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Synthesis and Haemolytic Activity of Oleanolic Acid Trisaccharides

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Summary. The first partial synthesis of monodesmosidic oleanolic acid trisaccharides is described. Their haemolytic index is lower compared to the corresponding disaccharide. The influence of the linkage and the configurations of the carbohydrate units on the haemolytic activity of these saponins was investigated.

Keywords. Haemolytic activity; Glycosidation; Oleanolic acid; Trisaccharides.

Synthese und hämolytische Wirkung von Oleanolsäuretrisacchariden

Zusammenfassung. Es wird die erste Partialsynthese monodesmosidischer Oleanolsäuretrisaccharide beschrieden. Ihr hämolytischer Index ist niedriger als der des entsprechenden Disaccharids. Untersucht wurde der Einfluß der Verknüpfung sowie der Konfiguration der Kohlehydrateinheiten auf die hämolytische Aktivität dieser Saponine.

Introduction

Saponins are glycosides of steroids or terpenes. They are widely distributed in nature and have several important biological and pharmacological activities [1]. One of these well known and characteristic properties [2] is their haemolytic activity, which is used as a bioassay for saponin drugs. The haemolytic potency is strongly influenced by the carbohydrate residue [3]; however, it is mainly determined by the structure of the sapogenin [2]. Investigating the effect of different sugar moieties on the haemolytic properties we used oleanolic acid, one of the most widespread triterpene aglyca [4], as genin for a series of synthetic saponins. Up to now the haemolytic activity of oleanolic acid is known to be raised when position 3 is glycosylated [5]. Additional linkage of the carboxy group to a sugar residue decreases the haemolytic power [6, 7].

The influence of the length of the carbohydrate residue in saponins is documented contradictory: some authors have maintained the maximum of haemolytic strength for saponins with short [3] or branched [6] sugar moieties,

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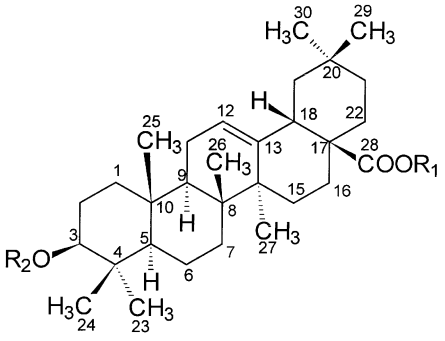
whereas others have supposed high activity for compounds with up to five monosaccharide units [8]. Unfortunately, saponins with different genins have been compared, disregarding the strong influence of the aglycon structure. A systematic research of the effect of sugar moieties on the haemolytic activity demands the comparison of haemolytic properties of different glycosides of exclusively one sapogenin. Therefore we decided to investigate the effect of the length and the linkage within the sugar residue, restraining our tests on saponins containing only glucose units.

Results and Discussion

Previously we have reported on the influence of the linkage between the glucose residues in oleanolic acid disaccharides [9]. This paper presents the first partial syntheses of monodesmosidic oleanolic acid trisaccharides **1**–**4** (Scheme 1) with different points of linkage of the terminal glucose units. The rest of the molecules, being identical, we were able to observe the influence of the linkage on the haemolytic activity. Besides, we compared the results to those of the corresponding disaccharide.



1: R_1 = H, R_2 = β-*D*-glcp-(1→2)-β-*D*-glcp-(1→6)-β-*D*-glcp-(1→
2: R_1 = H, R_2 = β-*D*-glcp-(1→3)-β-*D*-glcp-(1→6)-β-*D*-glcp-(1→
3: R_1 = H, R_2 = α-*D*-glcp-(1→4)-β-*D*-glcp-(1→6)-β-*D*-glcp-(1→
4: R_1 = H, R_2 = β-*D*-glcp-(1→4)-β-*D*-glcp-(1→6)-β-*D*-glcp-(1→
5: R_1 = H, R_2 = H
6: R_1 = $CH(Ph)_2$, R_2 = H
11: R_1 = H, R_2 = β-*D*-glcp-(1→6)-β-*D*-glcp-(1→

