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Novel mannitol based non-ionic surfactants from biocatalysis Part two: improved synthesis

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Abstract

The 1-*O*-lauroyl-D-mannitol, a non-ionic surfactant, was synthesised via a chemo-enzymatic pathway starting from the 1,2:4,5-di-*O*-isopropylidene-D-mannitol and vinyl laurate as acylation agent. The high hydrophobicity of the substrates allowed the enzymatic reaction to occur both in *n*-hexane and in solvent free conditions. The immobilised *Candida antarctica* lipase B was used as the catalyst of the enzymatic step. This enzyme acts differently depending on the position of the hydroxyls with respect to the isopropylidene groups. The acid selective hydrolysis of the isopropylidene groups gave the non-ionic surfactant without the presence of isomers.

Keywords: Carbohydrate ester; Non-ionic surfactant; Di-*O*-isopropylidene-D-mannitol; *Candida antarctica* lipase B

1. Introduction

Nowadays the environmental aspect of industrial transformations has become fundamental for a sustainable technological progress. In this context, biocatalysis represents the future of industrial processes because of its advantages of mild operative conditions and absence of by-products. Carbohydrate esters constitute an important class of biosurfactants largely used in food, cosmetics and drugs [1,2]. They have emulsifying properties, they are fully biodegradable, odourless, flavourless, non-toxic, non-skin irritant, and can be easily digested by the stomach as a sugar-fatty acid mixture [3]. Industrial synthesis of carbohydrate fatty acid esters are high energy consuming, not selective, producing a high amount of by-products, thus requiring a further purification step. For these reasons biocatalysis seems to be a very promising alternative. Many articles report the biocatalytic synthesis of carbohydrate esters following different strategies [4–9]. We have recently described the chemo-enzymatic syntheses of two mannitol lauric acid esters, starting from two different isopropylidene-D-mannitol isomers and vinyl laurate [10]. These syntheses were performed by using 2-methyl-2-butanol, a polar organic solvent that was able to dissolve both the substrates. Industrial processes tend to use solvents that are easily removable, for example by evaporation, or even better to not use solvents. The present work describes the improved synthesis of 1-*O*-lauroyl-D-mannitol. Starting materials are the 1,2:4,5-di-*O*-isopropylidene-D-mannitol and vinyl laurate. The presence of two protecting groups allowed to perform the enzymatic step both with an apolar solvent and in solvent free conditions.

2. Experimental

2.1. Chemicals

Immobilised lipase B from *Candida antarctica*, Novozym 435, was a kind gift of Novo Nordisk-Bioindustriale s.r.l. (Milano, Italy). 1,2:4,5-Di-*O*-isopropylidene-D-mannitol 98%, 1,2:5,6-di-*O*-isopropylidene-D-mannitol 98%, and $CHCl_3$ 99.9% were purchased from Aldrich. Vinyl laurate 99.0% and acetic acid 99.8% were purchased from Fluka. *n*-Hexane 99.0% and acetone 99.9% were purchased from Sigma.

2.2. Synthesis and purification of mannitol esters

A typical experiment was performed by dissolving 1,2:4,5-di-*O*-isopropylidene-D-mannitol (0.3733 g, 1.40 mmol)

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and vinyl laurate (0.7709 g, 3.40 mmol) in *n*-hexane (10 ml). After the addition of 0.50 g of Novozym 435 (lipase B from *C. antarctica*), the transesterification was carried out at 50 °C with magnetic stirring at 600 rpm. At the end of the reaction, the biocatalyst was removed by filtration and the solvent evaporated under reduced pressure.

Alternatively 1,2:4,5-di-*O*-isopropylidene-D-mannitol (0.5360 g, 2.04 mmol) and vinyl laurate (1.0603 g, 4.68 mmol) were mixed with the biocatalyst (0.50 g of Novozym 435) in a 10 ml flask. The transesterification reaction was carried out at 50 °C with magnetic stirring at 600 rpm.

Acid catalysed cleavage of the isopropylidene groups was carried out by using 10 ml of reaction mixture with 10 ml of acetic acid (90.0%) at 80 °C, with magnetic stirring at 600 rpm for 3 h. The solid reaction products were dissolved in $CHCl_3$ and then purified by column chromatography (silica gel 230–400 mesh) with a chloroform:methanol mixture as the mobile phase.

