Petersaponins III and IV, Triterpenoid Saponins from *Petersianthus macrocarpus*

Tiwalade A. Olugbade,[†,‡] Abiodun Ogundaini,[§] Nicolas Birlirakis,[†] Mary Païs,*[,†] and Marie-Thérèse Martin[†]

Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette Cedex, France, and Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

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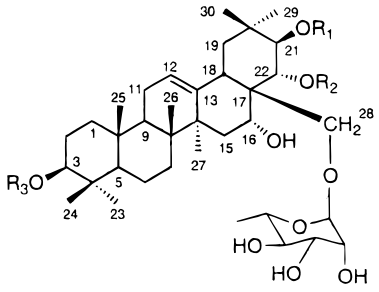
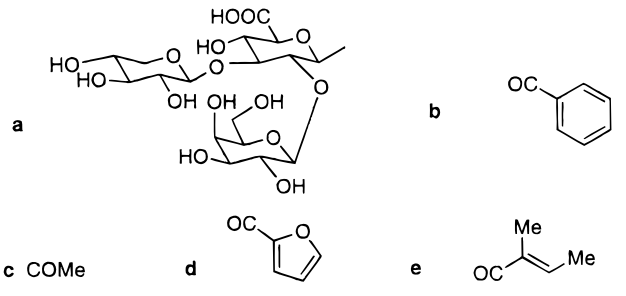
Two new triterpenoid saponins, petersaponins III and IV (**1** and **2**), were isolated from an *n*-butanol extract of the bark of *Petersianthus macrocarpus*. They possess 21-*O*-benzoyl-22-*O*-acetylbarringtogenol C and 21-*O*-2-furoxyl-22-*O*-tigloylbarringtogenol C as the aglycon, respectively. For both **1** and **2**, the trisaccharide moiety linked to C-3 of the aglycon consists of D-glucuronic acid, D-xylose, and D-galactose, while a L-rhamnose unit is linked to C-28. The structures of **1** and **2** were elucidated by extensive NMR experiments including ^{1}H−^{1}H (COSY, HOHAHA, NOESY) and ^{1}H−^{13}C (HMQC and HMBC) spectroscopy and by chemical evidence.

The stem bark of *Petersianthus macrocarpus* (P. Beauv.) Liben (Barringtoniaceae) is known for its hypotensive and arbortifacient properties.[1] In the course of previous work on a bioactive saponin fraction of the plant, two saponins named petersaponins I and II were isolated in the form of their peracetylated derivatives.[2] We report herein the isolation of two further saponins, petersaponins III (**1**) and IV (**2**). Their structures were established without derivatization with the aid of high-field NMR spectroscopy (800 MHz).



1 R_1 = **b**, R_2 = **c**, R_3 = **a**

2 R_1 = **d**, R_2 = **e**, R_3 = **a**



a **b** **c** COMe **d** **e**

The EtOH extract of the bark of *P. macrocarpus* was partitioned between *n*-BuOH and H_2O. The *n*-BuOH extract was dissolved in MeOH, further treated by precipitation from MeOH with diethyl ether, and subjected to Si gel chromatography to furnish a crude saponin mixture. Petersaponins III (**1**) and IV (**2**) were purified from this mixture using reversed-phase HPLC.

