Table I the herbicides studied may be divided into two groups.

Group I. Herbicides which produced tests rated poorer than the standard sample. This group included ACP 103, Benzac 103A, Chlorazine, Diuron, Emid, FW 450, Neburon, Niagara 5521, Randox, Simazine, and 2,4-D Amine.

Group II. Herbicides for which no tests scored lower than the standard sample. Included in this group were ACPM 118, ACPM 119, ACPM 622, Atrazine, Chloro-IPC, Crag-Sesone, Dalapon, Endothal, EPTC, Monuron, Natrin, Premerge, salt, Stoddard Solvent, Trietazine, URAB, and Vegedex.

Of the six slight off-flavor tests encountered, two occurred in sweet corn which had been treated with chemicals (Neburon and 2,4-D Amine) at their suggested rate of application. The remaining four slight off-flavor tests occurred in products which had been treated with chemicals (Neburon, Niagara, and Randox) applied at concentrations higher than their suggested rates. However, these chemicals also yielded products with flavor mean scores lower than the standard sample when treatments were at the suggested rate of application.

Treatments in excess of the suggested levels were not included for six of the chemicals listed in Group II. These six herbicides (ACPM 622, Atrazine, Dalapon, Natrin, Stoddard Solvent, and URAB) were somewhat favored by this bias.

The extent to which these herbicide flavor changes would influence consumer acceptance of the processed products cannot be determined from these data. Since experienced taste panels were employed for these analyses, the judges were undoubtedly more sensitive to flavor changes than most consumers would be. Thus, some of the flavor effects observed might not be detected by a consumer panel. Although none of the sample mean scores was sufficiently low to be considered unacceptable in flavor, any flavor impairment of processed foods is undesirable and should be controlled as closely as possible.

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ESSENTIAL OILS

Treatment of Compositional Data for the Characterization of Essential Oils. Determination of Geographical Origins of Peppermint Oils by Gas Chromatographic Analysis

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Mentha piperita and *M. arvensis* oils have been analyzed by gas-liquid partition chromatography. Compositional criteria thus established are utilized for determination of geographical origins, recognition of biochemical relationships governing formation of oil in the plant, evaluation of manufacturing processes, and detection of subtle adulterations. Data and their treatment should prove of value not only to processors of essential oils, but also in characterization, analysis, and quality control of complex natural and synthetic compositions produced by the food, drug, and cosmetic industries.

OIL OF PEPPERMINT is one of the few essential oils whose production and processing have assumed great commercial importance in many countries. Of all flavors competing for man's taste that of peppermint has long proved and remains one of the most popular. From less than 2000 pounds to well over 2 million pounds per year, such is the remarkable development of the peppermint oil industry in the United States since the early 19th century. Production has more than doubled during the last two decades (*3*).

To the food industry, oil of peppermint is an essential raw material for flavoring a wide range of consumer products, particularly baked goods, confectioneries, and alcoholic liqueurs. Large quantities are also used by the pharmaceutical industry to mask objectionable tastes or modify the nauseating, griping effects of many medicinals.

Canadian Food and Drug Regulations permit use of both *Mentha piperita* and *Mentha arvensis* oils of the required flavoring strength for these purposes (*13*) and are in this respect considerably broader in scope than those applying in the United States, where a ruling of the U. S. Department of Agriculture forbids mint oil to be designated as peppermint oil, and the U. S. Federal Food and Drugs Act requires that preparations containing *M. arvensis* oil must be labeled "flavored with corn mint" or "flavored with field mint" (*20*).

This situation requires some explanatory comments regarding the botanical classification of peppermint. According to Guenther (*18*), the plants used in

most areas of cultivation throughout the world today—e.g., those in Argentina, Belgium, Bulgaria, France, Holland, Hungary, Italy, Poland, Roumania, Russia, Spain, the United States, and Yugoslavia—are but different varieties and strains produced from "Black Mint," *M. piperita vulgaris* L., originally introduced into these countries from England. It is also designated as *M. piperita nigra* L. var. *Mitcham* or *M. piperita* Huds. var. *officinalis* sole forma *rubescens* Camus. At one time it flourished abundantly near Mitcham in Surrey which is now suburban London. English cultivations at present include the Fenlands of Lincolnshire and Cambridgeshire, and areas near Hitchin (Hertfordshire) and Long Melford (Suffolk).

In Japan, Brazil, and China an altogether different mint species is grown commercially—*Mentha arvensis* L.—comprising two characteristic types: *M. arvensis*, subsp. *haplocalyx* Briquet, var. *piperascens* Holmes or *M. arvensis* L. var. *piperascens* Malinvaud, cultivated predominantly in Japan and Brazil, and *Mentha arvensis* var. *glabrata* Holmes or *Mentha canadensis* var. *glabrata* Gray, grown in China (*25*).

A test considered to permit recognition of authentic peppermint oil (*M. piperita*) is described in both the United States and British Pharmacopoeias (*8*, *51*). It is based on development of a characteristic sequence of colorations following treatment of the oil with a solution of nitric acid in glacial acetic acid (1:300). Genuine *M. arvensis* oils fail to react, but the test may also be obtained on blends of *piperita* and *arvensis* oils even when the percentage of the latter is high. Hence, its value as a specific criterion of botanical origin is limited.

Additional identity tests set forth as pharmacopoeial standards include such conventional constants as specific gravity, refractive index, optical rotation, and solubility data. Quantitative determinations for only two of the more than 30 constituents of the oil isolated so far—namely, menthol, its major component, and menthyl acetate, its principal ester—have come to be officially recognized. Unfortunately, both methods lack specificity since alcohols other than menthol and esters other than menthyl acetate react similarly under experimental conditions of acetylation and titrimetric analysis. Numerous attempts have been made to improve these classical assays (*28*), but so far none of the procedures reported has been officially adopted.

This paper presents a method of analysis permitting quantitative determination of all major components occurring in peppermint oil, recognition of products of different geographical origins, establishment of biochemical relationships governing formation of the essential oil in the plant, evaluation of manufacturing processes—e.g., rectification or deterpenation, and detection of subtle adulterations. The novel treatment of experimental data should prove of value not only to producers and processors of peppermint oils, but also in the analysis and characterization of complex natural and synthetic compositions.

Experimental

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. in I. D., 215 cm. long.

Packing. Sucrose diacetate hexaisobutyrate (SAIB), 20 parts, deposited from ethanolic solution on 60- to 80-mesh, acid-washed Chromosorb W (80 parts) (*45*). Length of packed column, 200 cm.

Carrier Gas. Helium, inlet pressure 1.11 atm.; outlet pressure 1.00 atm.

Recorder. 0 to 1 mv.; 1 second; 0.5 inch per minute.

Materials. Peppermint oils. Commercial and experimental samples of different geographical origins.

Peppermint oil constituents. Reference specimens obtained from various sources of supply. (Details in Acknowledgment section.)

Gas Chromatographic Procedure. Samples ranging from 2 to 5 μl. 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 counter installed at the vapor exit. Chart speed was maintained at 0.5 inch per minute. A current of 200 ma. was applied to the hot wire detector and the thermal conductivity cell operated at 200° C.

Under these conditions, well resolved and informative chromatograms were charted by the instrument within 24 minutes, as shown in Figures 1 and 2 for a *M. piperita* and *M. arvensis* oil, respectively.

Calculation of Percentage Composition of Samples from Peak Areas of Their Gas Chromatograms

Determination of Areas. Quantitative data were assembled in accordance with the procedure developed recently in these laboratories by Bartlet and Smith (*6*). Based on studies by Keulemans (*34*) and Klinkenberg and Sjenitzer (*35*), chromatographic peaks as traced by a well designed instrument are considered to closely approximate normal or Gaussian distribution curves for which (*7*, *14*)

$$A = \sigma \times h \times \sqrt{2\pi} \qquad (1)$$

$$= \sigma \times h \times 2.507$$

where

A = peak area
h = peak height
σ = standard deviation, sigma

Sigma equals peak width measured at 0.882 h, $^1/_2$ the peak width measured at 0.607 h or $^1/_3$ the peak width measured at 0.324 h, its value being constant, or nearly so, to a peak width of 4 σ for any isolated, symmetrical peak produced by a single component, or compounds displaying identical retention times. Hence, any isolated area subtended by a symmetrical peak may be determined simply by measuring its peak height and sigma and multiplying the product by 2.507.

Further, in accordance with Keuleman's observation that bands broaden in a symmetrical manner during passage through the column (*33*), their sigma functions increase linearly with respect to retention times. Thus, a graph can be constructed by plotting sigma values of pure reference standards—or well resolved peaks occurring in a complex chromatogram—*vs.* retention times for a given column operated under controlled conditions. The straight-line graph, which may or may not pass through the origin, can then be used to obtain the sigma value for any peak observed when operating the same column under identical conditions, and to determine readily the area of any isolated peak in accordance with Equation 1.

To effectively process experimental data in a convenient fashion, it is best to express graphical information thus assembled by means of the following equation:

$$\text{Area} = (\text{slope constant} \times \text{retention time} + \text{intercept constant}) \times \text{peak height} \times \sqrt{2\pi}$$

where the constants are characteristics of the particular column operated under given conditions.

Peak areas insufficiently resolved for accurate measurement by any of the conventional methods may be similarly determined following evaluation of contributions afforded by adjacent peaks. Corrections to be applied are dependent upon separation of the peaks involved, their heights, and sigma functions (*7*). They can be expressed directly as percentages by reference to the calibration graph shown in Figure 3.

A given area may have to be corrected twice, provided its preceding and succeeding peak are both sufficiently intense and close to warrant such adjustments. If the interfering peak is separated by more than 4 σ from the peak to be assessed, the correction is generally less than 0.04% and, therefore, practically negligible. Based on the precision of duplicate determinations, such mutual corrections of peak heights

observed in chromatograms of peppermint oils obtained under the experimental conditions described need only be considered in the case of isomenthone.

Areas were determined in accordance with these procedures for all the chromatograms assembled. Unresolved regions of low concentration which could not be so treated were estimated either by counting through a square millimeter grid, or by carefully cutting and weighing (*32*).

A sample calculation based on the chromatogram of Yakima oil (Figure 1) is given in Table I.

Conversion of Areas to Weight Percentages. Areas as determined at this stage are functions of the thermal conductivities of the various components. They were converted to weight percentages by computing appropriate correction factors. This approach circumvented the introduction of exact volumes of standard substances required by conventional methods and any errors caused by impurities present in the reference compounds. It also avoided the addition of weighed amounts of an internal standard to each sample.

