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Three novel furospirostanol glycosides and a steroidal alkaloid glycoside from the bulbs of *Fritillaria camtschatcensis* (L.) Ker Gawl., and their cytotoxicity

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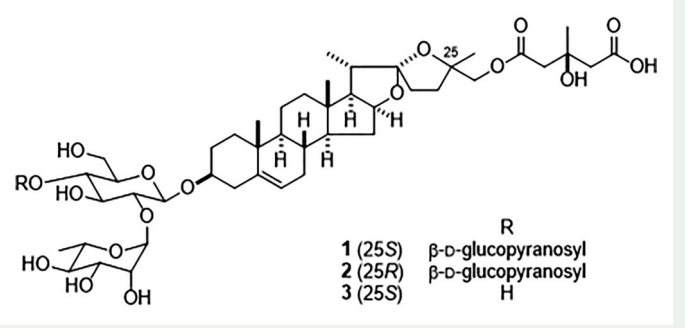
ABSTRACT

Three novel steroidal glycosides (**1**–**3**) and a previously described steroidal alkaloid glycoside (**4**) have been isolated from the bulbs of *Fritillaria camtschatcensis* (L.) Ker Gawl. (Liliaceae). The structures of novel compounds **1**–**3** were characterized based on NMR spectroscopy and chemical transformations. Compounds **1**–**3** are furospirostanol glycosides bearing a (3*S*)-3-hydroxy-3-methylglutaryl moiety at C-26 in the aglycone. Compounds **1**–**4** were evaluated in terms of their cytotoxic activities toward HL-60 human promyelocytic leukemia cells, A549 human lung adenocarcinoma cells, and SBC-3 human lung small cell carcinoma cells. Only **4** showed moderate cytotoxicity against HL-60, A549, and SBC-3 cells with IC_{50} values of 22.9, 13.3, and 11.9 μM, respectively. Compound **4** was found to cause necrotic-like cell death in HL-60 cells.



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Fritillaria camtschatcensis; Liliaceae; bulb; furospirostanol glycoside; steroidal alkaloid; cytotoxicity

1. Introduction

Fritillaria genus plants belonging to the Liliaceae family are distributed in the temperate regions of the Northern Hemisphere (Tsukamoto 1989). A literature survey revealed that *Fritillaria* species, including *F. anhuiensis* (Shou et al. 2010), *F. unibracteata* (Zhang et al. 2011), *F. pallidiflora* (Shen et al. 2012; Xu et al. 2014), *F. meleagris* (Matsuo

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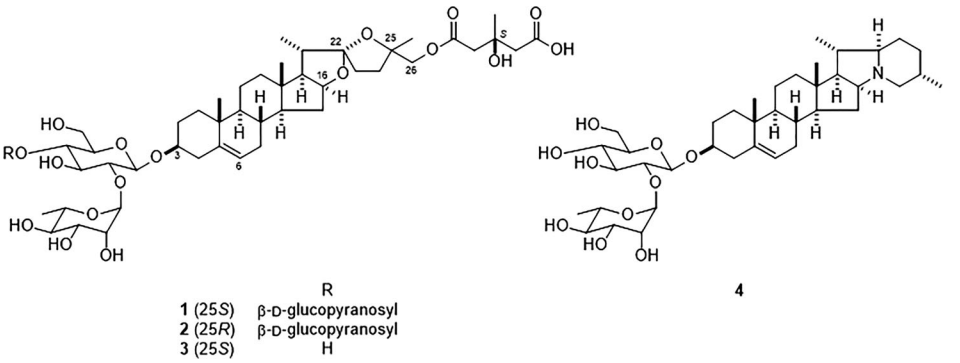

Figure 1. Structures of isolated compounds.

