Mar. Drugs **2012**, *10*, 1566-1571; doi:10.3390/md10071566

OPEN ACCESS

Marine Drugs

ISSN 1660-3397

www.mdpi.com/journal/marinedrugs

Article

Pseudoalteromone B: A Novel 15C Compound from a Marine Bacterium *Pseudoalteromonas* sp. CGH2XX

Yu-Hsin Chen [1,2,3], Jimmy Kuo [2,3], Jui-Hsin Su [2,3], Tsong-Long Hwang [4], Yung-Husan Chen [3], Chia-Hung Lee [1,2], Ching-Feng Weng [1,2,*] and Ping-Jyun Sung [1,2,3,5,*]

1 Department of Life Science and Institute of Biotechnology, National Dong Hwa University, Hualien 974, Taiwan; E-Mails: kb5634@yahoo.com.tw (Y.-H.C.); chlee016@mail.ndhu.edu.tw (C.-H.L.)

2 Graduate Institute of Marine Biotechnology, National Dong Hwa University, Pingtung 944, Taiwan; E-Mails: jimmy@nmmba.gov.tw (J.K.); x2219@nmmba.gov.tw (J.-H.S.)

3 National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan; E-Mail: tony_chen72001@yahoo.com.tw

4 Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan; E-Mail: htl@mail.cgu.edu.tw

5 Department of Marine Biotechnology and Resources and Division of Marine Biotechnology, Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan

* Authors to whom correspondence should be addressed; E-Mails: cfweng@mail.ndhu.edu.tw (C.-F.W.); pjsung@nmmba.gov.tw (P.-J.S.); Tel.: +886-3-863-3637 (C.-F.W.); Fax: +886-3-863-3630 (C.-F.W.); Tel.: +886-8-882-5037 (P.-J.S.); Fax: +886-8-882-5087 (P.-J.S.).

Received: 12 June 2012; in revised form: 12 July 2012 / Accepted: 12 July 2012 / Published: 20 July 2012

Abstract: A novel 15C compound, pseudoalteromone B (**1**), possessing a novel carbon skeleton, was obtained from a marine bacterium *Pseudoalteromonas* sp. CGH2XX. This bacterium was originally isolated from a cultured-type octocoral *Lobophytum crassum*, that was growing in cultivating tanks equipped with a flow-through sea water system. The structure of **1** was established by spectroscopic methods. Pseudoalteromone B (**1**) displayed a modestly inhibitory effect on the release of elastase by human neutrophils.

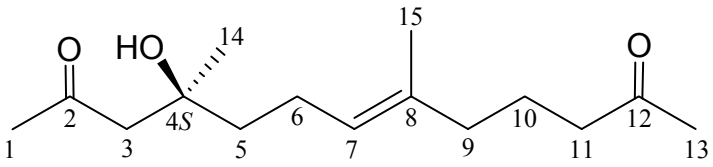
Keywords: pseudoalteromone; *Pseudoalteromonas*; anti-inflammatory; *Lobophytum crassum*; elastase

---

1. Introduction

Marine bacteria belonging to the genus *Pseudoalteromonas* sp. (family Pseudoalteromonadaceae) have proven to be not only an important source of various antibiotics, but have also played an interesting role in marine ecology [1–4]. In the continuing research aimed at the discovery of new natural substances from marine microorganisms, an organic extract of the bacterium identified as *Pseudoalteromonas* sp. CGH2XX, which was originally isolated from a cultured-type octocoral *Lobophytum crassum* (family Alcyonacea), exhibited significant cytotoxicity toward the HL-60 (human acute promyelocytic leukemia) and CCRF-CEM (human T cell acute lymphoblastic leukemia) tumor cells (IC_{50} = 0.9, 1.2 μg/mL) and displayed a significant inhibitory effect (inhibition rate 45.1%) on the release of elastase by human neutrophils at a concentration of 10 μg/mL. We isolated a novel 15C compound, pseudoalteromone B (**1**) (Figure 1), from this microorganism. The structure of **1** was established by spectroscopic methods and this compound displayed a modestly inhibitory effect on the release of elastase by human neutrophils.

Figure 1. The structure of pseudoalteromone B (**1**).



2. Results and Discussion

Pseudoalteromone B (**1**) was isolated as an oil and had the molecular formula $C_{15}H_{26}O_3$, as determined by HRESIMS ($C_{15}H_{26}O_3$ + Na, *m/z* found 277.1779, calculated 277.1780) indicating three degrees of unsaturation. The IR absorption bands at 3502 and 1706 cm^{-1} were characteristic for the hydroxy and ketone groups.

