

CHEMICAL EXAMINATION OF MEDICINAL PLANT “CARALLUMA UMBELLATE” (ASCLEPIADACEAE) ROOTS

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ABSTRACT: Two new pregnane compounds named as 3 β -hydroxy-pregn-5-ene (CRUR I) and 3 β ,14 β -dihydroxy pregn-5-ene (CRUR II) were isolated from *Caralluma umbellata* roots and their structures elucidated by extensive spectroscopic studies like IR, H¹, C¹³- NMR, and MS .

Key words: *Caralluma umbellata* roots, pregnane compounds, non polar solvents, extraction, isolation, spectral studies.

INTRODUCTION

The plant *Caralluma umbellata* Erect (Asclepiadaceae) is succulent leafless herb, up to 30 cm tall, flowers in terminal umbels. Fruit paired follicles, cylindric, green with small dark spots occasional in hilly regions of Orissa, Andhra Pradesh, Tamlil Nadu, Kerala and Karnataka. Stems of *Caralluma umbellata* are used in stomach disorders and abdominal pains (Vedavathy et al., 1997). The literature survey indicates plenty of pregnane glycosides were isolated from various parts of the plant (Ramesh Mullangi et al., 2005). With polar solvents like ethylacetate, methanol, ethanol, water, the compounds like C₂₁ steroidal glycosides with high molecular weight compounds were obtained (Lin et al., 1994, Qiu et al., 1997, Ramesh et al., 1999a, 1998, 1999b, 2005, G. Krishna Mohan et al., 1997, K.S. Babu et al 2008). When the same parts were extracted with non-polar solvents like n-hexane, benzene etc., and low molecular weight compounds like were terpenoids, steroids, cumarins were obtained. Sheng-Xiang *et al.*, (1997) first reported the isolation and identification of two new pregnane glycosides carumbellosides I and II and later C₂₁ steroidal glycosides carumbellosides III-V from the whole plant of *Caralluma umbellata*

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No Attempt were made to extract the compounds from the roots of *Caralluma umbellata* starting from n-hexane, benzene, acetone, methanol i.e., the extractions were carried out from the non-polar end to the polar end. An attempt was made to extract the compounds from *Caralluma umbellata* roots using non polar solvents like, n-hexane, benzene, acetone with a view to isolate compounds from different extracts and assign the structures using Mass, ^1H , ^{13}C -NMR, I.R spectra.

EXPERIMENTAL

The experimental part is described under three headings; a) Chemicals used and their purification, b) Instruments used and c) Extraction & isolation

a) Chemicals and their Purification: All chemicals used in this study were AR grade sold from Merck India Co. Ltd., and purified according to the standard procedures (A.I. Vogel 1971). Silica gel 60-120 and 200 meshes, silica gel G (ACME) were used for column and thin layer chromatography, vanillin-sulphuric acid is used as spray reagent.

b) Instruments used: Melting points (uncorrected) were determined on a Buchi capillary melting point apparatus. The FT-IR spectra were recorded on Perkin – Elmer 1605, using KBr pellets, the ^1H and ^{13}C NMR spectra were recorded on Varian Gemini 500 MHz, the mass spectra were recorded on Micro mass Platform-II (electron spray mode)

c) Chemical examination of roots of *Caralluma umbellata* plant:

- The roots of the plants of *Caralluma umbellata* was collected in Tirupathi, Andhra Pradesh, India. The air dried roots of *Caralluma umbellata* (50 g) were powdered.
- The plant material was extracted with benzene (1 lit), acetone (1 lit), and methanol (1 lit) in individual soxhlet extractor at reflux temperature for 10 hours. The extracts from the roots of the plant were filtered and then concentrated on water bath yielded a greenish semi-solid. These are also not examined further since they did not show positive test for tri-terpenoids, steroids and flavonoids and it was found to be fatty material.
- *n - Hexane extract:* The green semi-solid (9 g) obtained from n-hexane extract. Pink colour developed when the light green semi-solid dissolved in chloroform, and treated with acetic anhydride and sulphuric acid. The concentrated n-hexane extract was subjected to thin layer chromatography using silica gel. The eluant was benzene. After 20 min, the TLC plate is removed from the chamber, dried and sprayed using vanillin-sulphuric acid reagent.

