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Short Report

Triterpenoid Saponins from Stem Bark of *Pentaclethra macroloba*

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Duas novas e duas conhecidas saponinas triterpênicas foram isoladas da casca do caule de *Pentaclethra macroloba*. As estruturas foram determinadas usando uma combinação de técnicas de RMN homo- (1D: RMN^{1}H, RMN^{13}C-{^{1}H}, RMN^{13}C-DEPT135; 2D: ^{1}H-^{1}H-COSY, ^{1}H-^{1}H-TOCSY, ^{1}H-^{1}H-NOESY) and heteronuclear 2D (HMQC and HMBC), espectros de massas obtidos com ionização por pulverização eletrônica (ESIMS) e métodos químicos. As estruturas das duas novas saponinas triterpênicas foram estabelecidas como 3β-*O*-{[β-D-glicopiranosil-(1$\Downarrow$ 3)-α-L-ramnopiranosil-(1$\Downarrow$ 2)],β-D-glicopiranosil-(1$\Downarrow$ 4)}-α-L-arabinopiranosilhederagenina (**3**) e ácido 3β-*O*-{[β-D-glicopiranosil-(1$\Downarrow$ 4)-β-D-glicopiranosil-(1$\Downarrow$ 3)-α-L-ramnopiranosil-(1$\Downarrow$ 2)],β-D-glicopiranosil(1$\Downarrow$ 4)}-α-L-arabinopiranosiloleanólico (**4**).

Two new and two known triterpenoid saponins were isolated from the stem bark of *Pentaclethra macroloba*. Their structures were determined using a combination of homo- (1D ^{1}H NMR, ^{13}C NMR-HBBD and ^{13}C NMR-DEPT) and heteronuclear 2D NMR techniques (^{1}H-^{1}H-COSY, ^{1}H-^{1}H-TOCSY, ^{1}H-^{1}H-NOESY, HMQC and HMBC), ESIMS and chemical methods. The structures of the two new pentacyclic triterpenoid saponins were established as 3β-*O*-{[β-D-glucopyranosyl-(1$\Downarrow$ 3)-α-L-rhamnopyranosyl-(1$\Downarrow$ 2)],β-D-glucopyranosyl-(1$\Downarrow$ 4)}-α-L-arabinopyranosylhederagenin (**3**) and 3β-*O*-{[β-D-glucopyranosyl-(1$\Downarrow$ 4)-β-D-glucopyranosyl-(1$\Downarrow$ 3)-α-L-rhamnopyranosyl-(1$\Downarrow$ 2)],β-D-glucopyranosyl(1$\Downarrow$ 4)}-α-L-arabinopyranosyloleanolic acid (**4**).

Keywords: *Pentaclethra macroloba*, Mimosoideae, pentacyclic triterpenoid saponins

Introduction

Pentaclethra macroloba (Willd.) Kuntze, of the family Mimosoideae,[1,2] occurs in the northern region of Brazil, where it is known as "pracaxi" and is used by the local population to treat several afflictions, including snakebites.[3] The first chemical and biological investigations on *P. macroloba* has shown the presence of steroids (β-sitosterol, stigmasterol and campesterol)[4] and insecticidal properties.[5-7]

In this paper we report the isolation and characterization of two known, 3β-*O*-[β-D-glucopyranosyl-(1$\Downarrow$ 4),α-L-rhamnopyranosyl-(1$\Downarrow$ 2)]-α-L-arabinopyranosyloleanolic acid (**1**)[8,9] and 3β-*O*-{[β-D-glucopyranosyl-(1$\Downarrow$ 3)-α-L-rhamnopyranosyl-(1$\Downarrow$ 2)],β-D-glucopyranosyl-(1$\Downarrow$ 4)}-α-L-arabinopyranosyloleanolic acid (**2**),[8] and two new pentacyclic triterpenoid saponins, 3β-*O*-{[β-D-glucopyranosyl-(1$\Downarrow$ 3)-α-L-rhamnopyranosyl-(1$\Downarrow$ 2)],β-D-glucopyranosyl-(1$\Downarrow$ 4)}-α-L-arabinopyranosylhederagenin (**3**) and 3β-*O*-{[β-D-glucopyranosyl-(1$\Downarrow$ 4)-β-D-glucopyranosyl-(1$\Downarrow$ 3)-α-L-rhamnopyranosyl-(1$\Downarrow$ 2)], β-D-glucopyranosyl (1$\Downarrow$ 4)}-α-L-arabinopyranosyloleanolic acid (**4**). The two known saponins (**1** and **2**) are described for the first time from this species.

