Hydrocotylosides I−VII, New Oleanane Saponins from *Hydrocotyle sibthorpioides*

Aki Matsushita,[†] Yukimi Sasaki,[†] Tsutomu Warashina,[†] Toshio Miyase,*[,†] Hiroshi Noguchi,[†] and David Vander Velde[‡]

School of Pharmaceutical Sciences, University of Shizuoka, 52-1, Yada, Shizuoka 422-8526, Japan, and Department of Chemistry, University of Kansas, 1251 Wescoe Hall Drive, Lawrence, Kansas 66045

Received August 7, 2003

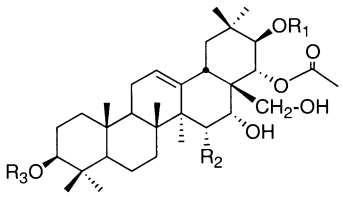
Seven new oleanane-type triterpenoid saponins, hydrocotylosides I−VII (**1**−**7**), and one known saponin, udosaponin B (**8**), were isolated from the methanol extract of the whole plants of *Hydrocotyle sibthorpioides*. The structures of the new compounds were elucidated on the basis of chemical and spectroscopic evidence.

Hydrocotyle sibthorpioides Lam. belongs to the Apiaceae. In this family, saponins are rare and have been reported only from members of the genera *Bupleurum*,[1] *Centella*,[2] *Hydrocotyle*, and *Sanicula*.[3,4] Previous investigations have led to the isolation of seven oleanane glycosides, ranuncosides I−VII, from *H. ranunculoides*.[5,6] *H. sibthorpioides* is known to have astringent, antifebrile, and diuretic activities;[7] however, no study of its chemical constituents has been performed. As a part of a research program on saponins in *Hydrocotyle* species, we have isolated seven new oleanane-type triterpenoid saponins named hydrocotylosides I−VII (**1**−**7**) and the previously known udosaponin B (**8**) from the whole plant of *H. sibthorpioides*.

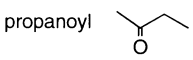
Results and Discussion

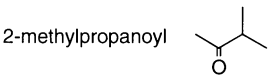
A methanol extract of the whole plants of *H. sibthorpioides* was dissolved in water and extracted with diethyl ether. The water layer was passed through a porous polymer gel Diaion HP-20 column, and the methanol eluate was separated by preparative HPLC to afford saponins (**1**−**7**) and udosaponin B (**8**).

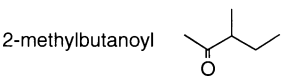
The FABMS of hydrocotyloside I (**1**) exhibited a quasi-molecular ion peak at *m/z* 965 $[M + Na]^+$, and the ^{13}C NMR spectrum revealed 47 carbon signals, indicating the molecular formula $C_{47}H_{74}O_{19}$. The ^1H NMR spectrum of **1** showed signals of an olean-12-ene-type aglycon [seven singlet methyl signals at δ 0.89, 1.03, 1.08, 1.14, 1.29 (CH_3-23 and CH_3-30, overlapped), and 1.80 and a trisubstituted olefinic proton signal at δ 5.50 (dd, J = 3, 3 Hz)], two anomeric proton signals [δ 5.01 (d, J = 7.5 Hz) and 5.40 (d, J = 7.5 Hz)], a methyl signal at δ 1.82 (s) of an acetyl moiety, and a triplet methyl signal at δ 1.18 (t, J = 8 Hz). Also, a quartet methylene signal at δ 2.43 (q, J = 8 Hz), which showed a HMBC correlation with a carbonyl carbon (δ 174.3), indicated the presence of a propanoyl moiety in the molecule. The ester groups were also identified by alkaline hydrolysis. The structure of the aglycon was determined by ^1H, ^{13}C NMR, HMQC, and HMBC experiments as 3,21,22-trisubstituted olean-12-en-3,15,16,21,22,-28-hexaol. The hydroxyl groups at C-15 (δ 67.5) and C-16 (δ 72.7) were assigned with 15α,16α-orientation by comparison with ^{13}C NMR data in the literature.[5,8] The coupling constants (10 Hz) between H-21 and H-22 indicated diaxial orientations. The sugar moieties of **1** were characterized as a β-D-glucuronopyranosyl unit and a β-D-glucopyranosyl unit from the difference HOHAHA spec-



