If the techniques of Ribéreau-Gayon are used, the authors' observations on the pigments of *Vitis labrusca* var. Concord more nearly compare with data presented by him for *Vitis labrusca*. Bearing in mind that the variety of *Vitis labrusca* used by Ribéreau-Gayon was not specified and may not have been Concord, the authors find the following differences. Fourteen anthocyanin pigments, instead of 11, are found in Concords. Cyanidin 3-monoglucoside is present in much larger amounts than found by Ribéreau-Gayon. Malvidin 3-glucoside is not the predominant pigment and is present in much smaller amounts, and a larger percentage of the pigments is acylated. Climatic differences could be responsible for the varying proportions of the pigments. Ribéreau-Gayon found wide differences between *Vitis vinifera* grown in France and California in the relative proportions of certain pigments present. A further possible reason for the differences in the amounts of acylated pigments is that Ribéreau-Gayon extracted grape skins with 1% aqueous HCl, while the authors used 1% methanolic HCl. The two major acylated pigments in Concords have a limited solubility in aqueous solutions and may not be completely extracted by 1% aqueous HCl.

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ESSENTIAL OILS

Determination of Botanical and Geographical Origin of Spearmint Oils by Gas Chromatographic and Ultraviolet Analysis

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OIL OF SPEARMINT is a popular flavoring agent, used extensively in chewing gums and tooth pastes. Its output in the United States has increased steadily during the last quarter of a century. In 1960, more than a million pounds were produced in Indiana, Michigan, and Washington (*11, 16*).

Taxonomists presently recognize two species of spearmints cultivated in the United States, namely *Mentha spicata* L. cultivar common or native American spearmint and *M. cardiaca* Gerard ex Baker cultivar Scotch or Highland spearmint. The former is not truly native to North America but was introduced from Europe (*7*) during the seventeenth century and has since been widely grown. Originally, it was the only source of oil of spearmint in the United States. Scotch or Highland spearmint was brought to America about 50 years ago. Its history, botanical characteristics, and nomenclature were discussed in detail by Hocking (*12*). Phylogenetically, it is wholly unrelated to *M. spicata* (*14*).

Criteria of identity and standards of quality have been established for spearmint oil by government agencies as well as trade organizations in various countries (*2, 3, 5, 13, 15*). Canadian Food and Drug Regulations specify at present that spearmint essence, extract, or flavor shall be prepared from spearmint or oil of spearmint, obtained from leaves and flowering tops of *M. spicata* L., and shall contain not less than 3% by volume of oil of spearmint (*5*). In view of the taxonomic classification of spearmint now generally recognized, these regulations are being revised to include preparations derived from *M. cardiaca* also.

The present study, a continuation of work on the genus *Mentha*, constitutes a detailed examination of spearmint oils of different geographical and botanical origins. It also demonstrates the application of gas chromatographic techniques to the characterization of these complex products, and relates carvone contents thus obtained to those determined by ultraviolet spectrophotometry.

Experimental

Gas Chromatographic Analyses. APPARATUS. Gas chromatograph. Burrell Kromo-Tog K-2 equipped with thermal conductivity detector cell and separate heating baths for column and detector, respectively.

Column. Glass tubing, 0.6 cm. inside diameter.

Packing. Ucon Polar 20% on Celite, 30-60 mesh obtained from Burrell Corp. Length of packing, 230 cm.

SAIB (sucrose diacetate hexaisobutyrate) 20% on acid-washed Chromosorb W, 60–80 mesh. Length of packing 200 cm.

Carrier gas. Helium, inlet pressure 1.1 atm.; outlet pressure 1.0 atm.

Recorder. 0 to 1 mv. full scale deflection.

Chart speed. 0.5 inch per minute.

MATERIALS. Spearmint oils. Commercial and experimental samples of different geographical and botanical origins.

Spearmint oil constituents. Reference specimens obtained from various sources of supply.

Spearmint oils were analyzed by gas-liquid partition chromatography. Compositional criteria established were used for the determination of geographical origins and the recognition of genetic variations between plants from which the products were obtained. Over 1% of menthone was found in American oils derived from *Mentha cardiaca* Gerard ex Baker cultivar Scotch or Highland spearmint, but only trace amounts (less than 0.5%) of the 3-oxygenated terpene were detected in oils distilled from *M. spicata* L. cultivar common or native American spearmint. Likewise, an unidentified component, occurring in American *M. spicata* to the extent of about 1% but present as trace amounts only in *M. cardiaca* oils, permitted reliable distinctions. Experimental results for carvone contents, illustrative of the selectivity of the gas chromatographic technique, are compared and correlated with those obtained by conventional ultraviolet analyses.

METHODS. Gas Chromatographic Procedure. Samples of 2.5 to 3 μl. (Ucon column) or 5 to 7 μl. (SAIB column) were injected from a Hamilton microliter hypodermic syringe through silicone seals into the column, maintained at 170° ± 2° C. Helium was passed through the apparatus at flow rates of 75 (±5) ml. per minute, kept constant to within ±0.5 ml. per minute for any run as recorded by means of a bubble type flowmeter installed at the vapor exit (*22*). A current of 200 ma. was applied to the hot wire detector in the thermal conductivity cell operated at 200° C. Under these conditions, relatively well resolved and informative chromatograms were charted by the instrument in less than half an hour as shown in Figures 1 and 2.

DETERMINATION OF PEAK AREAS AND CONVERSION TO WEIGHT PERCENTAGES. The procedures based on measurements of peak height and standard deviation (σ) as previously described were adopted to assess peak areas and correction factors computed from the chromatograms of synthetic mixtures of known compositions (*23*). Carvone served as reference standard, its correction factor being considered as unity (1.00).

IDENTIFICATION OF CONSTITUENTS. Genuine spearmint oils, diluted progressively from about 10 to 0.1% with a given constituent were chromatographed to permit peak assignments. The procedure proved particularly informative when only minute amounts of constituent were added, and the chromatographic characteristics of the original sample were, therefore, almost fully retained. Technically, this serial dilution was achieved by filling the hypodermic syringe with, e.g., 5 μl. of the oil, thus diluting the ca. 1 μl. of sample left in the dead space of the syringe from the previous run. Both Ucon and SAIB columns were employed for these experiments, and hence peak assignments could be effectively cross-checked.

Table I enumerates the various peaks observed in the order of their emergence from the Ucon column and lists components found (*10*) as well as suspected along with their boiling points, retention times, and relevant correction factors.

