JOURNAL OF NATURAL PRODUCTS

Note

pubs.acs.org/jnp

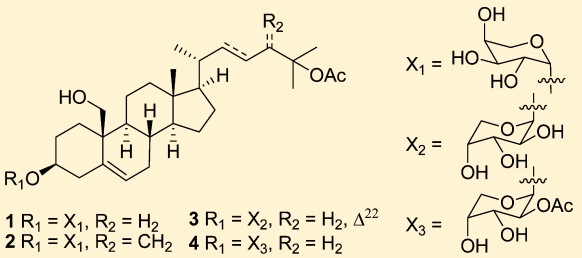
Steroids Glycosylated with Both D- and L-Arabinoses from the South China Sea Gorgonian *Dichotella gemmacea*

Mei Jiang, Peng Sun, Hua Tang, Bao-Shu Liu, Tie-Jun Li, Cui Li, and Wen Zhang*

Research Center for Marine Drugs and Department of Pharmacology, School of Pharmacy, Second Military Medical University, 325 Guo-He Road, Shanghai 200433, People's Republic of China

Supporting Information

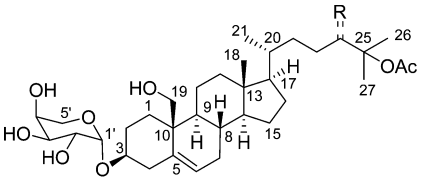
ABSTRACT: Three new 19-hydroxy steroidal glycosides, namely, junceellosides E–G (**2**–**4**), were isolated together with the known analogue junceelloside C (**1**) from the South China Sea gorgonian *Dichotella gemmacea*. The structures of these compounds were elucidated by a combination of detailed spectroscopic analyses, chemical methods, and comparison with reported data. These glycosides are found to have sugar moieties of both β-L- and β-D-arabinopyranoses by HPLC analysis of their thiocarbamoyl-thiazolidine derivatives and those of authentic D- and L-arabinoses, leading to the structure revision of junceelloside C (**1**). This is the first report of steroidal glycosides from the gorgonian *D. gemmacea* and the first report of glycosides with β-L-arabinopyranose from marine sources.

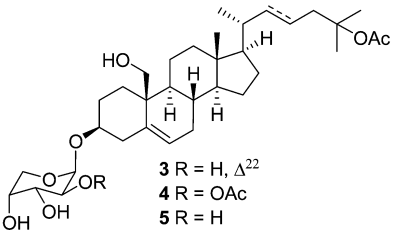


1 $R_1 = X_1$, $R_2 = H_2$ **3** $R_1 = X_2$, $R_2 = H_2$, Δ^{22}
2 $R_1 = X_1$, $R_2 = CH_2$ **4** $R_1 = X_3$, $R_2 = H_2$

Steroids with a 19-hydroxy group have received considerable attention since the first 19-hydroxylated steroid, 24-methylenecholest-5-ene-3β,7β,19-triol, was isolated from *Litophyton viridis* and helped to explain the biosynthesis of 19-nor steroids.[1] Since then, an increasing number of 19-hydroxy sterols have been found mainly from black coral,[2] soft corals,[3] sponges,[4] and gorgonians.[5] These metabolites were reported to exhibit cytotoxic activities toward the P-388, HT-29, A549, and KB tumor cell lines.[6] Recently, several 19-hydroxy steroidal glycosides were reported from gorgonian corals *Junceella juncea*[7,8] and *Dichotella fragilis*.[9] Some of the glycosides displayed brine shrimp lethality and antifouling activity.[9]

In the course of our ongoing screening for biologically active secondary metabolites from marine sources,[10−13] we made several collections of the gorgonian *Dichotella gemmacea* from the coast of Beihai, China. Chemical investigations of this species have uncovered briarane diterpenoids,[11−16] an alkane,[14] and fatty acids.[17] Our earlier investigation of the extract of *D. gemmacea* led to the isolation and structure determination of 28 briarane diterpenoids.[11−13] Our continuing investigation of the EtOAc-soluble fraction from the acetone extract of *D. gemmacea* has now led to the isolation of the 19-hydroxy steroidal glycoside junceelloside C (**1**) together with three new analogues, namely, junceellosides E–G (**2**–**4**). The structures of these compounds were elucidated by a combination of chemical methods and detailed analysis of their spectroscopic data, aided by the comparison with reported data. This is the first report of 19-hydroxy steroidal glycosides from the gorgonian *D. gemmacea* and the first report of glycosides with β-L-arabinopyranose from marine sources. We herein report the isolation, structure elucidation, and bioactivity of these compounds.





