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# A NEW SAPONIN FROM *DEERINGIA AMARANTHOIDES*

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ABSTRACT.—A new triterpenoid saponin, 3-*O*-[α-L-rhamnopyranosyl (1→3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl]-3β-hydroxyolean-12-en-28-oate [**3**] has been isolated together with two known saponins, 3-*O*-[α-L-rhamnopyranosyl (1→3)-β-D-glucuronopyranosyl]-3β-hydroxyolean-12-en-28-oic acid [**1**] and 3-*O*-[α-L-rhamnopyranosyl (1→3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl]-3β-hydroxyolean-12-en-oate [**2**], from the fruits of *Deeringia amaranthoides*.

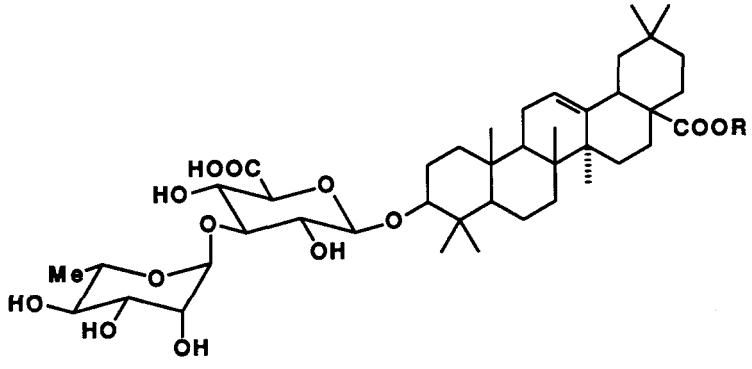
*Deeringia amaranthoides* L. Merrill (Amaranthaceae) grows in the Western Himalayas throughout the area between 1000 and 4000 feet (1). It has been reported to possess anticancer (2) and spermicidal activity (3). A thorough survey of the literature showed that there is no report of any chemical work in this genus. Herein we report a new oleanolic-acid-based saponin along with two known saponins.

## RESULTS AND DISCUSSION

The EtOH extract of the fruits of *D. amaranthoides*, on repeated cc, afforded three compounds which exhibited positive color tests for triterpenoid saponins.

Compound **1** in fabms (negative ion) displayed a molecular ion at *m/z* 777 $[M-H]^-$. The peaks at *m/z* 631 $[M-H-146]^-$ and 455 $[M-H-146-176]^-$ arose by the loss of a terminal methylpentose and a methylpentosyl hexose unit, respectively. Compounds **2** and **3** in fabms displayed molecular ions at *m/z* 939 $[M-H]^-$ and 1071 $[M-H]^-$ indicating the molecular weights to be 940 and 1072, respectively. Compound **3** exhibited peaks at *m/z* 939 $[M-H-132]^-$ and 925 $[M-H-146]^-$ by the loss of terminal pentose and methylpentose, respectively. The simultaneous loss of a methylpentose and a pentose unit was concluded from the ion recorded at *m/z* 793. The peak at *m/z* 631 was explained by the loss of a methylpentose, a pentose, and a hexose unit from the molecular ion.

Acid hydrolysis of **1–3** furnished oleanolic acid, D-glucuronic acid, and L-




**1** R=H
**2** R=β-D-glucopyranosyl
**3** R=β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl

rhamnose; in addition **2** afforded D-glucose and **3** gave D-glucose and D-xylose. Compounds **1–3** were treated with $CH_2N_2$ and hydrolyzed to afford the methylester of oleanolic acid from **1** and oleanolic acid from **2** and **3**. This confirmed that in compounds **2** and **3** the -COOH group was not free and glycosylation was at C-3 and C-28. The methylester of compounds **1–3** on $NaBH_4$ reduction resulted in the conversion of the -COOMe group to $CH_2OH$ forming the glucosyl derivatives. On permethylation (4) followed by hydrolysis, these glucosyl derivatives of **1–3** furnished 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose, in addition **2** gave 2,3,4,6-tetra-*O*-methyl-D-glucose and **3** gave 2,3,4-tri-*O*-methyl-D-xylose and 3,4,6-tri-*O*-methyl-D-glucose. 3,4,6-Tri-*O*-methyl-D-glucose gave a positive color test with Wallenfel's reagent (5) showing a free hydroxyl group at C-2.

