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REGIOSELECTIVE DEACYLATION OF 1,6-ANHYDRO-β-D-GALACTOPYRANOSE DERIVATIVES CATALYZED BY SOLUBLE AND IMMOBILIZED LIPASES

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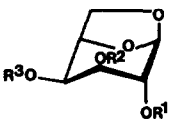
Abstract. The regioselectivity of the hydrolysis of secondary acyl esters of carbohydrates by lipases has been investigated. 1,6-Anhydro-2,3,4-tri-*O*-butanoyl-β-D-galactopyranose (**1**) and 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**8**), used as conformationally rigid model compounds, were treated with soluble lipases from pig pancreas and with lipases from *C. cylindracea* and *Mucor miehei* immobilized on agarose and ion exchange resin, respectively. Analysis of the reaction mixtures by ^{1}H-n.m.r. spectroscopy indicated that the axially oriented acyl esters at C-2 react faster than the equatorially oriented acyl esters at C-4, and these faster than the axially oriented acyl esters at C-3. The corresponding 3,4-di-, and 3-mono-*O*-acyl esters could be isolated in yields from moderate to excellent depending on the starting material and the reaction conditions.

Regioselective reactions are particularly needed in the synthesis of biologically relevant carbohydrates[1-4] where selective protection and deprotection of hydroxyl groups is a critical problem.[5] As a part of a project on the regioselectivity of some usual reactions in carbohydrate chemistry, we have recently investigated some new aspects of tributyltin ether mediated benzylation[6,7] and of heterogeneous catalytic transfer hydrogenolysis of benzyl groups.[8,9] We now report our results on the regioselectivity of deacylation of secondary acyl esters of carbohydrates catalyzed by soluble and immobilized lipases. Selective cleavage of the 1-*O*-acyl group of per-*O*-acylated derivatives of carbohydrates has previously been achieved in reasonable yield using native *Aspergillus niger* lipase[10] and selective hydrolysis of the acyl ester at the primary position of per-*O*-acylated derivatives of methyl glycopyranosides and glycofuranosides have been performed in good yield employing soluble lipase from *Candida cylindracea*.[11,12] We have recently succeeded in preparing an immobilized lipase very stable to temperature and to the presence of organic solvents. Since immobilization may improve or even change selectivity,[13] we have investigated the regioselectivity of the hydrolysis of the secondary acyl esters of 1,6-anhydrogalactopyranose (**1** and **8**), used as conformationally rigid model compounds, with lipase preparations both in the native and immobilized form.

RESULTS AND DISCUSSION

The lipase-catalyzed hydrolysis of compounds **1** and **8** was carried out at pH 7.5 and 30°C.



1 $R^1=R^2=R^3=COPr$	**6** $R^1=R^3=H$, $R^2=COPr$	**11** $R^1=R^3=Ac$, $R^2=H$
2 $R^1=H$, $R^2=R^3=COPr$	**7** $R^1=COPr$, $R^2=R^3=H$	**12** $R^1=R^2=H$, $R^3=Ac$
3 $R^1=R^2=COPr$, $R^3=H$	**8** $R^1=R^2=R^3=Ac$	**13** $R^1=R^3=H$, $R^2=Ac$
4 $R^1=R^3=COPr$, $R^2=H$	**9** $R^1=H$, $R^2=R^3=Ac$	**14** $R^1=Ac$, $R^2=R^3=H$
5 $R^1=R^2=H$, $R^3=COPr$	**10** $R^1=R^2=Ac$, $R^3=H$	**15** $R^1=R^2=R^3=H$

These were found to be the optimal conditions for good hydrolytic activity and reasonable enzyme stability during prolonged periods of time.[14] The course of the reaction was monitored by t.l.c. and ^{1}H-n.m.r. spectroscopy. The expected partially acylated derivatives were prepared using literature [18-20] or conventional procedures, as indicated in the experimental part, and used as reference compounds. Hydrolysis of **1** gave first the 3,4-di-*O*-butanoyl derivative (**2**) and then the 3-*O*-butanoyl derivative (**6**). The results are summarized in Table 1. The hydrolysis of **8** under the same conditions followed a similar course, although, as expected,[11,12,15] both yield and selectivity were lower. The results are summarized in Table 2. Enzyme preparations from different sources, including higher