Freshly collected specimens of *D. gemmacea* were immediately frozen at −20 °C and stored at this temperature before extraction. Frozen material was cut into small pieces and extracted with acetone. The EtOAc-soluble portion of the acetone extract was subjected to repeated column chromatography on silica gel, Sephadex LH-20, and RP-HPLC to afford four pure steroid glycosides (**1**–**4**).

Compound **1** was isolated as an optically active, white, amorphous solid. The molecular formula of **1** was established as $C_{34}H_{56}O_8$ from the pseudomolecular ion at m/z 615.3870 $[M + Na]^+$ in the HRESIMS spectrum, indicating seven degrees of double-bond equivalents. The IR spectrum showed the presence of hydroxy (3370 cm^{-1}) and ketone (1734 cm^{-1})

Received: December 25, 2012
Published: March 11, 2013

functionalities. This observation was in agreement with the signals in the ^{13}C and DEPT spectra (Table 1) for three sp^2 carbon atoms at lower field (1 × O═C, 1 × C═CH) and 31 sp^3 carbon atoms at higher field (1 × OC, 4 × OCH, 2 × OCH_2, 2 × C, 6 × CH, 11 × CH_2, 5 × CH_3), accounting for two double-bond equivalents. The *O*-bearing-methine protons resonating between δ 4.10 and 5.55 (Table 2) in conjunction with the presence of related secondary alcohol carbons (δ_C 71.1, 71.6, 70.7, 64.8) and an acetal carbon (δ_C 99.7) suggested the presence of a sugar moiety in the molecule, accounting for one additional double-bond equivalent. The remaining double-bond equivalents were due to the presence of four rings in the aglycone of **1**. The ^1H and ^{13}C NMR spectra of **1** in $CDCl_3$ (see Table S1 in the Supporting Information) were identical to those of junceelloside C (**5**), a 19-hydroxy steroidal glycoside isolated previously from the gorgonian coral *Junceella juncea*.[8] Detailed 2D NMR analysis revealed the same relative configuration for both compounds. In particular, a β-arabinopyranose was clearly indicated by the large coupling constant between H-2′ and H-3′ (^3J = 9.4 Hz) and the small coupling constant between H-1′ and H-2′ (^3J = 3.6 Hz) and between H-3′ and H-4′ (^3J = 3.4 Hz) (in pyridine-d_5), indicating antiaxial protons for H-2′ and H-3′ and equatorial protons for H-1′and H-4′, respectively. However, the absolute configuration of the monosaccharide in **1** was determined as L-

Table 1. ^{13}C NMR Data for Compounds 1−4 (in pyridine-d_5)[a]

