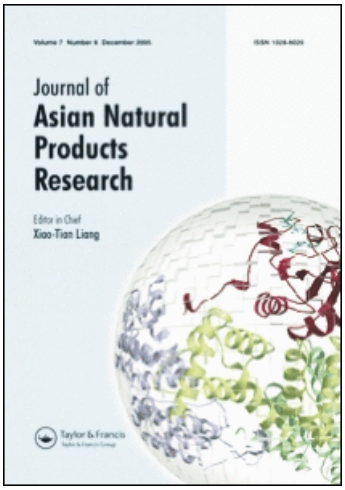


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# Two new triterpenes from the rhizomes of *Alisma orientalis*

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Two new triterpenoids, 25-anhydro-alisol F (**1**) and 11-anhydro-alisol F (**2**), were isolated from the rhizomes of *Alisma orientalis*. Their structures were elucidated by spectroscopic methods.

**Keywords:** *Alisma orientalis*; triterpenoids; 25-anhydro-alisol F; 11-anhydro-alisol F



## 1. Introduction

*Alisma orientalis* (Sam.) Juzep. is widely cultivated in China and Japan, and its dried rhizome has commonly been used as a crude drug for diuretics, blood fat reduction and diabetes.[1] Previous phytochemical investigations on the crude drug revealed the principal constituents of protostane-type triterpenes and sesquiterpenes.[2–8] During the search for anticancer compounds from Chinese herbal medicine, we investigated the constituents of the rhizomes of *A. orientalis*, which led to the isolation of two new triterpenes, 25-anhydro-alisol F (**1**) and 11-anhydro-alisol F (**2**). Herein, we describe the structural elucidation of the two new compounds **1** and **2**.

## 2. Results and discussion

An ethanolic extract of the rhizomes of *A. orientalis* was partitioned with petroleum ether, EtOAc and *n*-BuOH successively. The EtOAc part was repeatedly subjected to column chromatography over silica gel to yield two new compounds **1** and **2**.

Compound **1** was obtained as colourless prisms. The ESI-MS gave a quasimolecular ion peak at *m/z* 471 $[M + H]^+$. Its molecular formula was determined as $C_{30}H_{46}O_4$ by positive HR-ESI-MS at *m/z* 493.3288 $[M + Na]^+$. In the IR spectrum, absorption bands for hydroxy (3446 $cm^{-1}$) and carbonyl (1706 $cm^{-1}$) groups were observed. Its $^1H$ NMR (Table 1) spectrum showed two olefinic proton signals [$\delta_H$ 4.97, 4.93 (each 1H, br s)], four oxygenated methine proton signals at $\delta_H$ 4.46 (1H, dd, $J = 7.6$, 5.2 Hz), 3.85 (1H, d, $J = 7.6$ Hz), 3.80 (1H, m) and 3.66 (1H, m), as well as seven methyl proton signals at $\delta_H$ 0.90, 1.06, 1.07, 1.07, 1.25, 1.74 (each 3H, s), and 1.13 (3H, d, $J = 7.2$ Hz). The $^{13}C$ NMR (Table 1) spectrum revealed 30 carbons, including two double bonds, four oxygenated methines, a carbonyl and seven methyls. The NMR spectral analysis revealed that compound **1** should be a protostane-type triterpenoid. The NMR spectral data of **1** were very similar to those of alisol F[8] (Figure 1; Table 1) except that there were two more olefinic protons at $\delta_H$ 4.97 and 4.93 (each 1H, m, H-26). These spectral observations suggested the presence of one more double

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Table 1. $^{1}H$ and $^{13}C$ NMR spectral data of compounds **1**, **2**, and alisol F ($^{1}H$, 500 MHz; $^{13}C$, 125 MHz; in $CDCl_3$; *J* Hz).

