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Triterpenoid saponins and acylated prosapogenins from *Harpullia austro-caledonica*

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Abstract

Three new triterpenoid saponins have been isolated from the stem bark of *Harpullia austro-caledonica* and identified as 24-*O*-[α-L-rhamnopyranosyl-(1->2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1->2)-β-D-glucopyranosyl]-protoaescigenin, 24-*O*-[α-L-rhamnopyranosyl-(1->2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1->2)-β-D-glucopyranosyl]-16-desoxyprotoaescigenin, 24-*O*-[α-L-rhamnopyranosyl-(1->2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1->2)-β-D-glucopyranosyl]-24-oxo-camelliagenin D. The 21,22-di-*O*-angeloate esters of protoaescigenin and barringtogenol C were isolated in the acid hydrolysate of the saponin extract together with a new prosapogenin identified as 21β,22α-di-*O*-angeloyl camelliagenin D. The structures were established using one- and two- dimensional NMR and mass spectrometry.

Keywords: *Harpullia austro-caledonica*; Sapindaceae; Triterpenoid saponins; Protoaescigenin; Barringtogenol C; Camelliagenin D

1. Introduction

Harpullia austro-caledonica Baillon (Sapindaceae) is a tree or a shrub originating from New Caledonia and growing in the rain forest. In the phylogenetic and taxonomic systems of this genus, this isolated species is next to the most primitive species *H. pendula* and *H. arborea* (Leenhouts, 1985). The leaves consist of four to seven pairs of leaflets and the inflorescence is composed of yellow unisexual flowers that show an unusually wide range of variability (Leenhouts and Vente, 1982). This species was selected as a part of a screening program for potential cytotoxic compounds from plants collected in New Caledonia. The ethanolic extract of the stem bark of *H. austro-caledonica* exhibited in vitro a cytotoxic activity against KB cells (90% at 10 μg/ml). Primary chemical studies on the ethylacetate and ethanolic extracts of the leaves and the stem bark showed the presence of alkaloids and particularly saponins. This paper reports on the isolation and structural elucidation of three new and structurally unusual saponins from the stem bark of this plant and of three prosapogenins obtained after acid hydrolysis of saponin crude extract.

2. Results and discussion

H. austro-caledonica was collected in the reserve of Amieu Pass and Table Unio in New Caledonia. To obtain the saponin mixture, dried and powered stem bark was extracted with boiling 80% methanol. The methanolic extract was concentrated and precipitated into acetone. Then, the crude saponin precipitate was dialysed, chromatographed on a silicagel column and purified by reversed phase C-18 column chromatography. From the most polar fraction, three new compounds **1**, **2** and **3** were obtained in a pure state after a final purification by preparative TLC. Acid hydrolysis of the saponin extract gave a mixture of three major prosapogenins (**4**), (**5**) and (**6**) purified by silica gel column chromatography. The sugars were identified as

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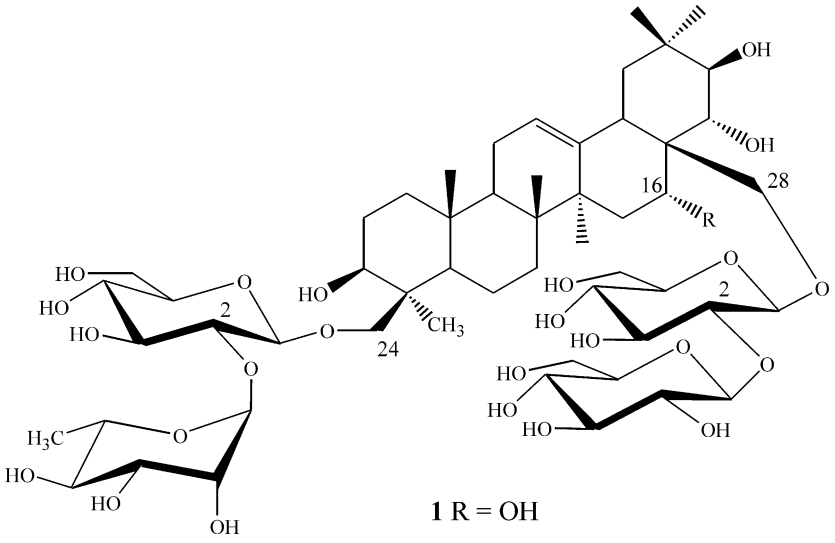

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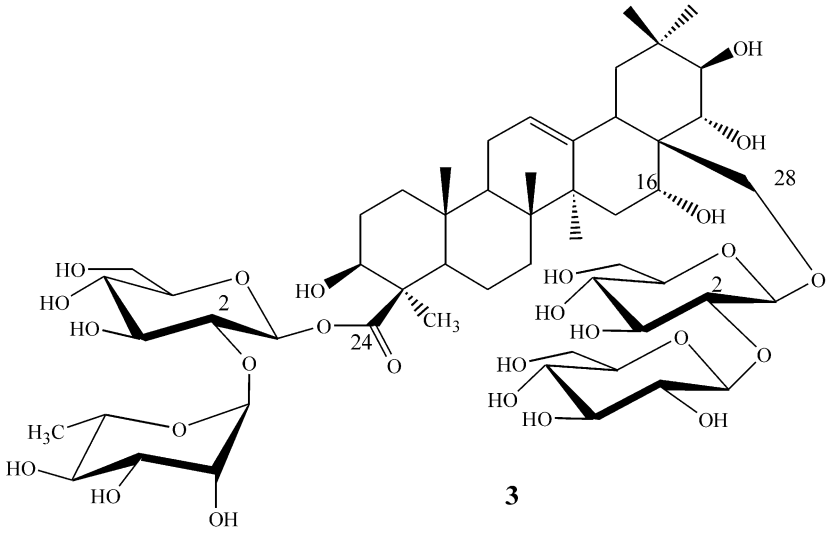
2 R = H



