Triterpenoid saponins from the stem bark of *Catunaregam spinosa*

Guang-Chun Gao, Zhong-Xian Lu, Shu-Hong Tao, Si Zhang, Fa-Zuo Wang, and Qing-Xin Li

Abstract: Four new triterpenoid saponins, Catunaroside E (**1**; 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}-siaresinolic acid), Catunaroside F (**2**; 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}-28-*O*-(β-D-glucopyranosyl)-oleanolic acid), Catunaroside G (**3**; 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]- 28-*O*-(β-D-glucopyranosyl)-siaresinolic acid), and Catunaroside H (**4**; 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}-28-*O*-(β-D-glucopyranosyl)-siaresinolic acid), and the known triterpenoid saponin Mussaendoside J (**5**), were isolated from the stem bark of *Catunaregam* spinosa. Their structures were elucidated on the basis of their spectral data and chemical evidence.

Key words: Catunaregam spinosa, Rubiaceae, triterpenoid saponins, Catunaroside E–H.

Résumé : À partir de l'écorce du tronc de *Catunaregam spinosa,* on a isolé quatre nouvelles saponines triterpénoïdes, le catunaroside E (**1**), l'acide 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}siarésinolique, le catunaroside F (**2**), l'acide 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}-28-*O*-(β-D-glucopyranosyl)oléanolique, le catunaroside G (**3**), l'acide 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-28-*O*-(β-D-glucopyranosyl)siarésinolique et le catunaroside H (**4**), l'acide 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-28-*O*-(β-D-glucopyranosyl)siarésinolique et une saponine triterpénoïde connue,le mussaendoside J (**5**). Leurs structures ont été élucidées sur la base de leurs propriétés chimiques et de leurs données spectrales.

Mots-clés : Catunaregam spinosa, rubiacée, saponines triterpénoïdes, caturanoside E-H.

[Traduit par la Rédaction]

Introduction

Catunaregam spinosa T. (Rubiaceae), which is mostly distributed in tropical and semitropical areas, was used in the traditional medicinal systems of India and Brazil for its antispasmodic, antidysenteric, anti-inflammatory, immunomodulatory, and antifertility properties.[1–4] Chemical investigation of *C. spinosa* led to the isolation of triterpene and saponins,[4–6] iridoid glucosides,[1] coumarin glucosides,[7] and norneolignans.[8] In continuation of our studies on the chemical diversity of mangrove plants on Hainan Island, this plant was investigated and four new triterpenoid saponins, Catunaroside E–H, together with Mussaendoside J, were obtained from the *n*-BuOH extract of its stem bark (Fig. 1). This paper deals with the isolation and structure elucidation of those triterpenoid saponins. Mussaendoside J was first obtained from this plant.

Results and discussion

Compound **1** was obtained as a colorless amorphous powder. Its molecular formula was assigned as $C_{48}H_{78}O_{19}$ based on the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS; *m/z*: 981.5080 $[M + Na]^+$). The infrared (IR) spectrum exhibited absorption bands at 3418 (OH), 1728 (C=O), and 1644 (C=C) cm^{-1}. The seven tertiary methyl groups (δ 1.26, 1.08, 0.81, 1.06, 1.66, 1.18, and 1.02 ppm) and one olefinic proton (δ 5.55, br s) were observed in the ^1H nuclear magnetic resonance (NMR) spectrum. The ^{13}C and distortionless enhanced polarization transfer (DEPT) NMR data of the aglycon part of **1** confirmed the presence of seven methyl carbons (δ 28.0, 16.7, 15.4, 17.5, 24.9, 28.9, and 24.9 ppm), two olefinic carbons (δ 123.1 and 144.9 ppm), and two oxygenated methine carbons (δ 89.5 and 81.3 ppm) (Table 2). The comparison of ^1H and ^{13}C NMR spectrum data of the aglycon part of **1** with that of Latifolioside H indicated that compound **1** possessed a 3β,19α-dihydroxyolean-12-ene-28-oic acidic aglycon.[9] The chemical shifts of C-3 (δ 89.5) and C-28 (δ 180.9) revealed that **1** was a monodesmosidic glycoside. Eighteen of

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Table 1. ^{1}H NMR data for the sugar moieties of compounds **1–5** in pyridine-d_5 (J in parentheses in Hz).

