Intensive neutral odourants of linden honey

Differences from honeys of other botanical origin

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Intensive neutrale Geruchsstoffe von Lindenhonig. Unterschiede zu Honigen anderer botanischer Herkunft

Zusammenfassung. Die Aromaextrakt-Verdünnungsanalyse der flüchtigen Verbindungen aus Lindenhonig ergab 21 Geruchsstoffe mit hohen FD-Faktoren; 18 davon wurden identifiziert: 1-Hexen-3-on, 2-Acetyl-1-pyrrolin, Dimethyltrisulfid, Methional, Phenylacetaldehyd, 2-Phenylethanol, Linalool, *p*-Kresol, 3,9-Epoxy-1-*p*-menthen, 4-Methylacetophenon, 3,9-Epoxy-1,4(8)-*p*-menthadien (Lindenether), 1,3-*p*-Menthadien-7-al, *p*-Anisaldehyd, 4-Vinylguajacol, (*E*)-β-Damascenon, Eugenol, Vanillin und *cis*-Rosenoxid. Lindenether und *cis*-Rosenoxid, die auch in einem Extrakt von Lindenblüten (*Tilia cordata*) vorkamen, fehlten in Honigen anderer botanischer Herkunft. Diese beiden Aromastoffe und das geruchlose *trans*-Limonen-1,2-diol werden als Indikatoren für Lindenhonig vorgeschlagen. Die Geruchsschwellen (in Luft) der 18 Aromastoffe wurden bestimmt.

Summary. Aroma extract dilution analysis of linden honey volatiles resulted in 21 odour compounds having high factors of dilution (FD); 18 of these compounds were identified as 1-hexen-3-one, 2-acetyl-1-pyrroline, dimethyl trisulphide, methional, phenylacetaldehyde, 2-phenylethanol, linalool, *p*-cresol, 3,9-epoxy-1-*p*-menthene, 4-methylacetophenone, 3,9-epoxy-1,4(8)-*p*-menthadiene (linden ether), 1,3-*p*-menthadien-7-al, *p*-anisaldehyde, 4-vinylguaiacol, (*E*)-β-damascenone, eugenol, vanillin and *cis*-rose oxide. Linden ether and *cis*-rose oxide, which were also found in an extract obtained from the blossoms of the lime tree (*Tilia cordata*), were absent in honeys of other botanical origin. These two odourants and the odourless *trans*-limonene-1,2-diol are proposed as indicators for linden honey. The odour thresholds (in air) of the 18 aroma compounds are reported.

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Introduction

The first analysis of the flavour components of honey was performed by Schmalfuß and Barthmeier [1] in 1929. Since then about 300 volatile compounds have been found in honeys of different botanical origin [2, 3]. A first approach to evaluate the potent aroma compounds of honey was carried out by Steeg and Montag [4, 5]. They compared the concentrations of aromatic acids, phenols and some other substances in honey with their taste or odour thresholds reported in the literature. Based on this, the authors reported that benzoic acid, phenylacetic acid, phenol, *p*-cresol, guaiacol and eugenol contributed to honey flavours. The aim of the following investigations was to identify, by use of aroma extract dilution analysis [6, 7], the primary neutral odourants of linden (lime tree) honey and to suggest a means of comparison with honeys of other botanical origin.

Experimental procedures

Materials

Honey samples were obtained from German importers. The botanical origin of each sample was confirmed by the Institut für Honigforschung, Bremen. Blossoms of linden (*Tilia cordata*) were collected and freed from insects, leaves and other green plant components. The following compounds were obtained commercially: phenylacetaldehyde, (±)-linalool, perillaldehyde, γ-terpinene, 1-hexen-3-ol,(*E*)-2-hexenal, (+)-*p*-menth-1-en-9-ol, rose Bengal, and *p*-cymene were from Aldrich (Steinheim, FRG); *p*-cresol, 4-methylacetophenone, and hexanal were from Fluka (Buchs, Switzerland); 4-vinylguaiacol was from Lancaster (Morecambe, UK); 2-phenylethanol, *p*-anisaldehyde, eugenol, vanillin, benzyl alcohol, and 3-methylbutanoic acid were from Merck (Darmstadt, FRG); limonene, terpinolene, and menthofuran were from Roth (Karlsruhe, FRG); methional was from Sigma (Deisenhofen, FRG). *cis*-Rose oxide, α-*p*-dimethylstyrene and (3*R*,4*S*,8*R*)-3,9-epoxy-1-*p*-menthene (dill ether) were gifts from Haarmann & Reimer (Holzminden, FRG); (*E*)-β-damascenone was a gift from Firmenich, SA (Genève, Switzerland). The solvents were purfied according to [8]. Silica gel 60 (Merck, Darmstadt, FRG) was treated with conc. HCl and deactivated with 7% by mass water [9]. Benzene and $CDCl_3$ (both 99.96% D) were from MSD Isotopes (IC Chemicals, Munich, FRG).

