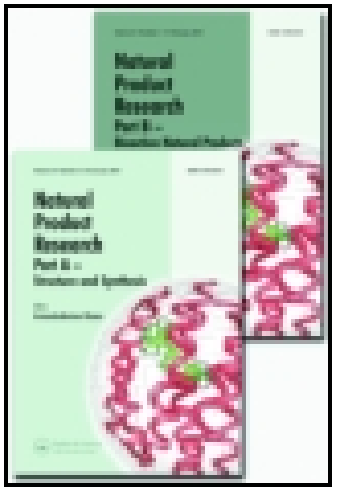


Natural Product Research: Formerly Natural Product Letters

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

New pregnane glycoside derivative from Caralluma retrospiciens (Ehrenb)

Mahmoud Fahmi Elsebai[ac] & Ietidal EL-Tahir Mohamed[b]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

[b] Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan

[c] Department of Chemistry, University of Oulu, Oulu, Finland

Published online: 23 Jan 2015.

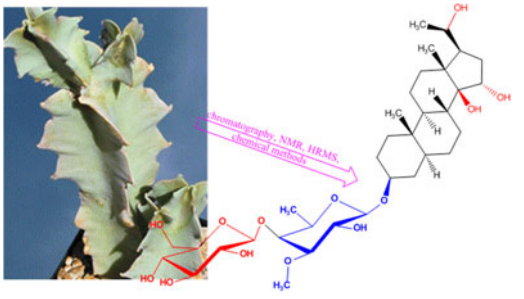
Natural Product Research, 2015
http://dx.doi.org/10.1080/14786419.2014.1003186

New pregnane glycoside derivative from *Caralluma retrospiciens* (Ehrenb)

Mahmoud Fahmi Elsebai[ac]* and Ietidal EL-Tahir Mohamed[b]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt;* [b]*Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan;* [c]*Department of Chemistry, University of Oulu, Oulu, Finland*

(*Received 1 October 2014; final version received 24 December 2014*)



Retrospinoside (**1**) is a new polyoxy pregnane glycoside which was isolated and characterised from the aerial parts of *Caralluma retrospiciens* (Ehrenb.) N. E. Br., family Apocynaceae. The structure was established as 3-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-(3-*O*-methyl-6-desoxygalactopyranosy)]-14,15,20-trihydroxy-4β-pregnane. Its structural elucidation was performed through extensive spectroscopic measurements including 1D- and 2D-NMR, and HRMS, in addition to chemical methods.

Keywords: plant natural products; pregnane glycosides; polyoxy pregnanes; *Caralluma retrospiciens*

1. Introduction

Caralluma retrospiciens (Ehrenb.), family Apocynaceae, is a rare succulent plant that grows wild on stony grounds. More than 200 species of *Caralluma* spp. grow throughout Asia and Africa, and the majority of these species are indigenous to the Arabian Peninsula and the Indian sub-continent (Al-Massarani et al. 2012). Several members of the genus *Caralluma* have found medicinal uses in the treatment of rheumatism, diabetes, snake and scorpion bites, tuberculosis and leprosy, scabies, fever and inflammation and as antiseptic and disinfectant (Al-Massarani et al. 2012). Many asclepiadaceous plants, example of the genus *Caralluma*, are rich in pregnane glycosides and their esters possessing a steroidal nucleus and function as precursors of the cardenolides (Halaweish et al. 2004; Avula et al. 2011; Abdel-Mogib & Raghib 2013; Adnan et al. 2014). The pregnane derivatives showed important pharmacological effects such as antitumor, cytotoxic, platelet pro-aggregating and anti-fungal activities. Pregnane-type glycosides were recently proved to induce differentiation of the mouse myeloid leukaemia

*Corresponding author. Email: elsebai72@yahoo.com

cells into phagocytic cells, appetite suppression and anti-obesity, and antitrypanosomal activity (Halaweish et al. 2004; Abdel-Sattar et al. 2009; Dutt et al. 2012). Only few pregnane glycosides have been isolated and characterised from *C. retrospiciens* (Khalil 1995; Halim & Khalil 1996; Halaweish et al. 2004).

