Four New Coumarin Derivatives from *Artemisia keiskeana*

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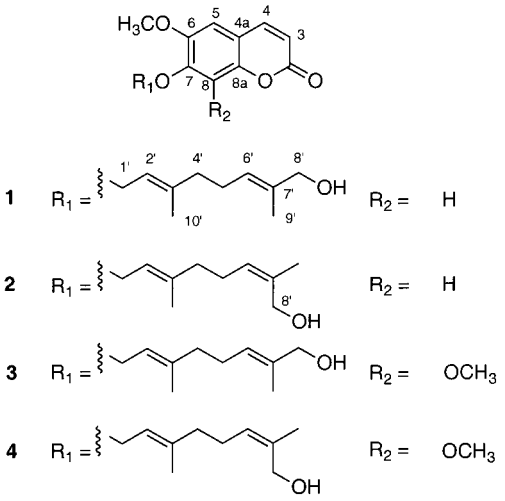
Four new coumarin monoterpene ethers, artekeiskeanols A−D (**1**−**4**), and three known coumarins, isofraxidin, fraxidin, and daphnoretin, were isolated from the whole plants of *Artemisia keiskeana*. All structures were determined from spectral data, and that of artekeiskeanol A (**1**) was confirmed by synthesis.

Coumarins are widely distributed in the plant kingdom and are present in notable amounts in the families Umbelliferae, Rutaceae, and Compositae. They have been reported to exhibit interesting biological activities (antiplatelet aggregation, cytotoxic activity, inhibition of various enzymes, antiviral, antibacterial, and antifungal activity, etc.).[1]

Artemisia keiskeana Miq. (Compositae) has been used as a traditional crude drug for the treatment of gynaecopathy, amenorrhea, bruising, and rheumatic disease.[2] The plant grows as a perennial herb in mountainous areas of Korea and is widely distributed.[3] Three coumarins and some sterols have been reported from *A. keiskeana*.[4] In this paper we report the structures of four new coumarin monoterpene ethers (**1**−**4**) and three known coumarins which have not been previously reported from this plant. We also report the isolation of the known coumarin, daphnoretin, from an *Artemisia* plant for the first time.



The MeOH extract of whole plants of *A. keiseana* was suspended in water and then partitioned consecutively with CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2-solubles were fractionated by Si gel chromatography. Selected fractions were rechromatographed on Si gel, RP-C_{18} chromatography, and reversed-phase C_{18} HPLC using different solvent combinations to yield compounds **1**−**4**. In addition, the known compounds isofraxidin,[5,6] fraxidin,[7] and daphnoretin[8,9] were also isolated and were identified by comparison of their spectral data with literature values.

Artekeiskeanol A (**1**) was obtained as an amorphous powder. The molecular formula $C_{20}H_{24}O_5$ was deduced from HREIMS. It has UV absorption maxima at 345, 295, 260 sh, 250 sh, and 230 nm, and the IR spectrum showed bands at 3420 and 1719 cm^{-1}, consistent with the presence of hydroxyl and conjugated ester carbonyl groups.

The ^1H NMR spectrum revealed signals indicative of the H-3 and H-4 protons of the coumarin nucleus [AB quartet at δ 6.24 and 7.59 (J = 9.4 Hz)],[1] two aromatic protons [δ 6.82 and 6.79 (H-5 and H-8, respectively)], and a methoxy group (δ 3.87). The ^1H NMR spectrum of **1** also showed eight more signals corresponding to two olefinic protons (δ 5.34 and 5.45), two methyleneoxy groups (δ 4.66 and 3.94), two methylene protons (δ 2.08 and 2.16), and two olefinic methyls (δ 1.63 and 1.74).

