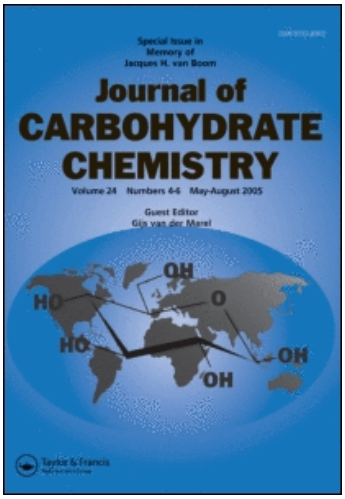

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ENZYMIC ACYLATION OF METHYL D- AND L-GLUCOPYRANOSIDES AND 6-DEOXY-GLUCOPYRANOSIDES*

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ABSTRACT

Methyl 6-*O*-butyryl-α-**D**- and **L**-glucopyranosides and methyl 6-deoxy-α-**D**- and **L**-glucopyranosides have been submitted to lipase catalyzed butyrylation, using porcine pancreatic, *Candida cylindracea*, and *Pseudomonas cepacia* lipases in organic solvents. The influence of the orientation of the secondary hydroxyl groups on the regioselectivity of the butyrylation is discussed.

INTRODUCTION

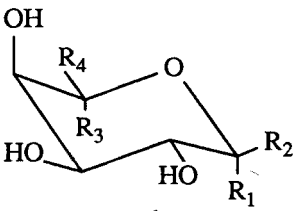
Recent studies[1-4] on the regioselective acylation of the secondary hydroxyl functions of carbohydrates through lipase-catalyzed transesterification have appeared in the literature; the configuration (**D** or **L**) of the sugar seems to be one important factor for determining the selectivity of this reaction.

In particular, when porcine pancreatic lipase or *Pseudomonas cepacia*[5] lipase are employed, the orientation of the sugar substrates at the active site of these enzymes

*This paper is dedicated to the memory of the late Prof. Alberto Fiecchi.

seems mainly determined by the sequence of the three free secondary hydroxyl groups.[3] In fact methyl 6-*O*-butyryl-α-**D**-galactopyranoside (**1a**) and methyl 6-*O*-butyryl-α-**L**-mannopyranoside (**1c**), as well as their 6-deoxy derivatives **1b** and **1d**, all having the same axial-equatorial-equatorial (AEE)[6] sequence of the alcoholic functions, are always acylated at the same equatorial terminus of this triplet[3,4] (2-OH for the **D**-series and 4-OH for the **L**-series).

On the contrary sugars having an equatorial-equatorial-axial (EEA) sequence, namely methyl 6-*O*-butyryl-α-**L**-galactopyranoside (**2c**) and methyl 6-*O*-butyryl-α-**D**-mannopyranoside[4] (**2a**), and the corresponding 6-deoxy derivatives[3] **2d** and **2b** usually give lower yields and poor regioselectivity.



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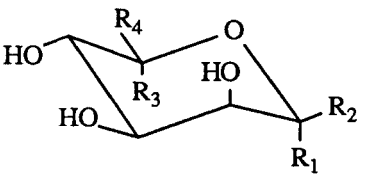


2

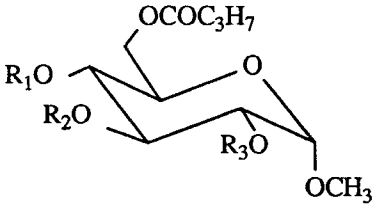
a: R_1=OCH_3, R_2=R_3=H, R_4=$CH_2OCOC_3H_7$
b: R_1=OCH_3, R_2=R_3=H, R_4=CH_3
c: R_3=OCH_3, R_1=R_4=H, R_2=$CH_2OCOC_3H_7$
d: R_3=OCH_3, R_1=R_4=H, R_2=CH_3

In principle, the differences found between the above mentioned sugars might be due either to the orientation of the OH at the right terminus of the triplet (*i.e.*, the acylation site) or to the orientation of the hydroxyl group in position β to it (*i.e.*, the left terminus), as the two triplets differ in the configuration of the two outer stereocentres.

