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A Pair of Enantiomeric Bis-*seco*-abietane Diterpenoids from *Cryptomeria fortunei*

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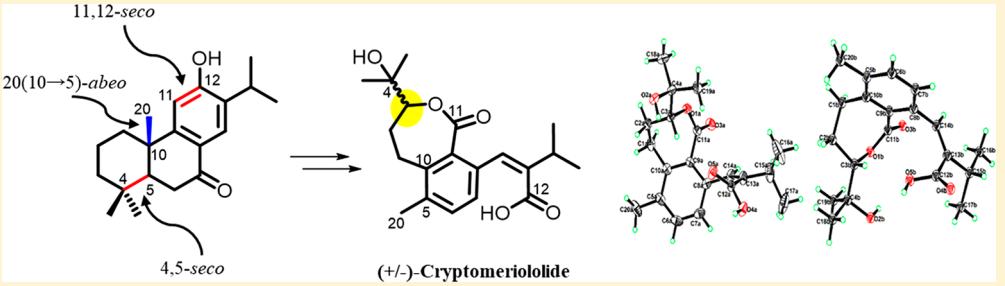
Supporting Information



ABSTRACT: (±)-Cryptomeriolide, a pair of racemic bis-*seco*-abietane diterpenoids, were isolated from the bark of *Cryptomeria fortunei*. The separation of enantiomers was achieved by using chiral stationary phase HPLC. Their structures including the absolute configuration and conformations in solution and solid state were determined by extensive analysis of spectroscopic data, single-crystal X-ray diffraction, and comparison of calculated and experimental electronic circular dichroism data. A bioinspired one-pot enantiomeric synthesis of **1a** and **1b** was accomplished via a readily made intermediate orthoquinone from sugiol. All compounds including the synthetic intermediates were assayed for their cytotoxic activities on human cancer cell lines HL-60, A549, and SGC7901.

Abietanes are a family of naturally occurring diterpenoids discovered from a variety of terrestrial plant sources and have displayed a wide spectrum of interesting biological activities, including cytotoxic, antimicrobial, antifungal, antiviral, antiulcer, cardiovascular, antioxidant, and anti-inflammatory activity.[1] Some drugs possessing an abietane skeleton, such as Ecabet Sodium, have been on the market for years. Ecabet Sodium is a 12-sulfodehydroabietic acid monosodium salt used in the management of gastritis and gastric ulceration due to its affinity to the gastric mucosa and to fibrinogen located on the gastric ulcer base.[2]

In recent decades, the abietanes have attracted broad attention from natural product chemists and pharmacological communities in their search for new lead compounds. Of the known abietane diterpenoids, *seco*-abietanes constitute a small but interesting group that originates from the ring cleavage of the core skeleton of the abietanes at unspecific positions.[3] They also showed remarkable bioactivity. For example, chlorabietins B and C, two 13,14-*seco*-abietanes, showed antineuroinflammatory effects by inhibiting the nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine BV-2 microglial cells.[4] To date, more than 80 naturally occurring *seco*-abietane diterpenoids have been reported.[3−15] Particularly, hyptisolode A is a bis-*seco*-abietane, and its C-7/C-8 bond (B-ring) and the C-11/C-12 bond (C-ring) both have been cleaved by oxidation.[7]

The genus *Cryptomeria* (Cupressaceae) comprises two species. Distributed mainly in China and Japan, *Cryptomeria* is known for its abundance in abietane diterpenoids.[16−20] Chinese cedar (*Cryptomeria fortunei* Hooibrenk), also known as Liu-Shan in Chinese, is an indigenous evergreen tree growing in the south of the Yangtze River region. As a traditional folk medicine, its root bark has been widely used as a detoxification and an insecticidal agent.[21] The essential oils of its wood and leaf were reported to show antibacterial and antitermitic activity.[22,23]

Our research group reported a series of abietane diterpenoids from *C. fortunei* previously.[19] In the course of our continuing effort to explore unique and bioactive

