Volatile Components and Odor Intensity of Four Phenotypes of Hyssop (*Hyssopus officinalis* L.)

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The volatile compounds of the four phenotypes of hyssop, *Hyssopus officinalis* L., differentiated by the color of the corolla, were investigated by Soxhlet extraction using pentane/diethyl ether and by supercritical carbon dioxide extraction. Pinocamphone, isopinocamphone, and pinocarvone were the main compounds among the 43 compounds observed, of which 33 were identified. Distinctly different percentage compositions of the volatiles were revealed. The phenotypes could be distinguished from another on the basis of the relative proportions of these three ketones despite notable annual variation during the three consecutive years of cultivation. The supercritical extract (10 MPa/40 °C) contained a lower amount of monoterpene hydrocarbons and a higher amount of oxygenated hydrocarbons than the solvent extract. The intensity of odor of the phenotypes and their respective CO_2 extracts was evaluated by sensory methods. The blue-flowered phenotype was assessed to be more intense in odor than the others.

INTRODUCTION

Flavor and fragrance preparations incorporated into various food, perfumery, and cosmetic products possess substantial value on the world market. The economic interests toward the fragrance components of aromatic plants direct the selection of commercially cultivated species to focus on both the amount and the quality of the volatile substances. The essential oil content may vary considerably within a single species from one growth season to another affected by climatic parameters and agrotechnical factors, such as fertilization, water supply, and harvesting, especially the phase of plant development at the time of harvest (Bernáth, 1986; Lawrence, 1986; Bernáth and Hornok, 1992). Many plants exist as various phenotypes, i.e., differing in their appearance, and both quantitative and qualitative diversity is often detected in the composition of essential oil obtained recorded, e.g., in basil (Simon et al., 1988) and oregano (Putievsky and Ravid, 1982). The essential oil of hyssop of various origins has been studied by Sharma et al. (1963), Joulain (1976), Joulain and Ragault (1976), Hilal et al. (1978), Lawrence (1984), Shah et al. (1986), von Schantz et al. (1986), Maheshwari et al. (1988), and Shah (1991). Schulz and Stahl-Biskup (1991) extended their study to cover the glycosidically bound volatiles in addition to essential oil from leaves, stems, flowers, and roots of hyssop during the growth season. Three phenotypes of hyssop with either red, blue, or white flowers cultivated in the Tashkent region analyzed by Khodzhimatov and Ramazanova (1975) were found to differ in their chemical composition. Due to evident dissimilarities in the volatiles identified in this study in comparison with previous investigations of hyssop, Lawrence (1980) considered these results to be tentative until verified.

The commonly used commercial methods to isolate volatile components from botanical material are distillation and solvent extraction. During the past 10 years applications of supercritical fluid extraction (SFE) have been introduced and adopted also in industrial scale. The volatile substances of aromatic plants are highly soluble in supercritical CO_2 already at moderate temperature and pressure (Brogle, 1982) and can therefore be selectively isolated. However, coextraction of other lipophilic compounds, e.g., fatty acids and their derivatives, waxes, and pigments, does occur to some extent. Comparison of chemical composition between hydrodistillates and supercritical carbon dioxide extracts of herbs and spices have been reported (e.g., Stahl and Gerard, 1982; Moyler and Heath, 1988; Udaya Sankar 1989; Nykänen et al., 1990; Reverchon and Senatore, 1991; Kollmannsberger et al., 1992; Hawthorne et al., 1993). A high yield of oxygenated hydrocarbons obtained by SFE compared with hydrodistillation has generally been associated with high quality of the aroma. Some studied have been published on differences between Soxhlet extraction and simultaneous distillation and extraction (SDE) compared with SFE (Hirvi et al., 1986; Bundschuh et al., 1986, 1988; Tateo and Chizzini, 1989; Kallio and Kerrola, 1992; Kerrola and Kallio, 1993).

To aid the selection of plants producing a high yield and quality essential oil, four phenotypes of hyssop, *Hyssopus officinalis* L., differentiated by the color of the corolla, were investigated. In this study we compare the chemical compositions of volatile compounds of the phenotypes cultivated during 3 consecutive years by Soxhlet extraction using pentane/diethyl ether. The volatile composition of the supercritical carbon dioxide extracts of the phenotypes is compared with the solvent extracts. The intensity of odor of the phenotypes and their respective CO_2 extracts are evaluated by sensory methods.

