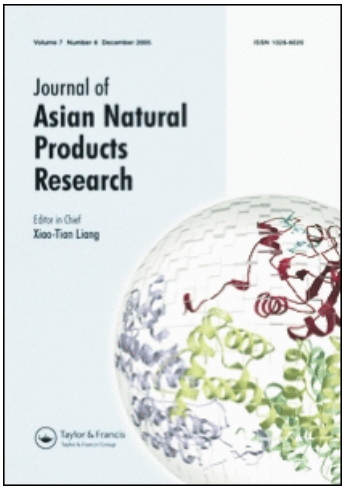

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THREE NEW TRITERPENOID SAPONINS FROM THE SEEDS OF *VACCARIA SEGETALIS*

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Three new triterpenoid saponins, named segetoside **G** (**1**), **H** (**2**) and **I** (**3**), have been isolated from the seeds of *Vaccaria segetalis*. On the basis of chemical reaction and spectral data, their structures have been established as: 28-O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl (1 → 3)]-β-D-(4-O-acetyl)-fucopyranosyl-gypsogenin-3-O-β-D-galactopyranosyl-(1 → 2)-β-D-(6-O-butyl ester)-glucuronopyranoside (**1**), 28-O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-(5-O-acetyl)-arabinofuranosyl-(1 → 3)]-β-D-(4-O-acetyl)-fucopyranosyl-gypsogenin-3-O-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranoside (**2**) and 28-O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-(5-O-acetyl)-arabinofuranosyl-(1 → 3)]-β-D-(4-O-acetyl)-fucopyranosyl-quillaic acid-3-O-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranoside (**3**).

Keywords: *Vaccaria segetalis*; Caryophyllaceae; Triterpenoid saponins

INTRODUCTION

In previous papers [1–3], we have reported the isolation and structural elucidation of segetoside **A**, **C**–**E** 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

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diuresis, activating blood circulation and relieving carbuncles [4]. Further investigation of the seeds led to the isolation of three new triterpenoid saponins, named segetoside **G** (**1**), **H** (**2**) and **I** (**3**). This paper deals with their isolation and structural elucidation.

RESULTS AND DISCUSSION

The n-butanol fraction from the ethanol extract of the seeds of *V. segetalis* was chromatographed on Diaion HP-20, silica gel and RP-18 silica gel to afford segetoside **G** (**1**), **H** (**2**) and **I** (**3**).

Segetoside **G** (**1**), an amorphous solid, had a molecular formula of $C_{70}H_{110}O_{32}$ determined by positive ion FABMS (at *m/z* 1486 $[M + Na]^+$) 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}CNMR 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 ^1H [δ 6.01, s; δ 6.00, d, $J = 8.0$ Hz; δ 5.74, brs; δ 5.21, d, $J = 7.6$ Hz; δ 5.02, d, $J = 7.1$ Hz; δ 4.85, d, $J = 7.2$ Hz] and ^{13}C [δ 111.7, 107.4, 106.2, 103.6, 102.1, 94.3] NMR data, respectively (Table I). Alkaline hydrolysis of compound **1** followed by acid hydrolysis gave fucose, xylose, arabinose, 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 monosaccharides and the sequence of the oligosaccharide chains were determined by a combination of DEPT, COSY, HMQC and HMBC. In the light of the assigned ^1H and ^{13}CNMR spectra (Table I), 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 glucuronic acid, galactose, fucose and xylose were judged from their large $^3J_{H1,H2}$ coupling constants (7–8 Hz). The HMBC spectrum showed that C_3 has cross peak with H_{GluA1}, and 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 $H_{\delta 4.23}$ ($-OCH_2-$ of n-butoxy); $C_{\delta 170.8}$ (C=O of acetyl) with H_{F4}, $H_{\delta 1.96}$ (CH_3 of acetyl). Thus, segetoside **G** (**1**) was determined to be 28-O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-β-D-(4-O-acetyl)-fucopyranosyl-gypsogenin-3-O-β-D-galactopyranosyl-(1 → 2)-β-D-(6-O-butyl ester)-glucuronopyranoside.

