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Two Novel Triterpenoid Pentasaccharides with an Unusual Glycosyl Glycerol Side Chain from *Ardisia crenata*

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Abstract: Two novel triterpenoid pentasaccharides, ardisicrenosides E and F, were isolated from the roots of *Ardisia crenata*. They are the first examples of a triterpenoid glycoside hybridized with a glycosyl glycerol. Their structures were mainly established on the basis of high field NMR studies. In addition, molecular mechanics and dynamics calculation studies showed that the lack of ^{13}C glycosylation shifts in the Rhamα1→2G'lcβ and G'lcβ1→4Araα fragments in ardisicrenoside E could be correlated with the distortion of the corresponding torsion angles. Both compounds exhibited moderate inhibitory activity on cAMP phosphodiesterase.

Ardisia crenata Sims (Myrsinaceae) is a widely occurring shrub in the south part of China. Its roots have been used in the treatment of respiratory tract infections and menstrual disorders in Chinese traditional medicine, and showed significant anti-fertility effects in modern pharmacological studies.[1] Previous chemical studies showed that triterpenoid glycosides were the main components of this genus.[2-4] Most recently, we have reported the isolation and structure study of several triterpenoid glycosides from *A. crenata*.[5,6] In this paper, we wish to report the isolation and structure study of two novel triterpenoid pentasaccharides, ardisicrenosides E (**1**) and F (**2**), from this source.

RESULTS AND DISCUSSION

Compound **1**, an amorphous solid, $[\alpha]_D$ + 30.4° (MeOH), has a molecular formula of $C_{63}H_{102}O_{31}$, as determined from its positive ion FABMS (*m/z* 1355 $[M+H]^+$, 1377 $[M+Na]^+$) and ^{13}C, DEPT NMR spectra. Its spectral features and physichemical properties suggested **1** to be a triterpenoid glycoside. Of the 63 carbons, 30 were assigned to the aglycone part, 29 to the oligosaccharide moiety, one to a methoxyl group, and the remaining 3 to a glycerol group (Tables 1 and 2). The six sp^3 quaternary carbon signals at δ 15.3, 16.3, 16.6,

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27.0, 27.8, 28.4 and the two sp^2 hybrid carbons at δ 122.7 (d) and 144.3 (s) indicated that the aglycone had an olean-12-en skeleton (Table 1). Its ^1H and ^{13}C NMR displayed five sugar anomeric signals at δ 4.84 (obscured by another signal), 5.09 (d, 7.6), 5.20 (d, 7.7), 5.35 (d, 3.6), 6.18 (s), and δ 101.2 (x 2), 102.8, 104.0 and 104.8, respectively (Tables 2 and 3). The nature of the monosaccharides and sequence of the oligosaccharide chain was determined by a combination of COSY, HOHAHA, HETCOR, HMBC and ROESY experiments. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were identified using COSY with the aid of the 2D-HOHAHA spectrum. On the basis of the assigned hydrogens, the ^{13}C resonances of each sugar unit were assigned by HETCOR and further confirmed by HMBC experiment. Interpretation of the COSY and 2D-HOHAHA spectrum revealed the presence of five sugar units and one of the spin systems, consisting of five protons, was assigned to a hexosyluronic acid residue. After mapping all of the spin systems for each individual sugar, a five-proton network ascribable to a glycerol residue was evident from the COSY spectrum in the region of δ 3.5-4.5 ppm. Correlated *via* a HETCOR experiment, the corresponding carbons were shown to be 65.4 (CH_2), 68.6 (CH) and 70.9 (CH_2), further substantiating the existence of the glycerol unit. Results from an HMBC (the mixing time was optimized at 5 Hz) cross-verified the glycerol fragment (Fig.1). After treatment with 3% sodium methoxide in MeOH, compound **1** resulted in a prosapogenin methyl ester (**3**) and a monoglycosyl glycerol. The ^1H and ^{13}C NMR spectra showed that compound **3** consisted of four sugar units (Table 2). Acidic hydrolysis of the prosapogenin in 1N HCl (MeOH-H_2O, 1:1) yielded a sapogenin identified to be 3β, 16α, 28-trihydroxy-12-olean-30-oic acid methyl ester (jacquinic acid methyl ester).[7] The monosaccharides were shown to be arabinose, glucose and rhamnose (1:2:1) based on GLC analysis. Hydrolysis of the glycosyl glycerol with HCl vapor on a TLC plate (both the sample and authentic monosaccharides were applied to the TLC plate at the same time. Hydrolysis condition: 65 °C, 1 hour),[8] followed by developing in the systems chloroform-methanol-water (10:5:1 or 7:3:1) revealed that the saccharide of the glycosyl glycerol moiety was a glucuronic acid. The above evidence showed that compound **1** was a pentasaccharide with a monoglycosyl glycerol moiety attached to the C-30 of the aglycone through an ester bond and the other four, in a chain, were ether-linked to the C-3 position of the aglycone.

