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Acylated pregnane glycosides from *Caralluma russeliana*

Essam Abdel-Sattar [a,*], Ahmed A. Ahmed [b], Mohamed-Elamir F. Hegazy [c], Mohamed A. Farag [d], Mohammad Abdul-Aziz Al-Yahya [e]

[a] *Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia*
[b] *Department of Chemistry, Faculty of Science, El-Minia University, El-Minia 61519, Egypt*
[c] *Department of Mathematical and life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan*
[d] *Center for Regulatory and Environmental Analytical Metabolomics, 2320 S. Brook St., University of Louisville, Louisville, KY 40208, USA*
[e] *Department of Pharmacognosy, Faculty of Pharmacy, King Saud University, Riyadh 11451, P.O. Box 2457, Saudi Arabia*

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To the spirit of Dr Ahmed A. Ahmed

Abstract

The chloroform extract of the aerial parts of *Caralluma russeliana* yielded four acylated pregnane glycosides, namely russeliosides **E**–**H**, three were found now. The structures of the compounds were elucidated using MS, ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, NOESY and HMBC experiments.

Keywords: *Caralluma russeliana*; Asclepiadaceae; Acylated pregnane glycosides

1. Introduction

Genus *Caralluma*, belongs to family *Asclepiadaceae*, which comprises some 200 genera and 2500 species; the members of the genus are small plants, erect and fleshy. They have 4 grooved stems that are almost round in shape. They are generally devoid of leaves and form small flowers in a variety of dark colors. The species of *Caralluma* found in India are edible and form part of the traditional medicine system of the country. *Caralluma fimbriata* is listed in The Wealth of India (1992) as medicinal plant used as an appetite suppressant and has also been used to treat diabete, pain, fever, and inflammation. *C. attenuata* is eaten raw as a cure for diabetes and the juice of the plant along with black pepper is recommended in the treatment of migraine (Ramesh et al., 1998). In addition, *Caralluma tuberculata* is consumed as food and is commonly used in the treatment of rheumatism, diabetes, leprosy and as antipyretic (Chopra et al., 1956; Ahmed and Shaikh, 1989).

Caralluma russeliana is succulent perennial herb growing wild in the rocky regions of Abha, south west of Saudi Arabia (Collenette, 1985). Several members of family *Asclepiadaceae* are reported to be rich in pregnanes and pregnane glycosides (Al-Yahya et al., 2000; Abdel-Sattar et al., 2001, 2002; Halim and Khalil, 1996; Lin et al., 1994; Tanaka et al., 1990; Deepak et al., 1989) which are drawing much attention in recent years due to their antitumor, (Qiu et al., 1999; Pan et al., 2003), platelet pro-aggregating (Piacente et al., 1998), anti-fungal (Hu et al., 1999), and a digitalis receptor binding activities (Templeton et al., 1993). The medicinal properties of *Caralluma* species have been attributed to the glycosides contained therein (Rajendran and Rajendran, 2004).

We have previously reported the isolation and structural elucidation of four new pregnane glycosides, namely russelioside **A**–**D** from the *n*-butanol extract of the over ground

* Corresponding author. Tel.: +966 2 6400000x22238; fax: +966 2 6951696.
E-mail address: abdelsattar@yahoo.com (E. Abdel-Sattar).

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parts of *Caralluma russeliana* (Al-Yahya et al., 2000). Further investigation of the chloroform extract of the plant afforded three new acylated pregnane glycosides namely russeliosides **E**–**G**.

2. Results and discussion

The chloroform fraction of the ethanol extract of the plant was chromatographed over silica gel column then subjected to HPLC on RP-18 column, to afford four pregnane glycosides [**1**–**4**], of which compounds **1**–**3** were new natural products. Compounds **1**–**4** gave positive tests for sterols (Liebermann-Bucrchard reagent) and sugars and/or glycosides (Molish reagent).

