Crude Drugs from Aquatic Plants. V.[1] On the Constituents of Alismatis Rhizoma. (3). Stereostructures of Water-Soluble Bioactive Sesquiterpenes, Sulfoorientalols a, b, c, and d, from Chinese Alismatis Rhizoma

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From the water-soluble portion of Chinese Alismatis Rhizoma, four bioactive sesquiterpenes, sulfoorientalols a, b, c, and d, were isolated and their structures having a sulfonic acid function were established on the basis of chemical and physicochemical evidence. In addition, two sulfoorientalol congeners were derived from alismol by sulfonation of the *exo*-methylene moiety. Sulfoorientalols and the synthetic congeners were found to inhibit the carbachol-induced contraction of isolated bladder smooth muscle of guinea pig.

Keywords sulfoorientalol; Alismatis Rhizoma; *Alisma orientale*; aquatic plant; water-soluble bioactive sesquiterpene; bladder smooth muscle contraction inhibitor

As a part of our chemical studies on the bioactive constituents of natural medicines originating from aquatic plants,[2] we have reported the isolation of various then-new constituents, namely four protostane-type triterpenes,[3] alisols E 23-acetate, F, and G, 13,17-epoxyalisol A and three guaiane-type sesquiterpenes,[1,4] orientalols A (**5**), B (**6**), and C (**7**), together with alismol (**12**) and alismoxide (**8**) from the lipophilic portion of Chinese Alismatis Rhizoma and have elucidated their chemical structures including structural revision of alismol (**12**) and alismoxide (**8**). In a continuation of these studies, we have isolated four water-soluble bioactive sesquiterpenes designated sulfoorientalols a (**1**), b (**2**), c (**3**), and d (**4**) from the polar fraction of the same Chinese Alismatis Rhizoma, the dried rhizome of *Alisma orientale* JUZEP., collected in Szechwan Province, China (Sentaku in Japanese).

In this paper, we present a full account of the structure elucidation of sulfoorientalols a (**1**), b (**2**), c (**3**), and d (**4**) and also describe syntheses of two sulfoorientalol congeners (**13**, **14**) from alismol (**12**)[1,4] which is a major sesquiterpene constituent in Chinese Alismatis Rhizoma. In addition, the inhibitory activity of sulfoorientalols (**1**—**4**) and the synthetic congeners (**9**, **13**, **14**) on the carbachol-induced contraction of isolated bladder smooth muscle is reported.[5]

The methanolic extract of Chinese Alismatis Rhizoma was partitioned into a mixture of ethyl acetate and water to furnish the ethyl acetate-soluble portion and the water-soluble portion as described in previous papers.[1,3] The water-soluble portion was extracted with 1-butanol and the 1-butanol-soluble portion was subjected to ordinary silica gel column chromatography to provide six fractions. Each fraction was further separated by ordinary and reversed-phase silica gel column chromatography finally to afford sulfoorientalols a (**1**), b (**2**), c (**3**), and d (**4**), daucosterin,[6] uridine, tymidine, and methyl β-D-fructofuranoside.[7]

Sulfoorientalols a (1) and d (4) Sulfoorientalol a (**1**) was obtained as a white powder and showed no optical activity. The infrared (IR) spectrum of **1** showed absorption bands due to hydroxyl (3350 cm^{-1}), olefin (1640 cm^{-1}), and sulfonic acid (1385, 1210 cm^{-1}) functions. The molecular formula $C_{15}H_{26}O_4S$ of **1** was confirmed from the quasimolecular ion peak (M−H+

