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THE CONVERSION OF 2,6-ANHYDRO-1-DEOXY-D-*galacto*-HEPT-1-ENITOL INTO 1-DEOXY-D-*galacto*-HEPTULOSE BY β-D-GALACTOSIDASE*

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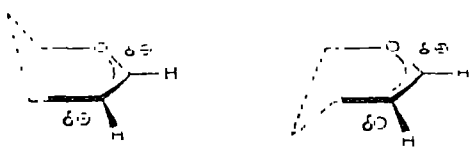
ABSTRACT

2,6-Anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**22**) was prepared by a multistep synthesis from 2,6-anhydro-D-*glycero*-L-*manno*-heptonic acid methyl ester (**9**). β-D-Galactosidase catalysed the conversion of **22** into 1-deoxy-D-*galacto*-heptulose (**24**), and in the presence of glycerol, glyceryl 2,6-anhydro-1-deoxy-β-D-*galacto*-heptuloside (**25**) was also formed; **25** was a substrate for β-D-galactosidase. The facile, enzyme-catalysed addition of water or glycerol to the double bond of **22** is compared with the normal β-D-galactoside cleavage in terms of a common triggering process. The enzyme-catalysed hydrolysis of **25** is the first example of a ketoside being cleaved by an aldosidase.

INTRODUCTION

β-D-Glucosidase from sweet-almond emulsin[1], β-D-galactosidase from *E. coli*[1,2], and maltase from *Candida tropicalis*[3] (but not that from yeast[1]) catalyse the addition of water or glycerol to D-glucal and D-galactal to give 2-deoxyhexoses and glyceryl 2-deoxyhexopyranosides. These reactions suggest that the glycal is held in a flattened conformation [^4E (**1**) or E_4 (**2**)], so that optimal resonance between the ring

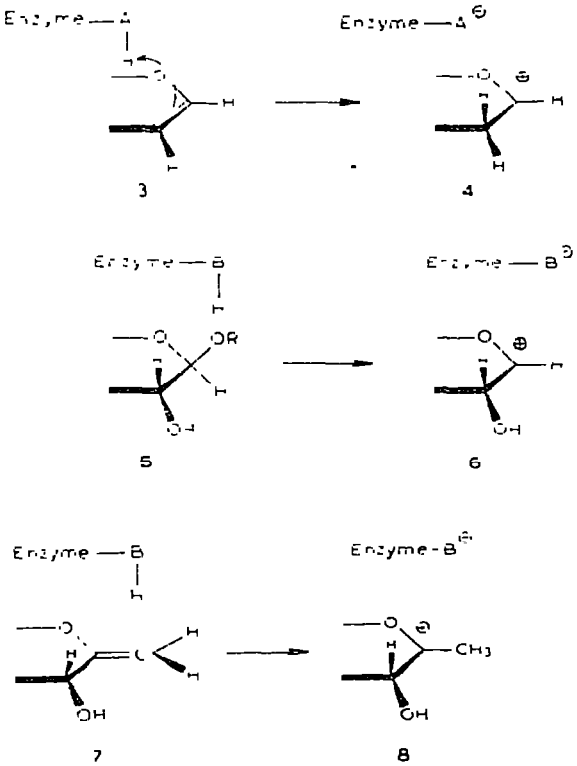


1 **2**

oxygen and double bond is possible, and so that a proton-donating group (AH) of the enzyme is situated in the vicinity of C-2 and mediates the formation of a 2-deoxyglycosyl cation (*e.g.*, **3** → **4**).

*Uncommon Results of Glycosidase Action: Part II. Part I: M. Brockhaus and J. Lehmann, *FEBS Lett.*, 62 (1976) 154–156.

It is probable that the pathways of enzymic β-D-glucoside and β-D-galactoside hydrolysis also include glycosyl cations, and it is likely that a group (BH) protonates the glycosidic oxygen, thereby making the aglycon a good leaving group and facilitating the production of a glycosyl cation (**5** → **6**). If this assumption is correct, the hypothetical grouping B–H might also be capable of protonating a nucleophilic carbon atom in a sterically comparable substrate, *e.g.* **7** → **8**.



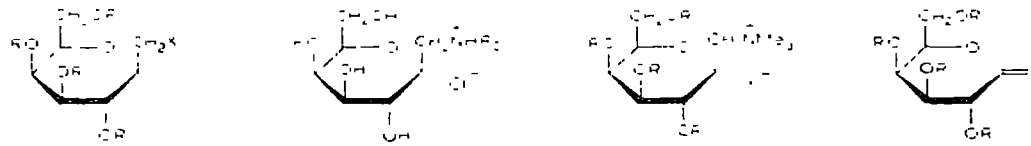
In order to demonstrate the validity of our reasoning, 2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**22**) was synthesised, and studied as a novel type of substrate for β-D-galactosidase.

