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RUMPHELLOLIDE K, A NOVEL C-3/8 ETHER LINKAGE CARYOPHYLLANE FROM *RUMPHELLA ANTIPATHES*

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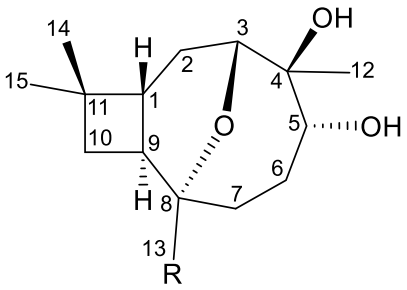
Abstract – Chemical examination of *Rumphella antipathes*, collected in the waters of Taiwan, led to the isolation of a novel caryophyllane sesquiterpenoid, rumphellolide K (**1**), with a C-3/8 ether linkage. The structure of **1** was established by spectroscopic analysis and this compound displayed inhibitory effects on the generation of superoxide anions and the release of elastase by human neutrophils.

The octocorals belonging to the genus *Rumphella* (family Gorgoniidae)[1] were found to be a rich source of caryophyllane sesquiterpenoids[2–6] and steroids,[7] and the compounds of these two types from *Rumphella* were found to exhibit antibacterial activity,[2,3] anti-inflammatory activity,[4,5] and cytotoxicity.[7] In our continuing studies of *R. antipathes*, a new caryophyllane sesquiterpenoid, rumphellolide K (**1**), was isolated (Chart 1). Herein, we described the isolation, structural characterization, and bioactivity of **1**.



1: R = Me, **2**: R = H

R. antipathes

Chart 1. Structures of rumphellolides K (**1**) and G (**2**), and a picture of *Rumphella antipathes*

Rumphellolide K (**1**) was isolated as a colorless oil that showed a sodiated adduct ion peak in its HRESIMS at *m/z* 277.1779 $(M + Na)^+$, accounted for the molecular formula, $C_{15}H_{26}O_3$ (Calcd for $C_{15}H_{26}O_3$ + Na, 277.1780) (unsaturation degrees = 3). Comparison of the ^{1}H NMR and DEPT data (Table 1) 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 ν_{max} 3397 cm^{-1}. From the ^{13}C NMR data of **1** (Table 1), there are no olefinic carbon and carbonyl group were observed. Thus, **1** must be a tricyclic compound.

Table 1. ^{1}H and ^{13}C NMR data for caryophyllane **1**

Position	δ_H (J in Hz)[a]	δ_C,[b] type
1	2.05 m	40.7, CH[c]
2/2´	2.06 m; 1.53 m	34.6, CH_2
3	3.86 dd (7.6, 1.2)	78.6, CH
4		81.4, C
5	4.12 dd (10.0, 4.8)	69.4, CH
6	1.69 m	25.0, CH_2
7/7´	1.82 m; 1.56 m	28.4, CH_2
8		74.0, C
9	1.88 ddd (10.8, 10.8, 8.0)	48.2, CH
10/10´	1.48 dd (10.0, 8.0); 1.16 dd (10.8, 10.0)	35.3, CH_2
11		35.7, C
12	1.28 s	23.3, Me
13	1.06 s	29.7, Me
14	1.03 s	30.2, Me
15	1.01 s	21.2, Me

[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] Multiplicity deduced by DEPT spectra.

The COSY spectrum of **1** enabled the determination of structural units, H-1/H_2-2/H-3, H-5/H_2-6/H_2-7, H-9/H-1, and H-9/H_2-10, which were assembled with the assistance of an HMBC experiment (Figure 1). The HMBC between protons and quaternary carbons, permitted elucidation of the main carbon skeleton of **1**. However, no correlation was observed between H-3 (δ_H 3.86) and C-8 oxygenated quaternary carbon at δ_C 74.0 in the HMBC experiment, so the cyclic ether ring between C-3/8 or C-4/8 cannot be determined by this method. Previous study showed that the NMR values of CH_2-2, CH-3, C-4, CH-5, and Me-12 resonating in a caryophyllane analogue, rumphellolide G (**2**) (Chart 1),[3] with a C-3/8 ether bridge moiety, are similar with those of **1**, suggested that the position of ether bridge in **1** should be assigned between C-3 and C-8 by chemical shift changes for deuterium-induced ^{13}C NMR spectrum (Table 2). Thus, the remaining hydroxy groups were attached at C-4 (an oxygenated quaternary carbon resonating at δ_C 81.4) and C-5 (an oxymethine resonating at δ_C 69.4), respectively, as indicated by the key COSY correlations and characteristic NMR signals analysis, although the hydroxy protons for OH-4 and OH-5 were not observed in the ^1H NMR spectrum of **1**.

