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Triterpenoidal saponins from *Cornulaca monacantha*

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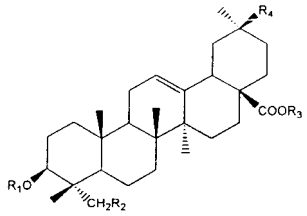
From the aerial parts of *Cornulaca monacantha*, three new triterpenoidal saponins have been isolated and their genuine structures were identified as 3-O-[β-xylopyranosyl-(1->3)-β-glucuronopyranosyl]-30-methylphytolaccagenate (**2**), 3-O-[β-xylopyranosyl-(1->3)-β-glucuronopyranosyl]-30-methylphytolaccagenate 28-O-β-gluco-pyranoside (**3**) and 3-O-[β-xylopyranosyl-(1->3)-β-glucurono-pyranosyl]-30-methylserjanate 28-O-βglucopyranoside (**4**) together with nine known saponins of oleanolic acid (**5**–**9**), hederagenin (**1**, **10**, **11**) and 30-methyl phytolaccagenate (**12**).

1. Introduction

Cornulaca monacantha Del. (Chenopodiaceae) is a wild desert Egyptian plant and known as Had [1]. Few reports revealed that the plant has potent molluscicidal, cercaricidal and miracidicidal effects [2]. The preliminary phytochemical screening of the plant showed the presence of alkaloids, coumarins and triterpenoidal saponins [3]. In addition, C-14 carboxy triterpenes (cornulacic acid and monocanthic acid) have been isolated from this plant [4–6]. The present study deals with the isolation and assignment of three new triterpenoidal saponins together with nine known compounds from the aerial parts of the plant.

2. Investigations, results and discussion

The ethanolic extract of the leaves and stems of *C. monacantha* was suspended in water and defatted with methylene chloride. The aqueous layer was subjected to a column of Diaion HP-20 and eluted successively with water, 50% aqueous methanol, methanol and acetone. Each of the methanol and 50% methanol eluates was devided into two parts (see exp.) and then further directed to two procedures. The first major part of the MeOH and 50% MeOH eluates was separately and repeatedly chromatographed on columns of silica gel and LiChroprep RP-18, then by HPLC to afford seven compounds from the methanol eluate (**5**–**9**, **11**, **12**) and five compounds from the 50% methanol eluate (**1**–**4**, **10**). Compounds **1**–**3**, **5**, **9** and **10** have been isolated after methylation with CH_2N_2. The second part containing a mixture of each of the prementioned eluates was subjected to acid hydrolysis, further isolation and identification of the genuine sapogenins, where it afforded oleanolic acid, hederagenin, 30-methyl serjanate and 30-methyl phytolaccagenate (comparison of their ^{13}C NMR with lit. data [10] and identical Rf values and m.p. with authentic sample).



	R1	R2	R3	R4
1	GlcA* —(3)— Xyl	OH	Glc	Me
2	GlcA* —(3)— Xyl	OH	Me*	COOMe
3	GlcA* —(3)— Xyl	OH	Glc	COOMe
4	GlcA —(3)— Xyl	H	Glc	COOMe
5	GlcA* —(3)— Glc	H	Glc	Me
6	Glc —(2)— Glc	H	Glc	Me
7	Glc —(4)— Glc	H	Glc	Me
8	H	H	Glc	Me
9	GlcA* —(3)— Xyl	H	Glc	Me
10	GlcA* —(3)— Glc	OH	Glc	Me
11	GlcA —(3)— Xyl	OH	H	Me
12	H	OH	Glc	COOMe

GlA: B-glucuronopyranose, Glc: B-glucopyranose, Xyl: B-xylopyranose GlcA*: B-methylglucuronate where the methyl group is introduced by CH_2N_2, Me*: Methyl group not genuine and introduced by CH_2N_2

2.1. Identification of saponin 1

^{13}C and ^{1}H NMR spectral data of compound **1** were coincident with those reported for hederagenin 3-O-[β-xylopyranosyl (1->3)-β-glucuronopyranosyl]−28-O-β-glucopyranoside previously isolated from *Chenopodium quinoa* [7].

