# Studies on the Constituents of *Amaranthus caudatus* Leaves: Isolation and Structure Elucidation of New Triterpenoid Saponins and Ionol-Derived Glycosides

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The isolation of seven triterpenoid saponins (**1**−**7**) is reported, among them three new natural products (**1**−**3**), together with three new ionol-derived glycosides (**8**−**10**) from the MeOH extract of the leaves of *Amaranthus caudatus* (Amaranthaceae). All structures were elucidated by spectroscopic methods, including the concerted application of one-dimensional ($^1$H, TOCSY, $^{13}$C, and $^{13}$C DEPT NMR) and two-dimensional NMR techniques (DQF−COSY, HSQC, HMBC, ROESY). A comparative study of seeds and leaves has been also carried out.

**Keywords:** *Amaranthus caudatus; Amaranthaceae; leaves; food source; triterpenoid saponins; ionol glycosides; $^1$H and $^{13}$C NMR analysis*

## INTRODUCTION

"Kiwicha" (*Amaranthus caudatus*) is a pseudograin that formed a major part of the diet of the Incas. Unlike other Andean crops such as beans, maize, and potato, kiwicha has not been cultivated in recent years on a wide scale in other countries. Recently, because of the excellent nutritional value of seed meals and leaves, there has been a renewed interest in this crop. This interest is due partly because of its high (14−18%) protein content and balanced amino acid composition and partly because of the tolerance of the plant to a wide range of unfavorable climatic conditions (Cheeke et al., 1981). However, kiwicha contains significant levels of saponins, which are potential antinutrients. Saponins are toxic compounds that protect the crop against attack by birds and, probably, other pests. The membranolytic activity of saponins has been appreciated for some time and has been exploited in the assay of such compounds by hemolysis of red blood cells. More recently, it has been demonstrated that saponins can damage intestinal mucosal cells by altering cell membrane permeability and interfering with active transport, an effect that is dependent upon the structure of the individual saponin molecule (Gee et al., 1993). The consequences of prolonged consumption of saponins are unknown, but it is possible that episodes of membranolytic activity might increase the uptake of antigens by the small intestine. Therefore, the presence of saponins in the seeds and leaves of *A. caudatus* could decrease the nutritional quality of this good plant.

In a previous paper (Rastrelli et al., 1995), we reported the isolation and the structure determination of seven new triterpene glycosides from the seeds of *A. caudatus*. We have now investigated the leaves of this plant to determine their saponin content. Here, we report the isolation and structural elucidation of seven triterpene saponins (**1**−**7**), among them three new natural products (**1**−**3**), together with three new ionol-derived glycosides (**8**−**10**), which are aroma compounds of the norisoprenoid class widely distributed in fruits and vegetables (Williams et al., 1993). The formation of these compounds has been attributed to the degradation of higher molecular weight terpenoids such as carotenoids during the curing and aging process (Wahlberg et al., 1977).

## EXPERIMENTAL PROCEDURE

**Material.** The leaves of *A. caudatus* were supplied by ESPOCH, Escuela Politecnica del Chimborazo, Riobamba, Ecuador, and collected in Ecuador in July 1994. A voucher sample is deposited at the ESPOCH.

**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 2−6 kV energy in a Kratos MS 902 spectrometer equipped with a Kratos FAB source.

A Bruker DRX-600 spectrometer, operating at 599.19 MHz for $^1$H and 150.858 for $^{13}$C, using the UXNMR software package was used for NMR experiments in $CD_3OD$. The DEPT (distortionless enhancement by polarization transfer) experiments were performed using 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. ROESY (Bax and Davis, 1985), $^1$H−$^1$H DQF−COSY (double quantum filtered COSY) (Boden-

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hausen and Ruben, 1986; Homans, 1990), $^1H-^{13}C$ HSQC, and HMBC (Martin and Crouch, 1991) experiments were obtained using the conventional pulse sequences as described in the literature, and 1D TOCSY (Davis and Bax, 1985) were acquired using waveform generator-based GAUSS-shaped pulse, mixing times ranging from 80 to 100 ms, and a MLEV-17 spin-lock field of 10 kHz preceded by a 2 ms trim pulse.

HPLC separations were performed on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector and with a Waters $\mu$-Bondapak C-18 column and a U6K injector.

GC were run using a Hewlett-Packard 5890 gas chromatograph equipped with mass-selective detector MSD 5970 MS and a fused-silica column HP-5 (25 m × 0.2 mm i.d., 0.33 mm film).

**Extraction and Isolation.** The powdered, dried leaves (1 kg) were defatted with petroleum ether (4.3 g) and $CHCl_3$ (5.50 g) in a Soxhlet apparatus and extracted successively at room temperature with $CHCl_3$−MeOH (9:1) (4.60 g) and MeOH (14.5 g). Part of the MeOH extract (9 g) was partitioned between *n*-BuOH and $H_2O$ to afford an *n*-BuOH soluble portion (4.6 g) that was chromatographed on a Sephadex LH-20 column using MeOH as eluent. Fractions (9 mL) were collected and checked by TLC [silica gel plates in, *n*-BuOH−HOAc−$H_2O$ (60:15:25)]. Fractions 18−28 (1.4 g), containing the crude saponin mixture, were submitted to reversed-phase HPLC on a C-18 $\mu$-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) using MeOH−$H_2O$ (60:40) as the eluent to yield pure compounds **1** (25.0 mg, $t_R$ = 18 min), **2** (17.7 mg, $t_R$ = 20 min), **3** (20.8 mg, $t_R$ = 14 min), **4** (25.5 mg, $t_R$ = 10 min), **5** (27.8 mg, $t_R$ = 33 min), **6** (19.4 mg, $t_R$ = 23 min), and **7** (8.4 mg, $t_R$ = 25 min). Fractions 32−38 (450 mg), containing the ionol glycosidic mixture, were also separated by HPLC using MeOH−$H_2O$ (30:70) to yield pure compounds **8** (12.5 mg, $t_R$ = 10 min), **9** (17.8 mg, $t_R$ = 13 min), and **10** (9.4 mg, $t_R$ = 20 min).

**Acid Hydrolysis of Compounds 1−7, Glycosidic Constituents.** A solution of each compound (4 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 with those of the authentic silylated sugars.

