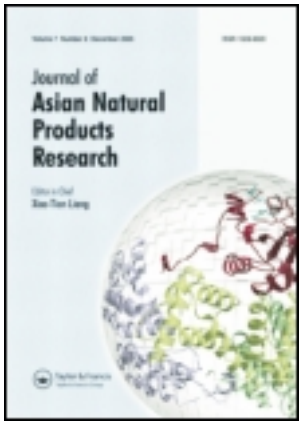

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Triterpenoids from *Viburnum betulifolium*

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Two new triterpenoids, ursa-12-sene-3β,11β-diol 3-*O*-palmitate (**1**) and ursa-12-sene-1β,3β,11α-triol 3-*O*-palmitate (**2**), were isolated from the 70% aqueous acetone extract of the aerial parts of *Viburnum betulifolium*, together with the artificial diene derivative of **2**, ursa-12-dien-1β,3β-diol 3-*O*-palmitate (**2a**). Their structures were characterized by various spectroscopic methods, including 1D NMR, 2D NMR, and HR-ESI-MS.

Keywords: *Viburnum betulifolium*; Caprifoliaceae; triterpenoids

1. Introduction

The genus *Viburnum*, belonging to the Caprifoliaceae family, consists of about 230 species distributed in subtropical and warm temperate regions, 80 of which are distributed in China [1]. *Viburnum* species have traditionally been used in China as a popular folk medicine for the treatment of diuretic, antispasmodic, sedative properties, and uterine excitability [2]. Phytochemical studies revealed that this genus characteristically contained triterpenoids, iridoids, vibsane-type diterpenes, lignans, coumarins, flavones, and phenolic glycosides [3–6]. *Viburnum betulifolium*, an evergreen shrub, is widely distributed throughout the southwestern part of China [1]. In our investigation on the components of this titled plant, the 70% aqueous acetone extract of the aerial part of *V. betulifolium* from Caojian town, Yunnan Province was studied. As a result, two new triterpenoids, named ursa-12-sene-3β,11β-diol 3-*O*-palmitate (**1**), ursa-12-sene-1β,3β,11α-triol 3-*O*-palmitate (**2**) and the artificial diene derivative of **2**, ursa-12-dien-1β,3β-diol 3-*O*-palmitate (**2a**), have been obtained. This paper deals with the isolation and structural elucidation of the two new triterpenoids from *V. betulifolium* on the basis of the spectroscopic analysis.

2. Results and discussion

The EtoAc fraction of the 70% aqueous acetone extract of *V. betulifolium* was purified by repeated column chromatography to afford compounds **1**, **2**, and **2a**. Their structures were characterized as two new triterpenoids, ursa-12-sene-3β,11β-diol 3-*O*-palmitate (**1**) and ursa-12-sene-1β,3β,11α-triol 3-*O*-palmitate (**2**), and an artificial diene derivative of compound **2** was identified as ursa-12-dien-1β,3β-diol 3-*O*-palmitate (**2a**). Their structures were characterized by various spectroscopic methods, including 1D NMR, 2D NMR, and HR-ESI-MS (Figure 1).

Compound **1**, obtained as a white amorphous solid, exhibited a quasi-molecular ion peak at *m/z* 703.6001 $[M + Na]^+$

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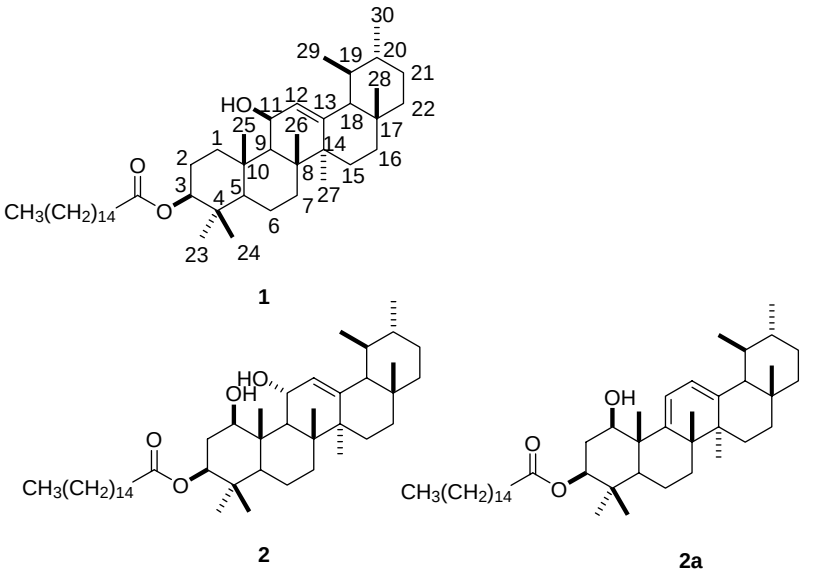

