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Saponins from *Ilex dumosa*, an Erva-maté (*Ilex paraguariensis*) Adulterating Plant

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Extraction of *Ilex dumosa* leaves, a material frequently used for the adulteration of erva-maté, resulted in the isolation of 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D-glucopyranosyl-3*β*-hydroxyolean-12-en-28-oic acid, 3-*O*-*β*-D-6-*O*-methylglucuronopyranosyl-28-*O*-*β*-D-glucopyranosyl-3*β*-hydroxyolean-12-en-28-oic acid, and three new saponins: 3-*O*-[*β*-D-glucopyranosyl-(1−2)-*β*-D-galactopyranosyl]-28-*O*-*β*-D-glucopyranosyl-3*β*,29-dihydroxyolean-12-en-28-oic acid, 3-*O*-[α-L-arabinopyranosyl-(1−2)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranosyl-3*β*-hydroxyolean-12-en-28-oic acid, 3-*O*-*β*-D-galactopyranosyl-28-*O*-*β*-D-glucopyranosyl-3*β*-hydroxyolean-12-en-28-oic acid. None of these saponins are present in the leaves of *Ilex paraguariensis* (genuine erva-maté).

Keywords: *Ilex paraguariensis; Ilex dumosa; erva-maté; saponins*

INTRODUCTION

Inherited from a Guarani (central South American natives) tradition, people from Uruguay, Paraguay, Argentina, and southern Brazil have shared for centuries the custom of drinking a mildly stimulant beverage called maté (or erva-maté or yerba-mate) (Moreau de Tours, 1904). This product, also named "Jesuit's tea" during the seventeenth and eighteenth centuries (Peckolt, 1943), is prepared by water infusion of the processed leaves and twigs of *Ilex paraguariensis* St. Hil. (Aquifoliaceae). Perpetuating the ritual, in South America, maté is nowadays generally still drunk in gourds made of the fruit of *Lagenaria vulgaris* Ser. In addition to substantial amounts of purine alkaloids (Moreau de Tours, 1904), the leaves of *I. paraguariensis* also contain triterpenoid saponins (Alikaridis, 1987). These bitter and highly water-soluble compounds are likely to be responsible in part for the taste of the beverage. Although the production of erva-maté is becoming more industrialized (Graham, 1984) and controlled, its adulteration by variable quantities of leaves of other South American *Ilex* species (mainly *Ilex dumosa* and, to a lesser extent, *Ilex theezans* and *Ilex brevicuspis*) is still frequent (Edwin and Reitz, 1967; Giberti, 1989). These added leaves might not only modify the physiological and pharmacological activities of maté [the leaves of *I. dumosa* do not contain caffeine (Lendner, 1913)] but also induce fluctuations in its content in saponin, substances whose nutritional and biological effects are still unclear (Milgate and Roberts, 1995). Strong modification of the beverage taste might also be associated with this adulteration. Recent efforts to promote the consumption of maté instead of (or in addition to) other stimulating beverages (coffee, tea, or stimulating carbonated drinks) in non-South American countries (South Asian countries especially) look promising. We initiated some years ago a program aimed at the structure determination of South American *Ilex* saponins, having as the ultimate goal the proposal of an efficient methodology based on the identification of some specific triterpenes and allowing the detection of any adulteration. Hence, we already reported the structure of several saponins isolated from erva-maté (Gosmann et al., 1989, 1995; Schenkel et al., 1997; Kraemer et al., 1996) and other *Ilex* species [*I. argentina* (Schenkel et al., 1995); *I. pseudobuxus* (Taketa and Schenkel, 1994); *I. theezans* (Athayde, 1993); *I. taubertiana* (Taketa and Schenkel, 1995); *I. integerrima* (Constantin, 1995)]. Recently, we began to study the saponin content of the caffeine-free species *I. dumosa* Reissek (Heinzmann and Schenkel, 1995). In this paper, we report the isolation and structure elucidation of five additional saponins from the leaves of *I. dumosa* together with a taste comparison between *I. paraguariensis* and *I. dumosa* saponins, and finally draw our primary conclusions concerning a possible maté adulteration simple detection.

