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ESSENTIAL OIL OF TWO CHEMOTYPES OF *MENTHA SUAVEOLENS* DURING ONTOGENESIS

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Key Word Index— *Mentha suaveolens* (= *M. rotundifolia*); Labiatae; essential oil; composition; chemotypes; piperitone oxide type; dihydrocarvone type; ontogenesis; biosynthetic scheme.

Abstract—The composition of the main constituents of the essential oil of two different chemotypes of *Mentha suaveolens* has been investigated during development. In the first chemotype (6) a definite increase of 1,2-epoxymenthylacetate was observed, but piperitone oxide was always the main constituent. In the second chemotype (12) an increase of neo- and dihydrocarveol and their corresponding acetates was observed and dihydrocarvone was the main constituent. The results have been used to formulate a new biosynthetic scheme for the cyclic oxygenated monoterpenes found in essential oils of the genus *Mentha*.

INTRODUCTION

In a previous paper a piperitone oxide type and a dihydrocarvone type of *Mentha suaveolens* Ehrh. (=*Mentha rotundifolia* (L) Hudson) have been described [1]. To ascertain that possible variations in the composition of the essential oil during the development of the plant were not the cause of the differences found between the two types, an investigation has been made into the composition of the essential oil during the vegetative period using a method described previously [2,3]. The results have also been used to discuss the relationship between the oxygenated monoterpenes present in the two chemotypes and to formulate a new biogenetic scheme for these compounds in species and hybrids of the genus *Mentha*.

RESULTS AND DISCUSSION

The main results are presented in Tables 1 and 2 and in Fig. 1. From the quantity of essential oil/leaf and from the percentage of the components, the quantity of the components/leaf can be calculated (further details see Experimental). In this way the quantities per leaf of the components of the essential oil can be followed for each insertion number during the vegetative period. In both chemotypes the total quantity of essential oil per leaf reaches a maximum after which a gradual decrease occurs. This maximum is reached first by the leaves with the lowest insertion number.

In chemotype 6 the quantity of 1,2-epoxymenthylacetate/leaf increases during development and after an initial increase that of piperitenone oxide gradually decreases. The quantity of piperitone oxide/leaf is maximal at the early stages of development after which it decreases.

In chemotype 12 a small quantity of carvone is present in leaves obtained at the first harvest. However, the GLC peak of carvone coincided with that of dihydrocarveol under the conditions used and a quantitative determination was therefore impossible. No carvone could be detected by TLC at the second or subsequent harvests. After an initial increase, the quantity of dihydrocarvone/leaf slowly decreases and in general the total quantities of neo- and dihydrocarveol/leaf and of neo- and dihydrocarveylacetate/leaf gradually increase during development. Monoterpenes with a hydroxyl or a keto function at C-3 were not detected.

The results obtained with *M. suaveolens* are comparable with those of earlier investigations with *Mentha* x *piperita* L. in which a distinct increase of the more reduced cyclic oxygenated monoterpenes (reduction of double bonds, conversion of ketones to corresponding alcohols with subsequent acetate formation) was demonstrated during the development of the plant [2,3]. Several biogenetic schemes have been proposed for the formation of monoterpenes in essential oils from the genus *Mentha* [4–7]. The biosynthesis of the hydrocarbon monoterpenes from linaloyl- geranyl- and from nerylpyrophosphate via carbonium ions [8–10] is generally accepted. These pyrophosphates originate from 2 molecules of 'active isoprene' (dimethylallyl- and isopentenylpyrophosphate) which in their turn have originated from 3 molecules of acetic acid via the mevalonic acid pathway. Linalool and geraniol, important constituents of the essential oil of respectively *M. citrata* Ehrh. [11] and *M. arvensis* subsp. *austriaca* (Jacq.) Briquet [12], evidently originate from the corresponding pyrophosphates.