	1[b]	2[c]	3[c]	4[b]
position	δ_C, type	δ_C, type	δ_C, type	δ_C, type
C-1	34.4, CH_2	34.4, CH_2	34.4, CH_2	34.4, CH_2
C-2	31.1, CH_2	31.1, CH_2	31.2, CH_2	30.9, CH_2
C-3	78.0, CH	78.0, CH	77.9, CH	77.9, CH
C-4	40.1, CH_2	40.1, CH_2	40.1, CH_2	39.9, CH_2
C-5	138.2, C	138.2, C	138.2, C	137.8, C
C-6	125.8, CH	125.7, CH	125.7, CH	126.0, CH
C-7	32.8, CH_2	32.8, CH_2	32.5, CH_2	32.5, CH_2
C-8	33.7, CH	33.7, CH	33.7, CH	33.7, CH
C-9	51.6, CH	51.6, CH	51.6, CH	51.6, CH
C-10	42.6, C	42.6, C	42.6, C	42.6, C
C-11	22.5, CH_2	22.5, CH_2	22.7, CH_2	22.7, CH_2
C-12	41.0, CH_2	41.0, CH_2	40.8, CH_2	40.8, CH_2
C-13	43.2, C	43.2, C	43.2, C	43.2, C
C-14	58.2, CH	58.2, CH	58.2, CH	58.0, CH
C-15	24.9, CH_2	24.9, CH_2	24.9, CH_2	24.9, CH_2
C-16	28.9, CH_2	28.9, CH_2	29.5, CH_2	28.9, CH_2
C-17	56.7, CH	56.7, CH	56.3, CH	56.8, CH
C-18	12.9, CH_3	12.9, CH_3	12.9, CH_3	12.7, CH_3
C-19	63.5, CH_2	63.5, CH_2	63.4, CH_2	63.3, CH_2
C-20	36.6, CH	36.6, CH	41.0, CH	36.4, CH
C-21	19.4, CH_3	19.4, CH_3	21.4, CH_3	19.2, CH_3
C-22	35.8, CH_2	35.8, CH_2	141.8, CH	36.9, CH_2
C-23	27.9, CH_2	27.9, CH_2	122.8, CH	21.2, CH_2
C-24	41.2, CH_2	154.2, C	44.1, CH_2	41.7, CH_2
C-25	82.7, C	82.4, C	82.4, C	82.7, C
C-26	26.5, CH_3	27.5, CH_3	26.5, CH_3	26.5, CH_3
C-27	26.5, CH_3	27.5, CH_3	26.5, CH_3	26.5, CH_3
C-28		108.8, CH_2		
1′	99.7, CH	99.7, CH	99.7, CH	96.2, CH
2′	71.1, CH	71.1, CH	71.1, CH	73.6, CH
3′	71.6, CH	71.5, CH	71.5, CH	68.2, CH
4′	70.7, CH	70.6, CH	70.6, CH	70.8, CH
5′	64.8, CH_2	64.7, CH_2	64.7, CH_2	64.5, CH_2
25-OAc	170.7, C	170.1, C	170.6, C	170.6, C
	21.1, CH_3	21.1, CH_3	21.1, CH_3	21.1, CH_3
2′-OAc				171.4, C
				21.3, CH_3

[a] δ in ppm, assignments made by DEPT, COSY, HSQC, HMBC, and NOESY. [b] At 100 MHz. [c] At 150 MHz.

Table 2. ^1H NMR Data for Compounds 1−4 (in pyridine-d_5)[a]

	1[b]	2[c]	3[c]	4[b]
position	δ_H (J in H_Z)	δ_H (J in H_Z)	δ_H (J in H_Z)	δ_H (J in H_Z)
1α	2.30 brd (13.2)	2.30 brd (13.2)	2.30 brd (13.2)	2.30 brd (13.2)
1β	1.10, ov	1.10, ov	1.06, ov	1.10, ov
2α	2.17, ov	2.15, ov	2.08, ov	2.10, ov
2β	1.85, ov	1.83, ov	1.90, ov	1.88, ov
3	3.87, m	3.87, m	3.87, m	3.80, m
4α	2.60, dd (10.8, 10.8)	2.60, dd (11.2, 11.2)	2.60, dd (11.2, 11.2)	2.60, dd (11.2, 11.2)
4β	2.75, dd (12.8, 2.8)	2.74, dd (13.2, 3.0)	2.74, dd (13.7, 3.0)	2.74, dd (13.2, 3.0)
6	5.66 brs	5.66 brs	5.66 brs	5.66 brs
7α	2.07, ov	2.15, ov	2.17, ov	2.07, ov
7β	1.62, ov	1.60, ov	1.57, ov	1.62, ov
8	2.06, m	2.06, m	2.06, m	2.06, m
9	0.96, m	0.96, m	0.96, m	0.96, m
11α	1.75, m	1.75, m	1.75, m	1.75, m
11β	2.04, m	2.06, m	2.03, m	2.04, m
12α	1.25, ov	1.30, ov	1.28, ov	1.25, ov
12β	2.12, ov	2.05, ov	2.05, ov	2.12, ov
14	0.93, m	0.93, m	0.93, m	0.93, m
15α	1.62, m	1.53, m	1.58, m	1.62, m
15β	1.11, m	1.15, m	1.12, m	1.11, m
16α	1.90, m	1.75, m	1.75, m	1.90, m
16β	1.28, m	1.50, m	1.50, m	1.28, m
17	1.17, m	1.17, m	1.17, m	1.17, m
18	0.83, s	0.83, s	0.83, s	0.83, s
19*a*	4.15, d (11.4)	4.15, d (11.4)	4.15, d (11.4)	4.15, d (11.4)
19*b*	3.89, d (11.4)	3.89, d (11.4)	3.89, d (11.4)	3.89, d (11.4)
20	1.52, m	1.52, m	2.05, m	1.52, m
21	0.99, d (6.6)	0.99, d (6.6)	1.08, d (6.6)	0.99, d (6.6)
22	1.06, 1.60, m	1.52, 1.82, m	5.43, dd (15.2, 8.3)	1.06, 1.60, m
23	1.35, 1.50, m	1.53, 1.53, m	5.45, ddd (15.2, 7.0, 6.9)	1.35, 1.50, m
24	1.75, 1.90, m		2.60, 2.60, ov	1.75, 1.90, m
26	1.53, s	1.65, s	1.52, s	1.53, s
27	1.53, s	1.65, s	1.52, s	1.53, s
28		5.18, 5.02, s		
25-OAc	2.01, s	2.02, s	2.02, s	2.01, s
1′	5.55, d (3.6)	5.55, d (3.4)	5.55, d (3.4)	5.71, d (3.6)
2′	4.65, dd (9.4, 3.6)	4.62, dd (9.4, 3.4)	4.62, dd (9.4, 3.4)	5.81, dd (9.4, 3.6)
3′	4.55, dd (9.4, 3.4)	4.52, dd (9.4, 3.4)	4.52, dd (9.4, 3.4)	4.62, dd (9.4, 3.4)
4′	4.43, brs	4.43 brs	4.43 brs	4.43 brs
5′α	4.10, dd (11.9, 2.8)	4.10, dd (11.9, 2.8)	4.10, dd (11.9, 2.8)	4.10, dd (11.9, 2.8)
5′β	4.25, d (11.9)	4.25, d (11.9)	4.25, d (11.9)	4.25, d (11.9)
OAc				2.02, s

