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Sesquiterpenes from *Artemisia argyi*: Absolute Configurations and Biological Activities

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Dedicated to Professor Xinsheng Yao on the occasion of his 80th birthday

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Nine guaiane-type sesquiterpenes and one eudesmane-type one – namely argyinolides A–J (**1**–**10**) – as well as nine known analogues were isolated from the leaves of *Artemisia argyi* Levl. et Vant. Their structures were determined by interpretation of spectroscopic data (MS, 1D and 2D NMR). A combination of X-ray crystal diffraction, specific optical rotations, CD spectroscopy, ECD calculation, and Mosher ester methods was employed to resolve the absolute configurations of the isolated compounds. Biological investigations into their cytotoxicities and anti-inflammatory effects showed that **1**, **13**, **16**, and **18** were remarkably cytotoxic against Bel-742 and/or A549 cells, with IC_{50} values of 3.3–6.0 μM, whereas **7**, **11**–**13**, **16**, and **18** exhibited sound inhibitory activity on LPS-stimulated NO production in BV-2 microglial cells, with IC_{50} values ranging from 3.2 to 8.6 μM. In addition, a brief discussion on the applicability of Geissman's rule for the sesquiterpene lactones, the probable reason for the presence of chlorine-containing sesquiterpenes, and preliminary structure–activity relationships (SARs) are included.

Introduction

Sesquiterpenes, a group of naturally occurring 15-carbon isoprenoid compounds, are mainly found in higher plants and are characterized by enormous diversity in structure, stereochemistry, biological function, and application.[1] Over the last several decades, phytochemical investigations of the Asteraceae (Compositae) family for chemically intriguing and medicinally significant sesquiterpenes have been an attractive topic for natural product and synthetic chemistry studies.[2,3]

Scrutiny of the literature , however, reveals that there are still some challenges facing the structural elucidation of sesquiterpenes, particularly for determination of stereochemistry. Many studies have confined themselves to the level of relative configuration determination, and assignments of the absolute configurations of sesquiterpene lactones have often been based on indirect evidence,[4] such as the application of the empirical Geissman rule to CD spectra.[5–7] Nevertheless, as we know, the empirical rules do not always work well, and it is better to confirm absolute configurations further by more comprehensive methods such as X-ray crystallography, chemical synthesis, NMR spectroscopy/chiral derivatization (Mosher esters), ECD calculation, or other chiroptical approaches.

We have recently reported a series of sesquiterpenes and dimeric guaianolides from *Artemisia* species, together with their anti-inflammatory effects and cytotoxicities.[8–11] Their structural diversity and significant bioactivities prompted us to research deeper into the active components of *A. argyi*, a traditional Chinese herb used for moxibustion and for curing eczema, diarrhea, hemostasis, and menstruation-related symptoms. As a result, ten new sesquiterpenes (**1**–**10**, Figure 1), along with nine known derivatives **11**–**19**, were isolated and identified by interpretation of spectroscopic data. The absolute configurations were resolved with the aid of a combination of single-crystal X-ray diffraction, specific optical rotations, CD spectra, ECD calculation, and the Mosher ester method. Moreover, the cytotoxic and anti-inflammatory activities of the isolated compounds were evaluated and are reported here.

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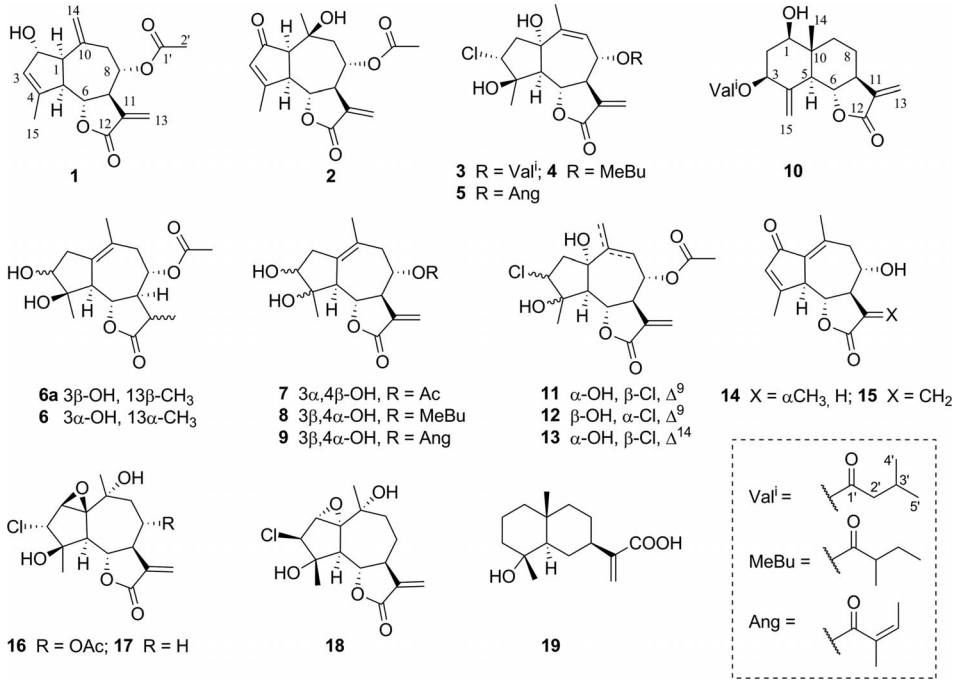

Figure 1. Structures of compounds **1**–**19** isolated from *Artemisia argyi*.

Results and Discussion

Compounds **1**–**10** showed bands at 3489 to 3404, 1781 to 1691, and 1663 to 1623 cm^{-1} in their FT-IR spectra, suggesting the presence of hydroxy and carbonyl groups and double bonds. Compound **1** was shown to have a molecular formula of $C_{17}H_{20}O_5$, as indicted by the observed ion at *m*/*z* 327.1208 [M + Na]$^+$ in its HRMS. The ^{1}H NMR spectrum (Table 1) exhibited a pair of signals typical of an exocyclic methylene group conjugated to a γ-lactone ring at δ_H = 6.30 and 5.73 ppm. Two methyl resonances were evident at δ_H = 2.16 and 1.96 ppm. The ^{13}C NMR spectrum (Table 2) indicated the presence of an acetoxy moiety at δ_C = 170.8 and 21.6 ppm (C-1′, 2′), a lactone carbonyl group at δ_C = 169.9 ppm (C-12), six olefinic carbons, and three oxygen-bearing carbons. The above information, coupled with biogenetic considerations, implied that **1** was a sesquiterpene lactone containing an acetoxy group.

The ^{1}H-^{1}H COSY and multiplicity-edited HSQC spectra indicated the C-1, C-2, C-5, C-6, C-7, C-8, C-9, C-11, and C-13 fragment sequences (Figure 2). The HMBC cross-peaks between 13-H$_2$ and C-7/C-11/C-12 further verified the existence of the α-methylene-γ-lactone moiety. The observable HMBC correlations from 15-Me to C-3/C-4/C-5 and from 14-H$_2$ to C-1/C-9/C-10 established the molecular skeleton of **1** as that of a guaiane-type sesquiterpene. The positioning of the C-2 hydroxy group was also confirmed from the HMBC correlations between 3-H and C-1/C-2. The esterification of 8-OH with acetic acid was secured by the key HMBC correlation from a deshielded proton signal at δ_H = 5.26 ppm (8-H) to δ_C = 170.8 ppm (C-1′). The molecular framework of **1** was thus established.

On the basis of an accepted principle that 7-H always has the α-orientation in natural guaianolides, 5-H, 6-H, and 8-H of **1** were deduced to be α-, β-, and β-oriented, respectively, from their large coupling constants ($J_{5,6}$ = 9.5, $J_{6,7}$ = 10.5, and $J_{7,8}$ = 9.5 Hz) and the allylic coupling constants ($^4J_{7,13a}$ = 3.5 and $^4J_{7,13b}$ = 3.0 Hz) based on the Samek lactone rule.[12] These postulated orientations were confirmed by the 1D-NOESY correlations of 5-H/7-H and 6-H/8-H. From the 3D molecular model, 9a-H (dd at δ_H = 2.83 ppm with $J_{8,9a}$ = 5.0 Hz) was assigned the β-orientation, adopting a dihedral angle with 8-H of about 40°, whereas 9b-H (dd at δ_H = 2.56 ppm with $J_{8,9b}$ = 3.5 Hz) was α-orientated, leading to a dihedral angle of about 70°. The NOESY cross-peaks of 1-H/5-H and 1-H/14a-H suggested the *cis* relationship of 1-H and 5-H. The NOESY correlations of 2-H/6-H and 9b-H indicated the α-orientation for the 2-hydroxy group. Furthermore, the preferred conformation by geometry optimization (Figure 2), was easily able to explain the NOEs observed above and thus to support the postulated configuration of **1**.

