Blepharocalyxins C−E, Three New Dimeric Diarylheptanoids, and Related Compounds from the Seeds of *Alpinia blepharocalyx*

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Three novel diarylheptanoids, blepharocalyxins C−E (**5**−**7**), together with four new (**1**−**4**) and one known (**8**) diarylheptanoids bearing a tetrahydropyran ring were isolated from the residual fraction of an EtOH extract of the seeds of *Alpinia blepharocalyx*. The structures and the stereochemistry at the chiral centers of the new diarylheptanoids were elucidated by spectroscopic techniques including 2D NMR spectroscopy. Blepharocalyxins C−E (**5**−**7**) have a novel carbon framework and are dimeric diarylheptanoids consisting of two diarylheptanoid units. Blepharocalyxin D (**6**) showed potent antiproliferative activity against murine colon 26-L5 carcinoma cells (ED_{50}, 3.61 μM), while against human HT-1080 fibrosarcoma cells, blepharocalyxin E (**7**) showed potent activity (ED_{50}, 9.02 μM).

The seeds of *Alpinia blepharocalyx* K. Schum. (Zingiberaceae) are medicinally important for their use in stomach disorders in the People's Republic of China.[1] In our work on the constituents of medicinal plants,[2] we have examined the constituents of *A. blepharocalyx*, which showed antiproliferative activity, and isolated 33 diarylheptanoids including 26 new ones, together with 12 known phenolic compounds. Their structures were deduced by spectroscopic analyses, and the diarylheptanoids are 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 the previous paper,[3] we reported the structures of the acyclic diarylheptanoids together with their antiproliferative activity. In this paper, we report the structures of the cyclic (**1**−**4**, **8**) and dimeric (**5**−**7**) diarylheptanoids (Chart 1) and their antiproliferative activity.[4]

Results and Discussion

Compound **1** was obtained as a colorless amorphous solid, $[\alpha]_D^{25}$ −12.3° (MeOH). Its molecular formula was determined as $C_{19}H_{20}O_3$ by HRFABMS, and its IR spectrum showed absorption at 3300 cm^{-1}. The ^1H and ^{13}C NMR spectra of **1** display signals for two *para*-substituted benzene rings, one *cis*-double bond, two oxymethines, and three methylenes (Table 1). These data are similar to those of (3*S*,7*S*)-5,6-dehydro-4″-de-*O*-methylcentrolobine (**8**),[5] which was also obtained from the same extract, but their chemical shifts and splitting patterns are slightly different. Thus, **1** was considered as an epimer of **8** either at C-3 or at C-7. Because the absolute configuration at C-3 was assumed to be *S* from the biogenetic point of view[6−8] and **8** has the 3*S*,7*S* configuration,[5] the absolute configuration at C-7 of **1** was concluded as *R*. Thus, compound **1** was determined as (3*S*,7*R*)-5,6-dehydro-1,7-bis(4-hydroxyphenyl)-4″-de-*O*-methylcentrolobine.

Compound **2**, $[\alpha]_D^{25}$ +28.5° (MeOH), was obtained as a yellow amorphous solid, and its molecular formula was determined to be $C_{19}H_{22}O_5$ by HRFABMS. Its IR spectrum also showed a broad hydroxyl absorption at 3300 cm^{-1}. The ^1H and ^{13}C NMR spectra of **2** resemble those of **1** (Table 1) and show the presence of two *para*-substituted benzene rings, four oxymethines, and three methylenes. However, the spectra were characterized by the disappearance of the olefinic signals and the presence of two additional oxymethines. From these data and the analyses of the COSY, HMQC, and HMBC spectra, compound **2** was considered to have two hydroxyl groups at C-5 and C-6 instead of the double bond (Figure 1a). The stereochemistry of **2** was determined by the analyses of coupling constants and ROESY data (Figure 1b). The large coupling constant (J = 10.0 Hz) between H-7 and H-6 and the small one of H-5 with H-6 and H-4_{ax} (J = 3.0 Hz) indicate the former two protons to have diaxial orientation and the latter to be equatorial. In the ROESY spectrum, the correlations H-3/H-7, H-3/H-4_{eq}, H-5/H-6, H-5/H-4_{ax}, H-5/H-4_{eq}, and H-6/H-4_{ax} were observed and indicated that H-3 and H-7 are *cis*; H-5 and H-6 are also *cis*; and both groups are *trans* with respect to one another. From these data and an assumption of 3*S* configuration, the structure of **2** was concluded to be (3*S*,5*S*,6*S*,7*R*)-5,6-dihydroxy-1,7-bis(4-hydroxyphenyl)-4″-de-*O*-methylcentrolobine.