	R_1	R_2	R_3
1	propanoyl	OH	-GlcA (2→) Glc
2	2-methylpropanoyl	OH	-GlcA (2→) Glc
3	2-methylpropanoyl	OH	-GlcA (2→) Glc; Glc (3→)
4	2-methylpropanoyl	OH	-GlcA (2→) Glc (2→) Glc
5	2-methylpropanoyl	OH	Ara (6→); -Glc (2→) Glc; Glc (4→)
6	2-methylbutanoyl	OH	Ara (6→); -Glc (2→) Glc; Glc (4→)
7	acetyl	H	Ara (6→); -Glc (2→) Glc; Glc (4→)







* To whom correspondence should be addressed. Tel/Fax: +81-54-264-5661. E-mail: miyase@ys7.u-shizuoka-ken.ac.jp.
[†] University of Shizuoka.
[‡] University of Kansas.

10.1021/np030356z CCC: $27.50
Published on Web 02/10/2004

trum and GC analysis.[11] The sugar units were assigned the β-configuration according to the anomeric proton coupling constants of each monosaccharide. The positions of the sugar moieties were identified from the HMBC and ROE NMR spectra (see Supporting Information). On the basis of these studies, the structure of hydrocotyloside I (**1**) was deduced as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-21-*O*-propanoyl-3β,15β,16α,21β,22α,-28-hexahydroxyolean-12-ene.

The FABMS of hydrocotyloside II (**2**) gave a quasi-molecular ion peak at *m/z* 979 $[M + Na]^+$ in accordance with the molecular formula, $C_{48}H_{76}O_{19}$. The ^1H NMR spectrum showed an acetyl moiety signal, two anomeric protons [δ 5.01 (d, $J = 7.5$ Hz) and 5.39 (d, $J = 8$ Hz)], two doublet methyl signals [δ 1.23 (d, $J = 7$ Hz) and 1.24 (dd, $J = 7$, 2 Hz)], and a methine signal at δ 2.67 (sept, $J = 7$ Hz) attributed to a 2-methylpropanoyl group. On comparison of the ^1H and ^{13}C NMR data of the sugar moieties with those of **1**, these were found to be the same as in **1**. Accordingly, hydrocotyloside II (**2**) was identified as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-21-*O*-(2-methylpropanoyl)-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

Hydrocotyloside III (**3**) was assigned a molecular formula of $C_{54}H_{86}O_{24}$, as determined from the FABMS data, which gave a quasi-molecular ion peak at *m/z* 1142 $[M + Na]^+$. The ^1H NMR spectrum showed the presence of an acetyl group and a 2-methylpropanoyl group and three anomeric protons [δ 4.97 (d, $J = 7.5$ Hz), 5.40 (d, $J = 8$ Hz), and 5.72 (d, $J = 8$ Hz)]. The HMBC spectrum indicated that the positions of the 2-methylpropanoyl group and the acetyl group were identical to those of **2**. The saccharide unit structure and linkage were determined as [β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranose by HMBC and ROE correlations (see Supporting Information) and by acid hydrolysis. Thus, the structure of hydrocotyloside III (**3**) was assigned as 3-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-21-*O*-(2-methylpropanoyl)-3β,15α,16α,-21β,22α,28-hexahydroxyolean-12-ene.

The FABMS of hydrocotyloside IV (**4**) exhibited a quasi-molecular ion peak at *m/z* 1142 $[M + Na]^+$, consistent with the molecular formula, $C_{54}H_{86}O_{24}$. The NMR spectra of the aglycon were similar to those of **2** and **3**. The ^{13}C NMR data showed the low-field shifts of C-2 of a glucuronopyranosyl unit and C-2 of a glucopyranosyl unit. These data led to the assignment of hydrocotyloside IV (**4**) as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-21-*O*-(2-methylpropanoyl)-3β,-15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

The FABMS data of hydrocotyloside V (**5**) afforded a quasi-molecular ion peak at *m/z* 1260 $[M + Na]^+$, which indicated a molecular formula of $C_{59}H_{96}O_{27}$. The ^1H NMR spectrum of **5** revealed signals for a 2-methylpropanoyl group, an acetyl group, and four anomeric protons [δ 4.71 (d, $J = 8$ Hz), 5.04 (d, $J = 7.5$ Hz), 5.28 (d, $J = 7.5$ Hz), and 5.44 (d, $J = 8$ Hz)]. On the basis of the difference HOHAHA spectrum and acid hydrolysis, the sugar moieties of **5** were determined to consist of three β-D-glucopyranosyl units and an α-L-arabinopyranosyl unit. The structures of the sugar moieties were deduced by ^{13}C NMR data and HMBC and ROE spectra (see Supporting Information). The glucopyranosyl unit at C-3 of the aglycon showed low-field shifts of its carbon resonances [C-2 (δ 81.0), C-3 (δ 80.5), and C-6 (δ 68.5)] due to glycosylation. On the basis of these results, the structure of hydrocotyloside V (**5**) was assigned as 3-*O*-[α-L-arabinopyranosyl-(1→6)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-22-*O*-acetyl-21-*O*-(2-methylpropanoyl)-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

