JOURNAL OF NATURAL PRODUCTS

Note

pubs.acs.org/jnp

Janadolide, a Cyclic Polyketide–Peptide Hybrid Possessing a *tert*-Butyl Group from an *Okeania* sp. Marine Cyanobacterium

Hidetoshi Ogawa,[†] Arihiro Iwasaki,[†] Shinpei Sumimoto,[†] Yuki Kanamori,[†] Osamu Ohno,[‡] Masato Iwatsuki,[§,⊥] Aki Ishiyama,[§,⊥] Rei Hokari,[§] Kazuhiko Otoguro,[§] Satoshi Ōmura,[§] and Kiyotake Suenaga*[,†]

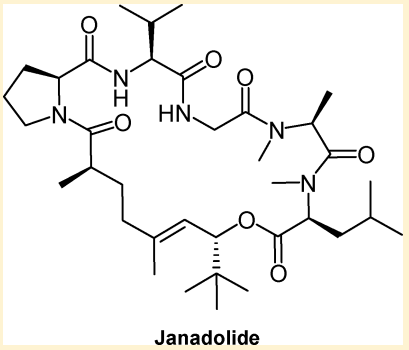
[†]Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

[‡]Department of Chemistry and Life Science, Kogakuin University, 2665-1 Nakano, Hachioji, Tokyo 192-0015, Japan

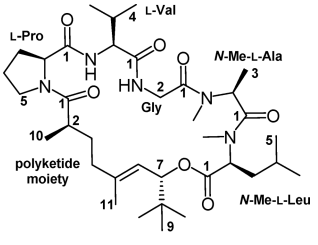
[§]Research Center for Tropical Diseases, Kitasato Institute for Life Sciences, and [⊥]Graduate School of Infection Control Sciences, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo 108-8641, Japan

Supporting Information

ABSTRACT: Janadolide, a new cyclic polyketide–peptide hybrid possessing a *tert*-butyl group, was isolated from an *Okeania* sp. marine cyanobacterium. The gross structure was elucidated by spectroscopic analyses, and the absolute configurations of the amino acid moieties were determined by acid hydrolysis and chiral-phase HPLC analyses. The absolute configuration of the two stereogenic centers in the polyketide moiety was elucidated based on a combination of degradation reactions and spectroscopic analyses including the phenyl-glycine methyl ester method. Janadolide showed potent antitrypanosomal activity with an IC_{50} value of 47 nM without cytotoxicity against human cells at 10 μM.



Marine prokaryotes produce diverse secondary metabolites, and many interesting compounds have been discovered from them to date.[1] In particular, marine cyanobacteria have been recognized as prolific producers of compounds with interesting structures.[2] Against this backdrop, we have isolated several natural compounds possessing new skeletons such as jahanyne,[3] bisebromoamide,[4] and kurahyne[5] from marine cyanobacteria. In our efforts to discover substances with unique structures, we investigated the constituents of an *Okeania* sp. marine cyanobacterium and isolated a new cyclic polyketide–peptide hybrid possessing a *tert*-butyl group, janadolide (**1**). So far, several *tert*-butyl-containing PKS–NRPS hybrid compounds, such as the antillatoxins[6,7] and the apratoxins,[8] have been isolated from marine cyanobacteria. Here, we report the isolation, structure elucidation, preliminary biological characterization, and molecular modeling of janadolide (**1**).



Janadolide (1)

An *Okeania* sp. marine cyanobacterium was collected at the coast near Janado, Okinawa. The collected cyanobacterium (700 g, wet weight) was extracted with MeOH, and the extract was filtered and concentrated. The residue was partitioned between EtOAc and H_2O. The material obtained from the organic layer was partitioned between 90% aqueous MeOH and hexanes. The aqueous MeOH fraction was separated by reversed-phase column chromatography (ODS silica gel, MeOH–H_2O) and reversed-phase HPLC (Cosmosil 5C_{18}AR-II, MeOH–H_2O) to give janadolide (**1**) (6.9 mg). The molecular formula of janadolide (**1**) was determined on the basis of the positive HRESIMS data. Table 1 shows the NMR data for **1**. An analysis of the ^{1}H NMR spectrum revealed the presence of two *N*-methyl groups (δ_H 2.30 and 2.82) and six protons corresponding to the α-positions of amino acids (δ_H 3.47, 4.02, 4.236, 4.241, 4.68, and 5.69). In addition, the presence of a *tert*-butyl group (δ_H 0.83), a vinyl methyl group (δ_H 1.75), and an olefinic proton (δ_H 5.20) was also clarified. The ^{13}C NMR spectrum indicated the existence of six carbonyl groups (δ_C 175.2, 172.7, 170.6, 170.1, 169.8, and 167.1) and one double bond (δ_C 144.3 and 120.0). Intensive interpretation of the COSY, HMQC, and HMBC spectra established five amino acid residues: *N*-methylleucine (*N*-Me-Leu), *N*-methylalanine (*N*-Me-Ala), glycine (Gly), valine (Val), and proline

