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Vaccariside A, Novel Saponin from *Vaccaria segetalis* (Neck.) Garcke

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Abstract: A new triterpenoid saponin, vaccariside A **1** was isolated from the seeds of *Vaccaria segetalis* using various chromatographic methods. The structure of compound **1** was elucidated by comprehensive spectroscopic analysis as 3-O-β-D-galactopyranosyl-(1-2)-[β-D-quinovpyranosyl-(1-4)-β-D-xylopyranosyl-(1-3)]-β-D-glucuronopyranosyl quillaic acid 28-O-β-D-glucopyranosyl-(1-3)-[β-D-xylopyranosyl-(1-4)]-α-L– rhamnopyranosyl-(1-2)-3-O-acetyl-β-D-fucopyranoside.

Keywords: Vaccariside A, saponin, *Vaccaria segetalis*.

The seeds of *Vaccaria segetalis* (Neck.) Garcke, belonging to caryophyllaceae family, is a well-known commonly-used traditional Chinese medicine (TCM) called 'wang-bu-liu-xing', and has been recorded in Chinese Pharmacopoeia as a folk medicine for the treatment of blood-stasis syndrome with amenia or dysmenorrhea, carbuncle, stranguria complicated by hematuria. Several triterpenoid saponins and eight cyclopeptides have been isolated from the seeds together with many other compounds [1-4]. During our further searching for bioactive secondary metabolites in this plant, a new triterpenoid saponin, Vaccariside A (**1**) was isolated. In this paper, we report the isolation and structural determination of **1**.

The ethanol extracts of the seeds of *Vaccaria segetalis* were partitioned between water and ethyl acetate. The water solubles was then subjected to D-101 marcoporous column, silica gel flash column, and C-18 column chromatography successively to obtain compound **1** (35 mg, isolation yield 0.00116%).

Vaccariside A (**1**) was obtained as an amorphous white powder, m.p. 215- 217°C, $[\alpha]_D^{18}$ -14.88 (CH_3OH, c 0.66), IR showed absorbence at ν 3429 and 1731 cm^{-1} indicating the presence of hydroxyl and carbonyl groups. The negative FAB mass spectrum of **1** possessed a quasi-molecular ion $[M-H]^-$ at *m/z* 1731 and the ESI mass spectrum displayed quasi-molecular ions: 1755$[M+Na]^+$, 1778$[M+2Na]^+$, 889$[M+2Na]^{2+}$ indicating the molecular weight of 1732. These data in combination with ^{13}C and ^1H NMR allowed assigning the molecular formula of **1** as $C_{78}H_{122}O_{42}$.

The ^1H NMR spectrum of **1** revealed the presence of six tertiary methyl groups (δ 0.87, 0.88, 0.90, 1.16, 1.46 and 1.71), three secondary methyl groups (δ 1.53, d, J=5.5Hz; 1.58, d, J=5.8Hz; 1.63, d, J=5.1Hz), one acetyl group (δ 2.12, s), one trisubstituted olefinic

proton (δ 5.52, m), one aldehydic proton (δ 9.45, s) and eight anomeric protons for the sugar moiety (δ 6.35, 5.88, 5.52, 5.48, 5.29, 5.22, 4.96 and 4.84). These data plus the fragment ion peaks at *m/z* 1567 $[M-H-162]^-$, 1442$[M-H-(162+142)]^-$, 910$[M-H-(176+132+162+146+162+42)]^-$ observed in the negative FAB mass spectrum suggested that compound **1** is a glycoside, which contains eight sugars, one acetyl group and a triterpenoid aglycone.

Table 1. ^{13}C and ^1H NMR chemical shifts of sugar moiety of **1** (in pyridine-d_5) [a]

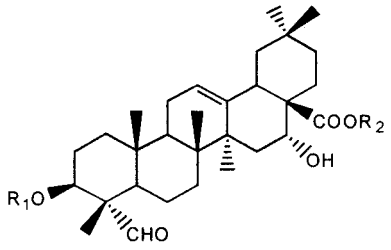
		C	H	HMBC			C	H	HMBC ($^3J_{CH}$)
3-O-					28-O-				
GlcA	1	103.82	4.84	aglycone H_3	Fuc	1	94.77	5.88	aglycone 28-CO
	2	**78.70**	4.32			2	**73.69**	4.61	
	3	**85.92**	4.22			3	**76.80**	4.19	
	4	71.32	4.42			4	71.32	4.12	
	5	77.20	4.43			5	71.84	3.97	
	6	171.86				6	17.20	1.58	
						CH3CO-	21.01	2.12	
						CH3**CO**-	169.42		
Gal	1	104.28	5.52	GlcA H_2	Rha	1	100.93	6.35	Fuc H_2
	2	73.69	4.44			2	71.32	4.95	
	3	75.38	4.13			3	**77.20**	4.62	
	4	70.17	4.52			4	**83.12**	4.53	
	5	73.24	3.64			5	68.34	4.55	
	6	61.69	4.39,			6	18.80	1.63	
Xyl	1	104.97	5.29	GlcA H_3	Xyl'	1	104.78	5.48	Rha H_4
	2	75.12	3.94			2	75.78	3.89	
	3	75.29	4.08			3	78.56	4.09	
	4	**84.08**	3.97			4	70.81	4.11	
	5	67.33	3.64,			5	67.20	3.36,	
Qui	1	106.70	4.96	Xyl H_4	Glc	1	105.43	5.22	Rha H_3
	2	78.40	4.01			2	76.70	3.95	
	3	75.82	3.99			3	78.20	4.10	
	4	76.60	3.60			4	71.68	3.88	
	5	70.81	3.62			5	79.14	4.07	
	6	18.50	1.53			6	64.40	4.64,	

