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VOLATILE CONSTITUENTS IN LEAVES OF PARSLEY

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Key Word Index—*Petroselinum sativum*; Umbelliferae; parsley leaves; essential oils; volatile constituents.

Abstract—Volatile chemicals obtained from the leaves of parsley, *Petroselinum sativum* by steam distillation, isopentane extraction, and head-space analysis were identified by GLC–MS. The presence in leaf oil of α-pinene, β-pinene, myrcene, β-phellandrene, *trans*-β-ocimene, γ-terpinene, 1-methyl-4-isopropenyl benzene, and 1,3,8-*p*-menthatriene as shown by earlier investigators was confirmed. In the present studies, the number of volatile chemicals detected in the leaves was extended by an additional 42. Sniffing tests of effluent from a gas chromatograph of a concentrate from parsley leaves showed that 1,3,8-*p*-menthatriene was only one of several compounds that gave a parsley-like aroma.

INTRODUCTION

PARSLEY, *Petroselinum sativum* Hoffm. (Umbelliferae), a biennial plant used widely as fresh green sprigs to garnish and aromatize foods, is grown extensively in North America and Europe[1] and used considerably in Sweden. The seeds of parsley may be steam-distilled to give an essential oil, which can be used as a spice, but it is only a substitute for the more expensive herb or leaf oil.[2] The chemical composition of the seed oil has been studied for many years by classical chemical methods and more recently by GLC.[3] We have found only one definitive study,[4,5] which was of a commercial leaf oil from Yugoslavia. GLC analyses showed the presence of about 30 volatile chemical components of which 8 were identified by IR spectrometry including a previously unknown substance identified as 1,3,8-*p*-menthatriene.[5] Five additional compounds were tentatively identified by retention times.

Concentration techniques and combined GLC–MS have had extensive application in this laboratory for the identification of volatile aroma compounds in food products, such as berries,[6,7] leaves,[8] and bread.[9] In the present study the methods were applied to green leaves of parsley, grown under known conditions in Sweden, so that results could be compared with the earlier work. Since only 8 chemical components were identified earlier[4,5] it was hoped that the methods of analyses used might extend the list of the known volatile constituents.

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* On transfer-of-work leave from: Canada Department of Agriculture, Research Station, Lethbridge, Alberta, Canada.

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RESULTS

Volatile compounds that were identified in head-space vapors from the high-vacuum distillate of parsley leaves are listed in Table 1 in order of increasing retention time from the GLC column. No attempts were made to record MS of all compounds because in certain regions of the chromatogram the amounts of volatile compounds were too large to be safely admitted into the ion-source of the mass spectrometer. Thus, many major components that could be found in the head-space vapors were not identified there, but in steam-distillation concentrates. Some compounds with low boiling points, which were identified in head-space vapors, were not detected in the concentrates obtained by steam distillation, presumably because during the processing they were lost by volatilization.

TABLE 1. VOLATILE COMPOUNDS IDENTIFIED IN THE HEAD-SPACE VAPORS FROM HIGH-VACUUM DISTILLATE OF PARSLEY LEAVES

Acetaldehyde*
Ethyl chloride*
Methanol*
Isopentane*
Acetone*
Diethyl ether
n-Pentane*
Dimethyl sulfide*
Carbon disulfide*
Dichloromethane*
Chloroform*
Benzene*
Trichloroethylene*
Dimethyl disulfide*
Toluene
trans-2-Hexenal
Ethyl benzene*
m- and/or *p*-Xylene
Styrene*
o-Xylene*
n-Nonane*
α-Thujene
α-Pinene
Camphene
Sabinene
Naphthalene
Tridecane*
α-Cubebene*
α-Copaene
Caryophyllene

* Identified only in head-space vapors from high-vacuum distillate.

TABLE 2. CONSTITUENTS IN CONCENTRATES OBTAINED FROM PARSLEY LEAVES BY STEAM DISTILLATION OR ISOPENTANE EXTRACTION

Peak No.	Identity	Peak No.	Identity
8	Toluene	37/38	1-Methyl-4-isopropenylbenzene + terpinolene
9	*trans*-2-Hexenal	41	1,3,8-*p*-Menthatriene
11	*m*- and/or *p*-Xylene	49a	Naphthalene
14	Cumene	51	α-Terpineol
15	α-Thujene	57	2,4-Dekadienal
16	α-Pinene	60	α-Copaene
17	Camphene	62	β-Elemene
21	Sabinene	65	Caryophyllene
23	β-Pinene	66	γ-Elemene
24	Myrcene	72	α-Cadinene
26	α-Phellandrene	78	Myristicin
29	*p*-Cymene	87	Carotol
30	β-Phellandrene	95	Apiole
31	*trans*-β-Ocimene	98	Benzyl benzoate
33	γ-Terpinene		

Major components are myristicin (RT 78·5 mins), 1,3,8-*p*-menthatriene (42·0 min), β-phellandrene (35·0 min), myrcene (31·0 min) and the mixture of 1-methyl-4-isopropenylbenzene and terpinolene (39·5 min).