For the formation of the cyclic oxygenated monoterpenes two possibilities exist; (a) by oxidation of monoterpene hydrocarbons, (b) via more oxidized precursors than those from which the cyclic hydrocarbon monoterpenes have originated. The results of infiltration experiments using labelled limonene and leaves of *Mentha* x *piperita* showed that labelled oxygenated monoterpenes could be formed [13]. The development of the acts in the hydrocarbon monoterpenes and the cyclic oxygenated monoterpenes, as observed by other investigators [14,15], seems to support possibility (a), but the fact that the formation of pulegone can take place via dimethylacrylic acid [16] also supports possibility (b). Without going further into the choice between the two possibilities, it appears from the results of the present

Table 1. Changes in the total quantity of essential oil per leaf during the development of *Mentha suaveolens* chemotype 6

Insertion No.†	1. Harvest 11.6.69 wt of 1 leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)	2. Harvest 25.6.69 buds wt of 1 leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)	3. Harvest 9.7.69 onset of flowering wt of 1 leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)
XV							67	0.370	249
XIII				54	0.291	158	143	0.359	514
XI				134	0.492	656	208	0.330	688
IX	108	*0.391	424	174	0.376	657	250	0.306	765
VII	212	0.300	636	270	0.294	794	286	0.232	663
V	240	0.086	207	290	0.194	564			

Insertion No.	4. Harvest 23.7.69 full bloom wt of 1 leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)	5. Harvest 6.8.69 full bloom/and beyond wt of 1 leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)
XV	78	0.412	320	88	0.345	303
XIII	146	0.391	570	168	0.292	490
XI	182	0.403	734	208	0.236	488
IX	190	0.420	802	217	0.253	548
VII	228	0.344	785	301	0.211	636

* 0.XXX is only a mathematical treatment and does not express the accuracy of the method of determination.
† First leaf pair above ground insertion No. I.

investigation, that at a more advanced stage of development the amounts of higher oxygenated compounds decline or occasionally disappear, whereas reduced terpenoid substances appear or increase in amount. Several investigators [13,15,17–19] have found a rapid incorporation of $^{14}CO_2$ in all group of terpenes which is incompatible with a slow development of reduced substances taking place. A system of equilibria seems much more acceptable where a shift takes place to the more reduced components under the influence of enzymes and the incorporated $^{14}CO_2$ distributes itself over all components that take part in each equilibrium. It should be noted that a definite relationship exists between the various cyclic oxygenated monoterpenes formed and that cell free extracts of young root tips of *Mentha arvensis* and *Mentha* x *piperita* are able to convert pulegone into menthone

Table 2. Changes in the total quantity of essential oil per leaf during the development of *Mentha suaveolens* chemotype 12

Insertion No.†	1. Harvest 11.6.69 wt of one leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)	2. Harvest 25.6.69 buds wt of one leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)	3. Harvest 9.7.69 buds/onset on flowering wt of one leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)
XII							139	0.303	420
X				129	0.417	537	276	0.282	779
VIII	76	*0.197	151	214	0.326	697	342	0.276	945
VI	170	0.240	408	314	0.242	761	360	0.232	835
IV	238	0.142	338						
II	126	0.058	73						

Insertion No.	4. Harvest 23.7.69 full bloom wt of one leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)	5. Harvest 6.8.69 full bloom wt of one leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)	6. Harvest 20.8.69 beyond full bloom wt of one leaf (mg)	% ess. oil	oil per leaf ($\mu l \times 10^{-3}$)
XII	146	0.340	495	163	0.276	450	173	0.177	307
X	217	0.300	652	206	0.276	566	201	0.174	349
VIII	276	0.280	773	243	0.250	605	253	0.158	401
VI	279	0.260	726	271	0.188	510	260	0.141	366

* 0.XXX is only a mathematical treatment and does not express the accuracy of the method of determination.
† First leaf pair above ground insertion No. I.

