Inhibition of Prostaglandin and Leukotriene Biosynthesis by Gingerols and Diarylheptanoids

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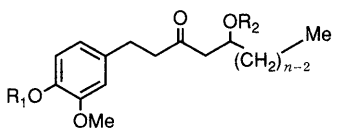
The rhizomes of *Zingiber officinale* (ginger) and *Alpinia officinarum* contain potent inhibitors against prostaglandin biosynthesizing enzyme (PG synthetase). Gingerols and diarylhepatanoids were identified as active compounds. Their possible mechanism of action which was deduced from the structures of active compounds indicated that the inhibitors would also be active against arachidonate 5-lipoxygenase, an enzyme of leukotriene (LT) biosynthesis. This was verified by testing their inhibitory effects on 5-lipoxygenase prepared from RBL-1 cells. A diarylheptanoid with catechol group was the most active compound against 5-lipoxygenase, while yakuchinone A was the most active against PG synthetase.

Keywords gingerol; diarylheptanoid; *Zingiber officinale*; *Alpinia officinarum*; prostaglandin; 5-lipoxygenase

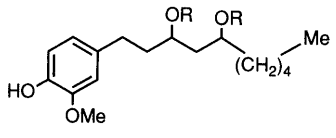
In vitro bioassay systems have been extensively used to monitor biological activities of extracts, fractions and isolated compounds during the isolation process of bioactive constituents in the studies of biologically active compounds of medicinal plants used in traditional medicines. A bioassay system that tests inhibitory effects on a prostaglandin (PG) biosynthesizing enzyme (PG synthetase) was used in our screening work to detect biological activities in the hot aqueous extracts of medicinal plants.[1] The inhibitors of PG biosynthesis are directly associated with anti-inflammatory[2] and anti-platelet aggregation activities,[3] and the efficacy of cyclooxygenase inhibitor contained in paeony root bark has been proved in a pre-clinical test in men.[3] The *in vitro* bioassays are advantageous in bioactivity oriented phytochemical studies where a large number of fractions must be tested by bioassays along with phytochemical separation work. Since we introduced this strategy, considerable success was obtained in studies using PG synthetase as a monitor of bioactivity. A variety of phenolic compounds was isolated as the potent inhibitors of PG synthetase from *Zingiber officinale*,[1a] *Alpinia officinarum*,[1b] *Arnebia euchroma*,[1c–e,j,k] *Dalbergia odorifera*,[1f] *Ipomoea aquatica*,[1g] *Allium chinense*,[1h] and *Mucuna birdwoodiana*.[1i] The isolated bioactive compounds have structural characteristics to contain phenolic and lipophilic groups. The substrate of PG synthetase is unsaturated fatty acids such as arachdonic acid, and hence the enzyme should have affinity to lipophilic groups. The phenol groups inhibit radical formation or quench a generated radical in endoperoxide synthetase reaction and the lipophilic groups assist the binding of the inhibitors to the enzyme. From this point of view the structures of gingerols and diarylheptanoids provide representative examples of the phenolic inhibitors of PG synthetase.[1a,b] Gingerols and diarylheptanoids cannot be the specific inhibitors of PG synthetase, but they are thought to act as general inhibitors of lipoxygenases since endoperoxide synthetase can be regarded as a kind of lipoxygenase, although the enzyme requires haem as a cofactor in the reaction, while lipoxygenases contain iron in their molecules. The phenolic inhibitors of PG biosynthesis were found to inhibit arachidonate 5-lipoxygenase, a responsible enzyme catalyzing the oxygenation of arachidonate to 5-HPETE (5-hydroperoxyeicosatetraenoic acid), which is the first product of the arachidonate cascade leading to leukotriene (LT) biosynthesis.[4] This paper describes full details of the studies on gingerols and diarylheptanoids as the inhibitors of PG and LT biosyntheses.

