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MINT OILS

Chromatographic Measurement of Variations in Essential Oils within a Single Plant

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Techniques have been developed for the study of essential oils from just one or two leaves. Leaves from different areas on the peppermint and spearmint plants have been steam distilled and the oils chromatographed. Measurement of the area of the chromatographic spots shows that the newer leaves contain considerably more oil per unit leaf area. The relative amounts of the constituents vary greatly from one area of the plant to another and it is possible to correlate these with increasing amounts of the more reduced forms of constituents in the older leaves.

THE FORMATION OF ESSENTIAL OILS in plants has been a subject of many proposed schemes of biogenesis. Some of these schemes have been based simply upon logical chemical reactions. Others have been the result of comparison of compounds in the oils isolated at different times of the year. A more direct method of comparison of oil of different ages would be desirable. At any one time on an actively growing plant, there are leaves formed several weeks earlier as well as leaves in the very young stages of growth. Presumably these leaves could contain oil of different ages also. Any differences between the oil from the newer leaves and the oil from the older leaves would give new insight into the processes of oil formation.

The chromatographic characterization of essential oils has been developed so that small amounts of oil can be used (*2, 3*). Peppermint and spearmint oils have been studied in detail both chemically and chromatographically and these have been used for the initial testing of the leaf comparison method. Steam distillation is a convenient method of separating the essential oils from other plant constituents. New distillation and isolation techniques were developed so that less than 1 λ of oil can be isolated efficiently from leaf areas of as small as 1.5 square inches.

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Experimental

Leaves were taken from various locations on the plant and their areas measured with a planimeter. As some leaves contained considerably less oil than others, it was convenient to use 3 to 6 square inches of leaf area to provide enough oil for chromatography from most plants, irrespective of the particular growth conditions or location of the leaves on the plant.

Three areas were arbitrarily defined to serve as a uniform basis of description of plant areas, as illustrated in Figure 1. The leaves on the central stem at the bottom of the plant (location *A*) are unquestionably the oldest leaves. The leaves on the same control stem toward the top of the plant (location *B*) are next in order of appearance. The newest leaves are those at the top of the plant and on side branches at the top of the plant (location *C*).

The leaves were ground in a mortar with a little sand, 2 ml. of hexane, and enough water to cover them. The ground mixture was added to a 50-ml. flask and about 15 ml. of water was used to rinse the contents into the flask. After addition of a foam inhibitor the mixture was then steam distilled, using a very short simple condenser, into a 30-ml. separatory funnel containing 1 ml. of hexane. About 10 ml. of condensate

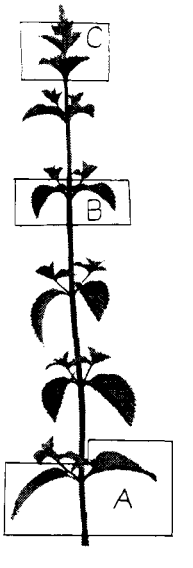

Figure 1. Mint plant illustrating location from which leaves were harvested

was collected, the condenser was rinsed down with 2 ml. of hexane, and this hexane was used to extract the aqueous layer. The combined organic layers were evaporated down nearly to dryness. Other solvents, such as toluene, can be used as the carrier liquid in the distillation.

The chromatography was carried out by the previously described method (*2*). The whole sample as prepared above was used for the chromatogram. In all cases, the identification of products was made through the three-step sequence of fluorescence, color with acidic dinitrophenylhydrazine spray, and subsequent heating. Certain compounds, such as menthol, were detected by fluorescein-bromine spray as described by Kirchner, Miller, and Keller (*1*). Identification of spots was by comparison with authentic materials and whole oils run on neighboring paths. Areas were measured with a planimeter.

Known amounts of materials were chromatographed to demonstrate that the amount of material present was directly related to the size of the chromatographic spot under standard conditions. Chromatoplates of 0.4-inch thickness were prepared and chromatographed under uniform conditions. Measurements of the area of the spots showed that the area of the spot was a direct function of the amount of compound which had been placed on the chromatoplate (Table I). Although methods have been developed to recover the material and run quantitative assays on some of the compounds after chromatography, the simple measurement of areas gives useful results on any compounds which can be located by the various techniques applied to these oils in the past.

