Oxepinochromenones, Furochromenone, and their Putative Precursors from the Endolichenic Fungus *Coniochaeta* sp.

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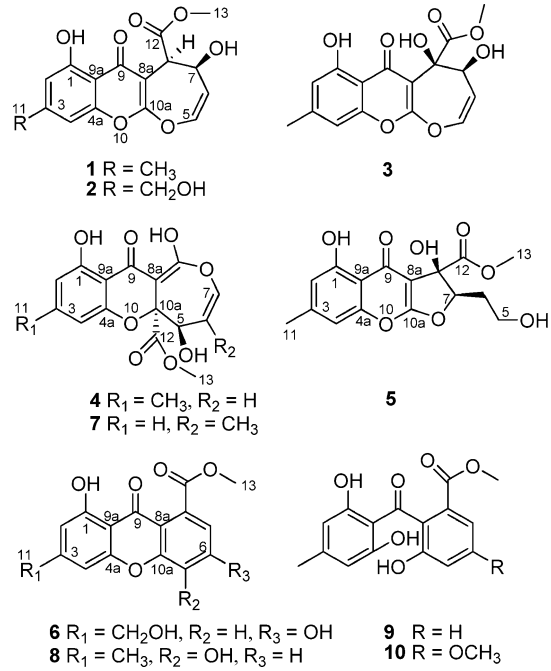
Six new polyketides including four oxepinochromenones, conioxepinols A−D (**1**−**4**), one furochromenone, coniofurol A (**5**), and one xanthone, conioxanthone A (**6**), have been isolated from the crude extract of the endolichenic fungus *Coniochaeta* sp. The absolute configurations of C-7 in **1** and the 7,8-diol moiety in **3** were assigned using the modified Mosher's and Snatzke's method, respectively, whereas that of C-8 in **5** was deduced via the circular dichroism data of the $[Rh_2(OCOCF_3)_4]$ complex. Compounds **2**−**4** showed modest cytotoxicity against a small panel of human tumor cell lines.

Although xanthone derivatives have been encountered frequently as the bioactive principles of plants and fungi,[1–9] only a limited number of oxepinochromenones and furochromenones (ring-expanded and ring-contracted xanthones, respectively) have been reported. Examples include fusidienol A, an FTPase inhibitory 6*H*-oxepino[2,3-*b*]chromen-6-one from an unidentified fungus of the genus *Phoma*;[10] the brocaenols, cytotoxic 5*H*-oxepino[4,3-*b*]chromen-11(5a*H*)-ones from the marine-derived fungus *Penicillium brocae*;[11] xanthepinone, an antimicrobial 4*H*-oxepino[2,3-*b*]chromen-6(5*H*)-one from a soil fungus closely related to *Phoma medicaginis*;[12] the microsphaeropsones, 4*H*-oxepino[2,3-*b*]chromen-6(5*H*)-ones from the endophytic fungus *Microsphaeropsis* sp. as antibacterial agents;[13] and the fukanefurochromenones isolated from the roots of *Ferula fukanensis* with in vitro anti-inflammatory effects.[14]

Endolichenic fungi living in the thalli of lichens are analogous to the plant endophytes inhabiting the intercellular spaces of the hosts.[15] However, they are chemically underexplored, with only three species previously investigated.[16–18] During an ongoing search for new cytotoxic natural products from this unique source, the fungus *Coniochaeta* sp. (Coniochaetaceae) was subjected to our chemical study. Fractionation of an organic solvent extract of its solid-substrate fermentation culture afforded six new compounds, including four oxepinochromenones, conioxepinols A−D (**1**−**4**), one furochromenone, coniofurol A (**5**), and one xanthone, conioxanthone A (**6**), together with four known ones, brocaenol A (**7**),[11] microxanthone (**8**),[13] moniliphenone (**9**),[19] and isosulochrin (**10**).[20] Details of the isolation, structure elucidation, and cytotoxicity of these metabolites are reported herein.



