Lipase-catalysed esterification of some α-D-glucopyranosides in dry media under focused microwave irradiation

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Immobilized *Candida antarctica* lipase (Novozym 435)-catalysed esterifications of methyl α-D-glucopyranoside **1**, α-D-glucose **2** and α,α-trehalose **3** with dodecanoic acid in dry media under focused microwave irradiation and classical heating conditions are described, under the same conditions of time and temperature. The advantages of performing the reactions in the microwave reactor are evident in all cases either in terms of yields and (or) purities of products.

Introduction

Fatty acid esters of mono- and di-saccharides have nowadays many applications related to their biodegradability and non-toxicity, as non-ionic detergents, ingredients in personal care products, food and feed additives or emulsifiers.[1] Some of them have pharmacological applications like α,α-trehalose-6,6'-dialkanoates.[2]

Lipases may be used as catalysts in order to introduce acyl groups regioselectively into carbohydrates without the use of protection–deprotection techniques.[3] Under the usual conditions, acylation is reversible and the composition of the reaction mixture at equilibrium is far from optimum. Complete conversion can be achieved by removing the co-products formed by chemical[3e,4] or physical means, *i.e.* working under vacuum[5] or adding zeolite.[6]

Lipase-catalysed esterification of sugars by long-chain fatty acids in organic solvents (pyridine, 2-methylbutan-2-ol) has been recently described in connection with great industrial interest.[7]

Björkling *et al.*[3f,5] have investigated esterification of several glucopyranosides with different fatty acids catalysed by Novozym 435 in a 'solvent-free' procedure under vacuum, where the molten fatty acid constituted the liquid phase. Under these conditions (0.01 bar and 70 °C), alkylglucopyranosides were much better substrates than unprotected glucose. After 24 h, less than 5% of glucose-6-alkanoate was formed and half time reaction ($t_{\frac{1}{2}}$) was more than one week. When the acyl acceptor was an alkyl glucopyranoside, $t_{\frac{1}{2}}$ depended on the nature of the alkyl group and varied from 1 h for butyl to 22 h for methyl α-D-glucopyranoside. However, the selectivity (6-*O*-monoester:2,6-*O*,*O*-diester) was better for methyl, ethyl and isopropyl glucopyranosides than for larger alkyl groups. Furthermore, yields obtained using shorter chain fatty acids (C_8–C_{10}) were lower (85%) than those obtained from higher homologues C_{16}–C_{18} (93–95%).

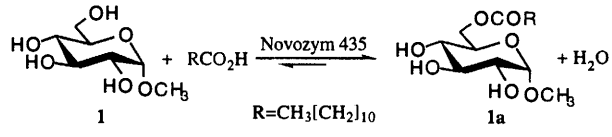
We have previously shown that kinetics of enzyme-catalysed reactions could be enhanced under microwave (MW) irradiation, so that the resolution of racemic alcohols and acids was efficiently realized in dry conditions, within short times and with better enantioselectivities when compared to classical heating.[8] Displacement of equilibrium under microwaves has already been observed in some chemical esterifications[9] and enzyme catalysed reactions in dry media are more efficient and allow higher reaction temperatures and easier water elimination.

Therefore, we have studied the esterification of methyl α-D-glucopyranoside **1**, α-D-glucose **2** and α,α-trehalose **3** at 95–110 °C in dry conditions, in order to obtain better regioselectivities, cleaner reactions or higher yields. Reactions had to be performed taking advantage of a monomode microwave reactor which is the best system able to allow measurements and control of temperature throughout the reaction which proceeds with a good homogeneity and high energetic yield. For these purposes, commercial *Candida antarctica* lipase immobilized on Accurel (a polypropylene resin, Novozym 435) was used and substrates were impregnated on it.

Results and discussion

Regioselective esterification of methyl α-D-glucopyranoside 1

In a typical experiment (Scheme 1), 1 g of Novozym 435 was



Scheme 1

impregnated with an aqueous solution of **1** (1–1.5 mmol, 0.5–1 ml) and the samples were dried overnight in a desiccator under vacuum (5 mmHg during 2 h). Dodecanoic acid (3 equiv.) in diethyl ether (1 ml) was then added (mode A). In some cases, wet diethyl ether (1 ml) was added at the beginning of the reaction (mode B).

