ELSEVIER

Carbohydrate Research 300 (1997) 103–108

CARBOHYDRATE RESEARCH

Chemoenzymatic synthesis of glucose fatty esters

Ines Redmann [a], Michel Pina [a], Bernard Guyot [a], Pierre Blaise [b], Marie Farines [b], Jean Graille [a,*]

[a] *Laboratoire de Lipotechnie, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Département Cultures Pérennes, BP 5035, F-34032 Montpellier, France*
[b] *Laboratoire de Chimie Organique des Substances Naturelles, Université de Perpignan, Avenue de Villeneuve, F- 66860 Perpignan, France*

Received 20 May 1996; accepted in revised form 18 December 1996

Abstract

D-Glucose fatty esters at C-6 were obtained by chemoenzymatic synthesis involving 1,2-*O*-cyclohexylidene-α-D-glucofuranose (**1**) followed by hydrolysis of the cyclohexylidene protecting group. The enzymatic esterification of **1** was performed with fatty acids of variable chain lengths (C8:0 to C18:0). The kinetic of the reaction was studied for each fatty acid and the structure of the octanoyl ester was determined by ^{1}H and ^{13}C NMR spectroscopy. © 1997 Elsevier Science Ltd.

Keywords: D-Glucose fatty esters; Lipase; Chemoenzymatic synthesis; NMR

1. Introduction

The regiospecific synthesis of sugar fatty esters is a difficult target. In this field, the esterification of D-fructose in a tertiary alcohol as solvent [1,2] and in a free solvent medium [3] are noteworthy. Other teams have developed several interesting modifications [4,5]. However, in order to facilitate the reaction between the sugar and the fatty acid, several processes have been proposed in order to enhance the reactivity of the latter by use of trichloroethyl esters in pyridine [6] or enol esters, such as vinyl esters [7].

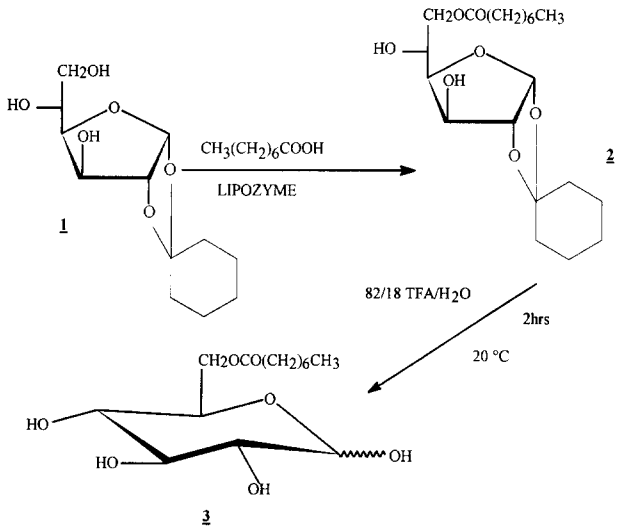
Another approach involves sugar derivatives more soluble in organic media. Among recent works, the esterification of methyl glucosides [8], the transacylation of D-glucose pentaacetate [9], the transesterification of methyl D-glucosides [10], and more recently, the esterification of isopropylidene monosaccharides [11,12] or disaccharides [13] should be mentioned. We have used this approach to synthesise D-glucose esters; the chemically prepared cyclohexylidene derivative was esterified enzymatically with the fatty acid and then the protecting group was chemically cleaved.

2. Results and discussion

The synthesis of glucose fatty esters from D-glucose and a fatty acid in a solvent-free system has not yet been successful. In fact, D-glucose is practically insoluble in fatty acids. In order to synthesise glucose esters, a combined chemical and enzymatic approach was chosen. For enzymatic acylation, 1,2-*O*-cyclohexylidene-α-D-glucofuranose **1** [14] proved to

* Corresponding author.

