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TRITERPENOID SAPONINS AND SAPOGENINS FROM *VACCARIA SEGETALIS*

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Key Word Index—*Vaccaria segetalis*; Caryophyllaceae; seeds; triterpenoid saponin; triterpene; nortriterpene; quillaic acid; gypsogenin; segetalic acid; vaccaric acid; vaccarosides E, F, G and H.

Abstract—Four new triterpenoid saponins, vaccarosides E, F, G and H were isolated from the seeds of *Vaccaria segetalis* and were respectively defined to be 3-*O*-*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl] quillaic acid 28-*O*-*β*-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-*β*-D-4-*O*-acetylfucopyranoside; 3-*O*-[*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl] 3*β*,4α,16α-trihydroxy-23-norolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-*β*-D-4-*O*-acetylfucopyranoside; 3-*O*-[*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl] gypsogenin 28-*O*-*β*-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-*β*-D-4-*O*-acetylfucopyranoside; and 3-*O*-[*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl] 3*β*,4α-dihydroxy-23-norolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-*β*-D-4-*O*-acetylfucopyranoside. Their structures were established on the basis of extensive NMR (DEPT, COSY, HOHAHA, HETCOR, HMBC AND NOESY), FAB-MS and ESI-MS studies as well as chemical strategies and enzymatic degradation. The new aglycones of two of the saponins have been designated as segetalic acid and vaccaric acid, respectively.

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. The seeds of the plant, popularly known as Wang-Bu-Liu-Xing in traditional Chinese medicine has been used widely to cure diseases associated with women after childbirth [1]. In our previous communication [2], we reported the isolation and characterization of four triterpenoid saponins, vaccarosides A, B, C, and D from the seeds of the plant. Further investigation on the polar fractions led to the isolation of four more new triterpenoid saponins designated vaccarosides E, F, G and H along with two new sapogenins, segetalic acid and vaccaric acid. In this paper, we report the isolation and structural elucidation of these compounds.

RESULTS AND DISCUSSION

The ethanol extract of the powdered seeds of the plant on chromatographic purification over Dianion HP-20, silica gel, followed by repeated MPLC and HPLC purification afforded four new bisdesmosidic triterpenoid saponins, vaccarosides E (**1**), F (**2**), G (**3**), and H (**4**).

Vaccaroside E (**1**), an amorphous solid, had a molecular formula of $C_{66}H_{102}O_{33}$ determined from its positive ion FAB-mass spectrum (at *m/z* 1445 $[M+Na]^+$, 1461 $[M+K]^+$) and negative ion ESI-mass spectrum (at *m/z* 1421 $[M-H]^-$) as well as ^{13}C, DEPT NMR data. Its spectral features and physicochemical properties suggested **1** to be a triterpenoid saponin. Of the 66 carbons, 30 were assigned to the aglycone part, 34 to the oligosaccharide moiety and the remaining two to an acetyl group (Tables 1 and 2). The IR spectrum showed absorptions at 3405 cm^{-1} (-OH) and at 1727 cm^{-1} (ester carbonyl). The six sp^3 hybrid carbons at δ 10.9, 15.8, 17.4, 24.4, 27.0, 33.1, and the two sp^2 hybrid carbons at δ 122.0 (*d*) and 144.5 (*s*) coupled with the information from ^1H NMR (six methyl proton singlets and a broad triplet vinyl proton at δ 5.60) indicated that the aglycone possessed an olean-12-ene skeleton. After an extensive 2D-NMR study, the aglycone was identified as quillaic acid (Table 1), a common aglycone of the triterpenoid

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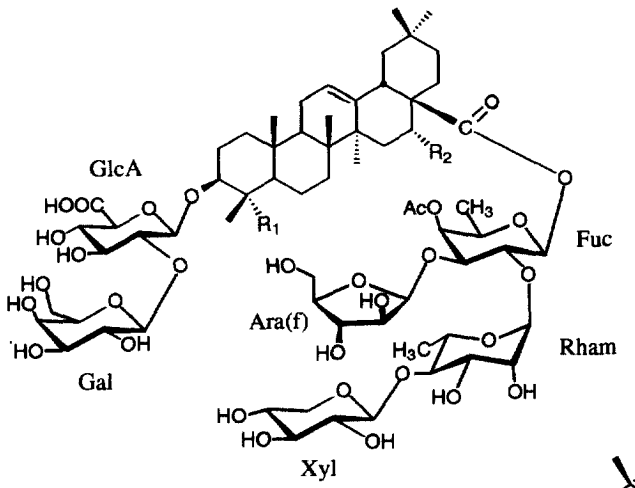
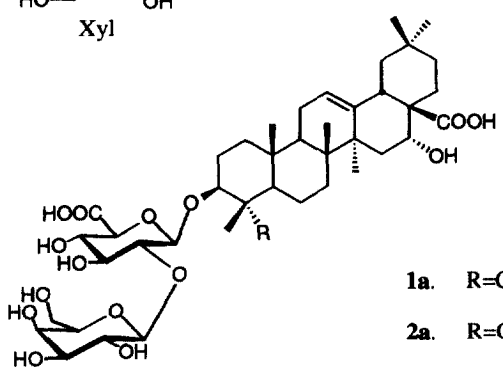
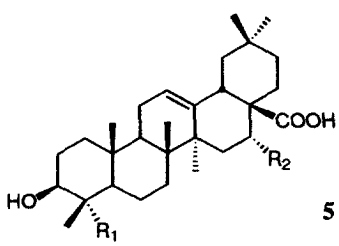