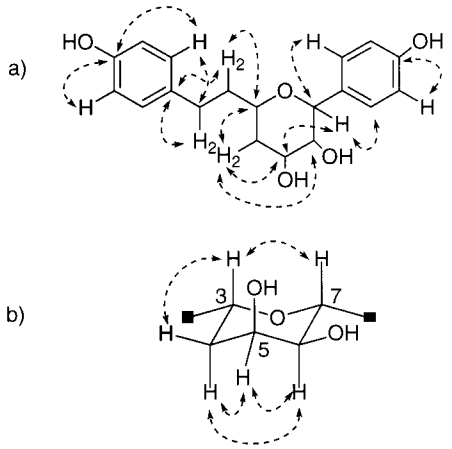

Figure 1. Significant HMBC (a) and ROESY (b) correlations of **2**.

Compounds **3** and **4** were obtained as an inseparable mixture with the same molecular formula ($C_{19}H_{22}O_5$) as for **2**. The ^1H and ^{13}C NMR spectra of the mixture show signals closely related to those of **2**, but they exhibit some differences in the chemical shifts in the ^{13}C NMR data as well as splitting patterns in the ^1H NMR data (Table 1). Thus, **3** and **4** were considered as stereoisomers of **2**. The COSY, HMQC, and HMBC data led to the unambiguous

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Chart 1

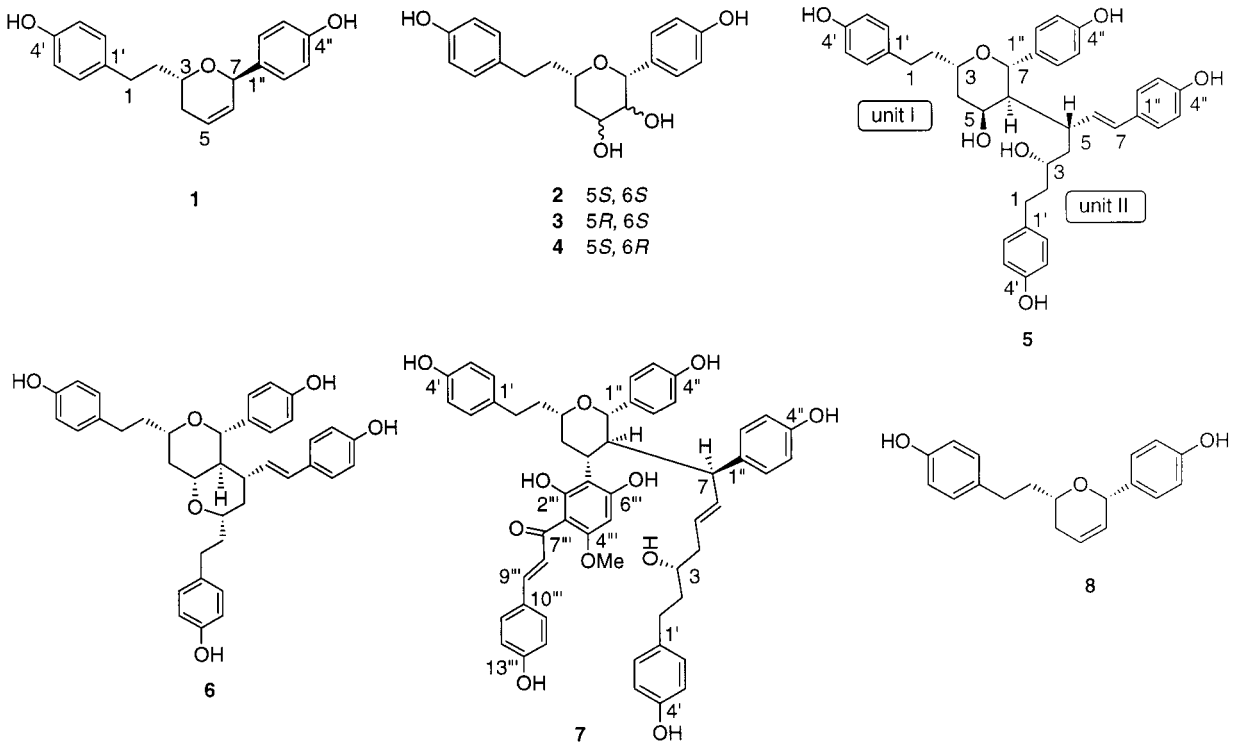

Table 1. ^{1}H and ^{13}C NMR Data for Diarylheptanoids **1**−**4** in CD_3OD[a]

