Antifungal Azaphilones from the Fungus *Chaetomium cupreum* CC3003

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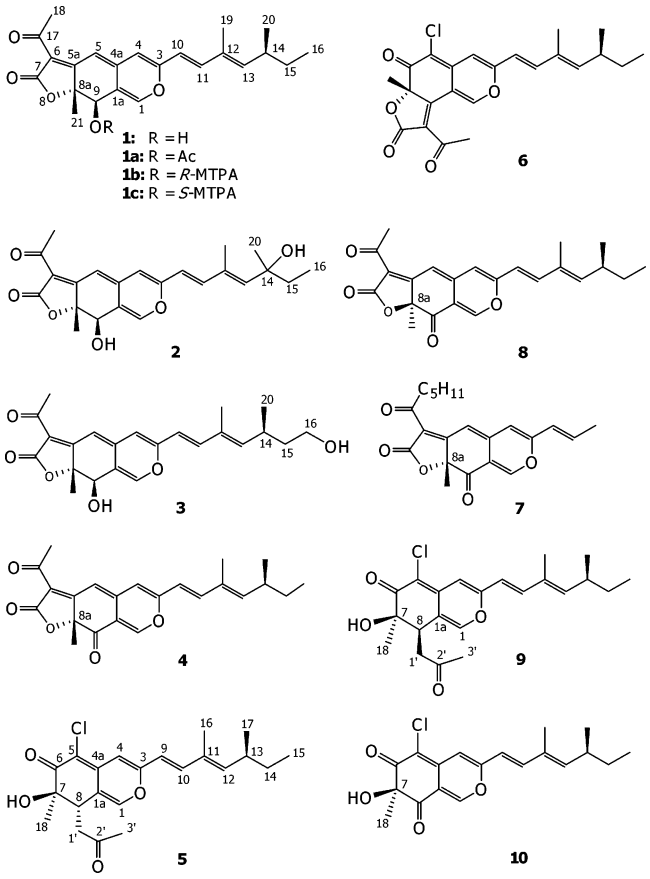
Three new azaphilones named rotiorinols A−C (**1**−**3**), two new stereoisomers, (−)-rotiorin (**4**) and *epi*-isochromophilone II (**5**), and a known compound, rubrorotiorin (**6**), were isolated from the fungus *Chaetomium cupreum* CC3003. Structures were established on the basis of spectroscopic evidence. The absolute configuration of **1** was determined by the modified Mosher's method along with an X-ray analysis of its acetate derivative, as well as by chemical transformation. Compounds **1**, **3**, **4**, and **6** exhibited antifungal activity against *Candida albicans* with IC_{50} values of 10.5, 16.7, 24.3, and 0.6 μg/mL, respectively.

Chaetomium is the largest saprophytic-ascomycetes genera. It belongs to the Chaetomiaceae family and comprises ca. 92 species,[1] of which ca. 20 species have been found in Thailand.[2,3] Previous investigations of secondary metabolites from *Chaetomium* species resulted in the isolation of compounds such as benzoquinone derivatives,[4] tetra-*S*-methyl derivatives,[5] azaphilones,[6] and chaetoglobosin analogues. Most of these compounds are mycotoxins.[7−12] In addition, *trans*-epoxysuccinyl peptides PF1138A and B,[13] anthraquinone-chromanones,[12] orsellinic acid, and globosumones[14] have also been reported. However, no phytochemical investigation of *C. cupreum* has been reported. As part of our ongoing project on natural medicines from microorganisms isolated from Thai soil[2], *C. cupreum* CC3003 was studied on the basis of its potential antifungal activity. Investigation of hexane, EtOAc, and MeOH extracts has led to the isolation of three new azaphilones named rotiorinols A−C (**1**−**3**), two new stereoisomers, (−)-rotiorin (**4**) and *epi*-isochromophilone II (**5**), and the known compound rubrorotiorin (**6**).



