Rumphellolides A—F, Six New Caryophyllane-Related Derivatives from the Formosan Gorgonian Coral *Rumphella antipathies*

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Six new caryophyllane-related natural products, including two carboxylated sesquiterpenoids, rumphellolides A (1) and B (2), and four norsesquiterpene alcohols, rumphellolides C—F (3—6), were isolated from the Formosan gorgonian coral *Rumphella antipathies*. The structures of the above new natural products were established on the basis of extensive spectral data analysis. Rumphellolides A (1), D (4), E (5), and F (6) showed weak antibacterial activity.

Key words rumphellolide; caryophyllane; sesquiterpenoid; gorgonian; *Rumphella*; antibacterial activity

In our screening for bioactive substances from the Formosan octocorals, we reported a series of interesting terpenoid and steroid metabolites from the octocorals *Briareum* sp.,[1] *Briareum excavatum*,[2—5] *Junceella fragilis*,[6—12] *Junceella juncea*,[8,13] and *Alcyonium* sp.[14] In continuation of our study of bioactive substances from Formosan marine invertebrates, from the gorgonian coral, *Rumphella antipathies* (phylum Cnidaria, order Gorgonacea, suborder Holaxonia, family Gorgoniidae),[15] collected from Taiwanese waters, we have isolated six new natural products including two carboxylated sesquiterpenoids, rumphellolides A (**1**) and B (**2**), and four norsesquiterpenoid alcohols, rumphellolides C—F (**3**—**6**) (Fig. 1). In previous studies, there was only one report focused on the chemical components of gorgonian coral *Rumphella aggregata*.[16] Organic extracts from the gorgonian belonging to the *Rumphella* genus in ecology and medical use, also were reported.[17,18] The caryophyllane-type natural products also were rarely found in marine organisms.[19—21] In this paper, we describe the isolation, structure characterization, and biological activity of the new natural products **1**—**6**. The structures of **1**—**6** were elucidated on the basis of extensive spectral data analysis. Antibacterial activity of these compounds toward the Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus* and the Gram-positive bacterium *Staphylococcus aureus* is also reported.

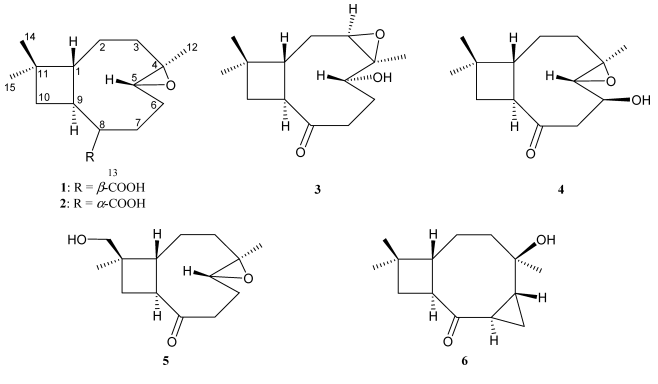

Fig. 1. Structures of Compounds **1**—**6**

Results and Discussion

The minced tissues of *R. antipathies* were successively extracted with a mixture of MeOH and CH_2Cl_2 (1 : 1). The residue was further partitioned between *n*-hexane and 9 : 1 MeOH–H_2O; the MeOH–H_2O phase 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 purified by HPLC to afford rumphellolides A—F (**1**—**6**).

Rumphellolide A (**1**) was obtained as a white powder. The HR-ESI-MS data recorded at *m/z* 275.1625 established the molecular formula of **1** as $C_{15}H_{24}O_3$ (Calcd for $C_{15}H_{24}O_3$+Na, 275.1623). Thus four degrees of unsaturation were determined for **1**. The IR spectrum showed bands at 3400—2400 (br.) and 1706 cm^{-1}, consistent with the presence of carboxylic acid in **1**. In the ^{13}C-NMR spectrum of **1** (Table 1), a carbonyl resonance appeared at δ 180.4 (s), supporting the presence of a carboxylic acid group. Thus the ^{13}C-NMR data accounted for one degree of unsaturation and required **1** to be tricyclic. The ^{1}H-NMR spectrum also showed the presence of three methyl groups (Table 2), including two methyls (δ 0.98, 3H, s, H_3-15; 0.96, 3H, s, H_3-14) attached to a quaternary carbon and a methyl (δ 1.29, 3H, s, H_3-12) attached to an oxygenated quaternary carbon. A trisubstituted epoxide group was confirmed by the signals of an oxymethine (δ_H 2.93, 1H, dd, J=11.2, 4.0 Hz, H-5; δ_C 64.7, d, C-5) and an oxygen-bearing quaternary carbon (δ_C 60.1, s, C-4) for the characteristic signals of an epoxide group. In addition, five pairs of methylene protons (δ 1.72, 1H, m, H-2α; 1.46, 1H, m, H-2β; 2.09, 1H, m, H-3α; 1.02, 1H, m, H-3β; 1.21, 1H, m, H-6α; 2.26, 1H, m, H-6β; 2.11, 1H, m, H-7α; 1.79, 1H, m, H-7β; 1.51, 1H, dd, J=10.4, 8.0 Hz, H-10α; 1.42, 1H, d, J=10.4 Hz, H-10β) and three aliphatic methine protons (δ 1.91, 1H, br t, J=9.6 Hz, H-1; 2.56, 1H, dt, J=6.4, 6.0 Hz, H-8; 2.51, 1H, m, H-9) were observed in the ^{1}H-NMR spectrum of **1**.