	R_1	R_2
1.	CHO	OH
2.	OH	OH
3.	CHO	H
4.	OH	H

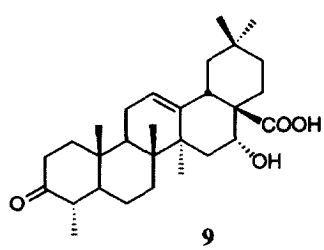


1a. R=CHO

2a. R=OH



	R_1	R_2
5	CHO	OH
6	OH	OH
7	CHO	H
8	OH	H



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glycosides, isolated from many sources [3, 4]. The chemical shifts of C-3 (δ 83.4) and C-28 (δ 175.9) indicated that **1** was a bisdesmosidic glycoside. The ^1H and ^{13}C NMR of **1** displayed six sugar anomeric protons at (δ 4.96 *d*, J = 7.3 Hz; 5.21 *d*, J = 8.0 Hz; 5.26 *d*, J = 7.3 Hz; 5.76 *s*; 6.01 *d*, J = 8.4 Hz; 6.06 *s*) and carbons (δ 94.4, 101,9, 103.4, 106.3, 106.6, 111.7), respectively (Tables 2 and 3). Alkaline hydrolysis of **1** furnished a prosapogenin (**1a**), identified as quillaic acid 3-*O*-β-D-galactopyranosyl-(1 $\rightarrow$2)-β-D-glucuronopyranoside from its spectral data (Tables 1, 2 and 3). Acid hydrolysis afforded quillaic acid (**5**) and the monosaccharide components, identified as arabinose, fucose, galactose, xylose, rhamnose (1 : 1 : 1 : 1 : 1) based on GLC analysis. The other monosaccharide was identified as glucuronic acid by TLC (solvent: $CHCl_3$–MeOH–H_2O, 6:4:1) on comparing with an authentic sample.

The sequence of the oligosaccharide chain at C-28 was determined by a combination of COSY, HOHAHA, DEPT, HETCOR, HMBC and phase-sensitive NOESY experiments. The spin systems for the glucuronic acid and galactose were weak and overlapped by other signals. Their proton and carbon assignments were later accomplished by comparing with that of the prosapogenin (**1a**). Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were delineated using COSY

Table 1. ^{13}C and ^{1}H data of the aglycone parts for vaccarosides E (**1**), F (**2**), G (**3**) and H (**4**), prosapogenins **1a** and **2a**, sapogenins **6** and **9** (150 or 125 MHz in pyridine-d_5)

	1	2	3	4	1a	2a	6	9	DEPT
1	38.1	38.5	38.1	38.3	38.1	38.4	38.8	40.2	CH2
2	25.2	27.5	25.0	27.2	24.9	27.4	28.6	37.6	CH2
3	83.4	92.4	83.6	92.1	83.4	92.2	79.8	212.0 (C)	CH
4	55.1	74.7	54.9	74.6	55.1	74.6	75.3	44.7	C
5	48.4	55.5	48.5	55.2	48.3	55.5	56.5	53.6	CH
6	20.5	18.0	20.6	17.8	20.4	17.6	17.9	24.3	CH2
7	32.7	32.9	32.5	32.5	32.8	33.0	33.1	32.3	CH2
8	40.2	40.3	40.1	39.9	40.1	40.0	40.1	39.6	C
9	46.7	47.1	47.8	47.8	47.0	47.1	47.3	44.7	CH
10	36.2	37.3	36.2	37.1	36.3	37.3	38.0	36.9	C
11	23.7	24.0	23.3	23.8	23.7	24.8	24.0	22.2	CH2
12	122.0	122.4	122.0	122.4	122.1	122.3	122.4	122.3	CH
13	144.5	144.5	144.0	143.9	145.2	145.2	145.2	145.3	C
14	42.1	42.4	42.2	42.3	42.1	42.2	42.2	42.3	C
15	36.3	36.3	28.3	28.4	36.2	36.3	36.2	36.2	CH2
16	73.9	73.9	23.6	23.3	74.7	74.7	74.7	74.7	CH
17	49.3	49.7	47.1	47.2	48.8	48.9	49.0	48.9	C
18	41.5	41.8	41.9	42.0	41.4	41.6	41.6	41.6	CH
19	47.5	47.7	46.3	46.3	47.2	47.3	47.3	47.3	CH2
20	30.7	30.8	30.6	30.6	31.1	31.1	31.1	31.1	C
21	35.9	36.1	33.9	33.9	36.1	36.1	36.1	36.1	CH2
22	31.9	32.0	32.2	32.2	32.9	32.9	32.8	32.9	CH2
23	209.4	—	209.6	—	209.3	—	—	12.0 (CH3)	C
24	10.9	15.4	10.9	15.1	10.9	15.3	15.4	—	CH3
25	15.8	18.9	15.7	18.8	15.7	18.9	17.9	13.1	CH3
26	17.4	17.6	17.3	17.4	17.4	17.5	17.6	17.5	CH3
27	27.0	27.0	25.8	25.7	27.2	27.2	27.2	27.2	CH3
28	175.9	176.2	176.4	176.5	179.9	180.0	180.0	180.0	C
29	33.1	33.2	33.0	32.9	33.3	33.4	33.4	33.4	CH3
30	24.4	24.5	23.6	23.6	24.7	24.8	24.7	24.8	CH3

