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Artaboterpenoids A and B, Bisabolene-Derived Sesquiterpenoids from *Artabotrys hexapetalus*

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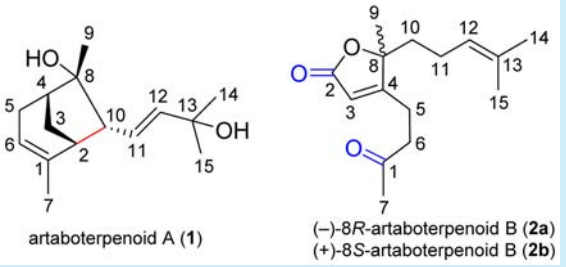
Supporting Information

ABSTRACT: Artaboterpenoids A and B (**1** and **2**), two novel bisabolene-derived sesquiterpenoids, were isolated from the roots of *Artabotrys hexapetalus*. Their structures with absolute configurations were elucidated by spectroscopic methods, and electronic circular dichroism (ECD) analyses. Notably, **1** featured a novel carbon skeleton with a new C-2−C-10 linkage, and **2a** and **2b**, a pair of enantiomers, represented the first examples of 1,2-seco-bisabolene-type sesquiterpene lactones. **2a** exhibited cytotoxic effects against HCT-116, HepG2, A2780, NCI-H1650, and BGC-823 cell lines with IC_{50} values of 1.38−8.19 μM. Plausible biogenetic pathways for artaboterpenoids A and B were proposed.



Bisabolane-type sesquiterpenoids are a very important family of natural products with structural diversity and various bioactivities, such as juvenile hormone,[1] antitumor,[2] and antimalarial[3] activities. Bisabolane was considered to be the biogenetic precursor of many sesquiterpenes.[4] For instance, amorphadiene, acoradiene, and cuprenene were derived from bisabolane by cyclization,[5] which was demonstrated by Tantillo with quantum chemical calculations.[6]

Artabotrys hexapetalus (L.f.) Bhandari was a Chinese folk medicine used for the treatment of malaria. Yingzhaosus A−D,[3,7] as representative bisabolane-type sesquiterpenoids, were discovered from the root of *A. hexapetalus*. In our continuing endeavor to discover structurally diverse and biologically interesting metabolites from traditional Chinese medicines, two bisabolene-derived sesquiterpenoids, artaboterpenoids A and B (**1** and **2**), were isolated from the root of *A. hexapetalus*. Artaboterpenoid A (**1**) possesses an unusual carbon skeleton formed by the new C-2−C-10 linkage (**1**), and (±)-artaboterpenoid B (**2a** and **2b**), a pair of enantiomers, were first reported as 1,2-seco-bisabolene-type sesquiterpene lactones. Herein, we report their isolation, structural elucidation, and cytotoxicity, as well as plausible biogenetic pathways.

Artaboterpenoid A (**1**) was isolated as a colorless oil with the molecular formula $C_{15}H_{24}O_2$, as determined by (+)-HRESIMS data (*m*/*z* 259.1670 $[M + Na]^+$, calcd for 259.1669), corresponding to four degrees of unsaturation. The ^{13}C and HSQC NMR data displayed three quaternary carbons (one olefinic and two oxygenated), six methines (three olefinic), two methylenes, and four methyls (Table 1). The ^1H NMR spectrum showed resonances attributable to a pair of *trans* olefinic protons [δ_H 6.23 (dd, J = 15.7, 9.9 Hz), 6.04 (d, J = 15.7 Hz)], one olefinic proton singlet (δ_H 5.33), and four tertiary methyls (δ_H 1.72, 1.51 × 2, and 1.44) (Table 1). These data combined with the degrees of unsaturation suggested a two-ring system in the structure of **1**.

Table 1. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of Artaboterpenoids A and B

	artaboterpenoid A[a]		artaboterpenoid B[b]	
no.	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	139.2		205.7	
2	47.6	2.17, br s	171.8	
3	34.5	a 1.79, m	114.5	5.65, t (1.8)
		b 1.75, m		
4	46.6	2.16, br s	174.9	
5	30.1	a 2.82, m	20.8	2.48, m, 2H
		b 2.16, m		
6	120.4	5.33, br s[c]	39.8	2.82, m, 2H
7	25.3	1.72, s	29.8	2.23, s
8	79.5		89.2	
9	32.9	1.44, s	23.8	1.45, s
10	60.8	2.52, dd (9.9, 5.8)	36.9	a 1.89, m
				b 1.66, m
11	126.7	6.23, dd (15.7, 9.9)	21.6	a 1.96, m
				b 1.79, m
12	141.6	6.04, d (15.7)	122.5	5.02, m
13	70.2		132.4	
14	31.2	1.51, s	17.5	1.56, br s
15	31.0	1.51, s	25.4	1.66, br s
8-OH		4.92, br s		
13-OH		5.86, br s		

[a]Data were recorded in pyridine-d_5. [b]Data were recorded in $CDCl_3$. [c]The peak width at half-height was 7.8 Hz.

