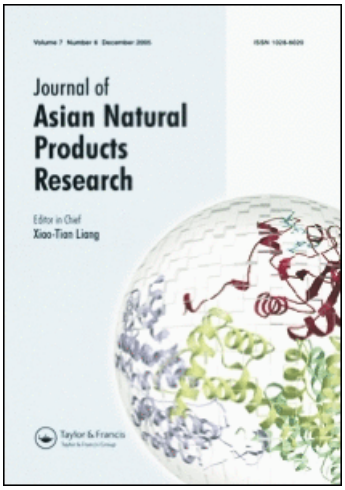

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Two new bioactive prenylated dihydroflavanoids from the medicinal plant *Dolichos tenuicaulis* (Baker) Craib

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Two new prenylated dihydroflavanoids have been isolated from the medicinal plant of *Dolichos tenuicaulis* (Baker) Craib. Their structures were elucidated as (2*S*)-5,2′,6′-trihydroxy-8-prenyl-6,7-(3-prenyl-2,2-dimethylpyrano)-3′,4′-(2,2-dimethyl-1-keone-cyclohexadiene)-flavanone (**1**) and (2*S*)-5,2′,6′-trihydroxy-8-prenyl-6,7-(3-prenyl-2,2-dimethyl-1-keone-cyclohexadiene)-flavanone (**2**) on the basis of spectroscopic analysis.

Keywords: *Dolichos tenuicaulis* (Baker) Craib; Prenylated flavanoid; Dihydroflava-noid; Anti-cancer activity

1. Introduction

Dolichos tenuicaulis (Baker) Craib (*Damayao* in Chinese) is mainly distributed in Guangxi, Guizhou, and Yunnan provinces and used in folk medicine as an anti-cancer, anti-virus, and anti-inflammation agent [1]. However, a literature search did not yield any references on the studies of chemical constituents from this plant. In order to look for some bioactive components, a systematic study on the chemical constituents of *D. tenuicaulis* has been undertaken. In our recent research, several compounds have been purified from the plant. Herein, we report the isolation, structure elucidation and anti-cancer activities of the two new prenylated dihydroflavanoids **1** and **2** (see figure 1) from the 80% aqueous ethanolic extract of the roots of *D. tenuicaulis*.

2. Results and discussion

Compound **1** was isolated as white powder. ESI-MS gave the pseudo-molecular ion peak at *m/z* 583.2 $[M - H]^-$. Its molecular formula was determined as $C_{36}H_{40}O_7$ by HREI-MS *m/z*

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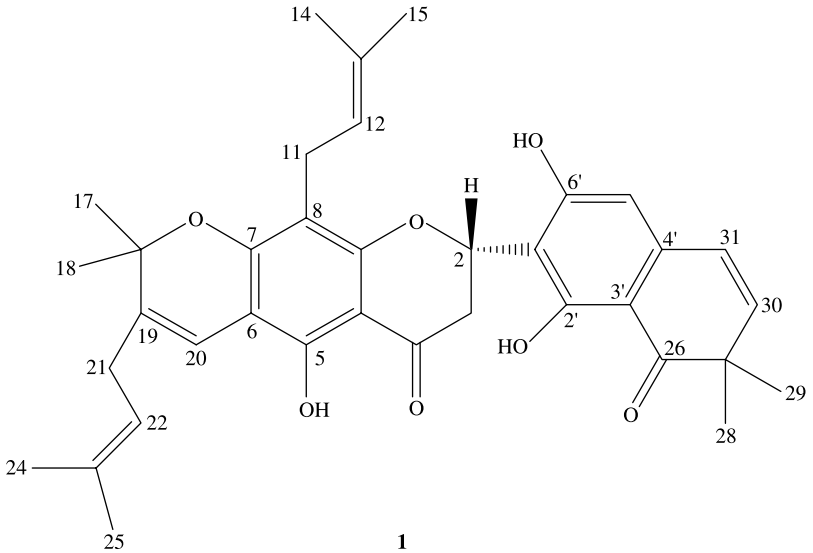

