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New triterpene and triterpenoid glycosides from *Ilex brevicuspis*

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From the leaves of Ilex brevicuspis were isolated a new triterpene, 20(S)-3β,19α-dihydroxyurs-12-en-23,28-dioic acid, named here brevicuspic acid, and two new triterpenoid glycosides, 3-O-α-L-arabinopyranosyl-20(S)-pomolic acid-28-O-β-D-glucopyranosyl ester, named brevicuspisaponin 3, and the 23-sodium salt of (20S)-3β,19α,24-trihydroxyurs-12-en-23,28-dioic acid-28β-O-β-D-glucopyranosyl ester, along with the known compound 3-O-α-L-arabinopyranosyl-20(S)-19α,24-dihydroxyursolic acid- 28-O-β-D-glucopyranosyl ester, already described for Ilex argentina. Their structures were established on the basis of chemical and spectroscopic methods.

Uniterms:
- Aquifoliaceae
- *Ilex brevicuspis*
- Triterpenes
- Saponins
- Brevicuspic acid
- Brevicuspisaponins 3 and 4

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INTRODUCTION

As previously reported, the leaves of *Ilex brevicuspis* Reissek (Aquifoliaceae) afforded the triterpenes ursolic acid, 23-methylester of 20(*S*)-rotundioic acid, and the new glycosides brevicuspisaponin 1 and 2(Taketa *et al*., 2000). Continuing the investigation on the leaves of *I. brevicuspis*, as a part of our studies on the adulterants of the genuine maté, *Ilex paraguariensis* (Taketa, Schenkel.,1994; Schenkel *et al.*,1995; Heinzmann, Schenkel,1995; Pires *et al.*, 1997; Schenkel *et al.*,1997; Athayde *et al.*, 1999; Reginatto *et al.*,1999; Athayde *et al.*, 2001), we report here further new triterpene and saponins from the title plant.

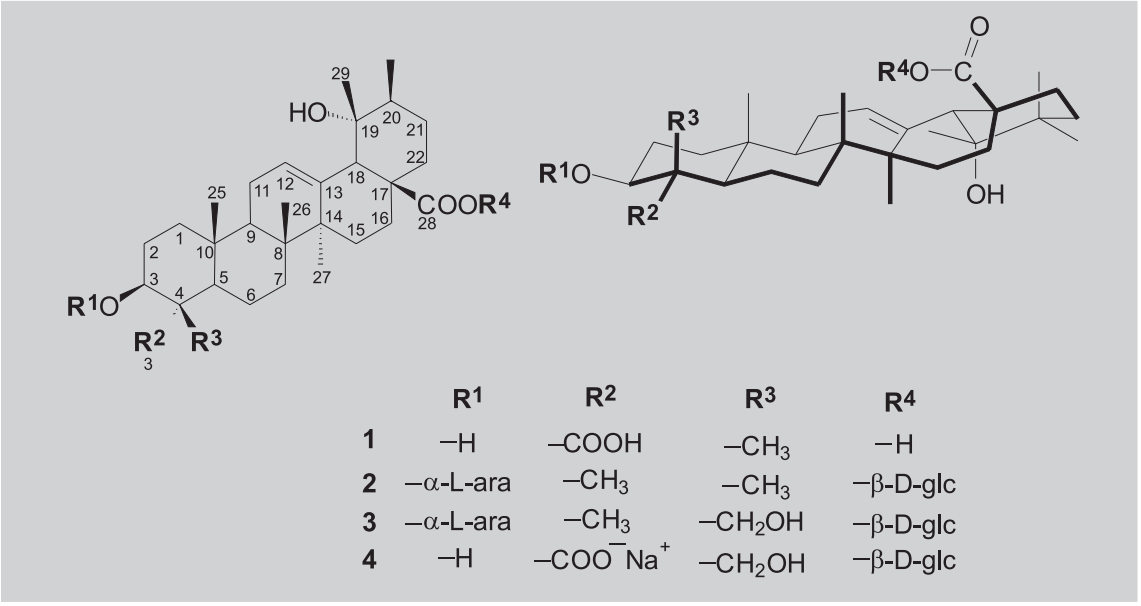
MATERIAL AND METHODS

Plant material

Leaves of *Ilex brevicuspis* Reissek were collected in Osório, State of Rio Grande do Sul, Brazil. A herbarium specimen (leg. Coelho 163) is on deposit in the Herbarium of the Botany Department of the Federal University of Rio Grande do Sul (Herbarium ICN, Porto Alegre, Brazil).

