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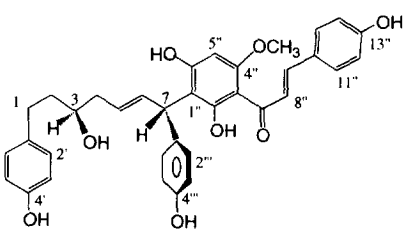
Six Novel Diarylheptanoids Bearing Chalcone or Flavanone Moiety from the Seeds of *Alpinia blepharocalyx*

Jeevan Kumar Prasain,[a] Yasuhiro Tezuka,[a] Jian Xin Li,[a] Ken Tanaka,[b] Purusotam Basnet,[a] Hui Dong,[c] Tsuneo Namba,[a] and Shigetoshi Kadota[a,*]

Research Institute for Wakan-Yaku (*Traditional Sino-Japanese Medicines*),[a] Toyama Medical and Pharmaceutical University, 2630-Sugitani, Toyama 930-01, Japan; National Research Institute of Police Science,[b] 6 Sanban-cho, Chiyoda-Ku, Tokyo 102, Japan; China Pharmaceutical University,[c] 24 Tong Jia Xiang Nanjing, China

Abstract: Six novel diarylheptanoids bearing chalcone or flavanone moiety (**1-6**) were isolated from the seeds of *Alpinia blepharocalyx* K. Schum. Their structures were determined by spectroscopic analysis. Stereo-chemical assignment of diarylheptanoid part of these compounds was done by NMR spectral analysis of their MTPA esters. These compounds inhibited nitric oxide (NO) production in endotoxin-activated murine macrophages, J774.1. © 1997 Elsevier Science Ltd.

Alpinia blepharocalyx K. Schum, a member of Zingiberaceae family has been used as an stomachic in South-West China including Yunnan, Shichuan provinces and Tibet. Medicinally, plants of this family are reputed to have values as antihepatotoxic, anti-inflammatory, and stomachic properties.[1] In the course of finding out the biologically active constituents from the seeds of *A. blepharocalyx*, six novel diarylheptanoids bearing a chalcone or flavanone moiety (**1-6**) were isolated. They had a novel carbon framework and showed inhibitory activity against nitric oxide (NO) production in activated murine macrophages. We now report the isolation and structure elucidation of them by the use of 2D NMR techniques and chemical methods.[2,3]



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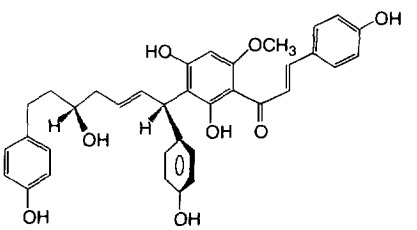

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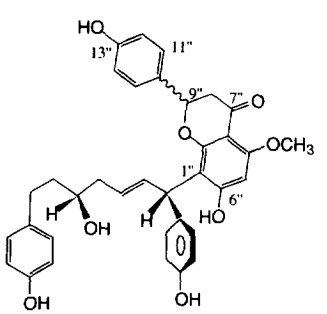
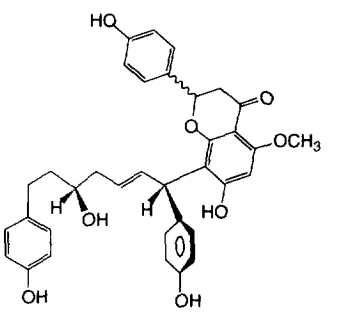


3 and 4



5 and 6

The seeds of *A. blepharocalyx* was extracted with 95% EtOH and the EtOH extract was partitioned into hexane- and ether-soluble fractions. From the ether-soluble fraction, two mixtures were isolated after a series of chromatographic separations. They showed a single spot in TLC analyses with various solvent systems but displayed some closely overlapping signals in the ^{13}C-NMR spectra, indicating that they were epimeric mixtures. Their separation was effected by HPLC using chiral column and six compounds (**1-6**) were isolated as shown in Fig. 1.

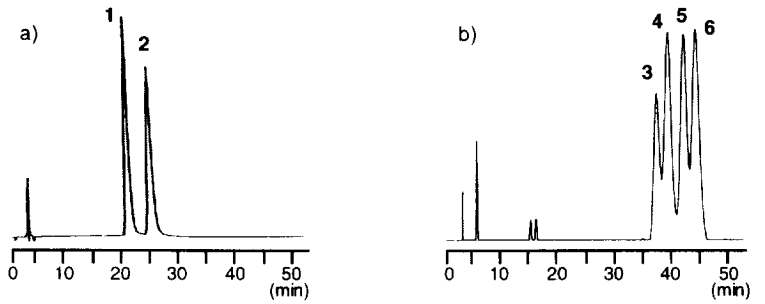


Fig. 1. HPLC Chromatograms of Novel Diarylheptanoids **1-6**
a) Mixture of **1** and **2**. b) Mixture of **3-6**.
[Column: Sumichiral OA-4700; Mobile phase: hexane–1,2-dichloroethane–ethanol–trifluoroacetic acid a) 70:20:10:0.2 b) 70:20:7:0.1; Flow rate: 1.0 ml/min; Detection: UV (254 nm)]

Calyxin B (**1**), a light yellow amorphous solid, showed $[\alpha]_D$ -24.7° (MeOH, c = 0.36), and its molecular formula was determined as $C_{35}H_{34}O_8$ [(M+H)$^+$ *m/z* 583.2340; calcd. 583.2332] by high resolution FAB-MS. In the IR spectrum, absorption bands attributable to hydroxyl (3225 cm^{-1}) and carbonyl (1605 cm^{-1}) groups were appeared. Extensive analysis of the ^{1}H- and ^{13}C-NMR spectra together with the use of the ^{1}H-^{1}H and ^{13}C-^{1}H COSY spectra of **1**, indicated the presence of a carbonyl group, three methylenes, two methines, a methoxy group, two sets of *trans*-double bonds, twelve *ortho*-coupled aromatic protons, a singlet aromatic proton and twelve quaternary carbons (Tables 1 and 2). These data indicated **1** to be a diarylheptanoid containing a chalcone moiety.

