A New Saponin from *Ilex argentina*

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**SUMMARY.** *Ilex argentina* Lillo is one of the species reported as adulterant or substitute of the genuine mate (*Ilex paraguariensis* St. Hil.). The main saponin found in the leaves was isolated and its structure elucidated through spectroscopic methods as the 28-β-D-glucopyranosylester of 3-O-α-L-arabinopyranosyl-20(S)-19α, 24-dihydroxyursolic acid.

**INTRODUCTION**

Several *Ilex* species have been reported as adulterant and/or substitutes of the genuine mate product 1. One of them is *Ilex argentina* Lillo ("roblo" or "palo de yerba"), an allopatric species from the subtropical subandean rainforest of Northwestern Argentina and Eastern Bolivia, geographically isolated and very distant from *I. paraguariensis* main distribution area. Because of this, *I. argentina* deserves a special attention. Continuing our work on the saponin content of *Ilex* species 2 we report herein our first results concerning the isolation and structural elucidation of the main saponin, named ILA-1 (1) obtained from the leaves of *Ilex argentina*.

**MATERIAL AND METHODS**

**Plant material**

Leaves of *Ilex argentina* Lillo were collected in Yerba Buena, Province of Tucumán, Argentina, in October 1992. A herbarium specimen (leg. Giberti 383) is deposited in BACP (Herbarium Cefaprin, Buenos Aires, Argentina).

**KEY WORDS:** Aquifoliaceae; *Ilex argentina*; Saponins.

**PALABRAS CLAVE:** Aquifoliaceae; *Ilex argentina*; Saponinas.

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General experimental procedures

FABMS spectra were performed on a Kratos MS-80 RF spectrometer. Optical rotation was determined on a Perkin-Elmer 141 polarimeter at 22 °C.

\(^1\)H- and \(^{13}\)C-NMR spectra were obtained on a Bruker AC 300 spectrometer (\(^1\)H-NMR:300 MHz, \(^{13}\)C-NMR:75 MHz). 1D and 2D NMR experiments were achieved using standard constructor procedures. ROESY experiments were done in phase sensitive mode with a spin lock delay of 300 ms. Acquisition was performed using 2K data point; 512 experiments of 48 accumulations were compiled to generate the second dimension. TLC was carried out on silica gel Merck GF 254 nm, using chloroform: ethanol (8:4:0.5) as eluant for the saponin and sugars; detection: anisaldehyde-H\(_2\)SO\(_4\). Acid hydrolysis: was performed on TLC, as described by Kartnig and Wegschaider.

Extraction and isolation

Air-dried leaves (100 g) were crushed and extracted with ethanol-water (6:4) at room temperature. The ethanol was removed under reduced pressure and the aqueous suspension was successively extracted with chloroform, ethyl acetate and n-butanol. The n-butanol layer was evaporated to dryness to give the crude saponin fraction (2.21 g). A part of this fraction was purified by phenolic compounds by dissolution in NaOH 1 %, followed by extraction of the saponin with n-butanol. A portion of the residue obtained after evaporation of the n-butanol (0.26 g) was repeatedly chromatographed on silica gel with chloroform:ethanol:water (8:4:0.5) to give 0.027 g of the main saponin ILA-1.

ILA-1

Amorphous powder; \([\alpha]_D^{22} = +7.0^\circ\) (c = 0.4, methanol). FAB-MS (positive, glycerine as matrix m/z: 805 [M+Na]+, 671 [M+Na]-pentose]+, 643 [M+Na]-hexose]+. \(^1\)H-NMR (C\(_5\)D\(_3\)N): 0.81; 1.12; 1.34; 1.49; 1.69 (3H each, all s, 5 x tert-Me); 0.97 (J = 7.0 Hz, H-30); 3.12 (1H, s, H-18); 3.46 (1H, dd, J = 11.5, 4.5 Hz, H-3); 3.58 (1H, br d, J = 11 Hz, H-24), 4.35 (1H, br d, J = 11 Hz, H-24), 4.82 (1H, J = 7.5 Hz, Ara H-1), 5.49 (1H,t-like, H-12), 6.31 (1H, d, J = 8.0 Hz) \(^{13}\)C-NMR data for the aglycone: see Table 1; for the sugar chain \(\beta\) (C\(_5\)D\(_3\)N) = 106.1; 72.5, 74.2; 69.0; 66.2 for C1 to C5 from the arabinopyranose; 95.5; 73.7; 78.6; 70.7; 78.9; 61.8 for C1 to C6 from the glucopyranose.