Received: February 26, 2016

DOI: 10.1021/acs.jnatprod.6b00171
J. Nat. Prod. XXXX, XXX, XXX–XXX

Table 1. ^{1}H and ^{13}C NMR Data for Janadolide (1) in C_6D_6

position	δ_C [a]	δ_H [b] (J in Hz)	position	δ_C [a]	δ_H [b] (J in Hz)
Pro			*N*-Me-Leu		
1	172.7, C		1	170.6, C	
2	62.4, CH	4.236, m	2	58.2, CH	4.68, dd (10.7, 4.3)
3a	31.8, CH_2	1.89, m	3a	39.1, CH_2	1.87, m
3b		1.72, m	3b		1.64, m
4a	22.9, CH_2	1.56, m	4	25.2, CH	1.42, m
4b		1.234, m	5	23.4, CH_3	0.82, d (6.7)
5a	46.8, CH_2	3.76, ddd (12.0, 8.5, 3.5)	6	21.7, CH_3	1.04, d (6.5)
5b		3.39, m	*N*-Me	29.7, CH_3	2.82, s
Val			polyketide moiety		
1	169.8, C		1	175.2, C	
2	58.0, CH	4.241, m	2	39.7, CH	2.40, m
3	33.0, CH	2.05, m	3a	35.4, CH_2	2.43, m
4	19.0, CH_3	0.92, d (6.6)	3b		1.01, m
5	18.5, CH_3	0.91, d (6.7)	4a	40.6, CH_2	2.15, m
NH		7.00, d (6.9)	4b		1.66, m
Gly					
1	167.1, C		5	144.3, C	
2a	41.8, CH_2	4.02, dd (18.3, 5.4)	6	120.0, CH	5.20, dq (10.1, 0.9)
2b		3.47, dd (18.3, 2.1)	7	79.3, CH	5.43, d (10.1)
NH		6.82, dd (5.4, 2.1)	8	35.8, C	
N-Me-Ala					
1	170.1, C		9	25.8, CH_3 × 3	0.83, s
2	49.8, CH	5.69, q (6.8)	10	19.2, CH_3	0.98, d (6.7)
3	14.9, CH_3	1.232, d (6.8)	11	16.7, CH_3	1.75, d (0.9)
N-Me	28.5, CH_3	2.30, s			

[a]Measured at 100 MHz. [b]Measured at 400 MHz.

(Pro). Moreover, the presence of a residue derived from 7-hydroxy-2,5,8,8-tetramethylnon-5-enoic acid (polyketide moiety) was elucidated. The configuration of the C-5−C-6 olefinic bond in the polyketide moiety was determined to be *E* on the basis of the two NOESY correlations in the polyketide moiety (H-4b/H-6 and H-7/H-11). The chemical shift of the vinyl methyl carbon at C-11 (δ_C 16.7) also supported this result.[9] The connectivity of these partial structures was determined based on the following six HMBC correlations: H-7 of the polyketide moiety/C-1 of *N*-Me-Leu, *N*-Me of *N*-Me-Leu/C-1 of *N*-Me-Ala, *N*-Me of *N*-Me-Ala/C-1 of Gly, NH of Gly/C-1 of Val, NH of Val/C-1 of Pro, and H-2 of Pro/C-1 of the polyketide moiety. As a result, the gross structure of **1** was established as shown in Figure 1.

