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# Triterpenoid glycosides and a triterpene from *Ilex brevicuspis*

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**Abstract**

Two saponins and a sapogenin were isolated from the leaves of *Ilex brevicuspis*. Their structures were established by means of spectroscopic methods as brevicuspisaponin 1 (3-*O*-α-L-arabinopyranosyl-20(*S*)-19α,24-dihydroxyursolic acid), brevicuspisaponin 2 (3-*O*-α-L-arabinopyranosyl-20(*S*)-19α,23,24-trihydroxyursolic acid) and 23-methylester of 20(*S*)-3β,19α,24-trihydroxyurs-12-en-23,28-dioic acid. 

*Keywords:* *Ilex brevicuspis*; Aquifoliaceae; Saponin; Sapogenin; Brevicuspisaponin 1 and 2, 20(*S*)-rotungenic acid, 20(*S*)-rotundioic acid; Ursolic acid

## 1. Introduction

*Ilex brevicuspis* Reissek is a native tree in South Brazil and more particularly in the State of Rio Grande do Sul, Brazil, where it is popularly known as "caúna-da serra", "congonha" or "orelha-de-mico". *I. brevicuspis* is one of the species reported as an adulterant of *Ilex paraguariensis* St. Hil., the genuine maté product whose leaves are traditionally used to prepare the stimulating beverage named maté (Giberti, 1989). Continuing our systematic phytochemical study on *I. paraguariensis* and other South American *Ilex* species (Taketa & Schenkel, 1994; Gosmann, Guillaume, Taketa & Schenkel, 1995; Schenkel, Athayde, Giberti & Guillaume, 1995; Pires, Guillaume, Gosmann & Schenkel, 1997), we report here the isolation and structural elucidation of two new saponins and a new sapogenin isolated from the leaves of the title plant.

## 2. Results and discussion

Solvent partition and chromatographic procedures allowed the isolation of the main triterpenoids from the leaves of *I. brevicuspis*: brevicuspisaponin 1 (**1**), brevicuspisaponin 2 (**2**) and one sapogenin (**3**). Ursolic acid was also isolated and identified through comparison of its spectral data with that of the literature (Tkachev, Denisov, Gatilov, Bagryanskaya, Shevtsov & Rybalova, 1994).

Acid hydrolysis of **1** and **2** afforded, in both cases, only one sugar, identified as arabinose by co-TLC. The sugar configuration in each case was determined as α-L by means of GLC analysis (Mihashi, Hara & Okabe, 1986).

FAB MS (positive ion mode) of compound **1** displayed a quasi-molecular ion peak at $m/z$ 621 $[M + H]^+$ suggesting a molecular formula of $C_{35}H_{56}O_9$. In addition, the fragment at $m/z$ 487 indicated the loss of an aldopentose moiety. The $^{13}$C-NMR spectrum of **1** showed 35 signals, whereas the DEPT spectrum revealed 6 methyl, 11 methylene, 10 methine and 8 quaternary carbon atoms (Table 1).

Careful comparison of $^{13}$C-NMR spectral data of **1**

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with that of ILA-1, isolated during a previous investigation (Schenkel et al., 1995), showed that **1** differs structurally from ILA-1 only by the absence of glucose signals. This assumption was confirmed by $^{1}H$–$^{1}H$ COSY, $^{1}H$–$^{13}C$ COSY, COLOC and ROESY experiments.

The most singularly important feature of this structure is the C-20 configuration, usually 20*R*, but in this case 20*S*. The axial configuration of $CH_3$-30 ($\delta_H$ 1.11, *d*, $J = 6$ Hz) was deduced from its connectivities with Hβ-18 ($\delta_H$ 3.26) and $CH_3$β-29 ($\delta_H$ 1.42) in the ROESY experiment. Therefore, compound **1** was identified as 3-*O*-α-L-arabinopyranosyl-20(*S*)-19α,24-dihydroxyursolic acid, and named brevicuspisaponin 1. To the best of our knowledge, this is the first report of the natural occurrence of this compound.

