Enzymatic Synthesis of Various 1′-*O*-Sucrose and 1-*O*-Fructose Esters

Giacomo Carrea, Sergio Riva,* and Francesco Secundo
Istituto di Chimica degli Ormoni, C.N.R. Via Mario Bianco 9, 20131 Milano, Italy
Bruno Danieli
Dipartimento di Chimica Organica ed Industriale, Centro C.N.R. di Studio delle Sostanze Organiche Naturali, Via Venezian 21, 20133 Milano, Italy

A crude preparation of the proteolytic enzyme subtilisin has been used to catalyse the regioselective esterification of sucrose in anhydrous dimethylformamide. In this way 1′-*O*-sucrose esters bearing acyl groups of different sizes and types have been synthesized. These sucrose derivatives have been hydrolysed by yeast α-glucosidase to the corresponding 1-*O*-fructose esters, not easily attainable by chemical methods.

Sucrose is a regenerable resource of great commercial importance.[1] In addition to its universal use as a sweetener, sucrose is used as a carbon source in several fermentation processes for making a wide class of compounds ranging from ethanol to complex biologically active molecules.[2] Several sucrose derivatives have also been synthesized and of these particular attention has been paid to selectively chlorinated compounds, which have proved to be several hundred times sweeter than the parent sugar,[3] and to fatty acid esters that are currently utilized as anticaries agents, biodegradable surfactants, and emulsifiers in foods and cosmetics. The fatty acid esters are prepared commercially either by aspecific esterification of sucrose with acyl chlorides or by random transesterification between sucrose and fatty acid esters, as described in the patent literature.[4]

Enzymes have also been used to obtain sucrose esters. In a first approach, lipases from different sources were used in aqueous buffers containing the disaccharide and fatty acids:[5] complex mixtures of mono-, di-, and poly-acylsucrose were obtained and the products were not characterized. More recently, Klibanov reported that hydrolytic enzymes in pure organic solvents can be successfully employed to catalyse the transesterification reaction between activated esters of fatty acids and alcohols.[6] Monosaccharides esterified regioselectively at the primary hydroxy group were obtained with porcine pancreatic lipase suspended in dry pyridine.[7] The same methodology was used to catalyse transesterification reactions between a number of sugar alcohols and various plant and animal oils. These enzymatically prepared sugar alcohol esters have proved to be excellent surfactants, reducing the interface and surface tension at a much lower concentration than their chemically produced counterparts.[8] In a later report,[9] several di- and poly-saccharides were also acylated regioselectively using proteases to catalyse the transesterification between sugars and the activated trichloroethyl butyrate in anhydrous dimethylformamide (DMF). In the case of sucrose the unusual esterification at the 1′-*O*-hydroxy group was observed.

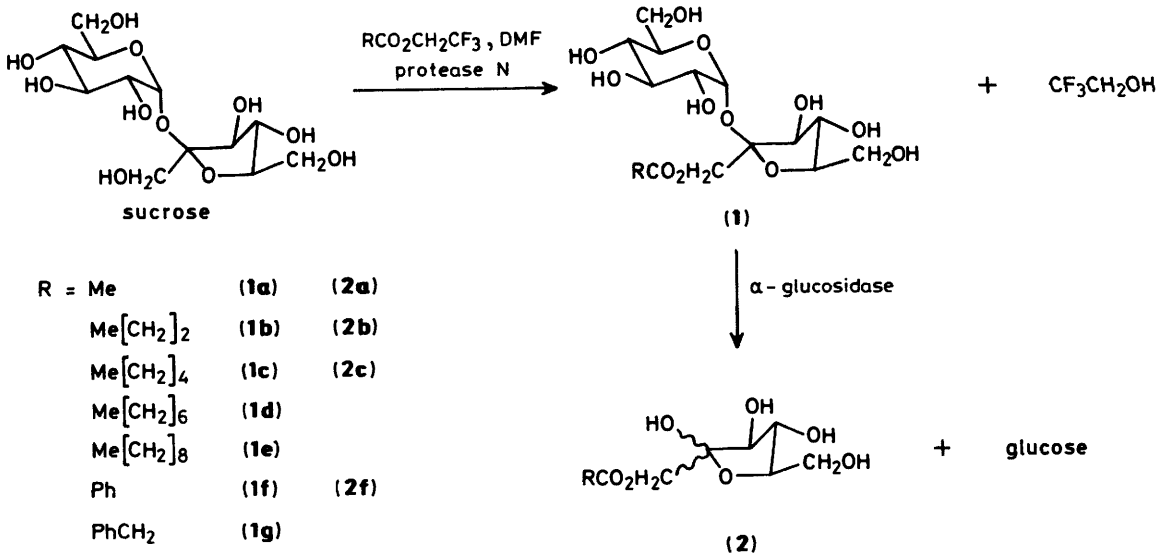
In this study we carried out a systematic investigation of the usefulness of this enzymatic approach for the synthesis of a large series of 1′-*O*-sucrose esters, bearing acyl groups of different sizes and types. Moreover, we found that these compounds could easily be converted, *via* α-glucosidase-promoted enzymatic hydrolysis, into the corresponding pure 1-*O*-acylfructose, which, surprisingly enough, had not yet been synthesized by conventional chemical reactions.