General experimental procedures

Melting points were obtained in a Kofler melting point apparatus and are uncorrected. IR spectra were recorded in a Perkin-Elmer 881 spectrophotometer. Optical rotations were measured in a Perkin-Elmer 241 polarimeter. EIMS and HRMS spectra were performed in a MS50 spectrometer and FAB-MS spectra on a Concept 1H spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in Bruker AMX and DRX 500 spectrometers. TLC were carried out on silica gel (Merck) GF_{254} and sugars and glycosides were eluted with EtOAc/MeOH/HOAc/H_2O 13:3:4:3. All compounds were visualized using the vanillin-sulfuric acid reagent/100 °C/10 min. Open CC were performed on a normal phase silica gel 40-60 mm using the eluants $CHCl_3$/MeOH 97:3 and cyclohexane/acetone 1:1 to **1a** and **2a**, $CHCl_3$/MeOH 9:1 to **1** and $CHCl_3$/EtOH/H_2O 8:4:0.5 to **2**, **3** and **4**. For **3**, the phase LiChroprep C-18, 40-63 mm, the eluant MeOH/H_2O 3:1 and Sephadex LH-20 in MeOH were used for final purifications.



	R1	R2	R3	R4
1	–H	–COOH	$–CH_3$	–H
2	–α-L-ara	$–CH_3$	$–CH_3$	–β-D-glc
3	–α-L-ara	$–CH_3$	$–CH_2OH$	–β-D-glc
4	–H	$–COO^-Na^+$	$–CH_2OH$	–β-D-glc

Extraction and isolation

Air-dried leaves (589 g) were crushed and extracted with ethanol (1.5 L) at room temperature (2 x 7 days). The ethanolic extract (2 L) was evaporated to dryness under reduced pressure and the residue (69 g) was then suspended in water (700 mL) and extracted with chloroform (5 x 500 mL). Between the water and chloroform phases an emulsified phase was formed. The emulsified phase was evaporated to dryness to give the fraction containing the saponins (19 g). Part of this residue was repeatedly chromatographed to give compounds **1** (17 mg), **2** (13 mg) and pure compounds **3** (26 mg) and **4** (17 mg).

Acid hydrolysis

Compounds **2, 3** and **4** were hydrolyzed on TLC plates in order to identify their sugars, as described by Kartnig and Wegschaider (1972).

Acetylation of compounds 1, 2 and 4

Compounds **1** (3 mg), **2** (3 mg) and **4** (6 mg) were acetylated with acetic anhydride/pyridine (1:1) at room temperature overnight, affording peracetylated compounds **1a**, **2a** and **4a**.

Acetylated derivative (**1a**):
*Acetylated brevicuspic acid [20(*S*)-3β,19α-dihydroxyurs-12-en-23,28-dioic acid] (**1**)*

White powder, mp 232-235 °C. $[\alpha]^{20}_{589}$ +33°, $[\alpha]^{20}_{578}$ +80°, $[\alpha]^{20}_{546}$ +94° and $[\alpha]^{20}_{436}$ +182° (MeOH, *c* 0.17). IR ν^{KBr}_{max} cm^{-1}: 3425, 2932, 1741, 1698, 1456, 1372, 1237, 1030. FAB-MS (positive-ion mode, mNBA) *m/z*: 567.3 $[M + Na]^+$, 467.2, 421.3, 338.3. EIMS *m/z*: 544.4 $[M]^+$, 426.4, 498.4, 482.4, 438.3, 426.4, 407.3. HRMS: 526.3291 $[M-H_2O]^+$. ^{1}H NMR (Table I) and ^{13}C NMR (Table II).

Brevicuspisaponin 3(**2**):
*[3-O-α-L-arabinopyranosyl-20(*S*)-pomolic acid-28-O-β-D-glucopyranosyl ester]*

FAB-MS (positive-ion mode, mNBA) *m/z*: 789.4 $[M+Na]^+$, 703.4, 687.4, 613.1, 599.1, 531.2.

