Anti-complementary Activity of Ursane-Type Triterpenoids from *Weigela subsessilis*

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A new ursane-type triterpenoid, weigelic acid (1), and seven known compounds, ursolic acid (2), ilekudinol A (3), corosolic acid (4), ilekudinol B (5), esculentic acid (6), pomolic acid (7), and asiatic acid (8) were isolated from the leaf and stem of *Weigela subsessilis*. The structure of the new triterpenoid was established as 1β,2α,3α,23-tetrahydroxyurs-12-en-28-oic acid on the basis of spectroscopic analyses. In addition, the isolated compounds were evaluated for their anti-complement activity against the classical pathway of the complement system. Of these, compounds 1—2 and 4—8 exhibited anti-complement activity with IC_{50} values of 152, 90, 130, 51, 56, 4, and 163 μM, respectively, whereas 3 was inactive. This shows that a carboxylic group of ursane-type triterpenoids seems to play an important role in inhibiting the hemolytic activity of human serum against erythrocytes.

Key words *Weigela subsessilis*; Caprifoliaceae; ursane-type triterpenoid; weigelic acid; anti-complementary

The complement system plays an essential role in innate immune defense against infectious agents and the inflammatory process. Activation of the complement system provides efficient means for protecting the host from the actions of invading antigens.[1,2] As already well known, this system is activated by one of three different pathways, the classical, alternative, or lectin pathway. Of these, the classical pathway is called antibody-dependent because it is activated by the binding of antibodies to invasive agents.[1,2] However, if the complement system is excessively activated, it may cause a variety of inflammatory injuries, such as rheumatoid arthritis, osteoarthritis, atopic dermatitis, atherosclerosis, and allergies.[1] Therefore, it is proposed that modulation of complement activity could be useful in the therapeutic treatment of inflammatory diseases. A number of natural products, such as phenolics,[3—6] lactones,[7] polyacetylen,[8] norlignans,[9] terpenoids,[9—15,20] and saponins,[9,11—15] have been reported recently to exhibit inhibitory effects on the complement system activated by the classical pathway.

The plant *Weigela subsessilis* L. H. BAILEY is an endemic species that grows throughout Korea.[16] With regard to the chemical constituents, flavonoids and coumarins have been isolated from the leaf and flower of this edible plant.[17—19] Our phytochemical study of the MeOH extract of the leaf and stem of this plant resulted in the isolation of a new and seven known ursane-type triterpenoids. Accordingly, although the anti-complement activities of oleanene-type,[9—13] dammarane-type,[14] and lanostane-type[15] have been well documented, little is known whether ursane-type triterpenoids are potent anti-complement compounds.[20] With the hope of gaining more agents for the treatment of inflammatory diseases, the anti-complementary activities of eight isolated triterpenoids against the classical pathway of the complement system were examined. This paper reports the characterization of a new triterpenoid as well as the anti-complement activities of eight ursane-type triterpenoids isolated from *W. subsessilis*.

MATERIALS AND METHODS

Plant Material The leaf and stem of *Weigela subsessilis* were collected at Gyeryong Mountain, Chungnam province, Korea in April, 2000. The plant material was identified by Prof. Bae KiHwan and a voucher specimen (CNU 2009) has been deposited at the herbarium of the College of Pharmacy, Chungnam National University, Daejeon, Korea.

General Experimental Procedure Melting point determinations were performed using a Kofler micro-hotstage. Optical rotations were measured on a JASCO DIP-370 polarimeter. IR spectra (KBr) were obtained on a Brucker spectrometer. FAB-MS and HR-FAB-MS (high resolution FAB-MS) were registered using a JEOL JMS-DX 300 spectrometer. NMR spectra including ^{1}H- and ^{13}C-NMR, ^{1}H–^{1}H COSY (homonuclear correlation spectroscopy), HMBC (heteronuclear multiplebond connectivity), HMQC (^{1}H-detected heteronuclear multiplequantum coherence), and NOESY (nuclear overhauser and exchange spectroscopy) were recorded on Bruker DRX-300 or 600 NMR spectrometers. Analytical TLC was performed on pre-coated silica gel 60 F_{254} plates (Merck) or RP-18 F_{254} (Merck). For column chromatography, silica gel (Kieselgel 60, Merck), and RP-18 (Merck) were used.

