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LIPID COMPONENTS *OF CARALLUMA EDULTS* (EDGEW.) HOOK

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ABSTRACT

Eighteen fatty acids (**A**-**R**), four hydrocarbons (**S**-**V**) and sterol (**W**) have been isolated from the ethyl acetate fraction of the vegetative part of the medicinal herb *Carallunta edulis.* Chemical and spectroscopic methods were used for the characterization of compounds as fatty acids, hydrocarbons and sterol.

Introduction

Caralluma edulis (Edgew.) Hook is one of the important member of the Asclepiadaceae family. This plant eaten raw or cooked as vegetable (S.I. Ali, 1983) and are reputed in the folk medicine for the cure of rheumatic fever and diseases of blood, it also useful as cooling, alterative and stimulant (Burkill, 1909; Chopra, 1956; Hoppe, 1975). Chemical investigations of plant C.edulis was carried out with the objective to isolate those agents which are medicinally active. Beside other components in this study, an attempt has been made to isolate fatty acid components from non-polar fraction of Caralluma edulis. As regard the biological significance of the naturally occurring fatty acids is concern they are necessary for the animal metabolism and represent a reserve of energy. Vast majority of fatty acids found in fats and oils are in the form of triglycerides. Dietary fatty acids can influence the utilization and metabolism of carbohydrates, proteins and certain vitamins and minerals. They serve as carrier for fat soluble, vitamins A, D, E and their precursor (Chow, 1989). They are also capable of influencing immune responses. For example linoleic acid has an effect on the lymphatic system and immune function. High consumption of essential fatty acid usually improve the humoral response (against T-cell dependent and independent antigen (Dewille, 1979; Wagner a *at,* 1982). The functional effect of polyunsaturated fatty acid content of neural cell membranes is to facilitate the capacity of the high affinity choline uptake system to transport low concentrations of choline whereas a sterol component, β-sitosterol has anti-inflammatory, antipyretic and analogsic activity (Gupta *et al*, 1980).

Materials and Methods

Melting points *are* determined on Electro thermal melting point apparatus mot SA-6797 and are uncorrected. Optical rotation of the some compounds were taken, Jasco DIP-140 digital polarimeter. The UV spectrum was scanned on Shimadzu UV 240 ultraviolet spectrophotometer and the IR spectra were recorded on Jasco IRA-1 Diffraction grating spectrophotometer. Mass *were* recorded on Varian MAT-312 mass spectrometer. ^{1}H- and^{13}C-NMR were scanned on Broker (Am-300 and Bruker Am-400 and 75.43, 100.6 MHz) NMR spectrometer. Lyophilization of compounds carried out by freeze dryer model FD I. Eyela Tokyo Rikakiai Co. Ltd. For analysis of fatty acids, gas chromatograph was used which was Shimadzu G model gas chromatograph equipped with a shimadzu C-R6A Chromatopac integrator The column length was 2 m, and the inner diameter was 3 mm and outer diameter 5 mm. The packing material used as GP 3%, SP-23102%, SP-2300 on 100/120 Chromasorb WAW, The column initial temperature was 150°C, while the temperature was 250°C with a rate of increase of 8°C/min. The detector as well injector temperature was 300°C. Nitrogen flow rate was 30 m1/min. Mass of fatty *were recorded* on Gas Chromatograph-Mass Spectrometer of Hewlett Packard with a 11/73 DEC computer system and a 1.2 m x 4 mm packed glass capillary coated with gas chrome Q (100-120 mesh), 0V 101, 1%. The column temperature programmed from 170-250°C with rate of increase of 8°C/min. The injector to ture was 250°C. The *carrier* gas helium (He) flow rate was 32 ml/min. The purification of compounds were carried out by repeated conventional column chromatography which *were* detected by various chromogenic reagents i.e. vanillin sulphate reagent and cerie sulfate reagent followed by heating for 10-15 minutes.

