J. Braz. Chem. Soc., Vol. 12, No. 1, 32-36, 2001.
Printed in Brazil

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0103 - 5053 $6.00+0.00

Article

Steroidal and Triterpenoidal Glucosides from *Passiflora alata*

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Cinco glicosídeos foram isolados a partir das folhas de *P. alata*. Após extensivas análises espectroscópicas, as estruturas **1-5** foram identificadas como sendo o 3-O-β-D-glicopiranosil-estigmasterol (**1**), o ácido 3-O-β-D-glicopiranosil-oleanólico (**2**), o ácido 3-O-β-D-glicopiranosil-(1→3)-β-D-glicopiranosil-oleanólico (**3**), o ácido 3-O-β-D- glicopiranosil-(1→2)-β-D-glicopiranosil-oleanólico (**4**) e 9,19-ciclolanost-24*Z*-en-3β,21,26-tri-hidróxi-3,26-di-O-gentiobiose (**5**). Adicionalmente, foram analisados, através de CCD, extratos hidroetanólicos de espécies de *Passiflora* existentes no sul do Brasil (*P. actinia*, *P. caerulea, P. edulis* var. *flavicarpa*, *P. elegans*, *P. foetida*, *P. misera* e *P. tenuifila*). A acumulação de saponinas foi verificada somente em *Passiflora alata*.

Five glycosides were isolated from leaves of *P. alata*. The structures **1**-**5** were obtained through extensive spectral analyses as 3-O-β-D-glucopyranosyl-stigmasterol (**1**), 3-O-β-D-glucopyranosyl-oleanolic acid (**2**), 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-oleanolic acid (**3**), 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-oleanolic acid (**4**) and 9,19-cyclolanost-24*Z*-en-3β,21,26-trihydroxy-3,26-di-O-gentiobiose (**5**). Comparison of the TLC profiles of the hydroethanolic extracts from leaves of other *Passiflora* species found in the south of Brazil (*P. actinia*, *P. caerulea, P. edulis* var. *flavicarpa*, *P. elegans*, *P. foetida*, *P. misera* and *P. tenuifila*) showed that only *P. alata* presented saponin accumulation.

Keywords: Passifloraceae, *Passiflora alata*, passionflower, saponins

Introduction

Passion fruits that are nowadays grown throughout the tropics are native to Latin America and Brazil is the leading exporter of passion fruit juice[1]. Additionally to this alimentary function as flavor and as juice in food industries, passionflower extract has an ancient tradition in the folk medicine of American and even European countries due its reputed sedative and tranquilizing properties[2].

Although *Passiflora alata* is an official drug of the Brazilian Pharmacopoeia[3] and its leaf extract is included as an active component in many Brazilian registered pharma-ceutical preparations[4], there are only a few investigations on its chemical components[5] and pharmacological properties[6,7].

In view of its pharmaceutical utilization, the knowledge of the chemical components of *P. alata* is important for the development of methods for the quality control of drug and phytopharmaceutical preparations. Moreover, since it has recently been shown that *P. alata* could induce respiratory allergy[8], quality control methods could be extended to analyze pretended hypoallergenic drugs or beverages containing passionflower extracts. Also, the phytochemical study of *P. alata* is justified in view of searching for the still unknown therapeutically active or allergenic substances.

This paper describes the isolation and structure elucidation of one steroid glycoside (**1**) and four triterpene saponins (**2**-**5**) from the leaves of *P. alata*. The use of these compounds as natural markers is also discussed.

Experimental

Plant material

Aerial parts of *Passiflora alata* Dryander were collected in São Leopoldo, State of Rio Grande do Sul, Brazil, in February

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1997. A herbarium specimen (ICN 8344) is on deposit in the Herbarium of the Botany Department of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. Aerial parts of native *P. actinia* Hooker, *P. caerulea* L., *P. edulis* Sims var. *flavicarpa*, *P. elegans* Masters, *P. foetida* L., *P. misera* H.B.K. and *P. tenuifila* Killip were collected in different cities of the State of Rio Grande do Sul.

