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Monoterpenoids from the aerial parts of *Aruncus dioicus* var. *kamtschaticus* and their antioxidant and cytotoxic activities

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ABSTRACT

The aerial parts of *Aruncus dioicus* var. *kamtschaticus* afforded five new monoterpenoids (**1**–**5**): 4-(*erythro*-6,7-dihydroxy-9-methylpent-8-enyl)furan-2(5*H*)-one (**1**, aruncin A), 2-(8-ethoxy-8-methylpropylidene)-5-hydroxy-3,6-dihydro-2*H*-pyran-4-carboxylic acid (**2**, aruncin B), 4-(hydroxymethyl)-6-(8-methylprop-7-enyl)-5,6-dihydro-2*H*-pyran-2-one-11-*O*-β-D-glucopyranoside (**3**, aruncide A), (3*S*,4*S*,5*R*,10*R*)-3-(10-ethoxy-11-hydroxyethyl)-4-(5-hydroxy-7-methylbut-6-enyl)oxetan-2-one-11-*O*-β-D-glucopyranoside (**4**, aruncide B), and (3*S*,4*S*,5*R*,7*R*)-5-(9-methylprop-8-enyl)-1,6-dioxabicyclo[3,2,0]heptan-2-one-7-(hydroxymethyl)-12-*O*-β-D-glucopyranoside (**5**, aruncide C). Compound **2** showed potent cytotoxicity against Jurkat T cells with an IC_{50} value of 17.15 μg/mL. In addition, compounds **7** and **10** exhibited moderate antioxidant activity with IC_{50} values of 46.3 and 11.7 μM, respectively.

Ulleungdo is located 130 km from the mainland of Korea and has special local products including edible wild vegetables and marine products.[1] *Aruncus dioicus* var. *kamtschaticus* (Rosaceae) is the major wild leafy vegetable produced in Ulleungdo.[2] Biological studies have revealed that this plant possesses numerous effects such as antioxidant,[3,4] antidiabetic,[5] and anti-AIDS[6] effects and can be used in the prevention and treatment of ischemic and degenerative brain diseases.[7] The dried sprouts of this plant have been used for foods and the aerial parts to treat detoxification and tonsillitis.[8] However, their chemical constituents have not been reported.

This study was conducted to identify the bio-active compounds in this plant. The aerial parts of *A. dioicus*[9] were extracted with 95% EtOH, concentrated and fractionated into *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH and H_2O fractions. The EtOH extract and fractions were examined for their DPPH radical scavenging activity.[10,11] Among the samples tested, the CH_2Cl_2 and EtOAc fractions showed radical scavenging activity.

Activity-directed isolation of the CH_2Cl_2 and EtOAc fractions resulted in the identification of five new monoterpenoids (**1**–**5**) (Fig. 1) and seven known compounds (**6**–**12**) by RP-C18 and silica gel column chromatographies.[12] In this Letter, we report the isolation and structural elucidation of these compounds, their cytotoxic activity against Jurkat T cells, and their DPPH radical scavenging activity.

Compounds **6**–**12** were identified as: prunasin (**6**),[13] 1-feruloyl-β-D-glucose (**7**),[14] 1-*p*-coumaroyl-β-D-glucose (**8**),[15] kaempferol 3-*O*-β-D-glucoside (**9**),[16] quercetin-3-*O*-β-D-glucopyranoside (**10**),[17] kaempferol 3-*O*-β-D-(6-*E*-*p*-coumarylglucoside) (**11**),[18] and 2-hydroxy-3-phenylpropanoic acid (**12**),[19] by direct comparison with authentic samples or by comparing their physical data with that in the literature.

