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NOTE

Two butenolides with PPARα agonistic activity from a marine-derived *Streptomyces*

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors classified into three subtypes known as PPARα, PPARβ (δ) and PPARγ.[1] PPARs regulate the systemic energy metabolism by modulating the expression of numerous genes involved in lipid and glucose metabolism. Therefore, these nuclear transcription factors are recognized as ideal targets for the treatment of dyslipidemia, diabetes, and the cardiovascular disorders associated with obesity and the metabolic syndrome. In particular, PPARα is considered as a therapeutic target of dyslipidemic diseases as it mediates lipid lowering by activating a transcriptional cascade that induces genes involved in the catabolism of lipids.[1]

Marine-derived microorganisms have attracted significant attention for their potential of producing a wide array of secondary metabolites.[2–4] During the course of our screening for bioactive compounds from marine-derived bacteria, glycosylated tetronates with apoptosis-inducing activity, a cytotoxic anthracycline derivative, herbicidal isocoumarins and adipogenic polyketides were discovered.[5–8] As a part of our continuing study in this field, we screened for new scaffolds for PPARα agonists using a reporter gene assay and found two butenolides (**1** and **2**, Figure 1) from a marine-derived *Streptomyces* strain. The presence of compounds **1** and **2** was indicated by the GC/MS analysis of volatile components released from *Streptomyces* but their complete spectroscopic characterization, including the absolute configuration, was not reported.[9] In this study, we attempted the absolute configuration assignment of these butenolides. We herein report the structure determination and biological activity of **1** and **2**.

The producing strain TP-A0873 was isolated from a seawater sample collected in Toyama Bay, Japan. The strain was cultured in A3M liquid medium and the whole culture broth was extracted with 1-butanol. The crude extract was fractionated by silica gel and octadecylsilyl (ODS) column chromatographies, followed by reversed-phase HPLC purification to yield butenolides **1** and **2**.

Compound **1** was obtained as a colorless oil, which had a molecular formula of $C_{12}H_{20}O_2$ (three degrees of unsaturation) by interpretation of high-resolution ESITOFMS ($[M+H]^+$ *m/z* 197.1535, $\Delta -0.1$ mmu, calcd for $C_{12}H_{21}O_2$) and NMR data (Table 1). The IR spectrum of **1** indicated the presence of carbonyl functionality (1749 cm^{-1}). ^1H and ^{13}C NMR analysis of **1** in combination with HSQC spectrum revealed the presence of 12 carbons including a carbonyl carbon, two olefinic carbons, an oxygen-bearing methine, five methylenes, a methine and two methyls (Table 2). As one double bond and one carbonyl accounted for two of the three double-bond equivalents, **1** must be a monocyclic compound. Analysis of ^1H-^1H COSY spectrum provided two spin systems consisting of protons from H2 to H7 and from H8 to H11. Two doublet methyl protons (H11 and H12) showed HMBC correlations to one another, to C10, and to C9, establishing the isopropyl terminus in the latter fragment. The two fragments were connected by HMBC correlations from H6 to C8 and H9 to C7. Furthermore, two olefinic protons (H2 and H3) showed correlation to the carbonyl carbon C1 (δ 173.2). Finally, consideration of the remaining unsaturation degree and the small coupling constant between the olefinic protons ($J = 5.7$ Hz) provided the α,β-unsaturated γ-lactone bearing a linear chain at the γ-position. This was consistent with the observed UV (λ_{max} 206 nm) and IR data.[10] The structure of **1** was thus assigned as 4-hydroxy-10-methylundec-2-en-1,4-olide.

Compound **2** was isolated as a colorless oil. Its molecular formula was deduced as $C_{13}H_{22}O_2$ from high-resolution ESITOFMS ($[M+H]^+$ *m/z* 211.1692, $\Delta -0.1$ mmu, calcd for $C_{13}H_{23}O_2$) and ^{13}C NMR data (Table 1). The NMR data for **2**, including chemical shifts and coupling constants, were almost identical to **1**, except for the loss of one methyl group in the isopropyl terminus and the presence of a triplet methyl (H12) and an additional methylene (H11) group (Table 2). The COSY analysis revealed two small fragments H11/H12 and H13/H10, which were joined by HMBC correlations from H12 to C10 and from H13 to C10 and C11. This four-carbon fragment was connected to C9 on the basis of an HMBC correlation from H13 to C9, thereby establishing a *sec*-butyl moiety at the linear chain terminus. The structure of **2** was thus determined as 4-hydroxy-10-methyldodec-2-en-1,4-olide.