EXPERIMENTAL PROCEDURES

Material. Aerial parts of *I. dumosa* were collected in Sapucaia, RS, Brazil. A herbarium specimen is on deposit in the Botany Department Herbarium of Rio Grande do Sul Federal University (ICN-106.334), Porto Alegre, Brazil.

Extraction. Air-dried powdered leaves (390 g) were extracted during 5 days at room temperature with 2.5 L of EtOH. The solvent was evaporated and the residue suspended in H_2O (200 mL). This suspension was partitioned first with CH_2Cl_2 (550 mL) and then with *n*-BuOH (320 mL). The alcoholic phase was concentrated yielding the crude saponin mixture (28.0 g, 7.0%) that was chromatographed on a Si gel column (Merck, particle size: 80−100 *μ*m) at atmospheric pressure. The column was successively eluted with mixtures of $CHCl_3$−MeOH−H_2O [80:40:5 (0.5 L); 70:40:5 (1 L); 60:40:5 (0.5 L); 50:40:5 (0.5 L); 40:40:5 (0.5 L); 20:40:5 (0.5 L)]; 20-mL fractions were collected and pooled according to thin-layer chromatography [Si gel GF 254, Merck, mobile phase $CHCl_3$−MeOH (90:

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Table 1. ^{13}C NMR Chemical Shift Data of 1 and 2a−5a in $CDCl_3$ (CD_3OD for 1)

genine carbon	1	2a	3a	4a	5a	sugars carbon	1	2a	3a	4a	5a
C-1	39.8	38.4	38.0	38.5	38.4	C_{28}-β-D-glucopyranose					
C-2	26.9	25.6	25.5	25.9	25.8	C-1	95.7	91.5	90.8	91.5	91.5
C-3	91.0	90.5	91.2	90.2	90.6	C-2	73.9	69.4	69.4	69.4	69.8
C-4	40.7	39.3	38.9	39.2		C-3	78.7	71.3	71.1	71.8	72.7
C-5	57.0	55.4	55.1	55.5	55.5	C-4	71.0	67.8	66.9	67.8	68.4
C-6	19.3	18.1	18.5	18.0	18.1	C-5	78.3	72.3	72.0	72.4	72.4
C-7	33.1	31.7	30.2	29.6	29.6	C-6	62.4	61.5	60.8	63.6	61.4
C-8	40.2	38.8	38.7	39.2		3-*O*-sugars					
C-9	49.0	47.5	47.0	47.5	47.5	β-D-galactopyranose					
C-10	37.9	36.6	36.2	36.3		C-1			103.5		103.5
C-11	24.6	23.4	22.9	22.9	22.8	C-2			74.6		70.3
C-12	123.8	122.8	122.9	122.0	122.8	C-3			72.9		69.2
C-13	144.8	142.7	141.8	142.8		C-4			67.7		67.9
C-14	42.9	41.6	41.2	41.6		C-5			72.3		70.9
C-15	28.9	27.6	27.1	27.5	27.5	C-6			61.4		61.4
C-16	24.0	22.8	22.4	23.3	23.9	β-D-glucopyranose					
C-17	48.5	46.7	46.4	46.7		C-1			100.1		
C-18	42.6	40.9	39.6	40.9	40.9	C-2			69.7		
C-19	47.2	45.7	39.6	45.6	45.7	C-3			71.1		
C-20	31.5	30.5	29.2	30.5		C-4			67.5		
C-21	34.9	33.7	27.9	33.7	33.7	C-5			72.3		
C-22	34.0	32.9	32.4	31.7	31.6	C-6			61.0		
C-23	28.5	27.6	27.1	27.5	27.5	β-D-glucuronopyranose					
C-24	16.0	15.2	15.6	15.9	16.3	C-1	106.7	102.8			
C-25	17.0	16.3	14.8	15.2	15.2	C-2	73.7	69.7			
C-26	17.7	16.9	16.5	16.8	16.9	C-3	77.9	72.0			
C-27	26.3	25.6	25.1	25.6	25.6	C-4	75.4	72.3			
C-28	178.0	175.5	174.8	175.6		C-5	76.6	72.7			
C-29	33.5	32.8	74.1	32.9	33.7	C-6	181.2	167.2			
C-30	24.0	23.3	19.9	23.5	23.9	OCH_3		52.8			
						α-L-arabinopyranose					
						C-1				103.6/101.0	
						C-2				74.3/69.8	
						C-3				72.7/70.5	
						C-4				67.9/67.9	
						C-5				61.5/61.9	