Scheme 1

Oleanolic acid

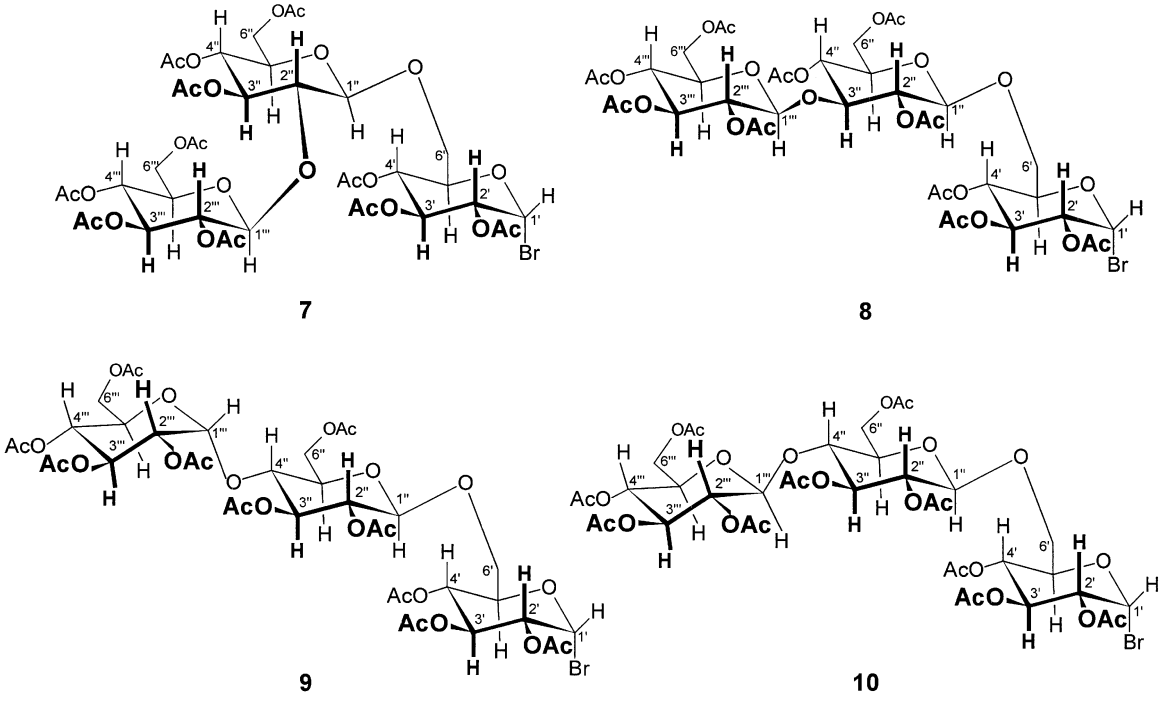
Oleanolic acid (**5**) was prepared from cloves according to a procedure given in Ref. [10]. The carboxyl group of oleanolic acid was protected by esterification with diphenyldiazomethane [11, 12], and the diphenylmethyl oleanolate (**6**) [13] was used as starting material for the glycosidation reactions.

Glycosidation

As glycosyl donors, peracetylglycosyl bromides were used (Scheme 2). These derivatives have been synthesized from the appropriate disaccharide bromides by a *Königs-Knorr* reaction with silver oxide as catalyst [14]. The syntheses of the disaccharide bromides have been described earlier [9]. For the glycosyl donor of saponin **1**, acetobromosophorose was condensed with 1,2,3,4-tetraacetylglucose to yield 6-sophorosido-α-*D*-glucopyranosyl undecaacetate which was converted to the 6-sophorosido-α-*D*-glucopyranosylbromide decaacetate **7** by means of HBr [15]. In the same way, the glycosyl donors **8**, **9**, and **10** were obtained using acetobromolaminaribiose, acetobromomaltose, or acetobromocellobiose as starting materials.

The NMR data of the intermediate trisaccharide undecaacetates correspond well with those given in Refs. [16, 17]. Coupling of the glycosyl donors **7**–**10** with diphenylmethyl oleanolate (**6**) took place employing the *Königs-Knorr* reaction with mercury II cyanide as the catalyst [18]. The acetyl groups were removed with sodium methylate [19]. Saponins **1**–**4** were obtained by hydrogenolysis of the benzhydrylic ester [13].

The resonances in the NMR spectra were assigned with the aid of *ge*-HMBC spectra which were optimized for 8 Hz. Within the glucose units, the ^{1}H NMR signals were determined by means of 1D TOCSY experiments. We conclude the β configuration for most anomeric protons because of the large vicinal coupling constants ($J \cong 8$ Hz) of the corresponding signals in ^{1}H NMR spectra. Only the anomeric proton of the terminal glucose of **3** exhibited a small coupling constant



Scheme 2

($J = 3.3$ Hz) indicating α configuration. For the respective resonance in the ^{13}C NMR spectrum we observed the typical 2.8 ppm upfield shift.

Determination of haemolytic activity

As mentioned above, the 3-O-monoglucoside of oleanolic acid has a higher haemolytic activity than the sapogenin [3]. Elongation of the carbohydrate residue with a further glucose unit in position 2, 4, or 6 of the sugar moiety decreased the haemolytic potency, whereas a linkage in position 3 raised it [9]. This paper deals with the investigation if further elongation of the carbohydrate residue also influences haemolytic potency dependent on the linkage. The haemolytic indices of the trisaccharide saponins **1**–**4** are compared with that of the corresponding disaccharide **11** (Table 1).

Table 1. Haemolytic Index (*HI*) of **1**–**4** and **11**

Saponin	Sugar combination	HI
1	$1\rightarrow2\rightarrow6$	22000
2	$1\rightarrow3\rightarrow6$	11000
3	$1\alpha\rightarrow4\rightarrow6$	19500
4	$1\beta\rightarrow4\rightarrow6$	<2000
11	$1\rightarrow6$	37500

From the results in Table 1 it is evident that the trisaccharides **1**–**4** are less potent than the corresponding disaccharide. Again we observe a dependence on the linkage: haemolytic activity decreases from linkage position 2 *via* position 3 to position 4. In oleanolic acid disaccharides we observed that the α configuration of the first glucose unit effects a lower potency [9]; an α-configuration of the terminal sugar residue in saponin **3**, however, raises the haemolytic activity by a factor 10 compared with saponin **4**.

In conclusion, it may be said that the influence of the carbohydrate moiety on the haemolytic activity of disaccharides and trisaccharides of oleanolic acid strongly depends on the linkage between the glucose units and on their configurations. The effects vary for different positions in the sugar residue.