Extraction and isolation

Air-dried powdered leaves of *P. alata* (750 g) were extracted by maceration in EtOH (plant:solvent, 1:10, w/v) (2 x 10 days). After evaporation of the ethanolic extract, the gummy residue was suspended in H_2O (500 mL) and extracted successively (4 x 200 mL) with chloroform, ethyl acetate and *n*-butanol. Evaporation of the *n*-butanol fraction yielded the crude saponin fraction (26 g) whose a part (12 g) was chromatographed on a Si gel column using $CHCl_3$:EtOH:AcOH (60:40:6, v/v) yielding pure **2** (7 mg), fractions A (40 mg), B (95 mg), C (180 mg) and D (840 mg). Fraction A was, after usual peracetylation[9], submitted to preparative TLC [Si gel GF_{254}, mobile phase AcOEt:cyclohexane (1:1, v/v)] yielding 17 mg of peracetylated **1** (**1a**) and 14 mg of peracetylated **2** (**2a**). Fraction B yielded pure **3** by CC (15 mg) using *n*-BuOH:AcOH:H_2O (5:3:1, v/v) and, after peracetylation and CC, peracetylated **3** (**3a**) (12 mg). Compound **4** (35 mg) was obtained from fraction C by precipitation. Compound **5** (230 mg) was isolated from fraction D through CC using *n*-BuOH saturated with H_2O.

Chromatographic analysis

Analytical TLC aluminum sheets coated with Si gel GF_{254} (Merck) were used. Air-dried powdered leaves of *Passiflora* species were extracted, separately, by maceration in EtOH (plant:solvent, 1:10, w/v) (2 x 10 days). After evaporation of the ethanolic extracts, the gummy residues were dissolved, separately, in MeOH for TLC comparison. Saponins were analyzed using $CHCl_3$:EtOH:AcOH (60:40:6, v/v) as the mobile phase and the spots were visualized by heating (100 °C) the anisaldehyde-H_2SO_4-sprayed plates.

General

FAB-MS analysis were performed in positive mode on a Kratos MS 80 instrument. NMR spectra were recorded on a Bruker AM 400 spectrometer. Compounds **2**, **3** and **4** were hydrolyzed as described by Kartnig and Wegschaider[10] in order to analyze the aglycone and sugar components.

Peracetylated compound **1 (1a)**

Peracetylated 3-O-β-D-glucopyranosyl-stigmasterol. ^1H NMR (400 MHz, $CDCl_3$) δ 0.69 (s, 3 H, CH_3-18), 0.79 (d, *J* 7.0 Hz, 3 H, CH_3-26 or CH_3-27), 0.80 (t, *J* 7.0 Hz, 3 H, CH_3-29), 0.84 (d, *J* 6.5 Hz, 3 H, CH_3-26 or CH_3-27), 0.98 (s, 3 H, CH_3-19), 1.01 (d, *J* 6.5 Hz, 3 H, CH_3-21), 1.25 (br, s, 2 H, H-25, H-16), 1.52 (m, 1 H, H-24), 2.00-2.10 (4 x OAc), 3.49 (m, Hα-3), 3.68 (ddd, *J* 9.9, 4.8, 2.5 Hz, 1 H, glc-H5), 4.10 (dd, *J* 12.0, 2.5 Hz, 1 H, glc-H6b), 4.25 (dd, *J* 12.0, 4.9 Hz, 1 H, glc-H6a), 4.60 (d, *J* 8.0 Hz, 1 H, glc-H1), 4.96 (dd, *J* 9.5, 8.0 Hz, 1 H, glc-H2), 5.05 (m, 1 H, H-23), 5.10 (m, 1 H, glc-H4), 5.15 (m, 1 H, H-22), 5.20 (t, *J* 9.5 Hz, 1 H, glc-H3), 5.35 (br, s, *J* 5.1 Hz, 1 H, H-6); ^{13}C NMR see Table 1.

