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# Guaiane-type sesquiterpenoids from *Alisma orientalis*

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**Abstract**

Two guaiane-type sesquiterpenoids named orientalol E (**1**) and orientalol F (**3**) were isolated from the rhizome of *Alisma orientalis* (SAM) JUZEP together with two known guaiane-type sesquiterpenoids alismol (**2**) and alismoxide (**4**). Their relative stereostructures were elucidated by spectroscopic methods, whereas absolute stereostructures were determined on the basis of chemical correlation.


*Keywords:* *Alisma orientalis*; Alismataceae; Sesquiterpenoids; Guaiane; Orientalol E and F; Stereostructure

## 1. Introduction

The plant *Alisma orientalis* (SAM) JUZEP has been widely cultivated in China and Japan, and its dried rhizomes have been used as folk medicines for diabetes, diuretics and they are an important crude drug component for several Chinese preparations. Our continuing phytochemical investigation on the sesquiterpenoids of the plant led to the isolation of two guaiane-type sesquiterpenoids named orientalol E (**1**) and F (**3**) along with two known compounds alismol (**2**) and alismoxide (**4**). Previously, it was reported to contain a few triterpenoids, sesquiterpenoids e.g. alismol (**2**), alismoxide (**4**), germacrene C, orientalols A–C and sulphate of orientalols (Nakajima et al., 1994; Oshima et al., 1983; Yoshikawa et al., 1992, 1993a, 1993b).

## 2. Results and discussion

### 2.1. Relative stereostructures of *1* and *3*

Orientalol E (**1**) was isolated as a pale yellow oil, whose molecular formula was determined to be $C_{15}H_{26}O_3$ by a combination of HR-ESI-MS at *m/z* 255.1938 $[M+H]^+$ (calc. 255.1955) and NMR spectroscopic analysis. The IR spectrum exhibited a broad absorption at 3400.6 $cm^{-1}$ due to hydroxyl. The $^1H$ MNR showed two tertiary methyls groups [$\delta_H$ 1.33 and 1.21 (each 3H, *s*)], one isopropyl group [$\delta_H$ 1.95 (1H, *sept*, $J=6.9$Hz), 1.03 (3H, *d*, $J=6.9$Hz), 1.01 (3H, *d*, $J=6.9$Hz)], and one oxygenated methine at $\delta_H$ 3.80 (1H, *d*, $J=9.3$Hz). The $^{13}C$ NMR and INEPT spectra revealed the presence of four methyl carbons, four methylene carbons, three methine carbons, one oxygenated methine carbon ($\delta_C$ 72.1), and three oxygenated quaternary carbons ($\delta_C$ 79.1, 86.4 and 83.0). These spectral features (Table 1) suggested **1** to be a guaiane-type sesquiterpenoid, and its $^{13}C$ NMR spectrum was similar to that of **4** except for C-5, C-6, C-7, C-10. The assignment of the protonated carbons was performed by application of a HMQC experiment. In the HMBC spectrum, the following correlations were noted: the proton signal at $\delta_H$ 1.33 ($H_3$-14) with the carbon resonance at $\delta_C$ 79.1 (C-4), 54.5 (C-5), 40.1 (C-3); the proton resonance at $\delta_H$ 1.03 ($H_3$-12) and $\delta_H$ 1.01 ($H_3$-13) with the carbon signals at $\delta_C$ 31.8 (C-7), 32.4 (C-11); the proton signal at $\delta_H$ 1.95 (H-11) with the carbon resonance at $\delta_C$ 86.4 (C-7), 72.1 (C-6), 29.0 (C-8), 17.4 (C-12), 18.3 (C-13); the proton resonance at $\delta_H$ 1.21 (H-15) with the carbon signal at $\delta_C$ 50.4 (C-1), 31.8 (C-9) and 83.0 (C-10); the proton signal at $\delta_H$ 3.80 (H-6) with the carbon signals at $\delta_C$ 79.1 (C-4), 54.5 (C-5), 86.4 (C-7), 29.0 (C-8), 32.4 (C-11). The correlations of **1** observed