In the HMBC spectrum of **1**, the long-range correlations, C-2',6'/1-H, C-1/2',6'-H and C-2'",6'"/7-H, indicated the presence of a diarylheptanoid part. Likewise, the correlations, C-2"/7-H and C-6"/7-H, enabled us to assign the position of linkage between the chalcone and the diarylheptanoid parts. A clear long-range correlation C-4"/OMe in the HMBC spectrum together with NOE between OMe and 8"-H in a difference NOE spectrum enabled us to assign the position of the methoxy group at C-4". Thus, a planar structure of calyxin B (**1**) was established.

Epicalyxin B (**2**) was obtained as a light yellow amorphous solid, $[\alpha]_D$ +11.5° (MeOH, c = 0.51). The high resolution FAB-MS data of **2** indicated that **2** has the same molecular formula as **1**, and the ^{1}H- and ^{13}C-NMR spectra of **2** were almost same as those of **1** (Tables 1 and 2) but differed from it in the ^{1}H-NMR splitting pattern of 4-H which was quartet-like in **2**, while it was triplet in **1**. Thus, the planar structure of **2** was considered to be identical with that of **1**; *i.e.* **2** is a stereoisomer of **1** either at C-3 or C-7.

The stereochemistry at chiral centres (C-3 and C-7) within **1** and **2** was determined through the NMR studies of MTPA esters of their methylated products **1a** and **2a**. In the ^{1}H-NMR spectra of *R*-(+)-MTPA esters (**1b** and **2b**), the protons 4-H, 5-H, 6-H and 7-H appeared upfield whereas 1-H and 2-H were in downfield in

comparison to those of *S*-(-)-MTPA derivatives (**1c** and **2c**), suggesting that in the *R*-(+)-MTPA esters 4-H, 5-H, 6-H and 7-H were more affected by the phenyl ring of the MTPA part. Thus, the absolute configuration of **1** and **2** at C-3 was determined to be *S*.[3,4]

Table 1. ^{1}H-NMR Spectral Data for **1-6** from *A. blepharocalyx* (in MeOH-d_4)

^{1}H	1	2	3	4	5	6
1	2.52 m	2.52 m	2.50 m	2.50 m	2.45 m	2.50 m
	2.62 m	2.62 m	2.55 m	2.55 m	2.60 m	2.55 m
2	1.61 m	1.62 m	1.57 m	1.60 m	1.55 m	1.60 m
	1.76 m	1.75 m	1.66 m	1.70 m	1.65 m	1.70 m
3	3.62 m	3.60 m	3.60 m	3.60 m	3.55 m	3.55 m
4	2.28 t (7.5)	2.27 q-like (7.5)	2.21 t (7.5)	2.25 t (7.5)	2.23 q-like (7.5)	2.20 q-like (7.5)
5	5.56 dt (15.0, 7.5)	5.56 dt (15.0, 7.5)	5.56 dt (15.0, 7.5)	5.56 dt (15.0, 7.5)	5.53 dt (15.0, 7.5)	5.53 dt (15.0, 7.5)
6	6.35 dt (15.0, 8.5)	6.33 dt (15.0, 8.5)	6.12 dt (15.0, 8.5)	6.12 dt (15.0, 8.5)	6.12 dt (15.0, 8.5)	6.16 dt (15.0, 8.5)
7	5.14 d (8.0)	5.14 d (8.0)	5.12 d (8.0)	5.12 d (8.0)	5.13 d (8.0)	5.10 d (8.0)
2',6'	6.95 d (8.5)	6.92 d (8.5)	6.88 d (8.5)	6.89 d (8.5)	6.87 d (8.5)	6.88 d (8.5)
3',5'	6.66 d (8.5)	6.65 d (8.5)	6.65 d (8.5)	6.65 d (8.5)	6.66 d (8.5)	6.63 d (8.5)
5"	6.03 s	6.03 s	6.17 s	6.17 s	6.18 s	6.19 s
8"	7.80 d (15.5)	7.80 d (15.5)	2.75 dt (16.5, 12.5)	2.90 dt (16.5, 12.5)	2.74 dt (16.5, 12.5)	2.89 dt (16.5, 12.5)
			2.57 dt (16.5, 4.0)	2.61 m	2.54 dt (16.5, 4.0)	2.61 dt (16.5, 4.0)
9"	7.66 d (15.5)	7.66 d (15.5)	5.16 dt (12.5, 4.0)	4.98 br d (12.5)	5.12 dt (12.5, 4.0)	4.97 br d (12.5)
11",15"	7.50 d (8.5)	7.50 d (8.5)	6.97 d (8.5)	7.01 d (8.5)	6.97 d (8.5)	6.99 d (8.5)
12",14"	6.82 d (8.5)	6.82 d (8.5)	6.71 d (8.5)	6.74 d (8.5)	6.70 d (8.5)	6.73 d (8.5)
2"',6"'	7.05 d (8.5)	7.05 d (8.5)	6.94 d (8.5)	6.92 d (8.5)	6.91 d (8.5)	6.92 d (8.5)
3"',5"'	6.63 d (8.5)	6.62 d (8.5)	6.63 d (8.5)	6.57 d (8.5)	6.63 d (8.5)	6.57 d (8.5)
OCH_3	3.91 s	3.91 s	3.85 s	3.85 s	3.85 s	3.85 s

Chemical shift (δ) in ppm and coupling constant (*J*) in Hz in parentheses. Assignments were done by ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and HMBC measurements.

