Synthesis and separation by tritylation of two isomers: 1,2-5,6-di-O-isopropylidene-α-d-galactofuranoside and 1,2-3,4-di-O-isopropylidene-α-d-galactopyranoside

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The two isomers – 1,2-5,6-di-O-isopropylidene-α-D-galactofuranoside and 1,2-3,4-d-O-iisopropylidene-α-D-galactopyranoside, have been obtained as a mixture, in different ratios, by heating D-galactose and acetone in a neutral or acidic medium; the neutral medium consisted in the two reagents and anhydrous copper sulfate while the acidic one contained p-toluene-sulfonic acid. The mixture of the two di-isopropylidenated isomers was submitted to tritylation by reacting it with trityl chloride. In this way a mixture consisting of 1,2-5,6-di-O-isopropylidene-α-D-galactofuranoside and 1,2-3,4-di-O-isopropylidene-6-O-trityl-α-D-galactopyranoside has been obtained. By this reaction, the difference in polarity between the two isomers was highly increased, their separation being possible simply by extraction. Separation of the two isomers have been also accomplished per se by column chromatography on silica gel in a gradient of methanol in chloroform. ^{1}H- and ^{13}C-NMR spectra confirmed the structure and purity of 1,2-5,6-diisopropylidene-α-D-galactofuranoside and of 1,2-3,4-diisopropylidene-6-O-trityl-α-D-galactopyranoside.

Key words: 1,2-5,6-diisopropylidene-α-D-galactofuranoside, 1,2-3,4-diisopropylidene-α-D-galactopyranoside, 1,2-3,4-diisopropylidene-6-O-trityl-α-D-galactopyranoside, ^{1}H NMR, ^{13}C NMR

Natural compounds containing D-galactose substituted on C-3, either in pyranosic or furanosic ring, are relatively widespread in living matter. Sulfatide (cerebroside 3-sulfate; sulfo-3-β-D-galactopyranosyl-1'ceramide) [1], ganglioside GM4 (sialosyl 2α-3-β-D-galactopyranosyl-1'ceramide) [2], acylated galactocerebroside (fatty acyl-3-β-D-galactopyranosyl-1'ceramide) [3, 4], seminolipid (1,2-di-O-acyl sn-3-β-D-galactopyranosyl(3'-sulfo)-glycerol) [5] are chemical constituents of essential organs endowed with an intense metabolism and exerting important physiological functions: brain, liver, kidney, testis. Numerous gangliosides - hundreds of different molecular species - contain the structural motif sialosyl 2α-3-β-D-galactopyranosyl [6]. Agelagalastatin, Galfα-2Galfβ-3Galpβ-1'Cer [7], a chemical constituent of a marine sponge *Agelas* sp., and a remarkably efficient anti-tumoral agent, is also based on a C-3 substitution of D-galactose. The specific capsular polysaccharide of *Streptococchus pneumoniae* type J20 is based on a hexasaccharide unit in which galactofuranosic ring is found in two hypostasis, C-3 substitution inclussively: Glcpβ-3Galfβ-3Glcpβ3(Galfβ-4)GlcNAcp [8]. In different strategies of synthesis, 1,2-5,6-di-O-isopropylidene α-D-galactofuranoside constitutes an extremely versatile precursor: isopropylidene fragment is remarkably resistant in alkaline and oxidative media, being easily cleaved by acids. The general scheme of work consists in the blockage of C-3 of di-isopropylidenated galactofuranoside (alternatively with benzyl, allyl, levulinoyl, etc.), removing of isopropylidene moieties succesively or concomitantly, adequate protection on the molecule and activation at C-1 [9]. On the other hand, D-galactofuranose has been associated with pathogenic or even extremely pathogenic microorganisms and metazoans [10]: *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Trypanosoma cruzzi*, *Streptococcus sanguis*, *Streptococcus mitis* etc. An affording ground for expectation in the combat against the diseases caused by these organisms is based on the fact that D-galactofuranose is absent in humans and other vertebrates. Consequently, drugs causing a controlled interpherence in the metabolism of parasites would not interphere in the metabolism of host - human beings or other vertebrates. In this context, chemists and biochemists have to be prepared for an increased and increasing demand for D-galactofuranosides.

