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Oleanane-triterpene Saponins from *Clinopodium urticifolium*

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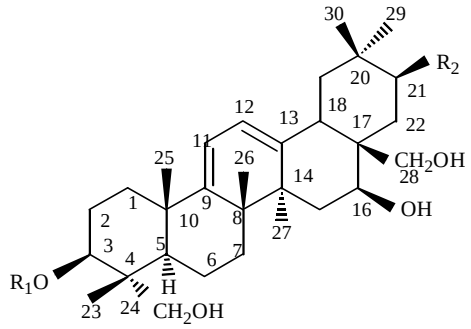
Abtract: Four new oleanane triterpene saponins were isolated and purified from the whole plant of *Clinopodium urticifolium.* They were 3β, 16β, 23, 28-tetrahydroxyoleana-9 (11), 12(13)-diene-3-yl-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]- β-D-fucopyranoside **1**; 3β, 16β, 21β, 23, 28-pentahydroxyoleana-9(11), 12(13)-diene-3-yl-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]- β-D-fucopyranoside **2**; 3β, 16β, 23, 28-tetrahydroxyoleana-9(11), 12(13)-diene-3-yl-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside **3**; 3β, 16β, 23, 28-tetrahydroxyoleana-9(11), 12(13)-diene-3-yl-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]- β-D-fucopyranoside **4**. Their structures were elucidated on the basis of interpretation of NMR and MS data and from chemical evidence.

Keywords: *Clinopodium urticifolium, Labiatae,* oleanane triterpene saponins.

The genus of *Clinopodium* is a popular Chinese traditional medicinal herb, used as salve for bruises and swelling, and is also purported to improve blood circulation[1]. In recent year, several papers have described phytochemical investigations of various species of *Clinopodium*[2, 3]. *Clinopodium urticifolium Labiatae* is native to Gansu province, chemically has not been investigated. During our systematic phytochemical investigation of this whole plant,fourteen oleanane-triterpene saponins were isolated from an *n*-BuOH portion of the MeOH extract of *C. Urticifolium*,among them four were new compounds. In this paper we describe the isolation and structure elucidation of four new oleanane-triterpene saponins **1**, **2**, **3** and **4.**



	R_1	R_2
1.	Fuc(2→1)Glu, Glu(3→1)	H
2.	Fuc(2→1)Glu, Glu(3→1)	−OH

	R_1	R_2
3.	Fuc(2→1)Glu, Glu(3→1)Glu(6→1)Glu	H
4.	Fuc(2→1)Glu, Glu(3→1)Glu(6→1)Glu(4→1)Glu	H

The HRESIMS of **1** showed pseudo-molecular ion peaks at *m/z* 965.5092$[M+Na]^+$ and 943.5250$[M+H]^+$, which, together with the NMR data, enabled the molecular formula to be determined as $C_{48}H_{78}O_{18.}$ IR absorption bands at 3300 cm^{-1} revealed the presence of hydroxyl groups. After acid hydrolysis, the modified aglycone, saikogenin H, was obtained as a white powder, which had a molecular ion peak at *m/z* 472 $[M]^+$ (using EIMS), a homoannular diene structure (from UV absorption at 281 nm), and was eventually characterized as a analogous saikogenin B[6] by comparison of its ^1H and ^{13}C-NMR data. In the ^1H-NMR spectrum, the proton signals were assigned by means of ^1H-^1H COSY, and showed six single methyl proton signals at δ 0.86, 0.89,1.08,1.20, 1.24, and 1.25, two olefin proton signals at δ 5.71 and 5.65(each 1H, d, J=5.8Hz, H-11 and H-12), and three sugar anomeric proton signals at δ 4.91(d, J=8.0Hz), 5.31(d, J=8.0Hz) and 5.57(d, J=8.0Hz). The position of two double bond at $\Delta^{9(11)}$ and $\Delta^{12(13)}$ were also secured by HMBC correlations of the H-11(δ5.71,d, J=5.8) with C-10 (δ 38.6), C-8(δ 42.0), and C-13(δ 144.6), H-12(δ 5.65, d, J=5.8) with C-9(δ 155.0), C-14(δ 42.1), and C-18(δ 42.7). On hydrolysis, the sugar units of **1** were identified as D-glucose and D-fucose by paper chromatography, comparing with authentic samples. The positions of linkage of the sugars were determined by comparing its NMR spectral data with buddlejasaponin IV, buddlejasaponin IVb[4] and were further established by HMBC cross-peaks between H-1′and C-3,H-1″and C-2′, H-1‴and C-3′ respectively. Based on the above results, it was concluded that saponin **1** has a similar structure to that of buddlejasaponin IVb[2, 4], except the different position of two double bonds, and the former has a homoannular diene structure, and the latter has a heterannular conjugated diene system. Thus, the structure of **1** is 3β, 16β, 23, 28-tetrahydroxyoleana-9(11), 12(13)-diene-3-yl-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]- β-D-fucopyranoside.