| | **1** | | **2** | | Alisol F | |
|---|---|---|---|---|---|---|
| No. | $\delta_C$ | $\delta_H$ (mult. *J*) | $\delta_C$ | $\delta_H$ (mult. *J*) | $\delta_C$ | $\delta_H$ (mult. *J*) |
| 1 | 30.9 | 2.16 (m), 2.08 (m) | 31.3 | 1.90 (m), 1.30 (m) | 30.6 | |
| 2 | 33.8 | 2.67 (m), 2.33 (m) | 33.8 | 2.70 (m), 2.28 (m) | 33.8 | |
| 3 | 220.7 | | 220.7 | | 220.0 | |
| 4 | 47.2 | | 46.6 | | 46.9 | |
| 5 | 48.4 | 2.14 (m) | 47.4 | 2.31 (m) | 48.1 | |
| 6 | 20.1 | 1.48 (m), 1.27 (m) | 19.5 | 1.50 (m), 1.36 (m) | 19.2 | |
| 7 | 34.1 | 2.06 (m), 1.28 (m) | 32.4 | 2.05 (m), 1.64 (m) | 33.5 | |
| 8 | 40.8 | | 38.3 | | 40.5 | |
| 9 | 49.8 | 1.76 (d, 10.4) | 47.4 | 2.29 (s) | 49.5 | 1.76 (d) |
| 10 | 37.2 | | 36.1 | | 36.9 | |
| 11 | 70.7 | 3.80 (m) | 130.2 | 5.67 (dd, 10.2, 2.0) | 70.3 | 3.79 (dt) |
| 12 | 34.0 | 2.62 (m), 2.06 (m) | 121.2 | 6.26 (dd, 10.2, 3.4) | 34.8 | |
| 13 | 136.7 | | 139.0 | | 136.9 | |
| 14 | 55.5 | | 55.2 | | 55.3 | |
| 15 | 39.6 | 2.26 (m), 1.30 (m) | 37.4 | 2.13 (m), 1.28 (m) | 39.4 | |
| 16 | 80.6 | 4.46 (dd, 7.6, 5.2) | 80.6 | 4.58 (br. d, 8.0) | 80.1 | 4.46 (dd) |
| 17 | 133.8 | | 135.1 | | 132.9 | |
| 18 | 24.6 | 0.90 (s) | 25.0 | 1.06 (s) | 24.3 | |
| 19 | 25.7 | 1.07 (s) | 25.0 | 0.91 (s) | 25.4 | |
| 20 | 26.8 | 2.78 (m) | 27.3 | 3.00 (m) | 26.5 | 2.86 (m) |
| 21 | 18.5 | 1.13 (d, 7.2) | 18.0 | 1.21 (d, 6.0) | 18.2 | 1.14 (d) |
| 22 | 34.8 | 1.48 (m), 1.36 (m) | 36.4 | 2.24 (m), 1.35 (m) | 33.8 | |
| 23 | 74.0 | 3.66 (m) | 73.3 | 4.12 (m) | 72.6 | 4.04 (br. d) |
| 24 | 79.5 | 3.85 (d, 7.6) | 77.3 | 3.03 (br. d, 4) | 77.2 | 3.05 (d) |
| 25 | 143.8 | | 73.6 | | 73.3 | |
| 26 | 114.9 | 4.97 (br. s), 4.93 (br. s) | 26.8 | 1.25 (s) | 26.5 | |
| 27 | 17.7 | 1.74 (s) | 27.6 | 1.31 (s) | 26.9 | |
| 28 | 29.8 | 1.07 (s) | 29.5 | 1.08 (s) | 29.5 | |
| 29 | 20.2 | 1.06 (s) | 19.5 | 1.05 (s) | 19.5 | |
| 30 | 23.9 | 1.25 (s) | 22.8 | 0.87 (s) | 23.5 | |



bond in compound **1**, which was confirmed by the carbon signals at $\delta_C$ 143.8 (C-25), 114.9 (C-26), 136.7 (C-13) and 133.8 (C-17). The long-range correlations between H-27 and C-24, C-25, C-26, H-26 and C-24, C-25, and C-27 in the HMBC spectrum of **1** indicated that the additional double bond should be present at C-25 (26). By further analysis of the HMQC, HMBC and $^{1}H$–$^{1}H$ COSY, NOESY spectra, all the proton and carbon signals were assigned unambiguously. Therefore, compound **1** was elucidated as 25-anhydro-alisol F.

