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TRITERPENOIDS FROM *AGRIMONIA PILOSA*

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Key Word Index—*Agrimonia pilosa*; Rosaceae; triterpenoid; 19α-hydroxyursolic acid.

Abstract—Two new triterpenoids have been isolated as a methylester from the whole plant of *Agrimonia pilosa*, along with 2α,19α-dihydroxyursolic acid (28-1)β-D-glucopyranoside from the roots of this plant. On the basis of chemical and spectral evidence, the structures were established as 1β,2α,3β,19α-tetrahydroxyurs-12-en-28-oic acid and 1β,2β,3β,19α-tetrahydroxyurs-12-en-28-oic acid.

INTRODUCTION

Agrimonia pilosa Ledeb is widely distributed in Asia. The chemical components of this plant have been extensively examined and agrimonolide [1], luteolin 7-*O*-β-D-glucoside, apigenin 7-*O*-β-D-glucoside [2, 3], agrimol A, B and D [4] agrimophol [5] and tannins [6, 7] were obtained. The antitumour activity of the extracts from the roots of this plant was also reported [8]. We report now on the constituents of *Agrimonia pilosa*.

RESULTS AND DISCUSSION

The methanol extract of the aerial parts of *A. pilosa* was fractioned by the usual procedure (Experimental) to afford a triterpenoid fraction. This was treated with diazomethane in methanol because of the difficulty of separation, and compounds **1** (45 mg) and **2** (112 mg) were isolated. The ^{1}H NMR spectrum of compound **1** $C_{31}H_{50}O_6$ (EIMS, *m/z* 518) showed the characteristic broad singlet at δ2.57, together with the tertiary methyl [δ0.68, 0.83, 1.02 (2Me), 1.22 (2 Me)], the secondery methyl (0.94, *d*, *J* = 6.4 Hz), the ester methyl (3.60) and the olefinic (5.35, *t*, *J* = 3.4 Hz) protons, all of which suggested a 19α-hydroxyurs-12-en type of triterpenoid. The olefinic carbon signals (δ130.0, C-12; 137.3, C-13) in the ^{13}C NMR spectrum of **1** also indicated that **1** had an urs-12-en skeleton [9]. Although the hydroxy methine protons were not obvious in the ^{1}H NMR spectrum of **1**, an acetate (**1a**) of **1** showed the signals of three acetoxyls and three acetoxy methine groups. The latter of which exhibited two doublets (δ4.79, *J* = 10.6 Hz; 4.88, *J* = 9.3 Hz) and a double doublet (δ5.22, *J* = 10.6 and 9.3 Hz), and were assignable to C-1 (or 3), C-3 (or 1) and C-2, respectively. Three hydroxy methine carbons were also indicated in the ^{13}C NMR spectrum of **1** (δ74.6, 74.9, 79.9). As the *J*-values of these signals indicated trans-diaxial correlated protons, the three acetoxyls must be equatorial. It was concluded that compound **1** was 1β,2α,3β,19α-tetrahydroxyurs-12-en-28-oate, and that the natural compound should be originally 1β,2α,3β,19α-tetrahydroxyurs-12-en-28-oic acid (**1b**).

The ^{1}H NMR spectrum of compound **2** ($C_{31}H_{50}O_6$; EIMS, *m/z* 518) showed a broad singlet at δ2.59 similar to that of **1**. As the other signals of the ^{1}H NMR and the ^{13}C NMR spectra of **2** were quite similar to those of **1**, compound **2** was considered to posses the same skeleton as **1**. An acetate (**2a**) of **2** showed in the ^{1}H NMR spectrum the signals of three acetoxyls and three acetoxy methine protons. These methine protons appeared at δ4.70 (*d*, *J* = 3.7 Hz, C-1 or 3), 4.75 (*d*, *J* = 3.7 Hz, C-3 or 1) and 5.44 (*t*, *J* = 3.7 Hz, C-2) compared to those of **1**, and these coupling constants were accommodated on an axial–equatorial–axial correlation for these protons. These findings indicated **2** is 1β,2β,3β,19α-tetrahydroxyurs-12-en-28-oate, and that the naturally occurring compound should be 1β,2β,3β,19α-tetrahydroxyurs-12-en-28-oic acid (**2b**).