**Compound 1:** $C_{53}H_{86}O_{25}N$; FABMS see Table 1; $^1H$ and $^{13}C$ NMR data see Tables 2 and 3.

**Compound 2:** $C_{48}H_{78}O_{21}$; FABMS see Table 1; $^1H$ and $^{13}C$ NMR data see Tables 2 and 3.

**Compound 3:** $C_{41}H_{62}O_{16}$; FABMS see Table 1; $^1H$ and $^{13}C$ NMR data see Tables 2 and 3.

**Compound 4:** $C_{35}H_{52}O_{11}$; FABMS see Table 1; $^1H$ NMR ($CD_3OD$) $\delta$ 0.84 (3H, s, Me-26), 1.22 (3H, s, Me-27), 1.31 (3H, s, Me-25), 1.42 (3H, s, Me-24), 3.62 (1H, d, $J$ = 3 Hz, H-3α), 4.12 (1H, ddd, $J$ = 4, 3, 3 Hz, H-2α), 5.40 (1H, m, H-12), 4.65 (2H, br s, =$CH_2$), 5.39 (1H, d, $J$ = 7.5 Hz, H-1 of glucose); $^{13}C$ NMR ($CD_3OD$) aglycon signals, $\delta$ 44.8 (C-1), 70.7 (C-2), 77.5 (C-3), 54.1 (C-4), 52.0 (C-5), 21.8 (C-6), 33.5 (C-7), 41.1 (C-8), 48.0 (C-9), 37.5 (C-10), 24.1 (C-11), 124.2 (C-12), 144.5 (C-13), 41.1 (C-14), 28.8 (C-15), 24.5 (C-16), 48.0 (C-17), 47.8 (C-18), 43.0 (C-19), 149.5 (C-20), 30.9 (C-21), 38.5 (C-22), 177.0 (C-23), 13.7 (C-24), 17.1 (C-25), 17.7 (C-26), 26.5 (C-27), 177.3 (C-28), 107.5 (C-29); sugar signals 95.8 (C-1′), 73.9 (C-2′), 78.7 (C-3′), 71.0 (C-4′), 78.3 (C-5′), 62.4 (C-6′).

**Compound 5:** $C_{42}H_{68}O_{13}$; FABMS see Table 1; $^1H$ NMR ($CD_3OD$) $\delta$ 0.84 (3H, s, Me-26), 0.88 (3H, s, Me-24), 0.95 (3H, s, Me-30), 0.97 (3H, s, Me-29), 0.99 (3H, s, Me-23), 1.11 (3H, s, Me-25), 1.19 (3H, s, Me-27), 2.89 (1H, dd, $J$ = 13.7, 5 Hz, H-18), 5.29 (1H, m, H-12), 4.46 (1H, d, $J$ = 7.5 Hz, H-1 glucose linked at C-3), 5.42 (1H, d, $J$ = 7.5 Hz, H-1 glucose linked at C-28); $^{13}C$ NMR ($CD_3OD$) aglycon signals, $\delta$ 39.6 (C-1), 26.7 (C-2), 90.7 (C-3), 40.6 (C-4), 56.7 (C-5), 19.3 (C-6), 33.1 (C-7), 40.1 (C-8), 48.0 (C-9), 37.7 (C-10), 24.4 (C-11), 123.6 (C-12), 144.8 (C-13), 42.6 (C-14), 28.2 (C-15), 26.2 (C-16), 47.8 (C-17), 42.3 (C-18), 47.2 (C-19), 31.5 (C-20), 34.7 (C-21), 33.2 (C-22),

**Table 1. FABMS Spectral Data for Compounds 1−10**

| compd | formula | spectral data |
|---|---|---|
| **1** | $C_{53}H_{86}O_{25}$ | 1122 $[M-H]^-$, 960 $[(M-H)-162]^-$, 944 $[(M-H)-178]^-$, 798 $[(M-H)-(162\times2)]^-$, 504 $[(M-H)-(162\times3)-132]^-$ |
| **2** | $C_{48}H_{78}O_{21}$ | 990 $[M-H]^-$, 828 $[(M-H)-162]^-$, 812 $[(M-H)-178]^-$, 666 $[(M-H)-(162\times2)]^-$, 504 $[(M-H)-(162\times3)]^-$ |
| **3** | $C_{41}H_{62}O_{16}$ | 810 $[M-H]^-$, 648 $[(M-H)-162]^-$, 632 $[(M-H)-178]^-$, 486 $[(M-H)-(162\times2)]^-$ |
| **4** | $C_{35}H_{52}O_{11}$ | 648 $[M-H]^-$, 486 $[(M-H)-162]^-$, 542 $[(M-H)-178]^-$ |
| **5** | $C_{42}H_{68}O_{13}$ | 779 $[M-H]^-$, 617 $[(M-H)-162]^-$, 601 $[(M-H)-178]^-$ |
| **6** | $C_{42}H_{68}O_{14}$ | 795 $[M-H]^-$, 633 $[(M-H)-162]^-$, 617 $[(M-H)-178]^-$ |
| **7** | $C_{42}H_{66}O_{25}$ | 826 $[M-H]^-$, 664 $[(M-H)-162]^-$, 648 $[(M-H)-178]^-$, 502 $[(M-H)-(162\times2)]^-$ |
| **8** | $C_{25}H_{44}O_{13}$ | 551 $[M-H]^-$, 389 $[(M-H)-162]^-$, 245 $[(M-H)-(162+144)]^-$ |
| **9** | $C_{19}H_{34}O_9$ | 405 $[M-H]^-$, 243 $[(M-H)-162]^-$, 227 $[(M-H)-178]^-$ |
| **10** | $C_{19}H_{30}O_8$ | 386 $[M-H]^-$, 224 $[(M-H)-162]^-$ |