The absolute configuration at C-7 was assigned as *R* from the negative Cotton effects (CEs) at 227 nm ($\Delta\varepsilon$ = –3.2) and 265 nm ($\Delta\varepsilon$ = –0.7) in the CD curves of **1**, according to the Geissman rule.[5–7] In parallel with the CD spectrum, the "in-NMR-tube" Mosher reaction[13] was applied to **1** for the assignment of the configuration at C-2. Analysis of ^{1}H NMR chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*S*)- and (*R*)-MTPA ester derivatives established an *S* configuration for C-2 (Figure 3). Compound **1** was therefore characterized as (+)-(1*R*,2*S*,5*R*,6*R*,7*R*,8*S*)-8-acetoxy-2-hydroxyguai-3,10(14),11(13)-trien-6,12-olide and assigned the trivial name argyinolide A.

Table 1. ^{1}H NMR spectroscopic data (500 MHz) for compounds **1**–**10**:[a] δ in ppm (J in Hz).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**[c]
1	3.32, dd (9.5, 6.0)	2.83, d (6.5)								3.64, dd (12.0, 4.5)
2	5.14, m		α 2.01, d (15.0) β 2.99, dd (15.0, 5.0)	α 2.01, d (15.0) β 2.99, dd (15.0, 5.0)	α 2.02, d (15.0) β 3.01, dd (15.0, 5.0)	α 2.37, d (16.5) β 2.83, br. d (16.5)	α 2.38, d (16.5) β 2.85, br. d (16.5)	α 2.66, dd (16.5, 8.0) β 2.17[b]	α 2.66, dd (16.5, 8.0) β 2.17, dd (16.5, 10.5)	α 2.25[b] β 1.61, q (12.0)
3	5.89, q (1.4)	6.12, q (1.5)	4.15, d (5.0)	4.15, d (5.0)	4.16, d (5.0)	3.86, d (4.5)	3.89, d (4.5)	4.08, dd (10.5, 8.0)	4.07, dd (10.5, 8.0)	5.24[b]
5	2.93, t (9.5)	3.31, dd (10.5, 6.5)	2.94, d (9.5)	2.94, d (9.5)	2.96, d (9.5)	2.72, d (10.5)	2.82, d (10.5)	2.72, d (10.5)	2.73, d (10.5)	2.14[b]
6	4.20, dd (10.5, 9.5)	5.17, dd (10.5, 9.0)	4.43, t (9.5)	4.43, t (9.5)	4.43, t (9.5)	4.02, t (10.5)	4.02, t (10.5)	3.99, t (10.5)	4.00, t (10.5)	4.09, t (10.5)
7	3.30[b]	3.30[b]	3.95, dddd (10.5, 9.5, 3.3, 3.0)	3.97, br. t (9.5)	4.03, br. t (9.5)	2.25, q (10.5)	3.16, tt (10.5, 3.0)	3.08, br. t (10.5)	3.12, tt (10.5, 3.0)	2.57, dd (12.0, 10.5)
8	5.26, ddd (9.5, 5.0, 3.5)	5.70, td (11.0, 4.5)	5.17, dd (10.5, 4.5)	5.15, dd (10.5, 4.5)	5.24, dd (10.5, 4.5)	4.80, td (10.5, 2.5)	4.87, td (10.5, 2.5)	4.94, dd (10.5, 4.0)	5.04, td (10.5, 4.0)	α 2.11[b] β 1.62, q (12.0)
9	β 2.83, dd (14.5, 5.0) α 2.56, dd (14.5, 3.5)	β 2.48, dd (13.5, 4.5) α 1.85, dd (13.5, 11.0)	5.60, d (4.5)	5.57, d (4.5)	5.65, d (4.5)	α 2.48, dd (14.0, 10.5) β 2.22, dd (14.0, 2.5)	α 2.48, dd (14.0, 10.5) β 2.32, dd (14.0, 2.5)	2.31–2.39 (m)	α 2.40, dd (15.0, 10.5) β 2.47, dd (15.0, 4.0)	α 1.37, td (13.5, 3.0) β 2.11[b]
11						2.50, m				
13	6.30, d (3.5) 5.73, d (3.0)	6.38, d (3.4) 5.86, d (3.0)	6.32, d (3.3) 5.78, d (3.0)	6.32, d (3.3) 5.80, d (3.0)	6.32, d (3.4) 5.77, d (3.0)	1.34, d (7.0)	6.24, d (3.2) 5.69, d (3.0)	6.27, d (3.3) 5.82, d (3.0)	6.25, d (3.2) 5.73, d (2.8)	6.12, d (3.2) 5.45, d (3.0)
14	5.32, d (2.0) 5.11, d (2.0)	1.87, s	1.97, s	1.96, s	1.98, s	1.78, t (2.0)	1.80, d (2.0)	1.71, br. s	1.72, s	0.86, s
15	1.96, br. s	2.21, br. s	1.89, s	1.89, s	1.90, s	1.58, s	1.61, s	1.28, s	1.28, s	5.23, s 5.03, s
2′	2.16, s	2.17, s	2.27, m	2.44, m		2.10, s	2.15, s	2.42, m		2.28, m
3′			2.15, m	1.74, dq (14.0, 7.5) 1.52, dq (14.0, 7.5)	6.21, q (7.5)			1.76, dq (14.0, 7.0) 1.50, dq (14.0, 7.0)	6.20, br. q (7.2)	2.15, m
4′			0.99, d (6.5)	0.94, t (7.5)	2.03, br. d (7.5)			0.95, t (7.0)	2.02, br. d (7.2)	0.99, d (6.5)
5′			0.99, d (6.5)	1.19, d (7.0)	1.94, br. s			1.21, d (7.0)	1.92, br. s	0.99, d (6.5)

[a] Diastereotopic methylene protons are referred to as Ha for the lower-field proton and Hb for the higher-field proton. Compounds **1** and **2** were examined in [D$_5$]pyridine, others in $CDCl_3$. [b] Overlapped signals are reported without designation of the multiplicity. [c] Measured at 600 MHz.

A molecular formula of $C_{17}H_{20}O_6$ was assigned to argyinolide B (**2**) on the basis of the $[M + Na]^+$ ion peak in the HRMS (ESI). ^{1}H and ^{13}C NMR spectroscopic data for **2** (Table 1 and Table 2) showed some similarity to those of compound **1**, with two characteristic α-methylene-γ-lactone doublets and an acetoxy group. The esterification of 8-OH could be established from the corresponding highly deshielded resonance at δ_H = 5.70 ppm,[16] even though the HMBC cross-peak between 8-H and C-1′ was invisible. The combined analysis of ^{1}H-^{1}H COSY and HMBC correlations implied a planar structure of **2**, as shown in the Supporting Information (Figure S19). The NOE effect of 6-H/8-H showed them to be *cis*-oriented. Additionally, the vicinal coupling constants between 8-H and 9b-H/9a-H (J = 4.5 and 11.0 Hz, respectively), in combination with the observed NOEs (Figure S19 in the Supporting Information), are only possible if the seven-membered ring adopts a chair geometry. The definite absence of NOE effect between 14-Me and 6-H or 8-H allowed 14-Me to be assigned as α-oriented with a more favored equatorial position, which was also corroborated by its chemical shift at δ_C = 31.3 ppm.[14] Similarly, the deshielding effects on 6-H and 8-H (δ_H = 5.17 and 5.70 ppm, respectively) supported a β-oriented axial 10-OH group.

The negative CE at 263 nm ($\Delta\varepsilon$ = –0.5) for **2** implied the same configuration as in **1** for 7α-H (7*R*). A strong positive CE at 226 nm ($\Delta\varepsilon$ = + 25.1) associated with the π–π* transition of an α,β-unsaturated cyclopentanone means the presence of a diene chromophore twisted in the sense of a right-handed helix (*P* helicity). The helicity rule[15] was thus ap-

Table 2. ^{13}C NMR spectroscopic data (125 MHz) for compounds **1**–**10**:[a] δ in ppm.

