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Short Communication

Determination of Positional Isomers of Monoenoic Fatty Acids Separated From Seed Oils of *Nicotiana tabacum* L. and *Nicotiana rustica*

Amran Waheed*, Muhammad Saleem and Shahid Mahmud
PCSIR Laboratories Complex, Lahore-54600, Pakistan

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Abstract. The octadecenoic acid separated from the seed oils of *Nicotiana tabacum* L. and *Nicotiana rustica* by the application of argentation thin layer chromatography was oxidized by modified Von Rudloff's technique. The liberated monofunctional and difunctional carboxylic acids were separated and identified by the application of thin layer chromatography and gas chromatography. The positional isomers determined in both species were *cis*-9-octadecenoic acid and *cis*-11-octadecenoic acid.

Keywords: isomers, monoenoic fatty acids, *Nicotiana tabacum, Nicotiana rustica,* seed oils

Nicotiana tabacum L. and *Nicotiana rustica*, yield tobacco and are commonly cultivated as crops (Kirtikar and Basu, 1984). The monoenoic fatty acid i.e. octadecenoic acid separated from the oils of both the species is oxidized to determine the positional isomers. The literature reveals that octadecenoic acid of *N. tabacum* consists of two positional isomers (Frega *et al*., 1991) *cis*-9-octadecenoic acid and *cis*-11- octadecenoic acid, whereas no work has been reported on the positional isomers of *N. rustica*. The present work reports the positional isomers of *N. tabacum* and *N. rustica.*

Liberation, methylation and purification of fatty acids from the plants were carried out according to the methods described by Waheed *et al.* (2001), Raie *et al.* (1989) and Devine and Williams (1961). The purified methyl esters were separated into four fractions (I to IV) with $AgNO_3$ impregnatede thin layer chromatography.

Separation and oxidation of octadecenoic acid were carried out by the methods described by Chaudri *et al*. (2001), Hamilton and Raie (1972) and Von Rudloff (1956).

The mixture of mono- and di-carboxylic acids produced in aqueous media was analyzed by gas chromatography using porapak "Q" column and short chain mono-carboxylic acids (C_4-C_9) were identified and later, the remaining acids (above C_{10}) were analyzed as described by Raie *et al.* (1989). The methyl esters of mono- and di-carboxylic acid were separated by preparative silica gel plates using mixture of hexane: diethyl ether (1 : 1 v/v) as a developing solvent.

The purified methyl esters of mono-carboxylic acid (C_9 and C_{11}) were analyzed and identified by the use of 5% SE-30 packed column at 110 °C to 150 °C and those of di-carboxylic acids were analyzed at 150 °C to 200 °C. Their identification was confirmed with the help of standards. The R_f values of the separated four fractions of fatty acids of the two species are reported in Table 1 and their composition in Table 2.

The percentages of saturated fatty acids and monounsaturated fatty acids in *N. tabacum* and *N. rustica* were 12.7% and 12.3%, respectively, whereas, the percentages of diunsaturated and triunsaturated fatty acids were 73.9% and 71.6% and 1.2% and 0.9%, respectively, in both the species (Table 2).

Table 3 and 4 show that the two positional isomers of the monoenoic fatty acid (octadecenoic acid) are present in the seed oil of *N. tabacum* i.e., *cis*-9-octadecenoic acid (11.1%) and *cis*-11-ctadecenoic acid (1.1%); similarly two positional isomers of monoenoic fatty acid (octadecenoic acid) i.e. *cis*-9-octadecenoic acid (14.4%) and *cis*-11-octadecenoic acid (0.8%) are present in *N. rustica.*

The oxidation of monounsaturated fatty acid (octadecenoic acid) was carried out to cleave double bonds and eventually to produce monobasic and dibasic acids, which helped to determine the positional isomers. Since complications arise by the production of a number of monobasic and dibasic

Table 1. R_f values of different fatty acid bands (as methyl esters) separated by the $AgNO_3$-TLC

Band no.	Bands	Fatty acid	R_f
I	saturated acids	$C_{12:0}$, $C_{14:0}$, $C_{16:0}$ $C_{18:0}$, $C_{20:0}$	0.65
II	monounsaturated acid	$C_{18:1}$	0.51
III	diunsaturated acids	$C_{18:2}$	0.30
IV	triunsaturated acids	$C_{18:3}$	0.18

*Author for corresponding; E-mail: dramranwaheed@yahoo.com

acids after the oxidation of polyunsaturated fatty acids, therefore, the oxidation technique was applied exclusively for monoenoic fatty acid although the diunsaturated fatty acids are present in large amounts as compared to monosaturated fatty acid in both the species of *Nicotiana* (Table 2).

A number of oxidizing agents (Bailey, 1958; Rigby, 1956; Swern, 1948; Milas, 1937) have been used for the cleavage of double bonds but potassium permanganate and sodium metaperiodate had been claimed to be the best oxidizing agents and the method was known as modified Von Rudloff's oxidation (Hamilton and Raie, 1972). The oxidation of unsaturated fatty acids by permanganate did not normally involve any change of configuration and the procedure had been applied to many other seed oils (Galun *et al.,* 1984; Hammond *et al.,* 1972; Wilson *et al.,* 1962).

Table 2. Percentage of different fatty acids (as methyl esters) separated on the $AgNO_3$-TLC

Band no.	Fatty acids	*N. tabacum* (%)	*N. rustica* (%)
I	saturated	12.7	12.3
II	monounsaturated	12.2	15.2
III	diunsaturated	73.9	71.6
IV	triunsaturated	1.2	0.9

Table 3. Positional isomers of octadecenoic acid of the oil of *N. tabacum*

Chain length of monobasic acid	Chain length of dibasic acid	Position of double bond	Percentage	Isomers	Referred work* percentage
C_9	C_9	Δ_9	11.1	*cis*-9-octa-decenoic	10.6
C_7	C_{11}	Δ_{11}	1.1	*cis*-11-octa-decenoic	0.6

* Frega *et al*., 1991

Table 4. Positional isomers in octadecenoic acid of the oil of *N. rustica*

Chain length of monobasic acid	Chain length of dibasic acid	Position of double bond	Percentage	Isomers
C_9	C_9	Δ_9	14.4	*cis*-9-octa-decenoic
C_7	C_{11}	Δ_{11}	0.8	*cis*-11-octa-decenoic

Since the major problem was the analysis of the water-soluble short chain acids produced as a result of the oxidation of monoenoic fatty acid, therefore, short chain acids in aqueous media were analysed directly on a column of Pora-Pak Q. The short chain mono-carboxylic acids and di-carboxylic acids produced by the oxidation of monoenoic fatty acid separated from the oil of *N. tabacum*, showed that there are two positional isomers i.e. *cis*-9-octadecenoic acid and *cis*-11-ctadecenoic acid showing double bond at $\Delta_{9:10}$ and $\Delta_{11:12}$, respectively (Table 3). The literature (Frega *et al.,* 1991) revealed that there were only two positional isomers among the monounsaturated fatty acids i.e. *cis*-9- octadecenoic acid commonly known as oleic acid (11.1%) and *cis*-11-octadecenoic acid usually known as vaceenic acid (0.7%). Similarly the positional isomers of *N. rustica* are *cis*-9-octadecenoic acid (14.4%) and *cis*-11-ctadecenoic acid (0.8%) having the double bond at $\Delta_{9:10}$ and $\Delta_{11:12}$ (Table 4). These studies reflect that positional isomers have a definite biosynthetic relationship.

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