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Structural Elucidation and Biomimetic Synthesis of (±)-Cochlactone A with Anti-Inflammatory Activity

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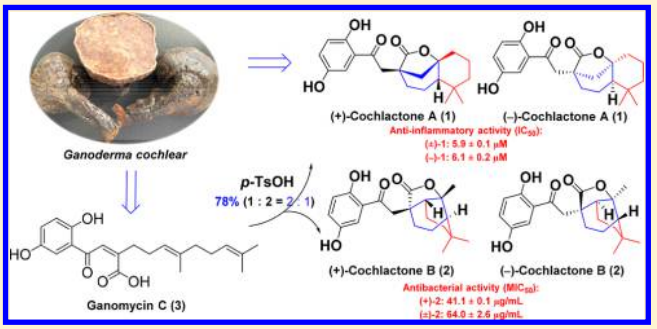
Supporting Information

ABSTRACT: A pair of new natural meroterpenoids, (±)-cochlactone A (**1**) possessing a bicyclo[4.4.0]decane ring system with a γ-lactone fragment, was isolated from *Ganoderma cochlear*. To further confirm their absolute configurations, a high-yielding, one-step biomimetic synthesis of (±)-cochlactone A (**1**) from ganomycin C (**3**) was conducted. In addition, a new compound, (±)-cochlactone B (**2**), featuring a bicyclo[3.3.1]decane fragment fused to a γ-lactone moiety was synthesized. Their structures were determined using spectroscopic data, X-ray diffraction crystallography, and electronic circular dichroism (ECD) analyses. Furthermore, a plausible reaction mechanism for the formation of **1** and **2** was proposed. Compounds (+)-**2** and (±)-**2** showed inhibitory effects against *Staphylococcus aureus* with MIC_{50} values of 41.1 ± 0.1 and 64.0 ± 2.6 μg/mL, respectively. Meanwhile, (±)-**1**, (−)-**1**, (+)-**2**, and (±)-**2** displayed significant anti-inflammatory activities (IC_{50}: 5.9 ± 0.1, 6.1 ± 0.2, 12.1 ± 0.4, and 18.7 ± 1.9 μM, respectively).



INTRODUCTION

Since the discovery of ganomycins A and B,[1] more than 80 aromatic meroterpenoids, derived from a hybrid of the shikimic acid and mevalonic acid biogenetic pathways, have been isolated from the genus *Ganoderma* (Ganodermataceae).[2] *Ganoderma* meroterpenoids (GMs) are attracting increasing attention because they have diverse structural skeletons and show a variety of bioactivities, such as NO inhibitory,[3] antioxidant,[4] antiallergic,[5] antifibrotic,[6] anti-AChE,[7] cytotoxic,[8] antimicrobial,[1] and aldose reductase inhibitory activities.[9] As a result, chemists have synthesized polycyclic meroterpenoids by pathways with many steps.[10−13]

We have previously reported the isolation of four pairs of polycyclic-meroterpenoid enantiomers, ganocins A−C, each possessing a spiro[4,5]decane ring system, and ganocun D, possessing an eight-membered ring from *Ganoderma cochlear*.[7] In our ongoing search for structurally unique and biologically important GMs, an in-depth investigation of the fruiting bodies of *G. cochlear* led to the isolation of a pair of new meroterpenoids, (±)-cochlactone A (**1**) (Figure 1). Due to the limited quantity of **1**, its absolute configuration could not be determined; therefore, we decided to synthesize this compound. Detailed analyses of the biosynthesis of **1** showed that it was derived from ganomycin C (**3**)[4a] (Figure 1) isolated from *G. cochlear* via a double-bond migration and intermolecular cyclization under acidic conditions. Finally, we obtained **1** (30 mg) from ganomycin C (**3**, 70 mg) via a single-step synthesis and successfully obtained a crystal of (+)-**1**. Interestingly,

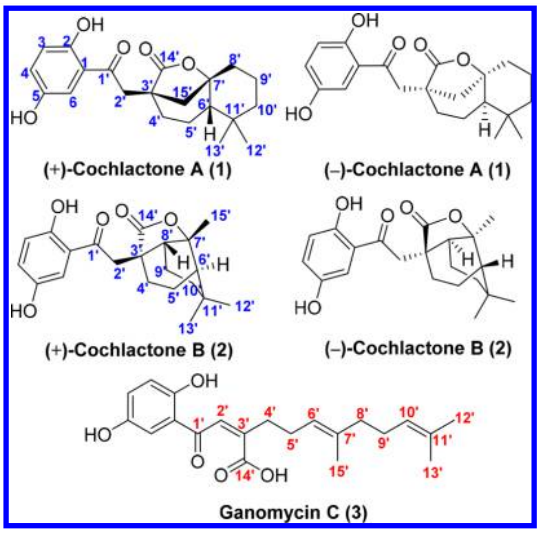


Figure 1. Structures of compounds **1**−**3**.

