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Sesquiterpene Lactones, Inhibitors of Farnesyl Protein Transferase, Isolated from the Flower of *Artemisia sylvatica*

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Abstract—Five sesquiterpene lactones, 8-acetylarteminolide (**1**), artanomaloide (**2**), arteminones (**3** and **4**), and dehydromatricarin (**5**), were isolated from the methanolic extract of the flower of *Artemisia sylvatica* and characterized on the basis of their spectral data. New sesquiterpene lactone **1** was identified as a configurational isomer of artanomaloide (**2**), and the new arteminones **3** and **4** are determined as stereoisomers. 8-Acetylarteminolide (**1**) strongly inhibited FPTase with an IC_{50} of 1.8 μM, however, the other sesquiterpene lactones mildly inhibited the transferase with an IC_{50} of 22–300 μM.

Introduction

During the course of screening medicinal plant extracts for antitumor activity, a methanolic extract of the flower of *Artemisia sylvatica* Maxim. (Compositae) exhibited a strong inhibition activity against a farnesyl protein transferase (FPTase). FPTase, a member of the prenyltransferase enzyme family, is a key post-translational modification step for Ras protein and this is a mandatory process for retention of transforming ability.[1,2] Therefore, inhibitors of Ras farnesylation have a promising to be effective antitumor agents.[3] Many synthetic FPTase inhibitors have been reported including peptidomimetics in the past few years,[3,4] however, a few examples of natural product inhibitors were reported.[3] We also recently reported a couple of FPTase inhibitors, which were isolated from medicinal plants.[5–7]

Members of the *Artemisia* genus are growing throughout the world and important medicinal plants.[8] Especially, artemisinin is well known an antimalarial agent,[9] which was isolated from *Artemisia annua* L. Isolated compounds **1**–**5** were identified as sesquiterpene lactones, which exhibited a wide range of biological activities[10–12] and were reported over 2,500 structures.[13] 8-Acetylarteminolide (**1**), artanomaloide (**2**), arteminones (**3** and **4**), and dehydromatricarin (**5**) were isolated from the methanolic extract of the flower of *Artemisia sylvatica* and characterized on the basis of their spectral data. Compounds **1**, **3**, and **4** were determined to be a new type of sesquiterpene lactone. The compounds **2** and **5** were identified as artanomaloide (**2**) and dehydromatricarin (**5**) by the comparison with the spectral data of them, respectively.[14,15] This paper describes the isolation and chemical and biological characterization of the metabolites with FPTase inhibitory activity produced the flower of *Artemisia sylvatica*.

Results and Discussion

The methanolic extract of the flower of *A. sylvatica* was fractionated by silica gel flash chromatography eluting with EtOAc/hexane. Fractions were monitored by FPTase inhibition activity. The active fractions were subjected to ODS column chromatography with aqueous MeOH and followed by a Sephadex LH-20 column chromatography eluting with MeOH Final purification of the sesquiterpene lactones was accomplished by ODS-HPLC with 70–80% MeOH to yield **1**, **2**, **3**, **4**, and **5**.

The structure of **1** was determined by the extensive analysis of NMR, IR, and mass spectral data. Analysis of HREIMS ($[M]^+$, *m/z* 548.2440, calcd. 548.2410) and ^{13}C NMR spectrum of **1** led to a molecular formula $C_{32}H_{36}O_8$, which indicated fifteen degrees of unsaturation and eight oxygen atoms (see Table 1 for ^1H and ^{13}C NMR spectral data). The ^{13}C NMR, HMQC experiment, and DEPT spectrum of **1** exhibited 32 carbons, which revealed carbon signals for five

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Table 1. NMR Data of 8-acetylarteminolide (**1**, 400 MHz, in $CDCl_3$)

