# Studies on the Constituents of *Amaranthus caudatus* (Kiwicha) Seeds. Isolation and Characterization of Seven New Triterpene Saponins

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Seven new triterpenoid saponins were isolated from the seeds of *Amaranthus caudatus* (Amaranthaceae). Their structures were elucidated on the basis of spectral and chemical data to be (**1**) 3-$\beta$-*O*-α-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$-dihydroxyolean-12-en-28-oic acid 28-*O*-$\beta$-D-glucopyranosyl ester; (**2**) 3-$\beta$-*O*-α-L-rhamnopyranosyl-(1→2)-6-*O*-methyl-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$-dihydroxyolean-12-en-28-oic acid 28-*O*-$\beta$-D-glucopyranosyl ester; (**3**) 3-$\beta$-*O*-α-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-$\beta$-D-glucopyranosyl ester; (**4**) 3-$\beta$-*O*-α-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$,6α-trihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-$\beta$-D-glucopyranosyl ester; (**5**) 2$\beta$,3$\beta$-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid 28-*O*-$\beta$-D-glucopyranosyl ester; (**6**) 2$\beta$,3$\beta$-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid 23,28-di-*O*-$\beta$-D-glucopyranosyl ester; (**7**) 3-*O*-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid 23,28-di-*O*-$\beta$-D-glucopyranosyl ester.

**Keywords:** *Kiwicha; Amaranthus caudatus; Amaranthaceae; South American crop; food source; triterpene saponins; $^1$H and $^{13}$C NMR; FABMS*

## INTRODUCTION

The *Amaranthus* plants are spread throughout the world, growing under a wide range of climatic conditions, and they are known to infest or to produce useful feed and food products.

The amaranth is a plant able to produce grains and leafy edible vegetables appreciated in southwestern United States (Sauer, 1950), in Central and South America, in Nepal, and in the Himalayas (Teutonico et al., 1985). The nutritional quality of both seed meals and leaves, rich in minerals and vitamins, suggested to the U.S. National Science Foundation to identify amaranth as one of six plants with promising potential as new crops (Cheeke et al., 1981). The flour is used to make pancakes, fermented syrups, or vegetable milk, resembling soya milk in its nutritional value. The plant has a higher content in proteins than other cereals; it is also rich in lisine, sulfurated amino acids, and essential fatty acids. Moreover, the amaranth is cultivated as an ornamental plant giving good looking leaves and natural dyes. This work on kiwicha (*Amaranthus caudatus*) is a part of a series of studies on chemical constituents of alimentary plants from Peru (De Simone et al., 1990; Dini et al., 1991a,b, 1992). Kiwicha was tamed by Andean people prior to conquest by Spain, is still cultivated by local people, but has not yet been utilized as a widespread crop in the Old World, unlike other Andean plants such as maize, potato, and tomato that radically changed human diet. This paper deals with the isolation and structural elucidation of triterpene saponins, compounds influencing the taste of meals from whole seeds as bitter principles in other plants (Mizui et al., 1988).

## EXPERIMENTAL PROCEDURES

**Material.** The plant material was supplied by Ce Pe Ser (Central Peruviane de Servicios) collected in Ayakawa, Peru, in 1990. A voucher specimen is deposited in the Herbario de Museo de Historia Natural "J. Prado" Un. H. S. Lima (Peru).

**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 902 spectrometer equipped with a Kratos FAB source.

The NMR spectra were obtained in $CD_3OD$ using a Bruker WM-250 Spectrospin or Bruker AMX-500 spectrometer. 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. The HETCOR experiment was performed on a data matrix 512 × 1024, using a CH coupling of 135 Hz and relaxation delay 1.5 s. The data matrix was processed using a *q* sine window function.

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 HP-5 column (25 m × 0.2 mm i.d., 0.33 $\mu$m film).

HPLC separations were performed on a Hewlett-Packard HP 1050 series pumping system with a Varian RI-4 refractive index detector equipped with a Whatman Partisil 10 ODS-2 column.

**Extraction and Isolation.** The whole flour from the seeds (1.0 kg) was defatted with petroleum ether and $CHCl_3$ and then extracted with MeOH to give 41 g of residue. Part of the MeOH extract (15 g) was partitioned between *n*-BuOH and $H_2O$ to afford an *n*-BuOH-soluble portion (7.5 g) which was chromatographed on a Sephadex LH-20 column (100 × 5 cm) with MeOH as eluent. Fractions (9 mL) were collected and checked by TLC [Si gel plates in *n*-BuOH–HOAc–$H_2O$ (60:15:25)]. Fractions 20–27 (950 mg) containing the crude

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glycosidic mixture were submitted to HPLC using MeOH–$H_2O$ (70:30) (flow rate 3 mL/min) as eluent to give pure compounds **1** (26 mg, $t_R$ 10 min), **2** (28 mg, $t_R$ 11.5 min), **3** (51 mg, $t_R$ 8 min), **4** (12 mg, $t_R$ 7 min), **5** (20 mg, $t_R$ 8.5 min), **6** (18 mg, $t_R$ 6 min), **7** (31 mg, $t_R$ 6.5 min).

