

A Highly Selective Enzyme-catalysed Esterification of Simple Glucosides

Fredrik Björkling,* Sven Erik Godtfredsen, and Ole Kirk

Department of Biochemical Synthesis, Novo Industri A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark

Regioselective 6-*O*-esterification of alkyl glucosides with long chain fatty acids, yielding more than 95% of 6-*O*-monoesters, can be achieved using lipases as catalysts in a solvent-free process.

Fatty acid esters of carbohydrates constitute an interesting group of nonionic surfactants. These surface-active materials exhibit highly useful properties; they are in principle conjugates of cheap and readily available raw materials and they are expected to be easily degraded in nature.¹ The possibilities for preparing esters of carbohydrates have, accordingly, been extensively studied in the past.² However, selective esterification of unprotected polyols has not proven practically feasible. Low yields only have been achieved and application of extensive purification procedures has been necessary to provide products of high purity. Likewise, specific transformations involving application of protection and deprotection procedures³ have proven not to be economically feasible for industrial processes. Furthermore, attempts by several groups to make use of enzymes for synthesis of carbohydrate esters have been unsuccessful. Yields reported are low and the amounts of enzyme utilized are too high to allow large-scale processing.⁴

In connection with our work on application of enzymes in organic synthesis, and the exploration of new sugar derivatives for industrial uses, we have developed a highly efficient lipase-catalysed process for regiospecific esterification of the primary hydroxy group in simple alkyl glycosides (Scheme 1). This procedure allows us, *e.g.*, to esterify ethyl *D*-glucopyranoside with C₈–C₁₈ fatty acids in yields of 85–95% of the 6-*O*-monoesters using an immobilized lipase from a species of *Candida antarctica*^{5†} as catalyst (Table 1). The reaction was performed conveniently simply by mixing the two reactants at 70 °C under reduced pressure in the presence of the thermostable immobilised lipase. The purity of the glucoside esters thus obtained is excellent. The surfactant properties have been found to be similar to those of common nonionic surfactants (Table 1).

Previous attempts by other groups to esterify enzymatically α -*D*-glucose and methyl α -*D*-glucopyranosides have not been successful.⁴ As indicated, however, we found a dramatic change in the reactivity of ethyl *D*-glucopyranoside as compared to either glucose or methyl α -*D*-glucopyranoside (Table 2). Presumably, the higher reactivity of the glucosides is due to higher solubilities of the reactants in one another and to the

substrate-selectivity of the enzyme used. Our best yields were obtained using C₈–C₁₈ fatty acids and ethyl or isopropyl glucoside as substrates and, as catalyst, a heat-stable lipase derived from a strain of *Candida antarctica* (Tables 1, 2, and 3). This particular enzyme is non-specific with regard to triglyceride hydrolysis reactions but exhibits a very high selectivity towards the primary hydroxy group in the syntheses described.

Table 1. Yield, critical micelle concentration (CMC), and surface tension (γ_{\min}) of fatty acid esters of ethyl *D*-glucopyranoside.⁹

Fatty acid	Yield of 6- <i>O</i> -monoester /%	CMC /mol l ⁻¹	γ_{\min} /dyn cm ⁻¹
Octanoic acid	86.9	2.0×10^{-3}	31
Decanoic acid	88.4	9.6×10^{-4}	31
Dodecanoic acid	85.8	5.1×10^{-5}	31
Tetradecanoic acid	89.1	5.3×10^{-5}	33
Hexadecanoic acid	93.1	1.8×10^{-4}	39
Octadecanoic acid	95.5	8.3×10^{-6}	44
Octadecenoic acid	91.8	3.4×10^{-4}	35

^a The standard reaction was performed by mixing 50 g (0.24 mol) of ethyl *D*-glucopyranoside[‡] at 70 °C and 0.01 bar, with 1.35 equiv. of fatty acid and 2.5% (w/w) of immobilized *Candida antarctica* lipase of an activity of 40 BIU/g. § The yields indicated refer to yields of product obtained after purification by chromatography on silica gel using a gradient of light petroleum, ethyl acetate, and methanol as eluant. The CMC and surface tension were determined using a Krüss tensiometer type K 10. Mass and n.m.r. (¹H and ¹³C) data corresponding to the expected reaction products were obtained for all compounds synthesized.

