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Anti-emetic Principles of *Alpinia katsumadai* Hayata

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Abstract – Bioassay-guided fractionation of anti-emetic constituents of *Alpinia katsumadai* Hayata was performed. Nine compounds including one novel compound, (3*R*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (**9**) were isolated from it. Among these compounds, four diarylheptanoids, one sesquiterpenoid and one flavonoid showed anti-emetic activity on copper sulfate induced-emesis in young chicks.

Key words – Anti-emetic, *Alpinia katsumadai* Hayata (Zingiberaceae), (3*R*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene .

Introduction

In the previous papers, we reported anti-emetic principles from Chinese herbal drugs (Kawai *et al.*, 1994; Tai *et al.*, 1995; Kinoshita *et al.*, 1996), and also established a new screening method for the anti-emetic activity using young chicks (Akita *et al.*, 1998). Using this method, we examined anti-emetic principles of *Alpinia katsumadai* Hayata, which was recorded in the Ben Cao Gang Mu as an anti-emetic agent and used for the treatment of stomach disease. It was reported that using marmot a small dose (0.25-0.75%) of its water extract stimulated the movement of dissected intestine while a large dose (1.00-1.25%) caused inhibition.

Experimental

Materials – Herbal drugs were obtained from Kotaro Pharmaceutical Co., Ltd.

Regents – Copper sulfate anhydride (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) was used as an emetic agent.

Spectroscopy and chromatography – ^{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 60 F_{254} (Merck) precoated plates were employed for thin-layer chromatography (TLC). Column chromatography was carried out on 70-230 mesh silica gel (Merck). HPLC was performed using an SSC-3100-J pump with an Oyo-Bunko Uvilog 7 UV detector. HR-MS and EI-MS were obtained using a JEOL JMX-DX 302.

Animals – Young male chicks (4 days of age) weighing 25-35 g were purchased from Goto Furanjo Co., Inc. (Saitama, Japan).

Bioassay of anti-emetic activity – The young chicks were divided into 1-3 groups consisting of six each. The young chicks were set aside for 10 min to stabilize in large beakers at 25°C. The sample solution was administered intraperitioneally at volume of 10 ml/kg. After 10 min, copper sulfate anhydride was administered orally 50 mg/kg, then the number of retching (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 compared with those of control. The inhibition (%) was calculated as follows:

Inhibition (%)=[(A-B)/A] × 100
A: control frequency of retching
B: frequency of retching after sample treatment

Statistical analysis – All numerical data were expressed as the mean±S. E. M. The statistical signif-

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icance of the difference was determined by an unpaired Student's t-test.

Isolation and purification of anti-emetic principles from the seeds of *A. katsumadai* Hayata – The crude drug (500 g) was extracted successively with *n*-hexane, $CHCl_3$, MeOH, and water. Each extract was examined by the anti-emetic bioassay using $CuSO_4$. The $CHCl_3$ extract showing anti-emetic activity was chromatographed on a silica gel column ($CHCl_3$-MeOH), and six fractions, fr. 1 to 6, were obtained. Among these fractions, fr. 2, 3, and 4 showed significant anti-emetic activities.

Fr. 2 was chromatographed on a silica gel column (*n*-hexane-acetone) and HPLC [silica-4251-N 10ϕ× 250 mm, *n*-hexane-acetone (9:1) and (20:1)] to give compound **1** (1.97 g) and **2** (0.10 g) which were identified as *trans,trans*-1,7-diphenyl-4,6-heptadien-3-one and *trans,trans*-1,7-diphenyl-5-hydroxy-4,6-heptadien-3-one, respectively, by comparison with published spectral data (Kuroyanagi *et al.*, 1983).

Fr. 3 was chromatographed on a silica gel column (*n*-hexane-acetone), and seven fractions, fr. 3-1 to 3-7, were obtained. Each fraction was tested for anti-emetic activity. Fr. 3-3 showed positive activity was chromatographed on a silica gel column (*n*-hexane-acetone) and PTLC [$CHCl_3$-MeOH (10:1)] to give *trans,trans*-farnesol (**3**) (0.89 g). In the same method, (5*R*) *trans*-1,7-diphenyl-5-hydroxy-6-hepten-3-one (**4**) (0.82 g) was obtained from active fraction, fr. 3-5. They were identified by comparison with published spectral data (Kuroyanagi *et al.*, 1983; Ngo *et al.*, 1998). Although fr. 3-6 showed moderate anti-emetic activity, the purification is under progress, and will be reported elsewhere.

