Crude Drugs from Aquatic Plants. IV.[1] On the Constituents of Alismatis Rhizoma. (2). Stereostructures of Bioactive Sesquiterpenes, Alismol, Alismoxide, Orientalols A, B, and C, from Chinese Alismatis Rhizoma

Masayuki YOSHIKAWA,* Shoko YAMAGUCHI, Hisashi MATSUDA, Yuko KOHDA, Hifumi ISHIKAWA, Nobumitsu TANAKA, Johji YAMAHARA, and Nobutoshi MURAKAMI

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan.
Received April 11, 1994; accepted May 13, 1994

Following the characterization of the triterpene constituents in Chinese Alismatis Rhizoma, we investigated the chemical structures of orientalols A, B, and C, isolated from the less polar fraction of the crude drug together with two known sesquiterpenes, alismol and alismoxide. On the basis of the chemical and physicochemical evidence, the structures of orientalols A, B, and C have been determined and those of alismol and alismoxide were revised.

All five sesquiterpenes were found to show an inhibitory effect on the contraction of isolated bladder smooth muscle induced by carbachol.

Keywords orientalol; alismol; alismoxide; Alismatis Rhizoma; *Alisma orientale*; Alismataceae

In the previous paper,[2] we reported the isolation of four protostane-type triterpenes, alisols E 23-acetate, F, and G, 13,17-epoxyalisol A, and five guaiane-type sesquiterpenes, orientalols A, B, and C, alismol, and alismoxide, from the less polar fraction of Chinese Alismatis Rhizoma [the dried rhizome of the aquatic plant *Alisma orientale* JUZEP. collected in Szechwan Province, China (Sentaku in Japanese)]. We have so far described the elucidation of the absolute stereostructures of the triterpene constituents, namely alisols E 23-acetate, F, and G and 13,17-epoxyalisol A. As a continuation of this work, we now present a full account of the structure elucidation of the remaining three sesquiterpenes, *i.e.* orientalols A (**1**), B (**2**), and C (**3**), and the structure revisions of alismol and alismoxide from **4'**[3] and **5'**[3] to **4** and **5**, respectively. All five sesquiterpenes (**1**—**5**) were found to show an inhibitory effect on the contraction of bladder smooth muscle induced by carbachol.[4]

Structures of Orientalols A (1) and B (2) Orientalol A (**1**) was obtained as a colorless oil and showed no significant optical activity. The molecula formula, $C_{15}H_{26}O_3$, of **1** was obtained from the quasimolecular ion peak $(M+Na)^+$ at *m/z* 277 in the positive FAB-MS and by high-resolution MS measurement. The IR spectrum of **1** suggested the presence of a hydroxyl group. The ^{1}H-NMR and ^{13}C-NMR spectra of **1** showed the presence of hydroxymethyl [δ 3.64, 3.80 (ABq, $J=11$ Hz, 14-H_2), δ_C 63.7], isopropyl [δ 1.00, 1.01 (3H each, both d, $J=7$ Hz, 12, 13-H_3), 2.30 (m, 11-H), δ_C 22.6, 22.7, 39.1], tertiary methyl [δ 1.11 (3H, s, 15-H_3), δ_C 22.2], trisubstituted olefin [δ 5.54 (br s, 6-H), δ_C 123.5, 150.4] and two tertiary hydroxyl (δ_C 77.3, 81.2) groups, together with four methylenes and two methines. These spectral properties led us to presume that **1** is a guaiane-type sesquiterpene analogue co-occuring along with alismol and alismoxide.

Acetylation of **1** with acetic anhydride (Ac_2O) in pyridine afforded the monoacetate (**1a**) which showed hydroxyl absorption in its IR spectrum. Comparison of the ^{13}C-NMR data for **1a** with those for **1** showed an acetylation shift around the C-14 position of **1a**. Oxidation of **1** with sodium metaperiodate ($NaIO_4$) gave the ketone (**6**),[5] which indicated the presence of an *exo*-vicinal diol moiety at C-10 and C-14 in **1**. In the NOESY spectrum of **1**, NOE correlations were observed between the signals