(±)-cochlactone B (**2**) (Figure 1) with a bicyclo[3.3.1]decane system fused to a γ-lactone moiety was also observed. Subsequently, a plausible reaction mechanism for (±)-**1** and (±)-**2** was proposed. In addition, the antibacterial and anti-

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Table 1. 1D NMR (600/150 MHz) Spectroscopic Data of Compounds 1 and 2

	1[a]		2[b]	
position	δ_H (J in Hz)	δ_C (type)	δ_H (J in Hz)	δ_C (type)
1		120.7 (C)		120.8 (C)
2		156.6 (C)		155.5 (C)
3	6.78 d (8.9)	119.7 (CH)	7.07 d (8.9)	119.2 (CH)
4	7.00 dd (8.9, 2.9)	126.0 (CH)	7.36 dd (8.9, 2.9)	125.7 (CH)
5		150.6 (C)		150.8 (C)
6	7.22 d (2.9)	115.4 (CH)	7.77 d (2.9)	115.8 (CH)
1′		204.2 (C)		203.0 (C)
2′	3.40 d (18.3); 3.25 d (18.3)	42.7 (CH_2)	3.52 s	40.6 (CH_2)
3′		46.9 (C)		50.4 (C)
4′	1.69 m; 1.79 m	33.9 (CH_2)	1.35 m; 1.95 m	28.3 (CH_2)
5′	1.45 m; 1.94 m	21.5 (CH_2)	1.80 m; 1.96 m	22.4 (CH_2)
6′	1.44 m	49.5 (CH)	1.44 m	46.5 (CH_2)
7′		86.3 (C)		86.4 (C)
8′	1.61 m; 1.92 m	37.2 (CH_2)	2.74 m	43.1 (CH)
9′	1.50 m; 1.73 m	19.6 (CH_2)	1.70 m	19.1 (CH_2)
10′	1.28 m; 1.47 m	42.6 (CH_2)	1.05 m; 1.47 m	33.7 (CH_2)
11′		33.6 (C)		34.6 (C)
12′	0.95 s	22.0 (CH_3)	0.90 s	30.1 (CH_3)
13′	0.89 s	31.4 (CH_3)	0.90 s	32.4 (CH_3)
14′		181.9 (C)		178.4 (C)
15′	1.76 m; 2.38 m	48.7 (CH_2)	1.58 (s)	27.2 (CH_3)

[a]Measured in CD_3OD. [b]Measured in C_5D_5N. The assignments were based on $^1H-^1H$ COSY, ROESY, HSQC, and HMBC experiments.

inflammatory effects of (±)-**1**, (±)-**2** and their pure enantiomers were evaluated.

RESULTS AND DISCUSSION

The dried fruiting bodies of *Ganoderma cochlear* were extracted with 95% EtOH under reflux at 80 °C three times. The combined ethanolic extracts were concentrated under reduced pressure. The residue was suspended in H_2O and extracted with EtOAc. Several chromatographic purifications of the EtOAc extract led to the isolation of compound **1**.

The molecular formula of (±)-cochlactone A (**1**) was determined to be $C_{21}H_{26}O_5$ from its HRESIMS data ([M + Na]$^+$, m/z 381.1681, calcd 381.1678), and this formula includes nine degrees of unsaturation. The IR spectrum of **1** showed the presence of hydroxy, unsaturated ketone carbonyl, and ester carbonyl groups (3424, 1750, and 1644 cm^{-1}). The 1D NMR spectroscopic data (Table 1) of **1** showed the characteristic signals at δ_H 7.22, d, J = 2.9 Hz, δ_C 115.4; δ_H 7.00, dd, J = 8.9 and 2.9 Hz, δ_C 126.0; and δ_H 6.78, d, J = 8.9 Hz, δ_C 119.7 for a 1,2,4-trisubstituted phenyl moiety. Additionally, two methyl groups, seven methylenes, one methine, and five quaternary carbons were assigned on the basis of the ^{13}C-DEPT NMR spectra and HSQC correlations. Considering the phenyl, ketone, and ester carbonyl groups, the remaining three degrees of unsaturation indicated the presence of three rings in **1**, suggesting that **1** is a polycyclic aromatic meroterpenoid similar to ganoresinain D.[2] However, a detailed comparison of the 1D NMR spectra of **1** and ganoresinain D showed the absence of an sp^2 quaternary carbon, an sp^2 methine, and an oxymethine but did show the presence of an oxy-quaternary carbon and two sp^3 methylenes in **1**. The aforementioned information hinted that C-7′ was an oxygenated quaternary carbon, while C-8′ and C-10′ were two sp^3 methylenes. Further evidence was established from the HMBC correlations (Figure 2) of H_3-12′ with C-12′, C-10′, and C-6′; and of H_3-13′ with C-12′, C-10′, and C-6′; of H_2-10′ with C-8′; and of H_2-8′ with C-10′, C-7′, and C-6′, together with the $^1H-^1H$ COSY correlations (Figure 2) of H-8′/H-9′/H-10′. Moreover, considering the molecular weight of **1**, an additional 7′ → 14′ γ-lactone ring was determined as shown in the planar structure of **1** in Figure 2.

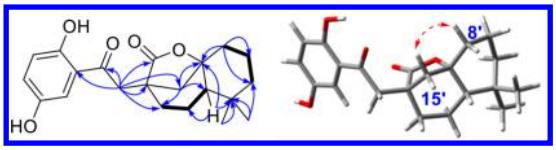

Figure 2. Key HMBC, $^1H-^1H$ COSY, and ROESY correlations of (±)-**1**.

