Glandular Trichomes and the Volatiles Obtained by Steam Distillation of *Quercus robur* Leaves

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Glandular trichomes in form of long stretched tubes are present on the lower leaf side of *Quercus robur* as shown by scanning electron microscopy. The glands contain an essential oil, which was isolated by steam distillation together with volatile waxy components of the leaves in an amount of 0.025% of fresh leaves. The product of steam distillation was analyzed by GC-MS. Identification of compounds is based on comparison of their mass spectral data with those of authentic samples in combination with retention indices and MS data using the SeKoMS (Search Kovats Indices and Mass Spectra) Library. Altogether 184 components of the product of steam distillation were separated, 155 of which could be identified, another 7 were tentatively assigned. Three groups of substances according to their chemical composition are found: hexenyl derivatives and some acetals (32%); terpenes including monoterpenes (4%), sesquiterpenes and diterpenes (21%); and alkane derivatives (35%). The residual 8% consist of benzyl alcohol, compounds which stem from the degradation of carotenes, and miscellaneous constituents.

Introduction

Leaves of the oak tree *Quercus robur* L. were studied recently in detail concerning their epicuticular waxes. Their chemical composition and their surface structure were analyzed during an entire vegetation period in two successive years. They show significant variations during leaf development and also upon ecological influences [1, 2]. Only the abaxial leaf side shows stomata and glandular trichomes. The glands are obviously filled with an essential oil. In the following we report on the composition of the product obtained by steam distillation of *Quercus robur* leaves.

A first investigation of the "essential oil" of oak leaves was reported by Palma-Fleming and Kepner, 1983 [3] for *Quercus agrifolia.*The oil consisted of large amounts of hexenal, some hexenols, derivates thereof and nonanal. Methylsalicylate, eugenol, the monoterpenes linalool and α-terpineol, and two sesquiterpenes were detected in low concentrations [3]. Further studies about the leaf oil of *Quercus dentata* are reported by Kameoka *et al.*, 1983 [4]; 60 compounds were found among them benzaldehyde, dihydroactinidiolid, caryophyllenoxid, eugenol, and tetradecanal [4, 5].

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Materials and Methods

Steam distillation

Leaf samples were collected from an approximately 12 years old isolated oak tree (*Quercus robur* L.) growing in the garden of the Botanical Institute of the University of Cologne [1]. 100 g mature fresh and entire oak leaves were used for steam distillation using the apparatus according to Hefendehl [6] for 6 h with ether in the graduate tube. The ether solution was dried over $MgSO_4$. The ether was carefully removed with a rotavapor yielding an oil of 0.025% of fresh leaf weight.

Gas chromatography

The volatile oil was analyzed on a GC Hewlett-Packard 5830 equipped with a FID. An OV-1 fused silica gel capillary column (25 m) was used for separation with a temperature program of 50–280 °C, 4 °C/min [7–9].

Gas chromatography/mass spectrometry

System 1 (Quadrupol): The volatiles were investigated by GC-MS on a non-polar methyl silicone

column using a Varian 3700 (splitless injection, 230°, 1 : 20), carrier gas helium (2 ml/min), equipped with 50 m × 0.32 mm i.d. (film thickness 0.52 μm) HP-1 (Hewlett-Packard) column; 50 – 290 °C, 4 °C/min, then 20 min isothermally; the GC was coupled with a Finnigan MAT 44 S (open split coupling – 200 °C), ion source 200 °C, EI = 70 eV, mass range 41 – 360.

System 2 (Iontrap): A DANI 6500 GC coupled directly to a Finnigan ITD mass spectrometer using a J & W DB-Wax fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness). The GC-ITD was operated under the following conditions: carrier gas helium (140 kPa), PTV-split/-splitless injector (in 0.2 min 50 – 240 °C), splitless time 2 min; temperature program: 50 – 220 °C, 1.7 °C/min, then 50 min isothermally; filament 15 mA; multiplier 1500 V; scan range 41 – 330. Linear retention indices were determined externally with a series of *n*-alkanes (C_7 – C_{27}) [10] and were calculated with a basic program included in SeKoMS.

Electron microscopy

Fresh and air-dried leaves were prepared by sputtering with gold using an Emscope sputter counter and examined under a Hitachi S-405 A scanning electron microscope at 25 kV [2].

