Six New Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*

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Chromatographic separation of part of an EtOH extract of the seeds of *Alpinia blepharocalyx* resulted in the isolation of six new (**1**−**6**) and two known (**7**, **8**) diarylheptanoids together with 12 known compounds. The structures of the new compounds, including their absolute stereochemistry, were elucidated by spectroscopic and chemical methods as (3*S*,5*S*)- (**1**) and (3*S*,5*R*)-3-hydroxy-5-methoxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene (**2**), (3*S*,5*S*)- (**3**) and (3*S*,5*R*)-3-hydroxy-5-ethoxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene (**4**), (3*S*)-methoxy-1,7-bis(4-hydroxyphenyl)-6*E*-hepten-5-one (**5**), and 1,7-bis(4-hydroxyphenyl)-hepta-4*E*,6*E*-dien-3-one (**6**). Among the isolated compounds, **5**, (3*S*,5*S*)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)heptane (**8**), 4′-hydroxy-5,6-dehydrokawain (**14**), and/or phloroglucinol (**20**) showed significant antiproliferative activity against murine colon 26-L5 carcinoma (ED_{50}: **5**, 5.2 μM; **8**, 12.8 μM; **14**, 20.7 μM; **20**, 26.4 μM) and human HT-1080 fibrosarcoma (ED_{50}: **5**, 10.1 μM; **14**, 20.1 μM; **20**, 20.9 μM) cells.

Alpinia blepharocalyx K. Schum. (Zingiberaceae) is widely distributed in southwest regions of the People's Republic of China including Yunnan and Shichuan Provinces and Tibet. Its seeds are used as an aromatic stomachic in mainland China.[1] Plants of this family have been reported to possess antioxidant,[2,3] antiinflammatory,[3] anticancer,[4] and hepatoprotective[5] activities. In a preliminary experiment, the 95% EtOH extract of the seeds of *A. blepharocalyx* was found to show significant antiproliferative activity against liver metastatic murine colon 26-L5 carcinoma[6] and human HT-1080 fibrosarcoma[7] cells (ED_{50} values of 33.2 and 7.3 μg/mL, respectively). The 95% EtOH extract was partitioned into hexane, diethyl ether, and residual aqueous fractions, among which the ether and residual aqueous fractions showed significant antiproliferative activity against both cell lines (ED_{50} values against colon 26-L5 and HT-1080 cells: ether fraction, 9.0 and 5.0 μg/mL; residual aqueous fraction, 12.3 and 6.8 μg/mL). Previously, we examined the constituents of the diethyl ether fraction and identified 15 novel diarylheptanoids bearing a chalcone or a flavanone moiety, namely, calyxins A−H, epicalyxins B−D, G, and H, and blepharocalyxins A and B, together with seven additional compounds.[8] We have now examined the residual aqueous fraction and have isolated 33 diarylheptanoids including 26 new compounds, together with 12 known compounds. Spectroscopic analysis has indicated that the diarylheptanoids may be classified into five groups: (1) acyclic diarylheptanoids, (2) cyclic diarylheptanoids, (3) dimeric diarylheptanoids, (4) novel diarylheptanoids having either a chalcone or a flavanone moiety, and (5) unusual diarylheptanoid derivatives. In this paper, we deal with the isolation and structure elucidation of the acyclic diarylheptanoids (**1**−**8**, Chart 1) and 12 known compounds, together with a consideration of the antiproliferative activity of these compounds.

Results and Discussion

The residual aqueous fraction of the 95% EtOH extact of the seeds of *A. blepharocalyx* was separated by a series of chromatographic separations, including passage over Sephadex LH-20, Si gel, and ODS, followed by normal- and reversed-phase preparative TLC and HPLC purification, to afford six new (**1**−**6**) and two known (**7**, **8**) acyclic diarylheptanoids, together with three known chalcones (**9**−**11**), one known flavanone (**12**), two known α-pyrones (**13**, **14**), one steroid (**15**), three cinnamic acid derivatives (**16**−**18**), and two phenolics (**19**, **20**). The structures of the known compounds were determined by comparing their spectral data with those of the authentic samples and/or with values in the literatures as follows: 1,2-dihydrobis(de-*O*-methyl)-curcumin (**7**),[8d] (3*S*,5*S*)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)heptane (**8**),[9] helichrysetin (**9**),[10] 2′,6′-dimethoxy-4,4′-dihydroxychalcone (**10**),[11] 4,4′-dihydroxychalcone (**11**),[12] 5-*O*-methylnaringenin (**12**),[13] 5,6-dehydrokawain (**13**),[14] 4′-hydroxy-5,6-dehydrokawain (**14**),[15] β-sitosterol glucoside (**15**),[16] methyl *p*-hydroxycinnamate (**16**),[17] methyl *p*-hydroxycinnamyl ketone (**17**),[18] *p*-hydroxycinnamic acid (**18**),[19] *p*-hydroxybenzaldehyde (**19**),[20] and phloroglucinol (**20**).[21]

