

Studies on the Essential Oils of Interspecific Hybrids in the Genus *Mentha*, Part VI*

On a New Chemical Strain in the Hybrids Involving *Mentha spicata* var. *crispa* Benth.

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It is well known that spearmint oils containing (–)-carvone as the major constituent have been produced by steam distillation of *Mentha spicata* var. *viridis* (number of chromosomes, $2n=36$) or *Mentha cardiaca* Gerarde (Syn. *Mentha gentilis* L. f. *cardiaca*, $2n=72$). According to Briquet, *Mentha spicata* var. *viridis* L. has been distinguished from *Mentha longifolia* (L.) Huds., as the former is glabrous, while the latter is more or less hairy. Ikeda, one of the authors, however, suggested that two species should be united into one species, *Mentha spicata* L. in accordance with Linnaei, since the difference was found insufficient from genetical and cytological point of view.¹⁾ By our chemo-taxonomical studies on the oils of Japanese and European *viridis* and *longifolia*, similar chemical strains have been found in both species.²⁾

Mentha spicata will be also divided into four groups in accordance with the number of chromosomes. That is, there are strains with $2n=24$, 36, 48 and 54 chromosomes in the somatic cells. *Mentha spicata* var. *crispa* Benth., being now widely spread in Japan, is said to have been introduced into this country from Europe about one hundred years ago, and has been named Oranda-hakka (Dutch mint) or Chirimen-hakka (Crisp leaved mint). It was reported by Ikeda, Udo and Nakamura that ten strains of *crispa* type found throughout Japan have been divided into two groups, namely three strains with $2n=54$ and seven strains with $2n=48$.³⁾ Any of them was determined to contain (–)-carvone predominantly in the essential oils, while *M. spicata* with $2n=24$ and 48 of European origin showed following several chemical strains; carvone, dihydrocarvone, menthone and pi-

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peritone, piperitone oxide, pulegone and piperitenone oxide strain, as indicated in our previous reports.⁴⁾

Recently, many biochemical studies have been published to demonstrate the relation between monoterpenes formation in various mint species and genes controlling the biogenesis of these terpenes.^{5) 6)} Analysis of the oils of various *Mentha* species revealed three types,^{6) 7)} in each of which a mixture of related monoterpenes predominated: the first type exemplified by *Mentha citrata* formed mainly acyclic compound such as linalool; the second type showed predominantly 2-oxygenated *p*-menthanes such as carvone or dihydrocarvone in the case of common species belonging to *Mentha spicata*; the third type, such as *Mentha arvensis* and *Mentha piperita*, mainly yielded 3-oxygenated *p*-menthanes (menthol, menthone, pulegone, piperitone, piperitone oxide or piperitenone oxide).

Murray studied on the oils of F₁ and F₂ of American *Mentha spicata* with 2n=48, belonging to the second type of oil. After self-pollination, he found the progenies producing oils of the second and third types in the ratio of

