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TRITERPENOID SAPONINS FROM *VACCARIA SEGETALIS*

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Key Word Index—*Vaccaria segetalis*; Caryophyllaceae; seeds; triterpenoid saponin: gypsogenic acid; 3,4-secogypsogenic acid; vaccarosides A, B, C, D.

Abstract—Four novel triterpenoid saponins were isolated from the seeds of *Vaccaria segetalis*. Their structures were established as vaccaroside A, gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 6)-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranoside; vaccaroside B, gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1 → 2)-[3-hydroxyl-3-methylglutaroyl-(1 → 6)]-*β*-D-glucopyranosyl-(1 → 6)-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranoside; vaccaroside C, 23-*O*-*β*-D-glucopyranosyl-gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 6)-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranoside and vaccaroside D, 3,4-secogypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1 → 3)-[*β*-D-glucopyranosyl-(1 → 6)]-*β*-D-glucopyranoside by a combination of extensive NMR (DEPT, COSY, HOHAHA, HETCOR, HMBC and NOESY) studies and chemical degradation.

INTRODUCTION

The plant *Vaccaria segetalis* (Neck.) Garcke (syn. *V. pyramidata* Medik) is an annual herb widely distributed in Asia, Europe and other parts of the world. In Japan, it has been cultivated as a garden plant for several centuries. However, the seeds of this plant, called Wang-Bu-Liu-Xing in traditional Chinese medicine have been prescribed frequently to cure the diseases associated with women after giving birth. According to traditional Chinese medicine, Wang-Bu-Liu-Xing has the capacity to activate blood flow and promote milk secretion. It is also used in the treatment of amennorrhea and breast infections [1]. Early chemical investigation in the 1970s on the seeds of this species led to the isolation of several triterpenoid saponins and their structures were studied by traditional chemical methods [2, 3]. The medicinal importance attached to this species prompted us to re-investigate the saponin components of *V. segetalis*. In this paper, we wish to report the isolation and structural studies of several novel triterpenoid saponins from the seeds of this plant.

RESULTS AND DISCUSSION

A 95% ethanol extract of the powdered seeds (2 kg) of *V. segetalis* was chromatographed on Dianion HP-20, silica gel, followed by repeated MPLC and HPLC purification to afford four saponins, vaccarosides A (**1**), B (**2**), C (**3**) and D (**4**).

Vaccaroside A (**1**), an amorphous solid, had a molecular formula of $C_{54}H_{86}O_{25}$ determined by positive ion FABMS (at *m/z* 1173 $[M+K]^+$, 1157 $[M+Na]^+$, 1135 $[M+H]^+$) as well as ^{13}C, DEPT NMR data. Its spectral features and physicochemical properties suggested **1** to be a triterpenoid saponin. Of the 54 carbons, 30 were assigned to the aglycone part, 24 to the oligosaccharide moiety (Tables 1 and 2). The IR spectrum showed a hydroxyl band at 3399 cm^{-1} and an ester band at 1729 cm^{-1}. The six sp^3 hybrid carbons at δ 12.4, 16.1, 17.4, 23.8, 26.1, 33.1 ppm, and the two sp^2 hybrid carbons at δ 122.8 (*d*) and 144.0 (*s*) together with the information from the ^1H NMR analysis (six methyl proton singlets and a broadened triplet-like vinyl proton at δ 5.41) indicated that the aglycone possessed an olean-12-ene skeleton. The ^{13}C NMR data for the aglycone part was assigned to gypsogenic acid, a common aglycone of the triterpenoid glycosides from the family Caryophyllaceae [4]. The C-3 signal at δ 75.5 revealed that no sugars were connected at this point. The C-23 carbonyl carbon was not observed in the 1D ^{13}C NMR spectrum but later located to be at δ 180.0 (indicative of an unsubstituted carboxylic group) from its HETCOR and HMBC experiments. The C-28 carbonyl carbon at δ 176.3 suggested that the sugar moiety is bound to this carbon. The tetrasaccharide nature of compound **1** was manifested by its ^1H [δ 4.99 *d* (J = 7.7 Hz), 5.30

