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Triterpenoid Saponins from *Clematis tangutica*

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Abstract: Two new triterpenoid saponins**,** tanguticoside A and B were isolated from aerial part of *Clematis tangutica*. Their structures were elucidated as 3-O-β-D-glucopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl- (1→4)-β-D-glucopyranosyl- (1→6)-β-D-glucopyranoside**1** and 3-O-β-D-glucopyranosyl- (1→2)-β-D-glucopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl- (1→4)-β-D-glucopyranosyl- (1→6)-β-D-glucopyranoside**2**, respectively.

Keywords: *Clematis tangutica,* ranunculaceae, triterpenoid saponins, hederagenin, tanguticoside A and B.

Clematis tangutica (Maxim). Korsh is a Tibetan herb and has been used to treat indigestion and skin diseases[1]. No phytochemical work on this plant has been reported in literature. In our work, some glycosides were obtained from aerial part of this plant. In this paper, the structure elucidation of two new triterpenoid saponins, tanguticoside A and B are described.

On mineral acid hydrolysis, both **1** and **2** yielded the same aglycone. By comparing Mp, MS, ^{1}HNMR and ^{13}CNMR spectra the aglycone was determined to be hederagenin[2]. Glucose and rhamnose were obtained from hydrolysis, and were identified by PC and TLC. These results showed that **1** and **2** are composed of hederagenin, glucose and rhamnose.

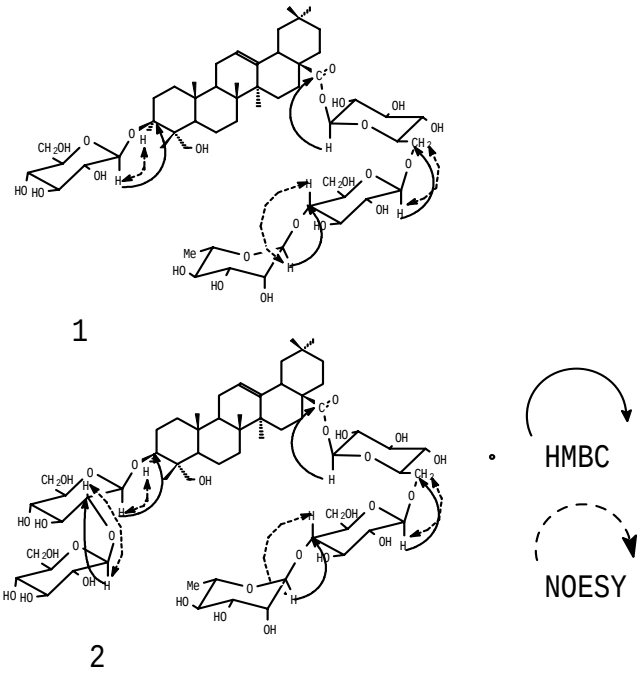
Saponin **1**, white amorphous powder, mp: 210-212℃, $[\alpha]_D^{26}$ -10.6 (c 0.308 MeOH). FABMS (negative): m/z 1104 $[M]^-$ combines with NMR data to give molecular formula ($C_{54}H_{88}O_{23}$). Its IR spectrum showed absorption at 3414, 1735, 1641and 1073 cm^{-1}. The ^{1}HNMR spectrum of **1** exhibited four anomeric proton signals at δ 4.98 (1H, d, J=7.88Hz), 5.12 (1H, d, J=7.22Hz), 5.85 (1H, s) and 6.22 (1H, d, J=8.12Hz), and the ^{13}CNMR spectrum showed four anomeric carbon signals at δ 105.8, 104.9, 102.8 and 95.7. These evidences revealed that **1** is a tetrasaccharide of hederagenin.

On alkaline hydrolysis, **1** gave a prosaponin **1**a, which gave peaks at m/z 633 $[M–H]^-$, 471 $[M–H–glc]^-$ in negative ion FAB mass spectrum. The ^{13}CNMR spectrum showed that C-3 signal of aglycone moiety was shifted to lowfield for 7ppm. This

indicated that sugar moiety was connected to C-3 of hederagenin. Anomeric proton signal at δ 5.10 [1H, d, J=7.64Hz] and carbon signals at δ105.9, 75.9, 78.8, 71.8, 78.3 and 62.9 in ^{1}H and ^{13}CNMR spectrum, showed that sugar moiety is β–D–glucopyranose. So**, 1**a is the 3–O–β–D–glucopyranoside of hederagenin, it is the known saponin caulosapin[3, 7].

