Triterpenoid Saponins from *Vaccaria segetalis*

MA, Ji[a](马骥) HE, Fa-Hu[b](何发虎) DENG, Jing-Zhen[a](邓京振) YE, Wen-Cai[a](叶文才)
ZHAO, Shou-Xun[a](赵守训) WU, Hou-Ming*[,b](吴厚铭)
[a]*Department of Phytochemistry, China Pharmaceutical University, Nanjing, Jiangsu 210009, China*
[b]*State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*

Four novel triterpenoid saponins, Vaccariside B—E (1—4), were isolated from the seeds of *Vaccaria segetalis* and their structures were elucidated as 3-*O*-β-*D*-galactopyranosyl-(1-2)-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-β-*D*-xylopyranosyl-(1-3)-α-*L*-rhamno-pyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-4-*O*-acetyl-β-*D*-fucopyranoside (1), 3-*O*-β-*D*-galactopyranosyl-(1-2)-3-*O*-acetyl-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-β-*D*-xylopyranosyl-(1-3)-α-*L*-rhamnopyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-4-*O*-acetyl-β-*D*-fucopyranoside (2), 3-*O*-β-*D*-galactopyranosyl-(1-2)-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-α-*L*-arabinopyranosyl-(1-3)-α-*L*-rhamnopyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-4-*O*-acetyl-β-*D*-fucopyranoside (3), 3-*O*-β-*D*-galactopyranosyl-(1-2)-[β-*D*-xylopyranosyl-(1-3)]-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-β-*D*-xylopyranosyl-(1-3)-α-*L*-rhamnopyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-4-*O*-acetyl-β-*D*-fucopyranoside (4), respectively.

Keywords *Vaccaria segetalis*, caryophyllaceae, saponin, quillaic acid

Introduction

Caryophyllaceae, which is divided into three subfamilies: Paronychioideae, Alsinoideae, and Silenoideae, is a large family distributed all over the world. Many plants of this family are used as folk medicines to treat various diseases. Among them, the seed of *Vaccaria segetalis* (Neck.) Garcke (Caryophyllaceae) is a kind of well-known Traditional Chinese Medicine (TCM) called 'wang-bu-liu-xing' and has been recorded in Chinese Pharmacopoeia (1995 Ed.). It has been used in China over a long time for the treatment of blood-stasis syndrome with anemia or dysmenorrhea, carbuncle and stranguria complicated by hematuria. Its chemical investigation has resulted in the isolation of several triterpenoid saponins, eight cyclopeptides, and many other compounds.[1-10]

During our further searching for bioactive secondary metabolites of this plant, four novel triterpenoid saponins, Vaccariside B—E (**1—4**) were isolated, and their structures were elucidated on the basis of spectroscopic evidences, especially 1D and 2D NMR data. In this paper, we report the isolation and structural determination of these compounds.

Results and discussion

The ethanol extract of the seeds of *V. segetalis* was partitioned between water and ethyl acetate. The water extract was subjected to chromatography over D-101 macroporous resin column, silca gel flash column and Lichroprep C-18 column successively to obtain **1—4**.

Vaccariside B (**1**) was obtained as an amorphous white powder, with the positive result of Liebermann-Burchard reaction, m.p. 205—207℃, $[\alpha]_D^{18}$ − 5.98 (*c* 0.65, CH_3OH). IR spectrum suggested the existence of carbonyl (1727 cm^{-1}) and hydroxyl (3423 cm^{-1}) groups. Electrospray ionized mass spectrum showed quasi-molecular ion peak 1445 $[M + Na]^+$ and indicat-

* Tel: (021)64163300-1431; Fax: (021)64166128; E-mail: hmwu@pub.sioc.ac.cn
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ed the molecular weight of 1422. These data in combination of ^{13}C and ^{1}H NMR allowed to assign the molecular formula of $C_{66}H_{102}O_{33}$.

The ^{1}H NMR spectrum clearly revealed the presence of six tertiary methyl groups (δ 0.81, 0.94, 0.97, 1.30, 1.39, 1.75), two secondary methyl groups (δ 1.16, 1.70), one acetyl methyl group (δ 1.96), one aldehydic proton (δ 9.88) and six anomeric protons (δ 6.0—4.8) for the sugar moieties. These data suggested that **1** was a triterpenoid glycoside.

