Triterpene Saponins from the Pericarps of *Akebia trifoliata*

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Eleven new triterpene saponin components (1–11) were isolated from the MeOH extract of pericarp of *Akebia trifoliata* (Thunb.) Koidz. Each of their structures was determined using NMR techniques and mass spectrometry.

Key words *Akebia trifoliata*; Lardizabalaceae; triterpene saponin; akemisaponin

Akebia trifoliata (Thunb.) Koidz. (Lardizabalaceae) is used as a diuretic and an antiphlogistic in traditional Chinese medicine. Use of the stem of *A. trifoliata*, known by the Japanese name *moku-tsu*, is described by the Pharmacopoeia of Japan. Phytochemical analyses were previously carried out on *Akebia quinata* (Houtt.) Decne[1–3] and stems of *A. trifoliata*[4,5] but only a few chemical papers have been published on other parts of *A. trifoliata*, therefore we analyzed pericarps of *A. trifoliata*. We now report on eleven new triterpene saponin components of the pericarps of *A. trifoliata*.

Results and Discussion

The dried pericarps of *A. trifoliata* were extracted with hot MeOH. The concentrated MeOH extract was separated into ether-soluble, ethyl acetate-soluble and water-soluble fractions. The water-soluble fraction was passed through a Diaion HP-20 column, eluted with MeOH–H_2O (1 : 1) and MeOH. A portion of the MeOH eluate was subjected to reversed-phase preparative HPLC, affording compounds **1**–**15** including eleven new compounds. By comparing their NMR data with those in the literature, compounds **12**–**15** were identified as scheffoleoside A (2*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid *O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester) (**12**),[4,6] asiaticoside (2*α*,3*β*,23-trihydroxyurs-12-en-28-oic acid *O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester) (**13**),[4,6,7] 2*α*,3*β*,23-trihydroxy-30-norolean-12,20(29)-dien-28-oic acid *O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**14**),[4] and 2*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**15**).[4]

Akemisaponin A (**1**) had the molecular formula $C_{53}H_{86}O_{22}$ as determined by positive mode high resolution (HR)-FAB-MS (*m*/*z* 1097.5518 $[M+Na]^+$). The ^{1}H-NMR spectrum showed signals for seven tertiary methyl groups [*δ* 1.24, 1.21, 1.10, 1.07, 1.03, 0.90×2 (each 3H, s)], an olefinic proton [*δ* 5.41 (1H, dd, *J*=3.0, 3.0 Hz)], two hydroxymethine protons [*δ* 4.08 (overlapped) and 3.37 (1H, d, *J*=9.0 Hz)], and a deshielding methine proton [*δ* 3.17 (1H, br d, *J*=14.0 Hz)] with four anomeric protons [*δ* 6.19 (1H, d, *J*=8.0 Hz), 5.80 (1H, br s), 5.20 (1H, d, *J*=8.0 Hz), and 4.93 (1H, d, *J*=8.0 Hz)]. Regarding the aglycone moiety of **1**, the ^{13}C-NMR and the ^{1}H-detected heteronuclear multiple-quantum coherency (HMQC) spectra indicated seven methyl carbon signals (*δ* 33.0, 29.2, 25.9, 23.6, 17.4×2, 16.8), a carbonyl carbon signal (*δ* 176.3), two olefinic carbon signals (*δ* 144.0, 122.6), and two hydroxymethine carbon signals (*δ* 83.6, 68.5). These results indicated that the aglycone of **1** was an olean-12-en-28-oic acid derivative. Based on the second dimensional (2D) NMR [^{1}H-detected heteronuclear multiple-bond connectivity (HMBC), HMQC, and ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY)] measurements of **1**, the methyl proton and carbon signals at *δ* 1.24, 29.2 and *δ* 1.07, 17.4 were assigned to H-23, C-23 and H-24, C-24, and additionally, two sets of hydroxymethine proton and carbon signals at *δ* 4.08, 68.5 and *δ* 3.37, 83.6 belonged to H-2, C-2 and H-3 and C-3. As the multiplicity and coupling constant of the H-3 signal (d, *J*=9.0 Hz) showed that H-2 and H-3 were both di-axial, the hydroxy groups at C-2 and C-3 were considered to retain the *α*- and *β*-orientations, respectively. Thus, the aglycone of **1** was determined as 2*α*,3*β*-dihydroxyolean-12-en-28-oic acid.[8] On acid hydrolysis, **1** gave a sugar moiety composed of D-glucose, L-rhamnose and D-xylose (2 : 1 : 1).[9] The ^{1}H-NMR signals of the sugar moiety were assigned as shown in Table 1 from the results of homonuclear Hartmann–Hahn (HOHAHA) difference experiments. The anomeric configurations of D-glucose and D-xylose were identified as the both *β*-form from the coupling constant of anomeric proton signals (*J*=8.0 Hz), and that of L-rhamnose, as the *α*-form from the chemical shift of an anomeric proton signal (*δ* 5.80) in the ^{1}H-NMR spectrum.[10] Observation of anomeric proton and carbon signals of the *β*-D-glucopyranosyl group at *δ* 6.19 and 95.5 indicated that this group was esterified at C-28 of the aglycone, which was confirmed by the long-range correlation between H-1 of this *β*-D-glucopyranosyl group (*δ* 6.19) and C-28 of the aglycone (*δ* 176.3). Other sugar linkages were determined based on the consequences of rotating frame nuclear Overhauser effect (ROE) difference experiments irradiating each anomeric proton signal. ROEs were observed between *δ* 5.20 (H-1 of *β*-D-xylopyranose) and *δ* 4.56 (H-3 of *α*-L-rhamnopyranose), *δ* 5.80 (H-1 of *α*-L-rhamnopyranose) and *δ* 4.39 (H-4 of *β*-D-glucopyranose), and *δ* 4.93 (H-1 of *β*-D-glucopyranose) and *δ* 4.62, 4.29 (H-6 of C-28-linked *β*-D-glucopyranose). On the basis of the above evidence, **1** was established to be 2*α*,3*β*-dihydroxyolean-12-en-28-oic acid *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

The molecular formula of akemisaponin B (**2**) was deduced as $C_{52}H_{82}O_{22}$ from positive mode HR-FAB-MS (*m*/*z* 1081.5209 $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectra of the sugar moiety in **2** were in good agreement with those of **1**. However, the NMR signals arising from the ring E part of the aglycone

The authors declare no conflict of interest.

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Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (400, 100 MHz) of Compounds **1**–**11**

