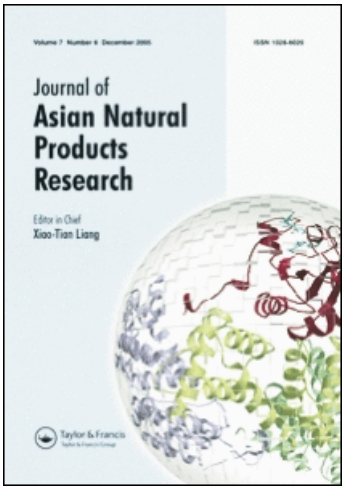

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Dammarane- and taraxastane-type triterpenoids from *Saussurea oligantha* Franch

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A new dammarane triterpenoid oliganthas A (**1**) and a new taraxastane triterpenoid oliganthas B (**2**), as well as five known taraxastane triterpenoids, ptiloepoxide (**3**), taraxast-20(30)ene-3β,21α-diol (**4**), 22-oxo-20-taraxasten-3β-ol (**5**), taraxast-20-ene-3β,30-diol (**6**), and taraxastane-3β,20α-diol (**7**), were isolated from the whole plant of *Saussurea oligantha* Franch. Their structures were elucidated by a series of spectroscopic methods. These compounds, especially taraxastanes **2**, **5**, and **6**, exhibited significant antibacterial activity against *Actinomyces viscosus* ATCC27044.

Keywords: *Saussurea oligantha* Franch; dammarane; taraxastane; triterpenoids; antibacteria

1. Introduction

More than 10 *Saussurea* species have long been used as herbal medicines in Chinese folk medicine, since they are efficacious in relieving internal heat or fever, harmonizing menstruation, invigorating blood circulation, stopping bleeding, alleviating pain, and increasing energy.[1] In our effort to find the biological activity of natural products as the lead molecule of drugs from Chinese medicinal plants, we isolated two new triterpenoids oliganthases A (**1**) and B (**2**) as well as five known taraxastane triterpenes, ptiloepoxide (**3**), taraxast-20(30)ene-3β,21α-diol (**4**), 22-oxo-20-taraxasten-3β-ol (**5**), taraxast-20-ene-3β,30-diol (**6**), and taraxastane-3β,20α-diol (**7**), from the ethanolic extract of *Saussurea oligantha* Franch (Figure 1). We report herein the isolation, structural elucidation, and antibacterial activity of these compounds. It was found that taraxastane triterpenoids **2**, **5**, and **6** possessed obvious antibacterial activities against *Actinomyces viscosus* ATCC27044.

2. Results and discussion

Compound **1** was isolated as colourless oil. The high-resolution electrospray ionization mass spectrometry (HRESIMS) of **1** showed an $[M + Na]^+$ ion at *m/z* 721.6111, corresponding to the molecular formula $C_{46}H_{82}O_4$. The IR spectrum showed absorption bands for hydroxyl (3387 cm^{-1}) and ester carbonyl (1739 and 1243 cm^{-1}) groups. The ester group in **1** could be further deduced as a palmitoyl moiety from the characteristic signal at δ 173.7 (C-1′) in the downfield region of the ^{13}C NMR spectrum (Table 1), as well as a saturated long-chain feature: a methyl signal at δ 0.86, several methylene signals at δ 1.23 and 2.26 (t, $J = 7.4$ Hz, H-2′)

