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Triterpenoid saponins from *Echinopsis macrogona* (Cactaceae)

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ABSTRACT

Triterpene saponins, pachanosides C1, E1, F1 and G1 (**1**–**4**), and bridgesides A1, C1, C2, D1, D2, E1 and E2 (**5**–**11**) were isolated from *Echinopsis macrogona*. Compounds **1**–**4** were saponins with pachanane type triterpene saponins, while the others (**5**–**11**) were oleanane type triterpene saponins. While the aglycones of **2**–**4** and **8**–**11** were hitherto unknown, the structure of pachanol C was revised in this paper. Their structures were elucidated on the basis of chemical and physicochemical evidence.

1. Introduction

Triterpene sapogenins from cacti were reported by C. Djerassi in 1953–1958 (Djerassi et al., 1953a,b, 1954a–c, 1955, 1956a,b, 1957; Djerassi and Lippman, 1954; Djerassi and Mills, 1958; Sandoval et al., 1957). Although 18 triterpene sapogenins were isolated from cacti, saponins had never been isolated by the Djerassi group. A new saponin from the cactus, *Pereskia grandifolia* had, however been reported, and is the only report about saponins from cacti (Sahu et al., 1974). In previous contributions, 17 new and 20 known triterpenee sapogenins were reported from several cacti (Kinoshita et al., 1992, 1995; Takizawa et al., 1993; Koyama et al., 1993; Yang et al., 1998), and also a saponin, dumortierinoside A, from *Isolatocereus dumortieri* (Kinoshita et al., 2000). Moreover another new one, stellatoside A, was isolated together with two known saponins from cultured and wild type cactus, *Stenocereus stellatus* (Imai et al., 2006) and stellatoside B and erucasaponin A were isolated from wild type cactus, *S. eruca* (Okazaki et al., 2007). Some triterpene sapogenins from cacti showed antinociceptive (Kinoshita et al., 1998) and antitumor-promotion activities (Kinoshita et al., 1999). In the study herein of saponins from cacti, 11 new triterpene saponins named pachanosides C1, E1, F1 and G1 (**1**–**4**), and bridgesides A1, C1, C2, D1, D2, E1 and E2 (**5**–**11**) were isolated from *Echinopsis macrogona.* Among them, compounds **1**–**4** were the first isolated saponins with aglycones possessing a pachanane skeleton triterpene. This paper deals with the isolation and structure elucidation of these new saponins (**1**–**11**).

2. Results and discussion

Dry *E. macrogona* plant tissue was extracted repeatedly with chloroform and then with methanol. The methanol extract was passed through a Diaion HP-20 column to adsorb saponins and remove sugar by eluting with H_2O. The methanol eluate was separated by silica gel and octadecyl silyl silica gel (ODS) column chromatography and HPLC, yielding pachanosides C1, E1, F1 and G1 (**1**–**4**), and bridgesides A1, C1, C2, D1, D2, E1 and E2 (**5**–**11**). Compounds **1**–**4** were the first isolated saponins with aglycones possessing a pachanane skeleton triterpene. Pachanane skeleton triterpenes, a new skeleton established in our previous studies of cacti, are characterized by translocation of the C-27 methyl from C-14 to C-15 (Takizawa et al., 1993; Kinoshita et al., 1995). The structures of compounds **1**–**11** were determined on the basis of spectroscopic data using DEPT, HMQC, HMBC, DQF-COSY(^{1}H–^{1}H COSY), HSQC-TOCSY, phase sensitive TOCSY, phase sensitive NOESY and NOE difference experiments. The ^{13}C and ^{1}H NMR spectroscopic data of compounds **1**–**11** are presented in Tables 1 and 2, respectively.

Pachanoside C1 (**1**), a colorless powder, consists of a pachanane skeleton triterpene, pachanol C, and three sugars, glucuronic acid, glucose and xylose. Its molecular formula $C_{49}H_{74}O_{21}$ was determined from the molecular ions observed in the ESI (Negative) Tof MS (*m*/*z* 997.4676 $[M-H]^-$) and ESI (positive) Tof MS (*m*/*z* 1021.4620 $[M+Na]^+$) and was confirmed by ^{13}C NMR and DEPT

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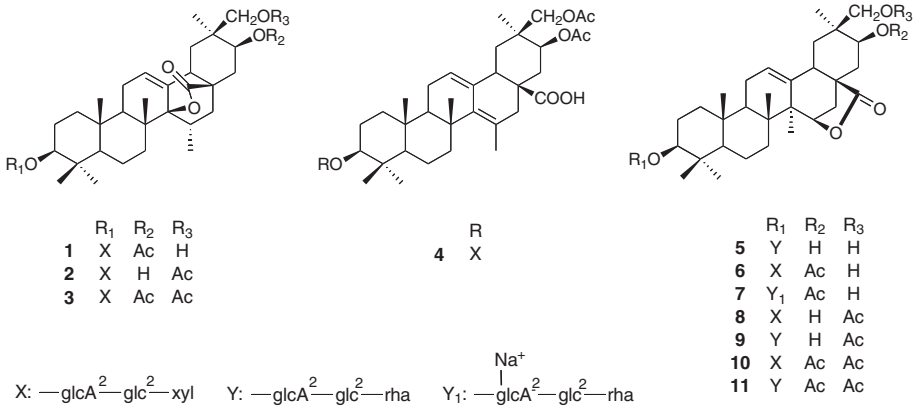


	R_1	R_2	R_3
1	X	Ac	H
2	X	H	Ac
3	X	Ac	Ac

	R
4	X

	R_1	R_2	R_3
5	Y	H	H
6	X	Ac	H
7	Y_1	Ac	H
8	X	H	Ac
9	Y	H	Ac
10	X	Ac	Ac
11	Y	Ac	Ac

spectroscopic analysis. The IR spectrum of 1 showed absorptions at 3400 cm^{-1} (hydroxy) and 1720 and 1738 cm^{-1} (ester carbonyl). The compound displayed 49 signals in its ^{13}C NMR spectrum. Its ^{1}H NMR spectrum showed the presence of six methyl groups characterized by singlets at δ 0.81, 1.04, 1.10, 1.28, 1.31, and a doublet at δ 1.03 (J = 7.6 Hz), an acetoxy methyl at δ 2.04 and an olefinic proton at δ 5.56 (br s). The ^{13}C NMR spectrum showed resonances of a quaternary carbon at δ 90.8, a pair of olefinic carbons at δ 124.4 and 133.1, and three carbonyl carbons at δ 170.5, 172.9 and 176.7. The methyl proton at δ 1.03 (d, J = 7.6 Hz) and quaternary carbon at δ 90.8 indicated that the aglycone possesses a pachanane skeleton (Takizawa et al., 1993; Kinoshita et al., 1995), and its structure was determined by HMQC, HMBC and DQF-COSY (^{1}H–^{1}H COSY) spectra correlations and phase sensitive NOESY spectroscopic correlations (Fig. 1).

HMBC correlations identified most of the structural elements of rings A and B, and these were gradually extended to cover the other rings. Regarding rings C–E, they were ascertained from the following extensive NMR experiments. ^{1}H–^{1}H COSY correlations between 2H-16 at δ 1.70 (2H, m) and H-15 at δ 2.27 (1H, m) and between H-15 and 3H-27 at δ 1.03 (J = 7.6 Hz), and by HMBC correlations between H-16 and C-27 and between 3H-27 and C-15. Moreover, on the basis of HMQC and HMBC results, the methyl proton with its doublet signal at δ 1.03 (d, J = 7.6 Hz) was located on C-27 which was attached to C-15. In the HMBC spectrum, the H-12 olefinic proton at δ 5.56 (1H, br s), H-18 at δ 2.86 (1H, br d, J = 11.0 Hz) and H-15 at δ 2.27 (1H, m) had cross-peaks with the quaternary carbon at δ 90.8. This information suggested that the quaternary carbon at δ 90.8 was assigned at C-14. From the chemical shift value, this quaternary carbon was assumed to be attached to an oxygen atom; the fact that **1** has a 6-membered ring lactone formed by the 14-C oxygen atom and 28-COO like pachanol D (Kinoshita et al., 1995) shown in Fig. 1 was confirmed by IR and its molecular formula.

The stereochemistry of the aglycone moiety of **1** was proven from analysis of the phase sensitive NOESY cross-peaks (Fig. 2). The olefinic proton at δ 5.56 showed a cross peak with H-18 at δ 2.86, which in turn was correlated with H-22 (ax.) at δ 2.51 and 2H-30 at δ 3.88 and 4.22, while 2H-30 gave a cross peak with H-22 (ax.) at δ 2.51. These results led to assignment of the hydroxy-methyl at C-30. The H-5 methine proton at δ 0.79 (m) showed an NOESY correlation with the H-3 and H-9 methine protons and the H-7 methylene axial proton. The 3H-27 showed important correlations with the H-7 axial proton at δ 1.77 (1H, m) and H-9 methine proton at δ 1.60 (dd, J = 11.1, 5.3 Hz) by phase sensitive NOESY. These results suggested the 3H-27 at δ 1.03 (3H, d, J = 7.6 Hz), which connects with C-15, has an α-orientation and only the 14-O is in a β-orientation. Thus, the aglycone **1** was determined as 21-acetyloxy-3β,30-dihydroxy-pachanan-12-en 28,14β-olide as shown in Fig. 2.

In a previous paper, we reported the structure of pachanol C isolated from *Echinopsis pachanoi* (*Trichocereus pachanoi*, Cactaceae) (Kinoshita et al., 1995), almost all the ^{1}H and ^{13}C NMR data of **1** were in good agreement with pachanol C. Although we previously reported the 14-OH of pachanol C to be in an α-orientation, its structure was the same as aglycone of **1**. Thus, we decided to re-investigate the structure of pachanol C. This time, when examining the NOESY correlations of pachanol C in great detail, the H-5 methine proton showed an NOE correlation with the H-3 (δ 3.36) and H-9 (δ 1.60) methine protons. The 3H-27 at δ 1.03 (d, J = 7.6 Hz) also showed correlations with the H-9 methine proton by phase sensitive NOESY. From the above, the 3H-27 connected to C-15 was established to be in an α-orientation. These NOESY correlations could only be observed if the 14-O functionally was in a β-orientation. Thus, pachanol C was determined to be the same as aglycone **1** as confirmed by its NMR, IR and MS data. Moreover, pachanol D, isolated from *I. dumortieri*, was determined by spectroscopic methods and X-ray diffraction analysis (Kinoshita et al., 1998), and has a six-membered ring lactone formed by the 14-O and 28-COO as shown in Fig. 3. The ^{13}C NMR chemical shift of the A–D rings of pachanol C are in good agreement with the data for pachanol D. Therefore, pachanol C is 21β-acetyloxy-3β,14β, 30-trihydroxypachan-12-en-28-oic acid 14β,28-lactone and has been corrected to that shown in Fig. 2.