Compound **1** was isolated as a colorless syrup. The FABMS values of **1** gave a $[M+Na]^+$ peak at *m/z* 221. The HRFABMS gave *m/z* 221.0793 for the $[M+Na]^+$, which corresponded to the molecular formula of $C_{10}H_{14}O_4Na$ (calcd 221.0790). Its IR (ν_{max} 3433, 1743 cm^{-1}) data suggested the presence of a hydroxy group and an α,β-unsaturated γ-lactone function. The ^1H NMR spectrum indicated an α,β-unsaturated γ-lactone moiety with two signals at δ_H 7.45 (1H, d, J = 1.2 Hz, H-3) and 4.87 (2H, d, J = 1.2 Hz, H_2-5), and there were corresponding ^{13}C NMR signals at δ_C 149.4 (C-3), 133.7 (C-4), 71.4 (C-5), and 174.2 (C-2). A combination of the ^1H, ^{13}C NMR, HMBC and COSY (Fig. 2) spectra suggested that the substituent at C-4 was a 1,2-dihydroxy-4-methyl-3-pentenyl side-chain [{δ_H 4.54 (1H, d, J = 4.4 Hz, H-6), δ_C 70.1 (C-6)}, {δ_H 4.60 (1H, dd, J = 9.0, 4.4 Hz, H-7), δ_C 69.6 (C-7)}, {δ_H 5.21 (1H, d, J = 9.0 Hz, H-8), δ_C 122.8 (C-8), δ_C 136.4 (C-9)}, {δ_H 1.70, 1.74 (3H each, s, H_3-10, 11), δ_C 17.4, 24.9 (C-10, 11)}]. The presence of two OH groups in **1** was suggested by the formation of a diacetate (**1a**) of **1**, which gave the expected molecular ion M^+ at *m/z* 282 in the EIMS and exhibited twos singlets at δ 2.08 and δ 2.04 in the ^1H NMR spectrum of **1a**. The absolute configuration at C-6 and C-7 in **1** could not be determined by Mosher's ester methodology[20–22] because of the decomposition

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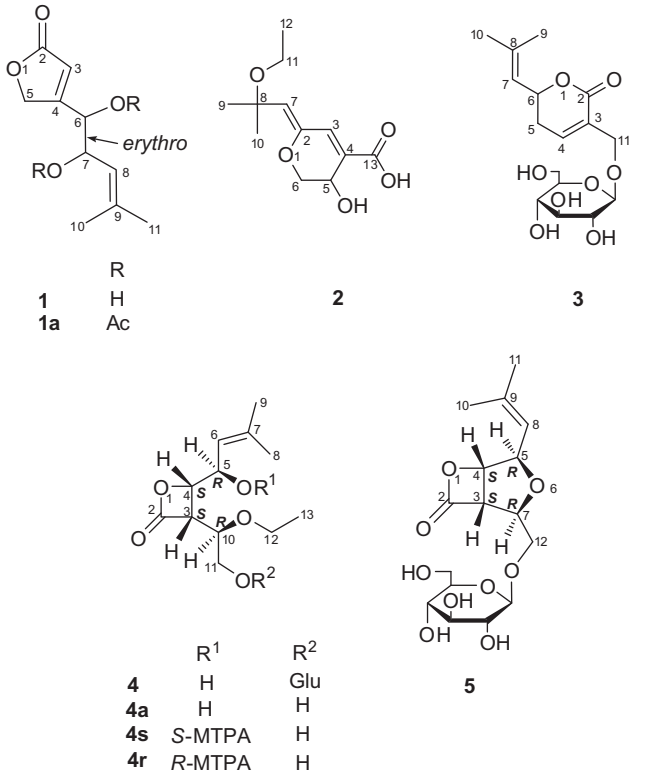

Figure 1. Structures of compounds **1**–**5** isolated from the CH_2Cl_2 and EtOAc fractions of *A. dioicus* var. *kamtschaticus*.

of **1** during the reaction. In order to deduce the absolute configuration of an acyclic 1,2-diol moiety in **1**, a CD method employing dimolybdenum tetraacetate [$Mo_2(AcO)_4$] developed by Snatzke[23] and Frelek[24] was applied to **1** to obtain its CD spectrum in the region of 550–250 nm. According to the rule proposed by Snatzke, the sign of the CD band around 305 nm, which has been assigned to a metal-to-ligand charge-transfer transition,[23] correlates with the absolute configuration of the acyclic 1,2-diol moiety in the ligating structure.[24] The rule states that a complex of a '*R*' or '*R,R*' 1,2-diol with dimolybdenum tetraacetate always gives rise to a negative CD band around 305 nm, whereas a complex having a '*S*' or '*S,S*' 1,2-diol always gives rise to a positive CD band around 305 nm. The CD spectrum of **1** did not show appreciable cotton effects in the range investigated, apart from occasional tails below 200–500 nm, which did not interfere significantly in the CD spectrum.[25] Therefore, the relative of C-6/C-7 in **1** was determined as erythro. Thus, compound **1** was confirmed to be 4-(*erythro*-6,7-dihydroxy-9-methylpent-8-enyl)furan-2(5*H*)-one and named aruncin A.