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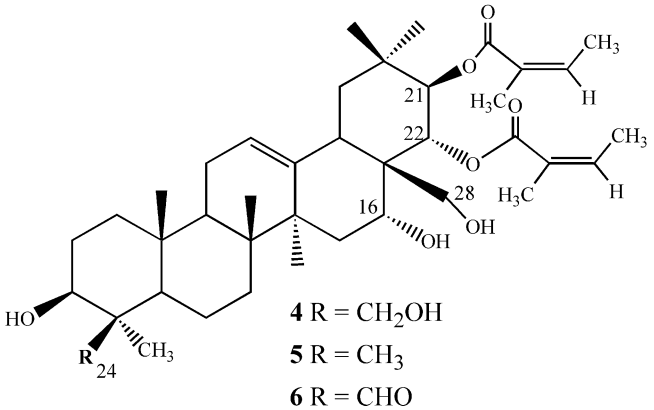

4 R = CH_2OH

5 R = CH_3

6 R = CHO

D-glucose, D-galactose, L-rhamnose, L-arabinose and D-xylose by TLC and their absolute configurations were determined by optical rotation measurement after separation by prep. TLC.

The positive ESI–MS of saponin **1** gave a quasi-molecular ion peak at m/z 1161 $[M+Na]^+$ and in the negative ESI–MS a molecular ion was detected at m/z 1137 $[M-H]^-$ in agreement with a M_r of 1138 amu ($C_{54}H_{90}O_{25}$). The MS^2 experiment of $[M-H]^-$ ion gave negative fragments at m/z 991 [M-146]$^-$ and 975 [M-162]$^-$ attributed to the losses of a terminal 6-desoxy-hexose and a terminal hexose, respectively (Table 1). The MS^3 fragmentation of ion at m/z 975 led to ions at m/z 829 [M-162-146]$^-$ and 667 [M-162-308]$^-$ attributed to the losses of the terminal 6-desoxy-hexose and of a disaccharide made of 6-desoxy-hexose and hexose, respectively. The MS^4 of ion fragment at m/z 829 gave rise to a product ion at m/z 505 [genin-H]$^-$ corresponding to the loss of a third hexose and suggesting a molecular formula of $C_{30}H_{50}O_6$ for the aglycone moiety (M_r of 506 amu).

Protoaescigenin was identified as the aglycone of saponin **1** by analysis of ^1H and ^{13}C NMR spectra (Table 2) and from observation of connectivities in COSY, HSQC and HMBC spectra. The set of data was in full agreement with those reported in the literature (Chen et al., 1985; Mahato and Kundu, 1994). The ^1H NMR spectrum showed four methylene protons linked to oxygen bearing carbons which constituted two AX spin systems at δ 3.67 and 4.13 (*d*, $J=10$ Hz, H-24) and at δ 3.45 and 3.66 (*d*, $J=10$ Hz, H-28), two hydroxymethine protons at δ 3.27 (*dd*, $J=12$-4.8 Hz, H-3) coupling to two protons at δ 1.64 and 1.76, and at δ 4.57 (*brs*, $W_{1/2}=8$ Hz, H-16) coupling to two protons at δ 1.89 (*dd*, $J=15$-4 Hz, H-15ax) and 1.43 (*d*, $J=15$ Hz, H-15eq), and two vicinal hydroxymethine protons corresponding to a *trans*-diequatorial diol which constituted an AB spin system at δ 3.79 (*d*, $J=10$ Hz, H-22) and 3.95 (*d*, $J=10$ Hz, H-21). In the ROESY experiment, the interaction observed between H-16 and H-28 confirmed the β-equatorial orientation for H-16 and the observation of ROE between β-axial H-2 at δ 1.76 and H-24 confirmed the configuration of C-4 with a β-axial orientation for C-24. The α-axial orientation of H-21 and the β-axial position for H-22 were also confirmed by the ROEs observed with H-29 and H-30, respectively.