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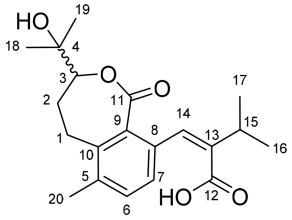
structures, this plant was investigated in depth. As a result, a pair of racemic bis-*seco*-abietane diterpenoids was isolated, and the two enantiomers (**1a** and **1b**) were obtained by chiral-phase HPLC separation. Their structures incorporate a rare architecture presumably derived from a common abietane diterpenoid skeleton. Their structures were characterized on the basis of spectroscopic data analysis, a single-crystal X-ray diffraction experiment, and comparison of experimental and computed electronic circular dichroism (ECD) data. A concise enantiomeric synthesis of **1a** and **1b** was accomplished following a plausible biosynthetic pathway. Herein, we present the isolation and structure elucidation of **1a** and **1b**, particularly the absolute configuration determination. The bioinspired one-pot method to construct **1a** and **1b** via a readily made intermediate orthoquinone from sugiol is also described.



(+)-(*S*)-cryptomeriolide (**1a**)
(−)-(*R*)-cryptomeriolide (**1b**)

RESULTS AND DISCUSSION

The acetone extract of the bark of *C. fortunei* was dissolved in water and partitioned with petroleum ether (PE), dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc), successively. After evaporation to dryness under vacuum of the solvent, the PE fraction dissolved in 80% aqueous MeOH. The CH_2Cl_2 fraction and the MeOH layer were combined together based on the TLC profiles and separated by repeated column chromatography and preparative HPLC to afford compound **1** as a white powder. Its molecular formula of $C_{20}H_{26}O_5$ was deduced from the sodium adduct ion at m/z 369.1662 $[M + Na]^+$ in the HRESIMS, corresponding to eight indices of hydrogen deficiency. The IR spectrum showed absorption bands for hydroxy (3446 cm^{-1}) and carbonyl (1713 cm^{-1}) groups. A total of 20 carbon resonances were well resolved in the ^{13}C NMR and DEPT spectra, ascribed to five methyls, two methylenes, five methines, five quaternary carbons, an oxygenated tertiary carbon, and two carbonyls (Table 1). The ^1H NMR data (Table 1), combined with the ^{13}C NMR data, displayed diagnostic signals for a 1,2,3,4-tetrasubstituted benzene [δ_H 7.25 (d, J = 8.0 Hz), 7.03 (d, J = 8.0 Hz); δ_C 135.3, 135.0, 134.3, 133.3, 130.4, 127.5], a trisubstituted double bond [δ_H 6.78 (s); δ_C 142.7, 130.2], an aromatic methyl [δ_H 2.35 (s, 3H); δ_C 19.5], an oxygenated isopropyl group [δ_H 1.24 (s, 3H), 1.16 (s, 3H); δ_C 72.2, 26.1, 24.0], an isopropyl [δ_H 2.80 (sept, J = 6.5 Hz, 1H), 1.17 (d, J = 6.5 Hz, 3H), 1.16 (d, J = 6.5 Hz, 3H); δ_C 32.1, 21.7, 21.2], and two ester carbonyl groups (δ_C 171.1, 170.6). These observations accounted for seven out of eight indices of hydrogen deficiency, and the remaining one implied the presence of an additional ring. The connectivity of $CH_2(1)-CH_2(2)-CH(3)$ could be well interpreted from correlations of H-1/H-2 and H-2/H-3 in the $^1H-^1H$ COSY spectrum (Figure 1). HMBC correlations from H-3 to the carbonyl C-11, from H-1 to the aromatic C-9 and C-5, and from H-2 to the aromatic C-10 facilitated the construction of a seven-membered ring. HMBC correlations from the olefinic H-14 to C-15 and C-12, and from H-15 to C-12 and C-14 established an isopentyl unit with a carboxylic group connecting to the olefinic C-13. HMBC correlations from H-14 to the aromatic carbons C-7 and C-9 confirmed the connection of C-14 to C-8. The key HMBC correlations from H-2 and H-3 to C-4, and from CH_3-20 to C-6 suggested that the oxygenated isopropyl and the methyl group were located at C-3 and C-5, respectively. The NOESY interaction between H-14 and H-15 indicated the Z configuration of the $\Delta^{13(14)}$ double bond. Therefore, the 2D structure of **1** was established and the compound was named cryptomeriolide.