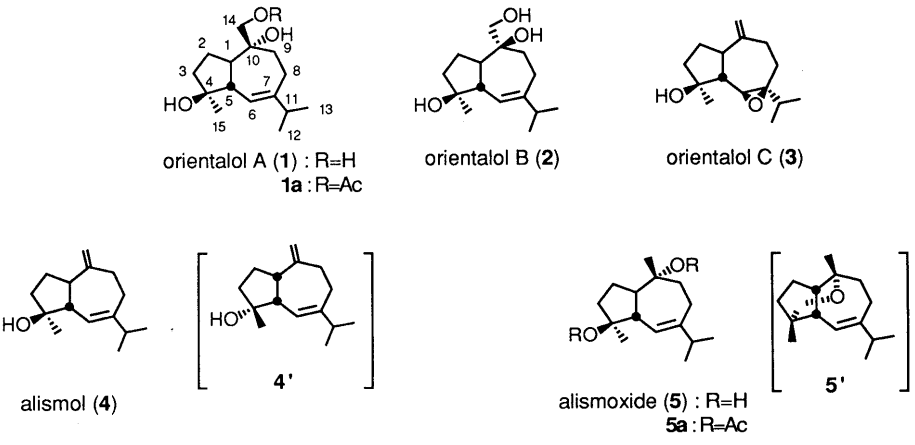
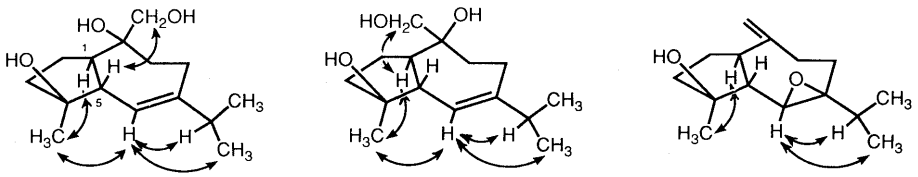


Chart 1



1 2 3

Fig. 1. NOE Enhancements of **1**, **2**, **3**

of the 1-proton and 15-protons and between those of the 5-proton and 14-protons (Fig. 1). Based on the above-mentioned evidence and comparison of the ^{1}H- and ^{13}C-NMR data for **1**, **1a**, and **6** with those for various known guaiane-type sesquiterpenes and their derivatives,[6,7] the relative stereostructure of orientalol A was concluded to be the 1,5-*trans*-guaiane structure **1**.

Orientalol B (**2**), obtained as a colorless oil, also showed no optical activity. The molecular formula, $C_{15}H_{26}O_3$, was identical with that of orientalol A (**1**) as determined by high MS measurement. The ^{1}H- and ^{13}C-NMR spectra of **2** showed signals assignable to the same functional groups as those of **1**. In particular, the carbon signals in the ^{13}C-NMR spectrum of **2** were superimposable on those of **1**, except for the signals assignable to C-1 and C-14, so that **1** and **2** were presumed to be epimeric isomers at the C-10 position. Furthermore, observation of NOEs (Fig. 1) in the following pairs of protons (1-H and 15-H_3; 1-H and 14-H_2) of **2** confirmed the above presumption and the structure of orientalol B was characterized as **2**.

Finally, oxidation of the sesquiterpene (**4**), which has been isolated from various soft corals[7] and shown to be identical with alismol (*vide infra*), with osmium tetroxide (OsO_4) provided **1** and **2** in a 1 : 1 ratio. Consequently, the stereostructures of orientalols A (**1**) and B (**2**) were determined to be as shown.

Previously, we proposed that alismol (**4**) and alismoxide (**5**), which were isolated from Alismatis Rhizoma, were mostly secondary products formed from germacrene C in the fresh rhizome of *Alisma orientale* during processing.[5] Orientalols A (**1**) and B (**2**) showed no optical activity and they were easily derived from alismol (**4**). Based on this evidence, **1** and **2** were also considered to be produced from alismol (**4**).