Atom no.	δ_C	δ_H	HMBC (C→H)
1	134.46		3, 6, 9, 15
2	195.11		3
3	136.60	6.30 (brs)	5, 14
4	170.34		3, 5
5	52.23	3.28 (d, 10.4)	3, 7
6	80.48	4.06 (t, 10.4)	5, 7
7	59.57	2.75 (t, 10.4)	9
8	68.66	4.78 (t, 10.4)	7
9	44.84	2.50 (t, 10.4)	15
		2.29 (m)	
10	144.06		15
11	61.62		7, 3′, 5′, 14′
12	178.88		13
13	40.55	2.57 (d, 11.6)	2′, 12
		1.90 (d, 11.7)	
14	20.59	2.32 (s)	
15	20.81	2.40 (s)	
16	169.25		8, 17
17	21.99	2.00 (s)	
1′	63.32		2′, 3′, 6′, 15′
2′	137.84	5.87 (brds)	13
3′	136.81	5.87 (brds)	5′, 14′
4′	58.27		2′, 3′, 14′
5′	67.25	3.08 (d, 10.4)	
6′	79.47	4.01 (t, 10.4)	5′
7′	43.48	3.36 (m)	13′
8′	23.88	2.24 (m)	
		1.43 (m),	
9′	34.93	1.77 (m)	15′
10′	72.88		15′
11′	140.94		12′
12′	170.71		13′
13′	119.12	6.07 (d, 2.8)	
		5.34 (d, 2.9)	
14′	17.33	1.55 (s)	
15′	30.14	1.31 (s)	

methyls, five methylenes, ten methines, eight quaternary carbons, and four carbonyl groups.

The partial structure **A** (Fig. 1) was determined by the cross-peaks in a COSY spectrum and HMBC correlations. COSY cross-peaks established the connectivity from H-5 through H-9. The chemical shift of H-6 (δ 4.06 for ^{1}H NMR and 80.48 ppm for ^{13}C NMR) suggested that it was substituted with the oxygen of lactone. In the HMBC experiment, correlations of a quaternary carbon (C-1 at 134.46 ppm) with H-3 and H-6, a quaternary carbon (61.62 ppm) with H-7, and that of an α,β-unsaturated carbonyl (195.11 ppm) with H-3 were observed. Hence, the partial structure **A** was

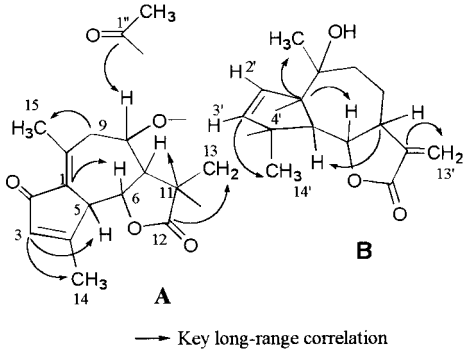


Figure 1. Partial structure of 8-acetylarteminolide (**1**).

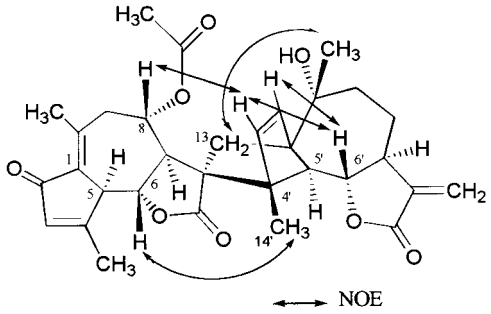


Figure 2. NOE cross peaks observed in NOESY spectrum (in $CDCl_3$) of **1**.

thought to be a sesquiterpene lactone. A partial structure **B** in Fig. 1 was elucidated by the chemical shifts of an olefinic carbon at 140.94 ppm and exocyclic olefinic protons at δ 6.07 and δ 5.34 for an α-methylene lactone and that of the olefinic protons at δ 5.87, and two- and three-bond HMBC correlations between carbons and their respective neighboring protons.

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**.

The relative stereochemistry of part **A** of **1** was determined by the analysis of NOESY experiment in which strong NOEs between H-6 and H-8 and between H-5 and H-7 were observed. Stereochemical assignments of part **B** were also based on NOEs between H-5′ and H-14′, H-7′, and between H-6′ and H-15′. The Diels–Alder product was verified by the NOE interactions between H-2′, H-3′ and H-8, and also a methyl proton (H-14′) with H-8 and H-6, and that of a methyl group (H-15′) with H-13 in the NOESY spectra of **1** (Fig. 2).

RetroDiels–Alder products **C** (*m/z* 302) and **D** (*m/z* 246) were observed in the EI mass spectra of **1** as shown in Fig. 3. The ion **C** undergoes further fragmentation and cleavage of ester bond to give a sesquiterpene lactone (**E**, *m/z* 260). These ions were confirmed by exact mass. HMBC, NOESY, and mass spectral data supported the proposed structure.

