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Varioxiranols A−G and 19-*O*-Methyl-22-methoxypre-shamixanthone, PKS and Hybrid PKS-Derived Metabolites from a Sponge-Associated *Emericella variecolor* Fungus

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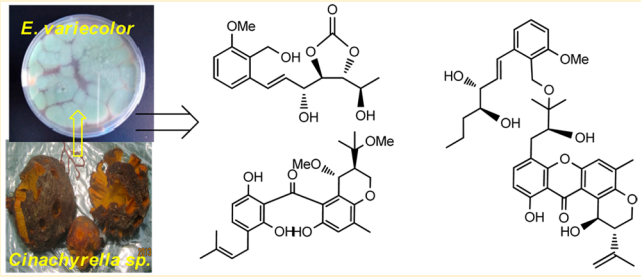
Supporting Information

ABSTRACT: Chemical examination of a sponge (*Cinachyrella* sp.)-associated *Emericella variecolor* fungus resulted in the isolation of seven new polyketide derivatives, namely, varioxiranols A−G (**1**−**7**), and a new hybrid PKS-isoprenoid metabolite, 19-*O*-methyl-22-methoxypre-shamixanthone (**8**), together with nine known analogues. Their structures were elucidated on the basis of extensive spectroscopic analyses, including ECD effects, Mosher's method, X-ray diffraction, and chemical conversion for the determination of absolute configurations. Varioxiranols F and G were found for the first time to link a xanthone moiety with a benzyl alcohol via an ether bond, while the dioxolanone group of **5** is unusual in nature. A cell-based lipid-lowering assay revealed that pre-shamixanthone (**12**) exerted significant inhibition against lipid accumulation in HepG2 cells without cytotoxic effects, accompanying the potent reduction of total cholesterol and triglycerides. Real-time quantitative PCR indicated that pre-shamixanthone (**12**) mediated the reduction of lipid accumulation related to the down-regulation of the expression of the key lipogenic transcriptional factor SREBP-1c and its downstream genes encoding FAS and ACC.



The fungus *Emericella variecolor*, a teleomorph of *Aspergillus variecolor* (synonym of *A. stellatus* and *A. stellifer*), is widely distributed in marine and terrestrial environments.[1] Previous chemical investigation of *E. variecolor* uncovered a variety of natural products with diverse and unusual scaffolds. A strain of *E. variecolor* derived from a Caribbean sponge produced benzyl alcohols (varitriol, varioxirane), prenylxanthones (varixanthone, shamixanthone, tajixanthone hydrate), and cyclopentanones,[2] while the same species associated with the sponge *Haliclona valliculata* generated anthraquinones, prenylxanthones (isoemericellin, shamixanthone), and stromemycin.[3] In addition, a strain from marine sediments afforded sesterterpenes with various scaffolds (ophiobolins,[4] astellatol,[5] andilesin A,[6] variecolin[7]) and the unique polyketide shimalactone A.[8] A UV-induced mutant strain of *A. variecolor* produced meroterpenoid metabolites such as andibenin B.[9] Alternatively, a strain of *A. variecolor* from terrestrial locations generated the secondary metabolites, which differed from those produced in marine-derived strains, as exemplified by the presence of isoechinulin-type alkaloids,[10] quinine-related derivatives,[11] xanthones,[12] and others.[13,14] These findings indicate that *E. variecolor* possesses diverse biosynthesis gene clusters and that ecological impacts may induce structural modifications of the secondary metabolites.[15,16]

In our program aimed at discovery of bioactive natural products from sponge-derived microorganisms, the fungus *E. variecolor* XSA-07-2, isolated from a *Cinachyrella* sp. sponge in the South China Sea, showed a remarkable diversity of secondary metabolites in a preliminary HPLC and NMR screening and was therefore subjected to detailed chemical analysis.

RESULTS AND DISCUSSION

Chromatographic separation of the EtOAc extract of *E. variecolor* XSA-07-2 cultured on solid rice medium, including semipreparative HPLC purification, resulted in the isolation of 17 derivatives, of which eight are new compounds.

Varioxiranol A (**1**) had a molecular formula of $C_{15}H_{22}O_4$ as deduced by HRESIMS and NMR data. The IR absorption suggested the presence of a hydroxy group (3251 cm^{-1}). The ^1H NMR spectrum exhibited aromatic signals for an ABC spin system at δ_H 6.87 (d, J = 8.0 Hz, H-3), 7.22 (t, J = 8.0 Hz, H-4),

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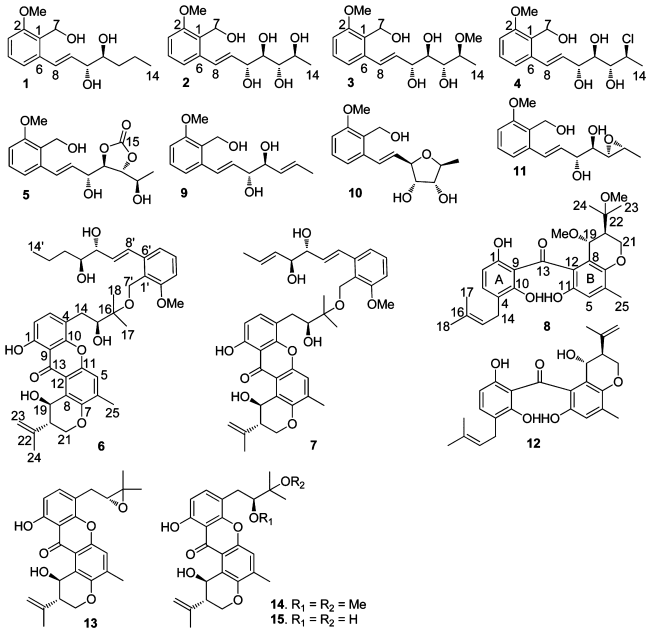

and 7.10 (d, J = 8.0 Hz, H-5), indicating the presence of a trisubstituted aromatic ring. The substitutions of a hydroxymethylene (δ_H 4.54 d, J = 5.2 Hz, H_2-7) and a methoxy group (δ_C 56.1, δ_H 3.78, s) at C-1 (δ_C 127.0) and C-2 (δ_C 158.0), respectively, were assigned by the HMBC interactions of the aromatic carbons with the protons of the substituted groups. In addition, COSY correlations established a linear side chain from C-8 to C-14, in which a double bond with E geometry ($J_{H\text{-}8/H\text{-}9}$ = 16 Hz) resided at C-8 (δ_C 127.5) and C-9 (δ_C 134.1). Additional COSY correlations of the oxymethine H-10 (δ_H 3.95, ddd, J = 6.0, 5.2, 4.0 Hz) and H-11 (δ_H 3.39, ddt, J = 5.6, 5.2, 4.0 Hz) with the D_2O exchangeable protons (δ_H 4.84 and 4.42), respectively, confirmed C-10 (δ_C 75.6) and C-11 (δ_C 74.0) to be hydroxylated. Thus, the side chain was identified as an (*E*)-hept-1-ene-3,4-diol segment, which was linked to C-6 (δ_C 138.9) of the aromatic ring, based on the HMBC interaction of H-8 (δ_H 6.94, d, J = 16 Hz) with C-1, C-5 (δ_C 118.5) and C-6 (δ_C 138.9). The relative configuration of C-10 and C-11 was determined by the $^3J_{H,H}$ value in association with the NOE interactions.[17,18] The $^3J_{H\text{-}10/H\text{-}11}$ value (4.0 Hz) was indicative of a *gauche* relationship of H-10 and H-11. The NOE

