THE OCCURRENCE OF MENTHOFURAN IN OIL OF PEPPERMINT*

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It is generally believed that menthofuran is formed exclusively in the floral organs of peppermint (*Mentha piperita* L.), and it has been detected in oils obtained by distillation of entire flowering plants from different origins (Italy, America, France, Russia, Holland)[1,2].

We have already shown that menthofuran is present in other parts of the plant as well as in the flowers; thus, for example, the very young plants and the purple stolons are rich in menthofuran[1,3]. An abnormally high content has also been found in oil of "basilic mint"[4]. Up to the present time no other evidence for the presence of menthofuran in oils from the foliaceous parts of the plant has been presented, although from a physiological point of view it would be of greatest interest to know if this substance is also secreted in the glandular hairs of the leaves.

Experimental

In order to demonstrate the presence of menthofuran in leaf oils we have employed a colour reaction and also the infra-red absorption curve of this substance. The colour reaction with trichloroacetic acid[5] may also be employed for quantitative estimations[3]. To show the presence of menthofuran in the glandular hairs of a leaf the following reagent was used: lactic acid 40, chloral hydrate 40, trichloroacetic acid 10. The mixture renders the leaves transparent and at the same time colours red the hairs containing menthofuran.

In Figure 1 the secretory glands with high content of menthofuran are more distinct than are those with a low content. It may also be noted that the unicellular glandular hairs are devoid of menthofuran. We may thus conclude that menthofuran is not secreted at the same time in all the glandular hairs. This is to be expected, for the production of essential oil does not commence at the same time in all the glandular hairs on a young leaf. We have already shown that 40 to 60 per cent of glandular hairs are free from oil in a leaf one to two weeks old[6].

The absence of menthofuran from some secretory hairs may also be explained by the fact that when the substance is secreted it is oxidised during the development of the leaf. Menthofuran is rapidly converted into the hydroxylactone which gives no reaction with trichloroacetic acid[6]. Naves[2] found this hydroxylactone in oil of peppermint in which menthofuran could not be detected spectrophotometrically and he believed that the lactone was derived from menthofuran.

Quantitative estimation of menthofuran by means of its colour reaction

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with trichloroacetic acid was rejected by Ohloff[7] because he believed it to be non-specific. Nevertheless it is sufficiently specific for oil of peppermint because there are practically no other substances present therein which react with trichloroacetic acid. This is shown by the following assay: a leaf oil, estimated by the method to contain 4·0 per cent of menthofuran, was exposed in thin layers to atmospheric oxidation for 6 months, after which it was found to contain 0·2 per cent of menthofuran by the same method. This latter figure may be regarded as a minimum value given by oils with trichloroacetic acid.

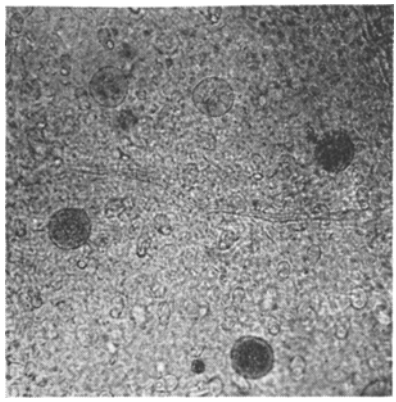

FIG. 1. Glandular hairs on leaf treated with the lactic acid-chloral hydrate-trichloroacetic acid reagent.

Further evidence for the absence of interfering substances is found in the fact that an oil, free from methofuran and giving no reaction with trichloroacetic acid, possesses the same constants as an oil distilled by us and examined by infra-red spectrophotometry.

We have shown conclusively by means of infra-red absorption curves that menthofuran is present in oils distilled from leaves. Naves[2] determined the absorption curve for menthofuran and the curve which we have recorded with the Leitz double beam spectrometer between 1 and 15 μ is reproduced as Figure 2. The absorption bands at 13·1 and 13·6 μ are well suited for the detection of menthofuran since oils free from this substance possess no marked absorption in this region. We have shown that a relation exists between the appearance of these characteristic absorption bands and a positive reaction with trichloroacetic acid. Figure 3 shows the appropriate absorptions between 11 and 15 μ for oils distilled from leaves (*a* and *b*) and from entire plants in full flower (*c*). Menthofuran contents determined by trichloroacetic acid were 0·02, 0·6 and 11 per cent respectively for the three oils. It is seen that a content of 0·02 per cent gives no absorption and that a 0·6 per cent content is already recognisable in the absorption curves. Hence it is concluded that the trichloroacetic acid reaction is sufficiently specific to be used to study the presence of menthofuran in oils of peppermint.