H	**1**	**2**	**3**	**4**	**5**
3-*O*	Glc	Glc	Glc	Glc	Glc
1′	4.83 d (7.5)	4.83 d (7.5)	4.94 d (7.0)	4.83 d (7.5)	4.94 d (6.0)
2′	4.38	4.02	4.29	4.36	4.29
3′	4.25	4.20	4.28	4.23	4.28
4′	4.01	4.09	4.17	4.01	4.16
5′	3.89	4.25	4.03	3.87	4.03
6′	4.53, 4.24	4.54, 4.39	4.53, 4.37	4.52, 4.24	4.54, 4.36
3′-*O*					
1″	5.37 d (7.6)	5.11 d (7.8)		5.36 d (8.0)	
2″	4.08	4.00		4.02	
3″	4.21	4.08		4.20	
4″	4.01	4.18		4.12	
5″	4.02	4.10		4.17	
6″	4.47	4.24		4.45	
2′-*O*					
1‴	5.72 d (7.6)	6.47 br s	6.56 br s	5.71 d (7.5)	6.56 br s
2‴	4.03	4.81	4.86	4.02	4.86
3‴	4.27	4.60	4.68	4.27	4.69
4‴	4.13	4.29	4.34	4.14	4.34
5‴	3.84	4.74	4.80	3.82	4.80
6‴	4.31	1.67 d (6.0)	1.71 d (6.0)	4.31	1.71 d (6.0)
28-*O*					
1⁗		6.31 d (8.1)	6.38 d (8.0)	6.36 d (8.0)	6.34 d (8.0)
2⁗		4.20	4.21	4.20	4.21
3⁗		4.28	3.98	4.01	4.03
4⁗		4.35	4.37	4.35	4.36
5⁗		3.95	4.10	4.06	3.94
6⁗		4.47, 4.43	4.48, 4.43	4.45, 4.39	4.47, 4.41

Note: Glc, glucose.

48 carbons were assigned to the oligosaccharide moieties. The ^{1}H and ^{13}C NMR spectra of **1** showed three sugar anomeric protons at δ 4.83 (d, J = 7.5 Hz), 5.37 (d, J = 7.6 Hz), and 5.72 (d, J = 7.6 Hz) and carbons at δ 105.1, 104.7, and 103.8 (Tables 1 and 2). The monosaccharides were identified as glucose by comparing the retention factor (R_f) value with that of D-glucose using thin-layer chromatography (TLC) after acid hydrolysis and a combination of DEPT, heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) experiments, respectively. All glucoses of **1** were in pyranose forms, as determined by their 1D and 2D NMR experiments. The β-anomeric configurations of the glucose units were established by its $^3J_{H1,H2}$ coupling constants (7.5–7.6 Hz). The signal at δ 88.6 in the ^{13}C NMR indicated a substitution at C-3′ of one of the glucoses. The sequence of the glycan part connected to C-3 of the aglycon was deduced from the following HMBC correlations: H-1′ (δ 4.83) of inner glucose with C-3 (δ 89.5) of sapogenin, H-1″ (δ 5.37) of one terminal glucose with C-3′ (δ 88.6), and H-1‴ (δ 5.72) of another terminal glucose with C-2′ (δ 79.3) (Fig. 2). The NMR data of the glycan part of **1** was in accordance with that of 3-*O*-[2′,3′-di-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl] oleanolic acid.[10] On the basis of the above results, compound **1** was elucidated as 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}-siaresinolic acid, named as Catunaroside E.