0008-6215/97/$17.00 © 1997 Elsevier Science Ltd. All rights reserved.
PII S0008-6215(96)00340-6



Scheme 1. Synthetic scheme for the preparation of 6-*O*-octanoyl-α-D-glucopyranose.

be soluble enough in an organic medium to allow the simultaneous access of both substrates to the catalytic site of the enzyme. The synthesis is followed by chemical hydrolysis of the protecting group, releasing the glucose ester. Different parameters were studied to optimize the reaction. The synthetic pathway for glucose octanoate is illustrated in Scheme 1. All synthesised products were identified by ^{1}H and ^{13}C NMR, allowing to confirm the fatty acyl substitution site.

Enzymatic synthesis of cyclohexylidene glucofuranose ester **2**.—This synthesis consists in treating **1** with octanoic acid in the absence of solvent. The fatty acid plays both the role of solvent and reactant. In a preliminary experiment, the influence of the molar ratio of the two reactants on the reaction was studied. Compound **1** was treated with variable quantities of octanoic acid at 70 °C in the presence of 10 or 20 mg of biocatalyst. The yield was almost always about 70% and independent of the molar ratio **1**/octanoic acid as shown in Table 1. This yield is better than previously obtained by Guillardeau [3] for the synthesis of D-fructose octanoate in a solvent free medium (26%). This is probably due to a sufficient solubility of the protected glucose in the fatty acid. Moreover, the effect of the amount of enzyme to be used was checked. In order to favour the phase contact and with reference to previous results [1,3], the **1**/octanoic acid molar ratio was fixed to 1/10 for this study, the other parameters being unchanged; above this ratio, the yield reaches a plateau. Moreover, as pointed in Table 2, for an enzyme amount above 30% (w/w) with respect to the ratio **1**/glucose, the yield reaches also a plateau.

Table 1
Effect of the **1**/octanoic acid molar ratio on esterification yield with a given amount of lipozyme

Molar ratio **1**/octanoic acid	Lipozyme amount (mg)	Esterification yields (%)
1/2	10	68
1/5	10	68
1/7.5	10	70
1/10	10	67
1/2	20	45
1/5	20	65
1/7.5	20	67
1/10	20	72

Table 2
Esterification yield of **1** versus lipozyme amount

Lipozyme (%) versus **1**	Esterification yields (%)
0	0.2
5	13
10	28
20	47
30	55
40	50
50	53

The effect of the chain length of the fatty acid on the reaction kinetic was studied by reacting **1** with octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid and octadecanoic acid, respectively. The kinetics are practically the same whatever the length of the fatty chains (Fig. 1). However, it should be noticed that the reactions involving short chain fatty acids give higher yields (C8; 75%); a yield of only 50% is observed with

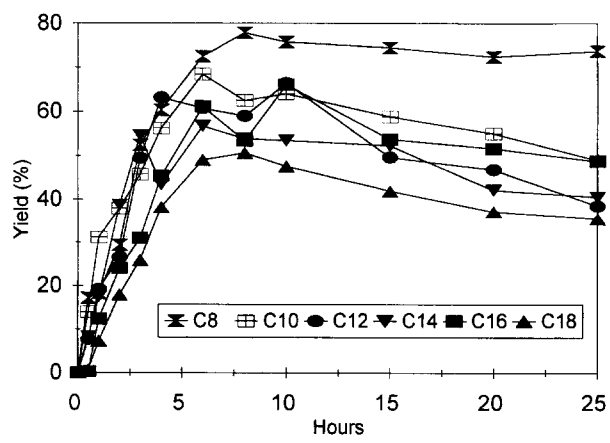


Fig. 1. Esterification kinetic of **1** by lipozyme for different fatty chain lengths.

C18. This difference can be explained by a better contact between the two substrates and the enzyme in the case of shorter fatty acids. The weak steric hindrance and the lower viscosity of these latters are certainly responsible for this enhanced reactivity.

In order to ascertain whether other lipases could also esterify **1**, another immobilized enzyme known for its performance in biocatalysis (the lipase of *Candida antarctica* SP435L) and two non immobilized enzymes (the devitalized mycelium of *Rhizopus arrhizus* and the lipase of *Candida cylindracea*) were tested. Esterification was carried out with **1** (1.61 g) and octanoic acid (10 mL) in the presence of enzyme (1 g) in a 50 mL reactor, under reduced pressure (21.3 kPa, the water vapor pressure at 60 °C). The reaction medium was analysed after 24 h. As shown in Table 3 free lipases, at least under the conditions used, are not efficient. On the other hand, comparable yields of about 55% are obtained in the presence of the two immobilized lipases.