The absolute configuration of **1** was determined by applying the CD exciton chirality method to acyclic dibenzoate **3b**.[11,12] Compound **1** was subjected to ozonolysis followed by $NaBH_4$ reduction and successive alkaline hydrolysis to give diol **3a**, which was converted

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to dibenzoate **3b** (Scheme 1). The CD spectrum of **3b** showed typical bisignate Cotton effects of a positive exciton chirality at 237 nm ($\Delta\varepsilon = +2.07$) and 221 nm ($\Delta\varepsilon = -1.18$) (Supplementary Figure S7). By combining the conformational analysis by ^{1}H NMR {$J_{1a,2}$ (*anti*) = 6.7 Hz, $J_{1b,2}$ (*gauche*) = 3.4 Hz} and CD data, the absolute configuration at C4 was clearly determined as *S*. Similarly, the absolute configuration at C4 of compound **2** was determined as *S* based on the CD and ^{1}H NMR data.

The absolute configuration at C10 of **2** was determined using the chiral fluorescent labeling reagents developed by Ohrui and Akasaka.[13–15] Diol **4a** derived from **2** was subjected to oxidative cleavage with $NaIO_4$, followed by Jones oxidation, yielding 7-methylnonanoic acid **5**. This *anteiso* fatty acid[16] (**5**) was then reacted with (*R*)-2-(anthracene-2,3-dicarboximido)-1-propanol [(*R*)-2A1P] to yield the ester **6a** (Scheme 2). The reference compounds (**6b** and **6c**) were prepared by the esterification of (*S*)-7-methylnonanoic acid with both enantiomers of 2A1P. In the ^{1}H NMR spectra, the methyl protons at C13 of **6a** appeared as doublet at δ_H 0.72, whereas those of **6b** and **6c** were observed at δ_H 0.72 and δ_H 0.73, respectively (Supplementary Figure S6). The absolute configuration at C10 of **2** was thus assigned to be *S*.

To assess the PPAR subtype specificity of butenolides **1** and **2**, two reporter cell lines that express luciferase gene in response to PPARα and PPARγ agonists were used. The agonist activity was determined as a relative potency to the positive controls, WY-14643[17] for PPARα and troglitazone[18] for PPARγ. Both **1** and **2** induced activation of PPARα

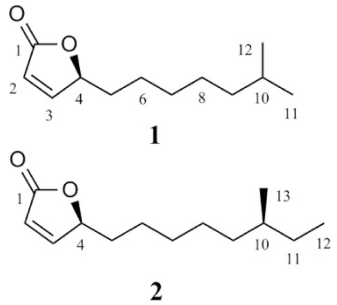
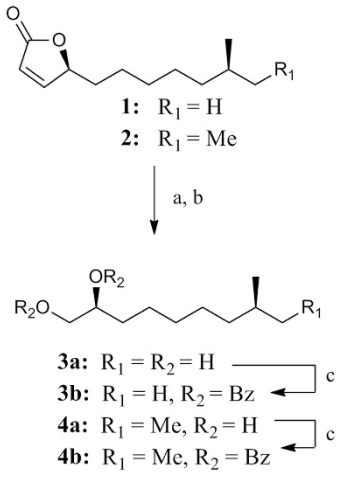


Figure 1 Structures of butenolides **1** and **2**.

Table 1 Physico-chemical properties of butenolides 1 and 2

	1	*2*
Appearance	Colorless oil	Colorless oil
Molecular formula	$C_{12}H_{20}O_2$	$C_{13}H_{22}O_2$
MW	196	210
HR-ESI-TOFMS (m/z)	$[M+H]^+$	
Calcd	197.1536 (for $C_{12}H_{21}O_2$)	211.1693 (for $C_{13}H_{23}O_2$)
Found	197.1535	211.1692
$[\alpha]_D^{24}$	+64 (*c* 0.50, $CHCl_3$)	+69 (*c* 2.6, $CHCl_3$)
UV (MeOH) λ_{max} (log ε) nm	206 (4.09)	206 (4.16)
IR (ATR) ν_{max} cm^{-1}	1749	1748

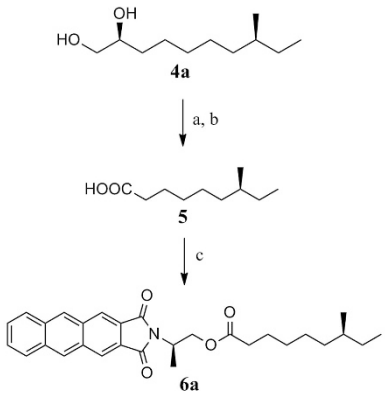


Scheme 1 Preparation of dibenzoates **3b** and **4b** from butenolides.

