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Synthesis and Hemolytic Properties of Arvensoside B Isomers

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Summary: The first partial syntheses of galactosyl-glucosyl oleanolic acid disaccharides are described. Arvensoside B and calenduloside A, which have earlier been isolated from *Calendula arvensis* and *Calendula officinalis*, and some further oleanolic acid glycosides were prepared from differently linked acetobromo sugars. The hemolytic properties of these saponins were investigated. Systematic variation of the carbohydrate structure and comparison with already synthesized glucosyl-glucosyl analogues enable general conclusions about structure-activity relationships.

Keywords. Arvensoside B; Calenduloside A; Oleanolic acid glycosides; Partial synthesis; Hemolytic properties.

Introduction

Some oleanolic acid disaccharides containing galactopyranosyl-glucopyranosyl residues have already been isolated from plants. The antimutagenic [1] and cytotoxic [2] saponin 3-O-(O-β-*D*-galactopyranosyl-(1 $\rightarrow$ 3)-β-*D*-glucopyranosyl) oleanolic acid called arvensoside B (**1**) was found in *Calendula arvensis* [3]. Its antiviral, antifungal, and molluscidal activities have been determined [4, 5, 6]. The 1 $\rightarrow$ 4 linked analogue, calenduloside A (**2**) has been isolated from *Calendula officinalis* [7] and tested for its antiviral activity [8].

The hemolytic activity is a well-known property of saponins, but only few oleanolic acid glycosides have yet been tested for it [9–11]. Therefore, general conclusions about structure-activity relationships are not possible. However, some authors have compared the hemolytic properties of oleanolic acid glycosides with those of compounds containing other aglyca, ignoring the strong influence of the aglycon structure [12–14] and the fact that the effect of the sugar residue on the hemolytic activity is not transferable from one aglycon to another [15]. The influence of the structure of the carbohydrate residue on the hemolytic power has been described contradictory: some authors have observed the maximum of hemolytic strength for saponins with short [12] or branched [16] sugar moieties, whereas

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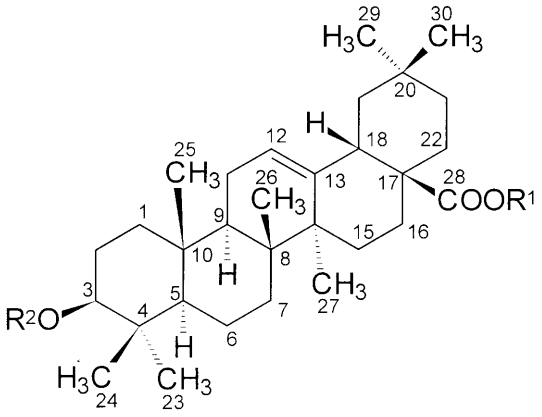


1: R^1 = H, R^2 = *β-D-galp*-(1→3)-*β-D-glcp*-(1→
2: R^1 = H, R^2 = *β-D-galp*-(1→4)-*β-D-glcp*-(1→
3: R^1 = H, R^2 = *α-D-galp*-(1→2)-*α-D-glcp*-(1→
4: R^1 = H, R^2 = *β-D-galp*-(1→2)-*α-D-glcp*-(1→
5: R^1 = H, R^2 = *α-D-galp*-(1→6)-*β-D-glcp*-(1→
6: R^1 = H, R^2 = *β-D-galp*-(1→6)-*β-D-glcp*-(1→
7: R^1 = H, R^2 = H
8: R^1 = $CH(Ph)_2$, R^2 = H

Scheme 1

others have supposed high activity for compounds with up to five monosaccharide units [13].

For the present we have focussed our investigations on the hemolytic power of glycosides of oleanolic acid, one of the most widespread triterpene aglyca, to determine the influence of the structure of the sugar moiety. Previously, we have described the remarkably high influence of the linkage of glucosyl-glucosyl residues on the hemolytic effect of synthetic oleanolic acid monodesmosides [17]. This paper deals with the same dependency in the isomeric galactosyl-glucosyl disaccharides **1–6** of oleanolic acid (**7**) as well as the first synthetic access to those compounds (Scheme 1).

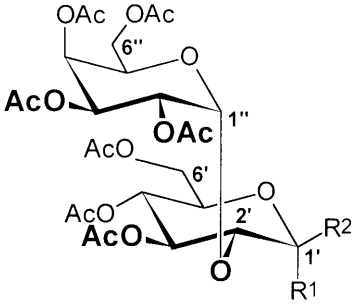
Results and Discussion

Oleanolic acid (**7**)

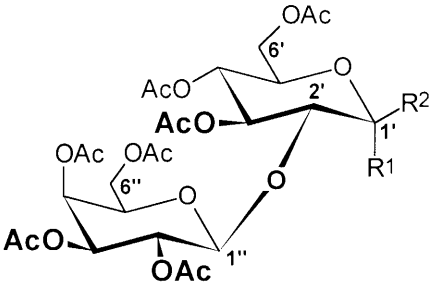
Oleanolic acid (**7**) was prepared from cloves according to a procedure given in the literature [18]. The carboxyl group of **7** was protected by esterification with diphenyldiazomethane [19–21], and the diphenylmethyl oleanolate **8** was used as the starting material for the glycosidation reactions.

Glycosyl donors (**9–14**)

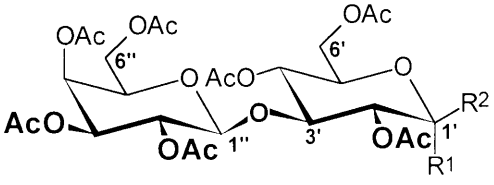
As glycosyl donors, the acetobromodisaccharides **9–14** (Scheme 2) were used which were usually prepared from the corresponding disaccharide octaacetates **15–20** by



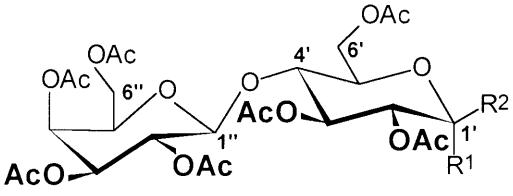
9: R^1 = Br, R^2 = H
15: R^1 = OAc, R^2 = H



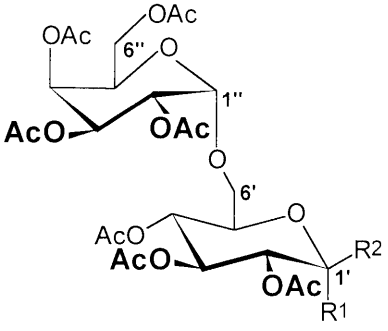
10: R^1 = Br, R^2 = H
16: R^1, R^2 = OAc, H



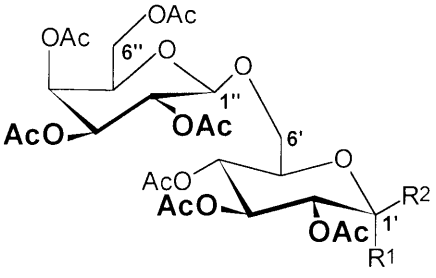
11: R^1 = Br, R^2 = H
17: R^1, R^2 = OAc, H



12: R^1 = Br, R^2 = H
18: R^1, R^2 = OAc, H



13: R^1 = Br, R^2 = H
19: R^1, R^2 = OAc, H



14: R^1 = Br, R^2 = H
20: R^1 = H, R^2 = OAc

Scheme 2

bromination with HBr using the method of *Brauns* [22]. Unfortunately, the reaction of galactopyranosyl-(1 → 6)-glucopyranose octaacetate **20** with HBr resulted in decomposition. Therefore, compound **14** was prepared following the method of *Zemplen* [23] using titanium tetrabromide.

