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SESQUITERPENOIDS FROM *ACORUS CALAMUS* AS GERMINATION INHIBITORS

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Key Word Index—*Acorus calamus*; anti-germinating activity; sesquiterpene; Araceae; acorane; eudesmane; lettuce seed.

Abstract—In the search for germination inhibitors from plant sources, the methanol extract of Acori rhizoma (*Acorus calamus*) was shown to inhibit germination of lettuce seeds. From Acori rhizoma, eight new sesquiterpenes were isolated together with some known sesquiterpenes. These sesquiterpenoids have cadinane, acorane and eudesmane skeletons and their structures were elucidated from the spectral evidence. Some of the compounds showed potent anti-germination activity. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

It can be considered that organisms are living together interactively in the natural world and make up the ecosystem. In the plant kingdom, many plants are probably living a system of mutual interaction. One type of interaction is the release of secondary metabolites from plants, the so called allelochemicals [1], which effect germination and growth of other plants. This phenomenon is called allelopathy. Well known allelochemical germination inhibitors include nagilactones from *Podocarpus nagi* [2] and 6-hydroxynaphthoquinone from *Juglus mandshrica* [3].

We have studied the germination inhibitors from various plant sources and screened about a hundred plant samples. We have identified an active material in the methanol extract of Acori rhizoma (*Acorus calamus* L.) and isolated the constituents to give eight new sesquinterpenes, tentatively named AC-1 (**1**) to AC-8 (**8**). In addition seven known compounds were identified as acorone [4], acoronene [5], isocalamediol [6], asarone [7], asaraldhyde [8], 1-(2,4,5-trimethoxyphenyl)-propane-1,2-dione [9] and 1-(2,4,5-trimethoxyphenyl)-1-methoxy-propan-2-ol [10].

RESULTS AND DISCUSSION

The molecular formula of AC-1 (**1**), $[\alpha]_D$ −44.9°, was determined to be $C_{15}H_{24}O_2$ from the EI-mass spectrum *m/z* 236 $[M]^+$. The ^1H NMR spectrum of **1** showed the presence of three secondary methyl groups at δ 0.94 (J = 7.5 Hz), 1.02 (J = 6.5 Hz) and 1.08 (J = 6.5 Hz), an olefinic methyl group (δ 1.75, *br s*), a carbinyl methine group (δ 4.28, *ddd*, J = 2.5, 5.2, 6.2 Hz), an olefinic proton (δ 6.62, *m*) and an isolated methylene group (δ 2.71, 1H, *dd*, J = 1.1, 17.0 Hz and 2.54, *d*, J = 17.0 Hz), which showed a long range coupling based on the *W*-character rule. The ^{13}C NMR spectrum of **1** showed the presence of two olefinic carbons (δ 135.0, 144.3), a carbonyl group (δ 200.9) and a quaternary carbon (δ 47.4), which was confirmed by ^1H–^{13}C COSY experiment. The ^{13}C NMR chemical shift (δ 200.9) of the carbonyl group and the UV spectrum (240 nm, ε 6570) showed the presence of α,β-unsaturated carbonyl group. These facts indicated that **1** had an acorane skeleton. The ^1H–^1H COSY experiment of **1** showed the correlations presented in Fig. 1 indicating a 2-hydroxy-7-en-6-one derivative of acorane. The ^1H–^{13}C COSY spectrum also supported the structure. The mass spectrum of **1** showed the characteristic fragmentation with ions at *m/z* 218, 154 and 136 from retro-Diels–Alder rearrangement and dehydration. These ions also supported the structure (Scheme 1.). The relative stereochemistry of **1** was examined by a difference NOE experiment. As shown in Fig. 2, the NOE effects were observed between: (i) Me-15 and H-6 *eq*; (ii) H-4 and H-1, H-3α; (iii) H-6*ax* and Me-12, Me-13, H-11, H-10*ax*; (iv) H-2 and H-1, H-3α; (v) H_{10}*eq* and H-1 and so on. These facts indicated that the relative configuration of AC-1 was as shown in Fig. 2. The absolute configuration of **1** was determined to be (1*S*, 2*R*, 4*S*, 5*S*) from a negative Cotton effect ($[\theta]_{320}$ −814) based on the n→π^* transition of an α,β-unsaturated ketone [11]. Compound **1** was given the name 2-hydroxyacoronene.

The molecular formula of AC-2 (2), $[\alpha]_D$ −75.8°, was determined to be $C_{17}H_{26}O_3$ from the EI-mass spectrum. The ^1H NMR spectrum of **2** showed almost the same signal pattern as that of **1** except for the

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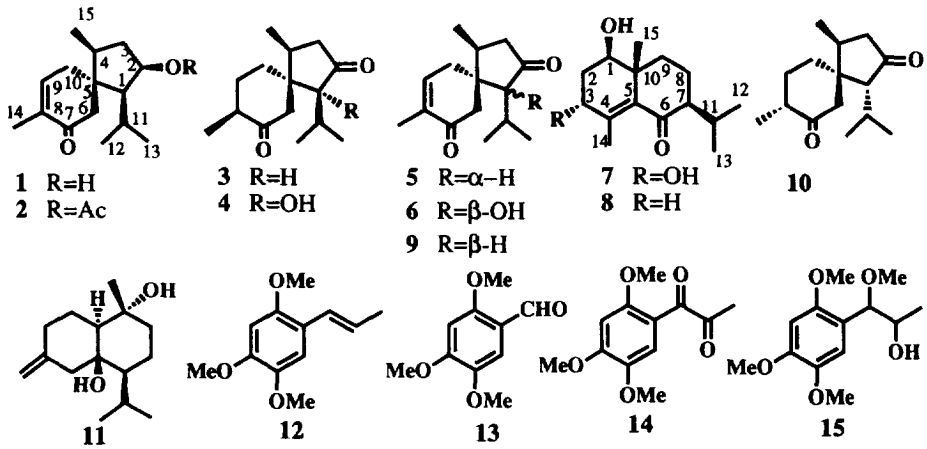
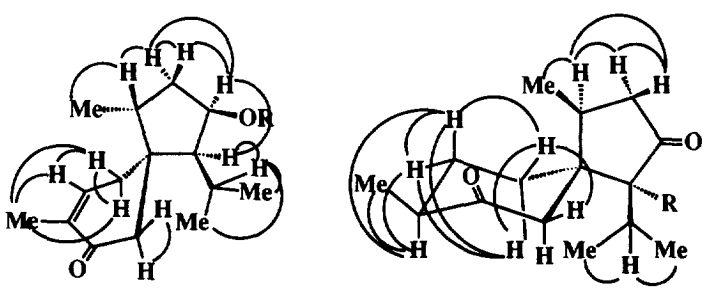