Results

Oak leaf surface structure

Only the lower (abaxial) side of *Q. robur* leaves shows numerous glandular trichomes as long stretched tubes with an ovally formed basis. The basis of the glands is covered with waxy crystalloids in form of platelets, which occur also on the upper leaf surfaces and on the stomata (Fig. 1 A, 1 B). In summer and autumn the waxy crystalloids on the leaf surface close to the top of the glandular trichomes seem to be melted away as shown in the SEM figures (Fig. 1 C, 1 D) [2]. To our interpretation this phenomenon is caused by the essential oil that dissolves the epicuticular wax crystalloids after it has been released from the glandular tubes. The glands are of unicellular cuticular material and covered with waxes. When these were washed off with chloroform, the glandular trichomes remained unchanged in their form (Fig. 1 E, 1 F). The glands have a length of 60 – 80 μm and a diameter of 15 – 20 μm (basis) and of 10 – 15 μm (tube). Compared to the glands the stomata are 25 – 30 μm in length and 15 – 20 μm in width.

Oil obtained by steam distillation

The entire fresh leaves of *Q. robur* were treated by a hydrodistillation for 6 h resulting in a volatile fraction of 0.025% fresh leaves. This oil was analyzed by GC and GC-MS. Identification of compounds is based on comparison of their mass spectra with those of authentic samples in combination with retention indices on a polar and an apolar column and comparison with mass spectra reported in the literature. The indices found match well with the data published by Jennings [11]. For identification the SeKoMS Library (Search Kovats Indices and Mass Spectra) was used which was established for the study of essential oil of *Cedronella* species [12, 13].

The volatile components identified in *Q. robur* leaf oil are listed in Table I including their linear retention indices, the percent composition, the molecular weight, the base peak and some prominent fragments of the mass spectra. The leaf oil of *Q. robur* consists of a complex mixture of different substances. They can be divided into three classes according to their chemical structure: Several alkanes and their derivatives (35% of the oil) were identified in homologous series especially in the higher boiling fraction: even and odd-numbered *n*-alkanes (C_{17} – C_{29}) which belong to the main constituents (29%), *e.g.* peak No. (= *) *109, *116, *120, *127, *131, and *138, followed by alkenes (C_6 – C_9, C_{20} – C_{28}), aldehydes (C_{18} – C_{26}), alcohols (C_5 – C_{22}), free fatty acids (C_2, C_8, C_9, C_{14} and C_{16}) and fatty acid methyl esters (C_{16} and C_{18}) as minor constituents. A special group form the hexenyl compounds and acetals (32% of the oil) which are found as free hexenols, their esters and as acetals (*1, *29, *58) with *cis*-3-hexenol (*6) as one of the main constituents. Hexenol esters are present in minor quantities in a series of homologues (*8, *13, *26, *33, *38, *44, *46, *51, *67, *87) starting with the formiate *via* the acetate up to the hexanoate, benzoate and decanoate. Of the terpenoids 19 monoterpenes (4% of the oil) were detected. Main components with more than 1% of the oil are 3-carene (*15), *cis*- and *trans*-linalooloxide (furanoid form, *24, *25). 32 substances were assigned to sesquiterpenes and diter-

