Enzyme and Microbial Technology 112 (2018) 14–21

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/enzmictec

D-Xylose and L-arabinose laurate esters: Enzymatic synthesis, characterization and physico-chemical properties

Thomas Méline[a], Murielle Muzard[b], Magali Deleu[c], Harivony Rakotoarivonina[a], Richard Plantier-Royon[b], Caroline Rémond[a,*]

[a] FARE laboratory, Chaire AFERE, Université de Reims-Champagne-Ardenne, INRA, 51686 Reims Cedex, France
[b] Institut de Chimie Moléculaire de Reims, CNRS UMR 7312, Université de Reims Champagne-Ardenne, 51687 Reims Cedex, France
[c] Université de Liège, Gembloux Agro-Bio Tech, Laboratoire de Biophysique Moléculaire aux Interfaces, 2 Passage des Déportés, B-5030 Gembloux, Belgium

ARTICLE INFO

Keywords:
D-Xylose laurate esters
L-Arabinose laurate esters
Lipase
Surfactants
Biorefinery

ABSTRACT

Efficient enzymatic synthesis of D-xylose and L-arabinose lauryl mono- and diesters has been achieved by transesterification reactions catalysed by immobilized *Candida antarctica* lipase B as biocatalyst, in organic medium in the presence of D-xylose or L-arabinose and vinyllaurate at 50 °C. In case of L-arabinose, one monoester and one diester were obtained in a 57% overall yield. A more complex mixture was produced for D-xylose as two monoesters and two diesters were synthesized in a 74.9% global yield. The structures of all these pentose laurate esters was solved. Results demonstrated that the esterification first occurred regioselectively onto the primary hydroxyl groups. Pentose laurate esters exhibited interesting features such as low critical aggregation concentrations values all inferior to 25 µM. Our study demonstrates that the enzymatic production of L-arabinose and D-xylose-based esters represents an interesting approach for the production of green surfactants from lignocellulosic biomass-derived pentoses.

1. Introduction

For several years, there has been an increasing interest for bio-based surfactants derived from annually renewable resources [1]. Among bio-based surfactants, alkyl glycosides and sugar fatty esters are non-ionic surface active compounds which present numerous advantages such as no toxicity for humans and for the environment, biodegradability, absence of odor and color [2–4]. The main fields of application of these non-ionic surfactants are related to personal care, cosmetics and pharmaceutical applications as well as food emulsification in case of sugar fatty esters [4–6]. The main alkyl glycosides and sugar fatty esters industrially produced or described in literature were generally obtained from hexoses, especially D-glucose, or hexose-based oligosaccharides such as sucrose, maltose and maltodextrins [1,7].

D-Xylose and L-arabinose are both pentoses abundantly present in lignocellulosic plant cell walls and are main components of xylans [8]. The production of new added-value molecules from pentoses represents a challenge for the valorisation of lignocelluloses in the context of development of biorefineries [1,7]. Xylose is currently reduced into xylitol, converted into furfural or fermented into ethanol [7]. Although few pentose-based surfactants were previously developed, some recent studies described the chemical synthesis of alkyl pentosides and pentose-based fatty esters [9–13]. These syntheses often require high energy and various catalysts that might not be compatible with green chemistry processes.

Classical chemical routes to the formation of fatty acid esters generally require esterification or transesterification reactions and the use of polar solvents (DMF, DMSO), a basic catalyst and high reaction temperatures (80–120 °C) leading to complex mixtures of monoesters, di- and higher esters as different regioisomers and unreacted sugar [14]. The use of fatty esters or acyl chlorides in the presence of an organic solvent and pyridine can improve the reaction yields but again with various degree of substitution [15]. Selective protection of the hydroxyl groups of the carbohydrate (acetyl, benzyl, isopropylidene) can orient the position of esterification reaction and also sometimes control the pyranose/furanose structure of the products [16].