Results and Discussion

Sucrose is a non-reducing disaccharide containing eight hydroxy groups, three of which are primary (6-OH, 1′-OH, and 6′-OH). Its fundamental chemistry has been carefully investigated[1a,10] and a profile of the reactivity of its hydroxy groups has been identified, allowing the modification of specific positions in the molecule. As expected, primary hydroxys are the most reactive, but with clear differences among them. Analysis of the products obtained after tritylation[11] or tosylation[12] indicated that reactivities are in the order 6-OH ≈ 6′-OH > 1′-OH. Reaction with sulphuryl chloride,[13] which involves an intramolecular nucleophilic substitution with insertion of chloride, showed the order 6-OH ≈ 6′-OH > 4-OH > 1′-OH. Reaction with PPh_3–CCl_4 in pyridine at 70 °C gave 6,6′-dichloro-6,6′-dideoxysucrose, in 92% yield.[14] Treatment of sucrose with acetic anhydride (1.1 mol equiv.) in pyridine at −40 °C gave 6-*O*-acetylsucrose in 40% yield.[1a] On the other hand, 6′-OH was the most reactive site towards silylating agents.[15] All these examples indicate clearly that the 1′-OH is less reactive than the other primary groups 6-OH and 6′-OH, presumably due to its neopentyl-like character. Despite this, 1′-*C*-modified sucrose derivatives have been prepared through multistep syntheses in order to study the role of this part of the molecule in the 'sweet response'[3,16] and in the binding of the disaccharide to carrier proteins in plant species.[17] Substitution of 1′-OH with a chloride resulted in an intensification of sweetness, while introduction of a fluorine or of an azide group enhanced the binding potency.

As we mentioned in the introduction, it has been recently reported[9] that subtilisin, a protease from *Bacillus subtilis*, is able to catalyse the regioselective esterification of disaccharides in anhydrous DMF. Using sucrose as substrate, this enzyme (either as a highly purified powder or as a crude preparation) promoted the unusual esterification of the 1′-OH and in this way 1′-*O*-butyrylsucrose has been prepared on a gram scale.

We were interested in exploring the ability of a cheap, crude preparation of this enzyme (protese N from Amano) to introduce a large variety of acyl residues other than the model butyryl moiety (Scheme). To that end, we first determined the rate of the transesterification reaction between sucrose and various trichloroethyl or trifluoroethyl esters in dry DMF. The data in Table 1 indicate that the enzyme tolerated different simple aliphatic and aromatic acyl groups but was quite sensitive to the length of the aliphatic chain, the reactivity of trifluoroethyl octanoate being only 8% that of the corresponding butyryl ester. These kinetic data were in accord with preparative-scale experiments. As reported in Table 2, we observed almost the same conversion with trifluoroethyl hexanoate and trifluoroethyl butyrate, but the yield of isolated sucrose monoesters dramatically dropped as the acyl group lengthened. In addition, reasonable yields were obtained with trichloroethyl acetate and trifluoroethyl benzoate, but no



Scheme. Enzymatic synthesis of 1′-*O*-acylsucroses and 1-*O*-acylfructoses

Table 1. Initial rates of the reaction between sucrose and various trichloroethyl (TCE) or trifluoroethyl (TFE) esters catalysed by 'protease N' in anhydrous DMF

Ester	Intial reaction rate[a] (μmol h^{-1})
Ac-TCE	0.48
$Me[CH_2]_2CO$-TCE	3.03
$Me[CH_2]_2CO$-TFE	3.34
$Me[CH_2]_4CO$-TFE	1.89
$Me[CH_2]_6CO$-TFE	0.26
CH_2=CHCO-TFE	0
PhCO-TFE	0.51

[a] Conditions: 0.15M-sucrose; 0.45M-ester; protease N (100 mg ml^{-1}); 45 °C. Suspensions in anhydrous DMF (1 ml) were shaken at 250 r.p.m. in an orbital shaker. At 15, 30, 45 min, aliquots (10 μl) were withdrawn, derivatized with HMDS (10 μl) plus trifluoroacetic acid (1 μl), and assayed by g.c. No appreciable reaction was detected in the absence of the enzyme.

