Rumphellclovane B, a Novel Clovane Analogue from the Gorgonian Coral *Rumphella antipathies*

Hsu-Ming Chung,[1,2] Tsong-Long Hwang,[3] Yung-Husan Chen,[2] Jui-Hsin Su,[2] Mei-Chin Lu,[2,4] Jih-Jung Chen,[5] Jan-Jung Li,[2] Lee-Shing Fang,[6] Wei-Hsien Wang,*[1,2,7] and Ping-Jyun Sung*[1,2,4,7,8]

[1]Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan

[2]National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan

[3]Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan

[4]Graduate Institute of Marine Biotechnology, National Dong Hwa University, Pingtung 944, Taiwan

[5]Department of Pharmacy, Tajen University, Pingtung 907, Taiwan

[6]Department of Sport, Health, and Leisure, Cheng Shiu University, Kaohsiung 833, Taiwan

[7]Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan

[8]Department of Life Science and the Institute of Biotechnology, National Dong Hwa University, Hualien 974, Taiwan

Received September 7, 2010; E-mail: pjsung@nmmba.gov.tw, whw@mail.nsysu.edu.tw

A novel clovane-type sesquiterpenoid derivative, rumphellclovane B (**1**), which possesses an unprecedented δ-lactone moiety, and a new natural clovane, 9α-hydroxyclovan-2-one (**2**), were isolated from the gorgonian coral *Rumphella antipathies*. The structures of clovanes **1** and **2** were elucidated by interpretations of spectral data. A plausible biosynthetic pathway between these two compounds was proposed. Compound **1** displayed inhibitory effects on superoxide anion generation by human neutrophils.

Previous chemical investigations on gorgonian coral *Rumphella antipathies* have yielded a series of interesting caryophyllane- and clovane-related sesquiterpenoid derivatives, including kobusone,[1] isokobusone,[2] rumphellatins A–C,[3–5] rumphellolides A–I,[6–9] and rumphellclovane A.[10] In our continuing studies on *R. antipathies*, a novel clovane-related sesquiterpenoid derivative, rumphellclovane B (**1**), along with a new natural clovane, 9α-hydroxyclovan-2-one (**2**) (Chart 1),[11–13] were isolated. In this paper, we describe the isolation, structure characterization, plausible biosynthetic pathway, and bioactivity of sesquiterpenoids **1** and **2**.

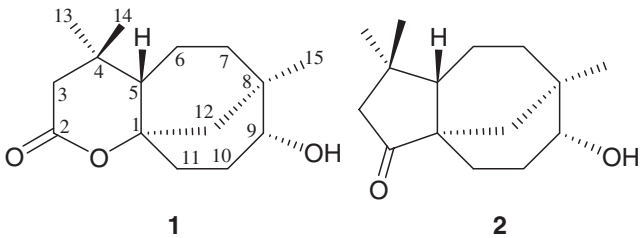


Chart 1.

Results and Discussion

Rumphellclovane B (**1**) was isolated as a colorless oil that gave a pseudomolecular ion $[M + Na]^+$ at m/z 275.1625 in the HR-ESI-MS, indicating the molecular formula $C_{15}H_{24}O_3$ (calcd for $C_{15}H_{24}O_3 + Na$, 275.1623) and implying four degrees of unsaturation. IR absorptions were observed at 3459 and 1730 cm^{-1}, suggesting the presence of hydroxy and δ-lactone groups in **1**. The ^{13}C NMR and DEPT spectra of **1** (Table 1) showed that this compound has 15 carbons, including three methyls, six sp^3 methylenes, two sp^3 methines, and four quaternary carbons. From the ^{13}C NMR data, a degree of unsaturation was accounted for (δ_C 172.0, s, an ester carbonyl) and **1** must be a tricyclic compound.