	1		**2**		**3**		**4**	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.50 ddd (15.0, 7.5, 4.7)	31.43	2.62 m	31.90	2.58 m	32.46	2.69 m	31.84
	2.35 ddd (15.0, 9.5, 7.5)		2.54 m		2.50 m		2.62 m	
2	1.72 m	38.82	1.76 m	38.83	2.21 m	34.01	1.87 m (2H)	39.26
	1.56 m		1.62 m		1.73 m			
3	3.48 m	66.78	3.80 m	71.99	3.96 m	73.95	3.85 m	72.72
4	1.97 m (2H)	32.22	1.89 ddd (13.6, 3.0, 2.0)	39.64	1.94 m	37.65	1.96 m	34.44
			1.69 m		1.84 m		1.55 dt (13.5, 3.2)	
5	5.90 m	128.64	4.09 q (3.0)	69.12	3.81 m	70.53	3.98 m	69.56
6	6.00 ddd (10.5, 5.0, 2.3)	126.82	3.51 dd (10.0, 3.0)	73.60	3.34 dd (9.5, 4.0)	78.22	3.55 t (2.0)	71.99
7	5.16 br s	75.49	4.38 d (10.0)	78.94	4.22 d (9.5)	76.61	4.71 br s	77.06
1′		134.04		134.30		133.84		134.44
2′,6′	6.79 d (8.5)	130.41	6.96 d (8.5)	130.32	6.99 d (8.5)	130.35[b]	6.98 d (8.5)	130.33
3′,5′	6.53 d (8.5)	115.99	6.65 d (8.5)	116.02	6.67 d (8.5)	116.13	6.66 d (8.5)	116.04
4′		156.08		156.25		156.42		156.23
1″		132.89		132.73		131.93		132.53
2″,6″	7.20 d (8.5)	131.13	7.24 d (8.5)	130.18	7.23 d (8.5)	130.33[b]	7.24 d (8.5)	128.64
3″,5″	6.62 d (8.5)	115.96	6.76 d (8.5)	115.80	6.76 d (8.5)	115.96	6.75 d (8.5)	115.68
4″		158.38		158.06		158.31		157.29

[a] The coupling constants (parentheses) are given in Hz. [b] Values may be interchanged.

assignments of the ^{1}H and ^{13}C signals, which revealed the planar structures of **3** and **4** to be the same as that of **2**. In the ROESY experiment, **3** and **4** showed correlations between H-3 and H-7, indicating the protons to be *cis*. Similarly, **3** showed the correlations H-3/H-5, H-7/H-5, and H-6/H-4_{ax}, and **4** showed the correlations H-7/H-6, H-5/H-4_{ax}, and H-5/H-4_{eq}. Thus, H-5 in **3** and H-6 in **4** were concluded to be *cis* to H-3 and H-7. On the other hand, the large coupling constant (9.5 Hz) between H-7 and H-6 in **3** indicated that they are *trans*, while that in **4** is small (2.0 Hz), suggesting the *cis* nature of H-7 and H-6. Thus, compounds **3** and **4** were shown to be (3*S*,5*R*,6*S*,7*R*)- and (3*S*,5*S*,6*R*,7*R*)-5,6-dihydroxy-1,7-bis(4-hydroxyphenyl)-de-*O*-methylcentrolobine, respectively.

Blepharocalyxin C (**5**) was obtained as a light yellow amorphous solid with $[\alpha]_D^{25}$ +63.5° (MeOH). The molecular formula of **5** was determined by negative ion HRFABMS to be $C_{38}H_{42}O_7$, and its IR spectrum showed the absorption for a hydroxyl group at 3350 cm^{-1}. The ^{1}H and ^{13}C NMR spectra (Table 2) indicated the presence of four *para*-substituted benzene rings, one *trans*-double bond, six methines including four oxymethines, six methylenes, and eight quaternary carbons, suggesting **5** to be a diarylheptanoid dimer. The analyses of the DEPT, COSY, TOCSY, HMQC, and HMBC spectra suggested the diarylheptanoid units to be 5-hydroxy-4″-de-*O*-methylcentrolobin (unit I) and 1,7-bis(4-hydroxyphenyl)-5-hydroxy-1-heptene (unit II). The COSY correlation between H-6 of unit I (H-I-6) and H-5 of unit II (H-II-5) and the HMBC correlations between C-II-5 and H-I-6 indicated the two units to be connected through the C−C bond between C-I-6 and C-II-5.[4] The stereochemistry at the chiral centers of **5** was determined by analyses of coupling constants and ROESY correlations. The large coupling constants of H-I-3 with H-I-4_{ax} (J = 11.3 Hz) and of H-I-6 with H-I-7 (J = 11.0 Hz) indicated the axial nature of H-I-3, H-I-6, and H-I-7, while H-I-5 must

