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Note

Synthesis of *p*-nitrophenyl β-D-galactofuranoside. A convenient substrate for β-galactofuranosidase*

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Nitrophenyl glycosides have been widely used as substrates for estimating the activity of glycosidases. Numerous studies on the kinetics and specificity of the enzymes have been conducted employing these compounds[1]. In particular, *o*- and *p*-nitrophenyl glycosides of D-galactopyranose are recommended substrates for the α- and β-galactopyranosidases isolated from several natural sources[1]. Galactofuranosidases have also been recently described[2,3]. Such an enzyme was considered responsible for the variation in galactofuranose content of an antigenic glycopeptide from *Penicillium charlesii*[2]. Methyl β-D-galactofuranoside was used as substrate for the enzyme, and the galactose released was estimated by the galactose oxidase method[2,3]. In our laboratory, galactofuranose was characterized as one of the components of glycoconjugates from the parasite *Trypanosoma cruzi*[4] and from the fungus *Ascobolus furfuraceus*[5]. In the latter instance, we also observed a variation in the ratio of sugars when different cultures were analyzed. In order to evaluate a probable β-galactofuranosidase activity, a suitable substrate was required. *p*-Nitrophenyl β-D-galactofuranoside (**6**) would be chromogenic and thus a useful substrate. Although many nitrophenyl glycosides have been described[1] and some of them are commercially available, no synthesis of **6** was found in the literature. Aryl glycopyranosides[6] are generally prepared by fusion of the phenol with the *O*-acetylated sugar in the presence of anhydrous zinc chloride (for the α anomer) or *p*-toluenesulfonic acid (for the β anomer). Some improvements of these techniques have been published[7], although the *p*-nitrophenyl glycosides are usually obtained in low yield. On the other hand, the solid-phase method[8] does not seem to be appropriate for the synthesis of a galactofuranoside, as the starting material

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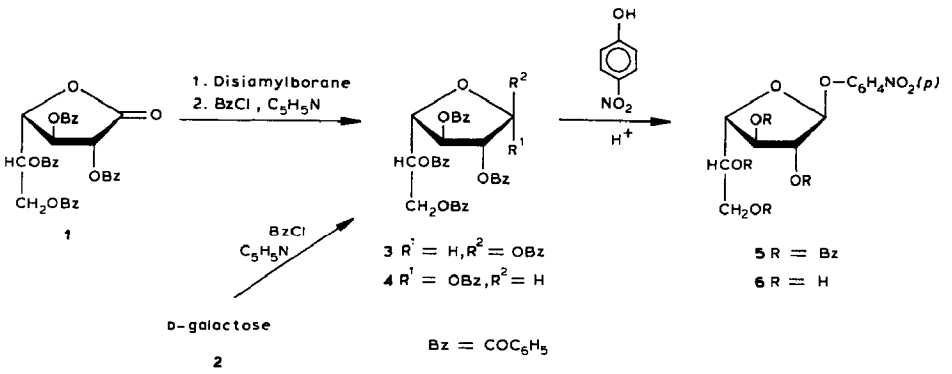
would be an unstable glycosyl bromide[9]. We report here a satisfactory procedure for the preparation of **6**.

