Application of Lipases to the Regioselective Synthesis of Sucrose Fatty Acid Monoesters

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ABSTRACT: A regioselective synthesis of 6′-*O*-acyl sucrose monoesters has been developed through the lipase-catalyzed esterification of sucrose acetals with fatty acids in both organic solvents and under solvent-free conditions. The products were obtained in overall yields of 20–27% after hydrolysis of the isopropylidene groups with aqueous acids. The strict selectivity of the enzymes used also enabled the preparation of a monoester fraction that was highly enriched in 6-*O*-acyl sucrose. This was accomplished by lipase-catalyzed transesterification of sucrose monoesters, prepared by conventional chemical methods, in propan-2-ol. After removal of the transesterification products (sucrose and fatty acid isopropyl esters) and column chromatography on silica gel, the obtained monoester product contained 80% of the single regioisomer, 6-*O*-acyl sucrose.
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KEY WORDS: Enzymatic esterification, lipase, regioselectivity, solvent-free acylation, sucrose acetal, sucrose ester, sugar fatty acid ester.

Sucrose esters are employed as surfactants in a wide range of food, pharmaceutical, and agri-chemical applications (1). Due to their manufacturing process, a typical preparation of sucrose monoesters contains a large number of individual regioisomers (1,2). However, the functional properties of sucrose monoesters, including their sensory characteristics and stability in particular formulations, depend on the position of acylation. Therefore, it is of interest to develop a facile regioselective synthesis of sucrose monoesters with the aim of undertaking a detailed structure–function relationship study. This communication describes the use of commercially available lipases for the preparation of specific regioisomers of sucrose monoesters.

The application of enzymes to the synthesis of sugar-based surfactants has been actively studied in recent years due to the mild reaction conditions and high regioselectivity typically displayed by biological catalysts (3). These investigations led to the identification of enzymes that can regioselectively acylate the primary hydroxyl groups of various mono- and disaccharides, including sucrose. In particular, subtilisin, a versatile serine esterase, and its variants have been used by several laboratories for the synthesis of 1′-*O*-acyl sucrose (4–7). However, subtilisin displays strict selectivity toward the 1′-OH group of sucrose and does not normally accept medium- and long-chain carboxylic acids as acyl donors (5), which limits the suitability of this enzyme for the preparation of surface-active agents. Conversely, lipases perform well with a wide range of fatty acids but display low catalytic activity in the highly aprotic solvents required to solubilize sucrose in sufficient concentrations. We have recently shown that the problem of substrate solubility/miscibility can be largely overcome by using mono- and disaccharide acetals in mixtures with fatty acids as solvent-free media for enzymatic esterifications (8–11). Furthermore, enzyme specificity was not compromised by performing the reaction with a crude mixture of sugar acetals, thus making the tedious prior isolation of a suitable sugar acetal substrate unnecessary (11). The main objectives of this study were to ascertain the feasibility of applying the same methodology to the regioselective synthesis of sucrose monoesters with an unresolved mixture of sucrose acetals and, if successful, to develop a facile synthetic protocol.

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EXPERIMENTAL PROCEDURES

Chemicals. Lipozyme IM-60™ (EC 3.1.1.3 lipase from *Mucor miehei*) and Novozym™ 435 (*Candida antarctica* lipase) were supplied by Novo-Nordisk A/S (Bagsvaerd, Denmark). *Chromobacterium viscosum* lipase was purchased from Biocatalysts Ltd. (Pontypridd, United Kingdom), partially purified according to Turner *et al.* (12) and immobilized on XAD4 (Sigma Chemicals, Poole, United Kingdom) at 1 g per mL of enzyme solution as previously described (13). The preparation of immobilized enzyme was dried in a laboratory fluid-bed drier (Palmer Research Laboratories, Flintshire, United Kingdom) and stored in a vacuum desiccator over phosphorus pentoxide. Fatty acids, disaccharides, Amberlite IRA-400 OH anion exchange resin, silica gel, and all organic solvents used in this study were obtained from Aldrich Chemical Co. (Gillingham, United Kingdom) and were of the highest available purity. Tetrafluoroboric acid (50% aqueous solution) and silica gel C60 TLC plates were supplied by Merck (Eastleigh, United Kingdom). Commercial preparations of Ryoto Sucrose Esters (L1695, M1695, P1695, and S1695)

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were obtained from Mitsubishi-Kasei Food Corporation (Tokyo, Japan).

