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Neonectrolide A, a New Oxaphenalenone Spiroketal from the Fungus *Neonectria* sp.

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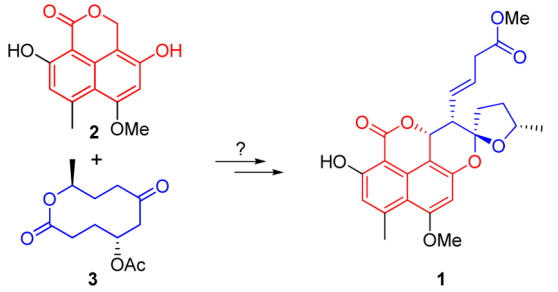
ABSTRACT



Neonectrolide A (1), an oxaphenalenone spiroketal with the previously undescribed (5,8′-dimethyl-5′-oxo-3a′,4,5,5′-tetrahydro-3*H*,3′*H*-spiro[furan-2,2′-isochromeno[3,4,5-*def*]chromene]-3′-yl)but-3-enoic acid skeleton, was isolated from cultures of the fungus *Neonectria* sp. Its absolute configuration was assigned by electronic circular dichroism (ECD) calculations. The skeleton of an oxaphenalenone fused with a 1,6-dioxaspiro[4.5]decane moiety in 1 could be derived from the coisolated putative precursors, corymbiferan lactone E (2) and 3-dehydroxy-4-*O*-acetylcephalosporolide C (3).

Oxaphenalenones have been isolated frequently from fungi and plants.[1−10] The notable structural feature for this class of natural products is the presence of either a benzo[*de*]isochromen-1(3*H*)-one or a benzo[*de*]chromen-2(3*H*)-one skeleton, in which the naphthalene unit fused with the δ-lactone moiety in A or B mode of junction (Figure 1), respectively. Oxaphenalenones are an important class of compounds showing various biological effects. Examples include bacillosporins A−C, the dimeric oxaphenalenones isolated from the fungus *Talaromyces bacillisporus* as mycotoxins and antibacterial agents;[3] coniosclerodorolide, an antibacterial and cytotoxic metabolite from a marine-derived fungus *Coniothyrium cereale*;[2] 2-(4′-hydroxyphenyl)-naphthalic anhydride, a phytoalexin from the unripe green fruit banana *Musa acuminata*;[7] and scleroderolide, a cholesteryl ester transfer protein (CEPT) inhibitor from a *Penicillium* sp. FO-5637.[10]

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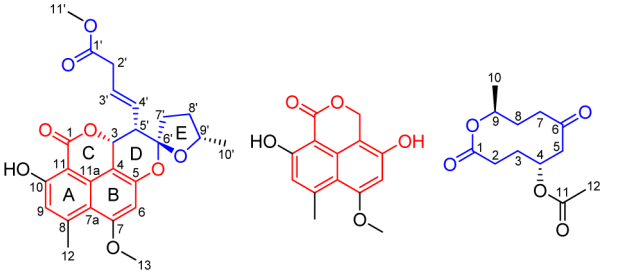
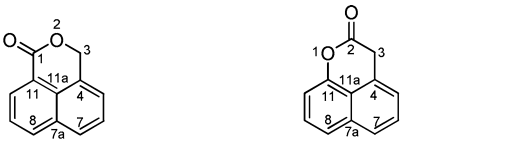
In a search for new cytotoxic metabolites from rarely studied fungi inhabiting unique environments, a strain of *Neonectria* sp. isolated from a soil sample that was collected from the Qinghai-Tibetan plateau (N: 28°27′, E: 97°02′), Chayu, Tibet, People's Republic of China, was chemically investigated. Although the *Neonectria* is a common fungal genus, its chemistry remained largely unexplored.[11] Fractionation of an EtOAc extract prepared from a solid-substrate fermentation culture afforded neonectrolide A (**1**), an oxaphenalenone spiroketal with the new skeleton of 4,5-dihydro-3*H*,3′*H*-spiro[furan-2,2′-isochromeno[3,4,5-*def*]chromen]-5′(3a′*H*)-one. Two new metabolites, corymbiferan lactone E (**2**) and 3-dehydroxy-4-*O*-acetylcephalosporolide C (**3**), were also isolated as the putative biosynthetic precursors of **1**. Details of the structure elucidation and cytotoxicity of **1**−**3**, as well as plausible biogenesis of **1** are reported herein.



