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Epicalyxin F and Calyxin I: Two Novel Antiproliferative Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*

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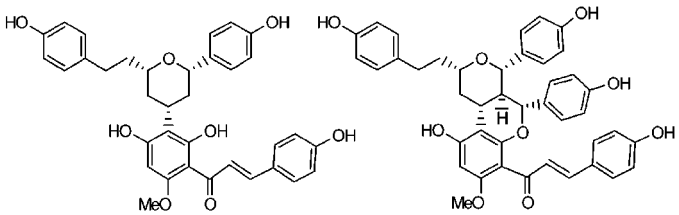
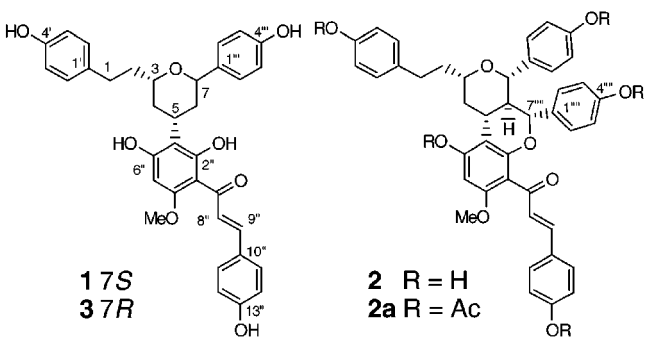
ABSTRACT



Epicalyxin F (1) and calyxin I (2), two novel diarylheptanoids, were isolated from a residual fraction of an EtOH extract of *Alpinia blepharocalyx*. Calyxin I (2) represented a new carbon skeleton, and epicalyxin F (1) possessed potent antiproliferative activity toward HT-1080 fibrosarcoma and colon 26-L5 carcinoma with ED_{50} values of 1.71 and 0.89 μM, respectively.

As a part of our ongoing research program to isolate bioactive compounds from Chinese medicinal plants, we have reported novel diarylheptanoids from an ether-soluble portion of an EtOH extract of seeds of *Alpinia blepharocalyx*,[1] which are used for the treatment of stomach disorders in The People's Republic of China. The isolated diarylheptanoids have an interesting structure: a chalcone or a flavanone moiety attached to a diarylheptanoid skeleton.[1] Further work on the residual fraction of the same extract afforded two novel diaryalheptanoids, epicalyxin F (**1**) and calyxin I (**2**). In this paper, we report the structure elucidation of these novel diaryalheptanoids together with their antiproliferative activity against human HT-1080 fibrosarcoma and murine colon 26-L5 carcinoma.



The seeds of *A. blepharocalyx* were extracted with 95% EtOH, and the EtOH extract was fractionated into hexane-

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soluble, ether-soluble, and residual fractions. Further investigation on the residual fraction gave two novel diaryalheptanoids, namely epicalyxin F (**1**) and calyxin I (**2**), along with helichrysetin,[2] 1,2-dihydro-bis(de-*O*-methyl)curcumin,[3] calyxin A,[1b] and calyxin F (**1**)[1b].

A series of chromatographies on Sephadex LH-20 and silica gel columns gave a mixture which showed a single spot on TLC with various solvent systems. The ^{1}H and ^{13}C NMR spectra of the mixture indicated that it contained two epimers. The epimers could be separated only by using HPLC on a chiral column (Figure 1a), and the spectral data

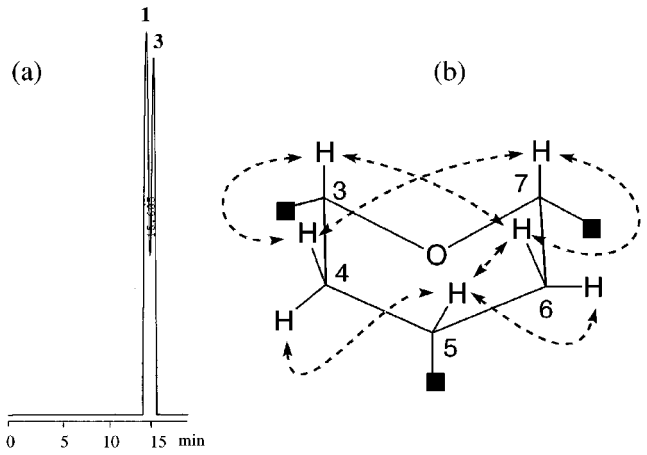

Figure 1. (a) HPLC chromatogram of a mixture of **1** and **3** [column Sumichiral OA-47100; mobile phase hexane−1,2-dichloroethane−ethanol (80:12:8); flow rate 1.0 mL/min; detection UV (254 nm)]. (b) ROESY correlations for the tetrahydropyran part of **1**.