Table 2. Transesterification reactions between various trichloroethyl (TCE) or trifluoroethyl (TFE) esters and sucrose catalysed by 'protease N' in anhydrous DMF[a]

Ester	Reaction time (h)	Conversion (%)[b]	Isolated yield of monoacyl sucrose[c]
Ac-TCE	48	39	1.40 g (36%)
$Me[CH_2]_2CO$-TFE	24	78	2.64 g (64%)
$Me[CH_2]_4CO$-TFE	48	73	2.70 g (61%)
$Me[CH_2]_6CO$-TFE	48	29	1.20 g (26%)
$Me[CH_2]_8CO$-TFE	48	7	0.16 g (4%)
CH_2=CHCO-TFE	48		
PhCO-TFE	48	35	1.42 g (32%)
$PhCH_2CO$-TFE	48	21	0.78 g (12%)[d]
PhCH=CHCO-TFE	48		

[a] Conditions: sucrose (3.42 g, 10 mmol); molar excess of the ester: 4-fold in all instances, except for TFE butyrate and TFE hexanoate where it was 2-fold and 3-fold respectively; solvent DMF (30 ml); enzyme (pH-adjusted, see Experimental section) (100 mg ml^{-1}); 45 °C; shaking at 250 r.p.m. in an orbital shaker. After the periods of time indicated in the second column of the Table, the enzyme was removed by filtration, the solvent was evaporated off under reduced pressure, and the remaining residue was subjected to silica gel chromatography with a mixture of ethyl acetate–methanol–water (90:10:5) as solvent. [b] Determined by g.c. [c] The isolated product came out of the silica gel column as a single peak and was pure by t.l.c. It was subsequently analysed (^{13}C n.m.r. and f.a.b.-m.e.) (see Experimental section) which showed overwhelming monoacylation at the 1′-*O* position of the fructose moiety. [d] This product was a 1:1 mixture of 1′-*O*- and 6′-*O*-monoacyl esters.

reaction at all with acryloyl and cinnamoyl activated esters was observed.

Products were isolated by silica gel chromatography and characterized as the 1′-*O*-acylsucrose by their ^{13}C n.m.r., ^{1}H n.m.r., and f.a.b.-m.s. spectra. Compared with the parent sucrose, the ^{13}C n.m.r. spectra of these derivatives showed the expected downfield shift of C-1′ and upfield shift of C-2′, in agreement with the general trend observed by Yoshimoto *et al.*[18] Small amounts (< 5%) of different monoester isomers were also indicated by minor peaks present in the spectra, but their acylation positions could not be assigned. Interestingly, major contamination was observed in octanoyl- and decanoyl-sucrose (roughly 10 and 25%, respectively) and thus elongation of the aliphatic chain influenced not only the rate and the yield but also the regioselectivity of the reaction. The ^{1}H n.m.r. spectrum (300 MHz) of the acetyl derivative (**1a**) confirmed the structural assignment. The methylene protons at C-1′ are the only AB system in the molecule: its doublets were clearly deshielded and were separated from the bulk of the oxymethyne and oxymethylene protons, thus showing the esterification site unambiguously. F.a.b.-m.s. spectra of compounds (**1a**—**g**) were recorded in the negative mode using glycerol as matrix; this soft ionization technique allowed direct analyses of these poorly volatile compounds with no need for chemical pre-derivatization.[19] The F.a.b.-m.s. spectrum of the hexanoate (**1c**) showed an intense pseudomolecular ion $(M - H)^-$ at 439 daltons. Other peaks were observed at *m/z* 341, 323, 115 [easily assigned as $(M - C_5H_{11}CO)^-$, $(M - C_5H_{11}CO_2H - H)^-$, and $C_5H_{11}CO_2^-$, respectively], while cleavage at the glycosidic oxygen produced the ions at *m/z* 161 and 179 (glucose moiety) and 277 (acylated fructose moiety). Finally, metastable ions were observed at a.u. 265 and 237.7, showing that the origin of the ions at *m/z* 341 and 323 was from the pseudomolecular ion at *m/z* 439. Similar fragmentation patterns were observed for all the other derivatives.

Since the recovered enzyme showed no residual activity after lyophilization, we decided to investigate the time course of inactivation of protease N suspended in dry DMF at 45 °C. As depicted in the Figure, the half-life for the enzyme shaken in the

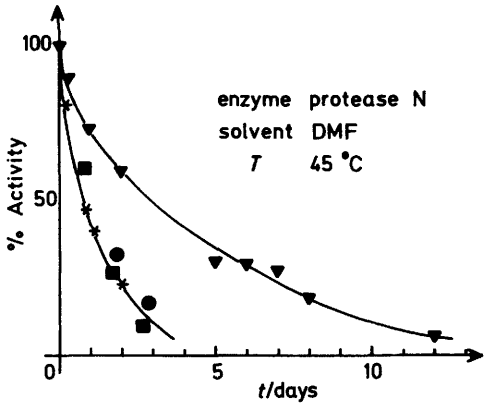
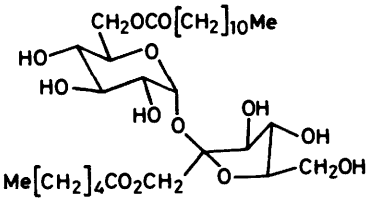