Experimental

General

Catalytic hydrogenations were run in a *Parr* hydrogenation apparatus shaker type 3911 at room temperature. Melting points were obtained 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, 600 (300 K), 5 mm tubes, solvent resonance as internal standard. ^1H and ^{13}C resonances were assigned using ^1H, ^1H and ^1H, ^{13}C correlation spectra. ^1H and ^{13}C resonances are numbered as given in the formulae. Assignments marked with an asterisk are interchangeable. Column chromatography (CC): silica gel 60 (Merck, 70–230 mesh), pore diameter 60 Å; preparative

TLC:TLC plates (Merck), silica gel 60, PF_{254}, 1 mm, 200×200 mm; thin layer chromatography (TLC): TLC plates (Merck), silica gel 60, F_{254}, 0.2 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. Acetobromomaltose and 1,2,3,4-tetraacetylglucose were purchased from Sigma. Nitromethane was deacidified with basic Al_2O_3. Oleanolic acid (**5**) was obtained from 200 g cloves as described in Ref. [10]. Diphenylmethyl oleanolate (**6**) was prepared from **5** and diphenyldiazomethane [11, 12] following the procedure given in Ref. [13].

Determination of haemolytic activity

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

β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-α-D-glucopyranosylbromide decaacetate (**7**; $C_{38}H_{51}BrO_{25}$)

Acetobromosophorose was linked with 1,2,3,4-tetra-O-acetyl-β-*D*-glucopyranose by the method of *Nichols* [14] giving β-*D*-glucopyranosyl-(1→2)-β-*D*-glucopyranosyl-(1→6)-β-*D*-glucose undecaacetate which was treated with HBr according to the method of *Brauns* [15] affording amorphous **7**.

Yield: 87.6%; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.96 (s, 6H, 2 CH_3COO), 1.98 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3COO), 2.03 (s, 6H, CH_3COO), 2.05 (s, 6H, 2 CH_3COO), 2.06 (s, 3H, CH_3COO), 2.08 (s, 3H, CH_3COO), 3.60–3.73 (m, 4H, 2″-H, 5″-H, 5‴-H, 6′-H), 4.02 (d, J = 11.5 Hz, 1H, 6′-H), 4.05 (d, J = 10.9 Hz, 1H, 6″-H), 4.18–4.28 (m, 4H, 5′-H, 6″-H, 6‴-H), 4.37 (d, J = 7.6 Hz, 1H, 1″-H), 4.61 (d, J = 7.5 Hz, 1H, 1‴-H), 4.81 (dd, J = 9.9, 4.6 Hz, 1H, 2′-H), 4.92 (t, J = 9.7 Hz, 1H, 4″-H), 4.98 (t, J = 8.8 Hz, 1H, 2‴-H), 5.04–5.14 (m, 3H, 3″-H, 3‴-H, 4‴-H), 5.26 (t, J = 9.9 Hz, 1H, 4′-H), 5.49 (t, J = 9.9 Hz, 1H, 3′-H), 6.61 (d, J = 3.8 Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.38 (*C*H_3COO), 20.51 (*C*H_3COO), 20.55 (3 *C*H_3COO), 20.58 (*C*H_3COO), 20.62 (*C*H_3COO), 20.66 (*C*H_3COO), 20.72 (2 *C*H_3COO), 61.97 (C-6″), 62.68 (C-6‴), 66.73 (C-6′), 67.68 (C-4′), 68.37 (C-4″), 68.90 (C-4‴), 70.39 (C-2′), 70.60 (C-3′), 70.99 (C-2‴), 71.54, 71.70 (C-5″, C-5‴), 72.73 (C-3″*), 73.00 (C-5′), 74.49 (C-3‴*), 76.57 (C-2″), 87.18 (C-1′), 100.21 (C-1‴), 101.00 (C-1″), 168.97 (CH_3*C*OO), 169.34 (CH_3*C*OO), 169.43 (CH_3*C*OO), 169.63 (CH_3*C*OO), 169.70 (CH_3*C*OO), 169.92 (CH_3*C*OO), 170.22 (CH_3*C*OO), 170.29 (CH_3*C*OO), 170.52 (CH_3*C*OO), 170.62 (CH_3*C*OO) ppm.

β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-α-D-glucopyranosylbromide decaacetate (**8**; $C_{38}H_{51}BrO_{25}$)

Acetobromolaminaribiose was linked with 1,2,3,4-tetra-O-acetyl-β-*D*-glucopyranose by the method of *Nichols* [14] giving β-*D*-glucopyranosyl-(1→3)-β-*D*-glucopyranosyl-(1→6)-β-*D*-glucose undecaacetate which was treated with HBr according to the method of *Brauns* [15] affording resinous **8**.

Yield: 97.9%; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.93 (s, 3H, CH_3COO), 1.96 (s, 3H, CH_3COO), 1.97 (s, 3H, CH_3COO), 1.98 (s, 3H, CH_3COO), 1.99 (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), 2.14 (s, 3H, CH_3COO), 3.48 (dd, J = 11.5, 5.0 Hz, 1H, 6′-H), 3.50–3.70 (m, 2H, 5″-H, 5‴-H), 3.85 (t, J = 9.5 Hz, 1H, 3″-H), 3.93 (dd, J = 11.5, 1.9 Hz, 1H, 6′-H), 3.99 (dd, J = 12.3, 2.2 Hz, 1H, 6‴-H), 4.08–4.18 (m, 3H, 5′-H, 6″-H), 4.31 (dd, J = 12.6, 4.6 Hz, 1H, 6‴-H), 4.35 (d, J = 8.1 Hz, 1H, 1″-H), 4.56 (d, J = 8.3 Hz, 1H, 1‴-H), 4.72 (dd, J = 9.8, 4.0 Hz, 1H, 2′-H), 4.85 (t, J = 8.8 Hz, 1H, 2‴-H), 4.88 (t, J = 10.4 Hz, 1H, 4″-H), 4.95 (dd, J = 9.7, 8.2 Hz, 1H, 2″-H), 5.00 (t, J = 9.8 Hz, 1H, 4′-H), 5.01 (t, J = 9.6 Hz, 1H, 4‴-H), 5.08 (t, J = 9.4 Hz, 1H, 3‴-H), 5.49 (t, J = 9.6 Hz, 1H, 3′-H), 6.57 (d, J = 3.8 Hz, 1H, 1′-H) ppm;