Compound **1**, $[\alpha]^{25}_{D}$ +21.0° (MeOH), and compound **2**, $[\alpha]^{25}_{D}$ +21.3° (MeOH), both were obtained as light brown amorphous solids by HPLC separation with a chiral column. They showed the same molecular formula, $C_{20}H_{24}O_3$, by HRFABMS, and their IR spectra both displayed hydroxyl group absorptions (3350 cm^{-1}). Their ^1H and ^{13}C NMR spectra were similar to each other and showed the presence of two phenyl rings (one monosubstituted and one *para*-substituted), one *trans*-double bond, two oxymethines, three methylenes, and a methoxyl group (Table 1). These data and their COSY and HMQC spectra suggested that **1** and **2** are diarylheptanoids with a hydroxyl and a methoxyl group at C-3 and C-5 and a *trans*-olefin between C-6 and C-7. The structures of **1** and **2**, including the locations of the substituents, were determined by their HMBC spectra (Figure 1). The long-range correlations H_2-1/C-2′,6′, H-2′,6′/C-1, H-7/C-2″,6″, and H-2″,6″/C-7 indicated the location of the *para*-substituted and the monosubstituted benzene rings at C-1 and C-7, respectively, while that between the methoxyl protons and C-5 revealed the position of the methoxyl group at C-5. Their stereochemistry was determined by spectral analyses of their MTPA derivatives. The absolute configuration at C-3 was determined as *S* by the advanced Mosher's method[22] (Figure 2), but the absolute configuration at C-5 was more difficult to assign due to possible free rotation around C-5. The magnitude of the $\Delta\delta$ ($=\delta_S - \delta_R$) value for the methoxyl group in the MTPA

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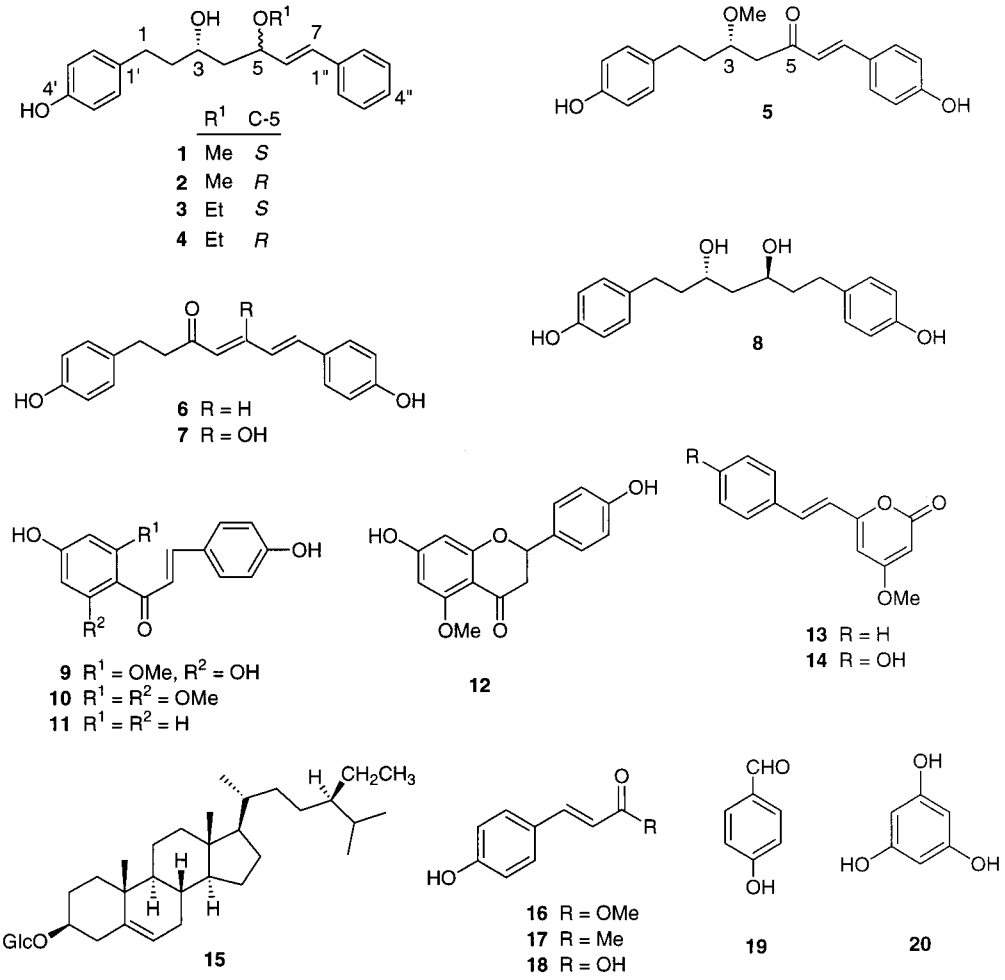
Chart 1