RESULTS AND DISCUSSION

2,6-Anhydro-D-*glycero*-L-*manno*-heptonic acid methyl ester (**9**) was the starting material in the synthesis of 2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**22**). The 5,7-*O*-benzylidene derivative (**10**) of **9** was reduced to 2,6-anhydro-5,7-*O*-benzylidene-D-*glycero*-L-*manno*-heptitol (**12**), which was tosylated and then debenzalated to give 2,6-anhydro-1-*O*-tosyl-D-*glycero*-L-*manno*-heptitol (**14**). Acetylation of **14** and subsequent iodine-exchange gave 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-1-deoxy-1-iodo-

D-*glycero*-L-*manno*-heptitol (**16**). Dehydrohalogenation of **16** yielded 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**23**) which was deacetylated to give **22**. For kinetic studies, ^{14}C-labelled **22** was prepared in a similar way, starting with tetra-*O*-acetyl-α-D-galactopyranosyl bromide and mercury(II) ^{14}C-cyanide.

Hofmann elimination of 1-amino-2,6-anhydro-1-deoxy-D-*glycero*-L-*manno*-heptitol (**17**), which seemed to offer a shorter route to **22**, did not yield the desired product.



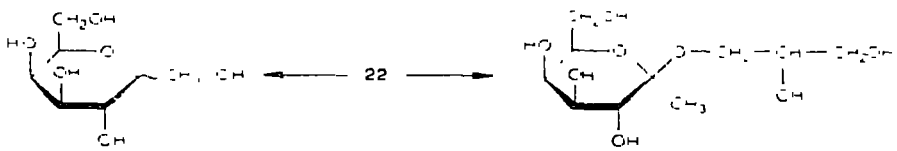
14 R = H, X = CF_3
15 R = Ac, X = CF_3
16 R = Ac, X = I

17 R = –
18 R = Me

19 R = H, X = I
20 R = H, X = OH
21 R = Ac, X = OAc

22 R = H
23 R = Ac

2,6-Anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**22**) is readily hydrated to give 1-deoxy-D-*galacto*-heptulose (**24**) on incubation with β-D-galactosidase: **24** is also formed at a much lower rate by non-enzymic addition of water to **22**. The structure of **24** was proved by preparing the known 2,5-dichlorophenylhydrazone[4]. The rates of enzymic and non-enzymic reaction are shown in Fig. 1. Rate determinations were carried out with 2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol-*1*-^{14}C (**22**)



24 **25**

The rate of enzymic hydration of **22** is inhibited by isopropyl 1-thio-β-D-galactopyranoside (Fig. 1), and preliminary experiments revealed that the inhibition is competitive. The apparent K_m is within the range 0.05–0.07M, and the velocity constant (k_{cat}) is 41–64 sec^{-1}. The data were calculated for a highly purified enzyme with a mol. wt. 525,000 and a specific activity of 360 U/mg. The reasonable value of k_{cat} (see Refs. 5 and 6) may be explained by the assumption that a stable, tertiary carboxonium ion is involved in the rate-determining step, which may support the hypothesis that the galactoside cleavage resembles an S_N1 (Ref. 7) or neighbouring-group reaction[8], rather than an S_N2 type reaction[9,10].

The enzymically induced transformation of glycals[1-3] and the conversion **22** → **24** illustrate the behaviour of glycosidases as specific protonating agents. Thus, if the glycosyl moiety possesses certain structural features, then a group (-AH or -BH)

