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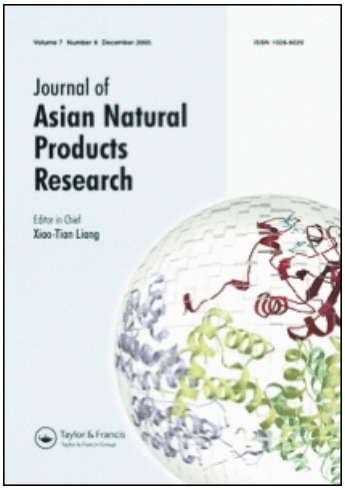

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
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Sheng-Min Sang[a]; Ai-Na Lao[a]; Ying Leng[a]; Lin Cao[a]; Zhong-Liang Chen[a]; Jun Uzawa[b]; Yoshida Shigeo[b]; Yasuo Fujimoto[c]
[a] Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China [b] The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama, Japan [c] College of Pharmacy, Nihon University, Funabashi, Chiba, Japan

Online publication date: 09 September 2010

To cite this Article Sang, Sheng-Min , Lao, Ai-Na , Leng, Ying , Cao, Lin , Chen, Zhong-Liang , Uzawa, Jun , Shigeo, Yoshida and Fujimoto, Yasuo(2002) 'A new triterpenoid saponin with inhibition of luteal cell from the seeds of *Vaccaria segetalis*', Journal of Asian Natural Products Research, 4: 4, 297 — 301
To link to this Article: DOI: 10.1080/1028602021000049096
URL: http://dx.doi.org/10.1080/1028602021000049096

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Journal of Asian Natural Products Research, 2002 VOL. 4 (4), pp. 297–301

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A NEW TRITERPENOID SAPONIN WITH INHIBITION OF LUTEAL CELL FROM THE SEEDS OF *VACCARIA SEGETALIS*

SHENG-MIN SANG[a], AI-NA LAO[a,*], YING LENG[a], LIN CAO[a], ZHONG-LIANG CHEN[a], JUN UZAWA[b], YOSHIDA SHIGEO[b] and YASUO FUJIMOTO[c]

[a]*Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China;* [b]*The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-0198, Japan;* [c]*College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba 274-8555, Japan*

(Received 28 January 2002; Revised 20 May 2002; In final form 27 May 2002)

A new triterpenoid saponin, named segetoside B, showing inhibition of luteal cell activity, has been isolated from the seeds of *Vaccaria segetalis*. On the basis of chemical reactions and spectral analyses, its structure has been established as 28-*O*-[β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)]-[α-L-(5-*O*-acetyl)arabinofuranosyl-(1 → 3)]-β-D-(4-*O*-acetyl)fucopyranosyl-gypsogenin-3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-(6-*O*-methyl ester)-glucuronopyranoside.

Keywords: *Vaccaria segetalis*; Caryophyllaceae; Triterpenoid saponins; Segetoside B; Luteal cell

INTRODUCTION

We have previously reported the isolation and structural elucidation of segetoside A, C-I from the seeds of *Vaccaria segetalis* (Neck) Garcke (Caryophyllaceae) [1–5] which is distributed all over China, except in southern China, and is used in Chinese folk medicine for promoting diuresis, activating blood circulation and relieving carbuncles [6]. Among them, segetoside F showed strong inhibition of luteal cell [5]. Further investigation of this seed led to the isolation of a new triterpenoid saponin, named segetoside B (**1**), which also has the inhibition of luteal cell activity. It is interesting that segetosides are the only naturally occurring triterpenoid saponins reported to have growth inhibition of luteal cell. This paper deals with the structural elucidation of segetoside B and its inhibitory activity against luteal cells.

RESULTS AND DISCUSSION

The *n*-butanol fraction from the ethanol extract of the seeds of *V. segetalis* was chromatographed on Diaion HP-20, silica gel, and RP-18 silica gel to afford segetoside B (**1**).