	1	2	3	4	5	6	7	8	9	10[b]
1	61.5	59.5	81.7	81.6	81.7	137.1	137.6	128.2	128.2	75.9
2	78.7	206.7	46.1	46.1	46.1	39.0	39.0	36.5	36.5	37.1
3	133.3	133.8	80.2	80.2	80.2	78.8	78.8	75.9	75.9	69.9
4	143.6	177.9	79.8	79.9	79.9	83.2	83.2	81.1	81.1	140.2
5	57.3	53.2	64.3	64.3	64.3	52.9	53.0	55.3	55.3	50.4
6	81.8	79.4	76.5	76.5	76.5	79.0	79.5	79.6	79.6	78.7
7	47.9	50.3	42.9	43.0	43.0	58.1	53.8	52.2	52.4	49.6
8	74.9	72.2	73.5	73.6	73.4	71.7	70.6	70.3	70.0	21.4
9	38.5	50.2	123.7	123.5	123.2	41.8	41.7	41.8	42.1	35.6
10	142.6	72.0	140.8	140.9	140.7	126.4	126.2	126.7	127.0	42.8
11	139.0	138.0	136.9	137.0	137.0	40.7	136.5	135.8	135.9	138.8
12	169.9	169.8	169.0	169.0	169.0	177.4	168.9	168.9	168.9	170.3
13	122.2	123.1	123.3	123.5	123.9	15.1	121.9	123.3	123.3	117.4
14	119.1	31.3	25.2	25.2	25.2	23.5	24.0	23.7	23.7	11.6
15	18.1	20.3	25.1	25.1	25.1	24.0	23.5	17.0	15.8	108.2
1′	170.8	170.3	172.7	176.3	167.1	169.9	169.8	175.5	166.5	172.7
2′	21.6	21.4	43.5	41.4	126.9	21.2	21.1	41.4	126.9	43.5
3′			25.6	26.5	140.7			26.3	140.1	25.8
4′			22.4	11.6	16.0			11.8	15.9	22.4
5′			22.4	16.5	20.5			15.8	20.5	22.3

[a] Compounds **1** and **2** were examined in [D_5]pyridine, the others in $CDCl_3$. [b] Measured at 150 MHz.

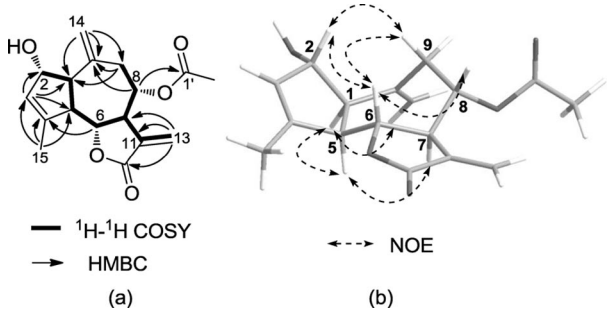

Figure 2. (a) ^{1}H-^{1}H COSY and key HMBC correlations of **1**. (b) Selected NOEs of geometry-optimized conformation for **1**.

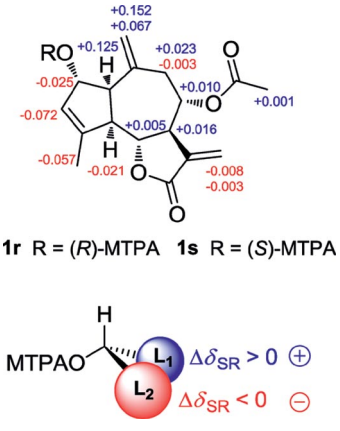

Figure 3. $\Delta\delta$ ($\delta_S - \delta_R$) values obtained from the ^{1}H NMR spectra of the MTPA esters of **1r** and **1s**.

plied to determine the *R* configurations for the C-1 and C-5 stereogenic centers, in accordance with the Geissman rule prediction (Figure S23 in the Supporting Information). Moreover, the quantum chemical ECD calculation method by time-dependent density functional theory (TDDFT), a recent approach increasingly applied for the determination of absolute configurations of natural products,[16] was used to give further verification of the absolute configuration of **2**. The overall predicted ECD of **2** was compared with the experimentally measured one, and the results revealed a good agreement between them (Figure 4). Argyinolide B (**2**) was thus determined to be (+)-(1*R*,5*R*,6*R*,7*R*,8*S*,10*S*)-8-acetoxy-10-hydroxy-2-oxoguai-3,11(13)-dien-6,12-olide.

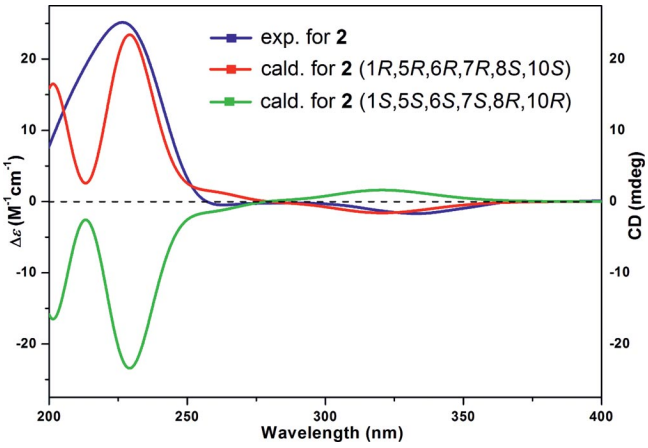

Figure 4. Calculated and experimentally measured CD spectra of **2**.

Argyinolides C–E (**3**–**5**) were each deduced to contain one chlorine atom, from the relative abundance ratios of 3:1 for $[M + Na]^+$ and $[M + Na + 2]^+$ in their MS (ESI) spectra. From their HRMS (ESI) spectra, compounds **3** and **4** were determined to share the same molecular formula of $C_{20}H_{27}O_6Cl$, whereas compound **5** was assigned the formula $C_{20}H_{25}O_6Cl$, two mass units lighter than **3** or **4**. Inspection of 1D-NMR spectroscopic data for **3**–**5** (Table 1 and Table 2) revealed that the gross framework of the three compounds closely resembled that of **11**,[17] with the only differences being in the C-8 side chain: an acetoxy moiety in compound **11** was replaced by an isovaleryloxy group in **3**, a 2′-methylbutyryloxy system in **4**, and an angeloyloxy group in **5**. The relative configurations of **3**–**5** were readily established by the vicinal coupling constants, which were identical to those observed in **11** ($J_{2b,3}$ = 5.0, $J_{5,6}$ = 9.5, $J_{6,7}$ = 9.5, $J_{7,8}$ = 10.5, and $J_{8,9}$ = 4.5 Hz), which suggested 3β-H, 5α-H, 6β-H, 7α-H, and 8β-H orientations, respectively. From comparisons with literature data,[17] the signal at δ_H = 2.01 ppm (d, *J* = 15.0 Hz) should be preferentially ascribed to 2α-H, and δ_H = 2.99 ppm (dd, *J* = 15.0, 5.0 Hz) to 2β-H, in good agreement with the favorable conformation of **3** (Figure S103 in the Supporting Information).

The consistency of the CD data for **3**–**5** at around 260 nm with those for **1**, due to the contribution of the α-methylene lactone, suggested the same absolute configuration at the C-7 stereocenter. In addition, the specific rotations for **3**–**5** were found to be $[\alpha]_D^{21}$ = +82.2, $[\alpha]_D^{21}$ = +88.0, and $[\alpha]_D^{21}$ = +91.8, respectively: the same direction and similar magnitudes as in the case of **11** ($[\alpha]_D^{21}$ = +52.5). These data implied identical stereocenters in their core skeleton. Unfortunately, a limited sample amount of **4** prevented us

from elucidating the configuration of the 2-methylbutyryloxy moiety by chemical means. Compounds **3**–**5** were hence established as (+)-(1*S*,3*R*,4*R*,5*R*,6*S*,7*R*,8*S*)-3-chloro-1,4-dihydroxy-8-isovaleryloxyguai-9,11(13)-dien-6,12-olide (argyinolide C, **3**), (+)-(1*S*,3*R*,4*R*,5*R*,6*S*,7*R*,8*S*)-3-chloro-1,4-dihydroxy-8-(2′-ξ-methylbutyryloxy)guai-9,11(13)-dien-6,12-olide (argyinolide D, **4**), and (+)-(1*S*,3*R*,4*R*,5*R*,6*S*,7*R*,8*S*)-8-angeloyloxy-1,4-dihydroxy-3-chloroguai-9,11(13)-dien-6,12-olide (argyinolide E, **5**).

