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Triterpenoids from *Gentiana veitchiorum*

Hong-Peng Yang[1], Sheng Que[2], Yan-Ping Shi[3] and Li-Tong Ban[1]*

[1]*Tianjin Agricultural College, Tianjin, China*
[2]*Minorities Teachers College, Qinghai Normal University, Xining, Qinghai, China*
[3]*Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, China*

ABSTRACT

*A thorough investigation of the flower part of Gentiana veithiorum afforded one new triterpene , 3□, 11β-dihydroxyurs-12-en-28-oic acid **(1),** as well as nine known triterpenoids, ursolic acid **(2)**, 3β, 28-dihydroxyurs-12-ene, **(3)**, Urs-12-en-28-hydroxy-3β-O- palmitate **(4)**, α-amyrin **(5)**, 2α, 3β-dihydroxyurs-12-en-28-oic acid **(6)**, oleanolic acid **(7)**, Lup-28-hydroxy -3β-O- palmitate **(8)**, lupeol **(9)**, 3-epi-betulinic **(10)** .The structures of the new compound were characterized by means of spectroscopic methods in cluding 1D, 2D NMR , ESI-MS and HRESI- MS, and the known ones were established on the basis of com- paring their NMR data with those of the corresponding compounds in the literature.Above all compounds were found in this plant for the first time.*

Keywords: *Gentiana veithiorum;* Triterpenoids; Tibetan medicine

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INTRODUCTION

The genus *Gentiana*(*Gentianaceae*) consists of about 400 species distributed throughout European, Asia, North of Australia, New Zealand, North America. There are about 247 species grown in China. *G. veithiorum* is a peculiar herbal plant grown in China, which has been used as a traditional Tibetan medicine for rheumatic arthritis.[1] Up to now, its chemical constituents have not been investigated. In order to obtain more evidence for chemotaxonomy and pharmacology, we carried on investigation of flower of *G. veithiorum*. Here we report constituent of flower of this plant.

RESULTS AND DISCUSSION

The alcoholic extract of the flower part of *G. veithiorum* were repeatedly separated by silica gel column chromatography to yield a new triterpenoids, 3□,11β-dihydroxyurs-12 -en-28-oic acid **(1)**, as well as nine known triterpenoids compounds, ursolic acid **(2)**,[2] 3β, 28-dihydroxyurs -12-ene, **(3)**,[3] Urs-12-en-28-hydroxy-3β-*O*-palmitate **(4)**,[4] α-amyrin (**5**),[5] 2α, 3β-dihydroxyurs -12-en-28-oic acid **(6)**,[6] oleanolic acid **(7)**,[2] Lup-28-hydroxy-3β-*O*-palmitate **(8)**,[7] lupeol **(9)**,[8] 3- epi-betulinic **(10)** [9] were isolated and purified by repeated chromatography over silica gel column. Every compound obtained was subjected to detail spectroscopic analysis to establish their chemical structures. To the best of our knowledge, compounds **1** are previously unreported naturally occurring. The structures of known compounds were identified by direct comparison of their spectral data (^{1}H NMR and ^{13}C NMR and DEPT) with those reported values in the corresponding literature.

Compound **1** was obtained as amorphous powder, mp 183-185 ^{o}C, $[\alpha]_D^{20}$ +34^o (C, 0.2, CH_3OH). Its molecular formula was assigned as $C_{30}H_{48}O_4$, indicated seven degrees of unsaturation, on the basis of ESI-MS data $[M-H]^-$ at m/z 471 and ESI-HR-MS date$[M-H]^-$ at m/z 471.3478(calc.471.6989) and results from ^{1}H, ^{13}C NMR and DEPT