MS and NMR data analysis of compounds **1**–**4** indicated that they were derivatives of the *C*/*D*-*cis*-polyoxypregnane boucerin by comparison with aglycon data previously reported in the literature (Braca et al., 2002; Halim and Khalil, 1996; Qiu et al., 1997). In addition, the ^{1}H and ^{13}C NMR spectra of compounds **1**–**4** showed signals due to tigloyl and/or acetyl and/or benzoyl groups. The ester linkage was located at C-12 and C-20 on the basis of the downfield shift of the respective protons and carbons. This was confirmed from the results of HMBC experiments which showed clear long-range correlation between the signal of carbonyl carbon of the tigloyl, acetyl or benzoyl groups and H-12 and/or H-20 of the aglycons (Yoshikawa et al., 1998; Braca et al., 2002).

Compound **1** was isolated as amorphous powder, $[\alpha]_D^{25} + 3.0^\circ$ (*c* 1.5, MeOH). The molecular formula was established to be $C_{57}H_{86}O_{21}$ by HRCIMS (*m*/*z* 1107.5752, $[M+H]^+$; calc. 1107.5740). This formula was confirmed by ^{13}C NMR and DEPT spectroscopic analysis suggesting the presence of pregnane derivative. The CI mass spectrum exhibited a base ion peak at *m*/*z* 1066 corresponding to the loss of an acetyl group which upon further loss of a water molecule yielded the ion peak at *m*/*z* 1048 (60%). The IR spectrum of compound **1** showed absorption bands due to hydroxyl group (3400 cm^{-1}) and acetyl groups (1725 and 1235 cm^{-1}).

The ^{1}H and ^{13}C NMR spectra of compound **1** showed the presence of an acetyl and a benzoyl moieties (see Tables 1 and 3). This finding was confirmed by comparing the spectral data of compound **1** with those reported in the literature (Yoshikawa et al., 1998; Braca et al., 2002). The presence of four anomeric protons and carbons in ^{1}H and ^{13}C NMR spectra of **1** (see Tables 1 and 2), suggested a tetrasaccharide glycoside. Acid hydrolysis of **1** yielded three sugars; two of them were identified as cymarose and glucose (TLC). The structure of the third sugar was determined as 6-deoxy-3-*O*-methyl-β-allopyranose (allom) on the basis of extensive NMR study (HMQC, HMBC and NOESY) and by comparison with data reported in literature (Braca et al., 2002; Yoshikawa et al., 1998; Abdel-Sattar et al., 2001). The strong long-range correlation between carbonyl groups at δ_C 169.3 and H-20 (δ_H 4.79), and 165.6 and H-12 (δ_H 4.66) in HMBC spectrum confirmed the acetylation position at C-20 and benzoylation at C-12. The NOESY correlation between H-12 and H-9 confirmed the β-configuration of the side chain at C-12. The relative stereochemistry at C-17 was deduced from the NOESY correlations between H-17 and H-9, with no evidence of spatial correlation between H-17 and Me-18, suggested the α-configuration of H-17. A double bond located at C-5/C-6 was deduced from the broad signal at δ_H 5.41 (H-6), which showed long range correlation with C-10 (δ_C 36.8). From the analyses of the 1D and 2D NMR experiments, the aglycon part was identified as 12-β-*O*-benzoyl-20-*O*-acetylboucerin a previously reported aglycon in *C. negevensis* (Braca et al., 2002). The direct evidence for the sugars sequence and their linkage sites was determined from the results of HMBC experiment which showed unequivocal correlation peaks between $H\text{-}1_{cym\ I}$-C-3, $H\text{-}1_{cym\ II}$-$C\text{-}4_{cym\ I}$, $H\text{-}1_{allom}$-$C\text{-}4_{cym\ II}$, and $H\text{-}1_{glc}$-$C\text{-}4_{allom}$. It is also interesting to note that the C-1 of the inner cymarose-I unit characteristically resonate upfield (δ_C 95.1) when linked to C-3 of the aglycon in contrast to C-1 of cym-II (δ_C 99.4) linked to the hydroxyl group of a sugar unit. The β-configuration of the anomeric

