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SAPONINS FROM *ILEX DUMOSA*

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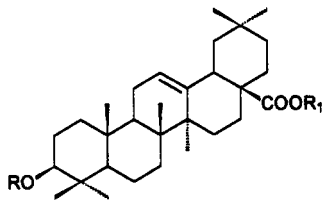
ABSTRACT.—Four new triterpene saponins, 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid [**1**], 3-*O*-α-L-arabinopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid [**2**], and their 28-*O*-β-D-glucopyranosides [**3** and **4**], together with the known ursolic acid and 3-*O*-β-D-galactopyranosyl-oleanolic acid, were isolated from the leaves of *Ilex dumosa*. Their structures were established on the basis of chemical and spectral data.

Ilex dumosa Reissek (Aquifoliaceae) is used to adulterate maté (*Ilex paraguariensis* St. Hil.), which is widely used as a traditional beverage in south Brazil, Argentina, Paraguay, and Uruguay. In the course of our systematic studies with maté and other *Ilex* species (1–5), it was observed that the saponins of *I. dumosa* have hemolytic activity (1). Recent press reports have suggested the association of gastrointestinal problems with the ingestion of maté adulterated with *I. dumosa*. This suspicion prompted us to undertake a chemical investigation of the latter plant.

The EtOH extract, subjected to different partition and chromatographic procedures, gave ursolic acid, 3-*O*-β-D-galactopyranosyl-oleanolic acid (saponin E1), and four new saponins (code-numbered E6, E3, E7, and E8). Saponin E6 [**1**], on acid hydrolysis, yielded oleanolic acid, glucose and galactose. The eims of the peracetylated saponin showed peaks for a terminal hexose (*m/z* 331) and a fragment [hexose-hexose] (*m/z* 619). The eims also exhibited retro-Diels-Alder fragments characteristic of the Δ^{12}-oleanane or ursane skeletons (6). The ^{1}H-nmr spectrum of compound **1** exhibited signals for two anomeric protons at δ 4.85 and 5.25 assigned to galactose and glucose, respectively, and indicated a β-linkage ($^4C_1$ conformation) for both sugars. The anomeric configuration was further confirmed by the application of Klyne's rule of molecular rotation (7). The ^{13}C-nmr spectrum of **1** confirmed that the sugar chain was linked to C-3 of the oleanolic acid (6,8,9) and also confirmed the nature of the sugar moieties (Table 1). The interglycosidic linkage in saponin E6 was established by comparison of its ^{13}C-nmr data with those of the corresponding methylated sugars, applying known chemical-shift rules and glycosylation shifts (10), with the help of ^{1}H-^{1}H COSY and HETCOR nmr experiments and with literature data (11). The resonance at 82.0 ppm, corresponding to C-2 of the galactose unit, indicated that the interglycosidic linkage is 1→2. Thus, saponin E6 [**1**] was characterized as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid.



	R	R_1
1	glc-(1→2)-gal	H
2	ara-(1→2)-gal	H
3	ara-(1→2)-gal	glc
4	glc-(1→2)-gal	glc

glc: β-D-glucopyranosyl
gal: β-D-galactopyranosyl
ara: α-L-arabinopyranosyl

Saponin E3 [**2**] yielded oleanolic acid, galactose, and arabinose on acid hydrolysis. The eims of the peracetylated compound exhibited peaks for a terminal pentose at *m/z* 259 and a fragment [hexose-pentose] at *m/z* 547. ^{1}H-Nmr signals for two anomeric protons at δ 4.91 and δ 5.12, corresponding to β-galactose and α-arabinose, respectively, were observed. The

TABLE 1. ^{13}C-Nmr Spectral Data of the Sugar Moieties of Compounds **1–4**.

	Compounds			
	1	**2**	**3**	**4**
O-Galactose				
1	105.3	105.4	105.5	105.3
2	82.0	82.1	82.3	81.8
3	75.3	75.1	75.3	75.3
4	69.8	69.5	69.8	69.8
5	76.4	76.3	76.5	76.3
6	62.1[a]	61.9	62.2[a]	62.0[a]
Glucose (terminal)				
1	106.1			106.0
2	76.9			76.8
3	78.0			78.0
4	71.6			71.6
5	78.0			77.8
6	62.5[a]			62.6
Arabinose (terminal)				
1		106.5	106.6	
2		73.3	73.5	
3		74.2	74.4	
4		68.8	69.1	
5		66.6	66.9	
28-*O*-Glucose				
1			95.7	95.7
2			74.2	74.0
3			78.9	78.7
4			71.1	71.0
5			79.4	79.1
6			62.2[a]	62.1[a]

[a]Assignments in columns may be reversed.

