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RUMPHELLOLIDE H, A NEW NATURAL CARYOPHYLLANE FROM THE GORGONIAN *RUMPHELLA ANTIPATHIES*

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Abstract – A new natural caryophyllane-type sesquiterpenoid, rumphellolide H (**1**), which possesses a tetrahydropyran moiety in structure, was isolated from the gorgonian coral *Rumphella antipathies*. The structure of **1** was established by spectral data analysis and this metabolite showed moderate inhibitory effects on elastase release by human neutrophils.

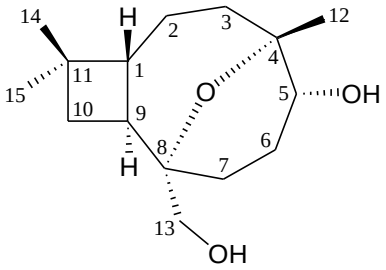
INTRODUCTION

Previous chemical investigations on *Rumphella antipathies* have yielded a series of interesting caryophyllane derivatives, including kobusone,[1] isokobusone,[2] rumphellatins A–D,[3–5] and rumphellolides A–G.[6,7] We describe herein the isolation, structure determination, and bioactivity of a new natural sesquiterpenoid, rumphellolide H (**1**), a caryophyllane derivative that possesses a tetrahydropyran moiety, from the further studies on *R. antipathies*.

RESULTS AND DISCUSSION

Rumphellolide H (**1**) was isolated as a colorless oil. The molecular formula for **1** was determined to be

$C_{15}H_{26}O_3$ by HRESIMS at *m/z* 277.1782 ($C_{15}H_{26}O_3$+Na, calcd, 277.1780). Comparison of the ^{1}H NMR (Table 1) 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 3424 cm^{-1}. From the ^{13}C NMR data of **1** (Table 1), no olefinic carbon and carbonyl carbon was observed. Thus, caryophyllane **1** must be tricyclic. In the ^{13}C NMR spectrum of **1**, the signals for four downfield carbons appeared between δ_C 69–79 ppm, including an oxymethylene (δ_C 69.5, t, CH_2-13), an oxymethine (δ_C 70.5, d, CH-5), two oxygenated quaternary carbons (δ_C 78.1, s, C-4; 75.6, s, C-8), and 11 aliphatic sp[3] carbon signals (a quaternary carbon, two methines, five methylenes, and three methyls) were observed. The ^{1}H NMR spectrum showed that all three methyl groups are isolated (δ_H 1.21, 3H, s, H_3-12; 1.02, 2×3H, s, H_3-14 and H_3-15). Furthermore, five pairs of aliphatic methylene protons (H_2-2, H_2-3, H_2-6, H_2-7, H_2-10), two aliphatic methine protons (H-1, H-9), an oxymethine proton (H-5), and a pair of hydroxymethyl protons (H_2-13) were observed in the ^{1}H NMR spectrum of **1**.



rumphellolide H (**1**)

Table 1. ^{1}H and ^{13}C NMR Data and HMBC (H→C) Correlations for Caryophyllane **1**

Position	δ_H	δ_C		HMBC
1	1.91 ddd (10.4, 10.0, 1.8)[a]	47.2	(d)[b]	C-3, -9
2α/β	1.44 m; 1.53 m	23.6	(t)	C-1, -4
3α/β	1.81 dd (6.8, 6.4); 1.70 m	38.7	(t)	C-1, -2, -4, -5, -12
4		78.1	(s)	
5	3.57 dd (6.4, 2.8)	70.5	(d)	n.o.[c]
6α/β	1.71 m; 2.11 m	25.4	(t)	C-4, -7, -8
7α/β	1.58 m; 1.84 m	19.4	(t)	C-5, -6, -8, -9
8		75.6	(s)	
9	2.04 ddd (10.0, 7.2, 5.2)	44.6	(d)	C-8, -10
10α/β	1.19 dd (10.0, 5.2); 1.48 dd (10.0, 7.2)	34.9	(t)	C-1, -9, -11, -14, -15
11		36.9	(s)	
12	1.21 s	27.8	(q)	C-3, -4, -5
13a/b	3.26 d (11.2); 3.17 d (11.2)	69.5	(t)	C-7, -8, -9
14	1.02 s	30.4	(q)	C-1, -10, -11, -15
15	1.02 s	20.6	(q)	C-1, -10, -11, -14

[a] *J* values (in hertz) in parentheses. [b] Attached protons were deduced by DEPT and HMQC experiments. [c] n.o. = not observed.

