A Novel Cyclopentene Derivative and a Polyhydroxylated Steroid from a South China Sea Gorgonian *Menella* sp.

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A chemical investigation on a South China Sea gorgonian, *Menella* sp. resulted in the isolation and elucidation of menellin A (1), a highly oxygenated racemate with C_8 skeleton, and a polyhydroxylated steroid, menellsteroid C (2), along with eight known compounds (3—10). The structures of the new compounds were elucidated by means of MS, 1D and 2D NMR spectra, and the relative stereochemistry of 1 was determined by X-ray single-crystal diffraction analysis. In addition, compound 7 was isolated as a new natural product. Compounds 1—3 and 7 were selected to test the anti-inflammatory inhibition against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages. 1 and 3 exhibited modest inhibitory effects with IC_{50} of 71.3, 33.9 μM, respectively, compared to the positive control aminoguanidine (IC_{50} 25.0 μM).

Key words *Menella* sp.; gorgonian; menellin A; menellsteroid C; X-ray diffraction; anti-inflammatory inhibition

Gorgonians, with trivial names of Sea Fan or Sea Whip *etc.*, are one of the important sources recognized the new and significant biological activity of metabolites and are arousing the interest of chemists all over the world. There are a large number of papers every year on secondary metabolite research of the gorgonians.[1—4] However, one of the prolific gorgonian in the South China Sea, the *Menella* sp., has been given comparatively less attention, with the exception of four papers that described the guaiane sesquiterpenoids, polyhydroxylated steroids and other known compounds.[5—8]

As a part of our systematic research on the structurally novel and significantly bioactive metabolites from South China Sea invertebrates, we undertook a chemical investigation on an unidentified gorgonian species *Menella* sp., collected on Hainan Island. This resulted in the isolation and structure elucidation of a novel constituent, we named menellin A (**1**), and a polyhydroxylated steroid menellsteroid C (**2**), together with eight known compounds (**3**—**10**), including a new natural product (**7**). The structures of these new compounds were elucidated by spectrum analysis of MS, 1D and 2D NMR. The relative stereochemistry of **1** was determined by X-ray single-crystal diffraction analysis.

Results and Discussion

Menellin A (**1**) was obtained as a colorless quadrate crystal ($CHCl_3$–MeOH). Its molecular formula was determined as $C_{11}H_{14}O_8$ by an $[M-H]^-$ ion peak at *m/z* 273.0618 (Calcd 273.0616) in the high-resolution-electrospray ionization (HR-ESI)-MS, indicating 5 degrees of unsaturation. The IR spectrum exhibited the characteristic absorption for a hydroxyl (3485 cm^{-1}) and carboxyls [1736, 1706 (br) cm^{-1}]. The ^{1}H- and ^{13}C-NMR spectra (Table 1) showed the signals for an olefin [δ_H 6.82 (t, 1.5); δ_C 142.4, 137.0] , three methoxys [δ_H/δ_C 3.78/53.1 (9), 3.45/59.6 (10), 3.77/52.5 (11)], three carboxyl carbons [δ 173.6 (C-8), 172.6 (C-7), 165.7 (C-6)], an oxygenated methine at δ 4.65/94.9, and a methine at δ 4.21/58.9, as well as an oxygenated quaternary carbon at δ 86.7. These data implied **1** to be a highly oxygenated compound with a single ring. These assignments and the planar structure were achieved by heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC) experiments. The HMBC correlations from two methoxys (OMe-9, 11) to two carboxyls (C-8, 6) respectively suggested the appearance of two methyl esters in the structure of **1**. An HMBC correlation from H-4 (δ 6.82) to C-6 established the connection of one methyl ester with the olefin. The HMBC correlations from additional methoxy proton to C-3, from H-3 to the quaternary olefin carbon (C-5), C-8 and C-2, as well as from H-1 to C-7 and C-8 showed the planar structure of **1** to be a derivative of cyclopentene with three carboxyl groups attached at the ring (Fig. 1).

