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Analysis and purification of *O*-decanoyl sucrose regio-isomers by reversed phase high pressure liquid chromatography with evaporative light scattering detection

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ABSTRACT

Purification of seven regio-isomers of *O*-decanoyl sucrose, 2-*O*-, 3-*O*-, 4-*O*-, 6-*O*-, 3′-*O*-, 4′-*O*- and 6′-*O*-decanoyl sucrose, were performed by LC followed by preparative RP-HPLC with ELSD. Using an optimized gradient of acetonitrile in water 2-*O*-, 3-*O*-, 6-*O*- and 3′-*O*-decanoyl sucrose were purified in yields (w/w) of 52.5%, 34.7%, 45.0% and 36.9%, respectively. In the purified preparations of the 2-*O*- and 3′-*O*-decanoyl sucrose respectively, acyl migration was observed as a result of the drying process. Lyophilization resulted in the highest purities (w/w) of 96% and 100% for the 2-*O*- and the 3′-*O*-decanoyl sucrose, respectively.

1. Introduction

Sugar fatty acid esters are non-ionic surfactants with application particularly in the food, cosmetic and pharmaceutical industries. These molecules can be used as emulsifiers by forming water in oil (sucrose polyester) or oil in water (sucrose monoester) microemulsions [1]; moreover, they are essential ingredients of natural aromas in a great variety of food formulations [2]. The most important types of these compounds are the monoesters as their water solubility is superior to the corresponding oligoesters. Depending on the carbohydrate moiety and the fatty acid chain length they are available in a wide range of hydrophilic–lipophilic balance ratios with different emulsifying and dispersing properties. Hence, the physical and chemical properties depend both on the position and degree of esterification (DE) [3]. The critical micelle concentration (CMC) of different sucrose regio-isomers has been reported to be significantly different. Thus the CMC of 1′-*O*-tetradecanoyl-sucrose and 6-*O*-tetradecanoyl sucrose was 9.1×10^{-5} and 1.3×10^{-4} M, respectively [4], while the CMC of 6-*O*-dodecanoyl-sucrose was 2.6 times greater than 1′-*O*-dodecanoyl-sucrose [5,6].

Fatty acid substitution of sucrose potentially involves eight available positions of reactive hydroxyl groups including three primary hydroxyl groups at the C-6 and C-1′ and C-6′ position and five secondary hydroxyl groups at the C-2, C-3 and C-4 position and C-3′ and C-4′ position (Fig. 1). The reactivity of the hydroxyl groups differs and is affected by the reaction conditions including the solvents [7] and the catalyst [8]. Lichtenthaler [9] reported that three primary hydroxyl groups are preferentially acylated and displaced by halogens in the order C-6 ≈ C-6′ ≫ C-1′ and when sucrose is dissolved in DMF or DMSO, the secondary hydroxyl group at the C-2 position is preferentially acylated due to the electrophilic properties induced by these conditions [10]. With the available hydroxyl groups on the sucrose molecule and the occurrence of intra molecular acyl migration, a maximum of 255 possible isomers, from mono to octa esters can theoretically be formed by acylation of sucrose with a single fatty acid [11]. The common analytical methods of sucrose fatty acid esters including TLC [12], GC [13], and HPLC [14–18] have been developed to separate and identify the sucrose fatty acid esters according to their DE. The purification of each positional isomer from a complex reaction mixture prepared by chemical or bio-catalysed synthesis has so far been inherently challenging as good separation of the different positional isomers has been difficult to obtain. Thus the methods reported so far were insufficient for preparing pure defined structures of sucrose fatty acid esters in larger scale, which is a prerequisite for further studies of their physico-chemical properties. Therefore efficient purification methods must progressively be developed. Large-scale purification of sucrose esters by liquid chromatography was performed on Sephadex LH 20 gel filtration, with 50 mg loading size, using DMF as eluent [19]. However, this method required 12 h for a single run

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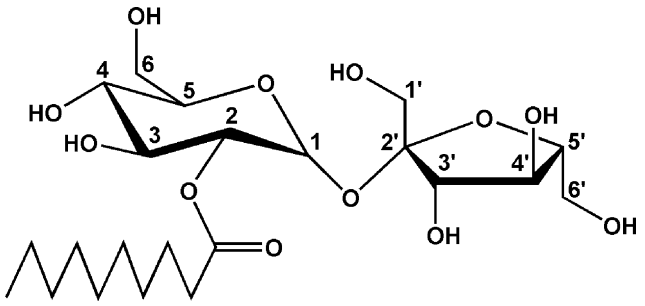

Fig. 1. Chemical structure of 2-*O*-decanoyl sucrose.

and could not separate the regio-isomeric mixture. The separation of sucrose monostearate regio-isomers by a combination of HPLC and TLC was reported to improve the resolution of the individual regio-isomers [11].

In this work the regio-isomeric distribution of *O*-decanoyl sucrose monoesters was investigated by RP-HPLC and ^{13}C NMR, and a purification procedure was developed to prepare seven different positional isomers of *O*-decanoyl sucrose in milligram scale. Furthermore acyl migration as affected by the post-chromatographic drying process of the purified monoesters was investigated.

