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Note

Synthesis of 2-fluoroethyl β-D-galactopyranoside and 2-fluoroethyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside from lactose using β-D-galactosidase

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The most cost-effective method for controlling mammalian pests in New Zealand, such as rabbits and possums, is the distribution of "baits" containing either of the poisons sodium fluoroacetate or sodium cyanide[1]. A major drawback in the use of these poisons is the phenomenon of "poison shyness". It appears that target animals which have previously ingested sublethal doses of poison can subsequently detect and therefore avoid poisoned baits[2]. We reasoned that decomposition of the food in the bait leads to lowering of the pH and the release of the free acid forms of the poisons, which are both volatile and odorous, and that it is this odour which is detected by the "poison shy" animals. We are, therefore, seeking to use the "prodrug" concept in order to design odourless compounds which can be metabolised by mammalian pests to form active poisons in vivo. The use of the title compounds (**1** and **2**) provides one possible approach. When ingested, they should be hydrolysed by digestive β-D-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23), to liberate 2-fluoroethanol which should then be absorbed in the same manner as ethanol. After absorption, the fluoroethanol should be transported to the liver and oxidised to fluoroacetate in the same manner as ethanol is converted into acetate[3]. The toxic species is therefore generated in vivo and there is little chance of the target animals detecting the "protoxin" in the bait. Similar approaches have been used against termites[4,5].

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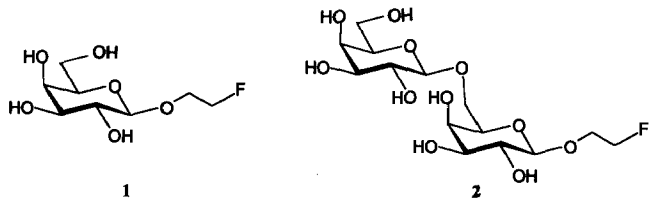

1 2

There are many reports on the use of the reverse reaction of glycosidases to prepare glycosides[6], so the use of β-D-galactosidase to prepare the target compound from lactose as the galactosyl donor and fluoroethanol as the acceptor seemed the best way to proceed. This type of reaction also produces galactobiosides (6-*O*-β-D-galactopyranosyl-β-D-galactopyranosides) by further galactosylation of the initial galactoside product[7,8].

We have previously demonstrated that, in general, galactoside formation from lactose and an alcohol works best with the largest possible concentration of *either* lactose *or* alcohol[9]. Since 2-fluoroethanol is relatively expensive, we chose to use an excess of lactose. The reaction time is also important since the enzyme can hydrolyse the product galactoside as well as synthesise it[6,9]. The reaction time-course was followed and the optimum time was found to be 80 min under the conditions used (Fig. 1).

When the reaction was scaled up and the products were isolated and purified by ion-exchange and flash chromatography (silica gel), the galactoside was obtained in 19% yield (relative to 2-fluoroethanol), about that expected from the time-course

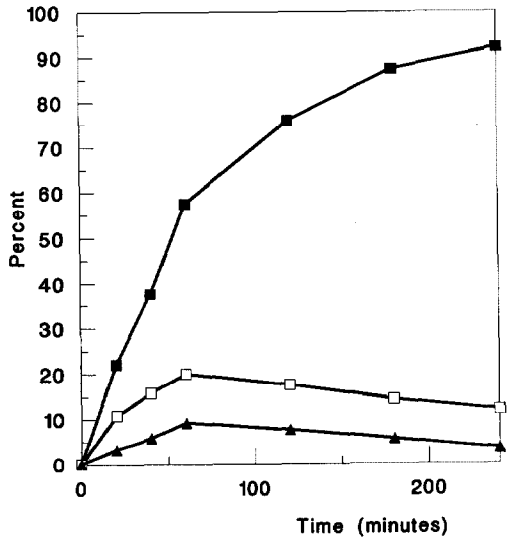

Fig. 1. Time course of the reaction of lactose with 2-fluoroethanol, catalysed by β-D-galactosidase. The reaction was monitored by GC; the graph shows degree of hydrolysis (lactose consumption, ■), galactoside **1** yield (□), and galactobioside **2** yield (▲).

experiment. The yield of galactobioside (5.4%) was lower than expected, probably due to losses during the silica column purification step.

A small-scale acute oral toxicity study in the possum (*Trichosurus vulpecula* Kerr) indicated that the galactoside is highly toxic. When administered by gastric intubation, three out of three possums receiving a dose of 10 mg/kg died within 6 h and two out of three animals receiving 5 mg/kg died within 12 h of dosing. Further testing is under way at present to determine the LD_{90} and also whether animals will voluntarily eat poisoned baits.

The use of either the title compounds in the purified form is almost certainly impractical and the production of 2-fluoroethanol at a reasonable cost will need to be addressed before large-scale use of these toxins will be practical. If, however, the crude reaction mixture was simply dried to remove water and unreacted 2-fluoroethanol, a mixture of underivatised sugars and toxins would be obtained which could be used directly. In practice, this mixture would probably be used in a second stage of control. Once cyanide or fluoroacetate has been used to kill most of the pest animals in an area, the bait-shy ones could then be dealt with by using the galactoside mixture which should be far more effective than a second treatment with the original poison.

