Two New Coumarin Derivatives from the Roots of *Heracleum rapula*

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Abstract

Two new coumarins, 13-*O*-[*β*-D-apiofuranosyl(1→6)-*β*-D-glucopyranosyl]-(12*R*)-heraclenol (**1**) and (12*R*,12″*R*)-diheraclenol (**2**) were isolated from the acetone extract of the fresh roots of *Heracleum rapula*. Their structures were determined by means of spectroscopic analysis and, in the case of compound **1**, the structure elucidation was supported by acid hydrolysis. Compound **1** is a coumarin glycoside while **2** is a coumarin dimer. The inhibitory effects of **1**, its aglycone (**3**), and **2** on rabbit platelet aggregation induced by PAF, AA and ADP were tested. Weak activities were observed for each compound with the percentages of inhibition in the range of 0.7 – 24.8 %.

Heracleum rapula Franch (Umbelliferae) is a frequently used Traditional Chinese Medicine for rheumatic disease, lumbago, gastralgia, and injuries from falls, fractures, contusions and strains; it has also been reported to dispel wind, remove dampness, expel cold and relieve pain, dredge all the channels and vessels, promote blood circulation and relax muscles and tendons [1]. A number of closely related furocoumarins were isolated from the roots of the plant in the previous reports [1], [2], [3]. In our continuing investigations, careful examination for minor coumarins of the acetone extract of the fresh roots of this plant has now led to the isolation of a new coumarin glycoside 13-*O*-[*β*-D-apiofuranosyl(1→6)-*β*-D-glucopyranosyl]-(12*R*)-heraclenol (**1**), and a new coumarin dimer (12*R*,12″*R*)-diheraclenol (**2**).

Compounds **1** and **2**, showing strong yellowish-green fluorescence under 265 nm ultraviolet light, exhibited UV absorptions (300, 260, 250 nm), IR bands due to hydroxy groups (3419 – 3460 cm^{-1}) and a coumarin ring (1710, 1624, 1587, 1465, 1439 cm^{-1}), and a base ion peak at *m/z* = 201 in its MS arising from an oxygen-bearing furocoumarin fragment ($C_{11}H_5O_4$). All these spectral findings are typical of linear furocoumarins [4].

Compound **1**, showed a molecular ion peak at *m/z* = 598 in its negative FABMS, consistent with a molecular formula of $C_{27}H_{34}O_{15}$, which was confirmed by its HRFABMS and NMR data. A comparison of the NMR data of **1** with those of heraclenol [4] revealed that the two compounds were alike except for the appearance of two additional sugars in **1**, which was further verified by the result that, on acid hydrolysis, compound **1** afforded heraclenol (**3**) as the aglycone, as well as D-apiose and D-glucose. The stereochemistry of C-12 in the aglycone was established as the *R*-configuration on the basis of the fact that its optical rotation value ($[\alpha]_D^{18.7}$: + 26.40°) was in good accordance with the data reported in the literature ($[\alpha]_D^{23}$: + 16.5°) [5]. In addition, the outstanding change of the C-13 signal [δ_C = 79.6 (s)] gave a hint that the sugar chain was attached to C-13. In the HMBC spectrum (Fig. **1**), H-1 of the glucose showed a ^1H-^{13}C long-range correlation with C-13, and H-1 of the apiose demonstrated a ^3J correlation with C-6 of the glucose and *vice versa*. The above facts revealed that two ether linkages existed between the aglycone and C-1 of the glucose, and between the C-6 of the glucose and the C-1 of the apiose, respectively. Finally, **1** was assigned as 13-*O*-[*β*-D-apiofuranosyl(1→6)-*β*-D-glucopyranosyl]-(12*R*)-heraclenol. Compound **2** displayed very similar ^1H-NMR signals to those of heraclenol [5]. The difference observed was that there was only one proton signal of a hydroxy group appearing at δ_H = 3.20 (br s) in **2**, while two such signals [δ_H = 3.62 (br s) and 2.79 (br s)] are seen in heraclenol. The more obvious distinctions came from their ^{13}C-NMR spectra, in which the chemical shift of the oxygenated quaternary carbon (C-13) moved downfield from δ_C = 71.8 (s) in heraclenol to δ_C = 78.4 (s) in **2**. All the above spectral findings indicated that **2** was a dimer of heraclenol, which was in agreement with the molecular ion peak at *m/z* = 590 $[M]^+$ in the negative FABMS and was confirmed by HRFABMS [*m/z* = 590.1744 (calcd. for $C_{32}H_{30}O_{11}$: 590.1788)]. The stereochemistry at C-12 and C-12″ of **2** were concluded to be the *R* configuration because its optical rotation value ($[\alpha]_D^{19.2}$: + 28.31°) was positive and identical with that of *R*-heraclenol [5]. Accordingly, compound **2** was established as (12*R*,12″*R*)-diheraclenol.

