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Saponins from European Licorice Roots (*Glycyrrhiza glabra*)

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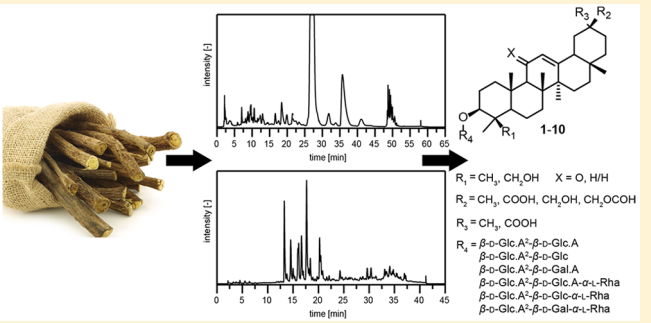
Supporting Information

ABSTRACT: European licorice roots (*Glycyrrhiza glabra*), used in the food and beverage industry due to their distinctive sweet and typical licorice flavor, were fractionated, with the triterpenoid saponins isolated and their chemical structures determined by means of ESIMS, ESIMS/MS, HRESIMS, and 1D/2D NMR experiments. Next to the quantitatively predominant saponin glycyrrhizin (**11**) and some previously known saponins, the structures of 10 monodesmosidic saponins were assigned unequivocally for the first time, namely, 30-hydroxyglycyrrhizin (**1**), glycyrrhizin-20-methanoate (**2**), 24-hydroxyglucoglycyrrhizin (**3**), rhaoglycyrrhizin (**4**), 11-deoxorhaoglycyrrhizin (**5**), rhaoglucoglycyrrhizin (**6**), rhaogalactoglycyrrhizin (**7**), 11-deoxo-20α-glycyrrhizin (**8**), 20α-galacturonoylglycyrrhizin (**9**), and 20α-rhaoglycyrrhizin (**10**).



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Several herbaceous perennial species from the genus *Glycyrrhiza*, also known as licorice, are well accepted for their attractive flavor properties as well as other biological activities that are due to oleanane-type triterpenoid saponins.[1−5] The legume genus *Glycyrrhiza* consists of about 30 species native to Europe, Asia, North and South America, and Australia, including *G. glabra*, *G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi*, and *G. eurycarpa*, respectively. Owing to the distinctive sweet and licorice sensation of their roots, *Glycyrrhiza* spp. are named after the Greek words "glycys" (sweet) and "rhize" (roots) and are used for production of food, confectionary, beverage, and cosmetic products, as well as to flavor cigarettes.[2] Besides uses as a low-caloric sweetener and flavor additive, licorice extracts have a long-standing history as a botanical drug in Europe and Asia, dating back to at least 2100 B.C.[6−10] For centuries, licorice extracts have been used in China to treat respiratory (asthma), gastrointestinal (chronic gastritis), urogenital (bladder infection), and skin diseases (atopic dermatitis), respectively.[2,9,11]

Various polyphenols and saponins are reported to be responsible for the pharmacological activity of licorice.[2,9,11−23] Among the oleanane triterpenoid saponins, glycyrrhizin has been reported as the major secondary metabolite in licorice; this monodesmosidic saponin exhibits a 18β-glycyrrhetinic acid skeletal structure, derived from β-amyrin, linked to a disaccharide unit bearing two glucuronic acid moieties at position C-3.[7,8,24−28] In addition to its anti-inflammatory, antimicrobial, antiviral, and antitumor activities,[2,9,11−23] glycyrrhizin has been reported to inhibit 11β-hydroxysteroid dehydrogenase (11βHSD2) and to induce mineralocorticoid excess syndrome,[29,30] leading to hypokalemia, hypertension, and hypernatremia in humans.[2,9,11,31]

Although a total of 43 saponins have been reported in *G. uralensis*,[19,27,32−38] which is mostly used in oriental medicine systems, mass-spectrometry-based metabolome profiling indicated a series of not yet fully characterized saponins in several *Glycyrrhiza* species.[15,18,22,39−42] In particular, the present knowledge on the saponin composition in European licorice (*Glycyrrhiza glabra* L.), mainly used in the food and beverage industry, is rather fragmentary. Therefore, the objective of the present study was to isolate and identify the chemical structure of oleanane-type triterpenoidic saponins in European licorice roots by means of ESIMS, ESIMS/MS, HRESIMS, and 1D/2D NMR experiments.

RESULTS AND DISCUSSION

In order to isolate and identify oleanane-type saponins from the roots of *G. glabra*, a MeOH−H_2O extract was fractionated by means of an offline multidimensional preparative strategy (Figure S1, Supporting Information). An initial preseparation of different classes of phytochemicals in licorice roots was achieved by taking up the MeOH−H_2O extractables in H_2O and preseparating them into four fractions by means of MPLC.

To obtain pure compounds, fractions II/III and IV were separated by HPLC into 23 and 18 subfractions, respectively. While compounds **11**, **12**, and **19** could be obtained as pure substances from fractions II/II-19, IV-11, and II/III-22, respectively, additional semipreparative and preparative HPLC separation was performed to afford compounds **1**−**10**, **13**−**18**, **20**, and **21** from fractions II/III-15−II/III-18, II/III-20, II/III-21, II/III-23, IV-6, IV-7, IV-9, IV-14, IV-15, and IV-16, respectively (Figure S1, Supporting Information, colored in

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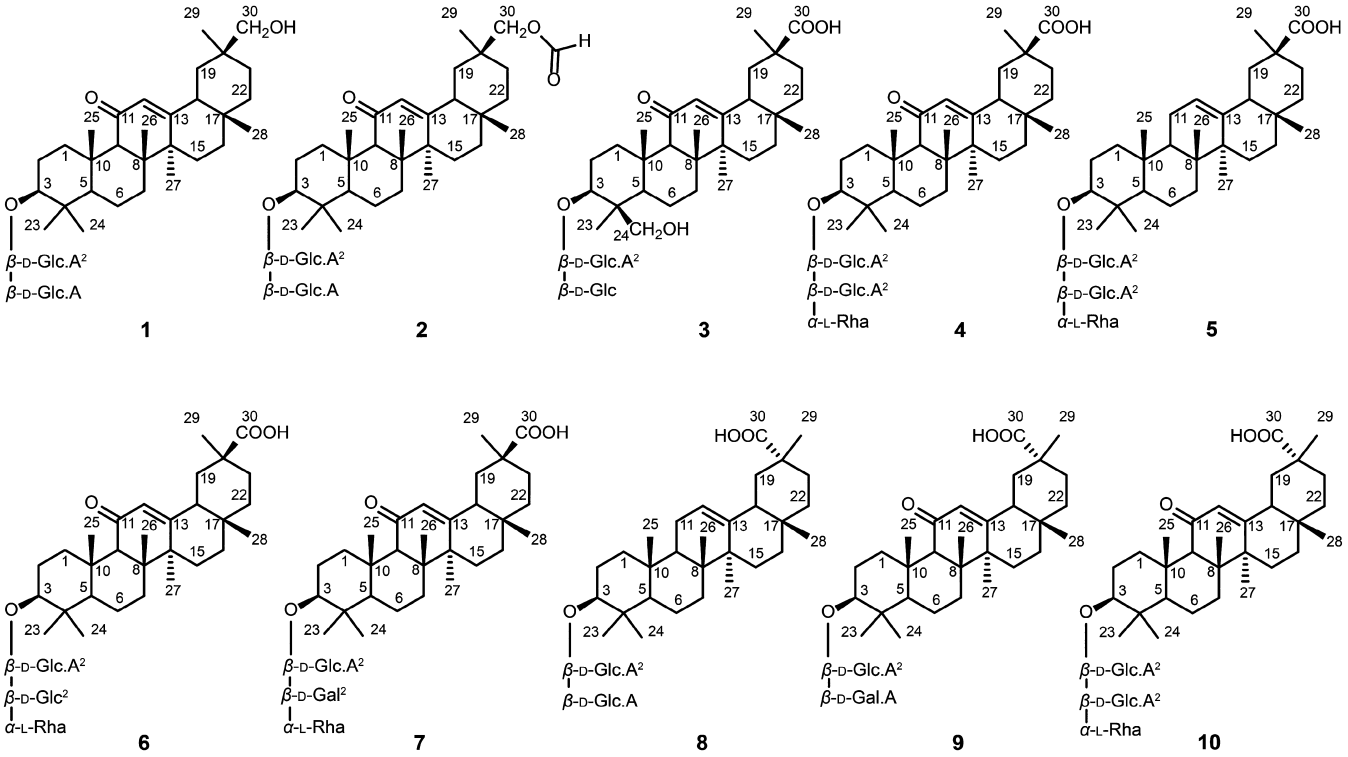

Figure 1. Structures of new triterpenoid saponins **1**−**10** isolated from *G. glabra*.

black). Preparative fractionation, followed by HRESIMS, LC-MS/MS, and 1D and 2D NMR experiments, led to the identification of 21 triterpenoid saponins in European licorice roots (Figure 1). To the best of our knowledge, the chemical structures of 10 triterpenoid saponins (**1**−**10**) were determined unequivocally for the first time (Figure 1). In addition, the presence of saponins **12**, **13**, **15**, **16**, **17**, **18**, and **21**, previously isolated from *G. uralensis* and *G. inflata*, is reported for the first time in *G. glabra*. Furthermore, the NMR data of compounds **12**, **15**, and **20** could be complemented.[22,37,38,43,44] Mass spectrometric and NMR spectroscopic data (Tables S1−S11) of several previously identified saponins corresponded well with published data: glycyrrhizin (**11**),[45] LS-B2 (11-deoxoglycyrrhizin, **12**),[22,37,38] LS-G2 (24-hydroxyglycyrrhizin, **13**),[38,44] LS-J2 (11-deoxo-24-hydroxyglycyrrhizin, **14**),[33] glucoglycyrrhizin (**15**),[43] araboglycyrrhizin (**16**),[32,38] apioglycyrrhizin (**17**),[32,38] glycyrrhetinic acid-monoglucuronide (**18**),[46,47] LS-H2 (20α-glycyrrhizin, **19**),[38,44] 24-hydroxy-20α-glycyrrhizin (**20**),[38,44] and LS-C2 (11-deoxo-11,13-glycyrrhizin diene, **21**).[22,37,38] A comparison of selected key NMR data, shown in Figure 2, were used to support the structure elucidation of unknown saponins and might be useful in the future to determine new saponins.

