A study of the monoterpene interrelationships in the genus mentha with special reference to the origin of pulegone and menthofuran

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SUMMARY

The object of this thesis is to provide a study of the monoterpene interrelationships that exist in the *Mentha* genus. To achieve this, a chemosystematic study of the *Mentha* genus was undertaken. Within this study, a number of taxonomically authenticated species and natural hybrids of *Mentha* were grown in an experimental garden. The essential oils from each of the populations were obtained by laboratory distillation using a modified Clevenger technique. Each oil was subjected to analysis using a combination of analytical techniques such as column chromatography, analytical and preparative gas chromatography, infra red spectroscopy and, on occasion, polarimetry.

During this chemosystematic study, it became evident that a number of chemotypes in the strains of *Mentha* examined could be realized. In the treatise these chemotypes were categorized according to their structural dissimilarities (qualitative chemotypic differentiation). Within a number of these chemotypes important quantitative differences could be found. In the case that these quantitative differences were so important that the real character of the oil was changed, we accepted these quantitative different strains as real chemotypes. In other cases, where it was not certain that the differences were not the consequence of a different state of development as of differences in the relation of buds, stems, leaves or flowers, we brought these quantitatively different strains into groups within the chemotype. It should not be excluded that by breeding, pure chemotypes could arise out of these groups.

Using the chemotypic categorization alluded to above, the following populations, which were analysed by this author, can be summarized in the following way: *Mentha requienii* (1 clone, 1 chemotype); *Mentha dimenica* (1 clone, 1 chemotype); *Mentha japonica* (1 clone, 1 chemotype); *Mentha pulegium*(8 clones, 3 chemotypes); *Mentha gattefossei* (2 clones, 1 chemotype); *Mentha cervina* (1 clone, 1 chemotype); *Mentha arvensis* - European (12 clones, 7 or possibly 8 chemotypes); *Mentha arvensis* - N. American (78 clones, 4 chemotypes, 3 groups of chemotype 1,2 groups of chemotype 2, 2 or possibly 3 groups of chemotype 3 and 2 or possibly 3 groups of chemotype 4); *Mentha arvensis* var. *piperascens* (2 clones, 1 chemotype, 2 groups); *Mentha aquatica* (12 clones, 1 chemotype); *Mentha suaveolens* (20 clones, 3 chemotypes, 3 groups of chemotype 1); *Mentha longifolia* including subspecies, "*Mentha incana*" and "*Mentha longifolia* var. *candicans*" (23 clones, 4 chemotypes, 3 groups of chemotype 1); *Mentha spicata* - 2n = 48 (15 clones, 3 chemotypes, 3 groups of chemotype 1, 3 groups of chemotype 2 and 2 groups of chemotype 3); *Mentha spicata* - 2n = 36 (6 clones, 1 chemotype); *Mentha x citrata* (4 clones, 2 chemotypes); *Mentha x cordifolia* (11 clones, 2 chemotypes); *Mentha x dalmatica* (1 clone, 1 chemotype); *Mentha x dumetorum* (5 clones, 3 chemotypes); *Mentha x gentilis* (19 clones, 4 chemotypes); *Mentha x maximilianea* (5 clones, 2 chemotypes); *Mentha x muellerana* (3 clones, 1 chemotype); *Mentha x niliaca* (11 clones, 2 chemotypes); *Mentha x piperita* (9 clones 1 chemotype); *Mentha x pyramidalis* (1 clone, 1 chemotype); *Mentha x smithiana* (4 clones, 2 chemotypes); *Mentha x verticillata* (12 clones, 5 chemotypes); *Mentha x villosa* (3 clones, 1 chemotype); "*Mentha asiatica*" (2 clones); "*Mentha caucasica*" (1 clone); "*Mentha dahurica*" (1 clone); "*Mentha insularis*" (1 clone); "*Mentha lavanduliodora*" (1 clone); "*Mentha x nemorosa*" (1 clone)' "*Mentha palustris*" (1 clone); "*Mentha sacchalinensis*" (1 clone); "*Mentha tomentosa*" (1 clone) and "*Mentha x suavis*" (1 clone).

To obtain some further information about the possible biosynthetic origin of some of the compounds that were isolated during the above listed analyses, a number of artificial hybrids were obtained for this purpose. Using the techniques described earlier, these hybrids, which were made by M.J. Murray, were subjected to analysis. The object of these particular analyses was to provide pertinent information related to specific compound interrelationships that occur between components. For example,

the following artificial crosses were examined:

Mentha aquatica x
- *Mentha spicata* (carvone-rich)
- *Mentha spicata* (piperitenone-rich)
- *Mentha spicata* (isomenthone-rich)
- *Mentha longifolia* (piperitenone oxide-rich)
- *Mentha suaveolens* (piperitone oxide-rich)
- *Mentha x cordifolia* (carvone-rich)
- *Mentha arvensis* (isopulegone-rich)
- *Mentha arvensis* (pulegone-rich)
- *Mentha x citrata* (linalool/linalyl acetate-rich)