From the ^1H–^1H COSY experiment of **1** (Figure 1), it was possible to establish the spin systems that map out the proton sequences from H-5/H_2-6/H_2-7 and H-9/H_2-10/H_2-11, which were assembled with the assistance of an HMBC experiment. The HMBC correlations between protons and quaternary carbons of **1**, such as H-6a, H_2-10, H_2-11/C-1; H_2-3/C-2; H_2-3, H-5, H-6a/C-4; H_2-7/C-8, permitted elucidation of the main carbon skeleton. The tertiary methyl at C-8 was confirmed by the HMBC correlations between H_3-15/C-7, -8, -9. Moreover, the two tertiary methyls at C-4 were elucidated by the HMBC correlations between H_3-13/C-3, -4, -5, -14 and H_3-14/C-3, -4, -5, -13. The C-12 methylene bridge between C-1 and C-8 was linked by the HMBC correlations between H_2-7, H_2-11, H_3-15/C-12; and H_2-12/C-1, -5, -7, -8, -9, -15 (Figure 1). Based on the consideration of molecular formula, an additional oxygen atom had to be placed between C-1 and C-2 to form a δ-lactone moiety.

The relative configuration of **1** was established from the interactions observed in a NOESY experiment (Figure 2). Because of the β-orientation of H-5, and the correlations of this

Published on the web December 25, 2010; doi:10.1246/bcsj.20100253

Table 1. ^{1}H and ^{13}C NMR Data for Clovanes **1** and **2**

Position	**1**		**2**	
	δ_H[a)]	δ_C[b)]	δ_H[a)]	δ_C[b)]
1		83.2 (s)[d)]		49.4 (s)
2		172.0 (s)		221.3 (s)
3a/b	2.33 d (16.0)[c)], 2.24 d (16.0)	44.4 (t)	2.27 d (16.0), 2.13 d (16.0)	24.2 (t)
4		34.0 (s)		36.3 (s)
5	1.58 m	45.7 (d)	1.70 m	48.9 (d)
6a/b	1.36 m, 1.61 m	21.0 (t)	1.46 m, 1.58 m	20.5 (t)
7a/b	1.47 m, 1.13 m	32.2 (t)	1.42 m, 1.18 m	32.8 (t)
8		36.1 (s)		33.7 (s)
9	3.32 br s	73.1 (d)	3.56 br s	73.9 (d)
10a/b	1.97 m, 1.75 m	26.6 (t)	1.98 m, 1.67 m	26.4 (t)
11a/b	1.98 m, 1.48 m	35.4 (t)	1.91 m, 1.12 m	29.7 (t)
12α/β	1.75 d (12.4), 1.47 d (12.4)	36.3 (t)	1.53 d (12.8), 1.01 br d (12.8)	31.6 (t)
13	0.95 s	22.6 (q)	0.97 s	24.2 (q)
14	1.08 s	29.7 (q)	1.05 s	30.6 (q)
15	1.04 s	27.8 (q)	0.96 s	28.1 (q)

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. c) *J* values (in hertz) in parentheses. d) Attached protons were deduced by DEPT and HMQC experiments.

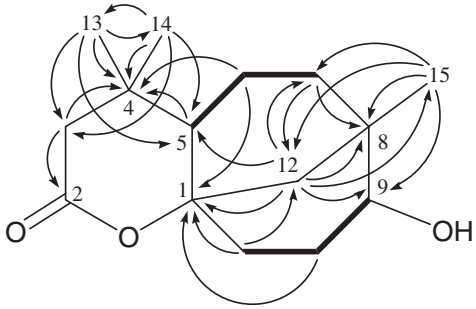


Figure 1. The ^{1}H–^{1}H COSY and selective key HMBC correlations of **1**.

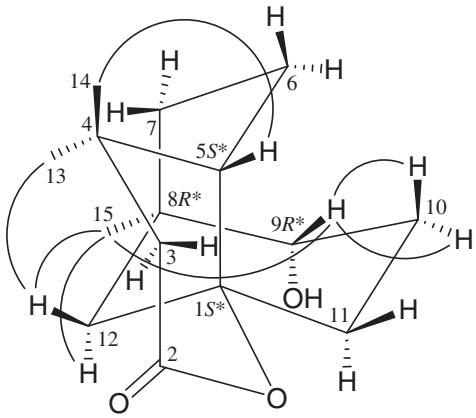
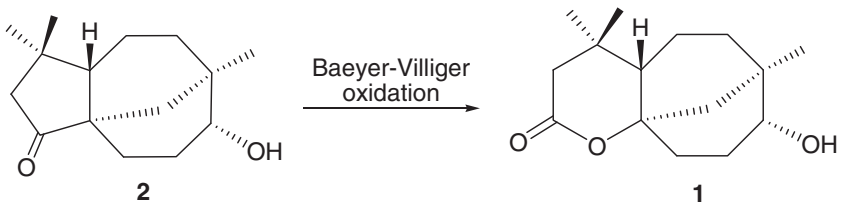


Figure 2. Selective NOESY correlations of **1**.



