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A NEW SAPONIN FROM MATE, *ILEX PARAGUARIENSIS*

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ABSTRACT.—Matesaponin 1, a new saponin obtained from the leaves of *Ilex paraguariensis*, has been characterized by ^{1}H nmr, ^{13}C nmr, eims, fabms, and chemical reactions. The structure has been determined as ursolic acid-3-*O*-[β-D-glucopyranosyl-(1↦3)-α-L-arabinopyranosyl]-(28↦1)-β-D-glucopyranosyl ester.

Ilex paraguariensis St. Hil. (Aquifoliaceae) is widely used as a traditional beverage known as mate (Paraguay tea) in southern Brazil, Argentina, Paraguay, and Uruguay. It is also used in popular medicines and employed in commercial herbal preparations as a stimulant to the central nervous system, a diuretic, and an antirheumatic. Apart from the well-known xanthines, very little is known about its secondary metabolites (1). This paper reports the isolation and elucidation of the structure of a new saponin, named matesaponin 1, from the leaves of this medicinal plant.

Matesaponin 1 was obtained from the *n*-BuOH extract through cc. After acid hydrolysis, the sugar components were identified as glucose and arabinose by tlc, whereas the identity of the aglycone moiety as ursolic acid was confirmed by ^{1}H and ^{13}C nmr (2–5).

The eims of the acetyl derivative of matesaponin 1 showed peaks at *m/z* 438 and 331, which were attributed to the aglycone and the terminal hexose, respectively.

The molecular mass of matesaponin 1 was shown to be 912 through fabms, which exhibited molecular ion peaks at *m/z* 919 $[M + Li]^+$ and 935 $[M + Na]^+$. The fab mass spectrum, obtained in the negative ion mode, confirmed the molecular mass and gave information about the sequence of the sugars by the peaks at *m/z* 911 $[M - H]^-$, 749 $[(M - H) - \text{hexose}]^-$, 587 $[(M - H - \text{hexose}) - \text{hexose}]^-$, and 455 $[(M - H - \text{hexose} - \text{hexose}) - \text{pentose}]^-$.

The ^{1}H-nmr spectrum of the acetyl derivative of matesaponin 1 showed three anomeric proton signals at δ 4.63 ppm (d, $J_{1,2} = 8$ Hz) and δ 4.31 ppm (d, $J_{1,2} = 8$ Hz) attributed to glucose and arabinose, respectively, at C-3, and at δ 5.53 ppm (d, $J_{1,2} = 8$ Hz) corresponding to glucose at C-28 (6). These assignments were supported by the ^{13}C-nmr spectrum of matesaponin 1, which showed three anomeric proton signals at δ (ppm) 107.2, 106.0, and 95.5, the latter in full agreement with the presence of a β-glucopyranose linked in the form of an ester (7–9).

The β configuration for the two glucopyranosyl units and the α configuration for the arabinopyranoside were inferred from the *J* values of the respective anomeric protons.

The interglycosidic linkage was determined by the observed downfield shift (Δδ 9.3) for C-3 of the inner α-L-arabinose, by comparing with the corresponding methylglycoside (10). This deshielding clearly indicated that the terminal glycosyl unit is attached at this position; the rest of the carbons were unaffected (Table 1).

Matesaponin 1 on partial hydrolysis afforded a prosapogenin. Its fabms spectrum indicated the molecular mass at *m/z* 750, shown through the fragments 757 $[M + Li]^+$ and 773 $[M + Na]^+$ (positive ion mode) and 749 $[M - H]^-$

TABLE 1. ^{13}C-nmr Chemical Shifts of Matesaponin 1 and its Prosapogenins in Pyridine-d_5, ppm.

