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SAPONINS FROM *HARPULLIA CUPANIOIDES*

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Key Word Index—*Harpullia cupanioides*; Sapindaceae; triterpene saponins.

Abstract—Five new saponins have been isolated from the stem bark of *Harpullia cupanioides* and identified as 3-*O*-*β*-D-glucopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 22-*O*-angeloyl-A1-barrigenol, 3-*O*-*β*-D-glucopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 28-*O*-angeloyl-A1-barrigenol, 3-*O*-*β*-D-galactopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 28-*O*-angeloyl-A1-barrigenol, 3-*O*-*β*-D-galactopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 16-*O*-*β*,*β*-dimethylacryloyl-camelliagenin A and 3-*O*-*β*-D-glucopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 28-*O*-angeloyl-camelliagenin A. The structures were elucidated by analysis of 2D-NMR spectra and mass spectra. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Harpullia cupanioides Roxb. is a large tree originating from Asia and shown to contain saponins whose hydrolysis led to the identification of six prosapogenins, 22-*O*-angeloyl A1-barrigenol, 22-*O*-*β*,*β*-dimethylacryloyl A1-barrigenol, 16-*O*-tigloyl camelliagenin A, 22-*O*-angeloyl camelliagenin A, 16-*O*-*β*,*β*-dimethylacryloyl camelliagenin A and 22-*O*-*β*,*β*-dimethylacryloyl camelliagenin A [1]. *H. cupanioides* was selected for further studies because of the high haemolytic activity of its saponins and we report here on the isolation and structural elucidation of five new saponins from the stem bark of this tree.

RESULTS AND DISCUSSION

H. cupanioides was collected in the Kisantu Botanical Gardens in the Democratic Republic of Congo (former Zaire). Crude saponin extract was purified by reversed-phase C-18 column chromatography followed by preparative TLC on reversed-phase RP-18. Three new compounds **1**, **4** and **5** were obtained in a pure state accompanied by an inseparable mixture of isomers **2** and **3**. Acid hydrolysis of the saponin extract gave the aforementioned mixture of prosapogenins and sugars identified by TLC as D-glucose, D-galactose, L-rhamnose and D-glucuronic acid.

The negative FAB mass spectrum of saponin **1** and of the mixture of **2** and **3** were similar and displayed the same molecular ion peak at *m*/*z* 1055 $[M–H]^-$ suggesting an M_r of 1056 amu ($C_{53}H_{84}O_{21}$). Losses of a C_5H_9O fragment, of terminal 6-desoxyhexose and of hexose from $[M–H]^-$ led to ions at *m*/*z* 973, 909 and 893, respectively.

The structure of the triterpene part of saponin **1** was recognized to be A1-barrigenol by ^1H NMR and ^{13}C NMR analysis (Table 1) using the connectivities observed in COSY, HMQC and HSQC spectra and was in full agreement with literature data [2, 3]. The ^1H NMR spectrum showed six singlets for seven angular methyl groups, one olefinic proton at *δ* 5.42 (*brt*, *J* = 3 Hz) and one shielded oxygen bearing methine proton at *δ* 3.20 (H-3, *m*) coupled with two protons at *δ* 1.69 and 2.02. The protons attached to C-15, C-16, C-22 and C-28 were identified as a broad signal at *δ* 3.78, a sharp doublet at *δ* 3.92 (*J* = 4.5 Hz), as one deshielded doublet of doublets at *δ* 5.45 (*J* = 12 and 5 Hz) [coupling to protons of a methylene at *δ* 1.56 (*brd*, *J* = 12 Hz) and 2.22 (*t*, *J* = 12 Hz)] and as one isolated AB system at *δ* 3.12 and 3.32 (*J* = 11 Hz). The deshielding of H-22 suggested that the position was esterified. Signals for the ester connected onto