—, Not observed.

with the aid of 2D-HOHAHA and NOESY spectra. Information from COSY and 2D-HOHAHA furnished most of the assignments. On the basis of the assigned protons, the ^{13}C NMR resonances of each sugar unit were identified by HETCOR and further confirmed by HMBC. Interpretation of the COSY and 2D-HOHAHA spectra revealed the presence of six monosaccharide units and one of them was α-rhamnose from its typical pattern in the COSY spectrum. In the light of the assigned ^{1}H NMR and ^{13}C NMR signals, as well as the information from NOESY, the other three sugar units were identified as β-fucose, β-arabinofuranose and β-xylose, which were further confirmed by the GLC analysis of the acid hydrolysate of **1**. From the above evidences it was concluded that **1** was a bisdesmosidic triterpenoid glycoside with glucuronic acid and galactose linked to the C-3 position of the aglycone and the other four monosaccharides were linked to the C-28 of the aglycone through an ester bond. The linkage of the sugar units at the side chain was established from the following HMBC correlations: H-1 of the xylose (X) with C-4 of rhamnose (R); H-1 rhamnose with C-2 of the fucose (F); H-1 arabinose (A) with C-3 of the fucose, while the attachment of the tetrasaccharide chain to C-28 of the aglycone was based on a correlation between H-1 of arabinose and the C-28 of the aglycone (Fig. 1). The appended acetyl group to H-4 of the fucose moiety was established from the HMBC correlation. Moreover, the low field signal of H-4 (δ 5.81) of the fucose also indicated that it was acylated. The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiment (Fig. 1). The linkage was also supported from the fragmentation patterns observed in the ESI-MS/MS experiment. MS/MS analysis of the deprotonated molecular ion $[M-H]^-$ (*m/z* 1421) gave a daughter ion at *m/z* 1289 $[(M-H)^-$-132] by the loss of one of the terminal pentanoses (xylose or arabinose) and at *m/z* 1143 $[(M-H]^-$-277] with the loss of the xyl-rham fragment from **1**. The most prominent fragment observed at *m/z* 823 was due to the loss of the tetrasaccharide chain linked to C-28 of vaccaroside E (**1**). Further loss of the terminal galactose at C-3 afforded another ion at *m/z* 643 confirming the attachment of glucuronic acid to C-3 of the aglycone (Fig. 1).

It was apparent that the arabinose existed in a furanose form as indicated from its C-2 and C-4 ^{13}C

Table 2. ^{13}C data of the sugar parts for vaccarosides E (**1**), F (**2**), G (**3**) and H (**4**), and prosapogenins **1a** and **2a** (150 or 125 MHz in pyridine-d_5)

	1	**2**	**3**	**4**	**1a**	**2a**
3-*O*-GluA						
GA-1	103.4 ($^1J_{CH}$, 146.7 Hz)	105.4	103.5	105.2	103.4	105.3
GA-2	82.2	85.1	82.2	84.9	82.5	85.2
GA-3	77.6	77.3	77.6	77.5	77.5	77.5
GA-4	72.9	72.9	72.8	72.8	72.9	73.0
GA-5	77.4	77.0	76.9	77.2	77.1	77.4
GA-6	172.6	172.0	170.7	172.4	172.6	172.0
Galactose						
G-1	106.3 (158.5)	107.8	106.2	107.6	106.3	107.8
G-2	73.4	74.3	74.4	74.3	74.5	74.4
G-3	74.6	74.4	74.7	74.5	74.8	74.7
G-4	70.2	69.9	70.1	69.8	70.2	70.1
G-5	77.6	77.6	78.0	77.9	77.7	77.8
G-6	62.1	61.7	62.1	62.0	62.1	62.1
28-*O*-Fucose						
F-1	94.4 (164.3)	94.4	94.3	94.2		
F-2	73.2	74.3	73.7	73.7		
F-3	82.9	81.1	80.8	81.0		
F-4	73.8	73.9	73.8	73.8		
F-5	70.6	70.6	70.4	70.4		
F-6	16.5	16.5	16.4	16.4		
$\underline{CH_3}CO$	20.7	20.8	20.7	20.7		
$CH_3\underline{C}O$	170.8	170.9	170.7	170.7		
Rhamnose						
R-1	101.9 (171.7)	102.0	102.0	102.0		
R-2	71.6	71.6	71.4	71.4		
R-3	72.3	72.3	72.3	72.3		
R-4	82.9	83.9	84.7	84.8		
R-5	68.8	68.9	68.7	68.5		
R-6	18.6	18.6	18.5	18.5		
Arabinose						
A-1	111.7 (173.1)	111.8	111.7	111.7		
A-2	83.5	83.6	83.5	83.5		
A-3	78.0	78.0	78.0	78.0		
A-4	85.7	85.7	85.8	85.7		
A-5	61.9	61.6	61.9	61.9		
Xylose						
X-1	106.6 (157.0)	106.6	107.4	107.4		
X-2	76.0	76.0	76.0	76.0		
X-3	78.5	78.5	78.5	78.7		
X-4	70.9	70.6	70.7	70.7		
X-5	67.3	67.4	67.4	67.4		

