J. Am. Oil. Chem. Soc. 40, 28-30 (1963)

Petroselinic Acid and Nonsaponifiable Constituents of Parsley Seed Oil[1]

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Abstract

Selective extraction of parsley seed oil *(Petroselinum sativum)* facilitates the isolation of myristicin (5-allyl-1-methoxy-2,3-methylenedioxybenzene) and is also useful for the preparation of petroselinic acid from the triglycerides of this oil. Coriander seed oil *(Coriandrum sativum)* is also a satisfactory source for the isolation of petroselinic acid.

Introduction

PARSLEY SEED OIL may contain up to 75% petroselinic (6-octadecenoic) acid and, therefore, appears to constitute an excellent starting material for the preparation of this acid (1, 2, 4).

During a large-scale preparation of petroselinic acid from parsley seeds, we observed that the extraction procedure for obtaining the total lipids was of crucial significance, and various batches of oil differed widely in their composition.

The average parsley seed is 2–3 mm long and less than 1 mm wide. Their small size and refractory nature make them difficult to grind. The degree of grinding has an important bearing on the yield and composition of the oil. The oil obtained from thoroughly ground seeds contains a much higher percentage of triglycerides than that from partially ground seeds. In fact, it is possible to extract virtually all of the nonglyceride constituents by merely washing the whole (unground) seeds with solvent. Furthermore, extraction of the washed seeds after they are thoroughly ground yields virtually pure triglyceride.

Experimental

Extraction of Parsley Seeds. Parsley seed *(Petroselinum sativum)* was obtained from the Burpee Seed Co., Clinton, Iowa.

a) *Whole Seeds.* A one hundred g batch of whole seeds was extracted four times in a 2-liter flask by refluxing with 1-liter portions of methanol-acetone 4:1 (v/v) for periods of six hr. The total extracted material was 5.9 g, I.V. (Wijs), 181. The extract, after removal of the nonsaponifiable portion, yielded only 0.16 g of fatty acids. Thus, the material extracted in this manner consisted of about 97% nonsaponifiable lipids. Thin-layer chromatography (TLC) (Figure 1A) showed that it consisted of five components. The major component was isolated and identified, as described below, as the phenolic ether, myristicin (5-allyl-1-methoxy-2,3-methylenedioxybenzene).

b) *Ground Seeds.* One hundred g of seed was ground to a fine powder in a porcelain ball mill and extracted four times in a 2-liter flask by refluxing with 1-liter portions of chloroform-methanol 2:1 (v/v) for six-hr. periods. The yield of material was 20.3 g (I.V. [Wijs], 115). After saponification, removal of the nonsaponifiables and acidification, 14.4 g fatty acids was obtained. The TLC of the nonsaponifiable fraction (5.4 g) (Figure 1E) was very similar to the methanol-acetone extract of the whole seed (Figure 1A).

Analysis of Parsley Seed Oil

Gas-liquid chromatography (GLC) of the various fractions described above and the derived methyl esters of the fatty acids of parsley seed oil was performed using an Aerograph instrument equipped with a thermal conductivity detector and a 6′ x ¼″ column packed with Chromosorb W containing 30% diethylene glycol succinate polyester at 200C.

The fatty acids of parsley seed oil consisted of a trace of palmitoleic, 5% palmitic, 82% octadecenoic (oleic and petroselinic), and 13% linoleic (Figure 2A). The GLC of the methanol-acetone extract of the whole seeds and the nonsaponifiable fraction (Figure 2, C and D, respectively) were very similar. Furthermore, the TLC and GLC analyses corresponded well, each showing four major components, with myristicin making up about 60% of the total. Figure 2B shows that methyl petroselinate and myristicin have almost the same retention time. From these data (Figure 2), it was estimated that about 14% of the total oil consisted of myristicin.

