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TRITERPENOID SAPONINS FROM *ILEX PARAGUARIENSIS*

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ABSTRACT.—The leaves of *Ilex paraguariensis* have yielded three new saponins named matesaponins 2, 3, and 4 [**1–3**], which have been characterized by chemical and nmr methods as ursolic acid 3-*O*-{β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]}-α-L-arabinopyranosyl]-(28→1)-β-D-glucopyranosyl ester, ursolic acid 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]-(28→1)-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, and ursolic acid 3-*O*-{β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]}-α-L-arabinopyranosyl]-(28→1)-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, respectively.

Ilex paraguariensis St. Hil. (Aquifoliaceae) is a widely distributed tree of southern Brazil, Argentina, Paraguay, and Uruguay, where it is called "maté." In these areas its leaves are used to prepare a traditional beverage and are included in medicinal preparations as a stimulant, diuretic, and antirheumatic. Earlier, we reported preliminary findings on maté saponins, identifying a three-sugar residue bidesmoside (matesaponin 1: ursolic acid 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]-(28→1)-β-D-glucopyranosyl ester) (1). Continuing our efforts, we also reported the partial structure of three additional new saponins of higher molecular weight (2). The present work deals with the full structural determination of these novel compounds (**1–3**).

Repeated cc of the *n*-BuOH fraction led to the isolation of compounds **1**, **2**, and **3** in order of increasing polarity. Peracetylation of a **1**,**2**-mixture followed by further cc led to **1a** and **2a** in an amount sufficient to allow nmr characterization.

Careful comparison of the ^{13}C-nmr spectrum of **1a**, **2a**, and **3** with that of native and peracetylated matesaponin 1 (1), as well as with those of other ursolic acid-containing saponins (3) identified this latter acid as the genin of the three novel saponins.

Acid hydrolysis of pure aliquots allowed the characterization, by tlc, of the sugar components of **1** and **3** as glucose (glc), arabinose (ara), and rhamnose (rha), and glc and ara for **2**.

The molecular formula $C_{53}H_{86}O_{21}$ was deduced for **1** from its fabms, which displayed quasimolecular ion peaks at *m/z* 1065 $[M+Li]^+$ and *m/z* 1081 $[M+Na]^+$, while the fabms spectrum of **1a** displayed an ion peak at *m/z* 1585 $[M+Na]^+$. The presence in the ^{1}H- and ^{13}C-nmr spectra of **1a**, compared to the ^{1}H- and ^{13}C-nmr spectra of the peracetylated derivative of matesaponin 1, of one extra anomeric signal [δ (H-1) 5.35; δ (C-1) 96.0] and one extra methyl signal [δ (CH_3) 1.48; δ (CH_3) 16.7] established, together with observations from the fabms data, that **1** was substituted by one more rha unit than matesaponin 1. The sugar residue [δ (H-1) 5.52; δ (C-1) 91.5] bond at C-28 was identified by COSY and ^{13}C-^{1}H correlated 2D nmr as a glc moiety. Thus, as in the case of matesaponin 1, a terminal glc residue was linked at C-28 via an ester bond while an ara, glc, rha-constituted

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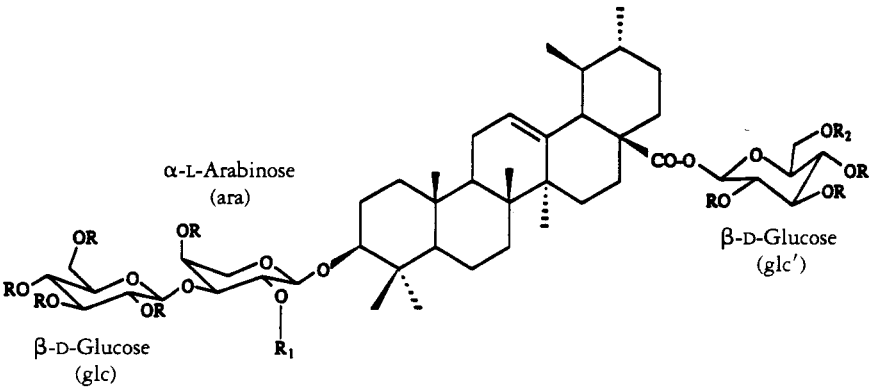

1 R_1=α-L-Rhamnose (rha); $R=R_2=H$
1a R_1=peracetylated rha; $R=R_2=Ac$
2 $R=R_1=H$; R_2=β-D-Glucose (glc″)
2a $R=R_1=Ac$; R_2=peracetylated glc″
3 $R=H$; R_1=rha; R_2=glc″

oligosaccharide was substituted at C-3. Identification of the sugar proton resonances of **1a** (COSY) showed that the glc and the rha moieties were at the terminal positions while the ara unit was substituted at its C-2 and C-3 positions. A first attempt to determine the structure of the branched side-chain using the NOESY technique was unsuccessful. However, use of the ROESY (4) experiment allowed observation of a correlation between H-1 of glc and H-3 of ara (Figure 1). Thus, **1** was determined to be ursolic acid 3-*O*-{β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]}-α-L-arabinopyranosyl]-(28→1)-β-D-glucopyranosyl ester.