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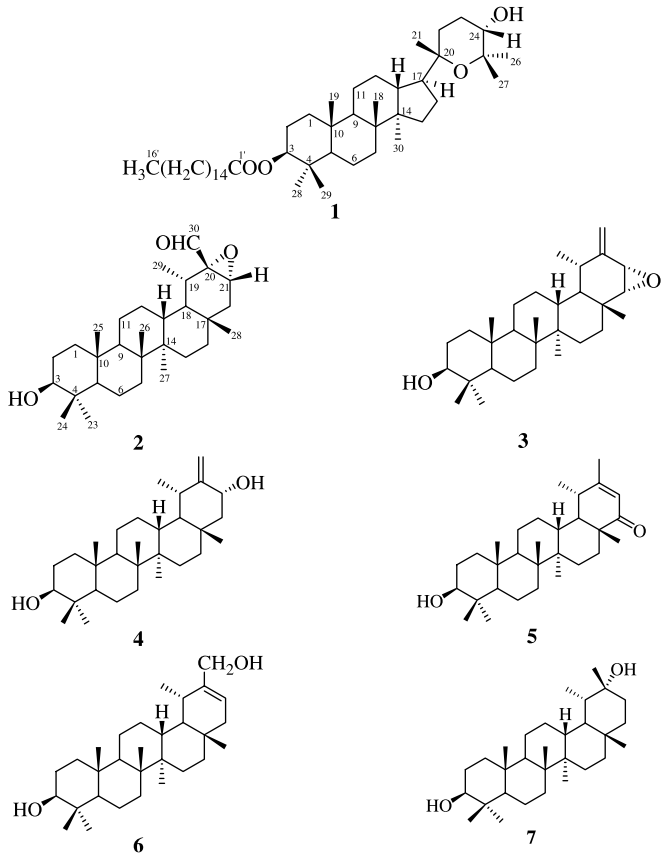

Figure 1. Structures of triterpenoids **1**–**7**.

in the ^{1}H NMR spectrum.[2,3] In addition to the long-chain ester group, the multiplicities of carbon signals were determined by the DEPT experiment, which revealed the presence of eight methyls, 10 methylenes, six methines, and six quaternary carbons. The ^{13}C NMR spectral data of **1** showed close similarity to 3β-acetyl-20,25-epoxydammarane-24α-ol.[4] The ^{1}H NMR spectrum of **1** showed eight tertiary methyl singlets, two oxymethine protons at δ_H 3.71 (1H, t, $J = 7.2$ Hz) and 4.45 (1 H, dd, $J = 10.8$, 5.2 Hz), and a series of resolved and unresolved multiplets from δ_H 0.64 to 1.78. These above structural features revealed that compound **1** was a triterpenoid. Therefore, the unsaturation index exhibited by the molecular formula $C_{46}H_{82}O_4$ of **1** was satisfied by the four carbon rings of a dammarane skeleton, the cyclized side chain at C-17, and a palmitoyl moiety (δ_C 173.7).

The ester group (palmitoyl) is linked at C-3β position because of the HMBC correlation between H-3 (δ_H 4.45, dd, $J = 10.8$, 5.2 Hz) and C-1′ (δ 173.7), C-28 (δ 16.3), and C-29 (δ 27.9) (Figure 2), together with the comparison of the H-3 chemical shift (δ_H 4.45), its peak pattern (double doublet), and the coupling constant ($J_{ae} = 5.2$ and $J_{aa} = 10.8$ Hz) of **1** with those of known triterpenoids.[2] The location of the hydroxyl group at C-24 was confirmed by the HMBC correlations from H-24 to δ 24.2 (C-26) and δ 27.4 (C-27). The axial (α-) orientation of the hydroxyl function

Table 1. ^{13}C NMR spectral data for compounds **1** and **2** (100 MHz; $CDCl_3$ as a solvent and TMS as an internal standard).

C	1	2	C	1	2
1	38.7	38.7	19	16.4	25.9
2	23.7	27.7	20	86.4	66.8
3	80.5	79.0	21	23.5	56.3
4	37.9	38.8	22	35.7	41.3
5	55.9	55.3	23	26.1	15.4
6	18.1	18.3	24	83.3	27.9
7	35.2	34.3	25	71.5	16.3
8	40.4	41.0	26	24.2	15.9
9	50.7	50.1	27	27.4	14.4
10	37.0	37.1	28	16.3	17.7
11	21.5	21.4	29	27.9	19.8
12	27.3	27.4	30	16.5	200.5
13	42.9	39.3	1′	173.7	
14	50.0	42.4	2′	34.8	
15	31.4	26.4	3′	25.2	
16	25.7	36.2	4′–14′	29.0–29.8	
17	49.5	34.4	15′	22.7	
18	15.4	44.9	16′	14.1	

Assignments were aided by spin splitting patterns, DEPT, COSY, HMQC, and HMBC experiments, and chemical shift values (δ). The δ values are given in ppm and are referenced to either the residual $CDCl_3$ (7.26 ppm) or $CDCl_3$ (77.00 ppm) signals.