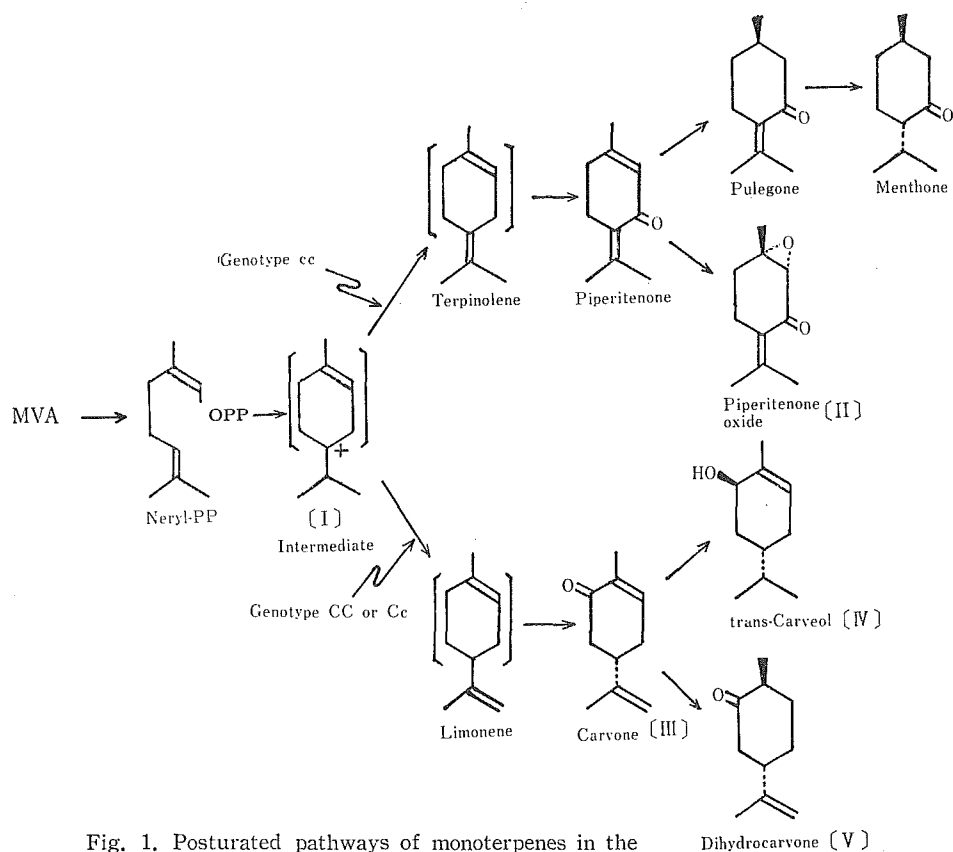


Fig. 1. Postulated pathways of monoterpenes in the hybrids of *spicata*, based on genetic evidence.

3:1.⁵⁾ From genetical studies on *M. spicata* (2n=48) and *M. crispa* (2n=48) with the genotype *Cc Aa*, he also showed that the dominant gene *C* caused the formation of carvone and the 2-oxygenated compounds of limonene, while the recessive gene *c* caused 3-oxygenated compounds such as menthone or pulegone. He further found that double recessive *caa* genotype produced piperitone which was mostly converted to piperitone oxide, and that *ccAA* genotype produced pulegone which was always partly converted to menthone. Any individual having the dominant gene *C* produced carvone without regard to whether the other gene was *AA*, *Aa* or *aa*.⁵⁾ These biogenetical formation of terpenes found in various species in *Mentha* will be shown in Fig. I.

In this paper we wish to report on the essential oils of four strains of the hybrids involving Japanese *Mentha spicata* var. *crispa* Benth. with 2n=48 and with 2n=54, compared with the results from American *Mentha crispa* mentioned above.

Results and Discussion

Origins of the parent *Mentha spicata* used for crossing:

Native places of the strains used as the parents of crossing are as following:

Morphological characteristics and genome constitutions of these three *spicata*

Table 1. Origins of parent *spicata*

Strain	Introduced from	Name of variety	Number of chromosome (2n)
[1]	Okayama Experimental Station for Agriculture	<i>var. longifolia</i>	48
[4]	"	<i>var. crispa</i>	48
[56]	Grows wild at Fukuyama, Hiroshima	<i>var. crispa</i>	54

Table 2 Physical properties of the oils from hybrids and parents

Name of hybrids or parent	2n	$[\alpha]^{20}_D$	λ_{max}	Absorbance (100r/10ml)	Principal component, (%)
<i>F</i> ₁ 8, [1] × [4]	48	- 51.8°	235 μ	0.40	(-)carvone(50)
<i>F</i> ₁ 55-4, [4] × [56]	51	-120.7	230	0.03	(-)trans carveol (85)
<i>F</i> ₁ 56-4, [56] × [4]	51	+ 87.2	260	0.32	(+)piperitenone oxide (53)
<i>F</i> ₁ 56-9, [56] × [4]	51	+ 89.4	260	0.37	(+)piperitenone oxide (61)
<i>spicata</i> [1]	48	- 60.0	235	0.35	(-)carvone (41)
" [4]	48	- 58.8	235	0.42	(-)carvone (51)
" [56]	54	- 55.5	235	0.31	(-)carvone (40)

have been reported by Ikeda and Udo^{3) 8)}. Crossing among these varieties of *spicata* was carried out in Okayama University. The essential oils were respectively obtained by usual steam distillation of air dried herbs. Physical properties of these oils are compared with those of the parent oils, as shown in Table 2. The contents of the terpenes in the essential oils were determined by peak area in gas chromatogram. Piperitone oxide was also determined by the ultra-violet absorption method.¹⁴⁾

