Carbohydrate Research 379 (2013) 18–20

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

A facile method for the synthesis of partially O-methylated alditol acetate standards for GC–MS analysis of galactofuranose-containing structures

Jian-yu He [a], Yu-na Guo [b], Ling-ling Zhang [c], Lin-hong Huang [c,*]

[a] Department of Pharmacology, Xi'an Jiaotong University College of Medicine, Xi'an 710061, China
[b] Northwest University, Xi'an 710069, China
[c] Hong-Hui Hospital, Xi'an Jiaotong University College of Medicine, Xi'an 710054, China

ARTICLE INFO

Article history:
Received 1 April 2013
Received in revised form 3 June 2013
Accepted 10 June 2013
Available online 18 June 2013

Keywords:
Partially O-methylated alditol acetates
GC–MS standards
Galactofuranose-containing structures
Methylation analysis

ABSTRACT

Mixtures of partially O-methylated alditol acetate standards of galactofuranose were synthesized rapidly. Methyl galactofuranosides were obtained with a yield of 79.9% within 4 h under optimized reaction conditions. Methylation of methyl glycosides was carried out in the presence of $BaO/Ba(OH)_2{\cdot}8H_2O$, giving rise to mixtures of partially methylated glycosides. The batch containing the most diverse structures of methyl ethers was converted into partially O-methylated alditol acetates (PMAAs) and then subjected to GC–MS. These PMAAs could be used as GC–MS standards for simultaneous identification of galactofuranose units with diverse linkages in complex carbohydrates.

Galactofuranose (Gal*f*)-containing structures are widespread in bacteria, fungi and protozoans. Their function as the structural determinants of the cell walls of bacteria and fungi, and their role as a broad range of storage polymers have been well verified.[1] Although they appear to play different roles, the Gal*f*-containing molecules that occur in organisms pathogenic or allergenic to man are frequently antigentic and immunodominant. Enzyme linked immunosorbent assay and immunofluorescence microscopy indicated that the immunodominant epitopes were present in the terminal Gal*f* residues, and the linkage type of each Gal*f* was closely correlated with their roles in cell recognition.[2] Methylation analysis is the most facile method for the determination of the substitution patterns of monosaccharide units in oligo- and polysaccharides or carbohydrate moieties of glycoconjugates.[3] Thus, rapid and facile synthesis of partially O-methylated alditol acetate standards of galactofuranose for methylation analysis is necessary.

Methyl glycosides are usually used as start materials for the synthesis of partially O-methylated alditol acetate standards. Under the experimental conditions described previously[3,4] methyl glycopyranosides were the main products while only small amounts of methyl glycofuranoside were formed. Sassaki et al.[5] prepared methyl galactofuranoside using galactose stirred in 0.5% w/w MeOH–HCl or 0.5% w/w MeOH–H_2SO_4 at 25 °C for 16 h. We attempted to develop an alternative set of reaction conditions to prepare methyl glycofuranosides and used them as start materials for the synthesis of their partially O-methylated alditol acetates (PMAAs).

Several methods have been described for the methylation of methyl glycosides, such as those developed by Hakomori,[6] Purdie[5] and Kuhn[7]. Taking into account the disadvantage of Purdie procedure, Wang[3] has developed a $BaO/Ba(OH)_2{\cdot}8H_2O$ system to prepare partially methylated glycosides (PMGs), and almost all the substitution patterns necessary for the analysis of galactopyranose substitutions in the nature have been obtained. We applied this approach to the methyl galactofuranosides, and almost all possible variously O-methylated methyl galactofuranosides were synthesized. The products were then converted into PMAAs for GC–MS analysis. Now, we report on the synthesis of PMAA standards of galactofuranose and their characterization.

For the synthesis of methyl galactosides, galactose was treated with a reflux of 2% hydrogen chloride in methanol at 70 °C for 12 h.[3,4] Under above reaction conditions, the mother liquor contained a mixture of methyl α/β-pyranosides (78%) and methyl α/β-furanosides (28%). To obtain higher yields of galactofuranosides, the methanolyses of galactose have been carefully studied by monitoring (by GC and GC–MS examination of derived volatile TMS-ethers) the loss of galactose, the formation of galactofuranosides, and the formation of galactopyranosides.

* Corresponding author at present address: Hong-Hui Hospital, 76 Nan-guo Road, Nan-guo Gate, Xi'an, Shaanxi 710054, China. Tel.: +86 29 8780 0002; fax: +86 29 8789 4724.
E-mail address: huanglin824@163.com (L.h. Huang).