Petersaponin III (**1**) exhibited a $[M + Na]^+$ peak in the ESIMS at *m/z* 1275, which in conjunction with the analysis of the ^{13}C NMR spectrum was shown to correspond to the molecular formula $C_{62}H_{92}O_{26}$. The 1D NMR spectrum revealed the presence of seven tertiary methyl groups between δ 0.90 and 1.51 and a double bond with typical ^{13}C NMR resonances at δ 125.8 and 142.9, indicating an olean-12-ene triterpene. The genin was decomposed on acid hydrolysis, but four sugars were detected: glucuronic acid, xylose, galactose, and rhamnose, which corresponded to four anomeric carbons in the ^{13}C NMR spectrum in the region of 100 ppm (δ_C 105.5, 104.9, 104.2, 102.0). The presence of an α-rhamnose unit was readily supported by the characteristic methyl doublet at δ 1.22 (J = 6.2 Hz) and the singlet of an anomeric proton at δ 4.52. In addition, the spectrum showed a methyl singlet at δ 1.79 assigned to an acetyl group with the carbonyl appearing at δ 172.6 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum also exhibited two doublets at δ 5.82 and 6.02 (J = 10.2 Hz), suggesting the presence of acylated oxymethine units at C-21 and C-22, and in the downfield region the resonances of an unsubstituted benzoyl group (Table 1). The 2D NMR spectra (Table 1) confirmed the preceding structural features of the aglycon and indicated the presence of a C-28 oxymethylene (δ_C 69.2) and a C-16 oxymethine (δ_C 69.4). The benzoyl and acetyl groups were located at C-21 and C-22, respectively, as shown from the HMBC cross-peaks from H-21 to the benzoyl C═O, and from H-22 to the acetyl C═O. The H-16β, H-21α, and H-22β configurations were deduced from the NOESY correlations H-16/H-28b, H-21/H-19α, and H-22/H-18, respectively, while the NOESY correlation between H-3 and H-5 supported a H-3α configuration.

As for the saccharide portion, careful analysis of the ^{1}H NMR coupling values (800 MHz) and various 2D experiments confirmed the structure of each monosaccharide and

* To whom correspondence should be addressed. Tel.: 33169823090. Fax: 33169077247. E-mail: Mary.Pais@icsn.cnrs-gif.fr.
† Institut de Chimie des Substances Naturelles, CNRS.
‡ Present address: Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.
§ Department of Pharmaceutical Chemistry, Obafemi Awolowo University.

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Table 1. ^{13}C (62.5 MHz) and ^{1}H (800 MHz) NMR Data for Petersaponin III (**1**) (CD_3OD)[a]

position	δ_C	δ H (*J* Hz)	HMBC[b]	NOESY	position	δ_C	δ_H (*J* Hz)	HMBC[b]	NOESY
Genin					Rha				
1	41.1	α 1.02 m		3	1	102.0	4.52 br s	rha-3, rha-5	rha-2
		β 1.65 m			2	72.1	3.97 m	rha-3, rha-4	rha-3
	27.1	α 2.00 m		3	3	72.8	3.72 dd	rha-4	
		β 1.74 m					(9.5, 3.5)		
3	91.8	3.20 dd	glcA-1,23,24	5,23,glcA-1	4	73.9	3.39 dd	rha-3, rha-5,	
		(4.2,11.7)					(9.5,9.4)	rha-6	
4	40.1				5	70.1	3.63 m		
5	57.1	0.80 br d(12.1)			6	18.2	1.22 d (6.2)	rha-4, rha-5	
6	19.3	1.40 m							
	1.59 m								
7	34.1	1.36 m			GlcA				
		1.65 m			1	105.5	4.50	3	glcA-3, glcA-5
							d (7.5)		
8	40.5				2	79.4	3.79 dd	glcA-3, gal-1	gal-1
9	48.0	1.70 m					(9.3,7.5)		
10	37.8				3	87.0	3.76 m	glcA-2, xyl-1	gal-1, xyl-1
11	24.8	1.95 m		12	4	72.1	3.60 m		
12	125.8	5.45 br s	9,14,18	18,19α*β*,	5	76.8	3.70 m		
				rha-5	6	180.1			
13	142.9								
14	42.4								
15	35.2	1.42 m		16	Gal				
		1.75 m		16,28b	1	104.2	4.85 d (7.7)	glcA-2	gal-3, gal-5
16	69.4	4.00 m		28ab	2	73.6	3.54 m	gal-1, gal-3	
17	48.0				3	75.1	3.50 dd	gal-1, gal-2	
18	41.3	2.85 br d (4.3)	12,13,14,17,28	22,30			(9.8,3.5)		
19	48.0	α 2.72 br t (13)	18,20,29,30	19*β*,21,27,29	4	70.1	3.85 dd (3.4)	gal-2, gal-3	
		β 1.24 dd	14,17,20,21,30		5	76.8	3.50 m	gal-4, gal-6	
		(13,4.4)			6	62.4	3.69 dd	gal-4, gal-5	
20	37.2						(11.0,5.8)		
21	81.1	6.02 d (10.2)	20,22,29,30,	29			3.76 dd	gal-4, gal-5	
			Bz-CO				(11.0,6.3)		
22	73.9	5.82 d (10.2)	16,17,21,28,	28ab,30					
			Ac-1						
23	28.5	1.11 s	3,4,5,24	glcA-1	Xyl				
24	17.0	0.90 s	3,4,5,23		1	104.9	4.65 d (7.4)	glcA-3	xyl-3, xyl-5α
25	16.3	1.00 s	1,5,9,10		2	75.2	3.30 m	xyl-1, xyl-3, xyl-4	
26	17.8	0.99 s	7,8,9,14	28ab	3	78.2	3.35 dd	xyl-2, xyl-4	
27	27.7	1.51 s	8,13,14,15				(9.2,8.4)		
28	69.2	a 2.66 d (9.2)	17,18,rha-1,22	28b, rha-1	4	71.0	3.54 ddd	xyl-3, xyl-5	
		b 3.52 m					(10.4,8.4,5.0)		
29	29.7	0.92 s	19,20,21,30		5	67.2	α 3.29 m	xyl-3	
30	20.3	1.16 s	19,20,21,29				*β* 4.00 m		
Bz-CO	168.1								
Bz-1	131.6								
Bz-2,6	130.6	7.96 d (7.5)	Bz-4, Bz-CO						
Bz-3,5	129.6	7.46 t (7.5)	Bz-1, Bz-4						
Bz-4	134.2	7.59 t (7.5)	Bz-2,6						
Ac-1	172.6								
Ac-2	20.8	1.79 s	Ac-1						