As the aglycone of **1** or the monomer of **2**, heraclenol (**3**) has been found to be the major constituent in the 70 % acetone extract of the roots of this plant [3]. Compounds **1 – 3** were evaluated for their *in vitro* inhibitory activity against rabbit platelet aggregation induced by PAF (platelet activating factor), AA (arachidonic acid), and ADP (adenosine diphosphate), using the same bioassay methods as previously described [6] (Table **1**). Ginkgolide B (BN52021) and acetylsalicylic acid (ASA) were used as positive controls, and 2 % PEG (polyethylene glycol) was used as contrast. Only compound **3** exhibited weak inhibitory activity on PAF-induced rabbit platelet aggregation.

Materials and Methods

General: General experimental procedures utilized were the same as previously described [3].

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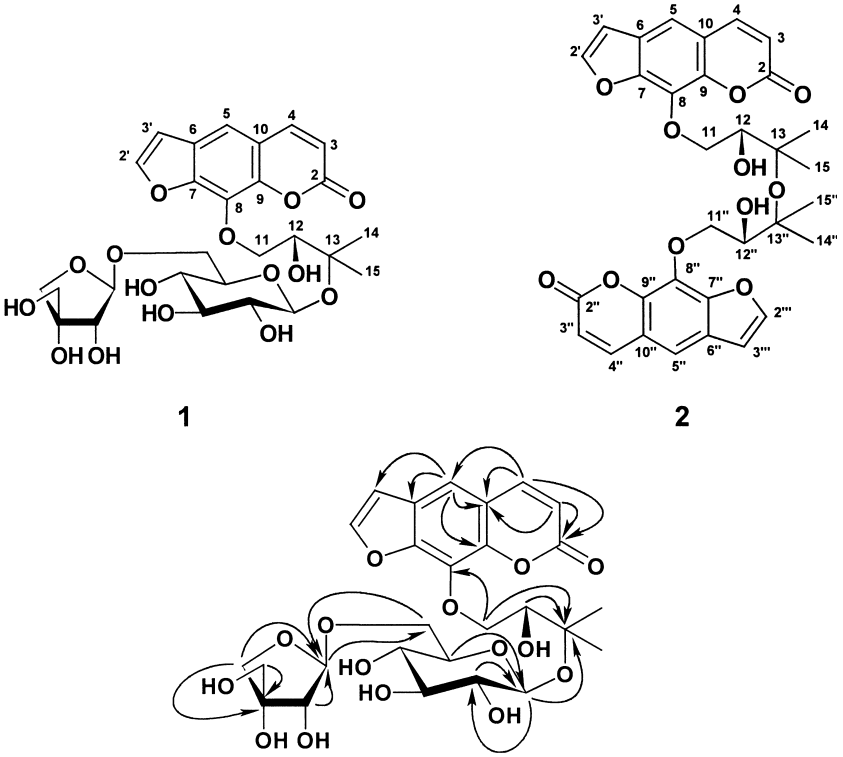

Fig. 1 The key correlations of compound **1** in the HMBC spectrum.

