Enzymatic Synthesis of Monosaccharide Fatty Acid Esters and Their Comparison with Conventional Products

Giuseppe Fregapane, Douglas B. Sarney, Sydney G. Greenberg, Dorothy J. Knight and **Evgeny N. Vulfson***

Department of Biotechnology and Enzymology, AFRC Institute of Food Research, Reading RG6 2EF, United Kingdom

5-*O*-Acyl-1,2-*O*-isopropylidene-D-xylofuranose and 6-*O*-acyl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose were enzymatically prepared from the corresponding monosaccharide acetals and commercial (crude) fatty acid mixtures. Subsequent acid-catalyzed hydrolysis of the isopropylidene group(s) gave monosaccharide esters with overall yields of 59–88%, where the monoester content was at least 80% (galactose oleate) and typically 90% for the other preparations. In contrast to sugar fatty acid esters prepared by conventional, high-temperature (trans)esterification, the enzymatically obtained monosaccharide esters contained no appreciable quantities of undesirable side products, and the only contaminants were monosaccharides and fatty acids.

KEY WORDS: Lipase, sorbitan esters, sugar fatty acid esters.

Sugar fatty acid esters are widely used as industrial detergents and as emulsifiers in a variety of food formulations. Current chemical methods of their manufacture are typically based on high-temperature (trans)esterification carried out in the presence of an alkaline catalyst (1). The main drawbacks of these conventional methods are a high consumption of energy and the formation of undesirable side-products. Additionally, a whole range of structural isomers are usually obtained due to the presence of multiple hydroxyl groups in the carbohydrate substrates. Consequently, numerous attempts have been made to apply enzymes to the synthesis of sugar fatty acid esters.

Two main approaches have been pursued so far with the aim of developing an alternative enzymatic method. The first was based on the use of organic solvents, suitable for solubilization of both substrates (2–5), while the second relied upon esterification of hydrophobic sugar derivatives with fatty acids under solvent-free conditions (6,7). Although the former approach appears to be more facile, the reaction kinetics are poor, as is the overall productivity. Hence, the latter methodology is currently more attractive from a technological standpoint. Indeed, the synthesis of 6-*O*-acyl-glucopyranosides from simple alkyl-glucosides, developed by Novo-Nordisk (6), has recently entered pilot-scale trials. A similar process has been described by Fregapane *et al.* (7) who used monosaccharide acetals as starting materials. Compared with alkyl glycosides, the use of isopropylidene sugars is a slightly more expensive option, but the latter methodology provides an efficient route to the synthesis of monosaccharide fatty acid esters that can be readily extended to disaccharides.

This paper describes the preparation of several monosaccharide fatty acid esters from commercial (crude) fatty acids and compares the overall composition of the products obtained *via* this route with that of functionally similar, commercial sorbitan esters. The preliminary results of this investigation have been reported at the symposium Biocatalysis in Non-Conventional Media (8).

EXPERIMENTAL PROCEDURES

Chemicals. Lipozyme IM-60 (EC 3.1.1.3 from *Mucor miehei*) was supplied by Novo-Nordisk A/S (Bagsvaerd, Denmark). 1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (di-ipGal) and 1,2-*O*-isopropylidene-D-xylofuranose (ipXyl) were obtained from Aldrich Chemical Company Ltd. (Gillingham, England). Sorbitan monoesters were kind gifts from ICI (Leatherhead, England), Grinstead (Bury St. Edmunds, England) and Croda Ltd. (North Humberside, England). Fatty acid mixtures used for the manufacture of corresponding sorbitan esters were obtained from the same manufacturers. The compositions of the fatty acid preparations used in this study are summarized in Table 1.

Synthesis of monosaccharide fatty acid esters. Enzymatic esterification was carried out as previously described (7) in a solvent-free process, with lipase as a catalyst, at 1:1 molar ratio of sugar acetal to fatty acid at 75°C in a stirred 100 mL flask under vacuum to remove the water produced during the reaction. Acid-catalyzed cleavage of the isopropylidene group(s) was carried out under conventional conditions (9) at 20% (wt/vol) of sugar acetal esters with aqueous trifluoroacetic acid at room temperature (di-ipGal esters) and formic acid at 75°C (ipXyl esters), as described in Table 2. In the case of galactose esters, the reaction mixture was evaporated to dryness with absolute ethanol. Xylose esters were recovered