Results and Discussion

Rotiorinol A (**1**) was obtained as red crystals and was assigned the molecular formula $C_{23}H_{26}O_5$, as deduced from the HRESITOFMS (observed *m*/*z* 383.1847 $[M + H]^+$), indicating 11 degrees of unsaturation. The IR spectrum of **1** indicated the presence of hydroxyl (3393 cm^{-1}), α,β-unsaturated-γ-lactone (1751 cm^{-1}), and conjugated ketone (1643 cm^{-1}) groups. The UV spectrum showed absorption maxima at 492, 284, and 256 nm, indicating the presence of an extended conjugated system. ^1H and ^{13}C NMR spectra (Tables 1 and 2) and DEPT experiments indicated the presence of two carbonyl, six sp^2 quaternary, six sp^2 methine, one sp^3 quaternary, two sp^3 methine, one sp^3 methylene, and five methyl carbons. The ^{13}C NMR spectrum showed conjugated ketone and lactone carbonyl carbons at δ 195.1 and 171.6, respectively. The ^1H NMR spectrum displayed a low-field resonance of an olefinic proton at δ 7.48 (d, $J = 2.0$ Hz, H-1), which had an allylic coupling to an oxymethine proton at δ 4.78 (d, $J = 2.0$ Hz, H-9), two olefinic protons at δ 6.72 (s, H-5) and 6.20 (s, H-4), and two singlet signals of methyl and acetyl groups at δ 1.40 (H-21) and 2.52 (C-6), respectively. The structure of **1** was then elucidated to be an azaphilone bearing a five-membered-ring lactone by analysis of the 2D data. The HMBC spectrum confirmed the connection by showing ^3J correlations of H-1 to C-3, C-4a, and C-9; H-4 to C-1a, C-5, and C-10; H-5 to C-1a, C-4, C-6, and C-8a; H-9 to C-1, C-4a, C-5a, and C-21; H-18 to C-6; and H-21 to C-5a and C-9. The 3,5-dimethylheptadienyl unit was established by COSY correlations between H-10 and H-11, H-13 and H-14, H-14 and H-15, and H-15 and H-16. The configurations of the C-10,11 and C-12,13 double bonds were both found to be *E*, on the basis of coupling constants (Table 1) and observation of NOESY correlations between H-10 and C-12

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Table 1. ^{1}H NMR Spectral Data (δ, ppm) of Compounds **1**−**5** in $CDCl_3$[a]

position	1	2	3	4	5
1	7.48 d (2.0)	7.40 d (2.0)	7.46 d (2.0)	7.89 brs	6.96 d (1.5)
4	6.20 s	6.19 s	6.18 s	6.24 s	6.57 s
5	6.72 s	6.71 s	6.70 s	6.45 s	6.76 s
8					3.33 brd (9.6)
9	4.78 d (2.0)	4.75 d (2.0)	4.74 d (2.0)		6.07 d (15.7)
10	6.00 d (15.7)	6.06 d (15.6)	5.98 d (15.7)	5.98 d (15.7)	7.02 d (15.7)
11	7.02 d (15.7)	6.96 d (15.6)	6.98 d (15.7)	7.00 d (15.7)	
12					5.64 d (9.7)
13	5.65 d (9.8)	5.84 s	5.65 d (9.8)	5.68 d (9.6)	2.46 m
14	2.46 m		2.75 m	2.46 m	1.41 m, 1.28 m
15	1.42 m, 1.31 m	1.67 m	1.70 m, 1.59 m	1.42 m, 1.28 m	0.85, t (7.4)
16	0.85 t (7.4)	0.92 t (7.4)	3.60 m	0.83 t (7.4)	
17					1.84 s
18	2.52 s	2.51 s	2.50 s	2.51 s	0.95 d (6.4)
19	1.82 s	2.06 s	1.82 s	1.81 s	1.10 s
20	1.00 d (6.6)	1.37 s	1.02 d (6.6)	0.98 d (6.6)	
21	1.40 s	1.37 s	1.37 s	1.70 s	
1′					3.23 dd (2.0, 17.4), 2.76 dd (4.9, 17.4)
3′					2.30 s

[a] Figures in parentheses are coupling constants in Hz.