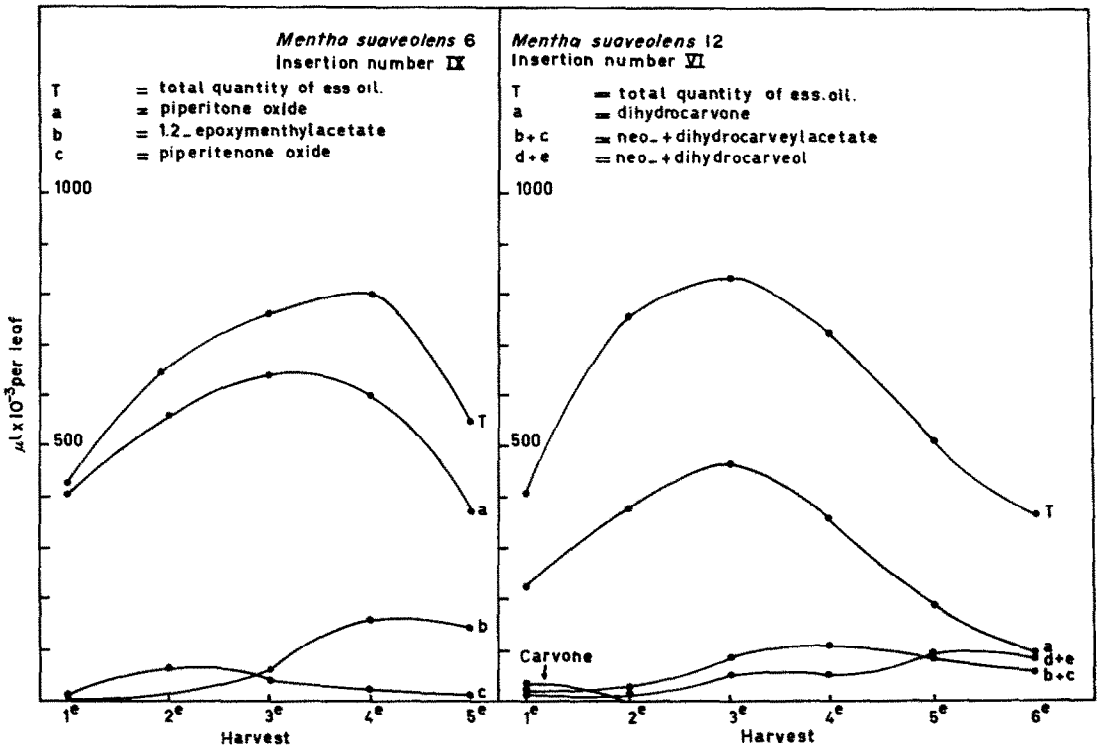

Fig. 1. Course of the total quantity of essential oil and of some constituents per leaf of an insertion number of *Mentha suaveolens* 6 and 12 during the development of the plant.

