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## Quadrangulosides A–F: new pregnane glycosides from *Caralluma quadrangula*†

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The phytochemical investigation of *Caralluma quadrangula* aerial parts yielded six new pregnane glycosides, quadrangulosides A–F (1–6), in addition to nine known pregnane glycosides and three known flavone glycosides. Structures of isolated phyto-constituents were elucidated *via* spectroscopic 1D-, 2D-NMR and spectrometric ESI-MS spectra.

### Introduction

The genus *Caralluma* comprises a group of tender, perennial, succulent plants belonging to the family Apocynaceae, subfamily Asclepiodoidae.<sup>1</sup> *Caralluma* classified to 2500 species which are distributed throughout Africa and Asia.<sup>2</sup> *Caralluma quadrangula* (Forssk.) is a wild medicinal succulent leafless perennial, edible herb that grows in hilly and stony regions.<sup>3–6</sup> Some of the Arabian folklore uses of *C. quadrangula* include treatment of liver diseases, diabetes, hypertension, snake venom, scorpion bite, cancer, tuberculosis, skin disorders, fever, inflammation, rheumatic arthritis, ulcers, leprosy and as antiseptics and disinfectants,<sup>7</sup> freckles, vitiligo, melisma, and to fulfill hunger and thirst.<sup>3,8</sup> Several pregrane glycosides including acylated boucerosides<sup>9</sup> and russeliosides,<sup>10</sup> in addition to the flavonoid luteolin 4'-O-β-D-neo-hesperidoside<sup>10</sup> were elucidated previously from *Caralluma quadrangula*.<sup>11</sup> Pregnane glycosides, flavone glycosides, terpenoids, and sterols are the most frequently bioactive constituents characterize the genus *Caralluma* with significant therapeutic activities<sup>8</sup> such as antioxidant, antidiabetic, anticancer, antimicrobial, anti-malarial, anti-inflammatory, and antieczema.<sup>4,7</sup> We aimed in this article to attain further investigation of this important species.

### Results and discussion

Application of various chromatographic separations for the methanolic extract of *C. quadrangula* aerial parts afforded

fifteen pregnane glycosides and three flavone glycosides. Structure elucidation of these compounds were performed using extensive spectroscopic (<sup>1</sup>H, <sup>13</sup>C, APT, H,H COSY, HSQC, HMBC and NOESY NMR) data and spectrometric (ESI MS) analyses. The new pregnane glycosides quadrangulosides A–F (1–6) as well as the two compounds (12, 13) are possessing the boucerin aglycon, compounds (11, 15, 16) having the calogenin aglycon, compounds (8, 10, 18) having the ketocalogenin skeleton and compound (9) having the 5α-dihydrocalogenin structure (Fig. 1).

Compound (1) was isolated from the CHCl<sub>3</sub> fraction as a white amorphous powder and exhibited molecular formula C<sub>40</sub>H<sub>58</sub>O<sub>9</sub> based on negative mode ESIMS (*m/z* 699.4245 [M + H<sub>2</sub>O – H]<sup>–</sup>). <sup>13</sup>C NMR spectrum (Tables 1 and 2) has confirmed its molecular formula by indicating the presence of 40 carbon signals that were assigned, by APT experiments, to six methyl, one methoxy, nine methylene, seventeen methine groups and seven quaternary carbon atoms. <sup>1</sup>H and <sup>13</sup>C-NMR data of (1) (Table 1) displayed resonances for a pregnane glycoside with two singlet signals characteristic for two angular methyl groups appeared at δ<sub>H</sub> 1.17 (δ<sub>C</sub> 9.74) and 0.99 (δ<sub>C</sub> 19.49) assigned for CH<sub>3</sub>-18 and CH<sub>3</sub>-19, respectively, in addition to, an olefinic proton signal at δ<sub>H</sub> 5.43 (brs, 1H, H-6) (δ<sub>C</sub> 121.62) located at C-5/C-6 and showed long range correlation in HMBC spectrum with C-5 (δ<sub>C</sub> 139.60), C-10 (δ<sub>C</sub> 29.84) and C-8 (δ<sub>C</sub> 37.41) characteristic for the Δ<sup>5</sup> pregnanes type. <sup>10</sup> <sup>13</sup>C and APT NMR spectra also displayed three resonances at δ<sub>C</sub> 77.54, 73.69 and 78.38 characteristic for oxygenated methine carbons and correlated in the HSQC spectrum with protons at δ<sub>H</sub> 3.55 (m, 1H, W<sub>1/2</sub> 23.4 Hz), 4.82 (dd, 1H, 4.3, 12.0 Hz) and 4.91 (dq, 1H, 12.0, 6.0 Hz) which attributed to H-3, H-12 and H-20, respectively. In addition to another oxygenated quaternary carbon at δ<sub>C</sub> 86.61 was given for C-14 identical with the C/D-*cis* junction of boucerin polyoxypregnane.<sup>12–15</sup> The <sup>1</sup>H and <sup>13</sup>C NMR spectra of (1) displayed characteristic resonances for two carbonyl carbon esters at (δ<sub>C</sub> 166.56 and 172.61) corresponding to benzoyl and isovaleroyl groups, respectively (see Table 1), which were further