Figure 1. The structures of compounds **1**, **2**, and **2a**.

in the high-resolution mass spectrometry, which corresponded to the molecular formula $C_{46}H_{80}O_3$, with seven degrees of unsaturation. Its IR spectrum showed absorption bands for hydroxyl (3422 cm^{-1}) and ester carbonyl (1732 and 1255 cm^{-1}) groups. The ester group in compound **1** could be further deduced as a palmitoyl moiety, because of the characteristic signal at δ_C 173.7 (C-1′) in the downfield region of the ^{13}C NMR spectrum, as well as saturated long-chain features: a methyl signal at δ_H 0.88 (t, $J = 6.6$ Hz, H-16′), several methylene signals at δ_H 1.28 (br s, H-15′ to H-4′, 24H), 1.62 (m, H-3′), and 2.28 (t, $J = 6.6$ Hz, H-2′) in the ^1H NMR spectrum (Table 1). In addition to the long-chain ester group, the left 30 carbon signals ($8 \times CH_3$, $8 \times CH_2$, $8 \times CH$, $6 \times C$) in the ^{13}C NMR spectrum combined with the DEPT experiment, implied the presence of a pentacyclic triterpene moiety in compound **1** (Table 2) that could also be supported by eight methyl signals in the ^1H NMR spectrum, including six singlets (δ_H 0.80, 0.83, 0.88, 0.91, 0.93, 1.07) and two doublets at δ_H 0.89 (d, $J = 6.6$ Hz) and 0.90 (d, $J = 6.6$ Hz) (Table 1). These results and typical resonances at δ_C 124.7 (C-12) and 146.0 (C-13) further indicated the pentacyclic triterpene resembled α-amyrin [7], except for the presence of an oxygenated methine at δ_H 4.54 (1H, H-11) and δ_C 81.7 (d, C-11). Furthermore, the ^1H–^1H COSY spectrum of **1** implied the connectivity for H-12 to the oxygenated methine proton described above, and suggested that —OH group was linked to C-11. This was further confirmed by the HMBC correlations from H-11 to C-8, and C-10 and C-12 (Figure 2). The HMBC correlations between H-3 at δ_H 4.52 (t, $J = 2.7$ Hz) and C-1′ (δ_C 173.7), C-2 (δ_C 23.7), C-4 (δ_C 38.0) suggested that the ester group (palmitoyl) was linked at the C-3 position. It was reported that the chemical shift of C-11 with β-oriented OH group in ursa-12-en-3β,11β,16β-triol 3-*O*-palmitate was δ_C 81.5, whereas that of C-11 with α-oriented OH group in ursa-12-dien-3β,11α-diol 3-*O*-palmitate was δ_C 68.4 [8–10]. The chemical shift of C-11 (δ_C 81.7) in compound **1**, together with the coupling constant of H-9 ($J_{H\text{-}9/H\text{-}11} = 8.8$ Hz) and the NOESY corre-

Table 1. ^{1}H NMR spectral data of compounds **1** and **2a** (^{1}H: 500 MHz in $CDCl_3$).