It is obvious that for the isolation of myristicin and/or petroselinic acid, an initial washing of the parsley seed with methanol-acetone should be carried out in order to remove practically all of the myristicin. The seeds should then be finely ground and extracted with chloroform-methanol to obtain the tri-

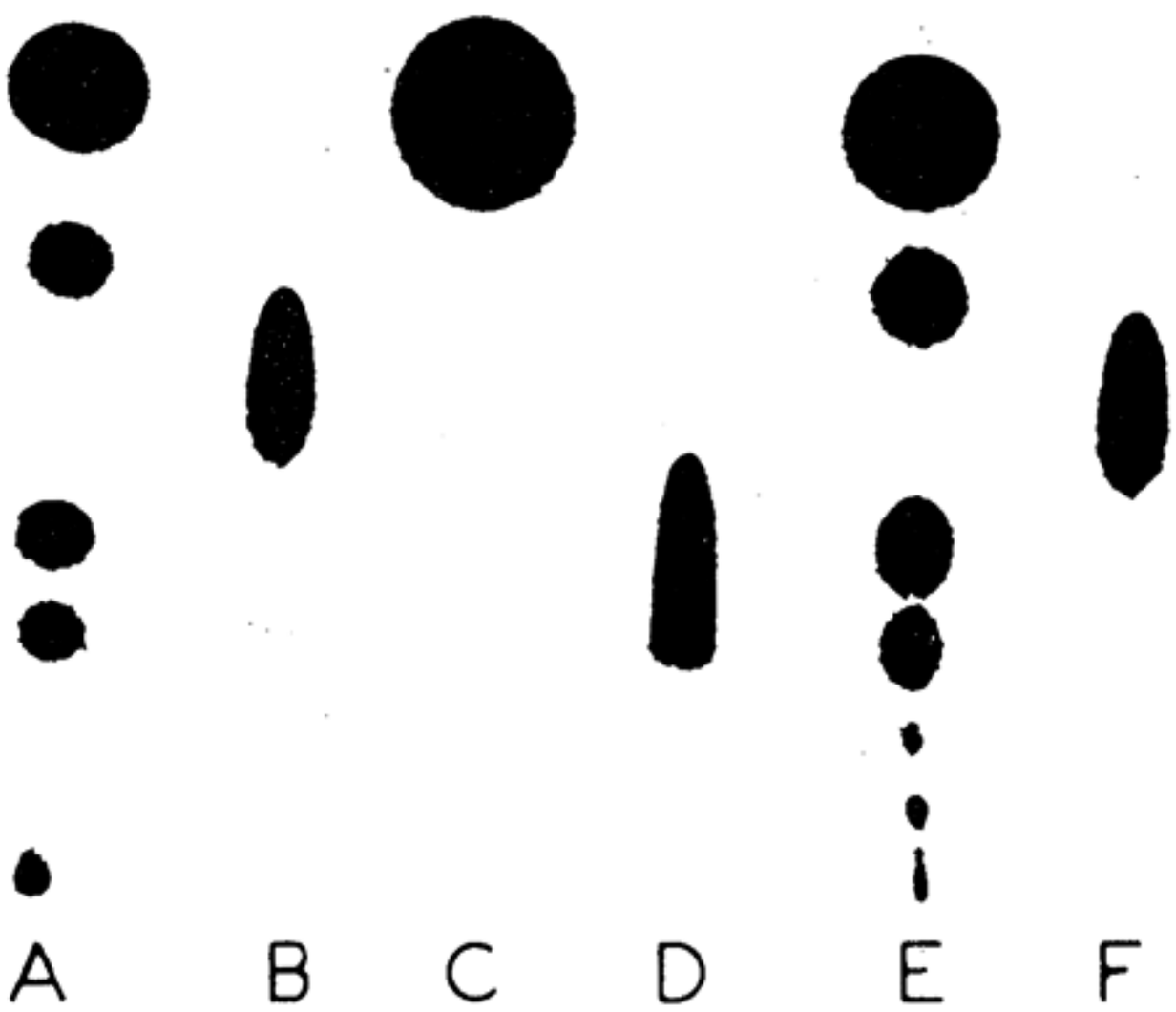

FIG. 1. Photostat of a thin-layer chromatoplate. A, nonglyceride extract of parsley seed; B, triolein; C, myristicin; D, oleic acid; E, nonsaponifiable material of parsley seed oil; F, triglyceride extract of parsley seed. Adsorbent: Silica Gel G, solvent system: petroleum ether (b.p. 30–60C), diethyl ether, acetic acid (90:10:1) (v/v/v). Indicator—charring with chromic-sulfuric acid solution.