The current phytochemical investigation on *C. retrospiciens* led to the isolation of a new polyoxy pregnane glycoside, retrospinoside (**1**). Retrospinoside (**1**) is a new polyoxy pregnane glycoside which was isolated from the aerial parts of *C. retrospiciens* (Ehrenb.) N. E. Br., family Apocynaceae. The identification of **1** was done using extensive spectroscopic data including 1D- and 2D-NMR, and HRMS, in addition to chemical means to determine the absolute identity of sugars and positions of hydroxyl groups.

2. Results and discussion

The methanolic extract of the aerial parts of *C. retrospiciens* yielded compound **1** after fractionation and repeated chromatographic separation on Si gel columns. Compound **1** is a glycoside polyoxy pregnane derivative of the two sugars D-glucose and 6-deoxy-3-*O*-methyl-β-D-galactose with the steroidal pregnane nucleus (Figure 1). Compound **1** was obtained as white powder with a molecular formula $C_{34}H_{58}O_{13}$ on the basis of FAB-MS mass measurement (*m/z* 673 $[M-H]^-$, 675 $[M+H]^+$) and (+)-HR-ESI-MS (*m/z* 697.3769 $[M+Na]^+$). The presence of various signals in the aliphatic proton region (0.5–2.5 ppm and 10–45 ppm) supported the presence of a steroidal compound. The ^1H and ^{13}C NMR spectra showed various resonance peaks through the $^1H-^{13}C-OH$ signal in the regions 3.10–5.20 ppm and 60–85 ppm, respectively, and the presence of two anomeric protons (4–5 ppm and 90–110 ppm) suggested a disaccharide glycoside. The acid hydrolysis of **1** with 1% HCI yielded two sugars, one is D-glucose which was identified by TLC, and the second sugar was determined as 6-deoxy-3-*O*-methyl-β-D-galactose based on the extensive spectroscopic data as described further. The IR spectrum showed peaks at 3455 cm^{-1} for the OH group. Compound **1** showed no absorption in the UV region indicating the absence of double bond characters.

Due to the presence of many hydroxyl groups both in the aglycone and sugar parts, and in order to obtain additional information and better quality of spectra for assignments, compound **1** was acetylated with acetic anhydride-pyridine. Extensive 1D and 2D NMR experiments and all detailed assignments, therefore, were performed on the acetylated product **2**.

Compound **2** showed a molecular formula of $C_{48}H_{72}O_{20}$ on the basis of the number of signals in both ^1H-, ^{13}C- and DEPT-NMR spectra (Figures S3–S5) and accurate mass measurement

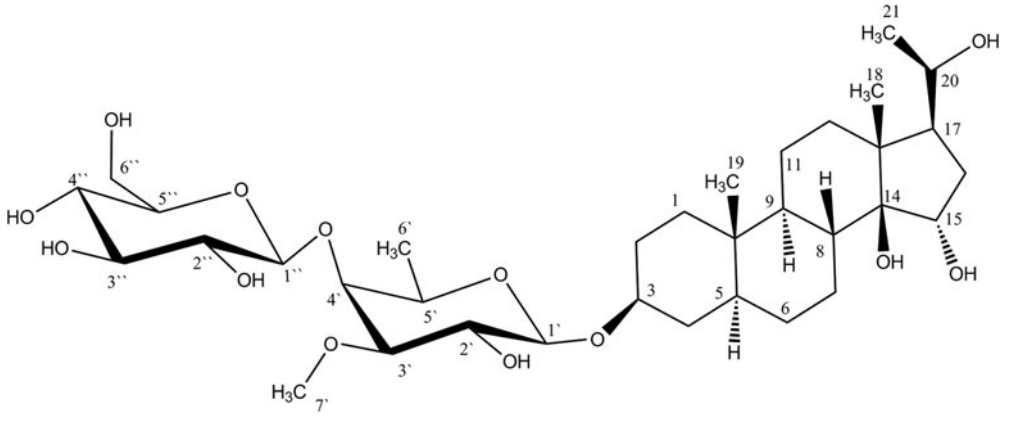


Figure 1. The structure of compound **1** isolated from *C. retrospiciens* (Ehrenb).