at C-24 was determined from the coupling constant (7.2 Hz) between H-24β (equatorial) and H-23.[4]

The relative stereochemistry of the C-17 side chain and the hydroxyl substituent at C-24 in **1** was finally determined by the NOESY experiment. One NOESY correlation of H-17 with H-30 indicated an α-configuration of the H-17, and another clear cross-peak between H-24β, H-21, and H-27 indicated their close proximity. On the basis of the above conclusions, the structure of compound **1** was deduced to be 20,25-epoxydammarane-3β,24α-diol-3-*O*-palmitate.

Compound **2** was obtained as colourless crystals. The molecular formula $C_{30}H_{48}O_3$ was deduced from the HRESIMS spectrum at *m/z* 474.3940 $[M + NH_4]^+$. Its ^{13}C NMR and DEPT experiments showed 7 × CH_3, 9 × CH_2, 8 × CH, and 6 × C. The IR spectrum indicated the presence of hydroxyl (3396 cm^{-1}) and aldehyde (1714 cm^{-1}) groups. The ^1H NMR spectrum demonstrated the presence of seven methyl groups, of which six were singlets (δ 0.67, 0.74, 0.83, 0.87, 0.95, 1.01) and one was doublet (δ 0.98, d, $J = 6.4$ Hz), and a proton (δ 8.84, s), two protons on oxygenated carbons at δ 3.17 (dd, 1H, $J = 11.2$, 4.8 Hz) and 3.44 (dd, 1H, $J = 6.4$, 2.0 Hz). In the ^{13}C NMR spectrum, there were three oxygenated carbons at δ 79.0 (correlated with the ^1H signal at δ 3.17), 56.3 (correlated with the ^1H signal at δ 3.44), and 66.8 (a quaternary carbon). From these spectral data together with seven degrees of unsaturation, compound **2** was deduced to be a taraxastane triterpenoid with an epoxy moiety, a hydroxyl, and an aldehyde group. Comparison of the ^{13}C NMR spectral data (Table 1) with those of known tarast-20-en-3β,30-diol [5] showed that, except for the ring E carbons, the chemical shifts were almost identical. This suggested that the epoxy moiety and the aldehyde groups must be located on ring E. The definitive location of the epoxide on C-20 and C-21 was established by 2D NMR spectroscopic

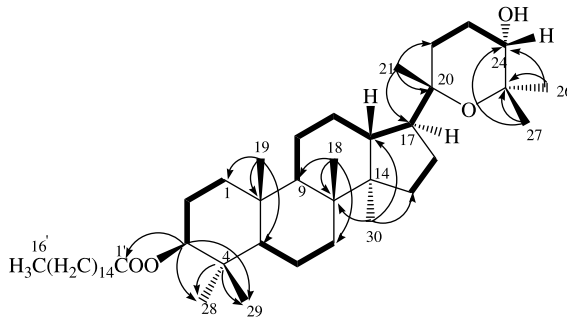

Figure 2. The ^1H–^1H COSY (bold bond) and key HMBC (from H to C) correlations of **1**.

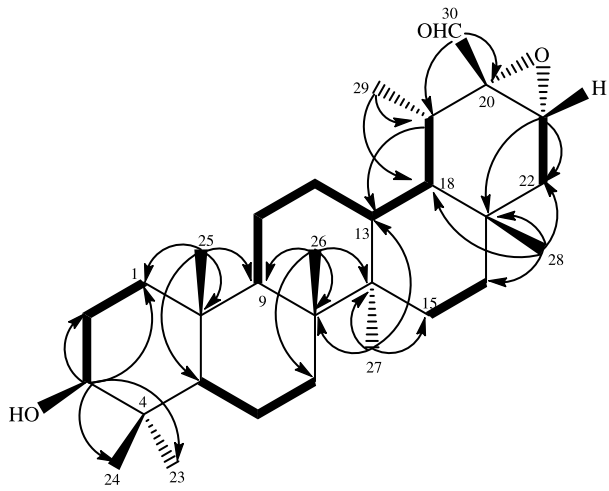

Figure 3. The $^{1}H-^{1}H$ COSY (bold bond) and key HMBC (from H to C) correlations of **2**.