As shown from analysis of ESI–MS, the sugar part of **1** consisted of four residues, with anomeric carbons at δ 100, 101.7, 102.4 and 103.4 in the ^{13}C NMR spectrum, attached to proton doublets at δ 5.38, 4.23, 4.32 and 4.66 respectively (HSQC exp.). The proton system of each sugar was completely assigned on the basis of COSY and TOCSY experiments (Table 3). The methyl carbon at δ 16.9 which correlated in HSQC spectrum with a methyl proton doublet at δ 1.29 ppm ($J=6$ Hz) was assigned to an α-L-rhamnose whose anomeric proton appeared as a narrow doublet at δ 5.38 ($J=1.3$ Hz). The sugars with their anomeric protons at δ 4.23 ($J=7.7$ Hz), 4.32 ($J=7.7$ Hz) and 4.66 ($J=7.8$ Hz) corresponded to three hexoses with hydroxymethyl carbons at δ 61.1, 61.4 and 60.6, respectively, and were identified as three β-D-glucoses (Table 3). The absolute configurations of these sugars were chosen in keeping with those mostly encoutered among other plant saponosides. Sequencing of the two sugar chains was achieved by analysis of HMBC and ROESY experiments. HMBC spectrum showed correlations between C-28 (δ_C 77) of protoaescigenin and H-1 of a glucose (δ_H 4.23) and between

Table 1
ESI–MS and MS^n data of saponins **1**–**3** (m/z)[a]

MS^n (parent ion)	Fragments	**1**	**2**	**3**
ESI–MS $^+$	$[M+Na]^+$	1161	1145	1176
ESI–MS$^-$	$[M-H]^-$	1137	1121 (obs. at 1122)	1151 (obs. at 1152)
MS^2 ($[M-H]^-$)	$[M-H\text{-rha}]^-$ $[M-H\text{-glc}]^-$ $[M-H\text{-rha-glc}]^-$	991 975 829	975 959 813	– 989 843
MS^3 ($[M-H\text{-rha}]^-$)	$[M-H\text{-rha-glc}]^-$ $[M-H\text{-rha-2glc}]^-$	829 667	813 651	
MS^3 ($[M-H\text{-glc}]^-$)	$[M-H\text{-rha-glc}]^-$ $[M-H\text{-rha-2glc}]^-$	829 667		
MS^3 ($[M-H\text{-rha-glc}]^-$)	$[M-H\text{-rha-2glc}]^-$ $[genin-H]^-$			681 519
MS^4 ($[M-H\text{-rha-glc}]^-$)	$[M-H\text{-rha-2glc}]^-$ $[genin-H]^-$	667 505	651 489	
MS^4 ($[M-H\text{-rha-2glc}]^-$)	$[genin-H]^-$			519

[a] rha, Rhamnopyranosyl ($C_6H_{11}O_4$); glc, glucopyranosyl ($C_6H_{11}O_5$).

Table 2
^{1}H and ^{13}C NMR data of genin part of saponins **1**–**3** in CD_3OD

	1				**2**				**3**			
Genin	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)
1 eq	1.70	*m*	–	38.7	1.69	*m*	–	38.5	1.77	*m*	–	39
1 ax	1.03	*m*	–		1.03	*m*	–		1.10	*m*	–	
2 eq	1.64	*m*	–	26.6	1.65	*m*	–	26.6	1.69	*dm*	12	na[a]
2 ax	1.76	*m*	–		1.76	*m*	–		2.30	*dd*	12/3.7	
3	3.27	*dd*	12/4.8	79	3.27	*m*	–	79	3.17	*dd*	12/4.5	78.5
4	–	–	–	42.6	–	–	–	42.6	–	–	–	49
5	0.86	*d*	8.3	56.1	0.93	*d*	12	56	1.03	*m*	–	56.7
6 ax	1.61	*m*	–	19.2	1.62	*m*	–	19	1.84	*m*	–	na[a]
6 eq	1.75	*m*	–		1.76	*m*	–		1.58	*m*	–	
7 eq	1.42	*m*	–	33	1.40	*m*	–	33.8	1.42	*m*	–	na[a]
7 ax	1.59	*m*	–		1.54	*m*	–		1.60	*m*	–	
8	–	-	–	39.4	–	–	–	39.6	–	–	–	40
9	1.68	*m*	–	46.7	1.62	*m*	–	47.7	1.66	*dd*	15/8.5	47
10	–	–	–	36.5	–	–	–	36.5	–	–	–	37.1
11	1.93	*m*	–	23.5	1.94	*m*	–	23.3	1.94	*m*	–	23.5
12	5.31	*brt*	3	122.9	5.27	*brt*	3.5	123.2	5.31	*brt*	3	123
13	–	–	–	142.1	–	–	–	143	–	–	–	142.3
14	–	–	–	41.1	–	–	–	41.4	–	–	–	41.2
15 ax	1.89	*dd*	15/4	33	–	–	–	25	1.90	*dd*	14.5/5	32.7
15 eq	1.43	*d*	15	–	–	–	–		1.44	*dm*	14.5	
16	4.57	*brs*	$W_{1/2=}8$	66.3	–	–	–	42.2	4.58	*brs*	$w_{1/2=}8$	66.3
17	–	–	–	45.7	–	–	–	na[a]	–	–	–	na[a]
18	2.18	*dd*	13.5/4.5	41.3	2.16	*dd*	15/4.1	41.5	2.17	*dd*	14/7.7	41.1
19 ax	2.52	*t*	13.5	47.4	1.98	*m*	–	46	2.51	*t*	13.5	47.3
19 eq	1.06	*m*	–	–	1.15	*dd*	14/4.1		1.05	*dd*	13.5/4.5	
20	–	–	–	35.1	–	–	–	35.4	–	–	–	35.1
21	3.95	*d*	10	77.5	3.29	*d*	10	76.2	3.95	*d*	9.6	77.6
22	3.79	*d*	10	77.7	3.74	*d*	10	76.2	3.79	*d*	9.6	77.6
23	1.22	*s*	–	22.4	1.22	*s*	–	22.4	1.49	*s*	–	22.9
24	4.13	*d*	10	71.1	4.14	*d*	10	70.7	–	–	–	175
24′	3.67	*d*	10	–	3.67	*d*	10					
25	1.04	*s*	–	14.7	1.03	*s*	–	14.6	0.96	*s*	–	13
26	0.97	*s*	–	16	1.01	*s*	–	15.9	0.99	*s*	–	15.9
27	1.44	*s*	–	26	1.21	*s*	–	25	1.45	*s*	–	25.9
28	3.66	*d*	10	77	3.75	*d*	10	75	3.65	*d*	10.5	77.2
28′	3.45	*d*	10	–	3.66	*d*	10		3.46	*d*	10.5	
29	0.97	*s*	–	28.7	0.98	*s*	–	28.6	0.93	*s*	–	28.7
30	0.94	*s*	–	18	0.92	*s*	–	17.8	0.97	*s*	–	18