TABLE 1

Composition of Commercial Fatty Acid Mixtures

Commercial preparation	Fatty acids (% w/w)								
	C10:0	C12:0	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2
Lauric acid	7.6	58.5	20.5	9.6	0	0	3.8	0	0
Stearic acid	0	0	2.6	29.0	0	0	68.4	0	0
Oleic acid	0	3.2	4.1	5.0	7.9	1.1	0	71.5	7.6

*To whom correspondence should be addressed at Department of Biotechnology and Enzymology, AFRC Institute of Food Research, Earley Gate, Whiteknights Road, Reading RG6 2EF, United Kingdom.

TABLE 2

Preparative-Scale Syntheses of Galactose and Xylose Esters[a]

	Yield after enzymatic step[b]	Deacetalization conditions[c]	Monoester content (%)	Overall yield (%)
Galactose-6-'laurate'	91	90% TFA (vol/vol) 10 min	87	86
Galactose-6-'stearate'	92	90% TFA (vol/vol) 5 min	88	88
Galactose-6-'oleate'	90	50% TFA (vol/vol) 16 h	80	79
Xylose-5-'laurate'	80	50% Formic acid (vol/vol) 1 h	94	59
Xylose-5-'stearate'	79	50% Formic acid (vol/vol) 1 h	93	67

[a]Reported yields were the average of at least three different preparations, where the standard deviation was ± 3-5%.
[b]After Lipase-catalyzed esterification.
[c]For further details refer to Experimental Procedures. TFA, trifluoroacetic acid.

by precipitation with an equal volume of acetonitrile at room temperature (xylose stearate) or at 0°C (xylose laurate). The remaining acid present in the filtered product was removed by azeotropic distillation with toluene, and no further purification or fractionation of the products was attempted. Acetone formed during the deacetalization was removed during the evaporation to dryness of the galactose and xylose ester preparations in the presence of ethanol and toluene, respectively. An overall yield of 59-88% (Table 2) was typically obtained. Individual 6-*O*-acylgalactoses and 5-*O*-acylxyloses were prepared by similar protocols and were fully characterized by nuclear magnetic resonance and mass spectrometry.

Gas chromatography (GC) analysis. GC analysis was performed on a Hewlett-Packard series 5890A gas chromatograph (Palo Alto, CA) equipped with flame-ionization detector (FID). Trimethylsilyl derivatives (1 μL), prepared according to Sweeley *et al.* (10), were applied to a Hewlett-Packard Ultra 2, 25 m × 0.22 mm fused-silica capillary column with 5% phenylmethyl silicone phase, 0.33 μm film thickness. A 60-cm length of deactivated fused silica, 0.32 mm i.d., was attached as a retention gap between the injector and the analytical column. The carrier gas was helium with a linear flow rate of 25 cm/s with a head pressure of 20 psi. The temperature of both the injector and the detector was 285°C. The temperature program was: 100°C for 0.5 min, then increased to 240°C at 8°C/min, held for 3 min, increased to 300°C at 10°C/min and held for 30 min.

Product identification. Identification of individual compounds on the GC chromatograms was effected with the corresponding standards, when available (the majority of fatty acids, monosaccharides and their acetals as well as individual galactose and xylose esters) and/or by GC-mass spectrometry (MS). The latter was carried out in the chemical ionization (CI) mode with a Hewlett-Packard series 5988 mass spectrometer with isobutane or ammonia as the reagent gas (source temperature, 200°C; source pressure, 1 torr; scan rate, 30-800 amu at 0.5 scan/s; unit resolution) and in electron ion mode with a HP TRIO-1 GC-MS (source temperature, 250°C; electron energy, 70 kev; unit resolution).

RESULTS AND DISCUSSION

Several galactose and xylose esters were prepared on a 20-g scale according to the method described, and the data are summarized in Table 2. Lipase-catalyzed esterification with either di-ipGal or ipXyl yielded practically no diester as detected by high-performance liquid chromatography analysis. The molecule of di-ipGal contains only one free hydroxyl group; therefore, only monoesters are feasible. For ipXyl, the esterification rate of the primary hydroxyl group was approximately 250 times higher than that of the secondary (as calculated from the data reported in our previous communication (7), and consequently, only traces of ipXyl diester were found over the incubation time used. A satisfactory product recovery of 67-88% was obtained for esters of long-chain fatty acid mixtures. The recovery of xylose laurate was somewhat lower, due to a partial loss of the product at the precipitation step (see Experimental Procedures). The lowest monoester content of the preparations was at least 80% (galactose oleate) and typically about 90% for the other preparations studied (Table 2). The composition of the crude products was further analyzed by GC and GC-MS, and the data are presented in Figures 1 and 2.