The ^{13}C NMR spectrum showed signals corresponding to two olefinic carbons (δ 144.51, 121.99), one aldehydic carbon (δ 209.38), one carboxylic carbon (δ 170.75), two ester carbons (δ 175.86, 170.68) and six anomeric carbons (δ 111.67, 106.50, 106.23, 103.24, 101.82, 94.42). Among them, the signal δ 111.67 suggested the existence of a furanose residue, while the signal δ 94.42 was attributed to an anomeric carbon of a sugar having an ester linkage. These evidences were indicative of the presence of six sugars (including a glucuronic acid, a furanose), an acetyl group and an aglycone of quillaic acid, which featured an aldehyde group at C-23, a tri-substituted double bond at C-12 and C-13, a carboxyl group at C-28. The aglycone was finally confirmed as quillaic acid by the comparison of its ^{13}C NMR data with those of analogous quillaic acid glycoside (Table 1).[11]

The structure elucidation was further proceeded by 2D NMR experiments due to the complicated sugar moieties. The ^{1}H NMR sub-spectra of each sugar were obtained from rows corresponding to the signals of their anomeric protons and other characteristic protons (such as Me-6 for fucose and rhamnose, methylene-5 for xylose) in the TOCSY spectrum. In combination of the DQF-COSY experiment, the signals of each proton of sugar moieties were assigned. The vicinal coupling constants between sugar ring protons obtained from these experiments determined the relative stereochemistry of each asymmetric center and thus identified each individual monosaccharide (Table 2). Each monosaccharide was confirmed by intra-residue NOE connectivities observed in ROESY spectrum (Table 3). The assignment of ^{13}C NMR of each monosaccharide was achieved directly by high-resolved HMQC experiment (Table 4). Compared to the typical values of corresponding sugar, the down-fielded signals (underlined in Table 4) were attributed to the glycosylation shifts, which were used for the determination of linkages between sugars.[12]

Table 1 ^{13}C NMR data of aglycones of **1**—**4** and analogue in reference 11 (in pyridine-d_5)

Position	1	2	3	4	Ref. 11
1	38.13	38.17	38.09	38.04	37.8
2	24.93	24.88	24.94	24.32	23.5
3	83.32	83.55	83.47	83.38	84.4
4	55.03	55.02	54.94	54.95	54.9
5	47.47	47.51	48.52	49.18	47.7
6	20.68	20.69	20.65	20.57	20.3
7	31.86	31.88	33.00	31.76	32.3
8	40.23	40.26	40.10	40.09	39.8
9	46.88	46.90	46.33	46.76	48.4
10	36.20	36.24	36.14	36.06	36.1
11	23.73	23.76	23.30	22.76	23.5
12	121.99	122.02	122.31	121.89	122.1
13	144.51	144.58	144.05	144.38	144.7
14	42.10	42.14	42.20	41.94	42.0
15	36.19	36.15	36.14	36.06	35.6
16	73.94	73.97	74.70	71.43	73.5
17	49.33	49.36	48.52	47.33	46.3
18	41.52	41.56	41.88	41.37	41.8
19	47.47	47.51	47.11	47.33	46.5
20	29.91	30.69	28.37	29.80	30.8
21	36.20	36.24	36.14	36.06	34.1
22	30.66	30.69	30.61	30.55	33.0
23	209.38	209.54	210.01	210.12	210.0
24	10.89	10.98	11.09	11.01	10.9
25	15.81	15.87	15.71	15.68	15.4
26	17.34	17.41	17.35	16.90	17.2
27	27.04	27.05	25.88	26.83	26.0
28	175.86	175.86	176.40	175.73	172.2
29	33.08	33.14	33.01	33.04	33.1
30	24.39	24.46	23.70	23.58	23.6

As the ^{1}H and ^{13}C signals of each sugar residue have been unambiguously assigned, the sequence and the linkages of sugar residues and the linkage between the sugar chain and the aglycone were established smoothly by the inter-residue NOE correlations and the inter-residue C—H long-range connectivities derived from ROESY and HMBC experiments, respectively. The inter-residue NOE correlations in the ROESY spectrum (Table 3) revealed two oligosaccharide chains. One had the sequence of β-*D*-galactopyranosyl-(1-2)-β-*D*-glucuronopyranose and attached with aglycone at C-3, and the other had the sequence of β-*D*-xylopyranosyl-(1-3)-