Compound **2** was determined to have the molecular formula of $C_{54}H_{88}O_{22}$ by its HR-ESI-MS spectrum (*m*/*z*: 1111.5702 [M + Na]$^+$). In the ^{1}H NMR spectrum, seven methyl protons (δ 1.23, 1.15, 0.83, 1.07, 1.23, 0.89, and 0.87 ppm), one olefinic proton (δ 5.41, br s), and four sugar anomeric protons (δ 4.83, d, J = 7.5 Hz; 5.11, d, J = 7.8 Hz; 6.47, br s; and 6.31, d, J = 8.1 Hz) (Table 1) were observed. The signals of seven methyl carbons at δ 28.1, 17.1, 15.7, 17.5, 26.1, 33.1, and 23.7 ppm, two olefinic carbons at δ 122.9 and 144.1 ppm, four sugar carbons at δ 104.9, 103.9, 101.7, and 95.8 ppm, and two carbons at δ 176.4 and 88.7 ppm linked to the glycan part were also shown in the ^{13}C NMR spectrum. The above evidence revealed that **2** was a bisdesmosidic glycoside. The aglycon part of this compound was determined to be oleanolic acid by comparing the ^{1}H, ^{13}C, and DEPT NMR signals of **2** with those of 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl]-olean-12-en-3β-ol-28-oic acid.[5] Acid hydrolysis of **2** suggested that the monosaccharides of this compound were L-rhamnose and D-glucose. In combination with the characteristic proton signal at δ 1.67 (3H, d, J = 6.0 Hz) in the ^{1}H NMR spectrum, **2** could be deduced to contain one rhamnose and three glucose. By comparing with the NMR spectra of Mussaendoside J (**5**), the presence of glucose linked to C-3′ (δ 89.6) in **2** was disclosed. The sequence of the oligosaccharide chain was confirmed by HSQC, HMBC, and ^{1}H-^{1}H double quantum filtered correlation spectroscopy (DQFCOSY) experiments. The key HMBC correlations were observed: H-1⁗ (δ 6.31) of a glucose and C-28 (δ 176.4) of the aglycon part, H-1′ (δ 4.83) of

Table 2. ^{13}C NMR data of compounds **1–5** in pyridine-d_5.

C	1	2	3	4	5	C	1	2	3	4	5
1	38.5	39.1	38.9	38.6	39.0	3-*O*	Glc	Glc	Glc	Glc	Glc
2	26.6	26.7	26.8	26.6	26.8	1′	105.1	104.9	105.4	105.1	105.4
3	89.5	88.7	88.9	89.5	88.8	2′	79.3	79.3	78.1	79.3	78.1
4	39.6	39.5	39.5	39.6	39.5	3′	88.6	89.6	79.8	88.7	79.9
5	55.9	56.1	56.2	56.0	56.1	4′	70.1	69.8	72.2	70.1	72.2
6	18.6	18.5	18.7	18.8	18.5	5′	77.7	77.0	79.3	77.7	79.3
7	33.3	33.1	33.0	33.1	33.1	6′	63.4	62.7	62.9	63.5	62.9
8	40.0	39.9	40.2	40.2	39.9	3′-*O*	Glc	Glc		Glc	
9	48.2	48.0	48.2	48.3	48.0	1′′	104.7	103.9		104.7	
10	37.1	37.0	37.1	37.1	37.0	2′′	76.4	75.2		76.4	
11	24.1	23.4	24.1	24.1	23.4	3′′	78.6	78.7		78.6	
12	123.1	122.9	123.1	123.2	122.9	4′′	71.6	71.4		71.6	
13	144.9	144.1	144.3	144.4	144.1	5′′	78.6	78.4		78.6	
14	42.1	42.2	42.1	42.2	42.2	6′′	62.6	62.3		62.6	
15	29.2	28.3	29.0	29.1	28.3	2′-*O*	Glc	Rha	Rha	Glc	Rha
16	28.4	23.8	28.0	28.1	23.8	1′′′	103.8	101.7	101.7	103.9	101.7
17	46.1	47.0	46.5	46.5	47.0	2′′′	75.4	72.4	72.4	75.4	72.4
18	44.8	41.8	44.6	44.6	41.8	3′′′	78.6	72.5	72.5	78.6	72.5
19	81.3	46.3	81.1	81.2	46.2	4′′′	72.6	73.9	74.2	72.7	74.2
20	35.7	30.8	35.5	35.6	30.8	5′′′	77.8	69.8	69.6	77.8	69.6
21	29.2	34.0	29.0	29.0	34.0	6′′′	62.4	18.6	18.7	62.2	18.7
22	33.7	32.6	33.2	33.3	32.5	28-*O*		Glc	Glc	Glc	Glc
23	28.0	28.1	28.1	28.8	28.1	1′′′′		95.8	95.9	95.9	95.8
24	16.7	17.1	17.1	16.7	17.1	2′′′′		74.1	74.2	74.2	74.2
25	15.4	15.7	15.5	15.5	15.6	3′′′′		78.9	78.9	79.0	78.9
26	17.5	17.5	17.6	17.6	17.5	4′′′′		71.1	71.1	71.2	71.1
27	24.9	26.1	24.9	25.0	26.1	5′′′′		77.8	77.8	77.8	77.8
28	180.9	176.4	177.2	177.3	176.4	6′′′′		62.3	62.2	62.4	62.2
29	28.9	33.1	28.7	28.1	33.1						
30	24.9	23.7	24.7	24.7	23.6						