Structure of Orientalol C (3) Orientalol C (**3**) was obtained as a colorless oil and its IR spectrum showed a hydroxyl absorption band. The molecular formula $C_{15}H_{24}O_2$ was obtained from the ion peak $(M+H-H_2O)^+$ observed in the positive FAB-MS and by high MS measurement. The ^{1}H-NMR spectrum of **3** showed signals due to oxymethine protons at δ 2.91 (d, $J=7$ Hz, 6-H), one tertiary methyl on a carbon having a hydroxyl group at δ 1.44 (s, 15-H_3), and two secondary methyls in an isopropyl group at δ 0.97 and 1.00 (both d, $J=7$ Hz, 12, 13-H_3). Comparison of the ^{13}C-NMR data for **3** with those of **1**, **2**, and known guaiane-type sesquiterpenes[6,7] led us to presume the plain structure of orientalol C (**3**). The relative stereostructure of **3** was clarified by NOE experiments as depicted in Fig. 1 and also by comparison of the ^{1}H–^{1}H coupling constants with those reported for related sesquiterpenes.[6,7]

Finally, epoxidation of the known sesquiterpene (**4**) from soft coral[7] with *m*-chloroperbenzoic acid (*m*-CPBA) furnished **3** and the α-epoxide (**7**) in a 1 : 1 ratio. Based on the above-mentioned evidence, the relative structure of orientalol C (**3**) was determined to be as shown. Since orientalol C (**3**) showed a little optical activity, $[\alpha]_D$ +2.5°, a part of **3** may be biosynthesized in the plant.

Revised Structures of Alismol (4) and Alismoxide (5) As described above, all orientalols (**1**—**3**) were found to have the 1,5-*trans* guaiane skeleton. This led us to reinvestigate the 1,5-*cis* guaiane structure (**4′**, **5′**) in alismol and alismoxide. The physicochemical properties of alismol and alismoxide isolated by us in the present investigation were found to be the same as those reported previously[3] except for their specific rotations and the IR data of alismoxide.[8] Since the 1,5-*cis* guaiane structure (**4′**) of alismol was presumed on the basis of chemical transformation to alismoxide having a 4,10-oxide ring in the structure (**5′**),[3] we began with a reinvestigation of the structure of alismoxide.

The EI-MS and FAB-MS of alismoxide showed the ion peak at *m/z* 220 ($C_{15}H_{24}O$) which was previously interpreted as the molecular ion peak for the structure **5′** of alismoxide. On the other hand, the secondary ion mass spectrometry (SIMS) spectrum of alismoxide showed the quasimolecular ion peak at *m/z* 261 [$(M+Na)^+$, $C_{15}H_{26}O_2Na$], whose elemental composition was determined by high MS measurement. In addition, a hydroxy absorption band was observed at 3325 cm^{-1} (Nujol) in the IR spectrum. These findings indicated the presence of a diol moiety instead of an oxide ring in the structure of alismoxide. Furthermore, acetylation of alismoxide with Ac_2O in pyridine in the presence of 4-dimethylaminopyridine (DMAP) afforded the diacetate (**5a**). Comparison of the ^{13}C-NMR data for **5a** with those for **5** showed acetylation shifts around the 4- and 10-positions. Detailed examination of the ^{1}H-NMR data of **5** led us to formulate the revised structure of alismoxide (**5**) and consequently, the structure of alismol (**4**) was also clarified. Finally, alismol and alismoxide were identical with the sesquiterpenes (**4**, **5**) which were isolated from Australian soft corals *Nephthea chabrolii* and *Lemnalia africana*[7a] and Okinawan soft coral *Xenia* sp.,[7b] so that their structures were revised from **4′** and **5′** to **4** and **5**.

Inhibitory Effect of Orientalols (1—3), Alismol (4), and Alismoxide (5) on the Carbachol-Induced Contraction of Isolated Bladder Smooth Muscle of Guinea Pig Alismatis Rhizoma is an important component of various Chinese traditional preparations such as Hachimi-Jio-Gan, which