[a] NA: not assigned.

C-2 (δ 80.3) of this glucose and H-1 (δ_H 4.66) of a second glucose unit, thus confirming the presence of a sophorose residue attached to C-28 of protoaescigenin. The HMBC cross-peaks observed between C-24 (δ_C 71.1) of the protoaescigenin and H-1 of the third glucose unit (δ_H 4.32) and between C-1 (δ 100) of the rhamnose and H-2 of this glucose (δ_H 3.41), allowed to locate a neohesperidosyl chain at C-24 of the aglycone. The C-3 position of the protoaescigenin part was not substituted as proven by its carbon chemical shift at δ 79 (Chen et al., 1985). These particular sequences were confirmed by the observation of ROE interactions between protons involved in the interglycosidic linkages. Thus, the structure of saponin **1** is 24-*O*-[α-L-rhamnopyranosyl-(1->2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1->2)-β-D-glucopyranosyl]-protoaescigenin.

Saponin **2** exhibited an intense $[M+Na]^+$ ion peak at *m*/*z* 1145 in its positive ESI–MS and a molecular ion was detected at *m*/*z* 1121 in the negative mode corresponding to a molecular formula of $C_{54}H_{90}O_{24}$ (M_r of 1122). All the fragmentations observed in the MS/MS^n experiments followed the pattern observed in saponin **1**, thus suggesting the similarity of the sugar chains and a missing oxygen atom in the genin (Table 1).

The lack of a substitution of C-16 in **2** was suggested by the absence of NMR signal for any deshielded signal for a H-16 proton near δ 4.57 ppm and by the replacement of the C-16 hydroxymethine of **1** by a supplementary methylene at δ 42.2 (Table 2). Thus the genin was identified as 16-desoxy-protoaescigenin. Proton and carbon spectra, and 2D NMR experiments (COSY, HSQC and HMBC) permitted assignments of all proton

Table 3
^{1}H and ^{13}C NMR data of osidic part of saponins **1**–**3** in CD_3OD

	1				**2**				**3**			
	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)
24-β-D-Glucose												
1	4.32	*d*	7.7	102.4	4.32	*d*	7.7	102.4	5.65	*d*	7.6	93.9
2	3.41	*dd*	8.5/7.7	77.8	3.42	*dd*	8.4/7.7	77.1	3.64	*dd*	8.5/7.6	76.8
3	3.5	*t*	8.5	77	3.5	*t*	8.4	77.8	3.58	*t*	8.5	7.8
4	3.32	*t*	8.5	70.6	3.31	*t*	8.4	70.6	3.43	*t*	8.5	70.2
5	3.28	*m*	–	76.8	3.29	*m*	–	76.3	3.38	*m*	–	76.4
6	3.69	*dd*	11.5/5.3	61.4	3.7	*dd*	11.5/6.9	61.4	3.70	*dd*	12/4.9	61.2
6′	3.89	*dd*	11.5/1.6		3.89	*dd*	11.5/2		3.82	*dd*	12/2.3	
α-L-Rhamnose												
1	5.38	*d*	1.3	100	5.38	*d*	1.3	100	5.34	*d*	1.5	96
2	3.95	*dd*	3.2/1.8	70.6	3.93	*dd*	3.3/1.8	70.6	3.95	*m*	–	70.8
3	3.76	*dd*	9.3/3.2	70.4	3.77	*dd*	9.4/3.3	70.5	3.68	*dd*	10/1.5	70.5
4	3.38	*t*	9.3	72.8	3.38	*t*	9.4	72.8	3.39	*t*	10	72.5
5	3.98	*m*	–	68.6	3.97	dq	9.4/62	68.6	3.78	*m*	–	68.8
6	1.29	*d*	6	16.9	1.29	*d*	6.2	16.9	1.27	*d*	6.5	16.9
28-β-D-Glucose												
1	4.23	*d*	7.7	101.7	4.31	*d*	7.8	101.7	4.24	*d*	7.6	102.1
2	3.47	*dd*	9.1/7.7	80.3	3.5	*dd*	9.1/7.8	80.3	3.47	*dd*	9/7.6	80.4
3	3.58	*t*	9.1	76.7	3.59	*t*	9.1	76.7	3.58	*t*	9	76.7
4	3.35	*t*	9.1	69.8	3.37	*t*	9.1	69.8	3.35	*m*	–	69.8
5	3.23	*m*	–	72.8	3.26	*m*	–	72.8	3.24	*m*	–	74.5
6	3.69	*dd*	11.7/5.3	61.1	3.72	*dd*	12/6.2	61.1	3.68	*dd*	11.8/5	61.2
6′	3.86	*dd*	11.7/1.9		3.87	*dd*	12/2		3.86	*dd*	11.8/2.3	–
β-D-Glucose												
1	4.66	*d*	7.8	103.4	4.68	*d*	7.8	103.3	4.66	*d*	7.8	104
2	3.24	*t*	7.8	76.5	3.21	*dd*	10.5/7.8	73.9	3.24	*t*	7.8	76.5
3	3.40	*m*	–	76.5	3.38	*m*	–	76.5	3.39	*m*	–	76.6
4	3.41	*m*	–	69.5	3.32	*m*	–	69.5	3.28	*m*	–	69.4
5	3.27	*m*	–	76.3	3.26	*m*	–	76.8	3.41	*m*	–	76.9
6	3.92	*dd*	10.6/1.6	60.6	3.89	*dd*	11.5/6	60.8	3.92	*dd*	12/1.6	60.6
6′	3.76	*dd*	11/5		3.74	*dd*	11.5/2		3.76	*dd*	12/4.6	–

