Calyxin H, Epicalyxin H, and Blepharocalyxins A and B, Novel Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*

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Four unprecedented diarylheptanoids—calyxin H (**1**) and epicalyxin H (**2**), possessing a diarylheptanoid unit and a chalcone moiety, and blepharocalyxins A (**3**) and B (**4**), possessing two diarylheptanoid units and a chalcone moiety—were isolated from the seeds of *Alpinia blepharocalyx*. The structures of **1**−**4**, including absolute stereochemistry, were elucidated by spectroscopic means and after a consideration of their biogenesis.

Alpinia species (Zingiberaceae) have been extensively investigated from both a phytochemical and a biological point of view.[1−3] Recently, we reported the isolation and structure elucidation of calyxins A−G from the seeds of *Alpinia blepharocalyx* K. Schum. (Zingiberaceae).[4−6] Further efforts toward the isolation of bioactive diarylheptanoids have resulted in the isolation of four additional unprecedented diarylheptanoids bearing a chalcone moiety, which have been named calyxin H (**1**), epicalyxin H (**2**), and blepharocalyxins A (**3**) and B (**4**). Compounds **3** and **4** occurred as unusual structures having two diarylheptanoids and a chalcone moiety. We report herein the isolation and structure elucidation of these intriguing natural products (**1**−**4**) based on the analysis of 2D NMR spectroscopy.[7]

Results and Discussion

The seeds of *A. blepharocalyx* were extracted with 95% EtOH, and the EtOH extract was partitioned into hexane- and Et_2O-soluble fractions. From the ether-soluble fraction, four mixtures were isolated after a series of chromatographic separations. Each of these mixtures showed a single spot on TLC analysis with various solvent systems but displayed some closely overlapping signals in their ^{13}C-NMR spectra, indicating that they were epimeric mixtures. These epimeric mixtures were separated by HPLC using a chiral column, and two of these afforded calyxins B (**5**), C, D, and epicalyxins B (**6**), C, D,[5] while the others yielded four new compounds named calyxin H (**1**), epicalyxin H (**2**), and blepharocalyxins A (**3**) and B (**4**) (Figure 1).

Calyxin H (**1**), a light yellow amorphous solid, showed $[\alpha]_D$ −4.7° (*c* 0.18, MeOH), and its molecular formula was determined by HRFABMS to be $C_{35}H_{34}O_7$, one oxygen atom less than calyxin B (**5**). The ^{1}H- and ^{13}C-NMR spectral data of **1** were similar to those of **5** except for the signals of some aromatic protons and carbons

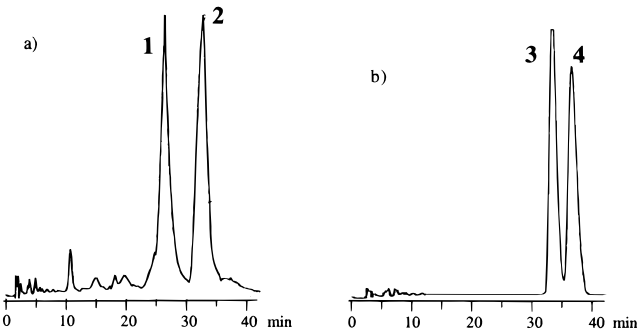

Figure 1. HPLC chromatograms of the diarylheptanoids **1**−**4**. (a) Mixture of **1** and **2**. (b) Mixture of **3** and **4**. [Column: Sumichiral OA-4700; mobile phase: (a) hexane−1,2-dichloroethane−EtOH (70:20:8), (b) hexane−1,2-dichloroethane−EtOH−trifluoroacetic acid (70:20:10:0.1); detection: UV (254 nm).

(Tables 1 and 2). The ^{1}H-NMR spectrum of **1** showed signals corresponding to four sets of *ortho*-coupled aromatic protons and a monosubstituted benzene ring. Thus, calyxin H was considered to differ from **5** by the absence of a hydroxyl group in one of the benzene rings. The HMBC spectrum of **1** showed long-range correlations similar to those of **5** including that between the protons of the monosubstituted benzene ring at δ_H 7.12 and the C-1 carbon (δ_c 33.49) (Figure 2). Thus, the position of the monosubstituted benzene ring was determined to be at C-1.