Thirty-two out of the 49 carbons signals total observed in the ^{13}C NMR spectrum were assigned to the aglycone structure, with the remaining 17 carbons to the oligosaccharide moiety. The ^{1}H and ^{13}C NMR spectra of **1** showed three anomeric carbons at δ 102.9, 104.9 and 106.4, three anomeric protons at δ 5.02 (d, J = 7.6 Hz), 5.39 (d, J = 7.0 Hz), and 5.58 (d, J = 7.9 Hz), together with two methylene carbons at δ 62.9 and 67.3, and a carbonyl carbon at δ 172.9. The unknown sugar residue possessing a carboxyl carbon was concluded to be a glucuronic acid on the basis of analysis of the 2D NMR spectroscopic data (Fig. 3). From the analysis of the HMQC, HMBC and DQF COSY spectra, the carbonyl carbon at δ 172.9 was assigned to C-6′, and the methine protons at δ 4.33 (d, J = 10.1 Hz), 4.48 (t, J = 10.1 Hz), 4.43 (t, J = 10.1 Hz), 4.23 (m), and 5.02 (d, J = 7.6 Hz) were assigned to H-5′, H-4′, H-3′, H-2′ and H-1′, respectively. For the second sugar moiety, the methylene carbon at δ 62.9, which demonstrated HMQC correlations with protons at δ 4.36 (dd, J = 10.1, 5.2 Hz) and 4.49 (d, J = 10.1 Hz), was assigned to the C-6″ of glucose. From the DQF COSY analysis, methylene protons at δ 4.36 (dd, J = 10.1, 5.2 Hz) and 4.49 (d, J = 10.1 Hz) and methine protons at δ 3.87 (m), 4.19 (t, J = 9.4), 4.29 (t, J = 9.4), 4.19 (m) and 5.58 (d, J = 7.9 Hz) were assigned as H-6″ to H-1″, respectively. For the third sugar moiety, the methylene carbon at δ 67.3, which exhibited HMQC correlations with protons at δ 3.69 (t, J = 12.6 Hz) and 4.33 (dd, J = 12.6, 2.7 Hz), was assigned to C-5‴ of xylose. From the HMQC, HMBC and DQF COSY analyses,

Table 1
^{13}C NMR spectral data for the aglycone moiety of **1**–**11** in pyrideine-d_5 or DMSO-d_6* or methanol-d_4 (temp. 50 °C)**.

Position	1	2	3	4	5a	6	7*	7**	8	9	10	11
C-1	38.9	38.9	38.9	38.8	39.0	39.0	38.3	40.4	39.1	39.1	39.1	39.1
C-2	26.4	26.5	26.5	26.5	26.3	26.4	25.2	26.9	26.5	26.4	26.5	26.4
C-3	89.2	89.2	89.2	89.1	89.8	89.0	88.6	91.7	89.0	89.7	89.0	89.7
C-4	39.6	39.6	39.6	39.2	39.7	39.5	38.7	40.7	39.6	39.6	39.6	39.6
C-5	55.9	55.9	55.9	54.6	56.0	55.8	54.6	57.2	55.8	55.9	55.8	55.9
C-6	18.0	18.0	18.0	19.0	18.2	18.1	17.4	19.1	18.1	18.1	18.1	18.1
C-7	33.5	33.5	33.5	40.9	33.7	33.7	32.9	34.7	33.7	33.7	33.7	33.7
C-8	40.1	40.1	40.1	40.8	40.5	40.3	39.8	41.6	40.5	40.5	40.4	40.4
C-9	47.7	47.7	47.7	56.8	48.5	48.4	45.2	49.7	48.4	48.5	48.4	48.4
C-10	37.1	37.2	37.2	37.2	36.7	36.7	36.1	38.0	36.7	36.8	36.8	36.8
C-11	22.8	22.8	22.8	22.0	23.5	23.4	22.7	24.5	23.5	23.5	23.5	23.5
C-12	124.4	124.4	124.8	124.7	127.9	128.1	127.0	129.7	128.0	128.0	128.4	128.4
C-13	133.1	133.3	132.7	136.9	138.1	137.8	137.4	138.6	137.6	137.8	137.3	137.3
C-14	90.8	90.6	90.8	139.0	45.8	45.8	45.0	46.9	45.8	45.7	45.7	45.7
C-15	37.2	37.2	37.1	126.2	80.1	80.1	79.5	82.1	80.1	80.1	80.1	80.1
C-16	34.3	34.5	34.1	38.7	34.6	34.3	33.5	35.2	34.5	34.5	34.1	34.1
C-17	42.5	42.4	42.3	46.8	46.5	46.2	45.2	47.5	46.2	46.2	46.0	46.0
C-18	37.1	37.2	39.6	40.9	41.6	41.3	40.1	42.3	41.3	41.3	41.0	41.0
C-19	33.4	33.9	33.9	39.2	42.5	41.0	39.6	41.8	41.4	41.4	41.1	41.1
C-20	39.4	39.3	38.1	38.4	41.0	40.3	39.5	41.0	40.4	40.4	39.1	39.1
C-21	75.8	72.2	75.0	75.4	74.5	76.3	75.1	77.6	73.2	73.2	75.6	75.6
C-22	31.7	35.6	31.6	34.8	36.8	32.3	31.3	32.8	36.4	36.4	32.3	32.3
C-23	28.2	28.1	28.2	27.7	28.3	28.0	27.6	28.8	28.0	28.3	28.0	28.3
C-24	16.7	16.7	16.8	16.3	16.7	16.7	16.1	17.0	16.8	16.7	16.8	16.7
C-25	16.3	16.2	16.3	16.6	16.1	16.1	15.8	16.7	16.1	16.1	16.1	16.1
C-26	17.5	17.5	17.5	19.5	19.7	19.6	19.1	20.2	19.7	19.7	19.6	19.6
C-27	20.1	20.2	20.1	24.3	25.2	25.2	24.6	25.7	25.2	25.2	25.3	25.2
C-28	176.7	177.7	176.3	177.9	179.5	178.9	178.3	181.4	179.2	179.0	178.5	178.5
C-29	24.0	24.6	23.6	23.6	25.0	24.0	23.2	24.1	24.7	24.7	23.7	23.6
C-30	61.7	65.1	64.6	64.8	63.2	61.2	60.0	62.3	64.6	64.6	64.0	64.0
21-CH_3CO	170.5		170.3	170.3		170.4	169.9	172.4			170.3	170.2
21-CH_3CO	20.9		20.7	20.6		20.8	20.7	20.9			20.7	20.7
30-CH_3CO		171.1	170.8	170.8					171.1	171.1	170.8	170.8
30-CH_3CO		20.8	20.7	20.6					20.8	20.8	20.7	20.7
β-D-glcA												
1′	104.9	104.9	105.0	104.8	105.3	104.9	103.7	105.7	105.9	105.1	105.0	105.3
2′	81.9	81.9	82.0	81.8	78.5	82.1	76.3	78.8	82.0	78.5	82.1	78.5
3′	78.0	78.0	78.0	77.7	78.6	78.0	72.3	79.0	78.1	78.9	78.0	78.7
4′	72.7	72.6	72.6	72.3	73.2	72.6	77.7	74.1	72.7	73.9	72.6	73.5
5′	77.4	77.5	77.6	77.3	76.8	77.6	73.5	76.6	77.3	76.6	77.6	77.3
COOH	172.9	172.6	172.5	172.3		172.5		n.d.	173.1	174.6	172.5	176.3
$COOCH_3$					170.6							
$COOCH_3$					51.9							
COO^-Na^+							172.5					
β-D-glc												
1″	102.9	102.9	103.0	102.7	102.0	103.0	99.6	102.3	102.9	101.9	103.0	102.0
2″	84.6	84.5	84.6	84.3	78.4	84.5	77.0	79.7	84.5	78.5	84.5	78.5
3″	77.8	77.8	77.9	77.9	79.4	77.9	77.6	79.5	77.9	79.4	77.9	79.4
4″	71.8	71.8	71.8	71.5	72.7	71.8	71.2	73.0	71.8	72.8	71.8	72.8
5″	77.8	77.8	77.8	77.7	77.8	77.7	76.4	78.0	77.7	77.6	77.7	77.6
6″	62.9	62.9	62.9	62.6	63.3	62.9	61.9	63.8	62.8	63.4	62.9	63.4
β-D-xyl												
1‴	106.4	106.4	106.5	106.1		106.4			106.4		106.4	
2‴	75.8	75.8	75.8	75.5		75.8			75.8		75.8	
3‴	77.6	77.6	77.6	77.3		77.6			77.6		77.8	
4‴	70.7	70.7	70.7	70.4		70.7			70.7		70.8	
5‴	67.3	67.3	67.3	67.0		67.3			67.3		67.3	
α-L-rha												
1‴					102.0		99.9	102.0		101.9		102.0
2‴					72.4		70.5	72.5		72.4		72.3
3‴					73.2		70.5	72.5		72.6		72.6
4‴					74.3		72.3	74.4		74.3		74.3
5‴					69.4		67.9	69.8		69.4		69.3
6‴					19.0		18.0	18.4		18.9		18.9

n.d: signal was not detected.

the methylene protons at δ 3.69 (t, J = 12.6 Hz) and δ 4.33 (dd, J = 12.6, 2.7 Hz), and methine protons at 4.14 (m), 4.13 (m), 4.09 (m) and 5.39 (d, J = 7.0 Hz) were assigned to H-5‴ to H-1‴, respectively. The coupling constants attributed to the *trans*-diaxial and phase sensitive NOESY spectrum suggested these three sugars had β-configurations (β-glcA, β-glc and β-xyl).

The configurations of glucuronic acid, glucose and xylose were determined using the HPLC method (Tanaka et al., 2007). **1** was hydrolyzed and converted into the thiazolizine derivatives to arylthiocarbamate using L-cysteine methyl ester and *O*-tolylisothiocyanate. Then the reaction mixture was analyzed by C18 HPLC and the retention time (T_R) was compared with derivatives of each D-

Table 2.1
^{1}H NMR spectroscopic data [δ_H, mult. (*J* in Hz)] for the aglycone moieties of **1**–**11**. (500 MHz, in pyridine-d_5 or DMSO-d_6* or methanol-d_4 on temp. 50 °C**).