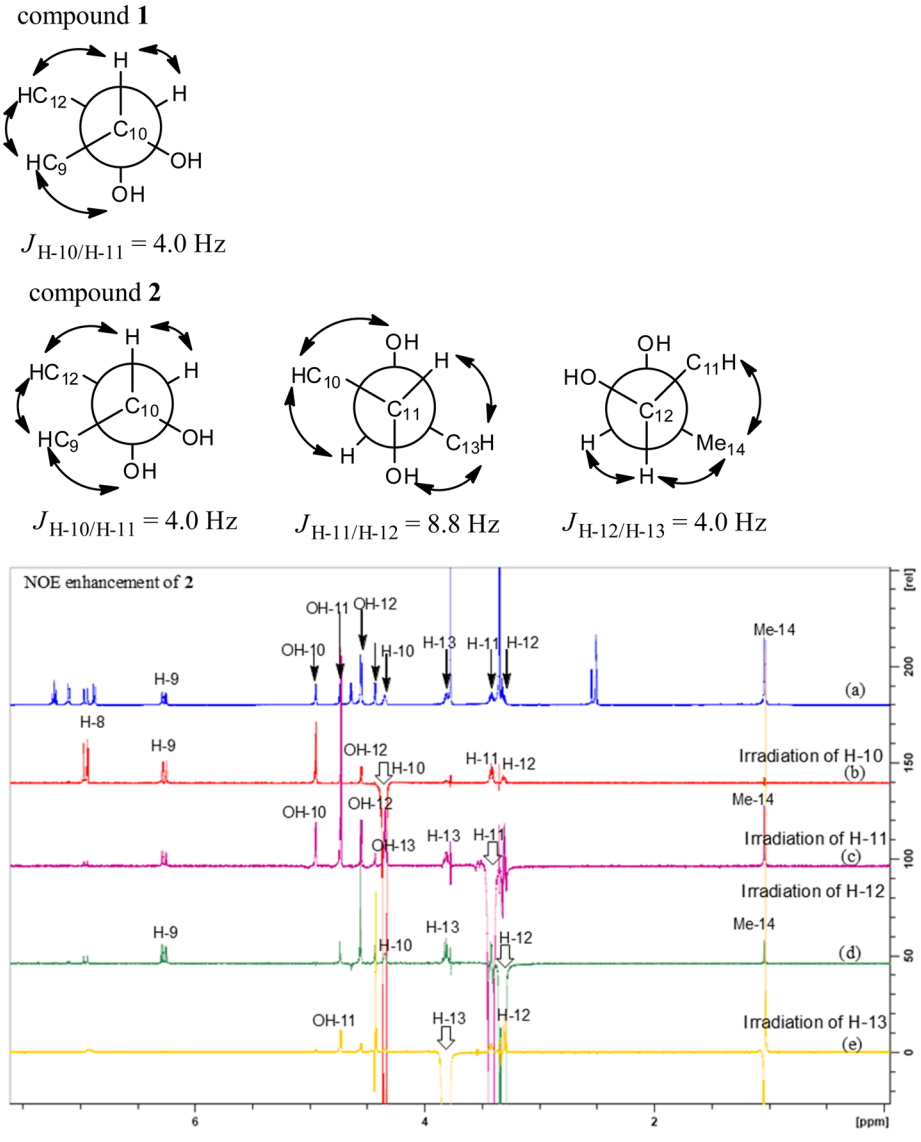

Figure 1. Key NOE interactions of compounds **1** and **2**.

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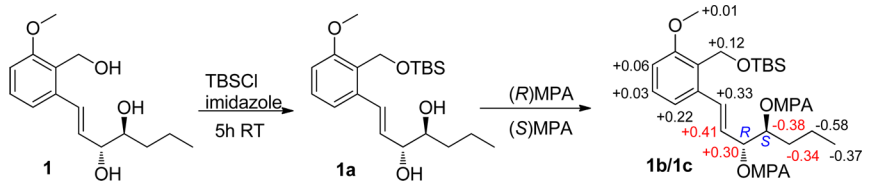

Figure 2. $\Delta\delta^{RS}$ ($\delta_R - \delta_S$) values (in ppm) for the MPA esters of **1a**.

Table 1. ^{1}H and ^{13}C NMR Data of 1−5[a,b]

	1		2		3		4		5	
no.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	127.0, C		127.0, C		127.0, C		127.0, C		127.3, C	
2	158.0, C		158.0, C		158.0, C		158.0, C		157.9, C	
3	110.1, CH	6.87, d (8.0)	110.1, CH	6.92, d (8.0)	110.1, CH	6.87, d (8.0)	110.2, CH	6.88, d (8.0)	110.6, CH	6.90, d (8.0)
4	128.8, CH	7.22, t (8.0)	128.8, CH	7.23, t (8.0)	128.8, CH	7.23, t (8.0)	128.9, CH	7.23, t (8.0)	129.0, CH	7.23, t (8.0)
5	118.5, CH	7.10, d (8.0)	118.5, CH	7.19, d (8.0)	118.4, CH	7.10, d (8.0)	118.5, CH	7.10, d (8.0)	118.4, CH	7.06, d (8.0)
6	138.9, C		138.9, C		138.9, C		138.7, C		138.1, C	
7	54.0, CH_2	4.54, d (5.2)	53.9, CH_2	4.55, d (5.3)	53.9, CH_2	4.55, s	53.9, CH_2	4.56, d (5.1)	53.8, CH_2	4.55, s
8	127.5, CH	6.94, d (16.0)	127.9, CH	6.95, d (16.0)	128.0, CH	6.95, d (16.0)	128.4, CH	6.95, d (16.0)	129.4, CH	7.15, d (16.0)
9	134.1, CH	6.27, dd (16.0, 6.0)	133.0, CH	6.27, dd (16.0, 7.2)	132.9, CH	6.26, dd (16.0, 7.2)	132.3, CH	6.24, dd (16.0, 7.6)	129.7, CH	6.11, dd (16.0, 4.8)
10	75.6, CH	3.95, ddd (6.0, 5.2, 4.0)	73.4, CH	4.34, ddd (7.2, 5.0, 4.0)	73.5, CH	4.34, dd (7.2, 4.0)	73.4, CH	4.34, ddd (7.6, 5.1, 4.0)	70.7, CH	4.54, dd (4.8, 3.0)
11	74.0, CH	3.39, ddt (5.6, 5.2, 4.0)	76.1, CH	3.40, ddd (8.8, 5.0, 4.0)	74.9, CH	3.36, dd (8.8, 4.0)	75.2, CH	3.58, ddd (8.4, 5.9, 4.0)	78.3, CH	4.67, dd (3.6, 3.0)
12	35.3, CH_2	1.30, m; 1.48, m	75.3, CH	3.30, ddd (8.8, 5.0, 4.0)	72.1, CH	3.59, dd (8.8, 3.2)	75.2, CH	3.41, ddd (8.4, 5.9, 3.6)	79.6, CH	4.44, dd (3.6, 3.0)
13	19.0, CH_2	1.30, m; 1.48, m	67.8, CH	3.80, ddq (6.3, 5.0, 4.0)	77.3, CH	3.52, qd (6.3, 3.2)	61.3, CH	4.48, dq (6.8, 3.6)	65.8, CH	3.84, dq (6.5, 3.6)
14	14.6, CH_3	0.88, t (6.9)	17.9, CH_3	1.04, d (6.3)	13.0, CH_3	1.04, d (6.3)	17.9, CH_3	1.39, d (6.8)	18.3, CH_3	0.92, d (6.5)
15									155.3, C	
7-OH		4.64, t (5.2)		4.63, t (5.3)				4.65, t (5.1)		4.55, br
10-OH		4.84, d (5.2)		4.94, d (5.0)				5.00, d (5.1)		5.89, br
11-OH		4.42, d (5.6)		4.73, d (5.0)				4.90, d (5.9)		3.50, br
12-OH				4.54, d (5.0)				5.23, d (5.9)		
13-OH				4.42, d (,5.0)						5.31, brs
2-OMe	56.1, CH_3	3.78, s	56.1, CH_3	3.78, s	56.1, CH_3	3.77, s	56.1, CH_3	3.78, s	56.1, CH_3	3.77 s
13-OMe					55.8, CH_3	3.20, s				

[a]Chemical shifts are in ppm; J values in Hz are in parentheses. [b]Measured at 400 MHz in DMSO-d_6.

