ORGANIC LETTERS

2013
Vol. 15, No. 21
5400–5403

Actinoranone, a Cytotoxic Meroterpenoid of Unprecedented Structure from a Marine Adapted *Streptomyces* sp.

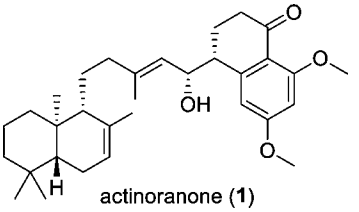
Sang-Jip Nam,[†] Christopher A. Kauffman, Lauren A. Paul, Paul R. Jensen, and William Fenical*

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0204, United States

wfenical@ucsd.edu

Received July 23, 2013; Revised Manuscript Received October 5, 2013

ABSTRACT



actinoranone (**1**)

The isolation and structure elucidation of a new meroterpenoid, actinoranone (1), produced by a marine bacterium closely related to the genus *Streptomyces* is reported. Actinoranone is composed of an unprecedented dihydronaphthalenone polyketide linked to a bicyclic diterpenoid. The stereochemistry of 1 was defined by application of the advanced Mosher's method and by interpretation of spectroscopic data. Actinoranone (1) is significantly cytotoxic to HCT-116 human colon cancer cells with an LD_{50} = 2.0 μg/mL.

The variability of environmental chemical and physical conditions in the oceans has clearly played a major role in the evolution of a great diversity of microorganisms.[1] Our evidence suggests that obligate marine microorganisms have, in part, adapted to life in the sea through the production of unique secondary metabolites that distinguish them from their terrestrial counterparts.[2] Thus, investigations of the chemistry and biology of these unique molecules could lead to the discovery of a significant number of new drug leads.[3]

The chemically prolific genus *Streptomyces*, which is abundant and well-known in terrestrial habitats, also appears to be represented in marine ecosystems. In recent years, expanding studies of marine-derived and obligate marine actinomycetes have yielded bioactive molecules with diverse chemical structures.[4] Phylogenetic classification, by analysis of 16S rRNA gene sequence data, has indicated that new taxa at both the genus and species levels are present.[5]

As part of our continuing program to explore the biomedical potential of marine actinomycetes, we have previously isolated and reported the structures of several highly modified peptides, actinoramides A–C, from our marine actinomycete strain CNQ-027 (GenBank accession number EU214912).[6] This seawater-requiring strain shares only 97.6% 16S rRNA gene sequence identity with an obligate marine *Streptomyces* species, *S. marinus*, suggesting it may represent a new *Streptomyces* species adapted to live in the sea.[7]

A more extensive study of the secondary metabolite composition of this strain has now yielded a new meroterpenoid, actinoranone (**1**), which possesses a new carbon skeleton and significant cancer cell cytotoxicity. Details of

[†] Current address: Department of Chemistry and Nano Science, Global Top 5 Program, Ewha Womans University, Seoul, South Korea.

(1) Colin, M. *Marine Microbiology: Ecology and Applications*; Garland Science: New York, 2012; Chapter 1.

(2) Murphy, B. T.; Maloney, K. N.; Fenical, W. In *Handbook of Marine Natural Products*; Fattorusso, E., Gerwick, W. H., Taglialatela-Scafati, O., Eds.; Springer: New York; Vol. 1, p 153.

(3) (a) Harvey, A. *Drug Discovery Today* **2000**, *5*, 294. (b) Zotchev, S. B. *J. Biotechnol*. **2012**, *158*, 168.

(4) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol*. **2006**, *2*, 666 and references therein.

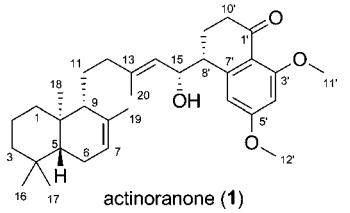
(5) (a) Jensen, P. R.; Gontang, E. A.; Mafnas, C.; Mincer, T. J.; Fenical, W. *Environ. Microbiol*. **2005**, *7*, 1039. (b) Gontang, E. A.; Fenical, W.; Jensen, P. R. *Appl. Environ. Microbiol*. **2007**, *73*, 3272.

(6) Nam, S.-J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Tetrahedron* **2011**, *67*, 6707.