Argyinolide F (**6**) had a molecular formula of $C_{17}H_{24}O_6$, as elucidated from its HRMS (ESI) spectrum. Inspection of 1D-NMR spectroscopic data and the specific optical rotation of **6** suggested that our sample corresponded exactly to indicumolide B (**6a**), a previously reported guaianolide from *Chrysanthemum indicum*.[18] The authors of ref.[18] had proposed incorrect configurations at the C-3 and C-11 positions, however, and the structure should in both case be revised to 8α-acetoxy-3α,4β-dihydroxyguai-11β-H-1(10)-en-6α,12-olide.

1D-TOCSY analysis was used to establish the assignments of 11-H and 7-H, which had been oppositely assigned in the original structural elucidation of **6a**. In a 1D-NOESY experiment (Figure 5) the cross-peaks of 6-H/11-H and 7-H/13-Me, as well as 5H/15-Me, were clearly detected; meanwhile, irradiation of 15-Me faintly enhanced the signal of 3-H, indispensable for defining 11-H as having the β-orientation and 15-Me the α-orientation. Furthermore, the stereochemistry of C-3 could be tentatively established by the expected Karplus-type relationship for vicinal coupling constants in combination with molecular modeling simulations (Figure 5). A doublet at δ_H = 3.86 ppm (J = 4.5 Hz, 3-H) suggested that 3-H should be equatorial (β-oriented) and flanked by two vicinal hydrogen atoms (2-H_2): 2b-H at δ_H = 2.37 ppm (d, J = 16.5 Hz) did not show a detectable coupling with 3-H, whereas a broadened 2a-H signal at δ_H = 2.83 ppm (br. d, J = 16.5 Hz) tended to, so the former could be assigned as having an α-orientation. Finally, confirmation of the proposed structure and evidence for the relative stereochemistry of **6** were confirmed by X-ray crystallography (Figure 6).

Nevertheless, the refinement of the Flack parameter for **6** [0.1(2)] led to an inconclusive result with regard to the absolute structure and the handedness of the chiral centers

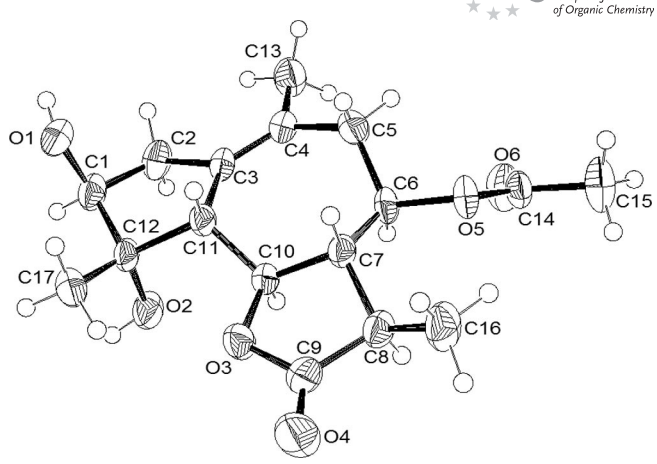

Figure 6. ORTEP plot for the molecular structure of **6** drawn with 50% probability displacement ellipsoids (Note: a different numbering system is used for the structure in the text).

due to a high standard deviation (0.2).[19,20] Moreover, the Bijvoet pair analysis of Hooft's y = 0.3(2) also could not enhance the precision and allow the safe assignment of the absolute configuration.[21]

In addition, preparation of the diastereomeric MTPA and MPA esters of **6** failed to confirm the configuration, due to identical signs for $\Delta\delta^{SR}$ at both sides of the asymmetric centers (Figure S54 and S59 in the Supporting Information). This anomalous behavior could be explained in terms of the steric hindrance of the axially oriented 3-OH group modifying the conformation of the ideal Mosher ester.[22,23] In this case, the empirical lactone octant and sector rules were applied to determine the absolute configuration of the lactone group (Figure 7).[24] On the grounds of the preferred conformation, a positive CE centered at 230 nm ($\Delta\varepsilon$ = +0.2) was found for the n–π* transition of the lactone, indicting the *R* configuration at C-7. Additionally, the absolute configuration of **6** was further checked by comparing experimentally measured and calculated ECD spectra in the same way as for compound **2**. This comparison revealed proportional agreement between them, whereas the enantiomer of **6** showed the opposite result (Figure S63 in the Supporting Information). Argyinolide F (**6**) was therefore reassigned as (–)-(2*R*,3*R*,5*S*,6*S*,7*R*,8*S*,11*S*)-8-acetoxy-3,4-dihydroxyguai-11-H-1(10)-en-6,12-olide.

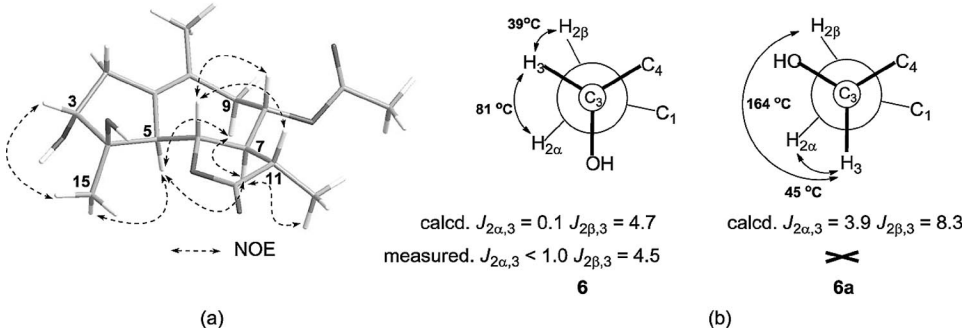

Figure 5. (a) Selected NOEs of geometry-optimized conformation of **6**. (b) Karplus-type relationship analysis for $^3J_{H,H}$ couplings of the C1–C4 moiety.

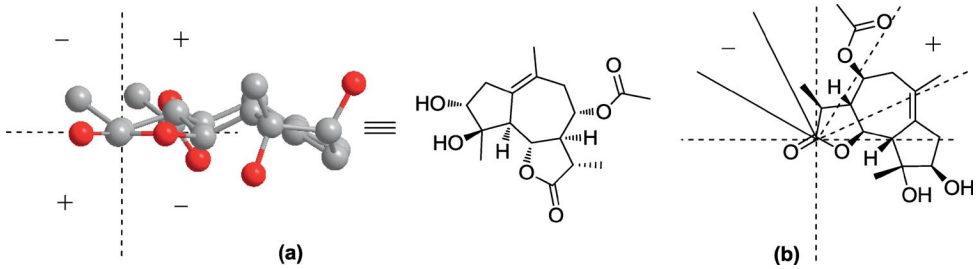

Figure 7. Application of Klyne's octant rule (a), viewed in the plane of the lactone along the bisectrix of the O–C–O angle and sector lactone rule (b), viewed from above for prediction of the sign of the n–π* transition in the CD spectrum (230 nm) of **6**.

The HRMS (ESI) spectrum of argyinolide G (**7**) gave a sodiated molecular ion peak at *m/z* 345.1306, consistent with a molecular formula of $C_{17}H_{22}O_6$, two hydrogen atoms fewer than in **6**. The NMR spectra of **6** and **7** (Tables 1 and 2) were nearly superimposable, with the main differences evident at C-11, C-13, and their immediate vicinity. The existence of signals at δ_H = 6.24 and 5.69 ppm suggested that **7** was an 11,13-exomethylene derivative, which was confirmed by the 2D-NMR spectroscopic data. On biogenetic grounds the absolute configuration of the lactone moiety of **7** was believed to be identical to that of **6**. To verify this assumption, quantum chemical ECD calculations were undertaken. As shown in Figure S75 in the Supporting Information, the overall pattern of the experimentally measured CD of **7** only matched the ECD curve calculated for (3*R*,4*R*,5*S*,6*S*,7*R*,8*S*)-**7**. Argyinolide G (**7**) was thus established as (+)-(3*R*,4*R*,5*S*,6*S*,7*R*,8*S*)-8-acetoxy-3,4-dihydroxyguai-1(10),11(13)-dien-6,12-olide.