The gross structure of **1** and all of the ^{1}H- and ^{13}C-NMR

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Table 1. ^{13}C-NMR Data for Sesquiterpenoids **1**—**6**[a]

Position	1	2	3	4	5	6
1	45.7 (d)[b]	45.6 (d)	45.7 (d)	51.6 (d)	45.3 (d)	58.2 (d)
2	27.2 (t)	27.2 (t)	29.0 (d)	26.4 (t)	27.1 (t)	26.5 (t)
3	38.3 (t)	38.3 (t)	64.3 (d)	39.0 (t)	38.8 (t)	46.9 (t)
4	60.1 (s)	60.0 (s)	64.7 (s)	58.5 (s)	59.0 (s)	73.7 (s)
5	64.7 (d)	64.7 (d)	72.2 (d)	67.0 (d)	61.6 (d)	33.8 (d)
6	28.1 (t)	28.1 (t)	37.5 (t)	69.0 (d)	24.6 (t)	5.8 (t)
7	21.1 (t)	21.2 (t)	30.1 (t)	46.9 (t)	37.9 (t)	25.3 (d)
8	45.9 (d)	45.7 (d)	212.7 (s)	213.1 (s)	214.5 (s)	209.3 (s)
9	42.5 (d)	42.6 (d)	49.0 (d)	52.6 (d)	51.6 (d)	52.3 (d)
10	35.3 (t)	35.4 (t)	33.2 (t)	35.1 (t)	30.0 (t)	32.0 (t)
11	34.5 (s)	34.5 (s)	34.8 (s)	34.6 (s)	38.9 (s)	36.3 (s)
12	17.1 (q)	17.1 (q)	16.4 (q)	17.2 (q)	16.3 (q)	20.2 (q)
13	180.4 (s)	178.6 (s)				
14	29.8 (q)	29.8 (q)	29.4 (q)	29.3 (q)	70.1 (t)	29.1 (q)
15	21.2 (q)	21.2 (q)	22.2 (q)	22.1 (q)	17.7 (q)	21.0 (q)

a) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. *b*) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. The values are downfield in ppm from TMS.

Table 2. ^{1}H-NMR Chemical Shifts for Sesquiterpenoids **1**—**6**[a]

Position	1	2	3	4	5	6
1	1.91 br t (9.6)[b]	1.92 br t (9.6)	2.17 m	1.97 br t (9.6)	2.28 m	1.81 m
2α	1.72 m	1.72 m	2.22 m	1.53 m	1.58 m	1.85 m
β	1.46 m	1.46 m	2.01 m	1.69 m	1.68 m	1.52 m
3α	2.09 m	2.10 m	3.12 dd (4.8, 4.8)	2.14 dt (12.8, 3.6)	2.15 dt (13.2, 4.0)	2.00 ddd (13.6, 6.4, 1.6)
β	1.02 m	1.03 m		1.00 dt (12.8, 4.8)	0.99 td (13.2, 4.8)	1.67 br t (13.6)
5	2.93 dd (11.2, 4.0)	2.93 dd (11.2, 4.0)	3.51 dd (10.4, 4.4)	2.68 d (8.0)	2.71 dd (10.0, 4.8)	1.01 m
6α	1.21 m	1.21 m	2.40 m	3.74 ddd (8.0, 4.8, 4.8)	1.48 m	0.82 ddd (7.2, 6.8, 5.6)
β	2.26 m	2.25 m	2.50 m		2.41 m	1.37 ddd (8.8, 6.4, 5.6)
7α	2.11 m	2.12 m	2.04 m	2.85 dd (12.0, 4.8)	2.56 d (5.6)	1.73 m
β	1.79 m	1.79 m	1.87 m	2.72 dd (12.0, 4.8)	2.58 dd (5.6, 1.6)	
8	2.56 dt (6.4, 6.0)	2.58 m				
9	2.51 m	2.52 m	2.95 td (9.6, 7.6)	3.05 td (9.6, 8.0)	3.09 td (9.2, 8.4)	3.15 td (10.4, 7.6)
10α	1.51 dd (10.4, 8.0)	1.52 dd (10.8, 8.0)	2.00 m	1.68 m	1.58 dd (11.2, 7.6)	1.48 dd (10.8, 7.6)
β	1.42 d (10.4)	1.41 d (10.8)	1.57 m	2.09 br t (10.0)	2.24 dd (11.2, 9.6)	1.91 br t (10.8)
12	1.29 s	1.30 s	1.31 s	1.30 s	1.32 s	0.98 s
14	0.96 s	0.97 s	1.02 s	1.04 s	3.39 s	0.98 s
15	0.98 s	0.98 s	1.08 s	1.04 s	1.08 s	1.04 s

a) Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. *b*) *J* values (in Hz) in parentheses. The values are downfield in ppm from TMS.

data associated with the molecule were determined by 2D-NMR studies, including ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. The ^{1}H-NMR coupling information in the ^{1}H–^{1}H COSY spectrum of **1** enabled identification of the C-1/C-2/C-3, C-5/C-6/C-7/C-8/C-9, and C-1/C-9 units (Fig. 2). These data, together with the HMBC correlations between H-1/C-3, C-8, C-9; H_2-2/C-1, C-3, C-4, C-9; H_2-3/C-1, C-2, C-4, C-5; H-5/C-6; H_2-7/C-5, C-6, C-8; and H-8/C-6, C-7, C-9 (Fig. 2, Table 3), established the connectivity from C-1 to C-9 within the nine-membered ring. The C-12 methyl attached at C-4 was confirmed by the HMBC correlations between H_3-12/C-3, C-4, C-5 and H-3/C-12. The cyclobutane ring, which is fused to the nine-membered ring at C-1 and C-9, was elucidated by the ^{1}H–^{1}H COSY correlations between H-9 and H_2-10 and by the key HMBC correlations between H-1/C-11; H-2/C-11; H-8/C-10; and H_2-10/C-1, C-8, C-9. The carboxylic acid group positioned at C-8 was confirmed by the HMBC correlation between H-8 (δ_H 2.56) and the acid

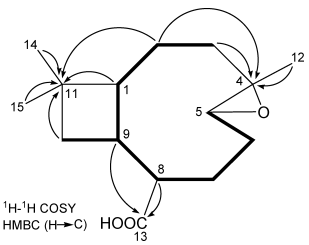

Fig. 2. ^{1}H–^{1}H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **1** and **2**

carbonyl (δ_C 180.4, s, C-13). These data, together with the HMBC correlations between H-1/C-14, C-15; H_2-10/C-11, C-14, C-15; H-9/C-13; H_3-14/C-1, C-10, C-11, C-15; and H_3-15/C-1, C-10, C-11, C-14, unambiguously established the planar structure of **1**.

The relative configurations of five chiral centers at C-1, C-

Table 3. The HMBC Correlations for Sesquiterpenoids **1**—**6** (H→C)

