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Cytotoxic steroidal glycosides from *Allium flavum*

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ABSTRACT

Three new spirostane-type glycosides (**1**–**3**) were isolated from the whole plant of *Allium flavum*. Their structures were elucidated mainly by 2D NMR spectroscopic analysis and mass spectrometry as (20*S*,25*R*)-2α-hydroxyspirost-5-en-3β-yl *O*-β-D-xylopyranosyl-(1 → 3)-[β-D-galactopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**1**), (20*S*,25*R*)-2α-hydroxyspirost-5-en-3β-yl *O*-β-D-xylopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**2**), and (20*S*,25*R*)-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside (**3**). The three saponins were evaluated for cytotoxicity against a human cancer cell line (colorectal SW480).

1. Introduction

The phytochemistry of *Allium* species is very described for their sulphur compounds, phenols and steroidal saponins [1–3]. The aglycon part of these glycosides is constituted of a furostane or spirostane-type skeleton, rarely cholestane. These saponins are known for their interesting pharmacological activities such as antifungal, anti-ischemia, antispasmodic, cytotoxic, haemolytic, and platelet antiaggregating [1,4–7]. Many species were known for their uses as foods, spices and herbal remedies. The genus *Allium* belongs now to the Amaryllidaceae family, Allioideae subfamily, according to the APG III classification [8]. In the field of our researches on steroidal glycosides from *Allium nigrum* [9] and *Allium schoenoprasum* [10], we have now examined the saponin fraction of a third species: *Allium flavum*. *A. flavum* L. subsp. *flavum*, called small yellow onion, is native to Europe and Western and Central Asia [11,12]. This perennial plant with tiny yellow flowers is traditionally used as a spice in the Balkan region [12]. Phenolic compounds of *A. flavum* extracts and their biological activities were previously investigated but not the saponin fraction [11,12]. Therefore, the aim of our researches was the isolation and structural elucidation of the steroidal saponins from the whole plant (stem, leaves, flowers and bulbs). Three compounds (**1**–**3**) were found and their structures were elucidated by a detailed spectral analysis by 600 MHz 2D-NMR (COSY, TOCSY, ROESY, HSQC, HMBC), and mass spectrometry. Cytotoxic effects of the three isolated compounds were examined against a human cancer cell line (colorectal SW480).

2. Experimental

2.1. General experimental procedures

Optical rotation values were recorded on a AA-OR automatic polarimeter. The 1D and 2D NMR spectra (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, TOCSY, ROESY, HSQC and HMBC) were performed using a Varian VNMR-S 600 MHz spectrometer equipped with 3 mm triple resonance inverse and 3 mm dual broadband probeheads. Spectra are recorded in 150 μl pyridine-d_5. Solvent

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signals were used as internal standard (pyridine-d_5: $\delta_H = 7.21$, $\delta_C = 123.5$ ppm), and all spectra were recorded at T = 35 °C. The carbon type (CH_3, CH_2, CH) was determined by DEPT experiments. Chemical shifts were reported as δ values (ppm) and coupling constants (J) were measured in Hz. HRESIMS (positive-ion mode) and ESIMS (negative-ion mode) were carried out on a Q-TOF 1-micromass spectrometer. Compound isolations were carried out using column chromatography (CC) on Sephadex LH-20 (GE Healthcare Bio-Sciences AB), and vacuum liquid chromatography (VLC) on reversed-phase RP-18 silica gel (75–200 μm, Silicycle). Medium-pressure liquid chromatography (MPLC) was performed on reversed-phase RP-18 silica gel (75–200 μm, Silicycle) with a Gilson Pump Manager C-605, having two pumps (2× Büchi pump modul C-601). Thin-layer chromatography (TLC, Silicycle) and high-performance thin-layer chromatography (HPTLC, Merck) were carried out on precoated silica gel plates $60F_{254}$, solvent system $CHCl_3$/MeOH/H_2O 14:6:1. The spray reagent for saponins was vanillin reagent (1% vanillin in EtOH/H_2SO_4, 50:1).

2.2. Plant material

The whole plant (stem, leaves, flowers and bulbs) of *A. flavum* was collected in May 2009 in Les Fléaux (Hautes-Alpes, France) and identified by Gérard Ducerf, Promonature, Briant, France. A voucher specimen (No. 20090720) is deposited in the herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, Burgundy University, Dijon, France.

2.3. Extraction and isolation

Dried powdered whole plant of *A. flavum* (91.8 g) was extracted two times under reflux by MeOH/H_2O (7:3, 2 L × 2) for 1 h. After filtration, the solvent was evaporated in vacuum yielding 23.3 g of extract. An aliquot of the resulting extract (10 g) was subjected to VLC (RP-18 silica gel, H_2O, MeOH/H_2O 50:50, and MeOH). The fraction eluted with MeOH (192.2 mg) was fractionated by CC (Sephadex LH-20, MeOH) to give a fraction rich in saponin (100 mg). The latter was submitted to a MPLC (RP-18 silica gel, MeOH/H_2O gradient 40% to 100%) yielding 12 fractions (F1–F12) and a pure compound, **1** (3.6 mg), in the fraction F8. The remaining fractions were combined and fractionated again by successive MPLC (RP-18 silica gel, MeOH/H_2O gradient 40% to 100%) to give compounds **2** (4.4 mg) and **3** (3.7 mg).

