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A [2 + 2] Cycloaddition Dimer and a Diels–Alder Adduct from *Alpinia katsumadai*

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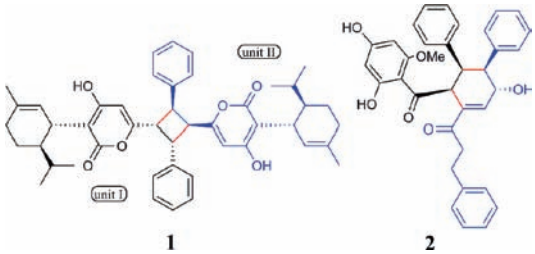
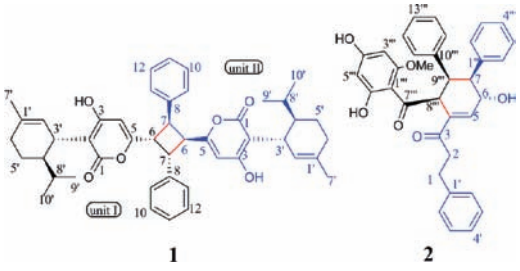
ABSTRACT



An unusual katsumadain dimer via a [2 + 2] cycloaddition, katsumadain C (1), and a unique chalcone-diarylheptanoid adduct via a Diels–Alder reaction, calyxin Y (2) with novel carbon frameworks, were isolated from the seeds of *Alpinia katsumadai*. Their structures and relative configurations were determined by spectroscopic evidence.

Plants of the genus *Alpinia* (Zingiberaceae) are herbs that have been traditionally used in China and some southeast Asian countries for relieving stomachache, treating colds, invigorating the circulatory system, and reducing swelling.[1] Previous investigations on the genus *Alpinia* led to the isolation of some new diarylheptanoids bearing a chalcone or a flavanone moiety[2−4] and monocyclic sesquiterpenes adducted by a chalcone.[5] In our continuing endeavor to discover new natural products from *Alpinia katsumadai*, a novel katsumadain dimer named katsumadain C (**1**), and a unique chalcone diarylheptanoid adduct named calyxin Y (**2**) were isolated from the seeds of this species. Herein, details of the isolation, structural elucidation, and postulated biogenetic origin are described.



Katsumadain C (**1**)[6] had the molecular formula of $C_{46}H_{52}O_6$ based on the HRESIMS (*m*/*z* 699.3641 $[M - H]^-$).

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(4) Gewali, M. B.; Tezuka, Y.; Banskota, A. H.; Ali, M. S.; Saiki, I.; Dong, H.; Kadota, S. *Org. Lett.* **1999**, *1*, 1733–1736.

(5) Hua, S. Z.; Wang, X. B.; Luo, J. G.; Wang, J. S.; Kong, L. Y. *Tetrahedron Lett.* **2008**, *49*, 5658–5661.

(6) Katsumadain C (**1**): white amorphous powder; $[\alpha]^{28}_D$ +173.2 (c 0.050, $CHCl_3$/MeOH = 1:1); UV ($CHCl_3$/MeOH = 1:1) λ_{max} (log ε) 237 (2.19), 301 (4.43) nm; IR (KBr) ν_{max} 3446, 2957, 2934, 1655, 1577, 1409, 1292, 1111, 1022, 839, 752, 699 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; negative ESI-MS *m*/*z* 699.5 $[M - H]^-$; HRESIMS *m*/*z* 699.3641 $[M - H]^-$ (calcd for $C_{46}H_{51}O_6$, 699.3691).

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1** (in DMSO-d_6)