presence of an acetyl group (δ 2.03, 3H, *s*, and δ 170.6, 21.4) and downfield shift of H-2 (δ 5.15, 1H, *ddd*, J = 2.7, 5.7, 6.7 Hz). The mass spectrum and ^{1}H NMR spectrum suggested that AC-2 should be an acetate of **1**. This was further supported from the mass spectrum fragmentation (Scheme 1), and H–H COSY spectrum and ^{13}C NMR data (Table 1). The difference NOE experiment of **2** gave the same results as that of **1**. The absolute configuration was determined to be the same as that of **1** by the similarity of the CD spectrum ($[\theta]_{310} - 778°$ (MeOH). The structure of **2** was also identified by acetylation from **1**. In order to confirm the structures of **1** and **2** unambiguously, the HMBC experiment of **2** was carried out as shown in Fig. 3 and the result was compatible with the structure. Compound **2** was named 2-acetyloxyacoronene.

The molecular formula of AC-3 (**3**), $[\alpha]_D - 53.9°$, was determined to be $C_{15}H_{24}O_2$ from the EI-mass spectrum. The ^{1}H NMR spectrum of **3** showed the presence of four secondary methyl groups [δ 0.72 (J = 7.5 Hz), 1.03 (J = 6.5 Hz), 1.09 (J = 6.5 Hz), 1.17 (J = 7.0 Hz)], an isolated methylene protons at C-6 (δ 1.94, 1H, *d*, J = 13.0 Hz and 2.14, 1H, *dd*, J = 2.5, 13.0 Hz), characteristic for a 7-keto-acorane skeleton, and a methylene group at C-3 (δ 1.59, *br d*, J = 19.0 Hz and 1.99, *dd*, J = 19.0, 8.5 Hz). The assignment of the ^{1}H NMR spectrum was achieved from the ^{1}H–^{1}H COSY spectrum of **3** (Fig. 1). The position of the second carbonyl group was confirmed to be C-2 from the mass spectral fragmentation (Scheme 1), in which the propene moiety was given by McLafferty rearrangement to give *m/z* 194 and 166. These data showed that the planer structure of **3** was the same as that of acorone, but their optical rotations were different from each other in sign. This indicated that **3** must be the stereoisomer of acorone.

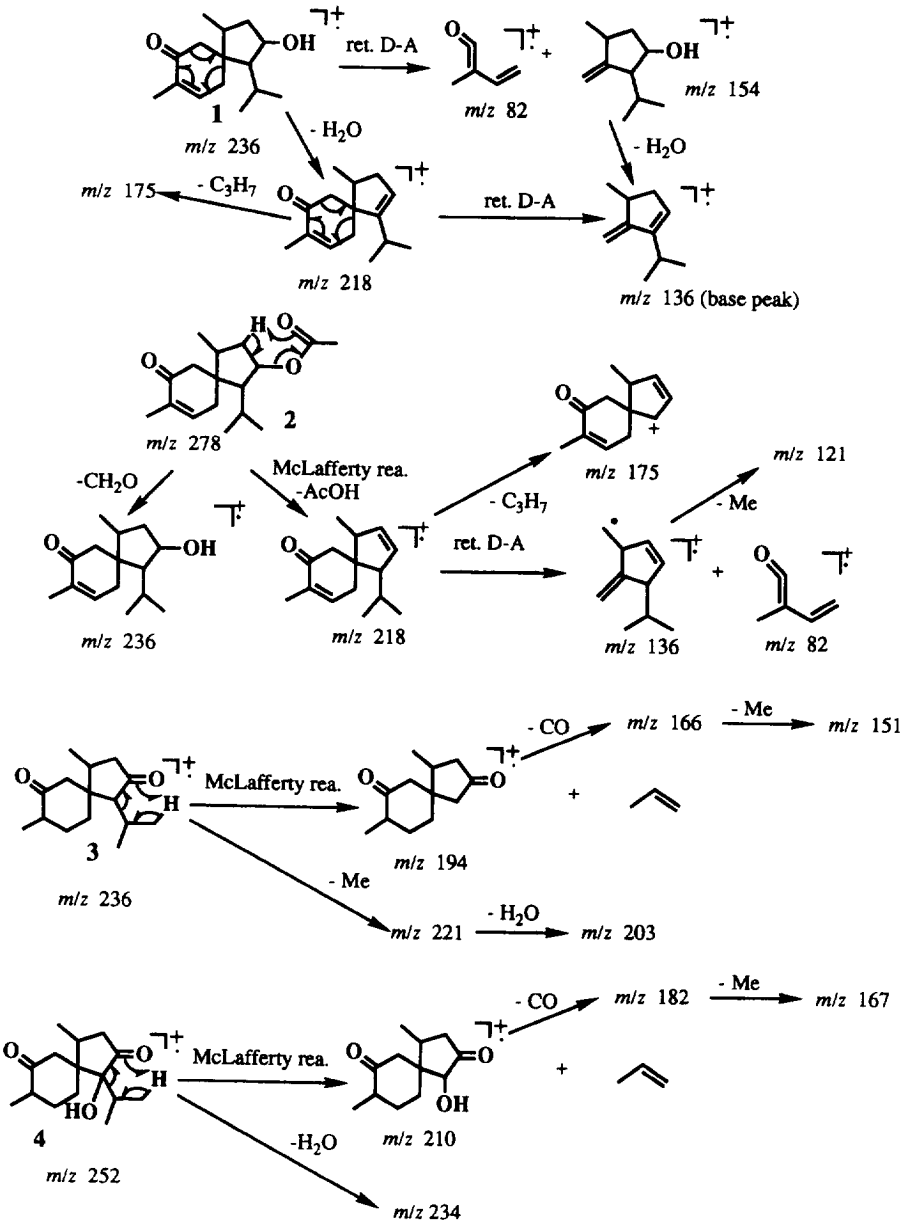
The molecular formula of AC-4 (**4**), mp 128–131°, $[\alpha]_D - 61.0°$, was determined to be $C_{15}H_{24}O_3$ from its EI-mass spectrum. The ^{1}H NMR and ^{13}C NMR spectra of **4** showed almost the same signal patterns as those in the spectra of **3** except for the presence of a tertiary hydroxyl group (δ 84.4). The position of the tertiary hydroxyl group was determined to be at C-1 from the presence of four secondary methyl groups. The structure of **4** was also supported by the mass spectral fragmentation based on the McLafferty rearrangement (Scheme 1). The structure of **4** was determined to be a 1-hydroxy derivative of **3** and it was unambiguously confirmed by the HMBC experiment shown in Fig. 3. The relative configurations of **3** and **4** were determined by difference NOE experiments as shown in Fig. 2. The NOEs were observed between: (i) H-6*eq* and CH_3-15, CH_3-12, CH_3-13, H-11; (ii) H-6*ax* and H-8, H-10*ax*, CH_3-12, CH_3-13; (iii) H-9*ax* and H-4. From these