TABLE 2. ^{13}C-NMR Spectral Data for **1-6** from *A. blepharocalyx* (in MeOH-d_4)

^{13}C	1	2	3	4	5	6
1	32.74 (t)	32.58 (t)	32.61 (t)	32.61 (t)	32.64 (t)	32.52 (t)
2	40.51 (t)	40.45 (t)	40.29 (t)	40.35 (t)	40.63 (t)	40.48 (t)
3	72.69 (d)	72.54 (d)	72.50 (d)	72.53 (d)	72.47 (d)	72.41 (d)
4	42.24 (t)	42.33 (t)	41.96 (t)	41.96 (t)	42.42 (t)	42.27 (t)
5	128.61 (d)	128.64 (d)	128.76 (d)	128.91 (d)	128.94 (d)	129.03 (d)
6	136.44 (d)	136.44 (d)	135.95 (d)	135.98 (d)	136.19 (d)	136.10 (d)
7	44.15 (d)	44.15 (d)	44.06 (d)	44.21 (d)	44.09 (d)	44.21 (d)
1'	135.41 (s)	135.35 (s)	135.31 (s)	135.38 (s)	135.38 (s)	135.34 (s)
2',6'	131.07 (d)	131.04 (d)	131.03 (d)	131.03 (d)	131.03 (d)	131.00 (d)
3',5'	116.89 (d)	116.83 (d)	116.80 (d)	116.67 (d)	116.64 (d)	116.31 (d)
4'	156.96 (s)	156.90 (s)	156.93 (s)	156.96 (s)	156.96 (s)	156.93 (s)
1"	113.04 (s)	112.98 (s)	113.94 (s)	114.03 (s)	114.00 (s)	114.03 (s)
2"	167.01 (s)	166.95 (s)	164.88 (s)	164.88 (s)	164.85 (s)	164.82 (s)
3"	107.45 (s)	107.42 (s)	106.96 (s)	107.23 (s)	106.96 (s)	107.23 (s)
4"	163.58 (s)	163.55 (s)	162.88 (s)	163.00 (s)	162.91 (s)	162.97 (s)
5"	92.88 (d)	92.88 (d)	94.59 (d)	94.73 (d)	94.54 (d)	94.70 (d)
6"	164.58 (s)	164.58 (s)	164.52 (s)	164.82 (s)	164.52 (s)	164.76 (s)
7"	194.97 (s)	194.94 (s)	193.63 (s)	193.97 (s)	193.63 (s)	193.64 (s)
8"	126.70 (d)	126.70 (d)	47.49 (t)	46.43 (t)	47.55 (t)	46.37 (t)
9"	144.21 (d)	144.18 (d)	80.52 (d)	80.61 (d)	80.52 (d)	80.58 (d)
10"	129.28 (s)	129.25 (s)	132.19 (s)	131.79 (s)	132.22 (s)	131.70 (s)
11",15"	132.10 (d)	132.07 (d)	129.27 (d)	129.85 (d)	129.24 (d)	129.82 (d)
12",14"	117.68 (d)	117.65 (d)	116.92 (d)	116.89 (d)	116.92 (d)	116.89 (d)
13"	161.82 (s)	161.79 (s)	159.21 (s)	159.45 (s)	159.21 (s)	159.45 (s)
1"'	137.38 (s)	137.29 (s)	137.26 (s)	137.41 (s)	137.17 (s)	137.29 (s)
2"',6"'	130.31 (d)	130.28 (d)	130.21 (d)	130.06 (d)	130.18 (d)	130.00 (d)
3"',5"'	116.25 (d)	116.25 (d)	116.31 (d)	116.34 (d)	116.34 (d)	116.31 (d)
4"'	156.66 (s)	156.63 (s)	156.72 (s)	156.72 (s)	156.75 (s)	156.69 (s)
OCH_3	57.03 (q)	56.99 (q)	56.84 (q)	56.87 (q)	56.84 (q)	56.87 (q)

The multiplicities of carbon signals were determined by means of the DEPT method, and are indicated as s, d, t and q. Assignments were based on ^{1}H-^{13}C COSY and HMBC spectra.

