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Note

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Varitatin A, a Highly Modified Fatty Acid Amide from *Penicillium variabile* Cultured with a DNA Methyltransferase Inhibitor

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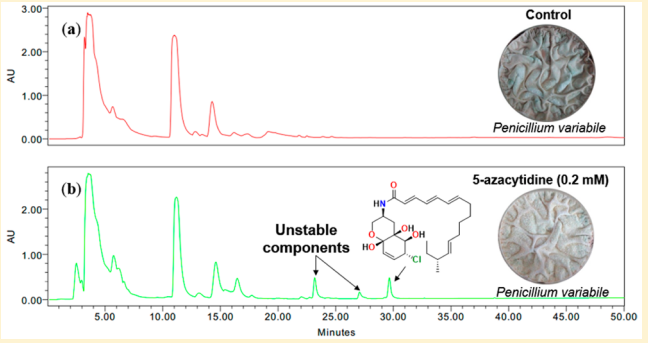
Supporting Information

ABSTRACT: A new, highly modified fatty acid amide, varitatin A (**1**), was isolated from the fungus *Penicillium variabile* HXQ-H-1 cultivated with the DNA methyltransferase inhibitor 5-azacytidine. The structure including the absolute configuration of **1** was established by analysis of NMR and MS data, together with chemical degradation and Mosher's method based on MPA esters. Compound **1** showed cytotoxicity against HCT-116 cells with an IC_{50} value of 2.8 μM and also inhibited the effects of protein tyrosine kinases.

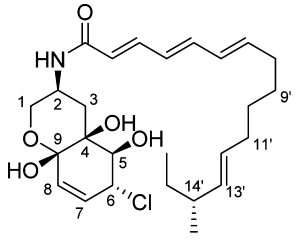


Marine-derived fungi are an excellent source of novel molecules for drug discovery and have attracted great attention of chemists and biologists.[1] Given that most of the fungal biosynthetic gene clusters are cryptic under classic laboratory culture conditions,[2] many efforts such as the OSMAC approach, epigenetic modification, and genome mining, have been devoted to activate cryptic pathways for exploration of new molecules.[3−5] Among them, epigenetic manipulation has proved to be effective by inhibiting the activities of histone deacetylase (HDAC) and DNA methyltransferase (DNMT), which are involved in gene silencing.[6,7]

During our efforts to tap the potential of marine-derived fungi to produce diverse secondary metabolites, several HDAC and DNMT inhibitors were tested on some selected fungal strains. Among them, the *Penicillium variabile* strain HXQ-H-1 was found to be sensitive to 5-azacytidine, which led to the appearance of new peaks in the HPLC-UV profile, indicating that additional molecules were induced by the DNMT inhibitor. The chemical investigation of the fermentation extract led to the discovery of a new compound, named varitatin A (**1**), which showed cytotoxicity against the HCT-116 cell line (IC_{50} = 2.8 μM) and also inhibited the effects of protein tyrosine kinases (PTKs). Herein, we describe the isolation, structure elucidation, and bioactivity.

The *P. variabile* strain was cultured at 20 °C under static conditions with the presence of 0.2 mM 5-azacytidine. The EtOAc extract of the fementation (15 L) was injected to repeated silica gel, Sephadex LH20, and preparative HPLC, yielding the new compound **1** (100 mg).

Varitatin A (**1**) was isolated as a colorless oil, and the molecular formula was determined as $C_{26}H_{38}ClNO_5$ based on the protonated peak at m/z 480.2520 in the HRESIMS spectrum. The ^1H and ^{13}C NMR data of compound **1** displayed



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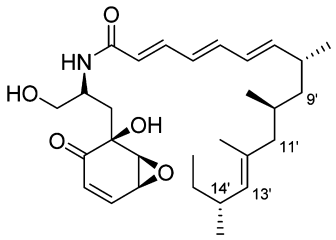

