Pergamon

Tetrahedron: Asymmetry Vol. 5, No. 7, pp. 1377-1384, 1994
Elsevier Science Ltd
Printed in Great Britain
0957-4166/94 $7.00+0.00

0957-4166(94)00189-8

Regio- and Diastereoselective Lipase-Catalyzed Preparation of Acetylated 2-*O*-Glucosylglycerols

Diego Colombo, Fiamma Ronchetti,* Antonio Scala, and Ida M. Taino

Dipartimento di Chimica e Biochimica Medica, Università di Milano, Via Saldini 50, 20133 Milano (Italy)

Franca Marinone Albini and Lucio Toma

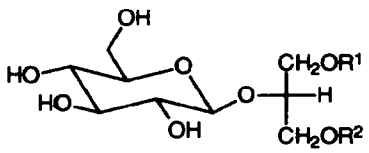
Dipartimento di Chimica Organica, Università di Pavia, Via Taramelli 10, 27100 Pavia (Italy)

Abstract: 2-*O*-(β-D-Glucopyranosyl)glycerol and 2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol have been submitted to lipase-catalyzed acetylation using *Pseudomonas cepacia* (LPS) and *Candida antarctica* (LCA) lipases in organic solvent. The reactions involved the glycerol moiety and were highly diastereoselective: LPS yielded the (2S)-1-*O*-acetyl-derivative, while, more interestingly, LCA yielded the (2R)-1-*O*-acetyl-derivative; in this way the natural compound lilioside A could be obtained. Conversely, lipase-catalyzed hydrolysis of the fully acetylated 1,3-di-*O*-acetyl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol using LCA furnished the (2S)-1-*O*-acetyl-derivative showing the same steric preference as the reverse reaction.

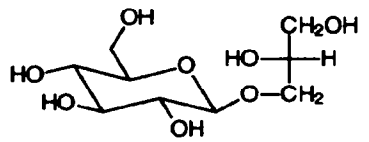
In recent years glyceroglycolipids have been found[1] in plants, animal tissues and many kind of bacteria. Various functions have been assigned to these glycerol derivatives, e.g. mediation of the cell surface recognition, but till now their role is not well understood Moreover, they are in general obtained from the natural sources in very small quantities; the chemical synthesis could supply larger amounts for their use in biological studies or as authentic standards in extraction and characterization procedures.

In particular, several glycerol glucosides, named liliosides and regalosides, have been isolated from different plants, e.g. from bulbs of the genus *Lilium*[2] or, recently,[3] from *Sporobolus stapfianus*, a typical desiccation-tolerant plant. The glycosidic bond between glucose and glycerol may involve the 2-position of the latter, such as in lilioside A (**1**) and lilioside B (**2**), whereas in other cases glucose is bound to a primary hydroxyl group of glycerol, such as in lilioside C (**3**) and in liliosides D (**4**) and E (**5**).

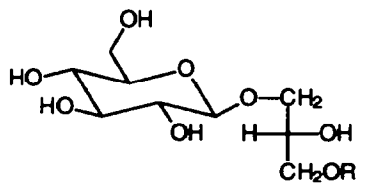
A major problem, in the synthesis of the monoacetylated liliosides like **1** or **5**, is the regio- and stereoselective introduction of an acetyl group on the suitable polihydroxylated substrate. This is a very difficult task with conventional chemical tools, because it is necessary to differentiate among three (in the case of **1**) or two (in the case of **5**) primary hydroxyl functions. As a valuable alternative could be used the enzymatic approach which, in the last years, has been shown more and more useful for the solution of such a kind of problems.



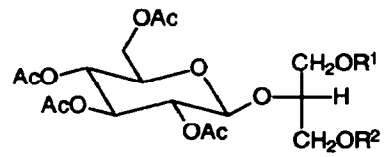
1 : R^1= H, R^2= Ac
2 : R^1= R^2= H
10 : R^1= Ac, R^2= H



3



4 : R = H
5 : R = Ac



6 : R^1= R^2= H
7 : R^1= R^2= Ac
8 : R^1= Ac, R^2= H
9 : R^1= H, R^2= Ac
11 : R^1= Ac, R^2= $C(C_6H_5)_3$
12 : R^1= $C(C_6H_5)_3$, R^2= Ac

In fact, regioselective acylation of polihydroxylated substrates, and in particular of carbohydrates, has greatly stridden forward with the introduction of procedures utilizing hydrolytic enzymes, such as lipases, in organic solvents.[4]

In preceding papers we have studied the regioselectivity of the acylation of secondary hydroxyl groups of various methyl α-D and L-glycopyranosides.[5] The selectivity in the acylation of primary hydroxyl groups has been less studied and liliosides are suitable substrates for this purpose. The proper choice of the experimental conditions could allow to obtain the natural acetylated liliosides by direct acylation of unprotected substrates.