Alkaline hydrolysis of **2** and **3** with $K_2CO_3$ provided a prosapogenol, which was found to be identical to compound **1** (mmp, co-tlc, and co-ir).

The partial acidic hydrolysis of **3** furnished prosapogenols $PS_1$, $PS_2$, $PS_3$, and $PS_4$, in addition to oleanolic acid. $PS_2$ and $PS_3$ were identical to compounds **1** and **2**, respectively. $PS_1$ on acidic hydrolysis afforded D-glucose and glucuronic acid, while $PS_4$ gave D-glucose and D-xylose. Permethylated $PS_4$ on methanolysis followed by hydrolysis afforded 2,3,4-tri-*O*-methyl-D-xylose and 3,4,6-tri-*O*-methyl-D-glucose.

The types of linkages at the glycosidic bonds were confirmed by the $^1$H- and $^{13}$C-nmr spectra. In the $^{13}$C-nmr spectra of **1**, the C-3 of glucuronic acid was observed at δ 83.9, revealing deshielding of ca. 6 ppm for this carbon resonance in comparison to the reported values for methyl-*O*-β-D-glucoside (6) as a result of glycosidation at C-3. The anomeric signals of the D-glucuronic acid and L-rhamnose were observed at δ 5.23 (d, $J = 7$ Hz) and δ 4.98 (s), respectively, in the $^1$H-nmr. Thus, **1** was characterized as 3-*O*-[α-L-rhamnopyranosyl (1↦3)-β-D-glucuronopyranosyl]-3β-hydroxy-olean-12-en-28-oic acid.

$^{13}$C nmr of **2** indicated that the inner glucuronic acid was substituted at C-3 (δ 83.6 ppm, revealing deshielding of ca. 6 ppm for this carbon resonance) with a terminal rhamnose. $^1$H nmr showed anomeric signals at δ 5.79 (d, $J = 8.5$ Hz), 5.22 (d, $J = 6.5$ Hz), and 4.88 (s) showing the presence of β-linked glucuronic acid and D-glucose and α-linked rhamnose. Thus, **2** was characterized as 3-*O*-[α-L-rhamnopyranosyl (1↦3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl]-3β-hydroxyolean-12-en-28-oate. Methyl ethers of **1** and **2** have also been reported for the first time from the aerial part of *Zexmenia buphthalmiflora* (8).

C-3 of glucuronic acid and C-2 of glucose appeared at δ 83.9 and δ 78.1, respectively, in the $^{13}$C-nmr of **3**, revealing deshielding ca. 6 ppm and ca. 5 ppm for these carbon resonances in comparison to the reported values (6). Anomeric signals in the $^1$H nmr appeared at δ 6.32 (d, $J = 7$ Hz), 6.28 (d, $J = 8$ Hz), 4.91 (d, $J = 8$ Hz), and 4.26 (br s). Thus, **3** was characterized as 3-*O*-[α-L-rhamnopyranosyl (1↦3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-xylopyranosyl (1↦2)-β-D-glucopyranosyl]-3β-hydroxyolean-12-en-28-oate.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were recorded with a Boetius microscopic apparatus, fabms JEOL, JMS-HX-110 instrument in the negative and positive ion mode using MeOH and glycerol as solvent. $^1$H-nmr spectra were recorded at 400 MHz using $C_5D_5N$, and $^{13}$C-nmr spectra were recorded at 100 MHz using $CD_3OD$, with TMS as an internal standard. Cc was performed on Si gel 60G (Merck). The spots on tlc were visualized by spraying with 10% aqueous $H_2SO_4$ followed by heating. Pc was carried out on Whatman paper no. 1 using the descending mode and visualized with aniline hydrogen phthalate. The following chromatographic solvents were used: (A) petroleum ether (60–80°)-$Me_2CO$ (1:1), (B) $CHCl_3$-MeOH (60:40), (C) $C_6H_6$-$Me_2CO$ (9:1), (D) *n*-BuOH–HOAc–$H_2O$

(4:1:5), (E) *n*-BuOH–EtOH–$H_2O$ (5:1:4), (F) $CHCl_3$-MeOH-$H_2O$ (65:40:10), and (G) $C_6H_6$-EtOAc (8:2).

