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ENZYME CATALYZED REGIOSELECTIVE SYNTHESIS OF SUCROSE FATTY ACID ESTER SURFACTANTS

Tulay Polat, Hélène G. Bazin and Robert J. Linhardt*

Division of Medicinal and Natural Products Chemistry and
Department of Chemical and Biochemical Engineering, PHAR-S328
University of Iowa, Iowa City, IA 52240, USA

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ABSTRACT

A commercial subtilisin preparation was used in pyridine to catalyze the regioselective conversion of sucrose and fatty acid vinyl esters into the 1'-*O*-acyl sucrose derivatives. The 1'-*O*-lauryl sucrose, 1'-*O*-myristyl sucrose and 1'-*O*-stearyl sucrose were obtained as the major products of these reactions. The 1',6-di-*O*-acyl sucrose derivatives were also obtained as minor products. The critical micellar concentration (CMC) of each of these sucrose monoesters was determined.

INTRODUCTION

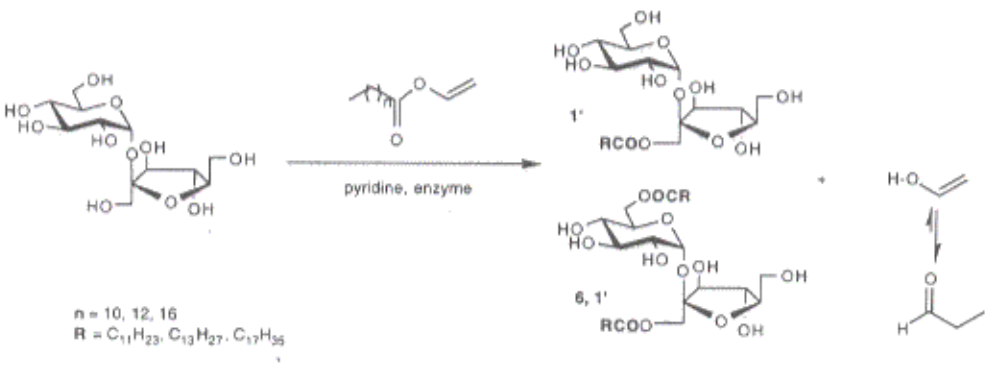
A number of carbohydrates, including sucrose, have been used for the chemical synthesis of surfactants.[1-6] Regioselective acylation of sucrose has been achieved by using organotin compounds,[7] and recently, our research group described the regioselective synthesis of 6-*O*-fatty ester sucrose derivatives using a dibutylstannylene intermediate.[8] An alternative approach for the regioselective synthesis of carbohydrate esters has involved the use of enzymes in organic solvents. The monoacylation of various monosaccharides,

using the trichloroethyl ester of acetic, butyric, caprylic and lauric acids, is catalyzed by porcine pancreatic lipase in organic solvents such as anhydrous pyridine.[9] These esterification reactions result in the acylation of the primary hydroxyl group of the monosaccharide. Disaccharides, such as sucrose, had very low reactivity towards this enzyme, probably the result of steric hindrance.[9] The transesterification of glycosides of common disaccharides such as lactose or maltose, using vinyl esters and catalyzed by lipases also leads to the acylation of the primary hydroxyl group of the disaccharide non-reducing end.[10] Lipases can also catalyze transesterification of the secondary hydroxyl groups of D-glucose, in which the C-6 hydroxyl group has been blocked by prior enzymatic acylation or chemical alkylation.[11] The transesterification of these secondary hydroxyl groups is also highly regioselective with positional specificity and is primarily dependent on the particular lipase (i.e., porcine pancreatic, bacterial, yeast, etc.) selected as the catalyst. Proteases also catalyze the transesterification of sugars.[12] The 1'-*O*-butyryl ester of sucrose was prepared from sucrose and trichloroethyl butyrate with the protease, subtilisin, in anhydrous dimethylformamide. This approach has been examined using subtilisin BPN and subtilisin Carlsburg in a variety of organic solvents, to prepare sucrose monoesters using various vinyl esters.[12] While selectivity for the primary hydroxyl groups was observed, both the 1'- and 6-*O*-acyl derivatives of sucrose were obtained. In addition, the esters of long-chain fatty acids ($C \geq 10$) were found to be poorly reactive with sucrose in anhydrous pyridine. It is these long chain fatty acid monoesters of sucrose that are particularly promising as potential surface active agents. This paper reports the regioselective preparation of sugar monoesters and diesters of lauric (C_{12}), myristic (C_{14}) and stearic (C_{18}) acids and the evaluation of their properties as surface active agents.