Position	1	2	3	4	5	6
H-1	C-3, 8, 9, 11, 14, 15	C-3, 8, 9, 11, 14, 15	C-3, 8, 9, 14, 15	C-9, 11, 14, 15	C-3, 9, 11, 14, 15	C-3, 9, 11, 14, 15
H-2	C-1, 3, 4, 9, 11	C-1, 3, 4, 9, 11	C-1, 3, 9, 11	C-1, 3, 4	C-1, 3	C-1, 3, 4, 9, 11
H-3	C-1, 2, 4, 5, 12	C-1, 2, 4, 5, 12	C-1, 2, 4, 12	C-1, 2, 4, 12	C-2, 4, 12	C-1, 2, 4, 5, 12
H-5	C-6	C-6	C-3, 4, 6, 7, 12	C-3, 4	C-6	C-3, 4, 6, 8, 12
H-6	n.o.[a)]	n.o.[a)]	C-4, 5, 7, 8	C-5, 8	C-4, 5	C-4, 5, 7, 8
H-7	C-5, 6, 8	C-5, 6, 8	C-5, 6, 8	C-5, 6, 8	C-5, 6, 8, 9	C-4, 5, 8
H-8	C-6, 7, 9, 10, 13	C-6, 7, 9, 10, 13				
H-9	C-13	C-13	C-1, 2, 8, 10	C-1, 2, 10	C-1, 2, 10	C-1, 7
H-10	C-1, 8, 9, 11, 14, 15	C-1, 8, 9, 11, 14, 15	C-1, 8, 9, 11, 14, 15	C-1, 8, 9, 11, 14, 15	C-9, 11, 14, 15	C-1, 8, 9, 11, 14, 15
H-12	C-3, 4, 5	C-3, 4, 5	C-3, 4, 5	C-3, 4, 5	C-3, 4, 5	C-3, 4, 5
H-14	C-1, 10, 11, 15	C-1, 10, 11, 15	C-1, 10, 11, 15	C-1, 10, 11, 15	C-1, 10, 11, 15	C-1, 10, 11, 15
H-15	C-1, 10, 11, 14	C-1, 10, 11, 14	C-1, 10, 11, 14	C-1, 10, 11, 14	C-1, 10, 11, 14	C-1, 10, 11, 14

a) n.o.=not observed.

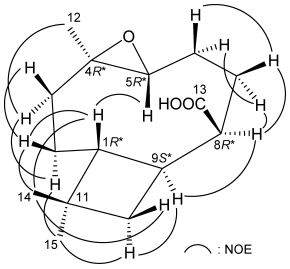

Fig. 3. Selective NOE Correlations of **1**

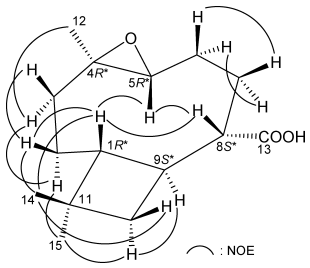

Fig. 4. Selective NOE Correlations of **2**

4, C-5, C-8, and C-9 in **1** were elucidated by the following NOE analysis, as shown in Fig. 3. It was found that H-1 showed strong NOE correlations with H-5 and H_3-14. Thus assuming the β-orientation of H-1, H-5 and Me-14 should be positioned on the β-face as well. One of the methylene protons at C-2 (δ 1.46) exhibited NOE correlations with H-1 and was assigned as H-2β, while the other (δ 1.72) was denoted as H-2α. The NOE correlation observed between H-2α and H_3-15; H-2α and one proton of C-3 methylene (δ 2.09, H-3α); H_3-15 and one proton of C-10 methylene (δ 1.51, H-10α); and H-10α and H-9, reflected the α-orientation of these protons. Also, H_3-12 was found to interact with H-3α but not with H-1 and H-5, revealing the *trans* geometry of the trisubstituted epoxide. Furthermore, H-8 showed an NOE correlation with H-9, but not with H-1, suggesting that the carboxylic acid group attaching at C-8 was positioned on the β face in the nine-membered ring. Thus by the above findings, the structure of **1** was established and the configurations of all chiral centers of **1** were assigned as 1*R**,4*R**,5*R**,8*R**,9*S**.

Rumphellolide B (**2**) had the same molecular formula as that of **1**, $C_{15}H_{24}O_3$, as determined by HR-ESI-MS, with four degrees of unsaturation. By detailed analysis, the spectral data (IR, MS, 1D-, and 2D-NMR) of **2** were very similar to those of **1** (Tables 1—3). However, the physical state (a colorless oil) and optical rotation value ($[\alpha]_D^{25}$ −3° (c=0.05, $CHCl_3$)) of **2** were substantially different from those of **1** (a white powder, mp 145—146 °C, $[\alpha]_D^{25}$ −29° (c=0.27, $CHCl_3$)), indicating that these two compounds are isomers. Comparison of the ^{13}C-NMR chemical shift of C-13 of **2** (δ 178.6, s) with that of **1** (δ 180.4, s) showed that the relative stereochemistry of C-8 in **2** is of *S** form. This observation was further supported by the NOE correlations observed among H-1, H-5, and H-8, in the NOESY experiment of **2** (Fig. 4). It has to be noted that sesquiterpenoids **1** and **2** are the first caryophyllane-type natural products possessing carboxylic acid groups.