The absolute configurations of the amino acids were determined by chiral-phase HPLC analyses and Marfey's analysis[10] of the acid hydrolysate of **1**. These analyses revealed that all of the amino acids had L-configurations (Supporting Information S16−19). The absolute configuration of the two stereogenic centers in the polyketide moiety was elucidated based on a combination of degradation reactions and spectroscopic analyses as follows. Ozonolysis and oxidative workup of **1** followed by acid hydrolysis afforded 2-methyl-5-oxohexanoic acid and 2-hydroxy-3,3-dimethylbutanoic acid derived from the polyketide moiety. Condensation of both compounds with (*S*)- or (*R*)-PGME gave the corresponding phenyl-glycine methyl ester (PGME) amides **2a** and **2b** from 2-methyl-5-oxohexanoic acid and **3a** and **3b** from 2-hydroxy-3,3-dimethylbutanoic acid, respectively. The ^{1}H NMR chemical shifts of **2a** and **2b** were compared, and calculated $\Delta\delta$ values revealed that the absolute configuration of C-2 was 2*R* (Scheme 1; S15).[11] To determine the absolute configuration of the remaining stereogenic center at C-7, we synthesized the (*R*)-PGME amide of (*S*)-2-hydroxy-3,3-dimethylbutanoic acid[12] as an authentic sample, and its ^{1}H NMR spectrum was compared with those of **3a** and **3b**. As a result, the spectrum of the (*S*)-PGME amide (**3a**) matched with that of the authentic sample, and it was clarified that the stereogenic center at C-2 of the 2-hydroxy-3,3-dimethylbutanoic acid from **1** was *R* (S11−13). Therefore, the absolute configuration of C-7 was determined to be 7*S*, and the absolute configuration of janadolide was established as shown in **1**. On the basis of the NMR data, we conducted preliminary molecular modeling of janadolide and proposed its lowest energy conformation (S23−24).

With regard to the biological activity of janadolide (**1**), it showed potent antitrypanosomal activity against *Trypanosoma brucei brucei* GUTat 3.1 strain (causative agent of Nagana disease in animals) with an IC_{50} value of 47 nM, which was more potent than a commonly used therapeutic drug, suramin (IC_{50} 1.2 μM). On the other hand, **1** did not inhibit the growth

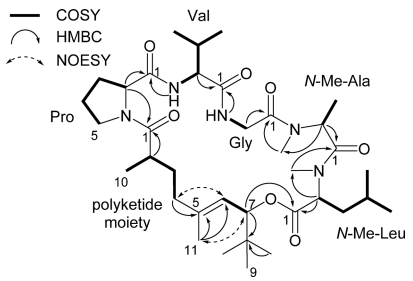
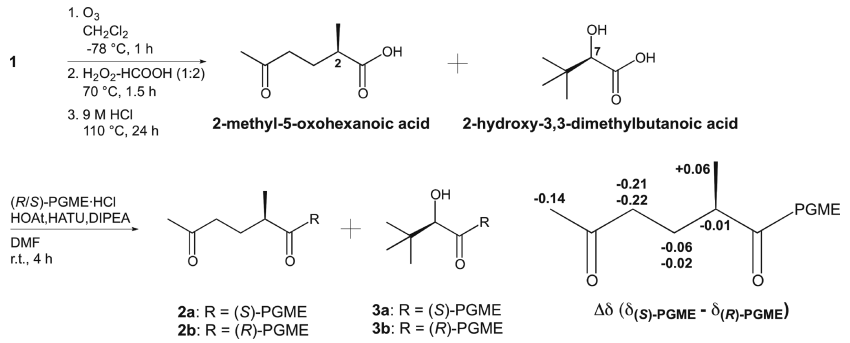


Figure 1. Gross structure of janadolide (**1**), based on 2D NMR correlations.

DOI: 10.1021/acs.jnatprod.6b00171
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 1. Preparation of the PGME Amides Derived from the Polyketide Moiety of Janadolide (1)



of human cells, such as MRC-5, HL60, and HeLa cells, even at 10 μM.

Janadolide (**1**), a new cyclic polyketide−peptide hybrid possessing a *tert*-butyl group, was isolated from an *Okeania* sp. marine cyanobacterium. The gross structure was elucidated by spectroscopic analyses, and the absolute configuration of the amino acid moieties was determined by acid hydrolysis and chiral-phase HPLC analyses. The absolute configuration of two stereogenic centers in the polyketide moiety was elucidated based on a combination of degradation reactions and spectroscopic analyses including the PGME method.

So far, approximately 20 *tert*-butyl-containing metabolites, such as bastimolide A,[13] nuiapolide,[14] and the amantelides,[15] have been discovered from marine cyanobacteria, and some of these compounds are classified as PKS−NRPS hybrids, including the antillatoxins[6,7] and the apratoxins.[8] The discovery of these *tert*-butyl-containing compounds including janadolide (**1**) indicates the usefulness of marine cyanobacteria as good sources of natural products with interesting structures.

