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Constituents of the Aerial Parts of *Agrimonia pilosa*

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Abstract – Four ursane type triterpenes have been isolated from the methanolic extract of the aerial parts of *Agrimonia pilosa* Ledeb. (Rosaceae) through repeated silica gel and reverse-phase C-18 column chromatography. Their chemical structures were elucidated as ursolic acid (**1**), pomolic acid (**2**), tormentic acid (**3**), and corosolic acid (**4**) on the basis of their MS, ^{1}H-, and ^{13}C-NMR spectral data. Compounds **2** and **4** were isolated from the genus of *Agrimonia* for the first time.
Keywords – *Agrimonia pilosa*, ursolic acid, polmolic acid, tormentic acid, corosolic acid

Introduction

Agrimonia pilosa Ledeb. (Rosaceae) is a perennial herbaceous plant, and widely distributed in Asia. The aerial parts of this plant have been used as an antihaemorrhagic, anthelmintic, and anti-inflammatory agent in Chinese herbal medicine (Zhu, 1998). Previous phytochemical studies on this plant have led to isolation of flavones (Kimura *et al.*, 1968), tannins (Okuda *et al.*, 1982), phloroglucinols (Yamaki *et al.*, 1989), isocoumarins (Pei *et al.*, 1989), and triterpenoids (Kouno *et al.*, 1988). In this paper, we describe the isolation and structural elucidation of four ursane type triterpenes including pomolic acid and corosolic acid, which were firstly isolated from the genus of *Agrimonia*.

Experimental

Plant material – The aerial parts of *Agrimonia pilosa* were collected in June 2001 at Mt. Hamra, Jeonbuk Province, Korea, and identified by Dr. Kyu-Kwan Jang, botanical garden, Wonkwang University. A voucher specimen (no. WP 523) was deposited at the herbarium of the College of Pharmacy, Wonkwang University (Korea).

Extraction and isolation – Dried aerial parts of *A. pilosa* (1.0 kg) were extracted twice with MeOH (3 L) under the ultrasonic condition for 3 h. The MeOH extract (37.8 g) was partitioned between equal volumes of *n*-hexane and 60% aqueous MeOH, and the aqueous MeOH layer extracted subsequently with $CHCl_3$. The $CHCl_3$-soluble fraction (9.6 g) was subjected to silica gel column chromatography with a CH_2Cl_2-MeOH mixture (MeOH 5-20%, step gradient) to give four fractions (Fr. 1-4). Fraction 2 (1.5 g) was subjected to silica gel column chromatography (elution with hexane-EtOAc, 4 : 1 to 1 : 1) to give four subfractions (Fr. 21-24). Fraction 21 (0.5 g) was subjected to YMC gel column chromatography with MeOH-H_2O (5 : 1) to yield compound **1** (61.5 mg) and compound **2** (15.2 mg). Fraction 3 (3.5 g) was fractionated by YMC gel column chromatography with MeOH-H_2O (1:1) to afford seven subfractions (Fr. 31-37). Fraction 37 (1.1 g) was subjected to silica gel column chromatography (CH_2Cl_2-MeOH, 15 : 1) to obtain two subfractions (Fr. 371 and 372). Fraction 371 (0.5 g) was chromatographed to YMC gel column chromatography with MeOH-H_2O (8 : 1) to give compounds **3** (12.0 mg) and **4** (131.8 mg).

Ursolic acid (**1**): White amorphous powder; (–)-ESI-MS *m/z* 455 $[M-H]^-$, ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 5.49 (1H, t-like s, H-12), 3.46 (1H, dd, J = 6.0, 10.1 Hz, H-3α), 2.64 (1H, d, J = 11.4 Hz, H-18), 1.25 (3H, s, H-23), 1.23 (3H, s, H-27), 1.06 (3H, s, H-26), 1.03 (3H, s, H-24), 1.00 (3H, d, J = 6.5 Hz, H-30), 0.95 (3H, d, J = 6.0 Hz, H-29), 0.89 (3H, s, H-25); ^{13}C-NMR data: see Table 1.