The molecular formula of argyinolide H (**8**) was determined to be $C_{20}H_{28}O_6$ by HRMS (ESI, +). Analysis of the compound's NMR spectroscopic data (Table 1 and Table 2) revealed a similar structure to **7**. Compound **8** incorporated a 2′-methylbutyryloxy fragment instead of the acetoxy unit in **7**. Meanwhile, there was an obvious discrepancy between these two compounds in their C-2–C-4 moieties. A doublet of doublets at δ_H = 4.08 ppm (*J* = 10.5, 8.0 Hz, 3-H) indicated that the proton 3-H in **8** was axially oriented, contrary to the equatorial orientation in **7**, and this was further confirmed by the NOESY cross-peak between 3-H and 5-H (Figure S103 in the Supporting Information). A positive CE at 261 nm ($\Delta\varepsilon$ = +0.4) in the CD spectrum indicated a 7*S* configuration according to the Geissman rule, which did not agree with biogenetic considerations. In this case, a practical and reliable method based on the CD data for the $Mo_2(OAc)_4$ complex formed in situ was employed to determine the absolute configuration of the 3,4-diol moiety in **8**.[25,26] According to Snatzke's theory, the positive CEs at 328 nm ($\Delta\varepsilon$ = +0.14, band IV) and 406 nm ($\Delta\varepsilon$ = +0.09, band II) in the CD spectrum (Figure 8), which correspond to a positive dihedral angle of the O–C–C–O moiety, indicated 3*S* and 4*S* configurations for **8** by the empirical helicity rule. The configuration of the 2-methylbutyryloxy unit remained unassigned, due to limitations of sample quantity. Argyinolide H (**8**) was hence characterized as (+)-(3*S*,4*S*,5*S*,6*S*,7*R*,8*S*)-3,4-dihydroxy-8-(2′-ξ-methylbutyryloxy)guai-1(10),11(13)-dien-6,12-olide.

Argyinolide I (**9**) had a molecular formula of $C_{20}H_{26}O_6$, established on the basis of HRMS (ESI, +). The ^1H and ^{13}C NMR spectra of **8** and **9** displayed near-identical similarity for all signals, with the exception of the resonances for the C-8 ester substituents. The methylbutyryloxy group in **8** was replaced by the angeloyloxy group in **9**, and this was further confirmed by 2D-NMR spectra. Comparison of the $[\alpha]_D^{21}$ value of **9** (+41.6) with that of **8** ($[\alpha]_D^{21}$ = +23.0) revealed that they shared the same stereogenic centers in the structural skeletons. In the CD spectrum of **9**, a strong positive CE ($\Delta\varepsilon$ = +13.5) appeared at 222 nm for the π–π* transition of the unsaturated ester moiety in position C-8, similar to that in the case of **5**. In an extension of the Geissman rule,[7] the absolute configuration of **9** could also be correlated through the C-8 chiral center, which was deduced to be 8*S* from the positive CE in the 220–240 nm range. Argyinolide I (**9**) was therefore established as (+)-(3*S*,4*S*,5*S*,6*S*,7*R*,8*S*)-8-angeloyloxy-3,4-dihydroxyguai-1(10),11(13)-dien-6,12-olide.

Argyinolide J (**10**) had a molecular formula of $C_{20}H_{28}O_5$, as determined by HRMS (ESI, –). Comparison of its ^1H NMR spectroscopic data (Table 1) with literature reports indicated that it closely resembled 3β-acetylridentin B, featuring an eudesmane-type sesquiterpene,[27] with a difference in the C-3 substituent. The acetoxy moiety in the side chain of 3β-acetylridentin B was replaced in **10** by an isovaleroyloxy group, as was supported by 2D-NMR correlations. Model building of **10** by geometry optimization provided a valuable insight into its stereochemical features, and the NOESY correlations matched the constructed model well (Figure S103 in the Supporting Information). A negative CE at 250 nm ($\Delta\varepsilon$ = –2.1) implied an α-configuration for 7-H (7*S* configuration). Argyinolide J (**10**) was therefore deduced to be (+)-(1*R*,3*S*,5*S*,6*S*,7*S*,10*R*)-1-hydroxy-3-isovaleroyloxyeudesman-4 (15),11(13)-dien-6,12-olide.

By comparison of their spectroscopic data with previously reported observations, the known compounds were identified as 8α-acetoxy-3β-chloro-1α,4α-dihydroxyguai-9,11(13)-dien-6α,12-olide (**11**),[17] 8α-acetoxy-3α-chloro-1α,4β-dihydroxyguai-9,11(13)-dien-6α,12-olide (**12**),[17] 8α-acetoxy-3β-chloro-1α,4α-dihydroxyguai-10(14),11(13)-dien-6α,12-olide (**13**),[17] deacetylmatricatin (**14**),[28] 14-deoxy-actucin (**15**),[29] 8α-acetoxy-3α-chloro-1β,2β-epoxy-4β,10α-dihydroxy-5α,7αH-guai-11(13)-en-12,6α-olide (**16**),[30] 3α-chloro-1β,2β-epoxy-4β,10α-dihydroxy-5α,7αH-guai-11(13)-

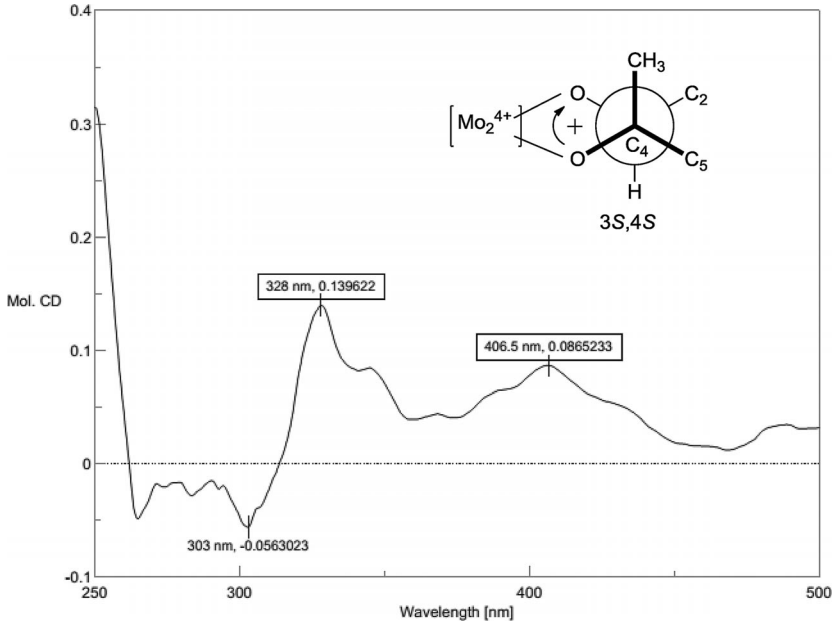

Figure 8. CD spectrum of compound **8** in a DMSO solution of $Mo_2(OAc)_4$ with the inherent contribution subtracted and the O–C–C–O dihedral in the compound **8**–$[Mo_2]^{4+}$ complex.

en-12,6α-olide (**17**),[31] 3β-chloro-1α,2α-epoxy-4α,10α-dihydroxy-5α,7αH-guai-11(13)-en-12,6α-olide (**18**),[30] and ilicic acid (**19**).[32] The absolute configurations were determined by comparison of the CD spectra (Figures S104–S111 in the Supporting Information) with those of **1**–**10**.