The whole plant of Caralluma edulis (Edgew.) Hook (10 kg) was purchase from the vegetable market of Karachi, and identification was kindly carried out by Prof. Dr. S.I. Ali, Department of Botany, University of Karachi Karachi. Chopped and pieces of fresh plant material were percolated in MeOH at room temperature month. Evaporation of MeOH from the extract under reduced pressure, gave a dark green thick semisolid residue *(155 g). For* fractionation, H_2O and EtOAc *were* in equal proportion as a result of this lipophilic components were separated lyophilized. Powder residue was then proceeded for analysis in two different shown in (Scheme 1). Powder residue A (22.01 g) was subjected to silica gel chromatography elated first with n-hexane; thereafter EtOAc and MeOH were gradually added. Three major fractions I, II and III were eluted from (100%), n-hexane-:EtOAc (95:5) and n-hexane-:EtOAc (90:10) respectively from fraction-I mixture of fatty acids were obtained in hexane which was then subject to gas chromatography followed by GC-MS. Eighteen components were determined. The mass spectroscopic data of all the eighteen fatty acids *were* given in (Table 1) which was found identical with the literature data (Pouchert, 1975; Heller, 1978). Fraction II (n-hexane- EtOAc, 90:10) afforded a mixture of four different compounds (490 mg) which was further purified by silica gel column and eluted with n-hexane and EtOAc in a gradient fashion, as a result of which four

hydrocarbons,hentriacontane (73 mg), tritriacontane (90 mg), triacontane (60 mg) and nonacosane (113 mg) were obtained in a pure state (Gascard, 1921; Landa, 1929).

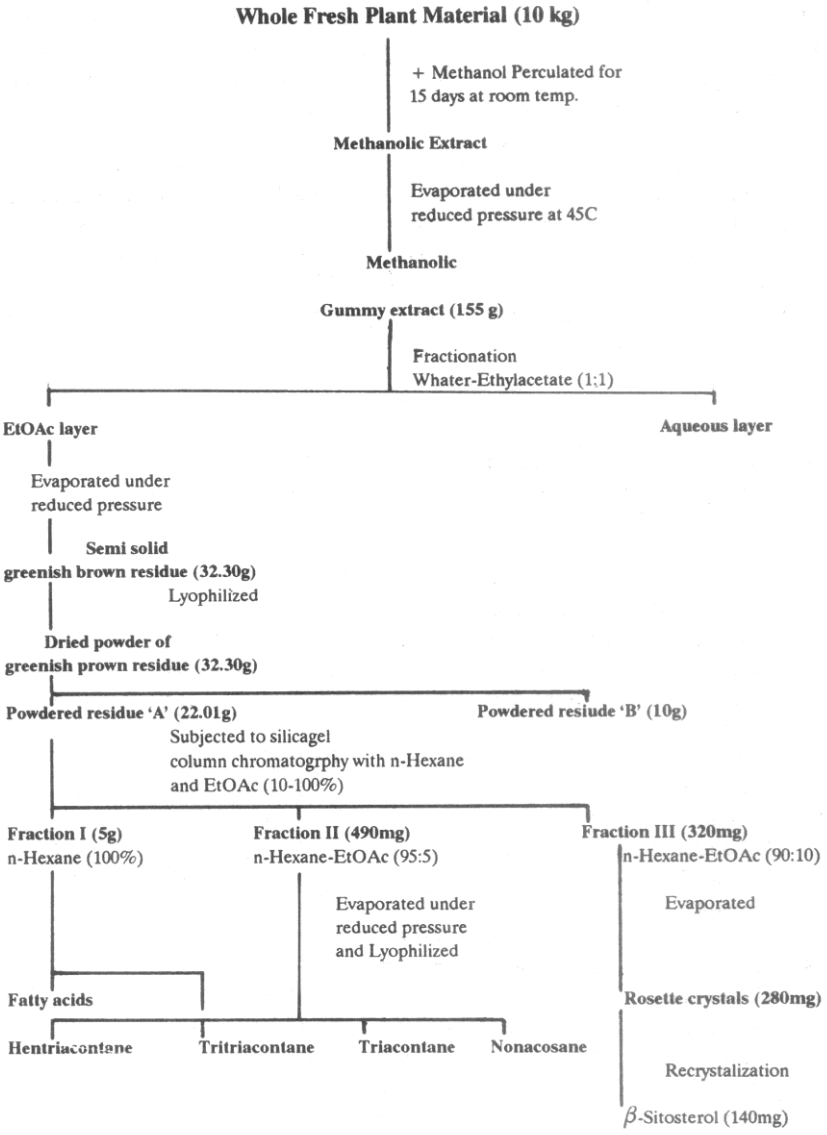
Fraction III (n-hexane-EtOAc, 90:10), the eluate was evaporated under vacuum as result of which rosette crystals (280 mg) were obtained. The crystals upon recrystallization in $CHCL_3$ furnished a pure compound (140 mg). It was identified as ß-sitosterol on the basis of spectroscopic evidences and by comparison with authentic sample (Co-TLC and Co-MPS). The spectroscopic data (UV, IR, Mass, NMR) of the compound was also compared with reported values for ß-sitosterol and was found to be identical (Rubinstein *et al*, 1976).