Carbon	Matesaponin 1	Compound	
		3-*O*-[β-D-Glucopyranosyl-(1↦3)-α-L-arabinopyranosyl]-ursolic acid	3-*O*-α-L-Arabinopyranosyl ursolic acid
3-*O*-sugar			
ara-1	107.2	107.2	107.5
ara-2	71.7	71.7	72.9
ara-3	83.9	83.9	74.6
ara-4	69.1	69.1	69.5
ara-5	66.7	66.8	66.7
glu-1	106.0	106.0	
glu-2	75.5	75.5	
glu-3	78.1	78.2	
glu-4	71.4	71.4	
glu-5	78.4	78.5	
glu-6	62.5	62.5	
28-*O*-sugar			
glu-1	95.5		
glu-2	73.9		
glu-3	78.7		
glu-4	71.0		
glu-5	78.9		
glu-6	62.1		
aglycone			
C-3	88.6	88.6	88.7
C-12	125.9	125.5	125.6
C-13	138.3	139.1	139.2
C-28	176.1	179.8	179.8

(negative ion mode). Comparison of the ^{13}C-nmr data for the prosapogenin and matesaponin 1 indicated for the prosapogenin the structure 3-*O*-[β-D-glucopyranosyl-(1↦3)-α-L-arabinopyranosyl]-ursolic acid.

The prosapogenin was further degradated through oxidation with $NaIO_4$ (Smith-de-Mayo degradation) to confirm the 1↦3 linkage between glucose and arabinose. An isolated product was shown to be 3-*O*-α-L-arabinopyranosyl-ursolic acid (by ^{1}H and ^{13}C nmr), supporting the above sites of glycosidation.

The structure of matesaponin 1 is, thus, suggested to be ursolic acid-3-*O*-[β-D-glucopyranosyl-(1↦3)-α-L-arabinopyranosyl]-(28↦1)-β-D-glucopyranosyl ester.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were taken with Kofler's Apparatus and are uncorrected. Optical rotation: Perkin-Elmer 141 polarimeter at room temperature (22°). Eims: MAT 44 spectrometer and fabms on KRATOS MS 80 RFA, using Xe/7-8 kV. ^{1}H- and ^{13}C-nmr spectra: Brucker WM 400 MHz and Varian Gemini 200 MHz, with TMS as an internal standard. Tlc (saturated chamber): Si gel GF, EtOAc-petroleum ether (2:8) (aglycones); Si gel GF, CH_2Cl_2-EtOH-H_2O (80:40:5) (sugars); Si gel GF, CH_2Cl_2-EtOH-H_2O (120:40:5) (prosapogenins); detection: anisaldehyde-H_2SO_4/uv 360 nm for aglycones and saponins and aniline phthalate/uv 360 nm for sugars (11).

PLANT MATERIAL.—The leaves of *I. paraguariensis* were collected in Veranopolis, State of Rio Grande do Sul, Brazil, in July 1986. A herbarium specimen (voucher no. ICN-68648) is on deposit in the Herbarium of the Botany Department of the Federal University of Rio Grande do Sul.

ISOLATION OF MATESAPONIN 1.—The air-dried leaves (750 g) were extracted by decoction (3 times/30 min) with EtOH-H_2O (4:6). Evaporation of the solvent afforded 230 g of a residue.

Part of this gum (180 g) was redissolved in H_2O and extracted successively with CH_2Cl_2 and *n*-BuOH. The *n*-BuOH fraction yielded 74 g (13.7%), of which 2.5 g was column chromatographed (over Si gel) using CH_2Cl_2-EtOH-H_2O (80:40:5) to give matesaponin 1 (0.225 g, 1.23%): mol wt 912; mp 225–228° (dec.); $[\alpha]^{22}D + 19.05°$ (pyridine, $c = 1.68$); [M] D obs. = +173.74°; fabms (positive mode) *m/z* [aglycone]$^+$ 439, [(M + Li) − glucose]$^+$ 757, [M + Li]$^+$ 919, [M + Na]$^+$ 935; fabms (negative mode) *m/z* [(M − H − glucose − glucose) − arabinose]$^-$ 455, [(M − H − glucose) − glucose]$^-$ 587, [(M − H) − glucose]$^-$ 749, [M − H]$^-$ 911; ^{13}C nmr see Table 1.