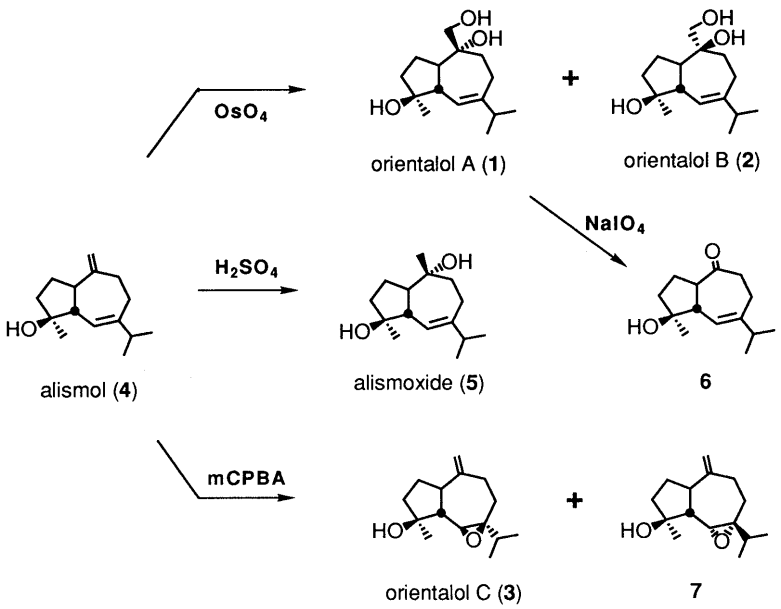


Chart 2

TABLE I. ^{13}C-NMR Data for **1**, **1a**, **2**, **3**, **4**, **5**, **5a**, **6**, and **7**

	1[a]	**1a**[a]	**2**[a]	**3**[b]	**4**[a]	**4**[c]	**5**[b]	**5a**[b]	**6**[b]	**7**[b]
C-1	50.9	51.1	46.6	47.9	55.8	55.3	50.5	48.3	53.1	42.7
C-2	22.5	22.2	22.2	26.3	25.7	25.3	21.5	22.4	19.1	25.5
C-3	41.5	41.4	41.4	41.5	40.7	40.6	40.4	36.5	39.4	40.3
C-4	81.2	81.1	81.5	80.3	81.0	79.6	80.1	88.9	80.5	81.0
C-5	50.6	50.7	49.3	57.5	48.9	47.3	50.2	46.9	51.9	55.7
C-6	123.5	123.4	124.0	62.3	123.2	123.8	121.3	121.0	120.6	61.2
C-7	150.4	150.4	151.6	65.1	150.3	148.6	149.5	149.3	148.6	65.1
C-8	25.9	25.7	26.1	29.4	31.0	30.3	25.0	24.6	25.7	27.1
C-9	38.4	38.6	37.3	34.1	38.2	37.5	42.5	36.9	42.2	33.9
C-10	77.3	76.2	77.0	151.7	155.2	154.4	75.2	87.5	211.9	152.4
C-11	39.1	39.1	39.0	34.6	38.7	37.7	37.2	37.3	37.4	37.1
C-12	22.6[d]	22.6[d]	22.5[d]	17.8[d]	21.7[d]	21.5[d]	21.3[d]	21.1[d]	21.2[d]	17.4[d]
C-13	22.7[d]	22.7[d]	22.7[d]	18.8[d]	22.0[d]	21.7[d]	21.4[d]	21.4[d]	21.5[d]	18.0[d]
C-14	63.7	68.5	70.0	107.4	107.0	106.7	21.1	19.0		107.5
C-15	22.2	22.2	23.2	25.0	24.1	24.6	22.4	19.4	22.1	24.6

a—*c*) The spectra were taken in CD_3OD,[a] $CDCl_3$,[b] or d_5-pyridine[c] at 67.5 MHz. *d*) The assignments may be interchangeable within the same column.

TABLE II. Inhibitory Effect of Sesquiterpenes (**1**—**5**) from Alismatis Rhizoma on Contractile Response of Guinea Pig Bladder Induced by Carbachol

Compound	Concentration	Contractile response (%) CCh (3×10^{-7} M)
Control	0.1% EtOH	100.0±8.1
Orientalol A (**1**)	10^{-4} M	44.3±4.0[a]
Orientalol B (**2**)	10^{-4} M	39.4±0.9[a]
Orientalol C (**3**)	10^{-4} M	52.1±3.7[a]
Alismol (**4**)	10^{-4} M	24.6±7.5[a]
Alismoxide (**5**)	10^{-4} M	69.9±8.0[a]
Atropine	10^{-7} M	0.0±0.0[a]

Each value represents the mean±S.E. of 5 or 6 experiments. *a*) $p<0.01$.

has been used to treat obstruction of micturition and is believed to have diuretic, anti-diabetic and antiflammatory effects. Recently, triterpene constituents of this natural medicine were tested for diuretic activity and, among them, alisol A monoacetate and alisol B were found to have diuretic activity.[9] In this study, we have investigated the effect of sesquiterpenes (**1**—**5**) from Alismatis Rhizoma on the contraction of isolated bladder smooth muscle of guinea pig induced by carbachol.