Table 2 ^{1}H NMR data of sugar moieties of **1** (in pyridine-d_5)

Position	δ_H
3-*O*-	
GlcA 1	4.86 (d, J = 9.2 Hz)
2	4.18 (dd, J = 8.4, 9.2 Hz)
3	4.26
4	4.40
5	4.43
Gal 1	5.18 (d, J = 9.2 Hz)
2	4.52
3	4.10
4	4.53 (dd, J = 4.1, 2.2 Hz)
5	4.11
6	4.12, 4.30
28-*O*-	
Fuc 1	5.95 (d, J = 8.3 Hz)
2	4.50
3	4.24
4	5.76
5	3.95 (dd, J = 5.8, 1.9 Hz)
6	1.16
CH_3CO-	1.97
Rha 1	5.97 (d, J = 2.4 Hz)
2	2.47
3	4.36 (dd, J = 8.3, 2.2 Hz)
4	4.58
5	4.41
6	1.70
Ara(f.) 1	5.68 (d, J = 7.2 Hz)
2	4.85 (dd, J = 6.3, 7.2 Hz)
3	4.80
4	4.68
5	4.14, 4.51
Xyl 1	5.15 (d, J = 7.9 Hz)
2	3.98
3	4.04
4	4.12 (dd, J = 8.4, 2.0 Hz)
5	3.45, 4.16

Table 3 Intra-residue and inter-residue NOE correlations of sugar moieties of **1** observed in ROESY spectrum

	Intra-residue NOE	Inter-residue NOE
βGlcA	H_1-H_3 H_1-H_5 H_2-H_4 H_3-H_5	H_1-aglycone H_3
βGal	H_1-H_3 H_3-H_4	H_1-GlcA H_2
βFuc	H_1-H_3 H_1-H_5 H_3-H_5 H_5-H_6	
αRha	H_1-H_2 H_2-H_3 H_5-H_6	H_1-Fuc H_2
αAra(f.)	H_1-H_2 H_1-H_3 H_1-H_5	H_1-Fuc H_3
βXyl	H_1-H_3 H_1-H_5 H_5-$H_{5'}$	H_1-Rha H_3

Table 4 ^{13}C NMR data (δ) of sugar moieties of **1**—**4** in pyridine-d_5 (Data for glycosylated carbons are underlined)

	1	**2**	**3**	**4**
3-*O*-				
GlcA 1	103.24	103.14	103.11	103.99
2	<u>82.22</u>	<u>81.88</u>	<u>81.97</u>	<u>80.66</u>
3	77.65	<u>82.09</u>	78.48	<u>86.20</u>
4	68.80	68.24	68.61	68.68
5	76.97	77.56	76.86	77.86
6	170.75	170.71	170.76	170.63
CH_3CO-		20.69		
CH_3CO-		170.63		
Gal 1	106.23	106.49	106.00	105.31
2	74.44	74.41	74.26	75.21
3	76.97	76.98	76.86	76.47
4	70.12	70.14	70.45	70.56
5	74.72	74.75	76.04	75.89
6	61.93	62.12	61.99	61.79
Xyl 1				104.71
2				75.21
3				78.35
4				71.43
5				67.17
28-*O*-				
Fuc 1	94.42	94.43	94.26	94.31
2	<u>73.27</u>	<u>73.17</u>	<u>73.52</u>	<u>73.69</u>
3	<u>80.78</u>	<u>81.16</u>	<u>81.15</u>	<u>82.91</u>
4	<u>73.80</u>	<u>73.84</u>	<u>74.26</u>	<u>74.60</u>
5	70.55	70.14	69.97	70.56
6	16.49	16.47	16.39	16.38
CH_3CO-	20.69	20.69	20.64	20.57
CH_3CO-	170.68	170.63	170.67	170.63
Rha 1	101.82	101.75	101.88	101.70
2	71.57	71.49	71.38	72.10
3	<u>82.92</u>	<u>82.47</u>	<u>81.97</u>	<u>83.38</u>
4	72.24	72.27	72.22	73.16
5	73.02	73.17	73.19	73.55
6	18.62	18.25	18.54	18.43
Ara(f.) 1	111.67	111.66	111.68	111.56
2	83.51	83.43	83.46	84.11
3	78.01	78.24	77.55	78.35
4	85.76	85.35	84.50	85.60
5	78.56	78.48	78.56	78.36
Xyl 1	106.50	106.49		106.42
2	76.01	75.75		76.47
3	78.45	78.59		78.35
4	70.89	70.59		70.03
5	67.30	67.31		67.17
Ara′ 1			107.25	
2			70.69	
3			69.98	
4			67.30	
5			64.29	