The FABMS data for hydrocotyloside VI (**6**) showed a quasi-molecular ion peak at *m/z* 1274 $[M + Na]^+$, consistent with the molecular formula, $C_{60}H_{98}O_{27}$. On acid hydrolysis, the sugar components, D-glucose and L-arabinose, were detected. The ^1H NMR spectrum showed four anomeric protons [δ 4.71 (d, $J = 8$ Hz), 5.05 (d, $J = 7.5$ Hz), 5.28 (d, $J = 7.5$ Hz), and 5.45 (d, $J = 8$ Hz)]. The sugar moieties were the same as in **5**, but the ester moieties differed. The ^1H NMR showed a triplet methyl signal at δ 0.95 (t, $J = 7.5$ Hz), a methylene signal at δ 1.51 (overlapped), a methine signal at δ 2.51 (m), a doublet methyl signal at δ 1.23 (d, $J = 7$ Hz), and an acetyl group signal at δ 1.81 (s). These data agreed with the signals of a 2-methylbutanoyl moiety, which was identified also by alkaline hydrolysis. Hence, the structure of hydrocotyloside VI (**6**) was concluded to be 3-*O*-α-[L-arabinopyranosyl-(1→6)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-22-*O*-acetyl-21-*O*-(2-methylbutanoyl)-3β,15α,16α,21β,-22α,28-hexahydroxyolean-12-ene.

Hydrocotyloside XI (**7**) was assigned the molecular formula $C_{57}H_{92}O_{26}$, as determined from the quasi-molecular ion peak at *m/z* 1216 $[M + Na]^+$ in its FABMS. The NMR data showed that the aglycon of **7** was somewhat different from compounds **1**−**6**. The ^1H NMR spectrum showed signals for an olean-12-ene-type aglycon, four anomeric protons, two methyl singlets of acetyl groups (δ 1.99 and 2.13), and methylene protons at δ 1.61 (br d, $J = 14$ Hz) and 1.87 (dd, $J = 14$, 4 Hz). The HMQC spectrum was used to assign the methylene signals to C-15 of the aglycon (δ 34.7). Further supporting evidence was obtained from the long-range HMBC correlations between the proton at δ 1.61 and C-13 (δ 142.9) and C-17 (δ 48.1), between the proton at δ 1.87 and C-13, and between H-27 (δ 1.81) and C-15. Thus, the structure of the aglycon was determined as a 3,21,22-trisubstituted olean-12-en-3,16,21,22,28-pentaol. The positions of the two acetyl groups were deduced at H-21 and H-22, respectively, from the HMBC experiment (see Supporting Information). The ^1H and ^{13}C NMR data were identical to those of **5** and **6**, so the sugar moieties of **7** are the same in all these compounds. Therefore, the structure of hydrocotyloside VII (**7**) was concluded to be 3-*O*-[α-L-arabinopyranosyl-(1→6)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-21-*O*-acetyl-22-*O*-acetyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene.

The known compound **8** was identified as udosaponin B or 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranosylolean-12-en-28-oic acid (isolated from *Steganotaenia araliacea* Hochst[9] and as the methyl ester from *Aralia cordata* Thumb.[10]), from ^1H, ^{13}C, HMQC, and HMBC NMR experiments.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a JASCO DIP 1000 digital polarimeter. NMR spectra were recorded in C_5D_5N on a JEOL α-400 instrument at 400 MHz for ^1H and 100 MHz for ^{13}C NMR at 35 °C. FABMS and HRFABMS data were obtained on a JEOL JMS 700 mass spectrometer. Preparative HPLC was performed on a JASCO system 800 instrument. GC was performed on a Hitachi G-3000 instrument.

Plant Material. The plant was collected in Shizuoka, Japan, in April 2002 and identified by Prof. Akira Ueno, School of Pharmaceutical Sciences, University of Shizuoka. A voucher specimen is deposited at the herbarium of the University of Shizuoka, No. 20021015.