Reference compounds available were chromatographed under conditions identical to those used for analysis of peppermint oils. Peak areas corresponding to each standard and its impurities were calculated and expressed as percentages of the total. Mixtures of reference substances were then made up by weight and similarly assayed. Their components were selected so that no overlapping of main peaks could occur. Utilizing data obtained for both the individual reference compounds and their impurities, true weight percentages of the various constituents making up a given mixture were calculated.

Menthol was considered the primary standard and its correction factor set at 1.00. For all other compounds, correction factors were then established by bringing the relative areas of their chromatographic peaks in line with the relative amounts originally weighed out. Five mixtures were so processed. A sample calculation illustrating the analysis of one of the mixtures used is reproduced in Table II. Average correction factors obtained are shown in Table III. It should be emphasized that these factors, although representing specific criteria for the components when chromatographed in accordance with the procedures described, are not applicable to other column packings or different experimental conditions.

Peak areas determined for the various components in accordance with the procedure described under Determination of Areas were converted to corresponding weights by multiplying by the respective correction factors. Relative percentage compositions of the different products were subsequently established using the internal normalization technique as illustrated in Table I (steps VIII and IX, respectively).

Results and Discussion

Identification of Constituents. Relative retention times, specific retention volumes, and partition coefficients of both major and minor components of peppermint oil were reported in a previous communication from this laboratory (*45*) and these data utilized in interpreting the chromatograms obtained. Columns of different polarities —e.g., Resoflex LAC-IR-296 (an adipate polyester of diethylene glycol) and Ucon (a polyalkylene glycol ether)— were employed in some instances to confirm the identifications made.

In addition, fractions were collected during analysis by means of microsampling cells mounted at the column exit (*1*) and their infrared spectra in carbon tetrachloride solution were compared with those of authentic reference compounds known to occur in peppermint oils.

These measurements confirmed the presence of 3-octanol as well as linaloöl in *M. arvensis* oils, and showed the presence of trace amounts of impurities (possibly γ-terpinene) in the cineole fraction. They revealed, furthermore, the simultaneous emergence of some other component or components along with piperitone. Experimental evidence for this phenomenon was also deduced from chromatograms. They showed that σ of the piperitone peak was not a constant and that its value was always greater than expected when plotted *vs.* retention time in accordance with the procedure described.

For the cineole-terpinene peak, this phenomenon was hardly noticeable because only trace amounts of hydrocarbon contaminated the oxide and, furthermore, sigma values were not measurable with the same degree of accuracy (sigma for cineole <1.5 mm.; sigma for piperitone >5.5 mm.).

Infrared measurements indicated the possible presence of carvone in the piperitone fraction. As yet, the occurrence of both 2- and 3-oxygenated terpenes in the same mint plant has not been proved (*11*) and the presence of small amounts of carvone in peppermint oil might be due to mere admixture. Most mint farms cultivate both peppermint and spearmint plants and often the fields lie side by side. Unless sufficient precautions are taken, intercontamination can and does occur. Moreover, the spearmint crop is generally distilled prior to the peppermint crop and if the same still is used for the operation, any spearmint oil (hence carvone) remaining in the plumbing will contaminate the peppermint oil (*11, 47*).

No major components of *M. piperita* emerged following appearance of cineole and preceding elution of menthofuran. The profile of this ill-defined region is probably due to traces of *p*-cymene, terpinolene, 3-octanol, sabinene hydrate, and related minor components known to occur in peppermint oil.

Menthyl acetate, observed to emerge together with *d*-neomenthol prior to menthol when employing polyester or polyether as partitioning agent, was found to elute after menthol when using SAIB as liquid phase, the alcohol evidently being held less firmly than its ester by the acetate groups of the strongly polar substrate.

A small shoulder following the menthone peak was observed to emerge irrespective of the nature of the column, its intensity increasing after treatment of the oil with dilute hydrochloric acid. The same phenomenon was observed when purified menthone was similarly analyzed before and after such treatment. This indicated the presence of isomenthone in both *piperita* and *arvensis* oils. Under the experimental conditions, limonene was well separated from cineole and menthone from menthofuran. However, none of the columns used separated cineole from γ-terpinene or improved resolution of the piperitone peak. Corresponding areas calculated from chromatograms are, therefore, not indicative of individual components.

Various constituents identified by means of the techniques described are shown in Table III along with their boiling points and respective area correction factors.

Per Cent Composition of Peppermint Oils

Tables IV and V show percentages of the main constituents of *M. piperita* and *M. arvensis* oils volatile under the experimental conditions used.

Some of the *M. piperita* oils included in this study were received as blends. Analytical data for these products are not reproduced in the tables, but are entered in Figures 4 and 5, respectively, with provenances considered to make up the greater portion of the oils shown in parentheses. Terpeneless oils and preparations which appeared to have deteriorated are indicated in the illustrations by means of an asterisk (Figures 4 to 8).

Constituent Ratios

From the crude data given in Tables IV and V, ratios of constituents were calculated in order to evaluate the properties of genuine products and assess the geographical origins of different species. Pertinent results are shown in Tables VI and VII for *M. piperita* and *M. arvensis* oils, respectively.

Terpenes (ratio *A*). *M. piperita* oils are generally produced from plant

material by steam distillation, which process yields the so-called natural oils. Redistillation to remove objectionable low boiling constituents such as acetaldehyde, dimethyl sulfide, color, and resinous matter produces oils meeting U.S.P. (U. S. Pharmacopoeia) requirements. Some manufacturers market triple-distilled oils which are practically colorless, and of exquisite odor and flavor. If the purification processes are carried out as fractional distillations involving removal of foreruns and high-boiling components, terpeneless oils are obtained (*16*). Different procedures and techniques of rectification and extraction are followed by different producers and as a rule technical details are kept confidential.

Natural *M. arvensis* oils are usually not purified by distillation, but dementholized by freezing, which treatment removes menthol (40 to 50%) as well as small and varying amounts of other oxygenated, monocyclic terpenes.

Products subjected to these processes—i.e., deterpenation and dementholization—may be recognized readily by determining their terpene and menthol content, respectively. Rectification or redistillation in toto will not appreciably affect ratio *A*, unless terpene constituents are being removed during purification.

Natural *M. piperita* and *M. arvensis* oils were always found to contain traces of low boiling components emerging prior to α-pinene at the column exit. Terpeneless oils invariably failed to show the presence of these substances. Rectification of *M. piperita* oils and dementholization of *M. arvensis* oils could not be so detected. Some of their chromatograms exhibited peaks indicating that these low boiling components had survived the purification procedure, while others displayed comparable areas of indefinite profiles which could easily be confused with instrument noise.

Menthone/Isomenthone (ratio *B*). Isomenthone can be produced via biochemical transformations, but could also be generated during distillation and subsequent purification of the oils, for it is known that menthone isomerizes readily to isomenthone in the presence of trace amounts of acids. *M. piperita* oils showed menthone/isomenthone ratios ranging from 2.7 to 6.6 (median 4.7), *M. arvensis* oils from Brazil and China displayed ratios varying from 2.8 to 6.3 (median 3.4), while for products originating in Japan, values ranging from 5.6 to 8.6 (median 7.4) were observed. Experimental data would therefore suggest that genetic and ecological factors determine, at least in part, the menthone/isomenthone ratio.

Limonene/Cineole (ratio *C*). This ratio was found to be particularly informative. Values ranging from 0.2 to 0.7 were characteristic for *M. piperita* oils, values greater than 2 were characteristic for *M. arvensis* oils. Evidently, the ratio is genetically controlled. Its determination therefore affords a more specific criterion for recognizing genuine *M. piperita* oils than the official glacial acetic–nitric acid test (*49*). It will, in addition, also identify authentic *M. arvensis* oils.

Experimental results indicated that the ratio increased with maturation of the plant. Unfortunately, only two pertinent samples (EO-534 and EO-532) representing U. S. Yakima oils from an early and late harvest, respectively, were available to demonstrate this trend.

Only little, if any, physical significance can be attached to the ratio for terpeneless oils because the different processes by which these products are manufactured distort the relationship to varying degrees.

Menthofuran (ratio *D*). In order to eliminate compositional effects resulting from deterpenation of oils, experimental data were processed by calculating the ratio of a given component to one or more biochemically related compounds. Thus, a consistent basis for menthofuran relationships between the various products was established by determining ratios for menthofuran to "menthone related constituents" (menthone + isomenthone + menthofuran) (ratio *D*). This treatment of experimental data yielded the following information about *M. piperita* oils.

Values up to, but not exceeding, 0.09 were characteristic of oils from the American Midwest, and possibly, England. Values ranging from 0.09 to 0.13 were characteristic of American oils from the western part of the states of Washington and Oregon. Values ranging from 0.14 to 0.60 were characteristic of oils from the eastern part of the state of Washington (area near Sunnyside, Yakima county), Italy, or other regions with a hot, sunny and dry growing season.

The ratio is undoubtedly influenced by climatic conditions and affected by differences in plant material (leaves or blossoms) used for production of the oil (*37*). Effects of environmental conditions were demonstrated by the analysis of two samples derived from the root stock of English Mitcham-type mints obtained from Germany and grown in the arid, sunny climate of South Africa. They proved indistinguishable from U. S. Yakima oils. Likewise, Italian oils which are generally considered descendants, via vegetative propagation, of Mitcham root stock (*M. piperita* var. *vulgaris* L.) brought to Italy by Carles at the beginning of this century (*24*) resembled Yakima oils with regard to menthofuran content. Climatic factors, no doubt, codetermine also the menthofuran variations observed between American oils of different provenance.

Ratio *D*, like the limonene/cineole ratio, evidently changes during maturation of the plant. Thus the "early" Yakima oil (EO-534) showed a lower menthofuran content, indicating that the plants had not yet blossomed fully, which observation is in accord with previous findings (*17*, *26*).

Chromatograms of *M. arvensis* oils exhibited occasionally "menthofuran" peaks amounting to as much as 0.8% of the oil and corresponding to an apparent menthofuran/menthone + isomenthone + menthofuran ratio of 0.03. However, chemical tests for menthofuran (*8*, *49*, *51*) were always negative. Fractions collected from several runs displayed the characteristic odor of linaloöl, and infrared analyses confirmed the presence of the unsaturated, tertiary alcohol. Trace amounts of the terpenoid occur probably also in *M. piperita* oils, but escape detection because the column fails to separate the linaloöl-menthofuran system.

Neomenthol/Menthyl Acetate (ratio *E*). This ratio proved useful for the assignment of provenance, as will be discussed in the section entitled "Distribution Plotting." Experimental results obtained on samples produced from plants at different stages of development revealed that the ester content increased with the maturation process.

