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New sesquiterpene–monoterpene lactone, artemisolide, isolated from *Artemisia argyi*

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Abstract—A new sesquiterpene–monoterpene lactone, artemisolide, was isolated from the aerial parts of *Artemisia argyi*. The structure of artemisolide was elucidated by spectroscopic data including HMBC and NOESY. The structure was confirmed by X-ray crystallographic analysis. Artemisolide exhibited in vitro cytotoxic activity with GI_{50} values of 2–8 μM against cancer cell lines.

Artemisia species, widespread throughout the world, are important medicinal plants, which are receiving phytochemical attention due to the biological and chemical diversities.[1] *Artemisias* (Compositae) are one of the most popular plants in Chinese traditional preparations and frequently used for the treatment of diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses. Extensive studies of the chemical components of *Artemisia* have led to the identification of many compounds, such as monoterpenes, sesquiterpenes, triterpenes, and flavones from the dry leaves.[2] One of the compounds, artemisinin, isolated from the aerial parts of *Artemisia annua* L., is clinically used as an antimalarial drug.[3,4] During the intensive investigation of the biologically active compositions of *Artemisia* genus, we have isolated a couple of sesquiterpene lactones from *A. sylvatica*.[5,6] The interesting biological activities of the isolated compounds led us to further investigate the chemical components of *Artemisia*.

In this report, we described the isolation and structure elucidation of a new sesquiterpene–monoterpne lactone **1**, named artemisolide from the aerial parts of *Artemisia argyi* Levl. et Vant. Artemisolide is a new class of sesquiterpene lactone with a cyclopropane ring system.

The acetone–chloroform extract of the aerial parts of *A. argyi* was concentrated under reduced pressure and fractionated by C18 column chromatography with 50% aqueous CH_3OH to remove chlorophyll fractions. The aliquot was concentrated and chromatographed successively on silica, Sephadex LH-20 and prepared TLC plate to afford compound **1** (Fig. 1). The isolated compound was further purified by recrystallization from acetone and hexane.

Analyses of HREI MS ($[M+H]^+$, m/z 397.2378, calcd 397.2379) and ^{13}C NMR spectrum indicated that compound **1**[7] possesses the molecular formula $C_{25}H_{32}O_4$. Structure of **1** was determined by the extensive study of

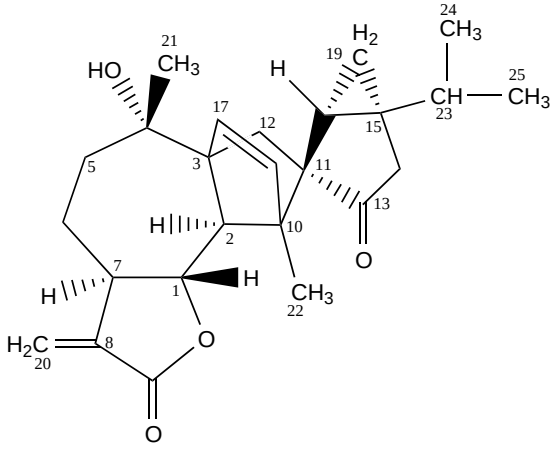

Figure 1. Structure of compound **1**.

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NMR experiments (^{1}H, ^{13}C, DEPT, COSY, HMQC, and HMBC), IR, and mass spectral data (Table 1). The analysis of NMR, IR, and mass spectral data indicated ten degrees of unsaturation and showed that the compound **1** had four methyls, six methylenes including an exocyclic methylene, seven methines, and eight quaternary carbons including two carbonyl groups.

