Studies on the Constituents of *Cyclanthera pedata* (Caigua) Seeds: Isolation and Characterization of Six New Cucurbitacin Glycosides

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Six new cucurbitacin glycosides were isolated from the seeds of *Cyclanthera pedata* Schrab (Cucurbitaceae). Their structures were elucidated on the basis of spectral and chemical data to be 2-[(6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-3,16α,20,22,25-pentahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one (**1**), 25-acetoxy-2-[(6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-3,16α,20,-22-tetrahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one (**2**), 25-acetoxy-2-(*β*-D-glucopyranosyloxy)-3,16α,20,22-tetrahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one (**3**), 25-acetoxy-2-[(4-*O*-α-L-rhamnopyranosyl-6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-3,16α,20-trihydroxy-29-norcucurbita-1,3,5-(10) triene-11,22-dione (**4**), 3*β*-[(6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-16α,20,22,25-tetrahydroxycucurbit-5-en-11-one (**5**), 3-*β*-(*β*-D-glucopyranosyloxy)-25-acetoxy-16α,20,22,-trihydroxy-cucurbit-5-en-11-one (**6**).

Keywords: *Caigua; Cyclanthera pedata; Cucurbitaceae; South American crop; food source; cucurbitacin glycosides; ^1H and ^{13}C NMR; FABMS*

INTRODUCTION

Cyclanthera pedata Scrabs (Caigua), a plant cultivated by ancient Peruvians, is largely used to make salad soup and has the reputation of being antiinflammatory, hypocholesterolemic, and hypoglycemic. This work on "Caigua" (*C. pedata*) is a part of a series of studies on chemical constituents of alimentary plants from Perù (De Simone et al., 1990; Dini et al., 1992; Rastrelli et al., 1995). No investigation of secondary metabolites has been reported prior to our own work on this plant. In the present report we describe the purification and structure elucidation of six novel tetracyclic triterpene glycosides **1**−**6** and the known compound **7** from seeds of *C. pedata*.

The wide natural distribution and structural diversity of triterpenoids have evoked considerable interest in the chemistry and biological activity (Galiano et al., 1983) of these compounds. Many of the tetracyclic and pentacyclic triterpenes isolated from plants in the Cucurbitaceae family possess the biogenetically unusual 10α-cucurbit-5-ene-[19(10→9*β*)-*abeo*-10α-lanostane] skeleton (Pryzek, 1979). These compounds and their glycosides have long been known to display a host of interesting biological activities which at least partly account for the extensive use of Cucurbitaceae in the food and folk medicine of most tropical or semitropical regions (Lyndon et al., 1989).

EXPERIMENTAL PROCEDURES

Material. The plant material was supplied by IPIFA, Instituto Peruano Investigaciones Fitoterapica Andina (collected in Perù, 1990). A voucher specimen was deposited at the Herbario de Museo de Historia Natural "*J.* Prado" Un. Lima (Perù).

Apparatus. The FABMS spectra 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 energy 2−6 kV in a Kratos MS spectrometer equipped with a Kratos FAB source. The NMR spectra were obtained in CD_3OD using a Bruker AMX-500 spectrometer. Two-dimensional homonuclear proton chemical shift correlation (COSY) experiments were measured by employing the conventional pulse sequence. The COSY spectrum was obtained using a data set ($t_1 \times t_2$) of 1024 × 1024 points for a spectral width of 1165 Hz (relaxation delay 1 s) The data matrix was processed using an unshifted sine bell window function followed by transformation to give a magnitude spectrum with symmetrization (digital resolution in both F2 and F1 dimensions 1.13 Hz/ point). The 2D HOHAHA experiment was performed in the phase sensitive mode (TPPI) using a MLEV-17 sequence for mixing. The spectral width (t_2) was 1002 Hz; 512 experiments of 80 scans each (relaxation delay 1.5 s, mixing time 100 ms) were acquired in both dimensions before transformation. The resulting digital resolution in F2 was 0.48 Hz/point. The ROESY experiment was performed in the phase sensitive mode (TPPI). The spectral width (t_2) was 1002 Hz; 512 experiments of 80 scans each (relaxation delay 1.5 s, mixing time 300 ms) were acquired in 2K data points. For processing a sine bell window function was applied in both dimensions before transformation. The HETCOR experiment was performed on a data matrix of 1024 × 512, using CH coupling of 135 Hz and relaxation delay of 1.5 s. The COLOC experiments were performed on a data matrix of 1024 × 512, using CH coupling of 8 and 6 Hz and relaxation delay of 1.5 s (Agrawal, 1992).

GC−MS were run using a Hewlett-Packard 5890 apparatus, a gas chromatograph fitted with an HP 5970B mass detector, and a HP 59970 MS Chemstation, equipped with a HP-5 column (25 × 0.2 mm i.d., 0.33 μm film). Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. Droplet counter current chromatography (DCCC) was performed on an apparatus manufactured by Buchi, equipped with 300 tubes. HPLC separations were performed on a Waters 590 series pumping system with a Waters R401 refractive index detector equipped with a Waters μ-Bondapak C18 column.