2. Experimental

2.1. Reagents and chemicals

All solvents were of analytical or HPLC grade. Vinyl decanoate was kindly donated by Japan Vam and Poval Co. (Osaka, Japan). Molecular sieves (3 Å, 8–12 mesh) and n-dodecyl-β-D-maltoside were purchased from Merck. Sucrose and other chemicals were all analytical grade supplied by Sigma–Aldrich. Anhydrous DMF and DMSO were dried over 3 Å, (8–12 mesh) molecular sieves before use.

2.2. Synthesis and purification of sucrose monodecanoate isomers

Sucrose decanoate esters were synthesised by an enzyme catalysed esterification in DMF/DMSO mixture (1/1, v/v) using alkaline protease AL 89 as biocatalyst in a total reaction volume of 10 ml as described by Ritthitham et al. [20]. After terminating the reaction, the un-reacted vinyl decanoate was removed by extraction three times with 2 volumes of hexane to 1 volume of reaction mixture. 1 ml of the crude residue was then loaded onto a glass column (300 mm × 60 mm) packed with Accubond^{II}SPE ODS (C18) (Agilent, UK) equilibrated with Milli Q water. Sucrose, sucrose monoesters, sucrose oligoesters were eluted by a step-wise gradient of methanol in water at 50%, 80% and 100% (v/v) methanol, respectively at ambient temperature with a gravitational flow. The monoester fraction was dried by evaporation under atmospheric pressure at room temperature prior to further purification by preparative RP-HPLC as described below.

2.3. Preparative HPLC

Sucrose monoester isomers were separated on a Hewlet Packard HP 1100 chromatography system with evaporative light scattering detector (Alltech model 500 ELSD) with the drift tube temperature maintained at 50 °C and neubrilizer gas flow rate of 3.0 l/min. A Lichrosorb RP18 stainless steel column (250 mm × 10 mm, 7 μm particle size, Merck Germany) was used for separation of the sucrose monoester isomers. The column was maintained at 45 °C and a gradient of acetonitrile in water was applied (see Table 1) at a flow rate of 2 ml/min. The dried monoester fraction (148.7 mg) was resolubilized in methanol (0.5 ml) and centrifuged at 14,000 × *g* for 2 min. 30 μl supernatant aliquots were injected onto the column with 25 min single run. Based on the retention times obtained (see Fig. 4), fractions were collected from a tube bypassing the detector. The preparative HPLC procedure was run repeatedly and identical monoester regio-isomer fractions were pooled and the solvent was then removed either by rotary vacuum concentrator (ambient temperature, <1 mbar) (Heto, Alleroed, Denmark) or evaporation at atmospheric pressure and 60 °C. The concentration of the regio-isomer fractions was determined by analytical HPLC by the calibration standard curve of n-dodecyl-β-D-maltoside and further analysed by NMR as previously described [20]. The yields of the purified regio-isomers were calculated based on concentration determinations.

Table 1
Gradient elution programme for preparative RP-HPLC column: Lichrosorb RP18 stainless steel 250 mm × 10 mm, 7 μm particle size.

Time (min)	Acetonitrile (%)	Water (%)	Flow (ml/min)
0	40	60	2
4	40	60	2
5	35	65	2
9	35	65	2
15	55	45	2
20	55	45	2
20.5	100	0	2
25	100	0	2

2.4. Analytical HPLC

The analysis of sucrose esters during the purification process was performed by HPLC equipped with C 18 Chromolith Performance RP-18e analytical column (4.6 mm × 100 mm Merck, Germany). The HPLC conditions and the ELSD detector operation were followed as previously reported [20]. Samples were dissolved or appropriately diluted with methanol prior to injection (10 μl).

2.5. NMR

The acylation position of the purified sucrose esters was determined by ^{1}H and ^{13}C NMR. Assignments were based on double-quantum filtered correlation spectroscopy (DQF-COSY) and multiplicity-edited heteronuclear single quantum coherence (HSQC) spectra as previously described [20].

2.6. Nano-electron spray mass spectrometry analysis

The lyophilized powder of purified 3-*O*-decanoyl sucrose (0.32 mg) was analysed by nano-electron spray mass spectrometry. The samples were dissolved in pure acetonitrile, diluted 200-fold in 50% (v/v) acetonitrile and 1% (v/v) formic acid and injected directly onto a hybrid quadropole time of flight (QTOF) mass spectrometer (MicroTOFq, Bruker Daltronics, Bremen, DE) with offline nanoelectronspray emitters (Proxeon Biosystems, Odense, Denmark). The mass spectra were recorded in positive ion mode.

3. Results and discussion

After termination of the protease catalysed synthesis of sucrose decanoate, the reaction mixture was analysed by analytical RP-HPLC with ELSD (see Fig. 2) and the presence of three major fractions were observed as sucrose (R_t 0.85 min), and its mono- and oligoester products. In the monoester fraction four peaks with retention time, R_t 4.79, 4.99, 5.28 and 5.48 min were detected and in the oligoester fraction peaks at R_t 9.01, 9.20, and 9.31 min were observed, respectively.