

Rumphellatins B and C, Two New Caryophyllane-Type Hemiketal Norsesquiterpenoids from the Formosan Gorgonian Coral *Rumphella antipathies*

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Two new caryophyllane-type hemiketal norsesquiterpenoid alcohols, designated as rumphellatins B (**1**) and C (**2**), were obtained from the Formosan gorgonian coral *Rumphella antipathies*. The structures of natural products **1** and **2** were established by analyzing the spectral data. Rumphellatin B (**1**) showed antibacterial activity toward the Gram-positive bacterium *Staphylococcus aureus*.

Gorgonian corals of the genus *Rumphella* (phylum Cnidaria, class Anthozoa, order Gorgonacea, suberorder Holaxonia, family Gorgoniidae), which are distributed in the tropical waters of the Indo-Pacific Ocean, have been investigated for ecological and medical uses.[1,2] Studies on the chemical constituents of gorgonian corals of *Rumphella* were initiated in 1995 with the discovery of a new steroid, (24*S*)-24-methylcholest-4-ene-3β,6β-diol, from an Indian gorgonian *R. aggregata*, collected at Natkal Island of the Andaman and Nicobar Islands.[3] In our screening for bioactive natural products from Formosan marine invertebrates, we have isolated a series of briarane and steroid metabolites from the octocorals *Alcyonium* sp.,[4] *Briareum* sp.,[5] *Briareum excavatum*,[6] *Ellisella robusta*,[7] *Junceella fragilis*,[8] *Junceella juncea*,[8c,9] and the tunicate *Eudistoma* sp.[10] Two caryophyllane-type natural products, kobusone and rumphellatin A (**3**), have been isolated from *R. antipathies*.[11,12] In this paper, we describe the isolation, structure determination, and antibacterial activity of two new caryophyllane-type hemiketal norsesquiterpenoids, rumphellatins B (**1**) and C (**2**), from *R. antipathies*. Caryophyllane-type natural products are a group of sesquiterpenoids having a bicyclo[7.2.0] system. The compounds of this type exist widely in terrestrial plants,[13] but rarely found in marine organisms.[14] The structures of compounds **1** and **2** were established by analyzing spectral data. Antibacterial activity of caryophyllanes **1** and **2** toward the Gram-positive bacterium *Staphylococcus aureus* is also reported.

Experimental

General Experimental Procedures. Melting points were determined on a FARGO apparatus and are uncorrected. Optical rotation values were measured with a JASCO P-1010 digital polarimeter at 25 °C. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrometer. The NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively, in $CDCl_3$ or C_5D_5N, using TMS as an internal standard. ESI-MS and HR-ESI-MS data were recorded on a BRUKER APEX II mass spectrometer. Column chromatography was performed using silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and spots were visualized by spraying with 10% H_2SO_4 solution, followed by heating. HPLC was performed by using a system comprised of a HITACHI L-7100 pump, a HITACHI photo diode array detector L-7455 and a RHEODYNE 7725 injection port. A semi-preparative column (Hibar 250–25 mm, LiChrospher Si 60, 5 μm) was used for HPLC.

Animal Material. Specimen of the gorgonian coral *R. antipathies* was collected in May 2004, off the southern coast of Taiwan. A voucher specimen was deposited in the National Museum of Marine Biology and Aquarium (NMMBA), Taiwan.

Extraction and Isolation. A freeze-dried and minced sample of *R. antipathies* (wet weight 402 g, dry weight 144 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1:1) at room temperature. The residue was partitioned between hexane and 9:1 MeOH–H_2O. The MeOH–H_2O layer was diluted to 1:1 MeOH–H_2O and partitioned against CH_2Cl_2. The CH_2Cl_2 layer was separated on silica gel and eluted using hexane/EtOAc (stepwise, 0–100% EtOAc) to yield four fractions A–D. Fraction A was separated on silica gel and eluted using CH_2Cl_2/acetone (stepwise, 20:1–1:1) to yield fractions A1–A14. Fractions A13 and A14 were combined and purified by normal-phase HPLC, using mixtures of hexane and acetone as a mobile phase to afford caryophyllanes **2** (4:1) and **1** (4:1–3:1).

Rumphellatin B (1). White powder (2.5 mg); mp 63–64 °C; $[\alpha]_D^{25}$ −23 (*c* 0.13, $CHCl_3$); IR (neat) ν_{max} 3410 cm^{-1}; ^{1}H ($CDCl_3$ or C_5D_5N, 400 MHz) and ^{13}C ($CDCl_3$ or C_5D_5N, 100 MHz) NMR data, see Table 1; ESI-MS *m*/*z* 297 $(M + Na)^+$, 299 $(M + 2 + Na)^+$; HR-ESI-MS *m*/*z* 297.1233 (calcd for $C_{14}H_{23}ClO_3$ + Na, 297.1233).

