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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-dihydroxy-ursolic acid.**

RESUMEN. "Una nueva saponina de *Ilex argentina* ". *Ilex argentina* Lillo es una de las especies que ha sido mencionada como substituto de la yerba mate verdadera *(Ilex paraguariensis* St. Hil.). La principal saponina de las hojas fue aislada y su estructura química elucidada a través de métodos espectroscópicos como el éster 28-β-D-glucopiranósido del ácido 3-O-α-L-arabinopiranosil-20(S)- 19α, 24-dihidroxiursólico.

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 ("roble" 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: water (8:4:0.5) as eluant for the saponin and sugars; detection: anisaldehyde-H_2SO_4. Acid hydrolysis: was performed on TLC, as described by Kartnig and Wegschaider [3].

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 from 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° (c= 0.4, methanol). FAB-MS (positive, glycerine as matrix m/z: 805 $[M+Na]^+$, 671 $[(M+Na)\text{-pentose}]^+$, 643 $[(M+Na)\text{-hexose}]^+$. ^{1}H-NMR (C_5D_5N): 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 δ (C_5D_5N) = 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(CDCl3) = 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 (s, 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 δ (C_5D_5N) = 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.

Carbon N°	DEPT	1 (C_5D_5N)	1a ($CDCl_3$)	2 (C_5D_5N)	3 (C_5D_5N)
1	CH_2	38.3	38.6	38.8	39.1
2	CH_2	26.4	25.8	28.5	26.9
3	CH	88.8	89.6	80.3	89.0
4	C	43.9	41.5	43.2	39.8
5	CH	56.0	56.0	56.5	56.2
6	CH_2	18.7	19.7	19.3	18.9
7	CH_2	33.4	33.6	34.0	33.9
8	C	40.0	39.8	40.4	40.6
9	CH	47.3	47.5	47.9	47.7
10	C	26.4	36.6	37.2	37.2
11	CH_2	24.3	23.8	24.3	24.2
12	CH	127.2	130.9	127.9	127.6
13	C	139.0	136.9	140.0	139.0
14	C	41.8	41.1	42.1	42.5
15	CH_2	28.8	28.1	29.1	30.0
16	CH_2	26.4	25.6	26.5	26.9
17	C	48.0	47.5	48.4	48.7
18	CH	46.9	46.3	54.7	48.0
19	CH	73.0	74.0	72.8	73.6
20	CH	42.5	42.1	42.4	42.9
21	CH_2	26.4	25.6	27.0	24.9
22	CH_2	31.5	31.9	38.6	31.8
23	CH_3	23.1	22.5	23.7	28.4
24	CH_2	63.0	65.9	64.6	17.2
25	CH_3	15.1	15.8	17.2	16.0
26	CH_3	17.0	15.6	16.8	17.6
27	CH_3	23.9	23.8	24.7	24.2
28	C	176.6	175.6	180.8	177.2
29	CH_3	29.4	30.0	27.2	29.8
30	CH_3	15.7	15.0	16.1	16.3

Table 1. ^{13}C NMR Spectral data for the aglycone of compound 1, peracetylated 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 δ = 95.5, establishing that this residue is bond to the aglycone via an ester bond, while arabinose C1 resonance was observed at δ = 106.1 .

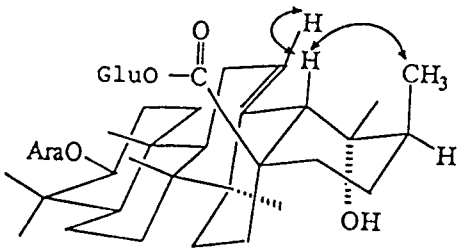

Figure 1. Structurally useful rOe's observed for the aglycone of compound *1*.

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 δ 3.12 and on the ^{13}C-NMR spectrum of a quaternary resonance at δ 73.0. These two signals are characteristic of a 19-hydroxylated-ursane derivative. The glycosilation shift observed for the aglycone C-3 signal (δ 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 β-(axial) in place of the α -(equatorial)methyl group in rotungenic acid [5,6]. This can be explained in terms of the γ-effect of the 30-β-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 β-configuration. Hence, the aglycone of ILA-1, on E ring, is stereochemically similar to ilexgenin B[5] and ilexoside XII (compound *3* in Table 1) [6], and can be formulated as 20(S)-3β, 19α, 24-trihydroxyurs-12-en-28-oic acid or 20(S)-19α, 24-dihydroxyursolic acid. Thus, the structure of the new saponin ILA-1 was deduced as the 28-β-D-glucopyranosylester of 3-O-α-L-arabinopyranosyl-20(S)-19α, 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].

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