Reaction mixtures were introduced into a focused microwave reactor and irradiated order different conditions of time and power. The results are given in Table 1. For the sake of comparison, reactions were performed in a thermostated oil bath under conditions identical to the optimal ones under microwave irradiation. The main product obtained was methyl 6-*O*-dodecanoyl-α-D-glucopyranoside **1a**. In a few cases small amounts (≤5%) of a lighter product was obtained, as judged by gas chromatography (GC).

Almost complete conversions were obtained under microwave irradiation (run 3), whereas a conversion of only 55% was obtained under classical heating conditions after 5 h. The reac-

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Table 1 Esterification of methyl α-D-glucoside **1** with dodecanoic acid

Run	*t*/h	*T*/ °C	Equiv. of acid	Power range/W	Conversion (%)	Mode of sample preparation
1	2	95	3	120–60	62	A
2[a]	2	95	3	120–60	67	A
3	5	95	3	120–40	95	A
4	5	95	3	Oil bath	55	A
5	3	95	3	120–60	95	B
6	2	110	3	120–60	97	B
7	4.5	95	1.5	120–40	48	A
8[b]	5	110	3	150–90	81	A

[a] Reaction conducted with recovered enzyme. [b] Five-fold larger scale preparation.

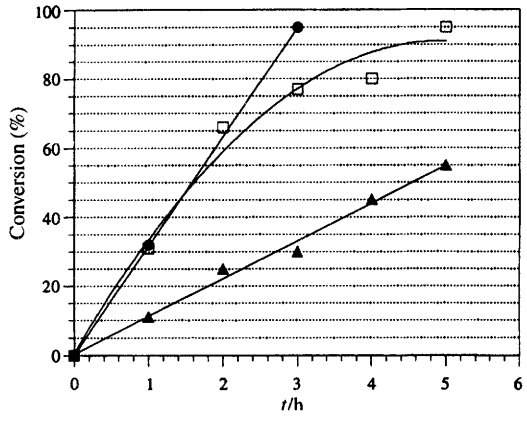


Fig. 1 Esterification of **1** with dodecanoic acid in the monomode reactor (□ mode A, ● mode B) and in an oil bath (▲ mode A)

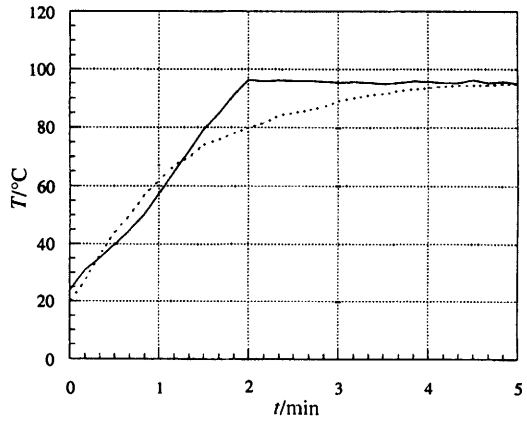


Fig. 2 Temperature profile for mutarotation of **2** in an oil bath (– – –) and in the microwave reactor (——)

tion time could be shortened when wet diethyl ether had been previously added (mode B, runs 5,6). The same results could be obtained with less catalyst, using a non-commercial enzyme preparation ST389 which had ten times lower activity than its parent Novozym 435.

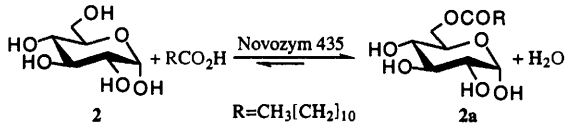
Furthermore, after several hours of heating in the microwave reactor at 95 °C, then extraction of the products and subsequent washings of enzyme with distilled water, the enzyme could be reused without loss of activity (runs 1 and 2). Scaling up of the best conditions on a five-fold larger scale gave 81% of conversion (run 8).

We have therefore obtained a noticeable enhancement in the reaction conditions and yield for methyl α-D-glucopyranoside **1** ($t_{\frac{1}{2}}$ = 1.5 h and final yield 95% within 5 h).

Fig. 1 shows the progress curves for the esterification of **1** with dodecanoic acid. both under MW and classical heating conditions.

Regioselective esterification of α-D-glucose 2

As unprotected glucose **2** is known to be a very poor substrate for 'solvent-free' lipase-catalysed esterifications, we thus examinated the esterification of **2** with dodecanoic acid catalysed by Novozym 435 in dry conditions under microwave irradiation (Scheme 2).



