Studies on the Constituents of *Cyclanthera pedata* Fruits: Isolation and Structure Elucidation of New Triterpenoid Saponins

Nunziatina De Tommasi,*,† Francesco De Simone,† Giovanna Speranza,‡ and Cosimo Pizza†

Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università di Salerno, Ponte Don Melillo Invariante 11C, 84084 Fisciano (SA), Italy, and Dipartimento di Chimica Organica ed Industriale, Università di Milano, via Venezian 21, 20133 Milano, Italy

The isolation of nine triterpenoid saponins (**1**−**9**), among them six new natural compounds (**1**−**6**), from the MeOH extract of the fruits of *Cyclanthera pedata* is reported. All of the structures were elucidated by spectroscopic methods, including the concerted application of one-dimensional ^{1}H−^{1}H total correlation spectroscopy, ^{1}H−^{1}H nuclear Overhauser effect spectroscopy), and ^{13}C−^{13}C DEPT-NMR and two-dimensional NMR techniques (double-quantum filtered correlated spectroscopy, rotating-frame Overhauser enhancement spectroscopy, heteronuclear single quantum coherence, and heteronuclear multiple bond correlation). A comparative study of seeds and fruits has been also carried out.

Keywords: *Cyclanthera pedata; Cucurbitaceae; fruits; food source; triterpenoid saponins; ^{1}H and ^{13}C NMR analysis*

INTRODUCTION

Cyclanthera pedata Scrabs (Caigua), a plant cultivated by ancient Peruvians, is largely used to make salad or soup and has the reputation of being anti-inflammatory, hypocholesterolaemic, and hypoglycemic.

This work on "caigua" (*C. pedata*) is a part of a series of studies on chemical constituents of alimentary plants from South America (De Simone et al., 1990; Dini et al., 1992; Rastrelli et al., 1995; De Tommasi et al., 1996).

In a previous paper (De Tommasi et al., 1996) we reported the isolation and structure determination of six new cucurbitacin glycosides from the seeds of *C. pedata*. We have now investigated the fruits of this plant to determine their saponin content. Here we report on the isolation and structural elucidation of nine triterpene saponins (**1**−**9**), among which six are new natural products (**1**−**6**).

EXPERIMENTAL PROCEDURES

Material. The fruits of *C. pedata* were supplied by IPIFA (Instituto Peruano Investigaciones Fitoterapica Andina) and were collected in Peru, 1995. A voucher sample is deposited at the Herbario de Museo de Historia Natural "J. Prado", Un. Lima (Peru).

Apparatus. The fast atom bombardment mass spectrs (FABMS), in negative ion mode, were obtained by dissolving the samples in a glycerol/thioglycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms of 2−6 kV energy in a Kratos MS 902 spectrometer (AEI Manchester, U.K.) equipped with a Kratos FAB source.

A Bruker DRX-600 spectrometer (Spectroscpin AG, Fallanden, Switzerland) operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C, using the UXNMR software package, was used for nuclear magnetic resonance (NMR) experiments in CD_3-OD. The distortionless enhancement by polarization transfer (DEPT) experiments were performed using a transfer pulse of 135° to obtain positive signals for CH and CH_3 and negative ones for CH_2. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. Rotating-frame Overhauser enhancement spectroscopy (ROESY) (Bax and Davis, 1985), ^{1}H−^{1}H double-quantum filtered correlation spectroscopy (DQF-COSY) (Agrawal, 1992; Homans, 1990), two-dimensional homonuclear Hartmann Hahn (2D-HOHAHA) (Agrawal, 1992), ^{1}H−^{13}C heteronuclear single-quantum coherence (HSQC) (Bodenhausen and Ruben, 1986; Bodenhausen et al., 1976), and heteronuclear multiple-bond correlation (HMBC) (Martin and Crouch, 1991) experiments were performed using the conventional pulse sequences as described in the literature; one-dimensional total correlation spectroscopt (1D-TOCSY) spectra (Davis and Bax, 1985) were acquired using a waveform generator-based GAUSS-shaped pulse, mixing time ranging from 60 to 100 ms, and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse, and one-dimensional nuclear Overhauser effect spectroscopy (1D-NOESY) (Agrawal, 1992) were acquired using mixing time ranging from 400 to 600 ms.

High-performance liquid chromatography (HPLC) separations were performed on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector and with a Waters μ-Bondapak C18 column and a U6K injector.

Gas chromatography (GC) analyses employed a Hewlett-Packard 5890 gas chromatograph equipped with mass-selective detector MSD 5970 MS, a split/splitness injector, and a fused-silica column HP-5 (25 m × 0.2 mm i.d.; 0.33 mm film).

Extraction and Isolation. The powdered, dried fruits (500 g) were defatted with petroleum ether (6.3 g) and $CHCl_3$ (2.5 g) in a Soxhlet apparatus and extracted successively at room temperature with $CHCl_3$/MeOH (9:1) (6 g) and MeOH (30 g). Part of the MeOH extract (12 g) was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH soluble portion (5 g), which was chromatographed on a Sephadex LH-20 column using MeOH/H_2O (9:1) as eluent. Fractions (8 mL) were collected and checked by thin-layer chromatography (TLC) [silica gel plates in *n*-BuOH/HOAc/H_2O (60:15:25)]. Fractions 15−23 (900 mg), containing the crude saponin mixture, were purified by droplet counter current chromatography (DCCC) using BuOH/Me_2CO/H_2O (30:9:11) in which the stationary phase consisted of the higher phase (discending mode, flow =

* Author to whom correspondence should be addressed (telephone 39 089962647; fax 39 089962828; e-mail detommasi@unisa.it).

† Università di Salerno.

‡ Università di Milano.