interactions between H-9/H-12, H-10/H-12, and H-9/OH-11 (Figure 1) in association with the absence of an NOE interaction between H-10/OH-11 and OH-10/H-12 suggested the relative configuration at the side chain of **1** to be 10*R** and 11*S**, which were the same as those of the known analogue (1*E*,5*E*)-1-(2-(hydroxymethyl)-3-methoxyphenyl)hepta-1,5-diene-3,4-diol (**9**).[19] The absolute configuration of **1** was determined on the basis of the modified Mosher's method used for the determination of the configuration of the acyclic 1,2-diol.[20] The hydroxy group OH-7 was first protected by TBSCl to yield the analogue **1a**. Treatment of **1a** with (*R*)- and (*S*)-MPA, respectively, afforded (*R*)- and (*S*)-MPA diesters (**1b** and **1c**). Diagnostic $\Delta\delta^{RS}$ ($\delta_{(R)\text{-MPA ester}} - \delta_{(S)\text{-MPA ester}}$) data (Figure 2) resulted in positive $\Delta\delta^{RS}$ values for H-9 and H-10, whereas H-11 and H-12 showed negative $\Delta\delta^{RS}$ values. These data were in accordance with 10*R* and 11*S* configurations, which were the same as those of **9**, whose enantiomer was previously obtained using total synthesis.[19]

Varioxiranol B (**2**) had a molecular formula of $C_{15}H_{22}O_6$ according to its HRESIMS and NMR data. The NMR data of **2** (Table 1) were comparable to those of **1**, while 2D NMR spectroscopic analyses established both **1** and **2** contained the same backbone. The significant differences were attributed to the side chain, where two additional oxymethines were present. The COSY and HMQC cross-peaks assigned the oxymethines to C-12 (δ_C 75.3) and C-13 (δ_C 67.8), while the COSY relationships between H-12 (δ_H 3.30)/OH-12 (δ_H 4.54 d, J = 5.0 Hz) and H-13 (δ_H 3.80)/OH-13 (δ_H 4.42 d, J = 5.0 Hz) allowed the substitution of OH groups at C-12 and C-13, respectively. The HMBC interaction from H_3-14 (δ_H 1.04, d, J = 6.3 Hz) to C-12 and C-13 further supported the assignments. The $J_{H\text{-}8/H\text{-}9}$ value (16.0 Hz) was indicative of the *E* geometry of the double bond. The coupling constants of $^3J_{H\text{-}10/H\text{-}11}$ (4.0 Hz), $^3J_{H\text{-}11/H\text{-}12}$ (8.8 Hz), and $^3J_{H\text{-}12/H\text{-}13}$ (4.0 Hz) reflected the *gauche* relationships between H-10/H-11 and H-12/H-13, as well as the *anti*-orientation for H-11/H-12. These data suggested the side chain of **2** adopted a single dominant conformer.[18] The NOE interactions in association with the $^3J_{H,H}$ values helped to assign the relative configuration of the side chain. In the 1D NOE experiment, irradiation of H-10 induced the NOE enhancement of H-8, H-11, H-12, and OH-12 (Figure 1b); irradiation of H-11 resulted in the NOE enhancement of OH-12, H-13, CH_3-14, and OH-12 (Figure 1c); in addition the

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NOE enhancements of H-10, H-13, and H_3-14 (Figure 1d) were observed when H-12 was irradiated in the 1D NOE experiment. These findings in association with the J values assigned the relative configuration to be 10*R**, 11*R**, 12*S**, and 13*S**. Acidic hydrolysis[21] of varioxirane (**11**) yielded a product (Figure 3) whose NMR data and specific rotation were

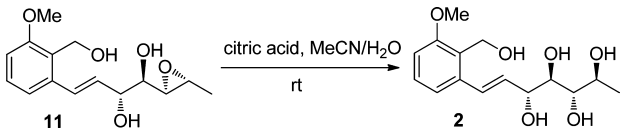

Figure 3. Acidic hydrolysis of **11** to **2**.

identical to those of **2**. As the absolute configuration of varioxirane was determined by total synthesis,[19] the stereogenic centers in **2** were confirmed as 10*R*, 11*R*, 12*S*, and 13*S*.

The HRESIMS and NMR data determined the molecular formula of varioxiranol C (**3**) ($C_{16}H_{24}O_6$) to be one CH_2 unit more than that of **2**. Apart from an additional methoxy group (δ_H 3.20, s) observed in **3**, the NMR spectroscopic data of both **2** and **3** were very similar. The 2D NMR (COSY, HMQC, and HMBC) data assigned **3** to be a 13-*O*-methyl analogue of **2**, as evident from the methoxy protons correlated to C-13 (δ_C 77.3) in the HMBC spectrum. The similar NOE interaction and J values of the side chain in both **2** and **3** (Table 1) indicated **3** shared the same relative configuration as **2**.

Varioxiranol D (**4**) was found to possess a molecular formula of $C_{15}H_{21}ClO_5$, as determined by the HRESIMS data, with a Cl atom to replace an OH group of **2**. The NMR data for the aromatic nucleus in **4** were identical to those of **2**, while the NMR data for the side chain of both **4** and **2** were mostly similar. The difference was attributed to the shielded C-13 (δ_C 61.3) and the deshielded H-13 (δ_H 4.48, dq, J = 6.8, 3.6 Hz) in comparison with those of **2**. These findings in association with the absence of OH at C-13 allowed assignment of a chlorinated C-13. On the basis of $^3J_{H,H}$ values between adjacent methines from H-8 to H-13 (Table 2) and NOE interactions similar to those of **2**, the configurations of the stereogenic centers in **4** were assumed to be the same as those of **2**.

The molecular formula of varioxiranol E (**5**) was determined as $C_{16}H_{20}O_7$ on the basis of its HRESIMS and NMR data, requiring seven degrees of unsaturation. The IR absorption at 1784 cm^{-1} suggested the presence of a dioxolanone functionality.[22,23] Inspection of the ^{13}C NMR spectrum revealed six aromatic carbons for a phenyl ring and two olefinic carbons for a double bond, which was similar to the benzyl alcohol skeleton such as **2**. The 2D NMR data established the molecular backbone of **5** to be the same as that of **2**, while the side chain carbons from C-10 (δ_C 70.7) to C-13 (δ_C 65.8) were oxygenated. The COSY correlations between H-10 (δ_H 4.54)/OH (δ_H 5.89) and H-13 (δ_H 3.84)/OH (δ_H 5.31) showed C-10 and C-13 to be hydroxylated. In addition, the HMBC interactions of the carbonyl carbon C-15 (δ_C 155.3) with both H-11 (δ_H 4.67) and H-12 (δ_H 4.44) established a 1,3-dioxolan-2-one ring, which was located between C-11 (δ_C 78.3) and C-12 (δ_C 79.6). The relative configuration of **5** was established by NOE and coupling constants of the side chain, while the stereogenic centers from C-10 to C-13 were all determined to be of the *R* configuration by the X-ray single-crystal diffraction analysis using the Flack parameter (Figure 4).