In addition, janadolide (**1**) showed potent antitrypanosomal activity without cytotoxicity against human cells, and this indicates that further investigation should be undertaken to evaluate the possibility of janadolide and its synthetic analogues being used to develop new antitrypanosomal drugs.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR spectra were recorded on a JASCORT/IR-4200 instrument. Chemicals and solvents were the best grade available and used as received from commercial sources. All NMR spectroscopic data were recorded on a JEOL ECX-400 spectrometer for ^{1}H (400 MHz) and ^{13}C (100 MHz). ^{1}H NMR chemical shifts (referenced to residual C_6HD_5 observed at δ_H 7.16 and CHD_2OD observed at δ_H 3.31) were assigned using a combination of data from COSY and HMQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to residual C_6D_6 observed at δ_C 128.06 and CD_3OD observed at δ_C 49.00) were assigned based on HMBC and HMQC experiments. ESI mass spectra were obtained on an LCT premier EX spectrometer (Waters). Chromatographic analyses were performed using an HPLC system consisting of a pump (model PU-2080, Jasco) and a UV detector (model UV-2075, Jasco). Semi-preparative HPLC was performed on a Cosmosil series (Nacalai Tesque).

Collection, Extraction, and Isolation. The marine cyanobacterium was collected at the coast near Janado, Okinawa Prefecture, Japan, at a depth of 0−1 m in May 2013. The collected cyanobacterium (700 g, wet weight) was extracted with MeOH (5 L) for a month. The extract was filtered and concentrated. The residue was partitioned between EtOAc (3 × 0.3 L) and H_2O (0.3 L). The material obtained from the organic layer was partitioned between 90% aqueous MeOH (0.3 L) and hexanes (3 × 0.3 L). The aqueous MeOH fraction (778 mg) was separated by column chromatography on ODS (7.8 g) eluted with 40% MeOH, 60% MeOH, 80% MeOH, MeOH, and $CHCl_3$−MeOH (1:1). The fraction (366 mg) that eluted with 80% MeOH was subjected to HPLC [Cosmosil 5C_{18}AR-II (ϕ 20 × 250 mm); flow rate 5 mL/min; detection, UV 215 nm; solvent 64% MeOH] in eight batches to give a fraction containing janadolide (288 mg, t_R = after 88.2 min, last collected fraction). The fraction was further separated by HPLC [Cosmosil 5C_{18}AR-II (ϕ 20 × 250 mm); flow rate 5 mL/min; detection, UV 215 nm; solvent 75% MeOH] in six batches to give a fraction containing janadolide (107 mg, t_R = before 116 min, first collected fraction). The fraction was further separated by HPLC [Cosmosil 5C_{18}AR-II (ϕ 20 × 250 mm); flow rate 5 mL/min; detection, UV 215 nm; solvent 78% MeOH] in three batches to give janadolide (**1**) (6.9 mg, t_R = 41.1 min, total yield 0.000 99% based on wet weight).

Janadolide (1): colorless oil; $[\alpha]^{28}_D$ −88 (*c* 0.05, MeOH); IR (film) ν_{max} 2959, 1733, 1652, 1635, 1456 cm^{-1}; ^{1}H NMR and ^{13}C NMR, Table 1; HRESIMS *m*/*z* 676.4650 (calcd for $C_{36}H_{62}N_5O_7$, 676.4649).

Identification of the Marine Cyanobacterium. The marine cyanobacterium was morphologically classified into the genus *Okeania* sp.,[16] because the cells were 14.5 ± 1.3 μm wide (n = 10) and 3.3 ± 0.7 μm long (n = 10), the sheaths were 1.8 ± 0.2 μm thick (n = 10), and the apical cells were sometimes attenuated with calyptra. A voucher specimen, named 1305-10, has been deposited at Keio University. In addition, we tried to identify the cyanobacterium based on the 16S rRNA gene. Despite our efforts, we could not obtain any sequences corresponding to marine cyanobacteria.

To verify the fact that janadolide was produced by an *Okeania* sp. marine cyanobacterium, we collected a janadolide-producing cyanobacterium, named 1504-15, at Minnajima Island, Okinawa, Japan, at a depth of 0−1 m in April 2015. The morphological features of this sample perfectly matched those of the previous sample collected at Janado, and janadolide was isolated from this sample by the same procedures described above. Therefore, the identification of this cyanobacterium based on the 16S rRNA gene was carried out as follows.

