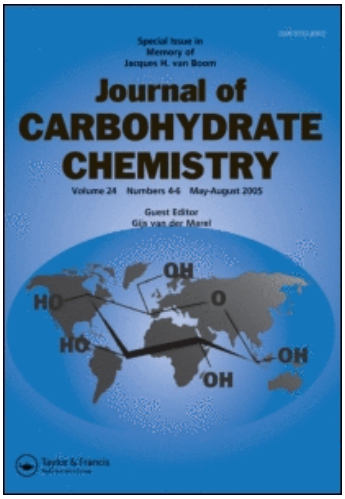


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Regioselective Hydrolysis of Carbohydrate Secondary Acyl Esters By Lipases[1]

M. Kloosterman[a]; M. P. De Nijs[a]; J. G. J. Weijnen[a]; H. E. Schoemaker[a]; E. M. Meijer[a]
[a] DSM Research, Bio-Organic Chemistry Section, MD, Geleen, The Netherlands

To cite this Article Kloosterman, M. , De Nijs, M. P. , Weijnen, J. G. J. , Schoemaker, H. E. and Meijer, E. M.(1989) 'Regioselective Hydrolysis of Carbohydrate Secondary Acyl Esters By Lipases[1]', Journal of Carbohydrate Chemistry, 8: 3, 333 — 341
To link to this Article: DOI: 10.1080/07328308908048563
URL: http://dx.doi.org/10.1080/07328308908048563

J. CARBOHYDRATE CHEMISTRY, 8(3), 333-341 (1989)

REGIOSELECTIVE HYDROLYSIS OF CARBOHYDRATE SECONDARY ACYL ESTERS BY LIPASES[1]

M. Kloosterman, M.P. de Nijs, J.G.J. Weijnen, H.E. Schoemaker and E.M. Meijer

DSM Research, Bio-Organic Chemistry Section, P.O. Box 18, 6160 MD, Geleen, The Netherlands

Received April 12, 1988 - Final Form November 15, 1988

ABSTRACT

Treatment of 1,6-anhydro-2,3,4-tri-O-n-butanoyl-β-D-glucopyranose (3) with lipase preparations of Pseudomonas sp., Mucor miehei or Chromobacterium viscosum in aqueous media resulted in regioselective removal of the acylester at C-4 to afford 1,6-anhydro-2,3-di-O-n-butanoyl-β-D-glucopyranose (5). The C-2 acylester of compound 5 was efficiently removed with lipase of Candida cylindracea to give 1,6-anhydro-3-O-n-butanoyl-β-D-glucopyranose (6). For 1,6-anhydro-2,3,4-tri-O-n-butanoyl-β-D-galactopyranose (4) a different pattern of enzymatic hydrolysis was observed, indicating that the stereochemistry at C-4 is important for enzymatic hydrolysis.

INTRODUCTION

Carbohydrates can be used as cheap, renewable raw materials[2] in the preparation of e.g. acylated glycosides,[3] alkyl (poly)glycosides[4] and microbial biosurfactants.[5] Such compounds vary widely in their hydrophilicity-lipophilicity balance (i.e. HLB values),[6] are usually biodegradable,[7] and are used mainly in feed, cosmetics and in the manufacture of pharmaceutical preparations.

Lipases (triacylglycerol ester hydrolases, E.C. 3.1.1.3) are versatile hydrolytic enzymes which are active at interfacial oil-water microemulsions.[8] In this respect lipases are distinguished from esterases, which show higher hydrolytic activity in homogeneous acyl ester solutions in water. Having in general a broad substrate specificity, together with a high regio- and stereo-selectivity, lipases have been used in the preparation of chiral intermediates to optically active pharmaceuticals and agrochemicals.[9-10] We have already shown that for lipase-catalysed regioselective hydrolysis of acylated glycosides it is not always necessary to use an oil in water emulsion. Thus, sometimes it may be advantageous in terms of selectivity and enzyme-activity to use the substrate in an (amorphous) flocculated form, or to use hydrophobic cosolvents.[11] We now report the treatment of 1,6-anhydro-2,3,4-tri-O-n-butanoyl-β-D-glucopyranose (3)[12] with various lipolytic enzymes originating from yeast (Candida cylindracea), mold (Mucor miehei), bacteria (Chromobacterium viscosum, Pseudomonas sp.), plant (wheat germ) and animal tissue (porcine liver and pancreas)[13] in order to investigate:

- if regioselective hydrolysis of one out of three secondary axially orientated acyl esters occurs,
- if the compound thus produced shows interesting surface-tension lowering properties.

1,6-Anhydro-2,3,4-tri-O-n-butanoyl-β-D-galactopyranose (4) was used for comparison.