Table 1. ^{1}H NMR Data (δ) of Compounds **1**−**7** in Pyridine-d_5

position		1	2	3	4	5	6	7
aglycon	3	3.34 (dd, 11.5, 4)	3.34 (dd, 11.5, 4)	3.33 (dd, 11.5, 4)	3.36 (dd, 11.5, 4)	3.18 (dd, 11.5, 4)	3.18 (dd, 12, 4)	3.18 (dd, 12, 4)
	5	0.83 (br d, 12.5)	0.82 (br d, 12)	0.83[a]	0.84 (br d, 12)	0.77 (br d, 11.5)	0.78 (br d, 12)	0.72 (br d, 11.5)
	12	5.50 (dd, 3, 3)	5.50 (dd, 3, 3)	5.50 (dd, 3,3)	5.50 (dd, 3, 3)	5.51 (dd, 3, 3)	5.52[a]	5.41 (dd, 3, 3)
	15	4.18 (d, 4.5)	4.18 (d,4)	4.18[a]	4.18[a]	4.18[a]	4.19[a]	1.61 (br d, 14)
	15							1.87 (dd, 14, 4)
	16	4.39[a]	4.31[a]	4.38[a]	4.38[a]	4.38[a]	4.39[a]	4.45[a]
	18	3.03 (br d, 11)	3.01[a]	3.02 (br d, 11)	3.03 (br d, 11)	3.10[a]	3.02[a]	3.07 (br d, 11)
	21	6.48 (d, 10)	6.46 (d, 10.5)	6.46 (d, 10)	6.46 (d, 10.5)	6.46 (d, 10.5)	6.48 (d, 10)	6.49 (d, 10)
	22	6.16 (d, 10)	6.16 (d, 10.5)	6.16 (d, 10)	6.16 (d, 10.5)	6.16 (d, 10.5)	6.17 (d, 10)	6.18 (d, 10)
	23	1.29 (s)	1.28 (s)	1.26 (s)	1.30 (s)	1.20 (s)	1.21 (s)	1.23 (s)
	24	1.14 (s)	1.13 (s)	1.11 (s)	1.15 (s)	1.09 (s)	1.09 (s)	1.10 (s)
	25	0.89 (s)	0.89 (s)	0.85 (s)	0.87 (s)	0.89 (s)	0.89 (s)	0.86 (s)
	26	1.03 (s)	1.03 (s)	1.02 (s)	1.03 (s)	1.03 (s)	1.03 (s)	0.88 (s)
	27	1.80 (s)	1.82 (s)	1.83 (s)	1.82 (s)	1.80 (s)	1.80 (s)	1.81 (s)
	28	3.45 (d, 11)	3.45 (d, 11)	3.44 (d, 10.5)	3.45 (d, 11)	3.45 (d, 11)	3.45 (d, 11)	3.39 (d, 11)
	28	3.70 (d, 11)	3.70 (d, 11)	3.69 (d, 10.5)	3.70 (d, 11)	3.70 (d, 11)	3.70 (d, 11)	3.63 (d, 11)
	29	1.08 (s)	1.07 (s)	1.07 (s)	1.07 (s)	1.08 (s)	1.10 (s)	1.08 (s)
	30	1.29 (s)	1.28 (s)	1.28 (s)	1.29 (s)	1.30 (s)	1.30 (s)	
ester moiety								
acetyl at C-21								2.13 (s)
acetyl at C-22		1.82 (s)	1.82 (s)	1.82 (s)	1.82 (s)	1.82 (s)	1.82 (s)	1.99 (s)
propanoyl	2	2.43 (q, 8)						
	3	1.18 (t, 8)						
2-methyl-propanoyl	2		2.67 (sept, 7)	2.67 (sept, 7)	2.68 (sept, 7)	2.68 (sept, 7)		
	3		1.23 (d, 7)	1.23 (d, 7)	1.24 (d, 7)	1.24 (d, 7)		
	4		1.24 (dd, 7, 2)	1.24 (d, 7)	1.25 (d, 7)	1.25 (d, 7)		
2-methyl-butanoyl	2						2.51 (m)	
	3						1.51[a]	
	4						0.95 (t, 7.5)	
	5						1.23 (d, 7)	

