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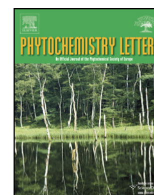

Prenylated chalcones from *Desmodium renifolium*

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ABSTRACT

Three new prenylated chalcones, renifolins A–C (**1**–**3**), together with seven known ones (**4**–**10**), were isolated from whole *Desmodium renifolium* plants. All of their structures were determined by spectroscopic methods including 1D and 2D NMR. Compounds **1** and **2** are the first naturally occurring chalcones possessing a 4-methylfuran-2(5*H*)-one unit. All of the isolates were evaluated for cytotoxicity using five tumor cell lines. Compounds **3**–**8**, and **10** exhibited moderate cytotoxicity against certain cell lines with IC_{50} values from 4.2 to 8.8 μM.

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1. Introduction

Prenylated flavonoids are excellent examples of natural products with important pharmaceutical activities (Marin and Manez, 2013; Yazaki et al., 2009), such as cytotoxic (Akihisa et al., 2012; Li et al., 2009), antioxidant (Dufall et al., 2003; Okoth et al., 2013; Ramli et al., 2013), anti-inflammatory (Peluso et al., 2010), antifungal (Ammar et al., 2013), anti-allergic (Quan et al., 2008) and other activities (Bourjot et al., 2010; Morel et al., 2013; Tala et al., 2013). *Desmodium renifolium* (Linn.) Schindl is a dwarf shrub belonging to the *Desmodium* genus of the Leguminosae family. Its range extends from India, Burma, Thailand, Vietnam, Laos, and Malaysia, to Indonesia and across the Pacific Islands (Editorial Committee of China Flora, 1955). The Dai people of Xishuangbanna prefecture in China's Yunnan province use it extensively as a diuretic, anti-inflammatory, and detoxifying agent (Zhao and Dao, 1981). Despite its uses in traditional medicine, no study on the secondary metabolites of *D. renifolium* has previously been presented in the literature. However, a range of bioactive compounds including flavonoids (Ma et al., 2011; Sasaki et al., 2012a,b), alkaloids (Ma et al., 2011), terpenoids (Ma et al., 2011), steroids (Ma et al., 2011) and phenylpropanoids (Ma et al., 2011) have been found in other plants from the *Desmodium* genus. Therefore, in our program aimed at identifying bioactive compounds with potential pharmaceutical applications from local plants, we investigated the secondary metabolites produced by *D. renifolium* plants collected in Xishuangbanna prefecture. Three new (**1**–**3**) and seven known (**4**–**10**) prenylated chalcones were isolated in this work (Fig. 1). Compounds **1** and **2** are the first known naturally occurring chalcones that feature a 4-methylfuran-2(5*H*)-one unit. This paper describes the elucidation of the structures of compounds **1**–**10** and a preliminary evaluation of their biological activity.

2. Results and discussion

Compound **1** was obtained as a pale yellow gum. Its HRESIMIS data suggests that it has the molecular formula $C_{21}H_{18}O_7$ ($[M+Na]^+$ m/z 405.0958; 405.0950 calcd. for $C_{21}H_{18}NaO_7$), with 13 degrees of unsaturation. Its ^{1}H NMR spectrum (Table 1) indicates the presence of an ABX system [δ_H 6.42 (1H, dd, 8.6, 2.2 Hz), 7.84 (1H, d, 8.6 Hz), and 6.34 (1H, d; 2.2 Hz)], as well as a 1,3,4,5-tetrasubstituted benzene ring [δ_H 6.78 (1H, d, 2.2 Hz) and 7.00 (1H, d, 2.2 Hz)]. In addition, the spectrum showed resonances due to an AB spin system at δ_H 7.70 (1H, d, 15.1 Hz, H-α) and 8.06

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Table 1
^{13}C NMR and ^{1}H data of compounds **1**–**3**.