Peracetylated compound **2 (2a)**

Peracetylated 3-O-β-D-glucopyranosyl-oleanolic acid. ^1H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 4 H, CH_3-26, H-5), 0.91 (s, 6 H, CH_3-29, CH_3-24), 0.92 (s, 6 H, CH_3-30, CH_3-23), 1.10 (s, 3 H, CH_3-27), 1.25 (s, 3 H, CH_3-25), 2.02-2.08 (4 x OAc), 2.81 (dd, *J* 4.3, 13.6 Hz, H-18), 3.11 (dd, *J* 4.8, 11.5 Hz, 1 H, H-3), 3.69 (ddd, *J* 2.7, 5.5, 10.0 Hz, glc-H5), 4.12 (dd, *J* 3.0, 12.0 Hz, glc-H6b), 4.26 (dd, *J* 5.3, 12.0 Hz, glc-H6a), 4.55 (d, *J* 8.0 Hz, glc-H1), 5.03 (dd, *J* 9.8, 8.0 Hz, glc-H2), 5.06 (dd, *J* 9.0, 8.5 Hz, glc-H4), 5.22 (t, *J* 9.5 Hz, glc-H3), 5.26 (t, *J* 3.5 Hz, H-12); ^{13}C NMR see Table 1.

Peracetylated compound **3 (3a)**

Peracetylated 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-oleanolic acid. ^1H NMR according to the literature[11]; ^{13}C NMR see Table 1.

Compound **4**

3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-oleanolic acid. ^1H NMR (400 MHz, C_5D_5N) δ 0.81 (s, 3 H, CH_3-24), 0.94 (s, 3 H, CH_3-29), 0.98 (s, 3 H, CH_3-26), 1.00 (s, 3 H, CH_3-30), 1.08 (s, 3 H, CH_3-25), 1.25 (s, 3 H, CH_3-23), 1.28 (s, 3 H, CH_3-27), 3.29 (m, 2 H, Hα-3, H-18), 3.89 (m, 2 H, glcI-H5, glcII-H5), 4.11 (m, 1 H, glcII-H2), 4.14 (m, 1 H, glcI-H4), 4.23 (m, 1 H, glcII-H3), 4.25 (m, 1 H, glcI-H2), 4.30 (m, 2 H, glcI-H6a, glcII-H6a), 4.31 (m, 1 H, glcI-H3), 4.33 (m, 1 H, glcII-H4), 4.46-4.52 (m, 2 H, glcI-H6b, glcII-H6b), 4.90 (d, *J* 7.6 Hz, glcI-H1), 5.36 (d, *J* 7.6 Hz, glcII-H1), 5.46 (br, s, 1 H, H-12); ^{13}C NMR see Table 1; fab-ms (positive ion mode) *m/z* 803 $(M+Na)^+$, 273, 203.

Compound **5**

(quadranguloside): 9,19-cyclolanost-24*Z*-en-3β,21,26-trihydroxy-3,26-di-O-gentiobiose (quadranguloside). ^1H

Table 1. ^{13}C NMR (100 MHz) chemical shift data of **1a**, **2a**, **3a**, **4** and **5**.