Position	^{1}H NMR (multi., *J* Hz)	
	1	**2a**
H (1)	1.28, 0.87 (m)	3.96 (dd, 5.5, 14.1)
H (2)	1.64, 1.45 (m)	1.95, 1.80 (m)
H (3)	4.52 (t, 2.7)	4.57 (dd, 5.5, 15.3)
H (5)	0.82 (m)	0.81 (m)
H (6)	1.55, 1.42 (m)	1.53, 1.40 (m)
H (7)	1.54, 1.28 (m)	1.51, 1.25 (m)
H (9)	1.89 (d, 8.8)	–
H (11)	4.54 (m)	6.54 (d, 4.8)
H (12)	5.36 (d, 4.8)	5.49 (d, 4.8)
H (15)	2.03–1.85 (overlapped)	2.04–1.90 (overlapped)
H (16)	2.03–1.85 (overlapped)	2.04–1.90 (overlapped)
H (18)	1.35 (m)	1.35 (m)
H (19)	0.98 (m)	1.02 (m)
H (20)	1.16 (m)	1.19 (m)
H (21)	1.41, 1.26 (m)	1.43, 1.28 (m)
H (22)	1.43, 1.26 (m)	1.45, 1.30 (m)
H (23)	0.83 (s)	0.87 (s)
H (24)	0.88 (s)	0.88 (s)
H (25)	0.93 (s)	0.90 (s)
H (26)	0.91 (s)	0.80 (s)
H (27)	1.07 (s)	0.92 (s)
H (28)	0.80 (s)	0.93 (s)
H (29)	0.89 (d, 6.6)	0.85 (d, 6.6)
H (30)	0.90 (d, 6.6)	0.82 (d, 6.6)
H (2′)	2.28 (t, 6.6)	2.32 (t, 6.6)
H (3′)	1.62 (m)	1.69 (m)
H (4′–15′)	1.28 (br s)	1.27 (br s)
H (16′)	0.88 (t, 6.6)	0.88 (t, 6.6)

lations (H-11/H-9), clearly indicated that the OH group at C-11 of compound **1** was determined as β-oriented. The NOESY correlations of H-3 with H-23, H-5, and H-1α suggested that the long-chain ester group at C-3 should be β-oriented also. From the above data, the structure of **1** was determined to be ursa-12-sene-3β,11β-diol 3-*O*-palmitate.

Compound **2** was obtained as a white amorphous solid. The HR-ESI-MS showed a quasi-molecular ion peak at *m*/*z* 719.5954 $[M + Na]^+$, in accordance with the molecular formula $C_{46}H_{80}O_4$. The ^{1}H NMR and ^{13}C NMR spectral data of compound **2** closely resembled those of ursa-12-sene-3β,11α-diol 3-*O*-palmitate except that a methylene signal of ursa-12-sene-3β,11α-diol 3-*O*-palmitate was replaced by the signals of an oxygenated methane [8,10]. When compound **2** was dissolved in $CDCl_3$ and left in a refrigerator (12°C) for 1 week, the signal of an allylic hydroxyl group was completely eliminated, and an artificial diene derivative (**2a**) was obtained. The structure of **2a** was determined to be ursa-12-dien-1β,3β-diol 3-*O*-palmitate, respectively, by 1D NMR, 2D NMR spectral analysis (Tables 1 and 2) (Figure 2), and by comparison of the spectroscopic data with those of ussuriensin A [8], which implied that the hydroxyl group at C-11 in **2** was completely eliminated to form the artificial 9(11),12-diene derivative (**2a**) by photolysis. In the ^{1}H–^{1}H COSY spectrum of **2a**, the correlations between H-1 at δ_H 3.96 (dd, 5.5, 14.1) with H-2 at δ_H 1.80 and 1.95

Table 2. ^{13}C NMR spectral data of compounds **1** and **2a** (^{13}C: 125 MHz, in $CDCl_3$).

	^{13}C NMR			^{13}C NMR	
Position	**1**	**2a**	Position	**1**	**2a**
C (1)	39.3, t	75.6, d	C (24)	16.8, q	16.2, q
C (2)	23.7, t	34.7, t	C (25)	16.7, q	18.6, q
C (3)	80.3, d	76.7, d	C (26)	17.5, q	17.8, q
C (4)	38.0, s	38.0, s	C (27)	22.0, q	22.7, q
C (5)	55.2, d	48.7, d	C (28)	28.2, q	27.7, q
C (6)	18.1, t	18.2, t	C (29)	18.1, q	17.4, q
C (7)	33.4, t	31.0, t	C (30)	21.4, q	21.5, q
C (8)	42.0, s	43.2, s	C (1′)	173.7, s	173.5, s
C (9)	48.8, d	152.0, s	C (2′)	34.9, t	34.5, t
C (10)	37.7, s	44.6, s	C (3′)	25.2, t	25.1, t
C (11)	81.7, d	117.7, d	C (4′)	29.71, t[a]	29.65, t[b]
C (12)	124.7, d	123.4, d	C (5′)	29.70, t[a]	29.64, t[b]
C (13)	146.0, s	141.6, s	C (6′)	29.68, t[a]	29.63, t[b]
C (14)	43.1, s	40.9, s	C (7′)	29.67, t[a]	29.62, t[b]
C (15)	26.7, d	26.2, d	C (8′)	29.65, t[a]	29.61, t[b]
C (16)	27.8, t	28.6, t	C (9′)	29.61, t[a]	29.60, t[b]
C (17)	33.4, s	33.7, s	C (10′)	29.5, t[a]	29.5, t[b]
C (18)	58.4, d	57.2, d	C (11′)	29.4, t[a]	29.4, t[b]
C (19)	39.4, d	39.4, d	C (12′)	29.3, t[a]	29.3, t[b]
C (20)	39.3, d	39.0, d	C (13′)	29.2, t[a]	29.2, t[b]
C (21)	31.9, t	31.9, t	C (14′)	31.1, t	31.2, t
C (22)	41.3, t	41.3, t	C (15′)	22.7, t	22.9, t
C (23)	28.1, q	28.2, q	C (16′)	14.1, q	14.2, q