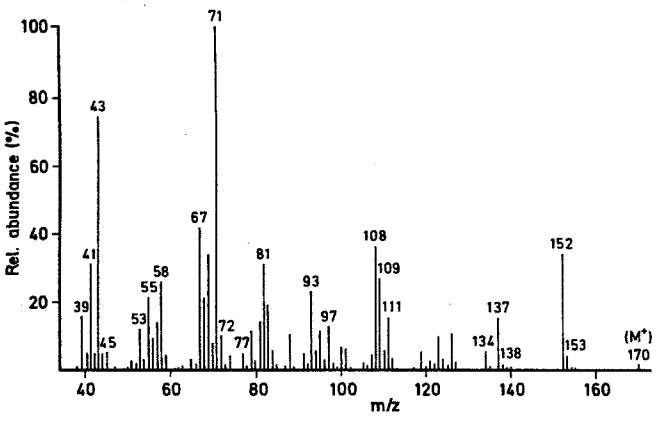

Fig. 1. MS(EI) of *trans*-8-*p*-menthen-1,2-diol

Syntheses

2-Acetyl-1-pyrroline and dimethyl trisulphide were prepared according to [10, 11]. 1-Hexen-3-one was obtained by oxidation of 1-hexen-3-ol with MnO_2 as described for 1-octen-3-ol [12], and was separated from the 1-hexen-3-ol by high resolution gas chromatography (HRGC) on capillary OV-1701. The MS(EI) of 1-hexen-3-one agreed with the data reported by Buttery et al. [13]. A mixture of (3*R*,4*S*,8*R*)- and (3*R*,4*S*,8*S*)-3,9-epoxy-1-*p*-menthene ("dill ether") was prepared by photooxidation of (+)-*p*-menth-1-en-9-ol according to the procedure reported by Ohloff et al. [14] using some modifications. A stirred solution of (+)-*p*-menth-1-en-9-ol (3.3 mmol) and rose Bengal (2 μmol) in methanol (10 ml) was irradiated for 10 h at 20 °C in the apparatus earlier described [15]. After cooling the reaction mixture to 0 °C in an ice bath, 50 ml of 0.2 mol/l sodium sulfite were added under stirring, and the temperature was raised to 20 °C during a period of 4 h. The pH of the solution was adjusted to 4 and the 3,9-epoxy-1-*p*-menthenes were extracted with diethyl ether (2 × 50 ml). The organic layer was washed with brine (100 ml) and dried over anhydrous Na_2SO_4. The mixture of the diastereomers was separated by HRGC on capillary SE-54 [RI for (3*R*,4*S*,8*R*) 1187, for (3*R*,4*S*,8*S*) 1235]. The MS(EI) and MS(CI, isobutane) data of both diastereomers were identical with the corresponding data of the reference substance (3*R*,4*S*,8*R*)-3,9-epoxy-1-*p*-menthene. 1,3-*p*-Menthadien-7-al was prepared from perillaldehyde [16]. ^{1}H-NMR [(d_6)-benzene, 360 MHz]: δ/ppm = 0.78 (d, J = 6.8 Hz, 6H, H-9.10), 1.72 (t, J = 9.7 Hz, H-5), 1.97 (quintet, J = 7 Hz, H-8), 2.36 (t, J = 9.7 Hz, H-6), 5.53 (dd, J = 5.5 Hz, J = 1,3 Hz, H-3), 6.15 (d, J = 5.45 Hz, H-2), 9.43 (s, CHO). MS(EI): 79 (100), 107 (75), 150 (65, M^+), 121 (50), 77 (40), 91 (40), 105 (30), 93 (25), 135 (25), 43 (20), 39 (20), 51 (20), 65 (10), 108 (10). *trans*-Limonene-1,2-diol was prepared according to Newhall [17] and Bonaga et al. [18] using some modifications. (+)-Limonene (0.2 mol), dissolved in 100 ml dichloromethane, was epoxidized with 3-chloroperbenzoic acid (0.25 mol) at room temperature. After a reaction time of 16 h, the solution was treated, successively, with 200 ml aqueous 5% sodium sulfite solution and with aqueous 5% sodium carbonate solution until pH 7.0. The organic layer was washed with water (three 100-ml portions). The MS(EI) in Fig. 1 agreed with the signals reported by Tsuneya et al. [19]; MS(CI, isobutane): 171 (15%; M^++1), 153 (100%; M^++1-H_2O), 135 (70%, M^++1-2H_2O). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ/ppm = 1.28 (s, CH_3), 1.74 (s, CH_3), 1.93 (m, H-3), 2.27 (m, H-4), 3.65 (dd, J_{ae} = 3.6 Hz; J_{aa} = 7.1 Hz, H-2, axial), 4.74 (s, H-9).

