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Pregnane Glycosides from *Caralluma russeliana*

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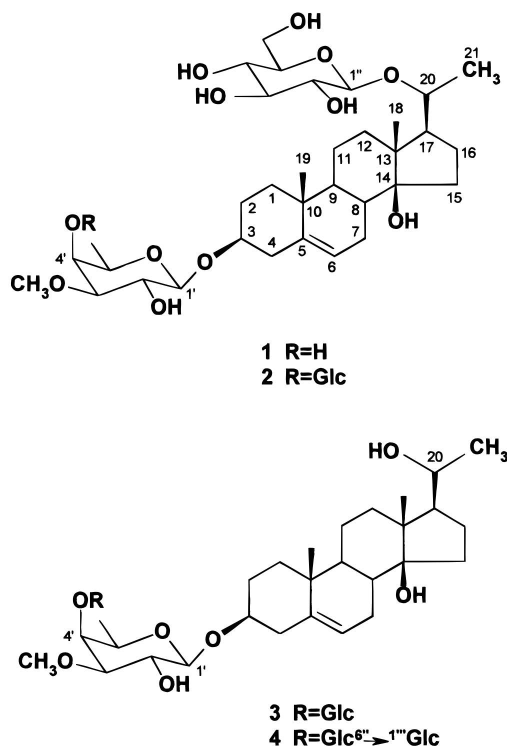
The aerial parts of *Caralluma russeliana* yielded four new pregnane glycosides, russeliosides A−D (**1**−**4**), in addition to a known flavone glycoside, luteolin 4′-*O*-*β*-D-neohesperidoside. The structures of compounds **1**−**4** were elucidated using a combination of spectroscopic methods.

The genus *Caralluma* belongs to the family Asclepiadaceae, which comprises some 200 genera and 2500 species. Plants belonging to the genus *Caralluma* are normally leafless and succulent perennial herbs that grow wild in stony habitats.[1] Several members of the genus *Caralluma* have found medicinal uses in the treatment of rheumatism, diabetes, and leprosy and as antiseptics and disinfectants.[2] Previous studies on plants in the genus *Caralluma* have reported the isolation of several pregnane glycosides or their esters,[3−7] of which some showed antitumor activity,[8,9] and others were postulated as precursors of cardenolides.[8] We report here the isolation and structure determination of four new pregnane glycosides (**1**−**4**) and a known flavone glycoside from the aerial parts of *Caralluma russeliana* (Courb. Ex Brongn.) Cufod.[10]



The *n*-butanol fraction of the ethanol extract of the aerial parts of *C. russeliana* was chromatographed repeatedly to afford five compounds, namely, four pregnane glycosides (**1**−**4**) and a known flavone glycoside. Compounds **1**−**4** gave a positive test for sterols (Liebermann-Burchard reagent), and all five compounds gave a positive test for sugars and/or glycosides (Molish reagent).

Compound **1** was isolated as colorless needle crystals, mp 170−172 °C, $[\alpha]^{25}_{D}$ −13°, and was assigned the molecular formula $C_{34}H_{56}O_{12}$, as shown by its ESMS data (*m*/*z* 679 [M + Na]$^+$) in combination with the ^{13}C NMR spectral data. Its IR spectrum showed the presence of a hydroxyl group (3450 cm^{-1}) and the absence of carbonyl groups. Compound **1** showed two anomeric protons and carbons at δ_H 4.39 and 4.32 and δ_C 104.5 and 102.9 in the ^1H and ^{13}C NMR spectra, respectively, suggesting it to be a diglycoside. Acidic hydrolysis of **1** yielded two sugars, of which one was identified as glucose (TLC). The structure of the second sugar was determined as 3-*O*-methyl-6-deoxygalactose by comparison with NMR data reported in the literature.[3,6,7] The signals at δ_H 1.29 (3 H, d, J = 6.2 Hz) and δ_C 17.1 and at δ_H 3.46 (3 H) and δ_C 57.3 were assigned to the secondary methyl (C-6′) and OMe groups, respectively, in 3-*O*-methyl-6-deoxygalactose. The genin was identified as calogenin from a careful analysis of its NMR spectra (COSY, HMQC, HMBC) and by comparison with literature values.[7,11−13] The double bond was positioned between C-5 and C-6 on the basis of long-range HMBC correlations between H-6 and C-4 and C-7 and between H-5 and C-4 and C-7. This genin is common to russeliosides A−D (**1**−**4**). The glycosidation site at C-3 was deduced from the ^{13}C NMR spectrum of **1** as a result of the downfield shift of C-3 and the upfield shifts of C-2 and C-4.[14] A long-range correlation between C-3 (δ_C 79.9) and H-1′ (δ_H 4.32) in the HMBC spectrum indicated the attachment of the 3-*O*-methyl-6-deoxygalactose sugar unit to C-3. The glucose unit was shown to be attached to C-20 from the downfield shift (+12 ppm) of the C-20 signal, relative to analogous data for compound **4**. Further structural proof for **1** was achieved by observation of a long-range correlation between C-20 (δ_C 79.1) and H-1″ (δ_H 4.39) in the HMBC spectrum. Compound **1** was assigned with H-17 in the α-configuration (δ_H 1.70, dd, J = 11.1, 4.7 Hz) and the side chain with an *β*-configuration, as deduced from the NOESY spectrum and by comparison with related compounds.[5−7,15] From the foregoing evidence, the structure of compound **1** was established as calogenin 20-*O*-*β*-D-glucopyranosyl-3-*O*-*β*-D-(3-*O*-methyl-6-deoxy)-galactoside and has been named russelioside A.