Pomolic acid (**2**): White amorphous powder; (–)-ESI-MS *m/z* 471 $[M-H]^-$, ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 5.63 (1H, t-like, H-12), 3.46 (1H, dd, J = 5.5, 11.0 Hz, H-3α), 3.16 (1H, dt, J = 4.6, 12.8 Hz, H-16α), 3.08 (1H, s, H-18), 2.37 (1H, dt, J = 4.6, 13.8 Hz, H-15β), 1.75 (3H, s, H-27), 1.47 (3H, s, H-29), 1.26 (3H, s, H-23), 1.14 (3H,

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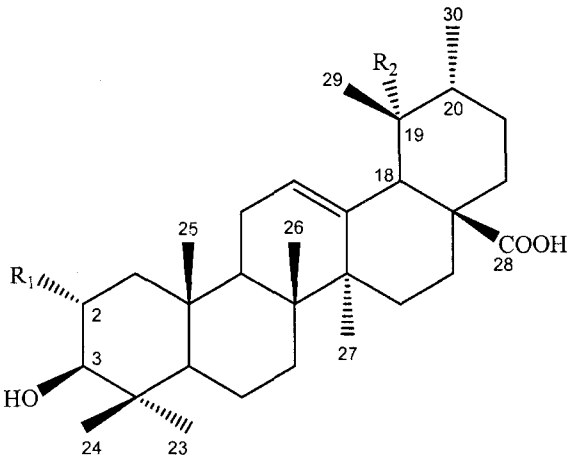

1 : R_1=R_2=H
2 : R_1=H, R_2=OH
3 : R_1=R_2=OH
4 : R_1=OH, R_2=H

Fig. 1. Chemical structures of compounds **1-4.**

d, J = 6.0 Hz, H-30), 1.13 (3H, s, H-26), 1.05 (3H, s, H-24), 0.93 (3H, s, H-25); ^{13}C-NMR data: see Table 1.

Tormentic acid (**3**): White amorphous powder; (−)-ESI-MS *m/z* 487 [M-H]$^-$, ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 5.60 (1H, br.s, H-12), 4.12 (1H, dt, J = 4.6, 9.2 Hz, H-2β), 3.40 (1H, d, J = 9.2 Hz, H-3α), 3.15 (1H, dt, J = 4.1, 12.8 Hz, H-16α), 3.06 (1H, s, H-18), 2.36 (1H, dt, J = 4.6, 13.8 Hz, H-15β), 2.26 (1H, dd, J = 4.1, 12.4 Hz, H-1), 1.73 (3H, s, H-27), 1.45 (3H, s, H-29), 1.28 (3H, s, H-23), 1.13 (3H, d, J = 6.0 Hz, H-30), 1.12 (3H, s, H-26), 1.09 (3H, s, H-24), 1.02 (3H, s, H-25); ^{13}C-NMR data: see Table 1.

Corosolic acid (**4**): White amorphous powder; (−)-ESI-MS *m/z* 471 [M-H]$^-$, ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 5.48 (1H, t-like, H-12), 4.11 (1H, dt, J = 4.1, 9.6 Hz, H-2β), 3.42 (1H, d, J = 9.6 Hz, H-3α), 2.64 (1H, d, J = 11.4 Hz, H-18), 2.34 (1H, dt, J = 4.6, 13.8 Hz, H-15β), 2.26 (1H, dd, J = 4.1, 12.4 Hz, H-1), 1.29 (3H, s, H-23), 1.22 (3H, s, H-27), 1.09 (3H, s, H-26), 1.06 (3H, s, H-24), 1.00 (3H, d, J = 5.0 Hz, H-30), 0.99 (3H, s, H-25), 0.96 (3H, d, J = 6.0 Hz, H-29); ^{13}C-NMR data: see Table 1.

Results and Discussion

The $CHCl_3$-soluble fraction of MeOH extract of the aerial parts of *A. pilosa* was subjected to silica gel and reverse-phase C-18 column chromatography to obtain four compounds.

The (−)-ESIMS spectrum of **1** showed a molecular ion peak at *m/z* 455 [M-H]$^-$. The ^{1}H-NMR spectrum of **1** showed five tertiary methyl groups (δ 0.89, 1.03, 1.06, 1.23, 1.25), together with two secondary methyl groups at δ 0.95 (d, J = 6.0 Hz) and 1.00 (d, J = 6.5 Hz). In addition, the doublet at δ 2.64 (J = 11.4 Hz) and the olefinic proton at δ 5.49 were exhibited. From these spectral data, it is suggested that **1** is to be the urs-12-en type triterpene. The doublet of doublets (J = 6.0, 10.1 Hz) of one proton with oxygenated carbon at δ 3.46 revealed the 3β-hydroxyl functionality. The ^{13}C-NMR spectrum of **1** showed two olefinic signals at δ 125.5 and 139.2, one carboxylic acid carbon at δ 179.8, one oxygenated carbon at δ 78.0, and seven methyl carbons (δ 15.6, 16.5, 17.3, 17.4, 21.3, 23.5, 28.6). Thus, the structure of **1** was expected to be ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) and confirmed by comparison of its spectral data with those reported in the literature (Hong *et al.*, 2002; Jin *et al.*, 2004).