Isolation of volatiles

Extraction at room temperature. Honey (200 g) was diluted with 400 ml of 0.2 mol/l sodium borate pH 9.0, saturated with NaCl. The pH of the solution was readjusted to 9.0 by addition of aqueous KOH (30% by mass) and, after addition of 250 ml dichloromethane, the mixture was vigorously shaken for 20 min (200 rpm) by a shaking machine (3D type EM4, Desaga, Heidelberg, FRG). The emulsion obtained was stored for 15 h at room temperature and then centrifuged (3000 rpm, 4 °C, 20 min). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and then concentrated to about 120 ml by distilling off the solvent on a Vigreux column (50 × 1 cm). The solution was poured into the distillation flask (250 ml) of the apparatus [20] used for the high-vacuum transfer. After freezing the sample for 30 min in liquid nitrogen, the volatiles and the solvent were sublimated in vacuo (3 Pa) for 3 h. The temperature of the distillation flask was then increased to 45 °C and the sublimation continued for a further 1 h. The condensates of the second and third cooling traps were combined and finally concentrated by micro-distilation [21] to about 200 μl for HRGC-effluent sniffing and MS studies.

Simultaneous distillation/extraction (SDE). In order to obtain enough material for the identification of trace components, the volatiles were isolated by SDE from larger amounts of linden honey. Each portion (250 g) of honey was diluted with 250 ml water and then distilled and continuously extracted for 4 h with 70 ml pentane/diethyl ether (1:1, by vol.) in the apparatus designed by Nickerson and Likens [22]. The extracts obtained from 5 kg honey were combined, dried over anhydrous Na_2SO_4 and then concentrated to 50 ml on a Vigreux column (50 × 1 cm). The solution of the volatiles was extracted with 0.5 mol/l sodium carbonate (three 100-ml portions), washed with water (three 100-ml portions), dried over anhydrous Na_2SO_4 and finally concentrated on a Vigreux column (50 × 1 cm) to 20 ml (neutral volatiles). The sodium carbonate solution was acidified with 5 mol/l HCl and then extracted with diethyl ether (three 100-ml portions). The organic layer was washed with brine (two 100-ml portions), dried over Na_2SO_4 and finally concentrated (acidic volatiles).

Volatiles from linden blossoms. Linden blossoms (300 g) were suspended in 1.2 l pentane/diethyl ether (1:2, by vol.) and then shaken vigorously for 15 h using the shaking machine described above. After filtration, drying over anhydrous Na_2SO_4 and concentration on a Vigreux column (50 × 1 cm), the volatiles were separated from the non-volatile material by high vacuum transfer as described above.

Column chromatography

The neutral volatiles obtained from linden honey were fractionated at 10–12 °C on a water-cooled column (30 × 1.6 cm) packed with a slurry of silica gel 60 in pentane. The elution was performed with 100 ml pentane/diethyl ether (95:5, by vol.; fraction A), 200 ml pentane/diethyl ether (75:25, by vol.; fraction B), 200 ml diethyl ether (fraction C), and finally 100 ml diethyl ether/methanol (95:5, by vol.; fraction D). Fraction B was rechromatographed on the same column. The elution was performed with 100 ml pentane/diethyl ether (95:5, by vol.; fraction not collected), 200 ml pentane/diethyl ether (90:10, by vol.; first 100 ml, subfraction BI; second half, subfraction BII) and 100 ml diethyl ether (subfraction BIII). The volatiles of linden blossoms were fractionated for HRGC-MS using the following elution sequence: 100 ml pentane (fraction E), 100 ml pentane/diethyl ether (80:20, by vol.; fraction F), and 100 ml diethyl/ether (fraction G).

HPLC

HPLC was performed with the column and the apparatus described [23]. The following solvents were used: pentane for fraction A, pentane/diethyl ether (97:3, by vol.) for subfraction BII, pentane/diethyl ether (95:5, by vol.) for subfraction BIII, pentane/diethyl ether (99:1,

Table 1. Elution range of the fractions obtained by HPLC

Separation of									
Fraction A		Subfraction BII		Subfraction BIII		HPLC fraction BIIf		HPLC fraction BIIg	
Fraction	Elution range (ml)	Fraction	Elution range (ml)	Fraction	Elution range (ml)	Fraction	Elution range (ml)	Fraction	Elution range (ml)
Aa	4.0– 9.6	BIIa	4 –14	BIIIa	6 –12	BIIf1	10 –40	BIIg1	4 –31
Ab	9.6–10.8	BIIb	14 –17	BIIIb	12 –13.2	BIIf2	40 –44.6	BIIg2	31 –36.2
Ac	10.8–12.4	BIIc	17 –19	BIIIc	13.2–16	BIIf3	44.6–50	BIIg3	36.2–39.8
Ad	12.4–14.8	BIId	19 –21.9	BIIId	16 –18.4	BIIf4	50 –58	BIIg4	39.8–72.2
Ae	14.8–26.0	BIIe	21.9–23.8	BIIIe	18.4–23				
		BIIf	23.8–27.9	BIIIf	23 –26.2				
		BIIg	27.9–50	BIIIg	26.2–50				