The fabms spectrum of **2** exhibited a peak at *m/z* 1097 $[M+Na]^+$, indicating a molecular formula of $C_{53}H_{86}O_{22}$, confirmed by a peak at *m/z* 1643 $[M+Na]^+$ in the fabms of **2a**. This molecular formula was consistent with the presence of one ara and three glc residues. Upon alkaline hydrolysis, **2** led to a prosapogenin identical to that previously obtained by alkaline hydrolysis of matesaponin 1 (1). Thus, **2** was esterified at C-28 by a glc-glc chain. The interglycosidic linkage of this disaccharide was deduced to be glc(1→6)glc from the deshielding in the ^{13}C-nmr spectrum of **2a** of one of the two CH_2 units of this moiety (δ 67.9), indicating its substituted character. Thus, **2** was identified as ursolic acid 3-*O*-[β-D-glucopyranosyl-

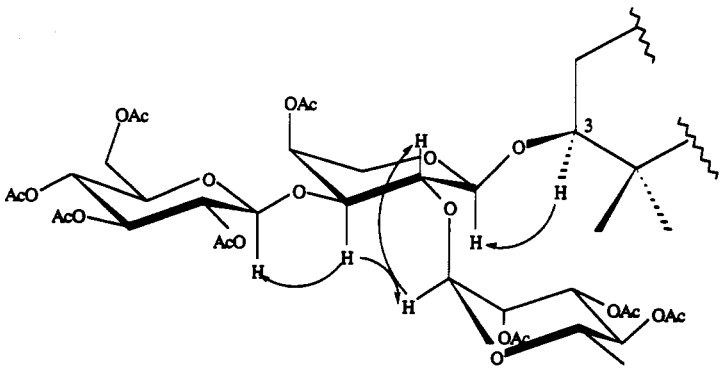

FIGURE 1. Structurally useful rOe's observed for **1a**.

(1→3)-α-L-arabinopyranosyl]-(28→1)-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

A molecular formula of $C_{59}H_{96}O_{26}$ was deduced for **3** from its positive-ion fabms, which displayed quasimolecular ion peaks at *m/z* 1227 $[M+Li]^+$ and *m/z* 1243 $[M+Na]^+$. The negative-ion fabms of **3** confirmed the molecular weight and gave information about the sequence of the sugars from the peaks at *m/z* 1219 $[M-H]^-$, 895 $[(M-H)-2\ glc]^-$, 733 $[(M-H-2\ glc)-glc]^-$, 587 $[(M-H-3\ glc)-rha]^-$ and 455 $[(M-H-3\ glc-rha)-ara]^-$. Alkaline hydrolysis of **3** afforded the same prosapogenin as that obtained by hydrolysis of **1**. The structure of the branched sugar side-chain at C-28 was deduced to be glc(1→6)glc from the presence of a CH_2 resonance at δ 69.1 in the ^{13}C-nmr spectrum of **3** and by comparison of the ^{13}C-nmr data of **2** and **3**. Taken together, these data indicated that **3** is ursolic acid 3-*O*-{β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]}-α-L-arabinopyranosyl]-(28→1)-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

The β configuration for the glucopyranosyl units and the α configuration for the arabinopyranosyl and rhamnopyranosyl residues were inferred from their ^{13}C-nmr data (Table 1), *J* values, and chemical shifts (see Experimental) of the anomeric protons. The new saponins **1**, **2**, and **3** have been named matesaponins 2, 3, and 4, respectively.

TABLE 1. Selected ^{13}C-Nmr Data of Compounds **1a**, **2a**, and **3** or Derivatives (75.4 MHz, ppm).