The structure of cryptomeriolide (**1**) has one stereogenic center at C-3. Although only one set of ^1H and ^{13}C NMR data was observed, the specific rotation value (−3) of **1** suggested that it could be racemic. Compound **1** was then analyzed on a chiral-phase HPLC column, and two peaks with an approximate ratio of 1:1 were observed (Figure S1, Supporting Information). Single crystals of the racemic cryptomeriolide (**1**) were obtained (CCDC 1446792), and the X-ray diffraction data (Figure 2) showed that **1** consisted of a racemic

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data for Compound 1 in $CDCl_3$ (δ in ppm, J in Hz)

position	δ_C	δ_H, mult (J in Hz)
1	25.0, CH_2	3.06, m; 2.55, ddd (13.8, 13.7, 7.0)
2	28.1, CH_2	2.14, m; 1.79, m
3	83.7, CH	3.88, dd (12.2, 4.5)
4	72.2, C	
5	135.3, C	
6	133.3, CH	7.25, d (8.0)
7	127.5, CH	7.03, d (8.0)
8	134.3, C	
9	130.4, C	
10	135.0, C	
11	171.1, C	
12	170.6, C	
13	142.7, C	
14	130.2, CH	6.78, s
15	32.1, CH	2.80, sept (6.5)
16	21.2, CH_3	1.16, d (6.5)
17	21.7, CH_3	1.17, d (6.5)
18	24.0, CH_3	1.24, s
19	26.1, CH_3	1.16, s
20	19.5, CH_3	2.35, s

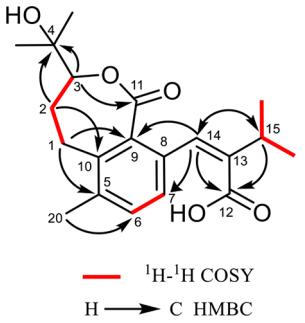


Figure 1. Key $^1H-^1H$ COSY (red bold line) and HMBC correlations (H → C) of **1**.

The structure of cryptomeriolide (**1**) has one stereogenic center at C-3. Although only one set of ^1H and ^{13}C NMR data was observed, the specific rotation value (−3) of **1** suggested that it could be racemic. Compound **1** was then analyzed on a chiral-phase HPLC column, and two peaks with an approximate ratio of 1:1 were observed (Figure S1, Supporting Information). Single crystals of the racemic cryptomeriolide (**1**) were obtained (CCDC 1446792), and the X-ray diffraction data (Figure 2) showed that **1** consisted of a racemic

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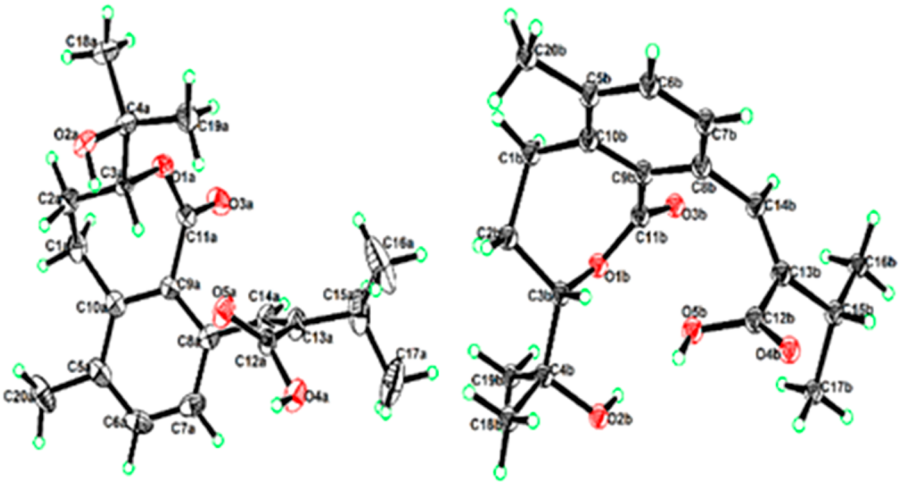

Figure 2. Perspective ORTEP drawing for **1**.

compound. Chiral-phase HPLC separation of *rac*-**1** eventually afforded the enantiomers **1a** and **1b**.

