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Two novel monoterpene–chalcone conjugates isolated from the seeds of *Alpinia katsumadai*

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ABSTRACT

Three monoterpene–chalcone conjugates, including two novel compounds isorubraine (**2**) and sumadain C (**3**), and a known compound rubraine (**1**) were isolated from the seeds of *Alpinia katsumadai*. Their structures and relative configurations were established by NMR spectroscopy and X-ray crystallography. Cytotoxic activities of these compounds were evaluated against cell lines HepG2, MCF-7 and MAD-MB-435, and compound **3** exhibited potent cytotoxic activity.

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As a part of our ongoing research program to isolate bioactive compounds from chinese medicinal plants, we have reported a pair of novel sesquiterpene–chalcone conjugates from the petroleum ether portion of an EtOH extract of seeds of *Alpinia katsumadai*,[1] which are used in traditional Chinese medicine (TCM) as an antiemetic agent and for the treatment of stomach disorders in China.[2] Further work on the chloroform portion of the EtOH extract afforded three monoterpene–chalcone conjugates, rubraine (**1**), isorubraine (**2**), and sumadain C (**3**) (Fig. 1). In this Letter, we describe the isolation and structure elucidation of new compounds **2**, **3**, and the cytotoxic activities of the three compounds.

The 20 kg seeds of *A. katsumadai* were extracted with 95% EtOH to give 2 kg extract which was suspended in water and then partitioned with petroleum ether, chloroform, ethyl acetate, and *n*-butanol successively. The chloroform extract (300 g) was chromatographed over a silica gel column eluting with gradient solvent petroleum ether/ethyl acetate (2:0–2:1), to yield 12 fractions. Fraction 2 was further subjected to repeated CC on silica gel and prep-HPLC (RP-18, MeOH/H_2O, 80:20) to afford rubraine (**1**, 10 mg), isorubraine (**2**, 3 mg). Fraction 5 afforded sumadain C (**3**, 15 mg) by repeated CC on silica gel and Sephadex LH-20).

The mixture of compounds **1** and **2** could be separated only by using RP-HPLC. The spectral data of **1** indicated that it was a known compound rubraine.[3] Compound **2**[4] was obtained as orange powder and the molecular formula was determined by HRESIMS to be $C_{25}H_{26}O_4$ with a quasi-molecular ion $[M+H]^+$ at *m/z* 391.1909 (calcd 391.1904). The IR spectrum of **2** showed absorption bands at 3426 and 1630 cm^{-1}, indicating the presence of hydroxyl and conjugated carbonyl groups. In the ^1H NMR spectrum, one phenyl group (δ 7.38, 1H, m; 7.40, 2H, m; 7.61, 2H, m), a single aromatic proton δ 6.07 (lH, s), two *trans*-olefinic protons (δ 7.79, 1H, d, J = 15.6 Hz; 8.10, lH, d, J = 15.6 Hz), three angular methyl groups at δ 1.12; 1.55, 1.56, and one chelated hydroxyl (δ 14.07) were observed. The ^{13}C NMR spectrum displayed 15 aromatic carbons, of them three were oxygenated (δ 158.5, 163.5, 166.4), two olefinic, and one carbonyl carbon (δ 192.1). These signals suggested the presence of a chalcone unit. Except signals of the chalcone, the ^{13}C NMR displayed 10 carbon signals, of which three were CH_3, three CH_2, two CH, and two oxygenated quaternary carbons (δ 76.9, 85.2), which suggested that the skeleton of **2** was a monoterpene–chalcone conjugate. Furthermore, the analysis of NMR spectra (^1H and ^{13}C NMR, HSQC, HMBC, see Table 1) data showed that the structure of **2** is composed of the monoterpene–chalcone conjugate moieties whose linkage pattern is identical to the sumadain B.[1] So the monoterpene and chalcone units of **2** were determined to form a $C_{3'}$–$C_{1''}$ bond direct linkage and two ether bridges between C-7″ and C-4′, and C-3″ and C-2′. And the C–C bond direct linkage between C-3′ of the chalcone unit with C-1″ monoterpene unit was further confirmed by HMBC correlations between H-2″ (δ 2.27), and C-3′ (δ 108.6) (Fig. 2). The relative configuration of the pyran ring was established on basis of the coupling constants of H-1″ (δ 2.87, t, J = 2.0 Hz) and ROESY experiment (Fig. 2).[1] Thus, the structure of **2** was deduced as isorubraine, an isomer of rubraine (**1**).