ACETYLATION OF MATESAPONIN 1.—Matesaponin 1 was acetylated in the usual manner, using pyridine and Ac_2O. The acetyl derivative: eims *m/z* (rel. int. %) 109 (46), 169 (74), 271 (8), [glucose(Ac)$_4$]$^+$ 331 (16), [aglycone]$^+$ 438 (1), [glucose(Ac)$_4$ − arabinose(Ac)$_2$]$^+$ 547 (4); ^1H-nmr ($CDCl_3$) 0.76, 0.77, 1.05 (s, 3 × Me), 0.85 (s, 2 × Me), 0.84 (d, $J = 7.0$ Hz, Me), 0.92 (d, $J = 7.0$ Hz, Me), 2.18 (d, $J = 11$ Hz, H-18), 1.99, 2.06, 2.08, 2.09, 2.12 (s, 5 × OAc), 2.01 (s, 3 × OAc), 2.02 (s, 2 × OAc), 3.03 (dd, $J = 11.5$, 5.5 Hz, H-3), 4.63 (d, $J = 8.0$ Hz, anomeric H, glucose C-3), 4.31 (d, $J = 8.0$ Hz, anomeric H, arabinose C-3), 5.53 (d, $J = 8.0$ Hz, anomeric H, glucose C-28), 5.24 (br t, $J = 3.5$ Hz, H-12).

ACID HYDROLYSIS OF MATESAPONIN 1.—Matesaponin 1 (20 mg) was refluxed in 10% H_2SO_4/96% 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 precipitate and the pyridine extract were analyzed by tlc for aglycone and sugar components, respectively.

ISOLATION OF A PROSAPOGENIN.—Matesaponin 1 was refluxed in 2 N H_2SO_4/96% EtOH for 10 min. After neutralization with 0.5 N KOH the mixture was extracted with *n*-BuOH and concentrated to yield the prosapogenin 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]-ursolic acid: mol. wt. 750; mp 274–277° (dec.); $[\alpha]^{22}D + 27.25°$ (pyridine, $c = 1.89$), [M] D obs. = +204.36°; fabms (positive mode) *m/z* [aglycone]$^+$ 439, [M + Li]$^+$ 757, [M + Na]$^+$ 773; fabms (negative mode) *m/z* [aglycone]$^-$ 437, [(M − H − glucose) − arabinose]$^-$ 455, [(M − H) − glucose]$^-$ 587, [M − H]$^-$ 749; ^{13}C nmr see Table 1.

ACETYLATION OF THE PROSAPOGENIN.—Acetylation was carried out in the usual manner, using pyridine and Ac_2O, and yielded the acetyl derivative: eims *m/z* (rel. int. %) 109 (28), 169 (28), [aglycone]$^+$ 203 (18), [aglycone]$^+$ 248 (20), [glucose(Ac)$_4$]$^+$ 331 (12), [aglycone]$^+$ 438 (4), [glucose(Ac)$_4$ − arabinose(Ac)$_2$]$^+$ 547 (16), [M − glucose(Ac)$_4$]$^+$ 671 (1).

The prosapogenin from above (100 mg) in 96% EtOH (5 ml) was treated with $NaIO_4$ (250 mg) in H_2O (3 ml) at room temperature overnight, yielding a precipitate which was separated by filtration. This precipitate was refluxed with 3% KOH/85% aqueous EtOH for 1 h, yielding another precipitate which was separated by filtration. This was column chromatographed over Si gel using $CHCl_3$/EtOH to afford a second prosapogenin 3-*O*-α-L-arabinopyranosyl ursolic acid mol wt 588; mp 245–255° (dec.); ^{13}C-nmr see Table 1; ^1H-nmr (pyridine) 3.35 (dd, $J = 11.5$, 4.5 Hz, H-3), 5.46 (t, $J = 3.5$ Hz, H-12), 0.83, 0.93, 1.10, 1.24, 1.28 (s, 5 × Me), 0.94 (d, $J = 7.0$ Hz, Me), 1.28 (d, $J = 8.0$ Hz, Me), 2.61 (d, $J = 11$ Hz, H-18), 4.76 (d, $J = 7.0$ Hz, arabinose H-1), 4.42 (dd, $J = 9.0$, 7.0 Hz, arabinose H-2), 4.15 (ddd, $J = 9.0$, 2.0, 4.0 Hz, arabinose H-3), 4.31 (m, arabinose H-4), 3.82 (m, arabinose H-5α), 4.31 (m, arabinose H-5β).

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