Tetrahedron Letters 49 (2008) 5658–5661

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A pair of unique sesquiterpene–chalcone conjugates isolated from the seeds of *Alpinia katsumadai*

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ARTICLE INFO

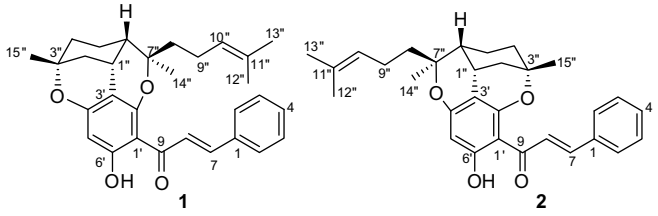
Article history:
Received 17 May 2008
Revised 11 July 2008
Accepted 15 July 2008
Available online 17 July 2008

Keywords:
Alpinia katsumadai
Sesquiterpene–chalcone conjugates
Sumadain

ABSTRACT

Sumadains A (**1**) and B (**2**), a pair of unique isomeric sesquiterpene–chalcone conjugates with unprecedented skeletons, were isolated from the seeds of *Alpinia katsumadai*. Their structures and relative configurations were established by NMR spectroscopy and X-ray crystallography.

The seeds of *Alpinia katsumadai* Hayata (Zingiberaceae) have been used in traditional Chinese medicine (TCM) as an antiemetic agent and for the treatment of stomach disorders,[1] and was coded in Chinese Pharmacopeia (volume I, 2005 edition) as an aromatic stomachic. Previous investigations of *Alpinia* genus have reported several new diarylheptanoids bearing a chalcone or a flavanone moiety,[2] and a labdane diterpene adducted by a chalcone.[3] In our continuing endeavor to discover new bioactive natural products from TCM, an investigation of *A. katsumadai* was undertaken. Sumadain A (**1**) and Sumadain B (**2**), a pair of novel sesquiterpene–chalcone conjugates with unprecedented carbon framework, were isolated from the seeds of this species. Herein, details of the isolation, structural elucidation, postulated biogenetic origin, and cytotoxic activities are described.



1 **2**

The dried powdered seeds (20 kg) were extracted with 95% EtOH (1 h × 4) to give 2 kg extract which was suspended in water and then partitioned with petroleum ether, chloroform, ethyl acetate, and *n*-butanol successively.

The petroleum ether extract (450 g) was subjected to chromatography over a silica gel column eluting with gradient solvent petroleum ether/ethyl acetate (1:0–2:1), to yield eight fractions. Fraction 2 was further subjected to repeated CC on silica gel, eluted with petroleum ether/acetone to afford **1** (25 mg) and **2** (15 mg).

Sumadain A (**1**)[4] was obtained as orange lamellar crystals ($CHCl_3/CH_3OH$) and gave an quasi-molecular ion $[M+H]^+$ at *m/z* 459.2538 (calcd 459.2530) in the HRESIMS, consistent with the elemental composition $C_{30}H_{34}O_4$. The IR spectrum of **1** showed absorption bands at 3443 and 1628 cm^{-1}, indicating the presence of hydroxyl and conjugated carbonyl groups. In the ^1H NMR spectrum, an aromatic proton (δ 6.09, lH, s), a phenyl group (δ 7.36, 3H, m; δ 7.61, 2H, m), a *trans*-olefinic group (δ 7.82, 1H, d, J = 15.7 Hz; δ 8.16, lH, d, J = 15.7 Hz), and one strongly chelated hydroxyl (δ 13.91) were observed. The ^{13}C NMR spectrum displayed fifteen aromatic carbons, of them three were oxygenated (δ 158.6, 162.8, 165.5), two olefinic, and one carbonyl carbon (δ 191.6). These signals suggested the presence of a chalcone unit.

