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Note

# Support of a cyclic versus acyclic intermediate in enzymatic glycoside cleavage: 1,3-(*R*)-*O*-benzylidene-D-threitol is a competitive inhibitor but not a substrate of β-D-galactosidase

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Some sugar derivatives other than glycosides can be specifically converted by glycosidases. Glycals [1,2] are susceptible to enzyme-catalysed water or alcohol addition; equally, hept-1-enitols and homologues thereof are substrates of the corresponding enzymes [3]. In each case, the enzyme acts as a general acid catalyst, initiating the reaction by a stereospecific proton transfer. This proton transfer is an essential part of the ordinary transglycosylation reaction, leading to hydrolysis or synthesis of a glycoside or oligosaccharide, depending on whether the acceptor is water or an alcohol.

Although early experiments demonstrating the enzymatic transfer of an intact 6-deoxy-α-L-*arabino*-hex-5-enopyranosyl residue to glycerol [4], as well as the aforementioned enzyme-catalysed additions to cyclic enol ethers [1–3] and the ease of enzyme hydrolysis of glycosylpyridinium ions [5,6], render the ring oxygen atom unlikely as the site of activation by glycosidases, the biologically plausible and widely accepted transfer of a cyclic oxocarbonium ion is still being questioned in favour of an acyclic intermediate [7,8]. The "acyclic mechanism" of hydrolysis does not require substrate distortion to take advantage of stereoelectronic assistance when an equatorial aglycon is to be cleaved off. A "cyclic mechanism" would require distortion in order to approach antiperiplanar lone pair-leaving group orientation [9].

Acetals of benzaldehyde are very sensitive to acid-catalysed cleavage due to efficient

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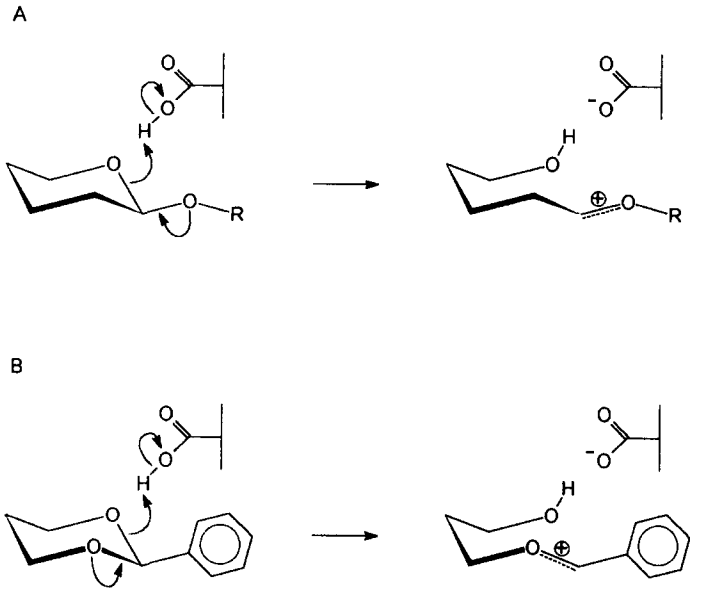
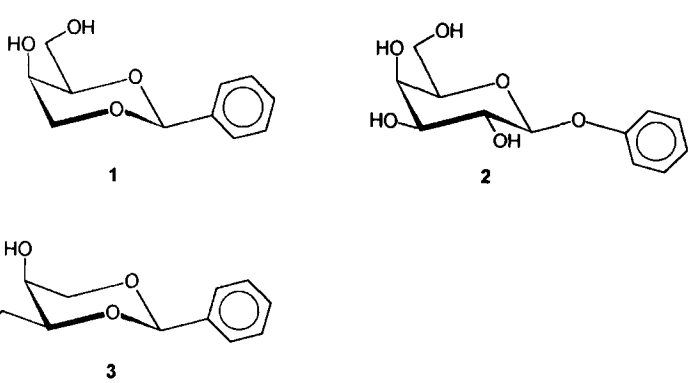



Fig. 1. Hypothetical glycoside cleavage via an acyclic intermediate (A) and hypothetical benzaldehyde acetal cleavage (1,3-dioxane system) by a glycosidase (B).

mesomeric stabilisation of a benzyloxonium ion. One would therefore expect a benzylidene derivative of a polyol structurally resembling a pyranoside to be hydrolysed by a stereochemically fitting glycosidase if the activating group has a chance to protonate one of the oxygen atoms of the 1,3-dioxane system (Fig. 1B). Such a cleavage mechanism would perfectly parallel the mechanism of glycoside cleavage via an acyclic intermediate (Fig. 1A).