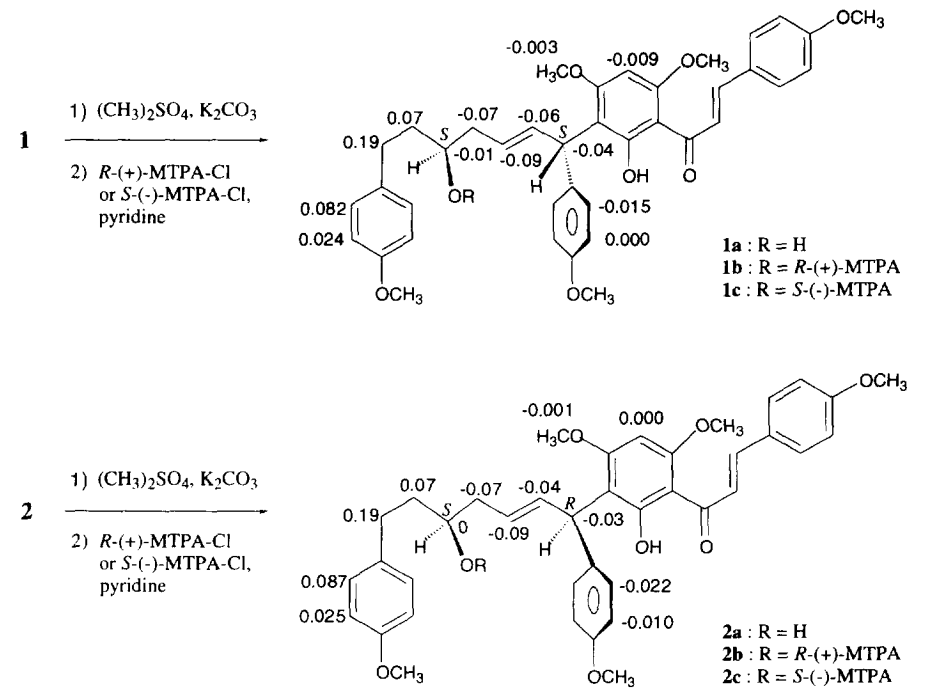


Fig. 2. $\Delta\delta$ (= δ^R-δ^S) Values Obtained from the MTPA Esters of Calyxin B (**1b** and **1c**) and Epicalyxin B (**2b** and **2c**) in $CDCl_3$ at 25℃.

The absolute configurations at C-7 were difficult to assign due to the possible free rotation around C-7. This problem was overcome by comparing the difference in the ^{1}H-NMR chemical shifts of aromatic protons at C-7 in the *R*-(+)- and *S*-(-)-MTPA esters of **1** and **2**. Ohtani *et al.*[4] pointed out that the $\Delta\delta$ (= δ^R–δ^S) values are proportional to the distance between the protons and the MTPA moiety. This rationale seems to be applicable in the prediction of the absolute configuration at C-7. As can be seen in Fig. 2, the $\Delta\delta$ values observed for 2'''-H and 3'''-H in the MTPA esters of **1** were -0.015 and 0.000 ppm, respectively, while in the MTPA esters of **2**, those were -0.022 and -0.010 ppm, suggesting that the aromatic protons in **1b** lie farther from the MTPA's phenyl ring than those of **2b**. Moreover, the observed $\Delta\delta$ values for 5''-H in the MTPA esters of **1** and **2** revealed reverse relation; *i.e.* 5''-H is nearer to the MTPA's phenyl ring in **1b** than in **2b**.

This trend of discrepancy in $\Delta\delta$ value was found to be more obvious when ^{1}H-NMR of these compounds were taken at low temperatures. Latypov *et al.*[5] noted that the aromatic shielding effect contributed by MTPA esters is constituted by the various conformers in close populations due to restricted rotation around the Cα-CO and Cα-Ph bonds and the alteration of their relative populations by varying temperature should cause change in average NMR chemical shifts. As anticipated, $\Delta\delta$ values for 2'''-H and 3'''-H were found more significant at lower temperatures. For example, the $\Delta\delta$ value obtained for 2'''-H at 25℃ was -0.0156 ppm whereas at -40℃, the value was augmented to -0.0271 ppm in **1**. Moreover, if we compare $\Delta\delta$ values for 2'''-H and 3'''-H of **1** to those of **2**, it is clear that the diamagnetic shielding effect experienced by these aromatic protons was more pronounced in the latter case (Table 3). This improvement in the $\Delta\delta$ values at lower temperature and consequently the seeming difference of the $\Delta\delta$ values between **1** and **2** rationally explain the stereochemistry of the benzene

Table 3. Selected Chemical Shifts of *R*-(+)- and *S*-(-)-MTPA Esters of Calyxin B (**1b** and **1c**) and Epicalyxin B (**2b** and **2c**) at Various Temperatures.

^{1}H	25℃			0℃			-20℃			-40℃		
	1b	**1c**	Δδ	**1b**	**1c**	Δδ	**1b**	**1c**	Δδ	**1b**	**1c**	Δδ
5"	5.9648	5.9747	-0.0090	5.9709	5.9824	-0.0115	5.9801	5.9923	-0.0122	5.9976	6.015	-0.0174
2"'	7.1175	7.1331	-0.0156	7.1175	7.1369	-0.0194	7.1194	7.1408	-0.0214	7.1213	7.1484	-0.0271
3"'	6.7507	6.7507	0.0000	6.7640	6.7659	-0.0019	6.7774	6.7785	-0.0011	6.7934	6.7953	-0.0019
	2b	**2c**	Δδ	**2b**	**2c**	Δδ	**2b**	**2c**	Δδ	**2b**	**2c**	Δδ
5"	5.9747	5.9747	0.0000	5.9824	5.9824	0.0000	5.9946	5.9923	-0.0023	5.9976	6.0100	-0.0124
2"'	7.1110	7.1331	-0.0221	7.1106	7.1369	-0.0263	7.1110	7.1408	-0.0298	7.1110	7.1427	-0.0317
3"'	6.7346	6.7453	-0.0107	6.7427	6.7560	-0.0133	6.7522	6.7663	-0.0141	6.7629	6.7797	-0.0168

δ values in $CDCl_3$.

ring at chiral centre C-7. Furthermore, the MTPA esters of **1** and **2** are likely to have similar conformation in view of similar splitting patterns in their ^{1}H-NMR spectra. Thus, the comparative studies on their Δδ values together with use of the Drieding stereomodel enabled us to predict the absolute stereochemistry of **1** and **2** at C-7 as being *S* and *R*, respectively. From the data presented above, the absolute structures of calyxin B and epicalyxin B were established as shown in **1** and **2**, respectively.