ester of **1** (0.044 ppm) was larger than that in the MTPA ester of **2** (0.032 ppm), while that for H-5 in the MTPA ester of **1** (0.124 ppm) was smaller than that in the MTPA ester of **2** (0.132 ppm). Thus, the methoxyl group in **1a** should lie nearer to the MTPA phenyl ring than that in **2a**; that is, C-3 and C-5 in **1** and **2** are *syn* (5*S*) and *anti* (5*R*), respectively. From these data, compounds **1** and **2** were determined as (3*S*,5*S*)- and (3*S*,5*R*)-3-hydroxy-1-(4-hydroxyphenyl)-5-methoxy-7-phenyl-6*E*-heptene, respectively.

Compound **3**, $[\alpha]^{25}_D$ +73.9° (MeOH), and compound **4**, $[\alpha]^{25}_D$ +49.5° (MeOH), were obtained as light yellow amorphous solids through HPLC separation with a chiral column, and their molecular formulas were determined by HRFABMS to be $C_{21}H_{26}O_3$, one methylene more than that of **1** and **2**. The ^{1}H and ^{13}C NMR spectra of **3** and **4** were similar to each other and to those of **1** and **2** (Table 1), but they showed the presence of an ethoxyl group instead of a methoxyl group in **1** and **2**. The COSY, HMQC, and HMBC data, including long-range correlations between the oxymethylene protons and C-5 and between H-5 and the oxymethylene carbon, indicate the location of the ethoxyl group to be at C-5. The ^{1}H and ^{13}C NMR data of **3** and **4** were similar to those of **1** and **2**, respectively. These data and the fact that compounds **1**−**4** all have positive optical rotation values suggested that **3** has the same absolute configuration as **1** (3*S*,5*S*) and **4** has the same one as **2** (3*S*,5*R*). Thus, compounds **3** and **4** were determined as (3*S*,5*S*)- and (3*S*,5*R*)-3-hydroxy-1-(4-hydroxyphenyl)-5-ethoxy-7-phenyl-6*E*-heptene, respectively.

Compound **5**, $[\alpha]^{25}_D$ +17.5° (MeOH), was obtained as a yellow amorphous solid, and HRFABMS determined its molecular formula to be $C_{20}H_{22}O_4$. The ^{1}H and ^{13}C NMR spectra were similar to those of **1**−**4** (Table 1) and showed the presence of two *para*-substituted benzene rings, a *trans*-olefin, a ketone-carbonyl, a methoxyl, an oxymethine, and three methylenes. The HMBC correlations between the carbonyl carbon and H-4, H-6, or H-7 indicated the replacement of the oxymethine at C-5 in **1**−**4** by the carbonyl group, and those between the methoxyl protons and C-3 and between H-3 and the methoxyl carbon suggested the location of the methoxyl group at C-3. These and other HMBC correlations similar to those of **1**−**4** (Figure 3) confirmed the planar structure of **5**. The configuration at C-3 was assumed to be *S*, because the other diarylheptanoids **1**−**4** all have the 3*S* configuration. Thus, compound **5** was assigned as (3*S*)-3-methoxy-1,7-bis(4-hydroxyphenyl)-6*E*-hepten-5-one.

Compound **6**, a yellow amorphous solid, showed IR absorptions at 3350, 1590, 1510, and 1440 cm^{-1}, and its molecular formula was determined to be $C_{19}H_{18}O_3$ by positive-ion HRFABMS. The ^{1}H and ^{13}C NMR spectra of **6** displayed signals similar to those of 1,2-dihydrobis(de-*O*-methyl)curcumin (**7**), but they were characterized by the presence of one more olefinic methine and the lack of any oxygenated olefinic carbon atoms. These data and interpretation of its COSY, HMQC, and HMBC spectra established the structure of **6** as 1,7-bis(4-hydroxyphenyl)hepta-4*E*,6*E*-dien-3-one.