R=H **1**
R=Ac **2**

R=H **3**
R=OH **4**

Fig. 1. ^{1}H–^{1}H COSY of compounds **1**–**4**.



Scheme 1. Mass spectral fragmentation of acorane type sesquiterpenes.

results the relative configurations of **3** and **4** were identified as shown in Fig. 2. The CD spectrum of **3** and **4** showed the same negative Cotton effects ($[\theta]_{302}$ − 9800 and $[\theta]_{300}$ − 12 000, respectively) based on the n→ π^* transition of carbonyl groups. Octant projection of the cyclohexanone parts of **3** and **4** showed a clear contribution to the CD sign. Octant projection of the cyclopentanone part also showed an ambiguous contribution to the CD sign, but the cyclohexanone system provided the stronger contribution to the CD spectrum. From the data, the absolute configurations of **3** and **4** were deduced as 1*S*,4*S*,5*S*,8*S* and 1*R*,4*S*,4*S*,5*S*,8*S*, respectively. Compounds **3** and **4** were named as epiacorone and 1-hydroxyepiacorone, respectively.

The molecular formula of AC-5 (5), $[\alpha]_D$ + 96°, was determined to be $C_{15}H_{22}O_2$ from mass spectrum. The ^{1}H NMR spectrum of **5** showed the presence of the characteristic isolated methylene (δ 2.11 *d*, J = 16.5 Hz and 2.23, *d*, J = 16.5, 1.5 Hz), a trisubstituted olefin conjugated to a carbonyl group, three secondary methyl groups and a vinyl methyl group (δ 1.79, *br s*). The ^{13}C NMR spectrum showed the presence of two carbonyl groups, of which one carbonyl (δ 198.9) was conjugated with an olefin group and the other one was non-conjugated (δ 216.2). There were also signals for two olefin carbons (δ 135.4 and 143.4). The ^{1}H–^{1}H COSY spectrum of **5** allowed the assignment of the ^{1}H NMR spectrum as shown in Table 1. The presence of an α,β-unsaturated carbonyl group was supported by

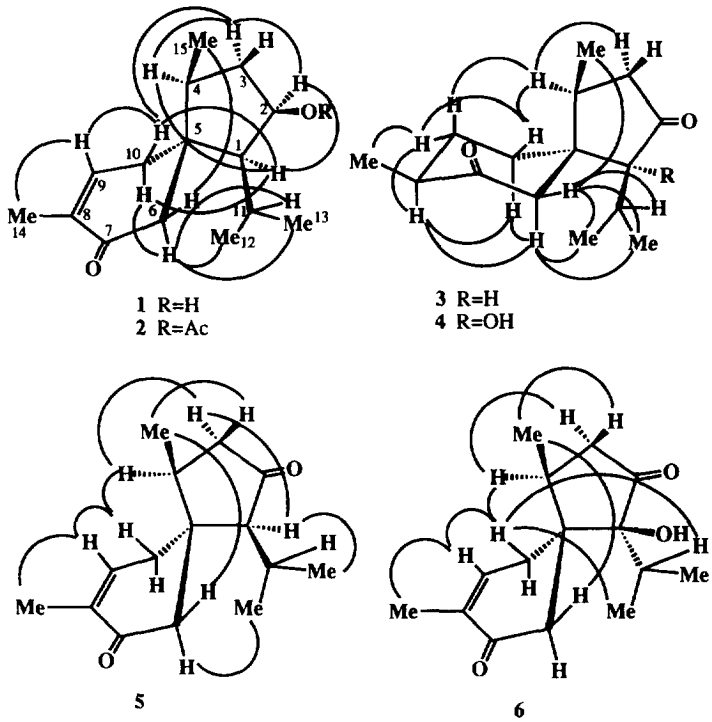


Fig. 2. Difference NOEs of compounds **1**–**6**.

