), and nitromethane (27 mL) was added a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (3.45 g, 5.65 mmol) in nitromethane (27 mL) with stirring for 10 min at −25° under Ar. Pyridine (2.7 mL) was added and the mixture was allowed to reach room temperature. Ethyl ether was added, salts were removed by filtration, and the solution was washed successively with aq. $Na_2S_2O_3$, water, 2M H_2SO_4, water, and aq. $NaHCO_3$. The solution was dried (Na_2SO_4) and concentrated. The syrup obtained was dissolved in methanol (40 mL) and treated with 4-toluenesulfonic acid (0.19 g) for 15 min at room temperature. Triethylamine (4 mL) was added and the solvent was evaporated. Column chromatography (2:1→1:1→2:3 hexane–ethyl acetate) of the residue gave **12** (1.7 g, 45%) as a solid, m.p. 90–91, $[\alpha]_D^{20}$ +68° (*c* 0.8, chloroform).

Anal. Calc. for $C_{46}H_{42}O_{14}$: C, 67.47; H, 5.17. Found: C, 67.74; H, 5.35.

Acetylation of **12** gave the diacetate **13**; ^{1}H-n.m.r. ($CDCl_3$): δ 8.10–7.20 (m, 25 H, 5 Ph), 5.97 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4′), 5.72 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.5 Hz, H-2′), 5.56 (dd, 1 H, H-3′), 5.18 (dd, 1 H, $J_{2,3}$ 7.8, $J_{3,4}$ 8.5 Hz, H-3), 4.91 (m, 2 H, H-1′,2), 4.78 (d, 1 H, *J* 12.3 Hz, PhC*H*), 4.57 (dd, 1 H, $J_{5',6'a}$ 6.7, $J_{6'a,6'b}$ 11.2 Hz, H-6′a), 4.50 (d, 1 H, PhC*H*), 4.44 (m, 2 H, H-1,6′b), 4.33 (t, 1 H, $J_{5',6'}$ 6.7 Hz, H-5′), 3.95 (m, 2 H, H-4,5*e*), 3.26 (dd, 1 H, $J_{4,5a}$ 10.6, $J_{5a,5e}$ 13.5 Hz, H-5*a*), and 1.99 (s, 6 H, 2 $OCOCH_3$).

O-*β*-D-*Galactopyranosyl-(1→4)*-D-*xylopyranose (***2***).* — Compound **12** (1.6 g, 2 mmol) was treated with methanolic 0.1M sodium methoxide (4 mL) for 15 min at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin and the solution concentrated after filtration. The syrup obtained was dissolved in ethanol (50 mL) and water (10 mL), and hydrogenolyzed (0.2 MPa) in the presence of 10% Pd–C (0.16 g) at room temperature. When t.l.c. (2:1 chloroform–methanol) indicated complete debenzylation, the catalyst was collected on Celite, and washed with methanol, and the combined filtrate and washings were concentrated. Column chromatography (12:10:1 chloroform–methanol–acetic acid) gave a first fraction (0.16 g, 26%) of **2** slightly contaminated, and then pure **2** (0.43 g, 70%) as a white solid, m.p. 160–168°, $[\alpha]_D^{20}$ +22° (initial) → +15.2° (24 h; *c* 1.0, water) {lit.[24], $[\alpha]_D$ + 18° (*c* 0.5, water)}; ^{1}H-n.m.r. (D_2O): δ 5.17, 4.58 (2d, 1 H, $J_{1,2}$ 3.8 and 7.9 Hz, H-1α,1*β*), 4.55, 4.45 (2d, 1 H, $J_{1',2'}$, 7.8 Hz, H-1′), 4.05 (dd, 1 H, $J_{4,5e}$ 5.3, $J_{5a,5e}$ 11.6 Hz, H-5*e*), 3.91 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4′), 3.38 (dd, 1 H, $J_{4,5a}$ 10.6 Hz, H-5*a*), and 3.25 (dd, 1 H, $J_{2',3'}$ 9.4 Hz, H-2′).

Anal. Calc. for $C_{11}H_{20}O_{10}$: C, 42.31; H, 6.46. Found: C, 42.16; H, 6.60.

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