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Triterpenoid Saponins from *Vaccaria segetalis*

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Abstract – Two new triterpenoid saponins, named segetoside D and E, have been isolated from the seeds of *Vaccaria segetalis*. On the basis of chemical reactions and spectral data, structures of segetoside D and E have been established as: 28-*O*-[*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)]-[5-*O*-acetyl-*α*-arabinofuranosyl(1→3)]-[4-*O*-acetyl-*β*-D-fucopyranosyl]-quillaic acid-3-O-[*β*-D-galactopyranosyl(1→2)]-6-*O*-methyl ester-*β*-D-glucuronopyranoside and 28-*O*-[*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)]-[5-*O*-acetyl-*α*-arabinofuranosyl(1→3)]-[4-*O*-acetyl-*β*-D-fucopyranosyl]-quillaic acid -3-*O*-[*β*-D-galactopyranosyl(1→2)]-6-O-n-butyl ester-*β*-D-glucuronopyranoside, respectively.

Key words – *Vaccaria segetalis*, Caryophyllaceae, triterpenoid saponins; segetoside D and E.

Introduction

The seeds of *Vaccaria segetalis* (Neck) Garcke which is distributed all over China except southern China, are used in Chinese folk medicine for promoting diuresis, activating blood circulation and relieving carbuncles (Jiangsu New Medical College, 1986). Previous studies on the seeds have led to the isolation of eight cyclic peptides (Morita, *et al.*, 1995, 1996; Yun, *et al.*, 1997) and several saponins (Amanmuradov, *et al.*, 1964; Litvinenko, *et al.*, 1967; Morita, *et al.*, 1997; Yun, *et al.*, 1998; Koike, *et al.*, 1998). We have reported the isolation and structural elucidation of a new phenylpropanoid glycoside, segetoside A and several new triterpenoid saponins from the seeds of *V. segetalis* (Sang, *et al.*, 1998). In our continued investigation of the seeds, two new triterpenoid saponins, named segetoside D (**1**) and E (**2**), were isolated. This paper deals with the isolation and structural elucidation of segetoside D (**1**) and E (**2**).

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Experimental

General – CC: silica gel 60H, TLC: HSGF 254 (Qingdao Haiyang Chemical Group CO. of China). Optical rotation: JASCO-DIP-181 polarimeter. IR: Perkin-Elmer 599 infrared spectrometer. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR: JEOL α600 with NM-AFG type field gradient unit, TMS as int. standard. FAB-MS: MAT-95 Mass spectrometer.

Plant material – The seeds of *Vaccaria segetalis* were purchased at Shijia Zhuang, Hebei Province (China) in 1995. The botanical identification was made by Professor Xuesheng Bao (Shanghai Institute of Drug Control). A voucher specimen has been deposited at the Herbarium of the Department of Phytochemistry, Shanghai

Institute of Materia Medica Chinese Academy of Sciences.

Extraction and isolation - The powdered seeds of *V. segetalis* (50 Kg) were extracted successively with petroleum ether $\times 2$ and 95% EtOH$\times 3$. After evaporation of ethanol in vacuo, the residue was suspended in water and then extracted successively with CH_2Cl_2, EtOAc and *n*-BuOH. The *n*-BuOH fraction (450 g) was subjected to Diaion HP-20 using a EtOH-H_2O gradient system (0%-100%). The fraction (70 g) eluted by 70% EtOH was subjected to silica gel CC with a CH_2Cl_2-MeOH-H_2O solvent system (5:1:0.1-2:1:0.2). The fraction eluted by CH_2Cl_2-MeOH-H_2O (4:1:0.15) was subjected to RP-18 silica gel CC with 75% MeOH to get compounds **1** (50 mg) and **2** (27 mg).

