

A New Triterpene Saponin from *Chenopodium ficifolium*

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Chenopodiaceae, *Chenopodium ficifolium*, Triterpenes

The new triterpene saponin 3-O- β -D-glucopyranoside, 28- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosiduronic acid oleanolate was isolated from the roots of *Chenopodium ficifolium*. The known compounds stigmasterol-3-O-glucoside and 3-O- β -D-glucopyranosiduronic acid, 28- β -D-glucopyranosyl oleanolate were also isolated. The latter compound, oleanolic acid, β -sitosterol and its glucoside were isolated from the aerial parts. The identity of these compounds was verified through different chemical and physico-chemical evidences including different 1D and 2D NMR experiments.

Introduction

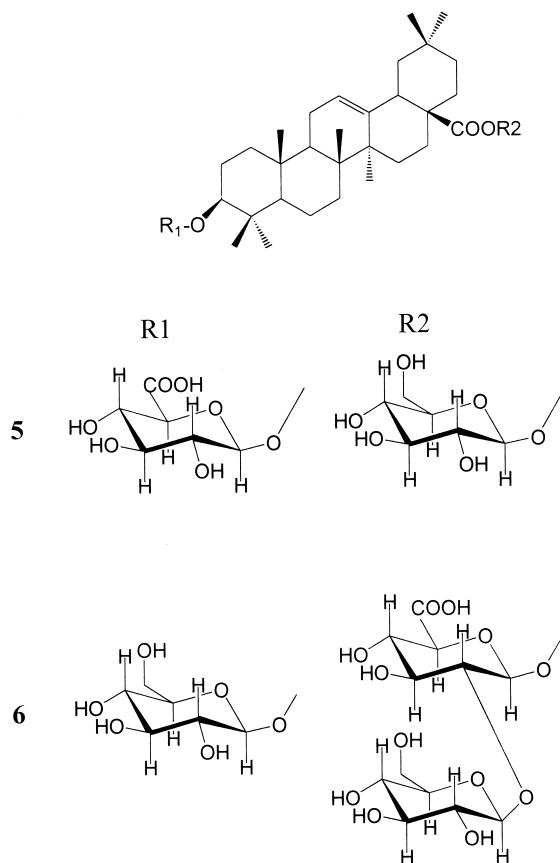
Plants of the family Chenopodiaceae are used for treatment of many ailments (Watt and Breyer-Brandwijk, 1962). Alkaloids, phenolic acids, saponins, glycosides and glycuronides were reported (Gibbs, 1974; Hussein, 1985). Saponins of Chenopodiaceae were found to exhibit hypoglycemic activity (Yoshikawa *et al.*, 1996; 1997). In a previous publication, *Chenopodium ficifolium* was reported to contain kaempferol-3,7-dirhamnoside (Gohar and El-Mazar, 1997). This report describes the isolation and the identification of triterpene saponins from *C. ficifolium*.

Results and Discussion

Solvent partition and repeated chromatographic purification over amberlite XAD-2, diion resin, normal phase and Rp-C18 silica of the methanol extracts of both roots and aerial parts of *C. ficifolium* afforded four compounds from the aerial parts (**1–3** and **5**) and three from the roots (**4–6**). Structural determination of compounds **5** and **6** was based on chemical reactions, 1-D- and 2-D NMR experiments (H-H-COSY, HMBC, HSQC, NOE).

Compound **5** gave positive Salkowski and Molisch's reactions indicating its triterpenoid glycosidic nature (Finar, 1991). Its R_f value is 0.18 and on alkaline hydrolysis (Debella *et al.*, 2000) it afforded prosaponin having R_f value 0.21 using **S2**.