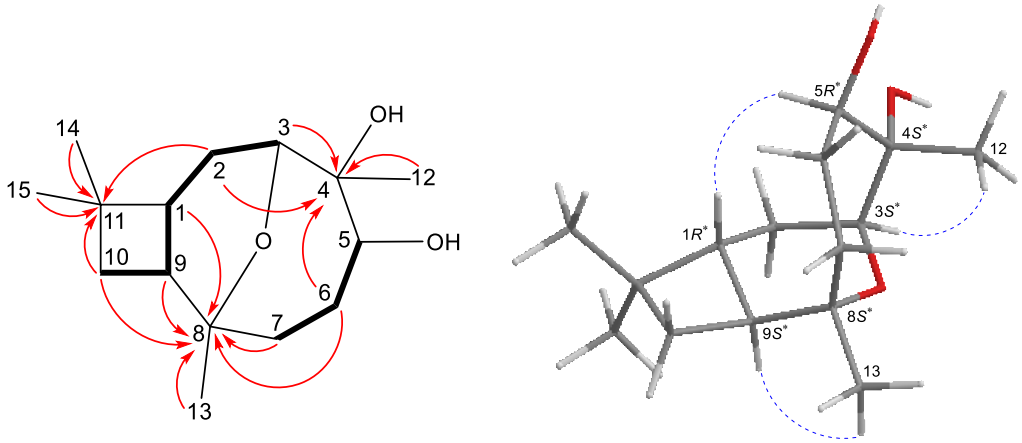


Figure 1. Key COSY (▬), HMBC ( ), and protons with NOESY ( ) correlations of **1**

Table 2. Key NMR data for caryophyllanes **1** and **2**

	1		**2**	
Position	δ_H (J in Hz)[a]	δ_C,[b] type	δ_H[a]	δ_C,[b] type
2/2´	2.06 m; 1.53 m	34.6, CH_2[c]	2.10 m; 1.56 m	34.9, CH_2
3	3.86 dd (7.6, 1.2)	78.6, CH	3.90 m	79.0, CH
4		81.4, C		81.9, C
5	4.12 dd (10.0, 4.8)	69.4, CH	4.17 m	69.8, CH
12	1.28 s	23.3, Me	1.29 s	23.0, Me

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 100 MHz in $CDCl_3$. [c] Multiplicity deduced by DEPT spectra.

The stereochemistry of **1** was determined by correlations observed in a NOESY experiment (Figure 1) and by vicinal proton coupling constants. The *trans* geometry of H-9 (δ_H 1.88) and H-1 (δ_H 2.05) is indicated by a 10.8 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 **1**. In the NOESY experiment, H-9 showed a NOE correlation with H_3-13, indicating that C-13 methyl having an equatorial direction in the tetrahydropyran ring by molecular modeling analysis. H-1 exhibited a strong NOE interaction with H-5, suggesting that the hydroxy group at C-5 was placed on the α-orientation. Moreover, H-3 showed an NOE correlation with H_3-12, but not with H-1, suggesting that these protons were in α-orientation at C-3 and C-4, respectively. Therefore, the configurations of stereogenic centers of **1** were elucidated as 1*R**,3*S**,4*S**,5*R**,8*S**, and 9*S**. As caryophyllane **1** was isolated along with rumphellaone A, a novel 4,5-*seco*-caryophyllane sesquiterpenoid from the same target organism, *R. antipathes*,[8] and the structure, including the absolute configuration, of rumphellaone A, was further confirmed by synthetic methods.[9–11] Therefore, it is reasonable on biogenetic grounds to conclude that caryophyllane **1** has the same absolute configuration as that of rumphellaone A, [9–11] and the configurations of the stereogenic carbons of **1** should be elucidated as 1*R*,3*S*,4*S*,5*R*,8*S*, and 9*S*.

