

SYNTHESIS OF TRITERPENE GLYCOSIDES

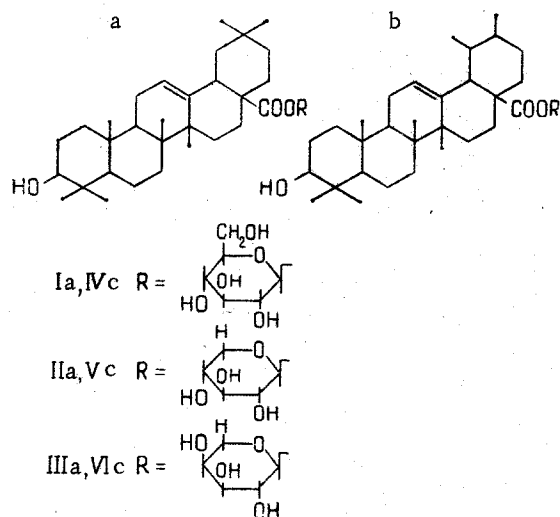
I. Synthesis of acyl glycosides of oleanolic and ursolic acids

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Synthetic glucosides of oleanolic acid [1] and glycosides of its esters [2] are known. There is only one paper on the synthesis of an acyl- β -D-glucopyranoside of acetyloleanolic acid [1] and a reference to a synthetic acyl- β -D-glucopyranoside of oleanolic acid [3]. However, there are many statements in the literature that acyl glycosides of triterpene hydroxyacids with a hindered carboxyl are widely distributed in nature because of their comparative stability [1, 4]. It is probable that they may be regarded as the starting materials in the synthesis of natural triterpene glycosides with a mixed glycosidic bond.

Here we present data on a synthesis of some acyl glycosides of oleanolic and ursolic acids which is based on a study of the glycosidation reaction of free oleanolic acid at various pH values. It has been found that in pyridine with aceto-bromosugars in the presence of silver oxide oleanolic acid gives predominantly acetates of acyl glycosides, while the secondary hydroxyl of oleanolic acid does not react. This has also been found for another triterpene hydroxy acid with a hindered carboxyl, ursolic acid. By this method, via their acetates, we have synthesized acyl- β -D-glucopyranosides (Ia, IVc), acyl- β -D-xylopyranosides (IIa, Vc), and acyl- α -L-arabinopyranosides (IIIa, VIc) of oleanolic acid (a) and of ursolic acid (b) in high yields.



In the case of the acyl- β -D-xylopyranosides, tri-O-acetyl- α -D-xylopyranosyl bromide was replaced by tri-O-benzoyl- α -D-xylopyranosyl bromide, which gave some increase in yield. All the acetates or benzoates of the acyl glycosides were purified on columns of silica with gradient elution by mixtures of benzene and ether. After the deacetylation of the acetates with sodium methoxide [5] or 10% caustic potash in methanol [1], the free acyl glycosides were obtained. In the case of the noncrystalline acetates of acyl- α -L-arabinopyranosides and benzoates of acyl- β -D-xylopyranosides, the free acyl glycosides obtained were purified on a column of silica with gradient elution first with mixtures of benzene and ether and then with mixtures of benzene and ether and then with mixtures of benzene and methanol.

The structure of the acyl glycosides was shown with an acyl- β -D-glucopyranoside of oleanolic acid as an example. On acetylation with acetic anhydride in pyridine, the latter gave a tetra-O-acetylacyl- β -D-glucopyranoside of acetyloleanolic acid. The same compound was obtained by the acetylation of a tetra-O-acetylacyl- β -D-glucopyranoside of oleanolic acid and by synthesis from acetyloleanolic acid under the conditions of the preparation of acetates of acyl glycosides. The tetra-O-acetylacyl- β -D-glucopyranoside of acetyloleanolic acid that we have prepared is probably identical with the compound synthesized by Khorlin et al. by another method [1]. Moreover, all the acyl glycosides of oleanolic and ursolic acids gave the corresponding triterpene acid and monosaccharide on methylation with diazomethane with subsequent hydrolysis by hydrochloric acid in methanol [6]. For all the acyl glycosides investigated, the specific rotation calculated by Klyne's method [7] and those found agreed satisfactorily.

Experimental

All the melting points were determined in a capillary and were not corrected. Chromatography was carried out with

silica gel of type KSK (100–150 mesh for columns and 200–250 mesh for plates). Chromatographic monitoring was carried out on plates (9 × 12 cm) with a thin fixed layer of silica gel in the following systems: 1) Benzene–ether (7:3) with traces of pyridine; 2) butanol–ethanol–18% ammonia (18:3.5:18); 3) toluene–butanol–25% ammonia (1:1:2); 4) benzene–methanol (7:3); 5) butanol–acetic acid–water (4:1:5). The chromatograms were revealed with antimony pentachloride in chloroform. The angles of rotation of the acyl glycosides were measured in absolute ethanol, and those of their acetates and benzoates in chloroform. Analytical samples were dried in a vacuum gun over phosphorus pentoxide at 83° C.