HRESIMS analysis of **1** revealed a molecular formula of $C_{42}H_{64}O_{15}$, based on the deprotonated molecule $[M - H]^-$ at m/z 807.4166, calcd for $C_{42}H_{63}O_{15}$ (m/z 807.4167). Compared to the spectrum of commercially available glycyrrhizin (**11**) with a determined empirical formula of $C_{42}H_{62}O_{16}$, the HRESIMS data of **1** indicated a lack of an oxygen atom and two protons. By means of additional ESIMS/MS experiments of the protonated molecule $[M + H]^+$, this resulted in fragments of m/z 633 and m/z 439, indicating the presence of two uronic acid moieties. The ^{13}C NMR spectroscopic data of **1**, isolated from fraction II/III-21-4-2, showed a total of 42 signals resonating between 17.1 and 199.8 ppm, among which 12 signals between 73.4 and 172.9 ppm were almost identical with the disaccharide moiety of glycyrrhizin (**11**), consisting of two β-D-glucuronic acids, as reported earlier.[43] Acid hydrolysis, followed by the determination of the monosaccharides, proved the presence of two D-glucuronic acids. For the characterization of the aglycone structure, carbon atoms with typical stand-alone ^{13}C NMR chemical shifts were used, such as 199.8 ppm for the carbonyl atom C-11, 170.2 ppm for the quaternary olefinic carbon atom C-13, and 89.5 ppm for the *O*-glycoside-bound carbon atom C-3, respectively (Table 1). Using C-3, C-11, and C-13 as starting points for NMR signal assignment and their corresponding HMBC correlations with H-1 (δ_H 3.05, δ_H 1.03), H-2 (δ_H 2.10, δ_H 2.33), Me-23 (δ_H 1.43), Me-24 (δ_H 1.27)/C-3 (δ_C 89.5); H-9 (δ_H 2.44), H-12 (δ_H 5.75)/C-11 (δ_C 199.8); and H-12 (δ_H 5.75), Me-27 (δ_H 1.42), H-18 (δ_H 2.26)/C-13 (δ_C 170.2), the adjacent functional groups at carbons 1, 2, 3, 9, 11, 12, 13, 18, 23, 24, and 27 were assigned. Compared to glycyrrhizin (**11**), compound **1** showed a high-field shift of H-12 with δ_H 5.75 (1H, s, **1**) and H-18 with δ_H 2.26 (1H, dd, J = 13.5, 4.5 Hz, **1**) with respect to H-12 with δ_H 5.98 (1H, s) and H-18 with δ_H 2.54 (1H, dd, J = 13.1, 3.7 Hz), indicating a less electronegative functional group at the triterpenoid ring E. With the absence of any signal at δ_C 179.5 (C-30), which was observed for glycyrrhizin (**11**), and the presence of the two signals δ_H 3.80 (1H, d, J = 10.8 Hz) and 3.72 (1H, d, J = 10.8 Hz) correlating with C-18 (δ_C 47.6) this revealed a CH_2OH group at position C-30 (Figure 2). Further HMBC correlations of Me-29 (δ_H 5.16)/C-30 (δ_C 65.7), H_2-30 (δ_H 3.72, δ_H 3.80)/C-29 (δ_C 28.6), and H-18 (δ_H 2.26)/C-29 (δ_C 28.6) also supported the presence of a CH_2OH group at position C-30. Moreover, ROE correlations were observed between the proton pairs H-3/Me-23, H-3/H-5, Me-23/H-5, H-5/H-9, and H-9/Me-27 of **1**, which indicated that the H-5 and H-9 protons as well as the methyl groups Me-

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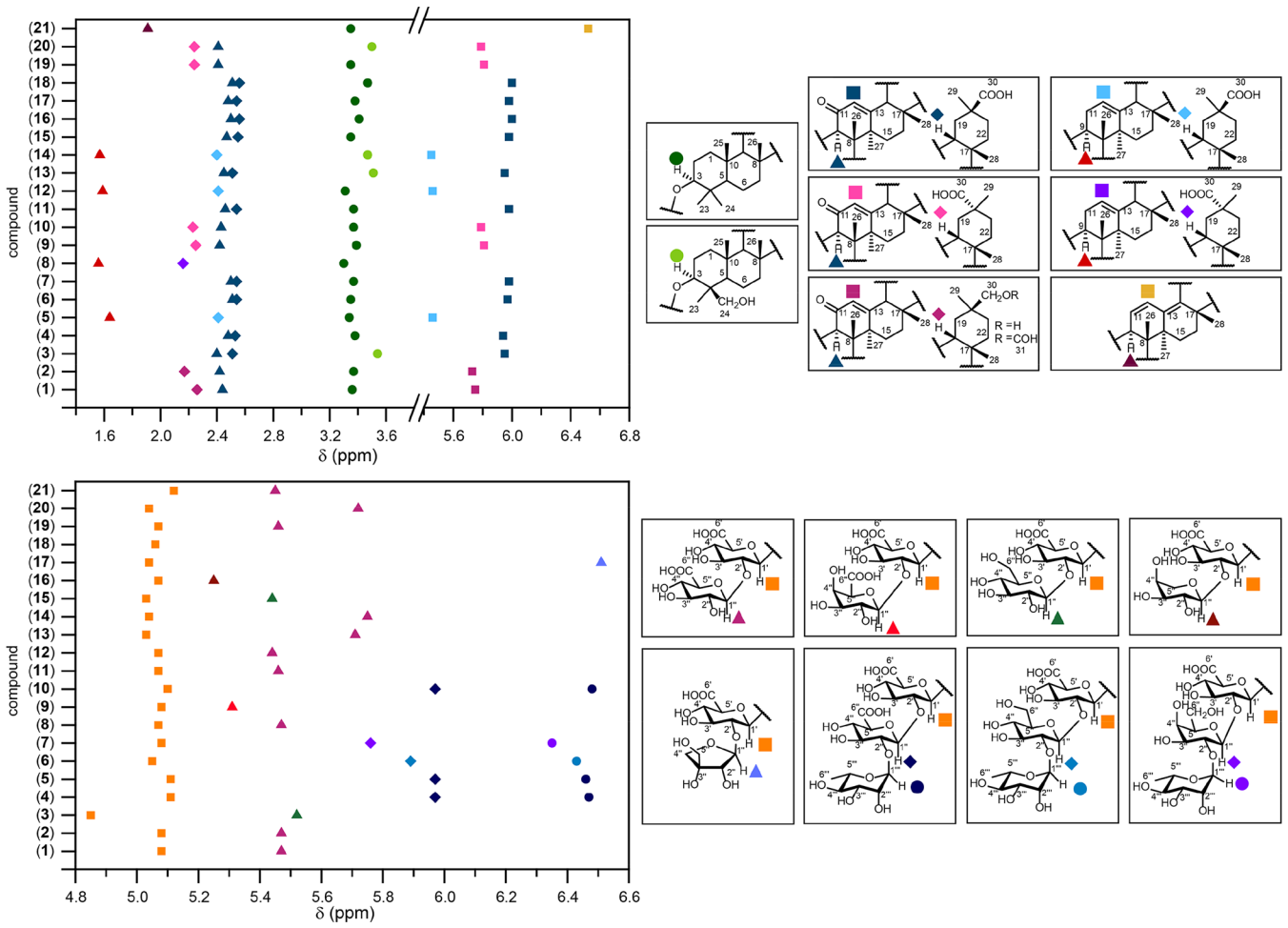

Figure 2. ^{1}H NMR chemical shifts of prominent positions in the aglycone and the carbohydrate moiety of saponins **1**–**21**.

23 and Me-27 are α-oriented. In addition, ROE correlations of protons Me-24/Me-25, Me-25/Me-26, Me-26/Me-28, H-18/Me-28, and H-18/H_2-30 established that H-18 and H_2-30 are β-oriented (Figure 3). Thus, compound **1** was determined as (3β,20β)-20-hydroxymethyl-11-oxo-30-norolean-12-en-3-yl 2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid (30-hydroxyglycyrrhizin).

Compound **2** gave a molecular formula of $C_{43}H_{64}O_{16}$, based on the $[M - COHH]^-$ ion with m/z 807.4195, calcd for $C_{42}H_{63}O_{15}$ (m/z 807.4167), in the HRESIMS spectrum. In contrast to the HRESIMS, the ESIMS data revealed a protonated peak of m/z 837 $[M + H]^+$, indicating an additional carbonyl group when compared to **1**. Further ESIMS/MS fragmentation of compound **2** showed patterns of m/z 809, 661, 457, and 442 with neutral mass losses of 176 + 219 Da, indicating the presence of two uronic acid moieties. The ^{1}H NMR spectrum of **2** displayed in contrast to **1** an additional resonance at δ_H 8.39 (1H, s) with a correlation to the carbon resonance at δ_C 162.1 in the HSQC spectrum, which could be attributed to an aldehyde group (Table 1). Comparison of ^{1}H and ^{13}C NMR resonances of compounds **1** and **2** revealed for both similar shifts for H-1 to H-28 and C-1 to C-28, including H-1′ to H-6″ and C-1′ to C-6″. With similar results of the monosaccharides after acidic hydrolysis, **2** was identical with **1** except for positions 29 and 30. For the methyl group at C-29, a high-field shift of δ_H 0.96 (3H, s, **2**), in contrast to δ_H 1.16 (3H, s, **1**), could be observed. In contrary, a downfield shift of the C-30 methylene group protons of δ_H 4.29 (1H, m, **2**) and 4.11 (1H, d, J = 11.0 Hz, **2**), in comparison to δ_H 3.80 (1H, d, J = 10.8 Hz, **1**) and 3.72 (1H, d, J = 10.8 Hz, **1**), was detected. By means of the HMBC correlation of H-30 (δ_H 4.29, δ_H 4.11)/C-31 (δ_C 162.1) the position of an aldehyde group could be determined at position C-30, and, consequently, compound **2** was assigned as (3β,20β)-20-methylformate-11-oxo-30-norolean-12-en-3-yl 2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid (glycyrrhizin-20-methanoate).