Table 2. ^{1}H and ^{13}C NMR Data for Blepharocalyxins C−E (**5**−**7**) in CD_3OD[g]

	5		**6**		**7**	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
I 1	2.54 m (2H)	31.73	2.57 m (2H)	31.74	2.56 m, 2.32 m	32.38
2	1.71 m, 1.61 m	39.26	1.82 m (2H)	39.11	1.58 m (2H)	39.39[e]
3	3.89 dtd (11.3, 5.5, 1.6)	72.08	3.54 m	76.40	3.27 m	71.09
4	1.80 dt (13.0, 1.6)	41.54	1.97 br d (12.0)	41.76	1.91 br t (11.2)	41.50[f]
	1.50 m		1.49 m		1.64 m	
5	4.31 q (1.6)	67.96	3.32 m[a]	80.64	3.04 dt (11.0, 4.9)	28.81
6	1.89 ddd (11.0, 5.0, 1.6)	51.69	1.57 q (10.0)	52.22	2.52 td (11.0, 9.5)	47.44
7	4.60 d (11.0)	79.58	3.95 d (10.0)	84.39	5.25 d (11.0)	83.82
1′		134.36		134.20		134.72
2′,6′	6.94 d (8.5)	130.34	6.98 d (8.5)	130.32	6.89 d (8.5)	130.14
3′,5′	6.64 d (8.5)	116.00	6.67 d (8.5)	116.10	6.69 d (8.5)	116.02
4′		156.12		156.30[b]		155.94
1″		133.60		133.51		133.60
2″,6″	7.19 d (8.5)	130.97	6.96 d (8.5)	130.36	7.13 d (8.5)	131.09
3″,5″	6.73 d (8.5)	115.97	6.66 d (8.5)	116.06	6.75 d (8.5)	116.02
4″		158.13		158.11		158.62
1‴						110.95
2‴						164.31
3‴						106.69
4‴						162.79
5‴					5.98 s	93.19
6‴						162.46
7‴						194.20
8‴					7.79 d (16.0)	125.46
9‴					7.69 d (16.0)	143.92
10‴						128.39
11‴,15‴					7.50 d (8.5)	131.35
12‴,14‴					6.82 d (8.5)	116.89
13‴						161.14
OMe					3.86 s	56.37
II 1	2.40 m (2H)	32.09	2.62 m (2H)	31.61	2.37 m, 2.24 m	31.37
2	1.57 m (2H)	41.41	1.71 m (2H)	39.32	1.47 m (2H)	39.06[e]
3	3.31 m	69.60	3.48 m	77.35	3.19 m	71.18
4	1.50 m (2H)	40.68	1.60 m	39.32	1.75 dt (13.0, 5.6)	38.51[f]
			1.25 m		1.50 dt (13.0, 6.0)	
5	2.46 m	40.91	2.17 m	43.14	4.87 m[c]	125.57
6	5.60 dd (15.9, 9.3)	131.56	5.00 dd (15.9, 8.5)	132.73	4.78 dd (15.5 9.5)	137.97
7	6.00 d (15.9)	131.11	5.74 d (15.9)	128.64	3.32 m[d]	51.51
1′		134.41		134.25		134.46
2′,6′	6.87 d (8.5)	130.26	6.98 d (8.5)	130.32	6.73 d (8.5)	130.22
3′,5′	6.58 d (8.5)	115.97	6.67 d (8.5)	116.10	6.62 d (8.5)	115.99
4′		156.21		156.35[b]		156.06
1″		130.89		130.98		135.44
2″,6″	6.97 d (8.5)	128.22	6.62 d (8.5)	128.03	6.98 d (8.5)	130.03
3″,5″	6.66 d (8.5)	116.06	6.53 d (8.5)	115.77	6.73 d (8.5)	116.61
4″		157.38		157.02		156.84