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Detailed analyses of the ^{1}H−^{1}H COSY and HSQC spectra were not able to lead to the assignment of the spin systems of C(10)H−C(2)H−C(3)H$_2$−C(4)H−C(5)H$_2$ because of the three overlapped signals at 2.17 ppm (H-2, 4, and 5b) (Figure 1). Afterward, a six-membered ring A was established by the

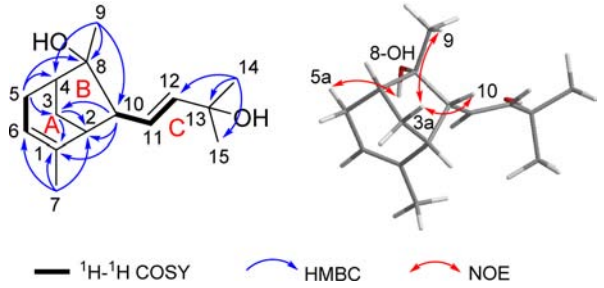

Figure 1. Key ^{1}H−^{1}H COSY, HMBC, and NOE correlations for **1**.

HMBC cross-peaks from H$_3$-7 to C-1, C-2, and C-6, from H-3b to C-1, C-2, and C-10, and from H-5a to C-3, C-4, and C-8, along with the ^{1}H−^{1}H COSY cross-peak between H-5a and H-6. Additionally, the HMBC cross-peaks from H$_3$-9 to C-4, C-8, and C-10 and from H-10 to C-1, C-2, and C-3 implied the presence of a five-membered ring B. Also, the side chain of ring B (fragment C) was deduced by the HMBC cross-peaks from H$_3$-14 to C-12, C-13, and C-15, along with the ^{1}H−^{1}H COSY cross-peaks of H-10/H-11/H-12. Thus, the planar structure of **1** was established as depicted (Figure 1).

The relative configuration of **1** was deduced from the interpretation of the nuclear overhauser effect (NOE) experiments. The remarkable NOE correlations from H$_3$-9 (δ_H 1.44) to H-3a (δ_H 1.79) and from H-10 (δ_H 2.52) to H-3a placed H$_3$-9, H-3a, and H-10 on the same face, while the strong NOE correlations from H-5a (δ_H 2.82) to C-8-OH (δ_H 4.92) placed these protons on the other side (Figure 1). Therefore, there were only two possible structures for **1**, with absolute configurations of **1A** (2*S*,4*R*,8*R*,10*S*) and **1B** (2*R*,4*S*,8*S*,10*R*) (Figure 2).

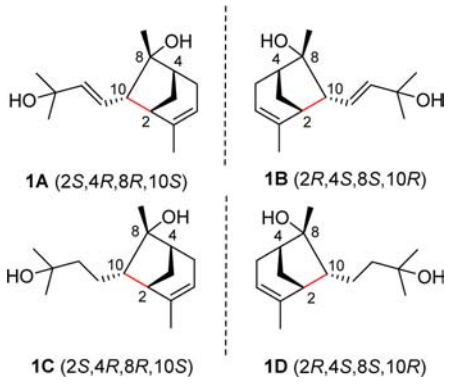

Figure 2. Structures of model compounds **1A** and **1C**, and **1B** and **1D** in the ECD calculations.

The absolute configuration of **1** was established by experimental and theoretical ECD after initially unsuccessful attempts to obtain single crystals of **1** and its *p*-bromobenzoate derivative. The ECD spectrum of **1** showed an intense negative Cotton effect at 208 nm ($\Delta\varepsilon$ − 18.04), which predominately resulted from the $\pi_x \rightarrow \pi_x^*$ electronic transition of the $\Delta^{1(6)}$ double bond of the cyclohexene ring,[8] rather than the Δ^{11} double bond. Time-dependent density functional theory[9] at the B3LYP/6-31G(d) level was employed to demonstrate the aforementioned deduction by comparing the calculated ECD spectra of **1A** and **1C**, and of **1B** and **1D** (Figure 2). The Cotton effects of **1A** and **1C**, and of **1B** and **1D** were the same in the positions and signs but different in the relative intensities, respectively (Figure 3). Thus, the absolute configuration of **1** was determined by