^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.27 (CH_3COO), 20.41 (CH_3COO), 20.47 (CH_3COO), 20.50 (CH_3COO), 20.53 (CH_3COO), 20.55 (CH_3COO), 20.58 (2 CH_3COO), 20.72 (CH_3COO), 20.93 (CH_3COO), 61.66 (C-6‴), 61.93 (C-6″), 66.13 (C-6′), 67.46 (C-4′), 67.99 (C-4″, C-4‴), 70.07 (C-3′), 70.55 (C-2′), 70.92 (C-2‴), 71.62 (C-5‴), 71.86 (C-5″), 72.31 (C-2″), 72.89 (C-3‴), 73.11 (C-5′), 78.61 (C-3″), 86.51 (C-1′), 100.68 (C-1″), 100.84 (C-1‴), 169.02 (CH_3COO), 169.10 (CH_3COO), 169.22 (CH_3COO), 169.30 (CH_3COO), 169.45 (CH_3COO), 169.67 (CH_3COO), 169.73 (CH_3COO), 170.29 (CH_3COO), 170.41 (CH_3COO), 170.68 (CH_3COO) ppm.

α-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-α-D-glucopyranosylbromide decaacetate (**9**; $C_{38}H_{51}BrO_{25}$)

Prepared using the method of *Brauns* [15] from *α-D*-glucopyranosyl-(1→4)-*β-D*-glucopyranosyl-(1→6)-*β-D*-glucose undecaacetate which was obtained from the reaction of acetobromomaltose with 1,2,3,4-tetra-O-acetyl-*β-D*-glucopyranose as described by *Nichols* [14]. NMR data of *α-D*-glucopyranosyl-(1→4)-*β-D*-glucopyranosyl-(1→6)-*β-D*-glucose undecaacetate and of **9** were identical with those given in Ref. [17].

β-D-Glucopyranosyl(1→4)-β-D-glucopyranosyl-(1→6)-α-D-glucopyranosylbromide decaacetate (**10**; $C_{38}H_{51}BrO_{25}$)

Acetobromocellobiose was linked with 1,2,3,4-tetra-O-acetyl-*β-D*-glucopyranose as described in Ref. [23] giving *α-D*-glucopyranosyl-(1→4)-*β-D*-glucopyranosyl-(1→6)-*β-D*-glucose undecaacetate. The latter was treated with HBr [15] affording amorphous **10**.

Yield: 53.4%; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 1.96 (s, 3H, CH_3COO), 1.98 (s, 3H, CH_3COO), 1.99 (s, 6H, 2 CH_3COO), 2.00 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3COO), 2.05 (s, 3H, CH_3COO), 2.06 (s, 3H, CH_3COO), 2.07 (s, 3H, CH_3COO), 2.11 (s, 3H, CH_3COO), 3.53–3.58 (m, 2H, 5″-H, 6′-H), 3.63 (d, b, $J = 9.8$ Hz, 1H, 5‴-H), 3.75 (t, $J = 9.5$ Hz, 1H, 4″-H), 3.92 (d, $J = 11.7$ Hz, 1H, 6′-H), 4.02 (d, $J = 11.3$ Hz, 1H, 6″-H), 4.05 (dd, $J = 11.7$, 4.9 Hz, 1H, 6″-H), 4.20 (dd, $J = 10.2$, 3.2 Hz, 1H, 5′-H), 4.34 (dd, $J = 12.5$, 4.4 Hz, 1H, 6‴-H), 4.43–4.52 (m, 3H, 1″-H, 1‴-H, 6″-H), 4.76 (dd, $J = 9.9$, 4.0 Hz, 1H, 2′-H), 4.88 (t, $J = 8.6$ Hz, 1H, 2″-H), 4.89 (t, $J = 8.6$ Hz, 1H, 2‴-H), 5.03 (t, $J = 9.5$ Hz, 1H, 4‴-H), 5.04 (t, $J = 9.8$ Hz, 1H, 4′-H), 5.12 (t, $J = 9.4$ Hz, 1H, 3‴-H), 5.15 (t, $J = 9.4$ Hz. 1H, 3″-H), 5.50 (t, $J = 9.6$ Hz, 1H, 3′-H), 6.58 (d, $J = 4.1$ Hz, 1H, 1′-H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 20.52 (5 CH_3COO), 20.60 (CH_3COO), 20.62 (CH_3COO), 20.63 (CH_3COO), 20.68 (CH_3COO), 20.86 (CH_3COO), 61.54 (C-6‴), 61.74 (C-6″), 66.73 (C-6′), 67.46, 67.77 (C-4′, C-4‴), 70.20 (C-3′), 70.53 (C-2′), 71.12 (C-2″), 71.59 (C-2‴), 71.95 (C-5‴), 72.32 (C-3″), 72.76 (C-5″), 72.90 (C-3‴), 73.10 (C-5′), 76.31 (C-4″), 86.58 (C-1′), 100.62, 100.76 (C-1″, C-1‴), 169.04 (CH_3COO), 169.24 (CH_3COO), 169.30 (CH_3COO), 169.73 (CH_3COO), 169.76 (2 CH_3COO), 169.88 (CH_3COO), 170.21 (CH_3COO), 170.31 (CH_3COO), 170.50 (CH_3COO) ppm.

