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GAS–LIQUID CHROMATOGRAPHY OF TERPENES

XV. THE VOLATILE OIL OF MENTHA ARVENSIS VAR. GLABRATA RAY[1]

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ABSTRACT

The volatile oil of the North American wild mint (*Mentha arvensis* L. var. *glabrata*) was found to consist mainly of *d*-pulegone (80–90%) and smaller amounts of α-pinene, β-pinene, sabinene, limonene, 1,8-cineole, 1-octen-3-ol, menthone, isomenthone, piperitone, *cis*- and *trans*-pulegone oxide, and piperitenone. Trace amounts of γ-terpinene, menthofuran, β-caryophyllene, and ε-, δ-, and γ-cadinene were also isolated, and camphene, *p*-cymene, terpinolene, sabinene hydrate, isopulegone or its stereoisomer, β-elemene, and α-terpineol were tentatively identified. A labile autoxidation product of pulegone was also detected.

A study of the seasonal variations in the oil composition showed that significant changes in the quantitative composition occurred only in very young plants. The maximum yield of oil was obtained at the start of flowering. Practically no variation in the composition of the oil was recorded for plants from different localities.

INTRODUCTION

For a biosynthetic study of some of the terpenes found in mint plants, a knowledge of the chemical composition of the oil of local wild mint, *Mentha arvensis* L. var. *glabrata* Ray (syn. *M. arvensis* var. *villosa* (1) and *M. canadensis* L. (2)), was required. This plant differs morphologically from the European *M. arvensis* varieties (1). Gildemeister (2, 3) lists a number of older investigations which indicate that pulegone is a major constituent and that the presence of menthol and isomenthol was suspected. Gage (4) and Kremers (5) report that the main constituent of the oil of *M. canadensis* L. is *d*-pulegone, and *l*-limonene was identified (5). However, Braun (6) could not confirm these findings. In view of these uncertainties and the lack of more modern data, a detailed analysis by means of gas–liquid chromatography (g.l.c.) was undertaken.

The present study was carried out in two parts. Since it is known that the composition of the oil of *Mentha piperita* L. changes during flowering (7), a study of any such variation in *M. arvensis* var. *glabrata* was made first. When the optimum yield and best harvest time for a representative composition of the oil had been determined, a larger amount of the oil was isolated and analyzed in detail. The composition of the oil from local plants was also compared with that of plants from the Cypress Hills and Peace River districts in Alberta to establish whether variation in the composition of the oil exists in plants from different locations.

The g.l.c. techniques used were essentially those described earlier (8–10). However, a number of new liquid phases and experimental parameters were studied in more detail to improve the resolution and to obtain data which could be compared directly with those found in the literature. Thus, Levi *et al.* (11–13) and Hefendehl (14) have reported good resolution of the mint terpenes on sucrose diacetate hexaisobutyrate (SAIB) columns. Previously a sample of this liquid phase had given a poorer separation, especially of some oxygenated monoterpenes (sabinene hydrate, menthofuran, and menthones), in this laboratory. However, when a sample of SAIB from another source was tried, results similar to those reported by Levi *et al.* and Hefendehl were obtained.

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EXPERIMENTAL

The g.l.c. analyses were carried out with a F & M model 500 (linear temperature programming; F & M Scientific Instrument Inc.) instrument and with one of conventional design for isothermal operation (15). Preparative g.l.c. was carried out with an Aerograph Autoprep A-700 instrument (Wilkens Instrument and Research Inc.). The carrier gas was helium, and thermal conductivity detectors were employed. For very small samples a model 600-D Aerograph instrument equipped with a flame-ionization detector was used. Retention times were measured from the time of injection to the initial emergence of the peaks (16, 17). The percentage composition of the oil was calculated by summation of the areas under the peaks (triangulation method); the errors were similar to those reported by Janak (18). Infrared spectra were recorded as films between sodium chloride plates on a Perkin–Elmer model 21 (double-beam) spectrophotometer. Ultraviolet spectra were obtained in isooctane on a Bausch and Lomb model 505 spectrophotometer. Nuclear magnetic resonance spectra were determined with a Varian HA-100 instrument, tetramethylsilane being used as the internal standard.