Table 1. Lipase-catalyzed deacylation of **1**

Enzyme from	Reaction time (h)	% Reaction products			
		2	**5**	**6**	**15**
C. cylindracea	47	70	<5	8	20
Pancreas	24	90		5	
	52	16	19	65	
C. cylindracea	14	87		<5	
immobilized	29	90	<5	8	
(agarose-lipase)	48	89	<5	8	
	219	60	8	22	10

organisms (pancreatic), yeast (*Candida*) and another fungi (*Mucor*), showing different specificity towards triacylglycerols, in different state (native *versus* immobilized, either by covalent multiple binding to agarose or by ionic binding and adsorption to a macroporous resin) were tested. In all

Table 2. Lipase-catalyzed deacylation of compound **8**

Enzyme from	Reaction time (h)	% Reaction products						
		9	**10**	**11**	**12**	**13**	**14**	**15**
C. cyclindracea	128	12			17	21	50	
Pancreas	21	37	10	>5				
	72	54			12	20	12	
C. cylindracea	15	8	<5	<5				
immobilized	48	28	10	7				
(agarose-lipase)	96	40	8	5	6	17	14	
	194	17			14	33	14	33
	264	3			10	21	8	58

cases the acyl group at C-2 was first hydrolyzed and then that at C-4. This reactivity order was independent of the enzyme preparation used, however, some interesting differences could be observed depending on the substrate and the nature and the state of the catalyst. Thus, the 3,4-di-*O*-butanoyl derivative **2** could be obtained in 87 % (in 14 h) or 90 % yield (in 29 h) using the lipase from *C. cylyndracea* immobilized on agarose, the reaction practically stopping at this stage. A similar result could be obtained using lipase from pig pancreas but, in this case, the reaction proceeded to give the 3-*O*-butanoyl derivative **6** in 65 % yield and the 4-*O*-butanoyl derivative **5** in 19 % yield after 52 h. Furthermore, in the cases where immobilized *Candida* lipase was used, the amount of enzyme was 3-fold lower than in reactions with soluble *Candida* lipase. Under the conditions of catalysis, agarose-lipase retained 64 % of the initial hydrolytic activity after 21 days, whereas the soluble counterpart dropped to 40 % after only 4 days. This increased stability allowed the reuse of the immobilized enzyme after removal from the reaction mixture.

We have previously found[14] that in the presence of 1.25 % of *n*-butanol the activity of the agarose-lipase increased to 380 % but its stability at 50°C dropped to 53 %. Addition of 1.25 % of *n*-butanol to the reaction mixture of **1** and **8** resulted in an almost complete loss of selectivity (data not shown). It has been recently reported that addition of methanol causes important effects on the lipase catalyzed regioselective deacylation of 1,6-anhydro-β-D-glucopyranose derivatives.[21]

EXPERIMENTAL

Candida cylindracea (now named *C. rugosa*) Type VII lipase and porcine pancreas Type II lipase (containing, respectively, 700 and 160 U/mg solid using olive oil as substrate) were purchased from Sigma and used as received. *Mucor miehei* lipase immobilized on a macroporous weak basic

anion exchange resin (Lipozyme TM) was kindly provided by Novo Industri A/S and contained 375 μg of enzyme per g. of support.[17] Lipase from *C. cylindracea* was covalently immobilized on agarose (Sepharose Cl-6B from Pharmacia) activated with 2,3-epoxy-1-propanol as previously reported;[14] the insoluble final preparation contained 31.2 mg solid (364.1 mkat) per mL agarose gel.

2,3,4-Tri-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**8**), 3,4-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**9**), 2,3-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**10**), 2,4-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**11**), and 2-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**14**) were prepared as reported in the literature.[18-20] T.l.c. was performed on silica gel GF_{254} (Merck) with detection by charring with sulfuric acid. ^{1}H-N.m.r. spectra (300 MHz) were recorded using a Varian XL-300 spectrometer.