β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3O-oleanolic acid (**1**; $C_{48}H_{78}O_{18}$)

1.8 g **6** (2.9 mmol) were dissolved in a mixture of 150 cm^3 dry benzene and 150 cm^3 nitromethane. Half of the amount of the solvent was evaporated at 50°C to remove last traces of moisture. After cooling to room temperature, 2.7 g of $Hg(CN)_2$ and 1.7 g **7** (1.7 mmol) were added to the solution. After refluxing 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 100 cm^3 of aqueous KI solution (5%). The solution was washed twice with 150 cm^3 of an aqu. $NaHCO_3$ solution (10%), three times with H_2O, and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* at 40°C gave 3.27g of a yellow residue which was purified by flash

chromatography on silica gel using CH_2Cl_2:ethylacetate = 9:1 as solvent to recover 880 mg of **6**. The mobile phase was changed to CH_2Cl_2:MeOH = 8:2 to get 1.68 g of the polar fraction containing the product of the *Königs-Knorr* reaction. This residue was dried over P_2O_5, dissolved in 10 cm^3 of dry $CHCl_3$, and cooled to −20°C. Then a cooled solution of 1.2 g Na in 60 cm^3 of dry CH_3OH was added. The mixture was allowed to stand for 75 min at this temperature. Then ice water and brine were added, and the mixture was extracted five times with $CHCl_3$. The combined organic layers were washed twice with a saturated solution of NH_4Cl, dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. The residue (480 mg) was dissolved in 55 cm^3 of dry CH_3OH, and 520 mg of Pd/C (10%) were added. Cleavage of the benzhydrylic ester was attained by shaking with H_2 overnight at a pressure of 50 psi at room temperature. Solids were filtered off, the solvent was evaporated *in vacuo* at room temperature, and the residue (383 mg) was recrystallized three times from CH_3OH:acetone: ether = 1:1:1 giving 160 mg (10%) **1**.

M.p.: 171–175°C decomp.; $R_f = 0.03$ (CH_2Cl_2:MeOH = 8:2); $[\alpha]_{546}^{20} = +18.0°$ ($c = 0.1$, CH_3OH); IR (KBr): $\bar{\nu} = 3388$ (s), 2944 (s), 1697 (m), 1388 (m), 1263 (m), 1163 (m), 1076 (s), 641 (w) cm^{-1}; ^{1}H NMR (400 MHz, δ, C_5D_5N): 0.80 (s, 3H, 25-H), 0.85 (d, $J = 11.4$ Hz, 1H, 5-H), 0.91 (s, 3H, 29-H), 0.94–0.99 (m, 9H, 24-H, 26-H, 30-H), 1.03–1.07 (m, 1H, 1-H), 1.14–1.52 (m, 15H, 1-H, 6-H, 7-H, 15-H, 19-H, 21-H, 22-H, 23-H, 27-H), 1.60–2.24 (m, 11H, 2-H, 7-H, 9-H, 11-H, 15-H, 16-H, 19-H, 22-H), 3.20–3.30 (m, 1H, 18-H), 3.48 (dd, $J = 11.6$, 4.0 Hz, 1H, 3-H), 3.80–3.90 (m, 2H, 5″-H, 5‴-H), 4.01–4.11 (m, 3H, 2′-H, 2″-H, 2‴-H), 4.13–4.39 (m, 10H, 3′-H, 3″-H, 3‴-H, 4′-H, 4″-H, 4‴-H, 5′-H, 6′-H, 6″-H, 6‴-H), 4.44 (d, $J = 11.4$ Hz, 1H, 6″-H), 4.51 (d, $J = 10.9$ Hz, 1H, 6‴-H), 4.68 (d, $J = 10.5$ Hz, 1H, 6′-H), 4.93 (d, $J = 7.6$ Hz, 1H, 1′-H), 5.14 (d, $J = 7.6$ Hz, 1H, 1″-H), 5.29 (d, $J = 8.1$ Hz, 1H, 1‴-H), 5.39 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, C_5D_5N): 15.15 (C-25), 16.71 (C-24), 17.02 (C-26), 18.12 (C-6), 23.30 (C-11, C-16), 23.39 (C-30), 25.90 (C-27), 26.34 (C-2), 27.95 (C-15, C-23), 30.59 (C-20), 32.85, 32.92 (C-7, C-22, C-29), 33.85 (C-21), 36.65 (C-10), 38.29 (C-1), 39.19, 39.36 (C-4, C-8), 41.55 (C-18), 41.73 (C-14), 45.99 (C-19), 46.26 (C-17), 47.64 (C-9), 55.50 (C-5), 61.86 (C-6‴), 61.98 (C-6″), 70.02 (C-6′), 70.54, 70.82 (C-4″, C-4‴), 71.13 (C-4′), 75.46 (C-2′), 75.91 (C-5′), 76.20 (C-2‴), 77.84, 77.93 (C-3″, C-3‴), 78.03, 78.10 (C-3′, C-5″), 78.51 (C-5‴), 84.45 (C-2″), 88.40 (C-3), 102.76 (C-1″), 106.27 (C-1‴), 106.41 (C-1′), 122.21 (C-12), 144.37 (C-13), 179.79 (C-28) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 941 [M-H^+] (71.7), 925 (13.6), 908 (6.8), 838 (9.1), 801 (6.8), 779 (39.2), 763 (9.8), 687 (7.2), 675 (7.5), 645 (12.8), 618 (19.6), 617 (41.5), 539 (13.2), 483 (21.1), 455 (43.0), 383 (18.1), 305 (15.1), 249 (25.3), 221 (49.1), 159 (46.4), 127 (46.8), 113 (59.6), 101 (63.8), 87 (71.3), 71 (100.0).