We have used this method of estimation to follow the production of menthofuran during the development of the plant. We believe that the menthofuran contents of oils prepared from entire plants to be of little value for this study because they are a summation of contents of different plant parts of different ages. Thus, in order to obtain a better idea of the production of menthofuran, it is necessary to examine separately oils prepared from leaves of different insertions on the plant and hence belonging to different stages in the vegetative cycle of the plant.

It should also be emphasised that the absolute quantities of menthofuran

in a plant organ must be determined rather than the percentage of it present in each oil; otherwise variations in menthofuran produced by the leaf may be masked by variations in total oil yielded. Thus the total menthofuran produced by each leaf at each insertion has been calculated.

Figure 4 shows the results of these analyses of oils obtained from leaves on the main stem (insertions V, VII, IX and X) and those of the branches ($IV_z + V_z$). Different samples were collected at different stages in the

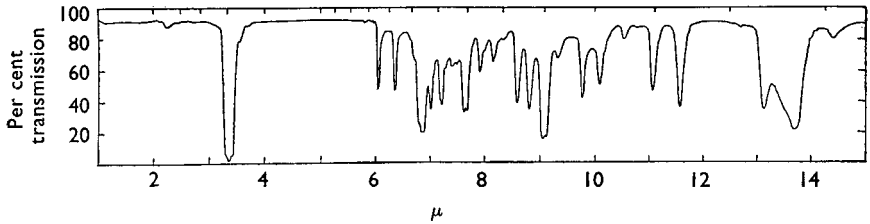

FIG. 2. Infra-red absorption curve of menthofuran.

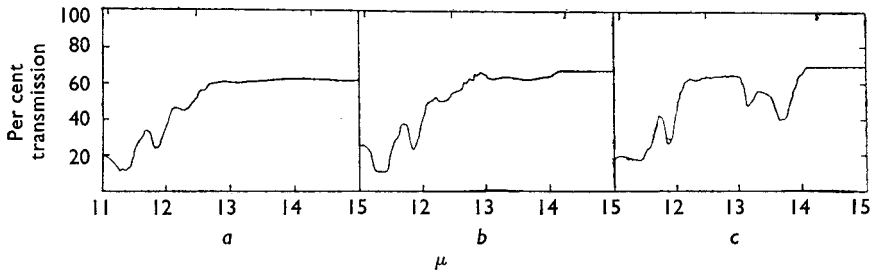

FIG. 3. Infra-red absorption curves of peppermint oils containing: *a*, 0·02; *b*, 0·6 and *c*, 11 per cent menthofuran.

vegetative cycle of the plant; A to E representing stages from young plant to development of flower buds; F to H covers the period of flowering. It is seen that the quantity of menthofuran is a maximum in the young leaves and it diminishes progressively with increased age of the leaf. This is most pronounced in leaves of the tenth insertion where the menthofuran decreases from 23 to 2 μg. For leaves from the branches ($IV_z + V_z$) the curve is more gradual because all the leaves of differing ages from the branch were distilled together.

If we consider the development of menthofuran in the tips of the branches (Table I) we find the converse phenomenon; the absolute

TABLE I

MENTHOFURAN CONTENTS OF OILS FROM BRANCH TIPS
PER CENT

Height of insertion of branches	Period of Collection					
	D	E	F	G	H	J
X	1·3	1·3	1·7	2·6	7·2	20·2
VI	2·0	2·0	2·3	6·3	13·2	—

TABLE II

MENTHOFURAN CONTENTS OF FLOWER OILS

Year	Flower buds		Freshly opened flowers		Full flowers		Fading flowers	
	Oil per cent	Menthofuran per cent	Oil per cent	Menthofuran per cent	Oil per cent	Menthofuran per cent	Oil per cent	Menthofuran per cent
1952	0·392	26·2	—	—	0·412	28·5	—	—
1953	0·480	36·7	0·386	37·2	0·320	36·8	0·289	32·0
1954	—	—	0·495	23·4	0·444	26·6	0·456	20·0

content of menthofuran increases due to the formation of flower buds possessing a very high content of this substance.

