Chinese Chemical Letters Vol. 11, No. 2, pp. 143–146, 2000

Two Triterpenoid Dimers from *Rubus pungens* Camb var. *oldhamii*

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Abstract: Two new triterpenoid saponin dimers, named rubupungenoside A (**1**) and B (**2**), have been isolated from the aerial parts of *Rubus pungens* Camb. var. *oldhamii*. Their structures have been established on the basis of spectroscopic methods and chemical transformations.

Keywords: *Rubus pungens* Camb. var. *oldhamii*, ursene, triterpenoid, dimer.

In the course of our continuous chemical studies of *Rubus* species[1-4], we have isolated two new triterpenoid dimers (**1**) and (**2**), in their methyl ester forms (**1a**) and (**2a**), from the aerial parts of *Rubus pungens* Camb var. *oldhamii*.

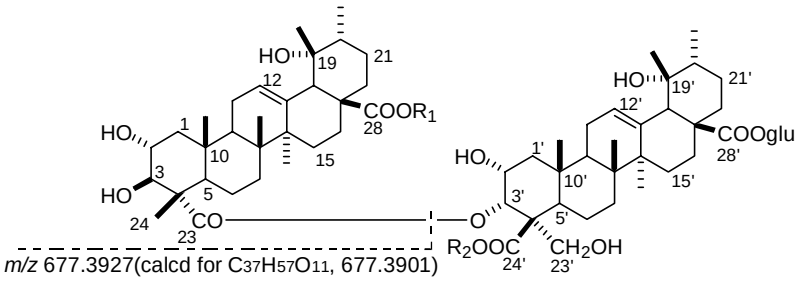
Compound **1a**, amorphous powder, m.p. 231-233°C, $[\alpha]_D^{21}$ + 12.32 (*c* 0.35, CH_3OH), gave a positive coloration in the Liebermann-Burchard and Molish tests which suggests that **1a** was a triterpenoid glycoside. The IR spectrum revealed the presence of hydroxyl (3435 cm^{-1}), ester carbonyl (1727 cm^{-1}), and double bond (1646 cm^{-1}) in the molecule. Its molecular formula was determined by negative HRFABMS as $C_{74}H_{114}O_{24}$ (1385.7638, $[M-1]^-$, calcd for $C_{74}H_{113}O_{24}$, 1385.7622). Most signals observed in the ^{13}C NMR spectrum as doublets (**Table 1)** implied that **1a** might be a triterpenoid dimer.

Hydrolysis of **1a** with 3% NaOH yielded two known triterpenoids **3** and **4**. This suggests that **1a** is a dimer related to **3** and **4**. By the detailed analysis of NMR and EIMS data, **3** and **4** were identified as 2α, 3β, 19α-trihydroxyurs-12-en-23, 28-dioic acid [2, 5] and 24-methyl ester of 2α, 3α, 19α, 23-tetrahydroxyurs-12-en-24, 28-dioic acid[6], respectively. Treatment of **3** and **4** with diazomethane yielded dimethyl ester **3a** and **4a**, which were acetylated with Ac_2O / pyridine afforded di-acetylated product **3b** and tri-acetylated product **4b**, respectively. These chemical transformations further confirmed structures of **3** and **4**.

In the ^1H NMR spectrum, H-3 signal appeared at δ 3.40 in **4**, and this signal shifted 1.73 ppm to the lower field at δ 5.13 in **1a**[7], which implied that 3-OH in **4** is one of the linkage positions. The carbon signal at δ 70.3 (d, C-3') in **4** shifted 3.6 ppm to the lower field at δ 73.9 in **1a**, also indicated that 3-OH in **4** is a linkage position to **1a**. Similarly, the C-23 carbon signal in **3** appeared as a carboxylic carbonyl group at δ 181.0 ppm, while in **1a** this signal appeared as an ester carbonyl group at δ 177.6 in the ^{13}C NMR spectrum, revealed that C-23 carboxylic group in **3** is another linkage position for **1a**. Its

HMBC spectrum exhibited cross peaks between C-24' and H-3' (δ 5.13) and 24'-$COOCH_3$ (δ 3.75), and between C-23 (δ 177.6) and H-3 (δ 4.38), H-24 (δ 1.47), and H-3' (δ 5.13) which further supported the above deduction.