β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3O-oleanolic acid (**2**; $C_{48}H_{78}O_{18}$)

3.0 g **6** (4.8 mmol) and 2.4 g **8** (2.4 mmol) gave in a similar procedure 134 mg (5.9%) of **2** after crystallization from ethanol/ether.

M.p.: 188–195°C decomp.; $R_f = 0.06$ (CH_2Cl_2:MeOH = 8:2); $[\alpha]_{546}^{20} = +0.8°$: ($c = 0.1$, CH_3OH); IR (KBr): $\bar{\nu} = 3146$ (s), 3049 (s), 2011 (w), 1692 (m), 1407 (s), 1161 (m), 1076 (s), 1043 (s), 587 (w) cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 0.80–0.83 (m, 1H, 5-H), 0.85 (s, 3H, 26-H), 0.89 (s, 3H, 24-H), 0.95 (s, 3H, 29-H), 0.98 (s, 3H, 30-H), 0.99 (s, 3H, 25-H), 1.02–1.05 (m, 1H, 1-H), 1.08–1.13 (m, 4H, 15-H, 23-H), 1.16–1.28 (m, 5H, 19-H, 21-H, 27-H), 1.32–1.86 (m, 13H, 1-H, 2-H, 6-H, 7-H, 9-H, 15-H, 16-H, 19-H, 21-H, 22-H), 1.92–2.00 (m, 3H, 2-H, 11-H), 2.05 (dt, $J = 10.1$, 3.4 Hz, 1H, 16-H), 2.89 (d, b, $J = 10.4$ Hz, 1H, 18-H), 3.20–3.26 (m, 2H, 3-H, 2′-H), 3.31–3.54 (m, 10H, 2″-H, 2‴-H, 3′-H, 3‴-H, 4′-H, 4″-H, 4‴-H, 5′-H, 5″-H, 5‴-H), 2.59 (t, $J = 8.8$ Hz, 1H, 3″-H), 3.70 (dd, $J = 12.0$, 6.0 Hz, 1H, 6‴-H), 3.75 (dd, $J = 12.0$, 5.2 Hz, 1H, 6″-H), 3.88 (dd, $J = 11.7$, 5.5 Hz, 1H, 6′-H), 3.90–3.95 (m, 2H, 6″-H, 6‴-H), 4.14 (d, $J = 12.0$ Hz, 1H, 6′-H), 3.36 (d, $J = 7.9$ Hz, 1H, 1′-H), 4.52 (d, $J = 7.8$ Hz, 1H, 1″-H), 4.60 (d, $J = 7.8$ Hz, 1H, 1‴-H), 5.29 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.27 (C-25), 17.33 (C-24), 18.03 (C-26), 19.63 (C-6), 24.27 (C-30), 24.34 (C-16), 24.91 (C-11), 26.76 (C-27), 27.30 (C-2), 28.84 (C-23), 29.12 (C-15),

31.91 (C-20), 33.87 (C-29), 34.11 (C-7), 34.27 (C-22), 35.21 (C-21), 38.23 (C-10), 40.11 (C-1), 40.42 (C-4), 40.86 (C-8), 42.99 (C-18), 43.15 (C-14), 47.44 (C-19), 47.90 (C-17), 49.30 (C-9), 57.31 (C-5), 62.79 (C-6″, C-6‴), 70.09 (C-6′), 70.31 (C-4″), 71.75 (C-4‴), 71.80 (C-4′), 74.77 (C-2″), 75.84, 75.90 (C-2′, C-2‴), 77.05 (C-5′), 77.86 (C-5″), 78.02 (C-3‴), 78.31 (C-3′), 78.44 (C-5‴), 88.40 (C-3″), 91.32 (C-3), 104.51 (C-1″), 105.64 (C-1‴), 106.97 (C-1′), 123.91 (C-12), 145.52 (C-13), 182.17 (C-28) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 941 [M-H$^+$] (76.6), 837 (10.9), 801 (11.7), 779 (18.9), 761 (7.5), 675 (6.8), 617 (21.1), 615 (9.8), 513 (9.1), 483 (13.2), 455 (30.9), 383 (8.3), 339 (7.5), 305 (11.7), 271 (12.5), 221 (15.1), 209 (21.1), 185 (27.9), 161 (32.5), 151 (44.5), 127 (100.0), 93 (64.2), 71 (43.8), 59 (41.5).

