Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 491–494
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Synthesis, intramolecular migrations and enzymic hydrolysis of partially pivaloylated methyl α-D-mannopyranosides

Srđanka Tomić,* Vesna Petrović, Maja Matanović

Department of Chemistry, Faculty of Science, University of Zagreb, Strossmayerov trg 14, HR-10000 Zagreb, Croatia

Received 19 September 2002; accepted 26 November 2002

Abstract

A series of methyl *O*-pivaloyl-α-D-mannopyranosides was synthesized using pivaloyl chloride in pyridine. The 3,6-di-*O*-pivaloyl derivative **6** undergoes intramolecular transesterification in neutral conditions (buffer, pH 7.2) to give its 2,6-di-*O*-pivaloyl analogue **5**. The course of this migration was followed using ^{14}C-labelled **6**. As opposed to **6** compound **5** was shown to be a good substrate for esterases present in rabbit serum. Thus, regioselective enzymic hydrolysis led to the preferential cleavage of the 2-*O*Piv group to yield a mixture of 2- and 6-*O*-monopivalates in a ratio of 1:2.6.

Keywords: Methyl α-D-mannopyranosides; Acylated; Esterases; Rabbit serum; Enzymic hydrolysis; Pivaloyl migrations

1. Introduction

Regioselective and/or chemoselective hydrolysis of pivaloylated and acetylated monosaccharides from the D-glucose,[1–3] 2-acetamido-2-deoxy-D-glucose,[4,5] and D-xylose[6] series of sugars, catalyzed by esterases from rabbit[2,7] and guinea pig[8] sera, were previously demonstrated. It was also shown that in many cases there is no need to isolate enzymes but native serum can be used to obtain satisfactory results, specially in larger scale syntheses. Furthermore, in course of our work with enzyme catalyzed reactions, migrations of the pivaloyl group were observed. These intramolecular migrations were caused by an enzyme present in some mammalian sera[9] but they were also observed, although in just a few cases, in neutral conditions such as buffered reaction solutions at pH 7.2.[3,6] Previous reports showed that in acetylated or benzoylated sugars migrations occur under acidic,[10] basic,[11] and occasionally neutral[11,12] conditions but the pivaloyl group was generally regarded as not prone to migrations. The mechanism of these intramolecular transesterifications was extensively studied, and the general conclusion is that it proceeds through orthoacid intermediates.[10] This is in accord with our results obtained with pivaloylated galactoses in which the intermediate orthoacid was stable enough to be isolated.[13]

We now report on the synthesis of unlabelled and ^{14}C-labelled pivaloylated methyl α-D-mannopyranosides in order to determine whether they could be used as substrates in hydrolyses catalyzed by esterases present in rabbit serum. Furthermore, labelled compounds were also used to determine possible intramolecular migrations of the pivaloyl group.

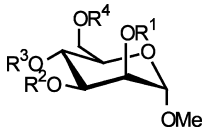
2. Results and discussion

Pivaloylation of methyl α-D-mannopyranoside (**2**) with a fivefold molar excess of pivaloyl chloride resulted in the formation of three products, methyl 2,6-di-*O*-pivaloyl-α-D-mannopyranoside (**5**, 11%), methyl 3,6-di-*O*-pivaloyl-α-D-mannopyranoside (**6**, 49%) and methyl 3,4,6-tri-*O*-pivaloyl-α-D-mannopyranoside (**7**, 26%) (Table 1).[14] Dipivalates **5** and **6** were then submitted to hydrolysis catalyzed by native rabbit serum. 2,6-Di-*O*-pivalate **5** was hydrolyzed in a mixture of phosphate buffer (pH 6.8) and DMSO to give methyl 2-*O*-pivaloyl-α-D-mannopyranoside (**3**, 23%) and methyl 6-*O*-pivaloyl-α-D-mannopyranoside (**4**, 33%) (Table 1). When 3,6-di-*O*-pivalate **6** was used as a substrate, under the same reaction conditions, monopivalates were not ob-

* Corresponding author. Fax: +385-1-4819288
E-mail address: srdjanka@rudjer.irb.hr (S. Tomić).