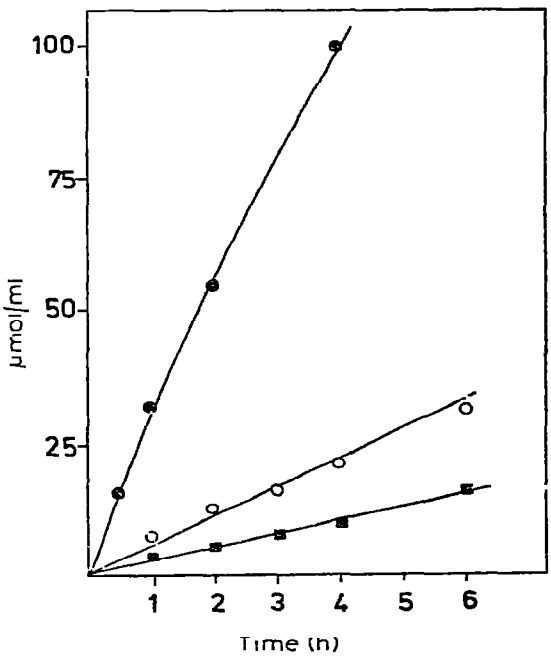


Fig. 1. Formation of 1-deoxy-D-*galacto*-heptulose-*1*-^{14}C (**24**) by the addition of water to 2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol-*1*-^{14}C (**22**) (350 µmole/ml): (*a*) in the presence of 9.3 U/ml of β-D-galactosidase using 0.1 M sodium phosphate buffer (pH 6.8) containing mM $MgCl_2$ at 30° (—●—); (*b*) as in (*a*), but with the addition of isopropyl 1-thio-β-D-galactopyranoside (50 µmole/ml) (—○—); (*c*) spontaneously (—■—).

at the active site of the enzyme will trigger a chemical reaction which need not be the ordinary cleavage of a glycosidic bond.

Incubation of 2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**22**) with glycerol-*1,3*-^{14}C and β-D-galactosidase yielded (*2R*)-glyceryl-*1,3*-^{14}C 1-deoxy-β-D-*galacto*-heptuloside (**25**). Periodate oxidation of **25** gave one mole of formaldehyde-^{14}C, thus proving that the glycosidic link involved C-1 of glycerol. The chirality of the glyceryl moiety was assumed from analogous transfer reactions[11] with β-D-galactosidase. β-D-Galactosidase hydrolysed **25** to give **24** and glycerol, thereby establishing **25** as a β-D-glycoside.

To our knowledge, the enzymic cleavage of **25** is the first example of ketoside hydrolysis mediated by β-D-galactosidase. A comparison was therefore made with glyceryl 2-deoxy-β-D-*lyxo*-hexopyranoside (Fig. 2). The release of glycerol from the latter compound was much slower than from **25**, and under the same conditions glyceryl β-D-galactopyranoside was hydrolysed completely within 5 min. Steric reasons may account for the lower rate of hydrolysis of **25** compared to that of glyceryl β-D-galactopyranoside. The much lower rate of hydrolysis of glyceryl 2-deoxy-β-D-*lyxo*-hexopyranoside, compared to that of **25** (Fig. 2), may be due to the anchimeric assistance by a neighbouring OH group, which is present in **25** but not in

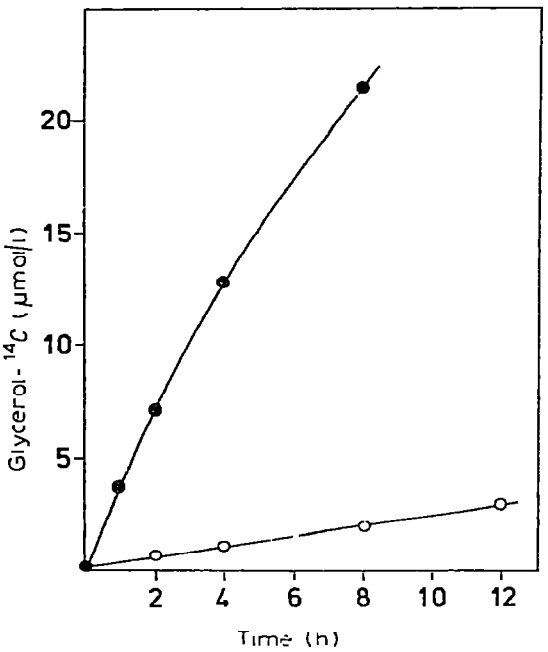


Fig. 2. Enzymic hydrolysis of glyceryl *1,3*-^{14}C-1-deoxy-*β*-D-*galacto*-heptuloside (**25**) (50 μmole/l; —●—) and glyceryl *1,3*-^{14}C-2-deoxy-*β*-D-*lyxo*-hexopyranoside (50 μmole/l; —○—) in 0.1M sodium phosphate buffer (pH 6.8) containing mM $MgCl_2$ at 30° by *β*-D-galactosidase (6.7 U/ml).

the former compound, and/or to the stabilisation of the intermediary carboxonium ion by the methyl group in **25**.