No.	**1**[a]		**2**[a]		**3**[b]	
	δ_C	δ_H (m, *J*, Hz)	δ_C	δ_H (m, *J*, Hz)	δ_C	δ_H (m, *J*, Hz)
1	128.5 s		128.2 s		126.8 s	
2	111.6 d	6.78, d (2.2)	111.2 d	6.70, d (2.2)	113.9 d	6.79, d 2.2
3	145.9 s		145.6 s		144.0 s	
4	148.5 s		148.7 s		150.2 s	
5	122.7 s		122.1 s		129.5 s	
6	116.3 d	7.00, d (2.2)	116.9 d	6.96, d (2.2)	120.0 d	6.98 d (2.2)
1′	115.3 s		129.1 s		114.4 s	
2′	166.8 s		130.2 d	7.88, d (8.7)	167.6 s	
3′	104.2 d	6.34, d (2.2)	115.4 d	6.83, d (8.7)	103.7 d	6.37, d (2.2)
4′	165.4 s		162.2 s		165.5 s	
5′	109.6 d	6.42, dd (8.6, 2.2)	115.4 d	6.83, d (8.7)	108.7 d	6.46, dd (8.8, 2.2)
6′	133.2 d	7.84, d (8.6)	130.2 d	7.88, d (8.7)	133.2 d	8.08, d (8.8)
1″	119.7 s		119.2 s		25.5 t	3.61, d (6.8)
2″	162.4 s		162.5 s		123.9 d	5.13, t (6.8)
3″	74.2 t	4.74, br s 4.79, br s	75.0 t	4.70, t (0.6) 4.75, t (0.6)	131.8 s	
4″	172.9 s		172.6 s		18.1 q	1.65, s
5″	16.0 q	2.19, s	15.5 q	2.15, s	25.9 q	1.85, s
α	121.5 d	7.70, d (15.1)	121.3 d	7.63, d (15.4)	119.3 d	7.65, d (15.2)
β	144.0 d	8.06, d (15.1)	143.9 d	7.99, d (15.4)	143.3 d	8.18, d (15.2)
C=O	193.0 s		192.2 s		192.8 s	
–OMe-4	60.8 q	3.82, s	60.8 q	3.82, s	60.0 q	3.82, s
–OMe-4′			55.8 q	3.78, s		
–OH-4						13.02, s
–OH-2′						13.32, s
–OH-4′						13.60, s

[a] Recorded at 400 MHz, methanol-d_4.
[b] Recorded at 500 MHz, acetone-d_6.

(1H, d, 15.1 Hz, H-β) together with one methoxyl group at δ_H 3.82 (3H, s) and one methyl at δ_H 2.19 (3H, s). The ^{13}C and DEPT NMR data (Table 1) for compound **1** indicate that its chalcone skeleton has the same substitution pattern as abyssinone D (Cui et al., 2008). The biggest difference between the two compounds is that in **1**, the prenyl group of abyssinone D is replaced by a 4-methylfuran-2(5*H*)-one unit (Imai et al., 1989) [δ_C 119.7 (C-1″), 162.4 (C-2″), 74.2 (C-3″), 172.9 (C-4″), and 16.0 (C-5″)]. The presence of the 4-methylfuran-2(5*H*)-one unit was confirmed by the observation of HMBC correlations from H_3-5″ to C-1″, C-2″, and C-3″, and from H_2-3″ to C-1″, C-2″, C-4″ and C-5″. The HMBC spectrum Fig. 2 of **1** showed correlations from the ABX spin system H-5′ (δ_H 6.42) to C-1′, C-3′, C-4′, and C-6′; H-6′ (δ_H 7.84) to C-1′, C-2′, C-4′, C-5′, and the carbonyl carbon (δ_C 193.0); and H-3′ (δ_H 6.34) to C-1′, C-2′, C-4′, and C-5′. Furthermore, the AB spin system of H-α showed HMBC correlations with C-1, C-1′, C-β, and the carbonyl carbon (δ_C 193.0), and of H-β showed HMBC correlations with C-1, C-2, C-6, C-α, and the carbonyl carbon (δ_C 193.0). Other HMBC correlations were

