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Occurrence of Volatile Mono- and Sesquiterpenoids in Highland and Lowland Plant Species as Possible Precursors for Flavor Compounds in Milk and Dairy Products

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This paper examines the distribution of mono- and sesquiterpene secondary metabolites in 47 plants present in grazing areas from which cheeses are produced. In total, 54 terpenoid volatiles, derived from both highland and lowland plants, were obtained by a gaseous purge-and-trap system without the use of organic solvents and then identified by automated GC/MSD-FID analysis using mass spectral libraries, retention indices, and data acquired by the authors with authentic substances. Single ion monitoring proved valuable in identifying overlapping compounds with similar spectra for the subsequent semiquantitative determination of compositions. These are displayed as radar plots. It is suggested that flavor/aroma differences in cheeses produced from animals grazing in different areas and seasons arise from species ($n = 38$), phenological stages ($n = 8$), locations ($n = 4$), and seasonal variation in terpenoids present (the most common were *trans*-β-ocimene, limonene, and *trans*-β-caryophyllene). This hypothesis is supported by the fact that distinct variations in terpenoids with differing odor descriptors and abundances were found. It is also possible that terpenes with a higher polarity are less efficiently recovered by the purge-and-trap method than compounds of lower polarity. As the radar plots display relative concentrations based on detection limits, the relative efficiency of detection for different classes of compounds needs to be taken into consideration.

Keywords: *GC/MS; flavor; volatile; monoterpene; sesquiterpene; plant; highland; lowland; pasture; retention index*

INTRODUCTION

In regions where production costs are high, agriculture needs to produce food of superior quality. The resulting products can then be labeled according to the specific conditions that characterize their origin and manufacture. These regions can then be designated for producing cream, butter, yoghurts, and cheeses with Protected Designation Origin (PDO). In the area of production of hard cheese such as a Swiss Gruyère type, the relationship between grass quality and cheese is not well understood. The secondary metabolites, often abundant in the grass of natural highland pastures, presenting a highly diversified botanical composition, are constituents that may influence milk and therefore cheese quality. A pasture rich in dicotyledons, mostly located in the highland, is said to give cheese with flavor different from that produced from a pasture rich in gramineae, located in the lowland (Sehovic, 1988,1991). Terpenoids in plants are products of secondary metabolism and may be considered as biochemical indicators or markers to characterize cheeses originating from highlands. Dumont and Adda (1978) found sesquiterpenes only in Beaufort cheeses made from summer milk when cows were grazing on high-altitude pastures. Dumont et al. (1981) have postulated that the aroma of mountain cheeses (Comté) was influenced by the presence of terpenes and sesquiterpenes. Bosset et al. (1994) have confirmed these results, showing a significantly higher occurrence of terpenes and aliphatic hydrocarbons in Swiss Gruyère and L'Etivaz highland cheeses than in the corresponding lowland cheeses.

The study of the relationships between pasture (starting material), milk or cream (intermediates), and ripened cheese (final product) represents a basically new approach in dairy research within the framework of PDO labeling. The aim and scope of this work was to establish the composition of secondary metabolites contained in cow pastures that could influence the volatile (flavor) compounds of cream and Gruyère and L'Etivaz cheeses. Because of its complexity, this study was divided into different parts, which will be published successively. The first paper in the series paper (Jeangros et al., 1997) describes the project and deals with the outlines, goals, procedures, study sites, and techniques adopted in this work. It also briefly describes the soil characteristics, the metereological conditions, and the characteristics of the pasture and herds of cows as well as the method of cheese manufacture used at every site.

The present paper deals exclusively with the characterization of the terpenoid composition of plants growing in the corresponding highland and lowland. The choice of plants was based on the acceptance by the animal or palatability. The palatability of a botanical species (Table 1) is a characteristic that is visually estimated after the grazing of the cattle. It depends on many factors including the following: (a) animal species (bovines are less selective than ovines); (b) the available grass quantity (we have observed that the available

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Table 1. Overview of All Plants Collected from Highland and Lowland Pastures[a]

plant family	species*	location[b]	class	palatability level[c]	phenol. stage[d]
Apiaceae	***Carum carvi***	1	d	2	2
	Heracleum sphondylium	2	d	2	1
	Ligusticum mutellina^{S} [e]	3	d	3	6
Asteraceae	***Aposeris foetida***^{S}	1	d	1–2	5
	Aster bellidiastrum^{S}	1	d	1–2	5
	Leontodon hispidus^{S} A	1	d	3	1–2
	Leontodon hispidus^{S} B	2	d	3	1–2
	Leontodon hispidus^{S} C	3	d	3	5–6
	Leontodon helveticus^{S}	3	d	3	5–6
	***Leucanthemum vulgare* A**	2	d	1–2	3–4
	***Leucanthemum vulgare* B**	3	d	1–2	5–6
	Taraxacum officinale^{S}	2	d	3	7–8
	Crepis aurea^{S}	3	d	3	6
Campanulacea	*Phyteuma spicata*	1	d	2	3
	Campanula rhomboidalis	1	d	2	3
	Campanula barbata^{S}	3	d	1–2	5
Fabaceae	***Anthylllis vulneraria***	1	d	2	4–5
	Trifolium pratense^{S} A	1	d	3	1–2
	Trifolium pratense^{S} B	3	d	3	6–7
	Trifolium pratense^{S} C	4	d	3	5
	Trifolium repens^{S}	4	d	3	6
	Trifolium thalii^{S}	3	d	3	6
	Trifolium badium^{S}	3	d	3	6–7
	Lotus corniculatus^{S}	2	d	3	4–5
	Lotus alpinus^{S}	3	d	3	5
Geraniaceae	***Geranium sylvaticum***	3	d	1	6
Lamiaceae	***Ajuga reptans***^{S}	1	d	1	5
	Mentha longifolia	2	d	0	3
	Prunella vulgaris^{S}	3	d	1–2	6
Plantaginaceae	*Plantago alpina*^{S} A	1	d	3	6
	Plantago alpina^{S} **B**	3	d	3	7
	Plantago lanceolata^{S} A	1	d	3	5
	Plantago lanceolata B	2	d	3	5
Poaceae	***Dactylis glomerata* A**	1	g	3	2
	Dactylis glomerata B	2	g	3	4–5
	***Dactylis glomerata* C**	4	g	3	1
	Phleum alpinum	3	g	3	6
	Phleum pratense	4	g	3	1
	Festuca pratensis	4	g	3	1
	Lolium perenne	4	g	3	4
Polygonaceae	***Rumex alpestris***	2	d	2	4
Ranunculaceae	***Ranunculus aconitifolius***	3	d	1–2	7
Rosaceae	***Alchemilla xanthochlora***	1	d	1	4
	Alchemilla conjuncta^{S}	3	d	1	5
	Potentilla erecta^{S}	3	d	1–2	6
Rubiaceae	***Galium anisophyllon***^{S}	3	d	2	5
Scrophulariaceae	*Bartsia alpina*^{S}	3	d	1	7

[a] Boldface type indicates plants containing terpenoids. *Aeschiman and Burdet (1994). [b] 1, collected in Les Arpilles on June 29, 1995, at 1685–1900 m (highland pastures); 2, collected in Le Van on June 29, 1995, at 1400–1510 m (highland pastures); 3, collected in Les Clés on August 2, 1995, at 1690–1920 m (highland pastures); 4, collected in Posieux (Grangeneuve) on July 21, 1995, at 630 m (lowland pastures). [c] 0, unpalatable; 1, somewhat palatable; 2, palatable; 3, highly palatable. [d] Phenological stages: 1, (gramineae = g) tillering, (dicotyledons = d) ground leaves; 2, (g) stem elongation, (d) appearance of flower buds; 3, (g) beginning of inflorescence emergence, (d) stem elongation; 4, (g) inflorescence emergence, (d) beginning of flowering; 5, (g) end of inflorescence emergence, (d) flowering; 6, (g) flowering, (d) end of flowering; 7, (g) seed ripeness, (d) seed ripeness; 8, (g) seed dispersal, (d) seed dispersal. [e] s, small plants.

vegetation on the pasture was well grazed, i.e. without big food selection and practically no grass refusal); (c) stage of plant development (plants are better consumed when they are young); (d) plant species morphology (small flowers cannot be selected/detected by the animal); (e) growing conditions of the plant (influencing the chemical composition); (f) botanical composition (mixture of plants presenting different palatabilities).

For this study we have chiefly selected botanical species having the following characteristics: dicotyledons (richer in secondary metabolites than gramineae); species that have been generally well consumed by the animals (palatability levels 2 and 3; cf. Table 1).

Terpenes are widely used as biochemical markers in forest genetics (Boscherini and Michelozzi, 1993). Monoterpene composition is under genetical control and varies between individuals within source and within species. The terpene content is often used for chemotaxonomy and biodiversity investigations (Dung et al., 1995). Although a large number of studies on the analysis of terpenoids have been done, these are more concerned with the characterization of the essential oils contained in some particular plants for industrial uses rather than with the characterization of terpenes in pastures.

The biogenesis of terpenoids has been exhaustively reviewed (Loomis and Croteau, 1980; Croteau, 1981, 1987, 1993; Cane, 1981; Cori, 1983; Croteau and Johnson, 1985; Gershenzon and Croteau, 1990; Croteau and Karp, 1991.

MATERIALS AND METHODS

Choice of Species and Location. Four observation sites were included in this study, which was performed during the summer (June 29, July 21, August 2, 1995). Three observation sites were located at altitudes between 1400 and 1920 m (Les

Arpilles, Le Van et Les Clés) and one at ~600 m (Posieux/Grangeneuve). A more detailed description of these geographic sites and operating conditions is published elsewhere (Jeangros et al., 1997). Forty-one samples corresponding to 38 different species growing in highland pastures as well as 6 samples corresponding to 6 different species coming from the lowland pastures (600 m) were selected for analysis. In total, 47 samples belonging to 13 families (Apiacea, Asteraceae, Campanulaceae, Fabaceae, Geraniaceae, Lamiaceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae, Rubiaceae and Scrophulariaceae) were harvested.

Reagents and Chemicals. Milli-Q water was boiled under a continuous nitrogen flow for ~15 min with an electric heating device to strip off all residual volatile trace components.

Sample Preparation and Analysis. Dynamic headspace analysis using GC/FID/MSD techniques was performed for the qualitative and semiquantitative determination of mono- and sesquiterpenoids in the different plant species. This method was used to facilitate comparison of further results obtained (by the same technique) with milk/cream and cheese samples. Entire plants including stem, leaf, and flower(s), but not root, were placed in a cool box with dry ice and deep frozen at −18 °C immediately after collection. Shortly before analysis, a representative sample of ~10 g of this plant material was roughly cut into small pieces with scissors and finely dispersed in 90 mL of water with a high-speed homogenizer (Polytron PT 3000 used with a PT-DA 6030-6060 cutting system, Kinematica) running at 5000 rpm for 5 min. Finally, 20 mL of this mixture was carefully introduced into a 25-mL non-fritted sparger of the purge-and-trap extraction system. This mixture was continuously stirred to avoid a separation of the suspension into two phases.

