SYNTHESIS OF β-D-GALACTOFURANOSE 1-PHOSPHATE

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ABSTRACT

β-D-Galactofuranose 1-phosphate (**2**) has been synthesised with high anomeric specificity, by a number of conventional routes. The product, isolated as an amorphous, hydrated barium salt, was characterised as a crystalline strychine salt. Periodate oxidation of **2**, followed by borohydride reduction, confirmed its furanosidic nature, Some mechanistic aspects of the phosphorylations are discussed. Improved procedures for the preparation of β-D-galactofuranose pentaacetate, directly from D-galactose, are also described.

INTRODUCTION

A synthesis of α-D-galactofuranose 1-phosphate (α-D-galactofuranosyl dihydrogen phosphate, **1**) was required as part of a study on the biosynthesis of galactofuranosyl units in the fungal polysaccharide galactocaralose. It was considered likely that, if the biosynthesis occurred in the manner usual for polysaccharides, a nucleoside 5-(α-D-galactofuranosyl dihydrogen pyrophosphate) would be involved. No chemical synthesis of a hexofuranose 1-phosphate has been accomplished so far. It was also required that a synthesis should be adaptable to ^{14}C-labelling techniques at a later date.

The formation of **1** was not achieved, and the problem was solved eventually by biochemical methods[1,2]. The product from the chemical studies was β-D-galactofuranose 1-phosphate (**2**), and some syntheses of **2** will now be described.

RESULTS AND DISCUSSION

When D-galactose was treated[3] with acidic methanol and the syrupy mixture of glycosides acetylated and then acetolysed[4], penta-*O*-acetyl-β-D-galactofuranose (**3**) was obtained in 37–41% overall yield. Treatment of ethyl β-D-galactofuranoside (**4**), derived from D-galactose diethyl dithioacetal, with an acetic anhydride–acetic acid–sulphuric acid mixture gave **3** in 40% yield; a previous[5] acetolysis of **4** gave only *ca.* 10% of **3**. Both procedures represent an improvement on the existing methods

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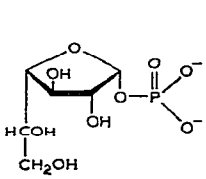
for preparing **3** directly from D-galactose. Anomerisation of **3** with zinc chloride in acetic anhydride gave the α-D anomer **5**, in the reported[8], low yield (10%).



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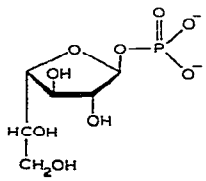

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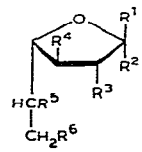


3 $R^1 = R^3 = R^4 = R^5 = R^6 = OAc$; $R^2 = H$
4 $R^1 = OEt$; $R^2 = H$; $R^3 = R^4 = R^5 = R^6 = OH$
5 $R^1 = H$; $R^2 = R^3 = R^4 = R^5 = R^6 = OAc$
6 $R^1 = Cl$; $R^2 = H$; $R^3 = R^4 = R^5 = R^6 = OAc$
7 $R^1 = Br$; $R^2 = H$; $R^3 = R^4 = R^5 = R^6 = OAc$
8 $R^1 = H$; $R^2 = SEt$; $R^3 = R^4 = R^5 = R^6 = OAc$

Both acetates **3** and **5** yielded crystalline tetra-*O*-acetyl-β-D-galactofuranosyl chloride (**6**) when treated with aluminium chloride in anhydrous ether[11]. The corresponding, syrupy bromide **7** was obtained by reaction of ethyl tetra-*O*-acetyl-1-thio-α-D-galactofuranoside (**8**) with bromine in dichloromethane[12,13].

Treatment of **6** or **7** with one equivalent of triethylammonium dibenzyl phosphate in benzene, followed by hydrogenolysis and mild alkaline hydrolysis to remove the benzyl and acetyl groups, respectively, gave β-D-galactofuranose 1-phosphate (**2**) which was isolated and purified as the barium salt. The product was further characterised as a crystalline strychine salt. The biscyclohexylammonium salt was a hygroscopic gum. Treatment[14] of **2** with 1 mol. of periodate preferentially cleaved the C-5–C-6 bond. Borohydride reduction of the aldehyde produced, followed by hydrolysis with dilute acid, yielded arabinose (identified by paper chromatography), thereby confirming the furanosidic nature of the product.