For compound **2**, the molecular formula $C_{35}H_{56}O_{10}$ was deduced based on the FAB MS, which displayed a quasi-molecular ion peak $[M + H]^+$ at *m/z* 637; the presence of 5 methyl, 12 methylene, 10 methine and 8 quaternary carbon atoms were also detected in the $^{13}C$-NMR spectrum (Table 1). The sugar position was located at C-3 through ROESY correlation observed between the anomeric proton at $\delta_H$ 5.05 (*d*, $J = 8$ Hz; $\delta_C$ 104.4) and the aglycone signal at $\delta_H$ 4.47 (Hα-3).

Careful comparisons of the $^{13}C$-NMR spectrum signals for **2** and **1** revealed the presence in the spectrum of **2** of one additional hydroxymethyl

Table 1
$^{1}H$- (500 MHz) and $^{13}C$-NMR (125 MHz) spectral data for compounds **1**, **2** (pyridine-$d_5$) and **3** ($CDCl_3$ : $CD_3OD$, 4 : 1)

| C | DEPT | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|---|
| | | $\delta_H$ (mult, $J$ = Hz) | $\delta_C$ | $\delta_H$ (mult, $J$ = Hz) | $\delta_C$ | $\delta_H$ (mult, $J$ = Hz) | $\delta_C$ |
| 1 | $CH_2$ | 0.89; 1.53 | 37.7 | 1.03; 1.59 | 37.0 | 0.88; 1.45 | 38.1 |
| 2 | $CH_2$ | 2.00; 2.20 | 25.9 | 2.14; 2.31 | 24.9 | 1.52; 1.52 | 26.7 |
| 3 | CH | 3.51 (*dd*, 11.0, 5.0) | 88.2 | 4.47 (*dd*, 13.0, 6.0) | 80.5 | 3.89 (*dd*, 11.0, 5.0) | 76.6 |
| 4 | C | – | 43.4 | – | 46.0 | – | 57.1 |
| 5 | CH | 0.95 | 55.4 | 1.88 | 46.3 | 1.19 | 51.7 |
| 6 | $CH_2$ | 1.35; 1.61 | 18.1 | 1.51; 1.90 (*t*, 13.0, Hβ) | 17.2 | 1.18; 0.91 | 21.1 |
| 7 | $CH_2$ | 1.35; 1.50 | 32.9 | 1.37; 1.73 (*dt*, 10.0, 4.0, Hα) | 31.9 | 0.99; 1.17 | 32.6 |
| 8 | C | – | 39.3 | – | 38.6 | – | 39.7 |
| 9 | CH | 1.78 | 46.7 | 1.88 | 45.8 | 1.42 | 47.4 |
| 10 | C | – | 35.9 | – | 34.9 | – | 36.3 |
| 11 | $CH_2$ | 1.78; 2.00 | 23.3 | 1.97; 2.05 | 22.6 | 1.65; 1.72 | 23.5 |
| 12 | =CH | 5.50 (*s*) | 126.3 | 5.52 (*s*) | 125.6 | 5.03 (*t*, 3.3) | 127.4 |
| 13 | =C | – | 138.6 | – | 137.9 | – | 137.9 |
| 14 | C | – | 41.3 | – | 40.5 | – | 41.0 |
| 15 | $CH_2$ | 1.25; 2.26 | 28.4 | 1.22; 2.24 | 27.6 | 0.75; 1.47 | 28.0 |
| 16 | $CH_2$ | 2.06; 3.25 (*dt*, 13.0, 4.0, Hα) | 26.2 | 2.05; 3.17 (*dt*, 13.0, 4.0, Hα) | 25.4 | 1.30; 2.31 (*dt*, 13.0, 4.0, Hα) | 25.8 |
| 17 | C | – | 47.1 | – | 46.4 | – | 47.0 |
| 18 | CH | 3.26 (*s*) | 46.6 | 3.26 (*s*) | 46.2 | 2.50 (*s*) | 46.3 |
| 19 | C | – | 72.5 | – | 71.7 | – | 73.5 |
| 20 | CH | 2.00 | 42.2 | 2.00 | 41.5 | 1.48 | 41.3 |
| 21 | $CH_2$ | 1.30; 2.70 (*tt*, 13.0, 4.0, Hα) | 24.0 | 1.27; 2.69 (*tt*, 10.0, 2.0, Hα) | 23.3 | 0.92; 1.98 (*tt*, 13.0, 4.0, Hα) | 23.7 |
| 22 | $CH_2$ | 1.95; 2.20 | 31.6 | 1.94; 2.24 | 30.8 | 1.28; 1.55 | 31.2 |
| 23 | $CH_3$ | 1.48 (*s*) | 22.6 | – | – | – | – |
| | O–$CH_2$ | – | – | 4.33 (*d*, 13.0); 4.90 (*d*, 13.0) | 59.4 | – | – |
| | O=C | – | – | – | – | – | 176.3 |
| 24 | O–$CH_2$ | 3.58 (*d*, 10.0); 4.35 | 62.5 | 3.89 (*d*, 13.0); 4.59 (*d*, 13.0) | 61.2 | 3.78 (*d*, 12.0); 4.01 (*d*, 12.0) | 60.8 |
| 25 | $CH_3$ | 0.81 | 14.5 | 0.91 | 13.9 | 0.68 | 15.7 |
| 26 | $CH_3$ | 1.05 | 16.3 | 1.08 | 14.5 | 0.48 | 16.0 |
| 27 | $CH_3$ | 1.73 | 23.5 | 1.68 | 22.7 | 1.00 | 23.8 |
| 28 | O=C–O | – | 179.9 | – | 179.1 | – | 181.0 |
| 29 | $CH_3$ | 1.42 (*s*) | 29.0 | 1.40 (*s*) | 28.2 | 0.90 (*s*) | 29.2 |
| 30 | $CH_3$ | 1.11 (*d*, 6.0) | 15.3 | 1.11 (*d*, 7.0) | 15.6 | 0.73 (*d*, 7.0) | 15.4 |
| 31 | O–$CH_3$ | – | – | – | – | 3.53 | 51.8 |
| 1′ | – | 4.73 (*d*, 7.0) | 105.6 | 5.05 (*d*, 8.0) | 104.4 | – | – |
| 2′ | – | 4.40 (*dd*, 8.0, 7.0) | 72.0 | 4.43 (*t*, 8.0) | 71.3 | – | – |
| 3′ | – | 4.20 (*dd*, 8.0, 3.0) | 73.6 | 4.07 (*dd*, 8.0, 4.0) | 73.0 | – | – |
| 4′ | – | 4.35 | 68.5 | 4.24 | 67.8 | – | – |
| 5′ | – | 3.85 (*d*, 10.0); 4.35 | 65.6 | 3.69 (*d*, 13.0); 4.31 | 65.1 | – | – |