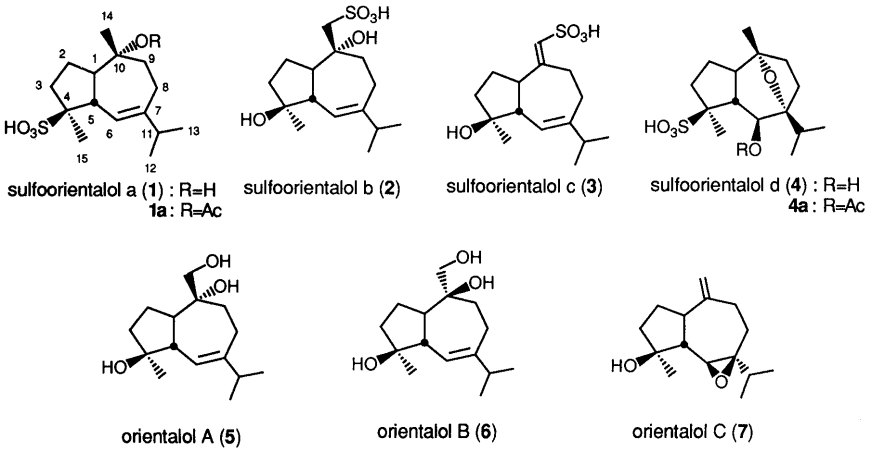

Chart 1

$2Na)^+$ at m/z 347 in the positive fast atom bombardment mass (positive FAB-MS) and high-resolution MS measurement. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Table I) of **1** showed signals due to a trisubstituted olefin proton [δ 5.75 (d, J=3 Hz, 6-H)], and two angular methine protons [δ 2.09 (ddd, J=6.7, 10.4, 10.4 Hz, 1-H), 2.83 (br d, J=*ca.* 11 Hz, 5-H)] together with two tertiary methyl groups, an isopropyl group, and four methylenes. The ^{13}C-NMR spectrum of **1** showed the presence of a quaternary carbon bearing a hydroxyl group (δ_C 75.8, C-10) and a quaternary carbon bearing a sulfonic acid group (δ_C 67.4, C-4) together with the above-mentioned functions, as shown in Table II. The ^{1}H- and ^{13}C-NMR spectra of **1** was assigned with the aid of homo and hetero correlation spectroscopy (^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY), distortionless enhancement by polarization transfer (DEPT) and heteronuclear multiple bond correlation (HMBC) experiments. The connectivities of the quaternary carbons (C-4, 7, 10) and the tertiary methyl groups were clarified by the following HMBC experiment. Namely, long-range correlations were observed between the following carbons and protons of **1** [C-1 and 6-H, 14-H; C-3 and 15-H; C-4 and 3-H, 6-H, 15-H; C-7 and 8-H, 9-H, 11-H; C-9 and 14-H; C-10 and 1-H, 2-H, 8-H, 9-H, 14-H]. Examination of these spectral properties including a detailed comparison of the ^{1}H–^{1}H coupling constants with those reported for related guaiane-type sesquiterpenes[1,9,10] led us to presume the chemical structure of sulfoorientalol a (**1**) having a sulfonic acid group at the C-4 position.

Furthermore, comparison of the ^{13}C-NMR data (Table I) for the monoacetate (**1a**), which was obtained from **1** by acetylation with acetic anhydride in pyridine in the presence of dimethylaminopyridine (DMAP), with those for **1** showed an acetylation shift[8] around the C-10 position of **1a**, so that the location of the sulfonic acid group was substantiated to be the C-4 position of **1**. Finally, the stereostructure of **1** was confirmed by the ^{1}H-NMR nuclear Overhauser effect spectroscopy (NOESY) spectrum, which showed nuclear Overhauser effect (NOE) correlations between the signals of following proton pairs (1-H and 15-H_3; 15-H_3 and 6-H; 5-H and 14-H_3), as depicted in Fig. 1. Based on the accumulated evidence, the stereostructure of sulfoorientalol a (**1**) has been determined to be as shown.

Sulfoorientalol d (**4**), obtained as a white powder, also

TABLE I. ^{1}H-NMR Data for **1**—**4**[a]

		1	2	3	4
1-H		2.09 (1H, ddd, J=6.7, 10.4, 10.4)	2.04 (m)[b]	2.61 (1H, m)	1.63 (1H, m)
2-H	α	1.80 (2H, m)	1.72 (m)[c]	1.80 (1H, m)	1.49 (1H, m)
	β		1.60 (m)[d]	1.73 (1H, m)	1.62 (1H, m)
3-H	α	1.62 (1H, ddd, J=3.7, 7.9, 12.5)	1.60 (m)[d]	1.71 (1H, m)	1.30 (1H, m)
	β	2.20 (1H, m)[e]	1.72 (m)[c]	1.77 (1H, m)	2.46 (1H, dd, J=9.4, 13.9)
5-H		2.83 (1H, br d, J=*ca.* 11)	2.24 (m)[f]	2.34 (1H, br d, J=*ca.* 11)	2.20 (1H, dd, J=10.2, 12.1)
6-H		5.75 (1H, d, J=3)	5.55 (1H, d, J=2)	5.56 (1H, d, J=1)	3.69 (1H, d, J=10.2)
8-H	α	2.16 (1H, m)	2.37 (1H, dd, J=9.2, 14.0)	2.10 (1H, dd, J=7.8, 16.0)	1.43 (1H, m)
	β	1.94 (1H, dd, J=10.4, 16.2)	2.04 (m)[b]	2.39 (1H, m)	2.00 (1H, ddd, J=5.2, 10.1, 15.3)
9-H	α	1.51 (1H, dd, J=10.4, 12.5)	1.72 (m)[c]	3.00 (1H, dd, J=10.4, 15.3)	1.71 (1H, ddd, J=3.7, 12.8, 12.8)
	β	1.73 (1H, m)	2.24 (m)[f]	2.81 (1H, dd, J=7.8, 15.3)	1.87 (1H, ddd, J=4.0, 10.1, 12.8)
11-H		2.20 (1H, m)[e]	2.24 (m)[f]	2.26 (1H, qq, J=7.7)	1.91 (1H, qq, J=7.7)
12-H		0.97 (3H, d, J=7.7)[g]	0.99 (3H, d, J=7.7)[g]	1.01 (3H, d, J=7.7)[g]	0.99 (3H, d, J=7.7)[g]
13-H		0.99 (3H, d, J=7.7)[g]	1.00 (3H, d, J=7.7)[g]	1.02 (3H, d, J=7.7)[g]	1.02 (3H, d, J=7.7)[g]
14-H		1.23 (3H, s)	3.16, 3.36 (2H, ABq, J=14.0)	6.21 (1H, s)	1.23 (3H, s)
15-H		1.35 (3H, s)	1.14 (3H, s)	1.17 (3H, s)	1.44 (3H, s)