Extraction and Isolation. The seeds (500 g) of *C. pedata* were defatted with petroleum ether (6 g) and $CHCl_3$ (2 g) and

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extracted successively with $CHCl_3$−MeOH (9:1) (6 g) and MeOH to give 25 g of residue. Part of the MeOH extract (8 g) was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH soluble portion (4.5 g) which was chromatographed on a Sephadex LH-20 column (100 × 5 cm) with MeOH−H_2O (8:2) as eluent. Fractions (9 mL) were collected and checked by TLC [Sigel plates in *n*-BuOH−HOAc−H_2O (60:15:25)]. Fractions 19−30 (600 mg) containing the crude glycosidic mixture were purified by DCCC using BuOH−Me_2CO−H_2O (30:9:11) in which the stationary phase consisted of the higher phase (descending mode, flow 15 mL/h) to give A (250 mg) and B (220 mg). Fractionation of each glycoside was achieved by HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm i.d.) with MeOH−H_2O (45:55) for A and MeOH−H_2O (50:50) for B, flow rate 3 mL/min, to yield pure **1** (30 mg, t_R = 30 min), **2** (25 mg, t_R = 32 min), **3** (20 mg, t_R = 34 min), **4** (32 mg, t_R = 15 min), **7** (10 mg, t_R = 18 min), **5** (20 mg, t_R = 8 min), and **6** (8 mg, t_R = 12 min).

Methanolysis of Compounds 1−6, Carbohydrate Constituents. A solution of each compound (2 mg) in anhydrous 2 N HCl/MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 12 h. After cooling, the solution was neutralized with Ag_2CO_3 and centrifuged. The supernatant was evaporated to dryness under N_2. The residue was reacted with TRISIL-Z (Pierce) and analyzed by GLC. Retention times were identical with those of the authentic methyl sugars.

Desacetylation of 2. Compound **2** (20 mg) dissolved in 2 mL of MeOH was treated with 50 mg of K_2CO_3 for 48 h at room temperature. Filtration and evaporation of the solvent gave a crude product, which was purified by CC [$CHCl_3$−MeOH (9:1)] to yield 10 mg of an amorphous powder identical in physicochemical data with **1**.

Compound 1: $C_{41}H_{64}O_{17}$; $[\alpha]^{25}_D = -45°$ (c = 1, MeOH); for FABMS see text; for ^1H and ^{13}C NMR signals, see Tables 1−3.

Compound 2: $C_{43}H_{66}O_{18}$; $[\alpha]^{25}_D = -40°$ (c = 1, MeOH); for FABMS, see text; ^1H NMR (for sugar moiety) δ 4.62 (1H, d, J = 7.5 Hz, H-1′), 4.41 (1H, d, J = 7.5 Hz, H-1″), 4.39 (1H, dd, J = 12.0, 2.5 Hz, H-6a′), 4.00 (1H, dd, J = 12.0, 2.5 Hz, H-6a″), 3.91 (1H, dd, J = 12.0, 4.0 Hz, H-6b′), 3.76 (1H, dd, J = 12.0, 4.0 Hz, H-6b″), 3.60 (1H, m, H-5″), 3.55 (1H, dd, J = 9.0, 7.5 Hz, H-2″), 3.50 (1H, dd, J = 9.0, 7.5 Hz, H-2′), 3.42 (1H, m, H-5″), 3.41 (1H, t, J = 9.0 Hz, H-3″), 3.36 (1H, t, J = 9.0 Hz, H-3′), 3.34 (2H, overlapped, H-4′, H-4″); ^1H NMR aglycon signals superimposable to ±0.02 on those reported for **1**; for ^{13}C NMR, see Table 3.

Compound 3: $C_{37}H_{56}O_{13}$; $[\alpha]^{25}_D = -30°$ (c = 1, MeOH); mp 140−145 °C; for FABMS, see text; ^1H NMR δ 1.94 (1H, s, Me of acetyl group), for sugar moiety see Table 2; ^1H NMR aglycon signals superimposable to ±0.02 on those reported for **1**; for ^{13}C NMR, see Table 3.

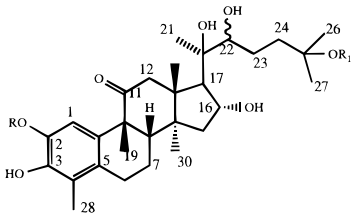
Compound 4: $C_{49}H_{74}O_{22}$; $[\alpha]^{25}_D = -70°$ (c = 1, MeOH); for ^1H and ^{13}C NMR, see Table 1−3.