Plant Material

Wild mint plants were collected from the Beaver Creek area (approximately 10 miles south of Saskatoon) and transplanted to experimental plots. The plants were identified as *Mentha arvensis* L. var. *glabrata* by Dr. G. W. Argus, Curator, W.P. Fraser Herbarium, University of Saskatchewan, and an herbarium specimen has been retained by us.

The seasonal variation in the composition of the oil was studied during the main growing season (July to September) of 1964. All other data were obtained from subsequent growth of the same plants. A number of wild plants from the Cypress Hills area just west of the Alberta border and from the Peace River district, Alberta, were collected during August 1965.

Recovery of Oil

The freshly harvested plants were steam-distilled for 3 to 5 h in a glass apparatus. The volatile oil was extracted with ether, washed with a little bicarbonate solution and water, and dried over anhydrous sodium sulfate. When less than 200 g plant material was available, the circulatory distillation apparatus of Kaiser and Lang (19), as modified by Hefendehl (20), was used. The latter apparatus gave a better recovery of monoterpene hydrocarbons.

Gas–Liquid Chromatographic Analyses

Column packings were prepared by the tray method, with nonacid-washed solid supports, including Chromosorb W, Gas Chrom P, and Anakrom ABS (60–80 mesh). Columns were packed by hand with gentle tapping or by applying a vibrator and vacuum. Both methods gave g.l.c. columns with the same resolving power (for the same packing), provided that only moderate vibration was used. Copper tubing was preferred over stainless-steel tubing, since the latter was found to contain an internal coating which was detrimental to the resolution of terpenes (on polar column packings) and which could not be removed easily. Comparison with a glass column of similar dimensions showed the copper column to perform equally well when the common terpenes were analyzed.

Aliquots (1 to 5 μl) of each oil sample were injected (injector at 125–140°) onto a 180 × 0.4 cm inside diameter column containing 15% polyethylene glycol (Carbowax 20M, PEG) on Chromosorb W. Linear temperature programming from 55 to 200° at 4°/min gave a satisfactory resolution of the major and most of the minor components. The percentage compositions of the oil at different stages of plant growth (as calculated from these chromatograms) are shown in Table I. The oils from the plants from both the Cypress Hills and Peace River districts had a composition similar to that of the local plants from August 5 to 13. More detailed analytical data were obtained by use of 300 × 0.3 cm PEG, SAIB (obtained from Wilkens Instrument and Research Inc.), SPEG (10% succinate polyethylene glycol polyester), and QF-1 (15% fluorinated silicone polymer on Anakrom ABS) columns, as well as a 180 × 0.4 cm EGPN (15% ethylene glycol bis-propionitrile on Chromosorb W) column. A typical chromatogram on the PEG column is shown in Fig. 1. Apiezon N, adipate, or succinate polyesters (NGA, APEG, SEG, BDS) and quadrol–SAIB mixtures did not give improved separation.

Prefractionation

The oil (10 g) was chromatographed on silicic acid (100 g, polyethylene glycol modified (9, 21)). Elution with petrol (b.p. 40–60°) gave a hydrocarbon fraction which still contained some of the less-polar oxygenated terpenes. Rechromatography gave an almost pure hydrocarbon fraction (0.8 g). Elution with methanol gave the oxygenated terpenes (9.2 g, by difference). Gas–liquid chromatographic analysis of the latter fraction showed that over three-quarters of peak 35 (see Fig. 1 and Table II) was lost in this procedure. New peaks, corresponding to the products of decomposition, could not be found. Some minor peaks also appeared to decompose.

Individual Components

Aliquots (10 to 50 μl) of the prefractionated terpenes were injected repeatedly onto a 180 or 300 × 0.4 cm PEG column. To facilitate recovery the detector current was reduced from 200 to 100 mA and the helium

flow from 110 to about 50 ml/min. Each peak was collected in a 15 × 0.2 cm inside diameter glass tube at the instrument exit, dry ice being applied at the middle of the tube. The infrared and ultraviolet spectra and physical constants were determined (see Table II), and these were compared with those of known terpenes to identify the individual components of the oil. Each fraction was analyzed on g.l.c. columns to determine the purity and relative retention times. Peaks were designated *a* and *b* if a contaminant was present (see Table II).