Rumphellatin C (2). White powder (1.8 mg); mp 94–95 °C; $[\alpha]_D^{25}$ −16 (*c* 0.09, $CHCl_3$); IR (neat) ν_{max} 3435 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 2; ESI-MS *m*/*z* 297 $(M + Na)^+$, 299 $(M + 2 + Na)^+$; HR-ESI-MS *m*/*z* 297.1233 (calcd for $C_{14}H_{23}ClO_3$ + Na, 297.1233).

Published on the web December 13, 2007; doi:10.1246/bcsj.80.2395

Table 1. ^{1}H and ^{13}C NMR Data and HMBC Correlations for Norsesquiterpenoid **1**

Position	^{1}H[a)]	^{1}H[b)]	^{13}C[c)]	^{13}C[d)]	HMBC (H → C)[e)]
1	1.50 dd (10.4, 10.4)[f)]	1.77 dd (10.4, 10.4)	46.1	45.4 (d)[g)]	C-3, 8, 9, 11, 13, 14
2α	1.84 m	2.11 m	36.9	37.6 (t)	C-1, 3, 4, 9, 11
β	2.04 m	2.28 dd (14.0, 2.0)			
3	4.31 dd (12.0, 2.4)	4.80 m[i)]	73.0	72.9 (d)	C-4, 12
4			76.9	76.2 (s)	
5	4.42 dd (9.6, 3.2)	4.83 m[i)]	83.1	84.5 (d)	C-3, 4, 8, 12
6α	2.33 m	2.44 m	25.0	25.3 (t)	C-4, 5
β	2.02 m	2.38 m			
7α	2.38 m	2.20 dd (10.4, 10.4)	32.4	32.1 (t)	C-5, 6, 8, 9
β	1.87 m	2.51 m			
8			108.8	108.9 (s)	
9	2.22 ddd (10.4, 10.4, 8.0)	2.64 ddd (10.4, 10.4, 8.0)	48.0	48.6 (d)	C-1, 2, 8, 10, 11
10α	1.65 dd (10.4, 8.0)	1.75 dd (10.4, 8.0)	35.5	35.7 (t)	C-1, 8, 9, 11, 13, 14
β	1.38 dd (10.4, 10.4)	1.54 dd (10.4, 10.4)			
11			35.9	35.2 (s)	
12	1.45 s	1.84 s	22.0	21.8 (q)	C-3, 4, 5
13	1.05 s	0.98 s	29.6	28.8 (q)	C-1, 10, 11, 14
14	1.01 s	0.93 s	20.7	20.1 (q)	C-1, 10, 11, 13
OH-4	n.o.[h)]	6.44 s			C-4, 5, 12
OH-8	n.o.	7.66 s			C-7, 8, 9

a) Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra recorded at 400 MHz in C_5D_5N at 25 °C. c) Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. d) Spectra recorded at 100 MHz in C_5D_5N at 25 °C. e) The ^{1}H–^{13}C long-range correlations cited in this table were by the interpretation of HMBC experiments of **1** measured in $CDCl_3$ and C_5D_5N. f) *J* values (in hertz) in parentheses. The values are downfield in parts per million from TMS. g) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. h) n.o. = not observed. i) Signals overlapping.

Table 2. ^{1}H and ^{13}C NMR Data and HMBC Correlations for Norsesquiterpenoid **2**

Position	^{1}H[a)]	^{13}C[b)]	HMBC (H → C)
1	2.06 dd (10.4, 10.4)[c)]	40.6 (d)[d)]	C-2, 3, 8, 11, 13, 14
2α/β	1.87 m; 2.12 m	32.7 (t)	C-1, 3, 4, 9, 11
3	4.08 d (4.8)	67.1 (d)	C-1, 2, 4, 5
4		75.8 (s)	
5	4.33 br t (8.8)	83.7 (d)	C-3, 4, 8
6α/β	2.87 m; 2.14 m	24.4 (t)	n.o.[e)]
7α/β	1.91 m; 2.51 dd (12.8, 6.0)	33.4 (t)	C-5, 8
8		109.3 (s)	
9	2.25 ddd (10.4, 10.4, 8.0)	47.7 (d)	C-8
10α/β	1.70 dd (10.4, 8.0); 1.44 dd (10.4, 10.4)	35.7 (t)	C-1, 8, 9, 11, 13, 14
11		35.1 (s)	
12	1.32 s	26.4 (q)	C-3, 4, 5
13	1.06 s	29.1 (q)	C-1, 10, 11, 14
14	1.03 s	20.7 (q)	C-1, 10, 11, 13

a) Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. c) *J* values (in hertz) in parentheses. The values are downfield in parts per million from TMS. d) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. e) n.o. = not observed.