Position	**1**	**2**	**3**	**4**	**5a**	**6**
1	0.73, 1.38 (each m)	0.74, 1.39 (each m)	0.76, 1.37 (each m)	0.67, 1.35 (each m)	0.88, 1.39 (each m)	0.81, 1.36 (each m)
2	1.84, 2.23 (each m)	1.87, 2.20 (each m)	1.88, 2.20 (each m)	1.81, 2.15 (each m)	1.82, 2.05 (each m)	1.84, 2.16 (each m)
3	3.36 (dd, 11.4, 3.8)	3.35 (dd, 11.6, 4.6)	3.36 (dd, 11.7, 4.4)	3.29 (dd, 11.7, 4.1)	3.30 (dd, 11.5, 4.2)	3.33 (dd, 11.6, 4.3)
5	0.79 (m)	0.83 (m)	0.79 (m)	0.67 (m)	0.80 (m)	0.76 (brd, 11.6)
6	1.33, 1.57 (each m)	1.36, 1.57 (each m)	1.31, 1.56 (each m)	1.32 1.51 (each m)	NA	1.33, 1.52 (each m)
7	1.52, 1.77 (each m)	1.81 (m)	1.52 1.79 (each m)	1.50 (m)	1.54 (m)	1.50 (m)
				2.56 (brd, 11.9)		
9	1.60 (dd, 11.1, 5.3)	1.63 (m)	1.60 (dd, 11.3, 5.5)	1.08 (m)	1.52 (m)	1.48 (m)
11	1.77, 1.84 (each m)	1.71, 1.87 (each m)	1.73, 1.88 (each m)	1.82 (m)	1.76 (m)	1.71 (dd, 8.1, 2.9)
12	5.56 (brs)	5.71 (brs)	5.66 (t-like, 2.1)	5.78 (brs)	5.50 (t-like, 3.5)	5.49 (t-like, 3.2)
15	2.27 (m)	2.31 (m)	2.28 (m)		4.58 (d, 6.1)	4.57 (d, 6.1)
16	1.70 (m)	1.68 (m)	1.69 (m)	2.72, 2.82 (each m)	1.93 (d, 12.6, 6.1)	1.94 (dd, 12.3, 3.2)
					2.72 (d, 12.6)	2.75, d (12.3)
18	2.86 (brd, 12.6)	2.81 (brd, 10.7)	2.76 (brd, 12.0)	3.33 (dd, 13.7, 3.4)	2.88 (dd, 6.8, 3.5)	2.93 (dd, 13.5, 3.2)
19	1.38 (m)	1.42, 2.23 (each m)	1.47 (brd, 12.0)	1.61 (t, 13.7)	1.85 (m)	1.81 (m)
	2.56 (dd, 12.6, 4.4)		2.18 (m)	1.96 (m)	2.11 (dd, 13.9, 3.5)	2.38 (dd, 13.5, 3.2)
21	5.20 (dd, 12.8, 4.7)	3.95 (dd, 13.2, 4.4)	5.13 (dd, 13.3, 4.6)	5.28 (dd, 12.2, 5.2)	3.95 (dd, 12.9.4.7)	5.13 (dd, 12.8, 4.9)
22	2.01 (dd, 12.8, 4.7)	2.14 (dd, 13.2, 4.4)	2.00 (dd, 13.3, 4.6)	2.06 (m)	1.97 (dd, 12.9, 4.7)	1.78 (dd, 12.8, 4.9)
	2.51 (t, 12.8)	2.60 (t, 13.2)	2.37 (t, 13.3)	2.20 (t, 12.2)	2.79 (t, 12.9)	2.53 (t, 12.8)
23	1.31 (s)	1.30 (s)	1.31 (s)	1.26 (s)	1.34 (s)	1.28 (s)
24	1.10 (s)	1.11 (s)	1.11 (s)	1.05 (s)	1.06 (s)	1.08 (s)
25	0.81 (s)	0.81 (s)	0.81 (s)	0.70 (s)	0.76 (s)	0.78 (s)
26	1.04 (s)	1.07 (s)	1.05 (s)	1.03 (s)	1.16 (s)	1.12 (s)
27	1.03 (d, 7.6)	1.09 (d, 6.4)	1.02 (d, 6.4)	2.02 (s)	1.22 (s)	1.22 (s)
29	1.28 (s)	1.39 (s)	1.13 (s)	1.10 (s)	1.53 (s)	1.23 (s)
30	3.88 (d, 11.0)	4.65 (s)	4.37 (d, 11.5)	4.42 (d, 11.8)	4.22 (d, 11.0)	3.91 (d, 10.7)
	4.22 (d, 11.0)		4.47 (d, 11.5)	4,81 (d, 11.8)	4.45 (d, 11.0)	4.39 (d, 10.7)
21-CH_3CO	2.04 (s)		2.02 (s)	2.04 (s)		2.03 (s)
30-CH_3CO		2.09 (s)	2.12 (s)	2.12 (s)		
β-D-glcA						
1′	5.02 (d, 7.6)	5.02 (d, 8.8)	5.04 (d, 8.8)	5.00 (d, 8.7)	4.98 (d, 7.1)	5.00 (d, 7.6)
2′	4.23 (m)	4.24 (t, 8.8)	4.24 (t, 8.8)	4.21 (t, 8.7)	4.50 (m)	4.22 (m)
3′	4.43 (t, 10.1)	4.43 (t, 8.8)	4.44 (t, 8.8)	4.40 (t, 8.7)	4.59 (t, 9.9)	4.43 (t, 9.3)
4′	4.48 (t, 10.1)	4.51 (t, 8.8)	4.52 (t, 8.8)	4.48 (t, 8.7)	4.29 (m)	4.52 (t, 9.3)
5′	4.33 (d, 10.1)	4.60 (d, 8.8)	4.62 (d, 8.8)	4.56 (d, 8.7)	4.48 (d, 9.9)	4.60 (d, 9.3)
6′-$COOCH_3$					3.72 (s)	
β-D-glc						
1″	5.58 (d, 7.9)	5.58 (d, 8.7)	5.58 (d, 7.9)	5.51 (d, 8.3)	5.85 (d, 7.5)	5.57 (d, 7.9)
2″	4.19 (m)	4.19 (t, 8.7)	4.19 (t, 9.2, 7.9)	4.14 (t, 8.3)	4.30 (dd, 9.0, 7.5)	4.19 (m)
3″	4.29 (t, 9.4)	4.29 (t, 8.7)	4.29 (t, 9.2)	4.24 (t, 8.3)	4.24 (t, 9.0)	4.29 (t, 9.0)
4″	4.19 (t, 9.4)	4.20 (t, 8.7)	4.20 (t, 9.2)	4.12 (t, 8.3)	4.07 (t, 9.0)	4.20 (m)
5″	3.87 (m)	3.87 (m)	3.87 (m)	3.83 (m)	3.85 (m)	3.86 (m)
6″	4.36 (dd, 10.1, 5.2)	4.37 (dd, 12.2, 5.2)	4.36 (dd, 11.7, 4.8)	4.30 (dd, 11.5, 5.2)	4.31 (m)	4.36 (dd, 11.6, 4.0)
	4.49 (d, 10.1)	4.50 (d, 12.2)	4.50 (dd, 11.7, 4.8)	4.45 (brd, 11.5)	4.50 (m)	4.48 (dd, 11.6, 4.0)
β-D-xyl						
1‴	5.39 (d, 7.0)	5.39 (d, 6.7)	5.39 (d, 7.0)	5.34 (d, 7.0)		5.40 (d, 6.7)
2‴	4.09 (m)	4.09 (m)	4.10 (dd, 9.3, 7.0)	4.06 (m)		4.11 (m)
3‴	4.13 (m)	4.15 (m)	4.13 (m)	4.12 (t, 8.4)		4.13 (m)
4‴	4.14 (m)	4.12 (m)	4.14 (m)	4.09 (m)		4.14 (m)
5‴	3.69 (t, 12.6)	3.70 (dd, 11.0, 4.6)	3.69 (m)	3.67 (t, 10.4)		3.69 (m)
6‴	4.33 (dd, 12.6, 2.7)	4.33 (dd, 11.0, 4.6)	4.33 (m)	4.32 (brs, 10.4)		4.33 (dd, 11.9, 4.8)
α-L-rha						
1‴					6.40 (brs)	
2‴					4.75 (dd, 3.5, 1.5)	
3‴					4.66 (dd, 9.3, 3.5)	
4‴					4.32 (t, 9.3)	
5‴					5.00 (m)	
6‴					1.78 (d, 6.1)	

N.A.: could not assignment.

or L-sugar. The peaks at 18.5, 19.2 and 21.4 minutes coincided with derivatives of D-glucose, D-glucuronic acid, and D-xylose (T_R of D-glucuronic acid: 19.4, T_R of D-glucose: 18.7, T_R of D-xylose: 21.1), respectively. The linkage of the sugar units at C-3 of pachanol C and the sequence of the sugar chains were established by the following HMBC and NOESY spectra. Analysis of the HMBC spectrum of **1** established that these were cross-peaks between H-1′ of glcA at δ 5.02 and C-3 of pachanol C at δ 89.2 and also between H-3 of the aglycone at δ 3.36 and C-1′ at δ 104.9 of glcA. These results indicated that the glcA was connected to C-3 of the aglycone. The linkage of the glc at C-2′ of glcA was indicated by cross-peaks between H-1″ of glc at δ 5.58 and C-2′ of glcA at δ 81.9 and between H-2′ of glcA at δ 4.23 and C-1′ of glc at δ 102.9. Similarly, the linkage of the xyl at C-2″ of glc was indicated by cross-peaks between H-1‴ of xyl at δ 5.39 and C-2″ of glc at δ 84.6, and H-2″ of glc at δ 4.19 and C-1‴ of xyl at δ 106.4. For the NOESY spectrum, NOE correlations between the methine proton at H-3 (δ 3.36) of the aglycone and the anomeric proton (δ 5.02) of glcA, and between H-2′ (δ 4.23) of glcA and the anomeric proton of glc (δ 5.58) were observed. On the basis of the above, the structure of pachanoside

Table 2.2
^{1}H NMR spectroscopic data [δ_H , mult. (*J* inHz)] for the aglycone moieties of **1–11**. (500 MHz, in pyridine-d_5 or DMSO-d_6* or methanol-d_4 on temp. 50 °C**).