α-*L*-rhamnopyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-β-*D*-fucopyranose (Fig. 1). Though the anomeric proton of the fucose showed no inter-residue NOE correlation, the second sugar moiety could be deduced to link to the 28-carboxyl carbon from the downfielded shift (δ 5.95) of anomeric proton and the up-fielded shift (δ 94.42) of anomeric carbon of the fucose. The direct evidence from the HMBC experiment confirmed the linkage: the anomeric proton of the fucose showed a long-range connectivity ($^3J_{CH}$) to 28-carboxylic carbon. The other inter-residue long-range connectivities ($^3J_{CH}$) observed in HMBC experiment (Table 5) further supported the assignment of the sequence and glycosylation linkages of the sugar moiety elucidated by the ROESY experiment. The acetyl group was also clarified to attach at C_4 of Fuc by the C—H long-range correlation between H_4 of Fuc and the carboxylic carbon of acetyl group from the HMBC experiment. Consequently, the structure of Vaccariside B (**1**) was determined as 3-*O*-β-*D*-galactopyranosyl-(1-2)-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-β-*D*-xylopyranosyl-(1-3)-α-*L*-rhamnopyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-4-*O*-acetyl-β-*D*-fucopyranoside.

Vaccariside C (**2**) was obtained as an amorphous white powder, with the positive result of Liebermann-Burchard reaction, m.p. 212—214℃ $[\alpha]_D^{18}$ − 30.98 (*c* 0.94, CH_3OH). IR spectrum suggested the existence of carbonyl (1727 cm^{-1}) and hydroxyl (3433 cm^{-1}) groups. Electrospray ionized mass spectrum showed quasi-molecular ion peak 1487 $[M+Na]^+$ and indicated the molecular weight of 1464. These data in combination of ^{13}C and ^1H NMR allowed to assign the molecular formula of $C_{68}H_{104}O_{34}$. The ^1H NMR was very similar to that of **1** except for an additional acetyl methyl group (δ 1.96), which suggested the presence of a second acetyl group. The aglycone was also confirmed to be quillaic acid by the careful comparison of its ^{13}C NMR data with those of **1** and other analogue.[12] Further analysis and comparison of the ^{13}C NMR data of sugar moieties with those of **1** revealed that they had the similar

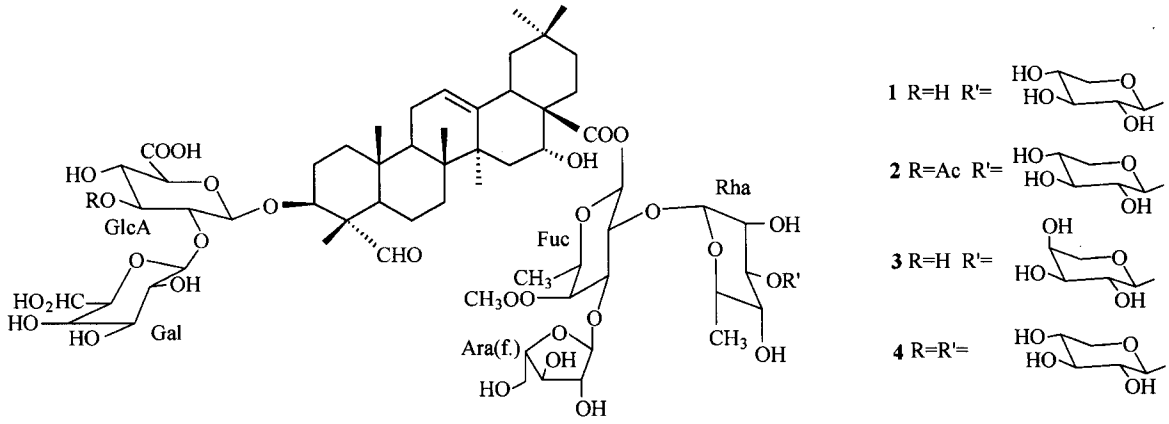

Fig. 1 Structures of vaccariside B-E (**1**—**4**).