Peracetylated derivative (**2a**):

White powder, mp 153-155 °C . $[\alpha]^{20}_{589}$ +17°, $[\alpha]^{20}_{578}$ +21°, $[\alpha]^{20}_{546}$ +23°, $[\alpha]^{20}_{436}$ +39° and $[\alpha]^{20}_{365}$ +61° ($CHCl_3$, *c* 0.21). IR ν^{KBr}_{max} cm^{-1}: 3481, 2946, 1752, 1370, 1224, 1056. FAB-MS (positive-ion mode, mNBA) *m/z*: 1083.4 $[M + Na]^+$, 904.4, 850.4, 783.3, 683.4, 667.2, 613.3. ^{1}H NMR (Table I) and ^{13}C NMR (Table II).

ILA-1(3):
*[3β-O-α-L-arabinopyranosyl-20(*S*)-19α,24-dihydroxyursolic acid-28-O-β-D-glucopyranosyl ester]*

White powder, mp 200-204 °C $[\alpha]^{20}_{589}$ +12°, $[\alpha]^{20}_{578}$ +13°, $[\alpha]^{20}_{546}$ +14°, $[\alpha]^{20}_{436}$ +24° and $[\alpha]^{20}_{365}$ +37° (MeOH, *c* 0.25). IR n^{KBr}_{max} cm^{-1}: 3437, 2932, 1734, 1637, 1382, 1074. FAB-MS (positive mode, mNBA) *m/z*: 805.3 $[M+Na]^+$, 642, 482, 411. ^{1}H NMR (Table I) and ^{13}C NMR (Table II).

Brevicuspisaponin 4 (**4**):
[23-sodium salt of (20S)-3β,19α,24-trihydroxyurs-12-en-23,28-dioic acid-28β-O-β-D-glucopyranosyl ester]

FABMS (positive-ion mode, thioglycerol) *m/z:* 725.3 $[M+Na]^+$, 703.3 $[M+H]^+$, 539.2 $[M-C_6H_{11}O_5]$. IR ν^{KBr}_{max} cm^{-1} : 3422, 2930, 1735, 1573, 1457, 1383, 1232, 1073.

Peracetylated derivative (**4a**):

White powder, mp 153-155 °C. FAB-MS (positive-ion mode, mNBA) *m/z*: 955.4 $[M + H]^+$ ^1H NMR (Table I) and ^{13}C NMR (Table II).

RESULTS AND DISCUSSION

Triterpene **1** and saponins **2**, **3** and **4** were isolated from the emulsified phase obtained from the leaves of the title plant, using the procedure described in the experimental section. Due to difficulty in purifications, compounds **1**, **2** and **4** were acetylated in order to afford pure compounds **1a**, **2a** and **4a**.

EIMS spectrum of **1a** display a molecular peak at *m/z* 544 $[M^+]$ as molecular ion, which together with the HRMS from the fragment ion with *m/z* 526.3291 $[M^+-H_2O]$ suggested the molecular formula $C_{32}H_{48}O_7$. The triterpenoid structure was confirmed by the ^1H broadband-decoupled ^{13}C NMR experiment which exhibited the presence of one acetyl group (δ_C 170.2 and 21.1) and thirty other carbon atoms. The DEPT subspectra revealed the presence of six methyl, nine methylene, six methane groups. It was possible to recognize the existence of two carboxy groups (δ_C 180.7 and 179.2), one double bond (δ_C 126.8 and 139.5), one acetylated *sec* alcohol group (δ_C 78.5) and one *tert* alcohol group (δ_C 73.3). The ^1H NMR spectrum showed the presence of five angular methyl groups (δ_H 0.94, 1.05, 1.42, 1.52 and 1.68) and one methyl group attached to CH (δ_H 1.10, *d*, ^3J= 7.0 Hz), suggesting an ursane or oleanane derivative. The multiplicities at δ_H 5.76 (3α-H, *dd*, ^3J= 12.0 and 3.4 Hz) indicated a 3β-hydroxy substitution on this skeleton. HMBC experiment allowed to locate 24-CH_3 (δ_C 12.8) and 23-COOH (δ_C 179.2) at C-4. NOE enhancements were detected for 25β-CH_3 (δ_H 0.94, s) and 24-CH_3 (δ_H 1.52, s) in a ROESY experiment, indicating the 4a-configuration of the carboxy group.

