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TRITERPENOID SAPONINS FROM THE SEEDS OF *AMARANTHUS CRUENTUS*

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Key Word Index—*Amaranthus cruentus*; Amaranthaceae; seeds; triterpenoid saponins.

Abstract—Four new saponins, 3-β-*O*-[α-L-rhamnopyranosyl(1 → 3)-β-glucuronopyranosyl]-2β,3β-dihydroxyolean-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl] ester, 3-β-*O*-[α-L-rhamnopyranosyl(1 → 3)-β-glucuronopyranosyl]-2β,3β,23-trihydroxyolean-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl] ester, 3-β-*O*-[α-rhamnopyranosyl(1 → 3)-β-glucuronopyranosyl]-2β,3β-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl] ester, 3-β-*O*-[β-glucuronopyranosyl]-2β,3β-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid 28-*O*-[β-D-glucopyranosyl] ester, together with known chondrillasterol (5α-stigmasta-7,22-dien-3β-ol) and its 3-*O*-glucopyranoside have been isolated from seeds of *Amaranthus cruentus*. The structures were established by LSI mass spectrometry and NMR spectroscopy. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Amaranth seeds have recently gained much attention as a "crop of the 21st century" due to their high nutritional quality [1]. No detailed research has been performed so far on the antinutritional principles that might be present in seeds. One of the groups of antinutrients are saponins, the presence of which, in amaranth seeds, has been mentioned in the literature. It is documented that seeds of *Amaranthus caudatus* and *A. hypocondiacus* contain saponins with a triterpene skeleton [2, 3]. No research has been performed on the saponins of *A. cruentus* which is one of the most often cultivated species. Thus, the aims of the present work were the isolation and structure elucidation of saponins from seeds of *A. cruentus* L.

RESULTS AND DISCUSSION

Extraction of amaranth seeds with CH_2Cl_2 gave an oil from which at low temperature a white substance, which on TLC showed two spots, was precipitated. This material on CC gave **1** and **2** (structures not shown). The EIMS spectrum of **2** contained a $[M]^+$ peak at *m/z* 412, which corresponded to molecular formula of $C_{29}H_{48}O$, a compound related to stigmasterol. Close examination of ^{13}C NMR shifts confirmed the compound to be chondrillasterol (5α-stigmasta-7,22-dien-3β-ol), which in a previous study had been isolated from *A. palmeri* aerial parts [4]. The second (**2**), more polar compound, showed a $[M]^+$ at 573 and a second ion *m/z* at 411 indicating the loss of one glucose unit from the parent molecule. Acidic hydrolysis afforded compound **1** and glucose. ^{13}C NMR confirmed the aglycone part of **2** was **1** and that a glucose unit was attached at the 3-*O* position. Thus, **2** was identified as the 3-*O*-glucopyranoside of chondrillasterol. To the best of our knowledge this compound has not previously been reported in amaranth seeds.

The methanolic extract of amaranth seeds provided a crude saponin mixture containing four compounds as shown by TLC. They were successfully separated by CC. LSI mass spectrometry of compounds **3**, **4** and **5** indicated very close structural relationship at least in the sugar substitution. Thus, compound **3** exhibited in negative ion mode LSIMS a peak at *m/z* 955 $[M-H]^-$. Negative fragmentations at *m/z* 793 and 809 corresponded to the loss of a terminal hexose and deoxyhexose, and an ion at *m/z* 647 reflected the loss of both terminal sugars. Additionally, an ion at *m/z* 471 corresponded to the aglycone moiety and was generated by the loss from the glycoside of hexose, deoxyhexose and uronic acid. Compound **5** exhibited a peak at *m/z* 969 $[M-H]^-$. Fragment ions at *m/z* 807