ISOLATION.—The fruits of *D. amaranthoides* were collected from Romdhar (Tehri Garhwal) in July and authenticated by the Department of Botany, University of Garhwal Srinagar. Voucher specimen is preserved in the Ethnobotanical Herbarium of the Department of Botany, University of Garhwal. The air-dried material (4 kg) was defatted with solvent A in a Soxhlet apparatus. The solvent-free mass (3 kg) was exhaustively extracted with 95% EtOH until the extracts became colorless. The concentrated mass was partitioned between *n*-BuOH–$H_2O$ (1:1) (4 × 500 ml). The *n*-BuOH extract, after concentration under reduced pressure, was dissolved in MeOH (50 ml) and precipitated in dry $Me_2CO$ (anhydrous $K_2CO_3$), to yield a complex mixture of saponins which on repeated cc (solvent B) afforded compounds **1** (10 g), **2** (12 g), and **3** (9 g).

COMPOUND **1**.—Colorless needles (10 g) from MeOH: mp 215–220°; ir max (CsI) $cm^{-1}$ 3400 (OH), 1690; fabms *m/z* 799, 777, 631, 455; $^1H$ nmr 0.70, 0.72, 0.81, 0.82, 0.84, 0.96, 1.04 (each s, 7 × tert-Me group of aglycone), δ 5.16 (1H, br s of H-12 of aglycone), δ 5.23 (1H, d, $J=7$ Hz), 4.98 (1H, br s) [anomeric protons] ppm; $^{13}C$ nmr (C-1–C-30 of aglycone) 39.7, 28.7, 90.9, 38.9, 57.0, 17.8, 33.4, 40.1, 48.2, 37.8, 24.5, 123.6, 145.1, 42.7, 28.6, 23.9, 47.6, 42.9, 47.5, 31.5, 34.8, 34.0, 27.7, 17.0, 15.9, 17.7, 26.4, 178.07, 34.7, 23.9; rhamnopyranosyl 102.4 (C-1), 72.0 (C-2), 72.3 (C-3), 73.8 (C-4), 69.5 (C-5), 19.2 (C-6); glucuronopyranosyl 106.4 (C-1), 76.2 (C-2), 83.9 (C-3), 72.1 (C-4), 78.1 (C-5), 178.0 (C-6).

COMPOUND **2**.—Crystallized from MeOH (12 g): mp 260–265°; ir max (CsI) $cm^{-1}$ 3400 (OH), 1690; fabms *m/z* 939, 793, 777, 631, 455; $^1H$ nmr 0.72, 0.82, 0.96, 0.97, 1.01, 1.24, 1.30 (each s, 7 × tert-Me group), 5.45 (br s of H-12 of aglycone), 5.79 (1H, d, $J=8.5$ Hz), 5.22 (d, $J=6.5$ Hz), 4.88 (s) [anomeric protons] ppm; $^{13}C$ nmr (C-1–C-30 of aglycone) 39.7, 28.6, 91.1, 37.8, 56.9, 17.8, 33.06, 40.0, 47.1, 37.8, 24.4, 123.7, 144.7, 42.8, 28.6, 23.98, 47.9, 42.8, 47.9, 31.4, 34.9, 34.8, 28.7, 17.0, 15.9, 17.7, 26.4, 178.0, 33.9, 23.9; glucuronopyranosyl 106.5 (C-1), 76.2 (C-2), 83.6 (C-3), 72.1 (C-4), 78.1 (C-5), 178.9 (C-6); rhamnopyranosyl 102.6 (C-1), 72.0 (C-2), 72.3 (C-3), 73.8 (C-4), 69.5 (C-5), 19.3 (C-6), glucopyranosyl 95.7 (C-1), 73.8 (C-2), 77.1 (C-3), 71.1 (C-4), 78.1 (C-5), 62.8 (C-6).