In the ^1H NMR spectrum, the deshielded methylene hydrogen proton at δ_H 3.19 showed characteristic multiplicities (*td*, *J*= 12.9 and 3.8 Hz) that established an axial relative stereochemistry for 16α-H. It was confirmed through the *W*-type correlation observed between proton signals at δ_H 2.02 (equatorial 16β-H) and δ_H 3.26 (equato-

TABLE I - ^{13}C-NMR data for the acetylated derivatives **1a**, **2a** and **4a**, and compound **3** (pyridine-d_5)

C	1a*	2a*	3	4a
1	38.2	38.7	38.7	38.8
2	23.5	26.7	27.0	24.0
3	78.5	89.5	89.2	77.9
4	52.1	39.4	44.4	54.9
5	52.0	55.9	56.4	52.6
6	21.3	18.7	19.1	23.1
7	33.2	33.8	33.8	34.2
8	40.5	40.4	40.5	40.6
9	47.8	47.8	47.8	48.1
10	36.7	37.2	36.9	37.0
11	23.9	24.1	24.8	24.2
12	126.8	128.1	127.7	127.7
13	139.5	138.4	139.0	138.4
14	42.1	42.3	42.3	42.1
15	29.2	29.3	29.3	29.2
16	27.0	26.8	26.9	26.6
17	47.9	48.6	48.5	48.6
18	47.4	47.2	47.3	47.1
19	73.3	73.4	73.5	73.3
20	43.2	42.9	43.0	42.9
21	24.9	24.7	24.4	24.7
22	32.4	31.8	32.0	31.7
23	179.2	28.1	23.6	176.5
24	12.8	17.0	63.5	63.9
25	15.8	15.7	15.6	15.5
26	17.1	17.5	17.5	17.1
27	24.3	24.3	24.7	24.1
28	180.7	176.5	177.2	176.4
29	29.8	29.8	30.0	29.6
30	16.1	16.1	16.2	16.0
Ara-1’		104.0	106.7	
Ara-2’		70.5	73.0	
Ara-3’		71.5	74.7	
Ara-4’		69.1	69.6	
Ara-5’		64.1	66.9	
Glc-1”		92.5	96.0	92.4
Glc-2”		71.1	74.3	71.0
Glc-3”		73.6	79.1	73.6
Glc-4”		69.0	71.2	69.0
Glc-5”		73.2	79.5	73.1
Glc-6”		62.3	62.3	62.3

*Values for the acetyl groups are not recorded in the table. Acetyl groups: $\delta_C \cong 20$ (CH_3) and $\delta_C \cong 168$ (C=O).

TABLE II - ^{1}H-NMR data for the acetylated derivatives **1a**, **2a** and **4a**, and compound **3** (pyridine-d_5) (multiplicities; *J*= Hz)

