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Segetoside F a new triterpenoid saponin with inhibition of luteal cell from the seeds of *Vaccaria segetalis*

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Abstract

A new triterpenoid saponin, named segetoside F, showing strong inhibition of luteal cell activity, has been isolated from the seeds of *Vaccaria segetalis*. Its structure has been established by chemical reactions and spectral analyses.

In previous papers,[1–3] we have reported the isolation and structural elucidation of segetoside A, C–E, G–I from the seeds of *Vaccaria segetalis* (Neck) Garcke (Caryophllaceae), which is distributed all over China, except southern China, and used in Chinese folk medicine for promoting diuresis, activating blood circulation and relieving carbuncles.[4] Further investigation of this seed led to the isolation of a new triterpenoid saponin, named segetoside F (**1**), which has the inhibition of luteal cell activity. In this paper we wish to report its structural elucidation and the inhibition of luteal cells.

The *n*-butanol fraction from the ethanol extract of the seeds of *Vaccaria segetalis* was chromatographed on Diaion HP-20, silica gel and RP-18 silica gel to afford segetoside F (**1**).

Segetoside F (**1**), an amorphous solid, has the molecular formula $C_{67}H_{104}O_{32}$ determined by positive ion FABMS (at m/z 1444 $[M+Na]^+$) as well as ^{13}C and DEPT NMR data. Its spectral features and physicochemical properties suggested **1** to be a triterpenoid saponin. Comparison of the signals from the aglycon moiety in the ^{13}C NMR spectra with those from gypsogenin[5] showed that the aglycon of compound **1** was gypsogenin and sugars were bound to the C-3 and C-28 positions of gypsogenin. The hexasaccharide nature of compound **1** was manifested by its

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Table 1
^{13}C (150 MHz) NMR of compound **1** and ^{1}H (600 MHz) NMR spectra data for the sugar moieties of **1** (C_5D_5N) (δ in ppm, J in Hz)

Position	δ_C	Position	δ_C	δ_H
1	38.2t	Glucuronic acid		
2	25.0t	1	103.5d	4.88 (1H, d, $J=7.2$)
3	83.8d	2	82.1d	4.17 (1H, m)
4	55.0s	3	77.4d	4.22 (1H, m)
5	48.8d	4	72.6d	4.40 (1H, m)
6	20.7t	5	76.8d	4.39 (1H, m)
7	32.5t	6	170.3s	
8	40.2s	6-OMe	52.1q	3.71 (3H, s)
9	47.8d	Galactose		
10	36.3s	1	106.3	5.21 (1H, d, $J=8.0$)
11	23.4t	2	74.5d	4.51 (1H, m)
12	122.4d	3	74.8d	4.11 (1H, m)
13	144.1s	4	70.1d	4.52 (1H, m)
14	42.3s	5	77.1d	4.10 (1H, m)
15	28.4t	6	62.2t[a]	4.50 (2H, m)
16	23.7t	Fucose		
17	47.2s	1	94.3d	6.00 (1H, d, $J=8.0$)
18	42.0t	2	73.8d	4.55 (1H, m)
19	46.4t	3	80.9d	4.25 (1H, m)
20	30.7s	4	73.9d	5.78 (1H, m)
21	34.0t	5	70.8d	3.96 (1H, m)
22	32.3t	6	16.5q	1.18 (3H, d, $J=6.0$)
23	209.8d	4-OAc; CH_3	20.7q	1.96 (3H, s)
24	11.1q	4-OAc; C=O	170.7s	
25	15.8q	Arabinose		
26	17.4q	1	111.8d	5.73 (1H, brs)
27	25.9q	2	83.6d	4.87 (1H, m)
28	176.4s	3	78.0d	4.80 (1H, m)
29	33.1q	4	85.8d	4.68 (1H, m)
30	23.7q	5	61.9t[a]	4.30 (1H, m)
				4.16 (1H, m)
		Rhamnose		
		1	102.1d	6.01 (1H, S)
		2	71.5d	4.72 (1H, m)
		3	72.4d	4.57 (1H, m)
		4	84.9d	4.28 (1H, m)
		5	68.7d	4.36 (1H, m)
		6	18.6q	1.78 (3H, d, $J=6.0$)
		Xylose		
		1	107.5d	5.02 (1H, d, $J=7.2$)
		2	76.2d	4.00 (1H, m)
		3	78.7d	4.02 (1H, m)
		4	70.5d	4.18 (1H, m)
		5	67.5t	3.51 (1H, t, $J=10.0$)
				4.22 (1H, m)

[a] Signals may be interchanged.