Results and Discussion

Two known (**1** and **2**) and two new triterpenoid saponins (**3** and **4**) were isolated from an ethanolic extract of the stem bark of *Pentaclethra macroloba* (Leguminosae-

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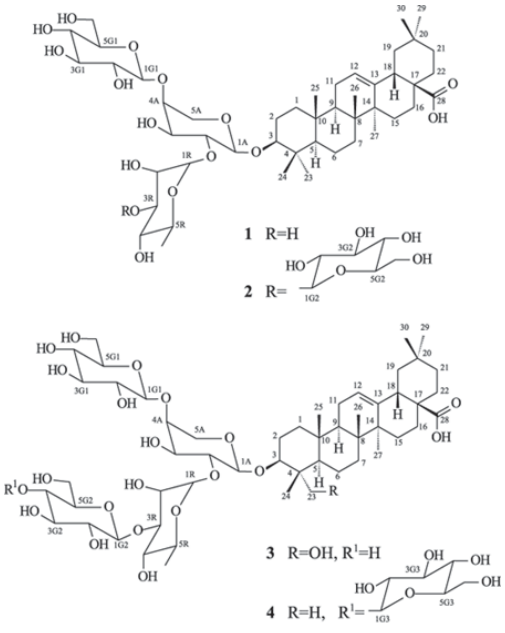
Mimosoideae) using a combination of silica gel, Sephadex LH-20 and reverse-phase chromatography (HPLC). The structures were established on the basis of chemical methods and spectral data. The IR spectra of the four compounds showed bands at ν_{max} 3400 (strong and broad, suggesting several OH groups) and 1700 cm^{-1} (carboxylic group). Additional spectral data, mainly NMR and ESMS spectra, led us to postulate the structures **1** – **4**. Oleanolic acid was identified as aglycone of saponins **1**, **2** and **3** after acid hydrolysis while hederagenin identified as aglycone of saponin **3** along with the carbohydrates arabinose, rhamnose and glucose for all compounds.



Comparative analysis of the ^{13}C NMR-HBBD and ^{13}C NMR-DEPT spectra of the four pentacyclic triterpenoid saponins was used to identify the number of signals attributed to quaternary, methine, methylene and methyl carbon atoms (Tables 1 and 2).

The known triterpene saponins **1** and **2** were identified as 3β-*O*-[β-D-glucopyranosyl-(1⇓4),α-L-rhamnopyranosyl-(1⇓2)]-α-L-arabinopyranosyloleanolic acid (**1**)[8,9] and 3β-*O*-{[β-D-glucopyranosyl-(1⇓3)-α-L-rhamnopyranosyl-(1⇓2)],β-D-glucopyranosyl-(1⇓4)}-α-L-arabinopyranosyloleanolic acid (**2**)[8] on the basis of spectral data, especially 1D and 2D NMR and ESIMS, and through comparison with the data available in the literature.[8,9]

Compound **3** was obtained as an amorphous powder. The ESIMS (negative ion mode) showed a quasimolecular ion peak at *m/z* 1073 ($[M-H]^-$), indicating a molecular weight of 1074 Da consistent with a molecular formula $C_{53}H_{86}O_{22}$. Other significant negative ion peaks were observed at *m/z* 911 ($[M-H-162]^-$, **3a** and **3b**), 765 ($[M-H-162-146]^-$, **3c**), 749 ($[M-H-162-162]^-$, **3d**), 603 ($[M-H-162-162-146]^-$ and/or $[M-H-162-146-162]^-$, **3e**) and 471 ($[M-H-162-162-162-146-132]^-$, **3f**), Scheme 1 (A). These peaks were attributed to fragments corresponding to the loss of one hexosyl (*m/z* 911), one hexosyl and one deoxyhexosyl (*m/z* 765), two hexosyl (*m/z* 749), two hexosyl and one deoxyhexosyl (*m/z* 603) and two hexosyl, one deoxyhexosyl and one pentosyl (*m/z* 471) radicals (Scheme 1). Based on these data we postulated the elimination of two glucopyranosyl, one rhamnopyranosyl and one arabinopyranosyl moieties to yield the aglycone ion at *m/z* 471 (hederagenin - H^- = $[C_{30}H_{48}O_4 - H]^-$= $[C_{30}H_{47}O_4]^-$, **3f**), in accordance with the sugars arabinose, rhamnose and glucose obtained by the acid hydrolysis experiment. The peak at *m/z* 911 ($[M-H-glucopyranosyl]^-$, **3a** and/or **3b**) suggested a terminal glucopyranosyl unit. Additional peaks at 765 ($[M-H-glucopyranosyl-rhamnopyranosyl]^-$ = *m/z* 911 - rhamnopyranosyl, **3c**) and 749 ([M-H-glucopyranosyl-glucopyranosyl, **3d**]$^-$ = *m/z* 911-glucopyranosyl) revealed a branched sugar chain involving a disubstituted arabinopyranosyl moiety (Schemes 1 and 2).[9,10]