analysis. The IR spectrum showed absorption bands for hydroxyl (3736, 3421 cm^{-1}) and a carbox- yl (1692 cm^{-1}) groups. The ^{13}C NMR and DEPT experiment of **1** shown 30 carbon signa- ls ascribable to 7×CH_3, 8×CH_2, 5×CH, two oxygenated methines (□c 76.6 and 79.7), seven quaternary and a carboxyl group (□c 178.2) (Table). The ^1H NMR spectrum of **1** displayed the characteristic signals of seven methyl groups (□$_H$1.07, 0.94, 0.91, 0.89, 0.88, 0.74, 0.67), two oxygenated methine (□$_H$ 2.98, dd, J= 4.8, 10.8Hz H-3; □$_H$ 4.28, dd, J= 3.2, 9.0 Hz H-11), an olefinic proton (□$_H$ 5.4, d, J= 3.2Hz, H-12), two hydroxyl proton (□$_H$ 12.03, 10.85, COOH, 11-OH). The above NMR results suggested compound **1** was a pentacyclic triterpenoids. Assignments of the ^1H and ^{13}C signals by 2D NMR revealed that **1** was an analogue of 3α, 11α-dihydroxyurs-12-ene and 3□, 11α -dihydroxyurs-12-en-28-oic acid(Torre et al. 1990; Mahato et al. 1994; Susana et al. 2005).[10, 11 ,12]The carboxyl group in **1** was attached at C-17, because of the correlation between □c178.2 and □$_H$1.93 (H-22) and □$_H$ 2.1 (H-18) in gHMBC. One hydroxyl group of **1** was attached at C-3β by comparison of the NMR date of H-3α (□$_H$ 2.98, dd, J=4.8, 10.8Hz, □c76.6) for **1** to those of 3α, 11α- dihydroxyurs-12-ene and 3□, 11α-dihydroxy- urs-12-en-28-oic acid. Another hydroxyl group was attached at C-11β by comparison of the NMR date of H-11α (□$_H$ 4.28, dd, J=3.2, 9.0 Hz, □c 79.7) for **1** to those of 3α,11α-dihydroxyurs-12-ene and 3□, 11α-dihydroxy- urs-12-en-28-oic acid, together with the cross peaks between H-11 with H-12 (□$_H$ 5.4, d, J=3.2Hz) and H-9 (□$_H$ 1.53, d, J=9.0Hz) in ^1H-^1H COSY, as well as H-11 with C-9, C-10, C-12, C-13; and H-12 with C-9, C-14, C-18 in gHMBC experiment(Fig1). Consequently, compound **1** was identified as 3□, 11β-dihydroxyurs-12-en-28- oic acid.

EXPEIMENTAL SECTION

General

Optical rotations were recorded in CH_3OH us ing aPerkin Elmer model 341 polarimeter. UV spectra were measured on a Spect 50-UV/Vis in strument (Analytic Jena AG). IR spectra were mea- sured on an FTS165-IR instrument (BioRad, USA). 1D NMR spectra and 2D NMR were recorded on a Varian INOVA-400 FT-NMR spectrometer (USA) in $CDCl_3$ with TMS as internal standard. HRESIMS were obtained on a Bruker Daltonics APEX II spectrometer. Silica gel (200- 300 mesh) used for column chromatography (CC) and silica GF_{254} for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, P. R. China. Spots were de tected onTLC by visual- ization under UV light or by spray ing with98% H_2SO_4-EtOH (v:v = 5:95) followed by heating at 110 ^oC.

Plant Material

3.2 ***Plant material***

The fresh air-dried flower of *Gentiana veithiorum* Hemsl was bought from Tibetan Hospital of Qinghai province, China, in October 2003 and was identified by Prof. Guo-Liang Zhang, College of Biology, Lanzhou University, Lanzhou, P. R. China. A voucher specimen has been deposited at key Laboratory of Natural Medicine for Gansu Province.

Extraction and Isolation

The air-dried flower of *G. veithiorum* (5.5 Kg) was extracted with C_2H_5OH (95%) at room temperature (30L×5, each extraction lasted 5 days). The combined extracts were evaporated to dryness under reduced pressure. The residue (510g) was then suspended in H_2O (1.5L), and extracted with petroleum ether (60-90 ^oC) (1.0 L × 5), EtOAc (1.0 L × 5) and *n*-BuOH (1.0 L × 5), respectively. The petroleum ether (60-90 ^oC) extract (63g) was subjected to column chromatography on silica gel (200~300 mesh, 650 g) using petroleum ether (60-90 ^oC) with increasing volume of acetone (v : v = 40:1, 20:1, 15:1, 10:1, 5:1, 2:1, 1:1, each about 3.5 L) as eluent. The fractions A1-A12 were collected according to TLC analysis. Fraction A2 (2.0 g) was further fractionated on a silica gel column (200-300 mesh, 100 g) eluting with petroleum ether-acetone (v : v = 20:1) to give a mixture of compounds **4** and **8** (500mg), that was further isolated and purified on a silica gel column (200-300 mesh, 15 g) using petroleum ether-ethyl acetate (v : v = 18:1) to give **4** (27 mg) and **8** (30 mg); Fraction A4 (300 mg) gave compound **5** (30 mg) after a CC on a silica gel eluting with petroleum ether-ethyl acetate (v : v=15 : 1); Fraction A6 (1.5 g) was purified by CC on a silica gel (200~300 mesh, 40 g) eluting with petroleum ether-acetone (v : v = 18:1) to yield a mixture of compounds **3** and **9** (300 mg), that was further isolated and purified on a silica gel (200-300 mesh, 15 g) eluting with petroleum ether-ethyl acetate (v : v = 12 : 1) to give **3** (20 mg) and **9** (30 mg); Fraction A8 (6.0 g) was subjected to column chromatography on a silica gel (200-300 mesh, 120 g) eluting with petroleum ether-acetone (v : v=5 : 1) to give four subfractions (A8a, 100 mg; A8b, 3.5 g; A8c, 60 mg; A8d, 50 mg). A8a (100 mg) was subjected to column chromatography on a silica gel (200-300 mesh, 20 g) eluting with petroleum chloroform-acetone (v : v = 30 : 1) to yield the compound **10** (20 mg); A8b (3.5 g) was further fractionated on a silica gel (200-300 mesh, 20 g) eluting with petroleum chloroform-ethyl acetate (v : v = 25 : 1) to give a mixture of compounds **2** and **7** (2.6 g) which couldn't be isolated by prep-TLC and CC; A8c (60 mg) was subjected to column chromatography on a silica gel (200-300 mesh, 10 g) eluting with petroleum chloroform -acetone(v : v = 15 : 1) to yield **1** (6 mg); A8d (50 mg) was further isolated and purified by CC over a silica gel (200-300 mesh, 10 g), eluting with petroleum chloroform-methanol (v: v = 20:1) to give **6** (20 mg).