A careful comparison of the ^1H and ^{13}C NMR data of **1a** with those of coreanoside F1, a triterpenoid dimer isolated from *Rubus coreanus*[6], showed that the structures of these two compounds were very similar, except that **1a** had two additional methoxy groups, one was the C-24' ester methyl at δ 51.5 (q), and another was at δ 59.1 (q), connected at C-6 position of the glucosyl moiety, which caused the C-6 signal of glucosyl moiety shifted from *ca* δ 62 ppm in glucosyl to δ 72.5 ppm in 6-methyl-glucosyl of **1a**. Meanwhile, the seven carbon signals of 6-methylglucosyl moiety observed in the ^{13}C NMR spectrum of **1a** apeared almost the same as those of 6-O-β-D-methylglucopyranose[8]. These spectral evidences indicated that one of the glucosyls in coreanoside F1 was substituted by a 6-O-methylglucosyl moiety in **1a**. The fragment ion peaks at *m/z* 677.3927($C_{37}H_{57}O_{11}$) and 501.3252 [677 - methylglucosyl] $^-$ suggest that 6-O-β-D-methylglucopyranosyl moiety must be attached to C-28 position, and then β-D-glucopyranosyl moiety must be attached to C-28' position. The anomeric proton signals at δ 6.25 (1H, d, J = 8.2 Hz) and δ 6.18 (1H, d, J = 8.2 Hz) in the ^1H NMR spectrum of **1a** indicated the β-configuration for both of the glucosyl and methylglucosyl moieties.



m/z 677.3927(calcd for $C_{37}H_{57}O_{11}$, 677.3901)

1 R_1 = 6'-O-β-D-methylglucopyranosyl, R_2 = H
1a R_1 = 6'-O-β-D-methylglucopyranosyl, R_2 = CH_3
2 R_1 = 3'-O-β-D-methylglucopyranosyl, R_2 = H
2a R_1 = 3'-O-β-D-methylglucopyranosyl, R_2 = CH_3

From all above-mentioned, the structure of **1a** was assigned as shown. The naturally occurring compound should be as **1**, named rubupungenoside A. Its NMR spectral data were assigned by detailed analysis of 2D NMR spectral data (^1H-^1H cosy, HMQC, HMBC, HMQC-TOCSY).

Compound **2a**, another amorphous powder, m.p. 216-218 °C, $[\alpha]_D^{21}$ +15.81 (*c* 0.25, CH_3OH), also gave a positive coloration in the Liebermann-Burchard and Molish tests suggesting that **2a** was another triterpenoid glycoside. The presence of hydroxyl, ester carbonyl and double bond in the molecule was suggested by the absorption bands at 3440, 1727, and 1648 cm^{-1} in the IR spectrum. Its negative FABMS showed the same molecular and fragment ion peaks as those of **1a** indicated that **2a** has the same molecular formula as **1a** ($C_{74}H_{114}O_2$). This suggestion was confirmed by its very closed NMR data as **1a** (**Table 1**). In the ^{13}C NMR spectrum, the differences of **2a** to those of

1a were observed with changes in chemical shifts for C-3 and C-6 positions of C-28 methylglucosyl moiety. The C-6 signal of 28-methylglucosyl in **2a** resonated at δ 62.2 (t), 10.3 ppm upfield compared to that of **1a**, indicated the de-etherification at C-6 position of methylglucosyl group. Furthermore, the signal at δ 88.8 (d) corresponding to the C-3 carbon of 28-methylglucosyl residue in **2a**, which shifted 11.0 ppm to lower field compared to that of **1a**, implied the etherification of C-3 hydroxyl group of 28-methylglucosyl moiety in **2a**. The other carbons of **2a** resonated in almost the same positions as in **1a** (**Table 1**).