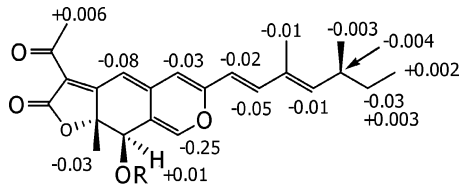
Table 2. ^{13}C NMR Spectral Data (δ, ppm) of Compounds **1**−**5** in $CDCl_3$

position	1	2	3	4	5
1	146.3 d[a]	146.1 d	146.3 d	152.9 d	143.5 d
1a	120.6 s	120.4 s	120.7 s	116.2 s	119.1 s
3	159.9 s	158.3 s	158.7 s	157.8 s	158.1 s
4	109.4 d	109.9 d	109.6 d	110.3 d	104.8 d
4a	145.8 s	145.2 s	145.9 s	145.0 s	143.4 s
5	102.9 d	103.2 d	103.0 d	103.9 d	107.9 d
5a	173.1 s	172.8 s	173.2 s	171.6 s	
6	110.4 s	110.7 s	110.3 s	112.7 s	192.1s
7	171.6 s	171.5 s	171.8 s	169.5 s	73.9 d
8					40.1 d
8a	83.2 s	83.1 s	83.3 s	85.7 s	
9	71.7 d	71.7 d	71.5 d	190.8 s	116.3 d
10	116.4 d	117.4 d	116.8 d	115.5 d	141.9 d
11	141.9 d	142.1 d	141.4 d	142.6 d	131.9 d
12	132.0 s	134.6 s	132.3 s	131.9 d	147.6 d
13	147.8 d	145.2 d	146.6 d	148.9 d	34.9 d
14	35.0 d	74.0 s	30.0 d	35.1 q	30.1 t
15	30.1 t	36.3 t	39.8 t	30.0 t	11.9 q
16	11.9 q	8.3 q	60.9 t	11.9 q	12.3 q
17	195.1 s	195.2 s	195.2 s	194.7 s	20.2 q
18	29.4 q	29.5 q	29.4 q	29.6 q	21.2 q
19	12.3 q	12.8 q	12.3 q	12.3 q	
20	20.2 q	28.6 q	20.6 q	20.2 q	
21	19.3 q	19.2 q	19.3 q	28.5 q	
1′					39.8 t
2′					206.9 s
3′					29.8 q

[a] Multiplicities were determined by analyses of the DEPT and/or HSQC spectra.

methyl protons (H-19), H-11 and H-13, and H-19 and H-20. The HMBC spectrum exhibited correlations of H-10 to C-3 and C-4 and of H-11 to C-3, confirming the connection of this unit at C-3.

Assignment of the absolute configuration at C-9 was carried out by the modified Mosher's ester method.[15−17] The reaction of **1** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) gave the (*S*)- and (*R*)-MTPA esters (**1c** and **1b**), respectively. Then, the differences in the ^{1}H NMR chemical shifts between the (*S*)- and (*R*)-MTPA ester ($\Delta\delta_H = \delta_S - \delta_R$) around the C-9 position were analyzed to determine the absolute configuration of this position, which was found to be *R* (Figure 1). Furthermore, rotiorinol A (**1**) exhibited a negative specific rotation similar to that of rubropunctatin (**7**) [−3670 (*c* 1.0, $CHCl_3$)],[18] which has an *R* absolute configuration at C-8a. Compound **1** was then oxidized with MnO_2·Al_2O_3 in CH_2Cl_2 to yield a product that was identical (mp, IR, NMR, specific rotation, and behavior on TLC)



1b: R = *R*-MTPA
1c: R = *S*-MTPA

Figure 1. $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$ in ppm) obtained for MTPA esters **1b** and **1c**.

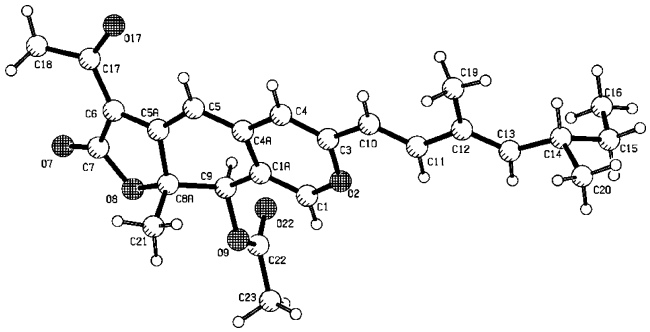


Figure 2. X-ray crystal structure of compound **1a**.

to natural (−)-rotiorin (**4**). The oxidation product of **1** and the isolated compound **4** displayed negative specific rotation values of −2251 and −2332, respectively, which supported the *R* configuration at C-8a, as in reference compound **7**. Finally, X-ray crystallographic analysis of the acetate derivative **1a** confirmed the absolute stereochemistry, including C-14 of the side chain, which was *S*, as was reported for (+)-rotiorin (**8**)[19] (Figure 2). Thus, the structure of rotiorinol A (**1**) was established as 6-acetyl-3-(3,5-dimethyl-1*E*,3*E*-heptadienyl)-9*R*-hydroxy-8a(*R*)-methyl-7*H*-furo-[2,3-*g*]-2-benzopyran-7-one.