Antiproliferative activity of the isolated compounds was examined against colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cells with the standard MTT assay,[23] and their ED_{50} values are summarized in Table 2. Most of

Table 1. ^{1}H and ^{13}C NMR Data for Diarylheptanoids **1**−**6** in CD_3OD[a]

	1		**2**		**3**		**4**		**5**		**6**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.62 ddd (14.0, 9.7, 6.3) 2.56 ddd (14.0, 8.7, 7.1)	32.0	2.67 ddd (15.0, 9.2, 5.8) 2.56 ddd (15.0, 8.1, 5.8)	32.1	2.62 ddd (14.0, 9.0, 6.0) 2.55 ddd (14.0, 9.0, 6.0)	32.0	2.66 ddd (14.7, 7.7, 5.6) 2.56 ddd (14.7, 7.0, 5.2)	32.1	2.60 m (2H)	31.5	2.82 t (7.4) (2H)	29.0
2	1.72 m (2H)	40.9	1.68 m (2H)	41.2	1.74 m (2H)	40.8	1.70 m (2H)	41.2	1.79 m (2H)	37.4	2.73 t (7.4) (2H)	41.6
3	3.64 m	69.1	3.78 m	68.1	3.67 m	69.3	3.80 m	68.3	3.75 quintet (6.5)	78.6		198.7
4	1.84 ddd (14.0, 9.1, 7.4) 1.66 m	44.0	1.76 ddd (15.0, 10.0, 4.0) 1.59 ddd (15.0, 8.0, 4.0)	44.8	1.84 m 1.67 m	44.1	1.77 ddd (14.0, 9.0, 4.5) 1.58 ddd (14.0, 8.0, 4.0)	44.8	2.96 dd (14.5, 6.5) 2.74 dd (14.5, 6.5)	45.9	6.18 d (15.5)	127.8
5	3.94 q-like (7.4)	82.2	3.98 dt (8.0, 4.0)	80.6	4.07 q-like (7.2)	80.4	3.98 dt (8.0, 4.0)	78.9		201.5	7.28 dd (15.5, 10.8)	143.3
6	6.00 dd (16.0, 8.0)	130.4	6.00 dd (16.0, 8.0)	130.9	6.03 dd (15.8, 8.0)	131.1	6.00 dd (15.8, 8.0)	131.6	6.66 d (15.5)	124.4	6.80 dd (15.5, 10.8)	123.6
7	6.59 d (16.0)	134.6	6.59 d (16.0)	133.4	6.59 d (15.8)	133.9	6.59 d (15.8)	132.7	7.56 d (15.5)	145.5	6.95 d (15.5)	141.4
1′		134.3		134.4		134.3		134.4		134.0		131.1
2′,6′	6.98 d (8.5)	130.3	7.00 d (8.5)	130.3	6.98 d (8.5)	130.3	7.00 d (8.5)	130.3	7.00 d (8.5)	130.3	6.98 d (8.5)	128.9
3′,5′	6.64 d (8.5)	116.1	6.66 d (8.5)	116.1	6.64 d (8.5)	116.1	6.66 d (8.5)	116.1	6.68 d (8.5)	116.2	6.63 d (8.5)	114.9
4′		156.3		156.3		156.3		156.3		156.4		155.4
1″		137.9		138.0		138.0		138.1		127.2		127.0
2″,6″	7.39 d (7.0)	127.6	7.39 d (7.0)	127.5	7.38 d (7.0)	127.5	7.38 d (7.0)	127.4	7.50 d (8.5)	131.6	7.35 d (8.5)	128.8
3″,5″	7.30 t (7.0)	129.6	7.30 t (7.0)	129.6	7.30 t (7.0)	129.6	7.30 t (7.0)	129.6	6.80 d (8.5)	116.9	6.62 d (8.5)	115.6
4″	7.22 t (7.0)	128.8	7.22 t (7.0)	128.7	7.22 t (7.0)	128.7	7.21 t (7.0)	128.6		161.6		158.7
OMe	3.30 s	56.3	3.30 s	56.7					3.32 s	57.3		
OCH_2					3.59 dq (10.5, 6.8)	64.8	3.62 dq (10.5, 6.8)	65.1				
					3.38 dq (10.5, 7.0)		3.40 dq (10.5, 7.0)					
CH_3					1.16 dd (7.0, 6.8)	15.6	1.18 dd (7.0, 6.8)	15.6				

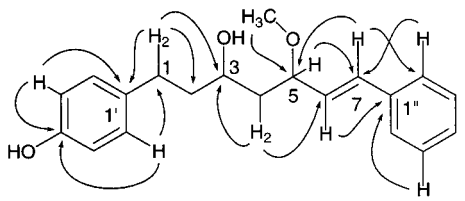


Figure 1. Significant HMBC correlations of **1**.

