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THE STEREOCHEMISTRY OF THE ADDITION OF GLYCEROL TO D-GALACTAL, CATALYZED BY β-D-GALACTOSIDASE*

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ABSTRACT

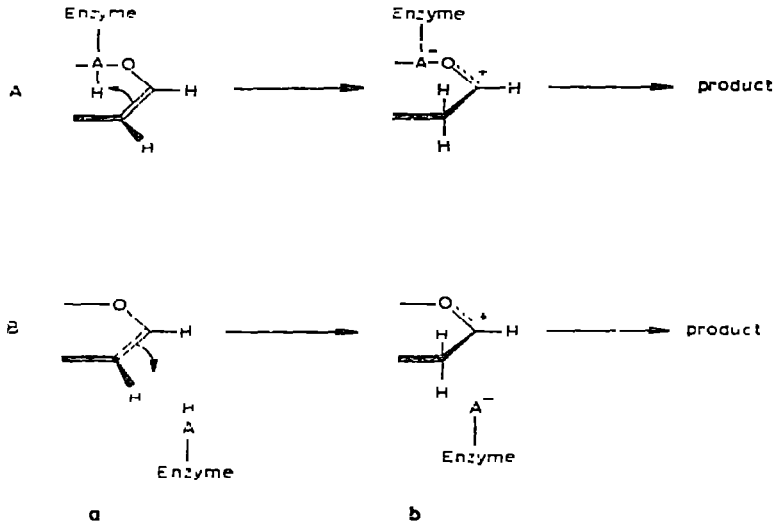
On incubation with β-D-galactosidase, D-galactal-*2-d* (**1**) plus glycerol yielded 1-deoxyglycerol-1-yl 2-deoxy-β-D-*lyxo*-hexopyranoside-*2*(*S*)-*d*. By ^{1}H-n.m.r. analysis, it was shown that the hydrogen atom introduced on C-2 is *trans*-related to the aglycon moiety. In contrast to this stereospecific, enzyme-catalyzed addition, the reaction of phenol with peracetylated **1**, catalyzed by *p*-toluenesulfonic acid, which yields phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*lyxo*-hexopyranoside-*2-d*, was shown to entail both a *trans* and a *cis* addition.

INTRODUCTION

β-D-Galactosidase from *Escherichia coli*[1,2] catalyzes the addition of water or glycerol to D-galactal (**2**) to give 2-deoxy-D-*lyxo*-hexose and 1-deoxyglycerol-1-yl 2-deoxy-β-D-*lyxo*-hexopyranoside (**3**), respectively. Corresponding reactions have been observed with β-D-glucosidase and D-glucal[1,3]. As the reaction probably requires, in the vicinity of C-2, a proton-donating group (AH) of the enzyme[1,4] in order to mediate the formation of a 2-deoxyglycosyl cation (*e.g.*, **a** → **b**), the question arises whether the protonation is stereospecific or not and, if it is, whether the protonation occurs from above or below the plane of the pyranoid ring.

Because of the stereospecific transfer to the acceptor water or glycerol, to yield a product having the β-D-anomeric configuration[5–7], the overall addition reaction would be either *cis* (as in A) or *trans* (as in B). In earlier, qualitative experiments[1], it was shown that 2-deoxy-D-*lyxo*-hexose and 2-deoxy-D-*arabino*-hexose exchange carbon-bound hydrogen (probably at C-2) when incubated in water-*t* (5 Ci/mol) for 48 h. As Hehre[8] could not find any exchange when using D_2O, and ^{1}H-n.m.r. spectroscopy as a probe, we assume that the exchange rate is extremely low and that exchange can only be detected when the highly sensitive, radiolabelling technique is applied. As 2-deoxy-D-*lyxo*-hexopyranosides did not show any exchange of carbon-bound hydrogen, even in solution in water-*t* of high specific activity, we preferred the enzymic synthesis of 1-deoxyglycerol-1-yl 2-deoxy-β-D-*lyxo*-hexopyranoside-*2-d* from