In fact, the hydrogen broad band decoupled (HBBD) ^{13}C NMR spectrum of **3** (Tables 1 and 2), showed 53 signals. Comparative analysis of the HBBD and DEPT ^{13}C NMR spectra allowed the identification of signals corresponding to eight quaternary [$(C)_{8:}$ six sp^3 and two sp^2 of an olefinic double bond at δ_C 144.95 and carboxyl group at δ_C 180.42 = $(C)_7(COOH)$], twenty four methine [$(CH)_{24}$: one sp^2 and twenty three sp^3, including twenty oxygenated = $(CH)_4(O-CH)_{20}$], fourteen methylene [$(CH_2)_{14}$: all sp^3, including four oxygenated = $(CH_2)_{10}(CH_2-O)_4$] and seven methyl [$(CH_3)_7$] carbon atoms. Consequently, expanded formula $(C)_7(COOH)(CH)_4(O-CH)_{20}(CH_2)_{10}(CH_2-O)_4[(CH_3)_7$ = $C_{53}H_{74}O_{26}$ was deduced, which after considering the presence of twelve hydroxyl groups and four ether functions was established as $C_{53}H_{86}O_{22}$ in accordance with the ESMS. The presence of four sugar moieties was confirmed by the HMQC spectrum, which showed the anomeric ^1H NMR signals at δ_H 4.90 (d, *J* 7.1 Hz, H-1A), 6.17 (br s, H-1R), 5.08 (d, *J* 7.9 Hz, H-1G1) and 5.46 (d, *J* 7.8 Hz, H-1G2) correlated with ^{13}C NMR signals at δ_C 105.09, 101.67, 106.97 and 106.90, respectively.[11] Complete ^1H and ^{13}C chemical shift assignments of each sugar unit was achieved by the ^1H-^1H-COSY, TOCSY,

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) spectral data for aglycones of the compounds **3** and **4** including results obtained by heteronuclear 2D shift-correlated HMQC and HMBC spectra, in C_5D_5N and TMS as internal standard. Chemical shifts (δ, ppm) and coupling constants (J in Hz, in parenthesis)[a]

	3				**4**			
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
C								
4	43.71	—			39.94	—	3H-23; 3H-24	
8	39.91	—			40.19	—	H-9; 3H-26	3H-27
10	37.05	—			37.44	—	3H-25	
13	144.95	—	H-18	H-11; 3H-27	146.13	—		3H-27
14	42.32	—			42.39	—	3H-27	H-18; 3H-26
17	46.82	—			47.00	—	H-18	
20	31.10	—			31.33	—	H-19b; 3H-29; 3H-30	
28	180.42	—		H-18	180.44	—		
CH								
3	81.47	3.20 (dd, 13.5, 3.8)		H-1A; 2H-23; 3H-24	90.02	3.27 (dd, 11.7, 4.1)		H-1A; 3H-23; 3H-24
5	48.31	1.70			57.37	0.81 (d, 11.4)		3H-25; 3H-26
9	47.74	1.72			49.36	1.67 (t, 8.6)		
12	122.76	5.45 (br s)	H-11	H-18	123.85	5.49 (br s)		
18	42.13	3.26 (dd, 13.6, 3.8)			43.30	3.32 (dd)		
CH_2								
1	39.12	1.52; 1.04			40.19	1.48, Hβ; 0.93, Hα		H-5, H-9; 3H-25
2	26.58	2.17; 1.97			28.01	2.10, Hα; 1.84, Hβ		
6	18.25	1.73; 1.32			18.91	1.52, Hβ; 1.20, Hα		
7	33.16	1.75; 1.20			34.51	1.51, Hβ; 1.29, Hα		3H-26
11	23.99	1.91 (dl, 10.8)			25.11	1.92	H-12	
15	28.47	2.12; 1.11			29.64	2.19, Hβ; 1.22, Hα		3H-27
16	23.93	2.08; 1.91			25.01	2.19; 1.98		
19	46.55	1.75; 1.12			47.79	1.83, Hβ; 1.32, Hα	H-18	3H-29; 3H-30
21	34.35	1.42; 1.10			36.55	1.58, Hα; 1.29, Hβ		3H-29; 3H-30
22	33.37	2.05; 1.80			34.51	2.10, Hβ; 1.84, Hα		
23	64.11	4.30 (d, 11.7); 3.89 (d, 11.7)			—	—	—	—
CH_3								
23	—	—	—	—	29.64	1.39 (s)		3H-24
24	14.26	1.09 (s)			18.43	1.16 (s)		H-5; 3H-23
25	16.24	0.91 (s)			16.86	0.86 (s)		H-9
26	17.61	1.00 (s0			18.69	1.01 (s)		H-9
27	26.33	1.23 (s)			27.49	1.39 (s)		
29	33.41	0.91 (s)			34.58	0.98 (s)		
30	23.83	0.98 (s)			25.08	1.03 (s)		3H-29

[a] 2D homonuclear ^{1}H-^{1}H-COSY and ^{1}H-^{1}H-TOCSY and heteronuclear HMBC (Table 2) spectra were also used for these assignments. Chemical shifts of hydrogen atoms obtained from 1D ^{1}H NMR spectra. Carbon signals corresponding to C, CH, CH_2 and CH_3 deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC and ^{1}H-^{1}H-COSY.