(HR-ESI-MS: *m/z* found = 991.4509 $[M+Na]^+$). The steroidal portion of **2** gave rise to ^1H and ^{13}C NMR signals (Figures S2 and S3, Table S11) very similar to those of a sterol compound related to a hydroxylated pregnane derivative which is also expected since it was isolated from a plant known to produce these types of steroids. Thus, the methyl groups of the sterol portion produced singlets at $\delta_{H/C}$ 0.92/15.1 and 0.68/12.4 for the angular tertiary CH_3-18 and CH_3-19, respectively. The ^1H NMR spectrum showed also two doublet methyl groups at δ_H 1.07 for CH_3-21 ($J = 5.90$ Hz) and at δ_H 1.83 for CH_3-6′ ($J = 6.60$ Hz). There were also further signals in the ^1H NMR spectrum for the multiple CH_2 groups between 0.5 and 2.5 ppm, in addition the down field resonance peaks of the sugars between 3.2 and 5.3 ppm. Interpretation of the COSY and HMBC spectra (Figure S7 and S8, Figure 2, Table S11) confirmed the structural features of compound **2** to be as drawn in Figure 1 and provided evidence for a steroidal skeleton similar to that of a pregnane type nucleus. The ^{13}C spectrum showed 15 oxygenated carbons C-3, C-14, C-15, C-20, C-1′, C-2′, C-3′, C-4′, C-5′, C-1″, C-2″, C-3″, C-4″ C-5″ and C-6″. The acetylation was performed on seven carbinols 15, 20, 2′, 2″, 3″, 4″ and 6″ to produce hepta-acetylated pregnane derivative as shown from their resonances in the ^1H and ^{13}C NMR spectra. Their positions were unambiguously determined by the HMBC correlation to the respective carbinols (Figure 2, Table S11). The oxygenated quaternary carbinol at 82.1 ppm was assigned to C-14 in accordance with reported data (Usmanghani & Rizwani 1988) and it was not acetylated due to the steric hindrance. OH-15 can be connected to C-15 or C-16, but it must be connected to C-15 based on the following: the COSY measurement (Table S11 and Figure 2) determined correlations between H-15 and H-16 and between H-16 and H-17 and no COSY correlation between H-15 and H-17, which means H-16 is centralised between H-15 and H-17. In addition, the multiplicity of H-15 as a triplet and H-16 (a and b) as multiplets and H-17 as a multiplet necessitates the presence of H-16 between H-15 and H-17 as illustrated in Figure 2.

The sugar residues of **2** were represented by two monosaccharide units as indicated by two anomeric carbon signals at δ_C 99.5 for CH-1′ and δ_C 100.4 for CH-1″, and two anomeric protons at δ_H 4.28 (1H, d, $J = 7.32$ Hz) for CH-1′, and δ_H 4.80 (1H, d, $J = 8.42$ Hz) for CH-1″, determined from the HMQC spectrum. The two sugars are β-configured due to their large values of coupling constant of the respective anomeric protons (Agrawal 1992). Glycosylation occurred at C-3 (Figures 1 and 2) as observed from the HMBC correlations between H-1′ and C-3, confirming that the sugar of galactose derivative is the one which is directly attached to the

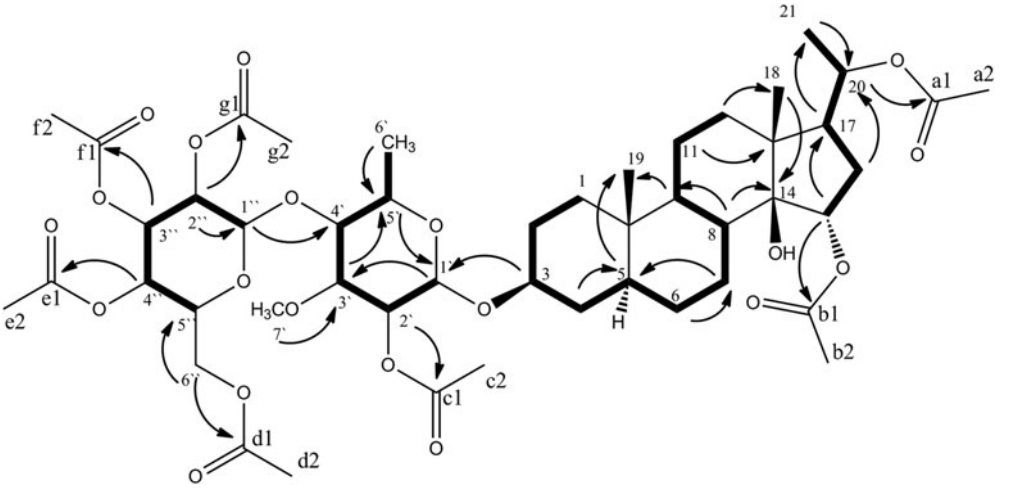


Figure 2. The key correlations of COSY (bold lines) and HMBC (arrows from H to C) for compound **2**.