Hydrolysis of the protecting group in **2**.—Neither sulfuric acid, nor hydrochloric acid, nor enzymatic hydrolysis resulted in the formation of the expected glucose ester. TLC analysis showed no hydrolysis reaction with acids whereas the enzymatic reaction cleaved the ester bond releasing the fatty acid and compound **1**.

Table 3
Esterification of **1** with octanoic acid and various biocatalysts

Lipases	Esterification yields (%)
Lipozyme NOVO	53
C. antarctica SP435L NOVO	56
C. cylindracea SIGMA	0
R. arrhizus GIST BROCADES	0

Table 4
Hydrolysis of **2** by TFA/water

TFA/water	Time	Hydrolysis yield (%)
98/2	10 min	20
98/2	1 h	26
98/2	16 h	23
82/18	1.5 h	69
50/50	1 h	31
50/50	16 h	15

The best results were obtained by using mixtures of trifluoroacetic acid (TFA) and water. We noticed that the degree of hydrolysis of the protecting group depends on the TFA/water ratio and of the reaction time. The results obtained for different TFA/water ratios (v/v) and for variable times of hydrolysis are shown in Table 4. The most convincing deprotection is obtained with 41:9 TFA–water for 2 h. Under these conditions, the yield approaches 70%. Shorter reaction times result in poor deprotection, whereas prolonged reaction times and/or larger proportions of water allowed simultaneous deprotection and ester hydrolysis regenerating the starting sugar. In TFA media containing low amounts of water (about 2%), the formation of products less polar than **2** with

Table 5
Comparative ^{1}H chemical shifts in Me_2SO-d_6 and C_5D_5N solution (δ ppm, J Hz)

	D-Glucose		**1**		**2**		**3**	
	Me_2SO-d_6	C_5D_5N	Me_2SO-d_6	C_5D_5N	Me_2SO-d_6	C_5D_5N	Me_2SO-d_6	C_5D_5N
δ_{H-1}	4.89	5.95	5.78	6.31	5.79	6.27	4.88	5.8
$J_{1,2}$	3.4	3.5	3.4	3.1			< 1	3.4
δ_{H-2}	3.09	4.25	4.436	4.93	4.38	4.9	3.1	4.13
$J_{2,3}$	9.7	9.3	5.6				6.4	
δ_{H-3}	3.38	4.81	4.026	4.82	3.87		3.39	4.67
$J_{3,4}$	9.3		8.8				9.8	
δ_{H-4}	3.02	4.31	3.817	4.97	4.04		3.02	4.05
$J_{4,5}$	9.3	9.3	2.4				9.8	9.3
δ_{H-5}	3.55	4.81	3.663	4.82	3.87		3.75	4.79
$J_{5,6}$		2.4/5.4					2/6.4	5.4
δ_{H-6}	3.55	4.59	3.536	4.42	4.2		4.25	4.99
	3.38	4.56	3.3	4.29	3.92		3.98	4.74
$J_{6a,6b}$		11.7		11.2			11.7	9.9
$\delta_{(CH_2)2'}$					2.29	2.3	2.26	2.22
$J_{2',3'}$							6.4	7.3
$\delta_{(CH_2)3}$						1.6	1.49	
$\delta_{(CH_3)8'}$					0.8	0.81	0.84	0.7
$J_{7',8''}$							7.3	

undetermined structures was noticed.

Products identification.—The IR spectrum of **2** displays an absorption band at 1747 cm^{-1} attributed to the carbonyl group of the ester.