10 or 85:15 or 80:20)]. Spots were visualized by heating (120 °C) the H_2SO_4-sprayed plates. Pooled fractions 1−2 (138 mg) and 3−5 (163 mg) contained compounds **4** and **5**, respectively, as main components. Separated acetylation (Gosmann et al., 1989) of these fractions followed by Si gel column chromatography [eluent AcOEt−hexane (1:1)] yielded pure acetylated derivatives **4a** (22 mg) and **5a** (31 mg), respectively. Fractions 35−38 (150 mg) were further purified by Si gel column chromatography eluting with *n*-BuOH−AcOH−H_2O (12:3:5) to yield **1** (23 mg) in pure form. Compounds **2** and **3** were the main components of the mixture obtained after evaporation of fractions 16−19 (380 mg). An aliquot of this latter mixture was acetylated, and pure peracetylated derivatives [**2a** (13 mg) and **3a** (47mg)] were obtained after separation by Si gel column chromatography [eluent AcOEt−hexane (2:1)].

Apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^1H and ^{13}C NMR spectra were recorded in CD_3OD at 300.13 and 75.47 MHz, respectively, on a Bruker AC 300-P instrument. DEPT experiments were performed using transfer pulses of 135 or 90° to obtain either positive signals for CH and CH_3 and negative ones for CH_2 or selectively CH resonances only. The heteronuclear correlation experiments (HMQC and HMBC) were performed on a data matrix 256 × 1024 or 512 × 2048, using optimal delays for a CH coupling of 135 Hz and a relaxation delay of 1 s. FAB mass spectra were recorded, after dissolution of the sample in a glycerol matrix, in positive mode on a Kratos MS-80 instrument, source temperature 250 °C, nominal ionizing voltage 70 eV, sample bombarded with a beam of xenon atoms.

Test for Bitterness. Preparation of the saponin solutions and evaluation of their bitterness was accomplished following established methods (Jisaka et al., 1992, 1993). Solutions (10 mg/5 mL) of *I. paraguariensis* and *I. dumosa* crude saponins were used to prepare filter papers impregnated with 10, 20, 30, and 40 μg of saponin mixtures. Seven volunteers tested each concentration, and the threshold value was considered the amount that tasted bitter to at least four volunteers. In the same trial, bitterness of quinine sulfate was found to be 10 μg.

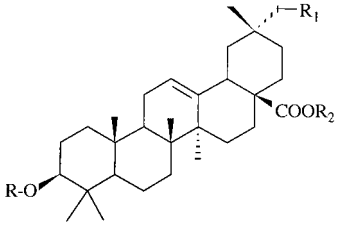
Hydrolysis of Saponins. Saponin **1** and peracetylated derivatives **2a** and **3a** (2.5 mg) were separately refluxed with 20% concentrated hydrochloric acid in ethanol (5 mL) for 3 h. The reaction mixture was neutralized using an aqueous Na_2CO_3 saturated solution and evaporated to dryness. The aglycon was dissolved in CH_2Cl_2 (3 × 2 mL), and its R_f was compared to those of authentic samples (TLC).

The sugar units were identified after TLC acid hydrolysis, carried out with Si gel GF 254 plates. The solvent used in the first development was $CHCl_3$−EtOH−H_2O (16:8:1). After hydrolysis of the saponins (HCl vapor, 1 h), the chromatogram was developed with EtOAc−MeOH−HOAc−H_2O (12:3:3:2) in the second direction and then compared with a reference sugar mixture.