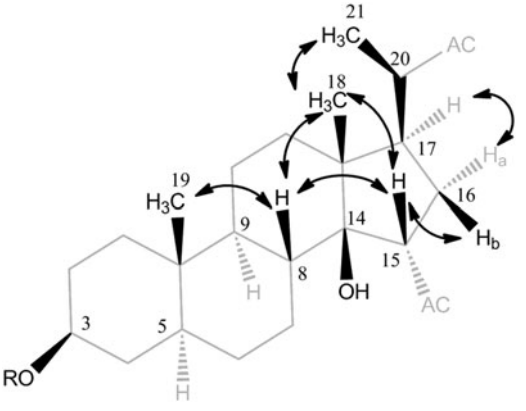


Figure 3. The key NOESY correlations of compound **2**.

pregnane nucleus, and from H-1″ to C-4′ indicating that the interglycosidic linkage is 1 → 4 connected disaccharide.

The ^{1}H NMR spectrum showed a resonance peak at $\delta_{H/C}$ 3.33/58.3 which is typical for a methoxy group which was assigned for CH_3O-7′, and it is attached to C-3′ due to HMBC correlation from CH_3O-7′ to CH-3′. Also in the ^{1}H NMR spectrum, it showed a doublet for the methyl group CH_3-6′ which is connected to CH-5′ due to mutual HMBC correlation. The 2D TOCSY experiment (Figure S10) showed a one spin system from CH-1′ to CH-6′, constructing a galactose unit containing one methoxyl group CH_3O-7′ and one methyl group CH_3-6′ instead of CH_2–OH to get a 6-desoxy-3-*O*-methyl galactose. We propose the D nature of the galactose derivative based on the literature which established the same type of sugar from the same plant. Moreover, the chemical shifts of galactose moiety in the ^{13}C data of compound **1** (Table S1) is almost identical to the same galactose moiety in the pregnane glycosides isolated from the same plant sp. (Halaweish et al. 2004).

For the stereochemistry of compound **2**, the methine multiplet at $\delta_{H/C}$ 3.47/78.1 has the expected complexity of steroidal 3-carbinol hydrogen which is characteristic for 3β-hydroxy sterols. The relative configuration of compound **2** was determined by interpretation of NOESY correlations and coupling constants. NOESY correlations between H_3-19 and H-8, between H-8 and both H_3-18 and H-15, and between H-15 and both H-18 and H-16b indicated all these protons to be on the same side of the molecule, i.e. in the β-position (Figure 3). A NOESY correlation between H-16a and H-17 and the absence of NOESY correlation between H-9 and any of H_3-19 and H-18, and absence of NOESY correlation between H-5 and any of H_3-19 and H-18 indicated that H-5, H-9, H-16a and H-17 are in the same other side of the molecule, i.e. in the α-position. We propose the β-orientation of OH-14 based on the chemical shift of the oxygenated quaternary C-14 at 82.1 ppm which was assigned to C-14 in accordance with the reported data (Usmanghani & Rizwani 1988; Agrawal 1992; Kunert et al. 2006, 2008, 2009; Oyama et al. 2007; Al-Massarani et al. 2012). These correlations indicated the *transoid* nature of rings A/B and B/C and the *cisoid* nature of rings C/D. The absolute configuration at C-20 was determined to be *R* based on its chemical shift value in the ^{13}C spectrum at 73.4 ppm (Al-Massarani et al. 2012) which was supported by the NOESY correlation between H_3-18 and H-20. We gave the name retrospinoside for compound **1**.