Except signals of the chalcone, the ^{13}C NMR displayed 15 carbon signals, of which four were CH_3, five CH_2, three CH, and three quaternary carbons (two of them bearing oxygen). The ^1H NMR exhibited four singlet methyl protons, five methylene protons, and three methine protons (including one olefinic proton). Careful analysis of the ^1H NMR and HSQC made the assignment of the two pyran ring methylene protons adjacent to the quaternary carbon as follows: H-2″α (δ 2.18, ddd, J = 13.3, 4.3, 3.2 Hz) and H-2″β (δ 1.84, br d, J = 13.3 Hz), and H-4″α (δ 1.84, br d, J = 13.4 Hz) and H-4″β (δ 1.45, m). The HMBC correlations (Fig. 1) from Me-15″ to C-2″ and C-4″, and Me-14″ to C-6″ and C-8″ confirmed the presence of a bisabolane sesquiterpene unit, which was coupled with the

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doi:10.1016/j.tetlet.2008.07.082

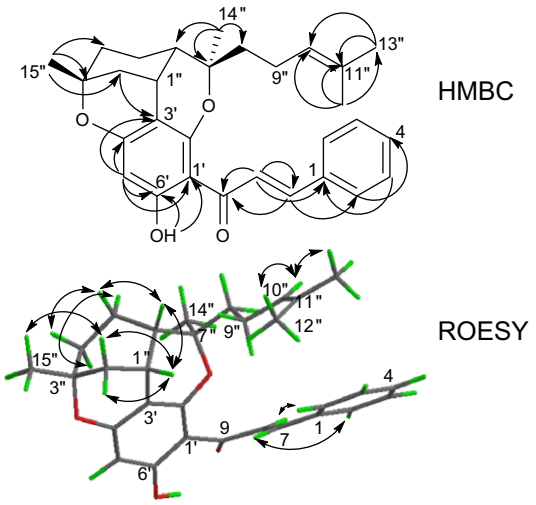

Figure 1. Selected 2D NMR correlations for Sumadain A (**1**).

chalcone moiety via the C-3′–H-2″ bond as suggested by the cross-peaks from C-3′ to H-2″β.

The only uncertainty for the planar structure of **1** was the remaining two degrees of unsaturation, which required the presence of two additional rings. As C-3″ and C-7″ were quaternary carbons and the 2D NMR spectra did not provide sufficient information to elucidate the pattern of connection of these carbons, a single-crystal X-ray diffraction of **1** (Fig. 2)[5] was performed and demonstrated that two ether bridges were present between C-7″ and C-2′, and C-3″ and C-4′.

The relative configuration of the pyran ring is based on the coupling constants of H-1″ (br s) and H-6″ (δ 2.12, ddd, 11.6, 5.2, 2.9), establishing an equatorial of H-1″ and an axial of H-6″, and the ROESY correlations (Fig. 1) of H-1″/H-6″ and H-2″β, H-6″/H-5″β and Me-15″/H-2″β. Single-crystal X-ray diffraction analysis[5] confirmed the above conclusion and demonstrated that Me-14″ was α-oriented though no signals correlated with it in the ROESY spectrum. The structure of **1** was thus established as depicted.

HRESIMS and ^{13}C NMR analysis of Sumadain B (**2**)[6] indicated that it has the molecular formula $C_{30}H_{34}O_4$, which was identical to that of **1**. The ^{1}H and ^{13}C NMR data (Table 1) as well as 2D correlations (Fig. 3) revealed that both the functional groups and the skeleton present in **2** were similar to those observed in **1**, suggesting that **2** was a conformation isomer of **1**. A single crystal of **2** was submitted to X-ray analysis (Fig. 4),[7] which confirmed the sesquiterpene and the chalcone units formed two ether bridges between C-7″ and C-4′, and C-3″ and C-2′.

Table 1
NMR data for **1** and **2** in $CDCl_3$[a,b]

