Notes

Antiemetic Principles of *Alpinia officinarum*

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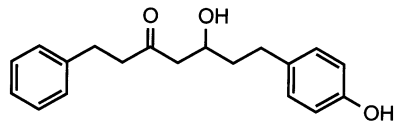
Bioasay-guided fractionation of the antiemetic constituents of *Alpinia officinarum* was performed, and eight compounds (**1**−**8**) including a new compound were isolated. Among the seven known compounds, two flavonoids (**1**, **2**), four diarylheptanoids (**3**, **5**, **6**, **8**), and one sterol (**4**) were obtained, with five (**2**−**6**) of those compounds showing antiemetic activity in a copper sulfate induced emesis assay in young chicks. The structure of the new compound **7**, which also showed antiemetic activity, was determined as 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-3-heptanone. The structure of **7** was established on the basis of spectroscopic data interpretation.

As part of an investigation on antiemetic principles from natural sources, we have reported several compounds that show antiemetic activities in a copper sulfate ($CuSO_4$) induced emesis assay in young chicks.[1−4] *Alpinia officinarum* Hance (Zingiberaceae), a Chinese herbal drug, has been used as an aromatic, stomachic, analgesic, and antiemetic.[5] Several constituents of the rhizomes of *A. officinarum* have been reported, namely, 1,8-cineole, methyl cinnamate, α-cadinene, galangin, kaempferide, alpinin, galangol, and some diarylheptanoids.[6,7] In this paper, we report the isolation and characterization of the antiemetic principles of *A. officinarum*, including a new diarylheptanoid (**7**).

Diarylheptanoids are common in the genus *Alpinia* and are known from *A. officinarum*,[6−10] *A. katsumadai*,[11] *A. oxyphlla*,[12,13] *A. conchigera*,[14] and *A. blepharocalyx*,[15] all of which have been used as antiemetics in Chinese traditional medicine. In a previous paper, we reported on the principles of *A. katsumadai*[11] and the structural relationships of some antiemetic diarylheptanoids and their analogues.[16]

The rhizomes of *A. officinarum* (5 kg) were extracted successively with *n*-hexane, $CHCl_3$, MeOH, and H_2O. Each extract was examined for antiemetic activity using a standard bioassay protocol. The $CHCl_3$ extract showed antiemetic activity (45.6% inhibition at a dose of 300 mg/kg). Inhibitions of the *n*-hexane, MeOH, and H_2O extracts were 15.7, 26.5, and 29.9% at a dose of 300 mg/kg, respectively (Table 1S in the Supporting Information). The $CHCl_3$ extract was separated into seven fractions (Fr-1−Fr-7), and Fr-5 and Fr-6 showed significant antiemetic activities (60.8 and 63.6% at a dose of 50 mg/kg) (Table 1S in the Supporting Information). Compounds **1**−**3** isolated from Fr-5 were identified by comparison of their published spectral data (UV, IR, ^{1}H and ^{13}C NMR, and MS data are listed in the Supporting Information) as galangin (**1**),[17] kaempferide (**2**),[17] and the diarylheptanoid 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-3-heptanone (**3**),[8] respectively. Compounds **4**, **5**, and **8** isolated from Fr-6 were identified as β-sitosterol 3-*O*-β-D-6-palmitoylglucoside (**4**)[18] and the diarylheptanoids (3*R*,5*R*)-1-(4-hydroxyphenyl)-7-phenyl-3,5-heptanediol (**5**)[9] and 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-3-heptanone (**8**),[19] respectively, by the same methods (spectral data are shown in the Supporting Information).



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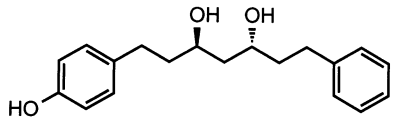

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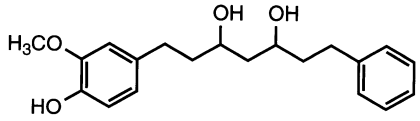

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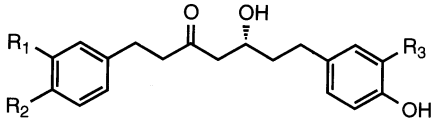

