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Synthesis and Hemolytic Properties of Glycyrrhetic Acid Glycosides

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Summary. The synthesis of monodesmosidic glycyrrhetic acid disaccharides *via* its diphenylmethyl ester is described. Their hemolytic activity is lower as compared to the corresponding oleanolic acid disaccharides. The influence of the structure of the aglycon on the hemolytic activity is discussed.

Keywords. Hemolytic activity; Glycosidation; Glycyrrhetic acid; Disaccharides.

Introduction

Glycyrrhetic acid disaccharides containing glucuronic acid are widely distributed in nature, whereas analogues with neutral sugar residues have only rarely been isolated [1]. Those have been tested for their cytoprotective effect on CCl_4-induced hepatic injury [2]. Other papers are dealing with their sweetness [3, 4]. However, there is no publication describing their hemolytic properties.

Although hemolytic properties of saponins, which are glycosides of steroids or terpenes, are well known [5], the molecular mechanism of this activity is not clear. Previously we have reported on the hemolytic power of oleanolic acid disaccharides [6]. Their hemolytic activity strongly depends on the linkage between the glucose units and on their configurations. This work represents a further step of our investigations on the hemolytic activity of triterpene saponins. Oleanolic acid (**1**) and glycrrhetic acid (**2**) have the same pentacyclic triterpene skeleton of the oleanane type. Glycyrrhetic acid differs from oleanolic acid only by the positions of a methyl and a carboxylic group in positions 17 and 20 and an additional keto group in position 11 (Scheme 1). Therefore we used the same sugar moieties for the synthesis of glycyrrhetic acid glycosides investigating if the influence of the carbohydrate residue is comparable.

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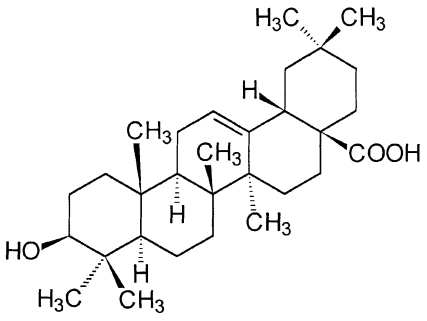

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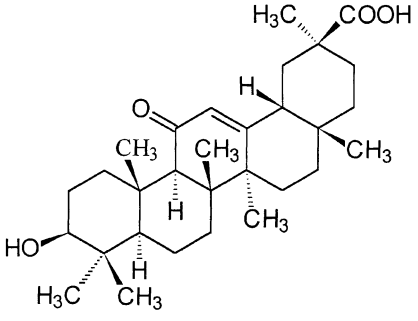

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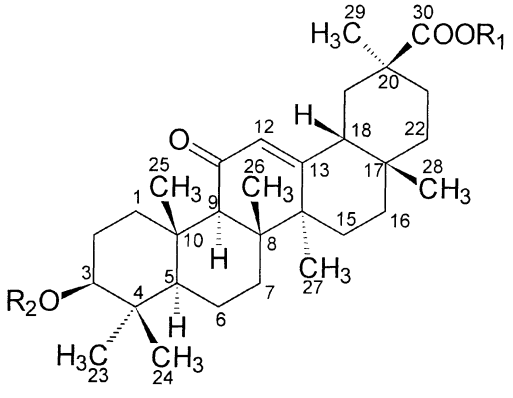
Scheme 1

Glycyrrhetic acid disaccharides **3**–**7** (Scheme 2) were synthesized *via* the diphenylmethylester **8**. The synthesis of **6** and **7** from glycyrrhetic acid methylester as sugar acceptor has already been described [3, 7].



3: R_1 = H, R_2 = β-*D*-glcp-(1→2)-β-*D*-glcp-(1→

4: R_1 = H, R_2 = β-*D*-glcp-(1→3)-β-*D*-glcp-(1→

5: R_1 = H, R_2 = α-*D*-glcp-(1→4)-β-*D*-glcp-(1→

6: R_1 = H, R_2 = β-*D*-glcp-(1→4)-β-*D*-glcp-(1→

7: R_1 = H, R_2 = β-*D*-glcp-(1→6)-β-*D*-glcp-(1→

8: R_1 = $CH(Ph)_2$, R_2 = H

Scheme 2

Results and Discussion

Glycyrrhetic acid

Glycyrrhetic acid is commercially available. The carboxylic group was protected by esterification with diphenyldiazomethane [8, 9] in the same way as described for oleanolic acid [10].

