Partial Methylation of Monosaccharides for the Elaboration of a Chromatographic Model Used in the Systematic Plant and Microorganisms as well as for Glycosylation

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1,2-5,6-Di-O-isopropylidene α-D-glucofuranose was prepared by acetonation of D-glucose in the presence of copper sulfate and sulfuric acid. The structure of 1,2-5,6-Di-O-isopropylidene α-D-glucofuranose has been confirmed by ^{1}H and ^{13}C NMR analysis as such or preceded by acetylation. Methylation of di-isopropylidene derivative, with methyl iodide/silver oxide in dimethylformamide, led to 1,2-5,6-Di-O-isopropylidene 3-O-methyl β-D-glucofuranose. Alternative peracetylation or perbenzoylation, the both at relative low temperature in order to prevent formation of furanosic ring, were preceded by removing of isopropylidene residues by acidic hydrolysis. 3-O-Methyl D-glucose was also chromatographically analyzed in comparison with D-glucose. Peracetylated derivatives were analyzed by ^{1}H and ^{13}C NMR spectra, in comparison with the corresponding peracetylated D-glucose. Perbenzoylated 3-O-methyl D-glucose was analyzed by IR spectroscopy in order to confirm complete benzoylation.

Keywords: 1,2-5,6-Di-O-isopropylidene α-D-glucofuranose, 3-O-methyl D-glucose, tetra-O-acetyl 3-O-methyl D-αβ-D-glucopyranose, NMR spectra

A series of partially methylated monosaccharides were found in plant tissues. Kiliani (1892) [1] discovered digitalose (3-O-methyl D-fucose) in digitalin, a mixture of glycosides from *Digitalum verum*, used for the treatment of some heart diseases. Other authors separated 3-O-methyl D-fucose from a hydrolysate of digitalin, in order to be used as a reference compound [2]. Practically, all common monosaccharides were found in natural materials as partially methylated forms. 6-O-methyl- and 3-O-methyl-α-D-glucopyranose were found in a polysaccharide from *Mycobacterium phlei* [3,4]. 3-O-methyl-D-glucose, as α-glucopyranose, is a constiuent of some glycosterols from marine organisms [5]. Partially methylated D-galactose was found in nature both as pyranosic and furanosic ring. 3-O-methyl-β-D-galactopyranose is a constiuent of some glycosphingolipids from the sea hare *Aplysia kurodai* [6,7] while 5-O-methyl-β-D-galactofuranose is a constituent of some saponins of the starfish *Astropecten indicus* [8]. 3-O-Methyl-α-D-mannopyranose was characterized as a monomeric unit of some polysaccharides from *Mycobacterium tuberculosis* [9,10]. Both 2-O-methyl-β-D-xylopyranose and 3-O-methyl-β-D-xylopyranose, as well as 2,3-di-O-methyl-β-D-xylopyranose were found in polar head of steroid glycosides from marine organisms [5,11,12]. 4-O-methyl-N-acetyl-α-D-glucopyranosaminide is a chemical constituent of the skin of the sea hare, *Aplysia kurodai* [7]. 2-O-methyl- and 4-O-methyl-α-L-rhamnopyranose are chemical constituents of the major phenolic glycolipid antigen from *Mycobacterium kansasii* [12]. Methylation of inositols, that are biosynthesized from D-glucose *via* D-glucose 6-phosphate, was also encountered [14-17]. Partially methylated acids (anions) were also identified and characterized in natural materials: 4-O-methyl-β-D-glucopyranosiduronic acid in polysaccharides [18] and glycosphingolipids [19] and 8-O-methyl- [20-23] and 9-O-methyl sialic acid [24] in gangliosides of marine organisms.

Monosaccharides having some hydroxy groups in a methylated state, played an important role in systematics [25,26] as well as in the knowledge of important physiological processes [16,17].

In this paper, we synthesized 3-O-methyl D-glucose and elaborated a chromatographical model for its identification in glycosides from plants and microorganisms.