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Table 1. FABMS Spectral Data for Compounds 1−6[a]

compd	formula	spectral data
1	$C_{53}H_{90}O_{23}$	1093 $[M-H]^-$, 947 $[(M-H)-146]^-$, 961 $[(M-H)-132]^-$, 815 $[(M-H)-(132+146)]^-$, 653 $[(M-H)-(162+146+132)]^-$, 491 $[(M-H)-(162+162+146+132)]$
2	$C_{47}H_{80}O_{19}$	947 $[M-H]^-$, 815 $[(M-H)-132]^-$, 653 $[(M-H)-132+162]^-$, 491 $[(M-H)-(162+162+132)]^-$
3	$C_{54}H_{92}O_{24}$	1123 $[M-H]^-$, 961 $[(M-H)-162]^-$, 815 $[(M-H)-(162+146)]^-$, 491 $[(M-H)-(162+162+146+162)]$
4	$C_{65}H_{110}O_{33}$	1417 $[M-H]^-$, 1255 $[(M-H)-162]^-$, 1123 $[(M-H)-(132+162)]^-$, 815 $[(M-H)-(162+162+146+132)]$, 653 $[(M-H)-(162\times3)-146-132)]$, 491 $[(M-H)-(162\times4)-(146+132)]^-$
5	$C_{48}H_{82}O_{18}$	945 $[M-H]^-$, 783 $[(M-H)-162]^-$, 621 $[(M-H)-(162+162)]^-$, 459 $[(M-H)-(162+162+162)]^-$
6	$C_{48}H_{80}O_{20}$	975 $[M-H]^-$, 829 $[(M-H)-146]^-$, 667 $[(M-H)-(146+162)]^-$

[a] The mass unit lost corresponded to fragments indicated in the text.

15 mL/h) to give A (200 mg), B (300 mg), C (180 mg). Fractionation of each glycoside was achieved by reverse-phase HPLC on a Waters μ-Bondapak C_{18} column (30 cm × 7.8 mm, flow rate = 2.5 mL/min) using MeOH/H_2O (2:3) for A and B and MeOH/H_2O (1:1) for C to yield pure compounds **1** (15.0 mg, t_R = 9.5 min), **2** (10.7 mg, t_R = 13 min), **3** (10.8 mg, t_R = 7 min), **4** (15.5 mg, t_R = 3.5 min), **5** (11.0 mg, t_R = 14 min), **6** (12.0 mg, t_R = 11 min), **7** (6 mg, t_R = 8 min), **8** (8 mg, t_R = 11.5 min), and **9** (4.5 mg, t_R = 25 min).

Acid Hydrolysis of Compounds 1−9, Carbohydrate Constituents. A solution of each compound (2 mg) in 10% H_2SO_4/EtOH (1:1, 3.5 mL) was refluxed for 4 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The Et_2O layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The H_2O layer was neutralized with Amberlite MB-3 ion-exchange resin and evaporated to dryness. The resulting monosaccharides were reacted with TRISIL-Z (Pierce) and analyzed by GC/MS. Retention times were identical to those of the authentic trisil sugars.

Compound ***1***. $C_{53}H_{90}O_{23}$; FABMS see Table 1; ^1H and ^{13}C NMR data see Tables 1−4.

Compound ***2***. $C_{47}H_{80}O_{19}$; FABMS see Table 1; ^1H and ^{13}C NMR data see Tables 2−4.

Compound ***3***. $C_{54}H_{92}O_{24}$; FABMS see Table 1; ^1H and ^{13}C NMR data see Tables 2−4.

Compound ***4***. $C_{65}H_{110}O_{33}$; FABMS see Table 1; ^1H and ^{13}C NMR data see Tables 2−4.

Compound ***5***. $C_{48}H_{82}O_{24}$; FABMS see Table 1; ^1H and ^{13}C NMR data see Tables 2−4.

Compound ***6***. $C_{48}H_{80}O_{20}$; FABMS see Table 1; ^1H NMR (for aglycon moiety) δ 5.63 (1H, d, J = 6.0 Hz, Ha-6), 4.42 (1H, t, J = 7.5 Hz, H-16), 3.46 (1H, dd, J = 9.0, 5.0 Hz, H-3), 3.20 (1H, t, d, J = 15 Hz, Ha-12), 3.31 (1H, dd, J = 8.5, 5.0 Hz, H-22), 2.70 (1H, d, J = 15 Hz, Hb-12), 2.60 (1H, d, J = 7.5 Hz, H-17), 2.46 (1H, ddd, J = 14.0, 7.0, 6.0 Hz, Ha-7), 2.42(1H, m, H-10), 2.10 (1H, dddd, J = 17.0, 10.0, 8.5, 8.0 Hz, Ha-23), 2.05 (1H, ddd, J = 14.0, 6.0, 3.0 Hz, Hb-7), 2.00 (1H, dd, J = 13.0, 7.5 Hz, Ha-15), 1.96 (1H, dddd, J = 14.0, 9.0, 8.0, 3.0 Hz, Hax-2), 1.88 (1H, dd, J = 3.0, 7.0 Hz, H-8), 1.78 (1H, dd, J = 13.0, 7.5 Hz, Hb-15), 1.78 (1H, dddd, J = 14.0, 5.0, 4.5, 3.0 Hz, Heq-2), 1.74 (1H, ddd, J = 16.0, 10.0, 7.0 Hz, Ha-24), 1.70 (1H, dddd, J = 17.0, 10.0, 8.0, 5.0 Hz, Hb-23), 1.58 (1H, m, Hb-1), 1.43 (1H, ddd, J = 16.0, 10.0, 7.0 Hz, Hb-24), 1.39 (3H, s, Me-27), 1.36 (3H, s, Me-26), 1.32 (1H, m, Ha-1), 1.31 (3H, s, Me-30), 1.25 (3H, s, Me-29), 1.24 (3H, s, Me-21), 1.07 (3H,s, Me-19), 1.06 (3H, s, Me-28), 0.95 (3H, s, Me-18); ^{13}C NMR (for aglycon moiety) δ 216.8 (C-11), 142.0 (C-5), 119.5 (C-6), 105.0 (C-1′), 104.3 (C-1″), 87.50 (C-3), 80.0 (C-20), 78.80 (C-22), 77.9 (C-5″), 77.8 (C-3″), 77.7 (C-3′), 77.1 (C-5′), 74.8 (C-2′), 74.7 (C-2″), 72.0 (C-25), 71.60 (C-16), 71.2 (C-4″), 70.7 (C-4′), 69.0 (C-6′), 62.4 (C-6″), 58.2 (C-17), 51.9 (C-14), 50.3 (C-9), 49.8 (C-12), 49.2 (C-13), 46.7 (C-15), 44.5 (C-8), 40.6 (C-4), 39.5 (C-24), 29.9 (C-27), 29.2 (C-26), 28.9 (C-2), 28.5 (C-28), 28.2 (C-23), 25.9 (C-21), 25.6 (C-29), 24.8 (C-7), 22.4 (C-1), 20.4 (C-18 and 19), 19.6 (C-30); ^1H and ^{13}C NMR data for sugar portion see Tables 3 and 4.