Table I. Separation of Spearmint Oil Components by Gas-Liquid Partition Chromatography

Peak No. (from Figures 1 & 2)	Constituent	B.P., ° C.[a]	Liquid Phase: Ucon Retention time[b]	Ucon Correction factor[c]	SAIB Retention time[b]	SAIB Correction factor[c]
1	α-Pinene	155–157	0.12	0.91	0.10	1.02
2	β-Pinene[d]	164–166	0.16	0.97	0.14	1.05
3	Limonene	177–178	0.19	1.46	0.17	1.55
4	Cineole[e]	176–177	0.21	1.33	0.19	1.48
5	3-Octanol	178–179	0.25	0.80	0.24	0.85
6	3-Octyl acetate	76/10 mm.[f]	0.25	0.80[g]	0.29	(1.0)[h]
7	Unknown	...	0.34	(1.0)[h]	0.60	(1.0)[h]
8	Linaloöl[i]	198–199	0.43	1.04	0.42	1.04
9	Menthone	209–210	0.46	0.96	0.47	0.97
10	Isomenthone	212	0.48	(1.0)[h]	0.49	(1.0)[h]
11	Linalyl acetate[i]	220 (decomp.)	0.56	...	0.59	...
12	Carvomenthone	220–221	0.61	1.10	0.65	1.02
13	Menthyl acetate[i]	227–228	0.65	...	0.67	...
14	Dihydrocarvone	221–222	0.69	1.55	0.70	1.56
15	Menthol[i]	216	0.69	...	0.63	...
16	Carvomenthol[i]	100/10 mm.[f]	0.72	...	0.70	...
17	Pulegone	221–223	0.80	1.35	0.78	1.45
18	Dihydrocarveyl acetate	105/10 mm.[f]	0.91	1.16	1.0	1.03
19	Dihydrocarveol	222–223	(1.0)	(1.0)[h]	0.87	(1.0)[h]
20	Carvone	230–231	1.00	1.00	1.00	1.00
21	*trans*-Carveyl acetate[i]	110/10 mm.[f]	1.0	(1.0)[h]	1.06	(1.0)[h]
22	*cis*-Carveyl acetate	113/10 mm.[f]	1.15	0.93	1.21	1.00
23	*trans*-Carveol[i]	104/10 mm.[f]	1.17	(1.0)[h]	1.0	(1.0)[h]
24	*cis*-Carveol	107/10 mm.[f]	1.29	(1.0)[h]	1.11	(1.0)[h]

[a] Literature values except where given by supplier. [b] Relative to carvone as 1.00. [c] Determined in accordance with procedure previously described (*23*). Carvone set arbitrarily at 1.00. [d] May contain trace amounts of camphene. [e] May contain trace amounts of γ-terpinene. [f] Courtesy J. M.De rfer, The Glidden Co., Jacksonville, Fla., U. S. A. [g] Not determined and assigned correction factor of main peak under which it appeared. [h] Value assigned arbitrarily as no pure reference material available. Correction probably insignificant as amount present very small. [i] Not positively identified in present study.

For all compounds shown and available in the authors' reference collection of essential oil constituents, relative retention times were determined on both column packings. The three terpenes listed—α-pinene, β-pinene, and limonene—are known to occur in various spearmint oils. A small, but reproducible peak was observed in several chromatograms between that of α- and β-pinene, possibly indicative of the presence of minute amounts of camphene. Traces of other terpenes which may conceivably occur in these products were left unresolved by either column and hence escaped detection. Limonene and cineole could not always be efficiently separated with the Ucon column, particularly if the sample contained only minute amounts of the terpenoid, but quantitative estimations were made using the more polar SAIB as liquid phase.

Likewise, 3-octanol and its ester emerged together from the Ucon column but were well resolved with SAIB. Tables II and III and Figures 1 and 2 illustrate typical column performances. Both compounds were identified by infrared spectroscopy following collec-

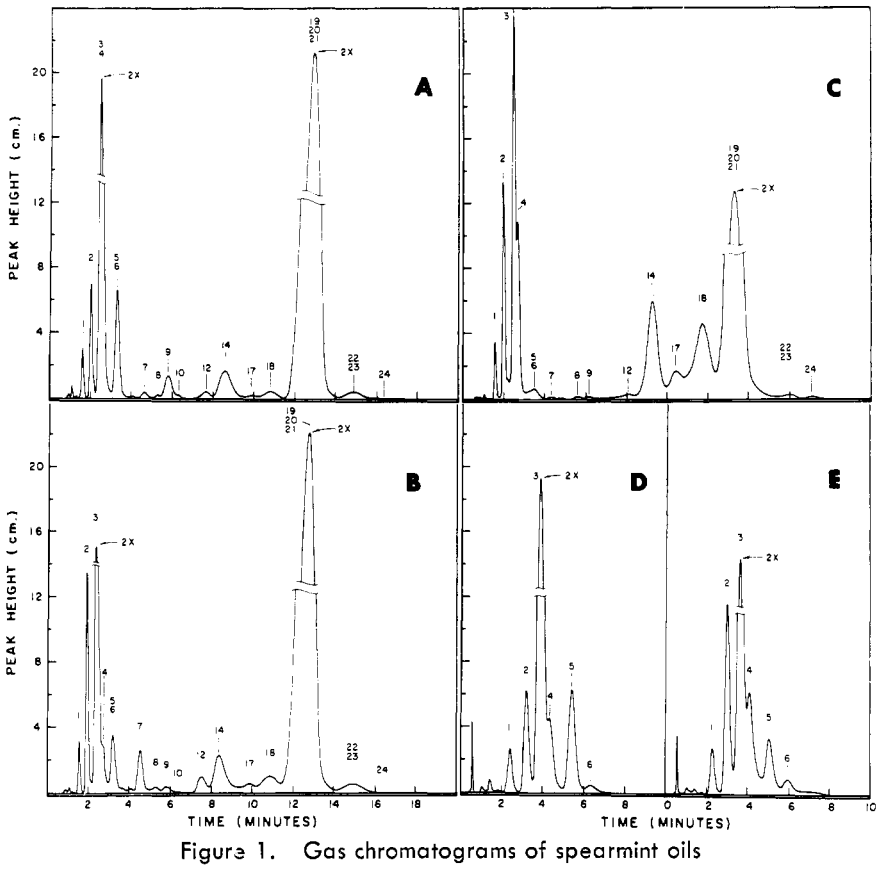

Figure 1. Gas chromatograms of spearmint oils

A. Oil of *M. cardiaca* Gerard ex Baker, cultivar "Scotch" or "Highland" spearmint grown in the U.S. Midwest; sample No. Sp-14; liquid phase, Ucon

B. Oil of *M. spicata* L. cultivar "Common" or "Native" spearmint grown in the U.S. Midwest; male sterile variety with $2n = 36$ chromosomes; sample No. Sp-16; liquid phase, Ucon

C. Oil of *M. spicata* L. grown in Canada; fertile variety with $2n = 48$ chromosomes; sample No. LL; liquid phase, Ucon

D. Oil of *M. cardiaca* and *E*, oil of *M. spicata*; sample No. Sp-14 and Sp-16, respectively; liquid phase, SAIB

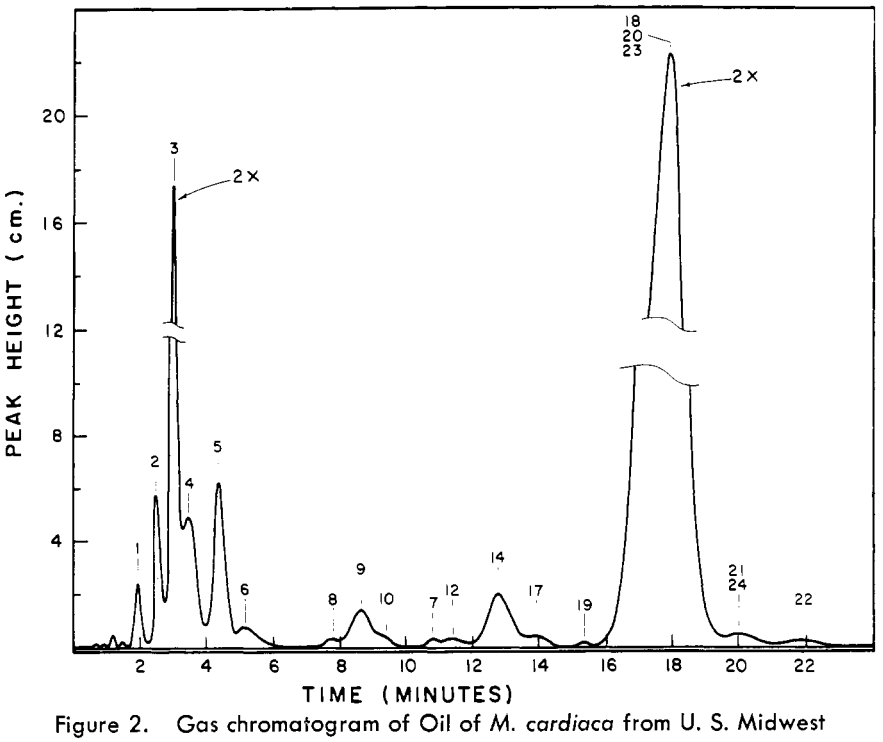

Figure 2. Gas chromatogram of Oil of *M. cardiaca* from U. S. Midwest

Sample No. Sp-14; liquid phase, SAIB

tion from three successive runs and comparison of the spectra obtained with those of authentic reference materials.