Table 2 NMR data for butenolides 1 and 2 in $CDCl_3$

	1			*2*		
Position	δ_H, *mult* (*J in Hz*)[a]	δ_C[b]	*HMBC*[a,c]	δ_H, *mult* (*J in Hz*)[a]	δ_C[b]	*HMBC*[a,c]
1		175.2			173.2	
2	6.11, dd (5.7, 2.0)	121.5	1, 3, 4	6.10, dd (5.6, 2.0)	121.4	1, 3, 4
3	7.45, dd (5.7, 1.4)	156.3	1, 2, 4	7.47, dd (5.6, 1.2)	156.4	1, 2, 4
4	5.04, dddd (7.2, 5.3, 2.0, 1.4)	83.4	2, 3, 5, 6	5.04, dddd (7.2, 5.6, 2.0, 1.2)	83.4	2, 3, 5, 6
5	1.77, m; 1.69, m	33.2	3, 4	1.77, m; 1.67, m	33.2	3, 4, 6, 7
6	1.45, m	25.0		1.45, m	25.0	7, 8
7	1.32, m	29.6		1.31, m	29.6	
8	1.31, m	27.1	7	1.28, m	26.8	
9	1.17, m	38.8	7, 8, 11, 12	1.27, m; 1.08, m	36.4	
10	1.52, m	27.9	9, 11, 12	1.29, m	34.3	
11	0.86, d (6.6)	22.6	9, 10, 12	1.28, m; 1.11, m	29.4	9, 10, 12, 13
12	0.86, d (6.6)	22.6	9, 10, 11	0.85, d (7.2)	11.3	10, 11
13				0.83, d (6.3)	19.1	9, 10, 11

[a]Recorded at 400 MHz.
[b]Recorded at 100 MHz.
[c]HMBC correlations are from proton(s) to the indicated carbon(s).



Reagents: (a) $NaIO_4$, MeOH; (b) NaClO, H_2O_2; (c) (*R*)- or (*S*)-2A1P, EDAC, DMAP.

Scheme 2 Degradation of diol **4a** and labeling with (*R*)-2A1P.

Table 3 PPAR activation activity of butenolides 1 and 2

		Activity relative to control	
Compounds	*Concentration (μM)*	*PPARα*	*PPARγ*
1	10	1.00	0.25
	1	0.19	0.06
2	10	1.58	0.45
	1	0.29	0.01
WY-14643	50	1.00	ND
Troglitazone	10	ND	1.00

Abbreviation: PPAR, peroxisome proliferator-activated receptor.

and PPARγ transcription in a concentration-dependent manner at 1–10 μM (Table 3). Their agonistic activity was more specific to PPARα *ca* 3–4 fold than to PPARγ.

It is known that some *Streptomyces*. species collected from marine environment produce butenolides bearing an alkyl side chain.[9,10,19,20] In particular, the presence of compounds **1** and **2** had been demonstrated by the GC/MS analysis of volatile components released from a marine-derived *Streptomyces*, although their isolation and complete spectroscopic characterization have not been accomplished.[9,20,21] In this study, we isolated two butenolides **1** and **2** as selective PPARα agonists and achieved the full spectroscopic characterization including the absolute configuration assignment. PPAR activation by butenolides is not known so far, making this class of natural products a new lead scaffold for designing hypolipidemic agents.