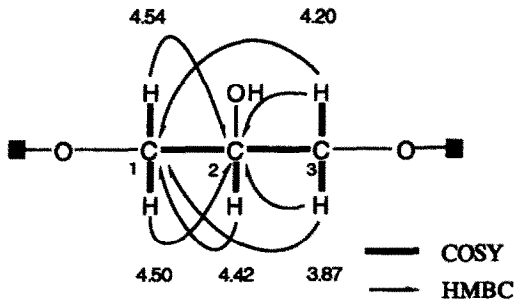


Fig.1. The Correlations of the Glycerol Fragment in COSY and HMBC.

All the monosaccharides in the pyranose forms were determined from their ^{13}C NMR data. The β anomeric configurations for the glucoses were judged from their large $^3J_{H1, H2}$ coupling constants (7-8 Hz). The ^1H non-splitting pattern along with its extremely low chemical shift of the rhamnose and the small $^3J_{H1, H2}$ coupling constants (3.6 Hz) of the glucuronic acid residue indicated α orientations. The anomeric proton of the

arabinose was obscured by another signal, thus no information was available from the ^1H NMR spectrum. However, information from the ROESY experiment showed that there were NOEs between H-1, H-3 and H-5 and this could be used as an indication that the arabinose adopts an α orientation at its anomeric carbon.[9] Results from a ^1H non-decoupled ^{13}C spectrum supported these conclusions (Table 2). The absolute configurations of these monosaccharides were chosen in keeping with those mostly encountered among plant glycosides.

The linkage of the sugar units was established using the following HMBC correlations: H-1 of rhamnose with C-2 of the inner glucose; H-1 of the inner glucose with C-4 of arabinose; and H-1 of the terminal glucose with C-2 of arabinose, while the position of the tetrasaccharide chain to C-3 of the aglycone was based on a correlation between H-1 of arabinose and the C-3 of the aglycone. The same conclusion was drawn from the ROESY experiment (Fig.2). The connectivities between the aglycone, the glycerol fragment, and the glucuronic acid residue were also established from the HMBC experiment. The correlations between the ^{13}C resonance at δ 177.5 (C-30, aglycone) and the ^1H resonance at δ 4.50, 4.54 (C_1-H, glycerol), and between δ 101.2 (C-1, glu A) and δ 3.87, 4.20 (C_3-H, glycerol) clarified the side chain structure (Fig.2). In addition, an HMBC correlation between δ 170.9 (C-6, glu.A) and δ 3.67 (3H, s, OCH_3) showed evidence that

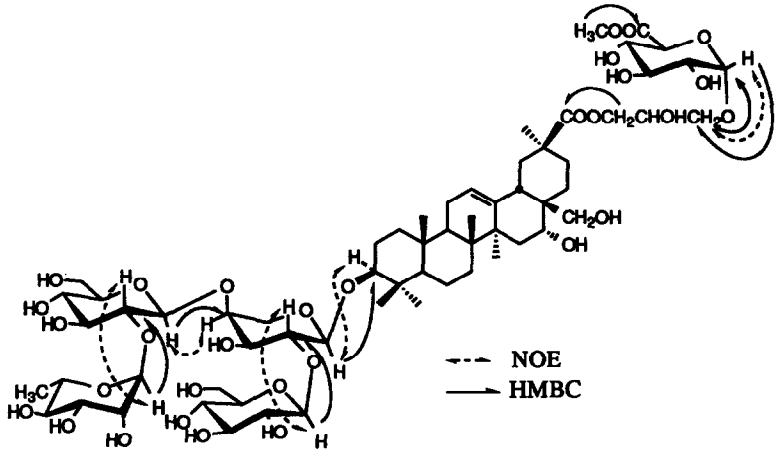
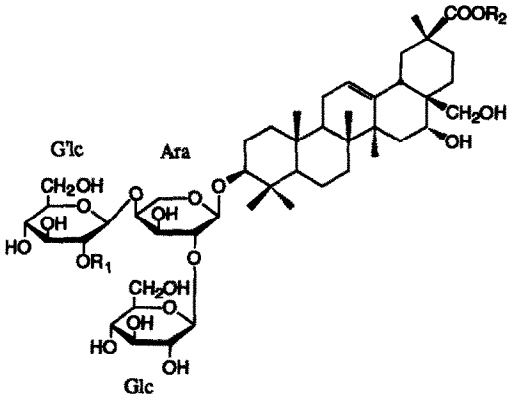


Fig. 2. Some Key Correlations of Ardisicrenoside E (1) Observed in ROESY and HMBC Experiments.

the glucuronic acid was in the form of a methyl ester. The stereochemistry of the glycerol remains to be determined. From the foregoing evidence, the structure of ardisicrenoside E (1) was elucidated to be 3β-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α, 28-dihydroxy-olean-12-en-30-oic acid 30-*O*-[3'-*O*-(methyl α-D-glucopyranuronate) glycerol (1'→30)] ester.

