Four Coumarins from *Heracleum yunngningense*

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Received January 13, 2005; accepted March 1, 2005

Four new coumarins (1—4) were isolated from the roots of *Heracleum yunngningense* HAND.–MASS. Their structures were established by spectral analyses.

Key words *Heracleum yunngningense*; Umbelliferae; coumarin

The roots of *Heracleum yunngningense* HAND.–MASS., "永寧独活", is a Chinese folk medicine used as an antipyretic, analgesic, and diaphoretic agent in local areas of Yunnan province, China.[1,2] In the course of our studies of the chemical constituents of Umbelliferous plants, we have isolated four new coumarins, yunngnin A (**1**), yunngnin B (**2**), yunngnoside A (**3**), and yunngnoside B (**4**), in addition to a polyacetylen, falcarindiol, two phenylpropanoids, ferulic acid, *p*-hydroxyphenethyl ferulate and fourteen Coumarins, imperatorin, phellopterin, moellendorffiline,[3] xanthotoxin, umbelliferone, unbelliprenine, vaginidiol,[4] (+)-heraclenol, 8-geranyloxypsoralen, apterin, heratomol-6-*O*-β-D-glucopyranoside,[5] isofraxidin, scopoletin and hermandiol (Fig. 1). This paper is concerned with the structural elucidation of compounds **1**—**4**.

The roots of *H. yunngningense*, collected from a mountainous area of Yunnan Province, China, were extracted five times with EtOAc and MeOH, successively. The EtOAc extract was subjected to a combination of silica gel and Sephadex LH-20 chromatography in the various solvent systems to afford compounds **1** and **2**. The MeOH extract was subjected to a combination of silica gel, ODS and Sephadex LH-20 to give compounds **3** and **4**.

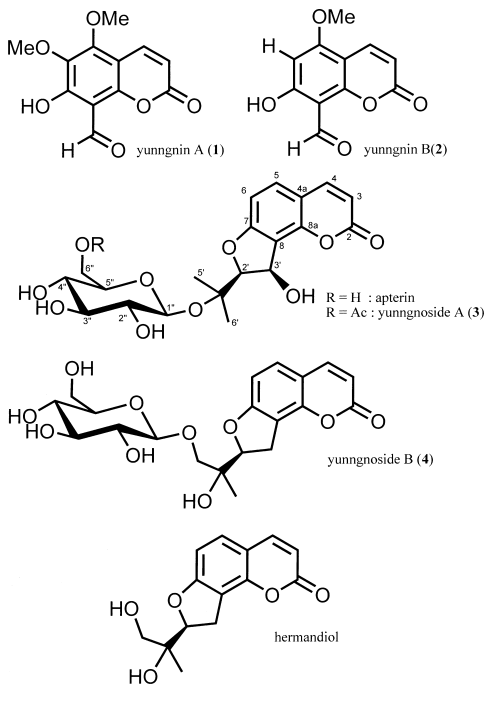

Fig. 1. Structures of **1**—**4**, Apterin, and Hermandiol

Compound **1**, colorless needles, mp 158—160 °C, was assigned the molecular formula $C_{12}H_{10}O_6$ ($[M]^+$ *m/z* 250.0465) by high resolution electron impact (HR-EI)-MS. The UV spectrum showed absorption maxima at 293.7, 216.2 and 206.6 nm and IR spectrum absorption bands at 2963, 1731, 1648, 1594, 1475 and 1424 cm^{-1}, suggesting the presence of an aromatic ring, an unsaturated lactone and hydroxyl groups. The ^{1}H- and ^{13}C-NMR spectra (Table 1) of **1** showed the presence of a 5,6,7,8-tetrasubstituted coumarin ring (δ_H 6.27 d, 7.95 d; δ_C 161.53, 159.33, 154.87, 153.32, 139.06, 134.84, 112.50, 105.30, 105.07), two methoxy groups (δ_H 4.24 s, 3.90 s; δ_C 61.73, 61.39), an aldehyde group (δ_H 10.43 s; δ_C 191.82) and a chelated hydroxyl group (δ_H 12.74 s), respectively. Successively, the analyses of ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond coherence (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) spectra of **1**, as shown in Fig. 2, clarified that the structure of **1** could be 8-formyl-7-hydroxy-5,6-dimethoxy coumarin.