Varioxiranol F (**6**) had a molecular formula of $C_{40}H_{48}O_{10}$, which was established by the HRESIMS data. Analyses of 1D and 2D NMR data (Table 2) revealed the structure of **6** to consist of two moieties. Moiety A was identical to compound **1** based on the comparison of their NMR spectroscopic data. In regard to moiety B, its NMR data including 12 aromatic carbons and a carbonyl carbon at δ_C 184.5 (C-13) were characteristic of a xanthone nucleus, structurally related to tajixanthone hydrate,[12] also isolated from the same fraction. The COSY correlations of H-20 (δ_H 2.76, ddd, J = 2.8,2.9,3.0 Hz) with H_2-21 (δ_H 4.44, 4.36) and H-19 (δ_H 5.43, brd, J = 2.8 Hz), in association with the HMBC interactions from H-19 to the aromatic carbons C-7 (δ_C 149.5), C-8 (δ_C 121.1), and C-12 (δ_C 116.9) and between H_2-21 and C-7, showed the fusion of a tetrahydropyran ring to the aromatic ring at C-7 and C-8. An isopropene unit linked to C-20 (δ_C 44.9) and a hydroxy group bonded to C-19 (δ_C 63.2) were defined by the HMBC correlations of olefinic methylene H_2-23 (δ_H 4.60, 4.82) and the methyl protons H_3-24 (δ_H 1.87, s) to C-21 and C-20, in addition to the COSY correlation between H-19 and OH-19. Moreover, a 15,16-dioxygenated isopentane unit was recognized by the COSY correlation between H_2-14 (δ_H 3.11, 2.72) and H-15 (δ_H 3.92, dd, J = 10.0, 2.0 Hz), as well as the HMBC interactions from the methyl protons H_3-17 (δ_H 1.43, s) and H_3-18 (δ_H 1.50, s) to C-16 (δ_C 78.0) and C-15 (δ_C 76.6). This unit was positioned at the aromatic carbon C-4 (δ_C 116.9) according to the HMBC interactions from H_2-14 to C-3 (δ_C 138.2), C-4, and C-10 (δ_C 153.0) and between H-3 (δ_H 7.60, d, J = 8.4 Hz) and C-14 (δ_C 31.1). The remaining OH (δ_H 12.64, s) and a methyl unit (δ_H 2.36, s) were located at C-1 (δ_C 160.2) and C-6 (δ_C 138.3), respectively, on the basis of their HMBC interactions (Figure 5). The NOE interaction between H_3-24 and H-19 indicated the same orientation of H-19 and the isopropene unit. Accordingly, moiety B was identical to tajixanthone hydrate.[12] The HMBC interaction between H_2-7′ (δ_H 4.58, 4.74) and C-16 allowed the linkage of both moieties A and B through an ether bond across C-16 and C-7′. Acidic hydrolysis[24] of **6** yielded two products, with one product being identical to compound **1** based on the same NMR data and specific rotation (Figure 6). The 2D NMR data including NOESY data indicated the gross structure of the second product to be the same as tajixanthone hydrate.[12] Mosher's method in association with the NOE relationships supported the absolute configuration of the second product to have 15*S*, 19*R*, and 20*S* configurations, which are the same as those of tajixanthone hydrate.

The HRESIMS and NMR data provided the molecular formula of varioxiranol G (**7**) as $C_{40}H_{46}O_{10}$, with 2 amu less than that of **6**. The NMR data of **7** were closely similar to those of **6** (Table 2), while the 2D NMR data indicated the presence of two moieties. Comparison of the NMR data revealed that **7** possessed the same xanthone moiety (moiety B) as that of **6**, while the remaining moiety was identical to compound **9** by the evidence that two additional olefin protons (δ_H 5.54 and 5.79) were observed in the ^1H NMR spectrum. The location of the double bond at C-12′ and C-13′ was confirmed by the COSY correlation. The connection of C-7′ and C-16 through an ether bond was assembled by the HMBC interaction from H_2-7′ (δ_H 4.59, 4.71) to C-16 (δ_C 78.0). Acidic hydrolysis of **7** to afford compound **9** and tajixanthone hydrate supported the structural assignment, while the similar ECD data of both **6** and **7** (Figure 7) reinforced the same absolute configuration of both compounds.

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Table 2. ^{1}H and ^{13}C NMR Data of 6−8[a,b]

	6		7		8	
no.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	160.2, C		160.2, C		162.9, C	
2	109.9, CH	6.78, d (8.4)	109.8, CH	6.83, d (8.3)	109.0, CH	6.42, d (8.4)
3	138.2, CH	7.60, d (8.4)	138.2, CH	7.61, d (8.4)	137.8, CH	7.25, d (8.4)
4	116.9, C		117.0, C		123.6, C	
5	119.2, CH	7.21, s	119.2, CH	7.21, s	120.5, CH	6.82, s
6	138.3, C		138.2, C		133.3, C	
7	149.5, C		149.5, C		147.0, C	
8	121.1, C		121.1, C		119.5, C	
9	109.2, C		109.2, C		112.8, C	
10	153.0, C		153.0, C		157.2, C	
11	152.1, C		152.1, C		155.3, C	
12	116.9, C		117.0, C		122.5, C	
13	184.5, C		184.5, C		199.2, C	
14	31.1, CH_2	3.11, dd (14.3, 2.0)	31.2, CH_2	3.11, dd (14.2, 2.3)	27.6, CH_2	3.26, d (6.9)
		2.72, dd (14.3, 10.0)		2.72, dd (14.2, 10.0)		
15	76.6, CH	3.92, dd (10.0, 2.0)	76.6, CH	3.93, dd (10.0, 2.3)	121.8, CH	5.28, t (6.9)
16	78.0, C		78.0, C		133.5, C	
17	19.4, CH_3	1.43, s	19.7, CH_3	1.42, s	17.8, CH_3	1.76, s
18	22.1, CH_3	1.50, s	22.1, CH_3	1.50, s	25.8, CH_3	1.71, s
19	63.2, CH	5.43, brd (2.8)	63.2, CH	5.43, brd (2.6)	70.4, CH	4.57, brs
20	44.9, CH	2.76, ddd (2.8, 2.9, 3.0)	44.9, CH	2.75, m	45.0, CH	2.19, dd (9.8, 3.6)
21	64.6, CH_2	4.44, dd (10.8, 2.9)	64.6, CH_2	4.36, dd (10.7, 2.7)	63.0, CH_2	4.26, dd (10.5, 3.6)
		4.36, dd (10.8, 3.0)		4.44, dd (10.7, 3.2)		4.30, dd (10.5, 9.8)
22	142.6, C		142.6, C		75.0, C	
23	112.3, CH_2	4.60, s; 4.82, s	112.3, CH_2	4.60, s; 4.83, s	23.9, CH_3	1.20, s
24	22.6, CH_3	1.87, s	22.6, CH_3	1.87, s	22.6, CH_3	1.05, s
25	17.4, CH_3	2.36, s	17.4, CH_3	2.36, s	16.8, CH_3	2.22, s
1′	123.8, C		123.9, C			
2′	157.7, C		157.7, C			
3′	110.1, CH	6.82, d (8.0)	110.1, CH	6.79, d (8.0)		
4′	129.1, CH	7.26, t (8.0)	129.1, CH	7.25, t (8.0)		
5′	119.4, CH	7.09, d (8.0)	119.3, CH	7.07, d (8.0)		
6′	138.7, C		138.3, C			
7′	54.4, CH_2	4.58, d (9.4)	54.4, CH_2	4.59, d (9.5)		
		4.74, d (9.4)		4.71, d (9.5)		
8′	130.8, CH	6.99, d (15.8)	130.6, CH	7.00, d (15.8)		
9′	130.2, CH	6.20, dd (15.8, 6.7)	130.4, CH	6.16, dd (15.8, 5.8)		
10′	75.7, CH	4.26, dd (6.7, 3.7)	75.5, CH	4.31, dd (5.8, 4.0)		
11′	73.9, CH	3.78, m	75.5, CH	4.18, dd (7.1, 4.0)		
12′	34.5, CH_2	1.43, m	129.0, CH	5.54, ddd(15.5,7.1, 1.5)		
13′	19.1, CH_2	1.54, m; 1.38, m	130.0, CH	5.79, dq (15.5, 6.1)		
14′	14.1, CH_3	0.92, t (7.3)	17.9, CH_3	1.71, d (6.1)		
1-OH		12.63, s		12.64, s		13.92, s
2′-OMe	55.8, CH_3	3.82, s	55.9, CH_3	3.82, s		
10-OH						10.87, s
11-OH						10.64, s
19-OMe					57.6, CH_3	3.17, s
22-OMe					48.7, CH_3	3.15, s

[a]Chemical shifts are in ppm; J values in Hz are in parentheses. [b]Measured at 400 MHz in $CDCl_3$.

Varioxiranols F and G (**6**, **7**) appear to be products derived from the intermolecular condensation of tajixanthone (**13**) with varioxiranol A (**1**) and (1*E*,5*E*)-1-(2-(hydroxymethyl)-3-methoxyphenyl)hepta-1,5-diene-3,4-diol (**9**), respectively. The highly regioselective nucleophilic attack of the hydroxy group at C-7 of **1** or **9** to C-16 of the oxirane in **13**, in addition to the observation of **6** and **7** in the HPLC chromatogram of the EtOAc extract, led to the hypothesis that an enzymatic step is necessary for the condensation.