7 R_1 = H, R_2 = OH, R_3 = OCH_3

8 R_1 = OCH_3, R_2 = OH, R_3 = H

Compound **6** was obtained as a yellow liquid. The molecular formula, $C_{20}H_{26}O_4$ (M^+ 330.1832, calcd 330.1832), was determined by HREIMS. The IR spectrum suggested the presence of one or more hydroxyl (3400 cm^{-1}) groups. The ^{1}H NMR spectrum (Table 1) of **6** indicated the presence of two phenyl groups [δ_H 7.17−7.30 (5H), 6.83 (1H, d, J = 7.9 Hz), 6.68 (1H, dd, J = 7.9, 1.8 Hz), and 6.69 (1H, d, J = 1.8 Hz)], two methine protons at δ_H 3.99, and a methoxy group at δ_H 3.87. From the ^{13}C NMR and DEPT spectra (Table 1), the presence of five methylenes (δ_C 32.0, 32.2,

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Table 1. ^{13}C and ^{1}H NMR Spectral Data of Compounds **6** and **7** in $CDCl_3$ (δ ppm)

	6		**7**	
position	δ_C	δ_H	δ_C	δ_H
1	32.2	2.60 (1H, ddd, J = 13.9, 9.5, 6.4 Hz) 2.79 (1H, ddd, J = 13.9, 9.8, 5.8 Hz)	28.7	2.82 (2H, t, J = 7.3 Hz)
2	39.2	1.70−1.90 (2H, m)	45.3	2.70 (2H, t, J = 7.3 Hz)
3	68.96	3.99 (1H, m)	211.4	
4	42.7	1.66 (2H, t, J = 5.5 Hz)	49.3	2.53 (2H, m)
5	68.98	3.99 (1H, m)	67.0	4.03 (1H, m)
6	39.4	1.70−1.90 (2H, m)	38.3	1.63, 1.77 (each 1H, m)
7	32.0	2.69 (2H, m)	31.4	2.59, 2.71 (each 1H, m)
1′	133.7		132.7	
2′	110.9	6.69 (1H, d, J = 1.8 Hz)	129.4[a]	7.01 (2H, d, J = 8.7 Hz)
3′	146.4		115.4[b]	6.73 (2H, d, J = 8.7 Hz)
4′	143.7		154.1	
5′	114.6	6.83 (1H, d, J = 7.9 Hz)	115.4[b]	6.73 (2H, d, J = 8.7 Hz)
6′	120.8	6.68 (1H, dd, J = 7.9, 1.8 Hz)	129.4[a]	7.01 (2H, d, J = 8.7 Hz)
1″	141.8	7.17−7.30 (5H, m)	133.7	
2″	128.3[a]		111.2	6.69 (1H, d, J = 1.9 Hz)
3″	128.4[b]		146.4	
4″	125.9		143.8	
5″	128.4[b]		114.3	6.82 (1H, d, J = 8.1 Hz)
6″	128.3[a]		121.0	6.66 (1H, dd, J = 8.1, 1.9 Hz)
−OCH_3	55.9	3.87 (3H, s)	55.9	3.86 (3H, s)

[a,b] Overlapping peaks.

39.2, 39.4, and 42.7), one methoxy (δ_C 55.9), two methines (δ_C 68.96 and 68.98), and 12 sp^2 carbons was inferred. In the $^{1}H-^{1}H$ COSY experiment, two methine protons at δ_H 3.99 (2H, H-3 and H-5) showed correlations with methylene protons at δ_H 1.70−1.90 (2H, H-2) and 1.66 (2H, H-4). The four methylene protons at δ_H 1.70−1.90 (4H, H-2 and H-6) showed correlations with methylene protons at δ_H 2.60 and 2.79 (each 1H, H-1) and 2.69 (2H, H-7). Moreover, correlations from the $^{1}H-^{1}H$ COSY spectrum and the coupling constants of the aromatic protons in the ^{1}H NMR spectrum suggested the presence of a monosubstituted benzene unit and a 1,3,4-trisubstituted benzene unit. In the ^{2}J and ^{3}J HMBC experiment, the methylene protons at δ_H 2.60 (H-1) showed correlations with the methylene carbons at δ_C 39.2 (C-2), 68.96 (C-3), 133.7 (C-1′), 110.9 (C-2′), and 120.8 (C-6′), and the methylene protons at δ_H 2.69 (H-7) showed correlations with the methylene carbons at δ_C 68.98 (C-5), 39.4 (C-6), 141.8 (C-1″), and 128.3 (C-2″ and C-6″). The methoxyl group and the hydroxyl group on the 1,3,4-trisubstituted benzene unit were determined from HMQC and ^{2}J and ^{3}J HMBC spectral correlations. Thus, the structure of **6** was assigned as 1-(4-hydroxy-3-methoxyphenyl)-7-phenyl-3,5-heptanediol. The stereochemistry of the hydroxy groups at C-3 and C-5 has not been determined. Although this compound is known as a synthetic compound,[10] full structural elucidation and unambiguous assignments of ^{1}H and ^{13}C NMR are reported for the first time in this paper.