Identification of principal components in F₁8, F₁56-4 and F₁56-9 oils:

The major constituent of F₁8 oil seemed to be an α, β -unsaturated ketone from the intense absorption at 235 $m\mu$. It gave the deep red 2,4-dinitrophenylhydrazone of mp. 189°, which was not depressed on admixture with that of authentic (-)-carvone. By comparing the absorption extinction at λ max. 235 $m\mu$ of F₁8 oil with those of the parent oils, no significant difference of the carvone content between this hybrid and the parents was observed.⁹⁾

Two oils of F₁56-4 and F₁56-9 were highly dextro-rotatory and showed the

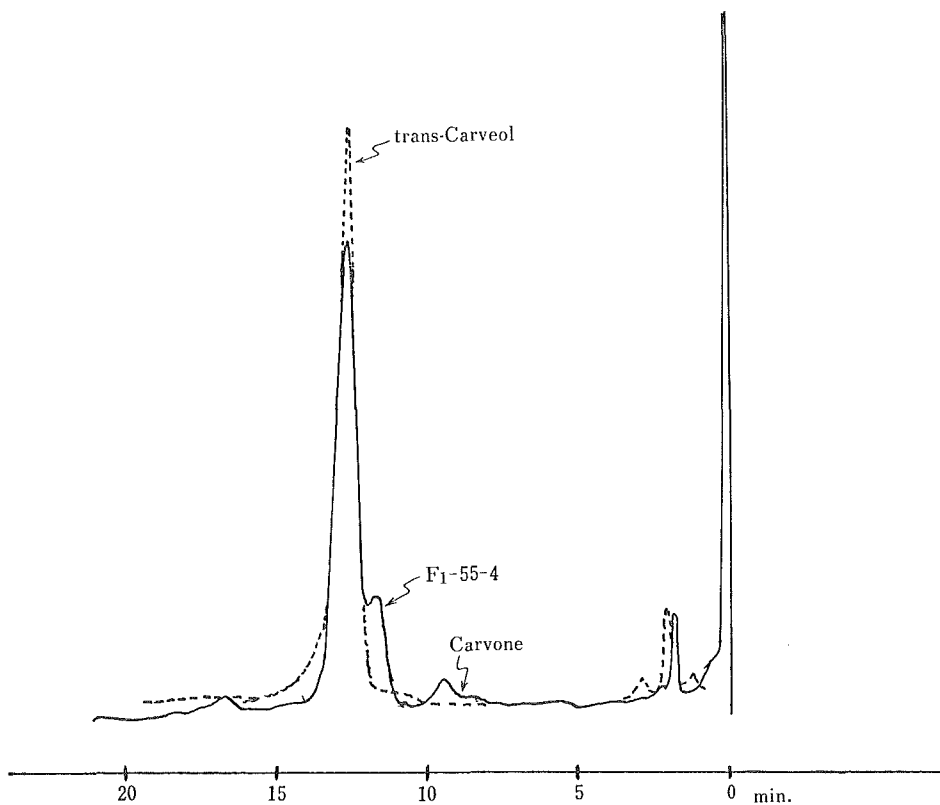


Fig. 2 Gas chromatogram of F₁55-4 oil (Fraction b. p. 110°C/20mm)

strong absorption at $260\text{ m}\mu$, indicating the presence of an α, β -unsaturated ketone with an exocyclic double bond. The semicarbazone of mp. 180° (with decomp.), λ max. $273\text{ m}\mu$ was obtained from the oils of both hybrids. It was not depressed on admixture with (+)-piperitenone oxide semicarbazone isolated from the oil of *Mentha rotundifolia*.⁴⁾