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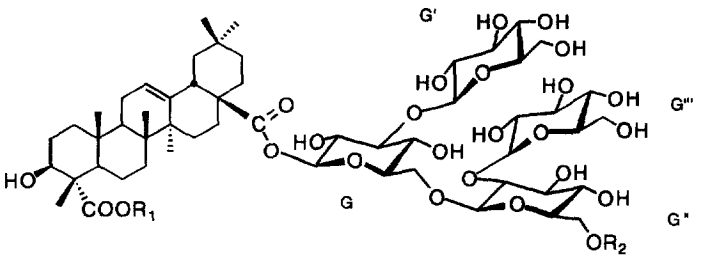

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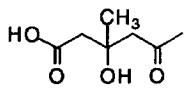
2. $R_1 = H$ $R_2 =$ 

3. $R_1 = glc$ $R_2 = H$

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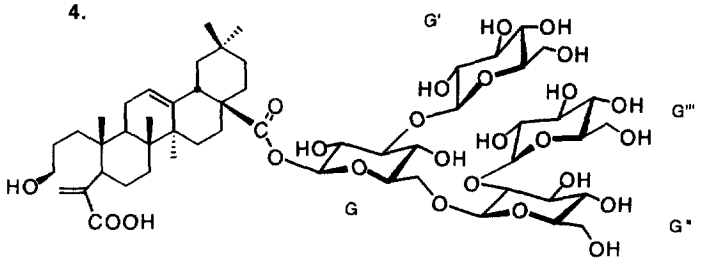

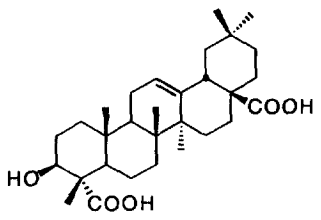

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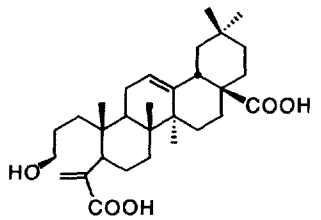

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d (7.7), 5.32 *d* (7.6), 6.20 *d* (7.7)] and ^{13}C [δ 94.8, 102.6, 105.7 (× 2)] NMR data, respectively (Tables 2 and 3). The identity of the monosaccharides and the sequence of the oligosaccharide chain were determined by a combination of COSY, HOHAHA, DEPT, HETCOR, HMBC and phase-sensitive NOESY experiments. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were delineated using COSY with the aid of 2D-HOHAHA and NOESY spectra. A NOESY experiment, in addition to the NOEs across the glycosidic bonds, also revealed the 1,3 and 1,5-diaxial relationships for glucoses, thus greatly simplifying the mapping of the spin systems. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by HETCOR and further confirmed by HMBC experiments. In the light of the assigned ^{1}H and ^{13}C NMR spectra, the four sugar units were identified as D-glucose and further confirmed by GLC analysis of the acid hydrolysate. From the completely assigned ^{13}C NMR data, the branched nature of the sugar moiety was evident, and the noticeable ^{13}C shift differences between inner sugars and terminal ones indicated that the glucose (designated as G) directly connected to C-28 was glycosylated at G-3 (Δ 9.1 ppm) and G-6 (Δ 6.5 ppm). Also, the glucose (G″) linked to G-6 was glycosylated at G″-2 (Δ 7.4 ppm). Information from HMBC and NOESY experiments confirmed these assignments (Fig. 1). All the monosaccharides in the pyranose forms were determined from their ^{13}C NMR data. The β anomeric configurations for these sugars were judged from their large $^{3}J_{H1,H2}$ coupling constants (7–8 Hz). The absolute configurations of these sugars were chosen in keeping with those mostly encountered among plant glycosides. Thus, vaccaroside A (**1**) was elucidated to be

Table 1. ^{13}C NMR data of the aglycone parts of vaccarosides A (**1**), B (**2**) and C (**3**) (125 MHz, pyridine-d_5)*