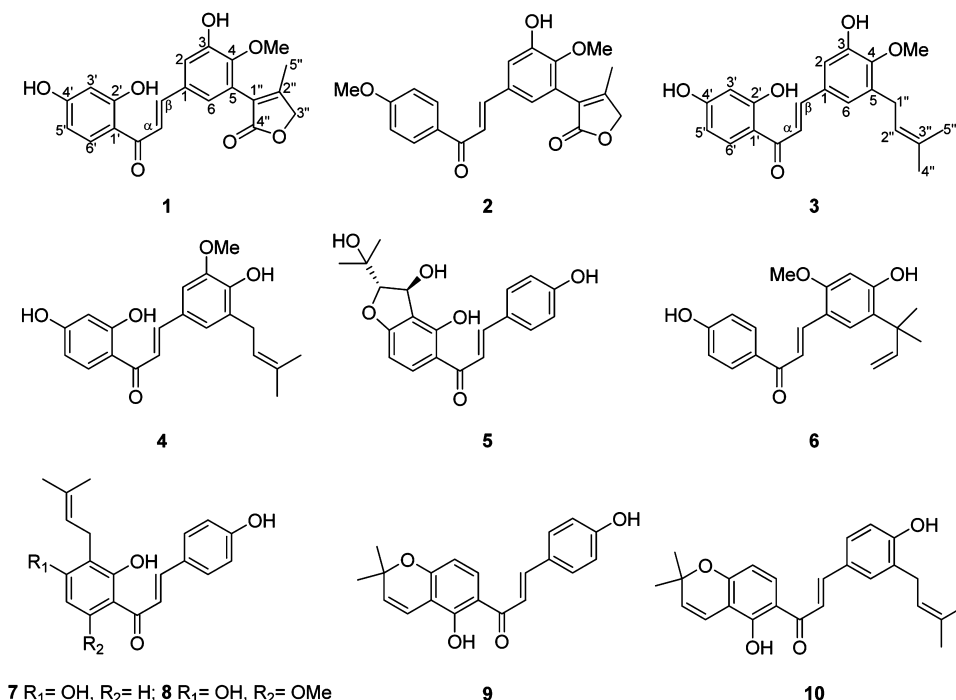

Fig. 1. The structures of chalcones isolated from *D. renifolium*.

noted between H-2 (δ_H 6.78) and C-1, C-3, C-4, C-6, and C-β and between H-6 (δ_H 7.00) and C-1, C-2, C-4, C-5, and C-β. The key HMBC correlation from the methoxyl group at δ_H 3.82 to C-4 (δ_C 148.5) assigned the position of the 4-MeO, and the location of the 4-methylfuran-2(5*H*)-one unit at C-5 was supported by the long range HMBC correlation from H-3″ to C-5. Compound **1** was thus assigned the structure shown in Fig. 1 and named renifolin A.

Compound **2** was isolated as a pale yellow gum with a molecular formula of $C_{22}H_{20}O_6$ and 13 degrees of unsaturation based on HRESIMS data ([M+Na]$^+$, *m/z* 403.1154; 403.1158 calcd. for $C_{22}H_{20}NaO_6$). The ^{13}C NMR and DEPT data for compound **2** indicate that like compound **1**, it has eleven quaternary carbons, six aromatic methines, two methines, one methylene, and two methyl groups (Table 1). The only difference between **1** and **2** is that the A ring of the latter is 1,4-disubstituted. This is demonstrated by the observation of an AABB-type spin system in the aromatic region of its ^{1}H NMR spectrum [δ_H 6.83 (2H, d, 8.7 Hz) and 7.88 (2H, d, 8.7 Hz)]. The position of 4-MeO and 4′-MeO were supported by the HMBC correlations of MeO-4 (δ_H 3.82) with C-4 (δ_C 148.7) and of MeO-4′ (δ_H 3.78) with C-4′ (δ_C 162.2), respectively. In addition, location of the 4-methylfuran-2(5*H*)-one unit at C-5 was assigned by the long range HMBC correlations of H-6 (δ_H 6.96) with C-1″ (δ_C 119.2). Compound **2** was therefore assigned the structure shown in Fig. 1 and named renifolin B. To the best of our knowledge, compounds **1** and **2** are the first identified naturally occurring chalcones that feature a 4-methylfuran-2(5*H*)-one unit.