	unit I		unit II	
no.	δ_H (mult, J, Hz)	δ_C	δ_H (mult, J, Hz)	δ_C
1		162.9		162.8
2		103.7		103.6
3		164.6		164.6
4	5.92 (s)	100.8	5.88 (s)	100.5
5		160.5		160.4
6	4.20 (dd, 9.8, 6.3)	44.2	4.26 (dd, 9.8, 6.3)	43.8
7	4.30 (dd, 9.8, 6.3)	42.8	4.32 (dd, 9.8, 6.3)	42.6
8		138.0		137.9
9, 13	7.32 (m)	127.5	7.30 (m)	127.3
10, 12	7.24 (m)	127.9	7.23 (m)	127.9
11	7.16 (m)	126.4	7.15 (m)	126.4
1′		131.9		131.8
2′	4.85 (s)	125.0	4.85 (s)	125.0
3′	3.33 (m)[a]	35.0	3.33 (m)[a]	34.9
4′	1.84 (m)[a]	39.7	1.84 (m)[a]	39.6
5′	1.13 (m)[a]	22.5	1.12 (m)[a]	22.5
	1.61 (m)[a]		1.60 (m)[a]	
6′	1.93 (m)[a]	30.3	1.93 (m)[a]	30.3
	1.86 (m)[a]		1.84 (m)[a]	
7′	1.53 (s)	23.2	1.53 (s)	23.1
8′	1.19 (m)[a]	27.8	1.19 (m)[a]	27.8
9′	0.60 (d, 6.8)	16.4	0.60 (d, 6.8)	16.4
10′	0.75 (dd, 6.8, 1.2)	21.2	0.75 (dd, 6.8, 1.2)	21.2
3-OH	10.78 (s)		10.78 (s)	

[a] Signal pattern unclear due to overlapping.

The IR spectrum indicated the presence of OH (3446 cm^{-1}), ester carbonyl (1655 cm^{-1}) groups, and aromatic rings (1577, 1514, and 1409 cm^{-1}). The ^{1}H, ^{13}C, and HSQC NMR spectra (Table 1) indicated the presence of a monosubstituted benzene ring (δ_H 7.15−7.33, 5H, overlapped), two methylenes, seven methines (including two olefinic methines), and three angular methyls [δ_H 0.60 (d, J = 6.8 Hz, 3H), 0.75 (dd, J = 6.8, 1.2 Hz, 3H), 1.53 (s, 3H); δ_C 16.4, 21.2, 23.1]. These spectral features were like those of katsumadain[7] except for the absence of *trans*-olefinic signals, and instead, the presence of two additional methines, which combined with 2-fold relationship of the molecular weight of **1** ($C_{46}H_{52}O_6$) and katsumadain ($C_{23}H_{26}O_3$), suggested **1** is a dimer of katsumadain, forming a cyclobutane ring between the two units. Two possible cycloadducts, head-to-tail or head-to-head, exists (Figure 1), which cannot be distinguished directly by NMR data. On the basis of the mass spectrometric studies on similar situations,[8,9] three typical fragments at m/z 350, 180, and 520 should be found for **1** in head-to-head mode, while only one fragment ion at m/z 350 could be found in head-to-tail mode. The EI-MS of **1** exhibited a very small molecular ion at 700 and the presence of a typical fragment at m/z 350 indicated **1** to be a head-to-tail dimer. In addition, as shown in Figure 1,

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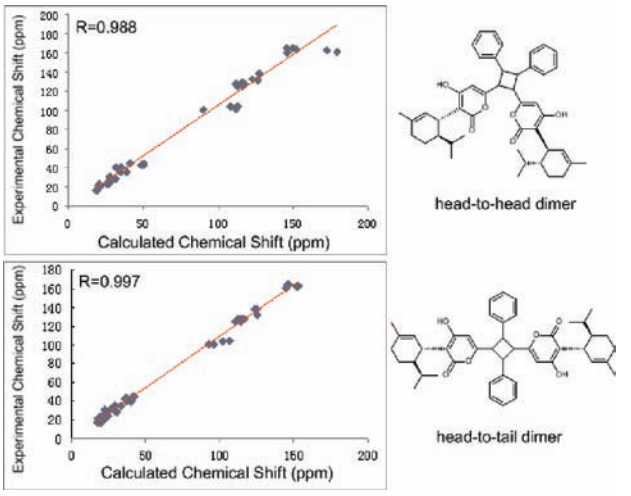

Figure 1. Two possible dimer modes for **1**. The correlations between the calculated and experimental chemical shifts for the head-to-head mode and the head-to-tail mode.

the closer fitting between the predicted and experimental ^{13}C NMR chemical shifts for the head-to-tail mode (R = 0.997) than the head-to-head mode (R = 0.988) also indicate the head-to-tail mode is the preferred one.[10]

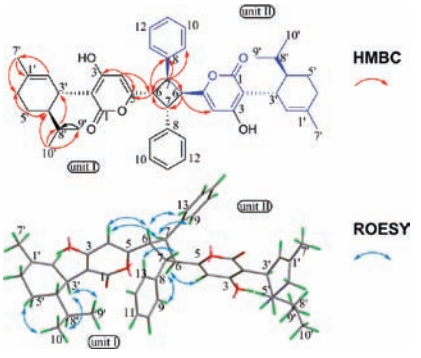

Figure 2. Selected HMBC (→) and ROESY (↔) correlations of **1**.

