

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 IC₅₀ values of 29.2 and 57.4 μ M, respectively. Cytotoxicity of **1**, **2**, **4**, and **9** against the NCI-H187 cell line showed respective IC₅₀ values of 22.9, 20.3, 1.8, and 56.6 μ M. Compounds **2** and **4** exhibited antimalarial activity with IC₅₀ values of 2.95 and 28.8 μ M, respectively. Substances **1**, **4**, **7**, **8**, and **9** possessed weak antimycobacterial activity (MIC 154.8–952.3 μ M), while compounds **2** and **3** showed potent antimycobacterial activity with respective MIC values of 1.24 and 7.14 μ M.

Our previous study on the seed fungus *Menisporopsis theobromae* BCC 4162¹ 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 μ M. 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 ¹H, ¹³C, DEPT, ¹H–¹H COSY, HMQC, and HMBC spectroscopic data and finally confirmed by literature data comparison.² The ¹H NMR spectrum of compound **1** in CDCl₃ is identical to reported data² (see Supporting Information). However, the ¹H 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 ¹H and ¹³C NMR data have not yet been assigned. We have completely assigned proton and carbon resonances in **1** (see Experimental Section).

The ¹H and ¹³C 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**, C₃₁H₃₆N₂O₉S₂, was deduced from the ESITOF mass spectrum. Signals of sp³ methines (C-12 and C-12a) in **1** were replaced by sp² 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-15 α), 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-15 α) 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 $J_{\text{H-5},\text{H-5a}}$ 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)-acetylpoaranotin³ 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

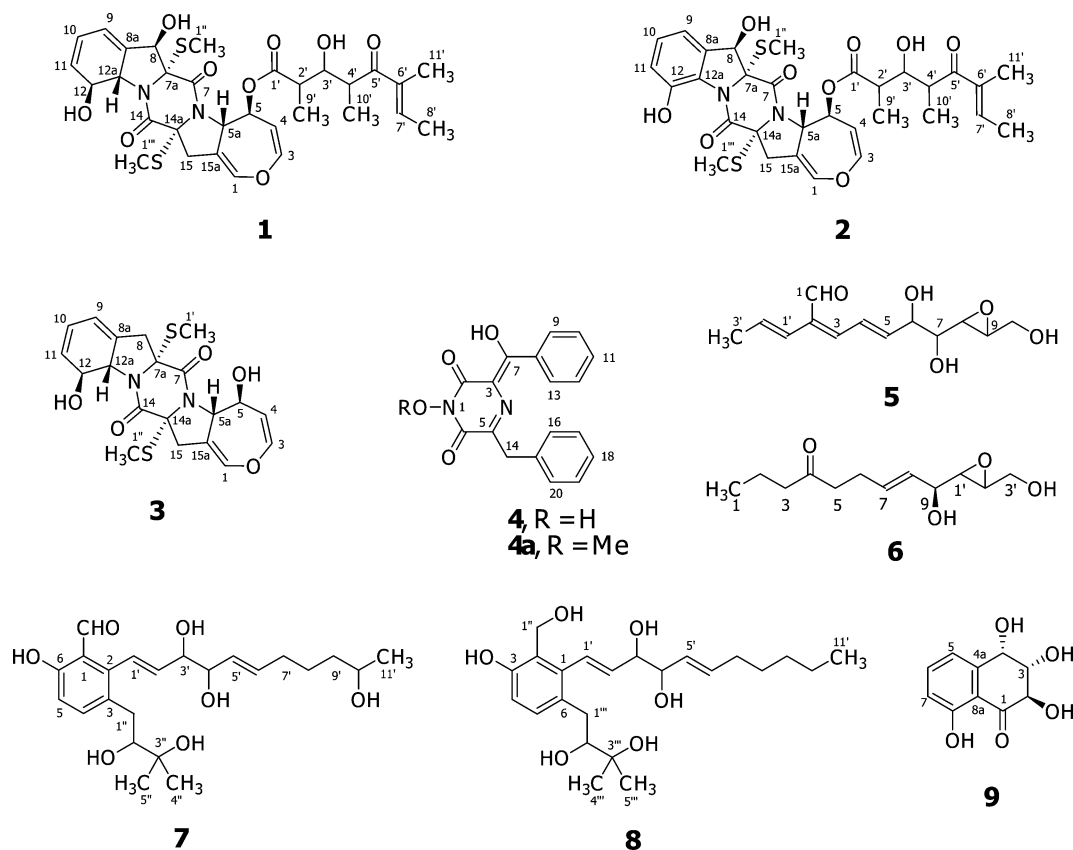
The molecular formula of compound **3**, C₂₀H₂₂N₂O₅S₂, was determined by ESITOFMS. In general, ¹H and ¹³C NMR data of **3** were similar to those of compound **1**, and analyses of these data revealed that compound **3** did not possess a 3-hydroxy-2,4,6-trimethyl-5-oxo-oct-6-enoic acid side chain, but 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 ¹H–¹H 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



