Bioactive Compounds from the Seed Fungus *Menisporopsis theobromae* BCC 3975

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Eight new compounds (**2**−**9**), together with a known dithiodiketopiperazine (**1**), were isolated from the seed fungus *Menisporopsis theobromae* BCC 3975. The structures of these substances were elucidated by analyses of spectroscopic data. Compounds **1** and **4** exhibited moderate cytotoxicity against BC-1 cell lines with IC_{50} values of 29.2 and 57.4 μM, respectively. Cytotoxicity of **1**, **2**, **4**, and **9** against the NCI-H187 cell line showed respective IC_{50} values of 22.9, 20.3, 1.8, and 56.6 μM. Compounds **2** and **4** exhibited antimalarial activity with IC_{50} values of 2.95 and 28.8 μM, respectively. Substances **1**, **4**, **7**, **8**, and **9** possessed weak antimycobacterial activity (MIC 154.8−952.3 μM), while compounds **2** and **3** showed potent antimycobacterial activity with respective MIC values of 1.24 and 7.14 μM.

Our previous study on the seed fungus *Menisporopsis theobromae* BCC 4162[1] implied that seed fungi may be a good source of bioactive compounds. Here we report bioactive metabolites from another strain (BCC 3975) of *M. theobromae*, which produced various classes of interesting bioactive compounds. Preliminary screening results revealed that a crude extract of the seed fungus *M. theobromae* BCC 3975 exhibited antimycobacterial activity with an MIC value of 1.24 μM. Chemical investigation of the fungus *M. theobromae* BCC 3975 led to the isolation of a known dithiodiketopiperazine (**1**),[2] together with eight new metabolites (**2**−**9**). Interestingly, the fungus *M. theobromae* BCC 3975 produced different classes of compounds from different culture batches. We report herein the isolation, structure elucidation, and biological activities of compounds **1**−**9**.

Results and Discussion

Compounds **1** and **2** were isolated from the first culture batch of broth and mycelia of the seed fungus *M. theobromae* BCC 3975, respectively. The structures of compounds **1** and **2** were very complex; however, the amounts of **1** and **2** obtained from the first culture batch were not sufficient for further studies. Therefore, we tried to isolate these substances in the second culture batch. Unfortunately, the fungus did not produce compounds **1** and **2** in the second culture batch, but instead, it produced compounds **4**−**7**. In the third culture batch, six metabolites, compounds **1**, **3**, **5**, **6**, **8**, and **9**, were isolated from the fungus extracts. Surprisingly, the fungus again produced compound **1** in the third culture batch, but only a small amount of compound **1** was obtained.

The structure of compound **1** was elucidated by analyses of ^{1}H, ^{13}C, DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC spectroscopic data and finally confirmed by literature data comparison.[2] The ^{1}H NMR spectrum of compound **1** in $CDCl_3$ is identical to reported data[2] (see Supporting Information). However, the ^{1}H NMR of **1** in acetone-d_6 showed better signal separation, which is more convenient to assign proton signals (see Experimental Section and Supporting Information). Although compound **1** is a known fungal metabolite, previously isolated from the fungus *Ramichloridium schulzeri* var. *schulzeri* F-2440 (FERM P-10187),[2] its ^{1}H and ^{13}C NMR data have not yet been assigned. We have completely assigned proton and carbon resonances in **1** (see Experimental Section).

The ^{1}H and ^{13}C NMR data of compound **2** were generally similar to those of compound **1**, and extensive analyses revealed that compound **2** was a derivative of **1**. The molecular formula of compound **2**, $C_{31}H_{36}N_2O_9S_2$, was deduced from the ESITOF mass spectrum. Signals of sp^3 methines (C-12 and C-12a) in **1** were replaced by sp^2 quaternary carbons in **2**. On the basis of these data and extensive analyses of 2D NMR data, compound **2** was a derivative of **1**, the cyclohexadiene unit in **1** being dehydrogenated to an aromatic moiety. In a similar fashion to that of **1**, proton and carbon resonances in compound **2** were successfully assigned by analyses of spectroscopic data. The stereochemistry of **2** was assigned by analysis of the NOESY spectrum, in which a correlation of H-7′ and H-4′, but none between H-7′ and H-11′ methyl protons, was observed, indicative of a *trans* configuration of the α,*β*-unsaturated ketone moiety. An intense cross-peak was observed between the H-1‴ methyl protons and the α-methylene proton (δ_H 3.35, H-15α), but none between the H-1‴ methyl protons and the *β*-methylene proton (δ_H 3.28, H-15*β*), implying that the H-1‴ methyl protons and the α-methylene proton (δ_H 3.35, H-15α) were cofacial. A cross-peak was observed between the H-1″ methyl protons and H-8 in the NOESY spectrum, suggesting a *cis* relationship between the H-1″ methyl protons and H-8. The coupling constant of 8.2 Hz for $J_{H-5,H-5a}$ indicated that the dihedral angle of H-5 and H-5a was close to 0° or 180°; however, a weak cross-peak correlation on the NOESY spectrum implied a *trans* relationship between H-5 and H-5a. Furthermore, compound **2** also showed a negative specific rotation similar to that of compound **1** and other known fungal metabolites, bis-dethiodi(methylthio)-acetylapoaranotin[3] and bis-dethiodi(methylthio)acetylaranotin.[3] Compound **2** is, therefore, more likely to possess the same absolute configuration as those of **1** and its derivatives.[3]

The molecular formula of compound **3**, $C_{20}H_{22}N_2O_5S_2$, was determined by ESITOFMS. In general, ^{1}H and ^{13}C NMR data of **3** were similar to those of compound **1**, and analyses of these data revealed that compound **3** did not possess a 3-hydroxy-2,4,6-trimethyl-5-oxo-oct-6-enoic acid side chain, but shared the same diketopiperazine core structure. In addition, an oxygenated H-8 methine signal in **1** (δ_H 4.84) was replaced by a methylene group in **3** (δ_H 2.98 and 3.03). Analyses of ^{1}H−^{1}H COSY and HMBC spectra of **3**, in combination with data comparison with those of **1** and **2**, led to the assignment of proton and carbon resonances in **3**. In a similar fashion to those of **1** and **2**, the stereochemistry of **3** was assigned by analysis of the NOESY spectrum. Furthermore, compound **3** showed a negative specific rotation similar to that of compounds **1** and **2**, suggesting that they shared the same absolute configuration.

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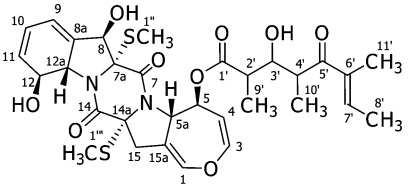
Chart 1



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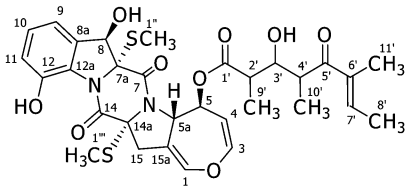

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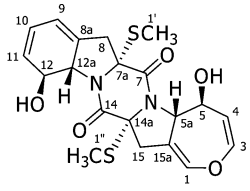

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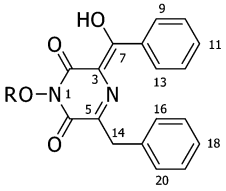
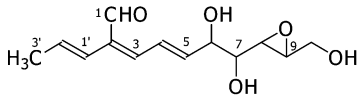


4, R = H
4a, R = Me



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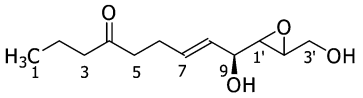

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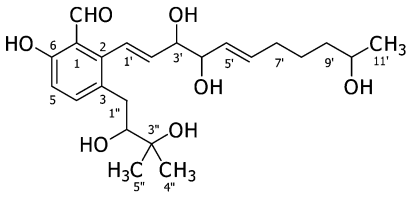

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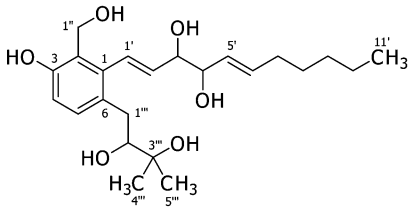