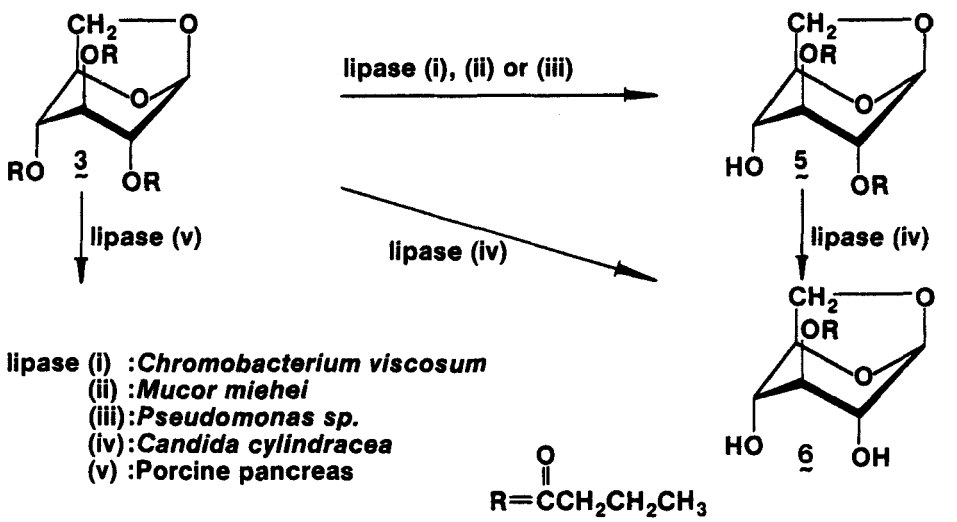
RESULTS AND DISCUSSION

Treatment of compound 3 (0.4 g, 1.07 mmol) with varying amounts of porcine pancreatic lipase (50-200 mg) in phosphate buffer (0.1 M, pH 8), resulted in a mixture of products [(5), (6) and 1,6-anhydro-2-O-butanoyl-β-D-glucopyranose]. With porcine liver esterase and an experimental esterase from NOVO-Industries (SP-122) hardly any conversion was observed. When treated with a lipase preparation of Chromobacterium viscosum (4.6 mg) at 20 °C in phosphate buffer (0.1 M, pH 8), compound 3 (2 g, 5.36 mmol) was transformed into one product exclusively after stirring overnight. After work-up, analysis

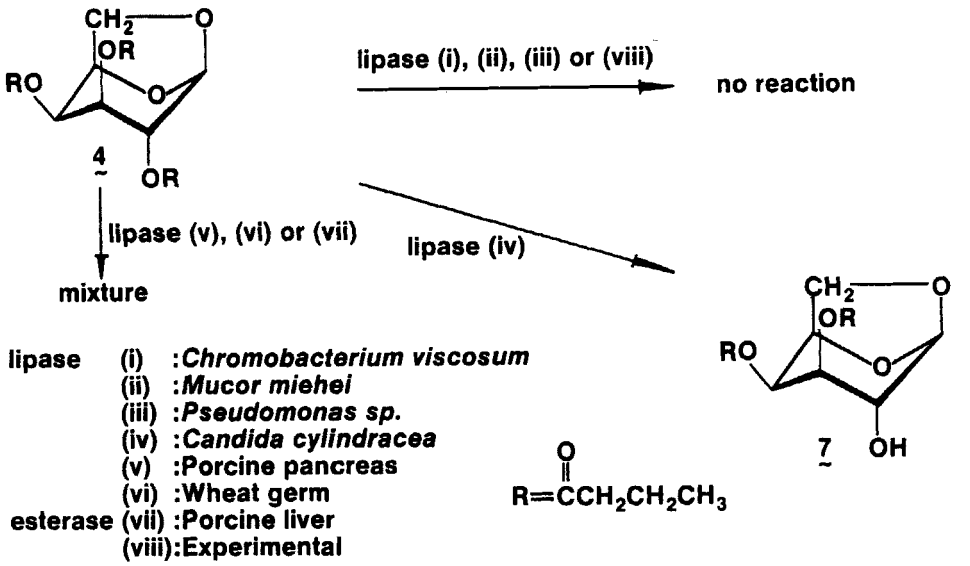
(^1H, ^{13}C, XH corr. NMR) proved that a free hydroxyl group was positioned at C-4, indicating that regioselective enzymatic hydrolysis of the acyl ester at C-4 had taken place, to afford compound 5 in a 91 % isolated yield. With crude lipase preparations from Mucor miehei, and Pseudomonas species the same phenomenon was observed, resulting in exclusive formation of 5. Simultaneous acyl migration through formation of acyl-oxonium ions on the vicinal trans-diaxial system[12,14] was not observed.