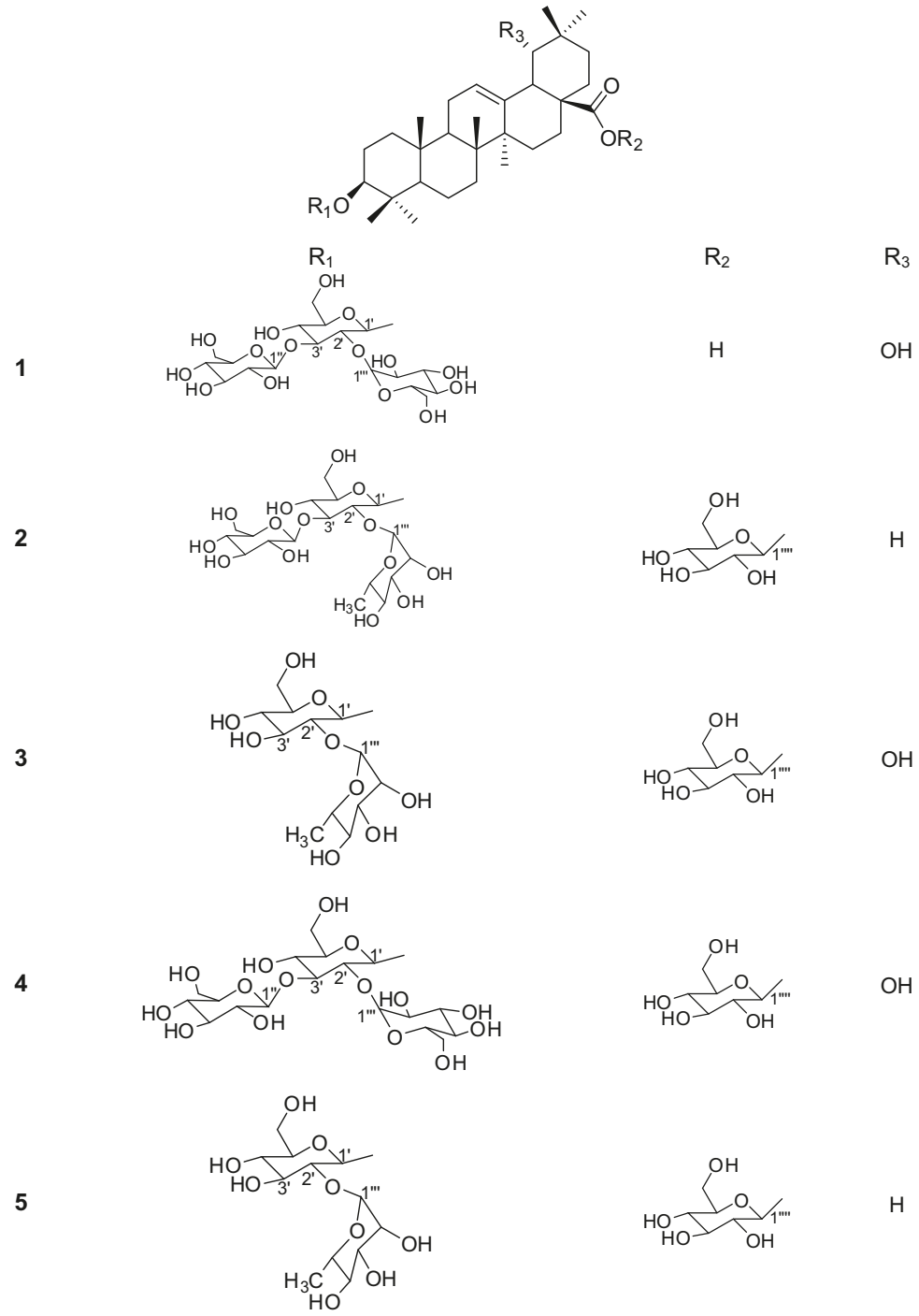
Note: Glc, glucose; Rha, rhamnose.

