

Two New Sesquiterpenes from *Alisma orientalis*

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Two new sesquiterpenoids named alismorientols A (1) and B (2) were isolated from the rhizomes of *Alisma orientalis* collected in Sichuan province, People's Republic of China. Their structures were elucidated based on spectroscopic analyses (1D and 2D NMR data including HSQC, HMBC, COSY, and ROESY) and X-ray crystallographic analysis. Anti-hepatitis B virus (HBV) bioassay revealed that compound 1 showed moderate anti-HBV activity *in vitro* with IC₅₀ for HBsAg: 1.1 μM, for HBeAg: 14.7 μM.

Key words *Alisma orientalis*; sesquiterpenoid; alismorientol A; alismorientol B; anti-hepatitis B virus activity

Alisma orientalis (SAM.) JUZEP. is widely cultivated in China and Japan, and the dried rhizomes are a crude drug for the treatment of diabetes and diuretics.¹⁾ Our previous paper²⁾ reported that a serious protostane-type triterpenes extracted from this plant showed anti-hepatitis B virus (HBV) activity. As a subsequent study on this plant, further investigation on the less polar part of the 90% EtOH extract of the dried rhizomes of *A. orientalis* led to the isolation of two new sesquiterpenes: alismorientols A (1) and B (2) (Fig. 1). Anti-HBV bioassay *in vitro* using Hep G 2.2.15 cell line revealed that alismorientol A (1) exhibited activities of suppressing HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) secretions. Herein, we report the isolation and structural elucidation of the new compounds 1 and 2, and anti-HBV activity of compound 1.

Compound 1 was obtained as a colorless prism. The molecular formula was deduced to be C₁₅H₂₈O₄, in agreement with the positive HR-ESI-MS experiment at *m/z* 273.1940 ([M+H]⁺, Calcd 273.1958). The IR spectrum of 1 showed a broad absorption due to OH groups at 3417 cm⁻¹. In the ¹H-NMR spectrum (Table 1) of 1, two tertiary Me groups and two secondary Me groups were observed, along with one oxygenated CH at δ_H 4.51 (br s, H-6). The ¹³C-NMR (DEPT) spectrum exhibited 15 carbon signals (Table 1). NMR spectral analysis revealed that compound 1 was a guaiane-type sesquiterpenoid. ¹³C DEPT experiment showed that compound 1 possessed the same numbers of methyls, methylenes, methines, and quaternary carbons as those of orientalol E.³⁾ However, the chemical shifts for the C-atoms of 1 were not identical with those of orientalol E,³⁾ especially for C-4 (δ 83.3), C-5 (52.0), C-7 (δ 76.5), C-9 (37.6), C-10 (δ 71.7), and C-14 (δ 32.8). The chemical shifts for other C-atoms in

1 were also shifted downfield about 1 to 2 ppm. Considering the different solvent, the down-shift chemical shifts of those carbons could be explained.

The differences between compound 1 and orientalol E are focused on C-4, 5, 7, 9, 10, and C-14. ESI-MS established that compound 1 had mass 18 units more than orientalol E, accounting for one molecule of H₂O was added to the epoxyl of C-10 and C-7 of orientalol E to form compound 1, deducing one additional OH group presented in compound 1, which could be supported by the HMBC experiment. In the HMBC spectrum (Fig. 2), no correlation between H-14 and C-7 could be found, demonstrating no epoxy attached between C-7 and C-10. The other long-range correlations between protons and carbons in HMBC were also given as follows: H-14 and H-5 with C-1, H-5, H-3, and H-15 with C-4, H-5 with C-6, H-6, H-11, H-12, H-13 with C-7, C-11, H-6 with C-11, H-12, and H-13 with C-11. The relative stereostructure of 1 characterized by ROESY experiment accounted for the very different chemical shifts of C-4, 5, 9, and C-14. As shown in Fig. 2, correlations between H-1, assumed to be α-oriented, and H-5, Me-14 were observed, besides correlation between H-5 and H-6, suggesting that H-5, 6, and Me-14 were in α-orientation. The α-configuration for H-6 could also be concluded by the coupling constant of H-6 at δ 4.51 (br s). Consequently, compound 1 was established as 4α,6β,7α,10β-tetrahydroxy-1,5-*cis*-guaiane (1), which was also confirmed by X-ray crystallographic analysis (Fig. 3).