The relative stereochemistry of **1** was finally determined by X-ray single-crystal diffraction analysis after the failure by nuclear Overhauser effect spectroscopy (NOESY) spectrum, where no useful NOESY correlation was observed between the H-1 with H-3, or between OMe-9 with OMe-10. However, the X-ray diffraction result showed that the **1** is a natural racemate in consideration of its space group (P-1), as P-1 is a racemic space group, one with 1*S*, 2*S*, 3*S* configurations (**1**) and another with all *R* configurations. This conclusion was further confirmed by the almost zero optical rota-

Table 1. The NMR Data of Compound **1**

No.	δ_C	δ_H
1	58.9	4.21 d (1.5)
2	86.7	—
3	94.9	4.65 br s
4	142.4	6.82 t (1.5)
5	137.0	—
6	165.7	—
7	172.6	—
8	173.6	—
9	53.1	3.78
10	59.6	3.45
11	52.5	3.77

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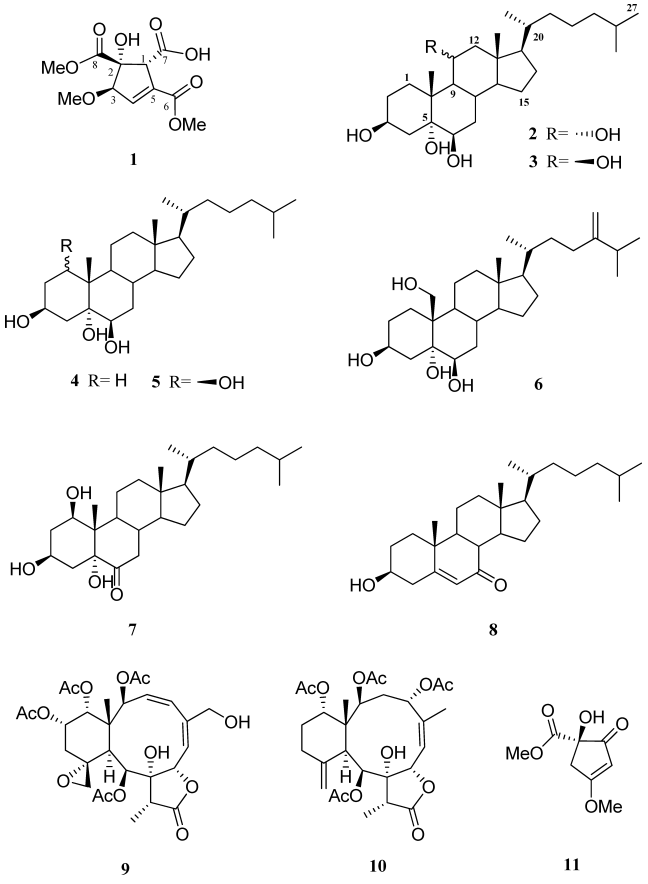

Fig. 1. Structures of Compounds from *Menella* sp. (**1**—**10**) and (+)-Kjellmanone (**11**)

tion value.

Menellsteroid C (**2**) was obtained as white powder. Its molecular formula was determined as $C_{27}H_{48}O_4$ by its ESI-MS ion $[M+Na]^+$ at *m*/*z* 459 and NMR data (Table 2). The ^{1}H- and ^{13}C-NMR spectra showed characteristic signals of five methyls, including two tertiary ones (C-18, 19) and three secondary methyls at δ_H/δ_C 0.99/19.2 (C-21), 0.89/22.9 (C-26) and 0.89/23.2 (C-27), which in collaboration with its typical C-3 oxygenated proton signal at δ_H 4.02 (m, H-3) and one at δ_H 3.46 (br s, H-6) due to both C-5 and C-6 hydroxylated in its ^{1}H-NMR, suggested **2** a polydroxylated cholestan-type steroid. A detailed comparison of its NMR data with those of menellsteroid A (**3**)[7,9] showed both are isomers with high structural similarity (Fig. 1), which was supported and confirmed by the HMBC spectra (Fig. 2). The differences be-

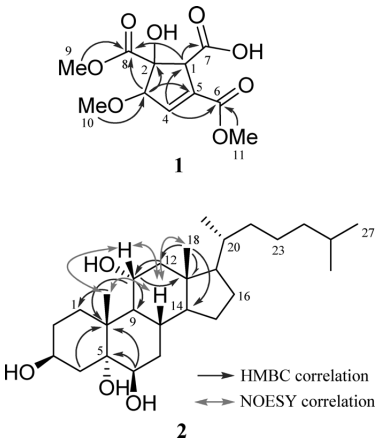

Fig. 2. Key HMBC and NOESY Correlations of **1** and **2**

Table 2. NMR Data (CD_3OD, 500/125 MHz) for **2**, **3** and **7**, δ in ppm and *J* in Hz