COMPOUND **3**.—Crystallized from MeOH (9 g): mp 270–274°; ir max (CsI) $cm^{-1}$ 3400 (OH), 1680; $^1H$ nmr 0.81, 0.89, 0.92, 0.99, 1.08, 1.26, 1.29 (each s, 7 × tert-Me group), 5.11 (1H, br s, H-12 of aglycone), 6.32 (1H, d, $J=7$ Hz), 6.28 (1H, d, $J=8$ Hz), 4.91 (d, $J=8$ Hz), 4.26 (br s) [anomeric protons] ppm; $^{13}C$ nmr (C-1–C-30 of aglycone) 39.8, 28.6, 91.1, 37.8, 56.8, 17.8, 33.0, 40.0, 47.1, 37.8, 24.4, 123.7, 144.7, 42.8, 28.6, 23.98, 47.9, 42.8, 47.9, 31.4, 37.8, 34.8, 28.7, 17.0, 15.9, 17.7, 26.4, 178.0, 33.9, 23.9; glucuronopyranosyl 106.5 (C-1), 72.5 (C-2), 83.9 (C-3), 71.1 (C-4), 76.9 (C-5), 177.9 (C-6); rhamnopyranosyl 102.6 (C-1), 72.0 (C-2), 72.3 (C-3), 73.8 (C-4), 69.5 (C-5), 19.3 (C-6); glucopyranosyl 99.7 (C-1), 79.2 (C-2), 78.3 (C-3), 71.4 (C-4), 78.0 (C-5), 64.0 (C-6); xylopyranosyl 98.7 (C-1), 74.07 (C-2), 75.9 (C-3), 71.8 (C-4), 65.9 (C-5).

ACIDIC HYDROLYSIS OF COMPOUND **1**.—Compound **1** (25 mg) was hydrolyzed with 2 M HCl-dioxane (1:1) (3 h) on a boiling $H_2O$ bath to afford the aglycone, crystallized from MeOH (5 ml) as colorless needles: mp 310–315°, identified as oleanolic acid by direct comparison (mmp, co-tlc, and co-ir) with an authentic sample: eims $[M]^+$ 456, 441, 411, 248, 208, 192.

The neutralized ($Ag_2CO_3$) and concentrated hydrolysate showed the presence of D-glucuronic acid and L-rhamnose (pc, solvent D, $R_f$ values 0.15 and 0.37, respectively). The estimation of sugars was performed using colorimeteric methods (7).

METHYLATION.—Compound **1** (250 mg) was dissolved in MeOH (5 ml) and was treated with excess ethereal $CH_2N_2$. The whole reaction mixture was allowed to stand for 12 h. Removal of the solvent under reduced pressure gave **1** methyl ester (220 mg).

$NaBH_4$ REDUCTION OF **1** METHYL ESTER.—The methyl ester of **1** (100 mg) was dissolved in dry $Et_2O$ (4 ml). $NaBH_4$ (500 mg) was added, and the mixture was ice-cooled, stirred for 20 h at room temperature, and evaporated in vacuo. The residue was diluted with an excess of $H_2O$ and extracted with *n*-BuOH. The *n*-BuOH layer was washed, dried, and concentrated. It afforded a reduced glycoside of **1** (75 mg), ir max (CsI) $cm^{-1}$ 3400 (OH), no COOMe.

PERMETHYLATION OF THE REDUCED GLYCOSIDE OF **1**.—A solution of the reduced glycoside of **1** (200 mg) in MeOH was treated with dry DMSO (0.4 ml), dry *t*-BuONa (40 mg), finely powdered dry NaOH (10 mg), and MeI (0.3 ml). The reaction mixture was stirred at room temperature for 1 h. The solution was poured into ice-$H_2O$ and extracted with $Et_2O$. The $Et_2O$ layer was washed with a saturated NaCl solution, dried, and evaporated. The product obtained was purified by cc ($SiO_2$, solvent C) to furnish the methylated reduced glycoside of **1** (190 mg).

METHANOLYSIS FOLLOWED BY HYDROLYSIS OF THE METHYLATED REDUCED GLYCOSIDE OF **1**.—A solution of the methylated reduced glycoside (50 mg) in 7% HCl/dry MeOH (1 ml) was treated under reflux for 3 h and acid-hydrolyzed with 6% $H_2SO_4$. The reaction mixture was neutralized with $Ag_2CO_3$ powder and filtered. The mother liquor furnished 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose ($R_G$ values 0.76 and 1.01, respectively) which were identified with authentic samples on pc (solvent E).