Extraction of the Volatiles. The purge-and-trap system LSC 2000 (Tekmar, Cincinnati, OH) included a 25-mL non-fritted sparger (Schmidlin Co., part 14-2333-4SL, Neuheim, Switzerland), a trap [No. 8, containing a mixture of Carbosieve SIII (0.05 g) and Carbopack B60/80 (0.2 g)], and a cryofocusing unit. The moisture control module was not used. The operating conditions were as follows: purge gas, nitrogen; purge flow (vent), 30 mL/min; prepurge, 1.5 min; water bath, 45 °C; purge, 10 min; dry purge, 10 min; cap cool down, −125 °C; desorb preheat to 210 °C; desorb, 4 min at 220 °C; inject, within 1 min from −125 to 200 °C; bake, 5 min at 260 °C; six-port valve, 150 °C; line, 150 °C; capillary union heater (= transfer line from purge-and-trap to gas chromatograph), 150 °C.

Gas Chromatography. The instrument used was a Hewlett-Packard (HP) 5890 Series II. The operating conditions were as follows: carrier gas, helium; inlet pressure, 40 kPa; flow, ~1.6 mL/min at 45 °C; transfer line (from GC to MS), 280 °C; interface, direct inlet; temperature program, 13 min at 45 °C, raised at 5 °C/min to 240 °C, and 5 min at 240 °C; capillary column, SPB1-sulfur (Supelco), 30 m × 0.32 mm i.d.; film thickness, 4 μm.

Detectors. Two detectors were mounted in parallel by splitting the flow at the end of the capillary column (split ratio = ~1:1 at 45 °C), i.e. a Hewlett-Packard flame ionization detector (FID) and a mass sensitive detector (MSD, Model HP 5972), operating in the scan mode (TIC) from 19 to 250 amu at 2.9 scan/s, ionization by EI at 70 eV by autotuning; MS scan after 3.5 min. The MSD was used for the identification of the volatile (flavor) compounds (Figure 1) and the FID for their semiquantitative determination.

Software. MassLib (Chemical Concepts, Stefan A. Körnig, Mass Spectrometry, Weinheim, Germany) was the software used for the identification of the terpenoid compounds. This software features SISCOM (= Search for Identical and Similar **COM**pounds), which is able not only to identify similar spectra but also to retrieve spectra of similar structures, revealing much of the complex relationship between spectra and structures (Henneberg et al., 1993). The SISCOM approach, featuring multiple rankings and also a neutral loss search, often allows at least the identification of the structural class of compounds, even if no reference spectrum is available.

Identification of Terpenoids. The aim of this work was limited to the unambiguous identification of terpenoid constituents of plants by gas chromatography/mass spectrometry (GC/MS). No separation of enantiomers was carried out. Because of their strructural similarity (high similarity of the MS fragmentation patterns), the use of retention indices and further data treatment was necessary: semiautomatic treatment of the chromatograms using the combined information of mass spectra and retention indices was also helpful.

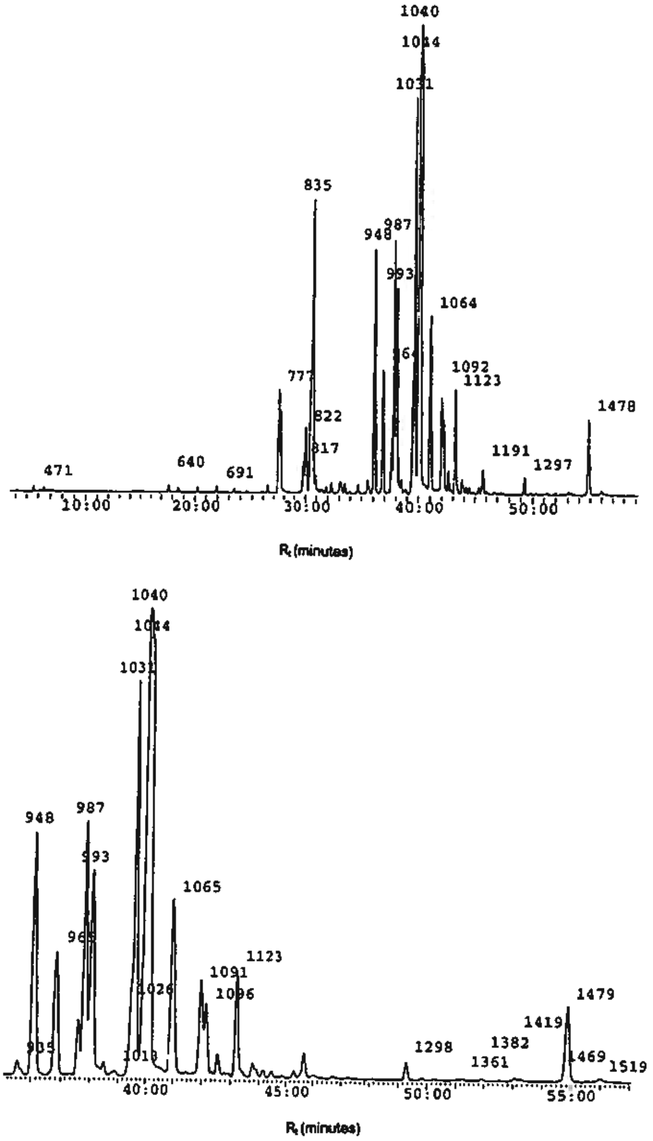


Figure 1. (A, top) Typical GC/MSD chromatogram of volatile compounds found in a highland plant (i.e. *Heracleum sphondylium*). (B, bottom) Expanded view of the section containing the terpenoids. The numbers indicate the relative retention indices, RI.

After the mass spectra were acquired and treated with the standard procedure of the GC/MS system (using the automated spectra treatment), certain peaks were not sufficiently identified. The mass spectra of several terpenes are very similar and different peaks were therefore identified as the same compound by the system. Some peaks showed a poor match with the library spectra. Therefore, a further peak treatment was necessary using the more sophisticated software package MassLib (Mariaca and Bosset, 1997). If available, authentic compounds were purchased and analyzed in the GC/MS system, and the resulting mass spectra and retention indices RI(m) were stored in our own spectral library. Due to the similarity of the mass spectra of several terpenes, a correct identification was only possible by using their retention indices such as RI(p), RI(l), and RI(m) (see Table 2).

The identification and semiquantitative determination of some peaks in the chromatograms proved difficult, especially when two or more compounds overlapped completely. Identification was made possible by examining the peaks for single

Table 2. List of Mono- and Sesquiterpenoids from Plants in Highland and Lowland Pastures

no.[a]	compound	RI(p) range (*n*)[b]	RI(l)[c]	RI(m)[d]	odor descriptors	CAS Registry No.[e]	ref material
1	*β*-myrcene	985−987 (8)	983^1	986	fragrant, fresh, hop note	123-35-3	Fluka 70049
2	*cis*-*β*-ocimene	1025−1030 (9)	1025^1	1029	fragrant, sweet, fresh, herbaceous note	13877-91-3	Fluka 74730
3	*trans*-*β*-ocimene	1039−1044 (16)	1038^1	1040	pleasant, warm herbaceous note	3779-61-1	Interchim 530685
4	4-*trans*-6-*trans*-alloocimene	1120−1124 (7)		1123	spicy herbaceous, diffusive	3016-19-1	Fluka 5700 [2 isomers] Fluka 5708 [4*E*−6*Z*]
5	linalool	1090−1093 (7)	1088^1	1090	flowery, citrus-like, sweet, woody	78-70-6	Fluka 62140
6	α-cyclocitral	1124 (1)		1124	lemon-like	432-24-6	Aldrich W 36,393-6
7	*β*-cyclocitral	1225 (1)	1219^2	1225	minty, fruity, green	432-25-7	Aldrich W 36,392-8
8	*β*-cyclogeraniumacid methyl ester	1235 (1)					
9	limonene	1037−1040 (13)	1023^1	1038	fruity, citrus-like, fresh, sweet	5989-27-5 (+) 2989-54-8 (−)	Fluka 62118 [*R*(+)] Fluka 62128 [*S*(−)]
10	*cis*-limonene 1,2-epoxide	1138 (1)		1137	fruity, eucalyptus-like, spicy, woody	4680-24-4	Fluka 62134
11	1,8-cineole	1039−1045 (4)	1033^2	1042	balsamic, sweet, fresh, eucalyptus	470-82-6	Fluka 46090
12	α-phellandrene	1011−1014 (4)		1013	citrus-like, fresh, herbaceous, sweet	4221-98-1	Fluka 77429 [*R*(−)]
13	α-terpineol	1197−1200 (2)	1177^1	1198	flowery-fruity, earthy, terpenic, lilac-like	7785-53-7 (+) 10482-56-1 (−)	Fluka 86481 [(+)] Fluka 86482 [(−)]
14	piperitone oxide	1259 (1)					
15	2-hydroxypiperitone	1309 (1)			powerful minty, tea-like, herbaceous		
16	2-hydroxyisopiperitone	1277 (1)					
17	α-terpinolene	1094−1096 (5)	1080^1	1096	flowery, fragrant, sweet	586-62-9	Interchim 0T667800
18	α-terpinene	1025 (1)	1009^1	1025	citrus-like, woody, terpeny	99-86-5	Fluka 86475
19	*γ*-terpinene	1061−1067 (9)	1049^1	1063	citrus-like, herbaceous, terpeny	99-85-4	Fluka 86478
20	*δ*-3-carene	1021−1025 (2)	1013^2	1022	sweet, fruity, pungent	498-15-7	Fluka 21986 [(+)]
21	*δ*-4-carene	1013 (1)	1001^2	1010	kerosene-, citrus-, and bergamot-like	554-61-0	Aldrich 23,238-6
22	*p*-cymene	1024−1027 (4)	1029^2	1027	weak, spicy herbaceous, citrus-like, fresh	99-87-6	Fluka 30040
23	sabinene	980−986 (9)	968^1	981	fragrant, woody, resinous	2009-00-9	Fluka 84085 [(+)]
24	*trans*-sabinene hydrate	1073 (1)	1060^1	1073	fresh, minty	17699-16-0	Interchim OS632782
25	α-thujene	933−935 (8)	924^1	934	woody, warm resinous	2867-05-2	Roth 5592.1
26	bornyl acetate	1297−1299 (2)	1272^1	1297	eucalyptus, minty, fresh, woody	5655-61-8	Aldrich B5,520-3 [(−)]
27	*endo*-borneol	1182−1184 (2)	1173^2	1182	camphor, dry woody	464-43-7	Fluka 15597 [(+)]
28	camphene	962−965 (5)	946^2	964	campherous, moth ball note	79-92-5 (+) 5794-04-7 (−)	Merck 820254 [(+)] Fluka 21290 [(−)]
29	tricyclene	936 (1)		936	woody, resinous, conifer-like	508-32-7	Fluka 91485
30	α-pinene	945−948 (9)	932^1	947	fragrant, fresh, conifer-like	7785-70-8 (+) 7785-26-4 (−)	Fluka 80599 [1*R*(+)] Fluka 80605 [1*S*(−)]
31	*β*-pinene	990−993 (8)	972^1	991	fragrant, fresh, conifer-like	19902-08-0 (+) 18172-67-3 (−)	Fluka 80607 [(+)] Fluka 80609 [(−)]
32	isopinocamphone	1171 (1)			warm camphoraceous, slightly spicy		
33	*trans*-pinocarveol	1155 (1)	1124^1	1155	warm woody balsamic, slightly fennel-like	547-61-5	Fluka 80613 [(−)]
34	myrtenol	1206 (1)		1207	camphor, malty, medicinal	19894-97-4	Fluka 70158 [(−)]
35	α-fenchene	959 (1)	940^1		fresh, camphor-like, fruity		
36	α-cubebene	1380−1382 (5)	1358^1	1382	fruity, sweet, citrus-like	17699-14-8	Fluka 28128 [(−)]
37	*β*-cubebene	1425−1427 (2)	1458^2				
38	α-copaene	1417−1420 (4)	1376^1	1419	fresh, earthy, mango-like	3856-25-5	Fluka 27814 [(−)]
39	*β*-elemene	1421−1422 (2)	1386^1				
40	α-humulene	1518−1519 (2)	1449^1	1518	hop-like, musty, woody	6753-98-6	Fluka 53675
41	*trans*-*β*-caryophyllene	1476−1479 (10)	1437^2		sickly sweet, wallflowers, woody-spicy	87-44-5	Fluka 22075
42	*β*-bourbonene	1431 (1)	1385^1				