In general, enzymatic hydrolysis by lipases is widely used in the case of glyceroglycolipids for the location of the acyl substituent linked to the glycerol moiety.[6] To the best of our knowledge, however, only once[7] lipases have been utilized in the reverse reaction of direct acylation of glyceroglycosides as such.

Here we describe the enzymic transformation, via lipase-mediated reaction in organic solvent, of 2-*O*-(β-D-glucopyranosyl)glycerol (**2**), easily synthesized by chemical means,[8] and of some related compounds, the tetraacetate 2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**6**) and the hexaacetate 1,3-di-*O*-acetyl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**7**).

RESULTS AND DISCUSSION

Prior to undertake the study of the acylation of compound **2** via lipase-catalyzed methods we investigated the relative reactivity of the two diastereotopic hydroxymethyl groups of the glycerol moiety on a model in which all the primary and secondary hydroxyl groups of the sugar moiety have been masked as acetates. This model, 2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**6**), was used as substrate for a screening of the experimental conditions. Two enzymes, *Pseudomonas cepacia* (LPS) and *Candida antarctica* (LCA) lipases, proved to carry out the reaction in good yields and high selectivity when used in THF at 45 °C with vinyl acetate as acyl donor (Table 1). Interestingly, these two enzymes displayed an opposite diastereoselectivity: while LPS preferentially acylates the pro-S hydroxymethyl group, LCA preferentially acylates the pro-R one yielding, as the main products, compounds **8** and **9**, respectively (for the determination of the configuration at C-2 see below).

Table 1. Enzymic Transesterification of **6** and **2** by LPS and LCA.[†]

SUBSTRATE	ENZYME	TIME (hours)	YIELD (%)	PRODUCTS	R/S ratio
6	LPS	2	85	**9/8**	9:91
6	LCA	2	45	**9/8**	98:2
2	LPS	2	68	**1/10**	10:90
2	LCA	2	43	**1/10**	82:18

[†]No acylation took place in the absence of the enzyme.

The same experimental conditions were then applied to the acylation of **2**, except for the fact that pyridine was the solvent of choice owing to the insolubility of **2** in the other organic solvents suitable for a transesterification reaction. Again the reaction proved to be highly regio- and diastereoselective; in fact, though in the presence of another possible site of reaction, i.e. the primary hydroxyl group of the glucosyl moiety, the results (Table 1) closely paralleled those already found for **6**, but with slightly lower yields and diastereoselectivity. The main products were, respectively, the monoacetate **10** with LPS and the natural compound **1** with LCA, which exhibited at C-2 the same stereochemistry of the pentaacetates **8** and **9**, respectively, previously obtained with the same enzymes. It is worthy pointing out that only trace amounts of the 6'-monoacetate were detected in the reaction mixtures.

To the monoacetate **10** was assigned the 2S configuration and to **1** was assigned the 2R one on the basis of the chemical correlation with the corresponding penta-*p*-bromobenzoyl derivative already described in literature.[9] The configuration of the pentaacetates **8** and **9** was also assigned through chemical correlation with **10** and **1**, respectively. To this aim, compound **8** was converted into its 3-*O*-trityl derivative **11**, alternatively obtainable from **10** through tritylation and isolation of the fraction corresponding to the monotrityl derivatives, followed by treatment with acetic anhydride. Analogously compound **12** could be achieved from both **9** and **1**.

The regioselectivity of LPS and LCA in the reverse hydrolytic reaction was also investigated in order to ascertain if the two enzymes maintain the same selectivity both in the forward and in the backward reactions. So, the hexaacetate **7** was submitted to lipase catalyzed hydrolysis in several experimental conditions. Whereas no transformation was observed with LPS (see Experimental), LCA suspended in THF-water saturated diisopropyl ether preferentially hydrolyzed the pro-R acetyloxymethyl group of the glycerol moiety affording in 44% yield the same pentaacetate **8** (in a 94:6 ratio with its isomer **9**) obtained in the transesterification reaction of **6** catalyzed by LPS.