of each compound indicated that one of the compounds was calyxin F (**3**); thus the other was named epicalyxin F (**1**). The molecular formula of epicalyxin F (**1**)[4] was determined by HR-FAB-MS to be $C_{35}H_{34}O_8$, the same as that of **3**. The IR spectrum of **1** showed absorption bands at 3400 (OH) and 1610 (C═O) cm^{-1}. The ^{1}H and ^{13}C NMR data of **1** were almost identical to those of **3**, showing six sets of *ortho*-coupled aromatic protons, a singlet aromatic proton, two *trans*-olefin protons, a methoxy group, two oxymethine protons, and four methylene groups. However, H-7 of **1** appeared at a slightly higher field (**1**, δ 5.05; **3**, δ 5.13), suggesting that **1** was an epimer of **3** at C-7. This was confirmed by a ROESY experiment. In the ROESY spectrum of **1**, significant correlations were observed between H-3$_{ax}$ and H-6$_{ax}$ and between H-4$_{ax}$ and H-7$_{ax}$. These correlations indicated that all the protons H-3, H-7, H-6$_{ax}$, and H-4$_{ax}$ must be oriented on the same side and the pyran ring must have a boat conformation (Figure 1b). Considering the absolute configuration of calyxin F (**3**) as 3*S*,5*R*,7*R* reported previously,[1b] the absolute configuration of epicalyxin F (**1**) was concluded to be 3*S*,5*R*,7*S*.

Calyxin I (**2**)[5] was obtained as a yellow amorphous solid having $[\alpha]^{25}_D$ −16.4° (*c* 0.05, MeOH). The HR-FAB-MS of **2** showed a *pseudo*-molecular ion at *m*/*z* 678.2598, indicating the molecular formula $C_{42}H_{38}O_9$. Its IR spectrum showed a broad absorption band at 3400 cm^{-1} and a sharp absorption band at 1700 cm^{-1}, indicating the presence of hydroxy and carbonyl groups, respectively. The ^{1}H NMR spectrum of **2** displayed signals corresponding to six sets of *ortho*-coupled aromatic protons [δ 7.62, 7.48, 6.94, 6.84, 6.64, 6.33 (all d, J = 8.5 Hz)], a singlet aromatic proton (δ 5.85), a methoxy group (δ 3.90), two *trans*-olefin protons [δ 7.78, 7.70 (both d, J = 16.0 Hz)], two oxymethine protons [δ 3.62 (m), δ 4.16 (d, J = 10.0 Hz)], and two methylene groups. These signals were almost identical to those of **1**, a tetrohydropyran-bearing diaryalheptanoid with a chalcone moiety at C-5 (Table 1). The extra signals of two sets of *ortho*-coupled aromatic protons [δ 7.65, 6.33 (both d, J = 8.5 Hz)] and an oxymethine proton at δ 4.64 (d, J = 10 Hz) suggested that **2** should have an extra *para*-substituted benzene group and an oxymethine group. This was further supported by the ^{13}C NMR spectrum which showed additional signals of an oxymethine carbon (δ 83.3) and six aromatic carbons including a phenolic one at δ 157.4. On the basis of detailed analyses of the ^{1}H−^{1}H COSY and TOCSY spectra, C-6 of the tetrahydropyran ring was shown to be bonded to the additional oxymethine carbon. The significant correlations observed between H-7′′′′ and C-5 and between H-7 and C-5 in the FG-pulsed HMBC spectrum also supported this conclusion. Moreover, correlation between H-7′′′′ and C-1′′′′ and C-2′′′′ in the FG-pulsed HMBC spectrum reveled the attachment of a *p*-hydroxybenzyl group at C-6 of the tetrahydropyran ring. The slight downfield shift of C-2′′ of **2** as compared to the corresponding signal of **1** (**2**, δ 167.4; **1**, δ 166.7) indicated a possible ether linkage between C-8 and C-2′′, which was confirmed by the fact that on acetylation **2** gave only pentaacetyl derivative **2a**.[6] From these data, the planar structure of calyxin I was determined.

The relative stereochemistry of the chiral centers in **2** was elucidated on the basis of coupling pattern of the protons in the rings A and B and by the ROESY data (Figure 2). Both H-7 and H-7′′′′ appeared as doublets with a coupling constant of 10.0 Hz, suggesting they should have diaxial interaction with H-6$_{ax}$ (quartet-like, J = 10.0 Hz). The triple doublet of H-5 was considered to be due to coupling with H-4$_{ax}$ (8.0 Hz), H-6$_{ax}$ (10.0 Hz), and H-4$_{eq}$ (4.0 Hz) in a chair

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(4) Light yellow amorphous solid; $[\alpha]^{25}_D$ +103.1° (*c* = 0.05, MeOH); HR-FAB-MS, *m*/*z* 583.2332 [calcd for $C_{35}H_{35}O_8$ (M + H)$^+$, 583.2333]; IR (KBr) ν_{max} 3400 (OH), 1610 (CO), 1510, 1440, 1340, 1140 cm^{-1}.