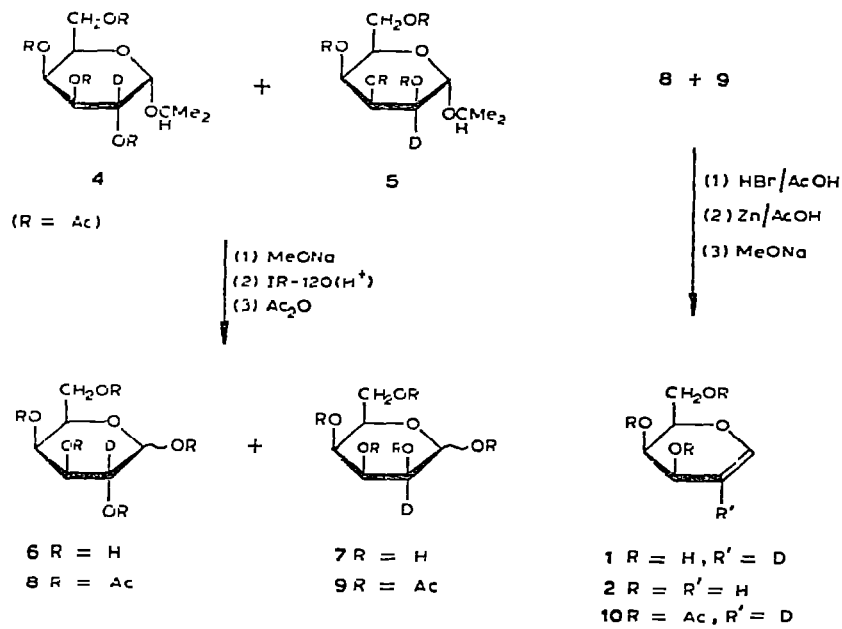
*Uncommon Results of Glycosidase Action, Part IV. For Part III, see J. Lehmann and E. Schroter, *Carbohydr. Res.*, 58 (1977) 65–72, (preceding paper).



1 and glycerol in order to elucidate the stereochemistry of the addition catalyzed by β-D-galactosidase.

RESULTS AND DISCUSSION

Compound 1 was prepared by starting with isopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside-*2-d* (4) plus its C-2 epimer[9], namely, isopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-talopyranoside-*2-d* (5), and applying the reaction sequence depicted.



Glycerol was enzymically added to **1**, and the resulting, crude glycoside 1-deoxy-glycerol-1-yl 2-deoxy-β-D *lyxo*-hexopyranoside-*2(S)-d* (**11**) was isolated by preparative t.l.c. Final purification was achieved by acetylating the crude glycoside **11**, and separating the acetylated product by preparative t.l.c. The 2,3-di-*O*-acetyl-1-deoxy-glycerol-1-yl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-*lyxo*-hexopyranoside-*2(S)-d* (**12**) so obtained was uniform according to analysis by g.l.c. Nonlabelled **12** (**13**) was obtained in the same way by using **2** instead of **1**. Comparison of the corresponding sections of the ^{1}H-n.m.r. spectra of **12** and **13** (see Fig. 1) clearly indicated that, in compounds **1** and **2**, the proton added through enzymic reaction occupies the equatorial position. Thus, it is proved that alternative pathway B is correct, and that the enzyme-catalyzed

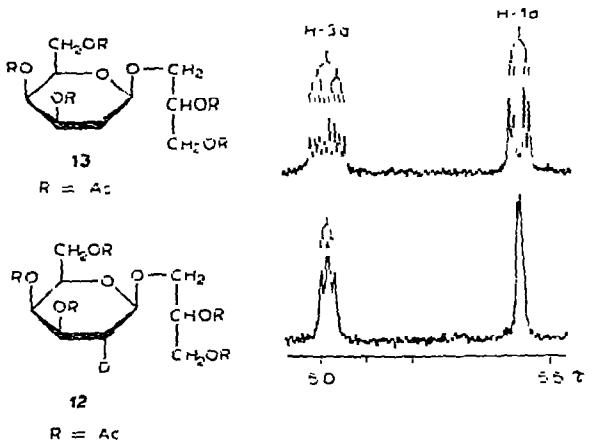


Fig. 1. Partial, 270-MHz, ^{1}H-n.m.r. spectra of **12** and **13** in chloroform-*d*.

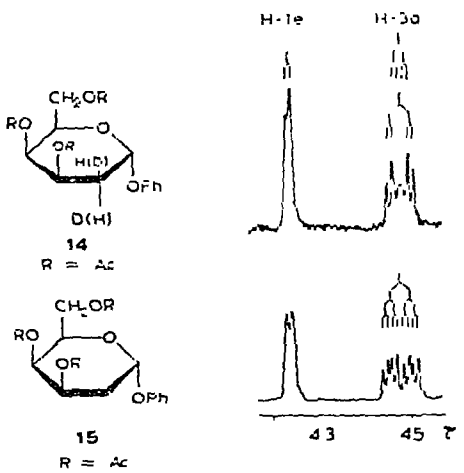


Fig. 2. Partial, 270-MHz, ^{1}H-n.m.r. spectra of **14** and **15** in chloroform-*d*.

reaction of glycerol, and, most probably, of water also, with **2** is a *trans* addition. This finding corresponds well with the results obtained by Hehre[8], who, by a different, experimental approach, showed that the reaction of D-glucal with water, catalyzed either by β-D-glucosidase or α-D-glucosidase (*Candida tropicalis*), is a *trans* addition. In order to exclude the possibility of a substrate-induced, chiral protonation of C-2 in **2**, phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*lyxo*-hexopyranoside-*2-d* (**14**) was synthesized by acid-catalyzed addition of phenol to 3,4,6-tri-*O*-acetyl-D-galactal-2-*d* (**10**). Compound **14** was compared with an authentic, unlabelled sample of phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*lyxo*-hexopyranoside[10] (**15**). Sections of the ^{1}H-n.m.r. spectra (see Fig. 2) indicated that **14** is a mixture of almost equal amounts of *cis*- and *trans*-addition products.

