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Pentacyclic triterpenes and steroids from the stem bark of uchi (*Sacoglottis uchi*, Humiriaceae)

Viviane Gomes da Costa ABREU[1*], Geone Maia CORRÊA[1,2], Izandina Aparecida dos Santos LAGOS[3], Roqueline Rodrigues SILVA[1], Antônio F. de C. ALCÂNTARA[1]

ABSTRACT

The ethanol extract from stem bark of *Sacoglottis uchi* Huber (popularly known as "uchi" in the Amazon Region) was submitted to chromatographic fractionation. The dichloromethane fractions provided the pentacyclic triterpene 3-oxo-friedelin (1). The dichloromethane:methanol fractions provided the pentacyclic triterpenes pseudotaraxasterol (2), lupeol (3), α-amyrin (4), betulin (5), and methyl 2β,3β-dihydroxy-urs-12-en-28-oate (6) and a mixture of the steroids sitosterol (7) and stigmasterol (8). Their chemical structures were determined by NMR spectroscopy and comparison with spectroscopic data from the literature. All compounds are described for the first time in this species.

KEYWORDS: Humiriaceae, medicinal plants, Amazon Region, triterpenes

Triterpenos pentacíclicos e esteróides da casca do uchi (*Sacoglottis uchi*, Humiriaceae)

RESUMO

O extrato etanólico da casca do caule de *Sacoglottis uchi* Huber (conhecida popularmente como "uchi" na Amazônia) foi submetido a fracionamento cromatográfico. As frações eluídas com diclorometano forneceram o triterpeno pentacíclico 3-oxo-friedelina (1). As frações em diclorometano:metanol forneceram os triterpenos pentacíclicos pseudotaraxasterol (2), lupeol (3), α-amirina (4), betulina (5) e 2β,3β-di-hidroxi-urs-12-en-28-oato de metila (6), além de uma mistura dos esteróides sitosterol (7) e estigmasterol (8). Suas estruturas químicas foram determinadas por espectroscopia de RMN e comparação com os dados espectroscópicos descritos na literatura. Todas as substâncias isoladas são descritas pela primeira vez nesta espécie.

PALAVRAS-CHAVE: Humiriaceae, planta medicinal, Região Amazônica, triterpenos

[1] Departamento de Química, ICEx, Universidade Federal de Minas Gerais, UFMG, 31270-901 Belo Horizonte – MG, Brazil – vivianegca@ufmg.br, roquelinersm@qui.ufmg.br, aalcantara@zeus.qui.ufmg.br

[2] Instituto de Ciências Exatas e Tecnologia, Universidade Federal do Amazonas, UFAM, 69100-000 Itacoatiara – AM, Brazil – geonemaia@ufam.edu.br

[3] Coordenação de Pesquisas em Produtos Naturais, Instituto Nacional de Pesquisas da Amazônia, INPA, 69080-971 Manaus, AM – Brazil – izandina@inpa.gov.br

The genus *Sacoglottis* (Humiriaceae) is represented by nine species which are mostly found in the Americas as large trees in tropical regions or small scrubs in neotropical ones (Gentry, 1977). Only the species *S. gabonensis* is found in the Western of the Africa. The species *S. uchi* Huber [sin. *Endopleura uchi*] is found in highland of the Amazon Forest and is popularly known as "uchi", "uxi", "uxi-amarelo", "pururu", "uxi-liso", "uxi-ordinário" or "uchi-pucu" (Politi *et al*. 2010). The wood of this species is widely used in civil and naval constructions and the fruits are an important food in rural communities of the Amazon Region (Shanley *et al.* 2002). Moreover, the macerated bark is popularly used for the treatment of arthritis, diabetes, and inflammations (Magalhães *et al.* 2007).

The literature reports few chemical studies about *S. uchi*. The fruit pulp contains oleic acid and *trans*-β-carotene as majority components, besides other carotenoid compounds (Silva *et al.* 2009). Recently, we described the isolation of (+)-bergenin from the bark of this species (Abreu *et al.* 2008). Bergenin exhibits high antioxidant activity and is widely used by oriental folk medicine for the liver disease treatment (Takahashi *et al.* 2003).

