Two Novel Anti-emetic Principles of *Alpinia katsumadai*

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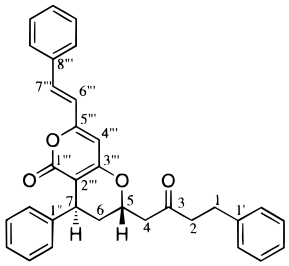
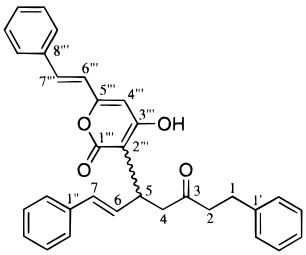
Two novel diarylheptanoids named katsumadain A (**1**) and katsumadain B (**2**) were isolated from the seeds of *Alpinia katsumadai*, and their structures were determined by spectroscopic analysis. Both katsumadains A (**1**) and B (**2**) showed anti-emetic activities on copper sulfate-induced emesis in young chicks.

Alpinia katsumadai Hayata (Zingiberaceae), a Chinese herbal drug, has been used as an anti-emetic agent and a stomachic.[1] During a survey of anti-emetic compounds from natural sources, we investigated anti-emetic principles in this plant. [2] In the present paper, we report the isolation and determination of two novel anti-emetic active diarylheptanoids named katsumadains A (**1**) and B (**2**).



1



2

Two novel diarylheptanoids named katsumadains A (**1**) and B (**2**) were obtained from the $CHCl_3$ extract of *A. katsumadai* Hayata. Katsumadain A (**1**), a light yellow amorphous solid, showed $[\alpha]^{25}_D$ +3.7° (*c* 0.40, EtOH). The molecular formula, $C_{32}H_{28}O_4$ (M^+ 476.1997, calcd 476.1988), was determined by HREIMS. The IR spectrum indicated the presence of a saturated ketone (1720 cm^{-1}). The ^1H NMR spectrum (Table 1) of **1** indicated the presence of three phenyl groups (*δ* 7.50−7.16), a pair of trans olefinic protons (*δ* 7.47 and 6.58, *J* = 16 Hz), and one proton (*δ* 4.49) of an oxygenated methine. The ^{13}C NMR spectrum (Table 1) indicated the presence of four methylenes (*δ* 29.8, 35.5, 45.6, 48.0), one methine (*δ* 35.4), one oxygenated methine (*δ* 70.0), one carbonyl (*δ* 206.5), seven unhydrogenated sp^2 carbons, and 18 sp^2 carbons bearing a hydrogen. ^1H homo-decoupling experiments showed that the methylene protons (*δ* 2.00) are neighboring the methine proton (*δ* 4.13) and the oxygenated methine proton (*δ* 4.49). The methylene protons (*δ* 2.49 and 2.83) neighbor the oxygenated methine proton (*δ* 4.49), while the methylene protons (*δ* 2.78) neighbor methylene protons (*δ* 2.89). These observations suggested that **1** has a conformable feature characteristic of (5*R*)-*trans*-1,7-diphenyl-5-hydroxy-6-hepten-3-one[3] and yangonin.[4] In HMBC experiments (Figure 1), the methine proton (*δ* 4.13) showed correlation with the carbons at *δ* 163.4, 165.5, 101.4, 143.5, and 70.0, and a pair of trans olefinic protons (*δ* 7.47, 6.58) showed correlation with a quaternary carbon at *δ* 158.1. Thus, the structure of **1** was determined as shown. The relative stereochemistry was elucidated based on the ^1H NMR coupling constants of each proton of **1**, which showed signals for the C-7 methine proton at *δ* 4.13 (1H, t, *J* = 4 Hz), the C-5 methine proton at *δ* 4.49 (1H, dddd, *J* = 8, 8, 4, 4 Hz), and the C-4 methylene protons at *δ* 2.49 (1H, dd, *J* = 16, 4 Hz) and 2.83 (1H, dd, *J* = 16, 8 Hz). These data suggested that the protons at C-5 and C-7 are anti.