EXPERIMENTAL

β-D-Galactosidase from *Kluyveromyces lactis* ("Biolactase") was a kind gift from Biocon (Australia) Pty. Ltd., Victoria, Australia. Solvents and other chemicals were obtained from Ajax Chemicals (Auburn, NSW, Australia), Aldrich (Milwaukee, WI, USA), or Sigma (St. Louis, MO, USA). Solvents used were the best available commercial grade. Melting points, accurate mass spectra, and NMR spectra were obtained as described previously[9].

Time course of reaction.—The assay mixture (0.975 mL) contained dithiothreitol (2 mM), lactose (2 M) and 2-fluoroethanol (0.5 M) in 25 mM phosphate buffer, pH 6.8. The reaction was started by the addition of enzyme (chromatographed on Sephadex to remove glycerol[9], 25 μL, 100 units in 25 mM phosphate buffer, pH 6.8, containing 5 mM $MgCl_2$) and the mixture was incubated in a water bath at 40°C. Aliquots (50 μL) of the mixture were removed at timed intervals and quenched by addition to 50 mM HCl (100 μL). The samples were then analysed by gas chromatography (GC) of their pertrifluoroacetate derivatives as described previously[9] and by thin-layer chromatography (TLC)[9,10].

Preparative scale reaction.—The small-scale trial reaction was scaled up directly to 20 mL and left under the same conditions for 80 min. The reaction was quenched by addition of 1 M HCl (1 mL) and then neutralised with 1 M NaOH. The scaled-up reaction was carried out three times in all. Each mixture was diluted to 100 mL with distilled water and applied to a 2.5 × 40 cm column of Amberlite IRA-900 (HO^-) (Serva, Heidelberg, Germany). The column was eluted with distilled water and 10-mL fractions were collected and monitored by TLC until all

of the galactoside products were eluted, the unwanted reducing sugars remained bound to the column. Product-containing fractions were pooled, neutralised with 1 M HCl, and evaporated to dryness. GC analysis showed that not all of the reducing sugars had been removed, so the products from the three column runs were combined and rerun. In order to separate the mixture of galactoside and galactobioside (2.2 g of solid), flash chromatography on a 2.5 × 30 cm column of silica gel was used[9]. The column was eluted successively with 20, 30, 40, and 50% MeOH in CH_2Cl_2, and 30-mL fractions were collected and monitored by TLC until all of the galactobioside had been eluted. Product-containing fractions were pooled, evaporated, and crystallised from EtOH–2-propanol. Both products were pure according to TLC and GC analysis.

2-Fluoroethyl β-D-galactopyranoside (**1**) was obtained as colourless crystals (1.21 g); mp 130.5–132°C; $[\alpha]_D$ −1.7° (*c* 1.0, H_2O); ^{13}C NMR (δ, D_2O): 105.6 (C-1), 86.1 (d, $J_{C,F}$ 163.4 Hz, aglycon CH_2F), 77.8 (C-5), 75.4 (C-3), 73.4 (C-2), 71.7 (d, $J_{C,F}$ 18.2 Hz, aglycon CH_2O), 71.3 (C-4), 63.6 (C-6). MS accurate mass (peracetate derivative, $M + NH_4^+$); calcd for $C_{16}H_{27}FNO_{10}$: 412.1619, obsd: 412.1629.

2-Fluoroethyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**2**) was also obtained as colourless crystals (0.6 g); mp 149.5–151.5°C; $[\alpha]_D$ −8.7° (*c* 1.0, H_2O); ^{13}C NMR (δ, D_2O, a prime refers to the nonreducing terminal galactosyl residue): 106.0 and 105.6 (C-1 and C-1′), 86.1 (d, $J_{C,F}$ 163.3 Hz, aglycon CH_2F), 77.8 (C-5), 76.5 (C-5′), 75.4 and 75.2 (C-3 and C-3′), 73.4 and 73.3 (C-2 and C-2′), 71.9 (d, $J_{C,F}$ 18.3 Hz, aglycon CH_2O), 71.6 (C-6), 71.3 and 71.29 (C-4 and C-4′), 63.6 (C-6′). MS accurate mass (peracetate derivative, $M + NH_4^+$); calcd for $C_{28}H_{43}FNO_{18}$: 700.2464, obsd: 700.2459.

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REFERENCES

1 D.R. Morgan, *NZ J. Ecol.*, 5 (1982) 36–43.
2 D.R. Morgan, *Aust. Wildl. Res.*, 17 (1990) 601–613.
3 A.L. Lehninger, *Principles of Biochemistry*, Worth, New York, 1982, pp 761–762.
4 G.D. Prestwich, U.S. Pat. 4,582,901 (1986); *Chem. Abstr.*, 105 (1986) 37516k.
5 R. Yamaoka, K. Hayashiya, T. Yoshimura, E. Seki, T. Masutani, and K. Kitahara, Eur. Pat. EP 331,089 (1989); *Chem. Abstr.*, 112 (1990) 99135p.
6 E.J. Toone, E.S. Simon, M.D. Bednarski, and G.M. Whitesides, *Tetrahedron*, 45 (1989) 5365–5422.
7 K.G.I. Nilsson, *Carbohydr. Res.*, 167 (1987) 95–103.
8 K.G.I. Nilsson, *Carbohydr. Res.*, 180 (1988) 53–59.
9 D.E. Stevenson, R.A. Stanley, and R.H. Furneaux, *Biotechnol. Bioeng.*, 42 (1993) 657–666.
10 C. Szustkiewicz and J. Demetriou, *Clin. Chim. Acta*, 32 (1971) 355–359.