**Acid Hydrolysis of Compounds 1–7, Carbohydrate Constituents.** A solution of each compound (8 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.

**Alkaline Hydrolysis.** Glycosides **1**, **3**, **5**, and **7** (10 mg) were separately heated in 0.5 aqueous KOH (1 mL) at 110 °C in a stoppered reaction vial for 2 h. The reaction mixture was adjusted to pH 7 with HCl and then extracted with *n*-BuOH. The organic phase was evaporated to dryness, dissolved in $CD_3$-OD, and analyzed by $^1H$ and $^{13}C$ NMR.

**Compound 1:** $C_{48}H_{78}O_{18}$; FABMS, see Table 1; $^{13}C$, see Tables 2 and 3; $^1H$ NMR $\delta$ 0.94 (3H, s, Me-30), 1.04 (3H, s, Me-29), 1.06 (3H, s, Me-26), 1.20 (6H, d, sharp s, Me-23 and Me-24), 1.28 (3H, s, Me-27), 1.38 (3H, s, Me-25), 3.44 (1H, d, $J$ = 4 Hz, H-3α), 4.37 (1H, ddd, $J$ = 4, 3, 3 Hz, H-2α), 5.40 (1H, m, H-12), 1.37 (3H, d, $J$ = 6 Hz, Me-Rha), 4.51 (1H, d, $J$ = 7.5 Hz, H-1 of glucose linked at C-3), 5.33 (1H, s, H-1 of rhamnose) 5.51 (1H, d, $J$ = 7.5 Hz, H-1 of glucose linked at C-28).

**Compound 2:** $C_{49}H_{80}O_{18}$; FABMS, see Table 1; $^{13}C$, see Tables 2 and 3; $^1H$ NMR $\delta$ 0.94 (3H, s, Me-30), 1.04 (3H, s, Me-29), 1.06 (3H, s, Me-26), 1.20 (6H, d, sharp s, Me-23 and Me-24), 1.28 (3H, s, Me-27), 1.38 (3H, s, Me-25), 3.44 (1H, d, $J$ = 4 Hz, H-3α), 4.37 (1H, ddd, $J$ = 4, 3, 3 Hz, H-2α), 5.40 (1H, m, H-12), 1.36 (3H, d, $J$ = 6 Hz, Me-Rha), 4.47 (1H, d, $J$ = 7.5 Hz, H-1 of glucose linked at C-3), 5.33 (1H, s, H-1 of rhamnose) 5.51 (1H, d, $J$ = 7.5 Hz, H-1 of glucose linked at C-28).

**Compound 3:** $C_{48}H_{76}O_{19}$: FABMS, see Table 1; $^{13}C$, see Tables 2 and 3; $^1H$ NMR $\delta$ 0.94 (3H, s, Me-30), 1.04 (3H, s, Me-29), 1.06 (3H, s, Me-26), 1.30 (3H, s, Me-27), 1.43 (3H, s, Me-24), 1.45 (3H, s, Me-25), 4.46 (1H, ddd, $J$ = 4, 3, 3 Hz, H-2α), 5.40 (1H, m, H-12), 9.49 (1H, s, C*H*O), 1.36 (3H, d, $J$ = 6 Hz, Me-Rha), 4.41 (1H, d, $J$ = 7.5 Hz, H-1 of glucose linked at C-3), 5.29 (1H, s, H-1 of rhamnose), 5.51 (1H, d, $J$ = 7.5 Hz, H-1 of glucose linked at C-28).

**Compound 4:** $C_{48}H_{76}O_{20}$; FABMS, see Table 1; $^{13}C$, see Tables 2 and 3; $^1H$ NMR $\delta$ 0.94 (3H, s, Me-30), 1.04 (3H, s, Me-29), 1.06 (3H, s, Me-26), 1.41 (3H, s, Me-27), 1.43 (3H, s, Me-24), 1.44 (3H, s, Me-25), 4.46 (1H, ddd, $J$ = 4, 3, 3 Hz, H-2α), 4.05 (1H, ddd, $J$ = 10, 10, 3 Hz, H-6β), 5.40 (1H, m, H-12), 9.49 (1H, s, C*H*O), 1.36 (3H, d, $J$ = 6 Hz, Me-Rha), 4.41 (1H, d, $J$ = 7.5 Hz, H-1 of glucose linked at C-3), 5.30 (1H, s, H-1 of rhamnose) 5.50 (1H, d, $J$ = 7.5 Hz, H-1 of glucose linked at C-28).