the UV spectrum [238 nm (ε 3640)]. The mass spectral fragment representing $[M-42]^+$ arose from a McLafferty rearrangement and showed the presence of a carbonyl group adjacent to an isopropyl group. From these data the planer structure of **5** was deduced to be the same as that of acoronene. The relative configuration of **5** was determined on the basis of the difference NOEs of **5**. NOEs were observed between: (i) H-10*eq* and H-3α, H-4; (ii) CH_3-15 and H-6; (iii) H-6 and CH_3-12, as shown in Fig. 2. The absolute configuration of **5** was deduced to be 1*S*, 4*S*, 5*S*, from the CD spectrum, which showed a positive Cotton effect based on the $n \rightarrow \pi^*$ transition of cyclopentane. The absolute stereochemistry was further confirmed by oxidation of **1** with CrO_3–pyridine to give **5** which had a CD spectrum identical with that of natural **5**. Compound **5** was named epiacoronene.

The molecular formula of AC-6 (**6**), $[\alpha]_D$ + 115.0°, was determined to be $C_{15}H_{22}O_3$ from its EI-mass spectrum. A mass spectral fragment (*m/z* 208 $[M-42]^+$) also showed the presence of an isopropyl group adjacent to a carbonyl group. The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) of **6** showed the presence of three secondary methyl groups, two carbonyl groups, a trisubstituted olefinic group, an olefinic methyl group and a tertiary hydroxyl group. The presence of an α,β-unsaturated carbonyl group was shown by the UV data [234 nm (ε 6700)]. From these results and a ^1H–^1H COSY experiment, the structure was determined and the relative configuration was deduced from a difference NOE experiment as shown in Fig. 2. The absolute configuration of 6 was concluded to be 1*S*, 4*S*, 5*S* from the CD spectrum ($[\theta]_{242}$ + 15 276) based on the $\pi \rightarrow \pi^*$ transition of a conjugated cyclohexanone [12].

The molecular formula of AC-7 (**7**), $[\alpha]_D$ + 67.0°, was determined to be $C_{15}H_{24}O_3$ from its EI-mass spectrum. The ^1H NMR spectrum of **7** showed the presence of two secondary hydroxyl groups (δ 3.84, *dd*, J = 11.5, 5.0 Hz and 3.69, *br s*), two secondary methyl groups (δ 0.94, *d*, J = 7.0 Hz and 0.97, *d*, J = 7.0 Hz) attributed to an isopropyl group, a tertiary methyl group (δ 0.87, s) and an olefinic methyl group (δ 1.87, *br s*). The ^{13}C NMR spectrum of **7** showed the presence of two carbinyl carbons (δ 72.9, 69.9), a carbonyl group (δ 207.8) and a tetra-substituted olfin group (δ 133.7, 142.5). The presence of an α,β-unsaturated carbonyl group was also shown by the UV data [240 nm (ε 6260)]. From these results and a ^1H–^1H COSY experiment, the planar structure of **7** was deduced to have an eudesmane skeleton. This was further confirmed by an HMBC experiment as shown in Fig. 4. The relative stereostructure of **7** was determined from the coupling constants of H-1, H-3, H-7, H_2-8 and H_2-9 (Table 1), and the NOE experiments as shown in Fig. 5.

The molecular formula of AC-8 (**8**) was determined to be $C_{15}H_{24}O_2$ from the EI-mass spectrum. The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) of **8** showed that **8** was supposed to be a 3-dehydroxy derivative of **7**, and the relative configuration of **8** was supposed to be the same with that of **7** from the coupling constants at H-1, H-8 and H-9. The mass

Table 1. ^{1}H NMR data of sesquiterpenes (in $CDCl_3$)

