Triterpenoid Glycosides from the Leaves of *Ilex pernyi*

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Ten triterpenoid glycosides, including five new ones (1—5), were isolated from the leaves of *Ilex pernyi*. The chemical structures of 1—5 were determined on the basis of the chemical and spectroscopic evidence.

Key words *Ilex pernyi*; Aquifoliaceae; triterpenoid glycoside

Plants of the genus *Ilex* belong to the medicinally important Aquifoliaceae family. More than 10 *Ilex* species are wildly used as traditional Chinese medicine (TCM) in China, which have a broad spectrum of biological properties ranging from reducing fever, detoxification, relieving cough to expectorant and angiocardiopathy.[1)] In continuation of our systematic research on the chemical constituents of the plants of genus *Ilex*,[2—4)] we investigated the leaves of *Ilex pernyi* Franch,[5—7)] an evergreen shrubs distributed mainly in the southern region of the People's Republic of China.[8)] In this paper, we report the isolation and identification of five new triterpenoid glycosides, 3β,21α,23-trihydroxy-urs-12-en-28-oic acid 21-*O*-β-D-glucopyranoside (**1**), 28-*O*-β-D-glucopyranosyl-3β,23-dihydroxy-urs-12-en-28-oic acid 3-*O*-β-D-glucuronopyranoside 6′-*O*-butyl ester (**2**), 3β,19α,23-trihydroxy-olean-12-en-28-oic acid 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**3**), 3-*O*-β-D-glucopyranosyl-3β,21α,23-trihydroxy-urs-12-en-28-oic acid 21-*O*-β-D-glucopyranoside (**4**), and 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-3β,19α,23-trihydroxy-urs-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**5**), together with five known triterpenoid glycosides, ilexoside XXX (**6**), 3-*O*-(methyl-β-D-glucuronopyranosiduronoate)-28-*O*-β-D-glucopyranosyl oleanolate (**7**), collinsonidin (**8**), quinoa-saponin-9 (**9**), and 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**10**) from this plant.

Results and Discussion

Compound **1** was obtained as colorless gum, with positive results for both Libermann–Burchard and Molish test. The HR-ESI-MS (positive mode) spectrum exhibited a quasimolecular ion peak $[M+Na]^+$ at *m*/*z* 673.3925, corresponding to the molecular formula $C_{36}H_{58}O_{10}$, in accordance with eight degrees of unsaturation. The ^{1}H-NMR spectrum of aglycon for **1** revealed four singlet methyl groups [δ_H 0.94 (3H, s), 1.02 (3H, s), 1.05 (3H, s), 1.05 (3H, s)], two doublet methyl groups [δ_H 0.96 (3H, d, J=7.0 Hz), 1.20 (3H, d, J=7.0 Hz), an olefinic proton [δ_H 5.51 (1H, br s)], an oxygenated CH_2 group [δ_H 3.65, 4.12 (2H, AB, J=10.5 Hz)], and two oxygenated CH groups [δ_H 4.14 (1H, dd, J=10.5, 5.0 Hz), 4.23 (1H, overlap)]. Moreover, the ^{13}C-NMR spectrum of aglycon indicated characteristic signals of one carboxyl group [δ_C 179.7 (s)], three oxygenated C-atoms [δ_C 76.6 (d), 73.6 (d) and 68.1 (t)], and two olefinic C-atoms [δ_C 139.2 (s), 125.5 (d)]. The above data revealed an urs-12-ene aglycon with three hydroxyl functions. In the ^{13}C-NMR spectrum of the aglycon for **1**, the carbon signals due to the A and B rings were in close resemblance to those of 3β,23-dihydroxy-urs-12-en-28-oic acid and those due to the C, D and E rings were almost superimposable with those of latifoloside J.[9,10)] The results above, together with the observed ROESY (rotating frame Overhauser effect spectroscopy) correlations between H-3/H-5, H-21/H-30, H-21/H-22α and H-21/H-22β revealed the structure and relative configuration for the aglycon of **1**, which was elucidated as 3β,21α,23-trihydroxy-urs-12-en-28-oic acid. The NMR spectra for the sugar unit indicated it to be a D-glucose, and acid hydrolysis together with GC analysis confirmed this suggestion. Meanwhile, the anomeric configuration of D-glucose was deduced to be β from the coupling constant value observed for the H-1 of Glc (J=8.0 Hz). The location of the hydroxyl group at C-21 and the site of glycosylation were confirmed by heteronuclear multiple