Epicalyxin H (**2**), a light yellow amorphous solid, showed an $[\alpha]_D$ +11.6° (*c* 0.12, MeOH), and its molecular formula ($C_{35}H_{34}O_7$) revealed that **2** was an isomer of **1**. Indeed, the ^{1}H- and ^{13}C-NMR data of **2** were almost the same as those of **1** (Tables 1 and 2) but differed in the ^{1}H-NMR splitting pattern of H-4, which was quartet-like in **2** but a triplet in **1**.

The stereochemistry, including the absolute stereochemistry, of **1** and **2** at C-3 and C-7 was assigned by comparison of the splitting patterns in the ^{1}H-NMR spectra and optical activity with **5** and epicalyxin B (**6**). The specific rotation of **1** was levorotatory (the same as **5**), and the splitting pattern of H-4 was the same as that of **5** (triplet), while in **2** it was dextrorotatory (the same as **6**), and the splitting pattern of H-4 was the same as

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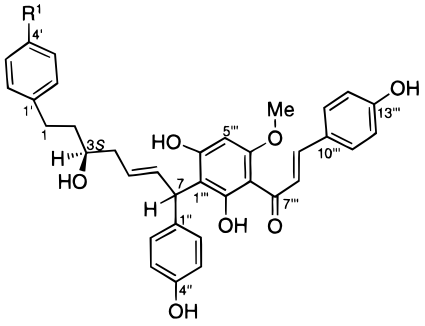
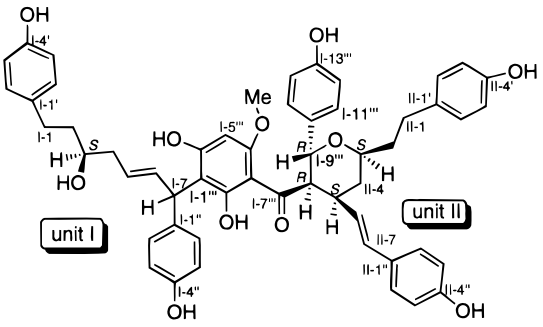
Table 1. ^{1}H-NMR Data for Diarylheptanoids **1**−**4** from *A. blepharocalyx* (MeOH-d_4)[a]

unit	proton(s)	**1**	**2**	**3**	**4**
I	1	2.60 m	2.60 m	2.52 m	2.51 m
		2.72 m	2.70 m	2.61 m	2.60 m
	2	1.62 m	1.62 m	1.62 m	1.61 m
		1.80 m	1.80 m	1.78 m	1.76 m
	3	3.62 m	3.61 m	3.61 m	3.59 m
	4	2.28 t (7.5)	2.28 q-like (7.5)	2.26 t (6.5)	2.26 q-like (6.5)
	5	5.57 dt (15.5, 7.5)	5.57 dt (15.5, 7.5)	5.55 dt (16.0, 7.5)	5.53 dt (16.0, 7.5)
	6	6.35 dd (15.5, 8.5)	6.34 dd (15.5, 8.5)	6.30 dd (16.0, 7.5)	6.30 dd (16.0, 7.5)
	7	5.13 d (8.5)	5.14 d (8.5)	5.12 d (7.5)	5.12 d (7.5)
	2′,6′	7.12 m[b]	7.10 m[b]	6.94 d (8.0)	6.91 d (8.0)
	3′,5′	7.20 t (7.0)	7.19 t (7.0)	6.63 d (8.0)[c]	6.63 d (8.0)[c]
	4′	7.13 m[b]	7.11 m[b]		
	2″, 6″	7.04 d (8.5)	7.05 d (8.5)	7.01 d (8.0)	7.01 d (8.0)
	3″, 5″	6.61 d (8.5)	6.61 d (8.5)	6.61 d (8.0)	6.61 d (8.0)
	5‴	6.01 s	6.01 s	5.91 s	5.90 s
	8‴	7.81 d (15.5)	7.81 d (15.5)	4.65 dd (4.5, 1.5)	4.66 dd (4.5, 1.5)
	9‴	7.66 d (15.5)	7.66 d (15.5)	5.22 br s	5.22 br s
	11‴,15‴	7.49 d (8.5)	7.49 d (8.5)	7.36 d (8.0)	7.36 d (8.0)
	12‴,14‴	6.82 d (8.5)	6.82 d (8.5)	6.86 d (8.0)	6.86 d (8.0)
	OMe-4‴	3.91 s	3.91 s	3.53 s	3.53 s
II	1			2.48 m	2.48 m
				2.70, m	2.70 m
	2			1.70 m	1.70 m
				1.84 m	1.84 m
	3			3.56 m	3.56 m
	4			1.50 m	1.50 m
				2.31 m	2.31 m
	5			2.84 m	2.84 m
	6			6.18 dd (16.0, 7.5)	6.18 dd (16.0, 7.5)
	7			6.31 d (16.0)	6.31 d (16.0)
	2′,6′			6.95 d (8.0)	6.95 d (8.0)
	3′,5′			6.64 d (8.0)[c]	6.64 d (8.0)[c]
	2″,6″			7.08 d (8.0)	7.07 d (8.0)
	3″,5″			6.66 d (8.0)[c]	6.66 d (8.0)[c]

[a] Chemical shifts (δ) are in ppm with coupling constants (J in Hz) in parentheses. [b] These were overlapped each others. [c] Values may be interchanged in each column.



