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ISOLATION OF A NEW TRITERPENIC ALCOHOL FROM *CARISSA CARANDAS*

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Chemical constitutents of fresh fruits of *Carissa carandas* have been investigated. Thus a new triterpenic alcohol has been isolated from the fresh fruits of this plant. The compound has been provisionally named "Carissol" and its structure is elucidated on the basis of spectral studies which shows that it is hitherto an unreported epimer of α-amyrin.

INTRODUCTION

Carissa carandas, a member of Apocynaceae family, is most commonly cultivated in Pakistan for hedges and is called "Kakronda" or "Karonda" in this country. Bisset [1] reported that *Carissa carandas* contained cardiac glycosides, a minor component which could be an odoroside whereas Vohra and coworkers [2] reported that maximum cardiotonic activity was found in roots of this plant. Rastogi *et al* [3] reported almost similar results. Blood reducing properties was also observed in the plant [4] and have been mentioned in old chemical literature as purgative, stomachic and antidote for snakebite while the leaves have been mentioned as useful in remittent fever [5, 6]. A C_{31} terpene "Carindone" [7]. β-sitosterol [3], carissone [3], and a lignan carinol [8] were isolated from this plant. No work has been done on the fresh fruits of *Carissa carandas* but few fatty acids were reported in the seeds by Shrivastava [9]. Our present investigation deals with isolation and elucidation of the structure of a triterpenic alcohol isolated from the fresh fruits of this plant.

DISCUSSION

Fresh fruits were used for this purpose. The fresh fruits were extracted with hexane. The extract was freed off the solvent and yellowish gummy mass thus obtained was chromatographed over a column of silica gel PF 254. Six fractions were obtained, out of which only one was found to be crystalline and was studied in detail. Our studies show that this compound is not identical with any known triterpene and has been provisionally named as "Carissol". This compound melted at 118-120° and analysed for $C_{30}H_{50}O$. Carissol gave a positive test for triterpenoids. TLC studies indicated that the structure of carissol corresponded to β-amyrin; however, nuclear magnetic resonance spectroscopy demonstrated that carissol is not β-amyrin whereas mass spectroscopy demonstrated it to be an olean-12-ene or urs-12-ene type pentacyclic triterpenic alcohol. The reaction of hydrogen peroxide with carissol showed that it belong to α-amyrin group.

The infra red spectrum of carissol showed a broad peak at 3400 cm^{-1} for hydroxyl group in carissol. It formed a monoacetate which confirmed the hydroxyl group in carissol. The hydroxylic proton resonated at δ 4.6 in the nuclear magnetic resonance spectrum.

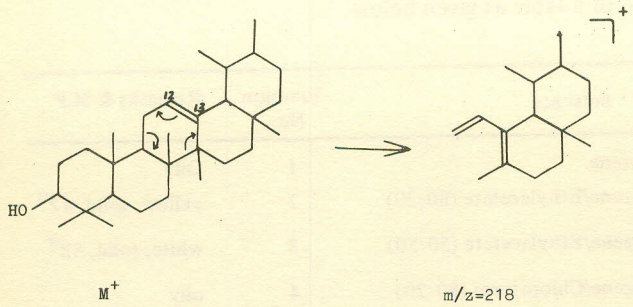
The presence of double bond was proved by infra red, nuclear magnetic and mass spectroscopy. The C=C stretching vibration occurred at 1635 cm^{-1} in infra red spectrum. The single olefinic proton resonated at δ 5.1 in nuclear magnetic resonance spectrum and the position of double bond at Δ^{12-13} was also confirmed by mass spectrum due to the appearance of the base peak m/z = 218 i.e. retro-Diels Alder fragment.

The peaks at 1380 cm^{-1} and 1360 cm^{-1} in infra red spectrum indicated the presence of gem-dimethyl groups in carissol which was more prominent in its acetyl derivative.

The proton nuclear magnetic resonance spectrum of carissol in $CDCl_3$ at 60 MHz showed eight singlet at δ 0.77, 0.79, 0.95, 1.00 1.10, 1.23, 1.40 1.63 were assigned for C-CH_3 at C-28, C-24, C-25, C-23, C-27, C-26, C-29 and C-30 respectively.