Rumphellolide K (**1**) represents the first caryophyllane-related sesquiterpenoid possessing a C-3/C-8 ether bridge moiety and this compound was found to exhibit 10.36 and 10.91% inhibitory effects on human neutrophils superoxide anions generation and elastase release, respectively, at a concentration of 10 μg/mL.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured using a Jasco P-1010 digital polarimeter. IR spectra were measured on a Thermo Scientific Nicolet iS5 FT-IR spectrophotometer. NMR spectra were taken on a Varian NMR Mercury Plus spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$ using the residual $CHCl_3$ signal (δ_H 7.26 ppm) and $CDCl_3$ (δ_C 77.1 ppm) as the internal standard for ^{1}H and ^{13}C NMR, respectively; coupling constants (*J*) are given in Hz. ESIMS and HRESIMS were recorded using a Bruker APEX II FTMS system. Column chromatography was carried out with silica gel (230–400 mesh, Merck). TLC was performed on plates precoated with Kieselgel 60 F_{254} (0.25-mm-thick, Merck), then sprayed with 10% H_2SO_4 solution followed by heating to visualize the spots. Normal-phase HPLC (NP-HPLC) was performed using a system comprised of a Hitachi L-7100 pump, a Hitachi L-7455 photodiode array detector, a Rheodyne 7725i injection port, and a semi-preparative normal-phase column (Supelco Ascentis Si, Cat #:581514-U, 25 cm × 10 mm, 5 μm; Sigma-Aldrich).

Animal Material. Specimens of *R. antipathes* were collected in May 2004 by hand with SCUBA divers off the coast of Southern Taiwan. A voucher specimen was deposited in the National Museum of Marine Biology and Aquarium, Taiwan (NMMBA-TWGC-010).

Extraction and Isolation. *R. antipathes* (wet/dry weight = 402/144 g) were sliced and then extracted with a solvent mixture of MeOH and CH_2Cl_2 (1:1). The extract was partitioned between EtOAc and H_2O. The EtOAc layer (1.23 g) was then applied on silica gel column and eluted with gradients of *n*-hexane/EtOAc (from 25:1 to 100% EtOAc) to furnish 29 subfractions. Among them, fraction 22 was further purified by NP-HPLC, using a solvent mixture of CH_2Cl_2/EtOAc (10:1) to yield 13 fractions 22A–22M. Fraction 22M was further purified by semi-preparative NP-HPLC using a solvent mixture of CH_2Cl_2 and acetone to give **1** (2.3 mg, 10:1).

Rumphellolide K (1): colorless oil; $[\alpha]_D^{26}$ –14 (*c* 0.1, $CHCl_3$); IR ν_{max} 3397, 2948, 1457, 1037 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; ESIMS *m/z* 277 $(M + Na)^+$; HRESIMS *m/z* 277.1779 (Calcd for $C_{15}H_{26}O_3$ + Na, 277.1780).

Anti-inflammatory Test. Human neutrophils were obtained from healthy human volunteers and were isolated by Ficoll centrifugation and dextran sedimentation. Purified neutrophils were re-suspended in calcium (Ca^{2+})-free Hank's balanced salt solution (HBSS) buffer at pH 7.4, and were maintained at 4 °C before use. For superoxide anion generation assay, neutrophils (6×10^5 cell/mL) were equilibrated in ferricytochrome c (0.6 mg/mL) and Ca^{2+} (1 mM) at 37 °C for 5 min and incubated with DMSO (0.1%) or tested compounds for another 5 min.[12] Cells were activated with fMLF (0.1 μM) for 10 min after the priming with CB (1 μg/mL) for 3 min. The change in absorbance was monitored continuously at 550 nm with a spectrophotometer (Hitachi U-3010). For elastase release assay, neutrophils (6×10^5 cell/mL) were equilibrated in MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide (100 μM) and Ca^{2+} (1 mM) at 37 °C for 5 min and incubated with DMSO (0.1%) or test compounds for another 5 min. Cells were activated with fMLF (0.1 μM) for 10 min after the priming with CB (0.5 μg/mL) for 3 min. The change in absorbance was monitored continuously at 405 nm with a spectrophotometer.[12] The results are recorded as the mean ± SEM of three measurements. The inhibition % was measured at 10 μM concentration of each compound and IC_{50} values were estimated from dose-response curves. Statistical analysis using Student's t-tests with SigmaPlot (Systat Software).

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