Compound 2 was obtained as a colorless prism. The IR spectrum of 2 exhibited absorptions for OH groups at 3406 cm⁻¹ and an olefinic moiety at 1634 cm⁻¹. The molecular formula was deduced to be C₁₅H₂₆O₃ by HR-ESI-MS (*m/z* 255.1860 ([M+H]⁺); Calcd 255.1854). In the ¹H-NMR spectrum of 2 (Table 1), four tertiary methyl signals were observed, together with an olefinic proton at δ_H 5.87 (1H, d, *J*=2.9 Hz, H-6). The ¹³C-NMR spectrum of 2 revealed the presence of 15 carbon signals, of which two olefinic carbons [δ_C 121.6 (C-6, d), 150.0 (C-7, s)] were observed. The above spectral features suggested 2 to be a guaiane-type sesquiterpenoid, and all ¹³C-NMR (Table 1) data of 2 were essentially the same as those of alismoxide^{3–5)} except for C-11, C-12,

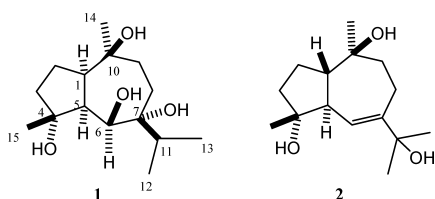


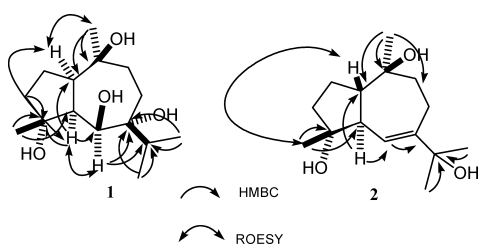
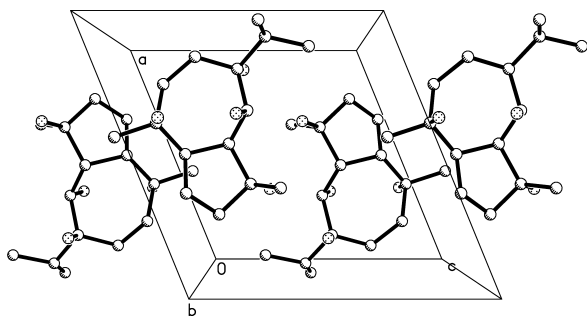
Fig. 1. Structures of Compounds 1 and 2

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Table 1. ^1H - (500 MHz) and ^{13}C - (125 MHz) NMR Data for Compounds **1** and **2**^{a)}

Position	1 ^{b)}		2 ^{c)}	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	52.3 (d)	3.15 (1H, dd, 9.7, 9.7 Hz)	50.4 (d)	1.80—1.88 (overlapped)
2	24.8 (t)	2.54—2.57 (overlapped)	21.5 (t)	1.85 (1H, m)
		2.28 (1H, m)		1.20—1.25 (overlapped)
3	41.3 (t)	2.38 (1H, m), 2.11 (1H, m)	40.4 (t)	1.88 (1H, m), 1.65 (1H, m)
4	83.3 (s)	—	80.1 (s)	—
5	52.0 (d)	3.38 (1H, br d, 9.6 Hz)	50.1 (d)	2.18 (1H, dd, 10.8, 3.0 Hz)
6	74.1 (d)	4.51 (1H, br s)	121.6 (d)	5.87 (1H, d, 2.9 Hz)
7	76.5 (s)	—	150.0 (s)	—
8	30.7 (t)	2.56—2.58 (overlapped)	24.6 (t)	2.30 (1H, m), 2.05 (1H, m)
		1.95—1.98 (1H, m)		—
9	37.6 (t)	2.36—2.38 (overlapped)	42.6 (t)	1.82 (1H, m)
		1.83—1.85 (1H, m)		1.42—1.48 (1H, m)
10	71.7 (s)	—	75.2 (s)	—
11	36.0 (d)	2.44 (1H, m)	73.8 (s)	—
12	18.0 (q)	1.21 (3H, d, 6.7 Hz)	28.5 (q)	1.30 (3H, s)
13	18.2 (q)	1.29 (3H, d, 6.7 Hz)	28.6 (q)	1.31 (3H, s)
14	32.8 (q)	1.43 (3H, s)	21.2 (q)	1.22 (3H, s)
15	25.9 (q)	1.78 (3H, s)	22.7 (q)	1.27 (3H, s)

a) Assignments based on HSQC and HMBC correlations. b) Measured in $\text{C}_5\text{D}_5\text{N}$. c) Measured in CDCl_3 .