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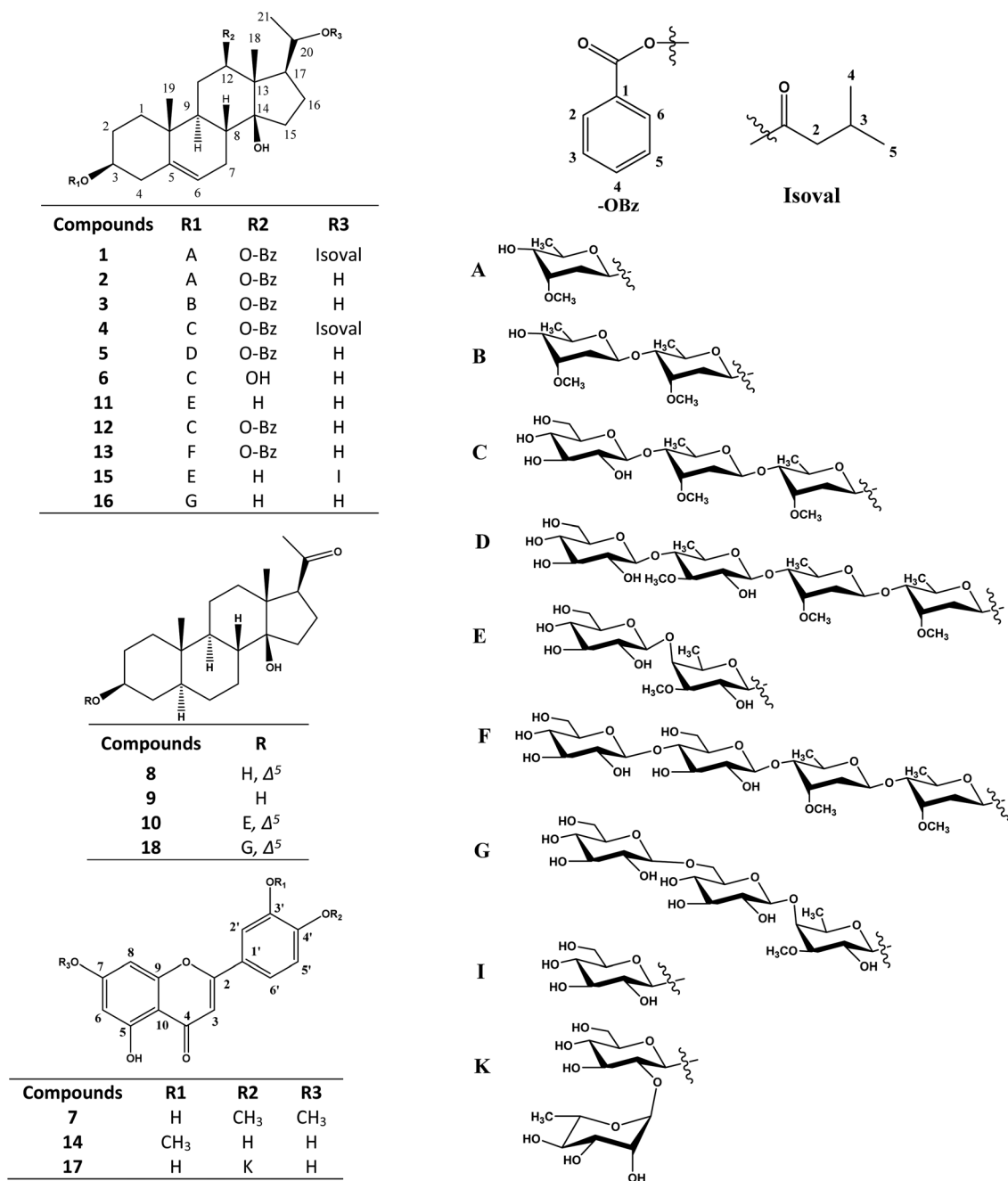



Fig. 1 Structure of the isolated compounds 1–18.

confirmed by comparing with the previously published spectra.<sup>10,11,13–15</sup> The esterification of C-12-OH of boucerin with benzoic acid was approved by the long ranged HMBC correlation of H-12 proton at  $\delta_{\text{H}}$  4.82 (dd, 1H, 4.3, 12.0 Hz) with  $\delta_{\text{C}}$  166.56, while the ester linkage at C-20-OH showed a long-range correlation between carbonyl groups at  $\delta_{\text{C}}$  172.61 of isovaleroyl moiety and H-20  $\delta_{\text{H}}$  4.91, (dq, 1H, 12.0, 6.0 Hz) (Fig. 2).<sup>12,14,16–19</sup>

The  $\beta$ -configuration of the benzoyl group at C-12 ( $\delta_{\text{H}}$  4.82) was confirmed by NOESY cross-correlation (Fig. 2) with H-9 ( $\delta_{\text{H}}$  1.33) also, large coupling constant of H-12 (dd, 1H, 4.3, 12.0 Hz) has proved the  $\alpha$  (axial)-orientation of this proton.<sup>10,12,17,20</sup> The relative configuration at C-17 was determined through the

correlation between H-17 ( $\delta_{\text{H}}$  2.05) and H-12 ( $\delta_{\text{H}}$  4.82) suggesting the  $\beta$ -configuration of the side chain and  $\alpha$ -orientation of H-17.<sup>4,14,21</sup> Stereochemical assignment of C-20 was established to be S, from the NOESY cross-peaks between H-17/H-21, H-20/H-18 and H-20/H-21 as well as the absence of any correlation between H-18 and H-21, along with comparing the spectral data with the related compounds russeleosides A–D, penicillosides A–C and russeleosides E–H.<sup>17,19,22</sup> So, the aglycone moiety was established as 12 $\beta$ -O-benzoyl-20-O-isovaleroyl-3 $\beta$ ,12 $\beta$ ,14 $\beta$ ,20 $\beta$ -tetrahydroxy-(20S)-pregn-5-ene.<sup>12,16,19,22</sup>