Although the ROESY correlation of H_2-15′ with H_2-8′ was observed, it was not useful for assigning the configurations of C-3′, C-6′, and C-7′. Surprisingly, its low optical rotation value ($[\alpha]^{18}_D$ = −4.3) was indicative of a racemic mixture, which was subsequently separeted by chiral HPLC column to afford two enantiomers, (+)-**1** and (−)-**1** (7:5), and these compounds showed opposite Cotton effects in their CD spectra (Figures S14 and S15). After many attempts, we failed to obtain a crystal for X-ray diffraction analysis due to the limited quantity of **1** (1.5 mg). This further informed our decision to synthesize this compound.

We analyzed the possible biogenetic pathway of **1** (Scheme 1) and deduced that cochlatone A could be derived from precursor ganomycin C (**3**) under acidic conditions. Moreover, the cyclization of the polyene fractions can be realized in the presence of *p*-toluenesulfonic acid (*p*-TSA).[14,15] On the basis of these observations, we could mimic this transformation, and we optimized the reaction conditions by changing the solvents, reaction temperature, and acids (Table 2). Notably, when toluene and trifluoroacetic acid (TFA) were used as the solvent and acid catalyst, respectively, compounds **1** and **2** were obtained in <10% yield under the refluxing conditions (entries 1−5). To improve the yield, other acids were screened. When

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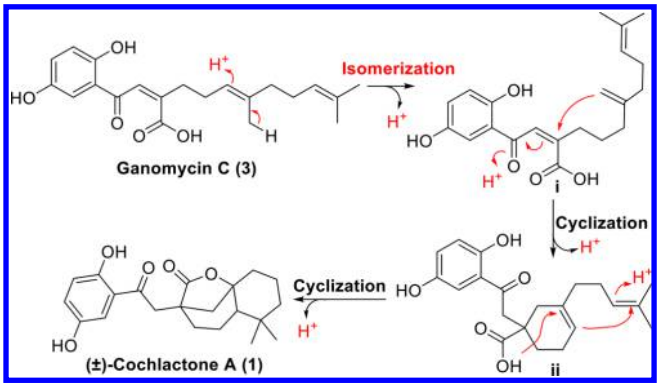
Scheme 1. Plausible Biosynthetic Pathway for 1



p-TSA was used as the catalyst, the yield increased to 50% (entry 6). However, the poor solubility of **4** in toluene resulted in an incomplete reaction. Satisfyingly, when acetonitrile (MeCN) was used as cosolvent (50% in toluene), the yield increased to more than 78% (entry 7). Notably, the higher or lower ratio of MeCN to toluene consistently led to lower yields (entries 8 and 9). It is interesting that the ratio of compounds **1** and **2** was 2:1 in all of the entries.

Finally, using the optimized conditions, a sufficient quantity of **1** was obtained, and X-ray diffraction analysis (Figure 3) of (+)-**1** confirmed that the absolute configuration of the compounds was 3′*S*,6′*S*,7′*S*.

In this reaction, another meaningful molecule, (±)-cochlactone B (**2**), possessing a bridged ring structure, was also obtained. (±)-Cochlactone B (**2**) was isolated as a yellow powder, and its molecular formula, $C_{21}H_{26}O_5$, was determined from its HRESIMS and ^{13}C-DEPT NMR spectra of **2**, indicating nine degrees of unsaturation. The IR, UV, and 1D NMR spectra of **2** showed the presence of 1,2,4-trisubstituted phenyl, a ketone carbonyl, and an ester carbonyl moiety. Additionally, the 1D NMR data (Table 1) of **2** also exhibited three singlet methyl groups, five methylenes, two methines, and two sp^3 quaternary carbons (one oxygenated). The above information indicated that **2** has the same structure as **1**, except for the presence of a methyl group (δ_H 1.58, s, H-15′; δ_C 27.2, C-15′) and a methine (δ_H 2,74, m, H-8′; δ_C 43.1, C-8′), as well

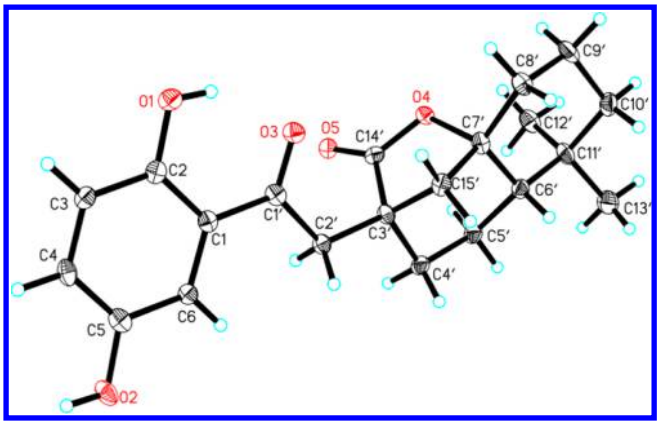


Figure 3. X-ray crystallographic structure of (+)-**1**.

as the absence of two methylenes in **2**. The HMBC spectrum of **2** displayed correlations (Figure 4) of the methyl protons (δ_H

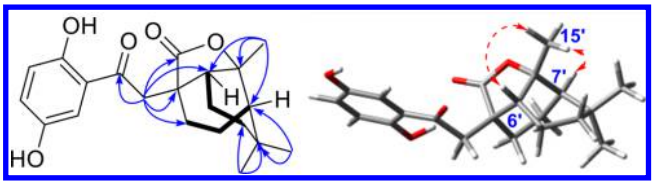
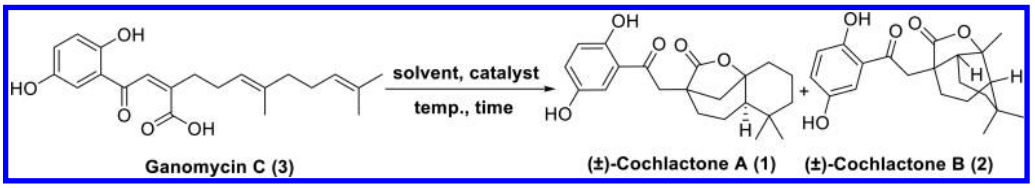


Figure 4. Key HMBC, ^{1}H−^{1}H COSY, and ROESY correlations of (±)-**2**.