[a,d] Overlapped with the solvent signal but in acetone-d_6 appeared at δ 3.37 (td, J = 10.2, 4.5 Hz) and 3.42 (t, J = 9.5 Hz), respectively. [b,e,f] Values may be interchanged in each column. [c] Overlapped with H_2O signal but in acetone-d_6 appeared at δ 5.04 (ddd, J = 15.5, 9.5, 5.3 Hz). [g] The coupling constants (parentheses) are given in Hz.

be equatorial, based on the small coupling constant of H-I-5 with H-I-6 and H-I-4$_{ax}$ (J = 1.6 Hz). In the ROESY spectrum, H-I-3 and H-I-5 showed correlations with H-I-4$_{eq}$ and H-I-7 and with H-I-4$_{ax}$, H-I-4$_{eq}$, and H-I-6, respectively, indicating the configuration depicted in Figure 2. Regarding the configuration at C-II-5, the small coupling constant between the vicinal protons H-I-6 and H-II-5 (J = 5.0 Hz) and the ROESY correlations of H-II-5 with H-I-5 and H-I-6 revealed H-I-6 and H-II-5 to have a *gauche* relationship. Thus, the intense ROESY correlations between H-II-6 and H-I-5 and between H-II-4 and H-I-7 should support the configuration as shown in Figure 2. Because the diarylheptanoids isolated so far from *A. blepharocalyx* all have *S* configuration at the C-3 position,[5−8] the absolute configuration at C-I-3 and C-II-3 is assumed to be *S*. Thus, the absolute configuration of blepharocalyxin C (**5**) was concluded to be (I-3)*S*,(I-5)*S*,(I-6)*S*,(I-7)*S*,(II-3)-*S*,(II-5)*S*.

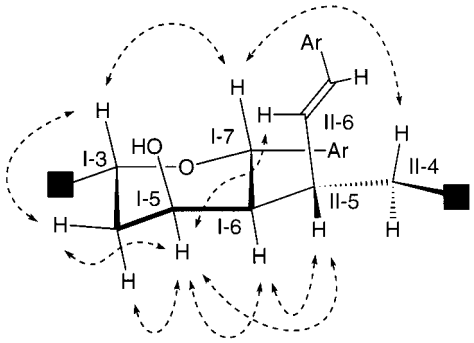

Figure 2. Significant ROESY correlations of blepharocalyxin C (**5**).

Blepharocalyxin D (**6**), $[\alpha]_D^{25}$ +18.5° (MeOH), was obtained as a light yellow amorphous solid, and its molecular formula was determined to be $C_{38}H_{40}O_6$, one water molecule less than that of **5**. The IR spectrum showed a broad absorption band at 3400 cm^{-1}, indicating the presence of a hydroxyl group. The ^{1}H and ^{13}C NMR spectra of **6** are similar to those of **5** and indicated the presence of two diarylheptanoid units, which was confirmed by the COSY, HMQC, and HMBC spectra. The molecular formula and

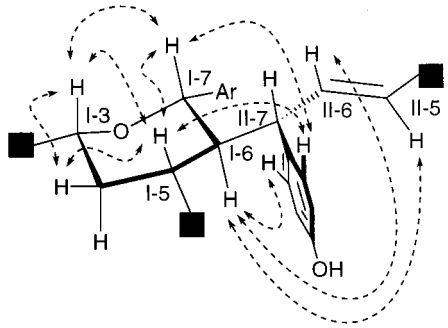

Figure 3. Significant ROESY correlations of blepharocalyxin E (**7**).

the low-field shift of C-I-5 (δ_C 80.64) and C-II-3 (δ_C 77.35) compared to C-I-5 (δ_C 67.96) and C-II-3 (δ_C 69.60) of **5** suggested the presence of an ether linkage between C-I-5 and C-II-3. The large coupling constants (10.0 Hz) of H-I-5, H-I-6, H-I-7, and H-II-5 indicated their axial nature, while the ROESY correlations among H-I-3, H-I-5, and H-I-7 and among H-I-5, H-II-3, and H-II-5 indicated these protons to be *cis*. From these data and the assumption that C-I-3 and C-II-3 have *S* configuration, the stereochemistry of **6** was concluded to be (I-3)*S*,(I-5)*R*,(I-6)*S*,(I-7)*S*,(II-3)*S*,(II-5)*S*.

