Diarylheptanoids from the Rhizomes of *Alpinia officinarum*

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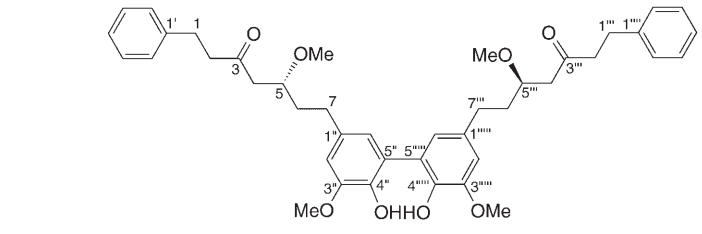
A novel dimeric diarylheptanoid, (5*R*,5′*R*)-7,7′-(6,6′-dihydroxy-5,5′-dimethoxy[1,1′-biphenyl]-3,3′-diyl)bis[5-methoxy-1-phenylheptan-3-one] (**1**), and two new diarylheptanoids, (4*E*,6*R*)-6-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one (**2**) and (4*E*,6*R*)-6-hydroxy-1,7-diphenylhept-4-en-3-one (**3**), together with seven known diarylheptanoids, were isolated from the rhizomes of *Alpinia officinarum*. Their structures were elucidated by application of extensive spectroscopic analyses and the modified *Mosher* method.

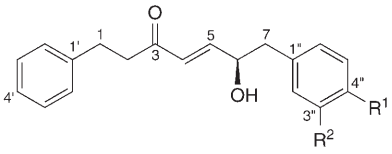
Introduction. – *Alpinia officinarum* HANCE (Zingiberaceae) is a medicinal herb native to East Asia, well known in Japan under the name of 'Ryokyo'. Its rhizomes are used as stomachic, analgetic, and antiemetic [1]. Diarylheptanoids, which are widely distributed in the genus *Alpinia*, possess cytotoxic, anti-inflammatory, antiplatelet, antioxidant, and antiproliferative activities [2–6]. Within the scope of our research on *Alpinia officinarum* HANCE, we report herein the isolation and the structure elucidation of three new diarylheptanoids named alpinoid A (**1**), B (**2**), and C (**3**), together with seven known diarylheptanoids, (5*R*)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenylheptan-3-one (**4**), (5*R*)-7-(4-hydroxy-3-methoxyphenyl)-5-methoxy-1-phenylheptan-3-one (**5**), (5*R*)-5-hydroxy-1,7-diphenylheptan-3-one (**6**), (4*E*)-7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one (**7**), (4*E*)-7-(4-hydroxyphenyl)-1-phenylhept-4-en-3-one (**8**), (4*E*)-1,7-diphenylhept-4-en-3-one (**9**), and (5*S*)-7-(4-hydroxyphenyl)-5-methoxy-1-phenylheptan-3-one (**10**). The known compounds **4**–**10** were identified by NMR and mass spectra and by comparison with published data [7][8]. The structures of the new diarylheptanoids were elucidated by analysis of NMR and MS data, and the absolute configurations of **2** and **3** were assigned by using the modified *Mosher* method.

Results and Discussion. – Alpinoid A[1]) (**1**) was obtained as a colorless oil, and its molecular formula was established to be $C_{42}H_{50}O_8$ by HR-FAB-MS ($[M+Na]^+$ at m/z 705.34035) and NMR spectroscopic data (*Table 1*). The complete structure of **1** was elucidated by the analysis of 2D-NMR data (*Table 1*) and by comparison with the NMR and MS data of **5** [7].

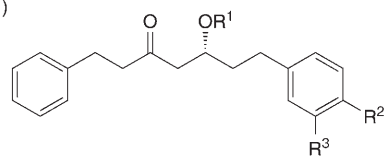
The NMR and MS data revealed that **1** and **5** were similar, but that **1** was approximately double the size of **5**, suggesting that **1** was a dimer of **5** through a C–C bond at C(5″)[1]). The symmetrical dimer **1**

[1]) Arbitrary atom numbering; for the systematic name, see *Exper. Part.*

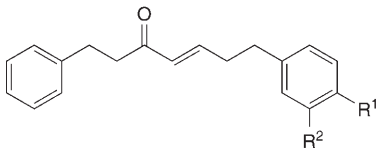




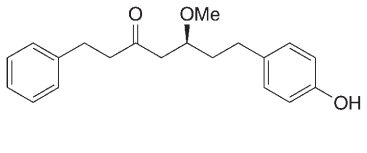
2 R^1 = OH, R^2 = MeO
3 R^1 = H, R^2 = H