et al. 2013), *F. shuchengensis* (Huang et al. 2013), and *F. thunbergii* (Suh et al. 2018) are rich sources of steroidal alkaloids. *F. camtschatcensis* (L.) Ker Gawl. grows in Northern Japan and a number of steroidal alkaloids have been isolated from this plant (Kaneko et al. 1978; Kaneko, Nakaoka, et al. 1981; Kaneko, Tanaka, et al. 1981). In a previous study, we accomplished the isolation of two phenolic compounds, regaloside A and 3,6′-di-*O*-feruloylsucrose, which are widely found in plants of the *Lilium* genus, and two steroidal alkaloids, solanidine glycoside and kuroyurinidine. Kuroyurinidine is a C-nor-D-homo steroidal alkaloid with three axial hydroxy groups at the C-2, C-3, and C-6 positions in the steroidal skeleton (Sashida et al. 1989; Mimaki and Sashida 1990). As a continuation of our previous study, a phytochemical investigation was conducted on the bulbs of *F. camtschatcensis* with a focus on steroidal glycosides, which resulted in the isolation of three novel furospirostanol glycosides (**1**–**3**) and a previously described steroidal alkaloid glycoside (**4**). The structures of the novel compounds were characterized based on one- and two-dimensional NMR spectroscopy and chemical transformations. Compounds **1**–**4** were evaluated for their cytotoxic activities toward HL-60 human promyelocytic leukemia cells, A549 human lung adenocarcinoma cells, and SBC-3 human lung small cell carcinoma cells.

2. Results and discussion

The bulbs of *F. camtschatcensis* (fresh weight, 2.3 kg) were extracted using MeOH (20 L) at 60 °C, and the solvent was removed under reduced pressure. The MeOH extract (130 g) was loaded onto a Diaion HP-20 porous polymer polystyrene resin column and eluted using MeOH/H_2O (3:7, v/v), EtOH, and EtOAc. The EtOH eluted portion (25 g) was repeatedly fractionated using silica gel column chromatography (CC), octadecylsilanized (ODS) silica gel CC, and preparative ODS HPLC to collect 4 compounds (**1**–**4**). Compound **4** was identified as solanidine 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (Kitajima et al. 1982) (Figure 1).

Compound **1** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{51}H_{80}O_{22}$ based on high resolution-electrospray ionization-time of flight mass spectroscopy (HR-ESI-TOF-MS) and ^{13}C-NMR spectroscopy. The IR spectrum of **1** indicated the presence of hydroxy (3539 cm^{-1}) and carbonyl (1739 and 1709 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra of **1** showed signals for four steroidal methyl groups [δ_H 1.30 (s, Me-27), 1.06 (d, $J = 6.9$ Hz, Me-21), 1.02 (s, Me-19), and

