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Floral origin markers of heather honeys: *Calluna vulgaris* and *Erica arborea*

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Abstract

'Heather' is commonly used to qualify honeys issuing from the *Ericaceae* family. As mellissopalynology and sensory assessments alone were not sufficient to authenticate their floral origin, volatile compounds from *Erica arborea* and *Calluna vulgaris* unifloral honeys were investigated. Flavours were isolated via dichloromethane solubilisation, followed by a Likens-Nickerson simultaneous steam-distillation/solvent extraction. The extracts exhibited an intense honey aroma, representative of their floral origin. Four hundred compounds were separated by gas chromatography and mass spectrometry. Among them, and in comparison with 11 other honey types, 4 proved to be markers of the *Ericaceae* family. Moreover, 3 were specific for *Calluna vulgaris* species and 3 others discriminant for the *Erica arborea* samples.

1. Introduction

The botanical origin of a honey, which greatly influences consumer preference, remains difficult to determine. Mellissopalynology and sensory assessments are routinely used but they are tedious and very dependent on expert ability and judgement (Persano Oddo et al., 1995). In some cases, moreover, pollen analysis is of no use, as with honeys derived from sterile plants. Another way to determine the floral origin is to identify molecules characteristic of the nectar of a certain type of flower, compounds which may or may not be biochemically modified by the bee (Bonaga and Giumanini, 1986). Volatile compounds (Steeg and Montag, 1988a, b; Blank et al., 1989; Häusler and Montag, 1990; Rowland et al., 1995; Bouseta et al., 1996; D'Arcy et al., 1997), flavonoids (Ferreres et al., 1993; Soler et al., 1995) and degraded carotenoids (Tan et al., 1989a,b) have proved adequate for identifying some floral origins.

In the case of heather honeys, much research has been devoted to identifying floral markers. Flavonoids such as myricetin, myricetin 3-methyl ether, myricetin 3′-methyl ether, and tricetin, have been considered possible markers of botanical origin (Ferreres et al., 1994). The floral source can be reliably authenticated on the basis of the presence of phenolic constituents such as ellagic acid (Ferreres et al., 1996a) or abscissic acid (Ferreres et al., 1996b). As regards volatile compounds, the norisoprenoid (S)-(+)-dehydrovomifoliol (4-hydroxy-4-[3-oxo-1-butenyl]-3,5,5-trimethylcyclohex-2-en-1-one) has been evidenced as an important quantitative marker of European heather honeys, where its concentration, ranging from 70 to 340 $\mu g\,g^{-1}$ 2,4-dichlorobenzaldehyde equivalent, was found to be 10–1000 times higher than in honeys of twelve other floral origins (Häusler and Montag, 1991). Similar levels have been measured in New Zealand heather (*Calluna vulgaris*) honeys (107–185 $\mu g\,g^{-1}$ methyl 3-phenyl-prop-2-enoate equivalent). In the latter, other 3,5,5-trimethylcyclohex-2-ene derivatives have been detected, their presence being currently attributed to carotenoid degradation (Tan et al., 1989a).

Some shikimate-pathway derivatives also seem adequate for authenticating heather honeys. Heather honeys could be distinguished from clover, Tasmanian leatherwood, rape, lime tree, and acacia honeys by their high phenylacetic acid and benzoic acid contents (Speer and Montag, 1984) and from rape, lime tree, chestnut, acacia, buckwheat, eucalyptus, orange, and sunflower honeys by their high 3,4,5-trimethoxybenzaldehyde and anisaldehyde contents (Häusler and Montag, 1990).

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In most of these studies, the authors were interested in authenticating honeys whose floral origin was 'heather', although this term is ambiguous. 'Heather' honeys, in fact, are produced from *Erica* species in Portugal, from either *Calluna* or *Erica* species in Spain and France, and from *Calluna* species in New Zealand (Ferreres et al., 1996b). Yet to our knowledge, no research has focused on establishing distinctive floral markers of different species within the *Ericaceae* family. In the present work, we have investigated the volatile compounds of heather honeys from two different species, *Calluna vulgaris* and *Erica arborea*, using an optimised Likens-Nickerson methodology that leads to organoleptically highly representative extracts (Bouseta and Collin, 1995). The aims were to compare concentrations of various compounds in these and 11 other types of honey and to find new, reliable markers for authenticating the floral origin of heather honeys.

2. Materials and methods

2.1. Honey samples

Twenty four heather unifloral honeys (18 *Calluna vulgaris* and 6 *Erica arborea*) were selected from various countries (*Calluna vulgaris* from France, Belgium, United-Kingdom, Norway and Germany; *Erica arborea* from France, Greece and Italy). Screening for floral purity was based on pollen analyses (Louveaux et al., 1978), sensory tests, conductivity, pH, titratable acidity (Journal Officiel, 1977), and sugar composition (Pourtallier and Rognone, 1977). All samples regarded as unifloral honeys met requirements defined in other studies (Maurizio, 1979; Crane et al., 1984; Gonnet and Vache, 1984; Accorti et al., 1986). The 11 samples of other unifloral origins (chestnut from France and Italy; *Eucalyptus* from Australia, Italy and Spain; fir from France; lavender from France and Spain; lime tree from France; orange blossom from France, Mexico and Spain; rape from Belgium and France; robinia from Canada, France, Hungary, Russia and Spain; rosemary from France and Spain; sunflower from Belgium and France; whiteclover from Canada and New Zealand; 10 of each) were also tested by the usual available physicochemical and sensory tests. All the samples were stored at 4°C until analysis.