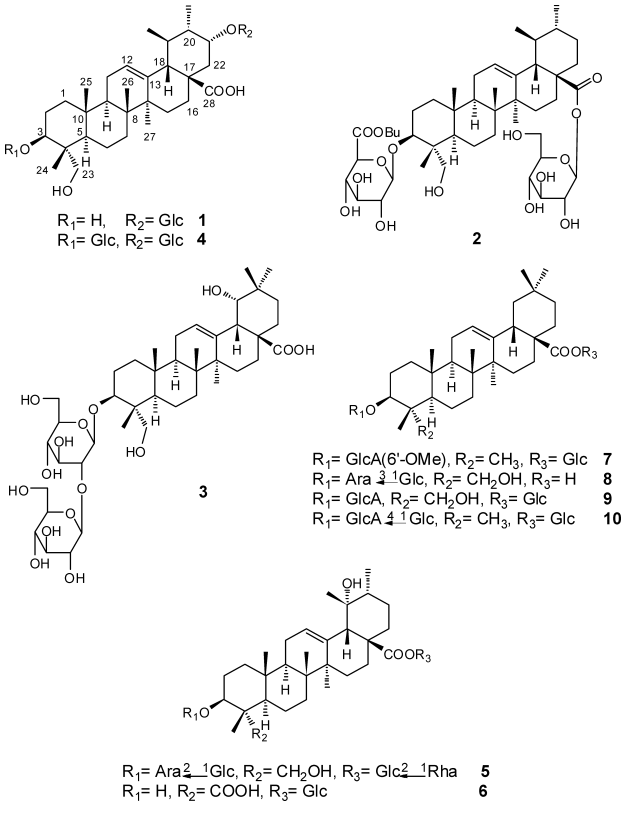

Fig. 1. Chemical Structures of Compounds **1**—**10**

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bond correlation (HMBC) experiments showing correlations between H-21 (δ_H 4.23, overlap) and both C-17 (δ_C 48.2) and C-19 (δ_C 33.2), H-1 (δ_H 4.97, d, J=8.0 Hz) of Glc and C-21 (δ_C 76.6). From the above evidence, the structure of compound **1** was established as 3β,21α,23-trihydroxy-urs-12-en-28-oic acid 21-*O*-β-D-glucopyranoside.

Compound **2**, colorless gum, was deduced the molecular formula $C_{46}H_{74}O_{15}$ (HR-ESI-MS m/z 889.4906 $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectra of **2** were almost superimposable with those of cynarasaponin E except that the former showed additional *O*-*n*-butyl signals [δ_H 0.71 (3H, t, J=7.3 Hz); δ_C 64.9 (t), 30.8 (t), 19.2 (t) and 13.7 (q)],[11] suggesting that **2** is a *n*-butyl ester derivative of the latter compound. Location of the *O*-*n*-butyl group and the site of glycosylation were also confirmed by HMBC experiments showing long-range correlations between H-1 (δ_H 4.22, overlap) of the *n*-butyl and C-6 (δ_C 170.3) of D-glucuronyl moiety, H-1 (δ_H 5.21, d, J=7.7 Hz) of the D-glucuronyl moiety and C-3 (δ_C 82.2) of aglycon, H-1 (δ_H 6.25, d, J=8.1 Hz) of the D-glucose and C-28 (δ_C 176.1) of aglycon. Therefore, the structure of **2** was determined as 28-*O*-β-D-glucopyranosyl-3β,23-dihydroxy-urs-12-en-28-oic acid 3-*O*-β-D-glucuronopyranoside 6′-*O*-butyl ester.