HMQC and the HMBC spectra (Table 2). The ^{1}H NMR spectrum of **3** displayed six singlet signals of seven methyl groups at δ_H 0.91, 0.91, 0.98, 1.00, 1.09 and 1.23, one doublet at δ_H 1.56 (J 6.2 Hz), one of olefinic hydrogen in a trisubstituted double bond (δ_H 5.45, br s) and one double-doublet (J 13.5, 3.8 Hz) at δ_H 3.20 attributed to the carbinolic hydrogen H-3 located at an axial position with a coupling constant value J=13.5 Hz (axial-axial interaction). These data suggested a pentacyclic triterpenoid skeleton type olean-12-ene as aglycone,[12] in accordance with the signals at δ_C 122.76 (CH-12) and 144.95 (C-13) observed in the ^{13}C NMR spectra (HBBD and DEPT). The ^{1}H and ^{13}C chemical shift assignments of CH-12 and CH-3 were unambiguously made by the cross peaks observed in the HMQC spectrum revealing correlation of the signals at δ_H 5.45 (H–12) with δ_C 122.76 (CH-12) and δ_H 3.20 (H-3) with δ_C 81.47 (CH-3), which were confirmed by heteronuclear connectivity to long-range coupling revealed by HMBC spectrum (Table 1). The lower ^{13}C chemical shifts of the CH-3 (δ_C 81.47), CH-5 (δ_C 48.31) and CH_3-24 (δ_C 14.26) when compared with those of **1**, **2** and **4** [*e. g.* δ_C 90.02 (CH-3), 57.37 (CH-5) and

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) for the carbohydrate moieties of the compounds **3** and **4** including results obtained by heteronuclear 2D shift-correlated HMQC and HMBC spectra, in C_5D_5N as solvent. Chemical shifts (δ, ppm) and coupling constants (J, Hz, in parenthesis)[a]