**Table 2. $^1$H NMR and $^{13}$C NMR Assignment ($\delta_C$ in $CD_3OD$) of Aglycon Moieties of Compounds 1 and 3[a]**

| proton | 1 | 3 | carbon | 1 | 3 |
|---|---|---|---|---|---|
| Ha-1 | 2.04 dd (14.4, 2.5) | 2.02 m | 1 | 44.97 | 44.90 |
| Hb-1 | 1.15 dd (14.4, 4.0) | 1.12 m | 2 | 69.92 | 70.71 |
| H-2 | 4.03 ddd (4.0, 2.5, 4.5) | 4.01 ddd (4.0, 4.0, 4.9) | 3 | 84.31 | 86.50 |
| H-3 | 3.53 d (4.0) | 3.48 d (4.0) | 4 | 39.98 | 53.00 |
| H-5 | 1.49 | 1.69 | 5 | 51.00 | 53.71 |
| H-6 | 4.00 br m | 3.99 br m | 6 | 69.10 | 21.74 |
| Ha-7 | 1.62 | | 7 | 42.80 | 33.54 |
| Hb-7 | 1.35 | | 8 | 40.00 | 41.00 |
| H-9 | 1.59 | 1.59 | 9 | 47.92 | 48.02 |
| Ha-11 | 1.92 m | 1.94 m | 10 | 37.57 | 37.41 |
| Hb-11 | 2.06 ddd (12.0, 9.7, 3.5) | 2.04 ddd (16.0, 11.0, 3.0) | 11 | 24.73 | 24.12 |
| H-12 | 5.28 m | 5.38 m | 12 | 123.88 | 124.31 |
| Ha-15 | 1.15 | 1.14 | 13 | 144.62 | 144.21 |
| Hb-15 | 1.18 | 1.19 | 14 | 43.40 | 41.20 |
| Ha-16 | 1.74 | 1.72 | 15 | 29.00 | 28.81 |
| Hb-16 | 0.93 | 0.92 | 16 | 25.21 | 24.72 |
| H-18 | 2.90 dd (4.3, 13.7) | 2.60 dd (4.0, 13.0) | 17 | 48.00 | 48.00 |
| Ha-19 | 1.78 m | | 18 | 43.04 | 47.51 |
| Hb-19 | 1.18 m | | 19 | 47.41 | 42.51 |
| Ha-21 | 1.88 m | | 20 | 31.94 | 149.41 |
| Hb-21 | 1.43 m | | 21 | 35.15 | 38.40 |
| Ha-22 | 1.76 m | | 22 | 33.51 | 30.92 |
| Hb-22 | 1.32 m | | 23 | 67.00 | 177.01 |
| Ha-23 | 3.42 d (11.0) | | 24 | 14.51 | 13.71 |
| Hb-23 | 3.68 d (11.0) | | 25 | 17.12 | 17.12 |
| Me-24 | 0.85 s | 1.42 s | 26 | 17.99 | 17.71 |
| Me-25 | 1.30 s | 1.31 s | 27 | 26.48 | 26.40 |
| Me-26 | 0.83 s | 0.84 s | 28 | 178.21 | 177.32 |
| Me-27 | 1.17 s | 1.22 s | 29 | 33.51 | 107.41 |
| Me-29 | 0.94 s | 4.65 br s | 30 | 23.99 | |
| Me-30 | 0.96 s | | | | |

[a] Signals for compound **2** are superimposable on those of **1** at 0.02 ppm. Chemical shift values are in ppm and *J* values in Hz presented in parentheses. All signals were assigned by DQF-COSY, HSQC, and HMBC studies.

**Table 3. $^{13}$C NMR and $^1$H NMR Data[a] of Sugar Moiety of Compounds 1−3 in $CD_3OD$**

| | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| position[c] | $\delta_C$ | $\delta_H$ ($J_{HH}$ in Hz)[b] | $\delta_C$ | $\delta_H$ ($J_{HH}$ in Hz)[b] | $\delta_C$ | $\delta_H$ ($J_{HH}$ in Hz)[b] |
| C-3 Glc-1 | 103.73 | 4.76 d (7.5) | 103.73 | 4.70 d (7.5) | 104.08 | 4.72 d (7.5) |
| Glc-2 | 83.20 | 3.42 dd (9.5, 7.5) | 74.07 | 3.38 dd (9.5, 7.5) | 74.94 | 3.40 dd (9.5, 7.5) |
| Glc-3 | 77.48 | 3.60 t (9.5) | 78.27 | 3.55 t (9.5) | 78.01 | 3.58 t (9.5) |
| Glc-4 | 71.25 | 3.28 t (9.5) | 71.22 | 3.30 t (9.5) | 71.00 | 3.30 t (9.5) |
| Glc-5 | 77.48 | 3.24 m | 77.48 | 3.26 m | 78.22 | 3.24 m |
| Glc-6 | 68.00 | 3.71 dd (11.0, 5.0) | 69.03 | 3.72 dd (11.0, 5.0) | 62.54 | 3.62 dd (11.0, 5.0) |
| | | 3.90 dd (11.0, 3.0) | | 3.90 dd (11.0, 3.0) | | 3.86 dd (11.0, 3.0) |
| Ara-1 | 104.37 | 4.84 d (5.2) | | | | |
| Ara-2 | 74.40 | 3.88 dd (5.2, 8.5) | | | | |
| Ara-3 | 72.36 | 3.82 dd (8.5, 3.0) | | | | |
| Ara-4 | 69.52 | 3.80 m | | | | |
| Ara-5 | 65.71 | 3.45 dd (12.0, 3.0) | | | | |
| | | 3.95 dd (12.0, 2.0) | | | | |
| Glc-1 | 103.76 | 4.65 d (7.5) | 103.40 | 4.66 d (7.4) | | |
| Glc-2 | 73.90 | 3.30 dd (9.5, 7.5) | 74.10 | 3.32 dd (9.5, 7.6) | | |
| Glc-3 | 77.91 | 3.45 t (9.5) | 77.80 | 3.44 t (9.5) | | |
| Glc-4 | 71.25 | 3.28 t (9.5) | 71.00 | 3.28 t (9.5) | | |
| Glc-5 | 77.78 | 3.34 m | 77.68 | 3.33 m | | |
| Glc-6′ | 63.00 | 3.65 dd (11.0, 5.0) | 62.98 | 3.64 dd (11.0, 5.0) | | |
| | | 3.86 dd (11.0, 3.0) | | 3.86 dd (11.0, 3.0) | | |
| C-28 Glc-1 | 95.26 | 5.41 d (7.5) | 94.89 | 5.47 d (7.5) | 95.89 | 5.40 d (7.5) |
| Glc-2 | 73.38 | 3.32 dd (9.5, 7.5) | 73.98 | 3.30 dd (9.5, 7.5) | 73.98 | 3.31 dd (9.5, 7.5) |
| Glc-3 | 77.50 | 3.64 t (9.5) | 78.07 | 3.43 t (9.5) | 78.07 | 3.43 t (9.5) |
| Glc-4 | 71.00 | 3.71 t (9.5) | 70.98 | 3.57 t (9.5) | 70.98 | 3.59 t (9.5) |
| Glc-5 | 78.01 | 3.40 m | 78.33 | 3.37 m | 78.33 | 3.37 m |
| Glc-6 | 62.41 | 3.68 dd (12.2, 5.0) | 62.13 | 3.70 dd (12.2, 5.0) | 62.13 | 3.70 dd (12.2, 5.0) |
| | | 3.84 dd (12.2, 3.5) | | 3.85 dd (12.2, 3.5) | | 3.86 dd (12.2, 3.5) |

[a] Assignments confirmed by 1D TOCSY and 2D DQF-COSY, HSQC, HMBC experiments. [b] $^1$H−$^1$H coupling constants in the sugar spin−spin were measured from TOCSY and COSY spectra in Hz. [c] Glc = β-D-glucopyranosyl, Ara = α-L-arabinopyranosyl.