0008-6215/03/$ - see front matter
PII: S0008-6215(02)00501-3

Table 1



	R^1	R^2	R^3	R^4
2	H	H	H	H
3	Piv	H	H	H
4	H	H	H	Piv
5	Piv	H	H	Piv
6	H	Piv	H	Piv
7	H	Piv	Piv	Piv

served as reaction products. Instead migration of 3-*O*Piv group occurred to yield 2,6-di-*O*-pivalate **5**. It was shown that this reaction was not catalyzed by the enzyme since the same product was obtained when the esterase was omitted from the reaction mixture.

Since 2,6-di-*O*-pivalate was shown to be a good substrate for an esterase present in rabbit serum we synthesized ^{14}C-labelled 2,6-di-*O*-pivalate **5** starting from [U-^{14}C]mannose, and submitted it to enzymic hydrolyses at different time intervals to obtain precise results on possible regioselectivity of these reactions. Thus, ^{14}C-labelled **5** was hydrolyzed in phosphate buffer (pH 6.8) and DMSO using native rabbit serum as the enzyme source. Incubations were stopped at 2 h and 22 h intervals. The 2-*O*Piv was hydrolyzed preferentially and the obtained ratio of 2-*O*-pivalate (**3**) and 6-*O*-pivalate (**4**) was 1:2.6 (2 h). Prolongation of reaction time to 22 h produced completely deprotected compound **2**. In all reaction mixtures traces (1–3%) of the 3,6-di-*O*-pivalate formed as a result of 2→3 migration of the pivaloyl group. No 3-*O*-pivalate was observed at any time. It is interesting to note that the ester group on a secondary hydroxyl in the sugar ring (2-*O*Piv) was cleaved faster than a chemically more labile ester on a primary hydroxyl (6-*O*Piv). Furthermore, this result is opposed to that obtained in the glucose series[3] in which 6-*O*Piv hydrolyses faster than 2-*O*Piv. This is probably due to the axial position of the 2-*O*Piv group in mannose derivatives.

When pure **6** was used as a substrate, the 3→2 migration of the pivaloyl group occurred first leading to the 2,6-*O*-dipivalate **5**, followed by hydrolysis leading to 2- and 6-*O*-pivalates. Again no 3-*O*-pivalate was detected indicating that either **6** is not a substrate for esterases in rabbit serum or that the hydrolysis of 3-*O*Piv occurs exclusively leading to the 6-pivalate. It is also possible that the 3-*O*-pivalate does form but then immediately migrates to the 2-OH position.

Parallel incubations were performed in all cases but without the presence of enzymes. It was observed that no spontaneous hydrolysis occurs, but 2→3 and 3→2 acyl migrations were noticed, indicating that they are not the result of enzymic catalysis but were caused by the reaction medium. We previously observed migrations of the pivaloyl group in reactions catalyzed by an enzyme present in some mammalian sera[9] but also in just a few cases in neutral conditions identical to those described for D-mannose. Thus, 1→2 migration in 1,6-di-*O*-pivaloyl-β-D-glucopyranoside[2] and 2→3 migration in methyl 2,4-di-*O*-pivaloyl-β-D-xylopyranoside were noticed.[6] Furthermore, unusual 2→3 and 3→2 migrations concurrent with 6→4 migrations in both, methyl 2,6- and 3,6-di-*O*-pivaloyl-α-D-galactopyranoside, were observed.[13] In the last case the 2→3 and 3→2 migrations of the pivaloyl group were only partially completed since an orthoacid, a presumed intermediate in the mechanism of transesterifications[10] was stable enough to be isolated as a major product. It was to be expected that 2- and 3-*O*-pivalates of mannose will be especially prone to migrations due to the fact that 2- and 3-OH groups are in a *cis* position which is suitable for the formation of orthoacids. In fact, it was noticed that in pivaloylated mannose, 3→2 migration in the 3,6-di-*O*-derivative **6** occurs rapidly giving the 2,6-di-*O*-pivalate in substantial yields, much higher than those obtained in direct pivaloylations. In contrast, 2→3 pivaloyl group migration in the 2,6-di-*O*-derivative **5** was minor. We then used ^{14}C-labelled **6** to follow the course of this migration (Table 2). It was shown that in 5 h an approximate 1:1 mixture of **5** and **6** was obtained, and prolongation of reaction times did not cause any significant changes in the ratio of two compounds.