Ardisicrenoside F (2) is an amorphous solid, $[\alpha]_D + 41.6°$, $C_{62}H_{100}O_{31}$ (positive FAB-MS ions at *m/z* 1341 $[M+H]^+$, 1363 $[M+Na]^+$). Its IR, ^1H and ^{13}C NMR spectra indicated that compound 2 had the same aglycone as that of 1 but differed in the oligosaccharide part (Tables 1 and 2). Alkaline hydrolysis of 2 as

carried out for **1** yielded a prosapogenin methyl ester (**4**) and a glycosyl glycerol. Further subjected to acidic hydrolysis, compound **4** afforded the same sapogenin as **1**, and the monosaccharides were identified to be xylose, glucose and arabinose in the ratio of 1:2:1 from GLC analysis. Extensive NMR studies using a combination of COSY, HOHAHA, HETCOR, ROESY and HMBC showed that the only difference between compounds **1** and **2** was the terminal rhamnose in **1** having been replaced by a xylose in **2**. Thus, the structure of ardisicrenoside F (**2**) was established to be 3β-*O*-{β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α, 28-dihydroxy-olean-12-en-30-oic acid 30-*O*-[3'-*O*-(methyl α-D-glucopyranuronate) glycerol (1'→30)] ester.



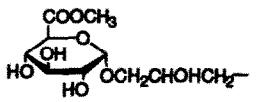
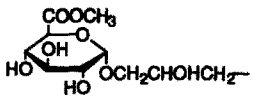
	R_1	R_2
1.	α-L-rham	
2.	β-D-xyl	
3.	α-L-rham	CH_3
4.	β-D-xyl	CH_3

Table 1 ^{13}C NMR Data for the Aglycone Parts of Ardisicrenosides E (**1**) and F (**2**) (125 MHz in pyridine-d_5)*

Carbon	1	2	Carbon	1	2
1	38.6 t	38.7 t	16	73.4 d	73.7 d
2	26.0 t	26.2 t	17	39.9 s	40.1 s
3	88.8 d	88.9 d	18	43.5 d	43.6 d
4	39.1 s	39.4 s	19	44.1 t	44.3 t
5	55.5 d	55.7 d	20	44.4 s	44.6 s
6	18.1 t	18.3 t	21	33.3 t	33.5 t
7	32.9 t	33.0 t	22	31.9 t	32.0 t
8	39.7 s	39.9 s	23	27.8 q	28.0 q
9	46.8 d	46.9 d	24	16.3 q	16.6 q
10	36.5 s	36.7 s	25	15.3 q	15.5 q
11	23.4 t	23.6 t	26	16.6 q	16.8 q
12	122.7 d	122.8 d	27	27.0 q	27.2 q
13	144.3 s	144.5 s	28	70.1 t	70.2 t
14	41.5 s	41.6 s	29	28.4 q	28.6 q
15	34.4 t	34.6 t	30	177.5 s	177.6 s

* Assignments were based upon COSY, HOHAHA, HETCOR, DEPT and HMBC experiments.

Table 2 ^{1}H and ^{13}C NMR Data for the Oligosaccharide Parts of Ardisicrenosides E (1) and F (2) (500 MHz in pyridine-d_5)[a]

	Ardisicrenoside E (1)		Ardisicrenoside F (2)	
Sugar Units	^{1}H shift	^{13}C shift	^{1}H shift	^{13}C shift
3-*O*-sugar				
Arabinose (A)				
A-1	4.84[b] ($^{1}J_{CH}$=164.3 Hz)	104.0 (d)	4.73 d (5.8 Hz)	104.5 (d)
A-2	4.42	80.0 (d)	4.45	79.5 (d)
A-3	4.37	71.7 (d)	4.20	73.0 (d)
A-4	4.48	74.5 (d)	4.19	78.4 (d)
A-5	3.74 d (11.0)	63.2 (t)	4.56	64.1 (t)
	4.31		3.64	
Glucose (G) (terminal)				
G-1	5.20 d (7.6) ($^{1}J_{CH}$=159.9)	104.8 (d)	5.12 d (7.6)	104.6 (d)
G-2	3.89	75.8 (d)	3.98	75.9 (d)
G-3	4.13	77.4 (d)	4.14	77.9 (d)
G-4	4.06	71.1 (d)	4.13	71.5 (d)
G-5	3.90	77.6 (d)	3.92	77.7 (d)
G-6	4.35	62.3 (t)	4.45, 4.31 dd	62.7 (t)
	4.23		(11.6, 4.9)	
Glucose (G') (inner)				
G'-1	5.09 d (7.6) ($^{1}J_{CH}$=155.5)	102.8 (d)	4.93 d (7.6)	103.9 (d)
G'-2	4.11	77.1 (d)	3.85 dd (8.2)	85.1 (d)
G'-3	4.04	78.9 (d)	4.12	77.3 (d)
G'-4	3.95 dd (9.1,9.2)	71.4 (d)	4.11	70.9 (d)
G'-5	3.67	77.8 (d)	3.73 m	78.0 (d)
G'-6	4.31	62.1 (t)	4.36	62.1 (t)
	4.13		4.22	
Rhamnose (R)				
R-1	6.18 (s) ($^{1}J_{CH}$=170.2)	101.2 (d)		
R-2	4.57	72.8 (d)		
R-3	4.49	72.1 (d)		
R-4	4.11	74.2 (d)		
R-5	4.87	69.1 (d)		
R-6	1.69 d (6.1)	18.4 (q)		
Xylose (X)				
X-1			4.86 d (6.7)	107.3 (d)
X-2			3.93	75.7 (d)
X-3			3.94	77.4 (d)
X-4			4.06 m	70.4 (d)
X-5			4.44	67.2 (t)
			3.65	
30-*O*-glycerol				
Gly-1	4.54 dd (11.2, 6.8)	65.4 (t)	4.60 dd (11.2, 4.9)	65.6 (t)
	4.50[b]		4.56 dd (11.2, 5.2)	
Gly-2	4.42[b]	68.6 (d)	4.48[b]	68.8 (d)
Gly-3	4.21 dd (10.2, 4.3)	70.9 (t)	4.25 dd (11.4, 4.0)	71.1 (t)
	3.87 dd (10.2, 7.1)		3.93[b]	
Glucuronic acid				
GA-1	5.35 d (3.6) ($^{1}J_{CH}$=170.2)	101.2 (d)	5.41 d (3.6)	101.4 (d)
GA-2	4.06	72.8 (d)	4.14	73.1 (d)
GA-3	4.44	74.0 (d)	4.50	74.3 (d)
GA-4	4.29	72.8 (d)	4.37	73.1 (d)
GA-5	4.78 d (10.0)	73.0 (d)	4.85 d (9.8)	73.2 (d)
GA-6	—	170.9 (s)	—	171.0 (s)
COOMe	3.67	51.6 (q)	3.68	51.8 (q)

