Arteminolide, an Inhibitor of Farnesyl Transferase from *Artemisia sylvatica*

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Ras proteins play an important role in intracellular signal transduction pathways involved in cell growth, and the mutated *ras* genes have been found in 30% of human cancers.[1] Ras proteins (H, K, and N) are small guanine nucleotide binding proteins that undergo a series of posttranslational modifications including the farnesylation onto cysteine 186 at C-terminal of Ras by farnesyl protein transferase (FPTase).[2] This is a mandatory process before anchoring to plasma membrane which is critical for its biological activity, e.g., cell proliferation and tumorigenesis.[3] Recent work has demonstrated that specific inhibitors of the FPTase might be interesting chemical leads to develop effective therapeutic agents for the treatment of cancer.[4]

We recently reported 2′-hydroxycinnamaldehyde,[5] solandelactones,[6] and rhombenone[7] as inhibitors of FPTase. In the course of our screening for potent inhibitors of FPTase from herbal medicines, we have isolated compound **1**, named arteminolide, from the leaves of *Artemisia sylvatica* Maxim (Compositae). Members of the *Artemisia* genus are important medicinal plants found throughout the world.[8] Artemisinin[9] is well-known as an antimalarial agent, which was isolated from *A. annua* L. Isolated compound **1** is a sesquiterpene lactone, which exhibits a wide range of biological activities.[10] Arteminolide (**1**) inhibits recombinant rat FPTase[11] with IC_{50} of 360 nM and appears to be selective for FPTase. It did not inhibit rat squalene synthase ($IC_{50} \gg 200\ \mu M$) and recombinant rat geranyl−geranyl protein transferase I ($IC_{50} \gg 200\ \mu M$). Isolation and structure elucidation of the compound are described.

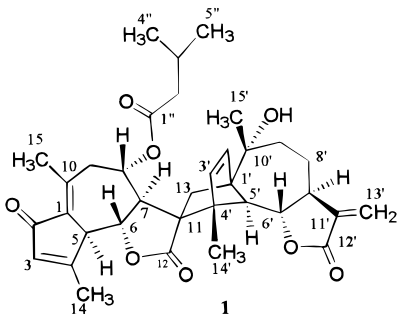

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Figure 1. Structure of arteminolide (**1**).

The chloroform extract of the dried leaves of *A. sylvatica* was fractionated by silica gel flash chromatography eluting with 10% MeOH in chloroform. Active fractions were further purified by C-18 column chromatography and gel filtration (Sephadex LH-20). Finally, the colorless solid arteminolide (**1**, 3 mg/kg) (Figure 1) was obtained by preparative reverse phase HPLC (YMC J'sphere ODS-H80, 250 × 20 mm i.d. column).

The structure of **1** was determined by interpretation of NMR, IR, and mass spectral data. Analysis of HR-FABMS ($[M + H]^+$, *m*/*z* 591.2904 (+ 1.7 ppm), and the ^{13}C NMR spectrum of **1** led to a molecular formula $C_{35}H_{42}O_8$, which indicated 15 degrees of unsaturation (see Table 1 for ^1H and ^{13}C NMR spectral data). The ^{13}C NMR of **1** exhibited 35 carbons, which revealed carbon signals for six methyls, six methylenes (including an exocyclic olefinic methylene), eight methines (three bearing oxygen), three olefinic methines, four quaternary carbons (one being oxygenated), four nonprotonated olefinics, and four carbonyls. The HMQC experiment and DEPT spectrum served in identifying the protons attached to a specific carbon.