Compound **3** was isolated as colorless gum. HR-ESI-MS analysis of **3** showed the $[M+H]^+$ signal at m/z 813.4630, consistent with the molecular formula $C_{42}H_{68}O_{15}$. The ^{1}H-NMR spectrum of aglycon for **3** showed six singlet methyl groups [δ_H 0.89 (3H, s), 1.02 (3H, s), 1.06 (3H, s), 1.07 (3H, s), 1.16 (3H, s) and 1.57 (3H, s)], an olefinic proton [δ_H 5.51 (1H, br s)], an oxygenated CH_2 group [δ_H 3.72, 4.33 (2H, AB, J=11.0 Hz)], and two oxygenated CH groups [δ_H 3.56 (1H, br s), 4.11 (1H, overlap)]. Meanwhile, the ^{13}C-NMR spectrum of aglycon indicated characteristic signals of one carboxyl group [δ_C 180.8 (s)], three oxygenated C-atoms [δ_C 82.6 (d), 81.1 (d) and 65.0 (t)]. Furthermore, a quaternary olefinic C-atom [δ_C 144.8 (s)] was observed. The data above indicated an olean-12-ene aglycon with three hydroxyl functions, as well as one carboxyl group at C-17, corresponding to 19α,23-dihydroxy-oleanolic acid, the same aglycon as randiasaponin VII.[12] A comparison of the ^{13}C-NMR spectrum of **3** with that of hederagenin 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside showed that the sugar moi-

Table 1. ^{1}H- and ^{13}C-NMR Data of the Aglycon Moieties of **1**—**3** (Pyridine-d_5)

Position	**1**		**2**		**3**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	0.99 (overlap)	38.8	0.86 (overlap)	38.8	0.84 (overlap)	38.4
	1.56 (overlap)		0.93 (overlap)		1.44 (overlap)	
2	1.87 (overlap)	27.6	1.99 (overlap)	26.1	1.90 (overlap)	25.8
	2.20 (overlap)		2.23 (overlap)		2.22 (overlap)	
3	4.14 (dd, 10.5, 5.0)	73.6	4.29 (overlap)	82.2	4.11 (overlap)	82.6
4		42.7		43.4		43.3
5	1.44 (overlap)	48.7	1.61 (overlap)	47.4	1.54 (overlap)	48.2
6	1.58 (overlap)	18.5	1.60 (overlap)	18.1	1.34 (overlap)	18.3
					1.69 (overlap)	
7	1.27 (overlap)	33.2	1.29 (overlap)	33.1	1.22 (overlap)	32.9
	1.60 (overlap)		1.63 (overlap)		1.58 (overlap)	
8		39.7		40.1		39.9
9	1.67 (t, 9.0)	48.0	1.69 (dd, 13.2, 3.7)	48.3	1.85 (overlap)	48.1
10		37.1		36.7		36.9
11	1.95 (overlap)	23.6	1.86 (overlap)	23.7	1.97 (overlap)	24.0
			1.97 (overlap)			
12	5.51 (br s)	125.5	5.42 (br s)	126.0	5.51 (br s)	123.2
13		139.2		138.3		144.8
14		42.6		42.4		42.0
15	1.11 (overlap)	29.0	1.06 (br d, 13.5)	28.6	1.20 (overlap)	29.0
	2.19 (overlap)		2.40 (dt, 13.5, 4.0)		2.28 (overlap)	
16	2.51 (br d, 14.0)	27.4	1.92 (overlap)	24.6	2.03 (overlap)	28.2
	2.95 (dt, 14.0, 4.0)		2.02 (overlap)		2.78 (dt, 14.0, 5.0)	
17		48.2		48.0		45.9
18	2.70 (d, 12.0)	54.1	2.48 (d, 11.2)	53.2	3.57 (br s)	44.6
19	2.13 (overlap)	33.2	1.37 (overlap)	39.3	3.56 (br s)	81.1
20	1.32 (overlap)	42.7	1.49 (overlap)	39.1		35.6
21	4.23 (overlap)	76.6	1.23 (overlap)	30.8	1.26 (overlap)	29.9
			1.32 (overlap)			
22	1.90 (overlap)	38.8	1.65 (overlap)	36.7	1.97 (overlap)	33.5
	2.70 (br d, 12.0)		1.90 (overlap)		2.16 (overlap)	
23	3.65, 4.12	68.1	3.68, 4.32	64.2	3.72, 4.33	65.0
	(AB, 10.5)		(AB, 10.5)		(AB, 11.0)	
24	1.02 (s)	13.0	0.91 (s)	13.6	1.06 (s)	13.2
25	0.94 (s)	16.1	0.85 (s)	21.2	0.89 (s)	15.8
26	1.05 (s)	17.5	1.13 (s)	17.6	1.02 (s)	17.4
27	1.05 (s)	23.1	1.11 (s)	23.6	1.57 (s)	24.7
28		179.7		176.1		180.8
29	0.96 (d, 7.0)	17.3	0.90 (d, 5.0)	17.3	1.16 (s)	28.7
30	1.20 (d, 7.0)	17.7	0.90 (d, 5.0)	16.3	1.07 (s)	24.7