Compound **2** was isolated as colorless crystals, mp 202−204 °C, $[\alpha]^{25}_{D}$ − 15.4°, with the molecular formula $C_{40}H_{66}O_{17}$ determined from its ^{13}C NMR and ESMS (*m*/*z* 841 [M + Na]$^+$) data. Its IR spectrum was similar to that of com-

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pound **1**. Acid hydrolysis of **2** showed the same aglycone and the same sugar units as those of **1** (glucose and 3-*O*-methyl-6-deoxygalactose). Compound **2** exhibited three anomeric protons and carbons at δ_H 4.35, 4.38, and 4.60 and δ_C 104.8, 104.7, and 103.4 in its ^{1}H and ^{13}C NMR spectra, respectively, suggesting it to be a triglycoside. The ^{1}H and ^{13}C NMR spectra showed the presence of an additional glucose unit attached through a 1 → 4 linkage to the 3-*O*-methyl-6-deoxygalactose unit. This was confirmed from the HMBC spectrum, which demonstrated a long-range correlation between C-1″ (δ_C 104.67) and H-4′ (δ_H 4.16) and between C-4′ (δ_C 75.5) and H-1″ (δ_H 4.60). Thus, compound **2** was deduced as calogenin 20-*O*-β-D-glucopyranosyl-3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-(3-*O*-methyl-6-deoxy)]galactoside and has been named russelioside B.

Compound **3** was isolated as colorless crystals, mp 188−190 °C, $[\alpha]^{25}_D$ − 39.3°, with the molecular formula $C_{34}H_{56}O_{12}$, as deduced from its ^{13}C NMR and ESMS (*m*/*z* 679 [M + Na]$^+$) data. Compound **3** showed only two anomeric protons and carbons at δ_H 4.42 and 4.67 and δ_C 105.5 and 106.8 in the ^{1}H and ^{13}C NMR spectra, respectively, suggesting it to be a diglycoside. The signal at δ_C 68.8 corresponding to C-20 appeared shifted upfield (−10.3 ppm) relative to those of compounds **1** and **2**, indicating the absence of glycosidation at C-20. The identity of the two sugars at C-3 and their linkages were similar to **2** and were confirmed from the ^{1}H−^{1}H COSY and HMBC spectra. Thus, compound **3** was assigned as calogenin 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-(3-*O*-methyl-6-deoxy)]galactoside and has been named russelioside C.

Compound **4** was obtained as amorphous powder, $[\alpha]^{25}_D$ − 42°, and was assigned a molecular formula of $C_{40}H_{66}O_{17}$ (^{13}C NMR data and ESMS, *m*/*z* 841 [M + Na]$^+$). Compound **4** was shown to be a triglycoside, as it exhibited three anomeric protons and carbons at δ_H 4.59, 4.38, and 4.34 and δ_C 103.7, 105.1, and 105.9, respectively, in its ^{1}H and ^{13}C NMR spectra. In a manner similar to compound **3**, no sugar unit was found to occur at C-20, as indicated by the upfield shift (−12 ppm) of C-20 (δ_C 67.1) when compared to **1**. The additional sugar moiety was identified as glucose and was shown to be linked to the other glucose unit through a 1 → 6 linkage, as indicated by a downfield shift (+7.7 ppm) of C-6″ (δ_C 71.2) relative to the corresponding carbon in **2** (δ_C 63.5). Further confirmation was achieved from the HMBC spectrum, which showed correlations between C-6″ (δ_C 71.2) and H-1‴ (δ_H 4.38) and between C-1‴ (δ_C 105.9) and H-6″ (δ_H 4.15, 3.78). From the previous discussion and by comparison with data reported in the literature,[5,6] compound **4** was assigned as calogenin 3-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl(1→4)-β-D-(3-*O*-methyl-6-desoxy)]galactoside and has been named russelioside D.