On the basis of these considerations, we have therefore submitted the methyl α-glycopyranosides of 6-*O*-butyryl-**D**- and **L**-glucose (**3a** and **4a**) and of 6-deoxy-**D**- and **L**-glucose (**5a** and **6a**) to lipase-catalyzed transesterification. In fact in these four sugars, all with the three secondary hydroxyl groups equatorially oriented, the EEE triplet allows the study of the influence of the orientation at the right terminus when compared with the EEA triplet and of the influence of the orientation at the left terminus when compared with the AEE triplet.

RESULTS AND DISCUSSION

The enzymic butyrylation of compounds **3a**, **4a**, **5a** and **6a**[7-9] was performed through the lipase-trifluoroethylbutyrate (TFEB) system[13] using crude porcine pancreatic lipase (PPL), *Pseudomonas cepacia* lipase (LPS) and *Candida cylindracea* lipase (CCL) at 45 °C in the conditions previously reported.[3]



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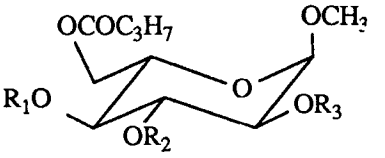

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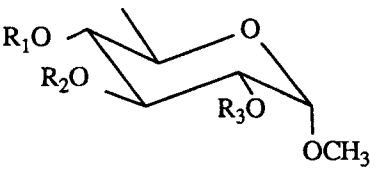

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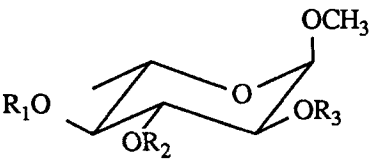

6

a: $R_1=R_2=R_3=H$ **b:** $R_1=R_2=H$, $R_3=COC_3H_7$

c: $R_1=R_3=H$ $R_2=COC_3H_7$ **d:** $R_2=R_3=H$, $R_1=COC_3H_7$

The monobutyrylation mixtures were obtained by flash chromatography and the relative ratios of the monoesters or diesters were obtained by ^1H NMR analysis. The signals used for the integration were previously assigned on the ^1H NMR spectra of each **D**-butyrate obtained pure[4,14](Table 2). Table 1 summarizes the enzymic butyrylation results.

The obtained data show that two enzymes, PPL and LPS always gave similar results, though the selectivity was higher with the former lipase. The enzymic acylation of the sugars of the **D** series either functionalyzed at C-6 (**3a**) or not (**5a**) gave high yields and an excellent selectivity toward 2-acylation (entries 1,2 and 7,8). For the **L** sugars a good regioselectivity toward 4-acylation was observed but with poorer yields than for the **D** sugars (entries 4,5 and 10,11).

Table 1. Enzymic butyrylation of **3a-6a**

entry	substr.	enzyme	time (days)	yield (%)	%esters **b** (C-2)	**c** (C-3)	**d** (C-4)
1	**3a**	PPL	2	88	99	1	0
2	**3a**	LPS	7	73	95	5	0
3	**3a**	CCL	2	70	88	12	0
4	**4a**	PPL	12	25	0	5	95
5	**4a**	LPS	12	35	13	10	77
6	**4a**	CCL	12	40	6	77	17
7	**5a**	PPL	2	93	100	0	0
8	**5a**	LPS	7	83	93	7	0
9	**5a**	CCL	7	74	86	14	0
10	**6a**	PPL	12	24	0	2	98
11	**6a**	LPS	12	38	6	10	84
12	**6a**	CCL	7	52	3	40	57

Table 2. ^{1}H NMR data[a] of compounds **3b-d** and **5b-d**

	chemical shifts, δ								
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	H_3-6	MeO
3b	4.99	4.70	3.95	3.41	3.75	4.28	4.48	--	3.37
3c	4.78	3.60	5.10	3.47	3.80	4.32	4.44	--	3.45
3d	4.80	3.61	3.82	4.87	3.89	4.11	4.22	--	3.44
5b	4.86	4.70	3.92	3.27	3.72	--	--	1.33	3.38
5c	4.74	3.62	5.02	3.24	3.71	--	--	1.32	3.45
5d	4.74	3.60	3.79	4.64	3.80	--	--	1.18	3.44
	coupling constants, Hz								
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{5,6}$	$J_{6a,6b}$	
3b	4.0	10.0	10.0	10.0	2.0	4.5	--	12.0	
3c	4.0	9.5	9.5	10.0	2.0	5.0	--	12.0	
3d	4.0	9.5	9.5	10.0	2.0	5.0	--	12.0	
5b	4.0	10.0	9.0	9.0	--	--	6.0	--	
5c	4.0	9.5	9.5	9.5	--	--	6.0	--	
5d	4.0	9.5	9.5	9.5	--	--	6.0	--	

[a]200 MHz, deuteriochloroform solutions.