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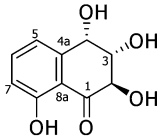


9

The ESITOFMS of **4** established its molecular formula as $C_{18}H_{14}O_4N_2$. The ^{1}H NMR spectrum ($CDCl_3$) showed signals of methylene protons at δ_H 4.18 (s) and a number of aromatic protons at δ_H 7.36−8.19. The ^{13}C NMR data ($CDCl_3$) revealed 14 resonances. However, analyses of the ^{13}C, DEPT, and HMQC spectra of **4** indicated that it possessed 18 carbons with four sets of overlapping signals, e.g., between C-9 and C-13, C-10 and C-12, C-16 and C-20, and C-17 and C-19. The DEPT spectrum demonstrated the presence of one methylene, 10 methine, and seven quaternary carbons. Analyses of the NMR data revealed that compound **4** consisted of a phenyl, a benzyl, and a central ring system. The COSY spectrum demonstrated the connectivity from H-9 to H-11 (or H-11 to H-13). The NMR signals of five protons for each aromatic ring were assigned from the COSY spectrum, indicating that **4** is bearing two monosubstituted aromatic rings. Therefore, these 1-substituted aromatic rings should link to the central core of compound **4**. The HMBC spectrum of **4** exhibited correlations from the benzylic protons (H-14) to the quaternary carbons of the central moiety (C-5 and C-6) and to C-15 and C-16 (or C-20). The HMBC spectrum showed the correlations from H-9 (or H-13) to the deshielded oxygenated sp^2 quaternary carbon (C-7), implying that C-7 must be located next to the phenyl ring. The downfield shift of 7-OH (δ_H 15.70) suggested that this OH was hydrogen-bonded with the C-2 carbonyl, therefore supporting the position of 7-OH in **4**. A molecular formula of $C_{18}H_{14}O_4N_2$ and the calculation of sites of unsaturation readily revealed that compound **4** had 13 sites of unsaturation. The two aromatic rings accounted for eight of these; therefore, the central part of compound **4** should contain five of these. The ^{13}C NMR spectrum showed four carbonyl-like signals at δ_C 152.7−180.3, implying the presence of at least one ring and a double bond. Comparison of IR data of compound **4** with those of scleriminol and flutimide[4−7] revealed that compound **4** may contain imide and C═N functionalities, suggesting that the middle chromophore of **4** is 1-hydroxy-2,6-pyrazinedione, as present in scleriminol and flutimide.[4−7] Methylation of **4** with MeI/K_2CO_3 in DMF afforded a methylated derivative (**4a**), showing an OMe signal at δ_H 4.08. It should be noted that the 7-OH group (δ_H 15.70) was not methylated and that no significant changes of chemical shifts in **4a** were observed when comparing with those of **4**. This information implied that the methylation occurred at the N-OH group. Therefore, compound **4** contained two hydroxyl groups, i.e., 7-OH and N-OH. On the basis of these data, together with spectral information from scleriminol and flutimide,[4−7] compound **4** was identified as 5-benzyl-1-hydroxy-3-(hydroxyphenylmethylene)-3*H*-pyrazine-2,6-dione. Compound **4** was subsequently crystallized from MeOH, and its crystals were subjected to X-ray crystallographic analysis, which readily confirmed the structure of **4** (Figure 1). Naturally occurring substances with a 1-hydroxy-2,6-pyrazinedione skeleton are rare, and a few examples are scleriminol and flutimide.[4−7] Scleriminol showed cytotoxicity against cancer cell lines, but demonstrated only mild

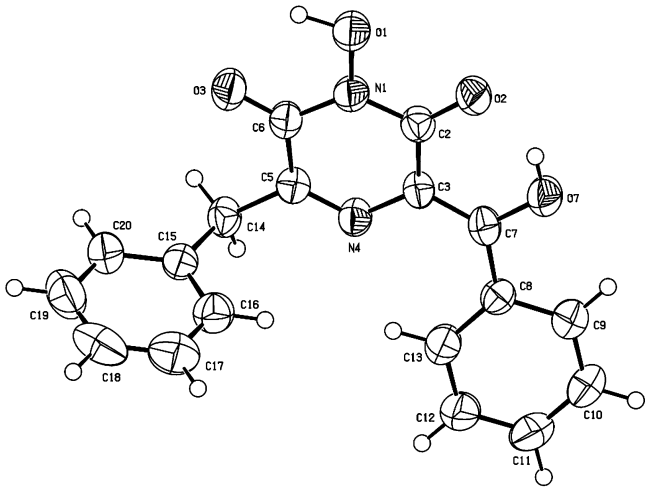


Figure 1. ORTEP plot of 5-benzyl-1-hydroxy-3-(hydroxyphenylmethylene)-3*H*-pyrazine-2,6-dione (**4**).

Table 1. Biological Activities of Compounds **1**−**9**

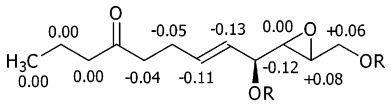
compound	cytotoxicity (IC_{50}, μM) KB[a]	BC-1[b]	NCI-H187[c]	antimalaria (IC_{50}, μM)	antimycobacteria (MIC, μM)
1	>30.9	29.2	22.9	>30.9	154.8
2	>31.0	>31.0	20.3	2.95	1.24
3	>46.0	>46.0	>46.0	>46.0	7.14
4	>62.1	57.4	1.8	28.8	310.5
5	>78.7	>78.7	>78.7	>78.7	>787.4
6	>30.3	>30.3	>30.3	>30.3	>303.0
7	>47.3	>47.3	>47.3	>47.3	473.9
8	>49.0	>49.0	>49.0	>49.0	245.1
9	>95.2	>95.2	56.6	>95.2	952.3
ellipticin[d]	5.41 ± 1.46 $n = 3$	5.93 ± 0.57 $n = 3$	1.59 ± 0.61 $n = 3$		
dihydroartemisinin[e]				0.004−0.014	
isoniazide[f]					0.29−0.66
kanamycin sulfate[f]					3.43−8.58

[a] Human epidermoid carcinoma in the mouth. [b]Human breast cancer cells. [c] Human small cell lung cancer cells. [d]Standard compound for cytotoxicity assay. [e]Standard antimalarial drug. [f]Standard antimycobacterial drug.

activity against influenza virus strains, while flutimide was an inhibitor of influenza virus endonuclease.[4−7] In the present work, compound **4** possessed antimalarial activity with an IC_{50} value of 28.8 μM and antimycobacterial activity with an MIC value of 310.5 μM (Table 1). Cytotoxicity of **4** against BC-1 (breast cancer) and NCI-H187 (small cell lung cancer) cell lines was shown by IC_{50} values of 57.4 and 1.86 μM, respectively (Table 1).

The molecular formula $C_{13}H_{18}O_5$ for compound **5** was determined by ESITOFMS spectrum. The ^{1}H NMR spectrum (CD_3OD) of **5** demonstrated an aldehydic proton resonance (δ_H 9.45), five olefinic protons (δ_H 7.03, 6.87, 6.59, 6.44, and 6.30), four oxymethine protons (δ_H 4.33, 3.43, 3.07, and 3.02), an oxygenated methylene group (δ_H 3.78 and 3.49), and methyl protons (δ_H 1.85). The ^{1}H and ^{13}C NMR spectra revealed the presence of an epoxide moiety, showing deshielded resonances of oxygenated methine protons and carbons at δ_H 3.02 and 3.07 (δ_C 57.4 and 57.7, respectively). The ^{13}C NMR data revealed 13 resonances, which could be classified by DEPT and HMQC spectra as 10 methine, one methylene, one methyl, and one quaternary carbon. The ^{13}C NMR spectrum showed two sets of carbon signals, presumably indicating a mixture of diastereoisomers. Analyses of the COSY spectrum of **5** led to the assignments of the partial structures from H-3 along the chain to H-10 and from H-1′ to H-3′. The HMBC spectrum showed correlations of the aldehydic proton H-1 to C-1′, C-2, and C-3 and from H-2′ to C-2, establishing the attachment of the aldehyde group and the propenyl side chain to C-2 in **5**. The HMBC spectrum also displayed correlations of the methyl protons H-3′ to C-1′; H-3 to C-1′; H-5 to C-3 and C-7; H-6 to C-4 and C-7; and H-8 to C-6. The J values of 15.0 and 15.8 Hz for $J_{H-4,H-5}$ and $J_{H-1',H-2'}$ indicated *trans* geometry of these double bonds, and the J value of 2.3 Hz for $J_{H-8,H-9}$ suggested the presence of a *trans*-epoxide in **5**.[8] The NOESY correlation between H-1′ and H-3 revealed *trans* geometry of the C-2/C-3 double bond. However, on the basis of available data, the absolute configuration in **5** could not be established. On the basis of these data, compound **5** was identified as 6,7-dihydroxy-7-(3-hydroxymethyloxiranyl)-2-propenylhept-2,4-dienal.