1 **2** **3**

Neonectrolide A (**1**) was assigned a molecular formula of $C_{25}H_{26}O_8$ (13 degrees of unsaturation) by HRESIMS (*m*/*z* 477.1525 $[M + Na]^+$; Δ −0.5 mmu). Analysis of its NMR data (Table 1) revealed one exchangeable proton (δ_H 11.64), four methyl groups (two methoxys), three methylenes, three methines (two oxymethines), 12 aromatic/olefinic carbons with four protonated, one doubly oxygenated sp^3 quaternary carbon (δ_C 111.3), and two carboxylic carbons (δ_C 170.9 and 171.9, respectively). The $^1H-^1H$ COSY NMR data of **1** defined the two isolated spin systems of C-7′−C-10′ and C-2′−C-3 (via C-5′), and the latter was attached to the C-1′ methyl formate on the basis of HMBC correlations from H_2-2′ and H_3-11′ to C-1′. HMBC cross peaks from H-9 to C-7a, C-10, C-11, and C-12, H-6 to C-4, C-5, C-7, and C-7a, H_3-12 to C-7a, C-8, and C-9, and from the intramolecularly hydrogen-bonded phenolic proton at 11.64 ppm to C-9, C-10, and C-11, plus chemical shift (δ_C 132.7) consideration of the remaining aromatic carbon (C-11a) in **1**, a naphthalene unit (rings A and B) was established with a methyl and a hydroxy group located at C-8 and C-10, respectively. A weak, but distinct four-bond *W*-type correlation from H-9 to C-1 connected the C-1 carboxylic carbon (δ_C 170.9) to C-11,[12] whereas that of H_3-13 with C-7 indicated that the C-13 methoxy unit is attached to C-7. Further correlations from H-3 to C-1, C-4, C-5, and C-11a enabled the connections of C-3 to C-4, and of C-1 and C-3 to the same oxygen atom, establishing a δ-lactone unit (ring C) fused with the naphthalene at C-11/C-11a/C-4. In turn, HMBC correlations from H-4′, H-5′, H_2-7′, and H_2-8′ to C-6′ located C-6′ between C-5′ and C-7′. Considering the doubly oxygenated nature of C-6′, and the chemical shifts for C-5 (δ_C 150.6) and C-9′ (δ_C 76.9), the two C-6′ bonded oxygen atoms were individually attached to C-5 and C-9′ to form a 1,6-dioxaspiro[4.5]decane moiety to satisfy the unsaturation requirement of **1**, even though no additional evidence for these linkages were provided by the HMBC data. Collectively, these data (Figure 2) permitted assignment of the planar structure of **1**.



A: benzo[*de*]isochromen-1(3*H*)-one B: benzo[*de*]chromen-2(3*H*)-one

Figure 1. Two modes of junction in oxaphenalenones.