Further, when compound 3 (2 g, 5.37 mmol) was treated with lipase from Candida cylindracea (1 g) in aqueous medium formation of two products was observed after 1.5 hours. After work-up, the product migrating faster on Kieselgel 60 was identified as compound 5, whereas the more polar product was shown to be 1,6-anhydro-3-O-n-butanoyl-β-D-glucopyranose (6) (47 % yield; $[\alpha]^{20}$ - 62.3 (C1, $CHCl_3$)).

Upon extension of reaction time compound 5 was further converted into (6) with concomitant formation of 1,6-anhydro-β-D-glucopyranose (1). These results indicate that for lipase of Candida cylindracea enzymatic deacylation of 3 first takes place at C-4 after which the



Scheme 1



Scheme 2

acyl ester at C-2 is being hydrolysed subsequently, the C-3 acyl ester being removed at a very low rate. In fact, when compound 5 (2.51 g, 8.30 mmol) was treated with lipase from Candida cylindracea (2 x 250 mg) for 8 hours in phosphate buffer (0.1 M, pH 7.5, 50 ml) compound 6 could indeed be isolated in 77 % yield.

Next we investigated the influence of the stereochemistry at C-4 on the rate of enzymatic hydrolysis, by using the butyrylated derivative of 1,6-anhydro-β-D-galactopyranose 2 (i.e. 4) instead of 3 as substrate. With the lipolytic enzymes from porcine pancreas, porcine liver and wheat germ compound 4 was transformed into a mixture of products.

Further experiments indicated that with lipase preparations from Chromobacterium viscosum, Mucor miehei, Pseudomonas sp. (or experimental esterase from NOVO) no conversion took place on compound 4. This suggests that the presence of an axially orientated n-butyryl ester at C-4 (as in 3) instead of an equatorial one (as in 4) is a prerequisite for recognition by lipases (i), (ii) and (iii) (see Schemes 1 and 2).

Thus these lipases function not only regio-, but also stereoselectively at C-4 of compounds 3 and 4. In conclusion, the results reported here indicate the following order of decreasing reactivity of n-butyryl esters in 1,6-anhydroaldohexopyranoses towards enzymatic lipolytic action:[15]

C-4 ax > C-2 ax > C-3 ax >> C-4 eq.

This pattern is distinct from the reported preferential order of removal of acetyl groups in per-O-acylated D-glucose exerted by lipase of wheat germ[16] and Aspergillus niger[17], which is most probably due to the different conformations adopted by compound 3 (^{1}C4 (D)) and acylated D-glucose (^{4}C1 (D)). Notably, the described pattern was observed for the chemical hydrolysis of 2,3,4-tri-O-acetyl-1,6-anhydro-β-D-glucopyranose in methanolic hydrogen chloride (though with less selectivity).[17]

On acid hydrolysis with methanolic hydrogen chloride the esterified hydroxyl group at C-3 was found to be the most stable one when compared with C-2 and C-4, but it was the most labile one upon hydrolysis with hydrazine hydrate.[18]

As the enzyme reactions described in this paper were performed at (nearly) neutral pH, it is more conceivable that the ester group at C-3 (in compounds 3 and 4) is less accessible for the investigated lipases due to steric hindrance exerted by the 1,6-anhydro bridge. This would explain the relative stability of the C-3 acyl ester towards lipolytic action.

Finally, some relevant data for the enzymatic hydrolysis of compound 3 into 5 by lipase from Pseudomonas sp. were investigated.

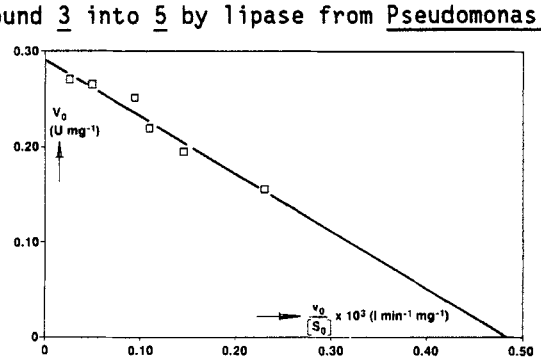


Fig. 1

Suspensions of given concentrations of compound 3 in 100 ml demineralised water (pH 7.5) were emulsified at 20.000 rpm with an Ultra-Turrax (Janke & Kunkel KG) for 20 seconds.

