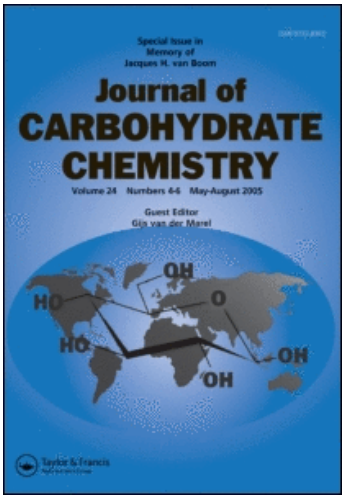

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THE REGIOSELECTIVITY OF THE LIPASE-CATALYZED ACYLATION OF 1,6-ANHYDRO-ß-D-GLYCOPYRANOSES

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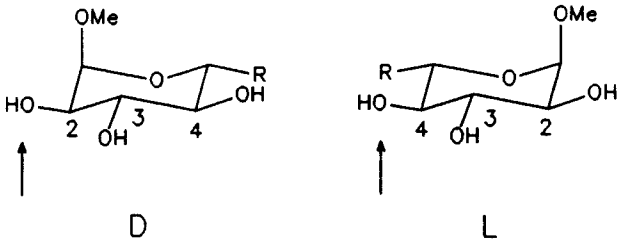
ABSTRACT

Transesterification of anhydroglucose, anhydrogalactose and anhydromannose with ethyl butanoate was catalyzed by *Candida antarctica* lipase in the presence of zeolite CaA. Anhydroglucose and anhydromannose were predominantly acylated at the 4-position whereas anhydrogalactose reacted slowly with low regioselectivity. The selectivity observed is in accordance with the selectivity rules of Ciuffreda *et al.* and Colombo *et al.*. That is, all pyranosides are acylated at the same terminus of the three secondary hydroxyl groups. If this specific hydroxyl group has the axial orientation then the regioselectivity is low. These results contribute to the prediction of the regioselectivity of the lipase-catalyzed acylation of glycopyranosides.

INTRODUCTION

It is known that lipases acylate preferentially the primary hydroxyl groups of carbohydrates; the secondary alcohol functions react much slower and often with some regioselectivity.[1] Selectivity rules have been developed by Ciuffreda *et al.*[2a,b]

and Colombo *et al.*[2c,d] based on investigations of the lipase-catalyzed acylation of methyl 6-*O*-butanoyl-α-D- and L-glycopyranosides and the 6-deoxy analogues. They found that the saccharides were all acylated at the same terminus of the three alcoholic functions if the pyranose ring was drawn in the same orientation, that is at the 2-position of the D-saccharides and at the 4-position of the L-saccharides. The regioselectivity is illustrated in Scheme 1 for the case of the glucopyranosides. When the orientation of this specific hydroxyl group was axial then the yields and regioselectivity of the esterification were lower than when it was equatorial. We have now examined the extension of these rules to the acylation of 1,6-anhydro-ß-D-glycopyranoses.



Scheme 1. Regioselective acylation of methyl 6-*O*-butanoyl-α-D- and L-glucopyranosides and 6-deoxy analogues, R = CH_2OCOPr or Me.

The lipase-catalyzed acylation of underivatized carbohydrates is hampered by the low solubility of the reactants in non-polar solvents. This solubility problem is diminished in the acylation of 1,6-anhydro-ß-D-hexopyranoses because of the presence of the 1,6-anhydro bridge. However the direct enzymatic acylation of these carbohydrates has been scarcely studied.[3] In contrast, the regioselective lipase-catalyzed hydrolysis of the peracylated derivatives has been studied extensively the last few years despite the additional acylation step.[4] The triester of anhydroglucose (**1**) is preferentially deacylated at the 4-position in most cases. The stereochemistry of C4 seems to be important for the regioselectivity. Thus, most hydrolyses of peracylated anhydrogalactose (**3**) gave a mixture of isomers, except for *Candida cylindracea* lipase, which deacylated the ester at the 2-position.