Disaccharide octaacetates (**15**–**20**)

The disaccharide octaacetates **15**–**20** were prepared by different procedures. α-*D*-Galactopyranosyl-(1 → 2)-α-*D*-glucopyranose octaacetate (**15**) was obtained by the

reaction of acetobromogalactose with 1,3,4,6-tetra-O-acetyl-α-*D*-gluco-pyranose using the method of *Helferich* and *Zirner* [24]. β-*D*-Galactopyranosyl-(1 $\rightarrow$ 2)-*D*-glucopyranose octaacetate (**16**) and β-*D*-galactopyranosyl-(1 $\rightarrow$ 3)-*D*-glucopyranose octaacetate (**17**) were synthesized by *Königs-Knorr* reaction of acetobromogalactose with methyl-4,6-O-benzylidene-α-*D*-glucopyranose [25, 26]. The resulting condensation products were separated by CC giving pure 1 $\rightarrow$ 2 and 1 $\rightarrow$ 3 linked disaccharide units. Hydrolysis and acetylation in one step yield mixtures of α and β isomers of **16** and **17**, respectively. β-*D*-Galactopyranosyl-(1 $\rightarrow$ 4)-*D*-glucopyranose octaacetate (**18**, lactose octaacetate) and α-*D*-galactopyranosyl-(1 $\rightarrow$ 6)-*D*-glucopyranose octaacetate (**19**, melibiose octaacetate) were obtained as a mixture of their α and β isomers by acetylation of lactose and melibiose with acetic anhydride in pyridine. β-*D*-Galactopyranosyl-(1 $\rightarrow$ 6)-β-*D*-glucopyranose octaacetate (**20**, allolactose octaacetate) was prepared by *Königs-Knorr* reaction of acetobromogalactose with 1,2,3,4-tetraacetyl-β-*D*-glucopyranose [27].

Glycosidation, deacetylation, and hydrogenation

Glycosidations were performed by application of *Königs-Knorr* procedures. Excess diphenylmethyl oleanolate (**8**) was recovered using column chromatography. For the condensation of **8** with the acetobromodisaccharides **9–11** and **13** we used silver oxide as catalyst and drierite as drying agent. The glycosidations of **8** with **12** and **14** were carried out using mercury (II) cyanide as catalyst. Cleavage of the acetyl groups with sodium methoxide [28] and catalytic hydrogenation with palladium on charcoal [21] yielded glycosides **1–6**.

The saponins **1**, **2**, **5**, and **6** have β-configuration at the inner anomeric position as indicated by a large coupling constant ($J \cong 8$ Hz). Only for glycosides **3** and **4** we conclude α-configuration at this position. This is evident from ^{1}H NMR spectroscopy: both proton signals exhibit a small coupling constant ($J \cong 3.5$ Hz). The formation of α-glycosides from 1 $\rightarrow$ 2 linked acetobromodisaccharides is due to sterical hindrance which has already been reported for comparable saponins [29].

Determination of hemolytic activity

The 1 $\rightarrow$ 4 linked saponin calenduloside A (**2**) shows the highest activity ($HI = 150700$). The hemolytic activity decreases from linkage position 4 *via* 3 to 6. The corresponding 1 $\rightarrow$ 2 linked oleanolic acid disaccharides are less active. Furthermore, β-configuration of the outer anomeric position effects higher potency in the 1 $\rightarrow$ 6 linked saponins, whereas both 1 $\rightarrow$ 2 linked glycosides **3** and **4** exhibit very low hemolytic activity. The hemolytic activities of the galactopyranosyl-glucopyranosyl oleanolic acid disaccharides **1–6** are given in Table 1.

Previously, we have reported on the hemolytic properties of oleanolic acid disaccharides containing differently linked glucose units [17]. Obviously, the influence of the linkage on the hemolytic power is similar to the above-mentioned results. Only the 1 $\rightarrow$ 3 linked randianin is more active than the 1 $\rightarrow$ 4 linked analogue, whereas the hemolytic potencies of the 1 $\rightarrow$ 6 and the 1 $\rightarrow$ 2 linked analogues are comparable to those of their galactosyl-glucosyl analogues.

Table 1. Hemolytic activity of compounds **1–6**

Saponin	Sugar combination	*HI*	*HI* of *glcp-glcp* analogue [17]
1	β-*galp* 1 → 3β-*glcp* →	100100	150000
2	β-*galp* 1 → 4β-*glcp* →	150700	80350
3	β-*galp* 1 → 2α-*glcp* →	3400	
4	β-*galp* 1 → 2α-*glcp* →	3400	<10000
5	β-*galp* 1 → 6β-*glcp* →	22000	
6	β-*galp* 1 → 6β-*glcp* →	35000	37500

In conclusion, it may be stated that the connectivity between the sugar units strongly influences the hemolytic activity of oleanolic acid disaccharides. Besides, linkage positions 3 or 4 are structural requirements for high potency.

Experimental

General

Catalytic hydrogenations were run in a shaker-type Parr hydrogenation apparatus 3911 at room temperature. Melting points were determined on a digital melting point apparatus (Electrothermal IA 9200) and are uncorrected. Optical rotation: polarimeter 241 MC (Perkin Elmer); MS: Varian MAT 711 spectrometer (70 eV, electron impact and field desorption); IR spectra: infrared spectrometer system 2000 FT (Perkin Elmer); UV/Vis: Lambda 17 UV/Vis spectrometer (Perkin Elmer); NMR spectra: Varian Inova 400 (300 K), 5 mm Tubes, solvent resonance as internal standard. ^{1}H- and ^{13}C-resonances were assigned using ^{1}H, ^{1}H- and ^{1}H, ^{13}C-correlation spectra and are numbered as given in the formulae. Materials: column chromatography (CC): silica gel 60 (Merck, 70 – 230 mesh, pore diameter 60 Å); thin-layer chromatography (TLC): TLC plates (Merck, silica gel 60 F_{254}, 0.2 mm, 200 × 200 mm); preparative TLC: PLC plates (Merck, silica gel 60 F_{254}, 1 mm, 200 × 200 mm); the substances were detected in UV light at 254 nm and by spraying with methanol/sulfuric acid (9:1) and subsequent heating with a hot gun. Nitromethane was deacidified with basic aluminum oxide. Assignments marked with an asterisk are interchangeable.