As we reported in a previous communication a hot aqueous extract of ginger, the rhizomes of *Zingiber officinale* ROSCOE (Zingiberaceae), was highly inhibitory against PG synthetase prepared from rabbit kidney medulla.[1a,c] Ginger has been commonly used not only as a spice but also as an important medicinal drug in traditional Japanese Kampo medicine, therefore extensive phytochemical studies have been undertaken to clarify the constituents of ginger.[5] [6]-Gingerol (**3**) has been identified as the main constituent responsible for the pungent taste of ginger.[6] [8]- and [10]-Gingerols (**4** and **5**), [6]-gingediol (**12**), [6]-gingediacetate (**13**) and hexahydrocurcumin (**15**) have been isolated as minor constituents.[6] Gas chromatography–mass spectrometry (GC-MS) analysis has shown that ginger contains the following compounds: [3]-, [4]-, [5]-, [6]-, [8]-, [10], and [12]-gingerols, [3]-, [4]-, [5]-, [6]-, [8]- and [10]-shogaols, [4]-, [6]-, [8]- and [10]-gingediols, [6]-methylgingediol, [4]- and [6]-gingediacetates, and [6]-methylgingediacetate.[7]

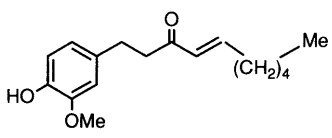
A preliminary small scale extraction and activity testing demonstrated that bioactive compounds contained in the hot aqueous extract were extractable with ethyl acetate. The ethyl acetate extract inhibited PG biosynthesis by 73% at a concentration of 150 μg/ml while no activity was observed in the remaining aqueous layer. Three kg of fresh ginger was therefore successively extracted with chloroform and methanol in order to isolate and characterize the active constituents. The concentrated methanol extract was successively extracted with hexane and ethyl acetate. Chromatographic separation of the ethyl acetate fraction afforded [6]- and [10]-gingerols (**3**, **5**), [6]-acetylgingerol (**10**), [6]-gingediol (**12**), [6]-gingediacetate (**13**), [6]-shogaol (**14**) and hexahydrocurcumin (**15**). The hexane soluble fraction gave four new gingerols tentatively called ZO-1, 2, 3 and 4. ZO-1 is a yellow compound with ultraviolet (UV) absorption maximum at 374 nm, indicating the presence of an extended conjugation. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum gave signals arising from a C_5 alkyl moiety corresponding to [6] series at δ 2.38 (2H, m), 1.20—1.80 (6H, m) and 0.93 (3H, t), and three olefinic protons at δ 5.62 (1H, s), 6.35 (1H, d, J=16 Hz) and 7.52 (1H, d, J=16 Hz) along with aromatic and methoxy protons as in [6]-gingerol (**3**). The data indicated ZO-1 to be [6]-dehydrogingerdione (**16**), which had been synthesized as an intermediate of [6]-gingerol biosynthesis.[8] ZO-2 gave very similar spectral data to those of ZO-1 and it was



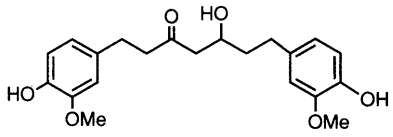
R_1	R_2	n		
H	H	2–16[n]-gingerol		(**1**–**8**)
Me	H	6	[6]-methylgingerol	(**9**)
H	Ac	6	[6]-acetylgingerol	(**10**)
Ac	Ac	6	[6]-diacetylgingrol	(**11**)



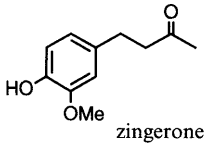
R=H [6]-gingediol (**12**)
R=Ac [6]-gingediacetate (**13**)



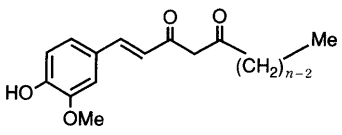
[6]-shogaol (**14**)