by vol.) for rechromatography of HPLC fraction BIIf, and pentane/diethyl ether (98:2, by vol.) for rechromatography of HPLC fraction BIIg. The effluents (flow rate 2 ml/min) were monitored at 220 nm: The elution ranges of the fractions collected are reported in Table 1. In order to obtain enough material for MS and NMR analysis, the fractions of 30 to 50 runs were collected and concentrated.

Gas chromatography

Preparative GC. This was performed using an SE-54 column [24]. The samples were chromatographed isothermally at 130–160 °C. The exact temperature depends on the volatility of the substances. The column effluent was split in a ratio of 1:13 (by vol.) to an FID and a glass trap cooled over liquid nitrogen. To avoid the condensation of water, the ends of the trap were closed between the GC runs with tubes containing phosphorous pentoxide (Merck, Darmstadt, FRG). The temperatures of the FID, of the injector and of the outlet, connected to the cooling trap, were held at 200 °C.

This was performed with a Carlo Erba gas chromatograph, using the capillaries OV-1701 and SE-54 (each 30 m × 0.32 mm, film thickness 0.3 µm). The AR glass capillaries were deactivated and coated according to Grob [25]. The flow of the carrier gas helium was 2.0 ml/min. The samples were applied by the on-column injection technique at 35 °C. After 2 min, the temperature of the oven was raised quickly (40 °C/min) to 50 °C, held 2 min isothermal, again raised at a rate of 6 °C/min to 230 °C and finally held at 230 °C for 10 min. At the end of the capillary, the effluent was split 1:1 (by vol.) into an FID and a sniffing port using deactivated but uncoated fused silica capillaries (40 cm × 0.3 mm). The FID and the sniffing port were held at a temperature of 220 °C. The splitter was flushed with helium for accelerating the split flows to 10 ml/min. Nitrogen (20 ml/min) was used as make-up gas for the FID. Retention indices (RI) were calculated from the retention times using a program for cubic spline interpolation [26, 27]. HRGC-MS analyses were performed using an MS-8230 (Finnigan MAT, Bremen, FRG) in tandem with the SE-54 or OV-1701 capillary [6].

Proton magnetic resonance spectra (^{1}H-NMR)

^{1}H-NMR spectra were recorded with Bruker spectrometers operating at 200 MHz and 360 MHz.

HRGC-effluent sniffing

The FD factors of the odourants were determined by HRGC-effluent sniffing [6] of the following dilution series: 200 µl of the original extract containing all the neutral volatiles from 200 g honey were diluted stepwise with dichloromethane. The HRGC-effluent sniffing was performed with aliquots of 0.3 µl using the capillary OV-1701. The TZ value of the capillary according to Kaiser [28] was >45 (for alkanes C_{10}/C_{11}). The dilution of the samples was determined on the basis of methyl decanoate as internal standard. The aromagram (logarithm of the FD factors of the odourants vs. their RI values) was plotted.

Odour threshold values

Odour threshold values were approximated by an olfactometric method [6] using (*E*)-2-decenal instead of hexanal as internal standard. HRGC was performed on the capillary OV-1701 or SE-54.

Results

Important odourants of linden honey

The volatile fraction of a sample of linden honey, which originated from Rumania, was isolated by extraction at room temperature and analyzed. The volatile fraction had the characteristic smell of this type of honey and its aromagram (Fig. 2) showed 20 odourants with FD factors of 16 and higher.

Extraction of the honey at room temperature did not yield enough material for the identification, especially, of the potent odourants 12, 13, and 17. Therefore, the volatile fraction of the linden honey was isolated by the SDE method which led to higher yields al-

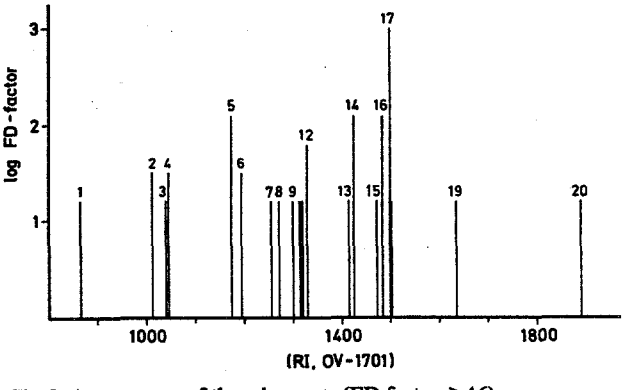

Fig. 2. Aromagram of the odourants (FD factor ≧16)