1.58, s, H-15′) with C-6′, C-7′ (δ_C 86.4), and C-8′; of H-6′ with C-4′, C-5′, C-7′, C-8′, C-11′, C-10′, C-12′, and C-13′; and of the methine protons (δ_H 2.74, m) with C-2′, C-3′, C-7′, C-8′, C-9′, and C-10′. In addition, the correlations (Figure 4) of H-6′/H-5′/H-4′ and of H-8′/H-9′/H-10′ were observed in the ^{1}H−^{1}H COSY spectrum. Taken together, these correlations suggest that compound **2** is a 3′,15′-*seco*-3′,8′-cyclic rearranged derivative of **1**.

The ROESY spectrum showed the correlations (Figure 4) of H-15′ with H-6′ and H-8′, suggesting that H-15′, H-6′, and H-8′ were on the same side. The optical rotation value and chiral analysis hinted that this was a racemic mixture. Furthermore,

Table 2. Optimization of the Reaction Conditions[a]



entry	solvent	acids	T (°C)	time (h)	yield[b] (%)	ratio[c] (1/2)
1	DCM	TFA (0.1 equiv)	rt	6		
2	THF	TFA (0.1 equiv)	rt	6		
3	MeCN	TFA (0.1 equiv)	rt	12		
4	toluene	TFA (0.1 equiv)	rt	12		
5	toluene	TFA (0.1 equiv)	reflux	12	<10	2:1
6	toluene	*p*-TSA (1 equiv)	reflux	12	50	2:1
7	toluene/MeCN (1:1)	*p*-TSA (1 equiv)	reflux	5	78	2:1
8	toluene/MeCN (1:3)	*p*-TSA (1 equiv)	reflux	5	60	2:1
9	toluene/MeCN (3:1)	*p*-TSA (1 equiv)	reflux	5	42	2:1

[a]Reaction conditions: ganomycin C (**3**, 0.2 mmol), solvent (2 mL), reflux, 5−12 h. [b]Combined yield of isolated products. [c]Ratio was determined from the HPLC charomatogram of the crude mixture.

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chiral HPLC separation led to the isolation of (+)-**2** and (−)-**2** (3:2); possessing opposite optical rotations and CD curves (see the Supporting Information). To confirm the absolute configuration of **2**, its ECD spectrum was calculated. As shown in Figure 5, the calculated ECD curve of the 3′*R*,6′*R*,7′*R*,8′*R* isomer corresponded well with the experimental curve of (+)-**2**. Thus, the structure of **2** was finally established.

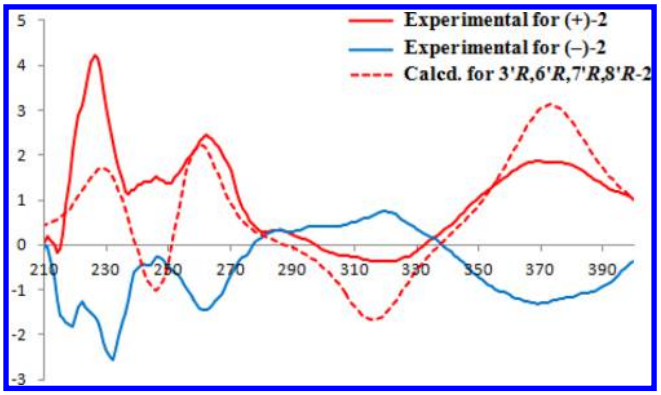


Figure 5. Experimental and calculated ECD spectra of (±)-**2**.

In addition, the concomitant production of compound **2** hinted that the mechanism of this reaction involved three key steps as depicted in Scheme 2. First, the double bond between C-6′ and C-7′ migrated to $\Delta^{7',15'}$ or $\Delta^{7',8'}$, which was confirmed by the isolation of ganoresinain D.[2] Then, a six-membered ring was formed via cyclization. The ketone carbonyl at C-1′ played an important role in this step, because when ganomycin F[4], which lacks the ketone carbonyl at C-1′, was used as the substrate, and no new products were formed (see the Supporting Information). The last step involved activation by the C-14′ carboxyl and led to the construction of the *δ*-lactone and another six-membered ring. To confirm this deduction, we tested the methyl ester derivative of ganomycin C, and this compound was not reactive under these conditions (see the Supporting Information). Moreover, we did not detect the formation of any intermediates during this process, suggesting that above three key steps were sequential reactions.

