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Further Pregnane Glycoside, Russelioside B, and Flavonoid Glycoside from *Huernia saudi-arabica*

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Abstract: Phytochemical investigation of *Huernia saudi-arabica* aerial parts has resulted in separation and identification of a pregnane glycoside, russelioside B, as its first report from this plant species, using chromatographic and spectroscopic techniques. Additionally, the flavonoid glycoside, luteolin 4'-O-*β*-D-neohesperidoside was also isolated and identified.

keywords: : Huernia saudi-arabica, Apocynaceae, aerial parts, russelioside B, flavonoid

1.Introduction

Huernia genus is widely cultivated as ornamental plants literally everywhere. They are native to Africa and Arabian Peninsula. A member of stapeliads family which belongs to subfamily Asclepiadoideae of Apocynaceae [1]. After *Caralluma*, *Huernia* is the second most varied section of stapeliads in terms of species and subspecies diversity [2], with about 70 currently known species.

Numerous pregnane glycosides or their esters have been isolated from *Huernia* plants in earlier research, with some exhibited anti-tumor activity. Several *Huernia* species have been used traditionally as antiseptics and disinfectants, as well as treatments for leprosy, diabetes, and rheumatism [3], in addition to famine-relief herbs [4]. Pregnane glycoside ester was isolated previously from *Huernia saudi-arabica* demonstrated a significant anti-schistosomal impact [3,4]. Furthermore, the inhibition of α-glucosidase and pancreatic lipase enzymes by extracts and pregnane glycosides from *Huernia saudi-arabica* was also reported previously [4].

In this paper, we discussed the results of further investigation of aerial parts of *Huernia Saudi-arabica* by comprehensive chromatographic separation and spectral identification that led to isolation of two compounds, a pregnane glycoside and a flavonoid glycoside.

2. Results and Discussion

The chemical investigation of butanol extract of *H. saudi-arabica* aerial parts yielded two glycoside compounds, pregnane glycoside and flavonoid glycoside which were characterized based on NMR analyses.

Compound **1** (Fig.1) was obtained as colorless amorphous powder. ^{13}C NMR (Table 1) confirmed the occurrence of 40 carbons divided into four methyl, one methoxyl, ten methylene, twenty-one methine groups and four quaternary carbon atoms. Two signals appeared in ^{1}H and ^{13}C NMR spectra at δ_H 1.12 /δ_C 15.30 and 1.04 /δ_C 19.93 that were referred to the angular methyl CH_3-18 and CH_3-19 of pregnanes, respectively. In addition to an olefinic proton at δ_H 5.44 (br.s, 1H, H-6) /δ_C 123.20 situated between C-5 and C-6 depending on HMBC correlation (Fig. 2) with C-5 (δ_C140.65) and H-4 and H-7, H-6 (δ_H 5.44) and C-4 and C-7. From analysis of 2D spectra (COSY, HSQC and HMBC) and comparing with previously reported data by Al-Yahya