Position	**1**				**2**			
	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE
1	1.27[a)]	47.6			1.27[a)]	47.9		
	2.24 (dd, 4.0, 12.0)		2, 3, 5, 10		2.24[a)]			
2	4.08[a)]	68.5			4.26[a)]	69.8		
3	3.37 (d, 9.0)	83.6	2, 4, 23, 24		3.37 (d, 9.0)	84.0	2, 4, 23, 24	
4		39.6				39.9		
5	1.01[a)]	55.8			1.01 (brd, 11.0)	56.1	24	
6	1.39[a)]	18.7			1.55[a)]	19.0		
	1.51[a)]							
7	1.37[a)]	33.9			1.34[a)]	33.0		
	1.53[a)]				1.51[a)]			
8		39.8				40.3		
9	1.77[a)]	48.0			1.78 (dd, 9.0, 9.0)	48.3	8, 10, 26	
10		38.4				38.7		
11	1.93[a)]	23.8			1.99[a)]	24.1		
	2.00 (brd, 8.0)		12, 13					
12	5.41 (dd, 3.0, 3.0)	122.6	9, 14, 18		5.45 (dd, 3.0, 3.0)	123.1	9, 14, 18	
13		144.0				143.7		
14		42.1				42.3		
15	1.15[a)]	28.1			1.10[a)]	28.7		
	2.30[a)]				2.32[a)]			
16	2.07[a)]	23.2			1.88[a)]	26.1		
					2.02[a)]			
17		46.9				45.3		
18	3.17 (brd, 14.0)	41.5	13		3.13 (brdd, 9.0,9.0)	42.4	12, 13, 14, 16, 17, 19	
19	1.25[a)]	46.1			1.90[a)]	36.9		
	1.74[a)]				2.33[a)]			
20		30.6				132.7		
21	1.12[a)]	32.4			5.22 (brs)	117.6		
22	1.76[a)]	33.0			2.22[a)]	36.9		
	1.91[a)]				2.52 (brd, 18.0)			
23	1.24 (s)	29.2	3, 4, 5, 24		1.24 (s)	29.4	3, 4, 5, 24	
24	1.07 (s)	17.4	3, 4, 5, 23		1.08 (s)	17.6	3, 4, 5, 23	
25	1.03 (s)	16.8	1, 5, 9, 10		1.04 (s)	17.0	1, 5, 9, 10	
26	1.10 (s)	17.4	7, 8, 9, 14		1.11 (s)	17.7	7, 8, 9, 14	
27	1.21 (s)	25.9	8, 13, 14, 15		1.26 (s)	27.0	8, 13, 14, 15	
28		176.3				176.2		
29	0.90 (s)	33.0	19, 20, 21, 30		1.61 (brs)	23.2	19, 20, 21	
30	0.90 (s)	23.6	19, 20, 21, 29					
Glc (at C-28)								
1	6.19 (d, 8.0)	95.5	28		6.18 (d, 8.0)	95.8	28	
2	4.09 (dd, 8.0, 9.0)	73.7			4.10 (dd, 8.0, 9.0)	73.9		
3	4.17 (dd, 9.0, 9.0)	78.5			4.18 (dd, 9.0, 9.0)	78.7		
4	4.26 (dd, 9.0, 9.0)	70.7			4.26 (dd, 9.0, 9.0)	71.3		
5	4.05[a)]	77.8			4.04 (m)	77.9		
6a	4.29[a)]	69.1			4.28[a)]	68.8		
6b	4.62 (brd, 11.0)				4.63 (brd, 11.0)			
Glc′ (at C-6 of Glc)								
1	4.93 (d, 8.0)	104.7	Glc-6	Glc-6a, 6b Glc′-2, 3, 5	4.91 (d, 8.0)	105.1	Glc-6	Glc-6a, 6b Glc′-2, 3, 5
2	3.89 (dd, 8.0, 9.0)	75.2			3.91 (dd, 8.0, 9.0)	75.4		
3	4.12 (dd, 9.0, 9.0)	76.2			4.14 (dd, 9.0, 9.0)	76.6		
4	4.39 (dd, 9.0, 9.0)	77.4			4.41 (dd, 9.0, 9.0)	78.1		
5	3.58 (m)	76.9			3.59 (m)	77.2		
6a	4.05 (dd, 2.0, 13.0)	61.1			4.05 (brd, 10.0)	61.6		
6b	4.17 (brd, 13.0)				4.16[a)]			
Rha								
1	5.80 (brs)	102.2	Glc′-4 Rha-2, 3, 5	Glc′-4, 6a Rha-2	5.83 (brs)	102.5	Glc′-4 Rha-2, 3, 5	Glc′-4, 6a, 6b Rha-2
2	4.81 (brs)	71.9			4.82 (brs)	72.1		
3	4.56 (dd, 3.0, 9.0)	83.0			4.58 (dd, 3.0, 9.0)	83.2		
4	4.45 (dd, 9.0, 9.0)	72.7			4.46 (dd, 9.0, 9.0)	72.9		
5	5.00 (dq, 9.0, 6.0)	69.8			5.03 (dq, 9.0, 6.0)	70.2		
6	1.63 (d, 6.0)	18.2			1.64 (d, 6.0)	18.4		
Xyl								
1	5.20 (d, 8.0)	107.0	Rha-3	Rha-3, 4 Xyl-3, 5	5.21 (d, 8.0)	107.1	Rha-3	Rha-3, 4 Xyl-3, 5
2	4.01 (dd, 8.0, 9.0)	75.4			4.02 (dd, 8.0, 9.0)	75.5		
3	4.08 (dd, 9.0, 9.0)	78.1			4.09 (dd, 9.0, 9.0)	78.2		
4	4.12 (m)	70.8			4.11 (m)	71.1		
5	3.57 (dd, 10.0, 10.0) 4.23 (dd, 5.0, 10.0)	67.1			3.56 (dd, 10.0, 10.0) 4.23 (dd, 5.0, 10.0)	67.2		

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (400, 100 MHz) of Compounds **1**–**11**

Position	**3**				**4**			
	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE
1	1.26[a)]	47.9			1.36[a)]	47.8		
	2.23[a)]				2.28[a)]			
2	4.08[a)]	68.7			4.23[a)]	69.0		
3	3.37 (d, 9)	83.9	2, 4, 23, 24		4.18[a)]	78.4		
4		39.9				43.7		
5	0.99[a)]	56.0			1.78 (brd, 12.0)	48.1	4, 10, 24, 25	
6		19.0			1.47 (m)	18.7		
7	1.33[a)]	33.0			1.31[a)]	32.6		
	1.49[a)]				1.65[a)]			
8		40.2				40.2		
9	1.77 (dd, 9.0, 9.0)	48.3	8, 10, 26		1.89[a)]	48.3		
10		38.7				38.5		
11	1.98 (brd, 9.0)	24.0	12, 13		1.89[a)]	24.0		
					2.01[a)]			
12	5.44 (dd, 3.0, 3.0)	123.1	9, 14, 18		5.44 (dd, 3.0, 3.0)	123.1	9, 11, 14, 18	
13		143.6				143.7		
14		42.2				42.2		
15	1.12[a)]	28.7			1.05[a)]	28.7		
	2.30[a)]				2.30[a)]			
16	1.87[a)]	26.0			1.84[a)]	26.0		
	2.02[a)]				2.01[a)]			
17		45.3				45.2		
18	3.15 (dd, 9.0, 9.0)	42.3	12, 13, 14, 16, 19		3.12 (dd, 9.0, 9.0)	42.2	14, 16, 17, 19	
19	1.89[a)]	36.9			1.88[a)]	36.9		
	2.25[a)]				2.24[a)]			
20		132.7				132.6		
21	5.23 (brs)	117.5			5.20 (brs)	117.5		
22	2.19[a)]	36.8			2.19[a)]	36.7		
	2.54 (brd, 18.0)				2.52 (brd, 18.0)			
23	1.24 (s)	29.4	3, 4, 5, 24		3.68 (d, 10.0)	66.7	3, 4, 5, 24	
					4.17[a)]			
24	1.08 (s)	17.7	3, 4, 5, 23		1.07 (s)	14.3	3, 4, 5, 23	
25	1.04 (s)	17.0	1, 5, 9, 10		1.12 (s)	17.5	1, 5, 9, 10	
26	1.11 (s)	17.7	7, 8, 9, 14		1.14 (s)	17.7	7, 8, 14	
27	1.25 (s)	27.1	8, 13, 14, 15		1.20 (s)	27.0	8, 13, 14, 15	
28		176.3				176.2		
29	1.62 (brs)	23.3	19, 20, 21		1.59 (brs)	23.3	19, 20, 21	
30								
Glc (at C-28)								
1	6.16 (d, 8.0)	95.8	28		6.18 (d, 8.0)	95.8	28	
2	4.12 (dd, 8.0, 9.0)	73.9			4.09 (dd, 8.0, 9.0)	73.9		
3	4.18 (dd, 9.0, 9.0)	78.7			4.19 (dd, 9.0, 9.0)	78.7		
4	4.25 (dd, 9.0, 9.0)	71.1			4.27 (dd, 9.0, 9.0)	71.1		
5	4.05 (m)	78.0			4.16[a)]	77.9		
6a	4.27[a)]	69.6			4.28[a)]	69.6		
6b	4.63 (brd, 12.0)				4.63 (brd, 11.0)			
Glc′ (at C-6 of Glc)								
1	4.94 (d, 8.0)	105.0	Glc-6	Glc-6a, 6b Glc′-2, 3, 5	4.91 (d, 8.0)	105.2	Glc-6	Glc-6a, 6b Glc′-2, 3, 5
2	3.91 (dd, 8.0, 9.0)	75.4			3.91 (dd, 8.0, 8.0)	75.5		
3	4.11 (dd, 9.0, 9.0)	76.6			4.14 (dd, 8.0, 9.0)	76.5		
4	4.34 (dd, 9.0, 9.0)	78.6			4.42 (dd, 9.0, 9.0)	77.6		
5	3.65 (m)	77.2			3.61 (m)	77.2		
6a	4.06 (dd, 3.0, 13.0)	61.5			4.06 (brd, 10.0)	61.4		
6b	4.19 (brd, 13.0)				4.19[a)]			
Rha								
1	5.78 (brs)	102.8	Glc′-4 Rha-2, 3, 5	Glc′-4, 6a Rha-2	5.83 (brs)	102.5	Glc′-4 Rha-2, 3, 5	Glc′-4, 6a, 6b Rha-2
2	4.62 (brs)	72.6			4.83 (brs)	72.2		
3	4.49 (dd, 3.0, 9.0)	72.8			4.59 (dd, 3.0, 9.0)	83.4		
4	4.27 (dd, 9.0, 9.0)	74.0			4.47 (dd, 9.0, 9.0)	73.0		
5	4.87 (dq, 9.0, 6.0)	70.4			5.03 (dq, 9.0, 6.0)	70.1		
6	1.66 (d, 6.0)	18.6			1.65 (d, 6.0)	18.5		
Xyl								
1					5.21 (d, 8.0)	107.3	Rha-3	Rha-3, 4 Xyl-3, 5
2					4.03 (dd, 8.0, 8.0)	75.7		
3					4.10 (dd, 8.0, 9.0)	78.4		
4					4.16 (m)	71.1		
5					3.57 (dd, 10.0, 11.0)	67.3		