The present work describes the isolation of other constituents from the bark of *S. uchi* (Figure 1). The pentacyclic triterpenes 3-oxo-friedelin (1), pseudotaraxasterol (2), lupeol (3), α-amyrin (4), betulin (5), and methyl 2β,3β-dihydroxy-urs-12-en-28-oate (6) and the mixture of the steroids sitosterol (7) and stigmasterol (8) were identified by IR and 1D and 2D NMR analyses. The compounds 1-8 were isolated for the first time in this species.

The stem barks of *S. uchi* were collected in February 2004 in the City of Manaus (State of Amazonas, Brazil). A voucher specimen of the plant was deposited in the herbarium of the Instituto Nacional de Pesquisas da Amazônia (INPA), under the code 82,627.

The stem barks were dried at room temperature and milled, giving 3.0 kg of powdered material. This material was submitted to extraction with ethanol at room temperature, providing the ethanol extract (EE; 11.83 g). The EE was submitted to column chromatography (CC) using silica gel as stationary phase and eluted with pure or mixtures of hexane, dichloromethane (DCM), and methanol. Chromatographic fractionation of EE was followed by thin layer chromatography (TLC) employing silica gel with fluorescence indicator F_{254} and monitored under UV light and iodine vapor, to give 74 fractions. The similar fractions were combined in groups based on TLC analysis.

The Group 1 (eluted with DCM) provided a white solid (1; 8.0 mg). The Group 2 (eluted with mixture of DCM/MeOH 10:1) provided a white solid (**2**; 11.0 mg). The Group 3 (eluted with mixture DCM/MeOH 3:1) provided a white crystalline solid (mixture of 3-6; 15.0 mg). The Group 4 (eluted with mixture DCM/MeOH 2:1) provided a white crystalline solid (mixture of 7-8; 13.0 mg).

The melting points (m.p.) were measured on a Mettler model FP62 apparatus. The IR spectra were recorded on the FT-IR Spectrometer Bomem-M102 and Perkin-Elmer Spectrum 2000 FTIR equipments, using KBr as support. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 – AVANCE equipment, with probes and inverse gradient of the operative field in 400.129 and 100.613 MHz, respectively. Samples (8.0-15.0 mg) were dissolved in 0.75 mL of $CDCl_3$ and transferred to a tube 5 mm. TMS was used as internal reference for NMR chemical shifts (δ_H = 0.00), scale in ppm and coupling constants (*J*) in Hertz. The experiments were performed using pulse sequences and programs provided by the manufacturer. The 1D NMR (^{1}H and ^{13}C NMR) data were acquired under normal conditions, using a direct detection 5 mm probe $^{1}H/^{13}C$ double.