Compound **1**: an amorphous solid, $[\alpha]_D^{24}$ -13.97° (MeOH, c 1.40). IR_{max}^{KBr} cm^{-1}: 3408, 1732, 1100-1000. FAB-MS: m/z 1502 $[M+Na]^+$, ^1H-NMR (C_5D_5N) of the triterpene moity of **1**: δ 9.90 (H-23, s), 5.59 (H-12, s), 5.20 (H-16, m), 4.07 (H-3, m), 3.38 (H-18, m), 2.74 (H-19a, t, J=13.4), 1.37 (H-19b, m), 1.76 (H-27, s), 1.41 (H-24, s), 1.07 (H-26, s), 1.02 (H-30, s), 0.97 (H-29, s), 0.85 (H-25, s); ^{13}C-NMR (C_5D_5N) of the triterpene moity of **1**: (see Table 1); ^1H-NMR (C_5D_5N) and ^{13}C-NMR (C_5D_5N) of the sugar moieties of **1**: (see Table 2).

Compound **2**: an amorphous solid, $[\alpha]_D^{24}$ -17.93° (MeOH, c 0.50). IR_{max}^{KBr} cm^{-1}: 3415, 1732, 1100-1000. FAB-MS (m/z): 1544$[M+Na]^+$, ^1H-NMR (C_5D_5N) of the triterpene moity of **2**: δ 9.90 (H-23, s), 5.59 (H-12, s), 5.20 (H-16, m), 4.07 (H-3, m), 3.37 (H-18, m), 2.74 (H-19a, t, J=13.4), 1.37 (H-19b, m), 1.76 (H-27, s), 1.42 (H-24, s), 1.08 (H-26, s), 1.02 (H-30, s), 0.97 (H-29, s), 0.87 (H-25, s); ^{13}C-NMR (C_5D_5N) of the triterpene moity of **2**: (see Table 1); ^1H-NMR (C_5D_5N) and ^{13}C-NMR (C_5D_5N) of the sugar moieties of **2**: (see Table 2).

Alkaline hydrolysis of **1** and **2**-Compound **1** and **2** (each 3 mg) was dissolved in 10% KOH/H_2O and kept at room temp. for 4 hr, respectively. The reaction mixt. was neutralized with 2N HCl and extracted with 1-butanol. The sugar fraction was concentrated and dissolved in 2N HCl and heated at 100°C for 2 hr, respectively. The reaction mixt was concentrated and then compared with standard sugars on HR-TLC silica gel plate developed with *n*-BuOH-Me_2CO-H_2O (4:5:1) and $CHCl_3$-MeOH-H_2O (7:3:0.5), detected by spraying with Aniline-phthalic acid reagent [Aniline:Phthalic acid:*n*-BuOH (2:3:200)] and then heating to 110°.

Acid hydrolysis of **1** and **2**-Compound **1** and **2** (each **3** mg) was dissolved in 2N HCl and heated at 100°C for 2 hr, respectively. The reaction mixt. was neutralized with 10% KOH and extracted with $CHCl_3$. The residue solution was concentrated and then compared with standard sugars on HR-TLC silica gel plate developed with *n*-BuOH-Me_2CO-H_2O (4:5:1) and $CHCl_3$-MeOH-H_2O (7:3:0.5), detected by spraying with Aniline-phthalic acid reagent [Aniline:Phthalic acid:*n*-BuOH (2:3:200)] and then heating to 110°.

Results and Discussion

Segetoside D (**1**), an amorphous solid, had a molecular formula of $C_{69}H_{106}O_{34}$ determined by positive ion FAB-MS (at m/z 1502 $[M+Na]^+$) as well as ^{13}C, DEPT NMR data. Its spectral features and physicochemical properties suggested **1** to be a triterpenoid saponin. Of the sixty nine carbons, thirty were assigned to the aglycone part, thirty four to the oligosaccharide moiety, four to the acetyl, and one to the methoxy (Tables 1 and 2). Its IR spectrum showed characteristic absorptions for hydroxyl (3408 cm^{-1}), ester (1732 cm^{-1}) and a glycosidic linkage (1000-1100 cm^{-1}). Comparison of the signals from the triterpene moiety in the ^{13}C-NMR spectra (Table 1) with those from quillaic acid (Mahato, *et al.*, 1994) showed that the