a) The spectra were taken in CD_3OD at 500 MHz. *b*—*f*) Completely overlapped with other signals. *g*) Assignments may be interchanged within the same column.

TABLE II. ^{13}C-NMR Data for **1**, **1a**, **2**, **3**, **4**, **4a**, **9**, **10**, **13**, and **14**[a]

	1	1a	2	3	4	4a	9	10	13	14
C-1	53.5	51.8	51.4	49.2	53.0	53.1	53.0	50.1	46.0	143.7
C-2	23.9	24.2	22.2	26.6	26.8	26.1	25.5	23.4	27.3	29.8
C-3	36.8	36.8	40.0	41.1	36.9	38.0	42.1	37.6	40.0	40.5
C-4	67.4	67.3	80.7	81.9	65.6	65.5	80.0	68.3[b]	80.0	81.7
C-5	45.9	45.2	50.0	57.3	50.0	50.1	56.3	46.7	54.1	55.0
C-6	124.8	124.7	122.8	123.0	71.8	72.7	73.0	64.2	122.5	121.3
C-7	148.8	149.0	150.8	150.7	84.0	83.8	84.8	67.5[b]	151.7	150.1
C-8	25.5	25.3	26.0	29.7	30.2	30.9	30.7	25.8	28.5	30.1
C-9	43.4	37.8	41.0	32.5	32.6	32.1	33.2	41.3	32.8	33.9
C-10	75.8	90.7	76.4	156.9	88.0	87.5	88.7	75.5	45.9	124.7
C-11	38.6	38.6	38.6	39.7	32.7	32.5	33.7	39.3	38.4	39.2
C-12	21.6[c]	21.7[c]	22.0[c]	22.5[c]	17.4[c]	17.5[c]	18.1[c]	19.0[c]	21.6[c]	22.6[c]
C-13	21.9[c]	21.9[c]	22.2[c]	22.7[c]	18.4[c]	18.5[c]	19.1[c]	21.0[c]	21.8[c]	22.8[c]
C-14	21.6	19.7	54.0	127.0	24.3	24.3	25.6	20.9	56.9	57.1
C-15	17.8	18.1	21.7	25.0	20.1	20.8	24.5	18.4	22.7	23.2

a) The spectra were taken in CD_3OD. *b*, *c*) Assignments may be interchanged within the same column.

