Artigo

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TRITERPENES AND NEW SAPONINS FROM *Ilex chamaedryfolia*: CHEMOTAXONOMIC TOOL TO *Ilex* SPECIES DIFFERENTIATION

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Three saponins were isolated from leaves of *Ilex chamaedryfolia*. Their structures were established by spectroscopic and mass spectrometry data as the new saponin 3β-*O*-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl-20(*S*)-19α-hydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl-(1-3)-β-D-glucopyranosyl ester, the new saponin 3β-*O*-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl-20(*S*)-19α-hydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester and the known saponin 3β-*O*-β-D-glucuronopyranosyl-20(*R*)-19α-hydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester. Ursolic acid and α-amyrin were also isolated.

Keywords: *Ilex chamaedryfolia*; Aquifoliaceae; triterpenes.

INTRODUCTION

We initiated some years ago a program aimed to identified saponins from South American *Ilex* species[1] considering the importance of this genera which includes *Ilex paraguariensis* A. St. Hil., named yerba mate. This species is widely used in South Brazil, Argentina, Paraguay and Uruguay to obtain the raw material used to prepare the traditional beverage called maté which is a very important economical crop. Hence, we already reported the structure of several saponins isolated from maté and other South American *Ilex* species: *I. affinis*. *I. argentina*. *I. brevicuspis*. *I. buxifolia*. *I. dumosa*. *I. integerrima*. *I. microdonta*. *I. psammophila*. *I. pseudobuxus*. *I. taubertiana* and *I. theezans*.[1-8]

Ilex chamaedryfolia Reissek is a native shrub in Southern Brazil, found in the states of Rio Grande do Sul, Paraná and Santa Catarina, where it is known as "congonha miúda", "congonhinha" or "congonha brava".[2,9] *I. chamaedryfolia* is one of the species eventually reported as an adulterant of the *I. paraguariensis*. Herein it is described the isolation and structural elucidation of three saponins and two triterpenes (ursolic acid and α-amyrin) from the leaves of *I. chamaedryfolia*. As far as we know, the saponins reported herein have not been yet described in the literature.

EXPERIMENTAL

Plant material

Leaves from *I. chamaedryfolia* Reissek were collected in Guaratuba, State of Paraná, Brazil. A specimen is on deposit in the Herbarium of the Department of Botany (ICN) at the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (M. Sobral and E. P. Santos 9308).

General

Optical Rotation was measured on a Perkin-Elmer® 341 polarimeter. FAB-MS analysis was performed in positive mode on a Kratos MS 80 instrument and HRMS MS spectra were recorded on a Q-Tof micro Waters® high resolution mass spectrometer, operating on eletronspray ionization mode. NMR spectra (^{1}H, 500 MHz; ^{13}C, 125 MHz) were recorded in CD_3OD and $CDCl_3$ on a Bruker® Avance 500 spectrometer. Thin-layer chromatography (TLC) was on Si gel GF_{254} Merck or Aldrich® using CH_2Cl_2:MeOH (98:2, v/v) and $CHCl_3$:MeOH:H_2O (80:40:5, v/v) as eluents for sapogenins and saponins, respectively. Compounds were visualised using anysaldehyde-sulphuric acid reagent and heating (100 °C).

Extraction and isolation

Air-dried powdered leaves (475 g) were submitted to maceration in aqueous 80% EtOH (1:10 plant:solvent, m/v) at room temperature (2 x 10 days). The ethanol extract was evaporated to dryness under reduced pressure and the residue (85 g, 18%) was suspended in water (1500 mL) and extracted successively with dichloromethane (3 x 500 mL), ethyl acetate (3 x 500 mL) and *n*-butanol (3 x 500 mL). Each organic phase was separately evaporated to obtain 5 g (0.7%), 5 g (0.7%) and 45 g (9.5%) of each fraction, respectively. The aqueous residue was lyophilised to obtain 27 g (5.6%).