Glycosidation

As glycosyl donors the acetobromodisaccharides 2-O-(2,3,4,6-tetraacetyl-β-*D*-glucopyranosyl)-3,4,6-triacetyl-α-*D*-glucopyranosylbromide (acetobromosophorose), 3-O-(2,3,4,6-tetraacetyl-β-*D*-glucopyranosyl)-2,4,6-triacetyl-α-*D*-glucopyranosyl bromide (acetobromolaminaribiose), 4-O-(2,3,4,6-tetraacetyl-β-*D*-glucopyranosyl)-2,3,6-triacetyl-α-*D*-glucopyranosylbromide (acetobromocellobiose), and 6-O-(2,3,4,6-tetraacetyl-β-*D*-glucopyranosyl)-2,3,4-triacetyl-α-*D*-glucopyranosylbromide (acetobromogentiobiose) were used; the preparation of these substances has been reported earlier [6]. 4-O-(2,3,4,6-Tetraacetyl-α-*D*-glucopyranosyl)-2,3,6-triacetyl-α-*D*-glucopyranosylbromide (acetobromomaltose) was purchased from Sigma. The glycosyl donors were condensed with **8** by a *Königs-Knorr* reaction with silver oxide or mercury(II) cyanide as catalyst [11, 12]. Easier handling and shorter reaction periods favour the usage of mercury(II) cyanide. Unfortunately, the reaction of the $1 \rightarrow 3$ linked acetobromolaminaribiose with mercury(II) cyanide gave a mixture of the α and β isomer, whereas catalysis with silver oxide led to the pure β product. Besides, $1 \rightarrow 2$ linked disaccharides usually are condensed with aglyca in better yields using silver oxide as catalyst.

The acetyl groups were removed with sodium methylate [13]. Saponins **3**–**7** were obtained by cleavage of the benzhydrylic ester *via* hydrogenation with Pd as catalyst [10].

The resonances in the NMR spectra were assigned using HMBC spectra which were optimized for 8 Hz. The proton signals within the glucose units were determined by means of 1 D TOCSY experiments. Most of the anomeric protons have β configuration, giving rise to large vicinal coupling constants ($J \cong 8$ Hz) of the corresponding signals in the ^{1}H NMR spectra. Only the anomeric proton of the outer glucose unit of compound **5** exhibits a small coupling constant ($J = 3.8$ Hz), thus indicating α configuration.

Determination of hemolytic activity

The aglyca show hemolytic power and have therefore been considered to be mainly responsible for the hemolytic activity [14]. Oleanolic acid (**1**) and glycyrrhetic acid (**2**) exhibit comparable activity [5]. The hemolytic properties of their glycosides with the same carbohydrate residues should be similar if the contribution of the carbohydrate residue is considered to be similar. In the case of oleanolic acid, the glycosides show enhanced hemolytic activity in comparison to the aglycon. This holds in particular for randianin, a disaccharide with a $1 \rightarrow 3$ linkage between the glucose units [6]. However, the glycyrrhetic acid disaccharides **3**–**7** possess no detectable hemolytic properties.

In most former studies dealing with the influence of the sugar moiety on the hemolytic activity of saponins, differing aglycon structures were compared [15]. Following this procedure, wrong conclusions are very likely, if one considers the results of the present investigation. Obviously, the effect of the sugar residue on the hemolytic activity is not transferable from one aglycon to another even for aglyca of similar structure and activity.

Experimental

General

Catalytic hydrogenations were performed 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, 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; they are numbered as given in the formulae. Assignments marked with an asterisk are interchangeable. Materials: column chromatography (CC): silica gel 60 (Merck, 70–230 mesh, pore diameter 60 Å); preparative TLC: PLC plates (Merck, silica gel 60 F_{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/H_2SO_4 (9:1) and subsequent heating with a hot gun). Acetobromomaltose was purchased from Sigma, Glycyrrhetic acid from Fluka. Nitromethane was deacidified with basic aluminum oxide.