Scheme 2

α-D-Glucose (98% of GC purity after silylation) was used as the starting material. Substrate and enzyme were conditioned as described above for **1**. The mutarotation of the remaining glucose was very fast in these reaction conditions. A ratio α:β = 47:53 was obtained after 5 min of heating of pure α-D-glucose both under microwave and classical heating conditions (contrary to a previous observation of Pagnotta *et al.*[10] who suggested an intrinsic effect of microwaves), and remained unchanged throughout the reaction time. However, only 6-*O*-dodecanoyl-α-D-glucopyranoside **2a** was obtained, a fact which indicates a low reactivity of β-D-glucose when compared to the α-isomer. Fig. 2 shows the temperature profile for classically and microwave heated glucose impregnated on Novozym 435.

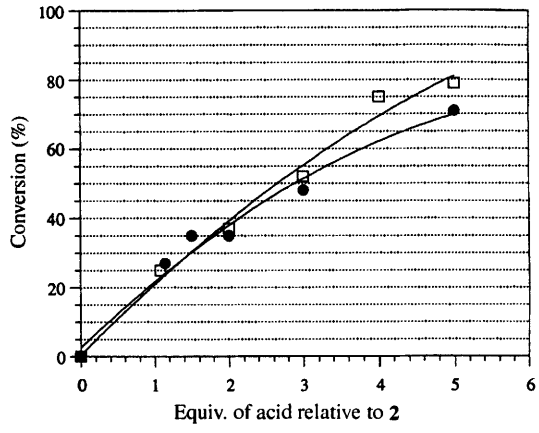


Fig. 3 Effect of the amount of dodecanoic acid on the esterification of **2** in the microwave reactor (●) and in an oil bath (□)

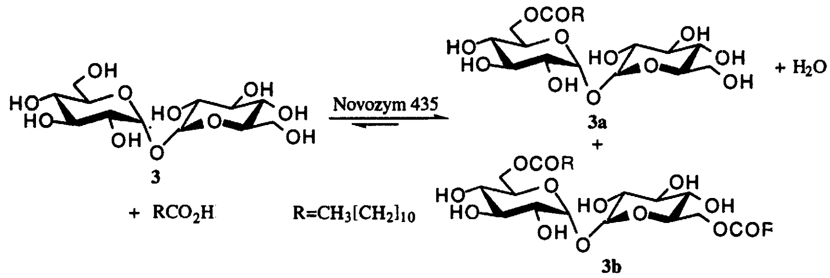
Next, the effects of relative amounts of dodecanoic acid *vs.* glucose and of reaction time were studied.

The results show (Fig. 3) that by increasing the amount of dodecanoic acid from 1 to 5 equiv., the initial rates were increased 3–4 times, but by extending the reaction time and using 3 equiv. of acid, 70–80% of conversion was obtained after 2 h (Fig. 4). Conversions obtained under classical heating conditions and microwave irradiation were of the same order of

Table 2 Esterification of α,α-trehalose **3** with dodecanoic acid at 110 °C

Run	t/h	Equiv. of acid	Power range/W	Conversion (%)	**3b**:**3a**	Mode of sample preparation
9	3	3	150–60	41	46:5	A
10[a]	3	3	150–90	39	37:2	A
11	13	3	150–60	83	80:3	A
12	13	3	150–60	92	88:4	B
13	13	3	Oil bath	78	75:3	B
14	13	3	120–60	32	30:2	C

[a] Reaction conducted with recovered enzyme.



Scheme 3

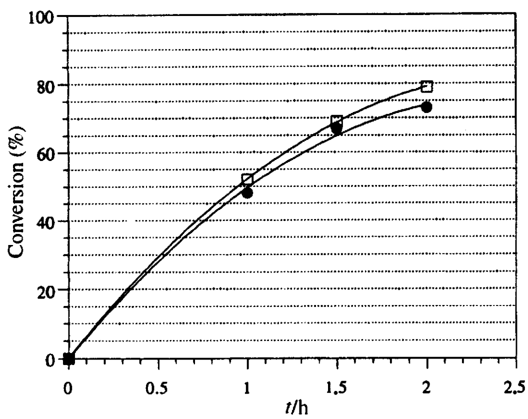


Fig. 4 Esterification of **2** with dodecanoic acid in the monomode reactor (●) and in an oil bath (□)