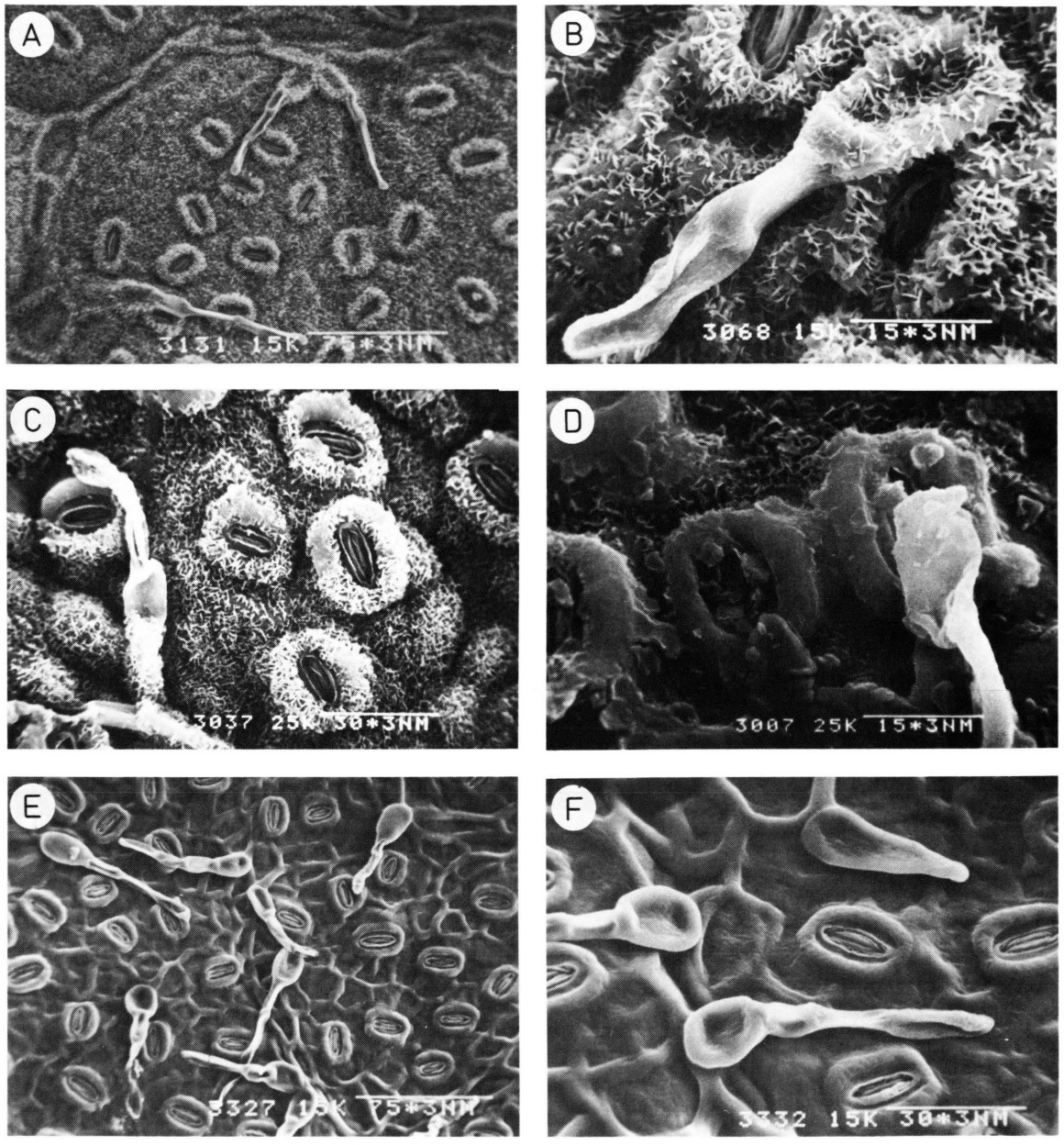

Fig. 1. Lower epidermal surface structures of a mature leaf of *Quercus robur*. A: The abaxial epidermis shows numerous stomata and glandular trichomes, uniform and of long stretched tubes; bar = 75 μm. B: The leaf surface is covered with a continuous wax layer superimposed with crystalloids in shape of fringed edged plateletts, also on the basis of the glands; bar = 15 μm. C and D: The glands contain an essential oil; this dissolves the wax crystalloids after it has been released onto the leaf surface; C: bar = 30 μm, D: bar = 15 μm. E and F: The wax layer with the wax crystalloids has been removed with chloroform. The entire structure of the cuticle and the glands remained unchanged by this procedure; E: bar = 75 μm, F: bar = 15 μm.

penes (21% of the oil), 21 of which were identified by the SeKoMS Library. In addition five were identified tentatively by their MS data [14]: four cadalene (*91, *96) and calamenene (*82, *89) derivatives and caryophyllen-13-al (*84) [15, 38]. The sesquiterpenes viridiflorol, T-cadinol, *a*-cadinol,

Table I. Volatile compounds in *Quercus robur* leaf oil.