Table 2 (Continued)

no.[a]	compound	RI(p) range (*n*)[b]	RI(l)[c]	RI(m)[d]	odor descriptors	CAS Registry No.[e]	ref material
43	not identified	1079 (1)					
44	not icentified	1112 (1)					
45	not identified	1128 (1)					
46	not identified	1181 (1)					
47	not identified	1186 (1)					
48	not identified	1191 (1)					
49	not identified	1201 (1)					
50	not identified	1204 (1)					
51	not identified	1348–1351 (3)					
52	not identified	1358–1361 (5)					
53	not identified	1457 (1)					
54	not identified	1467–1471 (5)					

[a] No., terpenoid number related to the radar plots. [b] RI(p), retention index of a specific terpenoid measured in plants harvested; (*n*), number of plant samples analyzed. [c] RI(l), retention index of a specific terpenoid found in the literature: 1, Kondjoyan and Berdagué (1996); 2, authors' library. [d] RI(m), retention index of a specific terpenoid measured with reference material. [e] CAS Registry No. were supplied by the authors.

ions (SIM) specific to one compound. Furthermore, MassLib offered the possibility of calculating the most probable composition of an overlapping peak by using regression analysis. The ratios are calculated as area percent. These values were used to calculate semiquantitative information based on the peak heights in the corresponding FID chromatograms. (All raw data are available from the last author.)

RESULTS AND DISCUSSION

Chromatographic Separation of the Volatile Compounds. Figure 1 show a typical GC/MS-FID separation of the volatiles found in plants analyzed with the dynamic headspace technique. The numbering of the compounds identified is given in the first column of Table 2, which is a list of the 54 terpenoids (mono- and sesquiterpenoids) identified in all of the plants analyzed (highland and lowland) as well as their relative retention indices or Kovats indices (RI) and odor descriptors.

Occurrence of Terpenoids in the Plants Analyzed. Table 1 lists the plant samples together with the dates and development stages (= phenological stages) at which they were sampled. The botanical composition of the highland pastures (locations 1–3) was richer in dicotyledoneous plants, while the composition of the pasture of the lowland (location 4) was richer in gramineae. Of the 47 plant samples analyzed, only 24 contained mono- and sesquiterpenoids (indicated by boldface type). Furthermore, 41 plant samples had a palatability level of >1, 26 had a palatability level of 3, and only 6 had palatability level of ≤1.

Figure 2 highlights the distribution of terpenoids found in all of the plants. Of the 41 plants occurring in the natural highland pasture, 22 plants contained 54 terpenoids. Of the 6 plants occurring in improved lowland grassland, only 2 species contained a common terpenoid.

The radar plot (Figures 3–5) overviews show the terpenoid fingerprints of each species based on its logarithmic relative peak intensities (heights), the black circle indicating the detection limit. Some plants of the highlands are very rich in terpenoids. Some of them, such as *Mentha longifolia*, contain up to 31 different terpenoids (see corresponding radar plot). This plant is certainly not consumed by cattle, but was nevertheless considered in this study as a reference for controlling the extraction procedure. Moreover, it is well documented in the literature due to its high terpenoid content. *Heracleum sphondylium* contains 21 terpenoids, etc.

Figure 6 shows the monoterpenoid distribution and Figure 7 the sesquiterpenoid distribution in the collected plants classified by terpenoid series. The acyclic monoterpene *trans*-*β*-ocimene in the volatile profile of 17 different plants seems to be an important plant constituent (Bergström et al., 1995), followed by limonene, occurring in 13 different species, and *trans*-*β*-caryophyllene (sesquiterpene), occurring in 11 different species. The occurrence of *β*-myrcene, *cis*-*β*-ocimene, *γ*-terpinene, sabinene, α-pinene, and *β*-pinene was also observed in 9 species. Only 8 species contained α-thujene and linalool.

Composition of the Same Botanical Species Collected at Different Locations and on Different Dates. Table 3 shows the terpenoid composition of some species collected on different dates and at different locations and phenological stages. *Leontodon hispidus* and *Trifolium pratense* gathered at three different sites on three different dates, as well as *Plantago lanceolata* collected at two different sites, did not contain any

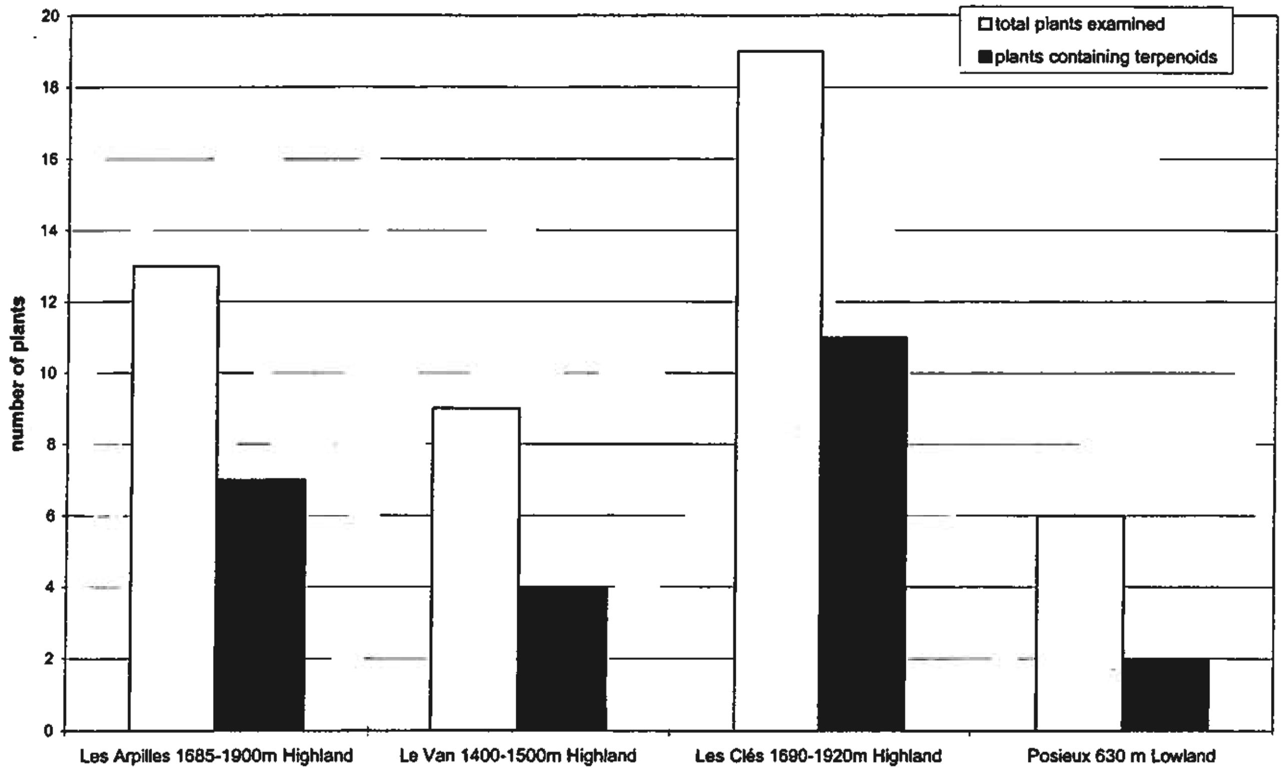


Figure 2. Comparison between total plants harvested and fraction of total plants containing terpenoids.

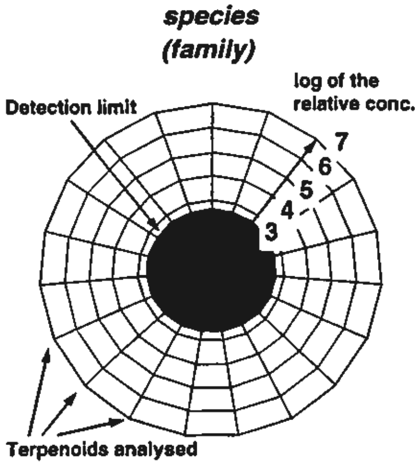


Figure 3. Description of the radar plots.