The ESITOFMS of **6** established a molecular formula of $C_{12}H_{20}O_4$. The ^{1}H NMR spectrum (acetone-d_6) of **6** showed signals of two *trans*-olefinic protons (δ_H 5.74 and 5.53), three oxymethine protons (δ_H 3.85, 2.97, and 2.83), methylene protons (δ_H 1.54−3.73), and methyl protons (δ_H 0.86). Analyses of ^{13}C and DEPT spectra of **6** revealed the presence of five methine, five methylene, one methyl, and one quaternary carbon. The deshielded carbon resonance at δ_C 209.3, together with the IR absorption at 1706 cm^{-1}, indicated the presence of a carbonyl carbon. The HMQC spectrum assisted in the assignment of protons attached to their corresponding carbon, while the COSY spectrum demonstrated the connectivities from H-1 to H-3 and from H-5 along the chain to H-3′. The HMBC spectrum of **6** exhibited long-range correlations of H-1 to C-3; H-2, H-3, H-5, and H-6 to C-4; H-7 to C-5 and C-9; and H-8 to C-6 and C-1′. The J values of 15.5 and 2.2 Hz for $J_{H-7,H-8}$ and $J_{H-1',H-2'}$ indicated *trans* geometry of a double bond and a *trans*-epoxide, respectively. On the basis of these data, compound **6** was identified as 9-hydroxy-9-(3-hydroxymethyloxiranyl)non-7-en-4-one. The absolute configuration at C-9, C-1′, and C-2′ in **6** could not be established on the basis of available spectroscopic data. However, the absolute configuration at C-9 in compound **6** was addressed through the use of Mosher esters.[9,10] Both (*R*)- and (*S*)-MTPA esters **6a** and **6b** were separately prepared and subjected to ^{1}H NMR analysis. The $\Delta\delta$ values [$\delta_{(-)} - \delta_{(+)}$] are shown in Figure 2, indicating that the absolute configuration at C-9 of compound **6** is *S*.



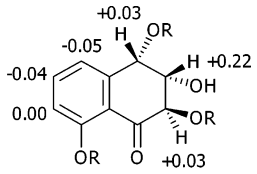
6a; R = (*S*)-(−)-MTPA

6b; R = (*R*)-(+)-MTPA

Figure 2. $\Delta\delta$ values [$\delta_{(-)} - \delta_{(+)}$] for the MTPA esters **6a** and **6b**.

The molecular formula of $C_{23}H_{34}O_7$ for compound **7** was deduced from the ESITOFMS spectrum. The ^{1}H NMR spectrum (CD_3OD) demonstrated resonances of an aldehydic proton (δ_H 10.21), two aromatic protons (δ_H 7.46 and 6.81), four olefinic protons (δ_H 6.89, 5.78, 5.75, and 5.61), four oxymethine protons (δ_H 4.24, 4.10, 3.70, and 3.45), eight methylene protons (δ_H 1.42−3.04), two methyl singlets (δ_H 1.25 and 1.23), and a methyl doublet (δ_H 1.12). The ^{13}C NMR spectrum exhibited 23 resonances, which were classified by the DEPT spectra as 11 methine, four methylene, three methyl, and five quaternary carbons. Analyses of the COSY spectrum allowed the assignment of the partial structure from H-1′ to H-11′ and also showed couplings between H-4 and H-5 and between H-1″ and H-2″. An aldehydic proton correlated to C-1 and C-6 in the HMBC spectrum. The HMBC correlations in **7** were also observed from H-4 to C-2 and C-6; H-5 to C-1 and C-3; H-1′ to C-1, C-2, and C-3′; H-2′ to C-2; H-1″ to C-2, C-3, and C-4; and both H-4″ and H-5″ to C-2″ and C-3″. The J values of 16.1 and 15.6 Hz for $J_{H-1'',H-2''}$ and $J_{H-5'',H-6''}$ indicated a *trans* geometry of these double bonds. On the basis of these data, compound **7** was identified as 3-(2,3-dihydroxy-3-methylbutyl)-6-hydroxy-2-(3,4,10-trihydroxyundeca-1,5-dienyl)benzaldehyde. Due to the limited amount of the material isolated, the absolute configuration of **7** could not be established.

The ESITOFMS of **8** established the molecular formula as $C_{23}H_{36}O_6$. The ^{1}H and ^{13}C NMR spectra of **8** were similar to those of **7**, except that the aldehydic signal at δ_H 10.21 (δ_C 198.0) in compound **7** was replaced by the oxygenated methylene resonances



9a; R = (*S*)-(−)-MTPA

9b; R = (*R*)-(+)-MTPA

Figure 3. $\Delta\delta$ values [$\delta_{(-)} - \delta_{(+)}$] for the MTPA esters **9a** and **9b**.

at δ_H 4.73 and 4.77 (δ_C 58.7) in compound **8**. In addition, the oxygenated methine signal at δ_H 3.70 (H-10′) in compound **7** was absent in compound **8**. In a similar fashion to that of **7**, protons and carbons in **8** were assigned by analyses of the COSY and HMBC spectra. Key HMBC correlations are as follows: H-1″ to C-1, C-2, and C-3; H-4 to C-2, C-3, C-5, and C-6; H-5 to C-1 and C-3; H-1′ to C-2 and C-3′; H-2′ to C-1; H-4′ and H-8′ to C-6′; H-5′ to C-7′; H-10′ and H-11′ to C-9′; H-1‴ to C-1, C-5, and C-6; H-2‴ to C-6, C-3‴, and C-5‴; and H-4‴ and H-5‴ to C-2‴ and C-3‴. On the basis of these data, compound **8** was identified as 1-[6-(2,3-dihydroxy-3-methylbutyl)-3-hydroxy-2-hydroxymethylphenyl]undeca-1,5-diene-3,4-diol, which was a reduced form of compound **7**. Due to the limited amount of the material isolated, the absolute configuration of **8** could not be established.