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 reference.

Diphenylmethyl glycyrrhetinate (**8**; $C_{43}H_{56}O_4$)

8 was obtained from glycyrrhetic acid and diphenyldiazomethane according to Refs. [8, 9].
$R_f = 0.52$ (benzene:Et_2O = 1:1); ^{1}H NMR (400 MHz, δ, $CDCl_3$): 0.64–0.69 (m, 4H, 5-H, 28-H), 0.78 (s, 3H, 24-H), 0.90–0.96 (m, 2H, 1-H, 15-H*), 0.98 (s, 3H, 23-H), 1.07 (s, 3H, 26-H), 1.11 (s, 3H, 25-H), 1.51 (s, 3H, 29-H), 1.17–1.32 (m, 4H, 7-H, 16-H*, 21-H, 22-H), 1.34 (s, 3H, 27-H), 1.35–1.45 (m, 2H, 6-H, 22-H), 1.55–1.68 (m, 5H, 2-H, 6-H, 7-H, 19-H), 1.78 (dt, J = 13.5, 4.2 Hz, 1H, 15-H*), 1.92–2.05 (m, 4H, 16-H*, 18-H, 19-H, 21-H), 2.30 (s, 1H, 9-H), 2.78 (dt, J = 13.5, 3.3 Hz, 1H, 1-H), 3.20 (dd, J = 10.7, 5.6 Hz, 1H, 3-H), 5.50 (s, 1H, 12-H), 6.91 (s, 1H, $CHPh_2$), 7.30–7.35 (m, 10H, aromatic H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 15.55 (C-24), 16.33 (C-25), 17.46 (C-6), 18.63 (C-26), 23.31 (C-27), 26.35, 26.43 (C-15, C-16), 27.26 (C-2), 28.07, 28.21, 28.26 (C-23, C-28, C-29), 31.13 (C-21), 31.71 (C-17), 32.73 (C-7), 37.03 (C-10), 37.45 (C-22), 39.09 (C-4), 39.15 (C-1), 41.10 (C-19), 43.12 (C-8), 43.96 (C-20), 45.28 (C-14), 48.02 (C-18), 54.90 (C-5), 61.74 (C-9), 76.60 (Ph_2CH), 78.69 (C-3), 126.95, 127.22, 127.79, 128.10, 128.44, 128.47, 128.60, 140.04, 140.08 (aromatic C, C-12), 168.80 (C-13), 175.16 (C-30), 200.03 (C-11) ppm.

β-D-Glucopyranosyl-(1→2-β-D-glucopyranosyl-3-O-18β-glycyrrhetic acid (**3**; $C_{42}H_{66}O_{14}$)

725 mg (1.1 mmol) of **8** were dissolved in 8 cm^3 $CHCl_3$, and 1.3 g of Ag_2O and 2.5 g drierite were added. This mixture was stirred in the dark in a dry flask for 2 h. After addition of 150 mg of I_2, a

solution of 660 mg (1.6 mmol) of acetobromosophorose in 8 cm^3 dry $CHCl_3$ was added. This mixture was stirred under Ar for 48 h, filtered, and the $CHCl_3$ was evaporated to yield 1.6 g of crude residue which was purified by CC over silica gel (160 g) eluting first with CH_2Cl_2:EtOAc (9:1) and subsequently changing to CH_2Cl_2:CH_3OH (8:2) to obtain 718 mg of product of the *Königs-Knorr* reaction. The residue was dried over P_2O_5, dissolved in 10 cm^3 of dry $CHCl_3$ and cooled to −20°C. Likewise, 700 mg of Na in 40 cm^3 MeOH cooled to −20°C were 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, dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. The residue (270 mg) was dissolved in 19 cm^3 of dry methanol, and 290 mg of Pd/C (10%) were 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 (130 mg) was subjected to preparative TLC eluting with CH_2Cl_2:CH_3OH (8:2) to give 70 mg (5%) of **3**.