3. Experimental

3.1. *General experimental procedures*

Melting points were measured on a YANACO apparatus (Yanaco New Science Inc., Kyoto, Japan). Optical rotations were measured on Schmidt + Haensch Polartronic D polarimeter

(Schmidt & Haensch GmbH & Co. Feinmechanik Optik, Berlin, Germany). Ultraviolet (UV) spectra were recorded in methanol on a Hitachi U-3200 spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan). IR spectrum was measured on a Jasco A-302 Infrared spectrophotometer (JASCO Corporation, Hachioji, Japan). All NMR spectra were recorded in CD_3OD and $CDCl_3$ employing Bruker Avance 400 DPX and 600 DRX spectrometers (Bruker, Rheinstetten, Germany). Column chromatography was performed on a glass column packed with silica gel (E. Merck (Darmstadt, Germany), type 60, 70–230 mesh) of particle size 70–230 mesh. The extract was chromatographed after being absorbed onto a small amount of the packing material. The aerial parts of the *Caralluma retrospiciens* (Ehrenb) were collected from Erkowit, Red Sea, Hills Eastern, Sudan, and identified at the Department of Botany, University of Khartoum with a voucher specimen (F-3-215) deposited at the herbarium of the Department of Botany.

3.2. *Extraction, isolation and characterisation*

The fresh plant material (0.52 kg) was chopped into small pieces, dried at room temperature and then soaked in MeOH for 3 days. The methanolic extract was evaporated under reduced pressure, which yielded a dark green thick residue 50.3 g. This was dissolved in distilled water (1 L) and defatted with pet. ether (3 L × 3 L) to afford 11.6 g extract. The defatted aqueous extract was further fractionated with CH_2Cl_2 (3 L × 3 L), EtOAc (3 L × 3 L) and then with *n*-BuOH (3 L × 3 L). On evaporation of the three fractions, 7.3, 5.4 and 13 g of extracts were obtained, respectively. The diethyl ether-soluble part of *n*-BuOH fraction was subjected to CC over polyamide using MeOH:H_2O as eluent. Fraction A was re-chromatographed over silica gel using EtOAc and then the polarity was increased by MeOH to afford compound **1**.

3.3. *Acid hydrolysis*

Compound **1** (10 mg) was refluxed with 1% HCI (10 mL) in a boiling water bath for 5 h. Thereafter, 10 mL H_2O was added and concentrated to 5 mL, and the reaction mixture was kept in a boiling H_2O bath for 30 min and extracted with $CHCI_3$. The aqueous phase of the hydrolysate was neutralised with saturated barium hydroxide solution, the mixture filtered and the filtrates concentrated, freeze-dried and subjected to TLC examination of the sugars against standard glucose and other monosaccharaides. Paper chromatography for sugars was run on Whatman paper No. 1 using solvent system EtOAc–MeOH–H_2O–HOAc (65:15:15:20). A saturated solution of aniline phthalate in *n*-BuOH was used as a staining agent.

3.4. *Acetylation procedure*

Acetylation of compound **1** (15 mg) was carried out in C_5H_5N (3 mL) and Ac_2O (6 mL), and the reaction mixture was left overnight at room temperature using magnetic stirrer, and then poured into ice-H_2O and extracted with EtOAc. The EtOAc extract was concentrated in vacuum followed by Si gel cc (1% MeOH in $CHCI_3$) of the residue to give compound **2** (6.3 mg). It forms white needles with $CHCI_3$.

3.5. *Physical characters of compound 1*

Amorphous powder; m.p. 208–210°C; $[\alpha]_D$ −2.3 (c, 2.2, MeOH); IR: 3455, 2923, 1452, 1375, 1076, 887 and 667 cm^{-1}; FAB-MS: *m/z* 673 $[M-H]^-$, 675 $[M+H]^+$, (+)-HR-ESI-MS found = 697.3769, clalc = 697.3775 for $C_{34}H_{58}NaO_{13}$; ^{13}C NMR data (125 MHz, $CDCl_3$), 38.4 (CH_2-1), 30.4 (CH_2-2), 71.8 (CH-3), 38.4 (CH_2-4), 45.6 (CH-5), 30.2 (CH_2-6), 27.9 (CH_2-7), 41.7 (CH-8), 50.5 (CH-9), 37.2 (C-10), 21.5 (CH_2-11), 41.4 (CH_2-12), 47.7 (C-13), 82.9 (C-14),

73.4 (CH-15), 35.3 (CH_2-16), 55.0 (CH-17), 12.7 (CH_3-18), 17.3 (CH_3-19), 71.4 (CH-20), 21.5 (CH_3-21), Gala: 102.7 (CH-1′), 75.9 (CH-2′), 85.7 (CH-3′), 77.8 (CH-4′), 71.6 (CH-5′), 17.3 (CH_3-6′), 58.4 (OCH_3-7′), Glu: 104.1 (CH-1″), 74.7 (CH-2″), 78.1 (CH-3″), 71.6 (CH-4″), 79.5 (CH-5″), 63.0 (CH_2OH-6″).