[a] δ in ppm, assignments made by DEPT, COSY, HSQC, HMBC, and NOESY. [b] At 400 MHz. [c] At 600 MHz.

dx.doi.org/10.1021/np300906b | J. Nat. Prod. 2013, 76, 764−768

arabinose instead of D-arabinose by comparison of the retention time of its thiocarbamoyl-thiazolidine derivative of the acid hydrolysate (20.9 min) with those of standard samples of L-arabinose (20.8 min) and D-arabinose (23.0 min), respectively.[10] Compound **1** was therefore determined as 25-*O*-acetyl-3-*O*-[β-L-arabinopyranosyloxy]cholest-5-ene-3β,19,25-triol. Although junceelloside C (**5**) was reported as a β-D-arabinoside, the structure of **5** was elucidated from NMR data only, and there were no data or experiments to support the absolute configuration of the arabinose.[8] Because the ^{1}H and ^{13}C NMR data are identical (see Table S1 in the Supporting Information) and the specific rotations can be considered the same based on general variations of these measurements [−116 (*c* 0.25, C_5H_5N) for **1** vs −114.0 (*c* 0.55, C_5H_5N) for **5**[8]], it is reasonable to conclude that **1** and junceelloside C are the same compound, and therefore **1** should be designated as junceelloside C. The original structure **5** should be revised to structure **1**.[18]

Junceelloside E (**2**) was isolated as an optically active, white, amorphous solid. Its molecular formula, $C_{35}H_{56}O_8$, was deduced from the HRESIMS spectrum. The ^{1}H and ^{13}C NMR spectra of **2** were closely related to those of **1** except for the appearance of an additional terminal double bond (δ_H 5.08, s and 5.12, s; δ_C 154.2, C and 108.8, CH_2). The location of the double bond at C-24 was clearly indicated by the distinct HMBC correlations from H_2-28 to C-23, C-24, and C-25. Configurations for the other stereogenic centers in **2** were proven to be the same as those in **1** by detailed 2D NMR analysis. The absolute configuration of the sugar moiety was identified as L-arabinopyranose by HPLC analysis of the thiocarbamoyl-thiazolidine derivative of the acid hydrolysate of **2** and those of authentic D- and L-arabinoses.[10] Compound **2** was thus assigned to be 25-*O*-acetyl-3-*O*-[β-L-arabinopyranosyloxy]cholest-5,24(28)-diene-3β,19,25-triol.

