

## Triterpenes and Saponins from *Ilex argentina* Leaves \*

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**SUMMARY.** From *Ilex argentina* Lillo leaves, a species reported as substitute or adulterant of the genuine erva-maté (*Ilex paraguariensis* A. St.-Hil.), two saponins and one triterpene have been isolated. By means of spectroscopic methods, this latter was identified as rotundic acid and the saponins as the 28-O- $\beta$ -D-glucopyranosylester of rotundic acid (pedunculósido) and the 20(S) isomer of 28-O- $\beta$ -D-glucopyranosylester of rotundioic acid.

**RESUMEN.** "Triterpenos y saponinas de las hojas de *Ilex argentina* Lillo". *Ilex argentina* es una de las especies que ha sido mencionada como adulterante o sustituto de la yerba mate verdadera (*I. paraguariensis*). De las hojas fueron aisladas dos saponinas y un triterpeno y sus estructuras químicas elucidadas a través de métodos espectroscópicos. El triterpeno fue identificado como el ácido rotúndico y las saponinas como el éster 28-O- $\beta$ -D-glucopiranosido del ácido rotúndico (pedunculósido) y el isómero 20(S) del éster 28-O- $\beta$ -D-glucopiranosido del ácido rotundioico.

### INTRODUCTION

*Ilex argentina* Lillo is one of the species reported as adulterant or substitute of the genuine maté <sup>1</sup> (*Ilex paraguariensis* A. St.-Hil.). *I. argentina*'s vegetative morphology is alike the one of the true erva-mate tree, so that it can be easily confused with *I. paraguariensis*, and it also receives one of its common names -"palo-de-yerba"- due to such resemblance. Historically, a Jesuit priest seems to have been the first to note the occurrence of "yerba mate" (very probable *Ilex argentina*) at the subtropical rainforests close to Tucumán, Northwestern Argentina, about 1750. He also reported that such Tucumán's trees yielded a "yerba mate" not only with different taste that the one coming from Paraguay, but that it also caused headache <sup>2</sup>. Additionally, in 1844, the governor of the Salta province authorized wild "yerba mate" harvest

in this territory for an eight year period <sup>3</sup>. However, no one in those days was able to differentiate *Ilex argentina*'s products from the genuine yerba mate, in spite that the problem of the bad quality of Northwestern Argentinian "yerba mate" seems to have already arisen. A few years later, Moussy <sup>4</sup> (1860) reported the occurrence of "*Ilex mate*" (botanically a synonym of *Ilex paraguariensis* A. St.-Hil.) for the San Francisco and Orán valleys, Salta (Northwestern Argentina). Actually, there is little doubt that *Ilex mate* was in fact *I. argentina*. Since 1936, Argentinian sanitary legislation forbids the industrialization of adulterants, among them alternative *Ilex* species, for the preparation of genuine yerba mate.

During the course of a large program aimed to identify the adulteration of maté by other *Ilex* species, the systematic identification of the

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triterpenoid content of the South-Brazilian *Ilex* species was undertaken<sup>5-15</sup>. In a previous paper we have reported the isolation of the main saponin present in the leaves of *I. Argentina*<sup>16</sup>. We report herein the isolation and structural elucidation of a novel saponin together with a known one and an acid triterpene from the same species.

## MATERIAL AND METHODS

### General experimental procedures

SIMS and CIMS spectra were performed on a Kratos MS-80 RF spectrometer. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were obtained on a Bruker AC 300. Analytical TLC was carried out on silica gel Merck G and GF 254 nm plates, using chloroform:ethanol (9:1, v/v), chloroform:ethanol (8:2, v/v) and chloroform:ethanol:water (6:4:0.5, v/v) as eluant for compounds **1**, **2** and **3**, respectively. Detection was performed with anisaldehyde-H<sub>2</sub>SO<sub>4</sub>/100 °C. For TLC different authentic samples of triterpenes were used.

### 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).

### Extraction and isolation

Air-dried leaves (100 g) were crushed and extracted with ethanol-water (6:4, v/v) at room temperature for seven days. The ethanol was removed under reduced pressure, the residue suspended in water and successively extracted with chloroform and ethyl acetate. Each extract was evaporated to dryness yielding 3.94 g and 2.0 g, respectively. Part of the chloroform fraction (1.0 g) was repeatedly chromatographed on a silica gel column using chloroform:ethanol (9:1 and 8:2, v/v) to afford compound **1** (13.5 mg). Ethyl acetate fraction (0.5 g) was repeatedly chromatographed on a silica gel column using chloroform:ethanol (8:2 and 7:3 v/v) resulting in 37.1 mg of pure compound **2**. Another part of the ethyl acetate fraction (1.5 g) was passed through an Amberlite XAD-2 column and eluted with EtOH to give a saponin mixture fraction. Repeated separation of this mixture on a silica gel column using chloroform:ethanol (9:1, v/v) and chloroform:ethanol:water (8:4:0.5, 6:4:0.5, and 4:4:0.5, v/v) furnished 21.7 mg of compound **3**.