Compound **2** was isolated as a colorless syrup. The molecular weight of **2** was suggested by a $[M+Na]^+$ peak at *m/z* 265 in the FABMS. The HRFABMS spectral analysis of **2** showed the $[M+Na]^+$ at *m/z* 265.1056, which corresponded to the molecular formula of $C_{12}H_{18}O_5Na$ (calcd 265.1052). The IR spectrum of **2** indicated absorption bands due to hydroxy and α,β,γ,δ-conjugated carboxylic acid functions at 3405 and 1724 cm^{-1}, respectively. The ^1H and ^{13}C NMR spectra of **2** showed signals of 5,6-dihydro-2*H*-pyran with carboxyl and hydroxy groups at δ_H 7.47 (1H, s, H-3), 4.29 (1H, dd, *J* = 5.6, 4.4 Hz, H-5), 3.76 (1H, dd, *J* = 12.0, 4.4 Hz, H-6a), and 3.65 (1H, dd, *J* = 12.0, 5.6 Hz, H-6b). The corresponding carbon resonances of these protons were observed at δ_C 147.4 (C-2), 143.1 (C-3), 132.1 (C-4), 76.7 (C-5), 64.2 (C-6), and 170.8 (C-13) in the HSQC and HMBC spectra. A combination of the ^1H, ^{13}C NMR, HMBC and COSY (Fig. 2) spectra suggested that the substituent at C-2 was a 2-ethoxy-2-methyl-3-propenyl side-chain [{δ_H 5.57 (1H, s, H-7), δ_C 125.0 (C-7)}, {δ_H 1.50 (6H, s, H_3-9,10), δ_C 30.3 (C-9,10)}, {δ_H 3.57 (2H, q, *J* = 7.2 Hz, H_2-11), δ_C 66.7 (C-11)}, {δ_H 1.22 (3H, t, H_3-12), δ_C 15.8 (C-12)}]. The absolute stereochemistry of C-5 in **2** couldn't be determined by Mosher's ester methodology[20–22] because of the decomposition of **2** during the reaction. Therefore, compound **2** was established as 2-(8-ethoxy-8-methylpropylidene)-5-hydroxy-3,6-dihydro-2*H*-pyran-4-carboxylic acid and named aruncin B.

Compound **3** was obtained as a colorless syrup. The molecular weight of **3** was suggested by a $[M+Na]^+$ peak at *m/z* 367 in the FABMS. The HRFABMS gave a molecular ion peak at *m/z* 367.1369, consistent with a molecular composition of $C_{16}H_{24}O_8Na$ (calcd 367.1374). The IR spectrum of **3** indicated absorption bands due to hydroxy and α,β-unsaturated lactone at 3401 cm^{-1} and 1744 cm^{-1}, respectively. The ^1H NMR spectrum indicated an α,β-unsaturated δ-lactone moiety with four protons at δ_H 6.40 (1H, dd, *J* = 2.8, 2.0 Hz H-4), 3.11 (1H, ddd, *J* = 16.0, 7.2, 2.0 Hz, H-5$_a$), δ_H 2.63 (1H, ddd, *J* = 16.0, 6.0, 2.8 Hz, H-5$_b$), and 5.28 (1H, ddd, *J* = 8.8, 7.2, 6.0 Hz, H-6), and there were corresponding ^{13}C NMR signals at δ_C 127.9 (C-3), 140.4 (C-4), 36.7 (C-5), 76.9 (C-6), and 171.9 (C-2). A combination of the ^1H, ^{13}C NMR, HMBC and COSY (Fig. 2) spectra of **3** suggested that the substituent at C-6 was an isobutenyl side-chain [{δ_H 5.25 (1H, d, *J* = 8.8 Hz, H-7), δ_C 124.6 (C-7), δ_C 141.1 (C-8)}, {δ_H 1.75 (3H, s, H_3-9), δ_C 18.5 (C-9)}, {δ_H 1.77 (3H, s, H_3-10), δ_C 26.0 (C-10)}]. Based on the ^1H, ^{13}C NMR and HMBC correlations (Fig. 2), a glycoside of hydroxymethyl {δ_H 4.82 (1H, dd, *J* = 12.0, 2.0 Hz, H-11a), δ_H 4.89 (1H, dd, *J* = 12.0, 2.8 Hz, H-11b), δ_C 67.0 (C-11)} was attached at C-3 (δ 127.9). The signals {δ_H 4.30 (1H, d, *J* = 8.0 Hz, H-anomeric), δ_C 104.4, 75.1, 77.9, 71.6, 78.1, 62.7} were identified as β-D-glucose,[26] which was further supported by GC analysis for sugar moiety obtained from the acid hydrolysis of **3**.[27,28] Thus, compound **3** was established as 4-(hydroxymethyl)-6-(8-methylprop-7-enyl)-5,6-dihydro-2*H*-pyran-2-one-11-*O*-β-D-glucopyranoside and named aruncide A.