The new caryophyllane norsesquiterpenoid, rumphellolide C (**3**), was isolated as a colorless oil and has a molecular formula $C_{14}H_{22}O_3$, as determined by HR-ESI-MS (*m*/*z* Calcd: 261.1467, Found: 261.1468, $[M+Na]^+$), indicating four degrees of unsaturation. The presence of hydroxyl and ketone groups in **3** was evidenced by IR absorption at 3442 and 1696 cm^{-1}. The ^{13}C-NMR data (Table 1) showed that **3** had a ketone carbonyl group appearing at δ 212.7 (s, C-8), and this compound must therefore be tricyclic to account for the remaining degrees of unsaturation. A trisubstituted epoxide containing a methyl substituent was deduced from the signals of an oxymethine (δ_H 3.12, 1H, dd, J=4.8, 4.8 Hz, H-3; δ_C 64.3, d, C-3), a quaternary oxygen-bearing carbon (δ_C 64.7, s, C-4), and a methyl singlet resonating at δ 1.31 (3H, s, H_3-12) (Table 2). Moreover, protons signals for two tertiary methyls, four methylenes, and three methines including an oxygenated one were further assigned by the assistance of HMQC spectrum. From the ^{1}H–^{1}H COSY and HMBC correlations (Fig. 5, Table 3), the epoxide group positioned at C-3/C-4 and the hydroxyl group positioned at C-5 were established. Furthermore, the C-8 ketone was confirmed by the key HMBC correlations between H-1 (δ 2.17), H_2-6 (δ 2.40, 2.50), H_2-7 (δ 2.04, 1.87), H-9 (δ 2.95), H_2-10 (δ 2.00, 1.57), and the C-8 ketone carbonyl carbon (δ 212.7, s).

The stereochemistry of **3** was elucidated by correlations observed in an NOESY experiment (Fig. 6). In the NOESY spectrum of **3**, H-1 gives NOE correlations to H-5 and H_3-14, but not with H-3 and H-9, indicating that H-1, H-5, and H_3-14 are situated on the same face of the structure and were

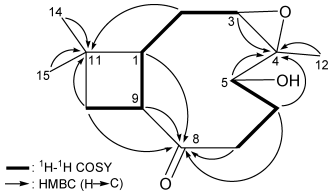

Fig. 5. ^{1}H–^{1}H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **3**

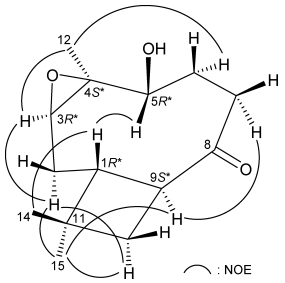

Fig. 6. Selective NOE Correlations of **3**

assigned as β protons since the H-9 proton is assigned as the α-substituent at C-9. H_3-12 was found to exhibit a strong NOE correlation with H-3. From consideration of molecular models, H_3-12 was found to be reasonably close to H-3, when C-12 was α-oriented in the epoxide ring. On the basis of the above observations, the structure of **3** was elucidated unambiguously. The relative configurations of all chiral centers of **3** were assigned as 1*R**,3*R**,4*S**,5*R**,9*S**.

Our present study has also led to the isolation of the new norsesquiterpenoid, rumphellolide D (**4**). Compound **4** has the same molecular formula as that of **3**, $C_{14}H_{22}O_3$, as determined by HR-ESI-MS, with four degrees of unsaturation, indicating compounds **3** and **4** are isomers. By detailed spectral data analysis, particularly with 1D- and 2D-NMR data and IR spectrum, compound **4** was found to possess the same substituents as those of **3** (a ketone, an epoxide, and a hydroxyl group). On the basis of ^{1}H–^{1}H COSY spectrum of **4** (Fig. 7), it was possible to establish the sequences of the protons attached to the carbon skeleton of **4**. In the HMBC experiment of **4** (Fig. 7, Table 3), the epoxide group was shown to be positioned at C-4/C-5 by the key HMBC correlations between H_2-3/C-4; H-5/C-3, C-4; and H-6/C-5. The hydroxyl group positioned at C-6 was further confirmed by the connectivity between the proton of an oxymethine (δ 3.74, H-6) and C-5 and C-8. The relative stereochemistry of **4** was deduced from an NOESY experiment (Fig. 8) and the configurations of five chiral centers including C-1, C-4, C-5, C-6, and C-9 were assigned as *R**,*R**,*R**,*S**,*S**.