2.3. HPLC analysis

HPLC analysis was performed using a C-18 column (Merck) and monitored by an evaporative light scattering detector, Sedex 75 (Sedere, France). Analyses were carried out at 35 °C, at constant flow of 1 ml min^{-1} with an isocratic elution of CH_3CN:MeOH (1:1).

2.4. ^{13}C NMR analysis

NMR measurements were performed using a Bruker Avance 300 MHz (7.05 T), equipped with a multinuclear probe operating at 75.475 MHz for the ^{13}C nucleus. The experiments were carried out at 25 °C using a standard variable-temperature control unity with an accuracy of ±0.5 °C. Appropriate amounts of sample (100 mg) were dissolved in a mixture 2:1 of $CDCl_3$:CD_3OD (2.5 ml) and such solutions were put inside NMR sample tubes with external diameter of 10 mm.

Additional experimental details were previously reported [10].

3. Results and discussion

3.1. Synthesis of 1-O-lauroyl-D-mannitol

The path of the synthesis of 1-*O*-lauroyl-D-mannitol is reported in Scheme 1. The enzymatic step was performed starting from a D-mannitol derivative having two isopropylidene protecting groups, and vinyl laurate as the acyl donor.

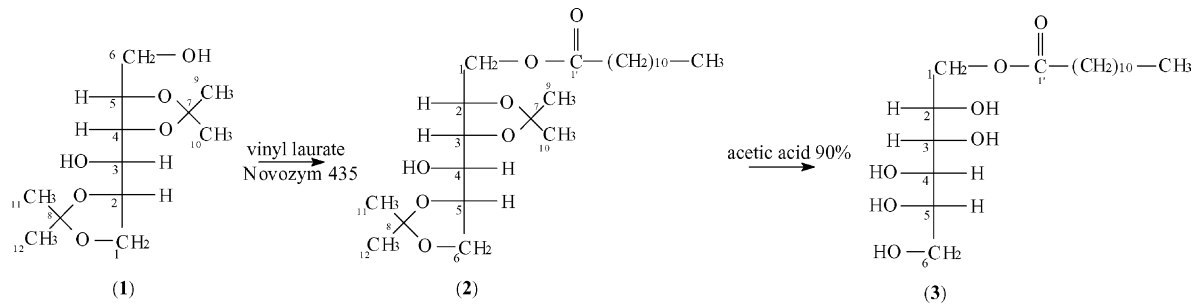
The enzyme used for the acylation step was the immobilised lipase B from *C. antarctica*, that is known with the commercial name Novozym 435. It was found that this enzyme was the most active towards isopropylidene-D-mannitol acylation [10].

In the previous work, since the substrate had only two protected hydroxyls, the 2-methyl-2-butanol was used as the solvent. The substrates showed in this solvent a good solubility, and this allowed a quite high enzymatic activity. However, some drawbacks occurred in that case. Here, such problems have been overcome with the new synthesis conditions. The starting compound 1,2:4,5-di-*O*-isopropylidene-D-mannitol has only two free hydroxyl groups. The low polarity of this compound allows some substantial improvements in the studied synthesis: the use of *n*-hexane and the use of solvent free conditions. Compared to 2-methyl-2-butanol, *n*-hexane has the advantage of an easier removal after the reaction, and in general it is safer for the enzyme. One of the biggest problems with the use of 2-methyl-2-butanol was the water removal from the solvent. Indeed, *C. antarctica* lipase B catalyses the hydrolysis of vinyl laurate even in presence of a low water amount [10]. The use of *n*-hexane or of solvent free conditions reduces this inconvenience significantly.

The product of the enzymatic acylation—the 1-*O*-lauroyl-2,3:5,6-di-*O*-isopropylidene-D-mannitol—gave, after 48 h, a yield of 92% in *n*-hexane and 65% in the solvent free syntheses, respectively. The selective acid hydrolysis of the isopropylidene groups gave the surfactant 1-*O*-lauroyl-D-mannitol with a yield of 80%.

3.2. ^{13}C NMR analysis

The various steps of the synthesis were followed through ^{13}C NMR spectroscopy. The chemical shifts of the carbons



Scheme 1. Synthesis of 1-*O*-lauroyl-D-mannitol.