Scheme 1. Plausible biogenetic relationships for compounds **1** and **2**.

proton shows with H_3-14 but not with H_3-13 one can surmise that H-5 and H_3-14 are located on the same face. One proton of C-12 methylene (δ_H 1.47) was found to exhibit a correlation with H_3-13 and assigned as H-12β and the other was assigned as H-12α (δ_H 1.75). H_3-15 showed correlations with H-12α/β, confirming the α-orientation for this tertiary methyl. Furthermore, H-9 showed correlations with H_2-10 and H_3-15, but not with H-12α. By molecular modeling analysis, H-9 was found to be reasonably close to H_2-10 and H_3-15 and can therefore be placed on the β face in **1**. Based on the above findings, the structure of **1** was elucidated and the chiral centers for **1** were assigned as 1*S**, 5*S**, 8*R**, and 9*R**.

Our present study has also led to the isolation of a new natural clovane **2** and this metabolite has the molecular formula $C_{15}H_{24}O_2$ as determined by HR-ESI-MS (*m/z* 259.1675, calcd for $C_{15}H_{24}O_2 + Na$, 259.1674), with four degrees of unsaturation. Its IR spectrum exhibits broad OH stretch at 3482 cm^{-1} and ketone carbonyl at 1729 cm^{-1}. It was found that the NMR data of **2** (Table 1) are similar with those of **1**, except that the signals corresponding to the δ-lactone group in **1** are replaced by a ketone group in **2**. The correlations from a NOESY experiment of **2** also showed that the relative stereochemistry of **2** is similar to those of **1** and the relative configurations of chiral centers of **2** were established as 1*S**, 5*S**, 8*R**, and 9*R**. It was found that clovane **2** had been obtained previously by chemical methods and named as 9α-hydroxyclovan-2-one (**2**).[11–13] To the best of our knowledge, clovane **2** has not been isolated previously from natural sources. The NMR and mass data for this compound were also reported for the first time.

A plausible biosynthetic pathway for **1** from **2** was proposed as illustrated in Scheme 1. Clovane **2** was further lactonized to **1** by Baeyer–Villiger oxidation. To the best of our knowledge, clovane-type derivative like **1** containing a δ-lactone moiety has not been found previously.

In biological activity experiments, the clovanes **1** and **2** displayed 44.2 and 4.3% inhibitory effects on superoxide anion generation by human neutrophils at 10 $\mu g\,mL^{-1}$, respectively.

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. 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 $CDCl_3$. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ_H 7.26). ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at δ_C 77.1. 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) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprising a HITACHI L-7100 pump, a HITACHI photo diode array detector L-7455, and a RHEODYNE 7725 injection port. A normal phase column (Hibar 250 × 25 mm, LiChrospher Si 60, 5 μm, MERCK) was used for HPLC.

Animal Material. Specimens of the octocoral *R. antipathies* were collected in May 2004, off the southern coast of Taiwan. A voucher specimen was deposited in the National Museum of Marine Biology and Aqaurium, Taiwan.

Extraction and Isolation. Sliced bodies of *R. antipathies* (wet weight 402 g, dry weight 144 g) were extracted with a mixture of MeOH and dichloromethane (DCM) (1:1). The extract was partitioned between EtOAc and H_2O. The EtOAc layer was separated on silica gel and eluted using *n*-hexane/EtOAc (stepwise, 25:1–pure EtOAc) to yield 29 fractions. Every fraction was checked by the ^{1}H NMR spectra. Fraction 22 was purified by normal-phase HPLC, using the mixtures of DCM and EtOAc as a mobile phase to afford compound **1** (10:1). Fraction 18 was purified by normal-phase HPLC, using the mixtures of *n*-hexane and EtOAc as a mobile phase to afford compound **2** (4:1).

Rumphellclovane B (1): Colorless oil (1.7 mg); $[\alpha]_D^{25}$ −9 (*c* 0.06, $CHCl_3$); IR (neat): ν_{max} 3459, 1730 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; 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).