(5) Light yellow amorphous solid; $[\alpha]^{25}_D$ −16.4° (*c* = 0.05, MeOH); HR-FAB-MS, *m*/*z* 687.2598 [calcd for $C_{42}H_{39}O_9$ (M + H)$^+$, 687.2594]; IR (KBr) ν_{max} 3400 (OH), 1610 (CO), 1510, 1440, 1340, 1200, 1140 cm^{-1}.

(6) light yellow amorphous solid; HR−FAB-MS, *m*/*z* 919.2917 [calcd for $C_{52}H_{48}O_{14}Na$ (M + Na)$^+$, 919.2942]; ^{1}H NMR ($CDCl_3$) δ 7.49 (2H, d, J = 8.5 Hz, H-11′′,15′′), 7.34 (1H, d, J = 16.0 Hz, H-9′′), 7.05 (4H, d, J = 8.5 Hz, H-12′′,16′′,2′,6′), 6.88 (2H, d, J = 8.5 Hz, H-3′,5′), 6.86 (1H, d, J = 16.0 Hz, H-8′′), 6.77 (2H, d, J = 8.5 Hz, H-3′′′,5′′′), 6.72 (2H, d, J = 8.5 Hz, H-3′′′′,5′′′′), 6.64 (2H, d, J = 8.5 Hz, H-2′′′′,6′′′′), 6.62 (2H, d, J = 8.5 Hz, H-2′′′,6′′′), 6.27 (1H, s, H-5′′), 4.73 (1H, d, J = 10.0 Hz, H-7′′′′), 4.20 (1H, d, J = 10.0 Hz, H-7), 3.41 (1H, m, H-3), 5.05 (1H, ddd, J = 10.0, 8.0, 4.0 Hz, H-5), 2.58 (2H, m, H-1), 2.52 (1H, m, H-4), 2.29 (1H, q like, J = 10.0 Hz, H-6), 2.24, 2.20, 2.18, 1.17, 1.12 (each 3H, s, H_3-OAc), 1.81 (1H, m, H-2), 1.70 (1H, m, H-2), 1.55 (1H, m, H-4).

Table 1. ^{1}H and ^{13}C NMR Data of Epicalyxin F (**1**) and Calyxin I (**2**)[a]

	1		**2**		
	δ_H	δ_C	δ_H	δ_C	HMBC[c]
1	2.65 m	33.0	2.57 m	31.4	2′, 6′
2	1.76 m	41.5	1.78 m	39.0	1
3	3.78 m	69.6	3.62 m	77.9	2, 4
4	1.78 m	42.8	1.32 m	37.3	
	2.02 m		3.32 m		
5	3.38 m	27.3	3.08 ddd (10.0, 8.0, 4.0)	40.0	7, 7′′′′
6	1.92 m	34.8	2.26 q like (10.0)	49.7	7′′′′
	2.25 dd (12.0, 2.0)				
7	5.05 dd (12.0, 2.0)	76.2	4.16 d (10.0)	83.0	2′′′, 6′′′, 6
1′		134.8		134.0	1
2′, 6′	7.02 d (8.5)	129.2	6.94 d (8.5)	130.4	1, 3′, 5′
3′, 5′	6.67 d (8.5)	117.6	6.64 d (8.5)	115.7	2′, 6′
4′		156.8		155.7	2′
1′′		109.0		106.9	5′′
2′′		166.7		167.4	
3′′		109.0		106.2	5′′
4′′		162.8		162.0	OMe
5′′	5.95 s	93.4	5.85 s	92.4	
6′′		161.7		163.5	5′′
7′′		194.7		193.9	8′′, 9′′
8′′	7.80 d (16.9)	126.2	7.78 d (16.0)	125.4	9′′
9′′	7.70 d (16.0)	144.7	7.70 d (16.0)	143.5	8′′, 11′′, 15′′
10′′		128.9		128.0	8′′, 9′′, 12′′, 14′′
11′′, 15′′	7.58 d (8.5)	130.9	7.48 d (8.5)	131.0	9′′
12′′, 14′′	6.82 d (8.5)	116.9	6.84 d (8.5)	116.6	
13′′		161.7		160.7	11′′, 12′′, 14′′, 15′′
1′′′		133.8		130.7	3′′′, 5′′′, 7
2′′′, 6′′′	7.58 d (8.5)	128.9	7.62 d (8.5)*	130.2	3′′′, 5′′′, 7
3′′′, 5′′′	6.79 d (8.5)	116.8	6.33 d (8.5)	115.1	2′′′, 6′′′
4′′′		158.3		156.7	2′′′, 3′′′, 5′′′, 6′′′
1′′′′				133.0	3′′′′, 5′′′′, 7′′′′
2′′′′, 6′′′′			7.65 d (8.5)[b]	130.2	3′′′′, 5′′′′, 7′′′′
3′′′′, 5′′′′			6.33 d (8.5)	115.0	2′′′′, 6′′′′
4′′′′				157.4	2′′′′, 3′′′′, 5′′′′, 6′′′′
7′′′′			4.64 d (10.0)	83.3	2′′′′, 6′′′′
OMe	3.90 s	57.1	3.90 s	56.0	