NMR data (Table 2). All other monosaccharides were in the pyranose forms as determined from their ^{13}C NMR data. The β-anomeric configuration for the fucose, galactose, glucuronic acid and xylose were based on their $^3J_{H1,H2}$ coupling constants (7–8 Hz) and their $^1J_{C1,H1}$ (Tables 2 and 3). The ^1H non-splitting patterns, large $^1J_{C1,H1}$ (171.7 Hz) and the ^{13}C NMR chemical shifts of the rhamnose indicated α-orientations. The anomeric proton of the arabinose was a broad singlet, indicating it to be α-arabinofuranose. The absolute configurations of these sugars were chosen in keeping with those mostly encountered among other plant glycosides. Thus, the structure of vaccaroside E is established as 3-*O*-[β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl] quillaic acid 28-*O*-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-β-D-4-*O*-acetylfucopyranoside (**1**).

Vaccaroside F (**2**), an amorphous solid, had a molecular formula of $C_{65}H_{102}O_{33}$ determined from its negative ESI-MS (*m/z*: 1409 $[M-H]^-$, 704 $[M-2H]^{2-}$) as well as ^{13}C DEPT NMR data. The ^1H NMR and ^{13}C NMR spectra of **2** revealed that the carbohydrate moiety was identical with that of vaccaroside E (**1**) but differed in the aglycone part (Tables 1 and 2). An extensive 2D-NMR analyses established the aglycone to be 3β,4α,16α-trihydroxy-23-norolean-12-en-28-oic acid (**6**), a new genuine nortriterpenoid sapogenin, designated as segetalic acid. Acid hydrolysis of **2** furnished a rearranged aglycone, and identified as 3-keto,16α-hydroxyl,24-noroleanolic acid (**9**). Alkaline hydrolysis resulted in a prosapogenin **2a**, which on enzymatic hydrolysis with β-glucuronidase afforded the genuine aglycone **6**. It was evident that during acid hydrolysis **6** had undergone facile dehydration of 4α-hydroxyl group, forming a double bond between C-3 and C-4, with concomitant migration of the proton furnishing the 3-keto compound (**9**). The 24β-methyl group changed into the energetically more favored 23α-position during the migration of the bond due to the steric influence as indicated by the significant highfield shift of the methyl protons at C-4 (Fig. 2).

The structure of **2** was mostly accomplished using the same protocol as that of **1**. The sugar arrangement was initially determined by the fragmentation patterns observed in the negative ESI-MS/MS experiment. Compound **2** gave the same fragmentation patterns as observed in **1** (Fig. 1). The exact linkage position for the tetrasaccharide unit was established using the HMBC and NOESY correlations as depicted for **1** in Fig. 1. The stereochemistry of each anomeric carbons were determined from the same observation as that of **1**. Thus, the structure of vaccaroside F was elucidated to be 3-*O*-[β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl] 3β,4α,16α-trihydroxy-23-norolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-β-D-4-*O*-acetylfucopyranoside (**2**).

Vaccaroside G (**3**) and H (**4**), amorphous solids, had a molecular formula of $C_{66}H_{102}O_{32}$, $C_{65}H_{102}O_{32}$, respectively, as determined from their positive FAB-MS and ^{13}C DEPT NMR data. ^1H NMR and ^{13}C NMR spectra indicated that compounds **3** and **4** had the same sugar arrangement as that of **1** but differed in the aglycone parts (Tables 1 and 2). Extensive 2D-

Table 3. ^{1}H NMR data of the sugar parts for vaccarosides E (**1**), F (**2**), G (**3**) and H (**4**), and prosapogenins **1a** and **2a** (600 or 500 MHz in pyridine-d_5)