et.al. [5], the aglycone was identified as calogenin. From the ^{1}H and ^{13}C NMR data of **1** (Table 1) three anomeric protons signals at δ_H 4.38, 4.62 and 4.41 correlated with carbons in HSQC at δ_C 102.94, 104.15 and 104.34, respectively, indicating it to be glycoside with three sugar units. One of them was identified as 3-O-methyl-6-deoxygalactose from the presence of signals for methyl and methoxyl at δ_H 1.30 (d, 3H, 6.3 Hz) (δ_C 17.36) and δ_H 3.52 (s, 3H) (δ_C 57.92), repectively. Along with detailed examination of 2D-NMR spectra, the two other sugar moieties were identified as glucose. One of glucose moiety attached by 1→4 link to the 3-O-methyl-6-deoxygalactose moiety which was approved by long range ^{3}J HMBC correlation among C-4` (δ_C 74.87) and H-1`` ($\delta_H$ 4.62) and C-1`` (δ_C 104.15) and H-4` ($\delta_H$ 4.20). By the same way, strong $^{3}J$ HMBC interconnection between H-1` (δ_H 4.38) and C-3 (δ_C 80.07). from HMBC, the connection of the 3-O-methyl-6-deoxygalactose unit to C-3 was shown besides the downfield shift of C-3 (δ_C 80.07) compared to upfield shifts of (C-2 at δ_C 30.67) and (C-4 at δ_C 39.54) in ^{13}C NMR spectrum, which was confirm the location of glycosidation linkage at C-3. The remaining glucose unit linked to C-20 which was achieved by noticing long-range attachment in HMBC between H-1``` (δ_H 4.41) and C-20 (δ_C 78.96) and between C-1``` (δ_C 104.34) and H-20 (δ_H 4.03). Large coupling constants of the anomeric protons have suggested them as β-D forms [6]. The relative configuration at C-17 (δ_H 1.7, m) was determined by comparing with literature NMR spectral data as α-orientation and *β*-configuration of the side chain [7,8].

Finally, by comparison with data previously published by Al-Yahya *et al* [5] compound **1** was identified as russelioside B which was reported from this plant species for the first time.

Russelioside B, is an intriguing bioactive alternative, have variety of pharmacological properties, comprising anti-diabetic, anti-obesity, anti-nociceptive, anti-ulcer, anti-inflammatory, anti-arthritis, anti-biofilm, antibacterial, antiparasitic, gastroprotective potentials and wound healing activities [9,10].

Compound **2** (Fig. 1) was isolated as yellow powder. ^{1}H NMR spectrum of compound **2** (Table 2) displayed signals characteristic of flavone glucoside pattern. the presence of ABX signals system at δ_H 7.44 (m, 1H, C-2`), 7.43 (m, 1H, C-6`), 7.26 (d, 1H, J 8.28 Hz, C-5`) attributed to trisubstituted ring B, besides two meta located protons at δ_H 6.30 (brd, 1H, J 1.5 Hz, C-8) and 6.09 (brd, 1H, J 1.5 Hz, C-6) attributed to 5,7-dihydroxyl substituted ring A together with a singlet at δ_H 6.52, which could be referred to H-3. In addition to the presence of two anomeric protons at δ_H 5.20 (1H, d, J 7.48 Hz H-1`) and δ_H 5.27 (1H, d, J 1.42 Hz H-1``), which were assigned to be β-D-glucopyranose and α-L-rhamnopyranose, respectively. That information along with previously reported in literature [11,12] were confirmed the structure of **2** as luteolin 4`-O-*β*-D-neohesperidoside (luteolin-4′-O-[α-(L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside), which was previously reported from this plant species [3].

luteolin 4`-O-*β*-D-neohesperidoside exhibited antibacterial activity [13], antinociceptive, anti-inflammatory [14] and antioxidant activity [15].