Analytical. High-performance liquid chromatography (HPLC) was carried out with a light-scattering mass detector as previously described (8). Gas chromatographic (GC) analysis was undertaken on a Carlo Erba (Milan, Italy) HRGC 5300 gas chromatograph equipped with flame-ionization detection. One µL of trimethylsilyl derivatives, prepared by adding a sample to a 1:1 mixture of *bis*(trimethylsilyl)trifluoroacetamide/pyridine at 50°C for 15 min, was applied to a 10 m Simdist wide-bore glass capillary column coated with PS 264 (Carlo Erba/Fisons, Loughborough, United Kingdom) with helium as a carrier gas (14). The oven temperature gradient began at 140°C and increased by 28°C/min up to 280°C, followed by 4°C/min up to 328°C and finally 23°C/min to reach 397°C. Thin-layer chromatography (TLC) was used for the qualitative analysis of sucrose acetal derivatives and sucrose esters on silica gel C60 plates with chloroform/methanol as the eluent. TLC plates were developed by spraying with Bial's reagent (Sigma) and heating at 100°C for 10 min. Column chromatography was performed on 60Å silica gel with a gradient elution of chloroform and methanol. Nuclear magnetic resonance (NMR) spectra were recorded on a Jeol (Tokyo, Japan) EX 270 Fourier transform spectrometer at 67.8 MHz (^{13}C) and 270.05 MHz (^{1}H) with d_4-methanol or d_6-dimethylsulfoxide as the solvent. All chemical shifts (δ) are reported in ppm relative to tetramethylsilane. Chemical shift values (δ) were determined by a combination of one-dimensional ^{1}H- and ^{13}C NMR spectroscopic analysis (including DEPT 90 and DEPT 135) and two-dimensional ^{1}H-^{1}H homonuclear COSY and ^{1}H-^{13}C heteronuclear COSY experiments. Negative FAB–MS (fast atom bombardment–mass spectrometry) spectra were obtained on a Kratos (Manchester, United Kingdom) MS9/50TC spectrometer with xenon at 5–8 KeV. Accurate mass measurements were recorded at 1.0 milli.amu resolution with PEG 600 ions as reference.

*Synthesis of sucrose acetals (**1–4**).* Four sucrose ketals (Fig. 1), *4,6-O*-isopropylidenesucrose (**1**), 2,1′:4,6-di-*O*-isopropylidenesucrose (**2**), 4,6-*O*-benzylidenesucrose (**3**), and 4,6-*O*-cyclohexylidenesucrose (**4**) were prepared according to published procedures (15–18) with the minor modifications described here. A solution of sucrose (8.55 g, 25 mmol) in dry *N,N*-dimethylformamide (100 mL), containing molecular sieve pellets (Type 3Å), was stirred with 2-methoxypropene (3.2 mL, 32.5 mmol, **1** and 12 mL, 125 mmol, **2**), benzaldehyde (17.1 mL, 114 mmol, **3**), or cyclohexanone dimethyl acetal (16.4 g, 114 mmol, **4**) in the presence of *p*-toluenesulfonic acid (*p*-TSA). The reaction was allowed to proceed for 40 min at 70°C (**1** and **2**) or 60 min at 40°C (**3** and **4**). The reaction mixture was then cooled, neutralized with sodium carbonate, filtered and the solvent was removed by rotary evaporation. The desired sucrose acetal was purified by column chromatography. The following yields were obtained: 3.2 g, 32% (**1**); 3.8 g, 36% (**2**); 3.4 g, 32% (**3**); and 3.3 g, 31% (**4**). NMR spectroscopic data for compounds **1–4** are summarized below.

*4,6-*O*-isopropylidenesucrose* (**1**). ^{1}H NMR (CD_3OD) δ ppm: 1.37, 1.49 (6H, 2*s*, 7-CH_3, 8-CH_3), 3.50–4.20 (13H, sucrose ring protons), 5.35 (1H, *d*, $J_{1,2}$ 4.0 Hz, 1-H). ^{13}C NMR (CD_3OD) δ ppm: 19.3, 29.5 (C-8, C-9), 62.9, 63.2, 63.7 (C-1′, C-6′, C-6), 65.4 (C-4), 71.9, 73.8, 73.8, 75.1, 78.8 (C-3′, C-4′, C-2, C-3, C-5), 83.8 (C-5′), 94.2 (C-1), 100.8 (C-7), 105.3 (C-2′).

*2,1′:4,6-di-*O*-isopropylidenesucrose* (**2**). ^{1}H NMR (CD_3OD) δ ppm: 1.37, 1.47, 1.50 (12H, 3*s*, 8-CH_3, 9-CH_3, 8′-CH_3, 9′-CH_3), 3.2–4.2 (13H, *m*, sucrose ring protons), 6.16 (1H, *d*, $J_{1,2}$ 3.6Hz, 1-H). ^{13}C NMR (CD_3OD) δ ppm: 19.3, 29.5 (C-8, C-9), 24.3, 25.5 (C 8′, C 9′), 63.2, 64.3, 67.1 (C-1′, C-6′, C-6), 64.6 (C-4),70.9, 74.7, 75.1, 75.6, 79.8 (C-3′, C-4′, C-2, C-3, C-5), 84.2 (C-5′), 92.4 (C-1), 100.9 (C-7), 102.8 (C-7′), 104.7 (C-2′).