The partial structures **A** and **B** (Fig. 2) were determined by the cross-peaks in a COSY spectrum and HMBC correlations. The structure **A** is a typical sesquiterpene lactone and was elucidated by cross signals H-5 through H-7 in COSY spectrum. And the spectrum of **1** revealed the oxygenated methine group (H-1) connected with these two methines (H-2 and H-7), while both of these two methine groups were attached to quaternary carbons. The HMBC correlations shown by quaternary carbons at δ_C 63.96 (C-3) and 72.99 (C-4) indicated that H-2 and H-5 was connected through the carbons. Furthermore, in HMBC spectrum, the olefinic protons of cyclopentene ring showed cross peaks with quaternary carbons at δ_C 63.96 (C-3) and 60.27 (C-10), and the correlations between at δ_C 137.99, 138.35 olefinic carbons and δ_H 2.67 (H-2) revealed that the cyclopentene and cycloheptane rings were connected through C-2 and C-3. The most upfield signals at δ_H 0.55 and −0.12 were assigned to the methylene group of cyclopropane ring based on the DEPT and COSY spectrum. One of protons at δ_H 0.55 of C-19 appeared multiplet, because it had a long range coupling with H-14 at δ_H 2.49. The connectivity of cyclopropane ring and isopropyl group was established by the correlations of a quaternary carbon C-15 at δ_C 28.46 with H-14 and H-23, and C-14 at δ_C 45.02 and H-19 in the HMBC experiment. The partial structure **B** was confirmed by the correlations between C-13 at δ_C 220.69 and H-12, H-14, and H-16 in HMBC spectrum.

The connection between two units **A** and **B** was established from the HMBC correlation of C-3 to H-12 and C-4 to H-17. It showed that the two partial structures **A** and **B** were connected by a Diels–Alder reaction between the cyclopentadiene ring of **C** and the olefinic methylene group of **D** as shown in Fig. 2. As shown in Fig. 3, the structure of **1** was also confirmed by EI mass spectral analysis. Retro-Diels–Alder product was observed at m/z 246 as a base peak. The ion undergoes further fragmentation and loses water to give a sesquiterpene lactone at m/z 203.

The relative stereochemistry of part **A** of **1** was determined by the analysis of NOESY experiment in which strong NOEs between H-2 and H-7, and between H-1 and H-17, 18, and 21 were observed. Stereochemical assignments of part **B** were also based on NOEs between H-16 and H-25, and between H-19 and H-14 (Fig. 4).

X-Ray analysis (Fig. 5) of the crystallized compound **1** obtained from acetone–hexane confirmed the proposed

Table 1. NMR data of artemisolide (**1**, 400 MHz, in $CDCl_3$)

Atom no.	δ_C	δ_H	HMBC (C→H)	COSY	NOESY
1	80.27	4.0 t, 10.0	2, 6	2, 7	17, 18, 21
2	65.07	2.67 d, 10.0		1	7
3	63.96		2, 5, 12, 17		
4	72.99		5, 17, 21		
5	34.98	1.83 m	6, 21	6	
6	24.01	2.24 m		5, 7	
		1.41 m			
7	43.40	3.29 m	2, 5	1, 6, 20	2
8	141.50		20		
9	170.96		20		
10	60.27		2, 18, 22		
11	64.44		19		
12	41.78	2.23 d, 8.0			
		1.27 d, 8.0			
13	220.69		12, 14		
14	45.02	2.49 dd, 2.4, 18.4		19	
		2.26 dd, 2.4, 18.4			
15	28.46		14, 23, 24, 25		
16	27.59	1.23 dd, 3.6, 8.0	12	19	19, 25
17	138.35	6.0 d, 5.6	2, 12	18	
18	137.99	5.97 d, 5.6	2, 22	17	
19	15.45	0.55 m	14	14, 16	14, 23, 25
		−0.12 dd, 3.6, 5.6		16	
20	118.64	6.04 d, 3.4		7	
		5.31 d, 3.4			
21	30.07	1.29 s			
22	15.04	1.35 s			
23	32.44	1.54 m		24, 25	19, 24, 25
24	19.64	0.84 d, 7.2	23, 25	23	23, 25
25	20.35	1.02 d, 6.4	23, 24	23	16, 19, 23, 24

