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Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification

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ABSTRACT

The direct esterification of palmitic acid with L-(+)-arabinose has been carried out. The use of *Candida antartica* lipase B as the catalyst and the choice of suitable solvent and experimental conditions allowed carrying out the reaction successfully. In particular 10% dimethyl-sulfoxide in *tert*-butanol was found to be the optimal solvent. The product has been fully characterized by means of FTIR, ESI-MS, DSC, mono and bidimensional ^{1}H and ^{13}C NMR. These techniques confirm that only the primary alcoholic group was involved in the esterification reaction.

1. Introduction

Sugar fatty acid esters are amphiphilic compounds widely employed as food-grade, odourless and tasteless, non-ionic surfactants in a wide range of industrial formulations (chemicals, pharmaceuticals, cosmetics, detergents, oral-care products, low-calorific sweeteners, medical supplies) in place of the synthetic ones [1]. The use of synthetic surfactants is, indeed, connected with several human health risks, including allergic reactions, eyes and skin irritations, intestinal disorders, cancer [2,3]. By contrast, sugar fatty acid esters are safe for both humans and the environment thanks to the lower toxicity and the higher biodegradability and biocompatibility. Moreover, they are easily digested as a mixture of sugars and fatty acids in the stomach [4] and some of them showed antimicrobial [5,6], anticancer [7] and insecticidal [8] activity. The surfactant properties of these compounds may be predicted by using the hydrophobic-lipophilic balance (HLB) that was introduced by Griffin [9]. The HLB of non-ionic sugar based surfactants depends on the degree of substitution and the ratio between the hydrophilic head (mono-, oligo- or polysaccharides) and the lipophilic tail (amount and length of the fatty acid chain). Interestingly, in contrast to conventional available food emulsifiers it is possible to produce sugar fatty acid esters that cover a very broad HLB range by modifying the sugar head and/or the length and the unsaturation of the fatty acid tail [10].

Thanks to their typical features, the production of sugar-based surfactants on an industrial scale has been attracting growing attention since 1990. Today, a wide number of them are commercially available as emulsifiers and stabilizers. For example, sucrose esters of lauric, palmitic or stearic acid (marked as E473) have been classified as Generally Recognized As Safe, GRAS [11] and can be used in cosmetic formulations because of their mild dermatological properties or in molecular cuisine to produce airs and foams [12]. Sugar fatty acid esters can be produced from renewable and low-cost raw materials (*viz* carbohydrates, fatty acids or oils) by using both chemical and enzymatic esterification. The chemical route, that is transesterification of the fatty acid methyl ester with the sugar, is the most widespread at an industrial level. However, not only this reaction is poorly selective, but it also involves the use of harmful solvent and production of organic and inorganic wastes [13] difficult to remove from the product. To overcome these limitations, the enzymatic route is an attractive and eco-friendly alternative that requires mild conditions of temperature and pressure. In addition, an important advantage is the higher regioselectivity which leads to a predominant reaction product [1,13].

Among the enzymes, lipases have been widely used to promote the ester bond formation because of their high substrate specificity. Lipases are efficient catalysts for the hydrolysis of esters; however, in the absence of water in neat organic solvents they can catalyse the reverse reaction. To enhance the stability in organic solvents, immobilized lipases have been employed. At the same time, immo-

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bilization allows an easy separation and recover of the enzyme [14].

Several studies had been reported about the enzymatic transesterification of mono- and disaccharide (*viz* D-(+)-glucose, D-(−)-fructose, sucrose) and fatty acid esters [7,15–17].

In this paper we wish to report on the preparation of L-(+)-arabinose-palmitic acid monoesters by using immobilized *Candida antarctica* lipase B (CALB, Novozyme 435) as a biocatalyst, following the previous research of some of us about the enzymatic synthesis of oligofructose fatty acids monoesters [1]. The effect of the reaction medium, temperature and molar ratio of reagents that strongly affect the activity and specificity of lipases has been investigated. The product was purified by flash chromatography and characterized by NMR, FTIR, ESI-MS analysis. The thermal properties were evaluated by DSC analysis.

Among the sugars, we chose L-(+)-arabinose because it is a constitutive component of arabinoxylans and other naturally occurring non-starch polysaccharides, such as pectin and lignocellulose. These biopolymers are easily extracted with water from several biomass residues of the agri-food industry, particularly flaxseed meal, rice bran, wheat bran, barley, corn fiber, and display interesting physiological and functional properties [18]. In this way, we have a natural source for biobased arabinose and the derivatives that can be esterified to give amphiphilic molecules for food and non-food industrial applications. To the best of our knowledge, the direct esterification of L-(+)-arabinose and free palmitic acid has never been investigated up to now. Only a few studies on the synthesis of D-(−)-arabinose esters [19,20] and the direct esterification of other underivatized sugars and free fatty acids have been reported [14].

2. Experimentals

2.1. Materials and methods

Commercial immobilized lipase from *Candida antartica* lipase B (CALB, Novozyme 435) was a generous gift from Novozymes (Bagsvard, Denmark). Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich and/or from VWR International and were used without further purification.

