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Note

Synthesis of 4-methylcoumarin-7-yl β-D-galactofuranoside, a fluorogenic substrate for galactofuranosidase

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Galactofuranosyl residues have been found as constituents of glycoconjugates from bacteria [1,2], fungi [3–6] and protozoa [7–10]. Particularly, galactofuranose is one of the components of the lipopeptidophosphoglycan (LPPG) from the parasite *Trypanosoma cruzi*. Molecular species with different proportions of galactofuranose have been characterized [11]. A β-galactofuranosidase activity could be responsible for this variation, although, as far as we know, there are no reports on the isolation of β-galactofuranosidases from protozoa. Action of this enzyme could be of physiological importance in *T. cruzi*, since galactofuranose, being absent from mammalian glycoconjugates, is a determinant of antigenicity [12]. A β-galactofuranosidase has been isolated and characterized from cultures of fungi such as *Penicillium charlesii* [13] and *Helminthosporium sacchari* [14]. In the latter, the release of galactose by the enzyme caused a decline in toxin activity of helminthosporoside, a sesquiterpenoid bis-β-D-galactofuranoside [14]. The enzyme is also interesting, as it could be used as a tool in determining minute amounts of β-D-galactofuranosyl residues.

The usual assay for glycosidases involves incubation of the enzyme solution with a suitable phenolic glycoside as substrate, followed by determination of the released phenol. 4-Methylumbelliferyl glycosides are widely used, since the released coumarin derivative can be determined by fluorimetry with very high sensitivity [15]. Thus, the

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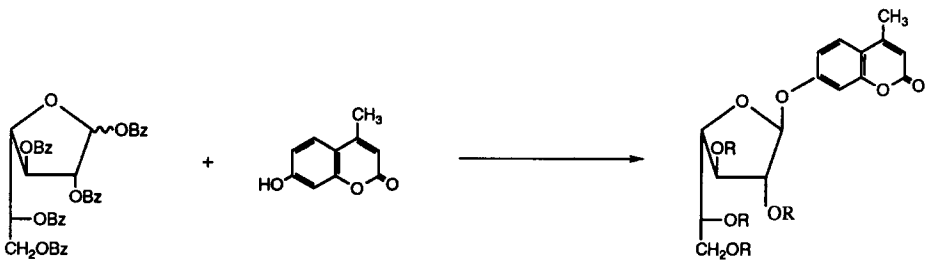
4-methylcoumarin-7-yl β-D-galactofuranoside (**4**), would constitute a valuable substrate for galactofuranosidase activity. Therefore, we report here some convenient procedures for the ready preparation of **4**.



1 **2** **3** R = Bz

4 R = H

The synthesis of **4** was first attempted employing the Koenigs–Knorr procedure. For that purpose, penta-*O*-benzoyl-α,β-D-galactofuranose (**1**) [16] was converted into tetra-*O*-benzoyl-β-D-galactofuranosyl bromide by treatment with bromotrimethylsilane [17]. However, the condensation of 7-hydroxy-4-methylcoumarin (**2**) with the crude bromide was unsuccessful, under the variety of conditions studied, and the only product isolated from the reaction mixture was spectroscopically identified as an α:β mixture (ratio 1:4) of 2,3,4,6-tetra-*O*-benzoyl-D-galactofuranoses. The C-1 signals for the anomers appeared at δ 96.0 and 101.0 respectively. However, the tin(IV) chloride-promoted glycosylation of **2** with **1**, which we have employed as a general strategy for the synthesis of galactofuranose derivatives [18–20], was successful when the tetrabutylammonium salt, instead of the phenolic form of **2**, was employed. The tetrabutylammonium salt of **2** has increased solubility in organic solvents and enhances the nucleophilicity of the phenol [21]. In fact, the salt completely dissolved when it was added to a dichloromethane solution of compound **1** anomerically activated with $SnCl_4$. TLC examination of the reaction mixture showed a strongly UV-active product which, after purification by column chromatography, afforded crystalline 4-methylcoumarin-7-yl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranoside (**3**) in 68% yield. The anomeric configuration of **3** was established as β, in accord with the $J_{1,2}$ value (< 1 Hz) [22] and the chemical shift (103.8 ppm) [23] of the anomeric signal, from the respective ^1H and ^{13}C NMR spectra.