TABLE I ^{13}C (150 MHz) NMR spectral data of the aglycon parts of compounds **1**, **2**, **3** (C_5D_5N), gypsogenin and quillaic acid ($CDCl_3$) (δ in ppm)

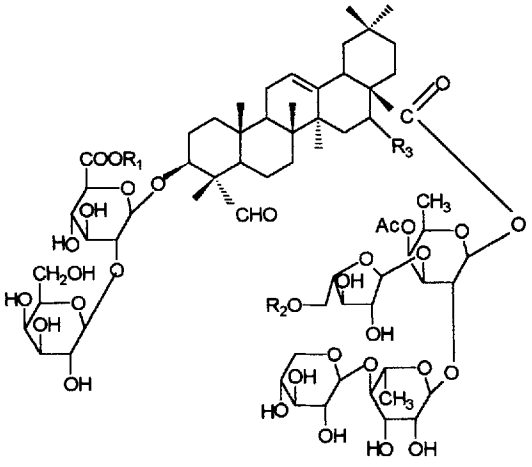
Position	**1**	**2**	*Gypsogenin*	**3**	*Quillaic acid*
1	38.1 t	38.1 t	38.4 t	38.2 t	38.1 t
2	25.1 t	24.9 t	26.0 t	25.0 t	26.0 t
3	83.8 d	83.5 d	71.6 d	83.4 d	71.7 d
4	55.0 s	54.9 s	55.2 s	55.0 s	55.2 s
5	48.7 d	48.5 d	48.0 d	48.3 d	48.1 d
6	20.7 t	20.7 t	20.7 t	20.6 t	20.7 t
7	32.6 t	32.6 t	32.5 t	33.0 t	32.3 t
8	40.2 s	40.1 s	40.0 s	40.2 s	39.7 s
9	47.8 d	47.7 d	47.7 d	46.8 d	46.7 d
10	36.3 s	36.1 s	36.2 s	36.1 s	35.9 s
11	23.4 t	23.2 t	23.0 t	23.8 t	23.2 t
12	122.4 d	122.3 d	122.2 d	122.1 d	122.2 d
13	144.1 s	144.1 s	144.8 s	144.5 s	142.8 s
14	42.3 s	42.2 s	42.2 s	42.1 s	41.4 s
15	28.4 t	28.4 t	28.2 t	36.0 t	35.4 t
16	23.7 t	23.4 t	23.8 t	73.9 d	74.7 d
17	47.2 s	47.1 s	46.6 s	49.3 s	48.7 s
18	42.0 t	41.9 t	41.9 t	41.5 t	40.5 t
19	46.4 t	46.3 t	46.5 t	47.5 t	46.4 t
20	30.7 s	30.6 s	30.9 s	30.6 s	30.0 s
21	34.0 t	33.9 t	34.2 t	36.1 t	35.4 t
22	32.3 t	32.2 t	32.1 t	31.9 t	30.3 t
23	209.8 d	210.0 d	207.1 d	209.5 d	207.0 d
24	11.1 q	11.0 q	9.6 q	11.0 q	9.0 q
25	15.8 q	15.7 q	15.7 q	15.8 q	15.7 q
26	17.3 q	17.3 q	17.4 q	17.3 q	16.9 q
27	25.9 q	25.9 q	26.1 q	27.0 q	27.0 q
28	176.4 s	176.4 s	180.0 s	175.9 s	177.2 s
29	33.1 q	33.0 q	33.2 q	33.1 q	32.7 q
30	23.7 q	23.6 q	23.8 q	24.4 q	24.6 q

Segetoside **H** (**2**) was obtained as an amorphous solid with the molecular formula $C_{68}H_{104}O_{33}$ which was deduced from the ESIMS (at *m/z* 1472 $[M+Na]^+$) as well as ^{13}C and DEPT NMR data. Spectral evidence indicated that compound **2** had the same aglycone, gypsogenin, and sugar arrangement as those of **1** (Tables I and II), but differed from the sugar substitution pattern. **2** has one acetyl group more, but one n-butoxyl group less than **1**. Comparing the ^{13}CNMR signals of the sugar part of compound **2** with those of **1**, the ^{13}CNMR data of A-5 and GluA-6 were shifted from δ 64.5 to δ 62.0 ppm and δ 170.7 to δ 169.8 ppm, respectively. Based on these spectral data, the additional acetyl group of **2** was located at C-5 of the arabinose unit, while in compound **2**, the hydroxyl substituted the n-butoxy of C_{glu6} of **1**. Consequently, the structure of segetoside **H** (**2**) was concluded to be 28-O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-(5-O-acetyl)-arabinofuranosyl-(1 → 3)]-β-D-(4-O-acetyl)-fucopyranosyl-gypsogenin-3-O-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranoside.