Compound **2**, colorless needles, mp 179–180 °C was as-

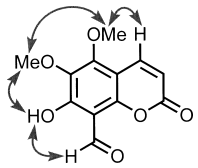

Fig. 2. NOE Correlations of **1**

Table 1. ^{1}H- and ^{13}C-NMR Data for **1** and **2** in $CDCl_3$

	^{1}H		^{13}C	
	1	**2**	**1**	**2**
2			159.33	159.42
3	6.27 d (9.8)	6.24 d (9.8)	112.50	111.64
4	7.95 d (9.5)	7.96 d (9.8)	139.06	138.56
4a			105.07	102.77
5			154.87	162.67
5-OCH_3	4.24 s	3.99 s	61.73	56.66
6		6.28 s	134.86	94.98
6-OCH_3	3.90 s		61.39	
7			161.53	168.09
7-OH	12.74 s	12.73 s		
8			105.30	103.89
8-CHO	10.43 s	10.38 s	191.82	191.25
8a			153.32	157.99

Chemical shifts are in δ values, followed by multiplicities and *J* values (in Hz).

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signed the molecular formula $C_{11}H_8O_5$ ([M]$^+$ *m/z* 220.0370) by HR-EI-MS. The UV spectrum showed absorption maxima at 319.3, 290.6 and 215.3 nm and IR spectrum absorption bands at 2964, 2363, 1731, 1639, 1596, 1475 and 1438 cm^{-1}. Comparison of the ^{1}H- and ^{13}C-NMR spectral data (Table 1) of **1** and **2** indicated that **2** is very closely related to **1**, except for the presence of an aromatic proton (δ_H 6.28 s; δ_C 94.98) and the lack of one of two methoxy groups. The location of each functional group was determined by the analyses of the ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra (Fig. 3).

Compound **3**, colorless needles, mp 158—160 °C, $[\alpha]_D^{25}$ +126.6° (*c*=0.51, MeOH) was assigned the molecular formula $C_{22}H_{26}O_{11}$ ([M]$^+$ *m/z* 466.1471) by HR-EI-MS. The UV spectrum showed absorption maxima at 322.6 and 204.0 nm, and IR spectrum absorption bands at 3447 br, 2926, 1737, 1617, 1577 and 1490 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **3** (Table 2) showed signals due to an angular type 2′,3′-disubstituted dihydrofuranocoumarin ring (δ_H 6.22 d, 7.66 d, 7.37 d, 6.81 d, 4.38 d, 5.82 dd; δ_C 161.05, 112.49, 144.18, 113.28, 130.64, 107.87, 163.07, 116.66, 151.92, 91.19, 69.50), a hydroxyl group (δ_H 5.00 br d) and a gem-dimethyl group (δ_H 1.58 s, 1.57 s; δ_C 78.36, 25.95, 23.30). In addition, signals assignable to a glucopyranosyl moiety [δ_H 4.66 (d, *J*=7.8 Hz, H-1″); δ_C 97.20] were observed. These above spectral data are closely related to those of apterin,[6,7] except for the presence of an acetyl group (δ_H 2.00 s; δ_C 171.30, 20.74), suggesting that **3** is apterin monoacetate. Location of the acetyl group is determined by analysis of the HMBC spectrum (Fig. 4).

Compound **4**, colorless viscous oil, $[\alpha]_D^{22}$ +110.6° (*c*=0.54, MeOH), was assigned the molecular formula $C_{20}H_{24}O_{10}$ ([M]$^+$ *m/z* 424.1365) by HR-EI-MS. The UV spectrum of **4** showed absorption maxima at 327.9, 261.2, 252.3 and 205.4 nm and IR spectrum absorption bands at 3413 br, 2926, 1713, 1616, 1490 and 1456 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **4** (Table 3) showed the presence of an angular type 2′-monosubstituted dihydrofuranocoumarin ring (δ_H 6.23 d, 7.97 d, 7.48 d, 6.82 d, 5.05 dd, 3.30 dd, 3.25 dd; δ_C 160.01, 111.12, 144.77, 112.36, 128.94, 106.38, 163.74, 113.58, 150.66, 87.36, 25.93), a methyl group (δ_H 1.13 s; δ_C 20.68), and a methylene group adjacent to an oxygen atom (δ_H 3.71 d, 3.58 d; δ_C 73.44) in addition to signals due to a glucopyranosyl moiety [δ_H 4.19 (d, *J*=7.8 Hz, H-1″); δ_C 103.59]. On acid hydrolysis with 5% HCl, **4** gave hermandiol,[8—10] whose spectral data were in perfect agreement with the authentic sample. The location of the acetyl group was confirmed by analysis of the HMBC spectrum (Fig. 5).