Katsumadain B (**2**), a light yellow amorphous solid, showed $[\alpha]^{25}_D$ +10.5° (*c* 0.25, EtOH). The molecular formula, $C_{32}H_{28}O_4$ (M^+ 476.1987, calcd 476.1988), was determined by HREIMS. The IR spectrum indicated the presence of a saturated ketone (1680 cm^{-1}) and hydroxyl (3400 cm^{-1}). In comparison with the NMR data of katsumadain A (**1**) (Table 1), the ^{13}C NMR spectrum of **2** showed that one methylene of **2** changed to one methine, and no oxygenated methine was detected. The ^1H NMR spectrum of **2** indicated the presence of an additional pair of trans olefinic protons relative to **1**. These suggested that the structure of **2** is similar to that of **1**. ^1H homo-decoupling experiments showed that the methine proton (*δ* 4.26) is neighboring the olefinic proton (*δ* 6.43) and methylene protons (*δ* 3.00 and 3.05). In HMBC experiments (Figure 2), the methine proton (*δ* 4.26) showed correlation with the carbons at *δ* 162.5 164.9, 104.2, 130.4, 128.9, and 208.1, and a pair of trans olefinic protons (*δ* 7.27 and 7.04) correlated with a quaternary carbon at *δ* 156.5. Thus, the structure of **2** was determined as shown. The stereochemistry at C-5 was not established.

These compounds were tested for anti-emetic activity on copper sulfate-induced emesis in young chicks. As shown in Table 2, katsumadain A (**1**) showed dose-dependent inhibition at the dosages of 10 mg/kg, 20 mg/kg, and 50 mg/kg. Katsumadain B (**2**) showed significant anti-emetic inhibition at a dosage of 50 mg/kg.

Diarylheptanoids are particularly common in the *Alpinia* spp. such as *A. officinarum*,[5,6] *A. oxyphylla*,[7,8] and *A. conchigera*.[9] The occurrences of metabolites consisting of diarylheptanoid and chalcone or flavonone moiety from *A. blepharocaly*, and a metabolite consisting of a labdane and a chalcone from *A. katsumadai* have been reported.[10−12] Katsumadains A and B from *A. katsumadai* are the first example of a diarylheptanoid combined with a monocyclic

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Table 1. ^{13}C and ^{1}H NMR Spectral Data of Compounds **1** and **2** (δ ppm)

	compound **1** (in $CDCl_3$)		compound **2** (in DMSO-d_6)	
position	δ_C	δ_H	δ_C	δ_H
1	29.8	2.89 (2H, t, J = 7 Hz)	28.9	2.73−2.78 (2H, m)
2	45.6	2.78 (2H, t, J = 7 Hz)	43.3	2.73−2.78 (2H, m)
3	206.5		208.1	
4	48.0	2.49 (1H, dd, J = 16, 4 Hz) 2.83 (1H, dd, J = 16, 8 Hz)	44.7	3.00 (1H, dd, J = 16, 7 Hz) 3.05 (1H, dd, J = 16, 7 Hz)
5	70.0	4.49 (1H, dddd, J = 8, 8, 4, 4 Hz)	33.4	4.26 (1H, q, J = 7 Hz)
6	35.5	2.00 (2H, m)	130.4	6.43 (1H, dd, J = 16, 7 Hz)
7	35.4	4.13 (1H, t, J = 4 Hz)	128.9	6.36 (1H, d, J = 16 Hz)
1′	141.1		141.1	
1″	143.5		136.9	
1‴	163.4		162.5	
2‴	101.4		104.2	
3‴	165.5		164.9	
4‴	101.3	5.93 (1H, s)	101.7	6.29 (1H, s)
5‴	158.1		156.5	
6″	119.2	6.58 (1H, d, J = 16 Hz)	119.7	7.04 (1H, d, J = 16 Hz)
7‴	135.6	7.47 (1H, d, J = 16 Hz)	133.3	7.27 (1H, d, J = 16 Hz)
8‴	135.8		135.2	
three phenyl				
126.2−129.3		7.50−7.16 (15H, m)	125.7−128	7.03−7.43 (15H, m)

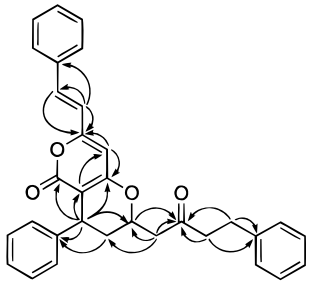

Figure 1. HMBC correlations ($^{1}H \rightarrow {}^{13}C$) of **1** in $CDCl_3$.

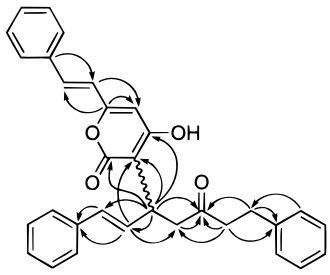

Figure 2. HMBC correlations ($^{1}H \rightarrow {}^{13}C$) of **2** in DMSO-$d_6$.