Carbon	1	2	3	DEPT
1	39.1	39.1	38.8	CH_2
2	27.7	27.8	27.5	CH_2
3	75.5	75.6	75.1	CH
4	54.4	54.5	55.1	C
5	51.8	51.9	52.3	CH
6	23.2	23.2	23.1	CH_2
7	32.9	32.9	32.8	CH_2
8	40.2	40.2	40.1	C
9	48.3	48.4	48.2	CH
10	36.8	36.8	36.8	C
11	23.8	23.8	23.7	CH_2
12	122.8	122.8	122.6	CH
13	144.0	144.1	144.1	C
14	42.1	42.1	41.9	C
15	28.2	28.2	28.1	CH_2
16	21.7	21.8	21.2	CH_2
17	46.9	47.0	46.9	C
18	41.7	41.7	41.6	CH
19	46.1	46.2	46.1	CH_2
20	30.7	30.8	30.6	C
21	33.9	33.9	33.8	CH_2
22	32.1	32.4	32.2	CH_2
23	180.0†	180.7	177.7	C
24	12.4	12.3	12.0	CH_3
25	16.1	16.1	16.1	CH_3
26	17.4	17.4	17.3	CH_3
27	26.1	26.0	25.9	CH_3
28	176.3	176.3	176.3	C
29	33.1	33.1	33.0	CH_3
30	23.8	23.7	23.6	CH_3
HMG				
1′		171.7		
2′		46.7		
3′		70.1		
4′		46.7		
5′		175.6		
1″		28.3		

*The assignments based upon ^1H NMR, ^{13}C NMR, COSY, HOHAHA, DEPT, HETCOR, HMBC and NOESY.

†Not observed in ^{13}C NMR, but located from HMBC experiment.

Table 2. ^{13}C NMR data of the sugar moieties (125 MHz in pyridine-d_5)*

Sugar units	1	2	3	4
28-*O*-sugar				
G-1	94.8	94.7	94.8	94.9
G-2	73.2	73.1	73.1	73.2
G-3	88.0	88.3	87.8	88.1
G-4	69.2	69.3	69.2	69.3
G-5	76.8	76.9	76.7	76.8
G-6	68.9	69.3	68.8	68.9
G′-1	105.7	105.9	105.9	105.8
G′-2	75.7	75.5	75.6	75.7
G′-3	78.0	77.8	77.9	78.0
G′-4	71.3	70.9	71.3	71.3
G′-5	78.5†	78.5†	78.5†	78.5†
G′-6	62.3	62.3	62.3	62.3
G″-1	102.6	102.6	102.6	102.6
G″-2	83.7	83.3	83.7	83.7
G″-3	78.0	78.0	78.0	78.0
G″-4	70.8	71.2	70.8	70.9
G″-5	78.3†	75.1	78.3†	78.3†
G″-6	62.1	64.3	62.1	62.1
G‴-1	105.7	105.7	105.8	105.8
G‴-2	76.3	76.3	76.3	76.3
G‴-3	78.1	78.1	78.1	78.1
G‴-4	71.1	71.4	71.1	71.2
G‴-5	78.4†	78.4†	78.4†	78.4†
G‴-6	62.4	62.4	62.4	62.5
23-*O*-sugar				
G⁗-1			96.5	
G⁗-2			74.4	
G⁗-3			78.6	
G⁗-4			70.7	
G⁗-5			79.5	
G⁗-6			62.0	

*The assignments based upon ^1H NMR, ^{13}C NMR, COSY, HOHAHA, DEPT, HETCOR, HMBC and NOESY.

†Assignment may be reversed in each column.

gypsogenic acid-28-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside.