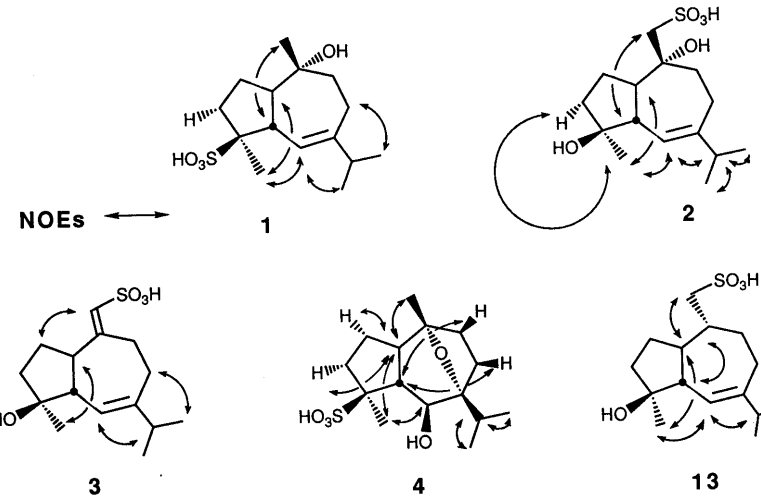

Fig. 1

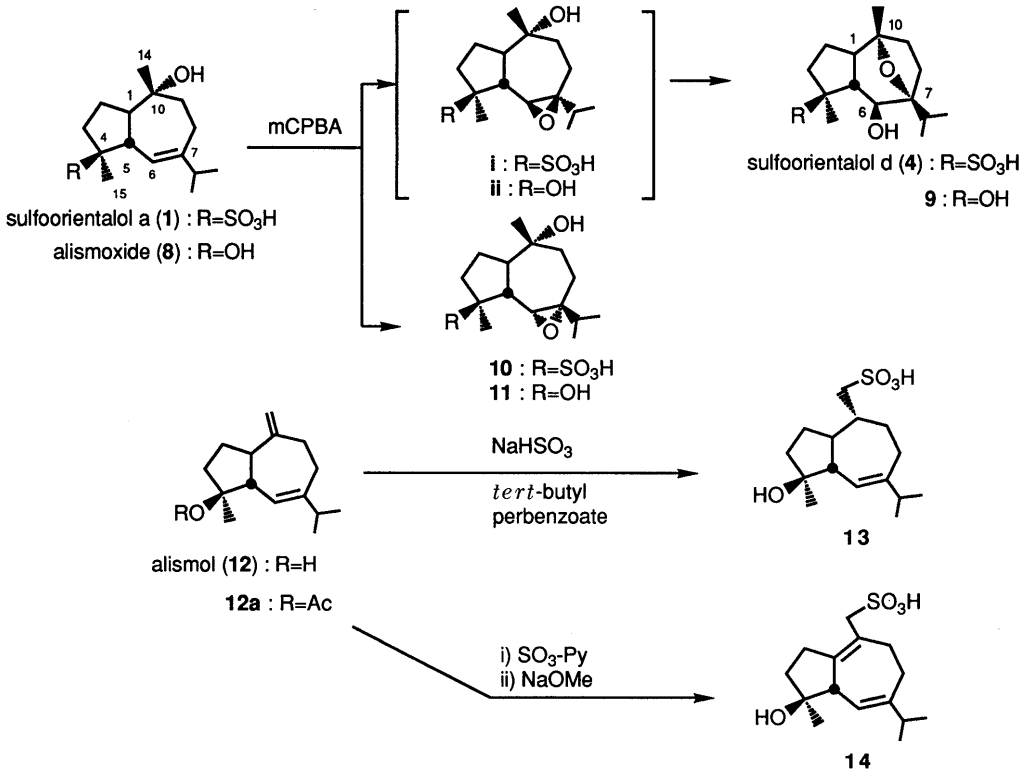

Chart 2

showed no optical activity. The molecular formula $C_{15}H_{26}O_5S$ was determined from the quasimolecular ion peak $(M-H+2Na)^+$ in the positive FAB-MS and by high-resolution FAB-MS measurement. The ^{1}H-NMR spectrum (Table I) of **4** showed signals due to a hydroxyl-bearing methine proton [δ 3.69 (d, $J=10.2$ Hz, 6-H)] and two angular methine protons [δ 1.63 (m, 1-H), 2.20 (dd, $J=10.2$, 12.1 Hz, 5-H)] together with two tertiary methyls and an isopropyl group. The ^{13}C-NMR spectrum (Table II) of **4** showed signals assigned to two quaternary carbons bearing an oxygen function (δ_C 84.0, 88.0, C-7, 10) and a quaternary carbon bearing a sulfonic acid group (δ_C 65.6, C-4).

Acetylation of **4** with acetic anhydride in pyridine furnished the monoacetate (**4a**) whose ^{1}H- and ^{13}C-NMR spectra showed signals due to an acetoxyl group [δ 2.00 (3H, s), 5.12 (δ, $J=12$ Hz, 6-H)] and a ^{13}C-NMR acetylation shift around the C-6 position, respectively. Furthermore, NOE correlations were observed in the following proton pairs of **4** (1-H and 15-H_3; 6-H and 15-H_3, 5-H and 8β-H, 9β-H), as shown in Fig. 1. The above-mentioned evidence and comparison of the physicochemical data for **4** with those for known guaiane-type sesquiterpenes[1,9,10] led us to presume the structure of **4**.

Finally, the stereostructure of **4** was established by chemical derivation from sulfoorientalol a (**1**). It was reported that the epoxidation of **8** with *meta*-chloro-

perbenzoic acid (*m*-CPBA) in CH_2Cl_2 furnished the 6,7-α-epoxide (**11**) and the 7,10-α-oxide (**9**) which was derived from the unstable β-epoxide (**ii**).[9a] Thus, the treatment of **1** with *m*-CPBA in dichloroethane under reflux afforded sulfoorientalol d (**4**, 45%) *via* **i** and the 6,7-α-epoxide (**10**, 22%), whose structure was clarified by comparison of the ^{1}H- and ^{13}C-NMR (Table II) data with those for **11**. Consequently, the stereostructure of sulfoorientalol d (**4**) was determined to be as shown.