a. Assignments were based upon COSY, HOHAHA, HETCOR, DEPT, ROESY and HMBC experiments.
b. Obscured by other signals.

From the detailed analysis and comparison of the assigned ^{13}C NMR data of compounds **1** and **2**, we found that a significant difference existed between the glycosylation shifts caused by the attachment of rhamnose (as in **1**) or xylose (in **2**) to the C-2 of the inner glucose. Compared with the corresponding carbons of the terminal glucose in the same molecule, the glycosylation shifts in compound **2** are +7.6 ppm for C-2 and -0.5 ppm for C-3, conforming to the established rule for a glycosylation shift.[10] However, such changes in **1** are +1.4 ppm for C-2, and +1.5 ppm for C-3, too small and abnormal to predict an interglycosidic site. Also, this irregular effect has been propagated through the inner glucose to the C-4 of arabinose, which was approximately 4 ppm upfield compared to its counterpart in compound **2** (Table 2, Fig. 4). A literature search showed that a similar situation existed in some glycosides possessing rhamnose 1→2 glucose or galactose units.[11-13] Therefore, application of the glycosylation shift rule here should be treated with caution, especially in the structure study of oligosaccharides with a branched chain. It seems that the 6-deoxy group in the terminal rhamnose is responsible for this irregular phenomenon. In order to obtain some information about the geometry and energy of compounds **1** and **2** and to reflect the ^{13}C NMR shift abnormality from a deeper theory basis, conformational analysis using the cff 91 force field[14a] was carried out on compounds **3** and **4**. The calculation results showed that the glycosidic torsion angles φ and ψ (defined as H1-C1-O1-Cx and C1-O1-Cx-Hx, respectively) of the preferred conformations of compounds **3** and **4** (Fig.3) are obviously different in the global minimum energy conformations between the Rhaα1→2Glcβ and Xylβ1→2Glcβ as well as between their Glcβ→4Araα fragments (Table 3). The conformations of the remaining parts are nearly the same.

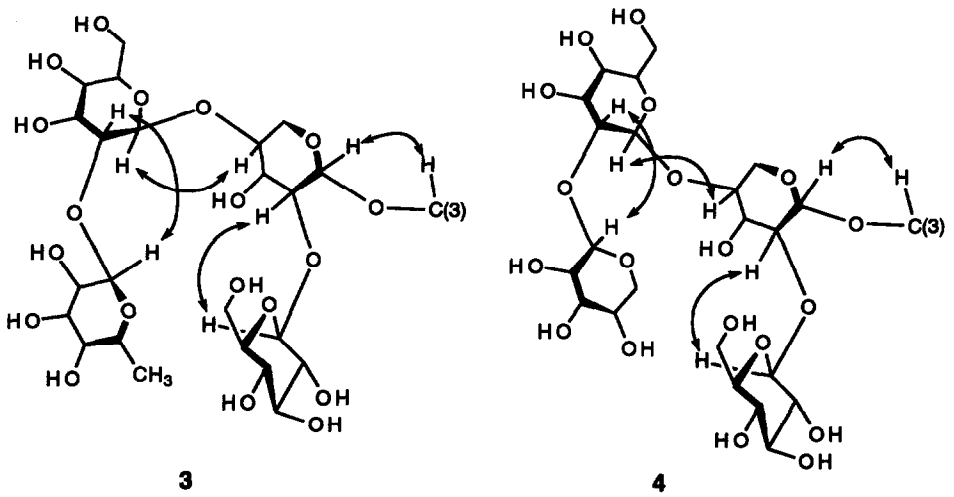


Fig. 3 The Calculated Preferred Conformations of Compounds **3** and **4**
(the arrows show the NOEs from NMR and from calculations)