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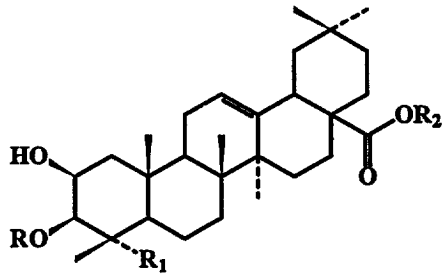
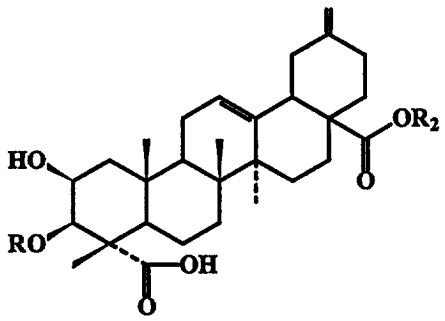


3: R = Rha (1→3) GlcA $R_1 = CH_3$ R_2 = Glc
4: R = Rha (1→3) GlcA $R_1 = CH_2OH$ R_2 = Glc
5: R = Rha (1→3) GlcA R_1 = CHO R_2 = Glc



6: R = GlcA R_2 = Glc

and 823 corresponded again to the loss of terminal hexose and deoxyhexose, respectively and an ion at *m/z* 485 reflected the aglycone moiety obtained by the loss of hexose, deoxyhexose and uronic acid. Similarly for compound **4** a peak at *m/z* 971 was due to the $[M-H]^-$ ion and peaks at *m/z* 809, 825 and 487 suggested the loss of terminal hexose and deoxyhexose and subsequent loss of uronic acid.

Structural elucidation began with the most abundant compound **4**. The ^{13}C NMR and DEPT ^{13}C spectra of **4** showed 48 signals of which 18 were assigned to a sugar portion made up of three hexoses and 30 which were assigned to an oleanene triterpene moiety.

The ^1H NMR spectrum exhibited for the aglycone moiety, in addition to six singlets assignable to tertiary methyls at δ 0.84–1.31, a doublet at δ 3.23 (1H, *d*, J = 12.5 Hz) typical of a proton involved in a —CH_2OH function, a signal at δ 4.36 (1H, *ddd*, J = 3.0, 3.7 and 4.0 Hz) ascribable to H-2 equatorial and an olefinic proton signal at δ 5.31 (1H, *t*, J = 3.4).

The ^{13}C NMR of **4** showed for the oleane skeleton signals at δ 123.8 and 144.8 assignable to a Δ^{12} double bound, at δ 178.0 indicative of a carboxylic group, at δ 70.8 and 83.7 ascribable to C-2 and C-3, respectively, and δ 65.4 suggesting the occurrence of a primary alcoholic function located at C-23.

Thus, the aglycone of **4** was identified as 2β,3β,23-trihydroxyolean-12-en-28-oic acid. Attachment of the sugar residues at C-3 and C-28 was indicated respectively by the significant downfield shift (δ_C 83.7) observed for the C-3 resonance and by the highfield shift exhibited by C-28 (δ_C 178.0) [5]. On the basis of a COSY-90 experiment, which allowed the complete sequential assignment of all proton resonances in each sugar residue, the three sugars were identified as β-D-glucuronic acid, α-L-rhamnose and β-D-glucose in the pyranoside form. The chemical shift of H-1 (δ 5.41) and C-1 of the β-D-glucopyranosyl unit suggested this residue was linked to the carboxylic group at C-28.

HETCOR experiments which correlated all proton resonances with those of the corresponding carbons, showed a glycosidation shift on C-3 of the β-D-glucopyranosyl unit.

On the basis of these data the structure of **4** was unambiguously established as 3-β-*O*-[α-L-rhamnopyranosyl (1 →)-β-D-glucuronopyranosyl]22β,3β,23-trihydroxyolean-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester.