4 R^1 = H, R^2 = OH, R^3 = MeO
5 R^1 = Me, R^2 = OH, R^3 = MeO
6 R^1 = H, R^2 = H, R^3 = H



7 R^1 = OH, R^2 = MeO
8 R^1 = OH, R^2 = H
9 R^1 = H, R^2 = H



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had the same negative optical rotation value as compounds **4**–**6** [8]. Therefore, *R* configuration of both C(5) and C(5″) can be assumed. This is further supported by the consideration that **1** is likely to result biosynthetically by the dimerization of a single enantiomer. The formula of **1** indicated 18 degrees of unsaturation. The ^{13}C-NMR, however, showed the presence of only 21 C-atoms, and the ^{1}H-NMR spectrum exhibited 24 proton signals. This clearly indicated that **1** is a symmetrical dimer. According to the DEPT-NMR spectrum, each half of **1** contained one C=O group, five aromatic quaternary C-atoms, seven aromatic and one oxygenated CH group, and two oxygenated Me and five CH_2 groups. The ^{1}H-NMR spectrum of **1** indicated the presence of both a tetrasubstituted benzene ring at δ(H) 6.73 (*d*, $J = 2.0$ Hz) and 6.71 (*d*, $J = 2.0$ Hz) and a monosubstituted benzene ring at δ(H) 7.24–7.27, 7.16–7.18, and 7.15–7.17 (3*m*). Proton signals from δ 2.88 to 1.78 corresponding to 10 H revealed the presence of five CH_2 groups. Also present in this spectrum were two MeO signals at δ(H) 3.91 and 3.30 (2*s*) and the signal of an oxygenated CH at δ(H) 3.69–3.73 (*m*). The correlations in the ^{1}H,^{1}H-COSY plot displayed the connectivities from CH_2(1) to CH_2(2) and from CH_2(4) to CH_2(7). The oxygenated CH group at δ(H) 3.69–3.73 (H–C(5)) showed HMBC correlations to the MeO at δ(C) 57.1 and two CH_2 at δ(C) 47.5 (C(4)) and 36.0 (C(6)). The CH_2 protons at δ(H) 2.76 (CH_2(2)) and 2.68 and 2.45 (CH_2(4)) also had HMBC correlations to the carbonyl C-atom at δ(C) 208.6 (C(3)). These observations established the positions of the MeO group and the carbonyl C-atom on the aliphatic chain. The linkages of two aromatic rings to the aliphatic chain were confirmed by the HMBC CH_2(1)/C(1′) and CH_2(7)/C(1″). Moreover, the two protons at δ 6.71 (H–C(2″)) and 6.73 (H–C(6″)) of the *AB* benzene-ring system showed HMBC with the MeO-substituted C(3″) at δ(C) 147.2, the OH-substituted C(4″) at δ(C) 140.7, and the quaternary C(5″) at δ(C) 124.5, respectively.

Alpinoid B (**2**) was obtained as a colorless oil and had a molecular formula of $C_{20}H_{22}O_4$ as determined by HR-EI-MS. The IR spectrum of **2** indicated the presence of

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp., $CDCl_3$) *of* **1**[1]). δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC	^{1}H,^{1}H-COSY
CH_2(1)	29.7	2.88 (*t*, *J* = 7.5)	C(2), C(3), C(1′)	CH_2(2)
CH_2(2)	45.4	2.76 (*t*, *J* = 7.5)	C(1), C(3), C(1′)	CH_2(1)
C(3)	208.6			
CH_2(4)	47.5	2.68 (*dd*, *J* = 15.8, 7.1), 2.45 (*dd*, *J* = 15.8, 5.1)	C(3), C(5), C(6)	H−C(5)
H−C(5)	76.0	3.69–3.73 (*m*)		CH_2(4), CH_2(6)
CH_2(6)	36.0	1.78–1.81 (*m*)	C(5), C(7)	H−C(5), CH_2(7)
CH_2(7)	31.2	2.67–2.72 (*m*)	C(5), C(6), C(1″)	CH_2(6)
C(1′)	141.0			
H−C(2′,6′)	128.3	7.16–7.18 (*m*)		
H−C(3′,5′)	128.5	7.24–7.27 (*m*)[a])		
H−C(4′)	126.1	7.15–7.17 (*m*)		
C(1″)	133.7			
H−C(2″)	110.6	6.71 (*d*, *J* = 2.0)	C(7), C(3″), C(6″)	
C(3″)	147.2			
C(4″)	140.7			
C(5″)	124.5			
H−C(6″)	122.9	6.73 (*d*, *J* = 2.0)	C(2″), C(4″), C(5″)	
*Me*O−C(5)	57.1	3.30 (*s*)	C(5)	
*Me*O−C(3″)	56.2	3.91 (*s*)	C(3″)	