(7) Prieto-Davó, A.; Fenical, W.; Jensen, P. R. *Aquat. Microbial Ecol*. **2008**, *52*, 1.

10.1021/ol402080s
Published on Web 10/23/2013

the isolation, characterization, and biological activity of actinoranone are presented here.



The molecular formula of actinoranone (**1**)[8] was defined as $C_{32}H_{46}O_4$, based on analysis of HRESIMS data (a pseudomolecular ion peak at m/z 495.3450 $[M+H]^+$) and on interpretation of ^{13}C NMR data. The ^1H NMR spectrum of **1** displayed *meta*-coupled aromatic protons [δ 6.56 (d, J = 2.0 Hz), 6.50 (d, J = 2.0 Hz)], two olefinic protons [δ 5.38 (br s), 5.25 (d, J = 8.5 Hz)], and a downfield methine proton [δ 4.47 (dd, J = 8.5, 8.5 Hz)]. The ^1H NMR spectrum also showed two methoxyl groups [δ 3.87, 3.83] and five methyl singlets [δ 1.71, 1.63, 0.90, 0.86, 0.78] (Table 1). Analysis of ^{13}C NMR and gHSQC spectral data revealed seven methyl, eight methylene, eight methine, and nine fully substituted carbons. Analysis of the gCOSY spectroscopic data for **1** revealed the connectivity of four partial structures; substructure a (C-1 to C-3), substructure b (C-5 to C-7), substructure c (C-9 to C-11 and C-11 to C-12), and substructure d (C-15 to C-8′, and C-8′ to C-10′), as shown in Figure 1. Substructures (a and b) and gHMBC correlations from gem-dimethyl singlets (H-16 and H-17) to carbons C-3, C-4, and C-5, from a methyl singlet H-18 to carbons C-1, C-5, C-9, and C-10, and from a methyl singlet H-19 to carbons C-7, C-8, C-9 allowed the terpenoid bicyclic ring to be defined. The dihydronaphthalenone moiety was also constructed by interpretation of gCOSY and gHMBC spectroscopic data. The presence of *meta*-coupled aromatic protons [δ 6.56 (d, J = 2.0 Hz), 6.50 (d, J = 2.0 Hz)] indicated a 1,2,3,5-tetra substituted benzene ring. The composition of substructure d and gHMBC correlations from H-9′ to carbons C-1′, C-7′, from H-8′ to carbons C-2′, C-6′, and C-7′, from H-11′ to C-3′, from H-12′ to C-5′, and from H-4′ to C-2′, C-6′ confidently established the dihydronaphthalenone ring. Lastly, gHMBC correlations from the H-20 methyl singlet to carbons C-12, C-13, and C-14 permitted the connectivity of C-14 and C-15, thus completing the assignment of the planar structure of **1** (Figure 1).

The relative configuration of the bicyclic terpenoid ring in **1** was assigned by interpretation of ROESY NMR correlations. Correlations between the H-18 methyl singlet and the H-11 methylene protons, and between H-5 and H-9, allowed the relative configurations to be assigned as 5*R**, 9*R**, and 10*R**. The 7*Z* and 13*E* double-bond geometries were also assigned by the observation of ROESY NMR correlations between the H-7 olefinic proton and the H-19 methyl singlet, and between the H-12 methylene protons and the H-14 olefinic proton, respectively.

Next, application of the advanced Mosher's method allowed the absolute configuration at C-15 to be defined.[9] Treatment of **1** with (*R*)- and (*S*)-MTPA-Cl

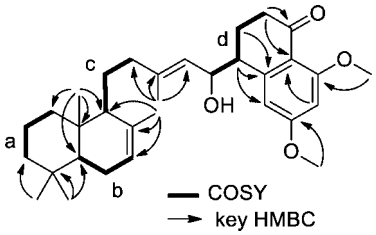


Figure 1. gCOSY and key gHMBC correlations assisting in the structure elucidation of actinoranone (**1**).