[a] The ^{1}H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively, in CD_3OD, and coupling constants (parentheses) are in hertz. [b] Values are interchangeable. [c] ^{1}H correlating with ^{13}C resonance.

conformation. By closer inspection of a Dreiding model, ring B was also concluded to have a distorted chair conformation because a 10.0 Hz coupling constant was observed between H-7′′′′ and H-6$_{ax}$. This was further supported by the intense cross-peaks observed in the ROESY spectrum: H-5 and H-7, H-5 and H-7′′′′, H-5 and H-3, H-4 and H-6. Because all the diarylheptanoids isolated from *A. blepharocalyx* possess the 3*S* configuration,[1] the stereochemistry of **2** was assumed to be 3*S*,5*R*,6*S*,7*S*,7′′′′*R*.

Curcumin, a simple diaryalheptanoid isolated from *Curcuma longa*, is a well-known antioxidant and also has potent anticancer activity.[7] We thus examined the antiproliferative

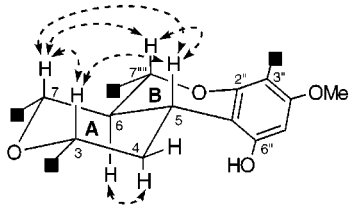

Figure 2. ROESY correlations of the protons on the rings A and B of calyxin I (**2**).

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activity of the newly isolated diarylheptanoids against human HT-1080 fibrosarcoma and highly liver-metastatic murine colon 26-L5 carcinoma.[8] Cellular viability in the presence and absence of experimental agents were determined using the standard MTT assay,[9] and results are summarized in Table 2. Among the isolated compounds, epicalyxin F (**1**) showed the strongest antiproliferative activity having ED_{50} values of 0.89 and 1.71 μM toward colon 26-L5 carcinoma and HT-1080 fibrosarcoma, respectively. The cytotoxicity of **1** against human HT-1080 fibrosarcoma is stronger than that of 5-fluorouracil (ED_{50} 8.0 μM), a clinically used drug for the treatment of human tumor[10] and falls within the range of the potent cytotoxic agent (ED_{50} < 4 μg/mL) made by Geran et al.[11] Other diarylheptanoids, except for 1,2-dihydro-bis(de-*O*-methyl)curcumin, also had interesting antiproliferative activity with ED_{50} values equal to or less than 10 μM in both tested cancer cells; calyxin A showed an ED_{50} of 13.14 μM against colon 26-L5 cells. It is interesting to note here that all the diarylheptanoids bearing chalcone moieties had stronger antiproliferative activity than curcumin toward both of the cell lines. In addition, both a free chalcone (helichrysetin) and a simple diarylheptanoid [1,2-dihydro-bis(de-*O*-methyl)curcumin] have weaker antiproliferative activities than the diaryalheptanoids bearing a chalcone moiety. This result indicates that the attachment of a chalcone moiety enhances the antiproliferative activity of diaryalheptanoids.

Table 2. Antiproliferative Activity of Isolated Compounds from Residual Fraction of *A. blrpharocalyx* (ED_{50} Values Are in μM)[a]

compound	colon 26-L5	HT-1080
epicalyxin F (**1**)	0.89	1.71
calyxin I (**2**)	8.39	9.08
calyxin F (**3**)	10.40	10.40
calyxin A	13.14	10.65
1,2-dihydro-bis(de-*O*-methyl)curcumin	62.61	105.51
helichrysetin	64.68	40.21
curcumin	23.23	23.42
5-fluorouracil	0.53	8.00

[a] ED_{50} values were calculated from the mean of data from six determinations.

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