Extraction and Isolation Dried and powdered plant material (6.7 kg) was extracted with MeOH (50 l×3) at room temperature for one month and filtered. The MeOH extract was concentrated to give a residue (684 g) that was suspended in H_2O (2 l) and partitioned sequentially against hexane (2 l×2), EtOAc (2 l×2), and BuOH (1 l×2) to give

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hexane-, EtOAc-, and BuOH-soluble fractions (90, 163, 164 g, respectively) and a water layer. The EtOAc fraction (163 g) was subjected to silica gel column chromatography (10×30 cm) eluted with gradient hexane–EtOAc (10 : 1, 9 : 1, 8 : 1 to 0 : 1) and separated into 8 fractions: fr. 1 (23 g), fr. 2 (26 g), fr. 3 (15 g), fr. 4 (9 g), fr. 5 (17 g), fr. 6 (33 g), fr. 7 (16 g), and fr. 8 (21 g). Fraction 1—2 (49 g) was further chromatographed on a silica gel column (5×40 cm) using hexane–EtOAc (1 : 4) as the eluting solvent to give compound **2** (323 mg). Fraction 3 (15 g) was rechromatographed on a Sephadex LH-20 column (3×30 cm) eluted with MeOH–H_2O (20 : 1) and separated into 5 fractions, fr. 3a (4.5 g), fr. 3b (compound **3**, 15 mg), fr. 3c, fr. 3d (compound **2**, 32 mg) and fr. 3e. Fraction 3a was subjected to an RP-18 column (2.5×30 cm) eluted with MeOH–H_2O (20 : 1) to yield compound **4** (181 mg) and **5** (234 mg). Repeat chromatography fraction 4 (7.5 g) on an RP-18 column (2.5×30 cm) eluted with MeOH–H_2O (20 : 1, 600 ml), and the collected subfraction (300—350 ml, 90 mg) was further chromatographed on an RP-18 column (1.6×25 cm) eluted with MeOH–H_2O (20 : 1) to yield compound **6** (6.7 mg). Fraction 6 (33 g) was rechromatographed on an RP-18 column (4×30 cm) eluted with MeOH–H_2O (2 : 1) to yield compounds **7** (7.1 mg) and **8** (21 mg). Fraction 7 (15 g) was subjected to a silica gel column (4×30 cm) eluted with hexane–EtOAc (2 : 1, 4 l), and the collected subfraction (1.8—2.5 l, 850 mg) was further chromatographed on an RP-18 column (2.5×30 cm) using MeOH–H_2O (2 : 1) as the eluting solvent to yield compound **1** (18.2 mg).

Weigelic Acid (**1**): White amorphous powder; mp 244—246 °C; $[\alpha]_D^{20}$: +8.8° (*c*=0.25, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400, 2925, 1700, 1445, 1390, 1050; FAB-MS *m/z*: 527 $[M+Na]^+$; HR-FAB-MS *m/z*: 527.3348 $[M+Na]^+$, Calcd for $C_{30}H_{48}O_6Na$: 527.3349; ^{1}H- and ^{13}C-NMR: see Table 1.

Ursolic Acid (**2**): White amorphous powder; mp 260—262 °C; $[\alpha]_D^{20}$: +62.0° (*c*=1.0, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400, 2920, 1680, 1450, 1380, 1185; FAB-MS *m/z*: 479 $[M+Na]^+$.

Ilekudinol A (**3**): White amorphous powder; mp 169—171 °C; $[\alpha]_D^{20}$: +63.9° (*c*=0.25, MeOH); IR (KBr) ν_{max} cm^{-1}: 3430, 2959, 2929, 2859, 1727, 1275; FAB-MS *m/z*: 477 $[M+Na]^+$ and 455 $[M+H]^+$.

Corosolic Acid (**4**): White amorphous powder; mp 274—276 °C; $[\alpha]_D^{20}$: +45.6° (*c*=0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 3430, 2927, 1692, 1565 1462, 1273, 1049; FAB-MS *m/z*: 495 $[M+Na]^+$.