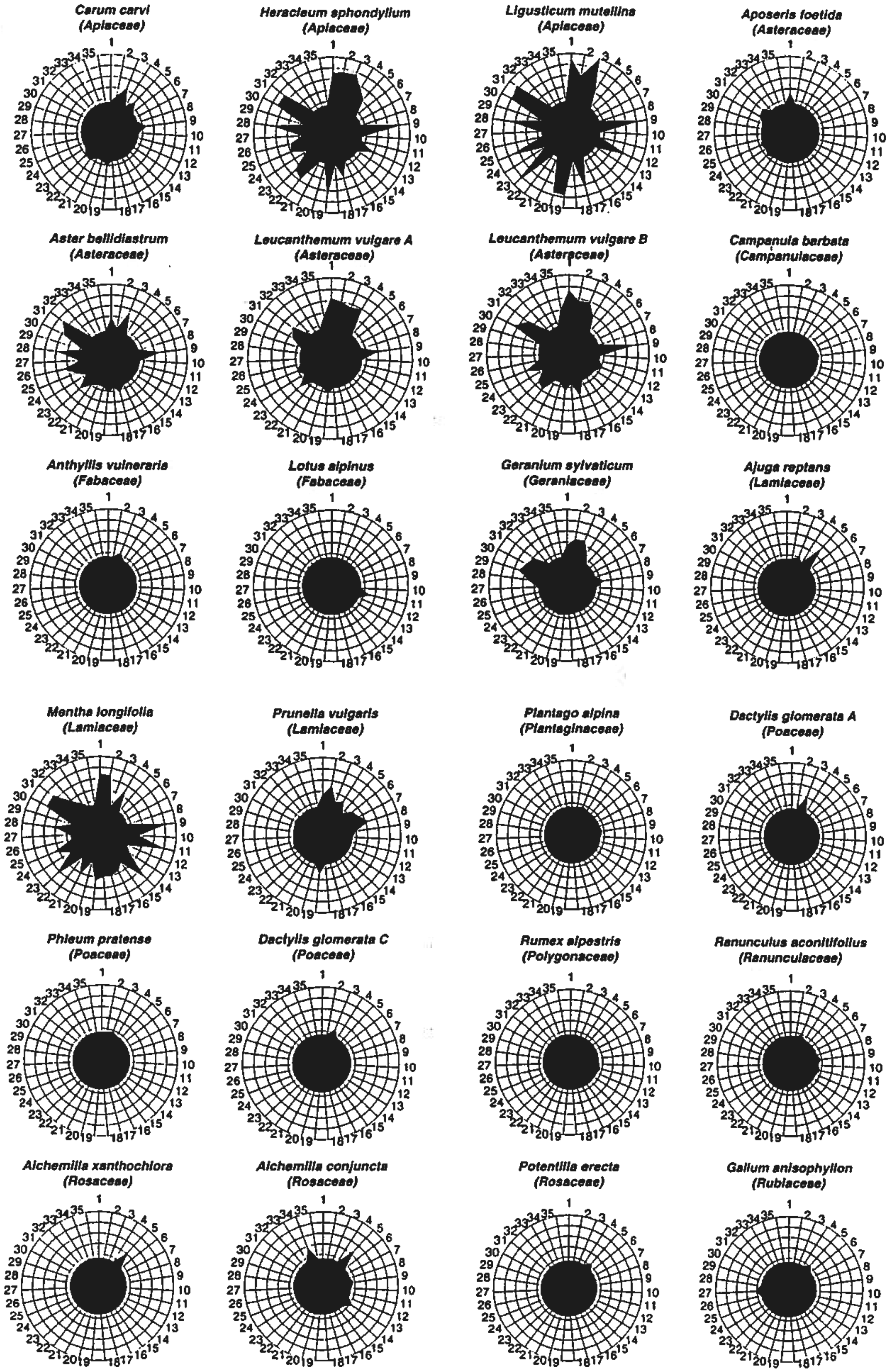
terpenoids. *Plantago alpina*, collected in Les Arpilles (location 1), had a different terpenoid composition from that collected in Les Clés (location 3) having a similar development stage. The former had no terpenoids, while the latter contained *trans*-β-ocimene and limonene in a 1:1 ratio. Since the concentration of these compounds is near the detection limit, the ratio is only approximate. With the harvest dates varying by about 1 month, *Dactylis glomerata* found in Les Arpilles (highlands) as well as the same species picked in Grangeneuve (lowlands) contained *trans*-β-ocimene. On the other hand, the local ecotype of Le Van (location 2) did not show any detectable amounts of terpenoids. As the phenological stages of the two varieties were not identical, a valid comparison of these plants is not possible.

Leucanthemum vulgare A found in Le Van (1400–1500 m) at the beginning of flowering contained 19 terpenoids as compared with 22 terpenoids in *L. vulgare* B found in Les Clés (1690–1920 m). The interesting similarity of the two plants of the same species but from different locations is well highlighted in the radar plot representation (see Figure 6). Both types have basically the same terpenoid profile. In addition to these common terpenoids, the plants picked in Les Clés also contain *p*-cymene and α-terpinolene. Since the phenological stages of both species were quite different, it is not clear whether the differences are caused by the location or by the phenological stages of these plants.

Review of Some Genera Considered in This Study. Study of the literature showed that the distribution of terpenoids in plants considered in this work is only partially known for the following genera:

Carum. Schantz and Ek (1971), in their investigation into *C. carvi* L., found a change in the terpenoid composition during the phenological development of the plant. Up to the stage of flowering limonene predominates. Thereafter carvone biosynthesis begins. In our study, we did not find any carvone, only limonene. As the species were harvested at an early stage, carvone biosynthesis had not yet begun (Schantz and Huhtikangas, 1971). The absence of carvone and/or carveole (Rothbächer und Suteu, 1972) could also be explained by the higher solubility of these substances in water. Being more polar, these compounds would be more difficult to purge. Fleisher and Fleisher (1988) also observed that the recovery rate of carvone decreased to almost zero during the steam distillation of the essential oil. However, we found that other mono- and sesquiterpenes such as *trans*-β-ocimene, *cis*-β-ocimene, *p*-cymene, γ-terpinene, sabinene, α-cubebene, β-elemene, β-humulene, and *trans*-β-caryophyllene did occur together in this species.

Heracleum. The family Apiaceae is a well-known source of pharmaceutically active compounds, such as the furocoumarins. The excretory canals, present in all of the aerial vegetative plant parts as well as in the fruits, produce mono- and sesquiterpenoids. The presence of mono- and sesquiterpenoids of such compounds in *H. sphondylium* L. subsp. *sphondylium* harvested in



Carum carvi (*Apiaceae*)

Heracleum sphondylium (*Apiaceae*)

Ligusticum mutellina (*Apiaceae*)

Aposeris foetida (*Asteraceae*)

Aster bellidiastrum (*Asteraceae*)

Leucanthemum vulgare A (*Asteraceae*)

Leucanthemum vulgare B (*Asteraceae*)

Campanula barbata (*Campanulaceae*)

Anthyllis vulneraria (*Fabaceae*)

Lotus alpinus (*Fabaceae*)

Geranium sylvaticum (*Geraniaceae*)

Ajuga reptans (*Lamiaceae*)

Mentha longifolia (*Lamiaceae*)

Prunella vulgaris (*Lamiaceae*)

Plantago alpina (*Plantaginaceae*)

Dactylis glomerata A (*Poaceae*)

Phleum pratense (*Poaceae*)

Dactylis glomerata C (*Poaceae*)

Rumex alpestris (*Polygonaceae*)

Ranunculus aconitifolius (*Ranunculaceae*)

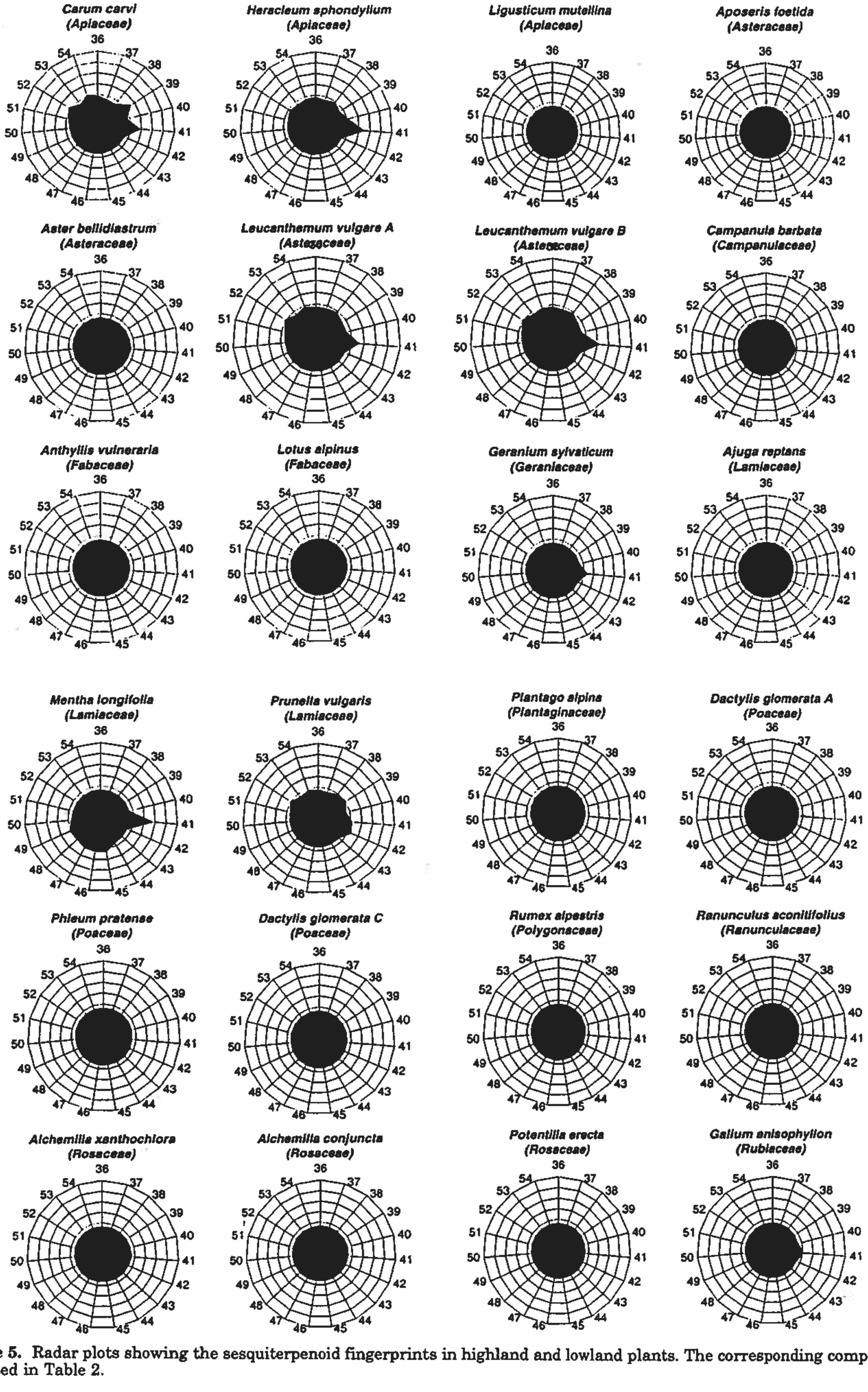
Alchemilla xanthochlora (*Rosaceae*)

Alchemilla conjuncta (*Rosaceae*)

Potentilla erecta (*Rosaceae*)

Galium anisophyllon (*Rubiaceae*)

Figure 4. Radar plots showing the monoterpenoid fingerprints in highland and lowland plants. The corresponding compounds are listed in Table 2.