Compound **2** was obtained as colourless prisms. The positive HR-ESI-MS established the molecular formula $C_{30}H_{46}O_4$ for **2** at *m/z* 493.3290 $[M + Na]^+$. Absorption bands for hydroxy (3474 $cm^{-1}$) and carbonyl (1706 $cm^{-1}$) groups were observed in its IR spectrum. The $^{1}H$ NMR (Table 1) spectrum showed two olefinic proton signals at $\delta_H$ 6.26 (1H, dd, $J = 10.2$, 3.4 Hz), 5.67 (1H, dd, $J = 10.2$, 2.0 Hz), three oxygenated methine proton signals at $\delta_H$ 4.58 (1H, br d, $J = 8.0$ Hz), 4.12 (1H, m) and 3.03 (1H, br d, $J = 4.0$ Hz), and eight methyl signals at $\delta_H$ 0.87, 0.91, 1.05, 1.06, 1.08, 1.25, 1.31 (each 3H, s), and 1.21 (3H, d, $J = 6.0$ Hz). The $^{13}C$ NMR (Table 1) spectrum revealed 30 carbon signals. The $^{1}H$ and $^{13}C$ NMR spectral analysis revealed that compound **2** should be a protostane-type triterpenoid. Comparison

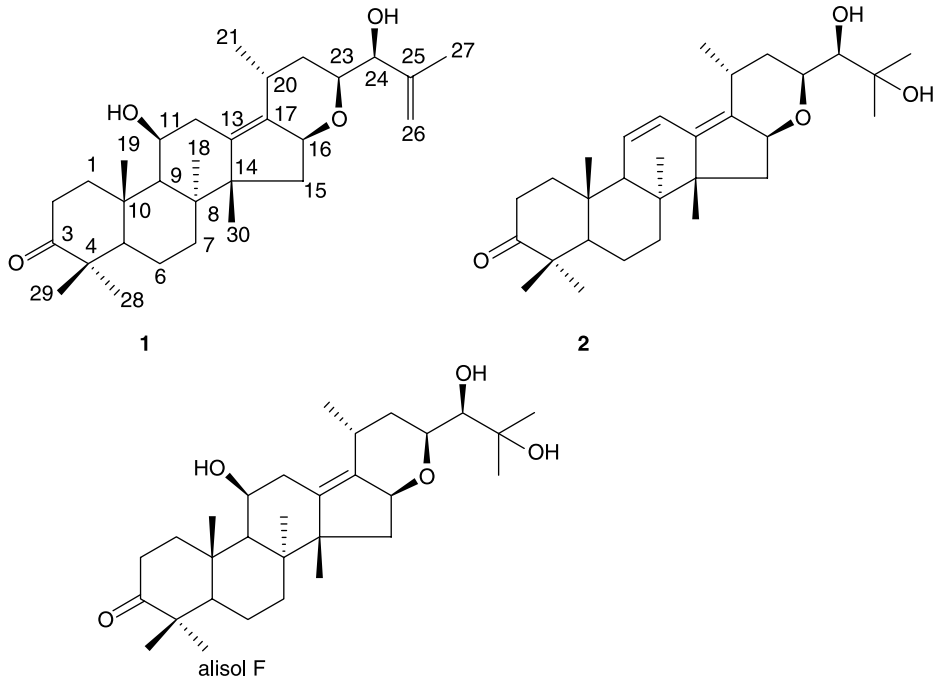


Figure 1. Structures of compounds **1**, **2** and alisol F.



