ABSOLUTE STEREOSTRUCTURES OF ALISMALACTONE 23-ACETATE AND ALISMAKETONE-A 23-ACETATE, NEW *SECO*-PROTOSTANE AND PROTOSTANE-TYPE TRITERPENES WITH VASORELAXANT EFFECTS FROM CHINESE ALISMATIS RHIZOMA

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New bioactive *seco*-protostane and protostane-type triterpenes, alismalactone 23-acetate and alismaketone-A 23-acetate, were isolated from Chinese Alismatis Rhizoma originating from Fukien province, the dried rhizoma of *Alisma orientale* JUZEP. Absolute stereostructures of the new triterpenes were determined on the basis of chemical and physicochemical evidence, which included chemical correlations with the known triterpene alisol B monoacetate. Alismalactone 23-acetate and alismaketone-A 23-acetate were found to show inhibitory activity on the contractions induced by a high concentration of K^+ in isolated aortic strips from rats.

KEY WORDS Chinese Alismatis Rhizoma; alismalactone 23-acetate; alismaketone-A 23-acetate; vasorelaxant effect; *seco*-protostane-type triterpene; *Alisma orientale*

In the course of our studies on bioactive constituents of Alismatis Rhizoma,[1] which has been prescribed for diuretic and antiinflammatory purposes in Chinese traditional medicine, we have reported the sesquiterpene constituents with inhibitory activity on the contraction of isolated bladder smooth muscle[2] and the triterpene constituents[3] from Chinese Alismatis Rhizoma originating from Szechwan province. As a continuing study, we have isolated ten new bioactive triterpenes from Chinese Alismatis Rhizoma originating from Fukien province, the dried rhizoma of *Alisma orientale* JUZEP (Alismataceae). In this communication, we describe evidence consistent with the absolute stereostructures of alismalactone 23-acetate (**1**) and alismaketone-A 23-acetate (**2**) and their vasorelaxant effect.

The MeOH extract of the rhizoma was partitioned into an AcOEt-water mixture. The AcOEt-soluble portion was purified by repeated ordinary and reverse-phase silica-gel column chromatography and finally HPLC to give alismalactone 23-acetate (**1**, 0.0031%), alismaketones-A 23-acetate (**2**, 0.0022%), -B 23-acetate (0.0019%), -C 23-acetate (0.0014%), -D 23-acetate (0.0010%), -E 23-acetate (0.0010%), -F (0.0012%), -G 23-acetate (0.0009%), -H (0.0012%), and -I (0.0011%) together with known protostane-type triterpenes and guaiane-type sesquiterpenes.

Alismalactone 23-acetate (**1**), a white powder, $[\alpha]_D$ -34.0°, $C_{32}H_{48}O_7$, IR (KBr, cm^{-1}) : 3500—2500, 1740, 1700, showed quasimolecular ion peaks at *m/z* 545 $(M+H)^+$ and 567 $(M+Na)^+$ in its positive-mode FAB-MS. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **1** indicated the presence of seven tert. methyls, a sec. methyl [δ 1.03 (d, *J*=7.0Hz, 21-H_3)], an acetyl [δ 2.06 (s), 4.59 (ddd, *J*=3.1, 8.5, 10.4Hz, 23-H)], a lactone ring [δ 2.55, 2.58 (ABq, *J*=14.0Hz, 1-H_2), 1.73 (δ, *J*=11.0Hz, 9-H), 4.10 (ddd, *J*=5.5, 11.0, 11.0Hz, 11-H)], an epoxide [δ 2.72 (d, *J*=8.5Hz, 24-H)], a carboxyl, and a tetra-substituted olefin. The connectivities of the H-H and the quart. carbons (C-2, 3, 4, 8, 10, 13, 14, 17, 25) in **1** were clarified by the H-H COSY and HMBC experiments as shown. Furthermore, the NOESY experiment of **1** showed NOE correlations between the following protons: 5-H and 30-H_3, 11-H and 30-H_3, 9-H and 18-H_3, and 9-H and 19-H_3. Methylation of **1** with CH_2N_2-etherate in MeOH furnished the monomethyl ester (**1a**).[4] Comparison of the ^{1}H-NMR and ^{13}C-NMR data for **1** and **1a** with those for alisol B monoacetate (**3**)[3,5] led us to depict the new 2,3-*seco*-protostane skeleton of **1**.