Saponin **3** showed the molecular formula $C_{42}H_{64}O_{16}$ based on the ion $[M - H]^-$ at m/z 823.4122, calcd for $C_{42}H_{63}O_{16}$ (m/z 823.4116), in its HRESIMS. When compared to the empirical formula of glycyrrhizin (**11**) ($C_{42}H_{62}O_{16}$), compound **3** showed two additional protons. An ESIMS/MS experiment of compound **3** revealed, based on the protonated molecular ion peak $[M + H]^+$ at m/z 825, fragments at m/z 663 and m/z 469, indicating a hexose as terminal sugar moiety and an uronic acid as the aglycone-bound sugar moiety. Further, the aglycone fragment m/z 469 of **3** showed in comparison with the corresponding fragment of glycyrrhizin (**11**) at m/z 453 an aglycone with a 16 Da increased mass, thus suggesting an additional hydroxy group linked to the sapogenin. The ^{13}C NMR spectrum displayed 42 resonances like glycyrrhizin (**11**), with two signals shifted to δ_C 63.6 and δ_C 61.9 (Table 1 and Table S1, Supporting Information). These signals gave cross-peaks with proton resonances at δ_H

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Table 1. NMR Spectroscopic Data for Compounds 1−5 in Pyridine-d_5 (500 MHz for ^{1}H and 125 MHz for ^{13}C, δ Values in ppm, J Values in Hz)

	1		2		3		4		5	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	39.9, CH_2	3.05, dd (13.6, 3.5)	39.8, CH_2	3.05, dt (13.2, 3.5)	39.4, CH_2	2.97, dd (9.3, 4.1)	39.8, CH_2	3.04, dd (9.5, 3.5)	39.1, CH_2	1.39, m
		1.03, m		1.05, m		1.06, m		1.12, m		0.91, m
2	27.1, CH_2	2.33, m	27.1, CH_2	2.34, dd (13.9, 4.2)	26.8, CH_2	2.60, m	26.5, CH_2	2.26, dd (12.1, 4.4)	26.8, CH_2	2.18, m
		2.10, m		2.09, m		2.09, m		2.05, m		1.84, m
3	89.5, CH	3.36, dd (11.7, 4.5)	89.5, CH	3.37, dd (11.8, 4.3)	90.8, CH	3.54, dd (12.3, 5.4)	89.8, CH	3.38, dd (11.7, 4.3)	90.4, CH	3.34, dd (11.8, 4.5)
4	40.3, C		40.3, C		44.5, C		40.5, C		40.0, C	
5	55.8, CH	0.74, d (11.8)	55.7, CH	0.73, d (11.6)	56.1, CH	0.87, d (11.9)	55.4, CH	0.83, dd (12, 2.6)	56.2, CH	0.83, m
6	18.0, CH_2	1.29, 1.08, m	17.9, CH_2	1.50, 1.32, m	18.0, CH_2	1.58, 1.28, m	18.0, CH_2	1.59, 1.36, m	18.9, CH_2	1.56, 1.37, m
7	33.3, CH_2	1.53, 1.27,m	33.2, CH_2	1.55, 1.23, m	33.2, CH_2	1.55, 1.24, m	33.3, CH_2	1.65, 1.29, m	33.3, CH_2	1.54, 1.30, m
8	45.9, C		45.9, C		45.7, C		45.9, C		40.4, C	
9	62.4, CH	2.44, s	62.4, CH	2.42, s	62.1, CH	2.40, s	62.5, CH	2.48, s	48.2, CH	1.64, t (8.9)
10	37.6, C		37.6, C		37.0, C		37.6, C		37.2, C	
11	199.8, C		199.8, C		199.8, C		199.9, C		24.1, CH_2	1.81, m
12	128.9, CH	5.75, s	129.1, CH	5.73, s	128.8, CH	5.95, s	129.0, CH	5.94, s	123.2, CH	5.46, t (4.0)
13	170.2, C		169.3, C		170.3, C		169.9, C		145.5, C	
14	44.0, C		43.8, C		43.7, C		43.8, C		42.1, C	
15	27.1, CH_2	1.68, 1.06, m	27.0, CH_2	1.68, 1.04, m	27.1, CH_2	1.68, 1.09, m	27.2, CH_2	1.73, 1.12, m	27.0, CH_2	1.81, 1.02, m
16	27.3, CH_2	2.09, m	27.0, CH_2	2.01, m	26.9, CH_2	2.09, m	27.0, CH_2	2.05, m	27.6, CH_2	2.10, dt (13.8, 4.3)
		0.90, m		0.85, m		0.94, m		0.95, m		0.92, m
17	32.9, C		32.8, C		34.4, C		32.5, C		32.7, C	
18	47.6, CH	2.26, dd (13.5, 4.5)	47.2, CH	2.17, m	49.0, CH	2.51, dd (13.3, 3.3)	49.1, CH	2.53, dd (16.9, 3.3)	49.0, CH	2.41, dt (13.7, 3.3)
19	41.2, CH_2	1.69, 1.49, m	40.6, CH_2	1.62, 1.22, m	42.0, CH_2	2.13, 1.72, m	42.0, CH_2	2.12, 1.74, m	43.8, CH_2	2.29, 1.85, m
20	36.4, C		34.8, C		44.3, C		44.5, C		44.7, C	
21	30.3, CH_2	1.69, 1.38, m	30.6, CH_2	1.35, 1.36, m	31.9, CH_2	2.26, 1.46, m	31.9, CH_2	2.29, 1.49, m	32.2, CH_2	2.30, 1.51, m
22	36.8, CH_2	1.54, 1.28, m	36.4, CH_2	1.41, 1.24, m	38.6, CH_2	1.68, 1.42, m	38.8, CH_2	1.69, 1.44, m	39.4, CH_2	1.81, 1.47, m
23	28.5, CH_3	1.43, s	28.5, CH_3	1.42, s	22.9, CH_3	1.37, s	28.8, CH_3	1.48, s	28.8, CH_3	1.47, s
24	17.2, CH_3	1.27, s	17.2, CH_3	1.26, s	63.6, CH_2	4.33, m	17.2, CH_3	1.27, s	17.3, CH_3	1.25, s
						3.39, d (12.3)				
25	17.1, CH_3	1.21, s	17.1, CH_3	1.21, s	16.9, CH_3	1.06, s	17.0, CH_3	1.19, s	15.9, CH_3	0.79, s
26	19.2, CH_3	1.05, s	19.1, CH_3	1.04, s	18.9, CH_3	1.00, s	19.4, CH_3	1.07, s	17.2, CH_3	0.92, s
27	23.9, CH_3	1.42, s	23.9, CH_3	1.38, s	23.8, CH_3	1.39, s	23.8, CH_3	1.43, s	26.5, CH_3	1.30, s
28	29.0, CH_3	0.83, s	28.8, CH_3	0.79, s	28.3, CH_3	0.76, s	29.1, CH_3	0.77, s	28.9, CH_3	0.89, s
29	28.6, CH_3	1.16, s	28.1, CH_3	0.96, s	29.1, CH_3	1.34, s	29.0, CH_3	1.37, s	29.5, CH_3	1.39, s
30	65.7, CH_2	3.80, d (10.8)	67.1, CH_2	4.29, m	179.9, C		179.5, C		180.0, C	
		3.72, d (10.8)		4.11, d (11.0)						
31			162.1, CH	8.39s						
1′	105.5, CH	5.08, d (7.8)	105.5, CH	5.08, d (7.6)	104.7, CH	4.85, d (8.0)	105.5, CH	5.11, d (7.8)	105.5, CH	5.11, d (7.7)
2′	84.9, CH	4.33, m	84.8, CH	4.32, m	82.1, CH	4.15, m	79.7, CH	4.55, dd (9.0, 7.8)	79.7, CH	4.55, dd (9.2, 7.6)
3′	78.1, CH	4.43, t (8.8)	78.1, CH	4.43, pt (8.7)	78.8, CH	4.31, m	79.1, CH	4.67, m	79.1, CH	4.68, t (9.0)
4′	73.4, CH	4.60, m	73.4, CH	4.60, m	73.6, CH	4.31, m	73.8, CH	4.47, m	73.9, CH	4.50, dd (9.8, 9.0)
5′	77.8, CH	4.63, m	77.8, CH	4.61, m	76.5, CH	4.28, m	77.9, CH	4.62, m	77.8, CH	4.68, d (9.6)
6′	172.9, C		172.9, C		175.5, C		172.9, C		173.0, C	
1″	107.3, CH	5.47, d (7.8)	107.3, CH	5.47, d (7.6)	105.2, CH	5.52, d (7.9)	103.1, CH	5.97, d (7.9)	103.1, CH	5.97, d (7.8)
2″	77.2, CH	4.28, m	77.2, CH	4.29, m	76.1, CH	4.09, dd (8.8, 7.8)	78.5, CH	4.48, m	78.5, CH	4.48, dd (9.1, 7.7)
3″	78.0, CH	4.35, m	78.0, CH	4.36, m	78.0, CH	4.20, t (9.1)	79.3, CH	4.35, m	79.3, CH	4.35, pt (9.1)
4″	73.7, CH	4.68, m	73.7, CH	4.65, m	70.3, CH	4.38, t (9.1)	74.0, CH	4.62, m	74.0, CH	4.62, dd (9.8, 8.9)
5″	78.8, CH	4.65, m	78.8, CH	4.68, m	78.6, CH	3.71, dt (9.6, 3.8)	77.8, CH	4.48, m	77.9, CH	4.48, d (9.7)
6″	172.5, C		172.6, C		61.9, CH_2	4.34, 4.38, m	172.6, C		172.5, C	
1‴							102.6, CH	6.47, d (2.3)	102.5, CH	6.46, d (1.9)
2‴							72.8, CH	4.8, dd (3.1, 2.1)	72.8, CH	4.80, dd (3.2, 1.6)

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Table 1. continued

	1		2		3		4		5	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
3‴							73.1, CH	4.73, dd (9.0, 3.3)	73.1, CH	4.74, dd (9.2, 3.7)
4‴							74.7, CH	4.38, t (9.0)	74.7, CH	4.37, pt (9.2)
5‴							70.0, CH	5.08, dd (8.8, 6.3)	70.0, CH	5.08, dd (9.3, 6.2)
6‴							19.1, CH_3	1.84, d (6.2)	19.4, CH_3	1.84, d (6.0)

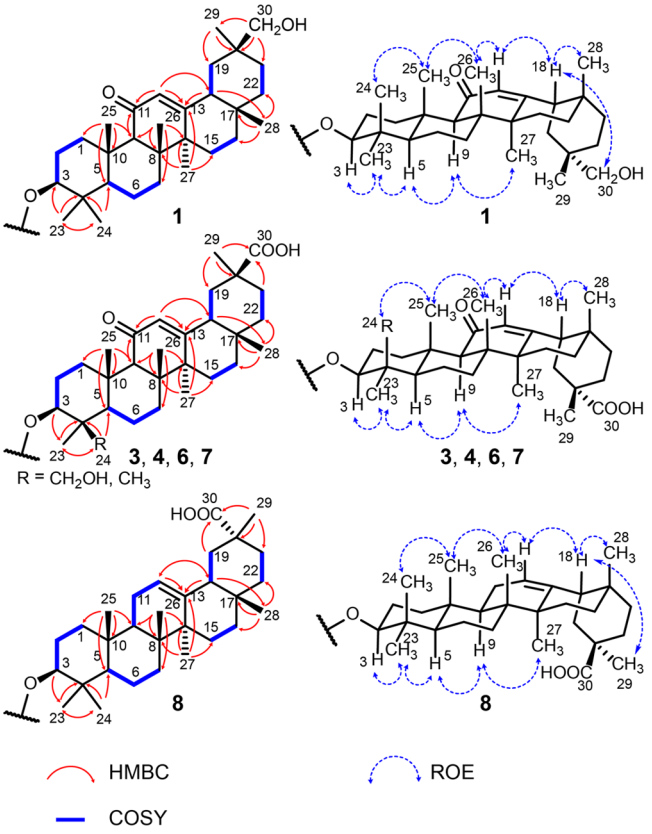

Figure 3. Key HMBC and COSY (left) as well as ROE (right) correlations of selected compounds.

