

C₂₁ Pregnane Steroid from *Caralluma umbellata* (Asclepiadaceae)

Ramesh Mullangi^{1*}, Bobbala Ravi Kumar¹, Chandrakanth Kokate¹, Sama Venkatesh², and A.V.N. Appa Rao¹

¹University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506 009, India

²G.Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad-500 028, India

Abstract – A C₂₁ steroid has been isolated from the whole plants of *Caralluma umbellata* (Asclepiadaceae) and its structure was unambiguously assigned by spectroscopy.

Keywords – *Caralluma umbellata*, Asclepiadaceae, isolation, steroid.

Introduction

Caralluma umbellata Haw. (Syn: *Bourcerosia umbellata* W&A, *Stapelia umbellata* Roxb., *Caralluma campanulata* N.E.Br.) (Asclepiadaceae), is a thick, erect, leafless, branching, succulent perennial herb. It grows wild in dry and arid regions of Chittoor district and several districts of Andhra Pradesh, India. Plant stem juice warmed and mixed with turmeric powder is given in stomach disorders and abdominal pains (Vedavathy *et al.*, 1997). From this plant we have isolated five novel pregnane glycosides viz., carumbelloside I and II (Lin *et al.*, 1994); carumbelloside III-V (Qiu *et al.*, 1997) and a known flavone glycoside, luteolin-4'-*O*-neohesperidoside (Ramesh *et al.*, 1999a). The flavone glycoside was found to possess potent anti-inflammatory activity (Ramesh *et al.*, 1998). Carumbelloside-I has significant antinociceptive activity and no anti-inflammatory activity (Ramesh *et al.*, 1999b).

To the best of our knowledge this steroid (**1**) from *Caralluma* genus has not yet been reported. This is the first report the isolation and identification of this steroid from *Caralluma umbellata*.

Experimental

Instrumentation – The melting points (uncorrected) were determined on a Buchi capillary melting point apparatus. The FT-IR spectrum was recorded in the solid state as KBr dispersion using a Perkin Elmer 1650 FT-IR spectrophotometer. Solutions in CDCl₃ were used for all the NMR studies. The ¹H (at 500 MHz), ¹³C NMR and DEPT (at 125 MHz) studies were performed on a Bruker DRX500

Spectrometer. PE Sciex API 3000 mass spectrometer equipped with a TurboionsprayTM interface was used to record mass and fragmentation pattern.

Plant Material – Fresh whole plants of *Caralluma umbellata* were collected from Tirupathi, Andhra Pradesh and identified by Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal). A voucher specimen (AVN/186) has been deposited at the herbarium of the University College of Pharmaceutical Sciences, Warangal.

Table 1. ¹³C NMR data of **1** in CDCl₃ [(ppm) relative to TMS]

Carbon	1
1	37.6
2	31.9
3	72.0
4	39.0
5	139.4
6	122.6
7	27.7
8	36.6
9	46.2
10	37.2
11	21.1
12	42.0
13	49.4
14	85.6
15	34.7
16	24.9
17	62.9
18	15.4
19	19.8
20	218.4
21	33.8

*Author for correspondence

Fax: +91-40-23045439; E-mail: Mullangiramesh@dreddys.com

Extraction and Isolation – Three kilograms of dried powder of the whole plant of *C. umbellata* was extracted with ethanol by reflux. After removal of ethanol at 50°C *in vacuo*, the ethanolic extract was suspended in water and fractionated with toluene, dichloromethane, ethyl acetate, butanone and n-butanol. The toluene extract on repeated column chromatography over silica gel (230-400 mesh) eluted with pet. ether and acetone mixtures yielded **1** (300 mg); ^{13}C NMR (at 125 MHz, CDCl_3 , δ)-see Table 1.

Caraumbellogenin (1) – Amorphous white powder, m.p: 226-227°C; IR (KBr) 3490 (OH), 2929 (C-H), 1680 (C=O) cm^{-1} ; ^1H NMR (500 MHz, ppm from internal standard CDCl_3). δ : 1.08 (1H, m, H-1 α), 1.70 (1H, m, H-1 β), 2.28 (1H, bd, H-2 α), 1.76 (1H, m, H-2 β), 3.94 (1H, m, H-3 α), 2.64 (1H, dd, $J=12.5$ Hz, H-4 α), 2.42 (1H, t, $J=12.5$ Hz, H-4 β), 5.28 (d, H-6), 1.08 (1H, m, H-7 α), 1.70 (1H, m, H-7 β), 1.82 (1H, m, H-8 β), 1.10 (1H, m, H-9 α), 2.10 (1H, m, H-11 α), 1.98 (1H, m, H-11 β), 1.38 (1H, m, H-12 α), 1.20 (1H, m, H-12 β), 1.96 (1H, m, H-15 α), 1.74 (1H, m, H-15 β), 1.97 (1H, m, H-16 α), 1.85 (1H, m, H-16 β), 2.00 (1H, m, H-17 α), 1.07 (3H, s, H-18), 0.88 (3H, s, H-19), 2.20 (3H, s, H-21).