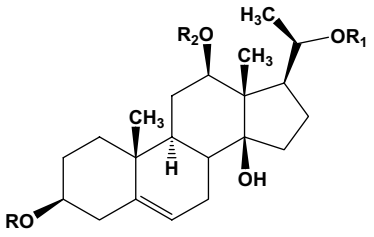
Table 1
^{13}C NMR data of the aglycon moiety of compounds **1**–**3**[a]

No	1	2	3
1	36.6, t	36.6, t	36.6, t
2	29.3, t	29.3, t	29.3, t
3	76.2, d	76.2, d	76.2, d
4	38.2, t	38.2, t	38.2, t
5	138.9, s	138.9, s	138.9, s
6	121.8, d	121.8, d	121.8, d
7	26.7, t	26.7, t	26.1, t
8	36.1, d	36.1, d	36.1, d
9	42.6, d	42.6, d	42.6, d
10	36.8, s	36.8, s	36.7, s
11	25.7, t	25.7, t	25.7, t
12	78.0, d	78.0, d	78.0, d
13	51.3, s	51.3, s	51.3, s
14	84.7, s	84.7, s	84.6, s
15	31.7, t	31.7, t	31.7, t
16	24.6, t	24.6, t	24.6, t
17	49.5, d	49.5, d	49.5, d
18	9.5, q	9.4, q	9.4, q
19	19.0, q	19.0, q	19.0, q
20	72.9, d	72.9, d	72.9, d
21	19.1, q	19.1, q	19.1, q
Acetyl			
CH_3–	21.3, q	21.3, q	21.3, q
C═O	169.8, s	169.7, s	169.7, s
Benzoyl			
1″	165.6, s	165.6, s	165.6, s
2″	130.2, s	130.2, s	130.2, s
3″, 7″	129.3, d	129.2, d	129.2, d
4″, 6″	128.7, d	128.7, d	128.7, d
5″	133.4, d	133.4, d	133.3, d

[a] Multiplicity was determined by DEPT experiments (s = quaternary, d = methine, t = methylene, q = methyl).

protons was indicated from their large $J_{H1,H2}$ coupling constant (8–10 Hz) (Agrawal, 1992) (see Table 2). From the aforementioned data, the structure of compound **1** was established as 12-β-*O*-benzoyl-20-*O*-acetylboucerin 3-*O*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside, named russelioside **E**.

Compound **2** was isolated as amorphous powder, $[\alpha]_D^{25} - 4.5^\circ$ (*c* 0.2, MeOH), and has a molecular formula $C_{50}H_{74}O_{17}$, as deduced from the ^{13}C NMR and the HRCI mass spectrometry (*m*/*z* 929.4831 ($[M+H]^+$–H_2O); calc. 929.4898). Its IR spectrum is similar to that of compound **1**. Compound **2** showed similar ^1H and ^{13}C NMR data to those of **1**, except the presence of three sugar units instead of four in **1**. Acid hydrolysis of **2** showed the presence of only two sugars (cymarose, and glucose) and similar aglycon to that of **1** (^1H and ^{13}C NMR). The ^1H and ^{13}C NMR spectra of **2** showed the presence of three anomeric protons and carbon signals at δ_H 4.73, 4.67, 4.18 and δ_C 95.1, 99.2, 10.4.8, respectively, suggesting **2** to be a trisaccharide glycoside consisting of two cymarose and one glucose units. The identification of the sugar part and their linkage sites (H-$1_{cym\ I}$-C-3, H-$1_{cym\ II}$-C-$4_{cym\ I}$, H-1_{glc}-C-$4_{cym\ II}$) was confirmed in similar way as in **1**. Thus, compound **2** was identified as 12-β-*O*-benzoyl-20-*O*-acetylboucerin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside, named russelioside **F**.