Table 1 ^1H-NMR data for saponin **1-4** in pyridine-d_5 at 400 MHz (δ_{ppm}, J_{Hz})

H	**1**	**2**	**3**	**4**	H	**1**	**2**	**3**	**4**
3	4.01(t)	4.00(t)	4.07(t)	4.10(t)	Fucose 1	4.91 (d,8)	4.90 (d,8)	4.76 (d,8)	4.75 (d,8)
11	5.71 (d,5.8)	5.73 (d,5.7)	5.72 (d,5.8)	5.70 (d,5.8)	Glu(C-2 of fuc) 1	5.57 (d,8)	5.59 (d,8)	5.53 (d,8)	5.53 (d,8)
12	5.65 (d,5.8)	5.65 (d,5.7)	5.62 (d,5.8)	5.60 (d,5.8)	Glu(C-3 of fuc) 1	5.31 (d,8)	5.32 (d,8)	5.17 (d,8)	5.17 (d,8)
21		3.61 (t, 4.4)			Glu(C-6 of glc,C-3 of fuc) 1			4.57 (d,8)	4.88 (d,8)
23	3.58 (d,11) 4.28 (d,11)	3.61 (d,11) 4.34 (d,11)	3.68 (d,11) 4.32 (d,11)	3.69 (d,11) 4.35 (d,11)	Term glu 1				4.93 (d,8)
24	1.08(s)	1.10(s)	1.08(s)	1.09(s)					
25	1.25(s)	1.26(s)	1.25(s)	1.25(s)					
26	1.24(s)	1.25(s)	1.24(s)	1.24(s)					
27	1.20(s)	1.21(s)	1.20(s)	1.20(s)					
29	0.89(s)	1.20(s)	0.96(s)	0.96(s)					
30	0.86(s)	1.25(s)	0.86(s)	0.85(s)					

Table 2 ^{13}C-NMR data for saponin **1-4** in pyridine-d_5 at 100 MHz (δ_{ppm}, J_{Hz})

C	1	2	3	4	C	1	2	3	4
1	37.3	37.6	37.6	37.5	Fucose				
2	26.0	26.8	26.7	26.7	1	103.9	104.0	104.0	104.0
3	82.5	82.5	84.8	84.9	2	77.1	77.1	77.3	77.2
4	38.7	38.7	40.6	40.5	3	84.7	84.2	85.0	85.1
5	47.8	44.1	43.8	43.7	4	71.5	71.4	71.9	71.9
6	18.4	18.1	18.1	18.1	5	70.4	70.1	70.0	70.6
7	32.1	32.1	34.1	34.1	6	17.1	17.3	17.3	17.3
8	42.0	43.3	43.3	43.2	Glu(C-2 of fuc)				
9	155.0	155.1	154.9	154.9	1	104.0	104.0	104.0	104.0
10	38.6	36.8	38.7	38.6	2	76.2	76.0	76.2	76.2
11	116.0	116.0	116.0	115.9	3	78.7	78.9	78.8	78.8
12	121.5	121.6	121.2	121.1	4	72.1	72.0	72.2	72.2
13	144.6	144.5	145.1	145.2	5	77.5	77.4	77.5	77.5
14	42.1	43.8	43.3	43.1	6	63.0	63.1	63.2	63.2
15	36.2	36.2	36.2	36.1	Glu(C-3 of fuc)				
16	67.8	67.5	66.8	66.7	1	105.1	105.1	104.9	104.8
17	40.5	43.1	44.2	44.2	2	75.3	75.2	75.2	75.2
18	42.7	42.2	42.7	42.6	3	78.3	78.4	78.4	78.3
19	47.5	47.6	42.0	46.9	4	71.9	71.8	72.0	71.9
20	30.5	30.3	31.0	31.0	5	78.4	78.3	77.1	77.1
21	34.9	72.5	32.1	32.1	6	62.4	62.4	70.3	70.3
22	27.0	35.0	26.0	26.0	Glu(C-6 of glc, C-3 of fuc				
23	64.5	65.1	65.1	65.2	1			105.4	104.8
24	17.0	17.2	17.2	17.2	2			75.4	74.9
25	21.0	21.0	21.0	21.0	3			78.4	76.6
26	21.3	21.2	21.3	21.2	4			71.7	80.9
27	26.0	26.1	26.0	26.0	5			78.4	76.5
28	73.1	69.0	69.3	69.3	6			62.7	62.0
29	31.6	29.8	33.2	33.1	Term glu				
30	26.0	17.9	24.0	24.0	1				104.8
					2				74.7
					3				78.2
					4				71.5
					5				78.5
					6				62.4