Compounds **1a** and **1b** gave the opposite specific rotations (+159 for **1a** *vs* −153 for **1b**) and mirror images of ECD curves (Figure S2, Supporting Information). To assign the absolute configuration of C-3, time-dependent density functional theory (TDDFT) ECD calculations were carried out on the arbitrarily chosen enantiomer (*S*).[24,25] A Merck Molecular Force Field (MMFF) conformational search in $CHCl_3$ resulted in 116 conformers in a 21 kJ/mol energy window. These conformers were reoptimized at the B3LYP/6-31G(d) level in vacuo and at the B97D/TZVP[26,27] PCM/MeOH and the CAM-B3LYP/TZVP[28,29] PCM/MeOH levels. ECD spectra were calculated with various functionals (B3LYP, BH&HLYP, CAM-B3LYP, and PBE0) and the TZVP basis set for conformers above 1% Boltzmann population (Figure 3). All

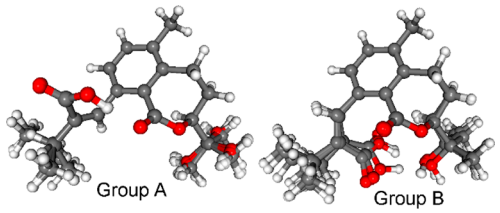

Figure 3. Low-energy conformers (≥1%) of (*S*)-**1** obtained by optimization of the MMFF conformers at the CAM-B3LYP/TZVP level with PCM for MeOH. Group A contains conformers A–D, F, G, I, J, and L–O; group B contains conformers E, H, K, P, and Q.

applied combinations of levels gave moderate to good agreement with the experimental ECD spectrum recorded in MeOH. In most cases the BH&HLYP and the CAM-B3LYP functionals gave better agreement than the B3LYP and the PBE0 ones. It is also noteworthy that ECD spectra computed for the two optimized solid-state X-ray conformers with different conformations exhibit a curve that is nearly a mirror-image of that of the solution conformers (Figure S3, Supporting Information and Figure 4).[30] In solution, the hydrogen of the carboxyl group forms a hydrogen bond with the oxygen of the carbonyl or OH groups, while in the solid state intermolecular hydrogen bonds are produced, which give rise to different orientations of the α,β-unsaturated carboxyl group. The orientation of the α,β-unsaturated carboxyl group

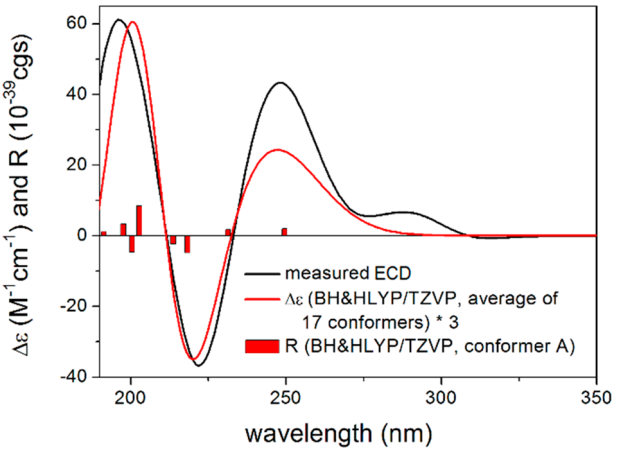

Figure 4. Experimental ECD spectrum of **1a** in MeOH compared with the Boltzmann-weighted BH&HLYP/TZVP PCM/MeOH ECD spectrum of (*S*)-**1** computed for the CAM-B3LYP/TZVP PCM/MeOH conformers. Bars represent the rotational strength values of the lowest-energy conformer.

relative to the conjugating benzene ring seems to play a decisive role in the ECD spectrum. In this case, the comparison of the experimental ECD spectrum with the weighted TDDFT-ECD spectrum computed for the optimized solid-state X-ray conformers would have given an incorrect absolute configuration, since different conformers with opposite ECD are prevalent in solution.