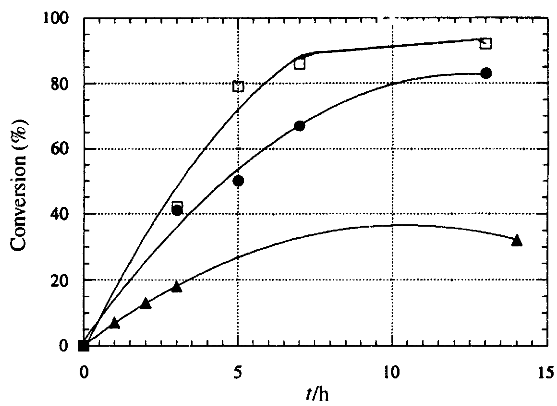


Fig. 5 Esterification of **3** with dodecanoic acid in the microwave reactor at 110 °C (● mode A, □ mode B and ▲ mode C)

magnitude. However the product obtained under classical heating conditions was accompanied by *ca.* 10% of impurities whereas it was pure under microwave irradiation.

The best yields ($t_{\frac{1}{2}}$ = 1 h, yield *ca.* 70%) were obtained after 1 h at 95 °C with 5 equiv. of acid or after 2 h with only 3 equiv. of acid. Reaction under microwave irradiation gives no by-products which makes isolation of the desired product easier.

Regioselective esterification of α,α-trehalose 3

Higher fatty acid esters (C_{18}–C_{80}) of α,α-trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) show many biological effects such as antitumour and antibiotic activities.[2] Recently, Novozym 435-catalysed esterification of **3** with ethyl dodecanoate has been realized under classical heating conditions, in refluxing *tert*-butyl alcohol.[11] A conversion of 80% and a ratio **3b**:**3a** = 80:3 were obtained using a large excess (50 equiv.) of the acyl donor (Scheme 3).

We have thus investigated the esterification of α,α-trehalose **3** with dodecanoic acid in dry media.

The samples were prepared as for **1** and dried overnight in a desiccator under vacuum (5 mmHg during 2 h, mode A). To this dry preparation, wet diethyl ether (1 ml) was added successively every 2 h (mode B), or the samples were dried in the microwave reactor at 95 °C during 30 min (mode C).

The major product obtained was the 6,6′-diester **3b** accompanied by <4% of 6-monoester **3a**. Results are given in Table 2 and Fig. 5.

Yields obtained under classical heating conditions, run 13 (78% within 13 h) were improved by as much as 92% under microwave irradiation. Here again the enzyme can be reused without significant loss of activity (runs 9,10).

Conclusions

We have taken the advantage of the complementarity of two recent technologies, enzymatic catalysis using immobilized enzymes in dry media and microwave activation in solvent-free conditions, to enhance reactivity and selectivity of the regioselective acylation of pyranose derivatives. Specific microwave effects when compared to classical heating are evident. They could result from an improvement in the irreversibility of the reaction due to more expeditious water removal under microwave irradiation and possibly due to some specific (non-thermal) effects of microwaves including entropic effects.[12]

Experimental

Materials and methods

Candida antarctica lipase immobilized on Accurel (Novozym 435) is a commercial product from Novo Nordisk. ST389 is a non-commercial preparation of *Candida antarctica B* lipase immobilized on Accurel with a 1:10 activity when compared to Novozym 435. It was a gift from Unilever Research Colworth Laboratory, UK. α-D-Glucopyranosides **1–3** and dodecanoic

acid were supplied by Fluka. All chemicals were commercial. Solvents were used without any further purification.

Microwave equipment. Reactions were performed in a monomode microwave reactor (Synthewave 402 from Prolabo) with focused electromagnetic field, fitted with a stirring system and an IR temperature detector which indicates the surface temperature.[13] Reaction conditions were controlled using the algorithm '*tout ou peu*' which allows temperature control at the given value during the reaction time by varying the power between an adequate value and the lower one fixed to 20 W (in order to operate always under an electromagnetic field during the reaction).

Analytical methods. NMR Spectra were recorded on Bruker equipment at 250 or 400 MHz with tetramethylsilane as an internal standard. Reactions were followed by GC or HPLC. After silylation of carbohydrates GC analysis was performed on a 6000 Vega Series with FID detector. Spectra-Physics SP 4290 integrator and a OV1 column (12 or 15 m) under the following conditions: detector temperature 300 °C, injector temperature 290 °C. Column temperature was programmed in the range 150–250 °C in the case of methyl α-D-glucopyranoside **1** and glucose **2**, and 220–290 °C for α,α-trehalose **3** with the heating rate 10 °C min^{-1}. Retention times (t_r/min) were: 3.05 (methyl α-D-glucopyranoside **1**). 10.43 (methyl 6-*O*-dodecanoyl-α-D-glucopyranoside **1a**). 3.20 (α-D-glucose **2**). 4.00 (β-D-glucose). 10.48 (6-*O*-dodecanoyl-α-D-glucose **2a**). 3.10 (α,α-trehalose **3**). 8.63 (6-*O*-dodecanoyl-α,α-trehalose **3a**) and 31.64 (6,6′-*O*,*O*-didodecanoyl-α,α-trehalose **3b**).