experiments. The $^{1}H-^{1}H$ COSY experiment of **2** (Figure 3) showed that the proton on oxygenated carbons (δ 3.44) correlated only with H-22. In the HMBC spectrum (Figure 3), H-28 clearly correlated with C-18, C-22, C-16, and C-17; H-21 correlated with C-22 and C-17; H-30 correlated with C-20 and C-19, indicating that the epoxide functionality was located at C-20 and C-21, and C-30 is an aldehyde. The NOESY spectrum showed a cross-peak between H-28 and H-21, indicating the α-configuration of the epoxide. Therefore, compound **2** is deduced to be 20α,21α-epoxytaraxastane-3β-hydroxyl-30-al.

Compounds **3**–**7** were identified as ptiloepoxide,[6] taraxast-20(30)ene-3β,21α-diol,[7] 22-oxo-20-taraxasten-3β-ol,[8] taraxast-20-ene-3β,30-diol,[5] and taraxastane-3β,20α-diol,[9] respectively, by comparing their physical and spectral data with those reported in the literature and from the chemical evidence.

The results of antibacterial activities of compounds **1**–**7**, based on the minimum inhibitory concentration (MIC) method, showed that compounds **2**, **5**, and **6** have strong activity against *A. viscosus* ATCC27044, when compared with the positive control, triclosan. The MIC values of all compounds were reported in Table 2.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-4 digital display micro-melting point apparatus, and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter, solvent $CHCl_3$. IR spectra were measured on an FTS 165-IR spectrometer (Bio-Rad, USA). ^{1}H NMR (400.13 MHz), ^{13}C NMR (100.62 MHz), and 2D NMR were recorded on a Varian INOVA-400 FT-NMR spectrometer (USA) in $CDCl_3$ with TMS as

Table 2. MIC values of compounds **1**–**7** against *A. viscosus*[a].

	MIC (ppm)							
Strain	**1**	**2**	**3**	**4**	**5**	**6**	**7**	Triclosan[b]
ATCC27044	>250	7.8	31.25	31.25	7.8	7.8	>250	3.9

[a] Data is expressed as the mean of triplicate determinations.
[b] Positive control.

an internal standard. HRESIMS were recorded on a Bruker APEX II spectrometer. Separation and purification were performed by column chromatography (CC) over silica gel. Silica gel (200–300 mesh) used for CC and silica gel (GF_{254}) for TLC were obtained from the Qingdao Marine Chemical Factory, Qingdao, China. Spots were detected on the TLC under UV light or by heating over 110°C after spraying with 98% H_2SO_4–EtOH (v:v = 5:95).

3.2 Plant material

The whole plant of *Saussurea oligantha* was collected from Huzhu Country, Qinghai province of China in August 2002, and was identified by Mrs Huan-Yang Qi, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou. A voucher specimen (2002004) has been deposited at the Key Laboratory for Natural Medicine of Gansu Province.

3.3 Extraction and isolation

The chopped whole plant material (5.1 kg) was extracted repeatedly (four times, 7 days each time) with 95% EtOH at room temperature. The combined extracts were evaporated to dryness under reduced pressure. The residue (220 g) was then suspended in H_2O (1.0 l) and extracted with petroleum ether (60–90°C) (1.0 l × 5), EtOAc (1.0 l × 5), and *n*-BuOH (1.0 l × 5), respectively. The petroleum ether (60–90°C) extract (98.2 g) was subjected to column chromatography on silica gel (200–300 mesh, 1000 g) using petroleum ether (60–90°C) 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 an eluent. The fraction of petroleum ether–acetone (40:1, 2.0 g) was separated by CC over 40 g silica gel (200–300 mesh) with petroleum ether–EtOAc (20:1) to yield crude **1**, which was further isolated and purified on a silica gel column (200–300 mesh) with petroleum ether–$(CH_3CH_2)_2O$ (50:1) to yield pure compound **1** (9.0 mg). The fraction of petroleum ether–acetone (15:1, 4.0 g) was separated by CC over silica gel (200–300 mesh, 180 g) with petroleum ether–EtOAc (8:1) to yield two subfractions A and B. Fraction A (1.4 g) was separated by CC over 20 g silica gel (200–300 mesh) with $CHCl_3$–EtOAc (15:1) to yield **4** (7.0 mg). Fraction B (2.0 g) was separated by CC over 40 g silica gel (200–300 mesh) with petroleum ether–EtOAc (8:1) to yield crude **3** and **5**, which was purified by rechromatography on a silica gel column (200–300 mesh) with petroleum ether–Me_2CO (15:1) to yield pure compounds **3** (5.5 mg) and **5** (12.0 mg). The fraction of petroleum ether–acetone (10:1, 2.0 g) was separated by CC on silica gel eluting with $CHCl_3$–EtOAc (20:1) and petroleum ether–EtOAc (10:1) to yield **7**. The fraction of petroleum ether–acetone (5:1, 3.5 g) was applied to a silica gel column chromatography eluting with $CHCl_3$—EtOAc (4:1) to yield **2** (4.5 mg) and crude **6**, which was purified by rechromatography on a gel column (200–300 mesh) with petroleum ether–acetone (3:1) to yield pure **6** (6.0 mg).