This paper presents the synthesis of 1,2-5,6-di-O-isopropylidene α-D-galactofuranoside and 1,2-3,4-di-O-isopropylidene α-D-galactopyranoside as well as a new approach for the rapid separation of the two isomers; the final products have been characterized by ^{1}H and ^{13}C NMR spectra as well as by other physical constants.

Experimental part

^{1}H and ^{13}C NMR spectra were registered in $CDCl_3$ containing TMS. Thin-layer chromatography (TLC) was performed on ready-to-use glass plates covered with silica gel 60 (E. Merck). The following solvent systems (SS) were used: toluene-ethanol, 3:1 (v/v, SS I), chloroform-methanol, 49:1 (v/v, SS II). Visualysation was made by dipping the plates in a solution of amonium molybdate, sulfuric acid and cerium(IV) sulfate, followed by heating. D-Galactose was exhaustively dried before synthesis, by keeping it in a high vacuum dessicator on phosphorus pentoxide. Two methods of synthesis have been used.

Method 1. A three necked flask containing 0.5 L of dry acetone and 2,8857 g p-toluene sulfonic acid monohydrate was brought to boiling. Separately, 15 g (83.3 mmole) of D-galactose was dissolved by heating in 130 mL dimethylformamide (DMF). Boiling of acetone was stopped and the hot solution of D-galactose was quantitatively

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transferred in the flask. Refluxing was immediately resumed and lasted 5 h; solution was cooled to room temperature and a slight excess of saturated sodium carbonate was added in the flask to neutralize the acidity. The precipitate that appeared was removed by filtration and from this point processing for the two methods was identical.

Method 2. To a suspension of 75 g anhydrous copper sulfate and 0.5 L of acetone that had been heated to boiling in a three necked flask protected against moisture, a hot solution of 15 g (83.3 mmole) D-galactose in 150 mL DMF was added under energetic stirring. Boiling under reflux was immediately resumed and after 24 h, heating was interrupted and 25 g anhydrous copper sulfate and 0.5 L dry acetone were added in the flask [11]. Boiling under reflux was continued for another 24 h and then solution was cooled to room temperature. Solids were removed by filtration and from this point processing of the filtrate was identical for the two methods. The thin syrup obtained by evaporation of solvents in vacuum was diluted with 3-4 vols of water and extracted 5-6 times with chloroform. Total chloroformic solution was dried with sodium sulfate, filtered and concentrated to dryness; the residue consists almost exclussively of 1,2-5,6-diisopropylidene-α-D-galactofuranoside and 1,2-3,4-diisopropylidene-α-D-galactopyranoside.

Tritylation. A mixture of 4 g (15.4 mmole) of di-isopropylidenated galactosides was dissolved in 80 mL pyridine and 7 g (25.1 mmole) trityl chloride was added [12]. The solution was heated to 50 ^{0}C for 5 h, then cooled to room temperature and concentrated to dryness in vacuum. Residue was carefully partitioned between water and hexane. After the equilibrium was established, the two layers were separated and water layer was extracted with a volume of hexane and organic layer with a volume of water. Finally, solutions in the same solvents were mixed. Water solution was evaporated to dryness and the residue, di-isopropylidene-galactofuranoside was crystalyzed from diethyl ether-hexane; m. p. 96-98 0C, $[\alpha]_D^{23}$ - 34.50 (c=1.5, methanol).

^{1}H-NMR ($CDCl_3$; δ, ppm; *J*, Hz): 5.865 (d, 3.6, H-1α of D-Galf), 4.543 (d, 2.4, H-2), 4.128 (m, H-3), 4.090-4.052 (m, 8.4, 6.8, H-4), 4.343 (m, 6.8, 6.4, 7.2, H-5), 3.869-3.837 (m, 4.8, 8.0, H-6a,b); 1.550, 1.454, 1.379, 1.354, methyl groups.