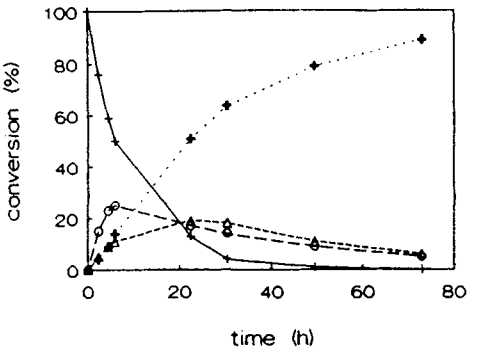


Figure 1. Course of the acylation of anhydroglucose (**1**) with ethyl butanoate at 40 °C + **1**, O 4-*O*-butanoyl-**1**, ▵ 2- or 3-*O*-butanoyl-**1**, + 2,4- and 3,4-di-*O*-butanoyl-**1**.

Here we report on the regioselectivity of the acylation of 1,6-anhydro-ß-D-glucopyranose (**1**), 1,6-anhydro-ß-D-mannopyranose (**2**) and 1,6-anhydro-ß-D-galactopyranose (**3**) with ethyl butanoate, catalyzed by *Candida antarctica* lipase. For convenience the systematic names of the 1,6-anhydro-ß-D-glycopyranoses studied are simplified to anhydroglucose, anhydrogalactose and anhydromannose. The influence of *tert*-butyl alcohol as a cosolvent on the course of the reaction was also examined. The results were compared with the above mentioned rationale.

RESULTS

Anhydroglucose, anhydrogalactose and anhydromannose were subjected to transesterification with ethyl butanoate catalyzed by *Candida antarctica* lipase in the presence of zeolite CaA. The reactions were performed on a small scale (40 mg substrate in 4 mL medium) while shaking at 40 °C.

Acylation of anhydroglucose (**1**) with ethyl butanoate gave mainly 4-*O*-butanoyl anhydroglucose besides a small amount of a 2- or 3-monoester. These products were transformed subsequently into a mixture of 2,4- and 3,4-diesters (Fig. 1) the ratio of which could not be determined by GC under our conditions. In 73 h the total yield of diesters was 89%.

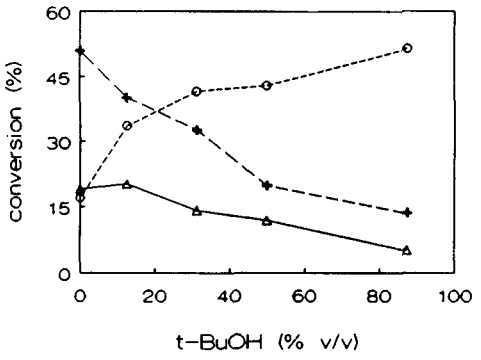


Figure 2. Effect of the concentration of *tert*-butyl alcohol on the acylation of anhydroglucose (**1**) with ethyl butanoate at 40 °C for 24 h. The legend is the same as in Fig. 1.

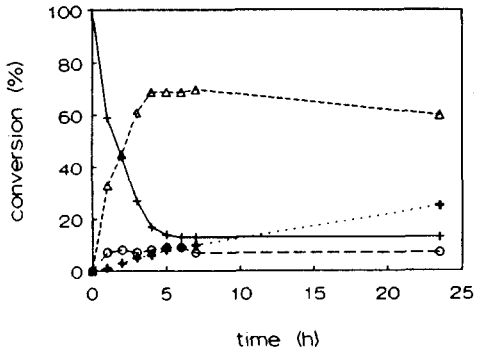


Figure 3. Course of the acylation of anhydromannose (**2**) with ethyl butanoate at 40 °C + **2**, ▵ 4-*O*-butanoyl-**2**, O 2- or 3-*O*-butanoyl-**2**, + 2,4-di-*O*-butanoyl-**2**.