^{13}C-nmr spectrum of **2** suggested that oleanolic acid was the aglycone and provided information on the nature of the sugar moieties. The glycosylation of oleanolic acid at C-3, and the interglycosidic linkage were determined by comparison of measured ^{13}C-nmr chemical shifts with those of saponin E6 [**1**] (Table 1) and with literature data. Thus, the structure of saponin E3 [**2**] was established as 3-*O*-α-L-arabinopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid.

Saponin E7 [**3**] yielded oleanolic acid, galactose, glucose, and arabinose on acid hydrolysis. Basic hydrolysis of **3** gave saponin E3 [**2**]. The mol wt of compound **3** was determined by fabms. The positive-ion fabms showed characteristic ions at *m/z* 919 and 758, assigned to $[M+Li]^+$ and $[(M+Li)-hexose-H]^+$. The negative-ion fabms showed ions at *m/z* 749, 617, and 455, ascribed to $[(M-H)-hexose]^-$, $[(M-H-hexose)-pentose]^-$, and $[(M-H-hexose-pentose)-hexose]^-$. The ^{1}H-nmr spectrum of **3** showed signals at δ 4.87, δ 5.10, and 6.33, attributed to 3-*O*-β-galactose, terminal α-arabinose, and 28-*O*-β-D-glucose, respectively. The ^{13}C-nmr spectrum of **3** confirmed the glycosylation of positions 3 and 28 of oleanolic acid. The ^{13}C-nmr signals indicated that the β-D-glucose unit was linked to the 28-carboxyl group of the aglycone through an ester bond and confirmed that the arabinose-(1→2)-galactose saccharide unit was linked to position 3 of oleanolic acid (Table 1). Thus, saponin E7 [**3**] was identified as 28-*O*-β-D-glucopyranoside of 3-*O*-α-L-arabinopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid.

Saponin E8 [**4**] yielded oleanolic acid,

galactose, and glucose on acid hydrolysis. Basic hydrolysis of **4** afforded saponin E6 [**1**]. The fabms spectrum indicated a mol wt of 942. The positive-ion fabms showed peaks at *m/z* 949 and 787, assigned to $[M+Li]^+$ and $[(M+Li)-hexose]^+$. The negative fab spectrum showed characteristic ions at *m/z* 779, 617, and 455, ascribed to $[(M-H)-hexose]^-$, $[(M-H-hexose)-hexose]^-$ and $[(M-H-hexose-hexose)-hexose]^-$. The ^{1}H-nmr spectrum showed signals at δ 4.90 and δ 6.35, attributed to H-1 of 3-*O*-galactose and H-1 of 28-*O*-glucose, respectively. The signal corresponding to the anomeric proton of the terminal glucose was obscured. The ^{13}C-nmr spectrum confirmed the glycosylation at positions 3 and 28 of oleanolic acid, with the signal at 95.7 ppm, corresponding to the anomeric carbon of one β-D-glucose unit, indicating the linkage of this sugar at position 28 of oleanolic acid through an ester bond. The other ^{13}C-nmr sugar signals confirmed a β-D-galactose unit at position 3, and a terminal β-D-glucose unit, and the interglycoside linkage as 1→2 (Table 1). Thus, saponin E8 [**4**] was characterized as the 28-*O*-β-D-glucopyranoside of 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer 298 instrument and optical rotations on a Perkin-Elmer 241 polarimeter (in pyridine). Fabms were taken on a MS 80 RFA instrument; eims on Finnigan MAT 1020 B and a Finnigan MAT 44S (70 eV); ^{1}H-, ^{13}C-, and 2D-nmr on a Varian XL 300 instrument (^{1}H nmr at 300 MHz, ^{13}C nmr at 75 MHz).

PLANT MATERIAL.—Aerial parts of *I. dumosa* were collected in Campo Bom, RS, Brazil, and identified by Rodney Schmidt. A herbarium specimen is on deposit in the Botany Department, Herbarium of Rio Grande do Sul, Federal University (ICN), Porto Alegre, Brazil.

EXTRACTION AND ISOLATION.—Fresh leaves of *I. dumosa* (255 g) were extracted with EtOH at room temperature. The solvent was removed under reduced pressure and the residue suspended in H_2O. This suspension was partitioned with CH_2Cl_2 and the precipitated monodesmosidic saponins (3.6 g) were separated by filtration. The suspension was extracted with *n*-BuOH; the organic layer gave a residue of 7.2 g. An aliquot (2.9 g) of the separated monodesmosidic saponins was chromatographed on a Si gel column (eluent, CH_2Cl_2-EtOH-H_2O, 160:40:5; 140:40:5, 120:40:5; 100:40:5; 80:40:5) to give ursolic acid (585 mg), E1 (3 mg), E3 (245 mg), and a mixture of E3 and E6 (255 mg) which was further chromatographed over a Si gel column eluting with CH_2Cl_2-EtOH-H_2O (80:40:5) to give E6 (47 mg). The *n*-BuOH fraction (1.7 g) was chromatographed on a Si gel column after purification by Amberlite XAD-2. Gradient elution was performed as described above for the isolation of the monodesmosidic saponins. The fractions containing the saponins E7 and E8 were purified on a Si gel column eluting with CH_2Cl_2-EtOH (80:40) to give 50 mg of E7 and 40 mg of E8.