The ^1H NMR and ^{13}C NMR data of **3** and **5** suggested for these compounds the same sugar portion as in **4** but different aglycones. The ^1H NMR spectrum of **3** showed the absence of the signals ascribable to CH_2OH-23 and the presence of a further methyl signal. These were confirmed in the ^{13}C NMR spectrum by the substitution of the signal at δ 65.4 in **4** with a signal at δ 30.0 in **3** and by significant differences in the carbon resonance of ring A (Table 1). Thus **3** was identified as 3-β-*O*-[α-L-rhamnopyranosyl(1 → 3)-β-glucuronopyranosyl]-2β,3β,dihydroxyolean-12-en-28-oic acid-28-β-D-glucopyranosyl ester. The aglycone of compound **5** was characterized by the occurrence of a CHO-23 group as suggested by the signal at δ 208.9 in the ^{13}C NMR spectrum. Thus **5** was identified as 3-β-*O*[α-L-rhamnopyranosyl(1 → 3)-β-D-glucuronopyranosyl]-2β,3β-dihydroxy-23-oxoolean-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester.

Compound **6** exhibited in the negative ion mode LSIMS a peak at *m/z* 823 $[M-H]^-$ and two other peaks at *m/z* 661 and 647 indicating the loss of hexose and uronic acid, respectively. A fragment at *m/z* resulted from the cleavage of two sugars and was ascribable to the aglycone. Acid hydrolysis revealed the presence of glucose and glucuronic acid in the sugar portion (TLC). The ^{13}C NMR and ^1H NMR spectra suggested that **6** was a noroleanane-type triterpenoid glycoside. Carbon signals observed at δ 144.0 (C=), 124.0 (CH=), 149.2 (C=) and 107.2 (=CH_2) and proton signals at δ 5.37 (H-12, *t*, J = 3.4 Hz) and 4.65 (H2-29, *s*) indicated, respectively, the presence of double bond between C-12 and C-13 and one *exo*-methylene group between C-20 and C-29. The ^1H NMR spectrum showed also the presence of only four methyl signals at δ 0.84, 1.23, 1.31 and 1.43 and two hydroxymethine signals at δ 4.31 (H-2, *ddd*) and 4.11 (H-3, *d*). In the ^{13}C NMR spectrum two carboxyl carbon signals at δ 181.6 and 177.2 were observed.

Table 1. ^{13}C NMR spectral data for compounds **1–6** in CD_3OD

	1	**2**	**3**	**4**	**5**	**6**
1	37.1	37.5	44.6	44.5	44.4	44.5
2	31.4	32.3	70.5	70.8	70.0	71.1
3	70.9	77.2	91.1	83.7	83.5	87.2
4	37.9	34.7	39.2	43.1	55.1	53.1
5	40.2	40.2	56.8	49.0	50.0	52.9
6	29.6	30.1	18.6	18.5	21.0	21.4
7	117.4	118.0	33.0	33.3	33.0	33.4
8	139.5	139.7	40.7	40.6	41.0	40.9
9	49.9	49.8	48.0	48.0	48.0	49.0
10	34.1	34.6	37.6	37.4	36.9	37.2
11	21.5	21.7	24.6	24.6	24.5	24.5
12	38.4	39.7	123.8	123.8	123.6	124.0
13	43.2	43.7	144.8	144.8	144.8	144.0
14	55.1	56.2	43.0	42.5	43.0	42.4
15	22.9	23.4	28.7	28.7	28.6	28.6
16	28.4	29.0	23.9	23.9	23.8	23.9
17	55.8	55.4	47.9	47.9	48.0	48.6
18	12.9	13.2	42.5	43.1	42.5	48.2
19	12.0	12.4	47.1	47.1	47.0	42.8
20	40.7	41.2	31.4	31.4	31.4	149.2
21	21.2	21.0	34.8	34.8	34.8	30.7
22	138.1	138.8	33.9	33.0	33.2	38.2
23	129.3	129.8	30.0	65.4	208.9	181.6
24	51.2	51.6	16.9	14.5	11.3	13.4
25	37.1	37.1	17.7	17.4	17.0	16.9
26	21.3	21.0	17.8	17.7	17.7	17.5
27	18.9	19.3	26.2	26.3	26.2	26.2
28	25.3	25.8	178.0	178.0	178.0	177.2
29	12.1	12.7	33.4	33.4	33.3	107.2
30			23.8	23.8	23.8	
1′ (GlcA)			105.5	104.9	104.1	105.5
2′			75.6	75.7	75.5	75.6
3′			83.2	83.0	82.6	83.2
4′			72.3	72.3	72.2	72.3
5′			76.6	76.4	76.5	76.6
6′			172.6	172.6	172.6	172.6
1″ (Rha)			104.4	102.2	102.2	
2″			72.3	72.2	72.2	
3″			72.3	72.3	72.3	
4″			73.8	73.8	73.8	
5″			69.8	69.7	69.6	
6″			17.8	17.7	17.7	
1‴ (Glc)		102.4	95.6	95.6	95.6	95.6
2‴		75.5	74.0	74.0	74.0	74.0
3‴		78.7	78.2	78.2	78.2	78.2
4‴		71.9	71.0	71.0	71.0	71.0
5‴		78.8	78.6	78.6	78.6	78.6
6‴		63.0	62.3	62.3	62.3	62.3