Table 1

No.	Type of Fatty Acid	Systematic Name	Mol. Formula	Mol. Weight	Relative Retention Time(R.R.T.)
1.	Saturated	n-Decenoic acid (A)	$C_{10}H_{18}O_2$	170	25%
2.	Unsaturated	n-Undecenoic acid (B)	$C_{11}H_{20}O_2$	184	22.5%
3.	Saturated	n-Dodecynoic acid (C)	$C_{11}H_{20}O_2$	196	18%
4.	Saturated	n-Dodacenoic acid (D)	$C_{12}H_{22}O_2$	198	28%
5.	Unsaturated	n-Tridecenoic acid (E)	$C_{13}H_{24}O_2$	212	19%
6.	Saturated	n-Tetradcenoic acid (F)	$C_{14}H_{26}O_2$	226	03%
7.	Saturated	n-Tridecenoic acid (G)	$C_{12}H_{22}O_2$	198	28%
8.	Unsaturated	n-Pentadecenoic acid (H)	$C_{15}H_{28}O_2$	240	05%
9.	Saturated	n-Hexadecenoic acid (I)	$C_{16}H_{30}O_2$	254	11%
10.	Unsaturated	n-Heptadecenoic acid (J)	$C_{17}H_{32}O_2$	268	06%
11.	Saturated	n-Octadecenoic acid (K)	$C_{18}H_{34}O_2$	282	03%
12.	Unsaturated	n-Nonadecenoic acid (L)	$C_{19}H_{36}O_2$	296	25%
13.	Unsaturated	n-Heneicosenoic acid (M)	$C_{21}H_{40}O_2$	324	05%
14.	Saturated	n-Docosenoic acid (N)	$C_{22}H_{42}O_2$	338	17%
15.	Unsaturated	n-Tricosenoic acid (O)	$C_{23}H_{44}O_2$	352	06%
16.	Saturated	n-Tetracosenoic acid (P)	$C_{24}H_{46}O_2$	336	13%
17.	Unsaturated	n-Pentacosenoic acid (Q)	$C_{25}H_{48}O_2$	380	12.5%
18.	Saturated	n-Hexacosenoic acid (R)	$C_{26}H_{50}O_2$	394	03%

Fatty acids of *Caralluma edulis* with their Relative Retention Time.

ISOLATION SCHEME - I



***n*-Decenoic acid (A):** 170 (M^+, $C_{10}H_{18}O_2$, 25%), 127 (M^+- 43, 7.5%), 113 ($M^{+'}$ -14, 8%), 99 (M^+ -14, 11%), 85 ($M^{+'}$-14, 49%), 71 ($M^{+'}$ -14, 69%), 57 (M^+ -14, 100%), 43 (M^+ -14, 28%).

***n*-Undecenoic acid (B):** 184 (M^+ $C_{11}H_{20}O_2$, 22.5%), 141 (M^+ 43, 5.5%), 127 (M^+ 14, 7.5%), 113 (M^+ -14, 8%), 99 (M^+ -14, 11%), 85 (M^+ -14, 51%), 71 (M^+-14, 71.8%), 57 (M^+ -14, 100%), 43 (M^+-14, 28%).

***n*-Dodecynoic acid (C):** 196 (M^+, $C_{12}H_{20}O_2$, 18%), 140 (M^+-56, 5%), 125 (M^+ -15, 16%), 111 (M^+-14, 37%), 97 (M^+ -14, 94%), 83 ($M^{+'}$ -14, 100%), 69 (M^+-14, 87%), 55 (M^+-14, 59%), 41 (M^+-14, 21%).

***n*-Dodecynoic acid (D):** 198 (M^+, $C_{12}H_{22}O_2$, 28%), 155 (M^+-43, 4%), 141 (M^+ -14, 5%), 127 (M^+-14, 6%), 113 (M^+ -14, 7%), 99 ($M^{+'}$ -14, 10.5%), 85 (M^+-14, 48%), 71 (M^+ -14, 62%), 57 (M^+-14, 100%), 43 (M^+-14, 30%).