From the above deduction, the structure of **2a** was established as shown. The related natural compound should be as **2**, which was named as rubupungenoside B.

Table 1. ^{13}C NMR (DEPT) data of compounds **1a**, **2a**, **3** and **4**

C no.	**1a** [a]			**2a** [a]		**3** [b]	**4** [b]
1	48.7 (t)	1'	42.8 (t)	48.7 (t)	43.0 (t)	48.4 (t)	42.8 (t)
2	69.5 (d)	2'	65.8 (d)	69.9 (d)	66.1 (d)	69.4 (d)	67.1 (d)
3	80.8 (d)	3'	73.9 (d)	81.0 (d)	73.4 (d)	81.1 (d)	70.3 (d)
4	55.5 (s)	4'	55.5 (s)	55.5 (s)	55.5 (s)	55.1 (s)	55.5 (s)
5	52.2 (d)	5'	54.4 (d)	52.4 (d)	54.5 (d)	52.7 (d)	47.7 (d)
6	21.2 (t)	6'	20.7 (t)	21.4 (t)	20.7 (t)	21.8 (t)	20.9 (t)
7	32.8 (t)	7'	33.3 (t)	33.1 (t)	33.6 (t)	33.7 (t)	34.1 (t)
8	40.4 (s)	8'	40.4 (s)	40.7 (s)	40.4 (s)	41.1 (s)	40.9 (s)
9	48.5 (d)	9'	48.5 (d)	48.9 (d)	47.3 (d)	48.8 (d)	46.8 (d)
10	38.7 (s)	10'	38.3 (s)	39.0 (s)	38.6 (s)	39.2 (s)	39.0 (s)
11	24.1 (t)	11'	24.2 (t)	24.3 (t)	24.2 (t)	24.7 (t)	24.8 (t)
12	128.2 (d)	12'	127.8 (d)	128.4(d)	128.1 (d)	129.1 (d)	129.4 (d)
13	139.2 (s)	13'	139.1 (s)	139.4 (s)	139.3 (s)	140.1 (s)	140.1 (s)
14	42.8 (s)	14'	40.4 (s)	42.3 (s)	40.7 (s)	42.7 (s)	42.8 (s)
15	29.0 (t)	15'	29.0 (t)	29.1 (t)	29.3 (t)	29.6 (t)	28.9 (t)
16	25.9 (t)	16'	26.0 (t)	26.2 (t)	26.2 (t)	26.6 (t)	26.7 (t)
17	48.1 (s)	17'	48.1 (s)	48.9 (s)	48.9 (s)	48.8 (s)	48.8 (s)
18	54.3 (d)	18'	54.3 (d)	54.6 (d)	54.5 (d)	55.1 (d)	55.2 (d)
19	72.4 (s)	19'	72.4 (s)	72.8 (s)	72.8 (s)	73.6 (s)	73.6 (s)
20	42.0 (d)	20'	42.0 (d)	42.3 (d)	42.2 (d)	43.1 (d)	43.1 (d)
21	26.8 (t)	21'	26.6 (t)	26.8 (t)	26.8 (t)	27.3 (t)	27.3 (t)
22	37.5 (t)	22'	37.5 (t)	37.7 (t)	37.7 (t)	39.0 (t)	39.5 (t)
23	177.6 (s)	23'	68.7 (t)	177.6 (s)	68.9 (t)	181.0 (s)	67.1 (t)
24	12.8 (q)	24'	175.1 (s)	12.9 (q)	175.3 (s)	13.1 (q)	177.1 (s)
25	17.3 (q)	25'	17.3 (q)	17.5 (q)	17.5 (q)	17.4 (q)	14.8 (q)
26	16.5 (q)	26'	16.5 (q)	17.5 (q)	16.8 (q)	17.4 (q)	16.6 (q)
27	24.4 (q)	27'	24.1 (q)	24.7 (q)	24.6 (q)	24.7 (q)	24.7 (q)
28	176.7 (s)	28'	176.7 (s)	177.1 (s)	177.1 (s)	181.0 (s)	181.2 (s)
29	26.8 (q)	29'	26.8 (q)	27.1 (q)	27.1 (q)	27.1 (q)	27.1 (q)
30	16.5 (q)	30'	14.4 (q)	16.8 (q)	14.6 (q)	16.6 (q)	17.4 (q)
24-CO_2 **Me**			51.5 (q)		51.6 (q)		51.8 (q)
Sugar moiety							
1	95.6 (d)		95.7 (d)	95.9 (d)	95.7 (d)		
2	73.8 (d)		73.8 (d)	74.1 (d)	74.1 (d)		
3	77.8 (d)		78.3 (d)	88.8 (d)	79.3 (d)		
4	71.0 (d)		71.1 (d)	70.9 (d)	71.4 (d)		
5	78.7 (d)		79.2 (d)	79.0 (d)	79.0 (d)		
6	72.5 (t)		62.2 (t)	62.2 (t)	62.5 (t)		
6-OMe	59.1 (q)						

