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Two Novel Antifungal Saponins from Tibetan Herbal Medicine *Clematis tangutica*

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Abstract: Antifungal assay-guided isolation of the ethanol extract of the aerial parts of *Clematis tangutica* yielded two novel triterpene saponins. Their structures were determined to be 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl ester (**1**) and 3-*O*-β-D-glucopyranosyl–(1→4)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl ester (**2**) on the basis of spectral data and chemical reactions.

Keywords: *Clematis tangutica*, triterpene saponins, hederagenin, antifungal assay.

Many species of the genus of *Clematis* are used in folk medicines and abundant in saponins. It has been reported that the Tibetan herb *Clematis tangutica* (Maxim.) Korsh. (Ranunculaceae) is used to treat indigestion and skin diseases, and phytochemically characterized by containing dozens of triterpene saponins[1, 2]. However, no work has been reported on the bioactivities of this species. During the screening test searching for antifungal agents from higher plants, the ethanol extract of the aerial parts of *C. tangutica* showed antifungal activity against the strain of *Penicillium avellaneum* UC-4376, and two novel antifungal triterpene saponins with oleanolic acid as aglycone moiety, 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl ester (**1**) and 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopy-ranosyl ester (**2**) were obtained through the antifungal assay-guided fractionation.

Compound **1**, obtained as colorless needles (MeOH), $[\alpha]_D^{25}$ +46.7 (c 0.1, MeOH), gave positive coloration in the Liebermann-Burchard and Molish tests. The IR spectrum of **1** revealed the presence of the hydroxyl (3435 cm^{-1}), ester carbonyl (1733 cm^{-1}), and double bond (1646 cm^{-1}) functionalities. The negative HRFABMS determined the molecular formula to be $C_{41}H_{66}O_{12}$ (*m/z* 749.4497 [M – H], calcd. 749.4476). It was evident that **1** was a triterpene saponin related to oleanolic acid based on the ^{1}H-NMR spectral signals assigned to seven tertiary methyl groups at δ 0.92, 0.92, 0.99, 1.00, 1.04, 1.22 and 1.63, and three anomeric protons at δ 5.11, 5.45 and 6.22, together with the ^{13}C-MR signals for olefinic carbons at δ 122.7 and 144.9, two anomeric carbons at δ 101.7 and 104.4, and a carbonyl carbon at δ 180.3. The presence and

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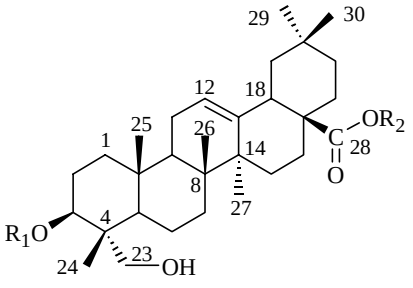
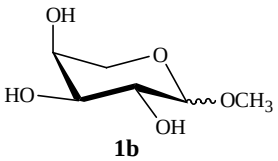
structures of two sugar moieties were determined by the hydrolysis of **1** with 5% HCl methanol solution, which yielded three compounds **1a**, **1b** and **1c** (**Figure 1**). The ^{1}H-, ^{13}C-NMR, DEPT and EIMS data of **1a** were identical to those of 3,23-dihydroxy-12-oleanen-28-oic acid[3]. The two methylated monosaccharides (**1b, 1c**) were chromatographied over silica gel and Sephadex LH-20, and determined to be methyl rhamnoside and arabinoside, respectively, by the comparison of their optical rotations, and R_f values on TLC with those of corresponding synthetic methylated monosaccharides. The sugar linkages were determined on the basis of HMBC experiments. A cross peak of ^{1}H-^{13}C long-range coupling was observed between the proton signal at δ 5.11 (ara-H-1) and the carbon signal at δ 81.1 (C-3), indicating that the arabinose linked at C-3. The ^{1}H-NMR singlet at δ 6.22 showed the presence of C-28 rhamnosyl ester, which was supported by the mild acid hydrolysis of **1** with 1% H_2SO_4 in 80% aqueous methanol solution at the ambient temperature for seven days to afford **1a** and **1d**. Therefore, **1** was determined to be 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl ester.