	δ (ppm)					
Carbon	**1a** $CDCl_3$	**2a** $CDCl_3$	**3a** $CDCl_3$	**4** C_5D_5N	**5** C_5D_5N	
1	38.9	38.8	38.4	38.7	32.3	
2	31.8	27.7	27.6	26.6	30.2	
3	80.0	90.5	90.5	89.0	88.9	
4	42.2	38.3	38.8	39.5	41.4	
5	140.3	55.4	55.5	55.8	47.6	
6	122.1	18.1	18.1	18.5	21.3	
7	31.8	29.7	32.6	33.3	27.9	
8	31.8	39.2	39.2	39.7	47.6	
9	50.2	47.6	47.6	48.0	20.1	
10	36.7	36.7	36.7	36.9	26.4	
11	21.2	23.6	23.4	23.8	26.4	
12	39.6	122.2	122.6	122.5	35.9	
13	42.2	143.9	143.5	145.0	45.6	
14	56.8	41.6	41.6	42.2	49.1	
15	24.3	25.8	25.7	28.3	32.4	
16	29.7	23.0	22.9	23.8	26.7	
17	55.9	46.5	46.4	46.7	43.1	
18	12.0	41.0	40.9	42.0	18.8	
19	19.3	46.0	45.9	46.5	30.0	
20	40.5	30.6	30.6	31.0	46.8	
21	21.2	33.9	33.8	34.3	61.8	
22	138.2	32.6	32.4	33.3	30.9	
23	129.3	27.7	27.7	28.2	25.2	
24	51.2	15.2	15.2	15.5	131.3	
25	31.8	16.3	16.2	16.8	132.0	
26	21.0[a)]	17.0	17.0	17.4	67.8	
27	19.0[a)]	25.7	25.8	26.2	22.3	
28	24.3	183.9	182.6	180.4	19.9	
29	12.2	33.1	33.0	33.2	25.9	
30	-	23.4	23.5	23.8	15.6	
					β-gentiobiose	
glcI-1	99.6	102.9	103.0	105.1	glcI-I'	103.4/106.9
glcI-2	71.5	71.6	73.2	83.4	glcI-I'	75.0/75.7
glcI-3	72.9	72.8	78.9	78.0	glcI-I'	78.4/78.4
glcI-4	68.5	68.7	68.7	71.7	glcI-I'	71.7/71.7
glcI-5	71.7	71.5	71.4	78.4	glcI-I'	77.3/77.2
glcI-6	62.1	62.2	61.7	62.8	glcI-I'	70.3/70.0
glcII-1	-		100.9	106.0	glcII-II'	105.4/105.5
glcII-2	-		71.6	77.1	glcII-II'	75.3/75.2
glcII-3	-		72.9	77.9	glcII-II'	78.6/78.5
glcII-4	-		68.1	71.7	glcII-II'	71.7/71.6
glcII-5	-		71.0	78.3	glcII-II'	78.5/78.5
glcII-6	-		62.4	62.7	glcII-II'	62.9/62.8

[a)]interchangeable attributions: Acetate **1a**: 170.7, 170.4, 169.4, 169.3, 20.8, 20.7, 20.6 (2x); Acetate **2a**: 170.6, 170.3, 169.4, 169.1, 20.7 (2x), 20.6 (2x); Acetate **3a**: 170.7, 170.5, 170.3, 169.3, 169.2 (2x), 168.7, 21.0, 20.7 (2x), 20.6, 20.5 (2x), 20.2.

NMR (400 MHz, C_5D_5N) δ 0.20 (br, s, 1 H, H-19a), 0.45 (br, s, 1 H, H-19b), 0.9 (s, 3 H, CH_3-28), 1.03 (s, 3 H, CH_3-18), 1.07 (s, 3 H, CH_3-30), 1.27 (s, 3 H, CH_3-29), 1.93 (s, 3 H, CH_3-27), 3.58 (m, 1 H, Hα-3), 3.82 (m, 1 H, H-21a), 3.90 (m, 4 H, glcII-H3, glcII'-H3, glcII-H5, glcII'-H5), 4.05 (m, 7 H, H-21b, glcI-H2, glcI'-H2, glcII-H2, glcII'-H2, glcI-H5, glcI'-H5), 4.20 (m, 6 H, glcI-H3, glcI'-H3, 4 x glc-H4), 4.33 (m, 4 H, glcI-H6, glcII'-H6), 4.48 (m, 2 H, glcII-H6), 4.55 (m, 2 H, H-26), 4.82 (m, 3 H, glcI-H1, glcI'-H6), 4.89 (d, *J* 8.0 Hz, 1 H, glcI'-H1), 5.10 (d, *J* 7.6 Hz, 1 H, glcII'-H1), 5.15 (d, *J* 8.0 Hz, 1 H, glcII-H1). 5.52 (t, *J* 6.8 Hz, H-24); ^{13}C NMR see Table 1.

Results and Discussion

The *n*-BuOH extract of *P. alata* was submitted to Si gel CC to yield compounds **1-5** and their identification was achieved through the combined use of mass and NMR spectroscopy.