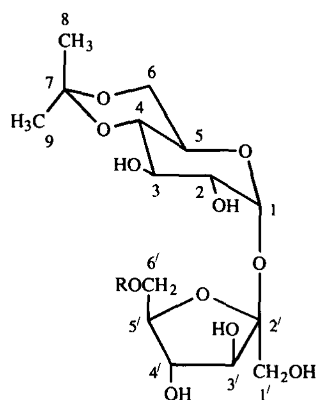
*4,6-*O*-benzylidenesucrose* (**3**). ^{1}H NMR (CD_3OD) δ ppm: 3.3-4.3 (13H, sucrose ring protons), 5.41 (1H, brd, 1-H), 5.56 (1H, *s*, 7-H), 7.20–7.70 (5H, aromatic protons).^{13}C NMR (CD_3OD) δ ppm: 63.0, 63.8, 69.7, (C-1′, C-6′, C-6), 64.5 (C-4),71.6, 73.8, 75.2, 78.9, 82.7 (C-3′, C-4′, C-2, C-3, C-5), 83.8 (C-5′), 94.2 (C-1), 103.0 (C-7), 105.3 (C-2′),127.0, 127.5, 129.0, 129.9 (aromatic carbon atoms), 139.2 (C-8).

*4,6-*O*-cyclohexylidenesucrose* (**4**). ^{1}H NMR [$(CD_3)_2SO$] δ ppm: 1.35–1.85 (10H, cyclohexane ring protons), 3.30–5.30 (20H, sucrose ring protons). ^{13}C NMR [$(CD_3)_2SO$] δ ppm: 22.3, 22.6, 25.5, 27.6, 37.9 (C-8–C-12), 61.3, 61.3, 62.1, (C-1′, C-6′, C-6), 63.9 (C-4), 70.0, 72.5, 73.2, 74.1, 76.9 (C-3′, C-4′, C-2, C-3, C-5), 82.7 (C-5′), 92.6 (C-1), 99.0 (C-7), 104.3 (C-2′).

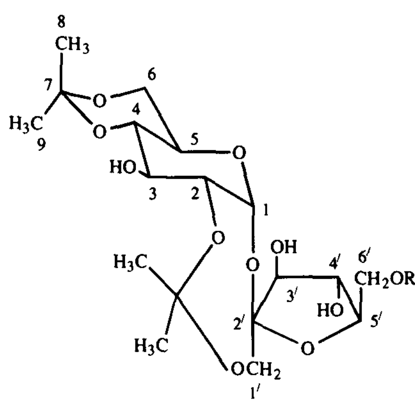
A crude mixture of isopropylidenesucrose was prepared as described for **2**. This typically contained some unreacted sucrose, 60–70% of **2**, 30-35% of **1** and a small quantity of a sucrose triacetal [2,1′:4.6-di-*O*-isopropylidene-6′-*O*-(1-methyl-1-methoxyethyl) sucrose] (18). This preparation was used for enzymatic esterification as obtained, after neutralization of the *p*-TSA catalyst with Amberlite IRA 400 OH anion exchange resin, filtration and removal of the solvent.

*Preparation of sucrose acetal 6′-*O*-monoesters.* Initially, enzymatic esterification of sucrose acetals **1–4** was performed on a 0.065 mmol analytical scale with an equimolar mixture of the corresponding sucrose acetal and fatty acid and 0.1 mL of organic solvent (see the Results and Discussion section for details) with 20 mg of lipase. The reaction was carried out at 75°C in glass vials in a shaker–incubator (180 rpm). After the indicated time intervals, aliquots of the reaction mixture were withdrawn and analyzed by HPLC and GC to ascertain the effectiveness of various lipases under the range of reaction conditions and solvents. The reaction was carried out on a larger scale (0.65 mmol), and the monoesters were isolated by column chromatography on silica gel as described above in order to establish the regioselectivity of acylation.

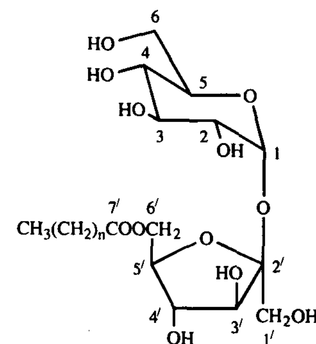
*General preparation of sucrose acetal 6′-*O*-monoesters (**2a–e**) and sucrose 6′-*O*-monoesters (**5a–e**).* The approach is exemplified by the enzymatic esterification of 2,1′:4,6-di-*O*-isopropylidene-sucrose (**2**) with octadecanoic acid (stearic acid, $C_{18:0}$) to form 6′-*O*-octadecanoyl-2,1′:4,6-di-*O*-iso-



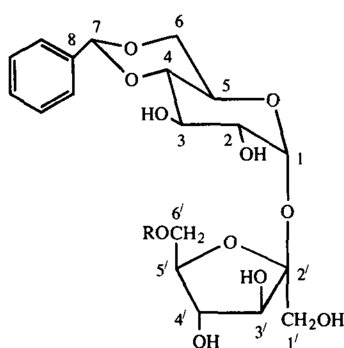
4,6-*O*-isopropylidenesucrose (**1**) R=H
6′-*O*-acyl-4,6-*O*-isopropylidenesucrose (**1a–e**)
a R= $CH_3(CH_2)_8CO$
b R= $CH_3(CH_2)_{10}CO$
c R= $CH_3(CH_2)_{12}CO$
d R= $CH_3(CH_2)_{14}CO$
e R= $CH_3(CH_2)_{16}CO$