3.39 (1H, d, J = 12.3 Hz), δ_H 4.33 (1H, m) for δ_C 63.6 and δ_H 4.34 (1H, m), δ_H 4.38 (1H, m) for δ_C 61.9 in the HSQC spectrum, respectively, indicating the occurrence of two CH_2OH groups. HMBC correlations of H-3 (δ_H 3.54) with δ_C 63.6 and H-5 (δ_H 0.87) with δ_C 63.6 indicated that one CH_2OH group is located at C-24. The ROE correlation of H-25 (δ_H 1.06)/H-24 (δ_H 3.39) substantiated this observation (Figure 3). The additional hydroxy group at the triterpenoid A ring resulted in a downfield shift of H-3 at δ_H 3.54 (1H, dd, J = 12.3, 5.4 Hz) in contrast to glycyrrhizin (**11**) at δ_H 3.37 (1H, dd, J = 12.0, 4.5 Hz) (Figure 3). Further, specification of the second CH_2OH group could be carried out by the COSY correlation of H-5″ (δ_H 3.71)/δ_H 4.34 and H-5″ (δ_H 3.71)/δ_H 4.38. These signals correlated with δ_C 61.9 (CH_2) in the HSQC spectrum, revealing the second CH_2OH group to occur at C-6″. The observed coupling constants $^3J_{2'',3''}$, $^3J_{3'',4''}$, and $^3J_{4'',5''}$ with 8.8 to 9.6 Hz as well as acidic hydrolysis leading to the determination of the absolute configuration of the monosaccharides revealed the terminal sugar moiety to be β-D-glucose. Thus, compound **3** was identified as (3β,20β)-20-carboxy-24-hydroxy-11-oxo-30-norolean-12-en-3-yl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranosiduronic acid (24-hydroxyglucoglycyrrhizin).

HRESIMS analysis of compound **4** afforded a molecular formula of $C_{48}H_{72}O_{20}$, based on a deprotonated molecular ion peak $[M - H]^-$ at m/z 967.4570, calcd for $C_{48}H_{71}O_{20}$ at m/z 967.4539. In comparison to glycyrrhizin ($C_{42}H_{62}O_{16}$, **11**) compound **4** showed a molecular formula that was increased by $C_6H_{10}O_4$, indicating a further methylpentose moiety, which could be substantiated by ESIMS/MS of this compound. The protonated molecule of saponin **4** (m/z 969, $[M + H]^+$) exhibited a fragmentation pattern with m/z 823, 647, and 453, thus indicating a methyl-pentose and two uronic acid moieties. Besides the typical ^1H and ^{13}C NMR shifts for the glycyrrhetinic acid moiety, all HSQC, HMBC, and ROE correlations corresponded to the glycyrrhizin (**11**) aglycone (Table 1 and Table S1, Supporting Information). Further, by means of NMR spectroscopy as well as by determination of the absolute configuration of the monosaccharide moieties after acidic hydrolysis, the presence was demonstrated of two 1″ → 2′ linked β-D-glucuronic acids attached to an additional 1‴ → 2″ glycosylated α-L-rhamnose unit. This was substantiated by means of the observed COSY and HMBC correlations: H-1′ (δ_H 5.11)/C-3 (δ_C 89.8), H-1′ (δ_H 5.11)/H-2′ (δ_H 4.55), H-2′ (δ_H 4.55)/C-1″ (δ_C 103.1), H-1″ (δ_H 5.97)/H-2″ (δ_H 4.48), H-2″ (δ_H 4.48)/C-1‴ (δ_H 6.47). α-L-Rhamnose could be identified as the terminal sugar moiety as a result of the COSY correlations at H-1‴ (δ_H 6.47)/H-2‴ (δ_H 4.80), H-2‴ (δ_H 4.80)/H-3‴ (δ_H 4.73), H-3‴ (δ_H 4.73)/H-4‴ (δ_H 4.38), H-4‴ (δ_H 4.38)/H-5‴ (δ_H 5.08), and H-5‴ (δ_H 5.08)/H-6‴ (δ_H 1.84) and the presence of a methyl group resonating at δ_H 1.84 (3H, d, J = 6.2 Hz, H-6‴) and by comparison of the coupling constants $^3J_{1''',2'''}$ = 2.3 Hz, $^3J_{2''',3'''}$ = 3.2 Hz, $^3J_{3''',4'''}$ = 9.0 Hz, $^3J_{4''',5'''}$ = 9.0 Hz, and $^3J_{5''',6'''}$ = 6.2 Hz with those reported earlier.[48] Consequently, compound **4** was assigned as (3β,20β)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-*O*-α-L-rhamnopyranosyl-2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid (rhaoglycyrrhizin). For the first time, the structure of this compound proposed previously by mass spectrometry was confirmed unequivocally.[49]

Compound **5** demonstrated a molecular formula of $C_{48}H_{74}O_{19}$ based on the deprotonated molecular ion peak $[M - H]^-$ at m/z 953.4777, calcd for $C_{48}H_{73}O_{19}$ at m/z 953.4746. In contrast to saponin **4** ($C_{48}H_{72}O_{20}$), compound **5** exhibited a molecular formula diminished by one oxygen and increased by two hydrogen atoms, indicating the substitution of a carbonyl group by a methylene group. Furthermore, the ESIMS/MS data of **5** showed fragments at m/z 655 and 439, representing cleavages of 322 Da (uronic acid + methylpentose) and 216 Da (uronic acid). Compared to glycyrrhizin (**11**) and rhaoglycyrrhizin (**4**), the aglycone fragment of **5** at m/z 439 Da indicated a mass difference of 14 Da, demonstrating the substitution of a carbonyl group by a

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Table 2. NMR Spectroscopic Data for Compounds 6−10 in Pyridine-d_5 (500 MHz for ^{1}H and 125 MHz for ^{13}C, δ Values in ppm, J Values in Hz)