MATERIALS AND METHODS

Materials. Four phenotypes of hyssop (*H. officinalis* L.) were cultivated at the Agricultural Research Center, South Savo Research Station in Mikkeli, Finland (61° 40′ N, 27° 15′ E) during the summers of 1990, 1991, and 1992. The phenotypes were characterized by the color of the corolla. The mixed color flowered plants were of Kekviragu strain from Hungary, and the red form originated from the mountainous regions of Romania. The origin

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of the other two phenotypes was less accurately known: The seeds of the white form were purchased at Suffolk Herbs, England, and the seeds of the blue-flowered plants of unknown origin were collected at Pukkila Manor, Piikkiö, Finland, grown there more than 10 years. Fertilization consisting of 35 kg of nitrogen, 120 kg of phosphorus, and 70 kg of potassium per hectare were applied on the coarse sand soil (pH 6.2) in the spring, with additional nitrogen dressing (30 kg/ha) in June each year. In the first year of the experiment the seeds were sown into peat pots (5 × 5 cm in size) in April and transferred into open field (4 plants/m^2) during the first week of June. The weed control was carried out by hand and no pesticides were used. The plants were harvested 10 cm above the ground by hand, at the mid-flowering stage in August. The plant biomass was dried at 35 °C for 48 h in a pilot-scale cabinet drier under continuous circulation of air. The material was stored in paper bags at ambient temperature protected from light until analyzed.

Solvent Extraction. Comminuted hyssop (5.00 g) was extracted for 6 h with 150 mL of the mixture of redistilled pentane/diethyl ether (1:2 v/v) using a modified Soxhlet technique (Huopalahti and Linko, 1983). The extract was concentrated at 45 °C, with a Widmer column, to ca. 2 mL. To remove coextracted waxes, carotenoids, and the major proportion of chlorophylls, purification of the concentrate was carried out at 8 °C using column chromatography with 5 g of silica gel (Merck, silica gel 60 extra pure, 70–230-mesh ASTM) as described by Scheffer et al. (1976, 1977) and by Chamblee et al. (1991). The compounds were eluted with 30 mL of the solvent mixture used for the extraction. The internal standards, *n*-tetradecane and *n*-eicosane (purity >99%; Nu Chek Prep, Elysian, MN), were added to the effluent. The effluent was concentrated with a Widmer column as described above to the final volume of 2 mL. The samples were dried over anhydrous sodium sulfate and stored at −20 °C in Teflon-capped glass vials. A 50-fold dilution of the concentrate was made prior to gas chromatographic analyses. Triplicate solvent extractions of each phenotype were carried out. The mean values were calculated on the basis of three replicate gas chromatographic determinations of the extracts obtained.

Supercritical Fluid Extraction. Hyssop phenotypes (harvested in 1991) were pulverized in a pin mill under cooling by injection of liquid CO_2. Batch procedure was applied using 10-L autoclave type extractors at 10 MPa and 40 °C. The volatile substances of ca. 1 kg of plant material were extracted with 18 kg of CO_2 in 1 h, and the extracts were collected at 6 MPa and 30 °C. After removal of coextracted water, the semisolid extract was transferred to brown-glass bottles and stored at −20 °C until analyzed. The total yields of extracts isolated by a commercial scale extractor were 0.65, 0.50, 0.49, and 0.66% of dry weight for the blue, red, white, and mixed color flowered phenotype, respectively. A 200-fold dilution of the extract in *n*-pentane/diethyl ether (1:2 v/v) was made, and internal standards were added prior to GC and GC–MS analyses.