position	1	2	3	4	5	6	7
sugar moiety	GlcA	GlcA	GlcA	GlcA	Glc[d]	Glc[d]	Glc[d]
1	5.01 (d, 7.5)	5.01 (d, 7.5)	4.97 (d, 7.5)	5.08 (d, 8)	4.71 (d, 7.5)	4.71 (d, 8)	4.72 (d, 7.5)
2	4.34[a]	4.33[a]	4.48[a]	4.20[a]	4.02[a]	4.04[a]	4.05[a]
3	4.39[a]	4.38[a]	4.36[a]	4.62[a]	4.14[a]	4.17[a]	4.18 (dd, 10, 10)
4	4.56[a]	4.55[a]	4.51[a]	4.54[a]	4.32[a]	4.35[a]	4.36[a]
5	4.61[a]	4.60[a]	4.54[a]	4.66[a]	3.84[a]	3.86[a]	3.87[a]
6					4.57 (dd, 11, 3.5)	4.59 (dd, 11, 4)	4.60 (dd, 11, 3.5)
6					4.74 (d, 10)	4.77 (br d, 10.5)	4.77 (br d, 11)
	Glc	Glc	Glc[c]	Glc[b]	Glc[e]	Glc[e]	Glc[e]
1	5.40 (d, 7.5)	5.39 (d, 8)	5.72 (d, 8)	5.55 (d, 8)	5.28 (d, 7.5)	5.28 (d, 7.5)	5.29 (d, 7.5)
2	4.12[a]	4.11 (dd,9, 8)	4.08[a]	4.18[a]	4.01[a]	4.00[a]	4.01[a]
3	4.24[a]	4.24 (dd, 9, 9)	4.27[a]	4.30[a]	4.20[a]	4.19[a]	4.20[a]
4	4.32[a]	4.32[a]	4.15[a]	4.18[a]	4.25[a]	4.26[a]	4.26[a]
5	3.93[a]	3.92[a]	3.89 (m)	3.86 (m)	3.87[a]	3.88[a]	3.88[a]
6	4.46[a]	4.44[a]	4.36[a]	4.36[a]	4.40[a]	4.40[a]	4.39[a]
6	4.46[a]	4.490	4.48[a]	4.47 (dd, 11.5, 3)	4.48[a]	4.48[a]	4.49 (dd, 11, 3)
			Glc[c]	terminal Glc	Glc[f]	GcI[f]	GIc[f]
1			5.40 (d, 8)	5.36 (d, 8)	5.44 (d, 8)	5.45 (d, 8)	5.45 (d, 8)
2			4.03[a]	4.08[a]	4.02[a]	4.02[a]	4.02[a]
3			4.21	4.20[a]	4.22[a]	4.34[a]	4.35[a]
4			4.14[a]	4.11[a]	4.35[a]	4.23[a]	4.22 (dd, 10, 10)
5			4.03 (m)	3.98[a]	4.13[a]	4.12[a]	4.12 (m)
6			4.31[a]	4.30[a]	4.24[a]	4.23[a]	4.25[a]
6			4.46[a]	4.59[a]	4.40[a]	4.41[a]	4.40[a]
					Ara	Ara	Ara
1					5.04 (d, 7.5)	5.05 (d, 7.5)	5.05 (d, 7)
2					4.45[a]	4.45[a]	4.45[a]
3					4.05[a]	4.04[a]	4.05[a]
4					4.23[a]	4.23[a]	4.23[a]
5					3.72 (br d, 11)	3.71 (br d, 11)	3.73 (br d, 11)
5					4.25[a]	4.25[a]	4.25[a]

[a] Overlapped with other signals. [b] Glc at C-2 of GIcA. [c] Glc at C-3 of GIcA. [d] Glc at C-3 of aglycon. [e] Glc at C-2 of Glc. [f] Glc at C-4 of Glc.

Extraction and Isolation. Dried whole plants of *Hydrocotyle sibthorpioides* (764 g) were extracted with MeOH. The MeOH extract was concentrated under reduced pressure to give 85 g of residue, which was dissolved in water and then extracted with ether. The water layer was applied to a Mitsubishi Diaion HP-20 column (7 × 35 cm), and the adsorbed material was eluted with 50% MeOH (3.5 L), 60% MeOH (3.5 L), 70% MeOH (5 L), and MeOH (5 L) after washing with water to give 50% MeOH (6.8 g), 60% MeOH (1.8 g), 70% MeOH (2.0 g), and MeOH eluates (2.5 g). The 70% MeOH eluate (2.0 g) was subjected to HPLC (column, ODS, 5 × 100 cm; solvent, CH_3CN-H_2O (25:75→45:55) linear gradient; flow rate, 45 mL/min; detection, UV 205 nm) to afford 17 fractions.