Blepharocalyxin E (**7**), $[\alpha]_D^{25}$ +145.5° (MeOH), was obtained as a light yellow amorphous solid, and its molecular formula was determined to be $C_{54}H_{54}O_{11}$ by negative ion HRFABMS. In the IR spectrum, an absorption band attributable to a hydroxyl group was observed at 3300 cm^{-1}. The ^1H and ^{13}C NMR spectra of **7** are partly identical with those of calyxin F, also isolated from the same extract, but in **7**, signals assignable to one more diarylheptanoid unit are present. Extensive analyses of the DEPT, COSY, TOCSY, and HMQC spectra indicated the two units to be connected through the C−C bond between C-I-6 and C-II-7, which was confirmed by the HMBC correlations H-I-7/C-II-7 and H-II-7/C-I-6.[4] The stereochemistry at the chiral centers in unit I (I-3, I-5, I-6, and I-7) was determined to be the same as that of **6** by the ROESY experiment (Figure 3). The large coupling constant between the vicinal protons H-I-6 and H-II-7 (J = 9.5 Hz) indicated the two protons to have an *anti* relationship. On the other hand, the intense ROESY correlations of H-II-2″(6″) with H-I-5 and H-I-7 and of H-II-6″(2″) with H-I-6 in methanol-d_4, together with those of H-I-6 with H-II-5 and H-II-6 in acetone-d_6, revealed the relative configuration depicted in Figure 3. From these data and the assumption that C-I-3 and C-II-3 have *S* configuration, the stereochemistry of **7** was concluded to be (I-3)*S*,(I-5)*R*,(I-6)*S*,(I-7)*S*,(II-3)*S*,(II-7)*S*.

Compounds **2**−**4** possess a tetrahydropyran ring with dihydroxyl functionality. On the other hand, blepharocalyxins C−E (**5**−**7**) have a novel carbon framework consisting of two diarylheptanoid units with a C−C bond between C-6 of one unit and C-5 or C-7 of the other unit. Blepharocalyxin E (**7**) also possesses an additional chalcone moiety. These compounds are interesting from a biogenetic point of view. The diarylheptanoid **9**, which was also isolated from the same extract,[3,9] would give the allylic carbocation through reduction and elimination of the allylic hydroxyl group. Intramolecular nucleophilic attack of the C-3 hydroxyl group to the benzylic (C-7) carbocation could give rise to the cyclic diarylheptanoids **1** and **8**, and subsequent dihydroxylation of **8** may result in the formation of **2**−**4**. On the other hand, reaction of **8** with the carbocation may give the dimeric diarylheptanoid cations, which could yield blepharocalyxins C−E (**5**−**7**) (Scheme 1).

We also examined the antiproliferative activity of diarylheptanoids **1**−**8** against human HT-1080 fibrosarcoma[10] and liver metastatic murine colon 26-L5 carcinoma[11] cells using the standard MTT assay method[12] (Table 3). Among the isolated compounds, blepharocalyxin D (**6**) showed the most potent antiproliferative activity against murine carcinoma cells with an ED_{50} value of 3.61 μM, which falls within the range of significantly active cytotoxic agent (ED_{50} < 4.0 μg/mL) introduced by Geran et al.[13] On the other hand, blepharocalyxin E (**7**) exhibited the most potent activity against human fibrosarcoma cells with an ED_{50} value of 9.02 μM, which is comparable with that of 5-fluorouracil (5-FU) (ED_{50}, 8.0 μM), a clinically used anticancer drug.[14]

Experimental Section

General Experimental Procedure. Optical rotations were determined in MeOH solutions on a Jasco DIP-140 digital polarimeter at 25 °C. IR spectra were recorded on a Shimadzu IR-408 spectrophotometer in KBr disks. ^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 were recorded in δ values. FABMS were measured with a JEOL JMS-700T spectrometer with glycerol as a matrix. 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 from 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, Academia Sinica, and a voucher specimen (CPU9008037) is preserved in the herbarium of the China Pharmaceutical University.

Extraction and Isolation. The seeds of *A. blepharocalyx* (10 kg) were extracted with 95% EtOH by percolation at room temperature, and the alcoholic extract was concentrated under reduced presure to give an EtOH extract (800 g), which was suspended in 10% H_2O−MeOH and partitioned into hexane, ether, and residual fractions. The residual fraction (60 g) was subjected to Sephadex LH-20 column chromatography (CC) with a H_2O−MeOH gradient system to provide 14 fractions.