<sup>1</sup>H- and <sup>13</sup>C-NMR spectra of (1) (Table 2) displayed an anomeric proton signal at 4.78 (brd 1H, 9.5 Hz) ( $\delta_{\text{C}}$  95.68)

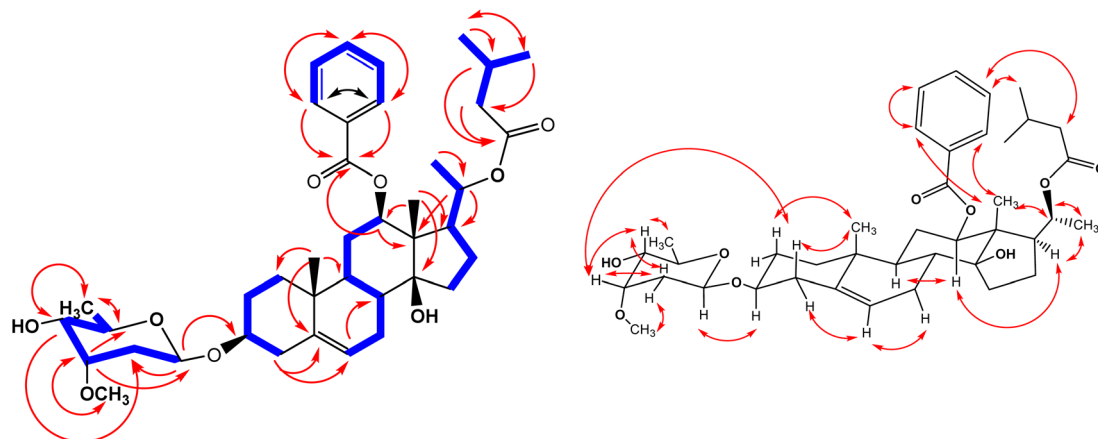


Fig. 2 H–H COSY, HMBC and NOESY correlations of quadranguloside A (1).

together with signals for methyl  $\delta_{\text{H}}$  1.28 (d, 3H, 6.4 Hz) ( $\delta_{\text{C}}$  95.68), one methoxy group 3.43 (s, 3H) ( $\delta_{\text{C}}$  57.38) and one methylene carbon ( $\delta_{\text{C}}$  34.19) characteristic for one 3-*O*-methyl-2,6-deoxyhexopyranose. Detailed examination of H–H COSY, HSQC, HMBC and NOESY spectra identified the sugar as cymarose and the anomeric proton coupling constant (brd, 1H, 9.5 Hz) suggested its  $\beta$ -D form. The strong  $^3J$  HMBC correlation between anomeric proton of cymaropyranose unit ( $\delta_{\text{H}}$  4.78) and the aglycone C-3 ( $\delta_{\text{C}}$  77.54) revealed the glycosylation site at C-3.<sup>20</sup>

By comparing the aforementioned data, with the previously published closely related pregnane glycosides, compound (1) was confirmed as 12 $\beta$ -*O*-benzoyl-20-*O*-isovaleroyl-3 $\beta$ ,12 $\beta$ ,14 $\beta$ ,20 $\beta$ -tetrahydroxy-(20*S*)-pregn-5-ene 3-*O*- $\beta$ -D-cymaropyranoside, that we named quadranguloside A.

Compound (2) was separated from the  $\text{CHCl}_3$  fraction as an amorphous solid with a molecular formula established  $\text{C}_{35}\text{H}_{50}\text{O}_8$  based on the sodium adduct ion at  $m/z$  311.1833 for  $[\text{M} + \text{H} + \text{Na}]^{2+}$  in the positive ESI MS spectrum. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR data of (2) (Tables 1 and 2) were closely related to those of (1), as all the observed signals are typically matched those of quadranguloside A, except the absence of the isovaleroyl group, indicating a free hydroxyl group at C-20. The signal at  $\delta_{\text{C}}$  71.76 was attributed to the HO–C-20 with its proton appeared as multiplet at  $\delta_{\text{H}}$  3.81 ppm. The benzoyl group exist at C-12, as deduced from comparing their spectral data and also by HMBC correlations. The  $\beta$ -orientation of side chain at C-17 and  $\alpha$ -configuration of H-17 was determined in similar way as in 1. Thus compound (2) was identified as 12 $\beta$ -*O*-benzoyl-3 $\beta$ ,12 $\beta$ ,14 $\beta$ ,20 $\beta$ -tetrahydroxy-pregn-5-ene 3-*O*- $\beta$ -D-cymaropyranoside, that we named quadranguloside B.

Compound (3) was obtained from the  $\text{CHCl}_3$  fraction as a white powder. Examination of negative ionization ESI MS of (3) showed an deprotonated intense ion  $[\text{M} + \text{HCOOH} - \text{H}]^-$  at  $m/z$  787.4901 and  $[\text{M} + \text{Cl}]^-$  at  $m/z$  777.4920, corresponding to the molecular formulas  $\text{C}_{42}\text{H}_{62}\text{O}_{11}$ . Careful examination of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of (3) displayed good similarity to those of (2), with the signals of the isovaleroyl group no longer present. In addition to the presence of two anomeric protons instead of one in (2), which appeared at  $\delta_{\text{H}}$  4.86 (dd, 1H, 2.1, 9.8 Hz) ( $\delta_{\text{C}}$

97.27) and 4.77 (dd, 1H, 2.0, 9.7 Hz), ( $\delta_{\text{C}}$  101.21). The additional sugar moiety was identified as cymarose. Both anomeric protons were appeared as  $\beta$ -configuration from their large coupling constants ( $\approx 10$  Hz). HMBC experiment showed direct evidences for the linkage sites between sugar moieties and the aglycone which showed correlation between H-1 $_{\text{cym-I}}$ –C-3 and H-1 $_{\text{cym-II}}$ –C-4 $_{\text{cym-I}}$ .