Antimicrobial Assays. Natural products **1** and **2** were assayed for their antibacterial activity against the Gram-positive bacterium *Staphylococcus aureus*. The standard agar diffusion assay were carried out according to the procedure described previously.[15]

Results and Discussion

Rumphellatin B (**1**) was isolated as a white powder. The molecular formula for **1** was determined to be $C_{14}H_{23}ClO_3$ (three degrees of unsaturation) by analysis of ^{1}H and ^{13}C NMR data (Table 1) in conjunction with DEPT results, and this conclusion was further supported by HR-ESI-MS ($C_{14}H_{23}ClO_3$ + Na: found, 297.1233; calcd, 297.1233). Comparison of the ^{1}H NMR and DEPT data with the molecular formula indicated that there must be two exchangeable protons, requiring the presence of two hydroxy groups, and this deduction was supported by a broad absorption in the IR spectrum at 3410 cm^{-1}. From the ^{13}C NMR data of **1**, no olefinic carbon and carbonyl groups were observed. Thus, rumphellatin B

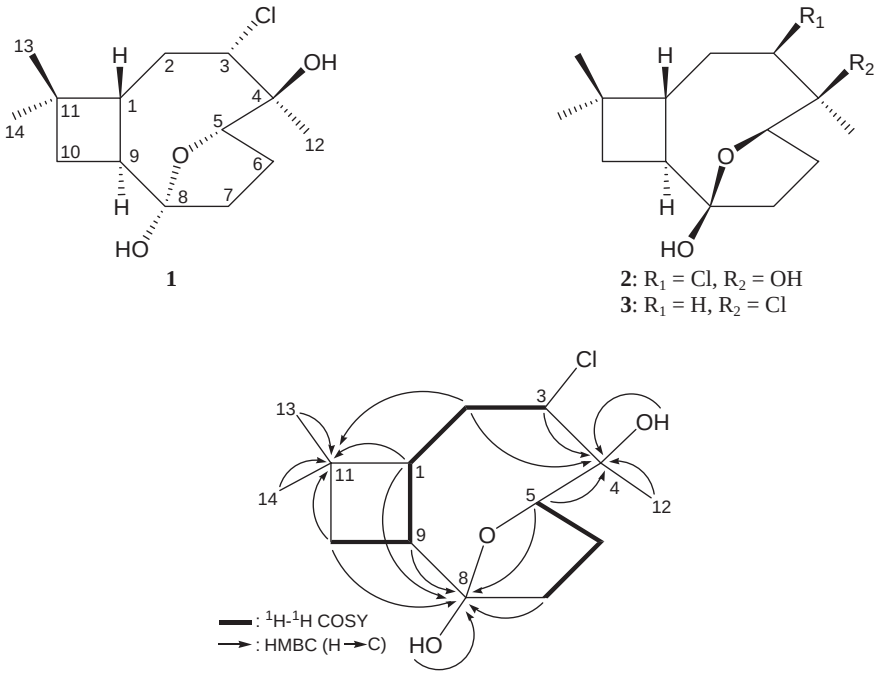

Fig. 1. The ^{1}H–^{1}H COSY and selective HMBC correlations (protons and the quaternary carbons) of **1** and **2**.

