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Rapulasides A and B: two novel intermolecular rearranged biiridoid glucosides from the roots of *Heracleum rapula*

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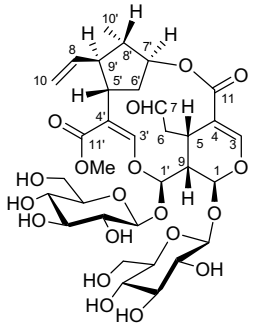
Abstract—Two novel intermolecular rearranged biiridoid glucosides, rapulasides A and B (**1** and **2**), have been isolated from the roots of *Heracleum rapula*. Their structures were identified by extensive spectral analysis especially different NMR techniques. NOESY experiment, with the help of Dreiding molecular model, was used to elucidate their relative stereochemistry. Both compounds were tested for their inhibitory effects on rabbit platelet aggregation induced by PAF, ADP, or AA, respectively. Only trends of inhibition were observed for them.

Heracleum rapula Fr. (Umbelliferae), widely distributed in Yunnan province of China, is commonly used in Chinese traditional medicine to dispel wind, remove dampness, expel cold, relieve pain, dredge all channels and vessels, promote blood circulation, and relax muscles and tendons.[1] According to the results of pharmacological experiments, the water-soluble extracts possessed antibiotic and antiasthmatic effects.[2] Previous phytochemical investigations from our group showed the presence of a series of coumarins in this plant.[1,3,4] Our present search for bioactive compounds from the water-soluble extracts of its roots resulted in the isolation of two novel rearranged biiridoid glucosides, namely rapulasides A and B (**1** and **2**). The structures of both compounds were identified by extensive NMR spectroscopic experiments including ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY techniques. Both compounds have been evaluated for their in vitro inhibitory activity against rabbit platelet aggregation induced by PAF, AA, and ADP, respectively. We describe herein the isolation, structure elucidation, and biological activity of compounds **1** and **2**.



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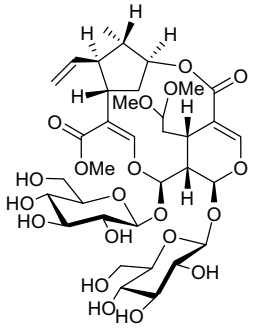

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The roots of *H. rapula* Fr. were collected from Dali prefecture of Yunnan province in 2003 and verified by Prof. Zhongwen Lin. The air-dried and powdered roots (4.0 kg) were extracted with acetone (4 × 15 L) at room temperature and concentrated in vacuo to give a crude extract (195 g), which was partitioned between H_2O and EtOAc. The water layer was directly subjected to column chromatography over Diaion 101 macroporous resin (800 g) eluting with H_2O, aqueous MeOH (30%, 40%, and 60%) and MeOH to give five parts. The 40% aqueous MeOH part (20 g) was repeatedly chromatographed on silica gel column, which was further purified with preparative HPLC (Agilent 1100 HPLC system; Zorbax SB-C-18, Agilent,

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9.4 mm × 25 cm, MeOH–H_2O 35:65) to yield rapulasides A (21 mg) and B (18 mg).