Junceelloside F (**3**) was isolated as an optically active, white, amorphous solid. The molecular formula of **3** was established as $C_{34}H_{54}O_8$ by an HRESIMS experiment. Analysis of the ^{1}H and ^{13}C NMR spectra of **3** revealed a strong similarity to those of **1**. However, an additional disubstituted double bond at lower field (AB system, δ_H 5.43, 1H, dd, J = 15.2, 8.3 Hz; 5.45, 1H, ddd, J = 15.2, 7.0, 6.9 Hz; δ_C 141.8, CH; 122.8, CH) was assigned as Δ^{22} due to the proton connectivity from H_3-21 to H_2-24 as established by the COSY experiment and the significant long-range correlations of H_3-21 with C-17, C-20, and C-22 observed in the HMBC spectrum. The *E* geometry of Δ^{22} was deduced from the large coupling constant (15.2 Hz) between the olefinic protons. A β-arabinopyranose was deduced from the large coupling constant between H-2′ and H-3′ (^3J = 9.4 Hz) and the small coupling constant between H-1′ and H-2′ (^3J = 3.4 Hz) and between H-3′ and H-4′ (^3J = 3.4 Hz). Its absolute configuration was identified as D-arabinopyranose in contrast to L-arabinopyranose by HPLC analysis of the thiocarbamoyl-thiazolidine derivative of the acid hydrolysate of **3** and those of authentic D- and L-arabinoses.[10] The structure of **3** was therefore determined as 25-*O*-acetyl-3-*O*-[β-D-arabinopyranosyloxy]-(22*E*)-cholesta-5,22-diene-3β,19,25-triol.

Junceelloside G (**4**), an optically active, white, amorphous solid, had a molecular formula of $C_{36}H_{58}O_9$, as established by HRESIMS, showing 42 more mass units than **1**. The ^{1}H and ^{13}C NMR spectra of **4** were almost identical to those of **1** (Tables 1 and 2), except for the presence of an additional acetyl group (δ_H 2.02, s; δ_C 21.3, CH_3; 171.4, C). The location of the acetoxy group at C-2′ was shown by the downfield shift of the respective proton signal at H-2′ from δ 4.65 in **1** to δ 5.81 in **4**. The sugar subunit of **4** was determined as a 2′-monoacetate of β-D-arabinopyranose by the HPLC analysis as described above.[10] The structure of compound **4** was thus assigned to be 2′,25-*O*-diacetyl-3-*O*-[β-D-arabinopyranosyloxy]cholest-5-ene-3β,19,25-triol.

The isolates were evaluated *in vitro* for the tumor growth inhibitory activity toward the A549 and MG63 cell lines.[19] None of the compounds were considered active (IC_{50} > 10 μM; see Table S2 in the Supporting Information).

This is the first report of 19-hydroxy steroidal glycosides from the gorgonian *D. gemmacea* and the first report of glycosides with a β-L-arabinopyranose moiety from marine sources. A series of sterol glycosides have been obtained from octocorals and a sponge,[20] including 4′-*O*-acetyl-3-*O*-[β-D-arabinopyranosyloxy]cholest-5-ene-3β,19-diol[7] and junceellosides A–D[8] from the gorgonian *J. juncea*, fragiliosides A and B from the gorgonian *Dichotella fragilis*,[9] and carijoside A from the octocoral *Carijoa* sp.[15] All of the isolates are simply claimed to have a β-D-arabinopyranose on the basis of an NMR analysis. Recently, two steroid glycosides, namely, sokodosides A and B (**6** and **7**) (Figure 1), were obtained from the Hachijo sponge

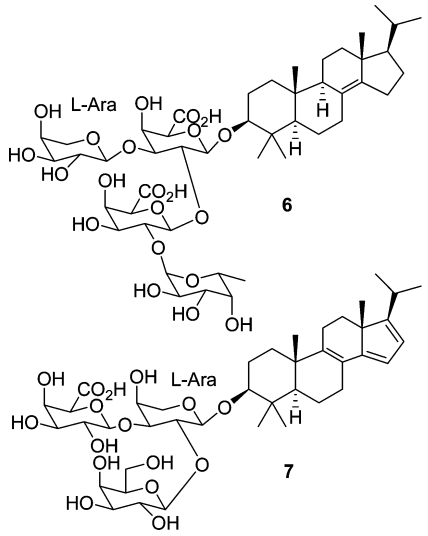


Figure 1. Structures of sokodosides A (**6**) and B (**7**).