Table 1 ^{13}C NMR (100.13 MHz) data for compound 1, 4 and 8

Carbon	**1**	**4**	**8**	Carbon	**1**	**4**	**8**
C-1	36.2	38.4	38.2	C-20	38.6	39.3	150.4
C-2	23.6	23.6	23.6	C-21	29.9	30.9	29.6
C-3	76.6	80.5	80.4	C-22	39.2	30.6	33.9
C-4	38.5	38.3	37.6	C-23	28.3	28.1	27.8
C-5	54.7	55.2	55.2	C-24	15.9	15.7	16.0
C-6	17.9	18.2	18.0	C-25	16.4	16.7	16.4
C-7	33.0	32.7	34.0	C-26	18.4	17.3	15.8
C-8	42.1	39.8	40.8	C-27	22.1	23.2	14.5
C-9	49.6	47.6	50.1	C-28	178.2	69.9	60.0
C-10	37.5	37.7	36.9	C-29	21.0	16.7	109.5
C-11	79.7	23.4	20.7	C-30	16.9	21.3	18.9
C-12	125.8	124.9	25.0	C-1′		173.6	173.6
C-13	142.0	138.7	37.1	C-2′		35.1	34.7
C-14	41.5	42.0	42.5	C-3′		25.1	25.0
C-15	27.7	29.2	26.8	C-4′ to		29.1	29.0
C-16	27.1	22.6	29.2	C-13′		29.7	29.5
C-17	46.4	36.8	47.6	C-14′		31.9	31.7
C-18	51.6	54.0	48.6	C-15′		22.7	22.5
C-19	38.2	39.4	47.7	C-16′		14.1	14.0

Assignments were aided by spin splitting patterns, DEPT, ^{1}H-^{1}H COSY, gHMQC, gHMBC experiments, and chemical shift values (□□. The・・・values are in ppm.

3α, 11β-dihydroxyurs-12-en-28-oic acid (1)

Amorphous powder. Mp.183-185℃, $[\alpha]_D^{20}$ +34^o (C, 0.2, CH_3OH). IR (film, ν_{max}, cm^{-1}) 3736 (COOH), 3421 (OH), 2929, 2869, 1692 (C=O), 1459, 1386, 1233, 1309, 997, 890, 765, 667. ESI-MS *m/z*: 471 [M-1]. ^{1}H NMR (400 MHz, TMS, DMSO-d_6) ・・ppm: ・12.03 (COOH), 10.85, (11-OH), 5.40 (1H, d, J = 3.2Hz, H-12), ・4.28 (1H, dd, J = 3.2, 9.0 Hz H-11)・・2.98 (1H, dd, J = 4.8, 10.8Hz H-3), ・$_H$1.53 (1H, d, J = 9.0Hz H-9); 1.07, 0.94, 0.91, 0.89, 0.88, 0.74, 0.67 (each 3H, s, H-23, 24, 25, 26, 27, 29, 30). ^{13}C NMR: see Table1.

ursolics acid (2) and oleanolic acid (7)