Carum carvi (*Apiaceae*)

Heracleum sphondylium (*Apiaceae*)

Ligusticum mutellina (*Apiaceae*)

Aposeris foetida (*Asteraceae*)

Aster bellidiastrum (*Asteraceae*)

Leucanthemum vulgare A (*Asteraceae*)

Leucanthemum vulgare B (*Asteraceae*)

Campanula barbata (*Campanulaceae*)

Anthyllis vulneraria (*Fabaceae*)

Lotus alpinus (*Fabaceae*)

Geranium sylvaticum (*Geraniaceae*)

Ajuga reptans (*Lamiaceae*)

Mentha longifolia (*Lamiaceae*)

Prunella vulgaris (*Lamiaceae*)

Plantago alpina (*Plantaginaceae*)

Dactylis glomerata A (*Poaceae*)

Phleum pratense (*Poaceae*)

Dactylis glomerata C (*Poaceae*)

Rumex alpestris (*Polygonaceae*)

Ranunculus aconitifolius (*Ranunculaceae*)

Alchemilla xanthochlora (*Rosaceae*)

Alchemilla conjuncta (*Rosaceae*)

Potentilla erecta (*Rosaceae*)

Galium anisophyllon (*Rubiaceae*)

Figure 5. Radar plots showing the sesquiterpenoid fingerprints in highland and lowland plants. The corresponding compounds are listed in Table 2.

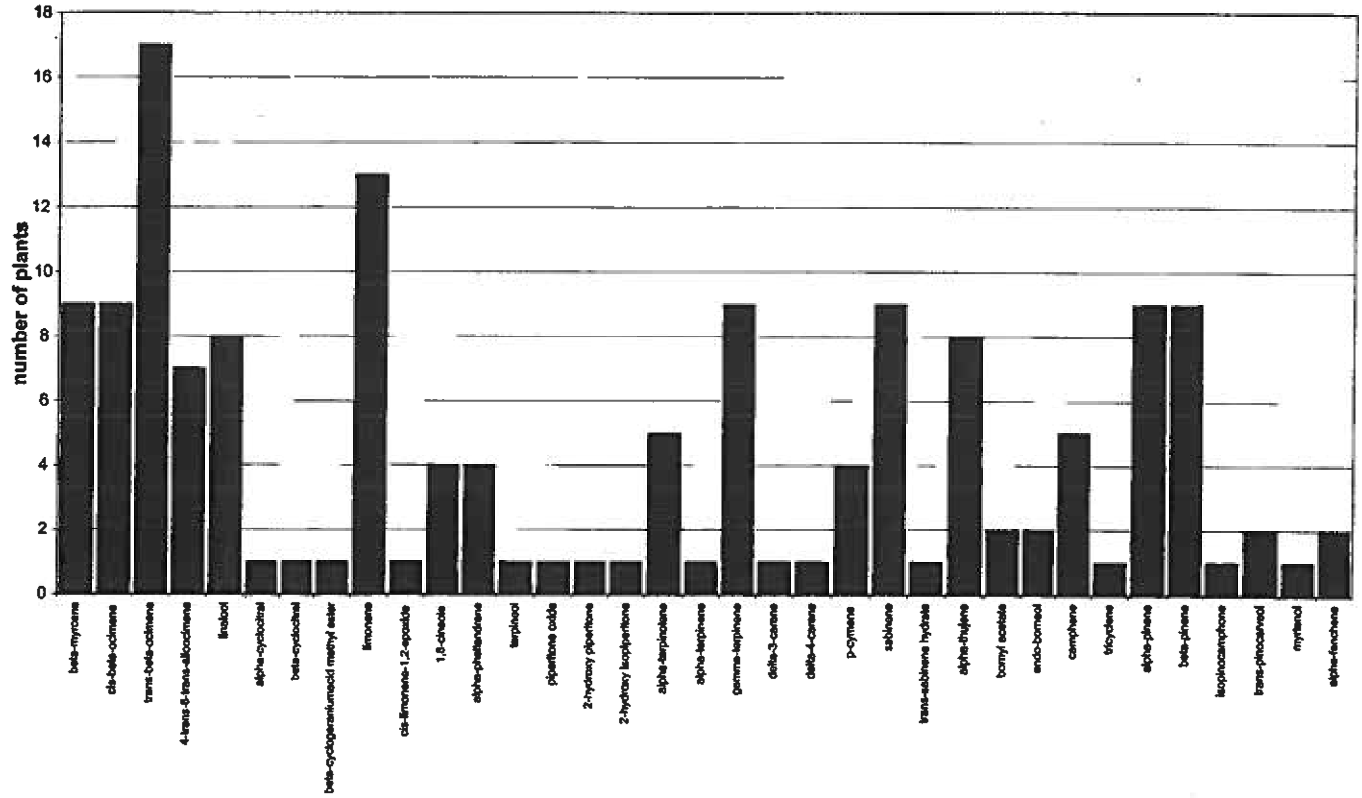


Figure 6. Distribution of monoterpenoids in all collected plants.

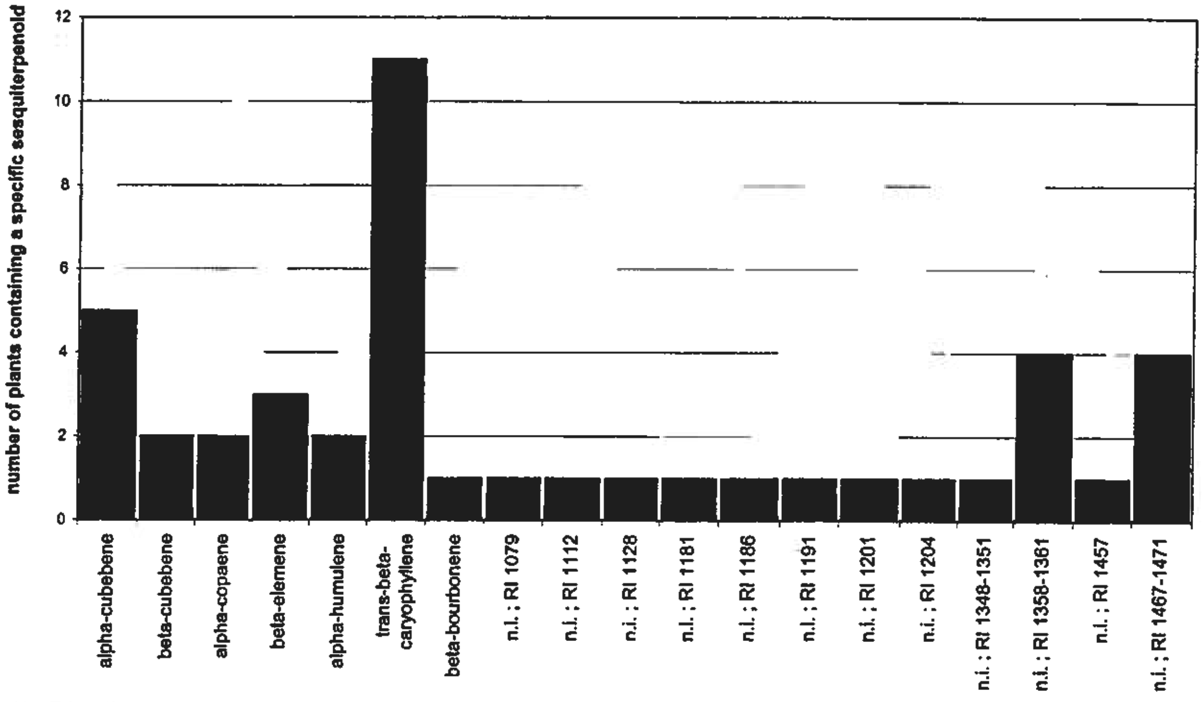


Figure 7. Distribution of sesquiterpenoids by chemical class in all plants collected. ni, not identified; RI, relative retention indices.

Trento, Italy, 1200 m, was reported by Bicchi et al. (1990). We found a similar qualitative terpenoid distribution as well as some additional terpenoids such as 4-*trans*,6-*trans*-alloocimene, linalool, δ-3-carene, and α-terpinolene. The relative distribution in our study was, however, quite different. The difference may be due to our extraction method (purge-and-trap extraction instead of steam distillation).

Mentha. The existence of different chemotypes, based on qualitative differences within a species, is a common feature in many genera of the Lamiaceae family (Kokkini, 1991). As far as the composition of *Mentha* essential oils is concerned, the differences may result in the definition of particular chemotypes. *cis-* and *trans*-piperitone oxide have been reported as main components of the essential oils of *M. longifolia* (L.) *Huds.* from Belgium, Italy (Shimizu and Ikeda, 1961), Germany (Baquar and Reese, 1965; Hefendhel and Nagell, 1975), Greece (Kokkini and Papargeorgiou, 1988), Egypt, France, Poland, southwestern Africa, and India (Lawrence, 1978). We also found 2-hydroxypiperitone (diosphenol) and its tautomer 2-hydroxyisopip-

Table 3. Terpene Composition of Some Species Harvested on Different Dates and at Different Locations and Phenological Stages

family	species[a]	location	class	phenol. stage	terpenes
Fabaceae	*Trifolium pratense* A	1	d	1–2	nd[b]
	B	3	d	6–7	nd
	C	4	d	5	nd
Plantaginaceae	*Plantago alpina* A	1	d	6	nd
	B	3	d	7	limonene (50%), *trans-β*-ocimene (50%)
	Plantago lanceolata A	1	d	5	nd
	B	2	d	5	nd
Asteraceae	*Leontodon hispidus* A	1	d	1–2	nd
	B	2	d	1–2	nd
	C	3	d	5–6	nd
	Leucanthemum vulgare A	2	d	3–4	19 different terpenes identified
	Leucanthemum vulgare B	3	d	5–6	same terpene composition as above plus α-terpinolene and *p*-cymene
Poaceae	*Dactylis glomerata* A	1	g	2	*trans-β*-ocimene
	B	2	g	4–5	nd
	C	4	g	1	*trans-β*-ocimene

[a] A, B, and C are local ecotypes. See Table 1 for details on location, class, and phenolic stage. [b] nd, not detected.

eritone (isodiosphenol), which are natural derivatives of piperitone oxide in *Mentha* (Shibata and Shimizu, 1974), as well as isopinocamphone, which is a *β*-pinene derivative. Research published to date suggests strongly that the qualitative production of mint essential oils is controlled by simple genetic systems (Kokkini, 1991). A dominant gene (*c*) is responsible for the formation of C-2 oxygenated compounds (such as carvone and dihydrocarvone), whereas the recessive cc genotype allows the formation of C-3 oxygenated compounds such as piperitenone and piperitone. The dominant gene (*Is*) causes the formation of *β*-pinene and isopinocamphone (Lincoln et al., 1986). Hefendehl and Murray (1976) suggested that the epoxidation of both piperitenone and piperitone is controlled by a recessive gene (*o*).