Results and Discussion

Conioxepinol A (**1**) gave a pseudomolecular ion $[M + Na]^+$ peak at *m*/*z* 341.0630 (Δ +0.2 mmu) by HRESIMS, consistent with a molecular formula of $C_{16}H_{14}O_7$ (10 degrees of unsaturation). Analysis of its ^{1}H, ^{13}C, and HMQC NMR data (Table 1) revealed two exchangeable protons (δ_H 4.30 and 12.19, respectively), two methyl groups including one *O*-methyl, two methines including one oxymethine, 10 olefinic/aromatic carbons (four of which are protonated), one carboxylic carbon (δ_C 172.5), and one α,β-unsaturated ketone carbon (δ_C 182.3). The ^{1}H−^{1}H COSY NMR data showed the isolated spin-system of C-5−C-8 (including OH-7). The H_3-11 protons were correlated to the C-2, C-3, and C-4 sp^2 carbons in the HMBC spectrum of **1**, suggesting the connection of the C-3 nonprotonated carbon to C-2, C-4, and C-11. HMBC correlations from the phenolic proton at δ_H 12.19 (OH-1) to C-1, C-2, and C-9a indicated that C-1 is attached to C-2 and C-9a, whereas those from H-2 to C-4 and C-9a completed the tetrasubstituted aryl ring. The four-bond *W*-type correlations from H-2 and H-4 to C-9 connected C-9 to C-9a,[21] which was supported by the downfield chemical shift of 12.19 ppm for OH-1 due to formation of an intramolecular hydrogen bond with the C-9 oxygen ketone group. Correlations from H-8 to C-8a, C-9, and C-10a indicated that C-8 and C-9 are attached to C-8a. Those from H-8 and the *O*-methyl protons H_3-13 to C-12 connected C-8 and the *O*-methyl group to C-12. A key correlation from H-5 to C-10a established the dihydrooxepine moiety. Considering the chemical shifts of C-4a (δ_C 153.4) and C-10a (δ_C 163.2) and the unsaturation requirement for **1**, they were attached to the remaining oxygen to establish the 4*H*-oxepino[2,3-*b*]chromen-6(5*H*)-one skeleton. On the basis of these data, the gross structure of **1** was elucidated as shown.

The relative configuration of **1** was determined by analysis of gHSQMBC and NOESY data. The $^3J_{C-H}$ coupling constant of

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Table 1. NMR Data of Compounds **1**–**5**

	1			**2**		**3**		**4**		**5**	
pos.	δ_C[a], mult.	δ_H[b] (*J* in Hz)	HMBC[a]	δ_C[c], mult.	δ_H[d] (*J* in Hz)	δ_C[c], mult.	δ_H[e] (*J* in Hz)	δ_C[a], mult.	δ_H[b] (*J* in Hz)	δ_C[c], mult.	δ_H[d] (*J* in Hz)
1	160.2, qC			161.1, qC		161.0, qC		161.7, qC		161.9, qC	
2	112.8, CH	6.66, s	1, 4, 9, 9a, 11	109.6, CH	6.78, s	113.1, CH	6.63, s	112.9, CH	6.63, s	114.2, CH	6.65, s
3	147.6, qC			153.2, qC		148.8, qC		148.0, qC		147.2, qC	
4	107.0, CH	6.66, s	2, 4a, 9, 9a, 11	104.4, CH	6.94, s	107.5, CH	6.79, s	106.8, CH	6.67, s	108.4, CH	6.84, s
4a	153.4, qC			154.4, qC		153.8, qC		152.8, qC		154.7, qC	
5	135.5, CH	6.29, dd (7.8, 2.4)	6, 7, 10a	136.2, CH	6.47, m	138.6, CH	6.68, dd (6.0, 2.4)	70.6, CH	4.90, m	58.2, CH_2	3.77, m
6	116.0, CH	5.36, dt (7.8, 1.8)	5, 7, 8	116.2, CH	5.32, m	122.3, CH	5.68, dd (6.0, 4.2)	118.1, CH	5.58, dd (6.0, 5.4)	31.7, CH_2	2.16, dt (7.0, 6.0)
7	66.0, CH	4.45, m	6, 12	66.6, CH	4.64, m	71.1, CH	4.82, m	140.2, CH	6.62, dd (6.0, 2.4)	91.6, CH	5.33, t (7.0)
8	42.7, CH	4.97, dd (4.2, 1.8)	6, 7, 8a, 9, 10a, 12	44.8, CH	4.83, m	78.3, qC		159.9, qC		79.4, qC	
8a	98.1, qC			98.6, qC		103.8, qC		102.4, qC		99.4, qC	
9	182.3, qC			183.5, qC		184.6, qC		185.1, qC		179.9, qC	
9a	106.2, qC			107.3, qC		106.7, qC		106.2, qC		107.9, qC	
10a	163.2, qC			164.4, qC		164.1, qC		77.2, qC		171.0, qC	
11	22.4, CH_3	2.40, s	2, 3, 4	63.8, CH_2	4.70, s	22.2, CH_3	2.41, s	22.4, CH_3	2.40, s	21.9, CH_3	2.39, s
12	172.5, qC			171.7, qC		172.7, qC		171.8, qC		172.5, qC	
13	52.8, CH_3	3.71, s	12	52.5, CH_3	3.65, s	53.0, CH_3	3.70, s	53.3, CH_3	3.81, s	53.6, CH_3	3.80, s
OH-1		12.19, s	1, 2, 3, 9a		12.39, s		12.11, s		11.80, s		12.57, s
OH-5											3.98, t (4.5)
OH-7		4.30, d (13)	7, 8		4.77, m		4.88, d (7.8)				
OH-8							4.76, s				5.17, s
OH-11					4.58, s						