CCL showed for the **D** sugars a behavior similar to that of the other two lipases, but reactions were less selective (entries 3 and 9); moreover, it showed a very poor selectivity with the **L** sugars and, in the case of **4a** a preference for the acylation of the 3-hydroxyl group (entry 6) was observed, a result which parallels the results obtained with **L**-galactose (66%),[4] **L**-mannose (54%)[4] and **L**-fucose (89%).[3]

Comparison of the PPL and LPS results obtained in this work (EEE triplets, **D**-series, **3a**,**5a**) with those on **D**-fucose[3] and **D**-galactose[4] (AEE triplets) shows that the left end of the triplet has no influence on the results given by the **D**-sugars. However, further comparison with the results on **4a, 6a** (EEE triplets, **L**-series) and with those on **L**-rhamnose[3] and **L**-mannose[4] (AEE triplets) shows that the yields are usually lower for the **L** sugars in consequence of exchange of 1-methoxyl and 5-acyloxymethyl (or 5-methyl) groups in each couple **D**-glucose *vs.* **L**-glucose (**3a**,**4a**), **D**-deoxyglucose *vs.* **L**-deoxyglucose (**5a**,**6a**), **D**-galactose *vs.* **L**-mannose (**1a**,**1c**), **D**-fucose *vs.* **L**-rhamnose (**1b**,**1d**); the lowering of yields is, however, more pronounced in the couples having the EEE triplet than in the couple having the AEE triplet, probably for steric reasons.

At last, comparison of the PPL and LPS results obtained on glucose and 6-deoxyglucose with those obtained on **L**-fucose,[3] **L**-galactose,[4] **D**-rhamnose[3] and **D**-mannose[4] (EEA triplets, **2a**-**2d**) shows the equatorial orientation of the hydroxyl group to be acylated usually allows better yields and selectivity. So the reason for the poor efficiency of the EEA with respect to the AEE triplet must be due to the difference on their right terminus.

It can be concluded that in all the cases studied there is a general tendency of lipases (PPL and LPS) to acylate at the right terminus (*i.e.*, position 2 in the **D** series and position 4 in the **L** series) of a sugar having three contiguous secondary hydroxyl groups. The orientation of the group involved in the reaction can heavily influence the outcome of the reaction, while the orientation of the β hydroxyl group has minor influence. The other substituents on the pyranoside ring exert their influence mainly on the yields but do not influence the regiochemical outcome of the reactions.[16]

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3. P. Ciuffreda, D. Colombo, F. Ronchetti, and L. Toma, *J. Org. Chem.*, **55**, 4187 (1990).

4. D. Colombo, F. Ronchetti, and L. Toma, *Tetrahedron*, **47**, 103 (1991).

5. In ref. 1-3 *Pseudomonas cepacia* lipase was incorrectly referred to as *Pseudomonas fluorescens* lipase by the manufacturer (Amano Pharmaceutical Co., private communication).

6. The AEE, EEA and EEE termina refer to the orientation (axial A, or equatorial E) of the three free hydroxyl groups of the sugars under investigation, which are indicated in the sequence 4-OH, 3-OH, 2-OH in the D-series, and 2-OH, 3-OH, 4-OH in the L-series.