Compound ***7***. $C_{42}H_{70}O_{16}$; identified as 3β-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)-16α,20,22,25-tetrahydroxycucurbita-5-en-11-one, previously isolated from seeds of *C. pedata*, by spectral data (De Tommasi et al., 1996).

Compound ***8***. $C_{38}H_{62}O_{12}$; identified as 3-β-(6-*O*-β-D-glucopyranosyloxy)-25-acetoxy-16α,20,22-trihydroxycucurbit-5-en-11-one, previously isolated from seeds of *C. pedata*, by spectral data (De Tommasi et al., 1996).

Table 2. ^{13}C and ^1H NMR Data of Aglycon of Compounds 1 and 5 in CD_3OD[a]

position	**1** δC	**1** δH (J in Hz)	**5** δC
1	41.4	1.75 m	41.0
		1.12 m	
2	29.7	2.00 m	26.8
		1.84 m	
3	80.0	3.26 dd, J = 10.0, 4.5	89.2
4	41.3		37.0
5	59.4	0.80 d, J = 10.0	55.3
6	77.2	3.95 ddd, J = 10.0, 10.0, 3.0	18.7
7	46.7	1.32 dd, J = 14.0, 3.0	35.1
		1.61 dd, J = 14.0, 10.0	
8	42.2		42.3
9	47.9	1.77 dd, J = 11.0, 3.0	49.0
10	38.3		38.3
11	31.6	1.48 m	31.2
12	68.7	3.60 d, J = 10.0	69.0
13	56.0	1.80 d, J = 10.0	49.4
14	49.0		51.0
15	26.7	1.50 m	30.0
		1.10 dd, J = 11.5, 8.0	
16	35.0	2.20 ddd, J = 12.0, 4.5, 3.0	27.0
		1.40 ddd, J = 12.0, 10.0, 4.0	
17	85.0		52.0
18	16.2	1.18 s	16.2
19	17.0	1.02 s	17.1
20	88.2		83.0
21	22.9	1.30 s	23.0
22	33.5	1.75 s.o.	35.5
		1.80 s.o.	
23	23.9	2.25 m	24.0
		2.00 m	
24	124.8	5.36 m	125.7
25	130.0		131.0
26	17.9	1.68 s	17.9
27	26.0	1.62 s	25.7
28	18.0	0.94	16.9
29	33.5	1.07 s	28.2
30	16.6	1.22 s	16.8

[a] Assignments are confirmed by $^1H-^1H$ COSY and HETCOR experiments.

Compound ***9***. $C_{41}H_{64}O_{17}$; identified as 2-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)-3,16α,20,22,25-pentahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one, previously isolated from seeds of *C. pedata*, by spectral data (De Tommasi et al., 1996).

RESULTS AND DISCUSSION

C. pedata fruits were successively extracted with petroleum ether, $CHCl_3$, $CHCl_3$/MeOH (9:1), and MeOH. The MeOH extract was then partitioned into a mixture of *n*-BuOH and H_2O to afford an *n*-BuOH soluble portion, which was subjected to a Sephadex LH-20. The fractions containing the triterpenic glycosides checked by TLC were further purified by DCCC and HPLC to obtain the glycosides **1**−**9** (Chart 1). The structures and molecular formulas of compounds **1**−**9** were determined

Table 3. ^{13}C NMR Data for the Sugar Moieties of Compounds 1−6 in CD_3OD[a]

		1 δC	**2** δC	**3** δC	**4** δC	**5** δC	**6** δC
Glc C-20	1	96.6	96.0	96.1	96.9	96.9	
	2	**82.5**	**82.7**	**82.5**	**83.0**	74.4	
	3	77.4	77.6	77.4	77.3	78.1	
	4	70.5	71.0	70.8	70.5	70.6	
	5	78.0	78.4	78.0	77.9	78.3	
	6	**68.2**	62.2	**68.0**	**68.5**	62.5	
Glc I	1	104.0	104.1	104.3	104.5		
	2	75.6	75.2	75.5	75.4		
	3	78.0	78.1	78.1	78.3		
	4	70.8	71.1	71.0	71.1		
	5	78.0	78.3	78.1	78.5		
	6	62.5	62.5	62.4	62.0		
Rha I	1	101.2		101.5	101.2		
	2	71.4		71.3	71.5		
	3	73.1		73.2	73.3		
	4	74.0		74.0	74.1		
	5	69.0		69.4	69.8		
	6	17.7		18.0	18.1		
Xyl at C-6	1	107.0	106.8		107.0		
	2	75.0	75.3		75.2		
	3	77.8	78.0		77.8		
	4	71.5	71.7		71.6		
	5	67.5	67.1		67.5		
Glc at C-6	1			105.0			
	2			75.8			
	3			78.7			
	4			71.8			
	5			78.3			
	6			62.2			
Glc II C-3	1				105.2	105.0	104.1
	2				74.8	75.1	74.2
	3				78.5	78.5	78.4
	4				70.6	70.7	79.1
	5				76.9	77.2	77.0
	6				**69.3**	**69.0**	**68.90**
Glc III	1				104.2	104.1	103.8
	2				75.0	74.7	74.8
	3				78.2	78.0	78.3
	4				71.0	71.0	**71.5**
	5				78.1	78.1	78.0
	6				62.6	62.4	62.5
Rha II	1						102.0
	2						72.9
	3						72.3
	4						75.0
	5						69.0
	6						18.0

[a] Assignments are confirmed by 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments.

by negative ion FABMS spectra (Table 1) and 1D- and 2D-^{1}H and ^{13}C NMR data.

Compound **1** ($C_{53}H_{90}O_{23}$) showed an $[(M - H)]^-$ ion at *m/z* 1093 $[M - H]^-$ and prominent fragment ions at *m/z* 947 $[(M - H) - 146]^-$ and 961 $[(M - H) - 132]^-$, which were interpreted as the cleavage of a deoxyhexose or pentose, respectively, and at *m/z* 815 $[(M - H) - (146 + 132)]^-$. Another fragment at *m/z* 491 $[(M - H) - (162 + 162 + 146 + 132)]^-$, resulting from the cleavage of two hexose, one pentose, and one deoxyhexose units, was ascribable to aglycon.