eties were identical in these two compounds.[13] All sites of glycosylation were comfirmed by HMBC experiments showing long-range correlations between H-1 (δ_H 5.06, d, J=6.0 Hz) of the inner D-glucose and C-3 (δ_C 82.6) of aglycon, H-1 (δ_H 5.36, d, J=7.5 Hz) of the terminal D-glucose and C-2 (δ_C 83.9) of inner D-glucose. Therefore, the structure of **3** was established as 3β,19α,23-trihydroxy-olean-12-en-28-oic acid 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside.

Compound **4** was obtained as colorless gum. The molecular formula was deduced from the peak at *m/z* 835.4455 $[M+Na]^+$ in the HR-ESI-MS as $C_{42}H_{68}O_{15}$. Analyses of the ^{1}H- and ^{13}C-NMR spectroscopic data indicated that **4** were superimposable with those of **1** except for an additional D-glucose. The long range correlation between the H-1 (δ_H 5.12, d, J=8.0 Hz) of the additional glucose and C-3 (δ_C 82.0) of the aglycon in HMBC indicated that the additional D-glucose was attached to C-3 of the aglycon. Hence, compound **4** was elucidated as 3-*O*-β-D-glucopyranosyl-3β,21α,23-trihydroxy-urs-12-en-28-oic acid 21-*O*-β-D-glucopyranoside.

Compound **5** was obtained as colorless gum. The molecular formula was determined as $C_{53}H_{86}O_{23}$ from the HR-ESI-MS (*m/z* 1091.5627 $[M+H]^+$), suggesting eleven degrees of unsaturation. The ^{1}H-NMR spectrum of aglycon for **5** showed five singlet methyl groups [δ_H 0.95 (3H, s), 0.96 (3H, s), 1.14 (3H, s), 1.37 (3H, s) and 1.60 (3H, s)], a doublet methyl group [δ_H 1.04 (3H, d, J=7.0 Hz)], an olefinic proton [δ_H 5.54 (1H, br s)], an oxygenated CH_2 group [δ_H 3.62, 4.13 (2H, AB, J=11.0 Hz)], and an oxygenated CH group [δ_H 4.09 (1H, dd, J=12.0, 4.5 Hz)]. Meanwhile, the ^{13}C-NMR spectrum of aglycon indicated characteristic signals of an ester carboxyl group [δ_C 176.8 (s)], and three oxygenated C-atoms [δ_C 82.2 (d), 72.5 (s), 64.7 (t)]. Furthermore, a quaternary olefinic C-atom [δ_C 139.2 (s)] was observed. The data above indicated an urs-12-ene aglycon with three hydroxyl functions, as well as one carboxyl group at C-17, corresponding to 19α,23-dihydroxy-urosic acid (rotundic acid), the same aglycone as randiasaponin II.[12] The NMR spectra for the sugar moieties indicated them to be two D-glucose, one L-arabinose and one L-rhamnose. Acid hydrolysis together with GC analysis confirmed this suggestion. All sites of glycosylation were established by HMBC experiments showing correlations between the H-1 (δ_H 5.13, d, J=6.5 Hz) of the L-arabinose and C-3 (δ_C 82.2) of the aglycon, H-1 (δ_H 5.14, d, J=8.0 Hz) of the D-glucose and C-2 (δ_C 81.1) of the L-arabinose, H-1 (δ_H 6.13, d, J=8.0 Hz) of another D-glucose and C-28 (δ_C 176.8) of the aglycon, H-1 (δ_H 6.63, br s) of L-rhamnose and C-2 (δ_C 75.2) of the D-glucose. Hence, the structure of **5** was established as 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-3β,19α,23-trihydroxy-urs-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside.