7. All the new compounds gave satisfactory elemental analyses.

8. Compounds **3a** and **4a**, prepared by enzymic butyrylation of methyl α-**D**-glucopyranoside and methyl α-**L**-glucopyranoside[10] in the conditions previously described,[4] were obtained pure by flash chromatography (9:1 methylene chloride-methanol); **3a**, oil, $[\alpha]_D^{20}$ +86.9° (*c* 0.9, $CHCl_3$), **4a**, oil, $[\alpha]_D^{20}$ -87.7° (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.33 (dd, 1H, $J_{3,4}$= 9.5 Hz, $J_{4,5}$= 10.0 Hz, H-4), 3.40 (s, 3H, MeO), 3.51 (dd, 1H, $J_{1,2}$= 4.0 Hz, $J_{2,3}$= 10.0 Hz, H-2), 3.70 (dd, 1H, $J_{2,3}$= 10.0 Hz, $J_{3,4}$= 9.5 Hz, H-3), 3.74 (ddd, 1H, $J_{4,5}$= 10.0 Hz, $J_{5,6a}$= 6.0 Hz, $J_{5,6b}$= 2.0 Hz, H-5), 4.25 (dd, 1H, $J_{5,6a}$= 6.0 Hz, $J_{6a,6b}$= 12.0 Hz, H-6a), 4.39 (dd, 1H, $J_{5,6b}$= 2.0 Hz, $J_{6a,6b}$= 12.0 Hz, H-6b), and 4.74 (d, 1H, $J_{1,2}$= 4.0 Hz, H-1).

9. Compounds **5a** and **6a** have been prepared by reduction with tributylstannane[11] of the corresponding methyl-6-deoxy-6-iodo-α-**D**-[12] and α-**L**-glucopyranosides.

10. R. H. Pater, R. A. Coelo, and D. F. Mowery, Jr., *J. Org. Chem.*, **38**, 3272 (1973).

11. G. O. Aspinall, R. C. Carpenter, and L. Khondo, *Carbohydr. Res.*, **165**, 281 (1987).

12. P. J. Garegg, and B. Samuelsson, *J. Chem. Soc. Perkin Trans. 1*, 2866 (1980).

13. Porcine pancreatic lipase (type II) (PPL) (specific activity 11.8 triacetin units/mg solid) and *Candida cylindracea* lipase (type VII) (CCL) (specific activity 665 triacetin units/mg solid) were purchased from Sigma; *Pseudomonas cepacia* lipase (lipase PS, LPS) (specific activity 30.5 triacetin units/mg solid) was a generous gift from Amano Pharmaceutical Co., Frankfurt. CCL was used as received; PPL and LPS were kept under vacuum prior to use in order to lower the water content to 0.5%. For the experimental details see ref. 3.

14. **3b** (0.55 g) was obtained by PPL butyrylation on **3a** (0.50 g) and was purified by flash chromatography (3:2 ethyl acetate-petroleum ether); oil, $[\alpha]_D^{20}$ +82.1° (*c* 1.0, $CHCl_3$). Analogously **5b** (0.65 g) was obtained by PPL butyrylation on **5a** (0.50 g) and was purified by flash chromatography (3:2 ethyl acetate-petroleum ether); mp 82-83 °C from ethyl acetate, $[\alpha]_D^{20}$ +121.0° (*c* 0.7, $CHCl_3$).
3c and **3d** were obtained by chemical butyrylation;[15] **3c**, oil, $[\alpha]_D^{20}$ +102.5° (*c* 1.2, $CHCl_3$); **3d** , oil, $[\alpha]_D^{20}$ +96.2° (*c* 0.9, $CHCl_3$). The same procedure afforded pure **5c** and **5d**; **5c**, oil, $[\alpha]_D^{20}$ +157.7° (*c* 1.1, $CHCl_3$), **5d**, oil, $[\alpha]_D^{20}$ +142.0° (*c* 1.0, $CHCl_3$).

15. Substrate (**3a** or **5a**, 1 g), butyric anhydride (0.61 mL for **3a** and 0.89 mL for **5a**), pyridine (10 mL), overnight at room temp. The reaction mixture from **3a** was purified by flash chromatography (3:2 ethyl acetate-petroleum ether) to afford (45% of isolated diester yield) pure **3c**, **3b** and **3d** eluted in the order, in the ratio 34:53:13.
Analogously, the reaction mixture from **5a** afforded (61% of isolated monoester yield) pure **5c**, **5b** and **5d** eluted in the order, in the ratio 40:42:18.

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