Compound **8** had a molecular formula of $C_{27}H_{34}O_7$, as deduced from HRESIMS data. The ^{13}C NMR spectrum displayed a total of 27 resonances, including 12 aromatic carbons for two phenyl rings (rings A and B). In regard to aromatic ring A, an AB spin system was observed by the COSY correlation between H-2 (δ_H 6.42, d, J = 8.4 Hz) and H-3 (δ_H 7.25, d, J = 8.4 Hz). The presence of an isoprenyl unit was recognized by the COSY correlation between methylene H_2-14 (δ_H 3.26, d, J = 6.9 Hz) and olefinic proton H-15 (δ_H 5.28, t, J =

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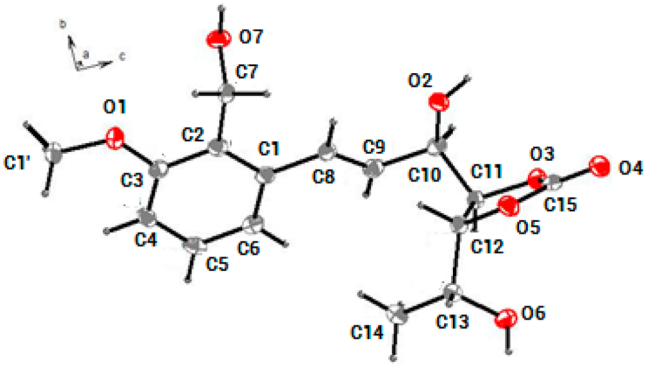

Figure 4. Structure of **5** determined by X-ray single-crystal diffraction analysis.

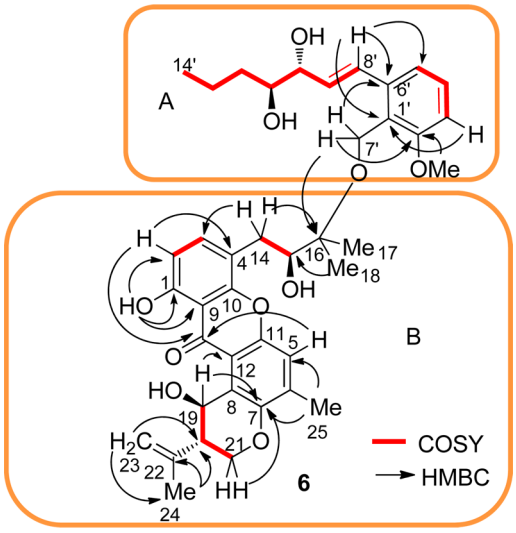

Figure 5. Key HMBC and COSY correlations of **6**.

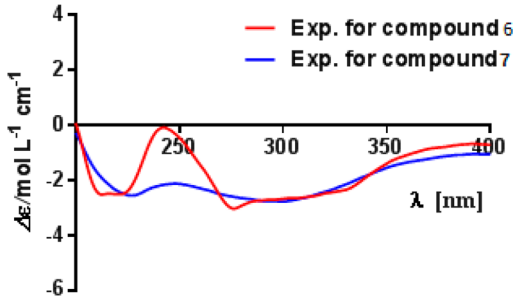

Figure 7. ECD spectra of **6** and **7**.

6.9 Hz), along with the HMBC relationships from methyl protons H_3-17 (δ_H 1.76, s) and H_3-18 (δ_H 1.71, s) to C-16 (δ_C 133.5) and C-15 (δ_C 121.8). The linkage of the isoprenyl unit to C-4 (δ_C 123.6) was confirmed by the HMBC interactions from H_2-14 to C-3 (δ_C 137.8), C-4, and C-10 (δ_C 157.2). Additional HMBC relationships from H-3 to C-1 (δ_C 162.9) and C-10 and from H-2 to C-9 (δ_C 112.8) and C-4 assigned all carbons in aromatic ring A, while the deshielded chemical shifts of C-1 and C-10 were indicative of their oxygen substitution. Aromatic ring B presented a lone proton H-5 (δ_H 6.82, s) for a pentasubstituted aromatic ring, while the formation of a chroman ring was based on the COSY relationships from H-20 (δ_H 2.19, brdd, J = 9.8, 3.6 Hz) to H_2-21 (δ_H 4.26, 4.30) and H-19 (δ_H 4.57, brs), in association with the HMBC interactions from H_2-21 to C-7 (δ_C 147.0) and from H-19 to C-7, C-8 (δ_C 119.5), and C-12 (δ_C 122.5). In addition, a methoxyisopropane unit linked to C-20 (δ_C 55.0) was evident from the HMBC correlation between MeO (δ_H 3.15, s) and C-22 (δ_C 75.0) and from both methyl singlets δ_H 1.20 (3H, s, H_3-23) and 1.05 (3H, s, H_3-24) to C-22 and C-20. An additional HMBC interaction between MeO (δ_H 3.17, s) and C-19 (δ_C 70.4) confirmed C-19 to be methoxylated. Moreover, the methylation and hydrox-

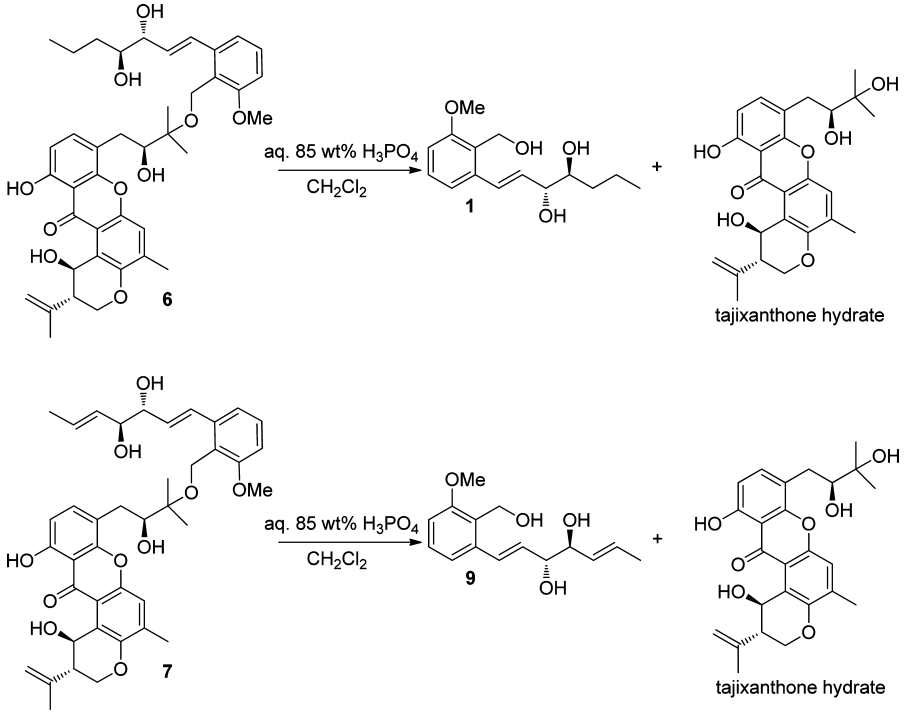

Figure 6. Acidic hydrolysis of **6** and **7**.

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ylation at C-6 (δ_C 133.3) and C-11 (δ_C 155.3), respectively, were defined by the HMBC correlations from methyl protons (δ_H 2.22, s, H_3-25) to C-5, C-6, and C-7 in addition to OH-11 (δ_H 10.87, s) correlating to C-5, C-11, and C-12. The linkage of both aromatic rings A and B to form a benzophenone across C-13 (δ_C 199.2) was confirmed by the observation of long-range HMBC interactions from the carbonyl C-13 to H-2 and H-5. Thus, the structure of **8** was determined as 19-*O*-methyl-22-methoxypre-shamixanthone. The NOE interaction between H-19 and H_3-23/H_3-24 determined a *trans* orientation of H-19 and H-20, while similar ECD effects of both **8** and pre-shamixanthone (**12**) (Figure 8) revealed both compounds shared the same configurations at C-19 and C-20.