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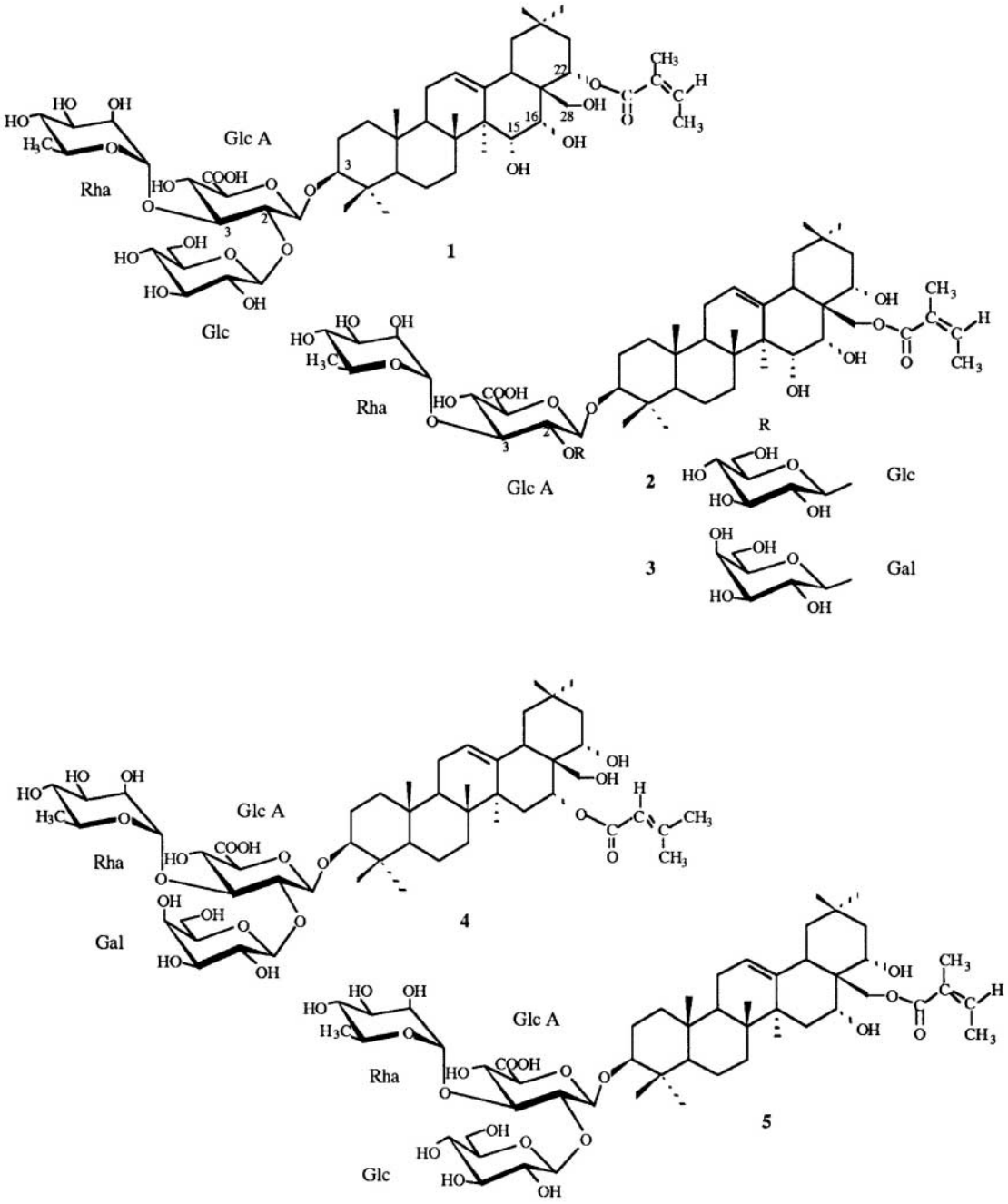