Determination of absolute configuration is one of the most challenging tasks in the sesquiterpene chemistry. It is a pity, however, that many studies have still left this problem unsolved or tacitly assumed absolute configurations on the basis of empirical rules.[33,34] For a long time, ECD spectroscopy, an experimentally very sensitive and non-destructive technique, has been demonstrated to be a powerful tool for the absolute configuration assignment of natural products. We have therefore made a brief summary of the CD spectra characteristics for the isolated sesquiterpenes. All the eudesmanolide and guainolides bearing α-methylene-γ-lactone chromophores displayed negative CEs at around 220 nm and/or 260 nm in their CD spectra, the latter diagnostic especially for the determination of configuration at C-7, which is often assumed to be the 7α-H orientation on the basis of the Geissman rule. As an extension of this rule, a second chiral indicator at around 220 nm for the lactones with unsaturated ester moieties at C-8, such as compounds **5** and **9**, also holds true. The empirical rule seems to be widely applicable, but in our study several exceptions were observed and should be mentioned. Compounds **7** and **8**, for example, showed positive CE signs at around 260 nm, whereas further ECD calculation and $Mo_2(OAC)_4$-induced CD allowed the definite assignment of the same 7*R* configuration as in the other isolated sesquiterpenes. For compound **9**, a weak, broad, positive CE without a maximum in the range from 250 to 280 nm was of little significance but observable. This might result from the overlap of n–π* transitions of the unsaturated ester at C-8 and lactone chromophores, which could not be an indicator of the C-7 configuration. The known compound **15** showed a split CE (positive at 249 nm and negative at 272 nm with UV maxima absorption at around 254 nm), due to the interaction between the conjugated enone and lactone chromophores. The absolute configuration of **15** was therefore determined by applying the exciton chirality CD method,[35] as shown. Moreover, it is important to note that the empirical rule only worked for the lactones with α-methylene groups, whereas the CD curve of **6**, a saturated lactone, showed only one weak but observable CE at 230 nm, which could conceivably be an indicator for the Klyne lactone octant and sector rule. In future studies of sesquiterpene lactones, the validity of the Geissman rule correlation thus deserves more attention. In order to allow a safe assignment, the configuration should be checked by more comprehensive methods, such as X-ray crystallography, formation of Mosher esters, ECD calculation, or other chiroptical approaches.

Besides the stereochemistry of the sesquiterpenes, attention should also be paid to several chlorine-containing guaianolides (see **3**–**5**, **11**–**13**, **16**–**18**); this has also been encountered during the chemical investigation of some other *Artemisia* species.[17,34,36] There are difficulties in explaining chlorine sources for the biosynthesis of chlorinated compounds in higher plants, and so it cannot be ruled out that such chlorohydrins might be artifacts, generated during the isolation procedure, in which chlorinated solvents ($CHCl_3$) might serve as sources of Cl^- to attack corresponding sensitive epoxide derivatives,[37] which have been frequently reported to be isolated from this genus plants.[17,38]

Of the isolated sesquiterpenes, most share the α-methylene-γ-lactone motif, a well-known Michael acceptor that has often represented a wide spectrum of bioactivities including antitumor, cytotoxicity, antimicrobial, and anti-inflammation.[39,40] In addition, prompted by the significant cytotoxic and anti-inflammatory effects of previous isolates from the genus *Artemisia*,[8–10] all sesquiterpenes obtained here were screened for their cytotoxicity against five human tumor cell lines, including lung adenocarcinoma (A549), stomach cancer (BGC-823), colon cancer (HCT-8), hepatoma (Bel-7402), and ovarian cancer (A2780) cell lines, as well as the inhibitory activity against LPS-stimulated NO production in BV-2 microglial cells. Compounds **1** and **13** were selectively active against the Bel-7402 cell line with IC_{50} values of 4.9 and 4.6 μM, respectively. Compounds **13**, **17**, and **18** showed strong inhibitory effect against the A549 cell line with IC_{50} values of 4.8, 6.0, and 3.3 μM, respectively, whereas no significant positive results were observed for other isolates ($IC_{50} > 10$ μM). The NO inhibitory effects of the isolates are listed in Table 3. Of the compounds screened, **7**, **11**–**13**, **15**, and **18** exhibited marked potencies in inhibiting NO production, comparable to that of the positive control indomethacin, and had no influence on LPS-activated BV-2 cell viability. As expected, many isolates containing the α-methylene lactone system exhibited sound inhibitory activities against NO production, whereas the absence of such a group significantly reduced the activity, as is shown by comparison of **7** with **6**. Additionally, the chlorine-containing compounds (**11**–**13**, **17**, and **18**) showed stronger inhibitory effects, and compounds **3**–**5** displayed much less activity, probably due to the presence of a larger group in place of the acetyl moiety at C-8. Although larger ester groups can increase lipophilicity, these moieties, beyond a certain size limit, might give rise to steric hindrance to the exocyclic methylene group, preventing it from approaching its target.[41] The structure–activity relationships suggested that the chlorohydrin moiety and a "size optimum" of lipophilic ester group as side chain in these α-methylene lactones might contribute to the NO inhibitory activities.

Table 3. Inhibitory effects of bioactive compounds against LPS-induced NO production in BV-2 cells.[a]

	IC_{50} (μM)	Cell viability (%, 40 μM)[b]
6	17.9	99.7
7	5.3	99.5
11	3.2	88.6
12	6.9	83.5
13	4.2	97.0
16	22.2	100.0
17	8.6	99.8
18	6.4	100.0
19	20.9	96.7
Indomethacin	7.1	100

[a] Only those compounds with strong or moderate inhibitory effects ($IC_{50} < 40$ μM) and low cytotoxities (cell viability > 80%) are listed here. [b] Cell viability was expressed as a percentage (%) of that of the LPS-only treatment group.

Conclusions

In summary, ten new sesquiterpenes, of the guaiane type (**1**–**9**) and the eudesmane type (**10**), along with nine known analogues (**11**–**19**) were isolated from the ethanolic extract of *A. argyi*. Their complete structures, including their absolute configurations, were elucidated by NMR and MS interpretation, X-ray crystal diffraction, specific optical rotations, CD spectroscopy, ECD calculation, and Mosher ester analysis. The Geissman rule in CD spectra has proven to be a general indicator for elucidation of the absolute stereochemistry of α-methylene lactones, but the irregularities in the sign of CE should be noticed. Deduction by comprehensive spectroscopic or chemical methods is thus to be preferred. The cytotoxicities and anti-inflammatory activities of the isolated sesquiterpenes were evaluated in vitro, and the preliminary SARs are also briefly discussed. Further investigation of the mechanism(s) of action of these sesquiterpenes is warranted, and additional studies are now underway in our laboratory.

Experimental Section

General Experimental Procedures: Optical rotations were measured with a Rudolph Autopol III automatic polarimeter. UV spectra were recorded with a Shimadzu UV-2401 UV/Vis recording spectrophotometer. IR spectra were recorded with a Thermo Nicolet Nexus 470 FT-IR spectrometer. CD spectra were measured with a JASCO J-810 spectropolarimeter. NMR measurements were performed with Varian INOVA-500 and Bruker Avance-600 FT NMR spectrometers and use of solvent peaks as references. HRMS (ESI) spectra were measured with Waters Xevo G2 Q-TOF, Shimadzu LC-MS-ITTOF, and Bruker APEX IV FT-MS spectrometers. Mass spectra were obtained with an Agilent 1200 Series LC/MSD ion-trap mass spectrometer (ESI). Analytical HPLC was performed with an Agilent 1100 system and Agilent Zorbax XDB-C_{18} column (5 μm, 4.6 × 250 mm), and semipreparative HPLC was conducted with a Waters 600 instrument and Grace® Prevail column (5 μm, 10 × 250 mm). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), ODS (50 μm, Merck, Germany), and Sephadex LH-20 (Amersham Biosciences, Sweden). TLC was carried out with precoated silica gel GF254 glass plates. Spots were visualized under UV light or by spraying with 2% vanillin-sulfuric acid.

Plant Material: Dried leaves of *A. argyi* were purchased in Anguo County, Hebei Province, China, in January 2010. The plant material was authenticated by one of the authors (P. T.). A voucher specimen (No. 20100119) was deposited at the Herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine.

Extraction and Isolation: Dried leaves (50 kg) of *A. argyi* were extracted with aqueous ethanol (95%, 400 L × 2.5 h × 3). The concentrated extract (7.0 kg) was suspended in H_2O (30 L) and partitioned successively with petroleum ether (PE) and chloroform. The $CHCl_3$ extract (1500 g) was transferred to a silica gel column and eluted with a stepwise gradient of PE/EtOAc (1:0, 9:1, 3:1, 1:1, and 0:1 v/v) to produce 10 fractions (F1–F10), which were examined by TLC.