Note: [a,b]Assignments may be interchangeable.

(m) suggested that a —OH group was linked to C-1, which was supported by the HMBC correlations between H-1 and C-9 (δ_C 152.0), C-10 (δ_C 44.6), and C-25 (δ_C 18.6). The NOESY correlations (H-1/H-2α), together with the comparison of the spectroscopic data of **2a** with those of ussuriensin A, implied the OH group at C-1 of **2a** and **2** should be β-oriented [8]. In addition, by comparing the chemical shift of C-11 in compound **2** with that of compound **1**, the upfield shift from δ_C 81.7 to 67.3 suggested that the OH group was assigned as α-oriented [8–10]. From these results and the spectral data, the structure of compound **2** was determined as ursa-12-sene-1β,3β,11α-triol 3-*O*-palmitate.

Kakuda and Machida reported that ursa-12-dien-3β-ol 3-*O*-palmitate derivatives were easily obtained by the photoly-

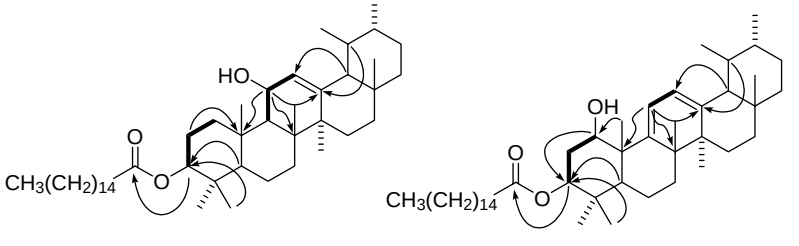

Figure 2. Key HMBC (↷) and $^1H-^1H$ COSY (—) correlations of compounds **1** and **2a**.

sis of ursa-12-sene-3β, 11α-diol 3-O-palmitate [10]. In fact, this photolysis was not observed for compound **1**. The only difference between compound **1** and ursa-12-sene-3β, 11α-diol 3-*O*-palmitate is that the OH group at C-11 in compound **1** is β-oriented, while the OH group at C-11 in ursa-12-sene-3β, 11α-diol 3-*O*-palmitate is α-oriented, which implied that the α-orientation of the OH group may be important for the photolysis reaction. However, it is likely that the trace HCl in the NMR solvent ($CDCl_3$) catalyzed the elimination reaction.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained using a JASCO-20C digital polarimeter. UV spectra were measured on a JASCO V-550 spectrophotometer. IR spectra were obtained with a 577 spectrometer. The ^1H and ^{13}C NMR DEPT-135 experiments were conducted on a Bruker (AM-500) FT NMR spectrometer with tetramethylsilane as the internal standard. 2D NMR experiments included HSQC, HMBC, ^1H–^1H COSY, and ROESY. MS were measured on a VG AutoSpec-3000 mass spectrometer. HR-ESI-MS were recorded on an API QSTAR Pulsar-1 mass spectrometer. Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden) and silica gel (200–300 mesh; Qingdao Ocean Chemical Factory, Qingdao, China) were used for column chromatography. Silica gel F_{254} (Qingdao Ocean Chemical Factory, Qingdao, China) was used for TLC.

3.2 Plant material

The aerial parts of *V. betulifolium* were collected in the Caojian town, Yunnan Province of China, in July 2006 and identified by Prof. Xiao Cheng of Kunming Institution of Botany, Kunming, China. A voucher specimen (20060801) has been deposited in the Kunming Institution of Botany, Chinese Academy of Sciences, Kunming, China.