Compound 1 (chikusetsusaponin IVa): ^1H NMR (300 MHz, CD_3OD) δ 0.98 (s, Me), 1.02 (s, Me), 1.10 (s, Me), 1.11 (s, Me), 1.12 (s, Me), 1.22 (s, Me), 1.33 (s, Me), 3.05 (dd, J = 10.0, 1.5 Hz, H-18), 4.53 (d, J = 7.6 Hz, GluA-1), 5.42 (m, H-12), 5.55 (d, J = 8 Hz, Glc-1); ^{13}C NMR see Table 1.

Compound 2a (chikusetsusaponin IVa methyl ester): ^1H NMR (300 MHz, $CDCl_3$) δ 0.65 (s, Me), 0.66 (s, Me), 0.85 (s, 3 × Me), 1.05 (s, Me), 1.15 (s, Me), 2.05 (OAc), 2.75 (dd, J = 10.8, 1 Hz, H-18), 3.05 (dd, J = 10.5, 4.9 Hz, H-3), 3.55 (m, Glc-5), 3.75 (s, OMe), 4.50 (d, J = 7.9 Hz, GluA-1), 5.27 (m, H-12), 5.52 (d, J = 7.8 Hz, Glc-1); ^{13}C NMR see Table 1.

Peracetylated dumosasaponin 5 (3a): $[\alpha]^{20}_D = +14°$ (c = 0.4, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.70 (s, Me), 0.80 (s, Me), 0.90 (s, Me), 0.96 (s, Me), 1.04 (s, Me), 1.10 (s, Me), 1.25 (s, Me), 2.00−2.20 (OAc), 2.78 (dd, J = 13.7, 3.9 Hz, H-18), 3.02 (dd, J = 10.8, 5.2 Hz, H-3), 3.85 (dd, J = 10.1, 7.6 Hz, Gal-2), 4.37 (d, J = 7.6 Hz, Gal-1), 4.66 (d, J = 8.1 Hz, Glc-1), 4.85 (t, J = 8.1 Hz, Glc-2), 4.90 (dd, J = 10.1, 3.4 Hz, Gal-3), 5.28 (m, H-12), 5.51 (d, J = 8 Hz, Glc-1); ^{13}C NMR see Table 1.



1 R = gluA, R_1 = H, R_2 = glc

2 R = gluAOMe, R_1 = H, R_2 = glc
2a R = $(OAc)_3$gluAOMe, R_1 = H, R_2 = $(OAc)_4$glc

3 R = glc(1→2)gal, R_1 = OH, R_2 = glc
3a R = $(AcO)_4$glc(1→2)$(AcO)_3$gal,
R_1 = OAc, R_2 = $(AcO)_4$glc

4 R = ara(1→2)ara, R_1 = H, R_2 = glc
4a R = $(AcO)_3$ara(1→2)$(AcO)_2$ara,
R_1 = H, R_2 = $(AcO)_4$glc

5 R= gal, R_1 = H, R_2 = glc
5a R= $(AcO)_4$gal, R_1 = H, R_2 = $(AcO)_4$glc

Figure 1.

Peracetylated dumosasaponin 6 (4a): $[\alpha]^{20}_D = +20°$ (*c* = 1.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.60 (s, Me), 0.75 (s, Me), 0.80 (s, 2 × Me), 0.95 (s, Me), 1.04 (s, Me), 1.15 (s, Me), 1.85−2.10 (OAc), 2.72 (dd, *J* = 13.2, 3.2 Hz, H-18), 3.02 (dd, *J* = 10.5, 4.4 Hz, H-3), 3.45 (d, *J* = 14.4 Hz, Ara-5), 3.52 (d, *J* = 14.0 Hz, Ara-5), 3.73 (m, Glc-5), 4.20 (dd, *J* = 11.8, 4.0 Hz, Glc-6), 4.40 (d, *J* = 6.1 Hz, Ara-1), 4.55 (d, *J* = 7.3 Hz, Ara-1), 5.5 (d, *J* = 7.9 Hz, Glc-1); ^{13}C NMR see Table 1.