Compound **7** was obtained as a pale yellow liquid. The molecular formula, $C_{20}H_{24}O_5$ (M^+ 344.1627, calcd for 344.1642), was determined by HREIMS. The IR spectrum suggested the presence of carbonyl (1700 cm^{-1}) and hydroxyl (3375 cm^{-1}) absorptions. The ^{1}H NMR spectrum (Table 1) of **7** exhibited seven aromatic protons [δ_H 6.66 (1H, dd, J = 8.1, 1.9 Hz), 6.69 (1H, d, J = 1.9 Hz), 6.73 (2H, d, J = 8.7 Hz), 6.82 (1H, d, J = 8.1 Hz), and 7.01 (2H, d, J = 8.7 Hz)], one methine proton at δ 4.03, and a methoxy group at δ 3.86. The ^{13}C NMR and DEPT spectra (Table 1) indicated the presence of five methylenes (δ_C 28.7, 31.4, 38.3, 45.3, and 49.3), one methoxy (δ_C 55.9), one methine (δ_C 67.0), one carbonyl (δ_C 211.4), and 12 sp^2 carbons. In the $^{1}H-^{1}H$ COSY experiment (Figure 1), two methylene protons at δ_H 1.63 and 1.77 (each 1H, m, H-6) and 2.53 (2H, m, H-4) were coupled with the signal at δ_H 4.03 (1H, m, H-5). The two methylene protons at δ_H 2.82 (H-1) and 2.70 (H-2) and two methylene protons at δ_H 1.63 and 1.77 (H-6) and 2.59 and 2.71 (H-7) also exhibited respective mutual correlations. Moreover, the correlation of $^{1}H-^{1}H$ COSY and the coupling constants of the aromatic protons suggested the presence of a 1,4-disubstituted benzene unit and a 1,3,4-trisubstituted benzene unit. In ^{2}J and ^{3}J HMBC experiments (Figure 1), the methylene protons at δ_H 2.82 (H-1) correlated with the quaternary carbon at δ_C 132.7 (C-1′), the carbonyl carbon at δ_C 211.4 (C-3), and the methine carbons at δ_C 111.2 (C-2′) and 121.0 (C-6′). The methylene proton at δ_H 2.70 (H-2) showed HMBC correlations with resonances at δ_C 28.7 (C-1), 211.4 (C-3), and 132.7 (C-1′). The location of the carbonyl carbon at δ_C 211.4 (C-3) was ascertained from HMBC correlations of the methylene protons at δ_H 2.82 (H-1), 2.70 (H-2), and 2.53 (H-4). The assignments at C-1″, C-2″, and C-6″ were determined from the HMBC correlations of the methylene protons at δ_H 1.63 (H-6) and 2.59 and 2.71 (H-7). From these HMQC and ^{2}J and ^{3}J HMBC experiments, assignments of all protons and carbons were elucidated as shown in Figure 1. Itokawa et al.[7] have reported on the absolute configuration assignment of the hydroxyl group of the two diarylheptanoids, hexahydrocurcumin and dihydroyashabushiketol, by application of the circular dichroism (CD) excition chirality method.[7,9] The hydroxy group absolute configuration of compounds showing negative first and positive second Cotton effects was determined to be 5*S*, and those showing first positive and second negative Cotton effects determined as 5*R* using CD spectra. The CD spectrum of **7** in $CHCl_3$ exhibited first positive (290.5 nm,

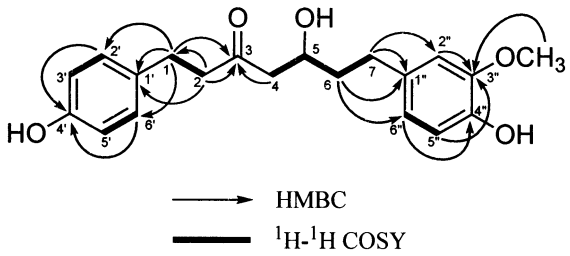

Figure 1. HMBC and $^{1}H-^{1}H$ COSY correlations of **7**.