RESULTS AND DISCUSSION

Reaction, isolation, characterization: Studies by others[12,13] on the regioselective enzymatic transesterification of sucrose suggested the starting conditions for the preparation of sucrose fatty acid monoesters. The catalyst selected was a commercially available, stabilized, insoluble polymer of subtilisin, called ChiroCLEC-BL. The 6-*O*-acyl sucrose esters of lauric (C_{12}), myristic (C_{14}) and stearic (C_{18}) acid had previously been regioselectively prepared by our laboratory using chemical methods.[8] These acids were

also used in this study. The trichloroethyl ester of lauric acid was used in preliminary studies to examine solvents that both solubilized sucrose and fatty acid ester and supported regioselective enzymatic transesterification. An initial screening of dimethyl sulfoxide, dimethylformamide and pyridine (all capable of dissolving both reactants) relied on small scale reactions (6 mg catalyst, 0.55 mmol ester, 0.58 mmol sucrose, 1.5 mL solvent) and was monitored by thin-layer chromatography (TLC) using conditions previously established.[8] Anhydrous pyridine gave a modest yield (~ 15%) of product while dimethylformamide afforded only trace amounts of this product and dimethyl sulfoxide gave no observable product. All further studies used dry pyridine as the solvent. Next, lauryl vinyl ester was used as reactant. Previous studies[13] had suggested that vinyl esters are able to drive the transesterification reaction to completion through the irreversible tautomerization of vinyl alcohol to unreactive aldehyde. The use of the vinyl ester of lauric acid (12 mg catalyst, 1.29 mmol ester, 1.12 mmol sucrose, 3 mL pyridine) markedly improved the reaction yield to ~80-90% by TLC (Scheme). Scale-up of this reaction (see general procedure) afforded sufficient product for characterization. It should be noted that the reaction yield was ~80-90%. The loss on flash chromatography, needed to obtain pure product, decreased the yield to 60%. The surface active properties of the sample did not allow aqueous extraction of the product.



In addition to the major product, two minor products were also observed. The elution positions of these minor products suggested they were another monoacyl ester of sucrose and a diacyl ester of sucrose. The identity of the minor monoacylester (~ 2%) was not the expected 6-*O*-acyl sucrose derivative[13] by its failure to co-migrate on TLC with chemically synthesized product.[8] The diacyl sucrose product was isolated and its structure

Table 1. ^{1}H NMR Assignments for the carbohydrate moiety of sucrose acylates.