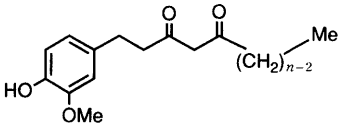
hexahydrocurcumin (**15**)



zingerone



n=6 [6]-dehydogingerdione (**16**)
n=10 [10]-dehydrogingerdione (**17**)



n=6 [6]-gingerdione (**18**)
n=10 [10]-gingerdione (**19**)

identified as [10]-dehydrogingerdione (**17**), a higher homologue of **16**. The ^{1}H-NMR spectra of **16** and **17** indicate that they are present as enol forms in solution. The ^{1}H-NMR spectrum of ZO-3 gave signals arising from two methylenes adjacent to carbonyls and benzylic methylene at δ 2.55 and 2.84, respectively. A signal at δ 3.52 of 0.2H integration indicated ZO-3 exists as a mixture of keto-enol tautomers in a deuterated chloroform solution, and the signal of the enol form appeared at δ 5.43 with 0.9H integration as an olefinic proton. The spectral data indicated ZO-3 to be [6]-gingerdione (**18**), a dihydro-derivative of **16**. ZO-4 was identified as [10]-gingerdione (**19**), a higher homologue of **18**. Dehydrogingerdiones (**16**, **17**) and gingerdiones (**18**, **19**) are the biosynthetic intermediates of gingerols and their isolation further supports the proposed biosynthetic scheme of gingerols.[8] It may be worth noting that the biosynthetic intermediates of [8]-gingerol were not isolated in this work. This may indicate that the [8]-series is present in ginger as a very minor constituent.

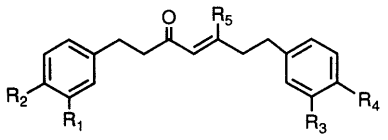
The isolated gingerol derivatives were subjected to tests their inhibitory effects on PG synthetase, and the results are summarized in Table I. Except for [6]-methylgingerol (**9**), gingerols (**3**, **5**), dehydrogingerdiones (**16**, **17**) and gingerdiones (**18**, **19**) exhibited strong inhibition, indicating that the presence of phenol group is essential for the inhibitory activity. [6]-Diacetylgingerol (**11**) was as active as [6]-gingerol. This is due to the hydrolysis of the acetyl groups by non-specific hydrolase contained in a microsomal preparation rather than acetylation of enzyme as in the case of aspirin (data not shown). Zingerone, a degradation product of ginerols without alkyl group, was significantly less active than gingerols, demonstrating that the hydrophobic alkyl groups play a very important role in the inhibition of PG synthetase by binding to the enzyme with hydrophobic interaction.

The other medicinal drug investigated together with ginger was the rhizomes of *Alpinia officinarum* HANCE (Zingiberaceae), which has been used in traditional Kampo medicine mainly for stomach trouble and diarrhea. The hot aqueous extract of the rhizomes significantly inhibited PG synthetase.[1c] To isolate and identify the inhibitors, the material was extracted with methanol and the methanol extract was then fractionated with organic solvents. The chloroform fraction inhibited PG synthetase by 99% at a

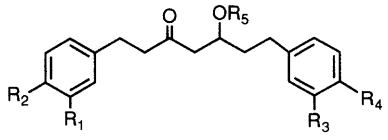
TABLE I. Inhibitory Effects of Gingerol Derivatives on PG Synthetase

Name	No.	IC_{50} (μM)
[6]-Gingerol	**3**	5.5
[10]-Gingerol	**5**	2.3
[6]-Methylgingerol	**9**	110
[6]-Acetylgingerol	**10**	1.9
[6]-Diacetylgingerol	**11**	2.8
[6]-Gingediacetate	**13**	4.3
[6]-Shogaol	**14**	1.6
[6]-Dehydrogingerdione	**16**	1.0
[10]-Dehydrogingerdione	**17**	2.3
[6]-Gingerdione	**18**	1.6
[10]-Gingerdione	**19**	2.0