of the NMR data of **2** with those of alisol F suggested the presence of one more double bond in compound **2**, which was confirmed by the carbon signals at $\delta_C$ 130.2 (C-11) and 121.2 (C-12) in the $^{13}C$ NMR spectrum of **2**. The long-range correlations between H-11 and C-8, -9, -10 and -13 in the HMBC spectrum of **2**, and the $^{1}H-^{1}H$ COSY spectrum of **2** revealed two separated spin–spin systems (H-11/H-12), indicating that the additional double bond should be present at C-11 (12). By further analysis of HMQC, HMBC, $^{1}H-^{1}H$ COSY, and NOESY spectra, the signals of compound **2** were assigned unambiguously. Consequently, compound **2** was elucidated as 11-anhydro-alisol F.

## 3. Experimental

### 3.1 General experimental procedures

Optical rotations were recorded on a PE 241 MC polarimeter. The IR spectra were recorded on a Bio-Rad FTS 6000 infrared spectrometer. The UV spectra were measured on a Shimadzu UV-2450 spectrophotometer. The NMR spectra were run on Bruker AVANCE-400 and Varian unity INOVA-500 spectrometers, using TMS as an internal standard. The mass spectra were obtained on an IonSpec 7.0 T FT-MS instrument. Silica gel (200–300 mesh, Qingdao Ocean Chemical Group Co., China) and Sephadex LH-20 (Merck Co., New York) for column chromatography as well as silica gel GF254 (Qingdao Ocean Chemical Group Co.) for TLC were used.

### 3.2 Plant material

The rhizomes of *A. orientalis* were purchased from Jian'ou, Fujian province, China and identified by Prof. Wen-Yuan Gao. A voucher specimen (No. 20050820) has been deposited in the School of Pharmaceutical Science and Technology, Tianjin University, China.

### 3.3 Extraction and isolation

The rhizomes of *A. orientalis* (8 kg) were extracted three times with 95% EtOH under reflux for 3 h. After removal of solvent under reduced pressure, the extract (690 g) was suspended in water and then partitioned with petroleum ether, EtOAc and *n*-BuOH successively. The EtOAc-soluble part (200 g) was subjected to column chromatography over silica gel eluting with petroleum ether–EtOAc to EtOAc–MeOH gradient system with increasing amounts of EtOAc and MeOH, respectively, to give 15 fractions. Fraction 6 was rechromatographed over a silica gel column eluting with $CHCl_3$–MeOH to afford two subfractions and further purified by HPLC to obtain compound **1** (12.7 mg). Compound **2** (11.2 mg) was isolated from fraction 5 by repeated column chromatograph over silica gel, eluting with petroleum ether–acetone.

#### 3.3.1 25-Anhydro-alisol F (*1*)

Colourless prisms; $[\alpha]_D^{26} + 23.4°$ (*c* 0.4, MeOH); UV $\lambda_{max}$ (MeOH) nm: 207; IR (KBr) $\nu_{max}$ $cm^{-1}$: 3446, 1706, 1655, 1375; $^1H$ NMR ($CDCl_3$, 500 MHz); and $^{13}C$ NMR ($CDCl_3$, 125 MHz) spectral data (Table 1); ESI-MS *m/z*: 471 $[M + H]^+$, positive HR-ESI-MS *m/z*: 493.3288 $[M + Na]^+$ (calcd. for $C_{30}H_{46}O_4Na$, 493.3294).

#### 3.3.2 11-Anhydro-alisol F (*2*)

Colourless prisms; $[\alpha]_D^{26} + 10.2°$ (*c* 0.4, MeOH); UV $\lambda_{max}$ (MeOH) nm: 242; IR (KBr) $\nu_{max}$ $cm^{-1}$: 3474, 1706, 1635, 1251; $^1H$ NMR ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz) spectral data (Table 1); ESI-MS *m/z*: 471 $[M + H]^+$, positive HR ESI-MS *m/z*: 493.3290 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3294)

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