In the HMBC spectrum (Figure 2), the proton signal of H-6 (δ_H 4.20) was correlated with the carbon signals of C-7 (δ_C 42.8), and the proton signals of H-7 (δ_H 4.30) with the carbon signals at C-6 (δ_C 44.2). The HMBC spectrum showed long-range correlations from the protons of the monosubstituted benzene ring at δ_H 7.30 (H-9 and H-13) to the carbon signals of C-7. Thus, the position of the monosubstituted benzene ring was determined to be at C-7 position. The proton signal at δ_H 4.20 (H-6) was correlated with the carbon signals in lactonic ring (γ-pyrone) at δ_C 100.8 (C-4) and 160.5 (C-5). Therefore, the planar structure of **1** was established as shown in Figure 1. The relative configuration

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of compound **1** was deduced from the analysis of its ROESY correlations (Figure 2) and the energy minimized molecular modeling using density functional theory (DFT) at the B3LYP/6-31G+(d,p) basis set level in Gaussian 09.[11] The ROESY correlation from H-3′ to H-8′ and OCH_3-9′ confirmed the partial stereostructure of **1**, which was consistent with katsumadain.[7] The key points were the assignments of the relative coupling system consisting of at least sixteen symmetrical peaks[8,12] was found for the four methine protons on the cyclobutane ring in the ^{1}H NMR spectrum, suggested a configurations of the cyclobutane ring. A typical AA′BB′ symmetrically substituted cyclobutane ring. A 2D *J*-resolved experiment determined the ^3J coupling constants of H−I-6/H−I-7 and H−II-6/H−I-7 to be 6.3 and 9.8 Hz, respectively, which implied that **1** to be a *trans*−*trans* fused dimer in head-to-tail mode. The computer modeled 3D structure analysis of **1** was compatible with the aforementioned relative configuration as shown in Figure 2, and thus established the whole stucture except for the absolute configuration.

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of **2** (in DMSO-d_6)

no.	δ_H (multi, *J*, Hz)	δ_C	no.	δ_H (multi, *J*, Hz)	δ_C
1	2.84 (m, 2H)[a]	30.2	2‴		163.1
2α	3.05 (m)[a]	38.5	3‴	5.81 (d, 1.8)	91.7
2β	3.28 (m)[a]				
3		199.9	4‴		165.6
4		137.6	5‴	5.91 (d, 1.8)	96.4
5	7.31 (br s)	147.1	6‴		167.6
6	4.41 (br dd, 10.2, 7.2)	64.8	7‴		203.5
7	3.11 (dd, 10.2, 4.2)	47.1	8‴	4.75 (s)	51.5
1′		141.7	9‴	3.18 (d, 4.2)	49.5
2′, 6′	7.28 (m)	128.8	10‴		141.3
3′, 5′	7.28 (m)	128.7	11‴, 15‴	6.47 (d, 7.2)	128.7
4′	7.18 (m)	126.4	12‴, 14‴	7.09 (m)	127.9
1″		140.5	13‴	7.15 (m)	126.9
2″, 6″	6.67 (m)	129.2	OCH_3	2.89 (s)	55.6
3″, 5″	7.10 (m)	128.1	6-OH	5.24 (d, 7.2)	
4″	7.12 (m)	126.6	4‴−OH	10.70 (s)	
1‴		103.5	6‴−OH	13.63 (s)	

[a] Signal pattern unclear due to overlapping.

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(13) Calyxin Y (**2**): white amorphous powder; $[\alpha]^{28}_D$ +2.75 (c 0.080, $CHCl_3$/MeOH = 1:1); UV ($CHCl_3$/MeOH = 1:1) λ_{max} (log ε) 238 (3.17), 292 (3.20) nm; IR (KBr) ν_{max} 3445, 2922, 2851, 1631, 1463, 1379, 1108, 810, 720, 696 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; negative ESIMS *m*/*z* 547.3 [M − H]$^-$; HRESIMS *m*/*z* 547.2108 [M − H]$^-$ (calcd for $C_{35}H_{31}O_6$, 547.2126).