Rotiorinol B (**2**) was obtained as a red, amorphous powder and had the molecular formula $C_{23}H_{26}O_6$, as deduced from the HRESITOFMS (observed *m*/*z* 399.1788 $[M + H]^+$), indicating 11 degrees of unsaturation. The UV spectrum showed absorption maxima at 480, 284, and 254 nm, indicating the presence of an extended conjugated system. The IR spectrum of **2** indicated the presence of hydroxyl (3400 cm^{-1}), α,β-unsaturated-γ-lactone (1750 cm^{-1}), and conjugated ketone (1654 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) were similar to those of **1** except for the absence of a methine proton resonance at C-14 of the side chain.

The ^{13}C NMR spectrum showed a signal at δ 74.0, which was assigned to C-14 bearing an OH group. The HMBC data supported the side chain by demonstrating correlations of H-10 to C-4 and C-12; H-11 to C-3, C-13, and C-19; H-13 to C-11, C-15, C-19, and C-20; H-15 to C-13, C-14, and C-20; and H-16 to C-14 and C-15. Compound **2** exhibited a negative specific rotation similar to that of rotiorinol A (**1**), implying that **2** possessed the same stereochemistry at C-8a. The configuration at C-9 was assigned as *R*, since the NOESY spectrum showed no correlation between C-8a methyl (H-21) and H-9 protons. However, the configuration at C-14 of the side chain remains unclear. Thus, the structure of **2** was assigned as 6-acetyl-3-(5-hydroxy-3,5-dimethyl-1*E*,3*E*-heptadienyl)-9*R*-hydroxy-8a(*R*)-methyl-7*H*-furo-[2,3-*g*]-2-benzopyran-7-one, which was named rotiorinol B.

Rotiorinol C (**3**) was obtained as a red, amorphous powder and had the molecular formula $C_{23}H_{26}O_6$, as deduced from the HRESITOFMS (observed *m*/*z* 399.1789 $[M + H]^+$), indicating 11 degrees of unsaturation. The UV spectrum showed absorption maxima at 485, 286, and 256 nm, indicating an extended conjugated system. The IR spectrum of **3** showed the presence of hydroxyl (3413 cm^{-1}), α,β-unsaturated-γ-lactone (1745 cm^{-1}), and conjugated ketone (1650 cm^{-1}) groups. The ^1H and ^{13}C NMR data of **3** (Tables 1 and 2) were similar to those of **1** except that the C-16 methyl signal was displaced by an oxymethylene group. The COSY correlations between H-10 and H-11, H-13 and H-14, H-14 and H-15, and H-15 and H-16 as well as the HMBC ^3J correlations of H-10 to C-4 and C-12; H-11 to C-3, C-13, and C-19; H-13 to C-11, C-15, C-19, and C-20; H-14 to C-12 and C-16; H-15 to C-13 and C-20; and H-16 to C-14 revealed the connectivity of this side chain. The stereochemistry of **3** was then assigned to be the same as compound **1** on the basis of the same sign of specific rotation and the NOESY correlations. Thus, **3** was defined as 6-acetyl-3-(3,5-dimethyl-1*E*,3*E*-heptadien-7-ol)-9*R*-hydroxy-8a(*R*)-methyl-7*H*-furo-[2,3-*g*]-2-benzopyran-7-one, which was named rotiorinol C.