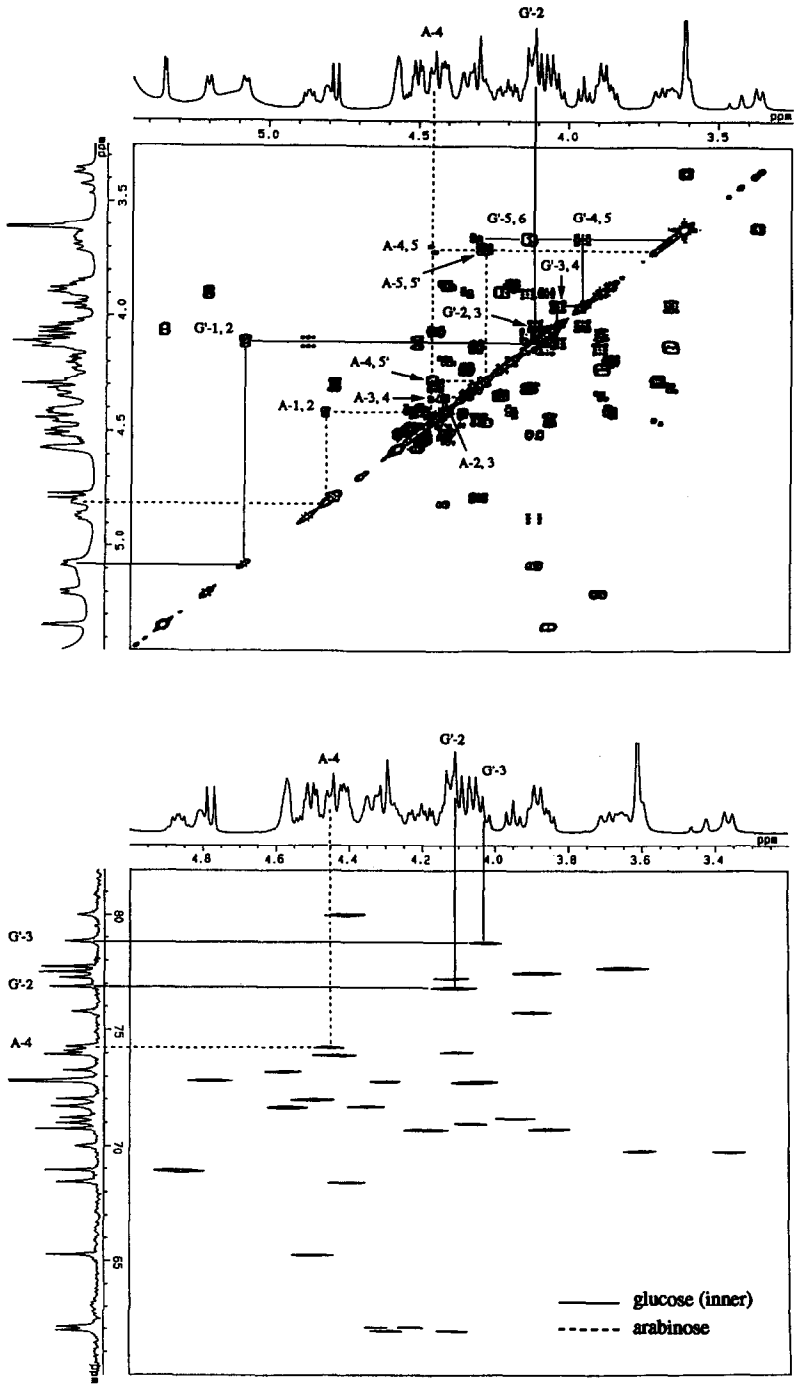


Fig. 4. COSY and HETCOR Spectra of the Oligosaccharide Moiety for Ardisicrenoside E (1)

Table 3. ^{13}C Chemical Shifts of the Anomeric and the Aglyconic Carbons and the Calculated Minimum Energy Conformations of the Tetrasaccharide Units of Compounds **3** and **4**

Compounds	Fragment[a]	^{13}C anomeric	^{13}C and GS[b] aglyconic	φ(°)	ψ(°)
	Rhamα(L)1→2G'lcβ(D)	101.2	77.1(+1.5)[c]	−32.76	−21.73
3	G'lcβ(D)1→4Araα(L)	102.8	74.5	+37.29	+9.02
	Glcβ(D)1→2Araα(L)	104.8	80.0	+63.07	+9.58
	Xylβ(D)1→2G'lcβ(D)	107.3	85.1(+7.6)[c]	+55.83	−4.04
4	G'lcβ(D)1→4Araα(L)	103.9	78.4	+52.55	−15.27
	Glcβ(D)1→2Araα(L)	104.6	79.5	+67.95	+4.06

[a]G'lc and Glc refer to inner and terminal glucose residues, respectively.
[b]GS: glycosylation shift.
[c]Compared with the corresponding carbons of the terminal glucose in the same molecule.

