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VACCAROID A, A NEW TRITERPENOID SAPONIN WITH CONTRACTILITY OF RAT UTERINE FROM *VACCARIA SEGETALIS*

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Abstract: Vaccaroid A, a new triterpenoid saponin, showing uterine contraction, has been isolated from the seeds of *Vaccaria segetalis* and its structure was elucidated to be 3β-hydroxyolean-12-en-23, 28-dioic acid-28-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside by using a combination of extensive 2D NMR techniques. © 1997 Elsevier Science Ltd.

Seeds of *Vaccaria segetalis* (Caryophyllaceae) have been used to activate blood flow and promote milk secretion, and also to treat amenorrhea and breast infection in China.[1] Previously, we have reported the isolation of cyclic peptides named segetalins A-H, having estrogen-like activity, from the seeds of *V. segetalis*.[2-5] Now we found that the *n*-butanol extract from the seeds of *V. segetalis* has the activity of rat's uterine contraction. Chromatographic purification of the extract led to the isolation of a new saponin, named vaccaroid A, as the active principle. This paper deals with its isolation, structure elucidation and uterine contraction activity of vaccaroid A.

The *n*-butanol soluble material prepared from the methanol extract was chromatographed on Diaion HP-20, followed by MPLC and HPLC on ODS to give a new saponin, vaccaroid A (0.007%). The negative FAB mass (at *m/z* 1133[M-H]$^-$) and ^{13}C NMR data established vaccaroid A[6] to have the molecular formula of $C_{54}H_{86}O_{25}$. The IR spectrum contained a hydroxy band at 3395 cm^{-1}, a carboxylic band at 1650 cm^{-1}, and an esteric band at 1719 cm^{-1}. The ^{1}H NMR spectrum showed the signals of six methyl groups, one olefinic proton, and four anomeric protons. The ^{13}C NMR spectroscopic data revealed a pair of olefinic carbon atoms at δ 122.7 and 144.0, two carbonyl carbons at δ 180.6 and δ 176.2, and four anomeric carbons at δ 105.89, 105.83, 102.56, and 94.81. These spectral data suggested that vaccaroid A is a saponin of oleanolic-type triterpene. Since both the ^{1}H and ^{13}C NMR spectra exhibited sugar signals, vaccaroid A was subjected to acid hydrolysis with 2N HCl to yield sugar components identified as all D-glucose by comparison with the authentic sample. Moreover, the FAB mass spectrum of vaccaroid A showed fragment ions at *m/z* 972, 810, 647, and 485 due to loss of glucose unit. The mode of glucoside linkages was regarded as β from the ^{1}H-^{1}H coupling

anomeric proton constants of ca. 8 Hz. Although all sugars were D-glucose and many proton signals were very congested, particularly in the δ 3.7 - 4.7 region, HSQC-TOCSY[7] spectrum permitted to assign all ^{1}H and ^{13}C signals of the sugars. The HMBC experiment[8] showed correlation of each anomeric proton of glucose **A**, **B**, **C** and **D** units with C-28 of genin, C-3, C-2 and C-6 of glucose **A**, **D**, and **A**, respectively. These results provided unambiguous information about the position of the glycosidic linkage and permitted us to conclude that glucoses sugar chain bound to C-28 of the sapogenin. Consequently, the structure of vaccaroid A was determined to be 3β-hydroxyolean-12-en-23,28-dioic acid-28-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside.

Vaccaroid A exhibited the activity of female rat's uterine contraction *in vitro* (0.97±0.23 g tension at a dose of 0.2 mg/ml, and 0.70±0.15 g tension at a dose of 0.06 mg/ml).[9] The observation that a saponin like vaccaroid A exhibits uterine contraction activity is unique.

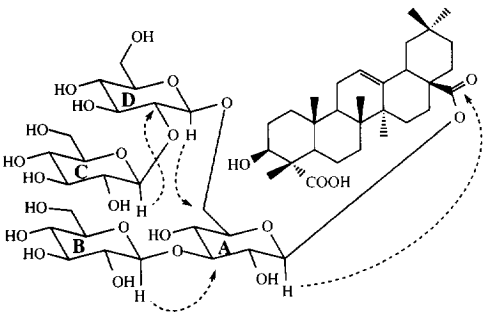

Fig. Structure of vaccaroid A; dashed arrows show selected HMBC correlations that indicate glycosidic linkage.

References and Notes

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6. Vaccaroid A: Colorless powder. $[\alpha]_D$ +2.2° (c 0.2, MeOH). ^{13}C NMR (100MHz, pyridine-d_5) δ 39.04 (C-1), 27.77 (C-2), 75.05 (C-3), 54.41 (C-4), 51.92 (C-5), 21.69 (C-6), 32.27 (C-7), 40.20 (C-8), 48.33 (C-9), 36.80 (C-10), 23.81 (C-11), 122.75 (C-12), 144.02 (C-13), 42.07 (C-14), 28.18 (C-15), 23.15 (C-16), 46.93 (C-17), 41.67 (C-18), 46.12 (C-19), 41.67 (C-20), 32.26 (C-21), 34.57 (C-22), 180.63 (C-23), 12.20 (C-24), 16.06 (C-25), 17.36 (C-26), 25.99 (C-27), 176.28 (C-28), 32.99 (C-29), 23.62 (C-30), 94.81 (Glc-A, C-1), 73.14 (Glc-A, C-2), 88.14 (Glc-A, C-3), 69.23 (Glc-A, C-4), 76.78 (Glc-A, C-5), 68.90 (Glc-A, C-6), 105.86 (Glc-B, C-1), 75.82 (Glc-B, C-2), 78.13 (Glc-B, C-3), 71.26 (Glc-B, C-4), 78.42 (Glc-B, C-5), 62.47 (Glc-B, C-6), 105.83 (Glc-C, C-1), 76.42 (Glc-C, C-2), 77.97 (Glc-C, C-3), 71.13 (Glc-C, C-4), 78.51 (Glc-C, C-5), 62.31 (Glc-C, C-6), 102.56 (Glc-D, C-1), 83.66 (Glc-D, C-2), 77.97 (Glc-D, C-3), 70.82 (Glc-D, C-4), 78.31 (Glc-D, C-5), 62.17 (Glc-D, C-6). ^{1}H NMR (400MHz, pyridine-d_5) δ 0.84 (H-29), 0.86 (H-30), 1.03 (H-25), 1.09 (H-26), 1.18 (H-27), 1.65 (H-24), 3.15 (H-18), 4.68 (H-3), 5.43 (H-12), 6.21 (Glc-A, H-1, J=7.5), 4.26 (Glc-A, H-2), 4.28 (Glc-A, H-3), 4.34 (Glc-A, H-4), 4.14 (Glc-A, H-5), 4.25/4.46 (Glc-A, H-6), 5.34 (Glc-B, H-1, J=8.4), 4.67 (Glc-B, H-2), 4.14 (Glc-B, H-3), 4.16 (Glc-B, H-4), 3.91 (Glc-B, H-5), 4.46/4.56 (Glc-B, H-6), 5.30 (Glc-C, H-1, J=8.1), 4.05 (Glc-C, H-2), 4.14 (Glc-C, H-3), 4.16 (Glc-C, H-4), 3.91 (Glc-C, H-5), 4.25/4.32 (Glc-C, H-6), 5.00 (Glc-D, H-1, J=7.7), 4.06 (Glc-D, H-2), 4.06 (Glc-D, H-3), 3.91 (Glc-D, H-4), 3.79 (Glc-D, H-5), 4.38/4.42 (Glc-D, H-6).
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