Calyxin C (**3**) and epicalyxin C (**4**) were obtained as pale yellow amorphous solids and their molecular formulas were determined as $C_{35}H_{34}O_8$ by high resolution FAB-MS. IR spectrum of both the compounds showed absorption bands at 3300 (OH) and 1610 (C=O) cm^{-1}. The ^{1}H- and ^{13}C-NMR data of **3** and **4** were found to be very similar to calyxin B (**1**) except for some chalcone signals (Tables 1 and 2). For example, the ^{1}H- and ^{13}C-NMR spectra of **3** showed the lack of the conjugated double bond and the presence of three proton signals as an ABX pattern at δ_H 5.16 (dd, J = 12.5, 4.0 Hz), 2.75 (dd, J = 16.5, 12.5 Hz) and 2.57 (dd, J = 16.5, 4.0 Hz) which were correlated with the ^{13}C signals at δ_C 80.52 and 47.49 in the ^{13}C-^{1}H COSY. These spectral evidences indicated the presence of a methylene group and an oxygenated carbon in the chalcone moiety. Moreover, the HMBC spectrum of **4** displayed the long-range correlations similar to those of **1** including that of between the ^{13}C signal at δ_C 80.61 (C-9") and the aromatic protons at δ_H 7.01 (11"-H, 15"-H), inferring C-9" is the oxygenated carbon. The observed vicinal coupling constants (12.5 and 4.0 Hz) in **3** were in close agreement with a typical value of flavanone[6] and not of hydroxydihydrochalcone,[7] suggesting the presence of a flavanone moiety rather than a β-hydroxydihydrochalcone one. Thus, the NMR data together with molecular formulas of these compounds suggested **3** and **4** to be diarylheptanoids bearing a flavanone moiety instead of a chalcone moiety of **1**. All the ^{1}H- and ^{13}C-NMR data of **3** and **4** were almost similar except for the chemical shifts of 8"-H_2 and 9"-H in the ^{1}H-NMR spectra (Tables 1 and 2), inferring that both are stereoisomeric to each other at C-9".

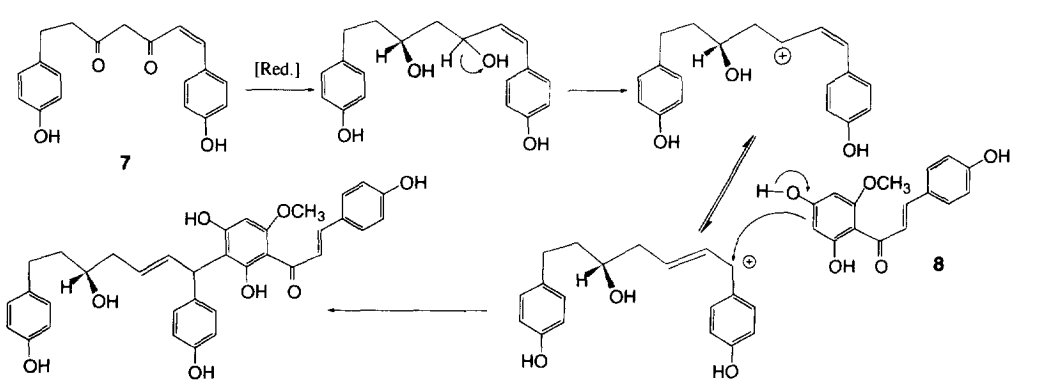
Calyxin D (**5**) and epicalyxin D (**6**) were also a pale yellow amorphous solids, whose mass spectra were found to be identical to that of previous compounds. The ^{1}H- and ^{13}C-NMR spectra of **5** and **6** were very similar to those of epicalyxin B (**2**) except for similar differences observed in the previous case (Tables 1 and 2).

Thus, **5** and **6** were also considered to be diarylheptanoids bearing a flavanone moiety. As in the previous case, **5** and **6** were deemed as stereoisomers at C-9" as the chemical shifts of 8"-H_2 and 9"-H of them were found to be different in their ^{1}H-NMR spectra.

The stereochemistry of **3**-**6** at C-3 and C-7 was assigned by comparison with **1** and **2** in view of their similar splitting patterns in the ^{1}H-NMR spectra and optical activity. The specific rotations of **3** and **4** were negative (same as **1**) and showed similar splitting pattern at 4-H to that of **1** (triplet), while the specific rotations of **5** and **6** were positive (same as **2**) and the splitting pattern of 4-H was same as that of **2** (quartet-like). These data suggested the absolute stereochemistry at C-3 and C-7 of **3** and **4** was 3*S*,7*S* and that of **5** and **6** was 3*S*,7*R*. Presently, the meager quantity of these compounds made us difficult to describe the stereochemistry at C-9" and thus it remained unsolved. Nevertheless, **3** and **5** were supposed to have the same stereochemistry at C-9" with regard to their similar chemical shifts and coupling constants in the ^{1}H-NMR spectra and the same is expected for **4** and **6**. From the data presented above, the structures of calyxins C and D and epicalyxins C and D were established as shown in **3**-**6**.