All *M. spicata* oils were found to contain approximately 1% of a component emerging at a relative retention time of 0.34 on Ucon and 0.60 on SAIB. Infrared measurements suggested—in agreement with these observations—the presence of an ester function. Further characterization of the compound is under way to establish its identity.

The occurrence of menthone was confirmed by infrared analysis of a sample collected from several runs and comparison of the spectrum with that of an authentic reference specimen. Thus, the presence of a 3-oxygenated terpene in the oil of a plant producing primarily and predominantly 2-oxygenated terpenes was demonstrated. Isomenthone was recognized by gaging the augmentation of its peak and simultaneous diminution of menthone following treatment of the oil with mineral acid. Dihydrocarvone was identified by infrared examination of a collected sample and comparison with a standard. Occasionally its chromatographic peak was followed closely by a shoulder indicating the presence of a small amount of some other component in the sample. Using both the Ucon and SAIB column carvomenthol, menthol and menthyl acetate were readily eliminated as likely components by means of the serial dilution technique. Also, oxidation of genuine products by potassium dichromate produced no augmentation of the carvomenthone peak, which observation confirmed the absence of any significant amount of carvomenthol.

Both *cis*- and *trans*-carveol and their esters were identified by infrared analyses. Chromatographic examinations with either Ucon or SAIB failed to yield quantitative results due to overlapping of some of the peaks. However, correlation of the experimental data obtained from both columns permitted reliable estimations of each isomer by difference. Table I shows relative retention times and Table III correlated quantitative data on a limited number of spearmint oils.

Saponification of an American *M. spicata* oil (sample No. Sp-16) by means of alcoholic potassium hydroxide to convert the esters to their alcohols and assessment of the peaks thus enhanced and decreased served, likewise, to detect and identify these compounds.

Ultraviolet Analyses. Apparatus. Beckman DK-2 ratio recording and Model DU spectrophotometer, equipped with photomultiplier.

Matched silica cells of 10.-cm. path length.

Materials. *l*-Carvone, commercial product purified via preparation of hydrogen sulfide addition compound (*25*)—m.p. 211.5–212.3° C. following three

recrystallizations—and subsequent regeneration of the ketone from the derivative by means of 20% aqueous alkali (*4, 6, 25*).

ϵ_{max} at 318 mμ = 42.95;

ϵ_{max} at 235 mμ = 9235

$$\frac{\epsilon_{max} \text{ at } 235 \text{ m}\mu}{\epsilon_{max} \text{ at } 318 \text{ m}\mu} = 215.02$$

METHODS. Approximately 75 (±10) mg. of sample were weighed accurately into 25-ml. volumetric flasks and ultraviolet spectra of the preparations charted by means of the recording spectrophotometer. Cells were then transferred to a Model DU instrument for accurate absorbance determinations at the observed maxima. Carefully measured 1-ml. sample aliquots were subsequently diluted to 200 ml. with anhydrous ethyl alcohol for similar ultraviolet examination throughout the 220- to 260-mμ region.

Results and Discussion

Compositional Criteria of Botanical and Geographical Origins. The experimental data recorded in Table II illustrate how American Scotch and Common spearmints may be distinguished from one another. Oils distilled from the former (*M. cardiaca*) contained about 1% of menthone and trace amounts of a constituent still to be characterized (relative retention time on Ucon 0.34). Those distilled from the latter (*M. spicata*) contained only trace amounts of menthone but up to 1.5% of the constituent still to be identified. Menthone to carvomenthone ratios supplied equally valuable criteria of identity. For *M. cardiaca* oils, this ratio varied from 1 to 6, and for *M. spicata* oils, from 0.1 to 0.6. Similarly, limonene to cineole ratios as determined by means of the SAIB column were never less than 8 for *M. cardiaca* oils, and never higher than 7 for *M. spicata* oils experimentally determined values ranging from 8.3 to 17.0 and 3.8 to 6.8, respectively.

Regional origins of the products could, likewise, be deduced with reasonable accuracy from the experimental data. Thus, *M. cardiaca* oils from the Midwest generally showed dihydrocarvone contents of less than 4%, limonene to cineole ratios of less than 10 and dihydrocarvone to ester (dihydrocarveyl acetate + *cis*-carveyl acetate) ratios ranging from about 1 to 4. Products from the Far West, on the other hand, generally contained somewhat more than 4% dihydrocarvone, displayed limonene to cineole ratios greater than 10 and showed dihydrocarvone to ester ratios ranging from about 3 to 10. Similarly, characteristic compositional criteria of identity were recognized for *M. spicata*. Oils from the Midwest displayed ester contents ranging from about 3 to 7% and dihydrocarvone to ester ratios ranging from about 0.8 to 1.3. Products from the Far West, on the other hand, showed ester contents ranging from about 1 to 3% and dihydrocarvone to ester ratios ranging from about 1.5 to 4.5. Oils produced in the Netherlands and experimental samples of Canadian origin appeared to be closely related. Both showed higher pulegone and ester contents than any other specimen examined (1.7 to 3.7% and 5.8 to 9.4%, respectively). Other compositional characteristics determined were the following: limonene to cineole ratio 1.8 to 2.5; menthone to carvomenthone ratio 0.4 to 1.0; and dihydrocarvone to ester ratio 0.5 to 1.8. The Formosan oil seemed to be of the same variety (limonene to cineole ratio 3.3; menthone to carvomenthone ratio 0.6; ester content 5% and dihydrocarvone to ester ratio 0.8), whereas the Chinese and Japanese specimens (EO-169 and EO-237) displayed more prominently Scotch characteristics (limonene to cineole ratios 11 and 32; menthone to carvomenthone ratios 2.0 and 3.7). Although the Chinese oil had been effectively deterpenated, it could thus still be classified.