Position	**7***	**7****	**8**	**9**	**10**	**11**
1	0.89, 1.56 (each m)	1.03, 1.67 (each m)	0.88, 1.42 (each m)	0.90, 1.40 (each m)	0.89, 1.43 (each m)	0.92, 1.45 (each m)
2	1.54, 1.98 (each m)	1.73, 2.05(each m)	1.85, 2.23 (each m)	1.84, 2.26 (each m)	1.86, 2.15 (each m)	1.80, 2.16 (each m)
3	2.97 (dd, 11.6, 4.3)	3.22 (m)	3.33 (dd, 11.6, 4.3)	3.32 (dd, 11.0, 3.4)	3.34 (dd, 11.6, 4.3)	3.30 (dd, 11.4, 3.2)
5	0.75 (m)	0.83 (m)	0.76 (m)	0.78 (m)	0.77 (brs, 11.6)	0.81 (m)
6	1.35, 1.53 (each m)	1.48, 1.61 (each m)	1.33, 1.53 (each m)	1.33, 1.53 (each m)	1.34, 1.53 (each m)	1.34, 1.54 (each m)
7	1.51 (m)	1.61 (m)	1.52 (m)	1.53 (m)	1.53 (m)	1.51 (m)
9	1.43 (m)	1.55 (t, 8.7)	1.50 (m)	1.52 (m)	1.49 (t, 8.1)	1.54 (m)
11	1.83 (m)	1.93 (m)	1.75 (m)	1.56 (m)	1.75 (m)	1.51, 1.75 (each m)
12	5.56 (brs)	5.64 (t-like, 3.5)	5.52 (t-like, 3.2)	5.51 (brs)	5.46 (t-like, 3.5)	5.46 (brs)
15	4.57 (d, 5.8)	4.64 (m)	4.58 (d, 5.8)	4.57 (d, 6.7)	4.56 (d, 6.1)	4.56 (d, 5.9)
16	1.85 (m)	1.99 (dd, 14.3, 5.3)	1.92 (m)	1.91 (m)	1.92 (d, 12.5, 6.0)	1.93 (dd, 12.5, 5.9)
	2.73 (d, 12.5)	2.78 (d, 5.3)	2.70 (d, 12.5)	2.69 (d, 12.5)	2.71 (d, 12.5)	2.70 (d, 12.5)
18	2.39 (brd, 13.1)	2.50 (dd, 13.1, 1.1)	2.84 (dd, 12.9, 3.1)	2.83 (dd, 13.0, 2.9)	2.75 (dd, 10.4, 7.3)	2.74 (t, 10.1)
19	1.67 (t, 13.1)	1.70, 1.90 (each m)	1.76 (m)	1.77 (m)	1.81 (m)	1.78 (m)
	1.85 (m)					
21	4.72 (dd, 13.0, 5.1)	4.77 (dd, 13.4, 4.8)	3.89 (m)	3.88 (dd, 13.3, 4.4)	5.04 (dd, 12.7, 4.7)	5.03 (dd, 13.0, 4.7)
22	1.40 (dd, 13.0, 5.1)	1.51 (dd, 13.4, 4.8)	1.90 (m)	1.92 (dd, 13.3, 4.4)	1.76 (m)	1.76 (m)
	1.90 (t, 13.0)	2.06 (t, 13.4)	2.65 (t, 13.3)	2.65 (t, 13.3)	2.38 (t, 13.1)	2.37 (t, 13.0)
23	1.03 (s)	1.12 (s)	1.28 (s)	1.33 (s)	1.29 (s)	1.34 (s)
24	0.76 (s)	0.88 (s)	1.09 (s)	1.07 (s)	1.10 (s)	1.08 (s)
25	0.89 (s)	0.99 (s)	0.81 (s)	0.78 (s)	0.81 (s)	0.79 (s)
26	0.91 (s)	1.04 (s)	1.15 (s)	1.15 (s)	1.13 (s)	1.12 (s)
27	1.13 (s)	1.22 (s)	1.23 (s)	1.23 (s)	1.20 (s)	1.20 (s)
29	0.85 (s)	0.97 (s)	1.32 (s)	1.33 (s)	1.05 (s)	1.05 (s)
30	3.28 (d, 10.6)	3.55 (d, 11.0)	4.55 (d, 12.1)	4.45 (d, 11.8)	4.26 (d, 12.1)	4.26 (d, 11.5)
	3.76 (d, 10.6)	3.94 (d, 11.0)	4.95 (d, 12.1)	4.95 (d, 11.8)	4.74 (d, 12.1)	4.73 (d, 11.5)
21-C*H*$_3$CO	2.00 (s)	2.03 (s)			2.03 (s)	2.02 (s)
30-C*H*$_3$CO			2.11 (s)	2.12 (s)	2.13 (s)	2.13 (s)
β-D-glcA						
1′	4.13 (d, 8.0)	4.43 (d, 8.3)	4.97 (d, 8.3)	4.95 (d, 7.6)	5.00 (d, 8.7)	4.99 (d, 8.8)
2′	3.49 (t, 8.0)	3.70 (t, 8.3)	4.22 (t, 8.3)	4.48 (t, 7.6)	4.22 (t, 8.7)	4.51 (t, 8.8)
3′	3.09 (t, 8.0)	3.62 (t, 8.3)	4.42 (t, 8.3)	4.58 (t, 7.6)	4.43 (t, 8.7)	4.60 (t, 8.8)
4′	3.40 (t, 8.0)	4.45 (m)	4.47 (t, 8.3)	4.29 (m)	4.52 (t, 8.7)	4.42 (t, 8.8)
5′	3.15 (d, 8.0)	3.57 (d, 7.6)	4.53 (d, 8.3)	4.41 (d, 9.8)	4.59 (d, 8.7)	4.57 (d, 8.8)
β-D-glc						
1″	4.82 (d, 8.9)	4.90 (d, 7.6)	5.57 (d, 7.6)	5.82 (d, 7.6)	5.58 (d, 8.4)	5.83 (d, 7.6)
2″	3.16 (t, 8.9)	3.39 (m)	4.12 (t, 9.1, 7.6)	4.28 (m)	4.19 (t, 8.4)	4.29 (m)
3″	3.27 (m)	3.47 (t, 9.2)	4.28 (t, 9.1)	4.22 (t, 9.1)	4.29 (t, 8.4)	4.23 (t, 9.2)
4″	2.90 (t, 9.2)	3.10 (t, 9.2)	4.03 (t, 9.1)	4.03 (t, 9.1)	4.21 (t, 8.4)	4.06 (t, 9.2)
5″	3.03 (m)	3.24 (m)	3.85 (m)	3.84 (m)	3.86 (m)	3.84 (m)
6″	3.35 (dd,)	3.55 (dd, 11.9, 3.3)	4.35 (m)	4.27 (m)	4.37 (dd, 11.6, 3.8)	4.31 (m)
	3.70 (brd, 11.3)	3.83 (dd, 11.9, 3.3)	4.47 (m)	4.49 (d, 8.5)	4.49 (dd, 11.6, 3.8)	4.49 (dd, 11.7, 3.2)
β-D-xyl						
1‴			5.39 (d, 6.7)		5.40 (d, 6.7)	
2‴			4.09 (m)		4.10 (m)	
3‴			4.12 (m)		4.12 (m)	
4‴			4.14 (m)		4.13 (m)	
5‴			3.68 (m)		3.69 (m)	
			4.33 (dd, 9.5, 4.6)		4.33 (dd, 11.9, 4.3)	
α-L-rha						
1‴	5.02 (s)	5.22 (d, 1.9)		6.37 (brs)		6.38 (brs)
2‴	3.66 (brs)	3.92 (dd, 3.3, 1.9)		4.74 (m)		4.74 (m)
3‴	3.52 (dd, 10.6, 4.0)	3.76 (dd, 9.5, 3.3)		4.69 (dd, 9.4, 3.1)		4.69 (dd, 9.1, 3.1)
4‴	3.16 (t, 10.6)	3.39 (t, 9.5)		4.32 (t, 9.4)		4.33 (t, 9.1)
5‴	3.96 (m)	4.12 (m)		5.03 (m)		5.04 (m)
6‴	1.09 (d, 6,1)	1.26 (d, 6.4)		1.77 (d, 6.1)		1.77 (d, 6.1)

N.A.: could not assignment.

C1 (**1**) was elucidated as 21β-acetyloxy-3β,14β,30-trihydroxypachan-12-en-28-oic acid 14β,28-lactone (pachanol C) 3-*O*-β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Pachanoside E1 (**2**) was isolated as a colorless powder. Its molecular formula $C_{49}H_{74}O_{21}$ was determined from its negative ion HRFABMS (*m*/*z* 997.4641 [M−H]$^-$) and confirmed by ^{13}C-NMR and DEPT analysis. Its ^{13}C NMR spectrum contained 49 signals. For the ^{1}H and ^{13}C NMR spectra of **2**, a doublet methyl proton at δ 1.09 (*J* = 6.4 Hz) and a quaternary carbon at δ 90.6 were observed, and it was assumed that the aglycone of **2** possesses a pachanane skeleton. In comparison with the ^{13}C NMR spectroscopic data of the aglycone moiety for **1**, **2** exhibited upfield shifts on the C-21 (-3.6 ppm) and acetoxy methyl carbon (−0.1 ppm) and showed downfield shifts on the C-22 (+3.9 ppm), C-30 (+3.4 ppm) and carboxyl carbon at δ 171.1 (+0.6 ppm). In the HMBC spectrum, the acetoxy methyl proton at δ 2.09 and the methylene proton on 2H-30 at δ 4.65 (2H, s) showed correlations with the carboxyl carbon at δ 171.1. These results indicated that an acetoxyl group was located at C-30 instead of at C-21 as in **1.** This aglycone is thus a new triterpene, 30-acetyloxy-3β,14β,21β-trihydroxypachan-12-en-28-oic acid 14β,28-lactone, named pachanol E. After an extensive NMR study, the oligosaccharide moiety of **2** was determined to be the same as that of **1**. The configurations of glucuronic acid, glucose and xylose were determined using the same method (Tanaka et al., 2007). Peaks at 18.4, 19.2 and 21.4 minutes

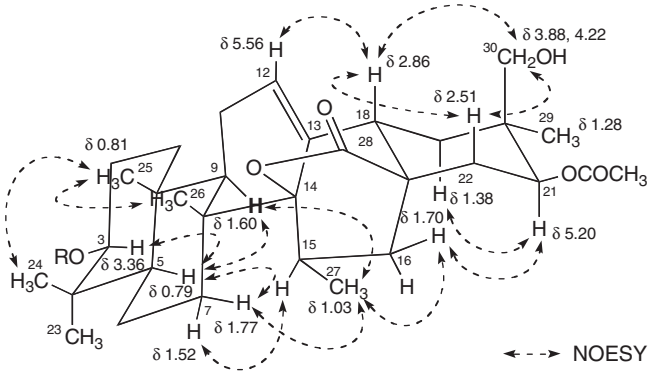


Fig. 1. Phase-sensitive NOESY correlations for the aglycone moiety of pachanoside C1 (**1**).