[a] The ^{13}C chemical shifts of substituted carbons were blacked

The ^{13}C NMR spectrum of **1** showed signals corresponding to a trisubstituted double bond (δ 144.57, 122.00), one aldehydic carbon (δ 210.10), one carboxylic carbon (δ 171.86), two carboxylic ester carbons (δ 176.22, 169.42) and eight anomeric carbons (δ 106.70, 105.43, 104.97, 104.78, 104.28, 103.82, 100.93 and 94.77. The latter was attributable to an anomeric carbon of a sugar, which is linked to the aglycone through an ester linkage. These evidences were indicative of the presence of eight sugars, including a glucuronic acid, an acetyl group and a quillaic acid aglycone, which is characterized by an aldehyde group at position 4, a tri-substituted double bond at position 12 and 13, as well as a carboxylic group at position 17. The aglycone of **1** was finally identified as

quillaic acid by comparison of the ^{13}C NMR data of the relevant part of **1** to those in literature [5].

Figure 1. Structure of Vaccariside A



R_1=β-D-gal(1-2)-[β-D-qui(1-4)-β-D-xyl(1-3)]-β-D-glcA-

R_2=β-D-glc(1-3)-[β-D-xyl(1-4)]-α-L-rha(1-2)-3-O-Ac-β-D-fuc-

Structure elucidation of the carbohydrate portion of **1** was more complicated due to the extensive overlap of their ^1H NMR resonances. In the first step, the interpretation was carried out through the identification of each pair of anomeric proton and anomeric carbon for eight sugars, which was relatively easy due to the well-resolved signals in its HMQC spectrum. Then, the ^1H sub-spectra of various sugar residues were obtained from the rows corresponding to their anomeric proton resonances and to their other structural relevent resonances of each sugar residue (such as Me-6 for fucose, rhamnose and quinovose, methylene-5 for xylose) in the TOCSY spectrum. The individual proton in each sugar residue was determined using direct connectivity between the sugar ring protons observed in the DQF-COSY experiment and their chemical shift data were listed in **Table 1**. The vicinal coupling constants between sugar ring protons obtained from these experiments with enough precision (Δ ±1Hz) were used to determine the relative stereochemistry of each asymmetric center (data not shown) and thus to identify the particular monosaccharide. The monosaccharide identification was confirmed by intra-residue NOE connectivities, which were observed by the NOESY spectrum (shown in **Table 2**). Since the chemical shifts of the carbohydrate moiety were unambiguously determined, the full assignment of ^{13}C NMR resonances of each monosaccharide was achieved directly by well-resolved HMQC experiment, and the data were summarized in **Table 1**. The ^{13}C NMR chemical shift values of each sugar were well coincident with typical values of relevant sugar type except for those attributable to the glycosylation shifts, which were used for the identification of linkages between sugars.

The sequence and the glycolinkages of sugar residues as well as the linkage between the sugar chain and the aglycone were determined by the inter-residue NOE correlation and the inter-residue C-H long-range connectivity observable through HMBC experiment. As the ^1H and ^{13}C resonances of each sugar moiety have been unambiguously assigned previously, the sequence and inter-residues linkages of the sugar chains of **1** were established quite straightforwardly. The inter-residue NOE correlations observed in the NOESY spectrum were summarized in **Table 2** and revealed the sequences and the

linkages of two oligosaccharide chains in the molecule as shown in **Figure 1** except for the anomeric proton of fucose, which did not show any inter-residue NOE correlation. However, the low-field shift (δ 5.88) of anomeric proton and the high-field shift (δ 94.77) of anomeric carbon of the residue suggested the fucose may link to the 28-carboxylic carbon by an ester linkage as mentioned above. Furthermore, the HMBC experiment afforded the crucial evidence to confirm the assignment: 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 was summarized in the last column of **Table 1**. These results supported the assignment of the sequence and glycosylation linkages of the carbohydrate moiety of the molecule made by the NOESY experiment and were well accounted for by the glycosylation shifts of C_2 and C_3 of GlcA, C_4 of xyl, C_2 and C_3 of Fuc, as well as C_3 and C_4 of Rha (blacked in **Table 1**). Finally, the attachment of the acetyl group to C_3 of Fuc was clarified by the C-H long-range correlation between H_3 of Fuc and the carboxylic carbon of acetyl group in the HMBC experiment.

Table 2. Intra- and Inter-residue NOE Correlations of sugar residues of **1**

	Intra-residue NOE				Inter-residue NOE
βGlcA	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
βXyl	H_1-H_3	H_1-H_5	H_5-H_5'		H_1-GlcA H_3
βQui	H_1-H_3	H_1-H_5	H_2-H_4	H_4-H_6	H_1-Xyl H_4
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
βXyl'	H_1-H_3	H_2-H_4	H_3-H_5		H_1-Rha H_4
βGlc	H_1-H_3	H_1-H_5			H_1-Rha H_3

Consequently, the structure of Vaccariside A (**1**) was determined as 3-O-β-D-galactopyranosyl-(1-2)-[β-D-quinovpyranosyl-(1-4)-β-D-xylopyranosyl-(1-3)]-β-D-glucuronopyranosyl quillaic acid 28-O-β-D-glucopyranosyl-(1-3)-[β-D-xylo-pyranosyl-(1-4)]-α-L-rhamnopyranosyl-(1-2)-3-O-acetyl-β-D-fucopyranoside.

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