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (400, 100 MHz) of Compounds **1**–**11**

					4.24 (dd, 5.0, 10.0)			
Position	**5**				**6**			
	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE
1	1.35[a)]	47.8			1.38[a)]	47.7		
	2.26[a)]				2.29[a)]			
2	4.17[a)]	69.0			4.17[a)]	68.1		
3	4.06[a)]	78.0			4.03[a)]	77.2		
4		43.7				56.6		
5	1.77 (brd, 12.0)	48.1	3, 4, 7, 9, 10, 23, 24, 25		1.62[a)]	48.2		
6	1.46 (m)	18.7			1.07[a)]	20.7		
					1.50[a)]			
7	1.31[a)]	32.6			1.22[a)]	32.2		
	1.65[a)]				1.51[a)]			
8		40.3				40.2		
9	1.88[a)]	48.3			1.90[a)]	48.2		
10		38.5				38.5		
11	1.87[a)]	24.1			1.92[a)]	24.0		
	1.99[a)]				2.00[a)]			
12	5.43 (dd, 3.0, 3.0)	123.1	11, 14, 18		5.45 (dd, 3.0, 3.0)	122.8	9, 11, 14, 18	
13		143.7				143.7		
14		42.2				42.2		
15	1.05[a)]	28.7			1.06[a)]	28.6		
	2.27[a)]				2.27[a)]			
16	1.84[a)]	26.0			1.89[a)]	25.9		
	1.98[a)]				2.03[a)]			
17		45.2				45.2		
18	3.12 (dd, 9.0, 9.0)	42.2	12, 13, 14, 16, 17, 19, 28		3.14 (dd, 9.0, 9.0)	42.2	7, 12, 13, 14, 19	
19	1.87[a)]	36.9			1.91[a)]	36.8		
	2.24[a)]				2.26[a)]			
20		132.7				132.5		
21	5.21 (brs)	117.5	17, 19, 29		5.23[a)]	117.5		
22	2.18[a)]	36.8			2.21[a)]	36.7		
	2.53 (brd, 18.0)		21		2.53 (brd, 18.0)			
23	3.66 (d, 11.0)	66.8	3, 4, 5, 24		9.62 (s)	206.5	4	
	4.15[a)]							
24	1.06 (s)	14.4	3, 4, 5, 23		1.43 (s)	10.7	3, 4, 5, 23	
25	1.11 (s)	17.6	1, 5, 9, 10		1.07 (s)	17.2	1, 5, 9, 10	
26	1.13 (s)	17.7	7, 8, 9, 14		1.09 (s)	17.5	7, 8, 9, 14	
27	1.19 (s)	27.1	8, 13, 14, 15		1.26 (s)	27.0	8, 13, 14, 15	
28		176.3				176.1		
29	1.60 (brs)	23.3	19, 20, 21		1.62 (brs)	23.3	19, 20, 21	
30								
Glc (at C-28)								
1	6.16 (d, 8.0)	95.8	28		6.18 (d, 8.0)	95.7	28	
			Glc-3					
2	4.09 (dd, 8.0, 9.0)	73.9			4.09 (dd, 8.0, 9.0)	73.9		
3	4.18 (dd, 9.0, 9.0)	78.4			4.18 (dd, 9.0, 9.0)	78.7		
4	4.25 (dd, 9.0, 9.0)	71.1			4.28 (dd, 9.0, 9.0)	71.0		
5	4.05[a)]	78.7			4.03 (m)	77.9		
6a	4.27[a)]	69.6			4.25[a)]	69.6		
6b	4.63 (brd, 11.0)				4.64 (brd, 10.0)			
Glc′ (at C-6 of Glc)								
1	4.94 (d, 8.0)	105.0	Glc-6	Glc-6a, 6b	4.91 (d, 8.0)	105.2	Glc-6	Glc-6a
			Glc′-2, 5	Glc′-2, 3, 5				Glc′-3, 5
2	3.91 (dd, 8.0, 9.0)	75.4			3.92 (dd, 8.0, 9.0)	75.4		
3	4.12 (dd, 9.0, 9.0)	76.6			4.14 (dd, 9.0, 9.0)	76.5		
4	4.34 (dd, 9.0, 9.0)	78.6			4.43 (dd, 9.0, 9.0)	77.5		
5	3.65 (m)	77.2			3.61 (m)	77.2		
6a	4.06 (dd, 2.0, 13.0)	61.5			4.07 (dd, 2.0, 13.0)	61.4		
6b	4.20 (brd, 13.0)				4.19 (brd, 13.0)			
Rha								
1	5.77 (brs)	102.8	Glc′-4	Glc′-4, 6a, 6b	5.84 (brs)	102.4	Glc′-4	Glc′-4, 6a
			Rha-2, 3, 5	Rha-2			Rha-2, 3, 5	Rha-2
2	4.61 (brs)	72.6			4.84 (brs)	72.1		
3	4.49 (dd, 2.0, 9.0)	72.8			4.59 (dd, 3.0, 9.0)	83.4		
4	4.27 (dd, 9.0, 9.0)	74.0			4.47 (dd, 9.0, 9.0)	73.0		
5	4.87 (dq, 9.0, 6.0)	70.4			5.04 (dq, 9.0, 6.0)	70.1		
6	1.66 (d, 6.0)	18.6			1.65 (d, 6.0)	18.4		
Xyl								
1					5.22 (d, 8.0)	107.3	Rha-3	Rha-3, 4
								Xyl-3, 5
2					4.03 (dd, 9.0, 9.0)	75.7		
3					4.09 (dd, 9.0, 9.0)	78.4		
4					4.17 (m)	71.1		
5					3.57 (dd, 9.0, 11.0)	67.3		