Table 2. Odourants (FD factor ≧ 16) identified in linden honey

No.	Compound	Fraction	RI on		Odour description	FD factor	Indentified earlier in honey
			OV-1701	SE-54			
1	1-Hexen-3-one	BIIb	865	775	metallic, like cooked vegetables[a]	16	–
2	2-Acetyl-1-pyrroline[b]	BIII	1013	923	roasty	32	–
3	Dimethyl trisulphide	Ad	1038	974	sulphurous, cabbage-like	16	–
4	Methional[b]	BIII	1041	909	like cooked potato	32	–
5	Phenylacetaldehyde	BIId	1174	1053	honey-like	128	[5, 29–31]
6	Linalool	BIIIg	1195	1100	flowery	32	[29, 32]
8	2-Phenylethanol	D	1272	1118	honey-like, spicy	16	[5, 29–34]
9	p-Cresol[c]	C	1302	1075	phenolic, musty	16	[5]
10	3,9-Epoxy-1-*p*-menthene[c,d]	BIIIe	1315	1235	flowery, mint-like	16	–
11	4-Methylacetophenone	BIIf3	1320	1191	spicy, almond-like	16	–
12	Linden ether[d]	BIIe	1330	1252	flowery, mint-like	64	–
13	1,3-*p*-Menthadien-7-al	BIIg2	1415	1293	fatty, spicy	16	–
14	*p*-Anisaldehyde	C	1424	1263	mint-like, sweet	128	–
15	4-Vinylguaiacol	BIIIf	1472	1323	spicy	16	–
17	(*E*)-*β*-Damascenone[e]	BIIc	1499	1395	honey-like, fruity, sweet	1024	–
18	Eugenol	BIIIe	1500	1364	spicy, honey-like	16	[5, 19, 30]
19	Vanillin	C	1635	1410	vanilla-like, sweet	16	–

The honey originated from Rumania. The compound numbers indicate peaks in the aromagram (Fig. 2). The fraction given is that in which most of the compound appeared after enrichment (column chromatography, HPLC). The odour description was assigned during aroma extract dilution analysis. Compounds were identified by comparison it with the reference substance on the basis of the following criteria: RI on the two capillaries, mass spectra obtained by MS (EI) and MS (CI) and the odour quality which was perceived at the sniffing port, unless indicated otherwise

[a] Impact compound of the artichoke aroma [13]

[b] The MS signals of the substance were too weak for an interpretation, therefore the compound was only identified by comparing it with the reference substance on the basis of the RI on the two capillaries and the odour quality which was perceived at the sniffing port

[c] Most likely the (3*S*, 4*R*, 8*R*)-diastereomer

[d] The compound was identified by NMR measurements (unpublished results)

[e] The honey-like odour quality of *β*-damascenone was also reported by Kovats [39]

though under more drastic conditions, since the extraction was performed at higher temperature. Higher amounts (up to 20 kg) of the honey were worked up in batches. The volatile fraction obtained was separated by column chromatography and HPLC. The fraction or subfraction in which the odourant was sufficiently enriched for the identification is reported in Table 2.

Of the 20 potent odourants 14 appearing in the aromagram (Fig. 2) were identified on the basis of HRGC and MS data and on the agreement of the odour quality with that of the corresponding reference substance (Table 2).The odourant 17 showing the highest FD factor and the potent odourants 5 and 14 belong to this group of compounds. They were identified as (*E*)-*β*-damascenone, phenylacetaldehyde and *p*-anisaldehyde (Table 2).

As discussed in the next section, compound 12 was a typical odourant of linden honey. The MS(EI) (Fig. 3) indicated that it was a monoterpene with a relative molecular mass of 150. To determine the structure, approximately 2 mg was isolated from 20 kg honey and then purified by column chromatography, HPLC and preparative GC. Application of various NMR techniques led to results from which its structure was elucidated as 3,9-epoxy-1,4(8)-*p*-menthadiene. The details of the NMR, MS and the microchemical experiments which demonstrate the chemical identity of compound 12 will be published elsewhere.