	6		7		8		9		10	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	39.8, CH_2	3.06, dd (9.5, 3.7)	39.9, CH_2	3.07, dd (9.5, 3.7)	39.1, CH_2	0.82, m	39.9, CH_2	3.09, dd (9.3, 3.7)	39.8, CH_2	3.05, dd (3.7, 3.4)
		1.11, m		1.12, m		1.40, m		1.09, m		1.11, m
2	26.9, CH_2	2.25, m	26.9, CH_2	2.27, m	26.8, CH_2	1.88, m	27.1, CH_2	2.34, dd (13.5, 4.8)	26.9, CH_2	2.26, m
		2.05, m		2.08, m		2.24, m		2.13, m		2.04, m
3	90.1, CH	3.35, dd (12, 4.6)	90.1, CH	3.37, dd (12, 4.6)	89.7, CH	3.30, dd (12.0, 5.0)	89.1, CH	3.39, dd (12, 4.9)	90.2, CH	3.37, dd (11.8, 4.5)
4	40.4, C		40.4, C		39.9, C		40.2, C		40.4, C	
5	55.8, CH	0.82, m	55.9, CH	0.81, m	56.1, CH	0.70, m	55.7, CH	0.73, dd (12, 2.3)	55.8, CH	0.80, d (11.7)
6	18.0, CH_2	1.58, 1.38, m	18.0, CH_2	1.57, 1.38, m	18.8, CH_2	1.43, 1.26, m	18.0, CH_2	1.52, 1.33, m	18.0, CH_2	1.56, 1.37, m
7	33.2, CH_2	1.65, 1.30, m	33.3, CH_2	1.61, 1.30, m	33.1, CH_2	1.40, 1.22, m	33.3, CH_2	1.54, 1.23, m	33.2, CH_2	1.61, 1.25, m
8	45.9, C		45.9, C		40.4, C		45.9, C		45.9, C	
9	62.5, CH	2.51, s	62.5, CH	2.50, s	48.1, CH	1.56, s	62.4, CH	2.42, s	62.4, CH	2.43, s
10	37.6, C		37.6, C		37.2, C		37.6, C		37.6, C	
11	199.9, C		199.9, C		24.0, CH_2	1.83 dd (8.9, 3.4)	199.8, C		199.8, C	
12	129.0, CH	5.97, s	129.0, CH	5.98, s	123.1, CH	5.27, t (3.7)	129.2, CH	5.81, s	129.1, CH	5.79, s
13	170.0, C		169.9, C		145.0, C		169.5, C		169.4, C	
14	43.8, C		43.8, C		42.2, C		43.9, C		43.9, C	
15	27.2, CH_2	1.72, 1.11, m	27.2, CH_2	1.72, 1.10, m	27.0, CH_2	1.74, 0.92, m	27.0, CH_2	1.68, 1.04, m	27.0, CH_2	1.69, 1.06, m
16	27.0, CH_2	2.09, 0.97, m	27.0, CH_2	2.06, 0.95, m	27.6, CH_2	2.14, 0.88, m	26.9, CH_2	2.12, 0.89, m	26.9, CH_2	2.05, 0.90, m
17	32.5, C		32.5, C		28.7, C		32.9, C	–	32.9, C	
18	49.1, CH	2.54, dd (13.3, 3.5)	49.1, CH	2.54, dd (13.3, 3.5)	46.9, CH	2.16, dd (13.0, 3.5)	47.0, CH	2.25, dd (14, 3.8)	47.0, CH	2.23, m
19	42.0, CH_2	2.13, 1.75, m	42.0, CH_2	2.14, 1.74, m	41.8, CH_2	2.56, 1.70, m	40.2, CH_2	2.49, t (13.8), 1.62, m	40.1, CH_2	2.47, 1.61, m
20	44.5, C		44.5, C		43.2, C		42.9, C		42.9, C	
21	31.9, CH_2	2.27, 1.48, m	32.0, CH_2	2.28, 1.47, m	30.3, CH_2	2.25, 1.71, m	30.1, CH_2	2.20, 1.73, m	30.0, CH_2	2.21, 1.72, m
22	38.8, CH_2	1.73, 1.45, m	38.8, CH_2	1.73, 1.46, m	36.9, CH_2	1.57, 1.38, m	36.2, CH_2	1.53, 1.36, m	36.2, CH_2	1.52, 1.39, m
23	28.7, CH_3	1.40, s	28.8, CH_3	1.45, s	28.4, CH_3	1.39, s	28.5, CH_3	1.45, s	28.8, CH_3	1.46, s
24	17.1, CH_3	1.16, s	17.2, CH_3	1.22, s	17.2, CH_3	1.24, s	17.1, CH_2	1.23, s	17.3, CH_3	1.27, s
25	17.0, CH_3	1.22, s	17.1, CH_3	1.24, s	16.0, CH_3	0.83, s	17.2, CH_3	1.26, s	17.0, CH_3	1.20, s
26	19.1, CH_3	1.08, s	19.2, CH_3	1.08, s	17.3, CH_3	0.92, s	19.2, CH_3	1.08, s	19.2, CH_3	1.07, s
27	23.8, CH_3	1.44, s	23.9, CH_3	1.43, s	26.5, CH_3	1.25, s	23.8, CH_3	1.38, s	23.8, CH_3	1.37, s
28	29.0, CH_3	0.79, s	29.1, CH_3	0.80, s	28.7, CH_3	0.94, s	28.9, CH_3	0.87, s	28.9, CH_3	0.86, s
29	29.1, CH_3	1.37, s	29.0, CH_3	1.38, s	20.3, CH_3	1.49, s	20.1, CH_3	1.43, s	20.1, CH_3	1.42, s
30	179.5, C		179.5, C		181.6, C		181.1, C		181.1, C	
1′	105.7, CH	5.05, d (7.5)	105.6, CH	5.08, d (7.6)	105.5, CH	5.07, d (8.0)	105.6, CH	5.08, d (7.7)	105.4, CH	5.1, d (7.3)
2′	78.8, CH	4.57, m	79.6, CH	4.54, m	84.9, CH	4.29, m	84.3, CH	4.40, m	79.7, CH	4.55, t (8.3)
3′	79.2, CH	4.65, m	79.2, CH	4.66, m	78.2, CH	4.44, t (8.9)	77.7, CH	4.42, m	79.1, CH	4.67, m
4′	73.9, CH	4.47, pt (9.3)	73.9, CH	4.52, m	73.4, CH	4.58, m	73.6, CH	4.60, m	73.8, CH	4.47, t (9.0)
5′	77.8, CH	4.64, m	77.8, CH	4.64, m	77.8, CH	4.62, m	77.6, CH	4.65, m	77.7, CH	4.63, m
6′	172.9, C		173.1, C		172.5, C		172.8, C		173.0, C	
1″	102.4, CH	5.89, d (7.6)	103.1, CH	5.76, d (7.6)	107.3, CH	5.47, d (7.9)	107.0, CH	5.31, d (7.8)	103.1, CH	5.97, d (7.6)
2″	79.0, CH	4.34, m	77.1, CH	4.68, dd (7.5, 9.5)	77.2, CH	4.27, m	74.8, CH	4.68, dd (9.8, 7.8)	78.4, CH	4.47, pt (9.0)
3″	79.8, CH	4.27, t (9.0)	76.6, CH	4.21, dd (9.5, 3.5)	78.0, CH	4.32, m	74.8, CH	4.35, dd (9.8, 3.3)	79.3, CH	4.35, m
4″	73.1, CH	4.1, t (9.2)	70.9, CH	4.50, m	73.7, CH	4.64, m	72.0, CH	5.05, dd (3.3, 1.9)	74.0, CH	4.62, m
5″	78.2, CH	3.89, ddd (9.2, 5.9, 3.0)	76.7, CH	3.93, dd (1.6, 5.4)	78.9, CH	4.65, m	77.1, CH	4.81, d (1.9)	77.8, CH	4.47, m
6″	63.7, CH_2	4.54, m	62.3, CH_2	4.49, m	172.8, C		171.8, C		172.7, C	
		4.35, m		4.42, dd (11.0, 5.4)						
1‴	102.5, CH	6.43, d (1.5)	102.5, CH	6.35, d (2.3)					102.5, CH	6.48, d (1.5)
2‴	72.8, CH	4.79, dd (3.4, 1.6)	72.8, CH	4.8, dd (3.2, 2.2)					72.7, CH	4.80, d (2.0)
3‴	73.1, CH	4.73, dd (9.2, 3.4)	73.1, CH	4.75, dd (9.1, 3.2)					73.1, CH	4.73, dd (9.2, 3.5)

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Table 2. continued

	6		7		8		9		10	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
4‴	74.7, CH	4.37, m	74.8, CH	4.34, pt (9.5)					74.7, CH	4.37, m
5‴	69.8, CH	5.09, m	69.8, CH	5.07, m					70.0, CH	5.08, dd (9.1, 6.3)
6‴	19.4, CH_3	1.84, d (6.2)	19.4, CH_3	1.80, d (6.0)					19.4, CH_3	1.84, d (6.2)

methylene group in the aglycone moiety. The ^{1}H and ^{13}C NMR signal assignment, as well as analysis of the monosaccharide units after acidic hydrolysis, showed for compound **5** the same sugar pattern as for **4**, with two 1″ → 2′ linked β-D-glucuronic acids attached to an additional 1‴ → 2″ glycosylated α-L-rhamnose unit. The ^{13}C NMR spectrum of compound **5** showed in contrast to rhaoglycyrrhizin (**4**) no signal at δ_C 199.9 (C-11) (Table 1). Although **5** had no carbonyl group signal at C-11, it exhibited an additional methylene group with ^{1}H NMR chemical shifts of δ_H 1.81 (2H, m, H-11) and showed COSY and HMBC correlations between H-11 (δ_H 1.81)/H-9 (δ_H 1.64), H-11 (δ_H 1.81)/H-12 (δ_H 5.46), H-11 (δ_H 1.81)/C-9 (δ_C 48.2), H-11 (δ_H 1.81)/C-12 (δ_C 123.2), and H-11 (δ_H 1.81)/C-13 (δ_C 145.5). Moreover, upfield shifts for H-9 from δ_H 2.48 (1H, s, **4**) to δ_H 1.64 (1H, t, J = 8.9 Hz, **5**), for H-12 from δ_H 5.94 (1H, s, **4**) to δ_H 5.46 (1H, t, J = 4.0 Hz, **5**), and for H-18 from δ_H 2.53 (1H, dd, J = 16.9, 3.3 Hz, **4**) to δ_H 2.41 (1H, dt, J = 13.8, 4.3 Hz, **5**) (Figure 2) occurred. Thus, compound **5** was assigned as (3β,20β)-20-carboxy-30-norolean-12-en-3-yl 2-*O*-α-L-rhamnopyranosyl-2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid (11-deoxo-rhaoglycyrrhizin).

HRESIMS analysis of **6** revealed a molecular formula of $C_{48}H_{74}O_{19}$, based on the deprotonated molecule $[M - H]^-$ peak at m/z 953.4775, calcd for $C_{48}H_{73}O_{19}$ (m/z 953.4805), indicating the same molecular formula as saponin **5** or a molecular formula reduced by one oxygen and increased by two hydrogen atoms when compared to **4** ($C_{48}H_{72}O_{20}$). Compound **6** showed ESIMS/MS fragments at m/z 809, 647, and 453, representing neutral mass losses of 146, 162, and 194 Da, respectively, which were assigned to sugar fragmentations comprising a methyl-pentose, a hexose, and a uronic acid residue. Similar aglycone fragments of m/z 453 for **6** and glycyrrhizin (**11**) as well as similar ^{1}H and ^{13}C NMR resonances, e.g., for the junction positions at δ_H 3.35 (1H, dd, J = 12.0, 4.6 Hz, H-3), 2.51 (1H, s, H-9), and 2.54 (1H, dd, J = 13.3, 3.5 Hz, H-18), were measured, as occurred in the glycyrrhizin aglycone (**11**) [δ_H 3.37 (1H, dd, J = 12.0, 4.5 Hz, H-3), δ_H 2.46 (1H, s, H-9), and δ_H 2.54 (1H, dd, J = 13.1, 3.7 Hz, H-18)] (Table 2, Figure 2). Thus, the aglycone of **6** was identified as glycyrrhetinic acid. The determination of the carbohydrate moieties as a 1′ → 3 linked β-D-glucuronic acid, a 1″ → 2′ linked hexose, and a 1‴ → 2″ glycosylated α-L-rhamnose unit was carried out by the HMBC and COSY correlations of H-1′ (δ_H 5.05)/C-3 (δ_C 90.1), H-1′ (δ_H 5.05)/H-2′ (δ_H 4.57), H-2′ (δ_H 4.57)/C-1″ (δ_C 102.4), H-1″ (δ_H 5.89)/H-2″ (δ_H 4.34), and H-2″ (δ_H 4.34)/C-1‴ (δ_C 102.5). Further, the ^{1}H NMR resonances and coupling constants of δ_H 5.89 (1H, d, J = 7.6 Hz, H-1″), 4.27 (1H, t, J = 9.0 Hz, H-2″), 4.10 (1H, t, J = 9.2 Hz, H-3″), 3.89 (1H, ddd, J = 9.2, 5.9, 3.0 Hz, H-5″), 4.35 (1H, m, H-6″), and 4.54 (1H, m, H-6″), as well as determination of the monosaccharide moieties after acidic hydrolysis, substantiated the presence of a 1″ → 2′ linked β-D-glucose moiety (Figure 2).[48] Consequently, saponin **6** was assigned as (3β,20β)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-*O*-α-L-rhamnopyranosyl-2-*O*-β-D-glucopyranosyl-β-D-glucopyranosiduronic acid (rhaoglucoglycyrrhizin).