The ESITOFMS of **9** gave the molecular formula $C_{10}H_{10}O_5$. The ^{1}H NMR spectrum (acetone-d_6) of **9** showed three aromatic protons (δ_H 6.92, d, J = 8.4 Hz; 7.07, d, J = 7.4 Hz; and 7.59, dd, J = 7.5 and 8.3 Hz) and three oxymethine protons (δ_H 4.06, dd, J = 3.1 and 8.7 Hz; 4.59, d, J = 8.9 Hz; and 4.98, d, J = 3.0 Hz). The ^{13}C NMR spectrum revealed 10 signals, and the DEPT spectrum indicated the presence of six methine and four quaternary carbons. The deshielded carbon resonance at δ_C 203.7, together with the IR absorption at 1645 cm^{-1}, suggested the presence of a carbonyl carbon. The deshielded 8-OH resonance (δ_H 12.01) suggested that this OH was hydrogen-bonded with a carbonyl, therefore supporting the positions of 8-OH and a ketone. Analyses of the COSY spectrum demonstrated the connectivities from H-2 to H-4 and from H-5 to H-7. HMBC correlations were observed from H-4 to C-4a, C-5, and C-8a; H-5 to C-7 and C-8a; H-6 to C-4a and C-8; H-7 to C-5, C-8, and C-8a; and 8-OH to C-7 and C-8. On the basis of these data, compound **9** was identified as 2,3,4,8-tetrahydroxy-3,4-dihydro-2*H*-naphthalen-1-one. Analysis of the NOESY spectrum provided little information concerning the relative configuration in **9** due to overlapping signals. However, the coupling constants of 8.9 and 3.0 Hz for $J_{H-2,H-3}$ and $J_{H-3,H-4}$ indicated that the dihedral angle of H-2 and H-3 was close to 0° or 180° and that the dihedral angle of H-3 and H-4 was close to 60° or 120°. The absolute configuration of compound **9** was addressed through the use of Mosher's esters.[9,10] Both (*R*)- and (*S*)-MTPA esters **9a** and **9b** were separately prepared and subjected to ^{1}H NMR analysis. The deshielded methine protons of **9a** at δ_H 6.69 (H-4) and 5.82 (H-2) and those of **9b** at δ_H 6.66 (H-4) and 5.79 (H-2) indicated that the hydroxyl groups at C-4 and C-2 in **9** were esterified. The ESITOFMS exhibited the molecular formula of **9a** as $C_{40}H_{31}O_{11}F_9$-Na [observed m/z 881.1635 (M + Na)$^+$], similar to that of **9b** [observed m/z 881.1661 (M + Na)$^+$]; therefore, esterification with Mosher reagent provided tris-*O*-MTPA esters (compounds **9a** and **9b**) with ester linkages at C-2, C-4, and C-8. Moreover, the NOESY spectrum of Mosher's esters **9a** and **9b** revealed the correlation between H-3 and H-4, but none between H-2 and H-3, implying that H-3 and H-4 were *cis* oriented, while H-2 and H-3 adopted a *trans* relationship. The $\Delta\delta$ values [$\delta_{(-)} - \delta_{(+)}$] for **9a** and **9b** are shown in Figure 3, indicating the *S* configuration at C-4. Therefore, the absolute configuration at C-2 and C-3 in **9** were established as *R* and *S*, respectively.

Biological activities of compounds **1**−**9** are shown in Table 1. Compounds **1** and **4** exhibited moderate cytotoxicity against BC-1 cell lines with IC_{50} values of 29.2 and 57.4 μM, respectively. Compounds **1**, **2**, **4**, and **9** showed cytotoxicity against the NCI-H187 cell line with respective IC_{50} values of 22.9, 20.3, 1.8, and 56.6 μM. Compounds **2** and **4** exhibited antimalarial activity with IC_{50} values of 2.95 and 28.8 μM, respectively. Substances **1**, **4**, **7**, **8**, and **9** possessed weak antimycobacterial activity (MIC 154.8−952.3 μM), while compounds **2** and **3** showed potent antimycobacterial activity, with respective MIC values of 1.24 and 7.14 μM (Table 1).

The seed fungus *M. theobromae* BCC 3975 produced different compounds in different culture batches. Compounds **1**−**3** are dithiodiketopiperazine, while compound **4** and compounds **5**−**9** are pyrazinedione and polyketide, respectively. No morphological changes of fungal culture were observed in each culture batch; however, mutation at genetic levels of the fungus BCC 3975 could not be evaluated at this stage. Although there is no clear explanation why the fungus produced different classes of compound in different cultures batches, this chemical investigation proved that seed fungi are rich sources of bioactive compounds.

Experimental Section

General Experimental Procedures. IR spectra and optical rotations were measured on a Vector 22 (Bruker) spectrometer and Jasco P-1030 polarimeter, respectively. UV spectra were recorded on a Cary 1E UV−vis spectrophotometer. ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, HMBC, TOCSY, NOESY, and DEPT spectra were recorded on a Bruker ADVANCE 500 D NMR spectrometer, operating at 500 MHz for proton and 125 MHz for carbon. ESITOFMS were obtained from a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of the accurate mass.[11]

Fungal Material. The seed fungus *M. theobromae* BCC 3975 was collected from Songkla Province, Thailand, and identified by Sayanh Somrithipol. The culture was deposited at the BIOTEC Culture Collection (registration no. BCC 3975), Thailand. The fungus was grown in a potato dextrose broth and incubated for 9 days at 25 °C, then transferred into 250 mL (1 L flask) of the same culture medium. The culture was subsequently incubated (at 25 °C) for 24 days on a rotary shaker (200 rpm) and harvested for further study.

Extraction and Isolation. Culture Batch 1. The culture (5 L) of *M. theobromae* BCC 3975 was filtered to separate cells and broth. The culture broth was extracted twice with an equal volume of EtOAc. EtOAc layers were combined and evaporated to dryness, yielding 1.3 g of a crude extract. The crude EtOAc extract was purified by a Sephadex LH-20 column (eluted with MeOH) to afford nine fractions (A−I). Fraction D was rechromatographed on a Sephadex LH-20 column (MeOH as eluent) and further subjected to semipreparative HPLC (C_{18} reversed-phase column and 50:50 MeCN−H_2O as eluent) to furnish compound **1** (35.6 mg).

Mycelia were macerated in MeOH for 2 days at room temperature. The extract was evaporated, and H_2O (100 mL) was added to the extract. The mixture was washed with hexane (300 mL × 2). The aqueous solution was then extracted twice with an equal volume of EtOAc. The EtOAc layers were combined and evaporated to dryness, yielding 653.9 mg of a crude extract. The crude EtOAc extract was fractionated using a Sephadex LH-20 column (eluted with MeOH) to provide eight fractions (A−H), each with a volume of 100 mL. Fractions E and F were combined and further rechromatographed on Sephadex LH-20 (eluted with MeOH) to furnish nine fractions (E_1−E_9). Fraction E_8 was purified by a semipreparative HPLC (C_{18} reversed-phase column and 50:50 MeCN−H_2O as eluent) to yield compound **2** (0.7 mg).

Culture Batch 2. The culture (6 L) of *M. theobromae* BCC 3975 was filtered to separate cells and broth. The culture broth was extracted twice with an equal volume of EtOAc. EtOAc layers were combined and evaporated to dryness, yielding 2.2 g of a crude extract. The crude EtOAc extract was subsequently chromatographed on a Sephadex LH-20 column (MeOH as eluent) to provide nine fractions (A−I). Each fraction was analyzed with ^{1}H NMR technique; however, no trace of compounds **1** and **2** was detected in any fraction.

Fraction C was repeatedly purified on a Sephadex LH-20 column (MeOH as eluent) and further purified by a semipreparative HPLC (C_{18}

reversed-phase column and 25:75 MeCN−H_2O as eluent) to yield 3-(2,3-dihydroxy-3-methylbutyl)-6-hydroxy-2-(3,4,10-trihydroxyundeca-1,5-dienyl)benzaldehyde (**7**) (1.5 mg).

Fraction E was rechromatographed on a Sephadex LH-20 column and eluted with MeOH to provide 13 fractions (E_1−E_{13}), which were further purified by a Sephadex LH-20 column and a semipreparative HPLC (C_{18} reversed-phase column and 25:75 MeCN−H_2O as eluent). Separation of fraction E_6 by a Sephadex LH-20 column (MeOH as eluent) and a semipreparative HPLC (C_{18} reversed-phase column and 25:75 MeCN−H_2O as eluent) yielded 12.5 mg of 9-hydroxy-9-(3-hydroxymethyloxiranyl)non-7-en-4-one (**6**). Fraction E_8 was subsequently subjected to a Sephadex LH-20 column (MeOH as eluent), followed by a semipreparative HPLC (C_{18} reversed-phase column and 25:75 MeCN−H_2O as eluent), to afford 6,7-dihydroxy-7-(3-hydroxymethyloxiranyl)-2-propenylhepta-2,4-dienal (**5**) (3.8 mg). Crystallization of fraction F from MeOH gave 5-benzyl-1-hydroxy-3-(hydroxyphenylmethylene)-3*H*-pyrazine-2,6-dione (**4**) (22.4 mg).