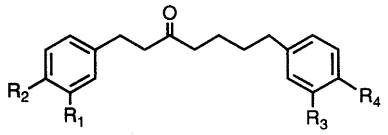
concentration of 150 μg/ml, and was therefore chromatographed on silica-gel and Sephadex LH-20 to afford four known flavonols, galangin, izalpin, kaempferide and kaempferol, and six diarylheptanoids. Of the isolated diarylheptanoids three are known compounds, 7-(4″-hydroxy-3″-methoxyphenyl)-1-phenylhept-4-ene-3-one (**20**),[9] 1,7-diphenyl-5-hydroxy-3-heptanone (**21**)[10] and 5-hydroxy-7-(4″-hydroxy-3″-methoxyphenyl)-1-phenyl-3-heptanone (**22**).[11] The other three new diarylheptanoids were tentatively designated as AO-1, 2 and 3. High resolution mass spectrum (MS) of AO-1 gave a molecular formula, $C_{20}H_{22}O_4$, and the ^{1}H-NMR spectrum revealed the presence of a phenyl and a 1,2,4-substituted benzene, a methoxy, four methylenes and a 1,3-diketone. The proton signals of the 1,3-diketone were observed at δ 3.47 (0.4H, s) and δ 5.38 (0.8H, s), indicating that AO-1 exists in a deuterochloroform solution as a mixture of keto-enol tautomers in a ratio of 1:4. AO-1 was identified as 7-(4″-hydoxy-3″-methoxyphenyl)-1-phenyl-3,5-heptadione (**23**). AO-2 was an oil with a molecular formula of $C_{21}H_{26}O_4$. The ^{1}H-NMR spectrum of AO-2 was very similar to that of **22** and differences were observed in the signal of additional methoxy at δ 3.28 and the shift of a methine signal of oxygen bearing carbon. AO-2 was then identified as 5-methoxy-7-(4″-hydroxy-3″-methoxyphenyl)-1-phenyl-3-heptanone (**24**), a methyl ether of **22**, however **24** had no optical rotation and could be formed from the corresponding α,β-unsaturated compound (**20**) by the addition of methanol. AO-3 was an oily compound of



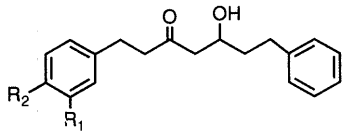
R_1	R_2	R_3	R_4	R_5	
H	H	OMe	OH	H	**20**
H	H	OMe	OH	OH	**23**



R_1	R_2	R_3	R_4	R_5	
OMe	OH	H	H	H	**15**
H	H	H	H	H	**21**
H	H	OMe	OH	H	**22**
H	H	OMe	OH	Me	**24**
H	H	H	OH	H	**25**
OH	OH	H	OH	H	**26**



R_1	R_2	R_3	R_4	
OMe	OH	H	H	**27**
OMe	OAc	H	H	**28**
OMe	OMe	H	H	**29**
H	H	OMe	OH	**30**



R_1	R_2	
OMe	OH	**31**
OH	OMe	**32**
H	OH	**33**
OH	OH	**34**

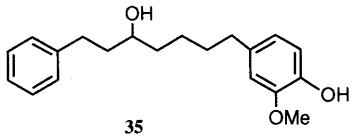


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TABLE II. Inhibitory Effects of Diarylheptanoids on PG Synthetase and 5-Lipoxygenase

Compd. No.	PG-ase[a] IC_{50} (μM)	5-Lipoxy[b] IC_{50} (μM)
15	23	3.2
20	2.0	N.T.[c]
21	170	N.T.[c]
22	4.4	1.6
23	2.0	N.T.[c]
24	2.3	—[d]
25	19	N.T.
26	N.T.[c]	0.26
27	0.5	0.41
28	0.6	0.94
29	>100	N.T.
30	N.T.[c]	0.24
31	2.3	0.26
32	N.T.[c]	—[d]
33	N.T.[c]	—[d]
34	N.T.[c]	0.018
35	N.T.[c]	0.21

a) PG synthetase. *b*) 5-Lipoxygenase. *c*) Not tested. *d*) Less than 10% inhibition at a concentration of 1 μM.

TABLE III. Inhibition of PG Synthetase and 5-Lipoxygenase by [2]- — [16]-Gingerols

Compounds	PG-ase[a] IC_{50} (μM)	5-Lipoxy[b] IC_{50} (μM)
[2]-Gingerol (**1**)	7% (100 μM)[c]	34% (10 μM)[c]
[4]-Gingerol (**2**)	35% (100 μM)[c]	39% (10 μM)[c]
[6]-Gingerol (**3**)	4.6	3.0
[8]-Gingerol (**4**)	5.0	0.36
[10]-Gingerol (**5**)	2.5	0.053
[12]-Gingerol (**6**)	4.1	0.046
[14]-Gingerol (**7**)	5.7	0.042
[16]-Gingerol (**8**)	8.6	0.055

a) PG synthetase. *b*) 5-Lipoxygenase. *c*) Per cent inhibition at the concentration in parentheses.