**Compound 5:** $C_{35}H_{52}O_{11}$; FABMS, see Table 1; $^{13}C$, see Tables 2 and 3; $^1H$ NMR $\delta$ 0.84 (3H, s, Me-26), 1.22 (3H, s, Me-27), 1.31 (3H, s, Me-25), 1.42 (3H, s, Me-24), 3.63 (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, =$CH_2$), 5.40 (1H, d, $J$ = 7.7 Hz, H-1 of glucose).

**Compound 6:** $C_{41}H_{62}O_{16}$; FABMS, see Table 1; $^{13}C$, see Tables 2 and 3; $^1H$ NMR $\delta$ 0.84 (3H, s, Me-26), 1.22 (3H, s, Me-27), 1.31 (3H, s, Me-25), 1.42 (3H, s, Me-24), 3.63 (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, =$CH_2$), 5.40 (1H, d, $J$ = 7.7 Hz, H-1 of glucose linked at C-28), 5.42 (1H, d, $J$ = 7.5 Hz, H-1 glucose linked at C-23).

**Compound 7:** $C_{47}H_{72}O_{21}$; FABMS, see Table 1; $^{13}C$, see Tables 2 and 3; $^1H$ NMR $\delta$ 0.84 (3H, s, Me-26), 1.22 (3H, s, Me-27), 1.31 (3H, s, Me-25), 1.42 (3H, s, Me-24), 5.40 (1H, m, H-12), 4.65 (2H, br s, =$CH_2$), 4.40 (1H, d, $J$ = 7.5 Hz, H-1 glucose linked at C-3), 5.40 (1H, d, $J$ = 7.7 Hz, H-1 of glucose linked at C-28), 5.42 (1H, d, $J$ = 7.5 Hz, H-1 glucose linked at C-23).

## RESULTS AND DISCUSSION

*A. caudatus* grains were successively extracted with petroleum ether, $CHCl_3$, and MeOH. The MeOH extract was then partitioned into a mixture of *n*-BuOH and $H_2O$ to afford the *n*-BuOH-soluble portion, which was subjected to Sephadex LH-20.

The fractions containing the glycosidic mixture, checked by TLC, were further purified by HPLC to obtain the glycosides **1–7** (Chart 1). The structures and molecular formulae (see Experimental Procedures) were determined by negative ion FABMS spectra (Table 1) and $^1H$, $^{13}C$, $^{13}C$ DEPT NMR data (Tables 2 and 3).

Compound **1** showed an $[M - H]^-$ ion at *m/z* 941 and prominent fragments at *m/z* 779 [(M − H) − 162] and *m/z* 763 [(M − H) − 178] which were interpreted as the cleavage of a glucose moiety without and with the glycosidic oxygen. The FABMS of compound **2** showed an $[M - H]^-$ ion at *m/z* 955, which was 14 mass units higher than that of **1**. On acidic hydrolysis **1** afforded a sapogenin **8**, D-glucose and L-rhamnose in the ratio 2:1.

The $^1H$ NMR spectrum of **1** exhibited seven tertiary methyl groups at $\delta$ 0.94, 1.04, 1.06, 1.20 (6H), 1.28, and 1.38, one secondary methyl group at $\delta$ 1.37, and two hydroxymethine signals at $\delta$ 4.37 (H-2 α, ddd, $J$ = 4, 3, 3 Hz) and $\delta$ 3.44 (H-3 α, d, $J$ = 4 Hz). The location of the secondary OH groups at C-2 and C-3 was deduced by direct 2D-$^1H$,$^{13}C$ chemical shift cross correlations (HETCOR). The configuration of the C-2 and C-3 hydroxyl groups was determined to be $\beta$ from the coupling constants (Kojima et al., 1989). Moreover it was found that the $^1H$ and $^{13}C$ NMR signals of **8** were composed of those due to the carbons of the A ring of asterogenic acid (Nagao et al., 1989) and those due to the carbon B–E rings of oleanolic acid (Tori et al., 1974), leading to the identification of **8** as 2-$\beta$-hydroxyoleanolic acid. For the sugar moiety the $^1H$ and $^{13}C$ NMR spectra (Table 3) confirmed the presence of two glucose in the $\beta$-D-pyranosyl form and a rhamnose in the α-L-pyranosyl form (Kasai et al., 1979). The presence of a $\beta$-glucose unit linked at the aglycon by an ether bond in compound **1** was suggested by the $^1H$ NMR ($\delta$ 4.51, 1H, d, $J$ = 7.5 Hz, H-1′) and by the $^{13}C$ NMR ($\delta$ 105.3) anomeric signals. The presence in **1** of a further $\beta$-glucose unit linked at carboxyl group (C-28) of the aglycon was consistent with the $^1H$ NMR anomeric signal at $\delta$ 5.50 (1H, d, $J$ = 7.5 Hz, H-1″) and in full agreement with the observed carbon resonance of C-1″ at $\delta$ 95.1. The ether glycosidation site was shown to be at C-3 by $^{13}C$ NMR (Table 1) absorptions of C-3 (91.2 ppm), C-2 (70.1 ppm), and C-4 (39.3 ppm), which were in agreement with a model of 2-$\beta$-hydroxyoleanolic acid substituted at C-3 (Kohda et al., 1991). The presence of an α-L-rhamnose unit linked at a glucopyranosyl group was suggested by the $^1H$ NMR ($\delta$ 5.33, 1H, s, H-1‴) and by the $^{13}C$ NMR (102.1 ppm, C-1‴) anomeric signals. The $^{13}C$ NMR signal of C-2 of a glucopyranosyl group appeared at $\delta$ 82.9 as expected for a 2-O-substituted glucose; these spectral data indicated that the sugar moiety is an *O*-α-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranosyl unit.