EXPERIMENTAL

General methods. — T.l.c. was performed on silica gel F_{254} (Merck) with ethyl acetate–propan-2-ol–water (125:70:35) for compounds having free hydroxyl groups, and ether–light petroleum (b.p. 60–70°) (4:1) for fully protected compounds. Detection was effected by charring with sulphuric acid. P.c. was performed on Whatman No. 1 paper with 1-butanol–pyridine–water (6:4:3). Radioactive compounds were located with a Packard 7200 radiochromatogram scanner, and inactive compounds containing vicinal hydroxyl groups with the periodate–Schiff's reagent[12]. G l c. was performed with glass columns containing 3% of SE 52 on Chromosorb G AW-DMCS, using nitrogen as carrier gas and flame-ionization detection.

I.r. and n.m.r. (internal Me_4Si) data were obtained with Perkin–Elmer Infracord Model 137 and Varian A-60 spectrometers, respectively. Optical rotations were measured with a Perkin–Elmer 141 polarimeter.

The radioactivity of samples in solution was measured with a Nuclear Chicago 724 liquid scintillation counter.

Enzyme reactions — β-D-Galactosidase (EC 3.2.1.23) from *E. coli* was purchased from Boehringer (Mannheim, Germany), and the suspension (5 mg/ml; specific activity, 200 U/mg) was used without further purification. All enzyme reactions were performed at 30° in 0.1M sodium phosphate buffer (pH 6.8) which contained 10^{-3}M magnesium chloride.

The units (U) of enzymic activity relate to the hydrolysis of *o*-nitrophenyl β-D-galactopyranoside (1.8mM) which was followed spectrophotometrically at 405 nm. One unit (U) of enzyme is defined as that amount which catalyses the formation of one μmole of *o*-nitrophenol per min

3,4-Di-O-acetyl-2,6-anhydro-5,7-O-benzylidene-D-glycero-L-manno-*heptonic acid 1-methyl ester* (**11**). — 2,6-Anhydro-D-*glycero*-L-*manno*-heptonic acid 1-methyl ester[13] (**9**, 75 g) and finely powdered $ZnCl_2$ (80 g) were shaken with benzaldehyde (400 ml) for 4 h. The main part of the benzaldehyde was removed by evaporation at 25°/10^{-3} Torr. The residue was treated with pyridine (500 ml) and acetic anhydride (400 ml). After 12 h, the mixture was stirred into ice-water (5 l), and the precipitate was collected and crystallised from methanol–ethanol (4:1) to give **11** (89 g, 66%), m.p. 201°, $[\alpha]_{578}^{25}$ +106° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): δ 2.07 and 2.12 (2 s, 6 H, 2 AcO), 3.83 (s, 3 H, OMe).

Anal. Calc. for $C_{19}H_{22}O_9$: C, 57.87; H, 5.62. Found: C, 57.83; H, 5.66.

2,6-Anhydro-5,7-O-benzylidene-D-glycero-L-manno-*heptonic acid 1-methyl ester* (**10**). — Compound **11** (12.5 g) was stirred with 0.01M sodium methoxide in methanol (100 ml). After 3 h, when dissolution was complete, concentration to 50 ml yielded a precipitate which was collected, and thrice recrystallized from ethanol to give **10** (7.5 g, 76%), m.p. 139° (sintering at 83–87°), $[\alpha]_{578}^{25}$ +34° (*c* 1, water); ν_{max}^{KBr} 3350 (OH) and 1720 cm^{-1} (CO).

Anal. Calc. for $C_{15}H_{18}O_7$: C, 58.06; H, 5.85. Found: C, 58.00, H, 6.11.

1,3,4-Tri-O-acetyl-2,6-anhydro-5,7-O-benzylidene-D-glycero-L-manno-*heptitol* (**13**). — To a solution of **11** (45.5 g) in either (600 ml), $LiAlH_4$ (10 g) was added cautiously. The mixture was boiled under reflux for 12 h and excess of reductant was destroyed by the addition of ethyl acetate (10 ml) and then water (50 ml). The mixture was concentrated to dryness, and the residue was stirred with pyridine (250 ml) and acetic anhydride (200 ml) for 14 h. Volatile material was evaporated *in vacuo*, and the residue was vigorously shaken with water (400 ml) and chloroform (350 ml). The organic layer (separated by centrifugation) was dried ($MgSO_4$) and concentrated, and the residue was crystallized from ether to give **13** (35 g, 63%), which contained 1 mol. of ether as shown by n.m.r. spectroscopy. Crystallization from methanol gave a product which contained 1 mol. of methanol of crystallization. Solvent of crystallization could be removed by heating to 80° *in vacuo* for several hours. The solvent-free product **13** had m.p. 121°, $[\alpha]_{578}^{25}$ +96° (*c* 1, chloroform). N.m.r. data ($CDCl_3$). δ 2.08 and 2.13 (2 s, each 6 H, 4 AcO).