Confirmation of (-)-trans-carveol in F₁₅₅₋₄ oil:

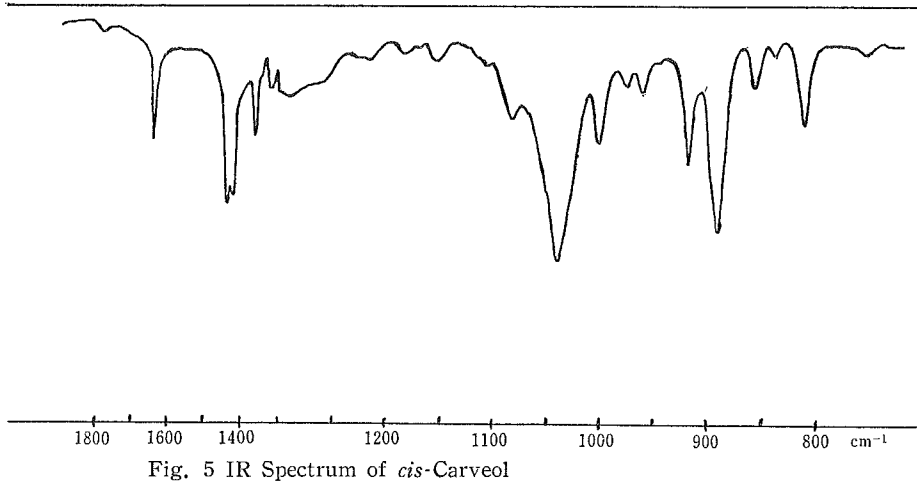
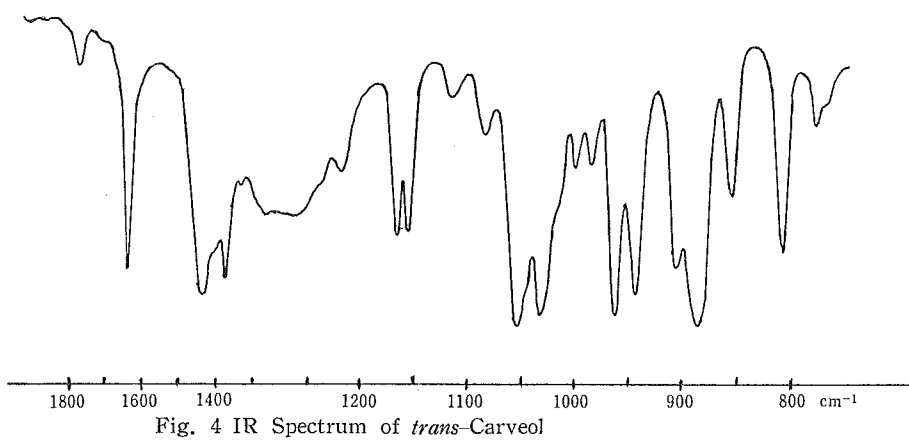
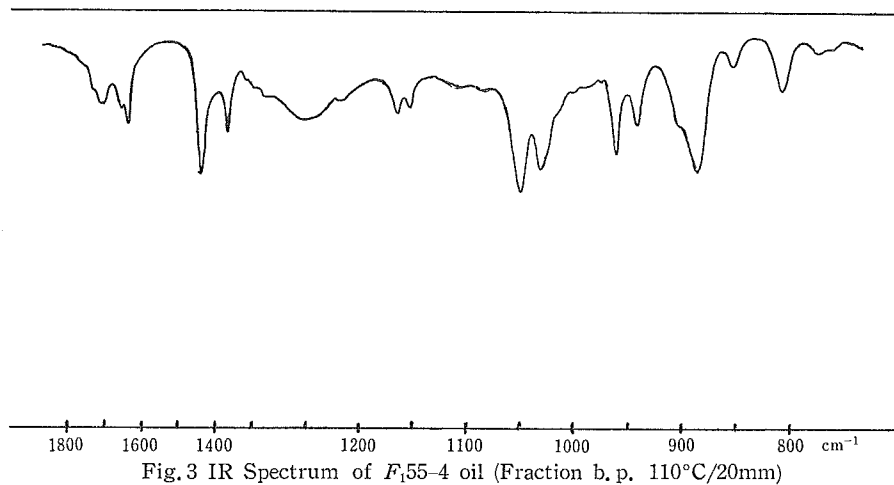
The oil of F₁₅₅₋₄ was highly levo-rotatory. The major fraction bp. $110^\circ/20$ mm of this oil showed a predominant peak of Rt. 13.3min. in the gaschromatogram (Fig. 2). It was assumed to be an alcohol, since a strong absorption was observed near 3300cm^{-1} in its IR spectrum (Fig. 3). Chromic acid oxidation of this fraction gave (-)-carvone, which was identified with the authentic sample as its 2,4-dinitrophenylhydrazone. Consequently, the major component of this hybrid oil should be one of stereoisomers of carveol reported by Read.¹⁰⁾ The *trans*-isomer was synthesized by the Meerwein Ponndorf reduction method and the *cis*-isomer was obtained by the lithium aluminum hydride reduction method. Our major component of the hybrid oil has been confirmed as (-)-*trans*-carveol by comparing with the IR spectra of the *trans*- and the *cis*-isomers (Fig. 4 and 5) and moreover, the 3,5-dinitrobenzoate of the hybrid oil (mp. 110°) was not depressed on admixture with the synthesized 3,5-dinitrobenzoate of *trans*-carveol.