HPLC Analysis was performed on a Spectra-Physics instrument fitted with a Knauer differential refractometer and a Spectra-Physics SP 4270 integrator using reversed phase column (Colosil C18 5 μm, dimension 250 × 4.6 mm). Methanol (flow rate 1 ml min^{-1} and pressure 60 bar) was used as mobile phase. Retention times (t_r/min) were: 2.61 (methyl α-D-glucopyranoside **1**). 3.17 (methyl 6-*O*-dodecanoyl-α-D-glucopyranoside **1a**). 2.57 (α-D-glucose **2**). 3.10 (6-*O*-dodecanoyl-α-D-glucose **2a**). 2.53 (α,α-trehalose **3**). 2.94 (6-*O*-dodecanoyl-α,α-trehalose **3a**) and 4.75 (6,6′-*O*,*O*-didodecanoyl-α,α-trehalose **3b**).

Preparation of silyl derivatives for GC. A sample (1–2 mg) was dissolved in dry pyridine (0.5–1 ml) followed by addition of Hydrox-Sil reagent [14] (150–300 μl). The mixtures were heated during 2 h at 80 °C except in the case of glucose where the mixture was heated for 30 min. Pyridine was removed by evaporation ($t \leq 50$ °C) and crude products were analysed by GC.

Esterification of glucopyranosides—general method

α-D-Glucopyranoside (1–1.5 mmol) was dissolved in the minimal amount of water (0.5–1 ml), impregnated on Novozym 435 (1 g) and dried by different modes (A, B, C as described in the text). Dodecanoic acid (1–5 mmol) in diethyl ether (1–1.5 ml) was added: the mixture was placed in the microwave reactor and irradiated as indicated in Tables 1 and 2, or introduced into a thermostated oil bath. After cooling to room temperature, the mixture was washed first with pentane in order to remove the excess of dodecanoic acid and then the products were dissolved in methanol and analysed by GC after silylation or by HPLC. To the crude products $CHCl_3$ was added and the starting glucopyranosides were washed out with water. The organic phase was dried over Na_2SO_4 and the pure esters were precipitated with pentane. The positions of acylation were established from ^{13}C NMR spectra according to the Yoshimoto method.[15] The NMR spectra were in agreement with literature data: methyl 6-*O*-dodecanoyl-α-D-glucopyranoside **1a**[16] and 6-*O*-dodecanoyl-α-D-glucose **2a**,[17] 6,6′-*O*,*O*-didodecanoyl-α,α-trehalose **3b**.[11]

Methyl 6-*O*-dodecanoyl-α-D-glucopyranoside 1a. δ_C([$^2H_6$]DMSO): 99.72 (C-1). 73.19 (C-2). 71.82 (C-3). 69.58 (C-4). 70.42 (C-5). 63.61 (C-6). 54.32 (OCH_3). 172.84 (C=O). 14.02 (CH_3). 22.18. 24.53. 28.49. 28.79. 28.98. 29.07. 31.37, 33.54 (methylene moiety).

6-*O*-Dodecanoyl-α-D-glucose 2a. δ_C([$^2H_5$]pyridine): 94.30 (C-1). 75.34 (C-2), 74.46 (C-3). 72.34 (C-4). 70.90 (C-5). 65.20 (C-6). 173.77 (C=O). 14.33 (CH_3). 22.10. 24.40. 28.50. 28.80. 29.00. 29.20. 31.30 (methylene moiety).

6,6′-*O*,*O*-Didodecanoyl-α,α-trehalose 3b. δ_C([$^2H_6$]DMSO): 93.41 (C-1.1′). 71.44 (C-2.2′). 72.72 (C-3.3′). 69.71 (C-4.4′). 70.10 (C-5.5′). 63.07 (C-6.6′). 172.77 (C=O). 13.95 (CH_3). 22.12. 24.49. 28.46. 28.75. 28.92. 29.04. 31.33. 33.57 (methylene moiety).

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