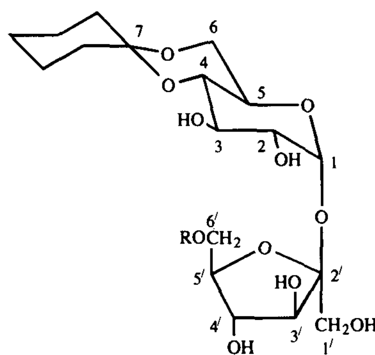
2,1′:4,6-di-*O*-isopropylidenesucrose (**2**) R=H
6′-*O*-acyl-2,1′:4,6-di-*O*-isopropylidenesucrose (**2a–e**)
a R= $CH_3(CH_2)_8CO$
b R= $CH_3(CH_2)_{10}CO$
c R= $CH_3(CH_2)_{12}CO$
d R= $CH_3(CH_2)_{14}CO$
e R= $CH_3(CH_2)_{16}CO$



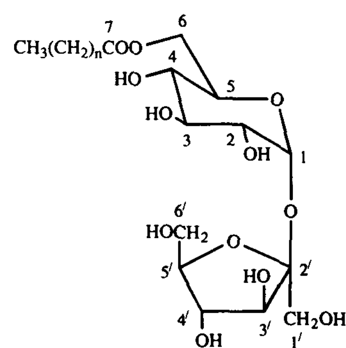
6′-*O*-acyl-sucrose (**5a–e**)
a $n = 8$
b $n = 10$
c $n = 12$
d $n = 14$
e $n = 16$



4,6-*O*-benzylidenesucrose (**3**) R=H
6′-*O*-acyl-4,6-*O*-benzylidenesucrose (**3a–e**)
a R= $CH_3(CH_2)_8CO$
b R= $CH_3(CH_2)_{10}CO$
c R= $CH_3(CH_2)_{12}CO$
d R= $CH_3(CH_2)_{14}CO$
e R= $CH_3(CH_2)_{16}CO$



4,6-*O*-cyclohexylidenesucrose (**4**) R=H
6′-*O*-acyl-4,6-*O*-cyclohexylidenesucrose (**4a–e**)
a R= $CH_3(CH_2)_8CO$
b R= $CH_3(CH_2)_{10}CO$
c R= $CH_3(CH_2)_{12}CO$
d R= $CH_3(CH_2)_{14}CO$
e R= $CH_3(CH_2)_{16}CO$



6-*O*-acyl-sucrose (**6b–e**)
b $n = 10$
c $n = 12$
d $n = 14$
e $n = 16$

FIG. 1. Chemical structures of sucrose acetals (**1–4**), sucrose 6′-*O*-acetal monoesters (**1–4**, **a–e**), sucrose 6′-*O*-monoesters (**5a–e**), and sucrose 6-*O*-monoesters (**6b–e**).

propylidenesucrose (**2e**), followed by cleavage of the acetal groups to yield 6′-*O*-octadecanoyl sucrose (**5e**). Crude 2,1′:4,6-di-*O*-isopropylidenesucrose syrup (20.66 g) was incubated with stearic acid (13.80 g) at 75°C for 48 h with Novozym™ 435 (1.70 g) with an initial addition of 21 mL of toluene to promote miscibility of the reagents. The solvent was allowed to evaporate during the first few hours of the reaction. After completion of the reaction (typically 48 h), the resultant mixture was dissolved in ethanol, and the enzyme was recovered by filtration. Unreacted fatty acid was then removed by passing the solution through a pad of basic alumina and, after evaporation of the ethanol *in vacuo*, the product was redissolved in ether (1:10, wt/vol). Unreacted sucrose acetals were then extracted with aqueous $NaCl/K_2CO_3$ (0.3 M each), and the organic solution was dried over $MgSO_4$, filtered and evaporated to dryness.

Hydrolysis of the isopropylidene groups to obtain the sucrose ester was carried out by dissolving the product (12.24 g) in a solution of acetonitrile/water/tetrafluoroboric acid (500:5.5:0.5, vol/vol/vol, 120 mL) and stirring at ambient temperature for 8 h. The solution was cooled in ice, and the resultant precipitate [9.91 g, (**5e**)] was filtered off. The reaction conditions for the acid-catalyzed hydrolysis of acetal groups were tailored for different esters (**2a–d**). Sucrose acetal monoesters **2a–c** (10% wt/vol) were hydrolyzed in ethyl acetate/trifluoroacetic acid (TFA)/water (95:3:2) at 60°C for

8 h. TFA was then removed by azeotropic distillation with ether. Reaction conditions for **2d** were identical to those described for **2e**.

Final purification was achieved by column chromatography. The structure of the resultant monoester (>98% pure by GC, Fig. 3A) was determined to be 6′-*O*-stearoyl sucrose (**5e**) by ^{1}H and ^{13}C NMR spectroscopy and high-resolution FAB–MS. This was in agreement with the structures assigned to the intermediate sucrose acetal ester (**2e**). Esters **5a–d** were prepared and characterized in an analogous manner.