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (400, 100 MHz) of Compounds **1**–**11**

Position	4.24 (dd, 5.0, 11.0)							
	7				**8**			
	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE
1	1.37 (dd, 12.0, 12.0)	47.7	25		1.30 (brdd, 12.0,12.0)	48.2	2, 3, 10, 25	
	2.27[a)]				2.29[a)]			
2	4.21[a)]	68.2			4.05[a)]	68.8		
3	4.01 (d, 9.0)	77.2	2, 4, 23		3.37 (d, 9.0)	84.0	2, 4, 23, 24	
4		56.6				39.8		
5	1.60[a)]	48.2			1.02[a)]	56.2		
6	1.04[a)]	20.8			1.39[a)]	18.9		
	1.48[a)]							
7	1.20 (brd, 10.0)	32.3	9, 26		1.39[a)]	33.9		
	1.49[a)]				1.53[a)]			
8		40.2				39.8		
9	1.87[a)]	48.2			1.75[a)]	48.4		
10		38.5				38.7		
11	1.89[a)]	24.0			1.92[a)]	24.0		
	1.99[a)]				1.97[a)]			
12	5.44 (dd, 3.0, 3.0)	122.8	9, 14, 18		5.55 (dd, 3.0, 3.0)[b)]		11, 14, 18	
13		143.7				143.0		
14		42.2				43.3		
15	1.06[a)]	28.6			1.19[a)]	28.1		
	2.26[a)]				2.28[a)]			
16	1.85[a)]	25.9			1.75[a)]	26.4		
	2.00[a)]				2.00[a)]			
17		45.2				46.1		
18	3.13 (dd, 9.0, 9.0)	42.2	12, 13, 17, 19		3.65 (brs)	46.0	19, 28	
19	1.90[a)]	36.9			5.23[a)]	129.5	18	
	2.25[a)]							
20		132.6				130.0		
21	5.23 (brs)	116.3				23.2		
22	2.19[a)]	36.7			1.77[a)]	32.7		
	2.53 (brd, 18.0)				2.02[a)]			
23	9.61 (s)	206.5	4, 24		1.24 (s)	29.4	3, 4, 5, 24	
24	1.41 (s)	10.8	3, 4, 5, 23		1.07 (s)	17.6	3, 4, 5, 23	
25	1.06 (s)	17.2	1, 5, 9, 10		1.04 (s)	17.3	1, 5, 9, 10	
26	1.07 (s)	17.6	7, 8, 9, 14		1.12 (s)	17.9	7, 8, 9, 14	
27	1.25 (s)	27.1	8, 13, 14, 15		1.16 (s)	23.6	8, 13, 14, 15	
28		176.2				176.3		
29	1.63 (brs)	23.3	19, 20, 21		1.60 (brs)	23.1	19, 20, 21	
30								
Glc (at C-28)								
1	6.16 (d, 8.0)	95.8	28		6.24 (d, 8.0)	95.9	28	
2	4.09 (dd, 8.0, 9.0)	73.9			4.09 (dd, 8.0, 9.0)	74.1		
3	4.18 (dd, 9.0, 9.0)	78.7			4.19 (dd, 9.0, 9.0)	78.7		
4	4.26 (dd, 9.0, 9.0)	71.0			4.29 (dd, 9.0, 9.0)	71.3		
5	4.05[a)]	78.0			4.03 (m)	78.0		
6a	4.30[a)]	69.5			4.26[a)]	69.8		
6b	4.63 (brd, 11.0)				4.63 (brd, 11.0)			
Glc′ (at C-6 of Glc)								
1	4.94 (d, 8.0)	105.0	Glc-6	Glc-6a, 6b Glc′-2, 3, 5	4.91 (d, 8.0)	105.1	Glc-6 Glc′-5	Glc-6a, 6b Glc′-2, 3, 5
2	3.91 (dd, 8.0, 9.0)	75.3			3.92 (dd, 8.0, 9.0)	75.4		
3	4.12 (dd, 9.0, 9.0)	76.6			4.15 (dd, 9.0, 9.0)	76.6		
4	4.35 (dd, 9.0, 9.0)	78.5			4.44 (dd, 9.0, 9.0)	77.9		
5	3.65 (m)	77.2			3.61 (m)	77.2		
6a	4.07 (dd, 2.0, 12.0)	61.5			4.04 (brd, 11.0)	61.6		
6b	4.20 (brd, 12.0)				4.18[a)]			
Rha								
1	5.79 (brs)	102.8	Glc′-4 Rha-2, 3, 5	Glc′-4, 6a Rha-2	5.82 (brs)	102.5	Glc′-4 Rha-2, 3, 5	Glc′-4, 6a, 6b Rha-2
2	4.63 (brs)	72.6			4.85 (brs)	72.1		
3	4.50 (dd, 2.0, 9.0)	72.8			4.60 (dd, 3.0, 9.0)	83.3		
4	4.28 (dd, 9.0, 9.0)	74.0			4.48 (dd, 9.0, 9.0)	72.9		
5	4.88 (dq, 9.0, 6.0)	70.4			5.05 (dq, 9.0, 6.0)	70.1		
6	1.67 (d, 6.0)	18.6			1.65 (d, 6.0)	18.4		
Xyl								
1					5.22 (d, 8.0)	107.1	Rha-3	Rha-3, 4 Xyl-3, 5
2					4.03 (dd, 9.0, 9.0)	75.5		
3					4.10 (dd, 9.0, 9.0)	78.2		
4					4.15 (m)	71.1		
5					3.57 (dd, 10.0, 10.0)	67.2		

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (400, 100 MHz) of Compounds **1**–**11**