Fr. 4 was divided into two fractions, $CHCl_3$ soluble and $CHCl_3$ insoluble ones. $CHCl_3$ insoluble fraction showing 48.3% inhibition was chromatographed on a silica gel column ($CHCl_3$-MeOH) and HPLC [silica-4251-N 10ϕ×250 mm, $CHCl_3$-acetone (10:1)], and compound **5** (1.00 g), **6** (1.80 g), and **7** (2.50 g) were obtained and identified as pinocembrin, alpinetin, and cadamomin by comparison with published spectral data (Kuroyanagi *et al.*, 1983). $CHCl_3$ soluble fraction showing 50.7% inhibition was chromatographed on a silica gel column ($CHCl_3$-acetone) and HPLC [silica-4251-N 10ϕ×mm, $CHCl_3$-acetone (90:1)], and compound **8** (1.40 g) and **9** (0.82 g) were obtained.

Compound **8** was obtained as colorless needles from *n*-hexane-$CHCl_3$, mp 76-78 °C, HREIMS m/z 282.1620 (M^+, calcd for $C_{19}H_{22}O_2$, 282.1620), $[\alpha]_D$ +16.0° (*c*=0.63, EtOH), and identified as (3*S*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene by comparison with published data (Kuroyanagi *et al.*, 1983). It was also supported by hydrogenation over palladium carbon to give a meso dihydro derivative, $C_{19}H_{24}O_2$, $[\alpha]_D$±0° (*c*=0.38, EtOH).

Hydrogenation of 8: A solution of **8** (3.8 mg) in MeOH (4.0 ml) was stirred with 5% Pd-C (15.0 mg) for 3 h at room temperature under an H_2 atmosphere, then the catalyst was removed by filtration and the filtrate was concentrated. The residual solid was crystallized from *n*-hexane to give colorless needles. $C_{19}H_{24}O_2$, $[\alpha]_D$±0° (*c*=0.38, EtOH).

Compound **9** was obtained as colorless needles from *n*-hexane-$CHCl_3$, mp 81-82 °C, HREIMS m/z 282.1618 (M^+, calcd for $C_{19}H_{22}O_2$, 282.1620), $[\alpha]_D$ -2.6° (*c*=0.47, EtOH). The MS, UV and IR spectra of compound **9** were identical to those of compound **8**. ^{13}C-NMR spectrum indicated the presence of three methylenes (δ 31.4, 39.7 and 45.0) and two hydroxylated methines (δ 65.9 and 67.7) (Table 1). ^{1}H-NMR spectrum indicated the presence of two phenyl groups (δ 7.14-7.38, 10H), a pair of *trans* olefinic protons (δ 6.29 and 6.49, *J*=16 Hz) and two protons of hydroxylated methines (δ 3.69 and 4.36) (Table 1). From ^{1}H-homo decoupling experiment, one (δ 6.29)

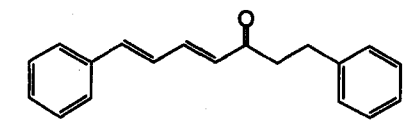
Table 1. ^{13}C- and ^{1}H-NMR spectral data of (3*R*,5*S*)-*trans*-3,5-dihydroxyl-1,7-diphenyl-1-hepetene (**9**) in DMSO-d_6 (δ ppm)

position	δ C	δ H
1	127.3	6.49 (1H, d, *J*=16 Hz)
2	135.0	6.29 (1H, dd, *J*=16, 6 Hz)
3	67.7	4.36 (1H, m)
4	45.0	1.52 (2H, t, *J*=6 Hz)
5	65.9	3.69 (1H, m)
6	39.7	1.64 (2H, m)
7	31.4	2.56-2.62 (1H, m)
		2.67-2.73 (1H, m)
1'	136.9	
2'/6'	126.1	7.38 (1H, d, *J*=7 Hz)
3'/5'	128.5	7.30 (1H, t, *J*=7 Hz)
4'	127.0	7.20 (1H, m)
1"	142.5	
2"/6"	128.3	7.20 (1H, m)
3"/5"	128.2	7.25 (1H, t, *J*=7 Hz)
4"	125.5	7.14 (1H, t, *J*=7 Hz)
3-OH		4.81 (1H, d, *J*=6 Hz)
4-OH		4.50 (1H, d, *J*=6 Hz)