No.	Compound	R_i ap.	R_i po.	%[1]	MW	Mass spectral data *m/z*[2]	Identification
1	Acetaldehyde-diethyl-acetal	737			118	45, 73, 103	[b]
2	n.i. (*m/z* 43/57/70/71)	768		0.3			
3	*n*-Octane	801			114	43, 57, 85	[a]
4	n.i., acetal or hemiacetal	802		14.35		45, 43, 73, 61	unid.-1
5	*trans*-3-Hexenol	836	1353		100	43, 41, 67, 82	[a]
6	*cis*-3-Hexenol	851	1373	11.47	100	41, 67, 55, 82	[a]
7	*n*-Nonane	898			128	43, 57, 85, 99	[a]
8	*cis*-3-Hexenyl-formiate	913		0.81	128	67, 41, 82	[b]
9	α-Pinene	939	1008	0.5	136	93, 92, 91, 77, 79	[a]
10	*tert*-Butylbenzene (contam.)	968			134	119, 91	[b]
11	β-Pinene	973	1090	0.36	136	93, 41, 69	[a]
12	n.i., isomer 1 of peak No. 4	991		0.26			
13	*cis*-3-Hexenyl-acetate	994		0.78	142	43, 67, 82	[b]
14	n.i., isomer 2 of peak No. 4	997		0.2			
15	3-Carene	1003	1135	1.13	136	93, 91, 79, 77	[a]
16	*p*-Cymene	1019			134	119, 91, 43, 77	[a]
17	2,6,6-Trimethyl-cyclohexanone	1021	1301		140	82, 56, 69, 41, 55	[b]
18	Limonene	1024	1184		136	68, 93, 41, 67	[a]
19	n.i. (*m/z* 43/84/57/71)	1025					
20	Benzyl alcohol	1028	1858	0.41	108	79, 91, 108	[a]
21	n.i. (*m/z* 43/57/70/84)	1044		0.13			
22	(Alkan)	1050					
23	1-Octanol	1053	1548		130	56, 41, 55, 84	[a]
24	*cis*-Linalool-oxid (furanoid)	1066	1413	1.1	170	59, 43, 55, 94	[a]
25	*trans*-Linalool-oxid (furanoid)	1075	1461	0.7	170	59, 43, 94, 55	[a]
26	*cis*-3-Hexenyl-propionate	1078	1398		158	57, 67, 82	[b]
27	*n*-Nonanal	1091	1377	0.54	142	57, 41, 43, 44	[a]
28	Linalool	1091	1536			43, 71, 55, 93, 80	[a]
29	Acetaldehyde-ethyl-*cis*-3-hexenyl-acetal	1096	1292	1.64	172	45, 73, 55, 83	[b]
30	n.i. (*m/z* 43/84/55/57)	1099					
31	n.i. (*m/z* 45/57/59/101/87)	1103	1635	0.29			
32	n.i. (*m/z* 43/84/57/85/71)	1119		0.31			
33	*cis*-3-Hexenyl-isobutyrate	1125			170	43, 67, 82, 71	[b]
34	n.i. (*m/z* 43/84/57/85/70)	1128					
35	n.i. (*m/z* 43/84/57/71/85)	1140		0.1			
36	*cis*-Linalool-oxid (pyranoid)	1152	1726		170	68, 59, 67, 94	[b]
37	*trans*-Linalool-oxid (pyranoid)	1165			170	59, 68, 43, 94	[b]
38	*cis*-3-Hexenyl-butyrate	1169	1448	0.61	170	67, 43, 82, 71	[b]
39	α-Terpineol	1177	1679	0.2	154	59, 93, 41, 81	[a]
40	Methylsalicylate	1191	1747		152	92, 120	[a]
41	n.i. (*m/z* 43/55/84/57/56)	1194					
42	β-Cyclocitral	1199	1600		152	81, 109, 137, 123	[b]
43	n.i. (*m/z* 43/84/57/45/55)	1202		0.11			
44	*cis*-3-Hexenyl-2-methylbutyrate	1217		0.31	184	57, 82, 67, 85	[b]
45	n.i. (*m/z* 91/45/41/73/134)	1252		0.19			unid.-2
46	*cis*-3-Hexenyl-pentanoate	1268			184	67, 43, 82, 57, 85	[b]
47	Vitispirane (*cis* and *trans*)	1272	1487/1488	0.37	192	93, 41, 192, 121	[b]
48	Riesling acetal	1295	1615	0.21	208	43, 138, 125, 148	[b]
49	1,1,6-Trimethyl-1,2-dihydro-naphthalene (TDN)	1338	1717		172	157, 142, 115	[b]
50	1,1,6-Trimethyl-1,2,3,4-tetrahydronaphthalene (TTN)	1339	1673		174	159, 128, 144	[b]
51	*cis*-3-Hexenyl-hexanoate	1362	1640		198	82, 43, 67, 99	[b]
52	(Alkane)	1370					
53	α-Copaene	1385		0.17	204	105, 119, 161, 93	[a]
54	β-Bourbonene	1391		0.23	204	81, 80, 123	[a]

Table I. (Continued).