the inner glucose and C-3 (δ 88.7) of the aglycon part, H-1′′ (δ 5.11) of the terminal glucose and C-3′ (δ 89.6) of the inner glucose, and H-1′′′ (δ 6.47) of the terminal rhamnose and C-2′ (δ 78.7) of the inner glucose (Fig. 2). The β-anomeric configurations of glucose units were determined by the relatively large $^3J_{H1,H2}$ coupling constants (7.5–8.1 Hz). The $^1J_{C1,H1}$ coupling constant (168 Hz) for the rhamnosyl unit confirmed that the anomeric proton was equatorial (α-pyranoid anomeric form).[11] On the basis of this evidence, compound **2**, named as Catunaroside F, was elucidated as 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}-28-*O*-(β-D-glucopyranosyl)-oleanolic acid.

Compound **3**, also an amorphous powder, has the molecular formula of $C_{48}H_{78}O_{17}$ as indicated by its HR-ESI-MS spectrum (*m/z*: 965.5129 $[M + Na]^+$). In the ^1H NMR spectrum, seven methyl protons (δ 1.26, 1.20, 0.88, 1.13, 1.63, 1.14, and 0.98 ppm), one olefinic proton (δ 5.50, br s), and three sugar anomeric protons (δ 4.94, d, J = 7.0 Hz; 6.56, br s; and 6.38, d, J = 8.0 Hz) (Table 1) were observed. The signals of seven methyl carbons at δ 28.1, 17.1, 15.5, 17.6, 24.9, 28.,7 and 24.7 ppm, two olefinic carbons at δ 123.1 and 144.3 ppm, three sugar carbons at δ 105.4, 101.7, and 95.9 ppm, and two carbons at δ 177.2 and 88.9 ppm linked to the glycan part were also shown in the ^{13}C NMR spectrum. The signals in the NMR spectrum of **3** were in agreement with those of Mussaendoside J, except for the signals of the aglycon part at δ 81.1 (d, C-19) ppm, which was oxygenated, and 35.5 (s, C-20) ppm.[12] The aglycon part of **3** was then established as siaresinolic acid by comparing the NMR spectrum data of **3** with that of **1**. From the above evidence, compound **3** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-28-*O*-(β-D-glucopyranosyl)-siaresinolic acid, named as Catunaroside G.

Compound **4**, obtained as an amorphous powder, was deduced to have the molecular formula of $C_{54}H_{88}O_{24}$ as indicated from the HR-ESI-MS data (*m/z*: 1143.5586 $[M + Na]^+$). Acid hydrolysis of **4** showed the existence of D-glucose. The signals at 177.3 and 89.5 ppm in the ^{13}C NMR spectrum suggested that **4** was a bisdesmosidic glycoside. In the ^1H and ^{13}C NMR spectrum, four anomeric carbon signals (δ 4.83, d, J = 7.5 Hz; 5.36, d, J = 8.0 Hz; 5.71, d, J = 7.5 Hz; and 6.36, d, J = 8.0 Hz and δ 105.1, 104.7, 103.9, 95.9 ppm) (Tables 1 and 2) were exhibited. Compound **4** had the same NMR signals as Aralia-saponin V except the signals of C-19 (δ 81.2 ppm) and C-20 (δ 35.6 ppm) of the aglycon part, which is just like the difference between compound **3** and Mussaendoside J.[13] Similarly, the aglycon moiety of **4** was determined to be siaresinolic acid. Thus, compound **4** was elucidated as 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}-28-*O*-(β-D-glucopyranosyl)-siaresinolic acid, named as Catunaroside H.