2.2. Reagents

Acetoin (>99%) and hexanal (98%) were from Fluka Chemika (Buchs, Switzerland). Benzaldehyde (99+%), butyric acid (99+%), γ-butyrolactone (99+%), *trans*-cinnamic acid (99%), decane (>99%), decanoic acid (99%), 2,3-dimethyl-2-cyclopenten-1-one (99%), 5-(hydroxymethyl)furfural (99%), isophorone (97%), 4-methoxybenzaldehyde (98%), 4-methoxybenzoic acid (99%), 5-methylfurfural (99%), methyl vanillate (4-hydroxy-3-methoxybenzoic acid methyl ester) (99%), nonanoic acid (98%), octanoic acid (99%), phenylacetaldehyde (95%), phenylacetic acid (99%), 2-phenylethanol (99%), pyridine-3-carboxaldehyde (98%), and 3,4,5-trimethylphenol (99%) were from Aldrich Chemie (Steinheim, West-Germany). Acetophenone (99%), 2-acetylfuran, benzyl alcohol (99+%), cinnamyl alcohol (98%), 2-furaldehyde (99%), furfuryl alcohol (99%), 3-methyl-1-butanol (98%), 3-methyl-2-buten-1-ol (99%), 3-methyl-3-buten-1-ol (97%), and γ-valerolactone (98%) were from Janssen Chimica (Geel, Belgium). Indole (>99%) and 3-phenyl-1-propanol (99%) were from Merck (Darmstadt, Germany). Dichloromethane (99.9%) was from Romil Chemicals (Leics, England). Benzoic acid and dehydrovomifoliol (4-hydroxy-4-[3-oxo-1-butenyl]-3,5,5-trimethylcyclohex-2-en-1-one) were from Sigma Chemical (St-Louis, MO, USA). *n*-Hexanol (>97%), isovaleric acid, phenylacetic acid, and toluene (99%) were from UCB Chemical (Leuven, Belgium). Phenol was from US Biochemical (Cleveland, Ohio, USA). 2-Methylbutanoic acid was from Alltech Associates, Inc. (IL, USA). Pyridine was from Analyticals Carlo Erba (Milano, Italy).

2.3. Honey flavour extraction

Aroma compounds isolation was performed by a dichloromethane solubilisation, followed by a Likens-Nickerson steam distillation/solvent extraction according to the procedure described by Bouseta and Collin (1995). For each sample, two replicates were obtained.

2.4. Gas chromatography-FID analytical conditions

A Hewlett Packard Model 5890 gas chromatograph was used, equipped with a Hewlett Packard Model 7673 automatic sampler, a cold on-column injector, a flame ionization detector (or a thermionic NPD detector), and a Shimadzu CR4A integrator. Analysis of honey volatile compounds was carried out on a 50 m × 0.32 mm, wall-coated, open tubular (WCOT) CP-SIL5 CB (Chrompack, Antwerpen, Belgium) capillary column (film thickness, 1.2 μm). The oven temperature was programmed to rise from 30 to 85°C at 55°C min^{-1} then to 145°C at 1°C min^{-1} and to 250°C at 3°C min^{-1}. The carrier gas was He at 1.5 ml min^{-1}. The injector temperature was maintained at 3°C above the oven temperature. The detector temperature was 275°C. The minimum peak area for data acquisition was set at 500 $\mu V\,s^{-1}$. Olfactometric analyses were operated under the same conditions, by means of a sniffing outlet. Retention indices were determined by GC/FID by interpolation of the retention times of a *n*-alkanes (C_6–C_{19}) mixture analysed under identical conditions.

Table 1
Volatile compounds in heather honeys: *Calluna vulgaris* and *Erica arborea*[a]