	1	**2**	**3**	**4**	**1a**	**2a**
3-*O*-GluA						
GA-1	4.96 *d* (7.3 Hz)	5.05 *d* (8.0)	4.92 *d* (7.4)	5.13 *d* (7.7)	4.95 *d* (7.6)	5.15 *d* (7.6)
GA-2	4.28	4.08	4.21	4.16	4.22 *dd* (7.6, 8.5)	4.19 *dd* (7.6, 8.5)
GA-3	4.32	4.25	4.28	4.35	4.31 *dd* (8.5, 8.8)	4.36 *dd* (8.5, 8.8)
GA-4	4.57	4.49	4.51	4.56	4.53	4.57
GA-5	4.55	4.50	4.52	4.60	4.55	4.60
GA-6	—	—	—	—	—	—
Galactose						
G-1	5.26 *d* (7.3)	5.09 *d* (8.1)	5.22 *d* (7.6)	5.19 *d* (7.6)	5.22 *d* (7.3)	5.20 *d* (7.6)
G-2	4.58	4.45	4.54	4.55	4.57	4.59
G-3	4.16	4.08	4.14	4.15	4.14	4.17 *dd* (9.5, 3.1)
G-4	4.60	4.45	N.A.	N.A.	4.57	4.58
G-5	4.28	4.03 *m*	N.A.	4.09	4.15	4.11 *t* (6.0)
G-6	4.57 (2H)	4.38, 4.50	4.52 (2H)	4.51, 4.54	4.55 (2H)	4.48 *dd* (12.0, 6.1) 4.56 *dd* (12.0, 6.5)
28-*O*-Fucose						
F-1	6.01 *d* (8.4)	5.92 *d* (8.1)	5.99 *d* (8.3)	6.00 *d* (8.0)		
F-2	4.41	4.40	4.52	4.54		
F-3	4.19	4.20	4.24	4.24		
F-4	5.81 *d* (2.7)	5.71 *d* (3.0)	5.78 *d* (3.3)	5.78 *d* (3.3)		
F-5	4.02	3.92	3.96	3.95		
F-6	1.20 *d* (6.2)	1.14 *d* (6.2)	1.18 *d* (6.5)	1.18		
$\underline{CH_3}CO$	2.00 *s*	1.99 *s*	2.01 *s*	2.02 *s*		
Rhamnose						
R-1	6.06 *s*	5.88 *s*	6.00 *s*	6.02 *s*		
R-2	4.74	4.61	4.74 *s*	4.75 *s*		
R-3	4.64 *dd* (8.8, 3.2)	4.49	4.58 *dd* (9.1, 3.0)	4.60		
R-4	4.44	4.23	4.30	4.30		
R-5	4.47	4.31 *m*	4.38	4.40		
R-6	1.75 *d* (5.9)	1.66 *d* (6.2)	1.78 *d* (6.1)	1.78 *d* (6.1)		
Arabinose						
A-1	5.76 *s*	5.62 *s*	5.72 *s*	5.72 *s*		
A-2	4.90	4.75	4.88 *m*	4.89 *m*		
A-3	4.87	4.69	4.82 *m*	4.83 *m*		
A-4	4.73	4.58 *m*	4.71 *m*	4.71 *m*		
A-5	4.16, 4.35	4.07, 4.21	4.18, 4.34	4.18, 4.33		
Xylose						
X-1	5.21 *d* (8.0)	5.01 *d* (7.0)	5.01 *d* (7.6)	5.02 *d* (7.3)		
X-2	4.03	3.92	4.02	4.05		
X-3	4.10	3.92	4.05	4.04		
X-4	4.11	4.07	4.16	4.04		
X-5	3.47 *t* (9.8)	3.40 *t* (9.8)	3.51 *t* (10.7)	3.50 *t* (12.2)		
	4.16	4.07	4.22	4.21		

N.A.; Not assigned.

NMR analyses established the aglycone for **3** as gypsogenin (**7**) and for **4** as 3β,4α-dihydroxy-23-norolean-12-en-28-oic acid (**8**), a new nortriterpenoid sapogenin, named vaccaric acid. As in the case of **2**, acid hydrolysis of **4** also furnished a rearranged aglycone, but due to the paucity of material, further investigation could not be pursued.

The structural assignments were mainly accomplished using a combination of COSY, HOHAHA, HETCOR, HMBC and NOESY. The presence of six sugars in **3** and **4** were indicated from the six anomeric protons and carbons from their ^{1}H NMR and ^{13}C NMR spectra (Tables 2 and 3). From the assigned aglycone (Table 1), it was apparent that the six sugars were present in two saccharide units, one attached to C-3 and the other at C-28. The exact linkage positions for the tetrasaccharide unit was established using the HMBC and NOESY correlations as depicted for **1** in Fig. 1. The stereochemistry of each of the anomeric carbons were deter-

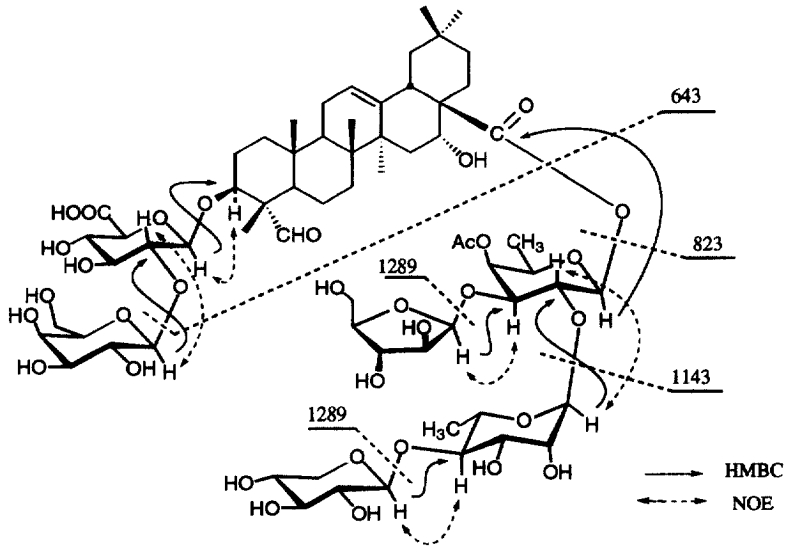

Fig. 1. Key HMBC and NOE correlations and ESIMS/MS fragmentation patterns for Vaccaroside E (**1**).