[a] Recorded at 100 MHz in $CDCl_3$. [b] Recorded at 500 MHz in $CDCl_3$. [c] Recorded at 100 MHz in acetone-d_6. [d] Recorded at 500 MHz in acetone-d_6. [e] Recorded at 600 MHz in acetone-d_6.

1a R= (*R*)-MPTA ester
1b R= (*S*)-MPTA ester

Figure 1. $\Delta\delta$ values (in ppm) = $\delta_S - \delta_R$ obtained for (*R*)- and (*S*)-MPTA esters **1a** and **1b**.

smaller than 1.0 Hz observed between H-7 and C-12 suggested a *cis* relationship of OH-7 and C-12,[11] which was partially supported by a NOESY correlation of H_3-13 with OH-7. The absolute configuration of **1** was assigned using the modified Mosher method.[22,23] Treatment of **1** with (*S*)- and (*R*)-MTPA-Cl afforded the (*R*)- (**1a**) and (*S*)-MTPA (**1b**) monoesters, respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for **1b** and **1a** was calculated to assign the 7*S* configuration. Therefore, the 7*S* and 8*R* absolute configuration was proposed for **1** on the basis of the $\Delta\delta$ results summarized in Figure 1.

Conioxepinol B (**2**) was isolated as a white, amorphous solid with a molecular formula of $C_{16}H_{14}O_8$ (10 degrees of unsaturation), established by HRESIMS (*m*/*z* 357.0578 $[M + Na]^+$; Δ +0.3 mmu). The ^1H and ^{13}C NMR spectra of **2** showed resonances similar to those of **1**, except that the C-11 methyl group (δ_H/δ_C 2.40/22.4) was replaced by an oxygenated methylene (δ_H/δ_C 4.70/63.8), which was confirmed by HMBC correlations from H_2-11 to C-2, C-3, and C-4 and from the exchangeable proton at δ_H 4.58 (OH-11) to C-3. Therefore, the gross structure of **2** was determined as shown.

The relative and absolute configuration of **2** was deduced by comparison of its NMR and CD data with those of **1**. The CD spectra of **1** and **2** (Figures S13 and S14; Supporting Information) both showed positive Cotton effects at 246 and 281 nm and a negative Cotton effect at 210 nm, suggesting a 7*S* and 8*R* configuration for both compounds.

Conioxepinol C (**3**) was assigned the same molecular formula, $C_{16}H_{14}O_8$ (10 degrees of unsaturation), as **2** by HRESIMS (*m*/*z* 357.0582 $[M + Na]^+$; Δ −0.1 mmu). Analysis of its NMR spectroscopic data revealed structural features similar to those of **1**, except that the C-8 methine (δ_H/δ_C 4.97/42.7) was replaced by an oxygenated sp^3 quaternary carbon (δ_C 78.3). This observation was confirmed by HMBC correlations from the exchangeable proton at δ_H 4.76 (OH-8) to C-8 and C-8a. A NOESY correlation of H-7 with H_3-13 suggested a *cis* relationship for the 7,8-diol moiety, implying that its absolute configuration could be assigned using the in situ dimolybdenum CD method developed by Frelek.[24,25] Upon addition of dimolybdenum tetraacteate $[Mo_2(OAc)_4]$ to a solution of **3** in DMSO, a metal complex was generated as an auxiliary chromophore. Since the contribution from the inherent CD resulting from the C-9 and C-12 carbonyls was subtracted to give the induced CD of the complex, the observed sign of the Cotton effect in the induced spectrum originates solely from the chirality of the *vic*-diol moiety expressed by the sign of the O−C−C−O torsion angle. The negative Cotton effects observed at around 310 and 400 nm, respectively, in the induced CD spectrum (Figure 2) permitted assignment of the 7*S* and 8*R* configuration on the basis of the empirical rule proposed by Snatzke.

