

POSITIONAL ISOMERS OF OCTADECENOIC ACID SEPARATED FROM OILS OF THREE CARUM SPECIES OF UMBELLIFERAE FAMILY

Tanweer Ahmad Chaudri*, Ijaz Ahmad, Din Muhammad and Manzoor Ahmed

PCSIR Laboratories Complex, Lahore-54600, Pakistan

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In the family *Umbelliferae* octadecenoic acid ($C_{18:1}$) is a phylogenetic character having distribution on the different positions of triacylglycerol molecules. It has a role as a key intermediate in fat metabolism during seed germination which created an interest to investigate further to find out its positional isomers in the oils of *Carum* species. Therefore, the octadecenoic acid separated from the seed oil of *Carum capticum*, *Carum carvi* and *Carum roxburghianum* of Umbelliferae family was oxidized separated by Von Rudloff's reagents. The liberated mono and difunctional fatty acids were separated and identified by the application of thin layer and gas liquid chromatography to determine positional isomers. The positional isomers determined among these three species were *cis*-6-octadecenoic acid, *cis*-9-octadecenoic acid and *cis*-12-octadecenoic acid. The percentage of these isomers varied from 4.5 to 46.0%

Key words: Octadecenoic acid, Von Rudloff's Oxidation, Positional isomers.

Introduction

The monoenoic fatty acid i.e. octadecenoic acid separated from the oils of resting seeds of *C. capticum*, *C. carvi* and *C. roxburghianum* of the Umbelliferae family is oxidized to determine the positional isomers. However, this type of work of separation of positional isomers has rarely been carried out previously (Vongerichten and Kohler 1938). The literature reveals that octadecenoic acid of different species of Umbelliferae family consists of two positional isomers (Placek 1963) i.e. *cis*-6-octadecenoic acid and *cis*-9-octadecenoic acid and low percentage of one more positional isomer *cis*-12-octadecenoic acid has been determined in *C. capticum* species. This has been studied by the oxidative, degradation of ethylenic bonds with the help of Von Rudloff's oxidation method (Von Rudloff 1956). The short chain mono-carboxylic acids have been analysed by gas liquid chromatography in aqueous media by the use of Porapak "Q" column. Then the mono-carboxylic and dicarboxylic acids produced as a result of oxidation are extracted, methylated and separated by thin layer chromatography and identified as methyl esters of acids by the use of gas liquid chromatography. The presented work on the positional isomers of octadecenoic acid of three species of *Carum* also confirms the presence of positional isomers carried out by previous workers (Vongerichten and Kohler 1938). These isomers clearly indicate that ethylenic bonds are present at different positions in the same chain length of monounsaturated fatty acids ($C_{18:1}$). The oxidative degradation of ethylenic bonds is carried out for the production of

mono and functional acids. Further application of thin layer and gas liquid chromatography for the separation and identification of mono and di-functional acids enabled us to find out positional isomers of octadecenoic acid separated from the oil of three *Carum* species of the Umbelliferae family. So, these studies reflect that nature produces positional isomers having double bonds at different position. The percentage of *cis*-12-octadecenoic acid has been found to be very low as compared to *cis*-5-octadecenoic and *cis*-9-octadecenoic acid in case of *Carum capticum*.

Materials and Methods

Liberation, methylation and purification of fatty acids: One gram oil of each specie (*C. capticum*, *C. roxburghianum* and *C. carvi*) was saponified with 20 ml of 0.5 N alcoholic potassium hydroxide (Devine and Williams 1961). The non-saponifiable material was removed with diethyl ether. The fatty acids were liberated from soap solution by the addition of HCl and then extracted with diethyl ether. The fatty acids (400 mg) were methylated (Kumar and Tsunada 1978), using a mixture (37 ml) of methanol:benzene:acetyl chloride 30:6:1.5 (v/v⁻¹) and purified on preparative silica gel plates using ether:hexane 1:9 (v/v⁻¹) as an eluent (Ijaz *et al* 1989).

Separation and oxidation of octadecenoic acid: The monoenoic fatty acid from the pure methyl esters of fatty acids of oils of three species was separated by preparative silica gel plates impregnated with 20% AgNO₃, using ether:hexane mixture (1:9) eluent as mentioned above (Shahina *et al* 1986). The monoenoic methyl octadecenoate (8 mg) of each specie was

* Author for correspondence

dissolved in purified t-butanol (3 ml) and 0.2 M solution of sodium and 0.005 M solution of potassium permanganate (2 ml) was used as Von Rudloff's oxidising reagent. The material was shaken for 1 h to accomplish the oxidation (Hamilton *et al* 1972).