Gas Chromatographic and GC–MS Analyses. The gas chromatographic analyses were carried out on a Varian 3300 gas chromatograph (Varian Associates, Walnut Creek, CA) equipped with a flame ionization detector connected to a Shimadzu Chromatopac C-R3A integrator (Shimadzu Corp., Kyoto, Japan). Fused silica columns (HNU-Nordion LTD, Helsinki, Finland) (25 m × 0.32 mm i.d., film thickness 0.20 μm) coated with NB-351 liquid phase (nitroterephthalate modified polyethylene glycol polymer, corresponding to OV-351) were used for the analyses. The oven temperature was programmed as follows: from 40 °C (isothermal for 5 min) to 170 °C at 4 °C/min, from 170 °C to 240 °C at 8 °C/min, and an isothermal period at 240 °C for 12 min. The temperature of the injector port and the detector was 240 °C. The split ratio was 1:20, and the average flow rate of carrier gas (helium) was 1.6 mL/min. The 70-eV electron impact mass spectra were obtained on a VG Analytical 7070E instrument and VG-11-250 data system (VG, Wythenshawe, Manchester, U.K.). A Dani 3800 HR ch gas chromatograph with the same capillary column and temperature program as in the gas chromatographic analysis was used in GC–MS. Qualitative analysis was based on comparison of the spectra obtained with mass spectral libraries (Stenhagen et al., 1974; TNO, 1979; Ramaswami et al., 1988) and Kovats indices (I_K) (e.g., Holm et al., 1988; Davies, 1990). The identifications were not verified by cochromatography combined with mass spectrometric analyses of pure reference substances.

Sensory Evaluation. *Assessors.* For the evaluations of the samples, 12 assessors (6 men, 6 women) were selected from the staff and graduate students of our department. All were nonsmokers, between 25 and 52 years of age, and four had previous experience in sensory analysis.

Procedure. The assessors were asked to evaluate the odor intensity of the samples by using a 12-point structured interval scale consisting of four word categories anchored as weak (values from 1 to 3), moderate (from 4 to 6), strong (from 7 to 9) and very strong (from 10 to 12) intensity. The herbal samples and the CO_2 extracts were rated in separate sessions. Analysis of variance was applied to the results of six replicas of each group of samples.

Samples. The samples evaluated were four phenotypes of hyssop (1991) as minced herbal material and as supercritical extract. The samples for each assessor were prepared simultaneously about 1 h prior to the sessions to allow the headspace to develop in the bottle. The dry herbal material was comminuted with a chopper mill (Moulinette S code 643, Moulinex, France) for 30 s in 5-s intervals; 0.50 g of freshly ground hyssop was weighed into 35-mL glass bottles wrapped in aluminum foil. The sample was covered with cotton wool to avoid visual identification and capped with a lid. The semisolid extracts (50 mg) were applied on a disk of Whatman No. 1 filter paper and covered as described above. All samples were coded with three-digit random numbers and served in randomized order for each assessor in every session.

The evaluation of the samples was performed in individual assessment booths at 2 p.m. throughout the study. The sessions were held twice a week during 3 consecutive weeks in May 1992.

RESULTS AND DISCUSSION

The differences among the four phenotypes of hyssop were assessed by comparing the relative proportions of the 43 compounds listed in Table 1. Thirty-three of them could be identified on the basis of their mass spectral data and retention indices (I_K). A typical chromatogram of pentane/diethyl ether (PE:DEE) extract of hyssop (Kekviragu) is presented in Figure 1. Large differences in the proportions of the major constituents of the extracts were detected, although annual variation gave rise to notable diversity.

Chemical Composition of the Phenotypes. The monoterpene hydrocarbons constituted 11–14% of the total extract of the blue- and white-flowered phenotypes, whereas the red-flowered phenotype was found to contain about 17% and the mixed flowered between 14 and 17% monoterpenes. The major compounds were β-pinene and β-phellandrene. We could not find an explanation for the exceptionally large proportion of β-pinene (17.5%) in the red-flowered plants in 1990. Largest year-to-year variation was detected in the proportion of β-phellandrene, which could be explained with the heterogeneity of the material and the stage of ontogeny at harvesting. Schulz and Stahl-Biskup (1991) reported higher amounts of β-phellandrene in the flowers than leaves or stems and at the beginning of flowering than after the flowering had ended.