0.79 (s, Me-18); δ_C 24.2 (C-27), 19.3 (C-19), 16.1 (C-18), and 15.1 (C-21)], an olefinic group [δ_H 5.26 (br d, $J = 4.7$ Hz, H-6); δ_C 140.7 (C-5) and 121.8 (C-6)], two oxygenated methine groups [δ_H 4.64 (q-like, $J = 7.6$ Hz, H-16) and 3.86 (m, H-3); δ_C 81.1 (C-16) and 78.1 (C-3)], an oxygenated methylene group [δ_H 4.40 (d, $J = 10.7$ Hz, H-26a) and 4.36 (d, $J = 10.7$ Hz, H-26b); δ_C 70.9 (C-26)], an acetal carbon [δ_C 120.3 (C-22)], an oxygenated quaternary carbon [δ_C 82.4 (C-25)], and three anomeric protons and carbons [δ_H 6.24 (br s), 5.13 (d, $J = 7.9$ Hz), and 4.94 (d, $J = 7.4$ Hz); δ_C 105.2, 101.7, and 100.0]. In addition, the ^{13}C-NMR spectrum showed the presence of a six carbon atom substituent, whose signals were observed at δ_C 171.5 (ester carbonyl, C-1′′′′), 46.7 and 46.4 (methylene carbons, C-2′′′′ and C-4′′′′), 70.0 (quaternary carbon with a hydroxy group, C-3′′′′), 174.6 (carbonyl carbon of a carboxy group, C-5′′′′), and 28.3 (methyl carbon, C-6′′′′). The signals in the ^{1}H-NMR spectrum that could be assigned to this substituent involved two isolated spin systems consisting of two methylene groups [δ_H 3.17 and 3.15 (each d, $J = 14.7$ Hz, H_2-2′′′′); δ_H 3.23 and 3.20 (each d, $J = 15.0$ Hz, H_2-4′′′′)] and a deshielded methyl group [δ_H 1.80 (s)]. These data suggested that the substituent was 3-hydroxy-3-methylglutaryl (HMG). Subsequent acid hydrolysis of **1** using 1 M HCl (dioxane/H_2O, 1:1, v/v) yielded (25*S*)-spirost-5-ene-3β,25-diol (**1a**) (Saijo et al. 1983) as the aglycone, and D-glucose and L-rhamnose as carbohydrate moieties. Identification of the monosaccharides was conducted by direct HPLC analysis of the hydrolysate, using a combination of refractive index and optical rotation detector. (25*S*)-26-Hydroxyfurospirostanols are known to be converted into their corresponding (25*S*)-hydroxyspirostanols under acidic conditions (Tschesche and Richert 1964). Thus, the genuine aglycone of **1** was elucidated to be (25*S*)-22,25-epoxy-furost-5-ene-3β,26-diol. ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) spectra of the sugar moieties in **1** revealed that it contained a 2,4-disubstituted β-D-glucopyranosyl unit [Glc (I): δ_H 4.94 (d, $J = 7.4$ Hz, H-1′); δ_C 100.0, 77.3, 77.7, 82.0, 76.2, and 61.9 (C-1′–C-6′)], a terminal α-L-rhamnopyranosyl unit [Rha: δ_H 6.24 (br s, H-1′′); δ_C 101.7, 72.4, 72.7, 74.1, 69.4, and 18.6 (C-1′′–C-6′′)], and a terminal β-D-glucopyranosyl unit [Glc (II): δ_H 5.13 (d, $J = 7.9$ Hz, H-1′′′); δ_C 105.2, 75.0, 78.2, 71.2, 78.5, and 62.1 (C-1′′′–C-6′′′)]. The heteronuclear multiple bond correlation (HMBC) spectrum of **1** exhibited $^3J_{C,H}$ correlations between H-1′′ of Rha (δ_H 6.24) and C-2′ of Glc (I) (δ_C 77.3), H-1′′′ of Glc (II) (δ_H 5.13) and C-4′ of Glc (I) (δ_C 82.0), and between H-1′ of Glc (I) (δ_H 4.94) and C-3 of the aglycone (δ_C 78.1). The ester linkage formed between the HMG group and C-26 atom in the aglycone was ascertained based on the HMBC correlations observed between the H_2-26 oxymethylene protons of the aglycone (δ_H 4.40 and 4.36) and the C-1′′′′ carbonyl group of the HMG moiety (δ_C 171.5). The absolute configuration at C-3′′′′ in the HMG moiety was determined using the following procedures. Compound **1** was treated with (*S*)-(–)-1-phenylethylamine (PEA) using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium (DMT-MM) to afford the (*S*)-(–)-PEA conjugate derivative (**1b**) of **1**. Compound **1 b** was then subjected to alkaline methanolysis with 1% NaOMe to obtain the methyl HMG amide derivative (**1c**). The physicochemical and spectroscopic data of **1c** were in agreement with those of the methyl HMG amide derivative (**1′a**) obtained from (25*R*)-6β-hydroxy-2-[[(3*S*)-3-hydroxy-3-methylglutaryl]oxy]-5α-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**1′**), which was previously

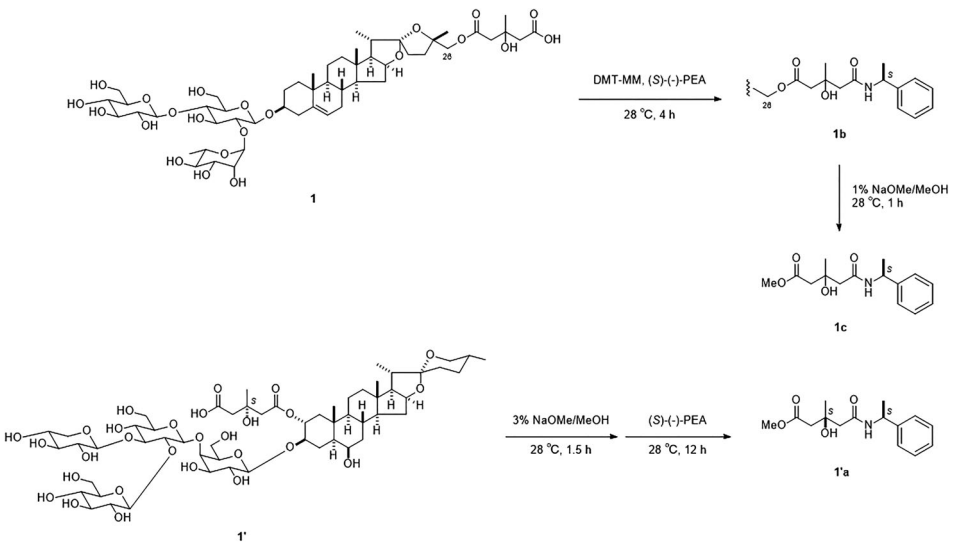

Figure 2. Chemical transformations of **1** and **1′**.