Biogenetic pathway of trans-carveol in the hybrid:

Though mevalonic acid MVA has been established as the very important precursor in terpenoid biosynthesis, the incorporation of mevalonic acid into monoterpenes by whole plants or plant tissues is generally low, in range of 0.01–0.1%, in some cases negligible⁷⁾. Monoterpenes have been considered to be produced from two isoprene units derived from mevalonic acid and geranyl pyrophosphate is well established as an intermediate in the biosynthesis of sterols and other isoprenoid compounds. However, neryl phosphate yielded principally the cyclic monoterpene, α -terpineol, while geranyl phosphate yielded no cyclic product by Ritterdorf.¹²⁾ So, we have shown the pathway (Fig. 1), in which a C₁₀ hypothetical intermediate [I] is used according to Ruzicka,¹³⁾ Murray⁵⁾ and Roomis.⁷⁾

Formation of (+)-piperitenone oxide [II] in the hybrids from the (-)-carvone producing parents will proceed by the same way as reported by Murray.⁵⁾ This interpretation means that Japanese *spicata* var. *crispa* with $2n=54$ and American *crispa* with $2n=48$ have the same dominant gene *C* causing (-)carvone formation, and piperitenone oxide in two hybrids will be yielded by combination of the recessive genes *cc aa* in crossing.

On the other hand, (-)-carvone [III] will be at first formed in our new hybrid F₁₅₅₋₄ as shown in Fig. 1 by the dominant gene *C* and then converted to *trans*-carveol [IV] by another gene *AA*, or *Aa*, in which the gene *A* is considered to be



concerned with the reduction of ketones by Murray.⁵⁾ It is noteworthy that this new hybrid was raised by back crossing [4]×[56], while F₁ [56]×[4] gave the (+)-piperitenone oxide dominant oil, being under influence of the recessive gene *cc aa* by Murray. Our hybrid having *trans*-carveol predominately in the oil seems to reduce (–)-carvone selectively to the *trans*-terpene alcohol but not to dihydrocarvone [V], which is several times found in American and European *spicata*.⁴⁾⁶⁾

The biosynthetic pathway of the terpenes in *spicata* hybrids will be concerned first with differentiation at the oxidation position of an intermediate such as [I], secondly with the selective reduction of the unsaturated ketones as shown in Fig. 1. These problems still await further detailed studies.

Experimental

All melting points and boiling points were uncorrected. UV absorption spectra and optical rotations were determined in methanol solution. Infrared absorption spectra were taken with a Hitachi EPI-G₃ Infrared Spectrophotometer. Gas chromatograms were obtained by using a column of 20% PEG 6000 on celite 545; Temp., 175°; carrier gas, Hydrogen, flow rate, 80 ml/min.. The essential oil of each hybrid was obtained from the herb harvested before flower by the usual steam distillation;¹⁴⁾ oil yields from fresh herbs, 0.15–0.30% (1958–1960).