TABLE II ^{13}C (150 MHz) NMR and ^{1}H (600 MHz) NMR spectral data for the sugar moieties of **1**, **2** and **3** (C_5D_5N) (δ in ppm, J in Hz)

	1		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-O-GluA						
1	103.6 d	4.85 d, 7.2	103.4 d	4.85 d, 7.2	103.0 d	5.00d, 7.1
2	82.1 d	4.16 m	81.9 d	4.16 m	82.2 d	4.14 m
3	77.4 d	4.21 m	77.5 d	4.21 m	77.7 d	4.20 m
4	72.5 d	4.41 m	73.1 d	4.41 m	72.9 d	4.35 m
5	76.9 d	4.38 m	76.0 d	4.38 m	76.9 d	4.40 m
6	169.8 s		170.7 s		170.7 s	
6-O Me						
6-OBu; CH_2	65.1 t	4.23 m				
6-OBu; CH_2	30.8 t	1.59 m				
6-OBu; CH_2	19.2 t	1.31 m				
6-OBu; CH_3	13.7 q	0.78 t, 7.2				
Galactose						
1	106.2 d	5.21 d, 7.6	106.0 d	5.21 d, 7.6	106.1 d	5.17 d, 7.6
2	74.3 d	4.50 m	74.7 d	4.50 m	74.3 d	4.49 m
3	74.8 d	4.11 m	74.8 d	4.10 m	74.7 d	4.10 m
4	70.1 d	4.52 m	70.1 d	4.51 m	70.0 d	4.51 m
5	77.0 d	4.08 m	77.0 d	4.08 m	76.9 d	4.09 m
6	62.1 t*	4.51 m	62.1 t	4.51 m	62.0 t	4.48 m
28-O-Fucose						
1	94.3 d	6.00 d, 8.0	94.3 d	6.00 d, 8.0	94.4 d	5.95 d, 8.0
2	73.8 d	4.55 m	73.8 d	4.55 m	73.2 d	4.50 m
3	80.8 d	4.24 m	80.8 d	4.23 m	81.2 d	4.19 m
4	73.9 d	5.79 m	73.9 d	5.78 m	73.8 d	5.75 d, 3.4
5	70.8 d	3.96 m	70.8 d	3.94 m	70.5 d	3.98 m
6	16.5 q	1.19 d, 6.1	16.5 q	1.20 d, 6.1	16.4 q	1.16 d, 6.4
4-OAc; CH_3	20.7 q	1.96 s	20.8 q*	1.96 s	20.7 q*	1.94 s*
4-OAc; C—O	170.8 s		170.8 s*		170.8 s*	
Arabinose						
1	111.7 d	5.74 brs	111.7 d	5.74 brs	111.6 d	5.68 brs
2	83.5 d	4.87 m	83.5 d	4.87 m	83.5 d	4.86 m
3	78.1 d	4.81 m	78.1 d	4.81 m	78.5 d	4.51 m
4	85.8 d	4.68 m	85.8 d	4.68 m	81.9 d	4.70 m
5	62.0 t*	4.31 m	64.5 t	4.31 m	64.3 t	4.51 m
		4.16 m		4.16 m		4.76 dd, 3.0, 11.8
5-OAc; CH_3			20.9 q*		20.6 q*	1.95 s*
5-OAc; C—O			170.9 s*		170.9 s*	
Rhamnose						
1	102.1 d	6.01 s	102.1 d	6.01 s	101.6 d	6.00 s
2	71.5 d	4.73 m	71.4 d	4.74 m	71.6 d	4.69 m
3	72.3 d	4.57 m	72.3 d	4.57 m	72.2 d	4.63 m
4	84.7 d	4.27 m	84.6 d	4.28 m	82.6 d	4.39 m
5	68.7 d	4.37 m	68.8 d	4.37 m	68.8 d	4.45 m
6	18.6 q	1.78 d, 6.1	18.6 q	1.78 d, 6.1	18.7 q	1.69 d, 6.0
Xylose						
1	107.4 d	5.02 d, 7.1	107.4 d	5.02 d, 7.1	106.4 d	5.17 d, 7.0
2	76.1 d	4.00 m	76.1 d	4.01 m	76.0 d	4.01 m
3	78.6 d	4.03 m	78.6 d	4.04 m	78.4 d	4.05 m
4	70.5 d	4.17 m	70.5 d	4.16 m	70.9 d	4.19 m
5	67.4 t	3.51 t, 10.3	67.4 t	3.51 t, 10.3	67.2 t	3.41 t, 10.0
		4.22 m		4.21 m		4.19 m

*Signals may be interchanged.



1: R_1=$CH_3CH_2CH_2CH_2$, R_2=H, R_3=H
2: R_1=H, R_2=Ac, R_3=H
3: R_1=H, R_2=Ac, R_3=OH

FIGURE 1 Structures of compounds **1**, **2** and **3**.

Segetoside **I** (**3**), an amorphous solid, was assigned a molecular formula of $C_{68}H_{104}O_{34}$ determined by ESIMS (at *m/z* 1488 $[M+Na]^+$) as well as ^{13}C and DEPT NMR data. The spectral data of **3** showed that it possessed the same saccharide structure as those of **2**, but differed from **2** only in the aglycon part. Comparison of the signals from the aglycon part in the ^{13}CNMR spectra (Table I) with those from quillaic acid [7] showed that the aglycon of compound **3** was quillaic acid, while the aglycon of **2** was gypsogenin. Thus, segetoside **I** (**3**) was identified as 28-O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-(5-O-acetyl)-arabinofuranosyl-(1 → 3)]-β-D-(4-O-acetyl)-fucopyranosyl-quillaic acid-3-O-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranoside (Fig. 1).