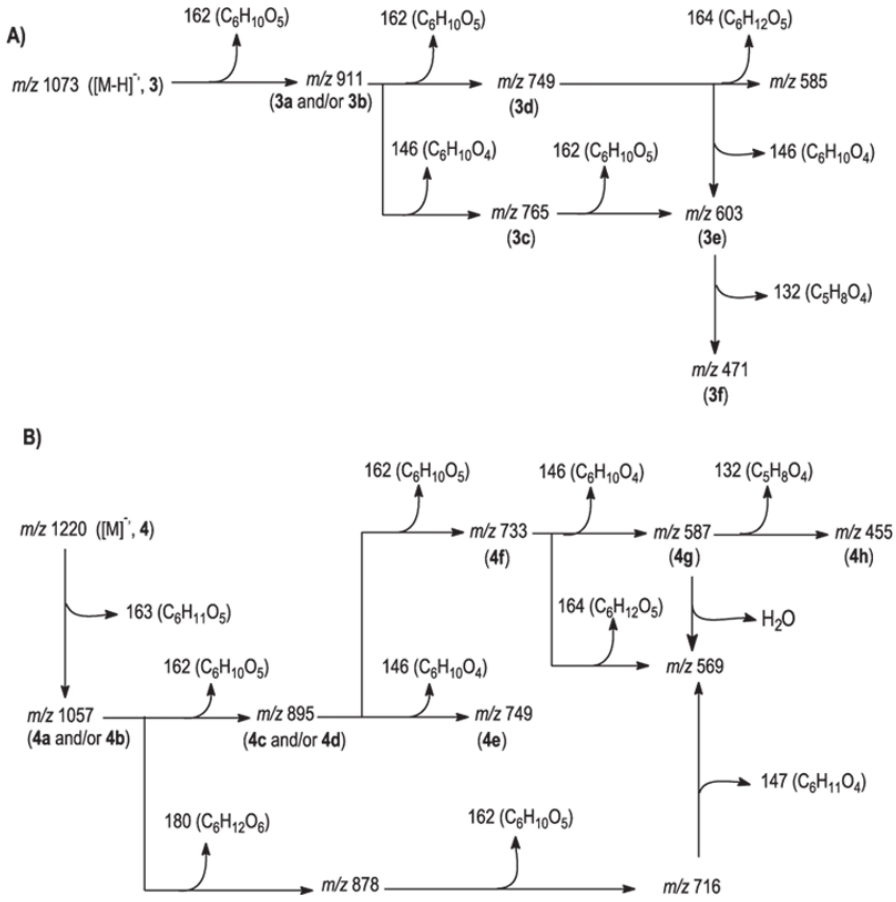
	3				**4**			
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
1A	105.09	4.90 (d, 7.1)	H-2A	H-3	105.60	4.73 (d, 6.7)		H-3
2A	76.39	4.40 (dd, 7.1, 8.2)	H-3A	H-4A,	76.39	4.46		H-1R
3A	75.16	3.88		H-5	74.57	4.18	H-2A	H-1A
4A	80.79	4.12 (br s)	2H-5	H-1G1	80.16	4.28		2H-5A, H-1G1
5A	66.10	4.36 (d, 11.7), 3.57 (d, 11.7)		H-1A	65.54	4.45 3.76 (d,11.4), Ha		H-1A
1R	101.67	6.17 (br s)		H-2A	101.92	6.18 (br s)	H-2R	H-2A
2R	71.83	4.96 (br s)			71.81	4.34 (br s)	H-1R	
3R	83.00	4.83 (dd, 2.8, 9.5)	H-2R, H-4R	H-1R, H-1G2	83.44	4.77 (dd, 9.3, 2.6)	H-2R, H-4R	H-1R, H-1G2
4R	73.05	4.47 (t, 9.5)	H-3R	H-2R, 3H-6R	73.08	4.48	H-3R	H-2R, 3H-6R
5R	70.02	4.69 (dq, 9.5, 6.2)		H-1R	70.02	4.65 (m)	3H-6R	
6R	18.74	1.56 (d, 6.2)	H-5R	H-4R	18.75	1.59 (d, 6.2)		H-4R
1G1	106.97	5.08 (d, 7.9)	H-2G1		106.79	5.14(d, 7.9)	H-2G1	
2G1	76.00	4.01 (dd, 7.9, 9.1)	H-3G1		75.67	4.04 (dd, 7.9, 8.2)		
3G1	78.62	4.19 (t, 9.1)	H-2G1, H-4G1		78.67	4.22	H-2G1	
4G1	71.41	4.17 (t, 9.1)			71.53	4.26		
5G1	78.93	3.88			78.91	3.92 (m)	H-3G3	
6G1	62.72	4.47, 4.34 (dd, 11.9, 4.9)		H-4G1	62.77	4.52, 4.39		
1G2	106.90	5.46 (d, 7.8)	H-2G2	H-3R	107.01	5.45 (d, 7.9)	H-2G2	H-3R
2G2	75.63	4.09 (dd, 7.8, 9.1)	H-3G2		76.07	4.11 (dd, 7.9, 8.4)	H-3G2	
3G2	78.62	4.21 (t, 9.1)	H-2G2	H-1G2	78.67	4.38	H-2G2, H-4G2	
4G2	71.67	4.19 (t, 9.1)			71.67	4.35	H-3G2	H-1G3
5G2	78.69	3.95			78.64	3.95 (m)	H-4G2	
6G2	62.72	4.49, 4.27 (dd, 11.6, 5.2)			63.29	4.56 (dd, 12.0, 3.5), 4.46		
1G3	—	—	—	—	106.27	5.19 (d, 7.8)	H-2G3	H-4G2
2G3	—	—	—	—	76.05	4.08 (dd, 7.8, 8.40		
3G3	—	—	—	—	79.55	4.20	H-4G3	
4G3	—	—	—	—	72.88	4.19	H-3G3	H-1G3
5G3	—	—	—	—	79.71	4.00 (m)		
6G3	—	—	—	—	63.29	4.28 4.52		

[a]Homonuclear ^{1}H-^{1}H-COSY, ^{1}H-^{1}H-TOCSY and ^{1}H-^{13}C-COSY-$^{1}J_{CH}$ (Table 1) spectra were also used for these assignments. Chemical shifts of hydrogen atoms obtained from 1D ^{1}H NMR spectra. Carbon signals corresponding to C, CH, CH_2 and CH_3 deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC, ^{1}H-^{1}H-COSY and ^{1}H-^{1}H-TOCSY.

18.43 (CH_3-24, Table 1] revealed the γ-effects,[13,14] of the hydroxy function presence in the hydroxymethylene group $HOCH_2$-23 [δ_C 64.11, δ_H 4.30 (d, J 11.7 Hz) and 3.89 (d, J 11.7 Hz)]. This result was confirmed by the HMQC and HMBC spectra (Table 2). The characterization of the [β-D-glucopyranosyl-(1⇓ 3)-α-L-rhamnopyranosyl-(1⇓ 2)],β-D-glucopyranosyl-(1⇓ 4)}-α-L-arabino-pyranosyl moiety and its placement at CH-3 were mostly based on the HMBC spectrum, which revealed cross peaks corresponding to heteronuclear spin-spin interaction between (Table 2): a) CH-3 (δ_C 81.47) and H-1A (4.90) and CH-1A (δ_C 105.09) and H-3 (δ_H 3.20); b) CH-1R (δ_C 101.67) and H-2A (δ_H 4.40); c) CH-4A (δ_C 80.79) and H-1G1 (δ_H 5.08); d) CH-3R (δ_C 83.00) and both H-1R (d_H 6.17) and H-1G2 (δ_H 5.46); e) CH-1G2 (δ_C 106.90) and H-3R (δ_H 4.83). In addition, we observed heteronuclear correlation of methyl signal at δ_H 1.57 (d, J 6.2 Hz) with d_C 18.74 in the HMQC and both CH-4R (δ_C 73.05, $^3J_{CH}$) and CH-5R (δ_C 70.02, $^2J_{CH}$) in the HMBC, in accordance with the presence of the rhamnose. Other heteronuclear long-range couplings observed in the HMBC spectrum of **3** are summarized in Table 2.