ACIDIC HYDROLYSIS OF COMPOUNDS **2** AND **3**.—Compounds **2** and **3** (25 mg each) were separately hydrolyzed with 2 M HCl-dioxane (1:1) (5 ml) for 3 h as usual to afford an aglycone identified as oleanolic acid by its mp, co-tlc, and co-ir with an authentic sample.

The neutralized ($Ag_2CO_3$) aqueous hydrolysates of **2** and **3** on pc (solvent D) showed the presence of D-glucuronic acid, D-glucose, and L-rhamnose ($R_f$ 0.15, 0.18, 0.37, respectively). Compound **3** showed D-xylose ($R_f$ 0.28) also.

ALKALINE HYDROYLSIS OF **2** AND **3**.—Solutions of **2** and **3** (250 mg each) in MeOH (5 ml) were separately treated with 5% aqueous $K_2CO_3$ (5 ml), and the whole mixture was heated under reflux for 3 h. The reaction mixture was neutralized with Dowex 50W × 8($H^+$) and filtered. Workup of the filtrate in the usual manner yielded a prosapogenal (210 mg), crystallized from MeOH as a colorless crystals. The prosapogenol was found to be identical to compound **1** (mmp, co-tlc, and co-ir).

METHYLATION OF COMPOUNDS **2** AND **3**.—Compounds **2** and **3** (100 mg each) were separately methylated with $CH_2N_2$ as described for compound **1** to furnish their methyl esters (80 and 85 mg, respectively).

$NaBH_4$ REDUCTION OF THE METHYL ESTERS OF **2** AND **3**.—The methyl esters of compounds **2** and **3** (60 mg each) were treated with dry $Et_2O$ and $NaBH_4$ (500 mg) as above to afford the respective methylated reduced glycosides.

PERMETHYLATION FOLLOWED BY HYDROLYSIS OF THE METHYLATED REDUCED GLYCOSIDES OF **2** AND **3**.—The methylated reduced glycosides of **2** and **3** (50 mg each) were separately permethylated as above. The permethyl ethers thus obtained were hydrolyzed as above to furnish oleanolic acid. The mother liquors afforded 2,4,6-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-L-rhamnose, and 2,3,4,6-tetra-*O*-methyl-D-glucose from the permethyl ether of reduced **2** and 2,4,6-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4-tri-*O*-methyl-D-xylose, and 3,4,6-tri-*O*-methyl-D-glucose (Wallenfels positive) from the permethyl ether of reduced **3**.

PARTIAL HYDROLYSIS OF COMPOUND **3**.—Compound **3** (200 mg) in 1 M HCl–*n*-BuOH (1:1) (50 ml) was heated at 70° for 4 h. The *n*-BuOH layer was washed with $H_2O$ and evaporated to dryness in vacuo. The residue after cc ($SiO_2$, 15 g, solvent C) yielded four prosapogenols tentatively named as $PS_1$ (40 mg), $PS_2$ (45 mg), $PS_3$ (45 mg), and $PS_4$ (35 mg), in addition to oleanolic acid (20 mg).

$PS_2$ and $PS_3$ were found identical to compounds **1** and **2**, respectively (mmp and co-tlc).

HYDROLYSIS OF $PS_1$.—On acidic hydrolysis with 3% $H_2SO_4$, $PS_1$ (10 mg) afforded oleanolic acid and D-glucuronic acid and D-glucose. $PS_1$ on alkaline hydrolysis gave D-glucose.

$NaBH_4$ REDUCTION AND PERMETHYLATION OF $PS_1$.—$PS_1$ was treated with $NaBH_4$ as above. Permethylation followed by hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-glucose ($R_G$ 1.00).

ACIDIC HYDROLYSIS OF $PS_4$.—$PS_4$ (10 mg) on acidic hydrolysis afforded oleanolic acid, D-glucose, and D-xylose.

PERMETHYLATION OF $PS_4$.—$PS_4$ (20 mg) was permethylated as above, followed by acid hydrolysis, and afforded 2,3,4-tri-*O*-methyl-D-xylose and 3,4,6-tri-*O*-methyl-D-glucose (pc, solvent D, $R_G$ 0.98 and 0.76, respectively).

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