Table 1. NMR Spectroscopic Data for **1** in $CDCl_3$

pos.	$\delta_H{}^a$ (*J* in Hz)	$\delta_C{}^b$	HMBC (H → C#)
1		170.9	
3	5.99, d (6.0)	72.9	1, 4, 5, 11a, 4′, 5′
4		98.1	
5		150.6	
6	6.31, s	95.7	3, 4, 5, 7, 7a
7		160.0	
7a		113.6	
8		147.7	
9	6.76, s	118.2	1, 7a, 10, 11, 12
10		163.2	
11		98.2	
11a		132.7	
12	2.80, s	25.4	7a, 8, 9
13	3.94, s	55.5	7
1′		171.9	
2′	3.09, m[c]; 3.01, dd (17.0, 8.0)	37.8	1′, 3′, 4′
3′	5.97, m[c]	128.9	1′, 2′, 5′
4′	5.44, dd (15.5, 10.0)	127.2	3, 2′, 5′, 6′
5′	3.08, dd (10.0, 6.0)	47.5	3, 4, 3′, 6′
6′		111.3	
7′	2.25, m	36.2	5′, 6′, 8′, 9′
8′	2.30, m; 1.62, m	30.9	6′, 7′, 9′, 10′
9′	4.46, m	76.9	
10′	1.25, d (6.0)	21.0	8′, 9′
11′	3.68, s	51.9	1′
OH-10	11.64, s		9, 10, 11

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Mutiplicity due to signal overlapping.

The C-3′/C-4′ olefin was assigned *E*-geometry on the basis of the large (15.5 Hz) coupling constant observed

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between H-3′ and H-4′.[13] NOESY correlations of H_2-7′ with H-3′ and H-4′ were used to place these protons on the same face of ring D, whereas those of H-9′ with H-5′ revealed their spatial proximity (Figure 2). The absolute configuration of **1** was deduced by comparison of the experimental and simulated electronic circular dichroism (ECD) spectra generated by time-dependent density functional theory (TDDFT).[14] Considering the above-mentioned NOESY data, one of the four stereoisomers, (3*S*,5′*R*,6′*R*,9′*R*)-**1**, (3*R*,5′*S*,6′*S*,9′*S*)-**1**, (3*S*,5′*S*,6′*S*,9′*S*)-**1**, and (3*R*,5′*R*,6′*R*,9′*R*)-**1**, should represent the actual configuration of **1**. Since the 6/6/6/5 ring system in **1** was relatively rigid, which would significantly affect the CD property, whereas the conformationally flexible side chain had insignificant effect on the CD spectrum of **1**, a

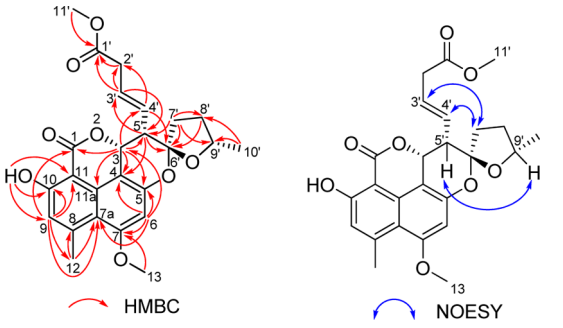

Figure 2. Selected key HMBC and NOESY correlations of **1**.

simplified structure **4** was used for ECD calculations (Figure 3). A systematic conformational analysis was performed for **4a**−**4d** by the Molecular Operating Environment (MOE) software package using the MMFF94 molecular mechanics force field calculation. The MMFF94 conformational search followed by reoptimization using TDDFT at B3LYP/6-31G(d) basis set level afforded three lowest-energy conformers for enantiomers **4a** and **4b** and two for **4c** and **4d**, respectively (Figures S7 and S8, Supporting Information). The overall calculated ECD spectra of **4a**−**4d** were then generated by Boltzmann-weighting of the conformers. The absolute configuration of **1** was extrapolated by comparison of the experimental and calculated ECD spectra of **4a**−**4d** (Figure 3). The experimental CD spectrum of **1** was nearly identical to the calculated ECD spectrum of (3*R*,5′*S*,6′*S*,9′*S*)-**4** (**4b**), both showing positive Cotton effects (CEs) in 230−265 nm, and negative CEs in the regions of 270−295 and 295−400 nm (Figure 3). The energy-minimized conformer of **4b** showed a dihedral angle of 47.0° between H-3 and H-5′ (Figure S21, Supporting Information), corresponding to a $^3J_{HH}$ value of 6.0 Hz from the Karplus equation ($^3J_{HH} = A + B\cos\Phi + C\cos2\Phi$; $A = 7$, $B = -1$, $C = 5$, Φ = dihedral angle).[15,16] Whereas the dihedral angle between H-3 and H-5′ was calculated as 174.4° in conformer **4d** (Figure S22, Supporting Information), with a theoretical $^3J_{HH}$ value of 13 Hz, the actual $^3J_{HH}$ value observed between H-3 and H-5′ in **1** was 6.0 Hz, matching that calculated in **4b**, supporting the absolute configuration deduced from the ECD spectra. Therefore, **1** was deduced to have the 3*R*, 5′*S*, 6′*S*, and 9′*S* absolute configuration.

