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Note

Chemical synthesis of cholesteryl β-D-galactofuranoside and -pyranoside

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Abstract—Two isomeric cholesteryl galactosides, cholesteryl β-D-galactofuranoside and -pyranoside, have been synthesized by the Koenigs–Knorr reaction. Glycosylation of cholesterol with 2,3,5,6-tetra-*O*-benzoyl-D-galactofuranosyl bromide, followed by Zemplén saponification with sodium methoxide, gave cholesteryl β-D-galactofuranoside. By using 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl bromide as the glycosyl donor, followed by alkaline hydrolysis, cholesteryl β-D-galactopyranoside was obtained. The title compounds were characterized by their IR spectra and by their ^{1}H and ^{13}C NMR spectra. Structure considerations of the two cholesteryl galactosides correlated with data in the literature, thus confirming that cholesteryl β-D-galactopyranoside is an antigenic lipid of Lyme disease agent, *Borrelia burgdorferi*.

Keywords: Cholesteryl β-D-galactofuranoside; Cholesteryl β-D-galactopyranoside; Sterylglycosides; Glycosylation

The isolation and/or synthesis of steryl glycosides have been motivated by reasons related to either physiological and biochemical phenomena or relative chemical reactivity of different hydroxyl groups on the same steroid.[1] One of the first chemical preparations of cholesteryl β-D-glucopyranoside, as well as of similar steroids derived from cholesterol, was carried out to explain structure–function relationships of saponins, which are natural compounds having a similar structure to that of the cholesteryl glucosides.[2] In this way, cholesterol became a model acceptor for glycosylation reactions.[3–5] About 45 glycosides, glucuronides, glucosides and *N*-acetylglucosaminides of the most prominent five naturally occurring bile acids, as well as a glucuronide conjugate of pregnandiol,[7] have been prepared by the Koenigs–Knorr reaction.[6] In the present paper the syntheses of cholesteryl β-D-galactofuranoside and -pyranoside are described, the latter compound being known as a constituent of the cell-wall membrane of *Borrelia burgdorferi*, the etiologic agent of Lyme disease.[8,9]

For the synthesis of cholesteryl β-D-galactofuranoside (**3**), a mixture of penta-*O*-benzoyl-α,β-D-galactofuranoses (**1**, Scheme 1) was prepared by repeated crystallization of the benzoylation products of D-galactose in hot pyridine, and the structure of each component was confirmed by its ^{1}H and ^{13}C NMR spectra.[10]

Bromination of this mixture with hydrobromic acid in glacial acetic acid[11,12] gave tetra-*O*-benzoyl-α-D-galactofuranosyl bromide (**2**, Scheme 1), which was used for the Koenigs–Knorr glycosylation of cholesterol in conjunction with cadmium carbonate.[6] The glycosylation mixture was submitted to alkaline hydrolysis, neutralization and Folch partition. The main reaction product was recovered, together with unreacted cholesterol, in the chloroform phase and the latter two compounds were separated by column chromatography on silica gel.[13] Finally, a compound (**3**, Scheme 1) containing equimolar amounts of cholesterol and galactose and

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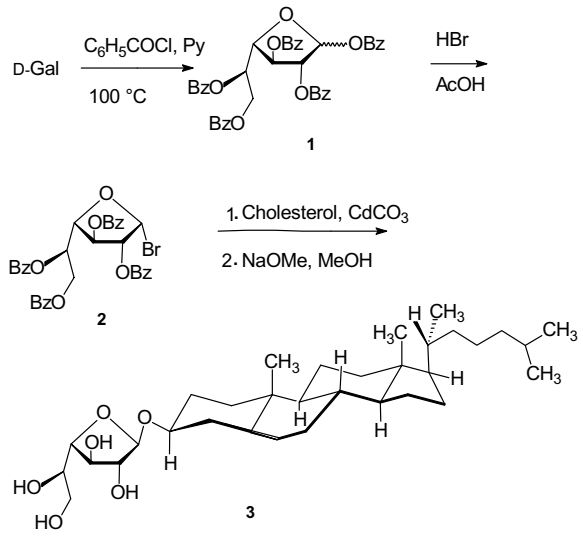

Scheme 1.

having the ^{1}H and ^{13}C NMR characteristics of cholesteryl β-D-galactofuranoside was obtained.