Scyphostatin

the resonances of three nonprotonated carbons including a carbonyl (δ_C 165.9), a hemiketal (δ_C 95.1), and an oxygenated (δ_C 75.0) one, seven methylenes, 14 methines including 10 olefinic and four sp^3 methines, and two methyls (Table 1). The planar structure of **1** was established by the 2D NMR data. A hydrogenated chromene ring was deduced by the COSY correlations (H-1/H-2/H-3, H-5/H-6/H-7/H-8) and the HMBC correlations from H-1 and H-7 to C-9, from 9-OH to C-4, C-8, and C-9, and from H-3 to C-4, C-5, and C-9. The additional COSY correlations between H-5 and 5-OH and the HMBC correlations from 4-OH to C-3, C-4, C-5, and C-9 located the two hydroxy groups. The fragment from C-1′ to C-17′ was constructed by the COSY correlations from H-2′ to H-17′ and HMBC correlations from H-2′ and H-3′ to C-1′. The fatty acid chain was further connected to the chromene ring via a nitrogen atom, evidenced by the COSY correlation between NH (δ_H 7.08) and H-2 and the HMBC correlations from NH to C-2 and C-1′. Finally, the gross structure was constructed by attaching the chlorine atom to C-6 with the consideration of

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Table 1. ^{1}H (500 MHz) and ^{13}C (150 MHz) NMR Data for 1 in Acetone-d_6

	varitatin A	
position	δ_C, type	δ_H (J in Hz)
1	63.2, CH_2	3.66, dd (10.2, 5.2), H-1α
		3.57, dd (10.7, 10.2), H-1β
2	44.1, CH	4.15−4.08, m
3	34.6, CH_2	2.52, dd (12.1, 4.5), H-3α
		1.76, dd (12.5, 12.1), H-3β
4	75.0, C	
5	74.7, CH	4.12, dd (8.0, 7.7)
6	63.1, CH	4.67, ddd (8.0, 2.1, 1.6)
7	129.5, CH	5.72, dd (10.2, 2.1)
8	130.5, CH	5.56, dd (10.2, 1.6)
9	95.1, C	
1′	165.9, C	
2′	124.6, CH	5.98, d (15.0)
3′	141.0, CH	7.16, dd (15.0, 11.4)
4′	129.3, CH	6.25, dd (14.8, 11.4)
5′	140.2, CH	6.56, dd (14.8, 10.8)
6′	131.1, CH	6.18, dd (15.1, 10.8)
7′	139.5, CH	5.93, dt (15.1, 6.9)
8′	33.4, CH_2	2.14, q (6.9)
9′	29.3, CH_2	1.48−1.34, m
10′	30.0, CH_2	1.48−1.34, m
11′	33.0, CH_2	2.00, q (6.8)
12′	137.1, CH	5.38, dd (15.3, 6.8)
13′	129.3, CH	5.27, dd (15.3, 7.6)
14′	39.2, CH	1.98−1.92, m
15′	30.5, CH_2	1.33−1.20, m
16′	12.1, CH_3	0.83, t (7.4)
17′	20.9, CH_3	0.94, d (6.7)
NH		7.08, d (7.6)
4-OH		3.75, s
5-OH		4.76, d (7.7)
9-OH		5.34, s

the chemical shift of CH-6 (δ_H 4.67 and δ_C 63.1) and the molecular formula.

The four double bonds in the fatty acid chain were all assigned the *E* geometry on the basis of the coupling constants ($^3J_{2'-3'}$ = 15.0 Hz, $^3J_{4'-5'}$ = 14.8 Hz, $^3J_{6'-7'}$ = 15.1, and $^3J_{12'-13'}$ = 15.3 Hz) and the NOESY correlations (Figure 1). The relative

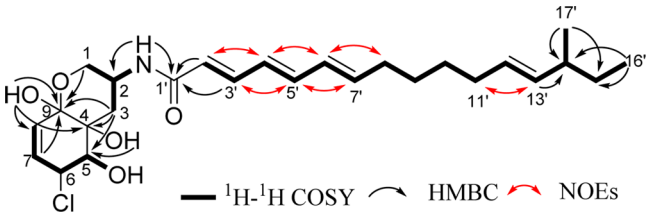

Figure 1. Selected 2D NMR correlations of **1**.