***n*-Tetradecenoic acid (E):** 226 (M^+ , $C_4H_{26}O_2$, 3%), 211 (M^+-15, 2.5%), 197 (M^+-14, 183 (M^+-14, 8%), 169 (M^+-14, 13%), 155 ($M^{+'}$-14, 4%), 141 (M^+-14, 155), (M^+-14, 7%), 113 (M^+-14, 17%), 99 (M^+- 14, 17.5%), 85 (M^+-14, 53%), 7(M^+-14, 90%), 57 (M^+-14, 100%), 43 (M^+-14, 30%).

***n*-Tridecenoic acid (F):** 212 (M^+, $C_{l3}H_{24}O_2$, 19%), 155 (M^+-14, 10%), 141 ($M^{+'}$-14, 6%), 127 (M^+ -14, 65%), 113 (M^+-14, 8%), 99 (M^+-14, 13%), 85 (M^+-14, 51%), 71 (M^{+-} -14 72%), 57 (M^+-14,100%), 43 (M^+-14,28%).

***n*-Trideconoic acid (G):** 224 (M^+, $C_{14}H_{24}O2$, 17%), 155 (M^+-69, 17%), 141 (M^+-14, 6%), 127 (M^+-14, 3%), 113 (M^+-14, 21%), 99 (M^+-14, 32%), 85 (M^+-14, 23%), 71 (M^+ -14, 64%), 57 (M^+-14, 87%), 43 (M^+-14, 34%).

***n*-pentadecenoic acid (H):** 240 (M^+, $C_{15}H_{28}O_2$,5%), 197 (M^+-43, 15%), 183 (M^+ 14, 2%), 169 (M^+ -14, 3%), 155 (M^+-14, 2%), 141 (M^+-14, 3%), 127 (M^+-14, 5%), 113 (M^+-14, 7%), 99 (M^+-14,115%), 85 (M^+-14, 45%), 71 (M^+ 14, 72%), 57 M^+-14,100%), 43 (M^+-14, 30%).

***n*-Heptadecenoic acid (I):** 208 (M^+ $C_{17}H_{32}O_2$, 6%), 225 (M^+-43, 1%), 211 (M^+-14, 1.3%), 197 (M^+-14, 2%), 183 (M^+14, 2%), 169 (M^+ -14, 2.5%), 155 (M^+-14, 2.7%), 141 (M^+-14, 3%), 127 (M^+-14, 4%), 113 (M^+ - 14, 6%), 57 (M^+-14, 100%), 43 (M^+-14, 31%).

***n*-Hexadecenoic acid (J):** 254 (M^+, $C_{16}H_{302}$, 11%), 211 (M^+-43, 2.5%), 197 (M^+-14, 3%), 183 (M^+-14, 4%), 169 (M^+-14, 4.5%), 155 (M^+-14, 5%), 141 (M^+-14, ^{6}%), 127 (M^+-14, 7%), 113 (M^+-14, 9%), 99 (M^+-14, 13%), 85 (M^+-14, 51%), 71 (M^+-14, 75%), 57 (M^+-14, 100%), 43 (M^+-14, 29%).

***n*-Octadccenok acid (K):** 282 (M^+, $C_{18}H_{34}O_2$, 3%), 239 (M^+ -43, 2.5%), 225 (M^+-14, 2%), 211 (M^+ -14, 2%), 197 (M^+-14, 14.5), 183 (M^+-14, 13.5%), 169 (M^+-14, 6.5%), 155 (M^+-14, 7%), 141 (M^+-14, 9%), 127 (M^+-14, 21%), 113 (M^+-14, 21.5%), 99 (M^+ -14, 18%), 85 (M^+ -14, 55%), 71 (M^+-14, 92%), 57 (M^+ -14, 100%), 43 (M^+-14, 21%).

***n*-Noaadecenoic acid (L):** 296 (M^+, $C_{I9}H_{16}O_2$, 25%), 253 (M^+-43, 4.5%), 239 (M^+-14, 63%), 225 (M^+-14, 7%), 211 (M^+-14, 7%), 197 (M^+-14, 6%), 183 (M^+-14, 75%), 169 (M^+-14, 7.5%), 155 (M^+-14, 8%), 141 (M^+-14, 9.5%), 127 (M^+-14, 10%), 113 (M^+-14,13%), 99 (M^+-14,18%), 85 (M^+-14, 61%), 71 (M^+-14, 81%), 57 (M^+-14,100%), 43 (M^+-14, 26%).