M.p.: 230–234°C (decomp.); $R_f = 0.18$ (CH_2Cl_2:CH_3OH = 8:2); $[\alpha]_D^{20} = +85.7°$, $[\alpha]_{546}^{20} = +70.2°$ ($c = 0.16$, CH_3OH); IR (KBr): $\nu = 3407$ (s), 2928 (s), 1650 (m), 1560 (m), 1463 (m), 1389 (m), 1287 (w), 1076 (s), 473 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 0.82 (d, $J = 11.4$ Hz, 1H, 5-H), 0.86 (s, 3H, 28-H), 0.92 (s, 3H, 24-H), 0.98–1.06 (m, 2H, 1-H, 15-H*), 1.12 (s, 3H, 29-H), 1.14 (s, 3H, 23-H), 1.18 (s, 6H, 25-H, 26-H), 1.22–1.38 (m, 4H, 6-H, 16-H*, 21-H, 22-H), 1.45 (s, 3H, 27-H), 1.49–2.02 (m, 10H, 2-H, 6-H, 7-H, 15-H*, 19-H, 21-H, 22-H), 2.14–2.22 (m, 1H, 16-H*), 2.38 (d, b, $J = 10.1$ Hz, 1H, 18-H), 2.48 (s, 1H, 9-H), 2.75 (d, b, $J = 13.8$ Hz, 1H, 1-H), 3.24–3.44 (m, 7H, 2″-H, 3-H, 3″-H, 4′-H, 4″-H, 5′-H, 5″-H), 3.59–3.73 (m, 4H, 2′-H, 3′-H, 6′-H, 6″-H), 3.85–3.90 (m, 2H, 6′-H, 6″-H), 4.48 (d, $J = 6.6$ Hz, 1H, 1′-H), 4.72 (d, $J = 7.4$ Hz, 1H, 1″-H), 5.76 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 17.22, 17.28 (C-24, C-25), 18.76 (C-6), 19.64 (C-26), 24.10 (C-27), 27.40 (C-2), 27.96, 28.03 (C-15, C-16), 28.73 (C-23), 29.67, 29.90 (C-28, C-29), 33.30 (C-17), 33.40 (C-21), 34.18 (C-7), 38.31 (C-10), 39.90 (C-22), 40.62 (C-1), 41.05 (C-4), 44.24 (C-19), 44.94 (C-8), 46.77 (C-20), 47.03 (C-14), 50.36 (C-18), 56.80 (C-5), 63.08 (C-6′*), 63.36 (C-9), 63.43 (C-6″*), 71.88 (C-4′), 72.23 (C-4″), 76.60 (C-2″), 77.95, 78.15, 78.61 (C-3″, C-5′, C-5″), 78.78 (C-3′), 81.48 (C-2′), 91.46 (C-3), 104.84 (C-1″), 105.69 (C-1′), 129.15 (C-12), 174.24 (C-13), 185.27 (C-30), 203.03 (C-11) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 793 [M-H^+] (100.0), 779 (13.5), 747 (9.0), 653 (9.8), 631 (48.9), 613 (15.8), 611 (8.3), 523 (11.2), 497 (21.8), 469 (49.6), 451 (30.1), 437 (18.7), 423 (11.2), 369 (13.5), 291 (11.2), 249 (12.8), 221 (18.0), 183 (33.1), 159 (30.0), 113 (36.8), 91 (63.9), 71 (72.9), 59 (55.6), 45 (16.5).

β-D-Glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-3-O-18β-glycyrrhetic acid (**4**; $C_{42}H_{66}O_{14}$)

The same procedure as described for **3** was used. 1.6 g (2.5 mmol) of **8** in 15 cm^3 $CHCl_3$ and 1.4 g (3.5 mmol) of acetobromolaminaribiose in $CHCl_3$ (15 cm^3) and 3.4 g of Ag_2O, 6.4 g of drierite, and 340 mg of l_2 were added. After purification, deacetylation, and hydrogenation, 75 mg (2.5%) of **4** were obtained by preparative TLC.