Table 1. NMR Data for Actinoranone **1** (methanol-d_4)[a]

no.	δ_C, #H.[b]	δ_H (mult, J (Hz))	HMBC
1	39.3, CH_2	1.85,[c] 0.94 dt (13.5, 3.6)	3, 18
2	25.4, CH_2	1.55, m 1.29, m	4, 10
3	42.3, CH_2	1.42, m 1.16[c]	5
4	32.7, C		
5	50.4, CH	1.16, m	7
6	23.7, CH_2	1.97, m 1.86, m	7, 8
7	122.2, CH	5.38, br s	6, 8, 19
8	135.0, C		
9	54.2, CH	1.61, m	11, 12
10	36.7, C		
11	25.4, CH_2	1.55, m 0.78, m	8, 13
12	42.1, CH_2	2.24, m 2.01, m	9, 13, 14
13	139.5, C		
14	126.8, CH	5.25, d (8.5)	15, 20
15	70.4, CH	4.47, dd (8.5, 8.5)	8′
16	32.5, CH_3	0.86, s	3, 4, 5, 17
17	21.0, CH_3	0.90, s	3, 4, 5, 16
18	12.8, CH_3	0.78, s	1, 5, 9, 10
19	21.5, CH_3	1.71, s	7, 8, 9
20	15.6, CH_3	1.63, s	12, 13, 14
1′	197.3, C		
2′	115.5, C		
3′	162.7, C		
4′	97.2, CH	6.50, d (2.0)	2′, 5′, 6′
5′	164.4, C		
6′	107.5, CH	6.56, d (2.0)	2′, 4′, 5′, 7′, 8′
7′	150.4, C		
8′	45.3, CH	2.97, m	15, 2′, 6′, 7′, 9′, 10′
9′	23.8, CH_2	2.12, m	1′, 7′
10′	36.3, CH_2	2.44, ddd (18.0, 11.0, 8.0) 2.59, dt (18.0, 4.5)	1′, 2′, 8′, 9′
11′	54.9, CH_3	3.83, s	3′
12′	55.0, CH_3	3.87, s	5′

[a] 500 MHz for ^1H NMR and 75 MHz for ^{13}C NMR. [b] The number of attached protons was determined by analysis of 2D spectroscopic data. [c] The coupling constant was not determined because of overlapping signals.

(8) Actinoranone: $[\alpha]_D^{21}$ +3 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3429, 2921, 1720, 1664, 1602, 1464, 1258, 1148 cm^{-1}; UV (MeOH) λ_{max} (log ε) 230 (3.2), 289 (2.8), 308 (2.0) nm; ^1H and ^{13}C NMR data, See Table 1; ESI-TOF m/z 495 $[M+H]^+$; HR ESI-TOF m/z 495.3450 (calcd for $C_{32}H_{47}O_4$, 495.3469). Details of the isolation of actinoranone are provided in the Supporting Information.

(9) Uhrin, D.; Barlow, P. N. *J. Magn. Reson.* **1997**, *126*, 248.

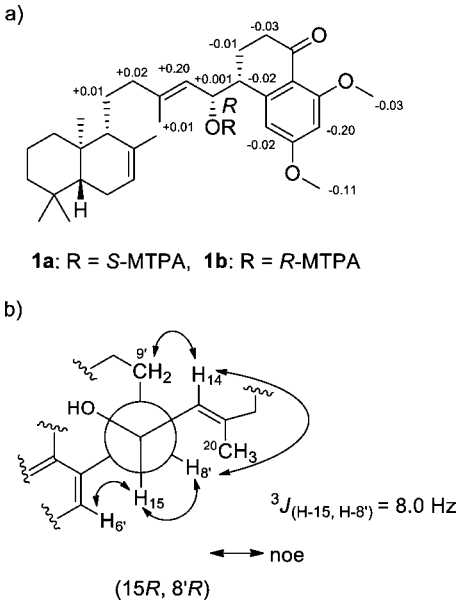
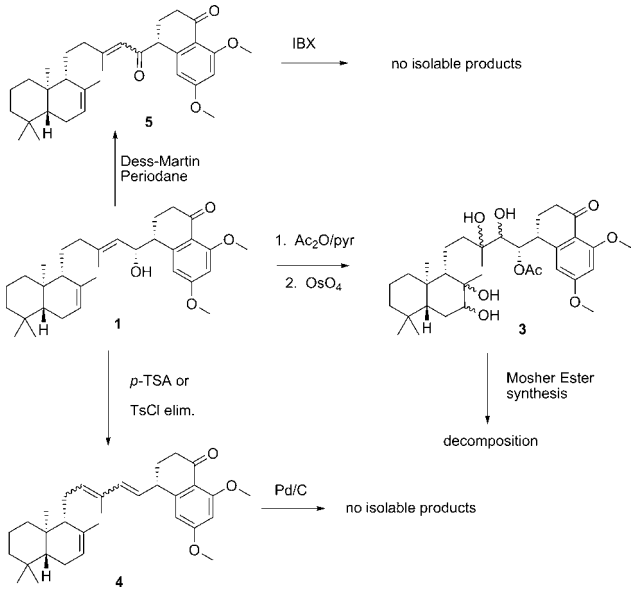