Glucose was detected as the sugar moiety after alkaline hydrolysis and glucuronic acid as the sugar of the prosaponin after acid hydrolysis using **S8** and **S9**. The aglycone was found to be oleanolic acid (co-chromatography with reference sample using **S4** and **S5**). Positive FAB-MS of **5** afforded pseudomolecular ion at m/z 833 ($M+K$)⁺, 817 ($M+Na$)⁺, calculated for C₄₂H₆₆O₁₄, and 633 (M -glucose)⁺, 617 (M -glucuronic acid)⁺, 477 (aglycone-H₂O+K)⁺, 439 (aglycone+1-H₂O)⁺. This fragmentation pattern indicated the presence of glucose, glucuronic acid and oleanolic acid moieties. Fragment at m/z 439 was characteristic for the presence of oleanolic acid (Konishi *et al.*, 1998). The ¹H and ¹³C-NMR data, shown in Table I and experimental and comparison with the reported data (Tori *et al.*, 1974; Schopke *et al.*, 1997), confirmed the previous deduction. Glycosidations at position-28 and -3 were concluded from the chemical shifts of both carbons, where an upfield shift of C-28 from δ 179–180 to δ 175–176 (Yoshikawa *et al.*, 1996; 1997) and a downfield shift of C-3 by ca. 5–12 ppm (Agrawal, 1992) were observed. Chemical shift of C-1" of the glucose at δ 94 confirmed its glycosilation at C-28 (Ye *et al.*, 2000). Moreover, correlation between the anomeric proton of this glucose moiety, H-1" at δ 5.24 and the C-28 at δ 175.1, in HMBC experiment, confirmed this conclusion. Location of the uronic acid at position-3 was concluded from the correlation of its H-1' at δ 4.12 with C-3 at δ 87.8 in HMBC experi-



ment and NOE of that proton with H-3 at δ 3.02. Thus, **5** was concluded to be 3-*O*- β -D-glucopyranosiduronic acid, 28- β -D-glucopyranosyl-oleanolate. This compound was previously reported in rhizomes of *Panax japonicum* (Lin *et al.*, 1976) and in the roots of *Beta vulgaris* (Yoshikawa *et al.*, 1996).

Compound **6** showed close similarity to **5** in its chemical reactions as well as its NMR and MS spectra. Positive FAB-MS displayed m/z 995 ($M+K$)⁺, 979 ($M+Na$)⁺, and 957 ($M+1$)⁺ ions, calculated for C₄₈H₇₆O₁₉, in addition to 833 ($M+K$ -hexose)⁺, 817 ($M+Na$ -hexose)⁺ fragments. Loss of sugar moieties from the Na or K fixed ions in FAB-MS is reported for similar compounds (Debella *et al.*, 2000). Since compound **5** displayed the latter two fragments (m/z 833 and 817) as K and Na fixed molecular ions, **6** should be **5** with one extraterminal hexose unit (Crow *et al.*, 1986). Alkaline hydrolysis of **6** afforded prosaponin having

R_f value 0.29 using **S2** indicating that the prosaponin of **6** was less polar than that obtained from **5** (R_f 0.21, **S2**). The aglycone of both **5** and **6** was oleanolic acid (co-chromatography, using **S4** and **S5**). Detection of glucose and glucuronic acid as the sugar moieties in **6** and only glucose in **5**, after alkaline hydrolysis using **S8** and **S9**, indicating that the first two sugar are attached to C-28 in **6**. Acid hydrolysis of the prosaponin of **6** afforded glucose, indicating that it is the sugar at position-3. This conclusion was confirmed by NOE, between the anomeric proton of glucose (H-1') at δ 4.15, and H-3 of the aglycone at δ 3.01 suggested that glycosilation of that hexose occurred at position-3. Moreover, H1' is correlated with C-3 at δ 87.8 in HMBC spectrum which confirmed that conclusion. H-1'' signal at δ 5.23 assigned for glucuronic acid, is correlated with C-28 at δ 175.1 in HMBC spectrum proved that the uronic acid is connected to C-28 of the oleanolic acid. Correlation between the anomeric proton of another glucose moiety at δ 4.41 and C-2'' of the uronic acid at δ 83.2 in HMBC experiment, confirmed that the terminal glucose is linked to position-2 of the glucuronic acid at position-28 of oleanolic acid. Thus, **6** was concluded to be 3-*O*- β -D-glucopyranoside, 28- β -D-glucopyranosyl-(1→2)- β -D-glucopyranosiduronic acid oleanolate which is a newly reported natural product.

Compounds **1**, **2**, **3** (from aerial parts) and **4** (from roots) were identified as β -sitosterol, oleanolic acid, β -sitosterol glucoside and stigmasterol glucoside, respectively. Their R_f values using different chromatographic systems **S2**, **S3**, **S4** and **S5**, were comparable with those of reference samples. These compounds gave positive reactions for sterols and/or triterpenes (Finar, 1991) and their IR spectra are superimposable with those of reference samples. Their ¹H, ¹³C-NMR and MS spectral data are comparable with the reported data (Tori *et al.*, 1974; Lin *et al.*, 1976; Patterson, 1984; Tandon *et al.*, 1990; Pauchert and Behnke, 1993; Gohar *et al.*, 2000). Moreover, as a result of acid hydrolysis of the glucosides **3** and **4**, β -sitosterol and stigmasterol were proved to be the aglycone moieties, respectively (co chromatography with reference samples using **S4** and **S5**) and glucose was the sugar moiety (PC using **S8** and **S9**).

