



HARPULOSIDE A TRITERPENOID SAPONIN FROM *HARPULLIA RAMIFLORA*

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Key Word Index—*Harpullia ramiflora*; Sapindaceae; Harpuloside; triterpenoid saponin; protoaescigenin; one- and two- dimensional NMR.

Abstract—A new triterpenoid saponin named Harpuloside was isolated from the stem bark of *Harpullia ramiflora*. Its structure was established as 3-*O* [α -L-rhamno-pyranosyl (1 $\rightarrow$ 3)- β -D-xylopyranosyl (1 $\rightarrow$ 2)- β -D-glucurono-pyranosyl]-3 β , 16 α , 24, 28-tetrahydroxy-21 β , 22 α -*O*-diangeloyl-olean-12-ene, using one- and two-dimensional NMR spectral data. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The stem bark of *Harpullia ramiflora* Radlk is used, by the traditional healers in Papua New-Guinea, as a topical medicine against skin diseases [1]. This paper reports on the isolation and structural elucidation of a new triterpenoid saponin, harpuloside (**1**) present in the stem bark of this plant.

RESULTS AND DISCUSSION

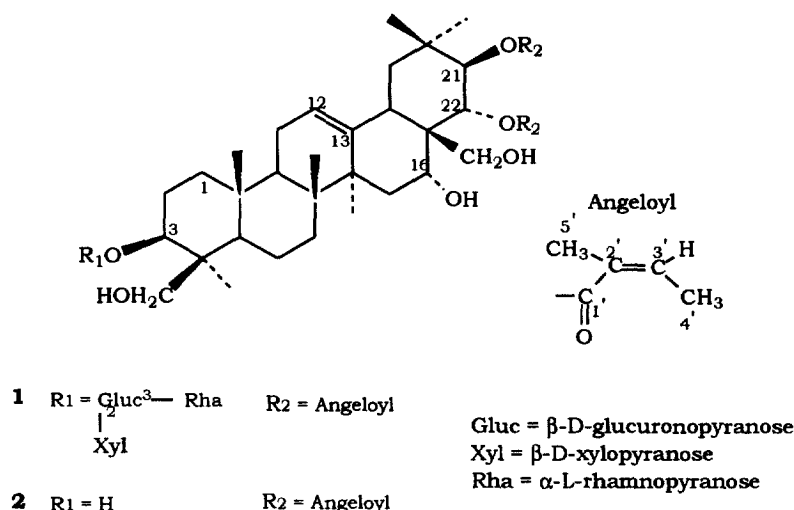
The powder was extracted with methanol-water (7:3). The residue of extraction was chromatographed on a silica gel column to give saponin (**1**) harpuloside, as a colourless powder, mp 190°C, $[\alpha]_D^{25} = -30^\circ$ (MeOH). The negative mode FAB-mass spectrum of **1** gave a molecular ion at m/z 1123 $[M-H]^-$ in agreement with a molecular formula of $C_{57}H_{88}O_{22}$. Negative fragments at m/z 991 and 977 were attributed to the losses of a terminal pentose and a terminal desoxyhesose, respectively. Acid hydrolysis of **1** yielded glucose, rhamnose and glucuronic acid as sugar components and compound **2** as the aglycone. Compound **2** showed a molecular ion peak at m/z 669 in the negative-ion FAB-mass spectrum corresponding to the molecular formula $C_{40}H_{62}O_8$.

The presence of two angeloyl groups in **2** was revealed by ten carbon signals at δ 169.1 and 167.5 (C-1'), 128.5 and 127.2 (C-2'), 140.2 and 138.1 (C-3'), 16.0 (2C, C-4') and 20.9 (2C, C-5'), and by characteristic 1H NMR resonances [2]: δ 6.08 and 6.00 (*br q*,

2H, $J=8$ Hz, H-3'), 1.97 and 1.93 (*d*, 6H, $J=8$ Hz, 6H, H-4') and 1.83 (6H, *br s*, H-5'). The 300 MHz 1H NMR spectrum of **2** showed six tertiary methyl groups at δ 0.88, 0.89, 0.93, 1.06, 1.26 and 1.45. Concerted application of one-dimensional (1H , ^{13}C and DEPT) and two-dimensional (COSY and HMQC) experiments indicated, in the 1H NMR spectrum of **2** the presence of one ethylenic signal located at δ 5.45 (*t*, $J=3.4$ Hz), of four methylene protons linked to oxygen bearing carbons which constituted two AX spin systems at δ 4.21 and 3.34 (each: 1H, $J=11$ Hz) and δ 3.26 and 2.91 (each: 1H, $J=11$ Hz), two hydroxymethine resonances at δ 3.95 (*br s*, $\Delta\nu/2=8$ Hz) and 3.46 (*dd*, $J=12.0, 5.0$ Hz) and a pair of protons corresponding to a vicinal diol substituted by the angeloyl groups and located at δ 5.86 (*d*, $J=10$ Hz) and 5.41 (*d*, $J=10$ Hz). In the ROESY experiment, the magnetization transfer observed between the hydroxymethine proton at δ 3.95 and the hydroxymethylene proton at δ 2.91, allowed assignment of these resonances to H-16 and H-28, respectively, and confirmed a β -equatorial orientation for H-16. These results suggested that the aglycone **2** was a new prosapogenin with the protoaescigenin skeleton substituted at C-21 and C-22 by angeloyl groups. Comparison of the ^{13}C NMR spectra of **2**, and of protoaescigenin [3, 4] showed that the signals of C-21 and C-22 in **2** were shifted by the acylation shift. Compound **2** was thus characterized as 21 β , 22 α -*O*-diangeloyl protoaescigenin.