9α-Hydroxyclovan-2-one (2): Colorless oil (18.4 mg); $[\alpha]_D^{23}$ +4 (*c* 0.15, $CHCl_3$) (Ref. 12 $[\alpha]_D$ +7 (*c* 1.50, EtOH)); IR (neat): ν_{max} 3482, 1729 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; ESI-MS: *m/z* 259 $[M + Na]^+$; HR-ESI-MS: *m/z* 259.1675 (calcd for $C_{15}H_{24}O_2 + Na$, 259.1674).

Human Neutrophil Superoxide Anion Generation. Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation was carried out according to procedures described previously.[14,15] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*.

This research work was supported by grants from the National Museum of Marine Biology and Aquarium (Grant Nos. 99200321 and 99200322); National Dong Hwa University; Asia-Pacific Ocean Research Center, National Sun Yat-sen University (Grant No. 97C031702); and the National Science and Technology Program for Biotechnology and Pharmaceuticals, National Science Council (Grant Nos. NSC 98-2323-B-291-001, 99-2323-B-291-001, and 98-2320-B-291-001-MY3), Taiwan, awarded to P.-J.S.

References

1 L.-F. Chuang, T.-Y. Fan, J.-J. Li, P.-J. Sung, *Biochem. Syst. Ecol.* **2007**, *35*, 470.
2 L.-F. Chuang, T.-Y. Fan, J.-J. Li, J. Kuo, L.-S. Fang, W.-H. Wang, P.-J. Sung, *Platax* **2007**, *4*, 61.
3 P.-J. Sung, L.-F. Chuang, J. Kuo, T.-Y. Fan, W.-P. Hu, *Tetrahedron Lett.* **2007**, *48*, 3987.
4 P.-J. Sung, L.-F. Chuang, W.-P. Hu, *Bull. Chem. Soc. Jpn.* **2007**, *80*, 2395.
5 P.-J. Sung, Y.-D. Su, T.-L. Hwang, L.-F. Chuang, J.-J. Chen, J.-J. Li, L.-S. Fang, W.-H. Wang, *Chem. Lett.* **2008**, *37*, 1244.
6 P.-J. Sung, L.-F. Chuang, J. Kuo, J.-J. Chen, T.-Y. Fan, J.-J. Li, L.-S. Fang, W.-H. Wang, *Chem. Pharm. Bull.* **2007**, *55*, 1296.
7 P.-J. Sung, L.-F. Chuang, T.-Y. Fan, H.-N. Chou, J. Kuo, L.-S. Fang, W.-H. Wang, *Chem. Lett.* **2007**, *36*, 1322.
8 T.-L. Hwang, Y.-D. Su, W.-P. Hu, L.-F. Chuang, P.-J. Sung, *Heterocycles* **2009**, *78*, 1563.
9 P.-J. Sung, Y.-D. Su, T.-L. Hwang, L.-F. Chuang, H.-M. Chung, J.-J. Chen, J.-J. Li, L.-S. Fang, W.-H. Wang, *Chem. Lett.* **2009**, *38*, 282.
10 H.-M. Chung, Y.-H. Chen, T.-L. Hwang, L.-F. Chuang, W.-H. Wang, P.-J. Sung, *Tetrahedron Lett.* **2010**, *51*, 2734.
11 A. Aebi, D. H. R. Barton, A. S. Lindsey, *J. Chem. Soc.* **1953**, 3124.
12 A. Aebi, D. H. R. Barton, A. W. Burgstahler, A. S. Lindsey, *J. Chem. Soc.* **1954**, 4659.
13 D. H. R. Barton, A. Nickon, *J. Chem. Soc.* **1954**, 4665.
14 T.-L. Hwang, G.-L. Li, Y.-H. Lan, Y.-C. Chia, P.-W. Hsieh, Y.-H. Wu, Y.-C. Wu, *Free Radical. Biol. Med.* **2009**, *46*, 520.
15 T.-L. Hwang, Y.-C. Su, H.-L. Chang, Y.-L. Leu, P.-J. Chung, L.-M. Kuo, Y.-J. Chang, *J. Lipid Res.* **2009**, *50*, 1395.