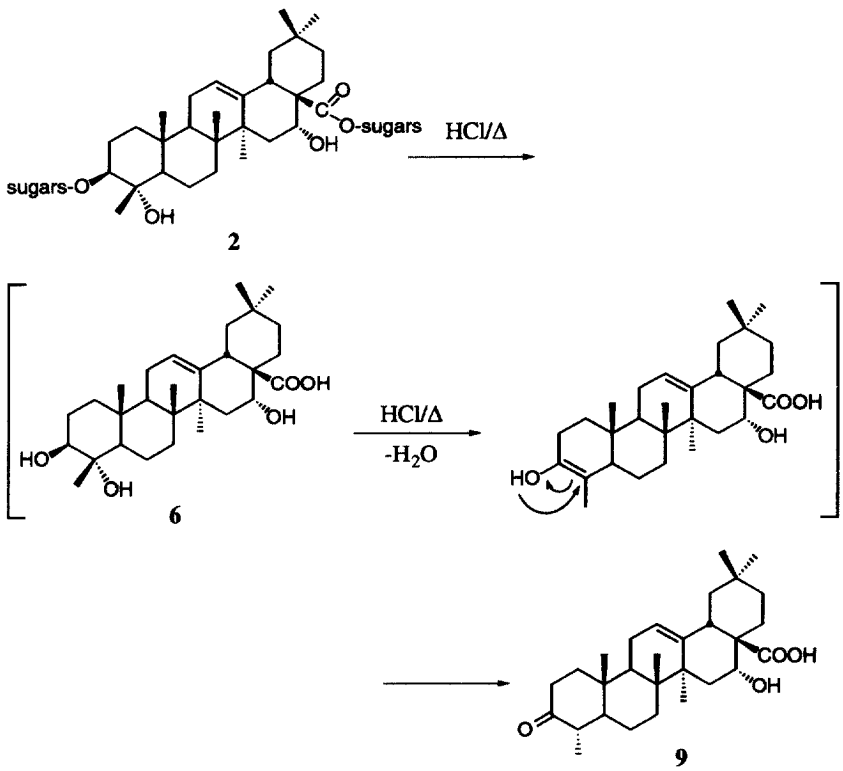

Fig. 2. The formation of **9** during acid hydrolysis of vaccaroside F (**2**).

mined from the same observation as that of **1**. Thus, vaccaroside G and H were elucidated to be 3-*O*-[β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl] gypsogenin 28-*O*-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-β-D-4-*O*-acetylfucopyranoside (**3**) and 3-*O*-[β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl] 3β,4α-dihydroxy-23-norolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-arabinofuranosyl-(1 → 3)]-β-D-4-*O*-acetylfucopyranoside (**4**), respectively.

It is noteworthy to mention that early phytochemical investigation [5, 6] on the seeds of this species led to the isolation and structural elucidation of several triterpenoid saponins containing 6–10 units with gypsogenic acid as the sapogenin. However, we could

not identify any of them using MPLC, HPLC, etc. and it is of doubt whether the saponins studied earlier were pure or mixtures of saponins.

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. FAB-MS were conducted using JEOL DX-303 mass spectrometer. ESI-MS were conducted using a Finnigan MAT LCQ mass spectrometer. ^{1}H NMR and ^{13}C NMR were recorded using a JEOL LA-600, JEOL α-500 or a JEOL EX-400 FT-NMR spectrometer. Chemical shifts were expressed in δ (ppm) referring to TMS. Dianion HP-20 (Mitsubishi Kasei), silica gel (Silica gel 60, Merck), and ODS (Chromatorex, 100–200 mesh, Fujisylisia) were used for CC. Prep. 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.

Extraction and isolation

Seeds of *Vaccaria segetalis* were purchased from a market in Beijing, China, in December 1993, and were identified by one of the authors (Z.J.). The seeds of the plant are kept in the herbarium of the Pharmacognosy Department of Toho University. Crushed seeds (2 kg) were extracted with 95%, 50% EtOH (5 l, each) 3 × under reflux for 2 h. The combined EtOH extract was concentrated under red. press. to give an extract (170 g), which was applied to a column of Dianion 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. HPLC purification of the high polar fraction (60–65% MeOH–0.06% TFA in H_2O, 1.5 ml min^{-1}, UV detector, 210 nm) gave vaccaroside E (85 mg), F (60 mg), G (15 mg) and H (10 mg), respectively.