Table 1
^{13}C NMR ($CDCl_3$:CD_3OD 2:1)

	δ (**1**) (ppm)	δ (**2**) (ppm)	δ (**3**) (ppm)	δ (**4**) (ppm)
C1	63.40	66.73	66.11	–
C2	70.24	69.57	69.08	–
C3	71.15	75.86	69.24	–
C4	75.20	75.41	69.27	–
C5	70.45	74.79	71.19	–
C6	66.74	63.70	63.35	–
C7, C8	108.81	109.18–108.52	–	–
C9, C10	26.02	26.13–26.23	–	–
C11, C12	24.70	24.29–24.50	–	–
C1′	–	173.92	174.62	171.05
C2′[a]	–	–	–	140.69
C3′[a]	–	–	–	97.21

Chemical shifts of C atoms of 1,2:4,5-di-*O*-isopropylidene-D-mannitol (**1**), 1-*O*-lauroyl-2,3:5,6-di-*O*-isopropylidene-D-mannitol (**2**), 1-*O*-lauroyl-D-mannitol (**3**), and vinyl laurate (**4**).

[a] These signals are due to the carbons of the double bond of the vinyl group in (**4**).

of reagents and products are reported in Table 1. The signals attributions were done in agreement to the carbons numeration reported in Scheme 1. The evidence of the occurred acylation is given by the new signal at 173.92 ppm, due to the CO group of the ester bond (C1′). Another evidence is the occurrence of two different signals for the quaternary carbons (C7 and C8) and also the signals of the $–CH_3$ of the isopropylidene groups (C9, C10, C11, and C12) which have become non-equivalents.

The selective hydrolysis of the isopropylidene groups is confirmed by the disappearance of the signals due to the quaternary C of the isopropylidene groups, and from the presence of the signal at 174.62 ppm due to the CO group (C1′) of the ester bond.

3.3. Formation of isomers in the acylation step

Another problem of the previously reported synthesis of 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol was the formation of different isomers [10]. These side products, that had a molar abundance of the 35%, are formed for two reasons. The former is the concurrent non-enzymatic reaction of acyl migration [11,12]; the latter is the acylation of secondary hydroxyls catalysed by the *C. antarctica* lipase B.

In the present case the absence of vicinal hydroxyls does not allow the acyl migration process, thus, we tried to investigate deeper the second cause. To this aim the enzymatic acylation 1,2:4,5-di-*O*-isopropylidene-D-mannitol to obtain the diester was tried. It was performed by using an excess of vinyl laurate. After a reaction time of 48 h only the monoester was obtained. This means that the position 3 is almost not available for the acylation. In this position the hydroxyl is a secondary alcoholic group. Moreover, the steric obstruction due to the two isopropylidene groups must be taken into account.

A further proof of this fact was found performing a similar synthesis through the use of 1,2:5,6-di-*O*-isopropylidene-D-mannitol and vinyl laurate to form 3-*O*-lauroyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol. The two free hydroxyls of this mannitol derivative are both secondary groups. The ^{13}C NMR of the reaction mixture showed that after 48 h, no new product was obtained. To have a detectable yield of the expected 3-*O*-lauroyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol a time of 66 h was necessary.

From the new and the old [10] results regarding mannitol backbone acylation it can be stated that:

1. *C. antarctica* lipase B acylates preferentially primary hydroxyl groups;
2. *C. antarctica* lipase B acylates also secondary hydroxyl groups but to a minor extent;
3. the presence of an isopropylidene group near to a secondary hydroxyl group reduces significantly the capacity of its acylation by *C. antarctica* lipase B;
4. the presence of two isopropylidene groups close to a secondary hydroxyl does not allow its acylation by *C. antarctica* lipase B.

4. Conclusions

In this work the pure 1-*O*-lauroyl-D-mannitol was obtained without the occurrence of unwanted isomers. The main reason is the absence of vicinal free hydroxyls in the starting substrate, and the reduced availability of the other free hydroxyl to the catalyst for steric reasons. The acylation step of the di-*O*-isopropylidene-D-mannitol was performed both in *n*-hexane and in solvent-free conditions, with a higher yield in first case. The *n*-hexane is a suitable organic solvent since it can be easily removed and it is not enzyme-denaturing.

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