The ^{13}C and DEPT ^{13}C NMR spectra showed 53 signals, of which 23 were assigned to the saccharide portion and 30 to a triterpene moiety.

The ^{1}H NMR spectrum of **1** showed, in addition to eight singlets assignable to tertiary methyls at δ 0.94−1.68 [two of which were diagnostic for methyl linked to sp^2 carbons (δ 1.62 and 1.70)], a signal at δ 5.36 indicating the occurrence of an olefinic proton. DQF-COSY and 1D- and 2D-TOCSY measurements showed coupling between H-24 (δ 5.36), H-23a (δ 2.00), H-23b (δ 2.25), H-22a (δ 1.75), and H-22b (δ 1.80). The linkage C22−C20 was deduced by correlations in an HMBC experiment (see Chart 2).

The remaining part of the molecule, as inferred from the molecular formula and inspection of the ^{13}C and DEPT ^{13}C NMR spectra, had to be composed of only sp^3 hybridized carbon atoms and must be tetracyclic. Furthermore, because all methyls were singlets in the ^{1}H NMR spectrum of **1**, they should be linked to quaternary carbon atoms. 2D-HOHAHA and DQF-COSY experiments provide evidence for the presence in the molecule of segments C1−C3, C5−C7, C9−C11−C12, and C15−C16. A signal in the ^{1}H NMR spectrum at δ 3.26 (1H, dd, J = 10.0 and 4.5 Hz) was typical for the axial oxymethine proton at C-3 of triterpene. Support for the β-OH stereochemistry at C-3 was derived from the ^{13}C NMR spectral data (Table 2). The location of other secondary hydroxyl groups at C-6 and C-12 was obtained by 1D-TOCSY and DQF-COSY experiments. The chemical shifts, the multiplicity, and the coupling constant values of the signal were consistent for two axial protons at C-6 and C-12, respectively (Matsuura et al., 1983).

The elucidation of the whole basic carbon skeleton from the above subunits was achieved on the basis of a series of HSQC and HMBC correlations, which also allowed the assignments of the resonances in the ^{13}C NMR to the pertinent carbon (Table 2; Chart 2). The tertiary hydroxyl group was placed at position C-17 by analysis of the chemical shift of carbon atoms of ring C,D and side chain and by HMBC correlations. Clear long-range shift correlations between H-12 and C-17; H-12 and C-14; H-12 and C-9; H-13 and C-17; H-13 and C-21; H-13 and C-16; H_3-21 and C-17; H_3-21 and C-22; and H_3-30 and C-13 confirmed the C-17 hydroxylation (Ahmad et al., 1990). The aglycon of compounds **1**−**5** identified as dammar-24-ene-3β,6α,12β,17α-tetraol has not be reported previously. Glycosidation of the alcoholic function at C-6 and C-20 was indicated by the significant downfield shift observed for the carbon resonances in **1** (Table 2) relative to the corresponding signals in model compounds in the literature (Matsuura et al., 1983).

Methanolysis and subsequent gas−liquid chromatography (GLC) analysis of methyl sugars of compound **1** showed the presence of xylose, glucose, and rhamnose in the ratio 1:2:1. Four anomeric protons were easily identified in the ^{1}H NMR spectra of **1**. They resonate at δ 4.80 (d, J = 7.5 Hz), δ 4.60 (d, J = 7.5 Hz), δ 4.83 (d, J = 1.6 Hz), and δ 4.55 (d, J = 7.6 Hz) and correlate to carbons at 96.6, 104.0, 101.2, and 107.0 ppm, respectively. The structure of the trisaccharide chain has been assigned by a combination of 1D-TOCSY, 2D-DFQ-COSY, HSQC, and HMBC experiments. The isolated anomeric signals resonanting at uncrowded regions of the spectrum, between 4.90 and 4.50 ppm, have been the starting point for the 1D-TOCSY experiments. Each subspectrum could be attributed to one set of coupled protons such as H-C(1) to H-C(5) for xylose or H-C(6) for glucose and rhamnose of a carbohydrate moiety. The irradiation of the signal at δ 4.55 showed a set of coupled resonances inside a sugar ascribable from H-1 to H-5 of the xylose unit. 1D-TOCSY subspectra obtained though irradiation at δ 4.80 and 4.60 allowed their easy

Table 4. ^{1}H NMR Data for the Oligosaccharide Moieties of Compounds 1−6 in CD_3OD[a]