Carbon	Compounds		
	1a[a]	**2a**[a]	**3**[b]
Aglycone			
3	89.1	90.0	87.8
12	125.8	126.1	125.7
13	136.8	137.0	138.1
28	175.0	175.2	176.0
3-*O*-Sugar			
Ara-1	104.1	103.7	104.8
Ara-2	72.5	73.1	73.5
Ara-3	79.0	76.9	81.7
Ara-4	72.0	73.1	67.8
Ara-5	63.8	64.3	64.4
Rha-1	96.0		101.5
Rha-2	68.2		72.0
Rha-3	70.3		72.1
Rha-4	70.7		73.5
Rha-5	66.0		69.7
Rha-6	16.7		18.2
Glc-1	99.1	100.6	104.3
Glc-2	69.5	70.1	74.6
Glc-3	72.0	72.1	77.5
Glc-4	66.8	68.4	71.1
Glc-5	72.3	71.6	77.8
Glc-6	61.2	61.7	62.2
28-*O*-Sugar			
Glc′-1	91.2	91.5	95.3
Glc′-2	69.5	70.3	74.7
Glc′-3	72.0	72.1	77.9
Glc′-4	66.7	68.5	70.6
Glc′-5	72.0	71.3	78.0
Glc′-6	60.8	67.9	69.1
Glc″-1		100.9	104.2
Glc″-2		71.2	74.3
Glc″-3		72.9	78.1
Glc″-4		69.1	71.0
Glc″-5		72.9	78.3
Glc″-6		62.1	62.1

[a]Recorded in $CDCl_3$.
[b]Recorded in C_5D_5N.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AC 300-P spectrometer. 1D and 2D nmr experiments were conducted using standard procedures, with the ROESY experiment carried out in a phase-sensitive mode (TPPI), using 2K points in the acquisition dimension and 512 experiments of 80 accumulations.

Two different spin-lock delays (200 and 300 msec) were used without showing significant differences. Other measurements, as well as the chromatographic methods used, were performed using techniques and instruments reported by Gosmann *et al.* (1).

PLANT MATERIAL.—See Gosmann *et al.* (1).

EXTRACTION AND ISOLATION.—The dried leaves of *Ilex paraguariensis* (200 g) were extracted with EtOH-H_2O (4:6). The gum obtained after evaporation of the solvent was dissolved in H_2O and successively extracted with $CHCl_3$ EtOAc, and *n*-BuOH. The *n*-BuOH fraction (9.0 g) was separated from phenolic compounds by extraction with a 1% NaOH solution. The residue obtained after evaporation of the *n*-BuOH was repeatedly

chromatographed over Si gel ($CHCl_3$-EtOH-H_2O, 8:4:0.5) to give pure matesaponin **2** (27 mg), matesaponin **3** (20 mg), matesaponin **4** (38 mg), and a mixture of matesaponins **2** and **3** (55 mg).

ACID HYDROLYSIS OF MATESAPONINS [**1–3**].—The isolated matesaponins [**1–3**] were refluxed in 10% H_2SO_4/90% EtOH for 1.5 h, yielding a precipitate, which was separated by filtration. The aqueous extract, after neutralization with 10% NH_4OH, was concentrated and extracted with pyridine. The pyridine extract was analyzed by tlc.

ACETYLATION OF MATESAPONINS **2** AND **3**.—A mixture of matesaponins **2** and **3** was acetylated using pyridine and Ac_2O. The solution was concentrated *in vacuo* and the residue extracted at neutral pH with EtOAc. Cc (EtOAc-petroleum ether, 1:1) afforded pure **1a** (21 mg) and **2a** (17 mg).

Matesaponin 2 [**1**].—White powder, $[\alpha]^{23}D$ +6.7° (*c*=0.7, pyridine); fabms (positive-ion mode) *m/z* 1081 $[M+Na]^+$, 1065 $[M+Li]^+$, (negative-ion) *m/z* 1057 $[M-H]^-$, 911 $[(M-H)-rha]^-$, 895 $[(M-H)-glc]^-$, 749 $[(M-H-glc)-rha]^-$, 733 $[(M-H-glc)-glc]^-$, 587 $[(M-H-2\ glc)-rha]^-$, 455 (aglycone).

