THREE NOVEL DIARYLHEPTANOIDS, CALYXIN A, CALYXIN B, AND 3-*EPI*-CALYXIN B FROM A CHINESE CRUDE DRUG "YUNNAN CAO KOU" (*ALPINIA BLEPHAROCALYX* K. SCHUM.)

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Three novel diarylheptanoids, calyxin A (**1**), calyxin B (**2**) and 3-*epi*-calyxin B (**3**), have been isolated from ethanolic extract of seeds of *Alpinia blepharocalyx* K. Schum. and their structures determined by the use of 2D NMR spectroscopy including NOE and HMBC experiments and chemical analyses.

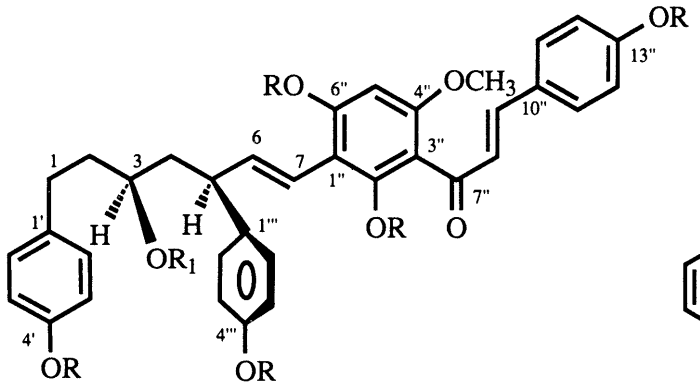
KEYWORDS calyxin A; calyxin B; 3-*epi*-calyxin B; *Alpinia blepharocalyx*; 3α-HSD

Alpinia blepharocalyx K. Schum. is a member of Zingiberaceae (ginger family); members of this family, including ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and cardamom (*Elettaria cardamomum*), have been used for centuries as foods, spices, dyes, and perfumes, and in traditional Chinese, Japanese and Indian medicines.[1] *A. blepharocalyx* has been used as a stomachic in South-West China including Yunnan and Shichuan Provinces and Tibet. During the course of a program to find the biologically active compounds, we isolated three novel diarylheptanoids bearing a chalcone moiety which had never been isolated before. This paper deals with the structure elucidation of three unique compounds, calyxin A (**1**), calyxin B (**2**) and 3-*epi*-calyxin B (**3**).

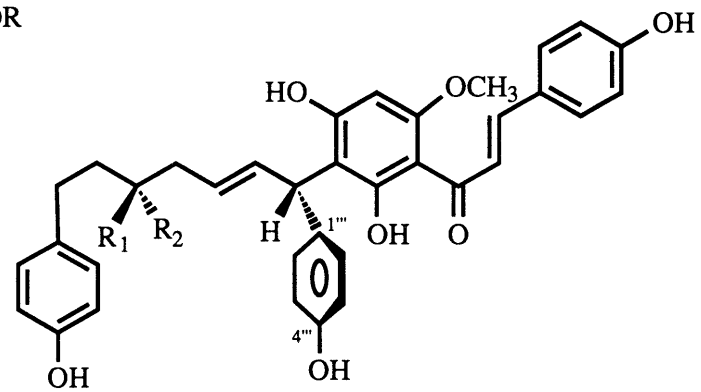
The seeds (10 kg) of *A. blepharocalyx* was extracted with 95 % EtOH, and the EtOH extract was suspended in water containing 10 % MeOH and partitioned with *n*-hexane and ether. From the ether extract on repeated silica gel column chromatography followed by Sephadex LH-20, preparative TLC and preparative HPLC using Sumichiral OA-4700 column,[2] three novel compounds **1**, **2** and **3** were isolated together with several other known compounds such as alpinatin, cardamonin and helichrysetin.