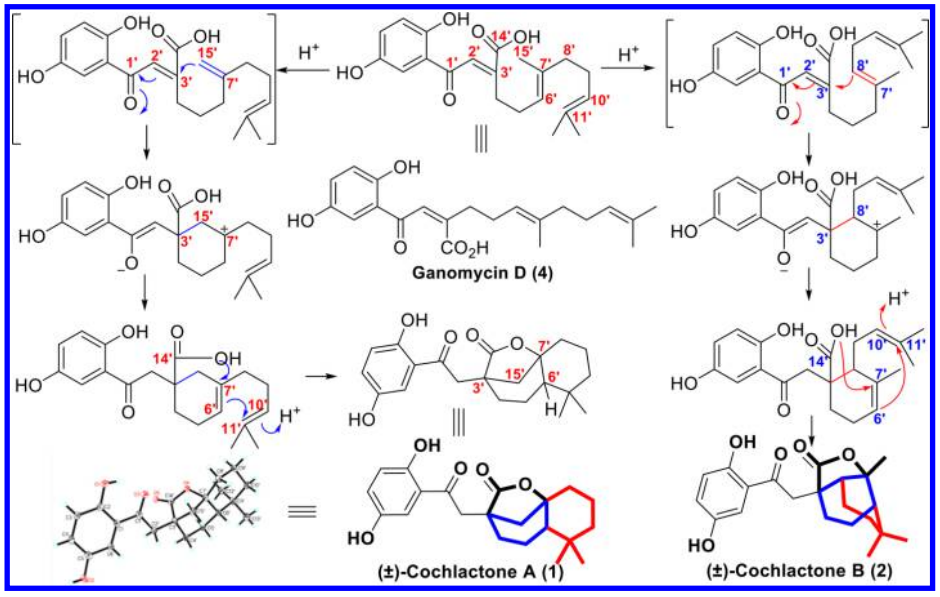
Although many research endeavors have focused on the cytotoxic, antioxidant, anti-AChE, and antifibrosis activities of farnesyl hydroquinones (aromatic meroterpenoids),[4,7,8] some previous reports have noted the antimicrobial and anti-inflammatory activities of aromatic meroterpenoids isolated from brown algae, sponges and *Ganoderma*.[1,3,16,17] Thus, in the current study, the antibacterial activities of compounds (±)-**1** and (±)-**2** and their pure enantiomers were evaluated on *Escherichia coli*, *Staphylococcus aureus* subsp. *eureus*, *Salmonella enteric* subsp. *enterica*, and *Pseudomonas aeruginosa* at a concentration of 128 *μ*g/mL. The inhibition rates of (±)-**2** and (+)-**2** reached 96.5 ± 0.2%, and 3.2 ± ± 1.6%, respectively. Furthermore, (±)-**2** and (+)-**2** showed inhibitory effects against *S. aureus* with MIC_{50} values of 64.0 ± 2.6 and 41.1 ± 0.1 *μ*g/mL, respectively (Table 3). Meanwhile, we tested their anti-inflammatory activities, and the result showed that (±)-**1**, (−)-**1**, (±)-**2**, and (+)-**2** displayed stronger NO production inhibitory activities (IC_{50}: 5.9 ± ± 0.1, 6.1 ± 0.2, 18.7 ± 1.9, and 12.1 ± 0.4 *μ*M, respectively) than that of the positive control (L-NMMA), which showed an IC_{50} value of 45.3 ± 1.8

Table 3. Anti-Bacterial and Anti-Inflammatory Activities of (±)-1, (±)-2, and Their Pure Enantiomers

compd	antibacterial activity (*Staphylococcus aureus* MIC_{50}: *μ*g/mL)	NO production inhibitory activity (IC_{50}: *μ*M)
penicillin G sodium[a]	0.8 ± 0.01	
L-NMMA[b]		45.3 ± 1.8
(±)-**1**	NA[c]	5.9 ± 0.1
(+)-**1**	NA[c]	NA[c]
(−)-**1**	NA[c]	6.1 ± 0.2
(±)-**2**	64.0 ± 2.6	18.7 ± 2.0
(+)-**2**	41.2 ± 0.13	12.1 ± 0.4
(−)-**2**	NA[c]	NA[c]

[a]Positive control for antibacterial activity. [b]Positive control for anti-inflammatory activity. [c]NA: no activity.

Scheme 2. Plausible Reaction Mechanism of the Formation of (±)-1 and (±)-2



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μM (Table 3). As mentioned previously, the chirality of these compounds can affect their bioactivities. For example, after chiral resolution of (±)-**2**, (−)-**2** did not show antibacterial and anti-inflammatory activities, indicating that the stereochemistry plays an important role in bioactivity. Moreover, compounds **1** and **2** possess an unprecedented skeleton, which can provide a new structural model for the design of antibacterial and anti-inflammatory agents.

CONCLUSION

Previous research showed that natural products can serve as lead compounds in the drug discovery process due to their multiple chiral centers, fused polycyclic systems, multiple degrees of unsaturation, and inclusion of heteroatoms.[18] The diversity-oriented chemical modification of compounds with a high content has been used to obtain privileged structures.[19] The present study described a one-step synthetic method to efficiently synthesize novel aromatic meroterpenoids, which will lay the foundation for the structural modification of compounds with a high content from *Ganoderma* to access more structurally diverse meroterpenoids in the next step. In addition, the one-step biomimetic synthesis of (±)-cochlactone A (**1**) also elucidated the formation of complex meroterpenoids from *Ganoderma*.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained with a JASCO P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were measured on Bruker AV-400 and DRX-500 instruments (Bruker, Zurich, Switzerland) using TMS as an internal standard for chemical shifts. Chemical shifts (δ) are expressed in ppm and referenced to the TMS resonance. ESIMS and HRTOF-ESIMS data were recorded on an API QSTAR Pulsar spectrometer. Infrared spectra were recorded on a Bruker Tensor-27 instrument by using KBr pellets. An Agilent 1100 series instrument equipped with an Agilent ZORBAX SB-C18 column (5 μm, 9.4 mm × 250 mm) was used for high-performance liquid chromatography (HPLC) analysis. Chiral chromatography using a CHIRALCEL OD-H column (5 μm, 4.6 mm × 150 mm) was used to resolve enantiomers.