The main compounds of hyssop extracts were terpene ketones, pinocamphone, isopinocamphone, and pinocarvone, contributing from 40 to 50% in the red-flowered phenotype and ca. half of the total extract in the three others. The blue-, red-, and white-flowered phenotypes were distinguishable on the basis of the abundance these ketones. Highest amounts of terpene ketones in blue- and red-flowered phenotypes were detected in 1992, i.e., in the third-year plants. In addition to the age of the plants, this could also be explained by the weather conditions. The only major difference found in meteorological data among the three growth seasons was the warm and dry weather in May and July of 1992. Up to 26% of the extracts

Table 1. Relative Composition of Solvent Extracts of Four Hyssop Phenotypes Cultivated in Finland during 3 Consecutive Years

		composition (%)												
		blue			red			white			mixed			I_K
no.[a]	compound	1990	1991	1992	1990	1991	1992	1990	1991	1992	1990	1991	1992	NB-351
1	α-thujene	0.3	0.2	0.3	0.3	tr[b]	0.6	0.5	tr	0.2	0.1	tr	0.5	948
2	α-pinene	0.8	0.2	0.3	0.8	0.7	0.4	0.6	tr	0.4	0.6	0.6	0.4	1018
3	camphene	0.4	0.2	0.2	0.4	0.4	0.3	0.4	0.4	0.2	0.3	0.3	0.2	1046
4	β-pinene	6.6	5.2	6.2	17.5	10.8	10.5	9.3	7.9	7.8	11.3	9.3	7.7	1091
5	sabinene	1.0	0.7	1.1	1.5	0.8	1.1	1.1	0.8	0.9	1.3	0.9	1.0	1117
6	β-myrcene	1.2	0.7	1.2	1.3	0.9	1.2	0.7	0.5	0.8	1.1	0.8	1.1	1166
7	D-limonene	0.5	0.4	0.5	0.6	0.4	0.6	0.4	0.4	0.4	0.5	0.4	0.5	1190
8	β-phellandrene	3.1	1.9	2.8	3.8	3.3	2.9	0.5	0.6	0.5	2.2	2.5	2.6	1195
9	unknown	0.1	0.1	0.2	tr	tr	tr	tr	tr	tr	0.1	tr	tr	1224
10	*cis*-β-ocimene	0.6	0.5	1.1	0.1	tr	0.1	1.0	0.8	2.0	0.4	tr	0.6	1255
11	myrtenol methyl ether	1.0	1.1	1.4	0.6	0.8	1.3	0.3	0.4	0.4	0.6	0.8	1.1	1372
12	*n*-tetradecane													1400
13	α-thujone	0.1	0.1	0.2	tr	tr	tr	0.1	tr	0.2	0.1	tr	0.2	1446
14	β-thujone	tr	0.2	0.2	0.2	tr	0.2	tr	tr	tr	0.2	tr	0.1	1460
15	unknown	0.1	0.1	0.2	0.3	0.3	tr	tr	tr	tr	tr	tr	0.1	1472
16	pinocamphone	18.0	19.5	19.7	1.6	1.4	2.7	48.7	46.0	46.2	25.4	7.5	12.8	1506
17	α-gurjunene	0.7	0.5	0.6	0.3	0.5	0.4	1.0	1.2	1.2	0.4	0.5	0.5	1515
18	isopinocamphone	29.2	29.4	32.6	13.8	14.1	22.2	1.1	2.0	1.3	16.4	16.7	22.1	1537
19	linalool	0.1	tr	0.2	0.2	tr	0.8	0.7	0.5	0.7	0.2	0.3	0.6	1553
20	pinocarvone	5.3	5.6	5.7	25.3	25.5	26.2	1.2	2.2	1.7	10.7	23.0	19.7	1563
21	β-caryophyllene	2.5	2.6	2.0	2.3	3.3	2.0	2.1	2.7	2.4	1.9	2.8	2.0	1582
22	*allo*-aromadendrene	0.2	0.3	0.1	0.3	0.3	tr	0.4	0.7	0.2	0.2	0.3	0.1	1625
23	α-caryophyllene	1.8	1.9	1.6	1.2	2.0	1.4	2.6	2.6	2.9	1.0	1.5	1.5	1640
24	*trans*-pinocarveol	0.6	0.4	0.4	0.6	0.5	0.4	0.9	0.6	0.7	0.6	0.4	0.5	1657
25	methyl myrtenate	0.4	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.3	0.3	1670
26	estragol	0.4	0.3	0.2	0.4	tr	0.3	0.4	tr	0.4	0.3	tr	0.3	1679
27	unknown	tr	0.4	0.4	tr	tr	tr	tr	tr	tr	tr	tr	tr	1688
28	germacrene D	10.2	9.0	6.7	9.9	15.5	10.3	9.5	9.7	9.2	8.9	14.0	10.1	1696
29	unknown	nd[c]	nd	nd	nd	nd	nd	0.6	1.2	1.0	nd	nd	nd	1700
30	bicyclogermacrene	4.6	4.8	4.2	5.1	7.6	4.6	4.3	5.4	5.2	4.1	6.8	4.9	1723
31	unknown	0.4	0.4	0.3	0.2	0.3	0.3	0.3	tr	0.3	0.3	0.3	0.3	1749
32	myrtenol	1.9	1.7	1.9	2.5	2.0	2.2	2.1	2.4	2.6	2.6	2.0	2.5	1796
33	1-(1,4-dimethyl-3-cyclohexen-1-yl)ethanone	0.1	0.2	tr	0.8	0.6	0.4	1.3	0.7	1.0	0.9	0.6	0.3	1860
34	unknown	0.8	1.2	0.7	1.0	0.4	0.6	0.5	0.3	0.2	0.8	0.5	0.4	1973
35	unknown	0.2	0.6	0.2	0.5	0.6	0.3	0.5	1.1	0.3	0.3	0.7	0.3	1985
36	*n*-eicosane													2000
37	ledol	0.3	0.4	0.3	0.3	0.5	0.6	0.3	0.4	0.4	0.2	0.4	0.6	2044
38	eugenol methyl ether	0.2	0.3	0.3	0.2	0.4	0.5	0.1	0.3	0.4	0.2	0.3	0.4	2063
39	hedycaryol	4.4	6.8	3.5	2.4	4.1	2.5	5.6	6.6	5.3	2.7	3.8	2.7	2100
40	β-selinenol	tr	0.2	tr	tr	tr	tr	tr	tr	tr	0.4	tr	tr	2114
41	spathulenol	1.0	1.3	1.0	1.6	1.2	0.9	1.2	1.1	1.3	1.0	1.1	1.1	2137
42	T-cadinol	0.2	0.3	0.3	tr	0.3	0.3	tr	0.2	0.3	0.2	0.3	0.3	2187
43	unknown	0.4	0.5	0.5	0.3	0.3	1.0	0.3	tr	0.8	0.2	0.3	0.7	2233