2.2. Identification of saponin 2

The molecular formula of compound **2** was determined as $C_{44}H_{68}O_{16}$ from HR-FAB mass spectrometry. Acid hydrolysis of **2** yielded 28, 30-dimethylphytolaccagenate as aglycone together with xylose and glucuronic acid methyl ester. The ^{13}C NMR spectral data of **2** (Table) were superimposable with those reported for 3-substituted 30-methyl phytolaccagenate [7]. The downfield shift of C-3 of the aglycone (+8.2 ppm) together with the upfield shift of C-2 (−1.6 ppm) indicated the attachement of the sugar moiety to this position [8]. Moreover, the downfield shift of C-3 of the glucuronopyranosyl unit (+8.2 ppm) proved its substitution with the xylopyranosyl unit [9]. ^{1}H NMR spectral analysis of **2** showed five singlets (δ 1.17, 1.15, 0.92, 0.89 and 0.81) for five methyl groups in addition to a multiplet at δ 3.2 for H-3 of the aglycone. The β configuration of the glucuronopyranosyl and xylopyranosyl units were determined from the coupling constants (each 7.6 Hz) of their anomeric proton doublets at δ 5.2 and 5.3 respectively [9]. The assignment was substantiated by negative FAB MS which revealed a quasi-molecular ion peak at m/z 851 $[M\text{-}H]^-$ together with other significant peaks at m/z 836 $[M\text{-}H\text{-}Me]^-$, 704 and 528 due to loss of xylose and glucuronic acid respectively. Consequently, compound **2** was assigned as 3-O-[β-xylopyranosyl-(1->3)-β-methylglucuronopyranosyl]-28,30-dimethylphytolaccagenate, and its genuine structure as 3-O-[β-xylopyranosyl-(1->3)-β-glucurono-pyranosyl]-30-methylphytolaccagenate.

2.3. Identification of saponin 3

The molecular formula of compound **3** was determined as $C_{49}H_{76}O_{21}$ from HR-FAB mass spectral analysis. By acid hydrolysis, **3** yielded 30-methylphytolaccagenate as aglycone together with xylose, glucose and glucuronic acid methyl ester. ^{13}C NMR spectral data of **3** (Table) were almost similar to those of **2**. However the signals at

Table: ^{13}C NMR Spectral data of compounds 2–4 (100 MHz, pyridine-d_5)

C	2	3	4	C	2	3	4
1	38.5	38.5	38.6	OMe	51.6	52.1	51.7
2	26.1	26.0	26.5		51.7	51.6	
3	82.2	82.2	89.2		52.1		
4	43.2*	43.4*	39.9*	GlcA			
5	47.3	47.2	55.7	1	106.1	105.9	106.2
6	18.1	18.0	18.2	2	74.5*	73.9	74.6*
7	32.7	32.7	33.1	3	86.2	86.1	86.4
8	39.6	39.8	39.5*	4	70.9	70.8	70.9
9	48.0	48.0	48.0	5	78.1	77.9	78.1
10	36.8	36.7	37.0	6	170.2	170.1	172.0
11	23.7	23.4	23.5				
12	122.9	123.4	124.0	Xyl			
13	143.8	143.7	143.8	1	106.3	106.1	106.8
14	41.8	41.9	42.0	2	75.4*	75.2	75.3
15	28.3	28.2	28.3	3	76.8	76.6	77.5
16	23.6	23.7	23.8	4	71.1	71.0	71.4
17	46.4	46.4	46.5	5	67.4	67.2	67.3
18	43.5*	42.6	44.0				
19	42.3	42.3	42.2	Glc			
20	43.9*	43.9*	43.1	1		95.6	95.8
21	30.5	30.4	30.6	2		74.4	74.1*
22	34.1	33.9	34.0	3		78.7	78.9
23	63.9	64.6	28.3	4		70.8	70.9
24	13.6	13.5	16.9	5		79.1	79.3
25	16.0	16.0	15.5	6		61.8	61.9
26	17.1	17.4	17.4				
27	26.1	26.0	26.1				
28	177.6	176.8	176.9				
29	28.2	28.2	28.1				
30	177.0	176.0	176.1				

* Values may be interchangeable in each column.