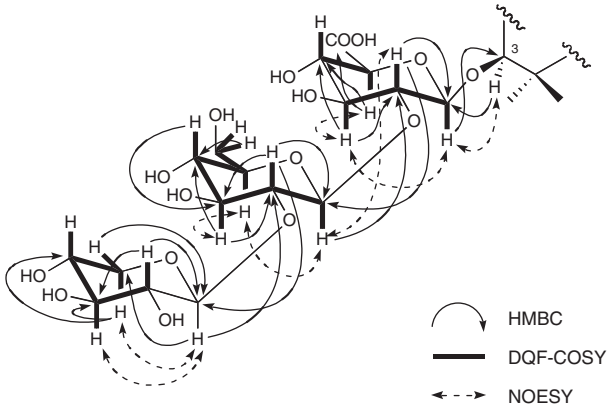
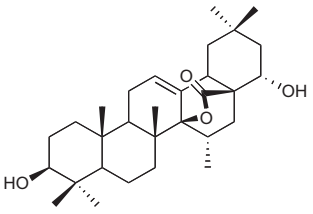


Fig. 3. HMBC, DQF-COSY and phase-sensitive NOESY correlations for the sugar moiety of pachanoside C1 (**1**).

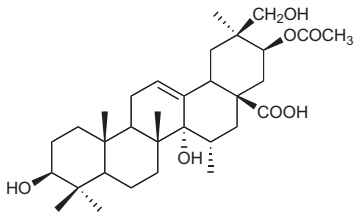
coincided with derivatives of D-glucose, D-glucuronic acid, and D-xylose (T_R of D-glucuronic acid: 19.4, T_R of D-glucose: 18.7, T_R of D-xylose: 21.1). Thus, pachanoside E1 (**2**) was elucidated as 30-acetyloxy-3β,14β,21β-trihydroxypachan-12-en-28-oic acid 14β,28-lactone (pachanol E) 3-*O*-β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Pachanoside F1 (**3**) was isolated as a colorless powder. Its molecular formula $C_{51}H_{76}O_{22}$ was determined from its negative ion HRFABMS (*m*/*z* 1039.4742) and confirmed by ^{13}C NMR and DEPT analysis. The compound displayed 51 signals in its ^{13}C NMR spectrum. For the ^{1}H and ^{13}C NMR spectra of **3**, a doublet methyl proton at δ 1.02 (J = 6.4 Hz) and a quaternary carbon at δ 90.8 were observed, and it was similarly assumed that the aglycone of **3** possessed a pachanane skeleton. Unambiguous assignment for the ^{1}H and ^{13}C NMR signals were made by a combination of 2D NMR experiments. In comparison with ^{1}H and ^{13}C NMR data of the aglycone moiety for pachanol C, an additional methyl proton signal at δ 2.12 and two carbon signals at δ 20.7 and 170.8 for an acetoxy group were observed on **3.** For the ^{13}C NMR spectrum, upfield shifts of C-21 (−0.8 ppm) and downfield shifts of C-30 (+2.9 ppm) were seen. From the HMBC spectrum, the carbonyl carbon at δ 170.3, which has a correlation with an acetoxy methyl proton at δ 2.02, had a cross-peak with H-21 at δ 5.13 (1H, dd, J = 13.3, 4.6 Hz). The additional carbonyl carbon at δ 170.8, which has a correlation with another acetoxy methyl at δ 2.12, had cross-peaks with 2H-30 at δ 4.37 and 4.47 (each 1H, d, J = 11.5 Hz). These results established that the two acetoxyl groups were attached to C-21 and C-30. Its aglycone moiety, 21β,30-diacetyloxy-3β,14β-dihydroxypachan-12-en-28-oic acid 14β,28-lactone, is a new triterpene named pachanol F. After an extensive NMR study, the oligosaccharide moiety of **3** was determined to be the same as that of **1**. The configurations of glucuronic acid, glucose and xylose were determined as D-glucuronic acid, D-glucose and D-xylose using the same method (Tanaka et al., 2007). Thus, pachanoside F1 (**3**) was elucidated as 21β,30-diacetyloxy-3β,14β-dihydroxypachan-12-en-28-oic acid 14β,28-lactone (pachanol F) 3-*O*-β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

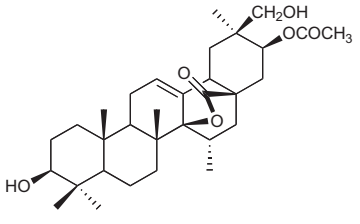
Pachanoside G1 (**4**) was isolated as a colorless powder. Its molecular formula $C_{51}H_{74}O_{21}$ was determined from its negative ion HRFABMS (*m*/*z* 1039.4747), and its ^{13}C NMR spectrum had 51 signals. The UV spectrum indicated the presence of a conjugated hetero system in the molecule (λ_{max} 242 and 248 nm). The ^{1}H NMR spectrum showed the presence of six methyl groups characterized by



pachanol D



reported structure of pachanol C



corrected structure of pachanol C

Fig. 2. Structures of pachanols C and D.

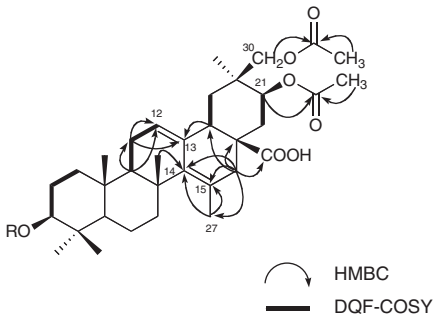


Fig. 4. Key HMBC and DQF-COSY correlations for the aglycone moiety of pachanoside G1 (**4**).

singlets at δ 0.70, 1.03, 1.05, 1.10, 1.26 and 2.02, two acetoxy methyl groups at δ 2.04 and 2.12, and an olefinic proton at δ 5.78 (br s). The ^{13}C NMR spectrum showed signals for two pairs of olefinic carbons at δ 124.7 (CH), 126.2 (C), 136.9 (C), 139.0 (C) and four carbonyl carbons at δ 170.3, 170.8, 172.3 and 177.9. Its structure was determined by HMQC, HMBC and DQF COSY spectra correlations (Fig. 4) and phase sensitive NOESY spectral correlations.

A-, B, and E rings of its structure were elucidated following the same method as for **1**. These results led to assignment of five methyl signals, δ 0.70, 1.03, 1.05, 1.10 and 1.26, at 3H-25, -26, -24, -29 and -23, respectively, while a carbonyl carbon at δ 177.9 could be assigned to C-28. The resonances of two methyl groups (δ 2.04 and 2.12) and carbonyl carbons suggested the presence of two acetoxyl groups. From the HMBC correlations, one is located at C-21 and the other at C-30. Thus, the methyl proton signals at δ 2.02 were deemed to be located on C-27. Regarding rings C and D, their structures were elucidated from the following extensive NMR experiments. On the HMBC, H-9 at δ 1.08 (1H, m) showed cross-peaks with C-11 (CH_2) at δ 22.0 and a sp^2 carbon at δ 124.7 (CH), and H-11 at δ 1.82 (1H, m) correlated with sp^2 carbons at δ 124.7 (CH) and δ 136.9 (C); thus, the sp^2 carbon at δ 124.7 (C) was assigned as C-12 and the quaternary carbon at δ 136.9 as C-13. Furthermore, the methyl proton at δ 2.02 (s), which has an HMQC correlations with carbon at δ 24.3, was assumed to be attached to a sp^2 carbon based on its chemical shift value. In the HMBC spectrum, the methyl proton at δ 2.02 has cross-peaks with two sp^2 carbond at δ 126.2 and 139.0 (Fig. 4). The 3H-26 at δ 1.03 showed a similar correlation with δ 139.0. Thus, the sp^2 carbons at δ 139.0 and 126.2 were assigned as C-14 and C-15, respectively. The above information confirmed that the methyl proton C-27 at δ 2.02 is connected to C-15. This aglycone is a new triterpene, 21β,30-diacetyloxy-3β-hydroxypachan-12,14(15)-dien-28oic acid which has a pachanane skeleton, named pachanol G. After an extensive NMR spectroscopic study, the oligosaccharide moiety of **4** was determined to be the same as that of **1**. The configurations of glucuronic acid, glucose and xylose were determined to D-configirations using the same method (Tanaka et al., 2007). Thus, pachanoside G1 (**4**) was elucidated as 21β,30-diacetyloxy-3β-hydroxypachan-12,14(15)-dien-28oic acid (pachanol G) 3-*O*-β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Bridgeside A1 (**5**) was isolated as a colorless powder. Its molecular formula $C_{48}H_{74}O_{20}$ was determined from its negative ion HRFABMS. The IR spectrum of **5** shows absorption at 3400 cm^{-1} (hydroxy) and 1760 cm^{-1} (five-membered ring lactone). The compound displayed 48 signals in its ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed the presence of six methyl groups characterized by singlets at δ 0.76, 1.07, 1.13, 1.22, 1.33, 1.51 and an olefinic proton at δ 5.49 (br s). The ^{13}C NMR spectrum showed signals for a pair of olefinic carbons at δ 127.9, 137.9 and a carbonyl carbon at δ 179.4. The sugar part contained, in the ^{13}C NMR spectrum, three anomeric signals at δ 101.8, 101.9 and 105.9. Since the ^{13}C NMR spectrum of **5** showed the presence of a carbonyl group consistent with an uronic acid, **5**. Since the ^{1}H and ^{13}C NMR spectroscopic data of compound 5 were broad, **5** was converted to its methyl ester (**5a**) by treatment with CH_2N_2. The ^{13}C NMR spectrum of **5a** displayed 50 signals, and two additional carbons at δ 51.9 (CH_3), and 170.6 (C) were observed. The six methyl protons characterized by singlets at δ 0.76, 1.06, 1.16, 1.22, 1.34 and 1.53 and an olefinic proton at δ 5.50 (t-like, J = 3.5 Hz), coupled with the information from ^{13}C NMR spectrum (six methyl carbon signals and a pair of olefinic carbons at δ 127.9, 138.1), indicated that the aglycone of **5a** possesses an olean-12-ene skeleton. The aglycone portion was identified as bridgesigenin A, which has a five-membered ring lactone by the HMQC and HMBC correlations of **5a**, a common aglycone that occurs in *Echinopsis bridgesii* (*Trichocereus bridgesii*, Cactaceae) (Kinoshita et al., 1992). The overall structural assignment was achieved following the same methodology as in **1**. The downfield chemical shift of C-3 (δ 89.8) indicated that **5a** was a monodesmoside glycoside. Out of 50 carbon signals observed in the ^{13}C NMR spectrum of **5a**, 30 were assigned to the aglycone part, and the remaining 20 carbons to the oligosaccharide moiety. The ^{1}H and ^{13}C NMR spectra of **5a** shows three anomeric carbon signals at δ 102.0, 102.0 and 105.3, and three anomeric proton resonances at δ 4.98 (d, J = 7.1 Hz), 5.85 (d, J = 7.5 Hz), 6.40 (br s), together with a methyl doublet proton signal at δ 1.78 (J = 6.1 Hz), suggesting the occurrence of three sugar units including one rhamnose unit. Two additional carbons at δ 51.9 and 170.6 suggested the presence of an uronic acid on the basis of the following data. From the analyses of the HMQC, HMBC and DQF COSY spectra (Fig. 5), the carbonyl carbon at δ 170.6 had a cross-peak with δ 3.72 (3H, s), which in turn has an HMQC correlation with a methoxyl carbon at δ 51.9. An extensive NMR study permitted assignment of the proton and the carbon resonances in the respective spectra and indicated presence of a terminal α-L-rhamnose (rha), a 2-substituted glucuronic acid methyl ester and 2-substituted glucose. The anomeric configuration for rhamnose was determined as the α-configuration from the large $^1J_{C,H}$ values (J = 173 Hz).