Determination of hemolytic activity

The hemolytic activity (expressed as Hemolytic Index, *HI*) was evaluated by the method of the Austrian Pharmacopoeia (OeAB 1994) using the *Austrian Saponinstandard* (*HI* = 30000) as a reference.

Preparation of **7** and **8**

Oleanolic acid (**7**) and diphenylmethyl oleanolate (**8**) were prepared as previously reported [17].

Preparation of **9–14**

Compounds **9–13** were prepared from their octaacetates **15–19** according to Ref. [22] by bromination with a solution of HBr in glacial acetic acid (saturated at 0°C). Compound **14** was prepared from the octaacetate **20** using titanium tetrabromide according to Ref. [23].

2α-Galactopyranosyl-α-D-glucopyranosylbromide heptaacetate (**9**; $C_{26}H_{35}BrO_{17}$)

1.4 g (2.1 mmol) 2α-galactopyranosyl-α-*D*-glucopyranose octaacetate were dissolved in 15 cm^3 of dry $CHCl_3$ and treated with 12 cm^3 of the above HBr solution. Crystallization from $CHCl_3$/ligroin yielded 980 mg (1.4 mmol, 66.7%) colourless needles of **9**.

M.p.: 169°C; corresponds well with reported data [30]; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.95 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO), 2.04 (s, 3H, CH_3COO), 2.05 (s, 3H, CH_3COO), 2.08 (s, 3H, CH_3COO), 2.10 (s, 3H, CH_3COO), 3.82 (dd, $J = 9.7$, 3.9 Hz, 1H, 2′-H), 3.94–3.99 (m, 1H, 6″-H), 4.07 (dd, $J = 12.2$, 1.6 Hz, 1H, 6′-H), 4.15–4.22 (m, 2H, 5″-H, 6″-H), 4.25–4.28 (m, 1H, 5′-H), 4.31 (dd, $J = 12.2$, 4.1 Hz, 1H, 6′-H), 5.01 (dd, $J = 10.9$, 3.6 Hz, 1H, 2″-H), 5.04 (t, $J = 9.7$ Hz, 1H, 4′-H), 5.18 (dd, $J = 10.9$, 3.3 Hz, 1H, 3″-H), 5.24 (d, $J = 3.6$ Hz, 1H, 1″-H), 5.43 (d, $J = 3.0$ Hz, 1H, 4″-H), 5.47 (t, $J = 9.6$ Hz, 1H, 3′-H), 6.40 (d, $J = 3.8$ Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.51, 20.57, 20.58, 20.98 (*C*H_3COO), 60.91 (C-6′), 61.13 (C-6″), 67.12 (C-3″), 67.22, 67.34 (C-4′, C-5″), 67.48 (C-4″), 67.72 (C-2″), 71.31 (C-3′), 72.16 (C-5′), 73.27 (C-2′), 86.89 (C-1′), 94.16 (C-1″), 169.55, 169.64, 169.76, 170.00, 170.15, 170.32, 170.63 (CH_3*C*OO) ppm.

2β-Galactopyranosyl-α-D-glucopyranosylbromide heptaacetate (**10**; $C_{26}H_{35}BrO_{17}$)

1.48 g (2.2 mmol) 2β-galactopyranosyl-α-*D*-glucopyranose octaacetate were dissolved in 15 cm^3 of dry $CHCl_3$, and 13 cm^3 of the above HBr solution were added. Treatment with ligroin yielded 940 mg (1.3 mmol, 61.1%) of **10** as amorphous solid.

^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.93 (s, 3H, CH_3COO), 1.97 (s, 3H, CH_3COO), 1.99 (s, 3H, CH_3COO), 2.02 (s, 6H, 2 CH_3COO), 2.04 (s, 3H, CH_3COO), 2.13 (s, 3H, CH_3COO), 3.79 (dd, $J = 9.4$, 3.7 Hz, 1H, 2′-H), 3.85 (t, $J = 6.5$ Hz, 1H, 5″-H), 4.03–4.14 (m, 3H, 6′-H, 6″-H), 4.25–4.31 (m, 2H, 5′-H, 6′-H), 4.60 (d, $J = 7.8$ Hz, 1H, 1″-H), 4.91 (dd, $J = 10.2$, 3.3 Hz, 1H, 3″-H), 5.05 (t, $J = 9.7$ Hz, 1H, 4′-H), 5.14 (t, $J = 9.9$ Hz, 1H, 2″-H), 5.33 (d, $J = 3.2$ Hz, 1H, 4″-H), 5.46 (t, $J = 9.6$ Hz, 1H, 3′-H), 6.37 (d, $J = 3.4$ Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.34, 20.47, 20.61, 20.68 (*C*H_3COO), 61.00 (C-6′), 61.12 (C-6″), 66.78 (C-4″), 67.08, (C-4′), 68.32 (C-2″), 70.59 (C-3″), 71.07 (C-5″), 71.93 (C-5′), 72.21 (C-3′), 75.77 (C-2′), 88.50 (C-1′), 101.37 (C-1″), 168.91, 169.40, 169.59, 170.08, 170.18, 170.29, 170.35 (CH_3*C*OO) ppm.

3β-Galactopyranosyl-α-D-glucopyranosylbromide heptaacetate (**11**; $C_{26}H_{35}BrO_{17}$)

635 mg (0.94 mmol) 3β-galactopyranosyl-α-*D*-glucopyranose octaacetate were dissolved in 7 cm^3 of dry $CHCl_3$, and 6 cm^3 of the above HBr solution were added. Yield: 578 mg (0.83 mmol, 88.3%) colourless resin.

^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.93 (s, 3H, CH_3COO), 1.95 (s, 3H, CH_3COO), 2.04 (s, 6H, 2 CH_3COO), 2.07 (s, 3H, CH_3COO), 2.12 (s, 3H, CH_3COO), 2.15 (s, 3H, CH_3COO), 3.92 (t, $J = 6.7$ Hz, 1H, 5″-H), 4.02–4.24 (m, 6H, 3′-H, 5′-H, 6′-H, 6″-H), 4.62 (d, $J = 8.0$ Hz, 1H, 1″-H), 4.78 (dd, $J = 9.8$, 4.1 Hz, 1H, 2′-H), 4.94 (dd, $J = 10.4$, 3.3 Hz, 1H, 3″-H), 5.05 (dd, $J = 10.4$, 8.0 Hz, 1H, 2″-H), 5.08 (t, $J = 10.4$ Hz, 1H, 4′-H), 5.33 (d, $J = 3.7$ Hz, 1H, 4″-H), 6.49 (d, $J = 3.9$ Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.44, 20.51, 20.57, 20.60, 20.63, 20.69, 20.77 (*C*H_3COO), 60.84 (C-6″), 61.10 (C-6′), 66.73 (C-4″), 66.77 (C-4′), 68.91 (C-2″), 70.51 (C-5″), 70.91 (C-3″), 72.31 (C-2′), 72.38 (C-5′), 76.11 (C-3′), 87.27 (C-1′), 101.01 (C-1″), 168.93, 169.02, 169.45, 170.13, 170.17, 170.35, 170.57 (CH_3*C*OO) ppm.