Table 1 Percentage inhibition of compounds **1 – 3** on the aggregation of rabbit platelets induced by PAF, AA, and ADP

		Aggregation % (Inhibition %)	
Compound (240 μM)	*PAF (4.5 nmol)*	*AA (350 μmol)*	*ADP (5 μmol)*
2% PEG	67.6 ± 2.2	80.5 ± 2.8	62.1 ± 3.4
1	61.5 ± 1.8 (9.0 ± 3.9)	75.9 ± 3.2 (5.7 ± 0.8)	58.3 ± 2.9 (6.2 ± 1.1)
2	59.6 ± 2.0 (11.8 ± 3.2)	79.9 ± 2.8 (0.7 ± 0.3)	54.9 ± 4.1 (11.6 ± 4.6)
3	50.8 ± 2.4* (24.8 ± 3.7)	79.6 ± 2.3 (1.1 ± 0.6)	53.6 ± 4.3 (13.7 ± 6.0)
BN52021	13.4 ± 2.1* (80.2 ± 4.4)		
Aspirin		6.3 ± 2.1* (91.3 ± 2.9)	

* $P < 0.01$, as compared with control (*t*-test).The data were expressed as means ± S.D. of 4 rabbits.

Plant material: The roots of *H. rapula* were collected and identified and a voucher specimen (KIB 99-7-10-014 Lin) has been deposited as previously described [3].

Extraction and isolation: The fresh roots of *H. rapula* (12.1 kg) were extracted with acetone (3×30 L). After evaporation of the acetone under vacuum, the concentrated extract was suspended in water and partitioned with petroleum ether (3×2000 mL) to afford 140.0 g of petroleum ether-soluble residue. The water-soluble fraction was directly subjected to column chromatography over Diaion 101 macroporous resin (800 g) eluting with H_2O, aqueous MeOH (30%, 40%, 50%) and MeOH (2000 mL each eluent) to provide four portions. The 50% MeOH portion (15.3 g) was chromatographed over MCI-gel CHP-20P (100 g) eluting with aqueous MeOH (30%, 50%, 80%) and MeOH (900 mL each eluent). The eluate from 80% aqueous MeOH was concentrated to dryness (4.1 g) and was further purified by medium-pressure column chromatography over silica gel (60 μm, 100 g) eluting with $CHCl_3$/MeOH (9 : 1, 1500 mL) to yield compound **1** (115 mg). 140.0 g of the petroleum ether-soluble residue were chromatographed over silica gel (200 – 300 mesh, 1500 g) eluting with chloroform, chloroform/acetone (9 : 1, 4 : 1) and acetone (3200 mL each eluent) to give fractions I – IV. The fractions were collected and combined by monitoring with silica gel TLC (petroleum ether/EtOAc, 4 : 1, 3 : 1, 2 : 1). Fraction II (62.2 g) was rechromatographed over silica gel (200 – 300 mesh, 500 g) developing with petroleum ether/EtOAc (9 : 1, 8 : 2, 7 : 3, 1 : 1) in a stepwise gradient mode (2000 mL each eluent). The petroleum ether/EtOAc (9 : 1) eluate (200 mg) was successively subjected to CC over silica gel (50 g, 200 – 300 mesh) with petroleum ether/EtOAc (6 : 1, 1000 mL) as eluting system to yield compound **2** (30 mg).

13-O-[β-D-apiofuranosyl(1→6)-β-D-glucopyranosyl]-(12R)-heraclenol (**1**): $C_{27}H_{34}O_{15}$, pale-yellow amorphous solid; $[\alpha]_D^{24.3}$: −34.52° (*c* 0.44, C_5H_5N); UV (H_2O): λ_{max} (log ε) = 303.5 (4.5), 260.5 (4.5), 247 (4.7), 217.5 (4.8), 198 (4.7) nm; IR (KBr):

ν_{max} = 3419, 2889, 1735, 1717, 1623, 1588, 1465, 1441, 1402, 1334, 1294, 1218, 1156, 1084, 999, 874, 824, 752, 705, 565, 495 cm^{-1}; ^{1}H-NMR data see Table **2**; ^{13}C-NMR data see Table **3**; negative FABMS: *m/z* = 598 $[M]^+$ (45), 201 (100); negative HRFABMS: *m/z* = 598.1954, calcd. for $C_{27}H_{34}O_{15}$: 598.1898.

