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SHORT COMMUNICATION

SEMISYNTHETIC LYSOGALACTOLIPIDS OF PLANT ORIGIN

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Abstract—Starting from the natural mono- and digalactosyl diglycerides, 1′-*O*-acyl-3′-*O*-β-D-galactopyranosyl-*sn*-glycerol and 1′-*O*-acyl-3′-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-*sn*-glycerol were synthesized. In an attempt to prepare the 2′-*O*-acyl-isomer, only a mixture of the 1′- and 2′-*O*-acyl-isomers was obtained.

INTRODUCTION

Lysoglycolipids have been recently found in small amounts in plants and bacteria,[1–4] but very little is known about their metabolism. Formally they are derived from the corresponding glycosyl diglycerides by the loss of one fatty acid. Lysoglycolipids are readily obtained from the corresponding glycosyl diglycerides by hydrolysis with pancreatic lipase, by which the positional distribution of fatty acids has been investigated.[5–8] Monogalactosyl monoglyceride has been demonstrated to be an intermediate product in the enzymatic hydrolysis of monogalactosyl diglyceride by galactolipase.[9] It is very likely that lysogalactolipids are also involved as intermediates in acyltransfer reactions leading to the formation of acyl galactosyl diglyceride[10] and acylated steryl glucoside.[11] Apart from these catabolic reactions it would be very interesting to see if lysogalactolipids in analogy to lysophospholipids[12] are involved in anabolic reactions, which may contribute to the specific esterification of different fatty acids at C-1′ and C-2′ of the glycerol moiety in galactolipids.[5,6,8] To start such investigations, we have prepared mono- and digalactosyl monoglycerides by a deacylation–reacylation sequence,[13] which also makes possible the preparation of doubly labelled compounds.

RESULTS AND DISCUSSION

1′-O-*Acyl*-3′-O-β-D-*galactopyranosyl*-sn-*glycerol*

With minor modifications, hydrogenated monogalactosyl diglyceride was carried through the reaction sequence described in detail previously to obtain 3′-*O*-(2,3,4,6-tetra-*O*-[1-methoxyethyl]-β-D-galactopyranosyl)-*sn*-glycerol.[13] The primary hydroxyl group of this

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compound was selectively acylated with acyl chloride at −20°,[14] and the blocking groups were subsequently removed by hydrolysis with boric acid.[15,16] For the final purification silicic acid chromatography was avoided, since it has been observed that 2′-*O*-acyl-3′-*O*-β-D-galactopyranosyl-*sn*-glycerol, which was produced by lipase hydrolysis of monogalactosyl diglyceride, was recovered as the 1′-*O*-acyl-isomer after purification by silicic acid column chromatography.[8] Therefore we used Sephadex LH-20 for the final purification, which has been used successfully in the isolation of lysophospholipids.[17] In this way 1′-*O*-acyl-3′-*O*-β-D-galactopyranosyl-*sn*-glycerol was obtained. No contamination by the 2′-*O*-acyl-isomer could be detected by NMR spectroscopy (Fig. 1c), which would have shown up as a multiplet due to the deshielded proton at C-2′ downfield of the doublet of the anomeric proton at 4·65 ppm.[18] By the same method 1′-*O*-acyl-3′-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-*sn*-glycerol was prepared.

