THREE NOVEL DIARYLHEPTANOIDS, CALYXIN A, CALYXIN B, AND 3-EPICALYXIN 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 an 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 Shihuan Provinces and Tibet. During the course of a program to find the biologically active compounds, we isolated these 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 Sumberchi OA-4700 column, 2 three novel compounds 1, 2 and 3 were isolated together with several other known compounds such as alpinatin, cardamom and helichrysin.

Compound 1, a light yellow amorphous solid, showed [α]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 C35H34O9 ([M+H]+ 599.2285, calcld. 599.2282) by high-resolution FAB-MS. The positive FeCl3 test and IR absorptions at νmax 3225, 1605 cm⁻¹ indicated that 1 contains phenolic and α,β-unsaturated carbonyl groups. The 1H- and 13C-NMR spectra of 1 indicated the presence of three methylenes (δH 1.64 (1H), 1.87 (1H), 2.11 (2H), 2.52 (1H), 2.62 (1H); δC 40.88, 42.48, 32.86), two methines (δH 3.51, 4.21; δC 71.23, 37.11), a methoxy group (δH 3.91; δC 56.97), two sets of trans double bond (δH 6.35, 6.53, 7.67, 7.78; δC 130.89, 131.77, 144.06, 126.79), twelve ortho coupling aromatic methines (δH 6.64, 6.69, 6.83, 6.96, 7.16, 7.49; δC 116.74, 116.98, 117.65, 128.97, 131.10, 132.04) and a single tetra aromatic methine (δH 6.00; δC 92.91) along with twelve quaternary carbons (δH 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, cardamom and helichrysin. 3 (Chart 1a [III]).

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, calcld. for C47H47O15 851.2915] along with some significant peaks at m/z 835, 810, 794, 749, 733 and six acetyl methyl signals at δH 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, calcld. for C35H35O8

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583.2332) and 835 [(M+H+O)⁺ (Found 835.2942, calcd. for C₄₂H₂₇O₁₄ 835.2966), respectively; in a qualitative test, I liberated iodine from the methanolic KI solution, suggesting the presence of hydroperoxide group.⁴ The chemical shift of C₃-H (δ₃ 3.51, m) of I was shifted downfield (δ₃ 4.99, m) in its acetate (Ia), indicating the position of hydroperoxide group at C₃. Also, NaBH₄ reduction of I gave 1b and the 13C-NMR of 1b showed hydroxy-bearing methine carbon at δ 63.34. Detailed analysis of 1H- and 13C-NMR spectra with the aid of 1H-1H COSY and 1H-13C COSY allowed us to deduce the partial structures shown in Chart 1a.

Next, we measured the HMBC of I 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 I. The 1H-NMR signals measured in acetone-d₆ showed the signals of the methylene protons at C₁₃ were positioned at δ 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 I is an erythro-type compound.⁵ On irradiating the proton at C₁₃, NOE were observed at the methylene protons at C₂ and C₄, and J-value analyses suggested that C₃-H is lying closer to the C₂ and C₄ methylene protons. In a similar way, on irradiating the C₃-H at δ, the NOE were observed at C₂ and only one proton of C₄. 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 I.

Compound 2, a light yellow amorphous solid, showed [α]D₂₀ -24.7⁰ (MeOH, c = 0.36), and its molecular formula was determined to be C₃₅H₃₂O₈ [(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 I. The IR spectrum of 2 was very similar to that of I. The number of proton and carbon signals of I and 2 were the same, but the signal patterns of the heptannoid chain were slightly different. From these spectral data this compound is considered to be the analog of I, and its partial structures (Chart 2) were deduced by the same methods as used for I. 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 [α]D₂₀ +11.5⁰ (MeOH, c = 0.51). The high resolution MS of 2 and 3 were identical to each other. The 1H- and 13C-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 1H-NMR spectrum was the quartet signal splitting pattern at C₄-H (δ₃ 2.27) in 3, while it was triplet in 2 (δ₃ 2.28). The complete assignment of all the signals was due to the 1H-1H, 1H-13C, 1H-13C long-range COSY and HMBC experiments.⁷ The structure was determined as represented by the formula 3 and named 3-epi-calyxin B.
These compounds were tested for their 3α-hydroxysteroid dehydrogenase (3α-HSD) inhibitory activity by the methods of Pennings. 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

2. Separation of epimeric mixture of 2 and 3 could not be achieved by normal- and reversed-phase HPLC.
5. The carbon signals of C10 and C14 overlapped with C11′ and C11′′ on measuring 13C-NMR spectrum in acetone-d6, but they were clearly separated in methanol-d4 so that the complete assignment was expressed due to NMR experiment measured in methanol-d4. In contrast, the J-values of 1H-NMR signals in acetone-d6 were clear so that the conformation of 1 was explained by the data measured in acetone-d6.
7. 3-Epi-Calyxin B (3): yellow amorphous solid, [α]D +11.5° (MeOH, c = 0.51); 1H-NMR (400 MHz, CD3OD): δH 1.62, 1.75 (each1H, m, C2-H), 2.27 (2H, q, J = 7.5 Hz, C4-H), 2.52, 2.62 (each 1H, m, C1-H), 3.60 (1H, m, C3-H), 3.91 (3H, s, C4-OCH3), 5.14 (1H, d, J = 8.5 Hz, C7-H), 5.56 (1H, dt, J = 15.0, 7.5 Hz, C5-H), 6.03 (1H, s, C3″-H). 6.33 (1H, dd, J = 15.0, 8.5 Hz, C6-H), 6.62 (2H, d, J = 8.5 Hz, C3-H), 6.65 (2H, dd, J = 8.5 Hz, C3-H), 6.82 (2H, d, J = 8.5 Hz, C12-H), 6.92 (2H, d, J = 8.5 Hz, C2-H), 7.05 (2H, d, J = 8.5 Hz, C2-H), 7.50 (2H, d, J = 8.5 Hz, C11-H), 7.66 (1H, d, J = 15.5 Hz, C8-H), 7.80 (1H, d, J = 15.5 Hz, C6-H); 13C-NMR (100 MHz, CD3OD): δC 32.58 (t, C-1), 40.45 (t, C-2), 42.33 (t, C-4), 44.15 (d, C-7), 56.99 (q, C-OCH3), 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″).

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