C-22 were two vinylic methyl groups at δ 1.90 and 1.97 correlated in the COSY spectrum with one ethylenic methine at δ 6.05 (*qq*, $J = 7$ and 1.5 Hz). This suggested their belonging to an angelic acid residue and its attachment at O-22 was confirmed by the observation of a HMBC correlation between H-22 and a carbonyl at δ 169.5 (itself coupled to one of the vinylic methyls). The sugar part of the molecule consisted of three residues giving signals for three anomeric carbons at δ 103.4, 103.7 and 105.4, respectively, attached to proton doublets at δ 5.06, 4.61 and 4.47 (HSQC). A methyl carbon at δ 17.8 was assigned to a 6-desoxyhexose therefore rhamnose, a carbonyl at δ 176.5 to a glucuronic acid and a hydroxymethyl at δ 63.6 corresponded to a hexose. COSY and HOHAHA experiments allowed the full identification of the spin systems of an α-L-rhamnose starting from the narrow doublet at δ 5.06, and of a β-D-glucose from the doublet at δ 4.61 and of a β-D-glucuronic acid from the doublet at δ 4.47 (Table 2). Sequencing of the sugar chain was achieved by observation of HMBC correlations between C-1 (δ 105.4) of glucuronic acid and H-3 of A1-barrigenol, C-1 (δ 103.7) of glucose and H-2 (δ 3.75) of glucuronic acid and between C-1 (δ 103.4) of rhamnose and H-3 (δ 3.67) of glu-

Table 1. ^{1}H and ^{13}C-NMR data of A1-barrigenol and camelliagenin A in saponins **1**–**5** (CD_3OD, ppm)

	1		**2** and **3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	40.1	1.02 *m*, 1.62 *m*	40.2	1.02 *m*, 1.65 *m*	39	1.03 *m*, 1.64 *m*	40.5	
2	27	1.69 *m*, 2.02 *m*	26.9	1.72 *m*, 2.05 *m*	27	1.73 *dd* (12–9), 2.03 *dd* (12–3)	26.9	1.74 *t* (12), 2.04 *dd* (12–6)
3	92	3.2 *m*	92	3.22 *m*	91.7	3.21 *dd* (11–3.6)	92	3.2 *dd* (12–4)
4	40.5		40.4		40.5		40	0.78 *brd* (11)
5	56.7	0.78 *brd* (12)	56.7	0.77 *m*	57	0.79 *brd* (11)	57	
6	19.5	1.54 *m*	19.5	1.57 *m*	19.3	1.41 *m*, 1.56 *m*	20.4	
7	37.2	1.72 *m*	37.2		34	1.35 *m*, 1.54 *m*	34	
8	42.2		42.2		41.2		40.9	
9	47.8	1.58 *m*	na	1.6 *m*	48.7	1.67 *dd* (15–7)	48	1.64 *m*
10	37.9		37.9		37.8		37.7	
11	24.8	1.92 *m*	24.7	1.94 *m*	24.6	1.94 *m*	24.6	1.88 *m*
12	126.3	5.42 *brt* (3)	126.3	5.35 *m* ($W_{1/2}$ = 8)	124.9	5.37 *brt* (3.5)	124.6	5.26 *m*
13	144.4		144.4		142.7		143.8	
14	48.2		46.8		42.8		46.8	
15	68.6	3.78 *m*	68.6	3.87 *d* (4.7)	31.8	1.47 *brd* (16), 1.98 *dd* (16–6)	35.5	1.83 *brd* (15.4), 1.4 *dd* (15.4–4.6)
16	74.2	3.92 *d* (4.3)	74	3.93 *d* (4.7)	71.2	5.51 *m* ($W_{1/2}$ = 8)	70.1	4.15 *m* ($W_{1/2}$ = 8)
17	45.4		na		na		45	
18	43	2.43 *brs*	43	2.43 *brs*	42.8	2.19 *m*	42.3	2.42 *dd* (12–6)
19	47.6	2.48 *m*, 1.06 *m*	na	2.45 *m*, 1.05 *m*	48	1.12 *m*, 2.3 *m*	48	
20	32.5		32.3		32.2		32.4	
21	45.9	1.56 *brd* (12), 2.22 *t* (12)	45.3	1.48 *brd* (12), 2.16 *t* (12)	44.5	1.42 *dd* (12–4), 1.71 *t* (12)	46.2	1.46 *m*, 2.1 *t* (12)
22	73.4	5.45 *dd* (12–5)	74.1	4.04 *dd* (11–6.5)	74.4	4.06 *m*	70.3	4.03 *dd* (11–6.5)
23	28.4	1.07 *s*	28.4	1.07 *s*	28.4	1.09 *s*	28.3	1.07 *s*
24	16.9	0.86 *s*	16.9	0.86 *s*	16.9	0.9 *s*	16.9	0.86 *s*
25	16.3	0.98 *s*	16.3	0.98 *s*	16.2	1.01 *s*	16.2	0.98 *s*
26	18	1.02 *s*	18	1.03 *s*	17.2	1 *s*	17.5	0.96 *s*
27	21	1.39 *s*	20.9	1.37 *s*	27.5	1.31 *s*	27.6	1.44 *s*
28	63.6	3.12 *d* (11), 3.32	69	3.23 *d* (10.7), 3.52 *d* (10.7)	70.3	3.32 *d* (12), 3.64 *d* (12)	67	3.85 *d* (11), 3.91 *d* (11)
29	33.7	0.9 *s*	33.6	0.89 *s*	34	0.99 *s*	33.7	0.9 *s*
30	25.4	0.98 *s*	25.1	0.98 *s*	25.5	1 *s*	25.4	0.97 *s*

