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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 3987–3989

Rumphellatin A, the first chloride-containing caryophyllane-type norsesquiterpenoid from *Rumphella antipathies*

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Received 23 March 2007; accepted 10 April 2007
Available online 14 April 2007

Abstract—A novel chloride-containing 14C norsesquiterpenoid, rumphellatin A (**1**), was isolated from the Formosan gorgonian coral *Rumphella antipathies*. The structure of metabolite **1** was determined on the basis of spectral data analysis and the configuration of **1** was further supported by molecular mechanics calculations. Rumphellatin A (**1**) showed activity against Gram-negative bacteria.

In our screening for bioactive substances from the Formosan soft corals, we have discovered a series of interesting terpenoid and steroid metabolites from the octocorals *Briareum* sp.,[1] *Briareum excavatum*,[2] *Junceella fragilis*,[3] *Junceella juncea*,[3c,4] and *Alcyonium* sp.[5] In previous studies, the compounds of caryophyllane-type exist widely in terrestrial plants,[6] however, the compounds of this type were shown to be rarely found in marine organisms.[7] Several studies have focused on the steroid components,[8] the organic extracts from the gorgonian belonging to the *Rumphella* genus in ecology and their medical use.[9,10] In this Letter, we wish to describe the isolation, structure characterization, and biological activity of a new chlorinated caryophyllane-type norsesquiterpenoid, rumphellatin A (**1**), from the gorgonian coral *Rumphella antipathies* (phylum Cnidaria, order Gorgonacea, suborder Holaxonia, family Gorgoniidae). The structure of **1** was elucidated by extensive spectral data analysis. Antibacterial activity of compound **1** toward the Gram-negative bacteria, *Escherichia coli* and *Vibrio parahaemolyticus*, is also reported.

The organisms of *R. antipathies* (wet weight, 402 g) were collected in May 2004, off the southern Taiwan coast, were successively extracted with a mixture of MeOH and DCM (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 DCM. The DCM layer was separated on silica gel and purified by HPLC to afford metabolite **1** (*n*-hexane–acetone, 7:1).

Rumphellatin A (**1**) was isolated as a white powder, 0.4 mg, mp 97–98 °C; $[\alpha]_D^{25}$ −5 (*c* 0.08, $CHCl_3$), The molecular formula for metabolite **1** was determined to be $C_{14}H_{23}ClO_2$ (three degrees of unsaturation) by analysis of ^{13}C and ^{1}H NMR data (Table 1) in conjunction with DEPT results, and this conclusion was further confirmed by HRESIMS ($C_{14}H_{23}ClO_2$+Na: found, 281.1287; calcd: 281.1284). Comparison of the ^{1}H NMR and DEPT data with the molecular formula indicated that there must be an exchangeable proton, requiring the presence of a hydroxy group and this deduction was supported by a broad absorption in the IR spectrum at 3395 cm^{-1}. From the ^{13}C NMR data of **1**, there are no olefinic carbon and carbonyl groups were observed. Thus, from above observations, rumphellatin A (**1**) must be a tricyclic compound. In the ^{13}C NMR spectrum of **1**, the signals for a hemiketal (δ 109.5, s, C-8), an oxygen-bearing methine (δ 83.9, d, CH-5), and a chlorinated

Keywords: Rumphellatin; Caryophyllane; Norsesquiterpenoid; *Rumphella*; Antibacterial activity.
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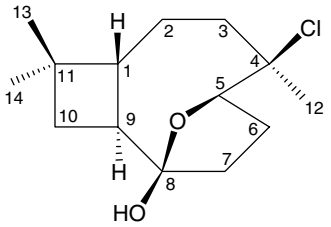
0040-4039/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2007.04.040

Table 1. ^{1}H and ^{13}C NMR data and HMBC correlations for sesquiterpenoid **1**