White powder. IR (film, ν_{max}, cm^{-1}) 3400 (OH), 1690(C=O); ^{1}HNMR (400MHz, DMSO-d_6) δ: 8.31 (1H, s), 5.14 (2H, t, H-12), 3.15 (2H, m, H-3) ; *ursolics acid* **(2)**: ^{13}CNMR (100 MHz, DMSO-d_6) δ: 38.2 (C-1), 27.4 (C-2), 76.7 (C-3), 38.3 (C-4), 54.7 (C-5), 17.9 (C-6), 32.7 (C-7), 39.0 (C-8), 37.9 (C-9), 36.4 (C-10), 23.3 (C-11), 124.5 (C-12), 138.1 (C-13), 41.5 (C-14), 28.1 (C-15), 23.8 (C-16), 46.7 (C-17), 52.3 (C-18), 38.4 (C-19), 38.3 (C-20), 30.1 (C-21), 36.2 (C-22), 28.1 (C-23), 15.9 (C-24), 15.1 (C-25), 16.9 (C-26), 23.2 (C-27), 178.1 (C-28), 22.9 (C-29), 20.9 (C-30); *oleanolic acid* **(7)**: ^{13}C NMR (100 MHz, DMSO-d_6) δ: 38.4 (C-1),26.9 (C-2), 79.0 (C-3), 38.8 (C-4), 55.1 (C-5), 17.9 (C-6), 32.7 (C-7), 39.2 (C-8), 47.0 (C-9), 37.8 (C-10), 23.2 (C-11), 121.4 (C-12), 143.7 (C-13), 41.2 (C-14), 27.9 (C-15), 23.3 (C-16), 46.7 (C-17), 41.2 (C-18), 45.6 (C-19), 30.6 (C-20), 33.2 (C-21), 32.6 (C-22), 27.9 (C-23), 15.9 (C-24), 15.1 (C-25), 16.8 (C-26), 25.5 (C-27), 178.4 (C-28), 33.2 (C-29), 23.5 (C-30).

3β, 28-dihydroxyurs -12-ene (3)

Amorphous powder. M.p.230- 231℃; ^{1}H NMR (400MHz, $CDCl_3$) δppm: 3.19 (1H, m, 3-H)，5.10 (1H, t, J = 3.6Hz, 12-H), 3.49 (1H, d, J = 10.4Hz, H-28), 3.20 (1H, d, J = 10.4Hz, H-28′), 0.75, 0.89, 0.91, 0.95, 1.06 (3H, each s, 5×CH_3), 0.78 (3H, d, J = 6.0Hz, H-29), 0.96 (3 H , overlap, H-30). ^{13}CNMR (100Hz, TMS, $CDCl_3$) δppm (order C-1~C-30): 38.7, 27.1, 78.9, 37.9, 55.0, 18.2, 32.7, 39.9, 47.6, 36.7, 23.2, 124.9, 138.6, 41.9, 28.0, 23.3, 38.6, 54.0, 39.2, 39.3, 30.5, 35.1, 28.0, 15.5, 15.6, 16.7, 23.1, 69.8, 17.3, 21.3.

Urs-12-en-28-hydroxy-3β-O–palmitate (4)

Colorless gum, ^{1}H NMR (400 MHz, TMS, $CDCl_3$)・・・ppm: δ 4.48 (2H, dd, J=11.0, 5.6Hz， H-3), 2.30 (2H, t, J = 8.0，H-11a), 5.10 (1H, t, H-12)，3.50 1H, d, J = 10.8Hz, H-28a), 3.16 (1H, d, J = 10.8Hz, H-28b), 0.85, 0.84×2, 0.90, 1.07 (3H, each s, 5×CH_3), 0.79 (3H, d, J = 6.0Hz, H-30), 0.96 (3H, d, J = 6.0Hz, H-29); 2.27 (t, J = 7.0Hz, H-2′). ^{13}C NMR: see Table1

α–amyrin (5)

Colorless needles. M.p. 184~185℃. ^{1}H-NMR (400 MHz, TMS, $CDCl_3$) ・ ppm: 3.19 (1H, dd, J = 5.2，8.8Hz，3β-H), 5.09 (1H, t, 12-H), 1.22, 1.04, 0.98, 0.97, 0.92, 0.88, 0.77, 0.76 (3H, each s, 8×CH_3);. ^{13}C-NMR (100 MHz, TMS, $CDCl_3$) ・・ppm (order C-1~C-30): 38.7, 27.2, 78.9, 38.7, 55.1, 18.3, 32.9, 39.9, 47.6, 36.8, 23.3, 124.3, 139.5, 42.0, 28.7, 26.5, 33.7, 58.9, 39.6, 39.6, 31.2, 41.5, 28.1, 15.6, 15.6, 16.8, 23.3, 28.0, 17.4, 21.4.