Results and Discussion

Variation of Oil from the Same Plant. The first problem was to determine whether any real difference existed in the oil from leaves of the different areas. These differences were found to exist—particularly with rapidly growing plants—and were so great that in many cases the minor constituents of one oil sample were actually the major ones in other oils. These differences were principally in the relative amounts of materials and not in the nature of the compounds themselves. Absence or presence of certain materials in many cases was due to the presence or absence of enough of the material in the sample to be detected by the standard methods. There is a measurable amount of time from the first appearance of the oil to the formation of the "mature" oil. As these oils are different and as the time of conversion is considerable, the order of formation of constituents may be studied by comparison of oil on the basis of age.

Characteristics of Oil from Various Locations. One significant difference observed in the oil from the three areas of the plants was that the newer areas had a much higher percentage of oil per unit leaf area than the older areas. The reason for this is not clear. However, the majority of oil may be present already in the first stages of leaf growth and later increase in leaf size may have little effect on the amount of oil. Another explanation is that oil is constantly being lost by evaporation during the life of the plant; therefore, the older leaves would have less oil if evaporation took place at a faster rate than any subsequent oil synthesis. This latter hypothesis appears to be a reasonable explanation although the present work offers no evidence for either view. Evaporation can not be used to explain the differences in the oil composition as the observed differences are not of a nature which would be the result of this process.

Oil, even in any one plant, is far from uniform. Oil obtained by steam distillation of a whole plant represents at best an average of the oil contained in the various leaves. A study of the oil from the individual leaves reveals large differences in relative amounts of the constituents. Typical results for some major peppermint constituents are given in Table II. On the basis of these experiments, it has been possible to establish a relative sequence based upon the age of the leaf by studying changes in relative size of the chromatographic areas. There is a progression from high pulegone in the newest leaves to menthone predominance in older leaves. The next logical step in the process—conversion of menthone to menthol—also could be observed.

Spearmint oil was studied in the same way. The most obvious difference between the oil from older and newer leaves was the increase in the amount of dihydrocarvone, at the expense of carvone in the older leaves. Thus, relatively new leaves from a spearmint plant (location *B*) of a 2.8-square inch area gave a chromatoplate with a 0.23-square inch area of the carvone spot and a 0.06-square inch area for dihydrocarvone. From 3.0 square inches of older-leaf area of the same plant (location *A*) at the same time the carvone area was only 0.10 square inch while the dihydrocarvone area had increased to 0.09 square inch. The terpene hydrocarbon spot at the top of the chromatoplate had decreased from 1.0 to 0.5 square inch in going to the older leaf area.

Conclusions

In both oils studied, the progression of change is in general from the unsaturated and ketonic to the saturated and alcoholic type constituents. This is in agreement with the results observed from comparison of oil distilled early in the season with that from late harvests (*4*). As plants vary among themselves, it is important to be able to demonstrate this variation as a feature of the individual mint plant. By a more complete knowledge of the components (oil from individual leaves) the nature of the average product (oil from many whole plants) can be evaluated more properly.

Other applications of the information derived from studies of this type are obvious. It may be possible to modify certain agricultural practices to control the properties of the essential oil as a result of an understanding of these variations within the plant. It is apparent that peppermint and spearmint are not the only essential oil plants which would show this difference and that this sort of comparison can be applied to other oils. It may be that many other types of agricultural products would be understood better if more consideration were made of the inhomogeneity of the product from an individual plant.

Table I. Area of Chromatogram Spots in Square Inches for Increasing Amounts of Various Materials

	Amount of Material Used			
Compound	1/4 λ	1/2 λ	1 λ	2 λ
Pinene	...	...	0.21	0.40[a]
Menthone	0.09	0.20	0.41	0.69
Pulegone	0.06	0.10	0.22	...
Menthofuran	0.14	0.21	0.39	...
Carvone	...	0.32	0.47	...

[a] 8 λ gave a 1.5-sq. in. area.

Table II. Oil from Constituents from Leaves from Various Parts of Peppermint Plants

Area of Leaves, Sq. In.	Location on Plant (Fig. 1)	Size of Chromatographic Spot, Sq. In.		
		Menthone	Pulegone	Piperitone area
4.8	*A*	0.07	0.11	0.12
3.9	*B*	0.19	0.16	0.05
1.6	*C*	0.20	0.11	...

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