Compounds	R.I.	*Calluna vulgaris*			*Erica arborea*			ID	Odour	FM
		Min	Max	Avg	Min	Max	Avg			
Acetoin	666	6	7549	1307	10	448	103	GC-MS		
3-Methyl-3-buten-1-ol	702	8	171	56	5	162	95	GC-MS		
3-Methyl-1-butanol	706	0	137	27	3	346	62	GC-MS		
Toluene	724	15	58	28	10	75	42	GC-MS		
Butanoic acid	745	9	587	109	7	193	122	GC-MS		
2-Methyl-2-buten-1-ol	754	0	58	14	0	24	9	GC-MS		
Hexanal	772	0	15	7	0	10	6	GC-MS	Cut grass	
2-Furaldehyde	805	46	333	128	118	348	240	GC-MS		
3-Methylbutanoic acid	812	0	553	130	9	240	73	GC-MS		
2-Methylbutanoic acid	819	31	577	177	1	57	26	GC-MS		
furfuryl alcohol	825	15	547	98	6	173	49	GC-MS		
1,3-Dimethylpyrazole[b] $M^+ = 96$	833	0	9	4	0	5	2	MS-NPD		
n-Hexanol	842	0	11	4	0	9	5	GC-MS		
γ-Butyrolactone	860	9	185	80	11	77	36	GC-MS		
2-Acetylfuran	883	3	54	13	6	182	59	GC-MS		
γ-Valerolactone	910	1	59	12	5	61	33	GC-MS		
5-Methylfurfural	935	3	29	10	0	27	14	GC-MS		
Benzaldehyde	940	41	259	103	7	98	41	GC-MS		
Phenol	956	0	267	70	0	18	5	GC-MS	Phenolic	
3-Pyridinecarboxaldehyde[b] $M^+ = 107$	968	0	227	26	0	9	4	MS		
Benzylalcohol	1015	100	725	353	32	265	143	GC-MS		
Phenylacetaldehyde	1021	64	508	165	5	122	46	GC-MS	Hyacinth	
Acetophenone	1043	1	54	22	0	8	3	GC-MS		
3-Methylfuranoate[b] $M^+ = 126$	1052	3	86	27	11	109	60	MS		
2-Phenylethanol	1087	78	1045	306	5	340	139	GC-MS	Rose	
3,5,5-Trimethyl-cyclohex-2-en-1-one (isophorone)	1097	50	1453	358	2	68	31	GC-MS		
Benzoic acid[c]	1155	2.83	61.1	23.2	1.95	63.8	15.1	GC-MS		H
Octanoic acid	1157	7	96	87	5	44	27	GC-MS		
5-(Hydroxymethyl)furfural	1175	10	272	61	20	394	99	GC-MS		
3-Phenyl-1-propanol	1201	4	174	79	2	61	21	GC-MS		
Benzoic acid hydrazone[b] $M^+ = 136$	1205	10	293	78	3	332	80	MS		
4-Methoxybenzaldehyde (*p*-anisaldehyde)	1224	0	0	0	66	2460	663	GC-MS	Gingerbread	Ea
Phenylacetic acid[c]	1230	7.5	168	75	0	0	0	GC-MS	Honey	Cv
Nonanoic acid	1253	0	59	5	0	0	0	GC-MS		
1-Methoxy-4-propyl-benzene[b] $M^+ = 150$	1266	0	0	0	65	1583	656	MS		
Indole	1269	5	106	31	0	15	6	GC-MS		
Cinnamyl alcohol	1275	23	628	165	5	56	173	GC-MS		
Decanoic acid	1348	4	418	68	8	294	72	GC-MS		H
Cinnamic acid[c]	1395	0.085	3.3	1.65	0.3	1.6	0.87	GC-MS		H
4-Methoxybenzoic acid (*p*-anisic acid)[c]	1396	0	0	0	13.1	1350	394	GC-MS		Ea
4-(3-Oxo-1-butynyl)-3,5,5-trimethyl-cyclohex-2-en-1-one[b] $M^+ = 204$	1474	8	890	180	0	0	0	MS		Cv
4-Hydroxy-3-methoxybenzoate methyl ester (methyl vanillate)[c]	1481	0	0	0	0.4	10	2.5	GC-MS		Ea
4-(3-Oxobut-1-enylidene)-3,5,5-trimethyl-cyclohex-2-en-1-one[b] $M^+ = 204$	1683	78	1079	537	9	773	27	MS		H
4-Hydroxy-4-(3-oxo-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one (dehydrovomifoliol)[c]	1757	31	310	124	0	0	0	GC-MS		Cv

[a] R.I. = retention indice; min, max, avg = minimal, maximal, average concentrations in the honeys expressed in $ng\,g^{-1}$; M^+ = molecular weight determined by chemical ionisation with methane; id = identification; GC = identification by gas chromatography (peak enhancement by coinjection of reference compound); MS = identification by mass spectroscopy (compared with NBS/EPA/NIH library); odour = results from the sniffing experiment; FM = floral marker of heather (H), *Calluna vulgaris* (Cv), or *Erica arborea* (Ea) honey.

[b] = concentrations in honeys expressed in $ng\,g^{-1}$ decane equivalent.

[c] = approximate concentrations in honeys expressed in $\mu g\,g^{-1}$ have been calculated taking into account the < 20% recovery factors.

2.5. Gas chromatography-mass spectrometry conditions

Chromatographic conditions were the same as those used for FID detection. The column was directly connected to an HP 5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV (filament current: 300 mA; electron multiplier voltage: 2500; scan rate: 4 s^{-1}; m/z range: 40–300). Spectral recording throughout elution was automatic using HP59970C software. Identification was on the basis of peak enhancement by coinjection with authentic standard compounds and comparison with the NBS/EPA/NIH mass spectra library. Chemical ionisation with methane was also achieved in order to determine the molecular weight of tentatively identified compounds.

2.6. Quantification of volatile compounds

Concentration of compounds in the honey samples was calculated with respect to the external standard, according to the equation:

$$C_i/C_e = (P_i/P_e)/(K_i \times 400) \quad (1)$$

where the suffix i and e refer, respectively, to the quantified compound and the external standard; P refers to the peak area obtained in GC; C refers to the concentration; 400 is the concentration factor calculated on the basis of volume ratio; K_i is the response factor at the FID detector of the compound i with regard to the external standard. In the case of tentatively identified compounds, quantifications were performed with regard to decane.