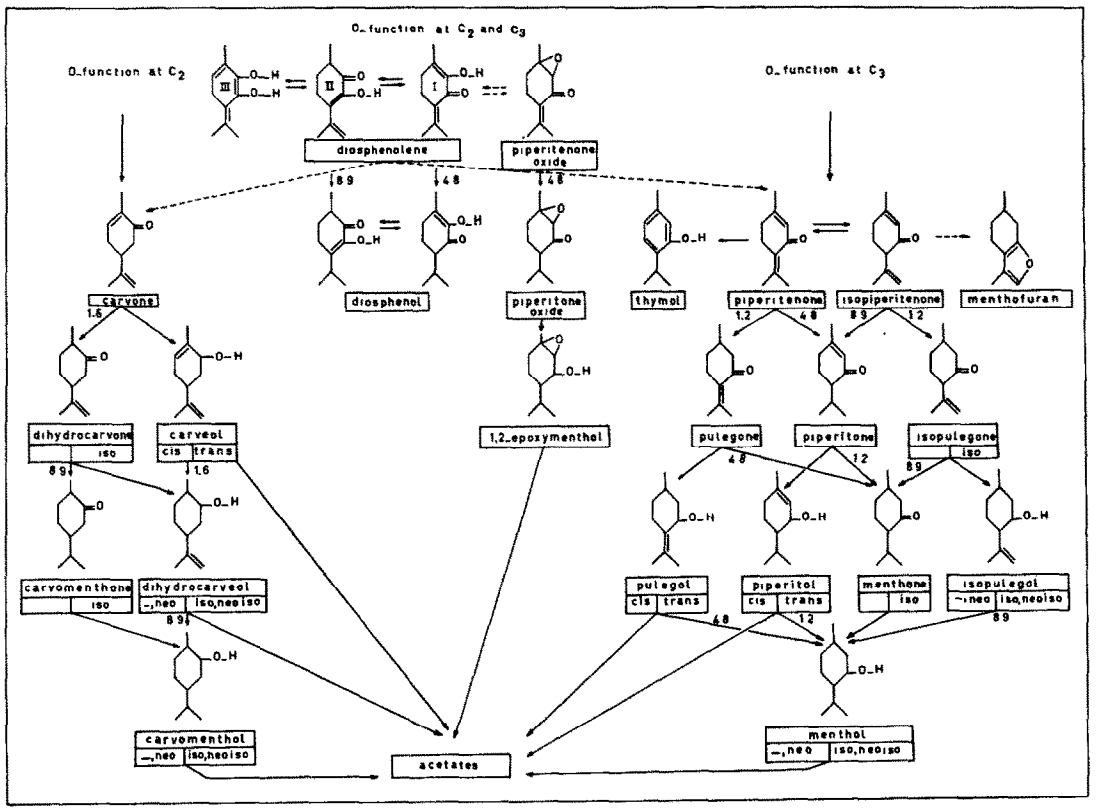

Fig. 2. Relation between the most important oxygen monoterpenes.

and isomenthone [20]. This phenomenon is not directly explained by assuming one common precursor [21] for all monoterpenes present in Mentha essential oils.

The relationship between the most important components of the essential oils from the genus *Mentha* is summarized in Fig. 2. Diosphenolene, which occurs in some oils of *Mentha* [22,23], with an oxygen function both at C-2 as at C-3 takes the central position. Enzymatic reduction and loss of H_2O at C-3 of diosphenolene (or a related precursor) influenced by the dominant gene C induces the formation of carvone followed by further reduction to other components of the carvone series. Reduction at C-2 and loss of H_2O, influenced by the presence of the dominant gene A leads to the formation of piperitenone and after further reduction to other components of the piperitenone series. When both dominant genes are present (genotype CCAA) only components of the carvone series are formed, so the gene C is epistatic over A. When both genes are recessively present (genotype ccaa) piperitenone oxide, closely related to diosphenolene is formed. Afterwards further reduction to other components of the piperitenone oxide series can take place. In another paper the genetical aspects will be discussed in more detail [24]. The scheme also includes a possible return route of constituents of the essential oil to the site of biosynthesis leading to the idea that the disappearance of monoterpenes from the essential oil during the latter stages of development of the plants could be caused by a conversion into higher, non-volatile components. This decrease cannot be satisfactorily explained by evaporation of the essential oil from the glandular trichomes [25] or by losses caused by rain [26]. It is remarkable that the essential oil of chemotype 6 does not contain free 1,2-epoxymenthol, but its acetate. This may result from an immediate conversion to the acetate because the alcohol is formed in the stage of development where acetylation dominates.

It is clear that during the development of *M. suaveolens* distinct changes occur in the composition of the essential oil and this fact must be recognized before ascribing the effects to those of a chemotype. Analyses have shown that both described types should be considered as distinct chemotypes, namely a piperitone oxide type (6) and a dihydrocarvone type (12).

EXPERIMENTAL

Every 2 weeks a part of the 2 strains of *Mentha suaveolens* (6 and 12) cultivated in the experimental garden at Buitenpost, was harvested by cutting just above ground level. Leaves were sorted accoring to their insertion number (first leaf pair above ground insertion, Number I). The leaves of each insertion were counted and weighed. The method of the Dutch Pharmacopoeia Ed. VI/2nd printing was used to determine the content of essential oil/insertion and the average quantity/leaf was calculated. The isolated essential oil was kept in ampoules under N_2 for further investigation. Investigation of the essential oil. (a) qualitative. As reported in ref. [1]. (b) quantitative. The essential oil of each insertion number was analysed by GLC using a katharameter detector. The columns were stainless steel, 4 mm × 2 m packed with 10% carbowax 20M on chromosorb, N_2 flow rate 60 ml/min. The injector was maintained at 250° and the column temp programmed from 80–200° at 4°/min. The amount of each component in the oils was calculated using the normalization method. The quantity of each component per leaf was calculated from the percentage and the average quantity of essential oil per leaf.

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