It remained, therefore, to establish the relative sugars position. The alkaline hydrolysis of **1** yielded a compound, whose $^1H$ and $^{13}C$ NMR spectra showed no signals ascribable to a $\beta$-D-glucose in an ester linkage but exhibited the signals ascribable to a *O*-α-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranosyl virtually unshifted with respect to **1**.

**Chart 1**

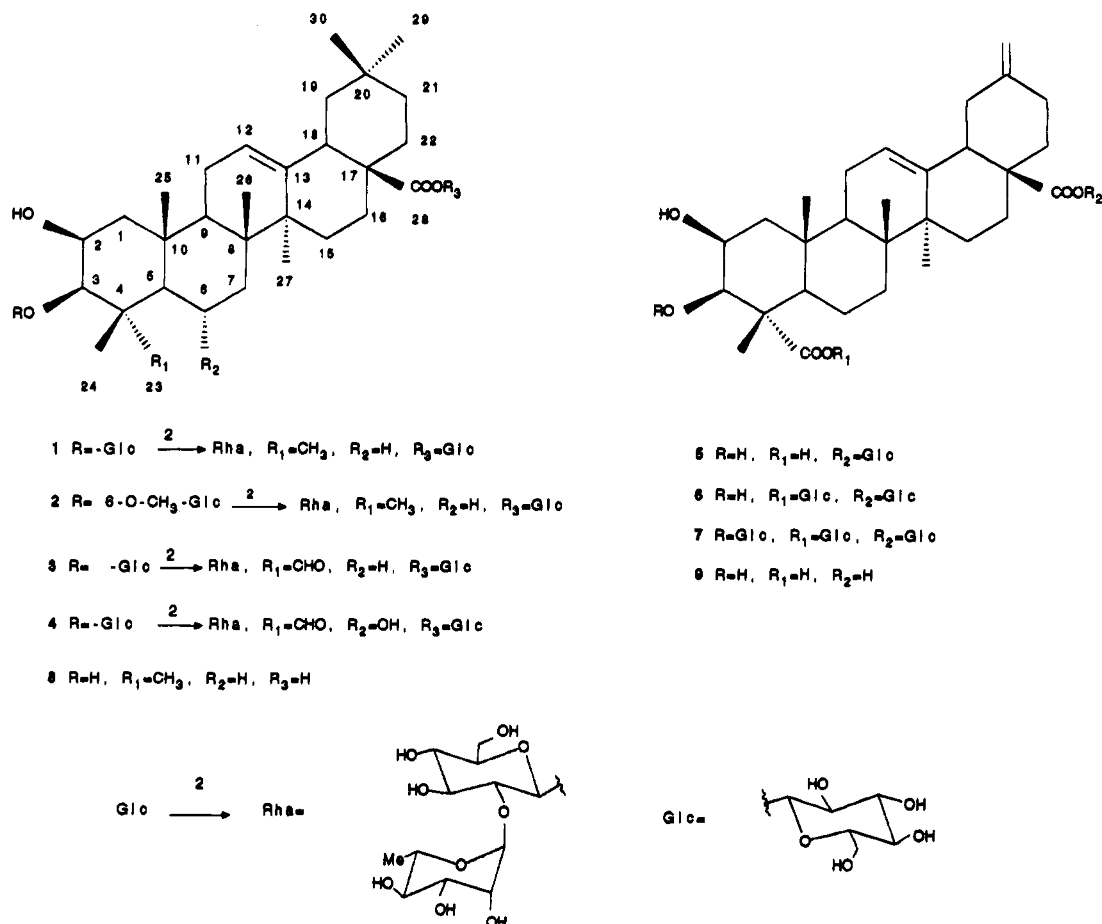


**Table 1. FABMS Spectral Data for Compounds 1–7**[a]

| | | |
|---|---|---|
| 1 | $C_{48}H_{78}O_{18}$ | 941 [M − H]⁻, 779 [(M − H) − 162]⁻, 763 [(M − H) − 178]⁻ |
| 2 | $C_{49}H_{80}O_{18}$ | 955 [M − H]⁻, 793 [(M − H) − 162]⁻, 777 [(M − H) − 178]⁻ |
| 3 | $C_{48}H_{76}O_{19}$ | 955 [M − H]⁻, 793 [(M − H) − 162]⁻, 777 [(M − H) − 178]⁻ |
| 4 | $C_{48}H_{76}O_{20}$ | 971 [M − H]⁻, 809 [(M − H) − 162]⁻, 793 [(M − H) − 178]⁻ |
| 5 | $C_{35}H_{52}O_{11}$ | 647 [M − H]⁻, 603 [(M − H) − COOH]⁻, 485 [(M − H) − 162]⁻, 469 [(M − H) − 178]⁻ |
| 6 | $C_{41}H_{62}O_{16}$ | 809 [M − H]⁻, 647 [(M − H) − 162]⁻, 631 [(M − H) − 178]⁻, 485 [(M − H) − (162 + 162)]⁻ |
| 7 | $C_{47}H_{72}O_{21}$ | 971 [M − H]⁻, 809 [(M − H) − 162]⁻, 793 [(M − H) − 178]⁻, 647 [(M − H) − (162 + 162)]⁻ |

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

From all these data the structure of **1** was concluded to be 3-β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-2β,3β-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester.

In the $^{13}C$ NMR spectra (Table 3), on going from **1** to **2**, the signals due to C′-6 and C′-5 of the glucose linked at the C-3 of the aglycon were displaced downfield by 6.1 ppm and upfield by 2.3 ppm, respectively, while other signals remained almost unshifted. In the $^{1}H$ NMR signal at δ 3.43 (3 H, s) and $^{13}C$ NMR signal at 54.6 ppm indicated the presence of a methoxyl group. These data indicated that a methyl group was located at the 6-hydroxy group of the glucosyl unit of the C-3 sugar moiety.