Compound 5: $C_{42}H_{70}O_{16}$; $[\alpha]^{25}_D = +25°$ (c = 1, MeOH); for FABMS, see text; ^1H NMR (for sugar moiety) δ 4.70 (1H, d, J = 7.5 Hz, H-1′), 4.45 (1H, d, J = 7.5 Hz, H-1″), 4.24 (1H, dd, J = 12.0, 2.5 Hz, H-6a′), 4.03 (1H, dd, J = 12.0, 2.5 Hz, H-6a″), 3.93 (1H, dd, J = 12.0, 4.0 Hz, H-6b′), 3.80 (1H, dd, J = 12.0, 4.0 Hz, H-6b″), 3.66 (1H, m, H-5″), 3.56 (1H, dd, J = 9.0, 7.5 Hz, H-2″), 3.54 (1H, dd, J = 9.0, 7.5 Hz, H-2′), 3.45 (1H, t, J = 9.0 Hz, H-3″), 3.41 (1H, t, J = 9.0 Hz, H-3′), 3.39 (2H, overlapped, H-4′, H-4″), 3.37 (1H, m, H-5″); for ^1H NMR aglycon signals and ^{13}C NMR see Tables 1 and 3.

Compound 6: $C_{38}H_{62}O_{12}$; $[\alpha]^{25}_D = +3°$ (c = 1, MeOH); ^1H NMR (for sugar moiety) δ 4.55 (1H, d, J = 7.5 Hz, H-1′), 3.90 (1H, dd, J =12.0, 2.5 Hz, H-6a′), 3.80 (1H, dd, J = 12.0, 4.0 Hz, H-6b′), 3.50 (1H, dd, J =7.5, 9.0 Hz, H-2′), 3.46 (1H, m, H-5′), 3.34 (2H, overlapped, H-3′, H-4′), 1.94 (3H, s, Me of acetyl group); ^1H NMR aglycon signals superimposable to ±0.03 on those reported for **5**; for ^{13}C NMR, see Table 3.

Compound 7: $C_{43}H_{64}O_{18}$; $[\alpha]^{25}_D = -36°$ (c = 1, MeOH); ^1H NMR δ 6.65 (1H, s, H-1), 4.60 (1H, d, J = 7.5 Hz, H-1′), 4.44 (1H, br dd, J = 7.5, 7.0 Hz, H-16), 4.40 (1H, d, J = 7.5 Hz, H-1″), 4.38 (1H, dd, J = 12.0, 2.5 Hz, H-6a′), 3.98 (1H, dd, J = 12.0, 2.5 Hz, H-6a″), 3.88 (1H, dd, J = 12.0, 4.0 Hz, H-6b′), 3.75 (1H, dd, J = 12.0, 4.0 Hz, H-6b″), 3.60 (1H, m, H-5″), 3.52 (1H, dd, J = 9.0, 7.5 Hz, H-2″), 3.50 (1H, dd, J = 9.0, 7.5 Hz, H-2′), 3.40 (1H, t, J = 9.0 Hz, H-3″), 3.36 (1H, t, J = 9.0 Hz, H-3′), 3.34 (2H, overlapped, H-4′, H-4″), 3.32 (1H, m, H-5″), 2.92 (1H, d, J =14.5 Hz, H-12a), 2.84 (1H, ddd, J = 18.0, 9.0, 9.0 Hz, H-6a), 2.80 (1H, ddd, J = 18.0, 10.0, 6.0 Hz, H-23a), 2.68 (1H, ddd, J = 18.0, 10.0, 6.0 Hz, H-23b), 2.66 (1H, d, J = 14.5 Hz, H-12b), 2.64 (1H, br dd, J = 18.0, 9.0 Hz, H-6b), 2.50 (1H, d, J = 7.0 Hz, H-17), 2.08 (3H, s, Me-28), 1.94 (3H, s, Me of acetyl group), 1.42 (3H, s, Me-26), 1.42 (3H, s, Me-27), 1.35 (3H, s, Me-21), 1.31 (3H, s, Me-19), 0.99 (3H, s, Me-30), 0.95 (3H, s, Me-18); ^{13}C NMR (for sugar moiety) 105.5 (C-1′), 74.70 (C-2′), 77.00 (C-3′), 71.50 (C-4′), 77.40 (C-5′), 69.50 (C-6′), 104.60 (C-1″), 75.10 (C-2″), 77.80 (C-3″), 71.00 (C-4″), 77.90 (C-5″), 62.70 (C-6″); ^{13}C NMR aglycon signals superimposable on those reported for **4**, see Table 3. This compound was identified by comparison of spectral data with literature values (Achenbach et al., 1993).