Compound **4** was obtained as a colorless syrup. The molecular weight of **4** was suggested by a $[M+Na]^+$ peak at *m/z* 429 in the FABMS. The HRFABMS spectral analysis of **4** showed the $[M+Na]^+$

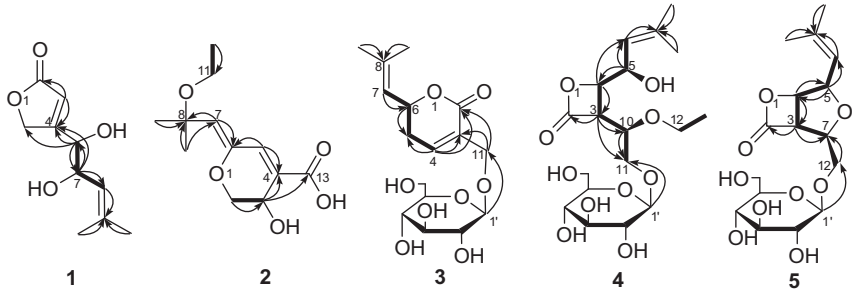

Figure 2. HMBC (single headed arrow) and COSY (bold) correlations of **1**–**5**.

at *m/z* 429.1734, which corresponded to the molecular formula of $C_{18}H_{30}O_{10}Na$ (calcd 429.1737). The IR spectrum showed absorption bands due to hydroxy (3,421 cm^{-1}), four-membered lactone (1749 cm^{-1}), and double bond (1646 cm^{-1}) groups. The ^1H NMR spectrum indicated a four-membered lactone moiety with two signals at δ_H 3.06 (1H, dd, *J* = 8.8, 2.4 Hz, H-3) and 4.84 (1H, dd, *J* = 8.8, 7.6 Hz, H-4), and there were corresponding ^{13}C NMR signals at δ_C 178.2 (C-2), 52.0 (C-3), and 82.7 (C-4). A combination of the ^1H, ^{13}C NMR, HMBC and COSY (Fig. 2) spectra of **4** suggested that the substituent at C-4 was a 1-hydroxyprenyl side-chain [{δ_H 4.28 (1H, dd, *J* = 9.2, 7.6 Hz, H-5), δ_C 73.9 (C-5)}, {δ_H 5.21 (1H, dq, *J* = 9.2, 1.2 Hz, H-6), δ_C 122.5 (C-6), δ_C 142.6 (C-7)}, {δ_H 1.80 (3H, d, *J* = 1.2 Hz, H_3-8), δ_C 18.8 (C-8)}, {δ_H 1.81 (3H, d, *J* = 1.2 Hz, H_3-9), δ_C 26.2 (C-9)}]. Furthermore, it was shown that a glycoside of the 2-hydroxyethyl substituent with 1-ethoxy was attached at C-3. This ethoxyl was observed at δ_H 3.74 (2H, q, *J* = 7.2 Hz, H_2-12), 1.13 (3H, t, *J* = 7.2 Hz, H_3-13), δ_C 68.0 (C-12), and 16.0 (C-13). Hydroxyethyl signals were present at {δ_H 3.72 (1H, dd, *J* = 9.6, 2.4 Hz, H-11a), 4.12 (1H, dd, *J* = 10.0, 9.6 Hz, H-11b), δ_C 70.0 (C-11)} and {δ_H 4.03 (1H, ddd, *J* = 10.0, 2.4, 2.4 Hz, H-10), δ_C 76.6 (C-10)}. Anomeric and glycosidic signals at δ_H 4.33 (1H, d, *J* = 8.0 Hz) and δ_C 104.8, 75.3, 78.0, 71.7, 78.3, 62.9 were established as β-D-glucose,[26] which was further supported by GC analysis for sugar moiety obtained from the enzymatic hydrolysis of **4**.[29,30]