Vaccaroside B (**2**) is an amorphous solid. The molecular composition, $C_{60}H_{94}O_{29}$ was determined from its FAB-MS pseudo-molecular ions at 1301 $[M+Na]^+$, 1317 $[M+K]^+$ and the ^{13}C NMR data. Of the 60 carbons, 30 were assigned to the aglycone part, 24 to the oligosaccharide moiety and the remaining six were assigned to a 3-hydroxy-3-methylglutaric acid (HMG) moiety. The presence of HMG in **2** was inferred from its ^1H and ^{13}C NMR data and further confirmed by HMBC long-range couplings. Spectral evidence indicated that compound **2** had the same aglycone, gypsogenic acid (**5**) and sugar arrangement as that of **1**. Acid hydrolysis afforded **5** and the monosaccharides were identified to be glucoses from GLC analysis of the acid hydrolysate. Among the four carbonyl carbons at δ 180.7, 176.3, 175.7 and 171.7 ppm observed in the ^{13}C NMR spectrum, the former two belonged to C-23 and C-28 of the aglycone, and the latter two were assigned to the HMG moiety (Table 1). Like compound **1**, the chemical shifts of C-3 (δ 75.6) and C-23 (δ 180.7) indicated that no sugars were connected at these points. The C-28 carbonyl carbon at δ 176.3 suggested that this carbon was in the esterified state. The site of HMG attachment was determined to be at C-6 of the inner glucose (G″) by observ-

Table 3. ^{1}H NMR data of the sugar moieties (500 MHz in pyridine-d_5)*

Sugar units	1	2	3	4
28-*O*-sugar				
G-1	6.20 *d* (7.7 Hz)	6.14 *d* (7.0)	6.18 *d* (7.9)	6.23 *d* (7.7)
G-2	4.31	4.20	4.29	4.32
G-3	4.34	4.20	4.29	4.36
G-4	4.34	4.29	4.31	4.37
G-5	4.13	3.90	4.10 *m*	4.14
G-6	4.30	4.25	4.50 *dd* (11.0, 1.0)	4.32
	4.52	4.52	4.29	4.54
G′-1	5.32 *d* (7.6)	5.25 *d* (7.7)	5.35 *d* (7.6)	5.36 *d* (7.6)
G′-2	4.08	4.00	4.09	4.10
G′-3	4.17	4.10	4.14	4.19
G′-4	4.17	4.10	4.14	4.19
G′-5	3.93	3.87	3.93	3.95
G′-6	4.46 *dd* (11.0, 2.5)	4.41 *dd* (12.0, 7.0)	4.46 *dd* (11.9, 2.6)	4.49 *dd* (11.7, 2.3)
	4.27	4.20	4.28	4.30
G″-1	4.99 *d* (7.7)	4.97 *d* (7.6)	5.00 *d* (7.9)	5.02 *d* (7.6)
G″-2	4.08	4.05	4.08	4.10
G″-3	4.27	4.18	4.26	4.29
G″-4	4.20	3.95	4.18	4.21
G″-5	3.80	3.87	3.79	3.82
G″-6	4.41 *dd* (11.0, 2.0)	4.94 *d* (13.1)	4.40 *dd* (11.9, 2.4)	4.44 *dd* (11.7, 2.4)
	4.34	4.62	4.31	4.36
G‴-1	5.30 *d* (7.7)	5.25 *d* (7.7)	5.30 *d* (7.6)	5.33 *d* (7.6)
G‴-2	4.08	4.00	4.07	4.10
G‴-3	4.17	4.10	4.16	4.19
G‴-4	4.16	4.10	4.14	4.19
G‴-5	3.92	3.87	3.91 *m*	3.95
G‴-6	4.58 *dd* (12.0, 2.0)	4.49 *dd* (11.7, 2.0)	4.56 *dd* (11.4, 2.3)	4.60 *dd* (11.7, 2.0)
	4.36	4.27	4.35	4.37
23-*O*-sugar				
G⁗-1			6.44 *d* (8.2)	
G⁗-2			4.20	
G⁗-3			4.27	
G⁗-4			4.35	
G⁗-5			4.01 *m*	
G⁗-6			N.A.†	

* The assignments based upon ^{1}H NMR, ^{13}C NMR, COSY, HOHAHA, DEPT, HETCOR, HMBC and ROESY.
† N.A.: Not assigned.