	1'-*O*-Lauryl	1'-*O*-Myristyl	1'-*O*-Stearyl	1'-6-di-*O*-Lauryl	1'-6-di-*O*-Myristyl	1'-6 di-*O*-Stearyl
H-1	5.19(d) $J_{1,2}$=3.6	5.18(d) $J_{1,2}$=3.5	5.19(d) $J_{1,2}$=3.8	5.20(d) $J_{1,2}$=3.5	5.19(d) $J_{1,2}$=3.8	5.19(d) $J_{1,2}$=3.8
H-2	3.18(dd) $J_{2,3}$=10	3.18(dd) $J_{2,3}$=9.5	3.18(dd) $J_{2,3}$=9.5	3.22(dd) $J_{2,3}$=9.5	3.19(dd) $J_{2,3}$=9.5	3.20(dd) $J_{2,3}$=9.5
H-3	3.46(t) $J_{3,4}$=9.5	3.47(t) $J_{3,4}$=9.0	3.45(t) $J_{3,4}$=9.0	3.47(t) $J_{3,4}$=9.5	3.47(t) $J_{3,4}$=9.5	3.47(t) $J_{3,4}$=9.3
H-4	3.13(dd) $J_{4,5}$=9.0	3.13(dd) $J_{4,5}$=9.5	3.14(dd) $J_{4,5}$=9.5	3.07(t) $J_{4,5}$=9.5	3.07(t) $J_{4,5}$=9.5	3.07(t) $J_{4,5}$=9.5
H-5	3.66(mm)	3.67(mm)	3.67(mm)	3.92(mm)	3.90(mm)	3.92(mm)
$H\text{-}6_{a,b}$	3.52(dd) $J_{a,b}$=12	3.51(dd) $J_{a,b}$=12	3.51(dd) $J_{a,b}$=12	- -	- -	- -
$H\text{-}6_{a}$	-	-	-	4.26(d) $J_{a,b}$=10	4.25(d) $J_{a,b}$=10	4.25(d) $J_{a,b}$=10
$H\text{-}6_{b}$	-	-	-	4.14(dd)	4.01(dd) $J_{a,b}$=12	4.03(dd) $J_{a,b}$=12
$H\text{-}1'_{a}$	4.18(d) $J_{a,b}$=12	4.17(d) $J_{a,b}$=12	4.18(d) $J_{a,b}$=12	3.98(d) $J_{a,b}$=12	3.96(d) $J_{a,b}$=12	3.98(d) $J_{a,b}$=12
$H\text{-}1'_{b}$	3.98(d)	3.98(d)	3.98(d)	4.17(d)	4.15(d)	4.15(d)
H-3'	3.82(d) $J_{3',4'}$=8.5	3.83(d) $J_{3',4'}$=8.5	3.84(d) $J_{3',4'}$=8.5	3.85(d) $J_{3',4'}$=8.5	3.84(d) $J_{3',4'}$=8.5	3.83(d) $J_{3',4'}$=8.5
H-4'	3.79(t) $J_{4',5'}$=7.0	3.80(t) $J_{4',5'}$=7.0	3.80(t) $J_{4',5'}$=7.0	3.77(t) $J_{4',5'}$=7.0	3.77(t) $J_{4',5'}$=7.0	3.77(t) $J_{4',5'}$=7.0
H-5'	3.59(m)	3.59(m)	3.59(m)	3.58(m)	3.59(m)	3.59(m)
H-6'	3.57(m)	3.57(m)	3.57(m)	3.57(m)	3.57(m)	3.57(m)

was characterized together with the major monoacyl derivative. The structures of both products were determined by high field 500 MHz ^{1}H NMR spectroscopy (see Table 1) to be the 1'-*O*-acyl sucrose and 1',6-di-*O*-acyl sucrose derivatives, respectively. The myristyl and stearyl esters of sucrose were similarly prepared and characterized (Table 1).

Table 2. Surface-activity characteristics of enzymatically synthesized esters compared with chemically synthesized esters.[8]

Compound	CMC [mol/L]
1'-*O*-Laurylsucrose	1.5×10^{-4}
1'-*O*-Myristylsucrose	9.1×10^{-5}
1'-*O*-Stearylsucrose	nd[a]
1',6-di-*O*-Laurylsucrose	nd
1',6-di-*O*-Myristylsucrose	nd
1',6-di-*O*-Stearylsucrose	nd
6-*O*-Laurylsucrose[8]	4.0×10^{-4}
6-*O*-Myristylsucrose[8]	1.3×10^{-4}
6-*O*-Stearylsucrose[8]	nd

a. value not determined because of water insolubility of sample.

Surface activity of esters synthesized. Sucrose esters of fatty acids, having 12 or more carbon atoms, are expected to display surface active properties. At a specific concentration called the critical micellar concentration (CMC) these molecules aggregate to form micellar particles. This value is of practical importance since it is the concentration of surfactant required to solubilize hydrophobic molecules in water. Our laboratory recently demonstrated[8] that a colorimetric method for CMC determination[14] was useful for the accurate analysis of sucrose based surfactants. The CMC values were obtained for the 1'-*O*-acyl sucrose esters (Table 2) but not for the 1'-*O*-stearyl and 1', 6-di-*O*-acyl sucrose esters because of their insolubility in water.

EXPERIMENTAL

General Methods: Subtilisin (ChiroCLEC-BL) was purchased from Altus Biologics Inc. (Cambridge, MA). Vinyl esters were purchased from TCI America (Portland, OR). All reactions were monitored by TLC on aluminum sheets, silica gel 60 F_{254} and detected by dipping the plates into staining solution (1.0 g ceric ammonium sulfate and 24.1 g ammonium molybdate in 31 mL sulfuric acid, 470 mL water) followed by heating. The elution system was 50:10:1, chloroform-methanol-water (developed two times). Flash chromatography was performed on silica gel 60 (230-400 mesh Aldrich) using solvent system 96:4 ethyl acetate-methanol. The fractions containing the 1'-*O*-acyl esters also contained a small amount of contaminant migrating with a slightly lower Rf on