### Compound 1 and Compound 2

Identified by direct comparison with the NMR data described previously<sup>7,14</sup>.

### Compound 3

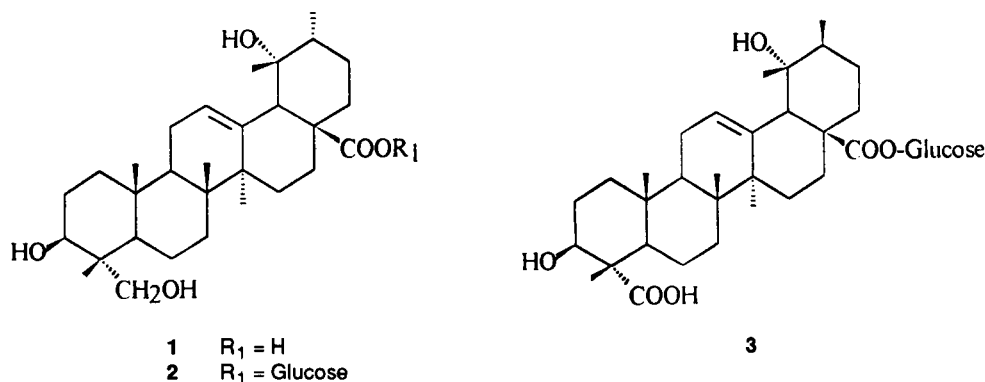
Amorphous powder,  $[\alpha]_D = + 31.85^\circ$  ( $c = 0.135$ , MeOH, 20 °C). SIMS  $m/z$  687 (M + Na)<sup>+</sup>. <sup>1</sup>H-NMR (C<sub>5</sub>D<sub>5</sub>N)  $\delta$ : 1.05, 1.25, 1.25, 1.41, 1.69, (3H each, s, CH<sub>3</sub> x 5), 1.0 (3H, d, J = 7.0, 30-CH<sub>3</sub>), 3.2 (1H, s, H-18) 4.7 (1H, dd, J = 6.6 and 8.5 Hz, H-3), 5.2 (1H s, 19-OH) 5.6 (1H, br t, H-12), 6.35 (1H, d, J = 8.0 Hz, Glc-1). <sup>13</sup>C-NMR (C<sub>5</sub>D<sub>5</sub>N): (C1 to C30) 39.0; 27.8; 75.5; 54.4; 51.9; 21.8; 33.3; 40.8; 48.0; 36.8; 24.0; 127.4; 138.8; 42.1; 29.1; 26.7; 48.2; 47.2; 73.3; 42.8; 24.6; 31.8; 180.7; 12.9; 16.1; 17.4; 24.2; 176.9; 29.6; 16.0; glucopyranosyl moiety (C1 to C6) 95.8; 74.0; 78.9; 71.0; 79.3 and 62.1.

## RESULTS AND DISCUSSION

The alcoholic extract of the leaves of *I. argentina* was concentrated and successively extracted with solvents of increasing polarity (chloroform and ethyl acetate). Compound **1** (13.5 mg) was isolated from the chloroform fraction. Its <sup>13</sup>C-NMR spectrum revealed the presence of 30 carbons indicating its triterpenic nature and the lack of sugar moiety. Careful analysis of the <sup>13</sup>C-NMR of **1** and co-TLC revealed that it was rotundic acid, a triterpene that we had previously isolated from *I. taubertiana*<sup>9</sup> and from *I. theezans*<sup>14</sup>.

Compound **2** (37.1 mg) was isolated from the ethyl acetate fraction. Its <sup>1</sup>H- and <sup>13</sup>C-NMR data indicated its triterpene nature (36 carbons). The hexose could be identified as one  $\beta$ -glucosyl moiety ( $\delta$  6.29, 1H, d, J = 8 Hz, H-1';  $\delta$  95.8, C-1'), both chemical shifts evidencing an ester linkage between the sugar portion and the aglycone. A careful comparison of the <sup>13</sup>C-NMR data obtained for **2** with the literature indicated that **2** was pedunculoside (Figure 1) a triterpenoid previously isolated from *I. rotunda*, *I. pedunculosa* and *I. oldhami*<sup>17,18</sup> and that we had previously identified from *I. taubertiana*<sup>7</sup> and *I. theezans*<sup>14</sup>.