H	1a*	2a*	3	4a
1	1.09/α - 1.53/β	0.91/α - 1.54/β	0.91/α - 1.52/β	1.12/α - 1.62/β
2	1.71/β - 1.92/α	1.84/β - 2.04/α	2.03/β - 2.18 (*td*; 13.8, 4.0)/α	1.12/β - 1.86/α
3	5.76 (*dd*; 12.0, 3.4)/α	3.23/α	3.50 (*dd*; 11.6, 4.8)/α	5.77/α
5	1.99/α	0.82/α	0.95/α	2.18/α
6	1.54/α - 1.65/β	1.37/β - 1.55/α	1.37/β - 1.61/α	1.94 - 2.10
7	1.30 - 1.68	1.34/β - 1.59/α	1.44/β - 1.54/α	1.37/β - 1.70/α
9	1.91/α	1.79/α	1.77/α	1.91/α
11	2.00 - 2.00	2.00 - 2.00	1.93 - 1.98	2.03 - 2.03
12	5.54	5.50	5.49	5.49
15	1.19/α - 2.22 (*td*; 13.5, 3.8)/β	1.24/α - 1.87/β	1.26/α - 2.47 (*td*; 13.6, 4.1)/β	1.15/α - 1.64/β
16	2.02/β - 3.19 (*td*; 12.9, 3.8)/α	1.87/β - 3.16 (*td*; 11.6, 4.3)/α	2.09/β - 3.23 (*td*; 13.1, 4.3)/α	1.82/β - 3.12 (*td*; 11.8, 3.5)/α
18	3.26 (*s*)/β	3.03 (*s*)/β	3.18 (*s*)/β	3.03 (*s*)/β
20	1.99/α	1.92/α	1.94/α	1.90/α
21	1.30/β - 2.68 (*tt*; 13.5, 3.3)/α	1.18/β - 2.57 (*tt*; 13.3, 4.2)/α	1.17/β - 2.59 (*tt*; 13.6, 3.9)/α	1.17/β - 2.55 (*tt*; 13.2, 3.5)/a
22	1.93/α - 2.22 (*td*; 13.5, 3.8)/β	1.80/a - 1.98/β	1.90/α - 2.09/β	1.78/α - 1.97/β
23	-	1.08 (*s*)	1.50 (*s*)	-
24	1.52 (*s*)	0.94 (*s*)	3.59 (*d*; 11.1) - 4.37 (ov.)	4.78 (*d*; 11.2) - 5.50 (ov.)
25	0.94 (*s*)	0.94 (*s*)	0.84 (*s*)	1.16 (*s*)
26	1.05 (*s*)	0.97 (*s*)	1.15 (*s*)	1.00 (*s*)
27	1.68 (*s*)	1.70 (*s*)	1.71 (*s*)	1.63 (*s*)
29	1.42 (*s*)	1.36 (*s*)	1.37 (*s*)	1.37 (*s*)
30	1.10 (*d*; 7.0)	0.98 (*d*; 6.6)	0.97 (*d*; 7.1)	0.96 (*d*; 6.8)
Ara-1’		4.83 (*d*; 7)	4.87 (*d*; 6.7)	
Ara-2’		5.77 (ov.)	4.42 (ov.)	
Ara-3’		5.56 (*dd*; 10.0, 3.3)	4.19 (*dd*; 8.2, 3.4)	
Ara-4’		5.64 (ov.)	4.35 (ov.)	
Ara-5’		3.90 (*d*;13.0) - 4.28 (*dd*; 13.0, 2.3)	3.85 (*d*; 10.9) - 4.36 (ov.)	
Glc-1”		6.35 (*d*; 8.0)	6.34 (*d*; 7.8)	6.36 (*d*; 8.0)
Glc-2”		5.75 (ov.)	4.23 (*t*; 8.5)	5.74 (*dd*; 8.6, 8.4)
Glc-3”		5.98 (*t*; 9.5)	4.31 (*t*; 8.5)	5.97 (*dd*; 9.5, 8.6)
Glc-4”		5.63 (ov.)	4.40 (ov.)	5.62 (*dd*; 9.6, 9.5)
Glc-5”		4.41 (*ddd*; 9.5, 4.2, 2.2)	4.05 (*ddd*; 9.7, 3.8, 3.0)	4.41 (*ddd*; 9.2, 4.1, 2.0)
Glc-6”		4.33 (*dd*; 12.1, 2.2) 4.63 (*dd*; 12.3, 4.2)	4.38 (ov.) 4.43 (ov.)	4.32 (*d*; 12.1) 4.60 (*dd*; 12.6, 4.3)

*Values for the acetyl groups are not recorded in the table.

rial 18β-H) in the HH COSY experiment. Further, evidence for an equatorial 18β-H was provided by heteronuclear long-range correlation *via* three bonds couplings, $^{3}J(^{13}C, ^{1}H)$, detected by the HMBC experiment. This proton displayed cross-signals with carbons C-14 (δ_C 42.1) and C-16 (δ_C 27.0), revealing a dihedral angle close to 180°, providing evidence for the *cis*-fusion between the rings D/E.