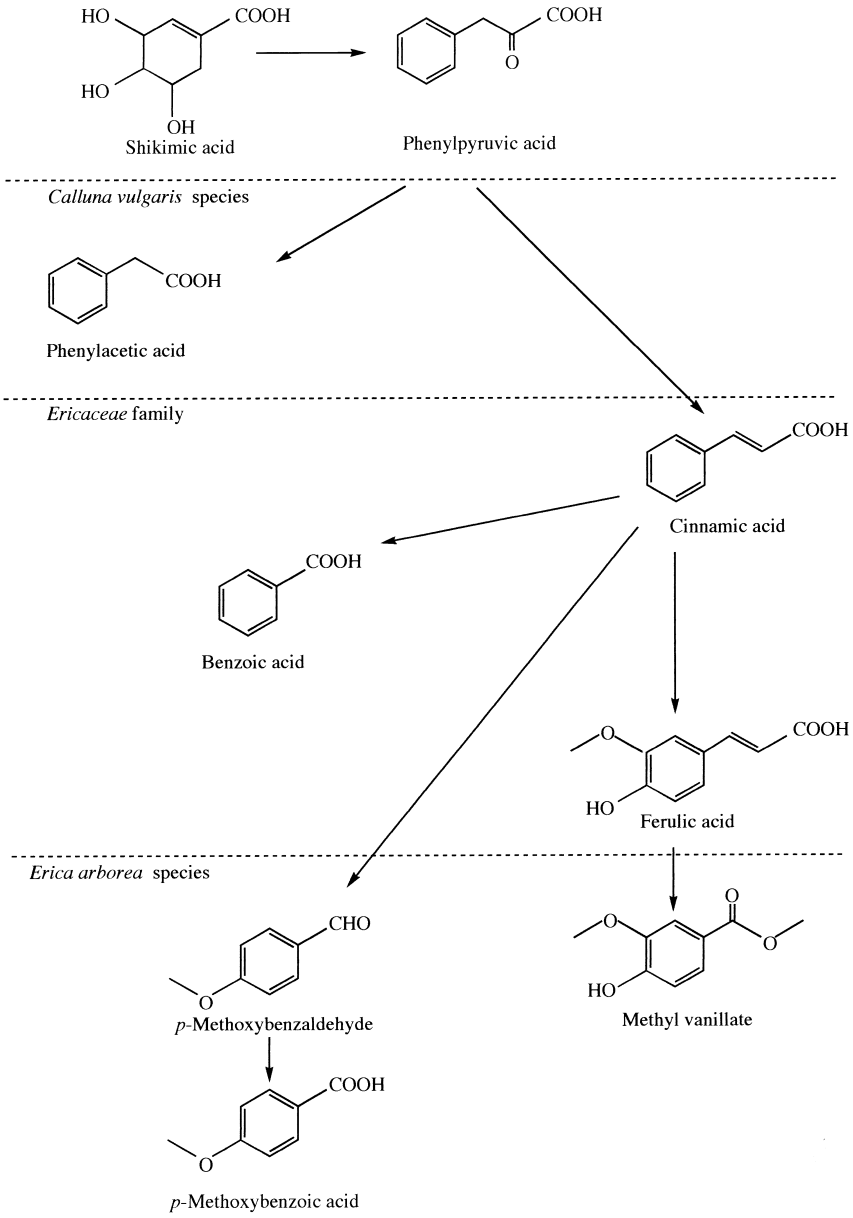

Fig. 1. Relationships between markers compounds issued from the shikimate pathway (adapted from Steeg and Montag, 1988a).

As previous analyses have shown that the Likens–Nickerson-derived method leads to recovery factors higher than 70% for most of the chemicals mentioned (Bouseta and Collin, 1995), concentrations were calculated with an extraction recovery factor equal to 100%. Similar values were here confirmed for three honey constituents: isophorone, 4-methoxybenzaldehyde and decanoic acid (respectively equal to 90, 84 and 70%). However, some polar compounds here investigated (benzoic acid, phenylacetic acid, 4-methoxybenzoic acid, cinnamic acid, methyl vanillate, and dehydrovomifoliol) are not accurately extracted with the Likens–Nickerson methodology (recovery factors lower than 20%). In that case, taking into account suspected losses, only approximate values are given, keeping in mind that other extraction techniques are needed for accurate quantification.

2.7. Statistical analyses

All the statistical analyses were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, NC). An analysis of variance (ANOVA) was used in order to determine significant differences among honeys. A Student-Newman-Keuls test was used to perform a multiple comparison of means.

3. Results and discussion

Forty-eight aroma compounds were identified by gas chromatography and/or mass spectrometry in *Calluna vulgaris* and *Erica arborea* honey extracts (Table 1). Most of the observed peaks do not constitute reliable markers, due to their presence in honeys of other origins. *Calluna vulgaris* and *Erica arborea* samples, however, can be authenticated on the basis of a few distinctive flavouring compounds, present at levels significantly different from those recorded in the other honeys ($p < 0.0001$; significance was determined by analysis of variance).