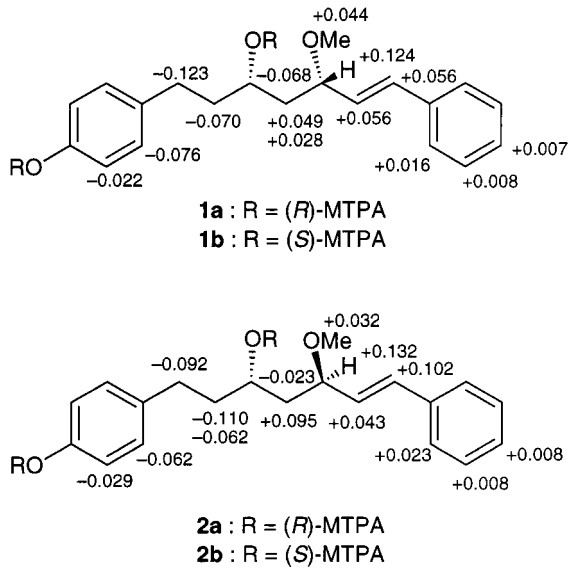


Figure 2. $\Delta\delta = (\delta_S - \delta_R)$ values obtained from the MTPA esters of **1** and **2** in $CDCl_3$ at 27 °C.

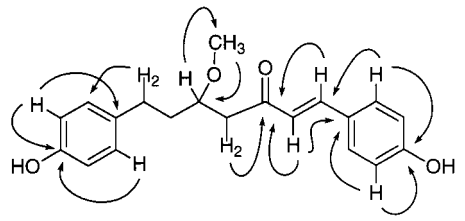


Figure 3. Significant correlations observed in the HMBC spectrum of **5**.

Table 2. Antiproliferative Activity for Compounds **1**−**20** (ED_{50} values in μM)[a]

compound[b]	colon 26-L5	HT-1080
1	86.4	>100
3	94.6	>100
5	5.2	10.1
6	57.7	78.8
7	62.6	>100
8	12.8	94.4
9	64.7	40.1
10	28.7	50.5
14	20.7	20.1
16	84.2	>100
20	26.4	20.9
5-fluorouracil	0.5	8.0

[a] ED_{50} values were calculated from the mean of data of six determinations. [b] Compounds **2**, **4**, **11**−**13**, **15**, and **17**−**19** were inactive (ED_{50} > 100 μM) in both cell lines.

the compounds showed more potent antiproliferative activity toward murine carcinoma than toward human fibrosarcoma with the exception of **9** and **20**. Compound **5** showed the most potent cytotoxicity against both the cell lines with ED_{50} values of 5.2 and 10.1 μM, respectively. In addition, the antiproliferative activity of compounds **5**, **8**, and **20** fell within the range of significantly active cytotoxic agents (ED_{50} < 4.0 μg/mL) as determined by Geran et al.[24]

Experimental Section

General Experimental Procedures. Optical rotations were determined in MeOH solution on a JASCO DIP-140 digital polarimeter at 25 °C. IR spectra were recorded on a Shimadzu IR-408 spectrophotometer in KBr disks or in $CHCl_3$ solution. ^1H and ^{13}C NMR spectra were measured in CD_3OD on a JEOL JNM-GX400 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are recorded as δ values. FABMS were measured with a JEOL JMS-700T spectrometer with glycerol as matrix. HPLC analysis was conducted with a Shimadzu LC-5A system using a Sumichiral OA-4700 column (4.6 mm i.d. × 25 cm; Sumika Chemical Analysis Service Ltd., Japan). The mobile phase was hexane-1,2-dichloroethane−EtOH (70:20:3) for the separation of **1** and **2** and hexane-1,2-dichloroethane−EtOH (70:20:2) for **3** and **4**, and UV (254 nm) was used for detection. Analytical and preparative TLC were conducted on precoated Merck Kieselgel 60F_{254} (0.25 and 0.50 mm) and RP-18F_{254} (0.25 mm) plates.