Anal. Calc. for $C_{20}H_{24}O_9$: C, 58.82; H, 5.92. Found: C, 58.62; H, 5.81.

2,6-Anhydro-5,7-O-benzylidene-D-glycero-L-manno-*heptitol* (**12**). — M Sodium methoxide in methanol (1 ml) was added to a solution of **13** (15 g) in methanol

(200 ml). After 3 h, the solution was concentrated to 50 ml, and the precipitate was collected and thrice crystallized from methanol to give **12** (5.5 g, 53%), m.p. 206–207°, $[\alpha]_{578}^{25}$ +38° (*c* 1, water); ν_{max}^{KBr} 3450 (OH) and 3300 cm^{-1} (OH)

Anal. Calc. for $C_{14}H_{18}O_6$: C, 59.97; H, 6.43. Found: C, 59.91; H, 6.18.

3,4,5,7-Tetra-O-acetyl-2,6-anhydro-1-deoxy-1-iodo-D-glycero-L-manno-*heptitol* (**16**). — Selective tosylation of **12** was performed according to the method of Fuchs and Lehmann[14]. To a solution of **12** (6 g) in pyridine (100 ml), hexamethyldisilazane (15 g) and chlorotrimethylsilane (10 g) were added. After 8 h, the solution was concentrated to a syrup, and a solution of this syrup in CCl_4 (150 ml) was washed with water (2 × 100 ml), dried ($MgSO_4$), and concentrated. The residue was dissolved in pyridine (100 ml), water (1 ml), and acetic acid (0.5 ml), and the reaction was followed by t.l.c. (benzene–methanol, 4:1). When the reaction was complete, CH_2Cl_2 (700 ml) was added and the solution was shaken with cold water (3 × 700 ml), then dried ($MgSO_4$), and concentrated. To the syrupy residue, dry pyridine (100 ml) and tosyl chloride (6 g) were added. Excess of tosyl chloride was decomposed after 8 h by the addition of water (2 ml), and the mixture was concentrated to dryness. The residue was debenzylidenated with 80% acetic acid (100 ml) at 90° for 4 h. The hydrolysate was concentrated *in vacuo* and the syrupy residue (19 g), which contained **14**, was esterified with acetic anhydride (130 ml) and pyridine (180 ml). After 14 h, the mixture was concentrated *in vacuo* and the syrupy residue of 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-1-*O*-tosyl-D-*glycero*-L-*manno*-heptitol (**15**, 27 g) was submitted to iodine exchange without further purification.

A mixture of impure **15** (27 g) and sodium iodide (12 g) in acetic anhydride (150 ml) was stirred at 125° for 30 min, then cooled and filtered, and the insoluble material was washed with acetone. The combined filtrate and washings were concentrated *in vacuo* to give a dark syrup, and a solution of this syrup in chloroform (200 ml) was washed with 2% aqueous sodium hydrogen carbonate (2 × 500 ml) containing sodium bisulphite (2 × 0.1 g), and then water (500 ml), dried ($MgSO_4$), and concentrated. The syrupy residue (10 g) was eluted from a column (6 × 100 cm) of silica gel with 4:1 ether–light petroleum (b.p. 60–70°) and recrystallized from 1:1 ether–hexane to give **16** (5.0 g, 50% with respect to **12**), m.p. 92°, $[\alpha]_{578}^{25}$ −7° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): δ 2.02 (s, 3 H, OAc), 2.11 (s, 6 H, 2 AcO), 2.20 (s, 3 H, OAc).

Anal. Calc. for $C_{15}H_{21}IO_9$: C, 38.15; H, 4.48. Found: C, 38.22; H, 4.45.

3,4,5,7-Tetra-O-acetyl-2,6-anhydro-1-deoxy-D-galacto-*hept-1-enitol* (**23**). — A mixture of **16** (4.8 g) and AgF (7 g) was shaken in pyridine (15 ml) for 1 h, then stirred into ether (1000 ml), and shaken vigorously for 1 h. The filtered mixture was washed with 1% aqueous sodium thiosulphate (100 ml) and water (3 × 100 ml), dried ($MgSO_4$), and concentrated *in vacuo* to give **23** (3.3 g, 94%), $[\alpha]_{578}^{25}$ +70° (*c* 1.1, chloroform), which decomposed on distillation at 135°/10^{-3} Torr. N.m.r. data ($CDCl_3$): δ 2.01–2.17 (4 s, 12 H, 4 AcO).