Rapulaside A (**1**),[5] pale yellow amorphous powder, $[\alpha]_D^{23.4}$ −69.2 (*c* 0.2, MeOH), showed a quasi-molecular ion peak at *m/z* 745 ($[M-H]^-$) in its negative FAB mass spectrum. The molecular formula of **1** was revealed as $C_{33}H_{46}O_{19}$ by HRFABMS data (found 746.2568, calcd 746.2633), corresponding to 11° of unsaturation in the molecule. The ^1H and ^{13}C NMR spectra showed the signals of one methoxyl group and two glucoses and twenty additional carbon signals as following: four quaternary carbons (including two ester carbonyl carbons and two olefinic quaternary carbons), 12 methines, three methylenes (including one olefinic methylene), and one methyl group. The obvious HMBC cross-peaks from the anomeric protons of two glucoses at δ_H 5.40 (d, $J = 7.8$) and 5.36 (d, $J = 7.9$) to δ_C 97.0 (C-1) and 96.5 (C-1′) showed that the two sugars were connected with C-1 and C-1′, respectively. Considering that both C-1 and C-1′ were connected with a glucose moiety and their low-field chemical shift, it is reasonable to deduce that both C-1 and C-1′ were ketal carbons. Further interpretation of HMBC spectrum showed the following correlations (Fig. 1): the methine proton at δ_H 5.82 (H-1) with C-3 (δ_C 153.0), C-5 (δ_C 27.2) and C-9 (δ_C 44.8); the methine singlet at δ_H 7.73 (H-3) with C-4 (δ_C 109.3) and C-11 (δ_C 166.5); the methine proton at δ_H 3.68 (H-5) with C-6 (δ_C 44.6), C-7 (δ_C 200.9) and C-11 (δ_C 166.5); the proton signal at δ_H 5.66 (H-1′) with C-5 and C-9. The above spectral evidences, along with the proton spin system H-1/H-9 (/H-1′)/H-5/H_2–6/H-7 deduced from ^1H–^1H COSY correlations, led to the establishment of the partial structure **1a** (Fig. 1), which possessed a substructure of a 7,8-*seco*-iridoid carbon skeleton. In addition, HMBC spectrum also showed the cross-couplings from H_2-10 to C-8 and C-9′, from Me-10′ to C-7′, C-8′, and C-9′, from H-9′ to C-4′, from H-5′ to C-3′ and C-11′, and from methoxyl singlet at δ_H 3.53 (3H, s) to C-11′. Moreover, C-7′ and C-3′ were deduced to be oxygenated due to their low-field chemical shift at δ_C 77.1 and 151.5, respectively. These spectral data, coupled with another proton spin system H-5′/H_2-6′/H-7′/H-8′/H_3-10′/H-9′/H-8/H_2-10 established by ^1H–^1H COSY correlations, gave rise to another partial structure **1b** (Fig. 1). Further HMBC correlations of H-7′ to C-11 and H/C-3′ to C/H-1′ indicated the linkages in the order of C-7′–O–C-11 and C-3′–O–C-1′, which permitted fragments **1a** and **1b** to be joined to get the planar structure of compound **1**.

Sugar analysis of **1** was carried out as follows. A solution of **1** (12 mg) in 2 M HCl (3 mL) was heated in a water bath at 70 °C for 6 h. After cooling, the reaction mixture was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. We were not able to get the aglycone of **1** unfortunately, because the TLC inspection of the $CHCl_3$ part indicated that there were yields of at least five products, which were not subjected to further isolation and identification due to the limited amount. However, through co-TLC with authentic sample using $CHCl_3$–MeOH–H_2O–HOAC (7:3:0.8:1) as a developing system, glucose was detected in the water layer ($R_f = 0.48$), which was further concentrated to dryness and subjected to a silica gel chromatography eluting with $CHCl_3$–MeOH (1:1) to give purified D-glucose (4.4 mg): $[\alpha]_D^{18.3}$ +24.5 (*c* 0.2, H_2O).

The relative stereochemistry of **1** was determined using a NOESY experiment, aided by a Dreiding molecular model. Stereochemically, H-5 and H-9 were biogenetically β-oriented and accordingly, CH_2-6 was α-oriented. NOESY correlations of H-1/H-6 and H-1′/H-6, and the fact that no correlation was observed between H-1/H-5 and H-1′/H-5, indicated that H-1 and H-1′ were α-oriented. The cross-peaks of H-6/H-7′ and H-6/Me-10′ suggested that H-7′ and Me-10′ were both in α-orientation and accordingly H-8′ was in β-orientation. The NOESY correlation of H-8′/H-5′ indicated that H-5′ possessed β-orientation. The α-orientation of H-9′ was established by the NOESY correlations of H-9′/H-7′ (Fig. 2).

Rapulaside B (**2**)[6] was obtained as pale yellow amorphous powder and has the molecular formula of $C_{35}H_{52}O_{20}$ as determined by analysis of ^1H, ^{13}C, and DEPT NMR spectral data, and verified by HRFABMS (found 792.3015, calcd 792.3052), requiring 10° of unsaturation. The ^1H NMR spectrum displayed a signal due to a secondary methyl. The close resemblance of the NMR spectra (Table 1) to those of **1** suggested **2** to be a related rearranged biiridoid glucosides. The two compounds are different only because the aldehyde at C-7 in **1** was replaced by a ketal carbon (δ_C 103.2) in **2** and two extra methoxyl groups were observed in **2**. In addition, these two methoxyl groups were found to be

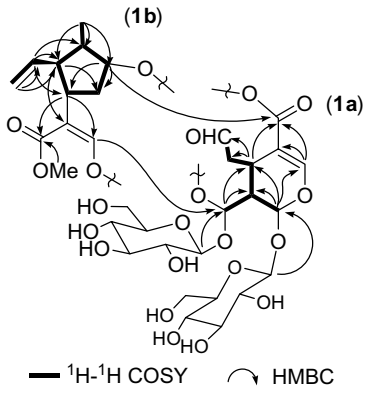

Figure 1. Key ^1H–^1H COSY and HMBC correlations of **1**.

