Isolation and Identification of Monoterpenes from Cultured Cells of Perilla Plant

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The tissue cultures of plant cells have attracted special attention as advantageous work for investigations concerning with plant growth and metabolism. The studies of such tissue culture have developed rapidly and their achievements have become a center of attention, but both of the identification and the bio-transformation of specific secondary metabolites must be still the subjects of investigation in this field.¹⁾ We have isolated and identified monoterpenes from the cultured cells of perilla plant, which was cultured in the liquid medium containing 2,4-dichlorophenoxy-acetic acid (2,4-D).

The callus was induced from the section (5 mm²) of the young leaf of *Perilla frutescens* Britt. var. *crispa* Deane²⁾ (seed from the Ehime Agricultural Experiment Station, Matsuyama, Japan) with Murashige and Skoog's basal medium³⁾ containing sucrose (30 g/liter), 2,4-D (1.0 ppm), kinetine (5.0 ppm) and Difco bacto-agar (0.9% w/v). The callus tissue was subcultured every 3 weeks during 6 months at 25°C in slightly dark. Suspension culture derived from the callus of eighth generations was maintained in a similar medium, without agar, on a rotary shaker at 25°C for 6 weeks. The growth was completed after 6 weeks with 10 g (fresh weight) of non-uniform aggregated cells per 100ml of medium. The growth rate was faster than in an agar medium and reached to a plateau in 4 weeks after inoculation.

The essential oil from the cultured cells was extracted with methylene chloride at 5°C, with avoiding an oxidation of terpenes, and separated with steam distillation under reduced pressure. The amount of essential oil from the cultured cells was nearly similar to that of original perilla plant, about 0.1% of fresh cells. The gas chromatogram of essential oil obtained from both of cultured cells and their mother plant has been shown in figure. The both columns of 3 mm × 5 m, 10% Carbowax 20M on Chromosorb P AW 60/80 mesh and 3 mm × 3 m, 10% Apiezon L (contained Igepal 5% w/w with respect to the stationary liquid) on Chromosorb W AW 60/80 mesh were used for determination of retentions.

As a major component of the oil from cultured cells,

Gas chromatogram of the essential oil from the cultured cells (I) and Perilla plant (II).

Column: 10% Carbowax-20M on Chromosorb P AW 60/80 mesh, 3 mm × 5 m.

Column Temp. 60~200°C at 2°C/min.

Carrier gas N₂ 20 ml/min.

limonene (peak 3) was identified with the combination of gas chromatography and mass spectrometer (GC-MS); M⁺ *m/e* 136, *m/e* 121, *m/e* 107, *m/e* 93 and *m/e* 68. Its amount was 21% of the oil. α -Pinene (peak 1) and linalool (peak 6) were identified tentatively with retention data. Moreover, the oily substances in the cultured cells were confirmed with the microscopic technique by using of pigments such as Sudan III and Sudan black.

In comparison of the essential oil from perilla plant with that of its cultured cells, isoamyl-3-furylketone (peak 8) and 1-(3-furyl)-4-methyl-2-penten-1-one (peak 10) were found as two major components of terpenes in perilla plant,⁴⁾ but they are not found in the oil from cultured cells. Linalool and limonene were found in both of plant and cultured cells. A few peaks corresponding to those of the oil from plant were found in the chromatogram of the oil from cultured cells, however, these peaks seem to be a mixture with mass spectrum data. Therefore, peaks 8~10 were fractionated all together by preparative gas chromatography and further identification of this fraction is still continuing with GC-MS, by using of 0.25 mm × 45 m capillary column. The detail will be published elsewhere.

This result clearly suggested that specific secondary metabolites were formed in such cultured cells and the desirable metabolites as terpenoids in the plant might be found in the cells cultured *in vitro*. Further investigations are now in progress to identify the minor component in the essential oil from cultured cells and to determine the possible relationship between terpenes and the action of chemicals in the nutrient medium.

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REFERENCES

- 1) "Plant Tissue Culture," ed. by M. Takeuchi, I. Ishihara and T. Furuya, Asakura shoten, Tokyo, Japan, 1972, pp. 349~379.
 - 2) N. Korezawa, T. Yoshida and S. Matsuo, *Nettai Nogyo*, **17**, 12 (1973).
 - 3) T. Murashige and F. Skoog, *Physiol. Plant.*, **15**, 473 (1962).
 - 4) H. Sugisawa and Y. Ohnishi, Abstracts of Papers, 50th National Meeting of Agricultural Chemical Society of Japan, Sapporo, July, 1975, p. 388.
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