From these results the structure of **2** was established as 3-β-*O*-α-L-rhamnopyranosyl-(1→2)-6-*O*-methyl-β-D-glucopyranosyl-2β,3β-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester.

Compound **3** showed an [M − H]⁻ ion at *m/z* 955, indicating its molecular weight to be 956, and prominent fragments at *m/z* 793 [(M − H) − 162] and 777 [(M − H) − 178] which were interpreted as the cleavage of a glucose moiety without and with the glycosidic oxygen; on acidic hydrolysis **3** afforded D-glucose and L-rhamnose in the ratio 2:1. The $^{1}H$ NMR spectrum of **3** exhibited six tertiary methyl groups at δ 0.94, 1.04, 1.06, 1.30, 1.43, 1.45, one secondary methyl group at δ 1.36, two hydroxymethine signals, and one formyl group at δ 9.49. It also showed three anomeric proton signals at δ 4.41 (1 H, d, *J* = 7.5 Hz, H-1′), δ 5.29 (1 H, s, H-1‴), δ 5.51 (1 H, d, *J* = 7.5 Hz, H-1″). In the $^{13}C$ NMR spectrum, all of the carbon signals of **3** were almost superimposable on those of **1**, except for the appearance of one formyl group at 208.9 ppm and loss of one methyl group; C-2, C-3, C-5, C-10, and Me-24 signals were shifted upfield by 1.0, 7.5, 6.6, 0.6, and 5.1 ppm respectively, and C-4 and C-6 were shifted downfield by 16.2 and 2.1 ppm respectively. These results suggested that the formyl group of **3** is located at C-23. The sugar moiety of compound **3** exhibited a good coincidence with those of **1** in the $^{13}C$ NMR spectrum and this observation was also supported by result of acidic and alkaline hydrolysis.

Therefore, the structure of **3** was concluded to be 3-β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-2β,3β-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester.