0008-6215/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2013.06.005

The TMS methyl glycoside derivatives of galactose were analyzed by GC, and six peaks were observed. The relative percentages were calculated on the basis of their peak areas in the GC spectra (Fig. S1 in Supplementary data). The acquired GC data of the TMS methyl glycoside derivatives were informative but disallowed unambiguous identification of the components. Complementary information, however, was obtained via MS analysis of the individual components, preferably performed as combined GC–MS. The major peaks were identified using their retention times and EIMS profiles, referring to previous reports[8].

Figure 1A shows the effect of variation of hydrogen chloride concentration ranging from 0.001 to 0.1 mol/L on the formation of Me Gal at 70 °C for 2 h. As presented in Figure 1A, 0.004 mol/L was the optimum HCl concentration for the formation of galactofuranosides. Figure 1B shows the effect of reaction time on the treatment result of galactose with methanol containing hydrogen chloride (0.004 mol/L) at 70 °C. With the reaction time prolonged, the yield of Me Gal*p* increased, and the concentration of galactose decreased. The percentage of Me Gal*f* was found to increase before 4 h. However, after 4 h, the formation of Me Gal*f* decreased. The effect of reaction temperature (i.e., 0–95 °C) on the galactoside formation in methanolic hydrogen chloride (0.004 mol/L) for 2 h is shown in Figure 1C. It was observed that the formation of galactosides increased as the reaction temperature increased. Galactose could not be converted into galactosides at the reaction temperature below 20 °C. When the reaction temperature was over 70 °C, the concentration of galactosides did not increase any more. The results suggested that galactose was converted into pyranosides via the intermediate furanosides and mild reaction conditions should be used for the preparation of methyl galactofuranosides. When galactose was treated at 70 °C using reflux of methanolic hydrogen chloride (0.004 mol/L) for 4 h, 79.9% galactofuranosides were obtained.

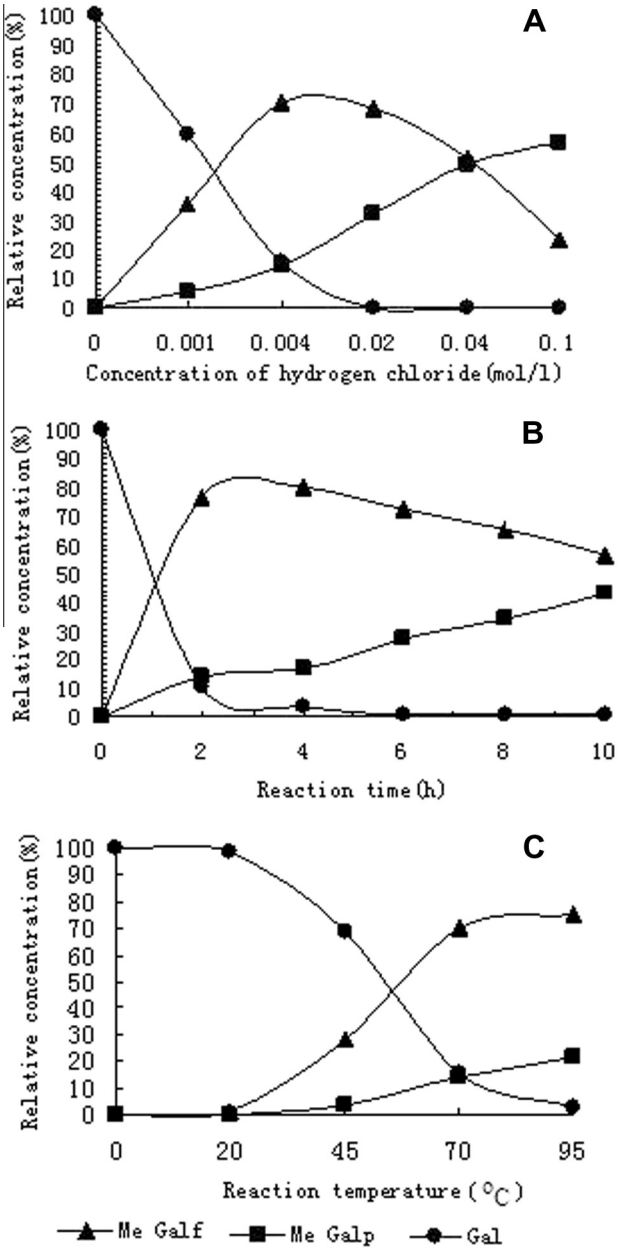

Figure 1. (A) Effect of hydrogen chloride concentration on the formation of Me Gal*f*. (B) Effect of reaction time on the formation of Me Gal*f*. (C) Effect of reaction temperature on the formation of Me Gal*f*.