[a] Numbers of the compounds correspond to peaks in Figure 1. [b] Trace amount, less than 0.1%. [c] None detected.

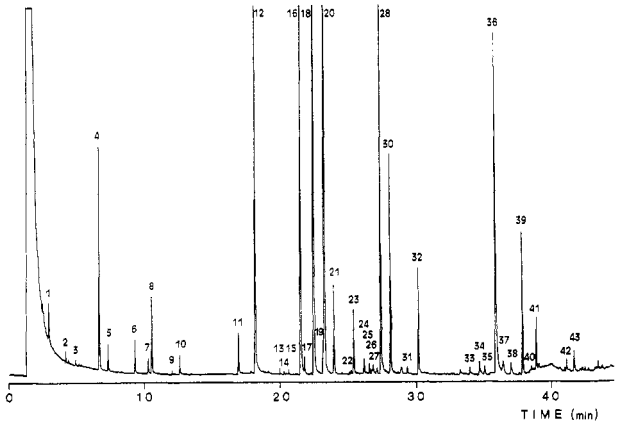

Figure 1. Gas chromatogram of pentane/diethyl ether extract of the mixed-color-flowered phenotype of hyssop (Kekviragu). Numbers of the peaks correspond to Tables 1 and 2.

of the red-flowered phenotype, from the mountain area of Romania, consisted of pinocarvone. Substantial amounts of pinocarvone in *H. officinalis* L. has previously been reported only by Maheswari et al. (1986) in their study of plants collected in the cold high-altitude arid desert of the Himalaya. High amount of pinocamphone and stability of the chemical composition was characteristic for the white flowered phenotype. The phenotype with mixed-colored flowers, Kekviragu strain, showed variation of chemical composition to the extent, which it could not merely be explained with annual variation of weather conditions. Instability of the strain was considered as the most likely source of the phenomenon. Previously, von Shantz et al. (1987) used headspace chromatography in determining the major substances of Kekviragu strain cultivated in Finland in 1984–1986. The relative proportions of the components and the large annual variation found in the volatile composition agree with our results.