Glycoside **4** showed an [M − H]⁻ ion at *m/z* 971, which was 16 mass units higher than that of **3**, and prominent fragments at *m/z* 809 [(M − H) − 162] and 793 [(M −

**Table 2. $^{13}C$ NMR Chemical Shift Assignments ($\delta$ in $CD_3OD$) of Aglycon Moieties of Compounds 1–7 and Related Compounds**

| carbon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 44.5 | 44.6 | 44.8 | 44.8 | 44.8 | 44.9 | 44.8 | 45.0 | 44.8 |
| 2 | 70.1 | 70.6 | 69.1 | 69.3 | 70.7 | 70.7 | 70.7 | 71.5 | 70.8 |
| 3 | 91.2 | 90.6 | 83.7 | 83.7 | 77.5 | 77.5 | 86.5 | 78.3 | 77.5 |
| 4 | 39.3[b] | 39.3[b] | 55.5 | 55.1 | 54.0 | 54.7 | 53.0 | 38.8 | 54.1 |
| 5 | 56.8 | 56.9 | 50.2 | 50.2 | 52.1 | 53.0 | 53.7 | 56.0 | 52.1 |
| 6 | 19.0 | 19.0 | 21.1 | 69.1 | 21.7 | 21.7 | 21.7 | 18.5 | 21.7 |
| 7 | 32.9 | 33.0 | 33.5 | 42.8 | 33.5 | 33.5 | 33.5 | 33.4 | 33.5 |
| 8 | 39.5[b] | 38.5[b] | 41.1 | 41.5 | 41.1 | 41.1 | 41.0 | 40.0 | 41.1 |
| 9 | 47.7 | 47.7 | 48.0[a] | 48.0[a] | 48.0[a] | 48.0[a] | 48.0[a] | 48.0[a] | 48.0[a] |
| 10 | 37.6 | 37.6 | 37.0 | 38.0 | 37.4 | 37.4 | 37.4 | 37.4 | 37.4 |
| 11 | 24.7[b] | 24.6[b] | 24.6[b] | 24.7[b] | 24.1 | 24.1 | 24.1 | 24.6[b] | 24.1 |
| 12 | 123.4 | 123.4 | 123.6 | 124.0 | 124.2 | 124.3 | 124.3 | 123.4 | 124.2 |
| 13 | 144.7 | 144.1 | 144.9 | 144.9 | 144.2 | 144.2 | 144.2 | 144.1 | 144.3 |
| 14 | 42.9 | 42.9 | 43.2 | 43.2 | 41.1 | 41.2 | 41.2 | 42.4 | 41.1 |
| 15 | 28.5 | 28.5 | 28.7 | 28.8 | 28.8 | 28.7 | 28.8 | 28.3 | 28.9 |
| 16 | 24.5[b] | 24.5[b] | 24.7[b] | 24.7[b] | 24.6 | 24.6 | 24.7 | 24.6[b] | 24.6 |
| 17 | 47.0 | 47.0 | 48.0[a] | 48.0[a] | 48.0[a] | 48.0[a] | 48.0[a] | 47.1 | 48.0[a] |
| 18 | 42.3 | 42.5 | 42.6 | 42.7 | 47.8 | 47.5 | 47.5 | 42.2 | 47.8 |
| 19 | 46.0 | 46.3 | 47.1 | 47.2 | 43.0 | 42.6 | 42.5 | 46.5 | 43.1 |
| 20 | 31.4 | 31.4 | 31.5 | 31.6 | 149.4 | 149.4 | 149.4 | 31.2 | 149.4 |
| 21 | 34.7 | 35.0 | 34.9 | 34.9 | 30.9 | 30.9 | 30.9 | 34.5 | 30.0 |
| 22 | 33.8 | 33.8 | 33.5 | 33.5 | 38.4 | 38.4 | 38.4 | 33.3 | 38.5 |
| 23 | 29.7 | 29.7 | 208.9 | 209.0 | 177.0 | 173.2 | 173.2 | 30.3 | 177.0 |
| 24 | 16.6 | 16.6 | 11.5 | 11.6 | 13.7 | 13.8 | 13.7 | 18.2 | 13.7 |
| 25 | 17.4 | 17.4 | 17.1 | 17.2 | 17.1 | 17.1 | 17.1 | 17.0 | 17.1 |
| 26 | 17.6 | 17.7 | 17.8 | 17.9 | 17.7 | 17.7 | 17.7 | 17.7 | 17.7 |
| 27 | 26.1 | 26.2 | 26.4 | 26.4 | 26.4 | 26.4 | 26.4 | 26.4 | 26.4 |
| 28 | 178.0 | 178.0 | 178.0 | 178.2 | 177.3 | 177.3 | 177.3 | 182.5 | 182.6 |
| 29 | 33.2 | 33.4 | 33.5 | 33.5 | 107.4 | 107.4 | 107.4 | 33.3 | 107.4 |
| 30 | 23.7 | 23.8 | 23.9 | 23.9 | | | | 23.8 | |

[a] Under methanol signal. [b] These assignments may be interchanged in each column.