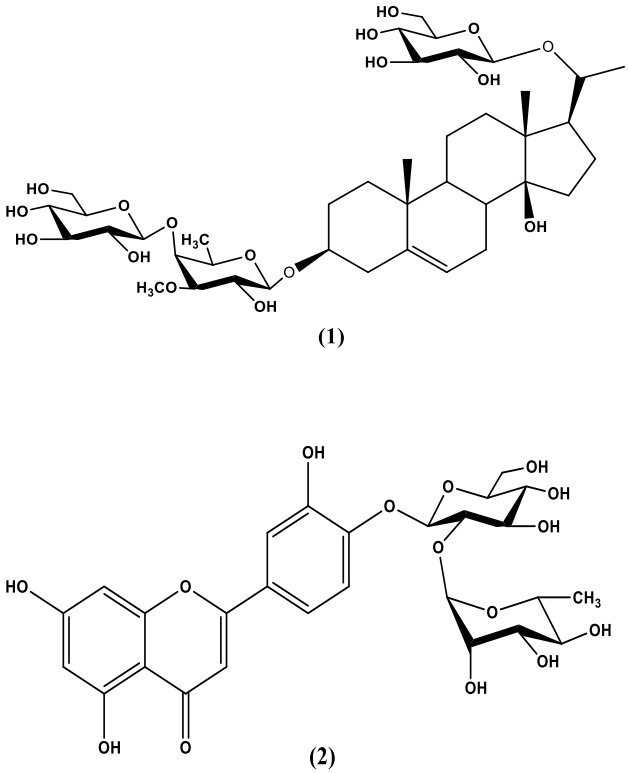

Fig. 1. Structures of isolated compounds **1, 2**

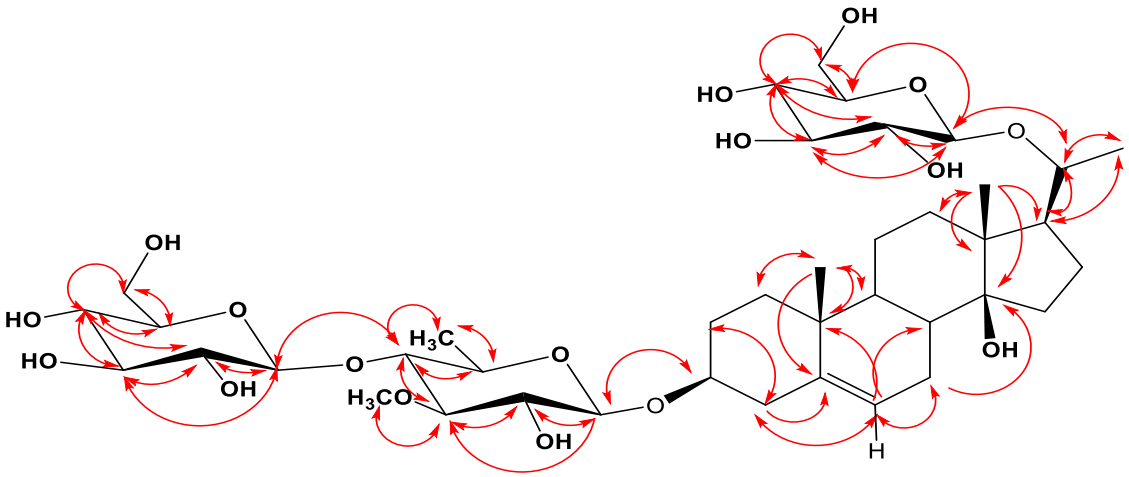

Fig. 2. HMBC correlations of **russelioside B (1)**

Table 1: ^{1}H, ^{13}C NMR for compound 1.

Position	^{1}H (multiplicity, J)	^{13}C	Sugars	^{1}H (multiplicity, J)	^{13}C
1	1.91, m, 2H	38.28	**Digitalose**		
2	1.93/1.64, m, 2H	30.67	1`	4.38, d, 1H, 7.8	102.94
3	3.54, m, 1H	80.07	2`	3.62, m, 1H	71.43
4	2.46, dd, 1H, 4.6, 12.6 /2.29, m, 1H	39.54	3`	3.28, m, 1H	85.71
5	-----	140.65	4`	4.20, brd, 1H, 3.6	74.87
6	5.44, brm, 1H	123.20	5`	3.66, m, 1H	71.61
7	1.87, 2.24, m, 2H	28.28	6`	1.30, brd, 3H, 6.4	17.36
8	1.77, m, 1H	37.81	3`-OCH_3	3.52, s, 3H	57.92
9	1.21, m, 1H	47.60	**Glucose**		
10	----	38.50	1``	4.62, d, 1H, 7.8	104.15
11	1.50/1.38, m, 2H	22.06	2``	3.23, m, 1H	75.89
12	1.40/1.49, m, 2H	41.48	3``	3.51, m, 1H	78.16
13	-----	48.32	4``	3.30, m, 1H	71.35
14	-----	85.98	5``	3.29, m, 1H	77.79
15	1.59/1.98, m, 2H	33.79	6``a	3.88, dd, 1H, 2.8, 12.4	62.97
16	1.86/ 1.95, m, 2H	20.13	6``b	3.68, m, 1H	
17	1.7, m, 1H	58.50	**Glucose at C-20**		
18	1.12, s, 3H	15.30	1```	4.41, d, 1H, 7.8	104.34
19	1.04, s, 3H	19.93	2```	3.16, t, 1H, 10.5	75.25
20	4.03, brq, 1H, 6.1	78.96	3```	3.46, m, 1H	78.55
21	1.30, brd, 3H, 6.1	21.25	4```	3.38, m, 1H	71.83
			5```	3.38, m, 1H	77.90
			6```a	3.88, dd, 1H, 2.8, 12.4	62.69
			6```b	3.68, m, 1H	