3.1. Ericaceae-family markers

'Heather' honeys (i.e. from floral sources belonging to the *Ericaceae* family) display high levels of aromatic carboxylic acids. Cinnamic acid, detected here at levels ranging approximately from 85 to 3300 ng g^{-1} (Table 1), is reported to be present in German and Scotch heather honeys at concentrations below 200 ng g^{-1} (Steeg and Montag, 1987). *Trans*-cinnamic acid is a shikimate-pathway derivative (Fig. 1), produced after deamination of L-phenylalanine by phenylalanine-ammonium lyase, an enzyme found exclusively in plants (Steeg and Montag, 1988a). Despite its absence in the 11 other investigated honeys (Fig. 2), cinnamic acid can be considered

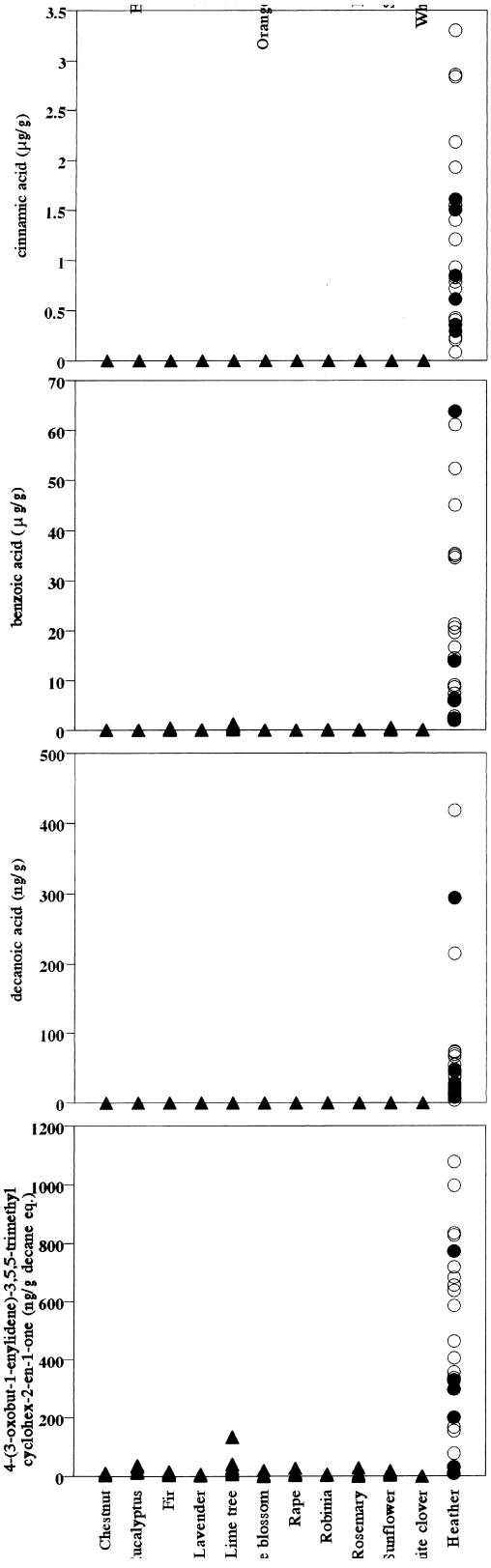

Fig. 2. Distribution of cinnamic acid, benzoic acid, decanoic acid and 4-(3-oxobut-1-enylidene)-3,5,5-trimethyl-cyclohex-2-en-1-one in the 134 honey samples. Keys: (●) *Erica arborea*; (○) *Calluna vulgaris*.; (▲) other monofloral honeys.

merely an indicator of floral origin because it is also present in buckwheat honey samples (where it is found at concentrations between 200 and 1000 ng g^{-1}) (Steeg and Montag, 1987).

Heather honeys could further be distinguished from non-heather samples on the basis of their content in benzoic acid, another aromatic carboxylic acid resulting mainly from cinnamic acid degradation (Fig. 1) (Steeg and Montag, 1988a). This compound was present in heather honeys at concentrations ranging from 2 to 64 μg g^{-1}, as opposed to less than 1.3 μg g^{-1} in the non-heather samples (Table 1 and Fig. 2). The approximate levels recorded here are similar to those previously reported by Speer and Montag (1984) and Steeg and Montag (1988c) for French, German, and Scotch heather honeys (11–216 and 5–90 μg g^{-1}, respectively) and by Tan et al. (1989a) for New Zealand *Calluna vulgaris* honeys (63–114 μg g^{-1}). *Calluna vulgaris* samples displayed higher levels of benzoic acid than *Erica arborea* samples, the difference being statistically significant ($p < 0.0001$). With a flavour threshold around 85 μg g^{-1} (Steeg and Montag, 1988a), benzoic acid could contribute to the aroma of heather honey. In our sniffing experiments, benzoic acid always appeared odourless, due to the low recovery of this compound in our extract.

Compounds derived from other metabolic pathways can also be used to distinguish heather honeys. Decanoic acid (4–418 ng g^{-1}; Table 1) was detected exclusively in heather honeys (Fig. 2). It has never been reported previously as a floral marker or even as a heather honey constituent, despite the presence of other linear fatty acids (Tan et al., 1988a). With a flavour threshold of 10 μg g^{-1} (Meilgaard, 1975), decanoic acid should not contribute to heather honey aroma.