$C_{19}H_{22}O_3$ and lacked the signal of methoxy group in the ^{1}H-NMR spectrum. A_2B_2 type signals of aromatic protons indicated AO-3 to be 5-hydroxy-7-(4″-hydroxyphenyl)-1-phenyl-3-heptanone (**25**).

Table II summarizes the inhibitory activities of diarylheptanoids which were isolated in this study as well as those obtained from other laboratories.[12,13] The presence of phenol groups is essential for inhibitory activity against PG synthetase and the alkyl groups of gingerols can be replaced by alkyl benzene groups of diarylheptanoids, which also act as hydrophobic groups binding to the enzyme. The most active compound was yakuchinone A (**27**), a diarylheptanoid isolated from the fruit of *Alpinia oxyphylla*,[12] and its IC_{50} value was less than 1 μM. The compound lacking methoxy group adjacent to the phenol were less active than those with the methoxy group, presumably because of the decrease of acidity of the phenol group.

Structural requirements for the phenolic inhibitors of PG biosynthesis suggest that gingerols and diarylheptanoids are not strictly specific inhibitors of PG biosynthesis but also act as inhibitors of other lipoxygenases such as arachidonate lipoxygenases, though the degree of inhibition would vary with each enzyme. Since LTs play an important role as chemical mediators in anaphylaxis[14] and inflammatory response,[15] an extensive search for inhibitors of arachidonate 5-lipoxygenase was carried out on natural products. Phenolic compounds such as dihydronorguaiaretic acid,[16] caffeic acid[17] and flavonoids[18] were found to inhibit arachidonate 5-lipoxygenase. Based on our view that the mechanism of inhibition of PG biosynthesis involves the phenolic compounds, we further explored the inhibitory effects of gingerols and diarylheptanoids on arachidonate 5-lipoxygenase. Samples of gingerols with different side chain lengths became available by their chemical synthesis,[19] and the effects of alkyl groups could therefore be evaluated quantitatively. Synthetic [2]- to [16]-gingerols were subjected to tests of their inhibitory effects on both PG synthetase and 5-lipoxygenase. The lysate of cultured RBL-1 cells was used as an enzyme source in the assay of

5-lipoxygenase. The inhibitory effects of gingerols on both enzyme systems are summarized in Table III. Gingerols show a clear maximum, *i.e.*, the minimum IC_{50} value, at [10]-gingerol, and both higher an lower homologues are less active, though the IC_{50} values of [6]- to [14]-gingerols (**4**—**8**) stay more or less at the same level. In contrast to the inhibition of PG synthetase, the effects of gingerols on arachidonate 5-lipoxygenase have a plateau in the homologues higher than [10]-gingerol (**6**) and the homologues lower than [8]-gingerol (**5**) were definitely less active. The contribution of alkyl group to the inhibitory effects on both enzyme systems are significantly different in their maximum activities, however, the alkyl groups of appropriate lengths are essential to higher activities in both systems.

Homologues with different carbon chain lengths were not available in the derivatives of diarylheptanoids, however, the effects of structural variation in the phenolic groups could be investigated on arachidonate 5-lipoxygenase and the results are summarized in Table II. It became quite clear that catechol or guaiachol (4-hydroxy-3-methoxyphenyl) group is essential for higher activities. Diarylheptanoids with *para*-hydroxyphenyl substitution were found to be inactive against LT biosynthesis, while they were active against PG synthetase. Diarylheptanoids with catechol group are very strong inhibitors and the results are well in accord with those reported on caffeic acid and flavonoids.[17,18] The IC_{50} value of AA-861,[20] a positive reference compound for the specific inhibition of 5-lipoxygenase, was 0.008 μM in our 5-lipoxygenase assay system, indicating that the assay system used in our study tended to give lower IC_{50} values. This is due to the assay condition of 5-liopoxygenase where the enzyme concentration was lower than those used in other studies.[21] The results obtained so far in our works may be another indication of possible functions of phenolic compounds in the efficacy of medicinal drugs used in Kampo medicine. Pharmacological activities of [6]-gingerol and [6]-shogaol have been studied in detail.[22] The antihepatotoxic action of gingerols was studied with carbon tetrachloride-induced cytoxicity in primary cultured rat hepatocytes and the results were surprisingly similar to those of the inhibition of PG biosynthesis.[22b] This may indicate that radical reaction is involved in the process of carbon tetrachloride-induced hepatotoxicity.

