Sesquiterpenoids from *Artemisia gilvescens* and an Anti-MRSA Compound

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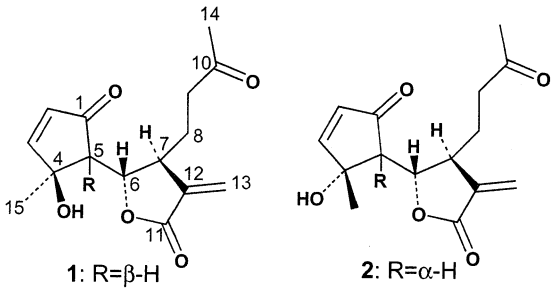
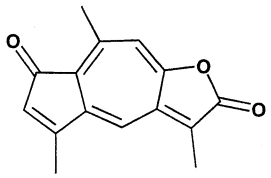
A new secoguaianolide sesquiterpene (**1**) was isolated along with its three stereoisomers (**2**−**4**) from the nonmedicinal plant *Artemisia gilvescens*. The structure of **1** was elucidated to be (4*S**,5*S**)-dihydro-5-[(1*R**,2*S**)-2-hydroxy-2-methyl-5-oxo-3-cyclopenten-1-yl]-3-methylene-4-(3-oxobutyl)-2(3*H*)-furanone on the basis of 2D NMR and other spectroscopic evidence. Five known sesquiterpenoids were also isolated from this plant, and one of them (**5**) showed activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

Artemisiae plants are very common and distributed in most parts of world, except for the Tropics, and many are used as medicinal plants. *Artemisia gilvescens* Miq. (Compositae) is in danger of extinction in the wild; it is found only in some provinces of China and Tokushima Prefecture, Japan. There are no reports of chemical research on *A. gilvescens*, and it has not been used as a medicinal plant. Recently, we have been studying nonmedicinal plants to discover new natural sources of medicines. As part of our continuing study of the Artemisiae, we herein describe some sesquiterpenoids obtained from this plant and their bioactivities.

The aerial part of *A. gilvescens* (3.3 kg) was dried and extracted with a Soxhlet extractor. Nine sesquiterpenes were isolated from the EtOAc extract. In the NMR spectrum, the signal patterns of compounds **1**−**4** were very similar, which suggested that they were closely related. In the IR spectrum, **1** showed absorbances characteristic of lactone (1764 cm^{-1}), carbonyl (1711 cm^{-1}), and hydroxyl groups (3437 cm^{-1}). Its ^{13}C NMR spectrum showed one pair of olefinic carbons, one vinyl moiety, and three carbonyl carbons. In the HREIMS spectrum, the molecular ion was detected at *m*/*z* 278.1174, consistent with a molecular formula of $C_{15}H_{18}O_5$. The degree of unsaturation (7) indicated that **1** had two rings. Together, these results suggested that **1** was a secoguaianolide. In the HMBC spectrum, long-range correlations were observed from protons of CH_3-15 (δ 1.61) to C-3, -4, and -5, from H-3 (δ 7.45) to C-1, -4, and -5, and from H-13 (δ 5.63, 6.31) to C-7, -11, and -12, and these correlations indicated that **1** had the same constitution as secotanapartholide A (**2**).[1−4]

Since compounds **2**−**4** were very similar to compound **1** on the basis of their NMR spectra, compounds **1**−**4** must be stereoisomers. By comparing the spectral data with the literature, we concluded that compounds **2** and **3** were secotanapartholides A and B, respectively, isolated from *Tanacetum parthenium*,[1−4] and compound **4** was one of the constituents of *Artemisia xerophytica*.[5] To determine the relative configuration of **1**, the NOE and proton long-range correlations of each compound were compared. In the COSY spectrum, compound **1** showed relatively strong long-range correlations between H-5 and CH_3-15, likely due to *W* coupling, and their configuration was determined to be





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trans. In contrast, the NOE spectra of compounds **3** and **4** for H-5 and CH_3-5 showed no long-range correlations. For compound **4**, the coupling pattern at H-6 was very close to that in **1**; that is, the *J* values for H-6 and -5 in compounds **1** and **4** were much smaller than those for H-6 and -7, while in compounds **2** and **3**, the *J* values for H-6 and -5 were greater than those for H-6 and -7. Consequently, the configuration of H-5, -6, and -7 in compound **1** must be the same as that in compound **4**, and the relative configuration of **1** was elucidated to be (4*S**,5*S**)-dihydro-5-[(1*R**,2*S**)-2-hydroxy-2-methyl-5-oxo-3-cyclopenten-1-yl]-3-methylene-4-(3-oxobutyl)-2(3*H*)-furanone.