Compound **3** was obtained as a pale yellow gum with the molecular formula $C_{21}H_{22}O_5$ as determined by HRESIMS ([M+Na]$^+$ *m/z* 377.1348; 377.1365 calcd. for $C_{21}H_{22}NaO_5$). Its IR spectrum reveals the presence of a hydroxyl group (3418 cm^{-1}), a conjugated ketone (1682 cm^{-1}), and an aromatic ring (1604, 1526 cm^{-1}). Its ^{1}H NMR spectrum indicates that it contains a 1,2,4-trisubstituted benzene ring [δ_H 6.37 (1H, d, 2.2 Hz), 6.46 (1H, dd, 8.8, 2.2 Hz), and 8.08 (1H, d, 8.8 Hz)], a prenyl group [δ_H 3.61 (2H, d, 6.8 Hz), 5.13 (1H, d, 6.8 Hz), and 1.65, 1.85 (each 3H, s)], a 1,3,4,5-tetrasubstituted benzene ring [δ_H 6.79 (1H, d, 2.2 Hz) and 6.98 (1H, d, 2.2 Hz)], and a double bond of the chalcones [δ_H 7.65 (1H, d, 15.2 Hz, H-α), 8.18 (1H, d, 15.2 Hz, H-β)]. The ^{13}C NMR spectrum of **3** contains 21 signals, representing two benzene rings, a prenyl group, a disubstituted alkene, a methoxy group and a carbonyl group. These findings suggest that compound **3** is a prenylated chalcone derivative. Its NMR data (Table 1) are very similar to those for 5′-prenylbutein (Yenesew et al., 2004): the two compounds only differ in that **3** has a methoxy group at the C-4 position while 5′-prenylbutein has a hydroxyl group there. The location of the methoxy group at C-4 was confirmed by HMBC correlations Fig. 2 between the 4-OMe group (δ_H 3.82, s) and C-4 (δ_C 150.2). The position of isopentyl group at C-5 was supported by the HMBC correlations of H-6 (δ_H 6.98) with C-1″ (δ_C 25.5) and of H-1″ (δ_H 3.61) with C-4, C-5, and C-6. Compound **3** was therefore assigned the structure shown in Fig. 1 and named renifolin C (**3**).

Table 2
The cytotoxicity data for the compounds **1**–**10** (μM).

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	>10	>10	>10	>10	>10
2	>10	>10	>10	>10	>10
3	6.4	>10	>10	8.5	>10
4	>10	>10	7.9	>10	8.8
5	6.8	>10	8.2	7.0	>10
6	4.2	8.5	6.8	>10	8.5
7	>10	9.4	>10	8.2	>10
8	8.2	>10	6.8	>10	7.8
9	>10	>10	>10	>10	>10
10	6.2	7.5	>10	>10	8.8
Paclitaxel	0.03	0.02	0.2	0.2	0.1

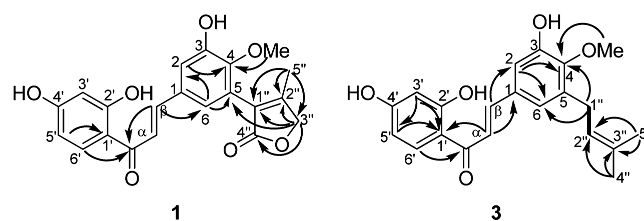

Fig. 2. Selected HMBC correlations of compounds **1** and **3**.

Many chalcone derivatives are known to be cytotoxic (Bandgar et al., 2012; Champelovier et al., 2011; Lee et al., 2007; Wu et al., 2011). Compounds **1–10** were therefore tested to determine their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) using the MTT method, using paclitaxel as a positive control (Mosmann, 1983). The results obtained are shown in Table 2. Compounds **3–8**, and **10** exhibited moderate cytotoxicity against certain cell lines with IC_{50} values in the range of 4.2–8.8 μM.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were acquired using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used to acquire IR spectra. 1D- and 2D NMR spectroscopic data were recorded on Bruker DRX-500 or DRX-400 spectrometers, using TMS as an internal standard. Chemical shifts (δ) are expressed in ppm relative to the TMS signal. HRESIMS analyses were performed using a VG Autospec-3000 mass spectrometer. Semi-preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma–Aldrich, Inc, USA), or MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC; individual TLC plates were visualized by spraying with 5% H_2SO_4 in EtOH and heating.

3.2. Plant material

Whole *D. renifolium* (Linn.) Schindl., plants were purchased from the Dai Minority Hospital in Xishuangbanna Prefecture, which was collected from Xishuangbanna Prefecture in Yunnan province during September of 2012. The species to which the samples belonged was determined by Prof. Yuan Ning. A voucher specimen (YNNI 12-9-36) was deposited in the herbarium of the Yunnan University of Nationalities.

