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Structure and cytotoxicity of steroidal glycosides from *Allium schoenoprasum*

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ABSTRACT

A phytochemical analysis of the whole plant of *Allium schoenoprasum*, has led to the isolation of four spirostane-type glycosides (**1**–**4**), and four known steroidal saponins. Their structures were elucidated mainly by 2D NMR spectroscopic analysis and mass spectrometry as (20*S*,25*S*)-spirost-5-en-3β,12β,21-triol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**1**), (20*S*,25*S*)-spirost-5-en-3β,11α,21-triol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**2**), laxogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**3**), and (25*R*)-5α-spirostan-3β,11α-diol 3-*O*-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside (**4**). Four of the isolated compounds were tested for cytotoxic activity against the HCT 116 and HT-29 human colon cancer cell lines.

1. Introduction

Plants of the genus *Allium* (Amaryllidaceae) are well known for their production of sulfur compounds, their culinary uses and also for their richness in steroidal saponins (Maisashvili et al., 2008; Eristavi et al., 2007; Ikeda et al., 2000). These glycosides have been found to have some various interesting biological and pharmacological activities (Chen et al., 2010; Ren et al., 2010). The genus *Allium* used to belong to the Alliaceae family, but according to the APG III classification, it is now placed in the Amaryllidaceae family, Allioideae subfamily (Chase et al., 2009). To complete our studies on steroidal glycosides of this genus (Jabrane et al., 2011), we have examined the saponin fraction of the whole plant of *Allium schoenoprasum* L. named also Chive. This commonly used household herb is native of northern Europe and parts of North America. The young leaves and bulbs are eaten as salad and are found to exert hypotensive and cardiac depressant properties (Daniel, 2006). In previous phytochemical analysis of *A. schoenoprasum* extracts, the major volatiles were found to be sulfide components (Jo and Kim, 2001), and thiosulfinate and zwiebelane derivatives were analyzed by GC–MS (Block et al., 1992).

In this paper, we report the isolation and structural determination of the steroidal saponins from *A. schoenoprasum* extract, by detailed analysis of their spectral data including 600 MHz 2D-NMR (COSY, TOCSY, NOESY, HSQC, HMBC), and mass spectrometry. Cytotoxic effects of some isolated compounds were examined against two human colon cancer cell lines, HCT 116 and HT-29.

2. Results and discussion

A concentrated fraction of the 70% aqueous MeOH extract of the whole plant of *A. schoenoprasum* was subjected to successive chromatographic steps like vacuum-liquid chromatography (VLC) and medium-pressure liquid chromatography (MPLC) on silica gel and reversed-phase silica gel RP-18 to provide four new spirostane-type glycosides (**1**–**4**), together with four known saponins identified as laxogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**5**) (Abbas, 2001), diosgenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside (Prosapogenin A of dioscin) (**6**) (Han et al., 1999), diosgenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (**7**) (deltonin) (Sautour et al., 2007), and (25*R*)-furost-5-en-3β,22α,26-triol 26-*O*-β-D-glucopyranosyl-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (deltoside) (**8**) (Sautour et al., 2007) (Fig. 1).

Compounds **1**–**4** were isolated as white, amorphous powders. The monosaccharides obtained by acid hydrolysis of each compound were identified by comparison on TLC with authentic samples as glucose and rhamnose for **1**–**3**, and glucose and galactose for **4**. The absolute configurations of the sugars were determined by GC analysis to be D for glucose and galactose, and L for rhamnose, by a method previously described (Hara et al., 1987).