As deduced from HREIMS and ^{13}C NMR, **2** had a same molecular formula of compound **1** ($C_{32}H_{36}O_8$). The ^{13}C NMR of **1** and **2** gave very similar chemical shifts, however, analysis of ^{1}H NMR of them showed a quite different chemical shifts at H-5′ (δ 3.08 for **1** and 1.98 for **2**, respectively). And the cross interactions between H-2′, H-3′, and H-5′ in the NOESY spectra were observed. Based on the spectral analysis and reported data, the structure of **2** was confirmed as artanomaloide (Fig. 4).[14] **2** is a regioisomer of compound **1**.

On the basis of the HREIMS ([M]+, *m/z* 278.1151, calcd 278.1154 for $C_{15}H_{18}O_5$), ^{13}C NMR, and DEPT of artemisinone **3** and **4**, they have the same molecular formula, $C_{15}H_{18}O_5$, which indicated 7 degrees of unsaturation (see

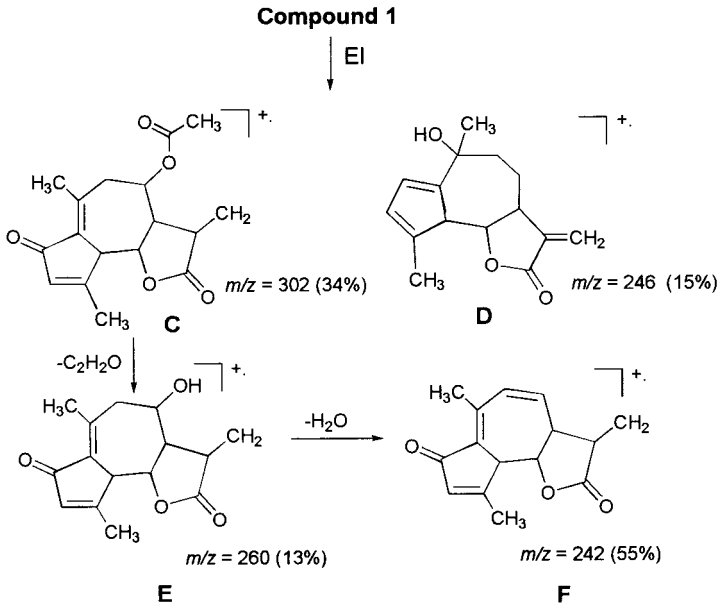


Figure 3. RetroDiels–Alder products in mass spectral fragmentations.

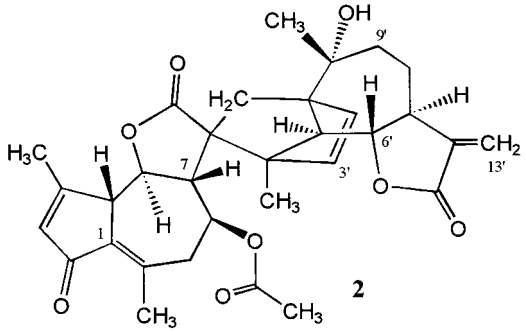


Figure 4. Structure of artanomaloide (**2**).

Table 2 for ^{1}H and ^{13}C NMR spectral data). The ^{13}C NMR of **3** and **4** exhibited 15 carbons, which consisted of three carbonyl groups, an exocyclic olefinic methylene, two methylenes, five methines, two methyl groups, and two quaternary carbons. The HMQC experiment, DEPT, and ^{1}H NMR spectrum of **3** revealed that it had an α,β-unsaturated group, two methines bearing oxygen, and a lactone with exocyclic olefinic methylene (at δ 6.29, 5.64 for ^{1}H and 138.13 ppm for ^{13}C). The oxygen of lactone was placed at C-1 based upon the low field proton and carbon shifts (at δ 4.57 for ^{1}H and 80.8 ppm for ^{13}C) observed. The structure of seven-membered ring in compounds **3** and **4** (Fig. 5) was assigned by COSY cross-peaks from 1 through 7 and HMBC correlation of carbonyl carbon (C-4 at 207.52 ppm) with H-2 and H-5. In the HMBC experiment, correlations of a quaternary carbon (C-3 at 77.66 ppm) with H-11 and H-15, and of C-7 at 40.05 ppm with an exocyclic olefinic proton (H-10) were also observed. These correlations suggested that the structure of arteminones was thought to be a sesquiterpene lactone with an α,β-unsaturated moiety. The relative configuration of olefinic protons in side chain was established as *cis* by the coupling constant between H-11 and H-12 (J=5.6 Hz).