No.	**2**		**3**	**7**	
	δ_H	δ_C	δ_C	δ_H	δ_C
1	2.07 t (11.5), 1.79 m	35.4	33.2	4.07 dd (10.5, 5.5)	71.7
2	1.78, 1.53 each m	32.0	31.6	2.05, 2.15 each m	42.7
3	4.02 m	68.2	68.3	3.95 m	65.6
4	1.57 m, 2.09 br d (11.5)	42.0	41.1	1.57 m, 1.80 br d (11.5)	36.7
5	—	77.5	77.5	—	82.2
6	3.46 br s	76.6	76.4	—	214.8
7	1.79, 1.53 m	35.2	36.4	2.79 t (12.5), 2.15 m	41.6
8	1.78 m	30.4	28.4	1.69 m	41.5
9	1.46 m	53.0	49.5	1.47 m	46.6
10	—	41.0	40.0	—	46.7
11	3.85 dt (5.0, 10.5)	69.5	69.5	1.86, 1.27 each m	23.2
12	1.24 m, 2.28 dd (12.5, 5.0)	52.8	50.5	1.99, 1.32 each m	40.7
13	—	44.3	43.1	—	43.6
14	1.19 m	57.6	59.3	1.17 m	58.0
15	1.39 br d (11.5), 1.18 m	25.0	25.0	1.40 d (11.5), 1.18 m	25.1
16	1.54, 1.30 each m	29.4	29.1	1.55, 1.30 each m	29.2
17	1.18 m	56.6	58.3	1.18 m	58.0
18	0.74 s	13.5	14.9	0.71 s	12.5
19	1.29 s	17.6	20.3	0.78 s	9.5
20	1.40 m	37.1	37.3	1.40 m	37.1
21	0.99 d (6.5)	19.2	19.3	0.98 d (6.5)	19.2
22	1.05 m	37.3	37.3	1.05 m	36.7
23	1.60 m	25.3	25.3	1.61 m	25.3
24	1.17 m	40.7	40.7	1.18 m	40.7
25	1.54 m	29.2	29.2	1.54 m	29.2
26	0.89 d (6.5)	22.9	23.0	0.89 d (6.5)	23.0
27	0.89 d (6.5)	23.2	23.2	0.90 d (6.5)	23.2

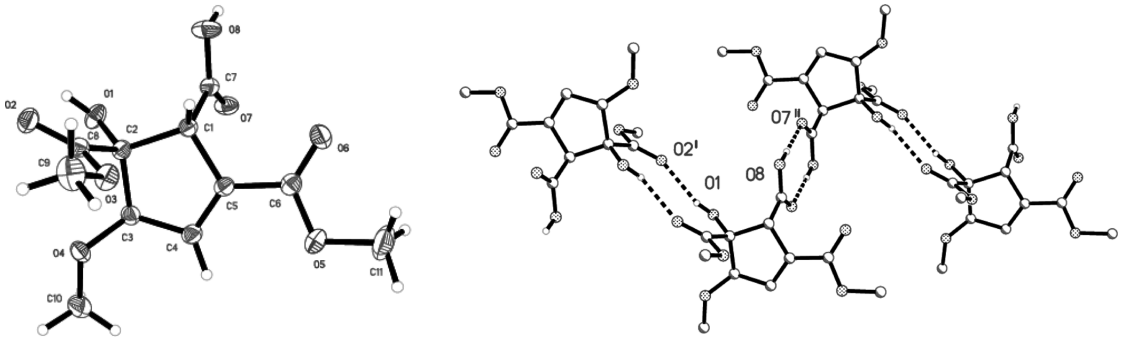

Fig. 3. Single-Crystal X-Ray Structure of **1**

tween them are the downfield chemical shift of 3.5 ppm C-9, and 2.3 ppm for C-12, and the upfield shift to δ 3.85 (H-11) compared to that of δ 4.14 in **3**, suggesting that **2** differs from **3** only at the probable stereochemistry of C_{11}–OH. This conclusion was supported by a comparision of their ^{13}C-NMR and confirmed by the NOESY correlation between H-11 with H-19. Also, the H-11β configuration could be deduced by its coupling pattern (dt, 5.0, 10.5 Hz) according to the literature.[7] Thus, compound **2** was determined to be cholestan-3β,5α,6β,11α-tetrol, and called menellsteroid C.

Compound **7** was obtained as a white solid, with its molecular formula of $C_{27}H_{46}O_4$ by its ESI-MS. The NMR data (Table 2) showed the typical signals of a polyoxygenated cholestan-type steroid: two tertiary methyls, three secondary methyls, and three oxygenated carbons. Its NMR data was closely similar to that of compound **5**,[10] including the coupling pattern of H-1 (4.07, dd, 5.0, 11.5 Hz), except for the appearance of an additional ketone signal at δ 214.8 in **7**, and the disappearance of the characteristic proton signal around δ 3.50, which suggests the hydroxyl group at C-6 in compound **5** was oxygenated to a ketone in **7**. All the NMR data was assigned by HSQC spectrum and was in agreement with references.[10,11] Thus, **7** was identified as 1β,3β,5α-trihydroxycholestan-6-one. Since it was isolated as a natural product for the first time, we propose its trivial name menellsteroid D.