and carbon signals for four sugars (Table 3). The TOCSY and ROESY experiments indicated that the osidic chains of saponins **1** and **2** were identical. The structure of the new saponin **2** was deduced to be 24-*O*-[α-L-rhamnopyranosyl-(1->2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1->2)-β-D-glucopyranosyl]-16-desoxy-protoaescigenin.

The positive ESI–MS of the new compound **3** gave a quasi-molecular ion at *m*/*z* 1176 amu $[M+Na+H]^+$ and the negative ESI–MS a molecular ion at *m*/*z* 1151 in agreement with a molecular formula of $C_{54}H_{88}O_{26}$ ($C_{54}H_{90}O_{25}$ for saponin **1**). The fragmentations were identical to those observed for saponins **1** and **2** and suggested the presence of the same osidic part (Table 1).

Comparison of the ^{1}H NMR spectra of compounds **1** and **3** showed signals for six angular methyl groups and for four hydroxymethine protons H-3, H-16, H-21 and H-22 in both compounds (Table 2) and absence of the AX spin system of H-24 protons in saponin **3**. HMBC spectrum of saponin **3** displayed a correlation between H-23 at δ 1.49 ppm and one carbonyl at δ 175 ppm suggesting that C-24 position of the genin was a carboxylic ester. The chemical shift of C-23 at δ 22.9 also suggested that this methyl group was in an α-equatorial position. The observation of ROE between α-axial H-3 and H-23 confirmed the configuration of C-4 with a β-axial carboxylic ester C-24. The aglycone of saponin **3** was concluded to be the new 3β, 16α, 21β, 22α, 28β-pentahydroxy-olean-12-en-24-oic acid named 24-oxo-camelliagenin D.

The ^{1}H and ^{13}C NMR spectra of **3** showed that this saponin contained four sugars with signals of anomeric carbons at δ_C 93.9, 96, 102.1 and 104 with corresponding anomeric protons at δ_H 5.65, 5.34, 4.24 and 4.66, respectively (Table 3). Analysis of 2D experiments (COSY, TOCSY and HSQC) permitted assignments of osidic ^{1}H and ^{13}C signals and identification of one β-D-glucose with shielded anomeric carbon (δ 93.9) and deshielded anomeric proton (δ 5.65) engaged in an ester linkage with the C-24 of genin, of one α-L-rhamnose and of two β-D-glucoses. The ROESY experiment provided information about glycosidic linkages and showed that the sugar chains were identical as in saponins **1** and **2**. Thus, saponin **3** was 24-*O*-[α-L-rhamnopyranosyl-(1->2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-