In conclusion, this study shows that the selectivity of LPS and LCA in the enzymatic transesterification reaction is independent from the fact that the glucose moiety has free or protected hydroxyl groups: LPS always transfers the acetyl group to the pro-S hydroxymethyl group of the glycerol moiety, whereas LCA transfers it to the pro-R one. The two enzymes have opposite selectivity, so it is possible to obtain the diastereoisomeric liliosides **10** or **1** (and **8** or **9**) simply by changing the enzyme. Moreover, LCA shows the same diastereoselectivity either in the transesterification or in the hydrolysis reaction, so it is possible to obtain the diastereoisomeric liliosides **8** or **9** only by changing the procedure of enzyme utilization on the proper substrate.

Work is in progress to extend the described procedures to the synthesis of glyceroglycolipids widely occurring as natural products.

EXPERIMENTAL

General procedures

^{1}H NMR spectra were recorded with a Bruker AM-500 spectrometer, in deuteriochloroform solutions, unless otherwise stated. Optical rotations were measured with a Perkin Elmer 241 polarimeter at 25 °C, as chloroform solutions. Melting points were recorded on a Büchi 510 capillary melting point apparatus and were uncorrected. Analytical thin layer chromatography (TLC) was carried out on Merck 60 F_{254} silica gel plates (0.25 mm thickness) and the spots were detected by spraying with 50% aqueous H_2SO_4 and heating at 110 °C. Flash chromatography was performed with Merck 60 silica gel (230-400 mesh). The elemental analyses of the new products were consistent with the calculated ones.

Compounds **2**, **6** and **7** were synthesized according to literature procedures.[8]

Pseudomonas cepacia lipase (lipase PS, LPS, specific activity 30.5 triacetin units/mg solid), a generous gift from Amano Pharmaceutical Co (Mitsubishi Italia), was supported on celite according to Bovara et al.;[10] *Candida antarctica* lipase SP 435 L, immobilized on a macroporous acrylic resin, (Novozym® 435, LCA, specific activity 9.5 PL units/mg solid), was a generous gift from Novo Nordisk A/S.

LPS and LCA were kept under vacuum prior to use in order to lower the water content to 0.5%. Tetrahydrofuran (THF) and pyridine were distilled just prior to use from, respectively, sodium/benzophenone and calcium hydride. Vinyl acetate was dried over 3Å molecular sieves. Evaporation under reduced pressure was always effected with the bath temperature kept below 40 °C.

LPS-catalyzed transesterification of **6**

2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**6**) (90 mg, 0.214 mmol) was dissolved in 2 mL of THF; vinyl acetate (0.2 mL, 2.14 mmol) and LPS (400 mg) were added in the order and the suspension was stirred at 45 °C for 2 h. The reaction, monitored by TLC using methylene chloride-acetone 8:2, was stopped by filtering-off the enzyme which was then washed with THF. The solvent was removed under reduced pressure to yield a crude product (S/R ratio 91:9 by ^{1}H NMR), which was purified by flash chromatography (methylene chloride-acetone 8:2) affording (2S)-1-*O*-acetyl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-glycerol (**8**) (77 mg) followed by its diastereoisomer **9** (8 mg). Compound **8** was crystallized from ethanol, mp 75-76 °C, $[\alpha]_D$ -2.1 (c 1.0); ^{1}H NMR δ 1.98, 2.00, 2.02, 2.04, 2.06 (5s, 15H, $OCOCH_3$), 2.68 (dd, 1H, $J_{OH,3a}$ = 9.0 Hz, $J_{OH,3b}$ = 5.0 Hz, OH), 3.60 (ddd, 1H, $J_{3b,3a}$ = 12.0 Hz, $J_{3b,2}$ = 6.5 Hz, H-3b), 3.64 (ddd, 1H, $J_{3a,2}$ = 3.0 Hz, H-3a), 3.74 (ddd, 1H, $J_{5',4'}$ = 9.5 Hz, $J_{5',6'a}$ = 3.0 Hz, $J_{5',6'b}$ = 6.0 Hz, H-5'), 3.86 (dddd, 1H, $J_{2,1a}$ = 6.0 Hz, $J_{2,1b}$ = 5.0 Hz, H-2), 4.06 (dd, 1H, $J_{1b,1a}$ = 12.0 Hz, H-1b), 4.09 (dd, 1H, H-1a), 4.14 (dd, 1H, $J_{6'b,6'a}$ = 12.0 Hz, H-6'b), 4.19 (dd, 1H, H-6'a), 4.60 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.98 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2'), 5.02 (dd, 1H, $J_{4',3'}$ = 9.5 Hz, H-4'), 5.20 (dd, 1H, H-3').