Compound **2**, white amorphous powder, showed the molecular ion peak at *m/z* 471 [M-H]$^-$ in (−)-ESIMS. Comparison of the ^{13}C-NMR spectrum of **2** with that of **1** indicates that **2** is the hydroxylated derivative at C-19

Table 1. ^{13}C-NMR spectral data of compounds **1-4** (125 MHz in pyridine-d_5)

position	1	2	3	4
1	39.3	39.0	47.9	48.1
2	28.0	28.1	68.6	68.6
3	78.0	78.2	83.9	83.8
4	39.3	39.4	42.2	39.9
5	55.7	55.9	56.0	55.9
6	18.7	19.0	19.0	18.9
7	33.5	33.6	33.6	33.5
8	39.9	40.4	40.4	40.0
9	47.9	47.8	47.9	48.0
10	37.3	37.4	38.5	38.4
11	23.8	24.0	24.1	23.8
12	125.5	128.1	128.0	125.6
13	139.2	140.0	139.4	139.3
14	42.4	42.1	39.9	42.6
15	28.7	29.3	29.4	28.7
16	24.8	26.4	26.4	24.9
17	47.9	48.3	48.3	48.1
18	53.4	54.7	54.6	53.5
19	39.4	72.7	72.7	39.4
20	39.0	42.4	42.4	39.5
21	31.0	27.0	26.9	31.1
22	37.2	38.5	38.5	37.5
23	28.6	28.8	29.3	29.4
24	15.6	15.6	16.9	17.7
25	16.5	16.5	17.3	17.0
26	17.3	17.2	17.7	17.5
27	23.5	24.7	24.7	23.9
28	179.8	180.7	180.7	179.9
29	21.3	27.1	27.1	21.4
30	17.4	16.8	16.8	17.5

position of **1**, since an oxygenated carbon signal at δ 72.7 appeared, and apart from the signals due to the A-D rings of this skeleton the carbon signals of **2** are in good agreement with those of **1** (Table **1**). Moreover, when the ^{1}H-NMR spectrum of **2** was compared with that of **1**, the signals ascribable to H-18 and H-29 in **2** were observed as two singlets at lower field (δ 3.08 and 1.47, respectively) than those in **1** (δ 2.64, d, J = 11.4 Hz and δ 0.95, d, J = 6.0 Hz, respectively). Thus, compound **2** was expected to pomolic acid (3β, 19α-dihydroxy-urs-12-en-28-oic acid) and verified by comparison of its spectral data with those reported (Liang *et al.*, 1989; Xu *et al.*, 2002).

Compound **3** was obtained as white amorphous powder. The (−)-ESIMS spectrum showed a molecular ion peak at *m/z* 487 $[M-H]^-$. The ^{1}H-NMR spectrum showed six tertiary methyl singlets at δ 1.02, 1.09, 1.12, 1.28, 1.45, 1.73, one methyl doublet at δ 1.13 (J = 6.0 Hz), and an olefinic proton at δ 5.60 (br. s). One singlet proton signal at δ 3.06 (s) and another proton at δ 3.15 (dt, J = 4.1, 12.8 Hz) assignable to 16α proton due to anisotropic effect of 19α-OH were also observed, which suggested to retain an 19α-hydroxy-ursenoic acid framework (Liang *et al.*, 1989). Two oxygenated methine protons at δ 4.12 (dt, J = 4.6, 9.2 Hz) and 3.40 (d, J = 9.2 Hz) were suggestive of 2α and 3β dihydroxy structure. The ^{13}C-NMR spectrum also suggested the trihydroxylated ursenoic acid structure with the presence one carboxyl carbon signals at δ 180.7 (C-28), two olefinic carbon signals at δ 128.0 (C-12) and 139.4 (C-13), and three hydroxylated carbons at δ 68.6 (C-2), 83.9 (C-3) and 72.7 (C-19). The identity of **3** as tormentic acid (2α, 3β, 19α-trihydroxy-urs-12-en-28-oic acid) was confirmed by comparison of reported spectral data (Jin *et al.*, 2004; Park *et al.*, 2002).

Compound **4** was obtained as white amorphous powder. The (−)-ESIMS spectrum showed a molecular ion peak at *m/z* 471 $[M-H]^-$. The ^{13}C-NMR spectrum was similar to that of **3**, except that, the peak at δ 39.4 was remarkably shifted in comparison with δ 72.7 (C-19) of **3**. It means that **4** could be deoxygenated at C-19 position of **3**. The ^{1}H-NMR spectrum also suggested the dihydroxylated ursenoic acid structure, and compound **4** was identified as corosolic acid (2α, 3β-dihydroxy-urs-12-en-28-oic acid) in comparison with its spectral data with those reported data (Park *et al.*, 2002; Taniguchi *et al.*, 2002). This is the first report on the isolation of compounds **2** and **4** from *Agrimonia* species.

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