δ 95.6, 74.4, 78.7, 70.8, 79.1 and 61.8 indicated the presence of an additional β-glucopyranosyl unit [8]. The upfield shift of the anomeric carbon signal (δ 95.6) of the glucopyranosyl unit indicated its attachment to C-28 of the aglycone [9]. Besides, ^{13}C NMR data of the sugar moiety were coincident with those of compound **1**. On the other hand, the alkaline hydrolysis of compound **3** followed by methylation with CH_2N_2 gave compound **2** confirming the attachment of the β-glucosyl unit to C-28. The ^{1}H NMR spectrum of **3** showed three doublets at δ 6.2 (J = 8.1 Hz), 5.2 (J = 7.3 Hz) and 5.1 (J = 7.3 Hz) for the anomeric protons of β-glucopyranose, β-xylopyranose and β-glucuronic acid respectively. Positive FAB MS of **3** exhibited peaks at m/z 1023 $[M+Na]^+$, 891 $[M+Na\text{-xyl}]^+$, 861 $[M+Na\text{-Glc}]^+$ and 729 [891-Glc or 861-Xyl]$^+$. Therefore, the structure of compound **3** was assigned as 3-O-[β-xylopyranosyl-(1->3)-β-glucuronopyranosyl]−30-methylphytolaccagenate 28-O-β-glucopyranoside.

2.4. Identification of saponin 4

The molecular formula of compound **4** was determined as $C_{48}H_{74}O_{20}$ from HR-FAB mass spectrometry. ^{13}C NMR spectral data (Table) revealed that **4** contains the same sugar moiety as **3** with different aglycone. The absence of the methylene signal at δ 64.6 (C-23) in addition to the downfield shift of C-3 from δ 82.2 in **3** to 89.2 in **4** indicated that this aglycone is 30-methylserjanate. In addition, the aglycone signals were superimposable with those reported for 3,28-disubstituted methylserjanate [7]. The acid hydrolysis of compound **4** afforded xylose, glucose, glucuronic acid together with 30-methylserjanate as aglycone. The ^{1}H NMR spectrum of **4** showed three doublets at δ 6.3 (J = 8.1 Hz), 5.3 (J = 7.6 Hz) and 5.0 (J = 7.8 Hz) for the anomeric protons of β-glucopyranose, β-xylopyranose and β-glucuronic acid respectively. The singlets at δ 1.3, 1.2, 1.1, 0.96 and 0.78 were obvious for the six methyl groups of the aglycone. Negative FAB MS of **4** exhibited a quasi-molecular ion peak at m/z 969 $[M-H]^-$ together with other peaks at m/z 837 $[M-H\text{-Xyl}]^-$, 807 $[M-H\text{-Glc}]^-$, 675 [837-Glc or 807-Xyl]$^-$ and 499 [675-GlcA]$^-$. Consequently, the structure of compound **4** was assigned as 3-O-[β-xylopyranosyl-(1->3)-β-glucuronopyranosyl]-30-methyl serjanate 28-O-β-glucopyranoside.

2.5. Identification of saponins 5–12

The structures of compounds **5–12** have been determined as shown by comparison of their spectroscopic data with those reported in the literature [10–14]. However, this is the first report for isolation of these compounds from the genus *Cornulaca*.