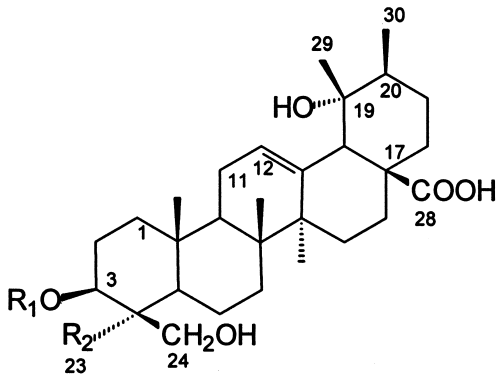
group ($\delta_C$ 59.4) that was located at C-23, in place of the methyl group at $\delta_C$ 22.6 (C-23) observed in **1**. The C-4 pattern could be established through the long range $^{13}C$–$^{1}H$ COSY spectrum, which displayed cross peaks between the signals at $\delta_C$ 80.5 (C-3), $\delta_H$ 4.33 (H-23) and at $\delta_H$ 5.05 (H-1 ara). One- and two-dimensional NMR spectroscopic data ($^{1}H$–$^{1}H$ COSY, $^{1}H$–$^{13}C$ COSY, COLOC, ROESY) confirmed **2** as 3-*O*-α-L-arabinopyranosyl-20(*S*)-19α,23,24-trihydroxyursolic acid, and named brevicuspisaponin 2. This is the first report of its natural occurrence.