3.3. Extraction and isolation

The combined samples (8.5 kg) were crushed and then filtered through a 30 mesh sieve. The resulting powder was extracted with 70% aqueous acetone (4× 10 L) at room temperature and then filtered again. The filtrate was evaporated under reduced pressure, and the crude extract (756 g) was passed through a silica gel (150–200 mesh) column, eluting with a $CHCl_3$–MeOH gradient (9:1, 8:2, 7:3, 6:4, 5:5) to afford six fractions (A–E). Further separation of fraction A (42.1 g) by silica gel column chromatography, eluting with petroleum ether–acetone (9:1–1:2), yielded subfractions A1–A6. Subfraction A1 (6.89 g) was loaded onto another silica gel

column and eluted using petroleum ether–EtOAc, followed by preparative HPLC (78% MeOH–H_2O, flow rate 12 mL/min) to obtain a pure sample of **10** (16.4 mg). Subfraction A2 (8.45 g) was separated on a silica gel column, eluting with petroleum ether–EtOAc, followed by preparative HPLC (65–70% MeOH–H_2O, flow rate 12 mL/min) to give **3** (10.4 mg), **4** (8.6 mg), **1** (12.6 mg), **2** (14.6 mg), **6** (18.2 mg), and **9** (18.2 mg). Subfraction A3 (6.5 g) was separated on a silica gel column, eluting with petroleum ether–EtOAc, followed by preparative HPLC (58–65% MeOH–H_2O, flow rate 12 mL/min) to give **5** (13.7), **7** (25.9), and **8** (22.4 mg).

3.4. Spectroscopic data

Renifolin A (**1**): was obtained as a pale yellow gum: UV (MeOH) λ_{max} (log ε) 210 (4.21), 256 (3.57), 368 (3.92) nm; IR (KBr): ν_{max} 3408, 3129, 3067, 2963, 2854, 1742, 1682, 1600, 1526, 1460, 1339, 1178, 1057, 890, 752 cm^{-1}; ^1H and ^{13}C NMR data (400 and 100 MHz, methanol-d_4), see Table 1; Positive ESIMS m/z 405 $[M+Na]^+$; Positive HRESIMS m/z 405.0958 $[M+Na]^+$ ($C_{21}H_{18}NaO_7$, calcd for: 405.0950).

Renifolin B (**2**): was obtained as a pale yellow gum: UV (MeOH) λ_{max} (log ε) 210 (4.14), 252 (3.38), 362 (3.87) nm; IR (KBr): ν_{max} 3410, 3126, 3065, 2967, 2850, 1745, 1680, 1602, 1531, 1458, 1337, 1175, 1054, 896, 761 cm^{-1}; ^1H and ^{13}C NMR data (400 and 100 MHz, methanol-d_4), see Table 1; Positive ESIMS m/z 405 $[M+Na]^+$; Positive HRESIMS m/z 403.1154 $[M+Na]^+$ ($C_{22}H_{20}NaO_6$, calcd for: 403.1158).

Renifolin C (**3**): was obtained as a pale yellow gum: UV (MeOH) λ_{max} (log ε) 210 (4.06), 248 (3.36), 356 (3.62) nm; IR (KBr): ν_{max} 3418, 3142, 3079, 2953, 2836, 1682, 1604, 1526, 1463, 1332, 1185, 1068, 891, 736 cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz, acetone-d_6), see Table 1; Positive ESIMS m/z 377 $[M+Na]^+$; Positive HRESIMS m/z 377.1348 $[M+Na]^+$ ($C_{21}H_{22}NaO_5$, calcd for: 377.1365).

3.5. The cytotoxicity assay

The IC_{50} values of the isolated compounds were measured using the MTT assay. The MTT assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole), converting it into an insoluble purple formazan compound. All cells were cultured in MEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO). All the cells were used within passage 5–6. In the assay, 2500 cells suspended in 100 μL MEM medium were seeded in each well of a 96-well plate and incubated for 24 h. The old medium in each well was then replaced with fresh medium containing various concentrations of each compound. The compound concentrations ranged from 100 μM to 1.5625 μM, which was achieved by doing twofold dilutions six times. The OD_{595} values of untreated control wells at 0 h and 72 h and of treated wells at 72 h were measured using a plate reader. The IC_{50} of each compound was then calculated as the concentration required to achieve 50% inhibition of cell growth.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2014.04.003.

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