The use of enzymes represents an interesting alternative for the preparation of surfactants from biomass [7,17]. For example, we previously described the enzymatic synthesis and the surfactant properties of alkyl xylosides and alkyl oligoxylosides from xylans and pretreated wheat bran using a transglycosylation approach with a xylosidase (EC 3.2.1.37) or xylanases (EC 3.2.1.8) [18,19]. Among well-known biocatalysts, lipases (EC 3.1.1.3) were widely used to catalyze the ester bond formation of sugar fatty esters [20,21]. Enzymatic synthesis of

* Corresponding author.
E-mail address: caroline.remond@univ-reims.fr (C. Rémond).

https://doi.org/10.1016/j.enzmictec.2018.01.008
Received 25 July 2017; Received in revised form 15 January 2018; Accepted 16 January 2018

sugar fatty esters is usually achieved by esterification reaction from a carbohydrate and a fatty acid or by a transesterification reaction from a carbohydrate and a fatty acid ester. Enzymatic synthesis of sugar fatty esters with lipases represents a green alternative compared to the conventional chemical approach [20]. Moreover, lipases can be used in immobilized form, then allowing a recyclability and reusability of the biocatalyst thus reinforcing the green alternative. Lipases display high regioselectivity compared to chemical acylation decreasing the complexity of mixtures of regioisomers produced [22–24]. During esterification, the amount of water present in reaction mixtures and formed during reaction must be highly controlled as water induces hydrolysis of esters products [25]. Hence, most of the lipase-based syntheses were performed in organic media as water quantity can be controlled by the use of salts or molecular sieves as desiccating agents [26,27]. Transesterification catalysed by lipases in presence of fatty acid esters, especially vinyl esters, represents an interesting strategy to overcome the water production during reaction and to induce better reactions yields. The main disadvantage in this latter case is the production of acetaldehyde as a by-product but the most widely employed lipases seem to be quite stable in the presence of acetaldehyde [28].

Although enzymatic synthesis of hexose-derived fatty esters was extensively described in the last twenty years, studies dealing with pentose-based fatty esters were less reported in literature [22,29–34]. Moreover, in most of the cases, structural data related to these molecules are not described.

In the present paper, we report the successful enzymatic synthesis of laurate pentose esters from D-xylose and L-arabinose catalysed by the lipase B from *Candida antarctica* (Novozym 435). Our strategy was based on transesterification reactions with vinyllaurate and allowed producing different mono- and diesters from D-xylose and L-arabinose. The structural features of these sugar esters were analysed by NMR and mass spectrometry and their surface-active properties were evaluated.

2. Experimental section

2.1. Materials

2-Methylbutan-2-ol (2M2B, 99%), molecular sieves (4 Å, beads, 8–12 mesh), hexane (> 95%), tetrahydrofuran (THF), Novozym 435 (immobilized lipase acrylic resin from *Candida antarctica*, Lot #SLBP0766V), vinyllaurate (> 99%), orcinol, chloroform (99%) and D-xylose (> 99%) were purchased from Sigma-Aldrich Corp. (St. Louis, USA). L-arabinose, acetic acid (AcOH, > 99%), ethylacetate (EA, > 99.8%), methanol (> 99.9%), petroleum ether (PE, > 99.9%) and *n*-butanol (BuOH, > 99%) were purchased from Roth (Karlsruhe, Germany). Sulfuric acid (H_2SO_4, 95%) was purchased from VWR (Radnor, USA). Acetonitrile (> 99.9%) and propan-2-ol (> 99%) were purchased from Carlo Erba Reagents (Dasit Group S.p.A, Cornaredo, Italy).

2.2. Methods

2.2.1. Enzymatic synthesis of D-xylose and L-arabinose laurate esters

Enzymatic syntheses were carried out in screwed glass bottles with magnetic stirrer, 400 × rpm, in an oil bath at 50 °C. Reactants, D-xylose or L-arabinose (50 mM) and vinyllaurate (150 mM) were incubated overnight with 2M2B and molecular sieves (10% w/v). Reaction started when Novozym 435 was added to the medium at 1% w/v. Reactions were stopped by incubating samples at 100 °C for 10 min and reaction mixtures were centrifuged at 500 × *g* for 5 min in order to pellet molecular sieves and enzymes. Supernatant was used to monitor sugar fatty esters production by thin layer chromatography and HPLC. Molecular sieves and enzymes were washed twice with ultrapure water in order to collect residual pentoses (HPLC quantification).

Kinetic studies were achieved at 50 °C with 20 mL reaction mix and sampling occurred at 1, 2, 4, 8, 24 and 48 h of incubation, 1 mL of reaction mixture was taken each time. These reactions were performed in triplicates.

Higher volume syntheses (100 mL) occurred in similar conditions in order to produce sufficient quantities of products for purification and characterization. Reactions were stopped after 4 h of incubation at 50 °C.

Recycling of the lipase was assessed in presence of D-xylose or L-arabinose (50 mM), vinyllaurate (150 mM), molecular sieves (10% w/v) and Novozym 435 (1% w/v). Reaction was conducted during 4 h at 50 °C under magnetic stirring (400 × rpm). After 4 h, reaction was centrifugated (45 × *g*) and pellets containing the lipase and the molecular sieves were further incubated with fresh D-xylose or L-arabinose and vinyllaurate. A total of 6 cycles of 4 h were performed.