In order to elucidate the total structure of **1** including the absolute configuration, we carried out chemical correlation of **1** with **3**. Thus **3** was subjected to ordinary acetylation followed by reduction of the 3-ketone group with $NaBH_4$ to yield the 3β-ol (**4**, quant.). Dehydration of **4** with $POCl_3$ furnished the 2-olefin (**5**, 43.0% yield), which was subjected to OsO_4 oxidation to give the 2β,3β-diol (**6**, 36.4%) and the 2α,3α-diol (**7**, 14.0%). The structures of **6** and **7** were clarified from examination of the ^{1}H-NMR data ($CDCl_3$) and NOESY experiment of **6a** [δ 5.24 (ddd, *J*=3.4, 3.9, 4.6Hz, 2-H), 4.71 (d, *J*=3.4Hz, 3-H)] and **7a** [δ 5.24 (ddd, *J*=3.1, 5.0, 11.0Hz, 2-H), 4.95 (d, *J*=3.1Hz, 3-H)], which were obtained by ordinary acetylation of **6** and **7**, respectively. The 2β,3β-diol moiety of **6** was cleaved with $Pb(OAc)_4$

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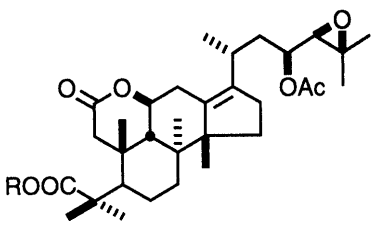
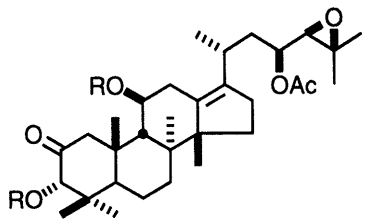
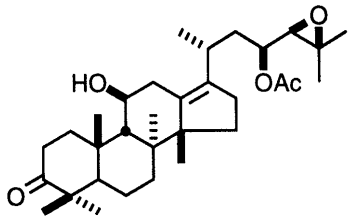


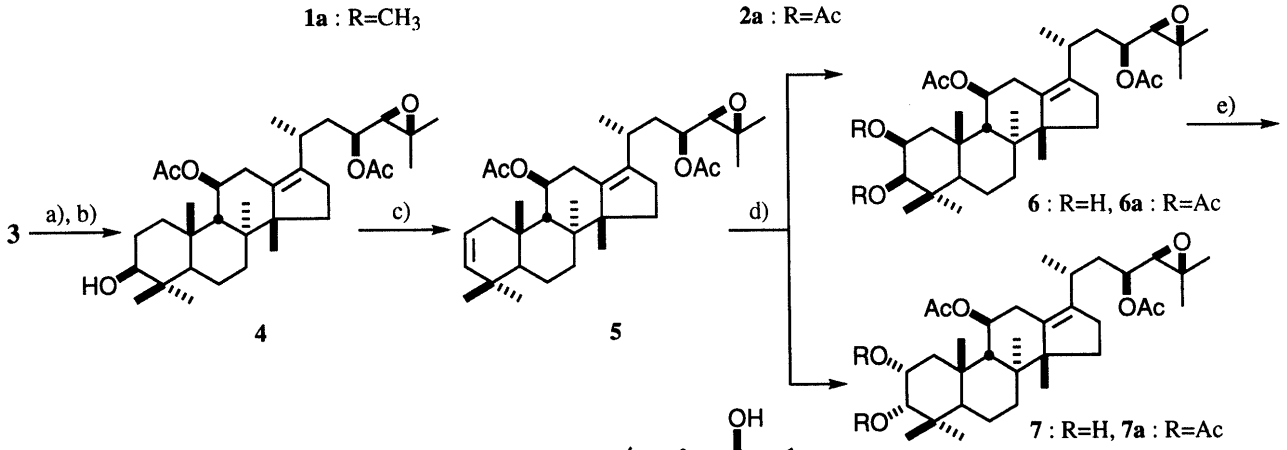
alismalactone 23-acetate (**1**) : R=H
1a : R=CH_3



