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SHORT COMMUNICATION

COMPOSITION OF THE VOLATILES OBTAINED BY STEAM DISTILLATION OF *QUERCUS ILEX* L. LEAVES

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ABSTRACT

The composition of holm oak (*Quercus ilex* L.) volatiles obtained by steam distillation of leaves was investigated with gas chromatography and gas chromatography/mass spectrometry. The volatile fraction amounted to 0.01 % of fresh leaves. 112 components were separated, of which 79 (comprising 85.0 % w/w of the sample) were positively identified and further 6 partially characterized. Fourty six substances could be assigned to terpenoids: 12 monoterpenes (3.6 %), 26 sesquiterpenes (47.6 %), β-cyclohomocitral (0.1 %), 6 C_{13}-norisoprenoids (1.1 %) and the diterpene phytol (0.2 %). A part of the detected substances like n-alkanes (8.0 %), 1-alkenes (2.5 %) and free fatty acids (2.3 %) originate from epicuticular lipids. Aldehydes (23.2 %) included short chain n-alkanals (C_6–C_9) and the most abundant single compound *trans*-2-hexenal (18.1 %). Terpene analysis is expected to be a valuable tool not only for characterizing populations of conifer species but also for oak provenances.

Key words: *Quercus ilex* L., steam distillation, volatiles, terpenoids

INTRODUCTION

The holm oak, *Quercus ilex* L., is the most common oak species in the Mediterranean area. On the basis of its wide natural distribution and the morphological variability *e.g.* of the leaf shape, significant genetic variation within *Quercus ilex* could be expected. An improved understanding of genetic differences between and within populations of holm oak could be valuable from the systematic and genetic points of view, and could also yield practical benefits.

A method which has proven to be applicable to the study of geographic variation within species, as well as possible hybridization and introgression between closely related species is the analysis of terpenes in various plant tissues by gas chromatography. This method has been successfully applied to characterize populations of conifer species (*e.g.* VON RUDLOFF 1975, LANG 1994, GERBER *et al.* 1995). However, so far only few oak species have been investigated with regard to the essential oil of their leaves: *Quercus agrifolia* Née (PALMA-FLEMING & KEPNER 1983), *Q. dentata* Thunb. (HEGNAUER 1989) and *Quercus robur* (ENGEL *et al.* 1993). Recently, holm oak (*Quercus ilex* L.) was shown to emit high amounts of monoterpenes and traces of isoprene (STAUDT & SEUFERT 1995, KESSELMEIER *et al.* 1996). However, data about the essential oil obtained by steam distillation are not available. In this paper the composition of holm oak volatiles obtained by steam distillation of entire leaves is reported. The present investigation precedes a study of the geographical variation of *Q. ilex* in Croatia where the volatiles will be analyzed by gas-liquid chromatography.

MATERIALS AND METHODS

Plant material and sample preparation

Leaf samples were collected from an approximately 20 years old holm oak tree (*Quercus ilex* L.) at Posedarje (Latitude 44°13' N, Longitude 15°27' E) 20 km NE of Zadar, Croatia. 70 g entire holm oak leaves were steam distilled for 6 hours. Volatiles were collected into 1.0 ml n-pentane. The yield of the volatile substances was calculated from a parallel sample to which a known amount of n-dodecane as internal standard was added.

Gas chromatography (GC) and GC/Mass spectrometry analysis

The volatiles were analyzed by GC with a flame ionization detector (FID) and by GC/mass spectrometry (Figure 1). A number of GC columns was used, but mainly fused silica capillary columns containing either bonded-phase Db-1 and CP Sil 5 CB (equivalent to OV 101) or Db-wax (equivalent to PEG 20M). Most components were identified by GC/MS with automatic peak identification using the Wiley275 data base. As an additional criterion of identification, retention indices according to VAN DEN DOOL & KRATZ 1963 were compared with those published in the literature (DAVIES 1990, ENGEL *et al.* 1993). Quantitative data given in Table 1 were obtained from GC-FID profiles using a 50 m fused silica column CP Sil 5 CB.

RESULTS

The volatile fraction obtained by hydrodistillation amounted to 0.01 % of fresh leaves. Quantitative data given in Table 1 were obtained from GC-FID profiles using a 50 m fused silica column CP Sil 5 CB. Overall, 112 components were regularely detected as volatiles of holm oak leaves, of which 79 (comprising 85.0% w/w of the sample) were positively identified, with a further 6 (8.5 % w/w) partially characterized. The 27 (6.5 % w/w) unidentified components are not included in Table 1. They were present in the sample in such low amounts that their mass spectra were too poor for interpretation.