Methylation of methyl galactosides was carried out in the presence of $BaO/Ba(OH)_2{\cdot}8H_2O$. The methylation proceeded gradually. After 30 min, domination of mono-*O*-methyl ethers was noticed, and another 30 min later, di-*O*-methyl derivatives were formed. The derivatives with higher degrees of methylation were obtained at later stages. As the reaction time exceeded 4 h, the reaction solution contained unsubstituted, mono-, di-, tri-, and tetra-*O*-methyl derivatives, which were subsequently converted into PMAAs via successive hydrolysis, reduction with $NaBH_4$, and acetylation in pyridine. The resulting PMAAs were identified via GC–MS analysis, using their retention times and EIMS profiles referring to previous reports.[4] Almost all possible PMAA structures were obtained in the rapid synthesis, except 2,5-Me_2Gal. They are tabulated in Table 1, with their relative proportion calculated on the basis of their peak areas in GC spectra. As shown in Figure 2, PMAAs of methyl Gal*f* were well separated by GC–MS using an Rtx-5ms capillary column and a temperature programing of 140 °C (3 min) −250 °C (40 min) at 5 °C/min. Some pairs of methylated derivatives (e.g., 2- and 5-*O*-methylhexitol, 3- and 4-*O*-methylhexitol, 2,4- and 3,5-di-*O*-methylhexitol), for symmetry reasons, gave alditols with the same substitution pattern. To distinguish them, we used $NaBD_4$ instead of $NaBH_4$ as the reducing agent, which can introduce deuterium at the C-1 position (Fig. S2 in Supplementary data).

Thus, a simple, efficient, and very useful procedure for the simultaneous synthesis of partially O-methylated alditol acetate standards (PMAAs) of galactofuranose was developed. It is of particular importance for the structural analysis of complex galactofuranose-containing carbohydrates.

Table 1
Partially O-methylated alditol acetates obtained of methyl galactosides which were synthesized with methanolic hydrogen chloride (0.004 mol/L) at 70 °C for 4 h

O-methylated altitol acetates	Rt (min)	Relative percentage (%)
2,3,5,6-Me_4-Gal*f*	12.93	1.14
2,3,4,6-Me_4-Gal*p*	13.20	1.49
2,5,6-Me_3-Gal*f*	14.66	6.16
2,3,6-Me_3-Gal*f*/*p*	14.79	3.29
3,5,6-Me_3-Gal*f*	14.97	1.62
2,3,5-Me_3-Gal*f*	15.84	3.50
5,6-Me_2-Gal*f*	15.92	6.34
2,6-Me_2-Gal*f*/*p*	16.25	10.30
3,6-Me_2-Gal*f*/*p*	16.64	2.70
2,3-Me_2-Gal*f*/*p*	17.31	16.12
6-Me-Gal*f*/*p*	17.56	2.41
3,5-Me_2-Gal*f*	17.83	4.88
2-Me-Gal*f*/*p* 5-Me-Gal*f*	18.54	28.46
3-Me-Gal*f*/*p*	19.14	5.90
Gal hexaacetate	19.81	5.70

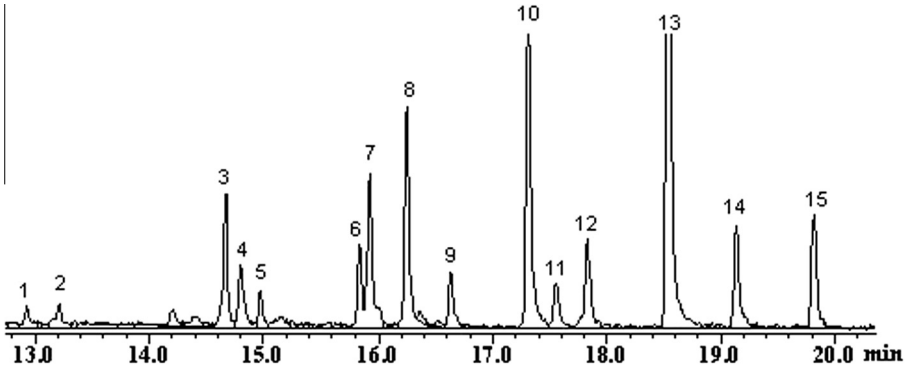

Figure 2. Total ion chromatogram from GC–MS analysis of PMAAs of galactose: (1) 2,3,4,6-Me4-Gal, (2) 2,3,5,6-Me4-Gal, (3) 2,5,6-Me3-Gal, (4) 2,3,6-Me3-Gal, (5) 3,5,6-Me3-Gal, (6) 2,3,5-Me3-Gal, (7) 5,6-Me2-Gal, (8) 2,6-Me2-Gal, (9) 3,6-Me2-Gal, (10) 2,3-Me2-Gal, (11) 6-Me-Gal, (12) 3,5-Me2-Gal, (13) 2-/5-Me-Gal, (14) 3-Me-Gal, (15) Gal hexaacetate.