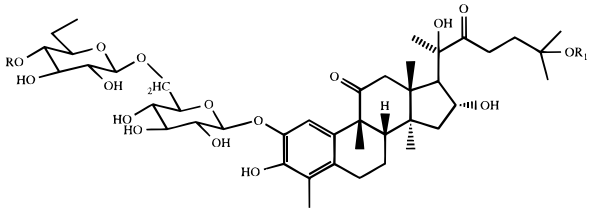
Chart 1



1 R= β-D-gentobiosyl R_1=H

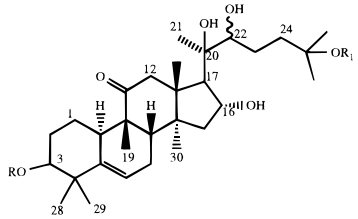
2 R= β-D-gentobiosyl R_1=Ac

3 R= β-D-glucosyl R_1=Ac



7 R= H R_1=Ac

4 R= α-L-rhamnopyranosyl R_1=Ac



5 R= β-D-gentobiosyl R_1=H

6 R= β-D-glucosyl R_1=Ac

RESULTS AND DISCUSSION

C. pedata seeds 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 column. The fractions containing the glycosidic mixture, checked by TLC, were further purified by DCCC and subsequent HPLC to obtain the glycosides **1**−**7** (Chart 1). The structures and molecular formulae were determined by negative ion FABMS spectra and 1D and 2D ^1H and ^{13}C NMR.

Table 1. ^{1}H NMR[a] Data of Aglycon of Compounds 1, 4, and 5 (J_{HH} in Hz)

	aglycon, δH		
proton	**1**	**4**	**5**
Ha-1	6.67 (s)	6.70 (s)	1.32 (m)
Hb-1			1.58 (m)
Hax-2			1.96 (dddd, J = 14.0, 9.0, 8.0, 3.0)
Heq-2			1.78 (dddd, J = 14.0, 5.0, 4.5, 3.0)
H-3			3.46 (dd, J = 9.0, 5.0)
Ha-6	2.98 (ddd, J = 15.0, 8.0, 8.0)	2.95 (ddd, J = 15.0, 8.0, 8.0)	5.61 (d, J =6)
Hb-6	2.80 (ddd, J = 15.0, 8.0, 3.0)	2.82 (ddd, J = 15.0, 8.0, 8.0)	
Ha-7	2.27 (dddd, J = 14.0, 8.0, 8.0, 7.0)	2.25 (dddd, J = 14.0, 8.0, 8.0, 7.0)	2.46 (ddd, J = 14.0, 6.0,7.0)
Hb-7	2.05 (so)	2.00 (so)	2.05 (ddd, J = 14.0, 6.0, 3.0)
H-8	2.15 (dd, J = 7.0, 2.0)	2.12 (dd, J = 7.0, 2.0)	1.88 (dd, J = 7.0, 3.0)
H-10			2.42 (m)
Ha-12	2.94 (d, J = 13.5)	2.90 (d, J = 13.5)	3.20 (d, J = 15)
Hb-12	2.67 (d, J = 13.5)	2.65 (d, J = 13.5)	2.70 (d, J = 15)
Ha-15	2.11 (dd, J = 13.0, 7.0)	2.07 (dd, J = 13.0, 7.5)	2.00 (dd, J = 13.0, 7.5)
Hb-15	1.78 (dd, J = 13.0, 7.5)	1.78 (dd, J = 13.0, 8.0)	1.78 (dd, J = 13.0, 7.5)
H-16	4.53 (br dd, J = 7.5, 7.0)	4.50 (dd, J = 8.0, 7.0)	4.42 (t, J = 7.5)
H-17	2.48 (d, J = 7.0)	2.45 (d, J = 7.0)	2.60 (d, J = 7.5)
Me-18	1.00 (s)	1.00 (s)	0.95 (s)
Me-19	1.23 (s)	1.23 (s)	1.07 (s)
Me-21	1.24 (s)	1.36 (s)	1.24 (s)
H-22	3.30 (dd, J = 8.0, 4.0)		3.31 (dd, J = 8.5, 5.0)
Ha-23	2.12 (dddd, J = 18.0, 10.0, 8.0, 7.0)	2.79 (ddd, J = 18.0, 10.0, 6.0)	2.10 (dddd, J = 17.0, 10.0, 8.5, 8.0)
Hb-23	1.74 (dddd, J = 18.0, 10.0, 7.0, 4.0)	2.69 (ddd, J = 18.0, 10.0, 6.0)	1.70 (dddd, J = 17.0, 10.0, 8.0, 5.0)
Ha-24	1.78 (ddd, J = 16.0, 10.0, 7.0)	1.72 (ddd, J = 14.0, 10.0, 6.0)	1.74 (ddd, J = 16.0, 10.0, 7.0)
Hb-24	1.48 (ddd, J = 16.0, 10.0, 7.0)	1.60 (ddd, J = 14.0, 10.0, 6.0)	1.43 (ddd, J = 16.0, 10.0, 7.0)
Me-26	1.34 (s)	1.40 (s)	1.36 (s)
Me-27	1.37 (s)	1.40 (s)	1.39 (s)
Me-28	2.12 (s)	2.06 (s)	1.06 (s)
Me-29			1.25 (s)
Me-30	1.03 (s)	1.00 (s)	1.31 (s)

[a] so, signals partially obscured by other signals. Assignments based on 2D COSY, 2D HOHAHA, HETCOR, and COLOC experiments.