	1 δH (*J* in Hz)	**2** δH	**3** δH	**4** δH (*J* in Hz)	**5** δH	**6** δH
Glc C-20 1	4.80 d, $J = 7.5$	4.80	4.76	4.82 d, $J = 7.5$	4.85	
2	3.55 dd, $J = 7.5, 9.0$	3.42	3.44	3.40 dd, $J = 7.5, 9.0$	3.40	
3	3.55 t, $J = 9.0$	3.54	3.53	3.53 t, $J = 9.0$	3.55	
4	3.28 t, $J = 9.0$	3.28	3.26	3.26 t, $J = 9.0$	3.28	
5	3.50 m	3.50	3.49	3.49 d, $J = 10.0$	3.48	
6a	3.89 dd, $J = 2.5, 12.0$	3.86	3.86	3.88 dd, $J = 2.5, 12.0$	3.93	
6b	3.70 dd, $J = 5.0, 12.0$	3.68	3.66	3.70 dd, $J = 5.0, 12.0$	3.74	
Glc I 1	4.60 d, $J = 7.5$	4.61	4.63	4.63 d, $J = 7.5$		
2	3.30 dd, $J = 7.5, 9.0$	3.28	3.25	3.26 dd, $J = 7.5, 9.0$		
3	3.46 t, $J = 9.0$	3.44	3.33	3.34 t, $J = 9.0$		
4	3.32 t, $J = 9.0$	3.33	3.36	3.35 t, $J = 9.0$		
5	3.48 m	3.49	3.49	3.46 m		
6a	3.83 dd, $J = 2.5, 12.0$	3.84	3.82	3.83 dd, $J = 2.5, 12.0$		
6b	3.66 dd, $J = 5.0, 12.0$	3.65	3.64	3.66 dd, $J = 5.0, 12.0$		
Rha I 1	4.83 d, $J = 1.6$		4.82	4.85 d, $J = 1.5$		
2	3.98 dd, $J = 3.0, 1.6$		3.97	3.97 dd, $J = 3.0, 1.5$		
3	3.74 dd, $J = 3.0, 9.5$		3.71	3.72 dd, $J = 3.0, 9.5$		
4	3.50 t, $J = 9.5$		3.53	3.50 t, $J = 9.5$		
5	4.00 m		3.99	4.02 m		
6	1.28 d, $J = 6.5$		1.28	1.30 d, $J = 6.5$		
Xyl at C6 1	4.55 d, $J = 7.6$	4.58	4.56	4.56 d, $J = 7.6$		
2	3.22 dd, $J = 7.6, 9.0$	3.24	3.22	3.21 dd, $J = 7.6, 9.0$		
3	3.30 t, $J = 9.0$	3.35	3.32	3.30 t, $J = 9.0$		
4	3.50 ddd, $J = 10.0, 9.0, 5.0$	3.53	3.51	3.51 ddd, $J = 10.0, 9.0, 5.0$		
5a	3.89 dd, $J = 10.0, 12.0$	3.86	3.88	3.90 dd, $J = 10.0, 12.0$		
5b	3.12 dd, $J = 5.0, 12.0$	3.12	3.14	3.14 dd, $J = 5.0, 12.0$		
Glc II at C-3 1				4.60 d, $J = 7.5$	4.73	4.59 d, $J = 7.5$
2				3.33 dd, $J = 7.5, 9.0$	3.35	3.55 dd, $J = 7.5, 9.0$
3				3.55 t, $J = 9.0$	3.45	3.45 t, $J = 9.0$
4				3.46 t, $J = 9.0$	3.36	3.37 t, $J = 9.0$
5				3.53 m	3.50	3.32 m
6a				3.86 dd, $J = 2.5, 12.0$	3.76	3.87 dd, $J = 2.5, 12.0$
6b				3.68 dd, $J = 5.0, 12.0$	3.65	3.70 dd, $J = 5.0, 12.0$
Glc III 1				4.45 d, $J = 7.5$	4.42	4.38 d, $J = 7.5$
2				3.28 dd, $J = 7.5, 9.0$	3.24	3.24 dd, $J = 7.5, 9.0$
3				3.42 t, $J = 9.0$	3.38	3.36 t, $J = 9.0$
4				3.40 t, $J = 9.0$	3.43	3.43 t, $J = 9.0$
5				3.53 m	3.56	3.56 m
6a				3.86 dd, $J = 2.5, 12.0$	3.78	3.77 dd, $J = 2.5, 12.0$
6b				3.66 dd, $J = 5.0, 12.0$	3.62	3.65 dd, $J = 5.0, 12.0$
Glc at C6 1			4.48			
2			3.22			
3			3.32			
4			3.40			
5			3.56			
6a			3.92			
6b			3.67			
Rha II 1						5.00 d, $J = 1.5$
2						3.90 dd, $J = 1.5, 3.0$
3						3.68 t, $J = 9.0, 3.0$
4						3.59 t, $J = 9.0$
5						4.08 m
6						1.28 d, $J = 6.5$

[a] Assignments are confirmed by ^{1}H−^{1}H COSY, HOHAHA, and HETCOR experiments.

identification as first and second glucopyranose units from their distinctive chemical shifts. The sequential assignments of these sugar protons as shown in Table 4 derived from their distinctive DQF-COSY patterns. The assignments of all proton resonances for the sugar moieties immediately allowed us to assign the resonances of the linked carbon atoms by HSQC (Tables 3 and 4). The absence of any glycosylation shift for the carbon resonances of the glucose II unit and of the rhamnose and xylose suggested these sugars to be terminal. Glycosylation shifts (∼6.0 ppm) observed on C-2 (82.50 ppm) and C-6 (68.20 ppm) of the first glucose unit allowed us to establish this as a C-2,6-substituted glucopyranose. Chemical shifts, multiplicity of the signals, absolute values of the coupling constant, and magnitude in the ^{1}H NMR spectrum as well as ^{13}C NMR data (Table 3) indicated the β-configuration at the anomeric positions for the two glucopyranosyl and xylopyranosyl units ($J_{H1-H2} = 7.5$ Hz), whereas the α-configuration for the rhamopyranosyl unit was established by the analysis of coupling constants between H1 and H2 and chemical shifts in the ^{13}C NMR spectrum (De Tommasi et al., 1990).

These data left two possible sequences for the trisaccharide chain of compound **1**: α-L-rhamnopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside or α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside. The HMBC spectrum allowed us to differentiate between the two proposed structures. Key correlation peaks were observed between the ano-