Sulfoorientalols b (2) and c (3) Sulfoorientalol b (**2**), obtained as a white powder, exhibited no optical activity. The IR spectrum of **2** showed absorption bands due to hydroxyl, olefin, and sulfonic acid groups. The positive FAB-MS of **2** showed the quasimolecular ion peak at *m/z* 363 $(M-H+2Na)^+$ and the molecular formula $C_{15}H_{26}O_5S$ was determined by the high-resolution MS measurement of the quasimolecular ion peak. The proton signals in the ^{1}H-NMR spectrum of **2** were shown to be superimposable on those of orientalol A (**5**)[1] and were assigned as shown in Table I, while the ^{13}C-NMR spectrum (Table II) of **2** closely resembled that of **6** except for a signal due to carbon linked to a sulfonic acid group. Namely, the signal assignable to C-14 (δ_C 54.0) of **2** bearing a sulfonic acid group was observed at higher field than that (δ_C 70.0)[1] of **6**. Furthermore, observation of NOEs (Fig. 1) in the following pairs of protons (1-H and 15-H_3; 15-H_3 and 6-H; 5-H and 14-H_2) established the stereostructure of sulfoorientalol b (**2**).

Sulfoorientalol c (**3**) was also obtained as a white powder and showed no optical activity. The molecular formula $C_{15}H_{24}O_4S$ of **3** was confirmed from the quasimolecular ion peak at *m/z* 345 $(M-H+2Na)^+$ in the positive FAB-MS and by high-resolution MS measurement. The IR spectrum of **3** showed absorption bands due to hydroxyl, olefin, and sulfonic acid functions. The ^{1}H-NMR (Table I) and ^{13}C-NMR (Table II) data of **3** indicated the presence of a trisubstituted olefin attached to a sulfonic acid group [δ 6.21 (s, 14-H), δ_C 156.9 (C-10), 127.0 (C-14)] together with many other signals resembling those of **2**. Comparison of the spectral data for **3** with those for **2** and various known sesquiterpenes such as alismol (**12**)[1,9,10] led us to presume that **3** was a dehydroxyl derivative of **2**. Observation of NOEs in the following pairs of protons in **3** (1-H and 15-H_3, 2α-H and 14-H_2, 15-H_3 and 6-H) confirmed the above presumption and established the geometry of the exocyclic olefin in **3**. Based on the above-mentioned evidence, the stereostructure of sulfoorientalol c (**3**) was determined to be as shown.

Chemical Transformation from Alismol (12) Leading to Sulfoorientalol Congeners (13, 14) In previous papers,[1,11] we suggested that most of alismol (**12**), alismoxide (**8**) and orientalols (**5**—**7**) isolated from Alismatis Rhizoma were secondary products formed from germacrene C in the fresh rhizome of *Alisma orientale* during processing. Since the sulfoorientalols showed no optical activity, sulfoorientalols were also considered to be secondarily produced from the sesquiterpenes in Alismatis Rhizoma.

In order to obtain chemical and spectral evidence for the structures of the 1, 5-*trans*-guaiane type sesquiterpenes such as sulfoorientalols b (**2**) and c (**3**) containing a sulfonic acid group at the C-14 position, sulfonation reaction of alismol (**12**) having a reactive *exo*-methylene group at the C-14 position was carried out. Namely, treatment of **12** with sodium bisulfite ($NaHSO_3$) in the presence of tert-butyl perbenzoate in 70% aqueous EtOH quantitatively afforded the sulfonated product **13**. The IR spectrum of **13** showed the presence of a sulfonic acid group, and in positive FAB-MS, the quasimolecular ion peak $(M-H+2Na)^+$ was observed at *m/z* 347. Comparison of ^{1}H- and ^{13}C-NMR data for **13** with those for **12** and examination of the sulfonation mechanism with $NaHSO_3$ and *tert*-butyl perbenzoate led us to formulate the structure of **13**. Furthermore, NOE correlations were observed between 1-H and 15-H_3, 14-H_2, and between 5-H and 10-H (Fig. 1). Consequently, the structure of **13** was concluded to be as shown.

On the other hand, the sulfonation of **12** with sulfur trioxide (SO_3)–pyridine complex gave a complex mixture, so monoacetylalismol (**12a**), which was obtained by ordinary acetylation of **12**, was subjected to sulfonation with SO_3–pyridine complex in *N*,*N*-dimethylformamide (DMF) and subsequently deacetylation reaction. However, it was found that the sulfonation followed by deacetylation reaction of **12a** gave **14** in a poor yield (12%). The IR spectrum of **14** showed absorption bands due to hydroxyl and sulfonic acid moieties and the quasimolecular ion peak $(M-H+2Na)^+$ was observed at *m/z* 345 in the positive FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **14** showed signals assignable to tetrasubstituted olefin (δ_C 127.4, 143.7) and a sulfonated methylene [δ 3.52, 3.53 (both br s), δ_C 59.1]. Furthermore, long-range correlations were observed at the following pairs of proton and carbons [15-H and C-3, C-4, C-5; 6-H and C-1, C-11, C-8; 11-H and C-6, C-7, C-8, C-12, C-13; 14-H and C-1, C-9, C-10]. Based on the accumulated evidence and comparison of the physicochemical evidence for **14** with those for **12** and **13**, the structure of **14** was determined to be as shown. The ^{1}H- and ^{13}C-NMR data of **13** and **14** were found to correspond satisfactorily to those of **2** and **3**. But no evidence for the chemical processing of sulfoorientalols could be obtained, and further investigation is in progress in our laboratory.