Norsesquiterpenoid **5** (rumphellolide E) had the same molecular formula as those of **3** and **4**, $C_{14}H_{22}O_3$, as determined by HR-ESI-MS with three degrees of unsaturation, indicating that compounds **3**, **4**, and **5** are isomers. By detailed 1D- and 2D-NMR data analysis, compound **5** has the same substituents as those of **3** and **4**. On the basis of ^{1}H–^{1}H COSY spectrum of **5** (Fig. 9), it was possible to establish the sequences of the protons attached to the carbon skeleton of **5**. Furthermore, by comparison the ^{1}H-, ^{13}C-NMR, ^{1}H–^{1}H COSY, and HMBC spectral data of **5** with those **4**, it was revealed that the signals corresponding to the tertiary Me-14 in

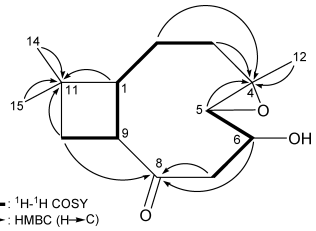

Fig. 7. ^{1}H–^{1}H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **4**

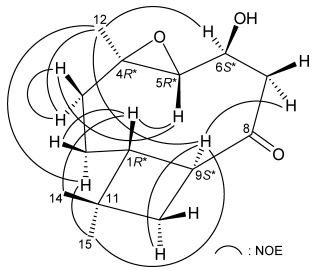

Fig. 8. Selective NOE Correlations of **4**

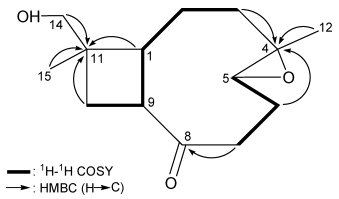

Fig. 9. ^{1}H–^{1}H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **5**

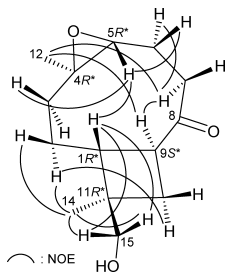

Fig. 10. Selective NOE Correlations of **5**

4 (δ_H 1.04, 3H, s, H_3-14; δ_C 29.3, q, C-14) disappeared and were replaced by a hydroxymethylene group in **5** (δ_H 3.39, 2H, s, H_2-14; δ_C 70.1, t, C-14) and the hydroxymethine in **4** (δ_H 3.74, 1H, ddd, J=8.0, 4.8, 4.8 Hz, H-6; δ_C 69.0, d, C-6) was replaced by an aliphatic methylene in **5** (δ_H 1.48, 1H, m, H-6α; 2.41, 1H, m, H-6β; δ_C 24.6, t, C-6). Furthermore, the proton signals of C-14 hydroxymethylene showed strong correlations with C-1, C-10, C-11, and C-15 in the HMBC experiment of **5** (Fig. 9, Table 3), confirming the 14-hydroxyl group in **5**. The relative stereochemistry of **5** was elucidated by an NOESY experiment (Fig. 10), and the results revealed that all the chiral centers of **5** were elucidated as 1*R**,4*R**,5*R**,9*S**,11*R**.

Rumphellolide F (**6**) was obtained as a white powder. The HR-ESI-MS data recorded at *m*/*z* 245.1516 established the molecular formula of **6** as $C_{14}H_{22}O_2$ (Calcd for $C_{14}H_{22}O_2$+Na, 245.1517). Thus four degrees of unsaturation

were determined for **6**. The IR spectrum showed bands at 3430 and 1702 cm^{-1}, consistent with the presence of hydroxyl and ketone groups in **6**. In the ^{13}C and DEPT spectra of **6** (Table 1), fourteen carbon signals, including three methyls (δ 29.1, 21.0, 20.2), four methylenes (δ 46.9, 32.0, 26.5, 5.8), four methines (δ 58.2, 52.3, 33.8, 25.3), a quaternary carbon (δ 36.3), an oxygen-bearing quaternary carbon (δ 73.7), and a ketone carbonyl (δ 209.3) appeared. Thus the ^{13}C-NMR data accounted for one degree of unsaturation and required **6** to be tricyclic. The ^{1}H-NMR spectrum of **6** (Table 2) also showed the presence of three methyl groups, including two methyls (δ 0.98, 3H, s, H_3-14; 1.04, 3H, s, H_3-15) attached to a tertiary carbon and a methyl (δ 0.98, 3H, s, H_3-12) attached to an oxygen-bearing quaternary carbon; four pairs of methylene protons (δ 1.85, 1H, m, H-2α; 1.52, 1H, m, H-2β; 2.00, 1H, ddd, J=13.6, 6.4, 1.6 Hz, H-3α; 1.67, 1H, br t, J=13.6 Hz, H-3β; 1.91, 1H, br t, J=10.8 Hz, H-10β; 1.48, 1H, dd, J=10.8, 7.6 Hz, H-10α; 1.37, 1H, ddd, J=8.8, 6.4, 5.6 Hz, H-6β; 0.82, 1H, ddd, J=7.2, 6.8, 5.6 Hz, H-6α); and four aliphatic methine protons (δ 1.81, 1H, m, H-1; 1.01, 1H, m, H-5; 1.73, 1H, m, H-7; 3.15, 1H, td, J=10.4, 7.6 Hz, H-9).