β-Pinene and sabinene could not be isolated separately, and their identity was confirmed by permanganate–periodate oxidation (22–24) to nopinone and sabina ketone. The terpene mixture (ca. 10 mg) was shaken vigorously with excess aqueous oxidant (100 mmoles/l, 5 ml) for 1 h. The product was extracted directly with ether (2 ml) and the ethereal solution was dried over anhydrous sodium sulfate. The ether was evaporated in a small vial (at 60–70°) and an aliquot of the residue (ca. 8 mg) was injected onto the PEG column. Two peaks with the same retention times as nopinone and sabina ketone were recorded.

Several sesquiterpene hydrocarbons were present in the oil (Fig. 1, solid black peaks in the peak 20 to peak 30 range). Of these, β-caryophyllene (peak 22), ε-cadinene (peak 26*a*), and γ- and δ-cadinene (peak 29, mixture (8, 10)) were identified by their spectral and retention characteristics. Peak 21*b* had the same retention time as β-elemene; peak 24 could not be correlated with a known sesquiterpene.

Analysis on the SAIB column showed that the menthone fraction (peak 15) contained a small amount of a contaminant with the same retention time as menthofuran. This was collected and had an ultraviolet spectrum similar to that of menthofuran. When the pulegone fraction (peak 23) was analyzed on the QF-1 column, a trace peak with the same retention time as α-terpineol was recorded.

Peaks 14, 18, 20, 30, 31, 35, 39, 40, and 41 appeared to decompose extensively during prefractionation. Attempts to isolate some of these components by g.l.c. directly from the oil met with similar difficulties, indicating that extensive decomposition of these components also takes place during g.l.c. However, enough of fractions 31 and 35 could be collected to record their spectra. The ultraviolet spectra were devoid of characteristic absorption, except for that of carbonyl groups. The infrared spectra showed the isolated fractions to consist mainly of ketones (1 715, 1 675, and 1 707 cm^{-1}, respectively). Weak to medium bands at 3 480 cm^{-1} indicated that contaminants containing hydroxyl groups were present. Absorption bands of variable (weak to medium) intensity near 3 040, 1 245, and 925 cm^{-1} could arise from epoxide groups, but such assignments are only tentative. The broad singlet near 1 375 cm^{-1} indicated that isopropyl or *gem*-dimethyl groups were absent. This suggested that these two components could be the isomeric pulegone oxides (25). The latter were synthesized by oxidation of *d,l*-pulegone with alkaline hydrogen peroxide solution (26) and separated by preparative g.l.c. The identity of the *cis*- and *trans*-isomers was established by their nuclear magnetic resonance spectra (25). If the presence of decomposition products is taken into account, there was good agreement between the spectra of components 31 and 35 and those of the synthetic oxides. Co-injection of the synthetic oxides confirmed the retention data.

In addition to *trans*-pulegone oxide, peak 35 was found to contain an autoxidation product of pulegone. These two components were partially separated on a 10% SPEG column. When the mint oil was allowed to stand in the light (daylight and fluorescent) for 24 h, peak 35*a* increased, with a concomitant decrease in the pulegone peak. When pulegone itself was allowed to autoxidize in the same manner, a second peak with the same retention time as peak 35*a* was recorded. Since polymeric autoxidation products (e.g. dimers) would not be eluted from the g.l.c. columns under the conditions used, the product must be a peroxide of pulegone. Whether this peroxide is formed in the plant or during isolation could not be established. However, the data in Table I suggest that only very small amounts can be present in the freshly distilled oil.