Chart 1. Structures of Triterpene Saponins 1−6 Isolated from *C. pedata* Fruits

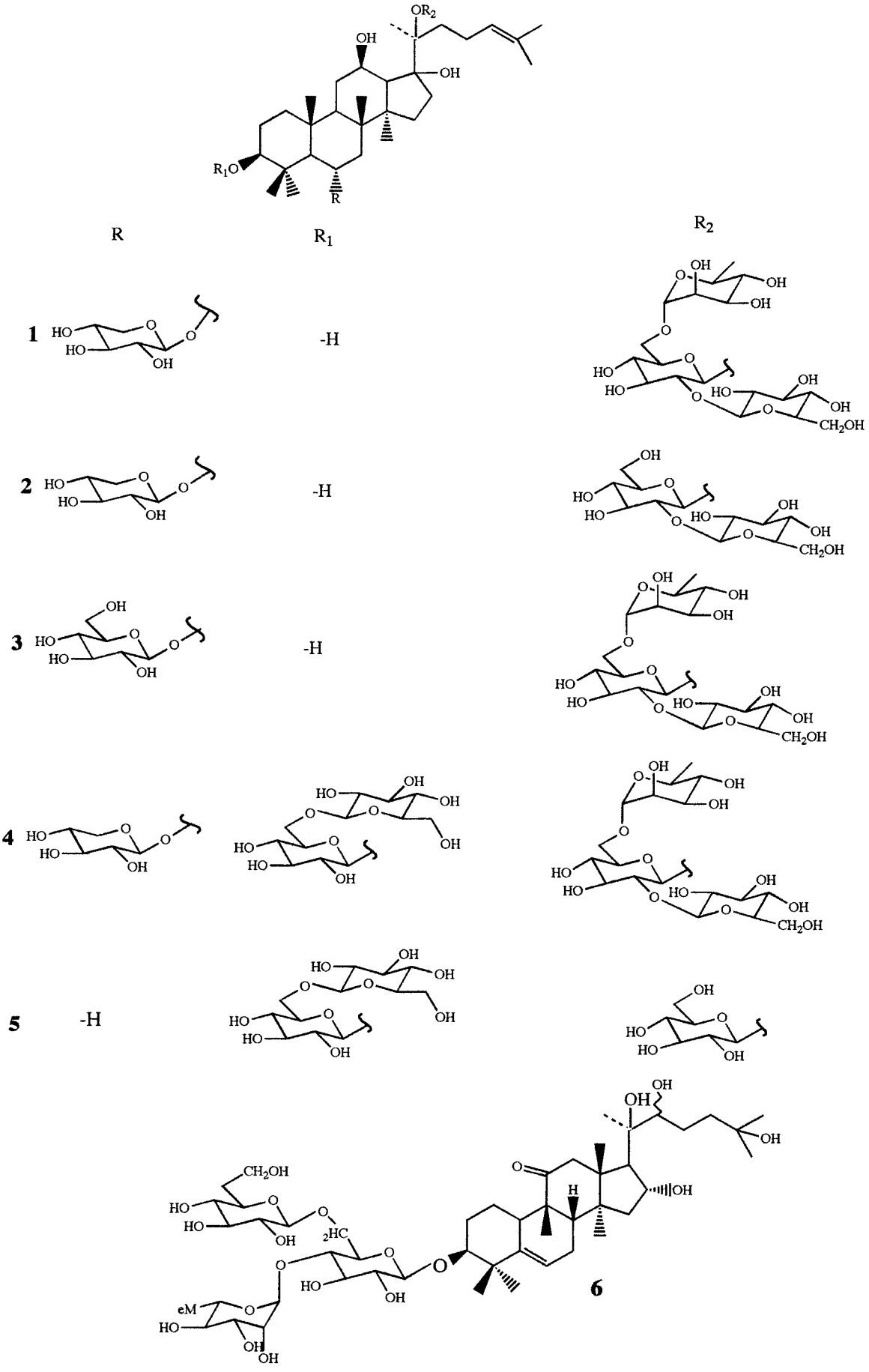
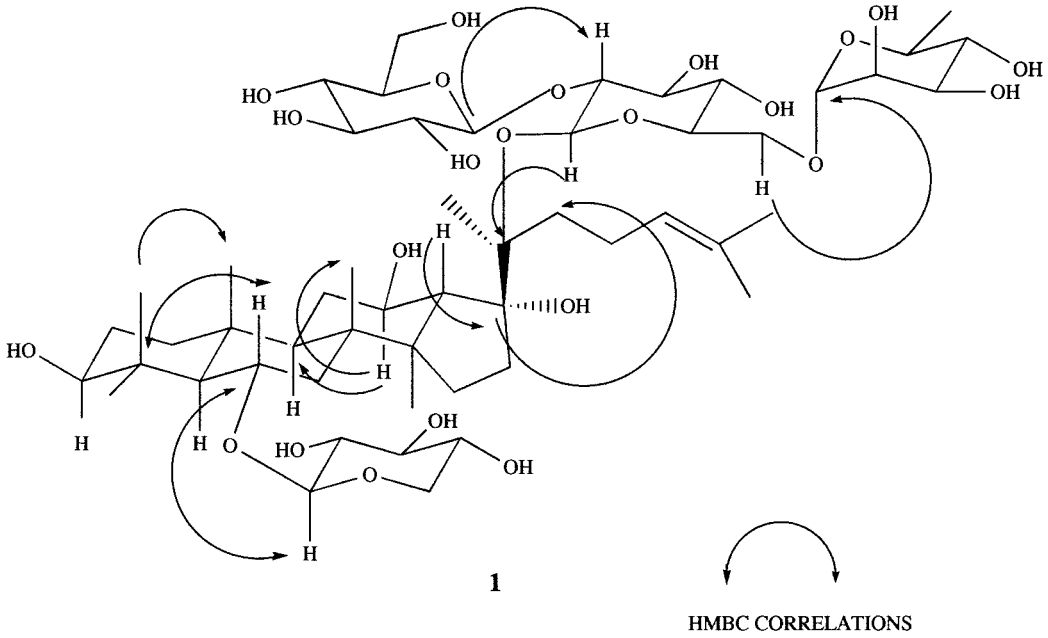


Chart 2. HMBC Correlations of Triterpene Saponin 1



meric proton of the glucose I unit at 4.80 ppm and the C-20 resonance (88.20 ppm) of the aglycon and between the anomeric proton of the glucose II unit (4.60 ppm) and C-2 of glucose I (82.50 ppm). A correlation peak observed between the anomeric proton of the xylose unit (δ 4.55) and C-6 (77.20 ppm) of the agyclon identified the β-D-xylopyranosyl unit linked to C-6, whereas the three-membered oligosaccharidic chain was confirmed to be bonded to C-20 through *O*-glycosidic linkage (Chart 1).

From these considerations the structure 17α-hydroxy-20(*S*)-protopanaxatriol-6-*O*-β-D-xylopyranosyl-20-*O*-β-D-glucopyranosyl-[(1→2)-β-D-glucopyranosyl]-(1→6)-α-L-rhamnopyranosyl was assigned to **1**.

Compound **2** showed, in the negative ion FABMS spectrum, a quasi molecular anion $[M - H]^-$ at *m/z* 947, 146 mass units higher than that of **1**, and gave ^{13}C and DEPT ^{13}C NMR consistent with a $C_{47}H_{80}O_{19}$ molecular formula. The FABMS of **2** exhibited fragments at *m/z* 815 $[(M - H) - 132]^-$ and 653 $[(M - H) - 132 + 162]^-$ corresponding to the sequential losses of one pentose unit and one hexose unit. The peak at *m/z* 491 $[(M - H) - (162 + 162 + 132]^-$ was ascribable to aglycon.