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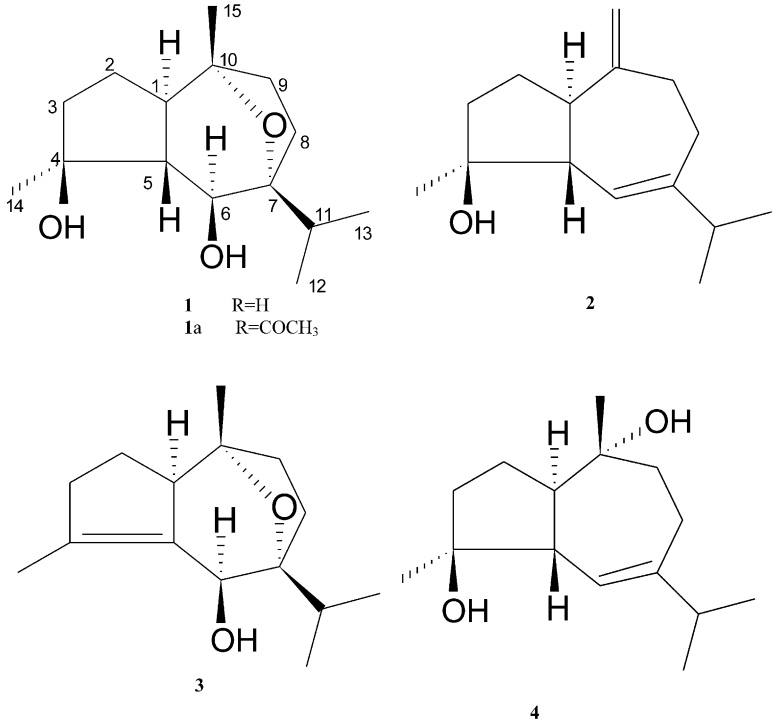
Table 1
$^{13}C$ NMR and $^{1}HNMR$ spectral data of compounds **1**, **1a**, **3**, **4** (400 or 300 MHz, δ, ppm, $CDCl_3$ as solvent)

| Compounds | Orientalol E | | Orientalol E 6-acetate | | Orientalol F | | Alismoxide | |
|---|---|---|---|---|---|---|---|---|
| No. | δC | δH | δC | δH | δC | δH | δC | δH |
| **1** | 50.4 | 1.72 | 50.5 | 1.67 *m* | 57.7 | 2.69 | 50.9 | |
| **2** | 23.5 | | 22.8 | | 24.0 | 1.21, 1.81 | 21.6 | |
| **3** | 40.1 | | 40.1 | | 39.1 | 2.31, 2.20 | 40.6 | |
| **4** | 79.1 | | 78.8 | | 133.6 | | 80.3 | |
| **5** | 54.5 | 1.57 | 52.8 | 1.76 *d* | 133.0 | | 50.0 | |
| **6** | 72.1 | 3.80 *d*, *J*=9.3 | 72.1 | 5.04 *d* | 74.0 | 4.44, *brs* | 121.4 | 5.52 |
| **7** | 86.4 | | 85.7 | | 86.7 | | 149.7 | |
| **8** | 29.0 | | 30.3 | | 28.6 | 1.71, 1.60 | 25.2 | |
| **9** | 31.8 | | 31.4 | | 31.7 | 1.83, 1.29 | 42.5 | |
| **10** | 83.0 | | 83.0 | | 84.5 | | 76.8 | |
| **11** | 32.4 | 1.95 | 32.9 | 1.78 | 31.8 | 1.95 | 37.3 | |
| **12** | 17.4 | 1.03 d *J*=6.9 | 17.4 | 0.93 *d* | 17.3 | 1.01, *d J*=6.9 | 21.2 | 0.97 |
| **13** | 18.3 | 1.01 *d*, *J*=6.9 | 18.2 | 0.98 *d* | 18.2 | 1.03, *d*, *J*=6.9 | 21.5 | 0.96 |
| **14** | 25.1 | 1.33 3H, *s* | 24.7 | 1.37 *s* | 14.7 | 1.89, 3H, *s* | 22.7 | 1.26 |
| **15** | 23.6 | 1.21 3H, *s* | 23.6 | 1.22 *s* | 24.1 | 1.19, 3H, *s* | 21.5 | 1.20 |
| $OCOCH_3$ | | | 170.7 | | | | | |
| | | | 21.5 | 2.04 *s* | | | | |