A representative gas chromatogram of the steam-distillation concentrate from parsley leaves showed over 100 components. Chemical components that were identified are listed in Table 2. The compounds present in largest quantities and in decreasing order of concentration were: myristicin No. 78, 1,3,8-*p*-menthatriene No. 41, β-phellandrene No. 30, myrcene No. 24, and peak No. 37/38. The latter peak contained 2 compounds that were not always separated by GLC. Nonetheless, the substances were positively identified by mass spectrometry as 1-methyl-4-isopropenyl benzene and terpinolene. It was estimated, from some chromatograms that did show separations, that these two compounds were present in a ratio of 2:3.

The concentrate, obtained by isopentane extraction, contained almost the same constituents as those found by the steam-distillation procedure. The obvious difference between the two processes were the substantial quantities of the unknown peak No. 100 in the isopentane extract and its near-absence in the steam distillate. In addition toluene and benzyl benzoate were not detected in the isopentane concentrate.

Comparison of concentrates prepared by either the isopentane-extraction or the steam-distillation methods for their contents of 21 major components, as measured by GLC (unpublished), showed no significant differences between the two methods. However, the quantitative comparison indicated certain trends. For compounds Nos. 9–50 inclusive and including myristicin, No. 78, values tended to be higher for the steam-distillation method. On the other hand, for compounds Nos. 62–100 the quantities were higher for the isopentane-extraction method.

The precision of the results from the steam-distillation concentrates as measured by the coefficient of variability was 16·2%, a value which was smaller ($P < 0{\cdot}05$) than the mean

TABLE 3. THE INTENSITY AND QUALITY OF ODOUR COMPONENTS FROM THE STEAM DISTILLATE OF PARSLEY LEAVES

Peak No.	Component	Odour intensity	Odour description
12	Unknown	3	Green, nasty
16	α-Pinene	2	Lumber-yard
24	Myrcene	4	Pungent, myrcene-like
30	β-Phellandrene	5	Spruce-resin, turpentine, pungent, parsley-like
37/38	Terpinolene plus 1-methyl-4-isopropenylbenzene	4	Burnt, after-fire, parsley-like
41	1,3,8-*p*-Menthatriene	5	Pungent, sweet, cooling, minty, eucalyptus, parsley-like
47,48,48a	Unknown	3	Green, sweet, pungent, burnt, hay
51a,52	Unknown	3	Fruity, sweet
58	Unknown	3	Green, metallic, minty
62	β-Elemene	3	Green, metallic, minty
77	Unknown	4	Fragrant, heavy, aromatic
78	Myristicin	5	Warm, snuff, pungent, burnt

of 25·2% for the isopentane extraction concentrates. For both the isopentane-extraction and the steam-distillation methods the average coefficients of variability for the compounds present in quantities of more than 1 ppm fresh wt were not different ($P > 0{\cdot}05$) for those below 1 ppm.

Table 3 shows qualitative descriptions and intensities of the aroma of some of the peaks representing compounds separated by GLC for a concentrate steam distilled from parsley leaves. No single peak was described as being uniquely parsley-like; however, the aroma of 3 of the 6 components that had strong (4) to very strong (5) odour intensities was reminiscent of parsley.

DISCUSSION

About 50 volatile chemicals were identified in the head-space vapors from the high-vacuum distillate and the concentrates obtained by steam distillation or isopentane extraction from parsley leaves. These results confirm and extend the earlier studies of parsley leaf oil produced under commercial conditions in Yugoslavia. Although our analyses were obtained from parsley leaves grown in southern Sweden, the results show, as far as can be determined, a great similarity to the Yugoslavian oil. Both oils contained α-pinene, β-pinene, myrcene, β-phellandrene, *trans*-β-ocimene, γ-terpinene, 1-methyl-4-isopropenylbenzene, and 1,3,8-*p*-menthatriene. However, there were some differences, since limonene and *cis*-β-ocimene occurred in the Yugoslavian but not in the Swedish oil. Oil from parsley seed contains both of these substances and since there may have been seed in the leaf material, contamination of the leaf oil could have occurred. The present results extend the number of volatile chemicals from the 8 found by the French investigators[4,5] by an additional 42. Twenty of these were identified with the head-space technique and the remainder were found in the steam-distillation concentrate.