Compound **1** ($C_{41}H_{64}O_{17}$) showed an $[M - H]^-$ ion at *m/z* 827 and prominent fragments at *m/z* 655 $[(M - H) - 162)]^-$ which were interpreted as the cleavage of hexose unit and *m/z* 477 $[(M - H) - (162 + 178)]^-$ due to loss of two hexose units. The FABMS of compound **2** ($C_{43}H_{66}O_{18}$)showed an $[M - H]^-$ ion at *m/z* 869, which was 42 mass units higher than that of **1** and is compatible with an additional acetyl group**.** Methanolysis of **1** and **2** and analysis in GLC afforded methylglucose.

The ^{1}H NMR spectrum of **1** showed seven singlet methyl signals in the range δ 1.00−2.12, two hydroxymethine signals at δ 4.53 (H-16, br dd, J = 7.5, 7.0 Hz) and 3.30 (H-22, dd, J = 8, 4.0 Hz), and a signal for one aromatic proton at δ 6.67 (H-1, s). Assignments for all protons of compound **1** are reported in Table 1. The ^{13}C NMR and DEPT−^{13}C NMR of **1** revealed 41 carbon signals, 12 ascribable to two hexose units and 29 due to a tetracyclic triterpene skeleton (Table 3).

These spectral evidences suggested that the aglycon of **1** was a norcucurbitacin derivative containing an aromatic ring (Achenbach, 1993). The location of two secondary −OH groups at C-16 and C-22 was deduced using a combination of a 2D ^{1}H−^{1}H correlations COSY and HOHAHA and direct 2D ^{1}H−^{13}C chemical shift cross-correlations (HETCOR).

A COLOC experiment was performed to discriminate between the similar resonances of C-2 and C-3 and to establish the position of glycosidation. The COLOC spectrum of **1** confirms the C-2 glycosidation showing a correlation between C-2 (146.0 ppm) and H-1′ (δ 4.60, 1H, d, J = 7.5 Hz) of the glucose unit and between C-3 (144.60 ppm) and Me-28 (δ 2.12 s).

For the sugar moiety the ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) confirmed the presence of two glucoses in the β-D-pyranosyl form (De Tommasi et al., 1990). The ^{13}C NMR signal of C-6 of a glucopyranosyl group appeared at 69.3 ppm as expected for a 6-O-substituted glucose; these spectral data indicated that the sugar moiety is a β-gentobiosyl unit (Table 3).

In the ^{1}H NMR spectrum of compound **2**, signal at δ 1.94 (3H, s) and ^{13}C NMR signals at 21.80 ppm and 172.0 ppm indicated the presence of an acetyl group. In the ^{13}C NMR spectra (Table 3) on going from **1** to **2**, the signals due to C-24, C-26, and C-27 of side chain were displaced upfield and the signal to C-25 downfield while other signals remained almost unshifted. These data indicated that the acetyl group was located at C-25 of the side chain. The structure of compound **2** as the acetyl derivative of **1** was also determined by alkaline cleavage of **2** to yield **1**.

On the basis of the foregoing data, the structures of compounds **1** and **2** are proposed to be, in turn, 2-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-3,16α,20,-22,25-pentahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one (**1**) and 25-acetoxy-2-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-3,16α,20,22-tetrahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one (**2**).

The FABMS of compound **3** ($C_{37}H_{56}O_{13}$) gave a quasi molecular anion peak at *m/z* 707 $[(M - H)]^-$ and a fragment at *m/z* 545 $[(M - H) - 162]^-$ corresponding to the loss of a glucose unit. Examination of NMR data and comparison with those of **2** showed **3** to differ from **2** only by the absence of the terminal glucopyranosyl unit (Tables 2 and 3). Thus compound **3** was determined as the new 25-acetoxy-2-(β-D-glucopyranosyloxy)-3,16α,20,22-tetrahydroxy-29-norcucurbita-1,3,5(10)-trien-11-one.

Compound **4** ($C_{49}H_{74}O_{22}$) showed a quasi molecular anion at *m/z* 1013 $[(M - H)]^-$ and prominent fragments at *m/z* 867 $[(M - H) - 146)]^-$, 851 $[(M - H) - 162)]^-$ (cleavage of a deoxyhexose unit with or without the glycosidic oxygen), 705 $[(M - H) - (146 + 162)]^-$, and

Table 2. ^{1}H NMR[a,b] Data of Sugar Moieties of Compounds 1, 3, and 4 (CD_3OD, 500 MHz)