	R^1	C-7
1:	H	*S*
2:	H	*R*
5:	OH	*S*
6:	OH	*R*



3: (I-7)*S*
4: (I-7)*R*

that of **6** (quartet-like). Based on these considerations, the absolute stereochemistry at C-3 and C-7 of **1** was determined to be 3*S*,7*S* and that of **2** was 3*S*,7*R*. Thus, the stereostructures of calyxin H and epicalyxin H are shown as **1** and **2**, respectively.

Blepharocalyxin A (**3**), a light yellow amorphous solid, showed $[\alpha]_D$ −56.4° (*c* 0.17, MeOH), and its molecular formula was determined to be $C_{54}H_{54}O_{11}$ by HRFABMS measurement. In the IR spectrum, absorption bands attributable to hydroxyl (3350 cm^{-1}) and carbonyl (1680 cm^{-1}) groups were apparent. Extensive analysis of the ^{1}H- and ^{13}C-NMR spectra, including DEPT and off-resonance measurements, indicated the presence of a ketone carbonyl, six methylenes, six sp^3 methines, a methoxyl group, two sets of *trans*-olefins, 20 *ortho*-coupled aromatic protons, a singlet aromatic proton, and 15 quaternary carbons (Tables 1 and 2).

The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of calyxin B (**5**), except for some chalcone signals, which indicated that part of its structure was similar to that of **5**. However, the lack of a conjugated olefin and the presence of two methine signals (δ_H 4.65 dd, J = 4.5, 1.5 Hz; δ_H 5.22 br s), having a correlation with the ^{13}C-NMR signals at δ_C 51.27 and 76.64 in the HMQC spectrum, suggested substitution at the conjugated olefin of a chalcone moiety. Furthermore, another

Table 2. ^{13}C-NMR Data for Diarylheptanoids **1**−**4** from *A. blepharocalyx* (MeOH-d_4)

unit	carbon (s)	**1**	**2**	**3**	**4**
I	1	33.49 t	33.50 t	32.08 t	32.09 t
	2	40.17 t	40.17 t	39.62 t	39.63 t
	3	72.53 d	72.66 d	71.83 d	71.75 d
	4	42.21 t	42.33 t	41.44 t	41.55 t
	5	128.57 d	128.51 d	127.84 d	127.92 d
	6	136.56 d	136.56 d	135.45 d	135.62 d
	7	44.15 d	44.15 d	43.23 d	43.01 d
	1′	144.48 s	144.48 s	134.60 s	134.61 s
	2′,6′	130.31 d	130.31 d	130.28 d	130.28 d
	3′,5′	127.36 d	127.36 d	116.06 d	116.07 d
	4′	130.06 d	130.06 d	156.19 s[a]	156.23 s[a]
	1″	137.38 s	137.29 s	136.55 s	136.45 s
	2″,6″	130.31 d	130.31 d	129.46 d	129.45 d
	3″,5″	116.28 d	116.28 d	115.46 d	115.48 d
	4″	156.66 s	156.66 s	155.76 s	155.82 s
	1‴	113.06 s	113.06 s	112.46 s	112.43 s
	2‴	166.98 s	166.98 s	166.20 s	166.64 s
	3‴	107.54 s	107.54 s	106.22 s	106.16 s
	4‴	163.55 s	163.55 s	162.07 s	162.11 s
	5‴	92.96 d	92.96 d	91.77 d	91.82 d
	6‴	164.55 s	164.55 s	163.53 s	163.53 s
	7‴	194.97 s	194.97 s	207.15 s	207.16 s
	8‴	126.72 d	126.72 d	51.27 d	51.30 d
	9‴	144.19 d	144.19 d	76.64 d	76.71 d
	10‴	129.27 s	129.27 s	131.65 s	131.67 s
	11‴,15‴	132.04 d	132.04 d	129.70 d	129.73 d
	12‴,14‴	117.68 d	117.68 d	116.31 d	116.33 d
	13‴	161.79 s	161.79 s	157.76 s	157.80 s
	OMe-4‴	57.02 q	57.02 q	56.17 q	56.17 q
II	1			31.89 t	31.90 t
	2			39.37 t	39.42 t
	3			70.94 d	70.94 d
	4			35.49 t	35.52 t
	5			38.84 d	38.88 d
	6			130.93 d	130.97 d
	7			130.76 d	130.79 d
	1′			134.41 s	134.41 s
	2′,6′			130.28 d	130.28 d
	3′,5′			116.06 d	116.07 d
	4′			156.12 s[a]	156.14 s[a]
	1″			130.76 s	130.79 s
	2″,6″			128.33 d	128.34 d
	3″,5″			116.24 d	116.26 d
	4″			157.62 s	157.65 s