Saponin **2** was obtained as an amorphous white powder and gave colorations in the Liebermann-Burchard and Molish tests for triterpenoid saponins. The FABMS of **2** revealed an $[M+Na]^+$ ion peak at *m/z* 981 and an $[M+Li]^+$ ion peak at *m/z* 965, which, together with the NMR data, enabled the molecular formula to be determined as $C_{48}H_{78}O_{19}$. The NMR assignments were performed by means of HMBC and HMQC methods. Comparing its ^{1}H, ^{13}C–NMR data with those of saponin **1**, there is the same sugars and the same arrangement of the sugar moieties. The difference is only that **2** has one more oxygen atom than in **1**, the DEPT spectrum showed the former has one more CH and one less CH_2, this exhibited one more OH substituent in the aglycone of **2 .** The δ_C of C-29 (-3 ppm) and C-30 (-6 ppm), when it compared with saikogenin G[5] strongly suggested that the additional hydroxyl group was located at C-21, since if its location on C-22 could not have the effects of the same magnitude[7]. On the basis of the splitting pattern of H-21 at δ3.61(t, J=4.4 Hz) and C-21 at δ 72.5 established the configuration of OH-21 to be β-orientation[2]. The HMBC correlations of the H-21 with C-29(δ29.8), C-

30(δ17.9), and C-17(δ43.1) were consistent with above results.The mutual HMBC correlations between the methine C-3 and an anomeric methine (δ_H 4.90, δ_C 104.0) allowed to place a glycosidic linkage at this position. Consequently, **2** was identified as 3β, 16β, 21β, 23, 28-pentahydroxyoleana-9(11),12(13)-diene-3-yl-[β-D- glucopyranosyl-(1→2)]-[β -D-glucopyranosyl-(1→3)]- β-D-fucopyranoside.

The molecular formula of saponin **3** was deduced as $C_{54}H_{88}O_{23.}$ On the basis of FABMS and the NMR results. The spectral data of this compound were very similar to those of **1**. Careful analysis of the ^{1}H and ^{13}C NMR data revealed that **3** was structurally identical to **1** with respect to the aglycone. The significant difference in the NMR spectra was that saponin **3** has one more sugar than that of saponin **1**. The arrangement of sugar units was determined by HMBC experiment. The cross-peak between H-1‴and C-6‴established that the additional glucose was linked to C-6‴ of saponin **1**. In addition, there are the same sugars and the same positions of linkage of sugars with clinoposaponin III[2]. Based on the above evidence, the structure of saponin **3** is 3β, 16β, 23, 28-tetrahydroxyoleana-9(11),12(13)-diene-3-yl-[β-D-glucopyranosyl-(1→6)- β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]- β-D-fucopyranoside.

The molecular formula of saponin **4** was deduced as $C_{60}H_{98}O_{28}$ on the basis of HREIMS and ^{13}C-NMR analysis. The spectral data of this compound were highly compatible with those of saponin **1** and **3.** A combination of 2D NMR experiments revealed that the aglycone of **4** was the same as that of saponin **1** and **3** , while the sugar residues as well as their arrangement patter n were totally identical to those of clinoposaponin V and clinoposaponin Vb[2]. Saponin **4** has one more sugar than that of **3** , the extra glucose was attached to C-4⁗ of saponin **3** by HMBC cross-peak between H-1⁗ and C-4⁗ The ion peaks at *m/z* 1105.5761[M-glu]$^+$, 943.5145[M+H-2glu]$^+$, 796.2876[M-2glu-fuc]$^+$, 633.2236[M-3glu-fuc]$^+$, and 71.1718[M-4glu-fuc]$^+$ in the ESIMS spectrum indicated further the presence of five sugars. Thus, the structure of saponin **4** is 3β, 16β, 23, 28-tetrahydroxyoleana-9(11), 12(13)-diene-3-yl-[β-D-gluco-pyranosyl- (1→4)- β-D-glucopyranosyl- (1→6)- β-D-glucopyranosyl- (1→3)]-[β -D-glucopyranosyl- (1→2)]- β-D-fucopyranoside.

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