na: not assigned.

curonic acid. Consequently the structure of saponin **1** was determined to be 3-*O*-*β*-D-glucopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 22-*O*-angeloyl-A1-barrigenol.

Despite repeated chromatography and apparent homogeneity on TLC, the next fraction consisted of two inseparable compounds **2** and **3**. The ^{1}H NMR and ^{13}C NMR spectra showed a set of sharp peaks corresponding to the common parts of the molecule and peaks of smaller intensity for the residues belonging to one isomer. Amongst the large peaks, those corresponding to the triterpene, A1-barrigenol, were readily identified. The chemical shift of proton H-22 at δ 4.04 (*dd*, J = 11 and 6.5 Hz) indicated that C-22 was not esterified. The ^{1}H NMR and ^{13}C NMR spectra showed the presence of one angelic acid with vinylic methyl groups at δ_H 1.96 and 1.92 (δ_C 16 and 20.9), ethylenic proton at δ_H 6.11 (δ_C 137) and two quaternary carbons at δ 127 (ethylenic) and δ 167.5 (carbonyl). The deshielding of H-28 suggested that this position was esterified by the angelic acid. Signals for four sugar residues were observed in the ^{13}C NMR spectrum with anomeric carbons at δ 103.3, 103.5, 104.4 and 105.4 which coupled to proton doublets at δ 5.07, 4.60, 4.55 and 4.47, respectively (HSQC). Examination of the intensities of the anomeric protons suggested that one of the sugars was a mixture. Analysis of COSY and HOHAHA experiments showed that three sugars were those of saponin **1**: α-L-rhamnose (sharp doublet at δ 5.07), *β*-D-glucose (doublet at δ 4.60) and glucuronic acid (doublet at 4.47). The fourth set of signals was identified as being due to a *β*-D-galactose with an anomeric doublet at δ_H 4.55 (*d*, J = 7.5 Hz) and δ_C 104.4 and characterised by a proton H-4 in equatorial position at δ 3.80 (*dd*, J = 3 and 1.4 Hz). Sequencing of the sugar chains was achieved by observation of HMBC correlations: saponins **1** and **2** had the same three sugar chain attached to C-3 of the aglycone while in saponin **3**, the glucose was replaced by galactose as suggested by the relative intensities of the signals of the anomeric protons. A HMBC correlation was found between H-1 of galactose and C-2 of glucuronic acid. Saponin **2** was therefore 3-*O*-*β*-D-glucopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 28-*O*-angeloyl-A1-barrigenol and saponin **3**, 3-*O*-*β*-D-galactopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 28-*O*-angeloyl-A1-barrigenol.

