SUPPLEMENTARY MATERIAL

A new pregnane glycoside from *Gomphocarpus fruticosus* growing in Egypt

Amani M. Marzouk^{a*}, Samir M. Osman^b and Ahmad A. Gohar^a

a Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

b Department of Pharmacognosy, Faculty of Pharmacy, 6th of October University, 6th of October City 12566, Egypt.

Fax: +2-050-2247496.

E-mail: amarzouk2003@yahoo.co.uk

* Author for correspondence and reprint requests

Abstract

Phytochemical investigation of *Gomphocarpus fruticosus* (L.) Ait. of Egyptian origin afforded the new pregnane glycoside lineolon-3-O- $[\beta$ -D-oleandropyranosyl-(1-4)- β -D-cymaropyranosyl-(1-4)- β -D-cymaropyranoside], along with six known compounds. The structures of the isolated compounds were elucidated on the basis of extensive spectroscopic evidences derived from 1D, 2D-NMR experiments, mass spectrometry and by comparing their physical and spectroscopic data to literature. These included the triterpenoids 3β -taraxerol, 3β -taraxerol acetate, and betulinic acid, which are identified for the first time in *G. fruticosus* and the cardenolides uzarigenin, gomphoside and calotropin.

Key words: *Gomphocarpus fruticosus*; Asclepiadoideae; Apocynaceae; pregnane glycosides; gomphofruticososide; cardenolides; triterpenoids

Table S1. NMR data of compounds 1-3*

No.	1**		2†		3†	
	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C
1		38.5		38.7		38.8
2		26.2		24.7		26.8
3	4.48, dd (7.0, 10.0)	81.1	4.76, m	80.2	3.14, m	78.6
4		39.2		40.7		38.9
5		55.5		55.6		55.5
6		18.3		18.1		18.3
7		34.2		34.1		34.3
8		41.2		39.7		40.7
9		50.4		50.5		51.0
10		37.8		38.3		37.0
11		16.6		16.2		20.9
12		34.3		33.9		25.6
13		38.9		39.4		38.3
14	5.25, m	154.7	5.12, m	155.4		42.4
15		118.9		117.6		30.5
16		38.5		38.1		32.2
17		38.4		36.7		56.2
18		48.4		48.7	2.96, m	47.0
19		42.3		41.8		49.1
20		29.7		29.4		150.7
21		36.8		36.7		29.6
22		37.1		37.7		37.1
23	1.01, s	28.0	0.97, s	28.0	0.89, s	27.7
24	0.83, s	16.6	0.83, s	16.2	0.70, s	15.2
25	1.01, s	16.4	1.04, s	16.0	0.78, s	15.7
26	1.02, s	26.2	1.13, s	27.3	0.93, s	15.9
27	1.00, s	23.8	1.02, s	23.6	0.90, s	14.5
28	0.84, s	29.7	0.84, s	29.6		179.1
29	0.92, s	34.3	0.89, s	34.3	4.53, br s 4.66, br s	109.3
30	0.87, s	23.8	0.87, s	23.4	1.64, s	19.0
COCH ₃	2.03, s	21.5				
COCH ₃		171.1				

* Spectra run in CH₂Cl₂-CD₃OD, 4:1. δ values in ppm and J values (between parentheses) in Hz.**at 400 MHz (¹H) and 100 MHz (¹³C).† at 500 MHz (¹H) and 125 MHz (¹³C).

Table S2. NMR data of compounds **4**, **6-7***

No.	4		6		7	
	H	C	H	C	H	C
1		37.7		41.6		34.2
2		29.5	3.94, m	68.5	4.00, dd (12.5, 4.0)	68.1
3	3.73, m	70.9	3.88, dd (12.5, 4.0)	71.5	3.85, ddd (9.5, 8.0, 2.0)	71.2
4		35.3		37.6		32.6
5		44.1		48.6		44.1
6		28.2		29.2		28.9
7		27.2		27.5		27.2
8		41.5		44.1		42.9
9		49.3		48.7		49.9
10		36.9		35.3		52.4
11		21.0		20.7		21.4
12		39.6		38.3		38.5
13		49.5		48.6		49.2
14		85.3		81.2		83.9
15		32.9		31.7		38.7
16		26.6		25.8		26.3
17	2.70, dd (9.2, 5.6)	50.6	2.58, dd (9.0, 4.5)	50.3	2.69, dd (9.0, 4.5)	50.1
18	0.75, s	15.53	0.69, s	16.1	0.89, s	15.1
19	0.81, s	12.0	0.58, s	13.3	0.79	207.7
20		175.8		175.3		175.7
21	4.73, dd (18.0, 1.0) 4.87, dd (18.0, 1.0)	73.2	4.66, dd (18.0, 1.0) 4.82, dd (18.0, 1.0)	73.6	4.63, dd (18.0, 1.0) 4.86, dd (18.0, 1.0)	73.5
22	5.81, br s	117.5	5.64, br s	117.2	5.69, br s	116.8
23		174.3		175.1		175.5
1`			4.86, s	95.3	4.78, s	95.5
2`				90.9		90.9
3`			3.67, m	72.4	3.72, m	72.3
4`				37.8		
5`			3.80, m	67.9	3.38, m	67.8
6`			1.20, d (6.0)	20.4	1.02, d (6.0)	20.4

*Spectra run in CDCl₃-CD₃OD (4:1) at 400 MHz (¹H) and 100 MHz (¹³C). δ values in ppm and J values (between parentheses) in Hz.