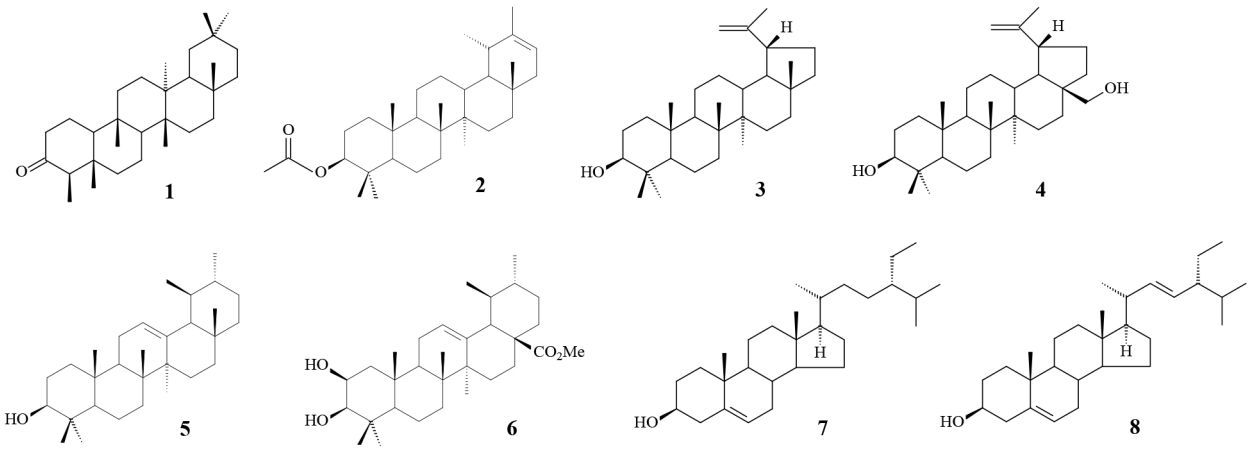

Figure 1 - Chemical structure of the pentacyclic triterpenes (1 to 6) and steroids (7 and 8) isolated from *Sacoglottis uchi*.

The IR data of 1 (m.p. 262-264 °C) show an intense absorption at 1730 cm^{-1} which was attributed to C=O stretch of ketones. The 1H NMR spectrum of **1** shows several overlapped signals in the characteristic region of aliphatic hydrogen atoms. 1H NMR (400 MHz; $CDCl_3$; ppm): δ_H 2.36–1.22 (overlapping signals), 1.25 (*s*, CH_3), 1.18 (*s*, CH_3), 1.05 (*s*, CH_3), 1.01 (*s*, CH_3), 0.95 (*s*, CH_3), 0.89 (*s*, CH_3), 0.84 (*s*, CH_3), and 0.73 (*s*, CH_3). The 13C NMR spectrum shows a signal at δ_C 213.2 (assigned to a carbonyl carbon of ketone) and other signals which are characteristic of saturated carbon atoms. The ^{13}C NMR data of **1** (Table 1) are in agreement with the corresponding data of the triterpene friedelin (Abreu *et al.* 2011).

Friedelin has been isolated from many vegetal species, such as roots of *Vismia laurentii* (Nguemeving *et al.* 2006) and leaves of *Maytenus salicifolia* (Miranda *et al.* 2006). Friedelin shows cytotoxicity against the human tumor cell lines (Mossi *et al.* 2004) and gastric antiulcerogenic and leishmanicidal activities (Surendra and Corey 2009; (Santos-Torres *et al.* 2004)

The 1H NMR spectrum of 2 shows a large signal at δ_H 5.26 (*d, J* = 6.2 Hz) which is characteristic of alkenyl hydrogen. The signal at δ_H 3.21 (*dd*, *J* = 10.0 and 5.6 Hz) was attributed to carbinolic hydrogen. The ^{13}C NMR spectrum shows signals at δ_C 140.1 and 119.7 which are attributed to alkenyl carbon atoms. The ^{13}C NMR data of 2 (Table 1) are in agreement with the correspondent data of the triterpene pseudotaraxasterol (Abreu *et al.* 2011). This compound exhibits antimicrobial activity against *Escherichia coli* and *Bacilus subtilis* (Xie *et al.* 2005) and are described for the first time in the genus *Sacoglottis*.

The ^{13}C NMR spectrum of Group 3 shows signals attributed to olefinic carbon atoms of triterpenes with lup-20(29)-ene-type skeleton (3: δ_C 150.9 and 109.3; 4: δ_C 150.7 and 109.7) and urs-12-ene-type skeleton (5: δ_C 124.5 and 139.9; 6: δ_C 126.9 and 138.3), as shown in Table 1. The signals at δ_C 78.9 and 79.1 are attributed to carbinol carbon atoms. The ^{13}C NMR data of 3 to 6 (Table 1) are in agreement with the corresponding data of the triterpenes lupeol, betulin, α-amyrin, and methyl 2β,3β-dihydroxy-urs-12-en-28-oate, respectively (Abreu *et al.* 2011).

Lupeol exhibits anti-inflammatory and antitumor activities (Geetha and Varalakshmi 1998). The compound α-amyrin exhibits anti-inflammatory (Hasmeda *et al.* 1999), insecticide, and anti-arthritic activities (Kweifio-Okai 1991). Betulin exhibits antitumor and antiviral activities (Mullaer *et al.* 2009; Bori *et al.* 2012).