Experimental part

Materials and methods

D-Glucose (Glc), D-galactose (Gal) and methyl iodide (MeI) were from Sigma. All reagents used were either from Fluka or from Merck and they were of analytical grade.

All chemical reactions (fig. 1) were followed by thin layer chromatography (TLC) analysis:

isopropylidenation was analyzed in solvent system 1(SS 1), toluene-ethanol 3/1 (v/v) or in SS 2, chloroform-methanol 19/1 (v/v) or in SS 3, hexane-ethyl acetate 1/1 (v/v). Crystallization of 1,2-5,6-di-O-isopropylidene α-D-glucofuranose (1,2-5,6-Di-O-Ipd α-Glcf) from chloroform-hexane 1:2 (v/v) gave a satisfactory product for the next step (fig. 2). SS 1 and SS 2 allowed also a comparison between Glc and 3-O-Me-Glc. Methylation of 1,2-5,6-Di-O-Ipd α-Glcf could be followed in SS 4, chloroform-methanol 49/1 (v/v) (fig. 3). Deisopropylidenation was monitored in SS 1 or SS 2. The acetonated methylated glycosides were separated by column chromatography on silica gel 60 (0.063–0.200 mm, Merck) in a gradient of ethyl acetate in hexane (fig. 4).

The ^{1}H and ^{13}C NMR spectra of synthesis intermediates and products were measured in $CDCl_3$ containing TMS.

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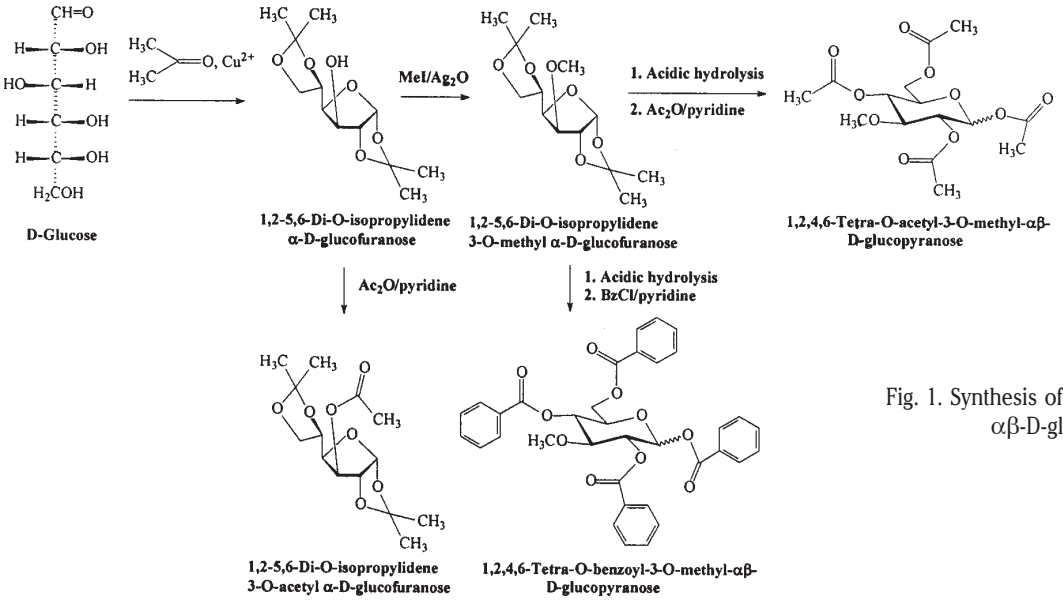

Fig. 1. Synthesis of peracylated 3-O-methyl αβ-D-glucopyranose

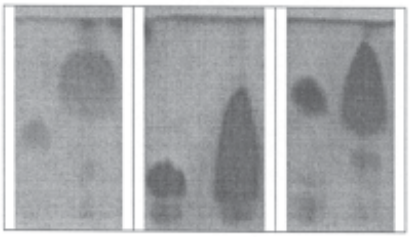

Fig. 2. A TLC comparison of a sample of 1,2:5,6-di-O-Ipd α-D-gulofuranose (start 1 in all chromatograms) and our 1,2:5,6-di-O-Ipd α-Glcf (start 2 in all chromatograms); plates were migrated with SS 3, SS 4 and SS 2, respectively; visualization with mostain.