	1		**2**	
	δ_H (mult, *J*, Hz)	δ_C	δ_H (mult, *J*, Hz)	δ_C
1		135.7		135.7
2/6	7.61 (m)	128.2	7.58 (m)	128.1
3/5	7.36 (m)	128.7	7.38 (m)	128.7
4	7.36 (m)	129.8	7.38 (m)	129.7
7	8.16 (d, 15.7)	127.1	8.08 (d, 15.7)	127.4
8	7.82 (d, 15.7)	142.1	7.77 (d, 15.7)	141.6
9		191.6		191.9
1′		108.2		106.5
2′		158.6		158.1
3′		108.6		107.1
4′		162.8		163.2
5′	6.09 (s)	97.5	6.06 (s)	99.0
6′		165.5		166.3
1″	2.79 (br s)	27.5	2.85 (t, 2.2)	27.3
2″α	2.18 (ddd, 13.3, 4.3, 3.2)	34.6	2.23 (ddd, 13.2, 4.5, 3.3)	33.9
2″β	1.84 (br d, 13.3)		1.87 (br d, 13.2)	
3″		76.0		76.6
4″α	1.84 (br d, 13.4)	37.5	1.87 (br d, 13.2)	37.5
4″β	1.45 (m)		1.48 (m)	
5″α	0.87 (m)	21.7	0.93 (m)	21.9
5″β	1.26 (m)		1.30 (m)	
6″	2.12 (ddd, 11.6, 5.2, 2.9)	45.5	2.10 (ddd,11.5, 6.2, 2.5)	44.5
7″		89.1		87.2
8″a	2.07 (m)	42.4	1.93 (m)	41.8
8″b	1.74 (m)		1.73 (m)	
9″a	2.38 (m)	22.9	2.20 (m)	22.7
9″b	2.25 (m)		2.12 (m)	
10″	5.19 (tq, 5.9,1.2)	123.5	5.14 (tq, 5.9, 1.3)	123.7
11″		132.1		131.9
12″	1.64 (s)	17.6	1.64 (s)	17.5
13″	1.73 (s)	25.5	1.70 (s)	25.5
14″	1.01 (s)	21.0	1.04 (s)	21.1
15″	1.38 (s)	28.5	1.52 (s)	28.8
6′-OH	13.91 (s)		14.13 (s)	

[a] ^{1}H was recorded in 500 MHz and ^{13}C at 125 MHz.
[b] The assignments were based on ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC experiments.

A plausible biogenetic pathway for Sumadains A (**1**) and B (**2**) is proposed as shown in Scheme 1. They might be derived through intracyclization of the intermediate C produced by *cis*, *trans*-FPP and 2,4,6-trihydroxychalcone. Saumadains A and B are the first

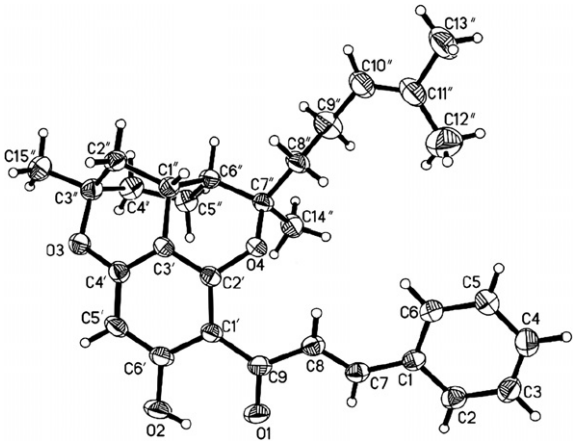

Figure 2. X-ray crystallographic structure of **1**.

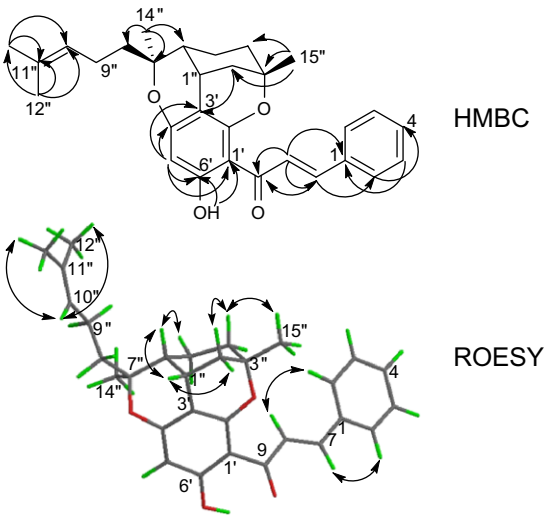

Figure 3. Selected 2D NMR correlations for Sumadain B (**2**).