A cyanobacterial filament was isolated under a microscope and crushed with freezing and thawing. The 16S rRNA genes were PCR-amplified from isolated DNA using the primer set CYA 106F, a cyanobacterial-specific primer, and 23S 30R, a cyanobacterial-specific primer. The PCR reaction contained DNA derived from a cyanobacterial filament, 0.5 μL of KOD-Multi & Epi- (TOYOBO), 12.5 μL of 2× PCR buffer for KOD-Multi & Epi- (TOYOBO), 1.0 μL of each primer (10 pM), and H_2O for a total volume of 25 μL. The PCR reaction was performed as follows: initial denaturation for 10 min at 94 °C, amplification by 40 cycles of 10 s at 98 °C, 10 s at 60 °C, and 1 min at 68 °C, and final elongation for 7 min at 68 °C. PCR products were analyzed on agarose gel (1%) in TBE buffer, visualized by ethidium bromide staining, and purified by a PCR Advanced PCR clean up system (VIOGENE). Sequences were determined with CYA 106F, 16S 1541R, and 23S 30R primers by a commercial firm (Macrogen Japan Corp.). These sequences are available in the DDBJ/EMBL/GenBank databases under accession number LC149728. From the phylogenetic tree inferred from 573 bp of the 16S-23S internal

DOI: 10.1021/acs.jnatprod.6b00171
J. Nat. Prod. XXXX, XXX, XXX−XXX

transcribed spacer (ITS) gene sequences revealed that the present cyanobacterium (accession no. LC149728) formed a clade with *Okeania* (S28, Figure S3). Therefore, the cyanobacterium was classified into the genus *Okeania*.

Acid Hydrolysis of Janadolide (1). Janadolide (**1**) (0.5 mg, 0.7 μmol) and 9 M HCl (0.1 mL) were charged in a reaction tube, which was sealed under reduced pressure and heated at 110 °C for 24 h. The mixture was evaporated to dryness and was separated into each component by HPLC. The retention times of components were as follows: *N*-Me-Ala (t_R = 3.0 min), *N*-Me-Leu (t_R = 6.0 min), Pro (t_R = 3.2 min), Val (t_R = 3.4 min) [Conditions for HPLC separation: column, Cosmosil 5C_{18}-PAQ (ϕ 4.6 × 250 mm); flow rate 1.0 mL/min; detection at 215 nm; solvent H_2O].

Chiral-Phase HPLC Analysis of Amino Acid Components, *N*-Me-Leu, Pro, Val. Each fraction that contained amino acids except for *N*-Me-Ala was dissolved in H_2O (50 μL) and analyzed by chiral-phase HPLC, and the retention times were compared to those of authentic standards. The retention times of *N*-Me-Leu, Pro, and Val in the hydrolysate matched those of *N*-Me-L-Leu (t_R = 17.9 min), L-Pro (t_R = 4.8 min), and L-Val (t_R = 6.0 min), but not *N*-Me-D-Leu (t_R = 10.3 min), D-Pro (t_R = 2.7 min), and D-Val (t_R = 3.4 min) [DAICEL CHIRALPAK (MA+) (ϕ 4.6 × 50 mm); flow rate 1.0 mL/min; detection 254 nm; solvent 2.0 mM $CuSO_4$].

Marfey's Analysis of *N*-Me-Ala. The fraction containing *N*-Me-Ala was dissolved in H_2O (100 μL). Marfey's reagent (1.0% 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide) solution in acetone (200 μL) and 50 μL of 1 M $NaHCO_3$ were added, and the mixture was heated at 80 °C for 3 min. The solution was cooled to room temperature (rt), neutralized with 1 M HCl, and evaporated to dryness. The residue was resuspended in 200 μL of MeCN−H_2O (1:1), and the solution was analyzed by reversed-phase HPLC.[17] The retention time of the derivatized *N*-Me-Ala in the hydrolysate matched that of the Marfey's derivative of the *N*-Me-L-Ala authentic sample (t_R = 12.7 min), but not Marfey's derivative of the *N*-Me-D-Ala authentic sample (t_R = 21.8 min) [Cosmosil Cholester (ϕ 4.6 × 250 mm); flow rate 1.0 mL/min; detection at 340 nm; solvent 0.02 M NaOAc aq−MeOH (45/55)].