[1] Supported in part by PHS Research Grant H-4601 from the National Institutes of Health, U.S. Public Health Service, and The Hormel Foundation.

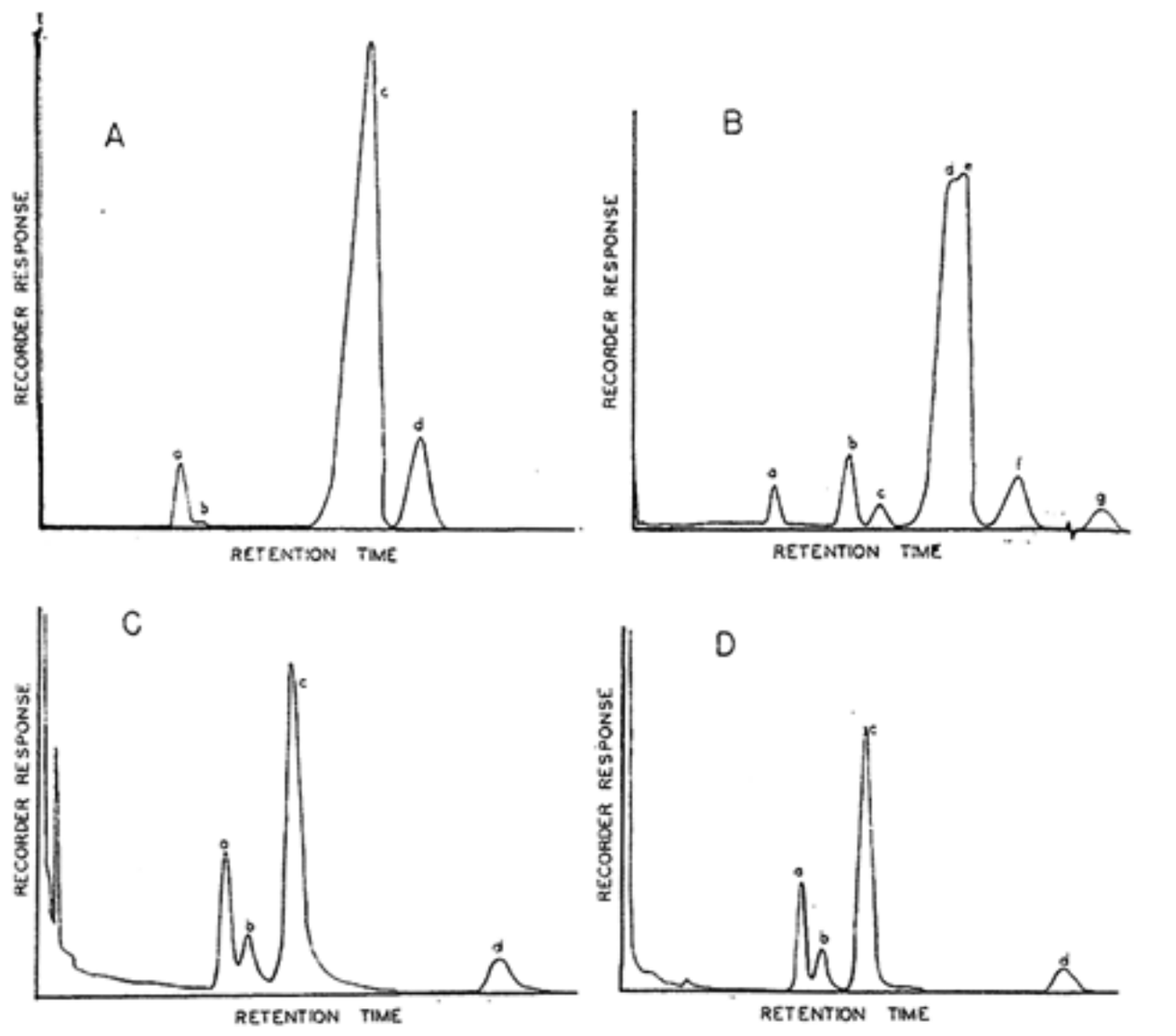

FIG. 2. GLC analysis of parsley seed oil constituents. A, Methyl esters of the fatty acids: a, palmitate, b, palmitoleate, c, oleate and petroselinate, d, linoleate. B, Nonsaponifiables plus methyl esters of the fatty acids: a, palmitate, b and c, unknown nonsaponifiable constituents, d, myristicin, e, oleate and petroselinate, f, linoleate, g, unknown nonsaponifiable constituent. C, Methanol-acetone extract of the whole seeds: a, b, d, unknown constituents, c, myristicin. D, Nonsaponifiable constituents: a, b, and d, unknown, c, myristicin.

glycerides which are virtually free of nonsaponifiable material.

Preparation of Myristicin and Petroselinic Acid

Myristicin. Myristicin was isolated from the nonglyceride fraction (extracted as described above) by low temp crystallization from petroleum ether, followed by fractional distillation. One part of the nonglyceride fraction was dissolved in 10 parts of petroleum ether, bp 30–60C (Skelly F) (v/v) and cooled to −30C. The crystalline precipitate was collected by filtration and recrystallized three times from petroleum ether at −30C. Fractional distillation of this material through a Podbielniak Hyper-Cal column yielded myristicin as the main fraction: bp 118C/2 mm; $n^{30/D} = 1.5380$; I.V. (Wijs), 258 (theo. 133.5 [addition]; 267 [addition plus substitution]).

Substitution of iodine is believed to occur at the 4,6 positions on the ring in accordance with the reaction of bromine on this compound (7). Elemental analysis: C: 68.65%, H: 5.95%, O (Direct): 24.98%. Calculated for $C_{11}H_{12}O_3$: C: 68.72%, H: 6.28%, O: 24.98%. These data are in accordance with the structure of myristicin, i.e., 5-allyl-1-methoxy-2,3-methylenedioxybenzene (6,7). The myristicin was shown to be pure by TLC (Figure 1C) and GLC analysis. This substance was further characterized as its bromide (dibromomyristicin dibromide), mp 128C in accordance with values reported in the literature (7). The infrared spectrum of myristicin is presented in Figure 3.

Petroselinic Acid. Approx 2 kg of triglycerides was obtained from seed, washed and ground as described above, and converted to methyl esters by interesterification with 3 liters of methanol and 9 g of metallic sodium. The methyl esters were recrystallized from 10 liters of acetone at −30C. The precipitate consisted mainly of methyl petroselinate and was collected by filtration. The filtrate contained most of the methyl oleate and linoleate. The crude methyl