A prominent feature in the ^{1}H NMR spectra of **1**−**4** was the very small value of the vicinal H-17 to H-20 coupling constants (see Experimental Section). A detailed analysis of the conformation of the side chain was performed on russelioside A (**1**), and NOESY cross-peaks were observed between H-17 and H-20, Me-18 and H-20, but not between Me-18 and Me-21. From these observations, it was concluded that the side chain is oriented with carbon C-20 having the *S* configuration.

The final isolate **5** was identified as luteolin 4′-*O*-β-D-neohesperidoside on the basis of the analysis of its spectral data and by comparison with NMR values reported in the literature.[16]

Experimental Section

General Experimental Procedures. Melting points were determined on an electrothermal melting point apparatus (Electrothermal Ltd., Southend-on-Sea, Essex, U.K.) and are uncorrected. Optical rotations were measured at ambient temperature, using a Perkin-Elmer 241 MC polarimeter. IR spectra were recorded on a Pye Unicam SP3-300 instrument. UV spectra were recorded on a Shimadzu 265 spectrophotometer. ^{1}H and ^{13}C NMR data were recorded in CD_3OD using a Bruker-400 NMR spectrometer (400.13 and 100.6 MHz, respectively) and a Varian Unity Plus 500 NMR spectrometer (500 and 125 MHz, respectively). Electrospray mass spectra (ESMS) were recorded on a quadrupolar Navigator mass spectrometer (Thermoquest).

Plant Material. The plant material was collected in March, 1995, from a rocky region south of Jeddah, in the 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 deposited in the herbarium of the College of Pharmacy, King Saud University (#11677-A).

Extraction and Isolation. The dried ground aerial parts (550 g) of *C. russeliana* were percolated with ethanol (5 L) at room temperature to give a dark greenish semisolid residue (76 g) after evaporation of the solvent. The ethanolic extract (55 g) was suspended in water and defatted with petroleum ether, then shaken with EtOAc (13 g) and *n*-BuOH (33 g). A portion of the *n*-BuOH fraction (7 g) was chromatographed on a Si gel column (6 × 19 cm) using a mixture of $CHCl_3$−MeOH−H_2O (78:20:2) to give four main fractions (A−D). Fraction A (250 mg) was rechromatographed on a Si gel column (3.5 × 17 cm) using 20% MeOH−$CHCl_3$ as solvent system to afford fractions A-1 and A-2. Fraction A-1 was purified by medium-pressure chromatography (MPLC) over C_{18} Si gel (LiChroprep RP-18, 25−40 μm, 2 × 18 cm) using 25% MeOH−H_2O (flow rate 4 mL/min), to give 40 mg of compound **1**. Compound **3** (17 mg) was isolated from fraction A-2 by chromatography over a Si gel column (3 × 16 cm) using 10% MeOH−$CHCl_3$ as solvent system. Fraction B (3.5 g) yielded pure compound **2** (2.1 g) by crystallization from MeOH/diethyl ether. Fraction C (2.2 g) was treated with MeOH/diethyl ether to give an additional amount of compound **2** (700 mg). The mother liquor left from fraction C was evaporated, and the residue left was rechromatographed on a Si gel column (2.5 × 15 cm) using EtOAc−MeOH−H_2O (100:20:12) as solvent system to remove a contaminating flavonoid. Fraction C-1 (310 mg) was further purified on another Si gel column (2 × 50 cm) using $CHCl_3$−MeOH−H_2O (90:10:1), to afford compound **4** (105 mg). Luteolin 4′-*O*-β-D-neohesperidoside (70 mg) was precipitated as a yellowish amorphous powder upon concentration of fraction D.