3.3.1 Oliganthas A (*1*)

Colorless oil. $[\alpha]_D^{20}$ + 17 (*c* 0.27, $CHCl_3$); IR (KBr) ν_{max} (cm^{-1}): 3387, 2920, 2851, 1729, 1464, 1375, 1243, 1173, 1082, 980; ^1H NMR: δ 1.18 (3H, s, H-27), 1.10 (3H, s, H-21), 1.09 (3H, s, H-26), 0.93 (3H, s, H-18), 0.83 (3H, s, H-28), 0.83 (3H, s, H-30), 0.82 (3H, s, H-29), 0.82 (3H, s, H-19), 4.45 (1H, dd, J = 10.8, 5.2 Hz, H-3), 3.71 (1H, t, J = 7.2 Hz, H-24), 2.26 (t, J = 7.2 Hz, H-2′), 1.75 (1H, m, H-17), 0.86 (3H, t, J = 7.2 Hz, H-16′), 1.23 (2H, m); ^{13}C NMR spectral data see Table 1. HRESIMS *m/z* 721.6111 $[M + Na]^+$ (calcd for $C_{46}H_{82}O_4Na$, 721.6105).

3.3.2 Oliganthas B (*2*)

Colorless crystals. $[\alpha]_D^{20}$ − 38 (*c* 0.36, $CHCl_3$); m.p. 236–238°C; IR (KBr) ν_{max} (cm^{-1}): 3396, 2866, 1714, 1678, 1449, 1382, 1190, 1113, 1040, 990, 856; ^1H NMR: δ 1.01 (3H, s, H-26), 0.98 (3H, d, J = 6.4 Hz, H-29),

0.95 (3H, s, H-24), 0.87 (3H, s, H-27), 0.83 (3H, s, H-25), 0.74 (3H, s, H-23), 0.67 (3H, s, H-28), 8.84 (1H, s, H-30), 3.44 (1H, dd, $J = 6.4$, 2.0 Hz, H-21), 3.17 (1H, dd, $J = 11.2$, 4.8 Hz, H-3), 2.33 (1H, dd, $J = 8.8$, 6.4 Hz, H-19), 1.84 (2H, dd, $J = 14.4$, 6.8 Hz, H-22); ^{13}C NMR spectral data see Table 1; HRESIMS *m/z* 474.3940 $[M + NH_4]^+$ (calcd for $C_{30}H_{48}O_{34}$ NH_4, 474.3942).

3.4 Antibacterial assay

Antibacterial activity was tested using MIC.[10] *A. viscosus* ATCC27044 was cultured at 37°C under a humidified atmosphere of 5% CO_2 for 24 h. Compounds **1**–**7** (2500–1.22 ppm) and triclosan (used as a positive control) were dispersed in replicate 96-well plates. The bacterium (1×10^4 cells/well) was then added. After 48 h exposure to the toxins, the antibacterial activity was determined by measuring the absorbance at 610 nm (Biotek, ELX808). The highest dilution, at which no growth ($OD \leq 0.05$) was observed, was defined as MIC. Each test was performed in triplicate.

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