Anal. Calc. for $C_{15}H_{20}O_9$: C, 52.32; H, 5.86. Found: C, 52.26; H, 5.97.

2,6-Anhydro-1-deoxy-D-galacto-*hept-1-enitol* (**22**). — M Sodium methoxide in

methanol (0.1 ml) was added to a solution of **23** (3.3 g) in methanol (20 ml). After 3 h, the solution was concentrated *in vacuo* and the residue was recrystallised twice from ethanol to give **22** (1.1 g, 65%), m.p. 174°, $[\alpha]_{578}^{25}$ +166° (*c* 1, water), R_F 0.63 (p.c.); ν_{max}^{KBr} 3500 (OH), 3250 (OH), and 1650 cm^{-1} (C=C).

Anal. Calc. for $C_7H_{12}O_5$: C, 47.72; H, 6.87. Found: C, 47.72; H, 6.68.

2,6-Anhydro-D-glycero-L-manno-*heptonic acid 1-methyl ester*-1-^{14}C (**9**). — A solution containing sodium cyanide-^{14}C (2 mCi; specific activity, 49 mCi/mmole; Farbwerke Hoechst, Germany) and sodium cyanide (5 mg) in water (0.3 ml) was frozen (liquid nitrogen) into a micro-distillation apparatus. A layer of water (0.3 ml) was then added and frozen, followed by a layer of 50% sulphuric acid (0.5 ml). The mixture was warmed to 50° and the hydrogen cyanide-^{14}C was distilled on to yellow mercury(II) oxide (8 mg) cooled in liquid nitrogen. The distillate was allowed to warm to room temperature and shaken until dissolution of mercuric oxide was complete. Excess of water and hydrogen cyanide were distilled off, and the crystalline residue was dried in a desiccator over KOH and then stirred for 24 h with a solution of tetra-*O*-acetyl-α-D-galactopyranosyl bromide (100 mg) in nitromethane (1 ml). The mixture was concentrated, the residue was dissolved in methanol (2 ml), and M sodium methoxide in methanol (0.5 ml) was added. Precipitated mercury salts were removed by centrifugation, the solution was concentrated, 25% aqueous sodium hydroxide (1 ml) was added, and the mixture was heated to 95° for 2 h. After cooling, the solution was acidified (to Methyl Orange) with 10% methanolic HCl, centrifuged, and concentrated to dryness. Methanol (3 ml) was added to the dry residue, and the mixture was boiled under reflux for 20 h. The solution (0.3 mCi total activity) was concentrated to 0.5 ml and centrifuged. P.c. (Whatman No. 3 paper) showed nearly pure title material which was cochromatographed with an unlabelled sample of **9**.

2,6-Anhydro-5,7-O-benzylidene-D-glycero-L-manno-*heptonic acid*-1-^{14}C *1-methyl ester* (**10**). — A freeze-dried eluate of **9**-*1*-^{14}C (~150 μCi) was stirred with freshly distilled benzaldehyde (1 ml) and anhydrous zinc chloride (40 mg). After 10 h, benzaldehyde was removed at 20^{-3}/10^{-3} Torr. The residue was chromatographed on Whatman No. 3 paper, and the product (R_F 0.82) was located by using a radio-chromatogram scanner.

2,6-Anhydro-5,7-O-benzylidene-D-glycero-L-manno-*heptitol*-1-^{14}C (**12**). —To a solution of the freeze-dried eluate of **10**-*1*-^{14}C (~150 μCi) in dry tetrahydrofuran (1 ml), $LiAlH_4$ (25 mg) was added, and the mixture was boiled under reflux for 2 h. Water (0.5 ml) was added cautiously, and the resulting pulp was centrifuged. The precipitate was washed with methanol (2 × 2 ml), the combined solutions were concentrated, and the residue was chromatographed on Whatman No. 3 paper. The main product **12**-*1*-^{14}C (~100 μCi) had R_F 0.78, and its identity was proved by cocrystallization with **12**.