An acetonide reaction of **2** with acetone and *p*-toluene sulphonic acid yielded two compounds **2d** and **2e** (trace), although the same reaction for **1** gave only the starting

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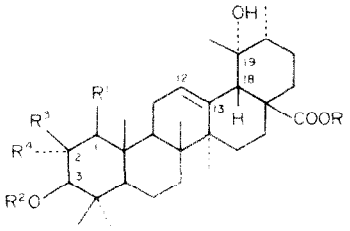
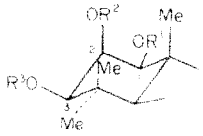


1 R = Me, R^1 = R^4 = OH, R^2 = R^3 = H
1a R = Me, R^1 = R^4 = OAc; R^2 = Ac; R^3 = H
1b R = R^2 = R^3 = H, R^1 = R^4 = OH

2 R = Me; R^1 = R^3 = OH; R^2 = R^4 = H
2a R = Me; R^1 = R^3 = OAc; R^2 = Ac; R^4 = H
2b R = R^2 = R^4 = H; R^1 = R^3 = OH

3 R = Glu, R^1 = R^2 = R^3 = H, R^4 = OH



2d (e) R^1 / R^2 = Me / Me, R^3 = H

2e (d) R^2 / R^3 = Me / Me, R^1 = H

material. These results supported the *cis*-relationships of the three hydroxy groups at C-1, C-2 and C-3 for **2** and the *trans*-relationship for **1**.

The methanol extract of the roots of *A. pilosa* was separated, as described in the Experimental, to give **1** (240 mg), **2** (15 mg) and **3** (250 mg). All the chemical and spectral evidences of **1** and **2** were identical with the above data. It is noteworthy that the quantities of these compounds were reversed from those of aerial parts.

The ^{1}H NMR and ^{13}C NMR spectra of compound **3**, $C_{36}H_{58}O_{10}$ (FABMS, *m/z* 673 $[M+Na]^+$, suggested a triterpenoic acid sugar ester structure for **3**, and they were completely identical with the data of rosamultin (2α,19α-dihydroxyursolic acid (28–1)β-D-glucoside) which was obtained from *Rosa multiflora* [10].

EXPERIMENTAL

The NMR spectra were measured in $CDCl_3$ at 90 MHz for the ^{1}H NMR and 22.5 MHz for the ^{13}C NMR unless otherwise specified. Chemical shifts are given on the δ (ppm) scale with TMS as int. standard. IR were taken in $CHCl_3$.

Extraction and isolation. The plant material was collected near Nagasaki (Japan) during the summer of 1986, and divided into the aerial parts and the roots. The air-dried aerial parts (1.5 kg) were extracted with MeOH to give the MeOH extract (270 g), and partitioned between *n*-hexane–H_2O and EtOAc–H_2O successively. The EtOAc soluble part (48 g) was partitioned again with $CHCl_3$–MeOH–H_2O (7:3:1) to afford a lower phase (17 g), which was separated by silica gel CC to give a triterpenoid fraction. This fraction was passed through an LH-20 column (eluted with MeOH) and the greenish soln was treated with a soln of CH_2N_2 in Et_2O and then evapd. The gum obtained was chromatographed repeatedly on silica gel using a solvent system of $CHCl_3$–MeOH to yield compounds **1** (45 mg) and **2** (112 mg).

Compound **1**. White amorphous powder. $[\alpha]_D^{16}$ +32.2° ($CHCl_3$ *c* 0.9). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1712, 1520. EIMS *m/z*: 518 $[M^+]$. HRMS found: 518.371; $C_{31}H_{50}O_6$ requires 518.360. ^{1}H NMR: δ 0.68, 0.83 (each *s*, 3H), 0.94 (*d*, *J* = 6.4 Hz, 3H, H-30), 1.02 (*s*, 6H), 1.22 (*s*, 6H), 2.57 (*br s*, 1H, H-18), 3.0–3.6 (*br m*, 3H, H-1, 2, 3), 3.60 (*s*, 3H, –OMe), 5.35 (*t*, *J* = 3.4 Hz, 1H, H-12); ^{13}C NMR δ: 11.4 (*q*, C-25), 16.1m 16.9, 17.1 (each *q*, C-24 and/or 26 and/or 30), 17.9 (*t*, C-6), 24.5 (*t*, C-11), 25.6 (*q*, C-27), 26.1 (*t*, C-16), 27.0 (*t*, C-21), 27.4 (*q*, C-29), 28.3 (*q*, C-23), 29.8 (*t*, C-15), 32.8 (*t*, C-7), 37.4 (*s*, C-10), 38.1 (*t*, C-22), 40.6 (*s*, C-4), 41.2 (*s*, 2C, C-8, 14), 42.9 (*d*, C-20), 48.0 (*d*, C-9), 48.5 (*s*, C-17), 51.5 (*q*, –COOMe), 52.6 (*d*, C-18), 53.2 (*d*, C-5), 73.2 (*s*, C-19), 74.6, 74.9 (each *d*, C-1 and/or 2), 79.9 (*d*, C-3), 130.0 (*d*, C-12), 137.3 (*s*, C-13), 178.3 (*s*, C-28).