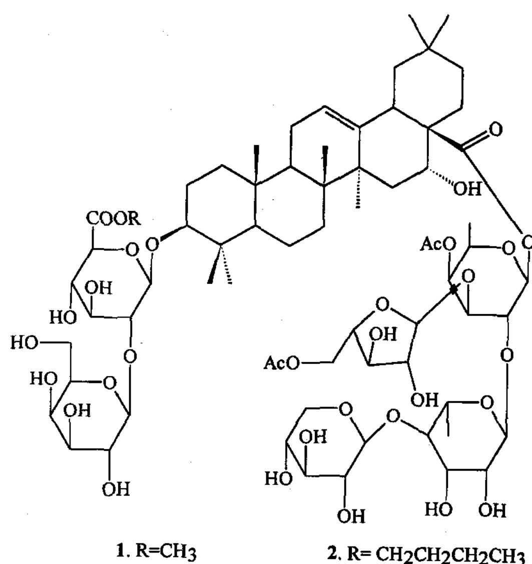
Table 1. ^{13}C (150 MHz) NMR spectra data of the triterpene moiety of **1**, **2** (C_5D_5N) and quillaic acid ($CDCl_3$) (δ in ppm)

	triterpene moiety of 1	triterpene moiety of 2	quillaic acid
1	38.2t	38.2t	38.1t
2	25.0t	25.0t	26.0t
3	83.5d	83.5d	71.7d
4	55.0s	55.0s	55.2s
5	48.6d	48.6d	48.1d
6	20.7t	20.7t	20.7t
7	33.2t	33.2t	32.3t
8	40.3s	40.3s	39.7s
9	47.0d	47.0d	46.7d
10	36.3s	36.3s	35.9s
11	23.8t	23.8t	23.2t
12	122.1d	122.1d	122.2d
13	144.6s	144.6s	142.8s
14	42.2s	42.2s	41.4s
15	36.1t	36.1t	35.4t
16	74.0d	74.0d	74.7
17	49.4s	49.4s	48.7s
18	41.6t	41.6t	40.5t
19	47.6t	47.6t	46.4t
20	30.7s	30.7s	30.0s
21	36.1t	36.1t	35.4t
22	31.9t	31.9t	30.3t
23	209.5d	209.5d	207.0d
24	11.0q	11.0q	9.0q
25	15.9q	15.9q	15.7q
26	17.5q	17.5q	16.9q
27	27.1q	27.1q	27.0q
28	175.9s	175.9s	177.2s
29	33.2q	33.2q	32.7q
30	24.6q	24.6q	24.6q

triterpene moiety of compound **1** was quillaic acid having glycosidic linkage at the C-3 and C-28 positions. The hexasaccharide nature of compound **1** was manifested by its ^{1}H [δ 6.00, s; δ 5.99, d, J=7.6 Hz; δ 5.70, brs; δ 5.21, d, J=7.6 Hz; δ 5.18, d, J=7.1 Hz; δ 4.90, d, J=7.1 Hz] and ^{13}C [δ 111.7, 106.6, 106.3, 103.4, 101.9, 94.5] NMR data, respectively (Table 2). Alkaline hydrolysis of compound **1** followed by acid hydrolysis gave D-fucose, D-xylose, L-arabinose, L-rhamnose. On the other hand, acid hydrolysis of **1** gave D-glucuronic acid, D-galactose, D-fucose, D-xylose, L-arabinose and L-rhamnose, so D-glucuronic acid and D-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, DQFCOSY, TOCSY, HMQC, HMQC TOCSY and HMBC. Starting from the anomeric proton of each sugar unit, all the hydrogens within each spin system were delineated using DQFCOSY with the aid of TOCSY spectra. On the basis of the assigned protons the ^{13}C resonances of each sugar unit were identified by HMQC, HMQC TOCSY and further confirmed by HMBC experiments. In the light of the assigned ^{1}H and ^{13}C-NMR spectra (Table 2), the arabinose sugar unit was identified as α-L-arabinofuranose (Yu, *et al.*, 1989) and other sugar units were in pyranose form. The α anomeric configuration for the rhamnose was judged by its C_5 data (δ 68.9) (Yu *et al.,* 1989). The β anomeric configurations for the glucuronic acid, the galactose, the fuctose and the xylose were judged from their large $^3J_{H1,H2}$ coupling constants (7-8 Hz). From the HMBC spectrum we can see that C_3 (δ 83.5) with H_{GluA1} (δ 4.90), C_{GluA2} (δ 82.2) with H_{Gal1} (δ 5.21), C_{28} (δ 175.9) with H_{F1} (δ 5.99), C_{F2} (δ 73.3) with H_{R1} (δ 6.00), C_{F3} (δ 81.1) with H_{A1} (δ 5.70), C_{R4} (δ 83.0) with H_{X1} (δ 5.18), C_{GluA6} (170.3) with $H_{\delta 3.71}$ (-OCH_3), $C_{\delta 170.8}$ (C=O of acetyl) with H_{A5} (δ 4.52, 4.80) and $H_{\delta 1.99}$ (CH_3 of acetyl) and $C_{\delta 170.7}$ (C=O of acetyl) with H_{F4} (δ 5.78) and $H_{\delta 1.96}$ (CH_3 of acetyl) have cross peaks. Thus, segetoside D (**1**) was determined to be 28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-[5-*O*-acetyl-α-