LCA-catalyzed transesterification of **6**

2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**6**) (90 mg, 0.214 mmol), processed as above, using LCA as enzyme, yielded a crude product (R/S ratio 98:2 by ^{1}H NMR), which, recovered and purified as described, afforded **8** (1 mg) followed by (2R)-1-*O*-acetyl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-glycerol (**9**) (44 mg). Compound **9** was crystallized from ethyl acetate, mp 122-124 °C, $[\alpha]_D$ -5.5 (c 1.0); ^{1}H NMR δ 1.98, 2.00, 2.03, 2.04, 2.06 (5s, 15H, $OCOCH_3$), 2.12 (dd, 1H, $J_{OH,3a}$ = 7.0 Hz, $J_{OH,3b}$ = 6.0 Hz, OH), 3.60 (ddd, 1H, $J_{3b,3a}$ = 12.0 Hz, $J_{3b,2}$ = 5.5 Hz, H-3b), 3.64 (ddd, 1H, $J_{3a,2}$ = 5.0 Hz, H-3a), 3.70 (ddd, 1H, $J_{5',4'}$ = 10.0 Hz, $J_{5',6'a}$ = 5.5 Hz, $J_{5',6'b}$ = 2.0 Hz, H-5'), 3.90 (dddd, 1H, $J_{2,1a}$ = 5.0 Hz, $J_{2,1b}$ = 6.0 Hz, H-2), 4.11 (dd, 1H, $J_{6'b,6'a}$ = 12.0 Hz, H-6'b), 4.11 (dd, 1H, $J_{1b,1a}$ = 12.0 Hz, H-1b), 4.21 (dd, 1H, H-6'a), 4.25 (dd, 1H, H-1a), 4.67 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.98 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, H-2'), 5.04 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4'), 5.19 (dd, 1H, H-3').

LPS-catalyzed transesterification of **2**

2-*O*-(β-D-glucopyranosyl)glycerol (**2**) (80 mg, 0.314 mmol) was dissolved in 2 mL of pyridine; vinyl acetate (0.29 mL, 3.14 mmol) and LPS (400 mg) were added in the order and the suspension was stirred at 45 °C for 2 h. The reaction, monitored by TLC using ethyl acetate-isopropanol-water 3:3:1, was stopped by filtering-off the enzyme which was washed with pyridine. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (methylene chloride-methanol from 10:1 to 7:3) affording 63 mg of a mixture (S/R ratio 90:10 by ^{1}H NMR) of (2S)-1-*O*-acetyl-2-*O*-(β-D-glucopyranosyl)-glycerol (**10**)[9] and its diastereoisomer **1**,[9] which was submitted to the *p*-bromobenzoylation procedure as such. ^{1}H NMR (C_5D_5N) of **10**: δ 1.96 (s, 3H, $OCOCH_3$), 3.95 (ddd, 1H, $J_{5',4'}$ = 8.5 Hz, $J_{5',6'a}$ = 2.5 Hz, $J_{5',6'b}$ = 5.5 Hz, H-5'), 3.99 (dd, 1H, $J_{2',3'}$ = 8.0 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 4.07 (dd, 1H, $J_{3b,3a}$ = 11.5 Hz, $J_{3b,2}$ = 5.0 Hz, H-3b), 4.14 (dd, 1H, $J_{3a,2}$ = 5.5 Hz, H-3a), 4.19 (dd, 1H, $J_{4',3'}$ = 8.5 Hz, H-4'), 4.22 (dd, 1H, H-3'), 4.32 (dd, 1H, $J_{6'b,6'a}$ = 11.5 Hz, H-6'b), 4.44 (dddd, 1H, $J_{2,1a}$ = 6.5 Hz, $J_{2,1b}$ = 5.0 Hz, H-2), 4.52 (dd, 1H, H-6'a), 4.59 (dd, 1H, $J_{1b,1a}$ = 11.5 Hz, H-1b), 4.65 (dd, 1H, H-1a), 5.06 (d, 1H, H-1').