3-OMe	60.9 (q)

[a] recorded in C_5D_5N. [b] recorded in CD_3OD.

Since diazomethane has been used to esterify the carboxylic group because of its difficulty of separation, and methanol has been used as elution in the procedures of purification, **1** and **2** are perhaps artifacts. To confirm whether they are natural products or artifacts, we selected a natural compound 2α, 3β, 19α - trihydroxyurs - 12 - en - 24, 28 - dioic acid - 28 - O - β - D - glucopyranosyl ester (trachelosperoside A-1, previously isolated from *Trachelospermum asiaticum*[9] and *Rubus pileatus*[4]) as a control test. This compound was treated with diazomethane to afford its 24-methyl ester derivative which was further subjected to CC on silica gel and eluting repeatedly with the same solvents as used in the separation procedures ($CHCl_3$: CH_3OH : H_2O, 20 : 5 : 1). The eluted compound was collected and its NMR and MS spectral data revealed that the glucosyl moiety remains unchanged, supporting that **1** and **2** are natural products.

Acknowledgments

Financial support by a grant from the National Natural Science Foundation of China is gratefully acknowledged. One of the authors, Dr Wang B. G., also wish to thank the K. C. Wong Education Foundation (Hong Kong) for partial support.

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7. ^{1}H NMR data (pyridine-d_5, 400 MHz): **1a** δ 6.25 (1H, d, *J* = 8.2 Hz, anomeric proton of methylglucosyl moiety), 6.18 (1H, d, *J* = 8.2 Hz, anomeric proton of glucosyl moiety), 5.13 (1H, br.s, H-3'), 3.75 (3H, s, 24'-$COOCH_3$), 3.50 (3H, s, methyl proton of methylglucosyl group), 1.47 (3H, s, H-24), 1.33 (3H, d, *J* = 6.8 Hz, H-30 or H-30'), 1.17 (3H, d, *J* = 8.3 Hz, H-30' or H-30), 1.60, 1.55, 1.07, 1.05, 1.03, 1.01, 0.99, 0.97 (each 3H, s, H-25, 26, 27, 29, 25', 26', 27', and 29'); **2a**: δ 6.28 (1H, d, *J* = 8.0 Hz, anomeric proton of methylglucosyl group), 6.23 (1H, d, *J* = 8.2 Hz, anomeric proton of glucosy moiety), 5.21 (1H, br. s, H-3'), 4.88 (2H, d, *J* = 9.5 Hz, H-23'), 4.34 (1H, d, *J* = 8.9 Hz, H-3), 3.89 (3H, s, 24'-$COOCH_3$), 3.75(3H, s, methyl proton of methylglucosyl group), 1.37 (3H, d, *J* = 7.1 Hz, H-30 or H-30'), 1.05 (3H, d, *J* = 6.0 Hz, H-30' or H-30), 1.68, 1.64, 1.63, 1.50, 1.19, 1.11, 1.09, 0.99, 0.98 (each 3H, s, H-24, 25, 26, 27, 29, 25', 26', 27', 29).
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Received 20 September 1999