Table 2

t/h	**6** (%)	**5** (%)
0.5	79.7	20.3
1	70.2	29.8
1.5	64.5	35.5
2	57.0	43
2.5	53.7	46.3
3	52.4	47.6
4	52.6	47.4
8	45.2	54.8
24	40.8	59.2

3. Experimental

3.1. General methods

All solvents were reagent grade and distilled before use. Column chromatography was performed on silica gel

(Merck). Analytical TLC was performed on Merck silica gel (60 F 254) plates (0.25 mm) with eluent A, EtOAc–C_6H_6 (proportions are given in the text), eluent B, EtOAc–C_6H_6–EtOH–$CHCl_3$ (100:20:1:1) and solvent C, MeCN–H_2O (5:1). Visualization was by charring with H_2SO_4. Melting points were determined with a Büchi B-540 apparatus and are uncorrected. ^1H and ^{13}C NMR spectra (300 MHz, $CDCl_3$, internal Me_4Si) were recorded with a Varian Gemini 300-BB spectrometer. The data presented are in accord with those previously reported for other acylated monosaccharides.[10,15,16] Optical rotations (in degrees) were recorded on the Optical Activity AA-10 Automatic Polarimeter at ~20 °C using $CHCl_3$ as a solvent. Radioactivity was measured by using a Beckman LS-100C liquid scintillation counter and Aquasol (NEN) as a scintillation cocktail.

3.2. Preparation of partially pivaloylated methyl α-D-mannopyranosides (5–7)

Pivaloyl chloride (1.54 mL, 12.5 mmol) was added dropwise to a solution of **2** (485 mg, 2.5 mmol) in dry Py (1.5 mL). The mixture was stirred at ambient temperature for 50 min. The course of reaction was monitored by TLC (solvent A, 1:1). The reaction was stopped by the addition of 96% EtOH (2 mL). Water was added and the mixture of solvents was evaporated under reduced pressure. The remaining traces of water were removed by co-distillation with toluene. The residue was dissolved in $CHCl_3$, the organic layer was washed three times with water, dried (Na_2SO_4) and the solvent evaporated. Column chromatography of the residue (eluent A, 1:1) gave, firstly, methyl 3,4,6-tri-*O*-pivaloyl-α-D-mannopyranoside (**7**; 82 mg, 26%) as an oil: $[\alpha]_D$ + 50.3° (*c* 0.88); R_f ~0.77; ^1H NMR ($CDCl_3$): 1.17 (s, 9 H, 4-*O*Piv), 1.19 (s, 9 H, 3-*O*Piv), 1.23 (s, 9 H, 6-*O*Piv), 3.42 (s, 3 H, *O*Me), 3.97 (m, 1 H, H-5), 4.03 (m, 1 H, H-2), 4.11 (m, 1 H, H-6b), 4.20 (m, 1 H, H-6a), 4.76 (d, 1 H, $J_{1,2}$ = 1.59 Hz, H-1), 5.23 (dd, 1 H, $J_{3,4}$ = 10.01 Hz, $J_{2,3}$ = 3.12 Hz, H-3), 5.37 (appt, 1 H, $J_{3,4}$ = 10.07 Hz, $J_{4,5}$ = 9.99 Hz, H-4); ^{13}C NMR ($CDCl_3$): 27.09, 27.16 (3 (CH_3)$_3$CCO), 38.91 (3 (CH$_3$)$_3$*C*CO), 55.19 (*C*H$_3$O), 62.33 (*C*-6), 65.36 (*C*-2), 68.68 (*C*-3), 69.33 (*C*-4), 71.36 (*C*-5), 100.40 (*C*-1), 176.87, 177.14, 178.10 (3 *C*=O). Anal. Calcd. for $C_{22}H_{38}O_9$: C, 59.18; H, 8.58. Found: C, 59.31; H, 8.75.