Culture Batch 3. The culture (6 L) of *M. theobromae* BCC 3975 was filtered to separate cells and broth. The culture broth was extracted twice with an equal volume of EtOAc. The EtOAc layers were combined and evaporated to dryness, yielding 2.4 g of a crude extract. The crude EtOAc extract was subjected to a Sephadex LH-20 column and eluted with MeOH, yielding nine fractions (A−I). Fraction C was rechromatographed on a Sephadex LH-20 column and eluted with MeOH to give fractions C_1−C_{10}. Fractions C_6 and C_7 were combined and further purified by a Sephadex LH-20 column (MeOH as eluent) to give fractions $C_{6,1}$−$C_{6,9}$. Separation of fraction $C_{6,5}$ by a semipreparative HPLC (C_{18} reversed-phase column and 25:75 MeCN−H_2O as eluent) yielded 4.35 mg of 1-[6-(2,3-dihydroxy-3-methylbutyl)-3-hydroxy-2-hydroxymethylphenyl]undeca-1,5-diene-3,4-diol (**8**). Fraction $C_{6,6}$ was purified by a silica gel column (95:5 CH_2Cl_2−MeOH as eluent) to provide 9-hydroxy-9-(3-hydroxymethyloxiranyl)non-7-en-4-one (**6**) (12.6 mg). Fraction C_8 was rechromatographed on a Sephadex LH-20 column (MeOH as eluent) and finally with a semipreparative HPLC (C_{18} reversed-phase column and 25:75 MeCN−H_2O as eluent), furnishing 6,7-dihydroxy-7-(3-hydroxymethyloxiranyl)-2-propenylhepta-2,4-dienal (**5**) (3.8 mg).

Fraction D was rechromatographed on a Sephadex LH-20 column (MeOH as eluent) followed by a semipreparative HPLC (C_{18} reversed-phase column and 25:75 MeCN−H_2O as eluent) to give compound **1** (3.4 mg). Fraction G was rechromatographed on a Sephadex LH-20 column (MeOH as eluent) followed by a silica gel column (95:5 CH_2Cl_2−MeOH as eluent) to yield compound **3** (3.2 mg) and 2,3,4,8-tetrahydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**9**) (9.3 mg).

Bioassays. Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), which was cultured continuously according to the method of Trager and Jensen.[12] Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[13] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth, as indicated by the in vitro uptake of [^{3}H]hypoxanthine by *P. falciparum*. An antimalarial drug, dihydroartemisinin, was used as a positive control (see Table 1). The cytotoxicity of compound **1** was determined employing the colorimetric method as described by Skehan and co-workers.[14] A standard compound, ellipticine, was used as a positive control in the cytotoxicity assay (see Table 1). The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the microplate Alamar Blue assay (MABA).[15] The reference compounds for the antimycobacterial assay were isoniazid and kanamycin sulfate (see Table 1).

Compound 1: colorless needles (MeOH−acetone); mp 87−89 °C; $[\alpha]^{24}_D$ −135.5 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.5), 224 (4.4), and 265 (3.8) nm; IR ($CHCl_3$ solution) ν_{max} 3425, 2927, 1728, 1642, 1392, 1191, and 1124 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ_H 6.90 (1H, br q, J = 6.9 Hz, H-7′), 6.79 (1H, dd, J = 2.1, 2.1 Hz, H-1), 6.42 (1H, dd, J = 8.3, 2.3 Hz, H-3), 6.19 (1H, dd, J = 3.2, 3.8 Hz, H-9), 5.94 (1H, ddd, J = 9.8, 4.7, 2.7 Hz, H-10), 5.82 (1H, ddd, J = 8.1, 2.1, 2.1 Hz, H-5), 5.71 (1H, br d, J = 9.8 Hz, H-11), 5.13 (1H, br d, J = 8.0 Hz, H-5a), 5.08 (1H, dd, J = 13.6, 2.1 Hz, H-12a), 4.92 (1H, s, OH-12), 4.87 (1H, br d, J = 13.7 Hz, H-12), 4.84 (1H, s, H-8), 4.74 (1H, dd, J = 8.3, 1.9 Hz, H-4), 4.20 (1H, dd, J = 6.0, 11.6 Hz, H-3′), 4.08 (1H, d, 5.6 Hz, OH-3′), 3.45 (1H, dq, J = 6.9, 6.9 Hz, H-4′), 3.21 (1H, br d, J = 15.6 Hz, H-15α), 3.04 (1H, ddd, J = 15.6, 2.0, 2.0 Hz, H-15*β*), 2.53 (1H, m, H-2′), 2.32 (3H, s, H-1″), 2.29 (3H, s, H-1‴), 1.89 (3H, d, J = 6.8 Hz, H-8′), 1.74 (3H, br s, H-11′), 1.17 (3H, d, J = 6.9 Hz, H-10′), 1.13 (3H, d, J = 7.0 Hz, H-9′); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C 204.5 (1C, s, C-5′), 174.2 (1C, s, C-1′), 168.7 (1C, s, C-14), 163.0 (1C, s, C-7), 140.2 (1C, d, C-3), 138.2 (1C, d, C-1), 138.3 (1C, d, C-7′), 137.7 (1C, s, C-6′), 135.6 (1C, s, C-8a), 132.3 (1C, d, C-11), 123.4 (1C, d, C-10), 121.5 (1C, d, C-9), 111.1 (1C, s, C-15a), 106.4 (1C, d, C-4), 77.5 (1C, s, C-7a), 74.7 (1C, d, C-12), 74.6 (1C, d, C-8), 73.4 (1C, d, C-3′), 72.2 (1C, d, C-5), 70.7 (1C, s, C-14a), 67.6 (1C, d, C-12a), 60.9 (1C, d, C-5a), 43.2 (1C, d, C-2′), 42.8 (1C, d, C-4′), 40.8 (1C, t, C-15), 14.6 (1C, q, C-8′), 14.5 (1C, q, C-1‴), 14.3 (1C, q, C-1″), 14.2 (1C, q, C-10′), 11.2 (1C, q, C-9′), 11.0 (1C, q, C-11′); ESITOFMS *m*/*z* 669.1923 (M + Na)$^+$, calcd for $C_{31}H_{38}N_2O_9S_2Na$ 669.1916.

Compound 2: colorless needles (MeOH−acetone); $[\alpha]^{24}_D$ −237.9 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (4.3), 261 (3.6), and 293 (3.5) nm; ^{1}H (acetone-d_6, 500 MHz) δ_H 7.27 (1H, dd, J = 7.4, 8.1 Hz, H-10), 7.11 (1H, d, J = 7.5 Hz, H-9), 6.96 (1H, d, J = 8.3 Hz, H-11), 6.91 (1H, br q, J = 6.3 Hz, H-7′), 6.84 (1H, br s, H-1), 6.44 (1H, dd, J = 8.3, 2.3 Hz, H-3), 5.86 (1H, ddd, J = 8.2, 2.0, 2.0 Hz, H-5), 5.21 (1H, br d, J = 8.6 Hz, H-5a), 5.15 (1H, br s, H-8), 4.78 (1H, dd, J = 8.3, 1.9 Hz, H-4), 4.22 (1H, dd, J = 6.3, 5.7 Hz, H-3′), 3.46 (1H, m, H-4′), 3.35 (1H, br d, J = 15.5 Hz, H-15α), 3.28 (1H, br d, J = 15.5 Hz, H-15*β*), 2.56 (1H, m, H-2′), 2.38 (3H, s, H-1‴), 2.29 (3H, s, H-1″), 1.89 (3H, d, J = 6.8 Hz, H-8′), 1.75 (3H, br s, H-11′), 1.18 (3H, d, J = 6.9 Hz, H-10′), 1.14 (3H, d, J = 7.0 Hz, H-9′); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C 204.5 (1C, s, C-5′), 174.3 (1C, s, C-1′), 168.7 (1C, s, C-14), 163.0 (1C, s, C-7), 148.5 (1C, s, C-12), 147.2 (1C, s, C-12a), 140.3 (1C, d, C-3), 138.6 (1C, d, C-1), 138.2 (1C, s, C-7′), 137.7 (1C, s, C-6′), 135.5 (1C, s, C-8a), 129.8 (1C, d, C-10), 119.8 (1C, d, C-11), 118.1 (1C, d, C-9), 110.8 (1C, s, C-15a), 106.6 (1C, d, C-4), 77.5 (1C, s, C-7a), 76.0 (1C, d, C-8), 73.4 (1C, d, C-3′), 72.4 (1C, d, C-5), 70.5 (1C, s, C-14a), 61.2 (1C, d, C-5a), 43.3 (1C, d, C-2′), 42.8 (1C, d, C-4′), 40.5 (1C, *t*, C-15), 14.6 (2C, q, C-8′, C-1″), 14.4 (1C, q, C-1‴), 14.3 (1C, q, C-10′), 11.2 (1C, q, C-9′), 11.0 (1C, q, C-11′); ESITOFMS *m*/*z* 667.1752 (M + Na)$^+$, calcd for $C_{31}H_{36}N_2O_9S_2$-Na 667.1760.