2, 3, 4, 6-Tetra-O-acetylacyl-β-D-glucopyranoside of oleanolic acid. One gram of tetra-O-acetyl-α-D-glucopyranosyl bromide and 0.5 g of active silver oxide were added to 0.3 g of oleanolic acid in anhydrous pyridine (5 ml). The mixture was shaken vigorously with water cooling for 15 min, and then at room temperature (6 hr). It was then diluted with methanol (100 ml) and passed through a column of KU-2 cation exchanger (H⁺ form). The eluates were distilled in vacuum and the residue was extracted with benzene (3 × 50 ml). The concentrated extracts were transferred to a column of 40 g of silica gel, which was eluted with benzene (100 ml) and subsequently with benzene–ethyl mixtures (9:1, 4:1, 3:1, 7:3, 3:2, and 1:1–100 ml of each mixture). The separation was monitored in system 1.

The combined fractions were distilled, and the residue was crystallized from a mixture of 4 ml of ether and 7 ml of petroleum ether (40°–60° C).

After recrystallization from the same solvent, the substance (0.38 g, or 74% of theoretical) became transparent at 133°–135° C (decomp.) and gave a meniscus at 150°–152° C. It had $[\alpha]_D^{20} +35 \pm 2^\circ$ (c 2.026). A figure of $[\alpha]_D^{20} +37.8^\circ$ was calculated by Klyne's method. In the calculations, the specific rotations were taken as $[\alpha]_D^{20} +80^\circ$ C (chloroform) for oleanolic acid [8] and $[\alpha]_D^{20} -18.7^\circ$ (chloroform) for the tetraacetate of methyl β-D-glucopyranoside [9].

Found, %: C 66.42; 66.58; H 8.48; 8.45. Calculated for C₄₄H₆₆O₁₂ · 1/2H₂O, %: C 66.39; H 8.46.

The acyl-β-D-glucopyranoside of oleanolic acid. A 0.01 N solution of sodium methoxide in methanol (2 ml) was added to 0.5 g of the acetate of the acyl-β-D-glucopyranoside of oleanolic acid in 5 ml of absolute methanol. The mixture was kept for a day at room temperature and then for 6 hr at 0° C. This gave 0.28 g of a substance with mp 221°–223° C (decomp.) and $[\alpha]_D^{20} +39 \pm 2^\circ$ (c 1.08). According to the literature: mp 216°–218° C (decomp.) and $[\alpha]_D^{20} +46.5^\circ$ (ethanol) [3]. An additional portion of substance (0.02 g) with mp 220°–222° C was isolated from the mother liquor. The total yield of pure acyl glycoside was 75% of theoretical. Its purity was checked in systems 2 and 3. The rotation for the acyl-β-D-glucopyranoside calculated by Klyne's method was $[\alpha]_D^{20} +47^\circ$. In the calculations, the specific rotations were taken as $[\alpha]_D^{20} +79^\circ$ (ethanol) for oleanolic acid [10] and $[\alpha]_D^{20} -34.2^\circ$ (water) for methyl β-D-glucopyranoside [11].

Found, %: C 70.04; 69.93; H 9.62; 9.55. Calculated for C₃₆H₅₈O₈, %: C 66.87; H 9.45.

Acyl-β-D-xylopyranoside of ursolic acid. 1.28 g of tri-O-benzoyl-α-D-xylopyranosyl bromide [12] and 0.5 g of active silver oxide were added to 0.53 g of ursolic acid in anhydrous pyridine (5 ml). The mixture was shaken with water cooling for 20 min and then at room temperature for 6 hr. After dilution with glacial acetic acid (20 ml) the mixture was poured into water (500 ml). The precipitate was dried and extracted with benzene. The extracts were chromatographed on a column of silica gel as in the preceding case. The sirupy product obtained was treated with 10% caustic potash in methanol (3 ml) at 3° C (24 hr) and passed through KU-2 cation exchanger (H⁺ form). The methanolic eluates were evaporated in vacuum to 10 ml and stirred with silica gel. The air-dried mixture was transferred to a column previously filled with silica gel (30 g) and this was subjected to gradient elution with mixtures of benzene and ether and benzene and methanol in ratios of 9:1, 4:1, 3:1, and 7:3. The separation was monitored in systems 3 and 4. The combined fractions were distilled and the residue was crystallized from 70% methanol (5 ml). This gave 0.22 g of a substance with mp 218°–220° C (decomp.) and $[\alpha]_D^{20} +31 \pm 3^\circ$ (c 0.85), which corresponds to 57% of theoretical. Calculations by Klyne's method gave $[\alpha]_D^{20} +34^\circ$. In the calculations $[\alpha]_D^{20} +66.5^\circ$ (ethanol) was taken for ursolic acid [13] and $[\alpha]_D^{20} -65.5^\circ$ for methyl β-D-xylopyranoside [14].