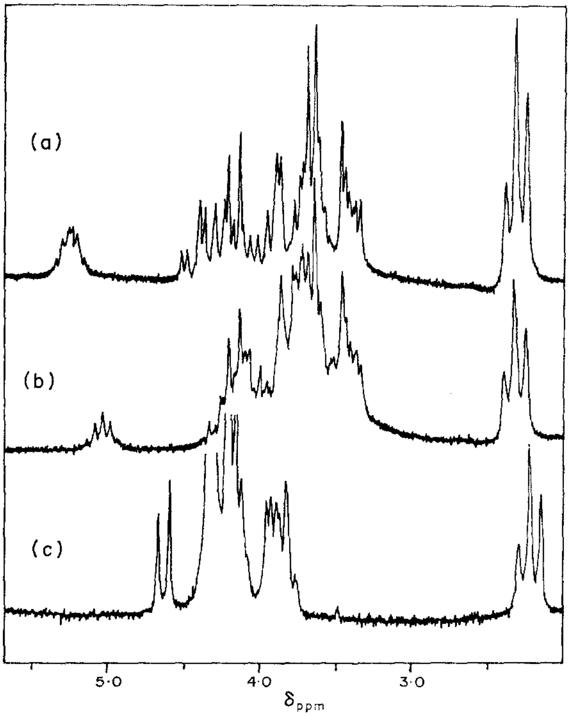

FIG. 1. NMR-SPECTRA OF (a) TMS-DERIVATIVE OF 1′,2′-DI-*O*-ACYL-3′-*O*-β-D-GALACTOPYRANOSYL-*sn*-GLYCEROL IN $CDCl_3$; (b) TMS-DERIVATIVE OF PARTIALLY ISOMERIZED 2′-*O*-STEAROYL-3′-*O*-β-D-GALACTOPYRANOSYL-*sn*-GLYCEROL IN $CDCl_3$; (c) 1′-*O*-STEAROYL-3′-*O*-β-D-GALACTOPYRANOSYL-*sn*-GLYCEROL IN PYRIDINE.

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2′-O-*Acyl*-3′-O-*β*-D-*galactopyranosyl*-sn-*glycerol*

For the preparation of the 2′-*O*-acyl-isomer, 3′-*O*-(2,3,4,6-tetra-*O*-[1-methoxyethyl]-*β*-D-galactopyranosyl)-*sn*-glycerol was reacted with triphenylchloromethane to protect the primary hydroxyl group. After purification by silicic acid column chromatography, the secondary hydroxyl group was acylated with acyl chloride. Subsequent removal of the blocking groups by boric acid hydrolysis and final purification by chromatography on Sephadex LH-20 gave a crystalline product, the melting points of which were lower than those of the 1′-*O*-acyl-isomer. According to the NMR-spectrum (Fig. 1b), it was a mixture of 1′- and 2′-*O*-acyl-isomers in a ratio of about 3:2. This ratio was calculated from the integrated signals of the tertiary proton of C-2′ at 5·05 ppm and the *α*-methylene protons of the acyl group at 2·32 ppm. Because of the missing deshielding acyl group at C-1′, the multiplet at 5·05 ppm, which is attributed to the proton at C-2′ of the 2′-*O*-acyl-isomer, appears at slightly higher field than the signal of the same proton in the 1′,2′-di-*O*-acyl-compound at 5·25 ppm (Fig. 1a). This upfield shift of the tertiary proton in glycerides can be observed going from triglycerides, through *α*, *β*-diglycerides to *β*-monoglycerides.[19–21] Although conditions which cause acyl migration[22] were avoided as far as possible, we were not able to obtain a pure 2′-*O*-acyl-isomer. These and the previous results[8] show that the 2′-*O*-acyl-isomer apparently is readily isomerized to the 1′-*O*-acyl-isomer. A similar situation is found with diglycerides, where acyl migrations cause the accumulation of 1,3-diglycerides, amounting to about 60% of an equilibrated mixture.[22] Wehrli and Pomeranz, who obtained a monogalactosyl monoglyceride as a byproduct in their synthesis of monogalactosyl diglyceride, were not concerned with this problem,[23] and in the other lysoglycolipids described so far, the location of the acyl group has not been investigated.[1–7,9]

EXPERIMENTAL

As solvents for TLC, $CHCl_3$–MeOH (13:7) for monogalactosyl monoglycerides and $CHCl_3$–MeOH (1:1) for digalactosyl monoglycerides were used. Compounds were detected by spraying with 50% aq. H_2SO_4 and subsequent charring. Monogalactosyl diglyceride (1′,2′-di-*O*-acyl-3′-*O*-*β*-D-galactopyranosyl-*sn*-glycerol), s.p. 95–96°, m.p. 152–153°, $[\alpha]_D^{21}$ − 2·31 (c, 4·1 in pyridine) and digalactosyl diglyceride [1′,2′-di-*O*-acyl-3′-*O*-(6-*O*-*α*-D-galactopyranosyl-*β*-D-galactopyranosyl)-*sn*-glycerol], s.p. 78–80°, m.p. 213–215°, $[\alpha]_D^{21}$ +38·2 (c, 1·3 in pyridine) were isolated from lyophilized leaves of *Sinapis alba* and hydrogenated. Reaction with methyl vinyl ether gave the *O*-(1-methoxyethyl)-derivatives as described before.[13]