Fig. 2. Selected HMBC and ROESY Correlations of Compounds **1** and **2**Fig. 3. X-Ray Crystal Structure of Compound **1**

and C-13, indicating compound **2** possessed the same rings A and B as those of alismoxide. Considering compound **2** has 4 methyl groups (all s) and one more oxygenated quaternary carbon signals than alismoxide at δ_{C} 73.8 (C-11, s) as the ^{13}C -NMR DEPT spectrum showed, it could be concluded that a substituted-isopropyl might be present. This assumption was confirmed by an HMBC experiment (Fig. 2) in which long-range correlations between H-12, H-13, and C-11 were observed, establishing that Me-12 and Me-13 are linked at C-11. The relative stereostructure of compound **2** was characterized by a ROESY experiment (Fig. 2) in which correlation between H-1, which was β -oriented by comparison with alismoxide, and H-15 was observed, revealing that H-1 and Me-15 are in β -configuration. This also was supported by comparing the J values with the known compound alismoxide.^{3–5} Thus the structure of **2** was deduced to be 4 α ,10 β ,11-trihydroxy-1,5-*trans*-guaiane-6,7-ene (**2**).

Bioassay against HBV of compound **1** obtained in a larger amount for *in vitro* was performed. Anti-HBV activity, cytotoxicity, and selectivity index (SI) are summarized in Table 2. It was exhibited that compound **1** showed higher SI of 16.73 for HBsAg, but lower activity for HBeAg under the toxic concentration. The activity of compound **2** can not be analyzed because of a trace amount isolated from the plant.

Experimental

General Experimental Procedures Column chromatography (CC): silica gel (200—300 mesh; Qingdao Marine Chemical Inc., China), MCI gel CHP-20P (70—150 μ ; Mitsubishi Chemical Corporation, Tokyo, Japan), Lichrospher Rp-18 gel (40—63 μ ; Merck, Germany) and Sephadex LH-20 (Pharmacia Biotech, Sweden). Detection was performed by TLC on silica gel sprayed with 10% H_2SO_4 in EtOH followed by heating. Optical rotations were carried out on a HORIBA SEPA-300 High Sensitive Polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets, ν in cm^{-1} . 1D- and 2D-NMR experiments were performed on a Bruker-AM-400 (^1H and ^{13}C , at 400 and 100 MHz, resp.) or DRX-500 (^1H and ^{13}C , at 500 and 125 MHz, resp.) spectrometer, and δ in ppm with TMS as internal reference, J in Hz. MS were taken on a VG-Auto-Spec-3000 instrument. mp was measured on an XRC-1 apparatus and uncorrected.

Plant Material The dried rhizomes of *A. orientalis* were collected in Sichuan province in October 2002 and identified by Prof. Jun Zhou, Kun-

Table 2. Anti-HBV Activity, Cytotoxicity and Selectivity Index of Compound 1

Compounds	CC ₅₀ (μ M)	HBsAg		HBeAg	
		IC ₅₀ (μ M)	SI	IC ₅₀ (μ M)	SI
1	>18.4	1.1	>16.73	>14.7	>1.25
ADFV	>1000	60.0	>16.7	100	>10.0

ADFV: adefovir dipivoxil, an antiviral agent used as positive control.

ming Institute of Botany, Chinese Academy of Sciences, where a voucher specimen was deposited.