Table 4
^1H and ^{13}C NMR data of prosapogenins **4**–**6** in $CDCl_3$

	4				**5**				**6**			
	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)	δ_H (ppm)	*m*	*J* (Hz)	δ_C (ppm)
Genin												
1 eq	1.65	*m*	–	38.3	1.67	*brd*	13	38.5	1.75	*brd*	13	38.4
1 ax	0.98	*ddd*	17/13/7		1.02	*m*	–		1.07	*m*	–	
2 eq	1.70	*dd*	13/3.7	27.5	1.62	*m*	–	27.1	1.86	*m*	–	28
2 ax	1.81	*m*	–		1.59	*m*	–		1.87	*m*	–	
3	3.44	*dd*	11.6/4.2	80.6	3.25	*dd*	12/5.2	78.9	3.23	*dd*	14/6.5	na[a]
4	–	–	–	42.6	–	–	–	38.7	–	–	–	52.6
5 ax	0.86	*d*	14.5	55.7	0.77	*d*	11.4	55.1	1.03	*d*	13.6	56.3
6 ax	1.29	*m*	–	18.3	1.42	*m*	–	18.2	1.64	*m*	–	18.3
6 eq	1.61	*m*	–		1.58	*m*	–		1.88	*m*	–	
7 ax	1.54	*m*	–	32.9	1.59	*m*	–	32.7	1.58	*m*	–	32.9
7 eq	1.30	*m*	–		1.31	*m*	–		1.41	*m*	–	
8	–	–	–	39.6	–	–	–	39.7	–	–	–	39.5
9	1.62	*m*	–	46.5	1.65	*m*	–	46.5	1.66	*m*	–	45.4
10	–	–	–	36.5	–	–	–	36.8	–	–	–	37.1
11	1.89	*m*	–	23.7	1.93	*m*	–	23.4	1.95	*m*	–	23.8
12	5.44	*brt*	3.5	124.5	5.48	*brt*	3.4	124.7	5.48	*t*	3.5	124.6
13	–	–	–	140.7	–	–	–	140.7	–	–	–	140.8
14	–	–	–	40.9	–	–	–	40.9	–	–	–	41.1
15 ax	1.65	*dd*	15.3/3.5	33.5	1.70	*dd*	15/4	33.6	1.69	*dd*	15/1.7	33.5
15 eq	1.35	*dd*	15.3/1.5		1.38	*dm*	15		1.40	*dd*	15/4	
16	3.94	*brs*	$W_{1/2}=8$	69.6	3.97	*brs*	$W_{1/2}=11$	69.8	3.96	*brs*	$W_{1/2}=10$	69.8
17	–	–	–	47.7			–	47.7		–	–	47.8
18 ax	2.72	*dd*	14/4.1	39.2	2.75	*dd*	14/4	39.2	2.74	*dd*	14/4	39.3
19 ax	2.57	*t*	14	46.3	2.59	*t*	14	46.3	2.56	*t*	14	46.3
19 eq	1.26	*dd*	14/4		1.30	*dd*	14/4		1.31	*dd*	14/4	
20		–	–	35.7			–	35.7		–	–	35.8
21	5.80	*d*	10.3	77.4	5.88	*d*	10.3	77.3	5.85	*d*	10.3	77.2
22	5.40	*d*	10.3	73.1	5.43	*d*	10.3	73.1	5.42	*d*	10.3	73.1
23	1.24	*s*	–	22.4	1.11	*s*	–	28	1.30	*s*	–	19.1
24	4.19	*d*	11	64.4	0.81	*s*	–	15.8	9.80	*s*	–	207.9
24′	3.32	*d*	11									
25	0.88	*s*	–	16.1	0.94	*s*	–	14.1	0.87	*s*	–	14.7
26	0.86	*s*	–	16.6	0.95	*s*	–	16.7	0.93	*s*	–	16.8
27	1.44	*s*	–	26.9	1.47	*s*	–	27	1.46	*s*	–	26.9
28	3.26	*d*	11	63.5	3.29	*d*	10	63.6	3.27	*d*	10.9	63.7
28′	2.89	*d*	11		2.92	*d*	10		2.92	*d*	10.9	
29	0.91	*s*	–	29	0.92	*s*	–	29	0.93	*s*	–	29
30	1.08	*s*	–	19.5	1.02	*s*	–	19.5	1.09	*s*	–	19.5
Angeloyls												
1′	–	–	–	167.5	–	–	–	167.5	–	–	–	167.6
2′	–	–	–	128	–	–	–	128	–	–	–	128
3′	6.01	*qq*	7.3/1.3	137.5	6.03	*qq*	7.3/1.3	137.5	6.02	*qq*	7.3/1.5	137.6
4′	1.92	*dq*	7.3/1.3	15.5	1.95	*dq*	7.3/1.3	15.5	1.93	*dq*	7.3/1.5	15.6
5′	1.82	*q*	1.3	20.4	1.85	*q*	1.3	20.4	1.83	*q*	1.5	20.4
1″	–	–	–	169.2	–	–	–	169.2	–	–	–	169.2
2″	–	–	–	127	–	–	–	127	–	–	–	127
3″	6.11	*qq*	7.2/1.4	140.1	6.14	*qq*	7.2/1.4	140.1	6.13	*qq*	7.2/1.5	140.3
4″	1.95	*dq*	7.2/1.4	15.5	1.98	*dq*	7.2/1.4	15.5	1.97	*dq*	7.2/1.5	15.8
5″	1.82	*q*	1.4	20.6	1.85	*q*	1.4	20.6	1.83	*q*	1.5	20.6

[a] *na*: not assigned.

(1−>2)-β-D-glucopyranosyl]-24-oxo-camelliagenin D. These three compounds **1**, **2** and **3** represent a new type of bidesmosidic triterpene saponins and to the best of our knowledge, the presence of a single sugar chain at C-24 instead of C-3 is unique.

The positive ESI–MS of compound **4** gave a quasi-molecular ion peak at *m*/*z* 693 $[M+Na]^+$ corresponding to a M_r of 670 and a molecular formula of $C_{40}H_{62}O_8$. The MS^2 displayed two fragmentation peaks at *m*/*z* 593 and 493 corresponding to the losses of two $C_5H_8O_2$

units, identified as angelic acids by analysis of ^{1}H and ^{13}C NMR spectra (Table 4) (Dizes et al., 1998).