LCA-catalyzed transesterification of 2

2-*O*-(β-D-glucopyranosyl)glycerol (**2**) (80 mg, 0.314 mmol), processed as above, using LCA as enzyme, yielded a crude product which, recovered and purified as described, afforded 40 mg of a mixture (R/S ratio 82:18 by ^{1}H NMR) of (2R)-1-*O*-acetyl-2-*O*-(β-D-glucopyranosyl)glycerol (**1**)[9] and its diastereoisomer **10**,[9] which was submitted to the *p*-bromobenzoylation procedure as such. ^{1}H NMR (C_5D_5N) of **1**: δ 1.96 (s, 3H, $OCOCH_3$), 3.92 (ddd, 1H, $J_{5',4'}$ = 9.0 Hz, $J_{5',6'a}$ = 2.0 Hz, $J_{5',6'b}$ = 5.0 Hz, H-5'), 4.03 (dd, 1H, $J_{2',3'}$ = 9.0 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 4.09 (dd, 1H, $J_{3b,3a}$ = 12.0 Hz, $J_{3b,2}$ = 5.0 Hz, H-3b), 4.10 (dd, 1H, $J_{3a,2}$ = 5.0 Hz, H-3a), 4.18-4.25 (m, 2H, H-3' and H-4'), 4.36 (dd, 1H, $J_{6'b,6'a}$ = 12.0 Hz, H-6'b), 4.46 (dddd, 1H, $J_{2,1a}$ = 5.5 Hz, $J_{2,1b}$ = 5.0 Hz, H-2), 4.51 (dd, 1H, H-6'a), 4.57 (dd, 1H, $J_{1b,1a}$ = 11.5 Hz, H-1b), 4.63 (dd, 1H, H-1a), 5.12 (d, 1H, H-1').

LPS-catalyzed hydrolysis of 7

Several attempts to hydrolyze 1,3-di-*O*-acetyl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**7**) with LPS were unsuccessful. Compound **7** was recovered unchanged after treatment with the nucleophilic acyl acceptor 1-pentanol[11] in THF, diisopropyl ether,[12] THF-diisopropyl ether 2:3 or THF-water saturated diisopropyl ether.[13]

LCA-catalyzed hydrolysis of 7

1,3-di-*O*-acetyl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**7**) (90 mg, 0.178 mmol) was dissolved in 9 mL of THF-water saturated diisopropyl ether[13] 2:3, LCA (180 mg) was added and the suspension was stirred at 45 °C for 24 h. The reaction, monitored by TLC using methylene chloride-acetone 8:2, was stopped by filtering-off the enzyme which was then washed with chloroform. The solvent was removed under reduced pressure to yield a crude product (S/R ratio 94:6 by ^{1}H NMR), which was purified by flash chromatography (methylene chloride-acetone 8:2 as eluant) affording (2S)-1-*O*-acetyl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**8**) (34 mg) followed by its diastereoisomer **9** (2 mg). **8** was identical, by ^{1}H NMR, mp, and $[\alpha]_D$, to the product obtained by transesterification of **6** with LPS.

No reaction occurred in the absence of the enzyme.

Assignment of the configuration of 1 and 10

a) The monoacetate **10** (in mixture with 10% of its diastereoisomer) (40 mg, 0.135 mmol), obtained from LPS-catalyzed transesterification of **2**, was dissolved in pyridine (6 mL), *p*-bromobenzoyl chloride (1.3 g, 5.936 mmol) was added and the mixture stirred at 95 °C for 5 h. After usual work-up, the resulting residue was purified by preparative TLC (hexane-ethyl acetate 2:1) to separate the predominant penta-*p*-bromobenzoate with the major R_f, which was identical to the (2R)-1-*O*-acetyl-3-*O*-*p*-bromobenzoyl-2-*O*-(2',3',4',6'-tetra-*O*-*p*-bromobenzoyl-β-D-glucopyranosyl)glycerol described in the literature.[9]

b) The monoacetate **1** (in mixture with 18% of its diastereoisomer) (30 mg, 0.101 mmol), obtained from LCA-catalyzed transesterification of **2**, was *p*-bromobenzoylated as described above. After usual work-up, the resulting residue was purified by preparative TLC to separate the predominant penta-*p*-bromobenzoate with the minor R_f, which was identical to the (2S)-1-*O*-acetyl-3-*O*-*p*-bromobenzoyl-2-*O*-(2',3',4',6'-tetra-*O*-*p*-bromobenzoyl-β-D-glucopyranosyl)glycerol described in the literature.[9]