Plant Material. The seeds of *A. blepharocalyx* were procured from Mengha (1800 m above sea level), Yunnan Province, People's Republic of China, in August 1991. The plant sample was identified by Prof. Wu Te-Lin, South China Institute of Botany, Academica Sinica, and a voucher specimen (CPU9008037) is preserved in the herbarium of China Pharmaceutical University, Nanjing, People's Republic of China.

Extraction and Isolation. The seeds of *A. blepharocalyx* (10 kg) were extracted with 95% EtOH by percolation at room temperature, and the combined extracts were concentrated under reduced pressure to give an EtOH extract (800 g). This was suspended in 10% H_2O−MeOH and partitioned in turn with hexane and diethyl ether, to afford hexane (44 g), ether (450 g), and residual aqueous fractions (280 g). A portion of the residual aqueous fraction (60 g) was subjected to Sephadex LH-20 column chromatography with a H_2O−MeOH gradient system to provide 14 fractions.

Fraction 7 (13.9 g) was further subjected to Si gel (700 g) column chromatography with a $CHCl_3$−MeOH (99:1→70:30) gradient system to give 24 subfractions. Subfraction 1 ($CHCl_3$−MeOH, 99:1 eluate, 120 mg) was purified by normal-phase preparative TLC ($CHCl_3$−MeOH, 97:3) to give **13** (70.0 mg), while reversed-phase preparative TLC (MeOH−H_2O−MeCN, 5:3:2) of subfraction 3 ($CHCl_3$−MeOH, 98:2 eluate, 90 mg) afforded **16**[17] (6.8 mg) and **19**[20] (9.1 mg). Subfraction 4 ($CHCl_3$−MeOH, 97:3 eluate, 150 mg), on reversed-phase preparative TLC (MeOH−H_2O−MeCN, 5:3:2), gave **14**[15] (34.0 mg), **16** (8.1 mg), and **17**[18] (6.8 mg), together with two mixtures. HPLC separation of the mixture having an R_f value of 0.60 ($CHCl_3$−MeOH, 9:1) on normal-phase TLC gave **1** (3.2 mg, t_R 11.5 min) and **2** (3.9 mg, t_R 13.5 min), whereas that of R_f 0.68 gave **3** (2.0 mg, t_R 11.5 min) and **4** (2.0 mg, t_R 13.0 min). Subfraction 7 ($CHCl_3$−MeOH, 94:6 eluate, 230 mg) was further purified by normal-phase preparative TLC ($CHCl_3$−MeOH, 9:1) to give **10**[11] (102.0 mg) and **12**[13] (23.7 mg). Subfraction 10 ($CHCl_3$−MeOH, 92:8 eluate, 1.5 g), on reversed-phase preparative TLC (MeOH−H_2O−MeCN, 6:3:1), afforded (5*S*,6*S*)-5,6-dihydroxy-4″-de-*O*-methylcentrolobine[25a] (25.2 mg), calyxin L[25b] (6.2 mg), and **15**[16] (5.0 mg). Subfractions 11 and 12 ($CHCl_3$−MeOH, 91:9 eluate, 1.3 g) were combined and separated by ODS column chromatography (MeOH−H_2O−MeCN, 5:3:2), followed by normal-phase (C_6H_6−$CHCl_3$−MeOH, 3:14:3) and reversed-phase (MeOH−H_2O−MeCN, 6:3:1) preparative TLC, to afford **10** (18.0 mg) and calyxins I (4.6 mg) and J[25b] (18.3 mg).

Fraction 9 (12.5 g) was chromatographed over Si gel with a $CHCl_3$−MeOH (99:1→50:50) gradient system to provide 14 subfractions. Normal-phase preparative TLC ($CHCl_3$−MeOH, 9:1), followed by reversed-phase preparative TLC (MeOH−H_2O−MeCN, 5:3:2), of subfraction 2 gave **14** (5.6 mg), while that of subfraction 4 gave **6** (10.0 mg) and **7**[8d] (15.0 mg).

Fraction 11 (10.5 g) was applied onto a Si gel column with $CHCl_3$−MeOH (9:1) to give eight subfractions, and further chromatographic separation of subfraction 1 (2.3 g) on Si gel ($CHCl_3$−CH_3COCH_3−MeOH, 8:1:1), followed by preparative TLC, afforded **5** (2.5 mg), **6** (1.2 mg), **8**[9] (37.3 mg), **10** (157 mg), **11**[12] (2.7 mg), **12** (400 mg), **13**[14] (13.6 mg), **16** (1.4 mg), **18**[19] (9.5 mg), **19** (19.2 mg), and **20**[21] (14.0 mg), together with (3*S*,7*R*)- (7.1 mg) and (3*S*,7*S*)-1,2-dehydro-4″-de-*O*-methylcentrolobine[25a] (1.4 mg).