Compound **3** could be obtained by an alternative route, which had been employed for the synthesis of *p*-nitrophenyl β-D-galactofuranoside [24]. Thus, the condensation of **1** with **2** took place in the presence of catalytic amounts of *p*-toluensulfonic acid, in boiling toluene, to afford **3** in a yield (~ 60%) slightly lower than that obtained by the procedure described above.

Debenzoylation of **3** with sodium methoxide gave crystalline **4** in 90% yield. The ^1H NMR spectrum of **4** showed for the anomeric proton a $J_{1,2}$ value (~ 1 Hz in D_2O, 1.8 Hz in CD_3OD) indicative of the *trans* relationship for H-1 and H-2 (β anomeric configuration). Accordingly, the anomeric carbon resonance [δ 103.5 in $(CD_3)_2SO$] was

similar to the chemical shift reported for analogous phenyl β-D-galactofuranosides [24]. The solubility of compound **4** in the buffer usually used for the enzymatic assays was satisfactory. The furanoid ring structure of **4** was confirmed by periodate oxidation under mild conditions that cleave selectively the C-5–C-6 bond [7], leading after reduction, to a product which showed identical chromatographic behavior as an authentic sample of 4-methylcoumarin-7-yl α-L-arabinofuranoside.

1. Experimental

General methods.—Melting points were determined with a Thomas–Hoover apparatus. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AC 200 spectrometer at 200 MHz (^{1}H) or 50.3 MHz (^{13}C). Column chromatography was performed on silica gel 60 (Merck). Thin-layer chromatography (TLC) was carried out on precoated aluminium plates of silica gel 60 F_{254} (Merck), using 9:1 toluene–EtOAc as solvent, unless otherwise indicated. The spots were visualized by exposure to UV light and by sprying the plates with 10% (v/v) H_2SO_4 in EtOH, followed by heating.

Synthesis of 4-methylcoumarin-7-yl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranoside (**3**).—(a) *By $SnCl_4$-promoted condensation of* **1** *with the tetrabutylammonium salt of* **2**. To an externally cooled (0°C) solution of **1** (0.25 g, 0.36 mmol) in dry CH_2Cl_2 (4 mL), $SnCl_4$ (60 μL, 0.5 mmol) was added. The mixture was stirred for 15 min followed by the addition of freshly prepared [21] tetrabutylammonium salt of **2** (0.45 g) After stirring at room temperature for 6 h, the mixture, which showed by TLC a main UV-active spot having R_f 0.32, was diluted with CH_2Cl_2 (30 mL), filtered and the filtrate extracted with satd aq NaCl (20 mL), dried ($MgSO_4$), and concentrated. Purification of the residue by column chromatography (49:1 toluene–EtOAc) afforded compound **3** (0.18 g, 68%), which after crystallization from EtOH gave mp 107–108°C (softening 104°C); $[\alpha]_D$ −63° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.22–7.10 (23 H-aromatic), 6.18 (H-3′), 6.08 (H-1,5, $J_{1,2} \sim 0.5$ Hz), 5.80 (H-2,3), 4.85–4.74 (H-4,6,6′), 2.39 (CH_3); ^{13}C NMR ($CDCl_3$) δ 165.9, 165.7, 165.6 (Ph*C*O), 160.9 (C-7′), 158.2 (C-2′), 154.9 (C-9′), 152.0 (C-4′), 133.7–128.3 (C-aromatic), 125.7 (C-5′), 115.1 (C-10′), 113.1, 113.0 (C-3′,6′), 104.8 (C-8′), 103.8 (C-1), 82.9 (C-4), 82.0 (C-2), 77.3 (C-3), 70.1 (C-5), 63.2 (C-6), 18.6 (CH_3). Anal. Calcd for $C_{44}H_{34}O_{12}$: C, 70.02; H, 4.54. Found: C, 70.23; H, 4.73.