According to their chemical structure the volatiles can be divided into different substance classes: Fourty six substances could be assigned to terpenoids, amounting to 52.6 % alltogether. Main constituent of the 12 monoterpenes (3.6 %) was (-)-α-terpineol (# 19, 1.3 %; # = peak number). Sesquiterpenes (47,6 %) comprised 20 fully identified and further 6 partially characterized compounds with α-copaene (# 27, 1.6 %), β-bourbonene (# 29, 8.8 %), aromadendrene (# 37, 2.7 %), germacrene-d (# 39, 1.0 %), (+)-ledene (# 40, 1.4 %), palustrol (# 44, 1.3 %), viridiflorol (# 48, 14.8 %), (+)-ledol (# 50, 1.8 %) and α-eudesmol (# 56, 1.4 %) as main constituents with more than 1 % of the oil. Minor terpene constituents were β-cyclohomocitral (# 21), six C_{13}-norisoprenoids (# 23, # 24, # 26, # 28, # 31, # 32), and the only diterpene compound found in the holm oak volatiles, phytol (# 64, 0.14 %).

The second main substance class of holm oak leaf volatiles were n-alkanes (8.0 %) and their derivatives 1-alkenes (2.5 %), aldehydes (23.2 %), free fatty acids (2.3 %) and fatty acid methyl esters (1.6 %). n-Pentacosane (# 74, 1.3 %), n-heptacosane (# 78, 1.9) and n-nonacosane (# 82, 1.2 %) were the most abundant compounds of even and odd-numbered n-alkanes (C_8, C_{16}–C_{18}, C_{21}–C_{31}). 1-alkenes (C_{22}–C_{30}) were present in concentrations up to 0.6 %. Aldehydes included n-alkanals (C_6–C_9, 5.1 %) and the most abundant single compound trans-2-hexenal (# 3, 18.1 %). Even numbered free fatty acids (C_{12}, C_{14}, C_{16}) amounted to 2.3 % together and the methyl ester of linolenic acid (# 65) to 1.6 %.

Further substances were 1-octanol (# 13), the phenols methylsalicylate (# 18), eugenol (# 22) and cis-isoeugenol (# 33), the C_{18} compound 6,10,14-trimethyl-