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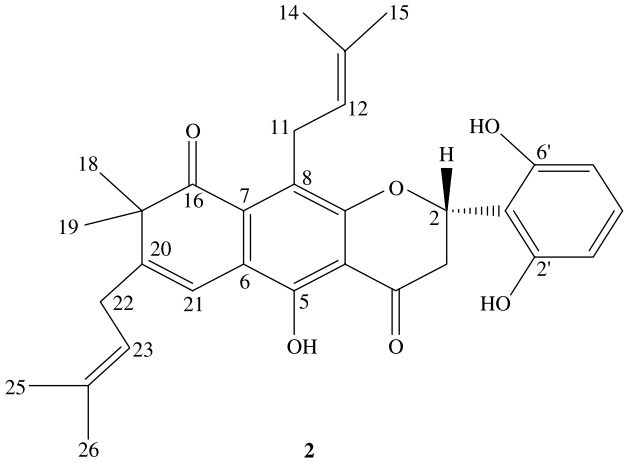

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Figure 1. Structures of compounds **1** and **2**.

584.2148 $[M]^+$. ^{13}C and DEPT NMR experiments of **1** showed eight methyls, three methylenes, seven methines and 18 quaternary carbons. The IR spectrum indicated the presence of hydroxyl (3414 cm^{-1}), conjugated carbonyl (1660 cm^{-1}), and aromatic (1608 and 1468 cm^{-1}) groups. The UV spectrum exhibited absorption maxima at 340, 294, 220 nm. The dihydroflavanone skeleton of **1** was deduced from the ^{13}C NMR signals, namely, an oxymethine, a carbonyl group and a methylene at δ 71.3 (C-2), 39.4 (C-3), and 198.1 (C-4), respectively, and the corresponding proton signals at δ 2.47 (dd, $J = 17.0$, 4.0 Hz, H-3e), 3.91 (dd, $J = 17.0$, 14.0 Hz, H-3a) and 5.81 (dd, $J = 14.0$, 4.0 Hz, H-2) [2]. The ^1H NMR signal at δ 12.27 was characteristic of 5-OH, and the proton signals at δ 3.17 (2H, d, $J = 7.0$ Hz, H-21), 5.38 (1H, m, H-22), 1.50 (3H, s, H-24), 1.64 (3H, s, H-25) and the corresponding carbon signals at δ 28.2 (C-21), 122.6 (C-22), 130.2 (C-23), 18.8 (C-24),

25.6 (C-25) were assigned to one γ,γ-dimethylallyl group. Another γ, γ-dimethylallyl group was deduced by the ^{1}H NMR signals at δ 3.13 (2H, d, $J = 8.0$ Hz, H-11), 5.14 (1H, m, H-12), 1.57 (3H, s, H-14), 1.42 (3H, s, H-15) and ^{13}C NMR signals at δ 21.5 (C-11), 122.8 (C-12), 129.8 (C-13), 18.5 (C-14), 24.8 (C-15). The dimethylchromene ring was elucidated by the proton signals at δ 1.35, 1.38 (s, each 3H, $CH_3 \times 2$), 6.15 (1H, brs, H-20), and the carbon signals at δ 77.6 (C-16), 25.8 (C-17), 26.4 (C-18), 139.1 (C-19), 112.5 (C-20), 103.5 (C-6), and 159.4 (C-7). A dimethyl-cyclohexadiene-ketone structure was deduced by the ^{1}H NMR signals at δ 1.41 (3H, s, 28-Me), 1.47 (3H, s, 29-Me), 6.35 (1H, d, $J = 10.0$ Hz, H-30), 6.11 (1H, d, $J = 10.0$ Hz, H-31), and ^{13}C NMR signals at δ 199.4 (C-26), 45.8 (C-27), 21.4 (C-28), 23.7 (C-29), 131.6 (C-30), 119.3 (C-31), 107.5 (C-3′), and 138.7 (C-4′). The ^{1}H NMR signal at δ 6.78 (1H, brs, H-5′) was attributed to the substituted aromatic ring. These data suggested that compound **1** has a flavanone skeleton with three hydroxyl groups, a dimethyl-cyclohexadiene-ketone moiety, one dimethylchromene ring, and two γ,γ-dimethylallyl groups. In the HMBC experiments, a singlet proton signal at δ 12.27 was correlated with C-5 signal at δ 157.1, and one set of doublet proton signals at δ 3.13 (2H, d, $J = 8.0$ Hz, H-11) was correlated with C-7 at δ 159.4 and C-9 at 157.2, and the other set of doublet proton signals at δ 3.17 (2H, d, $J = 7.0$ Hz, H-21) was correlated with C-16 at δ 77.6 and C-20 at 112.5, indicating that the two prenyl groups were attached at C-8 and C-19, respectively. Confirmation of the location of dimethylchromene ring at positions 6 and 7 was obtained from HMBC correlations between H-20 (δ 6.15) with C-5, C-6 and C-7, and the connection of the dimethyl-cyclohex-adiene-ketone unit to C-3′ and C-4′ was established by the long−range ^{1}H−^{1}C correlations between H-5′ (δ 6.78) with C-31 (δ 119.3), H-30 (δ 6.35) with C-4′ (δ 138.7) and C-31 (δ 119.3), and H-31 (δ 6.11) with C-5′ (δ 104.3), respectively. The coupling constant $J_{\text{H-2/H-3}}$ 14.0 Hz, indicating the axial-axial coupling, revealed H-2 is axial and the ring B equatorial. The chiral centre C-2 was assigned as S-configuration on the basis of its negative optical rotation, $[\alpha]_D^{25}$ −120 (*c* 0.80, CH_3OH) [3]. All ^{1}H and ^{13}C assignments (shown in table 1) for compound **1** were performed by ^{1}H−^{1}H COSY, HMQC and HMBC experiments (key HMBC and NOESY correlations are shown in figure 2). Thus the structure of **1** was determined to be (2*S*)-5,2′,6′-trihydroxy-8-prenyl-6,7-(3-prenyl-2,2-dimethylpyrano)-3′,4′-(2,2-dimethyl-1-keone-cyclohexadiene)-flavanone.