2.4. Acid hydrolysis and GC analysis

Each compound (3 mg) was hydrolyzed with 2N aq. CF_3COOH (5 ml) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 × 5 ml), the aq. layer was repeatedly evaporated to dryness with MeOH until neutral, and then analysed by TLC over silica gel ($CHCl_3$/MeOH/H_2O 8:5:1) by comparison with authentic samples. The trimethylsilyl thiazolidine derivatives of the sugar residue of each compound were prepared and analysed by GC [13]. The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich). The following sugars were detected: D-xylose and D-galactose for **1**, D-xylose, D-galactose and D-glucose for **2**, and D-glucose and L-rhamnose for **3**.

2.5. XTT cytotoxicity assay

The bioassay was carried out according to the XTT method [14] with human cancer cell line (colorectal SW480), provided by the Cohiro society, Dijon, France. Doxorubicin was used as a positive control, and exhibited an IC_{50} value of 1.47 μM.

3. Results and discussion

A concentrated fraction of the 70% aqueous MeOH extract of the whole plant of *A. flavum* was subjected to successive chromatographic steps like vacuum-liquid chromatography (VLC) and medium-pressure liquid chromatography (MPLC) on reversed-phase silica gel RP-18 to provide three new spirostane-type glycosides (**1**–**3**) (Fig. 1).

Compound **1**, a white amorphous powder, exhibited in the HRESIMS, the $[M + Na]^+$ peak at m/z 1071.4992 consistent with the molecular formula $C_{50}H_{80}NaO_{23}$. Its negative-ion ESIMS displayed a quasimolecular ion peak at m/z 1047 $[M–H]^-$ indicating a molecular weight of 1048. Other fragment ion peaks were observed at m/z 915 $[(M–H)–132]^-$, 885 $[(M–H)–162]^-$, 753 $[(M–H)–162–132]^-$, due to the loss of one terminal pentosyl moiety and one terminal hexoxyl moiety. The fragment ion peak at m/z 753 is followed by another one at m/z 591 $[(M–H)–162–132–162]^-$ which revealed the elimination of a second hexosyl moiety.

The (*R*)-isomer derivative of a $\Delta^{5,6}$-spirostene-type skeleton for the aglycon was deduced by the analysis of the ^1H and ^{13}C NMR spectra (Table 1) exhibiting two angular methyl groups at δ_H 0.82 (*s*) (H_3-18) and 0.94 (*s*) (H_3-19), two olefinic carbon signals at δ_C 140.1 (C-5) and 121.9 (C-6), and five characteristic carbon signals of a F-ring in a 22(*R*),25(*R*) form at δ_C 109.3 (C-22), 31.8 (C-23), 29.3 (C-24), 30.6 (C-25), 66.9 (C-26), and 17.3 (C-27) [15,16]. In the ^1H NMR spectrum, two oxygen bearing methine protons at δ_H 3.84 and δ_H 4.06 remained. The cross-peaks in the COSY spectrum between H-3 at δ_H 3.84 and H_2-4 at δ_H 2.56 (*t*, $J = 13.0$ Hz) and δ_H 2.69 (*dd*, $J =$ 13.0, 1.5 Hz), and between H-2 at δ_H 4.06 and H-1β at 2.31 (*dd*, $J = 12.6$, 4.5 Hz), revealed the location of secondary alcoholic functions at the C-3 and C-2 positions of the aglycon, respectively. The multiplicity of the H-4β at δ_H 2.56 as a triplet ($J = 13.0$ 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. A cross peak in the ROESY spectrum between H-2 at δ_H 4.06 and the β-axial H_3-19 at δ_H 0.94 (*s*), suggested a β-axial orientation of the H-2 and thus an α-equatorial orientation of the OH function. In the same way, the relative configuration of C-20 was determined from the ROESY experiment: the correlation between δ_H 1.13 (*d*, $J = 7.3$ Hz, H_3-21)/δ_H 1.80 (H-17α), suggested an α-orientation of the CH_3 group (*S*-configuration at C-20).

For the saccharidic part of this saponin, complete assignments of the resonances of each sugar were achieved by extensive 2D NMR analysis (COSY, TOCSY, HSQC, HMBC) (Table 2). The ^1H NMR spectrum of **1** displayed signals of four anomeric protons at δ_H 4.96 (*d*, $J = 7.7$ Hz), 5.12 (*d*, $J = 7.7$ Hz), 5.18 (*d*, $J = 7.7$ Hz), and 5.50 (*d*, $J = 7.7$ Hz), which gave correlations, in the HSQC spectrum, with four anomeric carbon signals at δ_C 103.2, 104.9, 105.3 and 105.3,