The structure of 1,3-(*R*)-*O*-benzylidene-D-threitol (**1**) resembles phenyl β-D-galactopyranoside (**2**) (Scheme 1). This is also supported by the fact that **1** competitively inhibits the hydrolysis of *o*-nitrophenyl β-D-galactopyranoside by *E. coli* β-D-galac-




Scheme 1. Comparative drawing of 1,3-(*R*)-*O*-benzylidene-D-threitol (**1**), phenyl β-D-galactopyranoside (**2**), and 1,3-(*R*)-*O*-benzylidene-L-threitol (**3**).

tosidase with a remarkable $K_i$ of $0.75 \pm 0.10$ mM, which is comparable to a $K_i$ of $0.45 \pm 0.03$ mM for β-D-galactopyranosylbenzene [10]. Even prolonged incubation of **1** in the presence of high concentrations of the enzyme does not cause any hydrolysis of the acetal **1**. Specificity of binding to β-D-galactosidase is demonstrated by the ineffectiveness of **1** as an inhibitor of coffee α-D-galactosidase. Surprisingly, the equally enzyme-resistant enantiomer of **1**, 1,3-(*R*)-*O*-benzylidene-L-threitol (**3**) [11], also competitively inhibits β-D-galactosidase, although to a lesser extent ($K_i$ $3.5 \pm 0.5$ mM).

## 1. Experimental

*General methods.*—Kinetic data were obtained with an Eppendorf photometer at 405 nm connected with a transformation unit (Samson Arugolanu) and an SE 120 recorder (BBC). All reactions were monitored by TLC on Silica Gel 60 $F_{254}$ (Merck). Melting points were measured with a Büchi apparatus and are uncorrected. Optical rotations were obtained with a Schmidt and Haensch Polartronic I polarimeter. $^1$H NMR spectra were recorded with a Bruker WM 250 spectrometer at 250 MHz for solutions in $CD_3OD$ (internal $Me_4Si$). Elemental analyses were obtained with a Perkin–Elmer 240 analyzer.

*Enzymes.*—β-D-Galactosidase (EC 3.2.1.23, 640 U/mg protein) from *E. coli* and α-D-galactosidase (EC 3.2.1.22, suspension of 5 mg/mL protein in 3.2 M $NH_4OAc$ with pH 6, 10 U/mg protein) from coffee beans were purchased from Boehringer Mannheim.

*Determination of the inhibition constants* ($K_i$).—*o*-Nitrophenyl β- and *p*-nitrophenyl α-D-galactopyranoside (Fluka) were used as substrate (0.1–1.0 mM, $K_M$ $0.11 \pm 0.01$ mM and 0.2–1.0 mM, $K_M$ $0.33 \pm 0.02$ mM) in 100 mM sodium–potassium phosphate buffer (pH 6.8, 1 mM $MgCl_2$) at 30°C (β-D-galactosidase) and 100 mM potassium-phosphate buffer (pH 6.5) at 25°C (α-D-galactosidase). Inhibitors were used in the following concentrations (mM): **1** 0.5, 0.75, 1.0, 2.0 (β-D-galactosidase), and 1.0, 5.0, 10.0 (α-D-galactosidase); **3** 2.0, 3.5, 5.0, 7.0 (β-D-galactosidase). Enzymes were used in the following activities (U/mL): β-D-galactosidase 0.100; α-D-galactosidase 0.042. $K_i$ values were determined by a Dixon plot [12]. After 1 h, no hydrolysis of **1** and **3** was detectable by TLC.

*1,3-(R)-O-Benzylidene-D-threitol* (**1**).—Compound **1** was synthesised from D-arabinitol, as described by Foster et al. for the L-enantiomer [11]; $[\alpha]_D^{23}$ −6°(*c* 1.0, MeOH); $R_f$ 0.57 (27:2:1 EtOAc–MeOH-$H_2O$); mp 123°C; $^1$H NMR data ($CD_3OD$): δ 7.60–7.28 (m, 5 H, phenyl), 5.62 (s, 1 H, CH-Ar), 4.12 (t, 2 H, $J_{4,4'}$ 1.5, $J_{4,3}$ 1.5 Hz, H-4, H-4′), 4.00 (ddd, 1 H, $J_{2,1'}$ 6.5, $J_{2,1}$ 5.7, $J_{2,3}$ 1.5 Hz, H-2), 3.76 (d, 1 H, $J_{1',2}$ 6.5 Hz, H-1′), 3.75 (d, 1 H, $J_{1,2}$ 5.7 Hz, H-1), 3.61 (dd, 1 H, $J_{3,4}$ 3.0, $J_{3,2}$ 1.5 Hz, H-3). Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.83; H, 6.73. Found: C, 62.60; H, 6.83.

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