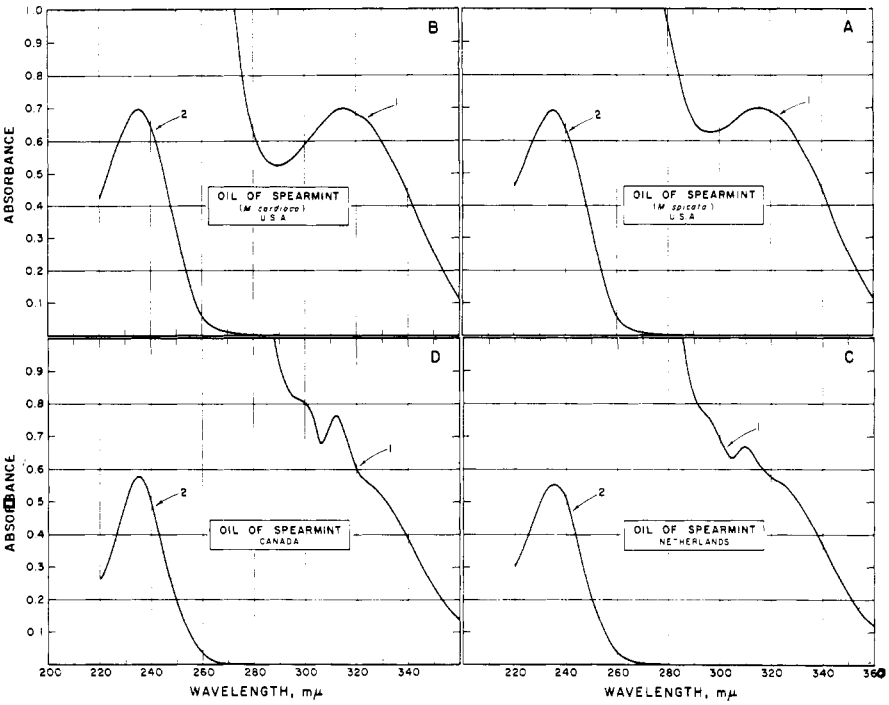

Figure 3. Ultraviolet spectra of typical spearmint oils

A. sample No. Sp-13; B, sample No. Sp-17; C, sample No. JCB; D, sample No. EO-556

Organoleptic Criteria. According to Guenther (*11*), Scotch or Highland spearmint has a milder flavor, more delicate, pleasant odor than the Common or Native American spearmint, and the quality of the Scottish spearmint from the Midwest is similar to that from the Far West. Only an expert could distinguish the latter two types. On the other hand, "native spearmint from the Midwest has a more pungent odor and flavor than that from the Far West." Thus instrumental data, because they permit simultaneous evaluation of many parameters, appear to be superior to organoleptic criteria for recognizing more readily *M. cardiaca* oils from the American Midwest and Far West. The odor of fractions collected during the course of the chromatographic study suggested that the carveylacetates probably impart most of the characteristic flavor notes to the oils. The milder flavor of Scotch or Highland spearmint might therefore well be associated with the smaller amounts of acetates occurring in these products and organoleptic differentiation of oils from the Midwest and Far West be difficult because both are quite comparable in ester content (0.4 to 2.9% and 0.4 to 1.9%, respectively). On the other hand, native spearmints from the Midwest, generally found to be richer in esters than those from the Far West, can be readily distinguished by the trained perfumer or flavor expert—the midwestern oils displaying the more pungent odor and flavor.

Essential Oil Composition and Plant Maturity. Experiments carried out with oils obtained from a patch of garden mint at different stages of plant maturity showed that both product yield and product composition varied with harvesting time. Oils distilled from plants harvested at full bloom were generally obtained in higher yields and contained lesser amounts of 2-oxygenated components other than carvone, especially acetates, than products obtained from late crops. When the leaves of full blooming plants were carefully separated