among protons were determined on the basis of $^{1}H$–$^{1}H$ COSY experiments. Treatment of **1** with $AC_2O$-pyridine afforded a monoacetate **1a** (Fig. 1). The acetylation shifts observed were as follows: the resonance due to C-8 (δ 30.3) appeared at a lower field than that in **1** (δ 29.0), while those of C-5 and C-7 (δ 52.8, 85.7) were at a higher field than those in **1** (δ 54.5, 86.7). In addition, the H-6 signal of **1a** (δ 5.04) was observed at lower field than that of **1** (δ 3.80) due to the acetylation shift. Thus the planar structure of orientalol E was determined as **1**.

The relative stereostructure of **1** was characterized by NOESY experiment. The correlations from H-1, assumed to be α oriented, to Me-14, from Me-14 to H-6, from H-6 to Me-14 and Me-12 suggested that these protons and methyls were all in an α-configuration. The correlations from H-5 to Me-15 and H-8β suggested that the protons and methyls were all in β-configur-




**1** R=H
**1a** R=$COCH_3$

**2**

**3**

**4**

Fig. 1. (**1**) R = H, (**1a**) R = $COCH_3$.

ation. These correlations indicated that the A/B ring junction is *trans* oriented. Therefore, the structure of **1** was established as 4β, 6β-dihydroxy-7α, 10α-epoxy-1, 5-trans-guaiane (Fig. 1).

Orientalol F (**3**) was isolated as pale yellow oil, whose molecular formula was determined to be $C_{15}H_{24}O_2$ by a combination of HR-ESI-MS at *m/z* 237.1844 $[M+H]^+$ (calc. 237.1849) and NMR. The IR spectrum exhibited an absorption band at 3476.4 $cm^{-1}$ due to a hydroxyl group. The $^1H$ MNR showed the presence of two tertiary methyl groups at $\delta_H$1.89 and 1.19 (each 3H, *s*), one isopropyl group at $\delta_H$ 1.95 (1H, *sept*, $J=6.9$Hz), 1.03 (3H, *d*, $J=6.9$Hz), 1.01 (3H, *d*, $J=6.9$Hz), and one oxygenated methine at $\delta_H$ 4.44 (1H, *d*, $J=9.3$ Hz). The $^{13}C$ NMR and DEPT spectrum revealed the presence of four tertiary methyl carbons, four methylene carbons, two methine carbons, one oxygenated methine carbon, a tetrasubstituted double bond ($\delta_C$ 133.62 and 133.04) and two oxygenated quaternary carbons ($\delta_C$ 85.75 and 84.47). These spectral features (Table 1) suggested **3** to be a guaiane-type sesquiterpenoid, when $^{13}C$ NMR spectra was similar to that of **1** except for a tetrasubstituted double bond ($\delta_C$ 133.62 and 133.04) and a tertiary methyl ($\delta_C$ 14.7). The assignment of the protonated carbons was performed by a HMQC experiment. Based on the above observations and by analyses of HMBC spectral data as shown in Fig. 2, the structure of orientalol F was established as **3** (Fig. 1). The relative stereostructure of **3** was characterized by NOESY experiment (Fig. 3), and it was established as 6β-hydroxy-7α, 10α-epoxyguaiane-4, 5-ene (Fig. 1).

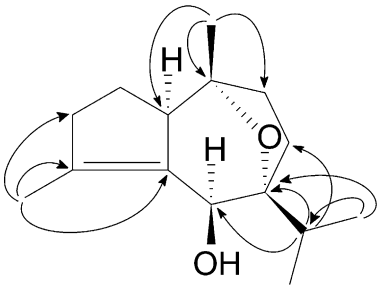



Fig. 2. HMBC correlations of **3**.

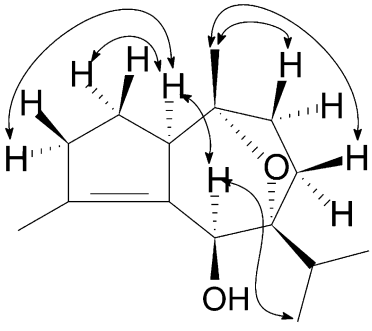



Fig. 3. NOESY correlations of **3**.