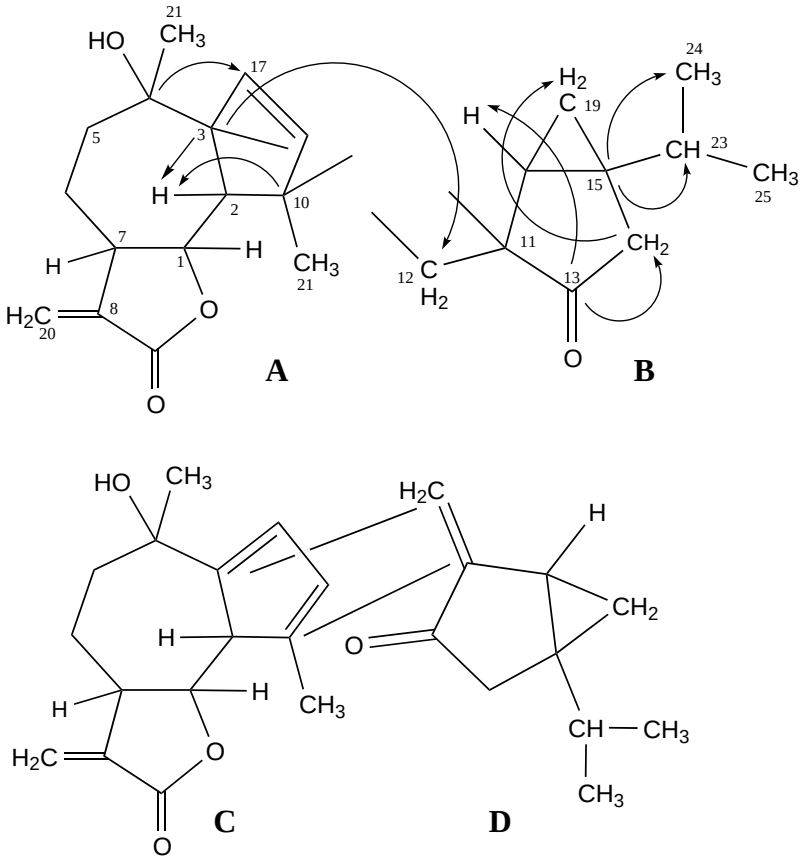

Figure 2. Significant HMBC and partial structures of compound **1**.

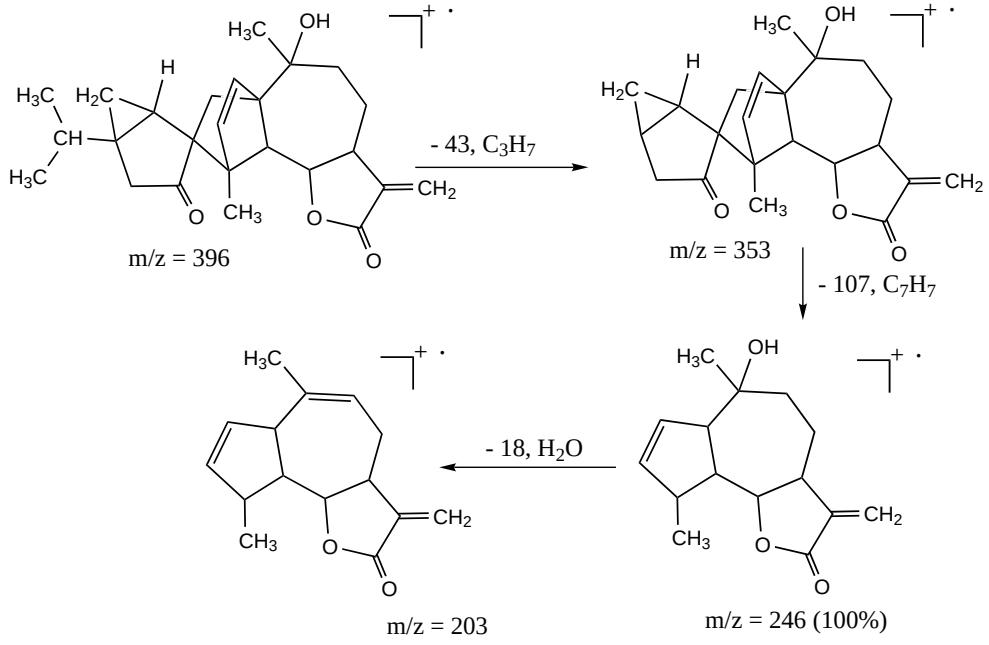

Figure 3. Retro-Diels–Alder product in mass spectral fragmentation.

structure with a unique fused-hexacyclic ring system. The relative stereochemistry of C-1, C-2, C-3, C-4, C-7, C-10, C-15, and C-16 was coincident with that deduced from NOESY correlations, as described above.