4β-Galactopyranosyl-α-D-glucopyranosylbromide heptaacetate (**12**; $C_{26}H_{35}BrO_{17}$)

3 g (4.4 mmol) of 4β-galactopyranosyl-α-*D*-glucopyranose octaacetate (octaacetyllactose) were dissolved in 30 cm^3 of dry $CHCl_3$, and 20 cm^3 of the above HBr solution were added. Crystallization from $CHCl_3$/ligroin yielded 2.8 g (4 mmol, 91.0%) colourless needles of **12**.

M.p.: 134°C; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.93 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO), 2.05 (s, 3H, CH_3COO), 2.09 (s, 3H, CH_3COO), 2.12 (s, 3H, CH_3COO), 3.80–3.87 (m, 2H, 4′-H, 5″-H), 4.02–4.19 (m, 4H, 5′-H, 6′-H, 6″-H), 4.46 (d, $J = 10.2$ Hz, 1H, 6′-H), 4.48 (d, $J = 7.8$ Hz, 1H, 1″-H), 4.72 (dd, $J = 9.9$, 4.0 Hz, 1H, 2′-H), 4.92 (dd, $J = 10.4$, 3.4 Hz, 1H, 3″-H), 5.09 (dd, $J = 10.4$, 8.0 Hz, 1H, 2″-H), 5.32 (d, $J = 3.3$ Hz, 1H, 4″-H), 5.51 (t, $J = 9.9$ Hz, 1H, 3′-H), 6.49 (d, $J = 4.1$ Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.44, 20.58, 20.60, 20.73, 20.75 (CH_3COO), 60.83 (C-6″), 61.00 (C-6′), 66.57 (C-4″), 68.98 (C-2″), 69.55 (C-3′), 70.74, 70.80 (C-2′, C-5″), 70.95 (C-3″), 72.94 (C-5′), 74.91 (C-4′), 86.36 (C-1′), 100.75 (C-1″), 168.89, 169.15, 169.90, 169.99, 170.07, 170.10, 170.27 (CH_3COO) ppm.

6α-Galactopyranosyl-α-D-glucopyranosylbromide heptaacetate (**13**; $C_{26}H_{35}BrO_{17}$)

3 g (4.4 mmol) 6α-galactopyranosyl-α-*D*-glucopyranose octaacetate (octaacetyl melibiose) were dissolved in 30 cm^3 of dry $CHCl_3$, and 20 cm^3 of the above HBr solution were added. Treatment with ligroin yielded 1.2 g (1.7 mmol, 38.8%) of **13** as an amorphous solid.

^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.95 (s, 3H, CH_3COO), 2.00 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO), 2.07 (s, 3H, CH_3COO), 2.08 (s, 3H, CH_3COO), 2.10 (s, 3H, CH_3COO), 3.60 (dd, $J = 11.9$, 2.2 Hz, 1H, 6′-H), 3.73 (dd, $J = 11.9$, 4.6 Hz, 1H, 6′-H), 4.00–4.08 (m, 2H, 6″-H), 4.14 (t, $J = 6.6$ Hz, 1H, 5″-H), 4.18–4.22 (ddd, $J = 10.2$, 4.4, 2.2 Hz, 1H, 5′-H), 4.75 (dd, $J = 9.9$, 4.0 Hz, 1H, 2′-H), 5.05 (dd, $J = 10.8$, 3.8 Hz, 1H, 2″-H), 5.11–5.16 (m, 2H, 1″-H, 4′-H), 5.30 (dd, $J = 10.8$, 3.3 Hz, 1H, 3″-H), 5.43 (d, $J = 3.3$ Hz, 1H, 4″-H), 5.52 (t, $J = 9.9$ Hz, 1H, 3′-H), 6.55 (d, $J = 4.0$ Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.54, 20.57, 20.60, 20.67, 20.74 (CH_3COO), 61.55 (C-6″), 65.35 (C-6′), 66.41 (C-5″), 67.39 (C-3″), 67.58 (C-4′), 67.91 (C-4″), 68.01 (C-2″), 70.17 (C-3′), 70.56 (C-2′), 72.86 (C-5′), 86.48 (C-1′), 96.21 (C-1″), 169.28, 169.73, 169.79, 169.82, 170.11, 170.30, 170.47 (CH_3COO) ppm.

6β-Galactopyranosyl-α-D-glucopyranosylbromide heptaacetate (**14**;$C_{26}H_{35}BrO_{17}$)

600 mg (0.88 mmol) 6β-galactopyranosyl-α-*D*-glucopyranose octaacetate (octaacetylallolactose) were dissolved in 2.5 cm^3 of dry $CHCl_3$ and treated with a solution of 1.5 g titanium tetrabromide in 2.2 cm^3 dry $CHCl_3$. Yield: 600 mg (0.86 mmol, 97.4%) colourless resin.

^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.97 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO), 2.04 (s, 6H, CH_3COO), 2.08 (s, 3H, CH_3COO), 2.09 (s, 3H, CH_3COO), 2.13 (s, 3H, CH_3COO), 3.59 (dd, $J = 11.6$, 5.1 Hz, 1H, 6′-H), 3.88 (dt, $J = 6.7$, 0.8 Hz, 1H, 5″-H), 3.98 (dd, $J = 11.6$, 2.1 Hz, 1H, 6′-H), 4.08–4.17 (m, 2H, 6″-H), 4.22 (ddd, $J = 10.3$, 4.8, 1.9 Hz, 1H, 5′-H), 4.48 (d, $J = 8.0$ Hz, 1H, 1″-H), 4.77 (dd, $J = 9.9$, 4.0 Hz, 1H, 2′-H), 5.00 (dd, $J = 10.4$, 3.4 Hz, 1H, 3″-H), 5.05 (t, $J = 9.6$ Hz, 1H, 4′-H), 5.19 (dd, $J = 10.5$, 7.9 Hz, 1H, 2″-H), 5.36 (dd, $J = 3.4$, 0.8 Hz, 1H, 4″-H), 5.52 (t, $J = 9.6$ Hz, 1H, 3′-H), 6.61 (d, $J = 4.0$ Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.49, 20.51, 20.54, 20.56, 20.57, 20.60, 20.71 (CH_3COO), 61.15 (C-6″), 66.64 (C-6′), 66.87 (C-4″), 67.50 (C-4′), 68.31 (C-2″), 70.13 (C-3′), 70.51 (C-2′), 70.71 (C-3″, C-5″), 73.10 (C-5′), 86.50 (C-1′), 101.25 (C-1″), 169.28, 169.54, 169.65, 169.78, 170.02, 170.13, 170.31 (CH_3COO) ppm.