(**1**) must be tricyclic. In the ^{13}C NMR spectrum of **1** (Table 1), the signals for a hemiketal (δ 108.8, s, C-8), that is, three downfield carbons appeared between δ 70–85 ppm, including two methines (δ 83.1, d; 73.0, d; C-3 + C-5) and an quaternary carbon (δ 76.9, s, C-4), and ten additional aliphatic sp^3 carbon signals (a quaternary carbon, two methines, four methylenes, and three methyl groups), were observed. The ^{1}H NMR spectrum showed that all three methyl groups are isolated (δ 1.45, 3H, s, H_3-12; 1.05, 3H, s, H_3-13; 1.01, 3H, s, H_3-14). In addition, four pairs of aliphatic methylene protons (δ 1.84, 1H, m, H-2α; 2.04, 1H, m, H-2β; 2.33, 1H, m, H-6α; 2.02, 1H, m, H-6β; 2.38, 1H, m, H-7α; 1.87, 1H, m, H-7β; 1.65, 1H, dd, J = 10.4, 8.0 Hz, H-10α; 1.38, 1H, dd, J = 10.4, 10.4 Hz, H-10β), two aliphatic methine protons (δ 1.50, 1H, dd, J = 10.4, 10.4 Hz, H-1; 2.22, 1H, ddd, J = 10.4, 10.4, 8.0 Hz, H-9), and two downfield protons (δ 4.42, 1H, dd, J = 9.6, 3.2 Hz, H-5; 4.31, 1H, dd, J = 12.0, 2.4 Hz; H-3) were observed in the ^{1}H NMR spectrum of **1**.

The gross structure of **1** was determined by 2D NMR studies, including ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. From the ^{1}H NMR coupling information in the ^{1}H–^{1}H COSY spectrum of **1**, it was possible to identify the C-1/2/3, C-5/6/7, C-9/1, and C-9/10 units (Fig. 1). These data, together with the HMBC correlations observed between H-1/C-3,8,9; H-2/C-1,3,4,9; H-3/C-4; H-5/C-3,4,8; H-6/C-4,5; H-7/C-5,6,8,9; and H-9/C-1,2,8 (Table 1 and Fig. 1), established the connectivity from C-1 to C-9 within the nine-membered ring. The presence of a methyl group attached at C-4 was confirmed by the HMBC correlations between H_3-12/C-3,4,5. A cyclobutane ring, which is fused to the nine-membered ring at C-1 and C-9, was elucidated by analyzing the key HMBC correlations between H-1/C-11,13,14; H-2/C-11; H-9/C-10,11; and H-10/C-1,8,9,11,13,14. The cyclic ether ring between C-5 and C-8 was established by analyzing the HMBC correlation between the proton of C-5 oxymethine (δ_H 4.42) and the C-8 quaternary carbon (δ_C 108.8). From above findings, the major molecular framework of **1** could be established. However, the signals for the hydroxy protons were not observed in the ^{1}H NMR spectrum of **1**, which was measured in $CDCl_3$, and no HMBC correlation was recorded for these hydroxy protons. Therefore, the positions for the hydroxy groups and the chlorine atom in **1** cannot be fully determined by using this method. Thus, to obtain the complete spectra, the 1D and 2D NMR experiments of **1** were measured at 25 °C in C_5D_5N (Table 1). Fortunately, it was found that the signals for the hydroxy protons could be observed and assigned with the assistance of 2D NMR data. It was concluded that the hydroxy groups should be positioned at the quaternary carbons, such as C-4 and C-8, based on the key HMBC correlations between OH-4/C-4,5,12, and OH-8/C-7,8,9. This was further supported by the fact that no ^{1}H–^{1}H COSY correlation was found between the hydroxy protons and any protons. Thus, the remaining chlorine atom should be positioned at C-3 by the key ^{1}H–^{1}H COSY correlations and characteristic NMR signals analysis.

The stereochemistry of **1** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 2) and by the vicinal ^{1}H–^{1}H coupling constants. The *trans* geometry of H-9 (δ 2.22, ddd, J = 10.4, 10.4, 8.0 Hz) and H-1 (δ 1.50, dd, J = 10.4, 10.4 Hz) is indicated by a 10.4 Hz coupling constant between these two ring juncture protons, and H-9 and H-1 were assigned as α- and β-oriented protons, respectively, in the structure of **1**. In the NOESY experiment of **1**, H-1 exhibited strong NOE correlations with H-3 (δ 4.31), H-2β (δ 2.04), H-6β (δ 2.02), H-7β (δ 1.87), and H_3-13 (δ 1.05), but not with H-9 (δ 2.22), indicating that these protons (H-1, H-3, H-2β, H-6β, H-7β, and H_3-13) are located on the same face. Thus, they were assigned as β protons, and H-9 was assigned to be α-oriented. Furthermore, H_3-12 (δ 1.45) exhibited correlation with H-2α (δ 1.84) and H-5 (δ 4.42), but not with H-3 and H-6β, and H-3 showed a strong NOE correlation with

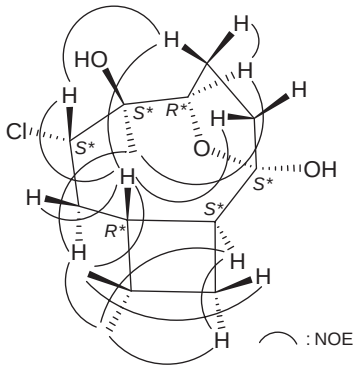

Fig. 2. Selective NOESY correlations of **1**.