The new compound **2** is regarded as genuine natural product and not artifact formed during extraction by *n*-BuOH, which was confirmed by an additional experiment in which quinoa-saponin-9 (**9**), a known compound isolated from *I. pernyi*, was treated by the method mentioned in literature.[14]

The structure characterizations of the known compounds, ilexoside XXX (**6**),[15] 3-*O*-(methyl-β-D-glucuronopyranosiduronoate)-28-*O*-β-D-glucopyranosyl oleanolate (**7**),[16] collinsonidin (**8**),[17] quinoa-saponin-9 (**9**),[18] and 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**10**)[19] were deduced by direct comparison of their spectroscopic data with those reported in the literature.

Experimental

General Procedure Optical rotations were measured on a Perkin-Elmer-243B digital polarimeter at 25 °C. IR spectra were measured on a NEXUS-470 FTIR (Nicolet) spectrometer, KBr pellets, in cm^{-1}. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured in pyridine-d_5 on a Varian Inova 500 NMR spectrometer and Varian Unity 500 NMR spectrometer. Chemical shifts were shown as δ-values (ppm) with TMS as an internal standard. ESI-MS were measured on a QSTAR (ABI, U.S.A.) mass spectrometer. HR-ESI-MS were measured on a Bruker APEX II FT-ICR-MS mass spectrometer. Semi-preparative HPLC was carried out using a Waters 600 Pump with 600 controller (Waters C18 Nova-Pak column, 300×7.8 mm, 5 μm), with ELSD detector (Alltech). GC were carried out on Agilent 6890N gas chromatograph, capillary column (28 m×0.32 mm i.d.; HP-5), FID detector, operated at 260 °C (column temp 180 °C); N_2 as carrier gas (40 ml/min). For the column chromatography, silica gel (200—300 mesh, Qingdao Marine Chemical Industry), Sephadex LH-20 (Pharmacia), ODS gel (25—40 μm, Merck), and D101 porous polymer resin (Tianjin Chemical Industry) were used.

Table 2. ^{1}H- and ^{13}C-NMR Data of the Aglycon Moieties of **4** and **5** (Pyridine-d_5)