"Menthol"/Neomenthol (ratio *F*). This ratio expresses the relationships observed between "menthol related constituents" (menthol + neomenthol + menthyl acetate) and neomenthol. It is probably governed by dynamic equilibrium conditions existing in the plant between menthol and neomenthol and is a function of the activity of specific enzyme systems. Undoubtedly it is controlled by both genetic and climatic factors.

"Menthone"/"Menthol" (ratio *G*). It has been reported that both menthol and menthyl acetate concentrations increase, while menthone content decreases during maturation of the plants and their exposure to sunlight (*2*, *4*, *15*, *21*, *30*). Ratio *G* ("menthone related constituents"/"menthol related constituents"—i.e., menthone + isomenthone + menthofuran/menthol + neomenthol + menthyl acetate) was calculated to detect these phenomena. Results obtained on two samples from an early and a late harvesting, respectively (EO-534 and EO-532), fully confirmed the observations referred to above.

The ratio is less meaningful for *M. arvensis* oils because of various degrees of dementholization these products undergo during manufacture. It is, likewise, distorted in terpeneless oils which are prepared by means of different techniques of distillation and extraction. The experimental data obtained served to indicate that deterpenation removed preferentially menthone related constit-

uents from most of the samples. Experimental data assembled for terpeneless oils are indicated by means of an asterisk in Figures 4 to 8.

3-Octanol, Pulegone, Piperitone, and Menthyl Acetate (ratios *H*, *J*, *K*, *L*). Utilization of appropriate ratios of these constituents proved of particular value for *M. arvensis* oils. Since these products are dementholized to varying extents by freezing, constituent ratios must be properly selected and suitably corrected to allow meaningful comparisons. For the percentages of 3-octanol, pulegone, and piperitone, respectively, this was achieved by using the sum total of the following "liquid oxygenated constituents" (3-octanol, menthone, isomenthone, neomenthol, pulegone, and piperitone) as the divisor.

For menthyl acetate, this treatment of experimental data was not sufficient. Apparently, some ester is removed from the oil, probably by mere adsorption, along with the crystallized menthol. In order to correct for this phenomenon, the amount of menthol precipitated must be taken into consideration. This was accomplished by further dividing the values obtained by the respective menthol contents as shown in Table VII for ratio *L*.

Distribution Plotting

It has been shown that experimental data obtained on products of similar botanical nature may be so processed as to permit fairly conclusive distinctions and classifications (*5*). The present study illustrates the type of information that can be gained by plotting characteristic constituent ratios, observed for peppermint oils of different geographical origins, against one another.

Differentiation of *M. piperita* and *M. arvensis* Oils. The distinction between these two products is shown by plotting ratio *C* (limonene/cineole) *vs.* ratio *D* (menthofuran/"menthone related constituents"), as illustrated in Figure 4. The reliability of this classification surpasses markedly the classical, chemical identification test (*49*) which confirms solely the presence of *M. piperita*, but does not detect even gross adulteration of *M. piperita* with *M. arvensis* oils. Moreover, the sensitivity of the color reaction diminishes markedly and the test may even fail for genuine *M. piperita* oils if applied to samples that have been stored for some length of time and therefore become depleted in menthofuran.

***M. piperita* Oils.** Figure 5 illustrates results obtained when plotting ratio *E* (neomenthol/menthyl acetate) *vs.* ratio *D* (menthofuran/"menthone related constituents"). Isolated areas thus produced show how U. S. Yakima, Midwestern, and Oregon oils may be differentiated and Italian oils be recognized. English Mitcham peppermint oils could not be grouped within a narrow area when so processed, but were moderately separated from other products when ratio *F* ("menthol related constituents"/neomenthol) was plotted *vs.* ratio *G* ("menthone related constituents"/"menthol related constituents") as illustrated in Figure 6. Similar treatment of experimental data obtained on blends of American oils showed that most of the samples contained Yakima oil as their chief component.

***M. arvensis* Oils.** Figure 7 shows the classification of these products on the basis of a plot of ratio *H* (3-octanol/"liquid oxygenated constituents") *vs.* ratio *J* (pulegone/"liquid oxygenated constituents"). Chinese and Japanese oils were well separated. A distinction between Brazilian and Formosan oils could also be made, but a larger number of samples would be required to demonstrate more convincingly the reliability of this identification scheme.

Figure 8 illustrates area assignments on the basis of a plot of ratio *K* (piperitone/"liquid oxygenated constituents") *vs.* ratio *L* (menthyl acetate/"liquid oxygenated constituents") $\times \frac{1}{\text{menthol}}$. Well defined regions and reliable separations were thus obtained for all products.

Compositional differences between these oils may well be genetic rather than climatic and one is tempted to speculate on the history of the respective plantations. Japanese settlers emigrating from their native land to Brazil after the great earthquake in 1923 began to cultivate the peppermints they had brought with them. The high-yielding variety, known as M.A. No. 701, was developed from these peppermints by plant breeding at the Agricultural Experiment Station in Campinas, São Paulo, and is now grown almost exclusively throughout Brazil. About 15 years ago, Dr. Alberto Tedin brought it to Argentina where it is presently also cultivated on a commercial scale (*26*).

Japanese growers may also have introduced, before World War II, a native strain of *M. arvensis* var. *piperascens* Malinvaud to Formosa. The same variety is apparently propagated in Korean and South African plantations. The Chinese plant has been recognized as another variety, *M. arvensis* var. *glabrata* Holmes (*25*) and this taxonomic differentiation is fully supported by chemical data obtained in this study.

Experimental Samples, Blends and Terpeneless Oils. Also included in the diagrams are data obtained on oils produced as experimental samples only. Since insufficient specimens of such products were available no area assignments were made. Results obtained on blends are shown in parentheses. The oil found to make up the greater portion of these products was considered indicative of sample origin. All experimental points thus reproduced were in agreement with information and comments subsequently received from the suppliers of these specimens. Data applying to terpeneless oils are indicated by means of an asterisk. Experimental points illustrating duplicate determinations are joined by straight lines (Figures 4 to 8). Distances between such points reflect analytical reproducibility. It should be noted, however, that they also depend on the parameters chosen and, therefore, illustrate the degree of precision with which the boundaries of a given area may be established.

Appraisal of Experimental Data

The composition of an essential oil is influenced by many factors—e.g., the genetic make-up of the plants from which it is isolated, the ecological conditions under which the plants are grown, their maturity at the time of harvest, and, finally, the manufacturing processes they have undergone. All of these variables are reflected in the final product. As a result each and every lot represents a mixture of considerable complexity and the disentanglement of these factors would seem to be a hopeless task. Yet, for samples which may be considered extremes, the effects of certain variables can clearly be recognized and from the experimental data thus assembled valid deductions and extrapolations may be made. Previous work from this laboratory demonstrated the successful examination of ylang-ylang oils by means of infrared spectroscopy (*41*) and the characterization of citrus oils by means of specific chemical analyses (*38*) for the purpose of recognizing products of different manufacture and geographical origins, respectively. The present investigation based on compositional analysis by means of gas-liquid partition chromatography and mathematical treatment of the data in terms of constituent ratios demonstrates a more fundamental approach to this objective and has confirmed that origin assignments of essential oils can be made with a reasonable degree of accuracy.

It should be realized, however, that this study is based on a rather limited number of commercial and experimental samples of whose history relatively little was known to us. They were obtained from different suppliers and presumed to be genuine; however, none of them was completely authenticated. The experimental data reported should, therefore, not be regarded as offering a final, unequivocal scheme of classification. Yet they serve to demonstrate the potentiality of a general, sensitive method of analysis and its value to industry as a selective technique for the manufacture and quality control

of essential oils and related products.

Because only a restricted number of samples have been examined, area distinctions made on the basis of any particular scatter diagram should be considered presumptive rather than conclusive evidence for geographical origin. Although based on two ratio parameters representing at least four individual criteria of identity, they are primarily probability functions indicative of sample provenance. Further processing of the experimental data to show that the characteristic criteria of origin remain valid considerably enhances the reliability of the identification scheme, for confirmatory evidence from a second scatter diagram serves to demonstrate that four ratio parameters (i.e., at least eight criteria of identity) are in agreement with the original deduction. The authors are pleased to report that origin determinations made on this basis on coded samples to evaluate the reliability of the method proved indeed highly successful (*11*, *26*).

Biochemical Considerations

Essential oils may be regarded as plant excretion products whose composition depends on a number of simultaneous, sometimes competitive, biosynthetic reactions, each of which determines the formation of a specific constituent. The present study has shown that various components occurring in peppermint oil are related to one another and the experimental data may, therefore, serve as a basis for speculation on the nature and course of some of the biochemical pathways controlling the generation and accumulation of specific constituents in plant tissues.

Production of menthofuran by *M. piperita*, for example, must be considered genetically determined, for the heterocyclic compound is not found in *M. arvensis* oils. Since, furthermore, it is never produced by *M. arvensis* under any climatic conditions, none of the essential oil constituents common to *M. arvensis* and *M. piperita* can be the parent material for the production of menthofuran under the sole influence of sun, heat, and oxygen.

Menthofuran is primarily generated in the young, growing tissues of *M. piperita*. When inflorescences develop, the menthofuran content of the oil increases due to formation of new growing tissue in the flowers. The present study has shown that the ratio of "menthone related constituents"/"menthol related constituents" remains fairly constant because any increase in menthofuran content appears to be compensated by an apparent decrease in menthone and isomenthone content. One may be tempted to conclude that menthone is being transformed to menthofuran. This interpretation does not necessarily hold true, however. The marked shift of ratio *D* (menthofuran/"menthone related constituents") in favor of a higher menthofuran content can to a large extent be attributed to maturation and flowering of the plant, for during these processes both the total yield of oil as well as its menthofuran content are increasing. The metabolic course of events probably entails concurrent production of menthone and menthofuran from a common precursor. When plants blossom, their menthone content reaches a maximum, but more oil is still being produced in the flowering foliage and in the new tissues the precursor is preferentially transformed to menthofuran.

Taking these concepts into consideration, differences in menthofuran content between peppermint oils of different geographical regions—e.g., products from the U. S. Midwest (low menthofuran content) and U. S. Far West (high menthofuran content)—may be ascribed to such plant characteristics as, for example, the vigor of bloom in relation to the rest of the plant; the larger flowering spikes produced in dry, irrigated, sunny climates; or the smaller spikes, but more enhanced leaf development of plants grown in moist, cloudy environments.