Fraction 11 (148 mg) was separated by preparative HPLC [Capcell Pak Ph, 20 mm × 25 cm, CH_3CN-H_2O-TFA (27.5:72.5:0.05), 6.5 mL/min, UV 205 nm] to give **2** (t_R 67.8 min, 7.5 mg), **7** (t_R 34.0 min, 55.3 mg), and **8** (t_R 54.0 min, 28.6 mg).

Fraction 12 (134 mg) was subjected to preparative HPLC [Capcell Pak Ph, 20 mm × 25 cm, CH_3CN-H_2O-TFA (30:70:0.05), 6.5 mL/min, UV 205 nm] to give **1** (t_R 42.2 min, 3.9 mg), **2** (t_R 65.0 min, 17.4 mg), **3** (t_R 59.1 min, 8.6 mg), and **4** (t_R 50.0 min, 14.8 mg).

Fraction 13 (90 mg) was purified by preparative HPLC [Capcell Pak Ph, 20 mm × 25 cm, CH_3CN-H_2O-TFA (32.5:67.5:0.05), 6.5 mL/min, UV 205 nm] to give **2** (t_R 54.0 min, 18.8 mg) and **5** (t_R 27.5 min, 34.9 mg).

Table 2. ^{13}C NMR Data (δ) of Compounds **1**−**7** in Pyridine-d_5

position		1	2	3	4	5	6	7
aglycon	1	39.1	39.1	39.0	39.1	39.1	39.0	38.9
	2	26.7	26.7	26.7	26.7	26.7	26.7	26.7
	3	89.2	89.2	89.5	89.2	89.5	89.4	89.5
	4	39.6	39.6	39.6	39.6	39.5	39.4	39.6
	5	55.6	55.7	55.6	55.9	55.8	55.7	55.9
	6	18.8	18.8	18.9	18.9	18.9	18.8	18.5
	7	36.7	36.7	36.7	36.7	36.7	36.7	33.2
	8	41.5	41.5	41.5	41.5	41.5	41.4	40.1
	9	47.2	47.2	47.2	47.2	47.3	47.2	47.0
	10	37.0	37.0	37.0	37.0	37.0	36.9	36.8
	11	24.0	24.0	24.0	24.0	24.1	24.0	23.9
	12	125.4	125.4	125.4	125.4	125.5	125.4	124.0
	13	143.8	143.8	143.7	143.7	143.7	143.7	142.9
	14	47.8	47.8	47.9	47.9	47.8	47.8	41.7
	15	67.5	67.5	67.5	67.5	67.5	67.5	34.7
	16	72.7	71.8	72.7	72.7	72.7	72.6	68.1
	17	48.4	48.4	48.4	48.4	48.4	48.4	48.1
	18	41.0	41.0	41.0	41.0	41.0	41.0	40.2
	19	46.9	46.9	46.9	46.9	46.9	46.9	47.3
	20	36.2	36.3	36.3	36.3	36.4	36.3	36.2
	21	79.2	78.9	78.9	78.9	78.9	78.8	79.6
	22	74.1	74.0	74.0	74.0	74.0	74.0	74.5
	23	28.1	28.1	27.9	28.2	28.1	28.0	28.1
	24	16.9	16.1	16.8	17.0	16.9	16.8	16.8
	25	15.8	15.9	15.8	15.9	15.8	15.9	15.7
	26	17.6	17.6	17.6	17.6	17.6	17.5	17.0
	27	21.1	21.1	21.1	21.1	21.1	21.1	27.5
	28	63.3	63.4	63.5	63.4	63.4	63.3	63.9
	29	29.4	29.4	29.4	29.4	29.5	29.6	29.5
	30	20.0	20.0	20.0	20.0	20.0	20.1	20.1
ester moiety								
acetyl at	1							170.9
C-21	2							21.1
acetyl at	1	170.8	170.9	170.8	170.9	170.9	170.8	171.1
C-22	2	20.7	20.8	20.8	20.8	20.8	20.8	20.9
propanoyl	1	174.3						
	2	28.3						
	3	9.8						