Erylus placenta.[21] The structures that were stated to have a β-L-arabinopyranose based on chiral-phase GC analyses of the hydrolyzed sugars in fact have an α-L-arabinopyranose moiety due to the large coupling constant of their anomeric protons (^3J = 6.5 Hz).[22] The structures as originally drawn in ref 21 and as depicted in Figure 1 correctly represent an α-L-arabinose moiety.

It is extremely interesting to observe that D- and L-arabinose modifications occur in the same gorgonian specimen. The isolation of steroids with both D- and L-arabinoses from the same species indicates it is inappropriate to determine the absolute configuration of a sugar moiety simply by an NMR analysis or biogenetic correlation. The use of an HPLC or GC analysis of a sugar derivative from the acid hydrolysate is therefore strongly recommended in the absolute structure determination of the saccharide portion of glycosides.

dx.doi.org/10.1021/np300906b | *J. Nat. Prod.* 2013, 76, 764–768

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ on an Anton Paar MCP 500 polarimeter at the sodium D line (590 nm). Infrared spectra were recorded in thin polymer films on a Nexus 470 FT-IR spectrophotometer (Nicolet). The NMR spectra were recorded at 300 K on Bruker DRX 400, DRX 500, and Avance 600 spectrometers. ^{13}C NMR and ^{1}H NMR chemical shift values were referenced to $CDCl_3$ (δ_C 77.0 ppm) and the residual $CHCl_3$ signals (δ_H 7.26 ppm), and pyridine-d_5 (δ_C 150.3, 135.9, 123.9) and residual pyridine (δ_H 8.74, 7.58, 7.22); assignments were supported by COSY, HSQC, HMBC, and NOESY experiments. The mass spectra and high-resolution mass spectra were obtained on a Q-TOF Micro mass spectrometer, resolution 5000. An isopropyl alcohol solution of sodium iodide (2 mg/mL) was used as a reference compound. Semipreparative RP-HPLC was performed on an Agilent 1100 system equipped with a refractive index detector using a YMC-Pack-ODS-A column (particle size 5 μm, 250 × 10 mm). Commercial silica gel (Yantai, P. R. China, 200−300; 400−500 mesh) was used for column chromatography. Precoated SiO_2 plates (HSGF-254; Yantai, China) were used for analytical TLC. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde−sulfuric acid reagent.

Animal Material. The gorgonian coral *Dichotella gemmacea* (3.5 kg, wet weight) was collected from the South China Sea in August 2008, at a depth of 16 m, and authenticated by Dr. Xiu-Bao Li (The South China Sea Institute of Oceanology, Chinese Academy of Sciences). A voucher specimen (ZS-5) was deposited in the Second Military Medical University.

Extraction and Isolation. The frozen animals were cut into small pieces and extracted ultrasonically with acetone (2.0 L × 3) and MeOH (1.5 L × 3). The combined residue was partitioned between H_2O and EtOAc to afford 16.1 g of an EtOAc extract. The EtOAc extract was further partitioned between MeOH and hexane, affording 11.2 g of a MeOH-soluble residue. The MeOH extract was subjected to column chromatography (CC) on silica to give 16 fractions, using hexane/acetone (from 100:0 to 0:100) as eluent. Fraction 4 was subjected to Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to give eight subfractions. Subfraction 3 was chromatographed on a silica gel column (gradient *n*-hexane/acetone, from 10:1 to 1:1) and HPLC (particle size 5 μm, 250 × 10 mm; 85% MeOH/H_2O; 1.5 mL/min) to yield **1** (10.0 mg, t_R 29.3 min), **2** (5.1 mg, t_R 27.1 min), **3** (7.8 mg, t_R 25.2 min), and **4** (3.5 mg, t_R 31.5 min).

Junceelloside C (1): white, amorphous solid; $[\alpha]_D^{25}$ −82.8 (*c* 0.27, $CHCl_3$) and −116 (*c* 0.25, C_5H_5N); IR (film) ν_{max} 3370, 2928, 1734, 1072, 1014 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 615.3870 $[M + Na]^+$ (calcd for $C_{34}H_{56}O_8Na$, 615.3873).