The linkage of the sugar units at C-3 of bridgesigenin A and the sequence of the sugar chains were established by HMQC, HMBC and NOESY spectra. The HMBC spectrum of **5a** indicated cross-peaks between H-1 of glcA at δ 4.98 and C-3 of bridgesigenin A at δ 89.8. This result indicated that the glcA was connected to C-3 of the aglycone. The linkage of the glc at C-2 of glcA was indicated by a HMBC cross-peak between H-2 of glcA at δ 4.50 and C-1 of glc

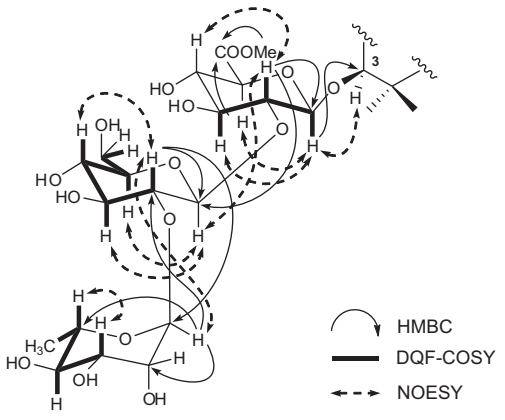


Fig. 5. HMBC, DQF-COSY and phase sensitive NOESY correlations for the sugar moiety of bridgeside A1 methyl ester (**5a**).

at δ 102.0. Similarly, the linkage of the rha at C-2 of glc was indicated by cross-peaks between the anomeric proton of rha at δ 6.40 and C-2 of glc at δ 78.4 and between H-2 of glc at δ 4.30 and C-1 of rha at δ 102.0. The configurations of glucuronic acid, glucose and rhamnose were determined using same method (Tanaka et al., 2007). In the case of rhamnose, L-rhamnose was converted into the thiazolizine derivatives to arylthiocarbamate using L-cysteine methyl ester hydrochloride instead of D-cysteine methyl ester hydrochloride, the HPLC retention times (T_R) coincided with derivatives of D-glucuronic acid, D-glucose and L-rhamnose. On the basis of the above evidence, the structure of bridgeside A1 methyl ester (**5a**) could be elucidated as bridgesigenin A 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranosyl(1 → 2)-6'-*O*-methyl-β-D-glucuronopyranoside. Thus, the structure of bridgeside A1 (**5**) was determined as bridgesigenin A 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Bridgeside C1 (**6**) was isolated as a colorless powder. Its molecular formula $C_{49}H_{74}O_{21}$ was determined from its negative ion HRFABMS. The IR spectrum of **6** shows absorptions at 3420 cm^{-1} (hydroxy) and 1760 cm^{-1} (five-membered ring lactone). The compound displayed 49 signals in its ^{13}C NMR spectrum. When compared with the ^{1}H NMR spectrum of bridgesigenin A (Kinoshita et al., 1992), an additional acetoxy methyl signal at δ 2.03 was observed on **6**. The ^{13}C NMR spectrum showed downfield shifts of C-21 (+1.8 ppm) and two additional signals at δ 20.8 and 170.4. From the HMBC spectrum, the carboxyl carbon at δ 170.4, which has a correlation with an acetoxy methyl at δ 2.03, showed a cross-peak with H-21 at δ 5.13 (1H, dd, J = 12.8, 4.9 Hz). These results indicated that an acetoxyl group is attached to C-21 as bridgesigenin C. Thus, the aglycone of **6** was identified as in bridgesigenin C, a common aglycone that occurs in *Echinopsis pachanoi* (*Trichocereus pachanoi*, Cactaceae) (Kinoshita et al., 1995). After an extensive NMR spectroscopic analysis, the oligosaccharide moiety of **6** was determined to be the same as that of **1**. The configuration of each sugars were also the same as **1**. Thus, the structure of bridgeside C1 (**6**) was elucidated as bridgesigenin C 3-*O*-β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Bridgeside C2 (**7**) was isolated as a colorless powder. Its IR spectrum showed absorptions at 3420 cm^{-1} (hydroxy) and 1760 cm^{-1} (five-membered ring lactone). Interestingly, **7** was insoluble in pyridine. While the compound displayed 49 signals in its ^{13}C NMR spectrum in methanol-d_4 (temp.50 °C), the ^{1}H and ^{13}C NMR spectra of **7** shows three anomeric carbon signals at δ 102.0, 102.3 and 105.7 and three anomeric proton resonances at δ 4.43 (d, J = 8.3 Hz), 4.90 (d, J = 7.6 Hz), 5.22 (d, J = 1.9 Hz), respectively. After an extensive NMR study, the stereochemistry of the aglycone moiety was identified as bridgesigenin C and two sugar moieties were confirmed as glucose and rhamnose. The remaining sugar unit seemed to be glucuronic acid from its chemical shifts. But the carbonyl carbon signal of glucuronic acid in **7** was not observed in methanol-d_4 (temp. 50 °C). Further analysis of the NMR data in DMSO-d_6, indicated the unknown sugar unit was glucuronic acid, and the sequence of the oligosaccharide chain was determined to be the same as those of **5**. The results of FABMS indicated the presence of a sodium ion in a sample of **7**. FABMS (positive ion mode) of **7** (magic bullet + NaCl as matrix) gave an ion $[M{\cdot}Na{+}Na]^+$ at m/z 1057, in accordance with molecular formula $C_{50}H_{75}O_{21}Na_2$. FABMS (negative ion mode) showed ions $[M{\cdot}Na{-}H]^-$ at m/z 1033, $[M{\cdot}Na{-}Na]^-$ at m/z 1011. Furthermore, HRFABMS (positive ion mode) gave ions $[M{\cdot}Na{+}Na]^+$ at m/z 1057.4602 (calcd for 1057.4596, $C_{50}H_{75}O_{21}Na_2$) and $[M{\cdot}Na{+}H]^+$ at m/z 1035.4784 (calcd for 1035.4777, $C_{50}H_{76}O_{21}Na$). The above information suggests that **7** had been isolated as mono sodium salt. The configurations of xylose, glucose and glucuronic acid were determined using the same method. The retention times (T_R) of HPLC were considered with derivatives of D-xylose, D-glucose and D-glucuronic acid. Thus, bridgeside C2 (**7**) was elucidated as 21β-acetyloxy-3β,15β,30-trihydroxyolean-12-en-28-oic acid 15β,28-lactone (bridgesigenin C) 3-*O*-β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside, mono sodium salt.

Bridgeside D1 (**8**) was isolated as a colorless powder. Its molecular formula $C_{49}H_{74}O_{21}$ was determined from its negative ion HRFABMS. The IR spectrum of **8** showed absorptions at 3420 cm^{-1} (hydroxy) and 1760 cm^{-1} (five-membered ring lactone). The compound displayed 49 signals in its ^{13}C NMR spectrum. On comparing with the ^{13}C NMR data of **6**, they shared the same sugar moiety. Its ^{13}C NMR spectrum showed upfield shifts of C-21 at δ 73.2 (−3.1 ppm) and downfield shifts of C-30 at δ 64.4 (+3.4 ppm) and carboxyl carbon at δ 171.1 (+0.7 ppm). The carboxyl carbon at δ 171.1, which has a correlation with an acetoxy methyl signal at δ 2.11, had cross-peaks with 2H-30 at δ 4.55 and 4.95 (each 1H, d, J = 12.1 Hz) by HMBC. These results indicated that an acetoxyl group is located at C-30 instead of at C-21 as in **6.** The aglycone is a new tritepene, 30-acetyloxy-3β,15β,21β-trihydroxyolean-12-en-28-oic acid 15β,28-lactone, named bridgesigenin D, but whose sugar moiety and configuration is the same as **1**. Thus, bridgeside D1 (**8**) was elucidated as 30-acetyloxy-3β,15β,21β-trihydroxyolean-12-en-28-oic acid 15β,28-lactone (bridgesigenin D) 3-*O*-β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Bridgeside D2 (**9**) was isolated as a colorless powder. Its molecular formula $C_{50}H_{76}O_{21}$ was determined from its negative ion HRFABMS. Its IR spectrum showed absorptions at 3410 cm^{-1} (hydroxy) and 1760 cm^{-1} (five-membered ring lactone). After an extensive NMR study, comparison of the signals of **8** and **9** indicated many similarities, except that signals due to the β-D-xylopyranosyl unit in **8** were replaced in **9** by a set of resonances assigned to a terminal α-L-rhamnopyranosyl unit. Its sugar moiety and configuration were the same as for bridgeside A1 (**5**). Thus, bridgeside D2 (**9**) was elucidated as 30-acetyloxy-3β,15β,21β-trihydroxyolean-12-en-28-oic acid 15β,28-lactone (bridgesigenin D) 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Bridgeside E1 (**10**) was isolated as a colorless powder. Its molecular formula $C_{51}H_{76}O_{21}$ was determined from its negative ion HRFABMS. The IR spectrum of **10** showed absorptions at 3400 cm^{-1} (hydroxy) and 1720 cm^{-1} (five-membered ring lactone). When compared with the ^{1}H NMR spectrum of **6**, an additional acetoxy methyl at δ 2.13 was observed. The ^{13}C NMR spectrum showed upfield shifts of C-21 at δ 75.6 (−1.3 ppm), downfield shifts of C-30 at δ 64.0 (+2.8 ppm) and two additional signals at δ 20.7 and 170.8. The carboxyl carbon at δ 170.3, which is correlated with an acetoxy methyl at δ 2.03, showed a cross-peak with H-21 at δ 5.04 (1H, dd, J = 12.7, 4.7 Hz), while another carboxy carbon at δ 170.8 which is correlated with an acetoxy methyl at δ 2.13 had cross-peaks with 2H-30 at δ 4.26 and 4.74 (each 1H, d, J = 12.1 Hz) by HMBC. These results indicated that acetoxyl groups were attached to C-21 and C-30. The aglycone part corresponds to a new genin, 21β,30-diacetyloxy-3β,15β-dihydroxyolean-12-en-28-oic acid 15β,28-lactone, named bridgesigenin E. After an extensive NMR study, the oligosaccharide moiety of **10** was determined to be the same as that of **6**. The sugar moiety and their configuration were same as **1**. Thus, bridgeside E1 (**10**) was elucidated as in 21β,30-diacetyloxy-3β,15β-dihydroxyolean-12-en-28-oic acid 15β,28-lactone (bridgesigenin E) 3-*O*-β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Bridgeside E2 (**11**) was isolated as a colorless powder. Its molecular formula $C_{52}H_{78}O_{22}$ was determined from its negative ion HRFABMS. The IR spectrum of **11** shows absorption at 3415 cm^{-1} (hydroxy) and 1740 cm^{-1} (five-membered ring lactone). After an extensive NMR study, comparison between the assigned signals

of **11** and **10** indicated that the only difference was the resonance due to the sugar unit attached to C-2″ of the ester-linked α-rhamnopyransly unit. The sugar moiety and their configuration were same as **5**. Thus, bridgeside E2 (**11**) was elucidated as bridgesigenin E 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside.