*General preparation of sucrose 6-O-monoesters (**6b–e**).* Commercial samples (5 g) of Ryoto™ sucrose monoesters—L1695 (laurate), M1695 (myristate), P1695, (palmitate), and S1695 (stearate)—were dissolved in 70 mL of dry (3Å molecular sieves) propan-2-ol with 2 g of Novozym™ 435 lipase. The reactions were carried out at 50°C for 72–96 h in an orbital shaker (180 rpm) until the 6′-*O*-monoester peak did not diminish further, as observed by GC. The reaction mixture was then cooled to ambient temperature to precipitate the required 6-*O*-monoester and leave the products of transesterification (isopropyl fatty acid esters and a large proportion of the diesters) in solution. Subsequent filtration yielded a precipitate of 6-*O*-monoesters, containing sucrose and immobilized enzyme. The latter was separated by dissolving this precipitate in methanol (200 mL) and decanting, whereafter methanol was removed by rotary evaporation to give 1.25–1.7 g of dry solid. The esters (**6b–e**) were isolated by column chromatography in 39–54% yields and 80% purity.

RESULTS AND DISCUSSION

Sucrose acetals **1–4** (Fig. 1) were prepared for assessment as substrates in enzymatic esterifications. Twenty lipase preparations were tested during the initial screening program to identify which lipases would be most suitable for the production of sucrose monoesters and to select the best sucrose acetal(s) for the subsequent preparative synthesis. The reactions were carried out in fifteen different organic solvents (a variety of tertiary alcohols, ketones, and ethers), as well as under solvent-free conditions at a later stage. These preliminary experiments were essential to elucidate which enzymes would be capable of accepting sterically hindered sucrose acetals (**1–4**) as substrates and, more importantly, to establish the regioselectivity of acylation with different preparations of lipase.

We found that most of the enzymes were only marginally active under the experimental conditions used, but three lipases consistently gave acceptable yields over the range of reaction conditions tested. These were *M. miehei* (Lipozyme™ IM60, 49 BIU/g), *C. antarctica* (Novozym™ 435, 7000 PLU/g), and *Chr. viscosum* lipases. We then established the regioselectivity of acylation for these three enzymes. The reactions were carried out with all four sucrose acetals (**1–4**) on a 0.65-mol scale with myristic acid and 3,3-dimethyl-2-butanone (pinacolone) or 2-hexanone as solvents. The products obtained were analyzed by ^{13}C NMR for each of the enzyme/solvent combinations. In all cases, one major monoester product could be isolated by column chromatography, although the formation of minor amounts of other regioisomers, particularly for **1** and **4**, was noted. Significant quantities of diesters were formed only when the reaction was continued for a long period (7 d). Characterization of the major monoester products by NMR spectroscopy revealed that, irrespective of the solvent or enzyme employed, the position of acylation was the same for all acetals (**1–4**), namely the primary hydroxyl group of the fructose ring. Thus, the products were identified as 6′-*O*-myristoyl-4,6-*O*-isopropylidenesucrose (**1c**), 6′-*O*-myristoyl-2,1′:4,6-di-*O*-isopropylidenesucrose (**2c**), 6′-*O*-myristoyl-4,6-*O*-benzylidenesucrose (**3c**), and 6′-*O*-myristoyl-4,6-*O*-cyclohexylidene-sucrose (**4c**). Therefore, regardless of the nature of sucrose acetals used for enzymatic esterification, 6′-*O*-acyl sucrose would be the same final product of the synthesis after cleavage of the acetal groups. This meant that the efficiency of enzymatic esterification, rather than the regioselectivity, was the dominating factor in the final selection of optimal combinations of enzyme and substrate.

The synthesis of sugar acetal esters **1–4, a–e** catalyzed by *M. miehei, C. antarctica* and *Chr. viscosum* lipases was then investigated in more detail, and the results obtained are summarized in Table 1. We found that among the three enzymes studied, the *M. miehei* lipase was generally the most effective at catalyzing the esterification of acetals **1–4** with myristic acid ($C_{14:0}$) and that, among all the sucrose acetals tested, 2,1′:4,6-di-*O*-isopropylidenesucrose **(2)** gave the highest yield of monoester. As evident from Table 1 and Figure 2, high yields were routinely obtained with this lipase after 48 h by using equimolar mixtures of **2** and myristic acid under the variety of experimental conditions used. *Candida antarctica* lipase (Novozym™ 435) showed a similar preference for **2** in solvent-free esterifications, but relatively poor yields were obtained with other sucrose acetals **1** and **3**. Among the enzymes studied, *Chr. viscosum* lipase showed the lowest esterification yields of **2** and was only marginally active against other sucrose acetals (**1, 3**, and **4**) in the absence of organic solvents. This enzyme was therefore excluded from subsequent studies.