Conioxepinol D (**4**) gave a pseudomolecular ion $[M + Na]^+$ peak at *m*/*z* 357.0584 (Δ −0.3 mmu) by HRESIMS, consistent with the molecular formula $C_{16}H_{14}O_8$ (10 degrees of unsaturation). A literature search identified brocaenol A (**7**),[11] which had the same elemental composition as **4**, and was co-isolated from the crude extract. Comparison of the NMR data of **4** and **7** indicated that they differ only in the substituents at C-3 and C-6. Therefore, the absolute configuration of **4** was deduced as shown by analogy with **7**.

Coniofurol A (**5**) was obtained as a pale yellow oil. Its elemental composition was determined to be $C_{16}H_{16}O_8$ (nine degrees of

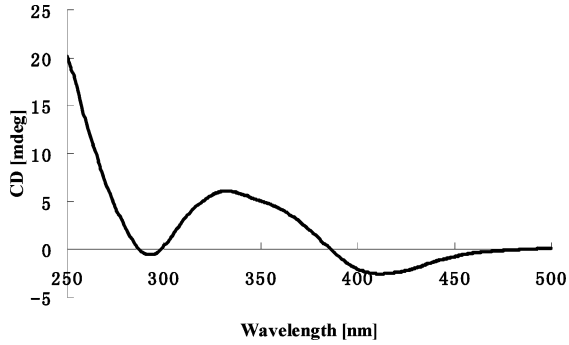


Figure 2. CD spectrum of **3** in DMSO containing $Mo_2(OAc)_4$ with the inherent CD spectrum subtracted.

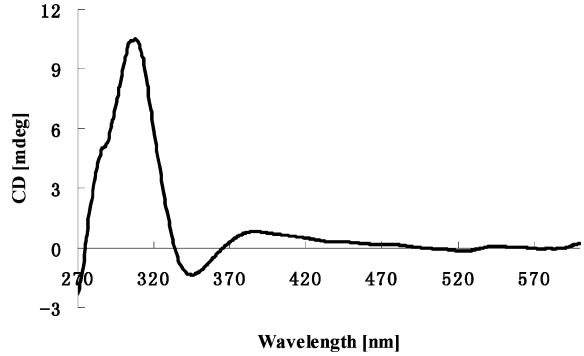


Figure 3. CD spectrum of Rh-complex of **5** with the inherent CD spectrum subtracted.

unsaturation) by HRESIMS (*m/z* 359.0740 $[M + Na]^+$; Δ −0.3 mmu). Analysis of its ^1H and ^{13}C NMR data (Table 1) revealed the same chromenone unit as found in **1**−**3**, but the resonances for the dihydrooxepine unit in **1** were significantly different from those for the remaining portion of **5**. The $^1H-^1H$ COSY NMR data showed the isolated spin-system of the C-5−C-7 (including OH-5) moiety. HMBC correlations from H-7 to C-8 and from OH-8 to C-7, C-8, C-8a, and C-12 indicated that C-7, C-8a, and C-12 are connected to C-8. A correlation from H_3-13 to C-12 attached the C-13 *O*-methyl to C-12. Although no correlation was observed from H-7 to C-10a, the chemical shifts of C-7 (δ_C 91.6) and C-10a (δ_C 171.0) and the molecular formula of **5** required the connection of C-7 and C-10a to the same oxygen to complete the 2*H*-furo[2,3-*b*]chromen-4(3*H*)-one unit.

The relative configuration of **5** was assigned on the basis of NOE data. Upon irradiation of H-7 in the NOE experiment, enhancement was observed for H_3-13, suggesting their *cis* relationship. The absolute configuration of C-8 was assigned via the CD data of the $[Rh_2(OCOCF_3)_4]$ complex,[26] with the inherent contribution subtracted. Upon addition of $[Rh_2(OCOCF_3)_4]$ to a solution of **5** in CH_2Cl_2, a metal complex was generated as an auxiliary chromophore. It has been demonstrated that the sign of the E band (at ca. 350 nm) can be used to correlate the absolute configuration of a tertiary alcohol by applying the bulkiness rule.[26,27] In this experiment, the Rh-complex of **5** displayed a negative E band (Figure 3), correlating with the 8*R* absolute configuration. Therefore, the 7*R* and 8*R* absolute configuration was assigned for **5**.

Conioxanthone A (**6**) was assigned the molecular formula $C_{16}H_{12}O_7$ (11 degrees of unsaturation) by HRESIMS (*m/z* 339.0477 $[M + Na]^+$; Δ −0.2 mmu). The ^1H and ^{13}C NMR spectra of **6** displayed resonances for the same xanthone skeleton as found in **8**,[13] which was co-isolated in the current work. Comparison of the NMR data of **6** and **8** indicated that **6** differs from **8** by having different substituents at C-3, C-5, and C-6, respectively.