No.	Compound	R_i ap.	R_i po.	%[1]	MW	Mass spectral data *m/z*[2]	Identification
55	*n*-Tetradecane	1400			198	57, 43, 71, 85	a
56	Hydroxydihydroedulan-1	1428	1902	0.44	210	126, 43, 70, 84	c
57	Hydroxydihydroedulan-2	1447	1986		210	126, 43, 70, 84	c
58	Acetaldehyde-di-(*cis*-3-hexenyl)-acetal	1456	1692	0.55	226	55, 83, 41, 127	b
59	γ-Muurolene	1474	1666	0.12	204	161, 41, 105, 148	a
60	n.i. (*m/z* 99/41/177/57/155/192)	1482					
61	α-Muurolene	1498	1703	0.67	204	105, 93, 94, 161	a
62	γ-Cadinene	1506	1760		204	161, 105, 119	a
63	*cis*-Calamenene	1509	1812		202	159	a
64	δ-Cadinene	1512	1740	0.29	204	161, 105, 134	a
65	α-Calacorene	1535	1889	0.32	200	157, 142, 141	a
66	n.i. (*m/z* 41/96/79/82/83/109)	1541					
67	*cis*-3-Hexenyl-benzoate	1546	2099	0.55	206	82, 67, 105, 77	b
68	(Oplopenone isomer)	1567	1898			43, 177	
69	Caryophyllene-oxid	1575	1961	0.52	220	41, 43, 79, 93	a
70	Viridiflorol	1596	2065	3.12	222	43, 41, 93, 105, 119	a
71	β-Oplopenone	1602	2048		220	43, 177	b
72	Ledol	1607	2006	0.29	222	43, 41, 93, 105, 122	a
73	Methyl-jasmonate isomer +	1627		0.54	224	83, 41, 151	a
74	Sesquiterpene alcohol	1629					
75	T-Muurolol (*cis*-α-Cadinol)	*	2166		222	43, 95, 161	a
76	T-Cadinol	1635	2149	3.05	222	161, 105, 81, 134	a
77	α-Cadinol (*trans*-α-Cadinol)	1643	2214	3.26	222	43, 95, 161	a
78	n.i. (*m/z* 57/43/71/85/95/111)	1663		0.17			
79	Cadalene	1666	2196		198	183, 198, 168	a
80	n.i. (*m/z* 188/173/43/203/228)	1675	2179	0.68	246	s. peak No. 96	unid.-3
81	*n*-Heptadecane	1700		0.19	240	57, 43, 71, 85	a
82	(7-Methoxy-calamenene)	1728	2135	0.4	232	189, 190, 174, 159	c
83	n.i. (*m/z* 57/56/69/70/83/111/126)	1732		0.18			
84	Caryophyllen-13-al/α-Betulenal	1736		0.4	218	41, 69, 147, 203	c
85	n.i. (*m/z* 187/172/129/128/213)	1740	2216	3.93	230		unid.-4
86	*n*-Tetradecanoic acid	1744		0.1	228	60, 43, 73	a
87	(*cis*-3-Hexenyl-decanoate)	1764				82, 67	
88	n.i., isomer of peak No. 85	1766	2216	0.12			unid.-5
89	(7-Hydroxy-calamenene)	*			218	175, 43, 60, 73	c
90	n.i. (*m/z* 191/43/176/71)	*			262		unid.-6
91	(7-Methoxy-1,2-dihydrocadalene)	*			216	173, 158, 145, 128	c
92	*n*-Octadecane	1798		0.1	254	57, 43, 71, 85	a
93	n.i. (*m/z* 82/67/41/55/91/153)	1810					
94	6,10,14-Trimethyl-pentadecan-2-one	1833	2111	1.31	268	43, 58, 71, 109, 250	a
95	n.i. (*m/z* 189/161/190/146)	1876	2669	0.14	232		unid.-7
96	(7-Methoxy-cadalene)	1880	2531	1.72	228	213, 198, 214	c
97	(Alkane)	1884		0.4			
98	Methyl-hexadecanoate	1905		0.12	270	74, 43, 87	a
99	Dibutyl-phthalate (contam.)	1918		0.28	278	149	a
100	Isophytol	1942			296	71, 43, 57, 82	a
101	Hexadecanoic acid	1947		0.44	256	43, 57, 73, 60	a
102	n.i., isomer 1 of peak No. 93	1958					
103	n.i. (*m/z* 213/43/228/191)	1985		0.4	262	s. peak No. 96	unid.-8
104	1-Eicosene	1992			280	43, 57, 97, 111	b
105	*n*-Eicosane	1997	2000	0.36	282	57, 43, 71, 85, 99	a
106	(Octadecanal isomer)	2004					
107	*n*-Octadecanol	2070		0.27	270	43, 57, 83, 69, 97	a
108	Heneicosene	2079		0.12	294	43, 57, 83, 97	b
109	*n*-Heneicosane	2098		1.56	296	57, 43, 71, 85, 99	a
110	*trans*-Phytol +	2105	2616	0.55	296	71, 57, 43, 81, 123	a
111	Methyl-octadecanoate	2110			298	74, 43, 87, 143, 185	a

Table I. (Continued).