1

C/H	^{1}H[a]	^{13}C[b]	HMBC (H–C)
1	1.42 br t (10.5)[c]	48.9 (d)[d]	C-2, C-3, C-9, C-11
2α	1.30 m	26.0 (t)	C-1, C-3, C-11
β	1.53 dt (10.5, 4.5)		
3/3′	2.06 dd (6.0, 6.0)	41.3 (t)	C-1, C-2, C-4, C-5, C-12
4		76.1 (s)	
5	4.50 dd (8.5, 7.5)	83.9 (d)	C-3, C-4, C-8
6α	2.37 m	26.3 (t)	n.o.[e]
β	2.12 m		
7α	1.85 dt (13.5, 11.0)	32.6 (t)	C-5, C-6, C-8, C-9
β	2.42 dd (13.5, 8.0)		
8		109.5 (s)	
9	2.15 m	47.9 (d)	C-8
10α	1.64 dd (10.5, 6.0)	35.6 (t)	C-1, C-8, C-9, C-11, C-13, C-14
β	1.35 br t (10.5)		
11		35.1 (s)	
12	1.71 s	28.9 (q)	C-3, C-4, C-5
13	1.02 s	29.7 (q)	C-1, C-10, C-11, C-14
14	0.99 s	20.7 (q)	C-1, C-10, C-11, C-13
OH-8	2.35 s		C-7, C-8, C-9

[a] Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C.
[c] *J* values (in Hz) in parentheses. The values are downfield in parts per million from TMS.
[d] Multiplicity was deduced by HMQC and DEPT spectra and indicated by usual symbol. The values are downfield in parts million from TMS.
[e] n.o. = not observed.

quaternary carbon (δ 76.1, s, C-4) were observed, along with eleven additional sp^3 signals (a quaternary carbon, two methine, five methylene, and three methyl groups) in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed that all three methyl groups are isolated (δ 1.71, 3H, s, H_3-12; 1.02, 3H, s, H_3-13; 0.99, 3H, s, H_3-14). In addition, five pairs of aliphatic methylene protons (δ 1.30, 1H, m, H-2α; 1.53, 1H, dt, $J = 10.5$, 4.5 Hz, H-2β; 2.06, 2H, dd, $J = 6.0$, 6.0 Hz, H_2-3; 2.37, 1H, m, H-6α; 2.12, 1H, m, H-6β; 1.85, 1H, dt, $J = 13.5$, 11.0 Hz, H-7α; 2.42, 1H, dd, $J = 13.5$, 8.0 Hz, H-7β; 1.64, 1H, dd, $J = 10.5$, 6.0 Hz, H-10α; 1.35, 1H, br t, $J = 10.5$ Hz, H-10β), two aliphatic methine protons (δ 1.42, 1H, br t, $J = 10.5$ Hz, H-1; 2.15, 1H, m, H-9), an oxygenated methine proton (δ 4.50, 1H, dd, $J = 8.5$, 7.5 Hz, H-5), and a hydroxy proton (δ 2.35, 1H, s, OH-8) 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 data associated with the molecule 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** enabled identification of the C1–C2–C3, C5–C6–C7, C9–C10, and C1–C9 units (Fig. 1). These data, together with the HMBC correlations between H-1/C-2, C-3, C-9; H_2-2/C-1, C-3; H_2-3/C-1, C-2, C-4, C-5; H-5/C-3, C-4, C-8; H_2-7/C-5, C-6, C-8, C-9; and H-9/C-8 (Table 1 and Fig. 1), established the connectivity from C-1 to C-9 within the nine-membered ring. A methyl attached at C-4 was confirmed by the HMBC correlations between H_3-12/C-3, C-4, C-5 and H_2-3/C-12. The cyclobutane ring, which is fused to the nine-membered ring at C-1 and C-9, was elucidated by the key HMBC correlations between H-1/C-11; H_2-2/C-11; and H_2-10/C-1, C-8, C-9. The cyclic ether ring between C-5 and C-8 was established by a strong HMBC correlation between the proton of C-5 oxymethine (δ_H 4.50) and the C-8 quaternary hemiketal carbon (δ_C 109.5, s). The hydroxy group positioned at C-8 was confirmed by the connectivity between the hydroxy proton (δ_H 2.35) and C-7, C-8, and C-9. These data, together with the HMBC correlations between H_2-10/C-11, C-13, C-14; H_3-13/C-1, C-10, C-11, C-14; and H_3-14/C-1, C-10, C-11, C-13, unambiguously established the planar structure of **1**.