Fraction 13 (12.0 g) was subjected to Si gel column chromatography using a $CHCl_3$−MeOH (99:1→50:50) gradient system to give 15 subfractions. Subfractions 2, 4, and 6 were separated by normal-phase preparative TLC ($CHCl_3$−MeOH, 9:1), followed by reversed-phase preparative TLC (MeOH−H_2O−MeCN, 5:3:2), and subfraction 2 afforded **19** (13.0 mg), subfraction 4 afforded **9**[10] (17.0 mg) and **18** (17.0 mg), and subfraction 6 afforded **12** (40.0 mg).

(3*S*,5*S*)-3-Hydroxy-5-methoxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene (1): light brown amorphous solid; $[\alpha]^{25}_D$ +21.0° (*c* 0.08, MeOH); IR ($CHCl_3$) ν_{max} 3350, 1600, 1505, 1460, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 311.1665 (calcd for $C_{20}H_{23}O_3$ [M − H]$^-$, 311.1647).

(3*S*,5*R*)-3-Hydroxy-5-methoxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene (2): light brown amorphous solid; $[\alpha]^{25}_D$ +21.3° (*c* 0.13, MeOH); IR ($CHCl_3$) ν_{max} 3350, 1600, 1505, 1460 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 311.1660 (calcd for $C_{20}H_{23}O_3$ [M − H]$^-$, 311.1647).

(3*S*,5*S*)-3-Hydroxy-5-ethoxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene (3): light yellow amorphous solid; $[\alpha]^{25}_D$ +73.9° (*c* 0.04, MeOH); ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 325.1784 (calcd for $C_{21}H_{25}O_3$ [M − H]$^-$, 325.1804).

(3*S*,5*R*)-3-Hydroxy-5-ethoxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene (4): light yellow amorphous solid; $[\alpha]^{25}_D$ +49.5° (*c* 0.10, MeOH); ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 325.1789 (calcd for $C_{21}H_{25}O_3$ [M − H]$^-$, 325.1803).

(3*S*)-Methoxy-1,7-bis(4-hydroxyphenyl)-6*E*-hepten-5-one (5): yellow amorphous solid; $[\alpha]^{25}_D$ +17.5° (*c* 0.13, MeOH); IR (KBr) ν_{max} 3300, 1720, 1590, 1500, 1430 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 325.1474 (calcd for $C_{20}H_{21}O_4$ [M − H]$^-$, 325.1440).

1,7-Bis(4-hydroxyphenyl)hepta-4*E*,6*E*-dien-3-one (6): yellow amorphous solid; IR (KBr) ν_{max} 3350, 1590, 1510, 1440 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 295.1332 (calcd for $C_{19}H_{19}O_3$ [M + H]$^+$, 295.1334).

Preparation of MTPA Esters. To a stirred solution of a mixture of **1** and **2** (6.5 mg) in $CHCl_3$ (0.5 mL) and pyridine (0.5 mL) was added (*S*)-(+)-MTPA chloride or (*R*)-(−)-MTPA chloride (50 μL), and the mixture was stirred overnight at room temperature. The reaction mixtures with (*S*)-(+)-MTPA chloride were subjected to reversed-phase preparative TLC (MeOH−H_2O−MeCN, 4:4:2), followed by normal-phase preparative TLC (hexanes−EtOAc, 8:2) to afford **1a** or **2a** (each 2.7 mg), while those with (*R*)-(−)-MTPA chloride were subjected to normal-phase preparative TLC with hexanes−EtOAc (8:2) and then with hexane−benzene (3:7) to afford **1b** or **2b** (each 2.8 mg).

Each MTPA ester (0.8 mg) was dissolved in MeOH (0.5 mL) and hydrolyzed with 0.5 M NaOH (0.5 mL) overnight at room temperature. The reaction mixture was extracted with EtOAc, and the product was identified as **1** or **2** by an HPLC analysis (hexane−1,2-dichloroethane−EtOH, 70:20:3).