Experimental

^{1}H-NMR spectra were measured on a JEOL JNM FX-100, MS on JEOL JMS-DX300, infrared (IR) on a JASCO model 701G, UV on a Hitachi spectrophotometer model 100-60 and optical rotation on a JASCO DIP-140 digital polarimeter. Melting points were determined on a Yanagimoto micro-melting point apparatus and were uncorrected.

Assay of PG Biosynthesizing Enzyme The assay of PG biosynthesis was carried out with an isotope method using enzyme preparation from rabbit kidney medulla as described in a previous paper.[1c]

Culture of RBL-1 Cells RBL-1 cells were purchased from Dainippon Pharmaceutical Co. and cultured stationarily in Eagle's MEM (Nissui Co., Japan) (5 ml) containing 5% FBS (fetal bovine serum, Flow Lab., U.S.A.), 2.5 % CS (calf serum, Flow Lab., U.S.A.), 2 mM L-glutamine and antibiotics (penicillin and streptomycin) with an initial cell volume of 1×10^5 cells/ml as maintenance cultures in a CO_2 incubator. For the preparation of enzyme the stationary cultures were transferred to shake culures (each 300 ml) with inoculation size of 3×10^5 cells/ml and the inoculated flasks were incubated at 37 °C at 150 rpm. After two days cultured cells were harvested by centrifugation at 1400 rpm and precipitated cells were washed twice with 50 mM phosphate buffer containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1% gelatin. The obtained cells were stored at −80 °C until use.

Preparation of Enzyme Solution Frozen RBL-1 cells were thawed by dipping tubes containing frozen cells in tap water and diluting them with 35 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA and 0.1% gelatin. The cell suspension solution was homogenized with a Teflon homogenizer at 4 °C and centrifuged at 10000 *g* for 10 min. The supernatant was diluted with the same buffer to a concentration of 3×10^7 cell equivalent/550 ml, frozen with dry ice and stored at −80 °C.

Assay of 5-Lipoxygenase The assay method was modified from that of Jackshik *et al.*[21] The standard assay mixture contained 29 mM phosphate buffer (pH 7.0), 2×10^6 cell equivalent lysate, 1.5 mM $CaCl_2$, 10 mM indomethacin, 0.1 μCi (58 Ci/mol, Amersham, U.K.) [^{14}C]arachidonic acid and test sample in a total volume of 600 μl. The test sample was added to the reaction mixture as dimethyl sulfoxide (DIMSO) solution. The reaction mixture was incubated at 37 °C for 15 min. The reaction was terminated by adding acetone (1.2 ml) and was acidified by adding 2 N formic acid (100 μl). Reaction products were extracted twice with $CHCl_3$ (1.8 ml) and the extract was evaporated with a centrifuge evaporator *in vacuo*. The extract was subjected to preparative thin layer chromatography (TLC) by developing with EtOAc–isooctane–AcOH–H_2O 110:50:20:100. The position of 5-HETE (5-hydroxyeicosatetraenoic acid) was deduced from the spot of indomethacin which gave almost the same *Rf* as 5-HETE. The zone of 5-HETE was scraped off and subjected to radioactivity measurement.

Extraction and Separation of Gingerol Derivatives from Ginger Fresh ginger (3 kg) purchased in a green grocery near The University of Tokyo was extracted with boiling $CHCl_3$ (4.5 l) for 4 h. The residue was further extracted twice with MeOH (5 l and 3.5 l). The MeOH extract was concentrated to 3.6 l and H_2O (500 ml) was added to the extract, which was fractionated with hexane to give MeOH and hexane layers. The MeOH layer was concentrated to 1.8 l and extract 3 times with EtOAc (each 1 l). The hexane fraction was subjected to repeated chromatographies with slica-gel and Lobar RP-8 to afford ZO-1 (76 mg), ZO-2 (19 mg), ZO-3 (10 mg) and ZO-4 (33 mg). The EtOAc layer was also fractionated repeatedly with silica-gel and Sephadex LH-20 column chromatographies to afford [6]-gingerol (1.3 g), [6]-gingediacetate (10 mg), [6]-acetylgingerol (20 mg), [6]-gingediol (63 mg), [10]-gingerol (8 mg) and hexahydrocurcumin (64 mg), which were identified by the comparison of spectral data.

