Fitoterapia 88 (2013) 25–30

Contents lists available at SciVerse ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Two new steroidal saponins from *Selaginella uncinata* (Desv.) Spring and their protective effect against anoxia ☆

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ARTICLE INFO

Article history:
Received 7 December 2012
Accepted in revised form 1 April 2013
Available online 12 April 2013

Keywords:
Selaginella uncinata
Steroidal saponins
Protective effect against anoxia

ABSTRACT

Four steroidal saponins were isolated from the anti-anoxic fraction of the 60% EtOH extract of *Selaginella uncinata*, including two new compounds, (3β, 7β, 12β, 25R)-spirost-5-ene-3, 7, 12-triol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside (**1**), (2α, 3β, 12β, 25R)-spirost-5-ene-2, 3, 12-triol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside (**2**) and two known compounds, (3β, 12β, 25R)-spirost-5-ene-3,12-diol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside, (**3**), (1α, 3β, 25R)-spirost-5-ene-2-diol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl(1 → 4)]-O-β-D-glucopyranoside (**4**). The four compounds showed potent protective effect against anoxia in the anoxic PC12 cells assay, among which compounds **1** and **2** were the most active. To our knowledge, this is the first study to report the steroidal saponins in the plant *S. uncinata* and demonstrate their protective effect against anoxia in PC12 cell assay.

1. Introduction

Selaginella uncinata (Desv.) Spring, mainly distributed in southwest China and used in traditional Chinese medicine for the therapy of jaundice, dysentery, edema and rheumatism diseases, have been found to possess many beneficial pharmacological effects such as antiviral, antitumor, antibacterial, antioxidation and anti-anoxic activities [1–7]. Previous phytochemical investigations on *Selaginella* genus revealed the presence of the biflavonoids as characteristic secondary metabolites. In addition, flavonoids, chromone glycosides and phenolic compounds have been isolated [8–13]. Herein, four steroidal saponin compounds found in this plant showed protective effect against anoxia on PC12 cells.

In our previous study, the 60% ethanolic extract of *S. uncinata* were found to show anti-anoxic effect and anti-anoxic biflavonoids were isolated from the EtOAc-soluble fraction of the 60% EtOH extract [6,7]. As a part of our continuous research for discovering anti-anoxic compounds, we investigated the BuOH-soluble fraction of the 60% EtOH extract of *S. uncinata*. Two new steroidal saponin compounds, (3β, 7β, 12β, 25R)-spirost-5-ene-3, 7, 12-triol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside (**1**) and (2α, 3β, 12β, 25R)-spirost-5-ene-2, 3, 12-triol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside (**2**), were isolated from the BuOH-soluble fraction of the 60% EtOH extract of *S. uncinata*, together with two known compounds (3β, 12β, 25R)-spirost-5-ene-3,12-diol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside,

☆ In commemoration of Professor Xinsheng Yao's 80th birthday.
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http://dx.doi.org/10.1016/j.fitote.2013.04.003

(**3**), (1α, 3β, 25R)-spirost-5-ene-2-diol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl(1 → 4)]-O-β-D-glucopyranoside (**4**) (Fig. 1). All the four compounds showed protective effect against anoxia in the anoxic PC12 cell assay.

2. Experimental section

2.1. General methods

Anal. TLC was performed on silica–gel plates (*Qingdao Haiyang Chemical Co.*), with $CHCl_3/CH_3OH$ as eluent; detection by spraying with 10% aq. H_2SO_4, followed by heating. Column chromatography (CC): silica gel (200–300 or 300–400 mesh; *Qingdao Haiyang Chemical Co.*). Optical rotations: *JASCO P-1020* digital polarimeter. UV spectra: *Shimadzu UV-2401PC* spectrophotometer, in anh. MeOH; λ_{max} (log ε) in nm. IR spectra: *Shimadzu FTIR-8900* spectrophotometer, as KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker AV-400* spectrometer, in (D_5) pyridine, δ in ppm, *J* in Hz. ESI-MS: *Bruker Esquire 2000* mass spectrometer; in *m/z* (rel. %). HR-ESI-MS: *Bruker APEX 7.0 TESLA FT-MS* apparatus. HR-EI-MS: Waters Micromass GCT. HR-ESI-MS: *Finnigan MAT95* mass spectrometer; in *m/z* (rel. %). Prep. HPLC (10 ml/min): Shimadzu Pak with RID detector; a *Shim-pack PREP-ODS* column (Ø 250 × 20 mm, 10 μm). HPLC analyses were carried out on an agilent series 1200 HPLC instrument equipped with a quaternary pump, a multiple wavelength detector, an auto sampler and a column compartment.