*Corresponding author. Tel.: +86-21-64311833-316. Fax: +86-21-64370269. E-mail: anlao@mail.shcnc.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1028602021000049096

Segetoside B (**1**) (Fig. 1), an amorphous solid, had a molecular formula of $C_{69}H_{106}O_{33}$ determined by negative ion HRFAB-MS (*m/z* 1461.6549 $[M - H]^-$, calcd. Mass 1461.6536 for $C_{69}H_{105}O_{33}$) as well as ^{13}C and DEPT NMR data. Its spectral features and physicochemical properties suggested compound **1** to be a triterpenoid saponin. Of the 69 carbons, 30 were assigned to the aglycone part, 34 to the oligosaccharide moiety, 4 to the acetyl and 1 to the methoxyl (see Table I). Comparison of the signals from the aglycon moiety in the ^{13}C NMR spectra with those from gypsogenin [7] showed that the triterpene

TABLE I ^{13}C (150 MHz) NMR of compound **1** and ^{1}H (600 MHz) NMR spectral data for the sugar moieties of **1** (C_5D_5N) (δ in ppm, *J* in Hz)

	δ_C		δ_C	δ_H
1	38.1t	Glucuronic acid		
2	25.1t	1	103.6d	4.89 (1H, d, $J = 7.2$)
3	83.8d	2	82.1d	4.20 (1H, m)
4	54.9s	3	77.4d	4.24 (1H, m)
5	48.7d	4	72.6d	4.37 (1H, m)
6	20.7t	5	76.8d	4.45 (1H, m)
7	32.6t	6	170.4s	
8	40.2s	6-OMe	52.1q	3.71 (3H, s)
9	47.8d	Galactose		
10	36.2s	1	106.3	5.21 (1H, d, $J = 7.6$)
11	23.4t	2	74.9d	4.55 (1H, m)
12	122.4d	3	74.5d	4.14 (1H, m)
13	144.1s	4	70.2d	4.55 (1H, m)
14	42.3s	5	77.1d	4.14 (1H, m)
15	28.4t	6	62.2t	4.55 (2H, m)
16	23.6t	Fucose		
17	47.2s	1	94.3d	6.00 (1H, d, $J = 8.0$)
18	41.9t	2	73.8d	4.55 (1H, m)
19	46.4t	3	81.2d	4.28 (1H, m)
20	30.7s	4	73.9d	5.77 (1H, m)
21	34.0t	5	70.5d	3.97 (1H, m)
22	32.3t	6	16.5q	1.18 (3H, d, $J = 6.0$)
23	209.8d	4-OAc; CH_3	20.7q	2.00 (3H, s)
24	11.1q	4-OAc; C = O	170.7s*	
25	15.7q	Arabinose		
26	17.4q	1	111.8d	5.70 (1H, brs)
27	25.9q	2	83.6d	4.90 (1H, m)
28	176.5s	3	78.7d	4.56 (1H, m)
29	33.1q	4	81.9d	3.95 (1H, m)
30	23.7q	5	64.3t	4.55 (1H, m)
				4.82 (1H, dd, $J = 3.2, 12.0$)
		5-OAc; CH_3	20.7q	2.00 (3H, s)
		5-OAc; C = O	170.8s*	
		Rhamnose		
		1	102.1d	5.99 (1H, S)
		2	71.5d	4.76 (1H, m)
		3	72.4d	4.60 (1H, m)
		4	84.8d	4.32 (1H, m)
		5	68.8d	4.41 (1H, m)
		6	18.5q	1.77 (3H, d, $J = 6.0$)
		Xylose		
		1	107.5d	5.01 (1H, d, $J = 7.0$)
		2	76.2d	4.02 (1H, m)
		3	78.6d	4.06 (1H, m)
		4	70.8d	4.19 (1H, m)
		5	67.5t	3.50 (1H, t, $J = 10.4$)
				4.25 (1H, m)

*Signals may be interchanged in the same column.

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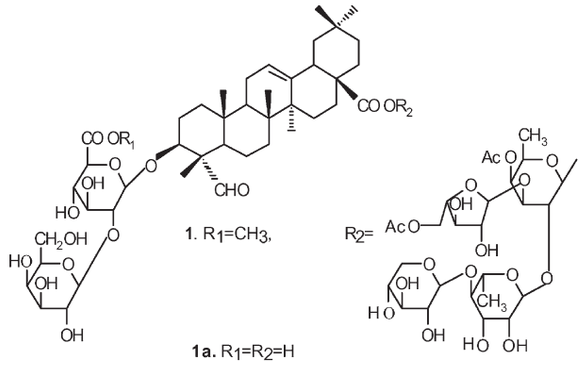

FIGURE 1 Structures of segetoside B (**1**) and **1a**.