Table 2. NMR data of arteminone **3** and **4** (400 MHz NMR in $CDCl_3$)

Position	**3**		**4**	
	δ_C	δ_H	δ_C	δ_H
1	80.08	4.57 (dd, 2.0, 6.3)	80.95	4.56 (dd, 2.4, 9.2)
2	56.98	2.55 (d, 6.3)	58.56	2.32 (d, 9.2)
3	77.66		78.48	
4	207.52		207.52	
5	39.81	2.56 (m)	39.87	2.53 (m)
6	26.43	1.93 (q, 6.9)	28.72	1.87 (q, 7.2)
7	40.05	3.65 (m)	41.24	3.53 (m)
8	138.13		138.13	
9	169.46		169.70	
10	123.07	6.29 (d, 2.8)	124.73	6.35 (d, 1.6)
		5.64 (d, 2.8)		5.75 (d, 1.6)
11	167.49	7.50 (d, 5.6)	165.94	7.46 (d, 5.6)
12	133.84	6.17 (d, 5.6)	133.49	6.17 (d, 5.6)
13	205.18		203.93	
14	30.37	2.18 (s)	30.35	2.18 (s)
15	27.49	1.54 (S)	29.29	1.59 (s)

The absolute stereochemistry of **3** was readily established by NOESY NMR experiment. Correlations from the proton at C-1 and C-2, and no through space correlation between H-1 and H-7 established the trans ring juncture. An additional correlation from H-2 to the C-15 methyl protons placed the α,β-unsaturated group in the down position.

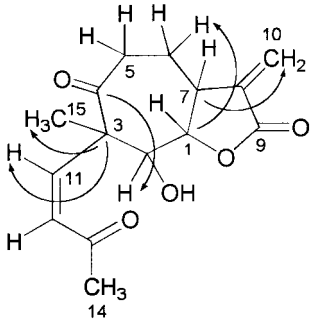


Figure 5. Some important HMBC correlations for compound **1** and **2**.

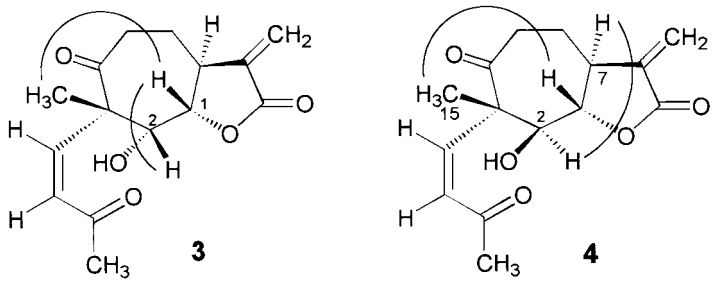
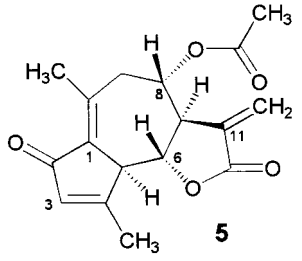


Figure 6. Important NOE interactions observed in the NOESY spectrum of **1** and **2**.

The structure of **4** was clarified by comparing its NMR data with the data of **3** and by NOESY spectrum. In compound **3**, the configuration of C-2 was assigned as S by the correlations of H-2 with H-1 and H-15, and no cross peaks of H-2 with H-7 in NOESY spectrum. Correlations between H-2 and H-1, H-15 were not detected, however, cross peaks were observed between H-2 and H-7 in the NOESY spectrum of **4**, which led to the assignment of C-2 configuration of **4** as R (Fig. 6). The compound **3** and **4** were named as an artemiinone.

Compound **5** was identified as dehydromatricarin by comparison of its spectral data with the reported one.[15]



8-Acetylarteminolide (**1**) inhibits recombinant rat FPTase with IC_{50} of 1.8 μM and appears to be selective for FPTase. It did not inhibit recombinant rat geranyl-geranyl protein transferase I ($IC_{50} \gg 100$ μM).

Compounds **2**, **3**, **4**, and **5** mildly inhibit recombinant rat FPTase with IC_{50} of 22, 85, 82, and 300 μM, respectively. Although these compounds fairly inhibit FPTase activity, the results are of interest in understanding the pharmaceutical activities of sesquiterpene lactones.

8-Aectylarteminolide (**1**) has an unique structure 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 pathways.

Experimental

General

Optical rotation was measured on a digital spectropolarimeter. High resolution mass spectra were obtained by Autospec-UltimaE, VG. NMR spectra were recorded on a Bruker AMX 400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer.