TABLE III. Inhibitory Effects of Sulfoorientalols (**1**, **2**, **3**, **4**) and the Synthetic Congeners (**9**, **13**, **14**) on Contractile Response of Guinea Pig Bladder Induced by Carbachol (CCh)

Compound	Concentration	Contractile response (%) CCh (3×10^{-7} M)
Control	0.1% EtOH	100.0±8.1
Sulfoorientalol a (**1**)	10^{-4} M	52.0±0.8**
Sulfoorientalol b (**2**)	10^{-4} M	51.3±1.5**
Sulfoorientalol c (**3**)	10^{-4} M	56.5±3.8**
Sulfoorientalol d (**4**)	10^{-4} M	46.2±4.4**
9	10^{-4} M	59.8±1.6**
13	10^{-4} M	53.3±0.4**
14	10^{-4} M	65.5±5.6**
Atropine	10^{-7} M	0.0±0.0**

Each value represents the mean±S.E. of 4—5 experiments ($p^{**}<0.01$).

Inhibitory Effect of Sulfoorientalols (1—4), the Congeners (13, 14), and 9 on the Carbachol-Induced Contraction of

Isolated Bladder Smooth Muscle of Guinea Pig In a previous paper,[1] we reported that the lipophilic sesquiterpene constituents such as orientalols (**5**—**7**), alismol (**12**), and alismoxide (**8**) inhibited the contraction of isolated bladder smooth muscle of guinea pig induced by carbachol. As summarized in Table III, sulfoorientalols (**1**—**4**), the synthetic congeners (**13**, **14**), and **9** also showed inhibitory effects on the contraction.

Alismatis Rhizoma is prescribed in many Chinese traditional preparations such as Hachimi-Jio-Gan, which has been used to treat obstruction of micturition. This activity may be beneficial in patients with micturition disorder. It is noteworthy that all sulfoorientalols were obtained as stable white powders with higher water-solubility than the oily lipophilic sesquiterpenes (**5**—**9**, **12**), since this suggests that they would be present in a decoction of the Chinese traditional preparation.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[3]

Isolation of Sulfoorientalols a (1), b (2), c (3) and d (4) from the Polar Fraction of Chinese Alismatis Rhizoma The MeOH extract (690 g) from Chinese Alismatis Rhizoma (Sentaku, 10 kg) was partitioned into a mixture of AcOEt–water. Isolation of the less polar constituents such as orientalols, alismol, alismoxide and alisols from the AcOEt-soluble portion was reported in previous papers.[1,3] The water-soluble portion was extracted with 1-BuOH and removal of the solvent from the 1-BuOH-soluble portion under reduced pressure gave the 1-BuOH extract (58 g). The 1-BuOH extract was subjected to silica gel column chromatography {[1.5 kg, $CHCl_3$–MeOH–H_2O [10:3:1, 7:3:1, 65:35:10 (each lower layer), 6:4:1)]} to furnish six fractions. Evaporation of the solvent under reduced pressure gave fr. 1 (13.4 g, lipid constituents), fr. 2 (2.2 g), fr. 3 (6.9 g), fr. 4 (5.6 g), fr. 5 (3.8 g), and fr. 6 (13.4 g).

Fraction 2 (2.0 g) was purified by reversed-phase silica gel column chromatography (30 g, 33% aqueous MeOH, MeOH) to furnish daucosterin (0.97 g). Fraction 3 (3.0 g) was purified by reversed-phase silica gel (45 g, 50% aqueous MeOH) and silica gel [200 g, $CHCl_3$–acetone (1:2)] column chromatography to afford thymidine (15 mg). Reversed-phase silica gel column chromatography (50 g, 20% aqueous MeOH) of fr. 4 (5.5 g) followed by silica gel column chromatography [50 g each, benzene–MeOH (4:1), benzene–acetone–MeOH (2:2:1)] furnished methyl β-D-fructofuranoside (0.9 g), uridine (0.3 g), and sulfoorientalol d (**4**, 43 mg).