Analysis of short chain fatty acids: An aliquot (50 μ ml) from the oxidized mixture of octadecenoic acid separated from the oil of each specie was injected into a parapak "Q" column (18 x 1/4) at 130°C and the temperature was increased from 130°C to 230°C at the rate of 6°C/minute by using the temperature programming (Hamilton *et al* 1972). The standard of short chain fatty acids (C₄-C₉) in aqueous media were also run on the same column under the same conditions for identification and comparison of the unknown short chain monocarboxylic acids produced as a result of oxidative degradation of octadecenoic acid. The results are shown in Table 1.

Extraction of mono and dicarboxylic acids from aqueous media and their methylation and separation by thin layer chromatography: Chloroform was used for the repeated extraction of mono and dicarboxylic acid from the above mentioned oxidised mixture of octadecenoic acid separated from the oil of each specie. These chloroform extracts were combined and the material, after the removal of chloroform was methylated (Ijaz *et al* 1992). The methyl esters of monocarboxylic and dicarboxylic acids were separated on preparative silica gel plates using mixture of hexane: ether 1: 1 (v/v⁻¹) as a developing solvent.

Analysis of monocarboxylic and dicarboxylic acids as methyl ester on 1% Apizon L column: The purified methyl ester of monocarboxylic acids (C₉ & C₁₂) produced as a result of oxidation of octadecenoic acid, separated from the oil of each specie were identified by the use of a 1% Apizon L (80-100 mesh) packed column.

The gas liquid chromatogram was operated at 110°C and 130°C for the identification of nonanoic and dodecanoic acids respectively. The identification of these acids was confirmed with the help of standards. The methyl esters of dicarboxylic acids of the respective part of monocarboxylic acid (having high boiling points) were also identified at 120-190°C. The dicarboxylic acids as methyl esters were confirmed by running the standard methyl esters of dicarboxylic acids under the same conditions.

Results and Discussion

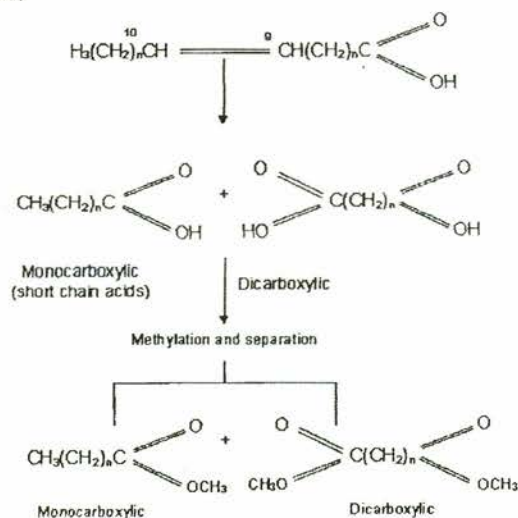
The oil yield of three species *C. capticum*, *C. carvi* and *C. roxburghianum* was 29% 24% and 26% respectively. The oil of each specie was saponified, methylated and separated into saturated, mono-unsaturated, di-unsaturated and tri-

unsaturated fatty acids as methyl esters by the application of silver nitrate impregnated thin layer chromatography. The percentage of monoenoic fatty acids as methyl ester were 75.5% 69.4% and 68.4% obtained from the oil of *C. capticum*, *C. carvi* and *C. roxburghianum*, respectively.

The oxidation of monounsaturated fatty acid is carried out to cleave double bonds and eventually to produce monofunctional and difunctional acids which help to determine positional isomers. Since complication arises by the production of a number of mono and difunctional acids after the oxidation of polyunsaturated fatty acids, therefore, the oxidation technique was applied exclusively for monoenoic fatty acids.

Different oxidising agents have been used for the cleavage of double bonds e.g. peracids (Sween 1949), Osmium tetroxide (Griegee 1938) hydrogen peroxide (Milas 1937) sodium chlorate (Hofman 1912), ozonolysis (Caxon and Tavs 1959) potassium permanganate (Kumar and Tsunoda 1978). However, potassium permanganate and sodium meta periodate have been claimed to be the best oxidising agents and the oxidation carried out by these reagents is known as Von Rudloff's oxidation.