M.p.: 226–228°C (decomp.); $R_f = 0.19$ (CH_2Cl_2:CH_3OH = 8:2); $[\alpha]_D^{20} = +62.9°$, $[\alpha]_{546}^{20} = +69.0°$ ($c = 0.07$, CH_3OH); IR (KBr): ν = 3420 (s), 2929 (s), 1642 (s), 1559 (s), 1464 (m), 1386 (m), 1363 (m), 1159 (w), 1078 (s), 1042 (s), 547 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 0.78–0.83 (m, 4H, 5-H, 28-H), 0.87 (s, 3H, 24-H), 0.95–1.04 (m, 2H, 1-H, 15-H*), 1.08 (s, 3H, 23-H), 1.12 (s, 3H, 29-H), 1.14 (s, 3H, 26-H), 1.15 (s, 3H, 25-H), 1.18–1.39 (m, 3H, 16-H*, 21-H, 22-H), 1.42 (s, 3H, 27-H), 1.45–2.02 (m, 11H, 2-H, 6-H, 7-H, 15-H*, 19-H, 21-H, 22-H), 2.11–2.19 (m, 1H, 16-H*), 2.28 (d, b, $J = 10.0$ Hz, 1H, 18-H), 2.45 (s, 1H, 9-H), 2.71 (d, b, $J = 13.1$ Hz, 1H, 1-H), 3.22 (dd, $J = 11.4$, 4.0 Hz, 1H, 3-H), 3.27–3.42 (m, 7H, 2′-H, 2″-H, 3″-H, 4′-H, 4″-H, 5′-H, 5″-H), 3.50–3.72 (m, 3H, 3′-H, 6′-H, 6″-H), 3.84–3.91 (m, 2H, 6′-H, 6″-H), 4.38 (d, $J = 7.6$ Hz, 1H, 1′-H), 4.58 (d, $J = 7.6$ Hz, 1H, 1″H); 5.66 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 17.30 (C-24, C-25), 18.74 (C-6), 19.61 (C-26), 24.16 (C-27), 27.24 (C-2), 27.81, 27.95 (C-15, C-16), 28.77 (C-23), 29.59 (C-28,

C-29), 32.86 (C-21), 33.28 (C-17), 34.13 (C-7), 38.34 (C-10), 39.63 (C-22), 40.57 (C-1), 40.80 (C-4), 43.48 (C-19), 44.91 (C-8), 46.01 (C-20), 47.04 (C-14), 50.27 (C-18), 56.79 (C-5), 62.83 (C-6′*) 62.97 (C-6′′*), 63.39 (C-9), 70.35 (C-4′), 71.80 (C-4′′), 75.33 (C-2′), 75.76 (C-2′′), 77.57 (C-5′), 78.03 (C-3′′), 78.42 (C-5′′), 88.36 (C-3′), 90.83 (C-3), 105.53 (C-1′′), 106.54 (C-1′), 129.14 (C-12), 173.79 (C-13), 183.88 (C-30), 202.98 (C-11) ppm; MS (pos. LSIMS, 5 kV): *m/z* (%) = 795 $[M+H]^+$ (2.1), 633 (7.4), 472 (6.7), 471 (19.7), 453 (12.3), 317 (4.2), 279 (9.2), 241 (4.9), 208 (14.1), 180 (59.2), 167 (100), 135 (9.9), 132 (50.7).

α-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl-3-O-18β-glycyrrhetic acid (**5**; $C_{42}H_{66}O_{14}$)

1.6 g (2.5 mmol) diphenylmethyl glycyrrhetinate (**8**) were dissolved in a mixture of 80 cm^3 dry benzene and 80 cm^3 nitromethane. Half of the amount of the solvent was evaporated at 50°C to remove traces of moisture. After cooling to room temperature, 2.8 g (11.0 mmol) of mercury(II) cyanide and 1.4 g (3.5 mmol) of acetobromomaltose 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. Mercury ions were removed by shaking the solution three times with 100 cm^3 of aqueous Kl solution (5%). The solution was washed twice with 150 cm^3 of aqueous $NaHCO_3$ solution (10%), three times with water, and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* at 40°C gave 2.85 g of a yellow residue. This was purified by flash chromatography on silica gel using CH_2Cl_2:ethylacetate = 9:1 as solvent system to recover 1.2 g of **8**. The mobile phase was changed to CH_2Cl_2:MeOH = 8:2 to get 1.3 g of the polar fraction containing the product of the *Königs-Knorr* reaction. This residue was dried over P_2O_5, dissolved in 8 cm^3 of dry chloroform, and cooled to −20°C. A solution of 1.3 g sodium in 64 cm^3 of dry MeOH was cooled and 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 (545 mg) was dissolved in 37 cm^3 of dry MeOH, and 540 mg of Pd/C (10%) were added. Cleavage of the benzhydrylic ester was achieved by shaking with H_2 overnight at a pressure of 50 psi at room temperature. Solids were filtered off, the solvent evaporated *in vacuo* at room temperature, and the residue (365 mg) was subjected to preparative TLC eluting with CH_2Cl_2:MeOH (8:2) giving 120 mg (4%) of saponin **5**.