The ECD results were also verified by OR calculations performed on the CAM-B3LYP/TZVP PCM/MeOH conformers, resulting in large positive rotation values in the range of +226 to +263.[31,32] Furthermore all low-energy conformers at all the applied combinations of levels had OR values in the range of +182 to +308, allowing the unambiguous elucidation of the absolute configuration (see Supporting Information).

Thus, the absolute configuration and preferred conformations of **1a** and **1b** were determined in solution and solid states. Compound **1a**, with a positive optical rotation and *S* absolute configuration, was named (+)-(*S*)-cryptomeriolide, while compound **1b** was named (−)-(*R*)-cryptomeriolide.

Cryptomeriolide (**1**) is an example of a 20(1→5)-*abeo*-4,5;11,12-bis-*seco*-abietane diterpenoid isolated from natural sources. The biosynthetic precursors of cryptomeriolide (**1**)

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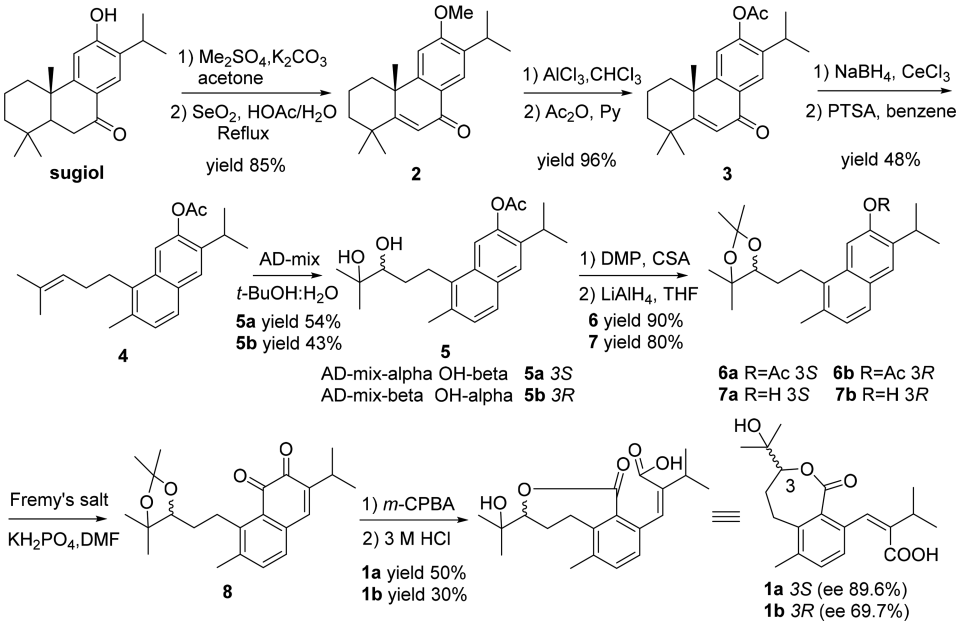
Scheme 1. Synthetic Pathway from Sugiol to 1a and 1b