Preparation of glycosides **1–6**

The saponins **1** and **3–5** were prepared by the following procedure. Diphenylmethyloleanolate (**8**) was dissolved in $CHCl_3$, and Ag_2O and drierite were added. This mixture was stirred in the dark in a dry flask for 2 h. After addition of I_2, a solution of the corresponding acetobromodisaccharide **9–11** or **13** in dry $CHCl_3$ was added. This mixture was stirred under Ar for at least 24 h (monitored by TLC, CH_2Cl_2:EtOAc = 9:1 as eluent), filtered, and the $CHCl_3$ was evaporated. The crude residue was purified by CC over silica gel eluting first with CH_2Cl_2:EtOAc (9:1) to recover **8** and subsequently

changing to CH_2Cl_2:CH_3OH (8:2) to obtain the product of the *Königs-Knorr* reaction. The residue was dried over P_2O_5, dissolved in dry $CHCl_3$, and cooled to $-20°C$. Likewise, Na in MeOH cooled to $-20°C$ was added. The mixture was allowed to stand for 1.5 h at this temperature; then, ice water and brine were added. The mixture was extracted five times with $CHCl_3$, and the combined organic layers were washed twice with a saturated solution of NH_4Cl and dried over Na_2SO_4. The solvent was evaporated *in vacuo*, and the residue was dissolved in dry MeOH. Pd/C (10%) was added and allowed to shake with H_2 overnight at a pressure of 50 psi at room temperature. After filtration, the solvent was evaporated *in vacuo* at room temperature, and the residue was subjected to preparative TLC eluting with CH_2Cl_2:CH_3OH (8:2) to give **1** or **3**–**5**.

3β-Galactopyranosyl-β-D-glucopyranosyl-3-O-oleanolic acid (**1**, arvensoside B; $C_{42}H_{68}O_{13}$)

1.0 g (1.6 mmol) of **8** was dissolved in 5 cm^3 dry $CHCl_3$ and treated with 2 g drierite, 1.7 g silver oxide, and 0.13 g iodine. 550 mg (0.79 mmol) of **11** dissolved in 5 cm^3 dry $CHCl_3$ were added, and the mixture was stirred for two days. After the usual workup the residue (400 mg) was dissolved in 10 cm^3 dry $CHCl_3$, treated with 0.4 g Na in 20 cm^3 dry MeOH, and then with 900 mg Pd/C (10%) in 50 cm^3 dry MeOH to give 51 mg (9.1%) of **1**.

M.p.: 252–258°C (decomp.); $R_f = 0.24$ (CH_2Cl_2:CH_3OH = 8:2); $[\alpha]_D^{20} = +50.2°$, $[\alpha]_{546}^{20} = +25.5°$ ($c = 0.126$, CH_3OH); IR (KBr): $\nu = 3419$ (s), 2942 (s), 1570 (m), 1461 (m), 1389 (m), 1079 (s), 780 (w), 638 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 0.83 (d, $J = 10.9$ Hz, 1H, 5-H), 0.88 (s, 3H, 24-H), 0.89 (s, 3H, 26-H), 0.93 (s, 3H, 29-H), 0.99 (s, 6H, 25-H, 30-H), 1.02–1.08 (m, 2H, 1-H, 15-H), 1.10 (s, 3H, 23-H), 1.14–1.26 (m, 5H, 19-H, 21-H, 27-H), 1.33–1.98 (m, 17H, 1-H, 2-H, 6-H, 7-H, 9-H, 11-H, 15-H, 16-H, 19-H, 21-H, 22-H), 2.93 (dd, $J = 13.2$, 2.9 Hz, 1H, 18-H), 3.23 (dd, $J = 11.6$, 4.3 Hz, 1H, 3-H), 3.31–3.36 (m, 1H, 5′-H), 3.43 (t, $J = 8.9$ Hz, 1H, 2′-H), 3.45 (t, $J = 10.0$ Hz, 1H, 4′-H), 3.53–3.67 (m, 4H, 2″-H, 3′-H, 3″-H, 5″-H), 3.70–3.75 (m, 2H, 6′-H, 6″-H), 3.79–3.86 (m, 2H, 4″-H, 6″-H), 3.89 (dd, $J = 11.8$, 2.1 Hz, 1H, 6′-H), 4.42 (d, $J = 7.8$ Hz, 1H, 1′-H), 4.56 (d, $J = 7.5$ Hz, 1H, 1″-H), 5.26 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.25 (C-25), 17.32 (C-24), 18.36 (C-26), 19.67 (C-6), 24.50 (C-30), 24.68, 24.86 (C-11, C-16), 26.75 (C-27), 27.28 (C-2), 28.82 (C-23), 29.41 (C-15), 32.03 (C-20), 34.10 (C-29), 34.42, 34.50 (C-7, C-22), 35.59 (C-21), 38.23 (C-10), 40.09 (C-1), 40.47 (C-4), 40.87 (C-8), 43.28 (C-14), 43.49 (C-18), 48.12 (C-19), 49.46 (C-9), 57.39 (C-5), 62.89, 63.00 (C-6′, C-6″), 70.40 (C-4′), 70.63 (C-4″), 73.33 (C-2″), 75.03 (C-3″), 75.31 (C-2′), 77.41, 77.57 (C-5′, C-5″), 88.24 (C-3′), 91.20 (C-3), 106.04 (C-1″), 106.61 (C-1′), 123.22 (C-12), 146.40 (C-13), 184.67 (C-28) ppm; MS (ES+): *m/z* (%) = 803 [M+Na^+] (21.5), 781 [M+H^+] (3.7), 636 (3.3), 635 (8.0), 513 (9.1), 472 (16.1), 440 (9.8), 439 (34.3), 342 (7.7), 325 (14.7), 271 (14.0), 241 (100.0), 204 (16.1), 163 (13.3), 130 (7.7); MS (ES-): *m/z* (%) = 893 [M+CF_3COO^-] (3.7), 792 (2.1), 656 (3.1), 520 (10.0), 385 (8.5), 249 (58.0), 227 (6.3), 167 (21.0), 155 (100.0) 153 (8.4).

2α-Galactopyranosyl-α-D-glucopyranosyl-3-O-oleanolic acid (**3**: $C_{42}H_{68}O_{13}$)

2.0 g (3.2 mmol) of **8** were dissolved in 10 cm^3 dry $CHCl_3$ and treated with 4 g drierite, 3 g silver oxide, and 0.2 g iodine. 1.0 g (1.43 mmol) of **9** dissolved in 10 cm^3 dry $CHCl_3$ were added, and the mixture was stirred overnight. After the usual workup the residue was dissolved in 30 cm^3 dry $CHCl_3$, treated with 1.3 g Na in 77 cm^3 dry MeOH, and then with 600 mg Pd/C (10%) in 42 cm^3 dry MeOH to give 155 mg (13.9%) of **3**.