Isolation

Flower of *Artemisia sylvatica* was collected near Incheon, Korea, in September 1999 and identified by Professor K. Bae, School of Pharmacy, Chungnam National University. The dried material (1 kg) was extracted with MeOH (2×2 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 strong inhibition activity against rat FPTase, which 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 70% MeOH to yield **1** (32 mg), **2** (40 mg), **3** (28 mg), **4** (20 mg), and **5** (32 mg), respectively.

8-Acetylarteminolide (1). HREIMS ($[M]^+$, *m/z* 548.2440, calcd 548.2410); mp 190–192°C; UV (MeOH) λ_{max} 254 (log ϵ=4.20) nm; $[\alpha]_D^{25}=-2.54°$ (*c* 0.15, MeOH); EI *m/z* 548 (43.2), 302 (34.1), 260 (13.3), 246 (15.1), 242 (55.9), 131 (66.1), 119 (100).

Artanomaloide (2). Mp 192–195°C; EI *m/z* 548 (90.3), 302 (70.2), 260 (17.6), 246 (72.5), 242 (71.3), 228 (100).

Arteminone (3). A white powder; HREIMS ($[M]^+$, *m/z* 278.1151, calcd 278.1154 for $C_{15}H_{18}O_5$); mp 180–181°C; UV (MeOH) λ_{max} 220 (log ϵ=3.65) nm; $[\alpha]_D^{25}=+7.1°$ (*c* 0.15, MeOH); IR (film) ν_{max} 3600–3400, 2927, 1768, 1711, 1650, 1370, 1147 cm^{-1}; DEI *m/z* 278 (43.2), 260 (9.3), 166 (52.5), 163 (84.73), 124 (46.7), 112 (88.1), 94 (100).; DCI *m/z* 591 (1.8), 345 (99.6), 247 (43.4), 243 (79.9), 229 (100).

Arteminone (4). A white powder; mp 183–185°C; UV (MeOH) λ_{max} 218 (log ϵ=3.68) nm; $[\alpha]_D^{25}=+2.4°$ (*c* 0.15, MeOH)); IR(film) ν_{max} 3600–3400, 2925, 1767, 1710, 1661, 1230, 1147 cm^{-1};DEI *m/z* 278 (45.1), 260 (12.6), 166 (47.8), 163 (44.27), 124 (28.1), 112 (93.2), 94 (100).

Dehydromatricarin (5). A white powder; mp 151–152°C; UV (MeOH) λ_{max} 254 (log ϵ=3.87) nm; $[\alpha]_D^{25}=+4.4°$ (*c* 0.12, MeOH); IR(film) ν_{max} 2935, 1725, 1698, 1664, 1120 cm^{-1}; HREIMS *m/z* 302.1153 (calcd for $C_{17}H_{18}O_5$, 302.1154) 302 (84.5), 260 (29.0), 242 (100), 227 (41.7),

199 (41.8), 136 (48.8), 91(45.8); ^{1}H NMR in $CDCl_3$ δ 6.19 (1H, s, H-3), 3.50 (1H, d, J=11.6 Hz, H-5), 3.71 (1H, t, J=10.2 Hz, H-6), 3.25 (1H, dt, J=2.0, 10.2 Hz, H-7), 4.91 (1H, dt, J=2, 10.4 Hz, H-8), 2.71, 2.46 (2H, t, J=11.2 Hz, H-9), 6.23, 5.65 (2H, d, J=3.0 Hz, H-13), 2.28 (3H, s, H-14), 2.44 (3H, s, H-15), 2.15 (3H, s, H-17); ^{13}C NMR in $CDCl_3$ δ 133.88 (C-1), 195.27 (C-2), 136.36 (C-3), 169.55 (C-4), 51.88 (C-5), 81.70 (C-6), 55.35 (C-7), 69.60 (C-8), 44.72 (C-9), 144.94 (C-10), 136.38 (C-11), 168.63 (C-12), 122.19 (C-13), 20.23 (C-14), 21.62 (C-15), 169.99 (C-16), 21.37 (C-17).

Biological activity

The FPTase assay was conducted by the Scintillation Proximity Assay (SPA), which was provided by Amersham Int. plc, UK (code TRKQ 7010). Farnesyl transferase was purified from rat brain homogenates by sequential ammonium sulfate fraction and Q-sepharose column chromatography,[1] and human FPTase was expressed in bacculovirus, purified by affinity column chromatography.

Acknowledgements

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