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Steroidal glycosides from the bulbs of *Bessera elegans* and their cytotoxic activities

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ABSTRACT

Examination of the bulbs of *Bessera elegans* (Liliaceae) led to isolation of nine new and five known steroidal glycosides. The structures of the nine compounds were determined based on the results of spectroscopic analysis, including two-dimensional NMR, and hydrolysis followed by chromatographic and spectroscopic analysis. The isolated compounds and derivatives were evaluated for cytotoxicity against HL-60 human promyelocytic leukemia cells, A549 human lung adenocarcinoma cells, and TIG-3 normal human diploid fibroblasts. One compound, the pseudo-furostanol glycoside, induced apoptosis in HL-60 and A549 cells in a time-dependent manner and cell-cycle arrest at the G0/G1 phase in A549 cells.

1. Introduction

Through our continuing search for bioactive natural products, a number of natural products have been discovered with antitumor activity in plants of the family Liliaceae, such as *Ornithogalum saundersiae* and *Galtonia candicans* (Kuroda et al., 2000), which are not used in traditional herbal medicine. For example, the acylated cholestane glycoside, OSW-1, which was isolated from the bulbs of *O. saundersiae*, is a promising lead for developing anticancer agents (Kubo et al., 1992; Mimaki et al., 1997). This current phytochemical study focused on *Bessera elegans* Schult. f., which belongs to the family Liliaceae and is indigenous to Mexico. It produces slender stems of 50 cm or taller, and is a popular ornamental garden plant (Tsukamoto, 1989). However, no systematic chemical analysis has been reported for *B. elegans*. Preliminary TLC analysis of the MeOH extract of *B. elegans* bulbs, however, suggested that it contained numerous steroidal glycosides. Herein is reported a detailed chemical study of the bulbs of *B. elegans*, and the isolation of nine new (**1**–**9**) and five known (**10**–**14**) steroidal glycosides. The structures of the new steroidal glycosides were determined based on the results of spectroscopic analysis, including two-dimensional NMR spectroscopic data, and hydrolysis followed by chromatographic and spectroscopic analysis. The isolated compounds and their aglycones were evaluated for cytotoxicity against HL-60 human promyelocytic leukemia cells, A549 human lung adenocarcinoma cells, and TIG-3 normal human diploid fibroblasts.

2. Results and discussion

2.1. Structural elucidation

The bulbs of *B. elegans* (2.8 kg fr. wt) were extracted with hot MeOH. The MeOH extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column, and the MeOH-eluted fraction was subjected to silica gel and octadecylsilanized (ODS) silica gel column chromatography (CC), and to reversed-phase preparative HPLC, giving compounds **1**–**14** (Fig. 1). Compounds **10**–**14** were identified as (25*R*)-5α-spirostan-3β-yl *O*-α-L-arabino-pyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-galactopyranoside (**10**) (Ikeda et al., 2000), (25*R*)-2α-hydroxy-5α-spirostan-3β-yl *O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**11**) (Perrone et al., 2005), (25*R*)-26-[(β-D-glucopyranosyl)oxy]-5α-furostan-2α,3β,22α-triol (**12**) (Barile et al., 2005), (25*R*)-26-[(β-D-glucopyranosyl)oxy]-22α-hydroxy-5α-furostan-3β-yl *O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**13**) (Perrone et al., 2005), and (25*R*)-26-[(β-D-glucopyranosyl)oxy]-2α,22α-dihydroxy-5α-furostan-3β-yl *O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-xylo-pyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**14**) (Perrone et al., 2005), respectively, by