As summarized in Table II, orientalols A (**1**), B (**2**), and C (**3**), alismol (**4**) and alismoxide (**5**) all inhibited contraction of the isolated bladder smooth muscle. This action may be beneficial to micturition disorders.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[2]

Isolation of Orientalols A (1), B (2), and C (3), Alismol (4), and Alismoxide (5) from the Less Polar Fraction of Chinese Alismatis Rhizoma The isolation procedure for the five sesquiterpenes was described in the previous paper.[2]

Orientalol A (**1**): A colorless oil, $[\alpha]_D^{22}$ ±0° (*c*=0.8, MeOH). High-resolution FAB-MS: Calcd for $C_{15}H_{26}O_3Na$ $(M+Na)^+$, 277.1779. Found, 277.1799. IR (KBr): 3600, 1640, 1460, 1040 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.00, 1.01 (3H each, both d, J=7 Hz, 12,13-H_3), 1.11 (3H, s, 15-H_3), 2.02 (1H, m, 1-H), 2.30 (1H, m, 11-H), 2.40 (1H, d, J=12 Hz,

5-H), 3.64, 3.80 (2H, ABq, $J=11$ Hz, 14-H_2), 5.54 (1H, br s, 6-H). ^{13}C-NMR (CD_3OD) δ_C: see Table I. Positive FAB-MS (*m/z*): 277 $(M+Na)^+$, 261 $(M+Li)^+$.

Orientalol B (**2**): A colorless oil, $[\alpha]_D^{22}$ $\pm 0°$ ($c=0.8$, MeOH). High-resolution FAB-MS: Calcd for $C_{15}H_{23}O$ $(M+H-2H_2O)^+$, 219.1749. Found, 219.1710. IR (KBr): 3550, 1460, 1045 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.99, 1.00 (3H each, both d, $J=7$ Hz, 12,13-H_3), 1.14 (3H, s, 15-H_3), 1.99 (1H, m, 1-H), 2.29 (1H, m, 11-H), 2.73 (1H, br d, $J=10$ Hz, 5-H), 3.28, 3.40 (2H, ABq, $J=11$ Hz, 14-H_2), 5.55 (1H, br s, 6-H). ^{13}C-NMR (CD_3OD) δ_C: see Table I. Positive FAB-MS (*m/z*): 277 $(M+Na)^+$, 261 $(M+Li)^+$, 219 $(M+H-2H_2O)^+$.

Orientalol C (**3**): A colorless oil, $[\alpha]_D^{22}$ $+2.5°$ ($c=0.6$, MeOH). High-resolution FAB-MS: Calcd for $C_{15}H_{23}O$ $(M+H-H_2O)^+$, 219.1748. Found, 219.1740. IR (KBr): 3445, 1470, 1050, 900 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.97, 1.00 (3H each, both d, $J=7$ Hz, 12,13-H_3), 1.44 (3H, s, 15-H_3), 2.91 (1H, d, $J=7$ Hz, 6-H), 4.71, 4.73 (1H each, both s, 14-H_2). ^{13}C-NMR ($CDCl_3$, δ_C: see Table I. Positive FAB-MS (*m/z*): 219 $(M+H-H_2O)^+$.

Acetylation of Orientalol A (1) A solution of **1** (15.8 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and the whole mixture was stirred at room temperature under an N_2 atmosphere for 6 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product which was purified by silica gel column chromatography [benzene–acetone (5 : 1)] to furnish **1a** (10.8 mg).

1a: A colorless oil, $[\alpha]_D^{22}$ $\pm 0°$ ($c=0.4$, MeOH). High-resolution FAB-MS : Calcd for $C_{17}H_{25}O_2$ $(M+H-2H_2O)^+$, 261.1856. Found, 261.1862. IR (KBr): 3400, 1740, 1460, 1040 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.00, 1.01 (3H each, both d, $J=7$ Hz, 12,13-H_3), 1.12 (3H, s, 15-H_3), 2.09 (3H, s, $COCH_3$), 2.41 (1H, br d, $J=11$ Hz, 5-H), 4.17, 4.37 (2H, ABq, $J=11$ Hz, 14-H_2), 5.54 (1H, br s, 6-H). ^{13}C-NMR (CD_3OD) δ_C: 21.3, 173.5 (Ac) and other signals as given in Table I. Positive FAB-MS (*m/z*): 261 $(M+H-2H_2O)^+$.