Many diarylheptanoids have been reported from the plants belonging to Zingiberaceae,[8] but the present results provided the first example of diarylheptanoids having a novel carbon skeleton. These compounds are of interest from a biogenetic view point. The biogenetic pathways of these compounds are still unclear, however, these compounds are supposed to be formed through the formation of carbonium ion at C-7 of a diarylheptanoid, followed by the nucleophilic attack of a chalcone or flavanone. The diarylheptanoid **7** and chalcone **8** have also been isolated from the ether extract of *A. blepharocalyx*. Thus, their combination in forming a novel carbon skeleton is very plausible. Furthermore, existence of these novel diarylheptanoids in stereoisomeric forms at C-7 also supported the proposed biogenetic pathway (Scheme 1).

The compounds **1**-**6** were evaluated for their role as an immunoregulator in activated murine macrophages, J774.1 by *in vitro* assay methods.[9] These compounds at a concentration of 100 μg/ml inhibited the production of NO at a range of 90-94%. The inhibitory effect of **1** and **2** was found to be slightly higher than those of **3**-**6**. Their cytotoxicity was assessed in terms of cell viability which was found to be 85-90% at a concentration of 100 μg/ml. These results suggest that these diarylheptanoids can modulate the immune responses by controlling production of NO. Studies as to whether these diarylheptanoids act as a direct inhibitor of NO synthase or act upon a control system are in progress in our laboratory and will separately be presented.



Scheme 1

Experimental

General: Optical rotations were measured in MeOH solution on a JASCO DIP-360 digital polarimeter at 25℃. IR spectra were recorded on a Hitachi 260-01 spectrometer in KBr discs. FAB-MS and desorption chemical ionization MS (DCI-MS) were measured with a JEOL JMS SX-102 spectrometer, and glycerol was used as a matrix for FAB-MS and isobutane as a reaction gas for DCI-MS. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM-GX400 spectrometer with tetramethylsilane as an internal standard and chemical shifts are recorded in δ values. Multiplicities of ^{13}C-NMR signals were determined by means of DEPT method and are indicated as s (singlet), d (doublet), t (triplet) and q (quartet). 2D-NMR spectra (^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, ^{13}C-^{1}H long-range COSY and HMBC) were measured by the JEOL standard software. Difference NOE spectra were obtained by the use of JEOL standard pulse sequence with 5 s irradiation. Column chromatography was performed with Wakogel C-200 (Wako Pure Chemical Co.). TLC and preparative TLC were carried out on precoated Kieselgel F_{254} plates (0.25 or 0.5 mm, Merck). HPLC analyses were carried out using Sumichiral OA-4700 column (4 mm I.D. x 25 cm or 10 mm I.D. x 25 cm, Sumika Chemical Analysis Service Ltd.). Mobile phase was hexane–1,2-dichloroethane–EtOH–trifluoroacetic acid (70:20:10:0.2 or 70:20:0.7:0.1) and detection was UV (254 nm). The murine monocyte macrophage cell line, J774.1 was obtained from the Japan Cancer Resource Bank (JCRB, Tokyo), and the bioassay was done according to the literature method.[9]

Isolation of Constituents from *Alpinia blepharocalyx*

The seeds (10 kg) of *A. blepharocalyx* was procured from Yunnan province in China and extracted with 95% EtOH. The EtOH extract was evaporated under reduced pressure and the residue (800 g) was suspended in 10% H_2O/MeOH and extracted successively with hexane and ether to provide hexane and ether extracts, respectively. The ether extract (450 g) was chromatographed over silica gel and eluted with $CHCl_3$-MeOH solvent system to give seven fractions.

Fraction 6 (10.3 g, 10% $CHCl_3$-MeOH eluate) was chromatographed over silica gel with a $CHCl_3$-MeOH gradient system and the fractions were further subjected to sephadex LH-20 column chromatography followed by preparativeTLC to give two epimeric mixtures (500 mg or 200mg). Separation of the epimeric mixtures (45 mg or 30 mg) were achieved by preparative HPLC to provide compounds **1** (20 mg), **2** (20 mg), **3** (4 mg), **4** (5 mg), **5** (4 mg) and **6** (5 mg).

Calyxin B (1): A light yellow amorphous solid, $[\alpha]_D$ -24.7° (MeOH, c = 0.36). IR ν_{max} cm^{-1}: 3225 (OH), 1605 (C=O). Positive ion FAB-MS m/z 583.2340 $[M+H]^+$ (calcd. for $C_{35}H_{35}O_8$, 583.2332). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Epicalyxin B (2): A light yellow amorphous solid, $[\alpha]_D$ +11.5° (MeOH, c = 0.51). IR ν_{max} cm^{-1}: 3225 (OH), 1605 (C=O). Positive ion FAB-MS m/z 583.2340 $[M+H]^+$ (calcd. for $C_{35}H_{35}O_8$, 583.2332). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Calyxin C (3): A pale yellow amorphous solid, $[\alpha]_D$ -55.1° (MeOH, c = 0.245). IR ν_{max} cm^{-1}: 3300 (OH), 1610 (C=O). Positive ion FAB-MS m/z 583.2327 $[M+H]^+$ (calcd. for $C_{35}H_{35}O_8$, 583.2332). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Epicalyxin C (4): A pale yellow amorphous solid, $[\alpha]_D$ -38.9° (MeOH, c = 0.81). IR ν_{max} cm^{-1}: 3300 (OH), 1610 (C=O). Positive ion FAB-MS m/z 583.2330 $[M+H]^+$ (calcd. for $C_{35}H_{35}O_8$, 583.2332). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Calyxin D (5): A pale yellow amorphous solid, $[\alpha]_D$ +43.0° (MeOH, c = 0.40). IR ν_{max} cm^{-1}: 3300 (OH), 1610 (C=O). Positive ion FAB-MS m/z 583.2323 $[M+H]^+$ (calcd. for $C_{35}H_{35}O_8$, 583.2332). ^{1}H- and

^{13}C-NMR: Tables 1 and 2.