1′-O-*Stearoyl*-3′-O-*β*-D-*galactopyranosyl*-sn-*glycerol*.1′,2′-di-*O*-acyl-3′-*O*-(2,3,4,6-tetra-*O*-[1-methoxyethyl]-*β*-D-galactopyranosyl)-*sn*-glycerol (5·8 g) was reduced with $LiAlH_4$ (0·6 g) in Et_2O (20 ml) at room temp. for 30 min.[24] After addition of wet ether and filtration the solution was dried over Na_2SO_4. Solvent removal and column chromatography on silicic acid (Merck Kieselgel 0·05–0·2 mm) gave 3′-*O*-(2,3,4,6-tetra-*O*-[1-methoxyethyl]-*β*-D-galactopyranosyl) *sn*-glycerol (0·6 g). This compound was acylated at − 20° in pyridine with 1 molar equivalent of stearoyl chloride exactly as described before.[13] Subsequently the blocking groups were removed with boric acid and the mixture was fractionated on a Sephadex LH-20 column (100 × 3 cm) by using $CHCl_3$–MeOH (1:1) for elution and collecting 10 ml fractions. Fractions containing the pure compound were pooled and yielded 180 mg (6% with respect to monogalactosyl diglyceride as starting material), s.p. 103–104°, m.p. 175–177° (crystallized from $CHCl_3$–MeOH), $[\alpha]_D^{21}$ − 4·86 (c, 1·8 in pyridine), reported s.p. 104–107°, m.p. 172–175°, $[\alpha]_D^{21}$ − 4·84.[8] (Found: C, 62·21; H, 10·11. $C_{27}H_{52}O_9$ required: C, 62·28; H, 10·07%.)

In the same way the following compounds were prepared: 1′-O-*Palmitoyl*-3′-O-*β*-D-*galactopyranosyl*-sn-*glycerol*. S.p. 99–101°, m.p. 126–128°, $[\alpha]_D^{21}$ − 4·60 (c, 0·3 in pyridine). (Found: C, 61·02; H, 9·92. $C_{25}H_{48}O_9$

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required: C, 60·95; H, 9·82%.) *1′-O-Palmitoyl-3′-O-(6-O-α-D-galactopyranosyl-β-D-galatopyranosyl)-*sn-*glycerol.* Overall yield 10%, $[\alpha]_D^{21}$ + 49·1 (c, 0·3 in pyridine) (Found: C, 56·66; H, 8·76 . $C_{31}H_{58}O_{14}$ required: C, 56·86; H, 8·93%.) According to the NMR spectrum, this compound was contaminated to 10% by the 2′-*O*-acyl-isomer, as indicated by the multiplet of H-2′ at 5·00 ppm of the TMS-derivative in $CDCl_3$. The doublet at 4·72 ppm (*J* 3 cycles/sec) is attributed to the α-anomeric proton of the second galactosyl residue. The remaining sugar and glycerol protons, forming a highly overlapping complex between 3·3 and 4·3 ppm, could not be assigned. *1′-O-Oleoyl-3′-O-(6-O-α-D-galactopyranosyl-β-D-galactopyranosyl)-*sn-*glycerol.* Overall yield 12%, $[\alpha]_D^{21}$ + 55·2 (c, 1·4 in pyridine). (Found: C, 58·07; H, 8·99. $C_{33}H_{60}O_{14}$ required: C, 58·22; H, 8·88%.)

*2′-O-Stearoyl-3′-O-β-D-galactopyranosyl-*sn-*glycerol.* 3′-*O*-(2,3,4,6-tetra-*O*-[1-methoxyethyl]-β-D-galactopyranosyl)-*sn*-glycerol (1·1 g) in pyridine (30 ml) at 5° was mixed with a cooled solution of Ph_3CCl (2·6 g) in CH_2Cl_2 (20 ml). After 4 days TLC with $CHCl_3$–MeOH (100:1) showed almost complete conversion to a more hydrophobic product. The mixture was diluted with ether, extracted several times with $NaHCO_3$-solution and dried over Na_2SO_4. After removal of the solvents the triphenylmethyl ether was purified by silicic acid column chromatography using $CHCl_3$–hexane (2:3) for elution. The purified compound (1·0 g) in pyridine was acylated with stearoyl chloride at room temp. overnight and hydrolysed with boric acid as described. Purification by chromatography on Sephadex LH-20 gave a product (400 mg, 6% overall yield) with s.p. 93–94°, m.p. 166–168° (crystallized from $CHCl_3$–MeOH) and $[\alpha]_D^{21}$ − 4·73 (c, 3·7 in pyridine). (Found: C, 62·15; H, 9·94 . $C_{27}H_{52}O_9$ required: C, 62·28; H, 10·07%.)

For NMR spectroscopy (Varian HA 100), the compounds were trimethylsilylated in pyridine with trimethylchlorosilane, since especially the digalactosyl monoglycerides were precipitated from pyridine solution by the addition of tetramethylsilane, which was used as internal standard.

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Key Word Index—Lysogalactolipids; synthesis; 1-*O*-acyl-3-*O*-β-galactopyranosyl-*sn*-glycerol.