Eluted next was methyl 3,6-di-*O*-pivaloyl-α-D-mannopyranoside (**6**; 227 mg, 49%) as white crystals: mp 89–91 °C (C_6H_6–EtOAc = 1:1); $[\alpha]_D$ + 50° (*c* 0.86); R_f ~0.65; ^1H NMR ($CDCl_3$): 1.24 (s, 9H, 3-*O*Piv), 1.26 (s, 9 H, 6-*O*Piv), 3.40 (s, 3 H, *O*Me), 3.80 (m, 2 H, H-4, H-5), 4.00 (m, 1 H, H-2), 4.40 (m, 2 H, H-6a, H-6b), 4.73 (d, 1 H, $J_{1,2}$ = 1.38 Hz, H-1), 5.05 (dd, 1 H, $J_{3,4}$ = 9.35 Hz, $J_{2,3}$ = 3.3 Hz, H-3); ^{13}C NMR ($CDCl_3$): 27.11 (2 (CH_3)$_3$CCO), 38.90 (2 (CH$_3$)$_3$*C*CO), 54.88 (*C*H$_3$O), 63.25 (*C*-6), 66.16 (*C*-2), 69.27 (*C*-3), 70.88 (*C*-4), 74.07 (*C*-5), 100.31 (*C*-1), 178.86 (2 *C*=O). Anal. Calcd. for $C_{17}H_{30}O_8$: C, 56.34; H, 8.34. Found: C, 56.64; H, 8.66.

Eluted last was methyl 2,6-di-*O*-pivaloyl-α-D-mannopyranoside (**5**; 44 mg, 11%) as white crystals: mp 104–106 °C (C_6H_6:EtOAc = 1:1); $[\alpha]_D$ + 22° (*c* 1.16); R_f ~0.42; ^1H NMR ($CDCl_3$):1.22 (s, 9 H, 2-*O*Piv), 1.25 (s, 9 H, 6-*O*Piv), 3.38 (s, 3 H, *O*Me), 3.59 (appt, 1 H, $J_{3,4}$ = 9.89 Hz, $J_{4,5}$ = 9.34 Hz, H-4), 3.94 (m, 1 H, H-5), 4.01 (dd, 1 H, $J_{3,4}$ = 9.34 Hz, $J_{2,3}$ = 3.57 Hz H-3), 4.16 (m, 1 H, H-6b), 4.59 (m, 1 H, H-6a), 4.67 (s, 1 H, H-1), 4.99 (m, 1 H, H-2). ^{13}C NMR ($CDCl_3$): 26.99, 27.16 (2 (CH_3)$_3$CCO), 38.92 (2 (CH$_3$)$_3$*C*CO), 55.01 (*C*H$_3$O), 63.15 (*C*-6), 67.62 (*C*-2), 69.95 (*C*-3), 70.44 (*C*-4), 71.19 (*C*-5), 98.79 (*C*-1), 178.03, 179.59 (2 *C*=O). Anal. Calcd. for $C_{17}H_{30}O_8$: C, 56.34; H, 8.34. Found: C, 56.37; H, 8.10.

3.3. Preparation of radiolabelled methyl α-D-mannopyranosides (2, 4, 6)

The solution of D-[U-^{14}C]mannose (**1**, 2.22 MBq) and D-mannose (50 mg, 0.28 mmol) in dry MeOH was boiled under reflux for 20 h in the presence of catalytic quantities of hydrogen chloride (0.2 mL of 3.3 M HCl in MeOH). The solvent was removed under reduced pressure and MeOH was distilled thrice from the residue in order to remove traces of HCl. TLC (solvent C) indicated that the residue consisted mainly of **2** (85% based on radioactivity), the rest being unreacted starting compound **1**. A solution of **2** in dry Py (1 mL) was treated with pivaloyl chloride (0.07 mL, 0.57 mmol) at room temperature for 3 h. The reaction was stopped by the addition of EtOH (96%, 2 mL). Water was added (1 mL) and the mixture of solvents evaporated under reduced pressure. The remaining traces of water were removed by co-distillation with toluene. The solution of the residual mixture in EtOH (0.5 mL) was applied to a column of silica gel and eluted with eluent A (3:1) to yield methyl 3,6-di-*O*-pivaloyl-α-D-[U-^{14}C]mannopyranoside (**6**; 16 mg, 16%, based on mannose) as an oil: R_f ~0.71; specific activity, 4.97 MBq/mmol. Eluted next was a mixture of **5** and **6** (7 mg, 7% based on mannose); R_f ~0.45 (for **5**).