Peracetylated dumosasaponin 7 (5a): $[\alpha]^{20}_D = +12°$ (*c* = 0.26, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70 (s, Me), 0.75 (s, Me), 0.90 (s, 2 × Me), 1.10 (s, Me), 1.25 (s, 2 × Me), 1.90−2.20 (OAc), 2.82 (dd, *J* = 13.4, 3.0 Hz, H-18), 3.10 (dd, *J* = 10.6, 4.1 Hz, H-3), 4.50 (d, *J* = 7.6 Hz, Gal-1), 5.57 (d, *J* = 7.5 Hz, Glc-1); ^{13}C NMR see Table 1.

RESULTS AND DISCUSSION

I. dumosa leaves were extracted with EtOH. Evaporation of the alcoholic extract furnished a residue that was suspended in H_2O and successively extracted with CH_2Cl_2 and then with *n*-BuOH. The dried *n*-BuOH-soluble portion subjected to silica gel chromatography afforded **1**, a mixture of **2** and **3** and a mixture of **4** and **5** (see Figure 1). After acetylation of these mixtures, peracetylated compounds (**2a**−**5a**, respectively) were obtained in pure form by silica gel column chromatography. Identification of the saponins in native or peracetylated form was mostly deduced from a combination of mass and NMR spectroscopy.

The FAB−MS (positive ion mode) of compound **1** exhibited ions at *m*/*z* (relative intensity) 839 (45) $[(M - H + Na) + Na]^+$ evidencing the presence within the molecule of one sodium carboxylate salt, 817 (20) $[M + Na]^+$, and 677 (20) $[(M - H + Na) + Na - 162]^+$, which was interpreted as the cleavage of a glucose moiety. The FAB−MS (positive ion mode) of compound **2a** showed ions at *m*/*z* 1141 (10) $[M + K]^+$, 1125 (40) $[M + Na]^+$, 795 (8) [M − peracetylated hexose]$^+$. For compound **3a**, ions at *m*/*z* 1485 (40) $[M + Na]^+$ and 619 (10) [two linked peracetylated hexose moieties] were observed. In the case of **4a**, ions at *m*/*z* 1283 (45) $[M + Na]^+$, 1024 [M − peracetylated pentose + $Na]^+$, and 331 (40) (peracetylated hexose) were detected. For compound **5a**, two important peaks were observed at *m*/*z* 1139 (30) $[M + Na]^+$ and 331 (100).

Acid hydrolysis of **1** and **2a** yielded oleanolic acid, glucose (Glc), and glucuronic acid (GluA), which were separately identified by direct comparison with authentic samples using TLC. Upon similar treatment, **3a** delivered a genine that did not fit, by co-TLC, with any of our authentics together with galactose (Gal) and Glc. Because **4a** and **5a** were only obtained in very minute amount, we did not attempt to characterize their aglycon or sugar residues by degradative methods and decided to use only spectroscopic data.

Attribution of all oleanolic acid resonances displayed on the ^{13}C NMR spectrum of **2a** was realized by direct comparison with literature data (Mahato and Kundu, 1994). This also established unambiguously the substitution, by a sugar moiety, of the genine 3-position (δ C-3 90.5) (see Table 1 for other ^{13}C NMR data) and also evidenced the presence of only two sugar residues within the molecule (δ 106.7, GluA C-1; 95.7, Glc C-1). The ^{1}H NMR of **2a** displayed a signal for a methyl ester function (δ 3.7, 3H, s), and the pattern of the sugar residues evidenced their β-D-pyranosyl form. From their anomeric proton chemical shift, the location of GluA was deduced to be at position C-3 of the aglycon (δ 4.6, 1H, d, *J* = 7.6 Hz) and this of Glc to be on an ester function (δ 5.48, 1H, d, *J* = 8 Hz). An HMBC experiment allowed the definitive location of Glc at position C-28 and concomitantly demonstrated that the GluA was esterified on its C-6 position. Thus compound **2a** was identified as the peracetylated derivative of the 3-*O*-β-D-6-*O*-methylglucuronopyranosyl-28-*O*-β-D-glucopyranosyl-3β-hydroxyolean-12-en-28-oic acid, an already known compound also named chikusetsusaponin IVa methyl ester (Shimizu et al., 1988; Penders and Delaude, 1994; Hu et al., 1995).

