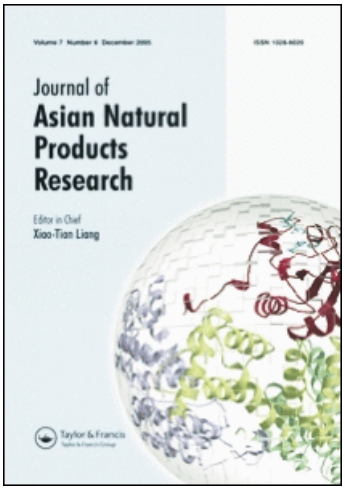

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TRITERPENOID SAPONINS FROM *VACCARIA SEGETALIS*

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A new triterpenoid saponin, named segetoside **C** (**1**), and two known saponins, vaccaroid **A** (vaccaroside **A**) (**2**) and dianoside **G** (**3**), have been isolated from the seeds of *Vaccaria segetalis*. On the basis of chemical reaction and spectral data, the structure of segetoside **C** (**1**) has been established as: gypsogenic acid-28-O-[β-D-glucopyranosyl-(1 → 3)]-[6-O-acetyl-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside.

Keywords: *Vaccaria segetalis*; Caryophyllaceae; Triterpenoid saponins; Segetoside **C**

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 [1]. Previous studies on the seeds of this plant have led to the isolation of eight cyclic peptides [2–5] and several saponins [6–10]. We have reported the isolation and structural elucidation of a new phenylpropanoid glycoside, segetoside **A**, and a new triterpenoid saponin, segetoside **B**, from the seeds of *V. segetalis* [11,12]. In our continued investigation of this seeds, a new

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triterpenoid saponin, named segetoside **C** (**1**), and two known compounds, vaccaroid **A** (vaccaroside **A**) (**2**) and dianoside **G** (**3**), have been isolated. This paper deals with their isolation and structural elucidation.

RESULTS AND DISCUSSION

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 **C** (**1**), vaccaroid **A** (vaccaroside **A**) (**2**) and dianoside **G** (**3**).

The known compounds, vaccaroid **A** [8] (vaccaroside **A** [10]) (**2**) and dianoside **G** [13] (**3**), were identified by comparison of their spectral data with those described in literatures.

Segetoside **C** (**1**), an amorphous solid, had a molecular formula of $C_{56}H_{88}O_{26}$ determined by positive ion FAB-MS (at *m/z* 1200 $[M+Na]^+$) as well as ^{13}C, DEPT NMR data. The molecular weight was 42 mass units greater than that of vaccaroid **A** (vaccaroside **A**) (**2**). Its spectral features and physicochemical properties suggested **1** to be a triterpenoid saponin. Of the 56 carbons, 30 were assigned to the aglycone part, 24 to the oligosaccharide moiety, and 2 to the acetyl (see Table I). Its **IR** spectrum showed characteristic absorptions for hydroxyl (3400 cm $^{-1}$), ester (1728 cm $^{-1}$) and a glycosidic linkage (1000–1100 cm^{-1}). The ^1H-NMR spectrum showed the signals of six methyl groups at δ 0.84, 0.94, 1.02, 1.08, 1.18, 1.64 ppm, and one olefinic proton at δ 5.43 ppm. The ^{13}C-NMR spectroscopic data revealed six methyl groups at δ 12.3, 16.1, 17.3, 23.8, 26.0, 33.1 ppm, a pair of olefinic carbon atoms at δ 122.9 and 144.1 ppm, and two carbonyl carbons at δ 176.3 and 180.7 ppm. All these proved that compound **1** closely resembled vaccaroid **A** (vaccaroside **A**) (**2**) and the triterpene moiety of compound **1** had the same aglycone, gypsogenic acid [14], as that of vaccaroid **A** (vaccaroside **A**) (**2**). Acid hydrolysis of compound **1** produced sugar components identified as all D-glucose by comparison with the authentic sample. The β anomeric configurations for the glucoses were judged from their large $^3J_{H1,H2}$ coupling constants (7–8 Hz). Comparing ^{13}C-NMR signals of the sugar part of compound **1** with those of vaccaroid **A** (vaccaroside **A**), the C-6 signal at G‴ unit was shifted from δ 62.3 to δ 64.7 ppm. Further, the ^{13}C signals at δ 21.0 and 171.2 ppm were additionally observed. These ^{13}C spectral data corresponded to those of acetyl moiety, which is also consistent with the increase of the mass unit. Based on these spectral data, the