Compound **2** gave a pseudomolecular ion $[M + H]^+$ peak at *m*/*z* 261.0755 by HRESIMS, consistent with the molecular formula $C_{14}H_{12}O_5$. Its ^{1}H and ^{13}C NMR spectra showed resonances for two exchangeable protons (δ_H 10.12 and 11.99, respectively), two methyl groups (one methoxy), one oxymethylene, 10 sp^2 carbons with two protoned, and one carboxylic carbon (δ_C 170.3). Analysis of its NMR data revealed structural similarity to corymbiferan lactone A,[1] except that the C-7 methoxy, C-5 hydroxy, and C-4 hydroxymethyl group in corymbiferan lactone A were replaced by a hydroxy, a methoxy, and a methyl group in **2**, respectively, which were supported by relavant HMBC data, completing the planar structure of **2** as shown. (Note: A different numbering system was used for **2**, in which C-7, C-5, and C-4 in corymbiferan lactone A corresponded to C-5, C-7, and C-8 in **2**, respectively.)

Compound **3** was assigned the molecular formula $C_{12}H_{18}O_5$ by HRESIMS (*m*/*z* 265.1056 $[M + Na]^+$; Δ −0.4 mmu). Its ^{1}H and ^{13}C NMR data were consistent with those for two methyls, five methylenes, two oxymethines, two carboxylic carbons (δ_C 172.1 and 170.0), and a ketone carbon (δ_C 208.4). The NMR data of **3** were nearly identical to those of cephalosporolide C,[17] both having the 10-methyloxecane-2,7-dione moiety. Interpretation of the 2D NMR data of **3** established its structure as 3-dehydroxy-4-*O*-acetylcephalosporolide C. The relative configuration of **3** was deduced by NOED data. Upon irradiation of H-4, enhancement was observed for H-7b in the NOE difference spectrum of **3**, whereas enhancement was observed for H-7a upon irradiation of H-9, suggesting a *trans* relationship between H-4 and H-9.

The absolute configuration of **3** was assigned using the modified Mosher's method on the semisynthetic product **5** (Figure S25, Supporting Information).[18] Specifically, treatment of **3** with NaOH−MeOH afforded **5**, and subsequent treatment of **5** with (*S*)- and (*R*)-MTPA Cl afforded the *R*- (**5a**) and *S*-MTPA (**5b**) esters, respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **5b** and **5a** was calculated to assign the 4*R* absolute configuration (Figure S25, Supporting