Compound **1**, a light yellow amorphous solid, showed $[\alpha]_D$ -58.9° (MeOH, c = 0.09). The positive ion FAB-MS of **1** exhibited the $[M+H]^+$ peak at *m/z* 599 along with other significant peaks at *m/z* 583 and 553. The molecular formula was determined to be $C_{35}H_{34}O_9$ [$(M+H)^+$ 599.2285, calcd. 599.2282] by high-resolution FAB-MS. The positive $FeCl_3$ test and IR absorptions at ν_{max} 3225, 1605 cm^{-1} indicated that **1** contains phenolic and α,β-unsaturated carbonyl groups. The ^1H- and ^{13}C-NMR spectra of **1** indicated the presence of three methylenes {δ_H 1.64 (1H), 1.87 (1H), 2.11 (2H), 2.52 (1H), 2.62 (1H); δ_C 40.88, 42.48, 32.86}, two methines (δ_H 3.51, 4.21; δ_C 71.23, 37.11), a methoxy group (δ_H 3.91; δ_C 56.97), two set of *trans* double bond (δ_H 6.35, 6.53, 7.67, 7.78; δ_C 130.89, 131.77, 144.06, 126.79), twelve *ortho* coupling aromatic methines (δ_H 6.64, 6.69, 6.83, 6.96, 7.16, 7.49; δ_C 116.74, 116.98, 117.65, 128.97, 131.10, 132.04) and a singlet aromatic methine (δ_H 6.00; δ_C 92.91) along with twelve quaternary carbons (δ_C 107.48, 112.16, 129.25, 132.12, 135.35, 156.75, 158.09, 161.67, 163.31, 164.62, 167.17 and 194.94). A part of its ^1H-NMR spectrum was similar to a substituted chalcone, cardamonin and helichrysetin[3] (Chart 1a [II]).

Acetylation of **1** gave an amorphous waxy hexa-*O*-acetate (**1a**), the high-resolution positive ion FAB-MS of which showed $[M+H]^+$ peak at *m/z* 851 {Found 851.2942, calcd. for $C_{47}H_{47}O_{15}$ 851.2915} along with some significant peaks at *m/z* 835, 810, 794, 749, 733 and six acetyl methyl signals at δ_H 1.99, 2.13, 2.25, 2.28 (Ac x 2), 2.31 were observed in the ^1H-NMR spectrum of **1a**. The mass spectra of **1** and **1a** showed the peaks at *m/z* 583 $[(M+H)-O]^+$ {Found 583.2300, calcd. for $C_{35}H_{35}O_8$



1: R = H, R_1 = OH
1a: R = Ac, R_1 = OAc
1b: R = R_1 = H



2: R_1 = H, R_2 = OH
3: R_1 = OH, R_2 = H

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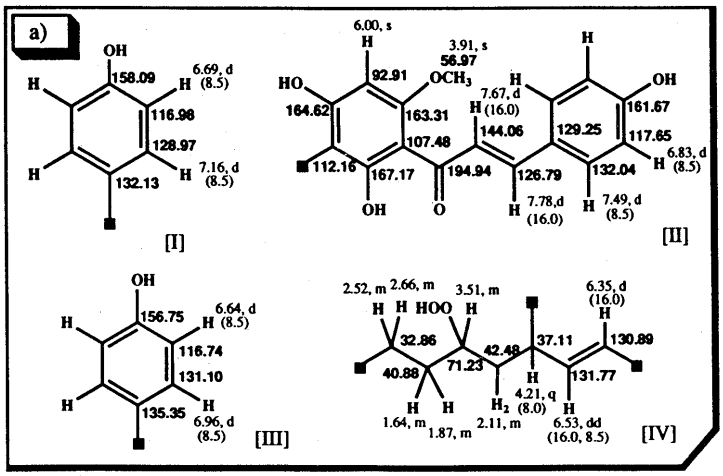
583.2332} and 835 [(M+H)-O]$^+$ {Found 835.2942, calcd. for $C_{47}H_{47}O_{14}$ 835.2966}, respectively; in a qualitative test, **1** liberated iodine from the methanolic KI solution, suggesting the presence of hydroperoxide group.[4] The chemical shift of C_3-H (δ_H 3.51, m) of **1** was shifted downfield (δ_H 4.99, m) in its acetate (**1a**), indicating the position of hydroperoxide group at C_3. Also, $NaBH_4$ reduction of **1** gave **1b** and the ^{13}C-NMR of **1b** showed hydroxy-bearing methine carbon at δ_C 63.34. Detailed analysis of ^{1}H- and ^{13}C-NMR spectra with the aid of ^{1}H-^{1}H and ^{1}H-^{13}C COSY allowed us to deduce the partial structures shown in Chart 1a.