Fraction 7 (13.9 g) was further subjected to CC on Si gel (700 g) with a $CHCl_3$−MeOH (99:1→70:30) gradient system to give 24 subfractions. Subfraction 9 ($CHCl_3$−MeOH = 92:8 eluate, 1.5 g) was separated by ODS CC (MeOH−H_2O−MeCN, 5:3:2), followed by normal-phase preparative TLC (C_6H_6−$CHCl_3$−MeOH, 10:78:12), to yield **6** (5.5 mg), calyxin I (14.0 mg), epicalyxin I (4.0 mg), and calyxin L (4.7 mg), together with an epimeric mixture of calyxin J and epicalyxin J (18.2 mg). Subfraction 10 ($CHCl_3$−MeOH = 92:8 eluate, 1.5 g) was separated by reversed-phase preparative TLC (MeOH−H_2O−MeCN, 6:3:1) to afford **2** (25.2 mg), calyxin L (6.2 mg), and β-sitosterol glucoside (5.0 mg). Subfractions 11 and 12 ($CHCl_3$−MeOH = 91:9 eluate, 1.3 g) were combined and separated by ODS CC (MeOH−H_2O−MeCN, 5:3:2), followed by normal-(C_6H_6−$CHCl_3$−MeOH, 3:14:3) and reversed-phase (MeOH−H_2O−MeCN, 6:3:1) preparative TLC, to afford 2′,6′-dimethoxy-4,4′-dihydroxychalcone (18.0 mg), calyxin I (4.6 mg), calyxin J (18.3 mg), and a mixture of **3** and **4** (7.0 mg). Subfraction 13 ($CHCl_3$−MeOH = 90:10 eluate, 2.3 g) was chromatographed over Si gel with C_6H_6−MeCN−EtOH (60:40:0→50:40:10) to provide eight subfractions, and reversed-phase preparative TLC of the subfractions 2−4 (C_6H_6−MeCN = 6:4 eluate, 190 mg) gave **5** (8.0 mg) and **7** (7.0 mg).

Fraction 11 (10.5 g) was applied on 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 **1** (7.1 mg) and **8** (1.4 mg).

(3*S*,7*R*)-5,6-Dehydro-1,7-bis(4-hydroxyphenyl)-de-*O*-methylcentrolobine (1): colorless amorphous solid; $[\alpha]_D^{25}$ −12.3° (*c* 0.335, MeOH); IR (KBr) ν_{max} 3300, 1600, 1510, 1450,