28.2 (C-23), 16.4 (C-24), 16.7 (C-25), 17.7 (C-26), 26.1 (C-27), 178.3 (C-28), 33.5 (C-29), 23.8 (C-30); sugar signals, Glu at C-3, δ 105.8 (C-1′), 73.8 (C-2′), 78.5 (C-3′), 71.6 (C-4′), 78.1 (C-5′), 62.4 (C-6′); Glu at C-28, δ 95.6 (C-1″), 74.9 (C-2″), 78.8 (C-3″), 71.4 (C-4″), 78.3 (C-5″), 62.6 (C-6″).

**Compound 6:** $C_{42}H_{68}O_{14}$; FABMS see Table 1; $^1$H NMR ($CD_3OD$) δ 0.94 (3H, s, Me-30), 1.01 (3H, s, Me-26), 1.04 (3H, s, Me-29), 1.18 (3H, s, Me-24), 1.20 (3H, s, Me-23), 1.28 (3H, s, Me-27), 1.34 (3H, s, Me-25), 2.89 (1H, dd, $J$ = 13.7, 5 Hz, H-18), 3.61 (1H, d, $J$ = 3 Hz, H-3α), 4.14 (1H, ddd, $J$ = 4, 3, 3 Hz, H-2α), 5.29 (1H, m, H-12), 4.44 (1H, d, $J$ = 7.5 Hz, H-1 glucose linked at C-3), 5.42 (1H, d, $J$ =7.5 Hz, H-1 glucose linked at C-28); $^{13}$C NMR ($CD_3OD$) aglycon signals, δ 44.6 (C-1), 70.1 (C-2), 91.2 (C-3), 39.3 (C-4), 56.7 (C-5), 19.3 (C-6), 33.1

**Table 4. $^1$H NMR and $^{13}$C NMR Assignment ($\delta_C$ in $CD_3OD$) of Compounds 8 and 10[a]**

| proton | **8**[b] | **10** | carbon | **8** | **10** |
|---|---|---|---|---|---|
| H-2a | 1.63 dd (14.0, 8.2) | 2.42 d (15.0) | 1 | 37.01 | 36.51 |
| H-2b | 1.57 dd (11.0, 4.5) | 2.22 d (15.07) | 2 | 41.31 | 50.12 |
| H-3 | 3.90 m | | 3 | 64.40 | 209.11 |
| H-4 | 1.26 dd (14.5, 4.5) | 4.52 s | 4 | 47.79 | 73.02 |
| | 2.29 dd (14.5, 6.5) | | 5 | 79.39 | 128.05 |
| H-7 | 5.95 d (15.5) | 6.06 d (15.5) | 6 | 67.43 | 135.00 |
| H-8 | 5.75 dd (15.5, 6.5) | 5.62 dd (15.5, 6.5) | 7 | 127.59 | 125.83 |
| H-9 | 4.44 q (6.5) | 4.15 q (6.0) | 8 | 137.00 | 138.89 |
| H-10 | 1.31 d (6.5) | 1.35 d (6.0) | 9 | 76.79 | 76.92 |
| H-11 | 1.15 s | 1.11 s | 10 | 20.89 | 21.22 |
| H-12 | 0.99 s | 1.05 s | 11 | 29.92 | 30.02 |
| H-13 | 1.22 s | 1.95 s | 12 | 24.91 | 28.21 |
| | | | 13 | 20.05 | 23.50 |
| Glu-1 | 4.38 d (7.5) | 4.36 d (7.5) | Glu-1 | 104.01 | 103.97 |
| Glu-2 | 3.28 dd (7.5, 9.5) | 3.26 dd (7.5, 9.5) | Glu-2 | 75.12 | 74.72 |
| Glu-3 | 3.43 t (9.5) | 3.42 t (9.5) | Glu-3 | 78.21 | 78.00 |
| Glu-4 | 3.34 t (9.5) | 3.34 t (9.5) | Glu-4 | 72.11 | 71.51 |
| Glu-5 | 3.30 m | 3.29 m | Glu-5 | 78.01 | 77.96 |
| Glu-6 | 3.64 dd (12.0, 5.1) | 3.62 dd (12.0, 5.1) | Glu-6 | 69.00 | 62.42 |
| | 3.98 dd (12.0, 3.2) | 4.01 dd (12.0, 3.2) | | | |
| Rha-1 | 5.20 d (1.5) | | Rha-1 | 102.28 | |
| Rha-2 | 3.94 dd (1.5, 3.0) | | Rha-2 | 72.31 | |
| Rha-3 | 3.82 dd (3.0, 9.0) | | Rha-3 | 72.51 | |
| Rha-4 | 3.40 m | | Rha-4 | 74.69 | |
| Rha-5 | 4.05 m | | Rha-5 | 69.81 | |
| Rha-6 | 1.28 d (6.5) | | Rha-6 | 18.20 | |

[a] Chemical shift values are in ppm and *J* values in Hz presented in parentheses. All signals were assigned by DQF-COSY, HSQC and HMBC studies. [b] Values of aglyon of compound **9** are almost superimposable to these of **8**.

(C-7), 39.5 (C-8), 47.5 (C-9), 37.7 (C-10), 24.4 (C-11), 123.4 (C-12), 144.7 (C-13), 42.7 (C-14), 28.4 (C-15), 25.2 (C-16), 47.6 (C-17), 42.3 (C-18), 46.2 (C-19), 31.5 (C-20), 34.7 (C-21), 33.6 (C-22), 28.9 (C-23), 16.4 (C-24), 17.4 (C-25), 17.6 (C-26), 26.1 (C-27), 178.3 (C-28), 33.2 (C-29), 23.7 (C-30); sugar signals, Glu at C-3, $\delta$ 105.8 (C-1′), 73.7 (C-2′), 78.4 (C-3′), 71.6 (C-4′), 78.0 (C-5′), 62.4 (C-6′); Glu at C-28, $\delta$ 95.6 (C-1″), 74.9 (C-2″), 78.8 (C-3″), 71.4 (C-4″), 78.3 (C-5″), 62.6 (C-6″).