could presumably be traced back to sugiol, which is widely distributed in the family Cupressaceae and also isolated previously from *C. fortunei*.[33] A concise enantioselective synthesis of **1a** and **1b** was effectively accomplished by using sugiol as a starting material (Scheme 1). First, sugiol was methylated in the presence of Me_2SO_4 in acetone and then oxidized with SeO_2 in acidic medium to form the α,β-unsaturated ketone (**2**). Demethylation and acetylation afforded the acetate (**3**). The acetate was reduced with $NaBH_4$ in the presence of cerium(III) chloride and subsequently treated with *p*-toluenesulfonic acid to yield a *seco* ring A precursor (**4**). Subsequently, the exocyclic double bond of **4** was oxidized to form an α- or β-orientated dihydroxy group by AD-mix-α or AD-mix-β according to the method of Sharpless.[34] The diol intermediate (**5**) was protected with 2,2-dimethoxypropane to afford compound **6**, which was hydrolyzed to remove the acetyl group. The resulting product (**7**) was oxidized to orthoquinone (**8**) by Fremy's salt.[35] The final product **1a** or **1b** with good enantiomeric excess was generated by treating **8** with *m*-CPBA and then hydrolyzed under acid conditions. The NMR data and specific rotation values of synthetic **1a** and **1b** were both in good agreement with those of the corresponding natural products, which further confirmed the structural assignments of naturally occurring compounds **1a** and **1b**.

The natural and synthetic bis-*seco*-abietane diterpenoids **1a** and **1b**, together with the intermediates from the synthetic procedures, were evaluated for cytotoxicity against three human cancer cell lines: HL-60, A549, and SGC7901. The results (Table 2) indicated that all intermediates (**3**, **4**, **5a**/**b**, **6a**/**b**, **7a**/**b**) showed moderate cytotoxic activities with IC_{50} values ranging from 16.0 to 88.9 μM except for compounds **1a**, **1b**, and **2**.

In summary, a pair of enantiomeric bis-*seco*-abietane diterpenoids from *C. fortunei* was isolated and fully characterized. They represent a rare carbon skeleton formed by migration of an angular methyl group and bond-breaking of rings A and C. Although different degrees of oxidation of rings A, B, or C of abietane diterpenoids have been reported,

Table 2. Cytotoxic Activities of Compounds 1−7 against the HL-60, A549, and SGC7901 Cell Lines

	IC_{50} (μM)		
compound	HL-60[a]	A549[b]	SGC7901[b]
1a	>100	>100	>100
1b	>100	>100	>100
2	>100	>100	>100
3	25.7 ± 0.7	26.9 ± 0.6	22.9 ± 0.5
4	20.8 ± 1.6	25.5 ± 1.8	34.4 ± 0.9
5a	21.7 ± 1.1	50.5 ± 0.3	45.8 ± 1.0
5b	41.2 ± 2.5	48.6 ± 0.9	47.5 ± 1.6
6a	16.0 ± 4.0	68.5 ± 0.2	88.9 ± 10.5
6b	17.2 ± 5.7	28.9 ± 0.7	36.7 ± 1.9
7a	45.5 ± 1.4	26.2 ± 0.7	31.7 ± 0.4
7b	49.4 ± 10.6	23.5 ± 0.6	33.1 ± 1.4
mitoxantrone	0.024 ± 0.01	0.036 ± 0.004	0.293 ± 0.005[c]

[a]Determined by the CCK8 assay. [b]Determined by the SRB assay. [c]Value given as nM.

resulting in a small group of *seco*-abietanes, this type of skeleton was in fact only described in a recent patent.[36] This is the first time that such a 20(10→5)-*abeo*-4,5;11,12-bis-*seco*-abietane skeleton was unambiguously defined by both single-crystal X-ray crystallography and synthesis.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin−Elmer 341 polarimeter. IR spectra were recorded on a Nicolet 6700 spectrometer in KBr pellets. ECD spectra were measured on a BRIGHTTIME Chirascan. NMR spectra were recorded on a Varian Mercury-500 NMR spectrometer. The chemical shift (δ) values are given in ppm with tetramethylsilane as internal standard, and coupling constants (J) are in Hz. ESIMS and HRESIMS data were recorded on Waters 2695−3100 LC-MS and Waters Xevo TOF mass spectrometers. Single-crystal X-ray diffraction measurements were conducted on a Bruker Smart Apex II diffractometer with a graphite monochromator. Column chromatography (CC) was performed with silica gel (100−200, 200−300, and 300−400 mesh, Qingdao Marine Chemical Industrials, Qingdao, People's Republic of