The ^{1}H NMR spectrum of **1a** displayed signals at δ 0.69 and 0.98 (s, 3 H each) for two tertiary methyl groups, two doublets (3 H each) at δ 0.79 (*J* 7.0 Hz) and 0.84 (*J* 6.5 Hz) assignable to an isopropyl group and the presence of another secondary methyl at δ 1.01 (d, *J* 6.5 Hz, 3 H). A triplet centered at δ 0.80 (*J* 7.0 Hz, 3 H) was attributed to a primary

methyl. These characteristic signals suggested a steroid skeleton. Three olefinic proton signals at δ 5.35 (br, d, J 5.1 Hz), δ 5.15 (m) and at δ 5.05 (m) were attributed to H-6, H-22 and H-23, respectively, and together with the signal at δ 3.49 (m, 1 H, Hα-3) they are characteristic for $\Delta^{5,22}$-3β-hydroxysterols[12,13,14]. Comparison of ^{13}C NMR spectrum (Table 1) and the 2D-HSQC spectrum with spectral data of related sterols found in the literature indicated that compound **1** is the sterol glycoside 3-O-β-D-glucopyranosyl-stigmasterol isolated as a peracetylated derivative. Its occurrence in nature has only been reported several times although the aglycone is widely found.

Acid hydrolysis of **2**, **3** and **4** furnished oleanolic acid and glucose (Glc). Both were identified through co-TLC with authentic samples.

The FAB-MS (positive ion mode) spectrum of **2** indicated the molecular mass 618 due to the quasi-molecular ion peak at *m/z* 641 (M+Na)$^+$. In addition, there were two characteristic peaks at *m/z* 203 and 248 denoting the retro-Diels-Alder fragmentation commonly found in the spectra of oleanane or ursane derivatives[15]. Careful comparison of the ^{13}C NMR data of peracetylated compound **2a** (Table 1) with those of peracetylated dumosasaponin 6[9], allowed the unambiguous assignment of the signals of the oleanolic acid aglycone. The presence of one β-D-Glc was evidenced through the anomeric signal at δ_c 102.9 (δ_H 4.55, d; J 8.0 Hz, 1 H) and the sugar substitution at C-3 of the aglycone was established (δ C-3 90.5). These conclusions were confirmed by ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and ROESY experiments. Therefore, compound **2** was identified as 3-O-β-D-glucopyranosyl-oleanolic acid, already isolated from *Chenopodium quinoa*[16].

For compound **3** the molecular formula $C_{42}H_{68}O_{13}$ was deduced based on the FAB-MS spectrum, which displayed a pseudo-molecular ion peak at *m/z* 803 (M+Na)$^+$ and it also showed fragments at *m/z* 203 and *m/z* 248. Detailed comparison of ^{13}C NMR data of **3a** and **2a** showed that **3a** structurally differs from **2a** only by the presence of signals for another hexose. Thus, as in the case of **2a**, compound **3a** presented a free carboxyl group at position C-28 while a Glc**,**Glc-constituted disaccharide was substituted at C-3.

Considering the sugar carbon chemical shifts, the correlation experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY) and literature data, we could identify **3a** as the peracetylated derivative of 3-O-β-D-glucopyranosyl-(1→3)-β-D-gluco-pyranosyl-oleanolic acid, an already known saponin[11].

The great similarity of the ^{13}C NMR spectrum of **4** with that of **3a** (Table 1) showed that **4** was also a derivative of oleanolic acid with two Glc residues substituted at carbon 3. The FAB-MS spectrum of **4** exhibited a peak at *m/z* 803 (M+Na)$^+$ and was consistent with the presence of these two Glc residues. The interglycosidic linkage of this disaccharide was deduced to be Glc(1→2)Glc from the deshielding in the ^{13}C NMR spectrum of **4** of one of the CH units of this moiety (δ 83.4). Thus, **4** was determined to be 3-O-β-D-gluco-pyranosyl-(1→2)-β-D-gluco-pyranosyl-oleanolic acid, already isolated from *Passiflora quadrangularis*[17] and *Luffa acutangula*[18].