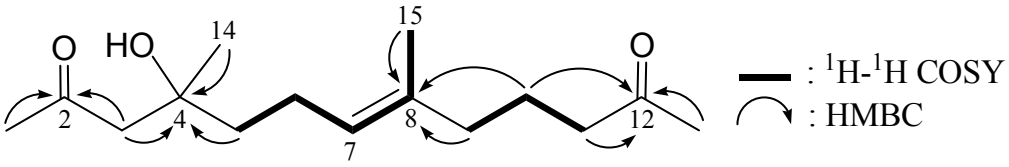
Table 1. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data for **1**.

Position	δ_H (*J* in Hz)	δ_C, Mult.
1	2.18 s	31.9, CH_3
2		211.0, qC
3a/b	2.58 d (17.2); 2.65 d (17.2)	52.3, CH_2
4		71.5, qC
5	1.51 m	41.9, CH_2
6	2.04 m	22.5, CH_2
7	5.09 tq (7.2, 1.2)	124.8, CH
8		134.6, qC
9	1.96 t (7.2)	38.8, CH_2
10	1.66 quintet (7.2)	21.8, CH_2
11	2.37 t (7.2)	43.0, CH_2
12		209.1, qC
13	2.12 s	29.9, CH_3
14	1.22 s	26.7, CH_3
15	1.58 br s	15.7, CH_3

The ^{1}H and ^{13}C NMR data of **1** (Table 1) showed the presence of 15 carbon signals, which were identified by the assistance of a DEPT spectrum as four methyls, six sp^3 methylenes, an sp^2 methine, an sp^3 quaternary carbon, and three sp^2 quaternary carbons including two ketone carbonyls. The ^{1}H NMR spectrum of **1** showed a signal of olefinic proton (δ_H 5.09, 1H, tq, J = 7.2, 1.2 Hz, H-7), two acetyl methyls (δ_H 2.18, 3H, s, H_3-1; 2.12, 3H, s, H_3-13), a vinyl methyl (δ_H 1.58, 3H, br s, H_3-15), a tertiary methyl attaching at an oxygenated quaternary carbon (δ_H 1.22, 3H, s, H_3-14) and six pairs of methylene protons (δ_H 2.65, 1H, d, J = 17.2 Hz; 2.58, 1H, d, J = 17.2 Hz, H_2-3; 2.37, 2H, t, J = 7.2 Hz, H_2-11; 2.04, 2H, m, H_2-6; 1.96, 2H, t, J = 7.2 Hz, H_2-9; 1.66, 2H, quintet, J = 7.2 Hz, H_2-10; 1.51, 2H, m, H_2-5).

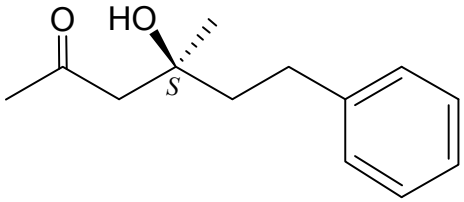
The constitution of the carbon skeleton of **1** was elucidated initially by the ^{1}H–^{1}H COSY and HMBC correlations of **1** (Figure 2), it was possible to establish the separate spin systems that map out the proton sequences from H_2-5/H_2-6/H-7 and H_2-9/H_2-10/H_2-11. These data, together with the HMBC correlations between H_3-1/C-2, C-3; H_2-3/C-2, C-4, C-5; H_2-5/C-4, C-6; H-7/C-9; H_2-9/C-7, C-8, C-10, C-11; H_2-10/C-8, C-9, C-11, C-12; H_2-11/C-9, C-10, C-12; and H_3-13/C-11, C-12, permitted elucidation of the main straight carbon skeleton. The vinyl methyl at C-8 was confirmed by the HMBC correlations between H-7, H_2-9/C-15; and H_3-15/C-7, C-8, C-9; and further supported by an allylic coupling between H-7 and H_3-15 (J = 1.2 Hz). Based on these data, together with the HMBC correlations between H_3-14/C-3, C-4, C-5 and H_2-3, H_2-5/C-14, the planar structure of **1** was established.

Figure 2. The ^{1}H–^{1}H COSY and selective HMBC correlations (protons→quaternary carbons) of **1**.



In the NOESY experiment of **1**, a correlation between H-7 with H_2-9, as well as the lack of correlation between H-7 and H_3-15, reflected the *E*-configuration of C-7/8 double bond. Furthermore, by comparison of the rotation value of **1** ($[\alpha]_D^{23}$ −20 (*c* 0.03, $CHCl_3$)) with that of a known synthetic compound, (*S*)-4-hydroxy-4-methyl-6-phenylhexan-2-one (**2**) ($[\alpha]_D^{25}$ −14.5 (*c* 1.1, $CHCl_3$)) (Figure 3) [5], the absolute configuration for the C-4 chiral center of **1** was determined as *S* form as that of **2**. Based on the above findings, the structure of **1** was determined unambiguously.

Figure 3. The structure of (*S*)-4-hydroxy-4-methyl-6-phenylhexan-2-one (**2**).