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China) and MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Industries, Tokyo, Japan). TLC was carried out on precoated silica gel GF254 plates (Yantai Chemical Industrials, Yantai, People's Republic of China), and the TLC spots were viewed at 254 nm and visualized with 5% H_2SO_4 in EtOH containing 10 mg/mL vanillin followed by heating. Analytical HPLC was performed on a Waters 2695 instrument with an Acquity ELSD detector. Preparative HPLC was performed on a Varian PrepStar system with an Alltech 3000 ELSD. Chromatographic separations were carried out on a Waters Sunfire RP C_{18}, 5 μm, 30 mm × 150 mm column, using a gradient solvent system composed of H_2O and CH_3CN, with a flow rate of 25.0 mL/min. Chiral-phase separation was performed by K-prep LAB100S (YMC, Japan) on a chiral-phase column Daicel Chiralpak AY-H, 10 μm, 5.0 cm i.d. × 25.0 cm L column (Daicel Chemical Industries, Ltd., Japan), using a gradient solvent system composed of hexane/EtOH/HAc (90:10:0.1). Analytical chiral-phase HPLC was performed by a Shimadzu LC 20A QA&QC-HPLC-02 on a chiral-phase Daicel Chiralpak AY-H, 5 μm, 0.46 cm i.d. × 15.0 cm L column (Daicel Chemical Industries, Ltd., Japan). All solvents used for CC and HPLC were of analytical grade (Shanghai Chemical Reagents Co. Ltd.) and gradient grade (Merck KGaA), respectively.

Plant Material. The bark of *C. fortunei* was collected in She County, Anhui Province, China, in October 2013, and identified by Professor Jin-Gui Shen of the Shanghai Institute of Materia Medica. A voucher specimen (no. 20130506) was deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered bark of *C. fortunei* (110 kg) was extracted with acetone (3 × 330L) at room temperature. After evaporation of the solvent, part of the residue (2 kg) was dissolved in water and partitioned with PE, CH_2Cl_2, and EtOAc, successively. The PE fraction was concentrated and dissolved in 80% aqueous MeOH. The CH_2Cl_2 fraction and the MeOH layer were combined together based on the TLC profiles and subjected to CC over MCI gel (EtOH/H_2O, 60% to 95%) to yield three fractions (A−C). Fraction A (75 g) was subjected to CC over MCI gel (EtOH/H_2O, 30% to 70%) to give 13 fractions (A1−A13). Fraction A10 (10.8 g) was subjected to CC over silica gel eluting with PE/acetone (10:1 to 1:2) in a stepwise manner to afford 16 fractions (A10A−A10P). Subsequently, fraction A10J (753 mg) was selected for separation by preparative HPLC (CH_3CN/H_2O, 35% to 45%) and further purified by CC over silica gel eluting with CH_2Cl_2/acetone (10:1) to yield cryptomeriolide (**1**) (44 mg).

Cryptomeriolide (1): white powder; $[\alpha]^{20}_D$ +159 (*c* 0.3, MeOH) (**1a**), $[\alpha]^{20}_D$ −153 (*c* 0.3, MeOH) (**1b**); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 248 (+30.35), 233 (0), 222 (−25.75), 212 (0), 196 (+42.76) nm (**1a**), ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 248 (−33.42), 233 (0), 222 (+28.43), 212 (0), 197 (−47.03) nm (**1b**); IR (KBr) ν_{max} 3446, 2965, 2928, 2871, 1713, 1466, 1384, 1261, 1182, 1090, 1053, 802 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS *m/z* 347.3 $[M + H]^+$, 345.2 $[M - H]^-$, 691.4 $[2M - 1]^-$; ESIHRMS *m/z* 369.1662 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_5Na$, 369.1672).

