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New triterpenoid saponins from *Leontice smirnowii*

Nino Tabatadze [a], Sok-Siya Bun [b], Badri Tabidze [a], Vakhtang Mshvildadze [a], Genri Dekanosidze [a], Evelyne Ollivier [b], Riad Elias [b,*]

[a] *Institute of Pharmacochemistry, Academy of Sciences of Georgia, 36, St. P. Sarajishvili, 0159, Tbilisi, Georgia*
[b] *Laboratory of Pharmacognosy and Ethnopharmacology, UMR-MD3, Faculty of Pharmacy, University of the Mediterranean Aix-Marseille II, 27 Bd Jean Moulin, 13385 Marseille cedex 5, France*

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ABSTRACT

Three new triterpene saponins, leonticins I (**1**), J (**2**) and L (**3**) were isolated from the tubers of *Leontice smirnowii*. On the basis of spectroscopic methods, including 2D NMR experiments (DEPT, gs-COSY, gs-HMQC, gs-HMBC and gs-HSQC-TOCSY), mass spectrometry (HR-ESI-MS) and chemical degradation, the structures of the new compounds were elucidated as 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-1→2)]-α-L-arabinopyranosyl-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid (**1**), 3-*O*-[β-D-xylopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid (**2**) and 3-*O*-[β-D-xylopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-[β-D-xylopyranosyl-(1→2)]-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid (**3**), respectively. The aglycone 3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid was observed for the first time in *Leontice* species.

1. Introduction

Four known compounds, leonticins D, F, H and K have been recently isolated from the tuber of *Leontice smirnowii* (Trautv.) Takht. (Berberidaceae), an endemic plant of Georgia by our team [1]. Previous studies have reported the presence of triterpene saponins and alkaloids in this plant [2–6]. Tubers of *L. smirnowii* are used as an antitubercular remedy in Georgian traditional medicine [7]. Recently, extracts from this plant have displayed antioxidant and anti-inflammatory effects [8,9].

This paper describes the isolation and the structure elucidation of three new nor-oleanediene triterpene saponins **1–3** (Fig. 1), from the tubers of *L. smirnowii*, on the basis of 2D NMR experiments (gs-COSY, gs-HMQC, gs-HMBC and gs-HSQC-TOCSY), mass spectrometry (HR-ESI-MS) and chemical degradation.

2. Experimental

2.1. General

Melting points were determined on a Buchi melting point B-540 apparatus. Optical rotations were measured on a Perkin-Elmer 341 Orot polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer in CD_3OD solutions. TMS was used as an internal standard for ^{1}H and ^{13}C measurements. Standard Bruker pulse sequences were used for two-dimensional experiments (gs-COSY, gs-HMQC, gs-HMBC and gs-HSQC-TOCSY). HR-ESI-MS were obtained on an Applied Biosystems MALDI-TOF Voyager Spec. instrument. Mass spectra were performed on a JEOL JMS-700 double-focusing mass spectrometer, equipped with an ESI source operating

* Corresponding author. Tel.: +33 4 91 83 55 42; fax: +33 4 91 83 55 30.
E-mail address: riad.elias@univmed.fr (R. Elias).