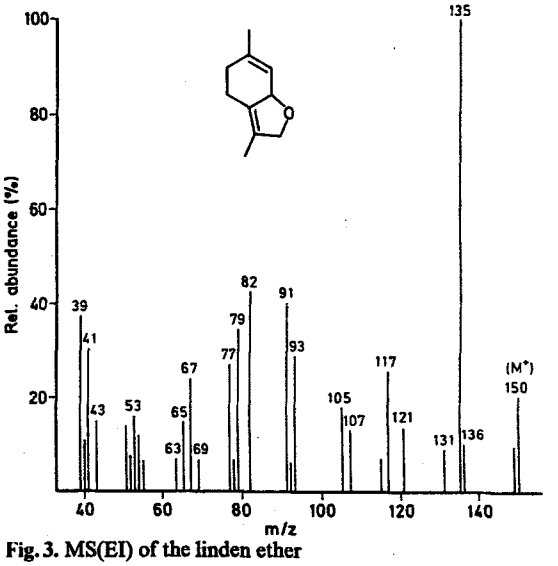

Fig. 3. MS(EI) of the linden ether

Compound 12 differed from compound 10, which was identified as 3,9-epoxy-1-*p*-menthene, by the occurrence of a second double bond. It was named "linden ether", since, to our knowledge, this monoterpene has not previously been described in the literature. As reported in Table 2, linden ether smells flowery and mint-like.

The MS(EI) data and the RI values of 3,9-epoxy-1-*p*-menthene (compound 10) on capillaries SE-54 and OV-1701 agreed with the corresponding data of (3*R*,4*S*,8*S*)-3,9-epoxy-*p*-menthene but, as shown in Table 5, the odour thresholds of both menthenes were different. It was therefore suggested that compound 10 was the (3*S*,4*R*,8*R*) stereoisomer.

Two odourants of the linden honey, 2-acetyl-1-pyrroline and methional, were identified only on the basis of HRGC data and the odour qualities (Table 2). The chemical structure of three odourants (7, 16, and 20) could not be clarified. Two volatile acids which smelled intensely (sweaty) were identified in the acidic fraction as 2-methyl- and 3-methylbutanoic acid (data not shown).

Differences from other honeys

The intensive odourants of the linden honey from Rumania were compared amongst others with those of a linden honey from China and with those of acacia and heath honeys. The results are displayed in Table 3. The linden honeys from Rumania and China differed only in the FD factors of the odourants, showing that their concentrations were different, e.g., the concentrations of phenylacetaldehyde, linden ether and *p*-anisaldehyde were higher in the linden honey from Rumania, whereas linalool, *p*-cresol and 4-vinylguaiacol predominated in the sample from China. The latter honey contained also a higher concentration of an odourant (compound 4a in Table 3), which, because of its low FD factor, was not noticed in the sample from Rumania. It smelled flowery and was identified as *cis*-rose oxide.

Most of the odourants occurring in the linden honeys were also found in the acacia and heath honeys. As shown in Table 3, (*E*)-β-damascenone, which appeared with the highest FD factor in all honey samples, belong to this group of compounds as well as 2-acetyl-1-pyrroline, methional, phenylacetaldehyde, 1-hexen-3-one, 2-phenylethanol, *p*-cresol, *p*-anisaldehyde, eugenol and vanillin.

cis-Rose oxide and the linden ether occurred in the linden honey samples in different concentrations (Table 3). These two monoterpenes were also detected in a total number of 11 linden honey samples which originated from different suppliers (data not shown).

Table 3. Comparison of linden, acacia and heath honey: FD factors of the primary odourants

No.	Compound	FD factor		
		Linden (Ru/Ch)	Acacia (Hu/Ch)	Heath (No/Uo)
1	1-Hexen-3-one	16/ 2	8/ 4	32/ 32
2	2-Acetyl-1-pyrroline	32/ 32	32/ 64	32/ 64
3	Dimethyl trisulphide	16/ 2	32/ 16	2/–
4	Methional	32/ 4	32/ 64	32/ 64
4a	*cis*-Rose oxide[a]	8/ 64	–/–	–/–
5	Phenylacetaldehyde	128/ 16	32/ 32	128/ 256
6	Linalool	32/ 128	32/ 32	–/–
7	U (nut-like, sweet)	16/ 2	16/ 16	32/ 32
8	2-Phenylethanol	16/ 16	64/ 32	32/ 32
9	*p*-Cresol	16/ 64	32/ 32	32/ 16
10	3,9-Epoxy-1-*p*-menthene	16/ 8	–/–	–/–
11	4-Methylacetophenone	16/ 8	–/–	–/–
12	Linden ether	64/ 16	–/–	–/–
13	1,3-*p*-Menthadien-7-al	16/ 2	–/–	–/–
14	*p*-Anisaldehyde	128/ 16	32/ 32	128/ 512
15	4-Vinylguaiacol	16/ 256	32/ 32	32/ 16
16	U (spicy)	128/ 256	64/ 64	256/ 16
17	(*E*)-β-Damascenone	≧1024/≧1024	≧1024/≧1024	≧1024/≧1024
18	Eugenol	16/ 8	16/ 16	32/ 32
19	Vanillin	16/ 4	16/ 16	16/ 32
20	U (spicy, fruity)	16/ 16	32/ 32	32/ 32

The numbers refer to Fig. 2. U indicates unknown odourants. Geographical origin of the honey: Rumania (Ru), China (Ch), Hungary (Hu), Norway (No), unknown origin (Uo)

[a] Not shown in Fig. 2

Neither compound was detected in the acacia and heath honeys, as reported in Table 3, and they were also absent in orange, rape and wild tobacco honeys (data not shown). 4-Methylacetophenone, 3,9-epoxy-1-*p*-menthene and 1,3-*p*-menthadien-7-al were also found only in linden honey, but they displayed quite low FD factors in most of the samples investigated.