Table I. ¹³C-NMR of compounds **5** and **6**.

C #	Compound 5	Compound 6	C #	Compound 5	Compound 6
1	38.2	38.2	R'3; 1	GlcA 105.3	Glc 104.7
2	25.3	25.3	2'	73.7	73.7
3	87.8	87.8	3'	77.6	77.1
4	40.0	40.0	4'	72.2	72.8
5	54.9	54.9	5'	76.5	75.9
6	17.7	17.7	6'	172.9	61.1
7	31.5	31.5			
8	38.9	38.9	R''28; 1''	Glc 94.0	GlcA 94.0
9	47.0	47.0	2''	73.9	83.2
10	38.6	38.6	3''	77.6	77.7
11	22.9	22.9	4''	72.2	72.3
12	121.6	121.6	5''	76.7	76.9
13	143.4	143.4	6''	60.6	175.1
14	41.2	41.2			
15	27.1	27.1	R'''28; 1'''		103.9
16	22.4	22.5	2'''		73.4
17	45.4	45.5	3'''		76.6
18	40.7	40.7	4'''		72.1
19	45.8	45.8	5'''		75.5
20	30.2	30.2	6'''		60.6
21	33.2	33.2			
22	32.2	32.2			
23	27.6	27.5			
24	16.4	16.4			
25	15.2	15.1			
26	16.6	16.6			
27	25.4	25.4			
28	175.1	175.1			
29	32.6	32.6			
30	23.3	23.3			

δ Value is expressed in ppm at 150 MHz in DMSO-*d*₆.

Experimental

General

Mps uncor., IR spectra in KBr discs, on Buck Scientific INC. Infrared Spectrophotometer Model 500. NMR spectra were run at 600 or 400 MHz (¹H) and 150 or 100 MHz (¹³C) in DMSO-*d*₆ or CD₃OD using solvent peak as internal standard (JEOL JNM A-600 & 400). Two-dimensional NMR experiments were preformed using standard programs. MS was obtained by positive and negative FAB (JEOL JMS MS-700). Planer chromatographic systems were performed on silica gel chromatoplates GF₂₅₄ using solvent mixtures as follows: CH₂Cl₂–CH₃OH (9.5:0.5 v/v) **S1**; EtOAc–CH₃OH–H₂O (100: 20:10 v/v/v) **S2**; CH₂Cl₂–MeOH–H₂O (10:4:0.5 v/v/v) **S3**; petroleum ether–EtOAc (7:3 v/v) **S4**; petroleum ether–EtOAc (1:1 v/v) **S5**; CH₂Cl₂–MeOH (9:1 v/v) **S6**; on silica gel Rp-C18 using H₂O–CH₃OH (4:6 v/v) **S7**; vanillin/H₂SO₄ spray; and on PC using What-

man filter paper No 1 and solvent mixtures consisted of butanol–benzene–pyridine–water (4:1:3:3 v/v/v/v) **S8** and butanol–acetic acid–water (5:1:4 v/v/v) **S9**, descending, aniline hydrogen phthalate spray. Reference samples of oleanolic acid, β-sitosterol, stigmasterol and their glucosides were obtained from materials previously isolated and identified in Pharmacognosy Department, Faculty of Pharmacy, Mansoura University.

Plant material, extraction and isolation

Chenopodium ficifolium was collected from the vicinity of Mansoura University in May 2000. Its identity was confirmed by Dr. I. Mashaly, Associate Prof. of systematic botany, Faculty of Science, Mansoura University. A voucher sample is kept at the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University. Powdered aerial parts (850 g) were extracted with methanol to afford 196 g semisolid greenish residue after solvent

evaporation. The methanol extract was defatted with petroleum ether to yield 156 g. Powdered root (600 g) was extracted with methanol to afford a 32 g residue after solvent evaporation. It was defatted with petroleum ether to yield 26 g as a semi-solid dark brown residue.