Position	**4**		**5**	
	δ_H	δ_C	δ_H	δ_C
1	0.85 (overlap)	38.8	0.91 (overlap)	38.7
	1.47 (br d, 13.0)		1.54 (overlap)	
2	1.91 (overlap)	25.8	1.90 (overlap)	25.8
	2.23 (overlap)		2.14 (overlap)	
3	4.26 (overlap)	82.0	4.09 (dd, 12.0, 4.5)	82.2
4		43.3		43.3
5	1.56 (overlap)	47.6	1.56 (overlap)	47.8
6	1.27 (overlap)	18.1	1.32 (overlap)	18.3
	1.62 (overlap)		1.63 (overlap)	
7	1.25 (overlap)	33.2	1.67 (overlap)	33.2
	1.59 (overlap)			
8		39.8		40.4
9	1.68 (overlap)	48.0	1.84 (dd, 11.0, 6.5)	47.6
10		36.8		36.7
11	1.89 (overlap)	23.6	2.02 (overlap)	24.0
12	5.49 (br s)	125.5	5.54 (br s)	128.2
13		139.1		139.2
14		42.6		42.1
15	1.10 (overlap)	29.0	1.52 (overlap)	29.4
	2.16 (overlap)		2.19 (dt, 13.5, 4.0)	
16	2.50 (br d, 14.0)	27.4	1.96 (overlap)	26.0
	2.95 (dt, 14.0, 4.0)		3.12 (dt, 12.5, 4.0)	
17		48.2		48.6
18	2.69 (d, 10.5)	54.1	2.85 (overlap)	54.6
19	2.12 (overlap)	33.2		72.5
20	1.32 (overlap)	42.7	1.42 (overlap)	41.7
21	4.23 (overlap)	76.6	1.23 (overlap)	26.5
			1.97 (overlap)	
22	1.86 (overlap)	38.8	1.94 (overlap)	37.4
	2.70 (overlap)		2.04 (overlap)	
23	3.66, 4.29	64.7	3.62, 4.13	64.7
	(AB, 11.0)		(AB, 11.0)	
24	0.93 (s)	13.6	0.95 (s)	13.2
25	0.88 (s)	16.2	0.96 (s)	16.1
26	1.02 (s)	17.5	1.14 (s)	17.4
27	1.06 (s)	23.1	1.60 (s)	24.1
28		179.7		176.8
29	0.95 (d, 6.5)	17.3	1.37 (s)	26.9
30	1.20 (d, 6.5)	17.7	1.04 (d, 7.0)	16.5

Table 3. ^{1}H- and ^{13}C-NMR Data of the Sugar Moieties of **1**—**3** (Pyridine-d_5)

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
	3-*O*-Glc[a]		3-*O*-GlcA[a]		3-*O*-Glc	
H-1	4.97 (d, 8.0)	101.6	5.21 (d, 7.7)	106.4	5.06 (d, 6.0)	103.7
2	4.05 (t, 8.5)	75.2	4.08 (t, 8.4)	75.4	4.14 (overlap)	83.9
3	4.25 (t, 8.5)	78.9	4.15 (overlap)	77.9	4.18 (overlap)	78.3
4	4.19 (t, 8.5)	72.1	4.46 (overlap)	73.0	4.16 (overlap)	71.1
5	3.90 (overlap)	78.3	4.45 (overlap)	77.3	3.77 (overlap)	77.9
6	4.35 (overlap)	63.1		170.3	4.29 (overlap)	62.6
	4.53 (dd, 12.0, 2.5)				4.45 (br d, 10.0)	
			Ester moiety COOBu			
H-1			4.22 (overlap)	64.9		
2			1.52 (m)	30.8		
3			1.27 (overlap)	19.2		
4			0.71 (t, 7.3)	13.7		
			28-*O*-Glc		Glc	
H-1			6.25 (d, 8.1)	95.6	5.36 (d, 7.5)	105.8
2			4.16 (overlap)	74.0	4.09 (overlap)	76.7
3			4.25 (overlap)	78.8	4.18 (overlap)	77.9
4			4.33 (overlap)	71.1	4.27 (overlap)	71.2
5			3.99 (overlap)	79.1	3.87 (overlap)	78.2
6			4.37 (dd, 12.0, 4.0)	62.2	4.40 (dd, 10.5, 3.5)	62.4
			4.43 (dd, 12.0, 1.5)		4.45 (br d, 10.5)	

a) Glc=β-D-glucopyranose; GlcA=β-D-glucuronopyranose.