Table 2. Cytotoxicity of Compounds **1**−**5**

	IC_{50} (μM)			
compound	HeLa	HepG2	A549	MDA-MB-231
1	103.8 ± 5.19	>120.0	>120.0	>120.0
2	36.2 ± 7.93	>120.0	>120.0	>120.0
3	>120.0	>120.0	83.6 ± 4.10	112.4 ± 5.60
4	>120.0	>120.0	40.9 ± 2.04	41.4 ± 2.07
5	>120.0	>120.0	>120.0	>120.0

The remaining two known compounds **9** and **10** were identified as moniliphenone and isosulochrin, respectively, by comparison of their NMR and MS data with those reported.[19,20]

Compounds **1**−**5** were tested for cytotoxicity against four human tumor cell lines: HeLa (cervical epithelium), HepG2 (human hepatocellular liver carcinoma), A549 (human lung carcinoma), and MDA-MB-231(human breast adenocarcinoma) (Table 2). Compound **2** showed modest cytotoxicity against HeLa cells, with an IC_{50} value of 36.2 μM, with the positive control 5-fluorouracil showing an IC_{50} value of 10.0 μM. Compound **4** showed cytotoxicity against the A549 and MDA-MB-231 cell lines, with IC_{50} values of 40.9 and 41.4 μM, respectively, while the positive control cisplatin showed IC_{50} values of 4.17 and 4.45 μM, respectively. Compounds **1**−**5** were not further evaluated for their antitumor effects due to their modest cytotoxicity.

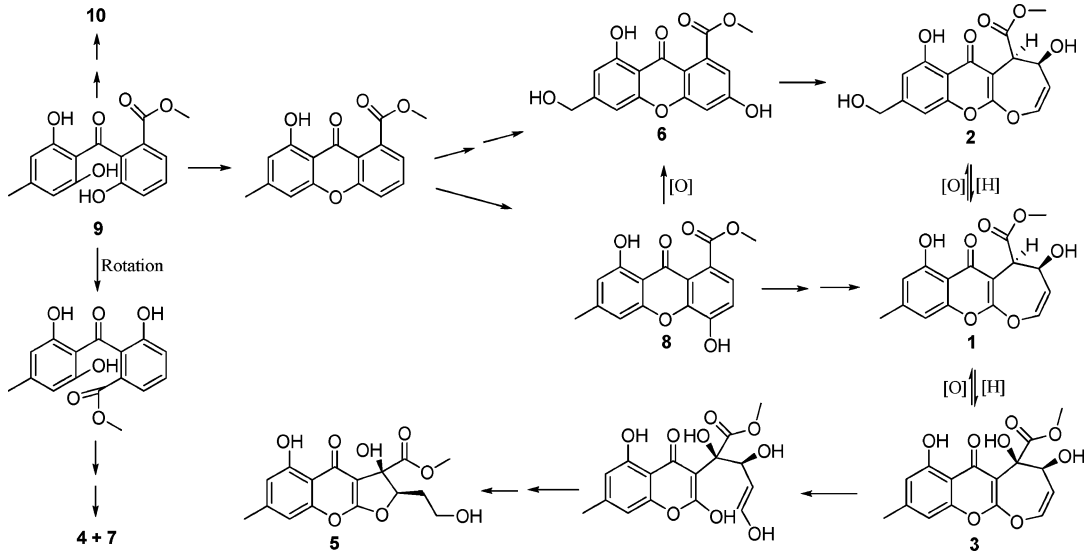
Conioxepinols A−C (**1**−**3**) are closely related to xanthepinone and the microsphaeropsones,[12,13] a relatively rare oxepinochromenone (ring-expanded xanthone) class of fungal metabolites with the 4*H*-oxepino[2,3-*b*]chromen-6(5*H*)-one skeleton, but differ from the known compounds by having different configurations at C-7 and C-8, as well as substitution patterns on the aryl and oxepine moieties. Conioxepinol D (**4**) is closely related to the brocaenols,[11] all possessing the 5*H*-oxepino[4,3-*b*]chromen-11(5a*H*)-one moiety, but differs in having different substituents at C-3 and C-6. Coniofurol A (**5**) is a new member of the furochromenone (ring-contracted xanthone) class of metabolites, possessing the same 2*H*-furo[2,3-*b*]chromen-4(3*H*)-one skeleton as found in the plant metabolites fukanefurochromenones.[14] However, **5** differs from the known analogues by having different substituents on the aryl and furan rings. Biosynthetic studies of some of the xanthones from fungi and lichens have demonstrated that they originated from the cyclization of benzophenones.[28,29] The biosyntheses of **1**−**10** could proceed in a similar manner, but with additional ring-expansion and ring-contraction steps involved (Scheme 1).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were obtained on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Varian Mercury-400, -500, and -600 spectrometers using solvent signals (acetone-d_6: δ_H 2.05/δ_C 29.8, 206.1; $CDCl_3$: δ_H 7.26/δ_C 76.7; DMSO-d_6: δ_H 2.49/δ_C 39.5) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0 T and APEX II FT-ICR spectrometers, respectively.