Figure. The time course of inactivation of the pH-adjusted, freeze-dried protease N suspended in anhydrous DMF. Crude enzyme (100 mg) was added to the solution (1 ml) in different vials and the suspensions were shaken at 45 °C. At the scheduled time the DMF was decanted, the solid was dissolved in TRIS buffer (1 ml; 0.1M, pH 8), and the residual activity was analysed spectrophotometrically with *N*-benzoyl-arginine ethyl ester (BAEE) as substrate in the hydrolytic reaction [1 ml cuvette; 1 cm optical path; TRIS buffer (950 μl; 0.5M; pH 8); enzyme solution (50 μl); BAEE (25 μg); monitoring of the increasing of absorbance at 255 nm]. ▼ pure DMF; ● 0.3M-sucrose in DMF; ■ 0.6M-TFE butyrate in DMF; * 0.3M-sucrose plus 0.6M-TFE butyrate in DMF

pure solvent was *ca.* 3 days. However, inactivation was much faster under reaction conditions (*i.e.*, in the presence of 0.3M-sucrose and 0.6M-trifluoroethyl butyrate): after 2 days the residual activity was only 26%. Almost the same data were obtained for protease N suspended in dry DMF containing either 0.3M-sucrose or 0.6M-activated ester. These experiments clearly show that under such harsh conditions for the enzyme, no reaction times longer than 2 days should be used.

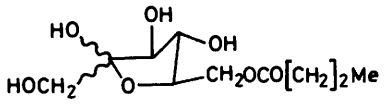
Since protease N failed to catalyse transesterification with long-chain fatty acids, we thought that lipases might be the right enzymes to obtain this type of acylated sucrose derivative. However, it has been shown that lipases are not catalytically active in DMF and no acylation of disaccharides takes place in pyridine.[6,7] On the other hand it is known that lipase activity is best retained in aprotic apolar or poorly polar solvents.[6,20] Exploiting the increased solubility of derivative (**1c**) in dry acetone, we have found that lipase from *Chromobacterium viscosum* catalyses the regioselective transesterification between trifluoroethyl laurate (dodecanoate) and (**1c**) to afford 6-*O*-dodecanoyl-1′-*O*-hexanoylsucrose (**3**) (31% yield) uncon-



(3)

taminated by other regioisomers. The negative f.a.b.-m.s. spectrum of compound (**3**) showed a pseudomolecular ion $(M - H)^-$ at 621 daltons and other peaks at *m/z* 523 $(M - C_5H_{11}CO)^-$ and 439 $(M - C_{11}H_{23}CO)^-$. As no other significative signals were present, the f.a.b.$^+$ spectrum was also recorded. In this case cationized pseudomolecular ions were observed at *m/z* 661 $(M + K)^+$ and 645 $(M + Na)^+$; other diagnostically useful peaks at 345 and 261 daltons were assigned to dodecanoyl- and hexanoly-monosaccharide respectively. The presence of these ions indicated that the second acylation step had occurred at the glucopyranosyl moiety and this conclusion was confirmed by ^{13}C n.m.r. analysis. Compared with (**1c**), the ^{13}C n.m.r. spectrum of compound (**3**) showed a clear downfield shift of C-6 and upfield shift of C-5 while all the other signals were practically unchanged.

1′-*O*-Sucrose esters appeared to be interesting starting materials for obtaining new 1-*O*-acylfructose derivatives. In fact compound (**1c**) was recognized as a substrate by yeast α-glucosidase, which is an enzyme that promotes hydrolysis of α-glucosides. A buffer solution of compound (**1c**) containing this enzyme was left at room temperature for 2 days and then freeze-dried. Silica gel chromatography furnished a pale yellow oil in good yield (81%), identifed as 1-*O*-hexanoylfructose (**2c**) by inspection of its ^{13}C n.m.r. spectrum which showed three sets of signals due to the three anomeric isomers with the expected downfield shift of C-1 and upfield shift of C-2. In the same way, other 1-*O*-acylfructoses were prepared from the parent sucrose esters (Scheme). It is worthwhile mentioning here that, in general, monosubstituted fructose derivatives, useful as chiral synthons, can be prepared only through tedious multistep procedures and that, to our knowledge, the chemical preparation of 1-*O*-acylfructoses has not been previously reported in the literature. In addition to this, direct enzymatic esterification of fructose with protease N and trichloroethyl butyrate in DMF was not selective, affording a mixture of two compounds running close together on t.l.c. They were identified as 1-*O*-butyrylfructose (**2b**) and 6-*O*-butyrylfructose (**4**), ratio 8:2, from