					4.24 (dd, 5.0, 10.0)			
Position	**9**				**10**			
	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE
1	1.25[a)]	47.9			1.25[a)]	47.9		
	2.22[a)]				2.22[a)]			
2	4.08[a)]	68.7			4.08[a)]	68.7		
3	3.38 (d, 9.0)	83.9	2, 4, 23, 24		3.38 (d, 9.0)	83.9	2, 4, 23, 24	
4		39.9				39.9		
5	1.00[a)]	56.0			1.00[a)]	56.0		
6	1.39[a)]	19.0			1.38[a)]	19.0		
7	1.36[a)]	33.2			1.35[a)]	33.2		
	1.52 (m)		6, 8		1.50 (m)		6, 8	
8		40.1				40.1		
9	1.74[a)]	48.2			1.73 (dd, .9.0, 9.0)	48.2	8, 10, 26	
10		38.6				38.6		
11	1.73[a)]	24.0			1.95 (brd, 9.0)	24.0	12, 13	
	1.96[a)]							
12	5.43 (dd, 3.0, 3.0)[b)]		9, 14, 18		5.42 (dd, 3.0, 3.0)[b)]		9, 14, 18	
13		143.6				143.6		
14		42.3				42.2		
15	1.17[a)]	28.3			1.17[a)]	28.3		
	2.30[a)]				2.29 (brdd, 13.0, 13.0)			
16	2.04[a)]	23.6			2.04[a)]	23.6		
	2.18[a)]				2.18[a)]			
17		47.4				47.5		
18	3.10 (dd, 5.0, 13.0)	47.7	14, 16, 17		3.10 (dd, 4.0, 8.0)	47.6	14, 17	
19	2.19[a)]	41.8			2.19[a)]	41.8		
	2.57 (dd, 13.0, 13.0)		18, 20, 29		2.57 (dd, 13.0, 13.0)		18, 20, 29	
20		148.5				148.4		
21	2.08[a)]	30.2			2.07[a)]	30.2		
	2.20[a)]				2.20[a)]			
22	1.72[a)]	37.7			1.73[a)]	37.7		
	2.03[a)]				2.03[a)]			
23	1.25 (s)	29.4	3, 4, 5, 24		1.25 (s)	29.4	3, 4, 5, 24	
24	1.07 (s)	17.7	3, 4, 5, 23		1.07 (s)	17.7	3, 4, 5, 23	
25	1.02 (s)	17.1	1, 5, 9, 10		1.02 (s)	17.1	1, 5, 9, 10	
26	1.09 (s)	17.7	7, 8, 9, 14		1.08 (s)	17.7	7, 8, 9, 14	
27	1.19 (s)	26.1	8, 13, 14, 15		1.19 (s)	26.1	8, 13, 14, 15	
28		175.9				175.9		
29	4.68 (brs)	107.4	19, 21		4.68 (brs)	107.5	19, 21	
	4.74 (brs)		19, 21		4.74 (brs)		19, 21	
30								
Glc (at C-28)								
1	6.16 (d, 8.0)	95.8	28		6.15 (d, 8.0)	95.8	28	
2	4.08 (dd, 8.0, 9.0)	73.9			4.08 (dd, 8.0, 9.0)	73.9		
3	4.17 (dd, 9.0, 9.0)	78.7			4.17 (dd, 9.0, 9.0)	78.7		
4	4.26 (dd, 9.0, 9.0)	71.0			4.25 (dd, 9.0, 9.0)	71.0		
5	4.04 (m)	78.0			4.03 (m)	78.0		
6a	4.27[a)]	69.5			4.28[a)]	69.5		
6b	4.61 (brd, 11.0)				4.62 (brd, 11.0)			
Glc′ (at C-6 of Glc)								
1	4.92 (d, 8.0)	105.0	Glc-6	Glc-6a, 6b Glc′-3, 5	4.94 (d, 8.0)	104.9	Glc-6	Glc-6a, 6b Glc′-2, 3, 5
2	3.90 (dd, 8.0, 9.0)	75.5			3.91 (dd, 8.0, 9.0)	75.3		
3	4.13 (dd, 9.0, 9.0)	76.5			4.12 (dd, 9.0, 9.0)	76.6		
4	4.41 (dd, 9.0, 9.0)	77.5			4.35 (dd, 9.0, 9.0)	78.5		
5	3.59 (brd, 9.0)	77.2			3.65 (m)	77.2		
6a	4.12 (dd, 2.0, 13.0)	61.4			4.07 (dd, 2.0, 12.0)	61.5		
6b	4.38 (brd, 13.0)				4.20 (brd, 12.0)			
Rha								
1	5.82 (brs)	102.4	Glc′-4 Rha-2, 3, 5	Glc′-4, 6a, 6b Rha-2	5.79 (brs)	102.8	Glc′-4 Rha-2, 3, 5	Glc′-4, 6a, 6b Rha-2
2	4.83 (brs)	72.2			4.63 (brs)	72.6		
3	4.49 (dd, 2.0, 9.0)	83.4			4.51 (dd, 2.0, 9.0)	72.8		
4	4.46 (dd, 9.0, 9.0)	73.0			4.29 (dd, 9.0, 9.0)	74.0		
5	5.01 (dq, 9.0, 6.0)	70.1			4.89 (dq, 9.0, 6.0)	70.4		
6	1.64 (d, 6.0)	18.5			1.67 (d, 6.0)	18.5		
Xyl								
1	5.22 (d, 8.0)	107.3	Rha-3	Rha-3, 4 Xyl-3, 5				
2	4.03 (dd, 8.0, 9.0)	75.7						
3	4.01 (dd, 9.0, 9.0)	78.4						
4	4.12 (m)	71.1						
5	3.57 (dd, 10.0, 10.0)	67.3						

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (400, 100 MHz) of Compounds **1**–**11**

	4.24 (dd, 5.0, 10.0)			
Position	**11**			
	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE
1	1.35[a)]	47.7	2, 5, 10, 25	
	2.27[a)]			
2	4.21[a)]	68.1		
3	4.05[a)]	77.2		
4		56.6		
5	1.61 (brd, 10.0)	48.2	4, 6, 7, 10, 24, 25	
6	1.07[a)]	20.7		
	1.49[a)]			
7	1.21[a)]	32.5		
	1.50[a)]			
8		40.1		
9	1.85 (brd, 8.0)	48.1		
10		38.5		
11	1.84[a)]	23.9		
	1.94 (brd, 8.0)		9, 12, 13	
12	5.42 (dd, 3.0, 3.0)	123.1	9, 11, 14	
13		143.6		
14		42.3		
15	1.05[a)]	28.2		
	2.27[a)]			
16	2.05[a)]	23.6		
	2.20[a)]			
17		47.4		
18	3.10 (dd, 4.0, 13.0)	47.6	12, 13, 14, 16, 17, 28	
19	2.20[a)]	41.7		
	2.56 (dd, 13.0, 13.0)		18, 20, 29	
20		148.4		
21	2.08[a)]	30.2		
	2.23[a)]			
22	1.74 (dd, 4.0, 13.0)	37.7	16, 17, 28	
	2.03[a)]			
23	9.64 (s)	206.5	4, 24	
24	1.42 (s)	10.8	3, 4, 5, 23	
25	1.05 (s)	17.2	1, 5, 9, 10	
26	1.05 (s)	17.6	7, 9, 14	
27	1.18 (s)	26.1	8, 13, 14, 15	
28		175.8		
29	4.69 (brs)	107.5	19, 21	
	4.75 (brs)		19, 21	
30				
Glc (at C-28)				
1	6.17 (d, 8.0)	95.8	28, Glc-5	
2	4.08 (dd, 8.0, 9.0)	73.9		
3	4.16 (dd, 9.0, 9.0)	78.7		
4	4.25 (dd, 9.0, 9.0)	71.0		
5	4.05 (m)	78.0		
6a	4.29[a)]	69.4		
6b	4.62 (brd, 12.0)			
Glc′ (at C-6 of Glc)				
1	4.95 (d, 8.0)	104.9	Glc-6	Glc-6a, 6b
			Glc′-5	Glc′-2, 3, 5
2	3.92 (dd, 8.0, 8.0)	75.3		
3	4.12 (dd, 8.0, 9.0)	76.6		
4	4.36 (dd, 9.0, 9.0)	78.5		
5	3.66 (m)	77.2		
6a	4.07[a)]	61.5		
6b	4.21 (brd, 11.0)			
Rha				
1	5.80 (brs)	102.8	Glc′-4	Glc′-4, 6a, 6b
			Rha-2, 3, 5	Rha-2
2	4.64 (brs)	72.6		
3	4.52 (dd, 3.0, 9.0)	72.8		
4	4.29 (dd, 9.0, 9.0)	74.0		
5	4.91 (dq, 9.0, 6.0)	70.4		
6	1.68 (d, 6.0)	18.5		
Xyl				
1				
2				
3				
4				
5				

Sugar proton signals were assigned from the HOHAHA difference spectra. *a*) Overlapped with other signals in each column. *b*) Overlapped with pyridine-d_5 signals at δ 123.