position		1	2	3	4	5	6	7
2-methyl-	1			76.9	176.8	176.8	176.8	
propanoyl	2			34.9	34.9	34.9	34.9	
	3			19.3	19.3	19.3	19.3	
	4			19.5	19.5	19.5	19.5	
2-methyl-	1							176.5
butanoyl	2							42.0
	3							27.2
	4							12.1
	5							17.2
sugar moiety		GlcA	GlcA	GlcA	GlcA	Glc[c]	Glc[c]	Glc[c]
1		105.4	105.4	105.3	105.1	105.0	105.0	105.0
2		83.0	83.0	79.1	83.0	81.0	80.9	81.0
3		77.8	77.8	87.8	77.8	76.3	76.3	76.3
4		73.2	73.2	71.9	72.5	80.5	80.3	80.5
5		77.5	77.4	77.3	77.7	74.6	74.6	74.6
6		172.4	172.5	171.9	172.4	68.5	68.4	68.5
		Glc	Glc[a]	Glc[a]	Glc[a]	Glc[d]	Glc[d]	Glc[d]
1		106.1	106.1	104.0	103.5	105.3	105.2	105.3
2		77.1	77.1	76.4	85.0	77.0	77.0	77.0
3		78.0	78.0	78.6	77.7	77.9	77.9	77.9
4		71.8	72.7	72.7	71.9	72.0	71.9	72.0
5		78.2	78.2	77.8	77.7	78.1	78.1	78.1
6		62.8	62.8	63.4	63.0	63.0	62.9	63.0
				Glc[b]	terminal Glc	Glc[e]	Glc	GIc[e]
1				104.7	106.4	104.8	104.8	104.8
2				75.5	76.8	75.2	75.2	75.2
3				78.6	77.8	78.1	78.2	78.1
4				71.7	71.4	71.8	71.7	71.8
5				78.6	79.3	78.5	78.5	78.5
6				62.4	62.8	62.4	62.2	62.4
						Ara	Ara	Ara
1						105.7	105.8	105.7
2						72.6	72.5	72.6
3						74.5	74.4	74.5
4						69.7	69.8	69.7
5						67.2	67.3	67.2

[a] Glc at C-2 of GlcA. [b] Glc at C-3 of GlcA. [c] CGlc at C-3 of aglycon. [d] Glc at C-2 of Glc. [e] Glc at C-4 of Glc.

Fraction 14 (128 mg) was subjected to preparative HPLC [Capcell Pak Ph, 20 mm × 25 cm, CH_3CN-H_2O−TFA (30:70:0.05), 6.5 mL/min, UV 205 nm] to give **2** (t_R 69.4 min, 48.2 mg).

Fraction 15 (13 mg) was separated by preparative HPLC [Capcell Pak Ph, 20 mm × 25 cm, CH_3CN-H_2O−TFA (27.5:72.5:0.05), 6.5 mL/min, UV 205 nm] to give **6** (t_R 79.3 min, 3.2 mg).

Hydrocotyloside I (1): 3.9 mg; amorphous powder; $[\alpha]_D^{23}$ −1.1° (*c* 0.46, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 965 $[M + Na]^+$; HRFABMS *m*/*z* 965.4713 $[M + Na]^+$ (calcd for $C_{47}H_{74}O_{19}Na$, 965.4722).

Hydrocotyloside II (2): 91.9 mg; amorphous powder; $[\alpha]_D^{23}$ −1.5° (*c* 0.91, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 979 $[M + Na]^+$; HRFABMS *m*/*z* 979.4890 $[M + Na]^+$ (calcd for $C_{48}H_{76}O_{19}Na$, 979.4879).

Hydrocotyloside III (3): 8.6 mg; amorphous powder; $[\alpha]_D^{23}$ −0.8° (*c* 0.86, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1142 $[M + Na]^+$; HRFABMS *m*/*z* 1141.5391 $[M + Na]^+$ (calcd for $C_{54}H_{86}O_{24}Na$, 1141.5407).

Hydrocotyloside IV (4): 14.8 mg; amorphous powder; $[\alpha]_D^{23}$ −1.1° (*c* 0.63, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1142 $[M + Na]^+$; HRFABMS *m*/*z* 1141.5388 $[M + Na]^+$ (calcd for $C_{54}H_{86}O_{24}Na$, 1141.5407).

Hydrocotyloside V (5): 34.9 mg; amorphous powder; $[\alpha]_D^{23}$ −1.5° (*c* 0.85, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1260 $[M + Na]^+$; HRFABMS *m*/*z* 1259.6033 $[M + Na]^+$ (calcd for $C_{59}H_{96}O_{27}Na$, 1259.6037).

Hydrocotyloside VI (6): 3.2 mg; amorphous powder; $[\alpha]_D^{23}$ −1.2° (*c* 0.34, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1274 $[M + Na]^+$; HRFABMS *m*/*z* 1273.6184 $[M + Na]^+$ (calcd for $C_{60}H_{98}O_{27}Na$, 1273.6193).