	sugars		
proton[c]	**1**	**3**	**4**
Glc-I H-1′	4.60 (d, J = 7.5)	4.64 (d, J = 7.5)	4.65 (d, J = 7.5)
H-2′	3.50 (dd, J = 9.0, 7.5)	3.50 (dd, J = 9.0, 7.5)	3.56 (dd, J = 9.0, 7.5)
H-3′	3.45 (t, J = 9.0)	3.36 (t, J = 9.0)	3.30 (t, J = 9.0)
H-4′	3.35 (so)	3.34 (so)	3.38 (t, J = 9.0)
H-5′	3.31 (ddd, J = 9.0, 4.0, 2.5)	3.32 (ddd, J = 9.0, 4.0, 2.5)	3.40 (ddd, J = 9.0, 5.0, 3.0)
Ha-6′	4.25 (dd, J = 12.0, 2.5)	4.38 (dd, J = 12.0, 2.5)	4.00 (dd, J = 12.0, 3.0)
Hb-6′	3.88 (dd, J = 12.0, 4.0)	3.90 (dd, J = 12.0, 4.0)	3.78 (dd, J = 12.0, 5.0)
Glc-II H-1″	4.40 (d, J = 7.5)		4.42 (d, J = 7.5)
H-2″	3.52 (dd, J = 9.0, 7.5)		3.42 (dd, J = 9.0, 7.5)
H-3″	3.40 (t, J = 9.0)		3.40 (t, J = 9.0)
H-4″	3.34 (so)		3.34 (so)
H-5″	3.50 (m)		3.51 (m)
Ha-6″	3.94 (dd, J = 12.0, 3.0)		3.98 (dd, J = 12.0, 3.0)
Hb-6″	3.70 (dd, J = 12.0, 4.5)		3.76 (dd, J = 12.0, 4.5)
Rha H-1‴			4.95 (d, J = 1.5)
H-2‴			3.90 (dd, J = 3.0, 1.5)
H-3‴			3.68 (dd, J = 9.5, 3.0)
H-4‴			3.60 (t, J = 9.5)
H-5‴			4.05 (m)
H-6‴			1.28 (d, J = 6.5)

[a] Assignments confirmed by HOHAHA, COSY-90, and HETCOR experiments. [b] ^{1}H−^{1}H coupling constants in the sugar spin−spin were measured from COSY and HOHAHA NMR spectra in Hz. [c] Glc = glucose, Rha = rhamnose. so, signals obscured by signals of CD_3OD.

543 $[(M - H) - (162 + 162 + 146)]^-$ due to the loss of two hexose units.

When **2** is used as a reference compound in the spectral analysis of compound **4**, close similarities are observed between the two molecules in spectral data of rings A−D, while the side chain and oligosaccharide moiety provide the points of difference. Main differences were, in the ^{1}H NMR spectrum, the chemical shifts of H_2-23 (δ 2.12 and 1.74 in **2** versus δ 2.79 and 2.69 in **4**) and Me-21 (δ 1.24 in **2** versus δ 1.36 in **4**) and the absence of the signal H-22; in the ^{13}C NMR spectrum, differences were observed for C-20, C-21, C-22, and C-23 (Table 3). Analysis of the observed shifts and comparison with some norcucurbitacin models from literature (Achenbach et al., 1993) promped us to established the aglycon of compound **4** as Fevicordin B, isolated from *Fevillaea cordifolia*. Glycosidation at C-2 was indicated by comparison of the carbon resonances observed in the ^{13}C NMR spectrum of **4** with those of compounds **1**−**3** and literature data (Matos et al., 1991). Anhydrous acid methanolysis of **4** gave methyl glucoside and methyl rhamnoside in a 2:1 ratio.

The structure of the oligosaccharide chain was achieved by 2D NMR spectroscopy. Even at high field (500 MHz) the 1D sugar spectral region of **4** was complex as most of the shifts were found between δ 4.95 and 3.10 and overlapped by aglycon signals. 2D-HOHAHA experiments allowed resolution of the overlapped spectra of oligosaccharide into a subset of inividual monosaccharide spectra. As in the HOHAHA method, the cross-peak represents both direct and relayed connectivities; we also recorded a 2D COSY-90 spectrum which established the proton sequence allowing the complete sequential assignments for all proton resonances starting from the well-isolated anomeric proton signals (Table 2) (Agrawal, 1992).

HETCOR experiment which correlated all proton resonances with those of each corresponding carbon permitted assignments of the interglycosidic linkage by comparison of the carbon chemical shifts observed with those of the corresponding methyl pyranosides and taking into account the known effect of glycosidation. The absence of any ^{13}C glycosidation shift for the rhamnopyranosyl residue suggested this sugar to be the terminal unit, while glycosidation shift on C-4 (∼7 ppm) of glucose II and on C-6 (∼6 ppm) of glucose I allowed to established C-4- and C-6-glycosylated glucopyranosyl units. These data left two possible sequences for the triglycoside chain of compound **4**: 25-acetoxy-2-[(4-*O*-α-L-rhamnopyranosyl-6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-3,16α,20-trihydroxy-29-norcucurbita-1,3,5(10)-triene-11,22-dione or 25-acetoxy-2-[(6-*O*-α-L-rhamnopyranosyl-4-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-3,16α,20-trihydroxy-29-norcucurbita-1,3,5-(10)-triene-11,22-dione.