The great similarity observed between the ^{13}C NMR spectra of **2a** and **1** (the only major difference was the lack of the methyl ester resonance at δ 52.8) allowed the direct identification of **1** as an already known saponin named glycoside D2 (Vidal-Ollivier et al., 1989) or chikusetsusaponin IVa (Kinjo et al., 1995).

The ^{13}C NMR spectrum of compound **3a** displayed signals for six angular methyl groups (δ 14.8, 15.6, 16.6, 19.9, 25.1, 27.1) together with signals corresponding to three hexose anomeric carbons (δ 90.8 Glc-1, 100.1 Glc′-1, 103.5 Gal-1) and four hydroxyl bearing methylene groups (δ 74.1, 61.4, 61.0, 60.8). Consequently, one of these groups had to belong to the aglycon. A survey of the literature showed that the aglycon of **3** was mesembryanthemoidigenic acid (Shao et al., 1989). The sugar substituting the position C-28 of the aglycon was identified as a β-D-Glc by analysis of the COSY spectrum starting from its anomeric proton signal (δ 5.45, 1H, d, *J* = 8 Hz). This spectrum also showed that the disaccharide unit linked by an ether function on position C-3 of the aglycon was composed of a β-D-Glc moiety substituting a β-D-Gal at its C-2 position (δ 3.85, 1H, dd, *J* = 10.1; 7.6 Hz, Gal-H2; 4.90, 1H, dd, *J* = 10.1; 3.4 Hz, Gal-H3). To the best of our knowledge, this compound has never been isolated before and is the peracetylated derivative of 3-*O*-[β-D-glucopyranosyl-(1−2)-β-D-galactopyranosyl]-28-*O*-β-D-glucopyranosyl-3β,29-dihydroxyolean-12-en-28-oic acid. We propose to name the native saponin dumosasaponin 5.

Comparison of the ^{13}C NMR spectrum of **2a** with this of **4a** and **5a** (DEPT spectrum) allowed the identification of the aglycon of these two latter compounds as oleanolic acid, its 3-position being substituted in both cases (see Table 1). For **4a**, the ^{13}C NMR spectrum also displayed

signals for three anomeric carbons (δ 91.5 Glc-1, 101.0 Ara-1, 103.6 Ara-1). The sugar residue esterifying the carboxyl at C-28 (δ H-1 5.5, d, 1H, J = 7.9 Hz) was identified as a non-substituted β-D-Glc residue from its COSY pattern. The same experiment allowed the identification of the other oligosaccharide residue as a diarabinose (Ara) unit whose interglycosidic linkage on C-2 was evidenced from the inner Ara H-2 chemical shift (δ 3.85). The large $^3J_{\text{H-1}-\text{H-2}}$ coupling constant (6.1 and 7.3 Hz) observed for the anomeric Ara protons was strongly in favor of two α-L-arabinoses in $^4C_1$ conformation. Thus, **4a** was identified as the peracetylated derivative of 3-*O*-[α-L-arabinopyranosyl-(1−2)-α-L-arabinopyranosyl]-28-*O*-β-D-glucopyranosyl-3β-hydroxyolean-12-en-28-oic acid, and the name dumosasaponin 6 was proposed for the corresponding new native product **4**. The stucture of compound **5a**, composed of an oleanolic acid molecule whose positions 3 and 28 were each substitued by one single sugar residue (δ 91.5 Glc-1, 103.5 Gal-1), was identified as the peracetylated derivative of 3-*O*-β-D-galactopyranosyl-28-*O*-β-D-glucopyranosyl-3β-hydroxyolean-12-en-28-oic acid by comparison with the ^{1}H and ^{13}C NMR data of saponin E8, a substance with an additional arabinopyranosyl residue in the C-3 chain that we previously isolated and identified from the same plant (Heinzmann and Schenkel, 1995). Compound **5** was named dumosasaponin 7.