The defatted residue of the aerial parts was loaded on top of a separating funnel containing 600 g of Rp-C18 and gradient elution was adopted using H₂O, 25% MeOH/H₂O, 50%, 75% and 100%, two liter each. Fractions eluted with 25% and 50% MeOH were combined and after solvent evaporation afforded 11.5 g residue which was dissolved in water and passed over Amberlite XAD-2 (non-ionic adsorbent, polymer, mesh size 20–60, moisture content 38%), after water elution the alcohol eluate afforded 8.5 g, after solvent evaporation. This residue was dissolved in water and passed over Dion resin, washed with water then eluted with alcohol. The alcohol eluate gave 4.8 g residue. This residue was loaded on Si gel column (150 g, 2.5 × 100 cm) and elution started with CH₂Cl₂ followed by MeOH/CH₂Cl₂ mixtures, gradient, 250 ml fractions were collected. Fractions 6–7 eluted with 5% MeOH contained single component *R_f* 0.38 (**S6**) and 0.80 (**S2**) (compound **3**, 35 mg, white needles). Fractions 30–32 eluted with 25% MeOH contained **5**.

Fractions 4–5 loaded on Si gel column (100 g, 2.5 × 80 cm), elution was started with petroleum ether, 5 × 100 ml; 5% EtOAc/ petroleum ether, 5 × 100 ml; 20% EtOAc, 9 × 100 ml; 30% EtOAc, 5 × 100 ml; 50% EtOAc, 8 × 100 ml. Fractions 2–3 and fractions 4–6 of 20% eluate, each contained single component *R_f* 0.52 and 0.43 (**S4**) and 0.82 and 0.75 (**S5**), respectively. Both components were recrystallized from hot methanol to yield compounds **1** (72 mg colourless needles) and **2** (15 mg, colourless needles) which are consistent with β -sitosterol and oleanolic acid, respectively. Fractions 1–5 of 50% eluate contained a single spot, *R_f* 0.33 and 0.43 using **S4** and **S5** respectively (not further investigated due to its poor yield).

The defatted root extract (25 g) was adsorbed on 50 g SiO₂ and loaded on top of silica gel column (350 g, 63–200 μ , 3 × 100 cm) using MeOH/EtOAc as solvent and adopting gradient elution technique (0%, 5%, 10%, 15%, 20, 25, 30, 35, 40, 50 and 100%), using 1 liter each. Fractions 300–500 ml were collected. Fractions eluted by 5–20%

contained terpenoid compounds, which gave red colour after spraying with vanillin sulfuric acid reagent followed by heating with heat gun for 5 sec. The combined fractions afforded 4.5 g residue, after solvent evaporation under reduced pressure. The residue was loaded on 100 g Si gel pre-packed column. Elution started with EtOAc followed by EtOAc–MeOH, gradient 50 ml fractions were collected: frs.1–10 (EtOAc), frs.11–16 (2.5% MeOH), frs.17–21 (5% MeOH), frs.22–33 (10% MeOH), frs.34–38 (15% MeOH), frs.39–56 [EtOAc–MeOH–H₂O (10:2:1 v/v/v)]. Fractions 12–16 contained compound **4**. Fractions 40–41 contained **5**; fractions 42–56 contained **5** and **6** mixture. The residue after solvent evaporation of fractions 42–56 was loaded on Si gel Rp-C18 (50 g), elution was continued with MeOH–H₂O (1:1 v/v) and 20 ml fractions were collected. Fractions 17–38 contained the same components. The residue after solvent evaporation was re-loaded on normal phase Si gel column (80 g) and elution was done isocratic using CH₂Cl₂–MeOH–H₂O (10:4:0.5 v/v/v) mixture and 20 ml fractions were collected. Fractions 22–32 contained **5**, fractions 33–41 contained mixture of **5** and **6** and fractions 42–50 contained **6**. After solvent evaporation of the mixed fractions the residue was subjected to repeated isolation by the same procedure to enrich the isolated **5** and **6** to 66 mg and 30.6 mg, respectively.