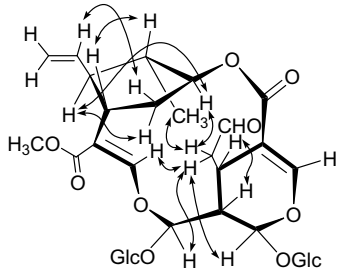

Figure 2. Key NOESY correlations of **1**.

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** in pyridine-d_5

Position	Compound **1**			Compound **2**		
	δ_H[b]	*J* (Hz)	δ_C[c]	δ_H[b]	*J* (Hz)	δ_C[c]
1	5.82	d, 4.6	97.0 d	5.89	d, 4.7	97.3 d
3	7.73	s	153.0 d	7.72	s	152.4 d
4	—	—	109.3 s	—	—	111.2 s
5	3.64–3.72	m	27.2 d	3.21–3.29	m	29.2 d
6	2.40–2.49	m	44.6 t	1.77–1.85	m	32.9 t
	2.81–2.90	m		2.27–2.35	m	
7	9.79	t, 4.4	200.9 d	4.69	t, 5.6	103.2 d
8	5.67–5.71	m	134.2 d	5.68–5.72	m	135.4 d
9	2.86–2.89	m	44.8 d	2.83–2.88	m	44.7 d
10	5.08	2H, overlapped by H_2O	120.0 t	5.10	2H, overlapped by H_2O	119.2 t
11	—	—	166.5 s	—	—	166.7 s
1′	5.66	d, 4.0	96.5 d	5.67	d, 3.9	96.4 d
3′	7.64	s	151.5 d	7.67	s	151.5 d
4′	—	—	112.5 s	—	—	112.7 s
5′	3.17–3.25	m	31.4 d	3.19–3.27	m	32.9 d
6′α	1.74–1.82	m	39.6 t	1.76–1.84	m	39.7 t
6′β	2.31–2.39	m		2.37–2.45	m	
7′	5.18–5.24	m	77.1 d	5.20–5.26	m	77.0 d
8′	2.04–2.12	m	39.9 d	2.05–2.13	m	39.9 d
9′	2.23–2.31	m	46.5 d	2.24–2.31	m	46.6 d
10′	0.89	3H, d, 6.8	13.2 q	0.91	3H, d, 6.7	13.2 q
11′	—	—	167.4 s	—	—	167.5 s
7-OMe	—	—	—	3.27	3H, s	53.2 q
7-OMe	—	—	—	3.28	3H, s	52.2 q
11′-OMe	3.53	s	51.0 q	3.54	s	51.1 q
1-Glc-1″	5.40	d, 7.8	100.7 d	5.39	d, 7.9	100.8 d
1-Glc-2″	4.13–4.19	m	74.9 d	4.13–4.19	m	74.9 d
1-Glc-3″	4.22–4.29	m	79.1 d[a1]	4.22–4.28	m	79.1 d
1-Glc-4″	4.28	Overlap	71.6 d	4.28	Overlap	71.5 d
1-Glc-5″	4.03	Overlap	78.6 d[a2]	4.02	Overlap	78.6 d
1-Glc-6″	4.40	Overlap	62.7 t	4.39	Overlap	62.7 t
	4.56	d, 11.0		4.54	d, 11.1	
1′-Glc-1‴	5.36	d, 7.9	100.9 d	5.33	d, 7.8	100.8 d
1′-Glc-2‴	4.11–4.17	m	74.7 d	4.10–4.16	m	74.6 d
1′-Glc-3‴	4.20–4.26	m	79.0 d[a1]	4.20–4.26	m	79.1 d
1′-Glc-4‴	4.28	Overlap	71.4 d	4.28	Overlap	71.3 d
1′-Glc-5‴	4.03	Overlap	78.5 d[a2]	4.02	Overlap	78.6 d
1′-Glc-6‴	4.40	Overlap	62.7 t	4.39	Overlap	62.7 t
	4.56	d, 11.0	—	4.54	d, 11.1	—

[a1,a2] Interchangeable.
[b] Measured at 500 MHz.
[c] Measured at 125 MHz.

connected to C-7 from the obvious HMBC cross-peaks from the methoxyl singlets at δ_H 3.27 (3H, s) and 3.28 (3H, s) to C-7. Sugar analysis of **2** was carried out with the same method used for **1** and the relative stereochemistry was established according to the NOESY experiment and aided by Drieding molecular model as well. Compound **2** was therefore identified as the 7,7-dimethyl ether of **1**.