The aforementioned results revealed that (3) could be identified as 12 $\beta$ -*O*-benzoyl-3 $\beta$ ,12 $\beta$ ,14 $\beta$ ,20-tetrahydroxy-pregn-5-ene 3-*O*- $\beta$ -D-cymaropyranose(1  $\rightarrow$  4)- $\beta$ -D-cymaropyranoside, (named quadranguloside C).

Compound (4) was obtained from butanol fraction as a white amorphous residue, showed a negative ESI MS  $[\text{M} - 3\text{H}_2\text{O} - \text{H}]^-$  ion at  $m/z$  933.5948, consistent with molecular formula  $\text{C}_{53}\text{H}_{80}\text{O}_{17}$ .  $^1\text{H}$  and  $^{13}\text{C}$ -NMR signals of the aglycone moiety of (4) were undistinguishable from those of (1) with the same (isovaleroyl and benzoyl) acyl groups at the same positions as in (1), defining the aglycone unit as 12 $\beta$ -*O*-benzoyl-20-*O*-isovaleroyl-3 $\beta$ ,12 $\beta$ ,14 $\beta$ ,20 $\beta$ -tetrahydroxy-(20*S*)-pregn-5-ene.  $^1\text{H}$ -NMR signals showed three anomeric protons characteristic to a trisaccharide glycoside at  $\delta_{\text{H}}$  4.86 (dd, 1H, 9.9, 1.7 Hz), 4.80 (dd, 1H, 9.7, 1.6 Hz) and 4.34 (d, 1H, 7.8 Hz), with their corresponding carbons resonances at  $\delta_{\text{C}}$  97.27, 101.16 and 106.21, respectively indicating that compound (4) having two cymarose and one glucose units, in agreement with 2D NMR (HSQC, HMBC, COSY) data. The sugar sequence of attachment was established from the  $^3J$  HMBC spectrum between the terminal D-glucose H-1''' ( $\delta_{\text{H}}$  4.34) and C-4'' of the middle D-cymarose ( $\delta_{\text{C}}$  83.82) and the H-1'' of the latter ( $\delta_{\text{H}}$  4.80) and C-4' of inner D-cymarose ( $\delta_{\text{C}}$  83.76), furthermore long-range correlation between H-1' ( $\delta_{\text{H}}$  4.86) and C-3 ( $\delta_{\text{C}}$  79.04) of the aglycone has confirmed that D-cymarose was the inner sugar moiety and C-3 was the only site of glycosylation. The  $\beta$ -configuration of sugar parts was deduced from the large  $J_{\text{H1,H2}}$  coupling constant (7–9 Hz) of the anomeric protons.<sup>9,12</sup> On the basis of extensive (HSQC, HMBC and COSY) NMR data the sugar parts were identified and the linkage sites (H-1 $_{\text{cym I}}$ –C-3, H-1 $_{\text{cym II}}$ –C-4 $_{\text{cym I}}$ , H-1 $_{\text{glc}}$ –C-4 $_{\text{cym II}}$ ) were confirmed which were identical with the data published previously in literature.<sup>12,23</sup> Therefore, the sugar chain was identified as  $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  4)- $\beta$ -D-

