SULFOORIENTALOLS a, b, c, AND d, FOUR NEW BIOLOGICALLY ACTIVE SESQUITERPENES, FROM ALISMATIS RHIZOMA

Masayuki YOSHIKAWA,* Youich FUKUDA, Shoko HATAKEYAMA, Nobumitsu TANAKA, Hisashi MATSUDA, Johji YAMAHARA, and Nobutoshi MURAKAMI

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan

Four new sesquiterpenes, sulfoorientalols a, b, c, and d, having a sulfonic acid function were isolated from Chinese Alismatis Rhizoma. Their structures were determined on the basis of chemical and physicochemical evidence. Sulfoorientalols inhibited the contraction of isolated bladder smooth muscle induced by carbachol.

KEYWORDS Alismatis Rhizoma; Alisma orientale; Alismataceae; aquatic plant; sulfoorientalol; bladder smooth muscle

During the course of our studies on biologically active constituents of naturally occurring drug materials,[1] we have investigated the chemical constituents of Alismatis Rhizoma (Takusha in Japanese), the dried rhizome of *Alisma orientale* Juzepczuk (Alismataceae), which is used as a diuretic in Chinese traditional medicine.[2] Recently, we reported the isolation of three new sesquiterpenes, orientalols A (**1**), B, and C, together with alismoxide (**2**) and alismol (**3**) from the lipophilic portion of Chinese Alismatis Rhizoma, which is now in common use in Japan, and described the structures of orientalols and revised those of alismoxide (**2**) and alismol (**3**).[3] As a continuing study, we have isolated four new sesquiterpenes, sulfoorientalols a (**6**), b (**9**), c (**10**), and d (**7**), from the water-soluble portion of the same Chinese Alismatis Rhizoma. This paper communicates the evidence consistent with the structures of sulfoorientalols a-d which exhibit an inhibitory effect for contraction on isolated bladder smooth muscle of the guinea pig induced by carbachol.

The MeOH extract of the Rhizoma was partitioned into an AcOEt-water mixture. Repeated separation of the water-soluble portion by normal and reversed phase column chromatography furnished **6** (0.0020% from the crude drug), **9** (0.0008%), **10** (0.0002%), and **7** (0.0004%) together with uridine (0.0030%) and tymidine (0.0003%).

Sulfoorientalol a (**6**), a white powder, $[\alpha]_D$ ±0,[4] was shown by its IR spectrum to have hydroxyl (3350 cm^{-1}), olefin (1640 cm^{-1}), and sulfonic acid (1385, 1210 cm^{-1}) functions. The molecular formula $C_{15}H_{26}O_4S$ was confirmed from the