4-(3-Oxobut-1-enylidene)-3,5,5-trimethyl-cyclohex-2-en-1-one (9–1079 ng g^{-1} decane equivalent), appears predominant in heather honeys, especially *Calluna vulgaris* honeys which display significantly higher concentrations of this compound than *Erica arborea* samples ($p < 0.0001$) (Table 1 and Fig. 2). Although decane equivalent concentrations up to 135 ng g^{-1} are found in lime-tree honeys, this compound can nevertheless be useful in authenticating the floral origin of heather-honey samples. Tan et al. (1989a) detected it in New Zealand *Calluna vulgaris* honeys. Despite its 'degraded-carotenoid-like' structure (3,5,5-trimethyl-cyclohex-2-ene), this substance probably arises through degradation of abscissic acid, a well-known growth hormone (Fig. 3) (Tan et al., 1988a). This hypothesis is supported by the fact that, to date, no intact carotenoids have been detected in heather honeys. Abscissic acid was recently reported as a potential marker of heather honeys due to its presence in the corresponding nectar (Ferreres et al., 1996b).

Isophorone (3,5,5-trimethylcyclohex-2-en-1-one) is another 3,5,5-trimethyl-cyclohex-2-ene-family derivative (Fig. 3) liable to contribute to authenticating heather honeys. This molecule is particularly abundant in *Calluna vulgaris* samples, where it can reach a concentration of 1453 ng g^{-1} (Table 1). High isophorone contents, however, were exceptionally observed in one sunflower and three eucalyptus honey samples (2353 ng g^{-1} and 711 ng g^{-1}, respectively), thus restricting the use of this compound as a floral marker.

3.2. Calluna vulgaris *markers*

Found exclusively in Calluna vulgaris honeys, phenylacetic acid appears as a major aroma compound, its

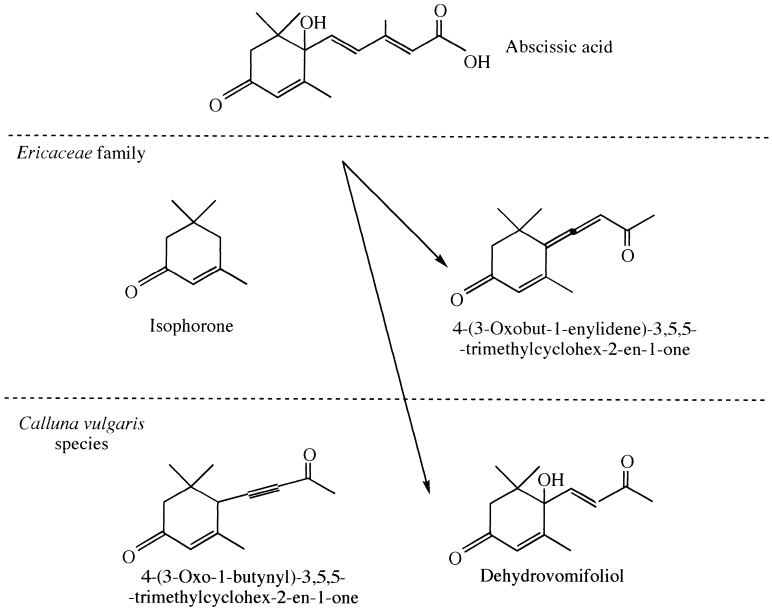

Fig. 3. Relationships between the 3,5,5-trimethylcyclohexene derivatives.

approximate concentration varying from 7.5 to 168 μg g^{-1} (Fig. 4 and Table 1). On several occasions, this molecule has been reported as a marker of heather honeys, but without any mention of differences between floral species. Concentrations of 47–977 μg g^{-1} (Speer and Montag, 1984), 0.6–242 μg g^{-1} (Steeg and Montag, 1987), and 12–242 μg g^{-1} (Steeg and Montag, 1988c) have been reported, in keeping with the present findings. Derived from the shikimate pathway (Fig. 1), phenylacetic acid exhibits a flavour threshold of 2.5 μg g^{-1} (Steeg and Montag, 1988a) and is currently described as displaying 'honey-like' notes. In agreement with these observations, this marker proved to be an odorant in our sniffing assessments of *Calluna vulgaris* honey extracts (Table 1).

From the data presented above, it emerges that two 3,5,5-trimethylcyclohex-2-ene derivatives are predominant in *Ericaceae*-family honey samples, especially *Calluna vulgaris* samples. Two other compounds of similar structure were found exclusively in *Calluna vulgaris* samples: dehydrovomifoliol (4-hydroxy-4-[3-oxo-1-butenyl]-3,5,5-trimethylcyclohex-2-en-1-one) and 4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohex-2-en-1-one (Table 1 and Fig. 4).

The dehydrovomifoliol contents determined in this study ranged from 31 to 310 μg g^{-1} (Table 1). Similar concentrations have been reported for European heather honeys (*Calluna* or unspecified): 70–340 μg g^{-1} 2,4-dichlorobenzaldehyde equivalent (Häusler and Montag, 1991) and for New Zealand *Calluna vulgaris* honeys: 107–185 μg g^{-1} 3-phenylprop-2-enoate equivalent (Tan et al., 1989a). Dehydrovomifoliol, the direct precursor of aroma compounds such as vitispirans and theaspirans (Häusler and Montag, 1991), is again suspected to result from abscissic acid degradation (Fig. 3) (Tan et al., 1989a).