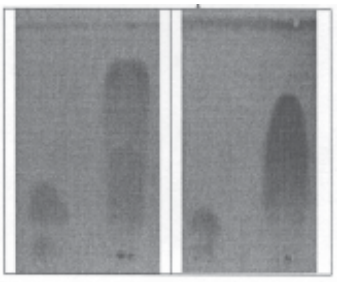

Fig. 3. TLC evidencing of kinetics of methylation reaction. Start 1, on both plates, 1,2:5,6-di-O-Ipd α-Glcf; start 2, methylation product. Migration with SS 4; visualization with mostain.

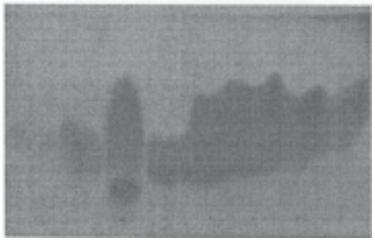

Fig.4. TLC analysis of fractions from column chromatography of 1,2:5,6-di-O-Ipd 3-O-Me α-Glcf in comparison with total methylation product (start 3, see text); migration with SS 4; visualization with mostain

One-dimensional NMR experiments were performed on a Bruker

Avance DRX 400 spectrometer using 400 and 100 MHz for the ^{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.

1,2:5,6-Di-O-isopropylideneα-D-glucofuranose (1,2:5,6-Di-O-Ipd α-D-Glcf) (fig. 1) was synthesized according to the recommendations of [27]: to a suspension consisting of 20 g (111 mmol) of dried, pulverized D-glucose, 400 mL of acetone and 50 g anhydrous cupric sulfate, 16 mL of concentrated sulfuric acid were added. The suspension was stirred at room temperature for 30 h and then filtrated on Celite. The material on the filter was washed with anhydrous acetone and then the whole filtrate was cooled on ice and made slightly alkaline by adding small portions from a 5 N solution of NaOH. The precipitated salts were removed by filtration and the filtrate was concentrated to a small volume by rotavapour and then extracted for three times with equal volumes of chloroform. The total chloroformic solution was washed with a small volume of water, dried with magnesium sulfate, and then evaporated to dryness by rotavapour, and the residue crystallized from chloroform-hexane 1:2 (v/v). The product, 24.55 g (94.4 mmol, yield 85%) had m. p. 106-108 °C, $[\alpha]_D^{20}$ – 18.1 (c=7, water).

^{13}C/^{1}H NMR. ($CDCl_3$; δ ppm; *J* Hz) (fig. 1): 105.24/5.914 (d, 3.6 Hz) (C1/H1); 85.08/4.503 (d, 3.6 Hz) (C2/H2); 73.31/4.293; 2.781 (HO-C-3) (C3/H3); 81.15/4.029 (dd, 5.2 Hz, 2.4 Hz) (C4/H4); 75.04/4.293 (C5/H5); 67.61/4.127 (dd, 6.4 Hz, 2.0 Hz) (C6/H6); 67.61/3.958 (dd, 5.2 Hz, 3.6 Hz) (C6/H6); 25.12, 26.14, 26.74, 26.81/1.299, 1.346, 1.425, 1.479 (*CH_3* groups of $>C(CH_3)_2$); 109.59, 111.79 ($>\!\!C(CH_3)_2$).