Ozonolysis and Acid Hydrolysis of 1 (2-Methyl-5-oxohexanoic Acid and 2-Hydroxy-3,3-dimethylbutanoic Acid). Janadolide (**1**) used in this reaction was additionally isolated from the same cyanobacterium collected at Minnajima Island by the same procedures described above. Ozone was bubbled through a stirred solution of janadolide (**1**, 9.7 mg, 14 μmol) dissolved in CH_2Cl_2 (1.0 mL) at −78 °C for 40 min. The reaction mixture was concentrated and treated with 1 mL of H_2O_2−HCOOH (1:2) at 70 °C for 4 h. After removal of the solvent *in vacuo*, the oxidized product and 9 M HCl (0.2 mL) were charged in a reaction tube, which was sealed under reduced pressure and heated at 110 °C for 24 h. Then, 2 mL of H_2O was added to the reaction mixture, and the mixture was extracted three times with EtOAc. The combined organic layers were concentrated *in vacuo* to afford the crude product containing 2-methyl-5-oxohexanoic acid and 2-hydroxy-3,3-dimethylbutanoic acid (2.0 mg, 2-methyl-5-oxohexanoic acid:2-hydroxy-3,3-dimethylbutanoic acid = 3:1).

PGME Derivatives of the Natural 2-Methyl-5-oxohexanoic Acid and 2-Hydroxy-3,3-dimethylbutanoic Acid (2a, 2b, 3a, and 3b). A half-portion of the crude product (1.0 mg) containing 2-methyl-5-oxohexanoic acid and 2-hydroxy-3,3-dimethylbutanoic acid was mixed with *O*-(7-azabenzotriazoll-yl)-l,l,3,3-tetramethyluronium hexafluorophosphate (HATU) (4.0 mg, 10 μmol), 1-hydroxy-7-azabenzotriazole (HOAt) (1.9 mg, 14 μmol), (*S*)-PGME·HCl (2.0 mg, 9.9 μmol), diisopropylethylamine (DIEA) (50 μL), and DMF (50 μL), which was allowed to stir at rt. After 3.5 h, 1.5 mL of EtOAc was added to the reaction mixture. The organic layer was washed with saturated aqueous NH_4Cl (3 × 1.0 mL), concentrated, and purified by reversed-phase HPLC to give the (*S*)-PGME amide of the natural 2-methyl-5-oxohexanoic acid (**2a**) (0.3 mg, 1 μmol, t_R = 11.3 min) and (*S*)-PGME amide of the natural 2-hydroxy-3,3-dimethylbutanoic acid (**3a**) (0.2 mg, 0.7 μmol, t_R = 12.6 min), respectively [Cosmosil 5C_{18}MS-II (ϕ 20 × 250 mm); flow rate 5 mL/min; detection, UV 215 nm; solvent 80% MeCN].

2a: ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.40−7.33 (5H, m), 5.48 (1H, s), 3.71 (3H, s), 2.46 (1H, m), 2.37 (1H, t, *J* = 7.8 Hz), 2.36 (1H, dd, *J* = 8.4, 6.3 Hz), 2.03 (3H, s), 1.75 (1H, m), 1.63 (1H, m), 1.14 (3H, d, *J* = 6.8 Hz); HRESIMS *m/z* 292.1553 (calcd for $C_{16}H_{22}NO_4$, 292.1549).

3a: ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.40−7.35 (5H, m), 5.47 (1H, s), 3.71 (3H, s), 3.69 (1H, s), 0.99 (9H, s); HRESIMS *m/z* 280.1540 (calcd for $C_{15}H_{22}NO_4$, 280.1549).

A portion of the crude product containing 2-methyl-5-oxohexanoic acid and 2-hydroxy-3,3-dimethylbutanoic acid (0.5 mg) was mixed with HATU (3.6 mg, 90 μmol), HOAt (1.9 mg, 14 μmol), (*R*)-PGME·HCl (1.2 mg, 5.9 μmol), DIEA (50 μL), and DMF (50 μL), which was allowed to stir at rt. After 4 h, the reaction mixture was separated as described above to give the (*R*)-PGME amide of the natural 2-methyl-5-oxohexanoic acid (**2b**) (0.1 mg, 0.3 μmol, t_R = 11.3 min) and (*R*)-PGME amide of the natural 2-hydroxy-3,3-dimethylbutanoic acid (**3b**) (0.1 mg, 0.3 μmol, t_R = 12.7 min), respectively.