ILA-1 peracetate

Usual acetylation performed at room temperature using pyridine and acetic anhydride yields 15 mg of the peracetylated compound as a colorless solid, m.p. 87-92 °C. 1H-NMR d(CDC\(_3\)) = 0.61 ; 0.99; 1.10; 1.18; 1.50 (3H each, all s, 5 x tert-Me); 0.84 (d, 3H, J = 7.0 Hz, H-30); 2.1-2.4 (7 OAc); 2.69 (3H, 1H, H-18), 3.31 (1H, dd, J = 11.5, 4.5 Hz, H-3), 3.51 (1H, br d, J = 14 Hz, Ara H-5), 3.7-3.73 (2H, m, Glu H-5, H-24), 3.90-4.0 (2H, br d, J = 14 Hz, Ara H-5, overlap with a d, J = 4 Hz, Glu H-6), 4.15-4.24 (2H, Glu H-6, H-24), 4.41 (1H, d, J = 7.5 Hz, Ara H-1), 4.49-5.2 (m, Ara H-2, Ara H-3, Ara H-4, Glu H-2, Glu H-3, Glu H-4), 5.49 (1H, J= 8.0 Hz, Glu H-1). \(^{13}\)C-NMR data for the aglycone: see Table 1; for the sugar chain \(\beta\) (C\(_5\)D\(_3\)N) = 102.8, 69.9, 70.0, 68.0, 62.5 for C1 to C5 from the arabinopyranose; 91.2, 69.4, 72.9, 67.5, 72.5, 61.5 for C1 to C6 from the glucopyranose.
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Table 1. $^{13}$C NMR Spectral data for the aglycone of compound 1, peracylated compound 1 (1a), rotungenic acid (2)$^a$ and ilexoside XII (3)$^b$. a) Cited from reference 4, b) cited from reference 6.
RESULT AND DISCUSSION

On acid hydrolysis (HCl), ILA-1 (compound 1) afforded two sugar residues identified as glucose (Glu) and arabinose (Ara) by TLC. $^1$H, $^{13}$C and 2D $^{13}$C-$^1$H correlation NMR spectra allowed the identification of glucose C1 resonance at $\delta = 95.5$, establishing that this residue is bound to the aglycone via an ester bond, while arabinose C1 resonance was observed at $\delta = 106.1$.

FABMS of 1 exhibited an intense molecular ion peak $[M+Na]^+$ at $m/z$ 805 together with two minor fragments at $m/z$ 643 and 671, corresponding respectively to the loss of one hexose (162) and one pentose unit (134).

The $^{13}$C-NMR spectrum of 1 displayed 41 resonances. From the DEPT spectrum, the 30 aglycone carbons could be identified (Table 1). Considering the presence of glucose, arabinose and the mass spectrum, the molecular formula $C_{41}H_{66}O_{14}$ could be deduced for 1.

For the aglycone, the main features were the presence on the $^1$H-NMR spectrum of a singlet at $\delta$ 3.12 and on the $^{13}$C-NMR spectrum of a quaternary resonance at $\delta$ 73.0. These two signals are characteristic of a 19-hydroxylated-ursane derivative. The glycosylation shift observed for the aglycone C-3 signal ($\delta$ 88.8) indicates that the arabinosyl unit was linked at this position.

The comparison of the $^{13}$C-NMR data of 1 with those reported for hydroxylated triterpenoid acids showed that the carbon signals due to the A, B and C rings of ILA-1 aglycone were almost superimposable with those of rotungenic acid (compound 2 in Table 1) 4 This suggested for the aglycone of ILA-1 a 19,24-dihydroxyursolic acid. Nevertheless, some $^{13}$C resonances of the D and E rings of 1 were significantly different (Table 1). The upfield shifts (1 vs 2) of the signals due to C-18 (-7.84 ppm) and C-22 (-7.10 ppm) revealed the C-30 methyl group to be $\beta$-(axial) in place of the $\alpha$-(equatorial)methyl group in rotungenic acid 5,6 This can be explained in terms of the $\gamma$-effect of the 30-$\beta$-axial methyl group in ILA-1.

Experiments using 2D-ROESY 7 showed cross signal between CH$_3$-30, H-18 and H-12 (Fig. 1). Therefore, it was concluded that H-18 and CH$_3$-30 have the same $\beta$-configuration. Hence, the aglycone of ILA-1, on E ring, is stereochemically similar to ilexigenin B 3 and ilexoside XII (compound 3 in Table 1) 6, and can be formulated as 20(S)-3$\beta$, 19$\alpha$, 24-trihydroxysurs-12-en-28-oic acid or 20(S)-19$\alpha$, 24-dihydroxyursolic acid. Thus, the structure of the new saponin ILA-1 was deduced as the 28-$\beta$-D-glucopyranosylester of 3-O-$\alpha$-L-arabinopyranosyl-20(S)-19$\alpha$, 24-dihydroxyursolic acid.

The here established structure for the main saponin of *Ilex argentina* is markedly different from the structure of the glycosides from *Ilex paraguariensis*, which are mono and bidesmosides of the oleanolic and ursolic acids 2,8,9.

REFERENCES