H	1	2	3*	4*	5	6	7*	8
1	1.39, *dd* *J*=5.1, 10.5	1.27, overlap	1.60, overlap		1.42, *br s*		3.84, *dd* *J*=5.0, 11.5	3.59, *dd* *J*=4.5, 11.5
2	4.28, *ddd* *J*=2.5, 5.2, 6.2	5.15, *ddd* *J*=2.7, 5.7, 6.7					1.60, *m*	
3	1.33, *ddd* *J*=2.7, 7.8, 14.0 2.17, *ddd* *J*=6.2, 8.9, 14.0	1.3, overlap 2.27, *ddd* *J*=6.5, 8.9, 14.6	1.59, *br d* *J*=19.0 1.99, *dd* *J*=8.5, 19.0	2.07, *dd* *J*=7.5, 19.0 1.57, *dd* *J*=5.5, 18.0	1.93, *ddd* *J*=1.5, 6.0, 18.0 1.54, *dd* *J*=11.5, 18.0	1.83, *dd* *J*=9.5, 19.5 2.60, *dd* *J*=10.5, 19.5	3.69, *br s*	1.60, *m*
4	1.75, *m*	1.81, *m*	1.72, *m*	1.68, *m*	1.30, *m*	2.27, *ddq* *J*=7.0, 9.5, 10.5		
6	2.54, *d* *J*=17.0 2.71, *dd* *J*=1.1, 17.0	2.54, *d* *J*=17.5 2.64, *dd* *J*=1.1, 17.5	1.94, *d* *J*=13.0 2.14, *dd* *J*=2.5, 13.0	2.35, *d* *J*=12.5 2.60, *dd* *J*=2.5, 12.5	2.11, *d* *J*=16.5 2.23, *dd* *J*=1.5, 16.5	2.19, *d* *J*=16.0 2.05, *dd* *J*=2.5, 16.0		
7							1.90, *dt* *J*=12.5, 6.0	
8				1.79, *m*			1.68, *ddt* *J*=6.0, 13.0, 3.5 1.49, *qd* *J*=3.5, 13.0	1.70, *m* 1.44, *qd* *J*=12.5, 3.0
9	6.62, *m*	6.64, *m*	1.48, *m* 1.10, *m*	1.48, *m* 1.03, *qd* *J*=3.5, 13.2	6.02, *br s*	6.52, *ddq* *J*=1.5, 1.5, 7.0	1.38, *dt* *J*=13.0, 3.5 2.04, *br d* *J*=13.0	2.15, *m* 1.56, *dt* *J*=13.0, 3.5
10	2.86, *qd* *J*=2.4, 20.0 2.02, *br dd* *J*=5.0, 20.0	2.90, *qd* *J*=2.4, 19.7 2.08, *br dd* *J*=5.7, 19.7	1.29, *dt* *J*=12.5, 3.5 1.04, overlap	0.56, *qd* *J*=3.0, 14.0 1.92, *dt* *J*=14.0, 4.5	1.97, *dquin* *J*=18.5, 2.0 1.50, overlap	2.55, *dquin* *J*=18.5, 2.5 2.48, *dd* *J*=5.7, 18.5		
11	1.94, *dhep* *J*=6.5, 10.5	1.93, *m*	1.78, *m*	1.79, *m*	1.56, *m*	2.05, sep *J*=7.0	2.37, *m*	2.30, *dhep* *J*=4.5, 6.5
12	1.08, *d* *J*=6.5	1.06, *d* *J*=6.2	1.09, *d* *J*=6.5	0.98, *d* *J*=6.5	1.13, *d* *J*=7.0	0.96, *d* *J*=7.0	0.97, *d* *J*=7.0	0.92, *d* *J*=7.0
13	1.02, *d* *J*=6.5	0.88, *d* *J*=6.2	1.03, *d* *J*=6.5	0.96, *d* *J*=6.5	1.00, *d* *J*=7.0	1.06, *d* *J*=7.0	0.94, *d* *J*=7.0	0.87, *d* *J*=7.0
14	1.75, *br s*	1.78, *br s*	1.17, *d* *J*=7.0	0.62, *d* *J*=6.5	1.79, *br s*	1.76, *br s*	1.87, *br s*	1.67, *br s*
15	0.94, *d* *J*=7.5	0.98, *d* *J*=7.0	0.72, *d* *J*=7.5	1.01, *d* *J*=7.5	0.68, *d* *J*=7.0	0.79, *d* *J*=7.0	0.87, *d*	0.90, *s*
$COCH_3$		2.03, *s*						

*Measured in C_6D_6.

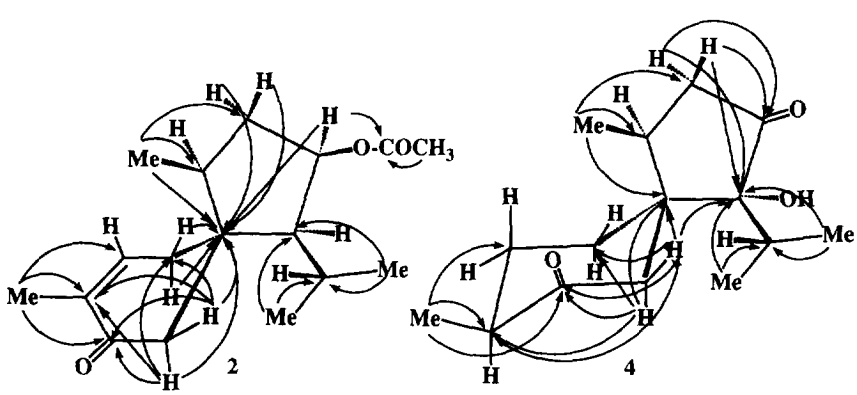


Fig. 3. HMBC of compounds **2** and **4**.

Table 2. ^{13}C NMR data of sesquiterpenes (in $CDCl_3$)*

C	1	2	3	4	5	6	7	8	9
1	62.0	60.2	60.3	84.4	66.4	87.1	72.9	76.8	60.8
2	73.4	76.4	212.5	213.1	216.2	218.3	35.9	26.7	218.4
3	41.8	39.9	40.3	40.3	44.8	40.5	69.9	32.1	45.1
4	46.7	46.6	31.5	31.5	41.5	30.2	142.5	139.2	45.2
5	47.4	47.7	48.1	51.6	48.1	51.4	133.7	136.4	48.7
6	40.5	39.1	45.5	45.2	39.8	39.1	207.8	206.8	31.6
7	200.9	200.4	217.7	218.0	198.8	198.1	58.2	57.5	198.8
8	135.0	135.1	44.4	44.5	135.4	136.1	22.6	21.7	136.2
9	144.3	144.1	29.9	30.4	143.4	139.4	37.0	37.0	143.1
10	39.5	39.3	32.7	30.9	37.8	29.1	44.0	43.0	36.3
11	25.3	25.4	25.0	31.7	25.8	33.9	25.8	25.8	28.1
12	22.8	22.8	24.6	18.5	16.0	17.9	17.8	18.2	15.3
13	23.3	22.9	16.3	18.0	18.9	19.3	21.1	21.0	19.5
14	15.8	15.7	14.3	14.1	15.6	15.6	16.3	20.7	15.5
15	18.6	18.1	19.6	19.6	24.8	15.7	18.4	18.3	23.6
CO		170.6							
CH_3		21.4							

*Assignments were confirmed by ^1H–^{13}C COSY and/or HMBC experiments.

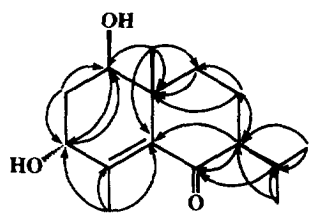


Fig. 4. HMBC of compound **7**.

spectral fragmentation (*m/z* 194 $[M-42]^+$) also supported the structure having an isopropyl group adjacent to a carbonyl group.