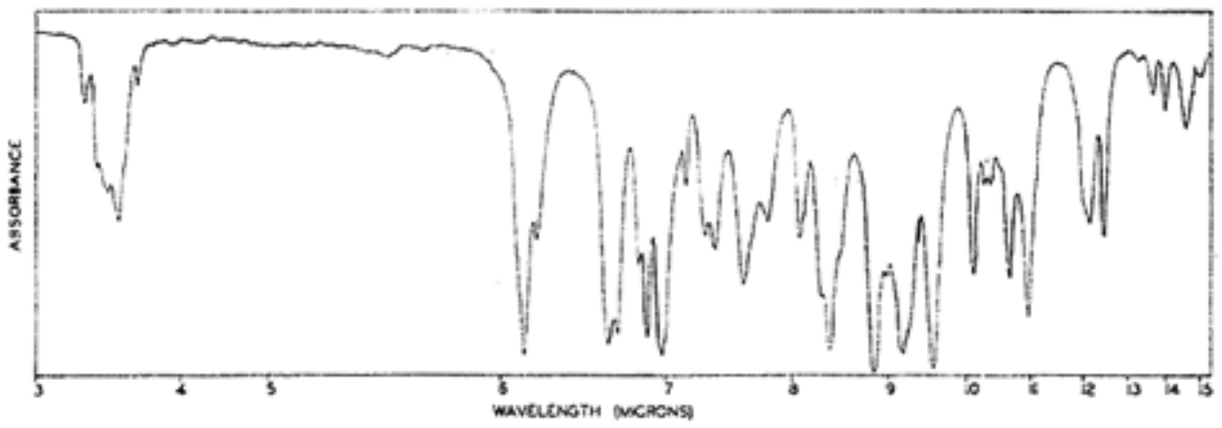

FIG. 3. Infrared spectrum of myristicin. 10% in CS_2 (680–1400 cm^{-1}, 2400–4000 cm^{-1}) and C_2Cl_4 (1400–2400 cm^{-1}).

petroselinate was further purified by repeated crystallization under identical conditions and by urea complex formation which removed all of the saturated esters. The methyl esters (approx 600 g) were recovered from the filtrate and distilled through a Podbielniak Hyper-Cal column. The C_{18}-fraction of the distillate consisted entirely of methyl petroselinate; the yield was about 500 g, which corresponded to approx one-third of the content of the seeds; bp 163C/2.5 mm; mp of petroselinic acid = 30C; $n^{30/D} = 1.4484$; I.V. (Wijs), 85.4 (theo. 85.6).

In order to determine the purity of the ester in regard to the position of the double bond, a 100-mg sample of the final product was ozonized (5). The aldehydes and half-ester aldehydes resulting from reductive ozonolysis were resolved by GLC in an F & M Model 609 instrument with a hydrogen-flame detector on a 12′ x ¼″ column containing 30% silicone in Chromosorb W.

Figure 4 shows that the final product consisted entirely of methyl 6-octadecenoate (methyl petroselinate). Fragments which could have been derived from methyl 9-octadecenoate (methyl oleate) could not be detected.

Discussion

Myristicin has been isolated from the nonsaponifiable fraction of parsley seed oil (1, 4), and it has also been synthesized (6, 7). We have demonstrated that the isolation of this compound is possible by selective extraction of total parsley seed with methanol-acetone. After removal of the myristicin and other nonglyceride constituents, virtually pure triglycerides can be obtained by extracting the thoroughly ground seed with chloroform-methanol.

Petroselinic acid is easily prepared from the triglycerides of parsley seed oil by the consecutive application of low temp crystallization of the fatty acid methyl esters, followed by urea complex formation