Volatiles of linden blossoms

To investigate whether odourants of the linden honey occurred also in the flowers of the lime tree, the volatile fraction of linden blossoms was extracted and then analyzed by HRGC/MS. The compounds numbered in the gas chromatogram obtained (Fig. 4) were identified.

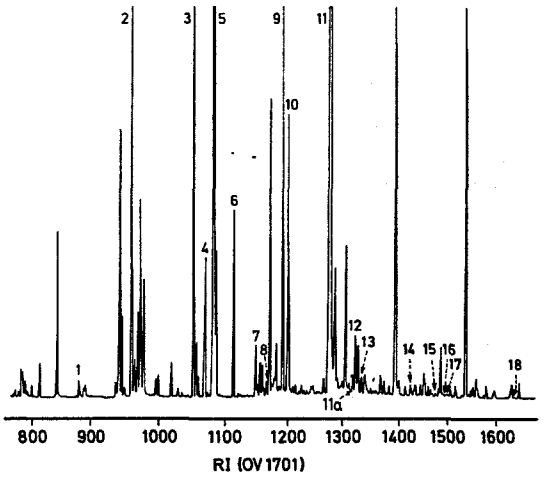

Fig. 4. Capillary gas chromatogram of the volatile fraction of linden blossoms. The numbered compounds were identified (Table 4)

The results, summarized in Table 4, indicated that the following odourants of the honey were also detected in the blossoms: *cis*-rose oxide, linalool, linden ether, 3,9-epoxy-1-*p*-menthene, *p*-anisaldehyde, 4-vinylguaiacol, (*E*)-β-damascenone, eugenol and vanillin. Some volatiles listed in Table 4 (e.g. *p*-cymene, benzyl alcohol, *a,p*-dimethylstyrene), were not investigated in the linden honeys, as they were such weak odourants they did not to appear in the aromagram (Fig. 2). On the other hand, γ-terpinene and terpinolene were only detected in the blossoms (Table 4) but not in the linden honey.

One of the major peaks in the gas chromatogram of the volatiles obtained from linden blossoms (no. 3 in Fig. 4) was identified as limonene. However, this monoterpene was lacking in the linden honeys (data not shown). Tsuneya et al. [19] have found *cis*- and *trans*-8-*p*-menthen-1,2-diol in linden honey. During the production of the honey from the nectar, the diol might be formed by hydroxylation of the limonene. We detected *trans*-8-*p*-menthen-1,2-diol (RI = 1545 on OV-1701 and 1348 on SE-54) whose MS is shown in Fig. 1, in all linden honey samples but not in the honeys of other botanical origin. The odourless diol was not found in the extract obtained from the blossoms.

Table 4. Volatile compounds of linden blossoms

No.	Compound	RI on		Identification		
		OV-1701	SE-54	CO	MS	OQ
1	Hexanal	881	797	+	+	+
2	(*E*)-2-Hexenal	957	854	+	+	+
3	Limonene	1051	1033	+	+	−
4	*p*-Cymene	1070	1027	+	+	−
5	γ-Terpinene	1087	1062	+	+	−
6	Terpinolene	1112	1090	+	+	−
7	*α,p*-Dimethylstyrene	1157	1118	+	+	−
8	*cis*-Rose oxide	1169	1117	+	−	+
9	Linalool	1195	1100	+	+	+
10	Benzyl alcohol	1220	1039	+	+	−
11	2-Phenylethanol	1272	1118	+	+	+
11a	3,9-Epoxy-1-*p*-menthene	1315	1235	+	−	+
12	*p*-Cymene-8-ol	1325	−−	−	+	−
13	Linden ether[a]	1330	1252	+	+	+
14	*p*-Anisaldehyde	1423	1263	+	−	+
15	4-Vinylguaiacol	1472	1323	+	−	+
16	(*E*)-β-Damascenone	1499	1395	+	−	+
17	Eugenol	1500	1364	+	−	+
18	Vanillin	1635	1410	+	−	+

The numbers refer to the gas chromatogram (Fig. 4). The compounds were identified by comparisons with the reference substance on the basis of the following: GC data on the two capillaries (*CO*), MS data (*MS*) and the odour quality (*OQ*), which was perceived in the sniffing port

[a] This compound was enriched by chromatography on silica gel (fraction F)

Odour thresholds

The odour thresholds of the aroma-active compounds of linden honey were evaluated (Table 5). Most of the compounds had an odour threshold below 1 ng/l (air). The lowest value was found for (*E*)-β-damascenone, which showed the highest FD factor of the volatiles of all honeys investigated. This result agrees with the very low value (0.002 µg/kg) reported by Buttery et al. [37] for the solution of (*E*)-β-damascenone in water. The odour threshold of 2-phenylethanol was approximately 10 times higher than that of phenylacetaldehyde. This suggests that reduction of the latter compound would lower the intensity of the honey flavour.