[a] Assignments based on 2D experiments (COSY, HOHAHA, HMQC, and HMBC). [b] Measured at 50 °C.

allowed us to establish the linkage of the sugars to the genin, as well as the interglycosidic linkages. Particularly diagnostic were the HMBC correlations from the H-1 of glucuronic acid to the C-3 of the genin, from the H-1 of xylose to the C-3 of glucuronic acid, and from the H-1 of galactose to the C-2 of glucuronic acid, establishing the linkages of the *β*-glcA unit to C-3 of the genin and of the *β*-galactopyranosyl group and the *β*-xylosyl group to C-2 of glcA and to C-3 of glcA, respectively. Finally, the HMBC correlation H-28a/C-1 of rha indicated that the α-rhamnose unit was linked to C-28 of the genin. The NOESY correlations H_2-28ab/H-1 of rha, H-1of glcA/H-3, H-1 of xyl/H-3 of glcA, and H-1 of gal/H-2 of glcA further supported the connectivities of the sugars with the genin and the linkages inside the saccharide moiety at C-3.

Thus, the structure of compound **1** was assigned as 3-*O*-(*β*-D-xylopyranosyl)(1→3)-*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl]-21-*O*-benzoyl-22-*O*-acetyl-28-*O*-α-L-rhamnopyranosylbarringtogenol C. Compound **1** possesses the same 21-*O*-benzoyl-22-*O*-acetyl-28-α-*O*-L-rhamnopyranosylbarringtogenol C moiety as the previously described peracetylated petersaponin II.[2] However, the authors assumed that the acetyl group was not present in the native genin.