RESULTS

Thin layer chromatography (TLC): From the TLC plate it was observed two spots by spaying vanillin – H₂SO₄ reagent with different R_f values (0.38 and 0.18). After that the n-hexane extract was subjected to silica gel column chromatography (10-40 μm, 150 g) and the column was eluted successively with benzene followed by benzene: methanol (95:5 v/v), benzene: methanol (90:10 v/v), methanol all together 20 fractions (each 50 ml) were collected. Fractions 1-9 on concentration followed by crystallization from benzene gave colorless crystalline solid (m.p 165°C). It was designated as CRUR-I. Gave appositive test to Libermann-Burchard reaction Salkowski test (LBS), indicating it may be a steroid. It has no absorption in U.V. region. Fractions 10-20 on concentration followed by crystallization from benzene gave white needles. m.p 180°C. It was also gave positives to the LBS test, so it was designated as CRUR-II.

Spectral studies

Mass (m/z values)-CRUR-I: 284.9, 195, 180.1, 178.0, 163.1, 151.0, 149.0, 102.1 and 101.0. **CRUR-II:** 318.0, 307.1, 297.1, 284.9, 239.6, 208.0, 195.0, 187.1, 180.9, 168.0, 170.1, 163.1, 156.1, 155.0, 151.1, 149.0, 135.0 and 120.0.

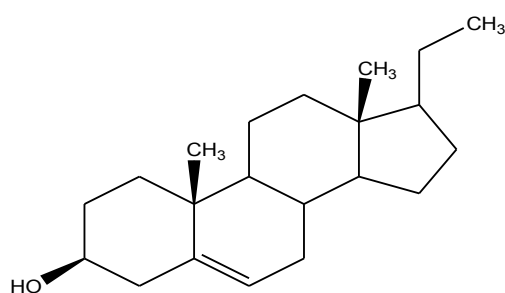
¹H-NMR (500 MHz, ppm from internal standard CDCl₃) δ: **CRUR-I:** 1.38 (1H, m, H-1α), 1.13 (1H, m, H-1β), 1.57 (1H, m, H-2α), 1.32 (1H, m, H-2β), 2.24 (1H, m, 3α), 3.45 (m, 3β-OH) 2.23 (1H, m, H-4α), 1.98 (1H, m, H-4β) 5.37 (1H, t, H-6α, J=7.22 Hz), 2.01 (1H, m, H-7α), 1.79 (1H, m, H-7β), 1.45 (1H, m, H-8α), 1.44 (1H, m, H-9α), 1.52 (1H, m, H-11α), 1.27 (1H, m, H-11β), 1.49 (1H, m, H-12α), 1.24 (1H, m, H-12β), 1.40 (1H, m, H-14α), 1.60 (1H, m, H-15 & 16α), 1.35 (1H, m, H-15 & 16α), 1.48 (1H, m, H-17α), 1.26 (3H, s), 1.16 (3H, s), 1.29 (2H, sextet, J=1.48 Hz), 0.96 (3H, t, J= 7.02). **CRUR-II:** 1.38 (1H, m, H-1α), 1.26 (1H, m, H-1β), 1.57 (1H, m, H-2α), 1.32 (1H, m, H-2β), 3.25 (1H, m, 3α), 2.23 (1H, m, H-4α), 1.98 (1H, m, H-4β) 5.37 (1H, t, H-6α), 2.04 (1H, m, H-7α), 1.79 (1H, m, H-7β), 1.53 (1H, m, H-8α), 1.44 (1H, m, H-9α), 1.57 (1H, m, H-11α), 1.27 (1H, m, H-11β), 1.49 (1H, m, H-12α), 1.24 (1H, m, H-12β), 1.75 (1H, m, H-15α), 1.50 (1H, m, H-15α), 1.60 (1H, m, H-16), 1.35 (1H, m, H-16α), 1.48 (1H, m, H-17α), 1.26 (3H, s), 1.16 (3H, s), 1.29 (2H, sextet, J=1.48 Hz), 0.96 (3H, t, J= 7.02), 3.45 (3α-OH), 2.25 (14α-OH).