Examination of the coupling information in the ^{1}H–^{1}H COSY spectrum of **1** enabled identification of the C-1/2/3, C-5/6/7, C-9/10, and C-1/9 units (Figure 1). These data, together with the HMBC correlations, established the carbon skeleton of **2** (Table 1 and Figure 1). A methyl attached at C-4 was confirmed by the HMBC correlations between H_3-12/C-3, -4, -5 and H_2-3/C-12. The hydroxymethyl group positioned at C-8 was confirmed by the connectivity between the downfield methylene protons (δ_H 3.26 and 3.17) and C-7, -8, and C-9. Based on the above findings, only one oxygen atom has not been assigned, and this atom had to be positioned between C-4 and C-8 (an ether bridge), there being two oxygenated quaternary carbons were observed in the ^{13}C NMR spectrum of **1**.

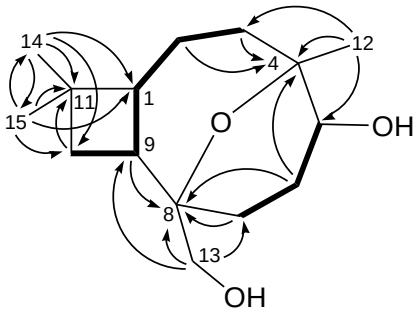


Figure 1. The ^{1}H–^{1}H COSY and selective key HMBC correlations of **1**

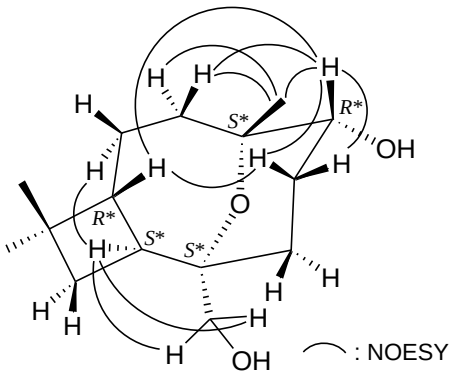


Figure 2. Selective NOESY correlations of **2**

The relative configurations of five chiral centers at C-1, C-4, C-5, C-8, and C-9 in **1** were elucidated by analysis of NOESY interactions (Figure 2) and vicinal ^{1}H–^{1}H coupling constants. The *trans* geometry of H-1 (δ_H 1.91, ddd, J=10.4, 10.0, 1.8 Hz) and H-9 (δ_H 2.04, ddd, J=10.0, 7.2, 5.2 Hz) is indicated by an 10.0 Hz coupling constant between these two ring junction protons, and H-9 and H-1 were assigned as α- and β-oriented, respectively. In the NOESY experiment of **1**, H-5 showed correlations with H-1, H_3-12, and one proton of C-3 methylene (δ_H 1.70), indicating that these protons (H-1, H-5, H_3-12, and H-3β) are located on the same face of the molecule and assigned as β-protons. Furthermore, H_3-12 exhibited responses with H_2-3, but not with H-1, suggesting the C-12 methyl should be placed on the equatorial direction in the tetrahydropyran ring. Furthermore, H_2-13 showed responses with H-9, suggesting the hydroxymethyl group attached at C-8 was α-oriented. On the basis of the above findings, the structure **1** was established and the configurations of the chiral centers of **1** were assigned as 1*R**, 4*S**, 5*R**, 8*S**, 9*S**. The compounds of caryophyllane-type natural products exist widely in terrestrial plant;[8] however, caryophyllane analogues were shown to be rarely found in marine organisms.[9–12] Caryophyllane **1** was first obtained as a synthetic product,[13,14] however, there has been no report of **1** (rumphellolide H) obtained from any natural source. In biological activity testing, caryophyllane **1** was found to show 32.7% inhibitory effect on human neutrophil elastase release at 10 μg/mL.

EXPERIMENTAL

General Experimental Procedures. Optical rotation values were measured with a JASCO P-1010 digital polarimeter. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrophotometer. 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 $CDCl_3$. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ_H 7.26 ppm) and ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at δ_C 77.1 ppm. ESIMS and HRESIMS 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) 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, MERCK) was used for HPLC.

Animal Material. Specimens of *Rumphella antipathies* were collected in May 2004, off the southern coast of Taiwan. This organism was identified by comparison with previous description.[15] A voucher specimen was deposited in the 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). The extract 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 fractions A–D. Fraction B was separated on silica gel and eluted using CH_2Cl_2/acetone (stepwise, 20:1–1:1) to yield fractions B1–B18. Fraction B8 was repurifed by normal-phase HPLC, using mixtures of hexane and acetone as a mobile phase to afford **1** (0.9 mg, 5:1).

Rumphellolide H (1): colorless oil; $[\alpha]_D$ –1 (*c* 0.04, $CHCl_3$); IR (KBr) ν_{max} 3424 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; ESIMS *m/z* 277 $(M+Na)^+$; HRESIMS *m/z* 277.1782 [$(M+Na)^+$, calcd for $C_{15}H_{26}O_3$+Na, 277.1780]. The related physical (optical rotation value) and spectral (^{1}H NMR) data of natural product **1** are in full agreement with those reported previously.[13,14]

Human Neutrophil Elastase Release. Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Elastase release was carried out according to the procedures described previously.[16] Elastase release experiments were performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate.

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