When **1** is used as a reference compound in the spectral analysis of compound **2** ($C_{47}H_{80}O_{19}$), close similarities are observed between the spectral data of the aglycon of both compounds, whereas the oligosaccharide moiety provides the points of difference (Tables 3 and 4). Main differences were the absence of the signals ascribable to the rhamnose unit and the absence of the glycosylation shift on C-6 (68.20 ppm in **1** and 62.20 ppm in **2**) of the glucose I unit, suggesting the presence of a β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyde chain linked at C-20 of the aglycon. Thus, **2** is 17α-hydroxy-20(*S*)-protopanaxatriol-6-*O*-β-D-xylopyranosyl-20-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **3** showed, in the negative ion FABMS spectrum, a quasi molecular anion $[(M - H)]^-$ at *m/z* 1123, 30 mass units higher than that of **1**, and gave ^{13}C and DEPT ^{13}C NMR consistent with a $C_{54}H_{92}O_{24}$ molecular formula. 2D-NMR analysis (2D-HOHAHA, DQF-COSY, HSQC) of both compounds indicated identical aglycon and saccharide chain at C-20 (Tables 2−4); in particular, the same glycosidation sites were deduced from the HSQC experiment. These observations led us to hypothesize that the structural difference between **1** and **3** was confined to the monosaccharidic unit at C-6. Analysis of ^1H and ^{13}C NMR data of **3** indicated the precence of a glucopyranosyl instead of a xylopyranosyl residue glycosylated at C-6. From these considerations the structure 17α-hydroxy-20(*S*)-protopanaxatriol-6-*O*-β-D-glucopyranosyl-20-*O*-β-D-glucopyranosyl-[(1→2)-β-D-glucopyranosyl]-(1→6)-α-L-rhamnopyranosyl was assigned to **3**.

Compound **4** ($C_{65}H_{110}O_{33}$) showed a quasi molecular anion at *m/z* 1417 $[(M - H)]^-$ and prominent fragments due to the loss of four hexose units, one deoxyhexose unit, and one pentose unit (Table 1). Methanolysis and subsequent GLC analysis of methyl sugars of compound **4** showed the presence of xylose, glucose, and rhamnose in the ratio 1:4:1. Comparison of NMR data for the aglycon moiety with those of **1** suggested structural similarity in the aglycon moiety. The main differences were in the chemical shift of carbon atoms of ring A. Signals in the ^{13}C NMR spectrum at 89.20 (C-3), 26.80 (C-2), and 38.00 (C-4) were typical for the glycosidation at C-3 of the triterpene (Yoshikawa et al., 1993) (Table 2).

The sugar portion of **4** contained in the ^1H NMR spectrum (Table 4) six anomeric proton signals (δ 4.85, d, J = 1.5 Hz; δ 4.82, d, J = 7.5 Hz; δ 4.63, d, J = 7.5 Hz; δ 4.60, d, J = 7.5 Hz; δ 4.56, d, J = 7.6 Hz; and δ 4.45, d, J = 7.5 Hz) and one methyl doublet (δ 1.30, d, J = 6.5 Hz), suggesting the occurrence of one deoxyhexose unit. The other sugar signals were overlapped in the region between δ 3.10 and 4.10. The structure of the oligosaccharide moieties was achieved using 1D-TOCSY and 2D-NMR experiments. Because of the selectivity of the multistep coherence transfer, the 1D-TOCSY method allowed the subspectrum of a single monosaccharide unit to be extracted from the crowded overlapped region. The isolated anomeric proton signals resonating at the uncrowded region of the spectrum (between δ 4.41 and 5.37) have been the starting point for the 1D-TOCSY experiments. Selected 1D-TOCSY

obtained by irradiating each anomeric proton signal yielded the subspectrum of each sugar residue with high digital resolution. Each subspectrum contained the scalar-coupled protons within each sugar residue. Because in the TOCSY method both direct and relayed connectivities occurr, we also recorded a DQF-COSY spectrum. The results of 1D-TOCSY and DQF-COSY experiments allowed the sequential assignments of all of the proton resonances to the individual monosaccharides as reported in Table 4. Thus, the shifts of the sugar resonances, summarized in Table 3, were attributable to α-L-rhamnopyranosyl (δH-1_{rha} = 4.85), β-D-glucopyranosy (δH-1_{glc} = 4.82), β-D-glucopyranosyl (δH-1_{glcI} = 4.63), β-D-xylopyranosyl (δH-1_{xyl} = 4.56), β-D-glucopyranosyl (δH-1_{glcII} = 4.60), and β-D-glucopyranosyl (δH-1_{glcIII} = 4.45) units.

HSQC experiments allowed the assignments of the interglycosydic linkages by comparison of the observed carbon chemical shifts with those of the corresponding methylpyranosides and accounted for the known effects of glycosidation. The absence of any ^{13}C NMR glycosidation shift for three β-D-glucopyranosyl moieties, one α-L-rhamnopyranosyl moiety, and one β-D-xylopyranosyl moiety suggested these sugars to be terminal units. Glycosidation shifts were observed for C-2glc (83.00), C-6glc (68.50), and C-6glcII (69.30) (Table 3).

The position of the sugar residues was unambiguously defined by the HMBC experiment. A cross-peak due to long-range correlations between C-3 (δ 89.20) of the aglycon and H-1glucII (δ 4.60) indicated that glucose is the hexose residue linked to C-3 of the aglycon; a cross-peak between C-6_{gluII} (δ 69.30) and H-1 of the terminal glucose III (δ 4.45) indicated that glucose is the second unit of the disaccharide chain at C-3 of the aglycon. Similarly, the sequence of the trisaccharide chain at C-20 was shown by the cross-peaks between C-2_{glc} (δ 83.00) and H-1glcI (δ 4.63) and between C-6_{glc} (δ 68.50) and H-1 of the terminal rhamnose (δ 4.85). A cross-peak between H-1 of xylose (δ 4.56) and the ^{13}C NMR resonance of the aglycon carbon atom (δ 77.00) provided definitive evidence for a xylopyranosyl residue glycosylated at C-6.

On the basis of these results, compound **4** was established as 17α-hydroxy-20(*S*)-protopanaxatriol-6-*O*-β-D-xylopyranosyl-3-{*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl}-20-*O*-β-D-glucopyranosyl-[(1→2)-β-D-glucopyranosyl]-(1→6)-α-L-rhamnopyranosyl.