Compound 3: yellow solid; $[\alpha]^{24}_D$ −103.3 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (4.0), 260 (3.5), and 295 (3.4) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 6.58 (1H, dd, J = 2.1, 2.1 Hz, H-1), 6.25 (1H, dd, J = 7.7, 2.4 Hz, H-3), 5.99 (1H, m, H-9), 5.93 (1H, m, H-10), 5.79 (1H, br d, J = 9.7 Hz, H-11), 4.95 (1H, dd, J = 8.3, 2.0 Hz, H-4), 4.94 (1H, m, H-12), 4.89 (1H, m, H-12a), 4.87 (1H, m, H-5a), 4.71 (1H, ddd, J = 7.7, 2.1, 2.1 Hz, H-5), 3.10 (1H, br d, J = 15.2 Hz, H-15α), 3.04 (1H, br d, J = 15.2 Hz, H-15*β*), 3.03 (1H, ddd, J = 15.5, 1.9, 1.9 Hz, H-8α), 2.98 (1H, br d, J = 15.3 Hz, H-8*β*), 2.31 (3H, s, H-1″); 2.27 (3H, s, H-1′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 167.3 (1C, s, C-14), 166.7 (1C, s, C-7), 138.1 (1C, d, C-1), 138.0 (1C, d, C-3), 131.2 (1C, s, C-8a), 130.6 (1C, d, C-11), 123.0 (1C, d, C-10), 120.7 (1C, d, C-9), 110.9 (1C, d, C-4), 107.8 (1C, s, C-15a), 74.5 (1C, d, C-12), 73.5 (1C, s, C-7a), 72.7 (1C, d, C-5), 68.9 (2C, d, C-12a and C-14a), 64.0 (1C, d, C-5a), 39.3 (1C, t, C-15), 38.7 (1C, t, C-8), 15.0 (1C, q, C-1′), 14.8 (1C, q, C-1″); ESITOFMS *m*/*z* 457.0869 (M + Na)$^+$, calcd for $C_{20}H_{22}N_2O_5S_2Na$ 457.0868.

Compound 4: yellow needles (MeOH); mp 168−171 °C; UV (MeOH) λ_{max} (log ϵ) 204 (4.6), 251 (4.2), and 365 (4.3) nm; IR ($CHCl_3$ solution) ν_{max} 2954, 2924, 2854, 1674, 1584, 1535, 1456, 1376, 1355, 1153, 761, 719, and 690 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 15.70 (1H, br s, 7-OH), 8.19 (2H, d, J = 8.3 Hz, H-9 and H-13), 7.62 (1H, *t*, J = 7.4 Hz, H-11), 7.43 (2H, *t*, J = 7.7 Hz, H-10 and H-12), 7.40 (1H, *t*, J = 6.35 Hz, H-18), 7.39 (2H, *t*, J = 6.7 Hz, H-17 and H-19), 7.36 (2H, d, J = 7.6 Hz, H-16 and H-20), 4.18 (2H, s, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 180.3 (1C, s, C-7), 161.6 (1C, s, C-2), 152.7 (1C, s, C-6), 147.3 (1C, s, C-5), 136.4 (1C, s, C-15), 133.5 (1C, d, C-11), 132.0 (2C, d, C-9 and C-13), 131.5 (1C, s, C-8), 129.8 (2C, d, C-16 and C-20), 128.7 (2C, d, C-17 and C-19), 128.2 (2C, d, C-10 and C-12), 126.9 (1C, d, C-18), 115.5 (1C, s, C-3), 39.1 (1C, t, C-14); ESITOFMS *m*/*z* 321.0865 (M − H)$^-$, calcd for ($C_{18}H_{14}O_4N_2$−H)$^-$ 321.0876.

Methylation of 5-Benzyl-1-hydroxy-3-(hydroxylphenylmethylene)-3*H*-pyrazine-2,6-dione (4). To a solution of 5-benzyl-1-hydroxy-3-(hydroxylphenylmethylene)-3*H*-pyrazine-2,6-dione (**4**) (2.5 mg) in DMF (1 mL) were added K_2CO_3 (10 mg) and MeI (0.3 mL), and the mixture was left stirring at room temperature for 20 h. The mixture was dried under vacuum, then dissolved in EtOAc (8 mL) and subsequently washed with H_2O (5 × 8 mL). A crude reaction mixture

was subjected to a Sephadex LH-20 column (eluted with MeOH) to obtain the methylated derivative (**4a**) (1.9 mg): ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 8.09 (2H, d, J = 8.5 Hz, H-9 and H-13), 7.55 (1H, *t*, J = 7.4 Hz, H-11), 7.37 (4H, *t*, J = 8.0 Hz, H-10, H-12, H-17, and H-19), 7.35 (1H, *t*, J = 8.0 Hz, H-18), 7.33 (2H, d, J = 7.5 Hz, H-16, and H-20), 4.11 (2H, s, H-14), 4.09 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 178.9 (1C, s, C-7), 164.9 (1C, s, C-2), 153.7 (1C, s, C-6), 149.7 (1C, s, C-5), 136.6 (1C, s, C-15), 135.1 (1C, s, C-8), 133.1 (1C, d, C-11), 131.7 (2C, d, C-9, and C-13), 129.9 (2C, d, C-16 and C-20), 128.6 (2C, d, C-17 and C-19), 128.1 (2C, d, C-10 and C-12), 126.7 (1C, d, C-18), 111.0 (1C, s, C-3), 64.6 (1C, q, OMe), 39.1 (1C, t, C-14); ESITOFMS *m*/*z* 337.1188 $(M + H)^+$, calcd for $(C_{19}H_{16}O_4N_2+H)^+$ 337.1188.

X-ray Crystal Structure Analysis of Compound 4. Crystal data for **4**: $C_{28}H_{14}N_2O_4{\cdot}CH_3OH$, MW 354.36, monoclinic, $P2_1/c$, a = 4.7417(11) Å, b = 17.886(9) Å, c = 20.692 (5) Å, β = 94.847(10)°, V = 1748.6(11) Å,^{3} D_x = 1.346 g/cm^3, Z = 4, $F(000)$ = 744. A total of 11 101 reflections, of which 2050 were unique reflections (1614 observed, $|F_o| > 4\sigma|F_o|$), were measured at room temperature from a 0.25 × 0.15 × 0.10 mm^3 yellow-orange crystal using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a Bruker-Nonius kappaCCD diffractometer. The crystal structure was solved by direct methods using SIR-97, and then all atoms except hydrogen atoms were refined anisotropically by full-matrix least-squares methods on F^2 using SHELXL-97 to give a final R-factor of 0.0530 (R_w = 0.1156 for all data). Crystallographic data of compound **4** have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 296815.

