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MATESAPONIN 5, A HIGHLY POLAR SAPONIN FROM *ILEX PARAGUARIENSIS*

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Key Word Index—*Ilex paraguariensis*; Aquifoliaceae; saponin; maté; matesaponin.

Abstract—The structure of matesaponin 5, a novel saponin isolated from the leaves of *Ilex paraguariensis*, was established as ursolic acid-3-*O*-{β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabino pyranosyl}-(28→1)-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

INTRODUCTION

Ilex paraguariensis St Hil. is a South American native, perennial tree belonging to the holly family. Its leaves and twigs are used to prepare the traditional beverage, maté. The widespread consumption of maté in South America, where it is also used for its claimed stimulant, diuretic and anti-inflammatory properties, prompted us to initiate, some years ago, a phytochemical and pharmacological investigation of this species. This work has already allowed us to isolate and identify four triterpenoid saponins (matesaponin 1–4 [1, 2]) and demonstrate the anti-oedematogenic activity of the crude saponin fraction (1.0 g kg^{-1}, *per os*) on carrageenin-induced oedema [3]. We report now the structure of a fifth and highly polar saponin, matesaponin 5 (**1**), isolated from the leaves of the title plant.

RESULTS AND DISCUSSION

The dried leaf alcoholic-maceration residue provided, after selective extraction, the crude saponin mixture (9%) from which, after repeated column chromatography, compound **1** was isolated as an amorphous powder (0.05%). Its FAB-mass spectrum showed a pseudo-molecular peak at *m/z* 1405 ($[M+Na]^+$), indicating the molecular formula $C_{65}H_{106}O_{31}$. Accordingly, its ^{13}C NMR spectrum (Table 1) contained signals for 65 carbons separated in eight methyl, 14 methylene, 36 methine and seven quaternary resonances from the DEPT spectra. Comparison of the ^{13}C NMR spectrum of matesaponins 1–4 with that of compound **1** indicated matesaponin 5 was closely related to matesaponin 4 and was constituted of an ursolic acid molecule substituted by six sugar residues. The latter moieties were identified, after acid hydrolysis and TLC, as glucose (glc), arabinose (ara) and rhamnose (rha). Combined mass and NMR data ($J_{H\text{-}1\text{-}H\text{-}2}$ and δ ^{13}C of the anomeric signals) showed that the sugar part of **1** was comprised of one α-L-rha, one α-L-ara and four β-D-glc units [4–6]; a methylene resonance at δ 69.4 indicating, in addition, that one of the glc residues was substituted at its position C-6.

Alkaline hydrolysis of **1** yielded **2**, identified by co-TLC as the prosapogenin previously isolated by alkaline hydrolysis of matesaponin 2 and 4 [2]. Therefore, the sugar side-chain substituting the C-3 position of the aglycone could be identified as glc(1→3)-[rha(1→2)]-ara and the sugar moiety branched at C-28 as three glucose units, one of which was substituted at C-6. The exact nature of this side-chain was deduced primarily from the presence of two methine resonances at δ 76.1 and δ 76.2 in the ^{13}C NMR spectrum of **1**, while no signal was observed around these chemical shifts in the ^{13}C NMR spectrum of matesaponin 4 [2]. Glycosylation causes a downfield shift of the α-carbon and an upfield shift of the β-carbon atoms of about 6 and 1 ppm, respectively [4, 5], and only a substitution at the glc C-4 position could explain these two resonances. The distinction between a linear or a ramified glycosidic side-chain could have been solved easily by attribution of the proton resonances of the different glc residues. Unfortunately, the overlapping of the signals of the four glc residues of the molecule at 300 MHz precluded their exact assignments and only the protons of the glc residue directly bond to C-28 (glcI) could be unambiguously assigned starting from its isolated

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Table 1. ^{13}C NMR spectral data (δ, ppm) of **1** (MeOD–DMSO-d_6, 4:1) and **3** ($CDCl_3$) (75 MHz)