Dichloromethane fraction (1 g) was submitted to CC on silica gel eluting with gradients of ciclohexane:ethyl acetate mixtures. Fractions were pooled according to TLC. Fractions 35-41 (88 mg) and 82-85 (30 mg) were subjected to crystallisation in yielding compounds **1** (19 mg) and **2** (25 mg) in a pure form, respectively. Part of the *n*-butanol fraction (10 g) was submitted to successive CC on silica

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gel eluting with gradients of CH_2Cl_2:EtOH:H_2O mixtures, or CC on LiChroprep™ RP-18 using H_2O:MeOH. Fractions were pooled according to their TLC profiles. Compounds **3** (13 mg), **4** (10 mg) and **5** (13 mg) were isolated (Figure 1). One fraction containing mainly compound **3** was acetylated[6] to obtain pure **3a** (14 mg).

α-*amyrin* (**1**). This compound was identified after comparison of its physical-chemical data with the literature[10] and by co-TLC with an authentic sample.

Ursolic acid (**2**). (3β)-3-hydroxyurs-12-en-28-oic acid. It was identified by co-TLC with an authentic sample.[11]

Compound **3** was identified as the deacylated form of **3a** after comparison of both NMR data.

Peracetylated 3β-O-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl-20(S)-19α-hydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl-(1-3)-β-D-glucopyranosyl ester (**3a**). Amorphous powder. FABMS (positive mode) *m/z*: 1659 $[M+Na]^+$ and HRMS *m/z* 1659.7831 $[M+Na]^+$ ($C_{79}H_{112}O_{36}$). $[\alpha]^{25}_D$ +14.3º ($CHCl_3$, *c* 0.35). ^1H NMR and ^{13}C NMR data ($CDCl_3$): see Table 1.

3β-O-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl-20(S)-19α-hydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl ester (**4**). Amorphous powder. FABMS (positive mode) *m/z*: 951 and HRMS *m/z* 951.4899 $[M+Na]^+$ ($C_{47}H_{76}O_{18}$). $[\alpha]^{25}_D$ -1.67º (MeOH, *c* 0.6). ^1H NMR and ^{13}C RMN data (CD_3OD): see Table 1.

3β-O-β-D-glucuronopyranosyl-20(R)-19α-hydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl ester (**5**). Amorphous powder. FABMS (positive mode) *m/z*: 833.6 $[M+Na]^+$. ^1H NMR and ^{13}C NMR data (CD_3OD): see Table 1.

RESULTS AND DISCUSSION

Solvent partition and chromatographic procedures allowed the isolation of five compounds from leaves of *I. chamaedryfolia* whose identification was accomplished through spectroscopic methods.

Compound **1** was identified as α-amyrin after comparison of its physical-chemical data with literature and by co-TLC with an authentic sample. Compound **2** was rapidly identified as ursolic acid by co-TLC analysis using authentic sample.

Along with the signals corresponding to acetyl groups, {^1H}-^{13}C-NMR spectrum of **3a** showed 53 signals, which after comparison with its DEPT-^{13}C-NMR spectrum, allow to recognize signals attributed to 7 methyl, 13 methylene and 25 methine groups, and 8 quaternary carbon atoms. The ^{13}C-NMR of this compound showed characteristic signals due to one carboxyl group (δ 175.7), a double bond (δ 128.0; δ 137.0) and two oxygenated carbons (δ 72.9; δ 89.9) beyond the acetyl characteristic signals.[12] A signal at δ_H 2.77 (*s*, 1H, H-18) suggesting the presence of a 19-O-substituted urs-12-en skeleton, specifically a 19α-hydroxyursolic acid derivative.[13] Chemical shifts of the C-18, C-22 and C-23 were found at upfield shift and indicate an unusual 20 *S* configuration to E ring of aglycone (Table 1).[8,12] The observation of chemical shifts to C-28 (δ 175.7) and C-3 (δ 89.9) dislocated to upfield and downfield respectively, cleared bisdesmosidic features. Thus, the compound **3a** (and **3**) has as aglycone ilexgenin B (Figure 1).[12]