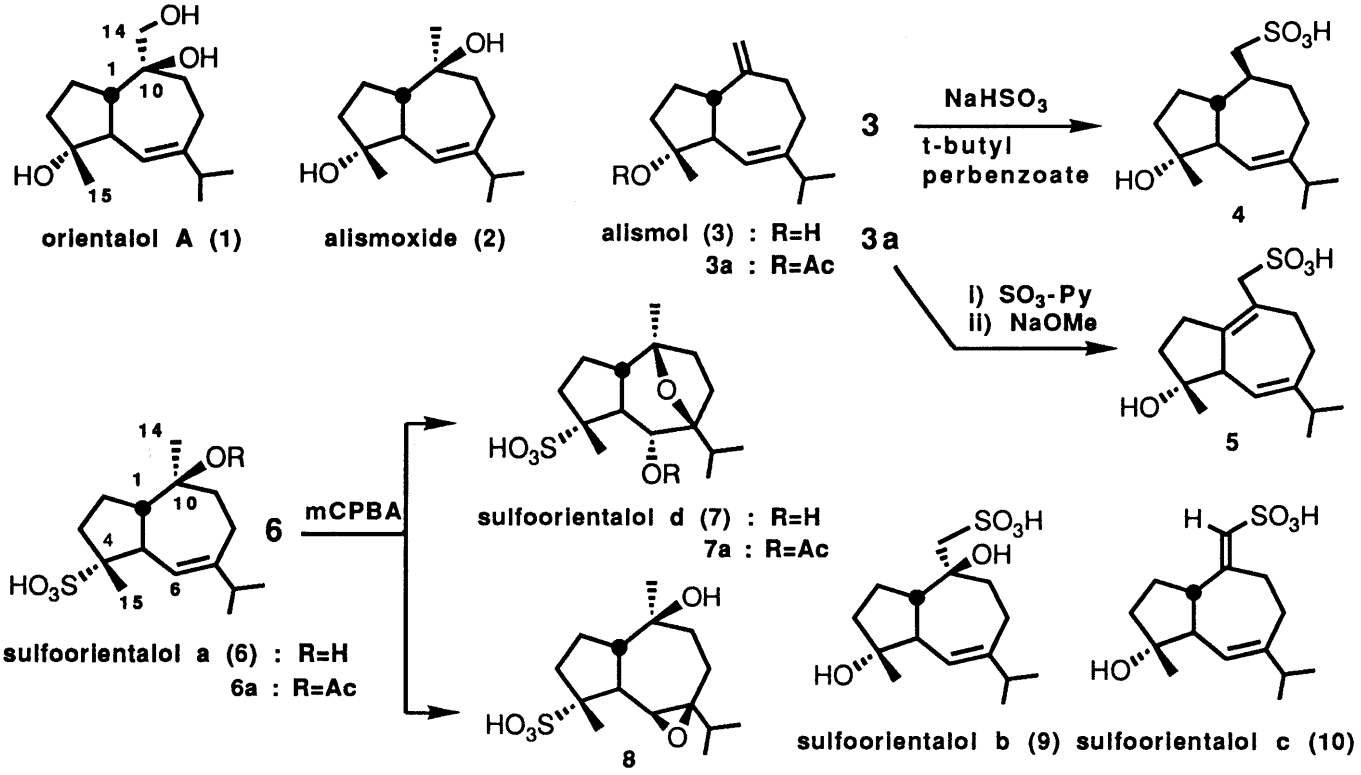

Table I. The ^{13}C NMR Data for **4**, **5**, **6**, **6a**, **7**, **9** and **10**[a)]

	4	5	6	6a	7	9	10
1	46.0	124.7	53.5	51.8	53.0	51.4	57.3
2	27.3	29.8	23.9	24.2	26.8	22.2	26.6
3	40.0	40.5	36.8	36.8	36.9	40.0[b)]	41.1
4	80.0	81.7	67.4	67.3	65.6	80.7	81.9
5	54.1	55.0	45.9	49.3	50.0	50.0	49.2
6	122.5	121.3	124.8	124.8	71.8	122.8	123.0
7	151.7	150.1	148.8	148.9	84.0	150.8	150.7
8	28.5	30.1	25.5	25.3	30.2	26.0	29.7
9	32.8	33.9	43.4	37.8	32.6	41.0[b)]	32.5
10	45.9	143.7	75.8	90.8	88.0	76.4	156.9
11	38.4	39.2	38.6	38.7	32.7	38.6	39.7
12	21.6[c)]	22.6[c)]	21.6[c)]	21.7[c)]	17.4[c)]	22.0[c)]	22.5[c)]
13	21.8[c)]	22.8[c)]	21.9[c)]	21.9[c)]	18.4[c)]	22.2[c)]	22.5[c)]
14	56.9	57.1	21.6	19.7	24.3	54.0	127.0
15	22.7	23.2	17.8	18.1	20.1	21.7	22.7

a)The spectra were taken in CD_3OD at 67.5 MHz. b),c)Assignments may be interchangeable within the same column.