Assignment of the configuration of 8 and 9

a) The pentaacetate **8** (40 mg, 0.084 mmol) was dissolved in pyridine (1 mL), trityl chloride (52 mg, 0.186 mmol) was added and the mixture was stirred at 100 °C for 5 h. The solution was repeatedly evaporated *in vacuo* with toluene and the resulting residue was purified by flash chromatography (ethyl acetate-petroleum ether 4:6) affording solid amorphous (2S)-1-*O*-acetyl-3-*O*-trityl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyrano-syl)glycerol (**11**) (54 mg), $[\alpha]_D$ -2.7 (c 0.6), ^{1}H NMR δ 1.96, 1.98, 1.99, 2.00, 2.01 (5s, 15H, $OCOCH_3$), 3.18 (dd, 1H, $J_{3b,3a}$ = 10.0 Hz, $J_{3b,2}$ = 6.5 Hz, H-3b), 3.31 (dd, 1H, $J_{3a,2}$ = 5.5 Hz, H-3a), 3.60 (ddd, 1H, $J_{5',4'}$ = 10.0 Hz, $J_{5',6'a}$ = 5.0 Hz, $J_{5',6'b}$ = 2.5 Hz, H-5'), 3.94 (dddd, 1H, $J_{2,1a}$ = 3.5 Hz, $J_{2,1b}$ = 7.0 Hz, H-2), 3.98 (dd, 1H, $J_{6'b,6'a}$ = 12.0 Hz, H-6'b), 4.14 (dd, 1H, $J_{1b,1a}$ = 12.0 Hz, H-1b), 4.17 (dd, 1H, H-6'a), 4.25 (dd, 1H, H-1a), 4.60 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 4.94 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, H-2'), 5.03 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4'), 5.16 (dd, 1H, H-3'), 7.19-7.42 (m, 15H, Ph).

b) The pentaacetate **9** (30 mg, 0.063 mmol) was tritylated and purified as described above, to yield (2R)-1-*O*-acetyl-3-*O*-trityl-2-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**12**) (45 mg), which was crystallized from mehylene chloride-hexane, mp 146-148 °C, $[\alpha]_D$ -21.0 (c 1.0), ^{1}H NMR δ 1.79, 1.94, 1.98, 2.01, 2.06 (5s, 15H, $OCOCH_3$), 3.18 (dd, 1H, $J_{3b,3a}$ = 10.0 Hz, $J_{3b,2}$ = 4.5 Hz, H-3b), 3.23 (dd, 1H, $J_{3a,2}$ = 6.0 Hz, H-3a), 3.60 (ddd, 1H, $J_{5',4'}$ = 10.0 Hz, $J_{5',6'a}$ = 5.0 Hz, $J_{5',6'b}$ = 2.0 Hz, H-5'), 3.94 (dddd, 1H, $J_{2,1a}$ = 6.0 Hz, $J_{2,1b}$ = 5.5 Hz, H-2), 4.08 (dd, 1H, $J_{6'b,6'a}$ = 12.0 Hz, H-6'b), 4.13 (dd, 1H, $J_{1b,1a}$ = 11.5 Hz, H-1b), 4.16 (dd, 1H, H-1a), 4.21 (dd, 1H, H-6'a), 4.62 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 5.00 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, H-2'), 5.05 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4'), 5.16 (dd, 1H, H-3'), 7.19-7.42 (m, 15H, Ph).

c) The (2S)-monoacetate **10** (in mixture with 10% of its diastereoisomer) (20 mg, 0.067 mmol) was tritylated as described for **8**. Flash chromatography (mehylene chloride-methanol 8:2) afforded a mixture (8 mg) of the 6'-*O*-trityl- and 3-*O*-trityl-1-*O*-acetyl derivatives, which was directly submitted to peracetylation. ^{1}H NMR analysis of the crude mixture showed the presence of 1,3-di-*O*-acetyl-2-*O*-(2',3',4'-tri-*O*-acetyl-6'-*O*-trityl-β-D-glucopyranosyl)glycerol, of the (2S)-3-*O*-trityl-1,2',3',4',6'-pentaacetate (**11**), and of the (2R)-3-*O*-trityl-1,2',3',4',6'-pentaacetate (**12**) in a 69:26:5 ratio.