RESULTS AND DISCUSSION

The seasonal variation in the chemical composition of the volatile oil from *M. arvensis* var. *glabrata* is shown in Table I. From the data obtained in the main growing season of 1964, it follows that no significant variation in the chemical composition of the oil occurs just before, during, or after flowering. This is similar to our findings for the oil from *Tanacetum vulgare* L. (10), but contrasts with those reported for the oil from *M. piperita* L. (7, and the literature cited in ref. 20). Battaile and Loomis (27) have reported that the *de novo* synthesis of terpenes occurs only in very young tissues of mint plants and that interconversion (e.g. menthone → menthol → menthyl acetate) takes place only in older ones. Hence, the oil from very young (5–10 cm high) wild mint plants was also investigated. The yield of oil was much lower than that of mature plants, and a markedly different percentage composition was recorded (cf. last column, Table I). Aside from a considerably higher content of monoterpene hydrocarbons (12.5% vs. 1.5 to 2%), the oil from the young plants contained much more menthone and isomenthone. This is surprising, because the

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TABLE I

Seasonal variations in the percentage composition of the volatile oil of *Mentha arvensis* var. *glabrata* Ray

	Date of harvest								
	1964								
	July 17	July 24	August 5*	August 10†	August 13	August 20	August 27	September 4	July 6, 1965
Yield of oil (g)	0.78	0.89	1.24	1.18	1.13	0.84	0.62	0.53	0.29
n_D^{23}	1.481	1.483	1.483	1.482	1.482	1.483	1.481	1.481	—
$[\alpha]_D^{23}$	+17.9°	+19.0°	+18.2°	+17.2°	+18.3°	+18.1°	+19.2°	+18.5°	—
Component (%)									
α-Pinene	Trace	0.1	0.4	0.2	0.3	0.2	0.1	0.1	1.5
β-Pinene + sabinene	Trace	0.4	0.8	0.4	0.5	0.3	0.2	0.2	2.7
Myrcene	0.1	0.2	0.4	0.3	0.3	0.2	0.1	0.2	1.0
Limonene	0.7	1.9	1.6	1.0	1.4	0.5	0.6	1.0	7.3
Cineole	0.2	0.2	0.1	Trace	0.1	Trace	0.1	Trace	
1-Octen-3-ol	0.1	0.2	0.3	0.4	0.4	0.1	0.1	0.1	
(Sabinene hydrate)	0.8	0.6	1.0	1.0	0.9	0.5	0.4	0.4	
Menthone	1.1	1.4	0.9	0.9	1.2	0.6	1.6	1.2	3.7
Isomenthone	3.0	3.1	1.9	2.0	2.4	1.5	4.2	2.7	7.4
Unidentified (peak 18)	0.9	0.4	0.6	0.5	0.6	0.4	0.3	0.3	0.8
Unidentified (peak 19)	0.6	0.4	0.3	0.3	0.3	0.2	0.3	0.2	0.7
(Isopulegone) (peak 20)	3.2	1.5	1.6	1.6	1.8	1.2	1.1	0.9	2.0
Pulegone	86	88	88	89	88	92	90	91	79
ε-Cadine + piperitone	0.9	0.5	0.5	0.5	0.6	0.5	0.4	0.3	1.0
γ-Cadinene + δ-cadinene	0.6	0.3	0.3	0.3	0.3	0.4	0.2	0.2	0.7
cis-Pulegone oxide	0.5	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.6
trans-Pulegone oxide‡	0.6	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.2
Piperitenone	0.8	0.5	0.4	0.4	0.5	0.4	0.4	0.4	

*Plants started to flower.
†Main flowering season.
‡Plus a peroxide of pulegone.

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biogenetic sequence advanced by Reitsema (28) and extended by Bataille and Loomis (27) predicts a higher content of the unsaturated ketones (piperitenone, piperitone, and pulegone) in young plants. This is of considerable interest for biogenetic studies, and is being investigated further by us.