Compound **2** was completely hydrolysed to galactose in 3 h at room temperature in 10mM hydrochloric acid. This lability to acid is comparable to that of the pentofuranose 1-phosphates[15,16]; under similar conditions, α-D-galactopyranose 1-phosphate underwent 9% hydrolysis.

Assignment of the β-D configuration to **2** was based on the optical rotations of the barium and strychnine salts (+17° and −28.5°), and by comparison with the corresponding values for the L-arabinofuranose 1-phosphates[16]. This assumption was substantiated by the fact that treatment[17] of **2** with dicyclohexylcarbodiimide (DCC) for periods of up to 3 days gave no *N*-phosphorylurea derivative. It is known[17] that, under these conditions, sugar 1-phosphates with vicinal *cis*-hydroxyl groups initially give 1,2-cyclic phosphates and then *N*-phosphorylureas.

The specific rotations (+15.5°–+16.2°) of various samples of **2** indicated that the amount of contaminating α-D-galactofuranose 1-phosphate (**1**) was very small. T.l.c. of the crude biscyclohexylammonium salt showed the presence of two compounds [R_F 0.19 (major) and 0.15 (minor)], both giving a rapid, purple colour with the periodate–Schiff's reagent, consistent with furanosidic structures. Attempted separation of the two components on Dowex 1-x8 (HCO_3^-) ion-exchange resin[18], using a linear, gradient elution of triethylammonium hydrogen carbonate solution, was unsuccessful.

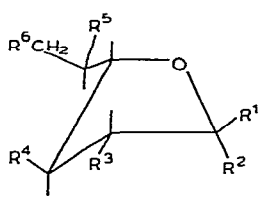
Treatment of **6** and **7** with silver dibenzyl phosphate, followed by hydrogenolysis

and mild alkaline hydrolysis, gave **2** in yields of 19 and 26%, respectively. Condensation of **6** or **7** with one equivalent of triethylammonium diphenyl phosphate or silver diphenyl phosphate, followed by hydrogenolysis (Adams' catalyst) and mild alkaline hydrolysis, did not afford any phosphorylated products. The formation of large amounts of inorganic phosphate indicated extensive decomposition during and/or after the hydrogenation stage, which was lengthy (14–20 h) compared with that for the corresponding benzylated products (*ca.* 1–2 h). The necessity for a short period of hydrogenation during the preparation of labile sugar phosphates has been noted[19] previously.

The low yield of **2** formed in the above reactions may be attributed, in part, to the lability of the product in the acidic conditions produced during hydrogenolysis. To minimise this effect, anhydrous sodium acetate was added to the hydrogenation mixtures.

Fusion[20] of the pentaacetate **3** with anhydrous phosphoric acid gave the barium salt of **2** in 46–51% yield. Treatment of the anomeric pentaacetate **5**, under the same conditions, gave **2** in 31% yield. Treatment of **3** with the reagent for 2 min at 75° yielded 39% of **2**. The reaction of a fully acetylated, reducing monosaccharide with anhydrous phosphoric acid normally leads to production of the thermodynamically more-stable isomer. In some circumstances, both isomers have been isolated[21]. Recent work has shown[22] that the ratio of anomers can be controlled by the length of heating the two reactants together. Traces of the α-D 1-phosphate **1** could be detected (t.l.c.) in the crude products from the above reactions.

