Changes in Fatty Acids during Maturation of *Coriandrum sativum* Seeds

G. LAKSHMINARAYANA[1], K.V.S.A. RAO, K. SITA DEVI and T.N.B. KAIMAL,
Regional Research Laboratory (CSIR), Hyderabad 500 009, India

ABSTRACT

Changes in fatty acids were studied during maturation of *Coriandrum sativum* seeds. The seeds matured in 50 days after flowering. Lipid synthesis proceeded at a steady rate up to 40 days after flowering. Reductive ozonolysis of the monoenes followed by gas liquid chromatographic analysis of the aldehydic fragments as dioxolanes of 1,3-propanediol was employed to estimate petroselenate (octadeca-*cis*-6-enoate) in the presence of oleate (octadeca-*cis*-9-enoate). Petroselenic acid was the major fatty acid at all stages.

INTRODUCTION

Changes in fatty acids, both common and unusual, during seed maturation have received much attention (1,2). However, only one report has dealt with the seeds containing petroselenic acid (octadeca-*cis*-6-enoic acid), i.e., of the ivy (*Hedera helix*) plant (3). The changes in fatty acids during maturation of another oilseed containing petroselenic acid, coriander (*Coriandrum sativum*, Umbelliferae) are reported here.

EXPERIMENTAL

Materials and Methods

Coriander seeds were purchased from the local market. Pure petroselenic acid was isolated (4) from coriander seed oil. Methylene chloride, dimethylsulfide and 1,3-propanediol were purchased from Matheson, Coleman and Bell, Norwood, Ohio. Silica Gel C (equivalent to Kiesel Gel G of E. Merck) was purchased from Acme Synthetic Chemicals, Bombay. Silver nitrate and *p*-toluenesulfonic acid (PTS), were reagent grade. Ozone was generated from oxygen in an ozonator.

Extraction of Lipids

Plants were grown in prepared plots. Flowers were tagged and the seeds collected at intervals of 10 days from the date of flowering. Moisture contents were determined by heating in an air-oven at 110 C to constant weight. Seeds were ground and extracted three times with chloroform/methanol (2:1, v/v) at room temperature. The extract was concentrated, diluted with water, reextracted with hexane, washed with sodium chloride solution and dried over sodium sulfate. The solvent was evaporated off. The residue was heated at 107 C for 4 hr at 6 mm of Hg with a nitrogen inlet leak to remove the volatile constituents. The lipid content was determined. The methyl esters were prepared by transesterification with 1% methanolic sodium methoxide solution and analyzed by gas liquid chromatography (GLC).

The GLC analysis was done on a Toshniwal unit equipped with dual columns and hydrogen flame ionization detectors. A column (6′ × 1/8″ stainless steel) packed with 10% EGSS-X on Gas Chrom Q (80/100 mesh) and maintained at 200 C was used for analysis of fatty acid methyl esters. The injector and detector blocks were maintained at 240 C. Flow rate of carrier gas (nitrogen) was 40 mL/min. Peaks were identified using reference compounds. Peak areas were measured by multiplying peak height with width at half-height. Since oleate and petroselenate were not separated on the EGSS-X column, composition of the monoenoic acids was determined by reductive ozonolysis followed by GLC analysis of the aldehydic fragments as dioxolanes of 1,3-propanediol according to the method of Lakshminarayana and Cornwell (5). To 1.0 mL of methylene chloride and two drops (ca. 0.05 mL) of 1,3-propanediol taken in a Teflon-lined, screw-capped Kimax culture tube (20 × 150 mm), cooled in a Dry Ice/acetone bath and saturated with ozone (until blue color persisted), 0.2-0.5 mL of a methylene chloride solution of the fatty acid methyl ester (ca. 2-5 mg) was added. Dimethylsulfide (1.0 mL), predried over activated neutral alumina and cooled in a Dry Ice/acetone bath was pipetted along the walls of the tube. A plug (ca. 7 mm) of PTS was made by introducing the capillary (ca. 7 cm) a few times into a mass of PTS crystals and the capillary slided gently into the tube such that the

[1]Author to address for correspondence.