**Compound 7:** $C_{42}H_{66}O_{16}$; FABMS see Table 1; $^1$H NMR ($CD_3OD$) $\delta$ 0.84 (3H, s, Me-26), 1.22 (3H, s, Me-27), 1.31 (3H, s, Me-25), 1.42 (3H, s, Me-24), 0.95 (3H, s, Me-30), 0.97 (3H, s, Me-29), 2.89 (1H, dd, $J$ = 13.7 and 5 Hz, H-18), 5.29 (1H, m, H-12), 4.46 (1H, d, $J$ = 7.5 Hz, H-1 glucose linked at C-3), 5.42 (1H, d, $J$ = 7.5 Hz, H-1 glucose linked at C-28); $^{13}$C NMR ($CD_3OD$) aglycon signal, $\delta$ 44.7 (C-1), 70.6 (C-2), 77.4 (C-3), 54.1 (C-4), 52.0 (C-5), 21.8 (C-6), 33.5 (C-7), 41.1 (C-8), 48.0 (C-9), 37.5 (C-10), 24.4 (C-11), 123.6 (C-12), 144.8 (C-13), 42.6 (C-14), 28.2 (C-15), 26.2 (C-16), 47.8 (C-17), 42.3 (C-18), 47.2 (C-19), 31.5 (C-20), 34.7 (C-21), 33.2 (C-22), 177.0 (C-23), 13.7 (C-24), 17.1 (C-25), 17.7 (C-26), 26.1 (C-27), 178.3 (C-28), 33.5 (C-29), 23.8 (C-30); sugar signals, Glu at C-3, $\delta$ 105.8 (C-1′), 73.9 (C-2′), 78.7 (C-3′), 71.8 (C-4′), 78.2 (C-5′), 62.6 (C-6′); Glu at C-28, $\delta$ 95.6 (C-1″), 74.9 (C-2″), 79.0 (C-3″), 71.5 (C-4″), 78.6 (C-5″), 62.6 (C-6″).

**Compound 8:** $C_{25}H_{44}O_{13}$; FABMS see Table 1; $^1$H and $^{13}$C NMR data see Table 4.

**Compound 9:** $C_{19}H_{34}O_9$; FABMS see Table 1; $^1$H and $^{13}$C NMR data see Table 4.

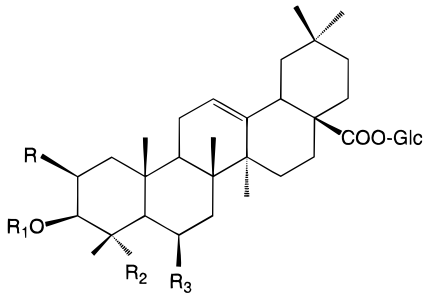
**Compound 10:** $C_{19}H_{30}O_8$; FABMS see Table 1; $^1$H and $^{13}$C NMR data see Table 4.

## RESULTS AND DISCUSSION

*A. caudatus* leaves 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 passage over Sephadex LH-20. The fractions containing the ionol and triterpenic glycosides checked by TLC were further purified by HPLC to obtain the glycosides **1**−**10** (Figures 1 and 2).

The structures and molecular formulas of compounds **1**−**10** were determined from their negative-ion FABMS spectra (Table 1) as well as 1D and 2D $^1$H and $^{13}$C NMR data.

Compound **1** showed an $[(M - H)]^-$ ion at *m/z* 1122 $[M - H]^-$ and prominent fragment ions at *m/z* 960 $[(M - H) - 162]^-$ and 944 $[(M - H) - 178]^-$, which were interpreted as the cleavage of a hexose unit without and with the glycosidic oxygen, respectively, and at *m/z* 798 $[(M - H) - (162 \times 2)]^-$, due to subsequent loss of a hexose unit. Another fragment at *m/z* 504 $[(M - H) - (162 \times 3) - 132]^-$, resulting from the cleavage of three hexose and a pentose units, was ascribable to the aglycon. The $^1$H NMR spectrum for the aglycon moiety of **1** exhibited six tertiary methyl group at $\delta$ 0.83, 0.85, 0.94, 0.96, 1.17, and 1.30 and three hydroxymethine signals at $\delta$ 4.03 (H-2α, ddd, $J$ = 4.0, 3.5, 4.5 Hz), $\delta$ 3.90 (H-6α, br m) and 3.53 (H-3α, d, $J$ = 4.0 Hz), and a $CH_2$-OH ($\delta$ 3.42 and 3.68, each d, $J$ = 11.0 Hz), suggesting a polyhydroxylated pentacyclic triterpene skeleton. The 30 $^{13}$C NMR resonances of the aglycon were easily identified by subtracting the sugar carbon resonances from the total spectrum of compound **1**. Some of them are characteristic for the $\Delta^{12}$ olean-28-oic acid skeleton (Rastrelli et al., 1995). Four more oxygenated carbons are present ($\delta$ 84.30, 69.92, 69.10, 67.00 ppm) that correlated, respectively, to the protons at $\delta$ 3.53, 4.03, 4.00, 3.42, and 3.68 by HSQC. The location of the −OH groups in the molecule has been made possible by the combination of the results of 2D DQF-COSY, HSQC, and HMBC that led to the assignment of the oxygenated positions as 2$\beta$, 3$\beta$, 6$\beta$, 23α (Table 2). The $\beta$-configuration of the C-2, C-3, and C-6 hydroxyl groups was determined by analysis of the coupling constants of the proton signals H-6 (br, m), H-3 (d, $J$ = 4.0 Hz), and H-2 (ddd, $J$ = 4.0, 2.5, 4.5 Hz) and the chemical shifts of the carbons. All the experiments were completely consistent with the proposed structure, 2$\beta$,3$\beta$,6$\beta$,23-tet-



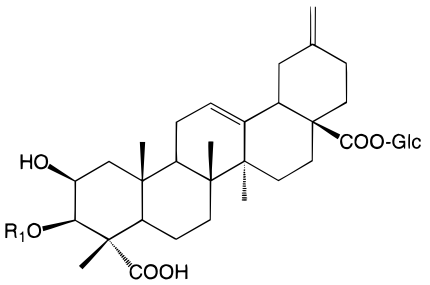

**1** R = OH, $R_1$ = Glc (2→ Ara; 6→ Glc), $R_2 = CH_2OH$, $R_3$ = OH

**2** R = OH, $R_1$ = Glc (6→ Glc), $R_2 = CH_2OH$, $R_3$ = OH

**5** R = H, R' = Glc, $R_2 = CH_3$, $R_3$ = H

**6** R = OH, R' = Glc, $R_2 = CH_3$, $R_3$ = H

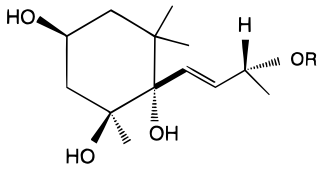
**7** R = OH, R' = Glc, $R_2$ = COOH, $R_3$ = H