Experimental

General ^{1}H- and ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT), HMQC, and HMBC spectra were recorded on a Varian UNITY INOVA-500 spectrometer, operating at 500 MHz for proton and 125 MHz for carbon, with tetramethylsilane (TMS) as an internal standard.

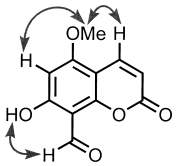

Fig. 3. NOE Correlations of **2**

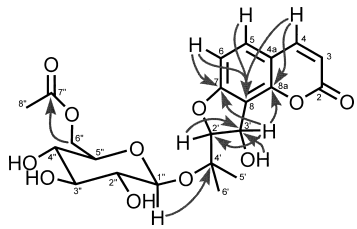

Fig. 4. The Main HMBC Correlations of **3**

Table 2. ^{1}H- and ^{13}C-NMR Data for **3** and Apterin in $CDCl_3$

	^{1}H		^{13}C		
	3	Apterin		**3**	Apterin
3	6.22 d (9.6)	6.20 d (9.6)	2	161.05	159.94
4	7.66 d (9.6)	7.65 d (9.6)	3	112.49	112.04
5	7.37 d (8.5)	7.37 d (8.4)	4	144.18	143.38
6	6.81 d (8.5)	6.80 d (8.4)	4a	113.28	112.72
2′	4.38 d (7.0)	4.39 d (7.0)	5	130.64	130.01
3′	5.82 dd (8.2, 7.0)	5.74 dd (8.6, 7.0)	6	107.87	107.32
3′-OH	5.00 d (8.2)	4.92 d (8.6)	7	163.07	162.28
5′	1.57 s	1.58 s	8	116.66	116.26
6′	1.58 s	1.60 s	8a	151.92	151.23
1″	4.66 d (7.8)	4.73 d (7.7)	2′	91.19	90.95
2″	3.24 dd (9.0, 7.8)		3′	69.50	69.27
3″	3.49 t (9.0)		4′	78.36	77.93
4″	3.32 dd (9.6, 9.0)		5′	25.95	25.45
5″	3.36 ddd (9.6, 5.1, 2.2)	3.20—3.50[a] m	6′	23.30	24.74
6″	4.11 dd (12.0, 5.1)		1″	97.20	97.20
	3.79 dd (12.0, 2.2)		2″	73.28	73.20
8″	2.00 s		3″	76.37	75.40
			4″	69.82	70.26
			5″	73.51	73.20
			6″	62.76	61.79
			7″	171.30	
			8″	20.74	

Chemical shifts are in δ values, followed by multiplicities and *J* values (in Hz). *a*) Overlapping signals.

Table 3. ^{1}H- and ^{13}C-NMR Data for **4** and Hermandiol in DMSO-d_6

	^{1}H		^{13}C		
	4	Hermandiol		**4**	Hermandiol
3	6.23 d (9.6)	6.15 d (9.5)	2	160.01	160.43
4	7.97 d (9.6)	7.94 d (9.5)	3	111.12	113.83
5	7.48 d (8.2)	7.45 d (8.2)	4	144.77	145.29
6	6.82 d (8.2)	6.78 d (8.2)	4a	112.36	113.06
2′	5.05 dd (9.6, 8.5)	4.93 dd (9.3, 8.8)	5	128.94	129.54
3′	3.30 dd (16.0, 8.5)	3.26—3.23[a] m	6	106.39	107.10
	3.25 dd (16.0, 9.6)		7	163.74	164.19
5′	3.71 d (9.8)	3.53 d (10.2)	8	113.58	114.43
	3.58 d (9.8)	3.28 d (10.2)	8a	150.66	151.15
6′	1.13 s	1.07 s	2′	87.36	88.42
1″	4.19 d (7.8)		3′	25.93	27.27
2″	2.99 dd (8.9, 7.8)		4′	72.06	73.79
3″	3.15 t (8.9)		5′	73.44	66.95
4″	3.06 dd (9.5, 8.9)		6′	20.68	22.12
5″	3.12 ddd (9.5, 5.9, 2.2)		1″	103.59	
6″	3.68 dd (11.8, 2.2)		2″	73.44	
	3.45 dd (11.8, 5.9)		3″	76.39	
			4″	69.99	
			5″	76.79	
			6″	60.94	