Table 1 NMR spectroscopic data (500 MHz) for the aglycones of isolated compounds

Position	1		2		3		4		5		6	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
1	1.14/1.84, m, 2H	37.35	1.15/1.85, m, 2H	38.42	1.17/1.84, m, 2H	38.40	1.14/1.83, m, 2H	38.31	1.14/1.83, m, 2H	38.38	1.13/1.89, m, 2H	38.32
2	1.96/1.56, m, 2H	29.54	1.91/1.52, m, 2H	30.66	1.90/1.51, m, 2H	30.65	1.90/1.53, m, 2H	30.64	1.89/1.52, m, 2H	30.64	1.90/1.54, m, 2H	30.67
3	3.55, m, 1H, W <sub>1/2</sub> 23.4	77.54	3.52, tt, 1H, 11.2, 4.6	79.06	3.51, tt, 1H, 11.4, 4.4	79.06	3.50, tt, 1H, 11.4, 4.4	79.04	3.51, m, 1H, W <sub>1/2</sub> 22.7	79.02	3.50, m, 1H, W <sub>1/2</sub> 20.3	79.07
4	2.37/2.23, m, 2H	38.73	2.18/2.37, m, 2H	39.77	2.17/2.37, m, 2H	39.76	2.16/2.35, m, 2H	39.75	2.16/2.36, m, 2H	39.72	2.17/2.34, m, 2H	39.72
5	—	139.60	—	140.33	—	140.49	—	140.50	—	140.44	—	140.43
6	5.43, brs, 1H	121.62	5.47, brd, 1H, 5.2	123.09	5.46, brd, 1H, 5.1	123.08	5.47, d, 1H, 5.0	123.00	5.46, brd, 1H, 4.9	123.10	5.44, brd, 1H, 5.0	123.17
7	1.88, 2.25, m, 2H	27.15	1.89/2.25, m, 2H	28.23	1.89/2.29, m, 2H	28.22	1.87/2.26, m, 2H	28.15	1.88/2.25, m, 2H	28.20	1.87/2.21, m, 2H	28.33
8	1.76, m, 1H	37.41	1.87, m, 1H	37.51	1.88, m, 1H	37.50	1.91, m, 1H	37.98	1.87, m, 1H	37.45	1.77, m, 1H	38.11
9	1.33, m, 1H	43.29	1.36, m, 1H	44.74	1.36, m, 1H	44.73	1.35, m, 1H	44.63	1.35, m, 1H	44.67	1.20, m, 1H	45.14
10	—	38.73	—	38.37	—	38.36	—	38.45	—	38.33	—	38.43
11	1.57/1.87, m, 2H	26.25	1.64/1.83, m, 2H	26.85	1.66/1.84, m, 2H	26.85	1.65/1.81, m, 2H	27.31	1.65/1.84, m, 2H	26.84	1.45/1.63, m, 2H	29.83
12	4.82, dd, 1H, 4.3, 12.0	78.38	4.81 dd, 1H, 4.3, 12.0	79.77	4.81 dd, 1H, 4.3, 11.9	79.76	4.88 dd, 1H, 4.2, 12.5	79.72	4.81, dd, 1H, 4.1, 12.0	79.70	3.27, m, 1H	76.83
13	—	51.87	—	53.88	—	53.87	—	53.26	—	53.81	—	54.39
14	—	86.61	—	86.81	—	86.81	—	87.35	—	86.80	—	87.07
15	1.81/1.68, m, 2H	32.39	1.84/1.68, m, 2H	33.46	1.84/1.68, m, 2H	33.45	1.82/1.69, m, 2H	32.91	1.83/1.67, m, 2H	33.42	1.62, m, 2H	33.76
16	1.95/1.66, m, 2H	25.33	1.98/1.67, m, 2H	26.66	1.98/1.67, m, 2H	26.66	1.83/1.59, m, 2H	25.89	1.96/1.63, m, 2H	26.65	1.87/1.61, m, 2H	27.13
17	2.10, m, 1H	49.89	1.92, m, 1H	53.61	1.91, m, 1H	53.61	2.08, m, 1H	51.23	1.92, m, 1H	53.57	2.09, m, 1H	54.91
18	1.17, s, 3H	9.74	1.38, s, 3H	11.27	1.38, s, 3H	11.26	1.15, s, 003H	10.28	1.38, s, 3H	11.27	1.08, s, 3H	9.59
19	1.00, s, 3H	19.49	1.05, s, 3H	19.83	1.04, s, 3H	19.83	1.04, s, 3H	19.82	1.04, s, 3H	19.85	1.03, s, 3H	19.92
20	4.91, dq, 1H, 12.0, 6.0	73.69	3.81, m, 1H	71.76	3.82, m, 1H	71.75	4.93, m, 1H	74.87	3.81, m, 1H	71.71	3.76, m, 1H	71.25
21	1.13, d, 3H, 6.3	19.37	1.18, d, 3H, 6.5	23.04	1.18, d, 3H, 6.6	23.04	1.09, d, 3H, 6.1	19.42	1.18, d, 3H, 6.3	23.05	1.20, d, 3H, 6.0	22.97
<b>Benzoyl – 12</b>												
COO	—	166.56	—	168.03	—	168.02	—	167.93	—	167.95	—	167.95
1	—	130.81	—	132.14	—	131.85	—	132.00	—	131.82	—	131.82
2, 6	8.10, brd, 2H, 7.5	129.81	8.08, m, 2H	130.61	8.08, m, 2H	130.62	8.10, m, 2H	130.66	8.07, brd, 2H, 7.5	130.63	—	130.63
3, 5	7.45, t, 2H, 7.5	128.48	7.49, m, 2H	129.59	7.49, m, 2H	129.60	7.51, m, 2H	129.59	7.49, t, 2H, 7.5	129.61	—	129.61
4	7.59, t, 1H, 7.5	133.19	7.61, m, 1H	134.25	7.61, m, 1H	134.26	7.64, m, 1H	134.33	7.61, t, 1H, 7.5	134.28	—	134.28
<b>Isovaleroyl – 20</b>												
COO	—	172.61	—	—	—	—	—	174.29	—	—	—	—
2	2.06, m, 2H	44.06	—	—	—	—	2.02, m, 2H	44.95	—	—	—	—
3	2.02, m, 1H	25.64	—	—	—	—	1.94, m, 1H	26.67	—	—	—	—
4	0.86, d, 3H, 6.1	22.49	—	—	—	—	0.83, d, 3H, 6.5	22.69	—	—	—	—
5	0.90, d, 3H, 6.1	22.59	—	—	—	—	0.88, d, 3H, 6.5	22.77	—	—	—	—

cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside. Based on these results, (4) was established as the new quadranguloside D, 12β-O-benzoyl-20-O-isovaleroyl-3β,12β,14β,20β-tetrahydroxy-(20S)-pregn-5-ene-3-O-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

Compound (5), an amorphous solid, showed a negative [M + 2HCOOH – 2H]<sup>−</sup> ion at *m/z* 557.1984 identical with molecular formula C<sub>55</sub>H<sub>84</sub>O<sub>20</sub>. The aglycone moiety of (5) was proved to be 12β-O-benzoyl-3β,12β,14β,20-tetrahydroxy-pregn-5-ene by

comparison of its NMR data (Table 1) with those of (2). Analysis of the <sup>1</sup>H and <sup>13</sup>C-NMR data (Table 2) of (5) has confirmed the presence of four anomeric protons at δ<sub>H</sub> 4.86 (dd, 1H, 9.5, 2.0 Hz) (δ<sub>C</sub> 97.23), 4.79 (d, 1H, 9.8, 2.1 Hz) (δ<sub>C</sub> 101.11), 4.34 (d, 1H, 7.6 Hz) (δ<sub>C</sub> 105.98) and 4.42 (d, 1H, 7.7 Hz) (δ<sub>C</sub> 104.28). Careful analysis of HMBC data concluded each monosaccharide spin system. The anomeric protons of the 3-O-methyl-2,6-dideoxyhexopyranoses appeared at δ<sub>H</sub> 4.86 and 4.79 were established as two cymaroses in addition to, one 3-O-methyl-6-deoxyhexopyranose with