Compound 5: yellow solid; $[\alpha]^{24}_D$ +5.9 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (3.6), 225 (3.6), and 271 (3.3) nm; IR (KBr) ν_{max} 3406, 2927, 1716, 1635, 1454, 1384, 1261, and 1087 cm^{-1}; ^{1}H and ^{13}C NMR data of **5** exhibited 2 sets of signals, designated as superscript a and b, respectively. ^{1}H NMR (CD_3OD, 500 MHz) δ_H 9.45^{a,b} (1H, d, J = 1.5 Hz, H-1), 7.04^a (1H, ddd, J = 15.0, 11.6, 1.5 Hz, H-4), 7.03^b (1H, ddd, J = 15.0, 11.6, 1.5 Hz, H-4), 6.88^a (1H, d, J = 11.5 Hz, H-3), 6.87^b (1H, d, J = 11.4 Hz, H-3), 6.59^b (1H, dq, J = 16.0, 6.7 Hz, H-2′), 6.58^a (1H, dq, J = 16.7, 6.7 Hz, H-2′), 6.50^a (1H, dd, J = 15.1, 5.4 Hz, H-5), 6.44^b (1H, dd, J = 15.0, 5.9 Hz, H-5), 6.30^{a,b} (1H, dd, J = 15.8, 1.4 Hz, H-1′), 4.33^b (1H, dd, J = 6.0, 6.0 Hz, H-6), 4.30^a (1H, dd, J = 7.1, 7.1 Hz, H-6), 3.80^a (1H, dd, J = 12.9, 2.7 Hz, H-10), 3.78^b (1H, dd, J = 12.8, 2.7 Hz, H-10), 3.50^a (1H, dd, J = 12.0, 6.0 Hz, H-10), 3.49^b (1H, dd, J = 12.6, 5.3 Hz, H-10), 3.43^b (1H, dd, J = 5.6, 5.7 Hz, H-7), 3.40^a (1H, dd, J = 5.2, 6.5 Hz, H-7), 3.11^a (1H, dd, J = 5.0, 2.3 Hz, H-8), 3.09^a (1H, dddd, J = 5.1, 2.5, 2.5, 2.5 Hz, H-9), 3.07^b (1H, dddd, J = 5.2, 2.5, 2.5, 2.5 Hz, H-9), 3.02^b (1H, dd, J = 5.6, 2.3 Hz, H-8), 1.85^{a,b} (1H, dd, J = 6.7, 1.6 Hz, H-3′); ^{13}C NMR (CD_3OD, 125 MHz) δ_C 196.3^{a,b} (1C, d, C-1), 148.8^a (1C, d, C-3), 148.6^b (1C, d, C-3), 145.7^a (1C, d, C-5), 144.8^b (1C, d, C-5), 137.4^b (1C, s, C-2), 137.3^a (1C, s, C-2), 134.6^b (1C, d, C-2′), 134.5^a (1C, d, C-2′), 127.4^b (1C, d, C-4), 127.0^a (1C, d, C-4), 122.2^b (1C, d, C-1′), 122.2^a (1C, d, C-1′), 75.5^b (1C, d, C-7), 75.1^a (1C, d, C-7), 74.8^b (1C, d, C-6), 74.4^a (1C, d, C-6), 62.7^a (1C, t, C-10), 62.5^b (1C, t, C-10), 57.7^b (1C, d, C-9), 57.6^a (1C, d, C-9), 57.4^b (1C, d, C-8), 57.4^a (1C, d, C-8), 19.6^{a,b} (1C, q, C-3′); ESITOFMS *m*/*z* 277.1051 $(M + Na)^+$, calcd for $(C_{13}H_{18}O_5+Na)^+$ 277.1052.

Compound 6: colorless oil; $[\alpha]^{24}_D$ −1.8 (*c* 0.08, MeOH); IR (KBr) ν_{max} 3399, 3018, 2964, 2933, 2877, 1706, 1408, 1377, 1216, 1125, 1079, 972, 751, and 667 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ_H 5.74 (1H, dtd, J = 15.5, 6.7, 1.3 Hz, H-7), 5.53 (1H, dddd, J = 15.5, 5.8, 1.4, 1.4 Hz, H-8), 3.85 (1H, br s, H-9), 3.73 (1H, dd, J = 2.1, 12.4 Hz, H-3′), 3.48 (1H, dd, 4.9, 12.4 Hz, H-3′), 2.97 (1H, ddd, J = 5.1, 2.2, 2.2 Hz, H-2′), 2.83 (1H, dd, J = 2.2, 5.8 Hz, H-1′), 2.50 (2H, t, J = 7.4 Hz, H-5), 2.41 (2H, t, J = 7.3 Hz, H-3), 2.26 (2H, dt, J = 6.8, 6.7 Hz, H-6), 1.54 (2H, sext, J = 7.3 Hz, H-2), 0.86 (3H, t, J = 7.4 Hz, H-1); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C 209.3 (1C, s, C-4), 131.1 (1C, d, C-7), 130.4 (1C, d, C-8), 72.6 (1C, d, C-9), 62.2 (1C, t, C-3′), 59.1 (1C, d, C-1′), 56.5 (1C, d, C-2′), 44.6 (1C, t, C-3), 42.1 (1C, t, C-5), 26.8 (1C, t, C-6), 17.4 (1C, t, C-2), 13.6 (1C, q, C-1); ESITOFMS *m*/*z* 251.1252 $(M + Na)^+$, calcd for $(C_{12}H_{20}O_4+Na)^+$ 251.1259.

Preparation of MTPA Ester Derivatives of 6. A reaction mixture consisting of **6** (ca. 2.3 mg), pyridine (300 μL), and (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (40 μL) was left standing at room temperature for 6 h. The mixture was dried under vacuum, then dissolved in 5 mL of EtOAc and subsequently washed with H_2O. The EtOAc layer was dried, yielding the (*S*)-(−)-MTPA ester of **6** (ca. 2.5 mg). Preparation of the (*R*)-(+)-MTPA ester of **6** from (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride was conducted in the same manner as that of the (*S*)-(−)-MTPA ester derivative. The *S*-(−)-MTPA ester (**6a**) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.52 (4H, m, aromatic protons of MTPA), 7.41 (6H, m, aromatic protons of MTPA), 5.82 (1H, ddd, J = 15.2, 6.7, and 6.7 Hz, H-7), 5.40 (1H, br dd, J = 15.4 and 7.3 Hz, H-8), 5.20 (1H, dd, J = 7.0 and 7.0 Hz, H-9), 4.61 (1H, dd, J = 12.3 and 3.3 Hz, H-3′), 4.26 (1H, dd, J = 12.3 and 6.8 Hz, H-3′), 3.59 (3H, s, OMe), 3.56 (3H, s, OMe), 3.20 (1H, m, H-2′), 3.08 (1H, dd, J = 6.9 and 2.0 Hz, H-1′), 2.45 (2H, t, J = 7.3 Hz, H-5), 2.37 (2H, t, J = 7.4 Hz, H-3), 2.30 (2H, dt, J = 7.0 and 7.0 Hz, H-6), 1.61 (2H, sext, J = 7.4 Hz, H-2), and 0.92 (3H, t, J = 7.4 Hz, H-1); ESITOFMS *m*/*z* 683.2076 $(M + Na)^+$, calcd for $(C_{32}H_{34}O_8F_6Na)^+$ 683.2056. The *R*-(+)-MTPA ester (**6b**) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.52 (4H, m, aromatic protons of MTPA), 7.41 (6H, m, aromatic protons of MTPA), 5.93 (1H, ddd, J = 15.2, 6.6, and 6.6 Hz, H-7), 5.53 (1H, br dd, J = 15.6 and 7.7 Hz, H-8), 5.32 (1H, dd, J = 6.1 and 6.7 Hz, H-9), 4.61 (1H, dd, J = 12.4 and 3.2 Hz, H-3′), 4.20 (1H, dd, J = 12.4 and 5.0 Hz, H-3′), 3.54 (6H, br s, OMe), 3.12 (1H, m, H-2′), 3.08 (1H, dd, J = 5.9 and 1.9 Hz, H-1′), 2.49 (2H, t, J = 7.3 Hz, H-5), 2.37 (2H, t, J = 7.3 Hz, H-3), 2.35 (2H, dt, J = 6.7 and 6.8 Hz, H-6), 1.60 (2H, m, H-2), and 0.92 (3H, t, J = 7.4 Hz, H-1); ESITOFMS *m*/*z* 683.2050 $(M + Na)^+$, calcd for $(C_{32}H_{34}O_8F_6Na)^+$ 683.2056.