Concluding remarks: Eleven new saponins from *E. macrogona* were isolated and their structures determined. The Pachanoside C1, E1, F1 and G1 are the first isolated saponins of the pachanane-type triterpene saponins. We had previously reported pachanol A as a triterpene sapogenin from *Trichocereus (Echinopsis) pachanoi* (Kinoshita et al., 1995), and considered that it must be an artifact by acid hydroloysis of saponins. The aglycone of saponin, pachanoside G1 (**4**), is a pachanol A derivative. Thus, pachanol A and pachanol G were considered as true aglycones of saponins.

3. Experimental

3.1. General experimental procedures

Melting points were determined on **a** Yanagimoto MP micromelting point apparatus, and $[\alpha]_D$ values were determined with a JASCO DIP-140 digital polarimeter. IR spectra were measured with a JASCO A-102 IR spectrophotometer, whereas ^1H and ^{13}C NMR spectra were recorded using a JEOL AL-400 or JNM LA-500 spectrometer in C_5D_5N, CD_3OD or DMSO-d_6 with tetramethylsilane as an internal standard. Kieselgel-60F_{254} (MERCK) or RP-18F_{254S} (MERCK)-precoated plates were employed for thin-layer chromatography (TLC). Column chromatography (CC) was carried out on 70–230 mesh silica gel (MERCK) and prep ODS-7515-12A. HPLC was performed using a PU-2080 Plus pump with a JASCO UV-2075 Plus UV detector. HREIMS and HRFABMS were obtained using a JOEL JMS-700.

3.2. Plant material

E. macrogona H. Friedrich and G.D. Rowley, was cultivated originally at the Japan Cactus Planning Co. (Fukushima City, Fukushima, Japan). The cactus was identified by Dr. H. Yuasa. A voucher specimen (MPU-C-02-1) is deposited at our laboratory.

3.3. Extraction and isolation

The dried and powdered whole plants of *E. macrogona* (229 g) were extracted with $CHCl_3$ (3 × 3.5 L) and then with MeOH (6 × 3.5 L). The dried MeOH extract (47.0 g, 20.5%) was then applied to a Diaion HP-20 column which was eluted with H_2O, MeOH H_2O (30:70, v/v) and 100% MeOH, successively. The MeOH eluted fraction (13.3 g, 5.8%) was separated by silica gel CC using a stepwise gradient [350 g, $CHCl_3$–MeOH (50:1) → $CHCl_3$–MeOH–H_2O → MeOH] to give eight fractions [Fr. A (0.34 g), Fr. B (0.81 g), Fr. C (0.34 g), Fr. D (4.18 g), Fr. E (3.80 g), Fr. F (0.96 g), Fr. G (0.22 g) and Fr. H (0.78 g)], respectively.

Fraction F (0.96 g) was further subjected to silica gel CC using a stepwise gradient [32 g, $CHCl_3$–MeOH–H_2O (60:10:1) → MeOH] to give bridgeside A1 (**5**, 0.27 g, 0.12%), and a portion of **5** (30.5 mg) was methylated with diazomethane (CH_2N_2) and purified by on silica gel CC [7 g, $CHCl_3$–MeOH–H_2O (60:20:1) → MeOH] to yield bridgeside A methyl ester (**5a**, 15.1 mg).

Fraction E (3.80 g) was subjected to silica gel CC using a stepwise gradient [120 g, $CHCl_3$–MeOH–H_2O (60:20:1) → MeOH] to afford five fractions [Fr. E1 (55.9 mg), Fr. E2 (0.45 g), Fr. E3 (0.85 g), Fr. E4 (1.64 g) and Fr. E5 (0.55 g)]. Fraction E3 (0.85 g) was further separated by ODS CC using a stepwise gradient [20 g, MeOH–H_2O (40:60, v/v) → MeOH] to give six fractions [Fr. E3-1 (66.8 mg), Fr. E3-2 (98.5 mg), Fr. E3-3 (0.24 g), Fr. E3-4 (0.10 g), Fr. E3-5 (0.19 g) and Fr. E3-6 (43.6 mg)]. Fraction E3-2 (98.5 mg) was purified by HPLC [$CHCl_3$–MeOH–H_2O (15:6:1), 3.0 mL/min, detector, 222 nm] to yield pachanoside C1 (**1**, 16.3 mg, 0.007%). Furthermore, fraction E3-3 (0.23 g) was separated by ODS CC using a stepwise gradient [20 g, MeOH–H_2O (20:80, v/v) → MeOH] to give three fractions [Fr. E3-3-1 (16.7 mg), Fr. E3-3-2 (0.21 g) and Fr. E3-3-3 (20.1 mg)]. Fraction E3-3-2 (0.21 g) was purified by HPLC [MeOH–H_2O (60:40, v/v), 2.0 mL/min, detector, 222 nm] to yield pachanoside C1 (**1**, 32.1 mg, 0.014%).

Fraction D (2.22 g) was applied to a silica gel column using a stepwise gradient [70 g, $CHCl_3$–MeOH–H_2O (60:10:1) → MeOH] to afford six fractions [Fr. D1 (19.5 mg), Fr. D2 (10.7 mg), Fr. D3 (0.52 g), Fr. D4 (0.49 g), Fr. D5 (0.88 g) and Fr. D6 (0.17 g)]. Fraction D5 (0.88 g) was further separated by ODS column chromatography using a stepwise gradient [20 g, CH_3CN–H_2O (30:70, v/v) → CH_3CN] to give six fractions [Fr. D5-1 (0.33 g), Fr. D5-2 (0.28 g), Fr. D5-3 (26.0 mg), Fr. D5-4 (12.6 mg), Fr. D5-5 (13.6 mg) and Fr. D5-6 (154.2 mg)]. Fraction D5-1 (0.33 g) underwent ODS CC using a stepwise gradient [20 g, MeOH–H_2O (40:60, v/v) → MeOH] to yield pachanoside B (**2**, 20.0 mg, 0.009%). Fraction D5-2 (0.28 g) was further purified by ODS CC using a stepwise gradient [20 g, MeOH–H_2O (40:60, v/v) → MeOH] to afford 10 fractions [Fr. D5-2-1 (5.1 mg), Fr. D5-2-2 (7.6 mg), Fr. D5-2-3 (1.4 mg), Fr. D5-2-4 (15.4 mg), Fr. D5-2-5 (29.7 mg), Fr. D5-2-6 (53.2 mg), Fr. D5-2-7 (0.12 g), Fr. D5-2-8 (11.6 mg), Fr. D5-2-9 (43.0 mg) and Fr. D5-2-10 (3.2 mg)]. Fraction D5-2-5 (29.7 mg) was identified as pachanoside C1 (**1**, 38.6 mg, 0.02%). Furthermore, fraction D5-2-6 (53.2 mg) was separated by ODS CC using a stepwise gradient [20 g, CH_3CN–H_2O (30:70, v/v) → CH_3CN] to give pachanoside C1 (**1**, 36.8 mg, 0.02%). Fraction D5-2-7 (0.12 g) was separated by ODS CC using a stepwise gradient [20 g, CH_3CN–H_2O (25:75, v/v) → CH_3CN] to afford bridgeside C1 (**6**, 77.1 mg, 0.03%).

Fraction E4 (1.64 g) was subjected to ODS CC using a stepwise gradient [35 g, CH_3CN–H_2O (20:80, v/v) → CH_3CN] to afford seven fractions [Fr. E4-1 (0.20 g), Fr. E4-2 (0.51 g), Fr. E4-3 (0.26 g), Fr. E4-4 (0.15 g), Fr. E4-5 (0.34 g), Fr. E4-6 (70.3 mg) and Fr. E4-7 (59.2 mg)]. Fraction E4-5 (0.34 g) was further separated by silica gel CC using a stepwise gradient [76 g, $CHCl_3$-MeOH-H_2O (20:7:1) → MeOH] to afford five fractions [Fr. E4-5-1 (19.9 mg), Fr. E4-5-2 (64.1 mg), Fr. E4-5-3 (58.6 mg), Fr. E4-5-4 (40.5 mg) and Fr. E4-5-5 (0.15 g)]. Fraction E4-5-3 (58.6 mg) was purified by HPLC [$CHCl_3$–MeOH–H_2O (15:6:1), 3.0 mL/min, detector, 222 nm] to give bridgeside C1 (**6**, 42.5 mg, 0.018%). Furthermore, fraction E4-5-5 (0.15 g) was purified by HPLC [MeOH–H_2O (35:65, v/v), 2.0 mL/min, detector, 222 nm] to yield bridgeside C2 (**7**, 0.12 g, 0.054%). Fraction E4-3 (0.26 g) was purified by HPLC [$CHCl_3$–MeOH–H_2O (15:6:1), 3.0 mL/min, detector, 222 nm] to give bridgeside D1 (**8**, 33.2 mg, 0.014%) and bridgeside D2 (**9**, 0.11 g, 0.047%).