Each saponin was refluxed with ethanolic HCl 10% for 3 h to furnish the aglycone, identified by authentic sample comparison (tlc). The sugar units were identified after tlc acid hydrolysis, which was carried out with Si gel GF 254 plates (20×20 cm). $CHCl_3$-EtOH-H_2O (80:40:5) was used as a solvent in the first development. After the saponin was hydrolyzed in HCl vapor for 1 h, the chromatogram was developed with EtOAc-MeOH-HOAc-H_2O (60:15:15:10) in the second direction, and then compared with a reference sugar mixture. The saponins and sugars were analyzed after tlc basic hydrolysis, according to the method described above, using NH_3 vapor to hydrolyze only the ester bond.

Saponin E6; 3-O-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid [**1**].—White amorphous powder, mp 262–298° (uncorrected); $[\alpha]^{20}D$ +23.84° (*c*=1.3, pyridine); ir (dry film) ν max 3410 (OH sugars), 1690 (CO_2H), 1630 (unsaturated) 1160–980 cm^{-1} (CO sugars); ^{1}H nmr (C_5D_5N, 300 MHz) δ 0.71 (1H, H-5), 0.79 (3H, s, Me-25), 0.93 (3H, s, Me-29), 0.95 (3H, s, Me-26), 0.97 (3H, s, Me-30), 1.06 (3H, s, Me-24), 1.27 (3H, s, Me-23), 1.27 (3H, s, Me-27), 1.58 (1H, t, H-9), 3.26 (1H, dd, *J*=4 and 11 Hz, H-3), 5.45 (1H, t, H-12), 4.85 (1H, d, *J*=7.5 Hz, gal H-1), 5.25 (1H, d, *J*=7.5 Hz, glc H-1); ^{13}C nmr (aglycone moiety) (C_5D_5N, 75 MHz) δ 180.0 (C-28), 144.8 (C-13), 122.5 (C-12), 88.8 (C-3), 55.9 (C-5), 48.0 (C-9), 46.7 (C-17), 46.4 (C-19), 42.2 (C-14), 42.0 (C-18), 39.8 (C-8), 39.6 (C-4), 38.8 (C-1), 37.0 (C-10), 34.3 (C-21), 33.4 (C-29), 33.3 (C-7), 33.3 (C-22), 31.0 (C-20), 28.4 (C-15), 28.3 (C-23), 26.8 (C-3), 26.2 (C-27), 23.9 (C-11), 23.9 (C-30), 23.8 (C-16), 18.6 (C-6), 17.5 (C-26), 16.9 (C-24), 15.6 (C-25); ^{13}C-nmr data of the sugar moieties, see Table 1; eims (70 eV, peracetylated saponin) *m/z* $[M]^+$ 619 (4), 331 (42), 248 (38), 203 (54), 169 (100), 133 (14).

Saponin E3; 3-O-α-L-arabinopyranosyl-(1→2)-

β-D-galactopyranosyl-oleanolic acid [**2**].—White amorphous powder, mp 249–252° (uncorrected), $[\alpha]^{20}D$ +34.66° (*c*=0.075, pyridine); ^1H nmr (C_5D_5N, 200 MHz) δ 5.50 (1H, s, H-12), 3.30 (1H, t, *J*=6.5 Hz, H-3), 0.82, 0.95, 0.96, 1.00, 1.10, 1.32 (21H, s, 7×Me), 4.91 (1H, d, *J*=7.5 Hz, gal H-1), 5.12 (1H, d, *J*=6.44 Hz, ara H-1), 3.70 (1H, s, ara H-5); ^{13}C nmr (aglycone moiety) (C_5D_5N, 75 MHz) δ 180.3 (C-28), 144.9 (C-13), 122.5 (C-12), 88.6 (C-3), 55.7 (C-5), 47.7 (C-9), 46.4 (C-17), 46.2 (C-19), 41.9 (C-14), 41.7 (C-18), 39.4 (C-8), 39.3 (C-4), 38.5 (C-1), 36.7 (C-10), 33.9 (C-21), 32.9 (C-29), 32.9 (C-22), 32.9 (C-7), 30.6 (C-20), 28.0 (C-15), 27.6 (C-23), 26.4 (C-27), 25.9 (C-2), 23.4 (C-30), 23.4 (C-11), 23.4 (C-16), 18.1 (C-6), 17.0 (C-26), 16.1 (C-24), 15.1 (C-25); ^{13}C-nmr data of the sugar moieties, see Table 1; eims (70 eV, peracetylated saponin) *m/z* $[M]^+$ 547 (9), 331 (11), 259 (90), 203 (54), 139 (84).