The ^{13}C NMR spectrum also revealed signals for two methyleneoxy groups (δ 66.2 and 68.7). The $^1H-^1H$ COSY (including long-range COSY 45) spectrum showed the correlation peaks H-3/H-4, H-4/H-5, H-5/OCH_3-6, H-8/H-1′, H-1′/H-2′ and H-10′, H-2′/H-4′ and H-10′, H-4′/H-5′/H-6′, and H-6′/H-9′; see structure **1**. These results, augmented by HMBC correlations (Table 3), confirmed the presence of an 8-hydroxygeranyloxy group attached to a coumarin skeleton. The methoxy and 8-hydroxygeranyloxy moieties were assigned to C-6 and C-7, respectively, because of the long-range couplings of H-5/OCH_3-6 and H-8/H-1′, and a NOE correlation of H-5/OCH_3-6. This substitution pattern was also supported by HMBC correlations, in particular, OCH_3-6/C-6 and H-1′/C-7. The configuration of the 8-hydroxygeranyloxy system was confirmed to be *E*,*E* by the NOE correlations of H-6′/H-8′, H-1′/H-10′, and H-2′/H-4′. Thus, the structure of **1** was assigned, and it is designated artekeiskeanol A.

For the purpose of structural confirmation, artekeiskeanol A was synthesized from scopoletin and *trans*,*trans*-2,6-dimethyl-2,6-octadiene-1,8-diol using the Mitsunobu reaction.[10] The spectral data of the synthetic compound were identical with that of natural artekeiskeanol A.

Compound **2** was assigned the molecular formula $C_{20}H_{24}O_5$ on the basis of HREIMS. The IR, UV, and ^1H and ^{13}C NMR data of **2** were nearly identical with those of **1** except for a few proton and carbon signals (H- and C-6′,8′,9′). In the ^{13}C NMR spectrum, signals for C-6′, -8′, and -9′ were shifted to δ 127.2, 61.5, and 21.1, respectively. These results suggested that **2** is a $\Delta^{6'}$ stereoisomer of **1**. The 2′*E*,6′*Z* configuration was assigned because of NOE correlations of H-6′/H-9′, H-5′/H-8′, and H-1′/H-10′. Comparison of the NMR data for **2** with literature values for

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

proton(s)	1	2	3	4
3	6.24 (d, 9.4)	6.25 (d, 9.3)	6.30 (d, 9.5)	6.31 (d, 9.7)
4	7.59 (d, 9.4)	7.59 (d, 9.3)	7.58 (d, 9.5)	7.58 (d, 9.7)
5	6.82 (s)	6.82 (s)	6.63 (s)	6.63 (s)
8	6.79 (s)	6.79 (s)		
1′	4.66 (d, 6.3)	4.65 (d, 6.5)	4.63 (d, 7.5)	4.62 (d, 7)
2′	5.45 (tdd, 6.5,2.4,1.2)	5.44 (tdd, 6.5,2.5,1.5)	5.52 (tdd, 6.4,2.3,1.3)	5.51 (tdd, 7.3,2.4,1.5)
4′	2.08 (br t, 7.5)	2.07 (br t, 7.5)	2.04 (br t, 7.5)	2.03 (br t, 7.3)
5′	2.16 (br q, 7.2)	2.18 (br q, 7.5)	2.11 (br q, 7)	2.13 (br q, 7.5)
6′	5.34 (tdd, 6.9,2.6,1.3)	5.21 (br t, 7)	5.32 (br t, 7)	5.21 (br t, 7.2)
8′	3.94 (s)	4.09 (s)	3.94 (s)	4.07 (s)
9′	1.63 (s)	1.73 (d, 0.8)	1.61 (s)	1.75 (d, 1)
10′	1.74 (s)	1.74 (s)	1.66 (s)	1.67 (s)
OCH_3-6	3.87 (s)	3.88 (s)	3.85 (s)	3.86 (s)
OCH_3-8			3.99 (s)	4.0 (s)

[a] Spectra were recorded in $CDCl_3$ at 500 MHz, referenced to $CDCl_3$ (δ 7.24); *J* values (Hz) are in parentheses.