3.3 Extraction and isolation

The dried and powdered aerial parts of *V. betulifolium* (5.5 kg) were cut into small pieces and ground, and then extracted with 70% aqueous acetone (20 liters × 3). The solvent was removed by rotary evaporation and the dark brown extract obtained was suspended in H_2O and extracted with ethyl acetate to afford ethyl EtOAc fraction (89 g). The EtOAc extract was subjected to silica gel chromatography with a gradient $CHCl_3$–MeOH to afford 90 fractions (F1–F90). F12–F30 (A) were permeated through Sephadex LH-20 using a MeOH–CH_3Cl (1:1) system to give 36 subfractions A1–A36. Fractions A12–A18 were further purified with silica gel chromatography eluted with CH_3Cl–MeOH (95:5 → 1:1) to afford **1** (10 mg). Fractions A19–A24 were further purified with silica gel chromatography eluted with CH_3Cl–MeOH (95:5 → 1:1) and Sephadex LH-20 eluted with MeOH–CH_3Cl (1:1) to afford **2** (25 mg).

3.3.1 *Ursa-12-sene-3β,11β-diol 3-O-palmitate (**1**)*

An amorphous powder; $C_{46}H_{80}O_3$; $[\alpha]_D^{20} + 15.49$ ($c = 0.040$, MeOH); UV λ_{max} ($CHCl_3$) nm (log ε): 252 (3.26); IR (KBr) ν_{max} cm^{-1}: 3422, 2924, 2854, 1732, 1642, 1460, 1380, 1255, 1019; ^1H (500 MHz in $CDCl_3$) and ^{13}C (125 MHz, in $CDCl_3$) NMR spectral data see (Tables 1 and 2), respectively; FAB-MS (pos.): *m/z* 681 $[M + H]^+$; HR-ESI-MS: *m/z* 703.6001 $[M + Na]^+$ (calcd for $C_{46}H_{80}O_3Na$, 703. 6005).

3.3.2 *Ursa-12-sene-1β,3β,11α-triol 3-O-palmitate (**2**)*

An amorphous powder; $C_{46}H_{80}O_4$; $[\alpha]_D^{20} + 243.47$ ($c = 0.032$, MeOH); UV

$\lambda_{max}(CHCl_3)$ nm (log ε): 283 (2.16); IR (KBr) ν_{max} cm^{-1}: 3449, 2923, 2853, 1734, 1639, 1462, 1377, 1366, 1177, 989; ^1H NMR (500 MHz in $CDCl_3$), δ (ppm): 5.23 (1H, t, 4.8), 4.56 (m), 4.32 (1H, dd, 5.5, 14.1), 3.88 (1H, dd, 5.5, 14.1), 1.99–1.80 (2H, m), 1.86 (1H, m), 1.80 (1H, d, 8.6), 1.75 (1H, m), 1.61 (1H, m), 1.52 (1H, m), 1.50 (1H, m), 1.40 (1H, m), 1.35 (1H, m), 1.38 (2H, m), 1.32 (1H, m), 1.26 (12H, br s), 1.23 (2H, m), 1.22 (1H, m), 1.12 (1H, m), 1.06 (1H, s), 1.02 (1H, m), 0.92 (1H, s), 0.91 (1H, s), 0.91 (1H, s), 0.89 (1H, d, 6.6), 0.88 (1H, t, 6.6), 0.85 (1H, s), 0.84 (1H, d, 6.6), 0.81 (1H, m), 0.81 (1H, s); ^{13}C NMR (125 MHz, in $CDCl_3$), δ (ppm): 173.5(s), 144.1(s), 126.6(d), 77.0(d), 76.8(d), 67.3(d), 57.7(d), 56.3(d), 52.2(d), 44.1(s), 43.6(s), 41.8(t), 39.4(d), 39.1(d), 37.8(s), 37.8(s), 34.7(t), 34.6(t), 33.4(s), 31.2(t), 31.0(t), 29.65(t), 29.64(t), 29.63(t), 29.62(t), 29.61(t), 29.60(t), 29.5(t), 29.4(t), 29.3(t), 29.2(t), 28.6(q), 28.3(t), 31.1(t), 27.9(q), 26.2(d), 25.1(t), 22.7(t), 22.6(q), 21.3(q), 18.4(t), 17.6(q), 17.5(q), 16.2(q), 15.8(q), 13.2(q); FAB-MS (pos.): *m/z* 696 $[M]^+$; HR-ESI-MS: *m/z* 719.5954 $[M + Na]^+$, (calcd for $C_{46}H_{80}O_4Na$, 719.5954).

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