Epicalyxin D (6): A pale yellow amorphous solid, $[\alpha]_D$ +26.6°(MeOH, *c* = 0.45). IR ν_{max} cm^{-1}: 3300 (OH), 1610 (C=O). Positive ion FAB-MS *m/z* 583.2342 $[M+H]^+$ (calcd. for $C_{35}H_{35}O_8$, 583.2343). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Methylation of Calyxin B (1) and Epicalyxin B (2)

To a solution of **1** or **2** (20 mg) in dry acetone (2 ml), K_2CO_3 (100 mg) was added under stirrring. After 5 min, $(CH_3)_2SO_4$ (100 µl) was added to the solution and the mixture was stirred for overnight at room temperature. The reaction mixture was applied over silica gel column and eluted with $CHCl_3$. The eluate was evaporated and separated by preparative TLC using benzene-EtOAc (8:2) as a developing solvent to give a penta-*O*-methylated compound **1a** (7.1 mg, 35.5 %) or **2a** (6 mg, 30%).

Calyxin B Methylate (1a): ^{1}H-NMR δ ($CDCl_3$): 1.74 (2H, q, *J* = 7.0 Hz, 2-H_2), 2.17, 2.34 (each 1H, m, 4-H_2), 2.61, 2.73 (each 1H, m, 1-H_2), 3.60 (1H, m, 3-H), 3.76, 3.77, 3.81, 3.85 and 3.95 (each 3H, s, 5 x OMe), 5.28 (1H, d, *J* = 8.0 Hz, 7-H), 5.52 (1H, dt, *J* = 15.0, 7.5 Hz, 5-H), 6.00 (1H, s, 5"-H), 6.36 (1H, dd, *J* = 15.0, 8.5 Hz, 6-H), 6.78 (2H, d, *J* = 8.5 Hz, 3'"-H, 5'"-H), 6.81 (2H, d, *J* = 8.5 Hz, 3'-H, 5'-H), 6.93 (2H, d, *J* = 8.5 Hz, 12"-H, 14"-H), 7.10 (2H, d, *J* = 8.5 Hz, 2'-H, 6'-H), 7.19 (2H, d, *J* = 8.5 Hz, 2'"-H, 6'"-H), 7.56 (2H, d, *J* = 8.5 Hz, 11"-H, 15"-H), 7.77 (2H, br s, 8"-H, 9"-H). Positive ion DCI-MS *m/z*: 639.2932 $[M+H]^+$ (calcd. for $C_{39}H_{43}O_8$, 639.2934).

Epicalyxin B Methylate (2a): ^{1}H-NMR δ ($CDCl_3$): 1.73 (2H, q, *J* = 7.0 Hz, 2-H_2), 2.17, 2.32, (each 1H, m, 4-H_2), 2.61, 2.74 (each 1H, m, 1-H_2), 3.63 (1H, m, 3-H), 3.76 (3H, s, OMe), 3.77 (3H, s, OMe), 3.81 (3H, s, OMe), 3.95 (6H, s, 2 x OMe), 5.27 (1H, d, *J* = 8.0 Hz, 7-H), 5.50 (1H, dt, *J* = 15.0, 7.5 Hz, 5-H), 5.99 (1H, S, 5"-H), 6.33 (1H, dd. *J* = 15.0, 8.5 Hz, 6-H), 6.78 (2H, d, *J* = 8.5 Hz, 3'"-H, 5'"-H), 6.81 (2H, d, *J* = 8.5 Hz, 3'-H, 5'-H), 6.92 (2H, d, *J* = 8.5 Hz, 12"-H, 14"-H), 7.10 (2H, d, *J* = 8.5 Hz, 2'-H, 6'-H), 7.20 (2H, d, *J* = 8.5 Hz, 2'"-H, 6'"-H), 7.56 (2H, d, *J* = 8.5 Hz, 11"-H, 15"-H), 7.76 (2H, br s, 8"-H, 9"-H). Positive ion DCI-MS *m/z*: 639.2932 $[M+H]^+$ (calcd. for $C_{39}H_{43}O_8$, 639.2934).

Preparation of MTPA Esters (1b, 1c, 2b and 2c)

To a stirred solution of penta-*O*-methylated compound **1a** or **2a** (3.5 mg) in CCl_4 (0.5 ml) and pyridine (0.5 ml), *R*-(+)-MTPA chloride or *S*-(-)-MTPA chloride (10 µl) was added and the mixture was stirred for overnight at room temperature. The reaction mixture was subjected to preparative TLC with benzene-EtOAc (8:2) to afford the MTPA esters (**1b**, **1c**, **2b** and **2c**, each *ca.* 4.5 mg).

