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TRITERPENOID SAPONIN FROM *VACCARIA SEGETALIS*

YOUNG SOOK YUN, KAZUHIRO SHIMIZU, HIROSHI MORITA, KOICHI TAKEYA, HIDEJI ITOKAWA* and OSAMU SHIROTA†

School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan; † Division of Pharmacognosy and Phytochemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan

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Key Word Index—Vaccaroid B; triterpenoid saponin; 3β-hydroxyolean-12-en-23; 28-dioic acid-28-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 6)-[6-*O*-(3-hydroxy-3-methylglutaryl)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside; *Vaccaria segetalis*.

Abstract—A new triterpenoid saponin, vaccaroid B, 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)-[6-*O*-(3-hydroxy-3-methylglutaryl)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside by spectroscopic methods. © 1997 Elsevier Science Ltd

INTRODUCTION

The seeds of *Vaccaria segetalis* have been used to activate blood flow and promote milk secretion, and also to treat amenorrhea and breast infection in China [1]. As part of our continuing investigation of new biologically active components from higher plants, we have previously reported eight cyclic peptides, named segetalins A–H [2–5], possessing estrogen-like activity, and a triterpenoid saponin, vaccaroid A [6], possessing the activity of rat's uterine contraction, from the seeds of *Vaccaria segetalis*. In our continued search, further chromatographic purification of the *n*-butanol extract prepared from the seeds of *V. segetalis* led to the isolation of a new saponin, named vaccaroid B. We now report the structure of vaccaroid B analysed by two-dimensional NMR methods.

RESULTS AND DISCUSSION

Vaccaroid B had a molecular formula of $C_{60}H_{94}O_{29}$ established by the negative FAB mass spectrum (*m/z* 1277 [M-H]$^-$) and ^{13}C NMR data; the molecular weight was 144 mass units greater than that of vaccaroid A. Acid hydrolysis of vaccaroid B with 2 N HCl produced sugar components identified as all D-glucose by comparison with the authentic sample. The IR spectrum exhibited a hydroxy band at 3431 cm^{-1}, a carboxylic band at 1670 cm^{-1}, and an esteric band at 1719 cm^{-1}. The NMR spectra of vaccaroid B closely resembled that of vaccaroid A. The ^1H NMR spectrum showed the signals of seven 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. Comparing the ^{13}C signals of the sugar part with those of vaccaroid A, the C-6 signal at Glc-D unit was shifted from δ 62.17 to δ 64.93. Further, the ^{13}C signals at δ 171.70, 46.17, 70.03, 45.40, 173.11 and 28.24 were additionally observed. These ^{13}C spectral data corresponded to those of a 3-hydroxy-3-methylglutaryl moiety [7], which is also consistent with the increase of the mass unit. The glucoside linkage was regarded as β from the ^1H-^1H coupling anomeric proton constants of *ca* 8 Hz. NMR signal assignments of all ^1H and ^{13}C signals of the sugars were conducted by HSQC-TOCSY [8] spectrum. The HMBC correlations as shown in Fig. 1, provided unambiguous information about the position of the glycosidic linkage and permitted us to conclude that the glucoses sugar chain was bound to C-28 of the sapogenin. Consequently, the structure of vaccaroid B was determined to be 3β-hydroxyolean-12-en-23,28-dioic acid-28-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 6)-[6-*O*-(3-hydroxy-3-methylglutaryl)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside, which is the same as vaccaroid A with the exception of an additional HMG moiety.

Though vaccaroid A showed activity in the *in vitro* female rat uterine contraction test [6], vaccaroid B did not show activity even at a dose of 0.2 mg ml^{-1}.

* Author to whom correspondence should be addressed.

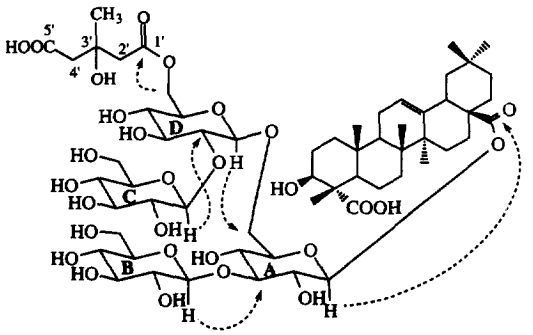

Fig. 1. Structure of vaccaroid B; dashed arrows show selected HMBC correlations that indicate glycosidic linkage.

EXPERIMENTAL

General. Optical rotations were measured with a JASCO DIP-4 spectrometer and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. FAB mass spectra were taken with a VG Autospec spectrometer. IR spectrum was recorded on a Perkin-Elmer 1710 spectrophotometer. High-pressure liquid chromatography (HPLC) was performed with an Inertsil PREP-ODS column (20 mm i.d. × 250 mm, GL Science Inc.) packed with 10 μm ODS. TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck) and the spots were detected by spraying 10% H_2SO_4. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer at 303 K.