Figure 2. (a) Mosher's ester analysis of actinoranone (**1**), $\Delta\delta_{S\text{-}R}$ of ^{1}H for *S*- and *R*-*mono*-MTPA esters of **1**, and (b) a Newman projection of the C-8′−C-15 bond illustrating the assigned C-8′ relative configuration.

[α-methoxy-α-(trifluoromethyl) phenylacetyl chloride] yielded the (*S*)- and (*R*)-MTPA ester derivatives, respectively. Calculation of the ^{1}H NMR $\Delta\delta_{S\text{-}R}$ values for the *mono*-MTPA esters of **1** established the absolute configuration at C-15 as *R* (Figure 2a). HETLOC[10] and HSQMBC[11] NMR experiments were undertaken to determine the configuration of C-8′, but we were unable to measure the coupling constants due to the complexity of signals (data not shown). As an alternative approach, we carefully analyzed the ROESY NMR data hoping to assign the absolute configuration of C-8′. There were only two possible stereoisomers, (15*R*, 8′*R*) or (15*R*, 8′*S*), for **1**. The median value of the coupling constant ($^3J_{\mathrm{H15-H8'}}$ = 8.0 Hz) and ROESY correlations from H-8′ and H-6′ to H-15′ and from H-8′ and H-9′ to H-14 supported our assignment of an 8′*R* configuration (Figure 2b).

In an attempt to determine the absolute configurations for C-5, C-9, and C-10, we performed a series of synthetic modifications. We protected the hydroxyl group at C-15 of **1** by acetylation, and then we treated the 15-acetoxyl derivative **2** with OsO_4 to afford a mixture of 7,8,13,14-tetra-ol products, which were then subjected to HPLC purification to obtain a major stereoisomer **3**.[12] The relative configuration of the bicyclic ring system of this stereoisomer was suggested as 5*R**, 7*S**, 8*R**, 9*S**, and 10*R** based upon ROESY correlations between H-7 and H-19, between H-19 and H-11, and between H-11 and H-18 (see Supporting Information). Treatment of this stereoisomer with (*R*)- and (*S*)-MTPA-Cl produced crude *bis*-(*S*)- and *bis*-(*R*)-MTPA ester derivatives, respectively. However, on HPLC purification these ester derivatives spontaneously decomposed under all conditions explored. Next, we attempted chemical reactions to eliminate the stereocenters at C-15 and C-8′ in an effort to compare the optical rotations of derivatives of **1** with the known (+)-isozonarol[13a] and (−)-isozonarol.[13b] Actinoranone A was treated with *p*-toluenesulfonic acid (TSA) hoping to obtain the $\Delta^{13,14,15,8'}$-diene.[14a] However, we obtained the undesired dehydration product possessing the $\Delta^{11,12,13,14}$-diene (**4**). We also attempted a base dehydration of actinoranone A using *p*-toluenesulfonyl chloride (TsCl),[14b] but this yielded the same result. In addition, attempted aromatization of these unexpected products with Pd/C[14c] did not produce any isolable products. Then, actinoranone A was treated with Dess−Martin periodinane (DMP)[14d] to oxidize the secondary alcohol to ketone **5** and then further oxidized by using 2-iodoxybenzoic acid (IBX)[14e] in hopes to obtain the C-8′−C-9′ α,β-unsaturated ketone. The reaction did not yield identifiable products.



All attempts defined above to eliminate the stereocenters at C-15 and C-8′ were unsuccessful (see Supporting Information for synthetic details). Thus, only the relative stereochemistry of **1** could be defined as 5*R**, 9*R**, 10*R**, along with the absolute configurations 15*R* and 8′*R*.

Traditionally, actinomycetes are not considered producers of terpenoids. However, strains of a largely marine

(10) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H.; Kover, K. E. *Magn. Reson. Chem.* **2000**, *38*, 265.