Table 2 NMR spectroscopic data (500 MHz) for the sugar moieties of isolated compounds

	1		2		3		4		5		6	
Position	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
<b>Cym</b>												
1'	4.78, brd, 1H, 9.5	95.68	4.86, dd, 1H, 9.7, 2.1	97.28	4.86, dd, 1H, 9.8, 2.1	97.27	4.86, dd, 1H, 9.9, 1.7	97.27	4.86, dd, 1H, 9.5, 2.0	97.23	4.86, d, 1H, 9.6, 1.3	97.22
2'	1.58/2.21, m, 2H	34.19	1.54/2.15, m, 2H	35.95	1.54/2.07, m, 2H	36.63	1.62/2.14, m, 2H	36.39	1.57/2.13, m, 2H	36.33	1.56/2.07, m, 2H	36.62
3'	3.63, m, 1H	77.64	3.59, q, 1H, 3.3	79.20	3.60, q, 1H, 3.1	79.18	3.84, q, 1H, 3.3	78.58	3.84, m, 1H	78.51	3.85, m, 1H	78.56
4'	3.22, m, 1H	72.64	3.17, dd, 1H, 9.5, 3.3	74.48	3.23, dd, 1H, 3.0, 9.6	83.81	3.34, dd, 1H, 3.3, 9.7	83.76	3.25, m, 1H	83.81	3.24, m, 1H	83.76
5'	3.60, m, 1H	70.90	3.72, dq, 1H, 9.6, 6.3	71.45	3.80, m, 1H	69.99	3.80, dq, 1H, 9.7, 6.3	69.98	3.81, m, 1H	70.03	3.80, m, 1H	69.98
6'	1.28, d, 3H, 6.4	18.42	1.22, d, 3H, 6.3	18.67	1.20, d, 3H, 6.2	18.49	1.19, d, 3H, 6.3	18.50	1.19, d, 3H, 6.3	18.52	1.19, d, 3H, 5.9	18.50
3'-OCH <sub>3</sub>	3.43, s, 3H	57.38	3.43, s, 3H	58.08	3.42, s, 3H	58.09	3.43, s, 3H	58.47	3.42, s, 3H	58.41	3.43, s, 3H	58.44
<b>Cym</b>												
1''				4.77, dd, 1H, 9.7, 2.0		101.21	4.80, dd, 1H, 9.7, 1.6	101.16	4.79, d, 1H, 9.8, 2.1	101.11	4.80, d, 1H, 9.7, 1.2	101.19
2''				1.57/2.23, m, 2H		35.61	1.56/2.06, m, 2H	36.66	1.57/2.07, m, 2H	36.55	1.61/2.14, m, 2H	36.36
3''				3.85, q, 1H, 3.3		78.57	3.93, m, 1H, 3.1	78.70	3.85, m, 1H	78.62	3.93, q, 1H, 3.4	78.69
4''				3.17, dd, 1H, 3.3, 9.7		74.46	3.23, dd, 1H, 3.2, 9.7	83.82	3.23, m, 1H	84.08	3.35, m, 1H	83.83
5''				3.72, dq, 1H, 9.7, 6.3		71.31	3.88, m, 1H	70.12	3.86, m, 1H	69.94	3.88, m, 1H	70.12
6''				1.23, d, 3H, 6.2		18.73	1.30, d, 3H, 6.3	18.69	1.30, d, 3H, 6.1	18.68	1.30, d, 3H, 6.4	18.68
3'- <sup>13</sup> C-OCH <sub>3</sub>				3.43, s, 3H		58.43	3.45, s, 3H	58.54	3.43, s, 3H	58.56	3.46, s, 3H	58.54
<b>Thev</b>												
1'''									4.34, d, 1H, 7.6	105.98		
2'''									3.30, m, 1H	74.88		
3'''									3.20, m, 1H	85.98		
4'''									3.37, brd, 1H, 2.7	82.69		
5'''									3.48, m, 1H	72.49		
6'''									1.37, d, 3H, 6.1	18.48		
3'''-OCH <sub>3</sub>									3.63, s, 3H	61.23		
<b>Glc</b>												
1'''							4.34, d, 1H, 7.8	106.21	4.42, d, 1H, 7.7	104.28	4.34, d, 1H, 7.6	106.25
2'''							3.20, dd, 1H, 7.8, 9.3	75.30	3.20, m, 1H	75.65	3.21, m, 1H	75.29
3'''							3.34, m, 1H	77.94	3.36, m, 1H	77.93	3.34, m, 1H	78.01
4'''							3.26, m, 1H	71.79	3.24 m, 1H	71.81	3.25, m, 1H	71.77
5'''							3.27, m, 1H	78.01	3.26, m, 1H	78.36	3.26, m, 1H	77.92
6''' a							3.64, dd, 1H, 5.8, 11.8	63.00	3.65, dd, 1H, 6.3, 12	63.08	3.65, dd, 1H, 5.8, 11.8	62.96
6''' b							3.88, dd, 1H, 2.0, 11.8		3.85, d, 1H, 6.3		3.89, dd, 1H, 2.0, 11.8	

anomeric proton at  $\delta_{\text{H}}$  4.34 was deduced as thevetose and a terminal sugar unit with anomeric proton appeared at  $\delta_{\text{H}}$  4.42 identified as glucopyranose. All the sugars moieties were assigned a  $\beta$ -conformations based on their coupling constants.<sup>24</sup> The anomeric proton signal at  $\delta_{\text{H}}$  4.86 showed HMBC correlation with C-3 of the aglycone at  $\delta_{\text{C}}$  79.02 suggesting the glycosylation linkage at C-3. The second cymarose anomeric proton at  $\delta_{\text{H}}$  4.79 exhibited a clear correlation with  $\delta_{\text{C}}$  83.81 (C-4'). Further, <sup>3</sup>J