3. Experimental

3.1. Instruments

NMR spectra were recorded in pyridine-d_5 using JEOL JNM A-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) with TMS as int. standard. MS were recorded by a JEOL JMS-SX 102 spectrometer. Optical rotations were measured by a Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (150 X 20 mm i.d., YMC) and polyamine II (250 × 20 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. The flow rate was 6 ml/min. TLC was carried out on precoated silica gel plates (kieselgel 60 F_{254}, Merck). For CC, silica gel G (E. Merck), LiChroprep RP-18 (40–63 m, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) CH_2Cl_2−MeOH−H_2O (6.5 : 3.5 : 0.5), (II) 30–50% MeOH, (III) 40% MeOH, (IV) 30% MeOH, (V) 90% MeCN, (VI) CH_2Cl_2−MeOH−H_2O (7 : 3 : 0.3), (VII) 45% MeOH, (VIII) 38% MeOH, (IX) 35% MeOH, (X) EtOAc-MeOH-H_2O (7 : 3 : 0.3), (XI) EtOAc-MeOH−H_2O (6.5 : 3.5 : 0.5), (XII) CH_2Cl_2−MeOH−H_2O (6 : 4 : 1) and (XIII) 90% MeOH. The spray reagent: 10% H_2SO_4 in EtOH. Reference samples of oleanolic, hederagen-

in, 30-methyl phytolaccagenate and 30-methyl serjanate were obtained from the Institute of Pharmaceutical Sciences, Faculty of Medicine, Hiroshima University, Hiroshima, Japan.

3.2. Plant materials

The plant material was collected in April, 1997 from Wady El-Assiuty area of the Eastern Desert of Egypt. The identity of the plant was confirmed by Prof. N. EL-Hadidi, Dept. of Botany and Plant Taxonomy, Faculty of Science, Cairo University. A voucher sample is kept in the Herbarium of the Faculty of Pharmacy, Assiut University, Egypt.

3.3. Extraction and isolation

The dried leaves and stems (3 kg) of *Cornulaca monacantha* were exrtacted with EtOH. After removal of the solvent by evapn, the residue (500 g) was defatted with CH_2Cl_2. The aqueous layer was subjected to a column of Diaion HP-20 and eluted with H_2O, 50% MeOH, MeOH and Me_2CO successively. The eluate of 50% MeOH (30 g) was divided into two parts. The first part was kept for acid hydrolysis and the second part was repeatedly chromatographed on silica gel (system I) and RP-18 (system II) columns followed by ODS MPLC (Tosoh MPLC system, pump HLC 803 D) using system III as eluent to afford five fractions. Fraction 4 was subjected to HPLC on ODS and polyamine columns (systems IV and V respectively) to give compound **4**. Fractions 1–3 have been methylated with CH_2N_2 then subjected to HPLC on polyamine column (system V) to afford four compounds (**1**–**3** and **10**). The MeOH eluate (20 g) was similarly monitored as the 50% MeOH eluate where the first part (5 g) was kept for acid hydrolysis and the second part (15 g) was chromatographed on silica gel (system VI) and RP-18 (system VII) columns followed by ODS MPLC (system VIII) to give four fractions. Fraction 3 was subjected to HPLC using ODS column (system IX) to provide five compounds (**6**–**8**, **11** and **12**). Fraction 2 was methylated by CH_2N_2 and subjected to HPLC on ODS column (system XIII) to afford two compounds (**5** and **9**).

3.4. Identification of compound 1

3-O-[β-Xylopyranosyl (1->3)-β-methylglucuronopyrano-syl]hederagenin-28-O-β-glucopyranoside. White powder (55 mg), R_f 0.78 (system I).

3.5. Identification of compound 2

3-O-[β-Xylopyranosyl-(1->3)-β-methyl glucuronopyrano-syl]-28,30-dimethyl-phytolaccagenate. White powder (12 mg), $[\alpha]_D^{20}$ − 34.2° (MeOH, c 3.0). R_f 0.85 (system I). ^{1}H NMR (pyridine-d_5): δ 5.3 (1 H, d, J = 7.6 Hz, H-1 Xyl), 5.2 (1 H, d, J = 7.6 Hz, H-1 GlcA), 3.7, 3.63 and 3.61 (9 H, each s, 3 OMe), 3.2 (1 H, m, H-3) and 1.17, 1.15, 0.92, 0.89, 0.81 (15 H, each s, Me-24, 25, 26, 27 and 29). Negative HR-FAB mass; Found: 851.4379 $[M-H]^-$ ($C_{44}H_{68}O_{16}$.H requires 851.4429).