F_1(*Mentha x citrata x Mentha aquatica*) x
- *Mentha arvensis* var. *piperascens* (menthol-rich)
- *Mentha aquatica* (menthofuran-rich)

F_1(*Mentha arvensis x Mentha aquatica*) x
- *Mentha arvensis* (pulegone-rich)

F_1(*Mentha arvensis x Mentha aquatica*) (menthofuran-rich)

Mentha arvensis var. *piperascens* x
- *Mentha arvensis* (isopulegone-rich)
- *Mentha arvensis* (pulegone-rich)
- *Mentha suaveolens* (piperitenone oxide-rich)
- *Mentha spicata* (piperitenone-rich)
- *Mentha spicata* (pulegone-rich)

Mentha x cordifolia x
- *Mentha x citrata* (linalool/linalyl acetate-rich)

Mentha spicata x
- *Mentha x piperita* (menthol/menthone-rich)

Before examining the results of these artificial hybrids and comparing them with those obtained from the natural species and hybrids, a discussion of monoterpene biosynthesis was considered mandatory. In this discussion, the subjects: compound co-occurrence, radioactive tracer studies, cell-free systems and genetic engineering techniques were briefly reviewed.

A combination of all of the previously published information and the results obtained by this author were used for the purpose of discussing and proposing a series of hypotheses related to the biosynthesis of monoterpenes in *Mentha*. In this context the following topics were discussed:

1. Substrates for monoterpene biosynthesis
2. Origin of α-terpineol
3. Origin of 1,8-cineole
4. Origin of limonene and terpinolene
5. Origin of carvone and piperitenone
6. Reduction of piperitenone to pulegone and piperitone
7. Origin of menthone
8. Origin of cis- and trans-dihydrocarvone
9. Origin of isomenthone
10. Origin of cis- and trans-isopulegone
11. Origin of menthofuran
12. Origin of 2- and 3-substituted alcohols
13. Origin of monoterpene acetates
14. Origin of piperitenone oxide
15. Origin of cis- and trans-piperitone oxide

16. Origin of 1,2-epoxymenthols and their acetates
17. Origin of borneol, camphor and camphene
18. Origin of α- and β-pinene
19. Origin of compounds based on β-pinene e.g. isopinocamphone
20. Origin of compounds based on α-pinene
21. Origin of linalool, cis- and trans-ocimene and myrcene

In addition, the biosynthetic origin of some other monoterpenoid and non-monoterpenoid compounds has been examined. The possibility that some of these compounds are not true constituents of *Mentha* oils but artifacts has also been discussed. It is of particular importance to note that this author has obtained unequivocal evidence that pulegone is the precursor of menthofuran (see 10.9.2). In addition, the existence of two stereospecific reductases capable of reducing monoterpene ketones to their corresponding alcohols has been demonstrated for the first time (see 10.10.2). During the discussion of the origin of the various monoterpene compounds, a genetic interpretation of the specific reactions has been considered. For example, it has been proposed that the genes control a series of biosynthetic reactions as shown below:

	Gene	Biosynthetic reactions
(a)	L_m	neryl pyrophosphate ⟶ limonene
(b)	L_t	neryl pyrophosphate ⟶ terpinolene
(c)	C	limonene ⟶ carvone
(d)	A_r	piperitenone ⟶ pulegone carvone ⟶ trans-dihydrocarvone piperitone ⟶ menthone
(e)	A_s	carvone ⟶ cis-dihydrocarvone
(f)	P_r	pulegone ⟶ isomenthone piperitenone oxide ⟶ cis-piperitone oxide
(g)	P_s	piperitenone ⟶ piperitone pulegone ⟶ menthone
(h)	R_r	menthone ⟶ menthol isomenthone ⟶ neoisomenthol cis-isopulegone ⟶ neoiso(iso)pulegol trans-isopulegone ⟶ isopulegol carvone ⟶ cis-carveol cis-dihydrocarvone ⟶ neoisodihydrocarveol trans-dihydrocarvone ⟶ dihydrocarveol trans-piperitone oxide ⟶ 1,2-epoxymenthol
(i)	R_s	menthone ⟶ neomenthol isomenthone ⟶ isomenthol cis-isopulegone ⟶ iso(iso)pulegol trans-isopulegone ⟶ neoisopulegol carvone ⟶ trans-carveol cis-dihydrocarvone ⟶ isodihydrocarveol trans-dihydrocarvone ⟶ neodihydrocarveol cis-piperitone oxide ⟶ 1,2-epoxymenthol

Gene		Biosynthetic reactions
(j)	H_r	pulegone ⟶ cis-isopulegone
(k)	H_s	pulegone ⟶ trans-isopulegone
(l)	f	pulegone ⟶ menthofuran
(m)	o	piperitone ⟶ trans-piperitone oxide piperitenone ⟶ piperitenone oxide
(n)	E	all monoterpene alcohols ⟶ corresponding monoterpene acetates