TABLE I ^{13}C-NMR (125 MHz, C_5D_5N, δ in ppm) data of compounds **1–3** and ^{1}H-NMR (600 MHz, C_5D_5N, δ in ppm, J in Hz) data of the sugar part of compound **1**

Carbon	**1**		**2**	**3**
1	39.1		39.0	39.1
2	27.8		27.7	27.8
3	75.5		75.5	75.5
4	54.5		54.3	54.4
5	52.0		51.8	51.9
6	21.8		21.6	21.7
7	32.9		32.9	32.9
8	40.2		40.2	40.2
9	48.4		48.3	48.4
10	36.9		36.8	36.8
11	23.8		23.8	23.8
12	122.9		122.7	122.8
13	144.1		144.0	144.1
14	42.1		42.0	42.1
15	28.3		28.2	28.2
16	23.3		23.1	23.3
17	47.0		46.9	47.0
18	41.7		41.6	41.7
19	46.2		46.1	46.1
20	30.8		30.6	30.7
21	33.9		33.8	33.9
22	32.5		32.2	32.4
23	180.7		180.8	180.9
24	12.3		12.3	12.3
25	16.1		16.0	16.1
26	17.4		17.3	17.4
27	26.0		26.0	26.0
28	176.3		176.3	176.3
29	33.1		33.0	33.1
30	23.8		23.6	23.7
	δ_C	δ_H		
G				
1	94.9	6.23, d, $J=7.6$	94.8	95.1
2	72.8	4.22, m	73.1	72.6
3	88.9	4.21, m	87.9	88.5
4	69.3	4.17, m	69.1	68.9
5	77.6	4.28, m	76.8	77.6
6	68.9	4.33, m	68.8	68.8
		4.55, m		
G′				
1	105.9	5.23, d, $J=7.8$	105.6	105.7
2	75.5	4.10, m	75.5	75.2
3	78.2	4.19, m	78.0	78.6
4	71.5	4.19, m	71.2	71.6
5	78.7	4.00, m	78.3	78.4
6	62.5	4.30, m	62.4	62.6
		4.51, m		
G″				
1	102.5	5.15, d, $J=7.9$	102.5	105.4
2	83.2	4.08, m	83.5	75.2
3	77.7	4.29, m	77.9	78.4
4	71.1	4.20, m	70.8	71.5

TABLE I (*Continued*)

Carbon	**1**		**2**	**3**
5	78.2	3.80, m	78.2	78.3
6	62.4	4.33, m 4.42, m	62.1	62.5
G‴				
1	105.0	5.32, d, $J = 7.6$	105.6	
2	76.0	4.10, m	76.2	
3	77.8	4.19, m	77.9	
4	71.2	4.19, m	71.0	
5	75.5	4.11, m	78.4	
6	64.7	4.83, dd, $J = 4.6, 11.5$ 5.11, d, $J = 11.3$	62.3	
6-OAc; CH_3	21.0	2.03, s		
6-OAc; C=O	171.2			

acetyl group of **1** was located at C-6 of G‴ unit. The identity of the single sugar chain and the sequence of the oligosaccharide chain were determined by a combination of DEPT, DQFCOSY, TOCSY, HMQC, HMQCTOCSY, HMQCRELAY, NOESY and HMBC. Starting from the anomeric protons of each sugar unit, all the protons within each spin system were delineated using DQFCOSY and TOCSY with the aid of NOESY and HMBC. Although many proton signals of compound **1** were very congested, particularly in the region of δ 4.00–4.60 ppm, HMQCTOCSY spectrum permitted to assign all ^1H and ^{13}C signals of the sugar part of **1** (see Table I). Moreover, from the HMBC spectrum of **1** we can see that C_{28} (δ 176.3) with H_{G1} (δ 6.23), C_{G3} (δ 88.9) with $H_{G'1}$ (δ 5.23), C_{G6} (δ 68.9) with $H_{G'1}$ (δ 5.15), $C_{G''2}$ (δ 83.2) with $H_{G'''1}$ (δ 5.33), $C_{\delta 171.2}$ (C=O of acetyl) with $H_{G'6}$ (δ 5.11, 4.83) and $H_{\delta 2.03}$ (CH_3 of acetyl) have cross peaks. These results of **1** provided unambiguous information about the position of the glycosidic linkage and further confirmed that the sugar chain was located at C-28 of the sapogenin. Thus, segetoside **C** (**1**) was determined to be gypsogenic acid-28-O-[β-D-glucopyranosyl-(1 → 3)]-[6-O-acetyl-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside (see Fig. 1).