### 2.2. Absolute stereostructures of orientalol E (*1*) and alismoxide (*4*)

Next, in order to clarify the absolute stereostructure of orientalol E (**1**) and alismoxide (**4**), we carried out the conversion of **2** into **4** and **1**. At first, **2** was naturally oxidized to a mixture contaning **2** and **4**, which was purified by prep. TLC to give pure **4**. Then, **4** was treated with *m*-chloroperbenzoic acid (mCPBA) to give pure **1** (Scheme 1). Since the absolute stereostructure of **2** had been determined by CD spectral analysis, and on the basis of the transformation from **2** to alismoxide (**4**) and orientalol E (**1**), the absolute stereostructures of orientalol E (**1**) and alismol (**3**) were determined to be 1R, 4S, 5R, 6S, 7R, 10R and 1R, 4S, 5R, 10R (Yoshikawa et al., 1993c).

## 3. Experimental

### 3.1. General experimental procedures

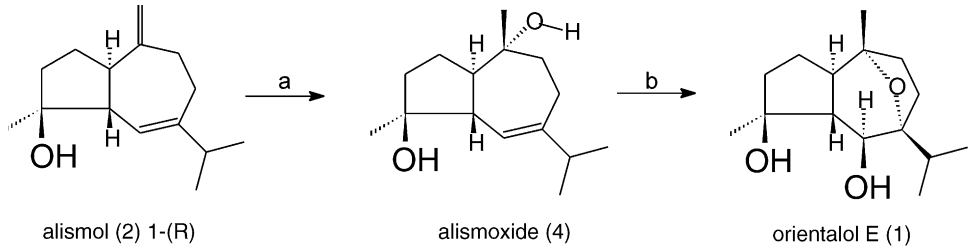
MPs: PHMK79/1212; uncorr. IR: Nicolet 170sx spectrometer. NMR: Burker ACF-300 or Burker ASR-400 spectrometer (400 or 300 MHz for $^1H$, 100 or 75 MHz for $^{13}C$), both with TMS as int. standard. EI-MS: Nicolet FTMS-2000; HR-ESI-MS: PE Biosystems Mariner System 5140 instrument. Optical rotations were measured with a JASCO DIP-181 spectropolarimeter in MeOH solution. CC: silica gel H (10–40 μ, made in QingDao Oceanic Chemical Industry). prep. TLC (thickness = 1 mm): silica gel H (10–40 μ), spots were visualized by spraying with 10% $H_2SO_4$ followed by heating.

### 3.2. Plant material

Rhizome of *Alisma orientalis* (SAM) JUZEP was collected in the province of the People's Republic of China. A voucher specimen (97-0721) was deposited at the Faculty of Pharmaceutical Sciences, Nanjing University of Traditional Chinese Medicine.

### 3.3. Extraction and isolation

The dried plant material (28 kg) was extracted with EtOAc (IOL, twice) at room temperature. The EtOAc solutions were combined and concentrated under reduced pressure to give an extract which was subjected to silica gel CC with petroleum ether–EtOAC (8:2–2:8) gradient to give four fractions (Fr. A-Fr. D). Fr. B was subjected to silica gel CC, eluted with petroleum ether–EtOAc (95:5, 85:15, 75:25, 70:30) to give 300 fractions. Frs. (85–96) were applied to a silica gel column to give pure alismol (**1**, 800 mg). Similarly, Frs. (105-122) were purified by prep. TLC to give pure orientalol F (**2**, 30 mg). Fr. D




Scheme 1. Reactions performed to elucidate the absolute configuration of alismoxide (**4**) and orientalol E (**1**) [Reaction conditions: (a) air, room temperature, 3 months; (b) *m*-chloroperbenzoic acid (mCPBA), $ClCH_2CH_2Cl$, room temperature, 30 h].

was subjected to silica gel CC, eluted with $CHCl_3$–MeOH (95:5, 85:15, 75:25, 70:30) to give 50 fractions. Frs (38–45) were applied to a silica gel column to give pure alismoxide (**4**, 300 mg) and orientalol E (**1**, 50 mg).