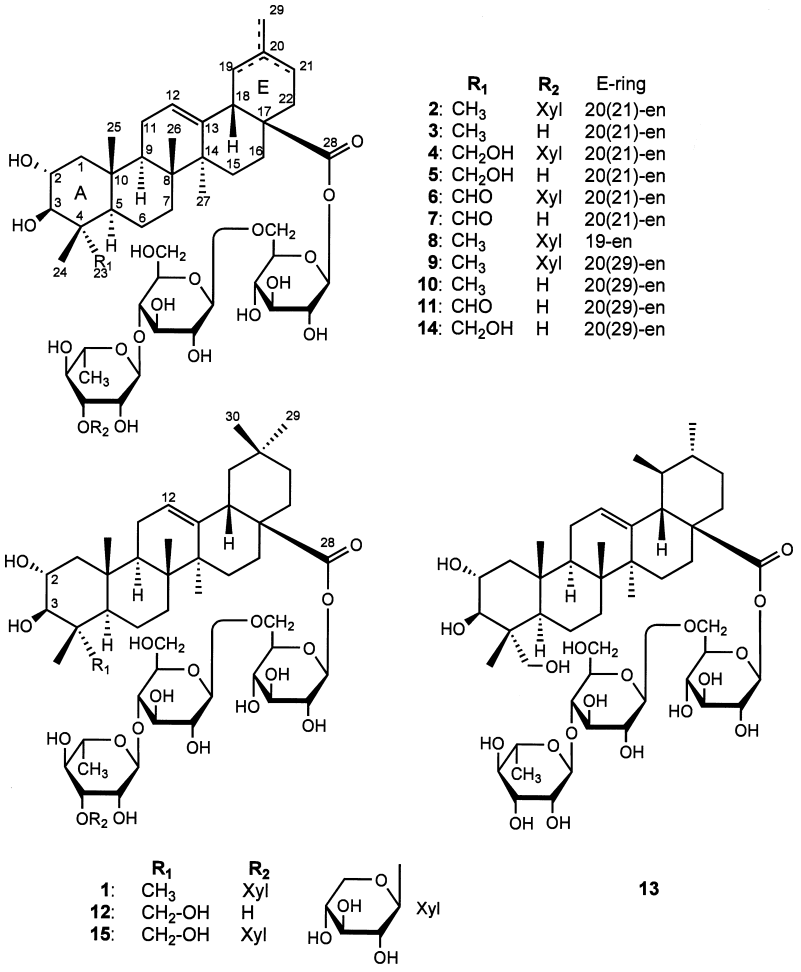

Chart 1. Structure of Compounds **1**–**15**

were different from those of **1**; the first difference was the lack of a tertiary methyl signal, and the second, the presence of a trisubstituted olefinic group [δ 5.22 (1H, brs) and δ 117.6, 132.7] other than that of 12-en and a methyl group on a double bond [δ 1.61 (3H, brs) and δ 23.2]. In the HMBC measurements, this methyl group (δ 1.61) showed long-range correlations with a methylene carbon (δ 36.9) as well as the olefinic carbons (δ 132.7, 117.6). In addition, a $^2J_{HCC}$ between the deshielding methine proton (δ 3.13, H-18) and the above methylene carbon (δ 36.9) suggested that this methylene carbon was assigned to C-19. Thus, the aglycone moiety of **2** was considered to possess the trisubstituted double bond between C-20 and C-21, and the methyl group at the C-29 position. These findings were quite consistent with those of 2α,3β-dihydroxy-30-norolean-12,20(21)-dien-28-oic acid.[4] Accordingly, **2** was determined as 2α,3β-dihydroxy-30-norolean-12,20(21)-dien-28-oic acid *O*-β-D-xylopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

The molecular formula of akemisaponin C (**3**) was revealed to be $C_{47}H_{74}O_{18}$ by positive HR-FAB-MS (*m*/*z* 949.4765 $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectra showed that **3** had the same aglycone, 2α,3β-dihydroxy-30-norolean-12,20(21)-dien-28-oic acid, as **2**, but differed in the oligosaccharide part related to C-28 of the aglycone. The deduced molecular formula was less than that of **2** by $C_5H_8O_4$, corresponding to the absence of a xylopyranosyl unit. Therefore, **3** was determined as 2α,3β-dihydroxy-30-norolean-12,20(21)-dien-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester. This sugar sequence was supported by the ROE difference experiments irradiating each anomeric proton and HMBC measurements.

Akemisaponins D (**4**) and F (**6**) were found to have the molecular formula $C_{52}H_{82}O_{23}$ and $C_{52}H_{80}O_{23}$, respectively, by positive HR-FAB-MS (*m*/*z* 1097.5139 $[M+Na]^+$ and *m*/*z* 1095.4987 $[M+Na]^+$). When the ^{13}C-NMR spectra of **4** and **6** were compared with that of **2**, the data for the sugar moiety and a majority of the aglycone were consistent. Moreover, the molecular formula of **4** had one more oxygen atom than that of **2**. The ^{1}H- and ^{13}C-NMR spectral data for **4** showed two hydroxymethyl proton signals at δ 3.68 (1H, d, J=10.0 Hz) and δ 4.17 (overlapped), and the corresponding hydroxymethyl carbon signal at δ 66.7. The above hydroxymethyl proton (δ 3.68) and the methyl protons (δ 1.07) exhibited long-range correlations to C-3 (δ 78.4), C-4 (δ 43.7), and C-5 (δ 48.1) in the HMBC measurement, suggesting that these hydroxymethyl and methyl groups were assigned to C-23 and C-24. Because the ^{13}C-NMR spectral data around the ring A part of **4** were

in good agreement with those of **12**[6] and **13**,[6] but were different from those of scheffoleoside F,[6] the hydroxymethyl group and methyl group were assigned to C-23 and C-24, respectively. Thus, **4** was elucidated to be 2*α*,3*β*,23-trihydroxy-30-norolean-12,20(21)-dien-28-oic acid *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester. For **6**, the ^{1}H- and ^{13}C-NMR spectral data suggested an aldehyde proton signal at *δ* 9.62 (1H, s), and the corresponding aldehyde carbon signal at *δ* 206.5. These signals were consistent with those of scheffursoside B[6] and scheffoleoside B.[6] Accordingly, this aldehyde group was also assignable to C-23, and **6** was identified as 2*α*,3*β*-dihydroxy-23-oxo-30-norolean-12,20(21)-dien-28-oic acid *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

Akemisaponins E (**5**) and G (**7**) were shown to have the molecular formula $C_{47}H_{74}O_{19}$ and $C_{47}H_{72}O_{19}$, respectively, on the basis of positive HR-FAB-MS (*m/z* 965.4739 $[M+Na]^+$ and *m/z* 963.4542 $[M+Na]^+$). The consistency of the ^{1}H- and ^{13}C-NMR spectral data due to the aglycone moieties of **5** and **7** with those of **4** and **6** suggested that **5** and **7** possessed 2*α*,3*β*,23-trihydroxy-30-norolean-12,20(21)-dien-28-oic acid and 2*α*,3*β*-dihydroxy-23-oxo-30-norolean-12,20(21)-dien-28-oic acid as each aglycone. Moreover, the sugar sequences in **5** and **7** were found to be the same as in **3** on comparison of NMR spectral data. Hence, the structures of **5** and **7** were established as shown in Chart 1.

