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Structural Elucidation of a New Novel Pentacyclic Triterpenoid Isolated from *Caralluma attenuata Root*

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ABSTRACT

From the root of Caralluma attenuata belonging to the family Asclepiadaceae, a novel pentacyclic triterpenoid was isolated. Its structure was elucidated on the basis of spectroscopic data. This is the first report of such novel pentacyclic triterpenoid from C. attenuata root.

Keywords: *Caralluma attenuata*, *Asclepiadaceae*, Pentacyclic triterpenoid.

Abbreviations: TLC: Thin Layer Chromatography; CC: Column Chromatography; MS: Mass Spectrometry; EI: Electron Ionisation; DEPT: Distortionless Enhancement by Polarisation Transfer; MPs: Melting Point; NMR: Nuclear Magnetic Resonance

INTRODUCTION

Recently, triterpenoids are known to exhibit several pharmacologically activities and extensively discussed in the literature. These compounds can be used as anticancer agents [1-8], anti HIV [9,10], antiinflammatory [11], antiviral [5] and against neurodegenerative disorders [12]. Triterpenoids are the class of compounds which include squalene derivatives, lanostanes, holostanes, cycloartanes cucurbitanes, dammaranes, euphanes, triucallanes, tetranortriterpenoids, quassoids, luphanes, oleananes, friedelanes urasanes, hopanes, serratanes, isomalabaricanes and saponins.

Various medicinal uses of *Caralluma* species have been reported in Indian traditional medicine system. Most of the *Caralluma* species are used as anticancer [13], antitubercular and anthelmintic [14], antibacterial [15], healing of ulcers [16], appetite suppressant [17], nootropic [18], nociceptive [19], antioxidant actions [20], ability to lower blood sugar [21]. Species like *Caralluma tuberculata*, *Caralluma Fimbriata*, and *Caralluma attenuata* are the most popular widely utilized species in the genus. Isolation and characterization of oxypregnane glycosides [22,23], pregnane gylcosides [24-31], triterpene saponins [32], flavonoids [33], pregnane esters [34], bisdesmosidic glycosides [35,36], flavone glycosides [37], were earlier reported from the same genus. In the course of our investigation on chemical constituents of *Carallumas* we have isolated two novel triterpenoids [38,39]. In this paper we report the structural elucidation of another new novel pentacyclic triterpenoid derivative from *C. attenuata.*

MATERIALS AND METHODS

Experimental

The plant material of *C. attenuata* was collected in Tirumala forests during January-2017. MPs uncorrected IR ν_{max}^{KBr}, Proton Nuclear Magnetic Resonance (^{1}H-NMR) δ ppm**,** 300 MHz $CDCl_3$, Carbon 13 Nuclear Magnetic Resonance (^{13}C-NMR) 150 MH, distortion less Enhancement by Polarization Transfer (DEPT) 135, Mass spectrometry (SHIMADZU 2000) Column Chromatography (CC) and Thin Layer Chromatography (TLC) on silica gel, TLC Chamber (Sigma Aldrich).

Preparation of TLC plates

A homogenous suspension of silica gel is prepared by mixing 20 g of 200 mesh silica gel G (Qualigens) in about 45 ml distilled water. This suspension is poured into TLC (UNDPLAN model) spreader, which was adjusted to 0.25 mm thickness. Glass plates (20 cm × 5 cm) are coated with this gel using spreader. These plates are air dried and activated in the oven at 110ºC for 30 min and then kept in a desiccator.

Extraction and isolation

The roots of *Carallluma attenuata* were air dried, powdered (1.8 kg) and extracted with 3 l of hexane, 3 l of benzene, 3 l of acetone and 3 l of methanol respectively using SOXHLET extractor. n-Hexane extract 50 g was subjected to column chromatography using silica gel 10-40 mesh. It is eluted with various fractions of benzene, acetone and methanol with increasing polarity. The various eluted fractions were observed time to time using TLC plate, benzene fractions (95-100) a yielded a white solid.