^{13}C-NMR ($CDCl_3$; δ, ppm; *J*, Hz): 104.940 (C-1α of D-Galf), 87.626 (C-2), 76.182 (C-3), 85.905 (C-4), 75.347 (C-5), 65.666 (C-6); 27.425, 26.730, 26.545, 25.255, methyl groups.

Column chromatography of di-isopropylidenated isomers. A mixture of 1,2-5,6-diisopropylidene-α-D-galactofuranoside and 1,2-3,4-diisopropylidene-α-D-galactopyranoside (1-2 g, 3.84-7.68 mmole) were dissolved in a few mL chloroform and loaded on a column of silica gel at the ratio 10-15 mg per g of chromatographic material. Elution was made with a gradient of methanol in chloroform (0-20 %), separation being monitored in SS II. The first compound eluted was 1,2-3,4-di-O-isopropylidene α-D-galactopyranoside. The respective fractions were mixed and evaporated to dryness; oily aspect, $[\alpha]_D^{23}$ - 45^0 (c=2.5, water).

^{1}H-NMR ($CDCl_3$; δ, ppm; *J*, Hz): 5.559 (d, 6.5, H-1α of D-Galp), 4.327 (dd, 2.4, H-2), 4.603 (dd, 2.0, H-3), 4.269 (d, 6.8, H-4), 3.818 (m, 6.8, H-5), 3.866 (m, 11.2, H-6a), 3.724 (m, 10.4, H-6b); 1.536, 1.490, 1.340, methyl groups.

^{13}C-NMR ($CDCl_3$; δ, ppm; *J*, Hz): 96.230 (C-1α), 70.520 (C-2), 70.690 (C-3), 71.466 (C-4), 68.143 (C-5), 62.131 (C-6); 25.951, 25.864, 24.872, 24.258, methyl groups.

1,2-3,4-Di-O-isopropylidene-6-O-trityl-α-D-galactopyranoside presented similar ^{1}H and ^{13}C NMR signals with untritylated compound; moreover it presented, as ^{1}H spectra, 7.233-7.375 (phenyl groups of trityl) and as ^{13}C spectra 128.26-133.60 (aromatic carbons of trityl).

Results and Discussion

The degree of isopropylidenation was relative high, 80-90 %. In a typical experiment, 18.85 g (72.5 mmole; 87 %) of mono- and di-isopropylidene-galactosides have been obtained from 15 g D-galactose. From this, about 40 % were di-isopropylidene galactosides in the ratio furanosic: pyranosic, 6:5. Consequently, 3.8-4.1 g (14.6-15.7 mmole; 17.5-19.4 %) 1,2-5,6-di-O-isopropylidene α-D-galacto-furanoside could be obtained from 15 g D-galactose.

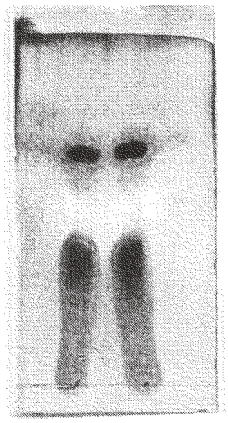

Fig. 1. TLC of the mixture of synthesis of 1,2-5,6-di-O-isopropylidene α-D-galactofuranoside after 5 h of refluxing. Both starts, total mixture of synthesis. Migration, SS I (toluene-etnaol, 3/1, v/v). Visualysation with mostain

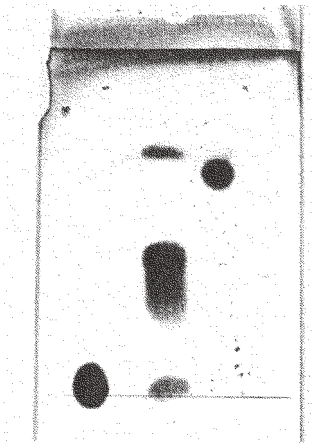

Fig. 2. TLC of the mixture of synthesis of 1,2-5,6-di-O-isopropylidene α-D-galactofuranoside after 5 h of refluxing (start 2), in comparison with D-galactose (start 1) and 1,2-5,6-di-O-isopropylidene α-D-gulofuranoside (start 3). Migration, SS I (toluene-ethanol, 3/1, v/v). Visualysation with mostain