The key intermediate involved is penta-*O*-benzoyl-α,β-D-galactofuranose (**4** and **3**), obtainable by two different routes. One starts from 2,3,5,6-tetra-*O*-benzoyl-D-galactono-1,4-lactone[10] (**1**), readily prepared from commercially available D-galactono-1,4-lactone. Reduction of the lactone group of **1** with disiamylborane[11] followed by benzoylation gave as a crystalline product only the β-anomer (**3**) of the perbenzoate, in 72% yield. The other route involves benzoylation of D-galactose at high temperature[12], affording a mixture of **3** and **4**. Either the benzoate **3** or the anomeric mixture (**3** and **4**) may be readily converted into the *p*-nitrophenyl glycoside **5**, by treatment with *p*-nitrophenol in the presence of catalytic amounts of *p*-toluenesulfonic acid, in boiling toluene. Debenzoylation of **5** with sodium methoxide afforded **6** in overall 67% yield from **3**. The anomeric configuration of compounds **5** and **6** was unambiguously established on the basis of the $J_{1,2}$ coupling constant (<1.0 Hz in **5**, and 2.2 Hz in **6**), which indicates[13] the *trans* configuration for H-1 and H-2. The signals of the ^{13}C-n.m.r. spectrum of **6** were assigned by comparison with the chemical shifts of methyl β-D-galactofuranoside[14]. As expected, the anomeric carbon resonance of **6** (106.0 p.p.m.) appeared shifted 3.2 p.p.m. upfield as compared to C-1 of the methyl glycoside, and 2.6 p.p.m. downfield with respect to C-1 of *p*-nitrophenyl β-D-galactopyranoside[15]. The furanoid ring structure of **6** was confirmed by periodate oxidation under conditions that cleave only the C-5–C-6 bond, leading, after reduction and hydrolysis, to arabinose[4].

The procedure described here constitutes a ready route of access to *p*-nitrophenyl β-D-galactofuranoside (**6**), a convenient substrate for β-D-galactofuranosidase.



EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. N.m.r. spectra (^{1}H and ^{13}C) were recorded with a Varian XL-100 spectrometer operating in the Fourier-transform mode with a 620

L-100 computer interfaced to a Sykes 7000 dual disk-drive. Tetramethylsilane was the internal standard. Optical rotations were recorded with a Perkin–Elmer 141 polarimeter. G.l.c. was performed with a Hewlett–Packard 5830A gas chromatograph equipped with a glass column (180 × 0.2 cm) packed with 3% ECNSS-M on Gas Chrom Q. Column chromatography was performed on silica gel 60 (Merck). T.l.c. was carried out on precoated aluminum plates (0.2 mm) of silica gel 60F-254 (Merck). Detection was effected by spraying the plates with 5% (v/v) H_2SO_4 in ethanol, followed by heating.

Penta-O-benzoyl-β-D-galactofuranose (**3**) *and its α anomer* (**4**). — *(a) Starting from 2,3,5,6-tetra-O-benzoyl-D-galactono-1,4-lactone*[10] (**1**). Compound **1** (1.2 g, 2 mmol) was reduced with disiamylborane, as previously described[16], to give syrupy 2,3,5,6-tetra-*O*-benzoyl-D-galactofuranose (1.1 g, 91%). This product (0.6 g, 1 mmol), was then benzoylated with benzoyl chloride (1.5 mL) in pyridine (3.0 mL) for 3 h at 0°. The mixture was purified conventionally[16], to give a colorless syrup that crystallized upon addition of ethanol (yield 0.51 g, 72%). The product showed by t.l.c. a single spot of R_F 0.45 (19:1 benzene–ethyl acetate) coincident with an authentic sample of **3**. Recrystallized from ethanol, it had m.p. 159–160°, $[\alpha]_D$ −25° (*c* 1.0, chloroform), in good agreement with literature values[12].

(b) Starting from D-*galactose.* Compounds **3** and **4** were obtained as described[12] as a ~1:1 mixture.