Detection by TLC

This white solid is dissolved in chloroform, spotted on TLC plate. The chromatograph was developed using benzene as a mobile phase. The dried plates were then sprayed with methanol-sulphuric acid reagent (98: 2) and heated in an oven for about 45 min, single spot (red colour) with R_f-0.1875 is observed. The solid obtained was recrystallised using benzene and acetone mixture and melting point was determined. MPs 240ºC. This was further analyzed by spectral data.

Spectral data IR ν_{max}^{KBr}

3264.10, 2956.08, 2844.25, 1641.65, 1412.24, 1112.06, 1015.02.

^{1}H-NMR (δ ppm)

1.381, 1.534, 1.436, 1.674, 1.68, 1.18, 1.367, 1.678, 1.42, 1.69, 1.329, 1.13, 1.396, 1.412, 1.712, 1.17, 1.396, 1.495, 1.36, 1.742, 1.14, 1.496, 1.702, 1.372, 1.762, 0.925, 1.432, 0.87, 1.015, 1.315, 0.882, 1.054, 0.79, 5.156, 2.302, 3.379, 1.602, 0.756.

^{13}C-NMR (δ ppm)

35.3611, 36.9137, 79.2, 41.0862, 44.3537, 29.3801, 30.0668, 45.0, 50.5015, 38.0, 21.4534, 26.8097, 47.6693, 42.4, 27.6043, 39.3620, 35.0, 47.8396, 41.391, 42.4, 37.2731, 36.9137, 18.9442, 27.3659, 17.3015, 15.603, 15.8027, 22.1138, 27.4352, 14.9319, 104.9132, 151, 40.187, 22.9581, 14.5294, 14.4079, 65.04.

DEPT

δ 35.3611, 36.9137, 41.0862, 44.3537, 29.3801, 30.0668, 50.5015, 21.4534, 26.8097, 47.66927.6043, 39.3620, 47.8396, 41.319, 37.2731, 18.9442, 27.3659, 17.3015, 15.6035, 15.8027, 22.1138, 27.4352, 14.9319, 104.9132, 40.187, 22.9581, 14.5294, 14.4079, 65.04.

MS EI+

[M+] m/z 538, 523, 537, 523, 510, 509 (100%), 495, 481, 466, 452, 410, 395, 353, 339, 227, 185, 171, 129, 116, 87, 73, 61.

RESULTS AND DISCUSSION

The compound was isolated as white crystalline needles with MP 240ºC and analysed for $C_{37}H_{64}O_2$ [M^+]m/z 538 [M-2H]. The compound showed positive test for Libermann-Burchard reaction, Salkowski test indicating it to be a steroid/terpenoid.

The IR spectrum (Table 1) showed strong absorption at ν_{max}^{KBr} 3264.10 cm^{-1} as a broad peak indicating the presence of -OH group. Absence of strong absorption between ν_{max}^{KBr} 1650-1800 cm^{-1} indicates the absence of >C=O. Thus from the molecular formula presence of two -OH groups can be expected which is supported by (M-2H) peak in mass spectra. A strong absorption at ν_{max}^{KBr} 1641.65 cm^{-1} indicates the presence of olefinic double bond.

Table 1: IR spectral data

The important absorption peaks are shown below.

S. No.	Absorption cm^{-1}	Group Assignment
1	3264.1	O-H stretching vibration, the peak is broad
2	2956.08, 2844.25	C-H stretching in CH_2
3	1412.24	$=CH_2$ absorbs due to scissoring vibration of the terminal methylene group
4	1641.65	C=C stretching mode of unconjugated alkenes

The ^{1}H-NMR spectrum showed the characteristic ring protons ranging from δ 0.6-1.8 indicating the presence of pentacyclic triterpenoid [40]. A septet at δ 1.602(1H) and a doublet at δ 0.756 (6H) indicates the presence of isopropyl group, loss of 43 units in mass spectra [M^+ m/z- 43] also gives support for the presence of isopropyl group.