Chemical shifts are in δ values, followed by multiplicities and J values (in Hz). *a*) Overlapping signals.

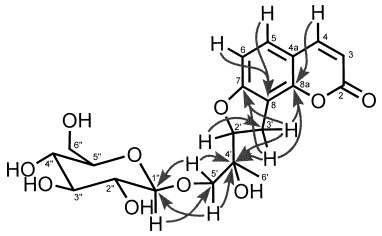

Fig. 5. The Main HMBC Correlations of **4**

HR-EI-MS spectra were obtained using a Hitachi M-4100H (70 eV) mass spectrometer. UV and IR spectra were recorded on a Shimadzu UV-2100 and a Perkin Elmer FT-IR 1720 spectrophotometer, respectively. Optical rotatory dispersion (ORD) spectra were recorded on a JASCO J820 digital polarimeter. Column chromatography was performed using Fuji silycia silica gel PSQ100B, Merck silica gel 60H, YMC GEL ODS-A 60-400/230 and Sephadex LH-20. TLC and prep. TLC was carried out on Merck Silica gel F_{254} plates (0.25 mm) and Whatmann silica gel 150A PLK 5F (1 mm). Spots and bands were detected by UV irradiation (254, 365 nm).

Plant Material Air-dried roots of *H. yungningense* (2.3 kg) were collected from plants grown in Lijiang, Yunnan Province, China, in September 2000. Voucher specimens are deposited in the Institute of Botany, Jiangsu Province, and Academia Sinica, Nanjing, China. The plant was identified by one of the authors (N.W.).

Extraction and Isolation The roots were chopped into small pieces and extracted with EtOAc and MeOH (each 31×5) under reflux. The EtOAc extracts were concentrated to dryness *in vacuo*. The residue (72 g) was subjected to column chromatography on silica gel, and eluted successively with a hexane–EtOAc solvent system with increasing polarity (3 : 1→1 : 2) to afford 19 fractions (fr.) [fr. 1 (2.3 g), fr. 2 (1.9 g), fr. 3 (1.1 g), fr. 4 (1.1 g), fr. 5 (1.2 g), fr. 6 (14.9 g), fr. 7 (6.2 g), fr. 8 (2.9 g), fr. 9 (12.4 g), fr. 10 (1.6 g), fr. 11 (1.3 g), fr. 12 (1.6 g), fr. 13 (0.3 g), fr. 14 (0.3 g), fr. 15 (0.3 g), fr. 16 (0.2 g), fr. 17 (0.1 g), fr. 18 (0.5 g) and fr. 19 (7.4 g)]. Each fraction was respectively rechromatographed on silica gel with successive hexane–EtOAc and CH_2Cl_2–MeOH solvent systems to give 22 compounds, including **1** and **2** [fr. 3: 6-isopentenyloxyisobergapten (1.2 mg), umbelliprenin (1.0 mg), 8-geranoxypsoralen (3.4 mg), fr. 4—7: pinpinellin (1.05 g), farcalindiol (605.5 mg), isobergapten (1.18 g), xanthotoxin (9.3 mg), isopimpinellin (1.81 g), mellendorffiline (144.6 mg), bergapten (216.8 mg), yunngnin A (**1**) (7.8 mg), yunngnin B (**2**) (5.1 mg), umbelliferone (2.0 mg), fr. 8—11: sphondin (1.67 mg), *p*-hydroxyphenethyl-*trans*-ferulate (45.9 mg), angelicin (22.0 mg), ferulic acid (241.8 mg), phelloptenin (1.5 mg), fr. 12—16: vaginidiol (10.6 mg), fr. 17: isoferaxidin (14.7 mg), fr. 19: apterin (757.5 mg)].