Table II. Composition of Spearmint Oils

Sample: Geographical origin and other information given by supplier	Code No.[a]	Component, %: α-Pinene	β-Pinene[b]	Limonene[c]	Cineole[c]	3-Octanol[d]	Unknown (rrt 0.34)	Linalool	Menthone	Isomenthone	Carvomenthone	Dihydrocarvone	Pulegone	Dihydrocarveyl acetate	Carvone[e]	cis-Carveyl acetate[f]	cis-Carveol	Others[g]
Mentha cardiaca GERARD EX BAKER CULTIVAR SCOTCH OR HIGHLAND SPEARMINT																		
U. S. Midwest[h]																		
Natural	Sp-14[i]	0.8	2.3	21.1	2.3	2.4	0.3	0.2	1.1	0.0[k]	0.4	3.0	0.1	0.5	64.7	0.4	...[l]	0.4
		0.6	1.9	17.7	2.4	2.2	0.2	0.2	1.1	0.1	0.4	2.9	0.4	0.8	68.2	0.5	...	0.4
Natural	Sp-11	1.0	1.6	20.3	2.2	2.0	0.3	0.1	1.8	0.2	0.5	2.9	0.7	1.3	62.8	1.6	0.5	0.2
		0.7	1.9	22.2	2.5	2.1	0.1	0.3	1.4	0.2	0.3	2.5	0.3	0.6	63.9	0.6	0.2	0.2
Natural	Sp-4	0.9	2.6	20.8	2.5	2.5	0.4	0.1	0.9	0.0	0.6	3.8	0.0	0.5	63.6	0.4	...	0.4
Natural	EO-254	0.7	2.0	21.6	2.5	2.2	0.2	0.1	1.2	0.2	0.4	2.7	0.5	0.5	63.5	1.1	0.3	0.3
Rectified	SP-3	0.3	1.4	23.2	2.1	2.0	0.1	0.2	1.2	0.1	0.2	4.7	0.0	0.4	64.0	0.0	...	0.1
Indiana	EO-558*	0.6	1.6	18.5	1.9	2.2	0.2	...	1.0	...	0.4	3.5	0.5	1.0	67.2	0.8	0.6	0.0
	EO-562*	0.7	1.9	19.2	2.0	2.2	0.2	...	1.2	0.2	0.3	3.7	0.6	1.3	64.5	1.0	0.9	0.1
Wisconsin	EO-560*	0.7	1.9	21.1	2.4	2.3	0.2	0.0	1.1	0.1	0.2	3.8	0.5	1.0	64.2	0.3	0.2	0.0
		0.8	2.0	21.1	2.4	2.3	0.1	0.0	1.1	0.2	0.3	3.9	0.4	0.9	63.5	0.6	0.3	0.1
	EO-567*	0.7	1.9	20.5	2.4	2.3	0.1	...	1.1	0.2	0.3	3.7	0.5	1.0	64.0	0.7	0.6	0.0
Michigan	EO-570*	0.6	1.8	22.9	2.6	1.6	0.1	...	1.0	0.1	0.2	2.5	0.3	0.7	65.0	0.5	0.1	0.0
	EO-566*	0.6	1.8	19.6	2.1	2.5	0.1	0.0	1.0	0.2	0.3	2.7	0.4	0.8	67.4	0.5	...	0.0
U. S. Far West[m]																		
Natural	Sp-13[i]	0.9	1.9	24.6	2.4	2.0	0.1	0.1	1.8	0.2	0.2	4.6	0.1	0.4	60.3	0.2	...	0.2
		0.6	1.6	22.8	1.9	1.8	0.1	0.1	1.2	0.2	0.3	4.4	0.3	0.8	63.5	0.3	...	0.1
Yakima[n]	EO-251	0.5	1.7	21.9	2.1	1.9	0.1	0.2	1.4	0.2	0.3	5.1	0.2	0.6	62.8	0.6	0.3	0.1
	EO-533*	0.5	1.3	19.2	1.8	1.7	0.1	0.0	1.1	0.1	0.4	6.9	0.5	1.1	64.4	0.8	...	0.1
	EO-740*	0.6	1.7	23.6	2.1	1.9	0.1	0.1	2.2	0.3	0.4	5.1	0.7	0.7	58.7	1.0	0.7	0.1
Fresh crop	EO-739*	0.7	1.8	29.7	2.4	1.7	0.1	0.1	1.3	0.2	0.3	5.6	0.5	0.6	53.9	0.7	0.2	0.2
Washington-Sunnyside	EO-561*	0.6	1.7	25.0	2.3	1.8	0.1	...	1.2	0.2	0.3	4.3	0.4	0.7	60.9	0.3	0.2	0.0
	EO-568*	0.5	1.6	24.7	2.3	1.7	0.1	...	1.1	0.2	0.1	4.1	0.1	0.3	62.9	0.1	...	0.2
China	EO-169	0.0	0.1	3.2	0.1	0.1	...	0.4	1.0	0.3	0.5	3.0	0.9	1.3	86.0	2.0	1.1	0.0
Japan	EO-237	0.6	1.6	20.0	1.8	2.6	0.1	0.1	1.1	0.3	0.3	2.9	0.2	1.2	66.7	0.3	0.1	0.1
Mentha spicata L. CULTIVAR COMMON OR NATIVE AMERICAN SPEARMINT																		
U. S. Midwest																		
Natural	Sp-16	0.6	3.3	13.3	3.0	1.2	1.5	0.3	0.3	0.0	1.0	3.6	0.7	1.5	68.1	1.3	...	0.3
		0.9	3.3	13.4	3.3	1.4	1.5	0.3	0.3	...	0.9	4.6	0.3	1.2	67.6	0.6	...	0.4
Natural	Sp-10	0.7	3.0	15.1	2.9	1.0	1.0	0.1	0.4	0.1	0.9	3.8	0.9	1.8	65.4	1.7	0.9	0.3
		0.8	3.4	17.1	3.3	1.1	1.1	...	0.4	...	1.3	3.4	0.5	1.5	63.0	2.0	1.0	0.1
Indiana	EO-557*	0.9	4.2	14.5	3.4	0.9	1.0	0.2	0.2	0.0	1.1	5.0	0.6	4.1	60.6	2.6	0.7	0.0
		0.9	4.3	15.9	3.8	0.9	1.0	0.1	0.1	0.0	1.3	5.5	0.9	3.3	59.1	2.4	0.5	0.0
	EO-563*	0.9	4.4	14.6	3.3	0.9	1.0	0.1	0.1	0.0	1.1	5.1	0.7	3.1	61.7	2.3	0.6	0.1
Michigan	EO-559*	0.8	3.6	13.9	3.7	1.1	1.3	0.2	0.2	0.1	1.2	3.8	0.7	1.8	65.6	1.8	0.2	0.0
	EO-564*	0.7	3.9	14.3	3.8	1.1	1.3	0.1	0.1	0.0	1.0	3.4	0.6	1.8	65.7	1.8	0.3	0.1
U. S. Far West																		
Natural	Sp-17[i]	0.8	2.7	17.8	3.4	1.3	1.0	0.1	0.3	0.0	0.7	4.7	0.1	1.8	64.4	0.5	0.2	0.2
		0.6	2.6	15.7	2.9	1.0	0.8	0.2	0.2	0.0	1.1	4.8	0.6	1.4	66.7	1.2	0.1	0.1
Fresh crop	EO-578	0.5	2.7	15.8	2.9	0.9	1.2	0.1	0.5	0.1	0.9	5.0	0.6	1.3	66.3	0.9	0.3	0.0
	EO-738*	0.7	1.8	17.5	4.4	1.0	1.4	0.4	0.2	...	0.9	5.3	0.5	0.5	64.0	0.7	0.3	0.4
Washington-Sunnyside	EO-565*	0.8	3.0	20.3	3.0	0.8	0.8	0.2	0.2	0.1	1.3	3.6	0.5	1.3	62.7	1.0	0.4	0.0
	EO-569*	0.8	3.0	20.5	3.3	0.7	0.8	0.2	0.2	0.1	1.1	3.5	0.3	1.3	62.1	1.8	0.3	0.0
Canada	LL[o]	1.0	4.9	14.9	5.8	0.6	0.1	0.2	0.2	...	0.4	12.2	2.8	8.1	48.2	0.5	0.1	0.0
	JCB[o]	1.2	5.2	13.3	7.5	0.6	...	0.3	0.2	...	0.5	13.1	3.7	6.2	46.3	1.0	0.1	0.8
Taiwan (Formosa)	Sp-18	0.5	0.9	16.4	5.0	0.3	...	...	0.3	0.2	0.5	3.8	1.6	3.9	64.4	1.1	0.1	1.0
Mentha viridis																		
Netherlands, crop year																		
1959[p]	EO-159	0.8	4.4	16.2	6.8	0.6	...	0.1	0.2	...	0.2	3.9	1.9	4.9	58.2	0.9	0.2	0.7
1960	EO-555	0.8	4.5	13.9	6.2	0.7	0.0	0.2	0.2	...	0.2	3.5	1.7	4.4	62.1	1.4	0.2	0.0
1960	EO-556	0.7	4.5	10.9	6.0	0.7	0.0	0.2	0.2	...	0.2	4.6	1.9	7.7	60.5	1.7	0.2	0.0

[a] When underlined, specimen was of known geographical origin but its botanical source deduced from the experimental data. When shown with an asterisk, test sample was received in code as a genuine American spearmint oil. Botanical species and, in some instances, also regional provenance of plant material used for its production were subsequently identified. [b] May include some camphene. [c] Limonene and cineole (which may contain trace amounts of γ-terpinene) not sharply separated by Ucon column under experimental conditions. Quantitative data derived as follows. Limonene/cineole ratio from SAIB = x; therefore, limonene = x; cineole = 1; and limonene + cineole = $x + 1$. To apportion components from the combined limonene + cineole peak, its percentage is set equal to $x + 1$. Hence, cineole % = $\frac{\text{peak } \%}{x+1}$ and limonene % = peak % − cineole %. [d] Includes 3-octyl acetate. [e] Includes small amounts of dihydrocarveol and *trans*-carveyl acetate. [f] Includes small amounts of *trans*-carveol. [g] Includes trace components emerging prior to α-pinene and those eluting between 3-octanol and unknown constituent. Value obtained by difference. [h] Comprises Mich., Ind., and Wis. [i] Experimental results shown do not represent duplicate determinations but were obtained at interval of 10 months during which time sample was stored under nitrogen at a temperature of 5° C. [k] Indicates that a small but reproducible peak corresponding to less than 0.1% of component was observed. [l] No peak or shoulder was observed in chromatogram for this component. [m] Comprises Ore. and Wash. [n] Reported to be a blend. [f] A fertile variety with $2n = 48$ chromosomes growing also in Europe. Different from Common or Native American spearmint which is male sterile and has $2n = 36$ chromosomes. [p] Distilled from herbs grown in the Noordoostpolder (the former Zuiderzee) now laid dry.