A COLOC experiment allowed us to differentiate between the two proposed structures. Key correlation peaks were obtained between anomeric proton of glucose I (δ 4.65, 1H, d, J = 7.5 Hz) and resonance of C-2 (144.80 ppm) and between anomeric proton of rhamnose (δ 4.95, 1H, d, J = 1.5 Hz) and resonance of C-4 of glucose II (79.00 ppm) (Nakano et al., 1994). 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 glucopyranosyl units (J_{H1-H2} = 7.5 Hz) and the α-configuration for the rhamnopyranosyl unit (J_{H1-H2} = 1.5 Hz) (Table 2). Thus compound **4** is 25-acetoxy-2-[(4-*O*-α-L-rhamnopyranosyl-6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-3,16α,20-trihydroxy-29-norcucurbita-1,3,5-(10)-triene-11,22-dione.

The FABMS of compound **5** ($C_{42}H_{70}O_{16}$) showed a quasi molecular anion $[(M - H)]^-$ at *m*/*z* 829 and peaks due to the fragmentation of the oligosaccharide portion at *m*/*z* 667 $[(M - H) - 162)]^-$ and 505 $[(M - H) - (162 \times 2)]^-$. The ^{13}C and DEPT−^{13}C NMR spectra showed 42 signals, of which 12 were assigned to the saccharide portion and 30 to a triterpenic moiety. Comparison of spectral data of compound **5** with those of **1** suggested the identical sugar portion. In fact the ^{1}H and ^{13}C NMR spectra (Table 3 and experimental) confirmed the presence of the *β*-D-gentobiosyl residue (Achenbach et al., 1993).

The ^{1}H NMR spectrum displayed resonances for eight methyls (δ 0.95−1.46), three of which are linked to oxygenated carbons (Table 1). In addition it was possible to observe an olefinic proton signal at δ 5.61 (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,

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

	δC					
carbon	**1**	**2**	**3**	**4**	**5**	**6**
1	114.00	113.00	113.50	113.20	22.40	23.00
2	146.00	145.70	145.60	144.80	28.90	28.50
3	144.60	144.70	144.50	144.50	87.50	87.10
4	125.00	124.90	124.80	124.60	40.60	40.00
5	131.50	131.20	132.00	130.30	142.00	142.10
6	24.90	25.00	24.80	24.70	119.50	120.00
7	20.00	20.10	20.30	20.31	24.80	25.10
8	43.70	44.00	43.71	44.00	44.50	44.50
9	51.50	52.00	51.60	52.00	50.30	50.30
10	129.00	129.70	129.50	129.90	36.60	36.80
11	218.00	217.30	217.00	218.00	216.80	217.00
12	52.50	52.10	52.00	51.90	49.80	50.00
13	51.70	51.80	51.80	51.30	49.20	49.30
14	49.70	50.00	49.50	49.80	51.90	51.90
15	45.80	45.50	45.40	46.00	46.70	46.80
16	71.90	72.00	71.80	71.30	71.60	71.70
17	57.00	56.50	57.00	60.10	58.20	58.00
18	19.20	19.00	19.10	20.50	20.40	20.40
19	29.90	29.00	29.80	29.00	20.40	20.40
20	80.00	79.90	80.00	82.90	80.00	81.10
21	25.60	25.20	26.00	27.40	25.90	25.90
22	79.00	79.00	78.80	216.00	78.80	78.90
23	28.00	27.80	27.80	32.76	28.20	28.00
24	39.40	37.40	37.00	35.70	39.50	37.10
25	72.10	83.10	82.50	83.20	72.00	83.10
26	29.00	25.10	25.00	26.20	29.20	25.00
27	29.00	26.40	26.50	26.40	29.90	26.00
28	11.80	12.00	11.80	11.40	28.50	28.80
29					25.60	25.50
30	19.70	20.00	19.70	20.10	19.60	19.70
CH$_3$CO		21.86	21.90	22.00		22.00
CH$_3$*CO*		172.00	172.00	172.30		173.00
1′	105.30	105.00	104.80	104.00	105.00	104.60
2′	74.80	75.00	74.30	74.20	74.80	74.40
3′	77.70	77.80	78.20	78.00	77.70	78.20
4′	70.60	70.60	71.00	71.80	70.70	71.70
5′	76.80	77.20	78.00	77.50	77.10	77.50
6′	69.30	69.10	62.50	69.30	69.00	62.30
1″	104.00	104.10		104.00	104.30	
2″	74.50	74.70		74.80	74.70	
3″	77.90	78.00		77.50	77.80	
4″	71.20	71.00		79.00	71.20	
5″	78.00	78.10		77.50	77.90	
6″	62.40	62.50		62.50	62.40	
1‴				102.00		
2‴				72.80		
3‴				72.20		
4‴				75.00		
5‴				69.00		
6‴				18.00		

[a] Assignments confirmed by DEPT-^{13}C NMR, COSY-90, and HETCOR experiments.

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 COSY experiments. Two proton signals at δ 3.20 (1H, d, J = 15 Hz, Ha-12) and 2.70 (1H, d, J = 15 Hz, Hb-12) were diagnostic for 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). The signal in the ^1H 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 (Table 3) and literature data (Lyndon et al., 1989).