Found, %: C 70.54; 70.22; H 9.46; 9.64. Calculated for C₃₅H₅₆O₇ · 1/2H₂O, %: C 70.31; H 9.61.

Information on the preparation of the acyl glycosides of oleanolic and ursolic acids is given in the table.

Tetra-O-acetylacyl-β-D-glucopyranoside of acetyloleanolic acid. 0.5 g of the acetate of the acyl-β-D-glucopyranoside of oleanolic acid in pyridine (5 ml) was acetylated with acetic anhydride (2.5 ml) at room temperature for 24 hr. The mixture was poured into water and the precipitate was recrystallized from a mixture of ether and petroleum ether (70°–100° C). A substance (0.4 g) was isolated which became transparent at 130°–132° C and gave a meniscus at 146°–148° C, $[\alpha]_D^{20} +40 \pm 1^\circ$ (c 2.518). According to the literature: mp 148° C and $[\alpha]_D^{20} +23 \pm 3^\circ$ (c 4; pyridine) [1]. Another portion of pure substance (0.05 g) was isolated from the mother liquor. The total yield was 85% of theoretical. Calculation by Klyne's method gave $[\alpha]_D^{20} +39^\circ$ for the acetate of the acyl-β-D-glucopyranoside of acetyloleanolic acid. In the calculations we took the specific rotations $[\alpha]_D^{20} +74.5^\circ$ (chloroform) for acetyloleanolic acid [16] and $[\alpha]_D^{20} -18.7^\circ$

Acylated glycosyl halide	Acyl glycoside	Yield, % of theoretical	[α]D, deg		Mp, °C	Solvent	Formula	Analysis, %			
			found	calculated				calculated		found	
								C	H	C	H
Tri-O-acetyl- β -L-arabinopyranosyl bromide	<u>Oleanolic acid</u> Acyl- α -L-arabinopyranoside	49	+54 \pm 3	+67	224°-226°	MeOH	$C_{35}H_{56}O_7 \times CH_3OH$	69.64	9.74	69.67	9.68
			(c 0.51)					71.39	9.58	71.33	9.69
Tri-O-benzoyl- α -D-xylopyranosyl bromide	<u>Ursolic acid</u> Acyl- β -D-glucopyranoside	54	+38 \pm 2	+45	238°-240°	MeOH	$C_{35}H_{56}O_7$	68.86	9.47	68.82	9.56
			(c 0.7)					67.14	8.43	66.95	8.56
Tetra-O-acetyl- α -D-glucopyranosyl bromide	Tetra-O-acetyl	78	+43 \pm 3	+31	153°-155°	(decomp.) H ₂ O-MeOH ether	$C_{44}H_{68}O_{12}$	71.39	9.56	71.01	9.50
			(c 1.08)								
Tri-O-acetyl- β -L-arabinopyranosyl bromide [15]	Acyl- α -L-arabinopyranoside	54	+65 \pm 3	+57	184°-186°	MeOH	$C_{35}H_{56}O_7$	71.39	9.56	71.01	9.50
			(c 0.53)								

for the tetraacetate of methyl β -D-glucopyranoside [9]. We prepared this compound similarly from the acyl- β -D-glucopyranoside of oleanolic acid (yield 75%) and also by the condensation of acetyloleanolic acid with tetra-O-acetyl- α -D-glucopyranosyl bromide, as in the preceding experiments (yield 50%). The compounds isolated in the three experiments were identical with respect to melting point, specific rotation, IR spectrum, and chromatographic behaviour in system 1.

Found, %: C 66.56; 66.85; H 8.39; 8.26. Calculated for $C_{46}H_{68}O_{13}$, %: C 66.63; H 8.27.

Methylation and hydrolysis of the acyl- β -D-glucopyranoside of oleanolic acid. 0.11 of the acyl- β -D-glucopyranoside of oleanolic acid in methanol (8 ml) was methylated with a 2% ethereal solution of diazomethane (25 ml) at room temperature for 24 hr. Chromatography in system 1 indicated that no methylation had taken place. The diazomethane was decomposed with acetic acid and the residue after the elimination of the solvent was hydrolyzed with 16 ml of 10% hydrochloric acid-methanol (1:1, 16 ml) in a steam bath for 22 hr. The precipitate that deposited was washed with water and dried (0.074 g). From its IR spectrum and chromatographic behaviour in systems 1 and 3 it was identical with oleanolic acid. Yield 93% of theoretical. The filtrate from the hydrolysate was passed through Dowex-2 anion exchanger (CO_3^- -form). A chromatographic investigation of the eluates in system 5, with the chromatogram revealed by α -naphthol [17], showed the presence of glucose alone.

Summary

1. The acyl- β -D-glucopyranosides, acyl- β -D-xylopyranosides, and acyl- α -L-arabinopyranosides of oleanolic and ursolic acids have been synthesized for the first time.

2. A method for the synthesis of acyl glycosides of triterpene hydroxyacids with hindered carboxy groups has been developed.

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