3.6. Identification of compound 3

3-O-[β-Xylopyranosyl-(1->3)-β-methyl glucuronopyrano-syl]-30-methylphytolaccagenate 28-O-β-glucopyranoside. White powder (60 mg), $[\alpha]_D^{20}$ − 17.6° (MeOH, c 2.80), R_f 0.73 (system I). ^{1}H NMR (pyridine-d_5): δ 6.2 (1 H, d, J = 8.1 Hz, H-1 Glc), 5.2 (1 H, d, J = 7.3 Hz, H-1 Xyl), 5.1 (1 H, d, J = 7.3 Hz, H-1 GlcA), 3.7 and 3.61 (6 H, each s, 2 OMe), 3.20 (1 H, m, H-3) and 1.2, 1.1, 1.0, 0.86, 0.85 (15H, each s, Me-24, 25, 26, 27 and 29). Negative HR-FAB mass; Found: 999.4731 $[M-H]^-$ ($C_{49}H_{76}O_{21}$.H requires 999.4800).

3.7. Identification of compound 4

3-O-[β-Xylopyranosyl-(1->3)-β-glucuronopyranosyl]-30-methylserjanate 28-O-β-glucopyranoside. White powder (45 mg), $[\alpha]_D^{20}$ − 8.2° (MeOH, c 0.95), R_f 0.55 (system I). ^{1}H NMR (pyridine-d_5): δ 6.3 (1 H, d, J = 8.1 Hz, H-1 Glc), 5.3 (1 H, d, J = 7.6 Hz, H-1 Xyl), 5.0 (1 H, d, J = 7.8 Hz, H-1 GlcA), 3.6 (3 H, each s, OMe), 3.4 (1 H, m, H-3) and 1.3, 1.2, 1.1, 0.96, 0.78 (18 H, each s, Me-23, 24, 25, 26, 27 and 29). Negative HR-FAB mass; Found: 969.4687 $[M-H]^-$ ($C_{49}H_{76}O_{21}$.H requires 969.4687).

3.8. Identification of compounds 5–12

5: 3-O-[β-Glucopyranosyl-(1->3)-β-methylglucurono-pyranosyl]-oleanolic acid 28-O-β-glucopyranoside. White powder (60 mg), R_f 0.75 (system X).
6: 3-O-[β-Glucopyranosyl-(1->2)-β-glucopyranosyl]-oleanolic acid 28-O-β-glucopyranoside. White powder (65 mg), R_f 0.40 (system X).
7: 3-O-[β-Glucopyranosyl-(1->4)-β-glucopyranosyl]-oleanolic acid 28-O-β-glucopyranoside. White powder (38 mg), R_f 0.42 (system X).
8: oleanolic acid 28-O-β-glucopyranoside. White powder (25 mg), R_f 0.65 (system X).
9: 3-O-[β-Xylopyranosyl-(1->3)-β-methyl glucurono-pyranosyl]-oleanolic acid 28-O-β-glucopyranoside. White powder (40 mg), R_f 0.79 (system X).
10: 3-O-[β-Glucopyranosyl-(1->3)-β-methyl glucurono-pyranosyl]-hederagenin 28-O-β-glucopyranoside. White powder (15 mg), R_f 0.70 (system XI).
11: 3-O-[β-Xylopyranosyl-(1->3)-β-glucuronopyranosyl]-hederagenin. White powder (15 mg), R_f 0.37 (system XI).
12: 30-Methylphytolaccagenate 28-O-β-glucopyranoside. White powder (25 mg), R_f 0.84 (system XI).The structure of these compounds have been determined by comparison of their spectroscopic data with those reported in literature [10–14].

3.9. Alkaline hydrolysis

Compound **3** (15 mg) was hydrolysed by heating with 1 M aq. NaOH (2 ml) in a sealed tube at 70 °C for 2 h. The reaction mixture was neutralized with Amberlite MB-3 resin and then partitioned between H_2O and *n*-BuOH. The n-BuOH layer was concentrated to dryness. The prosapogenin was isolated and purified by HPLC then identified by TLC and spectral analysis.