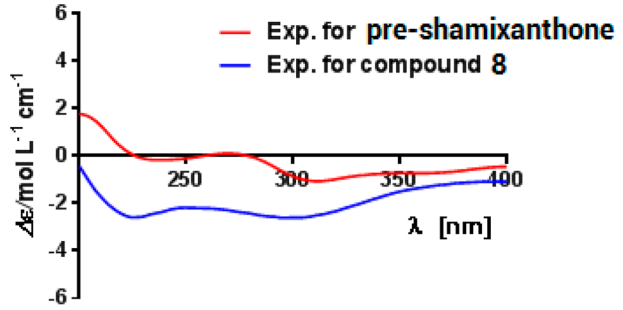

Figure 8. ECD spectra of **8** and pre-shamixanthone.

On the basis of the MS and NMR spectroscopic analyses and the comparison of the specific rotation, compound **9** was identified as the known fungal metabolite (1*E*,5*E*)-1-(2-(hydroxymethyl)-3-methoxyphenyl)hepta-1,5-diene-3,4-diol[25] and as the enantiomer of the synthesized product.[16] Natural **9** was initially reported with configuration information,[25] which has now been confirmed by Mosher's method (Figures S59 and S60, Supporting Information), indicating 11*R* and 12*S* configurations. Compounds **10** and **11** were identical to varitriol[2,26] and varioxirane,[2] respectively, according to the spectroscopic comparison. Analyses of 1D and 2D NMR spectroscopic data identified **12** as pre-shamixanthone, a product from *A. nidulans* when its cryptic PKS genes were activated.[19] In addition, five xanthone analogues were identical to tajixanthone (**13**),[24] tajixanthone methanoate (**14**),[12] tajixanthone hydrate (**15**),[12] 15-acetyltajixanthone hydrate,[20] and shamixanthone.[12]

Compounds **1**−**12** were tested for lipid-lowering effects against oleic acid (OA)-elicited lipid accumulation in HepG2 liver cells.[27,28] Three compounds, **1**, **9**, and **12**, exerted inhibitory effects against lipid accumulation at a dose of 10 μM as measured by Oil Red O staining (Figure 9) and showed no toxicity up to 100 μM toward HepG2 cells in the MTT assay (Figure S62, Supporting Information). Among the active compounds, compound **12** was the most active, with an inhibitory effect comparable to the positive control simvastatin. Further bioassay revealed that compound **12** exerted significant inhibition against intracellular triglyceride (TG) levels (Figure 10A) and dramatically reduced total cholesterol (TC) (Figure 10B) at a dose of 10 μM. It is noted that compound **1** mainly reduced TG levels with weak effects on TC, whereas compound **9** induced the reduction of TC with weak effect on TG. These findings suggested distinct mechanisms of **1** and **9** for the antihyperlipidemic effects, which is surprising considering that **9** differs from **1** only by having an extra double bond at C-12/

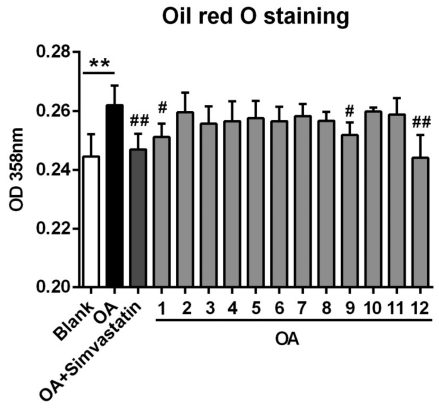

Figure 9. Effects of compounds on oleic acid-elicited intracellular lipid accumulation. Positive control: simvastatin; blank: DMEM; OA: oleic acid. Compounds were determined by spectrophotometry at 358 nm after Oil Red O staining. Bars depict the means ± SEM in triplicate. $^{**}p < 0.01$, OA vs blank; $^{\#\#}p < 0.01$, test group vs OA group.

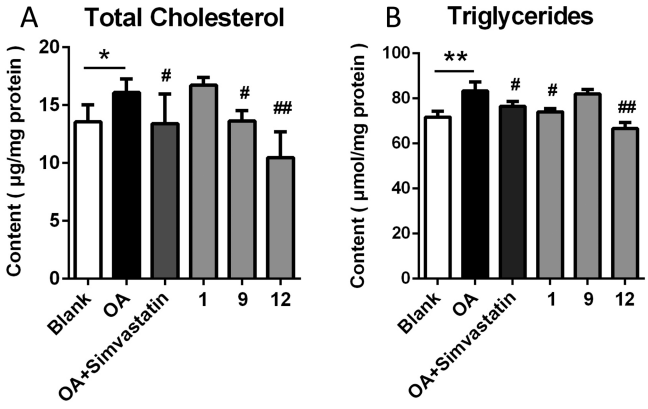

Figure 10. Inhibitory effects of compounds toward (A) total cholesteroland and (B) triglycerides. Positive control: simvastatin; blank: DMEM; OA: oleic acid. Intracellular levels of triglycerides and total cholesterol were measured by kits according to the manufacturer's instructions. Bars depict the means ± SEM in triplicate. $^{**}p < 0.001$, $^{*}p < 0.05$, OA vs blank; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compound vs OA.

C-13. Real-time quantitative PCR was performed[29] to determine how compound **12** regulated the expression of lipid metabolic genes. As shown in Figure 11, compound **12** dramatically down-regulated sterol regulatory element-binding transcription factor 1 (SREBP-1c) and the mRNA levels of

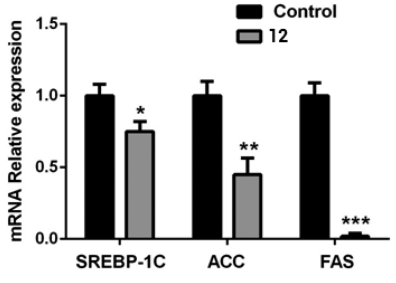

Figure 11. Effect of **12** on the transcription of lipogenic genes. Gene expression was quantified by quantitative real-time PCR analysis. The gene expression levels were normalized to β-actin mRNA levels. Values represent mean ± SD. Results are representative of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs control group.

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genes downstream of SREBP including genes encoding acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). SREBP-1c is a key transcriptional factor promoting lipid production,[30] while ACC and FAS are two sequential enzymes in the fatty acid biosynthetic pathway whose expression is regulated by SREBP-1c.[31] The experimental results indicated that **12** may decrease lipid accumulation through down-regulation of the SREBP-1 pathway.

The present work revealed additional chemical diversity from the marine sponge-associated fungal strain *E. variecolor* XSA-07-2. Varioxiranols F and G (**6**, **7**), featuring the linkage of a benzyl alcohol with a xanthone, were found for the first time from marine-derived fungi, while the 1,3-dioxolan-2-one unit in compound **5** is an unusual moiety, which was reported previously in diterpenes and anthrodioxolanone from plants,[32,33] as well as in cyclohexenes from fungi.[34] Cytosporin E is the only one bearing a dioxolanone unit from a marine-derived fungus. It was isolated from a strain of *Eutypella scoparia* associated with a marine pulmonate mollusc.[35] The potent inhibition of compound **12** against lipid accumulation by regulation of the SREBP pathway, with low cytotoxicity, suggested **12** to be a potential lead compound for the development of an antihyperlipidemic agent.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on an X-5 micro melting point apparatus (Kexian Co., China). Optical rotations were measured using an Autopol III automatic polarimeter (Rudolph Research Co.). UV spectra were recorded by a 3300-ELSD UV detector (Alltech Co.). IR spectra were measured on a Thermo Nicolet Nexus 470 FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX-400 NMR (or on a Bruker DRX-500 NMR) spectrometer in $CDCl_3$ or DMSO-d_6 with ^1H and ^{13}C nuclei observed at 400 and 100 MHz (500 and 125 MHz), respectively, using TMS as an internal standard. HRESIMS spectra were obtained on a Bruker APEX IV 70e FT-MS spectrometer and on a Thermo DFS spectrometer using a matrix of 3-nitrobenzyl alcohol. Column chromatography was carried out with silica gel (200−300 mesh), and HF_{254} silica gel for TLC was obtained from Qingdao Marine Chemistry Co. Ltd. ODS gel (50 μm) and Sephadex LH-20 (18−110 μm) were obtained from YMC (Japan) and Amersham Pharmacia Biotech AB, Uppsala, Sweden. HPLC was performed with an Alltech 426 pump employing a UV detector, and the Chromasil C_{18} column (semipreparative, 10 μm) was purchased from Pharmacia. All chemicals used herein were of analytical grade. HepG2 cells (ATCC CRL-10741) were obtained from the American Type Culture Collection. DMEM medium, fetal bovine serum, and penicillin/streptomycin were supplied by Gibco, while oleic acid and lovastatin were obtained from Sigma-Aldrich.