Petersaponin IV (**2**) exhibited a $[M + Na]^+$ peak in the ESIMS at *m/z* 1305 and gave a ^{13}C NMR spectrum consistent with the molecular formula $C_{63}H_{94}O_{27}$. The NMR spectra were close to those of compound **1**. Comparison of the 1D and 2D data indicated that the saccharide chains at C-3 of **1** and **2** were similar and that an α-rhamnose unit was linked at the C-28 oxymethylene of the genin as in **1**. Compound **2** had the skeleton of the sapogenin barringtogenol C[4] with typical oxygen substitutions at C-3, C-16, C-21, and C-22 (Table 2). In fact, **2** varied from **1** only in the nature of the groups linked to C-21 and C-22. Instead of the benzoyl group, there was a furan-2-carboxylic acid ester moiety, which showed ^1H and ^{13}C NMR resonances (Table 2) similar to those described for furan-2-

Table 2. ^{13}C (62.5 MHz) and ^{1}H (400 MHz) NMR Data for Petersaponin IV (**2**) (CD_3OD)[a]

position	δ_C	δ_H (*J* Hz)	HMBC[b]	NOESY	position	δ_C	δ_H (*J* Hz)	HMBC[b]	NOESY
Genin					Rha				
1	41.0	1.00 m			1	102.0	4.50 br s	rha-3, rha-5	rha-2
		1.65 m			2	72.1	3.98 m	rha-3	
2	27.0	1.70 m			3	72.8	3.72 m		
		2.00 m			4	73.9	3.37 m	rha-3, rha-6	
3	91.7	3.20 m	glcA-1	5,23,glcA-1	5	70.1	3.60 m		
4	40.1				6	18.2	1.21 d (6)	rha-4, rha-5	
5	57.1	0.80 br d (12)							
6	19.3	1.40 m							
		1.60 m			GlcA				
7	34.2	1.40 m			1	105.5	4.50 W1/2 10	3	glcA-3, glcA-5
	1.65 m				2	79.4	3.76 m	glcA-3	gal-1
8	40.5				3	86.8	3.74 m		gal-1, xyl-1
9	48.0	1.70 m			4	72.1	3.60 m		
10	37.8				5	76.8	3.67 m		
11	24.8	1.95 m		12	6	180.1			
12	124.5	5.45 br s		18, rha-5					
13	142.9								
14	42.4				Gal				
15	35.2	1.40 m		16	1	104.2	4.82 d (7)	glcA-2	gal-3, gal-5
		1.75 m		16	2	73.7	3.55 m	gal-1	
16	70.0	3.95 m		28ab	3	75.5	3.50 m		
17	48.0				4	70.1	3.80 m	gal-2, gal-3	
18	41.3	2.80 m		22,30	5	76.8	3.50 m		
19	48.0	α 2.75 m		19*β*,21,27	6	62.3	3.65 m		
		β 1.25 m					3.78 m		
20	37.4								
21	81.0	6.06 d (10)	20,22,29,30,Fur-CO	29	Xyl				
22	73.8	5.83 d (10)	17,21,28,Tig-1	30	1	104.8	4.64 d (7)		xyl-3, xyl-5-α
23	28.4	1.10 s	3,4,5,24		2	75.2	3.30 m		
24	17.0	0.89 s	3,4,5,23		3	78.2	3.35 m	xyl-2	
25	16.3	0.99 s	1,5,9,10		4	71.0	3.54		
26	17.8	0.98 s	7,8,9,14	28ab	5	67.3	α 3.30		
27	27.8	1.51 s	8,13,14,15				*β* 3.99		
28	69.5	α 2.60 d (9)		28b, rha-1					
		β 3.50 m							
29	29.7	0.91 s	19,20,21,30						
30	20.1	1.13 s	19,20,21,29						
Fur-CO	161.0								
Fur-2	146.0			Fur-3					
Fur-3	119.1	7.10 d (3)	Fur-2	Fur-4					
Fur-4	112.8	6.56 dd (1,3)							
Fur-5	148.3	7.70 d (1)	Fur-2						
Tig-1	169.4								
Tig-2	130.4	1.80 s							
Tig-3	137.8	6.77 q (7)		Tig-4					
Tig-4	14.3	1.67 d (7)	Tig-2,Tig-3						
Tig-5	12.1	1.65 s	Tig-1						

[a] Assignments based on 2D experiments (COSY, HOHAHA, HMQC, and HMBC). [b] Measured at 50 °C.