Fig. 1. Structures of compounds **1–5.**



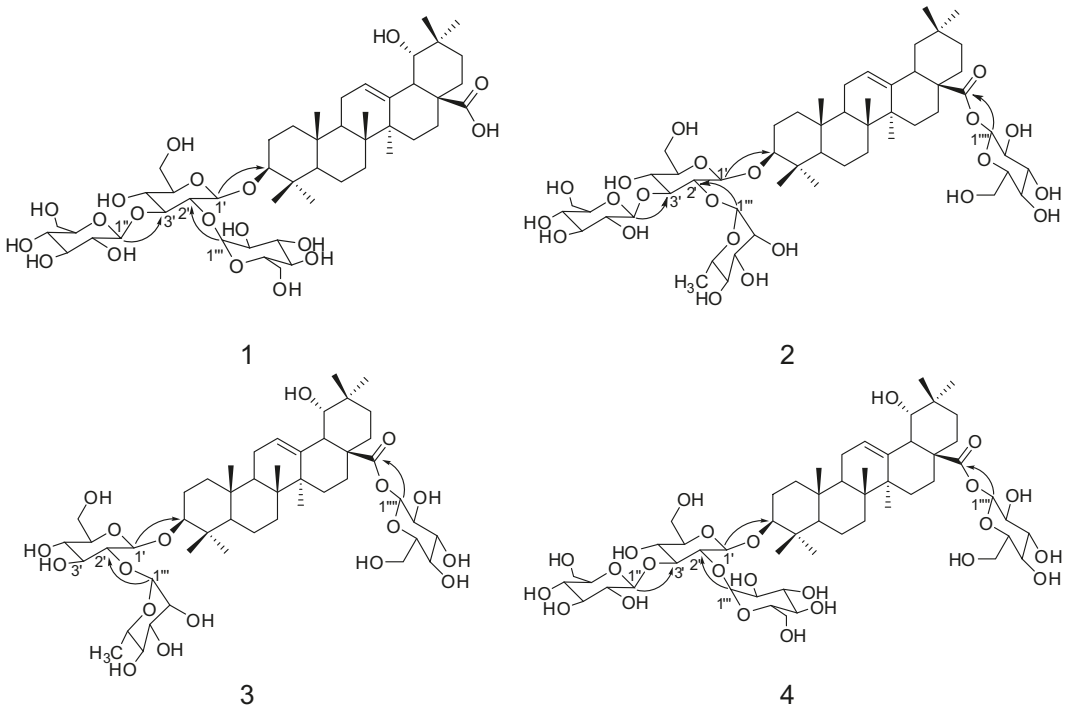
Compound **5** was elucidated as Mussaendoside J by comparing its spectra data with the data reported in the literature (IR, MS, and ^{1}H and ^{13}C NMR).[12]

Experimental

General

MS were recorded on a MDS SCIEX API 2000 LC/MS/MS instrument for ESI and a Bruker BioTOF Q spectrometer for HR-ESI. IR spectra were measured with a Bruker EQUINOX55 infrared spectrometer. ^{1}H and ^{13}C NMR spectra were acquired with a Bruker DRX-500 spectrometer ($SiMe_4$ as the internal standard). Silica gel (Qingdao Haiyang Chemical Co., Ltd., 100–200 mesh and 200–300 mesh) and LiChroprep RP-18 (Merck) were used for the silica gel column chromatography (CC) and medium-pressure liquid chromatography (MPLC). Sephadex LH-20 (Pharmacia) and macroporous resin D101 were also used for column chromatography. TLC was carried on precoated silica gel 60 F_{254} plates (Qingdao Haiyang Chemical Co., Ltd.), and detection was achieved by 10% H_2SO_4–EtOH for saponins. Semipreparative high-pressure liquid chromatography (HPLC) was performed using

Fig. 2. Key HMBC correlations for the sugar sequence of **1**–**4** (from H to C).



an octadecylsilane (ODS) column (YMC-Pack ODS-5-A, 250 mm × 10 mm inside diameter (i.d.), 5 μm; YMC Co., Ltd.) on a Waters-600 HPLC system equipped with a Waters-996 photodiode array detector.