We have previously shown that *tert*-butyl alcohol retards the formation of diesters in the enzymatic acylation of glucose and alkyl glycosides.[1c] Probably, *tert*-butyl alcohol competes for the active site of the lipase with the carbohydrate. Hence, in order to increase the selectivity of the acylation towards the formation of monoesters *tert*-butyl alcohol was used as a cosolvent and its concentration was varied. We found that when the concentration of *tert*-butyl alcohol was 87.5% (v/v) the conversion into 4-monoester was increased from 52% to 87% and the formation of diesters was reduced by 37% compared to the acylation with ethyl butanoate as solvent. In addition less 2- or 3-monoester was formed (Fig. 2).

The acylation of anhydromannose (**2**) in ethyl butanoate proceeded rapidly and regioselectively (Fig. 3). In 4 h 69% of 4-*O*-butanoyl ester was formed and the total conversion was 83%. Slow formation of 2,4-di-*O*-butanoyl anhydromannose was observed as the only diester. In 50% *tert*-butyl alcohol in ethyl butanoate the formation of both monoesters and diesters was reduced to a total conversion of 51% in the same period of time.

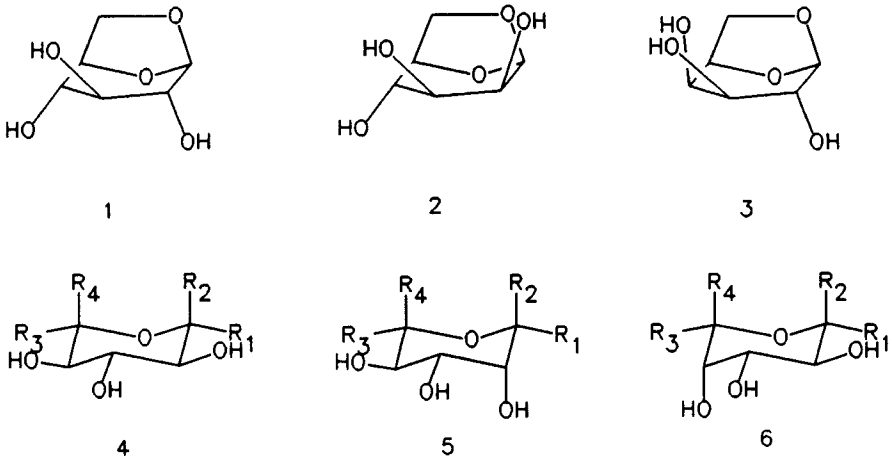
The transesterification of anhydrogalactose (**3**) in ethyl butanoate as solvent was not regioselective at all. All three possible monoesters were formed which reacted further to two diesters. Performing the acylation in 87.5% (v/v) *tert*-butyl alcohol resulted in a drop of the conversion in 24 h from 90% to 18% but the selectivity did not improve. Apparently, *tert*-butyl alcohol inhibites the acylation of anhydrogalactose and also of anhydromannose to a smaller extent.

DISCUSSION

1,6-Anhydro-ß-D-glycopyranoses lack the primary hydroxyl group at C6 which is preferentially acylated in the corresponding hexopyranoses. The smooth acylation of secondary hydroxyl groups in **1** and **2** which we experienced testifies to the potential of *Candida antarctica* lipase. When discussing the regioselectivity of the acylation of the three potential secondary acylation sites we should consider that according to X-ray[5] and ^{1}H NMR studies[6] the preponderant conformation of the 1,6-anhydro-ß-D-glycopyranoses is a chair which is flattened due to 1,3 diaxial interactions. The O2-O4 non-bonded distance increases from 2.5 Å in the unstrained conformation to 3.30 Å in anhydroglucose and the O2-C2-C3-O3 dihedral angle of 149.6° deviates 30.4° from the ideal chair.[7] In this way, the 1,6-anhydro-ß-D-glycopyranoses adopt a more boatlike conformation and the axial hydroxyl groups are in a more equatorial orientation and *vice versa*. The shift to a more boatlike conformation makes the former axial OH groups more reactive because equatorial OH groups react faster with bulky reagents. This explains why anhydroglucose, having only axial hydroxyl groups in the chair conformation, is readily converted.