The ESITOFMS of **4** established its molecular formula as $C_{18}H_{14}O_4N_2$. The 1H 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 sclerominol 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 sclerominol 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 sclerominol and flutimide,^{4–7} compound **4** was identified as 5-benzyl-1-hydroxy-3-(hydroxyphenylmethylene)-3H-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 sclerominol and flutimide.^{4–7} Sclerominol showed cytotoxicity against cancer cell lines, but demonstrated only mild

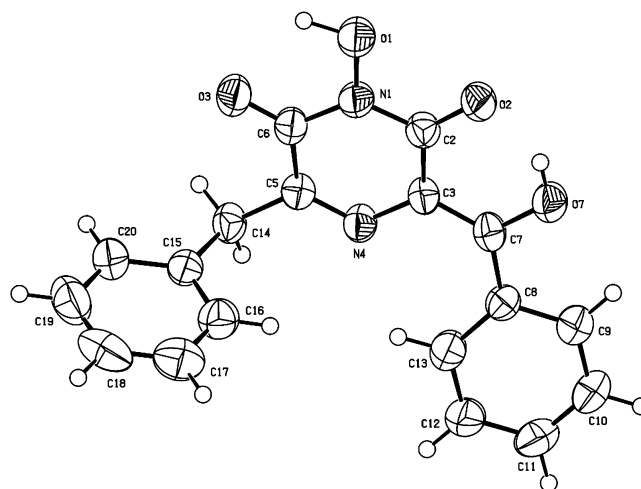


Figure 1. ORTEP plot of 5-benzyl-1-hydroxy-3-(hydroxyphenylmethylene)-3H-pyrazine-2,6-dione (**4**).

Table 1. Biological Activities of Compounds 1–9

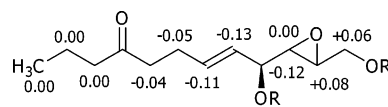
compound	cytotoxicity (IC ₅₀ , μ M)			antimalaria (IC ₅₀ , μ M)	antimycobacteria (MIC, μ M)
	KB ^a	BC-1 ^b	NCI-H187 ^c		
1	>30.9	29.2	22.9	>30.9	154.8
2	>31.0	>31.0	20.3	2.95	1.24
3	>46.0	>46.0	>46.0	>46.0	7.14
4	>62.1	57.4	1.8	28.8	310.5
5	>78.7	>78.7	>78.7	>78.7	>787.4
6	>30.3	>30.3	>30.3	>30.3	>303.0
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 $\pm$ 1.46	5.93 $\pm$ 0.57	1.59 $\pm$ 0.61		
	<i>n</i> = 3	<i>n</i> = 3	<i>n</i> = 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 IC₅₀ value of 28.8 μ M and antimycobacterial activity with an MIC value of 310.5 μ M (Table 1). Cytotoxicity of **4** against BC-1 (breast cancer) and NCI-H187 (small cell lung cancer) cell lines was shown by IC₅₀ values of 57.4 and 1.86 μ M, respectively (Table 1).

The molecular formula C₁₃H₁₈O₅ for compound **5** was determined by ESITOFMS spectrum. The ¹H NMR spectrum (CD₃OD) 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 ¹H and ¹³C 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 ¹³C 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 ¹³C 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 C₁₂H₂₀O₄. The ¹H 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 ¹³C 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



6a; R = (S)-(-)-MTPA

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

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

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 ¹H 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.

The molecular formula of C₂₃H₃₄O₇ for compound **7** was deduced from the ESITOFMS spectrum. The ¹H NMR spectrum (CD₃OD) 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 ¹³C 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 C₂₃H₃₆O₆. The ¹H and ¹³C 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

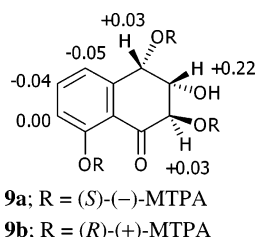


Figure 3. $\Delta\delta$ values [$\delta_{(-)} - \delta_{(+)}$] for the MTPA esters **9a** and **9b**.

