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Note

Synthesis of allyl β-D-galactopyranoside from lactose using *Streptococcus thermophilus* β-D-galactosidase

David E. Stevenson *, Richard H. Furneaux

Industrial Research Limited, PO Box 31-310, Lower Hutt, New Zealand

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Allyl glycosides are of interest for several reasons: the allyl group is convenient for protecting the anomeric hydroxyl group during the synthesis of sugar derivatives, especially oligosaccharides; it can be selectively removed in the presence of other protecting groups by initial base- or palladium-catalysed isomerisation to the 1-propenyl group, followed by mild acid hydrolysis [1]; the allyl moiety has been used in a radical-initiated polymerisation process to produce hydrophilic polymers [2].

Allyl glycosides are usually produced by the Fischer glycosidation, i.e., the acid-catalysed reaction of a free sugar in excess of allyl alcohol [3], or by directed synthesis using metal-ion-catalysed (Koenigs–Knorr type) or Lewis acid-catalysed glycosidation processes with O-protected glycosyl halides, sulfides, esters, or imidates as glycosyl donors and allyl alcohol as acceptor [4]. Since most alcohols are poor solvents for sugars, the Fischer glycosidation is usually carried out under reflux conditions in order to produce more rapidly an equilibrium mixture of anomeric pyranosides and furanosides. This process works well with such sugars as D-glucose and D-mannose, where the α-pyranoside is the dominant product and can be crystallised directly from the product mixture. Fischer glycosidations with D-galactose, however, yield mixtures containing significant amounts of α- and β-furanosides, in addition to the α- and β-pyranosides [5]. The ring size and anomeric configuration of the desired glycoside can generally be predetermined using one of the directed chemical synthesis methods referred to above, but this requires, as a minimum, a protection–glycosidation–deprotection sequence.

* Corresponding author.

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It is well known that, under favourable circumstances, glycosidase enzymes can be used to synthesise rather than hydrolyse glycosides [6–11]. In this way, a predetermined product can be obtained in a single step. β-D-Galactopyranosides are particularly accessible because there are commercially available β-D-galactosidases (β-D-galactoside galactohydrolase, EC 3.2.1.23) and lactose is a suitable cheap donor substrate. This type of reaction also produces small amounts of galactobiosides (6-*O*-β-D-galactopyranosyl-β-D-galactopyranosides) [7,8]. Optimised methods for the preparative production of alkyl β-D-galactopyranosides by this one-step procedure have been reported [10]. Allyl β-D-galactopyranoside has been prepared in good (30%) yield, from lactose, using *E. coli* β-D-galactosidase [7], but in only 13% yield using *A. oryzae* β-D-galactosidase [9], whereas the corresponding propargyl galactoside could be obtained in 42% yield [9], presumably because allyl alcohol inactivated the enzyme before it could effect complete conversion. Allyl alcohol also had a strong inactivating effect on the *K. lactis* enzyme [10].

We have recently discovered [12] that the β-D-galactosidase from *Streptococcus thermophilus* [13,14] is remarkably resistant (compared with other glycosidases) to denaturation by organic solvents in aqueous solution (e.g., 6 M or aq 38% v/v 2-fluoroethanol), and can be used to prepare ethyl and 2-fluoroethyl β-D-galactopyranosides in higher yields than obtainable using commercial enzymes from *Caldocellum saccharolyticum* [11] (recombinant, sold by Sigma as β-glucosidase), *Kluyveromyces lactis* [10] ("Biolactase" from Biocon), *Kluyveromyces fragilis* [10] ("Lactozym" from Novo), and *Aspergillus oryzae* [9,11] (Sigma). We now report that allyl β-D-galactopyranoside can be obtained in a higher yield than previously reported, by the use of this sturdier enzyme.

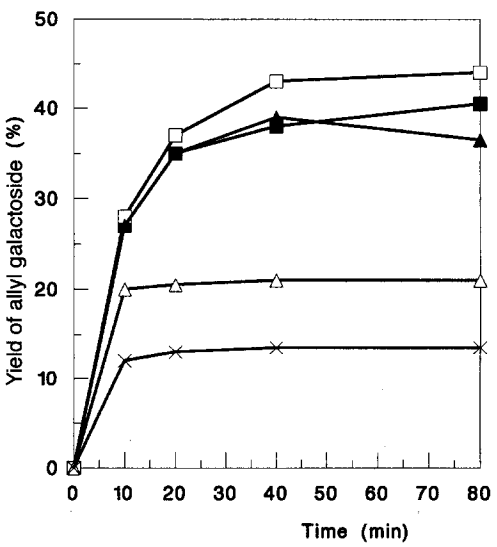

Fig. 1. Time-courses of β-D-galactosidase-catalysed reactions of lactose (10% w/v) with 2 M (■), 3 M (□), 4 M (▲), 5 M (△), and 6 M (×) allyl alcohol in phosphate buffer, pH 7. The reaction was monitored by GC; yields are expressed relative to lactose.