Although **1** was a less efficient substrate than the di-isopropylidene-derivative (**2**), the observation that **1** and **2** can be acylated at the same 6′ position [thus giving the same final product after removal of isopropylidene group(s)] prompted us to investigate the feasibiltity of enzymatic esterification of a mixture of **1** and **2**. To this end, a crude preparation of 2,1′:4,6-di-*O*-isopropylidenesucrose (**2**), containing up to 30% of **1** and some unreacted sucrose and higher acetals, was used directly as obtained after neutralization of *p*-TSA and evaporation of the solvent (see the Experimental Procedures section). When this was attempted with *M. miehei* enzyme as the catalyst, yields were significantly reduced (from >40% with pure **2** to 13% with the crude product). However, *C. antarctica* lipase worked well in the crude mixture of substrates and gave even better yields of **2c**, as determined by

TABLE 1
Enzymatic Yields of 6′-O-Myristoyl Sucrose Acetals[a]

Substrate	Solvent	Lipozyme™ IM-60[b]	*Chromobacterium viscosum* lipase	Novozym™ 435[b]
4,6-O-isopropylidene-sucrose (1)`	2-Heptanone	10	25	<10
	3,3-dimethyl-2-butanone,	30	<5	<10
	no solvent added	15	<5	<10
2,1′:4,6 di-O-isopropylidene-sucrose (2)	Toluene	40	n/d	17
	3,3-dimethyl-2-butanone,	32	33	43
	no solvent added	30	33[c]	44[c]
4,6-O-benzylidene-sucrose (3)	2-Methoxyethyl ether	<5	n/d[d]	<5
	3,3-dimethyl-2-butanone,	13	<5	<5
	no solvent added	14	<5	<5
4,6-O-cyclohexylidene-sucrose (4)	Tert-amyl alcohol	24	<5	24
	3,3-dimethyl-2-butanone,	26	<5	14
	no solvent added	24	<5	12
Crude 2,1′:4,6 di-O-isopropylidene-sucrose[e]	Toluene allowed to evaporate after 1 h of mixing	13	<5	57[f]

[a]Yields are based on percentage areas determined by high-performance liquid chromatography analysis after 48 h of reaction time at 75°C on a 0.056 mmol scale as described in the Experimental Procedures section. [b]From Novo Nordisk (Bagsvaerd, Denmark). [c]Yield after 7 d of incubation at 75°C. [d]n/d, Not determined. [e]Contains 30% of 1, some unreacted sucrose and higher acetals. [f]Yield after 72 h of incubation at 75°C.

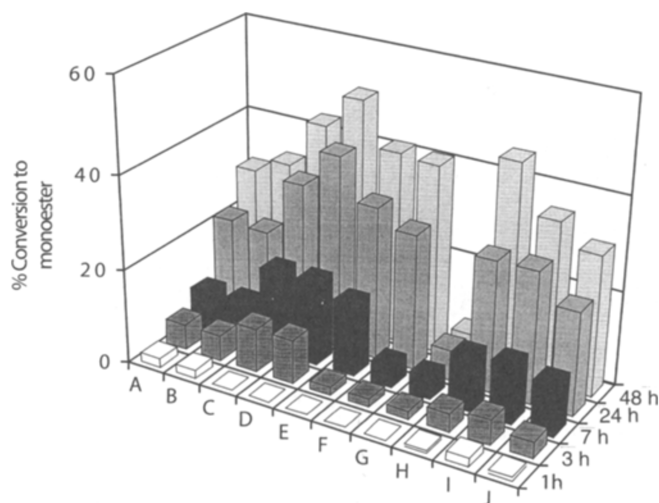


FIG. 2. Kinetics of Lipozyme™-catalyzed (Novo Nordisk, Bagsvaerd, Denmark) 6′-O-esterification of **2** under a range of conditions. In all cases, pure **2** (0.1 mmol) was mixed in a 1:1 molar ratio (1:2 for H) with myristic acid (methyl myristate for I) and 20 mg Lipozyme™ IM-60. The reactions were kept at 75°C and shaken at 180 rpm. A, toluene (200 μL) was added and allowed to evaporate from the beginning of the reaction; B, toluene was added, allowed to evaporate and, after 7 h, more toluene was added and allowed to evaporate after capping the vial for 1 h; C, toluene was added and allowed to evaporate after capping the vial for 1 h; D, toluene was added, the vial was capped for 1 h, and then the toluene was allowed to evaporate; after 7 h, the process was repeated; E, toluene was added and allowed to evaporate after 3 h; F, toluene was added and allowed to evaporate after 7 h; G, toluene added at the start of reaction and not allowed to evaporate by keeping the vial capped throughout; H, solvent-free, di-isopropylidene sucrose/myristic acid molar ratio 1:2, vial uncapped; I, solvent-free with methyl myristate; J, solvent-free with myristic acid.

GC, than the *M. miehei* enzyme and pure di-isopropylidene sucrose (**2**) (Table 1) with all fatty acids tested. Improved rates, observed with the mixture of 2,1′:4,6 di-O-isopropylidene-sucrose and *C. antarctica* lipase, were attributed to a better miscibility of this crude isopropylidene sucrose syrup with molten fatty acid. In accordance with our earlier results, the same major product 6′-O-monoester was obtained. Consequently, *C. antarctica* lipase and the crude preparation of 2,1′:4,6-di-O-isopropylidenesucrose (**2**) were selected for the preparation of sucrose-6′-O-monoesters.