Thus the aglycone of the compound **6** was identified as 2β,3β-dihydroxy-30-norolean-12,20(29)-diene-23,28-dioic acid and was identical to the aglycone reported previously in *A. caudatus* seeds [2]. The novelty of this compound resides in the sugar substitution of the aglycone being the glucuronic acid linked at C-3 and glucose unit at C-28.

EXPERIMENTAL

General

Mps: uncorr.; NMR spectra in CD_3OD: Bruker WH-250 Spectrospin and Bruker AMX-500 spectrometers with Bruker X-32 computer and a Bruker UXNMR software package. Negative ion LSIMS:

MAT 95 (Finnigan), glycerol as the matrix. EIMS: MAT 95, 70 eV. TLC: precoated silica gel 60 F254 (Merck); spots were detected by spraying with Liebermann-Burchard reagent ($MeOH–Ac_2O–H_2SO_4$, 5:1:1). Sugars were analysed by TLC: precoated cellulose CC: silica gel (70–230 mesh, Merck) and silanized gel (RP18, 40–60 μm).

Extraction and isolation

Milled seeds of *A. cruentus* (1 kg) were extracted in a Soxhlet apparatus with CH_2Cl_2. The extract was evapd to give an oil (135 g) which was stored at −10° for two weeks. The precipitate which formed was centrifuged (g), washed with petrol, dissolved in C_6H_6 and subjected to CC (45 × 4 cm) on silica gel washed sequentially with C_6H_6, $CHCl_3$, $MeOH–CHCl_3$ (1:9) and $MeOH–CHCl_3$ (1:19). The $CHCl_3$ eluate contained a single compound **1** ($C_{29}H_{48}O$, 112 mg), mp 154–157° on crystallization from $CHCl_3$–MeOH. EIMS *m/z* (rel. int.): 412 $[M]^+$ (72), $[M–C_6H_9]^+$ (62), 300 $[M–C_8H_{15}]^+$ (15), 273 $[M–C_{10}H_{19}]^+$ (64), 135 $[C_{10}H_{15}]^+$ (27), 107 $[C_8H_{11}]^+$ (63), 43 $[C_3H_7]^+$ (63); ^{13}C NMR: Table 1. The $MeOH–CHCl_3$ (1:9) fr. contained a single compound, **2** ($C_{35}H_{58}O_6$, 105 mg), trapezoidal plates from $MeOH–CHCl_3–Me_2CO$, mp 272–275°. LSIMS negative ion mode *m/z* (rel. int.): 573 $[M–H]^-$ (20), 411 $[M–H–Glc]^-$ (11). TLC of hydrolysis products revealed Glc and **1**. ^{13}NMR: Table.