α-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3O-oleanolic acid (**3**; $C_{48}H_{78}O_{18}$)

2.2 g **6** (3.5 mmol) and 2.2 g **9** (2.2 mmol) gave in a similar procedure 159 mg (7.6%) of **3** by use of preparative TLC (CH_2Cl_2:MeOH = 8:2).

M.p.: 179–185°C decomp.; $R_f = 0.07$ (CH_2Cl_2:MeOH = 8:2); $[\alpha]_{546}^{20} = +20.0°$ ($c = 0.3$, CH_3OH); IR (KBr): $\bar{\nu} = 3406$ (s), 2944 (s), 1697 (m), 1638 (w), 1460 (m), 1408 (m), 1307 (w), 1265 (w), 1150 (m), 1074 (s), 1032 (s), 534 (w) cm^{-1}; ^{1}H NMR (400 MHz, δ, C_5D_5N): 0.71 (d, $J = 10.9$, 1H, 5-H), 0.81 (s, 3H, 25-H), 0.86–0.92 (m, 1H, 1-H), 0.97 (s, 9H, 24-H, 26-H, 29-H), 1.00 (s, 3H, 30-H), 1.08–1.68 (m, 16H, 1-H, 6-H, 7-H, 9-H, 15-H, 19-H, 21-H, 23-H, 27-H), 1.76–2.24 (m, 9H, 2-H, 7-H, 11-H, 15-H, 16-H, 19-H, 22-H), 2.32–2.44 (m, 1H, 2-H), 3.25–3.36 (m, 2H, 3-H, 18-H), 3.72–3.80 (m, 1H, 5″-H), 3.97 (t, $J = 8.3$ Hz, 1H, 2′-H), 4.01 (t, $J = 7.6$ Hz, 1H, 2″-H), 4.08–4.28 (m, 7H, 2‴-H, 3′-H, 4′-H, 4‴-H, 5′-H, 5‴-H, 6′-H), 4.30–4.40 (m, 3H, 3″-H, 4″-H, 6‴-H), 4.43 (s, b, 2H, 6″-H), 4.48–4.62 (m, 2H: 3‴-H, 6‴-H), 4.86 (d, $J = 9.5$ Hz, 2H, 1′-H, 6′-H), 4.98 (d, $J = 7.6$ Hz, 1H, 1″-H), 5.46 (s, b, 1H, 12-H), 5.89 (d, $J = 3.3$ Hz, 1H, 1‴-H) ppm; ^{13}C NMR (100 MHz, δ, C_5D_5N): 15.11 (C-25), 16.67 (C-24), 17.04 (C-26), 18.14 (C-6), 23.34 (C-11, C-16), 23.43 (C-30), 25.83 (C-27), 26.30 (C-2), 27.86 (C-23), 27.96 (C-15), 30.62 (C-20), 32.86 (C-7, C-22), 32.95 (C-29), 33.87 (C-21), 36.61 (C-10), 38.28 (C-1), 39.12, 39.36 (C-4, C-8), 41.61 (C-18), 41.77 (C-14), 46.16 (C-19), 46.30 (C-17), 47.61 (C-9), 55.40 (C-5), 61.45 (C-6″), 62.36 (C-6‴), 70.34 (C-6′), 71.35 (C-4′), 71.48 (C-4‴), 74.14 (C-5‴), 74.31 (C-2″), 74.92 (C-3‴), 75.15, 75.17 (C-2′, C-2‴), 76.37 (C-5′), 76.47 (C-5″), 77.32 (C-3″), 78.21 (C-3′), 80.74 (C-4″), 88.70 (C-3), 102.70 (C-1‴), 105.04 (C-1″), 106.64 (C-1′), 122.29 (C-12), 144.37 (C-13), 179.81 (C-28) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 941 [M-H$^+$] (95.1), 925 (15.8), 843 (7.9), 800 (12.8), 779 (21.9), 693 (10.2), 645 (19.6), 617 (32.5), 539 (10.6), 525 (15.1), 513 (17.7), 483 (24.9), 455 (43.8), 437 (24.2), 325 (21.1), 311 (23.4), 265 (25.3), 221 (46.8), 141 (45.3), 127 (51.3), 119 (67.2), 87 (72.1), 71 (100.0), 59 (81.5).

β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3O-oleanolic acid (**4**; $C_{48}H_{78}O_{18}$)

1.5 g **6** (2.4 mmol) and 1.2 g of **10** (1.2 mmol) gave in a similar procedure 80 mg (7.1%) of **4** by use of preparative TLC (CH_2Cl_2:MeOH) = 8:2).

M.p.: 263°C decomp.; $R_f = 0.08$ (CH_2Cl_2:MeOH = 8:2); $[\alpha]_{546}^{20} = +1.4°$ ($c = 0.14$, CH_3OH); IR (KBr): $\bar{\nu} = 3424$ (s), 2944 (m), 1637 (m), 1460 (w), 1130 (w), 1072 (m), 1030 (m), 618 (w) cm^{-1}; ^{1}H NMR (400 MHz, δ, C_5D_5N): 0.75 (d, $J = 11.6$ Hz, 1H, 5-H), 0.83 (s, 3H, 25-H), 0.98 (s, b, 10H, 24-H, 26-H, 29-H, 1-H), 1.02 (s, 3H, 30-H), 1.07–1.66 (m, 16H, 1-H, 6-H, 7-H, 9-H, 15-H, 19-H, 21-H, 22-H, 23-H, 27-H), 1.78–2.22 (m, 9H, 2-H, 7-H, 11-H, 15-H, 16-H, 19-H, 22-H), 2.34–2.42 (m, 1H, 2-H), 3.28–3.38 (m, 2H, 3-H, 18-H), 3.83–3.87 (m, 1H, 5″-H), 3.97–4.31 (m, 14H, 2′-H, 2″-H, 2‴-H, 3′-H, 3″-H, 3‴-H, 4′-H, 4″-H, 4‴-H, 5′-H, 5″-H, 5‴-H, 6′-H, 6‴-H), 4.41 (d, $J = 9.2$ Hz, 1H, 6″-H), 4.46–4.54 (m, 2H, 6″-H, 6‴-H), 4.84 (d, $J = 9.2$ Hz, 1H, 6′-H), 4.87 (d, $J = 7.9$ Hz, 1H, 1′-H),