quasimolecular ion peak at *m/z*347$(M-H+2Na)^+$ in the positive FAB-MS and by high-resolution FAB-MS measurement. The ^1H-NMR spectrum [5)] of **6** showed the presence of a trisubstituted olefin, two tertiary methyl functions and one isopropyl function, while the ^{13}C-NMR spectrum (Table I) showed signals due to a quaternary carbon bearing a hydroxyl group (δc 75.8) and a quaternary carbon bearing a sulfonic acid group (δc 67.4) together with four methylene and three methine signals. The detailed comparisons of the ^1H and ^{13}C-NMR data for **6** with those for alismoxide (**2**) led us to assign the 1,5-trans-guaiane structure to sulfoorientalol a (**6**). The location of the sulfonic acid group in **6** has been demonstrated to be C-4 position by comparison of the ^{13}C-NMR data (Table I) of **6** and the monoacetate (**6a**)[6)] which was prepared by treatment of **6** with Ac_2O-pyridine in the presence of dimethylaminopyridine (DMAP). Furthermore, the NOEs were observed between the proton pairs of **6** [1-H & 4-CH_3, 5-H & 10-CH_3, 6-H & 4-CH_3] in its NOESY spectrum. Consequently the stereostructure of sulfoorientalol a (**6**) was determined.

Sulfoorientalol b (**9**),[7)] white powder, $[\alpha]_D$ ±0,[4)] $C_{15}H_{26}O_5S$, has hydroxyl, olefin, and sulfonic acid functions as shown by its IR spectrum. The positive FAB-MS of **9** showed the quasimolecular ion peak at *m / z* 363 $(M-H+2Na)^+$. The ^1H and ^{13}C-NMR spectra of **9** closely resembled those of orientalol A (**1**) except for the signal due to the carbon linked to a sulfonic acid group. Thus, the signal assignable to C-14 (δc 54.0) bearing a sulfonic acid group was observed at higher field than that (δc 70.0)[3)] in **1**. Finally, observation of NOEs in the following pairs of protons (1-H & 4-CH_3, 4-CH_3 & 6-H, 5-H & 14-H_2) established the stereostructure of sulfoorientalol b (**9**).

Sulfoorientalol c (**10**),[8)] white powder, $[\alpha]_D$ ±0,[4)] $C_{15}H_{24}O_4S$, is also a sulfonic acid-containing sesquiterpene as shown from the absorption bands in its IR spectrum and gave the quasimolecular ion peak $(M-H+2Na)^+$ at *m / z* 345 in the positive FAB-MS. The ^1H and ^{13}C-NMR (Table I) data for **10** indicated the presence of a trisubstituted olefin attached to a sulfonic acid group [δ 6.21 (s, 15-H), δc 156.9 (10-C), 127.0 (14-C)] together with many other signals resembling those of **9**. This finding led us to presume that **10** was a dehydroxyl derivative of **9**. Observation of NOEs in the following pairs of protons in **10** (1-H & 4-CH_3, 2α-H & 14-H, 4-CH_3 & 6-H) confirmed the above presumption and established the geometry of the exocyclic olefin in **10**.

Table II. Inhibitory Effect of Sulfoorientalols **a-d** and Their Analogs for the Carbachol-Induced Contraction on Isolated Bladder Smooth Muscle of the Guinea Pig

	Contractile response (%)
Sulfoorientalol a (**6**)	52.0±0.8**
Sulfoorientalol b (**9**)	51.3±1.5**
Sulfoorientalol c (**10**)	56.5±3.8**
Sulfoorientalol d (**7**)	46.2±4.4**
4	53.3±0.4**
5	65.5±5.6**

Each value represents mean±S.E. of 4-5 experiments (p**<0.01).

In order to obtain the chemical and spectral evidence for the 1,5-trans-guaiane type sesquiterpene containing a sulfonic acid group at C-14 position, sulfonation of alismol (**3**) was carried out. Namely, treatment of **3** with $NaHSO_3$ in the presence of t-butyl perbenzoate in 70% aq. EtOH quantitatively yielded **4**,[9)] white powder, $C_{15}H_{26}O_4S$. On the other hand, treatment of **3a** with SO_3-pyridine complex in DMF followed by deacetylation furnished **5**,[10)] white powder, $C_{15}H_{24}O_4S$. Comparison of the ^1H and ^{13}C-NMR data for **9** and **10** with those for **4** and **5** led us to confirm the structures of **9** and **10** bearing a sulfonic acid group at C-14 position to sulfoorientalols b and c, respectively.