Table 5 Inter-residue long range connectivity ($^3J_{CH}$) of sugar moieties of **1** observed in HMBC spectrum

	HMBC ($^3J_{CH}$)
3-*O*-	
βGlcA	C_1-aglycone H_3, H_1-aglycone C_3
βGal	C_1-GlcA H_2
28-*O*-	
βFuc	H_1-aglycone C_{28}
αRha	C_1-Fuc H_2, H_1-Fuc C_2
αAra (f.)	C_1-Fuc H_3, H_1-Fuc C_3
βXyl	H_1-Rha C_3, C_1-Rha H_3

signals except the signal of the C_3 of GlcA, which was downfield shifted by δ 4.4. These evidences indicated that **2** had the same sugar moieties as **1** except a second acetyl group attached at C_3 of GlcA. Therefore, the structure of **2** was determined as 3-*O*-β-*D*-galactopyranosyl-(1-2)-3-*O*-acetyl-β-*D*-glucuronopyranosyl quillaic acid 28-O-β-*D*-xylopyranosyl-(1-3)-α-*L*-rhamnopyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-4-*O*-acetyl-β-*D*-fucopyranoside.

Vaccariside D (**3**) was obtained as an amorphous white powder, with the positive result of Liebermann-

Burchard reaction, m.p. 209—211℃, $[\alpha]_D^{18}$ + 9.18 (*c* 0.77, CH_3OH). IR spectrum suggested the existence of carbonyl (1722 cm^{-1}) and hydroxyl (3427 cm^{-1}) groups. Electrospray ionized mass spectrum showed quasi-molecular ion peak 1445 $[M + Na]^+$ and indicated the molecular weight of 1422. These data in combination of ^{13}C and ^1H NMR allowed to assign the molecular formula of $C_{66}H_{102}O_{33}$. The aglycone was also identified as quillaic acid by the comparison of the ^{13}C NMR data with those of **1**. Further analysis and comparison of the ^{13}C NMR data of sugar moieties between **3** and **1** revealed that carbon signals for glucuronic acid, galactose, fucose, rhamnose, and furanoarabinose in **3** were well coincident with those in **1** within enough precision ($\Delta\delta < 0.25$). Instead of signals for xylopyranose of **1**, five unassigned signals (δ 107.25, 70.69, 69.98, 67.30, 64.29) appeared in the sugar-ring carbon range in the ^{13}C NMR spectrum of **3**. By the comparison with the data in literatures,[12,13] these signals were attributed to a pyranoarabinose. The sixth sugar residue of **3** was determined as arabinopyranose, which was attached to C_3 of rhamnose. Thus, the structure of **3** was established as 3-*O*-β-*D*-galactopyranosyl-(1-2)-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-α-*L*-arabinopyranosyl-(1-3)-α-*L*-rhamnopyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-4-*O*-acetyl-β-*D*-fucopyranoside.