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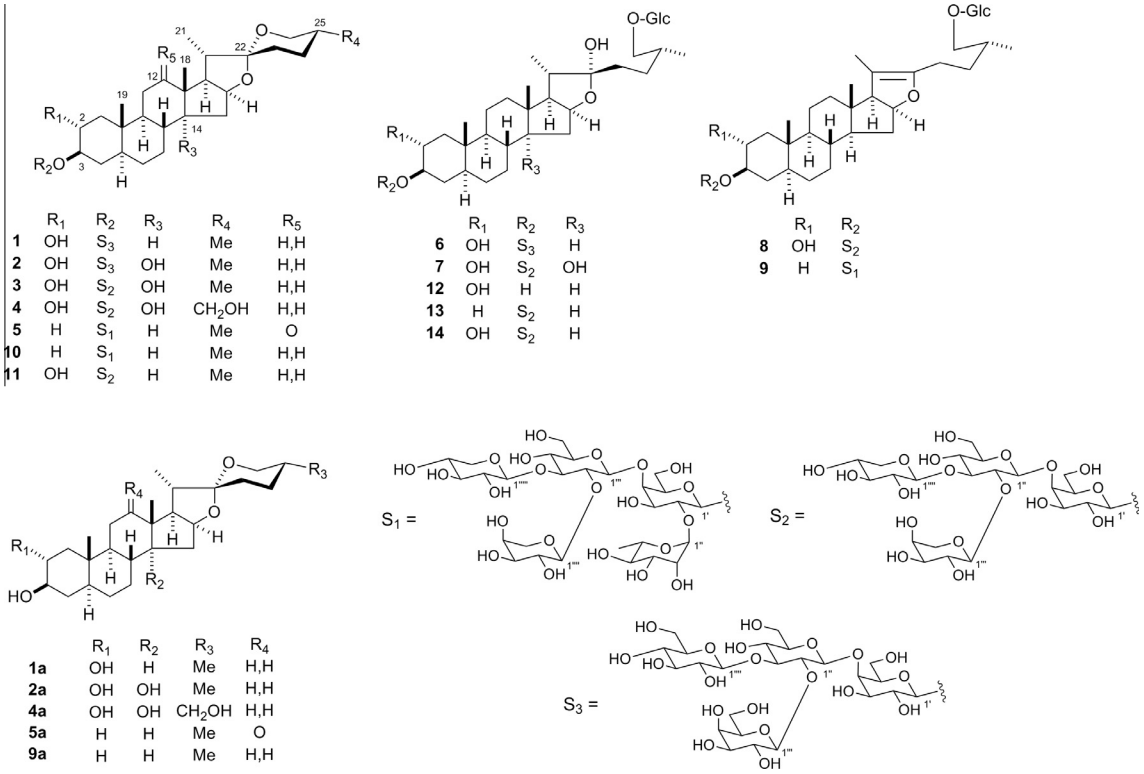

	R_1	R_2	R_3	R_4	R_5
1	OH	S_3	H	Me	H,H
2	OH	S_3	OH	Me	H,H
3	OH	S_2	OH	Me	H,H
4	OH	S_2	OH	CH_2OH	H,H
5	H	S_1	H	Me	O
10	H	S_1	H	Me	H,H
11	OH	S_2	H	Me	H,H

	R_1	R_2	R_3
6	OH	S_3	H
7	OH	S_2	OH
12	OH	H	H
13	H	S_2	H
14	OH	S_2	H

	R_1	R_2
8	OH	S_2
9	H	S_1

	R_1	R_2	R_3	R_4
1a	OH	H	Me	H,H
2a	OH	OH	Me	H,H
4a	OH	OH	CH_2OH	H,H
5a	H	H	Me	O
9a	H	H	Me	H,H

Fig. 1. Steroidal glycosides isolated from *Bessera elegans*.

comparing their physical and spectroscopic data with literature values.