Sumadain C (**3**)[5] was obtained as an orange needle crystal ($CHCl_3$) and gave a quasi-molecular ion $[M+H]^+$ at *m/z* 391.1890 (calcd 391.1904). The IR spectrum of **3** showed absorption bands at 3441

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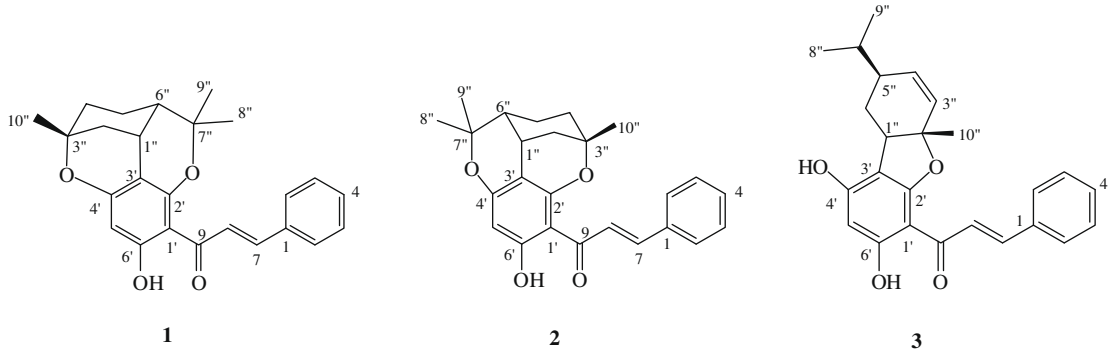

Figure 1. The structures of **1**–**3**.

Table 1
NMR data for **2** and **3** in $CDCl_3$[a,b]

	2		3	
	δ_H (mult, *J*, Hz)	δ_C	δ_H (mult, *J*, Hz)	δ_C
1		135.7		135.5
2/6	7.60 (m)	128.3	7.63 (m)	128.5
3/5	7.40 (m)	128.9	7.43 (m)	128.9
4	7.38 (m)	130.0	7.43 (m)	130.1
7	8.10 (d, 15.6)	127.5	8.14 (d, 15.6)	126.2
8	7.79 (d, 15.6)	141.9	7.82 (d, 15.6)	142.8
9		192.1		191.4
1′		106.9		103.1
2′		158.5		162.6
3′		106.9		107.4
4′		163.5		160.2
5′	6.07 (s)	98.8	5.91 (s)	96.2
6′		166.4		166.2
1″	2.87 (t, 2.0)	27.5	3.51 (t, 4.6)	43.8
2″α	2.27 (m)	34.8		89.1
2″β	1.91 (br d, 13.3)			
3″		76.9	5.71 (dd, 2.3, 10.3)	129.2
4″α	1.91 (br d, 13.3)	37.5	5.93 (dd, 5.9, 10.3)	135.3
4″β	1.51 (m)			
5″α	0.93 (m)	21.9	2.03 (m)	37.9
5″β	1.35 (m)			
6″α	2.06 (m)	45.9	2.39 (dt, 4.7, 13.5)	26.5
6″β			1.69 (m)	
7″		85.2	1.65 (m)	31.4
8″	1.56 (s)	24.6	0.95 (d, 2.5)	19.7
9″	1.12 (s)	29.5	0.93 (d, 2.5)	19.8
10″	1.55 (s)	29.0	1.68 (s)	26.1
6′-OH	14.07 (s)		13.87 (s)	

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

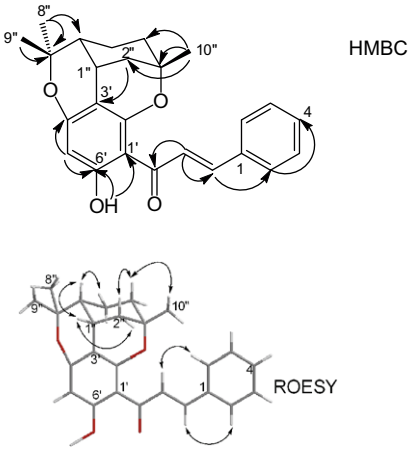

Figure 2. Selected 2D NMR correlations for isorubraine (**2**).

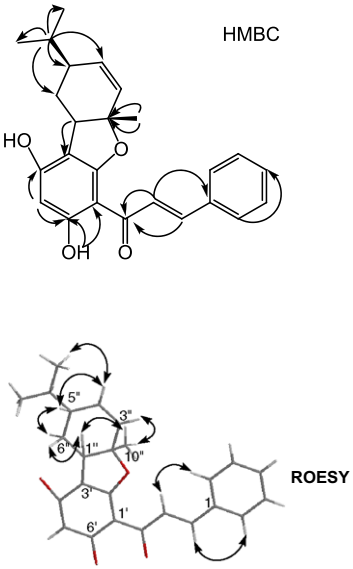

Figure 3. Selected 2D NMR correlations for **3**.