Vaccariside E (**4**) was an amorphous white powder, with the positive result of Liebermann-Burchard reaction, m.p. 218—220℃, $[\alpha]_D^{18}$ + 0.80 (*c* 0.94, CH_3OH). IR spectrum showed the existence of carbonyl (1723 cm^{-1}) and hydroxyl (3429 cm^{-1}) groups. Electrospray ionized mass spectrum showed quasi-molecular ion peak 1577 $[M + Na]^+$ and indicated the molecular weight of 1554, which was 132 Dalton higher than that of **1** and suggested the existence of another pentanose. These data in combination of ^{13}C and ^1H NMR allowed to assign the molecular formula of $C_{71}H_{110}O_{35}$. The ^1H NMR is more complicated than that of **1** due to signals for an additional sugar. Quillaic acid was confirmed as the aglycone by the analysis and comparison of ^{13}C NMR data with those of **1**. Comparison of the ^{13}C NMR data of each sugar moiety between **4** and **1** led to the identification and assignment of six sugar residues (glucuronic acid, galactose, fucose, rhamnose, xylose and arabinofuranose) of **4**. The rest carbon signals attributed to the seventh sugar (δ 104.71, 75.21, 85.62, 78.35, 71.43, 67.17) were well consistent to those of xylopyranose.[11-13] The substitution of xylopyranose at C_3 of GlcA was clearly indicated by the glucosylation shift of C_3 carbon signal of GlcA (Table 4). Thus the structure of **4** was elucidated as 3-*O*-β-*D*-galactopyranosyl-(1-2)-[β-*D*-xylopyranosyl-(1-3)]-α-*D*-glucuronopyranosyl quillaic acid 28-*O*-β-*D*-xylopyranosyl-(1-3)-α-*L*-rhamnopyranosyl-(1-2)-[α-*L*-arabinofuranosyl-(1-3)]-4-*O*-acetyl-β-*D*-fucopyranoside.

Experimental

General procedures

The NMR spectra were obtained with a Varian Unity Inova-600 spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C) using pyridine-d_5 as the solvent. IR spectra were recorded on Nicolet Impact 100, and optical rotations were measured on a Perkin-Elmer 241 MC Polarmeter. ESI/MS were performed with VG QUATTRO equipped with ESI source. TLC and HPTLC were employed on pre-coated silica gel plates 60 GF_{254} (Merck). Isolations were carried out using normal and reverse phase column chromatography. Silica gel 60 (200—400 mesh), Lichroprep C-18 (25—40 μm), MCI gel CHP 20P (75—150 μm), and D-101 macroporous resin (30—80 mesh) were used.

Plant material

The seeds of *V. segetalis* were purchased in Nanjing (Jiangsu, China) in July of 1996 and were identified by Tong Wu. A voucher specimen is deposited at Department of Phytochemistry, College of Traditional Chinese Medicine, China Pharmaceutical University, Nanjing 210009, China.

Extraction and isolation

The 95% ethanolic extract (500 g) obtained by reflux of 10 kg of dried and well powdered seeds was partitioned between EtOAc and H_2O. The water-soluble portion (400 g) was chromatographed over D-101 macroporous resin column eluted successively by H_2O-EtOH (100:0—0:100) to yield a crude saponin fraction (200 g), which was further subjected to flash column chromatography over silica gel eluted successively by $CHCl_3$-MeOH (95:5—0:100) and several saponin-con-

taining fractions were obtained. Each saponin-containing fraction was further purified by HPLC over reverse-phase Lichroprep C-18 and MCI gel CHP 20P (solvent: MeOH-H_2O, 3:7—9:1) to obtain **1**—**4** with the isolated yield of 0.000497% for vaccariside B (**1**, 15 mg), 0.000928% for vaccariside C (**2**, 28 mg), 0.000994% for vaccariside D (**3**, 30 mg) and 0.000895% for vaccariside E (**4**, 27 mg).

Vaccariside B (**1**) An amorphous white powder, m. p. 205—207℃, $[\alpha]_D^{18}$ - 5.98 (*c* 0.65, CH_3OH). ^1H NMR (600 MHz, pyridine-d_5) δ: aglycone: 0.81, 0.94, 0.97, 1.30, 1.39, 1.75 (s, each 3H, CH_3 of C-25, C-29, C-30, C-26, C-27, C-24), 3.37 (d, J = 10.2 Hz, 1H, H-18), 4.04 (m, 1H, H-3), 5.55 (m, 1H, H-12), 9.88 (s, 1H, H-23). ^{13}C NMR data for aglycone see Table 1; ^1H NMR and ^{13}C NMR data for sugar moieties see Tables 2 and 4, respectively; IR(KBr) ν: 3423, 2930, 1727, 1622, 1079, 1045 cm^{-1}. ESI/MS (positive mode) m/z(%): 1445 $[M+Na]^+$.