3-*O*-Acetyl- (13) and 4-*O*-acetyl- (12) -1,6-anhydro-β-D-galactopyranose.- Isolated as a mixture by partial acetylation of 1,6-anhydro-2-*O*-benzyl-β-D-galactopyranose followed by hydrogenolysis in the presence of 10% Pd/C. ^{1}H-N.m.r. data for compound **13** (chloroform-d): δ 5.38 (s, 1H, H-1), 5.08 (m, 1H, H-3), 4.44 (t, 1H, $J_{4,5} \simeq J_{5,6\ exo} \simeq 4.7$ Hz, H-5), 5.07 (m, 2H, H-4, H-6 endo), 3.74 (s, 1H, H-2), 3.71 (m, 1H, H-6 exo), 2.15 (s, 3H, Ac); (acetone-d_6): δ 5.21 (s, 1H, H-1), 5.04 (dd, 1H, $J_{2,3} \simeq 1.3$, $J_{3,4} \simeq 5.4$ Hz, H-3), 4.32 (d, 1H, $J_{6\ exo,\ 6\ endo} \simeq 7.2$ Hz, H-6 endo), 2.03 (s, 3H, Ac). ^{1}H-N.m.r. data for compound **12** (chloroform-d): δ 5.46 (s, 1H, H-1), 5.08 (m, 1H, H-4), 4.52 (t, 1H, $J_{4.5} \simeq J_{5,6} \simeq 4.6$ Hz, H-5), 4.36 (d, 1H, $J_{6\ exo,\ 6\ endo} \simeq 7.7$ Hz, H-6 endo), 4.14 (m, 1H, H-3), 3.85 (s, 1H, H-2), 3.71 (m, 1H, H-6 exo), 2.15 (s, 3H, Ac); (acetone-d_6): δ 5.27 (t, 1H, $J_{1,2} \simeq J_{1,3} \simeq 1.4$ Hz, H-1), 5.02 (m, 1H, H-4), 2.04 (s, 3H, Ac).

1,6-Anhydro-2,3,4-tri-*O*-butanoyl-β-D-galactopyranose (1).- From 1,6-anhydro-β-D-galactopyranose (**15**) by treatment with butanoyl chloride. $[\alpha]_D^{20}$ +6° (c 0.2, chloroform). ^{1}H-N.m.r. data (chlorofom-d): δ 5.43 (s, 1H, H-1), 5.27 (m, 2H, H-3, H-4), 4.47 (m, 1H, H-5), 4.34 (d, 1H, $J_{6\ exo,\ 6\ endo} \simeq 7.5$, H-6 endo), 3.74 (dd, 1H, $J_{5.6} \simeq 5.0$ Hz, H-6 exo), 2.36 (t, 4H, $2\,CH_2$), 2.25 (t, 2H, CH_2), 1.66 (m, 6H, $3\,CH_2$), 0.96 (m, 9H, $3\,CH_3$).

Anal. Calcd. for $C_{18}H_{22}O_8$: C, 58.05; H, 7.59. Found: C, 58.18; H, 7.59.

1,6-Anhydro-3,4-di-*O*-butanoyl-β-D-galactopyranose (2).- Prepared from 1,6-anhydro-2-*O*-benzyl-β-D-galactopyranose by treatment with butanoyl chloride followed by hydrogenolysis. $[\alpha]_D^{20}$ -25° (c 0.5, chloroform). ^{1}H-N.m.r. data (chloroform-d): δ 5.42 (s, 1H, H-1), 5.29 (m, 2H, H-3, H-4), 4.48 (t, 1H, $J_{4,5} \simeq J_{5,6\ exo} \simeq 5$ Hz, H-5), 4.32 (d, 1H, $J_{6\ exo,\ 6\ endo} \simeq 7.4$ Hz, H-6 endo), 3.74 (dd, 1H, H-6 exo), 3.72 (s, 1H, H-2), 2.35 (t, 2H, CH_2), 2.26 (t, 2H, CH_2), 1.65 (m, 4H, $2\,CH_2$), 0.96 (t, 6H, $2\,CH_3$).

Anal. Calcd. for $C_{14}H_{18}O_7$: C, 55.62; H, 7.33. Found: C, 55.67; H, 7.52.