Experimental[15,16] and theoretical[17,18] studies indicated that for glycosidic linkages the minimum energy value of φ is in the region of +60° in the case of β-D- or α-L-glycosides. Our experimental results seem fit quite well with these observations except for the Rhamα(L)1→2G'lcβ(D) and G'lcβ(D)1→4Araα(L) residues of **3**, which have a φ magnitude of -32.76 and +37.29, respectively (Table 3). These changes in conformations around the glycosidic bonds could be attributed to the repulsive nature between the bulky and lipophilic 6-deoxy group of the rhamnose and the hydroxyl groups of other sugars. Such important non-bonded interactions could cause appreciable distortion in the glycosidic valence angles. Lemieux et al.[15] have concluded that deshielding effects, which are the causes of the lack of glycosylation, are highly sensentive to the changes in the conformations of the atoms about the glycosidic linkage. Therefore, the lack of glycosylation shifts of C-2 of G'lc and C-4 of arabinose in **3** could be explained by these changes in the corresponding torsion angles. In addition, Bock et al.[19] reported that the ^{13}C chemical shifts for both the glycoside and the aglycone carbons in oligosaccharides can be directly correlated with the torsion angle ψ. In our case, the opposite sign of the ψ magnitude of the G'lcβ(D)1→4Araα(L) fragments of compounds **3** and **4** could be correlated with the ^{13}C shift discrepancy between the C-4 of the arabinoses.

Triterpenoid glycosides and glycosyl glycerides are very common natural products. The latter are the major membrane lipids in a wide variety of plants and microorganisms. However, to our knowledge, a triterpenoid glycoside hybridized with a glycosyl glycerol has never been reported before. Moreover, the occurrence of the α-D-glucuronosyl glycerol segments in nature is very rare. It is a little difficult to explain the existence of these compounds from a biosynthesis point of view. Inhibitory assay towards cAMP phosphodiesterase[20] showed that both compounds exhibited moderate activity with an IC_{50} of 55.7 and 104.0 x 10^{-5}M, respectively.

EXPERIMENTAL

***General Procedures*:** All melting points were measured using a Yanaco microscope apparatus and are uncorrected. IR spectra were determined using a JASCO 7300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. EI and FABMS were conducted using a JEOL D-300 and a DX-303 mass spectrometers, respectively. ^1H and ^{13}C NMR were recorded using a JEOL A-500 FT-NMR or a JEOL EX-400 FT-NMR spectrometer. Chemical shifts were expressed in δ (ppm) referring to solvent peaks: δ_H 7.20 and δ_C 135.50 for pyridine-d_5. TLC was carried out on silica gel 60 F_{254}, and spots were visualized by spraying with 10 % H_2SO_4 for triterpenoid glycosides and a mixture of aniline and o-phthalic acid in BuOH for monosaccharides. Diaion HP-20 (Mitsubishi Kasei), silica gel (Silica gel 60, Merck), and ODS (Chromatorex, 100-200 mesh, Fujisylisia) were used for column chromatography. Preparative HPLC was performed using an ODS column (Capcell pak ODS, Shiseido, 10 mm i.d.x 250 mm, detector: reflective index). GLC: 25 SE-30 on Chromsorb W (60-80 mesh), 3 mm i.d. x 1.5 m, 150°C column temperature, N_2 carrier gas, 15 ml/min flow rate.

Extraction and isolation of the triterpenoid glycosides. The roots of *Ardisia crenata* Sims were collected in Jiangxi, China in 1988. Dried powdered roots (5 kg) of *Ardisia crenata* were first defatted with petroleum ether, and then extracted with $CHCl_3$ and MeOH under reflux conditions. The MeOH extract (230 g) was applied to a column of Diaion HP-20 (1.5 kg) and washed with H_2O, 30, 50 ,70, and 100% MeOH to give 50 fractions. The fractions containing saponins were combined according to their TLC behaviors. Each combined fraction was repeatedly chromatographed on silica gel columns with the solvent system $CHCl_3$-MeOH-H_2O, and then purified on an ODS column with MeOH-H_2O. Further purification by HPLC on ODS with MeOH-H_2O (50:50) afforded the ardisicrenosides E (**1**, 61.0 mg) and F (**2**, 54.2 mg).

Ardisicrenoside E (1). An amorphous solid, mp 227-230°C (dec.), $[\alpha]_D$ + 30.4° (MeOH;*c*=1.00). Anal. Calcd for $C_{63}H_{102}O_{31}\cdot 2H_2O$: C, 54.36; H, 7.68. Found: C, 54.38; H, 7.71. IR ν^{KBr}max cm^{-1}: 3417, 2930, 1733, 1645, 1446, 1375, 1073, 1042. FAB-MS (positive ion mode) *m/z*:1355 $[M+H]^+$, 1377 $[M+Na]^+$. ^1H-NMR (pyridine-d_5, 500 MHz) δ 0.68 (1H, d, *J*=10.9 Hz, H-5), 0.83, 0.87, 0.94, 1.10, 1.28, 1.72 (each 3H, s, H_3 of C-25, C-26, C-24, C-23, C-29, C-27), 2.43 (1H, dd, *J*=14.1, 3.3 Hz, H-18), 2.68 (1H, dd, *J*=13.1, 13.0 Hz, H-19), 3.12 (1H, dd, *J*=11.6, 4.3 Hz, H-3), 3.39, 3.63 (each 1H, d, *J*=11.0 Hz, H_2-28), 4.58 (1H, br.s, H-16), 5.55 (1H, br.t, H-12).