Ciuffreda *et al.*[2a,b] and Colombo *et al.*[2c,d] studied the regioselectivity of the acylation of a number of methyl 6-*O*-butanoyl-α-D- and L-glycopyranosides and 6-

deoxy analogues catalyzed by four different lipases. These carbohydrates can be divided into three groups according to the sequence[7] of the orientations of the three hydroxyl groups. In this way the EEE group (**4**, methyl 6-*O*-butanoyl-α-D- and L-glucopyranosides and 6-deoxy analogues), the EEA group (**5**, methyl 6-*O*-butanoyl-α-D-galactopyranosides and methyl 6-*O*-butanoyl-α-L-mannopyranosides and 6-deoxy analogues) and the AEE group (**6**, methyl 6-*O*-butanoyl-α-L-galactopyranosides and methyl 6-*O*-butanoyl-α-D-mannopyranosides and 6-deoxy analogues) can be distinguished (Scheme 2). The groups showed the following selectivity: **4** and **5** were mostly acylated at the 2-position of the D-saccharides and at the 4-position of the L-saccharides (Scheme 1). It is quite remarkable that the regioselectivity of **4** and **5** was independent of the position of the substituent at C5 (R_1 or R_3) and of its structure (CH_2OCOPr or Me). Compound **4** differed from **5** by the lower yields of the acylation of the L-saccharides. In all cases the acylation of **6**, in which the hydroxyl group at the left terminus of the triplet was axial, proceeded sluggishly with low regioselectivity.



a. methyl 6-*O*-butanoyl-α-D-glycopyranosides: $R_1 = CH_2OCOPr$, $R_2 = R_3 = H$, $R_4 = OMe$.

b. methyl 6-deoxy-α-D-glycopyranosides: $R_1 = Me$, $R_2 = R_3 = H$, $R_4 = OMe$.

c. methyl 6-*O*-butanoyl-α-L-glycopyranosides: $R_1 = R_4 = H$, $R_2 = OMe$, $R_3 = CH_2OCOPr$.

d. methyl 6-deoxy-α-L-glycopyranosides: $R_1 = R_4 = H$, $R_2 = OMe$, $R_3 = Me$.

Scheme 2. Resemblance between the 1,6-anhydro-ß-D-glycopyranosides in the flattened chairform and the three groups of D- and L-glycopyranosides

We compared the hydroxyl sequences of the 1,6-anhydro-ß-D-glycopyranoses in the flattened chair form with those of the three groups of glycopyranosides each having the same regioselectivity in the lipase-catalyzed acylation and this showed that anhydroglucose (**1**) belongs to **4**. Apart from the fact that the axial-like hydroxyl groups point in different directions with regard to the pyranose ring, anhydromannose (**2**) and anhydrogalactose (**3**) have the same sequences as **5** and **6**, respectively (Scheme 2). From our results it appears that the three 1,6-anhydro-ß-D-glycopyranoses obey the general trend of regioselectivity of their respective groups. Thus anhydroglucose and anhydromannose are acylated preferentially at the 4-position whereas anhydrogalactose, whose hydroxyl group at this position has an axial-like ß-orientation, reacts slowly with low regioselectivity.

It is pertinent to note that the lipase-catalyzed hydrolysis of the corresponding peracylated saccharides is governed by these selectivity rules as well: 2,3,4-tri-*O*-acyl-anhydroglucose is hydrolysed at C4 and the selectivity of the hydrolysis of 2,3,4-tri-*O*-acyl anhydrogalactose is low.

We conclude that 1,6-anhydro-ß-D-glycopyranoses are acylated at the 4-position by *Candida antarctica* lipase. If the hydroxyl group at this position has a ß-orientation then the regioselectivity is low. These results fit in with the selectivity rules of Ciuffreda *et al.*[2a,b] and Colombo *et al.*[2c,d] for the acylation of methyl 6-*O*-butanoyl-α-D- and L-glycopyranosides and of the 6-deoxy analogues. The extension of the selectivity rules to the 1,6-anhydro-ß-D-glycopyranoses contributes to the prediction of the regioselectivity of the lipase-catalyzed acylation of glycopyranosides.