Compound **2** was established to have the molecular formula of $C_{47}H_{76}O_{17}$ (*m/z* 911.5002 [M – H], calcd. 911.5004) by negative HRFABMS. The ^{1}H- and ^{13}C-NMR spectra of **2** showed great similarities with those of compound **1** except for the glucosyl moiety. The FABMS fragments at *m/z* 766 [M – rha], 750 [M – glc], 603 [M –H – rha – glc] indicated the presence of two terminal sugar moieties. The ^{1}H-NMR singlet at δ 6.26 showed the presence of C-28 rhamnosyl ester, the same as that of **1**, which was confirmed by the molecular ion peak of the acetylated product of **2** at *m/z* 1331. A cross peak of ^{1}H-^{13}C long-range coupling was observed between the proton signal at δ 4.96 (ara-H-1) and the carbon signal at δ 81.1 (C-3), indicating that the arabinose linked at C-3. The proton at δ 5.09 (glc-H-1) had HMBC correlation with the carbon at δ 80.7 (ara-C-4), indicating that the glc-C-1/ara-C-4 linkage was between glucopyranosyl and arabinopyranoyl. So the structure of **2** was identified to be 3-*O*-β-D- glucopyranosyl-(1→4)-α-L-arabinopyranoyl hederagenin 28-*O*-α-L- rhamnopyranosyl ester.

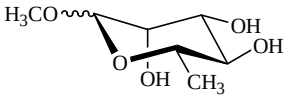
Figure 1



1 R_1 = Ara R_2 = Rha
1a $R_1 = R_2$ = H
1d R_1 = Ara R_2 = H
2 R_1 = Glc(1→4)Ara R_2 = Rha



1b



1c

Table 1 The NMR data of compounds **1** and **2** in C_5D_5N[a]

	1		**2**	
Position	^{13}C	^{1}H	^{13}C	^{1}H
1	39.0	1.51 (m), 1.08 (m)	39.0	1.48 (br d, 12.8), 0.97 (m)
2	26.5	2.12 (2H, m)	26.4	2.13 (2H, m)
3	81.1	4.24 (m)	81.1	4.22 (d, 9.2)
4	43.6	/	43.6	/
5	48.5	1.81(m)	47.8	1.74 (m)
6	18.2	1.72 (m), 1.34 (m)	18.2	1.72 (m), 1.34 (m)
7	33.3	2.02 (m), 1.78 (m)	33.3	1.98 (m), 1.78 (m)
8	39.8	/	39.8	/
9	48.2	1.78 (m)	48.2	1.68 (m)
10	37.0	/	37.0	/
11	23.7	1.92 (2H, m)	23.7	1.89 (2H, br d, 9.2)
12	122.7	5.45 (s)	122.7	5.43 (s)
13	144.9	/	144.9	/
14	42.2	/	42.2	/
15	28.4	2.05 (m), 1.08 (m)	28.4	2.06 (m), 1.10 (H, m)
16	23.9	2.08 (2H, m)	24.0	2.06 (2H, br s)
17	46.7	/	46.7	/
18	42.4	3.28 (m)	42.0	3.26 (m)
19	46.5	1.72 (m), 1.23 (m)	46.5	1.78 (m), 1.23 (m)
20	31.0	/	31.1	/
21	32.8	1.54 (m), 1.27 (m)	32.9	1.56 (m), 1.23 (m)
22	34.3	1.37 (m), 1.23 (m)	34.3	1.38 (m), 1.23 (m)
23	64.0	4.12 (m), 3.75 (d, 11.7)	63.9	4.11 (m), 3.70 (d, 10.4)
24	14.1	1.04 (3H, s)	14.2	1.05 (3H, s)
25	16.1	0.92 (3H, s)	16.2	0.88 (3H, s)
26	17.7	0.99 (3H, s)	17.5	0.97 (3H, s)
27	26.2	1.22 (3H, s)	26.3	1.19 (3H, s)
28	180.3	/	180.5	/
29	33.3	0.92 (3H, s)	33.4	0.88 (3H, s)
30	23.9	1.00 (3H, s)	23.9	0.95 (3H, s)
C-3 Ara-1	104.4	5.11 (d, 6.1)	104.6	4.96 (d, 6.8)
2	75.9	4.58 (m)	76.2	4.48 (m)
3	74.8	4.10 (m)	75.6	4.02 (d, 8.4)
4	69.3	4.16 (m)	80.7	4.14 (m)
5	65.9	4.24 (m), 3.72 (br d, 13.4)	65.7	4.35 (m), 3.63 (br d, 11.6)
Glc-1			106.9	5.09 (d, 7.6)
2			75.3	4.02 (d, 8.4)
3			78.6	4.20 (m)
4			71.2	4.18 (d, 8.8)
5			78.9	3.87 (m)
6			62.5	4.52 (dd, 7.6, 11), 4.32 (m)
C-28 Rha-1	101.7	6.22 (br s)	101.8	6.26 (br s)
2	72.6	4.71 (m)	72.4	4.68 (m)
3	72.2	4.65 (m)	72.5	4.62 (dd, 3.2, 9.2)
4	74.2	4.27 (m)	74.2	4.28 (m)
5	69.4	4.69 (m)	69.7	4.70 (m)
6	18.6	1.63 (3H, d, 6.1)	18.8	1.62 (3H, d, 6.0)

[a] The NMR data for compounds **1** and **2** were recorded on Bruker AM-400 and INOVA-400, respectively.

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