Ilekudinol B (**5**): White amorphous powder; mp 193—195 °C; $[\alpha]_D^{20}$: +70.2° (*c*=1.0, MeOH); IR (KBr) ν_{max} cm^{-1}: 3431, 2926, 1693, 1453, 1384, 1054; FAB-MS *m/z*: 479 $[M+Na]^+$.

Esculentic Acid (**6**): White amorphous powder; mp 270—272 °C; $[\alpha]_D^{20}$: +21.7° (*c*=0.25, MeOH); IR (KBr) ν_{max} cm^{-1}: 3433, 2937, 1692, 1565, 1049; FAB-MS *m/z*: 511 $[M+Na]^+$.

Pomolic Acid (**7**): White amorphous powder; mp 271—273 °C; $[\alpha]_D^{20}$: +17.8° (*c*=0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 3430, 2930, 2876, 1691, 1385, 1046; FAB-MS *m/z*: 473 $[M+H]^+$.

Asiatic Acid (**8**): White amorphous powder; mp 301—303 °C; $[\alpha]_D^{20}$: +50.3° (*c*=0.25, MeOH); IR (KBr) ν_{max} cm^{-1}: 3410, 2920, 2875, 1690, 1370, 1050; FAB-MS *m/z*: 511 $[M+Na]^+$.

Table 1. ^{1}H- (600 MHz), ^{13}C- (150 MHz), and HMBC NMR Spectral Data of Compound **1** in DMSO-d_6

Position	δ_C	δ_H (*J* in Hz)*a)*	HMBC (C→H)
1	80.0	3.31 d (9.0)	H-2, H-3, H-25
2	70.8	3.42 dd (9.0, 2.4)	H-1, H-3
3	77.3	3.50 d (2.4)	H-23, H-24
4	41.6	—	H-3, H-5, H-23, H-24
5	43.8	1.46 m	H-3, H-23, H-24
6	18.3	1.35 m, 1.39 m	H-5
7	33.5	0.92 m, 1.22 m	H-26
8	43.4	—	H-26
9	48.7	1.73 m	H-1, H-25, H-26
10	42.3	—	H-25
11	27.3	1.91 m, 1.95 m	H-9
12	126.7	5.14 br s	H-18
13	138.1	—	H-18
14	40.7	—	
15	28.4	0.98 m, 1.80 m	H-27
16	24.7	1.31 m, 1.54 m	
17	47.9	—	H-18
18	53.3	2.12 d (11.4)	H-29
19	39.9	1.50 m	H-30
20	39.6	1.74 m	H-29
21	31.1	1.41 m, 1.60 m	H-30
22	37.2	1.55 m	
23	70.0	3.20 d (10.2), 3.34 d (10.2)	H-24
24	17.8	0.73 s	H-5
25	13.6	0.95 s	H-1, H-5, H-9
26	17.9	0.77 s	
27	24.2	1.06 s	
28	179.3	—	H-18
29	17.9	0.84 d (6.6)	
30	22.0	0.94 d (6.6)	

a) Multiplicity signals were overlapped in the range 1.0—2.0 ppm; assignments made on the basis of ^{1}H–^{1}H COSY, HMQC, and HMBC.

Anti-complement Assay The *in vitro* anti-complement activity of the isolated compounds was examined according to a previously described method.[9,10] In brief, a diluted solution of normal human serum (80 μl) collected from a healthy male volunteer was mixed with gelatin veronal buffer (GVB^{2+}, 80 μl) with or without tested sample. The mixture was pre-incubated at 37 °C for 30 min and then 40 μl of sensitized erythrocytes (sheep red blood cells) was added. After incubation under the same conditions, the mixture was centrifuged at 1500 rpm and 4 °C for 10 min. The optical density of the supernatant was measured at 405 nm. Rosmarinic acid was used as a positive control.

RESULTS AND DISCUSSION

Repeat column chromatography of the EtOAc-soluble fraction of the MeOH extract of *W. subsessilis* resulted in the isolation of eight triterpenoids (**1**—**8**). The structures of seven known compounds (Fig. 1) were identified as ursolic acid (**2**),[21] ilekudinol A (**3**),[22] corosolic acid (**4**),[23] ilekudinol B (**5**),[22] esculentic acid (**6**),[24] pomolic acid (**7**),[25] and asiatic acid (**8**)[26] by comparing their spectral (IR, ^{1}H- and ^{13}C-NMR, FAB-MS) and physicochemical data with those reported in the literature.