Fraction D3 (0.52 g) underwent ODS CC using a stepwise gradient [35 g, MeOH–H_2O (40:60, v/v) → MeOH] to yield bridgeside E1 (**10**, 82.7 mg, 0.036%). Fraction D5-6 (0.15 g) was separated by ODS CC using a stepwise gradient [20 g, MeOH–H_2O (60:40, v/v) → MeOH] to afford three fractions [Fr. D5-6-1 (20.9 mg), Fr. D5-6-2 (0.13 g) and Fr. D5-6-3 (11.4 mg)]. Fraction D5-6-2 (0.13 g) was purified by HPLC [$CHCl_3$–MeOH–H_2O (15:6:1), 3.0 mL/min, detector, 222 nm] to yield bridgeside E2 (**11**, 80.7 mg, 0.035%).

Fraction C (4.18 mg) was separated by ODS CC using a stepwise gradient [125 g, CH_3CN–H_2O (10:90, v/v) → CH_3CN] to afford eight fractions [Fr. C1 (0.68 g), Fr. C2 (0.32 g), Fr. C3 (42.6 mg), Fr. C4 (0.12 g), Fr. C5 (46.4 mg), Fr. C6 (0.16 g), Fr. C7 (85.7 mg) and Fr. C8 (32.9 mg)]. Fraction C6 (0.16 g) was subjected to ODS CC using a stepwise gradient [70 g, MeOH–H_2O (10:90, v/v) → MeOH] to yield pachanoside F1 (**3**, 0.26 g, 0.111%) and bridgeside E1 (**10**,

71.8 mg, 0.031%). Furthermore, fraction C4 (0.12 g) was purified by ODS CC using a stepwise gradient [45 g, MeOH–H_2O (40:60, v/v) → MeOH] to afford pachanoside G1 (**4**, 51.1 mg, 0.022%).

*3.3.1. Pachanoside C1 (**1**)*

Colorless powder (124 mg); $[\alpha]_D^{25}$ −14.7 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3400, 1738, 1720, 1600, 1240, 1030 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESI (Negative) Tof MS *m/z*: 997.4676 $[M-H]^-$ (calcd for $C_{49}H_{73}O_{21}$, 997.4644 $[M-H]^-$), ESI (Positive) Tof MS *m/z*: 1021.4620 (calcd for $C_{49}H_{74}O_{21}Na$ $[M+Na]^+$, 1021.4620).

*3.3.2. Pachanoside E1 (**2**)*

Colorless powder (20.0 mg); $[\alpha]_D^{25}$ −14.6 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3430, 1740, 1720, 1250, 1080, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative FABMS *m/z*: 997 $[M-H]^-$, 865 [M-xyl-H]$^-$; negative HRFABMS *m/z*: 997.4641 (calcd for $C_{49}H_{73}O_{21}$ $[M-H]^-$, 997.4644).

*3.3.3. Pachanoside F1 (**3**)*

Colorless powder (255 mg); $[\alpha]_D^{25}$ −10.2 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3400, 1740, 1240, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative FABMS *m/z*: 1039 $[M-H]^-$, 907 [M-xyl-H]$^-$, 745 [M-xyl-glc-H]$^-$; negative HRFABMS *m/z*: 1039.4742 (calcd for $C_{51}H_{75}O_{22}$ $[M-H]^-$, 1039.4750).

*3.3.4. Pachanoside G1 (**4**)*

Colorless powder (51.1 mg); $[\alpha]_D^{25}$ −15.8 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3410, 1730, 1240, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative FABMS *m/z*: 1039 $[M-H]^-$, 907 [M-xyl-H]$^-$, 745 [M-xyl-glc-H]$^-$, 569 [aglycone moiety-H]$^-$; negative HRFABMS *m/z*: 1039.4747 (calcd for $C_{51}H_{75}O_{22}$ $[M-H]^-$, 1039.4750).

*3.3.5. Bridgeside A1 (**5**)*

Colorless powder (272 mg); $[\alpha]_D^{25}$ −33.5 (*c* 0.98, MeOH); IR ν_{max} (KBr) 3400, 1760, 1600, 1240, 1040 cm^{-1}; 969 $[M-H]^-$, 823 [M-rha-H]$^-$, 661 [M-rha-glc-H]$^-$, 485 [aglycone moiety-H]$^-$; ^1H NMR (pyridine-d_5) δ: 0.76, 1.07, 1.13, 1.22, 1.33, 1.51 (each 3H, s), 1.77 (3H, d, *J* = 5.6 Hz), 3.31 (1H, m), 4.97 (1H, d, *J* = 6.6 Hz), 5.49 (1H, br s), 5.82 (1H, d, *J* = 7.1 Hz), 6.37 (1H, br s); ^{13}C-NMR (pyridine-d_5) δ: 16.1, 16.8, 19.0, 19.7, 25.0, 25.3, 28.3 (each CH_3), 63.1 (CH_2), 63.4 (CH_2), 89.7 (CH), 101.8 (CH), 101.9 (CH), 105.1 (CH), 127.9 (CH), 137.9 (C), 179.4 (C); negative HRFABMS *m/z*: 969,4686 (calcd for $C_{48}H_{73}O_{20}$ $[M-H]^-$, 997.4644).

*3.3.6. Bridgeside A1 methyl ester (**5a**)*

Colorless powder (15.1 mg); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative FABMS *m/z*: 983 $[M-H]^-$.

*3.3.7. Bridgeside C1 (**6**)*

Colorless powder (105 mg); $[\alpha]_D^{25}$ −33.7 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3420, 1760, 1740, 1260, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative FABMS *m/z*: 997 $[M-H]^-$, 865 [M-xyl-H]$^-$, 703 [M-xyl-glc-H]$^-$; negative HRFABMS *m/z*: 997.4645 (calcd for $C_{49}H_{73}O_{21}$ $[M-H]^-$, 969.4644).

*3.3.8. Bridgeside C2 (**7**)*

Colorless powder (124 mg); $[\alpha]_D^{25}$ −44.6 (*c* 1.00, DMSO); IR ν_{max} (KBr) 3410, 1770, 1730, 1620, 1415, 1395, 1250, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; positive FABMS *m/z*: 1057 [(RCOONa)+Na]$^+$, 1035 [(RCOONa)+H]$^+$; negative FABMS *m/z*: 1033 [(RCOONa)-H]$^-$, 1011 [(RCOONa)-Na]$^-$, 887 [(RCOONa)-rha-H]$^-$, 865 [(RCOONa)-rha-Na]$^-$, 703 [(RCOONa)-rha-glc-Na]$^-$; positive HRFABMS *m/z*: 1057.4602 (calcd for 1057.4596, $C_{50}H_{75}O_{21}Na_2$), 1035.4784 (calcd for $C_{50}H_{76}O_{21}Na$ $[M+H]^-$, 1035.4777).

*3.3.9. Bridgeside D1 (**8**)*

Colorless powder (33.2 mg); $[\alpha]_D^{25}$ -17.9 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3420, 1760, 1730, 1240, 1120, 1080, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative FABMS *m/z*: 997 $[M-H]^-$, 865 [M-xyl-H]$^-$, 703 [M-xyl-glc-H]$^-$; negative HRFABMS *m/z*: 997.4646 (calcd for $C_{49}H_{73}O_{21}$ $[M-H]^-$, 997.4644).

*3.3.10. Bridgeside D2 (**9**)*

Colorless powder (107 mg); mp 198–201 °C; $[\alpha]_D^{25}$ -34.5 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3410, 1760, 1740, 1240, 1130, 1080, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative FABMS *m/z*: 1011 $[M-H]^-$, 865 [M-rha-H]$^-$, 703 [M-rha-glc-H]$^-$; negative HRFABMS *m/z*: 1011.4797 (calcd for $C_{50}H_{75}O_{21}$ $[M-H]^-$, 1011.4801).

*3.3.11. Bridgeside E1 (**10**)*

Colorless powder (155 mg); mp 205–208 °C; $[\alpha]_D^{25}$ −28.1 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3400, 1720, 1370, 1230, 1030 cm^{-1}; negative FABMS *m/z*: 1039 $[M-H]^-$, 907 [M-xyl-H]$^-$, 745 [M-xyl-glc-H]$^-$; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative HRFABMS *m/z*: 1039.4762 (calcd for $C_{51}H_{75}O_{22}$ $[M-H]^-$, 1039.4750).

*3.3.12. Bridgeside G (**11**)*

Colorless powder (80.7 mg); $[\alpha]_D^{25}$ −36.5 (*c* 1.00, MeOH); IR ν_{max} (KBr) 3415, 1740, 1380, 1240, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative FABMS *m/z*: 1053 $[M-H]^-$, 907 [M-rha-H]$^-$, 745 [M-rha-glc-H]$^-$; negative HRFABMS *m/z*: 1053.4902 (calcd for $C_{52}H_{77}O_{22}$ $[M-H]^-$, 1053.4907).

3.4. Determination of sugar configuration (Tanaka et al., 2007)

Saponins (**1**–**11**) (each 2 mg) were hydrolyzed by 3.5% HCl (0.4 mL) at 110 °C for 2.5 h. The reaction mixture was neutralized with 0.5 M NaOH. After drying under vacuum, the residue was dissolved in pyridine (0.4 mL) containing L-cysteine methyl ester hydrochloride (2 mg) and heated at 60 °C for 1 h. *O*-Toylisothiocyanate (2 μL) was then added and the mixture was heated at 60 °C for 1 h. Each reaction mixture was directly analyzed by reversed-phase HPLC using a SSC-3461 HPLC Pump with a SSC-5410 UV/VIS detector (Senshu Scientific Co. Ltd.). An Senshu Pak PEGASIL 4.6 $\phi \times 250$ mm HPLC column was used, (temp, 35 °C; flow, 0.8 ml/min; eluate, CH_3CN–H_2O (25:75) containing 50 mM H_3PO_4). The HPLC column was washed with MeOH after each injection. The reaction conditions for D, L-glucose, D, L-glucuronic acid, D, L-xylose and L-rhamnose were same as described above. For D-rhamnose, L-rhamnose dissolved in pyridine (0.4 mL) containing D-cysteine methyl ester hydrochloride (2 mg) was used for the reaction. The derivative was used for determining retention time instead of D-rhamnose. Retention times for authentic sugar derivatives, D-glucose (18.69), L-glucose (17.01), D-xylose (21.08), L-xylose (20.03), D-glucuronic acid (19.35), L-glucuronic acid (18.60), L-rhamnose (33.12) and as D-rhamnose (17.74) were used for comparison with retention times from reaction mixtures for each saponin. Peaks at 18.5, 19.2 and 21.4 minutes of the sugar derivatives from **1** coincided with derivatives of D-glucose, D-glucuronic acid, and D-xylose. Peaks at 18.4, 19.2 and 21.4 of the sugar derivatives coincided with derivatives of D-glucose, D-glucuronic acid, and D-xylose. For the other saponins (**3**–**11**), the same results as compounds **1** and **2** were obtained.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2010.10.004.

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