Large proportions of sesquiterpene hydrocarbons were observed in the PE:DEE extracts as compared with essential oils analyzed in the literature. Germacren D and bicyclogermacren were the most abundant sesquiterpenes in all phenotypes, both exhibiting relatively large annual variation. Hedycaryol and spathulenol were the major oxygenated sesquiterpenes. The mass spectrometric fragmentation pattern of compound number 39 presented greater resemblance with the MS spectra of hedycaryol (TNO, 1979) than with any MS data we could find for elemol. Elemol has been claimed to be an artefact

Table 2. Relative Composition of Solvent Extracts and Supercritical CO_2 Extracts of Hyssop Phenotypes

		composition (%)							
		blue		red		white		mixed	
no.[a]	compound	SC-CO_2	solv	SC-CO_2	solv	SC-CO_2	solv	SC-CO_2	solv
1	α-thujene	tr[b]	0.2	tr	tr	tr	tr	tr	tr
2	α-pinene	tr	0.2	tr	0.7	tr	tr	tr	0.6
3	camphene	tr	0.2	0.1	0.4	0.2	0.4	0.1	0.3
4	β-pinene	1.2	5.2	3.1	10.8	0.7	7.9	2.2	9.3
5	sabinene	0.1	0.7	0.3	0.8	tr	0.8	0.2	0.9
6	β-myrcene	0.2	0.7	0.2	0.9	tr	0.5	0.2	0.8
7	D-limonene	0.1	0.4	0.2	0.4	0.1	0.4	0.1	0.4
8	β-phellandrene	0.7	1.9	1.0	3.3	0.1	0.6	0.7	2.5
9	unknown	tr	0.1	nd[c]	tr	nd	tr	nd	tr
10	*cis*-β-ocimene	0.1	0.5	nd	tr	tr	0.8	nd	tr
11	myrtenol methyl ether	1.0	1.1	0.8	0.8	0.3	0.4	0.7	0.8
12	*n*-tetradecane								
13	α-thujone	0.2	0.2	0.2	tr	0.1	tr	0.1	tr
14	β-thujone	0.1	0.1	tr	tr	tr	tr	tr	tr
15	unknown	0.1	0.1	0.2	0.3	tr	tr	0.1	0.1
16	pinocamphone	21.5	19.4	2.7	1.4	42.7	46.0	12.0	7.5
17	α-gurjunene	0.2	0.5	1.0	0.5	tr	1.2	1.2	0.5
18	isopinocamphone	31.6	29.4	18.1	14.1	4.9	2.1	16.5	16.7
19	linalool	0.1	tr	0.1	tr	0.5	0.5	0.3	0.3
20	pinocarvone	5.9	5.6	26.5	25.5	6.7	2.2	20.3	23.0
21	β-caryophyllene	3.3	2.6	4.8	3.3	3.7	2.7	4.2	2.8
22	*allo*-aromadendrene	0.1	0.3	0.2	0.3	0.2	0.7	0.1	0.3
23	α-caryophyllene	2.6	1.9	3.3	2.0	4.0	2.6	2.5	1.5
24	*trans*-pinocarveol	0.8	0.4	1.2	0.5	1.6	0.6	1.0	0.4
25	methyl myrtenate	0.3	0.2	0.4	0.3	0.4	0.3	0.4	0.3
26	estragol	0.4	0.3	0.6	tr	0.6	tr	0.5	tr
27	unknown	0.6	0.4	0.3	tr	0.3	tr	0.3	tr
28	germacrene D	10.3	9.0	16.7	15.5	11.7	9.7	16.0	14.0
29	unknown	nd	nd	nd	nd	0.6	1.2	nd	nd
30	bicyclogermacrene	5.0	4.8	6.2	7.6	4.3	5.4	5.8	6.8
31	unknown	0.4	0.4	0.3	0.3	0.4	tr	0.3	0.3
32	myrtenol	2.7	1.7	3.2	2.0	3.1	2.4	3.2	2.0
33	1-(1,4-dimethyl-3-cyclohexen-1-yl)ethanone	0.1	0.2	0.1	0.6	0.1	0.7	0.1	0.6
34	unknown	2.9	1.2	1.4	0.4	1.2	0.3	1.6	0.5
35	unknown	0.4	0.6	0.5	0.6	0.9	1.1	0.6	0.7
36	*n*-eicosane								
37	ledol	0.4	0.4	0.5	0.5	0.5	0.4	0.5	0.4
38	eugenol methyl ether	0.3	0.3	0.3	0.4	0.2	0.4	0.4	0.3
39	hedycaryol	4.0	6.8	1.8	4.1	3.6	6.6	2.3	3.8
40	β-selinenol	0.1	0.2	0.1	tr	0.3	tr	0.1	tr
41	spathulenol	2.6	1.4	3.6	1.2	5.1	1.1	4.7	1.0
42	T-cadinol	0.3	0.3	0.7	0.3	0.6	0.3	0.4	0.3
43	unknown	0.6	0.5	0.7	0.3	1.4	tr	1.1	0.3