The 400 MHz 1H NMR spectrum of saponin **1** showed three anomeric protons at δ 5.08 (*br s*), 4.52 (*d*, $J=8$ Hz) and 4.49 (*d*, $J=8$ Hz). The proton system of each sugar was completely assigned on the basis of

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a one-dimensional TOCSY experiment using selective pulses [5]. From the HMQC diagram C-2 and C-3 of glucuronic acid were found at δ 78.6 and 86.9, respectively. Moreover, for this latter sugar, C-3 exhibited ^1H -C correlation signal with H-1 in the HMBC experiment. Assignments of other osidic carbons were achieved in the same manner from the analysis of HMQC and HMBC correlations. The deshielding of C-2 and C-3 carbons of glucuronic acid residue was indicative of the substitution at these positions.

This result was further confirmed by the observation of ROE effects between protons engaged in the interglycosidic linkages in the ROESY experiment. The location of the interglycosidic linkage was unambiguously determined by means of HMBC correlation in which cross peaks were observed between rhamnose H-1 and glucuronic acid C-3 and xylose H-1 and glucuronic acid C-2, indicating that the rhamnosyl and xylosyl moieties were attached to the C-3 and C-2 of the glucuronic acid moiety, respectively. Furthermore, the anomeric proton of the glucuronic acid part was correlated with the C-3 signal of the prosapogenin. Consequently the structure of harpuloside (**1**) was elucidated as 3-*O* [α -L-rhamnopyranosyl (1 $\rightarrow$ 3)- β -D-xylopyranosyl (1 $\rightarrow$ 2)- β -D-glucuronopyranosyl]-3 β , 16 α , 24, 28-tetrahydroxy-21 β , 22 α -*O*-diangeloyl-olean-12-ene. It should be noted that the NMR chemical shifts of the sugars were in good agreement with the values described for saponins containing a similar sugar moiety; in this condition, the absolute configurations of monosaccharides were determined unambiguously [6]. The ^{13}C NMR chemical shifts of **1** and **2** are presented in Table 1.

EXPERIMENTAL

General

FAB-MS were obtained on Nermag R10-10H mass spectrometer in the negative ion mode. ^1H and ^{13}C

NMR spectra were recorded on Bruker AC-300 and AMX-400 spectrometers in CDCl_3 or CD_3OD solutions; TMS was used as standard in ^1H and ^{13}C measurements. Two-dimensional homonuclear correlation experiments were performed using Bruker AC-300, one-dimensional HOHAHA and two-dimensional heteronuclear correlation experiments using Bruker AMX-400. COSY, HMQC and HMBC experiments were obtained using standard Bruker microprograms. ROESY experiments were performed with hardware modifications of the Bruker AC-300 spectrometer [7].

Extraction and isolation

Plant material was collected in the Urimo region (East-Sepik province) of Papua New-Guinea. A voucher specimen is kept in the Department of Pharmacognosy, Faculty of Pharmacy, Marseille, France. Dried and powdered bark (190 g) were extracted with H_2O -MeOH (3 : 7). After concentration, the H_2O layer was freeze-dried to give 41 g of residue; part of which (2 g) was submitted to preparative liquid chromatography on a silica gel column (silica gel 230–400 Mesh Merck, 200 g) eluted with CHCl_3 -MeOH- H_2O (6 : 4 : 1) to yield saponin (**1**) (670 mg). TLC analysis of saponins and sugars were performed on precoated silica gel plates (Kieselgel 60F 254, 0.25 mm; Merck) using the following solvent systems: CHCl_3 -MeOH- H_2O (60 : 40 : 10) [system 1]; *n*-BuOH-HOAc- H_2O (4 : 1 : 5) [system 2]; CH_2Cl_2 -MeOH- H_2O (10 : 5 : 1) [system 3]. Spots were visualised by spraying with phosphoric acid and naphthoresorcinol for sugars and H_2SO_4 for genin followed by heating at 110°C.