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 $\text{C}_{10}\text{H}_{10}\text{O}_5$. The ^1H 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-2H-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 $J_{\text{H-2,H-3}}$ and $J_{\text{H-3,H-4}}$ 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 ^1H 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 $\text{C}_{40}\text{H}_{31}\text{O}_{11}\text{F}_9\text{Na}$ [observed m/z 881.1635 ($M + \text{Na}^+$)], similar to that of **9b** [observed m/z 881.1661 ($M + \text{Na}^+$)]; therefore, esterification with Mosher reagent provided tri-*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 $57.4\text{ }\mu\text{M}$, 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 $56.6\text{ }\mu\text{M}$. Compounds **2** and **4** exhibited antimalarial activity with IC_{50} values of 2.95 and $28.8\text{ }\mu\text{M}$, 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 $7.14\text{ }\mu\text{M}$ (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. ^1H , ^{13}C , ^1H – ^1H 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 $\times$ 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 (E₁–E₉). Fraction E₈ 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 ^1H 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–H₂O as eluent) to yield 3-(2,3-dihydroxy-3-methylbutyl)-6-hydroxy-2-(3,4,10-trihydroxyundeca-1,5-dienyl)benzaldehyde (7) (1.6 mg).

Fraction E was rechromatographed on a Sephadex LH-20 column and eluted with MeOH to provide 13 fractions (E₁–E₁₃), which were further purified by a Sephadex LH-20 column and a semipreparative HPLC (C₁₈ reversed-phase column and 25:75 MeCN–H₂O as eluent). Separation of fraction E₆ by a Sephadex LH-20 column (MeOH as eluent) and a semipreparative HPLC (C₁₈ reversed-phase column and 25:75 MeCN–H₂O as eluent) yielded 12.5 mg of 9-hydroxy-9-(3-hydroxymethylxiranyl)non-7-en-4-one (6). Fraction E₈ was subsequently subjected to a Sephadex LH-20 column (MeOH as eluent), followed by a semipreparative HPLC (C₁₈ reversed-phase column and 25:75 MeCN–H₂O as eluent), to afford 6,7-dihydroxy-7-(3-hydroxymethylxiranyl)-2-propenylhepta-2,4-dienal (5) (3.8 mg). Crystallization of fraction F from MeOH gave 5-benzyl-1-hydroxy-3-(hydroxyphenylmethylene)-3H-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 C₁–C₁₀. Fractions C₆ and C₇ 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 (C₁₈ reversed-phase column and 25:75 MeCN–H₂O 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 CH₂Cl₂–MeOH as eluent) to provide 9-hydroxy-9-(3-hydroxymethylxiranyl)non-7-en-4-one (6) (12.6 mg). Fraction C₈ was rechromatographed on a Sephadex LH-20 column (MeOH as eluent) and finally with a semipreparative HPLC (C₁₈ reversed-phase column and 25:75 MeCN–H₂O as eluent), furnishing 6,7-dihydroxy-7-(3-hydroxymethylxiranyl)-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 (C₁₈ reversed-phase column and 25:75 MeCN–H₂O 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 CH₂Cl₂–MeOH as eluent) to yield compound 3 (3.2 mg) and 2,3,4,8-tetrahydroxy-3,4-dihydro-2H-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 (IC₅₀) represents the concentration that causes 50% reduction in parasite growth, as indicated by the in vitro uptake of [³H]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; [α]_D²⁴ –135.5 (c 0.13, CHCl₃); UV (MeOH) λ_{max} (log ε) 205 (4.5), 224 (4.4), and 265 (3.8) nm; IR (CHCl₃ solution) ν_{max} 3425, 2927, 1728, 1642, 1392, 1191, and 1124 cm^{–1}; ¹H 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-15β), 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'); ¹³C 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 C₃₁H₃₈N₂O₉S₂Na 669.1916.

Compound 2: colorless needles (MeOH–acetone); [α]_D²⁴ –237.9 (c 0.03, CHCl₃); UV (MeOH) λ_{max} (log ε) 206 (4.3), 261 (3.6), and 293 (3.5) nm; ¹H NMR (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-15β), 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'); ¹³C 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 C₃₁H₃₆N₂O₉S₂Na 667.1760.

Compound 3: yellow solid; [α]_D²⁴ –103.3 (c 0.19, CHCl₃); UV (MeOH) λ_{max} (log ε) 204 (4.0), 260 (3.5), and 295 (3.4) nm; ¹H NMR (CDCl₃, 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-15β), 3.03 (1H, ddd, *J* = 15.5, 1.9, 1.9 Hz, H-8α), 2.98 (1H, br d, *J* = 15.3 Hz, H-8β), 2.31 (3H, s, H-1''); 2.27 (3H, s, H-1'); ¹³C NMR (CDCl₃, 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 C₂₀H₂₂N₂O₅S₂Na 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 (CHCl₃ solution) ν_{max} 2954, 2924, 2854, 1674, 1584, 1535, 1456, 1376, 1355, 1153, 761, 719, and 690 cm^{–1}; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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 (C₁₈H₁₄O₄N₂–H)[–] 321.0876.