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1 Tyagi, S., Gupta, P., Saini, A. S., Kaushal, C. & Sharma, S. The peroxisome proliferator-activated receptor: a family of nuclear receptors role in various diseases. *J. Adv. Pharm. Technol. Res.* **2**, 236–240 (2011).
2 Subramani, R. & Aalbersberg, W. Marine actinomycetes: an ongoing source of novel bioactive metabolites. *Microbiol. Res.* **167**, 571–580 (2012).
3 Bhatnagar, I. & Kim, S.-K. Immense essence of excellence: marine microbial bioactive compounds. *Mar. Drugs* **8**, 2673–2701 (2010).
4 Jensen, P. R., Mincer, T. J., Williams, P. G. & Fenical, W. Marine actinomycete diversity and natural product discovery. *Antonie van Leeuwenhoek* **87**, 43–48 (2005).
5 Furumai, T., Takagi, K., Igarashi, Y., Saito, N. & Oki, T. Arisostatins A and B, new members of tetrocarcin class of antibiotics from *Micromonospora* sp. TP-A0316. I. Taxonomy, fermentation, isolation and biological properties. *J. Antibiot.* **53**, 227–232 (2000).
6 Furumai, T., Igarashi, Y., Higuchi, H., Saito, N. & Oki, T. Kosinostatin, a quinocycline antibiotic with antitumor activity from *Micromonospora* sp. TP-A0468. *J. Antibiot.* **55**, 128–133 (2002).
7 Azumi, M. *et al.* Bacilosarcins A and B, novel bioactive isocoumarins with unusual heterocyclic cores from the marine-derived bacterium *Bacillus subtilis*. *Tetrahedron* **64**, 6420–6425 (2008).
8 Kim, Y. *et al.* Nocapyrones: α- and γ-pyrones from a marine-derived *Nocardiopsis* sp. *Mar. Drugs* **12**, 4110–4125 (2014).
9 Dickschat, J. S., Martens, T., Brinkhoff, T., Simon, M. & Schulz, S. Volatiles released by a *Streptomyces* species isolated from the North Sea. *Chem. Biodiver.* **2**, 837–865 (2005).
10 Mukku, V., Speitling, M., Laatsch, H. & Helmke, E. New butenolides from two marine *Streptomyces*. *J. Nat. Prod.* **63**, 1570–1572 (2000).
11 Harada, N. & Nakanishi, K. *Circular dichronic spectroscopy: exciton coupling in organic stereochemistry*, University Science Books: Mill Valley, CA, USA, 1983.
12 Harada, N., Nakanishi, K. & Berova, N. in: *Comprehensive chiroptical spectroscopy.* (eds Berova, N., Polavarapu, P. L., Nakanishi, K., Woody, R. W.) Ch. 4, pp. 115–166, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2012.
13 Akasaka, K., Meguro, H. & Ohrui, H. Enantiomeric separation of carboxylic acids having chiral centers remote from the carboxyl group by labelling with a chiral fluorescent derivatization reagent. *Tetrahedron Lett.* **38**, 6853–6856 (1997).
14 Akasaka, K. & Ohrui, H. Enantiomeric separation of branched fatty acids after conversion with *trans*-2-(2,3-anthracenedicarboximido)cyclohexanol, a highly sensitive chiral fluorescent conversion reagent. *Biosci. Biotechnol. Biochem.* **63**, 1209–1215 (1999).
15 Matsuhashi, H. & Shimada, K. Chemical transformation of leustroducsins: synthesis of leustroducsin B. *Tetrahedron* **58**, 5619–5626 (2002).
16 Kaneda, T. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiol. Rev.* **55**, 288–302 (1991).
17 Murakami, K., Ide, T., Suzuki, M., Mochizuki, T. & Kadowaki, T. Evidence for direct binding of fatty acids and eicosanoids to human peroxisome proliferators-activated receptor alpha. *Biochem. Biophys. Res. Commun.* **260**, 609–613 (1999).
18 Kodera, Y. *et al.* Ligand type-specific interactions of peroxisome proliferator-activated receptor gamma with transcriptional coactivators. *J. Biol. Chem.* **275**, 33201–33204 (2000).
19 Cho, K. W. *et al.* New lactone-containing metabolites from a marine-derived bacterium of the genus *Streptomyces*. *J. Nat. Prod.* **64**, 664–667 (2001).
20 Schulz, S. *et al.* Biological activity of volatiles from marine and terrestrial bacteria. *Mar. Drugs* **8**, 2976–2987 (2010).
21 Citron, C. A., Rabe, P. & Dickschat, J. S. The scent of bacteria: headspace analysis for the discovery of natural products. *J. Nat. Prod.* **75**, 1765–1776 (2012).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)