Calyxin Y (**2**)[13] was isolated as white amorphous powder. Its molecular formula of $C_{35}H_{32}O_6$ was determined by the observed ion at *m*/*z* 547.2108 [M − H]$^-$ in HRESIMS, which indicated 20 degrees of unsaturation. The maximum UV absorptions at 237 and 292 nm indicated the presence of a conjugated system. In the IR spectrum, absorption bands at 3700−3200 cm^{-1} (hydroxyl group) and 1631, 1463, 720, 696 cm^{-1} (aromatic ring) were observed. The ^{13}C NMR spectra (Table 2) resolved 35 carbon resonances that came from three monosubstituted phenyl groups, a methoxyl group, two methylenes, four sp^3 methines, two aromatic protons, an olefinic methine, and two ketone carbonyls. The benzene ring with two aromatic protons [δ_H 5.81 (1H, d, *J* = 1.8 Hz), 5.91 (1H, d, *J* = 1.8 Hz); δ_C 91.7, 96.4], the ketone carbonyls [δ_C 203.5], two sp^3 methines [δ_H 3.18, 4.75; δ_C 49.5, 51.5], and a monosubstituted phenyl group formed a dihydrochalcone moiety. Its structure was indicated by the HMBC correlations (Figure 3) from the methine protons at δ_H 3.18 (H-9‴) to the aromatic carbons at δ_C 141.3 (C-10‴), 128.7 (C-11‴, C-15‴), and to the ketone carbon at δ_C 203.5 (C-7‴). The position of OMe at C-2‴ was confirmed by the correlation between the proton signals of methoxy group and C-1‴, C-2‴, C-3‴. The correlation between H-8‴ and H-9‴ was observed in the ^{1}H−^{1}H COSY spectrum. The remaining 19 signals including two other phenyl rings formed a diarylheptanoid moiety. The long-range HMBC correlations H-2′/C-1, H-1/C-3, H-5/C-3, H-6/C-7, H-2″/C-7, and the ^{1}H−^{1}H COSY correlation H-1/H-2, H-5/H-6, H-6/H-7 allowed us to assign the diarylheptanoid part. A hydroxyl signal at δ_H 5.24 (d, *J* = 7.2 Hz) has a correlation with the signals at δ_C 47.0 (C-7), 64.8 (C-6) and 147.1 (C-5), which means the hydroxyl group located at C-6. These data indicated **2** to be an adduct of a chalcone and a diarylheptanoid.

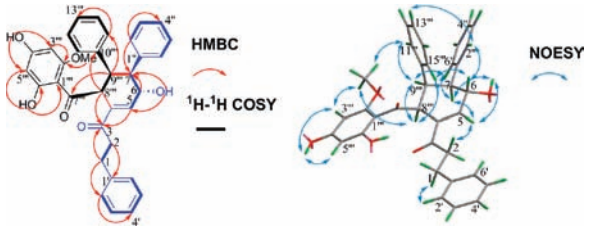


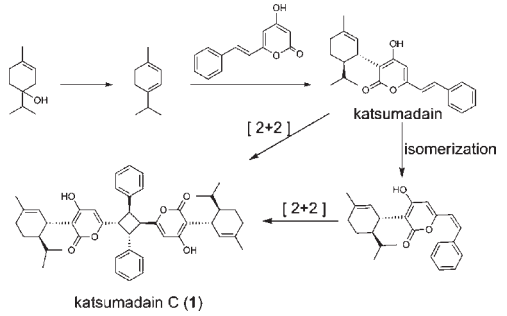
Figure 3. Key HMBC (→), ^{1}H−^{1}H COSY(—), and ROESY (↔) correlations for **2**.

The above-mentioned groups and structural fragments, four benzene rings, a double bond and two ketone carbonyls represented 19 degrees of unsaturation. The remaining one degree of unsaturation indicated compound **2** to have another ring. The ^{1}H−^{1}H COSY correlations (H-6/H-5 and H-7; H-9‴/H-7 and H-8‴) and HMBC correlations (H-5/C-7 and C-8‴; H-6/C-7; H-7/C-8‴; OH-6/C-5 and C-7) established a cyclohexene ring with a hydroxyl at C-6 between diarylheptanoid and chalcone. Thus, the planar structure of **2** was established as shown in Figure 3.