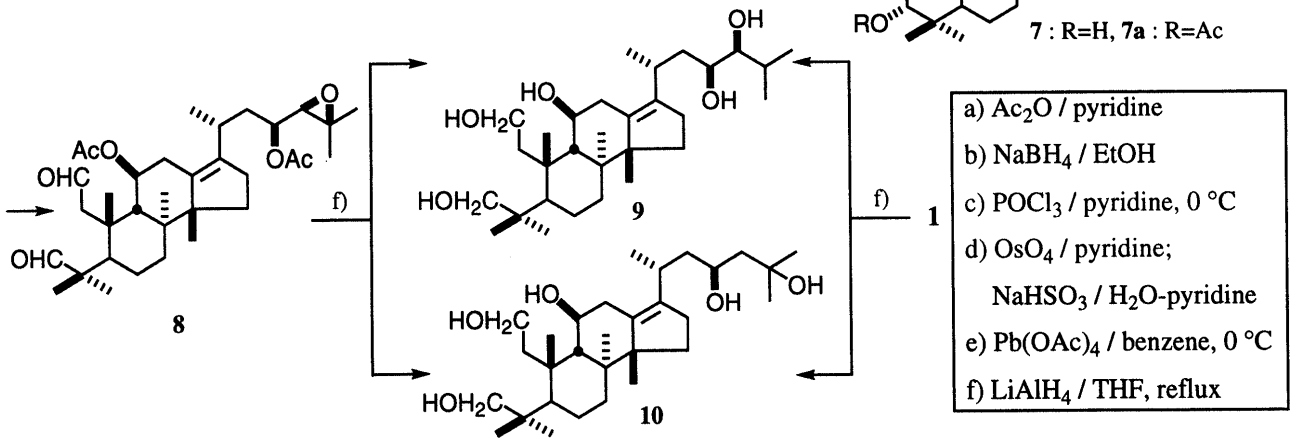
alismaketone-A 23-acetate (**2**) : R=H
2a : R=Ac



alisol B monoacetate (**3**)

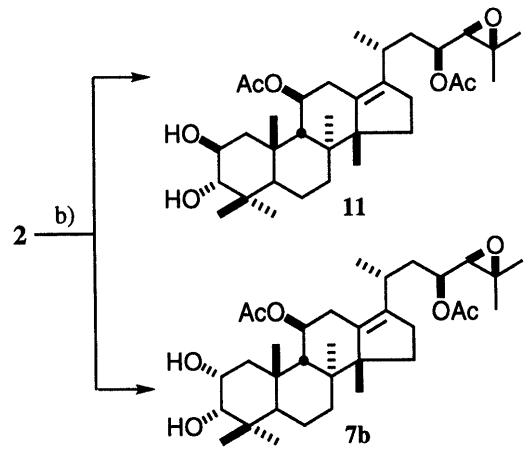


3 → a), b) → **4** → c) → **5** → d) → **6** : R=H, **6a** : R=Ac; **7** : R=H, **7a** : R=Ac; e)

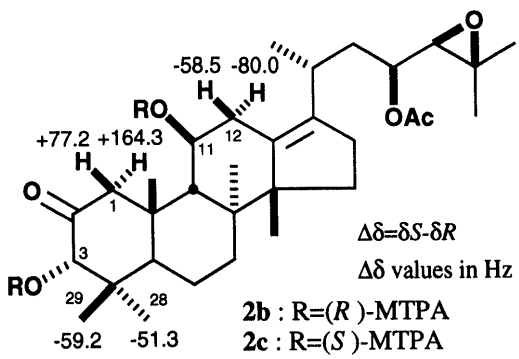


8 → f) → **9**, **10** → f) → **1**

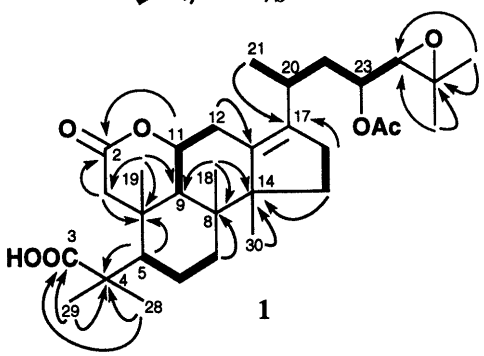
a) Ac_2O / pyridine
b) $NaBH_4$ / EtOH
c) $POCl_3$ / pyridine, 0 °C
d) OsO_4 / pyridine;
$NaHSO_3$ / H_2O-pyridine
e) $Pb(OAc)_4$ / benzene, 0 °C
f) $LiAlH_4$ / THF, reflux



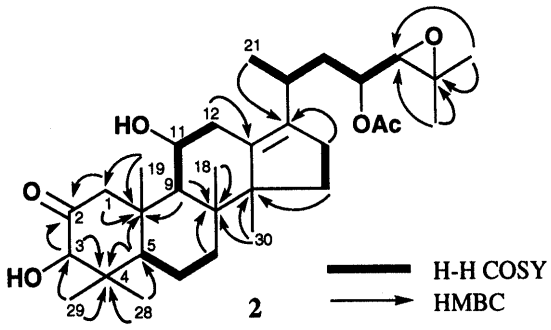
2 → b) → **11**, **7b**



$\Delta\delta=\delta S-\delta R$
$\Delta\delta$ values in Hz (500MHz)
2b : R=(*R*)-MTPA
2c : R=(*S*)-MTPA