The results of these experiments with β-D-galactosidase seem to suggest the presence, at the binding site of the enzyme, of a group that can trigger the addition reaction to **2**, and that normally serves as a binding, or even an activating, group[4] for the substituent at C-2.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Melting points are uncorrected. T.l.c. was performed on silica gel F_{254} (Merck), with the following solvent systems (v/v): *A*, 1:1 benzene–methanol; *B*, 4:1 benzene–methanol; *C*, 25:14:7 ethyl acetate–2-propanol–water; *D*, ether; and *E*, 4:1 ether–light petroleum (b.p. 60–70°). Detection was effected by charring with sulfuric acid. Paper chromatography was performed on Whatman No. 1 paper with 6:4:3 butanol–pyridine–water. G.l.c. was conducted in glass columns of 3% of SE 52 on Chromosorb G AW-DMCS, using nitrogen as the carrier gas, and flame-ionization detection. N.m.r. data ($CHCl_3$-*d*, internal Me_4Si) were obtained with a Varian 270-MHz, Fourier-transform spectrometer unless otherwise stated.

Enzyme reactions. — β-D-Galactosidase* from *Escherichia coli* was purchased from Boehringer (Mannheim, Germany), and the suspension (5 mg/ml; specific activity 30 U/mg) was used without further purification. All enzyme reactions were performed at 40° in 0.1M sodium phosphate buffer (pH 6.8) that contained 10^{-3}M magnesium chloride and 10^{-2}M 2-mercaptoethanol.

D-*Galactose*-2-d (**6**) *and* D-*talose*-2-d (**7**). — A mixture (25.3 g) of isopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside-*2-d* (**4**) and isopropyl tetra-*O*-acetyl-α-D-talopyranoside-*2-d*[9] (**5**) was stirred with M sodium methoxide in abs. methanol (100 ml). When the reaction was complete (t.l.c., solvent *A*), the solution was evaporated. The residual syrup was dissolved in water, and the solution was treated with Amberlite IR-120 (H^+) resin for 2 days at 90–95° (t.l.c., solvent *A*). Neutraliza-

*Commercial β-D-galactosidase was used for larger-scale preparations when proof was obtained that it did not show any difference in reaction pattern compared with pure β-D-galactosidase kindly donated by Prof. Dr. K. Wallenfels.

tion with sodium hydroxide, filtration, and lyophilization yielded **6** plus **7** (9.25 g) as a syrup, identified by g.l.c. and paper chromatography.

Penta-O-acetyl-β-D-galactopyranose-2-d (**8**) *and penta-O-acetyl-β-D-talopyranose* 2-d (**9**). — A mixture of **6** and **7** (9.25 g) was converted into a mixture of **8** and **9** with[11] sodium acetate (4.2 g) and acetic anhydride (45 g). The resulting solution was poured into ice–water (200 ml), with stirring, and the solid was filtered off, and washed successively with water and ice-cold ether. Compound **8** (6 g) was obtained as colorless needles, m.p. 141°; n.m.r. data ($CDCl_3$, 60 MHz): τ 4.16 (bs, 1 H, H-1), 4.40–4.90 (m, 2 H, H-3,4), 5.72–5.88 (m, 3 H, H-5, $-CH_2-$), and 7.75–8.10 (m, 15 H, $-OCOCH_3$).

The filtrate was extracted with dichloromethane (4 × 50 ml), and the extracts were combined, successively washed with aqueous sodium hydrogencarbonate (2 × 100 ml) and water (1 × 100 ml), dried ($MgSO_4$), and evaporated *in vacuo*, to give **9** (8.5 g) as a crude oil which was not purified.

3,4,6-Tri-O-acetyl-D-galactal-2-d (**10**). — A mixture of **8** and **9** (14.5 g) was converted with 40% HBr/glac. acetic acid (20 ml) into a mixture of the corresponding tetra-*O*-acetyl-α-D-hexopyranosyl bromides[12], which was reduced[13] with zinc (30 g) in 50% acetic acid (160 ml) to give **10** as a crude oil. Elution from silica gel with solvent *E* afforded **10** (3.8 g, 38%); n.m.r. data ($CDCl_3$, 60 MHz): τ 3.38 (d, 1 H, H-1, $J_{1,3}$ 1.7 Hz), 4.37–4.95 (m, 2 H, H-3,4), 5.50–6.20 (m, 3 H, H-5, $-CH_2-$), and 7.85–8.10 (m, 15 H, $-OCOCH_3$).