Similar considerations apply to the limonene/cineole relationships observed. Again, compositional differences suggest biochemical control. One may be tempted to conclude from experimental data that both the cineole content and the cineole/limonene ratio decline as the plant reaches maturity. Yet, such a deduction is not justified. The cineole content remains fairly constant, but the yield of oil and its limonene content increase with maturation of the plant, the terpene possibly being preferentially produced in the fresh flowering tissues. The experimental data presented would also favor a common precursor—possibly a terpineol derivative (*36*).

Valuable contributions to the biogenesis of compounds found in *M. arvensis* and *M. piperita* oils were made by Reitsema (*42*). Related work(*48*) suggests that up until their final deposition in and between the cells constituting plant tissues, essential oils remain as pyrophosphorylated complexes suspended in aqueous media. All precursors are considered to be water-soluble entities prior to production of the catabolite. Isomeric isopentenyl and β,β-dimethylallyl pyrophosphates are assumed to combine and form various open chain terpene pyrophosphates. These, in turn, are visualized as cyclizing to monocyclic and bicyclic terpene pyrophosphates serving as precursors of such constituents as limonene, cineole, α-pinene, β-pinene, and camphene.

Similar monocyclic terpene pyrophosphates may undergo oxidative cleavage and other reactions to yield oxygenated monocyclic terpenes occurring in peppermint oil. Thus, precursor terpenes oxygenated at the 3-position would lead to menthol or neomenthol, menthone or isomenthone or menthofuran, to pulegone, and to piperitone. Different stereospecific enzyme systems would control these metabolic processes and thus account for genetic differences between plants. Conceivably, most of the enzyme systems common to the *Mentha* genus are present in all oil-bearing parts of a given plant, but their activity is genetically geared to production of the constituents characteristic of that plant. Some enzymes may be inoperative or lacking. Absence of menthofuran in *M. arvensis* oils could thus be explained. Specific enzyme systems may also be accelerated or supersede other systems competing in different parts of the plant during different stages of development and under changing climatic conditions. In *M. piperita*, the menthofuran-producing enzyme system supersedes the menthone-producing system in the young tissue (*37*). Recently, radiocarbon tracer techniques were developed for the purpose of studying some of the complex phases of the biochemistry accompanying essential oil synthesis in mint including the order of formation of various constituent compounds (*43*).

Detection of Adulteration

Genuine specimens of *M. piperita* oils analyzed in accordance with the techniques described were experimentally adulterated with substances known to have been used for the sophistication of these products (*22*). With the possible exception of synthetic menthol and authentic *M. arvensis* oils, such additions can readily be recognized. From data obtained, the lowest amounts of adulterants which should be detected without difficulty were calculated and the predictions confirmed experimentally (Table VIII).

Detection of adulteration with menthol will depend on the original menthol content of the sample and whether the added amount places the relevant compositional parameters of the sophisticated preparation outside the normal ranges assigned. The effects of the addition of increasing amounts of menthol to a Yakima peppermint oil (Sample No. P-50, Figure 1) are illustrated in Figure 6 (squares and dash-dotted lines).

Addition of *M. arvensis* to a *M. piperita* oil affects of necessity most, if not all, the characteristic compositional parameters of the sample, the magnitude of the changes depending on the composition of both products. An example, illustrating the addition of various percentages of a Japanese *M. arvensis* oil (Sample No. P-21, (Figure 2) to a Yakima *M. piperita* oil (Sample No. P-50, Figure 1)

. shown in Figures 5 and 6, respectively (triangles and dashed lines).

Use and Limitations of the Method

This study does not represent a complete and final analysis of the composition of peppermint oil. It illustrates primarily gross compositional features due to major components volatile under the experimental conditions used. It does not detect any of the high-boiling components some of which impart characteristic flavor notes to the oil—e.g., menthyl isovalerate, hydroxylactone autoxidation product of menthofuran—and leaves all sesquiterpenes unaccounted for. It does not determine any of the minor and trace components, many of which contribute materially to the sweetness of genuine products. Refinements of the technique—e.g., application of capillary and preparative columns, use of different packings and temperature programmed gas chromatography, in conjunction with chemical pretreatment of the analytical samples—e.g., removal of specific compounds or classes of compounds for the purpose of realizing additional separations, will no doubt confirm the presence of many more components already discovered by classical techniques (*12, 23, 42, 44*)and establish the occurrence of hitherto unreported constituents.

The classifications arrived at reflect only dominant analytical sample characteristics and may not bear much relationship to those compounds which on the basis of organoleptic tests cause one area

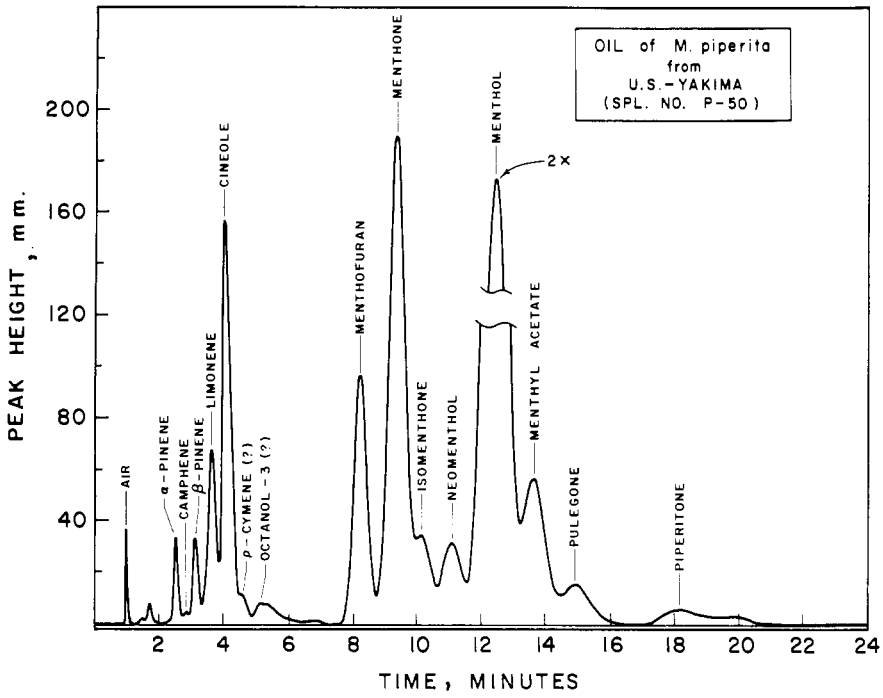

Figure 1. Gas chromatogram of oil of *M. piperita* from U.S., Yakima

Sample number P-50

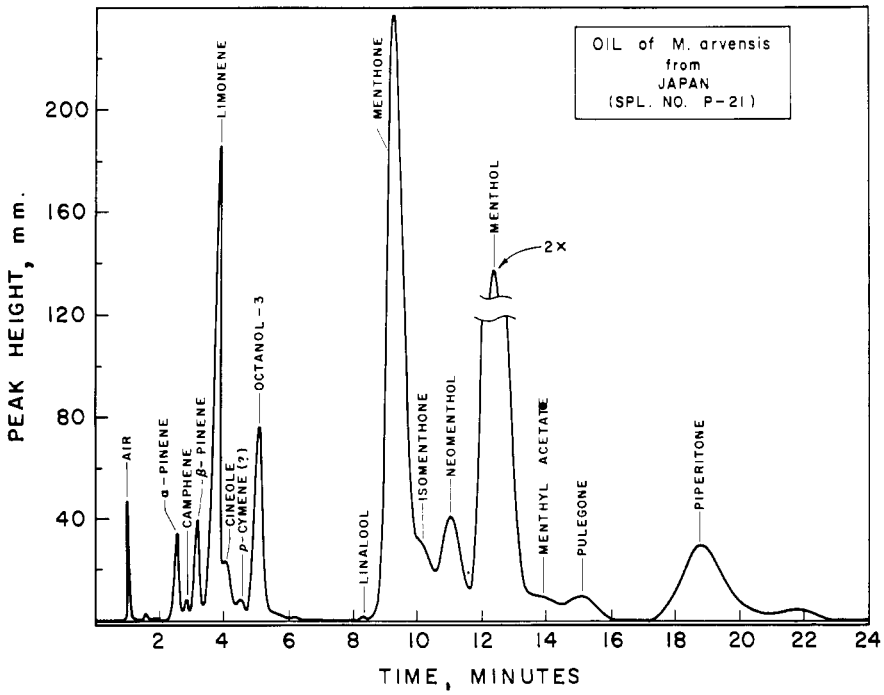

Figure 2. Gas chromatogram of oil of *M. arvensis* from Japan

Sample number P-21

Figure 3. Relationship between peak separation and peak height correction

Figure 4. Distribution plot of ratio C (limonene/cineole) *vs.* ratio D (mentofuran/"menthone related constituents") for peppermint oils

Figure 5. Distribution plot of ratio D (menthofuran/"menthone related constituents") *vs.* ratio E (neomenthol/menthyl acetate) for M. piperita oils

A.	Argentine	*O.*	U.S. Oregon
E.	English	*SA.*	South African
N.	Netherlands	*C.*	Canadian
S.	Spanish	*M.*	U.S., Midwest
B.	Bulgarian	*P.*	Polish
I.	Italian	*Y.*	U.S., Yakima

Figure 6. Distribution plot of ratio F ("menthol related constituents"/neomenthol *vs.* ratio G ("menthone related constituents"/"menthol related constituents") for *M. piperita* oils

Symbols have the same meaning as in Figure 5

Figure 7. Distribution plot of ratio H (3-octanol/"liquid oxygenated constituents") *vs.* ratio J (pulegone/"liquid oxygenated constituents") for *M. arvensis* oils

A. Argentine; K. Korean; SA. South African

Figure 8. Distribution plot of ratio K (piperitone/"liquid oxygenated constituents") *vs.* ratio L (menthyl acetate/"liquid oxygenated constituents" × 1/menthol) for *M. arvensis* oils