Acid hydrolysis of compound **1** *:* A solution of **1** (15 mg) in 0.25 mol/L H_2SO_4 (1 mL) was heated at 70 °C for 1 h. After cooling, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed subsequently with 10% $NaHCO_3$ and water, and dried over Na_2SO_4. The $CHCl_3$ was removed under vacuum and the residue was recrystallized from Me_2CO to afford 5 mg of **3** as pale yellow needles. Through silica gel TLC, glucose and apiose were detected in the water layer by comparison with authentic samples using $CHCl_3$/MeOH/H_2O (8 : 5 : 1) as developing system. The water layer was neutralized with Amberlite IRA-400 (OH^- form) resin, concentrated to dryness and subjected to silica gel chromatography [$CHCl_3$/MeOH/H_2O (6 : 4 : 1)] to afford D-glucose (3.0 mg): $[\alpha]_D^{15.3}$: +25.4° (*c* 0.15, H_2O) and D-apiose (2.5 mg): $[\alpha]_D^{15.1}$: +7.3° (*c* 0.10, H_2O).

Aglycone of **1** (**3**): Pale yellow needles (Me_2CO); $[\alpha]_D^{18.7}$: +26.40° (*c* 0.63, $CHCl_3$); EIMS: *m/z* = 304 $[M]^+$ (40), 202 (100). The NMR spectral data (Tables **3** and **4**) were consistent with those of *R*-heraclenol reported in the literature [4], [5].

(12R,12″R)-Diheraclenol (**2**): $C_{32}H_{30}O_{11}$; pale yellow amorphous solid; $[\alpha]_D^{19.2}$: +28.31° (*c* 0.36, $CHCl_3$); UV ($CHCl_3$): λ_{max} (log ε) = 331 (4.18), 300 (4.61), 261 (4.70), 250.5 (4.87) nm; IR (KBr): ν_{max} = 3462, 2990, 1710, 1624, 1587, 1465, 1439, 1404, 1361, 1337, 1310, 1297, 1217, 1179, 1150, 1110, 1098, 1030, 1008, 991, 876, 859, 759 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) data see Table **3**; EIMS: *m/z* = 344 (11), 304 (7), 286 (41), 269 (9), 245 (8), 227 (2), 215 (28), 202 (100), 187 (8), 174 (36), 157 (8), 145 (10), 129 (10), 118 (4), 101 (3), 89 (23), 71 (10), 59 (23); negative FABMS: *m/z* = 590 $[M]^+$ (30), 473 (100), 325 (38), 201 (72); negative HRFABMS: *m/z* = 590.1744, calcd. for $C_{32}H_{30}O_{11}$: 590.1788.

Table **2** The NMR data of compound **1** ([a] 500 MHz, [b] 125 MHz, δ in ppm, *J* in Hz, pyridine-d_5).