(4)

^{13}C n.m.r. analysis (see Experimental section). A similar mixture of compounds had been previously obtained using porcine pancreatic lipase in pyridine.[7]

The examples reported in this paper show that proteases or combinations of proteases and lipases or proteases and α-glucosidase can be successfully employed for the regioselective formation of mono- or di-acylsucrose and of monoacylated fructose derivatives. As these compounds are not easy to make by conventional chemical reactions, our results are another example of the usefulness of enzymatic methodology to organic synthesis. Enzyme-promoted acylation of more complex sugar derivatives, such as natural glycosides, is currently under investigation.

Experimental

Protease N, a crude preparation of subtilisin (EC 3.4.21.14, protease from *B. subtilis*), was obtained from Amano Pharmaceutical Co. (Frankfurt am Main, F. R. Germany); prior to use this enzyme preparation was dissolved in water, and the solution adjusted to pH 7.8 and freeze-dried.[6] Yeast α-glucosidase, type I (EC 3.2.1.20) was from Sigma. DMF (analytical grade) was used without further purification apart from drying by shaking with 3Å molecular sieves (Merck). Sucrose, (−)-β-D-fructose, 2,2,2-trichloroethanol, 2,2,2-trifluoroethanol, and the

acyl chlorides were purchased from Fluka. Enzymatic esterifications were followed by gas chromatography (g.c.) with a 5 m HP1 capillary silica gel column coated with methylsilicon gum (Hewlett Packard) (N_2 carrier gas 30 ml min^{-1}; detector and injector port temperature 300 °C); all reaction mixtures were subjected to precolumn derivatization with 1,1,1,3,3,3-hexamethyldisilazane (HMDS).[21] T.l.c. was performed on precoated silica gel 60 F plates from Merck; developer A = AcOEt–MeOH–water (75:25:5), 8 = AcOEt–MeOH–water (90:10:5). M.p.s are uncorrected and were measured in open-ended capillaries. Optical rotations were measured at 589 nm (sodium line) at 25 °C in a Perkin-Elmer 141 polarimeter. ^{13}C N.m.r. spectra were run on a Varian XL-200 (50.2 MHz) spectrometer, and ^1H n.m.r. spectra on a Bruker CPX-300 (300 MHz) spectrometer. F.a.b.-m.s. analysis was performed on a VG analytical 70-70 EQ-HF instrument equipped with its own source. DMSO is dimethyl sulphoxide.

Synthesis of Trichloroethyl and Trifluoroethyl Esters.—Trichloroethyl and trifluoroethyl esters were synthesized from the corresponding acyl chloride and 2,2,2-trichloroethanol or 2,2,2-trifluoroethanol according to a general methodology.[22] The b.p.s of the resultant esters were as follows: trichloroethyl acetate 168 °C, trichloroethyl butyrate 88—89 °C (18 mmHg), trifluoroethyl butyrate 112 °C, trifluoroethyl hexanoate 152—154 °C, trifluoroethyl octanoate 84 °C (18 mmHg), trifluoroethyl decanoate 137—139 °C (18 mmHg), trifluoroethyl dodecanoate 162 °C (18 mmHg), trifluoroethyl hexadecanoate 210 °C (18 mmHg), trifluoroethyl acrylate 85—87 °C, trifluoroethyl benzoate 120 °C (35 mmHg), trifluoroethyl phenylacetate 98 °C (18 mmHg), and trifluoroethyl cinnamate 162 °C (18 mmHg). Ester purity was at least 98.5% as determined by g.c.

Enzymatic Synthesis of 1′-O-*Acylsucroses.*—The experimental protocol is described in Table 1.

1′-O-*Acetylsucrose* (**1a**). R_F (A) 0.29; m.p. 220—221 °C (from MeOH); $[\alpha]_D^{25}$ +64.8 °C (*c* 1 in water); δ_H (300 MHz; D_2O) 5.47 (1 H, d, 1-H), 4.33 (1 H, d, 1′-H), and 4.25 (1 H, d, 1′-H); δ_C (50.2 MHz; D_2O) 102.2 (C-2′), 92.7 (C-1), 81.6 (C-5′), 76.8 (C-3′), 73.5 (C-4′), 72.6 (C-3 and -5), 71.0 (C-2), 69.2 (C-4), 63.0 (C-1′), 62.1 (C-6′), and 60.1 (C-6); for the acetyl moiety: δ_C 173.7 and 20.2; f.a.b.-m.s., *m/z* 383 [(*M* − H), 100%] and 341 (61).