Compound **1**, named weigelic acid, was obtained as a white amorphous powder with a positive optical rotation $[\alpha]_D^{20}$ +8.8° (*c*=0.25, MeOH). The molecular formula of **1**

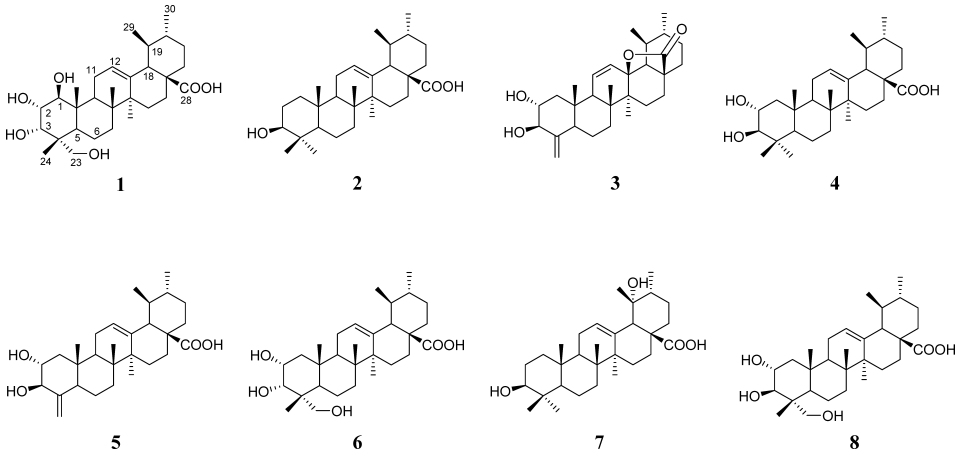

Fig. 1. Chemical Structures of Triterpenoids **1**—**8** Isolated from *W. subsessilis*

was assigned as $C_{30}H_{48}O_6$ by the molecular ion peak at *m*/*z* 527.3348 $[M+Na]^+$ in HR-FAB-MS. Its IR spectrum showed absorption bands deduced to be hydroxy groups at 3400 cm^{-1} and a carbonyl group at 1700 cm^{-1} attributed to a carboxylic group. The ^{1}H-NMR spectrum of **1** revealed an olefinic proton at δ 5.14 (br s), three methine protons at δ 3.50 (d, J=2.4 Hz), 3.42 (dd, J=9.0, 2.4 Hz), and 3.31 (d, J=9.0 Hz), a pair of methylene protons at δ 3.34 and 3.20 (each d, J=10.2 Hz), a doublet proton at δ 2.12 (J=11.4 Hz), four tertiary methyl protons at δ 0.73, 0.95, 0.77, and 1.06 (each 3H, s), and two secondary methyl protons at δ 0.84 and 0.94 (each 3H, d, J=6.6 Hz). In addition, the ^{13}C-NMR spectrum displayed a carboxylic carbon at δ 179.3, two signals of olefinic carbons at δ 126.7 and 138.1, three oxygenated methines at δ 70.8, 77.3, and 80.0, a hydroxymethylene at δ 70.0, and six methyl signals at δ 13.6, 17.8, 17.9, 17.9, 22.0, and 24.2. Moreover, from the ^{1}H–^{1}H COSY, HMQC and HMBC spectra, three methine protons exhibiting two doublets at δ 3.31 (d, J=9.0 Hz) and 3.50 (d, J=2.4 Hz), and a double doublet at 3.42 (dd, J=9.0, 2.4 Hz) were assignable to H-1, H-3, and H-2, respectively. The coupling constants were indicative of a *trans*-form of H-1/H-2 ($J^3_{\text{H-1/H-2}}$=9.0 Hz) and a *cis*-form of H-2/H-3 ($J^3_{\text{H-2/H-3}}$=2.4 Hz), in agreement with those for 1β,2α,3α,24-tetrahydroxyurs-12-en-28-oic acid isolated from *Prunus persica*.[27] These findings suggested a tetrahydroxyl substitution on ring A of an ursane-type triterpenoid for **1**. In comparison, the chemical shift of a C-23 hydroxymethylene group, about δ_C 68—71, is downfield to that of a C-24 hydroxymethylene group, which is about δ_C 63—66.[27,28] The chemical shift of the hydroxymethylene carbon of **1** was observed at δ 70.0, which is similar to esculentic acid (**6**),[24] and it was consequently assigned as C-23. Further comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of esculentic acid and 1β,2α,3α,24-tetrahydroxyurs-12-en-28-oic acid suggested that ring A was 1β,2α,3α,23-trihydroxyl substituted. This was additionally supported by the presence of NOE correlations between H-23 (δ 3.34/3.20) with H-3 (δ 3.50) and H-5 (δ 1.46), and correlations between H-24 (δ 0.73) with H-25 (δ 0.95) and H-2 (δ 3.42) in the NOESY spectrum (Fig. 2). On the basis of results obtained, the structure of weigelic acid (**1**) was established as 1β,2α,3α,23-tetrahydroxyurs-12-en-28-oic acid.