**Table 3. $^{13}C$ NMR Chemical Shift Assignments ($\delta$ in $CD_3OD$) of Sugar Moieties of Compounds 1–7**

| carbon | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| | | | | C-3 Sugar | | | | |
| Glc | 1 | 105.8 | 106.4 | 104.2 | 104.2 | | | 104.9 |
| | 2 | 82.9 | 83.1 | 82.8 | 82.7 | | | 74.5 |
| | 3 | 78.4 | 78.6 | 78.1 | 78.0 | | | 78.6 |
| | 4 | 70.9 | 70.9 | 71.4 | 71.3 | | | 71.1 |
| | 5 | 78.1 | 75.8 | 78.4 | 78.4 | | | 78.2 |
| | 6 | 62.2 | 68.9 | 62.6 | 62.7 | | | 64.4 |
| | $OCH_3$ | | 54.7 | | | | | |
| Rha | 1 | 102.1 | 102.3 | 102.3 | | | | 102.3 |
| | 2 | 72.1 | 72.2 | 72.3 | | | | 72.3 |
| | 3 | 72.2 | 72.2 | 72.3 | | | | 72.3 |
| | 4 | 73.7 | 73.8 | 74.0 | | | | 74.1 |
| | 5 | 69.6 | 69.9 | 69.7 | | | | 69.6 |
| | 6 | 18.4 | 18.5 | 18.0 | | | | 18.2 |
| | | | | C-23 Sugar | | | | |
| Glc | 1 | | | | | | 95.4 | 95.4 |
| | 2 | | | | | | 73.5 | 73.9 |
| | 3 | | | | | | 78.6 | 78.6 |
| | 4 | | | | | | 71.1 | 71.0 |
| | 5 | | | | | | 78.2 | 78.4 |
| | 6 | | | | | | 62.2 | 62.4 |
| | | | | C-28 Sugar | | | | |
| Glc | 1 | 95.7 | 95.6 | 95.8 | 95.8 | 95.8 | 95.7 | 95.7 |
| | 2 | 73.9 | 74.1 | 74.2 | 74.1 | 73.9 | 73.9 | 73.5 |
| | 3 | 78.4 | 78.6 | 78.7 | 78.6 | 78.7 | 78.6 | 78.4 |
| | 4 | 70.9 | 71.0 | 71.4 | 71.4 | 71.1 | 71.1 | 71.0 |
| | 5 | 78.1 | 78.2 | 78.3 | 78.2 | 78.3 | 78.0 | 78.2 |
| | 6 | 62.8 | 62.4 | 62.4 | 62.3 | 62.4 | 62.4 | 62.6 |

H) − 178] which were interpreted as the cleavage of a glucose moiety without and with the glycosidic oxygen. In the $^{1}H$ NMR, we observed an extra hydroxymethine group at $\delta$ 4.05 (1H, ddd, $J$ = 10, 10, 3 Hz). The chemical shifts, the multiplicity, and the coupling constants value of signal were consistent for an axial proton at C-6. Moreover, we also observed that the signal of 27-$CH_3$ was displaced downfield by 0.11 ppm in comparison with the resonance of 27-$CH_3$ of **3**. In the $^{13}C$ NMR spectrum of **4** all of the carbon signals were almost superimposable on those of **3** except for the downfield shift exhibited by C-6 from 21.1 ($CH_2$ by DEPT) to 69.1 (CH by DEPT) ppm. All these data were in agreement with a secondary hydroxyl group located at 6α position, while a hydroxyl group at the C-6 $\beta$ position would have a strong deshielding effect on the 24-, 25-, and 26-$CH_3$ due to 1−3 diaxial interactions (Liang et al., 1990; Khan et al., 1975).

The structure of **4** was concluded to be 3-$\beta$-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-2$\beta$,3$\beta$,6$\alpha$-trihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-$\beta$-D-glucopyranosyl ester.