configuration of the bicyclic core was determined based on the NOESY correlations and coupling constants. The NOESY cross-peaks of 9-OH/H-1β/H-3β/4-OH/H-6 suggested that these groups were on the same face of the chromene ring and the axial orientation of H-1β and H-3β. The coupling constant between H-1β and H-2 ($^3J_{1\beta\text{-}2}$ = 10.7 Hz) and between H-2 and H-3β ($^3J_{2-3\beta}$ = 12.5 Hz) suggested that H-2 was axial and oriented toward the other face of the chromene ring opposite H-1β and H-3β. The vicinal coupling constant between H-5 and H-6 ($^3J_{5-6}$ = 8.0 Hz) indicated their *anti* orientation (Figure 2).[16] However, the absolute configuration of C-14′ cannot be determined by NMR data.

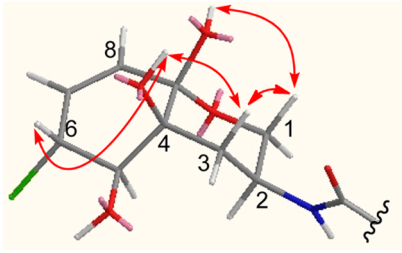

Figure 2. Key NOESY correlations of **1**.

The absolute configuration of the chromene ring was determined by making the MPA esters of **1**.[8] The $\Delta\delta^{RS}$ values between **2a** and **2b** (*R*- and *S*-MPA esters of **1** on 5-OH, respectively) were negative for H-5/6/7 and positive for H-1/2/3/8 (Figure 3), which indicated the 5*R* configuration. Then the 2*S*, 4*S*, 5*R*, 6*R*, 9*S* absolute configuration of the bicyclic core moiety of **1** was deduced and also confirmed by the proton spectra of **3a** and **3b** (Figure 3), which were obtained during the formation of **2a** and **2b**. For the two sets of proton signals for an ethanol group linked to a stereogenic carbon with the *S* configuration, Minale and co-workers have proved that those in the *R*-MTPA derivatives are closer than those in the *S*-MTPA ester, which agreed with the data for **3a** and **3b**.[9]

To determine the stand-alone stereogenic center C-14′ in the fatty acid chain, compound **1** was oxidatively digested by $RuCl_3$−$NaIO_4$ to form 2-methylbutyric acid, which was further esterified by 2-bromo-1-(4-bromophenyl)ethanone to generate 2-(4-bromophenyl)-2-oxoethyl 2-methylbutanoate (BOMB) (**4**).[10−12] By comparing the HPLC traces on a chiral-phase column of **4** (t_R = 9.6 min) with the BOMBs derived from commercially available (*S*)-(+)-2-methylbutyric acid (**5**: t_R = 10.4 min) and the racemic-2-methylbutyric acid (t_R = 10.4 and 9.6 min) (Figure 4), the absolute configuration of C-14′ was determined as *R*.

The cytotoxicity of compound **1** was evaluated against HCT-116 cells, showing an IC_{50} value of 2.8 μM. In addition, the inhibitory activities against a panel of PTKs were also screened *in vitro* using the enzyme-linked immunosorbent assay (ELISA),[13] indicating that compound **1** could inhibit the activity of PDGFR-β and ErbB4 kinases (inhibitory rates of 50% and 40% at a concentration of 1 μM, respectively) (Table S1 in the Supporting Information).

A literature survey showed that the most similar structure to compound **1** is scyphostatin, which was isolated from the fungus *Trichopeziza mollissima*.[14] Although the biogenetically related analogues such as the dankastatins, gymnastatins, and isariotins were discovered from various fungi including *Gymnascella dankaliensis*,[15] *Isaria tenuipes*,[16] *Arachniotus punctatus*,[17] *Ramaria madagascariensis*,[18] and *Gibellula formosana* (with the presence of a DNA methyltransferase inhibitor RG-108),[19] it is the first time that this kind of highly modified fatty acid amide was isolated from a *Penicillum* strain.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained on a JASCO-1020 digital polarimeter. UV spectra were