The *in vitro* cytotoxicity of pseudoalteromone B (**1**) toward HCT116 (human colorectal carcinoma), K-562 (human chronic myelogenous leukemia), HL-60 (human acute promyelocytic leukemia), CCRF-CEM (human T cell acute lymphoblastic leukemia), T-47D (human breast ductal carcinoma), and MDA-MB-231 (human breast adenocarcinoma) cells was tested. Unfortunately, the new compound **1**

described herein is not active toward the above cells (all IC_{50} values > 20 μg/mL). The *in vitro* anti-inflammatory effect of **1** was tested. Pseudoalteromone B (**1**) displayed a modestly inhibitory effect (inhibition rate 20.7%) on the release of elastase by human neutrophils at a concentration of 10 μg/mL.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were measured on a Jasco P-1020 polarimeter. IR spectra were recorded on a Jasco FT/IR-4100 infrared 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, in $CDCl_3$, respectively. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ_H 7.26 ppm). ^{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. Silica gel (Merck, 230–400 mesh) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck); spots were visualized by spraying with 10% H_2SO_4 solution followed by heating.

3.2. Marine Bacteria Isolation, Culture Conditions and Extract Preparation

A marine bacterium number CGH2XX was isolated from soft coral *Lobophytum crassum* that was growing in cultivating tanks equipped with a flow-through sea water system [4]. The bacterium strain CGH2XX was 98.3% identical with *Pseudoalteromonas* sp. H02P24-23 (Genebank accession no. HQ161380) on the basis of 16S rDNA gene sequence. The marine bacterium was cultured in 2.5 L flasks containing 1 L M1 broth (not containing agar) with 80% seawater. Flasks were incubated at 25 °C on a rotatory shaker at 120 rpm. After five days of incubation, extraction of the culture broth (10.0 L) with ethyl acetate (EtOAc, 2 × 10.0 L) yielded 1.71 g of crude extract. The extracts obtained were stored at −20 °C.

3.3. Separation

Crude extract was separated on Sephadex LH-20 and eluted using a mixture of dichloromethane and methanol (1:1) to yield 17 fractions. Fraction 6 was selected for further study and purified by silica gel, using a mixture of *n*-hexane and EtOAc (2:1) as a mobile phase to afford compound **1** (4.2 mg).

Pseudoalteromone B (**1**): colorless oil; $[\alpha]_D^{23}$ −20 (*c* 0.03, $CHCl_3$); IR (neat) ν_{max} 3502, 1706 cm^{-1}; ^{1}H ($CDCl_3$, 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).

3.4. Cytotoxicity Testing

The cytotoxicity of compound **1** was assayed with a modification of the MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to previously described procedures [6–8].

3.5. Elastase Release by Human Neutrophils

Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Elastase release experiments were performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate [9–11].

4. Conclusions

In a previous study [4], an ubiquinone derivative, pseudoalteromone A, was isolated from *Pseudoalteromonas* sp. CGH2XX, and this compound was found to be cytotoxic toward MOLT-4 (human acute lymphoblastic leukemia) and T-47D (human breast ductal carcinoma) cells (IC_{50} = 3.8, 4.0 μg/mL) and displayed moderately inhibitory effects on the generation of superoxide anion and the release of elastase (inhibition rates 38.0, 20.2%) by human neutrophils at a concentration of 10 μg/mL [12]. However, as described in the beginning of this communication, the organic extract of *Pseudoalteromonas* sp. CGH2XX showed significant cytotoxicity and anti-inflammatory activity. At this stage, the results showed that pseudoalteromone B (**1**) displayed a modestly anti-inflammatory activity and this compound was not cytotoxic toward HCT116, K-562, HL-60, CCRF-CEM, T-47D and MDA-MB-231 cells. We suggested that the other active components exist in the other fractions. The possible activity for pseudoalteromone B (**1**) will be studied if we can get enough material from *Pseudoalteromonas* sp. CGH2XX. Furthermore, to the best of our knowledge, compounds pseudoalteromones A and B, were the first two compounds from the marine bacterium belonging to the genus *Pseudoalteromonas* associated with octocorals.

Acknowledgments

This work was supported by grants from the National Dong Hwa University; the National Museum of Marine Biology and Aquarium (Grant No. 10120022); the Division of Marine Biotechnology, Asia-Pacific Ocean Research Center, National Sun Yat-sen University (Grant No. 00C-0302-05); and the National Science Council (Grant No. NSC 101-2325-B-291-001, 101-2320-B-291-001-MY3 and 98-2320-B-291-001-MY3), Taiwan, awarded to P.-J.S.