Table III. Gas Chromatographic Analysis of Typical Spearmint Oils Using Ucon and SAIB as Complementary Liquid Phases

Sample Provenance	Code	Liquid Phase	Component, % α-Pinene	β-Pinene	Limonene	Cineole	3-Octanol[a]	3-Octyl-acetate[b]	Unknown	Linalool	Menthone	Isomenthone	Carvomenthone	Dihydrocarvone	Pulegone	Dihydrocarveyl-acetate[c]	Dihydrocarveol[b]	Carvone[d]	trans-Carveyl-acetate[e]	cis-Carveyl-acetate[f]	trans-Carveol[g]	cis-Carveol[c]	Others
M. cardiaca																							
U. S. Midwest	Sp 14[h]	Ucon	0.7	2.1	19.4	2.4	2.0[a]	(0.3)[i]	0.3	0.2	1.1	0.1	0.4	3.0	0.3	0.6	(0.0)[i]	65.6	0.9	0.5	...	...	0.1
		SAIB	0.6	2.0	20.8	2.5	2.5	0.3	0.3	0.2	1.1	0.3	0.3	3.3	0.6	(0.6)[i]	0.0	63.1	0.9	0.6	...	...	0.0
U. S. Farwest	Sp 13[h]	Ucon	0.7	1.7	23.7	2.1	1.6[a]	(0.3)	0.1	0.1	1.5	0.2	0.2	4.5	0.2	0.6	(0.1)	61.1	0.7	0.3	...	...	0.3
		SAIB	0.7	1.6	23.3	2.0	1.9	0.3	0.2	0.1	1.1	0.3	0.2	5.0	0.6	(0.6)	0.1	61.0	0.7	0.3	...	...	0.0
M. spicata																							
U. S. Midwest	Sp 16[h]	Ucon	0.7	3.3	13.3	3.1	0.8[a]	(0.5)	1.5	0.3	0.3	0.0	0.9	4.1	0.5	1.3	(0.5)	66.4	1.0	1.0	...	...	0.5
		SAIB	0.5	2.7	11.9	2.9	1.2	0.5	0.8	0.1	0.1	0.0	0.4	4.1	1.0	(1.3)	0.5	69.5	1.0	1.2	...	...	0.3
U. S. Farwest	Sp 17[h]	Ucon	0.7	2.6	16.7	3.2	0.9[a]	(0.2)	0.9	0.1	0.2	0.0	0.9	4.8	0.4	1.6	(0.8)	64.0	0.8	(0.6)[i]	0.2	0.2	0.2
		SAIB	0.5	2.2	15.4	3.0	0.9	0.2	0.8	0.0	0.1	0.0	0.3	5.2	0.9	(1.6)	0.8	65.7	0.8	0.6	0.2	(0.2)[i]	0.6
Canada	JCB	Ucon	1.2	5.2	13.3	7.5	0.4[a]	(0.2)	...	0.3	0.2	...	0.5	13.1	3.7	6.2	(2.8)	42.7	0.8	1.0	...	0.1	0.8
		SAIB	0.9	4.0	13.8	7.8	0.2	0.2	0.2	0.0	0.1	0.1	0.3	13.6	2.6	(6.2)	2.8	45.5	0.8	0.7	...	...	0.2
M. viridis																							
Netherlands	EO-556	Ucon	0.7	4.5	10.9	6.0	0.4[a]	(0.3)	0.0	0.2	0.2	...	0.2	4.6	1.9	7.7	(4.8)	54.8	0.9	(1.2)	0.5	0.2	0.0
		SAIB	0.7	4.8	11.7	6.4	0.5	0.3	0.1	0.0	0.2	0.2	0.1	5.2	2.3	(7.7)	4.8	52.0	0.9	1.2	0.5	(0.2)	0.2

[a] (3-Octanol + 3-octylacetate)$_{Ucon}$ − (3-octylacetate)$_{SAIB}$. [b] Determined with SAIB column. [c] Determined with Ucon column. [d] Ucon: (Carvone + *trans*-carveylacetate + dihydrocarveol)$_{Ucon}$ − (*trans*-carveylacetate + dihydrocarveol). SAIB: (Carvone + *trans*-carveol + dihydrocarveylacetate)$_{SAIB}$ − (*trans*-carveol + dihydrocarveylacetate). [e] (*trans*-Carveylacetate + *cis*-carveol)$_{SAIB}$ − (*cis*-carveol)$_{Ucon}$. [f] Determined with SAIB column or expressed as (*cis*-carveylacetate + *trans*-carveol)$_{Ucon}$ − (*trans*-carveol). [g] (*cis*-Carveylacetate + *trans*-carveol)$_{Ucon}$ − (*cis*-carveylacetate)$_{SAIB}$. [h] Average value of results reported in Table II. [i] Bracketed values from chromatogram of complementary column.

from the flower spikes and each subjected to steam distillation, the leaf oil was richer in 2-oxygenated terpenes other than carvone and contained more cineole than limonene, whereas the flower oil, obtained in greater yield, was richer in carvone and contained more limonene than cineole. The observations appear to support previous concepts regarding the many possible transformations accompanying essential oil formation in plants and may illustrate the conversion of carvone to other 2-oxygenated terpenes and of limonene to cineole as the plant matures and its metabolic functions gradually slow down (*9*).

Comparison of Spearmint and Peppermint Oil Composition. Reitsema noted that spearmint oils were richer in unsaturated compounds and contained smaller amounts of saturated alcohols than peppermint oils (*18*).

The present study confirms these deductions but shows, in addition, that unlike the enhanced and apparently irreversible (*19*) production of menthol by peppermints, analogous production of carvomenthol by spearmints does not occur. Likewise, the relatively intense production of menthone (15 to 30%) in peppermints is not matched by comparable production of carvomenthone in spearmints. On the other hand, spearmints produce small amounts of unsaturated cyclic alcohols and their acetates, none of which are synthesized by peppermints.

The present study also demonstrates that the processes leading to formation of 2- and 3-oxygenated terpenes by any given plant (*18*) are not to be considered exclusive, for *M. cardiaca* was shown to contain menthone. Conceivably, contamination of spearmint fields with other plants, e.g., peppermint, could account for the observation. However, in view of the fact that a fairly large number of samples from different geographical areas were examined and relatively constant values were obtained for *M. cardiaca* (1 to 2%) and *M. spicata* (0.1 to 0.5%), this would not seem to be an adequate explanation. The absence of menthol is, likewise, a good indication of the absence of such contamination. The experimental results would, therefore, appear to indicate that cyclization leading to the 3-oxygenated terpene series is a metabolic side reaction in spearmint plants—a process perhaps governed by a specific enzyme system whose activity is suppressed by that controlling the production of 2-oxygenated terpenes. In all mint plants both systems could be operative, but in some, e.g., peppermints, that controlling formation of 3-oxygenated compounds has been given priority, whereas in spearmints that geared to production of 2-oxygenated compounds plays the major role. Not all the metabolic processes lead to cyclization, as demonstrated by

Table IV. Comparison of Ultraviolet and Gas Chromatographic Analyses for Carvone Content of Spearmint Oils