Akemisaponin H (**8**) was proposed to have the molecular formula $C_{52}H_{82}O_{22}$ on the basis of positive mode HR-FAB-MS (*m/z* 1081.5189 $[M+Na]^+$), which was the same as that of **2**. The compound **8** also exhibited a trisubstituted double bond [*δ* 5.23 (overlapped) and *δ* 129.5, 130.0] and a methyl group on a double bond [*δ* 1.60 (3H, brs) and *δ* 23.1] the same as **2** in its NMR spectra. Moreover, H-18, which was observed at *δ* 3.13 (1H, brdd, *J*=9.0, 9.0 Hz) in **2**, was displayed as a broad singlet signal at *δ* 3.65 in **8**. H-18 showed a long-range correlation to the above sp^2 methine carbon (*δ* 129.5), together with C-28 (*δ* 176.3) in the HMBC measurements, suggesting that **8** had a part of 19-en. Therefore, the aglycone of **8** was determined as 2*α*,3*β*-dihydroxy-30-norolean-12,19-dien-28-oic acid. The structure of **8** is shown in Chart 1.

The molecular formula of akemisaponin I (**9**) was deduced as $C_{52}H_{82}O_{22}$ from positive mode HR-FAB-MS (*m/z* 1081.5195 $[M+Na]^+$). Regarding the aglycone moiety of **9**, the ^{1}H-NMR spectrum revealed signals for five tertiary methyl groups [*δ* 1.25, 1.19, 1.09, 1.07, and 1.02 (each 3H, s)], an olefinic proton [*δ* 5.43 (1H, dd, *J*=3.0, 3.0 Hz)], and two characteristic exomethylene protons [*δ* 4.74 (1H, brs) and 4.68 (1H, brs)]. The ^{13}C-NMR spectrum also showed signals for the exomethylene group at *δ* 107.4 and 148.5. The long-range correlations from these exomethylene protons to C-19 (*δ* 41.8) and C-21 (*δ* 30.2) in the HMBC measurement indicated that this exomethylene group was assigned to C-29. Moreover, the ^{13}C-NMR spectral data due to the aglycone moiety of **9** were superimposable on those of 2*α*,3*β*-dihydroxy-30-norolean-12,20(29)-dien-28-oic acid[4] except for the C-28 position. The ^{1}H- and ^{13}C-NMR spectral data due to the sugar moiety were in good agreement with those of **1**, suggesting that **9** possessed the same sugar sequence as **1**. Hence, **9** was assigned as 2*α*,3*β*-dihydroxy-30-norolean-12,20(29)-dien-28-oic acid *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

The molecular formula of akemisaponin J (**10**) was determined by positive HR-FAB-MS (*m/z* 949.4748 $[M+Na]^+$) to be $C_{47}H_{74}O_{18}$, which was the same as that of **3**. The ^{1}H- and ^{13}C-NMR spectra showed that **10** had the same aglycone, 2*α*,3*β*-dihydroxy-30-norolean-12,20(29)-dien-28-oic acid, as **9**, and the sugar moiety was consistent with those of **3**. Thus, **10** was identified as 2*α*,3*β*-dihydroxy-30-norolean-12,20(29)-dien-28-oic acid *O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

Akemisaponin K (**11**) was shown to have the molecular formula $C_{47}H_{72}O_{19}$ by positive HR-FAB-MS (*m/z* 963.4584 $[M+Na]^+$). All the spectral properties of **11** showed a close similarity to those of **10**. On comparison of the ^{1}H- and ^{13}C-NMR spectra of **11** with those of **10**, the C-23 tertiary methyl group observed in **10** at *δ* 1.25 and *δ* 29.4 were replaced by signals assignable to an aldehyde group at *δ* 9.64 and *δ* 206.5 in **11**. Thus, the structure of **11** was characterized as 2*α*,3*β*-dihydroxy-23-oxo-30-norolean-12,20(29)-dien-28-oic acid *O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

We now report the isolation and structural elucidation of eleven new triterpene saponins from the pericarps of *A. trifoliata*. The stems of *A. trifoliata* afforded several bisdesmoside-triterpene saponins possessing sugar sequences at the C-3 and C-28 positions together with monodesmosides.[4,5] In spite of this detailed investigation, the pericarps of *A. trifoliate* yielded only monodesmoside-tritepene saponins having sugars at C-28. Bisdesmosides were not obtained. However, among the saponins in the stems and pericarps of *A. trifoliata*, the sugar sequences at C-28 were consistent. Of the 15 triterpene saponins from the pericarps of *A. trifoliate*, six belonged to the 30-norolean-20(21)-en type triterpene saponins. This type of triterpene saponins is known to exist the stems of *A. trifoliata*,[4] but few 30-norolean-20(21)-en type triterpene saponins are known from other plants.[11] Thus, this type-triterpene saponin may be characteristic of *A. trifoliate*. 30-Norolean-19-en type triterpene saponins had not been obtained from any other plants before, and this is the first report. 30-Norolean-20(29)-en type triterpene saponins were shown in the Araliaceaus[12] and Eupteleaceaus families,[13] together with the Lardizabalaceaus family.[4,5,14–16]

Experimental

General Procedures Optical rotations were measured on a JASCO P-2200 digital polarimeter. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were recorded on a JEOL *α*-400 FT-NMR spectrometer, and chemical shifts are given as *δ* values with tetramethylsilane (TMS) as the internal standard at 35°C in pyridine-d_5. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H}$=145 Hz) and HMBC (optimized for $^nJ_{C-H}$=8 Hz) pulse sequences with a pulse field gradient. HR-FAB-MS data were obtained on a JEOL JMS 700 mass spectrometer in the positive mode using *m*-nitrobenzyl alcohol as the matrix. Preparative HPLC was performed on a JASCO 800. GC was run on a Hitachi G-3000 gas chromatograph.

Plant Material The pericarps of *A. trifoliata* were collected in the medicinal plant garden of the University of Shizuoka, Shizuoka, Japan, in November 2008. The plant was identified by Dr. Toshio Miyase, a professor at the university.

A voucher specimen (No. 20081125) was deposited at the university's herbarium.

Extraction and Isolation The dried pericarps of *A. trifoliata* (dried weight 1.0 kg) were extracted with hot MeOH twice. The extract was concentrated under reduced pressure, and the dark brown concentrate (417 g) was suspended in hot water and extracted with ether continuously for 50 h. After the ether layer was removed and concentrated (concentrate weight 33 g), the water layer was extracted with ethyl acetate continuously for 120 h. The ethyl acetate layer was removed and concentrated (concentrate weight 43 g), and the water layer was fractionated on a Mitsubishi Diaion HP-20 column (9×30 cm), eluting with water (10 L), MeOH–H_2O (1 : 1, v/v) (5 L) and MeOH (5 L). The fraction eluted with MeOH was concentrated under reduced pressure to give a brown concentrate (30 g). Four grams of the MeOH eluate was subjected to preparative HPLC [column, Tosoh TSKgel ODS-80Ts 5.5×180 cm; solvent, CH_3CN–H_2O (75 : 25–65 : 35, v/v) linear gradient, UV 205 nm] to give 16 fractions (Frs. A–P). The 74 : 26 solvent gave Fr. E [akemisaponin E (**5**) (670.3 mg)], the 73 : 27 solvent gave Fr. G [2*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**15**)[4] (727.2 mg)], the 72 : 28 solvent gave Fr. H [scheffoleoside A (**12**)[4,6] (202.5 mg)] and Fr. I [asiaticoside (**13**)[4,6,7] (116 mg)], the 70 : 30 solvent gave Fr. K [akemisaponin G (**7**) (84.3 mg)], the 68 : 32 solvent gave Fr. L [akemisaponin I (**9**) (23 mg)], the 67 : 33 solvent gave Fr. N [akemisaponin J (**10**) (44.5 mg)], the 66 : 34 solvent gave Fr. O [akemisaponin C (**3**) (54.8 mg)], and the 65 : 35 solvent gave Fr. P [akemisaponin A (**1**) (243.9 mg)]. Then, Fr. M (83.7 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2×25 cm; solvent, CH_3CN–H_2O (70 : 30, v/v), UV 205 nm] to give akemisaponin B (**2**) (44.2 mg) and akemisaponin H (**8**) (15.1 mg). A part (150 mg) of Fr. C (408 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2×25 cm; solvent, CH_3CN–H_2O (75 : 25, v/v), UV 205 nm] to give akemisaponin D (**4**) (33.6 mg) and 2*α*,3*β*,23-trihydroxy-30-norolean-12,20(29)-dien-28-oic acid *O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**14**)[4] (71.2 mg). Fraction J (70.2 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, CH_3CN–H_2O (75 : 25, v/v), UV 205 nm] to give akemisaponin F (**6**) (20 mg) and akemisaponin K (**11**) (38.1 mg).