Physical and spectroscopic data of the isolated compounds:

3 β -taraxerol acetate (1): crystalline solid, m.p. 303-305 ° C (lit. 302-305 ° C), R_f : 0.61 (pet ether /EtOAc, 80:20). ^1H and ^{13}C -NMR (400 and 100 MHz, CDCl_3 - CD_3OD): (Table S1). EIMS 70 eV, m/z (rel. int.): 468 $[\text{M}]^+$ (16), 408 $[\text{M}-\text{HOAc}]^+$ (35), 393 $[\text{408}-\text{CH}_3]^+$ (43), 344 (46), 204 $[\text{C}_{15}\text{H}_{24}]^+$ (100).

13 α -methyl,27-norolean-14-en-3 β -ol(3 β -taraxerol) (2): crystalline solid, m. p.282-284 ° C (lit. 283-285 ° C), R_f : 0.48 (pet ether /EtOAc, 80:20). ^1H and ^{13}C -NMR (500 and 125 MHz, CDCl_3 - CD_3OD): (Table S1). EI-MS 70 eV, m/z (rel. int.): 426 $[\text{M}]^+$ (11), 411 $[\text{M}-\text{CH}_3]^+$ (14), 302 (52), 287 (40), 218 (37), 204 (100), 189 (30), 135 (19), 121 (24), 107 (22), 95 (23).

Betulinic acid (3): crystalline solid, m. p. 295-297 ° C (lit. 295-298 ° C), R_f : 0.27 (pet ether /EtOAc, 80:20), 0.44 (CH_2Cl_2 -MeOH, 95:5). ^1H and ^{13}C -NMR (500 and 125 MHz, CDCl_3 - CD_3OD): (Table S1). EIMS 70 eV: m/z (rel. int.) 456 $[\text{M}]^+$ (5), 441 $[\text{M}-\text{CH}_3]^+$ (10), 438 $[\text{M}-\text{H}_2\text{O}]^+$ (18), 248 (9.5), 85 (75.1), 83 (100).

uzarigenin (4): crystalline solid, m. p. 232-234 ° C (lit. 230-232 ° C) R_f : 0.52 (CH_2Cl_2 /MeOH, 95:5); 0.22 (MeOH/ H_2O , 70:30 on silica gel 60 RP-18). ^1H and ^{13}C -NMR (500 and 150 MHz, CDCl_3 - CD_3OD): (Table S2). EIMS 70 eV: m/z (rel. int.) 374 $[\text{M}]^+$ (4), 356 $[\text{M}-\text{H}_2\text{O}]^+$ (21), 338 $[\text{M}-2\text{H}_2\text{O}]^+$ (33), 219 (38), 201 (46), 111 (70), 83(100).

Lineolon-3-O- β -D-cymaropyranosyl-(1-4)- β -D-cymaropyranosyl-(1-4)- β -D-

Oleandropyranose (5): amorphous solid, m.p. 160 – 162 ° C. $[\alpha]_{\text{D}}^{25}$ - 7.6 (c 0.1, CHCl_3). R_f : 0.20 (pet ether / CH_2Cl_2 , 50:50 containing 5% MeOH). IR (KBr) ν_{max} : 3450, (-OH), 2920 (CH), 1780 (C=C ketone), 1705 (C=C), 1180 (C-O), 1100 cm^{-1} . ^1H NMR (400 MHz, CDCl_3 - CD_3OD): δ 0.99 (m, H-1), 1.51 (m, H-2a), 1.78 (m, H-2b), 3.80 (m, H-3), 2.12 (m,H-4a) 2.31 (m, H-4b), 5.27 (s, H-6), 2.15 (m, H-7), 1.40 (dd, J = 4.0, 11.0 Hz, H-9), 1.69(m, H-11a), 1.86 (m, H-11b), 3.16 (dd, J ,11.4, 4.1 Hz, H-12), 1.65 (m, H-15a) 1.73 (m, H-15b), 1.85 (m, H-16a), 1.95 (m, H-16b), 3.35 (m, H-17), 1.21 (s, CH_3 -18), 1.07 (s, CH_3 -19), 2.18 (s, CH_3 -21), 4.68 (dd, J = 9.6, 2.0, H-1`), 2.02 (m, H-2`), 3.07 (m, H-3`), 3.15 (m, H-4`), 3.61 (m, H-5`), 1.15 (d,  $J$  = 6.5 Hz,  $\text{CH}_3$ -6`), 3.41 (s, OMe), 4.47 (dd, J = 9.2, 2.0 Hz, H1``), 2.12 (m, H-2``), 3.09 (m, H-3``), 3.14 (m, H-4``), 3.80 (m, H-5``), 1.16 (d,  $J$  = 6.0 Hz,  $\text{CH}_3$ -6``), 3.41 (s, OMe), 4.43 (dd, J = 9.6, 2.0 Hz, H1``'), 2.25 (m, H-2``'), 3.49 (m, H-3``'), 3.10 (m, H-4``'), 3.21 (m, H-5``'), 1.25 (d, J = 6.0 Hz,