Hydrocotyloside VII (7): 55.3 mg; amorphous powder; $[\alpha]_D^{23}$ −1.5° (*c* 0.81, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1216 $[M + Na]^+$; HRFABMS *m*/*z* 1215.5748 $[M + Na]^+$ (calcd for $C_{57}H_{92}O_{26}Na$, 1215.5775).

Udosaponin B {3-*O*-[*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranosylolean-12-en-28-oic acid} (8): 28.0 mg; FABMS *m*/*z* 979 $[M + Na]^+$.

Acid and Alkaline Hydrolysis of Saponins. Each saponin (1 mg) was dissolved in 1 M NaOH (200 μL) and stirred for 3 h at room temperature. Then, the solution was acidified with 2 M HCl (300 μL) and extracted with EtOAc twice. For ester analysis, the EtOAc layer was washed with H_2O, *O*-(4-nitrobenzyl)-*N*,*N*′-diisopropylisourea (1 mg) was added, and the mixture was heated at 80 °C for 1 h. The reaction mixture was concentrated, and the residue was subjected to HPLC analysis [column, Develosil PhA (Nomura Chemical), 4.6 mm × 25 cm; solvent, CH_3CN-H_2O (45:55); flow rate, 1.0 mL/min; detection, UV 273 nm] for detection of esters. Acetic acid (t_R, 9.4 min) was detected from **1**−**7**, propanoic acid (t_R, 4.4 min) from **1**, 2-methylpropanoic acid (t_R, 15.2 min) from **2**−**5**, and 2-methylbutanoic acid (t_R, 20.1 min) from **6**. The H_2O layer was heated at 100 °C for 1 h. The solution was diluted with H_2O and extracted twice with EtOAc. Then, $AgCO_3$ (3 mg) was added to the H_2O layer, and the mixture was stirred and centrifuged. The supernatant was taken and concentrated. The residue was dissolved in 30 μL of pyridine with D-cysteine methyl ester (3 mg) and stirred for 1.5 h at 60 °C. After derivatization, a mixture of hexamethyldisilazane and trimethylsilyl chloride (9:1, 20 μL) was added to the solution and stirred for 30 min at 60 °C. The reaction solution was centrifuged, then the supernatant was analyzed by GC (column, Supelco SPB-1, 0.25 mm × 27 m; column temperature, 215 °C; carrier gas, N_2), and D-glucuronic acid (t_R, 15.3 min) was

detected from **1**−**4**, D-glucose (t_R, 20.9 min) from **1**−**7**, and L-arabinose (t_R, 11.8 min) from **5**−**7**, by comparison with the retention times of authentic samples.[11]

Supporting Information Available: Tables of HMBC and ROE correlations of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Barrera, A. F; Haïdour, A.; Sedqui, A.; Mansour, A. I.; Rodíguez-García, I.; López, A.; Muñoz Dorado, M. *Phytochemistry* **2000**, *54*, 741−745, and references therein.
(2) Matsuda, H.; Morikawa, T.; Ueda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, *49*, 1368−1371, and references therein.
(3) Arda, N.; Gören, N.; Kuru, A.; Pengsuparp, T.; Pezzuto, M. J.; Sheng-XIang, Q.; Cordell, A. G. *J. Nat. Prod.* **1997**, *60*, 1170−1173, and references therein.
(4) Schöpke, T.; Janka, M.; Nimtz, M.; Wray, V.; Hiller, K. *Planta Med.* **1998**, *64*, 83−85, and references therein.
(5) Greca, M. D.; Fiorentino, A.; Monaco, P.; Previtera, L. *Phytochemistry* **1994**, *36*, 1479−1483.
(6) Corsaro, M. M.; Greca, M. D.; Fiorentino, A.; Monaco, P.; Previtera, L. *Nat. Prod. Lett.* **1995**, *6*, 95−102.
(7) Mitsuhashi, H. *Illustrated Medicinal Plants of The World in Colour;* Hokuryukan Co., Ltd. Press: Tokyo, 1988; p 362.
(8) Greca, M. D.; Fiorentino A.; Monaco, P.; Previtera, L. *Phytochemistry* **1993**, *35*, 201−204.
(9) Lavaud, C.; Massiot, G; Oliver, M. L. L.; Viari, A.; Vigny, P.; Delaude, C. *Phytochemistry* **1992**, *31*, 3177−3181.
(10) Kawai, H.; Nishida, M.; Tashiro, Y; Kuroyanagi, M.; Ueno, A.; Satake, M. *Chem. Pharm. Bull.* **1989**, *37*, 2318−2321.
(11) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1986**, *34*, 1843−1844.

NP030356Z