1′-O-*Butyrylsucrose* (**1b**). Amorphous solid, R_F (A) 0.41; δ_C (50.2 MHz; D_2O) 102.4 (C-2′), 92.7 (C-1), 81.6 (C-5′), 76.7 (C-3′), 73.5 (C-4′), 72.6 (C-3 and -5), 71.0 (C-2), 69.2 (C-4), 62.5 (C-1′), 62.1 (C-6′), and 60.1 (C-6); for the butyryl moiety: δ_C 176.1. 35.7, 18.0, and 12.9; f.a.b.-m.s., *m/z* 411 (*M* − H, 100%), 341 (62), 323 (5), 249 (4), 179 (15), 161 (7), 159 (9), and 87 (91).

1′-O-*Hexanoylsucrose* (**1c**). Amorphous solid, R_F (A) 0.50; δ_C (50.2 MHz; D_2O) 102.4, 92.7, 81.6, 76.7, 73.5, 72.6, 71.0, 69.2 62.5, 62.1, and 60.1; for the hexanoyl moiety: δ_C 176.3, 33.7, 32.5, 24.0, 21.6, and 13.2; f.a.b.-m.s., *m/z* 439 (*M* − H, 43%), 341 (60), 323 (4), 277 (1), 179 (5), 161 (3), 159 (3), and 115 (100).

1′-O-*Octanoylsucrose* (**1d**). Amorphous solid, R_F (A) 0.53; δ_C (50.2 MHz; D_2O) 102.5, 92.7, 81.8, 76.8, 73.1, 72.6, 71.0, 69.2, 62.4, 61.7, and 60.2; for the octanoyl moiety: δ_C 174.9, 33.9, 31.6, 28.9, 28.8, 24.6, 22.5, and 13.8; f.a.b.-m.s., *m/z* 467 (*M* − H, 58%), 341 (100), 323 (8), 305 (2), 179 (15), 161 (7), 159 (8), and 143 (91).

1′-O-*Decylsucrose* (**1e**). Amorphous solid, R_F (A) 0.58; δ_C (50.2 MHz; D_2O) 102.6, 92.8, 81.9, 77.0, 73.1, 72.8, 71.1, 69.4, 62.5, 61.6, and 60.4; for the decyl moiety: δ_C 174.5, 34.0, 32.0, 29.7, 20.5, 29.3, 24.8, 22.7, and 13.9; f.a.b.-m.s., *m/z* 495 (*M* − H, 44%), 341 (64), 333 (1), 323 (5), 179 (12), 171 (100), 161 (6), and 159 (8).

1′-O-*Benzoylsucrose* (**1f**). Amorphous solid, R_F (A) 0.43; δ_C (50.2 MHz; D_2O) 102.5, 92.9, 81.7, 76.8, 73.5, 72.7, 71.0, 69.3, 63.1, 62.0, and 60.2; for the benzoyl moiety: δ_C 167.6, 134.0, 129.6, and 128.7; f.a.b.-m.s., *m/z* 445 (*M* − H, 51%), 341 (6), 323 (5), 283 (3), 179 (12), 161 (5), 159 (6), and 121 (100).

1′-O- *and* 6′-O-*Phenylacetylsucrose* (**1g**). Oil, R_F (A) 0.31; f.a.b.-m.s., *m/z* 459 (*M* − H, 71%), 341 (100), 325 (4), 323 (2), 297 (3), 207 (4), 179 (5), 161 (3), 159 (4), and 135 (35); δ_C (50.2 MHz; [$^2H_6$]DMSO) 102.0, 92.0, 82.6, 76.5, 73.1, 72.8, 72.7, 72.6, 70.0, 62.6, 61.6, and 60.7 (1′-*O*-phenylacetylsucrose); 104.2, 91.7, 79.0, 76.5, 74.7, 72.8, 72.7, 71.3, 69.7, 66.0, 61.8, and 60.4 (6′-*O*-phenylacetylsucrose); for the phenylacetyl moiety: δ_C 171.1 and 170.6, 134.2 and 134.1, 129.3, 128.3, and 126.7.

Enzymatic Synthesis of 1-O-*Acylfructoses.*—Compound (**1c**) (2.7 g) was dissolved in 0.02M-phosphate buffer (200 ml; pH 5). Yeast α-glucosidase (100 mg) was added, and the solution was left at room temperature for two days and then freeze-dried. The crude residue was purified by silica chromatography with AcOEt–MeOH–water (95:5:1) as eluant. The other 1-*O*-acylfructoses were prepared in the same way.