Compound **3** (21.7 mg) was isolated from the ethyl acetate fraction. Its <sup>1</sup>H-NMR indicated the presence of the  $\beta$ -glucosyl unit (H-1:  $\delta$  6.35, d, J = 8 Hz) linked to the aglycone by an ester linkage. Similarly to **2**, its <sup>13</sup>C-NMR spectrum displayed signals for 36 carbons, but the major differences between the two spectra include characteristic signals due to one additional carboxyl group in **3** ( $\delta$  180.7) instead of an hydroxymethylene group in **2**. Comparing the chemical

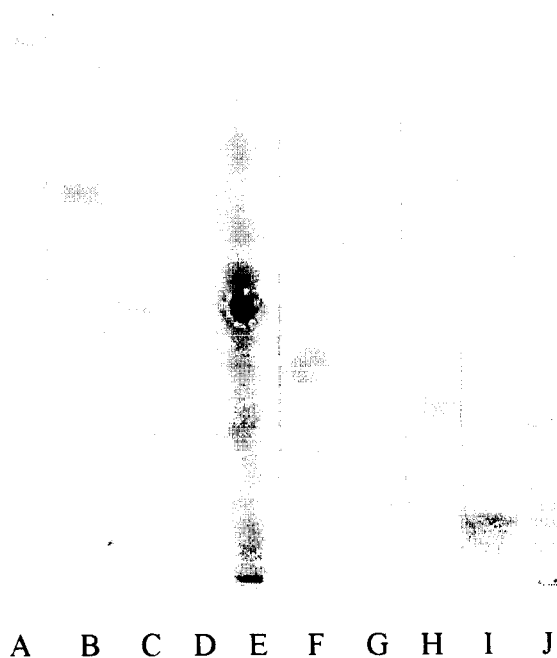


**Figure 1.** Compounds **1**, **2** and **3** isolated from *Ilex argentina* leaves.

shifts assigned in the A ring of **3** with those of **2**, we observed that the C4, C5 and C6 shifted from  $\delta$  42.8 to 54.4, 48.5 to 51.9 and 18.7 to 21.8 ppm respectively. These data are coherent with those observed<sup>19</sup> when C23- $\alpha$ CH<sub>3</sub> is replaced with COOH that resembled those of the 28-O- $\beta$ -D-glucopyranosylester of rotundioic acid that we had already isolated from *I. theezans*<sup>14</sup>. However, NMR comparison indicated that the two compounds, despite close similarities in A ring, were particularly different on E ring, suggesting another stereochemistry at C20. NMR data to discriminate between 20(S) and 20(R) series are numerous<sup>7,20-22</sup>, and the striking differences concern the chemical shifts of C18, C22 and C29. Since we had in hand both isomer, we could observe that indeed, in the case of **3**, C18' was at 47.2 ppm and C22 at 31.8 ppm, whereas we observed these signals at 52.8 and 36.2 ppm respectively in the case of the acetylated 28-O- $\beta$ -D-glucopyranosylester of rotundioic acid<sup>14</sup>. Also, the chemical shift observed for C29 was shifted by *ca.* 2.9 ppm downfield (29.6 ppm), when compared with those reported for the 20(R) series. Thus **3** was unambiguously identified as the 20(S) isomer of 28-O- $\beta$ -D-glucopyranosylester of rotundioic acid (Figure 1). To the best of our knowledge, this is the first report of the natural occurrence of this compound. Very few 19 $\alpha$ -hydroxyursolic acid derivatives with the singular 20S-configuration have been isolated, most of them from *Ilex species*<sup>7,15,20-23</sup>.

Considering the exomorphological resemblance, *I. argentina* has been provisionally classified in the same taxonomic group to which *I. paraguariensis* belongs, according to Loesener<sup>24</sup>, *i.e.*: subsection *Repandae*, section *Microdonatae*, series *Aquifolium*, subgenus *Euilex*. However, phytochemical analysis indicates a very different chemical composition, not only regarding the absence of xanthines or presence in

traces<sup>25,26</sup>, but also as a result of the present study on saponins. Both species, or their extracts, can be easily distinguished by the chromatographic profile of the saponins constituents, as shown in Figure 2, in which the substances were detected by spraying with anisaldehyde in sulfuric acid and heating, enabling maximal color development and visualisation in visible and long-wavelength UV light. In com-



**Figure 2.** TLC profile of *Ilex argentina* and *Ilex paraguariensis*. Eluent: chloroform:ethanol:water (60:40:5 by volume), detection with anisaldehyde-sulfuric acid followed by heating. A: compound **1** (rotundioic acid); B: compound **2** (pedunculoside); C: ILA<sub>1</sub> isolated from *I. argentina*<sup>16</sup>; D: compound **3**; E: n-butanol fraction of *I. argentina* leaves; F: isoquercitrin; G: rutin; H: matessaponin 1 (isolated from *I. paraguariensis* leaves); I: matessaponin 2 (isolated from *I. paraguariensis* leaves<sup>5,9</sup>); J: n-butanol fraction of *I. paraguariensis* leaves<sup>10</sup>.

parison with the glycosides isolated from *I. paraguayensis* which are derivatives of ursolic and oleanolic acids (deep purple color under visible and a bright yellow under UV light), the glycosides isolated from *I. argentina* (19 $\alpha$ -hydroxyursolic acid derivatives) displayed a blue color under visible light and a pale yellow color under UV conditions. These features could be helpful to allow the differentiation between the two species from the chemical point of view. Additionally, both chromosome numbers and DNA content were found to be different in both

species<sup>27</sup> -for *I. paraguayensis* n = 20 and DNA content 2C = 2.23  $\pm$  0.08 pg; whilst for *I. argentina* n = 40 and DNA content 2C = 4.27  $\pm$  0.07 pg.

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