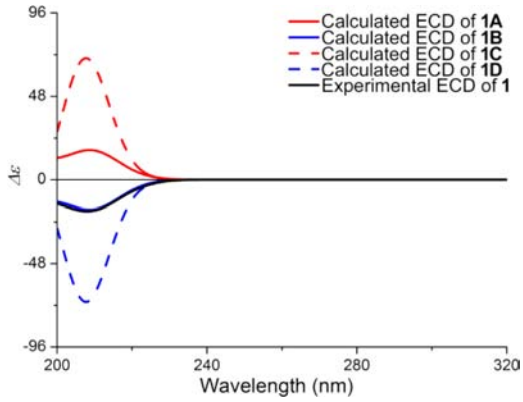

Figure 3. Experimental ECD spectrum of **1** and calculated ECD spectra of **1A** and **1C**, and **1B** and **1D**.

comparison of the experimental and calculated ECD spectra. The experimental ECD spectrum of **1** showed excellent agreement with the calculated spectrum of **1B** (2*R*,4*S*,8*S*,10*R*) (Figure 3).

Artaboterpenoid B (**2**) possessed a molecular formula of $C_{15}H_{23}O_3$ as established by (+)-HRESIMS data (*m*/*z* 251.1640 $[M + H]^+$, calcd for 251.1642). The IR spectrum exhibited the presence of ester and ketone carboxyl groups (1751, 1720 cm^{-1}) and a double bond (1639 cm^{-1}). The analysis of its ^{1}H, ^{13}C, and HSQC NMR data revealed the presence of five quaternary carbons [one ketone carboxyl (δ_C 205.7), one ester carboxyl (δ_C 171.8), two olefinic carbons (δ_C 174.9 and 132.4), and one oxygenated (δ_C 89.2)], two sp^2 methines [δ_H 5.65 (t, 1.8 Hz) and 5.02 (m); δ_C 122.5 and 114.5), four sp^3 methylenes (δ_C 39.8, 36.9, 21.6, and 20.8), and four tertiary methyls (δ_H 2.23, 1.66, 1.56, and 1.45; δ_C 29.8, 25.4, 23.8, and 17.5) (Table 1). These characteristic signals, with the five degrees of unsaturation, suggested that **2** was a sesquiterpene lactone with a single-ring system in the structure.

The planar structure of **2** was constructed by detailed analyses of its ^{1}H−^{1}H COSY and HMBC spectra. The ^{1}H−^{1}H COSY correlations from H-5 to H-6 as well as the HMBC correlations from H$_3$-7 to C-1 and C-6 established the connectivity of C-7−C-1−C-6−C-5. The ^{1}H−^{1}H COSY correlations from H-11 to H-10 and H-12, along with HMBC correlations from H$_3$-14 to C-12, C-13, and C-15, indicated the linkage of C-10−C-11−C-12−C-13(−C-15)−C-14. The HMBC correlations from H-3 to C-2, C-4, and C-8 and from H$_3$-9 to C-4 and C-8 implied the presence of an α,β-unsaturated γ-lactone unit (Figure 4). Furthermore, the HMBC correlations from H$_2$-5 to C-3, C-4, and C-8 and from H$_2$-6 to C-4 unambiguously confirmed the connectivity of C-4 and C-5, whereas the HMBC correlations from H$_2$-10 to C-8 and from H$_2$-11 to C-8 established the linkage of C-10 and C-8 (Figure 4). Thus, the planar structure of **2** is shown in Figure 4. It is particularly noteworthy that **2** is the first example of a 1,2-seco-bisabolene-type sesquiterpene lactone.

ECD has proven to be a powerful and reliable method for determining the absolute configuration of lactones.[10] However, Cotton effect resulting from α,β-unsaturated γ-lactone was not observed in the ECD spectrum of **2** (Figure S24, Supporting

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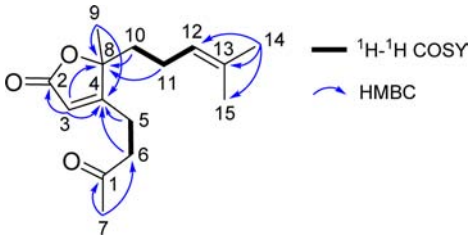


Figure 4. Key $^{1}H-^{1}H$ COSY and HMBC correlations for **2**.

Information). Furthermore, subsequent HPLC separation on a Chiralcel OZ-H column (Daicel Chemical Industries, Ltd., Tokyo, Japan) was successful in resolving two stereoisomers (**2a** and **2b**) (Figure 5), which were opposite in terms of the ECD

Figure 5. HPLC separation chromatogram of **2** on a Chiralcel OZ-H column.

curves (Figures S25 and S26, Supporting Information) and specific rotations {$[\alpha]_D^{20}$ −42.1 (*c* 0.1, MeOH) for **2a** and $[\alpha]_D^{20}$ +43.6 (*c* 0.1, MeOH) for **2b**} but identical in terms of HRESIMS and NMR data of **2** (Figures S12−S20, Supporting Information). The observed Cotton effect around 220 nm due to the $\pi \rightarrow \pi^*$ transition of the α,β-unsaturated γ-lactone chromophore was correlated directly to the chirality of the γ-carbon of butenolide. According to the helix rule,[11] a negative Cotton effect at 223.5 nm in the ECD spectrum of **2a** revealed that the C-10−C-8−C-4═C3 system possessed a left-handed helicity (counterclockwise) and suggested an 8*R* absolute configuration for **2a**, whereas a positive Cotton effect at 222.5 nm in the ECD spectrum of **2b** corresponded to a right-handed helicity (clockwise), indicating an 8*S* absolute configuration for **2b** (Figure 6). Therefore, **2a** and **2b** were named (−)-8*R*- and (+)-8*S*-artaboterpenoid B, respectively.