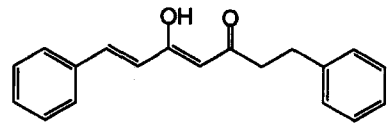
of the olefinic protons was coupled with one (δ 4.36) of the hydroxylated methine protons. Decoupling experiments showed that both the hydroxylated methine protons (δ 3.69 and 4.36) are neighboring to the protons at C-4 (δ 1.52, 2H, t, *J*=6 Hz). HMBC also showed correlations as shown in Fig. 2. Thus, the structure of **9** was established to be *trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene. Hydrogenation of compound **9** over palladium carbon gave a dihydro derivative, $C_{19}H_{24}O_2$, $[\alpha]_D$ -5.3° (*c*=0.46, EtOH), which was not *meso* type compound. This suggested that configuration of **9** was "3*R*,5*S*" or "3*S*,5*R*", not "3*R*, 5*R*" or "3*S*,5*S*". Reduction of (5*R*)-*trans*-1,7-diphenyl-5-hydroxy-6-heptene-3-one (**4**) with sodium borohydride in acetic acid afford two diol derivatives, compound **10**, $C_{19}H_{22}O_2$, $[\alpha]_D$ -14.7° (*c*=0.21, EtOH) and **11**, $C_{19}H_{22}O_2$, $[\alpha]_D$ -3.3° (*c*=0.74, EtOH). The ^{1}H- and ^{13}C -NMR spectra of compound **10** were same as (3*S*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (**8**), but the value of specific rotation was different, $[\alpha]_D$ -14.7° (*c*=0.21, EtOH). This suggested that compound **10** was (3*R*,5*R*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene and **11** was (3*R*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-hyptene. On the other hand, the ^{1}H- and ^{13}C-NMR spectra and the value of specific rotation of **9** were in good agreement with those of **11**, thus, compound **9** was determined to (3*R*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene as new compound.

Compound 9 [(3*R*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene]: colorless needles (*n*-hexane-$CHCl_3$), mp 81-82 °C; $[\alpha]_D$ -2.6° (*c*=0.47, EtOH); UV (MeOH) λ_{max} nm: 250; IR υ_{max} (KBr) cm^{-1}: 3400, 1500, 1445, 1395; HR-EIMS m/z: 282.1618 (calcd for $C_{19}H_{22}O_2$, 282.1620); EIMS m/z (rel. int. %): 282 (M^+, 0.89), 264 (32), 246 (73), 155 (100), 142 (26); ^{1}H- and ^{13}C-NMR: see Table 1.

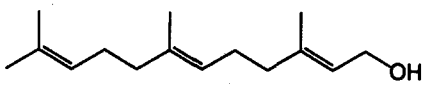
Hydrogenation of 9: A solution of **9** (4.6 mg) in



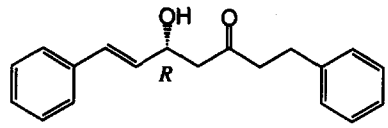
trans, trans-1,7-diphenyl-4,6-heptadien-3-one (**1**)



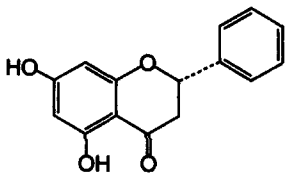
trans, trans-1,7-diphenyl-5-hydroxy-4,6-heptadien-3-one (**2**)



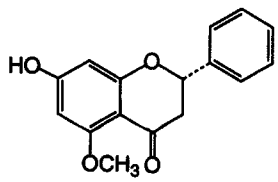
trans,trans-farnesol (**3**)



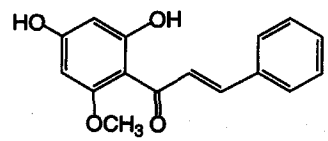
(5*R*)-*trans*-1,7-diphenyl-5-hydroxy-6-hepten-3-one (**4**)



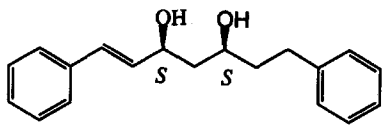
pinocembrin (**5**)



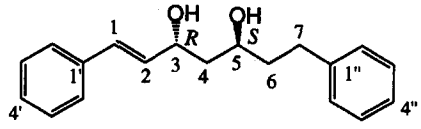
alpinetin (**6**)



cardamomin (**7**)



(3*S*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (**8**)



(3*R*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (**9**)

Fig. 1. The structures of compounds isolated from *Alpinia katsumadai* Hayata.

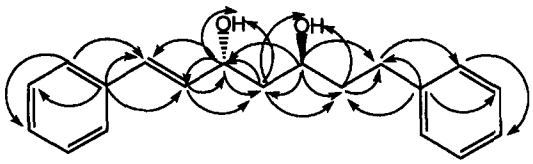


Fig. 2. HMBC correlations for (3*R*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (**9**) indicated by arrows from ^{13}C to ^{1}H.

MeOH (4.0 ml) was stirred with 5% Pd-C (15.0 mg) for 3 h at room temperature under H_2 atmosphere then the catalyst was removed by filtration and the filtrate was concentrated. The residual solid was crystallized from *n*-hexane to give colorless needles (4.6 mg). $C_{19}H_{24}O_2$, $[\alpha]_D$ -5.3° (*c*=0.46, EtOH).