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotation was obtained on a JASCO-DIP-181 polarimeter. IR was recorded on a Perkin-Elmer 599 infrared spectrometer. ^{1}H (400, 500 and 600 Hz), ^{13}C (100, 125 and 150 Hz) NMR and all 2D spectra were run on

Bruker AM-400, JEOL GSX-500 with NM-EFG type field gradient unit and JEOL α600 with NM-AFG type field gradient unit, TMS as int. standard. FAB-MS was recorded on a MAT-95 Mass spectrometer, the ESI-MS measured on Quattro mass spectrometer. LiChroprep RP-18 (25–40 μm, Merck) and Silica gel 60H for thin-layer chromatography (Qingdao Haiyang Chemical Group Co. of China) were used for column chromatography. TLC was performed on silica gel $HSGF_{254}$.

Plant Material

The seeds of *V. segetalis* were purchased at Shijia Zhuang, Hebei Province (China) in 1995. The botanical identification was made by Prof. 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 and 95% EtOH. 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 an EtOH–H_2O gradient system (0–100%). The fraction (70 g) eluted by 70% EtOH was subjected to silica gel column chromatography 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 (2 : 1 : 0.2) was subjected to RP-18 silica gel column chromatography with 70% MeOH to get compound (**1**) (40 mg), and with 65% MeOH to get compounds (**2**) (40 mg) and (**3**) (18 mg), (developed on TLC (silica gel) by CH_2Cl_2–MeOH–H_2O (2.0 : 1 : 0.2), detected by spraying with 10% H_2SO_4 in EtOH and then heating to 110°C, R_f of compounds **1**–**3**: (**1**) 0.41, (**2**) 0.31 and (**3**) 0.29).

Segetoside **G** (**1**) an amorphous solid, $[\alpha]_D^{24}$ −6.39 (c 0.36, MeOH). IR_{max}^{KBr} cm^{-1}: 3400, 1738, 1100–1000. FABMS: *m/z* 1486 $[M + Na]^+$. ^{1}HNMR (C_5D_5N) of the triterpene moiety of **1**: δ 9.95 (H-23, s), 5.39 (H-12, s), 4.02 (H-3, m), 3.09 (H-18, m), 1.48 (H-24, s), 1.25 (H-27, s), 1.05 (H-26, s), 0.90 (H-30, s), 0.88 (H-29, s), 0.85 (H-25, s); ^{13}CNMR (C_5D_5N) of the aglycon part of **1**: (Table I); ^{1}HNMR (C_5D_5N) and ^{13}CNMR (C_5D_5N) of the sugar moiety of **1**: (Table II).

Segetoside **H** (**2**) an amorphous solid, $[\alpha]_D^{24}$ −36.7 (c 0.14, MeOH). IR_{max}^{KBr} cm^{-1}: 3400, 1728, 1616, 1100–1000. ESIMS: *m/z* 1472 $[M+Na]^+$, ^{1}HNMR (C_5D_5N) of the aglycon part of **2**: δ 9.92 (H-23, s), 5.39 (H-12, s), 4.05 (H-3, m), 3.06 (H-18, m), 1.39 (H-24, s), 1.22 (H-27, s), 1.02 (H-26, s), 0.87 (H-30, s), 0.87 (H-29, s), 0.77 (H-25, s); ^{13}CNMR (C_5D_5N) of the aglycon part of **2**: (Table I); ^{1}HNMR (C_5D_5N) and ^{13}CNMR (C_5D_5N) of the sugar moiety of **2**: (Table II).

Segetoside **I** (**3**) an amorphous solid, $[\alpha]_D^{24}$ −13.9 (c 1.27, MeOH). IR_{max}^{KBr} cm^{-1}: 3400, 1726, 1616, 1100–1000. ESIMS: *m/z* 1488 $[M+Na]^+$, ^{1}HNMR (C_5D_5N) of the aglycon part of **3**: δ 9.83 (H-23, s), 5.55 (H-12, s), 5.20 (H-16, m), 4.03 (H-3, m), 3.32 (H-18, m), 2.70 (H-19a, t, $J = 13.3$ Hz), 1.35 (H-19b, m), 1.72 (H-27, s), 1.37 (H-24, s), 1.02 (H-26, s), 0.96 (H-30, s), 0.92 (H-29, s), 0.79 (H-25, s); ^{13}CNMR (C_5D_5N) of the aglycon part of **3**: (Table I); ^{1}HNMR (C_5D_5N) and ^{13}CNMR (C_5D_5N) of the sugar moiety of **3**: (Table II).

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