The deflated seeds (5 kg) were extracted with hot MeOH. The methanolic residue was partitioned between H_2O and *n*-BuOH. The butanolic fr. gave a crude saponin mixt. Sepn on silica gel 60 (70–270 mesh, Merck) eluting with *n*-BuOH afforded a fr. containing compounds **3**, **4** and **5** and subsequently with MeOH yielded compound **6** (80 mg). $[\alpha]^{25}$ +43.8; LSIMS negative ion mode *m/z* (rel. int.): 823 $[M–H]^-$ (33), 661 $[M–H–Glc]^-$ (10), 647 $[M–H–GlcA]^-$ (6), 615 $[M–H–Glc–HCOOH]^-$ (10), 485 $[M–H–Glc–GlcA]^-$; ^{1}H NMR: δ 0.84 (*s*, Me-26), 1.23 (*s*, Me-27), 1.31 (*s*, Me-27), 1.31 (*s*, Me–25), 1.43 (*s*, Me-24), 2.59 (*dd*, *J* = 13.6 and 13.9, H-19_{ax}), 2.77 (*dd*, *J* = 4.4 and 13.6, H-18), 4.11 (*d*, *J* = 3.7, H-3), 4.31 (*ddd*, *J* = 3.0, 3.7 and 4.0, H-2), 4.65 (*br*, H2-29), 5.37 (*t*, *J* = 3.4), 4.44 (*d*, *J* = 7.8, H-1, GlcA), 3.32, (*dd*, *J* = 7.8 and 9.0, H-2 GlcA), 3.37 (*dd*, *J* = 9.0 and 9.0, H-3 GlcA), 3.39 (*dd*, *J* = 9.0 and 9.0 H-4 GlcA), 3.40 (*d*, *J* = 9.0, H-5 GlcA), 5.40 (*d*, *J* = 8.1, H-1 Glc), 3.35 (*dd*, *J* = 8.1 and 9.1, H-2 Glc), 3.38 (*dd*, *J* = 9.1 and 9.1, H-3 Glc), 3.58 (*dd*, *J* = 9.1 and 9.1, H-4 Glc), 3.83 (*m*, H-5 Glc), 3.35 (*dd*, *J* = 12.5 and 3.0, H-6′ Glc), 3.70 (*dd*, *J* = 12.5 and 4.4, H-6″ Glc); ^{13}C NMR: Table 1.

The fr. containing compounds **3**, **4** and **5** was separated by CC (28/2 cm, octadecyl C18 40 μm, Baker) eluted sequentially with 40, 50 and 60% MeOH in H_2O. This yielded compounds:

3 (20 mg): $[\alpha]^{25}$ 0; LSIMS negative ion mode *m/z* (rel. int.): 955 $[M–H]^-$ (100), 793 $[M–H–Glc]^-$ (23), 809 $[M–H–Rha]^-$ (18), 647 $[M–H–Rha–Glc]^-$ (7), 471 $[M–H–Rha–Glc–GlcA]^-$ (22); ^{1}H NMR: δ 0.85 (*s*, Me-26), 0.94 (*s*, Me-29), 0.96 (*s*, Me-30), 1.11 (*s*, Me-23 and Me-24), 1.18 (*s*, Me-27), 1.26 (*d*, *J* = 6.5, Me-Rha), 1.29 (*s*, Me-25), 2.90 (*dd*, *J* = 4.4 and 13.6, H-18), 3.98 (*d*, *J* = 1.5, H-2 Rha), 4.14 (*d*, *J* = 8.0, H-5 Rha), 4.27 (*ddd*, *J* = 3.0, 3.7 and 4.0, H-2), 4.44 (*d*, *J* = 8.0, H-1 GlcA), 5.22 (*d*, *J* = 1.5, H-1 Rha), 5.30 (*t*, *J* = 3.4), 5.42 (*d*, *J* = 8.2, H-1 Glc); ^{13}C NMR: Table 1.