Compound 7: amorphous solid; $[\alpha]^{24}_D$ −53.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.3), 225 (4.2), 264 (3.8), and 349 (3.5) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ_H 10.21 (1H, s, CHO), 7.46 (1H, d, J = 8.6 Hz, H-4), 6.89 (1H, d, J = 16.1 Hz, H-1′), 6.81 (1H, d, J = 8.6 Hz, H-5), 5.78 (1H, dd, J = 6.6, 15.3 Hz, H-6′), 5.75 (1H, dd, J = 6.3, 16.0 Hz, H-2′), 5.61 (1H, dd, J = 6.8, 15.6 Hz, H-5′), 4.24 (1H, dd, J = 5.7, 5.1 Hz, H-3′), 4.10 (1H, dd, J = 5.8, 5.7 Hz, H-4′), 3.70 (1H, m, H-10′), 3.45 (1H, d, J = 10.1 Hz, H-2″), 3.04 (1H, br d, J = 13.9 Hz, H-1″), 2.50 (1H, dd, J = 10.8, 14.0 Hz, H-1″), 2.11 (2H, m, H-7′), 1.42 (4H, m, H-8′ and H-9′), 1.25 (3H, s, H-4″), 1.23 (3H, s, H-5″), 1.12 (3H, d, J = 6.2 Hz, H-11′); ^{13}C NMR (CD_3OD, 125 MHz) δ_C 198.0 (1C, d, CHO), 161.0 (1C, s, C-6), 142.6 (1C, s, C-2), 140.0 (1C, d, C-2′), 139.0 (1C, d, C-4), 133.1 (1C, d, C-6′), 129.8 (1C, s, C-3), 129.3 (1C, d, C-5′), 125.8 (1C, d, C-1′), 118.5 (1C, s, C-1), 115.2 (1C, d, C-5), 78.5 (1C, d, C-2″), 75.4 (1C, d, C-3′), 75.3 (1C, d, C-4′), 72.6 (1C, s, C-3″), 67.0 (1C, d, C-10′), 38.2 (1C, t, C-9′), 33.7 (1C, t, C-1″), 32.0 (1C, t, C-7′), 25.0 (1C, t, C-8′), 25.0 (1C, q, C-4″), 23.1 (1C, q, C-5″), 22.1 (1C, q, C-11′); ESITOFMS *m*/*z* 421.2222 $(M - H)^-$, calcd for $(C_{23}H_{34}O_7-H)^-$ 421.2227.

Compound 8: white needles (MeOH−$CHCl_3$); mp 79−81 °C; $[\alpha]^{24}_D$ −14.7 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.4) and 290 (3.5) nm; IR (KBr) ν_{max} 3375, 2955, 2927, 2855, 1646, 1585, 1457, 1384, 1282, 1061, 1000, and 976 cm^{-1}; ^{1}H NMR (acetone-d_6−D_2O, 9:1, v/v, 500 MHz) δ_H 7.01 (1H, d, J = 8.3 Hz, H-5), 6.67 (1H, d, J = 16.3 Hz, H-1′), 6.66 (1H, d, J = 8.2 Hz, H-4), 5.74 (1H, dd, J = 6.5, 16.3 Hz, H-2′), 5.72 (1H, ddd, J = 1.0, 6.8, 14.3 Hz, H-6′), 5.61 (1H, ddd, J = 1.3, 6.2, 16.8 Hz, H-5′), 4.77 (1H, d, J = 12.3 Hz, H-1″), 4.73 (1H, d, J = 12.3 Hz, H-1″), 4.14 (1H, ddd, J = 5.8, 5.2, 1.1 Hz, H-3′), 4.08 (1H, dd, J = 5.9, 5.7 Hz, H-4′), 3.45 (1H, dd, J = 1.8, 10.2 Hz, H-2‴), 2.97 (1H, dd, J = 1.8, 13.9 Hz, H-1‴), 2.41 (1H, dd, J = 10.2, 13.9 Hz, H-1‴), 2.00 (2H, m, H-7′), 1.36 (2H, m, H-8′), 1.27 (4H, m, H-9′ and H-10′), 1.18 (3H, s, H-4‴), 1.17 (3H, s, H-5‴), 0.84 (2H, t, J = 7.0 Hz, H-11′); ^{13}C NMR (acetone-d_6−D_2O, 9:1, v/v, 125 MHz) δ_C 154.8 (1C, s, C-3), 138.3 (1C, s, C-1), 135.6 (1C, d, C-2′), 132.1 (1C, d, C-6′), 130.3 (1C, d, C-5), 130.0 (1C, d, C-5′), 129.4 (1C, s, C-6), 128.6 (1C, d, C-1′), 124.0 (1C, s, C-2), 113.9 (1C, d, C-4), 78.7 (1C, d, C-2‴), 75.8 (1C, d, C-3′), 75.2 (1C, d, C-4′), 72.4 (1C, s, C-3‴), 58.7 (1C, t, C-1″), 34.8 (1C, t, C-1‴), 32.2 (1C, t, C-7′), 31.3 (1C, t, C-10′), 28.8 (1C, t, C-8′), 25.2 (1C, *q*, C-4‴), 24.0 (1C, *q*, C-5‴), 22.3 (1C, t, C-9′), 13.4 (1C, t, C-11′); ESITOFMS *m*/*z* 407.2425 $(M - H)^-$, calcd for $(C_{23}H_{36}O_6-H)^-$ 407.2434.

Compound 9: brown solid; mp 79−81 °C; $[\alpha]^{24}_D$ +31.4 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.1), 258 (3.8), and 335 (3.4) nm; IR (KBr) ν_{max} 3417, 2924, 2854, 1645, 1458, 1291, 1229, and 1087 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ_H 12.01 (1H, s, 8-OH), 7.59 (1H, dd, J = 7.5, 8.3 Hz, H-6), 7.07 (1H, d, J = 7.4 Hz, H-5), 6.92 (1H, d, J = 8.4 Hz, H-7), 4.98 (1H, d, J = 3.0 Hz, H-4), 4.59 (1H, d, J = 8.9 Hz, H-2), 4.06 (1H, dd, J = 3.1, 8.9 Hz, H-3); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C 203.7 (1C, s, C-1), 162.0 (1C, s, C-8), 143.5 (1C, s, C-4a), 137.1 (1C, d, C-6), 120.3 (1C, d, C-5), 116.9 (1C,

d, C-7), 114.3 (1C, s, C-8a), 73.4 (1C, d, C-2), 73.1 (1C, d, C-3), 69.5 (1C, d, C-4); ESITOFMS *m*/*z* 233.0422 $(M + Na)^+$, calcd for $(C_{10}H_{10}O_5+Na)^+$ 233.0426.

Preparation of MTPA Ester Derivatives of 9. A mixture consisting of **9** (ca. 1.2 mg), pyridine (300 μL), and (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (40 μL) was left standing at room temperature for 6 h. The mixture was dried under vacuum, then dissolved in 5 mL of EtOAc and subsequently washed with H_2O. The EtOAc layer was dried, yielding the (*S*)-(−)-MTPA ester of **9** (ca. 1.3 mg). Preparation of the (*R*)-(+)-MTPA ester of **9** from (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride was conducted in the same manner as that of the (*S*)-(−)-MTPA ester derivative. The *S*-(−)-MTPA ester (**9a**) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.71 (1H, dd, J = 7.9 and 8.0 Hz, H-6), 7.58 (1H, br d, J = 7.7 Hz, H-5), 7.44 (6H, m, aromatic protons of MTPA), 7.37 (9H, m, aromatic protons of MTPA), 7.18 (1H, dd, J = 7.9 and 1.0 Hz, H-7), 6.69 (1H, d, J = 3.5 Hz, H-4), 5.82 (1H, d, J = 11.1 Hz, H-2), 4.68 (1H, m, H-3), 3.65 (3H, s, OMe), 3.57 (3H, s, OMe), and 3.56 (3H, s, OMe); ESITOFMS *m*/*z* 881.1635 $(M + Na)^+$, calcd for $(C_{40}H_{31}O_{11}F_9Na)^+$ 881.1620. The *R*-(+)-MTPA ester (**9b**) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.75 (1H, dd, J = 7.9 and 7.9 Hz, H-6), 7.63 (1H, br d, J = 7.9 Hz, H-5), 7.46 (6H, m, aromatic protons of MTPA), 7.39 (9H, m, aromatic protons of MTPA), 7.18 (1H, dd, J = 7.8 and 1.1 Hz, H-7), 6.66 (1H, d, J = 3.4 Hz, H-4), 5.79 (1H, d, J = 11.1 Hz, H-2), 4.46 (1H, m, H-3), 3.77 (3H, s, OMe), 3.68 (3H, s, OMe), and 3.44 (3H, s, OMe); ESITOFMS *m*/*z* 881.1661 $(M + Na)^+$, calcd for $(C_{40}H_{31}O_{11}F_9Na)^+$ 881.1620.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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