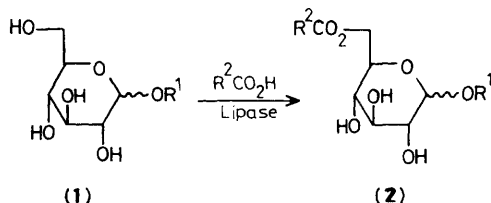
Table 2. Rate of reaction of selected carbohydrates with dodecanoic acid.^a

Carbohydrate [‡]	$T_{1/2}^b$	Conversion/%		Diester /%
		6 h	24 h	
α - <i>D</i> -Glucose	> 1 week	—	< 5%	—
Methyl α - <i>D</i> -glucopyranoside	22 h	20.0	53.3	3.5
Ethyl <i>D</i> -glucopyranoside	2.5 h	74.0	92.5	4.9
Isopropyl <i>D</i> -glucopyranoside	2.1 h	70.0	93.2	4.2
<i>n</i> -Propyl <i>D</i> -glucopyranoside	1.4 h	79.2	95.6	17.3
<i>n</i> -Butyl <i>D</i> -glucopyranoside	1.0 h	78.5	94.4	21.8

^a Conversion of different carbohydrates was performed at 70 °C and 0.01 bar, using 1.5 equiv. of dodecanoic acid and 6% (w/w) of immobilized *Candida antarctica* lipase of 40 BIU/g. § The progress of the reactions was monitored by h.p.l.c. ^b Time for conversion of 50% of the starting carbohydrate derivatives.

[‡] All glucosides except methyl α -*D*-glucopyranoside were mixtures of anomers obtained by glucosidation of the desired alcohol with α -*D*-glucose.⁸ The anomeric composition of the crude glucosides was determined by ¹H n.m.r. (400 MHz, D₂O). Ethyl *D*-glucopyranoside: $\alpha/\beta = 1/1$; *n*-propyl, iso-propyl, and *n*-butyl *D*-glucopyranoside: all $\alpha/\beta = 2/1$.

§ One Batch Interesterification Unit (BIU) corresponds to 1 mol of hexadecanoic acid incorporated (initial activity) into trioctadecenyl glycerol per minute.



R¹ = H, Me, Et, Prⁿ, Prⁱ, or Buⁿ. R² = C₇H₁₅, C₉H₁₉, C₁₁H₂₃, C₁₃H₂₇, C₁₅H₃₁, or C₁₇H₃₅.

Scheme 1

[†] Deposited at Deutsche Sammlung von Mikroorganismen (DSM) according to the Budapest Treaty under the deposit numbers DSM 3855, DSM 3908, and DSM 3909.

Table 3. Lipase activity, rate of conversion, and diester content in the synthesis using different immobilized lipases.^a

Lipase from	Activity BIU/g§	After 24 h		After 48 h	
		Conv. /%	Diester /%	Conv. /%	Diester /%
<i>Candida antarctica</i>	40	96.1	3.1	96.3	5.2
<i>Mucor miehei</i>	25	97.9	19.3	97.9	28.5
<i>Humicola sp.</i>	69	99.0	45.8	99.7	64.3
<i>Candida cylindracea</i>	29	21.2	1.2	39.6	6.1
<i>Pseudomonas sp.</i>	181	1.5	—	14.3	21.6

^a The conversion of ethyl D-glucopyranoside was performed at 70 °C and 0.01 bar, using 2 equiv. of dodecanoic acid and 6% (w/w) of different immobilized lipases. The conversions were measured by h.p.l.c.