Fraction 6 (3.5 g) was purified by silica gel [100 g, benzene–MeOH–acetone (2:1:2)] and reversed-phase silica gel column chromatography (50 g, H_2O) to give sulfoorientalol c (**3**, 24 mg). Reversed-phase silica gel column chromatography (200 g, H_2O, 50% aqueous MeOH) of fr. 7 (13.0 g) followed by silica gel column chromatography [50 g each, $CHCl_3$–MeOH–H_2O (65:35:10, lower layer) and benzene–acetone–MeOH (6:3:2—8:3:2)] afforded sulfoorientalols a (**1**, 170 mg) and b (**2**, 80 mg).

Sulfoorientalol a (**1**): A white powder, $[\alpha]_D^{22}$ ±0° (c=1.0, MeOH). High-resolution FAB-MS: Found: 347.132. Calcd for $C_{15}H_{25}Na_2O_4S$: 347.128 $(M-H+2Na)^+$. IR (KBr): 3350, 1640, 1385, 1210 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD)δ: see Table I. ^{13}C-NMR (75 MHz, CD_3OD) δ_C: see Table II. Positive FAB-MS m/z: 347 $(M-H+2Na)^+$.

Sulfoorientalol b (**2**): A white powder, $[\alpha]_D^{22}$ ±0° (c=1.0, MeOH). High-resolution FAB-MS: Found: 363.116. Calcd for $C_{15}H_{25}Na_2O_5S$: 363.122$(M-H+2Na)^+$. IR (KBr): 3450, 1650, 1385, 1200 cm^{-1}. ^{1}H-NMR (500 MHz): see Table I. ^{13}C-NMR (75 MHz): see Table II. Positive FAB-MS m/z: 363 $(M-H+2Na)^+$.

Sulfoorientalol c (**3**): A white powder, $[\alpha]_D^{22}$ ±0° (c=0.8, MeOH). High-resolution FAB-MS: Found: 345.114. Calcd for $C_{15}H_{23}Na_2O_4S$: 345.111 $(M-H+2Na)^+$. IR (KBr): 3430, 1630, 1380, 1170 cm^{-1}. ^{1}H-NMR (500 MHz): see Table I. ^{13}C-NMR (75 MHz): see Table II. Positive FAB-MS m/z: 345 $(M-H+2Na)^+$.

Sulfoorientalol d (**4**): A white powder, $[\alpha]_D^{22}$ ±0° (c=0.9, MeOH). High-resolution FAB-MS: Found: 363.117. Calcd for $C_{15}H_{25}Na_2O_5S$: 363.122 $(M-H+2Na)^+$. IR (KBr): 3550, 1640, 1385, 1190 cm^{-1}. ^{1}H-NMR (500 MHz): see Table I. ^{13}C-NMR (75 MHz): see Table II. Positive FAB-MS m/z: 363 $(M-H+2Na)^+$.

Acetylation of Sulfoorientalol a (1) A solution of **1** (10.1 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and DMAP (a catalytic amount) and the mixture was stirred at 20 °C for 11 h. Removal of the solvent from the reaction mixture under reduced pressure furnished a residue, which was purified by silica gel column chromatography [3 g, benzene–acetone–MeOH (7:3:2)] to give **1a** (7.3 mg).

1a: A white powder. IR (KBr): 3350, 1735, 1650, 1385, 1185 cm^{-1}. ^{1}H-NMR (270 MHz, CD_3OD) δ: 0.98, 0.99 (3H each, both d, J=7 Hz, 12,13-H_3), 1.34 (3H, s, 15-H_3), 1.57 (3H, s, 14-H_3), 2.21 (3H, s, $COCH_3$), 2.92 (1H, br d, J=*ca.* 12 Hz, 5-H), 5.77 (1H, d, J=3 Hz, 6-H). ^{13}C-NMR (67.5 MHz, CD_3OD) δ_C: 30.3, 168.3 (OAc × 1) and other signals as given in Table II. Positive FAB-MS m/z: 329 $(M-H+2Na-AcOH)^+$.

Acetylation of Sulfoorientalol d (4) A solution of **4** (15.0 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and DMAP (a catalytic amount) and the mixture was stirred at 20 °C for 12 h. Removal of the solvent from the reaction mixture under reduced pressure furnished a residue, which was purified by silica gel column chromatography [3 g, benzene–acetone–MeOH (7:3:1)] to give **4a** (9.8 mg).

4a: A white powder. IR (KBr): 3430, 1735, 1715, 1645, 1385, 1175 cm^{-1}. ^{1}H-NMR (270 MHz, CD_3OD) δ: 0.96 (6H, d, J=7 Hz, 12,13-H_3), 1.24 (3H, s, 14-H_3), 1.39 (3H, s, 15-H_3), 2.22 (3H, s, $COCH_3$), 2.48 (1H, dd, J=10, 12 Hz, 5-H), 5.12 (1H, d, J=12 Hz, 6-H). ^{13}C-NMR (67.5 MHz, CD_3OD) δ_C: 30.7, 169.5 (OAc × 1) and other signals as given in Table II. Positive FAB-MS m/z: 345 $(M-H+2Na-AcOH)^+$.