1



2

H-H COSY
HMBC

Table. 1 ^{13}C-NMR Data for **1** and **2** ($CDCl_3$, δc)

	1	2		1	2
1	42.9	47.7	17	137.6	134.7
2	173.6	214.3	18	21.5	23.1
3	184.7	80.0	19	28.9	28.3
4	45.3	40.8	20	27.7	27.9
5	44.8	49.8	21	20.1	20.1
6	18.8	20.5	22	36.7	36.8
7	28.8	34.0	23	71.1	71.5
8	38.9	40.4	24	65.1	65.1
9	52.2	51.7	25	58.5	58.5
10	38.3	38.3	26	19.5	19.4
11	76.6	70.0	27	24.7	24.7
12	29.8	34.5	28	29.2	25.3
13	137.0	137.9	29	20.9	23.1
14	56.7	57.1	30	20.2	23.8
15	30.2	30.5	OAc	170.0	170.0
16	28.9	29.1		21.1	21.2

to provide the dialdehyde (**8**) in 90.0% yield. The dialdehyde (**8**) was treated with $LiAlH_4$ to give the penta-ols (**9**, **10**), both of which were identical to the $LiAlH_4$ reduction product of **1**. Consequently, the absolute stereostructure of alismalactone 23-acetate (**1**) was determined as shown in the Chart. Alismalactone 23-acetate is the first example of a *seco*-protostane-type triterpene.

Alismaketone-A 23-acetate (**2**), a white powder, $[\alpha]_D$ +75.7°, $C_{32}H_{50}O_6$, CD ($CHCl_3$, nm) : +7400 (281), IR (KBr, cm^{-1}) : 3450, 1740, 1705, positive-mode FAB-MS (*m/z*) : 531 $(M+H)^+$, 553 $(M+Na)^+$, provided the triacetyl derivative (**2a**) by ordinary acetylation. The proton and carbon signals in the ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **2** and **2a** were very similar to those of **3** and its acetate, except for some signals due to the A ring part [**2** : δ 2.75, 2.80 (ABq, *J*=18.9Hz, 1-H_2), 4.55 (s, 3-H)]. The 2-keto-3-ol structure of **2** was characterized on the basis of detailed examination of its H-H COSY and HMBC experiment results as shown in the Chart. Namely, long-range correlations were observed between the 1-H_2 and the 2, 10-C and between the 3-H and 2, 4-C. The absolute configuration at the C-3 of **2** has been presumed to be the *S* configuration based on the application of modified Mosher's method as shown.[6] Finally, reduction of **2** with $NaBH_4$ provided the 2β-ol (**11**, 51.8%) and the 2α-ol (**7b**, 23.9%), whose ^{1}H-NMR spectrum showed signals due to the 2-axial proton at δ 3.96 (ddd, *J*=3.1, 4.6, 11.6Hz). The acetylation product of **7b** was identical to that of **7a** derived from **3** (*vide ante*), and the absolute stereostructure of alismaketone-A 23-acetate (**2**) has been determined as shown in the Chart.

The vasorelaxant effects of **1** and **2** were examined using a bioassay to test the inhibitory activity on the contraction induced by a high concentration of K^+ in isolated rat aortic strips. As is apparent from Table 2, **1** and **2** inhibited the contraction induced by high K^+ in a concentration-dependent manner (10^{-5}-10^{-4}M). Alismalactone 23-acetate (**1**) and alismaketone 23-acetate (**2**) seem to be the first examples of triterpenes with vasorelaxant activity, and their vasorelaxant activities may be related to the diuretic effect of this natural medicine.

Table 2. Inhibitory Effects of **1** and **2** from Alismatis Rhizoma on KCl-induced Contractions

Compounds	N	Relaxation (%) Concentration (M)		
		1.0×10^{-5}	3.0×10^{-5}	1.0×10^{-4}
Vehicle (DMSO)	9	2.7±0.7	3.8±0.8	4.4±0.9
Alismalactone 23-acetate (**1**)	4	11.7±2.1	34.4±3.6**	89.4±6.6**
Alismaketone-A 23-acetate (**2**)	5	21.4±2.2**	53.9±5.6**	99.6±3.8**

Each value represents the mean with S.E. (** $p<0.01$).

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