Vaccaroside E (**1**). An amorphous solid, mp 230° (dec.), $[\alpha]_D^{21}$ −22.4° (MeOH; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3405, 2932, 1727, 1079, 1051. FAB-MS (positive ion mode) *m/z*: 1445 $[M+Na]^+$, 1461 $[M+K]^+$. ESI-MS (negative ion mode) *m/z*: 1421 $[M-H]^-$. ^{1}H NMR (600 MHz, pyridine-d_5): δ 0.83, 0.98, 1.02, 1.09, 1.43, 1.78 (each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-24, C-27), 3.38 (1H, *dd*, *J* = 13.2, 4.4 Hz, H-18), 4.10 (1H, *m*, H-3), 5.22 (1H, *br t*, H-16), 5.76 (1H, *br s*, H-12), 9.92 (1H, *s*, H-23). For other NMR data, see Tables 1, 2 and 3.

Vaccaroside F (**2**). An amorphous solid, mp 200° (dec.), $[\alpha]_D^{20}$ −28.0° (MeOH, *c* 0.15). IR ν_{max}^{KBr} cm^{-1}: 3426, 2934, 1734, 1079, 1051. ESI-MS (negative ion mode) *m/z*: 1409 $[M-H]^-$, 704 $[M-2H]^{2-}$. ^{1}H NMR (600 MHz, pyridine-d_5): δ 0.78, 0.91, 0.98, 1.06, 1.36, 1.69, (Each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-24, C-27), 3.60 (1H, *m*, H-3), 5.56 (1H, *br t*, H-12), 5.06 (1H, *br s*, H-16). For other NMR data, see Tables 1, 2 and 3.

Vaccaroside G (**3**). An amorphous solid, mp 159° (dec.). $[\alpha]_D^{24}$ −9.5° (MeOH: *c* 1.77). IR ν_{max}^{KBr} cm^{-1} *m/z*: 3405, 2936, 1726, 1077. FAB-MS (positive ion mode) *m/z*: 1429 $[M+Na]^+$. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.82, 0.87, 0.89, 1.04, 1.25, 1.42 (each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-27, C-24), 3.08 (1H, *dd*, *J* = 14.1, 4.2 Hz, H-18), 4.05 (1H, *m*, H-3), 5.39 (1H, *br s*, H-12), 9.96 (1H, *s*, H-23). For other NMR data, see Tables 1, 2 and 3.

Vaccaroside H (**4**). An amorphous solid, mp 154°C (dec.), $[\alpha]_D^{24}$ −13.4°C (MeOH; *c* 1.28). IR ν_{max}^{KBr}: 3404, 2935, 1742, 1075, 1050. FAB-MS (positive ion mode) *m/z*: 1417 $[M+Na]^+$. ^{1}H NMR (500 MHz, pyridine-d_5); δ 0.79, 0.87, 0.88, 1.09, 1.23, 1.42 (each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-27, C-24), 3.10 (1H, *dd*, *J* = 14.4, 3.5 Hz, H-18), 3.68 (1H, *m*, H-3), 5.43 (1H, *br s*, H-12). For other NMR data, see Tables 1, 2 and 3.

Acid hydrolysis of vaccarosides E (**1**), *F* (**2**), *G* (**3**) *and H* (**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 temp. 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 arabinose, fucose, galactose, rhamnose and xylose (1:1:1:1:1) by co-GLC analyses with standard monosaccharides. The glucuronic acid was detected by co-TLC analysis with an authentic sample (solvent: $CHCl_3$–MeOH–H_2O, 6:4:1). By the same method, **2** (10 mg) resulted in **9** (2 mg), **3** (6 mg) gave **7** (1 mg). GLC analyses showed the monosaccharides of **2**, **3** and **4** were identical to that of **1**.

Alkaline hydrolysis of vaccarosides E (**1**) *and F* (**2**). Vaccarosides E (**1**, 20 mg) in 2 ml 1N KOH was heated at 80°C for 2 h. After cooling down, the reaction mixture was neutralized with 1N HCl and then extracted with *n*-BuOH (3 ×). The organic layers were combined and then evaporated to dryness *in vacuo*. The residue was subjected to HPLC purification (ODS column, 1.0 × 30 cm, 60% MeOH–0.06% TFA in H_2O, 1.5 ml min^{-1}., UV detector, 2.10 nm) afforded prosapogenin **1a** (8 mg). By the same method, **2** (30 mg) afforded **2a** (12 mg).