In the Von Rudloff's oxidation, the danger of over oxidation was minimized by the use of the sodium meta periodate, containing the small amount of potassium permanganate as a catalyst. Under this oxidation technique, the oxidation process was carried out for 2 h, the oxidants were destroyed by sulphur dioxide or sodium sulphite, neutralised by sodium carbonate to pH 7-8. In the present studies, the modified Von Rudloff's oxidation technique using sodium metaperiodate containing small amount of potassium permanganate was used. The major problem was the analysis of the water soluble short chain acids in aqueous media were analysed directly on a column of Porapak Q. The scheme for oxidation was as given below:



The short chain monocarboxylic and dicarboxylic acids produced by the oxidation of monoenoic fatty acids from the oil of *C.capticum* showed that there were three positional isomers i.e. *cis*-12-octadecenoic acid (4.5%), *cis*-9-octadecenoic acid (24.0%) and *cis*-6-octadecenoic acid (46.0%) cleaving double bond positions at 12:13, 9:10 and 6:7 respectively (Table 1). The literature revealed that there were two positional isomers among the monounsaturated fatty acids. i.e.

cis-9-octadecenoic acid commonly known as oleic acid (23.9%) and *cis*-6-octadecenoic acid known as petroselinic acid (48.1%). These acids were in close proximity of our findings except the low percentage of *cis*-12-octadecenoic acid. The finding of a third positional isomer, *cis*-12-octadecenoic acid is new and its presence has not been reported in the literature. Similarly the positional isomers of *C. carvi* were *cis*-9-octadecenoic acid (42.6%) and *cis*-6-octadecenoic acid (26.8%) hav-

Table 1
Positional isomers of octadecenoic acid in the oil of *C.capticum*

Monobasic		Dibasic		Position of double bond	Distribution of isomers		
Chain length	Wt (%)	Chain length	Wt (%)		Isomers	Referred work wt (%)	Presented work wt (%)
C ₆	2.2	C ₁₂	2.3	Δ ¹²	<i>Cis</i> -12-octadecenoic	** --	4.5
C ₉	11.8	C ₉	12.2	Δ ⁹	<i>Cis</i> -9-octadecenoic (Oleic acid)	23.9*	24.0
C ₁₂	24.6	C ₆	21.4	Δ ⁶	<i>Cis</i> -6-octadecenoic (Petroselinic acid)	48.1*	46.0

*JAOCS 40:320, 1963; *Nothing mentioned in the literature

Table 2
Positional isomers of octadecenoic acid in the oil of *C.carvi*

Monobasic		Dibasic		Position of double bond	Distribution of isomers		
Chain length	Wt (%)	Chain length	Wt (%)		Isomers	Referred work wt (%)	Presented work wt (%)
C ₉	20.2	C ₉	22.4	Δ ⁹	<i>Cis</i> -9-octadecenoic (Oleic acid)	40.0*	42.6
C ₁₂	12.8	C ₆	14.0	Δ ⁶	<i>Cis</i> -6-octadecenoic (Petroselinic acid)	26.0*	26.8

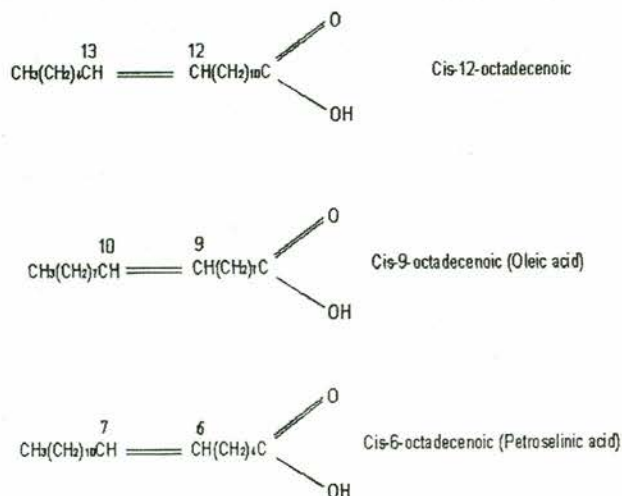
*JAOCS 40:320, 1963

Table 3
Positional isomers of octadecenoic acid in the oil of *C.carvi*

Monobasic		Dibasic		Position of double bond	Distribution of isomers		
Chain length	Wt (%)	Chain length	Wt (%)		Isomers	Referred work wt (%)	Presented work wt (%)
C ₉	20.4	C ₉	20.0	Δ ⁹	<i>Cis</i> -9-octadecenoic (Oleic acid)	*	40.4
C ₁₂	13.9	C ₆	14.1	Δ ⁶	<i>Cis</i> -6-octadecenoic (Petroselinic acid)	*	28.0

*JAOCS 40:320, 1963

ing the double bonds at 9:10 and 6:7, respectively (Table 2). The *C. roxburghianum* also showed double bond at the usual positions but its isomers, are being reported for the first time by these authors and are shown in Table 3. The structural formula of these positional isomers are given below:



These studies reflect that positional isomers have definitely biosynthetic relationship and further investigation may be carried out to know their specific uses and applications.

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