	R	R_1	R_2
1	**B**	**acetyl**	**benzoyl**
2	**C**	**acetyl**	**benzoyl**
3	**A**	**acetyl**	**benzoyl**
4	**B**	**acetyl**	**tigloyl**

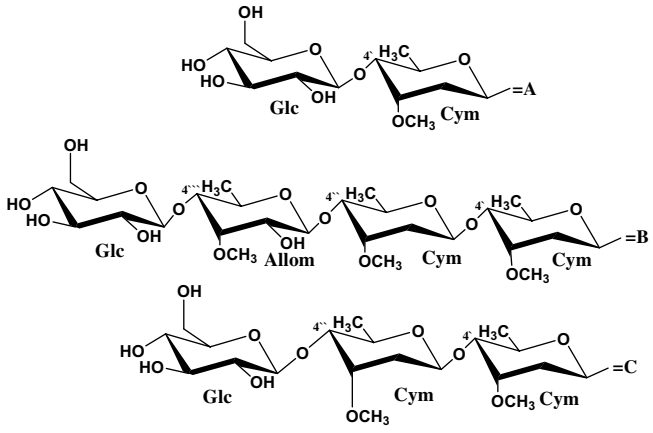


Table 2
^1H and ^{13}C NMR data of the sugar part of compounds **1**–**3**[a]

No	**1**				**2**		**3**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
	Cym		**Glc**		**Cym**		**Cym**	
1	95.1, d	4.73 (d, $J = 10$ Hz)	104.8 d	4.19 (*d*, $J = 7.5$ Hz)	95.1, d	4.73 (d, $J = 9$ Hz)	95.1, d	4.74 (d, $J = 10$ Hz)
2	35.6, t	1.45 (m), 1.96 (m)	73.7, d		35.6, t	1.44 (m), 1.95 (m)	35.7, t	1.44 (m), 1.90 (m)
3	76.7, d	3.66 (m)	76.8, d	2.93 (m)	76.7, d	3.70 (m)	76.6, d	3.67 (m)
4	82.1, d	3.15 (m)	70.2, d	3.09 (m)	82.0, d	3.20 (m)	82.3, d	3.15 (m)
5	68.2, d	3.66 (m)	76.7, d	3.00 (m)	68.2, d	3.76 (m)	68.3, d	3.73 (m)
6	18.1, q	1.06 (d, $J = 6$ Hz)	61.4, t	3.17 (m)	18.01,	1.19 (d, $J = 6$ Hz)	18.0, q	1.18 (d, $J = 6$ Hz)
OMe	57.8, q	3.31, s		3.44 (m), 3.68 (m)	57.8, q	3.32, s	57.7, q	3.35, s
	Cym				**Cym**		**Glc**	
1	99.4, d	4.66 (d, $J = 9.5$ Hz)			99.2, d	4.67 (d, $J = 9.5$ Hz)	104.9 d	4.17 (d, $J = 8$ Hz)
2	35.6, t	1.45 (m), 1.96 (m)			35.7, t	1.45 (m), 2.00 (m)	73.7, d	2.93 (m)
3	76.7, d	3.68 (m)			76.6, d	3.68 (m)	76.8, d	3.10 (m)
4	82.3, d	3.14 (m)			82.1, d	3.16 (m)	70.2, d	3.00 (m)
5	68.0, d	3.64 (m)			67.9, d	3.70 (m)	76.6, d	3.17 (m)
6	18.1, q	1.18 (d, $J = 6$ Hz)			18.0, q	1.21 (d, $J = 6$ Hz)	61.4., t	3.44 (m), 3.66 (m)
OMe	57.9, q	3.31, s			57.9, q	3.31, s		
	Allom				**Glc**			
1	102.7, d	4.44 (d, $J = 8.5$ Hz)			104.8 d	4.18 (d, $J = 7.5$ Hz)		
2	76.8, d	3.15 (m)			73.7, d	2.94 (m)		
3	81.6, d	3.78 (m)			76.8, d	3.08 (m)		
4	81.8, d	3.15 (m)			70.2, d	3.00 (m)		
5	70.9, d	3.16 (m)			76.6, d	3.17 (m)		
6	17.6, q	1.18 (d, $J = 6$ Hz)			61.4, t	3.42 (m), 3.68 (m)		
OMe	60.9, q	3.44, s						

[a] Multiplicity was determined by DEPT experiments (s = quaternary, d = methine, t = methylene, q = methyl).