[a]) Overlapped by the solvent signal.

an OH (3370 cm^{-1}) and an α,β-unsaturated C=O group (1654 and 1617 cm^{-1}). The NMR signals (*Table 2*) of two benzene rings for **2** matched those observed for **5**, with confirmation of the assignments from HMBC and COSY data. The absolute configuration at C(6) was assigned by application of the modified *Mosher* method [9][10]. *Mosher* acylation of **2** with both (αS)- and (αR)-α-methoxy-α-(trifluoromethyl)benzeneacetyl (MTPA) chloride yielded the C(6) (αR)- and (αS)-MTPA esters. The determination of $\Delta\delta$ values ($\delta_S - \delta_R$) for the protons neighboring C(6) led to the assignment of the (6*R*) configuration for **2** (*Table 3*), thus the structure of alpinoid B (**2**) was established as (4*E*,6*R*)-6-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one.

The ^{1}H-NMR spectrum of **2** (*Table 2*) showed protons at sp^2 C-atoms due to a 1,3,4-trisubstituted benzene ring at δ(H) 6.86 (*d*, *J* = 5.7 Hz), 6.70 (*dd*, *J* = 5.7 and 1.7 Hz), and 6.69 (*d*, *J* = 2.9 Hz), a monosubstituted benzene ring at δ(H) 7.28–7.17 (*m*), and a pair of olefinic *trans*-positioned protons at δ(H) 6.84 (*dd*, *J* = 15.7, 5.5 Hz) and 6.34 (*dd*, *J* = 15.7, 1.8 Hz). The presence of an OCH and an MeO group was evident from signals at δ(H) 4.48–4.49 (*m*) and 3.86 (*s*), confirmed by the ^{13}C-NMR signals for two oxygenated C-atoms at δ(C) 71.9 (C(6)) and 55.9 (MeO−C(3″)). The ^{13}C-NMR and DEPT spectra of **2** indicated the presence of two aromatic rings, one C=O group, a C=C bond, and three CH_2 and one OCH group. The observation of ^{1}H,^{1}H-COSY cross-peaks from the olefinic proton at δ(H) 6.84 (H−C(5)) to the OCH proton at δ(H) 4.48–4.49 (H−C(6)), in addition to those from H−C(6) to the CH_2(7) protons at δ(H) 2.88 and 2.69, confirmed the connection of both the C=C bond and the OH group. Further confirmation was provided by the HMBC cross-peaks (*cf. Fig.*) H−C(6)/C(4), C(5), and C(7). Moreover, the HMBC showed cross-peaks at δ(H) 6.34 (H−C(4))/δ(C) 199.2 (C(3)) and 71.9 (C(6)), and at δ(H) 2.87 (H−C(2))/δ(C) 29.9 (C(1)) and 199.2 (C(3)), yielding the aliphatic chain of **2**.

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp., $CDCl_3$) *of* **2** *and* **3**. δ in ppm, J in Hz.

	2		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	29.9	2.94 (*t*, J = 6.8)	29.9	2.94 (*t*, J = 6.9)
CH_2(2)	42.5	2.87 (*dt*, J = 6.8, 1.8)	42.5	2.88 (*dt*, J = 6.9, 1.5)
C(3)	199.2		199.2	
H–C(4)	128.2	6.34 (*dd*, J = 15.7, 1.8)	128.3	6.30 (*dd*, J = 15.8, 1.7)
H–C(5)	146.7	6.84 (*dd*, J = 15.7, 5.5)	146.6	6.83 (*dd*, J = 15.8, 4.6)
H–C(6)	71.9	4.48–4.49 (*m*)	71.8	4.51–4.53 (*m*)
CH_2(7)	43.0	2.88 (*dd*, J = 14.0, 5.2), 2.69 (*dd*, J = 14.0, 8.3)	43.3	2.93 (*dd*, J = 13.8, 5.1), 2.78 (*dd*, J = 13.8, 8.3)
C(1′)	141.0		141.0	
H–C(2′,6′)	128.4	7.17–7.19 (*m*)	128.3	7.18–7.21 (*m*)
H–C(3′,5′)	128.5	7.25–7.28 (*m*)[a])	128.4	7.28–7.30 (*m*)
H–C(4′)	126.1	7.18–7.20 (*m*)	126.1	7.17–7.20 (*m*)
C(1″)	128.3		136.6	
H–C(2″)	111.9	6.69 (*d*, J = 2.9)	128.7	7.30–7.33 (*m*)
C(3″)	146.6		129.4	7.20–7.22 (*m*)
C(4″)	144.7		127.0	7.25–7.27 (*m*)[a])
H–C(5″)	114.6	6.86 (*d*, J = 5.7)	129.4	7.20–7.22 (*m*)
H–C(6″)	122.1	6.70 (*dd*, J = 5.7, 1.7)	128.7	7.30–7.33 (*m*)
*Me*O–C(3″)	55.9	3.86 (*s*)		