Methylation of 5-Benzyl-1-hydroxy-3-(hydroxyphenylmethylene)-3H-pyrazine-2,6-dione (4). To a solution of 5-benzyl-1-hydroxy-3-(hydroxyphenylmethylene)-3H-pyrazine-2,6-dione (4) (2.5 mg) in DMF (1 mL) were added K₂CO₃ (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 H₂O (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): ^1H 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 ($\text{M} + \text{H}$) $^+$, calcd for $(\text{C}_{19}\text{H}_{16}\text{O}_4\text{N}_2 + \text{H})^+$ 337.1188.

X-ray Crystal Structure Analysis of Compound 4. Crystal data for **4**: $\text{C}_{28}\text{H}_{14}\text{N}_2\text{O}_4 \cdot \text{CH}_3\text{OH}$, MW 354.36, monoclinic, $P2_1/c$, $a = 4.7417(11)$ Å, $b = 17.886(9)$ Å, $c = 20.692(5)$ Å, $\beta = 94.847(10)^\circ$, $V = 1748.6(11)$ 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 $0.25 \times 0.15 \times 0.10$ mm 3 yellow-orange crystal using graphite-monochromated Mo K α radiation ($\lambda = 0.71073$ 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; $[\alpha]_D^{25} +5.9$ (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 $^{-1}$; ^1H and ^{13}C NMR data of **5** exhibited 2 sets of signals, designated as superscript a and b, respectively. ^1H 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 ($\text{M} + \text{Na}$) $^+$, calcd for $(\text{C}_{13}\text{H}_{18}\text{O}_5 + \text{Na})^+$ 277.1052.

Compound 6: colorless oil; $[\alpha]_D^{25} -1.8$ (c 0.08, MeOH); IR (KBr) ν_{max} 3399, 3018, 2964, 2933, 2877, 1706, 1408, 1377, 1216, 1125, 1079, 972, 751, and 667 cm $^{-1}$; ^1H 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 ($\text{M} + \text{Na}$) $^+$, calcd for $(\text{C}_{12}\text{H}_{20}\text{O}_4 + \text{Na})^+$ 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 $_2$ O. 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: ^1H 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 ($\text{M} + \text{Na}$) $^+$, calcd for $(\text{C}_{32}\text{H}_{34}\text{O}_8\text{F}_6\text{Na})^+$ 683.2056. The (*R*)-(+)-MTPA ester (**6b**) was obtained as a colorless oil: ^1H 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 ($\text{M} + \text{Na}$) $^+$, calcd for $(\text{C}_{32}\text{H}_{34}\text{O}_8\text{F}_6\text{Na})^+$ 683.2056.

Compound 7: amorphous solid; $[\alpha]_D^{25} -53.6$ (c 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.3), 225 (4.2), 264 (3.8), and 349 (3.5) nm; ^1H 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 ($\text{M} - \text{H}$) $^-$, calcd for $(\text{C}_{23}\text{H}_{34}\text{O}_7 - \text{H})^-$ 421.2227.

Compound 8: white needles (MeOH–CHCl $_3$); mp 79–81 $^\circ\text{C}$; $[\alpha]_D^{25} -14.7$ (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 $^{-1}$; ^1H 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 ($\text{M} - \text{H}$) $^-$, calcd for $(\text{C}_{23}\text{H}_{36}\text{O}_6 - \text{H})^-$ 407.2434.

Compound 9: brown solid; mp 79–81 $^\circ\text{C}$; $[\alpha]_D^{25} +31.4$ (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 $^{-1}$; ^1H 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 (C₁₀H₁₀O₅+Na)⁺ 233.0426.

Preparation of MTPA Ester Derivatives of 9. A mixture consisting of **9** (ca. 1.2 mg), pyridine (300 μ L), and (*R*)-(-)- α -methoxy- α -trifluoromethylphenylacetyl chloride (40 μ L) 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 H₂O. 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: ¹H NMR (CDCl₃, 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 (C₄₀H₃₁O₁₁F₉Na)⁺ 881.1620. The (*R*)-(+)-MTPA ester (**9b**) was obtained as a colorless oil: ¹H NMR (CDCl₃, 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 (C₄₀H₃₁O₁₁F₉Na)⁺ 881.1620.

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Supporting Information Available: ¹H and ¹³C 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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