galanthamine content, Ice Follies grew the most under conditions of different depth and had the highest galanthamine content. The data of a bulb increase (%), in this study, was lower than the increases reported for Fortune cultivar bulbs harvested after one year of growth at Rosewarne in England. Double nosed bulbs grown in beds, with a starting planting weight density of 2 kg/m^2, were reported to have a growth increase of 120% (5). At our starting planting densities, it is not expected that there should be any inhibition of a percentage weight increase based upon competition for nutrients or water. According to Anderson, 1989, Mississippi is not an ideal climate for growth of daffodils and planting in October may have affected by decreasing growth, each week delayed in planting schedule decreases growth.

Under high-density plantings, the growth rate of all four cultivars was reduced (data not shown). In contrast, planting density did not affect galanthamine content (Table **2**). Statistical analysis of other treatments such as flower bud removal and bulb size, reveled no effect on galanthamine content. There was a significant difference in the means for galanthamine content among cultivars (Table **1** and **2**). Ice Follies and Mount Hood had high content of galanthamine, 76 and 59.2 mg/100 g dry weight respectively, whereas White Cheerfulness and Geranium had reasonable growth, but poor galanthamine content. Galanthamine content was unaffected in all treatments.

Based on our results, Ice Follies bulb growth increased up to 69.3%, this should produce 1.43 kg/m^2 of new biomass per year. Extractable biomass production for Ice Follies per hectare would therefore be 14,300 kg, and the total yield of galanthamine per hectare would be 3.4 kg. In a commercial-scale drug isolation is predicted to be 65% efficiency which would result in a galanthamine yield of 2.21 kg per hectare. According to Rees (5), an important consideration in *Narcissus* biomass growth increases is planting densities; daffodil bulbs yield per plant falls as density increases but a biomass yield per unit area of land increases. Since galanthamine content per unit of biomass was unaffected by high density plantings, galanthamine yields per unit of production area might be the same. Depending on a growth increase, planting density may increase galanthamine yields per unit of production area. The economics of high density planting vs non dense plantings as for galanthamine yields will depend on the price of land, availability and price of propagation stock, labor and other associated agricultural costs. Any growth enhancement of the daffodil biomass should benefit total yield, and the result would be a decrease of the required land area and associated costs for commercial galanthamine production. Our results showed that *Narcissus* cultivars have low coefficients of variation which infer that galanthamine content is very stable. Plant sources with reduced variation make for more predictably and probably more economical commercial production.

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Artekeiskeanin A: A New Coumarin-Monoterpene Ether from *Artemisia keiskeana*

Jong Hwan Kwak[1,2], Woo Young Jang[1], Ok Pyo Zee[1], and Kang Ro Lee[1,3]

[1] Department of Pharmacognosy and Natural Products Chemistry, College of Pharmacy, SungKyunKwan University, Suwon 440-746, Korea
[2] Present address: Division of Applied Science, Korea Institute of Science and Technology, Seoul 136-650, Korea
[3] Address for correspondence

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Abstract: **A new coumarin-monoterpene ether, artekeiskeanin A (1) and two known coumarins, dracunculin (2) and scopoletin (3) were isolated from the aerial parts of *Artemisia keiskeana*. The structure of 1 was determined to be 7-(*trans*-8-oxogeranyloxy)-6-methoxycoumarin on the basis of spectroscopic studies.**

Artemisia keiskeana Miq. (Compositae) has been used as a traditional Chinese drug for the treatment fo gynaecopathy, amenorrhea, bruise, and rheumatic disease (1). This plant grows as a perennial herb in mountainous areas of Korea and is widely distributed (2). As a part of a continuing investigation of the genus *Artemisia* in Korea, we have examined the constituents of *Artemisia keiskeana*, which has not previously been studied.

In our studies of the aerial parts of this plant a new coumarin-monoterpene ether, artekeiskeanin A (**1**), was isolated from the EtOH extract. This paper deals with the isolation and structural elucidation of artekeiskeanin A (**1**). Two coumarins, dracunculin (**2**), scopoletin (**3**), a phytosterol mixture, and β-sitosterol 3-*O*-β-D-glucopyranoside were also isolated and identified.

The two known coumarins, dracunculin (**2**) and scopoletin (**3**) were characterized by direct comparison of the physical and spectroscopic properties with published data (3–5) and with commercially available scopoletin.