5.05 (d, $J = 7.3$ Hz, 1H, 1″-H), 5.15 (d, $J = 7.6$ Hz, 1H, 1‴-H), 5.45 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, C_5D_5N): 15.36 (C-25), 16.46 (C-24), 17.07 (C-26), 18.50 (C-6), 23.39 (C-30), 23.44 (C-11, C-16), 25.59 (C-27), 26.47 (C-2), 27.20 (C-23), 28.15 (C-15), 30.34 (C-20), 32.81 (C-7, C-22), 33.02 (C-29), 33.81 (C-21), 36.43 (C-10), 38.32 (C-1), 39.14 (C-8), 39.37 (C-4), 41.69 (C-18), 41.77 (C-14), 46.36 (C-19), 46.33 (C-17), 47.62 (C-9), 55.25 (C-5), 61.64 (C-6″), 62.07 (C-6‴), 70.17 (C-6′), 71.11, 71.40 (C-4′, C-4‴), 74.30 (C-2″, C-2‴), 75.25 (C-2′), 76.29 (C-5″), 76.44 (C-3′), 76.55 (C-5′), 77.79, 78.03, 78.17 (C-3″, C-3‴, C-5‴), 80.61 (C-4″), 88.58 (C-3), 104.48 (C-1‴), 104.67 (C-1″), 106.55 (C-1′), 122.30 (C-12), 144.29 (C-13), 179.96 (C-28) ppm; MS (neg. LSIMS, 5 kV): m/z (%) = 941 [M-H$^+$] (100.0), 871 (26.3), 859 (40.4), 837 (54.4), 801 (100.0), 713 (12.3), 675 (15.8), 617 (20.2), 565 (14.0), 497 (15.8), 483 (22.8), 455 (26.3), 423 (20.2), 355 (21.1), 323 (21.9), 301 (24.6), 265 (35.1), 247 (31.6), 209 (33.3), 183 (43.9), 149 (86.0), 127 (100.0), 99 (45.6), 91 (87.7), 59 (77.2).

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References

[1] Frohne D, Pfänder HJ (1987) In: Giftpflanzen, 3. Auflage. Wissenschaftliche Verlagsges, Stuttgart, p 32
[2] Tschesche R, Wulff G (1973) Progr Chem Organ Nat Prod **30**: 461
[3] Schlösser E, Wulff G (1969) Z Naturforsch B **24**: 1284
[4] Hiller K, Schöpke T, Franke P (1987) Pharmazie **42**: 67
[5] Hase J, Kobashi K, Mitsui K, Namba T, Yoshizaki M, Tomimori T (1981) J Pharmacobio-Dyn **4**: 833
[6] Woitke HD, Kayser JP, Hiller K (1970) Pharmazie **25**: 133
[7] Romussi G, Cafaggi S, Bignardi G (1980) Pharmazie **35**: 498
[8] Wulff G (1968) Dtsch Apoth Z **108**: 797
[9] Seebacher W, Haslinger E, Rauchensteiner K, Jurenitsch J, Presser A, Weis R (1999) Monatsh Chem **130**: 887
[10] Stahl E, Schild W (1986): In: Isolierung und Charakterisierung von Naturstoffen. Gustav Fischer, Stuttgart New York, p 132
[11] Staudinger H, Anthes E, Pfenninger F (1917) Ber Dtsch Chem Ges **49**: 1928
[12] Coleman GH, Gilman H, Adams CE, Pratt PE (1939) J Org Chem **3**: 99
[13] Hardegger E, El Heweihi Z, Rohinet FG (1948) Helv Chim Acta **31**: 439
[14] Nichols SH, Evans WL, Mc Dowell HD (1940) J Am Chem Soc **62**: 1754
[15] Brauns DH (1927) J Am Chem Soc **49**: 3170
[16] Bigler P, Ammann W, Richarz R (1984) Org Magn Reson **22**: 109
[17] Haslinger E, Seebacher W, Weis R (1997) Monatsh Chem **128**: 1009
[18] Janiszowska W, Wilkomirski B, Kaspyzyk Z (1980) Pol J Chem **54**: 2147
[19] Helferich B, Schäfer W (1926) Liebigs Ann Chem **450**: 229
[20] Segal R, Milo-Goldzweig I, Schupper H, Zaitschek DV (1970) Biochem Pharmacol **19**: 2501
[21] Tschesche R, Mercker HJ, Wulff G (1969) Liebigs Ann Chem **721**: 194
[22] Tschesche R, Wulff G (1964) Planta Med **12**: 272
[23] Zemplen G, Gerecs A (1931) Ber Dtsch Chem Ges **64**: 1545

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