The column was then eluted with MeOH, the solvent was evaporated under reduced pressure, and the residue rechromatographed on a column of silica gel with eluent C. Eluted firstly was methyl 6-*O*-pivaloyl-α-D-[U-^{14}C]mannopyranoside (**4**; 28 mg, 36% based on mannose); R_f ~0.76; specific activity, 5.86 MBq/mmol. Eluted next was the unreacted **2** (16 mg, 29%, based on mannose); R_f ~0.41, followed by **1**; 2 mg; R_f ~0.25.

3.4. Enzymic hydrolyses

(a) Native rabbit serum (1.0 mL) was added to the solution of **5** (100 mg, 0.28 mmol) in phosphate buffer (pH 6.8, 3.0 mL) and Me_2SO (400 mL). The reaction mixture was stirred for 48 h. The reaction was stopped by adding EtOH (2 mL) followed by the evaporation of solvents under reduced pressure. Column chromatography of the residue (eluent B) yielded, firstly, methyl 2-*O*-pivaloyl-α-D-mannopyranoside (**3**; 17.7 mg, 23%) as an oil; $[\alpha]_D$ +28° (*c* 1.09); R_f ~0.18; ^{1}H NMR ($CDCl_3$): 1.24 (s, 9 H, 2-*O*Piv), 3.39 (s, 3 H, *O*Me), 3.64 (m, 1 H, H-6b), 3.83–3.88 (m, 3 H, H-4, H-5, H-6a), 4.03 (dd, 1 H, $J_{3,4}=9.89$ Hz, $J_{2,3}=3.44$ Hz, H-3), 4.68 (s, 1 H, H-1), 5.06 (m, 1 H, H-2); ^{13}C NMR ($CDCl_3$): 26.97 ($(CH_3)_3CCO$), 38.95 ($(CH_3)_3CCO$), 55.02 (CH_3O), 62.35 (*C*-6), 68.35 (*C*-2), 70.22 (*C*-3), 71.43 (*C*-4), 71.63 (*C*-5), 98.60 (*C*-1), 178.19 (*C*=O). Anal. Calcd. for $C_{12}H_{22}O_7$: C, 51.79; H, 7.97. Found: C, 51.57; H, 8.11.

Eluted next was methyl 6-*O*-pivaloyl-α-D-mannopyranoside (**4**; 25.5 mg, 33%) as an oil; $[\alpha]_D$ +46° (*c* = 1.20); R_f ~0.14; ^{1}H NMR ($CDCl_3$): 1.25 (s, 9 H, 6-*O*Piv), 3.38 (s, 3 H, *O*Me), 3.53 (appt, 1 H, $J_{3,4}=9.54$ Hz, $J_{4,5}=9.48$ Hz, H-4), 3.68 (m, 1 H, H-5), 3.82 (dd, 1 H, $J_{3,4}=9.19$ Hz, $J_{2,3}=3.44$ Hz, H-3), 3.96 (m, 1 H, H-2), 4.24 (m, 1 H, H-6b), 4.57 (m, 1 H, H-6a), 4.75 (s, 1 H, H-1); ^{13}C NMR ($CDCl_3$): 27.09 ($(CH_3)_3CCO$), 38.80 ($(CH_3)_3CCO$), 55.08 (CH_3O), 63.35 (*C*-6), 69.73 (C-2), 70.15 (C-3), 71.87 (*C*-4), 73.99 (*C*-5), 99.10 (*C*-1), 178.96 (*C*=O). Anal. Calcd. for $C_{12}H_{22}O_7$: C, 51.79; H, 7.97. Found: C, 51.84; H, 8.14.