Table 2. Antiemetic Effects of Compounds **1**−**8** from *Alpinia officinarum* on Copper Sulfate Induced Emesis in Young Chicks

compound	dose (mg/kg)	no. of young chicks	no. of retching (mean ± SEM)	inhibition (%)
control		5	55.8 ± 2.03	
1	20	5	41.6 ± 2.32[a]	25.4
2	20	5	20.3 ± 1.80[b]	63.3
3	20	5	16.2 ± 3.06[b]	71.0
control		6	47.2 ± 1.08	
4	50	6	23.2 ± 1.25[b]	50.9
5	50	5	29.4 ± 1.91[b]	37.7
control		6	56.2 ± 1.14	
6	50	6	30.5 ± 2.86[b]	45.7
control		6	57.0 ± 2.21	
7	50	6	35.2 ± 2.89[b]	38.3
control		5	50.0 ± 1.92	
8	50	5	43.8 ± 1.53	12.4

[a,b] Significantly different from the control value, [a] $p < 0.01$, [b] $p < 0.001$.

$\Delta\epsilon$ $+1.3 \times 10^{-3}$) and second negative (270.6 nm, $\Delta\epsilon$ -1.2×10^{-3}) Cotton effects. Thus, the absolute configuration of the hydroxy group was 5*R* and the structure of **7** determined as (5*R*)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-3-heptanone. However, the $\Delta\epsilon$ values of (5*R*)-hexahydrocurcumin ($[\alpha]_D$ −10.0°) and (5*R*)-dihydroyashabushiketol ($[\alpha]_D$ −16.9°) were 7×10^{-2} and 4.5×10^{-2} as first positive Cotton effects at 300 nm, and the $\Delta\epsilon$ values of (5*S*)-hexahydrocurcumin ($[\alpha]_D$ +9.0°) and (5*S*)-dihydroyashabushiketol ($[\alpha]_D$ +9.0°) were 4.5×10^{-2} and 3.8×10^{-2} as first negative Cotton effects at 300 nm, respectively.[7] In contrast, the $\Delta\epsilon$ value of **7** was $+1.3 \times 10^{-3}$ as first positive Cotton effect at 290.5 nm. Since the $\Delta\epsilon$ value and $[\alpha]_D$ value of compound **7** were very small compared with hexahydrocurcumin and dihydroyashabushiketol, compound **7** was considered to be nearly racemic. The absolute configuration of the hydroxyl group of compound **8** was also examined by the same method. The CD spectrum of **8** in $CHCl_3$ exhibited a positive first (298.2 nm, $\Delta\epsilon$ $+9.9 \times 10^{-4}$) Cotton effect. Thus, the absolute configuration of the hydroxy group of **8** could be suggested as 5*R*, but compound **8** was assumed to have nearly the same optical purity as compound **7**.

Among the eight compounds (**1**−**8**) isolated from the antiemetic fraction, galangin (**1**) and 5-hydroxy-7-(4-hydroxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-3-heptanone (**8**) showed no antiemetic effects (Table 2). The other six compounds (**2**−**7**) showed significant antiemetic activity induced by $CuSO_4$. Since the yields of compounds **2** and **3** were relatively high and their antiemetic effects were potent, their dose responses were examined. Compound **2** showed dose-dependent inhibition of 23.1, 34.7, and 62.5%, respectively, at doses of 5, 10, and 20 mg/kg. Compound **3** showed 30.3, 48.8, and 64.7% dose-dependent inhibition at doses of 5, 10, and 20 mg/kg, respectively.

Although the aromatic units of the diarylheptanoids from *A. katsumadai* were not hydroxylated or methoxylated, all of the diarylheptanoids isolated from *A. officinarum* in the present investigation were hydroxylated and/or methoxylated in one or both of their phenyl groups. Accordingly, these hydroxy or methoxy groups may be assumed to have no influence on the resultant antiemetic activity.

In conclusion, six compounds (**2**−**7**) were isolated from *A. officinarum* as antiemetic principles using a standard bioassay. Since compounds **2** (kaempferide) and **3** [5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-3-heptanone] were obtained from the $CHCl_3$ extract of *A. officinarum* as the major constituents and showed potent inhibitory activities and dose-dependent inhibition, it is considered that these compounds are the major antiemetic principles of *A. officinarum* rhizomes.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO DIP-140 digital polarimeter. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. IR spectra were measured with a JASCO A-102 IR spectrophotometer. NMR spectra were recorded using a JNM-LA500 spectrometer in $CDCl_3$ with tetramethylsilane as the internal standard. HREIMS and EIMS were obtained using a JEOL JMX-DX 302. Kieselgel $60F_{254}$ (Merck) precoated plates were employed for thin-layer chromatography (TLC). Column chromatography was carried out on 70−230 mesh Si gel (Merck). HPLC was performed using an SSC-3461 pump with a JASCO UV-970 Galliver detector. Senshu Pak silica 4251-N (10 ϕ × 250 mm) or Senshu Pak PEGASIL silica 60−5 (10 ϕ × 250 mm) was used for HPLC.