A two proton doublet at δ 5.156 indicates the presence of a disubstituted olefenic bond, weak C-H absorption in IR at ν_{max}^{KBr} 1412.24 cm^{-1} indicates the presence of terminal double bond which is supported by ^{13}C-NMR spectra. The ^{13}C-NMR signal and δ 151 and δ 104.9 indicates

the presence of olefenic carbons, ^{13}C-NMR signal at δ 151 is not observed in DEPT clearly indicates this olefenic carbon to be a quaternary carbon. The signal at δ 104.9132 is seen in DEPT as CH_2 carbon indicating a disubstituted terminal olefinic double bond [> C=CH_2] .

A two protons downfield signal at δ 3.379d indicates that CH_2 is attached to electronegative group i.e., presence of 1^o alcoholic group -CH_2OH. This is further supported by ^{13}C-NMR and DEPT at δ 65.04 ppm and is further supported by [M^+- CH_2OH] at 509 in mass spectra.

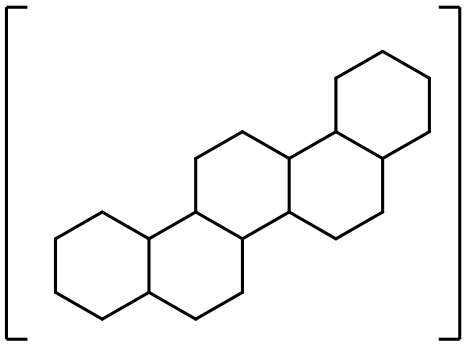
A one proton signal at δ 2.302(m) indicates the allylic proton. A singlet signal at δ 1.432 accounting for 3 protons indicates -CH_3 attached to a tertiary carbon with electronegative group accounting for a tertiary hydroxy group at C-3 is further supported by ^{13}C-NMR signal at δ 79.2 which is not seen in DEPT. A signal at δ 1.2 accounting for 12[H] indicating the presence of 4 angular methyl groups. It is evidenced in ^{13}C-NMR and DEPT at (δ 17.3015, 15.6035, 15.8027, 22.1138) and is supported by mass spectra through loss of 15 units.

^{1}H-NMR shows a doublet signal corresponding to 3H at δ 0.925 indicating presence of a methyl attached to methine [-CH-] carbon.

Table 2: ^{1}H-NMR and ^{13}C-NMR spectral analysis

Carbon Number	^{1}H-NMR Data	^{13}C-NMR Data	Carbon Number	^{1}H-NMR Data	^{13}C-NMR Data
1	α–1.381 β–1.534	35.3611	2	α–1.436 β–1.674	36.9137
3	-----	79.2	4	1.68	41.0862
5	1.18	44.3537	6	α–1.367 β–1.678	29.3801
7	α–1.42 β–1.69	30.0668	8	----	45
9	1.329	50.5015	10	-----	38
11	α-1.13 β–1.396	21.4534	12	α-1.412 β–1.712	26.8097
13	1.17	47.663	14	------	42.4
15	α–1.396 β–1.495	27.6043	16	α-1.36 β–1.742	39.362
17	------	35	18	1.14	47.8396
19	α-1.496 β–1.702	41.391	20	------	42.4
21	α-1.343 β–1.742	37.2731	22	α-1.367 β–1.762	36.9137
Carbon Number	**^{1}H-NMR Data**	**^{13}C-NMR Data**	**Carbon Number**	**^{1}H-NMR Data**	**^{13}C-NMR Data**
23	0.925	18.9442	24	1.432	27.3659
25	0.87	17.3015	26	1.015	15.6035
27	1.315	15.8027	28	0.882	22.1138
29	1.054	27.4312	30	0.79	14.9319
31	5.156	104.9	32	----	151
33	2.302	40.187	34	1.602	22.9581
35	0.756	14.5294	36	0.756	14.407
37	3.379	65.04			

It also shows a quartet at δ 1.054(2H) and a triplet δ 0.79 (3H) indicating an ethyl group attached to a quarternary carbon. Thus, based on IR, NMR (^{1}H-NMR and ^{13}C-NMR) (Table 2) partial structure of the compound can be as shown below (Figure 1).