Plant material

The stem bark of *C. spinosa* Tirveng was collected in February 2006 from Sanya, Hainan Province, P. R. China. The specimen was identified by professor Si Zhang, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, P. R. China. A voucher specimen has been deposited in the South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, P. R. China (accession number: GKLMMM020).

Extraction and isolation

The *n*-BuOH extract of air-dried and powdered plant material (9.0 kg) was extracted by the method as previously reported.[8] The *n*-BuOH soluble fraction (170 g) was passed through a macroporous resin (D101) column eluted with H_2O and EtOH–H_2O (3:7, 6:4, and 95:5), whereas the EtOH–H_2O (95:5, 10 g) eluted portion was subjected to silica gel CC eluted with $CHCl_3$–MeOH (98:2–50:50) to give fractions A–G. Fraction E was separated by preparative HPLC using MeOH–H_2O (68:32) to give compound **1** (10 mg). Fraction F was recrystallized to afford compound **2** (50 mg). The EtOH–H_2O (6:4, 35 g) eluted portion was subjected to silica gel CC eluted with $CHCl_3$–MeOH (90:10–50:50) to give fractions a–f. Fraction b was separated by Sephadex LH-20 (MeOH), then fractions 25–28 and 53–54 were purified by preparative HPLC eluting with MeOH–H_2O (55:45) and MeOH–H_2O (70:30) to obtain compounds **3** (22 mg) and **5** (12 mg) separately. Fraction f was rechromatographed to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (80:20:5), then fraction 12 was purified by preparative HPLC using MeOH–H_2O (50:50) to yield compound **4** (70 mg).

Catunaroside E (1)

Colorless amorphous powder. IR (KBr, cm^{-1}) υ_{max}: 3418, 1728, 1644. ^1H NMR (500 MHz, pyridine-d_5) δ: 3.25 (1H, dd, J = 4.4, 11.5 Hz, H-3), 5.55 (1H, br s, H-12), 3.62 (1H, br s, H-18), 3.60 (1H, br s, H-19), 1.26 (3H, s, H-23), 1.08 (3H, s, H-24), 0.81 (3H, s, H-25), 1.06 (3H, s, H-26), 1.66 (3H, s, H-27), 1.18 (3H, s, H-29), 1.02 (3H, s, H-30), 4.83 (1H, d, J = 7.5 Hz, H-1′), 5.37 (1H, d, J = 7.6 Hz, H-1′′), 5.72 (1H, d, J = 7.6 Hz, H-1′′′). ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2. ESI-MS *m/z*: 981 $[M + Na]^+$, 997 $[M + K]^+$. HR-ESI-MS *m/z*: 981.5080 $[M + Na]^+$. Calcd. for $C_{48}H_{78}O_{19}Na$: 981.5001.

Catunaroside F (2)

Colorless amorphous powder. IR (KBr, cm^{-1}) υ_{max}: 3426, 1739 1640. ^1H NMR (500 MHz, pyridine-d_5) δ: 3.38 (1H, dd, J = 3.9, 11.6 Hz, H-3), 5.41 (1H, br s, H-12), 3.18 (1H, dd, J = 3.4, 13.6 Hz, H-18), 1.23 (3H, s, H-23), 1.15 (3H, s, H-24), 0.83 (3H, s, H-25), 1.07 (3H, s, H-26), 1.23 (3H, s, H-27), 0.89 (3H, s, H-29), 0.87 (3H, s, H-30), 4.83 (1H, d, J = 7.5 Hz, H-1′), 5.11 (1H, d, J = 7.8 Hz, H-1′′), 1.67 (3H, d, J = 6.0 Hz, H-6′′′), 6.47 (1H, br s, H-1′′′), 6.31 (1H, d, J = 8.1 Hz, H-1′′′′). ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2. ESI-MS *m/z*: 1111 $[M + Na]^+$, 1127 $[M + K]^+$. HR-ESI-MS *m/z*: 1111.5702 $[M + Na]^+$. Calcd. for $C_{54}H_{88}O_{22}Na$: 1111.5691.