Oxidation of 1 with $NaIO_4$ A solution of **1** (10 mg) in [THF–H_2O (5 : 8, 1 ml)] was treated with $NaIO_4$ (20 mg) and the whole mixture was stirred at 20 °C for 36 h. The reaction mixture was poured into aqueous saturated Na_2SO_3 and the whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the product was purified by silica gel column chromatography [benzene–acetone (50 : 1)] to furnish **6** (9.1 mg).

The ketone (**6**) thus obtained was shown to be identical with an authentic sample[5] on the basis of ^{1}H-NMR ($CDCl_3$), ^{13}C-NMR ($CDCl_3$) and TLC comparisons.

Oxidation of 4 with OsO_4 A solution of **4** (100 mg) in [CH_3CN–H_2O (2 : 1, 3 ml)] was treated with OsO_4 (5 mg) in the presence of 4-methylmorpholine-*N*-oxide (120 mg) and the whole mixture was stirred at 60 °C for 16 h. The reaction mixture was poured into aqueous saturated Na_2SO_3 and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 1% aqueous HCl and brine, and dried over $MgSO_4$. After removal of the solvent from the AcOEt extract under reduced pressure, the product was purified by silica gel column chromatography [*n*-hexane–acetone (3 : 2)] and reversed-phase (ODS) HPLC to furnish orientalols A (**1**, 9.1 mg) and B (**2**, 7.9 mg).

Orientalols A (**1**) and B (**2**) thus obtained was shown to be identical with authentic samples isolated from Chinese Alismatis Rhizoma, by ^{1}H-NMR (CD_3OD), ^{13}C-NMR (CD_3OD) and TLC comparisons.

Oxidation of 4 with *m*-CPBA A solution of **4** (94 mg) in CH_2Cl_2 (10 ml) was treated with *m*-CPBA (77.7 mg) and the whole mixture was stirred at 0 °C under an N_2 atmosphere for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Work-up of $CHCl_3$ extract in the usual manner gave a product, which was purified by silica gel column chromatography [benzene–acetone (2 : 1)] to furnish orientalols C (**3**, 56 mg), and **7** (11 mg). Orientalol C (**3**) thus obtained was shown to be identical with authentic **3**, which was the natural product, by ^{1}H-NMR ($CDCl_3$), ^{13}C-NMR ($CDCl_3$) and TLC comparisons.

7: A colorless oil, $[\alpha]_D^{22}$ $+10.5°$ ($c=0.6$, MeOH). High-resolution FAB-MS: Calcd for $C_{15}H_{23}O$ $(M+H-H_2O)^+$, 219.1733. Found, 219.1661. IR (KBr): 3330, 1360, 1100, 890 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.92, 1.00 (3H each, both d, $J=7$ Hz, 12,13-H_3), 1.38 (3H, s, 15-H_3), 3.03 (1H, s, 6-H), 4.70, 4.73 (1H each, both s, 14-H_2). ^{13}C-NMR (CD_3OD) δ_C: see Table I. Positive FAB-MS (*m/z*): 219 $(M+H-H_2O)^+$.

Acetylation of Alismoxide (5) A solution of **5** (64 mg) in pyridine (2 ml) was treated with Ac_2O (2 ml) in the presence of DMAP (15 mg), and the whole mixture was stirred at room temperature under an N_2 atmosphere for 12 h. The reaction mixture was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product which was purified by silica gel column chromatography [benzene–AcOEt (5 : 1)] to furnish **5a** (31.7 mg).

5a: A colorless oil, $[\alpha]_D^{25}$ $\pm 0°$ ($c=0.5$, $CHCl_3$). IR (film): 2900, 1730, 1470 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.98, 0.99 (3H each, both d, $J=7$ Hz, 12,13-H_3), 1.41 (3H, s, 15-H_3), 1.53 (3H, s, 14-H_3), 1.96, 2.00 (3H each, both s, $COCH_3$), 5.49 (1H, d, $J=3$ Hz). ^{13}C-NMR (CD_3OD) δ_C: 22.1, 22.6, 170.4, 170.5 (Ac × 2) and other signals as given in Table I. Positive SIMS (*m/z*): 345 $(M+Na)^+$.