TABLE I

Composition of Monoenes from Mixtures of Methyl Petroselenate and Groundnut Oil Methyl Esters by Reductive Ozonolysis followed by GLC of Dioxolanes and Dioxolane Esters of 1,3-Propanediol

Petroselenic acid in total esters (mol %)	Composition of monoenes, calcd. (mol %)		Composition of monoenes by reductive ozonolysis and GLC (mol %)					
			Dioxolanes		Dioxolane esters		Dioxolanes + Dioxolane esters	
	Oleic	Petroselenic	Oleic	Petroselenic	Oleic	Petroselenic	Oleic	Petroselenic
52.9	32.6	67.4	34.4	65.6	37.8	62.2	35.5	64.5
42.9	41.4	58.6	41.5	58.5	44.3	57.7	42.8	57.2
32.5	53.0	47.0	51.4	48.6	57.5	42.5	53.0	47.0
			52.9	47.1	53.4	46.6	53.1	46.9
21.9	66.0	34.0	67.0	33.0	70.6	29.4	68.2	31.8
11.1	81.3	18.7	81.8	18.2	85.3	14.7	83.3	16.7
			82.9	17.1	80.6	19.4	82.1	17.9
			80.3	19.7	82.0	18.0	80.9	19.1
5.6	90.2	9.8	92.5	7.5	94.7	5.3	93.3	6.7
			89.8	10.2	89.6	10.4	89.7	10.3

TABLE II

Changes in Fatty Acid Composition during Maturation of *C. sativum* Seeds

Days after flowering	Lipids (% dry wt)	Fatty acid[a] (wt %)							
		14:0	16:0	16:1	18:0	18:1Δ9	18:1Δ6	18:2	18:3
10[b]	8.4	6.5	27.1	Tr	6.0	17.3	27.0	12.7	Tr
20	11.2	3.7	10.4	0.8	1.5	62.8[c]	62.8[c]	18.2	2.5
30	16.4	0.6	3.9	Tr	Tr	3.5	74.1	17.9	Tr
40	22.7	0.5	3.5	0.4	Tr	3.3	79.8	12.5	Tr
50	21.5	0.4	4.4	0.7	Tr	5.5	75.1	13.4	Tr

[a]The number before the colon denotes number of carbons and after the colon the number of double bonds in the chain. Double bond position in 18:1 is also shown.

[b]Also contains 3.5% of an unidentified acid eluting between 16:0 and 18:0.

[c]18:1Δ9 + 18:1Δ6.

empty end entered into the solution. The tube was capped tightly and removed from the Dry Ice/acetone bath and allowed to stand at room temperature for 2 hr with occasional swirling. The tube was gently tilted to bring the solvents into contact with PTS and dissolve it, kept in a water bath (25 C), heated to 75 C in 75 min, maintained at this temperature for 3 hr and allowed to cool overnight in the water bath. The PTS was neutralized with a pinch of sodium bicarbonate. The products were isolated by extraction with hexane. The hexane layer was washed three times with water to remove the propanediol, dried over sodium sulfate, concentrated and analyzed by GLC. The column temperature was programmed from 140-200 C (6 C/min) for the analysis of the dioxolanes. The method was standardized using monoenes isolated from mixtures (5-50%) of methyl petroselenate and methyl esters of groundnut oil by chromatography on silver nitrate (9%)-impregnated silica gel layers (0.5 mm) using hexane/diethyl ether (94:6, v/v).

RESULTS AND DISCUSSION

The utility of the ozonolysis procedure (5) for the determination of methyl oleate and petroselenate in mixtures is evident from Table I. The dioxolanes of the aldehyde and aldehyde ester fragments from a mixture of methyl oleate and petroselenate gave four well-defined peaks in GLC. The composition of the monoenes was calculated on the basis of corresponding dioxolanes, or dioxolane esters or dioxolanes plus dioxolane esters. Calculations based on the mole percentage of dioxolanes gave consistent and reasonably accurate results for the various mixtures of petroselenate and groundnut oil methyl esters (Table I).

Lipid contents, along with their fatty acid compositions at various stages of seed maturation, are given in Table II. Lipids accumulated at a steady rate up to the 40th day after flowering.

Except in the 10th-day sample, petroselenic acid was the major fatty acid at all stages. In this sample, palmitic and petroselenic acids were present in equal amounts. The palmitic acid content declined drastically from the 10th to the 20th day and continued to decrease thereafter. The same pattern of changes was noted with oleic acid. Small amounts of hexadecenoic acid were present at all stages.

The data, however, did not elucidate the precursor of petroselenic acid. This acid is believed to be synthesized by desaturation at the 6,7-position of stearic acid (3).

ACKNOWLEDGMENT

This work was supported by Grant No. FG-In-521, United States Department of Agriculture, Far Eastern Regional Research Office, American Embassy, New Delhi, India.

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[Received December 22, 1980]