Vaccariside C (**2**) An amorphous white powder, m. p. 212—214℃, $[\alpha]_D^{18}$ - 30.98 (*c* 0.94, CH_3OH); ^1H NMR (600 MHz, pyridine-d_5) for aglycone δ: 0.81, 0.94, 0.98, 1.01, 1.17, 1.75 (s, each 3H, CH_3 of C-25, C-29, C-30, C-26, C-27, C-24), 9.85 (s, 1H, H-23); ^{13}C NMR (150 MHz, pyridine-d_5) δ: see Tables 1 and 4; IR (KBr) ν: 3433, 2930, 1727, 1617, 1079, 1048 cm^{-1}; ESI/MS (positive mode) m/z(%): 1487 $[M+Na]^+$.

Vaccariside D (**3**) An amorphous white powder, m. p. 209—211℃, $[\alpha]_D^{18}$ + 9.18 (*c* 0.77, CH_3OH); ^1H NMR (600 MHz, pyridine-d_5) for aglycone δ: 0.78, 0.85, 0.86, 1.02, 1.16, 1.77 (s, each 3H, CH_3 of C-25, C-29, C-30, C-26, C-27, C-24), 9.92 (s, 1H, H-23); ^{13}C NMR (150 MHz, pyridine-d_5): see Tables 1 and 4; IR(KBr) ν: 3427, 2930, 1722, 1639, 1081, 1051 cm^{-1}; ESI/MS (positive mode) m/z(%): 1445 $[M+Na]^+$.

Vaccariside E (**4**) An amorphous white powder, m.p. 218—220℃, $[\alpha]_D^{18}$ - 0.80 (*c* 0.94, CH_3OH); ^1H NMR (600 MHz, pyridine-d_5) for aglycone δ: 0.79, 0.94, 0.96, 0.99, 1.04, 1.70 (s, each 3H, CH_3 of C-25, C-29, C-30, C-26, C-27, C-24), 9.86 (s, 1H, H-23); ^{13}C NMR (150 MHz, pyridine-d_5): see Tables 1 and 4; IR(KBr) ν: 3429, 2930, 1723, 1619, 1075, 1042 cm^{-1}; ESI/MS (positive mode) m/z(%): 1577 $[M+Na]^+$.

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References

1 Bayeba, R. T.; Karriev, M. O.; Abubakirov, N. K. *Izv. Akad. Nauk Turkm. SSR, Ser. Fiz.-Tekh., Khim. Geol. Nauk.* **1974**, *6*, 84 [*Chem. Abstr.* **1975**, *83*: 111088t].

2 Baeva, R. T.; Karryev, M. O.; Abubakirov, N. K. *Izv. Akad. Nauk Turkm. SSR, Ser. Fiz.-Tekh., Khim. Geol. Nauk.* **1975**, *4*, 83 [*Chem. Abstr.* **1976**, *84*: 59922q].

3 Morita, H.; Yun, Y. S.; Takeya, K.; Itokawa, H. *Tetrahedron Lett.* **1994**, *35*, 9593.

4 Morita, H.; Yun, Y. S.; Takeya, K.; Itokawa, H.; Yamada, K. *Tetrahedron.* **1995**, *51*, 6003.

5 Morita, H.; Yun, Y. S.; Takeya, K.; Itokawa, H. *Phytochemistry* **1996**, *42*, 439.

6 Kazmi, S. N.; Ahmed, Z.; Malik, A. *Heterocycles* **1989**, *29*, 1989.

7 Yun, Y. S.; Shimizu, K.; Morita, H.; Takeya, K.; Itokawa, H.; Shirota, O. *Phytochemistry* **1998**, *47*, 143.

8 Koike, K.; Jia, Z.; Nikaido, T. *Phytochemistry* **1998**, *47*, 1343.

9 Jia, Z.; Koike, K.; Kudo, M.; Li, H.; Nikaido, T. *Phytochemistry* **1998**, *48*, 529.

10 Ma, J.; Ye, W. C.; Wu, H. M.; He, F. H.; Deng, J. Z.; Zhao, S. X. *Chin. Chem. Lett.* **1999**, *10*, 921.

11 Liu, Z. M.; Li, D.; Owen, N. L.; Grant, D. M.; Cates, R. G.; Jia, Z. J. *J. Nat. Prod.* **1995**, *58*, 1632.

12 Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307.

13 Frechet, D.; Christ, B.; Sorbier, B.; Fischer, H.; Vuilhorgre, M. *Phytochemistry* **1991**, *30*, 927.

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