Acid Hydrolysis of Compounds 1−**4**. Compounds **1-4** were hydrolyzed according to the procedure reported by Ahmed et al.[5]

Russelioside A (1): colorless needles, mp 170−172 °C (MeOH/ether); $[\alpha]^{25}_D$ −13° (*c* 0.08, MeOH); IR (KBr) ν_{max} 3450 (OH), 2900, 1100 cm^{-1}; ^{1}H NMR (400.13 MHz, CD_3OD) δ 5.41 (1 H, br m, H-6), 4.39 (1 H, d, J = 7.9 Hz, H-1″), 4.32 (1 H, d, J = 7.9 Hz, H-1′), 4.01 (1 H, br q, J = 6.4 Hz, H-20), 3.84 (1 H, dd, J = 2, 12 Hz, H-6″A), 3.82 (1H, br d, J = 3.2 Hz, H-4′), 3.68 (1 H, dd, J = 12 Hz, 5.4, H-6″B), 3.55 (1 H, m, H-3), 3.46 (3 H, s, MeO), 3.17 (1 H, dd, J = 7.9 Hz, H-3′), 3.13 (1 H, dd, J = 9.6 Hz, 4, H-2″), 2.42 (1 H, dd, J = 13 Hz, 4.3, H-4α), 2.28 (1 H, bt, J = 12 Hz, H-4β), 2.24 (1 H, m, H-7α), 1.30 (3 H, d, J = 6.4 Hz, H-21), 1.29 (3 H, d, J = 6.2 Hz, H-6′), 1.11 (3 H, s, H-18), 1.02 (3 H, s, H-19); ^{13}C NMR, see Table 1; ESMS *m*/*z* 679 [M + Na]$^+$.

Russelioside B (2): colorless crystals, mp 202−204 °C (MeOH/ether); $[\alpha]^{25}_D$ −15.4° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3480 (OH), 2890, 1100 cm^{-1}; ^{1}H NMR (400.13 MHz, CD_3OD) δ 5.42 (1 H, br m, H-6), 4.60 (1 H, d, J = 7.7 Hz, H-1″), 4.38 (1 H, d, J = 7.9 Hz, H-1‴), 4.35 (1 H, d, J = 7.7 Hz, H-1′), 4.16 (1 H, br d, J = 2.1 Hz, H-4′), 4.01 (1 H, br q, J = 6.4 Hz, H-20), 3.85 (2 H, dd, J = 12, 3.5 Hz, H-6″, H -6‴A), 3.52 (1 H, m, H-3),

Table 1. ^{13}C NMR Data for Compounds **1**−**4** in CD$_3$OD (100.6 MHz)[a]

carbon	**1**	**2**	**3**	**4**
1	38.7, t	38.7, t	40.8, t	38.6, t
2	30.8, t	31.1, t	33.2, t	31.5, t
3	79.9, d	80.5, t	82.5, t	80.8, t
4	39.7, t	40.0, t	42.1, t	39.3, t
5	140.9, s	140.9, s	143.3, s	141.6, t
6	123.3, d	123.6, d	125.5, d	123.8, d
7	28.4, t	28.7, t	30.6, t	28.8, t
8	38.4, d	38.3, d	40.3, d	39.1, d
9	47.8, d	48.1, d	50.4, d	48.5, d
10	38.0, s	38.9, s	41.0, s	41.1, s
11	22.3, t	22.5, t	24.4, t	22.7, t
12	41.6, t	41.9, t	42.8, t	40.4, t
13	49.0, s	48.6, s	51.5, s	49.0, s
14	86.1, s	86.4, s	88.2, s	86.5, s
15	33.9, t	34.2, t	36.2, t	34.5, t
16	20.1, t	20.6, t	21.3, t	19.5, t
17	58.1, d	58.9, d	60.2, d	58.5, d
18	15.4, q	15.7, q	17.6, q	15.9, q
19	20.3, q	20.3, q	22.4, q	20.7, q
20	79.1, d	79.3, d	68.8, d	67.1, d
21	21.4, q	21.7, q	24.6, q	22.9, q
	MDG[b] at C-3	MDG at C-3	MDG at C-3	MDG at C-3
1′	102.9, d	103.4, d	105.5, d	103.7,d
2′	71.3, d	72.1, d	74.2, d	72.2, d
3′	84.7, d	86.2, d	88.2, d	86.5, d
4′	68.8, d	75.5, d	77.6, d	75.9, d
5′	71.8, d	72.1, d	73.9, d	72.5, d
6′	17.1, q	17.8, q	19.9, q	18.4, q
*C*H$_3$O	57.3, q	58.4, q	61.0, q	59.5, q
	Glc[c] at C-20	Glc (1→4)	Glc (1→4)	Glc (1→4)
1″	104.5, d	104.7, d	106.8, d	105.1, d
2″	75.4, d	75.7, d	78.4, d	76.6, d
3″	78.1, d	78.6, d	80.4, d	78.8, d
4″	71.4, d	71.8, d	74.4, d	72.7, d
5″	78.7, d	78.3, d	80.7, d	78.2, d
6″	62.8, t	63.5, t	65.5, t	71.2, t
		Glc at C-20		Glc (1→6)
1‴		104.8, d		105.9, d
2‴		76.3, d		75.9, d
3‴		79.0, d		78.8, d
4‴		72.3, d		72.5, d
5‴		78.3, d		78.8, d
6‴		63.2, t		63.6, t