The existence of cyclopentenone and lactone moieties shown in partial structure A (Figure 2) was determined as follows. Proton−proton connectivities, H-5 through H-7 and H-7 through H-9, were observed in the $^1H-^1H$ COSY spectrum. The chemical shifts of C-6 (80.13 ppm) and C-8 (68.13 ppm) suggested that they were substituted with oxygen. In the HMBC experiment, correlations of a nonprotonated olefinic carbon (134.15 ppm) with H-3 and H-6, a quaternary carbon (61.31 ppm) with H-7, and an α,β-unsaturated carbonyl (194.77 ppm) with H-3 were observed. Hence, the partial structure A was thought to be a sesquiterpene lactone. An α-methylene lactone functionality and a cyclopene in partial structure B were identified by the chemical shifts of a nonprotonated

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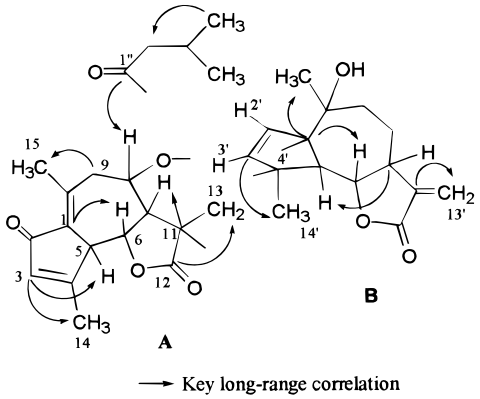


Figure 2. Partial structure of arteminolide (**1**).

Table 1. NMR Data of Arteminolide (1, 500 MHz, in $CDCl_3$)

atom	δ_C	δ_H	HMBC (C→H)
1	134.15		3, 6, 9, 15
2	194.77		3
3	136.25	6.19 (brs)	5, 14
4	170.08		3, 5
5	51.95	3.26 (d, 10.3)	3, 7
6	80.13	4.02 (dd, 10.7, 9.8)	5, 7
7	59.40	2.72 (dd, 11.3, 10.7)	
8	68.14	4.78 (ddd, 11.3, 10.7, 2.7)	7
9	44.55	2.48 (dd, 13.2, 10.3), 2.29 (dd, 13.2, 2.4)	15
10	143.65		15
11	61.31		7, 3′, 5′, 14′
12	178.41		13
13	40.33	2.59 (d, 11.2), 1.88 (d, 11.7)	2′, 12
14	20.28	2.32 (s)	
15	20.51	2.40 (s)	
1′	62.91		2′, 3′, 6′, 15′
2′	137.56	5.84 (AB system)	13, 5′, 14′
3′	136.40	5.84 (AB system)	14′
4′	57.93		14′
5′	66.92	3.08 (d, 9.8)	
6′	79.07	3.98 (dd, 9.3, 9.8)	
7′	43.14	3.29 (m)	5′, 13′
8′	23.57	2.23 (m)	6′
9′	34.67	1.84 (m), 1.77 (m)	15′
10′	72.57		15′
11′	140.60		12′
12′	170.31		13′
13′	118.76	6.06 (d, 3.4), 5.33 (d, 2.9)	
14′	17.02	1.48 (s)	
15′	29.87	1.30 (s)	
1″	171.04		8, 3″
2″	43.77	2.07 (m)	4″, 5″
3″	25.57	2.09 (m)	4″, 5″
4″	22.49	0.95 (d, 5.9)	
5″	22.59	0.98 (d, 6.4)	

olefinic carbon at 140.60 ppm and exocyclic olefinic protons at δ 6.06 and 5.33 for an α-methylene lactone and that of the olefinic protons at δ 5.84 with an AB system. Two- and three-bond HMBC correlations between the methyl groups and their respective neighboring carbons also supported the partial structures in Figure 2. C-8 and the isovaleryl group were connected by an ester bond between C-8 and C-1″, which was confirmed by the chemical shifts of C-8 (68.14 ppm) and H-8 (δ 4.78) and correlation of an ester carbonyl carbon (171.04 ppm, C-1″) with H-8 in the HMBC spectrum.