The absolute configuration of **7** was determined from the CD spectrum, which was based on the n→π* transition of the *S-cis* enone system of the conjugated ketone and showed negative Cotton effect. This could be attributed to the anticlockwise helicity as shown in Fig. 5. The CD spectrum based on π→π* transition of **7** gave a positive Cotton effect ($[\theta]_{247}$ + 25 536), which was the same as that of steroid derivatives having a 4-en-6-one moiety [13]. From these facts the absolute configuration of **7** was supposed to be 1*R*, 3*R*, 7*S*,10*R*. This was further confirmed by the exciton chirality rule [14, 15]. The dibenzoate of **7** showed a strong Cotton effect [$\Delta\varepsilon$ + 39 (249 nm)] attributed to clockwise helicity of the dibenzoate (Fig. 5). Compound **8** also showed the same Cotton effect ($[\theta]_{317}$ − 2246, $[\theta]_{245}$ + 27 629) and thus has the same configuration (1*R*, 7*S*, 10*R*) as that of **7**.

The known sesquiterpenes acorone, acoronene and isocalamendiol were isolated together with the aromatic compounds asarone, asaraldehyde, 1-(2,4,5-trimethoxyphenyl)-propane-1,2-dione (**14**) and 1-(2,4,5-trimethoxyphenyl)-1-methoxypropan-1-2-ol (**15**). The structures of these compounds were identified from their spectral data. Compounds **14** and **15** could be artificially transformed from asarone through oxidation, rearrangement and methanol addition.

The anti-germination activity of some of these

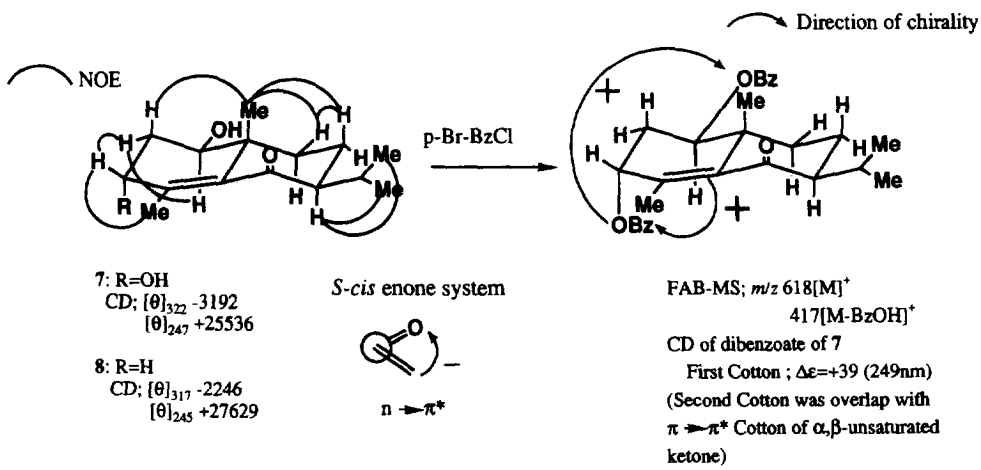


Fig. 5. NOE and CD data of compounds **7** and **8**.

Table 3. Germination ratio of lettuce seeds treated with *Acorous calamus* constituents (at 125 μg ml^{-1})*

Compounds	3 day (% control)	5 day (% control)	7 day (% control)
Control	100	100	100
1	90	100	110
2	95	100	100
4	0	0	0
5	0	0	42
7	30	82	88
9	0	0	75
12	0	0	11
13	140	95	95

*20 seeds were used for each experiment.

constitutents was examined using lettuce seeds. The results are shown in Table 3. Compounds **4** and **12** showed potent activity and **5** and **9** showed some activity, but the other compounds showed no activity. The most active compound (**4**) was tested against several kinds of seeds and the results are shown in Table 4. Compound **4** showed the most potent activity against lettuce seeds, and it was relatively potent against carrot seeds, but had no activity against burdock, white radish, Chinese cabbage and tomato seeds. Asarone showed potent anti-germination activity, but growth inhibitory activity was more potent against lettuce and carrot.

EXPERIMENTAL

Extraction and isolation of the constituents. Acori rhizoma (*Acorus calamus* L.), 2 kg, purchased from Niiya Co. Ltd. in Shimizu city, was extracted with MeOH under reflux to give a MeOH extract. The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc soluble fr showed anti-germination activity (completely inhibiting germination of lettuce seeds at a concentration of 100 μg ml^{-1}). The EtOAc (102 g) fr. was sepd by silica-gel CC to give eight frs of which fr. 3 and fr. 6 showed the most potent activity (completely inhibiting the germination of seeds at a concn of 100 μg ml^{-1}. These frs were further purified with silica-gel CC and prep. HPLC an ODS column successively, to give AC-1-8, acorone, acorenone, isocalamendiol, asarone, asaraldehyde, 1-(2,4,5-trimetoxyphenyl)- propane-1,2-dione and 1-(2,4, 5-trimethoxyphenyl) -1-methoxy-propane-2-ol.

Table 4. Germination ratio of several kinds of seeds treated with **4** (at 100 μg ml^{-1})*

Seed name	3 day (% control)	5 day (% control)	7 day (% control)
Control	100	100	100
Lettuce	0	0	0
Carrot	28	30	50
Burdock	100	100	100
White radish	115	100	100
Tomato	115	90	90

*20 seeds were used for each experiment.