CH₃-6''), 3.32 (s, OMe). ¹³CNMR (100 MHz, CDCl₃-CD₃O D): δ 38.7 (C-1), 27.2 (C-2), 78.0 (C-3), 38.8 (C-4), 141.2 (C-5), 117.4 (C-6), 34.2 (C-7), 74.6 (C-8), 44.1 (C-9), 37.1 (C-10), 28.9 (C-11), 68.5 (C-12), 55.9 (C-13), 85.7 (C-14), 33.1 (C-15), 23.2 (C-16), 60.7 (C-17), 13.0 (C-18), 18.9 (C-19), 214.8 (C-20), 31.8 (C-21), 96.1 (C-1'), 34.3 (C-2'), 78.0 (C-3'), 82.4 (C-4'), 68.3 (C-5'), 18.17 (CH₃-6'), 58.0 (OMe), 99.6 (C-1''), 34.4 (C-2''), 82.6 (C-3''), 75.3 (C-4''), 68.3 (C-5''), 18.23 (CH₃-6''), 58.2 (OMe), 101.4 (C-1'''), 37.1 (C-2'''), 75.3 (C-3'''), 80.6 (C-4'''), 71.5 (C-5'''), 18.0 (CH₃-6'''), 56.3 (OMe). HRFABMS (positive ion mode) m/z (rel. int.) 819.4511 (100) [M+Na]⁺ (calc. 819.4507), 796.4613 (26) [M]⁺ (calc. 796.4609). EI-MS 70 eV, m/z (rel. int.): 798 [M+2]⁺ (4), 797 [M+1]⁺ (2.5), 778 [M-H₂O]⁺, 753 [M-CH₃C=O]⁺ (1.5), 608 [M-CH₃C=O-Sugar]⁺ (9), 429 [M-CH₃C=O-2 Sugars-2H₂O]⁺ (57), 321 [M-CH₃C=O-3 Sugars]⁺ (29), 267 (20), 167 (31), 83 (50), 69 (100).

Gomphoside (6): crystalline solid, m. p. 236-238 ° C (lit. 235-238 ° C), *R_f*: 0.30 (pet ether/CH₂Cl₂/MeOH, 45:45:5). ¹H and ¹³C-NMR (500 and 125 MHz, CDCl₃-CD₃OD): (Table S2). FABMS (positive ion mode) m/z (rel. int.) 518 [M]⁺ (22), 519 [M + H]⁺ (100), 500 [M-H₂O]⁺ (26), 389 [M-sugar]⁺ (33).

Calotropin (7): crystalline solid, m. p 207-209 ° C (lit. 209-212 ° C). *R_f*: 0.23 (pet ether/CH₂Cl₂/MeOH, 45:45:5). ¹H and ¹³C-NMR (500 and 125 MHz, CDCl₃-CD₃OD): (Table S2). FABMS (positive ion mode) m/z (rel. int.) 532 [M]⁺ (4), 533 [M + H]⁺ (100), 514 [M-H₂O]⁺ (22), 404 [M-sugar]⁺ (34).

Fig. S1 ^1H -NMR spectrum of (5)

DR AMANY (60-85-2)

PROTON_BSU CDCl₃ {C:\data} nmr 19

Fig. S2 Expansion of ^1H -NMR spectrum of (5)

DR AMANY (60-85-2)

PROTON_BSU CDC13 {C:\data} nmr 19

Fig. S3. ^{13}C -NMR spectrum of (5)

AMANY-60-85-2

C13-BSU CDC13 {C:\data} nmr 19

Fig. S4. Expansion of ^{13}C -NMR spectrum of (5)

AMANY-60-85-2

C13-BSU CDCl₃ {C:\data} nmr 19

Fig. S5. Expansion of ^{13}C -NMR spectrum of (5)

AMANY-60-85-2

C13-BSU CDCl₃ {C:\data} nmr 19

Fig. S6. HMBC spectrum of (5)

Fig. S7. HMBC correlations of **(5)**

Fig. S8. Expansion HMBC spectrum of (5)

Fig. S9. Expansion HMBC spectrum of (5)

AMANY-60-85-2

HMBC CDCl₃ {C:\data} nmr 19

Fig. S10. HSQC spectrum of (5)

AMANY-60-85-2

HSQC CDCl₃ {C:\data} nmr 19

Fig. S11. Expansion of HSQC spectrum of (5)

AMANY-60-85-2

HSQC CDCl₃ (C:\data) nmr 19

Fig. S12. Expansion of HSQC spectrum of (5)

AMANY-60-85-2
HSQC CDCl₃ {C:\data} nmr 19

Fig. S13. EI Mass spectrum of (5)