As for 4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohex-2-en-1-one, it was detected in *Calluna vulgaris* honeys at concentrations ranging from 8 to 890 ng g^{-1} decane equivalent (Table 1). This compound has been isolated from New Zealand *Calluna* honeys where its concentration was found to range from 1100 to 1400 ng g^{-1} 3-phenylprop-2-enoate equivalent (Tan et al., 1989a). This 3,5,5-trimethylcyclohex-2-ene derivative can be considered a specific marker of *Calluna vulgaris* honeys.

3.3. Erica arborea *markers*

Erica arborea honeys can be distinguished from honeys of other origins essentially by the presence of certain shikimate-pathway derivatives. In the scope of the present study, 4-methoxybenzaldehyde (*p*-anisaldehyde) appears as an ideal marker because it is absent from honeys of other botanical origins (Fig. 5). This aroma compound might derive from cinnamic acids through cleavage of an acetate during formation of benzoic acids (Fig. 1) (Steeg and Montag, 1988a). Quantitatively, it is

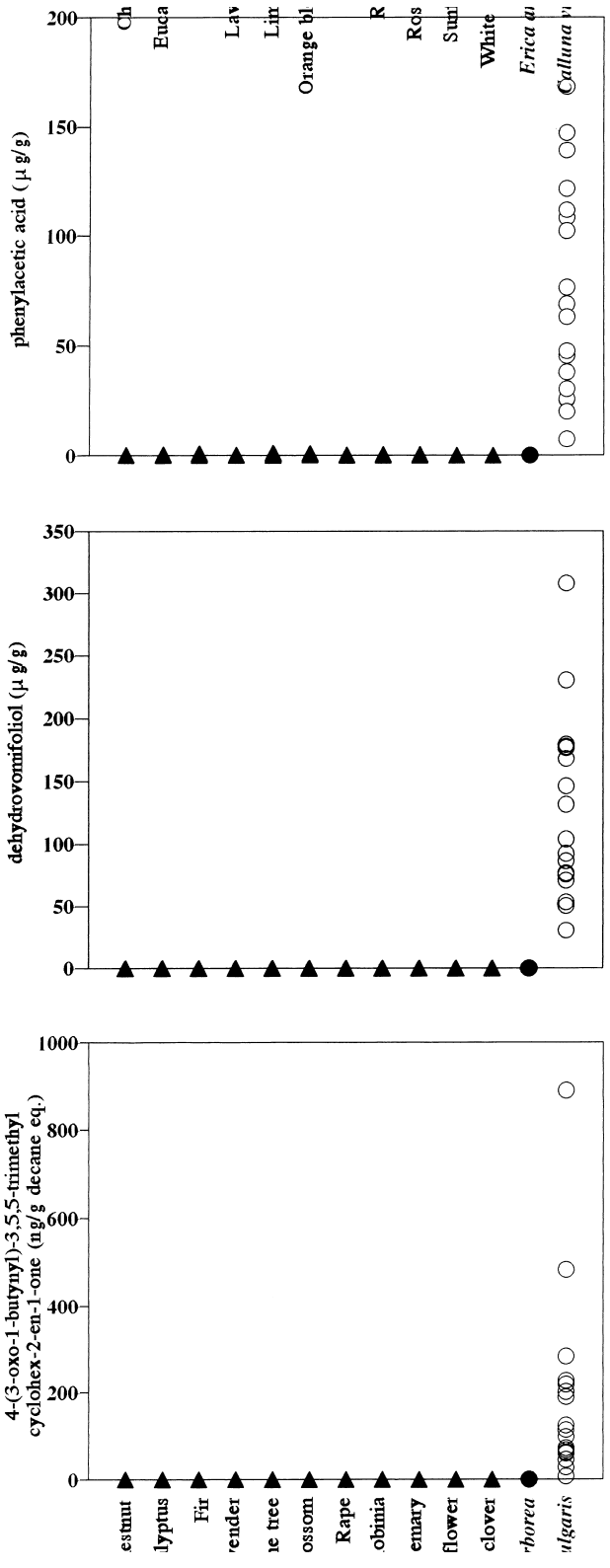

Fig. 4. Distribution of phenylacetic acid, dehydrovomifoliol, and 4-(3-oxo-1-butynyl)-3,5,5-trimethyl-cyclohex-2-en-1-one in the 134 honey samples. Keys: (●) *Erica arborea*; (○) *Calluna vulgaris*.; (▲) other monofloral honeys.