Comparisons of the negative FAB mass spectrum of saponins **4** and **5** showed that they were isomeric compounds with $C_{53}H_{84}O_{20}$ composition ([M–H]$^-$

Table 2. ^{1}H and ^{13}C NMR data of osidic moieties of saponins **1**–**5** (CD_3OD, ppm)

	1		**2** and **3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
β-D Glc A								
1	105.4	4.47 *d* (7.4)	105.4	4.47 *d* (7.7)	105.5	4.5 *d* (7.6)	105.4	4.47 *d* (7.4)
2	78.5	3.75 *dd* (8.7–7.4)	78.6	3.74 *dd* (9–7.7)	78.1	3.77 *dd* (8.9–7.7)	78.5	3.75 *t* (8)
3	86.6	3.67 *t* (8.5)	86.5	3.68 *m*	86.5	3.69 *t* (8.7)	86.5	3.67 *t* (8)
4	72.7	3.57 *m*	72.7	3.58 *m*	72.7	3.61 *dd* (9–5)	72.5	3.58 *t* (8)
5	77.6	3.56 *m*	77.5	3.58 *m*	77.7	3.59 *d* (6.4)	77.8	3.56 *d* (8)
6	176.5	–	176.5	–	no	–	176.4	–
α-L Rha								
1	103.4	5.06 *d* (1.6)	103.3	5.07 *d* (1.7)	103.3	5.1 *d* (2.4)	103.3	5.06 *brs* ($W_{1/2}$ = 4)
2	72.3	4.02 *dd* (3.4–1.6)	72.2	4.04 *dd* (4–1)	72.2	4.07 *dd* (3.8–2.4)	72.2	4.03 *brd* (4)
3	72.3	3.69 *dd* (9.5–3.4)	72.2	3.69 *dd* (9.1–3.8)	72.2	3.71 *dd* (9.6–3.8)	72.2	3.69 *dd* (9.5–2.6)
4	740	3.38 *t* (9.5)	74	3.38 *t* (9.1)	74.3	3.41 *t* (9.8)	74	3.38 *t* (9.5)
5	70.5	4.02 *dq* (9.5–6)	70.4	4.03 *dq* (9.1–6.3)	70.2	4.05 *m*	70.4	4.03 *dq* (9–6)
6	17.8	1.24 *d* (6)	17.8	1.24 *d* (6.3)	17.8	1.27 *d* (6.2)	17.8	1.24 *d* (6.1)
β-D Glc (in **2**)								
1	103.7	4.61 *d* (7.7)	103.5	4.6 *d* (7.8)			103.7	4.6 *d* (7.7)
2	75.7	3.21 *dd* (9.2–7.7)	75.7	3.2 *dd* (9.2–7.8)			75.7	3.21 *dd* (9.5–7.5)
3	77.8	3.36 *t* (9.2)	77.5	3.36 *t* (8.7)			77.5	3.37 *t* (9)
4	72.5	3.09 *t* (8.9)	72.5	3.09 *dd* (9.8–8.4)			72.7	3.09 *t* (9.5)
5	78.7	3.23 *ddd* (9–5–2)	78.5	3.25 *ddd* (9.8–7.7–2)			78.6	3.25 *m*
6	63.6	3.55 *dd* (12.4–2.3), 3.83 *dd* (12.2–4.9)	63.5	3.57 *dd* (11.9–9), 3.82 *dd* (11.6–2)			63.5	3.55 *dd* (12–7), 3.83 *dd* (12–2)
β-D Gal (in **3**)								
1			104.4	4.55 *d* (7.5)	104.4	4.58 *d* (7.4)		
2			73.1	3.55 *dd* (9.9–7.6)	73.2	3.56 *dd* (9.8–7.4)		
3			74.8	3.48 *dd* (9.9–3)	74.8	3.51 *dd* (10–3.3)		
4			69.9	3.8 *dd* (3–1.4)	70.2	3.83 *dd* (3.2–1.2)		
5			77.2	3.44 *brs*	76.8	3.47 *ddd* (7.2–5–1)		
6			62.6	3.62 *dd* (11.6–4.9), 3.80 *dd* (11.6–7.1)	62.7	3.65 *dd* (11.8–5), 3.79 *dd* (11.6–7.2)		
Ang								
1	169.5	–	167.5	–			169	–
2	128.9	–	127	–			129	–
3	139.4	6.05 *qq* (7–1.5)	137	6.11 *qq* (7.3–1.7)			139	6.1 *qq* (7–1.3)
4	16	1.97 *dq* (7.2–1.5)	16	1.96 *dq* (7–1.6)			16	1.96 *dq* (7–1.3)
5	21	1.9 q^t (1.5)	20.9	1.92 q^t (1.6)			20.9	1.91 *brd* (1.3)
dMA								
1					167	–		
2					117.5	5.69 *brs* ($W_{1/2}$ = 3)		
3					159	–		
4					20.3	2.22 *d* (0.8)		
5					27.5	1.95 *d* (0.7)		