[a] Multiplicities were determined by HMQC. [b] MDG: 3-*O*-methyl-6-deoxygalactose. [c] Glc: glucose.

3.51 (3 H, s, MeO), 3.16 (1 H, t, J = 8.5 Hz, H-2‴), 2.45 (1 H, dd, J = 13.6, 4.2 Hz, H-4α), 2.28 (1 H, br t, J = 13.6 Hz, H-4β), 2.22 (1 H, m, H-7α), 1.7 (1 H, dd, J = 11.1, 4.7 Hz, H-17), 1.28 (2 × 3 H, d, J = 6.4, H-6′, H-21), 1.12 (3 H, s, H-18), 1.02 (3 H, s, H-19); ^{13}C NMR, see Table 1; ESMS m/z 841 [M + Na]$^+$.

Russelioside C (3): colorless crystals, mp 188−190 °C (MeOH/ether); $[\alpha]^{25}_{D}$ −39.3° (c 0.08, MeOH); IR (KBr) ν_{max} 3450 (OH), 2900, 1090 cm^{-1}; ^{1}H NMR (400.13 MHz, CD$_3$OD) δ 5.48 (1 H, br m, H-6), 4.67 (1 H, d, J = 7.6 Hz, H-1″), 4.42 (1 H, d, J = 7.7 Hz, H-1′), 4.23 (1 H, brd, J = 2.8 Hz, H-4′), 4.07 (1 H, br q, J = 6.4 Hz, H-20), 3.94 (1 H, dd, J = 12, 2.3 Hz, H-6″A), 3.69 (1 H, dd, J = 12, 5.5 Hz, H-6″B), 3.59 (1 H, m, H-3), 3.57 (3 H, s, MeO), 3.37 (1 H, dd, J = 8.6 Hz, H-3′), 2.50 (1 H, dd, J = 13.1, 3 Hz, H-4α), 2.35 (1 H, br t, J = 13.1 Hz, H-4β), 2.28 (1 H, m, H-7α), 1.35 (3 H, d, J = 6.2 Hz, H-6′), 1.16 (3 H, d, J = 6.4 Hz, H-21), 1.12 (3 H, s, H-18), 1.10 (3 H, s, H-19); ^{13}C NMR, see Table 1; ESMS m/z 679 [M + Na]$^+$.

Russelioside D (4): amorphous powder, $[\alpha]^{25}_{D}$ − 42°(c 0.09, MeOH); IR (KBr) ν_{max} 3450 (OH), 2920, 1100 cm^{-1}; ^{1}H NMR (400.13 MHz, CD$_3$OD) δ 5.42 (1 H, br m, H-6), 4.59 (1 H, d, J = 7.9 Hz, H-1″), 4.38 (1 H, d, J = 7.9 Hz, H-1‴), 4.34 (1 H, d, J = 7.9 Hz, H-1′), 4.16 (1 H, br m, H-4′), 4.15 (1 H, dd, J = 12.2, 1.7 Hz, H-6′A), 4.0 (1 H, br q, J = 6.4 Hz, H-20), 3.87 (1 H, dd, J = 12, 2.3 Hz, H-6‴A), 3.78 (1 H, d, J = 12.2, 6.4 Hz, H-6″B), 3.65 (1 H, dd, J = 12, 5.6 Hz, H-6‴B), 3.52 (1 H, m, H-3), 3.51 (3 H, s, MeO), 2.43 (1 H, dd, J = 13.5, 3 Hz, H-4α), 2.27 (1 H, br t, J = 13.5 Hz, H-4β), 2.21 (1 H, m, H-7α), 1.30 (3 H, d, J = 6.5 Hz, H-6′), 1.10 (3 H, d, J = 6.5 Hz, H-21), 1.06 (3 H, s, H-18), 1.03 (3 H, s, H-19); ^{13}C NMR, see Table 1; ESMS m/z 841 [M + Na]$^+$.

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