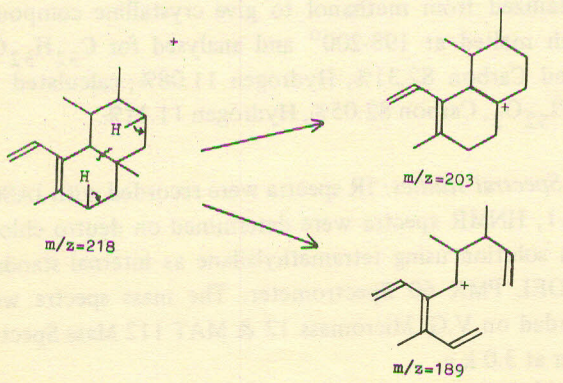
Further support for the structure of carissol was obtained by its mass spectroscopy. The molecular ion peak was recorded at m/z = 426 which corresponded to its molecular formula $C_{30}H_{50}O$ which was also confirmed by higher resolution mass spectroscopy. The prominent peaks in higher resolution mass spectrum were as m/z = 426.3885 ($C_{30}H_{50}O$), m/z = 411.3594 ($C_{29}H_{47}O$) and m/z = 218.1938 ($C_{16}H_{26}$).

The base peak occurred in single focusing mass spectrum at m/z = 218 showing that the compound contained Δ^{12-13} double bond. This peak is characteristic peak of α - and β-amyrin series which is obtained due to retro-Diels-Alder fragment.

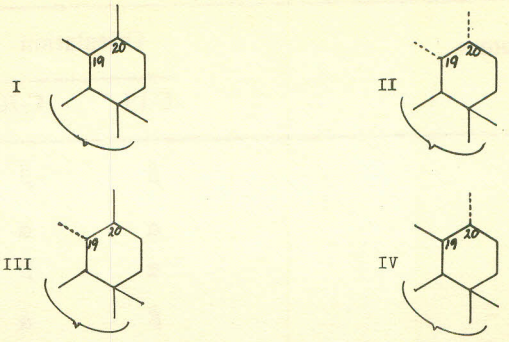


The base peak suggested that the most probable position of hydroxyl group is at C-3 in ring A or at C-6 in ring B and not in ring C or D. If an –OH group is present in ring C or D, the base peak would be shifted in 17 mass units higher. The position of hydroxyl group in ring B can be ruled out because carissol was easily acetylated since position C-6 is sterically hindered due to the presence of methyl group at C-25, C-26 and C-27 and would render acetylation difficult. The position C-3 is least hindered and hence the monoacetate is formed quite easily. The formation of monoacetate was also proved by mass spectroscopy and microanalysis.

The peak m/z = 203 is obtained due to the loss of a methyl group from fragment m/z = 218. The facile loss of methyl group is in accordance with its position at C-17. The peak at m/z = 189 is also obtained by the double hydrogen transfer.



The fragment m/z = 203 gave fragment m/z = 133 due to loss of 70 mass units. This cleavage results in the loss of



ring E yielding the stabilized ion.

The acetate of carissol melted at 198-200° and analyzed for $C_{32}H_{52}O_2$ The mass spectrum of carissol acetate showed the molecular ion peak at m/z = 468 in arrangement with its molecular formula.

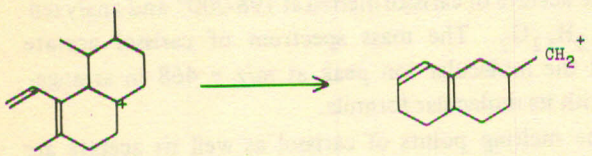
The melting points of carissol as well its acetate are quite different from those of α -amyrin and β -amyrin. (α-amyrin m.p. 186.5 –187° [10] β -amyrin m.p. 199 – 200° [11], carissol 118–120°, α -amyrin acetate m.p. 225–226° [10], β -amyrin acetate m.p. 241° [11] carissol acetate m.p. 200 – 202°).

α -Amyrin has eleven chiral centres and hence may have many stereo-isomers. The hydroxyl group is in β-disposition which is the preferred biogenetic orientation in natural ursene derivatives. Our literature survey does not show the presence of any natural epimer of α -amyrin. Out of the remaining chiral centres, biogenatic consideration permit us to assume that carissol may have the same stereochemistry as that of α -amyrin except at position C-19 or C-20. This is supported by the fact that C-CH_3 signals of carissol are located at a slightly different position from those of α -amyrin as shown by the following table.