The other seven known compounds were identified as menellsteroid A (**3**),[7,9] cholestan-3β,5α,6β-triol (**4**),[9] cholestan-1β,3β,5α,6β-tetrol (**5**),[10] nephalsterol (**6**),[12] and cholestan-3β-5-en-6-one (**8**),[13] and two briarane diterpenoids junceellolides B (**9**) and D (**10**),[14] by comparison of their NMR data with those of references.

Compound **1** is of special interest, as it is the first example with a highly oxygenated cyclopentene ring discovered from a gorgonian. Furthermore, **1** was a rarely reported and naturally occurring racemate. It was determined by the X-ray diffraction with a P-1 space group, and confirmed by its optical rotation value. It is structurally similar to (+)-kjellmanone (**11**),[15,16] a cyclopentenone derivative from the marine alga *Sargassum kjellmanianum*. Compound **7** was isolated as a natural product for the first time, and compounds **9** and **10** were the first two briarane diterpenoids found from *Menella* sp. to date.

Compounds **1**—**3** and **7** were chosen to test the anti-inflammatory against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages. The results showed that **1** and **3** exhibited modest inhibition effects with IC_{50} of 71.3 and 33.9 μM, respectively, compared to the positive control aminoguanidine with IC_{50} 25.0 μM. In addition, compounds **1**—**10** were evaluated by the anti-oxidant capacity in a radical 2, 2-diphenyl-(2,4,6-trinitrophenyl)hydrazyl (DPPH) free-radical assay, but no obvious scavenging effect was observed.

Experimental

General Experimental Procedures Melting points were determined on an X-5 digital micro melting point apparatus (Beijing Tech Instrument Co., Ltd., China) and are uncorrected. Optical rotations were recorded on a JASCO P-1020 digital polarimeter. IR spectra were measured on JASCO FT/IR-480 plus spectrometers. NMR spectra were recorded using Bruker 500 MHz NMR spectrometers. HR-ESI-MS spectra were recorded on an Applied Biosystems Mariner 5140 spectrometer. The column chromatographies were applied on the Buchi Sepacore (C-615/605) system. All solvents used were of analytical grade (Tianjing Fuyu Chemical and Industry Factory, China). Silica gel and preparative TLC plates (20×20×0.04 cm) (Qingdao Mar. Chem. Ind. Co., Ltd., China), Sephadex LH-20 gel (Pharmacia, Sweden) and C_{18} reverse-phased silica gel (150—200 mesh, Merck, Germany) were used for column chromatography.

Animal Material Fresh gorgonians was collected in April 2009 (7—10 m depth) off Meishan Island, Hainan province, China. The specimen was identified by Professor Hui Huang, the South China Sea Institute of Oceanology, Academia Sinica. A voucher specimen (No. M090402) was deposited in the Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, P. R. China.

Extraction and Isolation The fresh gorgonian (*ca.* 5 kg) were exhaustively extracted with 95%, 70% EtOH and $CHCl_3$–MeOH (1 : 1) at room temperature, and the solvent was evaporated *in vacuo*. The residue was partitioned in H_2O and extracted with petroleum ether (PE), EtOAc, and *n*-butanol, respectively, to provide the PE fraction (16 g), EtOAc fraction (9.5 g) and the *n*-butanol fraction (11 g). The EtOAc fraction was chromatographed on silica gel and eluted with PE–acetone (50 : 1), followed by a gradient of $CHCl_3$–MeOH (100 : 0, 30 : 1, 10 : 1, 1 : 1) to yield 8 fractions (Frs. A—G). Fr. B (2.1 g) was reseparated by Sephadex LH-20 (MeOH), followed by silica gel column chromatography (CC) and eluted with PE–acetone (5 : 3) to get 6 subfractions (Fr. Ba—f). A further isolation of Fr. Bc yielded **10** (4.6 mg) by preparative thin layer chromatography (p-TLC, two plates) with PE–EtOAc (1 : 1) as developer. The p-TLC (PE–acetone 5 : 4, one plate) of Fr. Bb provided **9** (1.8 mg). Fr. E (800 mg) was chromatographed by silica gel CC and eluted with PE–EtOAc (1 : 3), followed by a recrystallization ($CHCl_3$–MeOH 1 : 1) to obtain **1** (13.7 mg).