In general, β-D-galactosidase-catalysed galactoside formation from lactose and an alcohol works best with the largest possible concentration of either lactose or alcohol [10]. Since allyl alcohol is not expensive we chose to use it in excess. The reaction time is also important since the enzyme can hydrolyse the product galactoside as well as synthesise it [10]. The reaction time-course was followed, at different concentrations of allyl alcohol, and the optimum conditions for production of allyl galactoside from 10% w/v lactose were found to be 3 M (20% v/v) allyl alcohol, and a reaction time of 40–80 min (Fig. 1). In the presence of 5 or 6 M allyl alcohol, the enzyme was rapidly inactivated. A lactose concentration of 10% w/w is a good compromise between a high yield of product based on lactose and avoidance of an excessively large reaction volume.

When the reaction was scaled up and the products isolated and purified by ion-exchange and flash silica chromatography [15], the galactoside was obtained in 38.5% molar yield (relative to lactose).

This enzymic synthesis is a useful alternative to the chemical methods if the β-D-galactopyranoside, rather than the α anomer, is the desired product. The reaction is much simpler and quicker than a directed chemical synthesis. The product purification procedure is moderately time-consuming but no more so than the fractional crystallisation of a mixture of anomeric pyranosides and furanosides, required in a Fischer glycosidation procedure.

1. Experimental

General.—β-D-Galactosidase from fermentations of *Streptococcus thermophilus* 11F [13,14] was a kind gift from Dr. J. Smart of the NZ Dairy Research Institute. The dry enzyme preparation was a crude cell extract of activity 0.28 unit/mg at 40 °C (determined using a spectrophotometric assay [11], 1 unit = 1 μmol min^{-1} of galactose liberated from *p*-nitrophenyl β-D-galactopyranoside). Other chemicals were purchased from Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA). Solvents used were the best available commercial grade. Melting points and NMR spectra were obtained as described previously [10].

Time-course of reaction.—Assay mixtures were solutions of lactose and allyl alcohol, in buffer (50 mM phosphate, pH 7.0, total volume 1 mL). Reactions were started by addition of solid enzyme (10.8 mg, 3 units) and the mixture was incubated in a water bath at 40 °C. At timed intervals, aliquots of reaction mixture (10 μL) were removed and added to MeCN (0.5 mL) to quench the reaction. After evaporation of solvents under a stream of dry air, samples were analysed for sugar content by GC analysis of pertrifluoroacetylated derivatives, as described previously [11], and by thin-layer chromatography (TLC) [10,16].

Preparative scale reaction.—Allyl β-D-galactopyranoside was prepared by treating lactose (7.5 g) and allyl alcohol (15.3 mL) in 50 mM phosphate, pH 7.0 (total volume 75 mL) with *S. thermophilus* β-D-galactosidase (810 mg, 227 units) for 60 min at 40 °C. The mixture was boiled to terminate the reaction, diluted to 100 mL with distilled water, and applied to a column (2.5 × 40 cm) of Amberlite IRA-900 (OH^-) (Serva, Heidelberg, Germany). The column was eluted with distilled water and 10-mL fractions were

collected and monitored by TLC until all of the galactosidic products were eluted, the unwanted reducing sugars remaining bound to the column. Product-containing fractions were pooled, neutralised with 1 M HCl, and evaporated to dryness. In order to separate the mixture of galactoside and galactobioside, flash chromatography on a column (2.5 × 20 cm) of silica gel was used. The column was eluted successively with 20, 30, and 40% MeOH in CH_2Cl_2 (100 mL of each), and 30-mL fractions were collected and monitored by TLC. Product-containing fractions were pooled, the solvent was evaporated, and the residue was crystallised from EtOH–2-propanol. The galactoside was obtained as white crystals (1.77 g, 38.5% relative to lactose); mp 102–103.5 °C (lit. [17] 101–102 °C); $[\alpha]_D$ −11.1° (*c* 1.0, H_2O), lit. [17] −11°); ^1H NMR (δ, D_2O): 6.2–6 (m, 1 H, allyl H-2), 5.5–5.3 (2 d, 2 H, allyl H-3), 4.5 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.5–4.2 (2 d, 2 H, allyl H-1), 4.0 (d, 1 H, $J_{3,4}$ 3.1, $J_{4,5}$ ~0 Hz, H-4), 3.8 (d, 2 H, H-6), 3.8–3.6 (m, 2 H, H-3,5), 3.6–3.5 (2 d, 1 H, $J_{2,3}$ 9.8 Hz, H-2); ^{13}C NMR (D_2O) as reported previously [9,17].

A small amount (127 mg) of an impure, more polar (by TLC) syrup was also obtained; ^{13}C NMR (D_2O, a prime refers to the terminal galactosyl group): δ 136.5 (aglycon C–H), 121.8 (aglycon $=CH_2$), 106.2 (C-1′), 104.8 (C-1), 78.1 (C-5), 76.7 (C-5′), 75.65 (C-3), 75.5 (C-3′), 73.75 (C-2, aglycon CH_2O), 73.7 (C-2′), 71.9 (C-6), 71.7 (C-4,4′), 64.0 (C-6′) (plus several other signals of much lower intensity). These NMR data are consistent with the compound being 2-propenyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside. The compound resisted crystallisation and was not further characterised.

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