X-ray Crystallographic Analysis of Compound 1. From a mixture of CH_2Cl_2/*n*-hexane solution, white crystals were obtained, and a needle-like crystal was supplied for the X-ray crystallographic analysis. The structure was solved and refined using the Bruker SHELXTL Software Package. A total of 35 529 measurements yielded 9837 unique reflections (R_{int} = 0.0406), the final R_1 was determined as 0.0592, and wR_2 was 0.1687 ($I > 2\sigma(I)$). Crystal data for **1**: $C_{40}H_{52}O_{10}$, M = 692.82 g/mol, triclinic, space group *P*-1, *a* = 12.8671(3) Å, *b* = 14.1050(3) Å, *c* = 15.2457(3) Å; *α* = 64.1960(10)°, *β* = 89.7430(10)°, *γ* = 63.3900(10)°, *V* = 2163.93(8) $Å^3$, *Z* = 2, *T* = 170 K, μ(Mo Kα) = 0.076 mm^{-1}, *F* = 744, D_{calc} = 1.063 g/cm^3. Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre as deposit no. CCDC 1446792. Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html or on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [tel: (+44) 1223-336-408; fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk].

Cytotoxicity Bioassays. The antiproliferative activities of compounds **1**−**7** against HL-60 (human leukemia), A-549 (human non-small-cell lung adenocarcinoma), and SGC7901 (human gastric adenocarcinoma) tumor cell lines were evaluated.

Cell Culture and Reagents. Human acute myeloid leukemia cell line HL-60 was purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and cultured in media according to recommendations with 10% fetal bovine serum (FBS) (Gibco, New Zealand). The human non-small-cell lung cancer cell line A549 was purchased from the American Type Culture Collection (ATCC) and cultured in media according to recommendations with 10% FBS (Gibco, New Zealand). The human gastric cancer cell line SGC-7901 was purchased from Shanghai Sixth People's Hospital and cultured in RPMI-1640 medium with 10% FBS. The cells were cultured at 37 °C and 5% CO_2. Mitoxantrone, used as the positive control, was purchased from Selleck (TX, USA), and the purity was 99.36%.

Cell Viability Assay. The antiproliferative activity of the compounds against HL-60 cells was determined using the CCK8 assay (Dojindo, Japan).[37] Briefly, cells were seeded in 96-well plates (8×10^3 cells/well) overnight and then exposed to various concentrations of compounds in triplicates for 72 h. Subsequently, 10 μL of CCK8 reagent was added per well and incubated at 37 °C for 2 h. Cell proliferation was evaluated by optical density, which was measured using a SpectraMax 190 multiwell spectrophotometer (Molecular Devices, CA, USA) at 450 nm. The inhibitory rates (%) of compounds were calculated with the following formula: $[1 - (A_{450\ treated}/A_{450\ control})] \times 100\%$. Cell sensitivity was expressed as IC_{50} values, which were calculated by the Logit method. The experiment was repeated independently and shown as the average of two independent experiments.

The antiproliferative activity of the compounds against A549 and SGC7901 cells was evaluated using the sulforhodamine B (SRB) assay.[38] Cells (A549: 2×10^3 cells/well and SGC7901: 3×10^3 cells/well) were seeded into 96-well plates and grown for 24 h. Cells were then treated with increasing concentrations of compounds and grown for 72 h. DMSO was used as the vehicle control. At the end of the exposure time, 100 μL of ice-cold 10% trichloroacetic acid (TCA) was added to each well, left at 4 °C for 1 h, and washed five times with distilled water. The TCA-fixed cells were stained for 15 min with 100 μL of 4 mg mL^{-1} SRB in 1% HOAc. The plates were washed five times with 1% HOAc and air-dried overnight. After air-drying, protein-bound dye was dissolved in 150 μL of 10 mM Tris base for 5 min and was measured at 560 nm using a multiwall spectrophotometer (SpectraMax, Molecular Devices, USA). The inhibition rate was calculated as $(1 - A_{560}$ treated$/A_{560}$ control$) \times 100\%$. The experiment was repeated independently, and the results are shown with the average value of two independent experiments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00482.

1D and 2D NMR, IR, and ECD spectra, HRESIMS data of new compounds, chiral-phase HPLC separation, enantioselective synthesis, and the X-ray data for compound **1** (PDF)

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Notes

The authors declare no competing financial interest.

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