In FAB^+ mass spectrometry, the molecular ion at *m/z* 387 $[M + H]^+$ was observed, indicating that only one alcohol function was esterified. The base peak at *m/z* 289 ($M-C_6H_9O$) corresponds to the cleavage of the dioxolane ring. Ion at *m/z* 243 ($M-C_8H_{15}O_2-H$, 3%) is assumed to be due to the cleavage of the ester function with the transfer of one hydrogen atom; the ion at *m/z* 127 ($C_8H_{15}O$, 80%), corresponding to the fatty chain, was observed as well.

NMR spectra were studied in two different solvents, pyridine-d_5 and dimethyl sulfoxide-d_6 using $^1H/^1H$ and $^1H/^{13}C$ correlation spectra. The unambiguous determination of the chemical shifts confirmed the structure of the different compounds.

The ^1H NMR spectrum of **2** in C_5D_5N presents a single signal for hydrogen atoms of the glucofuranose ring. Conversely, in dimethyl sulfoxide-d_6, signals of each hydrogen atom were observed. For comparison of the chemical shift value in compounds **1** and **2**, the H-1 proton was not affected ($\Delta\delta = 0.01$ ppm). Based on the chemical shift of this hydrogen atom in the $^1H/^1H$ and $^1H/^{13}C$ correlation spectra of compound **2**, the attributions given in Tables 5 and 6 were ascertained. By comparison of the various resonance values of the different glucofuranose ring atoms, the diastereotopic protons H-6,6′ were found strongly unshielded ($\Delta\delta = 0.66$ and 0.62 ppm), due to the inductive effect of the acyl group in agreement with the presence of an acyl substitution at the primary alcohol function.

Compound **3**, obtained by hydrolysis of **2** with TFA–water showed a protonated molecular ion at *m/z* 307 (15%), indicating that the ester group had not been affected. This is confirmed by the presence of the CO ester band at 1730 cm^{-1} in the infrared spectrum.

The study of ^1H and ^{13}C NMR spectra of **3** and comparison with α-D-glucose, showed that the chemical shifts of atoms 1, 2, 3 and 4 were identical. This indicates that **3** adopts preferentially a pyranose rather than a furanose structure. All data are in a good agreement with location of the octanoyl moiety at the primary hydroxyl position. Moreover, the NMR values were in perfect agreement with those of 6-*O*-tetradecanoyl-α-D-glucopyranose reported previously [15]. The structure of **3** was then confirmed as 6-*O*-octanoyl-α-D-glucopyranose.

The ^1H and ^{13}C NMR chemical shift values of **3** are given in Tables 5 and 6. The chemical shifts values of the octanoic chain are obtained by comparison with data for trioctenoyl glycerol [16].

Conclusion.—Sugar solubility in organic media is a limitation to the synthesis of their fatty esters. Hence, the use of more hydrophobic sugar derivatives as substrates is required to achieve substantial acylation yields.

The main drawbacks of this chemoenzymatic method are, on one hand the formation of water in the esterification reactions and, on the other hand, the low yields of the protection–deprotection steps. In

Table 6
Comparative ^{13}C chemical shifts in Me_2SO-d_6 and C_5D_5N solution

δ	D-Glucose		**1**		**2**		**3**		
	Me_2SO-d_6	C_5D_5N	Me_2SO-d_6	C_5D_5N	Me_2SO-d_6	C_5D_5N	Me_2SO-d_6	C_5D_5N (α-D)	C_5D_5N (β-D)
C-1	92.3	94.3	104.1	105.5	104	105.6	92.3	94.3	99
C-2	72.4	74.6	84.3	86	94.1	85.9	72.2	74.4	76.8
C-3	73.1	75.5	80	81.7	80.1		72.9	75.3	78.5
C-4	70.6	72.7	73.3	75.4	72.8		70.5	72.3	71.8
C-5	72	73.8	68.4	70.6	65.1		69.1	70.9	75.3
C-6	61.3	63.3	63.7	65.3	66.4		63.8	65.2	65
C-1′					172.8	173.7	172.9	173.7	173.7
C-2′					33.5	34.5	33.5	34.4	34.4
C-3′					24.4	25.3	24.5	25.2	25.2
C-4′						29.2	28.4	29.1	29.1
C-5′						29.2	28.4	29.2	29.2
C-6′					31	31.8	31.1	31.8	31.8
C-7′					22	22.8	22	22.8	22.8
C-8′					13.8	14.2	14	14.2	14.2

spite of this, the acylation of glucose derivatives by fatty acids allows access to a wide variety of high purity glucose fatty esters. Probably, this synthesis could still be much improved.