2α, 3β-dihydroxyurs-12-en-28-oic acid (6)

Amorphous powder. M.p.249- 250℃; ^{1}H-NMR (400 MHz, TMS, DMSO-d_6) ·· ppm: 11.9 (1H, s, 28-COOH), 5.12 (1H, t, J = 4.0, 12-H), 4.40 (1H, d, J = 4.0,H-3-α), 4.28 (1H, d, J = 4.0, H-2-β), 2.10 (1H, d, J = 12, H-18), 0.69, 0.72, 0.90×2, 1.03, (each 3H, s, CH_3), 0.80 (3H,d, J = 6.4, H-29), 0.91 (3H, overlap, H-30); ^{13}C-NMR (100 MHz, TMS, $CDCl_3$) ·· ppm (order C-1~C-30): 46.9, 67.1, 82.2, 39.1, 54.7, 18.0, 32.9, 39.6, 47.0, 37.5, 23.8, 124.5, 138.2, 41.6, 27.4, 22.9, 46.8, 52.3, 39.0, 38.5, 30.1, 36.3, 28.8, 17.2, 16.4, 16.9, 23.2, 178.3, 17.0, 21.1.

Lup-28-hydroxy-3β-O- palmitate (8)

Colorless gum. $[\alpha]_D^{20}$ +16^o (C, 0.5, $CHCl_3$). IR (film, ν_{max}, cm^{-1}): 3445, 2924, 1729, 1641, 1460, 1371, 1247, 1175, 1028, 977, 880,822, 720. ^{1}H NMR (400MHz, TMS, $CDCl_3$) · ppm: δ 4.59 (1H, brs, H-I-29), 4.49 (1H, brs, H-II-29), 4.38 (1H, dd, J = 10.0, 6.0 H-3), 3.69 (1H, d, J = 10.4Hz, H-I-28), 3.22(1H, d, J = 10.4Hz, H-II-28), 1.60(3H,s, H-30), 0.94, 0.89, 0.76, 0.75×2(each 3H, s, H23-27), 0.79(t, *J* = 7.2Hz, H-16′), several methylene signals 1.17 (brs, H-15′ to H-4′) , 1.53 (m, H-3′), 2.20 (t, *J* = 7.2 Hz, H-2′··· ^{13}C NMR: see Table1

lupeol (9)

White needles. M.p.208~210℃. ^{1}H NMR (400 MHz, TMS, $CDCl_3$) · ppm: 0.75, 0.78, 0.83, 0.91, 0.96, 1.03, 1.12 (3H, each s, 8×CH_3), 3.17 (1H, m,3 -H)，δ 4.65 (1H, brs, H-29), 4.54 (1H, brs, H-29′), 4.38 (1H, dd, J=10.0, 6.0 H-3)。. ^{13}C-NMR (100 MHz, TMS, $CDCl_3$) ·· ppm (order C-1~C-30): 36.8, 27.9, 78.9, 38.8, 55.0, 18.2, 34.1, 40.8, 50.3, 37.9, 20.7, 25.1, 37.2, 42.0, 27.3, 35.1, 42.6, 48.6, 47.7, 150.4, 30.5, 38.7, 27.9, 15.3, 16.0, 15.9, 14.6, 17.3, 109.6, 20.7

3-epi-betulinic (10)

Amorphous powder. M.p.277-280℃; IR (film, ν_{max}, cm^{-1}): 3455 (OH), 1690(C=O), 885(end methylene). ^{1}H-NMR (400 MHz, TMS, DMSO-d_6) ·· ppm: 4.54, 4.67(each 1H, s, two protons of end methylene), 2.92(1H, t, J = 6.8Hz, C-3-H), 12.07(1H, brs, COOH), 0.63, 0.75, 0.85×2, 0.91 (each 3H, s, CH_3), 1.63 (3H, s, C=C-CH_3). ^{13}C-NMR (100 MHz, TMS, $CDCl_3$) ·· ppm (order C-1~C-30): 38.2, 27.1, 76.7, 39.0, 54.8, 18.0, 33.9, 40.2, 49.9, 38.4, 20.4, 25.1, 38.8, 41.9, 30.8, 31.6, 55.3, 46.8, 49.2, 150.3, 29.2, 37.5, 28.1, 15.7, 15.8, 15.9, 14.4, 177.1, 109.5, 19.4.

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