¹³C NMR(CDCl₃) relative to the TMS - CRUR-I: 45.2 (C₁), 23.5 (C₂), 130.3 (C₃), 127.4 (C₄), 141.3 (C₅), 124.2 (C₆), 30.1 (C₇), 32 (C₈), 50.9 (C₉), 36.4 (C₁₀), 22.8 (C₁₁), 36.9 (C₁₂), 46.2 (C₁₃), 56.2 (C₁₄), 27.4 (C₁₅), 29.5 (C₁₆), 50.1 (C₁₇), 23.3 (C₁₈), 20.4 (C₁₉), 23.6 (C₂₀), 12.5 (C₂₁) **CRUR-II :** 30.1 (C₁), 31.8 (C₂), 71.7 (C₃-OH), 41.9 (C₄), 140.9 (C₅), 121.9 (C₆), 30 (C₇), 31.9 (C₈), 50.9 (C₉), 37.4 (C₁₀), 22.7 (C₁₁), 36.9 (C₁₂), 46.2 (C₁₃), 56.2 (C₁₄), 27.4 (C₁₅), 27.5 (C₁₆), 50.1 (C₁₇), 23.2 (C₁₈), 20.4 (C₁₉), 23.6 (C₂₀), 12.5 (C₂₁): **IR (KBr pellet method):** **CRUR-I:** 3400-3250 cm⁻¹ (broad, -OH), 2929 cm⁻¹ (C-H), 2993 cm⁻¹ (=C-H). **CRUR II:** 3511-3405 cm⁻¹ (broad-OH), 2926 cm⁻¹ (C-H), 2992 cm⁻¹ (=C-H).

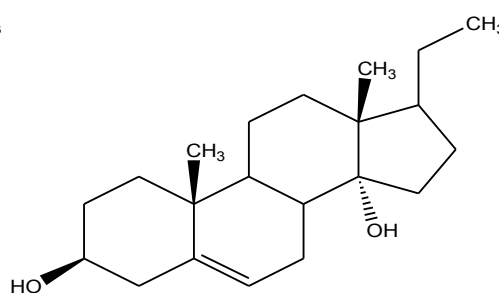
For a CRUR I the Mass peak at m/z 284 is considered as a base peak with a molecular formula $C_{21}H_{32}^+$, may be due to loss of H_2O molecule from original compound. The actual compound is with molecular weight 302 and molecular formula $C_{21}H_{34}O$ peak corresponding to this is not appeared in mass spectra. The IR spectrum of CRUR I showed broad peak in the region $3400-3250\text{ cm}^{-1}$ suggesting the presence of hydroxyl group (intramolecular hydrogen bonding). In 1H NMR spectra of the CRUR I the peak at δ 1.26 and 1.16 can be assigned to the angular methyl groups. The peak at δ 5.37 indicating the presence of tri substituted olefinic double bond. The peak at δ 3.45 indicating the presence of 3- α -H, 3- β -OH, The other $-CH_2$, $-CH$, protons present in the compound appeared as multiplet present at 1.2 to 2.01. The complex multiplets in the region δ 1.0 – 3.0 are characteristic of alicyclic units such as in a steroid.

CRUR II also has given positive test to steroid. The IR spectrum of this compound showed peak at 3511 cm^{-1} suggesting the presence of hydroxyl group ($-OH$). Mass spectra of the CRUR-II was shows a peak at m/z 318 is the molecular ion peak with a molecular formula $C_{21}H_{34}O_2$ and prominent fragment ions at m/z 284, 208, 180, 163, 181, 120.0 due to retro Diels-Alder (RDA) fragmentation. In the 1H NMR spectral data, some solvent impurity and some waxy impurity are recognized.

The developed two compounds recognized to be a typical C_{21} steroidal skeleton, from its characteristics spectral features. On MS/MS fragmentation, it has shown a typical steroidal RDA fragmentation, which is a characteristic to pregnanes having 3-OH Δ^5 system. The structures of CRUR I and CRUR II were established by comparing spectroscopic data (MS, 1H , ^{13}C -NMR and IR) with those reported in the literature and established as 3 β -hydroxy-pregn-5-ene (CRUR I) and 3 β ,14 β -dihydroxy pregn-5-ene (CRUR II). The ^{13}C NMR values of isolated products were found to match with genin portion of carumelloside I (Lin et al., 1994), 3 β , 14 β di hydroxy, 14 β pregn -5-en-20-one isolated from *Cynanchum paniculatum* (Sugama et al., 1986), Caraumbellogenin (Ramesh M et al., 2005) and also similar to that Lin, L.-J, Ling, L.-Z et al., (1994) Qiu, S.-X., Lin L.-Z. et al.(1997) in case of compounds extracted from *Caralluma umbellata* and also similar type of compounds 20-keto pregnane from the defensive secretion of a diving beetle (*Thermonectus marmoratus*) having this type of structure was reported by Jerrold Mein Wald et al., (1998).



CRUR I



CRUR II

CONCLUSION

To the best of our knowledge there is no report on the presence of these steroids from *Caralluma umbellata* through several steroid and steroidal glycosides. The presence of these steroids may be useful marker for *Caralluma* genus.

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