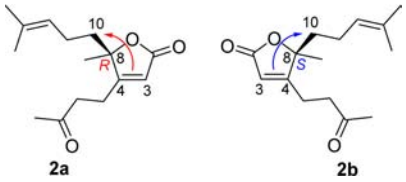
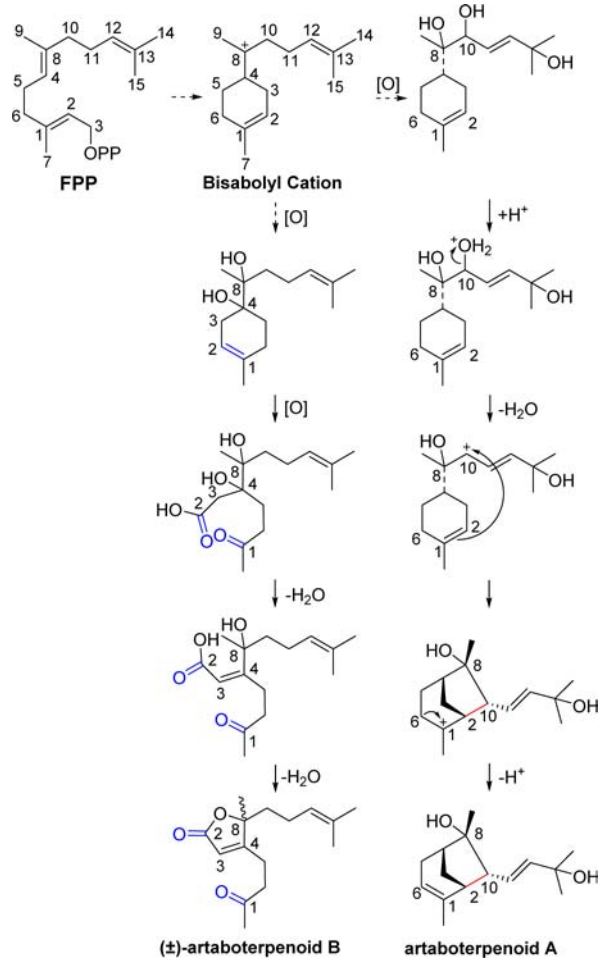


Figure 6. Helix rules for **2a** and **2b**.

Compounds **1**, **2a**, and **2b** were evaluated for their cytotoxicity against five human cancer cell lines (HCT-116, HepG2, BGC-823, NCI-H1650, and A2780) using the MTT method reported previously.[12] Taxol was used as a positive control (IC_{50} values of 31.6, 17.8, 1.17, 69.5, and 33.0 nM, respectively). Compound **2a** exhibited cytotoxicity against all five human tumor cell lines tested, with IC_{50} values of 1.38, 3.30, 6.51, 8.19, and 2.14 μM, respectively, and the others were inactive (IC_{50} > 10 μM).

Biosynthetically, artaboterpenoids A and B could be generated from bisabolene, as shown in Scheme 1. The route of

Scheme 1. Plausible Biosynthetic Pathways of Artaboterpenoids A and B



artaboterpenoid A is primarily involved in oxidation and cyclization, while the pathway of (±)-artaboterpenoid B is principally based on a ring-opening reaction by double bond oxidation and lactonization by dehydration.

In summary, we discovered two sesquiterpenoids from the roots of *A. hexapetalus*, including a novel endocyclic sesquiterpene with an unusual skeleton by the new C-2−C-10 linkage (**1**) and an unprecedented 1,2-secocyclic sesquiterpene lactone (**2**). By chiral chromatography, we achieved the chiral resolutions of a pair of enantiomers, (±)-artaboterpenoid B (**2a** and **2b**). The potent cytotoxicity and structural simplicity of **2a** make it a promising lead for the development of antitumor agents. We provide these examples of sesquiterpenes at the biosynthetical crossroad for bisabolane-type sesquiterpenoids.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01519.

Detailed experimental procedures, 1D and 2D NMR, MS, IR, UV, and ECD spectra (PDF)

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Notes

The authors declare no competing financial interest.

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