Koenigs-Knorr Synthesis of Natural and Potential Metabolites of Cholesteryl Hexosides

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The following glycosides of cholesterol have been synthesized: α-D-galactofuranoside, β-D-glucopyranoside, bis-sulfo-3,6-β-D-glucopyranoside. 1-α-Bromides of protected sugars were used as glycosylation donors: tetra-O-benzoyl-D-galactofuranosyl and tetra-O-acetyl-D-glucopyranosyl. Peracetylated sugars, i.e., precursors of sugar bromides, were synthesized in conditions that preferentially facilitated the envisaged ring: room temperature for pyranosic ring, high temperature for furanosic one. Constantly, cadmium carbonate was used as promoter. As a general scheme of work, glycosylation mixture was submitted to Zemplen saponification and the obtained glycolipids have been separated by characteristic methods. The glucoside of cholesterol was sulfated with chlorosulfonic acid in pyridine. Neutral or acidic glycolipids were characterized per se by chromatographic and chemical means as well as by IR spectroscopy. Sulfatide and galactocerebrosides of rat brain and ganglioside GM4 of rooster tests were used as reference compounds. Alternatively, the synthesized glycosides were peracetylated and their 1H and 13C NMR spectra registered. New NMR spectroscopic data are presented for cholesteryl-α-D-galactofuranoside and -bis-sulfo-6-β-D-glucopyranoside.

Key words: cholesteryl-α-D-galactofuranoside, cholesteryl-β-D-glucopyranoside, 3,6-bis-sulfo cholesteryl-β-D-glucopyranoside, 1H and 13C NMR spectra

More blamed than praised, cholesterol is, nonetheless, the most investigated sterol, both in free form and as ester with fatty acids or sulfuric acid. However, the approaches dealing with cholesteryl glycosides are less numerous [1]. Investigations concerning cholesteryl glycosides have been stimulated by a vast array of biochemical and physiological implications of sterol glycosides. As a rule, disclosing of these functions was preceded by the knowledge of their natural distribution. Surprisingly, in many cases the works linked with their widespread was preceded at its turn by steryl glycoside synthesis or closely intertwined with it [2].

The mechanism for avoiding desiccation in terrestrial vertebrates resides in a water barrier located in the outer portion of the integument [3]. This water barrier is formed especially of lipids and at least in mammalian, avian, and reptilian skin these lipids are packaged into lamellar granules within the viable epidermal cells. Besides free sterols, four other sterol derivatives were found in chicken epidermis: β-D-glucosylsterols, 6-O-acyl-β-D-glucosylsterols, steryl esters and cholesteryl sulfate [4]. Two types of glycolipids, acylglucosylsterol and glucosylsterol, have been described in snake epidermis by chemical, spectroscopic, and gas-liquid chromatographic methods [5]. Lyme disease is an infectious disease relatively widespread in the United States, Europe, and Northern Asia, whose causing agent is the spirochete Borrelia burgdorferi and its subspecies. Lyme disease affects sequentially or simultaneously the skin, joints, nervous system, and heart [6]. Glycolipids protruding outside B. burgdorferi cells are immunoreactive i. e., they give positive reaction with sera from Lyme disease patients. Their structure elucidation led to the conclusion that they are cholesteryl 6-O-acyl-β-D-galactopyranoside, the acyl group being a variety of fatty acids [7, 8]. Other spectacular biochemical and physiological roles of steryl glycosides prove they are more than cellular membrane constituents.

Cholesteryl β-D-glucopyranoside is the molecule of stress for lower and higher organisms, being the first molecule biosynthesized in case of this (ab)normal state [9]. Estradiol β-D-xylol(pyranoside) is a primer for glycosaminoglycan biosynthesis [10] and sitosterol β-D-glucopyranoside is the primer molecule for cellulose biosynthesis in plants [11]. Also in plants, steryl glucoside plays the role of special natural reagent, serving as a glucosyl donor to ceramides for glycosphingolipids biosynthesis [12].

In this paper, cholesteryl α-D-galactofuranoside, β-D-glucopyranoside, β-D-(bis-sulfo-3,6)glucopyranoside have been synthesized and characterized to be used as enzymatic substrates for furanosidases and glucosidases, respectively. The first and the third compounds are new and a new chemical condensing agent was used for the synthesis of cholesteryl β-D-glucopyranoside. Bis-sulfo derivative of cholesteryl glucoside is destined to investigate the influence of sulfate group(s) on glycosidases enzymatic activities acting upon steryl glycoside. At the same time, bis-sulfo glucoside structural motif is an excellent ligand for select in families.