2.2. Plant material

The dried herbs of *S. uncinata* were collected in Guangxi Province, China, in August of 2004, and identified by the professor Sun Qi-shi (Shenyang Pharmaceutical University, Shenyang, China). A voucher specimen (No. Y01156SU) is deposited at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

2.3. Extraction and isolation

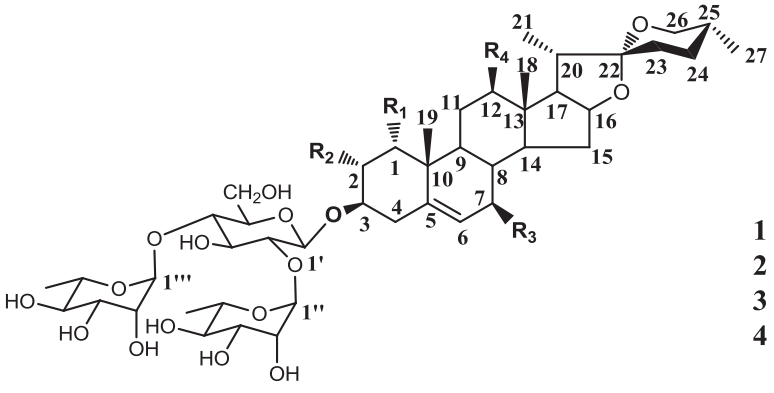
The air-dried whole herbs (4.2 kg) of *S. uncinata* were refluxed with 60% (v/v) ethanol (15 times volume) three times to afford an ethanol extract (856 g), which was suspended in H_2O (8000 ml), and extracted with ethyl acetate (3 × 8000 ml), and *n*-butanol (3 × 8000 ml), separately. The *n*-butanol extract (90.4 g) was subjected to CC (2 kg SiO_2; $CHCl_3$/MeOH gradient) to afford 12 fractions (Fr. 1–Fr. 12). Fr. 10, eluted with $CHCl_3$/MeOH 6:4, was subjected to repeated CC ($CHCl_3$/MeOH 6:4), and was further separated on a Sephadex LH-20 column ($CHCl_3$–MeOH, 1:1), an ODS column (MeOH–H_2O, 3:7), and finally purified by *Rp*-HPLC (Shimadzu, 20 × 250 mm, MeOH/H_2O 35:65, flow rate 10 ml/min), to give compounds **3** (10 mg) and **4** (15 mg). Fr. 12, eluted with $CHCl_3$/MeOH 5:5, was subjected to repeated CC ($CHCl_3$/MeOH 5:5), and was further separated on a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1), an ODS column (MeOH/H_2O, 4:6), and finally purified by *Rp*-HPLC (Shimadzu, 20 × 250 mm, MeOH/H_2O, 40:60, flow rate 10 ml/min), to give compounds **1** (30 mg) and **2** (25 mg).

3β, 7β, 12β, 25R)-spirost-5-ene-3,7,12-triol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside (**1**): White powder. $[\alpha]_D^{25} = -81.5$ ($c = 0.1$, MeOH). UV (MeOH): 202 (4.24). IR (KBr): 3420, 2931, 2874, 2359, 2341, 1716, 1697, 1635, 1558, 1541, 1456, 1375, 1242, 1041, 979, 952, 922, 900, (900 $cm^{-1} > 922$ cm^{-1} band) 864, 837, 812. HR-TOF-MS: 923.4611 ($[M + Na]^+$, $C_{45}H_{72}O_{18}Na^+$; calc. 923.4616). ESI-MS: 899 ($[M - H]^-$), 753 ($[M - H - C_6H_{10}O_4)^-$]), 607 ($[M - H - C_6H_{10}O_4)^-$]), 447 ($[M - H - C_6H_{10}O_4)^-$]). ^1H- and ^{13}C-NMR: see Table 1.

(2α, 3β, 12β, 25R)-spirost-5-ene-2,3,12-triol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside (**2**): White powder. $[\alpha]_D^{25} = -80.9$ ($c = 0.1$, MeOH). UV (MeOH): 202 (4.05). IR (KBr): 3420, 2934, 2874, 2359, 2330, 1716, 1635, 1558, 1541, 1456, 1373, 1244, 1049, 979, 922, 900, (900 $cm^{-1} > 922$ cm^{-1}band), 868, 841, 810. HR-TOF-MS: 923.4622 ($[M + Na]^+$, $C_{45}H_{72}O_{18}Na^+$; calc. 923.4616). ESI-MS: 899 ($[M - H]^-$), 753 ($[M - H - C_6H_{10}O_4)^-$]), 607 ($[M - H - C_6H_{10}O_4)^-$]), 447 ($[M - H - C_6H_{10}O_4)^-$]).

2.4. Acid hydrolysis and HPLC analysis

The absolute configuration of the sugar moieties in the structures was determined by the method of Tanaka et al. [14]. Compound **1** (2 mg) was hydrolyzed with 2 M HCl for 2 h at 90 °C. After the mixture evaporated to dryness under a vacuum, the residue was dissolved in H_2O and extracted with $CHCl_3$. The collected aqueous layer was then dried in vacuo,



	R_1	R_2	R_3	R_4
1	H	H	OH	OH
2	H	OH	H	OH
3	H	H	H	OH
4	OH	H	H	H

Fig. 1. The chemical structures of compounds **1**–**4**.