No.	Compound	R_i ap.	R_i po.	%[1]	MW	Mass spectral data *m/z*[2]	Identification
112	(Alkane)	2116					
113	n.i. (*m/z* 84/43/57/69/97/111	2119					
114	(Eicosanal isomer)	2145					
115	1-Docosene	2195		0.32	308	57, 43, 69, 97, 83	b
116	*n*-Docosane	2200	2200	2.95	310	57, 43, 71, 85, 99	a
117	(Eicosanal)	2206					
118	*n*-Eicosanol	2286			298	43, 55, 83, 97	b
119	1-Tricosene	2294		0.16	322	57, 43, 71, 83, 97	b
120	*n*-Tricosane	2300	2300	8.13	324	57, 43, 71, 85, 99	a
121	(Alkanal)	2323					
122	n.i. (*m/z* 57/99/43/71/83)	2335		0.1			
123	n.i. (*m/z* 41/203/175/187)	2342					
124	n.i., isomer 2 of peak No. 93	2365					
125	(Alkane)	2377					
126	Tetracosene	2379		1.57	336	57, 43, 97, 83, 111	b
127	*n*-Tetracosane	2400	2400	3.37	338	57, 43, 71, 85, 99	a
128	(Docosanal isomer)	2411		0.15			
129	*n*-Docosanol	2470		0.11	326	57, 43, 69, 83, 97	b
130	1-Pentacosene	2494			350	57, 43, 97, 83, 69	b
131	*n*-Pentacosane	2500	2500	8.29	352	57, 43, 71, 85, 99	b
132	Di-*n*-octyl-phthalate (contam.)	2508		0.34	390	57, 149, 167	b
133	(Alkene)	2517					
134	1-Hexacosene	2594		0.29	364	57, 43, 83, 97, 69	b
135	*n*-Hexacosane	2600		0.75	366	57, 43, 71, 85, 99	b
136	(Tetracosanal isomer)	2610		0.26			
137	1-Heptacosene	2689		0.12	378	57, 43, 83, 97, 69	b
138	*n*-Heptacosane	2700		2.36	380	57, 43, 71, 85, 99	b
139	(Pentacosanal isomer)	2725					
140	(Octacosene)	2731		0.12	392		b
141	*n*-Octacosane	2800		0.15	394	57, 71, 43, 85, 99	b
142	(Hexacosanal isomer)	2828		0.31			
143	(Alkane)	2829		0.18			
144	*n*-Nonacosane	2900		0.33	408	57, 71, 43, 85, 99	b

No.	Compound[3]	R_i po.	MW	Mass spectral data *m/z*[2]	Identification
145	2-Pentanone	981	86	43, 57, 58, 86	b
146	*n*-Hexanal	1066	100	41, 44, 43, 58	a
147	3-Pentanol	1103	88	59, 41	b
148	2-Pentanol	1117	88	45, 55	b
149	4-Methyl-3-penten-2-one	1123	98	83, 55, 98	b
150	*n*-Heptanal	1171	114	41, 55, 44, 70	a
151	*trans*-2-Hexenal	1209	98	41, 55, 69, 83	a
152	6-Methyl-2-heptanone	1226	128	43, 58	b
153	1-Pentanol	1246	88	42, 55, 41, 70	b
154	1-Hexyl-acetate	1275	144	43, 41, 56, 69	b
155	2-Ethoxyethyl-acetate	1286	132	43, 72, 59	b
156	1-Octen-3-one	1288	126	55, 70, 41, 97	a
157	*trans*-2-penten-1-ol	1312	86	57, 41, 43, 44, 67	b
158	6-Methyl-5-hepten-2-one	1325	126	43, 55, 108	a
159	1-Hexanol	1347	102	56, 41, 55, 69	a
160	2-Butoxy-ethanol	1391	118	57, 41, 45, 87	b
161	Acetic acid	1420	60	43, 60	b
162	Benzaldehyde	1485	106	77, 105, 51, 106	a
163	*(trans)*-2-Decenal	1615	154	41, 55, 70, 69	b
164	*trans*-Pinocarveol	1638	152	41, 55, 92, 91	a
165	1-Nonanol	1649	144	41, 55, 56, 70	a

Table I. (Continued).