Finally, the preparative synthesis of 6′-O-acyl sucrose was performed with decanoic ($C_{10:0}$), lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), and stearic ($C_{18:0}$) acids. The product of enzymatic esterification was extracted with diethyl ether and aqueous salt solution to remove unreacted sugar and fatty acid, respectively. Isopropylidene groups were then hydrolyzed with aqueous acids as detailed in the Experimental Procedures section. All five sucrose esters were obtained in gram quantities and fully characterized by NMR and FAB–MS (Tables 2 and 3). In all cases, a high degree of regioselectivity was observed where the final product contained up to 98% of 6′-O-monoester by GC (Fig. 3A).

The hydroxyl group at C-6 of the glucose moiety in the substrates **1–4** was protected so that esterification at this position was not possible. However, this hydroxyl group is the most reactive under the conditions of chemical acylation employed in industry. Thus, GC–MS analysis revealed that 6′-O-acyl sucrose accounts for 26% of sucrose monoesters (22% of total composition) in commercial preparations, compared with 46% of sucrose monoesters (40% of total composition) for 6-O-acyl sucrose (Fig. 3B). This observation led us to in-

TABLE 2
^{13}C NMR Chemical Shifts (δ) of Sucrose, Sucrose 6′-*O*-Monoesters and Sucrose 6-*O*-Monoesters in CD_3OD

	Sucrose	Sucrose -6′-C_{10} (**5a**)	Sucrose -6′-C_{12} (**5b**)	Sucrose -6′-C_{14} (**5c**)	Sucrose -6′-C_{16} (**5d**)	Sucrose -6′-C_{18} (**5e**)	Sucrose -6-C_{12} (**6b**)	Sucrose -6-C_{14} (**6c**)	Sucrose -6-C_{16} (**6d**)	Sucrose -6-C_{18} (**6e**)
C-1	93.6	93.4	93.1	93.4	93.4	93.4	93.3	93.4	93.4	93.4
C-2	73.2	73.2	73.2	73.2	73.2	73.2	71.8	71.9	71.9	72.0
C-3	74.4	74.2	74.1	74.2	74.2	74.2	74.3	74.4	74.4	74.4
C-4	71.3	71.4	71.6	71.5	71.4	71.4	71.5	71.6	71.6	71.6
C-5	74.6	74.7	74.6	74.7	74.6	74.6	73.0	73.1	73.1	73.1
C-6	62.2	62.4	62.7	62.5	62.4	62.4	64.6	64.6	64.6	64.6
C-7							175.5	175.5	175.5	175.5
C-1′	63.4	63.7	64.2	63.7	63.7	63.7	63.8	63.9	63.9	63.8
C-2′	105.3	105.5	105.4	105.5	105.5	105.4	105.1	105.2	105.2	105.3
C-3′	79.3	78.8	79.6	78.8	78.9	78.9	79.1	79.2	79.2	79.6
C-4′	75.7	76.8	76.9	76.8	76.8	76.8	75.7	75.8	75.8	76.0
C-5′	83.8	80.6	80.8	80.6	80.6	80.6	83.7	83.8	83.8	83.8
C-6′	64.0	66.9	66.8	66.9	66.9	66.9	63.9	64.1	64.0	64.1
C-7′		175.5	174.7	175.5	175.5	175.4				
CH_2 chain		34.9	34.8	34.9	34.9	34.9	34.9	34.9	34.9	34.9
CH_2 chain		33.0	32.9	33.1	33.0	33.0	33.0	33.1	33.1	33.0
CH_2 chain		30.6	30.6	30.8	30.8	30.8	30.7	30.8	30.8	30.7
CH_2 chain		30.4	30.5	30.6	30.6	30.6	30.6	30.6	30.6	30.5
CH_2 chain		30.2	30.4	30.5	30.5	30.5	30.4	30.5	30.5	30.4
CH_2 chain		26.0	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2
CH_2 chain		23.7	25.9	26.0	25.9	25.9	25.9	26.0	26.0	25.9
CH_2 chain			23.6	23.7	23.7	23.7	23.6	23.7	23.7	23.6
CH_3		14.5	14.6	14.5	14.5	14.5	14.5	14.5	14.5	14.4

vestigate the feasibility of selectively cleaving 6′-*O*-acyl sucrose in the commercial preparation with a 6′-specific lipase, so that 6-*O*-acyl sucrose could then be isolated by column chromatography. The same lipase (Novozym™ 435) proved to be the most suitable enzyme for this reaction and, after the optimization of the reaction conditions, propan-2-ol was selected as the solvent for transesterification.

As shown in Figure 3C, efficient cleavage of 6′-*O*-acyl sucrose was accomplished under these conditions, and 6-*O*-acyl sucrose was isolated in 39–54% yield. The product contained 80% of the required regioisomer (Fig. 3D), and its content could be increased to >90% by rechromatography under the same conditions. The purity of the resultant 6-*O*-acyl sucrose was sufficient for unambiguous characterization by NMR, and the chemical shifts are summarized in Table 2. A similar approach was previously described by Chauvin and Plusquellec (19), who successfully hydrolyzed the unwanted 6-*O*-regioisomer with *C. rugosa* lipase from a mixture of sucrose monoesters prepared by a chemical acylation method with 3-acyl-5-methyl-1,3,4-thiodiazole-2(3H)-thiones.