M.p.: 255–263°C (decomp.); $R_f = 0.1$ (CH_2Cl_2:CH_3OH = 8:2); $[\alpha]_D^{20} = +104.1°$, $[\alpha]_{546}^{20} = +115.4°$ ($c = 0.143$, CH_3OH); IR (KBr): $\nu = 3406$ (s), 2943 (s), 1552 (m), 1462 (m), 1390 (s), 1149 (s), 1055 (s), 1031 (s), 765 (w), 543 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 0.82 (d, $J = 11.3$ Hz, 1H, 5-H), 0.86 (s, 6H, 24-H, 26-H), 0.89 (s, 3H, 29-H), 0.91–0.94 (m, 1H, 1-H), 0.95 (s, 3H, 30-H), 0.96 (s, 3H, 25-H), 0.98–1.03 (m, 1H, 15-H), 1.06 (s, 3H, 23-H), 1.11–1.12 (m, 5H, 19-H, 21-H, 27-H), 1.28–1.94 (m, 17H, 1-H, 2-H, 6-H, 7-H, 9-H, 11-H, 15-H, 16-H, 19-H, 21-H, 22-H), 2.89

(dd, $J = 13.0$, 3.0 Hz, 1H, 18-H), 3.30–3.41 (m, 2H, 3-H, 4′-H), 3.58–3.63 (m, 1H, 2′-H), 3.64–3.82 (m, 8H, 2″-H, 3′-H, 3″-H, 5′-H, 6′-H, 6″-H), 3.92 (d, $J = 1.8$ Hz, 1H, 4″-H), 4.11 (t, $J = 5.9$ Hz, 1H, 5″-H), 5.04 (d, $J = 3.6$ Hz, 1H, 1″-H), 5.22 (s, b, 1H, 12-H), 5.27 (d, $J = 3.6$ Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.24 (C-25), 17.62 (C-24), 18.41 (C-26), 19.76 (C-6), 22.81 (C-2), 24.55 (C-30), 24.72, 24.87 (C-11, C-16), 26.79 (C-27), 29.44 (C-15), 29.82 (C-23), 32.04 (C-20), 34.15 (C-29), 34.44, 34.57 (C-7, C-22), 35.65 (C-21), 38.43 (C-10), 39.67 (C-1), 39.92 (C-4), 40.86 (C-8), 43.30 (C-14), 43.57 (C-18), 48.22 (C-19), 48.78 (C-17), 49.44 (C-9), 57.27 (C-5), 62.86 (C-6′), 63.12 (C-6″), 70.59 (C-2″), 71.46 (C-4″), 71.88 (C-3″, C-4′), 72.81 (C-5″), 73.76 (C-3′), 74.16 (C-5′), 77.99 (C-2′), 83.56 (C-3), 93.14 (C-1′), 98.28 (C-1″), 122.97 (C-12), 146.64 (C-13), 185.33 (C-28) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 779 [M-H$^+$] (64.3), 645 (14.3), 617 (49.2), 599 (19.5), 537 (13.9), 509 (14.3), 483 (27.8), 455 (43.6), 453 (36.1), 437 (25.9), 423 (20.3), 369 (11.7), 355 (15.4), 271 (12.8), 247 (23.3), 221 (40.2), 183 (16.9), 159 (36.5), 141 (43.6), 113 (51.1), 101 (56.0), 71 (100.0), 59 (78.9), 45 (29.3).

2β-Galactopyranosyl-α-D-glucopyranosyl-3-O-oleanolic acid (**4**; $C_{42}H_{68}O_{13}$)

1.6 g (2.6 mmol) of **8** were dissolved in 8 cm^3 dry $CHCl_3$ and treated with 3 g drierite, 2.6 g silver oxide, and 0.2 g iodine. 900 mg (1.29 mmol) of **10** dissolved in 8 cm^3 dry $CHCl_3$ were added and stirred for seven days. After the usual workup the residue was dissolved in 40 cm^3 dry $CHCl_3$, treated with 1.8 g Na in 100 cm^3 MeOH, and then with 910 mg Pd/C (10%) in 50 cm^3 dry MeOH to give 210 mg (20.9%) of **4**.

M.p.: 251–258°C (decomp.); $R_f = 0.13$ (CH_2Cl_2:CH_3OH = 8:2); $[\alpha]_D^{20} = +49.3°$, $[\alpha]_{546}^{20} = +54.7°$ ($c = 0.148$, CH_3OH); IR (KBr): $\nu = 3422$ (s), 2943 (s), 1630 (m), 1462 (m), 1387 (m), 1209 (w), 1078 (s), 768 (w) cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 0.82 (d, $J = 11.3$ Hz, 1H, 5-H), 0.86 (s, 6H, 24-H, 26-H), 0.90–0.92 (m, 1H, 1-H), 0.94 (s, 3H, 29-H), 0.98 (2s, 6H, 25-H, 30-H), 1.02–1.04 (m, 1H, 15-H), 1.07 (s, 3H, 23-H), 1.13–1.26 (m, 5H, 19-H, 21-H, 27-H), 1.30–2.08 (m, 17H, 1-H, 2-H, 6-H, 7-H, 9-H, 11-H, 15-H, 16-H, 19-H, 21-H, 22-H), 2.89 (dd, $J = 12.5$, 3.3 Hz, 1H, 18-H), 3.25 (dd, $J = 11.6$, 3.9 Hz, 1H, 3-H), 3.44 (t, $J = 9.3$ Hz, 1H, 4′-H), 3.49 (dd, $J = 9.9$, 3.4 Hz, 1H, 2′-H), 3.51–3.57 (m, 2H, 3″-H, 5″-H), 3.64 (dd, $J = 9.8$, 7.6 Hz, 1H, 2″-H), 3.72–3.82 (m, 5H, 5′-H, 6′-H, 6″-H), 3.89 (t, $J = 9.4$ Hz, 1H, 3′-H), 3.90 (s, 1H, 4″-H), 4.47 (d, $J = 7.6$ Hz, 1H, 1″-H), 5.24 (d, $J = 3.5$ Hz, 1H, 1′-H), 5.27 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.24 (C-25), 17.62 (C-24), 18.32 (C-26), 19.81 (C-6), 23.82 (C-2), 24.46 (C-30), 24.62, 24.86 (C-11, C-16), 26.75 (C-27), 29.35 (C-15), 29.81 (C-23), 31.99 (C-20), 34.06 (C-29), 34.44 (C-7, C-22), 35.52 (C-21), 38.38 (C-10), 39.84 (C-1), 39.91 (C-4), 40.86 (C-8), 43.25 (C-14), 43.43 (C-18), 48.03 (C-19), 49.42 (C-9), 57.38 (C-5), 62.67, 62.80 (C-6′, C-6″), 70.40 (C-4″), 71.75 (C-4′), 73.14 (C-2″), 73.93, 73.97 (C-3′, C-5′), 75.03 (C-3″), 76.85 (C-5″), 83.08 (C-2′), 87.06 (C-3), 98.38 (C-1′), 106.87 (C-1″), 123.28 (C-12), 146.29 (C-13), 184.50 (C-28) ppm; MS (ES+):*m/z* (%) = 803 [M+Na$^+$] (55.9), 781 [M+H$^+$] (3.5), 633 (4.2), 513 (7.0), 472 (21.0), 454 (7.0), 440 (15.4), 439 (47.6), 394 (7.0), 342 (8.4), 325 (16.1), 271 (9.1), 242 (9.8), 241 (100.0), 204 (23.1), 178 (12.6), 163 (18.9), 130 (11.9); MS (ES−): *m/z* (%) = 893 [M+CF_3COO^-] (8.9), 815 (3.4), 656 (1.6), 520 (7.1), 385 (8.5), 249 (57.3), 227 (6.3), 187 (5.6), 171 (9.1), 167 (21.0), 155 (100.0), 153 (8.4).