The ^{13}C NMR and DEPT−^{13}C NMR spectra (Table 3) showed, together with the resonances ascribable to two olefinic carbons (142.00 ppm, C-5; 119.50 ppm, C-6) and one carbonyl carbon (216.80 ppm C-11), signals indicating the occurrence of three tertiary hydroxyl groups (87.50, 71.60, and 78.00 ppm) and two quaternary hydroxyl groups (81.00 and 72.00 ppm) that were

Table 4. Selected Data from ROESY Experiments of 5 in CD_3OD

connectivities observed between δH	
H-proton	ROESY H-proton
3.46 (H-3)	1.06 (Me-28)
1.88 (H-8)	0.95 (Me-18), 1.07 (Me-19)
0.95 (Me-18)	4.42 (H-16)
1.31 (Me-30)	2.60 (H-17), 2.46 (H-7)
2.60 (H-17)	1.24 (Me-21)

[a] The experiments were optimized for dipolar couplings with mixing times of 300 ms.

located by 2D NMR spectroscopy. Thus the HETCOR spectrum shows that the signal at 58.20 (C-17) bears the hydrogen which resonated at δ 2.60, and from the COSY spectrum it can be seen that this proton couples to the other at δ 4.42, which from the HETCOR spectrum is attached to a carbon (C-16) at 71.60 ppm bearing a hydroxy group. The proton at δ 4.42 also coupled to the hydrogens at δ 2.00 and 1.78, which HETCOR reveals to be the methylenic group at C-15 (46.70 ppm). 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 side chain (C-22, C-23, C-24). The building up of the whole basic carbon skeleton from the above subunits was achieved on the basis of a series of data arising from 2D $^1H-^{13}C$ NMR heteronuclear correlations via both ^1J (HETCOR) and J long-range (COLOC) which also allowed to assign all the resonances in the ^{13}C NMR spectrum to the pertinent carbons (Table 3).

In fact the COLOC spectrum of **5** confirms the position of a double bond showing correlations between the chemical shift of H-6 and chemical shifts of C-4 and C-10 and the hydroxylation at C-22 showing correlations between the resonance of C-22 (78.80 ppm) and resonances of H-24a (δ 1.74) and Me-21 (δ 1.24). Similar interactions between the methylene protons at C-23 (δ 2.10 and 1.70) and the C-25 quaternary carbon (72.00 ppm), as well as between the H-26 (δ 1.36) and H-27 (δ 1.39) methyl protons and C-25, led us to locate at C-25 a quaternary hydroxyl group.

The relative stereochemistry was established taking into account the J values for H of the rings A−D and from a series of interproton contacts observed in the 2D ROESY experiment (Table 4). From all these data, it follows that the aglycon **5** is the never previously reported 16α,20,22,25-tetrahydroxycucurbit-5-en-11-one.

The attachment of the glycosidic chain at C-3 was at first deduced by significant downfield shift (87.50 ppm) observed for this carbon resonance in **5**, relative to the corresponding signal in model compounds (Lyndon et al., 1989), and subsequently confirmed by 2D NMR experiments. These observations led to the formulation of **5** as 3β-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-16α,20,22,25-tetrahydroxycucurbita-5-en-11-one.

The FABMS spectrum of compound **6** ($C_{38}H_{62}O_{12}$) gave a quasi molecular anion peak at m/z 709 $[(M - H)]^-$ and fragments at m/z 547 $[(M - H) - 162]^-$ and 531 $[(M - H) - 178]^-$ corresponding to the loss of a hexose unit with or without the glycosidic oxygen. On anhydrous acid methanolysis, **6** gave methyl glucoside.

The NMR spectra of **6** exhibited all resonances of unsubstituted β-D-glucopyranose (Stuppner et al., 1993) and the basic tetracyclic ring system of the compound **5**, but the spectra significantly differed in the signals of the side chain at C-17. In the ^{13}C NMR spectra (Table

3) on going from **5** to **6**, the signals due to C-24, C-26, and C-27 of the side chain were displaced upfield and the signal to C-25 downfield while other signals remained almost unshifted. In the ^{1}H NMR spectrum of **6**, a signal at δ 1.92 (3H, s) and ^{13}C NMR signals at 22.00 and 173.0 ppm indicated the presence of an acetyl group. These data indicated that an acetyl group was located at C-25 of the side chain. Thus the structure of **6** was concluded to be 3-β-(6-*O*-β-D-glucopyranosyloxy)-25-acetoxy-16α,20,22-trihydroxycucurbit-5-en-11-one.

Compounds **1**−**4** and **7** represent new natural products belonging to the rare class of 29-norcucurbitacins, while new compounds **5** and **6** belong to the class of cucurbitacins. The isolation of both 29-norcucurbitacins and cucurbitacins from *C. pedata* could be useful to confirm cucurbitacins as biogenetic precursors of the 29-norcucurbitacins. The presence of cucurbitacins has been reported frequently in fruit of Cucurbitaceae, and variation in the amount present in different cultivars is one of the criteria for selection of this fruit, since these compounds are responsible for the bitter taste of some of the cultivars.

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