Obviously, compounds **1** and **2** were derived from two iridoid glucoside monomers, loganic acid (**3**) and its 7,8-*seco*-analog (**4**), through an intermolecular

Table 2. Percentage inhibition of compounds **1**–**2** on the aggregation of rabbit platelets induced by PAF, AA, and ADP

Compound (10 mg/L)	Aggregation (%)		
	PAF (7.2 nmol/L)	AA (0.35 μmol/L)	ADP (3 μmol/L)
DMSO	60.3 ± 2.9	72.6 ± 3.3	69.5 ± 3.2
1	42.9 ± 3.5[b]	69.2 ± 3.6	68.9 ± 2.5
2	53.9 ± 4.0	73.6 ± 2.8	66.8 ± 2.4
BN52021	0.6 ± 0.1[b]		
Aspirin		4.7 ± 0.8[b]	65.9 ± 5.3

[a] $P < 0.05$.
[b] $P < 0.05$, as compared with control (*t*-test). The data were expressed as means ± SD of four rabbits.

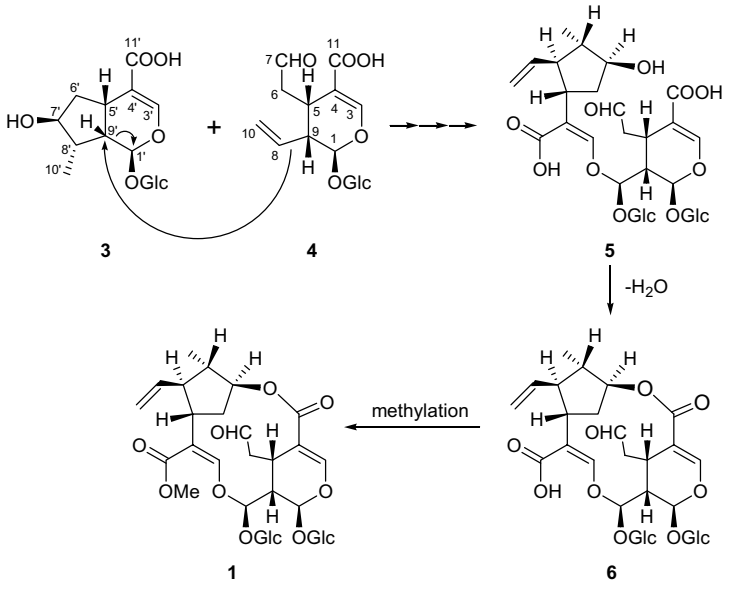

Figure 3. Plausible biogenetic pathway for **1**.

rearrangement (to **5**) and then dehydration (to **6**) and finally methylation, as shown for **1** in Figure 3. However, how the rearrangement realized in plants is unknown yet. Compounds **1** and **2** 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.[7] Ginkgolide B (BN52021) and acetylsalicylic acid (ASA) were used as positive controls, and 2% PEG. (polyethylene glycol) was used as contrast. Only the trends of inhibition were observed for them (Table 2).

Acknowledgements

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5. Rapulaside A (**1**): Pale yellow amorphous powder; $[\alpha]_D^{23.4}$ −69.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (lg ε): 198 (3.97), 235 (4.20), 389 (1.90) nm; IR (KBr) ν_{max}: 3426, 2922, 1701, 1631, 1439, 1385, 1287, 1154, 1074, 768, 575 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; negative FABMS *m/z* 745 $[M-H]^-$; HRFABMS *m/z*: Found 746.2568, calcd. 746.2633 for $C_{33}H_{46}O_{19}$.
6. Rapulaside B (**2**): Pale yellow amorphous powder; $[\alpha]_D^{25.2}$ −50.4 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (lg ε): 203 (4.10), 234 (4.17), 393 (1.70) nm; IR (KBr) ν_{max} 3424, 2931, 1701, 1633, 1440, 1384, 1289, 1155, 1074, 767, 633, 575 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; negative FABMS *m/z* 791 $[M-H]^-$; HRFABMS *m/z*: Found 792.3015, calcd 792.3052 for $C_{35}H_{52}O_{20}$.
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