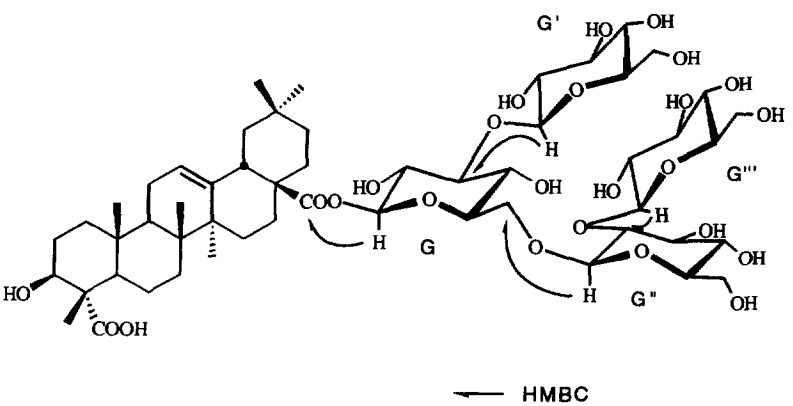

Fig. 1. Some key HMBC correlations observed in vaccanoside A (**1**).

ing the significant downfield shifts of H-6 and H-6′ and downfield shift (2 ppm) of C-6 of the glucose (Tables 2 and 3). Due to the limited amount of material available, the stereochemistry at C-3 of the HMG moiety remained undetermined. Accordingly, vaccaroside B (**2**) was elucidated to be gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1 → 2)-[3-hydroxyl-3-methylglutaroyl-(1 → 6)]-*β*-D-glucopyranosyl-(1 → 6)-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranoside.

Vaccaroside C (**3**), an amorphous solid, had the molecular formula $C_{54}H_{86}O_{25}$, determined from its FAB-MS pseudo-molecular ion at *m/z* 1157 $[M+Na]^+$ as well as the ^{13}C NMR data. Detailed analysis of the ^1H and ^{13}C NMR data showed that **3** had five sugar units and the aglycone was the same as that of compound **1** (gypsogenic acid) (Table 1). Acid hydrolysis afforded gypsogenic acid (**5**) and glucose as the component monosaccharide. Comparison of its ^{13}C and ^1H NMR data, COSY and HOHAHA patterns, with those of **1** indicated that they had the same sugar sequence at C-28 (Tables 2 and 3). This arrangement was further confirmed by a phase-sensitive NOESY experiment. The C-23 of the aglycone resonated at *δ* 177.7 indicating that the remaining glucose was connected to this carbon. On the other hand, the chemical shifts of the anomeric proton (*δ* 6.44) and carbon (*δ* 96.5) also revealed that this glucose connected to C-23 through an ester bond. Thus, the structure of vaccaroside C (**3**) were established to be 23-*O*-*β*-D-glucopyranosyl-gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 6)-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranoside.

Vaccaroside D (**4**), an amorphous solid, had the same molecular composition $C_{54}H_{86}O_{25}$ as that of vaccaroside A (**1**) as inferred from its $[M+Na]^+$ ion at *m/z* 1157 in the positive ion FAB-MS. The spectral feathers indicated that the two compounds possess the same sugar chain attached to C-28 of the aglycones. The aglycone part of **4**, unlike gypsogenic acid, showed five methyl proton singlets (^1H NMR: *δ* 0.90, 0.92, 0.99, 1.19 (×2) and two vinyl singlets (*δ* 5.60, 6.58) besides a triplet-like vinyl proton (*δ* 5.64). The characteristic *t*-like signal at *δ* 5.64 coupled with the two sp^2 hybrid carbons (*δ* 122.8 *d* and 143.9 *s*) indicated that the aglycone was of an olean-12-ene skeleton. Detailed analysis of the COSY, HOHAHA, HMQC, HMBC and NOESY data indicated that the aglycone in **4** possessed the same partial structure in the C, D, and E-rings as that of gypsogenic acid. However, a $-CH_2CH_2CH_2OH$ and an *α*-substituted-*α*,*β*-unsaturated carbonyl groups, which originated from the A-ring of gypsogenic acid was mapped out from the one bond and long-ranged couplings (see Fig. 2, Table 4). The above information suggested that the aglycone in **4** was the 3,4-seco derivative of gypsogenic acid. It should be pointed out that C-4 (*δ* 146.0), C-23 (*δ* 171.0) and C-24 (*δ* 124.0) were not observed in the 1D ^{13}C NMR spectrum, but were located from HMQC and HMBC experiments. The 3,4-seco A-ring had a strong influence on the chemical shifts of the B-ring. The C-5 and C-9 were shifted to highfield while C-6 and C-10 were shifted to lowfield. In particular, C-9 moved to *δ* 38.3, 10 ppm from its counterpart (*δ* 48.3) in vaccaroside A (**1**). 3,4-Seco-ogypsoginic acid (**6**) was first isolated as an aglycone of a triterpenoid saponin, called dianoside I from the whole plants of *Dianthus superbus* L. var. *longicalycinus* Williams (Caryophyllaceae) [5] in 1984 and the structure was established by UV, IR, ^1H-, ^{13}C-NMR and chemical conversion. However, most of the ^{13}C NMR assignments in the A and B-rings have to be revised (Table 4). Dianoside I was a trisaccharide with three moles of glucoses attached to C-28, while vaccaroside D had one more glucose in its sugar chain. Based upon the above information, vaccaroside D (**4**) was elucidated to be 3,4-seco-gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 6)-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranoside.