The FAB MS of compound **3** indicated the molecular formula $C_{31}H_{48}O_7$ through the positive molecular ion at *m/z* 533 $[M + H]^+$. Accordingly, the $^{13}C$-NMR spectrum displayed 31 signals (Table 1) including, as determined from the DEPT spectrum, 6 methyl, 10 methylene, 6 methine and 9 quaternary carbon atoms. In the $^{1}H$-NMR spectrum, the most salient features were the presence of a singlet at $\delta_H$ 2.50 (*s*, Hβ-18) suggesting a 19-hydroxy-ursene type skeleton and the presence of a singlet signal integrating for three protons at $\delta_H$ 3.53, and correlating in the $^{13}C$–$^{1}H$ COSY spectrum with a carbon atom at $\delta_C$ 51.8, characteristic of a methoxy group. Protons from this latter group correlated, in the long range $^{13}C$–$^{1}H$ COSY spectrum, with the carbon at $\delta_c$ 176.3, demonstrating the presence of a methyl ester group. In the same experiment, this carbonyl also correlated with protons at $\delta_H$ 3.78 and $\delta_H$ 4.01 ($\delta_C$ 60.8, $CH_2O$-24) demonstrating that the hydroxymethyl and methyl ester groups were both substituents of the same carbon. Finally, the hydroxymethyl proton at $\delta_H$ 3.78 also displayed a long range coupling with $\delta_C$ 76.6 (C-3).

The configuration of C-4 was deduced from the ROESY spectrum through the correlation between the β-$CH_3$ signal at C-25 (*s*, $\delta_H$ 0.68) and hydroxymethyl proton signals at $\delta_H$ 3.78 and at $\delta_H$ 4.01, confirming the β-position of this latter group at C-4.

Another important feature of the ROESY spectrum was the correlation between Hβ-18 ($\delta_H$ 2.50, *s*), $CH_3$-29 ($\delta_H$ 0.90, *s*) and $CH_3$-30 ($\delta_H$ 0.73, *d*, $J =$ 7.2 Hz) indicating their β-orientation. This demonstrated the axial position of methyl group at C-30, and hence, the 20*S*-configuration. Chemical shifts of C-18 ($\delta_C$ 46.3) and C-22 ($\delta_C$ 31.2) are in agreement with the proposed configuration and can be explained in terms of a γ-effect induced by the 30β-axial methyl group of **3**. Therefore, compound **3** was identified as 23-methylester of 20(*S*)-3β,19α,24-trihydroxyurs-12-en-23,28-dioic acid.

Very few 19α-hydroxyursolic acid derivatives with the singular 20*S*-configuration have been isolated, most of them from *Ilex species* (Hidaka et al., 1987; Kakuno, Yoshikawa & Arihara, 1992), including South-American species (Schenkel et al., 1995).




| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | α-L-ara | $CH_3$ |
| **2** | α-L-ara | $CH_2OH$ |
| **3** | H | $COOCH_3$ |

## 3. Experimental

### 3.1. General

Melting points were obtained with a Kofler melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 881 spectrophotometer. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. EI MS spectra were recorded on a MS50 spectrometer and FAB MS spectra on a VG ZAB HS spectrometer. $^{1}H$- and $^{13}C$-NMR spectra were obtained on Bruker AM 400 and AMX 500 spectrometers. TLC was carried out on silica gel (Merck) $GF_{254}$ using $CHCl_3$–MeOH (96 : 4) and $CHCl_3$–EtOH–$H_2O$ (8 : 4: 0.5) as eluants for sapogenins and saponins, respectively, and EtOAc–MeOH–HOAc–$H_2O$ (13 : 3 : 4 : 3) for sugars. Compounds were visualized using the vanillin–sulfuric acid reagent/100°/10 min.

### 3.2. Plant material

Leaves of *I. brevicuspis* Reissek were collected in Osório, State of Rio Grande do Sul, Brazil on April 1995. 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).