Compound **2**. White amorphous powder. $[\alpha]_D^{16}$ +30.0° ($CHCl_3$ *c* 0.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1735, 1520, 1370.EIMS *m/z*: 518 $[M^+]$. HRMS found: 518.364; $C_{31}H_{50}O_6$ requires 518.360. ^{1}H NMR δ 0.94 (*d*, *J* = 6.2 Hz, 3H, H-30), 0.70, 0.97, 0.99, 1.91, 1.21, 1.25 (each *s*, 3H), 2.59 (*br s*, 1H, H-18), 3.22, 3.31, 4.03 (each *br s*, 1H, H-1, 2, 3), 3.60 (*s*, 3H, –OMe), 5.38 (*t*, *J* = 3.6 Hz, H-12). ^{13}C NMR δ 11.4 (*q*, C-25), 16.1, 16.9, 17.1 (each *q*, C-24 and/or 26 and/or 30), 17.9 (*t*, C-6), 24.6 (*t*, C-11), 25.6 (*q*, C-27), 26.1 (*t*, C-16), 26.9 (*t*, C-21), 27.4 (*q*, C-29), 28.3 (*q*, C-23), 29.8 (*t*, C-15), 32.8 (*t*, C-7), 37.4 (*s*, C-10), 38.1 (*t*, C-22), 40.6 (*s*, C-4), 41.2 (*s*, 2C, C-8, 14), 42.9 (*d*, C-20), 48.0 (*d*, C-9), 48.5 (*s*, C-17), 51.5 (*q*, –COOMe), 52.6 (*d*, C-18), 53.2 (*d*, C-5), 73.2 (*s*, C-19), 74.6, 74.9 (each *d*, C-1 and/or 2), 79.9 (*d*, C-3), 130.1 (*d*, C-12), 137.2 (*s*, C-13), 178.4 (*s*, C-28).

The dried roots (1 kg) extracted with MeOH to give the MeOH extract, which was partitioned between Et_2O–H_2O and EtOAc–H_2O successively. The EtOAc soluble part (13 g) was chromatographed on silica gel column using a solvent system of $CHCl_3$–MeOH to give a triterpenoid and a triterpene glycoside fraction, respectively. The former was treated with CH_2N_2 as described above, and successive chromatography on silica gel gave compounds **1** (240 mg) and **2** (15 mg). The latter was passed through LH-20 column (MeOH) and chromatographed repeatedly on silica gel to afford **3** (250 mg).

Compound **3**. Colourless needles, mp 206–208°. $[\alpha]_D^{16}$ = +10° (EtOH *c* 1.0). FAB MS *m/z* 673 $[M+Na]^+$. ^{13}C NMR, δ 178.5 (*s*, C-28), 139.5 (*s*, C-13), 129.5 (*d*, C-12), 95.7 (*d*, anomeric carbon C-1), 84.5 (*s*, C-19), 78.4 (*d*, C-5'), 78.3 (*d*, C-3'), 73.8 (*d*, C-3), 73.6 (*d*, C-2'), 71.2 (*d*, C-4'), 69.5 (*d*, C-2), 62.5 (*t*, C-6'). All the data is consistent with that of rosamultin [10].

Triacetate (**1a**) *of compound* **1**. Prepared by treatment of **1** with Ac_2O–pyridine. Colourless oil. $[\alpha]_D^{16}$ +31.0° ($CHCl_3$ *c* 0.6). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1730, 1520. EIMS *m/z*: 644 $[M^+]$. ^{1}H NMR: δ 0.65, 0.89, 0.95 (each *s*, 3H), 0.92 (*d*, *J* = 5.1 Hz, 3H, H-30), 1.18, (*s*, 6H), 1.22 (*s*, 3H), 1.95, 1.98, 2.02 (each *s*, 3H,-OAc × 3), 2.55 (*br. s*, 1H, H-18), 3.59 (*s*, 3H, –COOMe), 4.79 (*d*, *J* = 10.6 Hz, H-1 or 3), 4.88 (*d*, *J* = 9.3 Hz, H-3 or 1), 5.22 (*dd*, *J* = 9.3 and 10.6 Hz, 1H, H-2), 5.30 (*t*, *J* = 3.4 Hz, 1H, H-12).