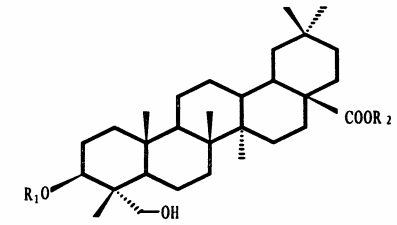
Acetate of **1** gave peaks at m/z 273 [terminal rha $(Ac)_3]^+$**,** 331 [terminal glc $(Ac)_4]^+$**,** 561 [rha $(Ac)_3$+glc $(Ac)_3]^+$, 849 [rha $(Ac)_3$+glc $(Ac)_3]^+$, in positive ion FAB mass spectrum. Obviously, peak 849 [rha $(Ac)_3$+glc $(Ac)_3]^+$ represents the other sugar chain moiety connected to C-28 of hederagenin, also it revealed that connection sequence of monosacchride in sugar chain is rha→glc→glc–. C-28 signal of aglycone δ 176 and anomeric carbon signal of inner glucose δ 95.7 indicated that the sugar chain is connected to C-28 of aglycone by a ester glycoside bond[4, 5]

Figure 1. NOE correlation and ^{13}C–^{1}H long range correlation of saponins 1 and 2



Anomeric proton and carbon signals of each sugar unit were related by HMQC experiment, there are correlation peaks at δ 105.88 (glc'C-1)/5.11 (glc'H-1), 104.85 (glc'''C-1)/4.97 (glc'''H-1), 95.75 (glc''C-1)/6.20 (glc''H-1) and 102.84 (rha C-1)/5.82 (rha H-1). Information about the sequence of oligosaccharide chain and linkage sites to the aglycone was obtained by NOESY and HMBC experiments (**Figure 1**). The NOESY spectrum of **1** showed cross-peaks between signals at δ 5.11 (glc'H-1)/4.24 (H-3 of aglycone), 4.97 (glc'''H-1)/4.32 (glc''H-6) and 5.82 (rhaH-1)/4.39 (glc'''H-4). While the HMBC spectrum revealed cross-peaks between signals at δ 176.72 (C-28 of genin)/5.20 (glc''H-1), 82.51 (C-3 of genin)/5.11 (glc'H-1), 69.28 (glc''C-6)/4.97

(glc'''H-1) and 78.33 (glc'''H-4)/5.82 (rhaH-1). On the basis of all evidences, the structure of saponin **1** was identified to be 3–O–β–D–glucopyranosyl hederagenin 28–O–α–L–O–rhamnopyransyl (1→4)–β–D–glucopyranosyl (1→6)–β–D–glucopyranoside, which was named tanguticoside A.



Saponin	R_1	R_2
1	glc –	rha(1 → 4) glc (1 → 6)glc –
2	glc (1 → 2) glc –	rha(1 → 4) glc (1 → 6)glc –
1a	glc–	H –
2a	glc (1 → 2) glc –	H –

Table 1. ^{13}CNMR chemical shifts of saponins 1, 2, 1a and 2a in pyridine-d_5 (ppm)

C	aglycone moieties				sugar moieties				
	1	2	1a	2a		1	2	1a	2a
1	38.9	39.0	38.8	38.8	3-o-glc-1	105.8	104.0	105.9	104.0
2	26.0	26.1	26.0	26.1	2	75.9	83.8	75.9	83.8
3	82.6	83.1	82.5	83.0	3	78.8[a]	78.6[a]	78.8[a]	78.6[a]
4	43.5	43.7	43.5	43.6	4	71.6	71.6	71.8	71.5
5	47.9	48.4	47.9	48.3	5	78.3[a]	78.2[a]	78.3[a]	78.2[a]
6	18.4	18.5	18.4	18.4	6	63.0	62.8	62.9	62.7
7	32.7	32.8	33.1[a]	33.1[a]	glc-1′		105.8		105.6
8	40.1	40.2	39.9	33.9	2′		76.8		76.8
9	48.3	49.0	48.3	48.7	3′		78.5[a]		78.5[a]
10	37.1	37.1	37.1	37.1	4′		71.5		71.4
11	23.5	23.6	23.9	23.6	5′		78.0[a]		78.1[a]
12	123.0	123.1	122.7	122.7	6′		62.8		62.7
13	144.3	144.4	145.0	145.0	28-o-glc-1″	95.7	95.8		
14	42.3	42.4	42.3	42.2	2″	74.1[b]	74.1[b]		
15	28.4	28.5	28.5	28.5	3″	78.8[a]	78.7[a]		
16	24.0	24.1	23.9	23.9	4″	71.1	71.0		
17	47.2	47.3	46.6	46.6	5″	77.2	77.2		
18	41.6	41.9	42.1	42.34	6″	67.4	69.4		
19	46.4	46.5	46.8	46.6	glc-1‴	104.9	104.8		
20	30.8	30.9	31.1	31.1	2‴	75.4	75.4		
21	34.2	34.2	34.4	34.4	3‴	76.7	76.7		
22	33.0	33.1	33.4	33.4	4‴	78.3[a]	78.7[a]		
23	65.1	65.3	65.0	65.2	5‴	78.1[a]	78.1[a]		
24	13.7	13.7	13.8	13.6	6‴	61.5	61.5		
25	16.3	16.4	16.2	16.2	rha-1	102.8	102.9		
26	17.7	17.8	17.6	17.6	2	72.8	72.8		
27	26.2	26.3	26.3	26.3	3	72.6	72.6		
28	176.6	176.7	180.3	180.4	4	74.0[b]	74.1[b]		
29	33.2	33.3	33.4[a]	33.4[a]	5	70.4	70.5		
30	23.8	23.8	23.9	23.9	6	18.6	18.6		

[a b]Signals may be interchangeable in the same column.