(*R*)-MTPA ester 1a: colorless amorphous solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.79 (1H, ddd, J = 14.0, 7.1, 4.7 Hz, H-4), 2.11 (1H, ddd, J = 14.0, 7.1, 6.7 Hz, H-4), 1.99 (2H, m, H_2-2), 2.62 (2H, m, H_2-1), 3.19 (3H, s, OMe), 3.62 (1H, q-like, J = 7.1 Hz, H-5), 5.20 (1H, m, H-3), 5.91 (1H, dd, J = 16.0, 8.0 Hz, H-6), 6.44 (1H, d, J = 16.0 Hz, H-7), 7.00 (2H, d, J = 8.5 Hz, H-3′,H-5′), 7.14 (2H, d, J = 8.5 Hz, H-2′,H-6′), 7.25 (1H, t, J = 7.0 Hz, H-4″), 7.31 (2H, t, J = 7.0 Hz, H-3″, H-5″), 7.35 (2H, d, J = 7.0 Hz, H-2″, H-6″); HRFABMS *m/z* 767.2416 (calcd for $C_{40}H_{38}O_7F_6Na$ [M + Na]$^+$, 767.2419).

(*R*)-MTPA ester 2a: colorless amorphous solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.85 (2H, td, J = 8.0, 4.0 Hz, H_2-4), 1.98 (1H, m, H-2), 2.03 (1H, m, H-2), 2.63 (2H, m, H_2-1), 3.22 (3H, s, OMe), 3.52 (1H, td, J = 8.0, 4.0 Hz, H-5), 5.42 (1H, m, H-3), 5.95 (1H, dd, J = 16.0, 8.0 Hz, H-6), 6.33 (1H, d, J = 16.0 Hz, H-7), 7.03 (2H, d, J = 8.5 Hz, H-3′,H-5′), 7.15 (2H, d, J = 8.5 Hz, H-2′,H-6′), 7.25 (1H, t, J = 7.0 Hz, H-4″), 7.31 (2H, t, J =

7.0 Hz, H-3″,H-5″), 7.35 (2H, d, J = 7.0 Hz, H-2″,H-6″); HRFABMS m/z 767.2416 (calcd for $C_{40}H_{38}O_7F_6Na$ $[M + Na]^+$, 767.2419).

(*S*)-MTPA ester 1b: colorless amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.82 (1H, m, H-4), 2.16 (1H, dt, J = 13.8, 7.1 Hz, H-4), 1.92 (2H, m, H_2-2), 2.50 (2H, m, H_2-1), 3.23 (3H, s, OMe), 3.74 (1H, q-like, J = 7.1 Hz, H-5), 5.26 (1H, m, H-3), 5.97 (1H, dd, J = 16.0, 8.0 Hz, H-6), 6.49 (1H, d, J = 16.0 Hz, H-7), 6.98 (2H, d, J = 8.5 Hz, H-3′,H-5′), 7.06 (2H, d, J = 8.5 Hz, H-2′,H-6′), 7.25 (1H, t, J = 7.0 Hz, H-4″), 7.32 (2H, t, J = 7.0 Hz, H-3″,H-5″), 7.37 (2H, d, J = 7.0 Hz, H-2″,H-6″); HRFABMS m/z 767.2458 (calcd for $C_{40}H_{38}O_7F_6Na$ $[M + Na]^+$, 767.2419).

(*S*)-MTPA ester 2b: colorless amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.92 (2H, m, H_2-2), 1.95 (2H, m, H_2-4), 2.54 (2H, m, H_2-1), 3.26 (3H, s, OMe), 3.65 (1H, td, J = 8.0, 4.0 Hz, H-5), 5.39 (1H, quint., J = 6.2, Hz, H-3), 5.99 (1H, dd, J = 16.0, 8.0 Hz, H-6), 6.44 (1H, d, J = 16.0 Hz, H-7), 6.99 (2H, d, J = 8.5 Hz, H-3′,H-5′), 7.09 (2H, d, J = 8.5 Hz, H-2′,H-6′), 7.26 (1H, t, J = 7.0 Hz, H-4″), 7.32 (2H, t, J = 7.0 Hz, H-3″,H-5″), 7.37 (2H, d, J = 7.0 Hz, H-2″,H-6″); HRFABMS m/z 767.2416 (calcd for $C_{40}H_{38}O_7F_6Na$ $[M + Na]^+$, 767.2419).

Antiproliferative Activity. Human HT-1080 fibrosarcoma and murine colon 26-L5 carcinoma cells were maintained in Eagle's minimum essential medium and RPMI-1640 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), respectively. These media were supplemented with 10% fetal calf serum (Gibco BRL Products, Gaithersburg, MD), 0.1% $NaHCO_3$, and 2 mM glutamine (Wako Pure Chemical Industries Ltd., Kyoto, Japan). Cellular viability in the presence and absence of the experimental agents was determined using the standard MTT (Sigma Chemical Co., Kyoto, Japan) assay as described previously.[26] 5-Fluorouracil (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) was used as a positive control in this assay.

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