Its ^1H-NMR and ^{13}C-NMR data indicated the presence of four sugar moieties. The sugar residue δ_H 5.55 (H-1'''); δ_C 91.7 (C-1''') evidenced an ester linkage between the anomeric carbon and the C-28 of the aglycone.[14] For the sugar chain, HH and HC correlation (COSY, HMQC, HMBC) (Figure 2) allowed to assign all carbon and proton signals. HMBC correlation spectrum allowed indicated the interglycosidic linkages, between the terminal glucose (δ_H 4.73, H-1; δ_C 99.7, C-1), attached at C-3''' of glucose II at C-28, as well as, glucose I and arabinose unit substituted at C-3. This spectrum showed a correlation between C-3' of arabinose and H-1'' of glucose I, C-3''' of glucose II and H-1'''' of glucose III (Figure 2). Besides H-1' of arabinose and C-3 of aglycone, H-1''' of glucose II and C-28 of aglycone. Thus **3a** was identified to be peracetylated form of 3β-*O*-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl-20(*S*)-19α-hydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl-(1-3)-β-D-glucopyranosyl ester (Figure 1).

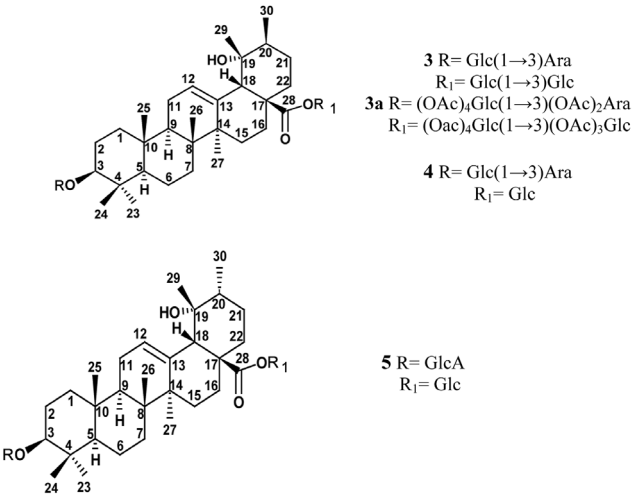

***Figure 1**. Compounds **3**, **4** and **5** isolated from leaves of Ilex chamaedryfolia*

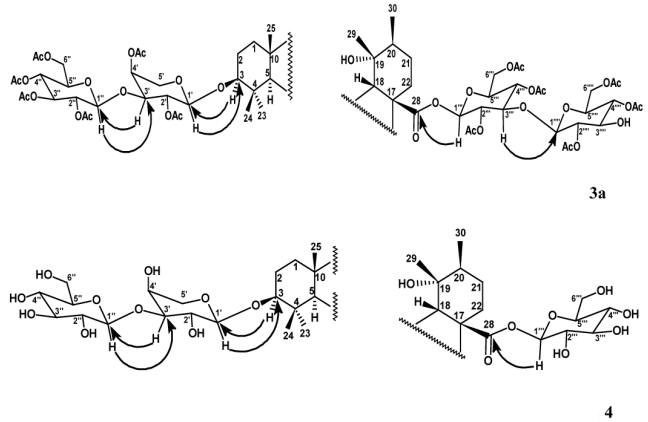

Figure 2.** Main long range correlations observed in HMBC spectra for **3a** and **4