		1				**3***	
C-1	39.5	glcI-1	95.4	C-1	35.8	glcI-1	91.5
C-2	27.1	glcI-2	74.1	C-2	29.6	glcI-2	69.5
C-3	89.2	glcI-3	77.6	C-3	89.4	glcI-3	71.9
C-4	39.9	glcI-4	71.2[c]	C-4	38.9	glcI-4	69.5
C-5	56.8	glcI-5	77.6	C-5	55.9	glcI-5	72.3
C-6	19.1	glcI-6	69.4	C-6	18.1	glcI-6	67.9
C-7	34.0	glcII-1	104.4[b]	C-7	35.8	glcII-1	100.5
C-8	39.9	glcII-2	74.1	C-8	39.0	glcII-2	72.8
C-9	48.7	glcII-3	76.1	C-9	47.5	glcII-3	73.5
C-10	37.6	glcII-4	76.2	C-10	36.6	glcII-4	76.5
C-11	24.2	glcII-5	77.6	C-11	24.0	glcII-5	76.5
C-12	128.5	glcII-6	61.5[a]	C-12	126.0	glcII-6	61.1[a]
C-13	139.0	glcIII-1	104.2[b]	C-13	137.1	glcIII-1	100.8[b]
C-14	43.0	glcIII-2	74.1	C-14	42.0	glcIII-2	72.3
C-15	29.1	glcIII-3	77.6	C-15	29.6	glcIII-3	72.3
C-16	25.0	glcIII-4	70.6[c]	C-16	26.1	glcIII-4	69.8
C-17	48.8	glcIII-5	77.6	C-17	48.0	glcIII-5	71.9
C-18	53.8	glcIII-6	62.0[a]	C-18	52.6	glcIII-6	61.4[a]
C-19	40.0	glcIV-1	104.2[b]	C-19	39.4	glcIV-1	99.4[b]
C-20	39.9	glcIV-2	74.6	C-20	39.0	glcIV-2	69.8
C-21	31.4	glcIV-3	77.6	C-21	30.4	glcIV-3	71.3
C-22	37.3	glcIV-4	71.2[c]	C-22	33.2	glcIV-4	66.4
C-23	28.5	glcIV-5	77.6	C-23	27.9	glcIV-5	72.3
C-24	16.3	glcIV-6	62.1[a]	C-24	15.6	glcIV-6	61.7[a]
C-25	17.3	ara-1	105.1	C-25	16.4	ara-1	104.4
C-26	17.8	ara-2	73.5	C-26	17.1	ara-2	72.5
C-27	23.8	ara-3	80.8	C-27	23.1	ara-3	79.3
C-28	17.2	ara-4	68.2	C-28	175.0	ara-4	71.9
C-29	17.7	ara-5	64.9	C-29	17.1	ara-5	64.1
C-30	21.6	rha-1	101.6	C-30	21.0	rha-1	96.3
		rha-2	71.4			rha-2	68.1
		rha-3	73.5			rha-3	70.6
		rha-4	74.6			rha-4	71.1
		rha-5	69.8			rha-5	67.7
		rha-6	18.2			rha-6	20.2

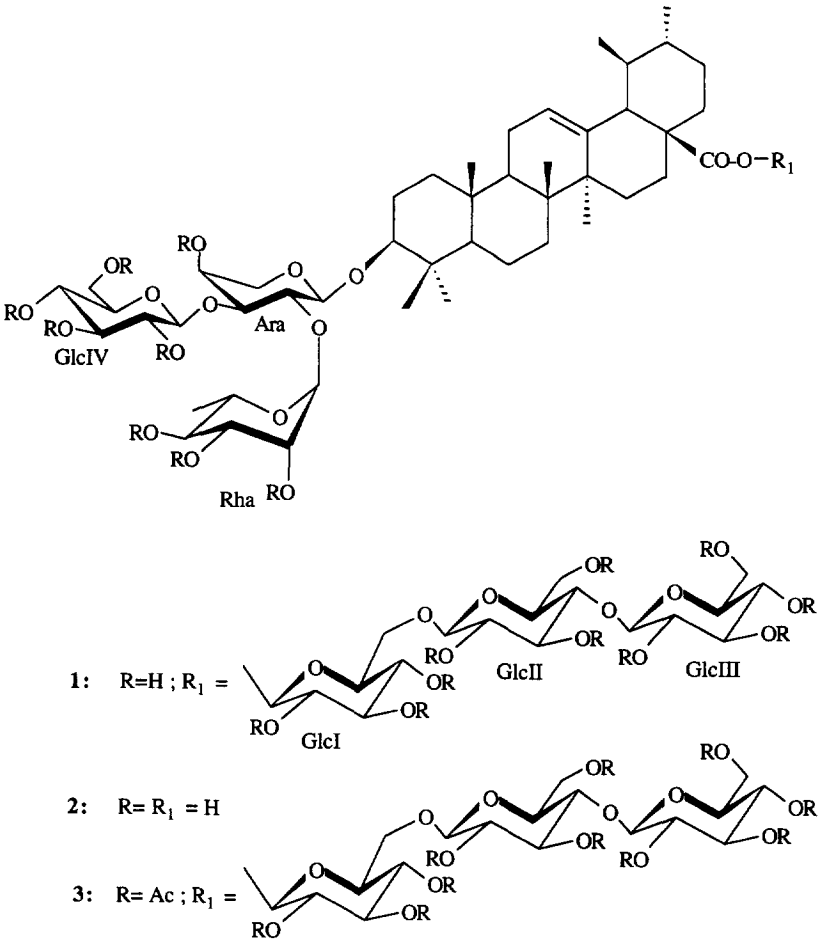
[a,b,c]Exchangeable signals in the same column.