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moiety of compound **1** was gypsogenin and sugars were bound to the C-3 and C-28 positions of gypsogenin. The hexasaccharide nature of compound **1** was manifested by its ^{1}H [δ 6.00, (d, $J = 8.0$ Hz), δ 5.99, (s), δ 5.70, (brs), δ 5.21, (d, $J = 7.6$ Hz), δ 5.01, (d, $J = 7.0$ Hz), δ 4.89, (d, $J = 7.2$ Hz)] and ^{13}C (δ 111.8, 107.5, 106.3, 103.6, 102.1, 94.3) NMR data, respectively (see Table I). Alkaline hydrolysis of compound **1** gave **1a** and the sugar fraction. Then, acid hydrolysis of the sugar fraction afforded D-fucose, D-xylose, L-arabinose, and L-rhamnose which were identified by comparison with the standard sugars on HP-TLC. Acid hydrolysis of **1a** gave D-glucuronic acid, and D-galactose (see "Experimental Section"). So D-glucuronic acid and D-galactose were connected to the C_3 position of the aglycon, the other four sugars were connected to the C_{28} position. The identity of the monosaccharides, and the sequence of the oligosaccharide chain were determined by a combination of DEPT, DQFCOSY, TOCSY, HMQC and HMBC experiments. In the light of the assigned ^{1}H and ^{13}C NMR spectra (see Table I), the arabinose sugar unit was identified as α-L-arabinofuranose [8], and the other sugar units were in the pyranose form. The α anomeric configuration for the rhamnose was assigned from its C_5 data (δ 68.8). The β anomeric configurations for the glucuronic acid, galactose, fucose and xylose were assigned from their large $^{3}J_{H1,H2}$ coupling constants (7–8 Hz). The HMBC spectrum showed that C_3 with H_{GluA1}, C_{GluA2} with H_{Gal1}, C_{28} with H_{F1}, C_{F2} with H_{R1}, C_{F3} with H_{A1}, C_{R4} with H_{X1}, C_{GluA6} with $H_{\delta\ 3.71}(-OCH_3)$, $C_{\delta\ 170.7}$ (or $C_{\delta\ 170.8}$) (C = O of acetyl) with H_{A5} (or H_{F4}) and $H_{\delta\ 2.00}$ (CH_3 of acetyl) have cross peaks. Thus, segetoside B (**1**) was determined to be 28-*O*-[β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)]-[α-L-(5-*O*-acetyl)arabinofuranosyl-(1 → 3)]-β-D-(4-*O*-acetyl)fucopyranosyl-gypsogenin-3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-(6-*O*-methyl ester)glucuronopyranoside.

Segetoside B exhibited inhibitory activity against luteal cell resulting in 60% at a concentration of 20 μg/ml. It is known that steroid saponins have inhibitory activity against luteal cell [9]. However, segetosides are unique examples of triterpenoid saponins that show inhibitory activity against luteal cell.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotation was measured using a JASCO-DIP-181 polarimeter. IR spectra were taken on a Perkin–Elmer 599 infrared spectrometer. ^{1}H (600 MHz), ^{13}C (125 MHz) NMR and all

2D spectra were recorded on a JEOL α-600 with NM-AFG type field gradient unit, and chemical shifts are expressed in δ (ppm) values with TMS as internal standard. FAB-MS and HRFAB-MS were obtained on MAT-95 and JEOL GC mate mass spectrometer, respectively. Diaion HP-20 (Mitsubishi Kasei), silica gel 60H (Qingdao Haiyang Chemical Group Co., China) were used for column chromatography. TLC and HP-TLC were performed on HSGF254 (Zhifu Huangwu Co. of Yantai, China).