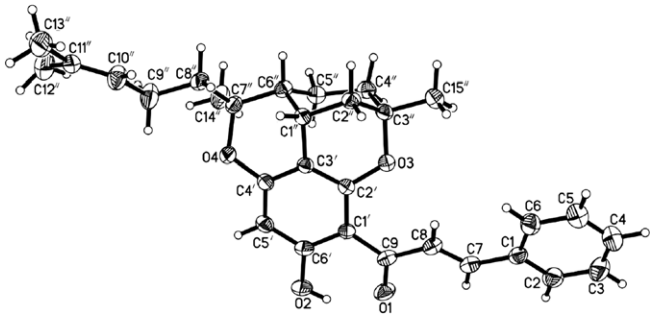

Figure 4. X-ray crystallographic structure of **2**.

sesquiterpene–chalcone conjugates with unprecedented carbon skeleton.

Compounds **1**, **2** and one chalcone helichrysetin isolated were evaluated for cytotoxic activities in one human liver cancer cell line HEPG2, and two human breast cancer cell lines MCF-7 and MDA-MB-435 with *cis*-platin (DPP) as positive control. Compounds **1** and **2** were found to be inactive in all tests, while helichrysetin showed inhibitory activity against the three cell lines with IC_{50} values 14.63 μg/mL, 24.22 μg/mL, and 1.83 μg/mL, respectively.

Chalcones exhibited varying degrees of anticancer properties.[8,9] However, the exact mechanisms of cytotoxic activity were not to be fully understood. Hydroxyl substituted chalcones were generally found to be the most potent cytotoxic in tumor cells. Introduction of bulky groups at positions C-2′ and C-3′ leads to a reduction of antileishmanial activity,[10] and bulky substituents at C-4′ also decrease the cytotoxicity activity of chalcones against leukemia cell line.[11]

Therefore, devoid of cytotoxity may be attributed to the etherfication of hydroxyls and hindered rotation of **1** and **2** due to the bridges formed between sesquiterpene and chalcone.

Acknowledgments

This research work was supported by the Program for Changjiang Scholars, Ministry of Education of China and the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of China (707033).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.07.082.

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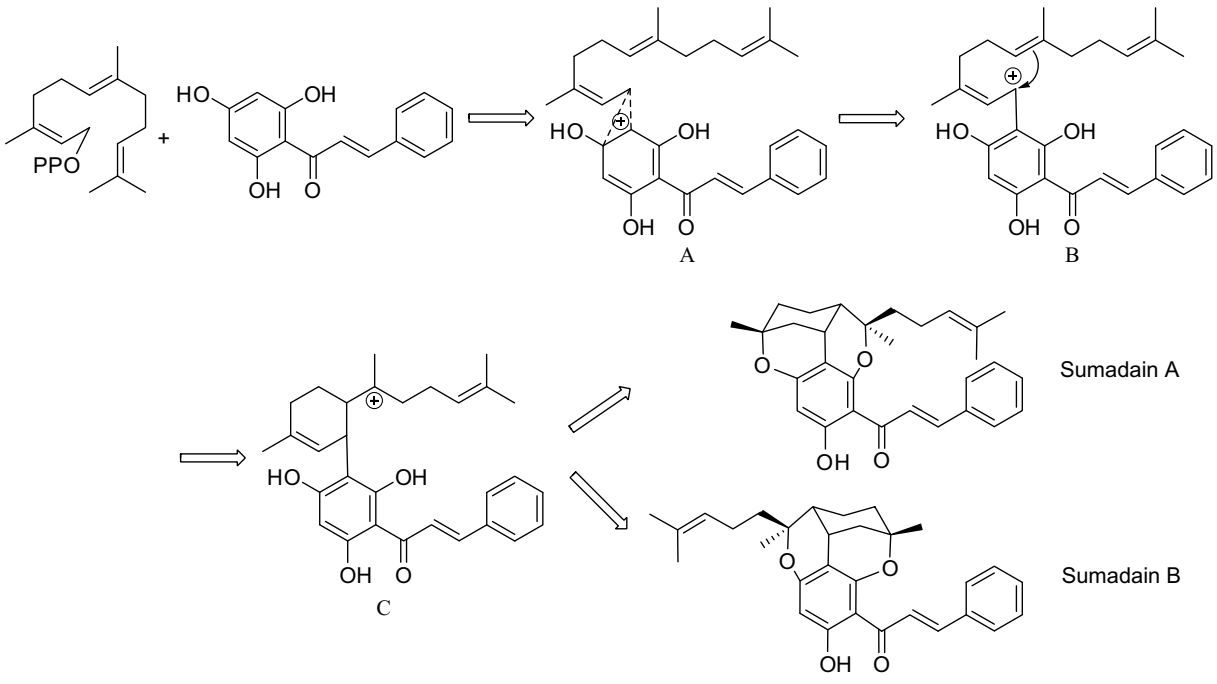
4. *Sumadain A* (**1**): Orange lamellar crystals ($CHCl_3$), mp: 160–162 °C; $[\alpha]_D^{13.6}$ −3.7 (*c* 0.066, $CHCl_3$); ^1H NMR and ^{13}C NMR see Table 1; UV ($CHCl_3$) λ_{max} (logε) 348 (3.42), 240 (3.15) nm; IR (KBr) ν_{max} 3443, 2923, 1628, 1554, 1164 cm^{-1}; NMR data, see Table 1; ESIMS *m/z* 457 $[M-H]^-$; HRESIMS *m/z* 459.2538 ($[M+H]^+$; calcd for $C_{30}H_{34}O_4$, 459.2530).
5. *Crystallographic data for* **1**: $C_{30}H_{34}O_4$, M = 458.57, monoclinic, space group $P\,2_1$, a = 14.1177(14) Å, b = 12.0014(12) Å, c = 15.630(2) Å, V = 2466.8(5) $Å^3$, Z = 4, Dc = 1.235 g/cm^3, crystal dimensions 0.47 × 0.46 × 0.35 mm were used for measurements on a Bruker Smart-1000 CCD with a graphite monochromator, Mo Kα radiation. The total number of reflections measured was 12130, of which 4320 were unique and were 1983 observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0698, ωR_2 = 0.1809 for observed reflections, and R_1 = 0.1277, ωR_2 = 0.2425 for all reflections. The crystal structure (**1**) was solved by direct methods using SHELX-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1990) and expanded using difference Fourier techniques, refined by SHELX-97 (Sheldrich, G. M. 1997). Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC-684851). Copies of these data can be obtained free of charge *via* the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax (+44) 1223 336 033; or deposit@ccdc.cam.ac.uk).