Experimental part

The following compounds or materials were purchased from Merck (Germany): cholesterol, D-glucose, D-galactose, pyridine, acetic anhydride, benzyl chloride, chlorosulfonic acid, silica gel for column chromatography, precoated glass plates with silica gel for thin layer chromatography (TLC). HBr, 32-33 % in glacial acetic acid was from Fluka. Galactocerebrosides (β-D-galactopyranosyl-1’ceramide) and sulfatide (galactocerebroside 3-sulfate) from rat brain and ganglioside GM4 (sialosyl-α3-β-D-galactopyranosyl-1’ceramide), ganglioside GM3 and lactosyl-ceramide of rooster tests have been isolated as indicated [13, 14]. Peracetylated monosaccharides – penta-O-benzoyl-αβ-galactofuranoside, penta-O-acetyl-αβ-
glucopyranoside, – have been synthesized from the respective sugars, as indicated [15-17]. The corresponding tetra-O-acyl-hexosyl bromides were prepared by the reaction of protected sugar with a 32% solution of HBr in glacial acetic acid [15]. Glycosylation reactions were accomplished in dry toluene by using C(OOCH3)2 as chemical condensing agent [15, 17]. Glycosylation mixture was diluted with chloroform while warm and filtered on a Celite pad. The filtrate was concentrated to dryness by rotavapor and the residue hydrolysed by Zemplen saponification [17, 18].

Glycolipids produced by synthesis were separated by column chromatography on silica gel and the separation was monitored by TLC in solvent 1 (SS1, chloroform-methanol-water, 50/10/1, v/v) or SS2 (chloroform-methanol-water, 60/25/4, v/v), in comparison with the total glycosylation mixture [17-19].

Sulfation of cholesteryl \(\beta\)-D-glucopyranoside was made in the cold (ice) by adding two moles of chlorosulfonic acid per mole of glycoside in a solution of pyridine [20]. Pure cholesteryl-hexosides were characterized per se or in peracetylated form.

Analytical methods

Partial or total acidic hydrolysis, periodic acid oxidation and determination of chemical constituents were made as indicated (13-19).

NMR Spectra Registration. \(^1\)H and \(^{13}\)C NMR spectra of all compounds, i.e., glycosylated sterols and aglycones, were registered in peracylated form in CDCl\(_3\) containing TMS. Constantly, the spectra of peracylated glycoside have been compared to the spectra of peracetylated aglycone. NMR experiments were performed on a Bruker Avance DRX 400 spectrometer using 400 and 100 MHz for \(^1\)H and \(^{13}\)C frequencies, respectively. The \(^1\)H-\(^1\)H correlation spectroscopy (COSY) and \(^1\)H-\(^{13}\)C heteronuclear multiple quantum coherence (HMQC) experiments were carried out with an inverse probe.

Cholesteryl-\(\alpha\)-D-galactofuranoside. \(^1\)H-NMR (CDCl\(_3\); \(\delta\) ppm; J Hz): 5.332 (d, 4.0, C-1), 4.919 (dd, 4.8, C-2), 5.585 (t, 7.2, 6.8, C-3), 4.059 (t, 6.0, C-4), 5.181 (m, 4.8), 4.363 (dd, 8.0, 4.0, C-6a), 4.136 (dd, 5.6, 6.4, C-6b), as well as the signals characteristic to cholesterol [7, 8, 15, 18]: 1.00 (H-1’\(\alpha\)), 1.83 (H-1’\(\beta\)), 3.47 (H-3’), 5.38 (H-6’), 0.88 (H-9’), 0.66 (H-18’), 0.94 (H-19’), 0.85 (H-27’) (fig. 1).

\(^1\)C-NMR (CDCl\(_3\); \(\delta\) ppm): 98.472 (C-1), 77.333 (C-2), 73.430 (C-3), 78.795 (C-4), 70.730 (C-5), 62.255 (C-6), as well as signals peculiar to cholesterol: 37.23 (C-1’), 77.33 (C-2’), 140.29 (C-3’), 122.04 (C-6’), 50.12 (C-9’), 11.85 (C-18’), 19.35 (C-19’), 22.81 (C-27’).

Fig. 1. Cholesteryl glycosides: cholesteryl \(\alpha\)-D-galactofuranoside, \(\beta\)-D-glucopyranoside and \(-(bis-sulfo-3,6)\)-\(\beta\)-D-glucopyranoside
was cholesteryl α-D-galactofuranoside. Configuration α was confirmed by the positive value of optical rotation.

Glucosylation reaction of cholesterol could be followed by TLC, by comparing the image of glucosylation mixture before and after Zemplen hydrolysis (fig. 3).

Diastereoisomeric cholesteryl glycosides were separated by silica gel column chromatography, the course of separation being also monitored by TLC (fig. 4). This way, amounts of reaction products of reasonable magnitude for physico-chemical analyses as well as for biochemical [19] and biological investigations have been obtained.

A new chemical condensing agent, cadmium carbonate, was used in this paper for the Koenigs-Knorr synthesis of cholesteryl β-D-glucopyranoside; reaction product was chromatographically separated (fig. 4) and completely characterized.

Chemical sulfation of cholesteryl β-D-glucopyranoside with chlorosulfonic acid in pyridine produced at least two sulfation product, besides unreacted cholesteryl β-D-glucopyranoside (Rf, 0.76) (fig. 5). The minor one (Rf, 0.49), being in trace amounts, could not be separated in order to be characterized. The compound was only tentatively identified, based on our results and others [13, 14, 20, 21], as being cholesteryl-β-D-(6-sulfo)glucopyranoside.