The gross structure of **6** was determined by 2D-NMR studies. From the ^{1}H–^{1}H COSY spectrum of **6**, it was possible to establish the separate spin system that maps out the proton sequences from H-1/H_2-2; H_2-2/H_2-3; H-5/H_2-6; H_2-6/H-7; H-5/H-7; and H-9/H-1 (Fig. 11). These data, together with the HMBC correlations between H-1/C-3, C-9; H_2-2/C-1, C-3, C-4, C-9; H_2-3/C-1, C-2, C-4, C-5; H-5/C-3, C-4, C-8; H-6/C-4, C-5, C-7, C-8; and H-9/C-1, C-7 (Fig. 11, Table 3), established the connectivity from C-1 to C-9. The cyclobutane ring, which is fused to the nine-membered ring at C-1 and C-9, was elucidated by the ^{1}H–^{1}H COSY correlations between H-9 and H_2-10 and by the key HMBC correlations between H-1/C-11, C-14, C-15; H_2-2/C-11; and H_2-10/C-1, C-8, C-9. The C-14 and C-15 methyls were positioned at C-11 from the HMBC correlations between H_3-14/C-1, C-10, C-11, C-15 and H_3-15/C-1, C-10, C-11, C-14. The cyclopropane ring, which is positioned between C-5 and C-7, was established by the ^{1}H–^{1}H COSY correlations among H-5, H_2-6, and H-7, and further supported by the key HMBC correlations between H-5 and a methylene carbon signal that appeared upfield (δ_C 5.8, t, C-6); and H_2-6/C-4, C-5, C-7, C-8. The ketone group positioned at C-8 was determined by the HMBC correlations between H-5, H_2-6, H-7, H_2-10 and the ketone carbonyl observed at δ 209.3 (s, C-8). The data, together with the HMBC correlations between H_3-12/C-3, C-4, and C-5, unambiguously established the planar structure of **6**.

The relative stereochemistry of **6** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 12). In the NOESY experiment of **6**, H-9 exhibited strong NOE correlations to H-7 and H_3-15, indicating that these protons (H-7, H-9, H_3-15) are located on the same face and are assigned as α protons. Furthermore, H-5 showed an NOE response with H-1, but not with H-7 and H_3-12, and H-7 exhibited correlation with H_3-12, supporting the β-orientation of H-5 and the α-orientation of H_3-12 in the nine-membered ring. Based on the above findings, the structure of **6** was established, and the configurations of all chiral centers were assigned as 1*R**,4*R**,5*R**,7*R**,9*S**. It was found that **6** has been

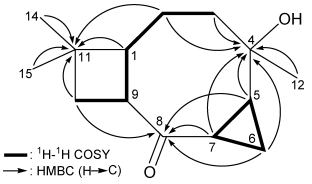

Fig. 11. ^{1}H–^{1}H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **6**

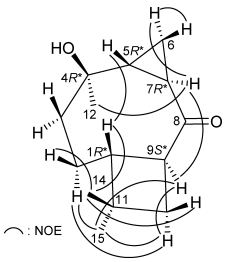

Fig. 12. Selective NOE Correlations of **6**

obtained previously by chemical methods.[22] However, to the best of our knowledge, rumphellatin F (**6**) is the first caryophyllane-type norsesquiterpenoid with a cyclopropane unit from nature.

In the biological activity testing, compounds **1** and **4** exhibited activity in standard agar disk diffusion assay against *Pseudomonas aeruginosa*, each causing a 10-mm zone inhibition (100 μg/ml). *Vibrio parahaemolyticus* was inhibited by **4** and **6**, the zone being 10- and 15-mm, respectively (100 μg/ml). Natural product **5** has been shown to exhibit antimicrobial activity toward *Escherichia coli* and *P. aeruginosa* at 200 μg/ml (inhibition zone 5-mm, respectively), and **6** was found to inhibit *Staphylococcus aureus* at 100 μg/ml (inhibition 15-mm).