2,6-Anhydro-1-O-tosyl-D-glycero-L-manno-*heptitol*-1-^{14}C (**14**). — To a freeze-dried eluate of **12**-*1*-^{14}C (~50 μCi), dry pyridine (0.15 ml) and tosyl chloride (5 mg) were added. After 15 min, a sample (1 μl) was withdrawn and the remainder was frozen (liquid nitrogen). The sample was treated with 50% acetic acid (20 μl) at 95° for

30 min. P.c. (Whatman No. 4 paper) then showed D-*glycero*-L-*manno*-heptitol-*1*-^{14}C (R_F 0.40) and **14**-*1*-^{14}C (R_F 0.82). The reaction mixture was then restored to room temperature and the above procedure was repeated. After a reaction time of 1 h, the selective tosylation was optimal. Water (5 μl) was then added to the reaction mixture which was concentrated to dryness, and the residue was treated with 50% acetic acid (200 μl) at 95° for 1 h. P.c. (Whatman No. 1 paper) then showed 33% of non-tosylated material (R_F 0.37), 54% of **14**-*1*-^{14}C (R_F 0.78), and 13% of ditosylate (R_F 0.91).

3,4,5,7-Tetra-O-acetyl-2,6-anhydro-1-deoxy-1-iodo-D-glycero-L-manno-*heptitol*-1-^{14}C (**16**). — The freeze-dried eluate of **14**-*1*-^{14}C (~27 μCi) was treated with pyridine (1 ml) and acetic anhydride (0.5 ml) for 8 h. The mixture was then concentrated, and the residue was stirred with acetic anhydride (1 ml) and sodium iodide (50 mg) at 125° for 30 min. The mixture was concentrated to dryness, the residue was extracted with chloroform (2 ml), and the extract was washed with water (3 × 2 ml) and dried ($MgSO_4$). The solution (14 μCi) contained (t.l.c.) nearly pure **16**-*1*-^{14}C.

2,6-Anhydro-1-deoxy-D-galacto-*hept-1-enitol*-1-^{14}C (**22**). — Unlabelled **16** (700 mg) was added to a solution of **16**-*1*-^{14}C (15 μCi) in chloroform. The preparation of **23**-*1*-^{14}C and **22**-*1*-^{14}C was analogous to that described for the unlabelled compounds. The specific activity of **22**-*1*-^{14}C remained constant during three crystallizations (7.6 μCi/mmol, yield 152 mg). A second crop (2.2 μCi/mmol, 206 mg) was obtained from the mother liquor by crystallization with **22** (200 mg).

1-Deoxy-D-galacto-*heptulose* (**24**). — β-D-Galactosidase (15 U) was added to a solution of **22** (70 mg) in buffer (1 ml). The course of the reaction was followed by t.l.c. The product was homogeneous on t.l.c. and p.c. (R_F 0.46, Whatman No. 1 paper).

A solution of 2,5-dichlorophenylhydrazine (100 mg) and a freeze-dried sample of **24** (80 mg) in methanol (5 ml) was boiled under reflux for 30 min. The precipitate was collected, and recrystallized from methanol to give 1-deoxy-D-*galacto*-heptulose 2',5'-dichlorophenylhydrazone (83 mg), m.p. 189–191° (dec.), $[\alpha]_{578}^{25}$ −22.5° (*c* 1, pyridine); lit.[4] m.p. 186.5° (dec.), $[\alpha]_D^{25}$ −22.6° (*c* 2.06, pyridine).

Kinetic studies on the hydration of **22**. — The concentration of **22**-^{14}C (7.6 μCi/mmol) was 10.0 mg in a total volume of 160 μl, and 1.50 U of β-D-galactosidase was used for each experiment. Isopropyl 1-thio-β-D-galactopyranoside (50 μmol/ml) was used in the inhibition experiment. The course of the reaction was followed by taking samples (25 μl) at suitable intervals, treating with pyridine (75 μl) at 95°, and analysing by p.c. (Whatman No. 1 paper). The product **24**-^{14}C was eluted, freeze-dried, and quantified by liquid scintillation counting. The same procedure was employed for estimating the apparent K_m.

Glyceryl-1,3-^{14}C *2,6-anhydro-1-deoxy*-β-D-galacto-*heptuloside* (**25**). — Glycerol (240 mg), glycerol-*1,3*-^{14}C (5 μCi, 26 mCi/mmol), and **22** (150 mg) were incubated with β-D-galactosidase (37 U) in buffer (3 ml) at 30°. After ~2 days, **22** had disappeared (t.l.c.). The solution was then heated to 95°, centrifuged, and chromatographed on a column (100 × 3 cm) of cellulose powder (Merck) with 1-butanol–pyridine–water (6:4:3). The first radioactive fraction contained glycerol. The second,

which contained **25**, was concentrated and the residue rechromatographed on Whatman No. 3 paper. The product was located by autoradiography (exposure time, 14 days) and eluted with water, and the solution was freeze-dried to give **25** (25 mg, 11%) which was pure by t.l.c. and p.c. The trimethylsilyl derivative[15] was homogeneous on g.l.c.