1,6-Anhydro-2,3- (3) and 2,4 (4) -di-*O*-butanoyl-1,6-anhydro-β-D-galactopyranose.- Isolated as a mixture by treatment of 1,6-anhydro-β-D-galactopyranose (**15**) with butanoyl chloride. ^{1}H-N.m.r. data for **3** (chloroform-d): δ 5.39 (t, 1H, $J_{1,2} \simeq J_{1,3} \simeq 1.4$ Hz, H-1), 5.08 (m, 1H, H-3), 4.77 (t, 1H, $J_{2,3} \simeq 1.4$ Hz, H-2), 4.44 (t, 1H, $J_{4,5} \simeq J_{5,6\ exo} \simeq 4.5$ Hz, H-5), 4.27 (d, 1H, $J_{6\ exo,\ 6\ endo} \simeq 7.4$ Hz,

H-6 endo), 4.21 (t, 1H, $J_{3,4} \simeq 4.7$ Hz, H-4), 3.70 (dd, 1H, H-6 endo), 2.38 (m, 4H, $2\,CH_2$), 1.68 (m, 4H, $2\,CH_2$), 0.97 (m, 6H, $2\,CH_3$). ^{1}H-N.m.r. data for **4** (chloroform-d): δ 5.46 (t, 1H, $J_{1,2} \simeq J_{1,3} \simeq 1.4$ Hz, H-1), 5.03 (t, 1H, $J_{3,4} \simeq J_{4,5} \simeq 4.4$ Hz, H-4), 4.84 (t, 1H, $J_{2,3} \simeq 1.4$ Hz, H-2), 4.51 (t, 1H, $J_{4,5} \simeq J_{5,6\,exo} \simeq 4.2$ Hz, H-5), 4.40 (d, 1H, $J_{6\,exo,\,6\,endo} \simeq 7.4$ Hz, H-6 endo), 4.11 (m, 1H, H-3), 3.70 (dd, 1H, H-6 exo), 2.38 (m, 4H, $2\,CH_2$), 1.68 (m, 4H, $2\,CH_2$), 0.97 (m, 6H, $2\,CH_2$).

1,6-Anhydro-4-*O*-(5) and 3-*O*-(6)-butanoyl-β-D-galactopyranose.- Isolated as a mixture by treatment of 1,6-anhydro-2-*O*-benzyl-β-D-galactopyranose with butanoyl chloride followed by hydrogenolysis. ^{1}H-N.m.r. data for **5** (chloroform-d): δ 5.48 (t, 1H, $J_{1,2} \simeq J_{1,3} \simeq 1.6$ Hz, H-1), 5.09 (m, 1H, H-4), 4.51 (t, 1H, $J_{4,5} \simeq J_{5,6\,exo} \simeq 4.5$ Hz, H-5), 4.35 (d, 1H, $J_{6\,exo,}J_{6\,endo} \simeq 7.5$ Hz, H-6 endo), 4.14 (m, 1H, H-3), 3.84 (t, 1H, $J_{2,3} \simeq 1.6$ Hz, H-2), 3.70 (m, 1H, H-6 exo), 2.39 (t, 2H, CH_2), 1.68 (m, 2H, CH_2), 0.97 (t, 3H, CH_3). ^{1}H-N.m.r. data for **5** (acetone-d_6): δ 5.28 (t, 1H, $J_{1,2} \simeq J_{1,3}$ 1.6 Hz, H-1), 5.04 (m, 1H, H-4), 4.40 (d, 1H, $J_{6\,endo,\,6\,exo} \simeq 7.5$ Hz, H-6 endo), 3.69 (m, 1H, H-2), 3.54 (m, 1H, H-6 exo), 2.34 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 0.94 (m, 3H, CH_3). ^{1}H-N.m.r. data for **6** (chloroform-d): δ 5.73 (t, 1H, $J_{1.2} \simeq J_{1,3} \simeq 1.6$ Hz, H-1), 5.09 (m, 1H, H-3), 4.44 (t, 1H, $J_{4,5} \simeq J_{5,6\,exo} \simeq 4.4$ Hz, H-5), 4.26 (t, 1H, $J_{3,4} \simeq 4.4$ Hz, H-4), 4.24 (d, 1H, $J_{6\,exo,}J_{6\,endo} \simeq 7.5$ Hz, H-6 endo), 3.73 (m, 1H, H-2), 3.70 (m, 1H, H-6 exo), 2.39 (t, 2H, CH_2), 1.68 (m, 2H, CH_2), 0.93 (m, 3H, CH_3). ^{1}H-N.m.r. data for **6** (acetone-d_6): δ 5.23 (t, 1H, $J_{1,2} \simeq J_{1,3} \simeq 1.6$ Hz, H-1), 5.04 (m, 1H, H-3), 4.32 (d, 1H, $J_{6\,exo,\,6\,endo} \simeq 7.5$ Hz, H-6 endo), 3.55 (m, 1H, H-2), 3.54 (m, 1H, H-6 exo), 2.34 (t, 2H, CH_2), 1.63 (m, 2H, CH_2), 0.94 (m, 3H, CH_3).