[a]) Overlapped by the solvent signal.

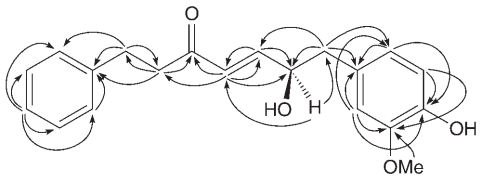

Figure. *HMBC for Alpinoid B* (**2**)

The aromatic proton at δ(H) 6.69 (H–C(2″)) correlated with δ(C) 146.6 (C(3″)) and 144.7 (C(4″)), and that at δ(H) 6.70 (H–C(6″)) with δ(C) 144.7 (C(4″)) and 114.6 (C(5″)), thus positioning the MeO and OH moieties at C(3″) and C(4″), respectively.

Alpinoid C (**3**) was also obtained as a colorless oil, with a HR-EI-MS molecular ion at m/z 280.14673, corresponding to a molecular formula $C_{19}H_{20}O_2$. A detailed analysis of its NMR data (*Table 2*) revealed that the structure was similar to that of alpinoid B (**2**), the difference being the presence of two monosubstituted benzene rings in **3** instead of one in **2**. By means of comparison of relevant ^{1}H-NMR $\Delta\delta$ values between the (αR)- and (αS)-MTPA esters of **3** as described above for **2**, the absolute configuration at C(6) of **3** was determined as (*R*) (*Table 3*). This allowed alpinoid C (**3**) to be assigned structurally as (4*E*,6*R*)-6-hydroxy-1,7-diphenylhept-4-en-3-one.

The ^{13}C-NMR data of **3** again showed two olefinic C-atoms at δ(C) 146.6 (C(5)) and 128.3 (C(4)), which were adjacent to the C=O group at δ(C) 199.2 (C(3)) and the oxygenated C-atom at δ(C) 71.8

Table 3. *Characteristic ^{1}H-NMR Data* (600 MHz, $CDCl_3$) *of the* Mosher *Esters Derived from* **2** *and* **3**. δ in ppm, J in Hz.

	From **2**			From **3**		
	δ_S[a])	δ_R[b])	$\Delta\delta$ ($\delta_S-\delta_R$)	δ_S[a])	δ_R[b])	$\Delta\delta$ ($\delta_S-\delta_R$)
H–C(4)	6.12	6.25	−0.13	6.06	6.18	−0.12
H–C(5)	6.69	6.72	−0.03	6.66	6.70	−0.04
H–C(6)	5.81	5.82	(*R*)	5.83	5.84	(*R*)
CH_2(7)	3.05, 2.93	2.99, 2.88	+0.06, +0.05	3.03, 2.92	2.95, 2.87	+0.08, +0.05

[a]) δ of (αS)-MTPA ester. [b]) δ of (αR)-MTPA ester.

(C(6)), respectively, as shown by HMBC. Further ^{1}H,^{1}H-COSY evidence for this arrangement was the presence of connectivities from the olefinic H–C(4) to CH_2(7).

There are several dimeric diarylheptanoids isolated from the genus of *Alpinia* [11][12]. Alpinoid A (**1**) is the first example of a C,C-linked dimeric diarylheptanoid from *Alpinia officinarum*.

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Experimental Part

General. Column chromatography (CC): silica gel (230–400 mesh; *Merck*), *Sephadex LH-20* (*Pharmacia*). TLC: silica gel GF_{254} (*Merck*). Prep. HPLC: *PU-2089-plus* pump (*Jasco*), *ODS-P* column (250 × 10 mm; *Inertsil*), *UV-2075-plus* detector (*Jasco*). Optical rotation: *Jasco DIP-360* polarimeter; in $CHCl_3$. UV Spectra: *Jasco V-550* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco FT/IR-300E* spectrometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Jeol ECX-500* (500 and 125 MHz, resp.) and *Jeol ECA-600* (600 and 150 MHz, resp.) spectrometers; J in Hz, δ in ppm rel. to $SiMe_4$ as an internal standard. MS and HR-MS: *JMS GC-mate* spectrometer; in m/z.