Sulfoorientalol d (**7**)[11], white powder, $[\alpha]_D$ ±0,[4] $C_{15}H_{26}O_5S$, gave the monoacetate(**7a**)[12], white powder, $C_{17}H_{28}O_6S$, by ordinary acetylation. Comparisons of the ^1H and ^{13}C-NMR (Table I) data for **7** with those for **7a** and **6** led us to presume the structure of sulfoorientalol d to be 7,10-epoxy-6-hydroxy-4-sulfoguaiane (**7**). Furthermore, NOE correlations were observed in the proton pairs of **7** (1-H & 4-CH_3, 4-CH_3 & 6-H). Finally, treatment of **6** with m-chloroperbenzoic acid (mCPBA) in $ClCH_2CH_2Cl$ yielded **7** (50%) and the 5,6-epoxide (**8**, 22%)[13], white powder, $C_{15}H_{26}O_5S$. Consequently, the stereostructure of sulfoorientalol d (**7**) was elucidated.

The inhibitory effect of sulfoorientalols a (**6**), b (**9**), c (**10**), and d (**7**) and their analogs (**4**, **5**) on the contraction of isolated bladder smooth muscle of guinea pig induced by carbachol are summarized in Table II. Alismatis Rhizoma has been prescribed in many Chinese traditional preparations such as Hachimi-Jio-Gan which has been used to treat the obstruction of micturition. Sulfoorientalols and their analogs inhibited the contraction on bladder smooth muscle, which may be beneficial to micturition disorders.[14]

REFERENCES AND NOTES

1) M. Yoshikawa, S. Hatakeyama, Y. Inoue, and J. Yamahara, *Chem. Pharm. Bull.*, **41**, 214 (1993).

2) a) J. Yamahara, H. Matsuda, H. Murakami, and H. Fujimura, *Chem. Pharm. Bull.*, **34**, 4422 (1986) ; b) H. Matsuda, G. Kobayashi, J. Yamahara, H. Fujimura, K. Kurahashi, and M. Fujiwara, *Life Sci.*, **41**, 1845 (1987) ; c) H. Matsuda, J. Yamahara, G. Kobayashi, H. Fujimura, K. Kurahashi, and M. Fijiwara, *Jpn. J. Pharmacol.*, **46**, 331 (1988) ; d) J. Yamahara, G. Kobayashi, M. Iwamoto, H. Matsuda, and H. Fujimura, *Phytother. Res.*, **3**, 57 (1989) ; e) J. Yamahara, H. Matsuda, G. Kobayashi, T. Katayama, and H. Fujimura, *ibid.*, **3**, 72 (1989).

3) M. Yoshikawa, S. Hatakeyama, N. Tanaka, Y. Fukuda, N. Murakami, and J. Yamahara, *Chem. Pharm. Bull.*, **40**, 2582 (1992).

4) Sulfoorientalols showed no optical activity, so that these sesquiterpenes would be considered to be formed during the processing procedure of this crude drug. The mechanism of these transformations during the processing is an interesting subject for further investigation.

5) The ^1H-NMR (CD_3OD) of **6** : δ 0.97, 0.99 (3H each, both d , J=7Hz, 12, 13-H_3), 1.23 (3H, s, 14-H_3), 1.35 (3H, s, 15-H_3), 2.08 (1H, ddd, J=5, 11, 11 Hz, 1-H), 2.83 (1H, br d, 5-H), 5.75 (1H, d, J=3 Hz, 6-H).

6) **6a** : IR (KBr, cm^{-1}) : 3350, 1735, 1720, 1650, 1385, 1200, ^1H-NMR (CD_3OD) : δ 0.98, 0.99 (3H each, both d , J=7Hz, 12, 13-H_3), 1.34 (3H, s, 15-H_3), 1.57 (3H, s, 14-H_3), 2.92 (1H, br d, 5-H), 5.77 (1H, d, J=3 Hz, 6-H), Positive FAB-MS : m / z 329 $(M-H+2Na-AcOH)^+$.