Then the steady state kinetics for the hydrolysis of compound 3 catalysed by lipase from Pseudomonas sp. (100 mg, lipase P from Amano) were determined when stirring at 37 °C and 250 rpm. From the Eadie-Hoffstee plot, depicted in Figure 1, the apparent Km value (6.0 mmol/l) and V_{max} (290 IU/g) were deduced, indicating that Michaelis-Menten kinetics are applicable for this heterogeneous lipolytic reaction.[19] Additionally the ability of compounds 3 and 5 to reduce the surface tension (air-water) in aqueous medium was examined. By using a γ/lnc plot, compound 5 was shown to be surface active and capable of reducing the surface tension of water to 47.2 mN/m. The critical micelle concentration, at which the minimum was reached, was 1.08 g/l. Thus, in order to reduce the critical micelle concentration and to increase the surface-activity, clearly acyl esters of longer chain-length should be used on compounds 1 and 2.

The results reported in this paper show that regioselective hydrolysis of one out of two (5 → 6) or three (3 → 5, and 4 → 7) secondary n-butyryl esters can be performed easily on 1,6-anhydroaldohexopyranoses by using lipases (i) - (iv). Furthermore, along with regioselectivity lipases (i) - (iii) also exhibit a high degree of stereoselectivity (e.g. 3 → 5; 4), features which are not limited to the described D-glucose and D-galactose derivatives 3 and 4.[20] The compounds thus obtained are valuable synthons for the preparation of aminosugars, oligosaccharides and macrolide antibiotics. At the moment we are investigating the factors that influence the regioselective and stereo-specific hydrolysis of acyl esters (cq. esterification) of carbohydrates and optically active pharmaceuticals mediated by lipases and esterases.[21] In fact, the regioselective hydrolysis of either two primary and one secondary acyl ester or only one secondary acyl ester on a per-O-acetylated disaccharide will be reported soon.[22]

EXPERIMENTAL

Compound 3 (2.0 g, 5.36 mmol) was suspended in 65 ml phosphate buffer (0.1 M, pH 7.5), after which lipase from Chromobacterium viscosum (4.6 mg) was added. After stirring overnight at room tem-

perature, the reaction mixture was extracted with ethyl acetate, dried ($MgSO_4$), and concentrated to dryness. The residue was purified by silica gel column chromatography (eluent dichloromethane/methanol, 99/1, v/v) to give 1.48 g of compound 5 as a syrup: yield 91 %; $[\alpha]^{20}$ -24.4° (c1, chloroform); ^{1}H-NMR ($CDCl_3$) δ 0.96 (dt, 6H, 2x CH_3), 1.66 (m, 4H, 2x CH_2CH_3), 2.33 (m, 4H, 2x CH_2C = O), 2.91 (d, 1H, OH, J H4, OH 10.27 Hz), 3.55 (dd, 1H, H4), 3.82 (dd. 1H, H6 exo, J6exo, 6endo -7.62 Hz; J5,6 exo 5.81 Hz), 4.10 (dd, 1H, H6 endo), 4.59 (d, 1H, H5), 4.61 (d, 1H, H2, J2,3 1.38 Hz), 4.80 (t, 1H, H3), 5.44 (s, 1H, H1); ^{13}C NMR ($CDCl_3$) δ 14.70 (CH_3), 19.43 (CH_3CH_2), 36.97, 37.21 (2x CH_2C = O), 66.06 (C6), 69.74 (C4), 69.93 (C2), 72.73 (C3), 77.26 (C5), 100.25 (C1) and 173.21 (C=O). For lipase-catalysed hydrolyses on substrate solutions more concentrated as reported in this example, it is preferable to perform the reaction under pH-stat conditions as otherwise the pH will drop too much, resulting in enzyme inactivation and incomplete conversion.

ACKNOWLEDGEMENT

We wish to thank Dr. N.K. de Vries and Mr. H. Linssen for the NMR analyses, Dr. J.G.H. Joosten for the γ/lnc plot Drs. P.E.F. Ketelaar for helpful discussions on the kinetic part and Mrs. Cox for typing the manuscript.

REFERENCES AND FOOTNOTES

1. Presented in a lecture at the Fourth European Carbohydrate Symposium, Darmstadt, July 12-17, 1987.

2. F. Rexen and L. Munck (Eds.), Cereal Crops for Industrial Use in Europe; EEC and Carlsberg Research Laboratory, Denmark (1984).

3. U.S. 3.059.011 (1962) Procter and Gamble; U.S. 4.614.718 (1986) Dai-Ichio Kogyo Seiyaka Co.; G. P. Rizzi and H. M. Taylor, J. Am. Oil. Chem. Soc., 55, 398 (1978); N. B. Desai and N. Lowicki, Parfümerie u. Kosmetik, 64, 463 (1983).