Fungal Material. Fungal strain *Emericella variecolor* XSA-07-2 was isolated from a marine *Cinachyrella* sp. sponge, which was collected from Yongxin Island in the South China Sea, in April 2013. The fungal clone germinated from the cut of sponge tissue was repurified under sterile conditions using standard methods. Morphological scrutiny of hyphae and spores combined with the 18S rDNA ITS sequence (GenBank number KP202154) led to the identification of its species. This strain (XSA-07-2) was deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, China.

Fermentation of the Fungus. Fermentation of the strain was initiated in 40 500 mL sized Erlenmeyer flasks, each preloaded with 80 g of rice and 100 mL of sterilized artificial seawater (NaCl 26.726 g, $MgCl_2$ 2.26 g, $MgSO_4$ 3.248 g, $CaCl_2$ 1.153 g, $NaHCO_3$ 0.198 g, KCl 0.721 g, NaBr 0.058 g, H_3BO_3 0.058 g, Na_2SiO_3 0.0024 g, $Na_2Si_4O_9$ 0.0015 g, H_3PO_4 0.002 g, Al_2Cl_6 0.013 g, NH_3 0.002 g, $LiNO_3$ 0.0013 g, H_2O 1 L). The seed was prepared by inoculating activated fungal cakes from an agar Petri dish into 200 mL of potato dextrose broth medium. Approximately 20 mL aliquots of the inoculum were then transferred to fermentation medium and further incubated for 30 days at 28 °C statically.

Extraction and Isolation. The rice cultures were extracted with EtOAc (4 times). The EtOAc solution was concentrated *in vacuo* (32 °C) to yield an extract (40 g), which was subsequently subjected to silica gel column chromatography eluting with petroleum ether/acetone (4:1, v/v) to afford four fractions, F1−F4. F1 (3.6 g) was further fractionated by silica gel column chromatography and eluted with a petroleum ether/acetone gradient (from 60:1 to 10:1) to obtain three fractions (F1a−F1c). F1b (2.5 g) was subjected to an ODS column (10 μm) eluting with constant MeOH/H_2O (75%) to yield **8** (7.1 mg), **12** (28.7 mg), **6** (14.6 mg), and **7** (8.0 mg). F1c (1.1 g) was separated through semipreparative HPLC (C_{18}) with MeCN/H_2O (55%) as a mobile phase to obtain **9** (28.3 mg), **10** (36.1 mg), and **4** (11.0 mg). A gel filtration over Sephadex LH-20 of F1c (30.2 mg) eluting with MeOH was employed to afford compound **2** (10.5 mg). Fraction F2 (200 mg) was separated by semipreparative HPLC with MeCN/H_2O (55%) as a mobile phase to yield **1** (12.0 mg), **11** (22.4 mg), **3** (9.3 mg), and **5** (15.5 mg). Fraction F4 (7.0 g) was subjected to a silica gel (300−400 mesh) column separation eluting with CH_2Cl_2/MeOH (10:1) to obtain tajixanthone (**13**, 2.0 g), tajixanthone methanoate (**14**, 3.0 g), and tajixanthone hydrate (**15**, 60.0 mg). The remaining collection (85 mg) was further separated on semi-preparative HPLC with acetonitrile/H_2O (65%) as a mobile phase to yield 15-acetyltajixanthone hydrate (9.3 mg) and shamixanthone (22.4 mg).

*Varioxiranol A (**1**):* white, amorphous powder; $[\alpha]^{20}_D$ +5.28 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 220 (2.8), 251 (2.3), 294 (1.7) nm; IR (KBr) ν_{max} 3251, 2957, 2906, 2868, 1575 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 289.1410 [M + Na]$^+$ (calcd for $C_{15}H_{22}O_4Na$, 289.1407).

*Varioxiranol B (**2**):* white, amorphous powder; $[\alpha]^{20}_D$ +15.0 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 220 (2.8), 251 (2.3), 294 (1.6) nm; IR (KBr) ν_{max} 3316, 2954, 2931, 2876, 1673, 1258 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 321.1327 [M + Na]$^+$ (calcd for $C_{15}H_{22}O_6Na$, 321.1322).

*Varioxiranol C (**3**):* white, amorphous powder; $[\alpha]^{20}_D$ +14.3 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 220 (2.5), 252 (1.7) nm; IR (KBr) ν_{max} 3439, 2978, 2897, 1681, 1575 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 335.1471 [M + Na]$^+$ (calcd for $C_{16}H_{24}O_6Na$, 335.1475).

*Varioxiranol D (**4**):* colorless gum; $[\alpha]^{20}_D$ +23.3(*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 220 (2.63), 252 (1.27) nm; IR (KBr) ν_{max} 3368, 2934, 1752, 1250 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 339.0975 [M + Na]$^+$ (calcd for $C_{15}H_{21}O_5ClNa$, 339.0982).

*Varioxiranol E (**5**):* colorless crystal; mp 76.5 °C; $[\alpha]^{20}_D$ +168 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 221 (2.63), 252 (1.99) nm; IR (KBr) ν_{max} 3315, 2957, 2875, 1784, 1677 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 347.1107 [M + Na]$^+$ (calcd for $C_{16}H_{20}O_7Na$, 347.1096).

*Varioxiranol F (**6**):* yellow gum; $[\alpha]^{20}_D$ +5.54 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.27), 241 (3.10), 254 (3.07), 268 (3.09), 294 (2.42) nm; ECD (*c* 3.8 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (−2.5), 242 (−0.1), 276 (−3.0) nm; IR (KBr) ν_{max} 3383, 2925, 2854, 1726, 1655, 1598, 1677, 1243 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m*/*z* 711.3145 [M + Na]$^+$ (calcd for $C_{40}H_{48}O_{10}Na$, 711.3136).

*Varioxiranol G (**7**):* yellow gum; $[\alpha]^{20}_D$ +8.57 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.61), 248 (3.44), 253 (3.40), 273 (3.42), 294 (2.89) nm; ECD (*c* 2.0 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 227 (−2.5), 248 (−2.1), 300 (−2.8) nm; IR (KBr) ν_{max} 3410, 2934, 2893, 1643, 1359, 1245 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m*/*z* 685.3013 [M − H]$^-$ (calcd for $C_{40}H_{45}O_{10}$, 685.3026).

*19-O-Methyl-22-methoxypre-shamixanthone (**8**):* yellow gum; $[\alpha]^{20}_D$ −8.10 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.16), 232 (2.73), 289 (2.61), 368 (2.14) nm; ECD (*c* 8.9 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 238 (−0.2), 270 (−0.1), 311 (−1.1) nm; IR (KBr) ν_{max} 3352, 2972, 2924, 1617, 1424, 1349, 1223 cm^{-1}; H and ^{13}C NMR

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Table 3. Primers Used in Real-Time Quantitative PCR Analysis

name	forward (5′−3′)	reverse (5′−3′)
SREBP-1c	CCATGGATGCACTTTCGAA	CCAGCATAGGGTGGGTCAA
FAS	CGGTACGCGACGGCTGCCTG	GCTGCTCCACGAACTCAAACACCG
ACC	TGATGTCAATCTCCCCGCAGC	TTGCTTCTTCTCTGTTTTCTCCCC

data, Table 2; HRESIMS m/z 469.2226 $[M - H]^-$ (calcd for $C_{27}H_{33}O_7$, 469.2215).