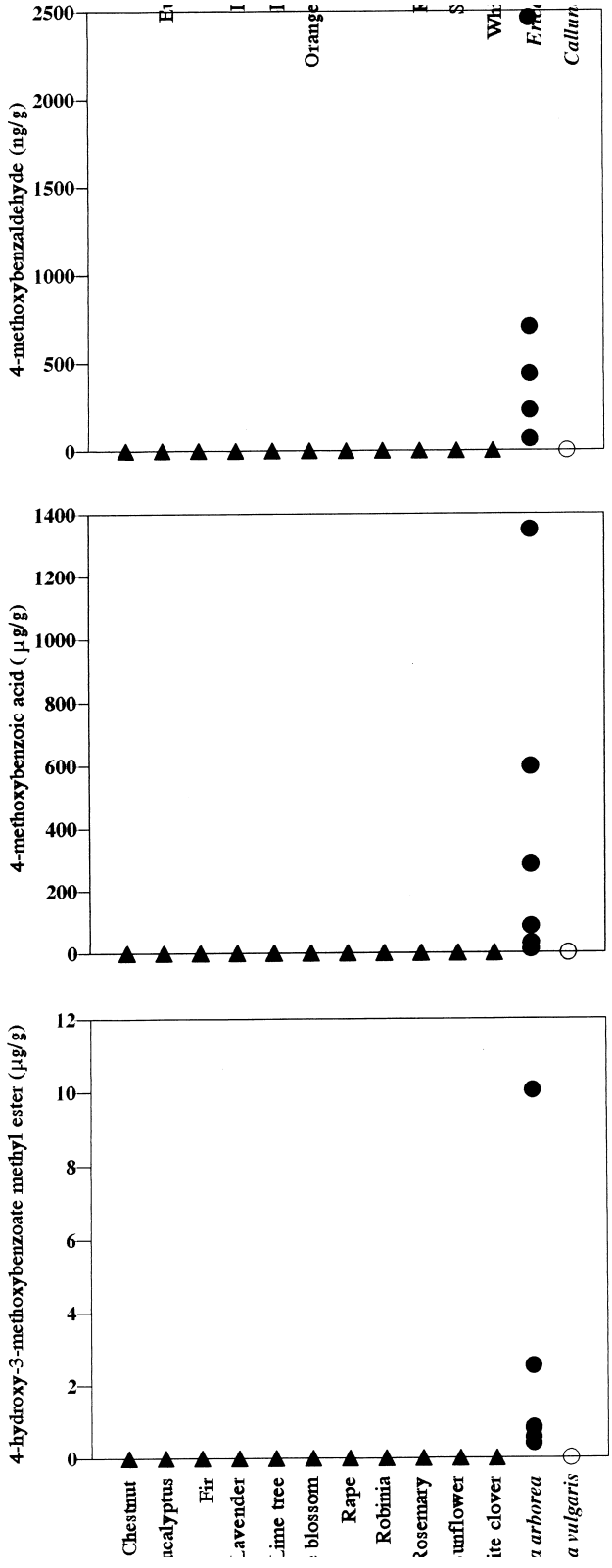

Fig. 5. Distribution of 4-methoxybenzaldehyde, 4-methoxybenzoic acid, and 4-hydroxy-3-methoxybenzoic acid methyl ester in the 134 honey samples. Keys: (●) *Erica arborea*; (○) *Calluna vulgaris*.; (▲) other monofloral honeys.

present in *Erica arborea* honeys at a concentration of 66–2460 ng g^{-1} (Table 1 and Fig. 5). Similar concentrations have been measured in European heather honeys surveyed by Häusler and Montag (1990). It is worth mentioning, however, that Tan et al. (1989b) found similar levels of this compound in New Zealand *Calluna vulgaris* honey samples. By aroma extract dilution analysis, Blank et al. (1989) determined it to be one of the most powerful odorants of heather honeys. Surprisingly, these authors also detected it in lime tree and acacia samples. At concentrations above the compound's perception threshold of 30 ng g^{-1} in aqueous solution (Häusler and Montag, 1990), gingerbread notes were detected in our sniffing experiments.

4-Methoxybenzoic acid (*p*-anisic acid) is another shikimate-pathway derivative. In all likelihood, its presence is related to the formation of its aldehyde homologue (Fig. 1) (Steeg and Montag, 1988a). Since this compound was not detected in any of the 128 other samples (Table 1 and Fig. 5), it emerges here, for the first time, as a specific marker of *Erica arborea* honeys (13–1350 µg g^{-1}).

4-hydroxy-3-methoxybenzoate methyl ester (methyl vanillate), evidenced for the first time in honey (Table 1 and Fig. 5), appears as another specific marker of *Erica arborea* samples, where its concentration was found to range from 0.4 to 10 µg g^{-1}. This aroma compound is probably related, like vanillic acid, to shikimate metabolism (Fig. 1) (Steeg and Montag, 1988a).

4. Conclusion

The presence of benzoic acid and decanoic acid in a honey reliably indicates a floral origin within the *Ericaceae* family. High levels of other compounds such as cinnamic acid, isophorone, and 4-(3-oxobut-1-enylidene)-3,5,5-trimethylcyclohex-2-en-1-one provide further authentication.

Honeys from different botanical species within the *Ericaceae* family are distinguishable on the basis of specific markers. Specific markers of *Calluna vulgaris* honeys are phenylacetic acid, dehydrovomifoliol, and 4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohex-2-en-1-one. These honeys are also usually characterised by higher levels of 3,5,5-trimethylcyclohexene derivatives than *Erica* honeys.

The presence of shikimate-pathway derivatives such as 4-methoxybenzaldehyde, 4-methoxybenzoic acid, and methyl vanillate would appear to prove irrefutably (within the limits of the floral origins studied) that the floral origin is *Erica arborea*.

To determine more accurate concentration limits for these specific markers, one should use another extraction protocol allowing high recovery of aromatic acids, methyl vanillate, and dehydrovomifoliol.

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