D-*Galactal*-2-d (**1**). — Compound **10** (525 mg) was deacetylated with 0.1M sodium methoxide in abs. methanol (20 ml), the reaction being monitored by t.l.c. (solvent *A*). The solution was made neutral with Amberlite IR-120 (H^+) resin, and filtered. Solvent removal left an oil which crystallized from ethyl acetate to give **1** (309 mg, 93%), m.p. 103–104°.

2,3-Di-O-acetyl-1-deoxyglycerol-1-yl 3,4,6-tri-O-acetyl-2-deoxy-β-D-lyxo-hexopyranoside (**13**). — β-D-Galactosidase (0.5 ml) was added to a solution of **2** (0.5 g) and glycerol (5 ml) in buffer (200 ml). After 14 days at 40°, **2** had almost disappeared (monitored by t.l.c. in solvent *C*). The solution was heated to 90°, filtered, cooled, and lyophilized. Compound **6** was obtained as a yellow oil after repeated preparative-layer chromatography (p.l.c.; solvent *C*); it was then acetylated with acetic anhydride (3 ml) in pyridine (3 ml). After 24 h, the mixture was evaporated *in vacuo*. Repeated addition of toluene and evaporation from the oily residue left a product which was purified on p.l.c. plates (solvent *D*) to give **13** (106 mg); n.m.r. data: τ 4.74 (m, 1 H, H-4), 5.02 (m, 1 H, H-3*a*, $J_{3a,2a}$ 12 Hz, $J_{3a,2e}$ 5.5 Hz), 5.44 (m, 1 H, H-1*a*, $J_{1a,2e}$ 3 Hz, $J_{1a,2a}$ 8 Hz), 6.21 (m, 1 H, H-5), and 7.85–8.05 (m, 15 H, $-OCOCH_3$).

2,3-Di-O-acetyl-1-deoxy-D-glycerol-1-yl 3,4,6-tri-O-acetyl-2-deoxy-β-D-lyxo-hexopyranoside-2-d (**12**). — Compound **12** was prepared and isolated as for compound **13**, by use of **1** (309 mg), glycerol (3 ml) in buffer (120 ml), β-D-galactosidase (1.5 ml), temperature 40°, incubation time: 7 days, affording **11**, and, by subsequent acetylation, **12** (73 mg); n.m.r. data: τ 4.74 (m, 1 H, H-4), 5.02 (m, 1 H,

H-3*a*, $J_{3a,2e}$ 5 Hz, $J_{3a,4e}$ 3 Hz), 5.44 (m, 1 H, H-1*a*, $J_{1a,2e}$ 2.5 Hz, $J_{1,2}$ 4.3 Hz), 6.21 (m, 1 H, H-5), 8.03 (m, 1 H, H-2*e*), and 7.85–8.0 (m, 15 H, $-OCOCH_3$).

Both **12** and **13** could be deacetylated with M sodium methoxide in abs. methanol to give chromatographically homogeneous **3** and **11**, respectively.

*Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-*α*-*D*-lyxo-hexopyranoside-2-d* (**14**). — The synthesis of **14** was performed according to a modified method by Wallenfels and Lehmann[10]. Part (75 mg) of a melt of phenol (1 g) and *p*-toluenesulfonic acid (0.05 g) was heated with **10** (70 mg) for 15 sec on a water bath (60°) with vigorous shaking; the melt became dark-colored. (The mixture must not be allowed to get black.) Ice-cold, aqueous sodium hydrogencarbonate (50 ml) and chloroform (30 ml) were then added; the aqueous layer was extracted several times with chloroform. The extracts were combined, washed with water (2 × 50 ml), dried ($MgSO_4$), and evaporated *in vacuo*, to give **14** as an oil. Crystallization from ethanol yielded **14** (55 mg, 58%) in chromatographically pure form, m.p. 127°; n.m.r. data: τ 2.79 (m, 5 H, –Ph–H), 4.24 (m, 1 H, H-1, $J_{1,2e}$ 1 Hz, $J_{1,2a}$ 3.5 Hz), 4.48 (m, 1 H, H-3, $J_{3,4}$ 3.0 Hz), 4.60 (m, 1 H, H-4), 5.74 (m, 1 H, H-5), 5.94 (m, 2 H, $-CH_2-$), 7.74 (m, 1 H, H-2*a*, $J_{2a,2e}$ 13 Hz, $J_{2a,3}$ 12.5 Hz), 7.89 (m, 1 H, H-2*e*, $J_{2e,3}$ 5.5 Hz, $J_{2e,4}$ 1 Hz), 7.84 (s, 3 H, $-OCOCH_3$), 7.97 (s, 3 H, $-OCOCH_3$), and 8.07 (s, 3 H, $-OCOCH_3$).

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