[a] Values may be interchanged in each column.

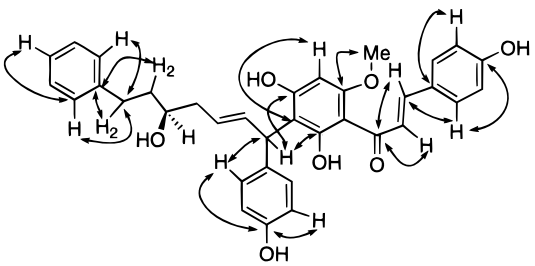


Figure 2. Significant long-range correlations observed in the HMBC spectrum of **1**.

set of signals in the ^{1}H- and ^{13}C-NMR spectra of **3**, assignable to a diarylheptanoid unit, was also observed. From these data and the analysis of the ^{1}H−^{1}H COSY spectrum, it was apparent that a new diarylheptanoid unit was attached to the chalcone portion of **3**, which was in accordance with the molecular formula obtained by FABMS.

Next, we measured the HMBC spectrum of **3** to elucidate the total structure.[7] The long-range correlations C-I-1/H-I-2′, C-I-1″/H-I-7, C-I-6‴/H-I-7, C-II-7/H-II-2″, and C-II-1/H-II-2′ allowed us to assign the diarylheptanoid parts. Further, the long-range correlation between δ_C 70.94 (C-II-3) and δ_H 5.22 (H-I-9‴) resulted in the formation of a tetrahydropyran ring between C-II-3 and C-I-9‴. Thus, the planar structure of blepharocalyxin A was deduced as that represented by **3**.

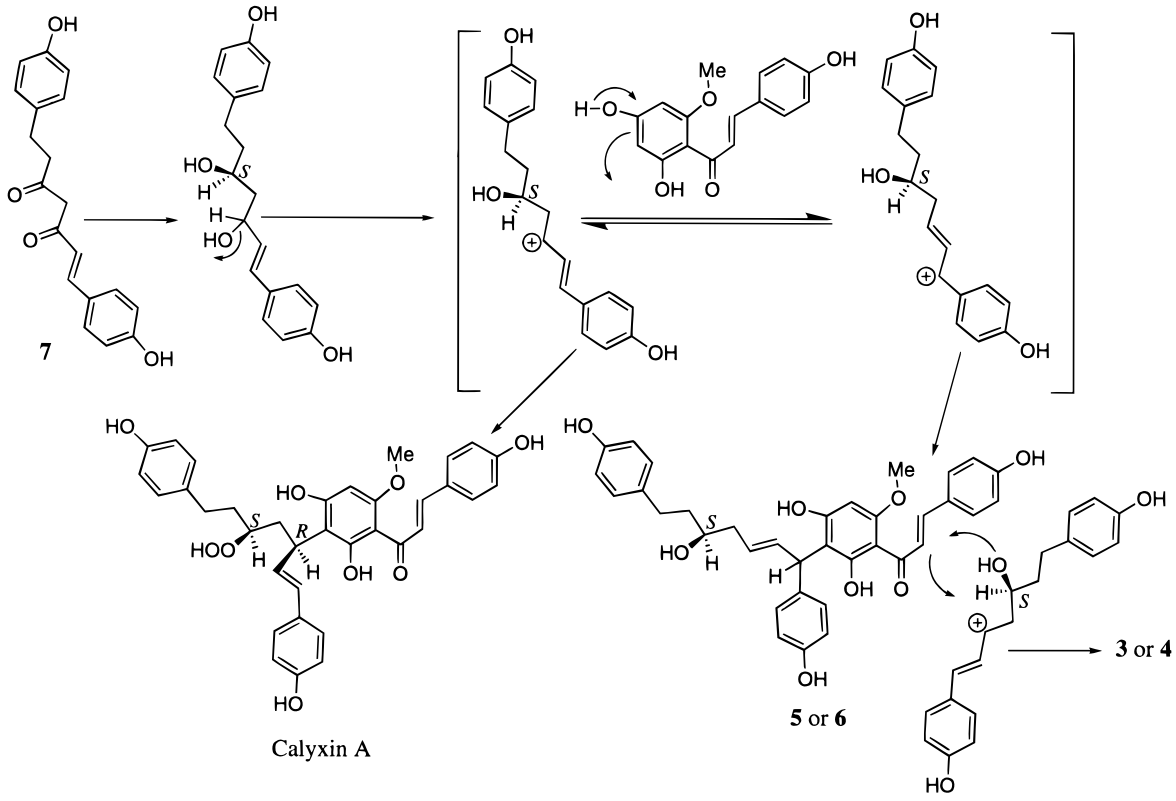
Blepharocalyxin B (**4**), a light yellow amorphous solid, showed $[\alpha]_D$ −97.7° (*c* 0.16, MeOH). The FABMS and the ^{1}H- and ^{13}C-NMR spectra of **4** were almost the same as those of **3** (Tables 1 and 2), but differed in the ^{1}H-NMR splitting patterns at H-4; that is, quartet-like in **4** and a triplet in **3**. Thus, **4** was considered to be a stereoisomer of **3**.