(−)-Rotiorin (**4**) was obtained as red crystals and had the molecular formula $C_{23}H_{24}O_5$, as deduced from the HRESITOFMS (observed *m*/*z* 381.1694 $[M + H]^+$). The IR spectrum of **4** indicated α,β-unsaturated-γ-lactone (1741 cm^{-1}) and conjugated ketone (1656 and 1641 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **4** were similar to those of rotiorinol A (**1**) except for the absence of an oxymethine proton at C-9, which was replaced by a carbonyl group (δ_C 190.8). The ^1H and ^{13}C NMR data of **4** (Tables 1 and 2) have not been previously reported. However, compound **4** has a negative sign of specific rotation, the same as rubropunctatin (**7**),[18] revealing the *R* configuration at C-8a, which is different from (+)-rotiorin (**8**) isolated from *Penicillium scerotiorum* [+5080 (*c* 0.002, $CHCl_3$)].[19] Thus, compound **4** was determined to be a new epimer, (−)-rotiorin.

Compound **5** was obtained as a yellow, amorphous powder and had the molecular formula $C_{22}H_{27}ClO_4$, as deduced from the HRESITOFMS (observed *m*/*z* 391.1676 $[M + H]^+$). The IR spectrum of **5** indicated hydroxyl (3447 cm^{-1}), ketone (1715 cm^{-1}), and conjugated ketone (1624 cm^{-1}) groups. The ^1H and ^{13}C NMR data and 2D NMR (COSY, HSQC, HMBC, and NOESY) of **5** indicated a structure similar to isochromophilone II (**9**).[20] However, there were some differences in chemical shifts between compound **5** and isochromophilone II (**9**).[20] Differences in the ^1H and ^{13}C spectra were noted at δ_H 6.96 and 7.42 (for H-1); 3.23, 2.76 and 3.08, 2.35 (for H_2-1′); and 2.30 and 2.08 (for H_3-3′), while the carbons signals differed at δ_C 143.5 and 145.4 (for C-1); 39.8 and 41.3 (for C-1′), and 29.8 and 30.5 (for C-3′) for **5** and **9**, respectively. This suggested an opposite configuration at C-8 between the two compounds. The absolute configuration of both C-7 and C-8 in **9** has been reported as *R*, without the reporting of specific rotation.[21] However, **5** exhibited a positive sign of specific rotation [+341 (*c* 0.15, $CHCl_3$)], comparable to the related compound (+)-deacetylsclerotiorin (**10**) [+480 (*c* 0.01, EtOH)],[22]

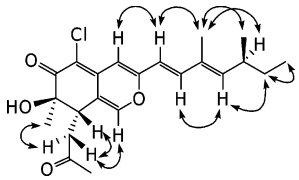


Figure 3. NOESY correlations for compound **5**.

indicating that **5** has the *R* configuration at C-7. The NOESY spectrum of **5** (Figure 3) showed no correlation between C-7 methyl protons (H-18) and H-8, indicating the *S* configuration at C-8. Thus, **5** was deduced to be *epi*-isochromophilone II.

The known azaphilone rubrorotiorin (**6**) was identified by IR and ^1H and ^{13}C NMR spectra including 2D NMR techniques (COSY, HMQC, and HMBC) as well as by comparison with data values reported in the literature.[23] The specific rotation of **6** [−448 (*c* 0.07, $CHCl_3$)] agrees well with the value reported in the literature [−368 (*c* 0.019, $CHCl_3$)].[23]

Compounds **1**, **3**, **4**, and **6** exhibited antifungal activity against *Candida albicans* with IC_{50} values of 10.5, 16.7, 24.3, and 0.6 μg/mL, respectively. Only **6** showed antifungal activity comparable to the reference drug, amphotericin (IC_{50} = 0.1−0.2 μg/mL). However, none of these compounds showed cytotoxicity against BC1 (breast cancer), KB (human epidermoid carcinoma), and NCI-H178 (human small cell lung cancer) cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Specific rotations were obtained using a JASCO DIP-1000 digital polarimeter. UV spectra were measured on an Agilent 8453 UV−visible spectrophotometer. IR spectra were taken on a Perkin-Elmer Spectrum One spectrophotometer. NMR spectra were recorded in $CDCl_3$ on a Varian Mercury Plus 400 spectrometer, using residual $CHCl_3$ as an internal standard. HRESITOFMS spectra were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of accurate masses. Column chromatography and preparative TLC were carried out on silica gel 60 (230−400 mesh) and PF_{254}, respectively.