2-Hydroxyacorenone (**1**). Oil (11 mg). HR-MS *m/z*: 236.1804 $[M]^+$, calcd 236.1776 for $C_{15}H_{24}O_2$. EI-MS *m/z*: 236 $[M]^+$ $C_{15}H_{24}O_2$, 221 $[M-CH_3]^+$ $C_{14}H_{21}O_2$, 218 $[M-H_2O]^+$ $C_{15}H_{22}$, 175 $[M-H_2O-C_3H_7O]^+$ $C_{12}H_{15}O$. $[\alpha]_D$ −44.9° [*c* = 0.29, MeOH). UV λ_{max}^{MeOH} nm (ε): 240 (6570). CD; $[\theta]_{320}$ −814 (MeOH). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

2-Acetoxyacorenone (**2**). Oil (80 mg). HR-MS *m/z*: 278.1900, calcd 278.1882 $[M]^+$ for $C_{17}H_{26}O_3$. EI-MS *m/z*: 278 $[M]^+$ $C_{17}H_{26}O_3$, 236 $[M-CH_2{=}CO]^+$ $C_{15}H_{24}O_2$, 218 $[M-AcOH]^+$ $C_{15}H_{22}O$, 175 $[M-C_3H_7]^+$, $C_{12}H_{15}O$, 136 $C_{10}H_{16}$, 82 C_5H_6O. $[\alpha]_D$ −75.8° (*c* = 0.31). CD; $[\theta]_{310}$ −778 (MeOH). UV λ_{max}^{MeOH} nm (ε): 240 (7070). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

Epiacorone (**3**). Oil (18 mg). EI-MS *m/z*: 236 $[M]^+$ $C_{15}H_{24}O_2$, 221 $[M-CH_3]^+$ $C_{14}H_{21}O_2$, 203 $[221-H_2O]^+$ $C_{14}H_{19}O$, 194 $[M-C_3H_6]^+$ $C_{12}H_{18}O_2$, 166 $[194-CO]^+$ $C_{11}H_{18}O$. $[\alpha]_D$ −53.9° (*c* = 0.54, MeOH). CD: $[\theta]_{302}$ −9800 (MeOH). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

1-Hydroxyepiacorone (**4**). Needles (90 mg) mp 128–131°C (MeOH). HR-MS *m/z*: 252.1680, calcd 252.1725 $[M]^+$ $C_{15}H_{24}O_3$. EI-MS *m/z*: 252 $[M]^+$ $C_{15}H_{24}O_3$, 234 $[M-H_2O]^+$ $C_{15}H_{24}O_2$, 234 $[M-H_2O]^+$ 210 $[M-C_3H_6]^+$ $C_{12}H_{18}O_3$, 182 $[210-CO]^+$ $C_{11}H_{15}O_2$. $[\alpha]_D$ −61.0° (*c* = 0.32, MeOH). CD: $[\theta]_{300}$ −12 000 (MeOH). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

Epiacoronene (**5**). Oil (20 mg). HR-MS *m/z*: 234.1662, calcd 234.1620 $[M]^+$ $C_{15}H_{22}O_2$. EI-MS *m/z*: 234 $[M]^+$ $C_{15}H_{22}O_2$, 219 $[M-CH_3]^+$ $C_{14}H_{19}O_2$, 192 $[M-C_3H_6]^+$ $C_{12}H_{16}O_2$, 191 $[M-C_3H_7]^+$ $C_{12}H_{15}O_2$. $[\alpha]_D$ −96.0° (*c* = 0.12, MeOH). CD; $[\theta]_{302}$ +8892 (MeOH). UV λ_{max}^{MeOH} nm (ε): 238 (3640). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

1-Hydroxyacoronene (**6**). Oil (30 mg). HR-MS *m/z*: 250. 1529, calcd 250.1569 for $C_{15}H_{22}O_3$. EI-MS *m/z*: 250 $[M]^+$ $C_{15}H_{22}O_2$, 208 $[M-42]^+$ $C_{12}H_{16}O_3$, 180 $[208-CO]^+$ $C_{11}H_{16}O_2$. CD; $[\theta]_{345}$ −192, $[\theta]_{205}$ +465, $[\theta]_{242}$ +15 276 (MeOH).UV λ_{max}^{MeOH}nm (ε): 234 (6700). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

Acorusdiol (**7**). Viscous oil (100 mg). HR-MS *m/z*: 252.1747, calcd 252.1725 $[M]^+$ $C_{15}H_{24}O_3$. EI-MS *m/z*: 252 $[M]^+$ $C_{15}H_{24}O_3$, 234 $[M-H_2O]^+$ $C_{15}H_{22}O_2$, 219 $[234-CH_3]^+$ $C_{14}H_{19}O_2$, 216 $[234-H_2O]^+$ $C_{15}H_{20}O$, 210 $[M-C_3H_6]^+$ $C_{12}H_{18}O_3$, 209 $[M-C_3H_7]^+$ $C_{12}H_{17}O_3$. $[\alpha]_D$ −67.0° (*c* = 0.03, MeOH). CD; $[\theta]_{322}$ −3192, $[\theta]_{247}$ +25 536 (MeOH). UV λ_{max}^{MeOH} nm (ε): 240 (6260). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

Acorusnol (**8**). Oil (30 mg). HR-MS *m/z*: 236.1747, calcd 236.1725 for $C_{15}H_{24}O_2$. EI-MS *m/z*: 236 $[M]^+$ $C_{15}H_{24}O_2$, 221 $[M-CH_3]^+$ $C_{14}H_{21}O_2$, 218 $[M-CH_3]^+$ $H_{14}H_{21}O_2$, 194 $[M-C_3H_6]^+$ $C_{12}H_{18}O_2$, 193 $[M-C_3H_7]^+$ $C_{12}H_{17}O_2$. CD; $[\theta]_{317}$ −2246, $[\theta]_{245}$ + 27 629 (MeOH). UV λ_{max}^{MeOH} nm (ε): 247 (8810). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

Acoronene (**9**). Oil (50 mg). EI-MS *m/z*: 234 $[M]^+$ $C_{15}H_{22}O_2$, 219 $[M-CH_3]^+$ $C_{14}H_{19}O_2$, 192 $[M-C_3H_6]^+$ $C_{12}H_{16}O_2$, 191 $[M-C_3H_7]^+$ $C_{12}H_{15}O_2$. $[\alpha]_D$ −104.0° (*c* = 0.12, MeOH). $[\theta]_{350}$ +2625 (MeOH). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