ZO-1: [6]-Dehydrogingerdione (16) Yellow needles (from hexane), mp 83.5—84.5 °C. *Anal.* Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.31; H, 7.71. UV λ_{max}^{EtOH} nm log (ε): 374 (4.44). MS *m/z* (rel. int.): 290 (M^+, 24), 272 (5), 219 (28), 216 (27), 201 (16), 191 (43), 177 (100), 145 ((32), 137 (27). IR ν_{max}^{KBr} cm^{-1}: 3320, 2920, 1625, 1600, 1510. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 0.90. (3H, t, J=7 Hz, Me), 1.20—1.80 (6H, m, $CH_2 \times 3$), 2.38 (2H, m, CH_2), 3.94 (3H, s, OMe), 5.62 (1H, s, =CH), 5.86 (1H, br s, OH), 6.35 (1H, d, J=16 Hz, =CH), 6.9—7.2 (3H, m, arom.), 7.52 (1H, d, J=16 Hz, =CH).

ZO-2: [10]-Dihydrogingerdione (17) Yellow needles from hexane, mp 69—69.5 °C. HR MS *m/z*: Calcd for $C_{21}H_{30}O_4$ 346.2145, Found 346.2154. UV λ_{max}^{EtOH} nm (log ε): 374 (4.62). MS *m/z* (rel. int.): 346 (M^+, 15). 328 (4), 219 (18), 216 (39), 201 (15), 191 (15), 177 (100), 145 (32), 137 (43). IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 2840, 1625, 1580, 1510. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 0.85 (3H, t, J=7 Hz, Me), 1.10—1.80 (14H, m, $CH_2 \times 7$), 2.36 (2H, m, CH_2), 3.19 (3H, s, OMe), 5.56 (1H, s, =CH), 5.82 (1H, br s, OH), 6.26 (1H, d, J=16 Hz, =CH), 6.8—7.2 (3H, m, arom.), 7.44 (1H, d, J=16 Hz, =CH).

ZO-3: [6]-Gingerdione (18) Colorless oil. HR MS *m/z*: Calcd for $C_{17}H_{24}O_4$ 292.1673, Found 292.1646. MS *m/z* (rel. int.): 292 (M^+, 22), 236 (2), 221 (3), 193 (3), 179 (4), 150 (130, 141 (8), 137 (100). ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 0.80 (3H, t, J=7 Hz, Me), 1.30—1.60 (6H, m, $CH_2 \times 3$), 2.55 (4H, m, $CH_2 \times 2$), 2.84 (2H, m, CH_2), 3.52 (0.2H, s, $CH_2 \times 0.1$), 3.85 (3H, s, OMe), 5.43 (0.9H, s, $=CH \times 0.9$), 5.45 (1H, br s, OH), 6.6—6.9 (3H, m, arom.).

ZO-4: [10]-Gingerdione (19) Colorless solid. HR MS *m/z*: Calcd for $C_{21}H_{32}O_4$ 348.2299, Found 348.2293. MS *m/z* (rel. int.): 348 (M^+, 17), 236 (6), 221 (2), 197 (4), 193 (3), 179 (4), 150 (16), 137 (100). IR ν_{max}^{cap} cm^{-1}: 3390, 2920, 2850, 1610, 1510. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 0.88 (3H, t, J=7 Hz, Me), 1.20—1.80 (14H, m, $CH_2 \times 7$), 2.5—2.6 (4H, m, $CH_2 \times 2$), 2.81 (2H, m, CH_2), 3.52 (0.2H, s, $CH_2 \times 0.1$), 3.87 (3H, s, OMe), 5.42 (0.9H, s, $=CH \times 0.9$), 5.49 (1H, br s, OH), 6.55—6.84 (3H, m, arom.).