The relative stereochemistry of part A of **1** was determined by an NOESY experiment in which strong NOEs between H-6 and H-8 and between H-5 and H-7 were observed (Figure 3). Stereochemical assignments

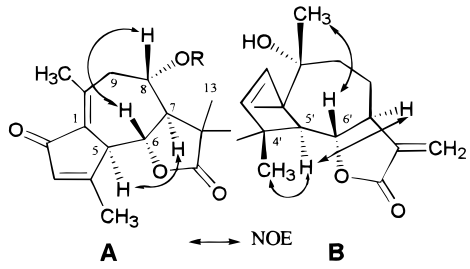
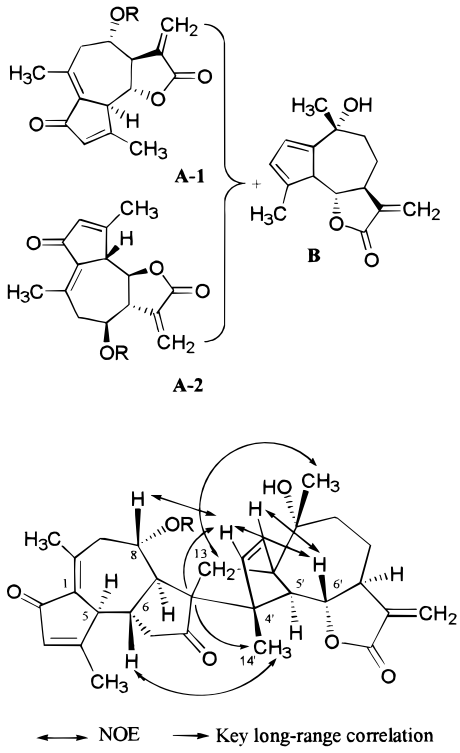


Figure 3. NOE cross-peaks observed in NOESY spectra (in $CDCl_3$) of **1**.

Scheme 1



of part B were also based on NOEs between H-5′ and H-14′, H-7′ and between H-6′ and H-15′.

The connection between units A and B was established from the HMBC data of H-3′ to C-11 and H-13 to C-2′. The observation of long-range couplings verified that the two partial structures A and B were connected by a Diels−Alder reaction between the exo-methylene of A and the cyclopentadiene of B as shown in Scheme 1. There are four possible orientations in the Diels−Alder reaction between two dienophiles (A-1 and A-2) and one diene (B) such as A-1 (down) + B (up), A-1 (up) + B (down), A-2 (down) + B (up), and A-2 (up) + B (down). The Diels−Alder reaction of A-1 (down), A-2 (down), and B (up) gave arteminolide (**1**) and artanomaloide,[12] respectively. Artanomaloide was also isolated from *A. sylvatica*. The Diels−Alder products were verified by the cross interactions between H-2′, H-3′, and H-6′ in the NOESY spectra of **1** and artanomaloide. However, the other orientations were excluded, because they should show the NOE effects between H-2′, H-3′, and H-5′ instead of H-6′, and the Diels−Alder reaction of A-2 (up) and B (down) cannot occur due to the steric hindrance between H-8 and CH_3-

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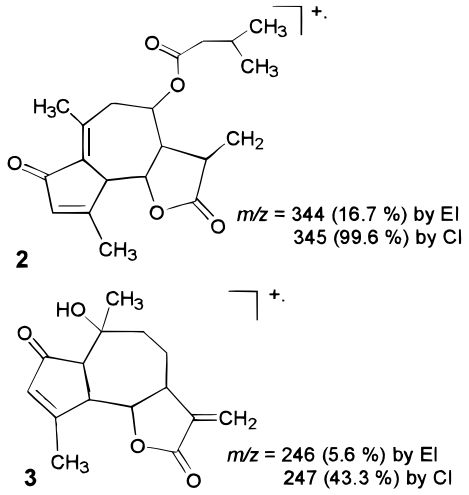

Figure 4. Retro-Diels–Alder products in mass spectral fragmentation.

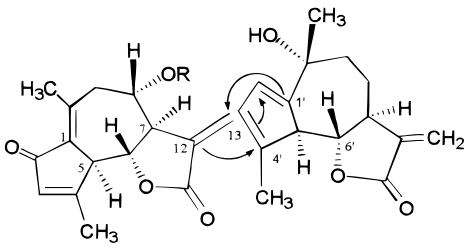

Figure 5. Biogenetic pathway for the carbon framework of **1**.