Comparative analysis of the ^{13}C-NMR-{^1H} and ^{13}C-NMR-DEPT spectra of **4** was also used to identify the presence of signals attributed to 7 methyl, 12 methylene and 20 methine groups and 8 quaternary carbon atoms. The NMR data (^{13}C; ^1H) of compound **4** were very similar to compound **3a** excepting the absence of the acetyl characteristic signals and those corresponding to one glucose. After detailed analysis of the spectroscopic data of **4** it was possible to conclude that this compound contained a single glucose residue in the ester chain at C-28, contrasting to **3a** and **3** that contained two glucose units. Thus, **4** was identified as 3β-*O*-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl-20(*S*)-19α-hydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (Figure 1). The HH and HC correlation (COSY, HMQC and HMBC) allowed to assign all carbon and proton signals and to identify all interglycosidic linkages (Figure 2). The 20*R* enantiomer of this compound has already been reported

Table 1. ^{1}H and ^{13}C NMR spectral data for triterpenoids **3a**, **4** and **5** compared to literature

	3a, $CDCl_3$		**4**, $CDCl_3$		**5**, CD_3OD	Mo-1, CD_3OD	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_C	δ_H
CH_2-1	38.6		38.5		39.9	39.90	1.64, 0.99
CH_2-2	26.0		25.8		21.1	27.05	1.84, 1.73
CH-3	89.9	3.10(m)	89.2	3.10 (m)	90.9	90.56	3.15 (dd, 11.5, 4.0)
C-4	39.9		38.8		40.2	40.20	
CH-5	55.6		55.7		57.0	57.03	0.79
CH_2-6	18.2		18.0		19.4	19.44	1.53
CH_2-7	33.0		32.8		34.2	34.14	1.54, 1.33
C-8	39.2		39.8		42.7	41.23	
CH-9	47.1		47.8		48.0	48.59	1.67
C-10	36.6		38.8		37.8	37.84	
CH_2-11	23.8		23.3		24.6	24.73	1.97
CH-12	128.0	5.35 (br s)	127.5	5.15 (br s)	129.8	129.66	5.33 (br s)
C-13	137.0		137.8		139.5	139.50	
C-14	41.2		41.3		42.9	42.59	
CH_2-15	28.0		28.2		29.7	29.63	1.83, 1.02
CH_2-16	25.8		25.7		26.5	26.51	2.16 (td), 1.63
C-17	47.5		48.8		49.0	49.41	
CH-18	46.3	2.77 (s)	46.4	2.65 (s)	54.9	54.88	2.51 (s)
C-19	72.9		72.9		72.9	73.63	
CH-20	41.4		41.8		41.3	42.87	1.36
CH_2-21	23.6		23.6		27.2	27.19	1.71
CH_2-22	30.6		30.8		32.2	38.24	1.77, 1.63
CH_3-23	27.8	1.10 (s)	27.1	0.95 (s)	28.5	28.59	1.05 (s)
CH_3-24	15.6	0.90 (s)	15.6	0.75 (s)	17.0	17.06	0.85 (s)
CH_3-25	16.2	0.85 (s)	14.6	0.85 (s)	16.0	16.06	0.95 (s)
CH_3-26	16.8	0.71 (s)	16.3	0.68 (s)	17.6	17.61	0.77 (s)
CH_3-27	24.0	1.20 (s)	22.9	1.22 (s)	24.7	24.73	1.33 (s)
C-28	175.7	-	177.1	-	178.5	178.46	
CH_3-29	29.9	1.30 (s)	28.9	1.06 (s)	28.9	27.14	1.20 (s)
CH_3-30	15.2	1.00 (d, 6.0)	14.8	0.89 (d, 5.6)	16.6	16.63	0.93 (d, 6.8)
sugars			3-*O*-Ara		3-*O*-GlcA		
CH-1'	104.2	4.30 (d, 6.9)	105.6	4.19 (d, 7.2)	106.7	107.01	4.29 (d, 7.0)
CH-2'	72.9		69.7		73.9	72.04	3.71
CH-3'	79.8		82.4		77.9	83.80	3.65
CH-4'	72.9		68.1		75.5	69.46	4.03 (br s)
CH-5'	-		-		78.3	-	
CH_2-5'	63.0		65.2		-	66.62	3.86 (dd), 3.55 (d)
C-6'	-		-		180.8	-	
CH_2-6'	-		-			-	
			3-*O*-Glc				
CH-1''	100.6	4.72 (d, 7.1)	103.9	4.45 (d, 7.8)		105.30	4.56 (d, 7.3)
CH-2''	70.0		73.9			75.25	3.31
CH-3''	72.0		76.9			77.80	3.31
CH-4''	68.0		69.8			71.13	3.37
CH-5''	71.9		76.5			77.56	3.49
CH_2-6''	61.5		61.0			62.34	3.82 (dd), 3.70
			28-*O*-Glc				
CH-1'''	91.7	5.55 (d, 8.1)	94.4	5.25 (d, 8.2)	95.8	95.70	5.33 (d, 8.3)
CH-2'''	70.2		72.5		73.9	73.78	3.33
CH-3'''	73.9		77.3		78.6	78.45	3.35
CH-4'''	67.9		70.7		71.1	71.05	3.37
CH-5'''	72.4		76.3		78.3	78.20	3.42
CH_2-6'''	62.9		61.0		62.4	62.39	3.82 (dd), 3.70
CH-1''''	99.7	4.73 (d, 7.8)					
CH-2''''	69.9						
CH-3''''	72.0						
CH-4''''	68.0						
CH-5''''	71.9						
CH_2-6''''	61.5						