The stereospecificity of the various phosphorylation reactions may be explained on the basis of the reagent's approaching from the less-hindered side of a carboxonium-ion intermediate. In β-D-galactofuranose compounds, the groups on C-2, C-3, and C-4 are in nearly equatorial orientation in conformation **9**. With the acetates **3** and **5**, and the two halides **6** and **7**, cleavage or displacement of the group on C-1, with neighbouring-group participation by the C-2 acetoxyl group, could lead to the acetoxonium-ion intermediate **10**. This would react with the phosphorylating reagent to give almost entirely product **2** which, because of the bulkiness of the C-5–C-6 side chain and the eclipsing between the C-1 and C-2 groups in the α-D anomer, will be the preferred isomer. Kjølberg[23,24] has obtained evidence that methyl α-D- and β-D-galactofuranosides adopt this conformation, and the greater stability of the β-D isomer is understandable on these grounds.



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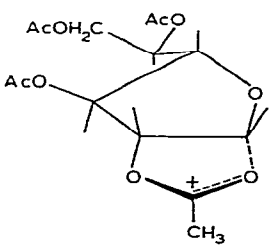

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EXPERIMENTAL

Paper chromatography was performed on Whatman No. 1 paper (previously washed with 2M acetic acid and distilled water, and air-dried) by the descending technique in the following solvents: (*A*) ethanol–M ammonium acetate (pH 7.2; 5:2 v/v)[25]; (*B*) propan-1-ol–ethyl acetate–water (7:1:2)[26]. The periodate–Schiff's[27], ammonium molybdate[28], and aniline hydrogen phthalate[29] sprays were used as location reagents. Thin-layer chromatography (t.l.c.) was effected on Silica Gel G (Merck) with ethanol–conc. ammonium hydroxide–water (5:3:1), and spots were detected by the anisaldehyde spray[30] and heating.

Penta-O-acetyl-β-D-galactofuranose (3). — (*a*) *From D-galactose.* Dry, sieved (100-mesh) D-galactose (50 g) was suspended in anhydrous methanol (1000 ml), and sulphuric and (7.5 ml) was added dropwise with cooling and stirring. The reaction was allowed to proceed at room temperature until samples (taken every 10–15 min) were practically non-reducing to Fehling's solution (3.5–4 h). The mixture was neutralised with Amberlite 1R-4B (HO^-) resin, previously washed with methanol. The resin was removed by filtration and washed with methanol (250 ml). The combined filtrate and washings were concentrated *in vacuo* to give a clear syrup which was treated with acetic anhydride (180 ml) and pyridine (225 ml) for 4 h at 0° and then for 2 days at room temperature. The usual working-up procedure yielded a clear, pyridine-free syrup, which was dissolved in acetic acid (350 ml) and acetic anhydride (75 ml), and treated with conc. sulphuric acid (15 ml) at −5°. After 12–15 h at room temperature, the brown solution was stirred with ice, the mixture was extracted with chloroform (3 × 200 ml), and the combined extracts were washed with water and saturated, aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated *in vacuo* to give a syrup which crystallised on addition of 2 vols. of propan-2-ol and storage at 0°. Recrystallisation from the same solvent gave **3** (40–44.5 g, 37–41%), m.p. 96–97°, $[\alpha]_D^{22}$ −41.5° (*c* 2, dichloromethane); lit.[7] m.p. 98°, $[\alpha]_D$ −42°.

(*b*) *From ethyl β-D-galactofuranoside* (4). Compound **4**[5] (15 g), dissolved in acetic anhydride (75 ml) and acetic acid (50 ml), was treated with conc. sulphuric acid (1.5 ml) at −5°, and then left at room temperature for 1 h. A further quantity of sulphuric acid (3.5 ml) was added with cooling, and the solution was then left at room temperature for a further 3 h. The mixture was treated with an excess of anhydrous sodium acetate (20 g) and co-evaporated several times with ethanol. Chloroform (300 ml) was added to the product, and the mixture was washed twice with water (200 ml), dried, and evaporated to give a syrup which was crystallised and recrystallised from propan-2-ol, as above, to give **3** (11.25 g, 40%), m.p. 95–97°, $[\alpha]_D^{22}$ −40.6° (*c* 2.7, dichloromethane).