Compound **5** was obtained as a yellow powder and showed IR absorptions due to a carbonyl at 1758 and 1686 cm^{-1}, while its ^{13}C NMR spectrum showed that it was comprised of 15 carbons. ^1H NMR spectroscopy showed only three protons at low field, which suggested a highly oxidized sesquiterpene. By measuring 2D NMR spectra, all of the carbons and protons were assigned and **5** was elucidated to be a known sesquiterpene that has been isolated from a marine gorgonian[6,7] and *Taraxacum wallichii*.[8] The assignments of ^1H and ^{13}C NMR data for compound **5** in the literature[8] were revised according to our present findings. By comparing physical data with those in the literature, the other sesquiterpenoids were elucidated to be 8-acetylarteminolide,[9] isosecotanapar-

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Table 1. ^{1}H NMR Data (δ) for Compounds **1**−**4**[a]

	1		2		3		4	
2	6.09	d, (4.3)	6.07	d, (5.8)	6.17	d, (5.8)	6.14	d, (5.6)
3	7.45	d, (4.3)	7.48	d, (5.8)	7.46	d, (5.7)	7.52	d, (5.6)
5	2.65	br s	2.70	d, (10.5)	2.32	d, (9.1)	2.56	d, (2.0)
6	4.49	br d, (6.3)	4.47	dd, (10.5, 2.0)	4.57	dd, (9.1, 2.5)	4.60	dd, (2.0, 6.4)
7	3.59	m	3.47	m	3.54	m	3.72	m
8	1.96	m	ca. 1.9	m	ca. 1.8	m	1.96	m
9	2.62	t, (5.4)	ca. 2.6	m	ca. 2.5	m	2.58	m
13a	5.63	d, (2.1)	5.78	d, (1.5)	5.75	d, (1.6)	5.65	d, (2.8)
13b	6.31	d, (2.1)	6.36	d, (1.8)	6.36	d, (1.9)	6.31	d, (3.2)
14	2.19	s	2.21	s	2.18	s	2.19	s
15	1.61	s	1.59	s	1.60	s	1.55	s

[a] Measured in $CDCl_3$. Coupling constants (Hz) in parentheses.

tholide,[10] 3-*O*-methylisotanapartholide,[11] and artecanin,[12] respectively.

In a test for activity against methicillin-resistant *Staphylococcus aureus* (MRSA), compound **5** showed good activity against one clinical strain with a minimum inhibitory concentration (MIC) of 1.95 μg/mL. Furthermore, in an MTT assay against the human colon carcinoma cell line HCT116, compounds **2**−**5**, 8-acetylarteminolide, isosecotanapartholide, and artecanin showed moderate cytotoxicity, i.e., IC_{50} values of 6.0, 3.3, 6.0, 16, 6.4, 13, and 14 μM, respectively.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a 1720 infrared Fourier transform spectrophotometer (Perkin-Elmer). Optical rotations were measured with a DIP-370 digital polarimeter (JASCO). NMR (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both in $CDCl_3$ referenced to TMS) spectra were measured on a ARX400 Fourier transform spectrometer (Bruker), and MS spectra were measured on a JMSD-300 mass spectrometer (JEOL). Column chromatographic supports: silica gel 60N (63-210 mm; Kanto Kagaku). TLC: silica gel $60F_{254}$ (Merck). HPLC supports: silica gel (Mightysil Si 60 250-20; Kanto Kagaku), gel-permeation column (H2001 and H2002; Shodex).