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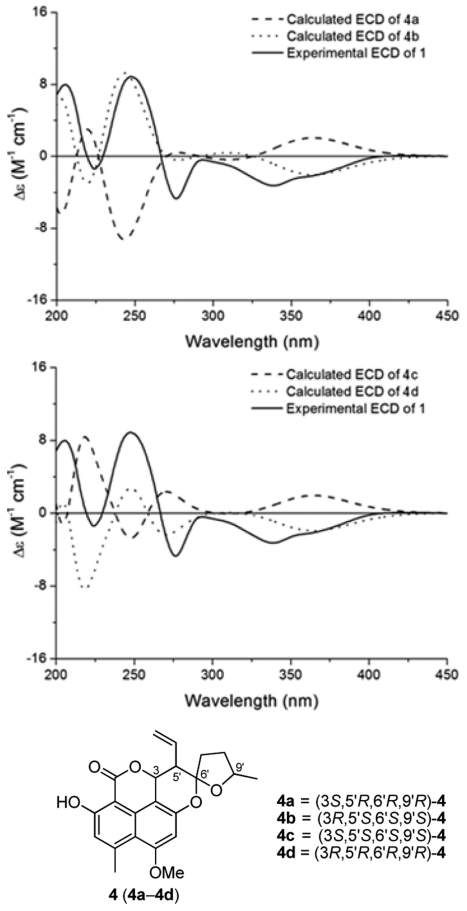


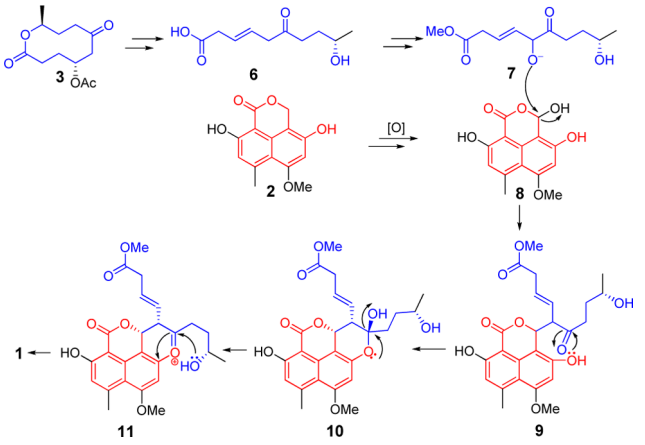
Figure 3. The experimental CD spectrum of **1** in MeOH and the calculated ECD spectra of **4a**–**4d**. Structures **4a**–**4d** represent four possible stereoisomers of **4**.

Information). Therefore, the 4*R* and 9*S* absolute configuration was proposed for **3**.

Compounds **1**–**3** were tested for cytotoxicity against human tumor cell lines, HeLa, A549, HCT116, and T24. Compounds **1** and **3** were cytotoxic to T24 cells, showing IC_{50} values of 47.1 and 19.0 μM, respectively (the positive control cisplatin showed an IC_{50} value of 22.1 μM), whereas **2** did not show detectable activity at 50 μg/mL.

Compound **1** is a new member of the oxaphenalenone-derived natural products. Although several synthetic compounds with partial structural similarity to **1** have been previously reported, which incorporated either a 3′,3a′,4,5,5′,6′,6a′,7′-octahydro-3*H*-spiro[furan-2,2′-pyrano [2,3,4-*de*]chromene][19–21] or a 1,2,4′,5′-tetrahydro-3′*H*-spiro[benzo[*f*]chromene-3,2′-furan] core,[22] compound **1** possesses the previously undescribed (5,8′-dimethyl-5′-oxo-3a′,4,5,5′-tetrahydro-3*H*,3′*H*-spiro[furan-2,2′-isochromeno[3,4,5-*def*]chromene]-3′-yl)but-3-enoic acid skeleton originated from fusion of an oxaphenalenone and an 1,6-dioxaspiro[4.5]decane unit. Biosythetically, compound **3**, the 10-membered lactone with the C-9 methyl group, could be derived from a 10-carbon phenol via a serious of oxadative reactions.[23] In addition, compound **1** could be generated from the coisolated **2** and **3** via the reaction cascades as illustrated in the hypothetical biosynthetic pathways (Scheme 1).

Scheme 1. Hypothetical Biosynthetic Pathways for **1**



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Supporting Information Available. Experimental procedures, characterization data, ^{1}H and ^{13}C APT NMR spectra of **1**–**3**, and UV and CD calculations for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.