We obtained the genin moiety (**4a**) through enzymatic hydrolysis of **4** to determine the absolute stereochemistry of the chiral center of C-5 in **4**. The (*R*)- and (*S*)-methoxytrifluoromethylphenylacetic acid (MTPA) esters (Mosher esters) {**4s** and **4r**} of **4a** were prepared.[31] ^1H-^1H COSY analysis of the Mosher ester derivatives was then performed. The ^1H NMR chemical shift data of **4r** and **4s** showed that the absolute configuration at C-5 was *R* (Fig. 3). On the basis of the established absolute configurations of compound **5** due to the similarity of spectral data of chiral centers in **4** and **5**, the absolute configurations of **4** were presumed as C-3*S*, C-4*S*, C-5*R*, and C-10*R*, respectively. From the above mentioned

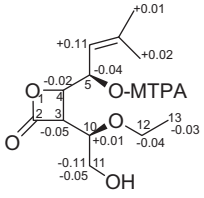

Figure 3. $\Delta\delta$ ($\delta_S - \delta_R$) values obtained from MTPA esters (**4s**, **4r**) of **4a**.

Table 1
MTT cytotoxic activity of **1**–**12** against Jurkat T cells and their DPPH radical scavenging activity

Compound	Cytotoxic activity IC_{50} (μg/mL)	Antioxidant activity IC_{50} (μM)
1	>100	>100
2	17.15	>100
3	>100	>100
4	>100	>100
5	>100	>100
6	>100	>100
7	>100	46.3
8	>100	>100
9	>100	>100
10	>100	11.7
11	98.3	>100
12	>100	>100
Auraptene [b]	55.36	–[a]
L-Ascorbic acid	–[a]	16.4

[a] NT: Not tested.
[b] Positive control for cytotoxicity.

Table 2
^1H NMR (400 MHz) data for **1** in $CDCl_3$ and **2**–**5** in CD_3OD[a]

No.	δ_H						
	1	**1a**	**2**	**3**	**4**	**4a**	**5**
3	7.45 d (1.2)	7.46 d (1.2)	7.47 s		3.06 dd (8.8, 2.4)	3.07 (9.0, 2.4)	2.72 dd (9.2, 2.4)
4				6.40 dd (2.8, 2.0)	4.84 dd (8.8, 7.6)	4.83 dd (9.0, 7.6)	4.83 dd (9.2, 7.6)
5	4.87 d (1.2)	4.86 d (1.2)	4.29 dd (5.6, 4.4)	3.11 ddd (16.0, 7.2, 2.0)	4.28 dd (9.2, 7.6)	4.29 dd (9.2, 7.6)	4.28 dd (9.2, 7.6)
				2.63 ddd (16.0, 6.0, 2.8)			
6	4.54 d (4.4)	5.77 d (4.4)	3.76 dd (12.0, 4.4)	5.28 ddd (8.8, 7.2, 6.0)	5.21 dq (9.2, 1.2)	5.21 dq (9.2, 1.2)	
			3.65 dd (12.0, 5.6)				
7	4.60 dd (9.0, 4.4)	5.97 dd (9.0, 4.4)	5.57 s	5.25 d (8.8)			3.98 ddd (11.6, 2.4, 2.4)
8	5.21 d (9.0)	5.18 d (9.0)			1.80 d (1.2)	1.80 d (1.2)	5.30 dq (9.2, 1.2)
9			1.50 s	1.75 s	1.81 d (1.2)	1.81 d (1.2)	
10	1.70 s	1.75 s	1.50 s	1.77 s	4.03 ddd (10.0, 2.4, 2.4)		1.78 d (1.2)
11	1.74 s	1.78 s	3.57 q (7.2)	4.82 dd (12.0, 2.0)	3.72 dd (9.6, 2.4)	3.62 dd (9.6, 2.4)	1.80 d (1.2)
				4.89 dd (12.0, 2.8)	4.12 dd (10.0, 9.6)	4.05 dd (10.0, 9.6)	
12			1.22 t (7.2)		3.74 q (7.2)	3.75 q (7.2)	3.87 dd (10.8, 2.4)
							4.40 dd (11.6, 10.8)
13					1.13 t (7.2)	1.14 t (7.2)	
$COCH_3$		2.08 s, 2.04 s					
β-D-glu							
1′				4.30 d (8.0)	4.33 d (8.0)		4.32 d (8.0)

[a] Data are δ (ppm), multiplicity, and *J* (in parentheses) in Hz.

evidence, **4** was found to be (3*S*,4*S*,5*R*,10*R*)-3-(10-ethoxy-11-hydroxyethyl)-4-(5-hydroxy-7-methylbut-6-enyl)oxetan-2-one-11-*O*-β-D-glucopyranoside and named aruncide B.