(1E,5E)-1-(2-(Hydroxymethyl)-3-methoxyphenyl)hepta-1,5-diene-3,4-diol (9): $[\alpha]^{20}_D$ +18.5 (c 0.22, EtOH) (lit.[25] $[\alpha]^{27}_D$ +20.2 (c 0.98, EtOH) and $[\alpha]^{24.5}_D$ +42.7 (c 0.22, $CHCl_3$)).

Varioxirane (11): $[\alpha]^{20}_D$ −23.6 (c 0.18, $CHCl_3$) (lit.[21] $[\alpha]^{30}_D$ −24.6 (c 0.13, $CHCl_3$)).

MPA Esterification of 9. Compound **9** (0.047 mmol) was dissolved in DMF (0.5 mL), and TBSCl (0.052 mmol) and imidazole (0.142 mmol) were added sequentially at 0 °C. Then the mixture was stirred for 5 h at room temperature (rt). The reacted mixture was washed with H_2O and then extracted with EtOAc and dried in *in vacuo*. The residue was purified on semipreparative HPLC with MeCN/H_2O (90%) as a mobile phase to give a colorless oil, **9a**. Both (*R*)- and (*S*)-MPA esters of **9a** were obtained by the treatment of **9a** (2.4 mg, respectively) with (*R*)- and (*S*)-MPA (3.16 mg) and dicyclohexylcarbodiimide (3.93 mg) in dry $CDCl_3$ (0.6 mL) catalyzed with dimethylaminopyridine (2.32 mg) and stirring at rt overnight. The MPA esters, **9b** (3.0 mg) and **9c** (2.1 mg), were purified by semipreparative HPLC using MeCN (100%) as a mobile phase.

MPA Esterification of 1. Following the same protocol as for **9**, the TBS protection and Mosher's reaction of **1** were performed to yield the (*R*)-MPA ester of **1a** (2.9 mg) and the (*S*)-MPA esters of **1a** (3.1 mg).

(R)-MPA ester of 1a: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 6.83 (1H, d, J = 8.1 Hz, H-3), 7.23 (1H, t, J = 8.1 Hz, H-4), 7.03 (1H, d, J = 7.6 Hz, H-5), 4.79 (2H, d, J = 3.80 Hz, H-7), 3.84 (3H, s, MeO-2), 7.15 (1H, d, J = 15.8 Hz, H-8), 6.06 (1H, dd, J = 15.9, 7.5 Hz, H-9), 5.69 (1H, ddd, J = 7.1, 4.2, 1.0 Hz, H-10), 4.95 (1H, td, J = 8.4, 4.4 Hz, H-11), 1.18 (2H, m, H-12), 0.74 (2H, m, H-13), 0.52 (3H, dd, J = 7.1 Hz, H_3-14); ESIMS m/z 697.83 $[M + H]^+$.

(S)-MPA ester of 1a: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 6.77 (1H, d, J = 7.6 Hz, H-3), 7.20 (1H, t, J = 8.0 Hz, H-4), 6.81 (1H, d, J = 7.7 Hz, H-5), 4.67 (2H, d, J = 11.8 Hz, H-7), 3.83 (3H, s, MeO-2), 6.82 (1H, d, J = 16.0 Hz, H-8), 5.65 (1H, dd, J = 16.3, 8.1 Hz, H-9), 5.39 (1H, dd, J = 8.1, 3.0 Hz, H-10), 5.33 (1H, td, J = 8.3, 3.0 Hz, H-11), 1.52 (2H, m, H-12), 1.32 (2H, m, H-13), 0.89 (3H, t, J = 7.2 Hz, H_3-14); ESIMS m/z 697.83 $[M + H]^+$.

Chemical Conversion of 11 to 2. To a solution of **11** (1.5 mg) in MeCN (3 mL) was added dropwise aqueous citric acid (20%, 2 mL). The mixture was stirred at rt until most of the starting material was transformed. A saturated aqueous $NaHCO_3$ solution (1 mL) was added to quench the reaction. The mixture was extracted by EtOAc (10 mL). The organic layers were combined and washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was subjected to flash chromatography on silica gel using EtOAc/petroleum ether (1:1.5) to give compound **2** (0.8 mg).

Acidic Hydrolysis of 6 and 7. To a solution of **6** (1.5 mg) in CH_2Cl_2 (1 mL) at rt was added aqueous H_3PO_4 (85 wt %, 0.12 mL) dropwise. The mixture was stirred for 14 h, and TLC assay showed the reaction was complete. Then 3 mL of H_2O was added. The mixture was extracted with EtOAc (4 mL). The combined EtOAc phase was dried over magnesium sulfate and concentrated *in vacuo* to give an extract, which was purified by semipreparative HPLC using 40% MeOH/H_2O as a mobile phase to yield compound **1** (0.4 mg) and tajixanthone hydrate (0.65 mg). Following the same hydrolysis protocol as for **6**, compound **7** (1.5 mg) was converted to **9** (0.5 mg) and tajixanthone hydrate (0.7 mg).

X-ray Crystallographic Analysis of 5. A white crystal of **5** was obtained in MeOH. The crystal data were recorded on an Oxford Diffraction Gemini E diffractometer with graphite-monochromated Cu Kα (λ = 1.5418 Å) radiation. The structures were solved by direct methods using SHELXS-97 and refined using full-matrix least-squares difference Fourier techniques. Crystal data of **5**: $C_{16}H_{20}O_7$, M = 324.32, crystal size 0.40 × 0.20 × 0.16 mm; orthorhombic, a = 9.1496(5) Å, b = 12.6638(4) Å, c = 13.4684(4) Å, α = 90.00°, β = 90.00°, γ = 90.00°, U = 1560.57(11) Å^3, T = 99.2 K, space group $P2_12_12_1$ (no.19), Z = 4, μ(Cu Kα) = 0.917 mm^{-1}, F(000) = 688; 5368 reflections measured, 2888 unique [(R_{int} = 0.0218(inf-0.9 Å)] were used in all calculations; the calculated density was 1.380 mg/mm^3; the final R indexes (all data) were R1 = 0.0357 and wR2 = 0.0852; the Flack parameter was −0.03(17). The crystallographic data for the structure of **5** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1019445). Copies of the data can be obtained free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (http://www.ccdc.cam.ac.uk; fax: +44-(0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

Cell-Based Lipid Accumulation Assay. HepG2 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 μg/mL). The cells with 70−80% confluence were incubated in DMEM + oleic acid (100 μM) for 12 h and then were treated with the compounds (each, 10 μM) and the positive control lovastatin in DMEM/100 μM oleic acid with DMEM/100 μM oleic acid as a blank for an additional 6 h. Subsequently, the cells were subjected to Oil Red O staining or TC and TG determination as described previously.[27,28] Each experiment (n = 8 for Oil Red O staining or n = 3 for TC and TG determination) was repeated in triplicate.

Quantitative Real-Time PCR. The mRNA levels of lipid metabolism-related genes were determined by real-time quantitative PCR. Total RNA extraction, cDNA synthesis, and quantitative PCR assays were performed as described previously.[28] Samples were cycled 40 times using a Fast ABI-7500 sequence detector (Applied Biosystems). ABI-7500 cycle conditions were as follows: 5 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. Cycle threshold (CT) was calculated under default settings for real-time sequence detection software (Applied Biosystems). At least three independent biological replicates were performed to check the reproducibility of the data. The gene-specific primers used for quantitative PCR are listed in Table 3.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00578.

NMR spectroscopic data for the new compounds (**1**−**8**) including ^{1}H, ^{13}C, and 2D NMR spectra, IR and ESIMS/MS data, and X-ray data for **5** (PDF)

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Notes

The authors declare no competing financial interest.

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