Oxidation of Sulfoorientalol a (1) with *m*-CPBA to Give Sulfoorientalol d (4) and 10 A solution of **1** (94.5 mg) in $C_2H_4Cl_2$ (5 ml) was treated with *m*-CPBA (165 mg) and the whole mixture was heated under reflux for 1 h. After cooling, removal of the solvent from the reaction mixture under reduced pressure furnished a residue, which was subjected to silica gel column chromatography [15 g, benzene–acetone–MeOH (7:3:2)] to afford **4** (49.7 mg), and **10** (21.1 mg). Sulfoorientalol d (**4**) thus obtained was shown to be identical with an authentic sample isolated from Chinese Alismatis Rhizoma, by comparisons of the ^{1}H-NMR (CD_3OD), ^{13}C-NMR (CD_3OD) and TLC [$CHCl_3$–MeOH–H_2O (10:3:1, lower layer)] behavior.

10: A white powder. IR (KBr): 3400, 1380, 1200, 1025 cm^{-1}. ^{1}H-NMR (270 MHz, CD_3OD) δ: 0.92, 0.96 (3H each, both d, J=7 Hz, 12,13-H_3), 1.24 (3H, s, 14-H_3), 1.44 (3H, s, 15-H_3), 3.36 (1H, br s, 6-H). ^{13}C-NMR (67.5 MHz): see Table II.

Conversion of 12 to 13 A solution of **12** (54.8 mg) in 70% EtOH (20 ml) was treated with $NaHSO_3$ (260 mg) and *tert*-butyl perbenzoate (23.8 μl), and then the whole mixture was heated under reflux for 4 h. After cooling, removal of the solvent from the reaction mixture furnished a residue, which was purified by silica gel column chromatography [7 g, benzene–acetone–MeOH (4:3:2)] to give **13** (73.5 mg).

13: A white powder, $[\alpha]_D^{22}$ ±0° (c=1.0, MeOH). High-resolution FAB-MS: Found: 347.123. Calcd for $C_{15}H_{25}Na_2O_4S$: 347.127 $(M-H+2Na)^+$. IR (KBr): 3450, 1650, 1380, 1190 cm^{-1}. ^{1}H-NMR (270 MHz, CD_3OD) δ: 0.98, 1.00 (3H each, both d, J=7 Hz, 12,13-H_3), 1.14 (3H, s, 15-H_3), 2.41 (1H, dd, J=3, 11 Hz, 5-H), 2.61 (1H, dd, J=9, 14 Hz), 2.91 (1H, dd, J=1, 14 Hz) (14-H_2), 5.54 (1H, d, J=3 Hz, 6-H). ^{13}C-NMR (67.5 MHz): see Table II. Positive FAB-MS m/z: 347 $(M-H+2Na)^+$.

Conversion of 12 to 14 A solution of **12** (500 mg) in pyridine (15 ml) was treated with Ac_2O (9 ml) and DMAP (a catalytic amount) and the mixture was stirred at 20 °C for 40 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 [25 g, *n*-hexane–AcOEt (20:1)] to give monoacetylalismol (**12a**, 490 mg).

A solution of **12a** (114 mg) in DMF (5 ml) was treated with SO_3–pyridine complex (1.4 g) and the mixture was heated under reflux for 30 min. After cooling, the reaction mixture was diluted with MeOH–water and the whole solution was neutralized with $BaCO_3$ powder, then filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by reversed-phase silica gel column chromatography (10 g, H_2O, MeOH) to furnish the crude product. This was dissolved in 5% NaOMe–MeOH and the solution was stirred at 20 °C for 15 min. The reaction mixture was neutralized with Dowex 50w × 8 (H^+ form) and then filtered to remove the resin.

Removal of the solvent from the filtrate under reduced pressure furnished **14** (16 mg).

14: A white powder, $[\alpha]_D^{22}$ $\pm 0°$ ($c=0.4$, MeOH). High-resolution FAB-MS: Found: 345.113. Calcd for $C_{15}H_{23}Na_2O_4S$: 345.111 $(M-H+2Na)^+$. IR (KBr): 3450, 1655, 1380, 1180 cm^{-1}. ^{1}H-NMR (270 MHz, CD_3OD) δ: 1.03 (6H, d, $J=7$ Hz, 12,13-H_3), 1.09 (3H, s, 15-H_3), 3.52, 3.53 (1H each, both br s, 14-H_2), 5.45 (1H, d, $J=3$ Hz, 6-H). ^{13}C-NMR (67.5 MHz) : see Table II. Positive FAB-MS m/z: 345 $(M-H+2Na)^+$.

Bioassay Tests The method of recording of inhibitory effects on the carbachol-induced contraction was the same as in our previous paper.[1)]

References and Notes

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