**3** $R_1$ = Glc

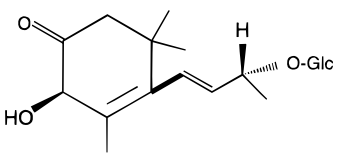
**4** $R_1$ = H

**Figure 1.** Structure of triterpene saponins **1**−**7** isolated from *A. caudatus* leaves.




**8** R = Glc (6→ Rha)

**9** R = Glc




**10**

**Figure 2.** Ionol glycosides **8**−**10** isolated from *A. caudatus* leaves.

rahydroxyolean-12-en-28-oic acid (protobassic acid) for the aglycon (Sahu et al., 1995).

Methanolysis and subsequent GLC analysis of the methylated sugars of compound **1** showed the presence of glucose and arabinose in a ratio of 3:1. Four anomeric protons were easily identified in the spectra of **1**. They resonated at $\delta$ 5.41 (d, $J$ = 7.5 Hz), 4.76 (d, $J$ = 7.5 Hz), 4.65 (d, $J$ = 7.3 Hz), and 4.84 (d, $J$ = 5.2 Hz) and correlated to carbons at 95.26, 103.73, 103.16, and 104.37 ppm, respectively. The first signals showed that one sugar residue must be linked to a carboxyl group by an ester bond. From the assigned aglycon and sugar values (Tables 2 and 3), it was apparent that a trisaccharide unit was attached to C-3 of the aglycon. The structure of the trisaccharide chain has been assigned by a combination of 1D TOCSY (Davis and Bax, 1985), 2D DFQ-COSY (Bodenhausen and Ruben, 1986), HSQC, and HMBC (Martin and Crouch, 1991) experiments. The isolated anomeric signals resonating at uncrowded regions of the spectrum between 5.41 and 4.64 ppm were the starting points for the 1D-TOCSY experiments. Because of the selectivity of multistep coherence transfer, the 1D-TOCSY subspectra of the single monosaccharide unit could be extracted from the overlapping region of the spectrum (between 3.0 and 4.0 ppm). Each subspectrum could be attributed to one set of coupled protons such as H−C(1) to H−C(4) for arabinose or H−C(6) for glucose of a carbohydrate moiety. Moreover, the 1D-TOCSY subspectra obtained irradiating at $\delta$ 4.03 and 4.00 recognized these protons as belonging to the triterpenoid skeleton as they showed set of coupled protons in the low-frequency region (above 2.04 ppm). The irradiation of the signal at $\delta$ 5.41 showed a set of coupled resonances inside a sugar ascribable from H-1 to H-6 of the third glucose unit linked at C-28 of the aglycon. 1D TOCSY subspectra obtained irradiating at $\delta$ 4.76 and 4.65 ppm allowed their easy identification as a first and a second glucopyranose units from their distinctive chemical shifts. The irradiation at 4.84 ppm showed connectivities to three methine groups (3.88, 3.82, and 3.80 ppm). The coherence transfer to H-5 was not obtained because of the small $J_{H4-H5}$ value of this arabinose unit. The sequential assignments of these sugar protons as shown in Table 2 derived from their distinctive DQF-COSY patterns. The assignments of all proton resonances for the sugar moieties immediately allowed the assignment of the resonances of the linked carbon atoms by HSQC (Table 3). The absence of any glycosylation shift for the carbon resonances of the second and third glucose units and of the arabinose suggested these sugars to be terminal. Glycosylation shifts (ca. 6.0 ppm) observed on C-2 (83.20 ppm) and C-6 (68.00 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 the magnitude in the $^1$H NMR spectrum as well as $^{13}$C NMR data (Table 3) indicated the $\beta$-configuration at the anomeric positions for the three glucopyranosyl units ($J_{H1-H2}$ = 7.5 Hz), while the α-configuration for the arabinopyranosyl unit ($J_{H1-H2}$ = 5.2 Hz) was established by the results of ROESY experiments as previously reported (De Tommasi et al., 1993).

These data left two possible sequences for the trisaccharide chain at C-3 of compound **1**: α-L-arabinopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranoside or α-L-arabinopyranosyl-(1→6)-[$\beta$-D-glucopyranosyl-(1→2)]-$\beta$-D-glucopyranoside. The HMBC spectrum allowed us to differentiate between the two proposed structures. Key correlation peaks were observed between the anomeric proton of the first glucose unit at 4.76 ppm and C-3 resonance (84.31 ppm) of the aglycon and between anomeric proton of the second

glucose unit (4.65 ppm) and C-6 of glucose (68.00 ppm). A correlation peak observed between the anomeric proton of the third glucose unit ($\delta$ 5.47) and C-28 (178.2 ppm) identified the $\beta$-D-glucopyranosyl unit linked to C-28 by an ester bond, while the three-membered oligosaccharidic chain was confirmed to be bonded to C-3 through an *O*-glycosidic linkage (Figure 1).

From these considerations, the structure 3-*O*-α-L-arabinopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-1→6]-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$,6$\beta$,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-$\beta$-D-glucopyranoside was assigned to **1**.

With **1** used as a reference compound in the spectral analysis of compound **2**, close similarities were observed between spectral data of the aglycon of both compounds, while their oligosaccharide moieties provided the points of difference (Tables 2 and 3). The main differences were the absence of the signals ascribable to the arabinose unit and the absence of the glycosylation shift on C-2 (83.20 ppm in **1** and 74.00 ppm in **2**) of the first glucose unit, suggesting the presence of a $\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyde chain linked at C-3 of the aglycone. Thus, **2** is 3-*O* -$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$,6$\beta$,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-$\beta$-D-glucopyranoside.