Saponin E7; 28-O-β-D-glucopyranoside of 3-O-α-L-arabinopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid [**3**].—White amorphous powder, $[\alpha]^{20}D$ +51.64° (*c*=0.25, pyridine); ^1H nmr (C_5D_5N, 300 MHz) δ 3.29 (1H, dd, *J*=4 and 12 Hz, H-3), 5.41 (1H, t, H-12), 0.84, 0.86, 0.88, 1.70, 1.80, 1.26, 1.30 (21H, s, 7×Me), 1.48 (1H, t, H-9), 0.62 (1H, s, H-5), 4.87 (1H, d, *J*=7.5 Hz, gal H-1), 5.10 (1H, d, *J*=6.5 Hz, ara H-1), 6.33 (1H, d, *J*=8 Hz, glc H-1); ^{13}C nmr (aglycone moiety) (C_5D_5N, 75 MHz) δ 176.3 (C-28), 144.1 (C-13), 122.8 (C-12), 88.8 (C-3), 56.0 (C-5), 48.1 (C-9), 47.1 (C-17), 46.3 (C-19), 42.2 (C-14), 41.8 (C-18), 40.0 (C-8), 39.6 (C-4), 38.9 (C-1), 37.0 (C-10), 34.1 (C-7), 33.2 (C-29), 32.6 (C-21), 32.6 (C-22), 30.9 (C-20), 28.1 (C-15), 28.1 (C-23), 26.9 (C-2), 26.2 (C-27), 23.8 (C-30), 23.5 (C-11), 23.5 (C-16), 18.6 (C-6), 17.6 (C-26), 16.6 (C-24), 15.7 (C-25); ^{13}C-nmr data of the sugar moieties, see Table 1; fabms (positive-ion) *m/z* $[M+Li]^+$ 919 (100), 758 (33), 711 (15), 607 (8), 509 (7), 440 (28); fabms (glycerol) (negative-ion) *m/z* 749 (100), 703 (12), 617 (29), 455 (25), 435 (15).

Saponin E8; 28-O-β-D-glucopyranoside of 3-O-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid) [**4**].—White amorphous powder; $[\alpha]^{20}D$ +16° (*c*=0.4, pyridine); ^1H nmr (C_5D_5N, pyridine) δ 5.45 (1H, s, H-12), 3.25 (1H, t, *J*=11 Hz, H-3), 1.60 (1H, t, H-9), 0.85, 0.90, 0.92, 1.11, 1.27, 1.30 (21H, s, 7×Me), 0.74 (1H, s, H-5), 4.90 (1H, d, *J*=7.63 Hz, gal H-1), 6.35 (1H, d, *J*=7.63 Hz, H-1 28-*O*-glc); ^{13}C nmr (aglycone moiety) (C_5D_5N, 75 MHz) δ 176.6 (C-28), 144.2 (C-13), 123.8 (C-12), 88.8 (C-3), 55.7 (C-5), 47.8 (C-9), 46.8 (C-17), 46.0 (C-19), 41.9 (C-14), 41.5 (C-18), 39.7 (C-8), 39.3 (C-4), 38.8 (C-1), 36.7 (C-10), 33.8 (C-21), 32.8 (C-7), 32.8 (C-22), 32.3 (C-29), 30.5 (C-20), 27.9 (C-15), 27.9 (C-23), 26.3 (C-2), 25.8 (C-27), 23.5 (C-11), 23.4 (C-30), 23.1 (C-16), 18.2 (C-6), 17.1 (C-26), 16.5 (C-24), 15.2 (C-25); ^{13}C-nmr data of the sugar moieties, see Table 1; fabms (positive-ion) *m/z* $[M+Li]^+$ 949 (100), 787 (28), 742 (17), 622 (14), 440 (24), 394 (18), 349 (38); fabms (negative-ion) *m/z* 779 (100), 617 (29), 455 (30), 437 (32).

Ursolic acid.—Identified by mp, co-tlc, and 2D tlc.

Saponin E1 (3-O-β-D-galactopyranosyl-oleanolic acid).—^1H nmr (C_5D_5N, 300 MHz) δ 5.46 (1H, s, H-12), 3.33 (1H, dd, *J*=4 and 12 Hz, H-3), 0.80, 0.94, 0.96, 1.00, 1.30 (21H, s, 7×Me), 4.86 (1H, d, *J*=7.5 Hz, gal H-1).

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