EXPERIMENTAL

General methods. Immobilized lipase from *Candida antarctica* was a gift from Novo Nordisk A/S, Denmark. 1,6-Anhydro-ß-D-galactopyranose and 1,6-anhydro-ß-D-mannopyranose were purchased from Sigma. 1,6-Anhydro-ß-D-glucopyranose was synthesized by pyrolysis of starch under reduced pressure.[8] Zeolite CaA from Uetikon was activated at 400 °C for 24 h before use.

GC analysis was performed on a Hewlet Packard 5890 chromatograph, equipped with a 25 m x 0.32 mm CP-Sil 5CB column. The carrier gas was nitrogen

at a flow of 2.3 mL/min. Temperature program: 60 °C (5 min) to 280 °C (10 °C/min). Peaks were detected using FID and were integrated on a HP 3396A integrator. Dodecane was used as an internal standard. Samples were prepared by withdrawing 20-μL-samples from the reaction mixture and treating them with 0.5 mL of a trimethylsilylating reagent. This reagent was a mixture of pyridine (104 mL), *N,N*-bis(trimethylsilyl)trifluoroacetamide (26 mL) and trimethylsilyl chloride (13 mL).

^{1}H and ^{13}C NMR spectra were recorded using a 400 MHz Varian-VXR 400S spectrometer with tetramethylsilane as internal standard. The ^{13}C NMR spectra were analyzed by comparing them with assigned spectra in the literature.[9]

General procedure for transesterification. A mixture of 1,6-anhydro-ß-D-glucopyranose, 1,6-anhydro-ß-D-galactopyranose or 1,6-anhydro-ß-D-mannopyranose (40 mg, 0.25 mmol), *Candida antarctica* lipase SP 435 (40 mg), zeolite CaA (0.4 g), and ethyl butanoate (4 mL) or 12.5%, 31.25%, 50% or 87.5% (v/v) *tert*-butyl alcohol in ethyl butanoate (4 mL) was shaken on an orbit shaker at 400 rpm at 40 °C. Analytical amounts of the products were isolated for characterization: the reaction mixtures were filtered and evaporated under reduced pressure. The remaining oil was chromatographed over silica gel with a mixture of CH_2Cl_2 and methanol (19:1).

4-*O*-Butanoyl 1,6-Anhydro-ß-D-mannopyranose (4-*O*-butanoyl-2). ^{1}H NMR ($CDCl_3$) δ 5.42 (t, 1H, $J_{1,2}=2.0$, $J_{1,3}=1.4$, H-1), 4.93 (t, 1H, $J_{3,4}=J_{4,5}=1.8$, H-4), 4.56 (dd, 1H, $J_{5,6en}=1.0$, $J_{5,6ex}=5.8$, H-5), 4.25 (dd, 1H, $J_{6,6}=7.5$, $J_{5,6en}=1.0$, H-6_{en}), 3.78 (dd, 1H, $J_{6,6}=7.5$, $J_{5,6ex}=5.8$, H-6_{ex}), 3.95 (m, 1H, H-3), 3.72 (dd, 1H, $J_{1,2}=2.0$, $J_{2,3}=5.8$, H-2); for the butanoyl moiety δ 2.37 (t, 2H, $J_{\alpha,\beta}=7.4$, CH_2), 1.68 (sextet, 2H, $J_{\alpha,\beta}=J_{\beta,\gamma}=7.4$, CH_2), 0.97 (t, 3H, $J_{\beta,\gamma}=7.4$, CH_3). ^{13}C NMR ($CDCl_3$) δ 101.66 (C1), 73.75, 73.55 (C4 and C5), 68.64 (C3), 66.67 (C2), 65.01 (C6); for the butanoyl moiety δ 173.09, 36.12, 18.43, 13.59.