Compound **5** was obtained as a colorless syrup. The molecular weight of **5** was suggested by a $[M+Na]^+$ peak at *m/z* 383 in the FABMS. The HRFABMS spectral analysis of **5** showed the $[M+Na]^+$ at *m/z* 383.1321, which corresponded to the molecular formula of $C_{16}H_{24}O_9Na$ (calcd 383.1318). The IR spectrum showed absorption bands due to hydroxy (3375 cm^{-1}), four-membered lactone (1757 cm^{-1}), and ether (1129 cm^{-1}) groups. The ^1H NMR spectrum indicated a four-membered lactone with two signals at δ_H 2.72 (1H, dd, *J* = 9.2, 2.4 Hz, H-3) and δ_H 4.83 (1H, dd, *J* = 9.2, 7.6 Hz, H-4), and there were corresponding ^{13}C NMR signals at δ_C 175.1 (C-2), 50.9 (C-3) and 82.5 (C-4). The ^1H and ^{13}C NMR (Tables 2 and 3), HMBC and COSY (Fig. 2) spectra of **5** showed some signals similar to **4**, confirming the presence of a four-membered lactone with a isobutenyl substituent [{δ_H 5.30 (1H, dq, *J* = 9.2, 1.2 Hz, H-8), δ_C 122.3 (C-8), δ_C 142.8 (C-9)}, {δ_H 1.78 (3H, d, *J* = 1.2 Hz, H_3-10), δ_C 18.8 (C-10)}, {δ_H 1.80 (3H, d, *J* = 1.2 Hz, H_3-11), δ_C 26.2 (C-11)}] at C-5 and a glucoside of hydroxymethyl {δ_H 3.87 (H, dd, *J* = 10.8, 2.4 Hz, H-12a), δ_H 4.40 (H, dd, *J* = 11.6, 10.8 Hz, H-12b), δ_C 68.8 (C-12)} at C-7. One doublet of doublet at δ 4.28 (1H, *J* = 9.2, 7.6 Hz, H-5), one doublet of doublet of doublet at δ 3.98 (1H, *J* = 11.6, 2.4, 2.4 Hz, H-7) and no ethoxyl group indicated that a tetrahydrofuran ring was formed. The signals {δ_H 4.32 (1H, d, *J* = 8.0 Hz, H-anomeric), δ_C 104.9, 75.0, 78.2, 71.9, 78.4, 62.6} were identified as β-D-glucose,[26] which was further supported by GC analysis for sugar moiety obtained from the acid hydrolysis of **5**.[28,30]

The ^1H and ^{13}C NMR spectra of compounds **4** and **5** were similar with differences observed in the side chain. Therefore, the configuration at C-5 of compound **5** should be presumed to be the same as that of compound **4**. In the NOESY spectrum of **5**, the correlation indicated connectivities between H-5 and H-7 and between H-3 and H-4. There were no correlations between H-4 and H-5, and between H-3 and H-7. Therefore, the absolute configurations of **5** were C-3*S*, C-4*S*, C-5*R*, and C-7*R*. Based on this evidence, **5** was established as (3*S*,4*S*,5*R*,7*R*)-5-(9-methylprop-8-enyl)-1,6-dioxabicyclo[3,2,0]heptan-2-one-7-(hydroxymethyl)-12-*O*-β-D-glucopyranoside and named aruncide C.

Twelve compounds isolated from *A. dioicus* var. *kamtschaticus* were tested for their cytotoxic activity against the Jurkat T cell line.[32,33] Compound **2** showed potent cytotoxic activity with an IC_{50} value of 17.15 μg/mL in Jurkat T cells. Cytotoxicity for **15** was not available because of its insolubility in DMSO (Table 1).

The radical scavenging activity of compounds **1**–**12** were evaluated in the DPPH radical scavenging assay.[11] Compounds **7** and **10** exhibited relatively strong antioxidant activity with IC_{50} values of 46.3 and 11.7 μM, respectively (Table 1), compared with the reference standard, L-ascorbic acid (IC_{50} of 16.4 μM). Silva et al.[34] had previously reported the antioxidant activity of quercetin-3-*O*-β-D-glucopyranoside (**10**). However, DPPH radical scavenging activity of 1-feruloyl-β-D-glucose (**7**) has never been reported.