Ligusticum. No references to the terpenoid composition of *L. mutelina*, considered in this paper, could be found, only references to other species of *Ligusticum. L. porteri* (Delgado et al., 1992) contains α-pinene, α-phellandrene, limonene, *β*-phellandrene, α-terpinene, and *p*-cymene. *L. stewartii* Hiroe E. Nasir, a wild Umbellifer of Pakistan (Ahmad et al., 1986), contains α-pinene, *β*-pinene, camphene, *β*-myrcene, *δ*-3-carene, limonene, *γ*-terpinene, α-terpineol, borneol, bornyl acetate, geranyl acetate, etc. *L. thomsonii* (Ashraf and Bhatty, 1977) contains coumarins and the following terpenoids: α-thujene, α-pinene, camphene, *β*-myrcene, *δ*-3-carene, *β*-phellandrene, α-phellandrene, limonene, *γ*-terpinene, linalyl acetate, geranyl acetate, α-terpineol, and borneol as well as unidentified sesquiterpenes.

Aster. A. bellidiastrum does not seem to be described in the literature, only the following genera: genus *Aster spp.*, which contains germacrene, a sesquiterpene (Williams, 1994); *A. tataricus*, a Chinese medicinal plant that contains new monoterpene glycosides (Cheng and Shao, 1994; Nagao et al., 1988); *A. scaber* Thunb, which contains *β*-myrcene as the most abuntant volatile (Chung et al., 1993); *A. prealtus*, which has in addition to the known coumarins four new 7-*O*-monoterpene ether derivatives of umbelliferone, prealtine A D (Wilzer et al., 1989). These authors also describe the occurrence of monoterpenes in *A. bakeranus* as well as the occurrence of sesquiterpene hydrocarbons in *A. umbellatus* and *A. exilis*.

Plantago. No reference was found for *P. lanceolata* or *P. alpina*. *P. aquatica* contains two sesquiterpenoids, alismol and alismoxide (Oshima et al., 1983).

Prunella. P. vulgaris also contains some sesquiterpene lactones (Dmitruk et al., 1985) in addition to the 16 terpenoids that we identified.

Phleum. Apart from *trans-β*-ocimene that we identified in *P. pratense*, sesquiterpenoids (chokols A–E) were also described by Yoshihara et al. (1985), Koshino et al. (1989), and Yamauchi and Kakinuma (1989).

Leucanthemum. Chrysanthemum leucanthemum (= *Leucanthemum vulgare*) is characterized in the literature by the presence of sesquiterpene lactones (Zeller et al., 1985; Blotzyk and Drozdz, 1978).

Ranunculus. No reference to *R. acontifolius* could be found. However, *R. acris* contains *trans-β*-ocimene and various sesquiterpenes (Bergström et al., 1995).

Geranium. No reference to *G. sylvaticum* was found, only a reference to the sesquiterpenes occurring in *G. macrorrhizum* (Piet et al., 1995).

CONCLUSION

Alpine pasture, which is much richer in dicotyledons, generally contains many terpenoids, which were identified and compared semiquantitatively using a dynamic headspace technique linked to a GC/MSD/FID system. Lowland pasture, which contains more gramineae than dicotyledons, is poor in terpenoids.

The different terpenoid compositions found within the same species may be related to the different dates and locations of the harvest as well as to the different phenological stages. The phenological stage of each species is probably the dominant factor for the biosynthesis of the secondary metabolites. Some terpenoids are formed only at an advanced stage of plant development. No traces of carvones were found in *C. carvi*, probably because of the early phenological stage of the plant (Schantz and Ek, 1971).

The high abundance of *trans-β*-ocimene as well as of limonene and *trans-β*-caryophyllene in plants suggests that these terpenes could be used as biochemical markers for the following sequence: plant → animal → milk → cheese. The occurrence of *γ*-terpinene, α-thujene, α-pinene, *β*-pinene, and limonene is also relatively high in plants and could explain their presence in some mountain cheeses (Dumont and Adda, 1978; Dumont et al., 1981; Bosset et al., 1994).

A further paper in this series will deal with the occurrence of terpenoids and sesquiterpenoids in cream and cheese made from summer milk when cows were fed on pastures containing the plants studied in the present paper.

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Occurrence of Volatile Mono- and Sesquiterpenoids in Highland and Lowland Plant Species as Possible Precursors for Flavor Compounds in Milk and Dairy Products

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This paper examines the distribution of mono- and sesquiterpene secondary metabolites in 47 plants present in grazing areas from which cheeses are produced. In total, 54 terpenoid volatiles, derived from both highland and lowland plants, were obtained by a gaseous purge-and-trap system without the use of organic solvents and then identified by automated GC/MSD-FID analysis using mass spectral libraries, retention indices, and data acquired by the authors with authentic substances. Single ion monitoring proved valuable in identifying overlapping compounds with similar spectra for the subsequent semiquantitative determination of compositions. These are displayed as radar plots. It is suggested that flavor/aroma differences in cheeses produced from animals grazing in different areas and seasons arise from species ($n = 38$), phenological stages ($n = 8$), locations ($n = 4$), and seasonal variation in terpenoids present (the most common were *trans*-β-ocimene, limonene, and *trans*-β-caryophyllene). This hypothesis is supported by the fact that distinct variations in terpenoids with differing odor descriptors and abundances were found. It is also possible that terpenes with a higher polarity are less efficiently recovered by the purge-and-trap method than compounds of lower polarity. As the radar plots display relative concentrations based on detection limits, the relative efficiency of detection for different classes of compounds needs to be taken into consideration.

Keywords: *GC/MS; flavor; volatile; monoterpene; sesquiterpene; plant; highland; lowland; pasture; retention index*

INTRODUCTION

In regions where production costs are high, agriculture needs to produce food of superior quality. The resulting products can then be labeled according to the specific conditions that characterize their origin and manufacture. These regions can then be designated for producing cream, butter, yoghurts, and cheeses with Protected Designation Origin (PDO). In the area of production of hard cheese such as a Swiss Gruyère type, the relationship between grass quality and cheese is not well understood. The secondary metabolites, often abundant in the grass of natural highland pastures, presenting a highly diversified botanical composition, are constituents that may influence milk and therefore cheese quality. A pasture rich in dicotyledons, mostly located in the highland, is said to give cheese with flavor different from that produced from a pasture rich in gramineae, located in the lowland (Sehovic, 1988,1991). Terpenoids in plants are products of secondary metabolism and may be considered as biochemical indicators or markers to characterize cheeses originating from highlands. Dumont and Adda (1978) found sesquiterpenes only in Beaufort cheeses made from summer milk when cows were grazing on high-altitude pastures. Dumont et al. (1981) have postulated that the aroma of mountain cheeses (Comté) was influenced by the presence of terpenes and sesquiterpenes. Bosset et al. (1994) have confirmed these results, showing a significantly higher occurrence of terpenes and aliphatic hydrocarbons in Swiss Gruyère and L'Etivaz highland cheeses than in the corresponding lowland cheeses.

The study of the relationships between pasture (starting material), milk or cream (intermediates), and ripened cheese (final product) represents a basically new approach in dairy research within the framework of PDO labeling. The aim and scope of this work was to establish the composition of secondary metabolites contained in cow pastures that could influence the volatile (flavor) compounds of cream and Gruyère and L'Etivaz cheeses. Because of its complexity, this study was divided into different parts, which will be published successively. The first paper in the series paper (Jeangros et al., 1997) describes the project and deals with the outlines, goals, procedures, study sites, and techniques adopted in this work. It also briefly describes the soil characteristics, the metereological conditions, and the characteristics of the pasture and herds of cows as well as the method of cheese manufacture used at every site.

The present paper deals exclusively with the characterization of the terpenoid composition of plants growing in the corresponding highland and lowland. The choice of plants was based on the acceptance by the animal or palatability. The palatability of a botanical species (Table 1) is a characteristic that is visually estimated after the grazing of the cattle. It depends on many factors including the following: (a) animal species (bovines are less selective than ovines); (b) the available grass quantity (we have observed that the available

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Arpilles, Le Van et Les Clés) and one at ~600 m (Posieux/Grangeneuve). A more detailed description of these geographic sites and operating conditions is published elsewhere (Jeangros et al., 1997). Forty-one samples corresponding to 38 different species growing in highland pastures as well as 6 samples corresponding to 6 different species coming from the lowland pastures (600 m) were selected for analysis. In total, 47 samples belonging to 13 families (Apiacea, Asteraceae, Campanulaceae, Fabaceae, Geraniaceae, Lamiaceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae, Rubiaceae and Scrophulariaceae) were harvested.

Reagents and Chemicals. Milli-Q water was boiled under a continuous nitrogen flow for ~15 min with an electric heating device to strip off all residual volatile trace components.

Sample Preparation and Analysis. Dynamic headspace analysis using GC/FID/MSD techniques was performed for the qualitative and semiquantitative determination of mono- and sesquiterpenoids in the different plant species. This method was used to facilitate comparison of further results obtained (by the same technique) with milk/cream and cheese samples. Entire plants including stem, leaf, and flower(s), but not root, were placed in a cool box with dry ice and deep frozen at −18 °C immediately after collection. Shortly before analysis, a representative sample of ~10 g of this plant material was roughly cut into small pieces with scissors and finely dispersed in 90 mL of water with a high-speed homogenizer (Polytron PT 3000 used with a PT-DA 6030-6060 cutting system, Kinematica) running at 5000 rpm for 5 min. Finally, 20 mL of this mixture was carefully introduced into a 25-mL nonfritted sparger of the purge-and-trap extraction system. This mixture was continuously stirred to avoid a separation of the suspension into two phases.

Extraction of the Volatiles. The purge-and-trap system LSC 2000 (Tekmar, Cincinnati, OH) included a 25-mL nonfritted sparger (Schmidlin Co., part 14-2333-4SL, Neuheim, Switzerland), a trap [No. 8, containing a mixture of Carbosieve SIII (0.05 g) and Carbopack B60/80 (0.2 g)], and a cryofocusing unit. The moisture control module was not used. The operating conditions were as follows: purge gas, nitrogen; purge flow (vent), 30 mL/min; prepurge, 1.5 min; water bath, 45 °C; purge, 10 min; dry purge, 10 min; cap cool down, −125 °C; desorb preheat to 210 °C; desorb, 4 min at 220 °C; inject, within 1 min from −125 to 200 °C; bake, 5 min at 260 °C; six-port valve, 150 °C; line, 150 °C; capillary union heater (= transfer line from purge-and-trap to gas chromatograph), 150 °C.