Periodate oxidation of **25**. — To an aqueous solution (50 µl) of **25**-*1',3'*-^{14}C (0.10 µCi, 26 mCi/mmol), 10% aqueous sodium metaperiodate (20 µl) was added. After 45 min, water (0.5 ml) and aqueous 35% formaldehyde (0.1 ml) were added, and the mixture was frozen. All volatile material was evaporated at 10^{-3} Torr into a liquid-nitrogen trap. An aqueous solution (150 ml) of dimedone (0.75 g) was added to the distillate. After 14 h, the formaldehyde dimedone derivative was collected (278 mg), and the radioactivity in an aliquot was measured by liquid scintillation counting. With quench correction, 50% of the initial activity in **25**-*1',3'*-^{14}C was detected in the precipitate.

Enzymic hydrolyses of **25**, *glyceryl β-*D*-galactopyranoside, and glyceryl 2-deoxy-β-*D*-lyxo-hexopyranoside*. — Glyceryl-*1,3*-^{14}C β-D-galactopyranoside[10] and glyceryl-*1,3*-^{14}C 2-deoxy-β-D-*lyxo*-hexopyranoside[1], prepared using glycerol-*1,3*-^{14}C (26 mCi/mmole) as acceptor, had R_F 0.30 and 0.41, respectively, on Whatman No. 1 paper. **25**-*1',3'*-^{14}C (26 mCi/mmole) was synthesized in an analogous manner. Enzyme solution (0.75 U in 10 µl) was added to each substrate solution (100 µl, 1.3 µCi/ml) at 30°. Samples were heated to 95°, chromatographed on Whatman No. 1 paper, and scanned. The glycerol-*1,3*-^{14}C (R_F 0.59) was eluted, dried in a desiccator over phosphorus pentaoxide, and quantified by liquid scintillation counting.

2,6-Anhydro-1-deoxy-1-dimethylamino-D-glycero-L-manno-*heptitol hydrochloride* (**18**). — A mixture of 1-amino-2,6-anhydro-1-deoxy-D-*glycero*-L-*manno*-heptitol hydrochloride[3] (**17**, 10 g), paraformaldehyde (35 g), and water (1 ml) was stirred at 160° for 16 h. The mixture was then boiled with water (200 ml) for 1 h, filtered, boiled for 2 days after adding conc. HCl (200 ml), and then concentrated *in vacuo*. The resulting syrup was crystallised from hot ethanol to give **18** (7.5 g, 67%), m.p. 205°, $[\alpha]_{578}^{25}$ +18° (*c* 1, water).

Anal. Calc. for $C_9H_{20}ClNO_5$: C, 41.94; H, 7.82; N, 5.44. Found: C, 41.75; H, 7.62; N, 5.26.

2,6-Anhydro-1-deoxy-1-trimethylammonio-D-glycero-L-manno-*heptitol iodide* (**19**). — A solution of **18** (4 g) in water (100 ml) was treated with Amberlite IRA-400 (HO^-) resin (30 ml) and then concentrated *in vacuo*. A solution of the residual syrup in ethanol (100 ml) and methyl iodide (7 ml) was boiled under reflux for 1 h and then cooled. The product which separated was recrystallized from 96% ethanol to give **19** (4.8 g, 85%), m.p. 133°, $[\alpha]_{578}^{25}$ +10.5° (*c* 1, water).

Anal. Calc. for $C_{10}H_{22}INO_5$: C, 33.07; H, 6.11; N, 3.86. Found: C, 32.95; H, 6.14; N, 3.60.

An aqueous solution of **19** (4 g, in 100 ml) was passed over Amberlite IRA-400 (HO^-) resin (50 ml), and then concentrated *in vacuo*. Pyrolysis of the resulting free-base (**20**) under nitrogen at various temperatures up to 160° for 5–30 min gave

(t.l.c. and p.c.) a mixture of products, the complexity of which depended on the time of pyrolysis. Unsaturated compounds (detected with permanganate) were formed only in minor amounts.

Acetylation of **20** (2 g) was achieved by stirring with acetic anhydride (15 ml) and pyridine (20 ml) until dissolution was complete. The mixture was concentrated *in vacuo*, and the residue was freed from traces of acetic anhydride by distillation of toluene (5 × 50 ml) therefrom. Pyrolysis of the product (**21**) under nitrogen, at temperatures up to 180°, also yielded a complex mixture of compounds.

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