### 3.3. Extraction and isolation

Air-dried leaves (589 g) were crushed and extracted with EtOH at room temperature (2 × 7 days). The ethanolic extract was evaporated to dryness under

reduced pressure and the residue (69 g) was suspended in water and extracted with chloroform. The chloroform and aqueous phases were evaporated to dryness to give the chloroform fraction (14 g) and the crude saponins fraction (19 g), respectively. Portions of these residues were repeatedly chromatographed on silica gel to give the pure compounds **1** (51 mg) and **2** (35 mg) from the saponin fraction, and **3** (58 mg) and **4** (13 mg) from the chloroform extract.

### 3.4. Acid hydrolysis

Compounds **1** and **2** were hydrolyzed on TLC plates in order to identify their sugar and aglycone moieties, as described by Kartnig and Wegschaider (1972). Compounds **1** and **2** were also hydrolyzed in 5% aq. $H_2SO_4$ at 100°C for 1 h in preparation for GLC analysis after derivatization.

### 3.5. Sugar derivatization

The sugar obtained through acid hydrolysis of **1** and **2** was derivatized as described by Mihashi et al. (1986) using L-cysteine ethyl ester hydrochloride in the thiazolidine reaction.

### 3.6. Gas–liquid chromatography analysis of sugar derivatives from *1* and *2*

Analysis employed a WCOT SE-30 column (30 m, 0.25 mm i.d.), column temperature at 220°C and injection temperature at 252°C with $H_2$ as carrier gas at 2.2 ml $min^{-1}$. The trimethylsilyl ether of the arabinose thiazolidine derivative obtained from **1** and **2** gave a retention time of 3.50 min (same value as L-arabinose pattern; retention time of D-arabinose: 4.08 min).

### 3.7. Brevicuspisaponin 1 [3-O-α-L-arabinopyranosyl-20(S)-19α,24-dihydroxyursolic acid] (*1*)

White powder, mp 237–238°C; $[\alpha]_{589}^{18}$ + 36° and $[\alpha]_{546}^{18}$ + 40° (MeOH, *c* 1.346); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3550–3050, 2966–2875, 1685, 1654, 1455, 1378, 1235, 1134, 865; FAB MS (positive-ion mode) *m/z*: 643.4 $[M + Na]^+$, 621.4 $[M + H]^+$, 487.4 $[M\text{-}C_5H_9O_4]^+$; $^1$H-NMR and $^{13}$C-NMR spectral data: Table 1.

### 3.8. Brevicuspisaponin 2 [3-O-α-L-arabinopyranosyl-20(S)-19α,23,24-trihydroxyursolic acid] (*2*)

White powder, mp 203–204°C; $[\alpha]_{589}^{16}$ + 29°, $[\alpha]_{578}^{16}$ + 29°, $[\alpha]_{546}^{16}$ + 34°, $[\alpha]_{436}^{16}$ + 57° and $[\alpha]_{365}^{16}$ + 72° (MeOH, *c* 0.64); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3550–3100, 2924–2854, 1718, 1684, 1454, 1377, 1260–1215, 1140; FAB MS (positive ion mode) *m/z*: 659.3 $[M + Na]^+$ and 637.3 $[M + H]^+$; $^1$H- and $^{13}$C-NMR spectral data: Table 1.

### 3.9. Compound *3* (23-methylester of 20(S)-3β,19α,24-trihydroxyurs-12-en-23,28-dioic acid)

Crystalline powder, mp 194–197°C; $[\alpha]_{589}^{20}$ + 68°, $[\alpha]_{578}^{20}$ + 71°, $[\alpha]_{546}^{20}$ + 82°, $[\alpha]_{436}^{20}$ + 145°. $[\alpha]_{365}^{20}$ + 227° ($CHCl_3$, *c* 0.1); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3434, 2936, 1710, 1456, 1382, 1236, 1066, 759; FAB MS (positive mode, matrix mNBA) *m/z* (rel. int.): 533.2 (40.3), 486.3 (9.3), 460.1 [3(mNBA + H) − H] (40.7), 391.2 (40), 307.0 [2mNBA − H] (100), 242.2 (5), 176.0 (13.5); EIMS *m/z*: 532, 514, 486, 470, 454, 437, 414, 269, 246, 232, 219, 201, 146. $^1$H-NMR and $^{13}$C-NMR spectral data: Table 1.

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