Plant Material. The dried rhizomes of *A. officinarum* Hance (as commercial crude drug, Lot. 95117F-9133) were donated from Kotaro Pharmaceutical Co., Ltd., Osaka, Japan. A voucher specimen (No. 00-09) is deposited in the laboratory of K.T.

Extraction and Isolation. The dried rhizomes of *A. officinarum* (5 kg) were extracted successively with *n*-hexane, $CHCl_3$, MeOH, and H_2O. The $CHCl_3$ extract was chromatographed over a Si gel column using a $CHCl_3$−MeOH gradient system (100:1 → 50:1 → 20:1 → 10:1 → 5:1, 2:1 → 1:1) guided by the antiemetic bioassay, resulting in seven pooled fractions. Fr-5, showing antiemetic activity, was purified by HPLC using *n*-hexane−acetone (3:1) as solvent system to give **1** (t_R = 15.2 min, 3.13 g), **2** (t_R = 18.4 min, 3.78 g), and **3** (t_R = 23.2 min, 2.04 g) (UV detection at 254 nm, flow rate 3.0 mL/min). Fr-6, also showing antiemetic activity, was chromatographed over a Si gel column using a *n*-hexane−acetone gradient system, resulting in five fractions (Fr-6-1−Fr-6-5). Fr-6-2 was purified by HPLC with *n*-hexane−acetone (3:1) to give **4** (t_R = 39.2 min, 43.4 mg) (UV detection at 222 nm, flow rate 2.5 mL/min). Fr-6-3 was purified by HPLC using *n*-hexane−EtOAc (1:2) to give **5** (t_R = 10.8 min, 83.0 mg) (UV detection at 254 nm, flow rate 2.5 mL/min) and using $CHCl_3$−MeOH (50:1) as solvent system to give **6** (t_R = 14.4 min, 15.4 mg) (UV detection at 254 nm, flow rate 2.5 mL/min). Fr-6-4 was purified by HPLC using $CHCl_3$−MeOH (60:1) to give **7** (t_R = 15.2 min, 109.6 mg) and **8** (t_R = 15.6 min, 34.1 mg) (UV detection at 254 nm, flow rate 2.5 mL/min), respectively.

1-(4-Hydroxy-3-methoxyphenyl)-7-phenyl-3,5-heptanediol (6): yellow liquid; $[\alpha]^{25}_D$ +6.87° (*c* 0.40, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 274 (3.11), 220 (3.06), 200 (3.04) nm; IR ν_{max} (KBr) 3400, 2950, 1600, 1510, 1030, 700 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 330 $[M]^+$ (78), 312 (66), 294 (3), 137 (100); HREIMS *m*/*z* 330.1832 (calcd for $C_{20}H_{26}O_4$, 330.1832).

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-3-heptanone (7): pale yellow liquid; $[\alpha]^{25}_D$ +1.05° (*c* 0.80, EtOH); UV (EtOH) λ_{max} (log ϵ) 273 (6.83), 217 (6.73), 197 (6.69) nm; IR ν_{max} (KBr) 3375, 2925, 1700, 1610, 1510 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 344 $[M]^+$ (82), 326 (27), 163 (10), 150 (24), 137 (100), 107 (58); HREIMS *m*/*z* 344.1627 (calcd for $C_{20}H_{24}O_5$, 344.1624).

Antiemetic Assay. Young male chicks weighing 25−35 g each (Goto Furanjo Co., Inc. Saitama, Japan) were divided into 1−3 groups consisting of six animals each. The chicks were set aside for 10 min and stabilized in large beakers at 25 °C. The sample solutions were administered intraperitoneally at a dose of 10 mL/kg. After 10 min, copper sulfate anhydride was administered orally at a dose of 50 mg/kg, then the number of animals retching (an emetic action without concomitant vomiting) was recorded during the next 10 min. The results were judged by the decrease in numbers of retching compared with those of the controls. The inhibition (%) was calculated as follows:

$$\text{Inhibition (\%)} = [(A - B)/A] \times 100$$

where A is the frequency of retching in control group, and B is the frequency of retching after sample treatment.

Statistical Analysis. All numerical data were expressed as the mean ± SEM. The statistical significance of the difference was determined by an unpaired Student's t-test.

Supporting Information Available: Antiemetic effects of the extracts and fractions from *A. officinarum* on copper sulfate induced emesis in young chicks are shown in Table 1S. The physical and spectral data of compounds **1**−**5** and **8** are also shown. Structures of the compounds (**1**−**8**) isolated from *A. officinarum* are shown in Figure 1S. This information is available free of charge via the Internet at http://pubs.acs.org.

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