Ardisicrenoside F (2). An amorphous solid, mp 225-228°C (dec.), $[\alpha]_D$ + 41.6°(MeOH;*c*=1.00). Anal. Calcd for $C_{62}H_{100}O_{31}\cdot 3H_2O$: C, 53.35; H, 7.66. Found: C, 53.35; H, 7.71. IR ν^{KBr}max cm^{-1}: 3427, 2926, 1733, 1646, 1456, 1374, 1074, 1043. FAB-MS (positive ion mode) *m/z* 1341 $[M+H]^+$, 1363 $[M+Na]^+$. ^1H NMR (pyridine-d_5, 500 MHz) δ 0.72 (1H, d, *J*=11.6 Hz, H-5), 0.83, 0.89, 1.04, 1.18, 1.31, 1.77 (each 3H, s, H_3 of C-25, C-26, C-24, C-23, C-29, C-27), 2.49 (1H, dd, *J*=13.7, 1.0 Hz, H-18), 2.73 (1H, dd, *J*=13.7, 13.1 Hz, H-19), 3.14 (1H, dd, *J*=11.6, 4.3 Hz, H-3), 3.44, 3.64 (each 1H, d, J=11.0 Hz, H_2-28), 4.64 (1H, br.s, H-16), 5.59 (1H, br.t, H-12).

Alakline treatment of ardisicrenosides E (1) and F (2). 1 (20 mg) was dissolved in 3% sodium methoxide in MeOH (2 ml) and then kept for 3 hours at room temperature (20 °C). After the reaction was finished, the solution was neutralized with a cation-exchange resin (Dowex 50W-X2) and concentrated in vacuum. Chromatography of the residue over ODS yielded a monoglycosyl glycerol and a prosapogenin (3), eluting with H_2O and 60% MeOH-H_2O, respectively.

Using the same method, 2 (20 mg) furnished a prosapogenin (4) and the same glycosyl glycerol.

Prosapogenin 3. An amorphous solid, mp 225 °C (dec.), $[\alpha]^{29}_D$ + 4.0° (MeOH;*c*=1.0). FAB-MS: *m/z* 1127 [M+ Na]$^+$. ^{1}H NMR (pyridine-*d5*, 400 MHz): δ 0.68 (1H, d, *J*=11.3 Hz, H-5), 0.83, 0.90, 0.97, 1.12, 1.25, 1.74 (each 3H, s, H_3 of C-25, C-26, C-24, C-23, C-29, C-27), 2.34 (1H, d, *J*=14.1 Hz, H-18), 2.70 (1H, dd, *J*=13.1, 13.0 Hz, H-19), 3.12 (1H, dd, *J*=11.5, 4.4 Hz, H-3), 3.38, 3.68 (each 1H, d, *J*=10.8 Hz, H_2-28), 3.70 (3H, s, COOMe), 4.64 (1H, br.s, H-16), 4.87 (1H, d, *J*=4.6 Hz, H-1 of ara.), 5.15 (1H, d, *J*=7.1 Hz, H-1 of inner glu.), 5.25 (1H, d, *J*=7.7 Hz, H-1 of term. glu.), 5.46 (1H, br.t, H-12), 6.24 (1H, s, H-1 of rha.). ^{13}C NMR data (pyridine-d_5, 100MHz): δ 38.7, 26.1, 88.9, 39.2, 55.6, 18.2, 32.9, 39.8, 46.8, 36.6, 23.5, 122.6, 144.5, 41.6, 34.5, 73.4, 40.0, 43.8, 44.2, 44.4, 33.4, 32.0, 27.9, 16.4, 15.4, 16.7, 27.1, 70.3, 28.4, 178.0 (aglycone C-1-C-30), 51.2 ($COOCH_3$), 104.2, 80.2, 71.8, 74.5, 63.4 (A-1-A-5), 104.9, 76.0, 77.6, 71.3, 77.7, 62.4 (G-1-G-6), 102.8, 77.2, 79.0, 71.5, 77.9, 62.3 (G'-1-G'-6), 101.3, 71.9, 72.3, 74.4, 69.2, 18.5 (R-1-R-6).