Saponin **2,** white amorphous powder mp 214–216 °C $[\alpha]_D^{18.7}$ -7.35 (c 0.510 MeOH). FABMS (negative): m/z 1266 $[M]^-$, combines with NMR data to give molecular formula $C_{60}H_{98}O_{28}$. Its IR spectrum showed absorption at 3401, 2940, 1729, 1695, 1025 cm^{-1}. The ^{1}HNMR of **2** showed five anomeric proton signals at δ 4.96 (1H, d, J=7.60Hz), 5.07 (1H, d, J=6.24Hz), 5.37 (1H, d, J=7.60Hz), 5.79 (1H, s), 6.18 (1H, d, J=7.92Hz). The positive FAB mass spectrum of its acetate gave fragment ions at m/z 273 $[rha\ (Ac)_3]^+$, 332 $[\ (Ac)_4]^+$, 561 $[rha\ (Ac)_3+glc\ (Ac)_3]^+$**,** 620 $[\ (Ac)_4+glc\ (Ac)_3]^+$**,** 849 $[rha\ (Ac)_3+2glc\ (Ac)_3]^+$. On alkaline hydrolysis, saponin **2** gave a prosaponin **2a,** which gave peaks at m/z 795 $[M–H]^-$**,** 633 $[M–H–glc]^-$**,** 471 $[M–H–2glc]^-$ in the negative FAB mass spectrum. The ^{13}CNMR and ^{1}HNMR spectrum of the **2**a showed the presence of two mono saccharide units by anomeric signals due to sugar moieties [anomeric carbon: δ 105.8, 104.0; anomeric proton: δ 5.41 (1H, d, J=7.52Hz), 5.11 (1H, d, J=6.88Hz)]. By 2DNMR experiment and comparison with carbon signals of β-sophorose in ^{13}CNMR spectrum[6], it is seen that the structure of the prosaponin is 3-O- [β-O-glucopyranosyl- (1→2)-β-D-glucopyranosyl]-hederagenin. It was isolated from *Hedera helix L.* by K.Hostettman[7]. The carbon signals due to 28-O-sugar moieties of saponin **2** were identical with those of the 28-O-sugar moieties of saponin **1**. While, proton signal related with carbon signal of sugar showed correlation peaks: δ 95.83/6.23, 102.89/5.82, 104.08/5.07, 104.84/4.98, 105.79/5.40, 69.29/4.67, 83.68/4.18, 75.46/3.99 in HMQC experiment The cross-peaks between signals at δ 176.91/6.23, 78.19/5.82, 83.68/5.40, 83.08/5.07, 69.29/4.98 in HMBC experiment and at δ 6.23/4.06, 5.82/4.39, 5.39/4.19, 5.07/4.15, 4.98/4.30 in NOESYexperiment revealed the sequence and linkage sites of sugar unit in oligosaccharide chain. The information obtained by FABMS, ^{1}HNMR, ^{13}CNMR, HMQC, HMBC and NOESY showed saponin **2** is 3-O-β-D-glycopyranosyl (1→2)β-D-glycopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl (1→4)-β-D-glycopyranosyl (1→6)glycopyranoside**,** it is named as tanguticoside B

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References

1. Northwest Plateau Institute of Biology**,** Academia Sinica. *"Zang Yao Zhi"*, Qinhai People's Press, Xining. **1991,** p. 249.
2. K. Tori, S. Seo, A. Shimaoka, and Y. Tomita, *Tetrahedron Letters.* , **1974,** *48*, 422.
3. T. Aoki, Y. Tanio, andT. Suga, *Phytochemistry***, 1976,** *15* (5)**,** 781.
4. a)M. A. Dubois, M. Ilyas, and H. wagner, *Planta Med*, **1986,** *52* (2), 80
   b) H. Kizu, S. Kitayama, F. Nakatani, and T. Tominor, *Chem. Pharm. Bull.* , **1985,** *33* (8)**,** 3324.
5. X-C. Li, D-Z. Wang, X-Gong. Wu, C-R. Yang, *Phytochemistry*, **1990**, *29* (2)**,** 595.
6. T. Usui, N. Yamaoka, K. Matsuda, and K. Tuzimura, *J. Chem. Soc. Perk I* . , **1973,** 2425.
7. K. Hostettmann, *Helv. Chim. Acta*. **1980,** *61* (3), 606.

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