Previous investigations [2, 3] on the same plants did not reveal the existence of the saponins reported here, but established chemically several complex triterpenoid saponins containing six or more different

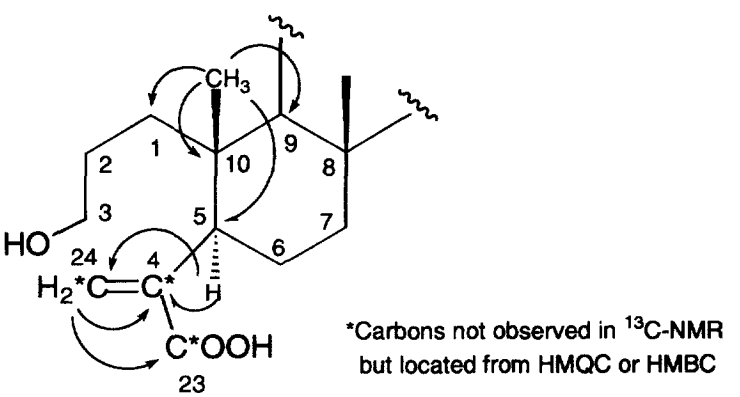

Fig. 2. The key long-ranged correlations of the aglycone part of vaccaroside D (**4**) observed from HMBC experiment (the parameter was optimized for 8 Hz).

Table 4. ^{13}C and ^{1}H data for the aglycone part of vaccarosides D (**4**) (500 MHz for ^{1}H and 125 MHz for ^{13}C in pyridine-d_5)

	Vaccaroside D (**4**)*			Dianoside I†
	^{13}C	DEPT	^{1}H	^{13}C
1	37.0	CH_2	1.21, 1.55	36.9
2	27.5	CH_2	1.79, 2.43	32.0
3	63.2	CH_2	3.82, 3.95	63.2
4	146.0‡	C	—	146.2
5	43.4	CH	3.26 *dd* (13.2, 1.0)	38.2
6	25.9	CH_2	1.42, 1.86	24.2
7	32.1	CH_2	1.22, 1.53	27.5
8	39.7	C	—	39.7
9	38.3	CH	2.24	43.4
10	39.6	C	—	39.5
11	24.4	CH_2	2.03 (×2)	26.0
12	122.8	CH	5.64 *t*-like	—
13	143.9	C	—	143.8
14	42.7	C	—	42.6
15	28.2	CH_2	1.12, 2.22	28.2
16	23.3	CH_2	1.92, 2.01	23.6
17	47.1	C	—	47.0
18	41.8	CH	3.19 *dd* (13.7, 3.8)	41.7
19	46.3	CH_2	1.23, 1.75	46.2
20	30.7	C	—	30.7
21	33.9	CH_2	1.12, 1.30	33.9
22	32.3	CH_2	1.73, 1.84	32.3
23	171.0‡	C	—	171.5
24	124.0‡	CH_2	5.60 *s*, 6.58 *s*	—
25	18.9	CH_3	0.99 *s*	18.8
26	17.6	CH_3	1.19 *s*	17.6
27	26.0	CH_3	1.19 *s*	26.0
28	176.4	C	—	176.3
29	33.1	CH_3	0.92 *s*	33.1
30	23.6	CH_3	0.90 *s*	23.6

*The assignments were based upon extensive NMR (DEPT, COSY, HOHAHA, HETCOR, HMBC and NOESY) analyses.