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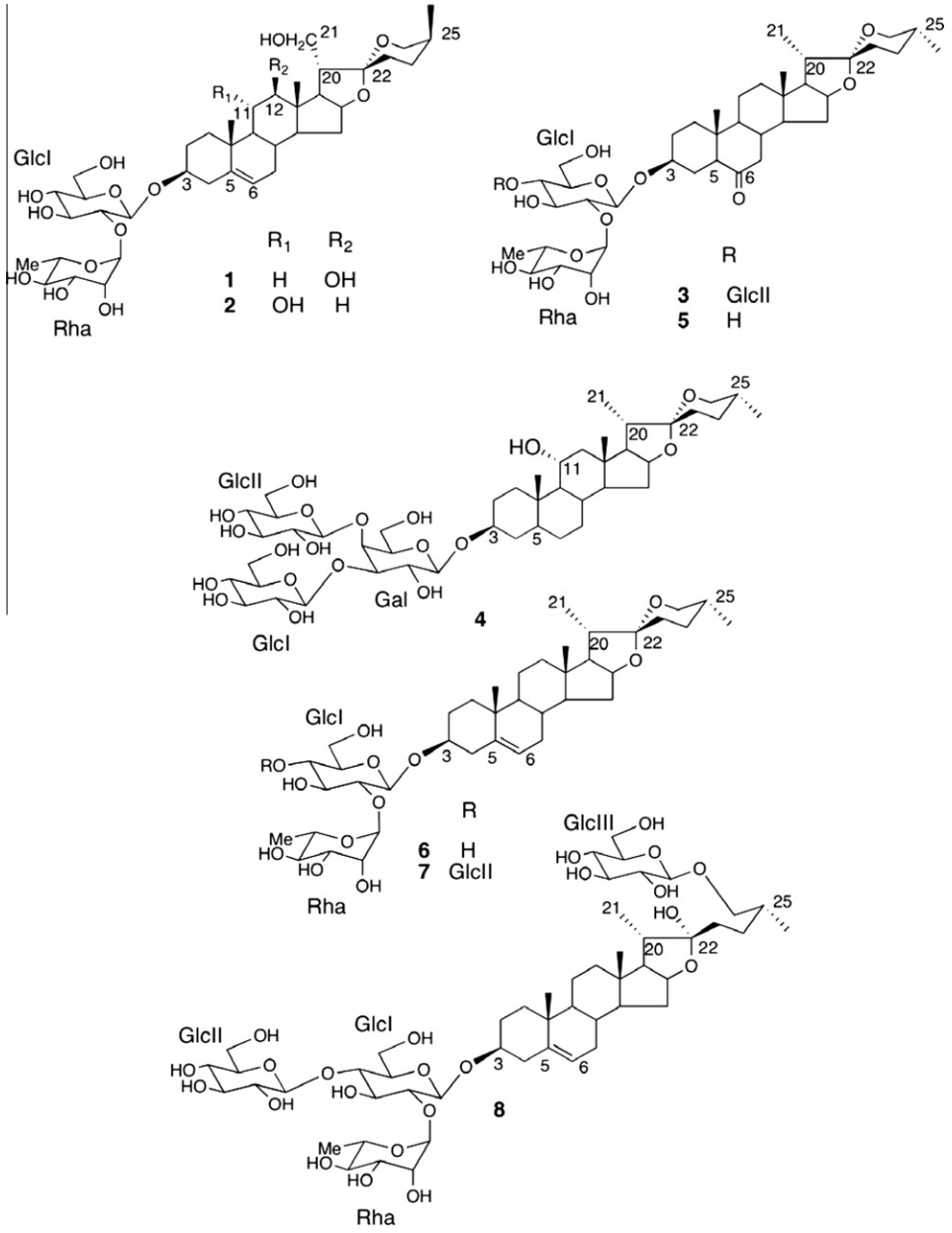

Fig. 1. Structures of compounds **1–8**.

The relatively large $^{3}J_{H\text{-}1,H\text{-}2}$ values of the glucose and galactose (6.9–7.8 Hz) in their pyranose form indicated their β anomeric orientation (Mimaki et al., 2004). The large $^{1}J_{H\text{-}1,C\text{-}1}$ values of the Rha (165–168 Hz), confirmed that the anomeric proton was equatorial (α -pyranoid anomeric form).

Compound **1** exhibited in the HRESIMS, the $[M+Na]^{+}$ peak at *m*/*z* 777.4049 consistent with the molecular formula $C_{39}H_{62}O_{14}$. Its negative-ion FABMS displayed a quasimolecular ion peak at *m*/*z* 753 $[M-H]^{-}$ indicating a molecular weight of 754. The (*S*)-isomer derivative of a $\Delta^{5,6}$-spirostane-type skeleton for the aglycon was deduced by the analysis of the ^{1}H and ^{13}C NMR spectra (Table 1): two angular methyl groups at δ_H 0.96 (*s*) (H_3-18) and 0.90 (*s*) (H_3-19), two olefinic carbon signals at δ_C 140.4 (C-5) and 121.3 (C-6), and five characteristic carbon signals of a F-ring in a 22(*R*),25(*S*) form at δ_C 109.4 (C-22), 26.9 (C-23), 25.4 (C-24), 26.7 (C-25), 64.7 (C-26), 15.7 (C-27) (Agrawal et al., 1985; Agrawal, 2003). In the ^{1}H NMR spectrum, two oxygen bearing methine protons at δ_H 3.54 (*dd*, *J* = 7.5, 2.0 Hz) and δ_H 3.78, and one primary alcoholic function at δ_H 4.03 (2H), remained. The cross-peak in the COSY spectrum between H-3 at δ_H 3.78 and H-4β at δ_H 2.53 (*t*, *J* = 11.7 Hz) and the downfield shift to δ_C 77.8 (C-3) confirmed the classical location of the first secondary alcoholic function and a glycosidic link at the C-3 position of the aglycon. The multiplicity of the H-4 at δ_H 2.53 as a triplet (*J* = 11.7 Hz) suggested an axial/axial coupling, with an α-axial orientation of the H-3 and thus a β-equatorial orientation of the OH group. The HMBC correlations between δ_H 0.96 (H_3-18), δ_H 2.31 (H-17), δ_H 1.49 (H-11) and δ_C 78.9 (C-12), suggested that the second secondary alcoholic function was attached at the C-12 position of the aglycon. The position of the primary alcoholic group at C-21 was determined by the HMBC correlations between δ_H 2.31 (H-17), δ_H 2.30 (H-20) and δ_C 62.8 (C-21). The relative configuration of C-12, and C-20 was determined from a NOESY experiment: cross-peaks at δ_H 3.54 (*dd*, *J* = 7.5, 2.0 Hz) (H-12)/δ_H 0.92 (H-9α) and 1.03 (H-14α), indicated an α-axial orientation of H-12, and the correlation between δ_H 4.03 (H_2-21)/δ_H 2.31 (H-17α), suggested an α-orientation