The composition of the fatty acids (A), transesterification products (B) and the final xylose 'laurate' esters (C) are depicted in Figure 1. It is evident that virtually no side-products were formed either in the course of lipase-catalyzed esterification or the subsequent removal of isopropylidene groups. Similar chromatograms were obtained while analyzing galactose 'stearate', xylose 'stearate', (Fig. 2A and B, respectively), galactose 'laurate' and galactose 'oleate' (not shown).

There have been numerous attempts to prepare monosaccharide fatty acid esters through acid- or base-catalyzed (trans)esterification at elevated temperatures (1). From an economic standpoint, this methodology appears to be the most suitable for the bulk manufacture of sugar-based surfactants. However, reducing monosaccharides were found to be unstable under these conditions and typically decomposed to give various side products. In the search for a more stable alternative, sorbitol was selected

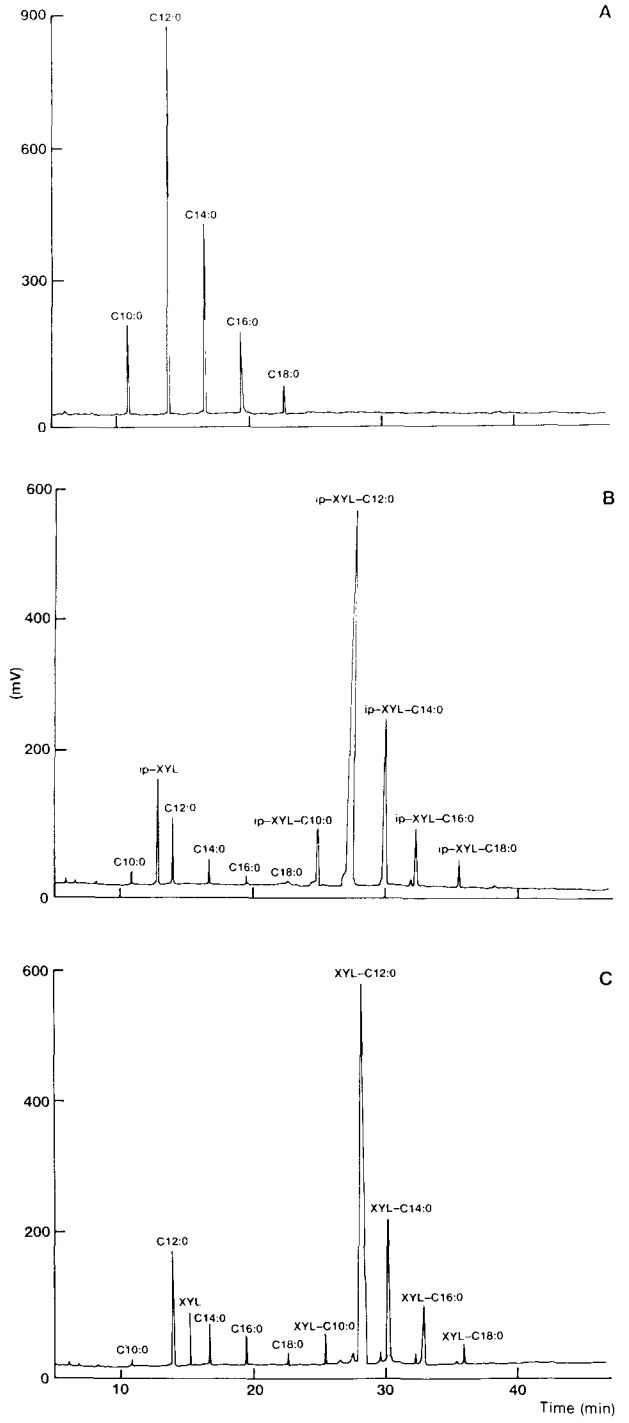

FIG. 1. Gas chromatographic analyses of crude lauric acid (A) and xylose fatty acid esters before (B) and after (C) cleavage of the isopropylidene group.