1,2:5,6-Di-O-isopropylidene 3-O-acetyl α-D-glucofuranose. A small portion (0.05 g; 0.192 mmol) of 1,2:5,6-Di-O-isopropylidene α-D-glucofuranose was acetylated by stirring overnight with an excess of pyridine (Py)-acetic anhydride (Ac_2O) 2:1 (v/v). The residue, 1,2:5,6-Di-O-isopropylidene 3-O-acetyl α-D-glucofuranose, produced by removing of solvents, partition with water and drying, had 0.0557 g (0.184 mmol, yield 96%).

^{13}C/^{1}H NMR. ($CDCl_3$; δ ppm; *J* Hz) (fig. 1): 104.87/5.804 (d, 3.6 Hz) (C1/H1); 83.18/4.428 (C2/H2); 75.95/5.173 (d, 2.4 Hz) (C3/H3); 79.52/4.140 (C4/H4); 72.29/4.140 (C5/H5); 66.96/4.002 (C6/H6); 66.96/3.954 (C6/H6); 25.09, 26.03, 26.54, 26.66/1.236, 1.253, 1.339, 1.446 (*CH_3* groups of $>C(CH_3)_2$); 109.14, 112.07 ($>\!\!C(CH_3)_2$); 20.66/2.033 (*CH_3* group of acetyl); 169.40 ($>\!\!C=O$ of acetyl).

1,2:5,6-Di-O-isopropylidene 3-O-methyl α-D-glucofuranose.Di-isopropylidene derivative was methylated as follows [28-30]: 2.133 g (8.2 mmol) 1,2:5,6-di-O-isopropylidene α-D-glucofuranose was mixed with 6.17 g (26.62 mmol) dry Ag_2O and 14 mL (224.9 mmol)

CH_3I. The suspension was stirred overnight at room temperature. The second day, TLC analysis in SS 4 indicated that the methylation had taken place, although unreacted material was also detected (fig. 3). In the methylation mixture, 2.5 g of molecular sieves (that had been dried for 2 h at 250 °C), 3.83 g (16.52 mmol) dry Ag_2O, 1.4 g (5.38 mmol) 1,2:5,6-di-O-isopropylidene α-D-glucofuranose, 10 mL (160.6 mmol) CH_3I and 20 mL DMF were added. The suspension was stirred for another 24 h at room temperature. After this time, TLC analysis indicated that all the methyl acceptor had reacted (fig. 3). The suspension was diluted with chloroform, filtrated and partioned with water. The methylated derivative was recovered in chloroformic phase: 3.16 g (11.54 mmol; yield 85%). The crude material was chromatographed on a column of silica gel, in a gradient of ethyl acetate in n-hexane (fig. 4).

$^{13}C/^{1}H$ NMR. ($CDCl_3$; δ ppm; *J* Hz) (fig. 1): 105.14/5.83 (d, 3.6 Hz) (C1/H1); 81.87/4.533 (d, 4.0 Hz) (C2/H2); 72.35/4.247 (dd, 6.0 Hz, 7.6 Hz) (C3/H3); 83.66/3.742 (d, 3.2 Hz) (C4/H4); 81.00/4.082 (C5/H5); 67.17/4.033 (C6/H6); 67.11/3.955 (dd, 5.6 Hz, 3.2 Hz) (C6/H6); 25.35, 26.18, 26.66, 26.81/1.294, 1.333, 1.404, 1.472 (*CH_3* groups of $>C(CH_3)_2$); 108.97, 111.69 ($>\!C(CH_3)_2$); 58.13/3.427 (s, 3H) (*OCH_3*).

3-O-Methyl D-glucose. Isopropylidene protecting groups were removed from 1,2:5,6-di-O-isopropylidene 3-O-methyl α-D-glucofuranose (3 g; 10.94 mmol) by heating the product for 3 h on a boiling water bath in a 80% solution of acetic acid. The solvents were removed by rotavapor and the residue was crystallized from AcOEt, 1.043 g (5.38 mmol; yield 65%) 3-O-Methyl D-glucopyranose, m. p. 166-169°C.