Extraction and Isolation The dried rhizomes (9.0 kg) were extracted three times with 90% EtOH for 2 h under reflux. The extract was concentrated under vacuum to give a residue that was suspended in H₂O and partitioned between CHCl₃ and H₂O to provide a CHCl₃ fraction (380 g). The CHCl₃ fraction was subjected to CC (silica gel, 2.0 kg, 200–300 mesh), gradient elution with CHCl₃ and CHCl₃–MeOH (98:2–80:20) to afford fractions 1–78 (500 ml each). Fractions 1–12 (21.5 g) were combined by TLC detection and submitted to CC (silica gel, petroleum ether–acetone 95:5, 90:10, 85:15, and 80:20) to provide 4 fractions. Fraction No. 4 (6.0 g) was chromatographed on silica gel (150 g, petroleum ether–acetone 90:10, 85:15, and 80:20) to provide four fractions: Fr. A (0.5 g), B (0.1 g), C (1.3 g), and D (1.2 g). Fr. A–D were submitted to CC (MCI gel CHP-20P, 100 g, MeOH–H₂O 60:40–90:10) to afford several sub-fractions: Fr. A1–3, Fr. B1–4, Fr. C1–5, and Fr. D1–5. Fr. B3 was submitted to CC (silica gel, CHCl₃–MeOH 92:8), followed by Rp-8 (Lobar, MeOH–H₂O 70:30) to afford compound **2** (6 mg). Compound **1** (70 mg) was obtained from Fr. C3 by repeatedly submitting to CC (silica gel, CHCl₃–MeOH 90:10) and Sephadex LH-20 (MeOH).

4 α ,6 β ,7 α ,10 β -Tetrahydroxy-1,5-*cis*-guaiane (1): Colorless prism. mp 186.5–189 °C; $[\alpha]_D^{18.6} +22.17^\circ$ ($c=0.20$, MeOH); IR (KBr) cm⁻¹: 3417, 2962, 2936, 2878, 1465, 1372, 1186, 1054; ¹H- and ¹³C-NMR data, see Table 1; EI-MS (70 eV) m/z : 254 [M–H₂O]⁺ (3), 236 (5), 218 (10), 71 (100). Positive FAB-MS m/z : 255 [M+H–H₂O]⁺ (45), 237 (100). Positive ESI-MS m/z : 273 [M+H]⁺ (40), 255 [M+H–H₂O]⁺ (100), 237 [M+H–2H₂O]⁺ (35). HR-ESI-MS m/z : 273.1940 [M+H]⁺ (Calcd for C₁₅H₂₈O₄+H: 273.1958).

4 β ,10 α ,11-Trihydroxy-1,5-*trans*-guaiane-6,7-ene (2): Colorless prism. mp 146–147.5 °C; $[\alpha]_D^{18.9} -3.41^\circ$ ($c=0.22$, MeOH); IR (KBr) cm⁻¹: 3406, 2972, 2936, 2869, 1634, 1377, 1126, 1096; ¹H- and ¹³C-NMR data, see Table 1; EI-MS (70 eV) m/z : 236 [M–H₂O]⁺ (10), 218 (70), 220 (8), 160 (100). HR-ESI-MS m/z : 255.1860 [M+H]⁺ (Calcd for C₁₅H₂₆O₃+H: 255.1854).

X-Ray Crystal Data of Compound 1 A colorless prism crystal was obtained from CHCl₃–MeOH (1:1). Crystal data: C₁₅H₂₈O₄; $M_r=272.38$; mon-

oclinic system, space group $P2_1$, $a=9.276$ (2) Å, $b=18.586$ (4) Å, $c=9.341$ (2) Å, $\beta=112.37$ (3)°, $V=1489.2$ (5) Å³, $Z=4$, $D_{calc}=1.215$ g/cm³. MoK α radiation, linear absorption coefficient $\mu=1.0$ cm⁻¹. A colorless prism of dimensions 0.40×0.60×0.90 mm was used for X-ray measurements on a MAC DIP-2030K diffractometer with a graphite monochromator set to a maximum 2θ value of 50.0°. The total number of independent reflections measured was 3143, 2842 of which were considered to be observed ($|F|^2 \geq 2\sigma|F|^2$). The structure was solved by the direct method SHELX-86 and expanded using difference Fourier techniques, refined by the program and method NOMCSDP⁶⁾ and full-matrix least-squares calculations. Final refinement: $R_1=0.0547$ (all data), $wR_2=0.1482$ (all data), $S=1.187$, $(\Delta/\sigma)_{max}=0.052$, $(\Delta\rho)_{min}=-0.155$ e/Å³, $(\Delta\rho)_{max}=0.257$ e/Å³.

Anti-HBV Assay Anti-HBV assay was performed in accordance with the method discussed in our previous report.²⁾

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