and 1632 cm^{-1}, indicating the presence of hydroxyl and conjugated carbonyl groups. The one ^1H, ^{13}C assignments (HSQC, HMBC, and ROESY spectra, Table 1) suggested that **3** also comprised an identical chalcone unit as 1. Besides the chalcone unit, two *cis*-olefinic protons (δ 5.71, 1H, dd, J = 2.3, 10.3 Hz; δ 5.93, 1H, dd, J = 5.9, 10.3 Hz); three methyl groups (δ 0.93, 3H, d, J = 2.5 Hz; δ 0.95, 3H, d, J = 2.5 Hz; δ 1.68, 3H, s), one methylene group (δ 2.36, 1H, dt, J = 4.7, 13.5 Hz; δ 1,69, 1H, m), three methine protons (δ 3.51, 1H, t, J = 2.0 Hz; δ 0.93, 1H, m; δ 1.65, 1H, m) were observed in ^1H NMR; And 10 carbon signals including two olefinic (δ 129.2, 135.3), an oxygenated quaternary carbon (δ 89.1) were found in ^{13}C NMR. These indicated the presence of a menthane monoterpene unit, which was further confirmed by HMBC correlations between Me-10″ with C-1″ and C-6″, H-7″ with C-8″, C-9″, C-4″ and C-8″, respectively (Fig 3). In addition, HMBC correlations between H-1″ and C-3′ indicated two moieties were connected via C-3′ of the chalcone unit to C-1″ monoterpene unit. Considering the 12 degrees of unsaturation of the identified structure, an extra degree of unsaturation strongly indicated that another cyclic moiety involved in the structure of **3**. However, 1D and 2D NMR spectra did not provide sufficient information to elucidate the pattern of connection. A single-crystal X-ray diffraction[6] of **3** was performed and demonstrated that the ether

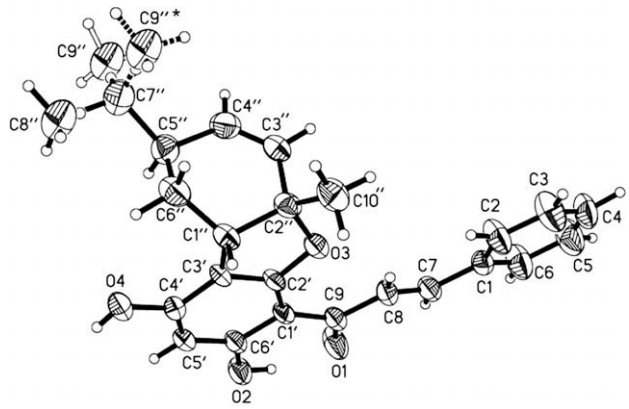

Figure 4. X-ray crystallographic structure of sumadain C (**3**), with the numbering of the atom (C9″* is disorder with C8″ and C9″).

bridge was present between C-2″ and C-2′ (Fig. 4), which is a new linkage pattern between monoterpene and chalcone.

The relative configuration of the furan ring was based on the coupling constants of H-1″ (δ 3.51, 1H, t, J = 4.6 Hz) and H-6″α (δ 2.39, 1H, dt, J = 4.7, 13.5 Hz), establishing equatorial H-1″ and H-6″α, and the ROESY correlations of H-1″/Me-10″ and H-6″α/H-5″ (Fig. 3). Single-crystal X-ray diffraction analysis confirmed the above conclusion (Fig. 4). The structure of **3** was thus established as depicted.

The isolated compounds **1**–**3** were evaluated for cytotoxic activities by MTT method[7] 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. The results indicated that compound **3** showed potent activity against the three cell lines, its IC_{50} values were 13.00, 15.93 and 12.78 μg/ml, respectively. Under the same test condition, the positive control (*cis*-platin) exhibited inhibitory activity against three cell lines with IC_{50} values 13.18, 31.91, and 16.39 μg/ml, respectively.

Acknowledgment

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4. Isorubraine (**2**). Orange powder ($CHCl_3$); mp: 197–200 °C, $[\alpha]_D^{13.6}$ 0 (c 0.06, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 348 (3.41), 240 (3.10); IR (KBr) υ_{max} 3426, 2924, 2853, 1629, 1122 cm^{-1}; NMR data, see Table 1; ESIMS m/z 389 $[M-H]^-$; HRESIMS m/z 391.1909 $[M+H]^+$ (calcd for $C_{25}H_{27}O_4$, 391.1904).
5. Sumadain C (**3**). Orange needle crystal ($CHCl_3$); mp: 210–213 °C, $[\alpha]_D^{13.6}$ +140 (c 0.07, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 340 (3.46), 240 (2.99); IR (KBr) υ_{max} 3441, 2923, 1630, 1551, 1147 cm^{-1}; NMR data, see Table 1; ESIMS m/z 389 $[M-H]^-$; HRESIMS m/z 391.1890 $[M+H]^+$ (calcd for $C_{25}H_{27}O_4$, 391.1904).
6. Crystallographic data for **3**: $C_{25}H_{26}O_4$, M = 390.46, monoclinic, space group $P2_1$, a = 13.3330(15) Å, b = 5.5574(5) Å, c = 15.310(2) Å, V = 1058.8(2) $Å^3$, Z = 2, D_c = 1.225 g/cm^3, crystal dimensions 0.70 × 0.43 × 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 5581, of which 3550 were unique and were 1983 observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0616, ωR_2 = 0.1237 for observed reflections, and R_1 = 0.1423, ωR_2 = 0.1399 for all reflections. The crystal structure (**3**) 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 721183). 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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