3-O- β -D-glucopyranosiduronic acid, 28- β -D-glucopyranosyl-oleanolate **5**: needles, m.p. 220–222°, *R_f* **S2**- 0.18, **S3**- 0.37 and **S7**- 0.23. Positive FAB-MS: *m/z* 833 (M+K)⁺, 817 (M+Na)⁺, 633 (M+1–glucose)⁺, 617 (M+1–glucuronic acid)⁺, 477 (aglycone–H₂O+K)⁺, 439 (aglycone+1–H₂O)⁺. IR (KBr) $\nu_{\max}$ cm^{−1} 3450, 3425, 1740, 1735, and 1075. ¹H-NMR (600 MHz, DMSO-*d*₆): δ 0.68, 0.75, 0.87, 0.96, 1.08 (3H, each, all s, 25, 30, 26, 23, 27-CH₃), δ 0.86 (6H, s, 24, 29 CH₃). δ 3.02 (1H, dd, *J* 4.5, 12 Hz, H-3 α), δ 5.16 s H-12. **Glc-28**: δ (ppm) 5.24 (1H, d, *J* 8 Hz, H-1''); 3.10 m, H-2''; 2.95 m, H-3''; 3.13 m, H-4''; 2.95 m, H-5''; 3.62 (1H, d, *J* 11.3 Hz, H-6''a); 3.43 (1H, dd, *J* 11.3, 4.5 Hz, H-6''b). **GlcA-3**: δ 4.12 (1H, d, *J* 7.8 Hz, H-1'); 3.10 m, H-2'; 3.18 m, H-3'; 3.28 m, H-4'; 3.45 m, H-5'.

3-O- β -D-glucopyranoside, 28- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosiduronic acid oleanolate **6**: amorphous powder, *R_f* **S2** 0.11, **S3** 0.21 and **S7** 0.38. FAB⁺-MS: *m/z* 995 (M+K)⁺, 979

(M+Na)⁺, 957 (M+1)⁺, 833 (M+K–glucose), 817 (M+Na–glucose)⁺, 633 (M+1–2 glucose units)⁺, 617 (M+1–glucuronic acid–glucose)⁺, 477 (aglycone–H₂O+K)⁺, 439 (aglycone+1-H₂O)⁺. IR (KBr) $\nu_{\max}$ cm⁻¹ 3450, 3425, 1740, 1735, and 1075. ¹H-NMR (600 MHz, DMSO-*d*₆): δ 0.68, 0.74, 0.75, 0.96, 1.07 (3H, each, all s, 25, 30, 26, 23, 27-CH₃), δ 0.87 (6H, s, 24, 29 CH₃). δ 3.01 (1H, m, H-3 α), δ 5.16 s H-12. **GlcA-28**: δ (ppm) 5.23 (1H, d, *J* 7.8 Hz, H-1''); 3.25 m, H-2''; 3.21 m, H-3''; 3.42 m, H-4''; 3.36 m, H-5''. **Glc** (terminal): δ 4.41 (1H, d, *J* 8.4 Hz, H-1'''); 3.19, H-2'''; 3.39, H-3'''; 3.41, H-4'''; 3.09, H-5'''; 3.62 (d, *J* 13.8 Hz, H-6'''b); 3.67 (d, *J* 10.2 Hz, H-6'''a). **Glc-3**: δ 4.15 (1H, d, *J* 8.4 Hz, H-1'); 3.14 m, H-2'; 3.38 m, H-3'; δ 3.53 m, H-4'; 3.05 m, H-5'; δ 3.62 (d, *J* 13.8 Hz, H-6'b); 3.67 (d, *J* 10.2 Hz, H-6'a).

Alkaline hydrolysis

Ten mg (glycoside **5** and **6**) were refluxed in 5% KOH (5 ml) for 2 h. The mixture was adjusted to pH 6 with 1 N HCl, then extracted twice with 3 ml *n*-BuOH (saturated with water). The organic phase yielded the corresponding prosapogenin, and the aqueous hydrolysates contained glucose for

compound **5** and glucose and glucuronic acid for compound **6**, using **S8** and **S9**, after the repeated acid hydrolysis of the resulted disaccharide (Debella *et al.*, 2000).

Acid hydrolysis

The prosapogenins of the alkaline hydrolysis were refluxed with 10 ml HCl (10%) on a steam water bath for 3 h. Extraction with CHCl₃ afforded aglycones. The aglycone of compounds **5** and **6** was found identical with oleanolic acid (co-TLC using **S4** and **S5**). The aqueous hydrolysates were subjected to paper chromatography to detect the sugar moieties which was glucuronic acid for **5** and glucose for **6** using **S8** and **S9** (Debella *et al.*, 2000). Compounds **3** and **4** were subjected to acid hydrolysis according to Tandon, *et al.*, 1990.

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