Table 3
^{13}C NMR (100 MHz) data for **1** in $CDCl_3$ and **2**–**5** in CD_3OD

No.	δ_C[a]				
	1	**2**	**3**	**4**	**5**
2	174.2	147.4	171.9	178.2	175.1
3	149.4	143.1	127.9	52.0	50.9
4	133.7	132.1	140.4	82.7	82.5
5	71.4	76.7	36.7	73.9	73.3
6	70.1	64.2	76.9	122.5	
7	69.6	125.0	124.6	142.6	75.2
8	122.8	71.2	141.1	18.8	122.3
9	136.4	30.3	18.5	26.2	142.8
10	17.4	30.3	26.0	76.6	18.8
11	24.9	66.7	67.0	70.0	26.2
12		15.8		68.0	68.8
13		170.8		16.0	
β-D-Glu					
1′			104.4	104.8	104.9
2′			75.1	75.3	75.0
3′			77.9	78.0	78.2
4′			71.6	71.7	71.9
5′			78.2	78.3	78.4
6′			62.7	62.9	62.6

[a] Data are δ (ppm) and multiplicity.

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9. The *A. dioicus* var. *kamtschaticus* were purchased in July 2006 from Ulleungdo in KyeongBuk, Republic of Korea. These materials were confirmed taxonomically by Professor Byung Sun Min, College of Pharmacy, Catholic University of Daegu, Korea. A voucher specimen (CUDP 200601) has been deposited at the College of Pharmacy, Catholic University of Daegu, Korea.
10. The DPPH radical-scavenging activity was measured using the method described by Tagashira and Ohtake[11] Briefly, 10 μL of each sample dissolved in EtOH was prepared in the 96-well microplate, and then 200 μL of 100 μM methanolic DPPH solution was added. After mixing and standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 517 nm. L-Ascorbic acid (Sigma-Aldrich; purity: >99%) was used as the positive control for DPPH radical-scavenging activity.
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12. The aerial part of *A. dioicus* var. *kamtschaticus* (6.90 kg) was extracted with 95% EtOH (180 L × 2) at room temperature for 36 h. The EtOH extract was concentrated under reduced pressure to make an EtOH extract (2.03 kg). The concentrated EtOH extract was suspended in H_2O (6.0 L) and partitioned successively with *n*-hexane (6 × 5 L, 308.0 g), CH_2Cl_2 (6 × 5 L, 41.7 g), EtOAc (6 × 5 L, 121.3 g), *n*-BuOH (6 × 5 L, 353.8 g) and H_2O (600.0 g), respectively. The EtOH extract, *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH, and H_2O-soluble fractions were assayed for DPPH radical scavenging activity. The active DPPH radical scavenging CH_2Cl_2 fraction (31.5 g) was subjected to open flash column chromatography over silica gel (6.5 × 25 cm) eluted with *n*-hexane–CH_2Cl_2 (100:0 to 0:100) and CH_2Cl_2–MeOH–H_2O (100:0:0.1 to 0:100:0.1) gradient. Fractions (M1 to M26) were collected and pooled according to their similar TLC patterns. Fraction M14 (0.1167 g) was chromatographed on a reverse-phase column (3.5 × 15 cm) using a MeOH–H_2O mixture as a solvent and eluted with a stepwise gradient (5% MeOH to 50% MeOH) to yield compounds **1** (20.6 mg) and **2** (29.2 mg). Fraction M17 (0.2 g) was chromatographed on a reverse-phase column (3.5 × 15 cm) using a MeOH–H_2O mixture as a solvent and eluted with a stepwise gradient (5% MeOH to 90% MeOH) to yield compounds **3** (20.2 mg) and **6** (21.1 mg). The most active DPPH radical scavenging EtOAc fraction (121.3 g) was chromatographed over a silica gel column (15 × 35 cm) and eluted with a gradient of CH_2Cl_2–MeOH–H_2O to afford 55 fractions (E1–E55). Fraction E2 (14.0 g) was chromatographed on a silica gel column (3.5 × 57 cm) and eluted with a gradient of CH_2Cl_2/MeOH/H_2O (55:1:0.1 to 10:1:0.1) to afford 23 subfractions (E2.1–E2.23). Subfraction E2.7 (200.0 mg) was chromatographed on a reverse-phase column (2.3 × 53 cm) using MeOH–H_2O mixture as a solvent and eluted with a stepwise gradient (100% H_2O to 100% MeOH) to yield compound **12** (10.0 mg). Subfraction E2.13 (2.0 g) was chromatographed on a reverse-phase column (3.5 × 57 cm) using MeOH–H_2O mixture as a solvent and eluted with a stepwise gradient (100% H_2O to 100% MeOH) to yield compound **4** (10.0 mg). Fraction E4 (3.5 g) was chromatographed on a reverse-phase column (3.5 × 57 cm) using MeOH–H_2O mixture as solvent and eluted with a stepwise gradient (100% H_2O to 100%