2b: ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.40−7.33 (5H, m), 5.42 (1H, s), 3.71 (3H, s), 2.58 (2H, t, *J* = 7.6 Hz), 2.47 (1H, m), 2.17 (3H, s), 1.77 (1H, m), 1.69 (1H, m), 1.08 (3H, d, *J* = 7.0 Hz); HRESIMS *m/z* 292.1552 (calcd for $C_{16}H_{22}NO_4$, 292.1549).

3b: ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.40−7.34 (5H, m), 5.48 (1H, s), 3.71 (3H, s), 3.70 (1H, s), 0.93 (9H, s); HRESIMS *m/z* 280.1539 (calcd for $C_{15}H_{22}NO_4$, 280.1549).

Synthesis of the Authentic Sample of (*R*)-PGME Amide of (*S*)-2-Hydroxy-3,3-dimethylbutanoic acid. (*S*)-2-Hydroxy-3,3-dimethylbutanoic acid[12] (1.1 mg, 8.3 μmol) was mixed with HATU (3.6 mg, 90 μmol), HOAt (1.5 mg, 11 μmol), (*R*)-PGME·HCl (1.6 mg, 7.9 μmol), DIEA (50 μL), and DMF (50 μL), which was allowed to stir at rt. After 4 h, 1.5 mL of EtOAc was added to the reaction mixture. The organic layer was washed with saturated aqueous NH_4Cl (3 × 1.0 mL), concentrated, and purified by reversed-phase HPLC to give the (*R*)-PGME amide of (*S*)-2-hydroxy-3,3-dimethylbutanoic acid (0.2 mg, 0.7 μmol, 9%, t_R = 12.4 min) [Cosmosil 5C_{18}MS-II (ϕ 20 × 250 mm); flow rate 5 mL/min; detection, UV 215 nm; solvent 80% MeCN].

(R)-PGME amide of (S)-2-hydroxy-3,3-dimethylbutanoic acid: ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.40−7.34 (5H, m), 5.47 (1H, s), 3.71 (3H, s), 3.69 (1H, s), 0.99 (9H, s); HRESIMS *m/z* 280.1544 (calcd for $C_{15}H_{22}NO_4$, 280.1549).

Cell Growth Analysis. Cell proliferation of HeLa cells and HL60 cells was measured by the MTT assay as described previously.[5] Measurement of cytotoxic activity against human fetal lung fibroblast MRC-5 cells was carried out as described previously.[18]

***In Vitro* Antitrypanosomal Assay.** The bloodstream forms of *T. b. b.* strain GUTat 3.1 parasites were used for experimentation, as described previously.[19] The strain GUTat 3.1 was cultured in IMDM with various supplements and 10% heat-inactivated fetal bovine serum at 37 °C under 5.0% CO_2−95% air. Subsequently, 95 μL of the parasite suspension ((2.0−2.5) × 10^4 trypanosomes mL^{-1}) was transferred to a 96-well microtiter plate, and 5.0 μL of a 25% MeOH solution of **1** was added, followed by incubation for 72 h at 37 °C. Then, 10 μL of Alamer Blue solution was added to each well. After incubation for 3−6 h, the resulting solution was read at 528/620 nm excitation wavelengths and 590/630 nm emission wavelengths by an FLx800 fluorescence microplate reader (BioTek Instruments, Inc.). The IC_{50} value was determined using fluorescent plate reader software (KC-4, BioTek Instruments, Inc.).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00171.

^{1}H, ^{13}C, COSY, HMQC, HMBC, and NOESY NMR spectra in C_6D_6 and ^{1}H, ^{1}H−^{1}H *J* resolved and NOESY NMR spectra in $CDCl_3$ for janadolide (**1**); ^{1}H NMR spectra in CD_3OD for **2a**, **2b**, **3a**, **3b**, and the authentic sample of (*R*)-PGME amide of (*S*)-2-hydroxy-3,3-

DOI: 10.1021/acs.jnatprod.6b00171
J. Nat. Prod. XXXX, XXX, XXX−XXX

dimethylbutanoic acid; HPLC chromatograms for determination of the absolute configurations; molecular modeling based on NMR data of janadolide (**1**) (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: suenaga@chem.keio.ac.jp.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (24310160 and 20436992), for the Promotion of Science, the Uehara Memorial Foundation, and the Keio Gijuku Fukuzawa Memorial Fund for the Advancement of Education and Research.

REFERENCES

(1) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2015**, *32*, 116−211.