Compound **3** showed an $[(M - H)]^-$ ion at *m*/*z* 809 and prominent fragment ions at *m*/*z* 647 and 485 resulting from the cleavage of two hexose units. These were identified as glucose by methanolysis and GLC analysis of methyl sugar derivatives. The NMR data for the aglycon moiety suggested that **3** is a norolean-12-ene-type triterpene with two carboxyl groups ($^{13}$C NMR 177.0 and 177.3 ppm). The signals typically due to C-20 and the geminal dimethyl group of an oleane were displaced in two carbon signals at $\delta$ 149.4 (C=) and 107.4 ($CH_2$=), the last one correlated to proton signal at 4.65 (2H, br s) by HSQC experiments, indicating the presence of one *exo*-methylene group. This was located at C-20 (29) by comparison of the chemical shifts of ring E carbons with model compounds (Rastrelli et al., 1995; Kamiya et al., 1997). The $^1$H NMR spectrum showed two hydroxymethine signals at $\delta$ 4.01 (H-2α) and 3.58 (H-3α) correlated with carbinol carbon at 70.7 and 86.5 ppm by HSQC. The coupling constant ($J$ = 3.5 Hz) of these protons demonstrated that both hydroxyl groups have a $\beta$-disposition. In the proton spectrum of **3** were also present only four methyl signals (0.84, 1.22, 1.31, and 1.42), the last one at considerably high field, thus indicating the location of a −COOH group at C-23 or C-24 positions (Rastrelli et al., 1995). The $^{13}$C NMR resonances of the key carbons C-3, C-4, C-5, C-6, and C-24 demonstrated that **3** has a C-23 equatorial carboxyl group. In light of these observations, the aglycon of compound **3** was identified as 2$\beta$,3$\beta$-dihydroxy-30-noroleane-12,20(29)-diene-23,28-dioic acid previously isolated from the seeds of *A. caudatus* (Rastrelli et al., 1995). The presence of two sugars in **3** was confirmed from two anomeric proton signals at $\delta$ 4.73 and $\delta$ 5.40 (each 1H, d, $J$ = 7.5 Hz) correlated to carbon resonances at 104.08 and 95.8 ppm in the HSQC NMR spectrum, respectively. Interpretation of DQF-COSY, 1D TOCSY, and HSQC spectra led to the identification of two D-glucopyranoses in which the $\beta$-anomeric configuration was evident from the large $^1$H−$^1$H coupling constants ($J$ = 7.5 Hz). From the assigned aglycon resonances (Table 2), it was inferred that one unit was attached to C-3 (86.5 ppm) by an *O*-glycosidic bond and the other at C-28 (177.3 ppm) by an ester bond, whereas C-23 (177.0 ppm) was a free carboxyl group. Definitive evidence in the structure of compound **3** was derived by the HMBC spectrum, which clearly showed cross-peaks due to $J_{2-3}$ long-range couplings between H-1′ (4.73 ppm) and C-3 (86.5 ppm) and between H-1″ (5.40 and C-28 (177.3 ppm) of the aglycon. From all these data the structure of compound **3** was determined as 3-*O*-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$-dihydroxy-30-noroleane-12,20(29)-diene-23,28-dioic acid 28-*O*-$\beta$-D-glucopyranosyl ester.

Compound **8** has a molecular formula ($C_{25}H_{44}O_{13}$) as suggested by $^{13}$C, $^{13}$C-DEPT NMR, and FABMS analysis in the negative-ion mode, which showed a $[M - H]^-$ ion at *m*/*z* 551 and prominent fragments at *m*/*z* 389 $[(M - H) - 162)]^-$ due to the cleavage of a hexose unit and *m*/*z* 245 $[(M - H) - (162 + 144)]^-$ due to the subsequent loss of a deoxyhexose unit (Table 1). The $^{13}$C NMR and DEPT-$^{13}$C NMR data of **8** revealed 25 carbon signals, with 12 of them ascribable to two hexose units and 13 to an α-ionol aglycon (Table 4) (De Tommasi et al., 1996).

The carbon signals of **8** for the aglycon moiety were sorted by DEPT-$^{13}$C NMR into four methyl groups ($\delta_C$ 20.89, 29.92, 24.91, and 20.05), two $CH_2$ group ($\delta_C$ 41.31 and 47.79), two olefinic CH ($\delta_C$ 127.59 and 137.00), three quaternary carbons, two of which were hydroxylated ($\delta_C$ 37.01, 67.43, and 79.39), and two oxygenated CH groups ($\delta_C$ 64.40 and 76.79). The $^1$H NMR spectrum of **8** confirmed the presence of four methyl groups; two of which ($\delta_H$ 1.31 d, $J$ = 6.5 Hz, and 1.22 s) were shifted downfield by the presence of an oxygen atom. Further features were two methylene signals [$\delta_H$ 2.29 dd ($J$ = 4.5, 15.5 Hz) and 1.26 dd ($J$ = 14.5, 6.5 Hz); 1.63 dd ($J$ = 14.0, 8.2 Hz), 1.57 dd ($J$ = 14.0, 4.5 Hz)], two olefinic protons ($\delta_H$ 5.95 and 5.75), and two signals diagnostic for >CHOH groups (3.90 br m and 4.44, q, $J$ = 6.5 Hz). The DFQ-COSY spectrum of **8** indicated, for the aglycon moiety, two different spin systems corresponding to the $-CH_2CHOHCH_2$ and $CH_3CHOHCH{=}CH-$ sequences, the last one typical of the side chain of ionol derivatives (De Tommasi et al., 1996).

A HSQC experiment established the association of the protons with the corresponding carbons and led to the location of the three remaining quaternary carbons at C-5 and C-6 for the oxygenated ones and C-1 for the unoxygenated one. The stereochemistry at the C-3 chiral center was assigned in accordance with the magnitude of the H-3-$H_2$-2 and H-3-$H_2$-4 proton coupling constants and by NOE difference spectra: upon irradiation of the signals at $\delta$ 1.63 (H-2ax, $\beta$), a NOE was observed with signals at $\delta$ 1.15 (C-11 Me, $\beta$); upon irradiation of the signal at $\delta$ 1.22 (C-13Me,α), we observed an NOE with signals at both $\delta$ 1.57 (H-2eq α) and $\delta$ 2.29 (H-4α). The irradiation at $\delta$ 1.15 (C-11Me,$\beta$) resulted in an NOE enhancement of H-7 ($\delta$ 5.95). Moreover, no NOE was observed between H-3 ($\delta$ 3.90) and any other signals. The results of the NOE experiments led to the establishment of the $\beta$-orientation of the side chain at C-6, the $\beta$-orientation of OH at C-3, and the α-orientation of Me-13. The downfield shift ($\beta$-effect) observed for the C-9 resonance ($\delta_C$ 76.79) and the upfield shift ($\gamma$-effect) experienced by the C-10 resonance ($\delta_C$ 20.89), by comparison with model compounds, suggested the attachment of the disaccharide chain at C-9 of the aglycon moiety. The $^1$H and $^{13}$C NMR data of the sugar portion of **8** (Table 4), together with the results from GLC, led us to conclude that the disaccha-