MeOH) to yield compound **5** (34.0 mg). Fraction E6 (8.0 g) was chromatographed on a reverse-phase column (3.5 × 57 cm) using MeOH–H_2O mixture as a solvent and eluted with a stepwise gradient (100% H_2O to 80% MeOH) to yield compounds **7** (40.0 mg), **8** (14.1 mg), **9** (280.0 mg), and **11** (8.2 mg), respectively. Fraction E9 (3.1 g) was chromatographed on a reverse-phase column (3.5 × 57 cm) using MeOH–H_2O mixture as a solvent and eluted with a stepwise gradient (100% H_2O to 80% MeOH) to yield compound **10** (48.0 mg).

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28. Compounds **3** and **5** (1 mg) were each refluxed with 2 mL of 1 N HCl (1,4-dioxane–H_2O, v/v, 1:1) at 80 °C for 3 h, respectively. Each mixture was extracted with CH_2Cl_2 to afford the aglycone, and the aqueous layer was neutralized with Na_2CO_3 and filtered. To the evaporate filtrates were added 1-(trimethylsilyl)imidazole (0.2 mL) and pyridine (0.2 mL), with the whole stirred at 60 °C for 5 min. After the reaction mixture was dried under a stream of N_2, each residue was partitioned between $CHCl_3$ and H_2O. Each $CHCl_3$ layer was analyzed by GC experiment [column: DB-Wax column (30 m × 0.25 mm), injection temperature: 200 °C, column temperature: 100–200 °C, rate: 4 °C/min, and retention time: D-glucose (14.31 min)]. Peaks of hydrolysates were detected by comparison to the retention time of an authentic sample (D-glucose for **3** and **5**) treated with 1-(trimethylsilyl)imidazole.
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30. Naringinase (100 mg, from *Penicillium decumbens*) was added to a suspension of **4** (3 mg) in 50 mm acetate buffer (pH 5.5), and the mixture was stirred at 37 °C for 5 h. The reaction mixture was extracted with EtOAc (10 mL × 3), and the organic layer was evaporated to dryness to obtain **4a** (1.5 mg). The H_2O layer was checked by silica gel TLC (EtOAc/MeOH/H_2O/AcOH, 65:20:15:15). The spot on the TLC plate was visualized with an anisaldehyde-H_2SO_4 reagent. The configuration of glucose was determined by a GC method, and the sugar derivative thus obtained showed a retention time of 14.31 min, identical to that of authentic D-glucose.
31. To 0.5 mg of **4a** in 0.5 mL of CH_2Cl_2 were added sequentially 0.2 mL pyridine, 0.5 mg 4-(dimethylamino)-pyridine, and 12 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (*R*-MTPA) chloride, separately. The mixture was left at room temperature overnight and purified over a micro-column (0.6 × 6 cm) of Si gel (230–400 mesh) eluted with 3–4 mL of *n*-hexane/CH_2Cl_2 (1:2); the eluate was dried, CH_2Cl_2 (5 mL) was added, and the CH_2Cl_2 was washed using 1% $NaHCO_3$ (5 mL × 2). The washed eluate was dried in vacuo to give the *S* Mosher ester (**4s**) of **4a**. Using (*S*)-MTPA chloride afforded the *R* Mosher ester (**4r**).
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33. Cytotoxicity was measured using a modified MTT assay. The Jurkat T cells (2.5×10^4 cells/well) were seeded on 96-well microplates and were precultured for 36 h. MTT solution (1.1 mg/mL) was added to each well and incubated for an additional 4 h. The colored formazan crystal produced from the MTT was dissolved in dimethyl sulfoxide (DMSO). The optical density (OD) values of the solutions were measured at 540 nm using a plate reader. All cell lines were purchased from the Korean Cell Line Bank (Seoul, Korea).
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