The major one (Rf, 0.31) was easily separated by the unreacted neutral glycoside, the difference between their Rf values being appreciable (0.45, fig. 5). Its chromatographical behaviour by TLC in comparison with neutral and acidic glycosphingolipids suggested two negative charges on the compound, rather than one: although cholesteryl β-D-glucopyranoside migrated very similar to galactocerebroside, the synthetic sulfate ester migrated much slower than cerebroside 3-sulfate (sulfatide) from rat brain (fig. 6).

Cholesteryl α-D-galactofuranoside was compared, in terms of chemical, physical and spectral properties with two diastereoisomeric glycosides, cholesteryl β-D-galactofuranoside and -β-D-galactopyranoside [15, 18]. The compound described in this paper clearly separated of the latter two compounds by TLC (fig. 2). Cholesteryl α-D-galactopyranoside was ruled out by two arguments: 1H and 13C spectral properties as well as production of formaldehyde by periodate oxidation.

There have been a good agreement between our results, concerning 1H and 13C NMR spectra, and the results of [22] about decyl α-D-galactofuranoside as well as the results of [23] about n-pentenyl α-D-galactofuranoside.

Lately only two natural glycosides containing α-D-galactofuranosic ring have been known, according to our knowledge: diacyl-glycerol-sn-3-α-D-galactofuranoside isolated from bacteria Axanthum [24] and agelagalastatin, α-D-galactofuranosyl-2-β-D-galactofuranosyl-3-α-D-galactopyranosyl-1’ceramide, isolated from the sponge Agelas sp., [25].

Synthetic glycosides based on α-D-galactofuranoside were represented more consistently. Ethyl α-D-galactofuranoside was produced by stirring a mixture consisting
of D-galactose diethyl dithioacetal, mercuric chloride and mercuric oxide in ethanol at room temperature [26, 27]. Boiling D-galactose, methanol and traces of hydrochloric acid produced a mixture of 2-O-methyl galactosides, from which methyl α-D-galactofuranoside was separated by column chromatography on cellulose powder [28]. Direct O-methylation of D-galactose by the Kuhn procedure, with methyl iodide and silver oxide in dimethyl formamide, gave predominantly methyl α-D-galactofuranoside [29]. Heating of D-galactose with acetic anhydride and copper acetate gave 1,2,5,6-di-O-isopropylidene-α-D-galactofuranoside [16, 30] and 1,2,6-O-isopropylidene-α-D-galactofuranoside [31]. α-D-Galactofuranosides of decyl, allyl and benzyl alcohol were synthesized by the reaction of unprotected sugar and cationic metals that forms the sugar into furanocyclic ring, pent-4-ethyl-α-D-galactofuranoside was prepared [23].

Sulfation of native cerebroside with a small excess of chlorosulfonic acid led to cerebroside 6-sulfate [21]. The order of the reactivity of the hydroxyl groups of methyl α-D-galactofuranoside, in the Koenigs-Knorr reaction, was found to be, 6-hydroxyl >> 3-hydroxyl > 4-hydroxyl > 2-hydroxyl, with relative yields of 6:4:1.7:1.2:1.0 [33]. Sulfation of unprotected 4-methylumbelliferyl β-N-acetyl-glucosamine, at a molar ratio chlorosulfonic acid:glycoside of 2:1, produced methylumbelliferyl β-N-acetylglucosaminide 6-sulfate [20]. In the present paper, the order of the reactivity of the hydroxyl groups of methyl α-D-galactofuranoside and the major product was cholesteryl β-D-(3,6-bis-sulfate)glucopyranoside. A similar structural motif, p-nitrophenyl β-D-(3,6-bis-sulfate) glucopyranoside. A similar structural motif, p-nitrophenyl β-D-(3,6-bis-sulfate) glucopyranoside, was synthesized by treating the glycoside with (Bu3Sn)2O, azeotropic removal of water, and reacting the stannylene acetal intermediate with SO3NMe3 [34].

In some preliminary experiments we have proved that a crude enzymatic extract of digestive tract of snail (Helix pomatia) hydrolyzed cholesteryl β-D-galactofuranoside that had been emulsified with biliary acids [19] and the action of the enzyme(s) on sulfated steryl glycoside follows to be tested.

Conclusions

Galactofuranosylation of cholesterol by using cadmium carbonate as promoter produces cholesteryl α-D-galactofuranoside and -β-D-galactofuranoside in the molar ratio 1:10. Cadmium carbonate is an excellent chemical condensing agent for glucosylation of cholesterol to cholesteryl β-D-glucopyranoside.

Sulfation of cholesteryl β-D-glucopyranoside, as pyridine solution with chlorosulfonic acid in the molar ratio 1:2, produces cholesteryl β-D-(3,6-bis-sulfate) glucopyranoside as the major product.

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