Recording of Inhibitory Effects on Carbachol-Induced Contraction Male Hartley guinea pigs (Kiwa Laboratory Animals Ltd., Wakayama, Japan) weighing about 300 g were used. Guinea pigs were killed with a blow on the back of the head and bled by severing both carotid arteries. The bladder was removed, then the surrounding connective tissue and the trigone and constrictor were cleaned off. The bladder preparations were cut into strips 10 mm long and 2 to 3 mm wide longitudinally.

Each preparation was mounted in a tissue bath containing Krebs-Henselite solution composed of 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 10 mM glucose. The solution was kept at 37 °C and gassed continuously with 5% CO_2-containing oxygen. The initial loading tension was set at 1 g. Contractile responses were detected isometrically with a force displacement transducer (Type 45196 A, NEC San-ei Instruments Ltd., Tokyo) and recorded on a polygraph (360 System, NEC Sai-ei Instruments Ltd.). After stabilization, carbachol (CCh, Sigma Chemical Company) was added to the bath at 3×10^{-7} M to obtain contractile responses and then the tissues were washed with the medium 3 times. Tissues were recontracted with CCh (3×10^{-7} M), and the response of each preparation was used as the reference for the following contraction of the corresponding tissues. The tissues were washed 3 times and stabilized, and then a test compound was added. After ten minutes, contractile responses induced by CCh (3×10^{-7} M) were recorded in the presence of test compounds. Each contractile response was expressed as a percentage of the control.

Test compounds and CCh were dissolved in ethanol and water, respectively, in the experiment. The final concentration of ethanol in the medium did not exceed 0.1% and had no effect on muscle contraction. Atropine sulfate (Wako Pure Chemical Industries Ltd.) was used as a reference drug. Statistical analysis was performed by the use of Student's or Welch's *t* test. Results are expressed as the mean ±S.E.

References and Notes

1) Part III : M. Yoshikawa, S. Yamaguchi, N. Chatani, Y. Nishino, T. Matsuoka, J. Yamahara, N. Murakami, H. Matsuda, M. Kubo, *Yakugaku Zasshi*, **114**, 241 (1994).
2) M. Yoshikawa, S. Hatakeyama, N. Tanaka, Y. Fukuda, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.*, **41**, 1948 (1993).
3) Y. Oshima, T. Iwakawa, H. Hikino, *Phytochemistry*, **22**, 183 (1983).
4) *a*) This work was reported in part in our preliminary communication; *b*) M. Yoshikawa, S. Hatakeyama, N. Tanaka, Y. Fukuda, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.*, **40**, 2582 (1992).
5) M. Yoshikawa, S. Hatakeyama, N. Tanaka, T. Matsuoka, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.*, **41**, 2109 (1993).
6) *a*) R. F. X. Bauer, I. A. Khan, H. Lotter, H. Wagner, *Helv. Chim. Acta*, **68**, 2355 (1985); *b*) M. Kuroyanagi, A. Ueno, K. Ujiie, S. Sato, *Chem. Pharm. Bull.*, **35**, 53 (1987).
7) *a*) B. F. Bowden, T. Iwakawa, J. Mitchell, *Aust. J. Chem.*, **33**, 1833 (1980); *b*) I. Kitagawa, M. Kobayashi, Z. Cui, Y. Kiyota, M. Ohnishi, *Chem. Pharm. Bull.*, **34**, 4590 (1986).
8) Previously, alismol and alismoxide were reported to have optical activities of $[\alpha]_D$ $+8.7°$ ($CHCl_3$) and $[\alpha]_D$ $+3.1°$ ($CHCl_3$), respectively.[3] In this investigation, we have found that alismol (**4**) and alismoxide (**5**) isolated from Chinese Alismatis Rhizoma showed no significant optical activity.
9) H. Hikino, T. Iwakawa, Y. Oshima, K. Nishikawa, T. Murata, *Syoyakugaku Zasshi*, **36**, 150 (1982).