Plant Materials The leaves of *I. pernyi* were collected in April 2005 at the Nature Protect Area of Shennongjia, Hubei Province, P. R. China. The identification of the plant was performed by Prof. P.-F. Tu, Peking University. A voucher specimen (MEC 0504) was kept in the herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

Extraction and Isolation The air-dried leaves (15 kg) of *I. pernyi* were extracted thrice with 70% aq. EtOH at 60 °C. The combined extract was concentrated under vacuum, and the residue was suspended in H_2O and extracted successively with EtOAc and *n*-BuOH after being defatted with petroleum ether. The *n*-BuOH extract (400 g) was dissolved in water and passed through a D101 porous polymer resin column and eluted with H_2O and 10, 30, 50, 70, and 95% aq. EtOH respectively. The fractions eluted with 50 and 70% aq. EtOH (97 g) were applied to silica gel column ($CHCl_3$–MeOH–H_2O, 10 : 1 : 0→1 : 1 : 0.1, v/v) to give eight fractions. Fr. 2 (2 g) was purified by column chromatography (Sephadex LH-20, MeOH; silica gel, $CHCl_3$–MeOH–H_2O, 10 : 1 : 0.1) followed by semi-preparative HPLC (MeOH–H_2O, 3 : 2) to afford **1** (10 mg) and ilexoside XXX (**6**, 8 mg). Fr. 3 (1.5 g) was subjected to column chromatography (Sephadex LH-20, MeOH; ODS, 70% aq. MeOH) followed by semi-preparative HPLC (MeOH–H_2O, 7 : 3) to give **2** (20 mg). Fr. 5 (3 g) was subjected to column chromatography (Sephadex LH-20, MeOH; ODS, 60→80% aq. MeOH) to afford 3-*O*-(methyl-β-D-glucuronopyranosiduronoate)-28-*O*-β-D-glucopyranosyl oleanolate (**7**, 8 mg) and collinsonidin (**8**, 10 mg). Fr. 6 (5 g) was subjected to column chromatography (silica gel, $CHCl_3$–MeOH–H_2O, 4 : 1 : 0.1; ODS, 60→70% aq. MeOH) to give subfractions 6-1 (110 mg) and 6-2 (25 mg). Each subfraction was isolated by semi-preparative HPLC [MeOH–0.05% TFA (53 : 47) for fraction 6-1; MeOH–0.05% TFA (1 : 1) for fraction 6-2] to yield quinoa-saponin-9 (**9**, 14 mg) and **3** (15 mg), **4** (8 mg) respectively. Purification of Fr. 7 (2.5 g) by column chromatography (silica gel, $CHCl_3$–MeOH–H_2O, 2 : 1 : 0.1; ODS, 50→65% aq. MeOH) and then by semi-preparative HPLC [MeOH–0.05% TFA (13 : 7) for fraction 7-1 (27 mg); MeOH–0.05% TFA (47 : 53) for fraction 7-2 (50 mg)] afforded 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**10**, 13 mg) and **5** (12 mg) respectively.

Compound **1**: Colorless gum; $[\alpha]_D^{26}$ +11.1° (*c*=0.009, MeOH); IR (KBr) cm^{-1}: 3415, 2928, 1678, 1451, 1204, 1075; ^{1}H- and ^{13}C-NMR (Tables 1, 3); ESI-MS *m/z*: 673 $[M+Na]^+$, 689 $[M+K]^+$; HR-ESI-MS *m/z*: 673.3925 $[M+Na]^+$ (Calcd for $C_{36}H_{58}NaO_6$: 673.3922).

Compound **2**: Colorless gum; $[\alpha]_D^{26}$ +0.55° (*c*=0.018, MeOH); IR (KBr) cm^{-1}: 3419, 2925, 1739, 1377, 1071; ^{1}H- and ^{13}C-NMR (Tables 1, 3). ESI-MS *m/z*: 867 $[M+H]^+$, 889 $[M+Na]^+$, 905 $[M+K]^+$; HR-ESI-MS *m/z*: 889.4906 $[M+Na]^+$ (Calcd for $C_{46}H_{74}NaO_{15}$: 889.4920).