1-O-*Acetylfructose* (**2a**). Pale yellow oil, R_F (B) 0.24; δ_C (50.2 MHz; D_2O) [a mixture of β-pyranoside (P) and α- and β-furanoside (F_α and F_β)] 103.3 (C-2, F_α), 99.9 (C-2, F_β), 97.0 (C-2, P), 82.0 (C-3, F_α), 81.7 (C-5, F_α), 80.9 (C-5, F_β), 76.5 (C-3, F_β), 75.7 (C-4, F_α), 74.3 (C-4, F_β), 69.4 (C-4, P), 69.0 (C-5, P), 68.1 (C-3, P), 65.8 (C-1, P), 65.2 (C-1, F_β), 64.1 (C-1, F_α), 63.7 (C-6, P), 62.2 (C-6, F_β), and 61.0 (C-6, F_α); for the acetyl moiety: δ_C 174.9 and 173.9, 20.4 and 20.3; f.a.b.-m.s., *m/z* 221 (*M* − H, 100%), 179 (30), 161 (39), and 149 (36).

1-O-*Butyrylfructose* (**2b**). Pale yellow oil, R_F (B) 0.38; δ_C (50.2 MHz; D_2O) 103.3, 100.0, 97.1, 82.0, 81.6, 80.9, 76.4, 75.8, 74.2, 69.4, 68.0, 65.6, 64.6, 64.1, 63.7, 62.1, and 61.1; for the butyryl moiety: δ_C 176.4 and 176.2, 35.8 and 35.6, 17.9, and 12.9; f.a.b.-m.s., *m/z* 249 (*M* − H, 14%), 179 (6), 177 (12), 161 (9), and 87 (100).

1-O-*Hexanoylfructose* (**2c**). Oil, R_F (B) 0.45; δ_C (50.2 MHz; D_2O) 103.3, 100.0, 97.0, 81.9, 81.8, 80.9, 76.4, 76.0, 74.1, 69.4, 69.0, 68.1, 65.6, 64.5, 64.2, 63.6, 62.0, and 61.3; for the hexanoyl moiety: δ_C 177.2 and 176.9, 34.5, 31.5, 24.9, 22.6, and 14.1; f.a.b.-m.s., *m/z* 277 (*M* − H, 12%), 205 (10), 179 (7), 161 (10), and 115 (100).

1-O-*Benzoylfructose* (**2f**). Oil, R_F (B) 0.45; δ_C (50.2 MHz; [$^2H_6$]DMSO) 102.8, 99.7, 96.7, 82.0, 81.1, 80.9, 76.1, 75.7, 74.1, 69.1, 68.6, 67.6, 65.6, 64.9, 64.6, 63.2, 62.1, and 61.1; for the benzoyl moiety: δ_C 166.3 and 166.1, 133.6 and 133.5, 129.3 and 129.2, 128.7 and 128.6; f.a.b.-m.s., *m/z* 283 (*M* − H, 38%), 211 (5), 179 (6), 161 (8), and 121 (100).

Esterification of Fructose Catalysed by Protease N in Dry DMF.—Fructose (1.8 g, 10 mmol) and trifluoroethyl butyrate (3.4 g, 20 mmol) were dissolved in dry DMF (30 ml). Protease N (3 g) was added and the suspension was shaken at 45 °C for 24 h. The enzyme was removed by filtration, the solvent was evaporated off, and the remaining residue was subjected to silica chromatography to yield a pale yellow oil (1.65 g, 6.59 mmol). ^{13}C N.m.r. analyses showed the presence of two monobutyrylfructose derivatives. Further purification by silica chromatography allowed the separation of the two compounds that were identified as 1-*O*-butyrylfructose (**2b**), major product, and 6-*O*-butyrylfructose (**4**). δ_C (50.2 MHz; D_2O) 176.4 and 176.2, 103.3, 100.0, 97.1, 82.0, 81.6, 80.9, 76.4, 75.8, 74.2, 69.4, 68.0, 65.6, 64.6, 64.1, 63.7, 62.1, 61.1, 35.8 and 35.6, 17.9, and 12.9 (1-*O*-butyrylfructose); 176.7, 104.6 (C-2, F_α), 101.8 (C-2, F_β), 81.9 (C-3, F_α), 78.7 (C-5, F_α), 77.9 (C-5, F_β), 76.4 (C-4, F_α), 75.1 (C-3, F_β), 74.7 (C-4, F_β), 64.8 (C-6, F_β), 63.8 (C-1, F_α), 62.7 (C-1, F_β), 35.7, 33.5, and 30.9 (6-*O*-butyrylfructose).