No.	*^{13}C[b]*	*^{1}H[a]*	*No.*	*^{13}C[b]*	*^{1}H[a]*
2	160.6 s		13	79.6 s	
3	114.9 d	6.40 (1H, d, *J* = 9.6 Hz)	14	24.2 q	1.64 (3H, s)
			15	22.7 q	1.69 (3H, s)
4	144.9 d	7.82 (1H, d, *J* = 9.6 Hz)	Glu-1	98.3 d	5.16 (1H, d, *J* = 7.8 Hz)
5	113.7 d	7.58 (1H, s)	Glu-2	75.4 d	3.97 (1H, overlap)
6	126.6 s		Glu-3	78.8 d	4.20 (1H, m)
7	148.1 s		Glu-4	72.0 d	3.95 (1H, overlap)
8	132.8 s		Glu-5	77.0 d	4.05 (1H, m)
9	143.8 s		Glu-6	69.1 t	4.67 (1H, d, *J* = 10.0 Hz), 4.09 (1H, d, *J* = 10.0 Hz)
10	117.1 s		Api-1	111.2 d	5.74 (1H, d, *J* = 1.0 Hz)
2′	147.5 d	7.84 (1H, d, *J* = 2.1 Hz)	Api-2	78.0 d	4.72 (1H, d, *J* = 1.0 Hz)
3′	107.3 d	6.85 (1H, d, *J* = 2.1 Hz)	Api-3	80.4 s	
11	76.1 t	5.07 (1H, dd, *J* = 1.8, 10.0 Hz) 4.98 (1H, dd, *J* = 7.7, 10.0 Hz)	Api-4	75.1 t	4.55 (1H, d, *J* = 9.2 Hz), 4.33 (1H, d, *J* = 9.2 Hz)
12	76.2 d	4.63 (1H, dd, *J* = 7.7, 1.8 Hz)	Api-5	65.9 t	4.16 (2H, s)

Table **3** The ^{1}H-NMR data of compounds **2** and **3** (400 MHz, δ in ppm, pyridine-d_5)

Proton	*2*	*3*
3 (3″)	6.38 (2H, d, *J* = 9.5 Hz)	6.38 (1H, d, *J* = 9.8 Hz)
4 (4″)	7.78 (2H, d, *J* = 9.5 Hz)	7.76 (1H, d, *J* = 9.8 Hz)
5 (5″)	7.39 (2H, s)	7.39 (1H, s)
2′ (2‴)	7.72 (2H, d, *J* = 2.2 Hz)	7.72 (1H, d, *J* = 2.2 Hz)
3′ (3‴)	6.63 (2H, d, *J* = 2.2 Hz)	6.63 (1H, d, *J* = 2.2 Hz)
11 (11″)	4.82 (2H, dd, *J* = 3.4, 10.3 Hz), 4.54 (2H, dd, *J* = 7.4, 10.3 Hz)	4.75 (1H, dd, *J* = 2.5, 10.2 Hz), 4.42 (1H, dd, *J* = 7.8, 10.2 Hz)
12 (12″)	3.90 (2H, dd, *J* = 3.4, 7.4 Hz)	3.88 (1H, dd, *J* = 2.6, 7.8 Hz)
14 (14″)	1.54 (6H, s, CH_3)	1.34 (3H, s)
15 (15″)	1.54 (6H, s, CH_3)	1.28 (3H, s)
OH	3.20 (2H, br s)	3.62 (1H, br s), 2.79 (1H, br s)

Table **4** The ^{13}C-NMR data of compounds **2** and **3** (100 MHz, δ in ppm, pyridine-d_5)

Carbon	*2*	*3*
2 (2″)	160.1 (2C, s)	160.4 (s)
3 (3″)	114.8 (2C, d)	115.1 (d)
4 (4″)	144.1 (2C, d)	144.4 (d)
5 (5″)	113.3 (2C, d)	113.9 (d)
6 (6″)	126.0 (2C, s)	126.3 (s)
7 (7″)	148.0 (2C, s)	148.4 (s)
8 (8″)	131.9 (2C, s)	132.2 (s)
9 (9″)	143.4 (2C, s)	143.7 (s)
10 (10″)	116.5 (2C, s)	116.8 (s)
2′ (2‴)	146.8 (2C, d)	147.0 (d)
3′ (3‴)	106.7 (2C, d)	107.1 (d)
11 (11″)	75.3 (2C, t)	76.0 (t)
12 (12″)	77.8 (2C, d)	76.4 (d)
13 (13″)	78.4 (2C, s)	71.8 (s)
14 (14″)	25.7 (2C, q)	26.8 (q)
15 (15″)	24.9 (2C, q)	25.4 (q)

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