15. The relative configurations of compound **1** and artanomaloide were assigned on the basis of NOSEY data. In compound **1**, the NOESY spectrum showed cross-peaks of a methyl proton (H-14′) with H-8 and H-6, a proton of the oxygenated carbon (H-8) with H-3′, and a methyl group (H-15′) with H-13. Those NOE effects were not detected in artanomaloide; however, NOE cross-peaks were observed between H-14′ and H-7 and also between H-13 and H-8.[12]

Retro-Diels–Alder products **2** and **3** were observed in the EI and CI mass spectra of arteminolide (**1**) as shown in Figure 4. The ion **2** undergoes further fragmentation and loses isovaleric acid to give a sesquiterpene lactone (*m*/*z* 242 by EI and 243 by CI). HMBC, NOESY, and mass spectral data supported the proposed structure.

All of the above data conclusively led to the structure of arteminolide (**1**). These results are consistent with a relative configuration of **1**, derived biogenetically by Diels–Alder reaction of A-1 (down) and B (up); therefore, C-11, C-1′, and C-4′ have the *S*-, *R*-, and *R*-configurations, respectively. Compound **1** is a configurational isomer of artanomaloide.[12] This structure can be also explained by the biogenetic pathway shown in Figure 5.

Although many FPTase inhibitors have been reported, most of them are structural analogues to CAAX box of Ras protein[13] or FPP.[14] Structure of **1** is quite different in comparison with reported FPTase inhibitors. Therefore, **1** may be a useful lead compound for the development of antitumor drugs through the control of Ras-mediated signal transduction.

Experimental Section

Optical rotation was measured on a digital spectropolarimeter. FAB mass spectra were supplied by the Mass Spectrometer Facility, Department of Chemistry, University of California at Riverside. NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

Isolation. Leaves of *A. sylvatica* was collected in summer near Incheon, Korea, and identified by Professor K. Bae, School of Pharmacy, Chungnam National University. The dried aerial parts (3 kg) were extracted with $CHCl_3$ (2 × 10 L). The combined extract was concentrated, and the dark residue was subjected to silica gel flash chromatography with $CHCl_3$/MeOH solvent pairs. Fractions were monitored by FPTase inhibition activity and silica gel TLC ($CHCl_3$–MeOH, 9:1). The active fractions ($CHCl_3$/MeOH, from 9:1 to 8:2) were subjected to C18 column chromatography with aqueous MeOH. The two fractions eluted with 70 and 80% MeOH were shown to have strong inhibition activity against rat FPTase and were collected and further purified by chromatography on a Sephadex LH-20 column eluting with MeOH. Final purification of arteminolide (**1**) was accomplished by ODS-HPLC with 80% MeOH to yield **1** (3 mg/kg).

Arteminolide (1): mp 195–196 °C; UV (MeOH) λ_{max} 215 (ϵ = 5904), 254 (6511) nm; $[\alpha]^{25}_D$ +15° (*c* 0.11, MeOH); IR (KBr) ν_{max} 3600–3400, 2927, 1768, 1697, 1650, 1617, 1230, 1147, and 908 cm^{-1}; DEI *m*/*z* 344 (16.7), 242 (42.7), 77 (22.0)57 (51.7); DCI *m*/*z* 591 (1.8), 345 (99.6), 247 (43.4), 243 (79.9), 229 (100).

Biological Activity. The FPTase assay was conducted by the scintillation proximity assay (SPA) which was provided by Amersham Int. plc, U.K. Arteminolide (**1**) inhibited an IC_{50} value of 360 nM against a recombinant rat FPTase; however, it did not inhibit a recombinant rat geranyl–geranyl protein transferase I ($IC_{50} \gg 200\ \mu$M) and a rat squalene synthase ($IC_{50} \gg 200\ \mu$M).[15]

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, mass, and HMBC spectra for arteminolide (**1**) and ^{1}H NMR of artanomaloide (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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