### 3.4. Orientalol E ((1R*, 4S*, 5R*, 6S*, 7R*, 10R*)-4, 6-dihydroxy-7, 10-epoxy-1, 5-trans-guaiane) (1)

Pale yellow oil; $[\alpha]_D^{25} = +3.7$ (MeOH; *c* 0.5); IR ($\upsilon_{max}^{KBr}$ $cm^{-1}$): 3400 (–OH), 2961, 2873, 1464, 1379, 1308, 1158, 1067, 1014, 813, 734; $^1H$ NMR and $^{13}C$ NMR spectral data see Table 1; HR-ESI-MS: *m/z* 255.1983 $[M+H]^+$, $C_{15}H_{27}O_3$ requires 255.1955.

### 3.5. Acetylation of orientalol E (1)

The oil **1** (10 mg) in pyridine (0.5 ml) was added to acetic anhydride (0.5 ml) and the mixture was stirred at room temperature for 24 h. After evaporation of excess reagent, the residue was separated by prep. TLC to give pure compound **1a**. Compound **1a** was pale yellow oil; IR ($\upsilon_{max}^{KBr}$ $cm^{-1}$): 3437 (–OH), 1736 (>C=O), 1461, 1377, 1243, 948; $^1H$ NMR and $^{13}C$ NMR data see Table 1; HR-EI-MS: *m/z* 296.3957 $[M]^+$, $C_{17}H_{28}O_4$ requires 296.3934; EIMS: *m/z* 296 $[M]^+$, 281 $[M–Me]^+$, 278 $[M–H_2O]^+$, 236 $[M–HAc]^+$.

### 3.6. Orientalol F (6β-hydroxy-7α, 10α-epoxyguaiane-4, 5-ene) (3)

Pale yellow oil; $[\alpha]_D^{25} = +4.3$ (MeOH, *c* 0.5); IR ($\upsilon_{max}^{KBr}$ $cm^{-1}$): 3476, 2965, 2932, 2876, 1488, 1375, 1177, 1073, 1016, 901; for $^1H$ NMR and $^{13}C$ NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 237.1844 $[M+H]^+$, $C_{15}H_{24}O_2$, requires 237.1849).

### 3.7. Alismol (2)

Colorless oil; $[\alpha]_D^{25} = +7.9°$ (MeOH; *c* 0.5); IR ($\upsilon_{max}^{KBr}$ $cm^{-1}$): 3400 (–OH), 1745(>C=O), 1705; $^1H$ NMR (400 or 300 MHz, δ, ppm, $CDCl_3$ as solvent): δ 5.56 (1H, s, H-6), 0.99 (3H, *d*, *J* = 6.9Hz, H-13), 1.00 (3H, *d*, *J* = 6.9 Hz, H-13), 1.25(3H, s, H-15), 4.76 (1H, s, H-14a), 4.70 (1H, s, H-14b); $^{13}C$ NMR (400 or 300 MHz, δ, ppm, $CDCl_3$ as solvent): δ 55.1, 24.8, 40.3, 80.6, 47.3, 121.4, 149.6, 30.0, 37.1, 153.9, 37.4, 21.3, 21.5, 24.1, 106.4.

### 3.8. Alismoxide (4)

Colorless prisms, mp 140–142°C; $[\alpha]_D^{25} = +5.2°$ (MeOH; *c* 0.5); $^1H$-NMR and $^{13}C$ NMR data see Table 1.

### 3.9. Conversion from 3 to 1 and 4

The oil **3** (200 mg) was exposed to air at room temperature for 3 months. The reaction mixture was purified by prep. TLC to furnish a pure sample of **4** (20 mg), identical (IR and NMR data) with the natural compound. A solution of **4** (100 mg) in $ClCH_2CH_2Cl$ (5 ml) was treated with *m*-chloroperbenzoic acid (mCPBA, 200 mg) and the whole mixture was stirred at room temperature for 30 h. The reaction mixture was separated by prep. TLC to give pure compound **1** (15 mg), identical (IR and NMR data) with the natural compound. The optical rotation of the semi-synthetic compound **1** ($[\alpha]_D^{25} = +3.4$ (concentration 0.5 mg/ml (MeOH)) is almost the same as natural compound.

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