[a] Numbers of the compounds correspond to peaks in Figure 1. [b] Trace amount, less than 0.1%. [c] None detected.

originating from hedycaryol as a result of a Cope rearrangement reaction during gas chromatography (Schulz and Stah-Biskup, 1991). Spathulenol and T-cadinol were tentatively identified by comparing the mass spectra recorded with identifications reported by Maurer and Hauser (1983) and Borg-Karlson et al. (1981), respectively.

The lack of genetically stable genotypes of medicinal plants gives rise to major variation in the chemical composition of their secondary products reviewed by Flück (1955) and more recently by Tétényi (1992a,b). The genetic heredity was considered the primary reason and the environmental factors a secondary reason for the differences in the composition of volatile substances. The hyssop phenotypes of this study were not cultivars but represented strains of hyssop differing in the color of corolla and also in their origins. The four phenotypes varied significantly in their volatile composition. The compounds isolated were essentially the same as identified by Joulain (1976) in hyssop cultivated in France and Hungary and by Schulz and Stahl-Biskup (1991) in hyssop of unknown origin. Although qualitative differences were minor, the phenotypes could be distinguished from one another on the basis of the relative proportions of the terpene ketone compounds, i.e., pinocamphone, isopinocamphone, and pinocarvone. Our results confirm in part the findings of Khodzhimatov and Ramazanova (1975) that hyssop strains with blue, red, or white flowers cultivated at the same location differ in the chemical composition of volatiles. Any conclusion on neither direct nor indirect connections between phenotypic and chemotypic differences in hyssop cannot be based on these results. Larger number of strains originating from different environmental locations must be included in the investigations to determine the existence of chemical races or chemotypes of hyssop.

Supercritical Carbon Dioxide Extraction. In order to compare the supercritical CO_2 extracts with PE:DEE extracts, the total peak area of the same 43 compounds as above was chosen to represent the total amount of extracted substances. The relative amounts of compounds are given as percentages calculated from the total amount (Table 2). When the SC-CO_2 extracts were analyzed by GC, several other peaks eluted in the latter part of the chromatogram beyond the last peak included in our calculations. Some of them were identified as fatty acid esters and ethers, and also phytol was detected. The SC-CO_2 extract represents about 1 kg of plant material in comparison with triplicate analysis of a 5-g sample of the same batch used in solvent extract analyses. However, the representativeness of the SC-CO_2 extracts suffers to some extent from the effects associated with long storage

time prior to isolation of the extract, i.e., quantitative changes in the amounts of volatile compounds of herbs known to occur during storage (Shah et al., 1986). In agreement with the previous results the supercritical extracts contained less monoterpene hydrocarbons and more oxygenated monoterpenes than respective solvent extracts (Hirvi et al., 1986; Kallio and Kerrola, 1992; Kerrola and Kallio, 1993). The percentage of sesquiterpene hydrocarbons was higher in supercritical extracts than solvent extracts, although bicyclogermacrene showed a different trend. The differences in extraction efficiencies toward oxygenated sesquiterpenes remained inconclusive.

