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 IC_{50} 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 paper[2)] 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 $C_{15}H_{28}O_4$, 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 ^{1}H-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 ^{13}C-NMR (DEPT) spectrum exhibited 15 carbon signals (Table 1). NMR spectral analysis revealed that compound **1** was a guaiane-type sesquiterpenoid. ^{13}C DEPT experiment showed that compound **1** possessed the same numbers of methyls, methylenes, methines, and quaternary carbons as those of orientaol E.[3)] However, the chemical shifts for the C-atoms of **1** were not identical with those of orientaol 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.

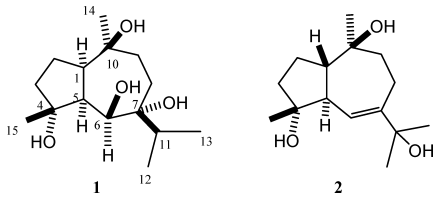

Fig. 1. Structures of Compounds **1** and **2**

The differences between compound **1** and orientaol 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 H_2O 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 $C_{15}H_{26}O_3$ by HR-ESI-MS (*m*/*z* 255.1860 ([M+H]$^+$); Calcd 255.1854). In the ^{1}H-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 ^{13}C-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 ^{13}C-NMR (Table 1) data of **2** were essentially the same as those of alismoxide[3—5)] except for C-11, C-12,

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Table 1. ^{1}H- (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) 2.28 (1H, m)	21.5 (t)	1.85 (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) 1.95—1.98 (1H, m)	24.6 (t)	2.30 (1H, m), 2.05 (1H, m)
9	37.6 (t)	2.36—2.38 (overlapped) 1.83—1.85 (1H, m)	42.6 (t)	1.82 (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 C_5D_5N. *c*) Measured in $CDCl_3$.

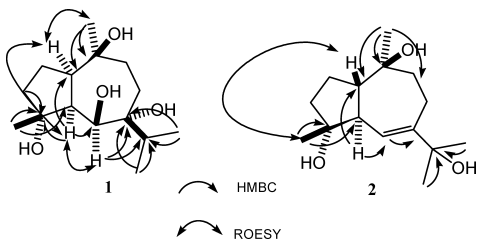

Fig. 2. Selected HMBC and ROESY Correlations of Compounds **1** and **2**

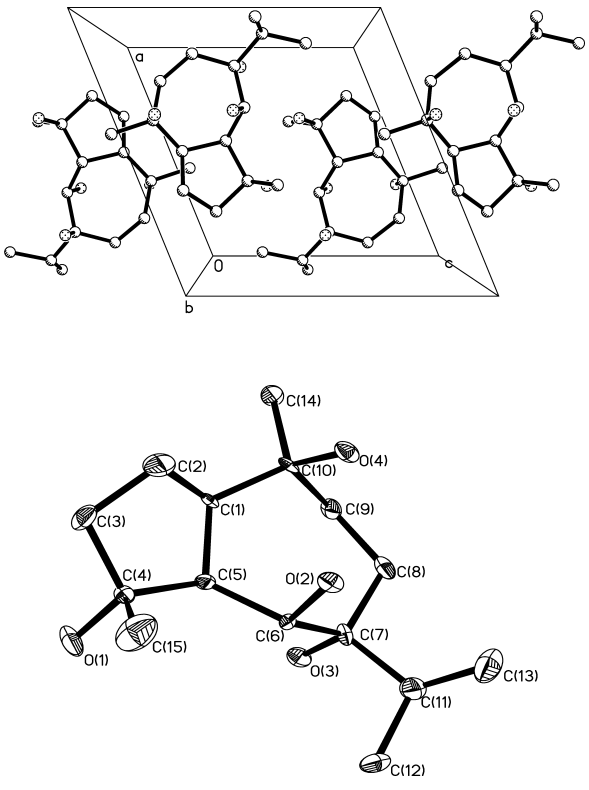

Fig. 3. X-Ray Crystal Structure of Compound **1**

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 4α,10β,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 (^{1}H and ^{13}C, at 400 and 100 MHz, resp.) or DRX-500 (^{1}H 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} (μM)	HBsAg		HBeAg	
		IC_{50} (μM)	SI	IC_{50} (μM)	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 H_2O and partitioned between $CHCl_3$ and H_2O to provide a $CHCl_3$ fraction (380 g). The $CHCl_3$ fraction was subjected to CC (silica gel, 2.0 kg, 200—300 mesh), gradient elution with $CHCl_3$ and $CHCl_3$–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–H_2O 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, $CHCl_3$–MeOH 92 : 8), followed by Rp-8 (Lobar, MeOH–H_2O 70 : 30) to afford compound **2** (6 mg). Compound **1** (70 mg) was obtained from Fr. C3 by repeatedly submitting to CC (silica gel, $CHCl_3$–MeOH 90 : 10) and Sephadex LH-20 (MeOH).

4α,6β,7α,10β-Tetrahydroxy-1,5-*cis*-guaiane (**1**): Colorless prism. mp 186.5—189 °C; $[\alpha]_D^{18.6}$ +22.17° (c=0.20, MeOH); IR (KBr) cm^{-1}: 3417, 2962, 2936, 2878, 1465, 1372, 1186, 1054; ^{1}H- and ^{13}C-NMR data, see Table 1; EI-MS (70 eV) *m/z*: 254 $[M-H_2O]^+$ (3), 236 (5), 218 (10), 71 (100). Positive FAB-MS *m/z*: 255 $[M+H-H_2O]^+$ (45), 237 (100). Positive ESI-MS *m/z*: 273 $[M+H]^+$ (40), 255 $[M+H-H_2O]^+$ (100), 237 $[M+H-2H_2O]^+$ (35). HR-ESI-MS *m/z*: 273.1940 $[M+H]^+$ (Calcd for $C_{15}H_{28}O_4$+H: 273.1958).

4β,10α,11-Trihydroxy-1,5-*trans*-guaiane-6,7-ene (**2**): Colorless prism. mp 146—147.5 °C; $[\alpha]_D^{18.9}$ −3.41° (c=0.22, MeOH); IR (KBr) cm^{-1}: 3406, 2972, 2936, 2869, 1634, 1377, 1126, 1096; ^{1}H- and ^{13}C-NMR data, see Table 1; EI-MS (70 eV) *m/z*: 236 $[M-H_2O]^+$ (10), 218 (70), 220 (8), 160 (100). HR-ESI-MS *m/z*: 255.1860 $[M+H]^+$ (Calcd for $C_{15}H_{26}O_3$+H: 255.1854).

X-Ray Crystal Data of Compound 1 A colorless prism crystal was obtained from $CHCl_3$–MeOH (1 : 1). Crystal data: $C_{15}H_{28}O_4$; M_r=272.38; monoclinic system, space group $P2_1$, a=9.276 (2) Å, b=18.586 (4) Å, c=9.341 (2) Å, β=112.37 (3)°, V=1489.2 (5) Å^3, Z=4, D_{calc}=1.215 g/cm^3. Mo$K\alpha$ radiation, linear absorption coefficient μ=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 NOMCSDP[6] 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/Å^3, $(\Delta\rho)_{max}$=0.257 e/Å^3.

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

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