Acorone (**10**). Oil (120 mg), $[\alpha]_D$ 132 (*c* = 0.2, MeOH), HR-MS *m/z*: 236.1798, calcd 236.1776 $[M]^+$ $C_{15}H_{24}O_2$. ^{1}H NMR ($CDCl_3$); δ 0.90 (3H, *d*, *J* = 6.5 Hz, Me), 1.01 (3H, *d*, *J* = 6.5 Hz, Me), 1.04 (3H, *d*, *J* = 6.0 Hz, Me), 1.06 (3H, *d*, *J* = 7.0 Hz, Me), 1.32 (1H, *dq*, *J* = 3.5, 13.0 Hz, H-9). 2.45 (1H, *m*, H-8). ^{13}C NMR ($CDCl_3$); δ 14.2, 14.4, 19.4, 23.7, 26.4, 29.2, 32.9, 37.7, 44.5, 45.1, 47.1, 51.3, 59.7, 212.0, 218.9.

Isocalamendiol (**11**). Viscous oil (110 mg). HR-MS *m/z*: 238.1978, calcd 238.1933 $[M]^+$ $C_{15}H_{26}O_2$. $[\alpha]_D$ −20.0° (*c* = 0.3, MeOH). ^{1}H NMR (benzene-d_6; δ 0.95 (3H, *d*, *J* = 7.0 Hz, Me), 1.09 (3H *d*, *J* = 6.5 Hz, Me), 1.28 (3H, *s*), 1.69 (1H, *dd*, *J* = 13.0, 2.0 Hz, H-4), 2.39 (1H, *dd*, *J* = 13.0, 1.2 Hz, H-4), 4.64 (1H, *br s*, H-14), 4.74 (1H, *br s*, H-14). ^{13}C NMR benzene-d_6); δ 18.7, 19.8, 23.1, 23.3, 2.1, 25.5, 35.0, 43.6, 47.1, 51.6, 54.2, 71.8, 75.0, 111.4, 146.4.

Asarone (**12**). Oil (500 mg). EI-MS *m/z*: 208 $[M]^+$ $C_{12}H_{16}O_3$, 193 $[M-CH_3]^+$ $C_{11}H_{13}O_3$, ^{1}H NMR ($CDCl_3$); δ 1.83 (3H, *dd*, *J* = 7.0, 2.0 Hz, Me), 3.79, 3.83, 3.88 (3H each, *s*, OMe), 5.75 (1H, *dq*, *J* = 17.0, 7.0 Hz), 6.47 (1H, *dq*, *J* = 17.0, 2.0 Hz), 6.52 (1H, *s*), 6.83 (1H, *s*).

Asaraldehyde (**13**). Oil. EI-MS *m/z*: 196 $[M]^+$ $C_{10}H_{14}O_5$. ^{1}H NMR ($CDCl_3$); δ 3.86, 3.92, 3.96 (3H each, *s*, OMe), 6.49 (1H, *s*), 7.32 (2H, *s*), 10.3 (1H, *s*).

1-(2,4,5-*Trimethoxyphenyl*)-*propane*-1,2-*dione* (**14**). Oil (90 mg), EI-MS *m/z*: 238 $[M]^+$ $C_{12}H_{14}O_5$. ^{1}H NMR ($CDCl_3$); δ 2.38 (3H, *s*, Me), 3.80, 3.87, 3.95 (3H each, *s*, OMe), 6.47 (1H, *s*), 7.32 (1H, *s*).

1-(2,4,5-*Trimethoxyphenyl*) - 1-*methoxypropan*-2-*ol* (**15**). Oil (80 mg), $[\alpha]_D$ −0° (*c* = 0.2, MeOH). EI-MS *m/z*: 256 $[M]^+$ $C_{13}H_{20}O_5$. 238 $[M-H_2O]^+$ $C_{13}H_{18}O_4$, 221 $[M-C_2H_5O]^+$. ^{1}H NMR ($CDCl_3$); 0.99 (3H, *d*, *J* = 6.5 Hz, Me), 3.22 (3H, *s*, alcoholic OMe), 3.83 (1H, *br quin*, *J* = 6.5 Hz), 3.80, 3.83, 3.89 (3H each, *s*, OMe), 4.40 (1H, *d*, *J* = 8.1 Hz), 6.52 (1H, *s*), 6.80 (1H, *s*.)

Acetylation of **1**. Compound **1** (15 mg) was acetylated with Ac_2O in pyridine. The reaction product was purified by prep. TLC to give **2** (16 mg), whose NMR and CD data were identical with those of natural **2**.

p-*Bromobenzoylation of* **7**. Compound **7** was benozylated with excess *p*-bromobenzoyl chloride in pyridine at 80° overnight. The reaction soln was poured into H_2O and extracted with hexane. The hexane soln was concd and purified by prep. TLC, and further purified by HPLC. The collected benzoate fr. was directly measured by UV and CD. (From the UV absorption, the concn of the benzoate was obtained and then the A-value was calculated).

CrO_3 *oxidation of* **1**. To a soln of **1** (10 mg) in pyridine (1 ml), CrO_3 (50 mg, excess) in pyridine (1 ml) was added at 0° and the mixture stirred for 30 min at room temp. The reaction soln was poured into H_2O and extracted with EtOAc. The EtOAc soln was washed with dil. HCL and then H_2O, and concd to give **5**. The product was purified with prep. TLC to give pure **5** (6 mg), which was identified with natural **5** by ^{1}H NMR and CD.

Anti-germination activity. Samples were dissolved in distilled H_2O at several concentrations. The sample solns were poured into Petri-dishes with filter papers and 20 lettuce seeds were put on to the moistened filter papers, and left at 24° in the dark. The seeds were examined each day for 1 week and the germination ratio was obtained at each day.

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