6α-Galactopyranosyl-α-D-glucopyranosyl-3-O-oleanolic acid (**5**; $C_{42}H_{68}O_{13}$)

2.0 g (3.2 mmol) of **8** were dissolved in 10 cm^3 dry $CHCl_3$ and treated with 4 g drierite, 3 g silver oxide, and 0.25 g iodine. 1.1 g (1.57 mmol) of **13** dissolved in 10 cm^3 dry $CHCl_3$ were added and stirred for seven days. After the usual workup the residue was dissolved in 33 cm^3 dry $CHCl_3$, treated with 1.4 g Na in 77 cm^3 dry MeOH, and then with 927 mg Pd/C (10%) in 510 ml dry MeOH to give 74 mg (6.1%) of **5**.

M.p.: 245–250°C (decomp.); $R_f = 0.16$ (CH_2Cl_2:CH_3OH = 8:2); $[\alpha]_D^{20} = +23.7°$, $[\alpha]_{546}^{20} = +21.5°$ ($c = 0.135$, CH_3OH), IR (KBr): $\nu = 3387$ (s), 2943 (s), 1559 (m), 1460 (m), 1389 (m),

1310 (w), 1245 (w), 1078 (s), 1034 (s), 773 (w), 631 (w), 564 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 0.78 (d, $J = 11.2$ Hz, 1H, 5-H), 0.86 (s, 3H, 24-H), 0.87 (s, 3H, 26-H), 0.88 (s, 3H, 29-H), 0.95 (m, 7H, 1-H, 25-H, 30-H), 0.98–1.01 (m, 1H, 15-H), 1.06 (s, 3H, 23-H), 1.09–1.10 (m, 1H, 19-H), 1.14 (s, 4H, 21-H, 27-H), 1.28–1.77 (m, 12H, 1-H, 2-H, 6-H, 7-H, 9-H, 16-H, 19-H, 21-H, 22-H), 1.84–1.95 (m, 5H, 2-H, 11-H, 15-H, 16-H), 2.90 (dd, $J = 13.7$, 3.7 Hz, 1H, 18-H), 3.18 (dd, $J = 11.8$, 4.4 Hz, 1H, 3-H), 3.21 (t, $J = 8.4$ Hz, 1H, 2′-H), 3.30–3.38 (m, 2H, 3′-H, 4′-H), 3.47–3.50 (m, 1H, 5′-H), 3.60–3.76 (m, 5H, 2″-H, 4″-H, 6′-H, 6″-H), 3.89–3.93 (m, 3H, 3″-H, 5″-H, 6′-H), 4.35 (d, $J = 7.7$ Hz, 1H, 1′-H), 4.84 (d, $J = 1.2$ Hz, 1H, 1″-H), 5.21 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.26 (C-25), 17.33 (C-24), 18.47 (C-26), 19.70 (C-6), 24.61 (C-30), 24.86 (C-11, C-16), 26.76 (C-27), 27.48 (C-2), 28.87 (C-23), 29.52 (C-15), 32.07 (C-20), 34.21 (C-29), 34.45, 34.65 (C-7, C-22), 35.75 (C-21), 38.26 (C-10), 40.09 (C-1), 40.43 (C-4), 40.85 (C-8), 43.32 (C-14), 43.69 (C-18), 48.39 (C-19), 48.88 (C-17), 49.48 (C-9), 57.45 (C-5), 62.96 (C-6″), 67.89 (C-6′), 70.83 (C-4″), 71.37, 71.91, 72.10 (C-2″, C-3″, C-4′), 72.46 (C-5″), 75.93 (C-2′), 76.32 (C-5′), 78.58 (C-3′), 91.66 (C-3), 100.31 (C-1″), 107.16 (C-1′), 122.92 (C-12), 146.75 (C-13), 185.84 (C-28) ppm; MS (neg. LSIMS, 5 kV): m/z (%) = 779 [M-H^+] (60.9), 645 (5.3), 617 (17.7), 513 (6.0), 483 (9.0), 455 (23.3), 437 (10.9), 397 (20.3), 355 (7.5), 275 (18.0), 247 (10.5), 221 (15.4), 183 (100.0), 141 (15.0), 119 (30.5), 91 (98.1), 71 (48.9), 59 (45.1), 58 (16.2), 45 (10.5).

Preparation of glycosides **2** *and* **6**

Diphenylmethyl oleanolate (**8**) was dissolved in a 1:1 mixture of dry benzene and nitromethane. Half of the amount of the solvent was evaporated at 50°C to remove last traces of moisture. After cooling to room temperature, $Hg(CN)_2$ and the acetobromodisaccharide **12** or **14** were added to the solution. After refluxing for 4 h at 100°C with vigorous stirring the mixture was cooled to room temperature, filtered, and the same amount of $CHCl_3$ was added. Hg ions were removed by shaking the solution three times with aqueous KI solution (5%). The solution was washed twice with an aqueous $NaHCO_3$ solution (10%), three times with H_2O, and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* at 40°C gave a yellow residue which was treated with sodium methoxide and Pd/C (10%) as described above to give the glycosides **2** or **6**.

4β-Galactopyranosyl-β-D-glucopyranosyl-3-O-oleanolic acid (**2**, *calenduloside A;* $C_{42}H_{68}O_{13}$)

2 g (3.2 mmol) of **8** were dissolved in 200 cm^3 of a mixture of benzene and nitromethane and treated with 3 g of $Hg(CN)_2$ and 1.46 g (2.1 mmol) of **12** as described above. After the usual workup the residue was dissolved in 18 cm^3 dry $CHCl_3$, treated with 0.8 g Na in 45 cm^3 dry MeOH, and hydrogenated on 550 mg Pd/C (10%) in 40 cm^3 dry MeOH to give 65 mg (4.0%) of **2** after crystallization from MeOH:EtOH (1:1).