Plant Material. The *Alpinia officinarum* rhizomes were collected in Jinping (Yunnan Province, P. R. China) in April 2001, and identified by Prof. *S. Kitanaka*. The voucher specimen was deposited in the self-medication laboratory of Nihon University.

Extraction and Isolation. Air-dried rhizomes of *Alpinia officinarum* HANCE (1 kg) were ground and extracted with MeOH (3 × 3 l for 24 h) at r.t. After the combined extract was concentrated at 40°, the residue (115.8 g) was suspended in H_2O (2 l) and then partitioned with AcOEt (2 l). The AcOEt-soluble extract (46.1 g) was again partitioned between hexane (1 l) and MeOH/H_2O 19:2 (1.1 l). The hexane extract (12.4 g) was subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): *Fractions 1.1–1.4* (by TLC). *Fr. 1.3* was purified by reversed-phase prep. HPLC (*RP-18*, MeOH/H_2O 65:35): **1** (2 mg), **3** (2 mg), **5** (270 mg), and **7** (36 mg). The MeOH/H_2O extract (32.2 g) was also subjected to CC (*Sephadex LH-20*, MeOH): *Fractions 2.1–2.6* (by TLC). *Fr. 2.2* was then purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1); then silica gel, 15 → 30% AcOEt/hexane): **4** (97 mg), **6** (156 mg), **8** (24 mg), and **9** (40 mg). *Fr. 2.3* was further separated by reversed-phase prep. *HPLC* (*RP-C18*, MeOH/H_2O 58:42): **2** (6 mg) and **10** (12 mg).

Alpinoid A (=*(5*R*,5′*R*)-7,7′-(6,6′-Dihydroxy-5,5′-dimethoxy[1,1′-biphenyl]-3,3′-diyl)bis[5-methoxy-1-phenylheptan-3-one]*; **1**). Colorless oil. $[\alpha]_{25}^{D} = -6.08$ (c = 0.01, $CHCl_3$). UV (MeOH): 211 (3.6), 220 (3.5), 283 (0.9). ^{1}H- and ^{13}C-NMR, HMBC, and ^{1}H,^{1}H-COSY: *Table 1*. FAB-MS: 705 ($[M + Na]^+$). HR-FAB-MS: 705.34035 ($C_{42}H_{50}O_8^+$, $[M + Na]^+$; calc. 705.34096).

Alpinoid B (=*(4*E*,6*R*)-6-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one*; **2**). Colorless oil. $[\alpha]_{25}^{D} = -3.93$ ($c = 0.05$, $CHCl_3$). UV (MeOH): 214 (3.9), 241 (3.5), 281 (2.3). IR (KBr): 3370, 1654, 1617, 1514, 1451. ^{1}H- and ^{13}C-NMR: *Table 2*. HMBC: *Figure*. EI-MS: 326 (M^+), 308 ($[M - H_2O]^+$), 137 ($[M - 189]$). HR-EI-MS: 326.15182 ($C_{20}H_{22}O_4^+$, M^+; calc. 326.15179).

Alpinoid C (=*(4*E*,6*R*)-6-Hydroxy-1,7-diphenylhept-4-en-3-one*; **3**). Colorless oil. $[\alpha]_{25}^{D} = -3.92$ ($c = 0.02$, $CHCl_3$). UV (MeOH): 238 (3.4), 279 (2.8). ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 280 (M^+), 262 ($[M - H_2O]^+$), 189 ($[M - C_6H_5{-}CH_2]^+$), 159 ($[M - C_6H_5{-}CH_2{-}CHOH]^+$). HR-EI-MS: 280.14673 ($C_{19}H_{20}O_2^+$, M^+; calc. 280.14632).

Mosher *Esters of Alpinoids B* (**2**) *and C* (**3**). (*αS*)-*α*-Methoxy-*α*-(trifluoromethyl)benzeneacetyl (MTPA) chloride (10 µl) was added to the soln. of **2** or **3** (1 mg) in pyridine (500 µl). After being stirred at r.t. for 4 h, the mixture was concentrated and purified by prep. HPLC (*RP-18*, 84% MeOH to give the (*αR*)-MTPA ester. By means of the same method, (*αR*)-MTPA chloride afforded the (*αS*)-*Mosher* esters. ^{1}H-NMR: *Table 3*.

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