TLC was performed on precoated TLC plates (200−250 μm thickness, F254 Si gel 60, Qingdao Marine Chemical, Inc.), and compounds were visualized by spraying the dried plates with 10% aqueous H_2SO_4 followed by heating until dryness. Silica gel (200−300 mesh, Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40−63 μm, Merck) and Sephadex LH-20 (20−150 μm, Pharmacia) were used for column chromatography. Methanol, dichloromethane, ethyl acetate, acetone, and *n*-butanol were purchased from Tianjin Chemical Reagent Co. (Tianjing, China). All other materials were of the highest grade available.

Fungal Material. The fruiting bodies of *G. cochlear* were purchased in July 2013 from Juhuacun Traditional Chinese Medicine Market in Kunming. The mushroom was identified by Prof. Liu Peigui, a fungal taxonomist at the Kunming Institute of Botany, Chinese Academy of Science.

Extraction and Isolation. *G. cochlear* (68 kg) mushrooms were chopped and extracted three times with 95% EtOH under reflux at 80 °C. The combined ethanolic extracts were concentrated under reduced pressure. The residue was suspended in H_2O and extracted with EtOAc. The volume of the combined EtOAc extracts was reduced to one-third of its initial volume under vacuum. The residue was fractionated by column chromatography (CC, macro resin D-101; MeOH−H_2O; fractions I−IV 50:50, 70:30, 90:1 and 100:1). Fraction II (480 g) was subjected to CC (silica gel; $CHCl_3$−MeOH step gradients) to obtain three subfractions (Fr. II-1−Fr. II-4; 100:0, 80:1, 50:1, 20:1, 5:1). Fr. II-3 (50:1, 50 g) was purified by CC (RP-18, MeOH-H_2O, 40:60 → 70:30 step gradients) to obtain six fractions (Fr. II-3−1 → Fr. II-3−6). Fr. II-3−5 was further separated by Sephadex LH-20 (MeOH) to give three subfractions (a, b, and c). Subsequently, the above three subfractions were analyzed by HPLC. By analyzing the absorption peaks in the three HPLC chromatograms, the negative peak of λ = 285 nm was observed in the HPLC chromatogram of Fr. II-3−5c. Thus, Fr. II-3−5c was further purified by P-TLC ($CHCl_3$−MeOH, 50:1) to afford compound **1** (1.5 mg).

Synthesis of Compounds 1 and 2. Ganomycin C (**3**, 70 mg, 0.2 mmol) was dissolved in a mixture of toluene and MeCN (2 mL, 1:1, v/v), and 1 equiv of *p*-TSA was added to the above mixture. The reaction was stirred at reflux for 5 h, and at this point, most of the start material had disappeared according to TLC analysis. Then the mixture was dried under reduced pressure. The residue was suspended in the water (50 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined $CHCl_3$ extracts were concentrated and purified by semipreparative HPLC (MeCN/H_2O = 50:50, v/v, flow rate: 3 mL/min) to yield **1** (36.4 mg, t_R = 22.5 min) and **2** (18.2 mg, t_R = 15.7 min).

Compounds **1** and **2** were isolated as racemates, which were then subjected to chiral HPLC to yield (+)-**1** (2.1 mg, t_R = 14.8 min) and (−)-**1** (1.6 mg, t_R = 21.9 min) (OD-H, *n*-hexane/2-propanol, 92:8, v/v, flow rate: 1 mL/min), and (+)-**2** (3.5 mg, t_R = 15.2 min) and (−)-**2** (2.8 mg, t_R = 23.5 min) (AD-H, *n*-hexane/2-propanol, 90:10, v/v, flow rate: 1 mL/min)

Cochlactone A (1): colorless crystals (MeOH); $[\alpha]^{18}_D$ −4.3 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε) 365 (3.42), 257 (3.69), and 213 (3.98); IR (KBr) ν_{max} 3424, 2948, 2933, 1750, 1644, 1623, 1486, 1457, and 1280 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Table 1; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{21}H_{26}O_5Na$ 381.1681, found 381.1678).

Crystal data for (+)-1: $C_{21}H_{26}O_5$, *M* = 358.42 g/mol, monoclinic, space group $P2_1$ (no. 4), *a* = 6.5047(3) Å, *b* = 17.1549(7) Å, *c* = 16.2889(4) Å, α = 90.00°, β = 91.085(4)°, γ = 90.00°, *V* = 1817.32 (13) Å^3, *Z* = 4, μ(CuKα) = 0.754 mm^{-1}, *d* = 1.310 mg/m^3, crystal dimensions of 0.14 × 0.13 × 0.12 mm were used for measurements on a Bruker APEX DUO diffractometer with a graphite monochromator (Φ/ω scans, $2\theta_{max}$ = 148.54°), Cu Kα radiation. The total number of independent reflections measured was 20837, of which 7178 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: R_1 = 0.0936, wR_2 = 0.02747 ($w = 1/\sigma|F|^2$), *S* = 1.081. The crystal structure of (+)-**1** was solved by a direct method (SHELXS-97, Sheldrich, G. M. University of Gottingen, Gottingen, Germany, 1997), and the full-maxtrix least-squares data were deposited in the Cambridge Crystallographic Data Centre (deposition no. 1589272). Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

Cochlactone B (2): yellow powder; $[\alpha]^{18}_D$ −12.7 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 362 (3.31), 258 (3.61), 208 (4.01); IR (KBr) ν_{max} 3425, 2926, 2873, 1749, 1643, 1485, 1282 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{21}H_{26}O_5Na$ 381.1674, found 381.1678.