Akemisaponin A (**1**): Amorphous solid. $[\alpha]_D^{22}$ −17 (*c*=1.09, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 1097.5518 $[M+Na]^+$ (Calcd for $C_{53}H_{86}O_{22}Na$: 1097.5510).

Akemisaponin B (**2**): Amorphous solid. $[\alpha]_D^{22}$ −35 (*c*=0.98, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 1081.5209 $[M+Na]^+$ (Calcd for $C_{52}H_{82}O_{22}Na$: 1081.5197).

Akemisaponin C (**3**): Amorphous solid. $[\alpha]_D^{22}$ −29 (*c*=1.07, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 949.4765 $[M+Na]^+$ (Calcd for $C_{47}H_{74}O_{18}Na$: 949.4774).

Akemisaponin D (**4**): Amorphous solid. $[\alpha]_D^{22}$ −33 (*c*=0.98, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 1097.5139 $[M+Na]^+$ (Calcd for $C_{52}H_{82}O_{23}Na$: 1097.5146).

Akemisaponin E (**5**): Amorphous solid. $[\alpha]_D^{22}$ −32 (*c*=1.01, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 965.4739 $[M+Na]^+$ (Calcd for $C_{47}H_{74}O_{19}Na$: 965.4723).

Akemisaponin F (**6**): Amorphous solid. $[\alpha]_D^{22}$ −25 (*c*=1.84, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 1095.4987 $[M+Na]^+$ (Calcd for $C_{52}H_{80}O_{23}Na$: 1095.4989).

Akemisaponin G (**7**): Amorphous solid. $[\alpha]_D^{22}$ −16 (*c*=0.90, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 963.4542 $[M+Na]^+$ (Calcd for $C_{47}H_{72}O_{19}Na$: 963.4567).

Akemisaponin H (**8**): Amorphous solid. $[\alpha]_D^{22}$ −48 (*c*=1.56, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 1081.5189 $[M+Na]^+$ (Calcd for $C_{52}H_{82}O_{22}Na$: 1081.5197).

Akemisaponin I (**9**): Amorphous solid. $[\alpha]_D^{22}$ +1.0 (*c*=1.03, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 1081.5195 $[M+Na]^+$ (Calcd for $C_{52}H_{82}O_{22}Na$: 1081.5197).

Akemisaponin J (**10**): Amorphous solid. $[\alpha]_D^{22}$ +14 (*c*=1.02, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 949.4748 $[M+Na]^+$ (Calcd for $C_{47}H_{74}O_{18}Na$: 949.4774).

Akemisaponin K (**11**): Amorphous solid. $[\alpha]_D^{22}$ +24 (*c*=1.04, pyridine). ^{1}H- and ^{13}C-NMR: Table 1. HR-FAB-MS *m/z* 963.4584 $[M+Na]^+$ (Calcd for $C_{47}H_{72}O_{19}Na$: 963.4567).

Acid Hydrolysis and GC Analysis Compounds **1**–**11** (*ca.* 1 mg) were dissolved in dioxane (200 *μ*L) and 1 M HCl (200 *μ*L), respectively. The solution was heated at 100°C for 1 h. After hydrolysis, this reaction mixture was diluted with H_2O and extracted with EtOAc. The H_2O layer was neutralized with an Amberlite IRA-60E column, and the eluate was concentrated dry. The residue was stirred with D-cysteine methyl ester hydrochloride (3 mg) in pyridine (25 *μ*L) at 60°C for 1.5 h. After warming, the trimethylsilylation reagent (hexamethyldisilazane and trimethylsilyl chloride) was added, and the warming at 60°C was continued for another 30 min. The precipitates were centrifuged, and the supernatant was subjected to GC analysis. GC conditions: column, GL Sciences capillary column TC-1 0.25 mm×30 m; carrier gas, N_2; column temperature 230°C; t_R 18.7 min (D-glucose), 18.0 min (L-glucose), 10.9 min (D-xylose), 10.3 min (L-xylose), 12.6 min (L-rhamnose), 12.3 min (D-rhamnose). D-Glucose and L-rhamnose were detected in all compounds. D-Xylose was found in **1**, **2**, **4**, **6**, **8**, and **9**. Retention times for L-glucose, L-xylose and D-rhamnose were obtained from their enantiomer made by using L-cysteine methyl ester hydrochloride.

References

1) Ikuta A., Itokawa H., *Phytochemistry*, **27**, 3809–3810 (1988).
2) Higuchi R., Kawasaki T., *Chem. Pharm. Bull.*, **24**, 1021–1032 (1976).
3) Jiang D., Gao Q. P., Shi S. P., Tu P. F., *Chem. Pharm. Bull.*, **54**, 595–597 (2006).
4) Mimaki Y., Kuroda M., Yokosuka A., Harada H., Fukushima M., Sashida Y., *Chem. Pharm. Bull.*, **51**, 960–965 (2003).
5) Gao H., Wang Z., *Phytochemistry*, **67**, 2697–2705 (2006).
6) Maeda C., Ohtani K., Kasai R., Yamasaki K., Duc N. M., Nham N. T., Cu N. K. Q., *Phytochemistry*, **37**, 1131–1137 (1994).
7) Mahato S. B., Sahu N. P., Luger P., Müller E., *J. Chem. Soc., Perkin Trans. 2*, **1987**, 1509–1515 (1987).
8) Singh B., Singh V. P., Pandey V. B., Rücher G., *Planta Med.*, **61**, 576–577 (1995).
9) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **34**, 1843–1845 (1986).
10) Kasai R., Okihara M., Asakawa J., Mizutani K., Tanaka O., *Tetrahedron*, **35**, 1427–1432 (1979).
11) Gao H., Wang Z., Yao Z.-H., Wu N., Dong H.-J., Li J., Wang N.-L., Ye W.-C., Yao X.-S., *Helv. Chim. Acta*, **91**, 451–459 (2008).
12) Jiang W., Li W., Han L., Liu L., Zhang Q., Zhang S., Nikaido T., Koike K., *J. Nat. Prod.*, **69**, 1577–1581 (2006).
13) Murakami T., Oominami H., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 741–746 (2001).

14) Wang H.-B., Yu D.-Q., Liang X.-T., Watanabe N., Tamai M., Omura S., *Planta Med*., **55**, 303–306 (1989).
15) Gao H., Wang Z., Yang L., Yu Y., Yao Z.-H., Wang N.-L., Zhou G.-X., Ye W.-C., Yao X.-S., *Magn. Reson. Chem*., **46**, 630–637 (2008).
16) Gao H., Zhao F., Chen G.-D., Chen S.-D., Yu Y., Yao Z.-H., Lau B. W. C., Wang Z., Li J., Yao X.-S., *Phytochemistry*, **70**, 795–806 (2009).