Compound **5** ($C_{48}H_{82}O_{20}$) showed an $[(M - H)]^-$ ion at *m*/*z* 945 and prominent fragment ions at *m*/*z* 783, 621, and 459 resulting from the cleavage of three hexose units (Table 1). These were identified as glucose by methanolysis and GLC analysis of methyl sugar derivatives. The NMR data and FABMS and comparison with literature data for the aglycon moiety suggested that **5** is a 20(*S*)-protopanaxtetraol glycosylated at positions C-3 and C-20 (Besso et al., 1982). Interpretation of DQF-COSY, 1D-TOCSY, and HSQC spectra led to the identification of three β-D-glucopyranose residues. The absence of any ^{13}C NMR glycosidation shift for two β-D-glucopyranosyls suggested two units to be terminal (Table 4).

Definitive evidence of the structure of compound **5** was derived from the HMBC spectrum, which showed clearly cross-peaks due to J_{2-3} long-range couplings between H-1′ of the gentobiosyl unit (4.73 ppm) and C-3 (89.2 ppm) of the aglycon. From all of these data the structure of compound **5** was determined as 20(*S*)-protopanaxadiol-3-{*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl}-20-*O*-β-D-glucopyranoside.

Compound **6** ($C_{48}H_{80}O_{20}$) showed a quasi molecular anion at *m*/*z* 975 $[(M - H)]^-$ and peaks due to the fragmentation of the oligosaccharide portion at *m*/*z* 829 $[(M - H) - 146]^-$, 667 $[(M - H) - 146 + 162]^-$, and 505 $[(M - H) - (146 + 162 + 162)]^-$. The ^{13}C and ^{13}C DEPT NMR showed 48 signals, of which 18 were assigned to the saccharide portion and 30 to a triterpenic moiety.

The ^{1}H NMR spectrum displayed resonances for eight methyls (δ 0.94−1.45), three of which are linked to oxygenated carbons (see Experimental Procedures). In addition, it was possible to observe an olefinic proton signal at δ 5.63 (1H, br d, J = 6 Hz, H-6) indicating the presence of a double bond connected to a methylene group at δ 2.46 (1H, ddd, J = 14.0, 6.0, 7.0 Hz, Ha-7) and 2.05 (1H, ddd, J = 14.0, 6.0, 3.0 Hz, Hb-7) as suggested by 1D-TOCSY and DQF-COSY experiments. Two proton signals at δ 3.20 (1H, d, J = 15.0 Hz, Ha-12) and 2.70 (1H, d, J = 15.0 Hz, Hb-12) were diagnostic for a methylene linked to a carbonyl group. The 3-β-OH substitution of this skeleton was evident from the chemical shift, and the J values of the proton were ascribable to C-3 (δ 3.46, 1H, dd, J = 9.0, 5.0 Hz, H-3). The signal in the ^{1}H NMR spectrum at δ 4.42 (1H, t, J = 7.5 Hz) indicated the presence of a C-16α hydroxyl group, which was supported by ^{13}C NMR data (see Experimental Procedures) and literature data (De Tommasi et al., 1996). The ^{13}C NMR and DEPT-^{13}C NMR spectra showed, together with the resonances ascribable to two olefinic carbons (142.0 ppm, C-5; 119.5 ppm, C-6) and one carbonyl carbon (216.8 ppm, C-11), signals indicating the occurrence of three tertiary hydroxyl groups (87.3, 71.5, and 78.1 ppm) and two quaternary hydroxyl group (81.1 and 72.0 ppm) that were located by 2D-NMR spectroscopy. Thus, the HSQC spectrum shows that the signal at δ 58.2 (C-17) bears the hydrogen which resonated at δ 2.60, and from the DQF-COSY spectrum it can be seen that this proton couples to the other at δ 4.42, which from the HSQC spectrum is attached to a carbon (C-16) at 71.6 ppm bearing a hydroxy group. Similar connectivities and assignments can be established for ring A (C-10, C-1, C-2, C-3), a portion of ring B (C-6, C-7, C-8), and the side chain (C-22, C-23, C-24). All of the resonances in the ^{13}C NMR spectrum to the pertinent carbons were achieved on the basis of a series of data arising from HSQC and HMBC (see Experimental Procedures).

The relative stereochemistry was established by taking into account the J values for H of the rings A−D and from 1D-NOESY experiments. In fact, irradiating the H-3 (δ 3.46) showed connectivity with Me-28 (δ 1.06), irradiating the H-8 (δ 1.88) signal showed connectivities with Me-18 (δ 0.95) and Me-19 (δ 1.07), and irradiating the Me-30 (δ 1.31) signal showed connectivities with H-17 (δ 2.60) and Me-H-7 (δ 2.46).

On the basis of reported data the aglycon of **6** was established to be 16α,20,22,25-tetrahydroxycucurbit-5-en-11-one, previously isolated from seeds of the same species (De Tommasi et al., 1996). Methanolysis and subsequent GLC analysis of the methyl sugars of compound **6** showed the presence of glucose and rhamnose in the ratio 2:1. Also in this case, the proton coupling network within each sugar residue was traced out using a combination of DQF-COSY, 2D-HOHAHA, and HSQC experiments, which indicated the presence

of one β-D-glucopyranosyl unit substituted at C-6 and C-4, respectively. Direct evidence for the sugar sequence was derived from 1D-NOESY experiments. In fact, irradiating the H-3 signal of the aglycon showed connectivity with the H-1 (δ 4.59) anomeric proton of glucose II, and irradiating the H-4 signal of glucose II showed connectivity with the H-1 (δ 5.00) anomeric proton of α-L-rhamnose. On the basis of reported data the structure of **6** was established to be 3β-[(4-*O*-α-L-rhamnopyranosyl-6-*O*-β-D-glucopyranosyl)-β-D-glucopyranosyl)oxy]-16α,20,22,25-tetrahydroxycucurbita-5-en-11-one.

The saponins distribution among the organs of *C. pedata* varies considerably. As reported in previous work (De Tommasi et al., 1996) seed saponins can be divided into two different groups containing 2,3,16α,20,22-pentahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one and 3β,16α,20,22,25-pentahydroxycucurbit-5-en-11-one as the aglycon.

Although saponins having 3β,6α,12β,17α-tetrahydroxy-20(*S*)-protopanaxatetraol (compounds **1**−**5**), 3β,16α,20,22,25-pentahydroxycucurbit-5-en-11-one (compound **6**), and 2,3,16α,20,22-pentahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one (compounds **7**−**9**) as the aglycons were also detected in the fruits, aglycon 3β,6α,12β,17α-hydroxy-20(*S*)-protopanaxatetraol was isolated for the first time from the fruits but was not found in the seeds.

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