***n*-Henekosenoic add (M):** 324 (M^+, $C_{21}H_4O_2$, 5%), 281 (M^+-43,1%), 225 (M^+-56, 1.5%), 211 (M^+-14, 2%), 197 (M^+-14, 2%), 183 (M^+-14, 2.5%), 169 (M^+-14, 3%), 155 (M^+-14, 4%), 85 (M^+-14, 51%), 71 (M^+-14, 76%), 57 ($M^{+'}$-14, 100%), 43 (M^+-14, 27%).

***n*-Docosenoic add (N):** 338 (M^+, $C_{22}H_{42}O_2$, 17%), 295 (M^+-43, 33%), 281(M^+-14, 5%), 267 (M^+-14, 6%), 253 (M^+-14, 6%), 239 (M^+-14, 6%), 225 (M^+-14, 6%), 211 (M^+-14, 5%), 197 (M^+-14, 7%), 183 (M^+-14, 7%), 169 (M^+-14, 7%), 155 (M^+-14,75%), 141 (M^+-14, 8%), 127 ($M^{+'}$, 10.5%),133 (M^+-14, 13%), 99 (M^+-14, 18%), 85 (M^+-14, 62%), 71 (M^+-14, 81.5%), 57 (M^+-14,100%), 43 (M^+-14, 25%).

***n*-Tdeosenoic acid (0):** 352 (M^+, $C_{23}H_{44}O_2$, 6%), 309 (M^+-43, 1%), 295 (M^+-14, 2%), 281 (M^+-14, 2%), 267 (M^+-14, 3%), 253 (M^+-14, 3%), 239 (M^+-14, 3%), 225 (M^+-14, 4%), 211 (M^+-14, 4%), 197 (M^+-14, 4%), 183 (M^+-14, 5%), 169 (M^+-14, 5%), 155 (M^+-14, 5%), 141 (M^+-14, 6%), 127 (M^+-14, 8%), 113 (M^+-14, 12%), 99 (M^+-14, 16%), 85 (M^+-14, 56%), 71 (M^+-14, 77), 57 (M^+-14, 100%), 43 (M^+-14, 26%).

***n*-Tetracosenoic acid (P):** 366 (M^+, $C_{24}H_{46}O_2$, 13%), 323 (M^+-43, 2.5%), 309 (M^+-14, 3.5), 295 (M^+-14, 4%), 281 (M^+-14, 4%), 267 (M^+-14, 4%), 253 (M^+-14, 4%), 239 (M^+-14, 4%), 225 (M^+-14, 5%), 211 (M^+-14, 4.5), 211 (M^+-14, 4.5%), 197 (M^+-14, 4.5%), 183 (M^+-14, 5%), 169 (M^+-14, 6%), 155 (M^+-14, 6%), 141 (M^+-14, 7%), 127 (M^+-14, 9%), 113 (M^+-14, 12%), 99 (M^+-14, 17%), 85 (M^+-14, 57%), 71 (M^+-14, 79157 (M^+-14,100%), 43 (M^+-14, 26%).

***n*-Pentacosenoic acid (Q):** 380 (M^+, $C_{25}H_{48}O_2$, 12.5%), 337 (M^+-43, 4%), 323 (M^+-14, 3%), 309 (M^+-14, 3%), 295 (M^+-14,3%), 281 (M^+ -14, 4%), 267 (M^+-14, 4%), 253 (M^+-14, 4%), 239 (M^+-14, 4%), 225 (M^+-14, 4.5%), 211 (M^+-14, 4.5%), 197 (M^+-14, 4.5%), 183 (M^+-14, 5%), 169 (M^+-14, 6%), 155 (M^+-14,6.5%), 141 (M^+-14, 7.5%),127 (M^+-14, 8%),113 (M^+-14, 12%), 99 (M^+-14, 22%).

***n*-Hexacosenoic acid (R)**: 394 (M^+, $C_{26}H_{50}O_2$, 3%), 323 (M^+-43, 1%), 309 (M^+-14, 1%), 295 (M^+-14, 1%), 267 (M'-14, 7%), 253 (M^+-14, 2.5%, 239 (M^+-14,

2.5%), 225 (M^+-14, 3%), 211 (M^+-14, 3%), 197 (M^+-14, 3%), 183 (M^+-14, 3%), 169 (M^+-14, 4%), 155 (M^+-14, 5%), 141(M^+-14, 6%), 127 (M^+-14, 7%), 113 (M^+-14, 10%, 99 (M^+-14, 15%), 85 (M^+-14, 51%), 71 (M^+-14, 74%), 57 (M^+-14, 100%, 43 (M^+-14, 27%).