F9 (152 g) was chromatographed on a column of silica gel, with elution with a gradient of PE/EtOAc (9:1, 4:1, 7:3, 3:2, and 1:1 v/

v), to give fourteen subfractions (F9a–F9n). F9m (21 g) was chromatographed on a reversed-phase C_{18} silica gel column, with elution with MeOH/H_2O (3:2, 4:1, 9:1, and 1:0 v/v), to yield seven subfractions (F9m1–F9m7). F9m1 (4.1 g) was separated again on a reversed-phase C18 silica gel column with elution with a gradient of MeOH/H_2O (3:2, 4:1, 9:1, and 1:0 v/v) to give ten subfractions (F9m1a–F9m1j).

F9m1e (840 mg) was repeatedly purified by semipreparative HPLC (25% CH_3CN/H_2O, 2 mL min^{-1}) to yield compound **15** (7.5 mg, t_R = 30 min). F9m1f (780 mg) was purified by semipreparative HPLC (25% CH_3CN/H_2O, 2 mL min^{-1}) to yield compounds **1** (4.1 mg, t_R = 23 min), **13** (2.0 mg, t_R = 34 min), and **10** (5.1 mg, t_R = 37 min). F9m1d (800 mg) was purified by semipreparative HPLC (25% CH_3CN, 2 mL min^{-1}) to yield compounds **14** (1.7 mg, t_R = 19 min), **16** (3.8 mg, t_R = 25 min), and **2** (8.3 mg, t_R = 28 min).

F9l (23 g) was transferred to a reversed-phase C_{18} silica gel column and eluted with MeOH/H_2O (9:11, 3:2, 3:1, and 9:1 v/v) to produce four subfractions (F9l1–F9l4). F9l1 (7.2 g) was further fractioned on Sephadex LH-20 with elution with MeOH to give two subfractions (F9l1a and F9l1b); subfraction F9l1a was crystallized with methanol to afford compound **17** (201 mg).

F9k (31 g) was chromatographed on a reversed-phase C_{18} silica gel column, with elution with MeOH/H_2O (2:3, 3:2, and 4:1 v/v) to yield three subfractions (F9k1–F9k3). Compound **18** (490 mg) was crystallized with methanol from subfraction F9k1 (10 g) during evaporation at room temperature. F9k3 (7.3 g) was fractioned on Sephadex LH-20 by isocratic elution with MeOH to give four subfractions (F9k3a–F9k3d). F9k3b (1.7 g) was purified repeatedly by semipreparative HPLC (55% CH_3CN/H_2O, 3 mL min^{-1}) to afford compounds **19** (8 mg, t_R = 13 min), **3** (1.5 mg, t_R = 20 min), **4** (1.0 mg, t_R = 22 min), **5** (0.8 mg, t_R = 23 min), and **10** (3.6 mg, t_R = 24 min).

F10 (96 g) was chromatographed on a column of silica gel, with elution successively with a gradient of PE/EtOAc (4:1, 7:3, 3:2, 1:1, and 0:1 v/v), to give eight subfractions (F10a–F10 h). F10g (23 g) was transferred to a reversed-phase C_{18} silica gel column and eluted with MeOH/H_2O (2:3, 3:2, and 4:1 v/v) to yield three subfractions (F10g1–F10g3). During natural evaporation a white crystalline-like powder precipitated from the mother liquor of F10g1. The residue was further purified by semipreparative HPLC (20% CH_3CN/H_2O, 3 mL min^{-1}) to afford compounds **6** (10 mg, t_R = 37 min) and **7** (2 mg, t_R = 35 min). F10g2 (5.7 g) was purified repeatedly by semipreparative HPLC (CH_3CN/H_2O, 3 mL min^{-1}) to afford compounds **12** (3.6 mg, t_R = 39 min, 25% CH_3CN/H_2O), **8** (2.0 mg, t_R = 42 min, 40% CH_3CN/H_2O), and **9** (3.0 mg, t_R = 26 min, 45% CH_3CN/H_2O).

Argyinolide A (1): Off-white amorphous powder. $[a]_D^{21}$ = +118.0 (*c* = 0.26, MeOH). ECD (MeOH): 227 ($\Delta\varepsilon$ = –3.2), 265 ($\Delta\varepsilon$ = –0.7). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3441, 2920, 1772, 1720, 1635, 1256, 1142, 1033 cm^{-1}. HRMS (ESI, +): calcd. for $C_{17}H_{20}O_5Na$ $[M + Na]^+$ 327.1203; found 327.1208, calcd. for $C_{17}H_{24}NO_5$ $[M + NH_4]^+$ 322.16490; found 322.16436.

Preparation of (*R*)- and (*S*)-MTPA Esters of 1 by the "in-NMR-tube" Mosher Ester Procedure: Compound **1** (0.45 mg) was transferred to a clean NMR tube and dried completely in a vacuum drying oven. Deuterated pyridine (0.5 mL) and (*R*)-MTPA chloride (5 μL) were added to the NMR tube immediately under a N_2 gas stream, and the NMR tube was then shaken rigorously to mix the sample and MTPA chloride evenly. The NMR tube was permitted to stand at room temperature and monitored every 2 h by ^{1}H NMR spectroscopy. The acylation reaction was found to be complete after 8 h. ^{1}H NMR spectroscopic data for the (*S*)-MTPA ester derivative (**1s**) of **1** (500 MHz, [D_5]pyridine): δ = 6.297 (d, *J* = 3.5 Hz, 1 H, 13a-H), 6.256 (m, 1 H, 2-H), 5.752 (d, *J* = 3.0 Hz, 1 H, 13b-H), 5.724 (q, *J* = 1.4 Hz, 1 H, 3-H), 5.259 (ddd, *J* = 9.5, 5.0, 3.5 Hz, 1 H, 8-H), 5.162 (d, *J* = 2.0 Hz, 1 H, 14a-H), 5.074 (d, *J* = 2.0 Hz, 1 H, 14b-H), 4.234 (dd, *J* = 10.5, 9.5 Hz, 1 H, 6-H), 3.369 (d, *J* = 9.5, 6.0 Hz, 1 H, 1-H), 3.290 (m, 1 H, 7-H), 2.948 (t, *J* = 9.5 Hz, 1 H, 5-H), 2.924 (dd, *J* = 14.5, 5.0 Hz, 1 H, 9β-H), 2.581 (dd, *J* = 14.5, 3.5 Hz, 1 H, 9α-H), 2.174 (s, 3 H, -OAc), 1.869 (s, 3 H, 15-H) ppm.

In the same manner, another portion of compound **1** (0.45 mg) was treated with (*S*)-MTPA chloride (5 μL) in a second NMR tube at room temperature for 8 h and in deuterated pyridine (0.5 mL) as solvent, to afford the (*R*)-MTPA derivative of **1** (**1r**). ^{1}H NMR spectroscopic data for **1r** (500 MHz, [D_5]pyridine): δ = 6.305 (d, *J* = 3.5 Hz, 1 H, 13a-H), 6.281 (m, 1 H, 2-H), 5.796 (q, *J* = 1.4 Hz, 1 H, 3-H), 5.756 (d, *J* = 3.1 Hz, 1 H, 13b-H), 5.248 (ddd, *J* = 9.5, 5.0, 3.5 Hz, 1 H, 8-H), 5.010 (d, *J* = 2.0 Hz, 1 H, 14a-H), 5.007 (d, *J* = 2.0 Hz, 1 H, 14b-H), 4.229 (dd, *J* = 10.5, 9.5 Hz, 1 H, 6-H), 3.244 (d, *J* = 9.5, 6.0 Hz, 1 H, 1-H), 3.274 (m, 1 H, 7-H), 2.969 (t, 1 H, *J* = 9.5 Hz, 5-H), 2.928 (dd, *J* = 14.5, 5.0 Hz, 1 H, 9β-H), 2.557 (dd, *J* = 14.5, 3.5 Hz, 1 H, 9α-H), 2.173 (s, 3 H, -OAc), 1.926 (s, 3 H, 15-H) ppm.

Argyinolide B (2): White powder. $[a]_D^{21}$ = +162.1 (*c* = 0.45, MeOH). ECD (CH_3CN): 227 ($\Delta\varepsilon$ = +25.1), 263 ($\Delta\varepsilon$ = –0.49), 322 ($\Delta\varepsilon$ = –1.7). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3436, 2964, 2931, 1767, 1721, 1691, 1628, 1378, 1259, 1164, 1121, 1028, 1006 cm^{-1}. HRMS (ESI, +): calcd. for $C_{17}H_{20}O_6Na$ $[M + Na]^+$ 343.1158; found 343.1152.