Computational Methods for Calculating the ECD Spectrum of Compound 2.[20] Conformational analyses were initially performed using Spartan'10 software (Wavefunction, Inc., Irvine, CA) with the MMFF94 force field. The conformers with Boltzmann populations over 5% were chosen for ECD calculations, and the conformers were initially optimized at the B3LYP/6-31+g(d,p) level in MeOH using the continuum polarizable continuum model (CPCM). Harmonic vibration frequencies were calculated to confirm the stability of these conformers. As revealed by the frequency analysis, no imaginary frequencies were observed in the ground states. The theoretical calculation of the ECD spectra was conducted in MeOH using time-dependent density functional theory (TD-DFT) at the CAM-B3LYP/6-311+g(d,p) level for all conformers of **2**. The CD spectra were generated by the program SpecDis 1.6 (University of Würzburg, Würzburg, Germany) using a Gaussian band shape with 0.3 eV exponential half-width from the dipole length and dipole and rotational strengths.

Anti-Bacterial Activity Assay.[21] MICs were determined using a broth microdilution assay as previously described. Briefly, 2-fold serial

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dilutions of PLA were prepared in sterile tryptic soy broth (TSB) to provide final volumes of 100 μL in 96-well microtiter plates. Each well was inoculated with 100 μL of the test organism in TSB to a final concentration of 5×10^5 CFU/mL. The MIC was taken as the lowest concentration of PLA at which growth was inhibited after 24 h of incubation with shaking at 37 °C. The optical density was measured at 625 nm (OD625) using a SpectraMax Plus 384 microtiter plate reader (Molecular Devices, San Jose, CA). Penicillin G sodium was used as the positive control. *Staphylococcus aureus subsp. aureus* (ATCC29213) was purchased from the China General Microbiological Culture Collection Center, CGMCC.

Anti-Inflammatory Activity Assay.[22] Nitric oxide (NO) inhibitory assays were carried out as previously described. DMEM containing 10% FBS, 100 units/mL penicillin, and 100 μg/mL of streptomycin was applied to cultured macrophage RAW264.7 cells. The RAW264.7 cell line was obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). All experiments were performed in triplicate. L-NMMA was used as the positive control.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b00525.

1D and 2D NMR spectra of natural and biomimetic synthesized **1** and **2**, the data for single-crystal X-ray diffraction of (+)-**1**, IR, HR-EIS-MS, $[\alpha]_D$ spectra and CD spectra for **1**, **2**, (+)-**1**, (−)-**1**, (+)-**2** and (−)-**2**, together with ECD data of (±)-**2** (PDF)

X-ray data for compound (+)-**1** (CIF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Mothana, R. A. A.; Jansen, R.; Julich, W. D.; Lindequist, U. Ganomycins A and B, new antimicrobial farnesyl hydroquinones from the Basidiomycete *Ganoderma pfeifferi*. *J. Nat. Prod.* **2000**, *63*, 416−418.

(2) Chen, X. Q.; Chen, L. X.; Li, S. P.; Zhao, J. Meroterpenoids from the fruiting bodies of higher fungus *Ganoderma resinaceum*. *Phytochem. Lett.* **2017**, *22*, 214−218.

(3) Wang, M.; Wang, F.; Xu, F. Two pairs of farnesyl phenolic enantiomers as natural nitric oxide inhibitors from *Ganoderma sinesnse*. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3342−3345.

(4) (a) Peng, X. R.; Liu, J. Q.; Wang, C. F.; Han, Z. H.; Shu, Y.; Li, X. Y.; Zhou, L.; Qiu, M. H. Unusual prenylated phenols with antioxidant activities from *Ganoderma cochlear*. *Food Chem.* **2015**, *171*, 251−257. (b) Peng, X. R.; Li, L.; Wang, X.; Zhu, G. L.; Zhou, L.; Qiu, M. H. Antioxidant farnesylated hydroquinones from *Ganoderma capense*. *Fitoterapia* **2016**, *111*, 18−23.

(5) (a) Luo, Q.; Di, L.; Dai, W. F.; Lu, Q.; Yan, Y. M.; Yang, Z. L.; Li, R. T.; Cheng, Y. X. Applanatumin A, a new dimeric meroterpenoid from *Ganoderma applanatum* that dispalys potent antifibrotic activity. *Org. Lett.* **2015**, *17*, 1110−1113. (b) Luo, Q.; Di, L.; Yang, X. H.; Cheng, Y. X. Applantumols A and B, meroterpenoids with unprecedented skeletons from *Ganoderma applantum*. *RSC Adv.* **2016**, *6*, 45963−45967.