† Data from ref. [5].

‡ These carbons were not observed in the ^{13}C NMR spectrum, but located from HMQC and HMBC experiments.

monosaccharides. We also isolated related saponins and their structures are under study.

EXPERIMENTAL

General procedures. All mps were measured using a Yanaco microscope apparatus and are uncorr. IR spectra were determined using a JASCO D-300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. FABMS were conducted using JEOL DX-303 mass spectrometer. ^{1}H and ^{13}C NMR were recorded using a JEOL α-500 FT-NMR or a JEOL EX-400 FT-NMR spectrometer. Chemical shifts were expressed in δ (ppm) referring to solvent peaks: δ_H 7.20 and δ_C 135.50 for pyridine-d_5. Diaion HP-20 (Mitsubishi Kasei), silica gel (Silica gel 60, Merck), and ODS (Chromatorex, 100–200 mesh, Fujisylisia) were used for column chromatography. Preparative HPLC was performed using an ODS column (PEGASIL ODS, Senshu Pak, 10 mm i.d. × 250 mm, detector: UV 210 nm). GLC: Shimadzu GC-7A. Column: Silicone OV-17 on Uniport HP (80–100 mesh), 3 mm i.d. × 2.1 m; column temperature, 160°C; carrier gas, N_2, flow rate 30 ml min^{-1}.

Extraction and isolation. Seeds of *Vaccaria segetalis* were purchased from a market in Beijing, China, in December 1993, and identified by one of the authors (Z. Jia). Crushed seeds (2 kg) were extracted with 95%, 50% EtOH (5 litres, each) three times under reflux for 2 h. The combined EtOH extract was concentrated under red. pres. to give an extract (170 g), which was applied to a column of Diaion HP-20 (2000 ml) and washed with 30, 50, 70, and 100% MeOH. The fractions containing saponins (from 70 and 100% MeOH) were combined and repeatedly chromatographed over silica gel and ODS columns to give several saponin fractions. Further HPLC purification (70% MeOH-0.06% TFA in H_2O, 1.5 ml min^{-1}, UV detector, 210 nm) afforded four triterpenoid saponins, vaccarosides A (**1**, 30 mg), B (**2**, 18 mg), C (**3**, 6.0 mg) and D (**4**, 10.0 mg), respectively.

Vaccaroside A (**1**). An amorphous solid, mp 208–210°C (dec.), $[\alpha]_D^{20}$ 2.5° (MeOH; *c* 0.16). IR ν_{max}^{KBr} cm^{-1}: 3399, 2926, 1729, 1652, 1074. FAB MS (positive ion mode) *m/z*: 1135 $[M+H]^+$, 1157 $[M+Na]^+$, 1173 $[M+K]^+$. ^{1}H-NMR (400 MHz, pyridine-d_5): δ 0.86, 0.88, 0.99, 1.04, 1.19, 1.54 (each 3H, *s*, H_3 of C-29, C-30, C-25, C-26, C-27, C-24), 3.15 (1H, *dd*, *J* = 13.5, 3.8 Hz, H-18), 4.36 (1H, *m*, H-3), 5.34 (1H, *br.t*, H-12). For other NMR data, see Tables 1 and 2.

Vaccaroside B (**2**). An amorphous solid, mp 222–224°C (dec.), $[\alpha]_D^{20}$ 4.0° (MeOH; *c* 0.15). IR ν_{max}^{KBr} cm^{-1}: 3419, 2929, 1721, 1651, 1075. FAB MS (positive ion mode) *m/z*: 1301 $[M+Na]^+$, 1317 $[M+K]^+$. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.86, 0.92, 1.01, 1.06, 1.17, 1.59 (each 3H, *s*, H_3 of C-29, C-30, C-25, C-26, C-27, C-24), 4.52 (1H, *m*, H-3), 5.43 (1H, *br.t*, H-12). For other NMR data, see Tables 1 and 2.