no: not observed.

at *m*/*z* 1039.5). The fragmentation observed on these spectra was similar to those observed for compounds **1**, **2** and **3**, with losses of a C_5 acid (at *m*/*z* 957.5), desoxyhexose (*m*/*z* 893.3) and hexose (*m*/*z* 877.3). With regard to the first series of saponins, the novelty in compounds **4** and **5** seemed thus to be the absence of an oxygen atom in the genin part. The ^{1}H NMR and ^{13}C NMR spectra of saponins **4** and **5** pointed to a common aglycone, camelliagenin A also named dihydropriverogenin A [4, 5]. In compound **4**, C-16 was esterified by a *β*,*β*-dimethylacrylic acid as in prosapogenin B of Ref. [1]. This was clearly demonstrated by the deshielding of H-16 (at *δ* 5.51), although no correlation was observed between H-16 and the carbonyl carbon in the HMBC spectrum (Table 1). In compound **5**, the genin was esterified by an angelic acid at C-28 as in compounds **2** and **3**. This was demonstrated by the observation of three bond correlations between the carbonyl carbon of angelic acid at *δ* 169 and protons H-28. ^{13}C NMR spectra of compounds **4** and **5** (1D, HMQC and HMBC) allowed determinations of the chains of sugars as being identical to those of compounds **3** and **1**, respectively. Compound **4** was thus identified to be 3-*O*-*β*-D-galactopyranosyl-(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 16-*O*-*β*,*β*-dimethylacryloyl-camelliagenin A and compound **5** was 3-*O*-*β*-D-glucopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 3)] *β*-D-glucuronopyranosyl 28-*O*-angeloyl-camelliagenin A.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 300 (proton at 300 MHz and carbon at 75 MHz) or Bruker Avance DRX 500 (^{1}H at 500 MHz and ^{13}C at 125 MHz); 2D experiments

were performed using standard Bruker microprograms. FABMS were recorded on a ZABZ-SEQ mass spectrometer and MeOH solution was added to glycerol matrix. Preparative TLC were performed with RP-18 Whatman plates, 20×20, 500 μm.

Plant material

The stem bark of *H. cupanioides* was collected in the Botanical Garden of Kisantu in the Democratic Republic of Congo. A specimen was compared to herbarium H.B. 4300 from the Brussels National Botanical Gardens.