The amount of menthofuran in flower buds and flowers is very high but differs in different years of cultivation. After flowering both the oil content and the menthofuran content decreases as shown in Table II.

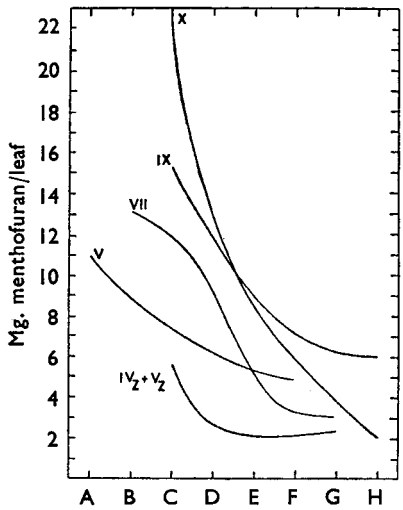

FIG. 4. Mg. of menthofuran present in the leaves of the main stem (insertions V, VII, IX, X) and the branches (IV + V), during the vegetative cycle of the plant (A–H).

Because of the variations outlined above, the percentage of menthofuran present in oils distilled from the entire plants is found to decrease and then to increase as shown in Figure 5. The curves represent the oil content and its menthofuran content as functions of the age of the plant in weeks. The oil of the youngest plants (first week) possesses the highest content of menthofuran. The amount decreases as the plant develops but increases again from the time of formation of flower buds (12th week), finally decreasing once more after flowering (24th week).

From these results it appears that menthofuran is only formed in the young parts of the plant. After a certain age it is secreted no more and that present in the glandular hairs is slowly oxidised to the hydroxylactone by the air. This is also confirmed by the fact that the basal part of a leaf contains a higher percentage of menthofuran than does the distal part, where the glandular hairs are not so young. From these cut leaves we have found the same yields of oils but with menthofuran contents of 1·9 and 0·6 per cent respectively.

DISCUSSION AND CONCLUSIONS

Menthofuran is a substance secreted in the young parts of the plant, that is where metabolism is most active. This explains the high content of the substance in flowers and stolons. The menthofuran content of

basilic mint oil is 20 to 25 per cent and at first sight this is abnormally high; it is, however, of the same order as that from young plants and stolons of healthy plants which contain 12 to 28 per cent.

Such values are quite normal for a young tissue. Now basilic mint is peppermint infected with the parasite *Eriophyes menthae*, family Acaridae, which results in atrophy of the plant, suppression of flowering and the production of abundant terminal masses of numerous, small, appressed leaves. Hence, such diseased basilic shoots are young tissues from the chemical point of view. Thus the composition of oil of basilic mint corresponds to that of young leaves and stolons of typical peppermint[2,6].

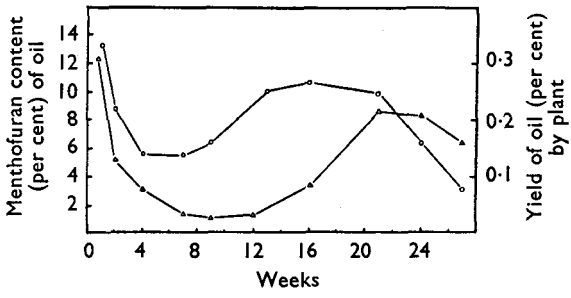

FIG. 5. Oils from entire plants.
○——○ yield of oil (per cent) by plant.
△——△ menthofuran content (per cent) of oil.

Menthofuran is thus shown to be secreted exclusively as a product of young tissues. Since this substance influences the quality of oil of peppermint and since its localisation and formation are understood, it will be possible to employ this information in producing oils of finest quality.

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