Plant material. The seeds of *V. segetalis* were purchased in Shanghai, the People's Republic of China in May, 1993. The botanical identification was made by Dr Zhi-Sheng Qiao, Department of Pharmacognosy, College of Pharmacy, Second Military Medical University, Shanghai, China. A voucher specimen has been deposited in the herbarium of the Tokyo University of Pharmacy and Life Science.

Extraction and isolation. The seeds of *Vaccaria segetalis* (5.0 kg) were extracted with hot MeOH × 4 to give a MeOH extract (180 g) which was partitioned between *n*-BuOH and H_2O. The *n*-BuOH soluble fr. (86 g) was subjected to Diaion HP-20 CC using a H_2O–MeOH gradient system (1:0–0:1). The fr. eluted with 80% MeOH was further subjected to ODS MPLC with MeOH–H_2O–CH_3CN (5:3.5:3) solvent system, followed by ODS HPLC with a 35% CH_3CN containing 1% TFA solvent system to give vaccaroid B (150 mg, 0.003%).

Vaccaroid B. Colourless powder. $[\alpha]_D$ +3.2° (*c* 0.25, MeOH). ^{13}C NMR (100 MHz, pyridine-d_5) δ 39.08 (C-1), 27.79 (C-2), 75.06 (C-3), 54.42 (C-4), 51.94 (C-5), 21.74 (C-6), 32.94 (C-7), 40.21 (C-8), 48.35 (C-9), 36.82 (C-10), 23.15 (C-11), 122.77 (C-12), 144.03 (C-13), 42.08 (C-14), 28.24 (C-15), 23.81 (C-16), 46.97 (C-17), 41.68 (C-18), 46.58 (C-19), 41.68 (C-20), 32.44 (C-21), 33.93 (C-22), 180.62 (C-23), 12.20 (C-24), 16.07 (C-25), 17.37 (C-26), 25.98 (C-27), 176.27 (C-28), 33.04 (C-29), 23.80 (C-30), 94.74 (Glc-A, C-1), 73.02 (Glc-A, C-2), 88.30 (Glc-A, C-3), 69.27 (Glc-A, C-4), 76.84 (Glc-A, C-5), 69.10 (Glc-A, C-6), 105.87 (Glc-B, C-1), 75.46 (Glc-B, C-2), 78.01 (Glc-B, C-3), 71.32 (Glc-B, C-4), 78.42 (Glc-B, C-5), 62.47 (Glc-B, C-6), 105.75 (Glc-C, C-1), 76.27 (Glc-C, C-2), 77.78 (Glc-C, C-3), 71.16 (Glc-C, C-4), 78.49 (Glc-C, C-5), 62.31 (Glc-C, C-6), 102.48 (Glc-D, C-1), 83.37 (Glc-D, C-2), 77.78 (Glc-D, C-3), 70.96 (Glc-D, C-4), 78.09 (Glc-D, C-5), 64.93 (Glc-D, C-6), 170.70 (C-1′), 46.17 (C-2′), 70.03 (C-3′), 45.40 (C-4′), 173.11 (C-5′), 28.24 (3′-CH_3). ^{1}H NMR (400 MHz, pyridine-d_5) δ 0.85 (H-29), 0.91 (H-30), 1.03 (H-25), 1.08 (H-26), 1.17 (H-27), 1.65 (H-24), 3.14 (H-18), 4.67 (H-3), 5.42 (H-12), 6.20 (Glc-A, H-1, J = 7.6 Hz), 4.24 (Glc-A, H-2), 4.24 (Glc-A, H-3), 4.31 (Glc-A, H-4), 4.12 (Glc-A, H-5), 4.31 and 4.55 (Glc-A, H-6), 5.30 (Glc-B, H-1, J = 7.6 Hz), 4.66 (Glc-B, H-2), 4.13 (Glc-B, H-3), 4.16 (Glc-B, H-4), 3.92 (Glc-B, H-5), 4.25 and 4.46 (Glc-B, H-6), 5.30 (Glc-C, H-1, J = 7.6 Hz), 4.05 (Glc-C, H-2), 4.23 (Glc-C, H-3), 3.92 (Glc-C, H-4), 3.92 (Glc-C, H-5), 4.34 and 4.55 (Glc-C, H-6), 5.00 (Glc-D, H-1, J = 7.7 Hz), 4.07 (Glc-D, H-2), 4.00 (Glc-D, H-3), 4.00 (Glc-D, H-4), 4.07 (Glc-D, H-5), 4.68 and 4.95 (Glc-D, H-6), 3.13 (H-2′), 3.13 (H-4′), 1.74 (3′-CH_3).

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