p-*Nitrophenyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranose* (**5**). — To a mixture of **3** and **4** (2.1 g, 3 mmol) suspended in toluene (20 mL), *p*-nitrophenol (2.1 g, 15 mmol) and *p*-toluenesulfonic acid (20 mg) were added, and the mixture was boiled under reflux for 2 h while water was removed from the system by a water-trap. The dark solution was evaporated at 50° under diminished pressure. The residue was diluted with dichloromethane (200 mL), extracted with 2% aqueous sodium hydroxide (50 mL, twice), washed with water to neutrality, dried (magnesium sulfate) and evaporated. T.l.c. of the syrup showed a main component having R_F 0.51 (19:1 benzene–ethyl acetate), slightly contaminated by starting material. The mixture was separated on a short column of silica gel (40 g) with 99:1 benzene–ethyl acetate. Fractions containing the product of R_F 0.51 were pooled and evaporated, affording 1.86 g (86.5%) of compound **5** as a chromatographically homogeneous syrup. By dissolution in hot acetone (5 mL) and addition of boiling ethanol (200 mL), an amorphous solid (**5**) was obtained upon cooling. Compound **5** had $[\alpha]_D$ −11° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.2–7.1 (m, H-aromatic), 6.06 (m, H-5), 6.04 (br. s, $J_{1,2}$ <1 Hz, H-1), 5.75 (m, H-2,3), and 4.76 (m, H-4,6,6′); ^{13}C-n.m.r. ($CDCl_3$): δ 165.6, 165.4, 165.3, 165.1 (CO of benzoates), 160.3 (C–O *p*-nitrophenyl), 142.5 (C–NO_2 *p*-nitrophenyl), 133.5–128.1 (C-aromatic), 125.6 (C-2,6 *p*-nitrophenyl), 116.3 (C-3,5 *p*-nitrophenyl), 103.7 (C-1), 83.0 (C-4), 81.8 (C-2), 77.2 (C-3), 70.0 (C-5), and 63.0 (C-6).

Anal. Calc. for $C_{40}H_{31}NO_{12}$: C, 66.94; H, 4.35; N, 1.95. Found: C, 67.17; H, 4.36; N, 1.77.

p-*Nitrophenyl β-D-galactofuranoside* (**6**). — To a suspension of compound **5**

(1.45 g, 2.0 mmol) in dry methanol (40 mL), 1-mL portions of M sodium methoxide in methanol were added every 5 min, until dissolution of **5** was complete (9 mL). The solution was made neutral with Dowex 50W (H^+) resin and evaporated, affording a crystalline product of R_F 0.54 (9:1 ethyl acetate–methanol). Debenzoylation could also be conducted directly on **5**, omitting the chromatographic purification. Recrystallization from ethyl acetate gave pure **6** (0.47 g, 77%); m.p. 152–154°, $[\alpha]_D$ −203° (*c* 1.0, methanol); ^{1}H-n.m.r. (C_5D_5N): δ 8.14 (d, $J_{2,3}$ 9.4 Hz, H-3 *p*-nitrophenyl), 7.20 (d, H-2 *p*-nitrophenyl), 6.21 (d, $J_{1,2}$ 2.2 Hz, H-1), 5.34–4.96 (m, H-2,3,4), and 4.70–4.28 (m, H-5,6,6′); ^{13}C-n.m.r. (C_2D_6SO): δ 161.8 (C–O *p*-nitrophenyl), 141.2 (C–NO_2 *p*-nitrophenyl), 125.5 (C-2,6 *p*-nitrophenyl), 116.4 (C-3,5 *p*-nitrophenyl), 106.0 (C-1), 83.5 (C-4), 81.6 (C-2), 75.8 (C-3), 69.9 (C-5), and 62.3 (C-6).

Anal. Calc. for: $C_{12}H_{15}NO_8$: C, 47.84; H, 5.02; N, 4.65. Found: C, 47.59; H, 5.09; N, 4.67.

Conversion of p-*nitrophenyl* β-D-*galactofuranoside* (**6**) *into* L-*arabinose.* — Compound **6** (3 mg, 0.01 mmol) dissolved in water (0.4 mL) was treated with 0.05M sodium metaperiodate (0.4 mL) for 20 min in the dark. Potassium borohydride (15 mg) was added and the mixture was kept for 2 h at room temperature. Hydrolysis of the glycoside was performed by adding 2M hydrochloric acid (0.4 mL) and heating for 2 h at 100°. The solution was evaporated several times with water in order to remove the acid, and then deionized by passing through Amberlite MB-3. Fractions that were positive in the phenol–sulfuric acid test[17] were combined and analyzed by paper chromatography (6:4:3 butanol–pyridine–water), showing a single spot of the same mobility as arabinose. No galactose was detected. Standard reduction ($NaBH_4$) and acetylation of the product gave by g.l.c. a single peak having the same retention time as arabinitol acetate (R_T 0.31, relative to glucitol acetate).

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