Fungal Material. The fungus was collected from Thai soil and was identified by Assoc. Prof. K. Soytong as *C. cupreum* CC3003. A voucher specimen (CC3003) was deposited at the Department of Plant Pest Management, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The fungus was cultivated in potato dextrose broth (PDB) at 25−28 °C for 4 weeks.

Extraction and Isolation. Air-dried mycelial mat (300 g) was ground and extracted successively with hexane (3 L × 3), EtOAc (3 L × 3), and MeOH (3 L × 3) at room temperature. The filtered samples were combined, and the solvents were evaporated in vacuo to yield crude hexane (6.1 g), EtOAc (2.5 g), and MeOH extracts (20.3 g), respectively. The hexane extract (6.0 g) was subjected to silica gel (120 g) flash column chromatography and eluted with increasing concentrations of EtOAc in hexane followed by MeOH in EtOAc. Each fraction (100 mL) was monitored by TLC; fractions with similar TLC patterns were combined to yield fractions F_1−F_{10}. Fraction F_3 was further separated by preparative TLC, eluting with EtOAc−hexane (20:80), to yield **6** (R_f = 0.66, 86.7 mg). Fraction F_4 was separated on a silica gel column eluted with a gradient of EtOAc−hexane, to furnish four subfractions, designated as $F_{5/1}$−$F_{5/4}$. Subfraction $F_{5/2}$ gave additional **6** (30 mg). Subfraction $F_{5/3}$ was separated by preparative TLC, eluting with EtOAc−CH_2Cl_2 (20:80), to yield **5** (R_f = 0.63, 22.4 mg). Subfraction $F_{5/4}$ was recrystallized from CH_2Cl_2−hexane to give red crystals of **1** (273 mg). The EtOAc extract (2.5 g) was separated on a silica gel column eluted with increasing EtOAc in hexane followed by MeOH in EtOAc. Each fraction (100 mL) was monitored by TLC; fractions with similar TLC patterns were combined (F'_1−F'_{10}). The solid from F'_5 was filtered to yield additional **1** (277 mg), and the filtrate was further purified by preparative TLC, eluting with EtOAc−hexane (30:70), to give **4** (15.5 mg). MeOH−CH_2Cl_2 (50:50) was added to the MeOH extract to give a solid, which was removed by filtration. The filtrate was evaporated in vacuo, and the residue (8.5 g) was

chromatographed on silica gel eluted with a gradient system of EtOAc−CH_2Cl_2 to give fractions F″$_1$−F″$_9$. Fraction F″$_2$ was recrystallized from CH_2Cl_2−hexane to obtain additional **1** (200 mg). Fraction F″$_6$ was further separated by preparative TLC, eluting with Et_2O−hexane (90:10), to yield **2** (8.9 mg). Fraction F″$_7$ was rechromatographed on silica gel eluted with EtOAc−CH_2Cl_2 (20:80) to give three subfractions, designated as F″$_{7/1}$−F″$_{7/3}$. Subfraction F″$_{7/3}$ was further purified by preparative TLC, eluting with Et_2O−hexane (90:10), to yield **3** (11.2 mg).

Rotiorinol A (1): red crystals; mp 134−137 °C; $[\alpha]_D^{31}$ −2261 (*c* 0.015, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 492 (4.58), 284 (4.47), 256 (4.25) nm; IR (film, $CHCl_3$) ν_{max} 3393, 2926, 2853, 1751, 1643, 1539, 1239, 1173 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* 383.1847 [M + H]$^+$ (calcd for $C_{23}H_{26}O_5$ + H, 383.1859).

Rotiorinol B (2): red, amorphous; $[\alpha]_D^{31}$ −1120 (*c* 0.015, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 480 (4.25), 284 (4.36), 254 (3.88) nm; IR (KBr) ν_{max} 3400, 2930, 2857, 1750, 1654, 1240, 1175 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* 399.1788 [M + H]$^+$ (calcd for $C_{23}H_{26}O_6$ + H, 399.1808).

Rotiorinol C (3): red, amorphous; $[\alpha]_D^{29}$ −1364 (*c* 0.015, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 485 (4.36), 286 (4.26), 256 (3.95) nm; IR (KBr) ν_{max} 3413, 2930, 2850, 1745, 1650, 1242, 1170 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* 399.1789 [M + H]$^+$ (calcd for $C_{23}H_{26}O_6$ + H, 399.1808).