The stereochemistry at six chiral centers (I-3, I-7, I-8‴, I-9‴, II-3, II-5) within **3** and **4** was determined as follows. The relative stereochemistry of **3** and **4** at C-I-3 and C-I-7 was assigned by comparison with **5** and **6** in view of their similar splitting patterns in their ^{1}H-NMR spectra. As alluded to earlier, these two compounds differed from each other in the splitting pattern of H-4 in their ^{1}H-NMR spectra, which was taken as an important clue to distinguish their stereostructure because similar differences were observed between the stereoisomers of the calyxin B type (i.e., **5** and **6**, and **1** and **2**). It is, therefore, convenient to describe the relative stereochemistry of both **3** and **4** and **5** and **6** at these centers.[8]

The relative stereochemistry of **3** and **4** at the positions I-8‴, I-9‴, II-3, and II-5 was proposed on the basis of ROESY correlations.[7] An intense correlation peak between H-I-8‴ and H-II-5 implied that these protons project on the same side of the molecules. On the other hand, H-I-8‴, H-I-9‴, H-II-5, and H-II-3 displayed mutual correlation with H-I-11‴ in their ROESY spectra, with those observations being further supported in their 1D ROESY difference spectra. Thus, the benzene ring at C-I-9‴, H-I-8‴, H-I-9‴, H-II-5, H-II-3, and H-I-11‴ were all cis. The conformation of the tetrahydropyran ring was determined to be a *quasi*-chair form through a Dreiding stereomodel using the observed coupling constants, corresponding dihedral angles, and ROESY correlations. The configurational relationship between unit I and unit II, however, could not be deduced by the available NMR data. Accordingly, the absolute configuration at these centers were assigned as (I-3)*S*, (I-7)*S*, (II-3)*S*, (II-5)*S*, (I-8‴)*R*, and (I-9‴)*R* in **3** and (I-3)*S*, (I-7)*R*, (II-3)*S*, (II-5)*S*, (I-8‴)*R*, and (I-9‴)*R* in **4** in view of the assumed biogenesis (see below).

A biogenetic pathway that might occur in the formation of the carbon skeleton of **3** or **4** is shown in Scheme 1. The diarylheptanoid **7**, which was also isolated from the ether extract, may result in the allylic carbocation through reduction and elimination of the allylic hydroxyl group. The carbocation may react with a chalcone[9] to give calyxin B (**5**) and epicalyxin B (**6**),[5] or calyxin A.[6] The absolute configuration at C-3 of **5**, **6**, **1**, **2**, and calyxin A is all *S*, indicating that the absolute configuration at C-3 of the allylic cation would also be *S*. Furthermore, an another carbocation would react with **5** or **6** leading to the formation of **3** or **4**. Thus, the absolute configuration at C-I-3 and C-II-3 was considered to be *S*. Accordingly, the absolute stereostructures of blepharocalyxins A and B were concluded to be **3** and **4**, respectively.

