A Novel Pregnan Glycoside From The *Caralluma Umbellata* Haw (Asclepiadaceae) Roots

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**Abstract:**
From the roots of *Caralluma umbellata* belonging to the family *Asclepiadaceae*, a novel pregnane glycoside was isolated. Its structure was elucidated 3-O-β-D-glucopyranosyl, 8β-isopropoxy, 14β-ethynyl, pregn-5-en-21,21-dimethyl-16,17-lactone on the basis of spectroscopic data. This is the first report from the genus *Caralluma* as well as its family *Asclepiadaceae*.

**Keywords:** *Caralluma umbellata* roots, *Asclepiadaceae*, pregnane glycoside, extraction, isolation, spectral studies.

**Introduction:**
*Caralluma umbellata* (Haw) is a succulent perennial herb grows up to 30 cm tall in dry and arid regions of Tamilnadu, Orissa, Andhra Pradesh, Karnataka. The genus *Caralluma* (*Asclepiadaceae*) is known to be a rich source of pregnanes1-7, pregnane glycosides3,8-27, flavonoidal glycosides28-30, megastigmane glycosides30, triterpenoids27,31,32. These pregnane glycosides were reported to be cytotoxic in the recent years12. In this paper we report isolation and characterization of a novel pregnane glycoside from the roots of *Caralluma umbellata*.

**Experimental:**
The plant material of *Caralluma umbellata* was collected from Tirumala hills, Tirupathi in December 2009. Plant material was identified by using regional flora conserved at herbarium, Dept of Botany Sri Venkateswara University. All chemicals used in this experimental work were AR grade sold from NSP Guntur, purified according to the standard procedures, methanolic sulfuric acid (98:2) is used as spraying reagent.

**Instruments used:**
Melting point was determined on a Buchi capillary melting point apparatus. The 1H NMR was recorded in CDCl3 solution by using 400 MHz spectrometer. IR spectra was recorded on FT-IR spectrometer. MS spectra was recorded on agilent 6430 series triplet Quard MS spectrometer.

**Extraction and isolation:**
The roots of the plant *Caralluma umbellata* (Haw) about 3kgs were extracted with ethanol in a soxhlet apparatus. The extract (about 150g) was impregnated on minimum amount of silica gel and washed successively with Hexane, Benzene and Methanol. Benzene and Methanol washes were impregnated on minimum amount of silica gel individually and washed with Hexane, Ether, Acetone and finally with Methanol.

The ether eluates of Benzene and Methanol extract have shown the similar spotting on TLC. Concentrated (10gms) and subjected to repeated column chromatography with Hexane, Ethylacetate and Methanol. Fractions of about 200 ml were collected and examined on TLC using different solvent systems, spraying with methanolic sulpheric acid (98:2). Fractions showing similar spot pattern were grouped and worked individually. The fractions 20&21 eluted using Hexane and Ethylacetate (9:1) has shown one single blue spot with Rf value 0.42 in pure Benzene solvent system. This on recrystallisation with methanol separated as white crystalline solid, gave a positive Liebermann-Burchard reaction showing the presence of a steroid.
Yield: 16 mg \hspace{1cm} m.p: 273-275 °C

Spectral studies:

I.R. (ν\text{max}) \text{ cm}^{-1}: 1793, 1601, 2253, 2869, 2931.

$^1$H NMR (CDCl$_3$)