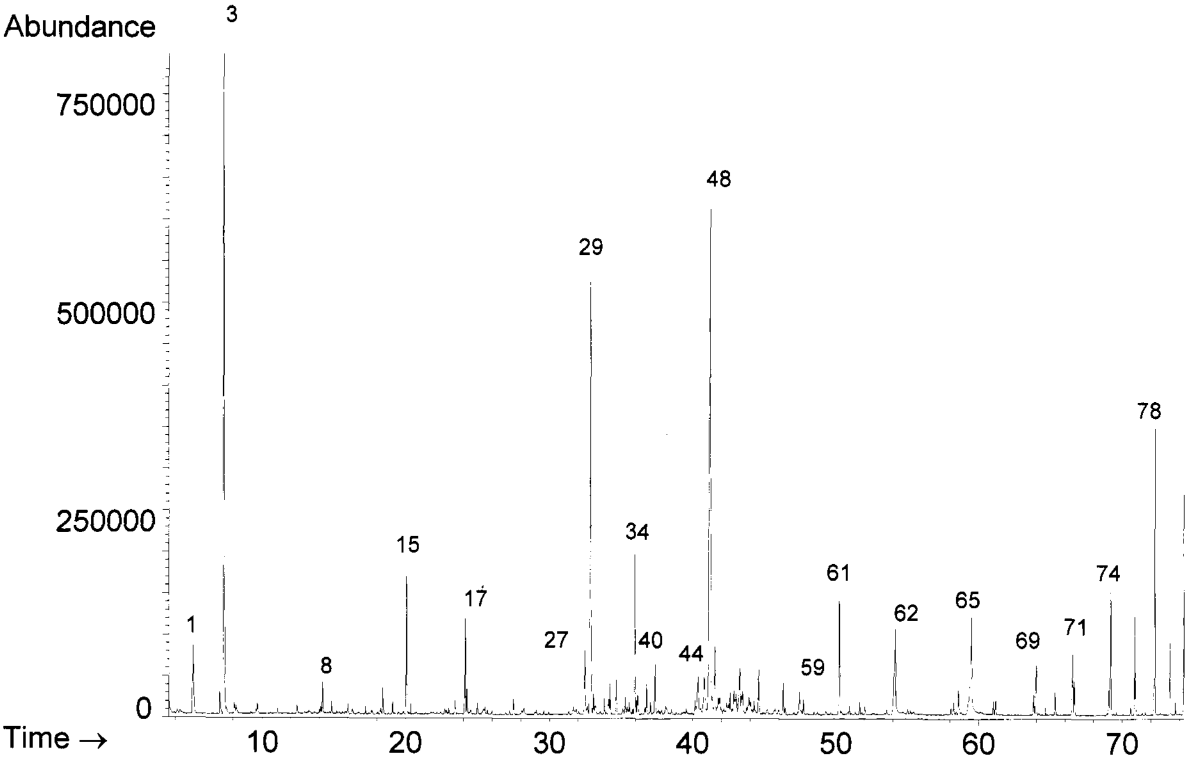

Figure 1. Typical total ion chromatogram (TIC) of holm oak leaf volatiles on HP-5 capillary column. See Table 1 for identification of the major peaks.

Table 1. Volatile compunds in distillates of *Quercus ilex* leaves. Linear retention indices and quantification according to the Area Percent Method for all compounds on the apolar column.

Peak	Compound	Retention index	Area [%]
1	Hexanal	784	3.21
2	Octane	803	0.14
3	*trans*-2-Hexenal	831	18.06
4	Heptanal	887	0.14
5	α-Pinene	929	0.15
6	Camphene	941	0.11
7	β-Pinene	981	0.15
8	Myrcene	985	0.53
9	Octanal	988	0.15
10	p-Cymene	1014	0.10
11	Limonene	1021	0.21
12	γ-Terpinene	1040	0.16
13	1-Octanol	1059	0.12
14	α-Terpinolene	1079	0.35
15	Nonanal	1089	1.60
16	endo-Borneol	1149	0.14
17	4-Terpineol	1162	0.12
18	Methylsalicylate	1171	0.43
19	(-)-α-Terpineol	1174	1.32
20	β-Cyclocitral	1199	0.27
21	β-Cyclohomocitral	1244	0.14
22	Eugenol	1334	0.13
23	1,2-Dihydro-1,1,6-trimethylnaphtalene	1341	0.06
24	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene	1345	0.12
25	α-Cubebene	1355	0.09
26	2-(1,3-Butadienyl)-1,3,5-trimethylbenzene	1360	0.15
27	α-Copaene	1370	1.60
28	6,10-Dimethyl-2-undecanone	1373	0.13
29	β-Bourbonene	1377	8.81
30	Bourbonen-isomere	1380	0.96
31	β-Damascone	1382	0.44
32	β-Ionone	1403	0.14
33	cis-Isoeugenol	1409	0.73
34	Sesquiterpene hydrocarbon	1420	1.09
35	*trans*-Caryophyllene	1423	0.31
36	Sesquiterpene hydrocarbon	1434	1.46
37	Aromadendrene	1450	2.73
38	α-Amorphene	1466	0.33
39	Germacrene-D	1469	0.96
40	(+)-Ledene	1485	1.43
41	γ-Cadinene	1506	0.21
42	δ-Cadinene	1512	0.24
43	α-Calacorene	1535	0.12
44	Palustrol	1553	1.33
45	Dodecanoic acid	1558	0.20
46	Sesquiterpene hydrocarbon	1563	0.52
47	Caryophyllene oxide	1569	0.83
48	Virdiflorol	1576	14.84
49	oxygenated Sesquiterpene	1578	3.51
50	(+)-Ledol	1586	1.78
51	Hexadecane	1600	0.26
52	Hinesol	1613	0.50
53	γ-Eudesmol	1618	0.65
54	Isospathunelol	1623	0.46
55	β-Eudesmol	1629	0.57
56	α-Eudesmol	1635	1.36
57	oxygenated Sesquiterpene	1666	0.93
58	n-Heptadecane	1702	0.12
59	Tetradecanoic acid	1750	0.34
60	n-Octadecane	1802	0.16
61	6,10,14-Trimethyl-2-pentadecanone	1833	1.47
62	Palmitic acid	1949	1.76
63	n-Heneicosane	2093	0.14
64	Phytol	2101	0.20
65	9,12,15-Octadecatrienoic acid methyl ester	2117	1.64
66	1-Docosene	2193	0.14
67	n-Docosene	2201	0.23
68	1-Tricosene	2293	0.21
69	n-Tricosene	2301	0.61
70	4,8,12,16-Tetramethyl-heptadecan-4-olide	2326	0.29
71	1-Tetracosene	2393	0.62
72	n-Tetracosene	2401	0.56
73	1-Pentacosene	2493	0.23
74	n-Pentacosene	2500	1.25
75	1-Hexacosene	2592	0.57
76	n-Pentacosene	2600	0.50
77	1-Heptacosene	2693	0.14
78	n-Pentacosene	2700	1.90
79	1-Octacosene	2793	0.33
80	n-Octacosene	2800	0.38
81	1-Nonacosene	2893	0.05
82	n-Nonacosene	2900	1.19
83	1-Triacontene	2994	0.17
84	n-Triacontene	3000	0.27
85	n-Hentriacontane	3098	0.37

2-pentadecanone (# 61, 1.5 %) and the C_{21} compound 4,8,12,16-tetramethylheptacecan-4-olide (# 70) as minor constituents.

