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Determination of regioisomeric distribution in carbohydrate fatty acid monoesters by LC–ESI-MS

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Abstract—A new LC–ESI-MS method for characterizing the regioisomeric distribution in carbohydrate monoesters with long-chain fatty acids is described. Sucrose monolaurate mixtures were used for development of the method. The surfactant nature and high polarity of these compounds make them appropriate analytes for being detected by electrospray-ionization mass spectrometry (ESI-MS). Despite the structural similarity of regioisomers, an excellent resolution of all regioisomers present in the different samples studied (sucrose monodecanoates, sucrose monolaurates, sucrose monopalmitates and melezitose monolaurates) is achieved. Reversed-phase liquid chromatography with isocratic acetonitrile–water mixtures was used for a proper separation of the analytes. Finally, the superiority of this chromatographic method for determining the regioselectivity in enzymatic carbohydrate acylation reactions, with respect to the typical methodology based on routine ^{13}C NMR spectroscopy, is also discussed.

Keywords: Oligosaccharide; Oligosaccharide fatty acid monoesters; Regioisomeric separation; Transesterification; Sucrose ester; LC–ESI-MS

1. Introduction

Sugar fatty acid esters are nonionic surfactants with broad applications in food, cosmetic and pharmaceutical industries.[1] Carbohydrate fatty acid monoesters are the most important group of this type of compounds because of their better water solubility compared to di-, tri- and higher ester derivatives. It is important to note that some of their properties depend on the acylation position. For example, regioisomeric sucrose fatty acid monoesters have different surfactant properties since they possess different CMC values.[2,3] Biological properties may also be different between regioisomers. In fact, better antimicrobial activity has been observed for 6-*O*-palmitoylraffinose than for 1″-*O*-palmitoylraffinose when tested against *Bacillus subtilis*.[4] On the other hand, when the monoacylation of a carbohydrate is a protective step within a synthetic sequence, the achievement of the maximum regioselectivity is a fundamental goal.[5] Consequently, it is important to have precise and sensitive analytical methodologies for the determination of the regioisomeric distribution in samples of carbohydrate fatty acid monoesters prepared by chemical or enzymatic synthesis.[6,7] The information about the regioselectivity of the acylation reaction is obtained from such type of analysis.

Although an example has been published of sucrose fatty acid ester analysis by high-temperature gas chromatography,[8] HPLC is a more convenient technique since it does not require previous derivatization of the sample. As this type of compound cannot be detected by UV absorption, refractive index or evaporative light-scattering detection is typically employed. Curiously, the majority of analytical methods that have appeared in the literature describing the synthesis of these types of compounds are focused on the monitoring of the starting carbohydrate and on the analysis of the degree of substitution rather than on the analysis of

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the regioisomeric compounds that are obtained.[9] Queneau and co-workers have described with certain detail the HPLC analysis of regioisomeric sucrose fatty acid monoesters in a study about acyl group migrations in basic media.[10] Nevertheless, the information on the enzymatic regioselectivity in acylation reactions of carbohydrates reported by majority of authors is usually based exclusively on routine ^{13}C NMR spectra.[11]

In this work, a new method for the analysis of regioisomeric distribution in carbohydrate long-chain fatty acid monoesters based on HPLC with ESI mass spectrometry detection is described. At the same time, the superiority of this method in the determination of the regioselectivity of an enzymatic carbohydrate acylation with respect to the classic methodology based on routine ^{13}C NMR spectra is also discussed.

2. Results and discussion

2.1. HPLC–ESI-MS of carbohydrate fatty acid mono esters

HPLC chromatography coupled to mass spectrometry detection is a very useful analytical technique due to its high sensitivity and the structural information that can be obtained about the analytes.[12] The electrospray-ionization (ESI) interface is very appropriate for the analysis of polar nonvolatile compounds. For example, underivatized oligosaccharides have been separated and characterized by LC–ESI-MS.[13] Due to the ESI mechanism, compounds of surfactant nature present a high response in this class of mass spectrometry detectors, and very low detection limits can be achieved.[14] For example, nonionic surfactants of the family of polyethers have been analyzed by HPLC–ESI-MS.[15] Carbohydrate monoesters with long-chain fatty acids are another type of nonionic surfactant, and, consequently, their HPLC analysis with ESI-MS detection is very appropriate and presents obvious advantages in terms of operability and sensitivity with respect to the refractive index detection.

The development of an HPLC–ESI-MS method for characterizing the regioisomeric distribution in sugar fatty acid monoesters prepared by chemical or enzymatic synthesis involves, on one hand, an optimization of the ionization conditions for the analytes in the ESI source, and, on the other hand, an optimization of the chromatographic separation of the different regioisomers.

For development of the method we employed two commercial sucrose monolaurates, each one comprising a different regioisomeric mixture, and sucrose monolaurates prepared in our laboratory using chemical or enzymatic catalysis following previously described procedures (see Experimental).

2.2. Ionization conditions

In order to optimize the ionization conditions, a diluted aqueous sample (ca. 0.1 mg L^{-1}) of commercial sucrose monolaurate (Fluka) was injected directly in the mass spectrometer with the ESI probe operating in the positive-ionization mode. Under these conditions, polar analytes without basic functional groups are usually detected as adducts with sodium ions, even though sodium salts have not been added to the sample (low concentration of sodium can be derived from glassware and storage bottles, or can be present as impurities even in analytical grade solvents).[14] Consequently, it is expected that the observable molecular ion peak for sucrose monolaurate has m/z 547 ($[M+Na]^+$).

Figure 1 confirms that, without adding sodium salts to the sample, sucrose monolaurate forms stable sodium adducts. Pseudomolecular ions and very few fragments are produced when working in ESI conditions since this is a very soft ionization method. Although we are working with a single quadrupole mass spectrometer in which tandem MS is not possible, in-source fragmentation may be induced by increasing the voltage on the sampling cone. The low-energy collisions in the CID region (the intermediate vacuum region between the sampling and extraction cones) often involve breaking of the weakest bonds in the original ion.[16] In the case of sucrose

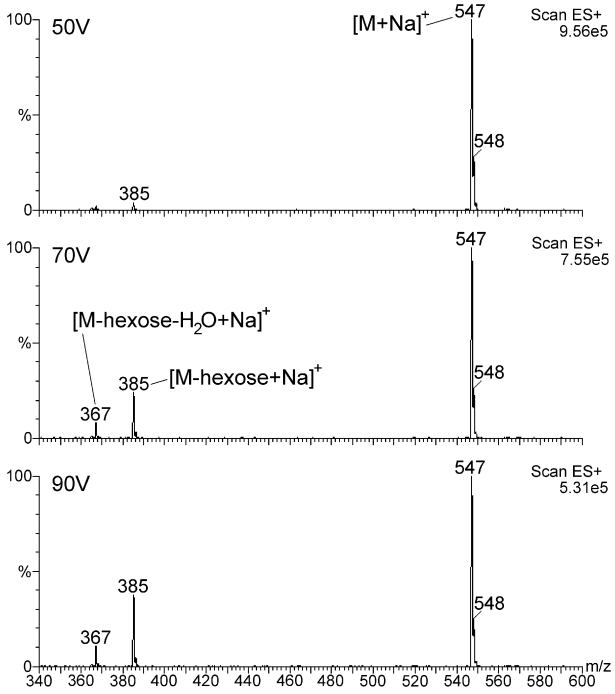

Figure 1. Mass spectra in the ESI positive-ion mode showing the in-source cone voltage induced fragmentation of commercial sucrose monolaurate (Fluka). Numbers in the upper right corner reflect the total ion counts.