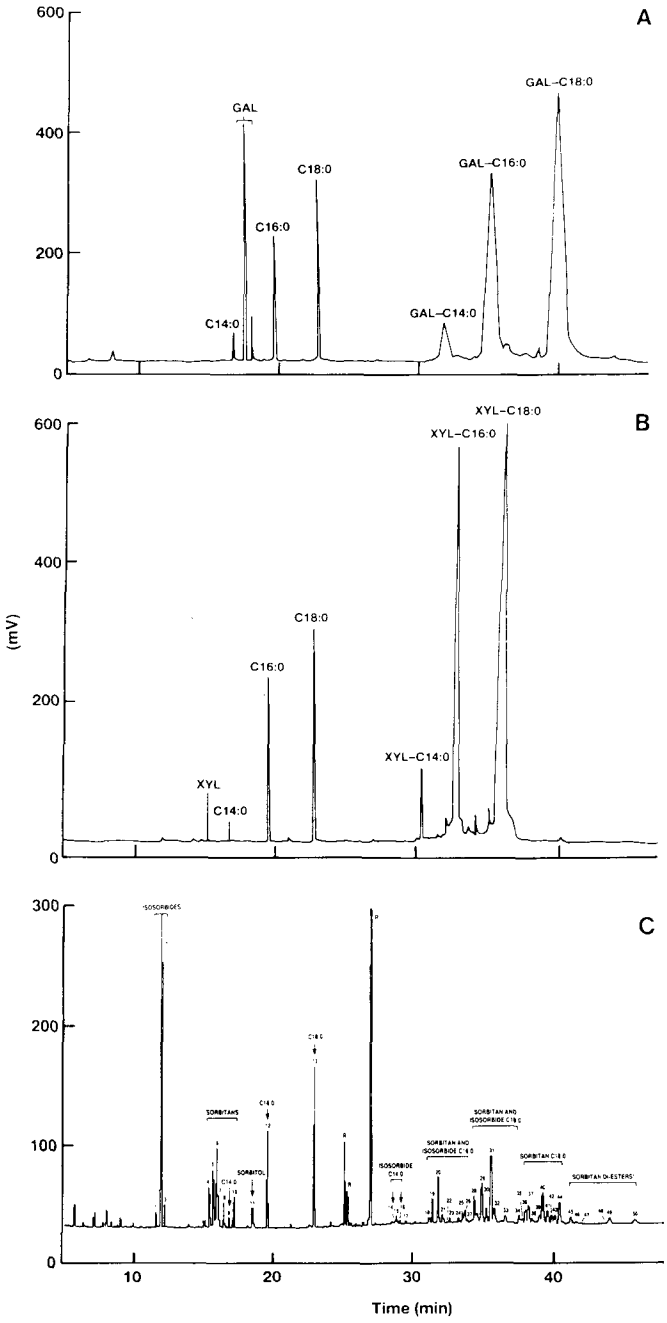

FIG. 2. Gas chromatographic analyses of galactose-'stearate' (A), xylose-'stearate' (B) and sorbitan-mono-'stearate' (C). Peak assignment: C. 1-3: isosorbides, 2-8: sorbitans, 9: C14:0 (myristic acid), 10: sorbitan, 11: sorbitol, 12: C16:0 (palmitic acid), 13: C18:0 (stearic acid), 14: isosorbide myristate, 15: ?, 16: isosorbide myristate, 17: ?, 18-20: isosorbide palmitates, 21-27: sorbitan palmitates, 28: isosorbide stearate + sorbitan palmitate, 29-30: isosorbide stearate, 31: isosorbide stearate + sorbitan stearate, 32: isosorbide stearate + sorbitan palmitate + sorbitan stearate, 33: isosorbide stearate + sorbitan stearate, 34: sorbitan stearate, 35: sorbitan palmitate, 36-44: sorbitan stearates, 45-50: sorbitan di-esters, R: reagent.

for the manufacture of this type of surfactant, and the resulting sorbitan esters are currently used in a wide variety of applications, including numerous food products. Nevertheless, a large number of by-products and regio-isomers are still formed, as illustrated by GC analysis of sorbitan esters prepared from fatty acid mixtures, the compositions of which are depicted in Table 1. Thus, more than 50 individual components were identified by MS, as various isomers of sorbitan, isosorbide and their mono- and di-esters in sorbitan stearate (Fig. 2C), and even more (65) were detected in sorbitan laurate (not shown). For