1,6-Anhydro-2-*O*-butanoyl-β-D-galactopyranose (7).- Prepared from 1,6-anhydro-3,4-*O*-isopropylidene-β-D-galactopyranose by treatment with butanoyl chloride followed by acidic hydrolysis. M.p. 78-80°C, $|\alpha|_D^{20}$ -4° (c 1, chloroform). ^{1}H-N.m.r. data (chloroform-d): δ 5.42 (t, 1H, $J_{1,2} \simeq J_{1,3} \simeq 1.4$ Hz, H-1), 4.86 (t, 1H, $J_{2,3} \simeq 1.4$ Hz, H-2), 4.46 (t, 1H, $J_{4,5} \simeq J_{5,6\,exo} \simeq 4.5$ Hz, H-5), 4.27 (d, 1H, $J_{6\,exo,\,6\,endo} \simeq 7.7$ Hz, H-6 endo), 3.99 (t, 1H, $J_{3,4} \simeq 4.5$ Hz, H-4), 3.91 (m, 1H, H-3), 3.67 (dd, 1H, H-6 exo), 2.35 (t, 2H, CH_2), 1.66 (m, 2H, CH_2), 0.96 (t, 3H, CH_3). ^{1}H-N.m.r. data (acetone-d_6): δ 5.27 (t, 1H, $J_{1,2} \simeq J_{1,3} \simeq 1.4$ Hz, H-1), 4.75 (t, 1H, $J_{2,3} \simeq 1.4$ Hz, H-2), 4.34 (m, 2H, H-5, H-6 endo), 3.91 (t, 1H, $J_{3,4} \simeq 4.6$ Hz, H-4), 3.86 (m, 1H, H-3), 3.52 (dd, 1H, H-6 endo), 2.31 (t, 2H, CH_2), 1.62 (m, 2H, CH_2), 0.93 (t, 3H, CH_3).

Anal. Calcd. for $C_{10}H_{14}O_6$: C, 51.72; H, 6.94. Found: C, 52.09; H, 6.94.

Lipase catalyzed hydrolysis of 1,6-anhydro-2,3,4-tri-*O*-butanoyl-β-D-galactopyranose (1) and 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (8).

a) With *Candida* and pancreatic lipases.

The enzyme (450 mg) and Amberlite CG-120 (Na^+) resin were added to a solution of **1** or **8** (120 mg) in 0.1 M phosphate buffer 0.1 M NaCl, pH 7.2 (22.5 mL for **1** or 15 mL for **8**). The pH was adjusted to 7.5 by addition of 0.1 N NaOH and the mixture was stirred at 30°C. The reaction progress was monitored by t.l.c. Aliquots of 5 mL for **1** or 3 mL for **8** were taken at different reaction times. These aliquots were lyophilized and the residue was extracted first with dichloromethane (2 x 15 mL) and then with acetone (2 x 15 mL). The extracts were separately

concentrated *in vacuo*, treated with chloroform-d and acetone-d_6, respectively, and analyzed by ^{1}H-n.m.r. spectroscopy.

b) With agarose-lipase

Agarose-lipase gel (6 mL) and Amberlite CG-120 (Na^+) (120 mg) were added to a solution of **1** or **8** (120 mg) in phosphate buffer (15 mL for **1** or 10 mL for **8**). The hydrolysis and the analysis were carried out as in a).

c) With lipozyme

Lipozyme (450 mg) and Amberlite CG-120 (Na^+) (120 mg) in phosphate buffer (15 mL) were treated as in a).

d) In the presence of butanol

The experiments were performed as in a) (soluble enzymes) or in b) (immobilized) with addition of 0.2 mL of *n*-butanol (final concentration 1.25 % v/v).

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