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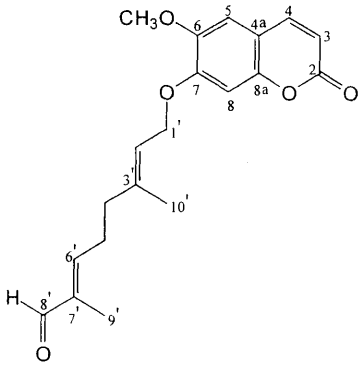
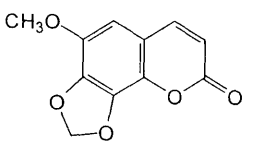


1



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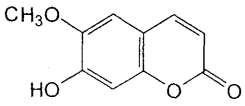

3

The new coumarin-monoterpene ether **1** was obtained as an amorphous powder, identified and characterized by spectroscopic methods. It has UV absorption maxima at 343, 294, and 227 nm and observed on TLC as a bluish white fluorescent spot under UV light (365 nm), suggestive of a coumarin (6). The empirical formula was found to be $C_{20}H_{22}O_5$ from high-resolution EIMS data. The ^{1}H-NMR spectrum (Table **1**) revealed the typical H-3 and H-4 protons of the coumarin nucleus which appeared as an AB quartet at δ = 6.26 and 7.62 (6). Two signals at δ = 6.81 and 6.87 (singlets) indicated aromatic protons of H-8 and H-5, respectively and revealed the presence of a methoxy group at δ = 3.92.

The proton signals of H-5 and H-8 were confirmed by an nOe difference experiment, where irradiation of the signal at δ = 7.62 (H-4) enhanced the signals for the H-5 and H-3 protons. Eight more signals indicated two olefinic protons (δ = 5.54 and 6.42), two methyls (δ = 1.74 and 1.82), an aldehyde group (δ = 9.33), methyleneoxy protons (δ = 4.71), and two methylene protons (δ = 2.29 and 2.52). In the ^{1}H-^{1}H homonuclear COSY spectrum (Table **1**), an olefinic proton at δ = 5.54 showed coupling to methyleneoxy protons (δ = 4.71), methylene protons (δ = 2.29), and a methyl group (δ = 1.82), and another olefinic proton at δ = 6.42 also was observed to couple to methylene protons (δ = 2.52) and a methyl group (δ = 1.74). The methyl protons at δ = 1.82 also showed coupling to methyleneoxy protons (δ = 4.71), an olefinic proton (δ = 5.54), and methylene protons (δ = 2.29). These observations including other ^{1}H-^{1}H homonuclear correlations (Table **1**) required the presence of 8-oxogeranyloxy system. The methoxy group and 8-oxogeranyloxy system were located at C-6 and C-7 of the coumarin, respectively, as shown by long-range couplings of H-5/OCH_3 and H-8/H-1′. These attachments were further supported by nOe difference experiments. When the methoxy group and H-1′ methyleneoxy protons were irradiated, the nOe were observed in the protons at δ = 6.87 (H-5, 12.7%) and 6.81 (H-8, 15.2%), respectively. Also, configuration of *trans*-8-oxogeranyloxy system was confirmed by the nOe difference experiment, when the aldehyde group was irradiated, an nOe (16%) was observed in the proton at δ = 6.42 (H-6′). In the HMQC spectrum of compound **1**, all direct ^1J connectivities between carbons and protons were determined. The assignments of the ^{1}H- and ^{13}C-NMR chemical shift values of compound **1** were based on the HMQC and HMBC correlation (Table **1**). On this basis the new coumarin-monoterpene ether has been assigned structure **1** (7-[*trans*-8-oxogeranyloxy]-6-methoxycoumarin).

Materials and Methods

Melting points were measured on a Gallenkamp melting point apparatus (uncorr.). ^{1}H- and ^{13}C-NMR were recorded on a Bruker AM-300 or Bruker AMX-500 spectrometer. UV spectra were obtained on a Shimadzu UV_{240} UV-Visible recording spectrophotometer. IR spectra were measured on Shimadzu IR-435 Infrared spectrophotometer (KBr disc). Mass spectra were recorded on a Hewlett-Packard 5985B GC/MS system equipped

Table 1 ^{13}C- (125 MHz) and ^{1}H-NMR (500 MHz) data for compound **1** (in $CDCl_3$).

Position	δ^{13}C	δ^1H (multiplicity, J {H})	^{1}H-^{1}H COSY[a]	^{1}H-^{13}C long range correlations[b]
2	161.4			
3	113.5	6.26 (d, 9.5)	4	C-2, 4a
4	143.3	7.62 (d, 9.5)	3, 5, 8	C-2, 5, 8a
4a	111.5			
5	108.3	6.87 (s)	4, 6-OCH_3	C-4, 6, 7, 8a
6	146.7			
7	151.9			
8	101.2	6.81 (s)	4, 1′	C-4a, 6, 7, 8a
8a	149.9			
1′	66.0	4.71 (br. d, 6.4)	8, 2′, 4′, 10′	C-7, 2′, 3′
2′	119.8	5.54 (br. t, 6.4)	1′, 4′, 10′	C-4′, 10′
3′	140.4			
4′	37.8	2.29 (br. t, 7.5)	1′, 2′, 5′, 10′	C-2′, 3′, 5′, 6′, 10′
5′	26.9	2.52 (br. q, 7.5)	4′, 6′, 9′	C-4′, 6′, 7′
6′	153.1	6.42 (br. t, 7.5)	5′, 9′	C-4′, 5′, 8′, 9′
7′	139.8			
8′	195.0	9.33 (s)	9′	C-6′, 7′, 9′
9′	9.2	1.74 (s)	5′, 6′, 8′	C-6′, 7′, 8′
10′	16.8	1.82 (s)	1′, 2′, 4′	C-2′, 3′, 4′
OCH_3	56.4	3.92 (s)	5	C-6