The chemicals found in the concentrates from parsley leaves are not novel compounds nor are they peculiar to this plant. They occur widely in essential oils[2] and particularly in oils from other members of the Umbelliferae, such as celery[10] and carrots.[11] However, the compound 1,3,8-*p*-menthatriene, which was found previously in parsley leaves by Garnero *et al.*[4] seems to be unique to this species. Most of the other volatile compounds found in the vapors from the high-vacuum distillate that were analysed by the head-space technique occur in other plant materials.[6,12,13] Although naphthalene and benzene have been reported in plants they could arise from contaminated air or soil.[14,15] The extensive use of diethyl ether and isopentane in the laboratory for extractions leaves little doubt that the environment was the source of these compounds. The apparent presence of trichloroethylene, chloroform, and carbon disulfide in parsley leaves is suspect because these compounds appeared in the head-space vapors of many other products that were analysed in this laboratory. Furthermore, halogen compounds have particularly characteristic mass spectra that are easily and, therefore, probably more frequently recognized than the spectra of other compounds in complicated mixtures.

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Similarities between the composition of the oils produced by the different methods of steam distillation and isopentane extraction indicate that few compounds were produced solely by the isolation procedures. Relatively large quantities of *trans*-2-hexenal were obtained in the steam-distillation concentrate but only traces were found in the isopentane-extraction concentrate. Since this substance is produced extensively from green plant material in the presence of air during distillation[16] it is likely that this compound was an artefact formed during the steam-distillation process. Nonetheless, the occurrence of *trans*-2-hexenal in the vapor of the high-vacuum distillate and in the concentrate from isopentane extraction suggests that at least some of the compound occurred naturally in the leaves. Head-space vapors from the high-vacuum distillate contained some volatile substances that were not detected in the other concentrates. In preparing concentrates from the isopentane extracts or steam distillates of the leaves the distillation procedures used doubtlessly caused some losses of the more volatile compounds.

For studies of the volatile chemical components in parsley leaves the steam distillation method was favoured over the isopentane extraction method because the former gave greater recovery of low boiling constituents, was simpler and faster, and in general was more precise. Our sniffing tests of effluent from gas chromatography of a concentrate from parsley leaves showed that 1,3,8-*p*-menthatriene was only one of several compounds that gave a parsley-like aroma. Garnero *et al.*[4] also described 1,3,8-*p*-menthatriene as having the odour of parsley leaves; the present results suggest that the aroma of parsley leaves is caused by a mixture of at least 3 naturally occurring volatile compounds.

EXPERIMENTAL

Plants. Plants were grown in a field on a uniform, sandy marine soil in southern Sweden. Seeds were sown at standard rates on 27 May in blocks about 3·0 m wide and 5·0 m long, each containing 8 rows. The plants were collected 14 October before the first autumn frost when about 30 cm high. Only centre rows, with plants at each end excluded, were harvested. The plants were excised 2–3 cm above the soil; yellow and crushed leaves were discarded. The remaining leaves were wrapped tightly with Al foil in bundles of about 1 kg and immediately packed in dry ice in insulated boxes for storage and transportation, which required about 1 day. Finally the bundles were stored at −40° for up to 4 months before being examined. Leaves from the parsley variety Extra Mosskrusig Hg (*Petroselinum sativum* Hoffm.) were used to provide the concentrates that were analysed qualitatively. Leaves of parsley, variety Bravour were used to quantitatively compare the isopentane-extraction and steam-distillation concentration methods.

Isolation and concentration of the volatile components from leaves. The following 3 procedures were used to prepare concentrates of the volatile compounds present in the green frozen leaves of parsley obtained from different packages: (a) *Isopentane extraction.* Leaves were homogenized and extracted in a Sorvall 'Omnimixer' modified by replacement of the bronze bearings and rubber gasket with Teflon parts. Air was flushed from the jar and solvents were added through 2 small Teflon-stoppered holes in the lid. 50 g of frozen leaves were crushed and homogenized in a jar with 250 ml of freshly boiled H_2O at 0° in a He atmosphere for 15 min. Chilled isopentane (150 ml) was added to the mixture, which was stirred for 90 min at about 0·5°. The slurry was centrifuged for 5 min at 1000 *g* and 0·5° and then the isopentane layer was decanted. An additional second and third centrifugation gave a 90–95% recovery of isopentane. The mixing and centrifugation was repeated with 100 ml of isopentane. In separate experiments it was determined that pretreatment of parsley leaves for 24 hr with commercial pectinases did not increase the recovery of isopentane. Furthermore, a third isopentane extraction recovered only negligible amounts of volatile substances, therefore only 2 extractions were made. The combined extracts were dried and stored overnight in a refrigerator. Isopentane was distilled from the extract in a Vigreux column at a reflux ratio of about 12:1 to give a final volume of 1–2 ml. The concentrate was transferred with 1–2 ml Et_2O to a Markham-type apparatus and rapidly steam-distilled. Distillate (50 ml) was collected in an ice-cooled separatory funnel that contained 12 g of NaCl and 5·0 ml of Et_2O. The volatile compounds were extracted into ether by careful shaking. The ether was decanted and a second extraction was made with 10·0 ml of Et_2O. The extracts were bulked and dried overnight and concentrated to about 1 ml by distillation in a small Vigreux column at a reflux