Compound **7** afforded a molecular formula of $C_{48}H_{74}O_{19}$ based on the $[M - H]^-$ peak at m/z 953.4781 in the HRESIMS, calcd for $C_{48}H_{73}O_{19}$ (m/z 953.4746), revealing the same empirical formula as **6**. Moreover, the ESIMS/MS of **7** showed fragments at m/z 647 and m/z 453 with similar neutral mass losses of 308 Da (146 + 162 Da) and 194 Da to saponin **6**. The ^{1}H and ^{13}C NMR resonances for the aglycone, uronic acid, and methyl pentose units could be assigned as almost identical, establishing glycyrrhetinic acid, glucuronic acid, rhamnose, and an unknown sugar as partial structures. Although saponins **6** and **7** showed similar NMR shifts and fragment patterns by ESIMS/MS analysis, the ^{1}H and J-resolved NMR spectra suggested somewhat different sugar patterns. The ^{1}H NMR resonances and coupling constants of the second carbohydrate moiety of compound **7** showed resonances and coupling constants of δ_H 5.76 (1H, d, J = 7.6 Hz, H-1″), δ_H 4.21 (1H, dd, J = 9.5, 3.5 Hz, H-3″), and δ_H 3.93 (1H, dd, J = 5.4, 1.6 Hz, H-5″) (Table 2). After the determination of monosaccharides after acidic hydrolysis and their observed NMR data, a β-D-galactose moiety was identified as a second carbohydrate moiety.[48] Thus, compound **7** was identified as (3β,20β)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-*O*-α-L-rhamnopyranosyl-2-*O*-β-D-galactopyranosyl-β-D-glucopyranosiduronic acid (rhaogalactoglycyrrhizin).

HRESIMS analysis of compound **8** revealed a molecular formula of $C_{42}H_{64}O_{15}$, based on the deprotonated molecular ion peak $[M - H]^-$ at m/z 807.4183, calcd for $C_{42}H_{63}O_{15}$ (m/z 807.4167), having the same empirical formula as compounds **1**, **12**, and **15**. ESIMS/MS revealed a fragment at m/z 439 with a neutral mass loss of 370 Da due to a possible elimination of two uronic acid substituents. The same fragment at m/z 439 was detected for compounds **1**, **5**, and **12** bearing a CH_2OH group at C-30 or a methylene group at C-11. Compound **8** showed similar ^{1}H NMR resonances at δ_H 1.83 (2H, dd, J = 8.9, 3.4 Hz, H-11) and δ_H 5.27 (1H, t, J = 3.7 Hz, H-12) to saponins **5** and **12** with δ_H 1.79−1.85 (2H, H-11) and δ_H 5.46 (1H, H-12) and a methylene group at C-11 (Tables 1, 2, and S2, Supporting Information, Figure 2). With COSY and HMBC correlations of H-11 (δ_H 1.83)/H-9 (δ_H 1.56), H-11 (δ_H 1.83)/H-12 (δ_H 5.27), H-11 (δ_H 1.83)/C-9 (δ_C 48.1), H-11 (δ_H 1.83)/C-12 (δ_C 123.1), and H-11 (δ_H 1.83)/C-13 (δ_C 145.0), this inference could be substantiated. Further structure elucidation exhibited for H-18 a ^{1}H NMR resonance at δ_H 2.16 (1H, dd, J = 13.0, 3.5 Hz), an upfield shift, in contrast to δ_H 2.41 (1H, dd, J = 13.7, 3.3, **5**) and 2.41 (1H, dd, J = 13.5, 4.5 Hz, **12**), respectively (Figure 2). Although similar COSY and HMBC correlations of compound **8** and glycyrrhizin (**11**) for the triterpenoid E-ring could be observed, ROE correlations for the proton pairs H-28 (δ_H 0.94)/H-18 (δ_H 2.16) and H-18 (δ_H 2.16)/H-29 (δ_H 1.49) were different. While compounds **1**−**7**, **11**−**18**, and **21**

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exhibited a β-orientation of the carboxylic group C-30, compound **8** shared like saponins **19** and **20** an α-orientation (Figure 3). Since two 1″ → 2′ linked β-D-glucuronic acids were shown to be present, saponin **8** was elucidated as (3β,20α)-20-carboxy-30-norolean-12-en-3-yl 2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid (11-deoxo-20α-glycyrrhizin).

The molecular formula of compound **9** was analyzed by HRESIMS for $C_{42}H_{62}O_{16}$, based on the $[M - H]^-$ peak at *m/z* 821.3965, calcd for $C_{42}H_{61}O_{16}$ (*m/z* 821.3960), identical to the molecular formula of glycyrrhizin (**11**). Moreover, neutral mass losses of 176 and 194 Da were observed in the ESIMS/MS of **9**, based on fragments of *m/z* 647 and 453, thus indicating the presence of two uronic acid moieties. Structure elucidation by 1D and 2D NMR experiments of **9** revealed similar ^{1}H NMR resonances for the triterpenoid E-ring at δ_H 2.25 (1H, dd, *J* = 14.0, 3.8 Hz, H-18), 2.49 (1H, t, *J* = 13.8 Hz, H-19), and 1.62 (1H, m, H-19) compared to saponins **8**, **19**, and **20** (Figure 2). Moreover, the ROE correlations of H-28 (δ_H 0.87)/H-18 (δ_H 2.24) and H-18 (δ_H 2.24)/H-29 (δ_H 1.43) in a similar manner to saponins **8**, **19**, and **20** supported an α-orientation of the carboxylic acid group at C-30. Although compound **9** showed in the ESIMS/MS a comparable fragmentation pattern and had the same COSY and HMBC correlations as found for glycyrrhizin (**11**), the proton resonances δ_H 4.68 (1H, dd, *J* = 9.8, 7.8 Hz, H-2″), 5.05 (1H, dd, *J* = 3.3, 1.9 Hz, H-4″), and 4.81 (1H, d, *J* = 1.9 Hz, H-5″) were more downfield shifted compared to **11**, at δ_H 4.28 (1H, dd, *J* = 9.5, 7.6 Hz, H-2″), 4.64 (1H, m, H-4″), and 4.65 (1H, m, H-5″) (Table 2). In particular, the coupling constants $^3J_{2'',3''}$ = 9.8 Hz, $^3J_{3'',4''}$ = 3.3 Hz, and $^3J_{4'',5''}$ = 1.9 Hz and the determination of monosaccharides after acidic hydrolysis led to identification of β-D-galacturonic acid as the terminal carbohydrate moiety of saponin **9**. Thus, saponin **9** was elucidated as (3β,20α)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-*O*-β-D-galactopyranuronosyl-β-D-glucopyranosiduronic acid (20α-galacturonoylglycyrrhizin).

HRESIMS analysis of compound **10** showed a deprotonated molecular ion peak $[M - H]^-$ at *m/z* 967.4586 with a molecular formula of $C_{48}H_{72}O_{20}$ (calcd for *m/z* 967.4539, $C_{48}H_{71}O_{20}$). The same fragmentation pattern in the ESIMS/MS with *m/z* 823, 647, and 453 indicated in a similar manner to rhaoglycyrrhizin (**4**) a methylpentose and two uronic acid moieties as the saccharide units. The ^{1}H NMR resonances of **10** at δ_H 2.23 (1H, m, H-18), 2.47 (1H, m, H-19), and 1.61 (1H, m, H-19) were supporting the configuration of the triterpenoid E-ring similar to saponins **8**, **19**, and **20** (Table 2, Figure 3). ROE cross signals of H-28 (δ_H 0.86)/H-18 (δ_H 2.23) and H-18 (δ_H 2.23)/H-29 (δ_H 1.42) revealed an α-orientation of the C-30 carboxylic acid group. COSY and HMBC NMR experiments demonstrated the following correlations: H-1′ (δ_H 5.10)/C-3 (δ_C 90.2), H-1′ (δ_H 5.10)/H-2′ (δ_H 4.55), H-2′ (δ_H 4.55)/C-1″ (δ_C 103.1), H-1″ (δ_H 5.97)/H-2″ (δ_H 4.47), and H-2″ (δ_H 4.47)/C-1‴ (δ_H 6.48). Altogether, after the determination of the absolute configuration of the monosaccharides after acidic hydrolysis, the presence of two 1″ → 2′ linked β-D-glucuronic acids attached to an additional 1‴ → 2″ glycosylated α-L-rhamnose unit could be established. Consequently, the structure of compound **10** was determined as (3β,20α)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid (20α-rhaoglycyrrhizin).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 polarimeter at 25 °C using a 600 μL cell with 1 dm path length. The ^{1}H, ^{13}C, COSY, HSQC, HMBC, *J*-RESOLVED, ROE-, and selective TOCSY-NMR spectra were acquired on a Bruker 500 MHz Avance III spectrometer (Bruker, Rheinstetten, Germany) using pyridine-d_5 as solvent. Chemical shifts are reported in parts per million, relative to the solvent signals. Liquid chromatography/mass spectrometry (ESIMS/MS) for MS/MS experiments was carried out by acquisition of mass and product ion spectra on an API 4000 QTrap mass spectrometer (Sciex, Darmstadt, Germany). Fragmentation of the molecular ions $[M + H]^+$ into specific product ions was induced by collision with nitrogen (4.5×10^{-5} Torr). For analyzing the monosaccharides after hydrolysis of the saponins a Nexera X2 UHPLC system (Shimadzu Europe GmbH, Duisburg, Germany) connected to a QTRAP 5500 mass spectrometer (Sciex) was used. UPLC/time-of-flight mass spectrometry (HRESIMS) was achieved by an Acquity UPLC core system (Waters UK Ltd., Manchester, UK) connected to a SYNAPT G2 HDMS spectrometer (Waters) operating in the negative electrospray (ESI^-) mode. Medium-pressure liquid chromatography of the MeOH−H_2O extract prepared from licorice roots was performed on a Sepacore chromatography system (Büchi, Flawil, Switzerland). All preparative and semipreparative separations of fractions II/III and IV and all subsequent subfractions were conducted on an LC-2000Plus system (JASCO, Groß-Umstadt, Germany).

The following compounds were purchased from the sources given in parentheses: glycyrrhizin ammonical (Extrasynthése, Genay, France) and formic acid (Merck, Darmstadt, Germany). Deuterated solvents for NMR spectroscopy were obtained from Euriso-Top (Gif-Sur-Yvette, France), and solvents for HPLC separation (CH_3CN and MeOH) were of HPLC grade (Mallinckrodt Baker, Griesheim, Germany), with the solvents used for HRESIMS and ESIMS/MS analysis being of LC-MS grade (Honeywell, Seelze, Germany). Ultrapure water for HPLC separation and mass spectrometry was purified by means of a Milli-Q Water Advantage A 10 water system (Millipore, Molsheim, France).