Scheme 1



Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH solutions on a JASCO DIP-360 digital polarimeter at 25 °C. IR spectra were recorded on a Hitachi 260-01 spectrometer in KBr disks. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL JNM-GX400 or a JNM-LA400WB spectrometer with tetramethylsilane as internal standard, and chemical shifts are recorded in δ values. Multiplicities of ^{13}C-NMR signals were determined by means of the DEPT method and are indicated as s (singlet), d (doublet), t (triplet), and q (quartet). 2D NMR spectra (^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY, ^{1}H−^{13}C long-range COSY, HMBC, and ROESY) were measured by the JEOL standard software. FABMS were measured with a JEOL JMS-SX102 spectrometer with glycerol as matrix. HPLC analyses were carried out using a Sumichiral OA-4700 column (4 mm i.d. × 25 cm or 10 mm i.d. × 25 cm; Sumika Chemical Analysis Service, Ltd., Japan). The mobile phase was hexane−1,2-dichloroethane−EtOH (70:20:8) for the separation of **1** and **2**, and hexane−1,2-dichloroethane−EtOH−trifluoroacetic acid (70:20:10:0.1) for **3** and **4**, and UV (254 nm) detection was used.

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 sample was properly authenticated by a taxonomist at Toyama Medical and Pharmaceutical University, Japan.

Extraction and Isolation. The seeds (10 kg) of *A. blepharocalyx* were extracted with 95% EtOH, and the solvent was removed by evaporation under reduced pressure. The EtOH extract (800 g) so obtained was suspended in 10% H_2O−MeOH and extracted with hexane and Et_2O to provide hexane and ether extracts, respectively. The ether extract (450 g) was chromatographed over Si gel with a $CHCl_3$−MeOH solvent system to give seven fractions.

Fraction 6 (10 g, 10% $CHCl_3$−MeOH eluate) was further chromatographed over Si gel with a $CHCl_3$−MeOH gradient system, and fractions were further subjected to Sephadex LH-20 column chromatography (eluent, MeOH−H_2O) followed by preparative TLC, to give four epimeric mixtures having R_f values of 0.41, 0.25, 0.19, and 0.11 on TLC with $CHCl_3$−MeOH (9:1). From the mixtures having R_f 0.25 and 0.19 on TLC, calyxins B, C, and D and epicalyxins B, C, and D were obtained through HPLC separation.[5] Separation of the other two epimeric mixtures (R_f 0.41 and 0.11 on TLC) was also achieved by preparative HPLC, and the mixture at R_f 0.41 gave compounds **1** (2.0 mg, t_R 27.1 min) and **2** (2.0 mg, t_R 32.2 min), while that at R_f 0.11 gave **3** (4.0 mg, t_R 32.1 min) and **4** (5.0 mg, t_R 35.3 min).

Calyxin H (1): a pale yellow amorphous solid; $[\alpha]^{25}_D$ −4.7° (*c* 0.18, MeOH); IR (KBr) ν_{max} 3300, 1610 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ion FABMS *m/z* 567.2347 [M + H]$^+$ (calcd for $C_{35}H_{35}O_7$, 567.2382).

Epicalyxin H (2): a pale yellow amorphous solid; $[\alpha]^{25}_D$ +11.6° (*c* 0.18, MeOH); IR (KBr) ν_{max} 3300, 1610 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ion FABMS *m/z* 567.2387 [M + H]$^+$ (calcd for $C_{35}H_{35}O_7$, 567.2383).

Blepharocalyxin A (3): a pale yellow amorphous solid; $[\alpha]^{25}_D$ −56.4° (*c* 0.17, MeOH); IR (KBr) ν_{max} 3350, 1680 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ion FABMS *m/z* 879.3768 [M + H]$^+$ (calcd for $C_{54}H_{55}O_{11}$, 879.3745).

Blepharocalyxin B (4): a pale yellow amorphous solid; $[\alpha]^{25}_D$ −97.7° (*c* 0.16, MeOH); IR (KBr) ν_{max} 3350,

1680 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2; positive ion FABMS *m/z* 879.3768 $[M + H]^+$ (calcd for $C_{54}H_{55}O_{11}$, 879.3745).

References and Notes

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(8) In Kadota et al. (ref 7), blepharocalyxins A and B were assigned as stereoisomers at C-3; however, a series of study on the constituents of *A. blepharocalyx* (refs 5, 6) led to the conclusion that they are stereoisomeric at C-7.
(9) This chalcone is also present in the Et_2O extract of *A. blepharocalyx*.

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