It is noteworthy that the oil from mature wild mint plants from the Cypress Hills area (southeastern Alberta, August 1965) and the Peace River district (northwestern Alberta, August 1965) had virtually the same composition as that of the mature plants found locally. This indicates that the composition of the oil from *M. arvensis* var. *glabrata* is relatively stable over a wide geographical area. The yield of oil from the local plants was a maximum at the beginning of flowering (cf. Table I, August 5, 1964), and dropped slowly during and after flowering. Since the chemical composition of the oil did not change to any significant degree during the flowering period, a large amount of plant material was harvested in August 1965, and the oil obtained was analyzed in detail. A typical gas–liquid chromatogram on the PEG column (as shown in Fig. 1 and Table II) lists the percentage composition and the components identified. The composition is very similar to that recorded the previous year, except that the content of component 35 is somewhat higher (1.5 to 2.0% vs. 0.2 to 0.6%). Apparently the content of the pulegone autoxidation product (peak 35*a*; cf. Experimental) was higher in the large batch.

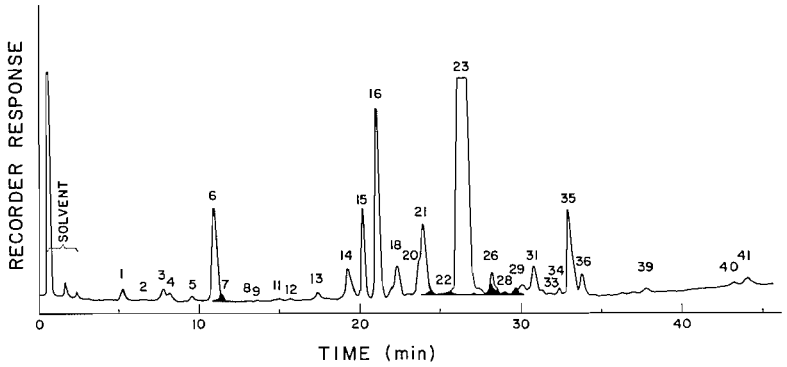

FIG. 1. Gas–liquid chromatogram of the volatile oil of *Menthaa rvensis* L. var. *glabrata* Ray (300 × 0.4 cm polyethylene glycol column, temperature programmed from 55° to 210° at 4°/min). The peaks in solid black were separated by silicic acid column chromatography; the peak identifications are shown in Table II.

Practically all of the components in the monoterpene hydrocarbon range (peaks 1–10) were identified, and α-pinene (peak 1), myrcene (peak 5), limonene (peak 6), and 1,8-cineole (peak 7) were isolated in a fairly pure state. The identity of β-pinene and sabinene (peaks 3 and 4) was confirmed by permanganate–periodate oxidation (22–24) to nopinone and sabina ketone, respectively. The infrared spectrum of peak 8 corresponded closely to that of γ-terpinene, but the optical rotation (+10°) requires the presence of an optically active contaminant. Components 9 and 10 were not obtained in sufficient amounts to record satisfactory spectra, but the retention data indicated these to be *p*-cymene and terpinolene, respectively. Ocimene isomers or phellandrenes were not detected.

The sesquiterpene hydrocarbon fraction makes up less than 0.5% of the oil, and isolation of the individual components was difficult. Peak 21*b* was only tentatively identified as β-elemene (by relative retention times), but β-caryophyllene (peak 22) and ε-cadinene (peak 26*a*) were sufficiently pure to permit identification by comparison of their infrared spectra. Component 29 showed an infrared spectrum typical of the mixture of δ- and

TABLE II

Percentage composition of the volatile oil of *Mentha arvensis* var. *glabrata* Ray