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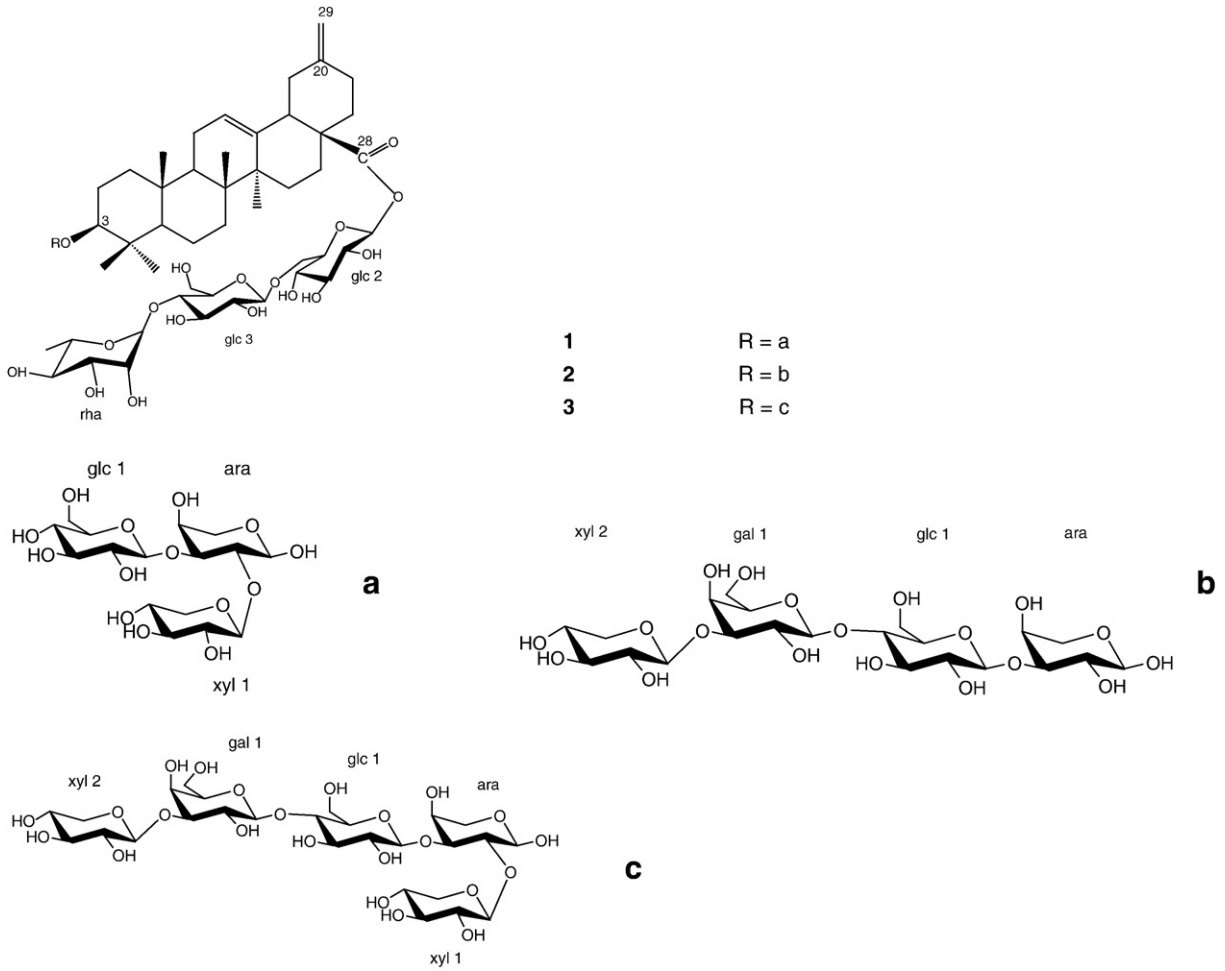

Fig. 1. Structures of leonticins I (**1**), J (**2**) and L (**3**).

under the positive-ion mode. Samples diluted in H_2O-CH_3OH (50:50) were introduced into the ESI interface via a syringe pump (PHD 2000 infusion, Harvard Apparatus, Holliston, MA) at a 30 μl/min flow rate. A 5-kV acceleration voltage was applied and the elemental composition of ions was checked at a typical resolving power of 8000 (10% valley) using a mixture of PEGs as internal standard. Low-pressure liquid chromatography (LPLC) was performed on a ChromatoSPAC Prep 100 using reversed-phase RP-18 (Lichroprep C_{18}, 15–25 μm). Silica gel (Kieselgel 0.040–0.063 mm, Merck) was used for column chromatography. TLC analysis was performed on precoated silica gel plates (Kieselgel 60F_{254}, Merck) using the following solvent systems: $CHCl_3$-MeOH-H_2O (26:14:3) (system 1; saponins); *n*-BuOH-HOAc-H_2O (4:1:5; upper layer) (system 2; saponins); and CH_2Cl_2-MeOH-H_2O (50:25:5) (system 3; sugars). Spots were detected by spraying the plates with phosphoric acid naphtoresorcinol for sugars and H_2SO_4 for saponins, followed by heating at 110 °C.

2.2. Plant material

The tubers of *L. smirnowii* were collected in the Borjomi region of Georgia, in May 2002, and dried in the shade. A voucher specimen (no. 95343) was identified by Pr. G. Dekanosidze and deposited in the Department of Pharmacobotany, Institute of Pharmacochemistry, Tbilisi, Georgia.

2.3. Extraction and isolation

Dried and powdered tubers of *L. smirnowii* (1.0 kg) were extracted with boiling 80% MeOH (7 l). After filtration, the solution was concentrated under a vacuum. The dried extract (298 g) was dissolved in MeOH (900 ml) and precipitated in acetone (5 l). The precipitate was filtered and dried to obtain a crude saponin fraction (205 g). Five g of this fraction was subjected repeatedly ($n = 8$; 40 g) to LPLC using reversed-phase RP-18 (Lichroprep C_{18}; 15–25 μm; 100 g), eluted with MeOH-H_2O (10% to 80% of MeOH; 1 l of each; fraction of 100 ml) to afford four fractions. Fraction 4 (24 g), containing the most polar triterpene saponins, was subjected repeatedly (3 g) to silica gel (Kieselgel 0.04–0.063 mm, Merck; 150 g) column chromatography, and eluted with CH_2Cl_2-MeOH-H_2O (26:14:3; 2 l; fraction of 50 ml), to give compounds **1** (90 mg), **2** (45 mg), **3** (80 mg), and leonticin D (60 mg), leonticin F (45 mg), leonticin H (75 mg), leonticin K (50 mg).