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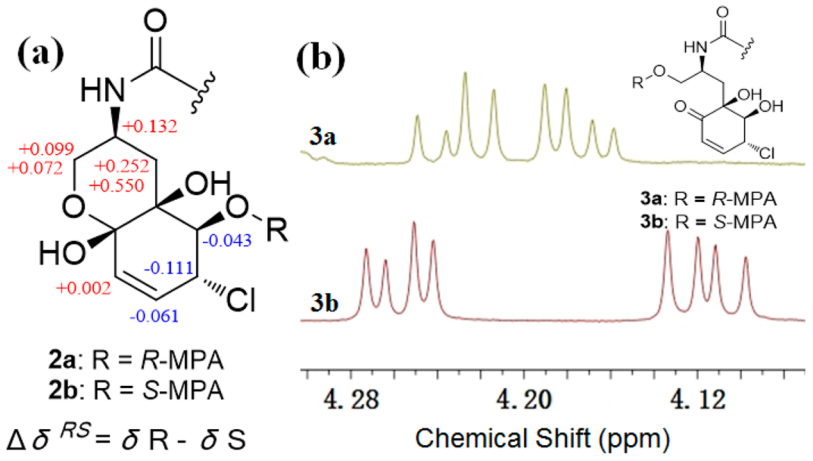

Figure 3. (a) $\Delta\delta^{RS}$ values of **2a** and **2b**. (b) Proton spectra of **3a** and **3b**.

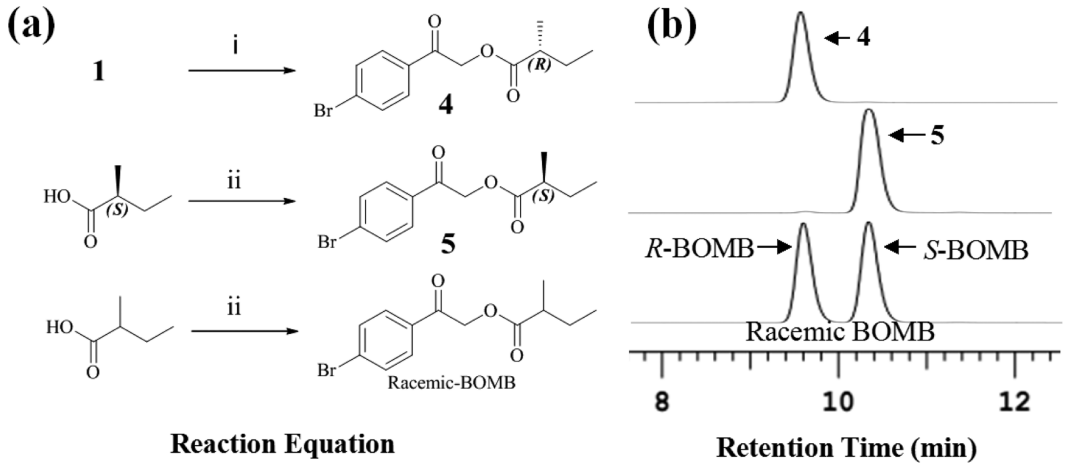

Figure 4. (a) Formation of compounds **4**, (*S*)-**5**, and *racemic*-BOMB. (i. $RuCl_3/NaIO_4$; ii. *p*-bromophenacyl bromide−KF−DMF). (b) HPLC analysis on a chiral Daicel Chiralpack IC column (detected at 254 nm).

recorded on a Waters 2487, while the ECD spectrum was measured on a JASCO J-815 spectropolarimeter. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer as KBr disks. ^{1}H NMR, ^{13}C NMR, DEPT, and 2D NMR spectra were recorded on an Agilent 500 MHz DD2 spectrometer. HRESIMS and ESIMS data were obtained using a Thermo Scientific LTQ Orbitrap XL mass spectrometer. Column chromatography (CC) was performed on silica gel (100−400 mesh, Qingdao Marine Chemical Factory), Sephadex LH-20 (Amersham Biosciences), and ODS resin (50 mm, Merck). Preparative HPLC collection used a C_{18} column (YMC-Pack ODS-A, 10 × 250 mm, 5 μm, 3 mL/min). Chiral-phase HPLC used a Daicel Chiralpack IC column (4.6 × 250 mm, 5 μm, 1 mL/min). (*S*)-(+)-2-Methylbutyric acid and *racemic*-2-methylbutyric acid were supplied by Acros Organics and Aladdin Industrial Corporation, respectively.