***R*-(+)-MTPA Ester (1b):** ^{1}H-NMR δ ($CDCl_3$): 1.93 (2H, q, *J* = 7.0 Hz, 2-H_2), 2.42 (2H, q-like, *J* = 7.5 Hz, 4-H_2), 2.58 (2H, m, 1-H_2), 3.76 (3H, s, OMe), 3.85 (3H, s, OMe), 3.94 (9H, s, 3 x OMe), 5.16 (1H, br t, *J* = 6.0 Hz, 3-H), 5.19 (1H, d, *J* = 8.0 Hz, 7-H), 5.43 (1H, dt, *J* = 15.0, 7.5 Hz, 5-H), 5.96 (1H, s, 5"-H), 6.31 (1H, dd, *J* = 15.0, 8.0 Hz, 6-H), 6.75 (2H, d, *J* = 8.5 Hz, 3'"-H, 5'"-H), 6.80 (2H, d *J* = 8.5 Hz, 3'-H, 5'-H), 6.93 (2H, d, *J* = 8.5 Hz, 12"-H, 14"-H), 7.01 (2H, d, *J* = 8.5 Hz, 2'-H, 6'-H), 7.12 (2H, d, *J* = 8.5 Hz, 2'"-H, 6'"-H), 7.56 (2H, d, *J* = 8.5 Hz, 11"-H, 15"-H), 7.76 (2H, br s, 8"-H and 9"-H). Positive ion DCI-MS *m/z*: 855.3343 $[M+H]^+$ (calcd. for $C_{49}H_{50}O_{10}F_3$, 855.3344).

***R*-(+)-MTPA Ester (2b):** ^{1}H-NMR δ ($CDCl_3$): 1.93 (2H, q, *J* = 7.0 Hz, 2-H_2), 2.42 (2H, q-like, *J* = 7.5 Hz, 4-H_2), 2.58 (2H, m, 1-H_2), 3.754, 3.756, 3.763, 3.85, 3.94 (each 3H, s, 5 x OMe), 5.14 (1H, br t, *J* = 6.0 Hz, 3-H), 5.21 (1H, d, *J* = 8.0 Hz, 7-H), 5.43 (1H, dt, *J* = 15.0, 7.5 Hz, 5-H), 5.97 (1H, s, 5"-H), 6.32 (1H, dd, *J* = 15.0, 8.0 Hz, 6-H), 6.73 (2H, d, *J* = 8.5 Hz, 3'"-H, 5'"-H), 6.79 (2H, d, *J* = 8.5 Hz, 3'-H, 5'-H), 6.93 (2H, d, *J* = 8.5 Hz, 12"-H, 14"-H), 6.99 (2H, d, *J* = 8.5 Hz, 2'-H, 6'-H), 7.11 (2H, d, *J* = 8.5 Hz, 2'"-H,

6'''-H), 7.56 (2H, d, J = 8.5 Hz, 11''-H, 15''-H), 7.76 (2H, br s, 8''-H and 9''-H). Positive ion DCI-MS *m/z*: 855.3404 $[M+H]^+$ (calcd. for $C_{49}H_{50}O_{10}F_3$, 855.3399).

***S*-(-)-MTPA Ester (1c):** ^{1}H-NMR δ ($CDCl_3$): 1.86 (2H, m, 2-H_2), 2.39 (2H, m, 1-H_2), 2.49 (2H, q-like, J = 7.5 Hz, 4-H_2), 3.75 (3H, s, OMe), 3.77 (3H, s, OMe), 3.85 (3H, s, OMe), 3.95 (6H, s, 2 x OMe), 5.17 (1H, br t, J = 6.0 Hz, 3-H), 5.23 (1H, d, J = 8.0 Hz, 7-H), 5.52 (1H, dt, J = 15.0, 7.5 Hz, 5-H), 5.97 (1H, s, 5''-H), 6.37 (1H, dd, J = 15.0, 8.0 Hz, 6-H), 6.75 (2H, d, J = 8.5 Hz, 3'''-H, 5'''-H), 6.77 (2H, d, J = 8.5 Hz, 3'-H, 5'-H), 6.923 (2H, d, J = 8.5 Hz, 12''-H, 14''-H), 6.925 (2H, d, J = 8.5 Hz, 2'-H, 6'-H), 7.13 (2H, d, J = 8.5 Hz, 2'''-H, 6'''-H), 7.56 (2H, d, J = 8.5 Hz, 11''-H, 15''-H), 7.76 (2H, br s, 8''-H and 9''-H). Positive ion DCI-MS *m/z*: 855.3338 $[M+H]^+$ (calcd. for $C_{49}H_{50}O_{10}F_3$, 855.3340).

***S*-(-)-MTPA Ester (2c):** ^{1}H-NMR δ ($CDCl_3$): 1.86 (2H, m, 2-H_2), 2.39 (2H, m, 1-H_2), 2.49 (2H, q-like, J = 7.5 Hz, 4-H_2), 3.746, 3.753, 3.76, 3.85, 3.95 (each 3H, s, 5 x OMe), 5.14 (1H, br t, J = 6.0 Hz, 3-H), 5.24 (1H, d, J = 8.0 Hz, 7-H), 5.52 (1H, dt, J = 15.0, 7.5 Hz, 5-H), 5.97 (1H, s, 5''-H), 6.36 (1H, dd, J = 15.0, 8.0 Hz, 6-H), 6.75 (2H, d, J = 8.5 Hz, 3'''-H, 5'''-H), 6.76 (2H, d, J = 8.5 Hz, 3'-H, 5'-H), 6.90 (2H, d, J = 8.5 Hz, 2'-H, 6'-H), 6.92 (2H, d, J = 8.5 Hz, 12''-H, 14''-H), 7.13 (2H, d, J = 8.5 Hz, 2'''-H, 6'''-H), 7.56 (2H, d, J = 8.5 Hz, 11''-H, 15''-H), 7.76 (2H, br s, 8''-H and 9''-H). Positive ion DCI-MS *m/z*: 855.3373 $[M+H]^+$ (calcd. for $C_{49}H_{50}O_{10}F_3$, 855.3375).

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