(b) A 13.5 mM solution of methyl 2,6-di-*O*-pivaloyl-α-D-[U-^{14}C]mannopyranoside (**5**) was prepared. An aliquot of this solution (10 mL) was added to the phosphate buffer (pH 6.8, 75 mL) followed by native rabbit serum (50 mL) to obtain the 1 mM solution which was used in hydrolysis experiments. The reaction mixtures were incubated at 37 °C. The reactions were stopped in 2 and 22 h intervals. Previously described work-up procedures were used, and the ratio of products was determined by TLC.[2,3,6,12] When rabbit serum was used the obtained ratio of the 2-*O*-pivalate (**3**) and 6-*O*-pivalate (**4**) was ~1:2.6 (2 h). Complete deacylation occurred in a 22 h period to yield compound **2**.

3.5. 3→2 Pivaloyl migration

(a) The solution of the methyl 3,6-di-*O*-pivaloyl-α-D-mannopyranoside (**6**, 200 mg, 0.55 mmol) in phosphate buffered saline (PBS, pH 7.2) was stirred at 60 °C for 24 h. The solvent was then evaporated under reduced pressure. Column chromatography of the residue (eluent A, 1:1) gave first starting compound **6** (36 mg, 18%) followed by the 2,6-di-*O*-pivalate **5** (136 mg, 68%).

(b) A 2 mM solution of methyl 3,6-di-*O*-pivaloyl-α-D-[U-^{14}C]mannopyranoside (**6**) was prepared. To an aliquot of this solution (10 μL) PBS (190 μL) was added to obtain the 0.1 mM solutions which was used in time course migration experiments. The reactions (200 μL) were kept at 37 °C at time intervals indicated in Table 2, and the ratio of products determined by TLC.[2,3,6,12]

Acknowledgements

We wish to thank the Ministry of Science and Technology of the Republic of Croatia for support of this work (project 119401).

References

1. Tomić, S.; Tomašić, J.; Sesartić, Lj.; Ladešić, B. *Carbohydr. Res.* **1987**, *161*, 150–155.
2. Tomić, S.; Treščec, A.; Ljevaković, Đ.; Tomašić, J. *Carbohydr. Res.* **1991**, *210*, 191–198.
3. Ljevaković, Đ.; Tomić, S.; Tomašić, J. *Carbohydr. Res.* **1992**, *230*, 107–115.
4. Ljevaković, Đ.; Tomić, S.; Tomašić, J. *Carbohydr. Res.* **1988**, *182*, 197–205.
5. Tomić, S.; Ljevaković, Đ.; Tomašić, J. *Carbohydr. Res.* **1989**, *188*, 222–227.
6. Petrović, V.; Tomić, S.; Ljevaković, Đ.; Tomašić, J. *Carbohydr. Res.* **1997**, *302*, 13.
7. Tomić, S.; Treščec, A.; Tomašić, J.; Petrović, B.; Simeon Rudolf, V.; Škrinjarić-Špoljar, M.; Reiner, E. *Biochim. Biophys. Acta* **1995**, *1251*, 11–16.
8. Tomić, S.; Krstanović, M.; Tomašić, J. *Period. Biol.* **1996**, *98*, 337–342.
9. Tomić, S.; Sesartić, Lj.; Tomašić, J. *Comp. Biochem. Physiol.* **1989**, *92B*, 681–684.
10. Horrobin, T.; Tran, C. H.; Crout, D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1069–1080.
11. Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 11–109.
12. Tomić, S.; Ljevaković, Đ.; Tomašić, J. *Tetrahedron* **1993**, *49*, 10977–10986.
13. Petrović, V.; Tomić, S.; Matanović, M. *Carbohydr. Res.* **2002**, *337*, 863–867.
14. Ljevaković, Đ.; Parat, D.; Tomašić, J.; Tomić, S. *Croat. Chem. Acta* **1995**, *68*, 477–484.
15. D'Accorso, N. B.; Thiel, I. M. E. *Carbohydr. Res.* **1983**, *124*, 177–184.
16. Hanessian, S.; Kagotani, M. *Carbohydr. Res.* **1990**, *202*, 67–79.