Fungal Material. The fungal strain HXQ-H-1 was isolated from the mangrove rhizosphere soil collected on the coast of Fujian Province (China) and identified as *Penicillium variabile* based on sequencing of the ITS region (GenBank no. KT429657) with 100% similarity to *P. variabile*. The strain was deposited at the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, People's Republic of China.

Fermentation. Erlenmeyer flasks (1 L) containing 300 mL fermentation media were directly inoculated with spores. The media contained maltose (20 g), mannitol (20 g), glucose (10 g), sodium glutamate (10 g), yeast extract (3 g), corn syrup (1 g), KH_2PO_4 (0.5 g), and $MgSO_4{\cdot}7H_2O$ (0.3 g) dissolved in 1 L of naturally collected seawater (Huiquan Bay, Yellow Sea) in the presence of 0.2 mM 5-azacytidine. The flasks were cultured under static conditions at 20 °C for 3 weeks.

Extraction and Purification. The whole fermentation broth (15 L) was filtered through cheesecloth to separate the supernatant from the mycelia. The supernatant was extracted with EtOAc (3 × 15 L), and the mycelia were macerated and extracted with acetone (3 × 5 L). All extracts were evaporated under reduced pressure to give a crude gum (10.0 g). The extract was separated by VLC on silica gel using CH_2Cl_2−MeOH (20:1) to give five fractions (fractions 1 to 5). Fraction 3 was further separated on a Sephadex LH-20 column with MeOH to provide five subfractions (fractions 3-1 to 3-5). Fraction 3-3 was separated by MPLC and then semipreparative HPLC eluted with MeOH−H_2O (80:20), to obtain compound **1** (100.0 mg, t_R = 30.5 min).

*Varitatin A (**1**):* colorless oil; $[\alpha]^{24}_D$ −22.0 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 300 (4.01) nm; ECD (1 mM MeOH) λ_{max} ($\Delta\varepsilon$) 259 (+1.0), 284 (+0.5), 293 (+0.7), 307 (+0.2), 312 (+0.3), 326 (−0.2), 369 (+0.2) nm; IR (KBr) ν_{max} 3296, 2958, 2926, 2854, 1657, 1605, 1369, 1279, 1161, 1045, 1003 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 480.2520 $[M + H]^+$ (calcd for $C_{26}H_{39}{}^{35}ClNO_5$, 480.2511).

Cytotoxicity Assay. The cytotoxic activity of **1** was evaluated using the HCT-116 cell line (human colon cancer) by using the SRB method,[20] with doxorubicin (IC_{50} = 0.2 μM) as the positive control.

Preparation of MPA Esters Derived from 1 (2a/2b and 3a/3b). The sample of varitatin A (**1**) (0.5 mg each) was treated with (*R*)- or (*S*)-MPA (0.4 mg) with DCC (0.5 mg) and DMAP (0.3 mg) in dry CH_2Cl_2 (0.5 mL). The reaction was stirred 3 h at 0 °C. The solvent

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was removed *in vacuo*, and the productions were individually purified by RP-HPLC eluting with MeOH−H_2O (85:15) to obtain the (*R*)-MPA ester (**2a**/**3a**) and (*S*)-MPA ester (**2b**/**3b**), respectively.