2,4-Di-*O*-Butanoyl 1,6-Anhydro-ß-D-mannopyranose (2,4-di-*O*-butanoyl-2). ^{1}H NMR ($CDCl_3$) δ 5.48 (t, 1H, $J_{1,2}=1.8$, $J_{1,3}=1.5$, H-1), 4.96 (t, 1H, $J_{3,4}=J_{4,5}=1.8$, H-4), 4.87 (dd, 1H, $J_{1,2}=1.8$, $J_{2,3}=5.2$, H-2), 4.62 (1H, m, H-5), 4.34 (dd, 1H, $J_{5,6en}=0.8$, $J_{6,6}=7.5$, H-6_{en}), 4.08 (m, 1H, H-3), 3.80 (dd, 1H, $J_{5,6ex}=5.8$, $J_{6,6}=7.5$); for the butanoyl moiety δ 2.39 (m, 4H, 2xCH_2), 1.70 (m, 4H, 2xCH_2), 0.98 (m, 6H, 2xCH_3). ^{13}C NMR ($CDCl_3$) δ 99.84 (C1), 74.15, 73.06 (C4 and C5), 69.04 (C2), 68.28 (C3), 65.42 (C6); for the butanoyl moiety δ 172.63, 172.34, 36.07, 35.86, 18.44, 18.37, 13.58.

The ^{1}H NMR data of 4-*O*-butanoyl 1,6-anhydro-ß-D-glucopyranose and 2,4- and 3,4-di-*O*-butanoyl 1,6-anhydro-ß-D-glucopyranose were in agreement with the data from the literature.[10]

ACKNOWLEDGEMENTS

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REFERENCES AND NOTES

1. a) M. Therisod and A.M. Klibanov, *J. Am. Chem. Soc.*, 109, 3977 (1987); b) A.T.J.W. De Goede, W. Benckhuijsen, F. Van Rantwijk, L. Maat and H. Van Bekkum, *Recl. Trav. Chim. Pays-Bas*, **112**, 567 (1993); c) A.T.J.W. De Goede, M. Van Oosterom, M.P.J. Van Deurzen, R.A. Sheldon, H. Van Bekkum and F. Van Rantwijk, *Biocatalysis*, **9**, 145 (1994).

2. a) P. Ciuffreda, D. Colombo, F. Ronchetti and L. Toma, *J. Org. Chem.*, 55, 4187 (1990); b) P. Ciuffreda and F. Ronchetti, *J. Carbohydr. Chem.*, **9**, 125 (1990); c) D. Colombo, F. Ronchetti and L. Toma, *Tetrahedron*, **47**, 103 (1991); d) D. Colombo, F. Ronchetti, A. Scala and L. Toma, *J. Carbohydr. Chem.*, **11**, 89 (1992).

3. C. Chon, A. Heisler, N. Junot, F. Levayer and C. Rabiller, *Tetrahedron: Asymmetry*, 4, 2441 (1993).

4. a) J. Zemek, S. Kučár and D. Anderle, *Collect. Czech. Chem. Comm.*, 52, 2347 (1987); b) J. Zemek, S. Kučár and D. Anderle, *Collect. Czech. Chem. Comm.*, **53**, 1851 (1988); c) M. Kloosterman, M.P. De Nijs, J.G.J. Weijnen, H.E. Schoemaker and E.M. Meijer, *J. Carbohydr. Chem.*, **8**, 333 (1989); d) R. Csuk and B.I. Glänzer, *Z. Naturforsch.*, **43b**, 1355 (1988); e) A. Ballesteros, M. Bernabé, C. Cruzado, M. Martín-Lomas and C. Otero, *Tetrahedron*, **45**, 7077 (1989).

5. Y.J. Park, H.S. Kim and G.A. Jeffrey, *Acta Cryst.*, B27, 220 (1971)

6. a) P.C. Wollwage and P.A. Seib, *J. Chem. Soc., C*, 3143 (1971); b) M. Buděšínský, T.Trnka and M. Černý, *Collect. Czech. Chem. Commun.*, 44, 1949 (1979).

7. The sequence starts from the left side of the pyranose ring as drawn in scheme 2 in which E stands for equatorial and A stands for axial.

8. R.B. Ward in *Methods in Carbohydrate Chemistry*, Vol. II; R.L. Whistler and M.L. Wolfrom, Eds.; Academic Press: New York, 1963, p 394.

9. K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41, 27 (1983).

10. S. Kučár, I. Tvaroška, J. Zemek, D. Anderle and M. Matulová, *Chem. Pap.*, 42, 389 (1988).