The course of isopropylidenation was followed in SS I (fig. 1 and 2). The faster running spots (R_F 0.65) represent the mixture of isomeric diisopropylidene galactosides and the slower migrating ones (R_F 0.35) represent the mixture of isopropylidene derivatives. The slowest spot (R_F 0.04) represents untransformed sugar (fig. 2). It must be underlined that SS I has a relatively low chromatographic resolution concerning diisopropylidene galactosides; in this solvent, the two isomers migrate as a unique spot. However, SS II, and other SS containing halogenated solvents (dichloromethane), clearly separate the two diastereoisomers (fig. 3). The synthesis of diisopropylidene galactopyranoside is accomplished at room temperature [13] and the synthesis of galactofuranoside isomer at 80-100 ^{0}C. However, in both hypostases, both isomers are formed, in different ratios. Initially was thought that at room temperature, exclusively diisopropylidene galacto-pyranoside is formed. In fact, at that time the low amount of diisopropylidene galactofuranoside fell under the sensitivity of analytical methods [14]; the latter compound could subsequently be determined either by mass spectrometry [15] or by TLC (fig. 3), especially in

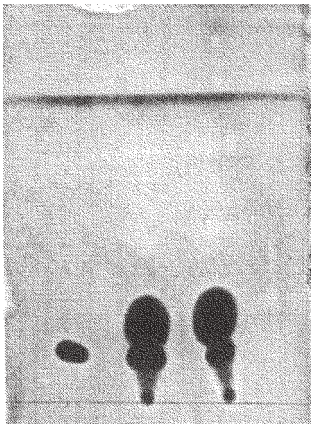

Fig. 3. TLC of total mixture of synthesis of 1,2-3,4-di-O-isopropylidene α-D-galactopyranoside acc. to [14], at room temperature. Start 1, 1,2-5,6-di-O-isopropylidene α-D-galactofuranoside; start 2 and 3, total mixture of synthesis in chloroformic solution (see text). Migration, SS II (chloroform-methanol, 49/1, v/v). Visualysation with mostain

chloroformic solution obtained by extraction of the watery syrup from synthesis. The mixture of two isomers appears as an oily material. Diisopropylidene furanosic derivative, as soon as is separated, crystalizes in an extremely spectacular manner when concentrated.

There is a good agreement between our results and the results of others [16] concerning ^{1}H and ^{13}C NMR spectra. At the same time, it has to be mentioned that this method unequivocally allows to distinguish between the two isomers, 1,2-5,6-di-O-isopropylidene α-D-galacto-furanoside and 1,2-3,4-di-O-isopropylidene α-D-galactopyranoside, as is evident from the above mentioned data.

Di-isopropylidene galactofuranoside served as precursor in the synthesis of 3'-O-sulfo ester of 8-methoxy-carbonyloct-1-yl-*O*-β-D-galactopyranosyl-(1-4)-2-acetamido-2-deoxy-β-D-glucopyranoside [17]. A synthesis of ceramides started with di-isopropylidene gluco-furanoside; isopropylidene fragment from C-5-C-6 was removed and C-6 was cleaved by a Malaprade reaction. Functional aldehyde on C-5 was ready to be submitted to a Wittig reaction in order to introduce the trans duble bond [18]. Di-isopropylidene glucofuranoside was also used to obtain 3-O-methyl-glucopyranoside and other structural motifs of the saponin Holotoxin A [19].

Conclusions

Heating of D-galactose with acetone in dimethyl-formamide, either in the presence of anhydrous copper sulfate or of *p*-toluene-sulfonic acid, produces a mixture of 1,2-5,6-di-O-isopropylidene α-D-galactofuranoside and 1,2-3,4-di-O-isopropylidene α-D-galactopyranoside.

The two isomers can be separated by tritylation of diisopropylidene galactopyranoside derivative.

^{1}H and ^{13}C NMR spectra constitute an adequate instrument to distinguish between the two isomers.

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