[a] Major ^{1}H-^{1}H correlations observed in COSY-45 and long range COSY-45 experiments (geminal coupling not shown).
[b] Major ^{1}H-^{13}C long range correlations determined from an HMBC experiment.

with direct inlet system, and high reslution mass spectrum was obtained on a VG70-VSEQ mass spectrometer (VG Analytical, UK). TLC and column chroamtography were carried out on Merck precoated silica gel F_{254} plates, Rp-18 F_{254s} plates and on Si gel 60 (Merck, 230–400 mesh) or Lichroprep RP-18 (Merck, 40–63 μm). All other chemicals and solvents were analytical grade and used without further purification.

Aerial parts of *Artemisia keiskeana* were collected in August, 1993 at Suwon, Kyunggido, Korea. The voucher specimen (SKKU 93-032) is deposited in the herbarium of college of pharmacy, SungKyunKwan University.

Fresh aerial parts of *Artemisia keiskeana* (3.0 kg) were extracted with 95% EtOH at room temperature (2 times). The concentrated EtOH extract (136 g) was dissolved in hexane/EtOAc (1 : 1, 700 ml). The concentrated extract (57 g) of the hexane/EtOAc soluble portion was subjected to column chromatography over SiO_2 (1.2 kg) eluting sequentially with hexane/EtOAc (1 : 1, 2 l), hexane/EtOAc (1 : 2, 1 l), hexane/EtOAc/MeOH (20 : 20 : 1, 1 l), hexane/EtOAc/MeOH (10 : 10 : 1, 1 l), and hexane/EtOAc/MeOH (5 : 5 : 1, 1 l), the eluates were fractionated by TLC to yield fractions designated as HE1-HE10: void volume (800 ml), HE1 (950 ml), HE2 (390 ml), HE3 (450 ml), HE4 (480 ml), HE5 (350 ml), HE6 (410 ml), HE7 (330 ml), HE8 (560 ml), HE9 (350 ml), and HE10 (770 ml). Compound **1** (47 mg) was obtained from repeated column chromatography of fraction HE5 on SiO_2 (200 g, hexane/$CHCl_3$/MeOH, 20 : 20 : 1, and 75 g, cyclohexane/EtOAc/MeOH, 35 : 15 : 3) and finally purified with reverse-phase column chromatography (RP-18, 40–63 μm, 1.7 × 35 cm, 60% MeCN). Fraction HE3 was chromatographed on SiO_2 (100 g) with hexane/EtOAc (1 : 1) and was recrystallized from MeOH to give compound **2** (54 mg). Fraction HE6 was purified by column chromatography on SiO_2 (125 g and 50 g) eluting with hexane/EtOAc (1 : 1) and hexane/$CHCl_3$/MeOH (10 : 10 : 1) and recrystallized from MeOH/EtOAc to give colorless needles (compound **3**, 32 mg). Fractions HE2 and HE9 were further chromatographed repeatedly on SiO_2 column and eluted with various solvent systems: HE2; I hexane/EtOAc (5 : 1), SiO_2 (225 g), II hexane : $CHCl_3$/ETOac (30 : 3 : 7), SiO_2 (100 g), HE9; I hexane/$CHCl_3$/EtOAc/MeOH (5 : 5 : 10 : 1), SiO_2 (200 g), II CH_2Cl_2/MeOH/H_2O (100 : 10 : 0.25), SiO_2 (75 g), which afforded a phytosterol mixture (135 mg, campesterol/stigmasterol/β-sitosterol, 10 : 36 : 100) and β-sitosterol 3-*O*-β-D-glucopyranoside (88 mg), resepctively, as colorless powders.

Artekeiskeanin A (**1**): amorphous powder, m.p. 102 °C; EI-MS: *m/z* (%) = 342 (M^+, 11.2), 192 (100), 177 (23.7), 164 (18.8), 149 (3.7); HR-EI-MS: *m/z* [M^+] 342.1471 calcd for $C_{20}H_{22}O_5$ 342.1467; IR: ν_{max}^{KBr} = 3100, 2910, 1710, 1680, 1610, 1550, 1510, 1430, 1380, 1270, 1140 cm^{-1}; UV: λ_{max}^{MeOH} = 213, 227, 257sh, 294, 343 nm; ^{1}H- and ^{13}C-NMR data are presented in Table **1**.

Dracunculin (**2**): amorphous powder, m.p. 218 °C; ^{13}C-NMR ($CDCl_3$): δ = 159.8 (C-2), 114.3 (C-3), 143.6 (C-4), 114.4 (C-4a), 105.1 (C-5), 141.1 (C-6), 133.9 (C-7), 135.0 (C-8), 139.8 (C-8a), 56.8 (OCH_3), 103.6 (-OCH_2O-). The IR, UV, EI-MS, and ^{1}H-NMR data were in good agreement with those of dracunculin (3, 4) while ^{13}C-NMR data have not yet been reported.

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