The MeOH extracts were concentrated to dryness *in vacuo*. The residue (206.1 g) was subjected to column chromatography on silica gel, and eluted successively with a hexane–EtOAc (2 : 1→1 : 2) and CH_2Cl_2–MeOH (20 : 1→1 : 1) solvent systems to afford 6 fractions [fr. 1 (2.0 g), fr. 2 (2.8 g), fr. 3 (3.1 g), fr. 4 (3.1 g), fr. 5 (24.3 g), fr. 6 (24.3 g), fr. 7 (170.2 g)]. Fractions 2—5 were rechromatographed on silica gel with CH_2Cl_2–MeOH (10 : 1) to afford five compounds, including compounds **3** and **4** [fr. 2: 8-geranoxy psoralen (3.4 mg), fr. 3: heratomol-6-*O*-β-D-glucopyranoside (45.8 mg), scopoletin (22.6 mg), hermandiol (12.0 mg), fr. 4: yunngnoside A (**3**) (17.8 mg), yunngnoside B (**4**) (328.0 mg)].

Yunngnin A (**1**): Colorless crystalline powder, mp 158—160 °C, HR-EI-MS: *m*/*z* 250.0465 $[M]^+$ (Calcd for $C_{12}H_{10}O_6$: 250.0477), UV λ_{max} (MeOH) nm (log ε): 293.7 (3.94), 216.2 (4.02), 202.6 (4.01), IR (KBr) cm^{-1}: 2963, 1731, 1648, 1594, 1475, 1424. ^{1}H- and ^{13}C-NMR data are shown in Table 1.

Yunngnin B (**2**): Colorless crystalline powder, mp 179—180 °C, HR-EI-MS: *m*/*z* 220.0370 $[M]^+$ (Calcd for $C_{11}H_8O_5$: 220.0372), UV λ_{max} (MeOH) nm (log ε): 319.3 (4.09), 290.6 (4.23), 215.3 (4.26), IR (KBr) cm^{-1}: 2964, 2363, 1731, 1639, 1596, 1475, 1438. ^{1}H- and ^{13}C-NMR data are shown in Table 1.

Yunngnoside A (**3**): Colorless crystalline powder, mp 240—242 °C, HR-EI-MS: *m*/*z* 466.1471 $[M]^+$ (Calcd for $C_{22}H_{26}O_{11}$: 446.1475), UV λ_{max} (MeOH) nm (log ε): 322 (4.15), 204.5 (4.62), IR (KBr) cm^{-1}: 3447 br, 2926, 1737, 1617, 1577, 1490, 1451, 1407, ORD (c=0.505, MeOH) $[\alpha]^{25}$ (nm): +62.9° (700), +105.3° (650), +126.6° (589), +213.2° (500), +313.9° (450), +536.4° (400). ^{1}H- and ^{13}C-NMR data are shown in Table 2.

Yunngnoside B (**4**): Pale yellow viscous oil, HR-EI-MS: *m*/*z* 424.1365 $[M]^+$ (Calcd for $C_{20}H_{24}O_{10}$: 424.1369), UV λ_{max} (MeOH) nm (log ε): 327 (4.11), 261.2 (3.62), 252.3 (3.59), 205.0 (4.56), IR (KBr) cm^{-1}: 3413 br, 2926, 1713, 1616, 1490, 1456, 1407, ORD (c=0.535, MeOH) $[\alpha]^{22}$ (nm): +27.8° (700), +79.3° (650), +110.6° (589), +158.5° (500), +223.4° (450). ^{1}H- and ^{13}C-NMR data are shown in Table 3.

Hydrolysis of 4 A solution of **4** (25.0 mg) in 5% HCl was heated on a boiling water bath for 2 h and subjected to an IRA-35 column. The eluate was concentrated *in vacuo* and chromatographed on ODS to give the aglycon (18.7 mg), which was identical with hermandiol by comparison of the spectral data.

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