isolated from *Allium albopilosum* bulbs in our laboratory (Mimaki et al. 1993) (Figure 2). Therefore, the absolute configuration of the asymmetric center in the HMG moiety was determined to be *S*, and **1** was elucidated to be (25*S*)-26-*O*-[(3*S*)-3-hydroxy-3-methylglutaryl]-22,25-epoxy-furost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **2** was obtained as an amorphous powder and its molecular formula ($C_{51}H_{80}O_{22}$; HR-ESI-TOF-MS *m/z*: 1045.5238 $[M+H]^+$, calculated for 1045.5219) was determined to be the same as **1**. The ^{1}H- and ^{13}C-NMR spectra obtained for **2** were very similar to those of **1**, except for the signals assigned to the H_2-26 methylene and Me-27 methyl protons, and the C-23, C-26, and C-27 carbon atoms in the aglycone moiety. All the other signals, including those corresponding to the triglycoside and HMG moieties, appeared at almost the same position in **1** and **2**. Thus, **2** was presumed to be a stereoisomer of **1** in regard to the C-25 configuration of the aglycone. Acid hydrolysis of **2** with 1 M HCl (dioxane/H_2O, 1:1, v/v) afforded (25*R*)-spirost-5-ene-3β,25-diol (**2a**) (Faini et al. 1984), D-glucose, and L-rhamnose. Accordingly, **2** was elucidated to be (25*R*)-26-*O*-[(3*S*)-3-hydroxy-3-methylglutaryl]-22,25-epoxy-furost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

The ^{1}H- and ^{13}C-NMR spectra of **3** were closely related to those of **1**, except for the signals corresponding to the sugar moiety attached to the C-3 atom of the aglycone. The molecular formula of **3** was lower than that of **1** by $C_6H_{10}O_5$, which corresponded to a hexosyl unit. Thus, **3** was proposed to share the same aglycone with **1**, including the HMG moiety attached at C-26, but differed in terms of the structure of the sugar moiety. Based on the ^{1}H- and ^{13}C-NMR spectral data, the sugar moiety of **3** was consisted of a 2-monosubstituted β-D-glucopyranosyl unit [Glc: δ_H 5.03 (d, $J = 7.4$ Hz); δ_C 100.4, 77.8, 79.6, 71.8, 78.2, and 62.6 (C-1′–C-6′)] and a terminal α-L-rhamnopyranosyl unit [Rha: δ_H 6.38 (br s, H-1′′); δ_C 102.0, 72.5, 72.8, 74.1, 69.4, and 18.7 (C-1′′–C-6′′)]. In the HMBC spectrum of **3**, long-range correlations were observed between H-1′′ of Rha (δ_H 6.38) and C-2′ of Glc (δ_C 77.8), and between H-1′ of Glc (δ_H 5.03) and C-3 of the aglycone (δ_C 78.0). Thus, **3** was

identified to be (25*S*)-26-*O*-[(3*S*)-3-hydroxy-3-methylglutaryl]-22,25-epoxy-furost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

The cytotoxic activities of **1**–**4** against HL-60, A549, and SBC-3 cells were evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. The dose-response curves shown in Figure S16 (Supplementary material) indicate that **4** exhibited moderate cytotoxic activity against HL-60, A549, and SBC-3 cells with IC_{50} values of 22.9, 13.3, and 11.9 μM, respectively. Compounds **1**–**3** did not exhibit any cytotoxicity at sample concentrations up to 40 μM. The morphology of HL-60 cells treated with **4** (50 μM) or cisplatin (3 μM) for 3, 6, 12, 24, 48, and 72 h were observed using a fluorescence microscopy after treatment with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Figure S17, Supplementary material). The characteristics of the apoptotic cells including nuclear chromatin condensation and nuclear disassembly were observed in HL-60 cells treated with cisplatin. On the other hand, cell membrane disruption was observed in HL-60 cells treated with **4**. Furthermore, HL-60 cells treated with **4** (50 μM) for 3, 6, 12, 24, 48, and 72 h were treated with oxazole yellow dimer (YOYO-1), which stains the impermeable cell membrane (Matsuo et al. 2017). The green fluorescence images suggest that the cell membrane of HL-60 cells was disrupted by **4** in a short period of time (Figure S18, Supplementary material). These observations suggested that **4** causes necrotic-like cell death in HL-60 cells.