Acid hydrolysis

Saponin **1** (5 mg) was heated with aq. 10% HCl (3 ml) in a sealed tube at 100°C for 4 hr. The sapogenin **2** was extracted with EtOAc; the aq. layer was neu-

Table 1. ^{13}C NMR chemical shifts (ppm) for **1** (CD_3OD) and **2** (CDCl_3)

	1	2		1	2
Aglycone carbon			Angeloyl carbon		
1	39.9	38.4	1'	169.8; 169.2	169.0; 167.5
2	27.0	28.0	2'	129.4; 129.2	128.5; 127.2
3	92.5	80.9	3'	139.5; 138.7	140.2; 138.1
4	44.8	43.0	4'	16.1; 16.0	16.0
5	57.5	56.0	5'	20.9; 20.8	20.9
6	19.4	18.6			
7	34.0	33.1	Sugar carbon	Glucuronic acid	
8	40.9	40.1	1	105.0	
9	48.3	46.7	2	78.6	
10	37.8	36.8	3	86.9	
11	24.9	23.9	4	76.9	
12	125.1	124.5	5	72.1	
13	143.1	141.0	6	172.4	
14	42.4	41.5			
15	34.9	33.7			
16	69.7	70.0		Rhamnose	
17	48.5	48.1	1	103.6	
18	40.9	39.3	2	72.2	
19	47.9	46.5	3	72.2	
20	36.9	36.2	4	73.8	
21	79.8	77.5	5	70.8	
22	74.4	73.0	6	18.0	
23	22.7	22.5			
24	64.5	64.6		Xylose	
25	16.2	16.1	1	104.7	
26	17.2	16.9	2	75.1	
27	27.8	27.0	3	77.8	
28	63.8	63.8	4	71.0	
29	29.7	29.1	5	66.9	
30	20.3	19.8			

tralized with N,N-diethylamine (10% in CHCl_3) and dried. The sugars were identified by TLC analysis with authentic samples in system 3.

Harpuloside (1): 3-O [α -L-rhamnopyranosyl (1 $\rightarrow$ 3)- β -D-xylopyranosyl (1 $\rightarrow$ 2)- β -D-glucuronopyranosyl]-21 β , 22 α -O-diangeloyl-protoaescigenin, colourless powder; $\text{C}_{57}\text{H}_{88}\text{O}_{22}$; TLC (system 1), R_f 0.63; (system 2), R_f 0.44; $[\alpha]_D^{25} = -30^\circ$ (MeOH); mp 190°C . FAB-MS m/z : 1123 $[\text{M}-\text{H}]^-$; 991 $[(\text{M}-\text{H})-132]^-$, 977 $[(\text{M}-\text{H})-146]^-$, 845 $[(\text{M}-\text{H})-132-146]^-$. ^1H NMR (CD_3OD , 400 MHz): rhamnose: 5.08 (*br s*, H-1), 4.09 (*br d*, $J=3.5$ Hz, H-2), 3.69 (*dd*, $J=9.0$, 3.0 Hz, H-3), 3.43 (*t*, $J=9$ Hz, H-4), 3.99 (*dq*, $J=9.0$, 6.0 Hz, H-5), 1.26 (*d*, $J=6.5$ Hz, H-6); xylose: 4.52 (*d*, $J=8$ Hz, H-1), 3.21 (*t*, $J=8$ Hz, H-2), 3.31 (*t*, $J=9$ Hz, H-3), 3.56 (*m*, H-4), 3.85 (*dd*, $J=10.0$, 5.0 Hz, H-5A), 3.12 (*t*, $J=10$ Hz, H-5B); glucuronic acid: 4.49 (*d*, $J=8$ Hz, H-1), 3.61 (*t*, $J=8.5$ Hz, H-2), 3.71 (overlapped, H-3), 3.70 (overlapped, H-4), 3.45 (overlapped, H-5). ^{13}C NMR (CD_3OD , 100.61 MHz): see Table 1.

21 β , 22 α -O-diangeloyl-protoaescigenin (**2**): white powder; $\text{C}_{40}\text{H}_{62}\text{O}_8$; $[\alpha]_D^{25} = -24^\circ$ (MeOH); mp 170°C .

FAB-MS m/z : 669 $[\text{M}-\text{H}]^-$. ^1H NMR (CDCl_3 , 300 MHz): 6.08 (1H, *br q*, $J=8$ Hz, H-3'), 6.00 (1H, *br q*, $J=8$ Hz, H-3'), 5.86 (1H, *d*, $J=10$ Hz, H-21), 5.45 (1H, *t*, $J=3.4$ Hz, H-12), 5.41 (1H, *d*, $J=10$ Hz, H-22), 4.21 (1H, *d*, $J=11$ Hz, H-24A), 3.95 (1H, *br s*, $\Delta\nu 1/2=8$ Hz, H-16), 3.34 (1H, *d*, $J=11$ Hz, H-24B), 3.26 (1H, *d*, $J=11$ Hz, H-28A), 2.91 (1H, *d*, $J=11$ Hz, H-28B), 2.73 (1H, *dd*, $J=14.0$, 4.0 Hz, H-18), 1.97 (3H, *d*, $J=8$ Hz, H-4'), 1.93 (3H, *d*, $J=8$ Hz, H-4'), 1.83 (6H, *br s*, H-5'), 1.45 (3H, *s*, H-27), 1.26 (3H, *s*, H-23), 1.06 (3H, *s*, H-30), 0.93 (3H, *s*, H-29), 0.89 (3H, *s*, H-25), 0.88 (3H, *s*, H-26). ^{13}C NMR (CDCl_3 , 75.3 MHz): see Table 1.

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