M.p.: 231–233°C (decomp.); $R_f = 0.17$ (CH_2Cl_2:CH_3OH = 8:2); $[\alpha]_D^{20} = +245.9°$, $[\alpha]_{546}^{20} = +81.1°$ ($c = 0.12$, CH_3OH); IR (KBr): $\nu = 3424$ (s), 2949 (s), 1651 (m), 1420 (m), 1558 (m), 1458 (m), 1391 (m), 1363 (w), 1077 (s), 795 (w), 471 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 0.81–0.86 (m, 4H, 5-H, 28-H), 0.91 (s, 3H, 24-H), 0.96–1.08 (m, 2H, 1-H, 15-H*), 1.12, 1.13 (2s, 6H, 23-H, 29-H), 1.18, 1.19 (2s, 6H, 25-H, 26-H), 1.20–1.38 (m, 4H, 6-H, 16-H*, 21-H, 22-H), 1.46 (s, 3H, 27-H), 1.49–2.06 (m, 10H, 2-H, 6-H, 7-H, 15-H*, 19-H, 21-H, 22-H), 2.15–2.22 (m, 1H, 16-H*), 2.37 (dd, $J = 13.1$, 3.8 Hz, 1H, 18-H), 2.48 (s, 1H, 9-H), 2.75 (d, b, $J = 13.8$ Hz, 1H, 1-H), 3.24 (dd, $J = 11.9$, 4.3 Hz, 1H, 3-H), 3.29–3.40 (m, 3H, 2′-H, 4′′-H, 5′-H), 3.48 (dd, $J = 9.6$, 3.8 Hz, 1H, 2′′-H), 3.60 (t, $J = 9.1$ Hz, 1H, 4′-H), 3.63–3.76 (m, 4H, 3′-H, 3′′-H, 5′′-H, 6′′-H), 3.83–3.91 (m, 3H, 6′-H, 6′′-H), 4.39 (d, $J = 7.9$ Hz, 1H, 1′-H), 5.21 (d, $J = 3.8$ Hz, 1H, 1′′-H), 5.75 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 17.32 (C-24, C-25), 18.77 (C-6), 19.64 (C-26), 24.13 (C-27), 27.34 (C-2), 27.95, 28.03 (C-15, C-16), 28.79 (C-23), 29.68, 29.92 (C-28, C-29), 33.31 (C-17, C-21), 34.19 (C-7), 38.36 (C-10), 39.88 (C-22), 40.62 (C-1), 40.83 (C-4), 44.14 (C-19), 44.94 (C-8), 46.65 (C-20), 47.05 (C-14), 50.37 (C-18), 56.85 (C-5), 62.49 (C-6′), 63.05 (C-6′′), 63.41 (C-9), 71.90 (C-4′′), 74.48 (C-2′′), 75.08 (C-5′′), 75.37 (C-3′′), 75.56 (C-2′), 76.67 (C-5′), 78.28 (C-3′), 81.53 (C-4′), 90.92 (C-3), 103.21 (C-1′′), 106.96 (C-1′), 129.17 (C-12), 174.18 (C-13), 184.97 (C-30), 203.03 (C-11) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 793 $[M-H^+]$ (100.0), 777 (9.0), 747 (6.7), 659 (11.2), 631 (50.2), 613 (33.0), 611 (13.5), 539 (6.7), 527 (7.5), 511 (15.0), 497 (39.0), 469 (44.9), 451 (28.5), 437 (20.2), 369 (10.5), 339 (8.2), 301 (10.4), 233 (9.7), 221 (14.2), 183 (18.7), 141 (27.0), 119 (46.4), 91 (47.2), 59 (59.2), 45 (17.2).