Triacetate (**2a**) *of Compound* **2**. Prepared by treatment of **2** with Ac_2O–pyridine. Colourless oil. $[\alpha]_D^{16}$ +29.1° ($CHCl_3$ *c* 0.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1713, 1520.EIMS *m/z*: 644 $[M^+]$. ^{1}H NMR: δ 0.69, 0.90 (each *s*, 3H), 0.93 (*d*, *J* = 6.4, 3H, H-30), 1.07, 1.18, 1.21, 1.30 (each *s*, 3H), 1.99, 2.02, 2.08 (each *s*, 3H, –OAc × 3), 2.55 (*br s*, 3H, H-18), 3.59 (*s*, 3H, –COOMe), 4.70, 4.75 (each *d*, *J* = 3.7 Hz, 1H, H-1 and/or 3), 5.26 (*t*, *J* = 3.6 Hz, 1H, H-12), 5.44 (*t*, *J* = 3.7 Hz, 1H, H-2).

Acetonide (**2d**) *of Compound* **2**. Prepared by treatment of **2** with Me_2CO, *p*-toluenesulphonic acid. Colourless oil. EIMS *m/z*: 558 [M^+]. ^1H NMR (400 MHz): δ0.73, 0.94 (each *s*, 3H), 0.95 (*d*, *J* = 6.5 Hz), 1.01, 1.16, 1.23, 1.27 (each *s*, 3H), 1.39, 1.51 (each *s*, 3H, Acetonide diMe), 2.61 (*s*, 1H, H-18), 3.60 (*s*, 3H, -COOMe), 3.58 (*d*, *J* = 4.1 Hz, 1H, H-1 or 3), 3.87 (*d*, *J* = 7.7 Hz, 1H, H-3 or 1), 4.18 (*dd*, *J* = 4.1, 7.7 Hz, 1H, H-2), 5.40 (*br s*, 1H, H-12). Another acetonide (**2e**) was detected by TLC but not identified with the spectral data.

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TRITERPENOIDS FROM *SALVIA PRZEWALSKII*

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Key Word Index—*Salvia przewalskii*; Labiatae; triterpenoids; przewanoic acid A; przewanoic acid B.

Abstract—Oleanolic acid and two novel triterpenoids, przewanoic acid A and przewanoic acid B, were isolated from the dried roots of *Salvia przewalskii*. Their structures were elucidated on the basis of chemical and spectral methods.

INTRODUCTION

The Tibetian folk drug 'Hong Qin Jiao' is the dried roots of *Salvia przewalskii* Maxim, which is widely distributed in the western areas of China [1–3]. It has been reported that its main chemical components are the *O*-naphthaquinone diterpenes [4, 5]. The present paper describes the isolation and elucidation of two novel triterpenoids, przewanoic acid A and przewanoic acid B, along with a known triterpenoid, oleanolic acid.

RESULTS AND DISCUSSION

Przewanoic acid A (**1**), white needles, mp 269–270°, $[\alpha]_D$ + 125° had the molecular formula $C_{30}H_{46}O_4$. Its UV spectrum λ_{max}^{MeOH} 211 nm (log ε3.65) showed the probable presence of a double bond conjugated cyclopropane [6]. The ^1H NMR spectrum showed the presence of six methyl groups in agreement with the ^{13}C NMR spectral data (δ17.5, 17.5, 22.2, 22.8, 29.5, 32.6; Table 1). Also the ^1H NMR spectrum revealed one allylic hydrogen and two secondary hydroxyls. The signal at high field (−0.08, *dd*, 1H, *J* = 4.8, 4.8 Hz) is characteristic of the CH_2 in a cyclopropane [6, 7].

The coupling relationships of related protons were assigned from the comparative study of the ^1H–^1H 2D COSY data. The signals at 5.82 (*dd*, 1H, *J* = 7.2, 3.6 Hz), 2.79 (*dd*, 1H, *J* = 13.2, 7.2 Hz) and 2.07 (*dd*, 1H, *J* = 13.2, 3.6 Hz) were a group of corresponding protons. This showed the presence of part structure $^{14}C = {}^{15}CH-{}^{16}CH_2-$. The signals at 4.29 (*ddd*, 1H, *J* = 10.7, 4.0, 2.6 Hz, CHOH) and 3.77 (*d*, 1H, *J* = 2.6 Hz, CHOH) were coupled with each other. This indicated two secondary hydroxy groups which should be placed at C-2 and C-3, respectively, in a *trans*-diaxial configuration ($J_{2H\text{-}3H}$ = 2.6 Hz).

The position of the carboxyl group at C-17 and the double bond at the Δ^{14}-position was established from the mass spectral fragmentation pattern of przewanoic acid A (**1**). Compound **1** exhibited a fragment peak at *m/z* 316 (**1a**). This ion peak was accompanied by a peak 15 mass units lower (**1b**) which was formed by the loss of the allylically activated methyl group at C-8. Moreover, the mass spectrum of **1** showed a peak at *m/z* 232 (**1c**) derived from rings D and E. Furthermore, the fragment **1c** loses the carboxyl substituent at C-17 giving rise to a fragment base peak at *m/z* 187 (**1d**). This type of fragmentation is