Compound **2** was isolated as white powder. Its molecular formula was determined as $C_{31}H_{34}O_6$ by HREI-MS at *m*/*z* 502.2456. ^{13}C and DEPT experiments showed six methyls, three methylenes, seven methines and fifteen quaternary carbons. The IR spectrum of compound **2** indicated the presence of hydroxyl (3432 cm^{-1}), conjugated carbonyl (1665 cm^{-1}), and aromatic (1618 and 1474 cm^{-1}) groups. The UV spectrum exhibited absorption maxima at 338, 294, 228 nm. In compound **2**, the dihydro-flavanone skeleton, two γ,γ-dimethylallyl groups and their linkage (C-8 and C-20), 5-OH, one dimethyl-cyclohexadiene-ketone moiety, and the configuration of the chiral centre C-2 (S-configuration) were confirmed, similar to compound **1**. Except for the signals assignable to the above structure moieties, the proton signals at δ 6.37 (d, 2H, $J = 8.0$ Hz, H-3′, 5′) and 6.94 (t, 1H, $J = 8.0$ Hz, H-4′) were attributed to three adjacent protons on an aromatic ring. The linkage of dimethyl-cyclohexadiene-ketone group was C-6 and C-7 according to the HMBC correlations between H-21 with C-5, C-6 and C-7. Furthermore, there was no dimethylchromene ring moiety in the structure of compound **2**, different from compound **1**.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compounds **1** and **2** in DMSO-d_6.