Scheme 1. Plausible biogenetic pathway for Sumadains A (**1**) and B (**2**).

6. *Sumadain B* (**2**): Orange lamellar crystals ($CHCl_3$), mp: 176–178 °C; $[\alpha]_D^{13.7}$ −2.4 (*c* 0.085, $CHCl_3$); ^1H NMR and ^{13}C NMR see Table 1; UV ($CHCl_3$) λ_{max} (logε) 348 (3.40), 240 (3.07) nm; IR (KBr) ν_{max} 3443, 2923, 1630, 1551, 1147 cm^{-1}; NMR data, see Table 1; ESIMS *m/z* 457 $[M-H]^-$; HRESIMS *m/z* 459.2531 ($[M+H]^+$; calcd for $C_{30}H_{34}O_4$, 459.2530).
7. *Crystallographic data for* **2**: $C_{30}H_{34}O_4$, *M* = 458.57, monoclinic, space group $P2_1$, *a* = 8.981(2) Å, *b* = 11.445(2) Å, *c* = 11.977(2) Å, *V* = 1225.9(3) $Å^3$, *Z* = 2, *Dc* = 1.2242 g/cm^3, crystal dimensions 0.41 × 0.30 × 0.18 mm were used for measurements on a Bruker Smart-1000 CCD with a graphite monochromator, Mo Kα radiation. The total number of reflections measured was 6179, of which 3708 were unique and were 1983 observed, $I > 2\sigma(I)$. Final indices: $R_1 = 0.0464$, $\omega R_2 = 0.0908$ for observed reflections, and $R_1 = 0.0854$, $\omega R_2 = 0.1110$ for all reflections. The crystal structure (**2**) was solved by direct methods using SHELX-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1990) and expanded using difference Fourier techniques, refined by SHELX-97 (Sheldrich, G. M. 1997). Crystallographic data for the structure of **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC-684852). Copies of these data can be obtained free of charge *via* the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223 336 033; or deposit@ccdc.cam.ac.uk).

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