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# GENETIC CONTROL OF ISOEGOMAKETONE FORMATION IN *PERILLA FRUTESCENS*

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**Key Word Index**—*Perilla frutescens*; Labiatae; volatile oil; chemotype; gene analysis; perillaketone; isoegomaketone.

**Abstract**—Genetic analysis of isoegomaketone formation in *Perilla frutescens* was carried out by intercrossing two different subgroups of the perillaketone chemotype. It was demonstrated that the accumulation of isoegomaketone in addition to perillaketone in the volatile oil is controlled by an inhibitor gene I, which presumably inhibits the isomerization of the possible precursor egomaketone leading to isoegomaketone.

## INTRODUCTION

Local varieties of *Perilla frutescens* cultivated in Japan [1, 2] are classified into five chemotypes according to the main components of their volatile oils, viz. perillaldehyde (PA), perillaketone (PK), elsholtziaketone (EK), citral (C) and phenylpropanoid (PP) types [3]. Genetic analysis of these chemotypes except for the C type demonstrated that the chemical variations are caused by two pairs of independent genes, G and H [4]. Furthermore, we found that strains of the PK type ($G_2G_2hh$) can be divided into two subgroups: one containing perillaketone (PK) and the other containing isoegomaketone (IK) in addition to PK as the main monoterpenes. Crossing experiments have been conducted in order to investigate the genetic basis of the difference in chemical composition between the two subgroups. This paper reports that the chemical difference is caused by a single gene.

## RESULTS AND DISCUSSION

Four parental strains of the PK type of *Perilla* used for crossing experiments were classified into two subgroups according to the monoterpenoid composition of volatile oils: (1) the 'PK-I type' containing mostly PK (80–90% of the volatile oil) and only a minute amount of IK (< 1%), and (2) the 'PK-II type' containing nearly equal amounts of IK (35–50%) and PK (40–50%). A small amount (10–14%) of caryophyllene, a sesquiterpenoid component, was also present in both types, but no egomaketone (EGK) was detectable by GC analysis (Table 1). Strains 8 and 11 of the PK-I type were crossed with strains 6 and 63 of the PK-II type. The $F_1$ and $F_2$ data obtained from the intercrosses are shown in Table 2.

In both crosses, 8 (PK-I) × 6 (PK-II) and 11 (PK-I) × 63 (PK-II), the $F_1$ hybrids were of the PK-I type and gave a 3:1 segregation ratio for PK-I and PK-II types in $F_2$. In $F_2$ plants of the PK-II type, the content of IK varied from 12 to 50% (average 24%) of volatile oils. These results suggest that the difference between the two types is due to a dominant allele I; the genotypes of PK-I and PK-II may be designated as $G_2G_2hhI$- and $G_2G_2hhii$, respectively, where I- stands for II and Ii.

Although we could not detect the presence of EGK in any of the *Perilla* plants examined by GC analysis, Fujita and Ueda reported two chemotypes of *Perilla* containing either PK [5] or EGK [6] as the main component of the volatile oil. On the other hand, Ito [7] isolated from *Perilla* leaves a mixture of IK and a very small amount of EGK which could not be detected by GC but its presence was suggested by $^1$H NMR and UV spectra. On the basis of such information, Hegnauer [8] proposed a biosynthetic scheme that PK and IK are derived separately from a common precursor, EGK (Scheme 1). The present genetic data are compatible with this hypothetical scheme, if gene I acts to inhibit the isomerization of EGK to form IK, without inhibiting the oxidative conversion of EGK into PK. Thus, gene I of *Perilla* seems to be analogous to genes I and Lm of *Mentha* which inhibit the conversions of linalool → limonene [9] and limonene → piperitone [10], respectively.

## EXPERIMENTAL

*Plant material.* Two strains, 8 and 11, of *P. frutescens* Britt. var. *acuta* Kudo f. *viridis* Makino, strain 6 of var. *acuta* Kudo and

Table 1. Main components of the volatile oils in parental strains of *Perilla frutescens*

| Strain no. | Chemotype | Total amount* of volatile oil | Relative amounts of constituents† | | |
|---|---|---|---|---|---|
| | | | PK | IK | CR |
| 8 | PK-I | 0.37 | 88 | 0.3 | 10 |
| 11 | PK-I | 0.32 | 81 | 0.5 | 14 |
| 6 | PK-II | 0.23 | 49 | 35 | 12 |
| 63 | PK-II | 0.25 | 38 | 48 | 12 |

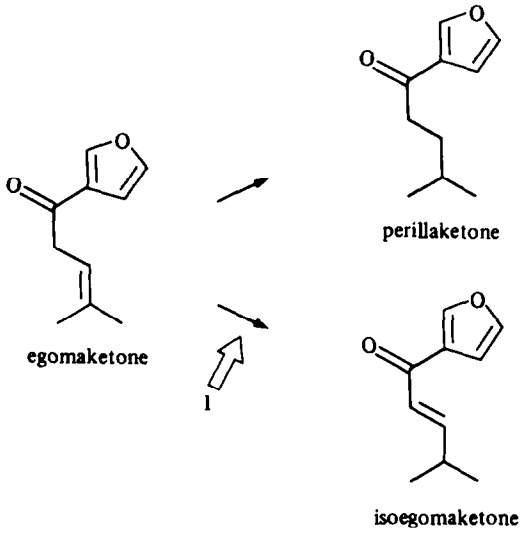
*Percentage of the fresh weight of leaves.
†Percentage of the total amount of volatile oils.
PK: perillaketone, IK: isoegomaketone, CR: caryophyllene.

Table 2. Segregation for monoterpenoid components in $F_2$ progenies of intercrosses between different chemotypes, PK-I and PK-II*, of *Perilla frutescens*

| Cross ($P_1 \times P_2$)† | Phenotype of $F_1$ | Segregation ratio in $F_2$ progeny: Phenotype | Observed ratio | Expected ratio | *P* value of $\chi^2$-test |
|---|---|---|---|---|---|
| 8 × 6 (PK-I × PK-II) | PK-I | PK-I:PK-II | 37:6 | 3:1 | 0.09 |
| 11 × 63 (PK-I × PK-II) | PK-I | PK-I:PK-II | 43:20 | 3:1 | 0.2 |
| Total | | | 80:26 | 3:1 | 0.9 |

*The PK-I type contains perillaketone (PK), which comprises 80–90% of the volatile oil together with a minute amount (< 1%) of isoegomaketone (IK). The PK-II type contains IK (12–50%) and PK (40–70%) as the main components of the volatile oil.

†$P_1$ and $P_2$ represent the female and male parents, respectively.




Scheme 1. Biosynthetic pathways of perillaketone and isoegomaketone postulated by Hegnauer [8] and the possible reaction step (indicated by arrow) controlled by gene I in *Perilla*.

strain 63 of var. *acuta* Kudo f. *crispidiscolor* Makino were used for crossing expts. Methods of artificial pollination and cultivation have been described elsewhere [11, 12].

*GC analysis.* Fresh leaves (2–3 g) were removed from individual plants in early July and extracted with $Et_2O$ (1.25 ml/g fr. wt) overnight at 4°. FID-GC analysis was carried out using a stainless steel column (3 mm × 2 m) packed with PEG-6000 Chromosorb W 17% (30–60 mesh) at a column temp. of 170°, $N_2$ carrier gas at 30 ml/min [3, 4]. Peaks of PK and IK were detected on the chromatogram at $RR_t$ (parillaldehyde) 0.93 and 1.43, respectively [3, 4, 7].

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