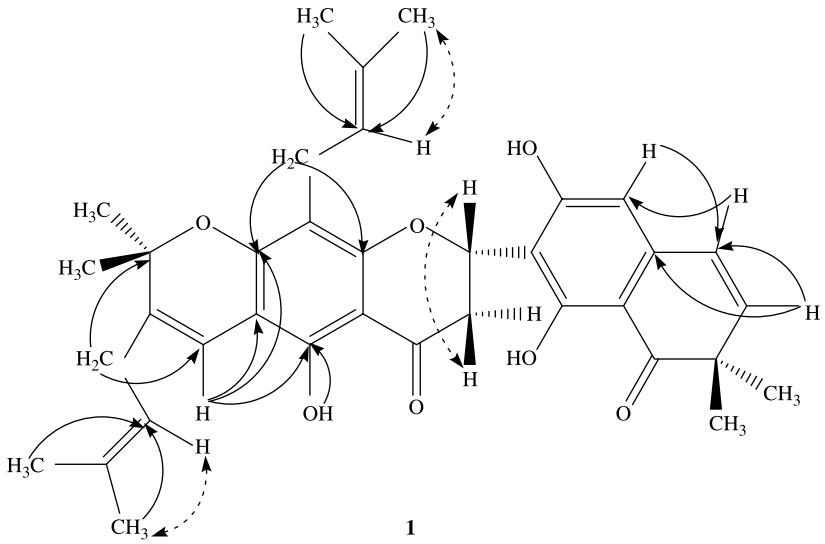
	1			***2***	
Position	δ_C	δ_H *(J in Hz)*	*Position*	δ_C	δ_H *(J in Hz)*
2	71.3	5.81 *dd* (14.0, 4.0)	2	71.6	5.76 *dd* (13.0, 3.0)
3	39.4	2.47 *dd* (17.0, 4.0)	3	39.1	2.43 *dd* (17.0, 3.0)
		3.91 *dd* (17.0, 14.0)			3.87 *dd* (17.0, 13.0)
4	198.1	–	4	198.4	–
5	157.1	12.27 *brs*	5	158.6	12.41 *brs*
6	103.5	–	6	111.8	–
7	159.4	–	7	139.4	–
8	104.6	–	8	125.3	–
9	157.2	–	9	159.6	–
10	102.1	–	10	113.2	–
11	21.5	3.13 *d* (2H, 8.0)	11	25.2	3.18 *d* (2H, 7.0)
12	122.8	5.14 *m*	12	124.2	5.36 *brs*
13	129.8	–	13	131.7	–
14	18.5	1.57 *s*	14	19.4	1.71 *s*
15	24.8	1.42 *s*	15	26.4	1.64 *s*
16	77.6	–	16	199.2	–
17	25.8	1.35 *s*	17	48.5	–
18	26.4	1.38 *s*	18	22.4	1.38 *s*
19	139.1	–	19	24.6	1.43 *s*
20	112.5	6.15 *brs*	20	139.1	–
21	28.2	3.17 *d* (2H, 7.0)	21	112.4	6.12 *brs*
22	122.6	5.38 *m*	22	28.7	3.12 *d* (2H, 8.0)
23	130.2	–	23	122.1	5.34 *brs*
24	18.8	1.50 *s*	24	131.2	–
25	25.6	1.64 *s*	25	18.4	1.51 *s*
26	199.4	–	26	25.2	1.68 *s*
27	45.8	–	1′	110.4	–
28	21.4	1.41 *s*	2′, 6′	157.2	–
29	23.7	1.47 *s*	3′, 5′	107.4	6.37 *d* (8.0)
30	131.6	6.35 *d* (10.0)	4′	129.6	6.94 *t* (8.0)
31	119.3	6.11 *d* (10.0)			
1′	114.2	–			
2′	159.4	–			
3′	107.5	–			
4′	138.7	–			
5′	104.3	6.78 *brs*			
6′	160.5	–			

Thus compound **2** was determined to be (2*S*)-5,2′,6′-trihydroxy-8-prenyl-6,7-(3-prenyl-2,2-dimethyl-1-keone-cyclohexadiene)-flavanone.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured using a JASCOP-1020 polarimeter. UV spectra were obtained with a Shimadzu UV 210A UV–Vis recording spectrophotometer. IR spectra were recorded on a Hitachi 275-50 spectrophotometer. The analytical HPLC was performed on a Shimadzu Pak with LC–10AT pump, SPD–10A UV–Vis detector using a Lichrospher C_{18} (150 × 4.6 mm) column, and the preparative HPLC system was composed of a 515 pump (Waters), 2487 detector (Waters), and a Lichrospher C_{18} (200 × 10 mm) column. Column chromatography was carried out on silica gel H60 (Qingdao Haiyang Chemical Group



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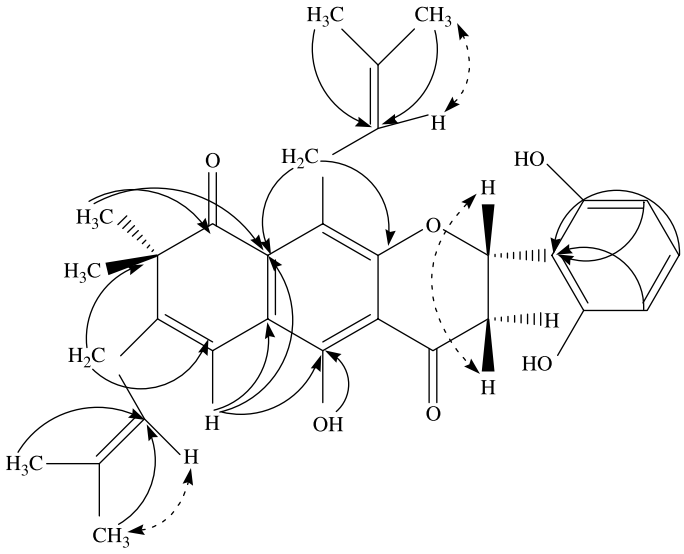

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Figure 2. Key HMBC and NOESY correlations of compounds **1** and **2**.