The PE fraction was chromatographed on silica gel and eluted with a gradient of PE–EtOAc (100 : 0, 50 : 1, 10 : 1, 2 : 1, 0 : 1) to yield 14 fractions (Frs. 1—14). Fr. 12 (2.5 g) was subjected to silica gel CC with elution by PE–EtOAc (3 : 1) to get 4 fractions (Frs. 12A—D), and twice to p-TLC of Fr. 12C (200 mg) ($CHCl_3$–acetone 10 : 1 and PE–EtOAc 1 : 1 as developer, respectively) which yielded **8** (2.2 mg). Fr. 14 (4 g) was resubjected to silica gel CC and eluted with a gradient of PE–EtOAc (3 : 1—1 : 4) to 9 fractions (Frs. 14A—I). A purification by Sephadex LH-20 (MeOH) to Fr. 14E (420 mg) produced **4** (32.0 mg) and 4 other portions (Frs. $14E_1$—E_4). Frs. $14E_3$ and E_4 were subjected to p-TLC ($CHCl_3$–EtOAc 1 : 5 and PE–acetone 2 : 3, respectively) to obtain **7** (3.9 mg) (from Fr. $14E_3$), and **3** (16.1 mg) and **6** (6.2 mg). Fr. 14C (600 mg) was applied to silica gel CC and eluted with

$CHCl_3$–MeOH (10 : 1) to yield 7 subfractions (1—7). Fr. $14C_6$ (40 mg) was applied to p-TLC (3 plates) and developed by $CHCl_3$–EtOH (5 : 1) to get **2** (4.2 mg) and **5** (8.5 mg).

Menellin A (**1**): Colorless quadrate crystal ($CHCl_3$–MeOH); mp 173.2—175.1 °C; $[\alpha]_D^{25}$ +0.0002 (c=0.13, MeOH); UV λ_{max} (MeOH) nm ($\log\varepsilon$): 222 (3.98) nm; IR (KBr) ν_{max} cm^{-1}: 3485, 3010, 2955, 1736, 1706 (br), 1645, 1436, 1258, 1146, 960; ^{1}H- and ^{13}C-NMR (CD_3OD, 500/125 MHz), see Table 1. ESI-MS m/z 275 $[M+H]^+$; HR-ESI-MS m/z 273.0618 $[M-H]^-$ (Calcd for $C_{11}H_{13}O_8$: 273.0616).

Menellsteroid B (**2**): White powder; $[\alpha]_D^{25}$ −18.5 (c=0.12, MeOH); IR (KBr) ν_{max} cm^{-1}: 3480, 2905, 2871, 1458, 1376, 1200; ^{1}H- and ^{13}C-NMR (CD_3OD, 500/125 MHz), see Table 2. ESI-MS m/z 459 $[M+Na]^+$; HR-ESI-MS m/z 437.3552 $[M+H]^+$ (Calcd for $C_{27}H_{49}O_4$: 437.3553).

1β,3β,5α-Trihydroxycholestan-6-one (Proposed Menellsteroid D) (**7**): White solid; ^{1}H- and ^{13}C-NMR (CD_3OD, 500/125 MHz), see Table 2. ESI-MS m/z 457 $[M+Na]^+$.

X-Ray Crystallographic Analysis of Menellin A (1) $C_{11}H_{14}O_8$, M=274.22, triclinic, space group P-1, a=7.9389(5) Å, b=9.1825(6) Å, c=9.7595(6) Å. V=625.73(7) $Å^3$, Z=2, D_{calcd}=1.455 Mg/m^3, crystal dimensions 0.46×0.43×0.42 mm^3, μ=0.126 mm^{-1}. 5216 Reflections measured, 2659 reflections independent (R_{int}=0.0154), R=0.0362, Rw=0.1068, X-ray diffraction experiments for this compound were carried out on a Bruker Smart 1000 CCD diffractometer at 273 K using Mo$K\alpha$ radiation (λ= 0.71070 Å). Absorption corrections were done by semi-empirical from equivalents. The structure was resolved using direct methods and refined with full-matrix least-squares on F^2. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 754564). Copies of the data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, U.K.; fax: t44 1223 336 033 or deposit@ccdc.cam.ac.uk].

Biological Assay The procedure reported by Yang *et al.*[17] was used for the measurement of anti-inflammatory inhibition against LPS-induced NO production in RAW 246.7 in macrophages, and the DPPH free-radical scavenging inhibitory activity was measured as described by Lee *et al.*[18]

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