Sesquiterpene lactones are a class of natural sesquiterpenoids, which occur in many plants, and the most characteristic secondary metabolites of *Artemisia*. These compounds, isolated from *Artemisia* genus, have a γ-

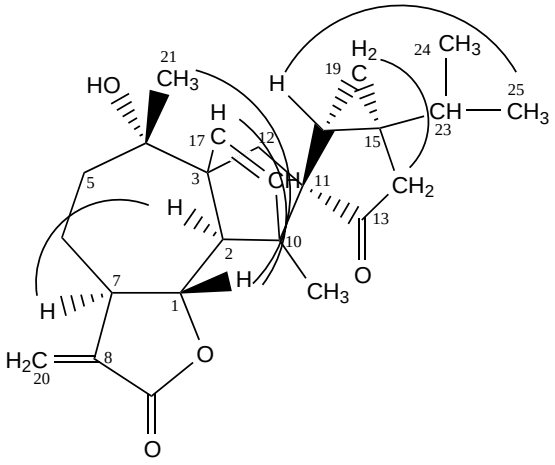

Figure 4. NOE correlations of compound **1**.

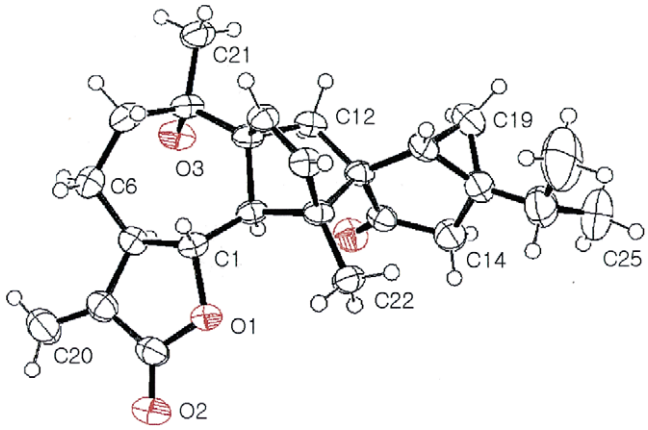

Figure 5. X-Ray structure of compound **1**.

lactone system with exocyclic methylene group and it is generally accepted that the mechanism of cytotoxicity due to the alkylation of biological nucleophiles on the α,β-unsaturated carbonyl moiety.[8] Activity of compound **1** on the cell growth of the three cell lines was examined using a cell proliferation assay kit. Artemisolide (**1**) exhibited a dose dependent inhibition of cell growth in a broad range of concentrations and the GI_{50} values of **1** toward SW620 (colon cancer cell), HL-60, and Molt-4 leukemia cancer cell lines were 7.7, 2.0, and 3.5 μM, respectively.[9]

Acknowledgements

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7. Compound **1**: crystalline solid, mp 200–201°C; UV (CH_3OH) λ_{max} 207 ($\log \varepsilon = 3.03$) nm; $[\alpha]_D^{25}$ −72 (*c* 0.5, CH_3OH); EI *m*/*z* 396 (1.2), 353 (13.6), 246 (100), 228 (95.8), 203 (84.9), 122 (35.3), 79 (25.5). IR (KBr) ν_{max} 3510, 3055, 2954, 2873, 1747, 1730, 1666, 1455, 1267, 1153, 990, and 906 cm^{-1}
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9. Tumor and transformed cells were maintained in DMEM supplemented with 10% heat-inactivated FBS. Cells were maintained at 37°C under a humidified atmosphere of 5% CO_2 and 95% air in a incubator. Cells (5000 cells/well) were seeded in a 96-well plates in DMEM containing 10% FBS. After 24 h, cells were replenished with fresh complete medium containing either a test compound or 0.1% DMSO. After incubation for 48 h, the cell proliferation reagent WST-1 (Roche Molecular Biochemicals) was added to each wells. The amount of WST-1 formazan produced was measured at 450 nm using an ELISA Reader (Bio-Rad).