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ISOLATION OF A NEW ISOMER OF URSOLIC ACID FROM FRUITS AND LEAVES OF *CARISSA CARANDAS*

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Our studies in the chemical investigations in *Carissa carandas* had led to the isolation of a new triterpenic alcohol which had already been reported [13]. Further studies in this respect resulted in the isolation of a new isomer of ursolic acid.

Key words: Carissa carandas, Apocynacea, Triterpenoid.

INTRODUCTION

Carissa carandas Linn. belongs to family apocynacae which consists of 300 genera and 100 species. It is a large shrub with simple thorns and commonly cultivated throughout Pakistan. The different parts of this plant have been used for various treatments in the indigenous system of medicine [1, 2]. Pharmacological properties of this plant have also been studied [3-7]. The chemical substances isolated from *Carissa carandas* and other species are tabulated in Table 1.

Table 1

Substances	Species	References
Odoroside-H	*C. carandas*	8
	C. lanceolata	9
	C. spinarium	8,15
Carindone	*C. carandas*	11
Carional	*C. carandas*	12
B-sitosterol	*C. carandas*	17
	C. congesta	10
Four crystalline substances designated A, B, C and D_1	*C. carandas*	6
Fatty acids	*C. carandas*	16
Carissol	*C. carandas*	13
Sugars and amino-acids	*C. carandas*	14
Carissone	*C. lanceolata*	9
	C. congesta	10
Evomonoside	*C. spinarium*	15
Odoroside-G	*C. spinarium*	15

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RESULTS AND DISCUSSIONS

A new compound was isolated from the fresh fruits and leaves of *Carissa carandas* according to the procedure described in the material and methods part. It melted at 230-232° and analysed for $C_{30}H_{48}O_3$. It gave a positive test for carboxylic acid group towards litmus as well as with sodium bicarbonate solution. This acid is triterpenoidal in nature as confirmed by Libermann – Buchardt reaction. It was characterized by preparation of acetyl and methyl ester derivatives as well as by spectroscopic methods and was provisionally named as carissic acid.

The infra red spectrum of carissic acid showed a broad peak at 3400 cm^{-1} indicative of hydroxy group. A peak at 1690 cm^{-1} was assigned for the presence of carboxylic group in this acid. A doublet at 1385-1370 cm^{-1} indicated the presence of gem-dimethyl groups. The mass spectroscopic studies demonstrated that carissic acid belonged to olean-12-ene or urs-12-ene type triterpenic acid. The molecular ion was recorded at m/z = 456 which corresponded to its molecular formula $C_{30}H_{48}O_3$. The base peak was found at m/z = 248 showing that carissic acid has a double bond at C 12-13. This base peak was due to retro-diels alder fragmentation 18-19. The easy loss of carboxylic group from the base peak indicated the position of -COOH group at C-17. The m/z = 203 was also decomposed to m/z = 133 by the loss of 70 mass units. The fragmentation pattern is shown in Chart I.

Carissic acid formed monoacetate when allowed to react with acetic anhydride in presence of pyridine. This formation of acetate was confirmed by mass and infra red spectra. The acetate of carissic acid melted at 190-192° and analyzed for $C_{32}H_{50}O_4$.

The infra red spectrum of acetyl derivative of carissic acid indicated a peak at 3400 cm^{-1} for -OH stretching of carboxylic group. Two peaks were obtained at 1720 cm^{-1}

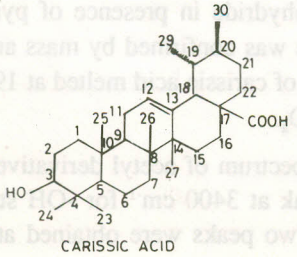
and 1685 cm^{-1} for a carbonyl functions of acetyl group as well as carboxylic acid function. The molecular ion peak of carissic acid acetate was recorded at m/z = 498 which confirmed its molecular formula $C_{32}H_{50}O_4$. The peaks m/z = 453 and 438 were due to loss of carboxylic acid group and acetic acid from the molecular ion respectively. The base peak obtained m/z = 248 was also a retro-diels alder fragmentation.

An examination of the structure of carissic acid showed that it is similar to that of ursolic acid. The melting point of carissic acid as well as its acetate are quite different from those of ursolic acid and its acetate respectively (carissic acid 230°-232°, carissic acid acetate 190°-192°, ursolic 298°, ursolic acid acetate 246°). Carissic acid may therefore be considered an isomer of ursolic acid. A large number of stereoisomers are possible due to the presence of ten chiral centres in ursolic acid. It is further supported by the fact that methyl signals of methyl ester of carissic acid are located at slightly different position from methyl ester of ursolic acid as shown in following Table 2.

Table 2

Position of methyl group	Methyl ester of ursolic acid	Methyl ester of carassic acid
C-23	0.99 ppm	1.05 ppm
C-24	0.78 ppm	0.70 ppm
C-25	0.92 ppm	0.90 ppm
C-26	0.75 ppm	0.66 ppm
C-27	1.08 ppm	
C-29/30	0.89/0.91 ppm	1.23/1.29 ppm

On the basis of evidence presented here we suggest that carissic acid may have same stereochemistry as ursolic acid except at position C-19 and C-20. However spectroscopic data and other chemical features such as Rf, melting point, mixed melting point, [α], and those of their derivatives respectively, indicated that carissic acid is not identical with ursolic acid. It can be assumed that carissic acid is an isomer of ursolic acid.