A triplet of triplets (^{3}J=13.3 and 3.3 Hz) observed for 21α-H (δ_H 2.68, axial) revealed the β-configuration of the C-30 methyl group (δ_H 16.1), corresponding to an ursane derivative with the 20(*S*)-configuration that could be confirmed by means of the ROESY experiment, indicating spatial correlation between the 18β-H (δ_H 3.26) and hydrogens 29β-CH_3 (δ_H 1.42) and 30β-CH_3 (δ_H 1.10). Compared with ursolic acid (Tkachev *et al.*, 1994), compound **1a** presented two important γ-effects in the ring E. The first one was caused by the axial 30β-CH_3, shielding the carbons C-18 and C-22 by 5 and 4 ppm, respectively, in the ^{13}C NMR. The second effect was observed due to the presence of the axial 19α-hydroxy group, that shielded C-21 by about 6 ppm. Thus, **1a** differs from the structure of rotundioic acid (Nakatni *et al.,* 1989) by a different configuration of C-20 and turns out to be the peracetylated derivative of 20(*S*)-3β,19α-dihydroxyurs-12-en-23,28-dioic acid, named brevicuspic acid.

Fast atom bombardment mass spectroscopy (FAB-MS, positive mode) of **2a** generated a fragment at *m/z* 1083 $[M+Na]^+$ as a pseudo molecular ion, in accordance with seven acetylated OH groups and with the spectrum obtained for the non-peracetylated compound **2**, showing a pseudo molecular ion at *m/z* = 789 $[M+Na]^+$. ^{13}C NMR of **2a** data revealed the occurrence of signals from one carboxy carbon (δ_C 176.5), seven acetate groups (δ_C 169 - 170 and δ_C 20.7 - 21.1), one double bond (δ_C 128.1 and

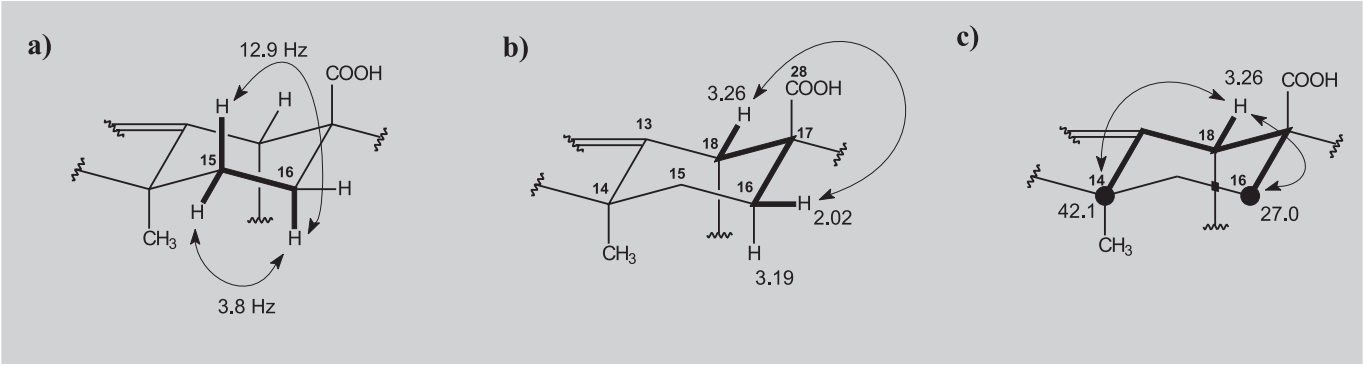


FIGURE 1 - Ring D from **1a**: additional evidence for the relative configuration by the ^{1}H NMR coupling constants (**a**), *W*-type correlation deduced from the HH COSY diagram (**b**) and long-range correlation $^{3}J(^{13}C,^{1}H)$ in the HMBC experiments (**c**).