(−)-Rotiorin (4): red crystals; mp 224−226 °C; $[\alpha]_D^{29}$ −2332 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 421 (4.62), 284 (4.34), 256 (4.20), 243 (4.25) nm; IR (KBr) ν_{max} 2949, 2925, 1741, 1656, 1641, 1564, 1524, 1502, 1237, 1169 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* 381.1694 [M + H]$^+$ (calcd for $C_{23}H_{24}O_5$ + H, 381.1702).

***epi*-Isochromophilone II (5):** yellow, amorphous; $[\alpha]_D^{26}$ +341 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 398 (3.87), 358 (3.85), 2.48 (3.82) nm; IR (KBr) ν_{max} 3447, 2960, 2927, 1715, 1624, 1559, 1519, 1248, 1177 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* 391.1676 [M + H]$^+$ (calcd for $C_{22}H_{27}ClO_4$ + H, 391.1677).

Acetylation of Rotiorinol A (1). To a solution of **1** (62.2 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) and pyridine (0.5 mL) was added acetyl chloride (0.5 mL), and the mixture was stirred for 2 h at room temperature. The mixture was evaporated to dryness and purified by preparative TLC, eluting with EtOAc−hexane to yield red crystals of **1a** (56 mg, 81%); $[\alpha]_D^{29}$ −3350 (*c* 0.012, $CHCl_3$); IR (KBr) ν_{max} 3108, 2958, 2924, 1750, 1742, 1654, 1573, 1523, 1506, 1222, 1169 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.00 (1H, s, H-1), 6.97 (1H, d, J = 15.7 Hz, H-11), 6.76 (1H, s, H-5), 6.19 (1H, s, H-4), 5.98 (1H, d, J = 15.7 Hz, H-10), 5.63 (1H, d, J = 9.6 Hz, H-13), 2.55 (3H, s, H-18), 2.47 (1H, m, H-14), 2.27 (3H, s, 9-OAc), 1.83 (3H, s, H-19), 1.46 (3H, s, H-21), 1.42 (1H, m, H-15a), 1.31 (1H, m, H-15b), 1.00 (3H, d, J = 6.4 Hz, H-20), 0.86 (3H, t, J = 7.2 Hz, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.4 (C-17), 171.9 (9-O*C*OCH$_3$), 171.0 (C-5a), 169.8 (C-7), 158.7 (C-3), 148.2 (C-13), 145.0 (C-4a), 144.9 (C-1), 142.2 (C-11), 132.2 (C-12), 118.2 (C-1a), 116.3 (C-10), 111.3 (C-6), 109.5 (C-4), 103.6 (C-5), 80.8 (C-8a), 72.0 (C-9), 35.3 (C-14), 30.3 (C-15), 29.8 (C-18), 21.0 (9-OCO*C*H$_3$), 20.9 (C-20), 20.5 (C-21), 12.6 (C-19), 12.2 (C-16); ESITOFMS *m*/*z* 425 [M + H]$^+$ (calcd for $C_{25}H_{28}O_6$ + H, 425).

Preparation of the (*R*)-α-Methoxy-α-(trifluoromethyl)phenyl Acetate of Rotiorinol A (1). To a solution of the alcohol (−)-**1** (10.0 mg, 26.0 μM) in CH_2Cl_2 (1.5 mL) were added (dimethylamino)pyridine (3.2 mg, 26 μM), triethylamine (3.9 mg, 39 μM), and (*S*)-MPTA-Cl (9.7 μL, 52 μM). The mixture was stirred under N_2 at room temperature for 15 h, then the solvent was removed in vacuo. The product was purified by preparative TLC (EtOAc−hexane, 50:50) to give the (*R*)-ester (**1b**, 10.2 mg, 64.0%): $[\alpha]_D^{29}$ −1856 (*c* 0.1, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, s, H-1), 6.18 (1H, s, H-4), 6.75 (1H, s, H-5), 6.18 (1H, brs, H-9), 5.95 (1H, d, J = 15.7 Hz, H-10), 6.98 (1H, d, J = 15.7 Hz, H-11), 5.64 (1H, d, J = 9.7 Hz, H-13), 2.449 (1H, m, H-14), 1.407 (1H, m, H-15a), 1.30 (1H, m, H-15b), 0.832 (3H, t, J = 7.2 Hz, H-16), 2.531 (3H, s, H-18), 1.80 (3H, s,19-H), 0.981 (3H, d, J = 6.6 Hz, H-20), 1.37 (3H, s, 21-H), α-methoxy-α-(trifluoromethyl)-phenyl acetate part had δ 7.60 and 7.46 (5H, m, C_6H_5), 3.55 (3H, s, −OCH$_3$); ESITOFMS *m*/*z* 599 [M + H]$^+$ (calcd for $C_{33}H_{33}F_3O_7$ + H, 599).