4. C. F. Putnik and N. F. Borys, Soap. Cos. Chem. Spec., June, (1986); A. D. Urfer, A. H. Malik, H. L. Kickle, G. M. Howell and N. F. Borys, Second World Conference on Detergents, p. 268, Montreux (1986).

5. D. Haferburg, R. Hommel, R. Claus and H-P. Kleber, Adv. Biochem. Engineer, 33, 55 (1986); P. A. Sandford, I. W. Cottrell and D. J. Pettitt, Pure & Appl. Chem., 56, 879 (1984); I. C. M. Dea and J. K. Madden, in: New Developments in Industrial Polysaccharides (V. Crescenzi, I. C. M. Dea and S. S. Stivala (Eds.)), p. 27, Gordon and Breach Publ., New York (1984).

6. W. C. Griffin, J. Soc. Cosmet. Chem., 1, 311 (1949).

7. R. D. Swisher, Surfactant Biodegradation, Marcel Dekker Inc., New York (1987).

8. B. Borgström and H. L. Brockman (Eds.) in: Lipases, Elsevier, Amsterdam (1984).

9. A. M. Klibanov, Chem. Tech., 355 (1986).

10. J. Kamphuis, M. Kloosterman, W. H. J. Boesten, H. E. Schoemaker and E. M. Meijer, Proc. IVth Eur. Congr. Biotechnol. (O. M. Neijssel, R. R. van der Meer and K. Ch. A. M. Luyben Eds.), 4, 331-348, (1987), Elsevier, Amsterdam.

11. M. Kloosterman, E. W. J. Mosmuller, H. E. Schoemaker and E. M. Meijer, Tetrahedron Lett., 28, 2989 (1987).

12. For a review on 1,6-anhydroaldohexopyranoses, see: M. Cerny and J. Stanek Jr., Adv. Carbohydr. Chem. Biochem., 34, 23 (1977). See also: M. Kloosterman, M. J. Dees, G. A. van der Marel and J. H. van Boom, Recl. Trav. Chim. Pays-Bas, 104, 116 (1985); M. Kloosterman, M. P. de Nijs and J. H. van Boom, J. Carbohydr. Chem., 5, 215 (1986).

13. Lipases from Chromobacterium viscosum (L-0763), Candida cylindracea (L-1754), porcine pancreas (L-3126), wheat germ (L-3001) and esterase from porcine liver (E-3128) were purchased from Sigma Chemical Co. (of St. Louis). Mucor miehei lipase (SP 225) and experimental esterase (SP 122) were gifts from NOVO Industries (Kopenhagen). Lipase from a Pseudomonas strain (lipase P) was kindly provided by Amano Pharmaceutical Co.

14. No acyl migration was noted when compound (5) was stirred for two days in aqueous buffer (pH 7.5) or when catalytic amounts of acid or base were added.

15. The regioselective lipase-catalysed hydrolyses of acyl esters on 1,6-anhydro-β-D-glycopyranoses has been further investigated by others. For acetyl esters see e.g. R. Csuk and B. Glänzer, Zeitschr. Naturforsch., accepted for publication (private communication), and M. Martin-Lomas (private comm.). See also: J. Zamek, K. Kucar and D. Anderle, Coll. Czech Chem. Commun., 52, 2347 (1987).

16. A. L. Fink and G. W. Hay, Can. J. Biochem., 47, 353 (1969).

17. J. F. Shaw and A. M. Klibanov, Biotechnol. Bioengin., 29, 648 (1987).

18. S. Kucár, J. Zámocký, J. Zemek, D. Anderle and M. Matulová, Coll. Czech. Chem. Commun., 49, 1780 (1984).

19. For reactions, in which insoluble substrates form aggregates surrounded by water, the reaction rate was found to be a function of the surface area of the substrate aggregate accessible to the enzyme. See B. Borgström and H. L. Brockman (Eds.), in: Lipases, Elsevier, Amsterdam (1984).

20. M. Kloosterman, N. K. de Vries, H. E. Schoemaker and E. M. Meijer, XIV Int. Carbohydr. Symp., August 14-19 (1988) Abst. D-36, Stockholm.

21. M. Kloosterman, V. E. Elferink, I. van Iersel, J. H. Roskam, E. M. Meijer, L. Hulshof and R. A. Sheldon, Trends in Biotechnology, in press.

22. M. Kloosterman, J. G. J. Weijnen, N. K. de Vries, J. Mentech, I. Caron, G. Descotes, H. E. Schoemaker and E. M. Meijer, J. Carbohydr. Chem., this volume.