4. Conclusion

Phytochemical investigation of *C. retrospiciens* (Ehrenb.) N. E. Br., family Apocynaceae led to the isolation of a new polyoxy pregnane glycoside retrospinoside (**1**). Retrospinoside (**1**) is a new polyoxy pregnane glycoside composed of multihydroxy pregnane aglycone to which a disaccharide is attached. The disaccharide is composed of D-glucose and 6-deoxy-3-*O*-methyl-β-D-galactose and the interglycosidic linkage is 1 → 4.

Supplementary material

Supplementary material for this article is available online, alongside Table S1 for compound **1** and caratuberside B, Figure S1 for compound **1**, Figures S2–S9 for compound **2** and NMR data in Table S2 for compound **2**.

Acknowledgements

The authors gratefully acknowledge the assistance from Dr Sampo Mattila (Department of Chemsitry, Oulu University, Finland) for fruitful support and measuring the spectroscopic data of compound **2**.

References

Abdel-Mogib M, Raghib H. 2013. Two new pregnane glycoside diesters from *Caralluma russeliana*. Nat Prod Res. 27:1287–1292.

Abdel-Sattar E, Shehab N, Ichino C, Kiyohara H, Ishiyama A, Otoguro K, Omura S, Yamada H. 2009. Antitrypanosomal activity of some pregnane glycosides isolated from *Caralluma* species. Phytomedicine. 16:659–664.

Adnan M, Jan S, Mussarat S, Tariq A, Begum S, Afroz A, Shinwari Z. 2014. A review on ethnobotany, phytochemistry and pharmacology of plant genus *Caralluma* R. Br. J Pharm Pharmacol. 66:1351–1368.

Agrawal P. 1992. NMR Spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry. 31:3307–3330.

Al-Massarani S, Bertrand S, Nievergelt A, El-Shafae A, Al-Howiriny T, Al-Musayeib N, Cuendet M, Wolfender J. 2012. Acylated pregnane glycosides from *Caralluma sinaica*. Phytochemistry. 79:129–140.

Avula B, Shukla Y, Wang Y, Khan I. 2011. Quantitative determination of pregnanes from aerial parts of *Caralluma* species using HPLC-UV and identification by LC-ESI-TOF. J AOAC Int. 94:1383–1390.

Dutt H, Singh S, Avula B, Khan I, Bedi Y. 2012. Pharmacological review of *Caralluma* R. Br. with special reference to appetite suppression and anti-obesity. J Med Food. 15:108–119.

Halaweish F, Huntimer E, Khalil A. 2004. Polyoxy pregnane glycosides from *Caralluma retrospiciens*. Phytochem Anal. 15:189–194.

Halim A, Khalil A. 1996. Pregnane glycosides from *Caralluma retrospiciens*. Phytochemistry. 42:1135–1139.

Khalil A. 1995. Pregnane esters from *Caralluma retrospiciens*. Fitoterapia. 66:261–264.

Kunert O, Rao B, Babu G, Padmavathi M, Kumar B, Alex R, Schuehly W, Simic N, Kuehnelt D, Rao A. 2006. Novel steroidal glycosides from two Indian *Caralluma* species, *C. stalagmifera* and *C. indica*. Helv Chim Acta. 89:201–209.

Kunert O, Rao V, Babu G, Sujatha P, Sivagamy M, Anuradha S, Rao B, Kumar B, Alex R, Schuehly W, et al. 2008. Pregnane glycosides from *Caralluma* adscendens var. fimbriata. Chem Biodivers. 5:239–250.

Kunert O, Simic N, Ravinder E, Venkatrao B, Rao A, Kumar B, Alex R, Kuehnelt D, Rao A. 2009. Steroidal glycosides from *Caralluma umbellata*. Phytochem Lett. 2:134–138.

Oyama M, Iliya I, Tanaka T, Iinuma M. 2007. Five new steroidal glycosides from *Caralluma dalzielii*. Helv Chim Acta. 90:63–71.

Usmanghani K, Rizwani G. 1988. New pregnane glycosides from *Caralluma tuberculata*. J Nat Prod. 51:1092–1097.