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ratio of 15:1. The concentrate was reduced further to about 100 μl in a microdistillation column similar to one described by Murray *et al.*[17] The concentrate was transferred with ether to a glass ampoule, made to a known volume, heat sealed, and stored at −20° for analyses. (b) *Steam distillation.* Leaves were steam distilled in a specially-made apparatus, in which the material was packed in a well insulated tower separated into four compartments by PTFE perforated plates. Steam was passed in from below. A sample, usually consisting of 50 g of frozen leaves, was distributed evenly throughout the 4 chambers of the apparatus. Distillate was collected in an ice-cooled separatory funnel, which contained 25 g of NaCl and 10 ml Et_2O. The steam generator, containing preheated and boiled H_2O, was attached and steam was quickly passed through the leaves. When drops of distillate appeared in the condenser the rate of distillation was reduced to 2–3 drops/sec. After 150 ml of distillate was collected the condenser was rinsed with a few ml Et_2O. The rinsings and the original Et_2O in the collector were vigorously shaken with the distillate and decanted. A second extraction with another 10 ml Et_2O was done. The combined extracts were dried, concentrated and stored as described for the isopentane extraction concentrate. The identifications of volatile constituents were done on concentrates prepared by the isopentane extraction of 300 g (6 × 50 g) of leaves and steam distillation of 150 g of leaves. (c) *High-vacuum distillation.* A speciallly made glass freeze-drying apparatus was used to obtain a high-vacuum distillate from the parsley leaves. Frozen leaves (131 g) were placed in a wide-mouth, 5 l., round bottomed flask and distilled for 7 hr at 30 μ to a final temperature of 20°. Most of the volatile compounds and H_2O were condensed on a 'cold-finger' trap that was cooled with dry ice in EtOH. Volatile components that passed the 'cold-finger' were condensed in traps cooled with liquid N_2 and, after the leaves were removed, back-distilled to the 'cold-finger' trap. The frozen distillate (80 ml) was melted, cooled, and stored at 0° and used to provide the head-space vapors which were collected in the pre-column concentration technique described[6] previously for analyses by either GLC or GLC–MS.

Gas chromatography. A gas chromatograph with an injector-splitter and a flame ionization detector was used for all analyses. An open tubular column, 103 m × 0·5 mm i.d. coated with 95% SF 96 + 5% Igepal CO-880 to give a film thickness of 0·7 μ was used for the analyses. Temperature was programmed from 65 to 175° at 2°/min after an initial isothermal period of 3 min. The carrier gas was He at a flow rate of 6·1 ml/min at 25°. Usually 2 μl of concentrate in Et_2O were injected with a split ratio of 1:13·5. The volatiles from the high-vacuum distillate were chromatographed on an open tubular column 105 m × 0·76 mm i.d. with the same phase as above. Temperature was programmed from 20 to 140° at 2°/min with an initial isothermal period of 8 min at 20°; He flow rate of 11 ml/min; 20 ml of distillate provided a 50·0-ml sample of head-space gas at 25°. Peak heights or integrated areas of peak were used for any quantitative comparisons.

Combined GLC–MS. All concentrates were analysed in an LKB 9000 GLC–MS instrument adapted to open tubular columns. The columns and temperature programs were the same as those described for the gas chromatography analyses. A make-up gas was added after the column to give a flow rate of 30 ml/min into the jet molecular separator. Because some compounds were decomposed at the normal separator temp. of 200°, additional analyses were made at 140° for the separator and connecting lines. Ion-source temperature was 270° and all spectra were recorded at 70 eV. MS were taken usually at the front, maximum, and tail of a chromatographic peak. Compounds were identified by comparing their spectra with reference spectra that had been recorded under similar conditions with the same instrument and with published spectra. Retention times also were used to assist in making identifications.

Odour assessment. The quality and intensity of odours for gas chromatography peaks of concentrates that were obtained by the steam-distillation method were measured as described.[6] Four experienced judges classified and quantitatively estimated the odours. The intensity of odours was measured on a scale ranging from no odour (0) to very strong (5). A comparison of the quantities of the 21 compounds found in the largest concentrations was made on 4 packages of parsley leaves for the isopentane extraction method and on 3 packages for the steam-distillation method.

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