Table 2. Anti-emetic Effects of Compounds **1** and **2** on $CuSO_4$-induced Emesis in Young Chicks

drugs	dose (mg/kg)	no. of chicks	no. of retching (mean ± S. E. M.)	inhibition (%)
control		6	75.2 ± 4.80	
katsumadain A (**1**)	10	6	68.6 ± 4.00	8.8
	20	6	64.3 ± 5.91	14.5
	50	6	37.4 ± 3.35[a]	50.2
control		6	72.8 ± 3.27	
katsumadain B (**2**)	50	6	42.7 ± 3.60[a]	41.4

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

α-pyrone moiety. Both katsumadains A and B showed anti-emetic activities.

Experimental Section

General Experimental Procedures. The IR spectra were measured with a JASCO A-102 IR spectrophotometer. Melting points were determined on a Yanagimoto MP micromelting point apparatus. The $[\alpha]_D$ values were determined with a JASCO DIP-140 digital polalimeter. The ^{1}H and ^{13}C NMR spectra were recorded using a JEOL GSX-400 spectrometer in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as internal standard. 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-3100-J pump with an Oyo-Bunko Uvilog 7 UV detector. HREIMS and EIMS were obtained using a JEOL JMX-DX 302.

Plant Material. The dry seeds of *A. katsumadai* Hayata were obtained from Kotaro Pharmaceutical Co., Ltd. (Japan).

Bioassay of Anti-emetic Activity. Young male chicks (4 days old) weighing 25−35 g (Goto Furanjo Co., Inc., Saitama, Japan) were placed in 1−3 groups of six each. The young chicks were set aside for 10 min to stabilize in large beakers at 25 °C. Katsumadains A and B were each dissolved in 0.9% saline containing 5% DMSO and 1% Tween-80, and the sample solution or control vehicle was administered intraperitoneally at a volume of 10 mL/kg. After 10 min, anhydrous copper sulfate was administered orally at a dose of 50 mg/kg, then the number of retching reflexes (an emetic action without vomiting gastric materials) was recorded during the next 10 min. The results were judged by the decrease in number of retching reflexes compared with those of the control. The inhibition (%) was calculated as follows:

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

where A is the frequency of retching after control vehicle treatment and B is the frequency of retching after sample treatment. All numerical data were expressed as the mean ± S. E. M. The statistical significance of the difference was determined by an unpaired Student's *t*-test.

Extraction and Isolation. The crude drug (500 g) was extracted successively with *n*-hexane, $CHCl_3$, MeOH, and H_2O. Each extract was examined by the anti-emetic bioassay using $CuSO_4$. The $CHCl_3$ extract with anti-emetic activity was chromatographed successively on a Si gel column ($CHCl_3$−MeOH, *n*-hexane−Me_2CO and *n*-hexanes−EtOAc) and HPLC [Senshu Pak. silica-4251-N 10 ϕ × 250 mm, *n*-hexanes−EtOAc (6:4)] guided by anti-emetic bioassay, and compound **1** (87 mg) and **2** (32 mg) were obtained.

Katsumadain A (1): light yellow amorphous solid ($CHCl_3$); mp 68−70 °C; $[\alpha]^{25}_D$ +3.7° (*c* 0.40, EtOH); UV (*n*-hexane) λ_{max} (log ϵ) 353 (3.36), 258 (3.30) nm. IR ν_{max} (KBr) 1720, 1645, 1610, 1565 cm^{-1}; EIMS *m/z* (rel int %) 476 $[M]^+$ (74), 343 (10), 327 (28), 301 (18), 157 (24), 131 (100), 115 (40), 105 (80); HREIMS *m/z* 476.1997 (calcd for $C_{32}H_{28}O_4$, 476.1988); ^{1}H and ^{13}C NMR, see Table 1.

Katsumadain B (2): light yellow amorphous solid ($CHCl_3$); mp 118−121 °C; $[\alpha]^{25}_D$ +10.5° (*c* 0.25, EtOH); UV (MeOH) λ_{max} (log ϵ) 355 (4.04), 254 (4.23) nm; IR ν_{max} (KBr) 3400, 1680, 1645,

1620, 1560 cm^{-1}; EIMS *m*/*z* (rel int %) 476 $[M]^+$ (74), 343 (10), 327 (28), 301 (18), 157 (24), 131 (100), 115 (40), 105 (80); HREIMS *m*/*z* 476.1987 (calcd for $C_{32}H_{28}O_4$, 476.1988); ^1H and ^{13}C NMR, see Table 1.

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