Compound **5** showed an $[M - H]^-$ ion at *m/z* 647 and prominent fragments at *m/z* 603 [(MH) − COOH], 485 [(M − H) − 162], and 469 [(M − H) − 178] which were interpreted as the cleavage of a glucose moiety without and with the oxygen. Acidic or alkaline hydrolysis of **5** yielded D-glucose and an aglycon identified as **9** from $^1$H and $^{13}$C NMR (Table 2). The $^{13}$C and $^{13}$C DEPT NMR spectra suggested that **5** is a noroleanane-type triterpenoid glycoside; in fact, carbon signals observed at $\delta$ 144.2 (C=), 124.2 (CH=), 149.4 (C=), and 107.4 (=$CH_2$) and proton signals at $\delta$ 4.65 (2H, s, C=$CH_2$) and 5.38 (1H, t, HC=C), indicated the presence of one trisubstituted double bond between C-12 and C-13 and one *exo*-methylene group present between C-20 and C-29. The $^1$H NMR spectrum also showed the presence of only four methyl signals at $\delta$ 0.84, 1.22, 1.31, and 1.42 and two hydroxymethine signals at $\delta$ 4.12 (H-2 $\alpha$, ddd, $J$ = 4, 3, 3 Hz) and $\delta$ 3.64 (H-3 $\alpha$, d, $J$ = 4 Hz). In the $^{13}$C NMR spectrum we also observed two carboxyl carbon signals at 177.0 and 177.3 ppm (Table 1). One of the COOH groups should be placed at the C-23 or C-24 positions, because in the $^1$H NMR the resonance of one methyl was shifted downfield from $\delta$ 1.20 in **8** to $\delta$ 1.42. The most significant features of the $^{13}$C NMR spectrum of **5**, which suggested the location of the carboxyl group at C-23, were the downfield shifts exhibited by C-4 (+15.2 ppm) and C-6 (+3.2 ppm) and the upfield shifts experienced by C-3 (−0.8 ppm), C-5 (−3.9 ppm), and Me-24 (−16 ppm) in comparison with those of **8**. Similar shifts were reported for arjunglucoside (Romussi et al., 1992), which has a 23-equatorial COOH group, when compared with 2$\beta$-hydroxyoleanolic acid.

These observations led to the formulation of **5** as 2$\beta$,3$\beta$-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid 28-*O*-$\beta$-D-glucopyranosyl ester.

Compound **6** showed an $[M - H]^-$ ion at *m/z* 809 and fragments at *m/z* 647 [(M − H) − 162], corresponding to the loss of a glucose unit without the oxygen, *m/z* 631 [(M − H) − 178], resulting from the cleavage of a glucose with the oxygen, and *m/z* 485, an aglycon ion [(M − H) − (162 + 162)] resulting from the cleavage of two glucose units. Acidic or alkaline hydrolysis of **6** yielded D-glucose and **9**. The $^{13}$C NMR resonances of **6**, in comparison with those of **5**, showed the presence of a further anomeric carbon signal ($\delta$ 95.4 ppm) in an ester linkage. The location at C-23 was supported by the spectral differences between **5** and **6** of the C-23 carboxyl group that was shifted upfield by 3.8 ppm from $\delta$ 177.0 to 173.2.

From these data the structure of **6** was concluded to be 2$\beta$,3$\beta$-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid 23,28-di-*O*-$\beta$-D-glucopyranosyl ester.

Compound **7** showed an $[M - H]^-$ ion at *m/z* 971 and prominent fragments at *m/z* 809 [(M − H) − 162] and 793 [(M − H) − 178] resulting from the cleavage of a glucose with and without the oxygen, and a fragment at *m/z* 647 [(M − H) − (162 + 162)] corresponding to the loss of a further glucose. Starting from the 809 and 647 peaks, the facile loss of a carboxyl group led to peaks at *m/z* 765 and 603 respectively (Table 1). Other fragment at *m/z* 485, resulting from the cleavage of a third glucose unit, was ascribable to aglycone and was also present in the spectra of **5**−**6**. In the $^{13}$C NMR spectra, on going from **6** to **7**, the signal due to C-3 was displaced downfield by 9 ppm. The $^1$H NMR of compound **7** showed a doublet at $\delta$ 4.43 (1H, d, $J$ = 7.5 Hz), correlated to $\delta$ 104.8 ppm in the $^{13}$C NMR spectrum, well adapted to an anomeric proton of a $\beta$-D-glucopyranosyl linked to C-3 of aglycon in an *O*-glycosydic bond. The other signals remained almost unshifted.

From all these data the structure of **7** was concluded to be 3-*O*-$\beta$-D-glycopyranosyl-2$\beta$,3$\beta$-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid 23,28-di-*O*-$\beta$-D-glucopyranosyl ester.

Saponins including such as aglycon 2$\beta$,3$\beta$-dihydroxyoleanolic acid and 2$\beta$,3$\beta$-dihydroxy-23-oxooleanolic acid have been isolated previously from *Amaranthus hypocondiacus* seeds (Kohda et al., 1991), the novelty of our compounds **1**−**3** resides in the combination of these aglycons with different sugar moieties, whereas the saponins **4** and **5**−**7** include respectively the new aglycon moieties 2$\beta$,3$\beta$,6$\alpha$-trihydroxy-23-oxooleanolic acid and 2$\beta$,3$\beta$-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid, which have never been isolated, as far as we know, from other natural sources.

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