Peak No.	Compound*	%	$[\alpha]_D^{25}$	Relative retention times† PEG‡	EGPN§	QF-1‖
1	α-Pinene	0.25	−2.0°	0.29	0.28	0.51
2	(Camphene)	Trace		0.42	0.44	0.64
3	β-Pinene	0.25	+8.7°	0.55	0.545	0.85
4	Sabinene	0.1		0.57	0.70	0.85
5	Myrcene	0.1	+2.0°	0.82	0.88	0.85
6	Limonene	1.8	−80.8°	1.00	1.00	1.00
7	1,8-Cineole	0.15	−1°	1.07	1.65	1.45
8	γ-Terpinene	Trace	+10°	1.40	1.42	1.24
9	(p-Cymene)	Trace	Negative	1.63	2.35	1.27
10	(Terpinolene)	Trace		1.79	1.73	1.25
				PEG¶	SAIB**	QF-1††
11	Unidentified	Trace	Negative	0.43	0.12	0.12
12	Alcohol + C=O	Trace	−1.9°	0.46		
13a	Ketone	Trace				
13b	1-Octen-3-ol	0.2		0.60	0.13	0.13
14	(Sabinene hydrate)	0.55		0.67‡‡	0.65‡‡	0.54‡‡
15a	Menthone	1.5	+2.3°	0.77	0.93	0.93
15b	Menthofuran	0.1	+66.8°	0.87	0.87	—
16	Isomenthone	3.9			0.99	1.00
17	Unidentified	0.1		(0.95)		
18	Unidentified	0.55	−6°	0.98	1.09	1.10
19	Unidentified	Trace				
20	Unidentified	(0.5)‡‡		1.25‡‡	1.15‡‡	
21a	(Isopulegone)	1.35	+33.9°	1.30	1.18	1.10
21b	(β-Elemene)	Trace				
22	β-Caryophyllene	0.1	−18.7°	1.65		
23a	Pulegone	81.5	+3.8°	1.71	1.39	1.32
23b	(α-Terpineol)	Trace		2.02		0.54
24	Unidentified	Trace	+15.3°	2.05		
25	Unidentified	0.1	−49.0°	2.10		
26a	ε-Cadinene	0.2	+5.6°	2.34		
26b	Piperitone	0.2	−2.2°	2.34	1.88	1.93
27	Unidentified	0.1				
28	Unidentified	Trace		2.51		
29	γ-Cadinene + δ-cadinene	0.15		2.66		
30	Unidentified	(0.2)‡‡				
31	*cis*-Pulegone oxide	0.6	−18.3°	3.48	2.43	
32	Unidentified	Trace				
33	Isopiperitone	Trace				
34	Unidentified C=O	0.2		4.47		
35a	A Pulegone peroxide	(1.5–2.0)‡‡	Negative	4.93	2.76	2.60
35b	*trans*-Pulegone oxide	0.5				
36	Piperitenone	0.3	−5.4°	5.48	3.28	3.18
37	Unidentified	Trace				
38	Unidentified	Trace				
39	Unidentified	0.15				
40	Unidentified	0.15				
41	Unidentified	0.35				

*Names in parentheses refer to compounds which were only tentatively identified; *a* and *b* peaks were resolved on columns other than PEG.
†Monoterpene hydrocarbons relative to limonene; others relative to camphor.
‡300 × 0.3 cm polyethylene glycol column at 65 °C.
§180 × 0.4 cm ethylene glycol bis-propionitrile column at 65 °C.
‖300 × 0.3 cm QF-1 fluorinated silicone column at 65 °C.
¶PEG column at 120°.
**300 × 0.3 cm sucrose acetate isobutyrate column at 170 °C.
††QF-1 column at 130 °C.
‡‡Decomposing; approximate values only.

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γ-cadinene encountered previously (8, 10). Katsuhara *et al.* (29) found in the sesquiterpene fraction of various mint oils β-elemene, β-caryophyllene, and δ- and γ-cadinene (as well as six unidentified trace components), which is in good agreement with the present findings.