β-D-Galactofuranose 1-phosphate (2). — (*a*) *Using trimethylammonium dibenzyl phosphate.* (*i*) The chloride[11] **6** (5.2 g) was treated in benzene solution (25 ml) with one equivalent of triethylammonium dibenzyl phosphate[32,33] [triethylamine (1.12 g), dibenzyl phosphate (3.79 g)]. The mixture was stirred for 1 h at room temperature

and for a further 3 h at 60–65°; triethylamine hydrochloride (745 mg, 49%) was then removed by filtration. The filtrate was concentrated *in vacuo* at 25°, and a solution of the residual syrup in anhydrous methanol (100 ml) was hydrogenated at 5–10°, in the presence of palladium oxide (1.5 g) and anhydrous sodium acetate (2 g), until the uptake of hydrogen ceased (*ca.* 1 h; 600 ml absorbed). The filtered mixture was concentrated *in vacuo* at 25°, and a solution of the resulting gum in tetrahydrofuran (15 ml) was added to ice-cold 2M lithium hydroxide (120 ml) which was then allowed to stand for 24 h at 0°. The precipitated trilithium phosphate was removed by centrifugation and washed with M lithium hydroxide (10 ml), and the combined supernatant and washings were adjusted to pH 8.2–8.5 with Dowex-50 (H^+) resin and concentrated *in vacuo* at 37° to *ca.* 10 ml. Addition of barium acetate (2 g), followed by ethanol (60 ml), to the clear solution gave a precipitate of the barium salt which was chilled for 30 min at 0°, collected by centrifugation, and washed with 60% aqueous ethanol, ethanol, and finally ether. The air-dried material was then extraced with water (4 × 5 ml) at 37°, and the combined extracts were concentrated *in vacuo* at 30° to *ca.* 6 ml and separated from a small amount of insoluble material by centrifugation. Ethanol (6 vol.) was added to the clear supernatant and the precipitate, after being kept for 2 h at 0°, was collected and washed in the manner described above. After two further reprecipitations, the completely water-soluble product was dried *in vacuo* (P_4O_{10}) to give **2** as the barium salt dihydrate (2.3 g, 38.5%), $[\alpha]_D^{22}$ +17° (*c* 1.2, water) (Found: C, 15.5; H, 3.3; P, 6.8. $C_6H_{11}BaO_9P \cdot 2H_2O$ calc.: C, 15.9; H, 3.8; P, 6.9%).

(*ii*) Compound **8**[31] (5 g) in dichloromethane (50 ml) was treated at 23° with a solution of bromine (0.8 ml) in dichloromethane (40 ml), over a period of 15 min with stirring. After a further 45 min at room temperature, the mixture was evaporated *in vacuo* at 25°, and then re-evaporated with dry toluene (4 × 50 ml) to yield tetra-*O*-acetyl-β-D-galactofuranosyl bromide (**7**) as an oil (5.4 g), $[\alpha]_D^{22}$ −37.6° (*c* 2, dichloromethane).

Treatment of the bromide **7**, in benzene solution, with one equivalent of triethylammonium dibenzyl phosphate for 2.5–3 h at room temperature gave triethylammonium bromide (72%, maximum yield). A working-up procedure identical to that in (*i*) gave **2** as the barium salt dihydrate (54%), $[\alpha]_D^{21}$ +16.7° (*c* 0.85, water).

Passage of a solution of **2** (barium salt, 250 mg) through Amberlite IR-120 (cyclohexylammonium) resin, followed by concentration of the effluent and washings *in vacuo*, gave a clear gum, $[\alpha]_D^{22}$ +15.5° (*c* 1, water).

To a solution of **2** (barium salt, 1 g) in water (100 ml) was added strychnine sulphate (1.9 g), and the mixture was stirred overnight at room temperature. The precipitated barium sulphate was removed by centrifugation and washed with water (20 ml), and the combined supernatant and washings were concentrated *in vacuo* to saturation, and then cooled overnight at 0°. The crystalline material was collected by filtration, and the filtrate was again evaporated to saturation and chilled. The com-

bined product was recrystallised twice from water to yield **2** as the strychnine salt dihydrate (1.12 g, 52%), $[\alpha]_D^{22}$ −28.5° (*c* 1.4, water) (Found: C, 48.8; H, 7.7; N, 7.1; P, 3.85. $C_{48}H_{57}N_4O_{13}P \cdot 2H_2O$ calc.: C, 49.2; H, 8.1; N, 7.3; P, 4.05%).