2.2. Synthesis of L-(+)-arabinose-palmitic acid esters

L-(+)-arabinose and palmitic acid (different sugar/acid molar ratios between 1 and 3) were mixed in a 20 ml glass reaction tube by using a magnetic stirrer (450 rpm) changing the solvent (*tert*-butyl alcohol, TBU, and methyl *tert*-butyl ether, MTBE, alone or in binary mixture with dimethyl sulfoxide, DMSO, or 200 mM phosphate buffer pH 7,) and the temperature (40–60 °C). After 30 min, molecular sieve (3 Å, 10% w/$w_{palmiticacid}$) and CALB (8% w/$w_{palmiticacid}$) were added to start the reaction and the mixture was stirred over night at the selected temperature. Control experiments were carried out without the enzyme. Then the mixture was cooled down to room temperature to stop the reaction and centrifuged. The solid was shown by IR to contain only the enzyme, molecular sieves and unreacted l-(+)-arabinose, therefore it was discarded. The solvent was removed under reduced pressure. The crude product was analysed by Thin Layer Chromatography (TLC), attenuated total reflection (ATR) Fourier transform infrared (FTIR) and GC-FID analysis.

For the semi-preparative scale, in a 250 ml round bottom flask L-(+)-arabinose (1.0 g, 6.7 mmol) and palmitic acid (1.7 g, 6.7 mmol) were mixed at 60 °C by using a magnetic stirrer (450 rpm) in TBU (35 ml) as the solvent, alone or in binary mixture with DMSO (7% and 10%). After 30 min, molecular sieves (3 Å, 10%) and CALB (140 mg) were added and the mixture was stirred o.n. at 60 °C. The reaction was controlled by using TLC. After cooling to room temperature, the mixture was centrifuged. The solid phase was discarded and the upper phase was dried under vacuum to give a white and odourless powder. The crude product was purified by flash chromatography and characterized by ATR-FTIR, ESI-MS, NMR and GC-FID analyses.

2.3. Thin layer chromatography

Analytical Thin Layer Chromatography TLC was performed on Silica Gel 60 F254 precoated aluminum sheets (0.2 mm layer; Merck, Darmstadt, Germany). Components were separated by using CH_2Cl_2:MeOH (12:1) and detected by spraying the sheet with a *p*-anisaldehyde/H_2SO_4 solution (ethanol:anisaldehyde:sulphuric acid:acetic acid, 90:5:5:1) followed by heating at 105 °C for 5–10 min for the detection of the sugars. At the same time, another plate was developed with a ceric sulfate/ammonium molybdate or with a $KMnO_4$ solution and heated at about 150 °C for few seconds to detect also palmitic acid. The R_f of monoester was 0.2.

2.4. Purification methods

Purification of products was accomplished by flash chromatography (silica gel 60, 40–63 mm, Merck). The crude product was eluted on a silica gel column with ethyl ether:*n*-hexane:formic acid (1:1:0.02), to remove unreacted palmitic acid and L-(+)-arabinose, followed by elution with ether:*n*-hexane:methanol (4.7:4.7:0.6).

2.5. Gas chromatography analysis

The monoester content in the crude of reaction was evaluated according to the GC methods of Cramer et al. [21] with slight modification. The analysis of arabinose, palmitic acid and reaction mixtures was performed on an Agilent 6890 Gas Chromatography system by using a Alltech Heliflex® AT-5 capillary column (30 m × 0.32 mm ID × 0.25 μm), with split injection and FID detection. Before injection, unsubstituted hydroxyl groups were silylated according to Degn et al. [22]. To 5–10 mg of crude reaction, 110 μL of heptane:pyridine (2:1 v/v) containing octyl-β-D-glucopyranoside (OGP, 5.9 mg/ml, 0.02 mol/ml) and heptadecane (4.66 mg/ml, 0.019 mol/ml) as internal standards was added together with 100 μL BSTFA (1%, v/v TMCS). The mixture was stirred at 70 °C for 30 min and, subsequently, 1 μL of the resulting solution was injected with a 10 μL glass syringe (Hamilton). The injection temperature was 220 °C and the detector temperature was 250 °C. A pressure of 21 psi, a gas flow of 1.7 mL/min (He) and a split flow of 106 mL/min were applied. The temperature profile of the analysis oven was: 1 min at 90 °C, heating to 250 °C at a rate of 30 °C/min and kept constant for 4 min, finally heating to 310 °C at a rate of 15 °C/min and kept constant for 5 min. OGP was used as internal standard to evaluate the monoester concentration and compare the yields of the crude products.

2.6. Structural characterization of 5-O-palmitoyl-L-(+)-arabinosyl ester

Mass Spectroscopy – Mass spectrum of monoester was recorded on a Q-TOF Micro-Waters® Mass Spectrometer interfaced to an Electro Spray Ionization (ESI) by using a positive ion mode. The sample was injected to the mass spectrometer in a MeOH solution.

NMR Spectroscopy – High-resolution ^1H and ^{13}C NMR spectra were acquired at 600.33 and 150.95 MHz, respectively, on a Bruker Advance 600 spectrometer (Bruker, Karlsruhe, Germany) interfaced with a workstation running a Windows operating system and equipped with a TOPSPIN software package. 20 mg of monoester was dissolved in 0.6 ml of DMSO-d_6 and the spectra were recorded