Extraction and isolation of saponins

The saponin extract was obtained as previously described [6]. A part of the extract (1 g) was fractionated on a reversed-phase RP-18 CC, eluted with a gradient of MeOH–H_2O (from 2:3 to 3:2). Fractions 103–123 and 143–153 were purified by prep. TLC on a reversed-phase RP-18 in MeOH–H_2O (65:35) (two migrations) to give saponin **1** (18 mg), the mixture of saponins **2** and **3** (10 mg) and saponin **4** (23 mg). Fraction 179–188 was purified by prep. TLC in the same conditions to give saponin **5** (10 mg).

Acid hydrolysis of saponin mixture

Crude saponin (200 mg) was dissolved in 8 ml of a mixture (1:1) of 6.5% aq. $HClO_4$ and 0.02 N H_2SO_4 and heated at 140° in a sealed tube for 2 h. After cooling, the saponin precipitate was filtered, rinsed with H_2O and dried *in vacuo* over P_2O_5. The acid aq. layer was neutralized with 0.5 M KOH and freeze-dried. Sugars were identified with authentic samples as glucose, galactose, rhamnose and glucuronic acid by TLC in MeCOEt–*iso*-PrOH–Me_2CO–H_2O (20:10:7:6).

Saponin **1**

$[\alpha]_D^{21°}$: −13.7° (c = 0.23, CH_3OH). Negative ion FABMS m/z: 1055.4 $[M–H]^-$, 973.6 $[M–H–Ang]^-$, 909.3 $[M–H–Rha]^-$, 893.3 $[M–H–Glc]^-$. ^1H NMR and ^{13}C NMR (CD_3OD): see Tables 1 and 2.

Saponins **2** *and* **3**

Negative ion FABMS m/z: 1055.4 $[M–H]^-$, 973.5 $[M–H–Ang]^-$, 909.3 $[M–H–Rha]^-$, 893.3 $[M–H–Glc]^-$ = 893.3 $[M–H–Gal]^-$. ^1H NMR and ^{13}C NMR (CD_3OD): see Tables 1 and 2.

Saponin **4**

$[\alpha]_D^{21°}$: −26.1° (c = 0.24, CH_3OH). Negative ion FABMS m/z: 1039.4 $[M–H]^-$, 893.3 $[M–H–Rha]^-$, 877.4 $[M–H–Gal]^-$. ^1H NMR and ^{13}C NMR (CD_3OD): see Tables 1 and 2.

Saponin **5**

$[\alpha]_D^{21°}$: −10,1° (c = 0.95, CH_3OH). Negative ion FABMS m/z: m/z 1039.4 $[M–H]^-$, 857.5 $[M–H–Ang]^-$, 893.3 $[M–H–Rha]^-$, 877.3 $[M–H–Glc]^-$, 731.2 $[M–H–(Glc + Rha)]^-$. ^1H NMR and ^{13}C NMR (CD_3OD): see Tables 1 and 2.

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REFERENCES

1. Dimbi, M. Z., Warin, R., Delaude, C., Huls, R. and Mpuza, K., *Bulletin des Societés chimiques de Belgique*, 1983, **92,** 473–484.
2. Furuya, T., Orihara, Y. and Tsuda, Y., *Phytochemistry*, 1990, **29,** 2539–2543.
3. Higuchi, R., Komori, T., Kawasaki, T. and Lassak, E. V., *Phytochemistry*, 1983, **22,** 1235–1237.
4. Kitagawa, I., Im, K. S. and Yosioka, I., *Chemical and Pharmaceutical Bulletin*, 1976, **24,** 1260–1267.
5. Yoshikawa, M., Murakami, T., Yoshizumi, S., Murakami, N., Yamahara, J. and Matsuda, H., *Chemical and Pharmaceutical Bulletin*, 1996, **44,** 1899–1907.
6. Kapundu, M., Kakera, L. K., Graftieaux, A. and Delaude, C., *Bulletin de la Société Royale des Sciences de Liège*, 1987, **56,** 125–128.