Symbols have the same meaning as in Figure 7

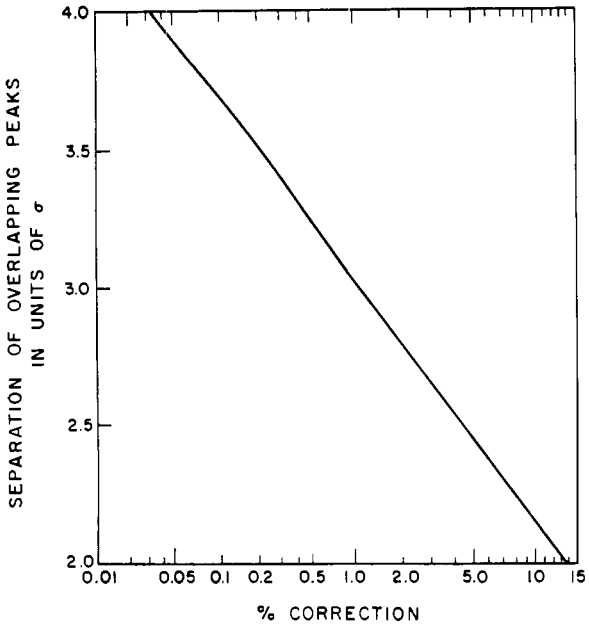

Figure 3

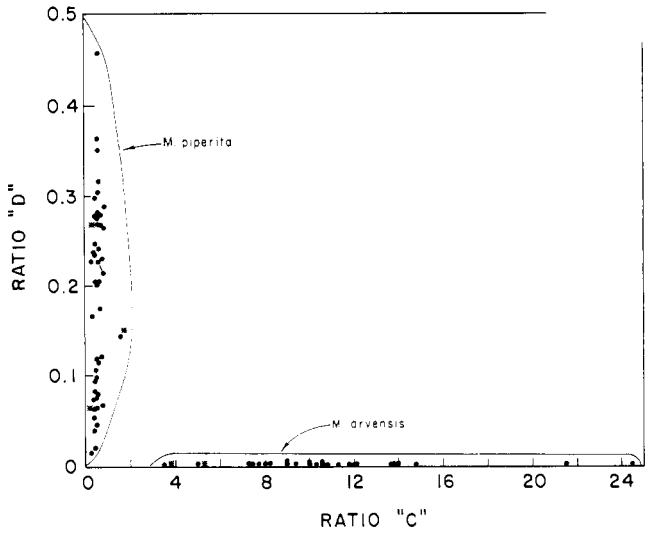

Figure 4

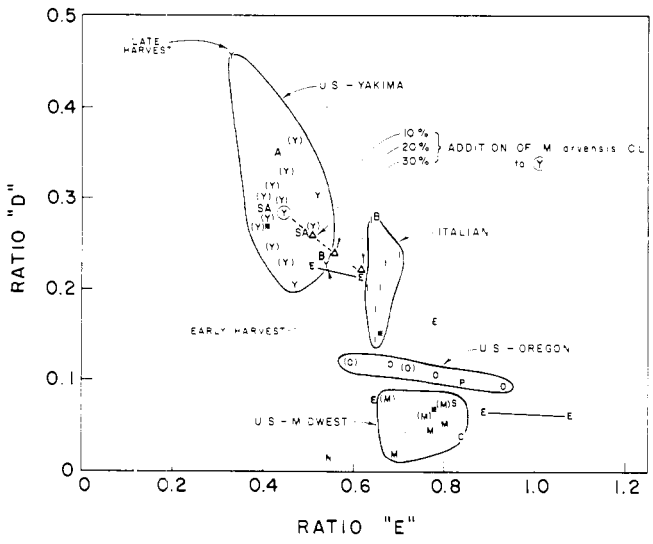

Figure 5

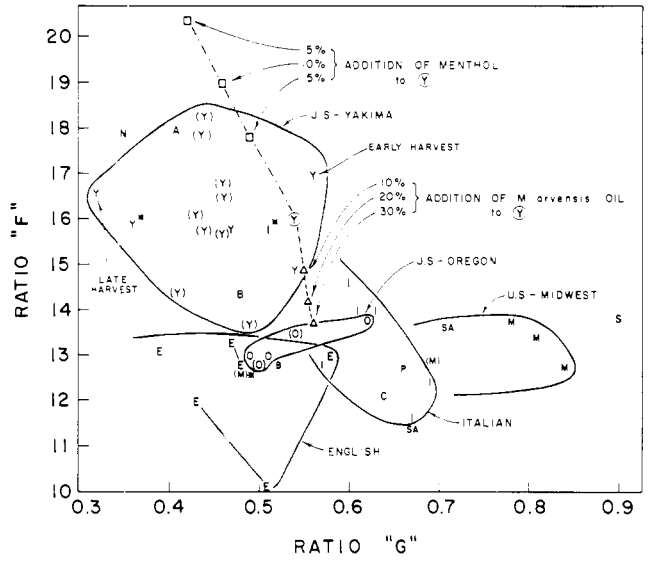

Figure 6

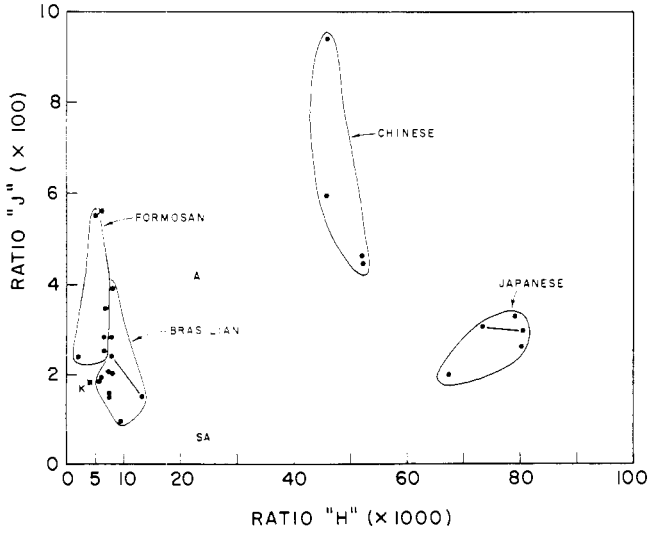

Figure 7

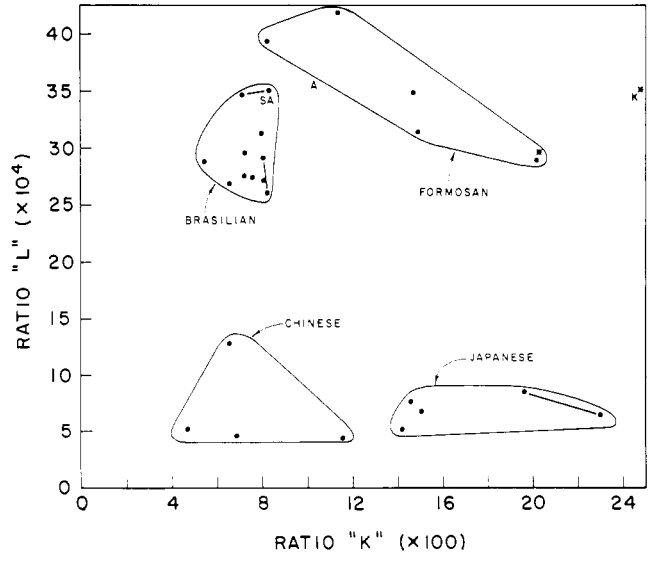

Figure 8

Table I. Mathematical Processing of Gas Chromatograms

Sample Calculation for Peak Areas and Their Conversion to Compositional Data, Based on Chromatogram of Yakima *M. piperita* Oil (Figure 1)

Stepwise Procedure	Experimental Results: α-Pinene	Camphene	β-Pinene	Limonene	Cineole	Menthofuran	Menthone	Isomenthone	Neomenthol	Menthol	Menthyl Acetate	Pulegone	Piperitone[a]	Others
I. Measure peak height, h, mm.	33.7	4.1	33.0	68.0	157.2	96.8	190.5	34.7	31.1	345.3	56.8	15.1	5.5	
II. Measure retention time, t, minutes[b]	2.55	2.85	3.15	3.7	4.15	8.25	9.4	10.2	11.1	12.5	13.7	14.9	18.2	
III. Measure or determine graphically σ, mm.[c]	0.89	0.98	1.07	1.24	1.38	2.64	3.00	3.24	3.52	3.95	4.32	4.69	5.71	
IV. Evaluate extent of overlapping peaks in terms of σ and correct for														
A. Effect of preceding peak														
1. Determine distance from preceding peak, mm., and divide by σ of preceding peak, mm.[d]	...	4.28	3.88	6.53	4.60	37.73	5.53	3.38	3.52	5.05	3.85	3.52	8.93	
2. Express peak separation as per cent correction by reference to calibration graph shown in Figure 3	...	0.01	0.06	[e]	[e]	[e]	[e]	0.33	0.2	[e]	0.06	0.2	[e]	
3. Convert per cent correction to mm. equivalent of preceding peak height	...	[e]	[e]	[e]	[e]	[e]	[e]	0.63	0.07	[e]	0.21	0.11	[e]	
4. Subtract value from peak height h to be corrected and obtain partially corrected peak height h', mm.	33.7	4.1	33.0	68.0	157.2	96.8	190.5	34.1	31.0	345.3	56.6	15.0	5.5	
B. Effect of succeeding peak														
1. Determine distance from succeeding peak, mm., and divide by σ of succeeding peak, mm.[f]	3.88	3.56	5.63	4.14	19.72	4.86	3.13	3.24	4.50	3.52	3.24	7.35	...	
2. Express peak separation as per cent correction by reference to calibration graph shown in Figure 3	0.06[e]	0.18[e]	[e]	0.018[e]	[e]	[e]	0.72	0.51	[e]	0.2	0.51	[e]	...	
3. Convert per cent correction to mm. equivalent of succeeding peak height	0.002[e]	0.06[e]	[e]	0.03[e]	[e]	[e]	0.25	0.16	[e]	0.11	0.08[e]	[e]	...	
4. Subtract value from partially corrected peak height h' to obtain fully corrected peak height h'', mm. [(*A*-4) − (*B*-3)]	33.7	4.0	33.0	68.0	157.2	96.8	190.3	33.9	31.0	345.2	56.5	15.0	5.5	
V. Compute peak area from measurements of height and sigma or from graphical data[g] $A = [(0.308 \times t) + 0.10] \times h'' \times \sqrt{2\pi}$ (mm.)^2	75	10	89	211	543	641	1429	275	273	3418	612	176	79	145[h]
VI. Account for thermal conductivity of component by using appropriate correction factor[i]	0.83	0.96	0.95	1.43	1.17	1.02	0.97	1.00	0.99	1.00	0.98	1.03	1.00	
VII. Multiply peak area with correction factor to obtain corrected area, (mm.^{2})[k]	62	9	85	302	635	654	1386	275	270	3418	600	181	79	145
VIII. Add areas of all components						8101								
IX. Normalize to obtain per cent (w./w.) composition of product[l]	0.8	0.1	1.1	3.7	7.8	8.1	17.1	3.4	3.3	42.2	7.4	2.2	1.0	1.8

[a] Including other components, possibly some carvone.
[b] Injection of sample taken as zero time.
[c] When plotting σ *vs.* t for isolated symmetrical peaks, a straight line is obtained. Under the experimental conditions, its equation was found to be $\sigma = [(0.308 \times t) + 0.10]$. This equation is generally applicable, but its specific constants must be experimentally determined for each column operated under a given set of conditions. It permits evaluation of σ for any peak, even if only partially resolved, provided retention time can still be measured with reasonable accuracy, or be otherwise determined (chromatographic analysis of reference compound under comparable conditions).
[d] $\frac{(t_{\text{peak to be corrected}} - t_{\text{preceding peak}})\text{ (min.)} \times 12.7\text{ (mm./min.)}}{\sigma_{\text{preceding peak (mm.)}}}$ = separation of peaks in σ units of preceding peak where 12.7 = chart speed in mm./minute.