Prosapogenins **1a** *and* **2a**. Compound **1a**, an

amorphous solid, mp 220° (dec.), $[\alpha]_D^{23}$ +18.0° (MeOH: *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3427, 2926, 1680, 1078. FAB-MS (positive ion mode) *m/z*: 847 $[M+Na]^+$. ^{1}H-NMR (400 MHz, pyridine-d_5): δ 0.82, 0.97, 1.06, 1.18, 1.41, 1.82 (each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-24, C-27), 2.82 (1H, *t*, *J* = 13.4, H-19), 3.59 (1H, *dd*, *J* = 13.1, 4.0 Hz, H-18), 4.09 (1H, *dd*, *J* = 11.9, 4.4, H-3), 5.22 (1H, *br s*, H-16), 5.60 (1H, *br t*, H-12), 9.92 (1H, *s*, H-23). For other NMR data, see Tables 1, 2 and 3. Compound **2a**, an amorphous solid, mp 225° (dec.), $[\alpha]_D^{23}$ 0° (MeOH; *c* 0.9). IR ν_{max}^{KBr} cm^{-1}: 3427, 2930, 1693, 1077. FAB-MS (positive ion mode) *m/z*: 835 $[M+Na]^+$, 851 $[M+K]^+$. ^{1}H NMR (400 MHz, pyridine-d_5): δ 0.82, 1.03, 1.06, 1.18, 1.43, 1.82 (each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-24, C-27), 2.83 (1H, *t*, *J* = 13.6, H-19), 3.60 (1H, *dd*, *J* = 14.0, 3.5 Hz, H-18), 3.70 (1H, *dd*, *J* = 12.0, 4.1 Hz, H-3), 5.23 (1H, *br s*, H-16), 5.62 (1H, *br t*, H-12). For other NMR data, see Tables 1, 2 and 3.

Enzymatic hydrolysis of prosapogenin **2a** [7]. To a soln of **2a** (10 mg) in an acetate buffer (pH 5.0, 0.5 ml) was added β-glucuronidase (0.25 ml) [Sigma No. G-0876 (Lot. 64H3370)], and the mixture was incubated at 37° for 4 h. EtOAc extraction afforded the genuine aglycone, which upon column purification (silica gel, $CHCl_3$–MeOH, 15:1) yielded **6** (5 mg). The galactose and glucuronic acid were detected from the water layer (TLC, $CHCl_3$–MeOH–H_2O, 6:4:1).

Quillaic acid (**5**). An amorphous solid, mp 206–208°, $[\alpha]_D^{23}$ +30.0 (MeOH; *c* 0.2). IR ν_{max}^{KBr} cm^{-1}: 3427, 2924, 2855, 1725, 1260. EI-MS *m/z*: 487 $[M+H]^+$, 469, 425, 355. ^{1}H NMR (500 MHz, pyridine-d_5): δ 0.94, 1.03, 1.07, 1.19, 1.36, 1.84 (each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-24, C-27), 2.85 (1H, *t*, *J* = 14.0, H-19), 3.63 (1H, *dd*, *J* = 14.5, 4.8 Hz, H-18), 4.11 (1H, *t*, *J* = 8.5, H-3), 5.24 (1H, *br s*, H-16), 5.66 (1H, *br t*, H-12), 9.62 (1H, *s*, H-23).

Segetalic acid (**6**). An amorphous solid, mp. 208–210°, $[\alpha]_D^{23}$ +29.0 (MeOH, *c* 0.2). IR ν_{max}^{KBr} cm^{-1}: 3428, 2928, 2856, 1693, 1395, 1200. FAB-MS *m/z*: 497 $[M+Na]^+$. ^{1}H NMR (400 MHz, pyridine-d_5): δ 0.94, 1.07, 1.08, 1.19, 1.45, 1.77 (each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-24, C-27), 2.84 (1H, *t*, *J* = 14.0, H-19), 3.64, (1H, *m*, H-18), 3.93, (1H, *dd*, *J* = 12.1, 4.4 Hz, H-3), 5.24 (1H, *br s*, H-16), 5.68 (1H, *br t*, H-12). ^{13}C NMR data see Table 1.

Gypsogenin (**7**). An amorphous solid, mp 215°. FAB-MS *m/z*: 471 $[M+H]^+$. ^{1}H NMR (400 MHz, pyridine-d_5): δ 0.90, 0.97, 1.00, 1.04, 1.30, 1.37 (each 3H, *s*, H_3 of C-25, C-29, C-30, C-26, C-27, C-24), 3.08 (1H, *dd*, *J* = 14.1, 4.2 Hz, H-18), 4.09 (1H, *m*, H-3), 5.50 (1H, *br s*, H-12), 9.64 (1H, *s*, H-23).

3-Keto,16α-hydroxy,24-noroleanolic acid (**9**). An amorphous solid, mp 145°C (dec.), $[\alpha]_D^{23}$ +31.3 (MeOH, *c* 0.3). IR ν_{max}^{KBr} cm^{-1}: 3516, 3428, 2926, 1681, 1218. EIMS *m/z*: 457 $[M+H]^+$, 439, 411, 395. ^{1}H NMR (500 MHz, pyridine-d_5): δ 1.00, 1.07 (*d*, overlapped), 1.08 (×2), 1.20, 1.79 (each 3H, *s*, H_3 of C-25, C-24, C-29, 30, C-26, C-27), 2.84 (1H, *t*, *J* = 13.1 Hz, H-19), 3.64 (1H, *dd*, *J* = 14.0, 3.8 Hz, H-18), 5.26 (1H, *br s*, H-16), 5.65 (1H, *br t*, H-12). ^{13}C NMR data see Table 1.

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