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

carbon	1	2	3	4
2	161.5 (s)	161.5 (s)	160.6 (s)	160.6 (s)
3	113.3 (d)	113.3 (d)	115.1 (d)	115.1 (d)
4	143.3 (d)	143.3 (d)	143.5 (d)	143.5 (d)
4a	111.3 (s)	111.3 (s)	114.4 (s)	114.4 (s)
5	108.1 (d)	107.9 (d)	103.5 (d)	103.5 (d)
6	146.6 (s)	146.5 (s)	150.6 (s)	150.6 (s)
7	152.1 (s)	151.9 (s)	144.7 (s)	144.7 (s)
8	101.2 (d)	101.1 (d)	141.6 (s)	141.7 (s)
8a	149.9 (s)	149.8 (s)	142.9 (s)	142.9 (s)
1′	66.2 (t)	66.2 (t)	70.1 (t)	70.1 (t)
2′	118.8 (d)	118.8 (d)	119.8 (d)	120.0 (d)
3′	141.5 (s)	141.6 (s)	142.1 (s)	142.1 (s)
4′	39.0 (t)	39.5 (t)	39.1 (t)	39.5 (t)
5′	25.5 (t)	25.6 (t)	25.7 (t)	25.8 (t)
6′	125.0 (d)	127.2 (d)	125.2 (d)	127.3 (d)
7′	135.4 (s)	134.9 (s)	135.1 (s)	134.8 (s)
8′	68.7 (t)	61.5 (t)	68.7 (t)	61.4 (t)
9′	13.7 (q)	21.1 (q)	13.6 (q)	21.2 (q)
10′	16.7 (q)	16.9 (q)	16.3 (q)	16.4 (q)
OCH_3-6	56.3 (q)	56.3 (q)	56.2 (q)	56.2 (q)
OCH_3-8			61.7 (q)	61.7 (q)

[a] Spectra were recorded in $CDCl_3$ at 125 MHz, referenced to $CDCl_3$ (δ 77); multiplicities were implied from DEPT experiments; assignments made by HMQC and HMBC experiments.

similar compounds[11] also supported this conclusion. Thus, artekeiskeanol B was confirmed to have structure **2**.

Compound **3**, artekeiskeanol C, was obtained as a yellowish, amorphous powder and was assigned the molecular formula $C_{21}O_{26}O_6$ from HRESIMS and NMR data. The ^{1}H and ^{13}C NMR data of **3** were similar with those of **1** except for the H- and C-8 signals of **1** that were replaced by those of a methoxy group (δ 3.99 and 61.7) and a quaternary carbon (δ 141.6), respectively, in **3**. Two methoxy groups and an 8-hydroxygeranyloxy group were located at C-6, C-8, and C-7 of the coumarin, respectively, as shown by long-range ^{1}H−^{1}H COSY 45 and NOE correlations of H-5/OCH_3-6 and HMBC correlations of H-5/C-6 and C-7, H-1′/C-7, OCH_3-6/C-6, and OCH_3-8/C-8. The *E*,*E* configuration was assigned to the 8-hydroxygeranyloxy moiety based on NOE correlations of H-6′/H-8′ and H-1′/H-10′ and close similarity between the NMR data for **3** and that for **1**. Thus, artekeiskeanol C was confirmed to have structure **3**.

The molecular formula $C_{21}H_{26}O_6$ was deduced for **4**, artekeiskeanol D, from HRESIMS and NMR data. The spectral data of **4** were nearly identical with those of **3** except for the signals for H- and C-6′, -8′, -9′ that in **4** were shifted a little. The configuration for the 8-hydroxygeranyloxy system was assigned 2′*E*,6′*Z* by comparison of the NMR data for **4** with that of **2**, and NOE correlations of H-6′/H-9′, H-5′/H-8′, and H-1′/H-10′. Thus, structure **4** was confirmed.