Experimental

Melting points were determined on a FARGO apparatus and were 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, in $CDCl_3$, respectively. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ 7.26 ppm). ^{13}C-NMR spectra were referenced to the center peak of $CDCl_3$ at δ 77.1 ppm. ESI-MS and HR-ESI-MS data were recorded on a BRUKER APEX II mass spectrometer. Column chromatography was performed on 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 using a system comprising 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 Specimens of the gorgonian coral *R. antipathies* were collected off the southern coast of Taiwan in May 2004. This organism was identified by comparison with previous description.[15] The voucher specimen was deposited in the National Museum of Marine Biology and Aquarium (NMMBA).

Extraction and Isolation The freeze-dried and minced material 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 *n*-hexane and 9 : 1 MeOH–H_2O. The MeOH–H_2O phase 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 *n*-hexane/EtOAc (stepwise, 0—100% EtOAc) to yield four fractions A—D. Fraction A was separated on silica gel eluted using DCM/acetone (stepwise, 20 : 1—1 : 1) to

yield fractions A1—A14. Fraction A4 was purified by normal-phase HPLC, using the mixtures of *n*-hexane and acetone as a mobile phase to afford caryophyllanes **3** (1.5 mg, 6 : 1) and **4** (1.6 mg, 6 : 1—5 : 1). The fraction A14 was separated again on normal-phase HPLC (*n*-hexane–EtOAc) to give caryophyllanes **1** (2.9 mg, 2 : 1—3 : 2) and **2** (1.3 mg, 3 : 2). Fraction B was chromatographyed on silica gel eluted using DCM/acetone (stepwise, 20 : 1—1 : 1) to yield fractions B1—B12. Fraction B9 was purified by normal-phase HPLC, using the mixtures of *n*-hexane and acetone as a mobile phase to afford caryophyllanes **6** (1.6 mg, 6 : 1) and **5** (0.5 mg, 4 : 1).

Rumphellolide A (**1**): White powder; mp 145—146 °C; $[\alpha]_D^{25}$ −29° (c=0.27, $CHCl_3$); IR (neat) ν_{max} 3400—2400 (br), 1706 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Tables 1 and 2; ESI-MS m/z 275 $(M+Na)^+$; HR-ESI-MS m/z 275.1625 (Calcd for $C_{15}H_{24}O_3$+Na, 275.1623).

Rumphellolide B (**2**): Colorless oil; $[\alpha]_D^{25}$ −3° (c=0.05, $CHCl_3$); IR (neat) ν_{max} 3400—2400 (br), 1705 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Tables 1 and 2; ESI-MS m/z 275 $(M+Na)^+$; HR-ESI-MS m/z 275.1625 (Calcd for $C_{15}H_{24}O_3$+Na, 275.1623).

Rumphellolide C (**3**): Colorless oil; $[\alpha]_D^{25}$ −10° (c=0.14, $CHCl_3$); IR (neat) ν_{max} 3442, 1696 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Tables 1 and 2; ESI-MS m/z 261 $(M+Na)^+$; HR-ESI-MS m/z 261.1468 (Calcd for $C_{14}H_{22}O_3$+Na, 261.1467).

Rumphellolide D (**4**): Colorless oil; $[\alpha]_D^{25}$ −66° (c=0.17, $CHCl_3$); IR (neat) ν_{max} 3423, 1687 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESI-MS m/z 261 $(M+Na)^+$; HR-ESI-MS m/z 261.1468 (Calcd for $C_{14}H_{22}O_3$+Na, 261.1467).

Rumphellolide E (**5**): Colorless oil; $[\alpha]_D^{25}$ −60° (c=0.03, $CHCl_3$); IR (neat) ν_{max} 3439, 1690 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESI-MS m/z 261 $(M+Na)^+$; HR-ESI-MS m/z 261.1466 (Calcd for $C_{14}H_{22}O_3$+Na, 261.1467).

Rumphellolide F (**6**): White powder; $[\alpha]_D^{25}$ +15° (c=0.08, $CHCl_3$); IR (neat) ν_{max} 3430, 1702 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESI-MS m/z 245 $(M+Na)^+$; HR-ESI-MS m/z 245.1516 (Calcd for $C_{14}H_{22}O_2$+Na, 245.1517).

Antimicrobial Assays Natural products **1**—**6** were assayed for antibacterial activity against the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus* and the Gram-positive bacterium *Staphylococcus aureus*. The standard agar diffusion assay was carried out according to the procedure described previously.[23)]

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