Continued on p. 239

Correction is negligible, considering accuracy of experimental measurements.

[f] $\frac{(t_{\text{succeeding peak}} - t_{\text{peak to be corrected}})\ (\text{min.}) \times 12.7\ (\text{mm./min.})}{\sigma_{\text{succeeding peak (mm.)}}}$ = separation of peaks in σ units of succeeding peak.

[g] For isolated, symmetrical peaks $A = \sigma \times h \times \sqrt{2\pi} = \sigma \times h \times 2.507\ (\text{mm.})^2$. Thus, measurements of peak height and sigma will suffice to carry out calculations. However, utilization of graphical information assembled,[e] simplifies the procedure still further. When substituting for sigma, the equation becomes $A = [(0.308 \times t) + 0.10] \times h'' \times 2.507 = [(0.772 \times t) + 0.251] \times h''(\text{mm.})$.[2] Hence, peak areas may readily be determined by means of two conveniently measurable variables, namely peak height and retention time.

[h] Measured by counting unassigned areas through square millimeter grid.

[i] Sample calculation for determination of these factors shown in Table III.

[k] This area bears a direct relationship to the weight of the constituent present, as determined by normalization (steps VIII and IX).

[l] Procedural details and experimental results are reproduced for the purpose of illustrating the method of data processing. In practice, only a fraction of the calculations need be made. Value of method lies in convenience it provides for securing quantitative, compositional parameters from well resolved gas chromatograms. Corrections to be made when dealing with only partially resolved peaks are easily computed from experimental data and quantitative results obtained, although the valley between adjacent peaks fails to return to the base line. In general, corrections are negligible when distance between neighboring peaks is greater than 4 σ—see columns A-1, A-2 and B-1, B-2, respectively. With some experience, mere visual inspection of a chromatogram will show whether or not corrections would be of any significance.

Table II. Sample Calculation of Correction Factors

Reference Compound	Composition, %																				Weight of Reference Compound in Mixture, Mg.
	α-Pinene		β-Pinene		Limonene		Cineole		Menthofuran		Menthone		Isomenthone		Neomenthol		Menthol		Menthyl acetate		Piperitone		
α-Pinene	98.9		1.1																		
Weight in mixture, mg.[a]		26.21		0.29																	26.50
Limonene			0.8		97.3		1.9														
Weight in mixture, mg.[a]				0.24		28.90		0.56													29.70
Menthone							0.2				95.3		3.6				0.8				
Weight in mixture, mg.[a]								0.04				18.82		0.71				0.16			19.75
Neomenthol			0.4				0.1		0.3		0.3				95.5		3.4				
Weight in mixture, mg.[a]				0.10				0.03		0.08		0.08				24.83		0.88			26.00
Menthol			0.3								0.8				1.5		97.2				
Weight in mixture, mg.[a]				0.12								0.31				0.58		37.57			38.65
Menthyl acetate																	0.5		99.5		
Weight in mixture, mg.[a]																		0.23		45.47	45.70
Piperitone					3.7		0.2										1.5		0.8		93.9	
Weight in mixture, mg.[a]						2.20		0.12										0.89		0.48	55.87	59.50
Weight of component, in mixture, mg.[b]		26.21		[c]		31.10		[c]		[c]		19.21		[c]		25.41		39.73		45.95	55.87	
Peak area, mm.^{2} as measured[d]		828		[c]		583		[c]		[c]		560		[c]		699		1080		1267	1654	
Peak area, mm.^{2} as calculated, considering menthol as primary standard and menthol area requiring no correction[e]		711				844						522				690		1080		1247	1517	
Correction factor[f]		0.86				1.44						0.93				0.99		1.00		0.98	0.92	

[a] Calculated from per cent composition and weight of reference compound used.

[b] Weight of component in mixture including contribution, as impurity, from other compounds.

[c] Contaminants shown in these columns need not be considered because their effects are eliminated by processing experimental data as described.

[d] Following method outlined in text and summarized in Table I.

[e] Calculated area (mm.^{2}) = total weight of component (mg.) $\times \frac{\text{menthol area (mm.}^2)}{\text{total weight of menthol (mg.)}}$ = total weight of component in mixture (mg.) $\times \frac{1080}{39.73}$ (for experiment carried out). It represents area component should have, if correction factor (thermal conductivity of component) were equal to that for menthol (set arbitrarily at 1.00).

[f] Ratio of calculated area/measured area. Per cent (w./w.) component in mixture = measured area × correction factor. Values shown for these factors in Table II represent average results obtained on five mixtures of different compositions and using three different sample sizes of each.

Table III. Constituents of *M. piperita* and *M. arvensis* Oils Separated by Gas-Liquid Partition Chromatography[a]

Column. SAIB on Chromosorb W Temperature. 170° C. Carrier gas (Helium). 75 ml./minute

Constituent	Boiling Point, ° C.	Area Correction Factor[b]	Constituent	Boiling Point, ° C.	Area Correction Factor[b]
α-Pinene	155–157	0.83	Linaloöl[d]	198–199	...
Camphene	158–160	0.96	Menthone	209–210	0.97
β-Pinene	164–166	0.95	Isomenthone	212	1.00
Limonene	177–178	1.43	Neomenthol	212–212.5	0.99
Cineole, γ-Terpinene[c]	176–177 / 183	1.17 / ...	Menthol	216	1.00
3-Octanol	178–179	0.84	Pulegone	221–223	0.98
Menthofuran	196	1.02	Menthyl acetate	227–228	1.03
			Piperitone[e]	235–237	1.00

[a] In order of emergence at column exit. [b] Menthol factor set at 1.00 (no correction). [c] Unresolved. [d] Unsaturated tertiary alcohol is partially decomposed under the experimental conditions. Recently, an SAIB-Quadrol column was used successfully for its detection in essential oils (*1*). [e] Containing other components, possibly some carvone.

Table IV. Composition of *M. piperita* Oils

Sample, Provenance and Other Information Given by Supplier	Code No.	Component, % α-Pinene	Camphene[a]	β-Pinene[a]	Limonene	Cineole	Menthofuran	Menthone	Isomenthone	Neomenthol	Menthol	Menthyl acetate	Pulegone	Piperitone[b]	Others[c]
U. S., Midwest															
Natural	P-54	0.8	...	1.0	3.0	8.3	1.9	29.9	4.8	3.4	39.3	4.2	0.5	1.3	1.8
Natural	P-5	0.8	...	0.9	3.4	7.1	1.7	30.7	5.3	3.5	38.8	4.5	0.8	0.9	1.7
Natural	P-43	0.8	(0.2)	0.9	3.6	8.7	0.7	31.6	5.9	3.6	37.0	5.1	0.8	0.5	0.8
Midwest-Oregon blend,[d] lightly rectified	P-51	0.5	...	0.8	2.7	7.3	2.6	27.5	4.8	3.9	41.7	4.8	1.5	1.4	0.7
Midwest-Oregon blend,[d] heavily rectified	P-48	0.1	...	0.1	0.7	1.9	1.9	24.4	4.2	5.0	51.4	6.6	1.1	2.1	0.4
U. S., Oregon															
Natural	P-45	0.8	...	0.9	4.3	8.3	3.2	19.5	4.5	4.1	43.1	6.1	2.3	1.7	1.2
Natural	P-49	0.7	...	1.0	3.6	8.0	3.3	23.8	4.0	3.6	41.8	4.4	2.1	2.3	1.5
Natural, Jefferson district	EO-531	0.6	(0.2)	1.6	3.2	7.5	2.6	21.3	3.2	4.2	46.2	4.5	0.9	3.1	1.1
U. S., Yakima															
Sunnyside, natural	P-44	0.5	(0.2)	1.0	4.3	8.1	8.8	16.7	3.5	3.6	43.2	6.9	1.9	0.7	0.8
Kennewick, natural, plants grown in Sunnyside area	P-50	0.8	(0.1)	1.1	3.7	7.8	8.1	17.1	3.4	3.3	42.2	7.4	2.2	1.0	1.8
Yakima, natural, early harvest	EO-534	0.6	(0.2)	1.7	3.5	13.5	6.2	17.9	3.0	2.8	40.2	5.3	2.6	1.6	1.2
Yakima, natural, late harvest	EO-532	0.7	...	1.6	3.7	6.4	9.4	8.9	2.2	3.9	48.7	11.6	0.9	1.1	0.9
Rectified, U. S. P.[e]	P-1	0.7	(0.5)	1.2	3.7	8.0	6.2	15.7	3.2	3.6	45.9	8.6	1.3	0.8	1.3
Terpeneless, produced from P-1 by vacuum fractionation[e]	OP-1	0.1	...	0.2	1.3	2.5	6.5	14.1	3.6	4.2	51.9	10.8	2.5	1.2	1.2
Italian															
Italo-Mitcham, rectified	P-41	0.7	(0.2)	0.9	4.2	8.0	6.3	21.0	3.6	3.5	40.2	5.3	3.3	1.7	1.2
Italo-Mitcham, rectified	P-36	0.7	(0.5)	1.0	4.6	7.7	8.4	16.8	5.1	3.9	35.2	6.1	4.6	2.9	3.0
Rectified	P-15	0.8	...	1.0	4.2	7.9	6.8	17.6	3.9	3.9	40.4	5.6	3.9	1.9	2.0
Italo-Mitcham, distilled	P-20	1.0	...	1.1	6.8	8.9	6.4	18.1	3.3	3.2	38.7	4.8	2.8	2.1	2.9
Italo-Mitcham, distilled, U. S. P., b.p.	P-23	0.9	(0.3)	1.0	6.2	9.9	5.5	19.6	5.6	3.6	35.3	5.6	2.7	2.1	2.2
Terpeneless	P-31	0.0	(0.0)	0.2	2.3	1.5	4.3	21.0	4.5	3.7	49.4	5.7	4.3	1.3	1.7
Italo-Mitcham, distilled, U. S. P.	EO-538	0.7	(0.2)	1.8	3.9	7.5	6.1	19.8	4.0	3.5	40.0	5.5	3.5	2.7	1.1
English															
Genuine, distilled	P-19	0.7	...	1.0	6.8	8.8	1.7	20.3	3.3	4.1	43.9	4.6	1.3	1.7	2.0
		0.7	...	1.0	4.0	9.4	1.6	21.0	3.3	4.1	46.7	3.8	0.8	1.6	2.0
Distilled, U. S. P., B. P.	P-29	0.6	(0.3)	0.7	4.3	8.7	1.8	16.9	4.1	4.5	47.6	6.9	1.3	1.4	1.2
Mitcham, distilled	P-22	0.5	...	0.7	3.9	6.1	5.8	15.9	3.8	4.9	44.4	9.6	1.9	1.9	0.6
		0.5	(0.6)	1.3	5.5	7.1	5.8	15.6	5.8	5.3	39.7	8.5	2.2	2.0	1.0
Mitcham, distilled, 1959; dry and sunny season	P-39	0.8	(0.2)	1.0	4.0	12.4	4.5	18.2	4.5	3.6	38.6	4.6	2.7	4.1	0.8
Bulgarian															
Bulgaro-Mitcham, natural	EO-180	0.7	...	1.6	3.5	7.5	6.2	16.9	3.1	3.8	43.9	7.2	2.7	1.9	1.1
Bulgaro-Mitcham, rectified	EO-181	0.2	...	1.0	2.7	5.2	8.0	17.1	3.5	4.3	44.3	6.6	2.8	2.8	1.5
South African															
Natural, Mitcham-type "*K*",[f] produced, 1957	P-25	0.6	...	0.7	5.8	7.4	8.8	19.1	5.1	3.4	36.1	6.9	2.1	2.3	1.8
Natural, Mitcham-type "*R*",[f] produced, 1958	P-27	0.4	...	0.5	6.3	7.4	9.2	17.9	4.9	4.2	33.2	10.5	2.1	2.0	1.4
Argentine	EO-224	0.6	(0.1)	1.5	3.8	6.7	8.4	12.8	2.7	3.2	46.9	7.5	2.1	2.2	1.7
Canadian															
Redistilled, U. S. P., produced from plants grown in province of Ontario	EO-459	0.8	(0.1)	1.9	3.0	8.9	1.2	26.5	4.1	4.2	40.6	5.0	0.6	1.7	1.6
Netherlands															
Experimental sample, 1959 crop	EO-158	0.5	(0.1)	1.3	1.0	4.4	0.3	17.8	4.2	3.6	54.5	6.5	0.9	3.6	1.4
Polish	EO-262	0.6	(0.1)	1.7	3.9	7.9	3.1	24.8	4.2	3.8	40.1	4.5	0.9	2.1	2.5
Spanish	EO-221	0.6	(0.1)	1.3	3.1	6.7	2.9	30.6	5.3	3.1	36.0	3.8	2.8	2.5	1.5