The brine shrimp lethality test[12] was carried out on the isolated compounds. Compounds **1** and **2** were weakly toxic to brine shrimp, with LC_{50} values of between 60 and 70 μg/mL. The other compounds were inactive at 100 μg/mL.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 16F-PC FT-IR spectrophotometer. UV spectra were obtained on a Hewlett-Packard HP 8453 diode array instrument. NMR experiments were performed on a Bruker AMX-500 spectrometer with the usual pulse sequences, and data processing was performed with the Bruker software. NMR signals are reported in parts per million (δ), referenced to $CDCl_3$. EIMS and HREIMS data were obtained on a VG70-VSEQ mass spectrometer. ESIMS and HRESIMS data were measured on a Micromass Q-TOF mass spectrometer. Column chromatography was carried out on Si gel 60 (Merck, 230−400 mesh) and RP-18 (Merck, 40−63 μm), and preparative HPLC was performed using a LiChroCART LiChrosorb RP-18 (250 × 10 mm) column and a UV detector (254 nm).

Plant Material. Whole plants of *Artemisia keiskeana* Miq. were collected in August 1995 at Suwon, Korea. A voucher specimen (95-SW-31) is deposited in the Department of Chemistry, Konyang University.

Extraction and Isolation. Fresh whole plants of *A. keiskeana* (2 kg) were extracted with MeOH twice at room temperature. The MeOH extract was evaporated to dryness under reduced pressure, and the residue was suspended in H_2O. The resulting solution was partitioned with CH_2Cl_2, EtOAc, and *n*-BuOH consecutively. A portion (15 g) of the CH_2Cl_2-solubles was fractionated on a Si gel column using a stepwise elution with hexanes−EtOAc (10:1, 5:1, 3:1, 2:1, 1:1, and 1:2). Fractions were combined based on their TLC pattern to yield fractions F01−F10. Fraction F08 was rechromatographed over a RP-C_{18} column with 50% H_2O−MeCN as eluent to obtain isofraxidin (15.8 mg), fraxidin (5.3 mg), daphnoretin (7 mg), and a mixture of compounds **1**−**4**. The mixture was resolved by C_{18} reversed-phase HPLC (37% H_2O−MeOH) to yield compounds **1**−**4** (16.9, 15.5, 15.0, and 7.2 mg, respectively).

Artekeiskeanol A (1): colorless, amorphous powder; IR (film) ν_{max} 3420, 1925, 1719, 1617, 1559, 1508, 1278, 1152, 994 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 230 (29 185), 250 sh, 260 sh, 295 (10 014), 345 (21 017); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* 344 $[M]^+$, 326, 245, 192 (100), 134; HREIMS *m*/*z* 344.1621 $[M]^+$ (calcd for $C_{20}H_{24}O_5$, 344.1624).

Synthesis of Artekeiskeanol A (1).[10] *trans*,*trans*-2,6-Dimethyl-2,6-octadiene-1,8-diol (Aldrich) (50 mg, 0.294 mmol) was dissolved in THF (0.6 mL) under an N_2 atmosphere, freshly distilled triphenylphosphine was added (126 mg, 0.482 mmol), and the mixture was cooled at −25 °C (in dry ice/20%

Table 3. Selected HMBC Correlations of Compounds **1**−**4**[a]

position	1	2	3	4
3	C-2,4a	C-2,4a	C-2,4a	C-2,4a
4	C-2,5,8a	C-2,4a,5,8a	C-2,4a,5,8a	C-2,4a,5,8a
5	C-4,6,7,8a	C-4,7,8a	C-4a,6,7,8a	C-6,7,8a
8	C-4a,6,7,8a	C-4a,6,8a		
1′	C-7,2′,3′	C-7,2′,3′	C-7,2′,3′	C-7,2′,3′
2′	C-4′,10′	C-4′,10′	C-4′,10′	C-4′,10′
4′	C-2′,3′,5′,6′,10′	C-2′,3′,5′,6′,10′	C-2′,3′,5′,6′,10′	C-2′,3′,5′,6′,10′
5′	C-3′,4′,6′,7′	C-3′,4′,6′,7′	C-3′,4′,6′,7′	C-3′,4′,6′,7′
6′	C-5′,8′,9′	C-8′,9′	C-5′,8′,9′	C-8′,9′
8′	C-6′,7′,9′	C-6′,7′,9′	C-6′,7′,9′	C-6′,7′,9′
9′	C-6′,7′,8′	C-6′,7′,8′	C-6′,7′,8′	C-6′,7′,8′
10′	C-2′,3′,4′	C-2′,3′,4′	C-2′,3′,4′	C-2′,3′,4′
OCH_3-6	C-6	C-6	C-6	C-6
OCH_3-8			C-8	C-8