The isolated triterpenoids **1**—**8** were evaluated for their anti-complement activity using the classical pathway com-

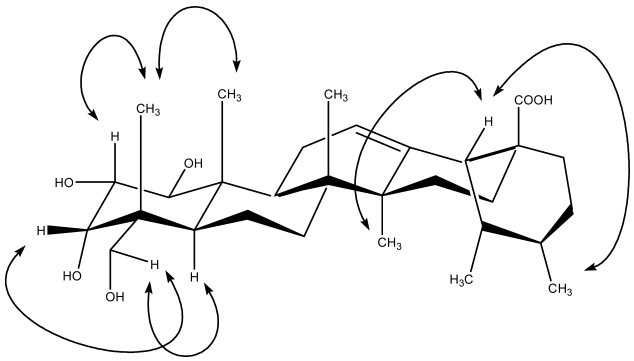

Fig. 2. Key NOE Correlations of Compound **1** in NOESY Spectrum

Table 2. Inhibitory Effects of Triterpenoids Isolated (**1**—**8**) from *W. subsessilis* on Complement System of Classical Pathway

Compound	IC_{50} (μM)[a]
Weigelic acid (**1**)	152±10.3
Ursolic acid (**2**)	90±5.8
Ilekudinol A (**3**)	>200
Corosolic acid (**4**)	130±10.8
Ilekudinol B (**5**)	51±4.5
Esculentic acid (**6**)	56±6.9
Pomolic acid (**7**)	4±0.3
Asiatic acid (**8**)	163±23.6
Rosmarinic acid[b]	182±27.7

a) The values represent the mean±S.D. of three experiments. *b*) Rosmarinic acid was used as a positive control.

plement inhibitory activity. As shown in Table 2, seven compounds **1**, **2**, **4**—**8** showed positive inhibitory effects with IC_{50} values of 152, 90, 130, 51, 56, 4, and 163 μM, respectively. All of these triterpenoids exhibited higher activity than that of rosmarinic acid, which was used as a positive control (Table 2). On the contrary, **3** was found to be inactive in this assay system when it has a lactone ring instead of a carboxylic group at C-28. This suggests that the carboxylic acid group at C-28 of the ursane-type triterpenoids may be important for their anti-complement activity. The carboxylic acid group is known to be essential for anti-complement activity in plant-derived triterpenoids, such as oleanolic acid and hederagenin.[10,13] In addition, Lee *et al.* reported that a hydroxy group at C-3 of oleanolic acid plays a significant role

in the anti-complement activity.[10] Compared with esculentic acid (**6**) and asiatic acid (**8**), the α-configuration of a hydroxy group at C-3 of ursane-type triterpenoid (**6**) manifested more potent activity than the β-configuration (**8**). Among the tested triterpenoids, pomolic acid (**7**) showed the most potent activity against complement-induced hemolysis *via* the classical pathway, while the others without a hydroxy group at the C-19 position were much less active. Therefore, it could be concluded that the hydroxy group at C-19 enhanced the anti-complement activity of this ursane-type triterpenoid.

In conclusion, although the structure–activity relationships of these compounds were not conclusively determined, this finding suggests that ursane-type triterpenoids may have potential for further development as anti-complementary substances.

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