Vaccaroside C (**3**). An amorphous solid, mp 220–224°C (dec.), $[\alpha]_D^{22}$ −7.0° (MeOH; *c* 0.2). IR ν_{max}^{KBr} cm^{-1}: 3426, 2920, 1740, 1655, 1078. FAB MS (positive ion mode) *m/z*: 1319 $[M+Na]^+$. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.82, 0.85, 0.95, 1.02, 1.08, 1.59 (each 3H, *s*, H_3 of C-29, C-30, C-25, C-26, C-27, C-24), 3.12 (1H, *dd*, *J* = 13.4, 4.2 Hz, H-18), 4.64 (1H, *dd*, *J* = 12.3, 5.2 Hz, H-3), 5.40 (1H, *br.t*, H-12). For other NMR data, see Tables 1 and 2.

Vaccaroside D (**4**). An amorphous solid, mp 225–228°C, $[\alpha]_D^{22}$ −4.0° (MeOH; *c* 0.2). IR ν_{max}^{KBr} cm^{-1}: 3409, 2927, 1721, 1622, 1073. FAB MS (positive ion mode) *m/z*: 1157 $[M+Na]^+$. For NMR data, see Tables 2, 3 and 4.

Acid hydrolysis of vaccarosides A (**1**), *B* (**2**), *C* (**3**) *and D* (**4**). Compound **1** (10 mg) was heated in 1 ml 1N HCl (dioxane-H_2O, 1:1) at 80°C for 2 h in a water bath. After dioxane was removed, the soln was extracted with EtOAc (1 ml × 3). The extraction was washed with H_2O and then combined to give an

amorphous powder (**5**, 4 mg). The monosaccharide portion was neutralized by passing through an exchange resin (Amberlite MB-3) column, concentrated (dried overnight) then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After the excess reagent was decomposed with water, the reaction product was extracted with hexane (1 ml × 2 times). The TMSi derivatives of the monosaccharides were identified to be D-glucoses by co-GLC analyses with standard monosaccharides. Using the same method, **2**, **3**, **4** resulted in **5** and the monosaccharides were identified to be D-glucose by GLC analyses.

Gypsogenic acid (**5**). An amorphous solid, mp 250–252°C, $[\alpha]_D^{22}$ +64.5° (MeOH; *c* 0.4). IR ν_{max}^{KBr} cm^{-1}: 3428, 2944, 1694, 1463, 1384, 1269, 1051. FAB MS (positive ion mode) *m/z*: 487 $[M+H]^+$. ^1H NMR (500 MHz, pyridine-d_5): δ 0.93, 0.98, 1.01, 1.04, 1.25, 1.66 (each 3H, *s*, H_3 of C-29, C-30, C-25, C-26, C-27, C-24), 3.31 (1H, *dd*, J = 12.8, 4.8 Hz, H-18), 4.70 (1H, *m*, H-3), 5.50 (1H, *br.t*, H-12).

3,4-*Secogypsogenic acid* (**6**). An amorphous solid, mp 198–200°C, $[\alpha]_D^{22}$ +37.3° (MeOH; *c* 0.3). IR ν_{max}^{KBr} cm^{-1}: 3427, 2929, 1702, 1637, 1395, 1260, 1060. FAB MS (positive ion mode) *m/z*: 487 $[M+H]^+$, 509 $[M+Na]^+$. ^1H NMR (500 MHz, pyridine-d_5): δ 0.92, 0.98, 1.03, 1.11, 1.26 (3H, *s*, H_3 of C-30, C-29, C-25, C-26, C-27), 3.29 (1H, *dd*, J = 13.2, 1.0 Hz), 3.31 (1H, *dd*, J = 13.7, 3.8 Hz, H-18), 3.81, 3.95 (each 1H, *m*, H-3, 3′), 5.50 (1H, *br.t*, H-12), 5.57, 6.57 (each 1H, *s*, H-24, 24′).

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