All of the major oxygenated terpenes, except for the double peak 20–21, were identified, as were also some of the minor constituents. The trace peak 12 could contain octan-3-ol (relative retention times), but its infrared spectrum showed the presence of major amounts of a ketone (1 710 cm^{-1}). Peak 13*a* was not identified, but peak 13*b* had an infrared spectrum closely resembling that of 1-octen-3-ol. Levi *et al.* (13) reported the presence of 3-octanol and its acetate in mint oils, and 1-octen-3-ol was identified in *M. pulegium* by Naves (30). The menthone fraction (peak 15) was found to contain a small amount of menthofuran (peak 15*b*) by rechromatography on the SAIB column. Isomenthone (peak 16) and pulegone (peak 23), the main constituent of the oil, were readily identified. Rechromatography of the latter indicated that a trace amount of α-terpineol could be present in the oil. Piperitone (peak 26*b*) and piperitenone (peak 36) were also obtained in a fairly pure state. Considerable difficulty was encountered with components 14, 18, 20–21, 31, 35, and 40, since these appeared to decompose or rearrange during isolation. Component 14 appears to be sabinene hydrate, which dehydrates easily to form sabinene. It cannot be ruled out that the oil in the plants contains only sabinene hydrate and that sabinene is an artifact. However, sabinene is frequently found in essential oils and may thus also be a true constituent of the wild mint. Levi *et al.* (13) have shown that sabinyl acetate may be a component of mint oils, but this thujane derivative could not be detected in the oil of our local wild mint. Sabinyl acetate was isolated from *Juniperus sabina* L. by our technique (31); hence, decomposition to sabinene may be ruled out. It is noteworthy that this oil does not contain any acetates in detectable amounts. Peaks 18 and 40, although present in 0.3 to 0.5% amounts, could not be isolated. Difficulties were also encountered with the double peak 20–21. Only the main portion of peak 21 was isolated with any degree of purity. Its infrared spectrum showed strong absorption bands at 1 707, 878, and 1 370 (broad) cm^{-1} and a weak band at 3 080 cm^{-1}. These absorption bands are similar to those expected for isopulegone, but no data for comparison could be found. The presence of isopulegone or its stereoisomer may be expected on biogenetic grounds.

Components 31 and 35 appear to be most interesting from a chemical point of view. Their infrared spectra suggest that these could be epoxides, but the isolated products also showed absorption in the hydroxyl region. It is possible that the epoxide ring is opened during passage through the detector (thermal conductivity type, at 220–250°) and that only part of the isolated fractions is the original oxides. The spectra and retention data suggested that these two components could be *cis*- and *trans*-pulegone oxide, respectively. This was confirmed by preparing the isomeric pulegone oxides (25, 26) and by comparing their properties with those of components 31 and 35. However, evidence was obtained (cf. Experimental) which shows that the *trans*-pulegone oxide (peak 35*b*) was accompanied by an autoxidation product (a pulegone peroxide, peak 35*a*) of pulegone. Since the latter is labile, it was not isolated. Peak 35 was partially resolved into these two components on the SPEG column. This appears to be the first time that these pulegone oxides were found in mint oils.

Shimizu (32) has isolated piperitone oxides from mint oils. These were prepared (26) and their retention data compared with the components of *M. arvensis* var. *glabrata*. The retention times were the same as those of peaks 26 and 27. However, since component 26 was found to consist mainly of ε-cadinene and piperitone, and peak 27 mainly of an unidentified sesquiterpene hydrocarbon, only traces, if any, of the piperitone oxides can be present.

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CONCLUSIONS

The chemical composition of the oil of *M. arvensis* var. *glabrata* resembles closely that of the oil of *M. pulegium* and *Hedeoma pulegioides* (American pennyroyal) (13), pulegone being the major constituent and smaller amounts of α-pinene, β-pinene, limonene, 1,8-cineole, menthofuran, menthone, isomenthone, piperitone, and piperitenone being present. However, the latter two oils are reported to contain also 3-octylacetate, linalyl acetate, and menthol (13), which were not found in the local mint oil. Another distinguishing feature appears to be the presence of the two pulegone oxides, the tentatively identified isopulegone, and possibly sabinene and its hydrate (in the oil of the local wild mint). The oils from other *M. arvensis* varieties differ markedly (13, 2, 7) from the local variety.

From the biosynthetic point of view it is noteworthy that ketones are the predominant components and that only traces of alcohols and no acetates were detected. On the basis of the scheme proposed by Reitsema (28) and extended by Bataille and Loomis (27), the hydrogenation step from pulegone to the menthones appears to be somewhat arrested and the reduction of the latter to the menthols may be blocked. This indicates a marked difference in the secondary changes as compared with *M. piperita* L., and may account for the remarkably consistent composition of the oil before, during, and after flowering of the plants.

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