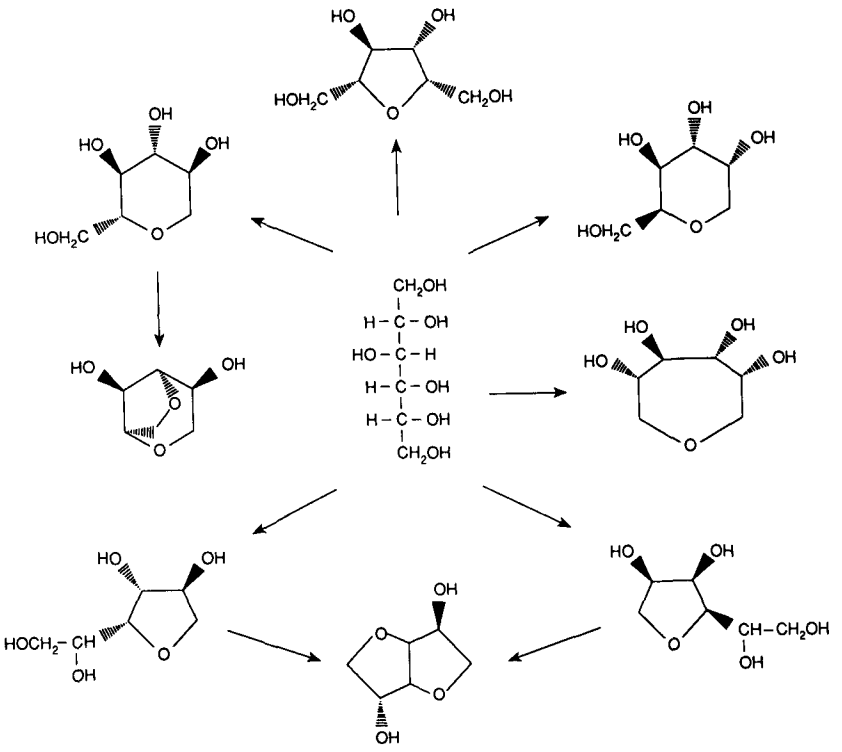

FIG. 3. Possible dehydration products of sorbitol in food-grade sorbitan esters.

example, up to ten sorbitan isomers have been detected on the basis of their molecular ions, and some possible structures are depicted in Figure 3. Similar results have been obtained with sorbitan esters produced by ICI (Spans), Grinstead (Famodans) and Croda (Crills).

The presence of sorbitan isomers (Fig. 3) and other side products (Fig. 2C) suggest reasons why regulating authorities have expressed some concern regarding the safety of sorbitan esters for human consumption. Recent studies of a related product, Polysorbate 80, have established that the administration of this emulsifier was associated with inflammation and squamous hyperplasia of the forestomach in male and female mice, and with ulcers of the forestomach in female mice (11). As a result, further investigations regarding the toxicity of sorbitan esters and other products of high-temperature esterification are being undertaken, and the search for improved manufacturing methods and/or functionally similar alternatives continues.

Although it would be rather premature to assume that the use of enzymes can at present provide an economically viable alternative to the chemical methods of bulk surfactant manufacture, the rapid advances in biotechnological methods, achieved in recent years, provide a foundation for cautious optimism. As far as the enzymatic synthesis described herein is concerned, it remains to be seen whether the additional steps of acetalization/deacetalization are justifiable by the improved quality of the final products. In principle, large-scale acetalization and subsequent deprotection should not present technological difficulties because these reactions are currently run on a pilot scale as a part of the vitamin C manufacturing cycle.

Production cost, however, cannot be assessed as an individual issue but should be considered in conjunction with actual product performance. If the emulsifying properties of monosaccharide fatty acid esters are found to be superior in specific applications (compared with currently used surfactants, particularly sorbitan esters), then additional production costs would be justified. We conclude that the monosaccharide fatty acid esters synthesized hold promise as alternatives to some currently used emulsifiers, particularly those at the more expensive end of the market (*e.g.*, in cosmetic formulations). The emulsifying properties and interfacial behavior of these compounds are currently under investigation.

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