During the past 10 years, we have systematically studied the saponins of the leaves of the South American *Ilex* species. This allowed us to work on genuine ervamaté (*I. paraguariensis*) and on the main adulterative species, looking for chemical differences in the saponin structure. Interestingly, the aglycons of all the major saponins are exclusively belonging to the ursane or oleanane series. Nevertheless, a closer look to the structures showed that all the saponins isolated from *I. theezans* (Athayde, 1993), *I. pseudobuxus* (Taketa and Schenkel, 1994), *I. argentina* (Schenkel et al., 1995), *I. integerrima* (Constantin, 1995), and *I. taubertiana* (Taketa and Schenkel, 1995) belong to the ursane series and have as aglycon 19-α-hydroxyursolic acid derivatives. Conversely, such aglycons have never been found in *I. paraguariensis* and *I. dumosa* saponins whose major genines are, with one exception [29-OH-oleanolic acid (mesembryanthemoidigenic acid) was found in an *I. dumosa* saponin], ursolic or oleanolic acid (Gosmann, 1989; Gosmann et al., 1995; Kraemer et al., 1996; Schenkel et al., 1997). Furthermore, and although we identified about 10 main saponins of *I. paraguariensis* and *I. dumosa*, we were unable to isolate one single saponin common to these two species. These data in addition to important taxonomical significances suggest that it should be possible to trace adulterations of *I. paraguariensis* and to develop easy methodologies for the quality control of maté products based on the characterization of the aglycons of the saponins isolated from commercialized maté. A second important point to rise is the high saponin content of *I. paraguariensis* and *I. dumosa* (6−10%) as compared to the other species (2 or 3%). This questioned us about the importance of the saponins on the taste of maté. To answer this question, we tested the crude saponin fractions of the two saponin-rich species using the filter paper method (Jisaka et al., 1992, 1993). The amount of *I. dumosa* saponin mixture required for bitter taste was 20 μg whereas it was 40 μg for *I. paraguariensis*. These results, in accordance with popular reports stating that beverages adulterated with *I. dumosa* are more bitter than genuine maté, seem to confirm the importance of the saponins in the taste of the beverage from a quantitative and qualitative point of view, ursolic acid-containing saponins looking less bitter than their oleanolic acid counterparts.

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LITERATURE CITED

Alikaridis, F. Natural Constituents of Ilex Species *J. Ethnopharmacol.* **1987**, *20*, 121−144.

Athayde, M. L. Saponinas e triterpenos em Algumas Espécies do Gênero Ilex. Master Thesis, Curso de Pos-Graduação em Farmácia de Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, 1993.

Constantin, M. B. Análise de saponinas em *Ilex integerrima* e comparação com *Ilex theezans*. Master Thesis, Curso de Pós-Graduação em Farmácia de Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, 1995.

Edwin, G.; Reitz, P. R. *Aquifoliáceas*. In *Flora Ilustrada Catarinense. I parte. Fasc. Aquifoliaceae*; Reitz, P. R., Ed.; Herbario Barbosa Rodrigues/CNPq: Itajai, 1967.

Giberti, G. C. Los Parientes Silvestres de la Yerba Mate y el Problema de su Adulteracion. *Dominguezia* **1989**, *7*, 3−21.

Gosmann, G.; Schenkel, E. P.; Seligmann, O. A New Saponin from Mate, *Ilex paraguariensis*. *J. Nat. Prod.* **1989**, *52*, 1367−1370.

Gosmann, G.; Guillaume, D.; Taketa, A. T. C.; Schenkel, E. P. Triterpenoid Saponins from *Ilex paraguariensis*. *J. Nat. Prod.* **1995**, *58*, 438−441.

Graham, H. N. Maté. In *The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects*; Spiller, G. A. Ed.; Liss: New York, 1984.

Heinzmann, B. M.; Schenkel, E. P. Saponins from *Ilex dumosa*. *J. Nat. Prod.* **1995**, *58*, 1419−1422.