Enzymatic Synthesis of 6-O-*Dodecanoyl*-1′-O-*hexanoylsucrose* (**3**).—Lipase (3.75 g) from *Chromobacterium viscosum* was added to dry acetone (25 ml) containing (**1c**) (1 g) and

trifluoroethyl laurate (3.1 g, 5 mol equiv.). The suspension was shaken at 45 °C and the formation of a new and less polar compound was soon observed on t.l.c. After 5 days the enzyme was filtered off, the solvent was evaporated off, and the crude residue was purified by silica chromatography to yield compound (**3**) (0.45 g, 31%) as an amorphous solid, δ_C (50.2 MHz; [$^2H_6$]DMSO) 102.1 (C-2′), 91.9 (C-1), 82.9 (C-5′), 76.5 (C-3′), 73.7 (C-4′), 72.5 (C-3), 71.2 (C-2), 70.1 (C-4 and -5), 63.5 (C-6), 62.4 (C-1′), and 62.1 (C-6′); for the dodecanoyl and hexanoyl moieties: δ_C 174.4, 172.9, and all the signals due to the aliphatic chains; f.a.b.-m.s. (negative), *m/z* 621 (*M* − H, 60%), 523 (65), 439 (9), 199 (100), and 114 (60); (positive), *m/z* 661 (*M* + K, 1), 645 (*M* + Na, 2), 345 (6), 261 (100), 183 (16), and 99 (85).

Acknowledgements

We thank the Consiglio Nazionale delle Ricerche, Rome, Progetto Finalizzato 'Biotecnologie e Biostrumentazioni' for financial support of this work.

References

1 (*a*) R. Khan, *Pure Appl. Chem.*, 1984, 56, 833; (*b*) H. Schiweck, K. Rapp, and M. Vogel, *Chem. Ind. (London)*, 1988, 228.
2 R. Khan and A. J. Forage, *Sugar Technol. Rev.*, 1979, **7**, 175.
3 L. Hough, *Chem. Soc. Rev.*, 1985, **14**, 357.
4 For instance, see T. Yamamoto and K. Kinami, *Chem. Abstr.*, 1987, **105**, P138727f; H. Konishi, *ibid.*, 1987, **106**, P120176r; R. O. Feuge, H. J. Zeringue, T. J. Weiss, and M. Brown, *J. Am. Oil Chem. Soc.*, 1970, **47**, 56; L. Osipow, F. D. Snell, W. C. York, and A. Finchler, *Ind. Eng. Chem.*, 1956, **48**, 1459.
5 H. Seino and T. Uchibori, *J. Am. Oil Chem. Soc.*, 1984, **61**, 1761.
6 A. M. Klibanov, *CHEMTECH*, 1986, **16**, 354.
7 M. Therisod and A. M. Klibanov, *J. Am. Chem. Soc.*, 1986, **108**, 5638.
8 J. Chopineau, F. D. McCafferty, M. Therisod, and A. M. Klibanov, *Biotechnol. Bioeng.*, 1988, **31**, 208.
9 S. Riva, J. Chopineau, A. P. G. Kieboom, and A. M. Klibanov, *J. Am. Chem. Soc.*, 1988, **110**, 584.
10 L. Hough and A. C. Richardson, *Pure Appl. Chem.*, 1977, **49**, 1069; L. Hough in 'Sucrochemistry,' ACS Symposium Series 41, ed. J. L. Hickson, American Chemical Society, Washington D. C., 1977, p. 9; Khan, *ibid.*, p. 40.
11 T. Otake, *Bull. Chem. Soc. Jpn.*, 1970, **43**, 3199; 1972, **45**, 2895.
12 J. M. Ballard, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, 1980, **83**, 138.
13 L. Hough, S. D. Phadnis, and E. Tarelli, *Carbohydr. Res.*, 1975, **44**, 37.
14 A. K. M. Anisuzzaman and R. L. Whistler, *Carbohydr. Res.*, 1980, **78**, 185.
15 F. Franke and R. D. Guthrie, *Aust. J. Chem.*, 1977, **31**, 639; 1978, **32**, 1285.
16 R. D. Guthrie, I. D. Jenkins, and J. J. Watters, *Aust. J. Chem.*, 1980, **33**, 2487.
17 P. J. Card, W. D. Hitz, and K. G. Ripp, *J. Am. Chem. Soc.*, 1986, **108**, 158.
18 K. Yoshimoto, Y. Itatani, and Y. Tsuda, *Chem. Pharm. Bull.*, 1980, **28**, 2065.
19 For a recent review on f.a.b.-m.s. analysis of carbohydrates see J. R. J. Paré, K. Jankowski, and J. W. ApSimon, *Adv. Heterocycl. Chem.*, 1987, **42**, 336.
20 M. Therisod and A. M. Klibanov, *J. Am. Chem. Soc.*, 1987, **85**, 3977.
21 C. C. Weeley, R. Beutley, M. Mikita, and W. W. Wells, *J. Am. Chem. Soc.*, 1963, **85**, 2497.
22 W. Steglich and G. Hoffe, *Angew. Chem., Int. Ed. Engl.*, 1969, **8**, 981.

Received 27*th October* 1988; *Paper* 8/04285C