Junceelloside E (2): white, amorphous solid; $[\alpha]_D^{25}$ −84.3 (*c* 0.17, $CHCl_3$); IR (film) ν_{max} 3379, 2931, 1735, 1068, 999 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 627.3885 $[M + Na]^+$ (calcd for $C_{35}H_{56}O_8Na$, 627.3873).

Junceelloside F (3): white, amorphous solid; $[\alpha]_D^{25}$ −78.6 (*c* 0.26, $CHCl_3$); IR (film) ν_{max} 3418, 2933, 1732, 1053, 1015 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 613.3719 $[M + Na]^+$ (calcd for $C_{34}H_{54}O_8Na$, 613.3716).

Junceelloside G (4): white, amorphous solid; $[\alpha]_D^{25}$ −86.5 (*c* 0.12, $CHCl_3$); IR (film) ν_{max} 3390, 2928, 1738, 1073, 1003 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 657.3976 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_9Na$, 657.3979).

Acid Hydrolysis and Absolute Configuration Determination of the Monosaccharides for Junceellosides C (1) and E−G (2−4). The glycosides **1**−**4** (each 0.8 mg) were dissolved in 2 M CF_3COOH(aq) (1.0 mL) at 120 °C for 2 h. The mixture was evaporated to dryness, and the residue was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was concentrated to furnish a monosaccharide residue. After drying under vacuum, the residue was dissolved in 0.4 mL of pyridine containing 2 mg of L-cysteine methyl ester hydrochloride and heated at 60 °C for 1 h. Phenyl isothiocyanate (2 μL) was then added, and the mixture was heated at 60 °C for 1 h. The reaction mixture was analyzed by reversed-phase HPLC, which was performed on an Agilent 1100 HPLC system (Agilent Technologies Inc.) equipped with a photodiode array detector and a Diamonsil-C_{18} column (particle size 5 μm, 250 × 4.6 mm) at 35 °C with isocratic elution of 25% CH_3CN in 50 mmol/L H_3PO_4 solution for 40 min and subsequent washing of the column with 90% CH_3CN at a flow rate of 0.8 mL/min. The injection volume was 4 μL, and peaks were detected at 250 nm. The reaction conditions for authentic L- and D-arabinose were the same as described above.[10] The absolute configurations of the monosaccharides were determined as L-arabinoae in **1** (t_R 20.9 min) and **2** (t_R 21.1 min) and D-arabinoae in **3** (t_R 23.2 min) and **4** (t_R 23.2 min) by comparison of the retention time of the thiocarbamoyl-thiazolidine derivative of the acid hydrolysate of steroidal glycosides (**1**−**4**) with those of standard samples of L-arabinose (20.8 min) and D-arabinose (23.0 min), respectively.

Cytotoxicity Assay. Cytotoxicity was tested against human lung adenocarcinoma (A549) and human osteosarcoma (MG63) cell lines, using a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[19] Adriamycin was used as a positive control; IC_{50} = 2.8 and 3.4 μM, respectively.

ASSOCIATED CONTENT

Supporting Information

HRESIMS and NMR spectra for **1**−**4**, NMR data in $CDCl_3$ for **1** and **4**, and HPLC spectra for thiocarbamoyl-thiazolidine derivatives of acid hydrolysates of **1**−**4** and reference compounds are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: 86 21 81871257. E-mail: wenzhang1968@163.com.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The research work was financially supported by NSFC (Nos. 41076082, 81202453), the National Marine "863" Project (No. 2013AA092902), and the Shanghai Pujiang Program (PJ2008).