TLC. Following evaporation of solvent, the residue was washed with ethyl acetate to remove this minor contaminant and to afford pure 1'-*O*-acyl ester for analysis. Optical rotations were measured on a Perkin Elmer 141 polarimeter at 22°C. Melting points were determined using an electrothermal melting point apparatus. ^{1}H NMR spectra were recorded at 25 °C on a Varian Unity 500 MHz spectrometer. All NMR samples were lyophilized and the remaining trace of water in the solid were exchanged in ^2H_2O (33.3% atom ^{2}H, Sigma Chemical Co., St. Louis, MO) by repeated lyophilization. After three exchanges, the solid was dissolved in 500 µL of DMSO (99.9% atom %D, $(CD_3)_2$ SO, Sigma Chemical Co., St. Louis, MO). The colorimetric CMC determination[14] used uniformly pre-coated plastic balls that were purchased from Pro Chem, Inc. (Rockford, IL). The absorption of the dye was measured at 612 nm on Shimadzu UV-60.

General Procedure. A mixture of 400 mg (1.170 mmol) sucrose, 1.23 mmol fatty acid vinyl ester, 3 mL pyridine and 12 mg subtilisin (Chiro CLEC-BL) was shaken at 250 rpm (37 °C - 40 °C). After 3 days, the enzyme was removed by filtration, solvent was evaporated under vacuum and the last traces of pyridine were removed by co-evaporation three times with 10 mL toluene. The remaining residue was subjected to flash chromatography with a mixture of ethyl acetate-methanol (96:4).

1'-*O*-Lauryl-β-D-fructofuranosyl α-D-glucopyranoside. Yield, 80-90% (TLC), 60% (isolated); Rf 0.32; $[\alpha]_D$ +45.1 (*c* 1, MeOH); mp 193-195 °C; ^{1}H NMR, Table 2; HRMS: Calcd for $C_{24}H_{44}O_{12}$ [m+Na$^+$]$^+$ 547.2731; Found 547.2714.

1'-*O*-myristyl-β-D-fructofuranosyl α-D-glucopyranoside. Yield, 80-90% (TLC), 60% (isolated); Rf 0.35; $[\alpha]_D$ +44.0 (*c* 1, MeOH); mp 203-206 °C; ^{1}H NMR, Table 2; HRMS: Calcd for $C_{26}H_{48}O_{12}$ [m+Na$^+$]$^+$ 575.3068; Found 575.3070.

1'-*O*-stearyl-β-D-fructofuranosyl α-D-glucopyranoside. Yield, 80-90% (TLC), 60% (isolated); Rf 0.38; $[\alpha]_D$ +42.0 (*c* 1, MeOH); mp 208-210 °C; ^{1}H NMR, Table 2; HRMS: Calcd for $C_{30}H_{56}O_{12}$ [m+Na$^+$]$^+$ Calcd 631.3694; Found 631.3688.

1'-*O*-lauryl-β-D-fructofuranosyl 6-*O*-lauryl-α-D-glucopyranoside. Yield, 10% (TLC), 5% (isolated); Rf 0.59; $[\alpha]_D$ +40.1 (*c* 1, MeOH); mp 135-137 °C; ^{1}H NMR, Table 2; HRMS: Calcd for $C_{36}H_{67}D_{13}$ [m+Na$^+$]$^+$ 729.4401: Found 729.4406.

1'-*O*-myristyl-β-D-fructofuranosyl 6-*O*-myristyl-α-D-glucopyranoside. Yield, 10% (TLC), 4.5% (isolated); Rf 0.65; $[\alpha]_D$ +40.0 (*c* 1, MeOH); mp 138-140 °C; ^{1}H NMR, Table 2; HRMS: Calcd for $C_{40}H_{75}O_{13}$ [m+Na$^+$]$^+$ 785.5027; Found 785.5029.

1'-*O*-stearyl-β-D-fructofuranosyl 6-*O*-stearyl-α-D-glucopyranoside. Yield, 10% (TLC), 5% (isolated); Rf 0.70; $[\alpha]_D$ +43.0 (*c* 1, MeOH); 140-142 °C; ^{1}H NMR, Table 2; HRMS: Calcd for $C_{48}H_{91}O_{13}$ $[m+Na^+]^+$ 897.6279; Found 897.6286

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