The most singular feature of **5** is the presence of two upfield shifted signals at δ 0.20 and 0.45 in the ^{1}H NMR spectrum which are characteristic of geminal cyclopropane protons. It also displayed signals corresponding to five methyl singulets (δ 0.9, 1.03, 1.07, 1.27, 1.93) and one olefinic proton at δ 5.52 (t, J 6.8 Hz) together with four anomeric protons (δ 4.82, 4.89, 5.10 and 5.15). These data, in accor-

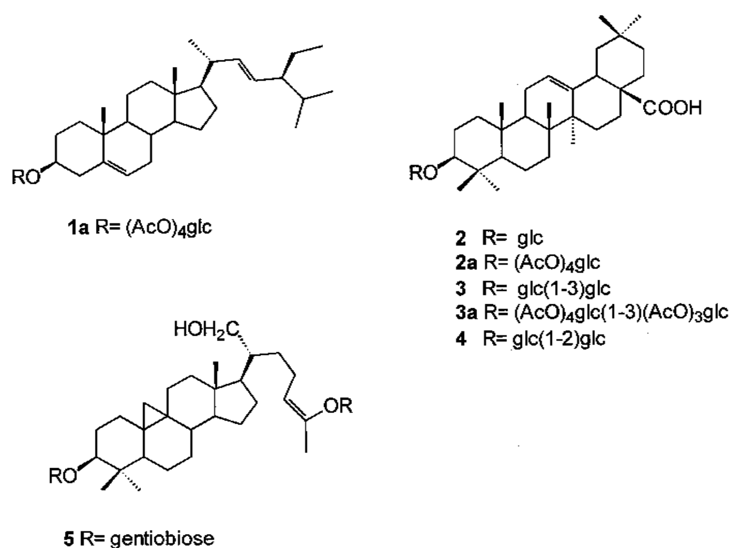

Figure 1.

dance with ^{13}C NMR spectrum (Table 1), suggested a cycloartane triterpene skeleton. ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY experiments showed the presence of two gentiobioses located at positions C-3 and C-26. Through detailed comparison of these data with those from the literature, **5** was identified as 9,19-cyclolanostan-24*Z*-en-3β,21,26-trihydroxy-3,26-di-O-gentiobiose, already isolated from *Passiflora quadrangularis*[19] and named quadranguloside.

This study allowed us to identify five glycosides from the leaves of *P. alata*. Although none of them are specific to *P. alata*, their association in a single plant is unique, so we wonder if we could use them as a phytochemical tracer to either authentify the exclusive use of *P. alata* in pharmaceutical preparations, or to certify the lack of *P. alata* in therapeutic preparations elaborated from other passionflower species. Furthermore, the TLC profiles of the hydroethanol extracts from the leaves of *Passiflora* species found in the State of Rio Grande do Sul (south of Brazil), *P. actinia*, *P. alata, P. caerulea, P. edulis* var. *flavicarpa*, *P. elegans*, *P. foetida*, *P. misera* and *P. tenuifila* showed accumulation of saponins only in *P. alata*. As far as we know, saponins have only been reported for *P. quadrangularis* L.[17,19] and *P. edulis* Sims[20].

Since secondary metabolite content can vary as a function of multiple factors (such as environmental conditions and harvest period), reproduction of this analysis over a long period of time is of course needed before the effectiveness of our method is totally demonstrated. We are currently pursuing this goal.

Acknowledgments

We are grateful to Marcos Sobral from the Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, and Cláudio Mondin from the Universidade do Vale do Rio dos Sinos, São Leopoldo, RS, for locating, identifying and helping to collect the plant material, to the Chemical Department of the Universidade Federal de Santa Maria, Santa Maria, RS, for recording part of the NMR spectra, and to the Centro Nacional de Ressonância Magnética Nuclear of the Departamento de Bioquímica, Universidade Federal do Rio de Janeiro, for access to the NMR facilities. This work was supported by research stipends from Capes and CNPq (Brazil).

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Received: May 5, 2000
Published on the web: October 25, 2000