^{1}H [δ 6.01, s; δ 6.00, d, J=8.0 Hz; δ 5.73, brs; δ 5.21, d, J=8.0 Hz; δ 5.02, d, J=7.2 Hz; δ 4.88, d, J=7.1 Hz] and ^{13}C [δ 111.8, 107.5, 106.3, 103.5, 102.1, 94.3] NMR data, respectively (Table 1). Alkaline hydrolysis of compound **1** followed by acid hydrolysis gave fucose, xylose, arabinose and rhamnose. On the other hand, acid hydrolysis of **1** gave glucuronic acid, galactose, fucose, xylose, arabinose and rhamnose, so glucuronic acid and galactose were connected to C_3 position of the aglycone, the other four sugars were connected to C_{28} position. The identity of the monosaccharide and the sequence of the oligosaccharide chain were determined by a combination of DEPT, COSY, HMQC, HMQC-RELAY, HMQC-TOCSY, HMBC-TOCSY and HMBC. In the light of the assigned ^{1}H and ^{13}CNMR spectra (Table 1), the arabinose sugar unit was identified as α-arabinofuranose,[6] and other sugar units were in pyranose form. The α anomeric configuration for the rhamnose was judged by its C_5 data (δ 68.7). The β anomeric configurations for the glucuronic acid, the galactose, the fucose and the xylose were judged from their large $^3J_{H1,H2}$ coupling constants (7–8 Hz). The HMBC spectrum showed that C_3 with H_{GluA1}, C_{GluA2} with H_{Gal1}, C_{28} with H_{F1}, C_{F2} with H_{R1}, C_{F3} with H_{A1}, C_{R4} with H_{X1}, C_{GluA6} with $H_{\delta 3.71}$(–OCH_3), $C_{\delta 170.7}$ (C=O of acetyl) with H_{F4} and $H_{\delta 2.00}$ (CH_3 of acetyl) have cross peaks. Thus, segetoside F (**1**) (Fig. 1) was determined to be 3-*O*-β-D-galactopyranosyl(1→2)-β-D-(6-*O*-methyl ester)-glucuronopyranosyl-gypsogenin-28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinofuranosyl(1→3)]-β-D-(4-*O*-acetyl)-fucopyranoside.

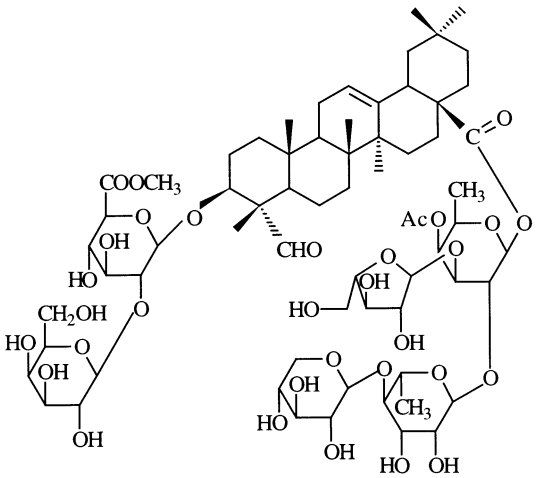

Figure 1. Structure of segetoside F

Segetalin F exhibited the strong activity of inhibition of luteal cell resulting in 100% at a concentration of 20 μg/ml, Its IC_{50} was 12.6 μg/ml. The observation that a saponin like segetoside F showed inhibition of luteal cell activity is unique.

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