To give an idea of the yields, we present an estimated amount of volatiles in the extracts calculated by the internal standard method. The estimations are used exclusively for comparing the solvent extracts with the SC-CO_2 extracts. The volatile compounds detected in SC-CO_2 extracts comprise 0.32, 0.11, 0.13, and 0.20 g/100 g of dried hyssop calculated of the blue, red, white, and mixed, respectively. The amount of the compounds analyzed in the PE:DEE extracts represented 0.40, 0.24, 0.20, and 0.34 g/100 g of the same material with standard deviation from 6 to 8%. The solvent extraction analyses were carried out 2 months after the harvest and the supercritical extractions three months later. It is probable that the total amount of volatiles had decreased during the long storage period. Shah et al. (1986) reported up to 0.45% loss in the essential oil yield after storing of the dried hyssop material for three months.

Sensory Evaluation of Odor Intensity. Differences among the four phenotypes in overall intensity and character of the odor were first observed during cultivation. Steinmetz et al. (1980) reported toxicity of the major components of hyssop (i.e., isopinocamphone and pinocamphone) in the rat. It was decided that sensory profiling of the different phenotypes would not be attempted, and only the odor intensity was to be evaluated. However, three assessor candidates reported a mild headache subsequent to evaluation sessions during preliminary testing, and they were exempted from the panel. To determine the magnitude of the differences in intensity of the odor, both dried hyssop and the supercritical fluid extracts isolated from the same batch were evaluated. A structured interval scale divided into 12 numerical and 4 word categories were used in scoring the samples. The scale was regarded to be linear (Amerine et al., 1965), and analysis of variance was considered applicable to the scores obtained. The dried herbal material and the extracts were analyzed separately to avoid bias caused by background effects originating from the plant matrix present in the herbal samples, but absent from the SFE samples. The mean scores and their standard deviations are presented in Figure 2. The means designated with letters in common were not significantly different at $p < 0.05$ using LSD test in analysis of variance. The odor of the blue flowered phenotype was assessed to be more intense in odor than the others regardless of the type of sample and was rated primarily as strong. The odor intensities of the other phenotypes were categorized as moderate, although statistically significant difference were detected between the mixed-color-flowered and the red-flowered phenotypes.

The assessors were a significant source of variance (F value = 5.62, $p < 0.001$). They were found to use the scale incoherently despite of training and instructions given, and they also displayed different association to the descriptive terms attached to the scale. When the odor intensities between two samples were very close to one another, the task of finding and quantitating the difference becomes more difficult. This phenomenon was observed as large variation of the scores of the white flowered phenotype exhibited by almost all assessors throughout the study. We could have reduced the number of categories, but we were skeptical about whether reliability would have increased in exchange for loss of discrimination efficiency (Land and Shepherd, 1988). In contradiction to our anticipation based on heterogeneity of the herbal material, significant differences between replicas (F value = 0.41, $p < 0.84$) were not detected. Larger standard deviations obtained for dried hyssop samples than for the extract samples were contributed to both matrix and heterogeneity. Interactions between assessors, samples, and replicas were not statistically significant as sources of variance.

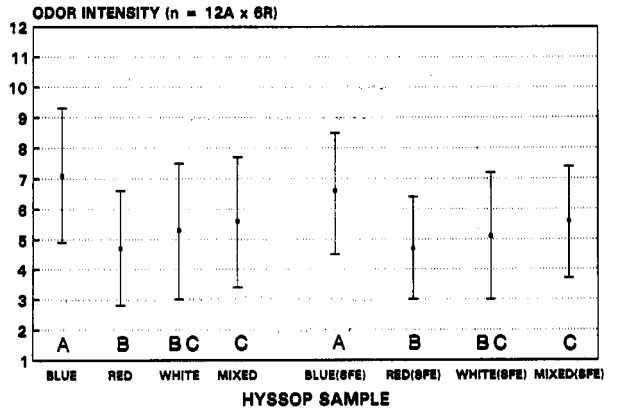

Figure 2. Mean scores and standard deviations for odor intensities of hyssop phenotypes and corresponding supercritical carbon dioxide extracts (calculated for 12 assessors and 6 replications). Means designated with the same letter did not differ at $p < 0.05$ based on LSD values obtained in analysis of variance.

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