<table>
<thead>
<tr>
<th>δ( ppm)</th>
<th>Multiplicity</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.05 - 1.03</td>
<td>[2H, m, 1β, 1α]</td>
<td></td>
</tr>
<tr>
<td>1.254</td>
<td>[2H, m, 2α, 2β]</td>
<td>2.279 [1H,m,3α], 5.12-5.26 [anomeric proton m, H-1’]</td>
</tr>
<tr>
<td>1.573-1.593</td>
<td>[2H,m,4α,4β]</td>
<td>5.35 [1H,m,H-6], 1.497-1.477 [2H,m,7β,7α], 1.64[H,m,H-9α], 2.0-1.75[2H,m, 11α,11β]</td>
</tr>
<tr>
<td>1.72-1.71</td>
<td>[2H,m,H-12α,12β], 1.18-1.17</td>
<td></td>
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<tr>
<td>1.51[1H,m, H-17], 0.75 [6H, S, H-18, 19], 0.87 [3H,m H-22], 0.95[3H,m,H-23], 3.81[1H,septetH-24], 1.23 [6H, d, H-25, 26], 2.11[H, m, H-28]</td>
<td></td>
<td></td>
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</tbody>
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Mass (ESIMS): 604, 559, 577, 397, 382, 354, 329, 301

Results and discussion

Compound obtained as white crystalline solid with m.p (273-275°C) analysed for C$_{34}$H$_{50}$O$_9$, showed positive test for Liebermann-burchard reaction indicating it to be a steroid. The m/z 604.5 indicated [M+2] peak, m/z at 397.4 [M’-C$_6$H$_{11}$O$_5$+H ] indicated that the compound contains one sugar unit. A clear septet in $^1$H NMR at δ 3.55 indicated the presence of isopropyl ether, which is further confirmed by a doublet at δ 1.23 corresponding to six hydrogens.

IR stretching frequency at ν\text{max} at 1793 cm$^{-1}$ indicated the presence of a lactone ring. Absence of any other downfield signal indicated that the ring is substituted. The stretching at 2253 cm$^{-1}$ and an acetylthion proton at δ 2.1 corresponding to [1H] indicated the presence of mono substituted acetelinic linkage. The $^1$H NMR spectrum showed a doublet at δ 5.12 to 5.26 integrating for one proton indicated that it has one mono saccharide moiety with the glycosidic linkage in β-configuration. The other hydrogens of the glycoside are not clearly visible in the spectrum very weak signals between δ 3.7 to 4.2 might be the other hydrogens of glycoside. This is further confirmed by a down field signal at δ 2.29[1H, m] indicated that the glycosidic bond is β-glycoside$^{4,57}$ with the above signal corresponding to H-3α. This is also supported from literature, by the isolation of caumbelloside-II from Caralluma umbellata$^4$ shows similar spectral data for glycoside ring.

One proton multiplet δ 5.35 indicated the presence of trisubstituted olefinic double bond. This olefinic double bond is assigned to 5,6 positions based on biogenic criteria$^{4,57}$. $^1$HNMR signals at δ 1.57-1.59[2H,m],1.47-1.49[2H,m] corresponds to allylic 4α,4β and 7α,7β respectively. The NMR spectra showed the presence of signals at δ 0.87[3H] and 0.95[3H]. The down field broad singlets corresponds to 0.87 and 0.95 respectively showed the presence of gem dimethyl groups in the lactone ring. The six hydrogen singlet at δ 0.75 indicated the presence of two angular methyl groups.

Therefore the partial structure of compound can be given as
The two angular methyl groups are assigned 10,13<sup>th</sup> carbons based on biogenetic criteria. A one proton clear septet at δ 3.81 indicated the presence of isopropyl ether group and absence of any other down field signals in the ring protons indicated this group to be attached to a tertiary carbon. Thus isopropyl ether group is allocated to C-8, this substitution also supported from the literature<sup>21,23</sup>.

A one proton multiplet at δ 1.51 and another one proton multiplet at δ 1.16 indicated the presence of lactone ring at H-16, 17 positions. A two proton multiplet at δ 1.18-1.17 is allocated to H-15α,15β with the acetylinic group at C-14. Thus the structure of compound is established as

The structure was further supported by MS spectral data.
Mass fragmentation pattern-1 of compound
Mass fragmentation pattern – 2 of compound
Conclusion:

The compound was assigned as 3-O-β-D-glucopyranosyl, 8β-isopropoxy, 14β-ethynyl, pregn-5-en-21, 21 dimethy16, 17 lactone. This is the first report from the genus Caralluma as well as family Asclepiadaceae.

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References


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