No.	Compound[3]	R_i po.	MW	Mass spectral data m/z[2]	Identification
166	*cis*-3-Hexenyl-pentenoate	1650	182	67, 55, 83, 82, 95	[b]
167	Benzyl-formiate	1663	136	91, 90, 79, 108, 136	[b]
168	Verbenon	1688	150	107, 79, 135, 91	[b]
169	Benzyl-acetate	1705	150	108, 43, 91, 150	[b]
170	2-Ethyl-3-methyl-maleic anhydride	1716	140	67, 53, 112, 68, 41, 140	[b]
171	*n*-Decanol	1732	158	41, 55, 70, 69, 83	[b]
172	1-Phenyl-ethanol (methyl-phenyl-carbinol)	1797	122	79, 107, 77, 43, 51, 122	[b]
173	*trans*-Carveol	1817	152	41, 109, 84, 137	[b]
174	*p*-Cymen-8-ol	1828	150	43, 135, 91, 115	[b]
175	(*p*-Cymen-9-ol)	1831	150	43, 135, 91, 117	[c]
176	Geranyl acetone (isomer)	1839	194	43, 41, 69, 107, 151	[b]
177	Dimethylsulfone	1883	94	79, 94, 45, 48, 63	[b]
178	β-Ionone	1917	192	43, 177	[b]
179	(Epoxy-β-ionone isomer)	1973		43, 123	[b]
180	Phenol	1984	94	94, 66, 65	[b]
181	Methyl-tetradecanoate	1990	242	41, 74, 55, 87, 143	[b]
182	Octanoic acid	2038	144	60, 43, 73, 101	[b]
183	Nonanoic acid	2146	158	41, 60, 57, 73, 69	[b]
184	δ-Cadinol	2183	220	43, 161, 119, 105, 79	[b]

[1] Quantification according to the Area Percent Method without consideration of calibration factors (F), *i.e.* F = 1.0 for all compounds on apolar column.
R_i = linear retention index, ap. = on the apolar, po. = on the polar column, MW = molecular weight.
[2] Fragmentation ions: base peak and characteristic ions in decreasing order of relative abundance.
[3] Compounds additionally detected on a iontrap MS run on DBW.
[a] Identification of compounds is based on comparison of their mass spectra with those of authentic samples in combination with their retention indices (SeKoMS –Search Kovats Indices and Mass Spectra library).
[b] Identification of compounds is based on comparison of their mass spectra in combination with retention indices reported in the literature (SeKoMS library).
[c] Identification based on mass spectra only.
* Detected on a separated iontrap MS run on OV-101 without determination of R_i.

7-methoxy-cadalene, and an unidentified sesquiterpenoid compound (*70, *76, *77, *96, *85) are present of more than 1% of the oil. Compounds with 13 carbons such as the vitispiranes, 1,1,6-trimethyl-1,2-dihydronaphthalene, rieslingacetal, 1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene (*47, *49, *48, *50) and two hydroxydihydroedulan isomers (*56, *57) [16] were altogether identified at 1.02%. 6,10,14-Trimethylpentadecan-2-one as well as isophytol and *trans*-phytol (*94, *100, *110) are found in reasonable amounts. Table II presents the data of unidentified components and those which have been tentatively assigned.

Discussion

The presence of glandular trichomes on the lower leaf surface of *Quercus robur* is an indication that essential oil components are synthesized and accumulated. As in many other plants with essential oil containing trichomes the glands are localized in the abaxial leaf epidermis. The occurrence of an essential oil is confirmed by the identification of a series of terpenoid compounds in the leaf oil which are typical for essential oils.

155 substances were identified in the product obtained by steam distillation; this is considerably more than described for *Q. agrifolia* [3] or *Q. dentata* [4]. Especially numerous mono-, sesqui- and diterpenes accounting for *ca.* 25% were identified; these compounds very likely stem from the content of the glandular trichomes on the abaxial leaf surface. Others such as the C-13 compounds (*47, *48, *49, *50) are rare essential oil components; they have been reported in the volatiles of grape juices, wines and brandies [17–21]. Geranylacetone, the

Table II. Unidentified and tentatively assigned compounds in *Quercus robur* leaf oil.