Table 2: ^{1}H NMR of compound 2

H atom	^{1}H (multiplicity, J)
3	6.52 (1H, s)
6	6.09 (1H, brd, J 1.5 Hz)
8	6.30 (1H, brd, J 1.5 Hz)
2`	7.44 (1H, m)
5`	7.26 (1H, d, J 8.3 Hz)
6`	7.43 (1H, m)
glucose	
1``	5.20 (1H, d, J 7.48 Hz)
rhamnose	
1```	5.27 (1H, d, J 1.4 Hz)
6```	1.21 (3H, d, J 6.3 Hz)

3. Experimental

General

NMR spectra were captured on a 400 MHz (Bruker) and 500 MHz (JEOL), Mansoura University, Egypt. Column chromatography was achieved by using polyamide 6. preparative TLC (PTLC) were carried out on silica gel with 0.25 thickness (Kieselgel 60, GF 254). Petroleum ether, chloroform, EtOAc, and MeOH were bought from Adwic Company Mansoura, Egypt.

Plant material

the aerial parts of *H. saudi-arabica* were collected from Amran Governorate, Yemen, on June 2021 which then were dried in shade. Dr. Hassan M. Ibrahim, head of the Herbarium, Bioligical Science Department, Faculty of Science, Sana'a University identify the plant.

Extraction process

Powderly air-dried *H. arabica*'s aerial parts were extracted using methanol. The extract of methanol was concentrated by evaporation under reduced pressure furnish 30 g of brown residue which was diluted with distilled water and fractionated successively with chloroform ($CHCl_3$) (3 x 2L), ethyl acetate (EtOAc) (3 x 2L) and n-butanol (4 x 3L) to give 2.15, 0.2 and 12.78 g, respectively.

Butanol fraction (12.78 g) was chromatographed over polyamide S6 column utilizing mobile phase of dist. H_2O, (dist.H_2O: methanol) and (methanol: ammonia), with raising polarities. Two main fractions were obtained after TLC examination. Compound (**1**) was obtained from Fraction **I** as a result of purification by repetitive PTLC using (silica gel, chloroform / methanol / H_2O (**73:28:5**) (41 mg, R_f =0.67). while, further purification for fraction **II** with the use of preparative TLC silica gel plates (silica gel, chloroform/ methanol/ H_2O **75:26:5**) produced compound (**2**) (9.5 mg, R_f =0. 29**).**

Calogenin 20-*O*-*β*-D-glucopyranosyl-3-*O*-[*β*-D-glucopyranosyl-(1 → 4)-β-D-(3-*O*-methyl-6-deoxy) galactoside] (**russelioside B):** Colorless amorphous powder, see Table 1 for ^{1}H and ^{13}C NMR (400 MHz, 100 MHz, CD_3OD).

Luteolin 4`-O-*β*-D-neohesperidoside: yellow powder, see Table 2 for ^{1}H NMR (500 MHz, CD_3OD).

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