Therefore, the structure of the new pentacyclic triterpenoid glycoside was characterized as 3β-*O*-{[β-D-glucopyranosyl-(1⇓ 3)-α-L-rhamnopyranosyl-(1⇓ 2)],β-D-glucopyranosyl-(1⇓ 4)}-α-L-arabinopyranosyl-hederagenin (**3**) on the basis of spectral data (including evaluation of spin-spin coupling deduced by 1D ^{1}H NMR analysis involving confirmation by ^{1}H-^{1}H-COSY, TOCSY and ^{1}H-^{1}H-NOESY) together with the identification of the aglycone hederagenin and sugars obtained (arabinose, rhamnose and glucose) from saponin hydrolysates.

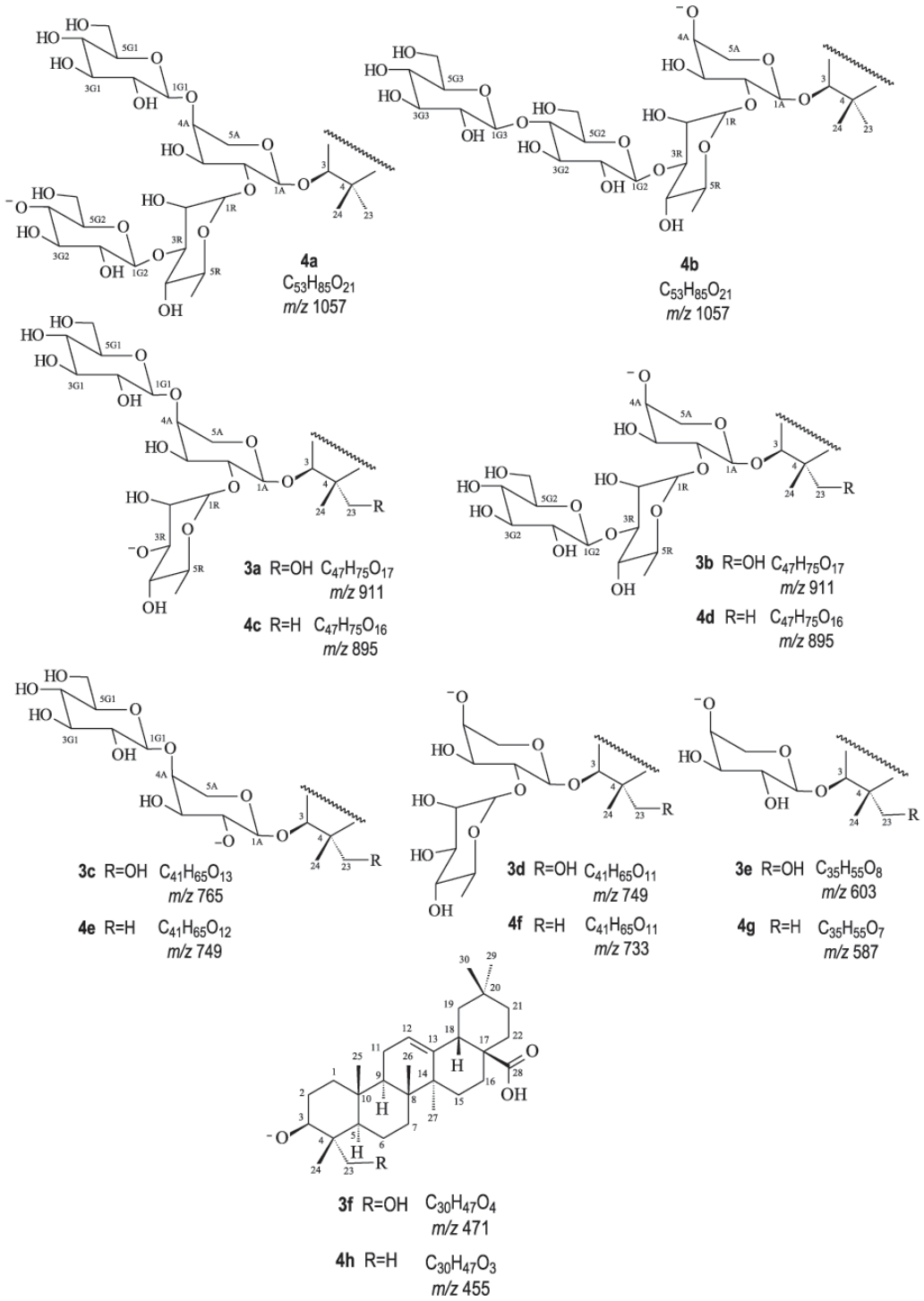
Comparison of the ^{1}H and ^{13}C spectral data of the saponin **4** (amorphous colorless solid) with those of **3** (Tables 1 and 2) indicated that the CH_2OH group [δ_C 64.11; δ_H 4.30 (d, J 11.7 Hz) and 3.89 (d, J 11.7 Hz)] in **3** was replaced by a methyl group (δ_H 1.39 and d_C 29.64) in **4**. This hypothesis was confirmed by the ^{13}C chemical shifts