Argyinolide C (3): White powder. $[a]_D^{21}$ = +82.2 (*c* = 0.22, MeOH). ECD (MeOH): 212 ($\Delta\varepsilon$ = –1.1), 265 ($\Delta\varepsilon$ = –0.4). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3404, 2958, 2926, 1755, 1725, 1663, 1632, 1459, 1376, 1278, 1160, 1058, 1006 cm^{-1}. HRMS (ESI, –): calcd. for $C_{20}H_{27}O_6Cl_2$ $[M + Cl]^-$ 433.1190; found 433.1199.

Argyinolide D (4): White powder. $[a]_D^{21}$ = +88.0 (*c* = 0.16, MeOH). ECD (MeOH): 227 ($\Delta\varepsilon$ = +2.9), 258 ($\Delta\varepsilon$ = –1.6). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3432, 2927, 1753, 1722, 1626, 1459, 1383, 1250, 1151, 1059 cm^{-1}. HRMS (ESI, –): calcd. for $C_{20}H_{27}O_6Cl_2$ $[M + Cl]^-$ 433.1190; found 433.1201.

Argyinolide E (5): White powder. $[a]_D^{21}$ = +91.8 (*c* = 0.24, MeOH). ECD (MeOH): 223 ($\Delta\varepsilon$ = +18.2), 258 ($\Delta\varepsilon$ = –0.4). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3433, 2927, 1756, 1712, 1644, 1455, 1231, 1146, 1066, 1038, 1008 cm^{-1}. HRMS (ESI, –): calcd. for $C_{20}H_{25}O_6Cl_2$ $[M + Cl]^-$ 431.1034; found 431.1047.

Argyinolide F (6): White powder. $[a]_D^{21}$ = –15.5 (*c* = 0.48, MeOH). ECD (MeOH): 230 ($\Delta\varepsilon$ = +0.2). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3489, 3390, 2923, 1772, 1717, 1657, 1452, 1376, 1260, 1168, 1049, 994 cm^{-1}. HRMS (ESI, –): calcd. for $C_{17}H_{24}O_6Cl$ $[M + Cl]^-$ 359.1267; found 359.1256.

X-ray Crystallographic Analysis of 6: Upon crystallization from methanol by use of the vapor diffusion method, colorless crystals of **6** were obtained. A crystal (0.31 × 0.38 × 0.51 mm) was separated from the sample and data were collected with a Rigaku MicroMax 002+ diffractometer and use of Cu-K_a radiation and the ω and κ scan technique to a maximum 2θ value of 144.48°. Crystal data: $C_{17}H_{24}O_6$, *M* = 324.37, monoclinic, $P2_1$, *a* = 10.160 (3) Å, *b*

= 8.059 (9) Å, c = 11.068 (9) Å, α = 90°, β = 115.983(16)°, γ = 90°, V = 814.6 (13) Å^3, Z = 2, $D_{calcd.}$ = 1.322 g cm^{-3}, 2892 reflections independent, 2422 reflections observed ($|F|^2 \geq 2\sigma|F|^2$), R_1 = 0.0404, wR_2 = 0.0888 ($w = 1/\sigma|F|^2$), S = 1.042. The crystal structure was solved by direct method with use of SHELXS-97, and all non-hydrogen atoms were refined anisotropically by use of the least-squares method. All hydrogen atoms were positioned by geometric calculations. The Flack parameter is 0.1(2) and the Hooft parameter is 0.3(2).

CCDC-926171 (for **6**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Argyinolide G (7): White powder. $[\alpha]_D^{21}$ = +43.1 (c = 0.44, MeOH). ECD (MeOH): 217 ($\Delta\varepsilon$ = +2.0), 234 ($\Delta\varepsilon$ = –3.2), 264 ($\Delta\varepsilon$ = +0.4). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3484, 3389, 2926, 2898, 1762, 1716, 1655, 1396, 1375, 1254, 1162, 1049, 968 cm^{-1}. HRMS (ESI, +): calcd. for $C_{17}H_{22}O_6Na$ [M + Na]$^+$ 345.1314; found 345.1306.

Argyinolide H (8): White powder. $[\alpha]_D^{21}$ = +23.0 (c = 0.24, MeOH). ECD (MeOH): 261 ($\Delta\varepsilon$ = +0.43), 230 ($\Delta\varepsilon$ = –4.43); $Mo_2(OAc)_4$-induced CD (DMSO): 328 ($\Delta\varepsilon$ = +0.14), 406 ($\Delta\varepsilon$ = +0.09). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3523, 2964, 2922, 2874, 1781, 1723, 1634, 1460, 1376, 1265, 1183, 1160 cm^{-1}. HRMS (ESI, +): calcd. for $C_{20}H_{28}O_6Na$ [M + Na]$^+$ 387.1778; found 387.1787.

Argyinolide I (9): White powder. $[\alpha]_D^{21}$ = +41.6 (c = 0.40, MeOH). ECD (MeOH): 222 ($\Delta\varepsilon$ = +13.5), 260 (sh, $\Delta\varepsilon$ = +1.8). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3420, 2919, 2851, 1765, 1715, 1377, 1242, 1056 cm^{-1}. HRMS (ESI, +): calcd. for $C_{20}H_{26}O_6Na$ [M + Cl]$^-$ 383.1627; found 383.1633.

Argyinolide J (10): White powder. $[\alpha]_D^{21}$ = +59.1 (c = 0.21, MeOH). ECD (MeOH): 250 ($\Delta\varepsilon$ = –2.1). ^{1}H NMR and ^{13}C NMR spectroscopic data: see Table 1 and Table 2. IR (KBr): $\tilde{\nu}_{max}$ = 3463, 2954, 2923, 1767, 1735, 1658, 1623, 1462, 1374, 1163, 1005, 971 cm^{-1}. HRMS (ESI, –): calcd. for $C_{20}H_{28}O_5Cl$ [M + Cl]$^-$ 383.1631; found 383.1635.

Determination of the Absolute Configuration of the 3,4-Diol Unit in 8 by Snatzke's Method: A diol **8**/$Mo_2(OAc)_4$ mixture (1:1.2) was subjected to CD measurement at a concentration of 0.1 mg mL^{-1} in anhydrous DMSO according to the published procedure.[26] 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 nm and 400 nm in the induced CD spectrum were used as key information to analyze the absolute configuration of the 3,4-diol unit.

Computational Methods: The 3D structures were firstly established with the aid of 1D-selective NOESY or 2D-NOESY spectra, which were subjected to random conformation analysis by use of the MMFF94s force field and the Sybyl-X 1.1 software package.[42] The lowest-energy conformers obtained, with relative energies within 6 kcal mol^{-1}, were preoptimized by ab initio calculation at HF/6–31G level in the Gaussian 09 program.[43] These minimum geometries were further optimized by DFT calculation at the B3LYP/6–31G(d) level in the gas phase; they were further checked by frequency calculation, which resulted in no imaginary frequencies. ECD calculations were performed by use of the TDDFT method at the B3LYP/6–311++G(2d, 3p) level by use of the CPCM model (in methanol or acetonitrile), and ECD spectra were then simulated by use of the SpecDis-V151 software.[44] The final ECD spectrum was obtained according to Boltzmann weighting of each conformer.

Bioactivity Assays: The purities of the isolated compounds (>95%) used for the biological assay in vitro were determined by ^{1}H NMR and HPLC techniques.

Cytotoxic Activity: Cell proliferation inhibition was determined by the MTT method.[45,46] Taxol was used as the positive control against HCT-8, Bel-7402, BGC-823, A549, and A2780 cell lines, with IC_{50} values of 0.051, 0.006, 0.003, 0.016, and 0.008 μM, respectively.

Inhibition of NO Production: The NO inhibitory activities of the isolated compounds were evaluated against BV-2 microglial cells by the MTT colorimetric method;[10] indomethacin was used as positive control. In addition, cell viabilities were also evaluated by MTT assay.

Supporting Information (see footnote on the first page of this article): Copies of IR, MS, 1D- and 2D-NMR, and ECD spectra for compounds **1**–**10**. ECD calculation details for compounds **2**, **6**, and **7**. Mosher esters procedure and NMR spectroscopic data for compound **6**. CD and UV spectra of known compounds.

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