HMBC correlations between the anomeric proton of thevetose at  $\delta_{\text{H}}$  4.34 and  $\delta_{\text{C}}$  84.08 (C-4'') and a correlation of the terminal glucopyranose anomeric proton at  $\delta_{\text{H}}$  4.42 with  $\delta_{\text{C}}$  82.69 (C-4''') were established. Therefore, compound (5) was identified as 12 $\beta$ -O-benzoyl-3 $\beta$ ,12 $\beta$ ,14 $\beta$ ,20-tetrahydroxy-pregn-5-ene-3-O- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-thevetopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranoside, that we named quadranguloside E.



Compound (6), white amorphous solid, gave in the ESI MS spectrum a deprotonated  $[M + \text{HCOOH} - \text{H}]^-$  ion at  $m/z$  845.5329 and  $[M + \text{Cl}]^-$  ion at  $m/z$  835.4434, corresponding to  $\text{C}_{41}\text{H}_{68}\text{O}_{15}$ .  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra (Tables 1 and 2) of (6) clearly displayed a close similarity to those of (4). Investigation of 1D and 2D NMR spectra of (6) showed the same aglycone unit without the benzoyl and isovaleroyl acyl groups, only two free hydroxyl groups at C-12 and C-20. The signals at  $\delta_{\text{C}}$  76.83 and 71.25 were attributed to hydroxylated C-12 and C-20, respectively. So, the aglycon of (6) was boucerin.<sup>24</sup> Thus compound (6) was assigned as boucerin 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranoside, that we named quadranguloside F.

Beside the new quadranguloside A–F (1–6), known previously published compounds were isolated and identified from *C. quadrangula* as arabinoside C (12), arabinoside D (13),<sup>23</sup> *cis*-polyoxypregnanes boucerin derivatives, isolated from the ethyl acetate fraction, russelioside C (11), russelioside B (15) and russelioside D (16), calogenin derivatives, that were identified previously from *C. russeliana*,<sup>4</sup> 3 $\beta$ ,14-dihydroxy-14 $\beta$ -pregn-5-en-20-one (8),<sup>25</sup> arabinoside A (10) and arabinoside B (18),<sup>23</sup> ketocalogenin derivatives, and 3 $\beta$ ,14-dihydroxy-5 $\alpha$ ,14 $\beta$ -pregnan-20-one (9),<sup>26</sup> a 5 $\alpha$ -dihydrocalogenin derivative and 5,3'-dihydroxy-7,4'-dimethoxyflavone (7),<sup>27</sup> chrysoeriol (14),<sup>28</sup> luteolin 4'-*O*- $\beta$ -D-neohesperidoside (17),<sup>29</sup> flavone glycosides. These isolated compounds were identified by comparing their 1D and 2D NMR spectral data with those previously reported in the literature. It is worthwhile mentioned that all the above mentioned compounds except (17) are reported for the first time from *C. quadrangula*.

## Experimental

### General

Optical rotations were assessed on WXG-4. UV polarimeter, and the spectra were recorded on Ati-Unicam-UV/Visible Vision. NMR spectra were performed on a JEOL 500 MHz and Bruker 400 MHz using deuterated ( $\text{MeOH-}d_4$  or  $\text{CDCl}_3$ ) solvents for NMR measurements, Mansoura University, Egypt. LC-DAD/ESI-MS analysis, A XEVO-TQD#QCA423 HPLC system coupled *via* an ESI interface consists of Waters ACQUITY FTN and controlled using MSD Trap Control Version 1.71.2686 software, Ain Shams University. Column chromatography (CC) was processed on silica gel F<sub>254</sub> (230–400 mesh), Sephadex LH-20 and polyamide 6. PTLC were carried out using 0.25 thickness silica gel (Kieselgel 60, GF 254). Chloroform, petroleum ether (60–80), methanol (MeOH), ethyl acetate (EtOAc) and anhydrous sodium sulphate were purchased from Adwic Company Mansoura, Egypt.

### Plant material

*C. quadrangula* (Forssk.) aerial parts were obtained in June 2021 from Amran Governorate, Yemen and were dried in shade. Botanical identification was made by Dr Hassan M. Ibrahim, head of the herbarium, Biological Science Department, Faculty of Science, Sana'a University. A herbarium specimen was deposited in the herbarium of Biological Science Department,

Faculty of Science, Sana'a University in special collection of the third author.

### Extraction and isolation

*C. quadrangula* powdered aerial parts (1395 g) were extracted using MeOH (8  $\times$  15 L), evaporated to give 750.63 g of brown residue and partitioned successively with  $\text{CHCl}_3$  (4  $\times$  2 L), ethyl acetate (4  $\times$  3 L) and *n*-butanol (5  $\times$  3 L) to yield 31.56, 57.71 and 228.62 g, respectively.