*(R)-MPA Ester (**2a**):* colorless oil; ^{1}H NMR (500 MHz, acetone) δ_H 7.53−7.28 (m, 5H), 7.16 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.56 (dd, *J* = 14.9, 10.6 Hz, 1H), 6.26 (dd, *J* = 14.8, 11.3 Hz, 1H), 6.18 (dd, *J* = 15.2, 10.7 Hz, 1H), 5.95 (d, *J* = 14.9 Hz, 1H), 5.96−5.89 (m, 1H), 5.72 (dd, *J* = 10.2, 2.1 Hz, 1H), 5.69 (d, *J* = 8.6 Hz, 1H), 5.63 (dd, *J* = 10.2, 1.9 Hz, 1H), 5.37 (dd, *J* = 14.3, 7.7 Hz, 1H), 5.27 (dd, *J* = 15.3, 7.7 Hz, 1H), 5.01 (s, 1H), 4.74 (dt, *J* = 8.6, 2.0 Hz, 1H), 4.25−4.15 (m, 1H), 3.70 (dd, *J* = 10.2, 4.7 Hz, 1H), 3.59 (t, *J* = 10.9 Hz, 1H), 3.49 (s, 3H), 2.15 (q, *J* = 7.0 Hz, 2H), 2.01 (dd, *J* = 13.6, 6.8 Hz, 2H), 1.98−1.92 (m, 1H), 1.85 (dd, J = 13.8, 6.5 Hz, 1H), 1.80 (t, *J* = 12.9 Hz, 1H), 1.50−1.34 (m, 4H), 1.33−1.20 (m, 2H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H); HRESIMS *m*/*z* 650.2852 $[M + Na]^+$ (calcd for $C_{35}H_{46}{}^{35}ClNO_7Na$, 650.2855).

*(S)-MPA Ester (**2b**):* colorless oil; ^{1}H NMR (500 MHz, acetone) δ_H 7.51−7.27 (m, 5H), 7.21 (dd, *J* = 14.9, 11.1 Hz, 1H), 6.59 (dd, *J* = 14.8, 10.8 Hz, 1H), 6.29 (dd, *J* = 14.8, 11.2 Hz, 1H), 6.20 (dd, *J* = 15.0, 10.4 Hz, 1H), 5.97 (d, *J* = 14.8 Hz, 1H), 5.97−5.90 (m, 1H), 5.78 (dd, *J* = 10.2, 2.0 Hz, 1H), 5.73 (d, *J* = 8.7 Hz, 1H), 5.63 (dd, *J* = 10.2, 2.1 Hz, 1H), 5.37 (dd, *J* = 14.6, 7.8 Hz, 1H), 5.27 (dd, *J* = 15.2, 7.6 Hz, 1H), 4.93 (s, 1H), 4.85 (dt, *J* = 8.5, 2.0 Hz, 1H), 4.10−4.05 (m, 1H), 3.63 (dd, *J* = 9.2, 4.8 Hz, 1H), 3.49 (t, *J* = 10.7 Hz, 1H), 3.40 (s, 3H), 2.16 (q, *J* = 6.6 Hz, 2H), 2.01 (dd, *J* = 13.4, 6.5 Hz, 2H), 1.98−1.93 (m, 1H), 1.55 (t, *J* = 12.8 Hz, 1H), 1.48−1.35 (m, 4H), 1.30 (m, 1H), 1.29−1.21 (m, 2H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H); HRESIMS *m*/*z* 650.2854 $[M + Na]^+$ (calcd for $C_{35}H_{46}{}^{35}ClNO_7Na$, 650.2855).

*(R)-MPA Ester (**3a**):* colorless oil; ^{1}H NMR (500 MHz, acetone) δ_H 7.46−7.32 (m, 5H), 7.12 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.83 (dd, *J* = 10.2, 2.8 Hz, 1H), 6.57 (dd, *J* = 14.8, 10.8 Hz, 1H), 6.26 (dd, *J* = 14.8, 11.3 Hz, 1H), 6.20 (dd, *J* = 15.1, 10.7 Hz, 1H), 5.99 (dd, *J* = 10.2, 1.8 Hz, 1H), 5.98−5.91 (m, 1H), 5.88 (d, *J* = 15.0 Hz, 1H), 5.37 (dd, *J* = 14.5, 7.5 Hz, 1H), 5.27 (dd, *J* = 15.3, 7.7 Hz, 1H), 4.95−4.90 (m, 1H), 4.86 (s, 1H), 4.37−4.29 (m, 1H), 4.23 (dd, *J* = 11.0, 6.6 Hz, 1H), 4.17 (dd, *J* = 11.0, 4.9 Hz, 1H), 3.95 (d, *J* = 5.8 Hz, 1H), 3.37 (s, 3H), 2.22 (dd, *J* = 14.6, 7.9 Hz, 1H), 2.15 (q, *J* = 6.8 Hz, 2H), 2.09 (dd, *J* = 11.4, 3.2 Hz, 1H), 2.01 (dd, *J* = 13.4, 6.5 Hz, 2H), 1.98−1.91 (m, 1H), 1.46−1.37 (m, 4H), 1.33−1.22 (m, 2H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H); ESIMS *m*/*z* 650.48 $[M + Na]^+$.