4 (295 mg): $[\alpha]^{25}$ 0; LSIMS negative ion mode *m/z* (rel. int.): 971 $[M–H]^-$ (70), 809 $[M–H–Glc]^-$ (29), 825 $[M–H–Rha]^-$ (17), 763 $[M–H–Glc–HCOOH]^-$ (15), 663 $[M–H–Glc–Rha]^-$ (18), 487 $[M–H–Glc–Rha–GlcA]^-$ (23); ^{1}H NMR δ 0.84 (*s*, Me-26), 0.94 (*s*, Me-29), 0.96 (*s*, Me-24 and Me-30), 1.19 (*s*, Me-27), 1.31 (*s*, Me-25), 2.88 (*dd*, *J* = 4.4 and 13.6, H-18), 3.23 (*d*, *J* = 12.5, H-23a), 3.62 (*d*, *J* = 3.7, H-3), 3.78 (*d*, *J* = 12.5, H-23b), 4.36 (*ddd*, *J* = 3.0, 3.7 and 4.0, H-2), 5.30 (*t*, *J* = 3.4), 4.48 (d, *J* = 8.0, H-1 GlcA), 3.45 (*dd*, *J* = 8.0 and 9.0, H-2 GlcA), 3.59 (*dd*, *J* = 9.0 and 9.0 H-3 GlcA), 3.50 (*dd*, *J* = 9.0 and 9.0, H-4 GlcA), 3.66 (*d*, *J* = 9.0, H-5 GlcA), 5.22 (*d*, *J* = 1.5, H-1 Rha), 3.97 (*dd*, *J* = 1.5 and 2.5, H-2 Rha), 3.75 (*dd*, *J* = 2.5 and 8.9, H-3 Rha), 3.41 (*dd*, *J* = 8.9 and 9.0), 4.16 (*m*, H-5 Rha), 1.26 (*d*, *J* = 6.5, H-6 Rha), 5.41 (*d*, *J* = 8.0, H-1 Glc), 3.36 (*dd*, *J* = 8.0 and 9.0, H-2 Glc), 3.44 (*dd*, *J* = 9.0, H-3 Glc), 3.45 (*dd*, *J* = 9.0 and 9.0, H-4 Glc), 3.38 (*d*, *J* = 9.0, H-5 Glc), 3.71 (*dd*, *J* = 12.0 and 5.0, H-6a Glc), 3.85 (*dd*, *J* = 12.0 and 3.5, H-6b Glc).

5 (50 mg): $[\alpha]^{25}$ 0; LSIMS negative ion mode *m/z* (rel. int.): 969 $[M–H]^-$ (100), 807 $[M–H–Glc]^-$ (23), 823 $[M–H–Rha]^-$ (15), 761 $[M–H–Glc–HCOOH]^-$ (15), 661 $[M–H–Glc–Rha]^-$ (6), 485 $[M–H–Glc–Rha–GlcA)^-$ (19); ^{1}H NMR: δ 0.85 (*s*, Me-26), 0.95 (*s*, Me-29), 0.97 (*s*, Me-30), 1.21 (*s*, Me-27), 1.26 (*d*, *J* = 6.5, Me–Rha), 1.33 (*s*, Me-25), 1.35 (*s*, Me-24), 2.90 (*dd*, *J* = 4.4 and 13.6, H-18), 3.85 (*dd*, *J* = 3.0 and 12.0, H-6 Glc), 3.92 (*d*, *J* = 3.7, H-2 Rha), 4.15 (*m*, H-5 Rha), 4.30 (*d*, *J* = 8.0, H-1 GlcA), 4.38 (*ddd*, *J* = 3.0, 3.7 and 4.0, H-2), 5.19 (*d*, *J* = 1.5, H-1 Rha), 5.31 (*t*, *J* = 3.4), 5.42 (*d*, *J* = 8.2, H-1 Glc), 9.39 (*s*, H-23); ^{13}C NMR: Table 1.

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