References and Notes

1. Bowman, J.P. Bioactive compound synthetic capacity and ecological significance of marine bacterial genus *Pseudoalteromonas*. *Mar. Drugs* **2007**, *5*, 220–241.
2. Fehér, D.; Barlow, R.S.; Lorenzo, P.S.; Hemscheidt, T.K. A 2-substituted prodiginine, 2-(*p*-hydroxybenzyl)prodigiosin, from *Pseudoalteromonas rubra*. *J. Nat. Prod.* **2008**, *71*, 1970–1972.
3. Fehér, D.; Barlow, R.; McAtee, J.; Hemscheidt, T.K. Highly brominated antimicrobial metabolites from a marine *Pseudoalteromonas* sp. *J. Nat. Prod.* **2010**, *73*, 1963–1966.
4. Chen, Y.-H.; Lu, M.-C.; Chang, Y.-C.; Hwang, T.-L.; Wang, W.-H.; Weng, C.-F.; Kuo, J.; Sung, P.-J. Pseudoalteromone A: a novel bioactive ubiquinone from a marine bacterium *Pseudoalteromonas* sp. CGH2XX (Pseudoalteromonadaceae). *Tetrahedron Lett*. **2012**, *53*, 1675–1677.
5. Chen, I.-H.; Kanai, M.; Shibasaki, M. Copper(I)—Secondary diamine complex-catalyzed enantioselective conjugate boration of linear β,β-disubstituted enones. *Org. Lett.* **2010**, *12*, 4098–4101.

6. Doxorubicin was used as a reference compound in cytototxicity testing. Doxorubicin showed cytotoxicity toward HCT116, K-562, HL-60, CCRF-CEM, T-47D, and MDA-MB-231 cells (IC_{50} = 1.8, 0.8, 0.2, 0.1, 1.5 and 1.7 μg/mL).
7. Alley, M.C.; Scudiero, D.A.; Monks, A.; Hursey, M.L.; Czerwinski, M.J.; Fine, D.L.; Abbott, B.J.; Mayo, J.G.; Shoemaker, R.H.; Boyd, M.R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589–601.
8. Scudiero, D.A.; Shoemaker, R.H.; Paull, K.D.; Monks, A.; Tierney, S.; Nofziger, T.H.; Currens, M.J.; Seniff, D.; Boyd, M.R. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res.* **1988**, *48*, 4827–4833.
9. In the *in vitro* anti-inflammatory bioassay, the inhibitory effect on the release of elastase by activated neutrophils was used as an indicator. For significant activity of pure compounds, an inhibition rate ≥ 40% is required (inhibition rate ≤ 10%, not active; 20% ≥ inhibition rate ≥ 10%, weakly anti-inflammatory; 40% ≥ inhibition rate ≥ 20%, modestly anti-inflammatory). Elastatinal was used as a reference compound in anti-inflammatory activity test (IC_{50} = 31.9 μg/mL).
10. Hwang, T.-L.; Wang, C.-C.; Kuo, Y.-H.; Huang, H.-C.; Wu, Y.-C.; Kuo, L.-M.; Wu, Y.-H. The hederagenin saponin SMG-1 is a natural FMLP receptor inhibitor that suppresses human neutrophil activation. *Biochem. Pharmacol.* **2010**, *80*, 1190–1200.
11. Yu, H.-P.; Hsieh, P.-W.; Chang, Y.-J.; Chung, P.-J.; Kuo, L.-M.; Hwang, T.-L. 2-(2-Fluorobenz-amido)benzoate ethyl ester (EFB-1) inhibits superoxide production by human neutrophils and attenuates hemorrhagic shock-induced organ dysfunction in rats. *Free Radic. Biol. Med.* **2011**, *50*, 1737–1748.
12. The authors regret that there is an error in pages 1 and 2 of [4] (pages 1675 and 1676 of the issue). In [4], the ubiquinone, pseudoalteromone A, was reported to display an inhibitory effect on the release of elastase (inhibition rate 45.1%) by human nuetrophils at a concentration of 10 μg/mL. However, after detailed collating, we found this data was cited incorrectly. The data (inhibition rate 45.1%) expressed an inhibitory effect of an organic extract from the marine bacterium *Pseudoalteromonas* sp. CGH2XX on the release of elastase by human nuetrophils as presented in this study. The *in vitro* anti-inflammatory effects of pseudoalteromone A were tested again. Pseudoalteromone A displayed moderately inhibitory effects on the generation of superoxide anion and the release of elastase (inhibition rates 38.0% and 20.2%) by human neutrophils at a concentration of 10 μg/mL. Diphenyl indonium (DPI) and elastatinal were used as reference compounds in anti-inflammatory activity testing. DPI displayed an inhibitory effect on superoxide anion generation (IC_{50} = 0.9 μg/mL), and elastatinal exhibited an inhibitory effect on elastase release (IC_{50} = 31.9 μg/mL) by human neutrophils, respectively. The authors apologize for any inconvenience caused by this error.

Samples Availability: Not available.