Plant Material. Dried and peeled *G. glabra* roots (Radix Liquiritae mundat conc EU. AB. 7.3) were obtained from Heinrich Klenk GmbH, Schwebheim, Germany. A sample (6381-A-140109) was deposited at the authors' laboratory at the Technical University Munich, Freising, Germany.

Extraction and Isolation. Peeled *G. glabra* roots (1.5 kg) were ground in a laboratory blender (Grindomix GM300, Retsch, Haan, Germany) at 4000 U/min for 120 s. Extraction of the ground roots was achieved six times with 15 L of MeOH and eight times with 15 L of MeOH−H_2O (7:3 v/v) with ultrasonification, followed by filtration (Schleicher and Schuell filter, 24 cm). The combined filtrates were collected, freed from solvent in a vacuum at 38 °C, and freeze-dried (yield: 470 g, 31%). For MPLC experiments, the MeOH−H_2O extract was dissolved in H_2O (336 g/840 mL). An aliquot (20 mL) of this solution was injected onto a self-packed MPLC polypropylene cartridge (150 × 40 mm i.d., Büchi) filled with LiChroprep RP-18 25−40 μm bulk material (Merck, Darmstadt, Germany). Chromatography was performed using H_2O as solvent A and MeOH as solvent B as well as the following gradient at a flow rate of 40 mL/min: 0 min, 0% B; 13.5 min, 0% B; 43.5 min, 50% B; 58.5 min, 50% B; 58.6 min, 60% B; 73.5 min, 60% B; 73.6 min, 70% B; 88.5 min, 70% B; 88.6 min, 80% B; 103.5 min, 80% B; 103.6 min, 100% B; 114 min, 100% B. Before each separation run, a conditioning step was conducted with 0 min, 100% B; 5 min, 100% B; 10 min, 0% B; 20 min, 0% B. Then, four fractions, I (42.5−58.5 min), II (58.5−73.5 min), III (73.5−88.5 min), and IV (88.5−103.5 min), were collected by monitoring the effluent using UV detection at 250 nm. Each fraction was freed from solvent under a vacuum and lyophilized. Combined fractions II and III were dissolved in CH_3CN−H_2O (3:7, 5.2 g/45 mL). HPLC separations were performed using a Nucleodur C18 Pyramid column (250 × 21 mm i.d., 5 μm, Macherey-Nagel, Düren, Germany) and 0.1% formic acid in H_2O (A v/v) and CH_3CN (B) as solvents.

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Separation was performed at a flow rate of 24 mL/min, starting with 0 min, 30% B, followed by the following steps: 3 min, 30% B; 5 min, 35% B; 45 min, 35% B; 50 min, 100% B; 55 min, 100% B; 58 min, 30% B; 65 min, 30% B. By monitoring the effluent at 250 nm, the extract was separated into fractions II/III-1 to II/III-24 to yield **11** (1.88 g, t_R 27.3 min, II/III-19) and **19** (178 mg, t_R 35.5 min, II/III-22).

Subfractionation of fractions II/III-15 (57 mg), II/III-16 (29 mg), II/III-17 (95 mg), II/III-18 (50 mg), II/III-20 (36 mg), II/III-21 (56 mg), and II/III-23 (64 mg) was carried out using semipreparative HPLC analysis with 0.1% formic acid (v/v) as solvent A and CH_3CN as solvent B. Fractions II/III-20, II/III-21, and II/III-23 were dissolved in CH_3CN-H_2O (3:7, 10 mL) individually and analyzed by means of a Synergi Hydro-RP 80A column (250 × 10 mm i.d., 4 μm, Phenomenex, Aschaffenburg, Germany). For HPLC analysis of fractions II/III-15, II/III-16, II/III-17, and II/III-18 a Nucleodur C_{18} Pyramid column (250 × 10 mm i.d., 5 μm, Macherey Nagel) was used. For chromatography of fractions II/III-15, II/III-16, and II/III-17 the following gradient was used: 0 min, 30% B; 15 min, 30% B; 40 min, 35% B; 45 min, 100% B. Semipreparative analysis of fraction II/III-18 was effected by the gradient 0 min, 30% B; 6 min, 30% B; 40 min, 38% B; 45 min, 100% B. In turn, fractionation of II/III-20 was conducted with the gradient 0 min, 30% B; 3 min, 30% B; 5 min, 35% B; 50 min, 50% B; 52 min, 100% B; 57 min, 100% B; 60 min, 30% B; 65 min, 30% B. For further purification of fractions II/III-21 and II/III-23 the following gradient was used: 0 min, 30% B; 3 min, 30% B; 5 min, 35%B; 50 min, 75% B; 52 min, 100% B; 57 min, 100% B; 60 min, 30% B; 65 min, 30% B. The effluent of each fractionation was monitored by UV detection at a wavelength of 250 nm. The following subfractions were collected: II/III-15-1 to II/III-15-10; II/III-16-1 to II/III-16-9; II/III-17-1 to II/III-17-8; II/III-18-1 to II/III-18-5; II/III-20-1 to II/III-20-8; II/III-21-1 to II/III-21-9; II/III-23-1 and II/III-23-2. From fractions II/III-15-6, II/III-21-9, and II/III-23-2 compounds **13** (36 mg, t_R = 27.1 min), **2** (2.2 mg, t_R = 25.7 min), and **9** (26 mg, t_R = 19.1 min) were obtained.

Further purification of fractions II/III-15-7 (9.2 mg), II/III-16-8 (23 mg), II/III-17-4 (53 mg), II/III-17-6 (22 mg), II/III-18-2 (22 mg), II/III-20-4 (28 mg), II/III-21-3 (7.2 mg), and II/III-21-4 (16.6 mg) was carried out by additional semipreparative HPLC steps. After dissolving the fractions in CH_3CN-H_2O (3:7 v/v) (2−6 mL), separately, fractionation was carried out using a Nucleodur C_{18} Pyramid column (250 × 10 mm i.d., 5 μm, Macherey Nagel), with a flow rate of 4.8 mL/min and solvents consisting of 0.1% formic acid in H_2O (v/v) as solvent A and CH_3CN as solvent B. All preparative HPLC analysis was carried out by UV detection at a wavelength of 250 nm. Separation of II/III-15-7 was conducted with the following solvent gradient: 0 min, 30% B; 3 min, 30% B; 5 min, 32% B; 40 min, 32% B. Accordingly, two subfractions, II/III-15-7-1 and II/III-15-7-2, were collected, in which **3** (4.3 mg, t_R 30.3 min) was afforded in II/III-15-7-1. Further separation of II/III-16-8 using the solvent gradient 0 min, 30% B; 3 min, 30% B; 5 min, 35% B; 50 min, 65% B; 52 min, 100% B; 57 min, 100% B; 60 min, 30% B; and 65 min, 30% B was carried out, yielding in subfractions II/III-16-8-1 and II/III-16-8-2 saponin **7** (3.7 mg, t_R 15.2 min) in fraction II/III-16-8-1. Chromatography of fraction II/III-17-4 and II/III-17-6 was achieved by applying the following gradient: 0 min, 30% B; 3 min, 30% B; 5 min, 35% B; 50 min, 52%, followed by a conditioning step with 30% B for 5 min, for II/III-17-4 and 0 min, 30% B; 3 min, 30% B; 23 min, 75% B, followed by a conditioning step with 30% B for 5 min for II/III-17-6, obtaining fractions II/III-17-4-1, II/III-17-4-2, II/III-17-6-1, and II/III-17-6-2 with **4** (32 mg, t_R 18.0 min) in II/III-17-4-2 and **6** (10.6 mg, t_R = 14.2 min) in II/III-17-6-2. Purification of fractions II/III-18-2 (22 mg) and II/III-20-4 (28 mg) was conducted by means of the gradient 0 min, 30% B; 3 min, 30% B; 5 min, 35% B; 35 min, 75% B followed by a conditioning step of 30% B for 5 min. Separation of fractions II/III-18-2-1, II/III-18-2-2, II/III-20-4-1, and II/III-20-4-2 led to compounds **20** (10.6 mg, t_R 15.8 min, II/III-18-2-2) and **15** (13 mg, t_R 15.5 min, II/III-20-4-1). For separation of fraction II/III-21-3 the following gradient was used: 0 min, 30% B; 3 min, 30% B; 5 min, 35% B; 35 min, 75% B, followed by a conditioning step of 30% B for 5 min to yield fractions II/III-21-3-1 to II/III-21-3-3, from which compound **10** (II/III-21-3-3, 3.8 mg, t_R 16.1 min) was obtained. Fractionation of II/III-21-4 was conducted with a gradient using 0 min, 30% B; 3 min, 30% B; 5 min, 35% B; 40 min, 38% B followed by a conditioning step of 30% B for 5 min to obtain fractions II/III-21-4-1 and II/III-21-4-2, from which saponin **1** (II/III-21-4-2, 10.1 mg, t_R 28.4 min) occurred.

A solution of fraction IV in CH_3CN-H_2O (3:7 v/v, 1.4 g/18 mL) was separated by means of preparative HPLC using a Nucleodur C_{18} Pyramid column (250 × 21 mm i.d., 5 μm, Macherey Nagel) and the following gradient: 0 min, 30% B; 30 min, 70% B; 33 min, 100% B; 38 min, 100% B, 40 min, 30% B; 45 min, 30% B. For this, solvent A was 0.1% formic acid in H_2O (v/v) and solvent B CH_3CN (flow rate: 21 mL/min). The effluent was detected using a DAD detector at 200/250 nm and separated into 18 subfractions (IV-1 to IV-18). After evaporation of the solvent under a vacuum and freeze-drying, compound **12** (78 mg, t_R 17.7 min) was obtained from fraction IV-11.