Sample	Carvone (% w./w.), from UV Absorbance at 313 (±3) mμ[a]	235 (±1) mμ[a]	$E^{1\%}_{1cm.}$ at 235 (±1) mμ / $E^{1\%}_{1cm.}$ at 313 (±3) mμ	Difference between UV and GLPC Carvone Data (%)[b]
		Mentha cardiaca		
Sp-14[c]	74.9	70.9	203.7	6.2
	75.4	69.7	198.7	1.5
Sp-11	74.5	68.9	198.6	6.1
Sp-4	75.3	71.7	204.7	8.1
EO-254	72.2	68.3	203.5	4.8
Sp-3	73.7	69.5	202.7	5.5
EO-558	75.2	70.5	201.1	3.3
EO-562	76.0	69.9	197.5	5.4
EO-560	73.5	68.3	200.1	4.4
EO-567	74.1	69.2	200.8	5.2
EO-570	76.4	70.4	198.3	5.4
EO-566	75.9	70.4	199.3	3.0
Sp-13[c]	74.1	70.0	202.8	9.7
	75.0	68.4	195.9	4.9
EO-251	71.8	68.7	205.9	5.9
EO-533	74.4	71.1	204.1	6.7
EO-740	75.4	64.2	183.4	5.5
EO-739	70.8	61.7	188.7	7.8
EO-561	73.3	66.8	195.9	5.9
EO-568	73.9	68.5	199.1	5.6
EO-169	92.0	87.8	205.2	1.8
EO-237	79.3	72.5	196.4	5.8
		Mentha spicata L.		
Sp-16	78.7	73.4	200.7	5.5
Sp-10	73.0	69.7	205.3	5.5
EO-557	72.8	67.0	197.7	7.2
EO-563	73.3	66.8	196.1	5.1
EO-559	75.8	70.4	199.9	4.8
EO-564	76.5	70.0	196.2	4.3
Sp-17[c]	74.1	70.4	204.4	6.0
	76.3	69.1	194.8	2.4
EO-578	77.3	70.3	195.4	4.0
EO-738[d]	85.3	62.7	158.1	−1.3
EO-565	71.9	69.4	207.3	6.7
EO-569	73.3	68.5	201.2	6.4
JCB (leaves)	85.1	59.7	151.0	13.4
(blossoms plus leaves)	98.8	77.3	168.2	
Sp-18[e]	127.0	54.3	92.0	−10.1
		Mentha viridis		
EO-159	77.3	64.1	178.6	5.9
EO-555	95.8	69.4	155.6	7.3
EO-556	83.6	65.5	164.9	5.0

[a] Experimental results averages of duplicate assays, carried out as described and differing less than 2%. [b] UV data based on measurements at 235 (±1) mμ; GLPC data taken from Table II. [c] Experimental results do not represent duplicate determinations but were obtained at interval of 10 months during which time sample was stored under nitrogen at a temperature of 5° C. [d] Sample colored faintly yellow. [e] Sample colored yellow and somewhat viscous.

the presence of small amounts of linaloöl in these products as well.

The occurrence of minute quantities of 2-oxygenated terpenes in plants producing predominantly 3-oxygenated compounds and conversely the occurrence of minute quantities of 3-oxygenated terpenes in plants producing predominantly 2-oxygenated compounds thus demonstrated should not affect their botanical classifications (*17*). It indicates that most stocks now at our disposal are natural hybrids in various degrees formed throughout the years. The scope and nature of these hybridizations may be more clearly understood as more of the minor constituents of the oils are detected and characterized.

Effects of Storage. As soon as received, all samples were assayed and subsequently kept under nitrogen in a refrigerator maintained at 5° C. Some of the products were re-examined about a year later (Table II) to appraise the effects of storage on their composition. With time, terpene contents decreased whereas carvone contents increased. Direct transformation of limonene to carvone may be partially responsible for this phenomenon (*1*), but more likely the terpenes undergo gradual oxidative polymerization to yield high boiling materials not detected by gas chromatography under the experimental conditions specified. Carvone results obtained for such products are therefore erroneous and no criterion of product quality. It should be emphasized in this connection that the degradations observed were taking place under relatively careful storage conditions. When keeping the products at room temperature in partially filled vials, deterioration occurred at accelerated rates.

Detection of Spearmint Oil in Food Products. "Mint Flakes" and "Rubbed Mint," two popular flavoring preparations on the Canadian market, were examined following extraction with pentane. Chromatographic analyses of the extracts showed that the essential oils present were rich in dihydrocarveylacetate and hence probably of the Canadian-Netherlands type. Similar processing of "Fresh Mint Jelly" or "Jellied Mint Sauce" failed to yield any oil. Both products were found to display but slight odor, indicating that most of their aromatic constituents were removed during manufacture. "Mint Sauce," a vinegar infusion-type preparation, was found to contain a spearmint oil (Scotch or Common American) of high carveylacetate and low terpene content which observation suggests that during processing, esterification of the carveols and degradation of the terpenes are likely to occur. Enrichment of carveylacetate, one of the most characteristic odoriferous principles of spearmint oil, by vinegar infusion probably explains the popularity the process enjoys in the flavor industry.

Ultraviolet Analyses for Carvone and Correlation of Results with Gas Chromatographic Data. Carvone, the major component of spearmint oil is traditionally determined by the neutral bisulfite method. The assay, based on volumetric measurement of the insoluble non-carvone fraction of the oil lacks specificity and as an absorption process suffers from a number of other disadvantages as well (*8*). Ultraviolet methods utilizing the characteristic absorptions of the ketone at 235 and 318 mμ, respectively, have been suggested as assays of greater simplicity, speed, and accuracy (*20*, *21*). Both spectrophotometric methods were examined during the course of the present study, and experimental results, correlated with those obtained by gas chromatography, are shown in Tables IV and V.

Qualitative examination of the ultraviolet spectra assembled revealed that all preparations displayed distinct carvone maxima at 235 (±1) mμ, but none exhibited peak absorption at 318 (±1) mμ. All *M. cardiaca* oils and the majority of the *M. spicata* oils showed maxima throughout the 214 to 216 mμ region. Four U. S. Midwestern oils (*M. spicata* EO-557, 559, 563, and 564) displayed maxima at 312 (±0.5) mμ, and oils of Canadian and Dutch provenance (samples EO-159, 555, 556, JCB, and LL) exhibited distinct peaks at still lower wavelengths [310.5 (±0.5) mμ]. Typical spectra are reproduced in Figure 3.

Comparison of the experimental results given in Table IV shows that carvone data based on absorbance measurements of maxima observed throughout the 313 (±3) mμ region are invariably higher than those based on absorbance determinations made at 235 (±1) mμ. Evidently, significant background absorption makes experimental measurements throughout the 313 (±3) mμ region suspect and unsuitable for quantitative interpretation.

Further experimental evidence for the greater specificity of measurements at 235 mμ stems from an appraisal of the absorbance ratio of the maxima observed throughout both wavelength regions. For pure carvone, this ratio was found to be 215.02. However, for none of the spearmint oils examined did this ratio exceed 207 which observation indicates that $E^{1\%}_{1\,cm.}$ at the longer wavelength was always greater than theoretical.

The magnitude assigned to this ratio appeared to be equally informative as a criterion of product origin and authenticity. Thus, genuine Dutch and Canadian oils, displaying particularly intense absorption at 310 (±0.5) mμ, yielded values ranging from 150 to 180, while American oils showed values varying from 180 to 207. For Dutch and Canadian oils, carvone data based on absorbance measurements at 310 (±0.5) mμ were accordingly up to 25% higher than those deduced from absorbance measurements at 235 (±1) mμ.

Furthermore, the ratio decreased during storage of the oils and hence reflected the degree of deterioration a given product had undergone. Evidently, during storage, carvone gradually decomposed, and, as comparison of the ultraviolet and gas chromatographic data obtained with specimens analyzed at approximately 10 months intervals would indicate (see experimental results for samples Sp-13, 14, and 17), high-boiling products contributing appreciably to background absorption throughout the 310 to 320 mμ region were formed. Oils stored for prolonged periods of time and without proper precautions failed, moreover, to display an absorption maximum throughout the 310 to 320 mμ region. Carvone values based on an arbitrarily selected wavelength (316 mμ) were therefore always abnormally high (see experimental results for samples EO-738 and Sp-18, respectively). For such samples, ultraviolet analyses based on measurements at 235 mμ should prove more informative than gas chromatographic examinations. For sample EO-738, kept under normal storage conditions for a period of 3 months, results obtained by the two methods were of the same order of magnitude. On the other hand, for sample Sp-18, stored for a period of 1 year without proper precautions, the gas chromatographic results—distinctly higher than those obtained by ultraviolet spectrophotometry—must be considered unreliable and erroneous. Applying, as they do, only to that fraction of the analytical specimen which is volatile under the experimental conditions, they are not representative of total sample composition.