Results and Discussion

Fatty acid components

Although the species of Carat/unto have been examined phytochemically, how-ever, there was no work published so far on the lipid components of *Caralluma* species, particularly from *C. edulis* prior to this work. While pursuing the investigations on *Caraiuma edulis*, it yielded eighteen fatty acids (saturated and unsaturated) four hydrocarbons (hentriacontane, tritriacontane, triacontane, nonaconsane) and one sterol (ß-sitosterol).

The methanolic extract of *C. edulis* followed by fractionation with ethyl acetate and water, then chromatographic separation with n-hexane resulted in the isolation of eighteen lipid components (Compounds **A**-**R**), which consist of eight unsaturated fatty and ten saturated fatty acids.

Analysis of lipid components were carried out by gas liquid mass spectrometric technique and they have been identified by methylene splitting and other characteristic fragmentation pattern as well as the differences in their relative retention times. As presented in Table 1.

The spectral data of different hydrocarbons (**S**-**V**) and sterols (**W**) obtained from fresh plants of C. edulis were confirmed by direct comparison with the authentic samples, through MP and MMP TLC, Co- TLC, superimposable IR and comparable mass values along with fragmentation patterns of mass spectra which are as follows:

Hentriacontane (S): m.p. 67° (Lit. m.p. 68°), IR ($CHCI_3$), 2820 $cm^{-1(}$C-H)molecular, formula: CH_3-CH_2-$(CH_2)_{31}$-CH_3,,mass at m/z 464 M^+ 421, 407, 393, 379, 365, 337, 323, 309, 295, 281, 267, 253, 239, 255, 211.

Tritriacontane (T): m.p. 71.8°C (lit. m.p. 72°C), IR ($CHCI_3$), 2845, 2920 cm (C-H); molecular formula ($C_{33}H_{68}$), structural formula: CH_3-$(CH_2)_{31}$-CH_3, Mass at Mz 464 M^+ 450, 408, 394, 380, 366, 352, 338, 310, 296, 282, 266, 254, 266, 211.

Triacontane (U): m.p. 65.9°C (lit. m.p. 65.8°C), IR ($CHC1_3$) 2880cm^{-1}C-H), molecular formula $_{(C3oH63),}$ structural formula: CH3-(CHZ)ze-CH3, Mass at m/z

422 M^+ 408, 394, 380, 366, 352, 338, 310, 296, 268, 254, 238, 212, 198, 184, 170, 156, 142, 128, 114.

Nanacosane (V): m.p. 63.4°C (lit. m.p. 64°C), IR (CHUL), 2820 cm^{-1} (C-H), molecular formula (CzsH6o), structural formula as CHs-(CH2)27-CH3, El-MS at m/z 408 M + 338, 323, 309, 295, 281, 267, 253, 239, 225, 211, 197, 183.

ß-Sitosterol (W): From the powdered residue, this compound was obtained from fraction III which was eluted with n- hexane (90:10, v/v) on evaporation in-hexane fraction yielded a pure crystalline compound. m.p. 138° (lit. m.p. 136.7°) **UV** max at 250 nm, IR (KBr), 3378 cm"' (OH), molecular formula (Cz9H5oO), Mass at m/z 414 M^+ 399, 396, 381, 329, 303, 273, 255, ^{t}H (300 MHz, CDCI3) ppm. 0.76 (311, s, C-18H), 0.83 H-26), 0.87 (H-27, 29), 0.99 (H-19), 1.05 (3H, s, C-21H), 3.20 (1H, m, C-3H), 5.34 (1H, t, J=6Hz, C-6H).

^{13}C-NMR 975.5 MHz, CDC13): ppm C-1 (37.30), C-2 (31.71), C-3 (71.62), C-4 (42.35), C-5 (140.76), C-6 (12.68), C-7 (31.95), C-8 (32.44), C-9 (50.21), C-10 (36.55), C-11 (21.12), C-12 (39.83), C-13 (42.55), C-14(56.61) C-15 (24.33), C-16 (28.25), C-17 (56.13), C-18 (11.68), C-19 (19.41), C-20 (36.14), C-21 (19.08), C-22(34.01), C-23 (26.21), C-24 (46.10), C-25 (29.25), C-26 (19.25), C-27 (20.21), C-28(23.130, C-29 (12.01).

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