7) **9** : IR (KBr, cm^{-1}) : 3450, 1650, 1385, 1200, ^1H-NMR (CD_3OD) : δ 0.99, 1.00 (3H each, both d , J=7Hz, 12, 13-H_3), 1.14 (3H, s, 15-H_3), 1.73 (m, 1-H), 2.25 (m, 5-H), 3.16, 3.36 (2H, ABq, J=14Hz, 14-H_2), 5.55 (1H, d, J=2 Hz, 6-H), Positive FAB-MS : *m / z* 363 $(M-H+2Na)^+$.

8) **10** : IR (KBr, cm^{-1}) : 3430, 1630, 1380, 1170, ^1H-NMR (CD_3OD) : δ 1.01, 1.02 (3H each, both d , J=7Hz, 12, 13-H_3), 1.17 (3H, s, 15-H_3), 1.63 (m, 1-H), 1.73 (m, 2α-H), 2.35 (1H, br d, 5-H), 5.56 (1H, d, J=1 Hz, 6-H), 6.21 (1H, s, 14-H), Positive FAB-MS : *m / z* 345 $(M-H+2Na)^+$.

9) **4** : IR (KBr, cm^{-1}) : 3450, 1650, 1380, 1190, ^1H-NMR (CD_3OD) : δ 0.98, 1.00 (3H each, both d , J=7Hz, 12, 13-H_3), 1.14 (3H, s, 15-H_3), 1.39 (m, 1-H), 2.03 (m, 10-H), 2.41 (1H, dd, J=3, 11 Hz, 5-H), 2.61 (1H, dd, J=9, 14 Hz), 2.91 (1H, dd, J=1, 14Hz) (14-H_2), 5.54 (1H, d, J=3 Hz, 6-H), Positive FAB-MS : *m / z* 347 $(M-H+2Na)^+$.

10) **5** : IR (KBr, cm^{-1}) : 3450, 1655, 1380, 1186, ^1H-NMR (CD_3OD) : δ 1.03 (6H, d , J=7Hz, 12, 13-H_3), 1.09 (3H, s, 15-H_3), 3.50, 3.55 (2H, ABq, J=14Hz, 14-H_2), 5.45 (1H, d, J=3 Hz, 6-H), Positive FAB-MS : *m / z* 345 $(M-H+2Na)^+$.

11) **7** : IR (KBr, cm^{-1}) : 3350, 1385, 1190, ^1H-NMR (CD_3OD) : δ 0.99, 1.02 (3H each, both d , J=7Hz, 12, 13-H_3), 1.23 (3H, s, 14-H_3), 1.44 (3H, s, 15-H_3), 1.63 (m, 1-H), 2.19 (1H, dd, J=10, 12 Hz, 5-H), 3.69 (1H, d, J=10Hz, 6-H), Positive FAB-MS : *m / z* 363 $(M-H+2Na)^+$.

12) **7a** : IR (KBr, cm^{-1}) : 3430, 1735, 1715, 1645, 1385, 1215, ^1H-NMR (CD_3OD) : δ 0.96 (6H, d, J=7Hz, 12,13-H_3), 1.24 (3H, s, 14-H_3), 1.39 (3H, s, 15-H_3), 2.48 (1H, dd, J=10, 12Hz, 5-H), 5.12 (1H, d, J=12Hz, 6-H), Positive FAB-MS : *m / z* 345 $(M-H+2Na-AcOH)^+$.

13) **8** : IR (KBr, cm^{-1}) : 3400, 1385, 1200, ^1H-NMR (CD_3OD) : δ 0.92, 0.96 (3H each, both d , J=7Hz, 12, 13-H_3), 1.24 (3H, s, 14-H_3), 1.44 (3H, s, 15-H_3), 3.36 (1H, d, J=10Hz, 6-H), Positive FAB-MS : *m / z* 363 $(M-H+2Na)^+$.

14) K. Nakase, I. Kimura, M. Kimura, and I. Kitagawa, *Phytother. Res.*, **5**, 67 (1991).

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