Table 2. ^{13}C (150 MHz) NMR and ^{1}H (600 MHz) NMR spectra data for the sugar moieties of **1** and **2** (C_5D_5N) (δ in ppm, J in Hz)

	1		**2**	
	δ_C	δ_H	δ_C	δ_H
3-O-GluA				
1	103.4d	4.90(1H, d, J=7.1)	103.6d	4.90(1H, d, J=7.2)
2	82.2d	4.19(1H, m)	82.3d	4.21(1H, m)
3	77.5d	4.25(1H, m)	77.5d	4.25(1H, m)
4	72.6d	4.38(1H, m)	72.6d	4.38(1H, m)
5	76.8d	4.45(1H, m)	76.9d	4.45(1H, m)
6	170.3s		169.9s	
6-OMe	52.1q	3.71(3H, s)		
6-OBu;CH_2			65.1t	4.23(2H, m)
6-OBu;CH_2			30.8t	1.59(2H, m)
6-OBu;CH_2			19.2t	1.32(2H, m)
6-OBu;CH_3			13.7q	0.78(2H, t, J=7.6)
Galactose				
1	106.3d	5.21(1H, d, J=7.6)	106.4d	5.24(1H, d, J=7.8)
2	74.5 d	4.55(1H, m)	74.5d	4.55(1H, m)
3	74.9d	4.13(1H, m)	74.9d	4.13(1H, m)
4	70.2d	4.56(1H, m)	70.2d	4.56(1H, m)
5	77.1d	4.12(1H, m)	77.1d	4.12(1H, m)
6	62.2t	4.52(2H, m)	62.2t	4.52(2H, m)
28-O-Fucose				
1	94.5d	5.99(1H, d, J=7.6)	94.5d	5.99(1H, d, J=8.0)
2	73.3d	4.55(1H, m)	73.3d	4.55(1H, m)
3	81.1d	4.28(1H, m)	81.1d	4.28(1H, m)
4	73.9d	5.78(1H, m)	73.9d	5.78(1H, m)
5	70.6d	4.00(1H, m)	70.6d	4.00(1H, m)
6	16.5q	1.19(3H, d, J=6.4)	16.5q	1.19(3H, d, J=6.4)
4-OAc;CH_3	20.7q	1.96(3H, s)	20.7q	1.96(3H, s)
4-OAc;C=O	170.7s		170.7s	
Arabinose				
1	111.7d	5.70(1H, brs)	111.7d	5.70(1H, brs)
2	83.6d	4.87(1H, m)	83.6d	4.90(1H, m)
3	78.7d	4.55(1H, m)	78.7d	4.55(1H, m)
4	82.0d	4.72(1H, m)	81.9d	4.73(1H, m)
5	64.3t	4.52(1H, m)	64.3t	4.53(1H, m)
		4.80(1H, dd, J=3.2, 11.7)		4.80(1H, dd, J=3.2, 12.0)
5-OAc;CH_3	20.7q	1.99(3H, s)	20.7q	1.99(3H, s)
5-OAc;C=O	170.8s		170.8s	
Rhamnose				
1	101.9d	6.00(1H, S)	101.9d	6.00(1H, S)
2	71.7d	4.70(1H, m)	71.7d	4.70(1H, m)
3	72.3d	4.63(1H, m)	72.3d	4.63(1H, m)
4	83.0d	4.39(1H, m)	83.0d	4.39(1H, m)
5	68.9d	4.44(1H, m)	68.9d	4.44(1H, m)
6	18.7q	1.73(3H, d, J=5.9)	18.7q	1.73(3H, d, J=5.9)
Xylose				
1	106.6d	5.18(1H, d, J=7.1)	106.6d	5.18(1H, d, J=7.0)
2	76.1d	4.02(1H, m)	76.1d	4.02(1H, m)
3	78.6d	4.05(1H, m)	78.6d	4.05(1H, m)
4	71.0d	4.20(1H, m)	71.0d	4.20(1H, m)
5	67.4t	3.46(1H, t, J=10.3)	67.4t	3.46(1H, t, J=10.3)
		4.20(1H, m)		4.21(1H, m)