1,2,4,6-Tetra-O-acetyl 3-O-methyl αβ-D-glucopyranose. 3-O-Methyl D-glucose, 1.5 g (7.73 mmol) were stirred overnight at room temperature in an excess of Py-Ac_2O (2:1, v/v). The solvent and the reagent were removed by evaporation at rotavapor by adding small volumes of dry toluene. The product, 2.62 g (7.26 mmol; 96 %), with oily aspect, was dried in vaccum on P_2O_5.

$^{13}C/^{1}H$ NMR. ($CDCl_3$; δ ppm; *J* Hz) (Fig. 1): 89.3/6.29 (d, 3.6 Hz) (C1/H1); 71.1/4.97 (dd, 4Hz, 6Hz) (C2/H2); 78.1/3.70 (C3/H3); 69.0/5.05 (C4/H4); 70.1/4.00 (C5/H5); 61.8/4.18 (dd, 4 Hz, 8 Hz, 1H) (C6/H6); 61.8/4.04 (C6/H6); 60.2/3.47 (s, 3H, CH3) (CH_3 etheric group); 20.6, 20.6, 20.7, 20.8/2.07, 2.08, 2.11, 2.17 (CH_3 of Ac groups); 168.7, 169.1, 169.2, 169.6, 170.6 (>C=O of Ac).

1,2,4,6-Tetra-O-benzoyl 3-O-methyl αβ-D-glucopyranose. 3-O-Methyl D-glucose, 2 g (10.3 mmol) were dissolved in a 30 mL mixture of chloroform-Py (1:1, v/v) and cooled on ice. The stoichiometric amount of benzoyl chloride plus an excess of 20% was added. The solution was stirred 2 h more on ice and then let on ice overnight in the refrigerator. The next day, the solvent was removed by rotavapor, the last traces being evaporated by adding small volumes of dry toluene. The residue was resumed in chloroform and washed successively with a solution of sodium bicarbonate, to remove unreacted benzoic acid, then with a dilued solution of sulfuric acid, in order to remove Py. After washing with water, the chloroformic solution was dried on $MgSO_4$, filtered and evaporated to dryness. The residue, 1,2,4,6-tetra-O-benzoyl 3-O-methyl αβ-D-glucopyranose (5.90 g; 9.68 mmol; 94 %) was analyzed for IR spectra.

Results and discussions

Acetonation (isopropylidenation) could be followed in SS 1 or in SS 2 (fig. 1). The following R_F values were obtained for di-, mono-O-Ipd-derivative and free sugar, respectively, in SS 1: 0.69; 0.38 ; 0.09. On the other hand, acetonation products depend on the chemical nature of the carbohydrate and the physico-chemical conditions used. By using a mixture of sulfuric acid-copper sulfate as an acetonation catalyst, D-Gal gives rise to a mixture of 1,2-3,4-di-O-Ipd α-D-Galp and 1,2-5,6-di-O-Ipd α-D-Galf [31], while D-Glc produces 1,2-5,6-di-O-Ipd α-D-Glcf and some mono-O-Ipd derivative [27]. Since obtaining of 1,2-5,6-di-O-Ipd α-D-Galf by separation from pyranosic isomer is relatively difficult, the schemes have been imagined for production of Galf from Glcf [32,33]. The following distribution is the result of partition of acetonation reaction mixture between water and chloroform: di-O-Ipd-derivative is extracted in chloroformic layer, unreacted sugar in the water layer and mono-O-Ipd-derivative in both. D-Glc could be clearly separated from 3-O-Me-Glc by TLC in SS 1: $R_{F\,Glc}$ 0.08, $R_{F\,3\text{-}O\text{-}MeGlc}$ 0.18. Methyl groups as well as isopropylidenic carbon could be recognized by their characteristic signals: 25.12, 26.14, 26.74, 26.81/1.299, 1.346, 1.425, 1.479 and 109.59 and 111.79 ppm, respectively. Furanosic ring of D-Glc, as well as its α-configuration could be recognized by their characteristic values: 105.24/5.914 ppm (d, 3.6 Hz). (C3/H3) and hydroxy group on C-3 were disclosed by the signals 73.31/4.293 ppm and 2.781 ppm, respectively. The other signals of D-glucofuranose were also present.