Position of methyl group	β - Amyrin (100-MHz)[12]	α-Amyrin (60 MHz)[13]	Carissol (60 MHz)
C-23	0.99	–	1.00
C-24	0.79	0.81	0.79
C-25	0.94	0.96	0.95
C-26	0.97	1.02	1.23
C-27	–	1.08	1.10
C-28	0.83	0.81	0.77
C-29/30	0.87	0.80/0.91	1.40/1.63

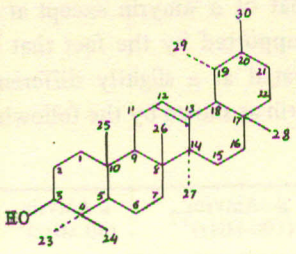
These evidences suggest that carissol might be a new stereoisomer of α -amyrin. It seems that the stereochemistry of carissol is different at C-19 and C-20 with respect to α-amyrin.

Possible isomer	Orientation	
	C-19	C-20
I	β	β
II	α	α
III	α	β
IV	β	α



m/z=203 m/z=133

Spectroscopic data and other chemical feature indicated that carissol is not identical with α-amyrin or β-amyrin. It can be claimed that carissol may be an epimer of α-amyrin. On the basis of the evidences presented here the stereochemistry of carissol can be depicted by the following structure.



Carissol

MATERIAL AND METHODS

10 kg. of fresh fruits of *Carissa carandas* were crushed and soaked in hexane. After three days of percolation the solvent was drained out and this process was repeated three times till the final extract was almost colourless. The combined extract was completely freed from the solvent under reduced pressure. A yellowish gummy mass was obtained which was chromatographed over a column of silica gel. For this purpose, silica gel PF-254 was shaken with distilled benzene. The resulting slurry was poured onto glass column of one inch diameter. The gummy mass was dissolved in minimum quantity of benzene and was poured over the column. It was eluted first with benzene followed by benezene/ethylacetate, benzene/chloroform and chloroform. The different fractions obtained were recorded in the form of a table as given below.

Solvents	Fraction No.	Remarks & M.P
Benzene	1	Oily
Benzene/Ethylacetate (80:20)	2	yellow, solid, 42°
Benzene/Ethylacetate (50:50)	3	white, solid, 68°
Benzene/Chloroform (80:20)	4	oily
Benzene/Chloroform (50:50)	5	white, crystalline, 118-120°
Benzene/Chloroform (20:80)	6	oily
Chlorofirm	7	oily

Fraction number 5 was studied in detail and was provisionally named carissol. It was re-crystallized from methanol till it showed a single spot on TLC (Solvent system, Benzene R_f = 0.58, Benzene-Chloroform 9:1, R_f = 0.65).

Acetylation of carissol: The compound (0.3 g.) was dissolved in 1 ml. of pyridine and 5 ml. acetic anhydride was added to it. The reaction mixture was then refluxed on water bath for five min. The resulting solution after being left overnight when checked on TLC was seen to change. it was then worked out by being pouring onto cold water and extracted with ether. The ethereal layer was washed with cold water. It was then dried over anhydrous sodium sulphate and heated to dryness. The dried mass repeatedly crystallized from methanol to give crystalline compound which melted at 198-200° and analysed for $C_{32}H_{52}O_2$. Found Carbon 82.31%, Hydrogen 11.08%; calculated for $C_{32}H_{52}O_2$, Carbon 82.05%, Hydrogen 11.11%.

Spectral studies: IR spectra were recorded with JASCO IRA-1, HNMR spectra were determined on deutro chloroform solution using tetramethylsilane as internal standard in JOEL PMX 60 Spectrometer. The mass spectra were recorded on V.G. Micromass 12 & MAT 112 Mass Spectrometer at 3.0 k.v.

H NMR of carissol: 0.77 (3 x H-28), 0.79 (3 x H-24), 0.95 (3 x H-25), 1.00 (3 x H-23) 1.10 (3 x H-27), 1.23 (3 x H-26). 1.40 (3 x H-29), 1.63 (3 x H-30), 5.1 (m, H-12), 4.63 (d, –OH), 3.14 (q, H-3).

MS of carissol: m/z 426 (M^+), 411 ($M^+ -CH_3$), 393 ($M^+ -CH_3$ & H_2O), 218 (M^+ – RDA fr.) 203 (RDA fr. $-CH_3$), 189 (RDA fr. $-CCH_3$ & 2H), 133 (RDA fr. $-CH_3$ & C_5H_{10}) MS of carissol acetate: M/z 468 (M^+), 453 ($M^+ -CH_3$, 425 ($M^+ -CH_3CO$), 408 ($M^+ -CH_3COOH$) 393 ($M^+ -CH_3COOH$ & CH_3), 357 (metastable peak), 218 (RDA fr.) Microanalysis of Carissol: Carbon 84.21%, Hydrogen 11.68% requires, C 84.50% H, 11.73%.

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