The chloroform fraction (31.56 g) was separated over silica gel CC using pet. ether:ethyl acetate and  $\text{CHCl}_3$ :MeOH as eluent with gradient increasing polarities. The sub-fractions were monitored using TLC after *p*-anisaldehyde sulfuric acid reagent spraying. Fractions with similar pattern gathered to give three main sub-fractions. Sub-fraction I was purified on PTLC plates using  $\text{CHCl}_3$ :MeOH (98:2) as eluent to give compound (1) (50 mg,  $R_f$  = 0.33). Fraction II was further purified on PTLC using  $\text{CHCl}_3$ :MeOH (96:4) to yield, compound (2) (30 mg,  $R_f$  = 0.56) and (3) (24 mg,  $R_f$  = 0.39). Sub-fraction III was also purified on PTLC using  $\text{CHCl}_3$ :MeOH (99:1) to yield, (7) (30 mg,  $R_f$  = 0.82), (8) and (9) (24 mg,  $R_f$  = 0.39).

Ethyl acetate fraction (42.71 g) was chromatographed on silica gel CC with  $\text{CHCl}_3$  and MeOH with increasing amount of MeOH (1–100%) as eluent. Similar collected fractions were gathered as indicated from their TLC profiling, to give ten sub-fractions. Sub-fraction I was further purified on PTLC plates using  $\text{CHCl}_3$ :MeOH:H<sub>2</sub>O (85:15:5) as eluent to give compound (5) (40 mg,  $R_f$  = 0.40), (6) (10 mg,  $R_f$  = 0.53), (10) (40 mg,  $R_f$  = 0.26) and (12) (30 mg,  $R_f$  = 0.46). Sub-fraction (II) purified by PTLC silica gel plates ( $\text{CHCl}_3$ :MeOH:H<sub>2</sub>O) (82:18:7) to give compound (11) (35 mg,  $R_f$  = 0.4) and (13) (40 mg,  $R_f$  = 0.63). Sub-fraction (III) was chromatographed on silica gel PTLC plates  $\text{CHCl}_3$ :MeOH:H<sub>2</sub>O (92:8:4) to yield compounds (4) (10 mg,  $R_f$  = 0.28) and decreasing polarity to (95:5) to give compound (14) (30 mg,  $R_f$  = 0.35).

Butanol fraction (65.0 g) was chromatographed over polyamide S6 column using mixture of dist. H<sub>2</sub>O, (dist. H<sub>2</sub>O:methanol) and (methanol:ammonia) as eluent, with increasing polarities. The collected sub-fractions were examined by TLC and gave four main sub-fractions. Sub-reaction I was further purified by PTLC using ( $\text{CHCl}_3$ :MeOH:H<sub>2</sub>O) (75:26:5) to yield compound (15), (16) (40 mg,  $R_f$  = 0.48) and (17) (30 mg,  $R_f$  = 0.29). Sub-fraction II purified using PTLC silica gel plates ( $\text{CHCl}_3$ :MeOH:H<sub>2</sub>O) (80:20:3) to give compound (18) (40 mg,  $R_f$  = 0.62).

**Quadranguloside A (1).** White amorphous powder,  $[\alpha]_{\text{D}}^{21} + 45.71$  (*c.* 0.01, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 253 (4.71), sh 282 (4.62); see Table 1 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR (500 MHz, 125 MHz,  $\text{CDCl}_3$ ) of aglycone moiety; and Table 2 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR of sugar moiety.

**Quadranguloside B (2).** Amorphous solid,  $[\alpha]_{\text{D}}^{21} + 1.89$  (*c.* 0.0065, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 248 (4.82), sh 274 (4.71); see Table 1 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR (500 MHz, 125 MHz,  $\text{CDCl}_3$ ) of aglycone moiety; and Table 2 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR of sugar moiety.

**Quadranguloside C (3).** White powder,  $[\alpha]_{\text{D}}^{21} + 34.28$  (*c.* 0.01, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 253 (4.76), sh 274 (4.69); see

Table 1 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR (500 MHz, 125 MHz,  $\text{CDCl}_3$ ) of aglycone moiety; and Table 2 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR of sugar moiety.

**Quadranguloside D (4).** White amorphous residue,  $[\alpha]_{\text{D}}^{21} + 57.14$  (c. 0.01, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 249 (4.87), sh 278 (4.78); see Table 1 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR (500 MHz, 125 MHz,  $\text{CD}_3\text{OD}$ ) of aglycone moiety; and Table 2 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR of sugar moieties.

**Quadranguloside E (5).** Amorphous solid,  $[\alpha]_{\text{D}}^{21} + 0.685$  (c. 0.04, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 252 (4.31), sh 287 (4.23); see Table 1 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR (500 MHz, 125 MHz,  $\text{CD}_3\text{OD}$ ) of aglycone moiety; and Table 2 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR of sugar moieties.

**Quadranguloside F (6).** White amorphous solid,  $[\alpha]_{\text{D}}^{21} + 74.28$  (c. 0.01, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 237 (4.76), sh 273 (4.50); see Table 1 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR (500 MHz, 125 MHz,  $\text{CD}_3\text{OD}$ ) of aglycone moiety; and Table 2 for  $^1\text{H}$ ,  $^{13}\text{C}$  NMR of sugar moieties.

## Conclusions

A unique new series of polyhydroxylated pregnane glycosides (1–6) were identified for the first time from *Caralluma quadrangula* by means of extensive chromatographic and spectral analyses.

## Author contributions

Ghada A. Ismail performed the chemistry experiments, AbelAziz M. Dawidar, Mamdouh Abdel-Mogib and Mohamed E. Mostafa performed experimental planning and data analysis. Ahmed Y. Mubarak collected the plant materials. Conceptualization and supervision performed by AbelAziz M. Dawidar and Mamdouh Abdel-Mogib. The manuscript was written by Ghada A. Ismail, Mohamed E. Mostafa and Ahmed Y. Mubarak. All authors reviewed the manuscript.

## Conflicts of interest

There are no conflicts to declare.

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