(b) *By* p-*toluenesulfonic acid-catalyzed condensation of* **1** *and* **2**. A solution of *p*-toluenesulfonic acid (0.01 g) in toluene (35 mL) was boiled under reflux for 1 h while water was removed by means of a water trap. To the solution, **1** (0.70 g, 1.0 mmol) and **2** (0.35 g, 2.0 mmol) were added, and the reflux was maintained for an additional 3 h. The dark solution was concentrated and the residue was dissolved in CH_2Cl_2 (60 mL), extracted with 2% aq NaOH (20 mL, twice), washed with water until neutrality, dried ($MgSO_4$) and concentrated. The resulting syrup showed by TLC the presence of **3** and some remaining starting material. The product was purified by column chromatography (49:1 toluene–EtOAc). Fractions containing the product of R_f 0.32 were combined and evaporated to afford a syrup (0.45 g, 60%), which crystallized from EtOH gave the same physical constants and spectral properties as **3**, described in (*a*).

β-7-Hydroxy-4-methylcoumarin-7-yl D-*galactofuranoside* (**4**).—Compound **3** (0.25 g, 0.33 mmol) was suspended in 0.5 M NaOMe in MeOH (10 mL) and stirred at room temperature until complete dissolution (2 h). The solution was made neutral with Dowex 50W(H^+) resin and concentrated affording crystalline **4** (0.10 g, 90%) having R_f 0.52 (4:1 EtOAc–MeOH). A commercial standard of the galactopyranose analogue showed R_f 0.43. After recrystallization from water, **4** had mp 86–88°C, $[\alpha]_D$ −195° (*c* 1, MeOH); ^{1}H NMR (D_2O) 7.69 (d, $J_{5',6'}$ 9.0 Hz, H-5'), 7.11 (d, H-6'), 7.04 (bs, H-8'), 6.23 (bs, H-3'), 5.81 (bs, $J_{1,2} \sim 1$ Hz, H-1), 4.63, 4.47, 4.29 (H-2,3,4), 4.22 (m, H-5), 3.77 (m, 2 H, H-6,6'); (CD_3OD) δ: 7.68 (d, $J_{5',6'}$ 8.5 Hz, H-5'), 7.07 (m, 2 H, H-6'), 6.18 (bs, H-3'), 5.63 (d, $J_{1,2}$ 1.8 Hz, H-1), 4.29 (dd, $J_{2,3}$ 4.0 Hz, H-2), 4.20 (dd, $J_{3,4}$ 6.4 Hz, H-3), 4.10 (dd, $J_{4,5}$ 3.0 Hz, H-4), 3.76 (m, H-5), 3.63 (m, 2 H, H-6,6'), 2.44 (s, 3 H, CH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.4, 160.0 (C-2',7'), 154.6 (C-9'), 153.6 (C-4'), 126.7 (C-5'), 114.1 (C-10'), 113.8 (C-6'), 111.8 (C-3'), 106.4 (C-8'), 103.5 (C-1), 83.6 (C-4), 82.0 (C-2), 76.1 (C-3), 70.4 (C-5), 62.7 (C-6), 18.3 ($\mathbf{C}H_3$). Solubility: 1 mg of compound **4** dissolves completely in 1 mL of sodium citrate buffer (pH 7.9). Anal. Calcd for $C_{16}H_{18}O_8 \cdot H_2O$: C, 53.93, H, 5.66. Found: C, 53.71, H, 5.47.

*Conversion of 7-hydroxy-4-methylcoumarin-7-yl β-*D-*galactofuranoside* (**4**) *into 7-hydroxy 4-methylcoumarin-7-yl α-*L-*arabinofuranoside.*—Compound **4** (5 mg, 0.01 mmol) dissolved in water (0.5 mL) was treated with 0.05 M $NaIO_4$ (0.5 mL) for 20 min in the dark. Sodiun borohydride (15 mg) was added and the mixture was kept for 2 h at room temperature. After deionization of the solution (Amberlite MB-3), TLC examination (4:1 EtOAc–MeOH) showed a single spot (R_f 0.59) having the same mobility as an authentic sample of 4-methylcoumarin-7-yl α-L-arabinofuranoside.

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