Compound **3**: Colorless gum; $[\alpha]_D^{26}$ +11.6° (*c*=0.013, MeOH); IR (KBr)

Table 4. ^{1}H- and ^{13}C-NMR Data of the Sugar Moieties of **4** and **5** (Pyridine-d_5)

Position	**4**		**5**	
	δ_H	δ_C	δ_H	δ_C
	3-*O*-Glc[a]		3-*O*-Ara[a]	
H-1	5.12 (d, 8.0)	105.8	5.13 (d, 6.5)	103.7
2	4.01 (t, 8.5)	75.8	5.54 (t, 8.0)	81.1
3	4.13 (t, 8.5)	78.6	4.24 (overlap)	73.5
4	4.19 (t, 8.5)	71.6	4.27 (overlap)	68.1
5	3.90 (overlap)	78.3	3.66 (d, 10.5)	64.8
			4.22 (overlap)	
6	4.35 (dd, 11.5, 6.0)	62.8		
	4.52 (br t, 11.5)			
	21-*O*-Glc		Glc	
H-1	4.97 (d, 7.5)	101.6	5.14 (d, 8.0)	105.7
2	4.05 (t, 8.5)	75.2	4.05 (t, 8.0)	76.1
3	4.24 (overlap)	78.9	4.14 (t, 8.5)	78.0
4	4.21 (t, 8.5)	72.0	4.21 (t, 9.0)	71.2
5	3.88 (overlap)	78.3	3.77 (overlap)	78.1
6	4.37 (dd, 12.0, 5.5)	63.1	4.33—4.36 (overlap)	62.3
	4.52 (br t, 12.0)		4.41—4.45 (overlap)	
			28-*O*-Glc	
H-1			6.13 (d, 8.0)	94.8
2			4.48 (t, 8.5)	75.2
3			4.32 (overlap)	79.9
4			4.25 (overlap)	71.4
5			4.00 (overlap)	78.8
6			4.33—4.36 (overlap)	62.2
			4.41—4.45 (overlap)	
			Rha[a]	
H-1			6.63 (br s)	101.3
2			4.78 (t, 3.5)	72.2
3			4.55 (dd, 9.5, 3.5)	72.4
4			4.30 (overlap)	73.7
5			4.59 (overlap)	69.6
6			1.72 (d, 6.5)	18.6

a) Glc=β-D-glucopyranose; Ara=α-L-arabinopyranose; Rha=α-L-rhamnopyranose.

cm^{-1}: 3451, 2925, 1696, 1457, 1077; ^{1}H- and ^{13}C-NMR (Tables 1, 3); ESI-MS *m*/*z*: 813 $[M+H]^+$, 835 $[M+Na]^+$; HR-ESI-MS *m*/*z*: 813.4630 $[M+H]^+$ (Calcd for $C_{42}H_{69}O_{15}$: 8813.4631).

Compound **4**: Colorless gum; $[\alpha]_D^{26}$ −8.6° (*c*=0.007, MeOH); IR (KBr) cm^{-1}: 3421, 2927, 1682, 1455, 1061; ^{1}H- and ^{13}C-NMR (Tables 2, 4); ESI-MS *m*/*z*: 835 $[M+Na]^+$; HR-ESI-MS *m*/*z*: 835.4455 $[M+Na]^+$ (Calcd for $C_{42}H_{69}O_{15}$: 835.4450).

Compound **5**: Colorless gum; $[\alpha]_D^{26}$ +15.1° (*c*=0.011, MeOH); IR (KBr) cm^{-1}: 3425, 2931, 1743, 1367, 1065; ^{1}H- and ^{13}C-NMR (Tables 2, 4); ESI-MS *m*/*z*: 1091 $[M+H]^+$, 1113 ($[M+Na]^+$; HR-ESI-MS *m*/*z*: 1091.5627 $[M+H]^+$ (Calcd for $C_{53}H_{87}O_{23}$: 1091.5633).

Acid Hydrolysis Each saponin (4 mg) was heated in 4 ml of 10% HCl–dioxane (1 : 1) at 80 °C for 4 h. After the dioxane was removed, water (5 ml) was added and the solution was extracted with EtOAc (5 ml×3). The aqueous fractions were evaporated and the residues were prepared to their derivatives for GC analysis according to the methods described in the literature.[2)]

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