(–)-*trans*-Carveol by the Meerwein-Ponndorf method:¹⁰⁾ (–)-carvone 14.5g was treated with 70ml of isopropanol solution containing 1 mol of aluminum isopropoxide by the usual method. The reduction product was washed with 10% soda and distilled with steam. This reduction product was found to be a mixture of *trans*- and *cis*-carveol (approximately 55:45 in ratio) by gas chromatography. From 1.5g of this mixture, 2.3g of 3,5-dinitrobenzoate (mp. 92–96°) was obtained. Pure dinitrobenzoate of the *trans*-isomer was separated by several recrystallization with a solvent mixture of methanol and ethyl acetate; yielded 0.9g, mp. 110° (lit.,¹⁰⁾ 111.5°) (–)-*trans*-carveol was regenerated from the dinitrobenzoate; bp. 100°/9mm., $[\alpha]^{20}_D$ –212°; Rt. 13.3 min. (PEG 6000 column, Temp., 175°); Rf. 0.57 (silica gel, benzene and petroleum ether 1:1)

(–)-*cis*-Carveol by the lithium aluminum hydride method:¹¹⁾ To 50ml ether solution containing 0.1g of lithium aluminum hydride 1.0g of (–)-carvone, $[\alpha]^{20}_D$ –61.0° was added under stirring; reduction product, the oil of bp. 102°/9mm., $[\alpha]^{20}_D$ –43.0°; 3,5-dinitrobenzoate of mp. 89° (lit.,¹⁰⁾ 92.5°); (–)-*cis*-carveol was regenerated from this dinitrobenzoate; bp. 102°/9mm., Rt. 14.5min. (PEG6000 column, Temp., 175°). Rf. 0.64 (silica gel, benzene and petroleum ether, 1:1).

The content of piperitenone oxide in the oils of the hybrids: (+)-piperitenone oxide in the oils of the hybrids was determined by the ultra-violet absorption method reported previously.¹⁴⁾

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Summary

The essential oils of four hybrids involving *Mentha spicata* var. *crispa* Benth., growing wild in Japan were investigated. One strain was found to contain (+)-piperitenone oxide predominantly, while another strain obtained by the back crossing was established to give (-)-*trans*-carveol as the major constituent of the essential oil. It is noteworthy from the chemotaxonomical point of view that a new *trans*-carveol strain was raised by crossing between *M. spicata* var. *crispa* with $2n=48$ and *crispa* with $2n=54$. The biosynthetic pathway of these terpenes in the hybrids derived from the carveone dominant *spicata* was discussed from the genetical point of view.

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薄荷属種間雑種精油成分に関する研究

(第6報) オランダハッカ交雑種の新化学成分系

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要 約

日本にひろく野生している、オランダハッカ *Mentha spicata* var. *crispa* BENTH. は帰化植物として知られている。このものの染色体数 $2n=48$ [4]と $2n=54$ [56]の2系統を交雑親に用いたが、精油主成分はともに(-)-carvoneであった。この交雑によって得られた F_1 [56]×[4]の系統は、両親の精油主成分とはことなり、(+)-piperitenone oxideが精油主成分であった。米国産の縮葉型薄荷($2n=48$)の F_1 , F_2 の精油成分の研究を行なって、Murrayが提出した、遺伝生化学的な考察は、日本のオランダハッカの場合にも可能であると考えられた。さらに、逆交雑種、 F_1 [4]×[56]の精油主成分が、(-)-*trans*-carveolであることを明らかにした。この系は、米国の実験には見出されておらず、chemotaxonomyの面から新しい系であると考えられる。なお本系は(-)-carvoneの還元により、従来知られているdihydrocarvoneを、ほとんど生成することなく、立体特異的に(-)-*trans*-carveolを高率(85%)に生成するものと考えられ、精油成分生合成研究上、注目すべき例であることを明らかにした。

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