[a] HMBC experiments maximized for $J = 9$ Hz.

Table 4. Selected NOE Correlations of Compounds **1**−**4**[a]

position	1	2	3	4
3	H-4	H-4	H-4	H-4
4	H-3,5	H-3,5	H-3,5	H-3,5
5	H-4 (OMe-6)	H-4 (OMe-6)	H-4 (OMe-6)	H-4 (OMe-6)
8	H-1′	H-1′		
1′	H-8,2′,10′	H-8,2′,10′	H-2′,10′	H-2′,10′
2′	H-1′,4′	H-1′,4′	H-1′,4′	H-1′,4′
4′	H-2′	H-2′	H-2′	H-2′
5′		H-8′		H-8′
6′	H-8′	H-9′	H-8′	H-9′
8′	H-6′	H-5′	H-6′	H-5′
9′		H-6′		H-6′
10′	H-1′	H-1′	H-1′	H-1′
OCH_3-5	H-5	H-5	H-5	H-5
OCH_3-8				

[a] NOESY spectra were obtained using a 0.3 s mixing time.

$CaCl_2$). Neat diethyl azodicarboxylate (DEAD) was injected (16.8 mg, 0.064 mmol), and the mixture was stirred for 10 min at −25 °C. After 10 min, exactly one-fifth of a solution of scopoletin (Aldrich) (47 mg, 0.244 mmol) in 0.5 mL of THF (with a few drop of DMF) was added dropwise. The resulting mixture was stirred for 1 h at −25 °C. The same sequential addition of DEAD and scopoletin was repeated four more times. The reaction mixture was stirred for 2 h following the last addition of scopoletin and then was warmed to room temperature. The white precipitate (diethyl hydrazodicarboxylate) was removed by filtration through a pad of Celite and Si gel. The filtrate was purified by RP-18 (40% H_2O−MeCN) and Si gel column chromatography [hexanes−EtOAc (1:1.2)] to give artekeiskeanol A (**1**) (58 mg, 68.8%).

Artekeiskeanol B (2): colorless, amorphous powder; IR (film) ν_{max} 3410, 1722, 1562, 1512, 1276, 1145 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 229 (27 685), 252 sh, 262 sh, 296 (10 150), 344 (18 227); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* 344 $[M]^+$, 326, 192 (100); HREIMS *m*/*z* 344.1626 $[M]^+$ (calcd for $C_{20}H_{24}O_5$, 344.1624).

Artekeiskeanol C (3): yellowish, amorphous powder; IR (film) ν_{max} 3400, 1723, 1605, 1563, 1292, 1152, 1125, 1041 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 230 sh, 297 (8766), 339 (6619); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* 374 $[M]^+$, 356, 222 (100); ESIMS *m*/*z* 397 $[M + Na]^+$; HREISMS *m*/*z* 397.1574 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_6Na$, 397.1627).

Artekeiskeanol D (4): yellowish, amorphous powder; IR (film) ν_{max} 3400, 1714, 1605, 1576, 1309, 1156, 1121, 1038 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 230 sh, 305 sh, 343 (5866); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* 374 $[M]^+$, 357, 275, 222 (100); ESIMS *m*/*z* 397 $[M + Na]^+$; HREISMS *m*/*z* 397.1600 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_6Na$, 397.1627).

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