Fungal Material. The culture of *Coniochaeta* sp. (Coniochaetaceae) was isolated by one of the authors (L.G.) from the lichen *Xanthoria mandschurica* (Zahlbr.) Asahina (Parmeliaceae) collected from Baihua Mountain, Beijing, People's Republic of China, in November 2005. The fungus was identified by L.G. and assigned the accession no. 6.2.2-1-1 in L.G.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. Agar plugs were used to inoculate in 250 mL Erlenmeyer flasks, each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for five days. The fungal strain was cultured on slants of PDA at 25 °C for 10 days.

Scheme 1. Plausible Biosyntheses of **1**−**10**



Fermentation was carried out in 12 Fernbach flasks (500 mL) each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Distilled H_2O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented material was extracted with EtOAc (4 × 1.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (5 0.0 g), which was fractionated by silica gel VLC using petroleum ether−EtOAc gradient elution. The fraction (136 mg) eluted with 20% EtOAc was separated by Sephadex LH-20 CC eluting with 1:1 $CHCl_3$−MeOH. The resulting subfractions were combined and further purified by semipreparative RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 55% MeOH in H_2O for 2 min, followed by 55−85% for 28 min; 2 mL/min) to afford **7** (3.0 mg, t_R 14.3 min), **1** (15.0 mg, t_R 15.8 min), **9** (2.0 mg, t_R 16.2 min), and **10** (2.5 mg, t_R 16.6 min). The fraction (150 mg) eluted with 25% EtOAc was separated by Sephadex LH-20 CC eluting with MeOH, and the resulting subfractions were purified by RP HPLC (55% MeOH in H_2O for 2 min, followed by 55−85% for 30 min; 2 mL/min) to afford **4** (12.5 mg, t_R 15.5 min), **6** (6.5 mg, t_R 16.4 min), and **8** (2.5 mg, t_R 16.9 min). Fractions (105 mg) eluted with 30% and 40% EtOAc were fractionated again by Sephadex LH-20 CC using 1:1 $CHCl_3$−MeOH as eluents. Purification of the resulting subfractions afforded **2** (10.0 mg, t_R 13.1 min; 40% MeOH in H_2O for 2 min, followed by 40−65% for 25 min), **3** (4.0 mg, t_R 15.6 min; 30% MeCN in H_2O for 2 min, followed by 30−50% for 20 min), and **5** (5.0 mg, t_R 13.5 min; the same gradient as in purification of **3**).

Conioxepinol A (1): white powder; $[\alpha]^{25}_D$ +25.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.11), 224 (3.05), 235 (3.06), 353 (2.95) nm; IR (neat) ν_{max} 3416 (br), 2922, 1735, 1659, 1602, 1493, 1266, 1173, 1055 cm^{-1}; ^{1}H, ^{13}C NMR, and HMBC data see Table 1; NOESY correlations ($CDCl_3$, 500 MHz) OH-7 ↔ H_3-13; H_3-13 ↔ OH-7; HRESIMS *m*/*z* 341.0630 (calcd for $C_{16}H_{14}O_7Na$, 341.0632).

Preparation of (*R*)- (1a) and (*S*)-MTPA (1b) Esters. A sample of **1** (1.5 mg, 0.004 mmol) was dissolved in CH_2Cl_2 (3.0 mL) in a 10 mL round-bottomed flask. DMAP (5.0 mg) and (*S*)-MTPA-Cl (10.0 μL, 0.052 mmol) were quickly added, the flask was sealed, and the mixture was stirred at room temperature for 12 h. The mixture was evaporated to dryness and purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 80% MeOH in H_2O for 2 min, followed by 80−100% for 30 min; 2 mL/min) to afford **1a** (0.8 mg): white powder; ^{1}H NMR (acetone-d_6, 600 MHz) δ 12.22 (1H, s, OH-1), 6.82 (1H, s, H-4), 6.67 (1H, s, H-2), 6.66 (1H, dd, J = 7.8, 2.4 Hz, H-5), 5.91 (1H, dt, J = 4.2, 2.4 Hz, H-7), 5.41 (1H, ddd, J = 7.8, 2.4, 1.8 Hz, H-6), 5.08 (1H, dd, J = 4.2, 1.8 Hz, H-8), 3.62 (3H, s, H_3-13), 2.41 (3H, s, H_3-11).