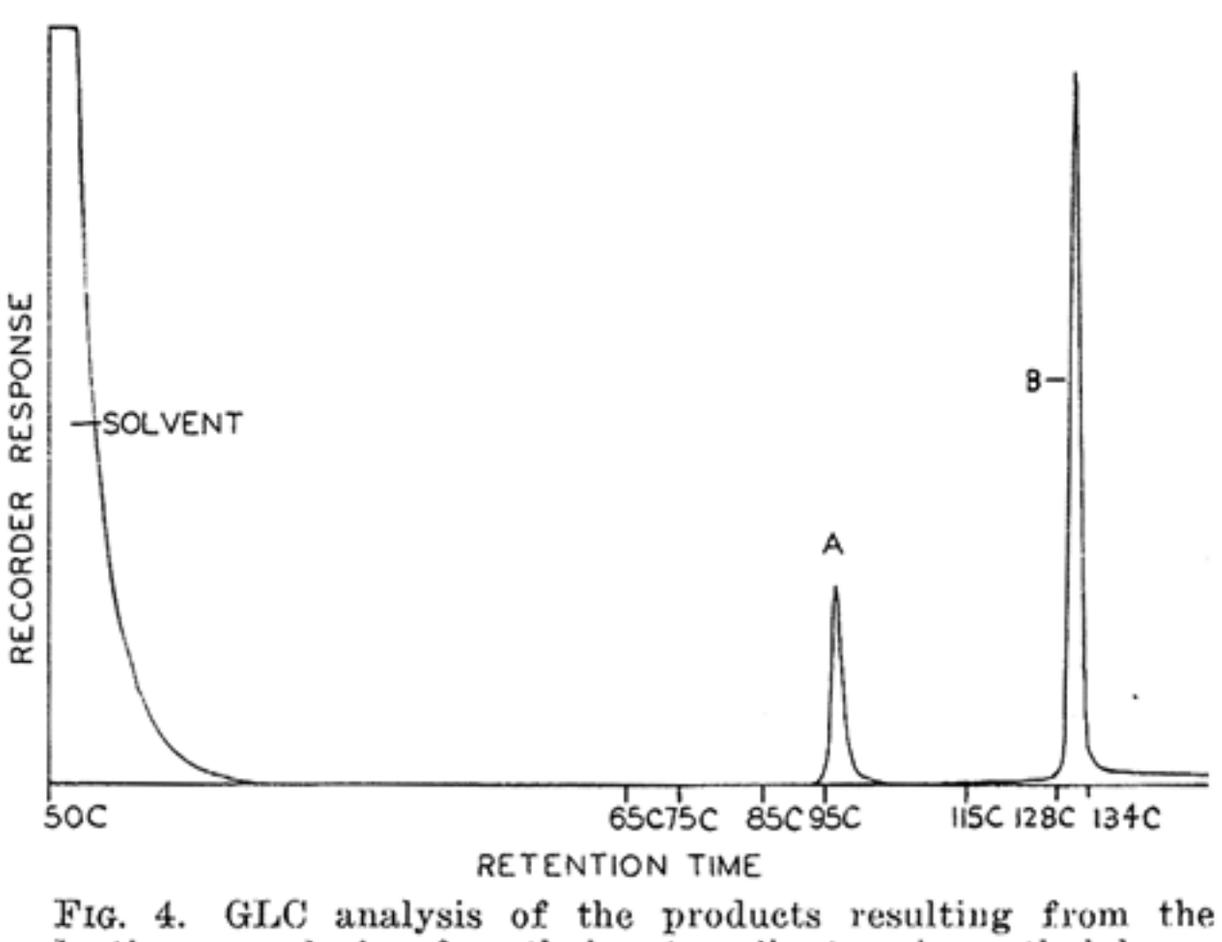

FIG. 4. GLC analysis of the products resulting from the reductive ozonolysis of methyl petroselinate. A, methyl hexanoate-6-al; B, dodecyl aldehyde.

and fractional distillation. A small amount of myristicin is collected in the forerun of the distillation; the C_{18}-fraction consists of pure methyl petroselinate.

In accordance with previously reported values (3), GLC analysis showed that the total lipid of coriander seed *(Coriandrum sativum)* contains about 50–55% petroselinic acid and only a small percentage of nonsaponifiable material. Therefore, it also constitutes a convenient source for the preparation of petroselinic acid.

Since the phenol ether, myristicin, overlaps in GLC analysis with methyl petroselinate and methyl oleate and has similar solubility properties, care must be exercised in the routine analysis and isolation of the "methyl esters" derived from seed oils. If the esters are prepared by methanolysis of the total oil without prior extraction of the nonsaponifiable material, certain peaks in the gas chromatogram may be interpreted erroneously as representing methyl esters of fatty acids.

Acknowledgment

The authors wish to thank E. Christense Nickell for her technical assistance.

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[Received April 20, 1962]