d) The (2R)-monoacetate **1** (in mixture with 18% of its diastereoisomer) (20 mg, 0.067 mmol) was tritylated, purified (11 mg) and acetylated as described in c). ^{1}H NMR analysis showed the presence of 1,3-di-*O*-acetyl-2-*O*-(2',3',4'-tri-*O*-acetyl-6'-*O*-trityl-β-D-glucopyranosyl)glycerol, of the (2S)-3-*O*-trityl-1,2',3',4',6'-pentaacetate (**11**), and of the (2R)-3-*O*-trityl-1,2',3',4',6'-pentaacetate (**12**) in a 63:8:29 ratio.

Acknowledgements

We thank M.U.R.S.T. (Rome) and Consiglio Nazionale delle Ricerche (Rome) for financial support.

REFERENCES

1. Ishizuka, I.; Yamakawa, T. *New Comprehensive Biochemistry,* Neuberger, A.; Van Deenen, L. L. M.; Wiegandt, H. Eds.; Elsevier: Amsterdam, Vol. 10, 1985; pp. 101-198. Koynova, R. D.; Tenchov, B. G.; Kuttenreich, H.; Hinz, H. J. *Biochemistry* **1993**, *32*, 12437-12445 and references cited therein.
2. Kaneda, M.; Mizutani, K.; Takahashi, Y.; Kurono, G.; Nishikawa, Y. *Tetrahedron Lett.* **1974**, *15*, 3937-3940. Kaneda, M.; Mizutani, K.; Tanaka, K. *Phytochemistry* **1982**, *21*, 891-893. Kaneda, M.; Kobayashi,

K.; Nishida, K.; Katsuta, S. *Phytochemistry* **1984**, *23*, 795-798. Shimomura, H.; Sashida, Y.; Mimaki, Y.; Iida, N. *Phytochemistry* **1988**, *36*, 451-454.

3. Marinone Albini, F.; Murelli, C.; Patritti, G.; Rovati, M.; Zienna, P.; Vita Finzi, P. *Phytochemistry*, in press.
4. Klibanov, A. M. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 3192-3196. Drueckhammer, D. G.; Hennen, W. J.; Pederson, R. L.; Barbas, C. F.; Gautheron, C. M.; Krach, T.; Wong, C. H. *Synthesis* **1991**, 499-526.
5. Ciuffreda, P.; Colombo, D.; Ronchetti, F.; Toma, L. *J. Org. Chem.* **1990**, *55*, 4187-4190. Colombo, D.; Ronchetti, F.; Toma, L. *Tetrahedron* **1991**, *47*, 103-110. Colombo, D.; Ronchetti, F.; Scala, A.; Toma, L. *J. Carbohydr. Chem.* **1992**, *11*, 89-94. Colombo, D.; Ronchetti, F.; Scala, A.; Taino, I. M.; Toma, L. *Bioorg. Med. Chem.* **1993**, *1*, 375-380.
6. Murakami, N.; Morimoto, T.; Imamura, H.; Nagatsu, A.; Sakakibara, J. *Tetrahedron* **1994**, *50*, 1993-2002 and references cited therein.
7. Nakano, H.; Kitahata, S.; Tominaga, Y.; Takenishi, S. *Agric. Biol. Chem.* **1991**, *55*, 2083-2089.
8. Marinone Albini, F.; Murelli, C.; Patritti, G.; Rovati, M. *Synthetic Commun.*, in press.
9. Kaneda, M. *Phytochemistry* **1990**, *29*, 3559-3564.
10. Bovara, R.; Carrea, G.; Ferrara, L.; Riva, S. *Tetrahedron: Asymmetry* **1991**, *2*, 931-938.
11. Lopez, R.; Perez, C.; Fernandez-Mayoralas, A.; Conde, S. *J. Carbohydr. Chem.* **1993**, *12*, 165-171.
12. Bevinakatti, H. S.; Banerji, A. A.; Newadkar, R. V. *J. Org. Chem.* **1989**, *54*, 2453-2455.
13. Hirose, Y.; Kariya, K.; Sasaki, I.; Kurono, Y.; Ebiike, H.; Achiwa, K. *Tetrahedron Lett.* **1992**, *33*, 7157-7160.

(*Received in UK* 3 *May* 1994)