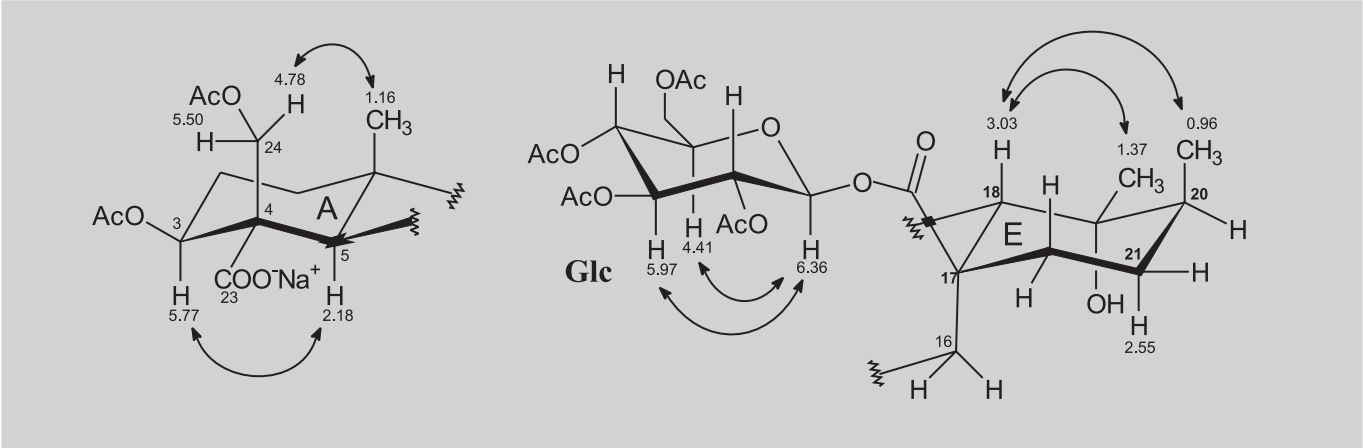


FIGURE 2 - Main ROESY correlation observed for compound **4a** in pyridine-d_5.

138.4), two anomeric carbons (δ_C 104.0 and 92.5), one glycosylated *sec* alcohol (δ_C 89.5) and one *tert* alcohol (δ_C 73.4). ^{1}H NMR confirmed the presence of six angular methyl groups (δ_H 2x 0.94, 0.97, 1.08, 1.36 and 1.70), one methyl group attached to CH (δ_H 0.98, *d*, ^3J= 6.6 Hz), one olefinic proton (δ_H 5.50) and two anomeric sugar protons (δ_H 4.83, *d*, ^3J= 8.0 Hz and δ_H 6.35, *d*, ^3J= 8.0 Hz). Both sugars presented antiperiplanar configuration of 1'-H and 2'-H (^3J= 8.0 Hz), reflecting the α-configuration of the L-arabinopyranose and the β-configuration of the D-glucopyranose. The glycosidic linkages were established using the HMBC techniques. It was possible to observe correlation between the anomeric proton of α-L-arabinose (δ_H 4.83) and C-3 (δ_C 89.5), and also between the anomeric proton of β-D-glucose (δ_H 6.35) and C-28 (δ_C 176.5).

ROESY experiments were used to establish the α-configuration for 1'-H (δ_H 4.83), 3-H (δ_H 3.23) and 23-CH_3 (δ_H 1.08, *s*). Moreover, the β-configuration of 29-CH_3 (δ_H 1.36, *s*) attached to C-19 (d_C 73.4) indicated the presence of a 19α-*tert* alcohol group. In the same way, the α-configuration of 20-H (δ_H 1.92) defined the configuration 30β-CH_3 (δ_H 0.98, *d*, ^3J = 6.6 Hz) at C-20. These data indicated the aglycone to be the 20(*S*)-isomer of pomolic acid (Brieskorn *et al.*, 1967) and compound **2a** was elucidated as the peracetylated derivative obtained from the 3-*O*-α-L-arabinopyranosyl-20(*S*)-pomolic acid-28-*O*-β-D-glucopyranosyl ester, named brevicuspisaponin 3.

Examination of compound **3** allowed its characterization as ILA-1, a saponin already isolated from the leaves of *Ilex argentina*(Schenkel *et al.*,1995). One- and two-dimensional NMR spectroscopic data confirmed **3** as 3-*O*-α-L-arabinopyranosyl-20(*S*)-19α,24-dihydroxyursolic acid-28-*O*-β-D-glucopyranosyl ester.

Compound **4** was characterized as a sodium salt by IR that shows a symmetric stretching band at 1573 cm^{-1}, a diagnostic frequency for a carboxylic acid salt. The presence of the sodium was determined by the flame ionization, employing a polarized filter for the D-sodium line, and compounds **3** as negative control. Acidic hydrolysis on TLC plates (Kartning, Wegschaider, 1972) indicated the presence of glucose. The FABMS of this genuine saponin (positive-ion mode) displayed peaks at *m/z*= 725.3 $[M+Na]^+$and 703.3 $[M+H]^+$. Compound **4a** showed the pseudo-molecular ion by FABMS (positive-ion mode) at *m/z*= 955.4 $[M + H]^+$.