The odour threshold of the linden ether was compared with those of compounds similar in chemical structure. It was found that the threshold of the linden ether (1–2 ng/l) equaled that of (3*S*,4*R*,8*R*)-3,9-epoxy-1-*p*-menthene (0.8–1.6 ng/l) and it lies between that of the menthofuran (0.1–0.2 ng/l) and that of the (3*R*,4*S*,8*R*)-3,9-epoxy-1-*p*-menthene (20–40 ng/l). *cis*-Rose oxide, but not the *trans*-stereoisomer, appeared as an aroma-active compound in the aromagram of linden honey (Fig. 2). The much lower odour threshold of the *cis*-compared to the *trans*-rose oxide (Table 5), might be the reason that only the *cis*-isomer was perceived during GC sniffing of the linden honey volatiles.

Table 5. Odour thresholds of the volatiles identified in linden honey and of some related compounds

No.	Compound	Threshold (ng/l; air)
1	1-Hexen-3-one	0.02 – 0.04
2	2-Acetyl-1-pyrroline	0.02 – 0.04
3	Dimethyl trisulphide	0.06 – 0.1
4	Methional	0.1 – 0.2
4a	*cis*-Rose oxide	0.1 – 0.2
–	*trans*-Rose oxide	80 –170
5	Phenylacetaldehyde	0.6 – 1.2
6	Linalool	0.4 – 0.8
8	2-Phenylethanol	12 – 24
9	p-Cresol	0.3 – 1
10	(3*S*, 4*R*, 8*R*)-3,9-Epoxy-1-*p*-menthene	0.8 – 1.6
11	4-Methylacetophenone	2 – 3
12	Linden ether	1 – 2
13	1,3-*p*-Menthadien-7-al	1 – 2
14	p-Anisaldehyde	0.1 – 0.2
15	4-Vinylguaiacol	0.4 – 0.8
17	(*E*)-β-Damascenone	0.002– 0.004
18	Eugenol	0.2 – 0.3
19	Vanillin	0.6 – 1.2
–	(1*R*)-3,9-Epoxy-3,8(9)-*p*-menthadiene (menthofuran)	0.1 – 0.2
–	(3*R*, 4*S*, 8*R*)-3,9-Epoxy-1-*p*-menthene[a]	20 – 40
–	(3*R*, 4*S*, 8*S*)-3,9-Epoxy-1-*p*-menthene	20 – 40

The numbers refer to Fig. 2 and Table 2. The threshold range was established by the lowest and the highest value found in triplicates
[a] This diastereomer is denoted as dill ether [35, 36]

Discussion

The results indicated that (*E*)-β-damascenone, *p*-anisaldehyde, phenylacetaldehyde and a compound of unknown structure were the major odourants of honey, independent of its botanical origin. The high FD factor and the pleasant honey-like odour quality suggest that (*E*)-β-damascenone was the most important odourant of the flavour of honey. 2-Acetyl-1-pyrroline, methional, 1-hexen-3-one, 2-phenylethanol, *p*-cresol, 4-vinylguaiacol, eugenol and vanillin were further important odourants. The concentrations of the aroma compounds in the honey samples were different; they depended not only on the botanical but also on the geographical origin of the honeys.

A comparison of linden honey with those of other botanical origin revealed differences, especially in the fraction of monoterpenes. *cis*-Rose oxide and linden ether were detected exclusively in linden honey even of different geographical origin. However, the FD factors of each of the two compounds varied in a broad range, indicating that the relative concentrations were different in the various linden honey samples. Both odourants also occurred in the linden blossoms. Obviously the bees transported them without changing the chemical structure in the combs. This was in contrast to limonene, which was found only in the blossoms but not in the honey. On the other hand, *trans*-8-*p*-menthen-1,2-diol was detected in the volatile fraction of linden honey. The compound, which appeared odourless, might be an oxidation product of limonene.

On the basis of the results reported, linden ether, *cis*-rose oxide and *trans*-8-*p*-menthen-1,2-diol are suggested as indicator substances for the detection of linden honey. However, since their concentrations varied in linden honey samples, further research is necessary to establish the lowest concentrations which can be tolerated for the designation of a honey sample as "linden honey". Honeys originating from citrus plants can be identified on the basis of a determination of methyl anthranilate [38]. This study enlarges the use of indicator substances for the detection of the botanical origin of a honey.

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