(2) Kleigrewe, K.; Gerwick, L.; Sherman, D. H.; Gerwick, W. H. *Nat. Prod. Rep.* **2016**, *33*, 348−364.

(3) Iwasaki, A.; Ohno, O.; Sumimoto, S.; Ogawa, H.; Nguyen, K. A.; Suenaga, K. *Org. Lett.* **2015**, *17*, 652−655.

(4) Teruya, T.; Sasaki, H.; Fukazawa, H.; Suenaga, K. *Org. Lett.* **2009**, *11*, 5062−5065.

(5) Iwasaki, A.; Ohno, O.; Sumimoto, S.; Suda, S.; Suenaga, K. *RSC Adv.* **2014**, *4*, 12840−12843.

(6) Orjala, J.; Nagle, D. G.; Hsu, V. L.; Gerwick, W. H. *J. Am. Chem. Soc.* **1995**, *117*, 8281−8282.

(7) Nogle, L. M.; Okino, T.; Gerwick, W. H. *J. Nat. Prod.* **2001**, *64*, 983−985.

(8) (a) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Corbett, T. H. *J. Am. Chem. Soc.* **2001**, *123*, 5418−5423. (b) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *Bioorg. Med. Chem.* **2002**, *10*, 1973−1978. (c) Gutiérrez, M.; Suyama, T. L.; Engene, N.; Wingerd, J. S.; Matainaho, T.; Gerwick, W. H. *J. Nat. Prod.* **2008**, *71*, 1099−1103. (d) Matthew, S.; Schupp, P. J.; Luesch, H. *J. Nat. Prod.* **2008**, *71*, 1113−1116. (e) Tidgewell, K.; Engene, N.; Byrum, T.; Media, J.; Doi, T.; Valeriote, F. A.; Gerwick, W. H. *ChemBioChem* **2010**, *11*, 1458−1466. (f) Thornburg, C. C.; Cowley, E. S.; Sikorska, J.; Shaala, L. A.; Ishmael, J. E.; Youssef, D. T. A.; McPhail, K. L. *J. Nat. Prod.* **2013**, *76*, 1781−1788.

(9) Carey, L.; Clough, J. M.; Pattenden, G. *J. Chem. Soc., Perkin Trans. 1* **1983**, *12*, 3005−3009.

(10) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(11) Nagai, Y.; Kusumi, T. *Tetrahedron Lett.* **1995**, *36*, 1853−1856.

(12) Boobalan, R.; Chen, C.; Lee, G.-H. *Org. Biomol. Chem.* **2012**, *10*, 1625−1638.

(13) Shao, C.-L.; Linington, R. G.; Balunas, M. J.; Centeno, A.; Boudreau, P.; Zhang, C.; Engene, N.; Spadafora, C.; Mutka, T. S.; Kyle, D. E.; Gerwick, L.; Wang, C.-Y.; Gerwick, W. H. *J. Org. Chem.* **2015**, *80*, 7849−7855.

(14) Mori, S.; Williams, H.; Cagle, D.; Karanovich, K.; Horgen, F. D.; Smith, R., III; Watanabe, C. M. H. *Mar. Drugs* **2015**, *13*, 6274−6290.

(15) Salvador-Reyes, L. A.; Sneed, J.; Paul, V. J.; Luesch, H. *J. Nat. Prod.* **2015**, *78*, 1957−1962.

(16) Engene, N.; Paul, V. J.; Byrum, T.; Gerwick, W. H.; Thor, A.; Ellisman, M. K. *J. Phycol.* **2013**, *49*, 1095−1106.

(17) Under typical Marfey's condition with L-FDAA, the L- and D-*N*-Me-Ala derivatives are not separable on a standard C-18 column. We resolved this problem by using L-FDLA and the Cosmosil-Cholester column.

(18) Otoguro, K.; Kohana, A.; Manabe, C.; Ishiyama, A.; Ui, H.; Shiomi, K.; Yamada, H.; Ōmura, S. *J. Antibiot.* **2001**, *54*, 658−663.

(19) Otoguro, K.; Ishiyama, A.; Namatame, M.; Nishihara, A.; Furusawa, T.; Masuma, R.; Shiomi, K.; Takahashi, Y.; Yamada, H.; Ōmura, S. *J. Antibiot.* **2008**, *61*, 372−378.

DOI: 10.1021/acs.jnatprod.6b00171
J. Nat. Prod. XXXX, XXX, XXX−XXX