Peracetylated matesaponin 2 [**1a**].—White powder; $[\alpha]^{23}D$ +4.2° (*c*=0.6, $CHCl_3$); fabms (positive-ion) *m/z* 1585 $[M+Na]^+$; ^1H nmr ($CDCl_3$) δ 1.10, 1.18 (2 Me), 1.19 (2 Me), 1.22, 1.39, 1.44, 1.48 (4 Me), 1.48 (1H, d, *J*=6.7 Hz, rha H-6), 2.1–2.4 (12 OAc), 2.51 (1H, d, *J*=11 Hz, H-18), 3.1 (1H, dd, *J*=11.8 and 4.6 Hz, H-3), 3.25 (1H, d, *J*=14 Hz, ara H-5), 3.48 (2H, m, glc H-5, glc′ H-5), 3.70 (1H, dd, *J*=14.2 and 4.6 Hz, ara H-3), 3.82 (1H, d, *J*=14.4 Hz, ara H-5), 3.97 (1H, m, ara H-2), 4.05 (2H, m, glc H-6, glc′ H-6), 4.21 (3H, m, ara H-1, glc H-6, glc′ H-6), 4.38 (1H, m, rha H-5), 4.63 (1H, d, *J*=8.1 Hz, glc H-1), 4.92 (1H, t, *J*=8.1 Hz, glc H-2), 5.0–5.4 (8H, m, glc′ H-2, glc′ H-3, glc′ H-4, rha H-3, rha H-4, glc H-3, glc H-4, ara H-4), 5.30 (1H, d, *J*=3 Hz, rha H-1), 5.48 (1H, dd, *J*=12 and 3 Hz, rha H-2), 5.52 (2H, m, H-12, glc′ H-1); ^{13}C-nmr data, see Table 1; *anal.*, calcd for $C_{77}H_{110}O_{33}$, C 59.13%, H 7.09%, found C 58.75%, H 7.09.

Matesaponin 3 [**2**].—White powder, $[\alpha]^{21}D$ +4.8° (*c*=0.46, pyridine), fabms (positive-ion) *m/z* 1097 $[M+Na]^+$, 1081 $[M+Li]^+$, (negative-ion) *m/z* 1073 $[M-H]^-$, 911 $[(M-H)-glc]^-$, 749 $[(M-H-glc)-glc]^-$, 587 $[(M-H-2\ glc)-glc]^-$, 455 (aglycone).

Peracetylated matesaponin 3 [**2a**].—White powder; $[\alpha]^{23}D$ +18.7° (*c*=0.2, $CHCl_3$); fabms (positive-ion) *m/z* 1643 $[M+Na]^+$; ^1H nmr ($CDCl_3$) δ 0.72 (2 Me), 0.95 (4 Me), 1.05 (Me), 1.95–2.20 (13 OAc), 3.05 (1H, dd, *J*=10.5 and 5.2 Hz, H-3), 3.50 (1H, d, *J*=13 Hz, ara H-5), 3.55–3.95 (5H, m, glc H-5, glc′ H-5, glc″ H-5, ara H-3, glc* H-6), 4.02 (1H, dd, *J*=13 and 1.1 Hz, ara H-5), 4.08–4.30 (5H, m, 5 glc H-6), 4.35 (1H, d, *J*=7.8 Hz, ara H-1), 4.55 (1H, d, *J*=8.0 Hz, glc″ H-1), 4.65 (1H, d, *J*=7.8 Hz, glc H-1), 4.90–5.30 (12H, m, H-12, glc H-4, glc H-3, glc H-2, ara H-4, ara H-2, glc′ H-2, glc′ H-3, glc′ H-4, glc″ H-2, glc″ H-3, glc″ H-4), 5.52 (1H, d, *J*=8.2 Hz, glc′ H-1); *exact assignment of this glc H-6 could not be determined; ^{13}C-nmr data, see Table 1; *anal.*, calcd for $C_{79}H_{112}O_{35}$, C 58.49%, H 6.96%, found C 58.32%, H 6.95%.

Matesaponin 4 [**3**].—White powder; $[\alpha]^{23}D$ −8.8° (*c*=1.2, pyridine); fabms (positive-ion) *m/z* 1243 $[M+Na]^+$, 1227 $[M+Li]^+$, (negative-ion) *m/z* 1219 $[M-H]^-$, 895 $[(M-H)-2\ glc]^-$, 733 $[(M-H-2\ glc)-glc]^-$, 587 $[(M-H-3\ glc)-rha]^-$, 455 (aglycone); ^1H nmr (pyridine-d_5) δ 0.92 (3H, d, *J*=6.5 Hz), 0.98 (3H, d, *J*=6.7 Hz), 1.08 (2 Me), 1.10, 1.19, 1.21, 1.59 (4 Me), 1.59 (1H, d, *J*=6.8 Hz, rha H-6), 2.52 (1H, d, *J*=12 Hz, H-18), 3.3–4.6 (26 H), [4.88 (2H, m), 4.95 (1H, d, *J*=6.8 Hz), glc″ H-1, ara H-1, glc H-1)], 5.42 (1H, br t, H-12), 5.79 (1H, br s, rha H-1), 6.05 (1H, d, *J*=7.2 Hz, glc′ H-1); ^{13}C-nmr data, see Table 1; *anal.*, calcd for $C_{59}H_{96}O_{26}$, 7 H_2O, C 52.57%, H 8.23%, found C 52.66%, H 8.67%.

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