Hu, M.; Ogawa, K.; Sashida, Y.; Xiao, P.-G. Triterpenoid Glucuronide Saponins from Root Bark of *Aralia armata*. *Phytochemistry* **1995**, *39*, 179−184.

Jisaka, M.; Ohigashi, H.; Takagaki, T.; Nozaki, H.; Tada, T.; Hirota, M.; Irie, R.; Huffman, A.; Nishida, T.; Kaji, M.; Koshimizu, K. Bitter Steroid Glycosides, verniosides A1, A2, and A3, and related B1 from a possible medicinal plant, *Vernonia amygdalyna*, used by Wild Chimpanzees. *Tetrahedron* **1992**, *48*, 625−632.

Jisaka, M.; Ohigashi, H.; Takegawa, K.; Hirota, M.; Irie, R.; Huffman, A.; Koshimizu, K. Steroid Glucosides from *Vernonia amygdalyna*, a possible Chimpanzee Medicinal Plant. *Phytochemistry* **1993**, *34*, 409−413.

Kinjo, J.; Suyama, K.; Nohara, T. Triterpenoidal saponins from *Dumasia truncata*. *Phytochemistry* **1995**, *40*, 1765−1769.

Kraemer, K.; Schenkel, E. P.; Gosmann, G.; Guillaume, D. Matesaponin 5, an Highly Polar Saponin from *Ilex paraguariensis*. *Phytochemisrty* **1996**, *42*, 1119−1122.

Lendner, A. L'*Ilex dumosa*, une falsification du Maté. *Mitt. Geb. Lebensmittelunters. Hyg.* **1913**, *4*, 42.

Mahato, S. B.; Kundu, A. P. ^{13}C NMR Spectra of Pentacyclic Triterpenoids−a Compilation and some Salient Features. *Phytochemistry* **1994**, *37*, 1517.

Milgate, J.; Roberts, D. C. K. The Nutritional and Biological Significance of Saponins. *Nutr. Res.* **1995**, *15*, 1223−1249.

Moreau de Tours, A. *Le Maté, Etude Historique, Chimique et Physiologique*; Steinheil, G., Ed.; Paris, 1904.

Peckolt, G. Herva mate. *Rev. Flora Med.* **1943**, *10*, 493−569.

Penders, A.; Delaude, C. Triterpenoid Saponins from *Melanthera scandens*. *Phytochemistry* **1994**, *37*, 821−825.

Schenkel, E. P.; Athayde, M. L.; Giberti, G. C.; Guillaume, D. A New Saponin from *Ilex argentina*. *Acta Farm. Bonaerense* **1995**, *14*, 5−9.

Schenkel, E. P.; Montanha, J. A.; Gosmann, G. Triterpene Glycosides from Maté, *Ilex paraguariensis*. In *Saponins Used in Traditional and Modern Medicine*; Waller, G. R., Yamasaki, K., Eds.; Plenum Publishing Co.: New York, 1997; in press.

Shao, C.-J.; Kasai, R.; Xu, J.-D.; Tanaka, O. Saponins from Leaves of *Acanthopanax senticosus* Harms., Ciwuja. II. Structures of Ciwujianosides A1, A2, A3, A4 and D3. *Chem. Pharm. Bull.* **1989**, *37*, 42−45.

Shimizu, S.; Ishihara, N.; Umehara, K.; Miyase, T.; Ueno, A. Sesquiterpene Glycosides and Saponins from *Cynara cardunculus* L. *Chem. Pharm. Bull.* **1988**, *36*, 2466−2474.

Taketa, A. T. C.; Schenkel, E. P. Saponins from *Ilex pseudobuxus. Acta Farm. Bonaerense* **1994**, *13*, 159−164.

Taketa, A. T. C.; Schenkel, E. P. Saponinas de *Ilex taubertiana. Rev. Bras. Farm.* **1995**, *76*, 9−11.

Vidal-Ollivier, E.; Balansard, G.; Faure, R.; Babadjamian, A. Revised Structure of Triterpenoid Saponins from the Flowers of *Calendula officinalis. J. Nat. Prod.* **1989**, *52*, 1156−1159.

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