arabinofuranosyl(1→3)]-[4-*O*-acetyl-*β*-D-fucopyrano-syl]-quillaic acid-3-*O*-[*β*-D-galactopyranosyl(1→2)]-6-*O*-methyl ester -*β*-D-glucuronopyranoside.

Segetoside E (**2**), an amorphous solid, had a molecular formula of $C_{72}H_{112}O_{34}$ determined by positive ion FAB-MS (at m/z 1544 $[M+Na]^+$) as well as ^{13}C, DEPT NMR data. Its spectral features and physicochemical properties suggested **2** to be a triterpenoid saponin. Of the seventy two carbons, thirty were assigned to the aglycone part, thirty four to the oligosaccharide moiety, four to the acetyl, and four to the butoxy (Tables 1 and 2). Its IR spectrum showed characteristic absorptions for hydroxyl (3415 cm^{-1}), ester (1732 cm^{-1}) and a glycosidic linkage (1000-1100 cm^{-1}). Spectral evidence indicated that compound **2** had the same aglycone and sugar arrangement as those of **1** (Tables 1 and 2), but differed from the substituent of C_{GluA6}, in compound **2**, the 1-butoxy substituted the methoxy of C_{GluA6} of **1**. Alkaline hydrolysis of compound **2** followed by acid hydrolysis gave D-fucose, D-xylose, L-arabinose, L-rhamnose while acid hydrolysis of **2** gave D-glucuronic acid, D-galactose, D-fucose, D-xylose, L-arabinose and L-rhamnose. The identity of the monosaccharide and the sequence of the oligosaccharide chain were determined by a combination of DEPT, HMQC, HMQC-TOCSY and HMBC. From the HMBC spectrum we can see that C_3 (δ 83.5) with H_{GluA1} (δ 4.90), C_{GluA2} (δ 82.3) with H_{Gal1} (δ 5.24), C_{28} (δ 175.9) with H_{F1} (δ 5.99), C_{F2} (δ 73.3) with H_{R1} (δ 6.00), C_{F3} (δ 81.1) with H_{A1} (δ 5.70), C_{R4} (δ 83.0) with H_{X1} (δ 5.18), C_{GluA6} (169.9) with $H_{\delta 4.23}$ (-OCH_2- of butoxy), $C_{\delta 170.8}$ (C=O of acetyl) with H_{A5} (δ 4.53, 4.80) and $H_{\delta 1.99}$ (CH_3 of acetyl) and $C_{\delta 170.7}$ (C=O of acetyl) with H_{F4} (δ 5.78) and $H_{\delta 1.96}$ (CH_3 of acetyl) Thus, segetoside E (**2**) was determined to be 28-*O*-[*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)]-[5-*O*-acetyl-*α*-arabinofuranosyl(1→3)]-[4-*O*-acetyl-*β*-D-fucopyranosyl]-quillaic acid-3-*O*-[*β*-D-galactopyranosyl(1→2)]-6-*O*-n-butyl ester -*β*-D-glucuronopy- ranoside.

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