Plant Material

The seeds of *V. segetalis* were purchased at Shijiazhuang, Hebei Province (China) in 1995. The botanical identification was made by Professor Xuesheng Bao (Shanghai Institute of Drug Control). A voucher specimen (No. 327) has been deposited at the Herbarium of the Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

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Extraction and Isolation

The powdered seeds of *V. segetalis* (50 Kg) were extracted successively with petroleum ether × 2 and 95% EtOH × 3. After evaporation of ethanol *in vacuo*, the residue was suspended in water and then extracted successively with CH_2Cl_2, EtOAc and n-BuOH. The n-BuOH fraction (450 g) was subjected to Diaion HP-20 using a EtOH–H_2O gradient system (0–100%). The fraction (70 g) eluted by 70% EtOH was subjected to silica gel column chromatography with a CH_2Cl_2–MeOH–H_2O solvent system (5:1:0.1–2:1:0.2). The fraction eluted by CH_2Cl_2–MeOH–H_2O (3:1:0.15) was subjected to RP-18 silica gel column chromatography with 70% MeOH to give compound **1** (30 mg).

Segetoside B (**1**): an amorphous solid, $[\alpha]_D^{24}$ − 8.70 (MeOH, c 0.52). IR_{max}^{KBr} cm^{-1} : 3400, 1730, 1100–1000. FAB-MS: *m/z* 1486$[M + Na]^+$, negative ion HRFAB-MS (*m/z* 1461.6549 $[M - H]^-$, calcd. Mass 1461.6536 for $C_{69}H_{105}O_{33}$). ^{1}H-NMR (C_5D_5N) of the triterpene moiety of **1**: δ 9.95 (H-23, s), 5.40 (H-12, s), 4.06 (H-3, m), 3.20 (H-18, m), 1.41 (H-24, s), 1.24 (H-27, s), 1.03 (H-26, s), 0.89 (H-30, s), 0.88 (H-29, s), 0.82 (H-25, s); ^{13}C-NMR (C_5D_5N) of the triterpene moiety of **1**: (see Table I); ^{1}H-NMR (C_5D_5N) and ^{13}C-NMR (C_5D_5N) of the sugar moieties of **1**: (see Table I).

Alkaline Hydrolysis of 1

Compound **1** (15 mg) was dissolved in 10% KOH/H_2O and kept at room temp. for 4 h. The reaction mixture was neutralized with 2 N HCl and was subjected to a Sephadex LH-20 column using MeOH as eluant to give compound **1a** (4 mg) and the sugar fraction. The sugar fraction was concentrated and then dissolved in 2 N HCl and heated at 100°C for 2 h. The reaction mixture was concentrated and then compared with standard sugars on HP-TLC silica gel plate developed with n-BuOH–Me_2CO–H_2O (4:5:1) and $CHCl_3$–MeOH–H_2O (7:3:0.5), detected by spraying with aniline-phthalic acid reagent [aniline:phthalic acid:n-BuOH (2:3:200)] and then heating to 110°C.

Acid Hydrolysis of 1a

MeOH solutions of compound **1a** together with standard sugar samples were applied at points ca. 1 cm from the bottom of a HP-TLC silica gel plate and hydrolysed with HCl vapour for 2 h at 50°C, the plate was then heated at 60°C for 4 h to remove residual HCl, sugar identification was performed as described under alkaline hydrolysis above.

Compound **1a**: an amorphous solid, ESIMS: *m/z* 848 $[M + K]^+$. ^{1}H-NMR (C_5D_5N): δ 5.48 (H-12, brs), 5.25 (H_{GluA}-1, d, $J = 7.5$ Hz), 4.94 (H_{Gal}-1, d, $J = 7.4$ Hz), 3.25 (H-18, m), 1.39 (H-24, s), 1.25 (H-27, s), 0.98 (H-26, s), 0.93 (H-30, s), 0.91 (H-29, s), 0.74 (H-25, s).

Luteal Cell Culture

Ovaries were excised from immature female rats treated with pregnant mare serum gonadotropin and human chorionic gonadotropin. Corpora lutea were separated from ovaries under a dissection microscope, then, stabbed with a needle to disperse the luteal cells. The remaining tissues were cut into pieces and digested with 100 U/ml collagenase at 37°C for 20 min. Dispersed luteal cells were washed with McCoy's 5A medium four times, and seeded at a density of $0.5-1 \times 10^5$ cells/well into 24-well plates in 0.5 ml of McCoy's 5A medium supplemented with 10% neonatal bovine serum. Cells were cultured in 5% CO_2 at 37°C for 24 h and then cultured cells were incubated with 1 of 20 ug/ml. After a 24 h incubation, cell viability was assessed with trypan blue dye exclusion.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 29632050).

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