ride chain was formed by one glucose and one rhamnose unit. The interglycosidic linkage was located at C-6′ of the glucose unit on the basis of the downfield shift exhibited by this carbon resonance ($\delta_C$ 69.00) by comparison with the shift in the unglycosylated models (De Tommasi et al., 1996). A 2D DQF-COSY experiment allowed the complete sequential assignment of all sugar proton resonances, starting from the well-isolated anomeric proton signals at $\delta_H$ 4.38 (H-1′ Glc) and 5.20 (H-1″ Rha). Chemical shifts, multiplicity of the signals, absolute values of the coupling constant, and the magnitude in the $^1$H NMR spectrum as well as $^{13}$C NMR data (Table 4) indicated the $\beta$-configuration at the anomeric positions for the glucopyranosyl unit ($J_{H1-H2}$ = 7.5 Hz) and the α-configuration for the rhamnopyranosyl unit ($J_{H1-H2}$ = 1.5 Hz) (Table 3) (De Tommasi et al., 1996).

The FABMS of compound **9** ($C_{19}H_{34}O_9$) gave a quasimolecular anion peak at *m/z* 405, and prominent fragments at *m/z* 243 $[(M - H) - 162)]^-$ and *m/z* 227 $[(M - H) - 178)]^-$ (cleavage of a hexose unit with or without the glycosidic oxygen), due to the loss of an hexose units. Comparison of NMR data of **9** with those of **8** indicated that both compounds have the same aglycon and compound **9** differed from compound **8** only in the absence of the terminal rhamnopyranosyl unit (C-6′ of glucose at $\delta_C$ 62.05 ppm).

On the basis of the foregoing data, the structures of compounds **8** and **9** are proposed as, in turn, 3,4,5-trihydroxydehydro-α-ionol-9-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**8**) and 3,4,5-trihydroxydehydro-α-ionol-9-*O*-*β*-D-glucopyranoside (**9**).

The composition of compound **10** was determined by FABMS spectrometry and $^{13}$C and DEPT-$^{13}$C NMR analysis as $C_{19}H_{30}O_8$. The $^{13}$C and $^1$H NMR spectra indicated that **10** was a glycoside of a *β*-ionol derivative (Perez et al., 1996). In the $^{13}$C NMR spectrum, 19 signals including six signals due to a *β*-D-glucopyranosyl moiety were observed. The resonances of the side-chain carbons and protons (from C-7 to C-10) remain nearly constant between **8** and **10**, and the glycosydation shift observed for C-9 was indicative of the linkage of glucose at this position. The chemical shifts of the carbon atoms of the *β*-ionol framework (from C-1 to C-6) indicated the presence of a methylene (50.12 ppm), a keto (209.11 ppm), and a carbinol (73.02 ppm) group as well as three quaternary carbons (two $sp^2$ at 128.05 and 135.00 ppm and one saturated at 36.51 ppm) and three methyls (30.02, 28.21, and 21.22 ppm). The $^1$H NMR spectrum exhibited the presence of a *gem*-dimethyl group ($\delta$ 1.11 and 1.05, each 3H, s), an olefinic Me signal ($\delta$ 1.95), an isolated methylene ($\delta$ 2.42 and 2.22, each d, $J$ = 15.5 Hz), and a carbinol hydrogen at $\delta$ 4.52 (1H, br s) α to a keto group. The absence of any further olefinic protons fixed the position of the double bond at C-5 (6). The relative position of the carbinol and keto groups were suggested by the chemical shifts and splitting pattern of H-4 and $H_2$-2 and by comparison of $^{13}$C resonances from C-1 to C-6 with those of *β*-ionol models (Rowan and Sykes, 1974; Miyase et al., 1988). Thus, compound **10** is 3-oxo-4-hydroxy-*β*-ionyl-9-*O*-*β*-D-glucopyranoside but the stereochemistry at C-4 was not defined.

The saponins **4**−**7** showed spectroscopic data identical with those of 2*β*,3*β*-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid 28-*O*-*β*-D-glucopyranosyl ester (**4**) isolated previously only from the seeds of *A. caudatus* (Rastrelli et al., 1995); oleanolic acid 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside (**5**); 2*β*-hydroxyoleanolic acid 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside (**6**); 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*-*β*-D-glucopyranosyl ester (**7**).

The saponins distribution among the organs of *A. caudatus* varies considerably. As reported in previous work (Rastrelli et al., 1995), seed saponins can be divided into four different groups containing 2*β*,3*β*-dihydroxyoleanolic acid, 2*β*,3*β*-dihydroxy-23-oxooleanolic acid, 2*β*,3*β*,6α-trihydroxy-23-oxooleanolic acid, and 2*β*,3*β*-dihydroxy-30-norolean-20(29)-ene-23,28-dioic acid as the aglycons. While saponins having 2*β*,3*β*-dihydroxyoleanolic acid (compounds **5** and **6**) and 2*β*,3*β*-dihydroxy-30-norolean-20(29)-ene-23,28-dioic acid (compounds **3** and **4**) as the aglycons were also detected in the leaves, aglycons of 3*β*-hydroxyoleane-23,28-dioic acid (compound **7**) and 2*β*,3*β*,6*β*,23-tetrahydroxyoleanolic acid (compounds **1** and **2**) were isolated for the first time from the leaves but were not found in the seeds.

Few *Amaranthus* species have been studied, and the occurrence of steroidal or triterpene saponins has already been reported, respectively, in *A. spinosus* (Banerji et al., 1980) and *A. hypocondriacus* (Kohda et al., 1991). It is interesting to observe that saponins both from *A. hypocondriacus* and *A. caudatus* possess common structural features, being 2*β*,3*β*-dihydroxyoleanolic acid or 2*β*,3*β*-dihydroxy-$\Delta^{20(29)}$-nor-oleanolic acid derivatives, but differ in the oxidation pattern at C-23 and C-6 as well as in the composition of the oligosaccharide chain at C-3. Aglycons characterized by the $-CH_3$ or $-CH_2OH$ or $-CHO$ group at C-23 have been previously described in *A. hypocondriacus*, whereas aglycons possessing a $-COOH$ group at C-23 and a $-OH$ group at C-6 are here reported for the first time in *A. caudatus*. Their isolation may be useful in chemotaxonomic studies.

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