Gas Chromatography. The instrument used was a Hewlett-Packard (HP) 5890 Series II. The operating conditions were as follows: carrier gas, helium; inlet pressure, 40 kPa; flow, ~1.6 mL/min at 45 °C; transfer line (from GC to MS), 280 °C; interface, direct inlet; temperature program, 13 min at 45 °C, raised at 5 °C/min to 240 °C, and 5 min at 240 °C; capillary column, SPB1-sulfur (Supelco), 30 m × 0.32 mm i.d.; film thickness, 4 μm.

Detectors. Two detectors were mounted in parallel by splitting the flow at the end of the capillary column (split ratio = ~1:1 at 45 °C), i.e. a Hewlett-Packard flame ionization detector (FID) and a mass sensitive detector (MSD, Model HP 5972), operating in the scan mode (TIC) from 19 to 250 amu at 2.9 scan/s, ionization by EI at 70 eV by autotuning; MS scan after 3.5 min. The MSD was used for the identification of the volatile (flavor) compounds (Figure 1) and the FID for their semiquantitative determination.

Software. MassLib (Chemical Concepts, Stefan A. Körnig, Mass Spectrometry, Weinheim, Germany) was the software used for the identification of the terpenoid compounds. This software features SISCOM (= **S**earch for **I**dentical and **S**imilar **COM**pounds), which is able not only to identify similar spectra but also to retrieve spectra of similar structures, revealing much of the complex relationship between spectra and structures (Henneberg et al., 1993). The SISCOM approach, featuring multiple rankings and also a neutral loss search, often allows at least the identification of the structural class of compounds, even if no reference spectrum is available.

Identification of Terpenoids. The aim of this work was limited to the unambiguous identification of terpenoid constituents of plants by gas chromatography/mass spectrometry (GC/MS). No separation of enantiomers was carried out. Because of their strructural similarity (high similarity of the MS fragmentation patterns), the use of retention indices and further data treatment was necessary: semiautomatic treatment of the chromatograms using the combined information of mass spectra and retention indices was also helpful.

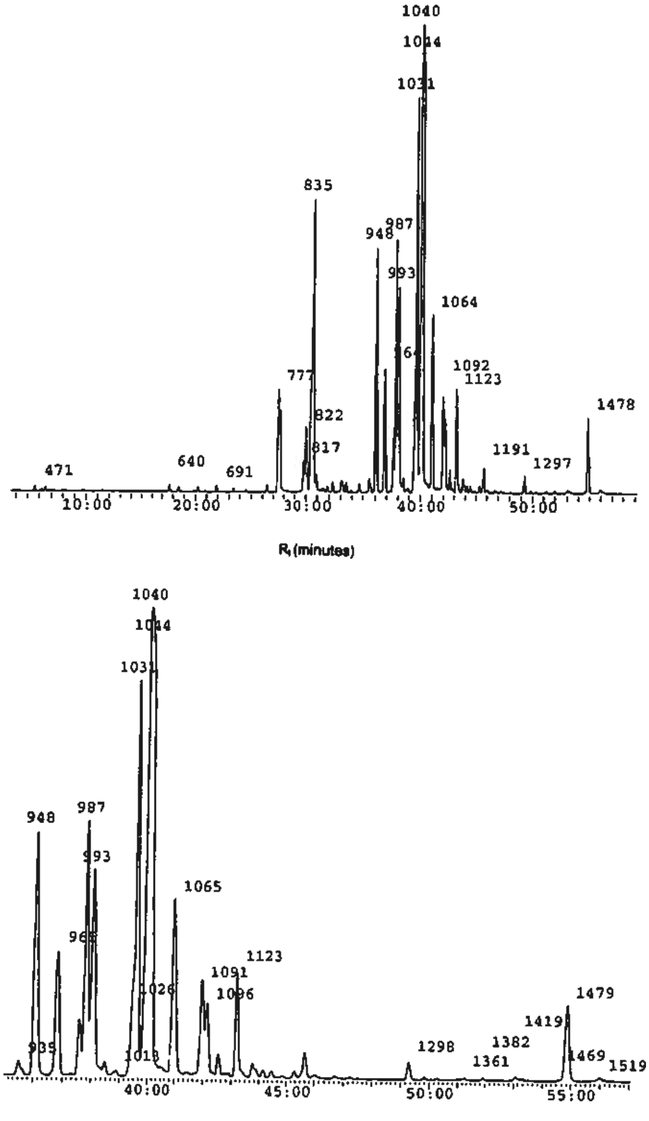

Figure 1. (A, top) Typical GC/MSD chromatogram of volatile compounds found in a highland plant (i.e. *Heracleum sphondylium*). (B, bottom) Expanded view of the section containing the terpenoids. The numbers indicate the relative retention indices, RI.

After the mass spectra were acquired and treated with the standard procedure of the GC/MS system (using the automated spectra treatment), certain peaks were not sufficiently identified. The mass spectra of several terpenes are very similar and different peaks were therefore identified as the same compound by the system. Some peaks showed a poor match with the library spectra. Therefore, a further peak treatment was necessary using the more sophisticated software package MassLib (Mariaca and Bosset, 1997). If available, authentic compounds were purchased and analyzed in the GC/MS system, and the resulting mass spectra and retention indices RI(m) were stored in our own spectral library. Due to the similarity of the mass spectra of several terpenes, a correct identification was only possible by using their retention indices such as RI(p), RI(l), and RI(m) (see Table 2).

The identification and semiquantitative determination of some peaks in the chromatograms proved difficult, especially when two or more compounds overlapped completely. Identification was made possible by examining the peaks for single

Table 2 (Continued)

no.[a]	compound	RI(p) range (n)[b]	RI(l)[c]	RI(m)[d]	odor descriptors	CAS Registry No.[e]	ref material
43	not identified	1079 (1)					
44	not identified	1112 (1)					
45	not identified	1128 (1)					
46	not identified	1181 (1)					
47	not identified	1186 (1)					
48	not identified	1191 (1)					
49	not identified	1201 (1)					
50	not identified	1204 (1)					
51	not identified	1348–1351 (3)					
52	not identified	1358–1361 (5)					
53	not identified	1457 (1)					
54	not identified	1467–1471 (5)					

[a] No., terpenoid number related to the radar plots. [b] RI(p), retention index of a specific terpenoid measured in plants harvested; (n), number of plant samples analyzed. [c] RI(l), retention index of a specific terpenoid found in the literature: 1, Kondjoyan and Berdagué (1996); 2, authors' library. [d] RI(m), retention index of a specific terpenoid measured with reference material. [e] CAS Registry No. were supplied by the authors.

ions (SIM) specific to one compound. Furthermore, MassLib offered the possibility of calculating the most probable composition of an overlapping peak by using regression analysis. The ratios are calculated as area percent. These values were used to calculate semiquantitative information based on the peak heights in the corresponding FID chromatograms. (All raw data are available from the last author.)

RESULTS AND DISCUSSION

Chromatographic Separation of the Volatile Compounds. Figure 1 show a typical GC/MS-FID separation of the volatiles found in plants analyzed with the dynamic headspace technique. The numbering of the compounds identified is given in the first column of Table 2, which is a list of the 54 terpenoids (mono- and sesquiterpenoids) identified in all of the plants analyzed (highland and lowland) as well as their relative retention indices or Kovats indices (RI) and odor descriptors.

Occurrence of Terpenoids in the Plants Analyzed. Table 1 lists the plant samples together with the dates and development stages (= phenological stages) at which they were sampled. The botanical composition of the highland pastures (locations 1–3) was richer in dicotyledoneous plants, while the composition of the pasture of the lowland (location 4) was richer in gramineae. Of the 47 plant samples analyzed, only 24 contained mono- and sesquiterpenoids (indicated by boldface type). Furthermore, 41 plant samples had a palatability level of >1, 26 had a palatability level of 3, and only 6 had palatability level of ≤1.

Figure 2 highlights the distribution of terpenoids found in all of the plants. Of the 41 plants occurring in the natural highland pasture, 22 plants contained 54 terpenoids. Of the 6 plants occurring in improved lowland grassland, only 2 species contained a common terpenoid.

The radar plot (Figures 3–5) overviews show the terpenoid fingerprints of each species based on its logarithmic relative peak intensities (heights), the black circle indicating the detection limit. Some plants of the highlands are very rich in terpenoids. Some of them, such as *Mentha longifolia*, contain up to 31 different terpenoids (see corresponding radar plot). This plant is certainly not consumed by cattle, but was nevertheless considered in this study as a reference for controlling the extraction procedure. Moreover, it is well documented in the literature due to its high terpenoid content. *Heracleum sphondylium* contains 21 terpenoids, etc.

Figure 6 shows the monoterpenoid distribution and Figure 7 the sesquiterpenoid distribution in the collected plants classified by terpenoid series. The acyclic monoterpene *trans*-β-ocimene in the volatile profile of 17 different plants seems to be an important plant constituent (Bergström et al., 1995), followed by limonene, occurring in 13 different species, and *trans*-β-caryophyllene (sesquiterpene), occurring in 11 different species. The occurrence of β-myrcene, *cis*-β-ocimene, γ-terpinene, sabinene, α-pinene, and β-pinene was also observed in 9 species. Only 8 species contained α-thujene and linalool.