For the synthesis of cholesteryl β-D-galactopyranoside, penta-*O*-acetyl-β-D-galactopyranose, obtained by acetylation of D-galactose in pyridine,[14] followed by column chromatography on silica gel, was converted to tetra-*O*-acetyl-α-D-galactopyranosyl bromide by treating it with hydrobromic acid. Koenigs–Knorr reaction of this compound with cholesterol, followed by alkaline hydrolysis with sodium methoxide, led to a compound containing equimolar amounts of cholesterol and galactose and ^{1}H and ^{13}C NMR spectra characteristic of cholesteryl β-D-galactopyranoside.[8,9]

Cholesteryl galactopyranosides have been characterized in the cell-wall membrane of *B. burgdorferi*, the agent of Lyme disease.[8,9] On the other hand, steryl glucosides present spectacular biochemical functions: sitosteryl β-D-glucopyranoside is a primer for cellulose synthesis in plants,[15] and steryl glucosides are glucosyl donors for ceramides that are involved in the biosynthesis of glucocerebrosides.[16]

1. Experimental

1.1. General methods

Thin-layer chromatography (TLC) was performed on E. Merck Silica Gel 60 plastic plates with the following mixtures: 7:1 toluene–MeOH (solvent 1) and 50:10:1 chloroform–MeOH–water (solvent 2). Visualization was accomplished by dipping the plates in a solution of ammonium molybdate, sulfuric acid and cerium(IV) sulfate, followed by heating. Column chromatography was conducted with Silica Gel (E. Merck, 5–40 μm). IR spectra (KBr) of the two cholesteryl galactosides were recorded on deacylated compounds. ^{1}H and ^{13}C NMR spectra were determined at 400 and 100 MHz, respectively, in $CDCl_3$, by using a Bruker ARX 400 spectrometer. For both cholesteryl galactosides synthesized in this paper, per-*O*-acetylated derivatives were used for recording the ^{1}H and ^{13}C NMR spectra. All reagents were purchased from E. Merck or Fluka Chemical Co.