*(S)-MPA Ester (**3b**):* colorless oil; ^{1}H NMR (500 MHz, acetone) δ_H 7.46−7.28 (m, 5H), 7.13 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.83 (dd, *J* = 10.2, 2.8 Hz, 1H), 6.58 (dd, *J* = 14.9, 10.7 Hz, 1H), 6.27 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.20 (dd, *J* = 15.1, 10.7 Hz, 1H), 6.00 (dd, *J* = 10.2, 1.8 Hz, 1H), 5.98−5.91 (m, 1H), 5.91 (d, *J* = 15.0 Hz, 1H), 5.37 (dd, *J* = 14.4, 7.7 Hz, 1H), 5.27 (dd, *J* = 15.3, 7.6 Hz, 1H), 4.96−4.89 (m, 1H), 4.84 (s, 1H), 4.40−4.30 (m, 1H), 4.26 (dd, *J* = 11.0, 4.4 Hz, 1H), 4.12 (dd, *J* = 11.0, 7.0 Hz, 1H), 3.96 (d, *J* = 5.9 Hz, 1H), 3.38 (s, 3H), 2.21 (dd, *J* = 14.6, 7.8 Hz, 1H), 2.16 (q, *J* = 7.2 Hz, 2H), 2.08 (dd, *J* = 6.1, 2.0 Hz, 1H), 2.01 (dd, *J* = 13.7, 6.8 Hz, 2H), 1.98−1.91 (m, 1H), 1.50−1.35 (m, 4H), 1.33−1.19 (m, 2H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H); ESIMS *m*/*z* 650.48 $[M + Na]^+$.

Preparation of 2-(4-Bromophenyl)-2-oxoethyl 2-Methylbutanoate. Varitatin A (**1**) (5 mg) was dissolved in MeCN−CCl_4−H_2O (2:2:3, 0.7 mL), and then $NaIO_4$ (20 mg) and $RuCl_3$ (1 mg) were added. After stirring overnight at room temperature (rt), the product was filtered through a small plug of silica eluting with CH_2Cl_2 (10 mL), then removed *in vacuo*. The mixture was further dissolved in DMF (0.5 mL) and treated with 2-bromo-1-(4-bromophenyl)-ethanone (20 mg) and KF (10 mg) at rt for 3 h. The product was purified by RP-HPLC eluting with MeOH−H_2O (70:30), to obtain 2-(4-bromophenyl)-2-oxoethyl 2-methylbutanoate (**4**). Similarly, the (*S*)-(**5**) and *racemic*-BOMB were obtained from the commercial (*S*)-(+)-2-methylbutyric acid and *racemic*-2-methylbutyric acid.

*(S)-2-(4-Bromophenyl)-2-oxoethyl 2-methylbutanoate (**5**):* white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.5 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 5.27 (s, 2H), 2.58−2.52 (m, 1H), 1.86−1.69 (m, 1H), 1.60−1.48 (m, 1H), 1.22 (dd, *J* = 14.5, 6.6 Hz, 3H), 0.98 (t, *J* = 7.4 Hz, 3H); HRESIMS *m*/*z* 299.0277 $[M + H]^+$ (calcd for $C_{13}H_{16}BrO_3$, 299.0277).

The NMR and MS data obtained for the racemic-2-(4-bromophenyl)-2-oxoethyl 2-methylbutanoate mixture were the same as those for compound **5**.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00742.