Continued on page 241

[a] Camphene and β-pinene were not always observed as two distinct peaks. Whenever separation was observed, values for both compounds were calculated. In order to allow, however, for uncertainty of trace amounts of camphene in some β-pinene fractions and ensure uniformity of data treatment percentages given for β-pinene always include those shown (in parentheses) for camphene.

[b] Including other components, possibly some carvone.

[c] Unassigned areas, due largely to minor components following the emergence of cineole and preceding that of menthofuran—e.g., *p*-cymene, terpinolene, 3-octanol, and sabinene hydrate.

[d] Product known to be a blend of Midwestern and Oregon oil. Analysis showed that Midwestern made up greater portion. Data also illustrate compositional effects due to different degrees of rectification.

[e] Products known to be blends of American peppermint oils. Analyses showed greater portion to be Yakima-type. Data also illustrate effects of deterpenation.

[f] Produced from *M. piperita* strains imported from Germany.

Table V. Composition of *M. arvensis* Oils

Sample, Provenance and Other Information Given by Supplier	Code No.	Component, % α-Pinene	Camphene[a]	β-Pinene[a]	Limonene	Cineole	3-Octanol	Linalool	Menthone	Isomenthone	Neomenthol	Menthol	Menthyl acetate	Pulegone	Piperitone[b]	Others[c]
Brazilian																
Partially dementholized	EO-173	1.0	...	2.5	8.5	0.7	0.4	...	29.9	8.5	3.9	33.2	5.3	1.1	3.9	1.1
Partially dementholized	P-46	0.5	...	1.1	4.9	0.2	0.3	...	21.5	6.6	2.8	53.1	5.7	0.6	2.6	0.2
Triple-distilled	P-34	0.6	...	1.9	7.1	0.6	0.4	...	29.3	9.1	3.8	36.8	5.3	0.8	4.3	0.1
Rectified	P-16	0.6	...	1.0	6.0	1.2	0.7	...	27.3	8.7	4.6	38.0	7.0	0.8	3.8	0.4
		0.4	...	1.1	5.1	0.7	0.4	0.1	26.2	8.9	4.4	39.5	7.4	1.3	4.4	0.3
Partially dementholized	P-53	0.7	...	0.7	5.0	0.5	0.4	...	22.8	7.0	3.4	50.3	6.2	0.4	2.3	0.4
	P-40	0.9	...	2.4	8.2	0.6	0.4	...	29.7	8.9	3.7	33.9	5.6	1.5	4.2	0.1
Natural	Sp-20	0.5	...	1.3	4.3	0.4	0.2	...	12.5	3.7	1.6	68.6	4.7	0.5	1.8	0.1
Natural	Sp-21	0.5	...	1.3	4.7	0.5	0.2	...	13.5	4.2	1.8	65.1	5.0	1.1	2.1	0.1
Rectified	P-37	1.0	...	2.4	8.9	0.6	0.4	...	29.6	8.9	3.8	34.6	4.7	0.7	4.3	0.1
		0.9	...	2.5	8.3	0.6	0.3	...	29.6	9.4	3.8	34.0	5.3	1.0	4.3	0.0
Formosan																
Rectified	P-33	1.0	...	2.2	6.8	0.6	0.3	0.1	21.3	5.9	3.3	43.5	8.4	1.3	5.2	0.1
	P-38[d]	0.1	...	0.2	0.9	0.1	0.1	...	22.1	7.9	5.0	48.4	9.6	1.2	4.1	0.1
	Sp-19	0.6	...	1.1	5.3	0.5	0.3	...	21.1	5.9	2.0	48.8	6.7	1.1	6.5	0.3
Partially dementholized	P-47[d]	0.1	...	0.1	0.7	0.2	0.2	...	15.5	2.8	2.4	60.8	6.7	2.1	7.7	0.7
Partially dementholized	EO-170	0.7	...	1.7	5.4	0.6	0.4	0.2	30.9	5.5	2.6	33.9	6.8	2.0	8.5	0.9
Japanese																
Rectified	P-17	1.2	...	2.2	12.4	1.2	3.7	...	25.0	3.4	5.0	36.9	1.3	1.2	6.7	0.5
Refined	P-21	0.9	...	1.9	12.0	1.2	3.8	0.0	24.5	3.3	4.9	36.8	1.2	1.4	7.1	1.2
		0.9	(0.1)	1.5	10.8	1.4	3.6	...	23.9	3.1	4.9	36.3	1.5	1.8	9.6	0.6
Partially dementholized	EO-171	0.7	...	2.1	8.5	0.8	3.4	0.7	22.0	4.0	4.6	37.4	1.2	1.0	11.6	2.1
	EO-238	0.9	...	2.7	11.2	0.8	3.6	0.8	24.9	2.9	4.4	38.9	0.9	1.5	6.4	0.2
Chinese																
Partially dementholized	EO-172	1.0	...	2.2	4.9	0.6	2.4	0.2	25.5	7.6	4.0	39.3	0.9	4.9	6.0	0.6
Rectified	P-18	1.4	...	2.1	7.2	0.9	2.7	0.1	29.4	8.7	5.0	36.5	1.0	2.4	2.4	0.4
Partially dementholized	P-52	1.2	...	1.8	7.2	0.8	2.7	...	28.4	8.8	5.0	37.3	0.9	2.3	3.5	0.3
	P-35	0.9	(0.6)	1.4	8.1	1 1	2.4	...	26.7	8.3	4.6	36.0	2.4	3.1	3.4	1.7
Argentine	EO-223	0.5	...	1.1	6.0	0.5	0.6	0.0	9.9	2.8	2.2	65.7	6.1	1.1	2.7	0.9
Korean	EO-554[d]	0.1	...	0.3	0.7	0.2	0.1	...	19.6	1.8	1.3	56.6	8.3	0.7	10.4	0.0
South African	P-26[e]	0.2	(0.1)	0.9	4.3	0.2	0.8	...	17.1	2.7	1.7	61.5	6.9	0.2	2.7	0.9

[a] See footnote[a] in Table IV.

[b] See footnote [b] in Table IV.

[c] Unassigned areas.

[d] Oil viscous and colored. Low terpene content indicative of partial polymerization.

[e] Oil produced from strain of Japanese mint imported from the United States.