Professor Itokawa, H. and Nikaido, T. have previously reported the structure of a new triterpenoid saponin (**4**), named vaccaroid **B** [9] (vaccaroside **B** [10]), respectively. It should be pointed out that a 3-hydroxy-3-methylglutaric acid (HMG) moiety in vaccaroid **B** (vaccaroside **B**) (**4**) was attached to C-6 of the inner glucose (G″), while an acetyl group in segetoside **C** (**1**) reported here was connected to C-6 of the terminal glucose (G‴) instead of G″ (see Fig. 1). The difference between segetoside **C** (**1**) and vaccaroid **B** (vaccaroside **B**) (**4**) is very interesting.

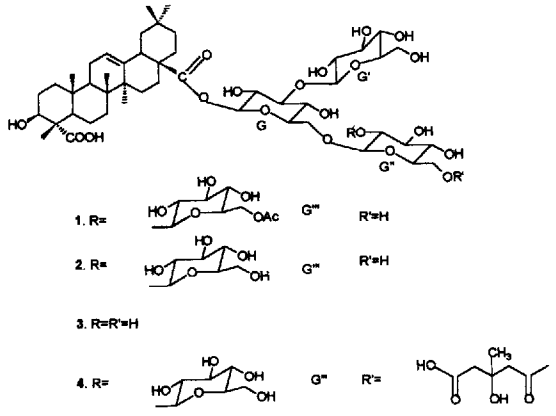

FIGURE 1 Structures of compounds **1**, **2**, **3** and **4**.

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 (500 Hz, 600 Hz), ^{13}C (125 Hz, 150 Hz) NMR and all 2D spectra were run on 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. Silica gel 60H for thin-layer chromatography (TLC) (Qingdao Haiyang Chemical Group CO. of China) was used for column chromatography. TLC was performed on silica gel $HSGF_{254}$.

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 × 2 and 95% EtOH × 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 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 (3 : 1 : 0.15) was subjected to RP-18 silica gel column chromatography with 75% MeOH to get compound (**1**) (20 mg), (**2**) (200 mg) and (**3**) (26 mg) [developed on TLC (silica gel) by CH_2Cl_2–MeOH–H_2O (2.5 : 1 : 0.2), detected by spraying with 10% H_2SO_4 in EtOH and then heating to 110°, Rf of compounds **1**–**3**: (**1**) 0.41, (**2**) 0.31 and (**3**) 0.47].

Segetoside **C** (**1**) It was obtained as white amorphous powder from CH_2Cl_2–MeOH–H_2O (3 : 1 : 0.15), 20 mg, $[\alpha]_D^{22}$ 8.86 (c 0.43, MeOH); IR (KBr) ν_{max} 3400 (OH), 1728 (ester), 1100–1000 (C–C) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N) data of the aglycon part of compound **1**, δ_{ppm} 0.84 (H-29, s), 0.94 (H-30, s), 1.02 (H-25, s), 1.08 (H-26, s), 1.18 (H-27, s), 1.64 (H-24, s), 3.20 (H-18, m), 4.67 (H-3, m), 5.43 (H-12, brs); ^{1}H-NMR (600 MHz, C_5D_5N) data of the sugar part of compound **1** and ^{13}C-NMR data of **1** see Table I; FAB-MS *m/z* $[M+Na]^+$ 1200 (75), $[M+H]^+$ 1178 (100), $[M+H-CH_3C{=}O]^+$ 1136 (20).

Acid Hydrolysis of **1** Compound **1** (5 mg) was dissolved in 1 ml 2 N HCl and kept at 80°C for 1 hr. The reaction mixture was neutralized with 10% KOH and was subjected to a Sephadex LH-20 column using MeOH as eluant to give the triterpene fraction and the sugar fraction. The sugar fraction was compared with standard sugar on HR-TLC silica gel plate developed with n-BuOH–Me_2CO–H_2O (4 : 5 : 1) and $CHCl_3$–MeOH–H_2O (2 : 1 : 0.2), detected by spraying with Aniline–phthalic acid reagent [Aniline : Phthalic acid : n-BuOH (2 : 3 : 200)] and then heating to 110°, Rf of glucose 0.33 and 0.39, respectively.

Vaccaroid **A** [8] (*vaccaroside* **A** [10]) (**2**) It was obtained as white amorphous powder from CH_2Cl_2–MeOH–H_2O (3 : 1 : 0.15), 200 mg, FAB-MS, ^{1}H and ^{13}C-NMR data were identical with published data.

Dianoside **G** (**3**) It was obtained as white amorphous powder from CH_2Cl_2–MeOH–H_2O (3 : 1 : 0.15), 26 mg, FAB-MS, ^{1}H and ^{13}C-NMR data were identical with published data [13].

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