1.2. Cholesteryl β-D-galactofuranoside

Penta-*O*-benzoyl-α,β-D-galactofuranoses (**1**) were prepared by benzoylation of D-galactose in hot pyridine, followed by repeated crystallization, and their structures were confirmed by ^{1}H and ^{13}C NMR spectroscopy.[10] Bromination of this mixture with HBr in glacial HOAc[11,12] gave tetra-*O*-benzoyl-α-D-galactofuranosyl bromide (**2**) (TLC, solvent 1). A solution of HBr (33%) in glacial HOAc (2.68 mL, 15 mmol) was cooled on ice under exclusion of moisture, and then 2.1 g (3 mmol) of penta-*O*-benzoyl-α,β-D-galactofuranoses and 10 mL of 1,2-dichloroethane were added. Tetra-*O*-benzoyl-α-D-galactofuranosyl bromide was extracted with cold $CHCl_3$ and processed in the usual manner[12] to give 1.25 g (1.89 mmol, 90%) of **2**. Product **2** was dissolved in 10 mL of dry toluene and added to a suspension consisting of 12 mL of dry toluene, 2 g of Drierite, 2 g of $CdCO_3$[6] and 0.608 g (1.58 mmol) of cholesterol and refluxed for 7 h. The suspension was then diluted with one volume of $CHCl_3$, mixed with Celite and filtered. The filtrate was concentrated to dryness in a vacuum, and the residue was dissolved in 40 mL of 0.15 M NaOMe and stirred overnight at room temperature. The solution was neutralized with methanolic HCl, and the product was partitioned as indicated by Folch.[13] The organic phase was evaporated, and the residue was submitted to column chromatography on silica gel in a continuous gradient of MeOH in $CHCl_3$. By mixing the appropriate fractions, 0.54 g (0.97 mmol, 61.6%) of cholesteryl β-D-galactofuranoside (**3**) was obtained. After crystallization from MeOH the product gave needles: mp 151–153 °C; $[\alpha]_D^{25}$ −79 (*c* 1.035, 1:4 $CHCl_3$–MeOH); R_f 0.58, solvent 2; IR (KBr): *v* 3364, 2934.38, 1463.64, 1379.20, 1078.45, 650.88; ^{1}H NMR: δ 5.17 ($J_{1',2'}$ <1; H-1′), 5.03 ($J_{2',3'}$ 2.0; H-2′), 5.00 ($J_{3',4'}$ 6.2; H-3′), 4.28 ($J_{4',5'}$ 3.6; H-4′), 5.37 ($J_{5',6'a}$ 3.2; H-5′), 4.21 ($J_{5',6'b}$ 3.6; H-6a′), 4.31($J_{6',6'b}$ 11.8; H-6b′) as well as values characteristic for the cholesteryl moiety;[8,9] ^{13}C NMR: δ 103.95 (C-1′), 81.93 (C-2′), 77.01 (C-3′), 79.43 (C-4′), 69.18 (C-5′), 62.70 (C-6′) as well as values characteristic of cholesterol.[8,9] Anal. Calcd for $C_{33}H_{56}O_6$: C, 72.26; H, 10.22. Found: C, 72.48; H, 10.47.

Acidic hydrolysis of the product (10 mg) in a boiling mixture of water–EtOH (10 mL) containing 1 M HCl needed less than 5 min for total reaction (TLC, solvent 2). The solution was concentrated to dryness and the residue was partitioned between water and $CHCl_3$. The organic phase was used for cholesterol

determination (Liebermann–Burchardt), and the water phase for galactose determination (anthrone). A molar ratio of cholesterol–galactose of 0.98:1.01 was found.

1.3. Cholesteryl β-D-galactopyranoside

Penta-*O*-acetyl-β-D-galactopyranose was obtained by acetylation of D-galactose in pyridine,[14] followed by column chromatography on silica gel, and it was converted to tetra-*O*-acetyl-α-D-galactopyranosyl bromide by treating it with HBr. Koenigs–Knorr reaction of this compound with cholesterol, followed by alkaline hydrolysis with NaOMe, led to a compound containing equimolar amounts of cholesterol and galactose and presenting the physicochemical characteristics of cholesteryl β-D-galactopyranoside.[8,9] The latter compound migrated slower than the preceding isomer (R_f 0.49, solvent 2). IR spectra disclosed the presence of sugar moiety as well as of cholesteryl alycone. ν_{max}^{KBr} cm^{-1}: 3421.35; 2930.33; 1733.27; 1636.55; 1461.57; 1375.46; 1046.47; 472.92. NMR spectra indicated signals very similar to those reported.[8,9] ^1H NMR: δ 4.540 ($J_{1',2'}$ 8.0; H-1′), 5.186 ($J_{2',3'}$ 10.2; H-2′), 5.031 ($J_{3',4'}$ 3.2; H-3′), 5.372 ($J_{4',5'}$ 3.2; H-4′), 3.889 ($J_{5',6'a}$ 6.4; H-5′), 4.116 ($J_{5',6'b}$ 6.8; H-6a′), 4.188 ($J_{6',6'b}$ 11.2; H-6b′) as well as values characteristic for the cholesteryl moiety; ^{13}C NMR: δ 100.27 (C-1′), 71.02 (C-2′), 77.01 (C-3′), 69.10 (C-4′), 77.33 (C-5′), 61.30 (C-6′) as well as values characteristic of cholesterol.

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