3.10. Acid hydrolysis of the total saponins

Five g from each of the MeOH and 50% MeOH eluates were refluxed with 10% aqueous HCl (300 ml). The total sapogenins were extracted with $CHCl_3$ and the residue of the $CHCl_3$ extract (ca 2.1 g) was directed for further isolation. The aqueous layer was neutralized by Amberlite MB-3 resin and dried. The total sugars were identified as glucuronic acid, glucose, and xylose by TLC against reference samples using system XII.

3.11. Isolation of the aglycones:

The residue of the total sapogenins (2.1 g) was chromatographed on a silica gel column using $CHCl_3$-MeOH gradient. Elution with $CHCl_3$−MeOH (99.5 : 0.5) afforded oleanolic acid (500 mg). Elution with $CHCl_3$−MeOH (99 : 1) yielded hederagenin (220 mg). While elution with $CHCl_3$−MeOH (98 : 2) furnished 30-methyl serjanate and further elution afforded 30-methyl phytolaccagenate. The four sapogenins were identified as oleanolic acid, hederagenin, 30-methyl serjanate and 30-methyl phytolaccagenate by direct authentication (m.p., cochromatography and comparison of their ^{13}C NMR with the lit. [10]).

3.12. Acid hydrolysis

Compounds **2** (8 mg), **3** and **4** (each 15 mg) were heated with 10% aq. HCl (2 ml) in a sealed tube at 80 °C for about 4 h. The sapogenins were extracted with Et_2O and identified as 28, 30-dimethyl phytolaccagenate, 30-methyl phytolaccagenate (**2**, **3** respectively) and methyl serjanate (**4**) by comparison of their ^{13}C NMR spectral data with those reported in the literature [10]. The aqueous layer was neutralized by Amberlite MB−3 resin and dried. Sugars were identified by comparison with authentic samples on TLC with system XII.

3.13. Methylation

The fractions containing compounds **1**–**3**, **5**, **9** and **10** were dissolved in MeOH and treated with ethereal CH_2N_2. The products were concentrated and purified by polyamine and ODS HPLC (systems V and XIII respectively).

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References

1 Tackholm, V.: Students Flora of Egypt; p. 128, Cairo Univ. Press, 1974
2 El-Sayed, M. M.; El-Nahas, H. A.: Egypt. J. Pharm. Sci. **43**, 539 (1993).
3 Rizk, A. M.: The Phytochemistry of Flora of Qatar. p. 33 Kingprint of Richmond 1986
4 Dawidar, A. M.; Reisch, J.; Amer, M. A.: Egypt. J. Chem. **23**, 31 (1980).
5 Amer, A. M.; Dawidar, A. M.; Fayez, M. B.: Planta Med. **26**, 289 (1974)
6 Dawidar, A. M.; Amer, M. A.: Egypt. J. Chem. **17**, 869 (1974)
7 Mizui, F.; Kasai, R.; Ohtani, K.; Tanaka, O.: Chem. Pharm. Bull. **38**, 375 (1990)
8 Braddbury, H.; Jenkins, J.: J. Carbohydr. Res. **126**, 125 (1984)
9 Agrawal, P.: Phytochemistry **31**, 3307 (1992)
10 Mahato, S. B.; Kundu, A.: Phytochemistry **37**, 1517 (1994)
11 Romussi, G.; Cafaggi, S.; Ringnardi, G.: Pharmazie **35**, 498 (1980)
12 Maria, L.; Pizza, C.: Planta Med. **57**, 250 (1991)
13 Mahato, S. B.; Sarkar, S. K.; Poddar, G.: Phytochemistry **27**, 3037 (1988)
14 Mahato, S. B.; Garai, S.: Progress in the Chemistry of Organic Natural Products, Vol. 74, p. 2, Springer-Verlag, Wien 1998

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