Scheme 1. Arithmetical analysis of ESMS of **3** (A) and **4** (B).

of the CH-3 at δ_C 90.02 (δ_C 81.47 in **3**), CH-5 at δ_C 57.37 (δ_C 48.31 in **3**) and CH_3-24 at δ_C 18.43 (δ_C 14.26 in **3**), which indicated absence of the γ-effects of a hydroxy function of hydroxymethylene group $HOCH_2$-23 in a 3β-*O*-glycosylated aglycone.[13,14] Significant additional differences observed in the comparative analysis of the NMR spectra of **3** and **4** were justified by oligosaccharide moiety, containing the triterpenoid **4** the same type and sequence the sugars chain as saponin **3** and one additional glucopyranosyl group (Table 2). These deductions were confirmed by ESIMS (negative ion mode) of **4**, which showed a molecular ion peak at *m/z* 1220 ($[M]^-$) compatible with a molecular formula $C_{59}H_{96}O_{26}$ (molecular weight 1220 Da). Other significant negative ion peaks were observed at *m/z* 1057 ($[M-163]^-$, **4a** and/or **4b**), 895 ($[M-163-162]^-$, **4c** and/or **4d**), 749 ($[M-163-162-146]^-$, **4e**), 733 ($[M-163-162-162]^-$, **4f**), 587 ($[M-163-162-162-146]^-$, **4g**) and 455 ($[M-163-162-162-146-132]^-$, **4h**), corresponding to the loss of one hexosyl, two hexosyl, three hexosyl, three hexosyl and one deoxyhexosyl and three hexosyl, one deoxyhexosyl and one pentosyl moieties, respectively, as revealed by the arithmetical analysis summarized in Scheme 1(B).

The hydrogen broad band decoupled HBBD-^{13}C NMR spectrum of **4** (Tables 1 and 2) showed 59 signals, which were identified as corresponding to quaternary, methine, methylene and methyl carbon atoms by comparative analysis involving the DEPT-^{13}C NMR spectrum (Tables 1 and 2). The presence of the additional sugar moiety bonded to carbon CH-4 of the glucopyranosyl G2 was deduced from the 2D NMR experiments [heteronuclear HMQC and HMBC (Table 2) and homonuclear ^{1}H-^{1}H-COSY, TOCSY



Scheme 2. Anion fragments proposed to justify the main peaks observed in the ESMS of **3** and **4**.

and NOESY). The single hydrogen spin systems for each sugar residue were delineated by ^{1}H-^{1}H-COSY, TOCSY and HMQC experiments and comparison with those of the depicted methyl pyranosides,[15] taking into account the known effects of glycosidation (Table 2).

The values corresponding to *vicinal* spin-spin interaction ($^3J_{H,H}$) between the anomeric hydrogens of arabinopyranosyl [*J* 7.1 (**3**) and 6.7 Hz (**4**)] and glucopyranosyl (*J* 7.8 to 7.9 Hz) moieties are consistent with axial-axial couplings and, consequently, the configuration of the anomeric carbons was defined as β for glucose and α for arabinose.

Thus, the structure 3-O-{β-D-glucopyranosyl-(1⇓ 4)-β-D-glucopyranosyl-(1⇓ 3)-α-L-rhamnopyranosyl-(1⇓ 2)]-[β-D-glucopyranosyl-(1⇓ 4)}-α-L-arabinopyranosyloleanolic acid (**4**) was established.

Experimental

General experimental procedures

NMR spectra were run on a Bruker Advance 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) in pyridine-d_5 (C_5D_5N) and residual C_5D_5N used as internal references (CH-2/CH-6: δ_H 8.64 and δ_C 149.80). ESI-MSMS data were collected in a triple quadrupole Micromass QuattroLC instrument equipped with a "Z-spray" ion source (Micromass, Wythenshawe, Manchester, UK). A Shimadzu LC10AD HPLC pump was used to deliver methanol-water [7:3] solutions at 70 μL/min to the mass spectrometer. The desolvation and ion source block temperatures were set, respectively, at 350 and 140 °C. Gaseous N_2 was used as nebulizer (80 L/h) and desolvation (450 L/h). The optimal voltages found for the probe and ion source components to produce maximum intensity of the ions [M-H]- were 3.2 kV for the stainless steel capillary, 39 V for the sample cone, and 9 V for the extractor cone. The tandem mass spectrometry experiments were performed by adding Ar in the collision cell to produce a pressure of 2 x 10^{-3} mBarr for CAD. The optimal collisional energies (CE) used for decomposition of the ions $[M-H]^-$ generated from saponins **1**-**4** were 35 eV.