*Attribution of the sugar resonances realized by analogy with the peracetylated derivatives of matesaponin 2 and 3 (unpublished data).

anomeric proton signal. A combination of COSY, Relay-COSY and HOHAHA NMR experiments showed that the chemical shifts of the protons of glcI were almost superimposable on those of the similar glc residue of matesaponin 4. Thus, a substitution of C-28 by a linear sugar side-chain could be proposed. In order to confirm this conclusion, we prepared compound **3**, the peracetylated derivative of **1**, whose FAB-mass spectrum displayed a quasi $[M]^+$ peak at m/z 2145 $[M + Li]^+$, indicating the complete peracetylation of the molecule. Two-dimensional NMR HOHAHA correlations of GlcI-H1, unambiguously assigned at δ 5.56, should easily discriminate a ramified sugar chain substituting C-28 (GlcI substituted at its positions C-4 and C-6) from a linear one (GlcI substituted at its positions C-6 and GlcII substituted at its position C-4). On the HOHAHA NMR spectrum, the protons H-2, H-3 and H-4 of three glc residues (glcI glcIII and IV) were observed between δ 4.9 and δ 5.5, indicating that these residues were acetylated at these positions. Conversely, in the same spectrum, the H-4 proton of the fourth glucose residue was observed at δ 3.82. Therefore, this residue could be assigned unambiguously to glcII. From these data, we propose for matesaponin 5 the structure: ursolic acid-3-*O*-{β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl}-(28 → 1)-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glu-copyranosyl] ester.

EXPERIMENTAL

Plant material. Plants were collected in Mato Leitão, State of Rio Grande do Sul, Brazil, in August 1994. A herbarium specimen (voucher leg. Sobral 7677) 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. Optical rotation were recorded on a Perkin-Elmer 141 polarimeter. FAB-MS were recorded on a Kratos MS 80 or a VG ZAB SPECT instrument. NMR spectra were recorded on a Bruker AC 300-P spectrometer. Hydrolysis and acetylation were performed as previously reported [1, 2].

Isolation. Dried and ground leaves of *I. paraguariensis* (218 g) were extracted by maceration in EtOH–H_2O (6:4). The dried maceration extract was dissolved in H_2O and extracted successively with CH_2Cl_2 and *n*-BuOH. Evapn of the latter fr. yielded the crude saponin fr. (19.8 g, 9%) that was purified by silicagel CC (CH_2Cl_2–EtOH–H_2O). Frs containing the more polar glycosides were combined and rechromatographed over silicagel (*n*-BuOH–HOAc–H_2O) yielding matesaponin 5 (100 mg).

Matesaponin 5 (**1**). White powder, $[\alpha]_D^{18}$ +1.5° (*c* = 1.9, MeOH); FAB-MS 1405 $[M+Na]^+$. ^1H NMR (MeOD–DMSO-d_6, (4:1): δ 0.66 (3H, *s*), 0.70 (3H, *s*), 0.75 (3H, *d*, *J* = 6.9 Hz), 0.80 (6H, *m*), 0.84 (3H, *s*), 0.91 (3H, *s*), 1.05 (3H, *d*, *J* = 6.8 Hz, rha-H6), 2.9–3.8 (31 H), 3.1 (gluI-H2), 3.28 (gluI-H3), 3.31 (gluI-H4)), 4.22 (1H, *d*, *J* = 7.3 Hz, glu-H1), 4.26 (1H, *d*, *J* = 7.5 Hz, glu-H1), 4.35 (1H, *d*, *J* = 6.8 Hz, ara-H1), 4.48 (1H, *d*, *J* = 7.6 Hz, glu-H1), 5.09 (2H, *m*, H-12, rha-H1), 5.17 (1H, *d*, *J* = 8 Hz, gluI-H1). (*assigned from the HOHAHA spectrum). ^{13}C NMR: see Table 1.

Peracetylated matesaponin 5 (**3**). $[\alpha]_D^{18}$ −3.3° (*c* = 0.7, $CHCl_3$); FAB-MS 2145 $[M+Li]^+$; ^1H NMR ($CDCl_3$): δ 0.82 (3H, *s*), 0.90 (6H, *s*), 0.99 (3H, *d*, *J* = 7.2 Hz), 1.11 (3H, *d*, *J* = 7.2 Hz), 1.22 (3H, *d*, *J* = 6.8 Hz), 1.29 (6H, *s*), 1.98–2.25 (CH_3CO–), 3.15 (1H, *dd*, *J* = 5.5, 10.4 Hz, H-3), 3.82 (glcII-H-4), 4.38 (ara-H1)*, 4.51 (1H, *d*, *J* = 7.8 Hz, glcII-H-1), 4.56 (1H, *d*, *J* = 7.9 Hz, glcIII-H-1), 4.79 (1H, *d*, *J* = 7.7 Hz, glcIV-H-1), 5.31 (rha-H-1)*, 5.31 (H-12)*, 5.56 (1H, *d*, *J* = 8.2 Hz, glcI-H-1), 3.5–4.6 [(4 × 2glc-H-6), 4 × glc-H5), ara-H2, -H3, 2 ara-H5, rha-H5], 4.9–5.5 (ara-H4, rha-H2, -H-3, -H-4, glcI-H-2, -H-3, -H-4, glcII-H-2, -H-3, glcIII-H-2, -H-3, -H-4, glcIV-H-2, -H-3, -H-4. (*overlapped by other signals). ^{13}C NMR: see Table 1.

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