Mo-1 = 20(*R*) = randiasaponin III[16] = ilekudinoside E[15]

in *Ilex kudincha*[15] and *Randia formosa.*[16] Thus, **4** is the epimer of ilekudinoside E and randiasaponin III.

In relation to **5**, through comparison of its ^{13}C-NMR-{1H} and ^{13}C-NMR-DEPT spectra, it was possible to identify the presence of 7 methyl, 10 methylene and 16 methine groups and 8 quaternary carbon atoms. ^{1}H-NMR joined to ^{13}C-NMR data indicated the presence of two sugar residues. The later spectrum also evidenced the presence of one glucose residue linked to C-28 via an ester linkage (anomeric carbon at δ_C 95.8) and the presence of glucuronic acid moiety (δ_C 106.7) attached to C-3. Comparison of the NMR data of compound **5** to those of the literature allowed the identification of its aglycone as pomolic acid, one 19α-hydroxyursolic acid derivative possessing a 20*R*-configuration.[17] The β configuration for the glucopyranosyl and glucuronopyranosyl units and the α configuration for the arabinopyranosyl residues were inferred from their ^{13}C-NMR data and *J* values.[18] These data suggested that **5** is the 3β-*O*-β-D-glucuronopyranosyl-20(*R*)-19α-hydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (Figure 1) which was already isolated from *Ilex kudincha* by Nishimura *et al*.[15] as ilekudinoside B.

To the best of our knowledge, the saponins **3** and **4** reported herein have not been yet described in the literature. Moreover, excepting few reports,[8,19] the co-occurrence of triterpenes with configuration 20*R* and 20*S* is not a common feature to the *Ilex* species already studied. Indeed, it is possible to differentiate *I. chamaedryfolia* from other South-American *Ilex* considering their saponins.[8]

CONCLUSIONS

Several works have demonstrated that the saponins isolated from leaves of *I. paraguariensis* (maté) are different from those found in other South-American *Ilex* species.[1,3-6,8] The aglycone pattern 19-hydroxyursolic and 29-hydroxyursolic acid found in saponins isolated from South-American *Ilex* species investigated up to now, allowed to differentiate all this species from *I. paraguariensis* leaves whose saponins are glycosides of ursolic or oleanolic acid. As suggested by our previous results,[8] saponin content of South-American *Ilex* species can be used as a chemotaxonomic tool to the genera. This was confirmed herein considering that saponins so far isolated from *I. chamaedryfolia* are based on 19-hydroxyursane triterpenes which are different from those presented in the leaves of *I. paraguariensis*.

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