Prosapogenin 4. An amorphous solid, mp 227 °C (dec.). $[\alpha]^{29}_D$ + 17.4° (MeOH;*c*=1.0). FAB-MS: *m/z* 1113 [M+Na]$^+$. ^{1}H NMR (pyridine-d_5, 400 MHz): δ 0. 73 (1H, d, *J*=11.5 Hz, H-5), 0.85, 0.92, 1.00, 1.16, 1.23, 1.69 (each 3H, s, H_3 of C-25, C-26, C-24, C-23, C-29, C-27), 2.65 (1H, dd, *J*=13.1, 12.0 Hz, H-19), 3.14 (1H, dd, *J*=11.5, 4.0 Hz, H-3), 3.33, 3.62 (each 1H, d, *J*=11.0 Hz, H_2-28), 3.70 (3H, s, COOMe), 4.55 (1H, br.s, H-16), 4.70 (1H, d, *J*=5.8 Hz, H-1 of ara.), 4.84 (1H, d, *J*=7.1 Hz, H-1 of xyl.), 4.87 (1H, d, *J*=7.8 Hz, H-1 of inner glu.), 5.25 (1H, d, *J*=7.7 Hz, H-1 of term. glu.), 5.60 (1H, br.t, H-12). ^{13}C NMR (pyridine-d_5, 100 MHz): δ 38.5, 26.0, 88.3, 39.2, 55.6, 18.1, 32.8, 39.7, 46.8, 36.5, 23.4, 122.5, 144.3, 41.4, 34.3, 73.3, 39.9, 43.7, 44.0, 44.3, 33.2, 31.8, 27.7, 16.4, 15.3, 16.5, 26.9, 70.2, 28.3, 177.9, (aglycone C-1-C-30), 51.0 ($COOCH_3$), 104.3, 79.3, 72.7, 78.2, 63.9 (A-1-A-5), 104.4, 77.1, 77.4, 70.6, 77.1, 62.4 (G-1-G-6), 103.7, 84.8, 77.0, 71.3, 77.5, 61.8 (G'-1-G'-6), 107.1, 75.4, 75.6, 70.2, 66.9 (X-1-X-5).

Acidic hydrolysis of prosapogenins 3 and 4. 3 (15 mg) was heated in 1ml 1N HCl (MeOH-H_2O, 1:1) at 80 °C for 4 hours in a water bath. After MeOH was removed, the solution was extracted with EtOAc (1 ml x 3). The extraction was washed with water, and then combined to give a white powder. Purification of the product over silica gel and crystallization from MeOH afforded a sapogenin (5 mg). The monosaccharide portion was neutralized by passing through an anion-exchange resin (Amberlite MB-3) column, concentrated and then treated with 1-(trimethylsilyl) imidazole at room temperature for 2 hours. After the excess reagent was decomposed with water, the reaction product was extracted with hexane (1 ml x 3 times). The TMSi derivatives of the monosaccharides were identified to be L-arabinose, D-glucose and L-rhamnose (1:2:1) from GLC analysis.

Using the same method, **4** was hydrolyzed to give the same sapogenin and the monosaccharides were shown to be L-arabinose, D-glucose and D-xylose (1:2:1).

The sapogenin. Colorless needles, mp 243-245 °C, $[\alpha]^{24}_D$ + 55.2 (MeOH;*c*=0.5). FAB-MS: *m/z* 503 $[M+H]^+$. EI-MS (rel. int. %): *m/z* 484 $[M-H_2O]^+$(3.0), 454 (45.3), 439 (28.9), 421 (6.46), 264 (93.9), 245 (81.5), 207 (30.0), 185 (69.7). ^{1}H NMR (pyridine-d_5, 400 MHz): δ 0.90 (1H, d, *J*=11.2 Hz, H-5), 0.97, 1.01, 1.05, 1.23, 1.31, 1.83 (each 3H, H_3 of C-25, C-26, C-24, C-23, C-29, C-27), 2.79 (1H, dd, *J*=13.3, 12.8 Hz, H-19), 3.47 (1H, dd, *J*=10.6, 5.4 Hz, H-3), 3.49, 3.78 (each 1H, d, *J*=10.8 Hz, H_2-28), 3.74 (3H, s, COOMe), 4.74 (1H, br.s, H-16), 5.55 (1H, br.t, H-12). ^{13}C-NMR (pyridine-d_5, 100 MHz): δ 39.2, 28.2, 78.1, 39.5, 55.9, 18.8, 33.4, 40.1, 47.3, 37.3, 23.9, 122.9, 144.9, 41.9, 34.9, 73.9, 40.4, 44.1, 44.6, 44.7, 33.9, 32.4, 28.8, 16.6, 15.9, 17.1, 27.5, 70.7, 28.8, 178.2, (C-1-C-30), 51.4 ($COOCH_3$).

Conformational calculations. The geometries chosen for the starting conformations were built for the tetrasaccharide fragments of compounds **3** and **4** and submitted to energy minimization by using Discover-cff 91 force field program.[14a] The local minima found for them were taken as starting structures for molecular dynamics (MD) calculations in vacuo at 300k and at a time step of 1 fs. The equilibration time was 10 ps and the total simulation time 1000 ps. Trajectory frames were saved every 1 ps. The trajectories were then examined with the Analysis module of INSIGHT II.[14b]

Assay of cAMP Phosphodiesterase Activity. The phosphodiesterase activity was assayed using the modified method of Thompson and Brooks as previously described.[20] The assay consisted of a two-step isotopic procedure. Tritium-labeled cAMP was hydrolyzed to 5'-AMP by phosphodiesterase, and the 5'-AMP was then further hydrolyzed to adenosine by snake venom nucleotidase. The hydrolysate was treated with an anion-exchange resin (Dowex AG1-X8; Bio-Rad) to adsorb all charged nucleotides and to leave [^{3}H]adenosine as the only labeled compound to be counted.

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