M.p.: 250–252°C (decomp.); $R_f = 0.18$ (CH_2Cl_2: CH_3OH = 8:2); $[\alpha]_D^{20} = +45.5°$, $[\alpha]_{546}^{20} = +38.9°$, ($c = 0.103$, CH_3OH); IR (KBr): $\nu = 3415$ (s), 2944 (s), 1695 (m), 1463 (m), 1387 (m), 1160 (s), 1073 (s), 890 (w), 635 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 0.80–0.84 (m, 4H, 5-H, 26-H), 0.87 (s, 3H, 24-H), 0.93 (s, 3H, 29-H), 0.96 (s, 3H, 30-H), 0.98 (s, 3H, 25-H), 0.98–1.00 (m, 1H, 1-H), 1.08 (s, 3H, 23-H), 1.03–1.17 (m, 2H, 15-H, 19-H), 1.18 (s, 3H, 27-H), 1.22–1.25 (m, 1H, 21-H), 1.30–1.98 (m, 16H, 1-H, 2-H, 6-H, 7-H, 9-H, 11-H, 15-H, 16-H, 19-H, 21-H, 22-H), 2.04 (dt, $J = 13.1$, 3.3 Hz, 1H, 16-H), 2.87 (dd, $J = 13.6$, 3.7 Hz, 1H, 18-H), 3.22 (dd, $J = 11.7$, 4.3 Hz, 1H, 3-H), 3.30 (t, $J = 8.3$ Hz, 1H, 2′-H), 3.39–3.43 (m, 1H, 5′-H), 3.49–3.66 (m, 5H, 2″-H, 3′-H, 3″-H, 4′-H, 5″-H), 3.72 (dd, $J = 11.4$, 4.6 Hz, 1H, 6″-H), 3.78–3.88 (m, 4H, 4″-H, 6′-H, 6″-H), 4.38 (d, $J = 7.4$ Hz, 1H, 1′-H), 4.40 (d, $J = 7.4$ Hz, 1H, 1″-H), 5.27 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.25 (C-25), 17.29 (C-24), 18.03 (C-26), 19.64 (C-6), 24.28 (C-30), 24.36 (C-16), 24.84 (C-11), 26.72 (C-27), 27.33 (C-2), 28.83 (C-23), 29.13 (C-15), 31.91 (C-20), 33.87 (C-29), 34.11, 34.32 (C-7, C-22), 35.20 (C-21), 38.20 (C-10), 40.09 (C-1), 40.46 (C-4), 40.88 (C-8), 43.03 (C-18), 43.19 (C-14), 47.54 (C-19),

47.92 (C-17), 49.30 (C-9), 57.35 (C-5), 62.37 (C-6′), 62.81 (C-6″), 70.63 (C-4″), 72.86 (C-2″), 75.11 (C-3″), 75.57 (C-2), 76.51 (C-5′), 76.88 (C-3′), 77.37 (C-5″), 81.02 (C-4′), 91.19 (C-3), 105.37 (C-1″), 106.90 (C-1′), 123.95 (C-12), 145.48 (C-13), 182.09 (C-28) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 779 [M−H$^+$] (85.0), 735 (8.3), 645 (9.8), 617 (37.6), 599 (16.2), 551 (10.5), 497 (10.5), 483 (16.5), 455 (30.5), 437 (16.2), 403 (9.8), 367 (34.2), 331 (10.2), 311 (15.4), 275 (14.7), 183 (100.0), 127 (8.3), 91 (79.7), 71 (13.9).

6β-Galactopyranosyl-β-D-glucopyranosyl-3-O-oleanolic acid (**6**; $C_{42}H_{68}O_{13}$)

1.2 g (1.93 mmol) of **8** were dissolved in 150 cm^3 mixture of benzene and nitromethane and treated with 4 g of $Hg(CN)_2$ and 640 mg (0.92 mmol) of **14** as described above. After the usual workup the residue was dissolved in 15 cm^3 dry $CHCl_3$, and treated with 0.7 g Na in 35 cm^3 dry MeOH. Hydrogenation with 640 mg Pd/C (10%) in 37 cm^3 dry MeOH gave 182 mg (25.5%) of **6**.

M.p.: 251–256°C (decomp.); $R_f = 0.2$ (CH_2Cl_2:CH_3OH = 8:2); $[\alpha]_D^{20} = +0.69°$, $[\alpha]_{546}^{20} = +0.31°$ ($c = 0.160$, CH_3OH); IR (KBr): $\nu = 3422$ (s), 2944 (s), 1637 (m), 1544 (m), 1461 (m), 1389 (m), 1252 (w), 1073 (s), 1043 (s), 917 (w), 541 (m) cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 0.79 (d, $J = 10.7$ Hz, 1H, 5-H), 0.85 (s, 3H, 24-H), 0.86 (s, 3H, 26-H), 0.89 (s, 3H, 29-H), 0.95 (s, 6H, 25-H, 30-H), 1.00–1.03 (m, 2H, 1-H, 15-H), 1.06 (s, 3H, 23-H), 1.07–1.12 (m, 1H, 19-H), 1.14 (s, 4H, 21-H, 27-H), 1.28–1.98 (m, 17H, 1-H, 2-H, 6-H, 7-H, 9-H, 11-H, 15-H, 16-H, 19-H, 21-H, 22-H), 2.90 (dd, $J = 13.6$, 3.5 Hz, 1H, 18-H), 3.19–3.22 (m, 2H, 3-H, 2′-H), 3.31–3.42 (m, 2H, 3′-H, 4′-H), 3.43–3.57 (m, 4H, 2″-H, 3″-H, 5′-H, 5″-H), 3.74–3.76 (m, 2H, 6″-H), 3.79 (dd, $J = 11.7$, 5.8 Hz, 1H, 6′-H), 3.84 (d, $J = 2.7$ Hz, 1H, 4″-H), 4.11 (dd, $J = 11.7$, 1.7 Hz, 1H, 6′-H), 4.33 (d, $J = 8.0$ Hz, 1H, 1′-H), 4.36 (d, $J = 7.7$ Hz, 1H, 1″-H), 5.22 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.27 (C-25), 17.32 (C-24), 18.40 (C-26), 19.69 (C-6), 24.54 (C-30), 24.74, 24.87 (C-11, C-16), 26.75 (C-27), 27.39 (C-2), 28.83 (C-23), 29.46 (C-15), 32.04 (C-20), 34.14 (C-29), 34.43, 34.58 (C-7, C-22), 35.65 (C-21), 38.24 (C-10), 40.11 (C-1), 40.47 (C-4), 40.86 (C-8), 43.31 (C-14), 43.59 (C-18), 48.25 (C-19), 49.58 (C-9), 57.35 (C-5), 62.75 (C-6″), 70.02 (C-6′), 70.61 (C-4″), 71.85 (C-3′*), 72.88 (C-2″), 75.21 (C-3″), 75.93 (C-2′), 76.97 (C-5″), 77.15 (C-5′), 78.38 (C-4′*), 91.10 (C-3), 105.65 (C-1″), 107.01 (C-1′), 123.11 (C-12), 146.54 (C-13), 180.59 (C-28) ppm; MS (ES+): *m/z* (%) = 803 [M+Na$^+$] (53.1), 781 [M+H$^+$] (6.9), 513 (7.7), 472 (16.8), 441 (4.2), 440 (16.8), 439 (52.3), 431 (7.0), 393 (4.9), 377 (4.2), 342 (9.8), 325 (23.8), 317 (8.4), 276 (5.6), 271 (29.3), 242 (12.6), 241 (100.0), 205 (7.0), 204 (44.1), 200 (18.2), 163 (20.2), 144 (11.2), 130 (17.5); MS (ES−): *m/z* (%) = 893 [M+CF_3COO^-] (8.2), 656 (2.4), 520 (8.9), 385 (8.2), 249 (62.2), 167 (22.3), 155 (100.0), 153 (7.7).

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