The esterification was found to be catalysed by a variety of enzymes, however, with quite different conversion rates and selectivities (Table 2). LipozymeTM, an immobilized *Mucor miehei* lipase, is a 1,3-specific lipase with respect to hydrolysis of triglycerides and as such preferentially catalyses reactions with primary hydroxy groups, e.g. in acidolysis reactions explored commercially using this enzyme. Even so, the activity and selectivity of this enzyme was lower than that of the unspecific *Candida* lipase when expressed in the synthesis of glucoside esters. To achieve the best enzyme activity the immobilized enzyme preparations were moisturized to a water content of about 10%.⁶

The yields obtained using short chain fatty acids, C₈—C₁₀, were generally lower than those realized using their higher homologues (Table 1). This may be due to an increased solubility in water of the short-chain fatty acids, which causes a

pH change in the water bound to the enzyme and which may also dissolve some water from the enzyme surface and thereby cause a loss of activity.⁷

Received, 20th February 1989; Com. 9100777F

References

- 1 H. Baumann, M. Bühler, H. Fochem, F. Hirsinger, H. Zoebelien, and J. Falbe, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 41.
- 2 E. Reinefeld and H.-F. Korn, *Die Stärke*, 1968, **20**, 181; K. Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa, and Y. Tsuda, *Chem. Pharm. Bull.*, 1979, **27**, 2661; E. Albano-Garcia, E. G. Loric, M. Pama, and L. de Leon, *Philipp. J. Coconut Stud.*, 1980, **5**, 51; D. Plusquellec and K. Baczkó, *Tetrahedron Lett.*, 1987, **28**, 41; D. V. Myhre, U.S. Pat. 3,597,417, 1971; G. N. Bollenback and F. W. Parrish, *Carbohydr. Res.*, 1971, **17**, 431.
- 3 J. M. Sugihara, *Adv. Carbohydr. Res.*, 1953, **8**, 1.
- 4 M. Therisod and A. M. Klibanov, *J. Am. Chem. Soc.*, 1986, **108**, 5638; H. M. Sweers and C.-H. Wong, *ibid.*, 1986, **108**, 6421; J. Chopineau, F. D. McCafferty, M. Therisod, and A. M. Klibanov, *Biotechnol. Bioeng.*, 1988, **31**, 208; S. Riva, J. Chopineau, A. P. G. Kieboom, and A. M. Klibanov, *J. Am. Chem. Soc.*, 1988, **110**, 584; J.-F. Shaw and A. M. Klibanov, *Biotechnol. Bioeng.*, 1987, **29**, 648; M. Therisod and A. M. Klibanov, *J. Am. Chem. Soc.*, 1987, **109**, 3977; M. Kloosterman, E. W. J. Mosmuller, H. E. Schoemaker, and E. M. Meijer, *Tetrahedron Lett.*, 1987, **28**, 2989.
- 5 H. P. Heldt-Hansen, I. Michiyo, S. A. Patkar, T. T. Hansen, and P. Eigtved, 1988, Proc. Symposium on Biocatalysis and Biomimetics: Aspects of Enzyme Chemistry for Agriculture-Enzyme Production of Speciality Products, in the press; Danish Pat. Appl. No. 3250/88.
- 6 A. Zaks and A. M. Klibanov, *J. Biol. Chem.*, 1988, **263**, 8017.
- 7 C. Laane, S. Boeren, K. Vos, and C. Veeger, *Biotechnol. Bioeng.*, 1987, **30**, 81; M. Norin, J. Boutelje, E. Holmberg, and K. Hult, *Appl. Microbiol. Biotechnol.*, 1988, **28**, 527.
- 8 E. Fischer and L. Bensch, *Ber.*, 1894, **27**, 2478; J. E. Cadotte, F. Smith, and D. Spriesterbach, *J. Am. Chem. Soc.*, 1952, **74**, 1501.