β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl-3-O-18β-glycyrrhetic acid (**6**; $C_{42}H_{66}O_{14}$)

The same procedure as described for **5** was used. 2.6 g (4.6 mmol) of **8**, 3.2 g of mercury(II) cyanide, and 1.8 g (4.5 mmol) of acetobromocellobiose were added. After work-up, purification, deacetylation, and hydrogenation preparative TLC afforded 140 mg (3.9%) of **6**.

M.p.: 230–231°C (decomp.); $R_f = 0.18$ (CH_2Cl_2:$CH_3OH = 8:2$); $[\alpha]_{546}^{20} = +57.8°$ ($c = 0.07$, CH_3OH); IR (KBr): $\nu = 3408$ (s), 2931 (s), 1650 (m), 1560 (m), 1460 (m), 1389 (m), 1076 (s), 624 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 078–0.83 (m, 4H, 5-H, 28-H), 0.87 (s, 3H, 24-H), 0.94–1.04 (m, 2H, 1-H, 15-H*), 1.08 (s, 6H, 23-H, 29-H), 1.14, 1.15 (2s, 6H, 25-H, 26-H), 1.17–1.38 (m, 3H, 16-H*, 21-H, 22-H), 1.42 (s, 3H, 27-H), 1.46–2.00 (m, 11H, 2-H, 6-H, 7-H, 15-H*, 19-H, 21-H, 22-H), 2.13–2.19 (m, 1H, 16-H*), 2.35 (d, b, $J = 10.3$ Hz, 1H, 18-H), 2.44 (s, 1H, 9-H), 2.71 (d, b, $J = 13.0$ Hz, 1H, 1-H), 3.18–3.40 (m, 7H, 2′-H, 2″-H, 3-H, 3″-H, 4″-H, 5′-H, 5″-H), 3.50 (t, $J = 9.0$ Hz, 1H, 3′-H), 3.58 (t, $J = 9.2$ Hz, 1H, 4′-H), 3.67 (dd, $J = 11.4$, 4.9 Hz, 1H, 6″-H), 3.82–3.92 (m, 3H, 6′-H, 6″-H), 4.36 (d, $J = 7.9$ Hz, 1H, 1′-H), 4.43 (d, $J = 7.6$ Hz, 1H, 1″-H), 5.72 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 17.29 (C-24, C-25), 18.76 (C-6), 19.62 (C-26), 24.09 (C-27), 27.33 (C-2), 27.95, 28.01 (C-15, C-16), 28.75 (C-23), 29.68, 29.95 (C-28, C-29), 33.30, 33.39 (C-17, C-21), 34.17 (C-7), 38.34 (C-10), 39.91 (C-22), 40.58 (C-1), 40.82 (C-4), 44.23 (C-19), 44.93 (C-8), 46.75 (C-20), 47.04 (C-14), 50.36 (C-18), 56.82 (C-5), 62.24 (C-6′), 62.69 (C-6″), 63.38 (C-9), 71.66 (C-4″), 75.24, 75.67 (C-2′, C-2″), 76.53 (C-3″), 76.86 (C-3′), 78.13, 78.41 (C-5′, C-5″), 80.96 (C-4′), 90.93 (C-3), 104.88 (C-1″), 106.84 (C-1′), 129.15 (C-12), 174.24 (C-13), 185.27 (C-30), 203.03 (C-11) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 793 [M-H^+] (63.9), 713 (9.8), 653 (18.0), 631 (69.9), 613 (26.3), 583 (16.5), 511 (18.0), 497 (46.6), 469 (60.9), 451 (44.4), 437 (23.3), 369 (18.8), 301 (18.8), 255 (20.3), 205 (20.3), 183 (27.1), 141 (36.1), 113 (55.6), 91 (65.4), 71 (100.0), 45 (27.1).