Corporation, Qingdao, China). NMR spectra were run on a Bruker AVANCE 500 NMR spectrometer (500 MHz for ^{1}H, and 125 MHz for ^{13}C) with TMS as internal standard. ESI-MS were performed on a Varian 1200 L mass spectrometer and HREI-MS were obtained on a Finnigan MAT 711 mass spectrometer.

3.2 Plant material

The roots of *Dolichos tenuicaulis* were purchased in July 2003 from a local drug store, Kunming, Yunnan, China, and identified by Professor Lu-Ping Qin (the Second Military

Medical University, Shanghai, China). A voucher sample (ZJU DMY0301) is deposited in the Herbarium of School of Pharmacy, Zhejiang University.

3.3 Extraction and isolation

Before extraction, the roots of *D. tenuicaulis* were ground into powder, and 4.0 kg powders were weighted and refluxed with 80% aqueous ethanol for 2 times. After evaporation of EtOH *in vacuo*, the residue (547 g) was diluted in water and extracted with chloroform, ethyl acetate, and *n*-butanol, respectively. The chloroform extract (78 g) was subjected to column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0 → 1:1) to produce 8 fractions. Fraction 2 (12.5 g) was further chromatographed on silica gel eluted with $CHCl_3$/MeOH (100:0 → 20:1) to afford 10 subfractions. Subfractions 4 (680 mg) and 6 (550 mg) were subjected to preparative HPLC separation to afford compounds **1** (56 mg) and **2** (48 mg).

3.3.1 Compound 1. was obtained as white powder; $[\alpha]_D^{25}$ −120 (*c* 0.80, CH_3OH); UV (MeOH) λ_{max} (nm): 338, 294, 228; IR (KBr) ν_{max} (cm^{-1}): 3414 (OH), 1660 (C═O), 1608, 1468, 1380, 1245; ^1H NMR and ^{13}C NMR data: see table 1; ESI-MS *m/z*: 583.2 $[M - H]^-$; HREI-MS *m/z* 584.2148 $[M]^+$ (calcd for $C_{36}H_{40}O_7$, 584.2142).

3.3.2 Compound 2. was obtained as white powder; $[\alpha]_D^{25}$ −150 (c 1.00, CH_3OH); UV (MeOH) λ_{max} (nm): 340, 294, 220; IR (KBr) ν_{max} (cm^{-1}): 3432 (OH), 1665 (C═O), 1618, 1474, 1375, 1278; ^1H NMR and ^{13}C NMR data: see table 1; ESI-MS *m/z*: 501.2 $[M - H]^-$; HREI-MS *m/z* 502.2456 $[M]^+$ (calcd for $C_{31}H_{34}O_6$, 502.2451).

3.4 Anti-cancer activity assay in vitro

MTT assay was performed as described in the literature [4]. In the assay, six kinds of typical human cancer cell lines, including human lung cancer cell A549, human liver cancer cell BEL-7402, human esophageal cancer cell HT-29, human breast cancer cell MCF-7, human leukemia cancer cell K562, and human kidney cancer cell A498, were selected to evaluate the cytotoxic activities of **1** and **2**. The results are reported in table 2, and the IC_{50} values ($IC_{50} < 4.0$ μg/ml) indicated that two compounds exhibited significant activities, especially to A549 and K562 cancer cells ($IC_{50} < 1.5$ μg/ml).

Table 2. Inhibition of compounds **1** and **2** on the growth of human cancer cells (IC_{50}, μg/ml).

	Cancer cells					
Compound	*A549*	*BEL-7402*	*HT-29*	*MCF-7*	*K562*	*A498*
1	0.45	1.10	2.32	1.42	0.84	1.24
2	1.36	2.54	3.56	3.61	1.28	2.68

Acknowledgements

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