The presence of protoaescigenin as aglycone in the structure of prosapogenin **4** was deduced from the analysis of its ^{1}H and ^{13}C NMR spectra (Table 4). The deshielded vicinal H-21 and H-22 protons at δ 5.80 and 5.40 suggested that these positions were esterified by the angeloyl groups. HMBC experiment confirmed this assumption by the correlations observed between H-21 (δ_H 5.80) and the carbonyl (δ_C 167.5) of the first angeloyl group and between H-22 (δ_H 5.40) and the carbonyl (δ_C 169.2) of the second angeloyl residue. Prosapogenin **4** was identified as 21β, 22α-*O*-diangeloyl-protoaescigenin previously described in *Harpullia ramiflora* (Dizes et al., 1998).

Compound **5** was characterized as 21β, 22α-*O*-diangeloyl barringtogenol C previously described in *Aesculus indica* (Sati and Rana, 1987) and *Maesa ramentacea* (Tuntiwachwuttikul et al., 1997). Its positive ESI–MS showed a quasi-molecular ion $[M+Na]^+$ at m/z 677 in agreement with a molecular formula of $C_{40}H_{62}O_7$. The MS^2 fragmentation gave two ion fragments at m/z 577 and 477 attributed to the losses of two angelic acids as in prosapogenin **4**. Comparison of ^{1}H NMR spectrum of compounds **4** and **5** indicated that **5** possessed one supplementary methyl singlet at δ 0.81. In the ^{13}C NMR spectrum of **5**, only one hydroxymethylene was detected at δ 63.6 (C-28) and a supplementary methyl was observed at δ 15.8 (Table 4). Consequently, the genin was identified as barringtogenol C (Ito and Ogino, 1967; Mahato and Kundu, 1994) also named theasapogenol B, which was substituted by two angeloyl groups on C-21 and C-22 in prosapogenin **5**.

Prosapogenin **6** presented in positive ESI–MS an ion peak $[M+Na]^+$ at m/z 691 ($C_{40}H_{60}O_8$) and two ion fragments due to the losses of two angeloyl groups (m/z 591 and 491). The ^{1}H and ^{13}C NMR spectra of prosapogenin **6** showed six quaternary methyls, two hydroxymethines at C-3 and C-16 positions, two hydroxymethines at C-21 and C-22 positions which were deshielded by esterification with angeloyl groups, one AX spin system (H-28) at δ_H 2.92 and 3.27 (*d*, $J=10.9$ Hz) and δ_C 63.7. Deshielded signals at δ_H 9.80 and δ_C 207.9 were detected and analysed for an aldehydic fonction at position C-24 (Table 4). The ROESY experiment confirmed this assignment with a ROE effect between axial H-3 and equatorial H-23 both in an α-orientation. The configurations of C-16, C-21, C-22 and C-28 were consistent with those reported for camelliagenin D (Ito and Ogino, 1967). Thus, the new prosapogenin **6** was identified as 21β, 22α-*O*-diangeloyl camelliagenin D.

At the moment, the biological activities of the isolated compounds have not been investigated. Further investigations are in progress to isolate and identify acylated saponins containing the prosapogenins **4**–**6**. The pure saponins will then be tested and compared to target cytotoxicity in the crude plant extract.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 500 (^{1}H at 500 MHz and ^{13}C at 125 MHz); 2D experiments were performed using standard Bruker microprograms. ESI–MS and MS–MS experiments were recorded on Bruker Esquire LC–MS instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

3.2. Plant material

Stem bark of *H. austro-caledonica* was collected in rain forest at an elevation of 600 m in the reserve of Amieu Pass and Table Unio, New Caledonia, in March 1997. The specimen of the plant (LIT 0250) is deposited in the herbarium of the botanical laboratory at the CNRS centre of Noumea (New Caledonia).

3.3. Extraction and isolation

Dried and powered stem bark (1110 g) was macerated in 20% aq. MeOH (10 l) for 17 h and boiled for 3 h. The hydromethanolic extract was filtered, evaporated and freeze-dried to give a residue (106 g) which was suspended in MeOH (400 ml). The methanolic fraction was added to 2 l of Me_2CO and the ppt. was filtered and dried over KOH in vacuo. This dried ppt. (57 g) was dissolved in pure H_2O and dialysed against H_2O in seamless cellulose tubing under agitation during 48 h. The contents of the tubes were freeze-dried to afford 32 g of a saponin mixt. (yield 3%).

Two aliquotes of the saponin mixt. (1 and 3 g) were fractionated on a silica gel CC, using a gradient of $CHCl_3$–MeOH–H_2O (8:2:0 to 15:10:1) for the first and (7:3:0 to 60:40:5) for the second. Frs. (15–21) of the first column and frs. (10–21) of the second column eluted with $CHCl_3$–MeOH (7:3) were similar in comparing by TLC ($CHCl_3$–MeOH–H_2O 60:40:5) and were purified on a reversed-phase RP-18 CC using a gradient of MeOH-H_2O (55:45 to 8:2). Frs. (14–16) eluted with MeOH–H_2O (55:45) was purified by preparative TLC in $CHCl_3$–MeOH–H_2O (60:40:5) to give saponin **1** (7.8 mg). Frs. (19–29) eluted with (55:45), contained saponins **2** (4.7 mg) and **3** (2.4 mg), which were purified by silica gel CC eluted with $CHCl_3$–MeOH–H_2O and followed by prep. TLC ($CHCl_3$–MeOH–H_2O, 60:40:5).