To a solution of **2** (barium salt, 100 mg) in water (5 ml) was added ammonium sulphate (34 mg) and the mixture was chilled for 15 min at 0°. The precipitated barium sulphate was removed by centrifugation, the clear supernatant was cooled to 0° and treated with 80mM sodium metaperiodate (3 ml), and the mixture was stirred for 40 min at 0°, in the total absence of light. Sodium borohydride (20 mg) was then added, the solution was left for 16 h at 25°, and then acetic acid (a few drops) was added to decompose the excess of borohydride. The solution was passed through a column of Dowex-50 (H^+) resin (5 ml), and the eluate and washings were combined and evaporated several times with small portions of methanol to remove boric acid. The residue was hydrolysed with 50mM hydrochloric acid (1 ml) for 30 min at 100°, and the products were separated by paper chromatography (solvent *B*) to give arabinose and a small proportion of galactose as the only reducing sugars.

(*b*) *Using silver dibenzyl phosphate.* Compound **6** (4.1 g) in benzene (40 ml) was treated with Drierite (2 g) and silver dibenzyl phosphate[34] (4.8 g), and the mixture was heated for 4 h under reflux with stirring. The precipitated silver salts were removed by filtration and washed with benzene (2 × 20 ml), and the combined filtrate and washings, which were not positive to silver nitrate dissolved in acetone, were concentrated under diminished pressure to give a clear syrup. Hydrogenolysis and alkaline hydrolysis, similar to that described in (*a*), then yielded **2** as the barium salt dihydrate (0.84 g, 19%), $[\alpha]_D^{22}$ +16.8° (*c* 2.4, water).

In a similar manner, the bromide **7** gave **2** (26%).

(*c*) *Using anhydrous phosphoric acid.* Finely powdered **3** (500 mg) was placed in the top section of a Thunberg tube, equipped with a vacuum side-arm, and anhydrous phosphoric acid (600 mg) was placed in the bottom section. After the whole tube and its contents had been thouroughly dried *in vacuo* over magnesium perchlorate for 5 days, the side arm was connected to a vacuum pump and the lower section was heated at 75°. After the phosphoric acid had melted, the two substances were mixed, and the reaction was allowed to proceed at this temperature for 5–7 min. The mixture was then rapidly cooled, dissolved in re-distilled tetrahydrofuran (5 ml), and added to ice-cold M lithium hydroxide (30 ml). The solution was kept for 18 h at room temperature and then for 4 h at 0°. Trilithium phosphate was removed by centrifugation and the pH of the clear supernatant was adjusted to 8.5 with Dowex-50 (H^+) resin. After concentration of the solution *in vacuo* at 25–30° to *ca.* 3 ml, barium acetate (0.5 g) was added followed by ethanol (15 ml). The precipitate was kept for 2 h at 0°, collected by centrifugation, washed successively with ethanol and ether, and dried *in vacuo* ($CaCl_2$). The product was dissolved in water (4 ml), faint traces of insoluble material were removed by centrifugation, and the solution was treated with ethanol (24 ml). The precipitate, after being stored for 1 h at 0°, was collected by centrifugation and immediately subjected to an identical re-precipitation. The product was washed successively with 60% ethanol, acetone, and ether, and then dried *in*

vacuo (P_4O_{10}) to yield **2** as the barium salt dihydrate (216 mg, 39%), $[\alpha]_D^{21}$ +16.7° (*c* 0.89, water).

Treatment of **3** (500 mg) with anhydrous phosphoric acid (600 mg) for 2 h at 60°, in a similar manner, yielded **2** (255–282 mg, 46–51%), $[\alpha]_D^{22}$ +16.65° (*c* 0.76, water).

In the same manner, **5** (500 mg) gave **2** (172 mg, 31%).

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