The MS and NMR spectra for compounds **1**, **2a**, **2b**, **3a**, **3b**, **5**, and the *racemic*-BOMB and the IR spectrum and proposed biosynthetic pathway of **1** (PDF)

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The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160−258. (b) Gerwick, W. H.; Moore, B. S. *Chem. Biol.* **2012**, *19*, 85−98.

(2) (a) Scherlach, K.; Hertweck, C. *Org. Biomol. Chem.* **2009**, *7*, 1753−1760. (b) Sanchez, J. F.; Somoza, A. D.; Keller, N. P.; Wang, C. C. *Nat. Prod. Rep.* **2012**, *29*, 351−371.

(3) (a) Bode, H. B.; Bethe, B.; Hofs, R.; Zeeck, A. *ChemBioChem* **2002**, *3*, 619−627. (b) Cai, S.; Luan, Y.; Kong, X.; Zhu, T.; Gu, Q.; Li, D. *Org. Lett.* **2013**, *15*, 2168−2171.

(4) Cichewicz, R. H. *Nat. Prod. Rep.* **2010**, *27*, 11−22.

(5) Wiemann, P.; Keller, N. P. *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 301−313.

(6) Asai, T.; Yamamoto, T.; Shirata, N.; Taniguchi, T.; Monde, K.; Fujii, I.; Gomi, K.; Oshima, Y. *Org. Lett.* **2013**, *15*, 3346−3349.

(7) Asai, T.; Otsuki, S.; Sakurai, H.; Yamashita, K.; Ozeki, T.; Oshima, Y. *Org. Lett.* **2013**, *15*, 2058−2061.

(8) Latypov, Sh. K.; Seco, J. M.; Quinoa, E.; Riguera, R. *J. Org. Chem.* **1996**, *61*, 8569−8577.

(9) De Ricciardis, F.; Minale, L.; Riccio, R.; Giovannitti, B.; Iorizzi, M.; Debitus, C. *Gazz. Chim. Ital.* **1993**, *123*, 79−86.

(10) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, L. B. *J. Org. Chem.* **1981**, *46*, 3936−3938.

(11) Clark, J. H.; Miller, J. M. *Tetrahedron Lett.* **1977**, *7*, 599−602.

(12) Saito, S.; Tanaka, N.; Fujimoto, K.; Kogen, H. *Org. Lett.* **2000**, *2*, 505−506.

(13) Lin, L. G.; Xie, H.; Li, H. L.; Tong, L. J.; Tang, C. P.; Ke, C. Q.; Liu, Q. F.; Lin, L. P.; Geng, M. Y.; Jiang, H. L.; Zhao, W. M.; Ding, J.; Ye, Y. *J. Med. Chem.* **2008**, *51*, 4419−4429.

(14) Tanaka, M.; Nara, F.; Suzuki-Konagai, K.; Hosoya, T.; Ogita, T. *J. Am. Chem. Soc.* **1997**, *119*, 7871−7872.

(15) Amagata, T.; Minoura, K.; Numata, A. *J. Nat. Prod.* **2006**, *69*, 1384−1388.

DOI: 10.1021/acs.jnatprod.5b00742
J. Nat. Prod. XXXX, XXX, XXX−XXX

(16) Bunyapaiboonsri, T.; Yoiprommarat, S.; Intereya, K.; Rachtawee, P.; Hywel-Jones, N. L.; Isaka, M. *J. Nat. Prod.* **2009**, *72*, 756−759.
(17) Phoon, C. W.; Somanadhan, B.; Heng, S. C. H.; Ngo, A.; Ng, S. B.; Butler, M. S.; Buss, A. D.; Sim, M. M. *Tetrahedron* **2004**, *60*, 11619−11628.
(18) Liu, D.-Z.; Li, J.-G.; Zhang, M.-W.; Liu, G. *J. Antibiot.* **2015**, *68*, 137−138.
(19) Asai, T.; Chung, Y.-M.; Sakurai, H.; Ozeki, T.; Chang, F.-R.; Wu, Y.-C.; Yamashita, K.; Oshima, Y. *Tetrahedron* **2012**, *68*, 5817−5823.
(20) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

DOI: 10.1021/acs.jnatprod.5b00742
J. Nat. Prod. XXXX, XXX, XXX−XXX