For further purification of compounds **14**, **16**, **17**, **5**, **18**, **8**, and **21** fractions IV-6 (23 mg), IV-7 (24 mg), IV-9 (79 mg), IV-14 (41 mg), IV-15 (25 mg), and IV-16 (21 mg) were separated by means of semipreparative HPLC. For the chromatography of fractions IV-6, IV-7, IV-9, IV-11, and IV-14 a Nucleodur C_{18} Pyramid column (250 × 21 mm i.d., 5 μm, Macherey Nagel) was used. Fractions IV-15 and IV-16 were fractionated using a Nucleodur C_{18} Pyramid column (250 × 10 mm i.d., 5 μm, Macherey Nagel). Both columns were run using 0.1% formic acid in H_2O (v/v) as solvent A and CH_3CN as solvent B. Chromatography of fractions IV-6, IV-7, and IV-9 was performed using a DAD detector at 200 and 250 nm and the following gradient operated at 21 mL/min: 0 min, 35% B; 3 min, 35% B; 5 min, 40% B; 30 min, 55% B; 33 min, 100% B; 38 min, 100% B; 40 min, 35% B; 45 min, 35% B, yielding subfractions IV-6-1, IV-6-2, IV-7-1, IV-7-2, IV-9-1, IV-9-2, and IV-9-3 with saponins **14** (8.6 mg, t_R 28.5 min) in fraction IV-6-2, **16** (7.2 mg, t_R 33.9 min) in IV-7-2, **17** (5.4 mg, t_R 39.4 min) in IV-7-3, and **21** (3.6 mg, t_R 35.3 min) in IV-9-1. Purification of fraction IV-14 was carried out by means of preparative HPLC (21 mL/min) using the following conditions: 0 min, 30% B; 3 min, 30% B; 18 min, 100% B; 22 min, 100% B; 25 min, 30% B; 27 min, 30% B. The effluent was monitored using a UV detector (200 nm), and subfractions IV-14-1 and IV-14-2 with compound **5** (6.1 mg, t_R 15.8 min) in fraction IV-14-1 were obtained. Separation of fractions IV-15 and IV-16 was accomplished by semipreparative HPLC at a flow rate of 4.8 mL/min and applying the gradient 0 min, 30% B; 3 min, 30% B; 22 min, 100% B; 25 min, 100% B; 28 min; 30% B; 30 min, 30% B. Collection of subfractions IV-15 and IV-16 was performed by monitoring the effluent using a UV detector (250 nm) and resulted in the following subfractions: IV-15-1, IV-15-2, and IV-16-1 to IV-16-3, leading to saponins **18** (9.7 mg, t_R 15.4 min) in fraction IV-15-1 and **8** (12 mg, t_R 16.8 min) in fraction IV-16-3.

*30-Hydroxyglycyrrhizin (**1**):* white powder; $[\alpha]^{25}_D$ +17 (*c* 0.05, MeOH); ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 809 $[M + H]^+$; ESIMS/MS (DP 20 V; CE 8 V) *m/z* 809 (8), 647 (3), 633 (6), 457 (4), 453 (2), 439 (100); HRESIMS *m/z* 807.4166 $[M - H]^-$ (calcd for $C_{42}H_{63}O_{15}$, 807.4167.

*Glycyrrhizin-20-methanoate (**2**):* white powder; $[\alpha]^{25}_D$ +22 (*c* 0.08, MeOH); ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 837 $[M + H]^+$; ESIMS/MS (DP 20 V; CE 11 V) *m/z* 837 (67), 809 (30), 661 (35), 457 (100), 442 (100); HRESIMS *m/z* 807.4195 $[M - COHH]^-$ (calcd for $C_{42}H_{63}O_{15}$, 807.4167).

*24-Hydroxy-glucoglycyrrhizin (**3**):* white powder; $[\alpha]^{25}_D$ +21 (*c* 0.05, MeOH); ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 825 $[M + H]^+$; ESIMS/MS (DP 40 V; CE 20 V) *m/z* 825 (14), 664 (4), 663 (5), 649 (3), 645 (15), 627 (52), 487 (62), 469 (100), 451 (20); HRESIMS *m/z* 823.4122 $[M - H]^-$ (calcd for $C_{42}H_{63}O_{16}$, 823.4116).

*Rhaoglycyrrhizin (**4**):* white powder; $[\alpha]^{25}_D$ −9 (*c* 0.18, MeOH); ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 969 $[M + H]^+$; ESIMS/MS (DP 40 V; CE 16 V) *m/z* 969 (5), 823 (6), 647 (35), 671 (25), 455 (55), 453 (100), 319 (14), 275 (10), 242 (9), 141 (18), 130 (15); HRESIMS *m/z* 967.4570 $[M - H]^-$ (calcd for $C_{48}H_{71}O_{20}$, 967.4539).

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*11-Deoxo-rhaoglycyrrhizin (**5**):* white powder; $[\alpha]^{25}_{D}$ −23 (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 977 [M + Na]$^{+}$; ESIMS/MS (DP 20 V, CE 50 V) *m/z* 977 (100), 655 (33), 521 (47), 439 (10), 345 (12), 261 (5), 191 (6); HRESIMS *m/z* 953.4777 [M − H]$^{-}$ (calcd for $C_{48}H_{73}O_{19}$, 953.4746).

*Rhaoglucoglycyrrhizin (**6**):* white powder; $[\alpha]^{25}_{D}$ +35 (*c* 0.16, MeOH); ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 955 [M + H]$^{+}$; ESIMS/MS (DP 20 V; CE 10 V) *m/z* 955 (7), 809 (5), 647 (41), 471 (27), 453 (100), 294 (4), 256 (6); HRESIMS *m/z* 953.4775 [M − H]$^{-}$ (calcd for $C_{48}H_{73}O_{19}$, 953.4805).

*Rhaogalactoglycyrrhizin (**7**):* white powder; $[\alpha]^{25}_{D}$ −18 (*c* 0.2, MeOH); ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 955 [M + H]$^{+}$; ESIMS/MS (DP 40 V; CE 21 V) *m/z* 955 (1), 647 (20), 629 (3), 471 (40), 453 (100); HRESIMS *m/z* 953.4781 [M − H]$^{-}$ (calcd for $C_{48}H_{73}O_{19}$, 953.4746).

*11-Deoxo-20α-glycyrrhizin (**8**):* white powder; $[\alpha]^{25}_{D}$ −4 (*c* 0.14, MeOH); ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 809 [M + H]$^{+}$; ESIMS/MS (DP 1 V, CE 110 V) *m/z* 809 (70), 439 (100), 205 (2), 203 (28), 191 (20), 189 (4), 149 (2) 147 (5), 141 (10), 95 (10), 93 (2); HRESIMS *m/z* 807.4183 [M − H]$^{-}$ (calcd for $C_{42}H_{63}O_{15}$, 807.4167).

*20α-Galacturonoylglycyrrhizin (**9**):* white powder; $[\alpha]^{25}_{D}$ +24 (*c* 0.01, MeOH); ^{1}H, and ^{13}C NMR data, see Table 2; ESIMS *m/z* 823 [M + H]$^{+}$ ESIMS/MS (DP 20 V; CE 7 V) *m/z* 823 (2), 805 (1), 647 (2), 471 (4), 453 (100); HRESIMS *m/z* 821.3965 [M − H]$^{-}$ (calcd for $C_{42}H_{61}O_{16}$, 821.3960).

*20α-Rhaoglycyrrhizin (**10**):* white powder; $[\alpha]^{25}_{D}$ +41 (*c* 0.13, MeOH); ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 969 [M + H]$^{+}$; ESIMS/MS (DP 20 V; CE 9 V) *m/z* 969 (1), 823 (3), 661 (2), 647 (11), 630 (1), 471 (11), 453 (100); HRESIMS *m/z* 967.4586 [M − H]$^{-}$ (calcd for $C_{48}H_{71}O_{20}$, 967.4539).

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharides. According to a literature protocol,[50] the absolute configuration of the monosaccharides was determined after acidic hydrolysis. A solution of each saponin (**1**−**10**, 50 μL of a 1 mM solution in CH_3CN−H_2O, 3:7) in HCl (2 M) was heated for 1 h at 100 °C. Afterward, the mixture was evaporated to dryness under reduced pressure. The residue was suspended in H_2O (750 μL) and extracted with EtOAc (2 × 750 μL). Next, the H_2O layer was evaporated to dryness to obtain the monosaccharide residue. Each residue was dissolved in a solution of L-cysteine methyl ester hydrochloride in anhydrous pyridine (2 mg/mL, Sigma-Aldrich, Steinheim, Germany). The mixture was shaken for 1 h at 60 °C with 1400 rpm (Thermo-Shaker PHMT-PSC24N, Grant Bio, Cambridge, UK). Then, to this solution was added phenylethyl isothiocyanate (5 μL, Sigma-Aldrich, Steinheim, Germany), and the resulting solution was shaken for 1 h at 60 °C. The mixture was dried under a stream of nitrogen and resolved in CH_3CN−H_2O (500 μL, 1/1 v/v), and each solution was analyzed by means of UHPLC-MS/MS using a Phenomenex Kinetex F5 column (100 × 2.1 mm i.d., 100 Å, 1.7 μm). Chromatography was performed with an injection volume of 0.5 μL and a flow rate of 0.4 mL/min. The mobile phase consisted of (A) formic acid (1% in H_2O) and (B) CH_3CN (1% formic acid). The following gradient was used: 0 min, 5% B; 3 min, 5% B; 5 min, 20% B; 25 min, 25% B; 27 min, 100% B; 30 min, 100% B; 31 min, 5% B; 35 min, 5% B. After optimizing instrument settings with authentic samples (Sigma-Aldrich, Steinheim, Germany) treated the same way as described above, the derivatized monosaccharides were analyzed using the MRM transitions Q1/Q3 of *m/z* 461.0/298.1 (DP = 86 V, CE = 17 V, CXP = 6 V) for D-glucose, Q1/Q3 of *m/z* 461.0/298.1 (DP = 71 V, CE = 17 V, CXP = 6 V) for L-glucose, Q1/Q3 of *m/z* 461.1/298.2 (DP = 71 V, CE = 17 V, CXP = 6 V) for D-galactose, Q1/Q3 of *m/z* 475.0/312.1 (DP = 91 V, CE = 19 V, CXP = 6 V) for D-galacturonic acid, Q1/Q3 of *m/z* 475.0/312.1 (DP = 61 V, CE = 19 V, CXP = 8 V) for D-glucuronic acid, and Q1/Q3 of *m/z* 445.0/282.1 (DP = 61 V, CE = 17 V, CXP = 6 V) for L-rhamnose. The following retention times were observed for the corresponding derivatives: D-glucose 13.28 min, L-glucose 13.04 min, D-galactose 12.38 min, D-galacturonic acid 13.70 min, D-glucuronic acid 13.47 min, and L-rhamnose 18.53 min.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00022.

MPLC, HPLC, LC-MS parameters; NMR, MS spectroscopic data for new and known compounds **1**−**21**; determination of absolute configurations of monosaccharides after acidic hydrolysis (PDF)

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Notes

The authors declare no competing financial interest.

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