(11) (a) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092. (b) Seco, J. M.; Quinoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17.

(12) VanReen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *17*, 1973.

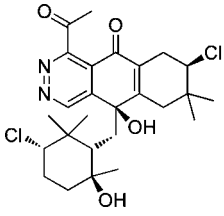
(13) (a) Schröder, J.; Magg, C.; Seifert, K. *Tetrahedron Lett.* **2000**, *41*, 5469. (b) Pérez-García, E.; Zubía, E.; Ortega, M .J.; Carballo, J. L. *J. Nat. Prod.* **2005**, *68*, 653.

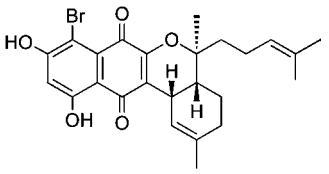
(14) (a) Stevens, R. V.; Albizati, K. F. *J. Org. Chem.* **1985**, *50*, 632. (b) Choudary, B. M.; Chowdari, N. S.; Kantam, M. *Tehtrahedron* **2000**, *56*, 7291. (c) Mosettig, E.; Duvall, H. M. 1937, *59*, 367. (d) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. (e) Nicolaou, K. C.; Zhong, Y.-L.; Baran, P. S. *J. Am. Chem. Soc.* **2000**, *122*, 7596. (f) Hirai, Y.; Doe, M.; Kinoshita, T.; Morimoto, Y. *Chem. Lett.* **2004**, *33*, 136.

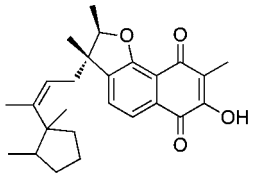
(15) Gallagher, K. A.; Fenical, W.; Jensen, P. R. *Curr. Opin. Biotechnol.* **2010**, *21*, 794.

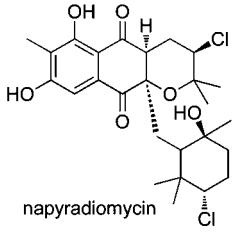
(16) Cho, J. Y.; Kwon, H. C.; Williams, P. G.; Jensen, P. J.; Fenical, W. *Org. Lett.* **2006**, *8*, 2471.

lineage we have classified as MAR4 consistently produce diverse meroterpenoids.[15] Three classes of meroterpenoids, exempified by azamerone,[16] marinone, and neomarinone,[17] and the many members of the napyradiomycin family,[18] have been isolated from these organisms. They commonly share a 2,3-dihydronaphthalene-1,4-dione or naphthalene-1,4-dione derived from the polyketide precursor 1,3,6,8-tetrahydroxynaphthalene (THN) and terpenoid substituents in the molecules. Given these comparisons, it is surprising that strain CNQ-027 does not belong to the MAR4 group of marine actinomycetes.









(17) (a) Pathirana, C.; Jensen, P. R.; W. Fenical, W. *Tetrahedron Lett*. **1992**, *33*, 7663–7666. (b) Hardt, I. H.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett*. **2000**, *41*, 2073.

To the best of our knowledge, this is the first observation of the common diterpenoid "labdane" bicyclic ring system in microbial natural products. This is also the first report of a meroterpenoid isolated from a marine-derived actinomycete possessing the dihydronaphthalenone moiety. Actinoranone (**1**) showed *in vitro* cytotoxicity against HCT-116 human colon carcinoma with an LD_{50} = 2.0 μg/mL. Testing in a broader number of diverse cancer cell lines is in progress.

Acknowledgment. Financial support was provided by the National Cancer Institute, NIH (under Grant R37 CA044848). We thank Chambers C. Hughes, Scripps Institution of Oceanography, UCSD, for his generous advice in the determination of the stereochemistry of actinoranone, and Katherine N. Maloney, Scripps Institution of Oceanography, UCSD for providing preliminary information about the strain CNQ-027.

Supporting Information Available. Details of the isolation procedures, reactions and ^{1}H, ^{13}C, and 2D NMR spectra of actinoranone, ^{1}H NMR spectra of *mono*-MTPA esters (**1a**, **1b**), and other derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

(18) Soria-Mercado, I. E.; Prieto-Davó, A.; Jensen, P. R.; Fenical, W. *J. Nat. Prod*. **2005**, *68*, 904.

The authors declare no competing financial interest.