Reduction of 4: A solution of **4** (30.0 mg) in neat acetic acid (1.0 ml) was stirred with $NaBH_4$ (8.0 mg) for 30 min at room temperature (Evans *et al.*, 1988). The reaction mixture was neutralized with 1 N NaOH and extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and concentrated to give a yellow solid, which was chromatographed on HPLC [silica-4251-N 10φ×250 mm, $CHCl_3$-acetone (90:1)] to give (3*R*,5*R*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (2.5 mg) (**10**), $C_{19}H_{22}O_2$, $[\alpha]_D$ -14.7° (*c*=0.21, EtOH), and (3*R*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (8.3 mg) (**11**), $C_{19}H_{22}O_2$, $[\alpha]_D$ -3.3° (*c*=0.74, EtOH).

Results and Discussion

As shown in Table 1, *n*-hexane, $CHCl_3$, and MeOH extracts of *A. katsumadai* showed an anti-emetic activity at the dose of 300 mg/kg on copper sulfate induced-emesis in young chicks. The $CHCl_3$ extract showing high inhibition (60.1%) was chromatographed, to give three flavonoids, one sesquiterpene and five diarylheptanoids (Fig. 1). Pinocembin (**5**) and alpinetin (**6**) showed no anti-emetic effects (Table 2). Cardamomin (**7**) showed anti-emetic activity at three doses of 10 mg/kg, 20 mg/kg and 50 mg/kg. *Trans*,*trans*-farnesol (**3**) showed anti-emetic activity at two doses of 20 mg/kg and 50 mg/kg. *Trans*,*trans*-1,7-diphenyl-4,6-heptadien-3-one (**1**), (5*R*)-*trans*-1,7-diphenyl-5-hydroxy-6-hepten-3-one (**4**), (3*S*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (**8**) and (3*R*,5*S*)-*trans*-3,5-dihydroxy-diphenyl-1-heptene (**9**) were diarylheptanoids and showed dose-dependent inhibition at the doses of 10 mg/kg, 20 mg/kg and 50 mg/kg, respectively. Although the analogous *trans*,

Table 2. Anti-emetic effects of compounds from *Alpinia katsumadai* Hayata on copper sulfate induced-emesis in young chicks

Drugs	Dose (mg/kg)	No. of young chicks	No. of retching (mean±S.E.M.)	Inhibition (%)
control		6	84.8±1.31	
compound **1**	10	6	67.0±3.29**	21.0
	20	6	57.5±3.68**	32.0
	50	6	53.8±4.01**	36.6
control		6	82.2±2.27	
compound **2**	50	6	64.7±5.22	17.3
control		6	81.7±1.52	
compound **3**	10	6	74.2±4.96	9.2
	20	6	40.0±6.85**	51.0
	50	6	48.3±6.75**	40.8
control		6	75.8±2.33	
compound **4**	10	6	60.3±3.00**	20.4
	20	6	54.7±4.74*	27.9
	50	6	33.5±3.71***	55.8
control		6	67.8±4.64	
compound **5**	10	6	57.2±5.11	15.7
	20	6	59.5±5.46	12.3
	50	6	63.3±5.06	6.6
control		6	79.2±2.18	
compound **6**	50	6	71.8±5.20	9.3
control		6	87.5±1.15	
compound **7**	10	6	57.5±8.05	34.3
	20	6	34.7±4.69***	60.4
	50	6	40.7±6.68***	53.5
control		6	82.2±2.27	
compound **8**	10	6	52.5±4.25**	36.1
	20	6	49.2±3.14**	40.2
	50	6	36.5±2.20**	55.6
control		6	74.2±4.76	
compound **9**	10	6	50.1±3.64*	32.4
	20	6	32.8±4.15**	55.7
	50	6	21.7±1.19***	70.8

Significantly different from the control value, *p<0.05, **p<0.01, ***p<0.001.

trans-1,7-diphenyl-5-hydroxy-4,6-heptadien-3-one (**2**) showed no effect. Diarylheptanoids are very common in the *Alpinia* spp. and are known from *A. officinarum* (Itokawa *et al.*, 1981 and 1985; Uehara *et al.*, 1987), *A. oxyphylla* (Itokawa *et al.*, 1982; Shoji *et al.*, 1984), *A. conchigera* (Athamprasangsa *et al.*, 1994), and *A. blepharocalyx* (Kadota *et al.*, 1994), which have been used as anti-emetics in Chinese traditional medicine. Therefore, based on the present study, dia-

rylheptanoids might be the main anti-emetic principles of *Alpinia* spp.

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