Plant Material. *Artemisia gilvescens* cultivated in The Herb Garden of University of Tokushima was obtained in August 2000 and identified by Dr. M. Ogawa, Tokushima Prefectural Museum. Specimens (HUT0041) were deposited in the Herbarium of University of Tokushima.

Extraction and Isolation. The dried aerial parts of *A. gilvescens* (3.3 kg) were crushed and extracted using a Soxhlet extractor with successive hexane and EtOAc. The EtOAc extract (58 g) was subjected to column chromatography eluted with hexane−EtOAc to obtain three fractions. Fraction 1 was separated by repeated column chromatography, HPLC, and gel permeation to yield **5** (5 mg). Fractions 2 and 3 were also separated using chromatography to give **1** (8 mg), **2** (9 mg), **3** (12 mg), **4** (12 mg), 8-acetylarteminolide (13 mg), isosecotanapartholide (29 mg), 3-*O*-methylisotanapartholide (4 mg), and artecanin (34 mg).

Compound 1, ((4*S,5*S**)-dihydro-5-[(1*R**,2*S**)-2-hydroxy-2-methyl-5-oxo-3-cyclopenten-1-yl]-3-methylene-4-(3-oxobutyl)-2(3*H*)-furanone):** colorless oil, $[\alpha]^{25}_D$ +53° (*c* 0.70 in $CHCl_3$); IR ($CHCl_3$) 3437, 1764, 1711 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Tables 1 and 2; HREIMS *m*/*z* 278.1174 (calcd for $C_{15}H_{18}O_5$, 278.1154).

Compound 2−4: NMR data in Tables 1 and 2.

Compound 5: yellow powder; IR ($CHCl_3$) 1758, 1686, 1620, 1523 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86 (1H, s, H-6), 6.54 (1H, s, H-9), 6.24 (1H, s, H-3), 2.71 (3H, s, H-14), 2.32 (3H, s, H-13), 2.12 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.2 (s, C-2), 169.2 (s, C-12), 160.8 (s, C-5), 156.1 (s, C-7), 146.0 (s, C-4), 144.7 (s, C-8), 143.4 (s, C-10), 132.7 (d, C-3), 127.1 (s, C-1), 116.9(d, C-9), 116.9 (s, C-11), 114.7 (d, C-6), 22.3 (q, C-14), 14.3 (q, C-13), 8.5 (q, C-15); HREIMS *m*/*z* 240.0816 (calcd for $C_{15}H_{12}O_3$, 240.0786).

Table 2. ^{13}C NMR Data (δ) for Compounds **1**−**4**[a]

	1	2	3	4
1	203.4	202.4	204.8	205.0
2	132.5	131.0	133.3	133.7
3	166.3	166.5	165.8	167.4
4	78.6	78.8	78.3	77.5
5	59.9	62.8	58.4	56.9
6	78.7	80.3	80.8	79.9
7	41.6	52.0	41.1	39.9
8	26.3	28.6	28.6	26.3
9	40.0	39.7	39.7	39.7
10	208.2	207.7	207.7	207.4
11	169.9	169.7	169.5	169.3
12	138.4	137.5	138.0	138.0
13	122.2	125.0	124.5	122.9
14	30.2	30.0	30.2	30.2
15	24.5	25.4	29.1	27.4

[a] Measured in $CDCl_3$.

Antibacterial Assay. Strains of methicillin-resistant *Staphylococcus aureus* (MRSA) were obtained from Tokushima University Hospital as clinical isolates. After culturing all strains on Mueller-Hinton agar (Difco, Detroit, MI) at 37 °C for 24 h, the cells were resuspended in Mueller-Hinton broth (Difco) to give 10^8 colony-forming units/mL; the suspended cells were then incubated as described above. MIC was determined using Mueller-Hinton agar according to the method described by the Japanese Society for Antimicrobial Chemotherapy (1981).[13] Cell suspensions (1×10^6 colony-forming units/mL) of MRSA were inoculated onto agar plates using a replicating device. Plates were read after 20 h incubation at 37 °C.

Measurement of Cytotoxicity Using MTT Method. Cytotoxicity was measured using the MTT method as described previously.[14]

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