3. Experimental

3.1. Compound 1

Amorphous powder; $[\alpha]_D^{25}$ −101.2 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3539 (OH), 1739, 1709 (C = O); ^{13}C-NMR spectral data of the aglycone moiety (150 MHz, C_5D_5N): δ_C 37.4, 30.1, 78.1, 38.9, 140.7, 121.8, 32.1, 31.6, 50.2, 37.1, 21.0, 39.8, 40.5, 56.4, 32.2, 81.1, 62.4, 16.1, 19.3, 38.5, 15.1, 120.3, 33.0, 33.5, 82.4, 70.9, 24.2 (C-1–C-27); ^{13}C-NMR spectral data of the sugar and HMG moieties (150 MHz, C_5D_5N): 100.0, 77.3, 77.7, 82.0, 76.2, 61.9 [C-1′–C-6′ of Glc (I)], 101.7, 72.4, 72.7, 74.1, 69.4, 18.6 (C-1″–C-6″ of Rha), 105.2, 75.0, 78.2, 71.2, 78.5, 62.1 [C-1‴–C-6‴ of Glc (II)], 171.5, 46.7, 70.0, 46.4, 174.6, 28.3 (C-1⁗–C-6⁗ of HMG).

3.2. Compound 2

Amorphous powder; $[\alpha]_D^{25}$ −60.4 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3450 (OH), 1728, 1715 (C = O); ^{13}C-NMR spectral data of the aglycone moiety (150 MHz, C_5D_5N): δ_C 37.5, 30.1, 78.1, 38.9, 140.8, 121.8, 32.2, 31.6, 50.2, 37.1, 21.0, 39.8, 40.5, 56.5, 32.2, 80.9, 62.6, 16.1, 19.4, 38.6, 15.1, 120.3, 34.0, 33.7, 82.3, 69.7, 26.3 (C-1–C-27); ^{13}C-NMR spectral data of the sugar and HMG moieties (150 MHz, C_5D_5N): 100.0, 77.4, 77.7, 82.0, 76.2, 61.9 [C-1′–C-6′ of Glc (I)], 101.8, 72.4, 72.8, 74.1, 69.4, 18.6 (C-1″–C-6″ of Rha), 105.2, 75.0, 78.3, 71.2, 78.5, 62.1 [C-1‴–C-6‴ of Glc (II)], 171.6, 46.5, 70.0, 46.4, 174.7, 28.3 (C-1⁗–C-6⁗ of HMG).

3.3. Compound 3

Amorphous powder; $[\alpha]_D^{25}$ −69.2 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3405 (OH), 1730 (C = O); ^{13}C-NMR spectral data of the aglycone moiety (150 MHz, C_5D_5N): δ_C 37.5, 30.2,

78.0, 39.0, 140.8, 121.7, 32.2, 31.6, 50.2, 37.1, 21.0, 39.8, 40.5, 56.4, 32.2, 81.1, 62.4, 16.1, 19.4, 38.5, 15.1, 120.3, 33.0, 33.5, 82.5, 70.9, 24.2 (C-1–C-27); ^{13}C-NMR spectral data of the sugar and HMG moieties (150 MHz, C_5D_5N): 100.4, 77.8, 79.6, 71.8, 78.2, 62.6 (C-1′–C-6′ of Glc), 102.0, 72.5, 72.8, 74.1, 69.4, 18.7 (C-1″–C-6″ of Rha), 171.5, 46.7, 70.0, 46.4, 174.6, 28.3 (C-1‴–C-6‴ of HMG).

See supplementary material for others.

4. Conclusions

Three novel steroidal glycosides (**1**–**3**) and a known steroidal alkaloid glycoside (**4**) were isolated from the bulbs of *F. camtschatcensis*. The structures of **1**–**3** including the absolute configuration of the HMG moiety were determined using one- and two-dimensional NMR spectroscopy and chemical transformations. Compound **4** exhibited moderate cytotoxicity against HL-60, A549, and SBC-3 cells, and caused necrotic-like cell death in HL-60 cells.

Disclosure statement

The authors declare no conflict interest.

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