Compound **1** was obtained as an amorphous solid, and its molecular formula was assigned as $C_{51}H_{84}O_{24}$ based on data from high-resolution electrospray ionization time-of-flight mass spectrometry (HR-ESI-TOF-MS; *m/z* 1103.5248 $[M+Na]^+$, calcd. 1103.5250) and ^{13}C NMR (51 carbon signals). The IR spectrum of **1** suggested the presence of hydroxy groups (3362 cm^{-1}). The ^{13}C NMR spectrum showed an acetal carbon signal at δ_C 109.1, and resonances for four methyl groups at δ_C 17.2, 16.5, 14.9, and 13.3 (Table 1), which were characteristic of the spirostan skeleton. Its ^1H NMR spectrum showed two singlet signals for tertiary methyl groups at δ_H 0.79 and 0.68, two doublet resonances for secondary methyl groups at δ_H 1.12 (J = 6.8 Hz) and 0.69 (J = 7.0 Hz), and four anomeric proton doublet signals at δ_H 5.50 (J = 7.8 Hz), 5.19 (J = 7.8 Hz), 5.14 (J = 8.0 Hz), and 4.94 (J = 7.8 Hz) (Tables 2 and 3). These NMR spectroscopic data suggested that **1** was a spirostanol tetraglycoside. Enzymatic hydrolysis of **1** with naringinase gave **1a** as the aglycone ($C_{27}H_{44}O_4$), and D-galactose and D-glucose as the carbohydrate moieties. Aglycone **1a** was identified as (25*R*)-5α-spirostane-2α,3β-diol (gitogenin) from its physical and spectroscopic data (Gvazava and Kikoladze, 2006). The monosaccharides and their absolute configurations were determined by direct HPLC analysis of the hydrolysate. The ^1H–^1H COSY analysis of **1** allowed sequential assignment of the signals from H-1 to H_2-6 of the monosaccharides. The signal multiplet patterns and coupling constants (Table 2) indicated the presence of two β-D-glucopyranosyl units (Glc (I) and Glc (II)) and two β-D-galactopyranosyl units (Gal (I) and Gal (II)). The proton signals were correlated with the carbon resonances through one-bond coupling in the HMQC spectrum. In the HMBC spectrum of **1**, long-range correlations were observed between the anomeric proton (H-1‴) of Gal (II) at δ_H 5.50 and C-2″ of Glc (I) at δ_C 80.9, between H-1⁗ of Glc (II) at δ_H 5.19 and C-3″ of Glc (I) at δ_C 87.4, between H-1″ of Glc (I) at δ_H 5.14 and C-4′ of Gal (I) at δ_C 79.6, and between H-1′ of Gal (I) at δ_H 4.94 and C-3 of the aglycone at δ_C 84.3. Thus, **1** was assigned as (25*R*)-2α-hydroxy-5α-spirostan-3β-yl *O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

Compound **2** ($C_{51}H_{84}O_{25}$) showed spectroscopic features similar to those of **1**, although the molecular formula of **2** contained one extra oxygen atom. Significant differences were also observed between the two compounds in the ^1H and ^{13}C signals for ring D (C-13 to C-17): the C-14 carbon at δ_C 56.2 (CH) in **1** was replaced by a downfield-shifted quaternary carbon at δ_C 87.0 (C) in **2**, and the H-14 proton resonance, which was observed at δ_H 0.99 (m) in **1**, disappeared. These spectroscopic data suggested that **2** was the C-14 hydroxy derivative of **1**. This was confirmed by long-range correlations between C-14 (δ_C 87.0) and H-8 (δ_H 1.88, ddd, J = 11.5, 11.5, 3.6 Hz), H-15a (δ_H 2.34, dd, J = 12.6, 7.5 Hz), δ_H H-15b (1.84, dd, J = 12.6, 6.0 Hz), and Me-18 (δ_H 1.03, s) in the HMBC spectrum of **2**. The significant downfield shifts of the H-9, H-12ax, H-16, and H-17 protons, and the upfield shifts of the C-9, C-12, and C-17 carbons could be explained by the 1,3-diaxial interactions and γ-effects with the C-14α hydroxy group. Enzymatic hydrolysis of **2** with naringinase gave the aglycone **2a** ($C_{27}H_{44}O_5$), together with D-galactose and D-glucose. The physical and spectroscopic data were consistent with the structure of **2a** (25*R*)-5α-spirostane-2α,3β,14α-triol. The ^1H and ^{13}C NMR spectroscopic data and the HMBC correlations of **2** confirmed that the tetraglycoside linked to C-3 of the aglycone was the same as that of **1**. Accordingly, **2** was assigned as (25*R*)-2α,14α-dihydroxy-5α-spirostan-3β-yl *O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

The spectroscopic data for compound **3** ($C_{50}H_{82}O_{24}$) showed it was a spirostanol tetraglycoside closely related to **2**, except for one terminal monosaccharide constituent. Enzymatic hydrolysis of **3** afforded **2a** as the aglycone, and D-xylose, D-galactose, and D-glucose were detected by HPLC analysis of the sugar fraction of the hydrolysate. When the ^1H and ^{13}C NMR spectra of **3** were