3. Experimental

General methods of purification and analysis of products by thin layer chromatography (TLC).—The quantification of **2** in a given reaction medium was carried out by TLC on HPTLC Silica 60F plates (ref. 5558, E. Merck) with the developing solvent 92:8 $CHCl_3$–MeOH, which was also used for the monitoring of the purification of products on preparative column chromatography. Samples were applied in 10 mm narrow bands with a Desaga AS 30 automatic applicator. After charring, the intensity of bands was evaluated by a Desaga CD 60 spectrophotodensitometer. TLC plates were scanned at 500 nm and concentrations were deduced from comparison with standards of **2** purified on a silica column according to the method indicated below.

The staining was obtained by charring in an oven at 180 °C during 5 min, after spraying an acid ethanolic vanillin soln [1 g of vanillin in 1:1 EtOH–water (50 mL) soln to which was added concentrated phosphoric acid (35 mL)].

Product **2** was purified on a silica gel column (230–400 mesh, ref. 9385 E. Merck) with a gradient elution hexane–Et_2O: 90:10 (500 mL); 80:20 (500 mL); 50:50 (700 mL); 0:100 (1000 mL).

Similarly, **3** was purified on a silica gel column (230–400 mesh, ref. 9385 E. Merck) (60 g for 0.5 g of reaction medium) by using a gradient elution Et_2O–EtOAc: 100:0 (400 mL); 1:1 (500 mL); 0:100 (700 mL). The resulting fractions were monitored by TLC.

Synthesis of 6-O-octanoyl-1, 2-O-cyclohexylidene-α-D-glucofuranose **2**.—Two immobilized lipases were used for this study: Lipozyme (Novo), a lipase of *Mucor miehei* immobilized on an anionic resin and *Candida antarctica* SP435L (Novo), a lipase immobilized on acrylic resin. Two free lipases were also used: *Candida cylindracea* (Sigma) and lipase S which is a devitalized mycelium of *Rhyzopus arrhizus* (Gist Brocades).

A soln of **1** (0.135 mmole, 35 mg), obtained according to [14], was treated with octanoic acid (1.34 mmole, 194 mg) in an open flask (20 mL) in the presence of lipase (10 mg) at 60 °C under magnetic stirring.

For synthesis of ester with a longer chain fatty acid such as hexadecanoic acid and octadecanoic acid, the temperature had to be increased to 75 °C. In all cases, the reactor can optimally be placed under reduced pressure (160 mm Hg) to eliminate the water formed continuously.

TLC monitoring of the hydrolysis of the protecting group of **2**.—The products of the reaction were analysed by TLC using a 80:20:8:2 $CHCl_3$–MeOH–AcOH–water mixture. Visualisation was carried out by charring at 180 °C after spraying with the vanillin reagent, described above.

Hydrolysis of **2** *by sulfuric acid.*—A 20% (w/v) soln of **2** (2 g) was prepared in 65:35 0.5 M sulfuric acid 2-propanol (10 mL) and stirred for 24 h at 20 °C [17].

Hydrolysis of **2** *by hydrochloric acid.*—A 20% (w/v) solution of **2** (2 g) was prepared in a 1:4 HCl (10% in water)–Et_2O mixture (10 mL) and stirred for 5 to 60 min at 25 °C [18].

Enzymatic hydrolysis of the protecting group of **2**.—All enzymes used were Sigma enzymes with glycosidase activity: invertase (ref. 19253), amyloglucosidase (ref. A7255 and A3176), α-amylase (ref. G5003). Solutions at 2% (w/v) of **2** in a 75:25 0.1 M buffer-2-propanol, were used with acetate buffer (pH 4.75) for invertase and amyloglucosidase and phosphate buffer (pH 6.9) for α-amylase.