Catunaroside G (3)

Colorless amorphous powder. IR (KBr, cm^{-1}) υ_{max}: 3419, 1730, 1644. ^1H NMR (500 MHz, pyridine-d_5) δ: 3.34 (1H, dd, J = 4.0, 11.5 Hz, H-3), 5.50 (1H, br s, H-12), 3.52 (1H, br s, H-18), 3.57 (1H, br s, H-19), 1.26 (3H, s, H-23), 1.20

(3H, s, H-24), 0.88 (3H, s, H-25), 1.13 (3H, s, H-26), 1.63 (3H, s, H-27), 1.14 (3H, s, H-29), 0.98 (3H, s, H-30), 4.94 (1H, d, J = 7.0 Hz, H-1′), 1.71 (3H, d, J = 6.0 Hz, H-6‴), 6.56 (1H, br s, H-1‴), 6.38 (1H, d, J = 8.0 Hz, H-1⁗). ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2. ESI-MS *m/z*: 965 $[M + Na]^+$, 981 $[M + K]^+$. HR-ESI-MS *m/z*: 965.5129 $[M + Na]^+$. Calcd. for $C_{48}H_{78}O_{18}Na$: 965.5117.

Catunaroside H (4)

Colorless amorphous powder. IR (KBr, cm^{-1}) υ_{max}: 3422, 1738, 1645. ^{1}H NMR (500 MHz, pyridine-d_5) δ: 3.26 (1H, dd, J = 3.5, 11.5 Hz, H-3), 5.50 (1H, br s, H-12), 3.52 (1H, br s, H-18), 3.57 (1H, br s, H-19), 1.26 (3H, s, H-23), 1.09 (3H, s, H-24), 0.84 (3H, s, H-25), 1.12 (3H, s, H-26), 1.63 (3H, s, H-27), 1.14 (3H, s, H-29), 0.98 (3H, s, H-30), 4.83 (1H, d, J = 7.5 Hz, H-1′), 5.36 (1H, d, J = 8.0 Hz, H-1″), 5.71 (1H, d, J = 7.5 Hz, H-1‴), 6.36 (1H, d, J = 8.0 Hz, H-1⁗). ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2. ESI-MS *m/z*: 1143 $[M + Na]^+$, 1159 $[M + K]^+$. HR-ESI-MS *m/z*: 1143.5586 $[M + Na]^+$. Calcd. for $C_{54}H_{88}O_{24}Na$: 1143.5598.

Mussaendoside J (5)

Colorless amorphous powder. IR (KBr, cm^{-1}) υ_{max}: 3422, 1736, 1643. ^{1}H NMR (500 MHz, pyridine-d_5) δ: 3.35 (1H, dd, J = 4.0, 11.5 Hz, H-3), 5.43 (1H, br s, H-12), 3.19 (1H, dd, J = 3.8, 10.5 Hz, H-18), 1.25 (3H, s, H-23), 1.19 (3H, s, H-24), 0.84 (3H, s, H-25), 1.09 (3H, s, H-26), 1.25 (3H, s, H-27), 0.91 (3H, s, H-29), 0.88 (3H, s, H-30), 4.94 (1H, d, J = 6.0 Hz, H-1′), 1.71 (3H, d, J = 6.0 Hz, H-6‴), 6.56 (1H, br s, H-1‴), 6.34 (1H, d, J = 8.0 Hz, H-1⁗). ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2. ESI-MS *m/z*: 949 $[M + Na]^+$, 965 $[M + K]^+$. HR-ESI-MS *m/z*: 949.5178 $[M + Na]^+$. Calcd. for $C_{48}H_{78}O_{17}Na$: 949.5183.

Acid hydrolysis of 1–5

Compounds **1–5** (5 mg each) were refluxed in 5 mL of 4 N HCl for 8 h (kept sealed) in a water bath (100 °C). After cooling, the reaction mixtures were extracted with EtOAc (5 mL). The aqueous layers were adjusted to pH 6 with $NaHCO_3$. After being concentrated under reduced pressure, each H_2O layer (monosaccharide portion) was identified by comparing the R_f value with that of the authentic samples on the TLC plate (eluted with $CHCl_3$–MeOH–H_2O (8:7:1) and EtOAc–MeOH–AcOH–H_2O (13:3:4:3) solvent system, visualized with ethanol and 10% H_2SO_4 spraying and then heating).

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