Preparation of the (*S*)-α-Methoxy-α-(trifluoromethyl)phenyl Acetate of Rotiorinol A (1). To a solution of the alcohol (−)-**1** (10.0 mg, 26 μM) in CH_2Cl_2 (1.5 mL) were added (dimethylamino)pyridine (3.2 mg, 26 μM), triethylamine (3.9 mg, 39 μM), and (*R*)-MPTA-Cl (9.7 μL, 52 μM). The mixture was stirred under N_2 at room temperature for 15 h, then the solvent was removed in vacuo. The product was purified by preparative TLC (EtOAc−hexane, 50:50) to give the (*S*)-ester (**1c**, 8.0 mg, 50.0%): $[\alpha]_D^{29}$ −1941 (*c* 0.10, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.73 (1H, s, H-1), 6.15 (1H, s, H-4), 6.67 (1H, s, H-5), 6.19 (1H, brs, H-9), 5.93 (1H, d, J = 15.7 Hz, H-10), 6.93 (1H, d, J = 15.7 Hz, H-11), 5.63 (1H, d, J = 9.7 Hz, H-13), 2.445 (1H, m, H-14), 1.41 (1H, m, H-15a), 1.27 (1H, m, H-15b), 0.834 (3H, t, J = 7.2 Hz, H-16), 2.537 (3H, s, H-18), 1.79 (3H, s, 19-H), 0.978 (3H, d, J = 6.6 Hz, H-20), 1.342 (3H, s, 21-H), α-methoxy-α-(trifluoromethyl)-phenyl acetate part had δ 7.65 and 7.50 (5H, m, C_6H_5), 3.61 (3H, s, −OCH$_3$); ESITOFMS *m*/*z* 599 [M + H]$^+$ (calcd for $C_{33}H_{33}F_3O_7$ + H, 599).

Oxidation of Rotiorinol A (1). To a solution of **1** (6.5 mg, 0.017 mmol) in CH_2Cl_2 (2 mL) and pyridine (0.5 mL) was added MnO_2·Al_2O_3 (15 mg), and the mixture was stirred for 1 h at room temperature. The mixture was filtered and solvent was removed in vacuo. The residue was further purified by preparative TLC (EtOAc−hexane, 30:70) to give **4** (R_f = 0.60 3.2 mg, 49.3%) and starting material **1** (1.5 mg): mp 225−227°; $[\alpha]_D^{26}$ −2251 (*c* 0.07, $CHCl_3$); IR and NMR spectra were identical to those of (−)-rotiorin (**4**).

Antifungal Assay. Antifungal assays were made against clinically isolated *Candida albicans* using a method modified from the soluble formazan assay described by Scudiero and co-workers.[24] The number of living cells was determined by measuring the absorbance of XTT formazan at 450 nm. The reference substance was amphotericin (IC_{50} = 0.1−0.2 μg/mL).

X-ray Structure Determination of 1a. Crystal data of **1a**: $C_{25}H_{28}O_6$, MW 424.79, monoclinic, $P2_1$, a = 8.7040(6) Å, b = 15.983(2) Å, c = 9.2734(9) Å, β = 114.978(5)°, V = 1169.4(2) Å^3, D_x = 1.206 g/cm^3, Z = 2, $F(000)$ = 432. A total of 5691 reflections, of which 1736 were unique reflections (1505 observed, $|F_o| > 4\sigma|F_o|$), were measured at room temperature from a 0.25 × 0.15 × 0.10 mm^3 red 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 0.0625 (R_w = 0.1869 for all data).

Crystallographic data of compound **1a** have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 251067. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk).

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Supporting Information Available: X-ray crystallographic tables of atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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