(6) Huang, S. Z.; Cheng, B. H.; Ma, Q. Y.; Wang, Q.; Kong, F. D.; Dai, H. F.; Qiu, S. Q.; Zheng, P. Y.; Liu, Z. Q.; Zhao, Y. X. Anti-allergic prenylated hydroquiones and alkaloids from the fruiting body of *Ganoderma calidophilum*. *RSC Adv.* **2016**, *6*, 21139−21147.

(7) Peng, X. R.; Liu, J. Q.; Han, Z. H.; Yuan, X. X.; Luo, H. R.; Qiu, M. H. Protective effects of triterpenoids from *Ganoderma resinaceum* on H_2O_2-induced toxicity in HepG2 cells. *Food Chem.* **2013**, *141*, 920−926.

(8) Niu, X. M.; Li, S. H.; Sun, H. D.; Che, C. T. Prenylated phenolics from *Ganoderma fornicatum*. *J. Nat. Prod.* **2006**, *69*, 1364−1365.

(9) Zhang, J. J.; Ma, K.; Chen, H. Y.; Wang, K.; Xiong, W. P.; Bao, L.; Liu, H. W. A novel polycyclic meroterpenoid with aldose reductase inhibitory activity from medicinal mushroom *Ganoderma leucocontextum*. *J. Antibiot.* **2017**, *70*, 915−917.

(10) Sharmah Gautam, K.; Birman, V. B. Biogenetically inspired synthesis of Lingzhiol. *Org. Lett.* **2016**, *18*, 1499−1501.

(11) Li, X. Y.; Liu, X. Y.; Jiao, X. Z.; Yang, H. G.; Yao, Y. Y.; Xie, P. An approach to (±)-Lingzhiol. *Org. Lett.* **2016**, *18*, 1944−1946.

(12) Chen, D.; Liu, H. M.; Li, M. M.; Yan, Y. M.; Xu, W. D.; Li, X. N.; Cheng, Y. X. Concise synthesis of (±)-Lingzhiol via epoxy-arene cyclization. *Chem. Commun.* **2015**, *51*, 14594−14596.

(13) Liu, H. X.; Hou, L. Q.; Yang, B.; Yuan, Y. F.; Zhang, W. M.; Xu, Z. F.; Qiu, S. X.; Tan, H. B. Biomimetic-inspired syntheses of myrtucommuacetalone and myrtucommulone J. *Org. Lett.* **2017**, *19*, 4786−4789.

(14) Barbero, A.; Castreno, P.; Pulido, F. J. Acid-catalyzed cycliztion of epoxyallylsilanes. An unusual rearrangement cycliztion process. *Org. Lett.* **2003**, *5*, 4045−4048.

(15) Mihopoulos, N.; Vagias, C.; Chinou, I.; Roussakis, C.; Scoullos, M.; Haravala, C.; Roussis, V. Dynamic solvent effects in the degenerate isomerization of hexafluoroacetone anil studied by high-presure ^{19}F NMR. *Z. Naturforsch., C: J. Biosci.* **1999**, *54*, 417−423.

(16) Chen, D.; Xu, W. D.; Liu, H. M.; Li, M. M.; Yan, Y. M.; Li, X. N.; Li, Y.; Cheng, Y. X.; Qin, H. B. Enatioselective total synthesis of (+)-Lingzhiol via tandem semipinacol rearrangement/Friedel-Crafts type cyclization. *Chem. Commun.* **2016**, *52*, 8561−8564.

(17) Bonny, M. L.; Capon, R. J. A sesquiterpene quinone and hydroquinone from the southern Australian marine sponge, Thorecta choanoides. *J. Nat. Prod.* **1994**, *57*, 539−540.

(18) Ramallo, I. A.; Salazar, M. O.; Mendez, L.; Furlan, R. L. E. Chemically engineered extracts: source of bioactive compounds. *Acc. Chem. Res.* **2011**, *44*, 241−250.

(19) (a) Kumar, N. N. B.; Kutateladze, A. G. Photoassisted diversity-oriented synthesis: intramolecular cycloadditions of photogenerated azaxylylenes with oxazole pendants, and subsequent postphotochemical multicomponent modifications. *Org. Lett.* **2016**, *18*, 460−463. (b) Bai, J.; Lu, Y. Y.; Xu, Y. M.; Zhang, W.; Chen, M.; Lin, M.; Gunatilaka, A. A. L.; Xu, Y. Q.; Molnar, L. Diversity-orented combinatiorial biosynthesis of hybrid polyketide scaffolds from azaphilone and benzenediol lactone biosynthons. *Org. Lett.* **2016**, *18*, 1262−1265. (c) Herrera, L.; Barrio, P.; Ibanez, I.; Roman, R.; Mateu, N.; Fustero, S. 8-Iodonaphthalene-1-carbaldehyde: a versatile building block for diversity-oriented synthesis. *Org. Lett.* **2016**, *18*, 4722−4725.

(20) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kito, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Millam, J. M.; Klene, M.; Knox, J. E.;

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J. Org. Chem. XXXX, XXX, XXX−XXX

Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision C.01; Gaussian, Inc.: Wallingford, CT, 2010.

(21) *Clinical and Laboratory Standards*; Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, 2010.

(22) Miranda, K.; Espey, M.; Wink, D. A rapid, simple sectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* **2001**, *5*, 62−71.

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J. Org. Chem. XXXX, XXX, XXX−XXX