Table V. Analysis of Typical Spearmint Oils

(Corrected gas chromatographic and ultraviolet data for carvone content)

	Gas Chromatographic Analysis			Ultraviolet Analysis $E^{1\%}_{1\,cm.}$ at 235 mμ				Difference between UV and GLPC Data (% Carvone)	
Sample	Liquid phase	Carvone content, %	$E^{1\%}_{1\,cm.}$ at 235 mμ Equivalent	Whole oil[a]	Constituents other than carvone[b]	Whole oil corr.	Carvone equivalent (%)	Corrected	Uncorrected[c]
Sp-14	Ucon	65.6	403.3	432.3	5.4	426.9	69.4	3.8	3.8
	SAIB	63.1	387.9	432.3	6.7	425.6	69.2	6.1	...
Sp-13	Ucon	61.1	375.6	424.9	6.2	418.7	68.1	7.0	7.3
	SAIB	61.0	375.0	424.9	7.9	417.0	67.8	6.8	...
Sp-16	Ucon	66.4	408.2	451.5	5.4	446.1	72.6	6.2	5.5
	SAIB	69.5	427.3	451.5	6.5	445.0	72.4	2.9	...
Sp-17	Ucon	64.0	393.5	428.8	6.0	422.8	68.8	4.8	4.2
	SAIB	65.7	403.9	428.8	7.3	421.5	68.6	2.9	...
EO-556	Ucon	54.8	336.9	405.4	10.9	394.5	64.2	9.4	5.1
	SAIB	52.0	319.7	405.4	12.3	393.1	63.9	11.9	...
JCB	Ucon	42.7	262.5	367.2	18.5	348.7	56.7	14.0	13.4
	SAIB	45.5	279.7	367.2	15.4	357.8	57.2	11.7	...

[a] Average results of duplicate determinations reported in Table IV.

[b] Values based on composition of products as shown in Table III and representing sum total of absorbances displayed by constituents other than carvone. Quantitative data obtained utilizing the following $E^{1\%}_{1\,cm.}$ values: α-pinene 1.83; β-pinene 0.48; limonene 16.95; cineole 0.14; 3-octanol 0.24; 3-octyl acetate 0.69; linaloöl 2.61; menthone 0.93; isomenthone 0.93; carvomenthone 2.67; dihydrocarvone 31.95; pulegone 275.4; dihydrocarveylacetate 15.46; dihydrocarveol 18.71; carvone 614.8; *cis*-carveol 17.47; *cis*-carveylacetate 3.9; *trans*-carveylacetate 2.68; *trans*-carveol 16.62.

[c] Results obtained from Table IV; average values used where two determinations reported.

The significance of the results given in Table IV may briefly be summarized as follows. Carvone analyses based on ultraviolet measurements of maxima throughout the 310 to 320 mμ region yield high values, while those based on ultraviolet measurements at 235 mμ are more accurate. Gas chromatography, because of its greater selectivity yields carvone results lower than those obtained by ultraviolet spectrophotometric measurements made at 235 mμ, the difference between both sets of values amounting usually to about 5 to 6% provided the analyses are carried out on freshly distilled or carefully stored samples. Loss of carvone during storage is associated with decreased absorption at 235 mμ but increased absorption throughout the 310 to 320 mμ region making measurements in this wavelength range unreliable. Deteriorated products fail to display maximum absorption throughout the 310 to 320 mμ region, and, as a result, such oils exhibit low maximal absorbance ratios. Also, because of the presence of high-boiling degradation products not detected by gas chromatography, ultraviolet data based on measurements at 235 mμ are the more meaningful criteria of carvone content in such instances.

In an attempt to assess further the agreement between the two analytical techniques applied, carvone results obtained by direct ultraviolet measurements at 235 mμ were corrected for background absorption as shown in Table V. Based on compositional criteria recorded in Table III and evaluation of the ultraviolet contributions afforded by the various constituents of the sample agreement between both methods was not significantly improved. Some components contributing to the ultraviolet absorption characteristics of the oils at 235 mμ are evidently not detected by gas chromatography under the experimental conditions applied. The observations made serve to demonstrate the pitfalls of assuming that gas chromatography gives a complete assay of the entire sample and show how the combination of different techniques with meaningful correlation of the experimental results obtained by each reveals the inherent advantages as well as shortcomings of conventional and modern methods of analysis.

General Applicability of Analytical Methods. The methods applied in this study as well as those reported previously (*23*) should prove of value to both producers and processors of essential oils, flavors, aromatic chemicals, and related materials. They should also be of considerable aid in the development and appraisal of plant breeding programs. Provided a great many such analyses have to be undertaken routinely, the entire operation of data collection and processing could be surrendered to a computer (*24*). Following assembly of the chromatograms, measurements of retention times and peak heights could

be fed into the instrument, so programmed as to permit establishment of the retention time–peak height relationship, correction factors, conversion of these data to weight percentages, and even designation of constituents present. Closely related and equally valuable authenticating criteria, such as sample purity and geographical provenance established on the basis of characteristic constituent ratios, could thus likewise be determined not only for essential oils but other natural and synthetic preparations amenable to similar analyses.

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Addendum

Sabinene Hydrate and Sabinene Acetate: Two New Constituents of American Spearmint Oil

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THE OCCURRENCE of a peak emerging between 3-octanol and menthone in spearmint oil gas chromatograms and permitting reliable distinctions between *Mentha spicata* and *M. cardiaca* was reported in the previous paper (*5*). Experiments completed recently established that this peak (marked 7) is generated largely by two components—*trans*-sabinene hydrate and *trans*-sabinene acetate. The tertiary alcohol is known to occur in American peppermint oil (0.8%) (*2*), but was not yet detected in any other essential oil. The ester has so-far not been found in nature. Characteristic criteria of identity of the compounds were assembled by gas chromatography (Ucon, Reoplex, and SAIB substrates) and comparison of infrared analyses of effluents with recorded data (*1–3*).

Marked decomposition of both terpenoids to phellandrene (α- and β-isomer), terpinene (α- and γ-isomer), and other hydrocarbons occurred during gas chromatography. Formation of these degradation products from sabinene, sabinene hydrate, and sabinene acetate at elevated temperatures has been reported (*2,4*).

The analytical peak was observed and found to be of similar composition in gas chromatograms of American peppermint oils. The presence of the ester in *M. piperita* was thus also established for the first time.

[1] National Research Council of Canada Postdoctorate Fellow, 1962.

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Correction

Biuret Formation in the Manufacture of Urea

In this article by P. J. C. Kaasenbrood, P. J. van den Berg, and L. J. Revallier [J. AGR. FOOD CHEM. **11,** 39 (1963)], the ordinate and abscissa labels were omitted from Figures 3 and 4 on page 42. The ordinate label for Figure 3 should read

→ % BY WEIGHT OF Bi CALCD. ON U + Bi

and for figure 4

→ % BY WEIGHT OF NH_3 CALCD. ON U + Bi + NH_3

The abscissa labels for both figures should read

→ p_{NH_3} IN ATM.