REFERENCES

(1) Bortolotto, M.; Braekman, J. C.; Daloze, D.; Losman, D.; Tursch, B. *Steroids* **1976**, *28*, 461−466.
(2) Aiello, A.; Fattorusso, E.; Menna, M. *J. Nat. Prod.* **1992**, *55*, 321−325.
(3) Xu, S. H.; Zeng, L. M. *Chin. Chem. Lett.* **2000**, *11*, 531−534.
(4) Van Altena, I. A.; Butler, A. J.; Dunne, S. J. *J. Nat. Prod.* **1999**, *62*, 1154−1157.
(5) Zhang, W.; Guo, Y.-W.; Mollo, E.; Fontana, A.; Cimino, G. *J. Nat. Prod.* **2004**, *67*, 2083−2085.
(6) Sarma, N. S.; Krishna, M. S.; Pasha, S. G.; Rao, T. S. P.; Venkateswarlu, Y.; Parameswaran, P. *Chem. Rev.* **2009**, *19*, 2803−2828.
(7) Qi, S.-H.; Zhang, S.; Xiao, Z.-H.; Huang, J. S.; Wu, J.; Li, Q. X. *Chem. Pharm. Bull.* **2004**, *52*, 1476−1478.
(8) Qi, S.; Zhang, S.; Huang, J.; Xiao, Z.; Wu, J.; Li, Q. *Magn. Reson. Chem.* **2005**, *43*, 266−268.
(9) Zhou, Y.; Shao, C.; Wang, C.; Huang, H.; Xu, Y.; Qian, P. *Nat. Prod. Commun.* **2011**, *6*, 1239−1242.
(10) Sun, P.; Meng, L.-Y.; Tang, H.; Liu, B.-S.; Li, L.; Yi, Y.; Zhang, W. *J. Nat. Prod.* **2012**, *75*, 1656−1659.
(11) Li, C.; La, M.-P.; Tang, H.; Pan, W.-H.; Sun, P.; Krohn, K.; Yi, Y. H.; Li, L.; Zhang, W. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4368−4372.
(12) Li, C.; La, M.-P.; Sun, P.; Kurtan, T.; Mandi, A.; Tang, H.; Liu, B.-S.; Yi, Y. H.; Li, L.; Zhang, W. *Mar. Drugs* **2011**, *9*, 1403−1418.
(13) Li, C.; La, M.-P.; Li, L.; Li, X.-B.; Tang, H.; Liu, B.-S.; Krohn, K.; Sun, P.; Yi, Y. H.; Zhang, W. *J. Nat. Prod.* **2011**, *74*, 1658−1662.
(14) He, H.; Faulkner, D. J. *Tetrahedron* **1991**, *47*, 3271−3280.

(15) Liu, C.-Y.; Hwang, T.-L.; Lin, M.-R.; Chen, Y.-H.; Chang, Y.-C.; Fang, L.-S.; Wang, W.-H.; Wu, Y.-C.; Sung, P. J. *Mar. Drugs* **2010**, *8*, 2014−2020.
(16) Sun, J.-F.; Han, Z.; Zhou, X.-F.; Yang, B.; Lin, X.; Liu, J.; Peng, Y.; Yang, X.-W.; Liu, Y. *Tetrahedron* **2013**, *69*, 871−880.
(17) Wang, C.-Y.; Zhao, J.; Liu, H.-Y.; Shao, C.-L.; Liu, Q.-A.; Liu, Y.; Gu, Y.-C. *Lipids* **2011**, *46*, 81−85.
(18) On the basis of the ^{1}H NMR chemical shifts of the A-ring protons for compounds **1**−**3**, it can be seen that there are no major shift variations when either L-arabinose (**1** and **2**) or D-arabinose (**3**) is present (Table 1). However, there are minor differences for the H-2 protons. Though there are only a few structures in this class that currently have definitive assignments of L- and D-arabinose, the H-2 shifts originally reported for junceelloside C are consistent with the presence of the L-arabinose, providing additional support for the identity of **1** as junceelloside C.
(19) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(20) Ivanchina, N. V.; Kicha, A. A.; Stonik, V. A. *Steroids* **2011**, *76*, 425−454.
(21) Okada, Y.; Matsunaga, S.; van Soest, R. W. M.; Fusetani, N. *J. Org. Chem.* **2006**, *71*, 4884−4888.
(22) The designation of a β-L-arabinose in the sokodosides is likely due to the challenges inherent in using the α/β nomenclature for various sugars. For the common D-sugar glucose, an equatorial oxygen at the anomeric center of the pyranose corresponds to the β configuration. In arabinose, however, an equatorial oxygen at C-1 of the pyranose corresponds to an α configuration. The arabinose units in the sokodosides are drawn with the equatorial oxygen linkages and therefore should be designated as α-L-arabinopyranose moieties.