+ four angular methyls, one 1^0 OH, one 3^0 OH
one isopropyl group, one disubsubstituted
olefin, two methyls and one ethyl groups

Figure 1: Partial structure of the compound

Based on the triterpenoids isolated from the same plant [16,17], the position of angular methyls and tertiary -OH can be given as shown in Figure 2.

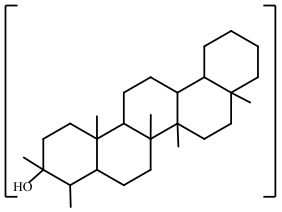


Figure 2: Placement of angular methyls and teritiary -OH

Based on ^{1}H-NMR, ^{13}C-NMR and DEPT (Tables 3 and 4), the structure of the compound can be given as shown below (Figure 3).

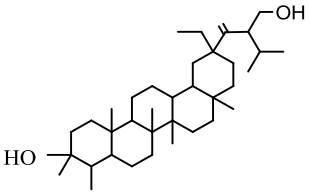
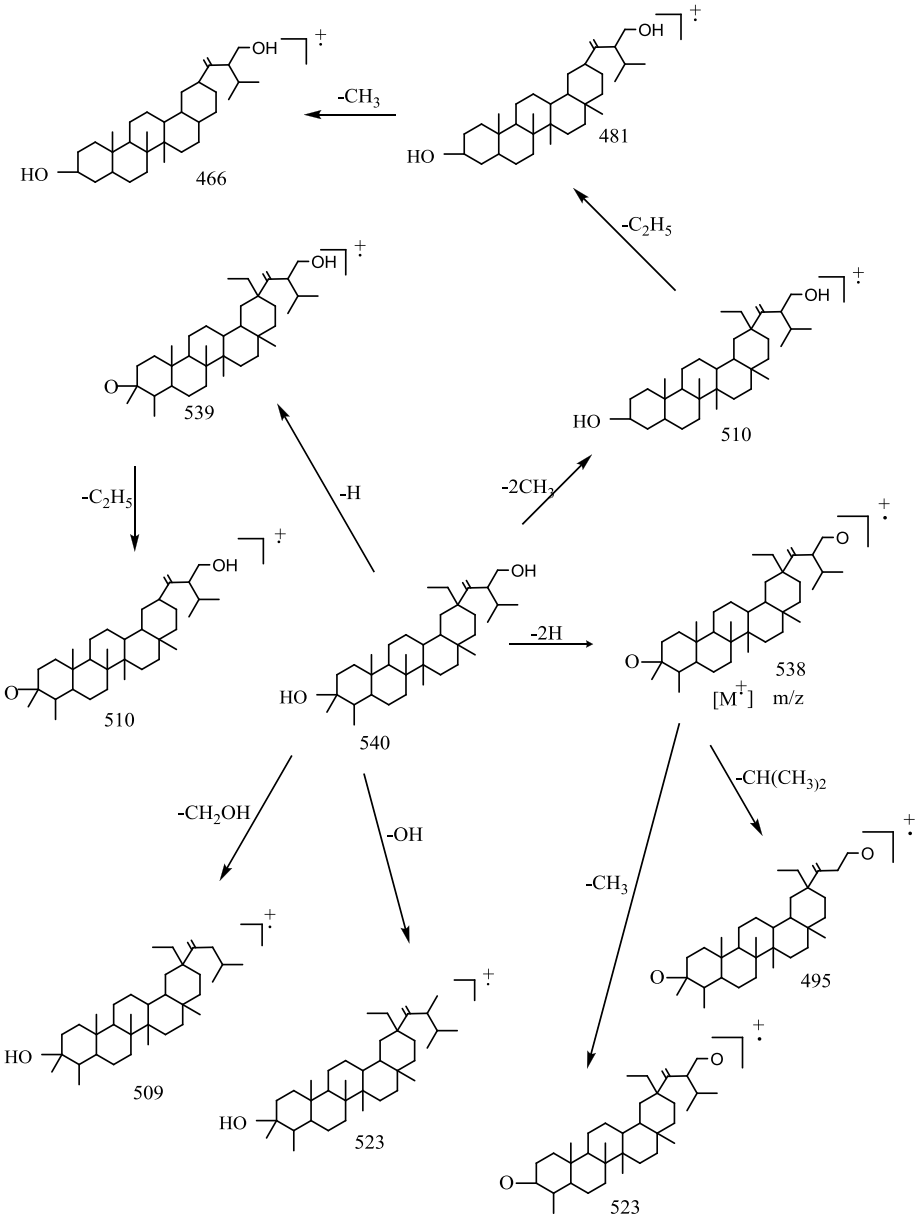