CARISSIC ACID

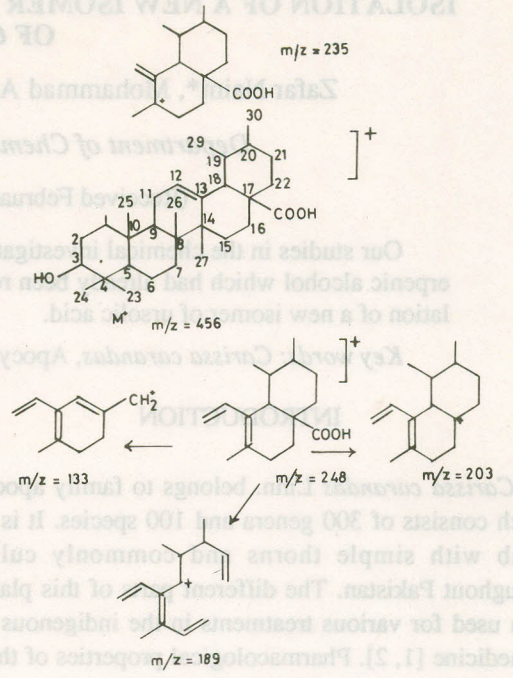


Chart 1.

MATERIAL AND METHODS

10 Kg of fresh fruits of carissa carandas were crushed and soaked in ethyl alcohol. After three days of percolation the solvent was drained out and this process was repeated three times. The combined alcohlic extracts were concentrated in vacuo in rotatory evaporator. During the evaporation of solvent a solid mass was precipitated out which was collected by filtration. This mass dissolved in ether and the etherial solution was evaporated and crystalized with hot methanol. This compound was further purified by thick layer chromatography. For this purpose, silica gel (HF 254) was used to prepare the plate (20 x 20 cms). The adsorbent was shaken with chloroform and the resulting slurry was spread over the glass plate smoothly with the help of spreader. The resulting plates were kept overnight and activated at 110° for two hours in an oven. The compound was dissolved in ether and loaded carefully on plate. This plate was run (chloroform and ethyl acetate, 9:1) in TLC tank which was presaturated with the solvent. The developed plate was dried. Another plate (5 x 20 cm) was also run side by side. This plate was placed in iodine tank. One major spot and two minor spots (Rf = 0.55, 0.17 and 0.32 respectively) were developed with the help of spots on small plate, the spots of first plate of 20 x 20 cms were marked. The major band was scraped and eluted with ethyl acetate. The combined solutes were then evaporated. It was re-ex-

amined on TLC using different solvent systems and was found to be pure. It melted at 230-232°.

Carissic acid was also obtained from the leaves of *Carissa carandas*. Dried leaves were soaked in ethyl alcohol for three days. The extracts was taken out and leaves were again treated with fresh quantity of ethyl alcohol. This operation was repeated three times. The combined alcoholic extracts were filtered and concentrated on rotatory evaporator under reduced pressure. A dark brown green residue was obtained. This residue was washed several times with cold ethyl alcohol. A little amount of this residue was tested on flame which was not completely burnt. This residue was dissolved in chloroform and then filtered. The filterate was evaporated on rotatory evaporator and crystallized with hot methanol and also recrystallized with the same solvent. The crystals obtained in this manner, melted at 230-232° $[\alpha]_D^{30} = +86°$ (ε + OH).

Microanalysis. Carassic acid analyzed for $C_{30}H_{48}O_3$. Found C 78.35%; H 10.81%; calculated for $C_{30}H_{48}O_3$ C 78.94%; H 10.52%.

Acetylation of carassic acid. The compound (0.1 g.) was dissolved in pyridine and 3 ml of acetic anhydride was added to it. The reaction mixture was then refluxed for five minutes on water bath. The resulting solution after being left over night, then was checked on thin layer chromatography and was found to have been changed. It was then worked out by pouring it into cold water. The resulting precipitate of the acetyl derivative was extracted from ethyl acetate. It was then dried over anhydrous sodium sulphate and heated to dryness. The dried mass was repeatedly crystallized from methanol to give crystalline compound which melted at 190-2° and analysed for $C_{32}H_{50}O_4$. Found: C 77.22%, H 10.34%. Calculated for $C_{32}H_{50}O_4$: C 77.01%; H10.04%.

Esterification of carissic acid. About 0.1 g. of the compound was dissolved in ether and cooled in ice bath. To this solution was added etherial solution of diazomethane (10 ml.). The reaction mixture was kept over night and was checked on thin layer chromatography and was found to be unchanged. 5 ml. of diazomethane solution was further added and again kept over night. It was rechecked on thin layer chromatography when it was seen to have been competely changed. The ether was evaporated from reaction mixture carefully. The residue was taken in ether and showed a single spot on TLC. It did not crystallize and remained an oily compound. The MS and IR spectra completely agreed with methyl ester of carissic acid.

Spectral studies. IR of Carissic acid: Jasco IRA-1 infra red spectrophotometer was used for infra red spectrum of carissic acid. The various peaks and their interpretation are as follows: 3400 broad -OH stret., 2940s C-H stret. of CH_3, 2850m C-H stret. of CH_2, 1690s C = 0 stret. of -COOH, 1385 and 1370 cm^{-1} d C-H stret. of gemdimethyl. Mass: V.G. Micro mass 12 and Mat 112 mass spectrometer were used at 3.0 KV. The peaks are interpreted as follows: M^+ = 456, m/z = 411 (M^+-COOH), m/z = 248 base peak retro DAR frg., m/z = 235, 207, 203, 189, 133. NMR of methyl ester of carissic acid. It was recorded on JOEL PMX 60 using TMS as internal standard and DMSO as solvent. The various peaks were assigned as follows:

PMR (DMSO, 60 MHz), 0.66 s 26-Me, 0.70 3H 24-Me, 0.90 3H 25-Me 1.05 3H 23-Me, 1.23, 1.29 6H d 29, 30-Me 3.5 H s COOMe.

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