carboxylic acid.[3] The HMBC cross-peak H-22/2-furoxyl C=O showed unambiguously that the latter substituent was at C-21. At C-22 there was a tigloyl group showing typical NMR chemical shift values (Table 2). This group is often found in oleanane saponins, especially those isolated from another plant of the family Barringtoniaceae, *Barringtonia acutangula*,[5] and in the sugar portion of petersaponin I. The linkage of the tigloyl moiety to C-22 was ascertained by the HMBC correlation H-22/tigloyl-C=O. Thus, the structure of compound **2** was assigned as 3-*O*-[*β*-D-xylopyranosyl)(1→3)-*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl]-21-*O*-2-furoxyl-22-*O*-tigloyl-28-*O*-α-L-rhamnopyranosylbarringtogenol C.

Experimental Section

General Experimental Procedures. Optical rotations at 20 °C were taken on a Perkin-Elmer 241 polarimeter. Spectra were recorded as follows: NMR, Bruker AC 250 (^{13}C NMR spectra), AMX 400 and AVANCE 800 (^{1}H and 2D NMR spectra); ESMS, VG-ZAB-SEQ spectrometer. Column chromatography: Si gel Merck 60 H. Semipreparative HPLC: column Waters Prep Nova-Pak C_{18}, 6 μm, 60 Å, 25 i.d. × 100 mm; eluent, MeOH−H_2O−AcOH (60:40:0.5); flow rate, 6 mL/min; UV and RI detection.

Plant Material. Stem bark of *Petersianthus macrocarpus* (P. Beauv.) Liben [=*Combretodendron macrocapum* (P. Beauv.) Keay = *Combretodendron africanum* (Welw.) Exell] (Barringtoniaceae) was collected on the Obafemi Awolowo University Campus, Ile-Ife, Nigeria. The sample was compared to a voucher specimen already deposited at the Herbarium of the Faculty of Phamacy, Obafemi Awolowo University.

Extraction and Isolation. The dried and powered plant material (320 g) was extracted with EtOH−H_2O (4:1), yielding a crude extract (50 g), which was partitioned beween H_2O and *n*-hexane, CH_2Cl_2, EtOAc, and BuOH, successively. The BuOH extract (10 g) was dissolved in MeOH (25 mL) by warming. The resulting solution was poured into diethyl ether (75 mL). The precipitate was collected by centrifugation at 3000 rpm (10 min) and dissolved in MeOH. Evaporation of the solvent yielded a crude saponin fraction (7.3 g). This fraction (1.5 g) was chromatographed on Si gel with mixtures of CH_2Cl_2−MeOH−H_2O containing increasing quantities of MeOH and H_2O. The fractions eluted with CH_2Cl_2−MeOH−H_2O (7:3:0 to

6:4:0.5) were further purified by semipreparative HPLC, yielding petersaponin IV (**2**) (16 mg, t_R 18.5 min) and petersaponin III (**1**) (20 mg, t_R 26.5 min).

Petersaponin III (1): white amorphous powder, $[\alpha]_D$ −8° (*c* 0.7, MeOH); ^{1}H and ^{13}C NMR, Table 1; ESIMS *m*/*z* 1275 [M + Na]$^+$.

Petersaponin IV (2): white amorphous powder, $[\alpha]_D$ −14° (*c* 0.5, MeOH); ^{1}H and ^{13}C NMR, Table 2; ESIMS *m*/*z* 1305 [M + Na]$^+$.

Acid Hydrolysis of 1 and 2. A solution of each compound (3 mg) in dioxane−5% H_2SO_4 1:1 (2 mL) was refluxed for 3 h. The reaction mixture was extracted with EtOAc. The aqueous layer was neutralized with $BaCO_3$ and filtered. The filtrate was checked by 2D TLC using CH_2Cl_2−EtOH−H_2O (16:8:1) for the first direction and EtOAc−MeOH−HOAc−H_2O (12:3:3:2) for the second one, together with the authentic sugar samples.

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References and Notes

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