The IR data of the Group 4 show weak absorptions between 1680 and 1650 cm^{-1} which are attributed to C=C stretchs. The absorptions at 1410 and 1185 cm^{-1} are attributed to the angular deformation of methyl groups and C-O stretch,

Table 1 - ^{13}C NMR data of the pentacyclic triterpenes isolated from *Sacoglottis uchi*

Carbon	Compound					
	1	2	3	4	5	6
C-1	22.3	39.0	38.7	38.8	38.7	44.9
C-2	41.2	27.6	27.4	27.2	27.2	71.9
C-3	213.2	79.2	79.1	79.1	79.1	79.1
C-4	58.2	39.1	38.9	38.9	38.9	38.1
C-5	42.1	55.5	55.2	55.3	55.2	55.0
C-6	41.2	18.5	18.4	18.4	18.4	18.0
C-7	18.2	34.6	34.3	34.3	33.9	33.0
C-8	53.0	41.3	40.0	40.0	40.0	39.6
C-9	37.4	50.6	50.2	50.5	47.8	47.8
C-10	59.4	37.3	37.3	37.3	37.2	46.9
C-11	35.6	21.8	21.1	21.6	23.1	23.4
C-12	30.4	27.9	25.2	25.2	124.5	126.9
C-13	39.6	39.4	38.1	37.3	139.9	138.3
C-14	38.2	42.4	42.3	42.3	42.3	42.2
C-15	32.4	27.3	27.5	27.2	28.0	27.9
C-16	35.9	36.9	35.6	29.2	26.7	24.3
C-17	29.9	34.7	43.1	47.8	33.9	48.4
C-18	42.7	48.9	48.4	48.8	59.1	53.0
C-19	35.3	36.5	48.0	47.8	39.6	39.1
C-20	28.1	140.1	151.0	150.6	39.6	38.9
C-21	32.7	11 9.1	29.7	29.8	31.5	30.7
C-22	39.2	42.5	40.0	33.9	41.5	36.2
C-23	6.8	28.2	28.0	28.0	28.0	29.8
C-24	14.6	15.6	15.4	15.4	16.0	17.4
C-25	17.9	16.5	16.1	16.1	16.0	16.1
C-26	20.2	16.3	16.0	16.0	16.8	16.9
C-27	18.6	15.0	14.2	14.6	23.1	23.1
C-28	32.0	17.9	18.0	60.4	28.0	178.7
C-29	35.0	22.8	109.3	109.7	17.5	17.0
C-30	31.7	21.8	19.4	19.1	21.1	21.1

respectively. The ^1H NMR spectrum shows signals at δ_H 5.35 (*d, J* = 4.4 Hz) and δ_H 5.21–4.94 attributed to alkenyl hydrogen atoms. The signal at δ_H 3.70–3.45 is characteristic of carbinol hydrogen atom. The ^{13}C NMR spectrum shows intense signals at δ_C 140.7 and 121.7 and less intense ones at δ_C 138.4 and 129.3 attributed to alkenyl carbon atoms, indicating that Group 4 is a mixture of two compounds with different proportions: sitosterol (**7**) and stigmasterol (8) (Costa *et al.* 2008). The integration of the ^1H NMR signals at δ_H 5.35 (assigned to H-6 of the sitosterol and stigmasterol) and δ_H 5.21–4.94 (assigned to H-22 and H-23 of the stigmasterol) indicates a ratio of 75.5% stigmasterol and 24.5% sitosterol. Both the steroids are described for the first time in the genus *Sacoglottis*.

Stigmasterol inhibits cholesterol biosynthesis via inhibition of sterol Δ24-reductase in human Caco-2 and HL-60 cell lines (Batta *et al.* 2006). Various action mechanisms of the sitosterol have been proposed, including anti-inflammatory effects,

alteration of cholesterol metabolism, and direct inhibition of prostate growth (Lowe and Ku 1996).

Therefore, some biological properties of the *S. uchi* can be attributed to the activities of the pentacyclic triterpenes 1 to 6 and steroids 7 and 8.

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