Extraction and Separation of Diarylheptanoids from the Rhizomes of *Alpinea officinarum* The rhizomes of *A. officinarum* (3 kg) purchased from

Uchida Wakanyaku (Tokyo) were extracted three times with MeOH (7.5, 4, 4 l) for 4 h each and the MeOH solution was concentrated to 3 l. H_2O (300 ml) was added to the solution, which was extracted with hexane three times (1.5 l each). MeOH–H_2O layer was extracted with $CHCl_3$ three times (1.5 l each) and the $CHCl_3$ extract obtained was subjected to repeated column chromatography with silica-gel and Sephadex LH-20 to afford three known diarylheptanoids, 7-(4″-hydroxy-3″-methoxyphenyl)-1-phenylhept-4-ene-3-one (**20**) (145 mg), 1.7-diphenyl-5-hydroxy-3-heptanone (**21**) (410 mg) and 5-hydroxy-7-(4″-hydroxy-3″-methoxyphenyl)-1-phenyl-3-heptanone (**22**) (1.1 g), and three new diarylheptanoids, AO-1 (5 mg), AO-2 (36 mg) and AO-3 (91 mg), along with flavonoids. Known compounds were identified by the comparison of spectral data with those reported.

AO-1: 7-(4″-Hydroxy-3″-methoxyphenyl)-1-phenyl-3,5-heptadione (23) Colorless oil. HR MS Calcd for $C_{20}H_{22}O_4$ 326.1515, Found 326.1463. MS *m/z* (rel. int.): 326 (M^+, 32), 310 (5), 221 (4), 193 (4), 150 (20), 137 (100), 105 (21), 91 (38). IR ν_{max}^{cap} cm^{-1}: 3430, 2930, 1720, 1700, 1600, 1512. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.40—2.64 (4H, m, $CH_2 \times 2$), 2.64—3.00 (4H, m, $CH_2 \times 2$), 3.47 (0.4H, s, $CH_2 \times 0.2$), 3.82 (3H, s, MeO), 5.38 (0.8H, s, CH=), 5.48 (1H, br s, OH), 6.52—6.82 (3H, m, arom.), 7.18 (5H, m, phenyl).

AO-2: 5-Methoxy-7-(4″-hydroxy-3″-methoxyphenyl)-1-phenyl-3-heptanone (24) HR MS *m/z*: Calcd for $C_{21}H_{26}O_4$ 342.1828, Found 342.1795. MS *m/z* (rel. int.): 342 (M^+, 12), 205 (5), 195 (5), 477 (9), 150 (12), 137 (100), 133 (9), 105 (38), 91 (46). IR ν_{max}^{cap} cm^{-1}: 3400, 2930, 1710, 1600, 1512. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 1.60—1.51 (2H, m, CH_2), 2.44—2.88 (8H, m, $CH_2 \times 4$), 3.28 (3H, s, OMe), 3.66 (1H, m, CH), 3.83 (3H, s, MeO), 5.48 (1H, s, OH), 6.52—6.79 (3H, m, arom.), 7.15 (5H, m, phenyl).

AO-3: 5-Hydroxy-7-(4″-hydroxyphenyl)-1-phenyl-3-heptanone (25) Colorless oil. HR MS *m/z*: Calcd for $C_{19}H_{22}O_3$ 280.1416, Found 280.1481. $[\alpha]_D^{30}$ −13.3° (*c* = 1.04, $CHCl_3$). MS *m/z* (rel. int.): 280 ($M^+ - H_2O$, 33), 151 (13), 148 (12), 147 (22), 133 (39), 121 (12), 120 (30), 107 (100), 105 (60), 94 (12), 91 (77). IR ν_{max}^{cap} cm^{-1}: 3360, 3030, 2930, 1703, 1610, 1595, 1513. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 1.40—1.80 (2H, m, CH_2), 2.40—2.90 (8H, m, $CH_2 \times 4$), 3.40 (1H, br s, OH), 4.00 (1H, m, CH), 6.48—6.92 (4H, A_2B_2, arom.), 7.08 (5H, s, phenyl).

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