2.2.2. Purification, characterization and quantification of the transesterification products

The production of pentose fatty esters was investigated by TLC, using pre-coated TLC-sheets ALUGRAM® Xtra SIL G/UV_{254} (Macherey-Nagel Gmbh & Co., Düren, Germany) and BuOH: AcOH: water (2/1/1) as the mobile phase. Products were revealed using 0.2% (w/v) orcinol in H_2SO_4 (20% v/v in water) and heating at 250 °C.

After removal of enzymes and molecular sieves, 2M2B was evaporated using a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) and crude products with remaining reactants were collected. Two hexane washings were then performed to eliminate the remaining vinyllaurate and a white paste was collected for both D-xylose and L-arabinose-based esters. Vinyllaurate removal was qualitatively assessed by HPLC. Finally, the residual pentose was precipitated in THF (100 mL) leading to soluble fractions containing mono- and diesters collected for further purification. Residual pentose was finally solubilized in water (50 mL) and quantified by HPLC.

The purification of D-xylose or L-arabinose laurate esters was performed by silica gel chromatography (9385 Merck Kieselgel 60, 230–400 mesh, 40–63 µm). Diesters were eluted using PE/EA (7/3, v/v) and monoesters were eluted using pure EA. All the products were obtained as a white crystalline powder.

NMR spectra were recorded on Bruker spectrometers (500 or 600 MHz for ^{1}H, 125 or 150 MHz for ^{13}C). Chemical shifts are expressed in parts per million (ppm) using tetramethylsilane as an internal standard. NMR spectra are presented as supplementary data. Mass spectra (ESI–MS) and high resolution mass spectra (ESI-HRMS) were performed on Q-TOF Micro micromass positive ESI (CV = 30 V).

2.2.2.1. 5-O-lauryl-L-arabinofuranose 1. White solid, mp 130 °C. ^{1}H NMR (500 MHz, $CDCl_3$): α/β = undetermined ratio, δ 5.30–5.35 (m, H-1α, H-1β), 4.20-4.35 (m, 2H-5α, 2H-5β, H-4α or β), 4.05-4.15 (m, H-2α, H-2β, H-4α or β), 2.37 (t, *J* = 7 Hz, 2H), 1.60-1.66 (m, 2H), 1.25-1.35 (m, 16H), 0.90 (t, *J* = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.8 and 174.4 (C=O α and β), 102.5 (C-1α), 95.9 (C-1β), 82.5 and 81.2 (C-4 α and β), 79.5 (C-3β), 77.6 (C-2α), 77.4 (C-3α), 76.1 (C-2β), 65.9 and 64.3 (C-5 α and β), 2D experiment (HMBC): correlations between C=O and H-5; ESI–MS: 355.2 $(M + Na)^+$; ESI-HRMS: *m/z* calcd for $C_{17}H_{32}O_6Na$ 355.2097, found 355.2089.

2.2.2.2. 3,5-Di-O-lauryl-L-arabinofuranose 2. White solid, mp 69 °C, ^{1}H NMR (500 MHz, $CDCl_3$): α/β = 1/1.56, δ 5.35–5.40 (m, H-1α, H-1β), 4.94 (t, *J* = 5 Hz, H-3β), 4.70 (dd, *J* = 5 Hz, *J* = 2 Hz, H-3α), 4.29-4.44 (m, H-5α, H-5β, H-4α), 4.23 (dd, *J* = 12 Hz, *J* = 5 Hz, H-5α), 4.13-4.19 (m, H-2α, H-2β), 4.07-4.11 (m, H-4β), 2.38 (m, 2H), 1.59–1.65 (m, 2H), 1.20-1.38 (m, 16H), 0.90 (t, *J* = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.6, 174.4, 173.9 and 173.5 (2C=O α and β), 102.9 (C-1α), 96.9 (C-1β), 81.2 (C-2), 81.0 (C-3 α), 79.9 and 79.8 (C-4α and C-3β), 79.1 (C-4β), 76.3 (C-2α and β), 65.0 and 63.4 (C-5 α and β), 2D experiments (HMBC): correlations between C=O 174.6 and 174.4 and H-3, C=O 173.9 and 173.5 and H-5; ESI–MS: 537.5 $(M + Na)^+$; ESI-HRMS: *m/z* calcd for $C_{29}H_{54}O_7Na$ 537.3767, found 537.3773.