Composition of the Same Botanical Species Collected at Different Locations and on Different Dates. Table 3 shows the terpenoid composition of some species collected on different dates and at different locations and phenological stages. *Leontodon hispidus* and *Trifolium pratense* gathered at three different sites on three different dates, as well as *Plantago lanceolata* collected at two different sites, did not contain any

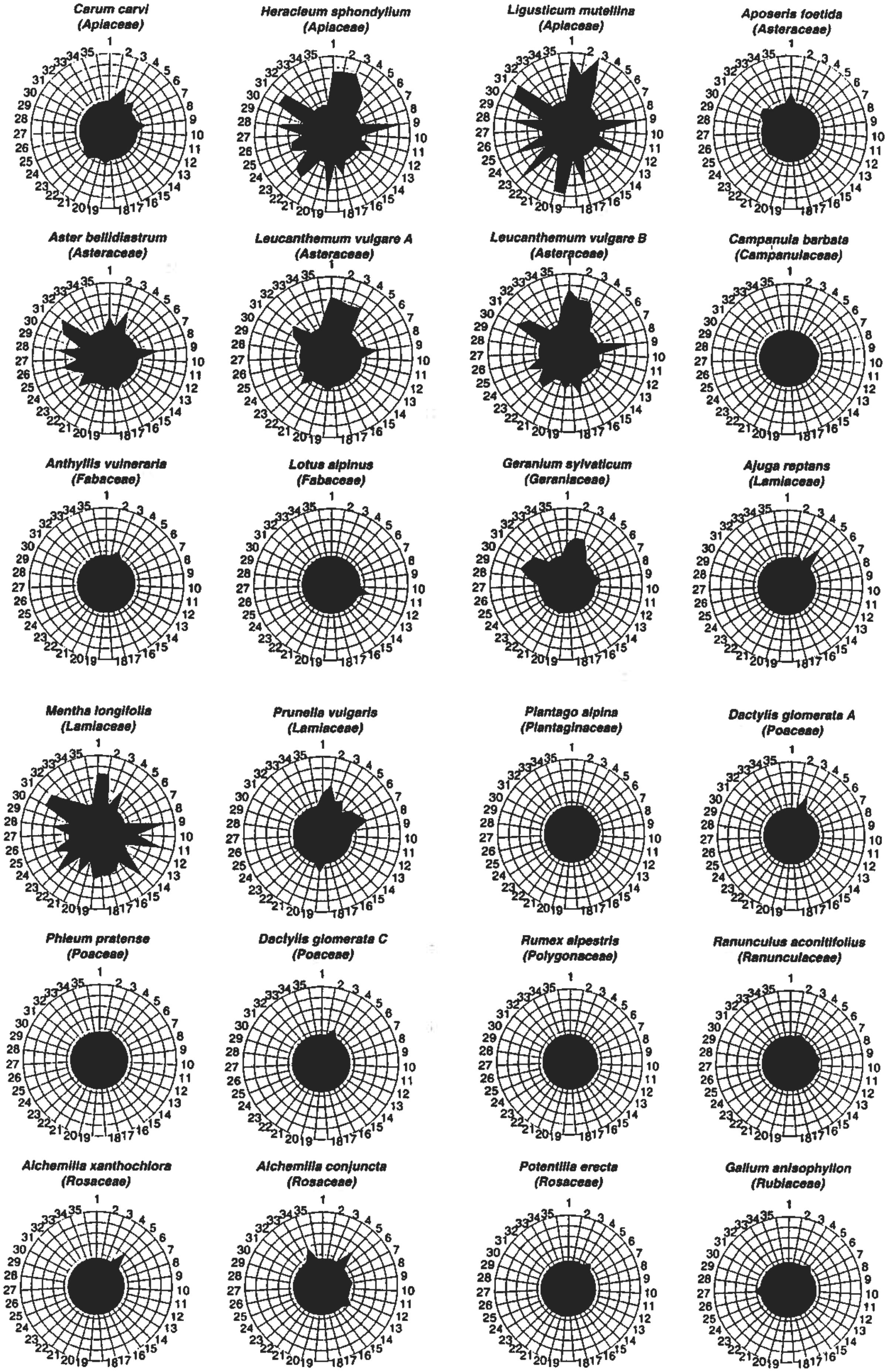

Figure 4. Radar plots showing the monoterpenoid fingerprints in highland and lowland plants. The corresponding compounds are listed in Table 2.

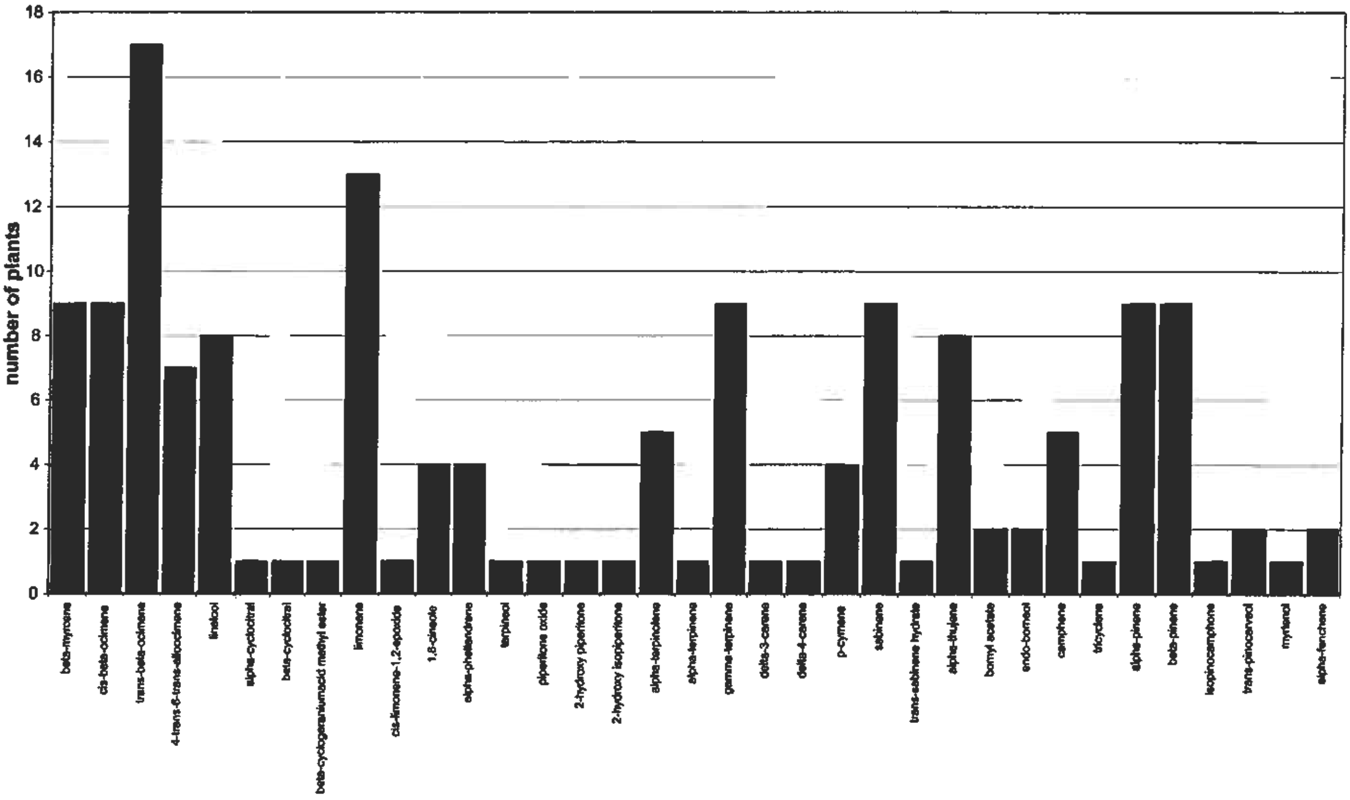

Figure 6. Distribution of monoterpenoids in all collected plants.

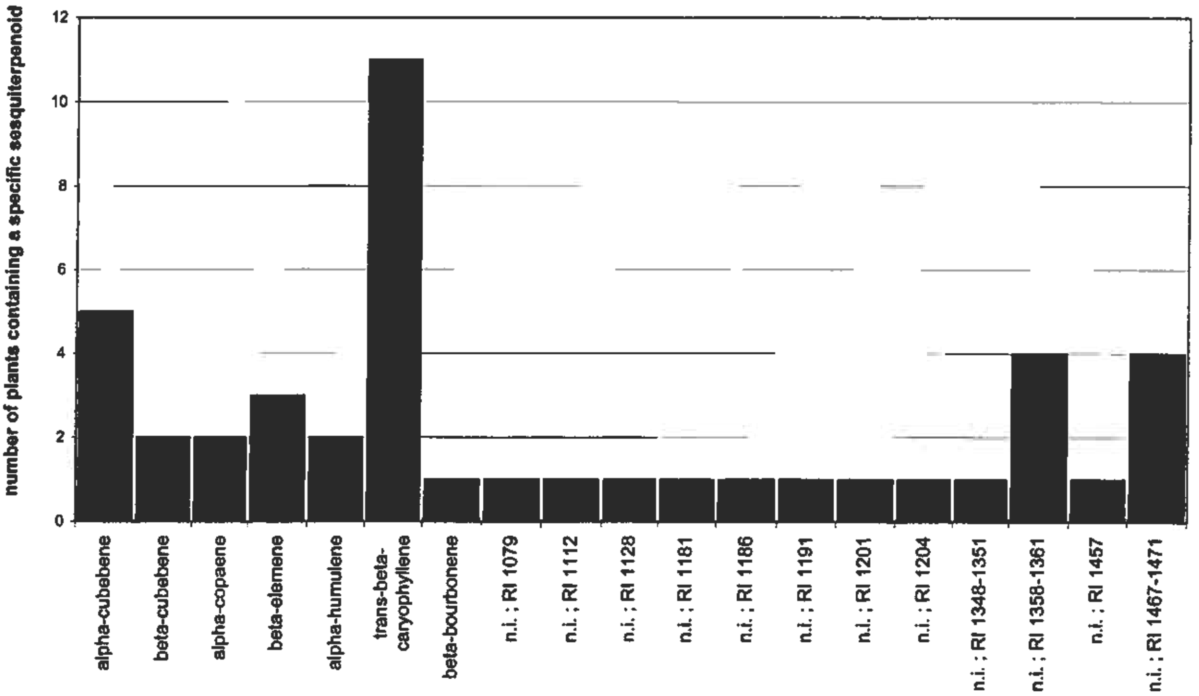

Figure 7. Distribution of sesquiterpenoids by chemical class in all plants collected. ni, not identified; RI, relative retention indices.

Trento, Italy, 1200 m, was reported by Bicchi et al. (1990). We found a similar qualitative terpenoid distribution as well as some additional terpenoids such as 4-*trans*,6-*trans*-alloocimene, linalool, δ-3-carene, and α-terpinolene. The relative distribution in our study was, however, quite different. The difference may be due to our extraction method (purge-and-trap extraction instead of steam distillation).

Mentha. The existence of different chemotypes, based on qualitative differences within a species, is a common feature in many genera of the Lamiaceae family (Kokkini, 1991). As far as the composition of *Mentha* essential oils is concerned, the differences may result in the definition of particular chemotypes. *cis*- and *trans*-piperitone oxide have been reported as main components of the essential oils of *M. longifolia* (L.) *Huds.* from Belgium, Italy (Shimizu and Ikeda, 1961), Germany (Baquar and Reese, 1965; Hefendhel and Nagell, 1975), Greece (Kokkini and Papargeorgiou, 1988), Egypt, France, Poland, southwestern Africa, and India (Lawrence, 1978). We also found 2-hydroxypiperitone (diosphenol) and its tautomer 2-hydroxyisopip-

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