Results and Discussion

Caraumbellogenin (**1**) on TLC gave positive test on steroid, showed one dark pink spot with R_f 0.25 (pet. ether-ethyl acetate, 4 : 1) after spraying with 10% H_2SO_4 . Caraumbellogenin, $\text{C}_{21}\text{H}_{32}\text{O}_3$, m/z : $(\text{M}+\text{H})^+$ 333, was recognized to be a typical C_{21} steroidal skeleton with a carbonyl from its characteristic spectral features. On MS/MS fragmentation, it has shown a typical steroidal retro Diels-Alder (RDA) fragmentation, which is characteristic to pregnanes having 3-OH- Δ^5 system. The CID spectrum of

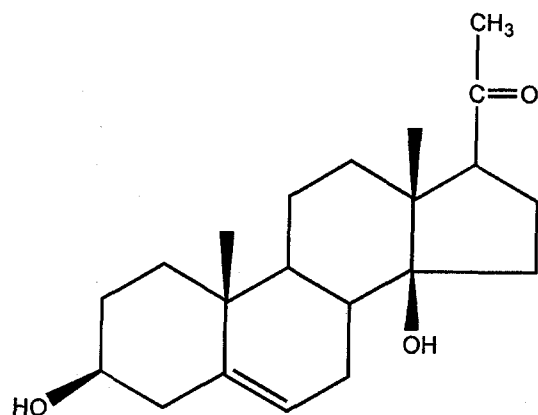


Fig. 1. 1.

m/z 333 has generated a series of product ions at m/z 315, 297, 255, 227 and 159. The sequential loss of 18 (H_2O), 18 (H_2O), 42 (COCH_3) and 28 (2 units of CH_3) from m/z 333 generated the product ions at m/z 315, 297, 255 and 227, respectively. The structure of **1** was established by comparing spectroscopic data (MS, ^1H , ^{13}C and DEPT) with those reported in the literature and established as 3 β ,14 β -dihydroxy-14 β -pregn-5-en-20-one. The ^{13}C NMR values of **1** were found to match with genin portion of carumbelloside-I (Lin *et al.*, 1994) and with 3 β ,14 β -dihydroxy-14 β -pregn-5-en-20-one isolated from *Cynanchum paniculatum* (Sugama *et al.*, 1986).

The presence of this steroid in the *Caralluma* species is being reported for the first time, although it has been reported from *Cynanchum paniculatum* (Sugama *et al.*, 1986). To the best of our knowledge there is no report on the presence of this steroid from *Caralluma* genus, though several steroid and steroidal glycosides were reported from various species of *Caralluma*. Presence of genin in the non-polar extract signifies the biosynthesis of glycosides from this genin in *Caralluma* species and also suggests that it is a precursor for the glycosides viz., carumbelloside I and II. Thus the presence of this steroid may be useful marker for *Caralluma* genus.

Acknowledgments

MR wishes to thank M. Ratna Sridevi for her support.

References

- Lin, L.J., Gil, R.R., Lin, L.Z., Cordell, G.A., Ramesh, M., Reddy, B.M., Srilatha, B., and Appa Rao, A.V.N., Pregnane glycosides from *Caralluma umbellata*. *Phytochemistry* **35**, 1549-1553 (1995).
- Qiu, S.X., Lin, L.Z., Cordell, G.A., Ramesh, M., Kumar, B.R., Radha Krishna, M., Mohan, G.K., Reddy, B.M., Rao, Y.N., Srinivas, B., Thomas, N.S., and Appa Rao, A.V.N., Acylated C-21 steroidal bisdesmodic glycosides from *Caralluma umbellata*. *Phytochemistry* **46**, 333-340 (1997).
- Ramesh, M., Nageshwara Rao, Y., Appa Rao, A.V.N., Prabhakar, M.C., Muralidhar, N., and Seshagiri Rao, C., Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. *J. Ethnopharmacol.* **62**, 63-66 (1998).
- Ramesh, M., Nageshwara Rao, Y., Rama Kumar, M., Krishna Mohan, G., Ravi Kumar, B., Appa Rao, A.V.N., Radha Krishna, M., and Madhava Reddy, B., Flavone glycoside from three *Caralluma* species. *Biochem. Syst. Ecol.* **27**, 85-86 (1999a).
- Ramesh, M., Nageshwara Rao, Y., Rama Kumar, M., Appa Rao, A.V.N., Prabhakar, M.C., and Madhava Reddy, B., Antino-

- ciceptive and anti-inflammatory activity of carumbelloside-I isolated from *Caralluma umbellata*. *J. Ethnopharmacol.* **68**, 349-352 (1999b).
- Sugama, K., Hayashi, K., Mitsuhashi, H., and Kaneko, K., Studies on the constituents of Asclepiadaceae plants. LXVI. The structures of three new glycosides, cynapanosides A, B, and C, from the Chinese drug "xu-chang-qing," *Cynanchum paniculatum* Kitagawa. *Chem. Pharm. Bull.* **34**, 4500-4507 (1986).
- Vedavathy, S., Mrudula, V., and Sudhakar, A., *Tribal Medicine of Chittoor district, A.P. (India)*. Herbal Folklore Research Centre, Tirupathi, A.P, India, p. 46 (1997).

(Accepted June 7, 2005)