In conclusion, a facile chemoenzymatic synthesis of 6′-*O*-acyl sucrose was developed using a crude mixture of sucrose acetals. Toluene was initially added to promote miscibility of the reactants and was subsequently evaporated to allow the reaction to continue in a solvent-free manner. Among the enzymes studied, *C. antarctica* lipase (Novozym™ 435) was the best catalyst to perform this reaction. The high purity of the final product and the fact that the whole synthesis can be accomplished in only three steps make this method attractive for the preparation of multi-gram quantities of 6′-*O*-acyl sucrose. This protocol and the described procedure of "upgrading" the commercial preparation of sucrose monoesters to provide a highly enriched fraction of 6-*O*-acyl-sucrose may prove useful in the synthesis of pure regioisomers for struc-

TABLE 3
Preparation and Analytical Data for Sucrose Monoesters (5a–e and 6b–e)

			6′-*O*-acyl-sucrose (**5**)		6-*O*-acyl-sucrose (**6**)	
Fatty acid	Molecular formula	Calculated mass	Measured mass (FAB–MS)[a]	Yield (%)	Yield (%)	6-Monoester content (%)
Caprate ($C_{10:0}$) (**a**)	$C_{22}H_{39}O_{12}$	495.2441	495.2424	23	n.a	n.a
Laurate ($C_{12:0}$) (**b**)	$C_{24}H_{43}O_{12}$	523.2754	523.2754	27	39	78
Myristate ($C_{14:0}$) (**c**)	$C_{26}H_{47}O_{12}$	551.3067	551.3014	26	54	80
Palmitate ($C_{16:0}$) (**d**)	$C_{28}H_{51}O_{12}$	579.3380	579.3370	27	40	80
Stearate ($C_{18:0}$) (**e**)	$C_{30}H_{55}O_{12}$	607.3693	607.3652	20	44	80

[a]Fast atom bombardment–mass spectrometry; n.a., not applicable.

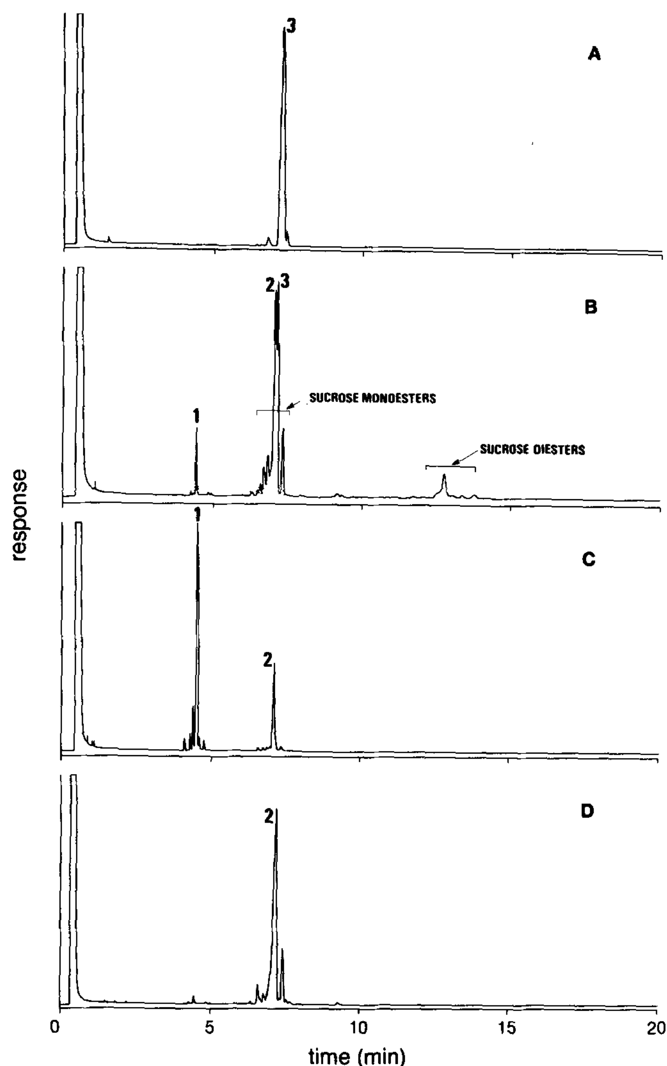


FIG. 3. Gas chromatograms of: A, 6′-*O*-lauroyl-sucrose (**5b**) synthesized by lipase-catalyzed esterification of **2** and acid-catalyzed hydrolysis of the isopropylidene groups; B, Ryoto™ L1695 (Mitsubishi-Kasei Food Corp., Tokyo, Japan) commercial preparation of sucrose monolaurate; C, the product obtained after Novozym™ (Novo Nordisk, Bagsvaerd, Denmark) 435-catalyzed transesterification of L1695 in 2-propanol to cleave 6′-*O*-lauroyl sucrose (**5b**) and subsequent precipitation of **6b** and sucrose from the reaction mixture; D, an enriched fraction of 6-*O*-lauroyl-sucrose (**6b**) obtained by column chromatography. Peak labelling: 1 = sucrose; 2 = 6-*O*-lauroyl sucrose (**6b**); 3 = 6′-*O*-lauroyl sucrose (**5b**).

ture–function relationship studies and, perhaps, some specialized applications.

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