1. Experimental

1.1. Preparation of methyl galactosides

Galactose (50 mg) was dissolved in methanolic hydrogen chloride and heated for some time. The solution was subsequently neutralized with excess powdered $NaHCO_3$, filtrated, and evaporated to dryness. Me αβ-Gal*p* and Me αβ-Gal*f* were obtained.

1.2. The silylation reaction of methyl galactoside mixtures

Silylation was performed by treating galactosides (10 mg) dissolved in pyridine (1 mL) with 0.4 mL of hexamethyldisilazane (HMDS) and 0.2 mL of trimethylchlorosilane (TMS-Cl). After stirring for 5 min at room temperature, the mixture was concentrated to dryness using a rotary evaporator in vacuo at 50 °C and the residue was dissolved in dichloromethane (5 mL). The sample solution was centrifugated, and the obtained supernatant was used for the analysis by GC and GC–MS.

1.3. GC and GC–MS analysis

GC: The TMS ethers of methyl galactosides were applied to a rtx-50 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm), using a temperature program of 180 °C (2 min) ~250 °C (10 min) at 6 °C/min; the temperatures of injector and flame ionization detector (FID) were set at 270 and 280 °C, respectively; the carrier gas was nitrogen at a flow rate of 0.88 mL/min.

GC–MS: GC–MS analysis of the TMS-ethers of galactosides was carried out on a Shimadzu QP2010 GC–MS, equipped with a capillary column of rtx-5ms (30.0 m × 0.25 mm × 0.25 μm), with the same programed temperature as that for GC; the temperature of injector was 270 °C; electron impact (EI) mass spectra were obtained from *m*/*z* 43 to 800 every 0.5 s in the total ion-monitoring mode using an ion source temperature of 200 °C; helium (1.24 mL/min) was used as the carrier gas.

1.4. Methylation of methyl galactosides

The methyl galactoside mixtures (53 mg) were dissolved in DMF (2 mL), and then BaO (200 mg), $Ba(OH)_2{\cdot}8H_2O$ (10 mg), and CH_3I (1 mL) were added. The suspension was vigorously stirred using a vibrational shaker in the dark to give PMGs. An aliquot (300 μL) of the sample solution was taken out as often as once every 30 min, poured into MeOH, filtered, and assayed by thin layer chromatography (10:1 CH_2Cl_2/MeOH) to monitor the degree of methylation using the orcinol-sulfuric acid staining reagent.

1.5. Preparation of PMAAs

The batch giving a maximum number of spots on the silica gel plate was evaporated, and the obtained residue was used to prepare mixtures of partially O-methylated alditol acetates (PMAAs). The procedure was carried out according to the methods described previously by Wang et al.[3]

1.6. GC–MS analysis of PMAAs

The PMAAs were applied to GC–MS equipped with a capillary column of rtx-5ms (30.0 m × 0.25 mm × 0.25 μm), using a temperature program of 140 °C (3 min) −250 °C (40 min) at 5 °C/min; routine analysis was performed with a scan range from *m*/*z* 43 to 500 in the electron impact mode.

Acknowledgments

The work of the authors described in this review was supported in part by New Century Excellent Talents in University (NCET-08-0893) from Ministry of Education of China and Shaanxi Science and Technology Research and Development Project Fund (2012K16-02-02).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.06.005.

References

1. Jones, C.; Todeschini, A. R.; Agrellos, A. O.; Previoto, J. O.; Mendonça-Previato, L. *Biochemistry* **2004**, *43*, 11889–11897.
2. Suzuki, E.; Toliedo, M. S.; Takahashi, H. K.; Straus, A. H. *Glycobiology* **1997**, *7*, 463–468.
3. Wang, Z.-F.; He, Y.; Huang, L.-J. *Carbohydr. Res.* **2007**, *342*, 2149–2151.
4. Sassaki, G. L.; Gorin, P. A. J.; Souza, L. M.; Czelusniak, P. A.; Iacomini, M. *Carbohydr. Res.* **2005**, *340*, 731–739.
5. Sassaki, G. L.; Iacomini, M.; Gorin, P. A. J. *An. Acad. Bras. Cienc.* **2005**, *77*, 223–234.
6. Hakomori, S. I. *J. Biochem.* **1964**, *55*, 205–208.
7. Kuhn, R.; Trischmann, H.; Low, I. *Angew. Chem.* **1955**, *67*, 32.
8. Bleton, J.; Mejanelle, P.; Sansoulet, J.; Goursaud, S.; Tchapla, A. *J. Chromatogr.* **1996**, *720*, 27–49.