Plant material

The stem bark of *Pentaclethra macroloba* was collected in September 1997 in Macapá, Amapá, Brazil. The plant was identified by Dr. Afrânio G. Fernandes and a voucher specimen (no. EAC-25947) is deposited in the Herbarium Prisco Bezerra of the Departamento de Biologia, Universidade Federal do Ceará, Brazil.

Extraction and isolation of constituents

The dried and powdered stem bark of *Pentaclethra macroloba* (800 g) was defatted with *n*-hexane and extracted with ethanol at room temperature. After removal of the solvent by evaporation under reduced pressure, the EtOH extract (26 g) was obtained. This extract was submitted to column chromatography on silica gel eluted with mixtures of $CHCl_3$-CH_3OH-H_2O (9:1:0.1) increasing the quantity of MeOH, yielding 126 fractions. Fraction 28/36 (2.4 g) was rechromatographed on silica gel column cromatography eluted with $CHCl_3$-CH_3OH (8:2), yielding saponin **1** (55 mg); fraction 41/52 (430 mg) was solubilized in MeOH (1.5 mL) and precipitated in Me_2CO (3 x 30 mL), yielding 295 mg of a crude saponin mixture, which was chromatographed on a Sephadex LH-20 column eluting by MeOH to yield a white power (160 mg); further separation of this fraction (150 mg) was performed by successive HPLC on a WATERS RP-8 semipreparative (7 μm, 7.8 x 150 mm) eluted with CH_3CN-H_2O [linear gradient 35:65 (v/v) to 65:35 (v/v) for time 25 min] to furnish compounds **2** (47.5mg), **3** (17.5 mg) and **4** (15.1 mg).

3β-O-[β-D-glucopyranosyl-(1⇓ 4)-α-L-rhamnopyranosyl-(1⇓ 2)]-α-L-arabinopyranosyloleanolic acid (***1***). Spectral data in agreement with literature values.[8,9]

3β-O-{[β-D-glucopyranosyl-(1⇓ 3)-α-L-rhamnopyranosyl-(1⇓ 2)]-β-D-glucopyranosyl-(1⇓ 4)}-α-L-arabinopyranosyloleanolic acid (**2**). Spectral data in agreement with literature values. [8]

3β-O-{[β-D-glucopyranosyl-(1⇓ 3)-α-L-rhamnopyranosyl-(1⇓ 2)],β-D-glucopyranosyl-(1⇓ 4)}-α-L-arabinopyranosylhederagenin (**3**). Amorphous colorless solid, mp 261-268 °C; $[\alpha]_D$ – 3.0° (*c* 0.5, MeOH); ESIMS: Schemes 1 and 2; ^{1}H and ^{13}C NMR spectral data: Tables 1 and 2.

3β-O-{[β-D-glucopyranosyl-(1⇓ 4)-β-D-glucopyranosyl-(1⇓ 3)-α-L-rhamnopyranosyl-(1⇓ 2)], β-D-glucopyranosyl (1⇓ 4)}-α-L-arabinopyranosyloleanolic acid (**4**). Amorphous colorless solid, mp 245-247 °C; $[\alpha]_D$ – 0.004° (MeOH); ESIMS: Schemes 1 and 2; ^{1}H and ^{13}C NMR spectral data: Tables 1 and 2.

Acid hydrolysis

A solution of the isolated saponin (5 mg) in 2N HCl – MeOH (8 mL) was refluxed for 3 h, the reaction mixture was cooled to room temperature, diluted with H_2O (20 mL) and extracted with EtOAc. The combined EtOAc extracts were washed with H_2O, dried over anhydrous Na_2SO_4 and then evaporated to dryness *in vacuo*. The aqueous layer was neutralized with aqueous NaOH 2% and concentrated under

reduced pressure; the residue was compared with a standard mixture of the sugars arabinose, glucose and rhamnose using silica gel TLC and CH_2Cl_2-MeOH-H_2O(6:4:0.5) as solvent. Furthermore, the mole ratio of each sugar was determined using RI detection in HPLC (Shodex RS pak DC-613, 75% CH_3CN, 1 mL min^{-1}, 50 °C) by comparison with authentic samples of the sugars (10 mmol L^{-1} each of Ara, Glc and Rha). The retention time of each sugar was as follows: Ara 6.0 min; Glc 7.4 min and Rha 4.8 min.

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