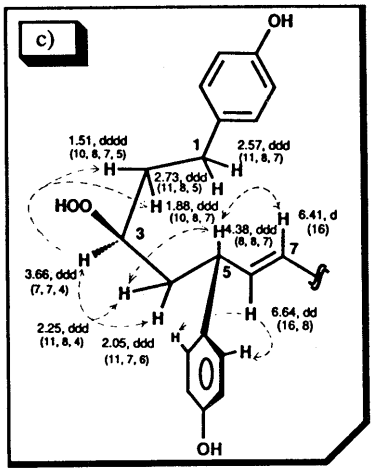
Next, we measured the HMBC of **1** in order to confirm the connectivities of the partial structures. As shown in Chart 1b, the carbon signal at δ 32.86 (C-1) is correlated with the proton at δ 6.96 (2'-H), and the signal at δ 37.11 (C-5) is correlated with the proton at δ 6.35 (7-H). Also, the carbon signals at δ 122.16 (C-1"), 131.10 (C-2"'), and 164.62 (C-6") are correlated with the proton signals at δ 4.21 (5-H), at δ 6.35 (7-H), and at δ 4.21 (5-H) and δ 6.00 (5"-H), respectively. Some of the other significant long-range correlations observed are also shown by arrows (Chart 1b).

The relative stereochemistry was elucidated on the basis of the coupling constants of each proton and NOE experiments of **1**. The ^{1}H-NMR signals measured in acetone-d_6[5] showed the signals of methylene protons at C_4 positions were δ_H 2.05 (ddd, J = 11.0, 7.0, 6.0 Hz) and 2.25 (ddd, J = 11.0, 8.0, 4.0 Hz), and it is suggested that **1** is an *erythro*-type compound.[6] On irradiating the proton at C_3, NOE were observed at the methylene protons at C_2 and C_4, and J-value analyses suggested that C_3-H is lying closer to the C_2 and C_4 methylene protons. In a similar way, on irradiating the C_5-H at δ_H the NOE were observed at C_7-H and only one proton of C_4. The position of the methoxy group was confirmed at C-4" by the NOE experiment. These observations led us to conclude the stereostructure of calyxin A to be **1**.

Compound **2**, a light yellow amorphous solid, showed $[\alpha]_D$ -24.7° (MeOH, c = 0.36), and its molecular formula was determined to be $C_{35}H_{34}O_8$ [(M+H)$^+$ *m/z* 583.2340; calcd. 583.2332] by high-resolution FAB-MS measurement. The mass spectrum of **2** clearly showed one oxygen less than that of **1**. The IR spectrum of **2** was very similar to that of **1**. The number of proton and carbon signals of **1** and **2** were the same, but the signal patterns of the heptanoid chain were slightly different. From these spectral data this compound is considered to be the analog of **1**, and its partial structures (Chart 2a) were deduced by the same methods as used for **1**. The connectivities of these partial structures were confirmed by the HMBC experiments, and the significant long-range correlations are shown by the arrows (Chart 2b). The relative stereochemistry was determined as **2** on the basis of the coupling constant of each proton and the NOE experiment in Chart 2c(A), and named calyxin B.

Compound **3**, a light yellow amorphous solid, showed $[\alpha]_D$ +11.5° (MeOH, c = 0.51). The high resolution MS of **2** and **3** were identical to each other. The ^{1}H- and ^{13}C-NMR spectra of **3** were almost the same as those of **2**.[7] We did not observe much differences between **2** and **3** by NOE experiments (Chart 2c). The only difference in the ^{1}H-NMR spectrum was the quartet signal splitting pattern at C_4-H (δ_H 2.27) in **3**, while it was triplet in **2** (δ_H 2.28). The complete assignment of all the signals was due to the ^{1}H-^{1}H, ^{1}H-^{13}C, ^{1}H-^{13}C long-range COSY and HMBC experiments.[7] The structure was determined as represented by the formula **3** and named 3-*epi*-calyxin B.





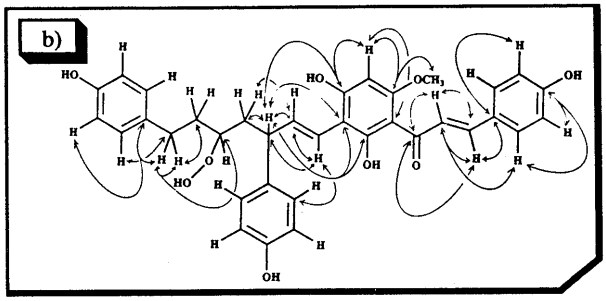


Chart 1

a) Partial structures and NMR data in methanol-d_4 for calyxin A (**1**).
b) Significant long-range correlations observed in HMBC experiment of calyxin A (**1**).
c) NOE observed in difference NOE experiments of calyxin A (**1**) in acetone-d_6.