Table VI. Significant Constituent Ratios for *M. piperita* Oils

Sample, Provenance and Code[a]	Percentage Ratio A: Terpenes[b] / All Constituents	B: Menthone / Isomenthone	C: Limonene / Cineole	D: Menthofuran / "Menthone Related Constituents"[c]	E: Neomenthol / Menthyl Acetate	F: "Menthol Related Constituents"[d] / Neomenthol	G: "Menthone Related Constituents"[c] / "Menthol Related Constituents"[d]
U. S., Midwest							
P-54	0.131	6.2	0.36	0.052	0.81	13.8	0.78
P-5	0.122	5.8	0.48	0.046	0.78	13.4	0.81
P-43	0.140	5.3	0.41	0.018	0.71	12.7	0.84
P-51	0.113	5.7	0.37	0.074	0.81	12.9	0.69
P-48	0.028	5.8	0.37	0.062	0.76	12.6	0.48
U. S., Oregon							
P-45	0.143	4.3	0.52	0.118	0.67	13.0	0.51
P-49	0.133	5.9	0.45	0.106	0.82	13.8	0.62
EO-531	0.129	6.6	0.43	0.096	0.93	13.1	0.49

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Table VI. Significant Ratios for *M. piperita* Oils (*Continued*)

Sample, Provenance and Code[a]	A: Terpenes[b] / All Constituents (Ratio, %)	B: Menthone / Isomenthone (Ratio, %)	C: Limonene / Cineole (Ratio, %)	D: Menthofuran / "Methone Related Constituents"[c] (Ratio, %)	E: Neomenthol / Menthyl Acetate (Ratio, %)	F: "Menthol Related Constituents"[d] / Neomenthol (Ratio, %)	G: "Methone Related Constituents"[c] / "Menthol Related Constituents"[d] (Ratio, %)
U. S., Yakima							
P-44	0.139	4.8	0.53	0.303	0.52	14.9	0.54
P-50	0.135	5.0	0.47	0.283	0.45	16.0	0.54
EO-534	0.193	6.0	0.26	0.229	0.53	17.3	0.56
EO-532	0.124	4.1	0.58	0.458	0.33	16.5	0.32
P-1	0.136	4.9	0.46	0.247	0.42	16.1	0.43
OP-1	0.041	3.9	0.52	0.269	0.39	15.9	0.36
Italian							
P-41	0.138	5.8	0.52	0.204	0.66	14.0	0.63
P-36	0.140	3.3	0.60	0.277	0.64	11.6	0.67
P-15	0.139	4.5	0.53	0.240	0.70	12.8	0.57
P-20	0.178	5.5	0.76	0.230	0.67	14.6	0.59
P-23	0.180	3.5	0.63	0.179	0.64	12.4	0.69
P-31	0.040	4.7	1.53	0.144	0.65	15.9	0.51
EO-538	0.139	5.0	0.52	0.204	0.63	14.0	0.61
English							
P-19	0.173	6.1	0.77	0.067	0.89	12.8	0.48
	0.151	6.4	0.43	0.062	1.08	13.3	0.47
P-29	0.143	4.1	0.50	0.079	0.65	13.1	0.39
P-22	0.112	4.2	0.64	0.227	0.51	12.0	0.43
	0.144	2.7	0.77	0.213	0.62	10.1	0.51
P-39	0.182	4.0	0.32	0.165	0.78	13.0	0.58
Bulgarian							
EO-180	0.133	5.5	0.47	0.237	0.53	14.4	0.48
EO-181	0.091	4.9	0.52	0.280	0.65	12.8	0.52
South African							
P-25	0.145	3.8	0.78	0.267	0.49	13.6	0.71
P-27	0.146	3.7	0.85	0.288	0.40	11.4	0.67
Argentine							
EO-224	0.126	4.7	0.57	0.351	0.43	18.0	0.41
Canadian							
EO-459	0.146	6.5	0.34	0.038	0.84	11.9	0.64
Netherlands							
EO-158	0.072	4.2	0.23	0.013	0.55	17.9	0.35
Polish							
EO-262	0.141	5.9	0.49	0.097	0.84	12.7	0.66
Spanish							
EO-221	0.117	5.8	0.46	0.075	0.82	13.8	0.90

[a] For key to sample codes see Table IV.
[b] "Terpenes": principal constituents emerging prior to and including cineole (α-pinene, camphene, β-pinene, limonene, and cineole). Inclusion of the oxide which followed closely the emergence of limonene and processing experimental data as shown enhanced considerably the value of various constituent ratios as characteristic criteria of identity.
[c] "Menthone related constituents": menthofuran + menthone + isomenthone.
[d] "Menthol related constituents": neomenthol + menthol + menthyl acetate.

of production to be preferred over another. Thus, a given sample may be identified as to its regional origin, but the experimental data need not be indicative of its flavor quality.

In spite of these limitations, the method described affords a valuable tool for analysis and characterization of these products. The wealth of compositional criteria which can be obtained using microsamples of extreme complexity is indeed astounding. Treatment of experimental data has shown, furthermore, that nature controls her creations quite effectively and that, in spite of many variables constantly at play, geographical criteria may often be evaluated with a great degree of reliability. Encouraging results along these lines have already been obtained on other essential oils (*46*). Thus, by means of equipment now available to any industrial and research laboratory, more information

Table VII. Significant Constituent Ratios for *M. arvensis* Oils

Sample, Provenance and Code[a]	A: Terpenes[b] / All Constituents (Percentage Ratio)	B: Menthone / Isomenthone (Percentage Ratio)	C: Limonene / Cineole (Percentage Ratio)	H: 3-Octanol / "Liquid" Oxygenated Constituents"[c] (× 10^3)	J: Pulegone / "Liquid Oxygenated Constituents"[c] (× 10^2)	K: Piperitone[d] / "Liquid Oxygenated Constituents"[c] (× 10^2)	L: Menthyl Acetate / "Liquid Oxygenated Constituents"[c] × 1/Menthol (× 10^4)
Brazilian							
EO-173	0.127	3.5	12.1	7.5	2.0	7.4	30.1
P-46	0.067	3.3	24.5	7.5	1.5	6.5	26.8
P-34	0.102	3.2	11.8	7.5	1.5	8.1	27.2
P-16	0.088	3.1	5.0	13.1	1.5	7.2	34.8
	0.073	2.9	7.3	7.5	2.4	8.3	35.3
P-53	0.069	3.3	10.0	9.4	0.9	5.4	29.0
P-40	0.121	3.3	13.7	7.4	2.8	7.8	30.6
Sp-20	0.065	3.4	10.8	8.0	2.0	7.2	27.4
Sp-21	0.070	3.2	9.4	7.2	3.9	7.5	27.5
P-37	0.129	3.3	14.8	7.6	1.3	8.2	25.9
	0.123	3.2	13.8	5.6	1.9	8.0	29.0

Table VII. (Continued)

Sample, Provenance and Code[a]	Percentage Ratio A: Terpene[b] / All Constituents	B: Menthone / Isomenthane	C: Limonene / Cineole	H: 3-Octanol / "Liquid" Oxygenated Constituents[c] (× 10^3)	J: Pulegone / "Liquid" Oxygenated Constituents[c] (× 10^2)	K: Piperitone[d] / "Liquid" Oxygenated Constituents[c] (× 10^2)	L: Menthyl Acetate / "Liquid" Oxygenated Constituents[c,e] × 1/Menthol (× 10^4)
Formosan							
P-33	0.106	3.6	11.3	6.5	2.8	11.3	42.2
P-38	0.013	2.8	9.0	2.0	2.4	8.2	39.7
Sp-19	0.075	3.6	10.6	6.9	2.5	14.9	31.5
P-47	0.011	5.5	3.5	5.3	5.6	20.6	29.5
EO-170	0.084	5.6	9.0	7.0	3.5	14.9	35.3
Japanese							
P-17	0.170	7.3	10.3	79.9	2.6	14.5	7.6
P-21	0.160	7.4	10.0	82.3	3.0	15.4	7.1
	0.146	7.7	7.7	73.9	3.7	19.8	8.5
EO-171	0.121	5.5	10.6	70.1	2.0	23.9	6.6
EO-238	0.156	8.6	14.0	79.3	3.3	14.1	5.1
Chinese							
EO-172	0.087	3.4	8.2	46.6	9.5	11.7	4.5
P-18	0.116	3.4	8.0	52.2	4.6	4.6	5.3
P-52	0.110	3.2	9.0	52.3	4.5	6.8	4.7
P-35	0.115	3.2	7.4	47.2	6.1	6.7	13.1
Argentine							
EO-223	0.081	3.6	12.0	23.6	4.3	10.6	36.6
Korean							
EO-554	0.013	10.9	3.5	2.4	1.7	24.5	34.7
South African							
P-26	0.056	6.3	21.5	24.9	0.6	8.4	34.9

[a] For key to sample codes see Table V.
[b] "Terpene": principal constituents emerging prior to and including cineole (α-pinene, camphene, β-pinene, limonene, and cineole); for further comments see footnote *b*, Table VI.
[c] "Liquid oxygenated constituents": all constituents less "terpenes" and menthol (solid) to circumvent rectification and dementholization effects.
[d] Including other components, possibly some carvone.
[e] Factor $\frac{1}{\text{menthol}}$ used to correct for extent of dementholization.

Table VIII. Detection of Adulteration of *M. piperita* Oils

Adulterant	Chromatographic Evidence	Test Parameter	Level of Detection, %
Propylene glycol	Appearance of peak between that of 3-octanol and menthofuran	Measurement of peak area	2
Terpineol	Appearance of peak between that of menthyl acetate and pulegone. Enhancement of pulegone peak	Ratio of "mentho" compounds/pulegone less than 15[a]	5
Benzyl alcohol	Enhancement of pulegone peak	Ratio of "mentho" compounds/pulegone less than 15[a]	5
Limonene	Enhancement of limonene peak	Ratio of limonene/cineole greater than 0.7	5
Phellandrene	Appearance of peak prior to that of limonene	Measurement of peak area	2
Menthol	Enhancement of menthol peak	Distribution plot as shown in Figure 6	10[b]
M. arvensis, Japanese corn mint oil	Variable enhancement of most peaks	Distribution plot as shown in Figures 5 and 6	10[c]

[a] "Mentho" compounds = menthofuran + menthone + isomenthone + neomenthol + menthol + menthyl acetate.
[b] Level of detection dependent on original composition of sample.
[c] Level of detection dependent on composition of both sample and adulterant.

about these heterogeneous products may be gained than by any other technique. Instrumentation is simple and results are readily assembled.

Because of its commercial importance, a vast amount of literature dealing with analysis and characterization of peppermint oil has accumulated during the last few decades. The field was critically reviewed by Guenther in 1948 (*19*), and has since been covered periodically in the Miltitzer Berichte (*39*). Recently, excellent contributions were made by eminent workers in Bulgaria, Formosa, India, Japan, and Yugoslavia (*9, 10, 27, 29, 31, 40, 50, 52*) where extensive experimental and commercial plantations are continuously being studied. Statistical treatment of data obtainable by the method described should prove of particular value to the appraisal of such agricultural research and development programs.

Important factors, such as composition of oils produced in different parts of the plant—e.g., stalks, buds, and flowers, respectively—proper selection of most advantageous harvesting times, quality evaluation of specimens at various stages of maturation, proper conditions for field drying plants prior to steam distillation, and appraisal of environmental effects may all be determined conveniently with no more than a few drops of sample being required. The techniques described should therefore find wide application for analysis and quality control of essential oils as well as characterization of complex natural and synthetic compositions produced continuously by the food, drug, and cosmetic industries.

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