In a similar fashion, a sample of **1** (1.5 mg, 0.004 mmol), CH_2Cl_2 (3.0 mL), DMAP (5.0 mg), and (*R*)-MTPA-Cl (5.0 μL, 0.026 mmol) were allowed to react in a 10 mL round-bottomed flask at room temperature for 12 h, and the reaction mixture was processed as described above for **1a** to afford **1b** (1.2 mg): white powder; ^{1}H NMR (acetone-d_6, 600 MHz) δ 12.22 (1H, s, OH-1), 6.82 (1H, s, H-4), 6.67 (1H, s, H-2), 6.66 (1H, dd, J = 7.8, 2.4 Hz, H-5), 5.94 (1H, dt, J = 4.2, 2.4 Hz, H-7), 5.25 (1H, ddd, J = 7.8, 2.4, 1.8 Hz, H-6), 5.08 (1H, dd, J = 4.2, 1.8 Hz, H-8), 3.63 (3H, s, H_3-13), 2.41 (3H, s, H_3-11).

Conioxepinol B (2): white powder; $[\alpha]^{25}_D$ +21.0 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.17), 220 (3.11), 232 (3.11), 351 (2.98) nm; IR (neat) ν_{max} 3369 (br), 2920, 1728, 1660, 1609, 1500, 1444, 1275, 1172, 1055 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HMBC data (acetone-d_6, 400 MHz) H-2 → C-1, 4, 9, 9a, 11; H-4 → C-1, 2, 3, 4a, 9, 9a, 11; H-5 → C-6, 7, 10a; H-6 → C-5, 7, 8; H-7 → C-6, 12; H-8 → C-6, 7, 8a, 9, 10a, 12; H_2-11 → C-2, 3, 4; H_3-13 → C-12; OH-1 → C-1, 2, 9a; OH-7 → C-6, 7, 8; OH-11 → C-3, 11; HRESIMS *m*/*z* 357.0578 (calcd for $C_{16}H_{14}O_8Na$, 357.0581).

Conioxepinol C (3): white powder; $[\alpha]^{25}_D$ −46.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.31), 220 (3.33), 234 (3.33), 350 (3.12) nm; CD (*c* 7.5 × 10^{-3} M, DMSO) λ_{max} ($\Delta\varepsilon$) 386 (−3.6), 315 (−10.4) nm, 268 (−21.2) nm; IR (neat) ν_{max} 3463 (br), 2955, 1740, 1655, 1600, 1491, 1435, 1292, 1105 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HMBC data (acetone-d_6, 400 MHz) H-2 → C-1, 4, 9, 11; H-4 → C-2, 4a, 9, 9a, 11; H-5 → C-6, 7, 10a; H-6 → C-5, 7, 8; H_3-11 → C-2, 3, 4; H_3-13 → C-12; OH-1 → C-1, 2, 3, 9a; OH-8 → C-8, 8a, 12; NOESY correlations (acetone-d_6, 400 MHz) H-7 ↔ H_3-13; H_3-13 ↔ H-7; HRESIMS *m*/*z* 357.0582 (calcd for $C_{16}H_{14}O_8Na$, 357.0581).

Absolute Configuration of the 7,8-Diol Moiety in 3. HPLC grade DMSO was dried with 4 Å molecular sieves. According to a published procedure,[30] a mixture of 1:1.3 diol−$Mo_2(OAc)_4$ for **3** was subjected to CD measurements at a concentration of 1.0 mg/mL. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed signs of the diagnostic bands at around 310 and 400 nm in the induced CD spectrum were correlated to the absolute configuration of the 7,8-diol moiety.

Conioxepinol D (4): pale yellow, amorphous solid; $[\alpha]^{25}_D$ +40.0 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.23), 219 (3.20), 231 (3.21), 345 (2.96) nm; IR (neat) ν_{max} 3454 (br), 2958, 2922, 1754, 1658, 1603, 1492, 1410, 1287, 1085 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HMBC correlations ($CDCl_3$, 400 MHz) H-2 → C-1, 4, 9a, 11; H-4 → C-2, 4a, 9a, 11; H-5 → C-6, 7, 10a, 12; H-6 → C-7, 10a; H-7 → C-5, 6, 8; H_3-11 → C-2, 3, 4; H_3-13 → C-12; OH-1 → C-1, 2, 3, 9a; HRESIMS *m*/*z* 357.0584 (calcd for $C_{16}H_{14}O_8Na$, 357.0581).