H-6β, along with the correlation observed between H-5 and H-6α (δ 2.33). Thus, Me-12 and H-5 are α-oriented in **1**. From molecular models, when the proton H-5 was positioned on the α-orientation of C-5, the cyclic ether ring between C-5 and C-8 should be attached in the opposite direction of H-5, and the relative configurations of C-5 and C-8 were assigned as R^* and S^* form, respectively. Based on above observations, the structure of **1** was elucidated unambiguously. The relative configurations of all chiral centers of **1** were assigned as 1R^*, 3S^*, 4S^*, 5R^*, 8S^*, and 9S^*.

Our present study also led to the isolation of a new norsesquiterpenoid, rumphellatin C (**2**). Compound **2** has the same molecular formula as that of **1**, $C_{14}H_{23}ClO_3$, as determined by HR-ESI-MS [$(M + Na)^+$, m/z calcd. 297.1233; found 297.1233], with three degrees of unsaturation, indicating compounds **2** and **1** are isomers. From detailed spectral data analysis, particularly with 1D, 2D NMR (Table 2), MS, and IR spectra, natural product **2** was found to possess the same substituents (a cyclic ether ring, a hemiketal, a chloride atom, and two hydroxy groups) and planar structure as those of **1** by 2D NMR (^{1}H–^{1}H COSY, HMQC, and HMBC) experiments (Fig. 1 and Table 2).

The relative stereochemistry of **2** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 3). Like as those of **1**, H-9 (δ 2.25) and H-1 (δ 2.06) were assigned as α and β protons, respectively, because there was no NOE interaction observed between these two key protons and because the coupling constant between these two ring juncture protons was 10.4 Hz. In the NOESY experiment of **2**, H-9 had strong NOE correlations to H-3 (δ 4.08), H-6α (δ 2.87), H-7α (δ 1.91), and H-10α (δ 1.70), suggesting that these protons (H-3, H-6α, H-7α, H-9, and H-10α) are located on the same face; thus, they were assigned as α protons. Furthermore, H-5 exhibited strong NOE correlations to H_3-12 and H-6β, but not with H-1. In addition, H_3-12 showed correlations with H-3. From molecular models, H_3-12 was found to be reasonably close to H-3 and H-5 when H_3-12 and H-5 were placed in the same direction and assigned as α and β protons, respectively. The cyclic ether ring should be positioned in the opposite direction of H-5 in the nine-membered ring. Furthermore, carbons C-5 and C-8 carbons should be assigned as S^* and R^* form, respectively, in **2**. Based on above findings, the structure, including the relative configuration of **2** was established, and the configurations of all chiral centers of **2** were assigned as 1R^*, 3R^*, 4S^*, 5S^*, 8R^*, and 9S^*.

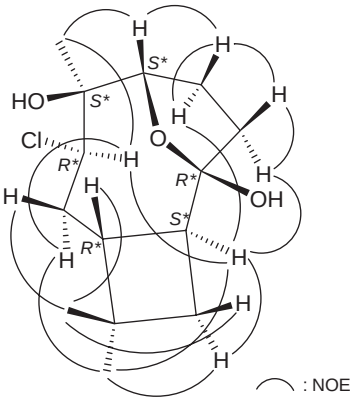

Fig. 3. Selective NOESY correlations of **2**.

To the best of our knowledge, the chlorinated caryophyllane-type natural products have only been found in *R. antipathies*.[12] The five-membered cyclic ether ring (furan ring) between C-5/C-8 and the C-8 hemiketal groups in natural products **1** and **2** have also been rarely found.[12]

In the tests for biological activity, compound **1** exhibited activity in standard agar disk diffusion assay against the Gram-positive bacterium *Staphylococcus aureus*, causing a 5 mm zone inhibition (200 μg mL^{-1}). Compound **2** did not show activity toward *S. aureus*.

The authors would like to thank Dr. Tung-Yung Fan and Jimmy Kuo, the National Museum of Marine Biology and Aquarium (NMMBA), Taiwan, for their contribution to identify the marine organisms and antibacterial activity testing, respectively. This research work was supported by grants from the National Science Council (NSC 95-2320-B-291-001-MY2) and by the intramural funding from the National Museum of Marine Biology and Museum, Taiwan, ROC, awarded to P.-J. Sung.

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