The stereochemistry of **1** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 2). Due to the α orientation of H-9, the ring juncture H-1 should be β-oriented as no NOE correlation was observed between H-1 and H-9. In the NOESY experiment of **1**, H-5 exhibited strong NOE correlations to H_3-12 and one proton of C-6 methylene, but not with H-1. From consideration of molecular models, H-5 was found to reasonably close to H_3-12 when H_3-12 and H-5 were placed on the equatorial direction and assigned as α and β protons, respectively, in the nine-membered ring. These observations were further supported by the NOE interactions between H_3-12 and H_2-3, but not with H_2-2. Based on above findings, the structure, including the relative stereochemistry of **1** was established, the configurations of all chiral centers of **1** were assigned as $1R^*,4R^*,5S^*,8R^*,9S^*$. Furthermore, geometrical optimization of **1** was performed with DISCOVER utilizing the consistent valence force field (CVFF) calculations for energy minimization. The calculated results were visualized using INSIGH II, running on a Silicon Graphics IRIS (SGI) Indigo XS24/4000 workstation.

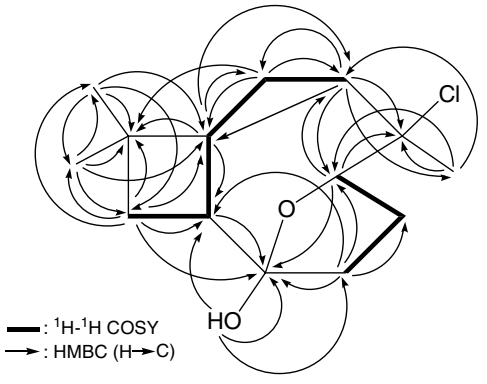


Figure 1. The ^{1}H–^{1}H COSY and HMBC correlations of **1**.

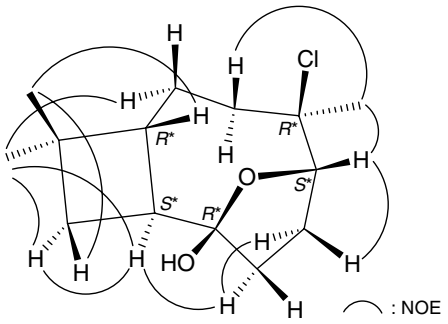
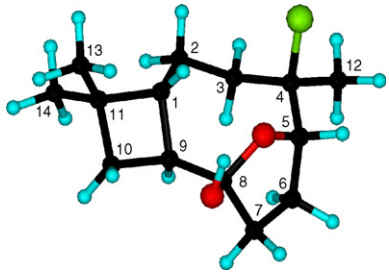


Figure 2. Selective NOESY correlations of **1**.

Table 2. The stereoview of **1** (generated from computer modeling) and the calculated distances (Å) between selected protons having key NOE correlations[a]

Rumphellatin A (**1**)	H/H	(Å)
	H-1/H_3-13	2.40
	H-2α/H_3-14	2.38
	H-3β/H_3-12	2.35
	H-5/H-6β	2.32
	H-5/H_3-12	2.43
	H-6α/H-7α	2.41
	H-7α/H-9	2.50
	H-9/H-10α	2.37
	H-9/H_3-14	2.89
	H-10α/H_3-14	2.57
	H-10β/H_3-13	2.50

[a] The calculated distance between H-1 (β) and H-9 (α) is 3.05 Å.

The conformation search suggested that the most stable conformation and the calculated distances between those protons having key NOE correlations of **1** are all shorter than 3 Å as shown in Table 2.[11] To the best of our knowledge, rumphellatin A (**1**) is the first chlorinated caryophyllane-type natural products. The five-membered cyclic ether ring (a tetrahydrofuran ring) between C-5/C-8 and the C-8 hemiketal group in **1** was also rarely found in caryophyllane derivatives.

In the biological activity testing, compound **1** exhibited activity in standard agar disk diffusion assay[12] against the Gram-negative bacteria *E. coli* and *V. parahaemolyticus*, each causing a 10 and 13 mm zone inhibition, respectively, at 100 μg/mL.

Acknowledgments

This research work was supported by grants from the National Science Council, Taiwan, ROC (NSC 94-2320-B-291-001 and NSC 95-2320-B-291-001-MY2), and by the intramural funding from the NMMBA awarded to P.-J.S.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.04.040.

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