Coniofurol A (5): pale yellow oil; $[\alpha]^{25}_D$ +57.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.19), 220 (3.15), 229 (3.15), 341 (3.00) nm; CD (*c* 7.5 × 10^{-3} M, CH_2Cl_2) λ_{max} ($\Delta\varepsilon$) 380 (−2.2) nm, 288 (−16.3) nm; IR (neat) ν_{max} 3447 (br), 2956, 1741, 1654, 1609, 1475, 1264, 1170, 1040 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HMBC data (acetone-d_6, 400 MHz) H-2 → C-1, 4, 9a, 11; H-4 → C-2, 4a, 9, 9a, 11; H_2-6 → C-5, 7, 8; H-7 → C-5, 6, 8, 12; H_3-11 → C-2, 3, 4; H_3-13 → C-12; OH-1 → C-1, 2, 3, 9a; OH-8 → C-7, 8, 8a, 12; NOED

data (acetone-d_6, 400 MHz) H-7 ↔ H_3-13; HRESIMS *m*/*z* 359.0740 (calcd for $C_{16}H_{16}O_8Na$, 359.0737).

Absolute Configuration of the Tertiary Alcohol Functionality in 5. According to the published procedure,[26,27] a sample of **5** (0.5 mg) was dissolved in a dry solution of the stock [$Rh_2(OCOCF_3)_4$] complex (1.5 mg) in CH_2Cl_2 (200 μL). The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at ca. 50 nm in the induced CD spectrum was correlated to the absolute configuration of the C-8 tertiary alcohol.

Conioxanthone A (6): white powder; UV (MeOH) λ_{max} (log ε) 202 (3.15), 235 (3.21), 308 (3.03), 357 (2.97) nm; IR (neat) ν_{max} 3310, 2953 (br), 1702, 1652, 1614, 1570, 1434, 1267, 1198, 1021 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 12.36 (1H, s, OH-1), 6.95 (1H, s, H-4), 6.90 (1H, s, H-5), 6.81 (1H, s, H-7), 6.73 (1H, s, H-2), 4.57 (2H, s, H_2-11), 3.86 (3H, s, H_3-13); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.1 (C, C-9), 168.4 (C, C-12), 164.8 (C, C-6), 160.6 (C, C-1), 157.8 (C, C-10a), 155.3 (C, C-4a), 153.5 (C, C-3), 135.0 (C, C-8), 113.1 (CH, C-7), 108.8 (C, C-8a), 107.6 (CH, C-2), 106.4 (C, C-9a), 103.9 (CH, C-4), 103.3 (CH, C-5), 62.3 (CH_2, C-11), 52.7 (CH_3, C-13); HMBC data (DMSO-d_6, 400 MHz) H-2 → C-1, 4, 9, 9a, 11; H-4 → C-2, 3, 9, 9a, 11; H-5 → C-6, 7, 8a, 9, 10a; H-7 → C-5, 6, 8a, 12; H_2-11 → C-2, 3, 4; H_3-13 → C-12; OH-1 → C-1, 2, 9a; HRESIMS *m*/*z* 339.0477 (calcd for $C_{16}H_{12}O_7Na$, 339.0475).

Brocaenol A (7): ^1H, ^{13}C NMR and the MS data were consistent with literature values.[11]

Microxanthone (8): ^1H, ^{13}C NMR and the MS data were consistent with literature values.[13]

Moniliphenone (9): ^1H, ^{13}C NMR and the MS data were consistent with literature values.[19]

Isosulochrin (10): ^1H, ^{13}C NMR and the MS data were consistent with literature values.[20]

MTT Assay. [18] The assay was run in triplicate. In a 96-well plate, each well was plated with 10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 μL of medium containing 0.2% DMSO, or appropriate concentrations of the test compounds and the positive controls 5-fluorouracil or cisplatin (10 mg/mL as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). Cells were treated at 37 °C for 4 h in a humidified incubator at 5% CO_2 first and were allowed to grow for another 48 h after the medium was changed to fresh Dulbecco's modified Eagle medium (DMEM). MTT (Sigma) was dissolved in serum-free medium or PBS at 0.5 mg/mL and sonicated briefly. In the dark, 50 μL of MTT/medium was added into each well after the medium was removed from the wells and incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 μL of DMSO was added to each well, and the plate was agitated at 60 rpm for 5 min to dissolve the precipitate. The assay plate was read at 540 nm using a microplate reader.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **1**−**6** and CD spectra of **1**, **2**, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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