Mass spectra of unidentified constituents				
Peak No.		Retention index R_i		MS *m/z* (rel. int.)
		apolar (HP1)	polar (DBW)	
4	unid.-1	802-isomer-1		[M⁺]?, 45 (100), 43 (72), 73 (39), 61 (37)
12		991-isomer-2		
14		997-isomer-3		
45	unid.-2	1252	1686	[M⁺]?, 134 (11), 91 (100), 73 (30), 45 (62), 41 (30)
80	unid.-3	1675	2179	246 [M⁺] (4), 188 (100), 173 (63), 43 (53), 203 (45), 228 (15), 213 (15), 211 (13), 226 (8)
85	unid.-4	1740-isomer-1	2216	230 [M⁺] (9), 187 (100), 172 (55), 129 (15), 128 (13) 213 (7), 228 (4), 198 (2)
88	unid.-5	1766-isomer-2	2216	
90	unid.-6			262 [M⁺] (22), 191 (100), 43 (43), 176 (26), 71 (9), 192 (9), 161 (4), 246 (<1)
95	unid.-7	1876	2669	232 [M⁺] (10), 189 (100), 161 (34), 190 (12), 146 (8), 131 (8), 174 (4)
103	unid.-8	1985		262 [M⁺] (9), 213 (100), 43 (91), 228 (49), 191 (42), 119 (38), 198 (25), 244 (2)

Mass spectra for constituents, which are only identified by MS				
Peak No.	Compound	Retention index R_i		MS *m/z* (rel. int.)
		apolar (HP1)	polar (DBW)	
56[1]	Hydroxydihydroedulan-1	1428	1902	210 [M⁺] (<1), 126 (100), 43 (75), 70 (51), 84 (42), 67 (34), 85 (32), 69 (30), 111 (23), 55 (13), 195 (2)
57	Hydroxydihydroedulan-2	1447	1986	
82	7-Methoxy-calamenene	1728	2135	232 [M⁺] (5), 189 (100), 190 (13), 174 (9), 159 (7), 128 (4)
84	Caryophyllen-13-al or cis-Caryophyllen-13-al	1736		218 [M⁺] (9), 41 (100), 69 (79), 147 (75), 91 (60), 105 (57), 79 (49), 55 (43), 175 (36), 119 (28), 190 (11), 203 (<1)
89	7-Hydroxy-calamenene			218 [M⁺] (3), 175 (100), 43 (17), 60 (13), 73 (13), 129 (4), 145 (3), 160 (3)
91	7-Methoxy-1,2-dihydro-cadalene			216 [M⁺] (9), 173 (100), 158 (26), 145 (7), 128 (4), 115 (2)
96	7-Methoxy-cadalene	1880	2531	228 [M⁺] (49), 213 (100), 198 (24), 214 (16)

[1] Determination of the isomers according to quantification.

β-ionone derivative 2,2,6-trimethylcyclohexanone, and β-cyclocitral are thought to be derived from the carotinoids [22]. Hexenyl esters and acetals are the most abundant components in *Q. agrifolia* leaf oil [3] and are also present in the steam distillate of *Q. robur* in a remarkable amount of 33%. Hexenols and hexenals are known to be formed by an enzymatic reaction from linoleic and linolenic acids when leaves are wounded or macerated [23]; they are responsible for the characteristic odor of damaged tissues of green leaves (*e.g. cis*-3-hexenol, *trans*-2-hexenal). Essential oils obtained from leaves usually contain these substances when the plant material was macerated before steam distillation [8, 24–29]. During the present work we carefully payed attention not to damage the leaves for steam distillation; thus we exclude at least a major portion of the hexenyl compounds being artificial and regard them as genuine compounds. The presence of homologous series of the corresponding esters and acetales may also be an indication for this assumption. 34% of the leaf oil account for alkane derivatives. Such compounds are constituents of the epicuticular leaf wax of *Q. robur* [1]. They usually occur in leaf oils obtained by hydrodistillation in different yield depending on the duration of steam distillation [9, 30].

Several sesquiterpenes of the cadinane type in the leaf oil are worth to be mentioned. Such compounds also accumulate in cultured cells of a liverwort *(Heteroscyphus planus)* [14]. Sesquiterpenes of the cadinane type, for example 7-hydroxy-calamenene or the mansonones, are found to have antifungal or antibacterial properties. Both compounds are reported as post-infectional antifungal compounds (phytoalexins) whose formation is induced in tissues after the infection by fungi, *e.g.* in the wood of *Ulmus glabra, Tilia europea* and other trees [31–37]. In this connection it may be indicative that the oak leaves harvested for steam distillation did not show any fungal infection except in late fall (October and November).

With the data presented here the volatile fraction of the leaves of *Q. robur* represents one of the best characterized and most extensively studied leaf oils of woody trees.

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