The relative configuration of **2** was elucidated by NOESY experiment (Figure 3). The intense correlations between H-8‴ (δ_H 4.75) and H-9‴ (δ_H 3.18) showed that they were cofacial and were arbitrarily assigned as α-oriented. The mutual correlations from H-9‴, H-6, and H- 7 to H-2″/6″ and from H-8‴, H-9‴ and H-6 to H-11‴/15‴ indicated that the two benzene rings at C-9‴ and C-7, and H-6 were on the same side, in β-orientation (Figure 3). Noteworthily, signals for OMe (δ_H 2.89) and the two phenyl groups (δ_H 6.67, 6.47) at C-7 and C-9‴ were severely upfield shifted due to the strong shielding effect of magnetic anisotropy, which was consistent with the observed NOE correlations of OMe/H-11‴ (or H-15‴) and H-2″/H-13‴. Thus, the structure of calyxin Y (**2**) except for the absolute configuration was established as shown in Figure 3.

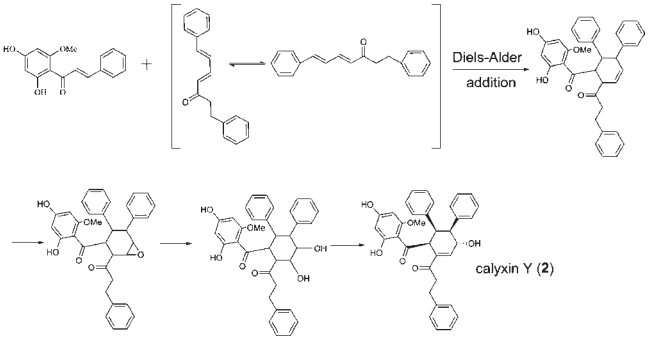
Katsumadain C (**1**) represented the first monoterpene substituted kavalactone dimer, conjugated in a head-to-tail mode; calyxin Y (**2**) was a unique chalcone-diarylheptanoid Diels–Alder adduct with a novel carbon framework of a cyclohexene ring, rather than of a tetrahydropyran ring such as blepharocalyxins A and B from *Alpinia blepharocalyx*.[14,15]

Scheme 1. Plausible Biogenetic Pathway of **1**



Since the two new adducts and key intermediates, diarylheptanoid,[16] chalcone,[17] and katsumadain,[7] were all found in the same plant, we could tentatively outline plausible biogenetic relationships of the isolates. Katsumadain C (**1**) may be derived through a [2 + 2] cycloaddition reaction of two ethylenic bonds between two katsumadain molecules (Scheme 1). Calyxin Y (**2**) could be formed through a Diels–Alder addition reaction between a diarylheptanoid and a chalcone (Scheme 2).

Scheme 2. Plausible Biogenetic Pathway of **2**



The cytotoxicities of katsumadain C (**1**) and calyxin Y (**2**) were evaluated against human tumor cell lines A375 (human melanoma cell line), MCF-7 (human breast cancer cell line), SMMC-7721 (human hepatic liver carcinoma cell line), and HCT-116 (human colon carcinoma cell line), using 5-fluorouracil as a positive control with IC_{50} values at 20.4, 33.8, 26.8, and 50.0 μM, respectively. They generally showed marginal to moderate activities. Compounds **1** and **2** both exhibited significant growth inhibitory effects against SMMC-7721 cells, with IC_{50} at 4.8, 9.7 μM, respectively, comparable to 5-fluorouracil.

Acknowledgment. This research work was supported by the National Natural Science Foundation of China (No. 21072230) and the Cultivation Fund of the Key Scientific & Technical Innovation Project, Ministry of Education of China (No. 707033), and the Scaling Project for Innovation Scholars, Natural Science Foundation of Jiangsu Province, China (No. BK2008039).

Supporting Information Available. Experimental procedures; IR, ESIMS, HRESIMS, and 1D and 2D NMR spectra of katsumadain C (**1**) and calyxin Y (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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