3.4. Saponin *1*

White powder $[\alpha]_D^{21^\circ}$ −13.6° (MeOH, *c* 0.45); ^{1}H and ^{13}C NMR (CD_3OD) see Tables 2 and 3; ESI–MS (positive ion mode) m/z 1161 $[M+Na]^+$; ESI–MS–MS: MS^2 (1161) m/z 1015 $[(M+Na)\text{-rha}]^+$, 997 $[(M+Na)\text{-glc}]^+$,

853 $[(M+Na)\text{-rha-glc}]^+$; MS^3 (1015) m/z 691 $[(M+Na)\text{-rha-2glc}]^+$; ESI–MS (negative ion mode) m/z 1137 $[M-H]^-$, 991 $[M-H\text{-rha}]^-$, 975 $[M-H\text{-glc}]^-$; ESI–MS–MS: see Table 1.

3.5. Saponin **2**

White powder $[\alpha]_D^{21}$ −7.46° (MeOH, *c* 0.25); ^1H and ^{13}C NMR (CD_3OD) see Tables 2 and 3; ESI–MS (positive ion mode) m/z 1145 $[M+Na]^+$, ESI–MS–MS : MS^2 (1145) m/z 999 $[(M+Na)\text{-rha}]^+$, 983 $[(M+Na)\text{-glc}]^+$, 837 $[(M+Na)\text{-rha-glc}]^+$, 675 $[(M+Na)\text{-rha-2glc}]^+$; ESI MS (negative ion mode) m/z 1122 $[M]^{-\cdot}$; ESI–MS–MS: see Table 1.

3.6. Saponin **3**

White powder $[\alpha]_D^{21}$ 0° (MeOH, *c* 0.108,); ^1H and ^{13}C NMR (CD_3OD) see Tables 2 and 3; ESI–MS (positive ion mode) m/z 1176 $[M+Na+H]^+$; ESI–MS–MS : MS^2 (1176) m/z 1129 $[(M+Na)\text{-rha}]^+$, 867 $[(M+Na)\text{-rha-glc}]^+$, MS^3 (867) m/z 705 $[(M+Na)\text{-rha-2glc}]^+$, MS^4 (705) m/z 543 $[\text{genine}+Na]^+$; ESI–MS (negative ion mode) m/z 1152 $[M]^{-\cdot}$; ESI–MS–MS : see Table 1.

3.7. Acid hydrolysis of saponins

The crude saponin mixture (500 mg) was dissolved in 16 ml of a mixture (1:1) of 6.5% $HClO_4$ and H_2SO_4 0.02N, and heated at 140 °C in a sealed tube for 2 h. After cooling, the sapogenin ppt. was filtered, rinsed with H_2O and dried in vacuo over P_2O_5. The acid aq. layer was neutralised with KOH 0.5 M and freeze-dried. Five sugars were identified with authentic samples by TLC in MeCOEt-*iso*-PrOH–Me_2CO–H_2O (20:10:7:6) as glucose, galactose, rhamnose, arabinose and xylose. After prep. TLC of the sugar mixt. (100 mg) in this solvent, the optical rotation of each purified sugar was measured. The sapogenin mixt. (260 mg) was purified by silica gel CC using a gradient of $CHCl_3$–MeOH (100:0 to 93:7). Prosapogenins **5** (3.7 mg) and **6** (3 mg) were found in frs. (7–13) eluted with $CHCl_3$ and purified by silica gel CC using a gradient of hexane-AcOEt (9:1 to 7:3). Frs. (27–30) eluted with (99:1) contained prosapogenin **4** (33.2 mg).

3.8. 21β,22α-O-Diangeloyl protoaescigenin **4**

$[\alpha]_D^{21°}$: +14.9° ($CHCl_3$, *c* 1); ^1H and ^{13}C NMR ($CDCl_3$) see Table 4; ESI–MS (positive ion mode) m/z 677 $[M+Na]^+$; ESI–MS–MS (677) m/z 577 $[(M+Na)\text{-Ang}]^+$, 477 $[(M+Na)\text{-2Ang}]^+$.

3.9. 21β,22α-O-Diangeloyl barringtogenol C **5**

$[\alpha]_D^{21°}$: +11° ($CHCl_3$, *c* 0.308); ^1H and ^{13}C NMR ($CDCl_3$) see Table 4; ESI–MS (positive ion mode) m/z 691 $[M+Na]^+$; ESI–MS–MS (691) m/z 591 $[(M+Na)\text{-Ang}]^+$, 491 $[(M+Na)\text{-2Ang}]^+$; ESI–MS (negative ion mode) m/z 667 $[M-H]^-$.

3.10. 21β,22α-O-Diangeloyl camelliagenin D **6**

$[\alpha]_D^{21°}$: +27° ($CHCl_3$, *c* 0.1); ^1H and ^{13}C NMR ($CDCl_3$) see Table 4; ESI–MS (positive ion mode) m/z 693 $[M+Na]^+$; ESI–MS–MS (693) m/z 593 $[(M+Na)\text{-Ang}]^+$, 493 $[(M+Na)\text{-2Ang}]^+$; ESI–MS (negative ion mode) m/z 669 $[M-H]^-$.

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