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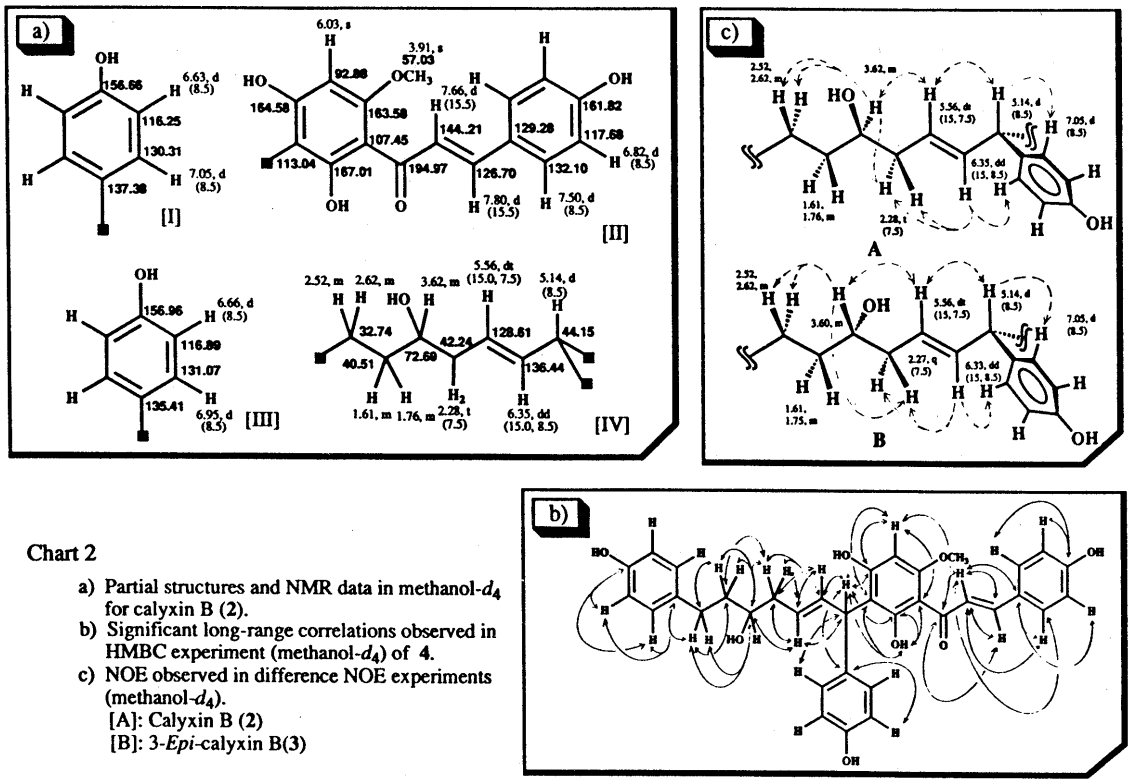

Chart 2

a) Partial structures and NMR data in methanol-d_4 for calyxin B (**2**).
b) Significant long-range correlations observed in HMBC experiment (methanol-d_4) of **4**.
c) NOE observed in difference NOE experiments (methanol-d_4).
[A]: Calyxin B (**2**)
[B]: 3-*Epi*-calyxin B(**3**)

These compounds were tested for their 3α-hydroxysteroid dehydrogenase (3α-HSD) inhibitory activity by the methods of Pennings.[8] The inhibitory activity of calyxin A (**1**) and an epimeric mixture of calyxin B (**2**) and 3-*epi*-calyxin B (**3**) were 50% and 62 % at the concentrations of 1.67 x 10^{-5} M and 1.72 x 10^{-5} M, respectively. These compounds showed a mild 3α-HSD inhibitory activity. The diarylheptanoids are the most common compounds found in Zingiberaceae; however, this is the first time we report a unique structure of natural products in which diarylheptanoids combine with a chalcone group. Other biological activities of these compounds are under investigation in our laboratory.