DISCUSSION

Distillation of holm oak (*Quercus ilex*) leaves afforded 0.01 % essential oil. The content of essential oil of fresh leaves is in the same order of magnitude as that of fresh *Quercus robur* leaves (0.025 %, ENGEL *et al.* 1993) and of *Quercus agrifolia* leaves (0.02 %, PALMA-FLEMING & KEPNER 1983). During this study 112 compounds were detected in holm oak leaf oil, of which 79 could be positively identified. Generally, the composition of holm oak essential oil resembles very much to that of *Quercus robur*, where 184 components could be detected and 155 of them could be identified (ENGEL *et al.* 1993). The essential oil of *Q. agrifolia* contained ca. 105 volatile compounds, but only 15 of them could be identified and 3 tentatively identified; the majority of the substances were present in amounts too small to permit identification (PALMA–FLEMING & KEPNER 1983). In *Q. dentata* leaf oil 60 compounds were found (HEGNAUER 1989).

The highest concentration in the steam distillate of *Quercus ilex* leaves had *trans*-2-hexenal. Striking high amounts of *trans*-2-hexenal were also detected in leaves of *Quercus agrifolia* (PALMA–FLEMING & KEPNER, 1983) and *Q. robur* (ENGEL *et al.* 1993).

Long chain alkanes and their derivatives are constituents of epicuticular lipids as it was shown for *Q. robur* leaves (PRASAD *et al.* 1990, GÜLZ & MÜLLER 1992). They usually occur in leaf oils obtained by hydrodistillation in different yield depending on the duration of the distillation (GÜLZ *et al.* 1984).

About one half of the oil obtained by distillation of holm oak leaves was composed of terpenes. The main classes of accumulated terpenes were sesquiterpenes (39.2 %) which had an about eleven fold higher concentration than monoterpenes (3.6 %). Both mono- and sesquiterpenes are main components of the essential oils that are synthesized and accumulated in various types of secretory structures such as glandular trichomes and resin ducts (CROTEAU & JOHNSON 1984, FAHN 1979). As resin ducts are not formed in oak tissues, glandular trichomes are expected to be the accumulation sites of the essential oil of holm oak. Capitate hairs have been observed on the adaxial surface of *Q. ilex* leaves (WESTERKAMP & DEMMELMEYER 1997). The essential oil of *Q. robur*, which has a similar composition to that of holm oak, was also shown to be accumulated in glandular trichomes which cover the abaxial side of *Q. robur* leaves (ENGEL *et al.* 1993).

Holm aok also emits large amounts of terpenes. However, in contrast to the composition of accumulated terpenes, the emitted substances consist mainly of monoterpenes, small amounts of isoprene and only traces of sesquiterpenes (STAUDT & SEUFERT 1995, KESSELMEIER *et al.* 1996). The emission rate depends on the availability of photosynthetic carbon (LORETO *et al.* 1996). According to STAUDT and SEUFERT (1995) the monoterpene pool or emission source should exhaust rapidly in the dark and recover in the light. No terpene accumulation of holm oak leaves was reported by STAUDT and SEUFERT (1995). However, these authors investigated only the gaseous emissions of oak leaves and they did not analyze leaf distillates.

Six minor compounds of the volatiles belong to C_{13}-norisoprenoids which usually accumulate in plant tissues as non-volatile glycosides (WINTERHALTER & SCHREIER 1994). Such glycosidally bound compounds might have been hydrolized during the steam distillation of the leaf samples and thus the volatile aroma compounds would have been generated. The diterpene phytol seems also to be a hydrolysis product, because in mature leaves practically all of the phytol is esterified in chlorophyll (PEISKER *et al.* 1989).

Irrespective of the mode of accumulation and the kind of accumulation sites, the analysis of the terpene pattern of *Quercus ilex* might be an important tool for revealing genetic variation within this species. Currently the value of terpene analyses is tested for characterizing holm oak populations in Croatia.

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