Hydrolysis of **2** *by trifluoroacetic acid.*—A 10% soln of **2** (w/v) was prepared in aq trifluoroacetic acid with variable proportions of water [19] and stirred at 20 °C for 1 to 16 h. Then, the solvent was evaporated under reduced pressure and the residue dissolved in abs EtOH. Unreacted **2** and the product **3** were separated by column chromatography and the latter recrystallized from water at 0 °C. Anal. Calc. for $C_{14}H_{26}O_7$: C, 54.93; H, 8.50. Found: C, 54.96; H, 8.46.

Products identification.—Infrared spectra were determined with soln in anhyd CCl_4 with a FTIR 3000 Mattson Unicam. Mass spectra were obtained with a Jeol DX 300 in FAB ionisation mode. ^1H and ^{13}C NMR spectra were obtained with a Jeol XJ 400 apparatus at the frequency of 400 MHz for ^1H and 100 MHz for ^{13}C. The spectra were performed in C_5D_5N and Me_2SO-d_6 (6 mg in 0.5 mL of solvent) with tetramethylsilane as internal reference. All spectra were measured in the same conditions for D-glucose, **1**, **2** and **3**.

References

[1] N. Khaled, D. Montet, M. Pina, and J. Graille, *Biotechnol. Lett.*, **13** (1991) 167–172.

[2] N. Khaled, D. Montet, M. Farines, M. Pina, and J. Graille, *Oléagineux*, **47** (1992) 181–190; *Chem. Abstr.*, **118**, 169442 m.

[3] L. Guillardeau, D. Montet, N. Khaled, M. Pina, and J. Graille, *Tenside*, **5** (1992) 342–344.

[4] G.B. Oguntimein, H. Erdmann, and R.D. Schmid, *Biotechnol. Lett.*, **15** (1993) 175–180.

[5] A. Schlotterbeck, S. Lang, V. Wray, and F. Wagner, *Biotechnol. Lett.*, **15** (1993), 61–64.

[6] M. Therizod and A.M. Klibanov, *J. Am. Chem. Soc.*, **108** (1986) 5638–5640.

[7] M. Degueil-Castaing, B. de Jeso, S. Drouillard, and B. Maillard, *Tetrahedron Lett.*, **28** (1987) 953–954.

[8] K. Adelhorst, F. Bjorkling, and S.E. Godtfredsen, *Synthesis*, (1990) 112–115.

[9] C.C. Akoh, *J. Am. Oil Chem. Soc.*, **71** (1994) 319–323.

[10] L.N. Mutua and C.C. Akoh, *J. Am. Oil Chem. Soc.*, **70** (1993) 43–46.

[11] G. Fregapane, D.B. Sarney, and E.N. Vulfson, *Enzyme Microb. Tech.*, **13** (1991) 796–800.

[12] G. Fregapane, D.B. Sarney, S.G. Greenberg, and D.J. Knight, *J. Am. Oil Chem. Soc.*, **71** (1994) 87–91.

[13] D.B Sarney, H. Kapeller, G. Fregapane, and E.N. Vulfson, *J. Am. Oil Chem. Soc.*, **71** (1994) 711–714.

[14] A. Vogel, *Textbook of Practical Organic Chemistry*, 4th ed., Longman Scientific and Technical, 1978, pp 463–464.

[15] K. Yoshimoto, Y. Itatani, and Y. Tsuda, *Chem. Pharm. Bull.*, **28** (1980) 2065–2076.

[16] F.D. Gunstone, in R.J. Hamilton and S. Hamilton (Eds.), *Lipid Analysis, a Practical Approach*, Oil Press, Oxford, 1991, pp 243–262.

[17] W. Heeswijk, J. Goedhart, and J. Vliegenthart, *Carbohyd. Res.*, **58** (1977) 337–344.

[18] G. Larson and A. Hernandez, *J. Org. Chem.*, **38** (1978) 3935–3936.

[19] S. Cook and J. Secrist, *J. Am. Chem. Soc.*, **101** (1979) 1554–1564.