Figure 3: Structure of the compound based on the analysis of ^{1}H-NMR, ^{13}C-NMR and DEPT

This structure is further supported by the mass spectral fragmentation as given in Scheme 1. This terpenoid is isolated and reported for the first time from this plant as well as from nature.



Scheme 1: Mass Spectral Fragmentation of Compound

The mass spectral data is given below.

Table 3: Mass spectral analysis

S. No.	m/z	Ion formed
1	538	[M-2H]
2	539	[M-H]
3	523	[M - OH], [M-2H-CH_3]
4	510	[M-2CH_3], [M-H-C_2H_5]
5	509	[M-CH_2OH]
6	495	[M-2H-$CH(CH_3)_2$]
7	481	[M-2CH_3-C_2H_5]
8	466	[M-2CH_3-C_2H_5-CH_3]

Table 4: DEPT spectral analysis

Carbon No.	-CH	0	-CH_3	C
1	--	35.36	--	--
2	--	36.91	--	--
3	--	--	--	79.2
4	41.086	--	--	--
5	44.333	--	--	--
Carbon No.	**-CH**	**0**	**-CH_3**	**C**
6	---	29.3801	--	--
7	--	30.668	--	--
8	--	----	--	45
9	50.501	---	--	--
10	--	----	--	38
11	---	21.4534	--	--
12	--	26.8097	--	--
13	7.663	---	--	--
14	--	--	--	42.4
15	--	27.6043	---	--
16	--	39.362	--	--
17	--	--	--	35
18	47.8396	---	--	--
19	--	41.39	--	--
20	--	--	---	42.4
21	--	37.2731	--	--
22	--	36.9137	--	---
23	--	--	18.9442	---
24	--	--	27.3659	--
25	--	--	17.3015	--
26	--	--	15.6035	--
27	--	--	15.8027	--
Carbon No.	**-CH**	**0**	**-CH_3**	**C**
28	--	--	22.1188	--
29	--	27.4312	--	--
30	--	--	14.9319	--
31	--	104.39	---	--
32	--	--	--	151
33	40.187	--	--	--
34	22.9581	--	--	--
35	--	--	14.4	--
36	--	--	14.5294	--
37	--	65.04	--	--

DEPT spectrum showed carbon resonances relating to -CH, -CH_2 and -CH_3 protons present in the compound which are in agreement with ^{13}C-NMR resonances.

CONCLUSION

In this paper, we report the isolation, purification and characterization of a new triterpenoid from *C. attenuata*. The available scientific research on *Caralluma* signifies its importance as medicinal plant used in a wide range of ethnomedicinal treatment especially for diabetes, obesity, gastro intestinal problems, blood disorders, skin problems and cancer. The medicinal properties of *Caralluma* are attributed to the presence of chemicals like pregnane glycosides, flavonoids, steroids, steroidal glycosides and terpenoids. Very few species of *Caralluma* are of these genus have been subjected to stringent scientific evaluation. The scientific evaluation of the triterpenoid isolated may help in exploring the medicinal importance of various *Caralluma* species. Since no sufficient information is available for the toxicity of the genus or its derivative medicines that need some extensive research. The diverse uses of *Caralluma* have made it susceptible to over exploitation and it may become threatened due to high trade. However *Carallumas* is not an invasive species, so modern cultivation techniques should be developed for commercial exploration. Tissue culture techniques must be developed to enhance the production and preserve natural germplasms.

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