Compound **3** was isolated as amorphous powder, $[\alpha]_D^{25} - 25^\circ$ (*c* 0.15, MeOH), and has a molecular formula $C_{43}H_{62}O_{14}$, as deduced from the ^{13}C NMR and the mass spectrometry data (*m/z* 785.41298 ($[M+H]^+$–H_2O); calc. 785.41122). Its IR spectrum is similar to those of compounds **1** and **2**. Acid hydrolysis of **3** showed the presence of similar sugars (cymarose, and glucose) and similar aglycon to that of **2** (NMR). The ^1H and ^{13}C NMR spectra of **3** showed identical NMR data to those of **3**, except in the presence of two sugar units instead of three in **2**, identified as cymarose and glucose (^1H and ^{13}C NMR spectra). The linkage sites ($H\text{-}1_{cym}$-C-3, $C\text{-}1_{glc}$-$H\text{-}4_{cym}$) were confirmed in similar way as in **1** and **2**. Thus, compound **3** was identified as 12-β-*O*-benzoyl-20-*O*-acetylboucerin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranoside, named russelioside **G**.

Compound **4** was identified as 12-β-*O*-tigloyl-20-*O*-acetylboucerin 3-*O*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside (russelioside **H**) by comparison of its spectral data with those reported for a pregnane glycoside previously isolated from *C. negevensis* (Braca et al., 2002).

The absolute configuration of the sugars were determined to be in the D-form on the basis of their optical rotation values (Abe et al., 1999), after acid hydrolysis of pregnane glycosides and chromatographic separation of the sugar mixture. The stereochemistry of C-20 was determined through a detailed analysis of the conformation of the side-chain as *S* in similar way as in russeleosides A–D (Al-Yahya et al., 2000), and penicillosides A–C (Abdel-Sattar et al., 2001) and was performed on russeliosides **E** and **F**. A NOESY cross-peaks were observed between H-17/H-20, H_3-18/H-20 and H_3-21/H-20 and was further supported from the absence of any correlation between H_3-21/H_3-18. From these observations, it can be safely concluded that the side-chain is indeed locked with carbon C-20 having the *S* configuration and with proton H-20 pointing to carbon 18.

3. Experimental

3.1. General experimental procedures

^1H NMR (500 MHz), ^{13}C NMR (125 MHz) and the 2D spectra were recorded in DMSO-d_6 on Varian 500 MHz, with TMS as an internal standard. HRCI-MS spectra were determined by VG-ZAB2E equipped with CI (70 eV) in positive ionization mode. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. TLC: precoated silica gel type 60 (Merck); CC: silica gel type 60 (Merck). HPLC was performed in the reversed phase on Knauer pump 64 and using preparative differential refractometer detector (column: Phenomenex RP-18, 250 × 25 mm, flow = 17 mL/min, elution with MeOH–H_2O mixtures).

3.2. Plant materials

The plant was collected in March, 1995, from a rocky regions of south of Jeddah, southwest of Saudi Arabia. The plant was kindly identified by Dr. Sultan Ul-Abedin, college of pharmacy, King Saud University, Riyadh, Saudi Arabia and a voucher specimen was deposited in the herbarium of the college of pharmacy, King Saud University (# 11677-A).

3.3. Extraction and isolation

The dried ground aerial parts (500 g) of *C. russeliana* were percolated with ethanol (5 l) at room temp to give a dark greenish semisolid residue (70 g) after evaporation of the solvent. The ethanolic extract (50 g) was suspended in water and defatted with petroleum ether, followed by shacking with $CHCl_3$ (10 g). A portion of the chloroform fraction (5 g) was chromatographed on silica gel column (3.5 × 16 cm) using a mixture of $CHCl_3$, followed by 4% MeOH/$CHCl_3$ to give four main fractions (**A**–**D**). Fraction **B** (19–23, 210 mg) was purified by HPLC (Phenomenex RP-18 column, 250 × 25 mm, flow = 17 mL/min) using MeOH–H_2O (75:25) to afford compounds **1** (6 mg), **2** (9 mg) and **4** (7 mg). Fraction **C** (24–34, 922 mg) also was purified by HPLC using MeOH–H_2O (85:15) to afford compounds **3** (5 mg).