Acetylation and methylation reaction of di-O-isopropyliden derivative could be also followed by TLC (fig. 3). In both cases, a less polar compound was obtained due to the blocking of a free hydroxy group. Acetyl ester on C-3 could be recognized due to the signals: 20.66/2.033 ppm and 169.40 ppm. Etheric methyl group could also be distinctively recognized due to the signals 58.13/3.427 ppm (s, 3H). In both cases, the other signals of di-isopropylidenic glucofuranose were also present.

De-isopropylidenation leads to a compound having high polarity, 3-O-methyl αβ-D-glucopyranose, still less polar than D-glucose. Due to this difference in polarity, 3-O-methyl αβ-D-glucopyranose has a higher R_F value in comparison with D-glucose, by paper and TLC [34]. In fact, it was this criterion that leads to finding of most natural compounds containing partially methylated sugars [35].

Perbenzoylated 3-O-methyl derivative do not present any no absorption at 3200-3500 cm^{-1}, by IR spectra, a proof that no free hydroxy group was present.

Peracetylation of 3-O-methyl D-glucose in the cold produced 1,2,4,6-tetra-O-acetyl 3-O-methyl αβ-D-glucopyranose. Methyl etheric group could be recognized due to the signals 60.2/3.47 ppm (s, 3H, CH3). There was a good agreement between our NMR data and the data from literature [32,33,36].

All linear isomeric monomethyl ethers of the common monosaccharides have been prepared. In numerous situations there are two or more options for this. 3-O-Methyl D-glucose has been synthesized as indicated above. 2-O-Methyl D-glucose was synthesized from 1,2-O-Ipd α-D-Glcf. The latter compound was perbenzoylated, then the isopropylidene group removed and the product exhaustively methylated. Zémplen saponification and an acidic hydrolysis gave 2-O-Me αβ-D-Glcp [37]. 4-O-Methyl D-glucose has been synthesized by methylation of 2,3:5,6-di-O-Ipd D-Glc dimethyl acetal, followed by the removal of the protective groups [38,39]. Isopropylidenation of γ-lactone of D-glucuronic acid gave 1,2-O-Ipd-α-D-glucurone; methylation of the latter compound, followed by reduction with $LiAlH_4$, produced 1,2-O-Ipd-5-O-Me-α-D-glucofuranose, and removing of protecting group led to 5-O-Me-αβ-D-Glcf [40]. Simultaneous deacetylation and methylation of 1,2-O-Ipd-3,5-O-Bzld-6-O-Ac-α-D-Glcf, and removal of protective groups, gave 6-O-Me-D-Glc [41].

Chemical means including phenylosazone formation [42], oxidation to onic/uronic/áric acid and the analysis of the lactones [43,44], periodate oxidation [45] have been used for the elucidation of carbon atom where methyl group was linked.

Conclusions

Isopropylidenation of D-glucose with acetone, in the presence of anhydrous copper sulfate and sulfuric acid gives rise to 1,2:5,6-Di-O-isopropylidene α-D-glucofuranose; reaction product can be characterized *per se* or as acetate.

Furanosic diisopropylidenated derivative of D-glucose is a suitable compound for selective methylation on C-3, with MeI/Ag_2O; a large excess of methylation reagent should be used.

Protective isopropylidene groups can be removed by acidic hydrolysis and the partially methylated sugar can be peracetylated or perbenzoylated. Both peracylated products can be activated as 1-bromo 1-deoxy derivatives and used in glycosylation reactions.

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