Scheme 1

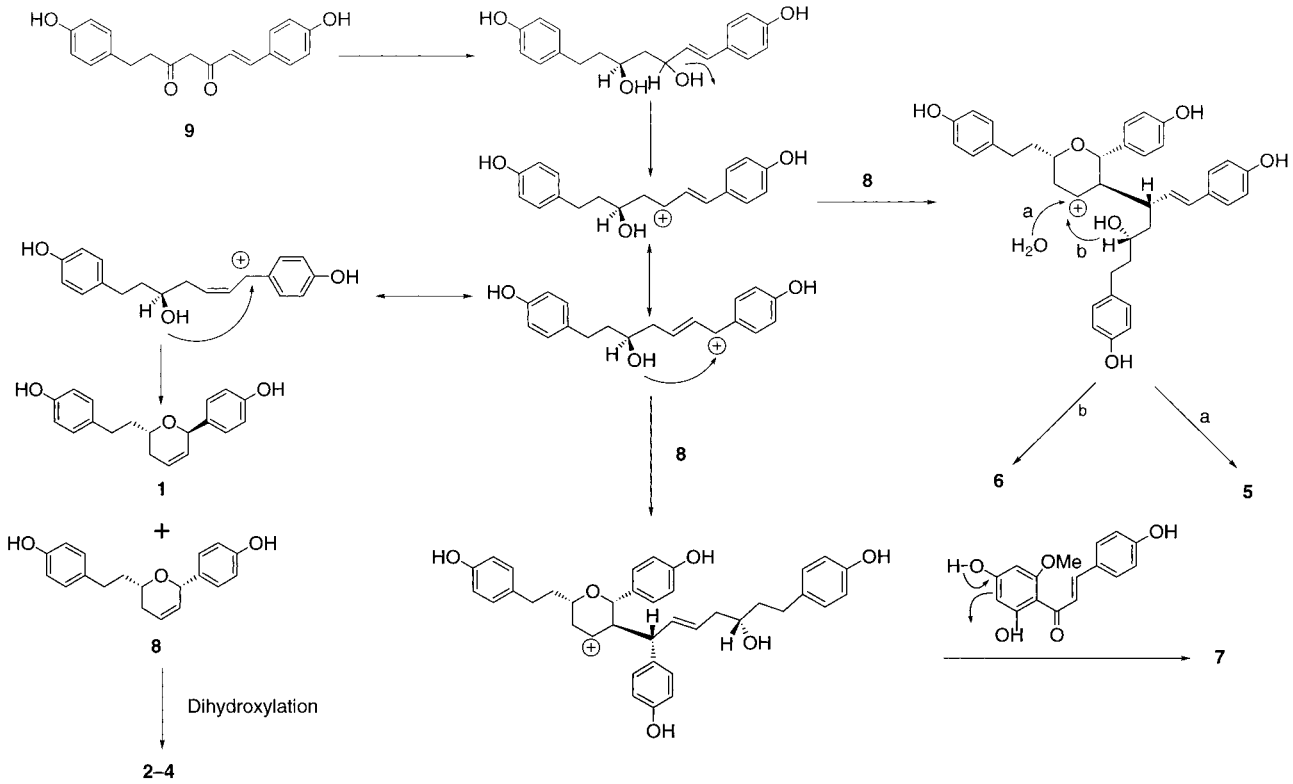

Table 3. Antiproliferative Activity for Diarylheptanoids **1**−**8** (ED_{50} values are in μM)[a]

compound	colon 26-L5	HT-1080
1	71.2	45.3
2	44.2	>100
mixture of **3** and **4**	49.4	83.7
5	29.6	54.3
6	3.61	25.7
7	32.2	9.02
8	>100	79.4
5-fluorouracil	0.53	8.00

[a] Values were calculated from the mean of data of six determinations.

1230 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 295.1352 (calcd for $C_{19}H_{19}O_3$ $[M - H]^-$, 295.1335).

(3*S*,5*S*,6*S*,7*R*)-5,6-Dihydroxy-1,7-bis(4-hydroxyphenyl)-de-*O*-methylcentrolobine (2): yellow amorphous solid; $[\alpha]_D^{25}$ +28.5° (*c* 0.040, MeOH); IR (KBr) ν_{max} 3300, 1610, 1590, 1510, 1445, 1220, 1030 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 329.1394 (calcd for $C_{19}H_{21}O_5$ $[M - H]^-$, 329.1399).

Mixture of (3*S*,5*R*,6*S*,7*R*)- (3) and (3*S*,5*S*,6*R*,7*R*)-5,6-dihydroxy-1,7-bis(4-hydroxyphenyl)-de-*O*-methylcentrolobine (4): yellow amorphous solid; IR (KBr) ν_{max} 3300, 1600, 1510, 1445, 1220, 1020 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 353.1393 (calcd for $C_{19}H_{22}O_5Na$ $[M + Na]^+$, 353.1365).

Blepharocalyxin C (5): light yellow amorphous solid; $[\alpha]_D^{25}$ +63.5° (*c* 0.035, MeOH); IR (KBr) ν_{max} 3350 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m/z* 609.2849 (calcd for $C_{38}H_{41}O_7$ $[M - H]^-$, 609.2852).

Blepharocalyxin D (6): light yellow amorphous solid; $[\alpha]_D^{25}$ +18.5° (*c* 0.025, MeOH); IR (KBr) ν_{max} 3400, 1610, 1510, 1450 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m/z* 591.2746 (calcd for $C_{38}H_{39}O_6$ $[M - H]^-$, 591.2748).

Blepharocalyxin E (7): light yellow amorphous solid; $[\alpha]_D^{25}$ +145.5° (*c* 0.025, MeOH); IR (KBr) ν_{max} 3300 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m/z* 877.3566 (calcd for $C_{54}H_{53}O_{11}$ $[M - H]^-$, 877.3588).

Antiproliferative Activity. Human HT-1080 fibrosarcoma and murine colon 26-L5 carcinoma cells were maintained in EMEM and RPMI-1640 medium (both Nissui Pharm. Co., Ltd., Tokyo, Japan), respectively. These media were supplimented with 10% fetal calf serum (Gibco BRL Products, Gaithersburg, MD), 0.1% sodium bicarbonate, and 2 mM glutamine (Wako Pure Chemicals Ind., Ltd., Kyoto, Japan). Cellular viability in the presence and absence of the experimental agents was determined using the standard MTT (Sigma Chemical Co., Japan) assay as described previously.[15] 5-Fluorouracil (Tokyo Kasei Kogyo Co., Ltd., Japan) was used as a positive control in this experiment.

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