REFERENCES AND NOTES

1) T. M. Jurgens, E. G. Frazier, J. M. Schaeffer, T. E. Jones, D. L. Zink, R. P. Borris, W. Nanakorn, H. T. Beck, M. J. Balick, *J. Nat. Prod.*, **57**, 230 (1994).
2) Separation of epimeric mixture of **2** and **3** could not be achieved by normal- and reversed-phase HPLC.
3) L.V. Puyvelde, N. D. Kimpe, J. Costa, V. Munyjabo, S. Nyirankuliza, E. Hakizamungu, N. Schamp, *J. Nat. Prod.*, **52**, 629 (1989); M. Kuroyanagi, T. Noro, S. Fukushima, R. Aiyama, A. Ikuta, H. Itokawa, M. Morita, *Chem. Pharm. Bull.*, **31**, 1544 (1983).
4) K. Ito, Y. Sakakibara, M. Haruna, *Chem. Lett.*, 1503 (1979); F. S. El-Feraly, Y. -M. Chan, E. H. Fairchild, R. W. Doskotch, *Tetrahedron Lett.*, 1973 (1977).
5) The carbon signals of C10 and C6 overlapped with C2''' and C11'' on measuring ^{13}C-NMR spectrum in acetone-d_6, but they were clearly separated in methanol-d_4 so that the complete assignment was expressed due to NMR experiment measured in methanol-d_4. In contrast, the *J*-values of ^{1}H-NMR signals in acetone-d_6 were clear so that the conformation of **1** was explained by the data measured in acetone-d_6.
6) T. Akihisa, H. Oinuma, T. Tamuara, Y. Kasahara, K. Kumaki, K. Yasukawa, M. Takido, *Phytochemistry*, **33**, 105 (1994); H. Kikuzaki, S. M. Tsai, N. Nakatani, *ibid*, **31**, 1783 (1992); P. L. Cazaux, P. Maroni, *Bull. Soc. Chem. Fr.*, 773 (1972).
7) 3-*Epi*-Calyxin B (**3**): yellow amorphous solid, $[\alpha]_D$ +11.5° (MeOH, c = 0.51); ^{1}H-NMR (400 MHz, CD_3OD): δ_H 1.62, 1.75 (each1H, m, C_2-H), 2.27 (2H, q, *J* = 7.5 Hz, C_4-H), 2.52, 2.62 (each 1H, m, C_1-H), 3.60 (1H, m, C_3-H), 3.91 (3H, s, $C_{4''}$-OCH_3), 5.14 (1H, d, *J* = 8.5 Hz, C_7-H), 5.56 (1H, dt, *J* = 15.0, 7.5 Hz, C_5-H), 6.03 (1H, s, $C_{5''}$-H), 6.33 (1H, dd, *J* = 15.0, 8.5 Hz, C_6-H), 6.62 (2H, d, *J* = 8.5 Hz, $C_{3'''}$-H), 6.65 (2H, d, *J* = 8.5 Hz, $C_{3'}$-H), 6.82 (2H, d, *J* = 8.5 Hz, $C_{12''}$-H), 6.92 (2H, d, *J* = 8.5 Hz, $C_{2'}$-H), 7.05 (2H, d, *J* = 8.5 Hz, $C_{2'''}$-H), 7.50 (2H, d, *J* = 8.5 Hz, $C_{11''}$-H), 7.66 (1H, d, *J* = 15.5 Hz, $C_{8''}$-H), 7.80 (1H, d, *J* = 15.5 Hz, $C_{9''}$-H); ^{13}C-NMR (100 MHz, CD_3OD): δ_C 32.58 (t, C-1), 40.45 (t, C-2), 42.33 (t, C-4), 44.15 (d, C-7), 56.99 (q, C-OCH_3), 72.54 (d, C-3), 92.88 (d, C-5''), 107.42 (s, C-3''), 112.98 (s, C-1''), 116.25 (d, C-3'''), 116.83 (d, C-3'), 117.65 (d, C-12''), 126.70 (d, C-9''), 128.64 (d, C-5), 129.25 (s, C-10''), 130.28 (d, C-2'''), 131.04 (d, C-2'), 132.07 (d, C-11''), 135.35 (s, C-1'), 136.44 (d, C-6), 137.29 (s, C-1'''), 144.18 (d, C-8''), 156.63 (s, C-4'''), 156.90 (s, C-4'), 161.79 (s, C-13''), 163.55 (s, C-4''), 164.58 (s, C-6''), 166.95 (s, C-2''), 194.94 (s, C-7'').
8) S. Kadota, S. Terashima, P. Basnet, T. Kikuchi, T. Namba, *Chem. Pharm. Bull.*, **41**, 487-490 (1993); T. M. Pennings, *J. Pharm. Sci.*, **74**, 651 (1985).

(Received September 21, 1994; accepted November 4, 1994)

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