β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl-3-O-18β-glycyrrhetic acid
(**7**; $C_{42}H_{66}O_{14}$)

The same procedure as described for **3** was used. 3.18 g (4.9 mmol) of **8** and 3 g (7.6 mmol) of acetobromogentiobiose in $CHCl_3$ (15 cm^3) and 7.7 g of Ag_2O, 12 g of drierite, and 630 mg of I_2 were added. After purification, deacetylation, and hydrogenation preparative TLC afforded 165 mg (4.2%) of **7**.

M.p.: 232–234°C (decomp.); $R_f = 0.19$ (CH_2Cl_2:$CH_3OH = 8:2$); $[\alpha]_D^{20} = +55.6°$, $[\alpha]_{546}^{20} = +37.7°$ ($c = 0.05$, CH_3OH); IR (KBr): $\nu = 3412$ (s), 2926 (s), 1652 (m), 1571 (s), 1457 (m), 1400 (m), 1047 (s), 622 (w) cm^{-1}; ^1H NMR (400 MHz, δ, CD_3OD): 0.82–0.86 (m, 4H, 5-H, 28-H), 0.91 (s, 3H, 24-H), 0.94–1.06 (m, 2H, 1-H, 15-H*), 1.12 (s, 6H, 23-H, 29-H) 1.18 (s, 6H, 25-H, 26-H), 1.22–1.42 (m, 4H, 6-H, 16-H*, 21-H, 22-H), 1.46 (s, 3H, 27-H), 1.49–2.01 (m, 10H, 2-H, 6-H, 7-H, 15-H*, 19-H, 21-H, 22-H), 2.14–2.24 (m, 1H, 16-H*), 2.38 (d, b, $J = 10.0$ Hz, 1H, 18-H), 2.49 (s, 1H, 9-H), 2.73 (d, b, $J = 13.2$ Hz, 1H, 1-H), 3.22–3.40 (m, 8H, 2′-H, 2″-H, 3-H, 3′-H, 3″-H, 4′-H, 4″-H, 5″-H), 3.44-3.50 (m, 1H, 5′-H), 3.72 (dd, $J = 11.7$, 5.1 Hz, 1H, 6″-H), 3.83 (dd, $J = 11.8$, 5.4 Hz, 1H, 6′-H), 3.91 (dd, $J = 11.8$, 1.8 Hz, 1H, 6″-H), 4.15 (dd, $J = 11.8$, 2.1 Hz, 1H, 6′-H), 4.48 (d, $J = 7.7$ Hz, 1H, 1′-H), 4.43 (d, $J = 7.9$ Hz, 1H, 1″-H), 5.75 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 17.31 (C-24, C-25), 18.75 (C-6), 19.60 (C-26), 24.07 (C-27), 27.36 (C-2), 27.95, 28.02 (C-15, C-16), 28.74 (C-23), 29.67, 29.94 (C-28, C-29), 33.29 (C-17), 33.39 (C-21), 34.16 (C-7), 38.34 (C-10), 39.90 (C-22), 40.57 (C-1), 40.82 (C-4), 44.22 (C-19), 44.94 (C-8), 46.74 (C-20), 47.04 (C-14), 50.34 (C-18), 56.76 (C-5), 63.02 (C-6″), 63.35 (C-9), 70.20 (C-6′), 71.82, 71.91 (C-4′, C-4″), 75.42, 75.93 (C-2′, C-2″), 77.12 (C-5′), 78.24, 78.27, 78.41 (C-3′, C-3″, C-5″), 90.80 (C-3), 105.18 (C-1″), 106.98 (C-1′), 129.18 (C-12), 174.27 (C-13), 185.25 (C-30), 203.14 (C-11) ppm; MS (neg. LSIMS, 5 kV): *m/z* (%) = 793 [M-H^+] (100.0), 653 (12.0), 631 (36.8), 613 (12.8), 511 (10.5), 497 (21.8), 469 (36.1), 451 (27.8), 437 (18.0), 369 (15.8), 325 (78.9), 311 (59.4), 255 (27.8), 205 (117.3), 183 (45.8), 141 (21.1), 113 (30.8), 91 (51.1), 71 (66.2), 59 (60.2), 45 (16.5).

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