^{13}C NMR spectra of compound **4a** revealed the presence of signals of two carboxy carbons (δ_C 176.4 and 176.5), one double bond (δ_C 127.7 and 138.4), one anomeric sugar carbon (δ_C 92.4), one *sec*-hydroxyl (δ_C 77.9), one *tert*-hydroxyl functions (δ_C 73.3) and two hydroxymethyl groups (δ_C 62.3 and 63.9). The ^{1}H NMR spectrum showed the presence of four angular methyl groups (δ_H 1.00, 1.16, 1.37 and 1.63), one methyl group attached to CH (δ_H 0.96, *d*, $^3J_{HH}$= 6.8 Hz), one olefinic proton (δ_H 5.49) and one anomeric sugar proton (δ_H 6.36, *d*, $^3J_{HH}$= 8.0 Hz). Its NMR data comparison with compound **2a** (table 2) indicated that the sugar β-D-glucose was esterified with the C-28 carboxy function in the aglycone.

The location of the carboxylate group at C-4 was derived from the HMBC correlation signals δ_C 176.5 ↔ δ_H 4.78 (24_A-H) and 5.50 (24_B-H). Moreover, the carbon resonance of this hydroxymethyl group presented long-range correlation with the signals at δ_H 5.77 (3α-H) and 2.18 (5α-H). The occurrence of a triplet of triplets at δ_H 2.55 (J_{HH}= 13.2 and 3.5 Hz) indicated an axial hydrogen 21α-H and thus, the (20*S*)-configuration. It was corroborated through spatial correlation observed between 18β-H (δ_H 3.03) and hydrogens 29β-CH_3 (δ_H 1.37) and 30β-CH_3 (δ_H 0.96; *d*, $^3J_{HH}$= 6.8 Hz) in the ROESY diagram (Figure 2). Furthermore, ROESY correlation between signals at δ_H 4.78 (24_Aβ-H) and δ_H 1.16 (25β-CH_3) also established the configuration on C-4 and located the sodium carboxylate group at C-23 (4α-configuration). This experiment also showed the correlation between 3α-H (δ_H 5.77) and 5α-H (δ_H 2.18). Thus, compound **4a** is a new saponin and was elucidated as the peracetylated derivative from of the 23-sodium salt of (20*S*)- 3β,19α,24-trihydroxyurs-12-en-23,28-dioic acid-28β-*O*-β-D-glucopyranosyl ester, named brevicuspisaponin 4.

Considering these and the previous published results (Taketa *et al*., 2000), the saponin profile of *I. Brevicuspis* leaves is markedly different from those found in *I. paraguariensis* leaves. The latter presents saponins derived from the oleanolic and ursolic acid without oxygenated functions at C-19, C-23 or C-24, as demonstrated to *I. brevicuspis*. These are important features that may be useful to develop methodologies for the quality control of maté products based on the characterization of the free triterpenes or the saponins.

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RESUMO

Novos triterpenos e glicosídeos triterpenóides de *Ilex brevicuspis*

Das folhas de Ilex brevicuspis *foram isolados e identificados um novo triterpeno, ácido 20(*S*)-3β,19α-diidroxiurs-12-en-23,28-dióico, denominado ácido brevicúspico, e dois novos glicosídeos, éster 28-*O*-β-D-glicopiranosil do ácido 3β-*O*-α-L-arabinopiranosil-20(*S*)-pomólico e o sal sódico em C-23 do ester 28β-*O*-β-D-glicopiranosil do ácido (20S)- 3β,19α,24-triidroxiurs-12-en-23,28-dióico. Foi isolado ainda o éster 28-*O*-β-D-glicopiranosil do ácido 3β-*O*-α-L-arabinopiranosil-20(*S*)-19α,24-diidroxiursólico, já descrito anteriormente para a espécie* Ilex argentina. *As estruturas foram estabelecidas por métodos espectroscópicos e químicos.*

UNITERMOS: Aquifoliaceae. Ilex brevicuspis. *Triterpenos. Saponinas. Ácido brevicúspico. Brevicuspisaponinas 3 e 4.*

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