3.4. Acid hydrolysis of compounds *1*–*4*

Compounds **1**–**4** were hydrolysed according to procedure reported by Halim and Khalil (1996).

3.5. Determination of the absolute configuration of the sugar moieties

The chloroform extract (2 g) was hydrolysed according to procedure reported by Halim and Khalil (1996). The H_2O layer was then concentrated and passed through a silica gel column using same procedure reported by Abe et al. (1999). The column afforded three sugars identified as D-cymarose, 6-deoxy-3-*O*-D-methylallose and D-glucose by comparison with the authentic samples on TLC and by comparison of their optical rotation values with those reported in the literature (Abe et al., 1999).

3.6. Russelioside *E* (*1*)

Amorphous powder $[\alpha]_D^{25} + 3.0^\circ$ (MeOH; *c* 1.5); IR (KBr) υ_{max} 3400, 1725 and 1235 cm^{-1}; CIMS *m/z* (rel. int): 1066 $[M+H-42]^+$ (100), 1048 $[M+H-60]^+$ (61), 1089 $[M+H-18]^+$ (16); HRCIMS $[M\text{-}18+H]^+$ *m/z* 1107.5752 (calc. for $C_{57}H_{86}O_{21}$, 1107.5740); ^1H NMR of aglycon (500 MHz, DMSO-d_6) δ: 8.03 (2H, *dd*, *J* = 1.5, 8.5, H-3′, 7′), 7.65 (1H, br *t*, *J* = 8.5, 1.5, H-5′), 7.53 (2H, *t*, *J* = 8.5 Hz, H-4′, 6′), 5.41 (1H, *m*, H-6), 4.79 (1H, *dq*, *J* = 6, 10 Hz, H-20), 4.66 (1H, br *d*, *J* = 10 Hz, H-12),

3.42 (1H, *m*, H-3), 2.16 (1H, *m*, H-7b), 1.90 (3H, *s*, COMe), 1.72 (1H, *m*, H-7a), 1.88 (1H, *m*, H-17), 1.01 (1H, *s*, H-18), 0.99 (3H, *d*, $J = 6$ Hz, H-21),0.91 (1H, *s*, H-19). See Table 1 for ^{13}C NMR of the aglycon and Table 2 for ^{1}H NMR and ^{13}C NMR of sugar moieties.

3.7. Russelioside F (2)

Amorphous powder $[\alpha]_D^{25} - 4.5^\circ$ (MeOH; *c* 0.2); IR (KBr) υ_{max} 3400, 1725 and 1235 cm^{-1}; CIMS *m*/*z* (rel. int): 931[M+H−16]$^+$ (90), 929 [M+H−18]$^+$ (42), 915 [M+H−32]$^+$ (65); HRCIMS [M+H−18]$^+$ *m*/*z* 929.4831 (calc. for $C_{50}H_{73}O_{16}$, 929.4898); ^{1}H NMR of aglycon: see compound **1**. See Table 1 for ^{13}C NMR of the aglycon and Table 2 for ^{1}H NMR and ^{13}C NMR of sugar moieties.

3.8. Russelioside G (3)

Amorphous powder $[\alpha]_D^{25} - 25^\circ$ (MeOH; *c* 0.15); IR (KBr) υ_{max} 3400, 1725 and 1235 cm^{-1}; CIMS *m*/*z* (rel. int): 785 [M+H−18]$^+$ (16), 743 [M+H−60]$^+$ (66); HRCIMS [M+H−18]$^+$ *m*/*z* 785.41298 (calc. for $C_{43}H_{61}O_{13}$, 785.41122); ^{1}H NMR of aglycon: see compound **1**. See Table 1 for ^{13}C NMR of the aglycon and Table 2 for ^{1}H NMR and ^{13}C NMR of sugar moieties.

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