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The Production of Capillen in Shoot Primordia of *Artemisia capillaris*

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Over the past decade there has been increased interest in production of secondary metabolites such as pigments, perfumes and flavours from cultured plant cells[1,2]. These secondary metabolites have been produced from immobilized plant cells by K. Lindsey[3] and by the new method of shoot primordia with *Haplopappus gracilis* by Tanaka and Ikeda[4].

Artemisia capillaris is a perennial plant that produces acetylenic compounds as secondary metabolites[5]. Isolation of acetylenic compounds such as capillin, capillen, capillanol and neocapillen from the whole plant of *A. capillaris* has already been reported in literature by Miyazawa and Kameoka[6-10]. However there is no report on the production of capillen from the callus or shoot primordia of *A. capillaris*.

Here we report the production of capillen(1), an acetylenic compound by a new method using shoot the primordia from *A. capillaris*.

Materials and Methods

GC conditions: OV-1 column, 0.25 mm id x 25 m; 90-280°, 4° min^{-1}, inj. and det. temps 280°. GC-MS analyses were carried out with the same conditions. The GC was coupled to a quadrupole MS. Electron energy, emission curret and ion acceleration voltage were 70 eV, 300 mA and 3.0 kV, respectively. The MS was operated in EI mode.

The preparation of shoot primordia from *A. capillaris*. The plant Material of *Artemisia capillaris* was collected in April 1989 at the suburbs of Okayama city. The shoot primordia was derived form the shoot apex of *A. capillaris* according to the following method described by Tanaka and Ikeda[4]. The uppermost portions of about 10 mm were removed from the stem of the plant and sterilized with 0.1% benzalkonium chloride, 1% sodium hypochloride solution and 70% alcohol. Domes of the shoot tips including 2-3 leaf primordia were removed from the stems. These domes were cultured in Murashige-Skoog medium (MS)[11] plus α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). The domes were planted in liquid medium and shaken slowly at 2 cycles/min. on a gyrated dram. The shoot primordia obtained was subcultured at 2 week intervals. All cultures were maintained at about 22-25°C under 2,000-8,000 lux illuminated by a fluorescent lamp.

The identification of capillen (1) from the shoot primordia of *A. capillaris*. To identify capillen, fresh shoot primordia of *A. capillaris* (25 g) was crashed using liquid nitrogen and subjected to steam distillation for 10 hours using the distillation apparatus. The essential oil (42.5

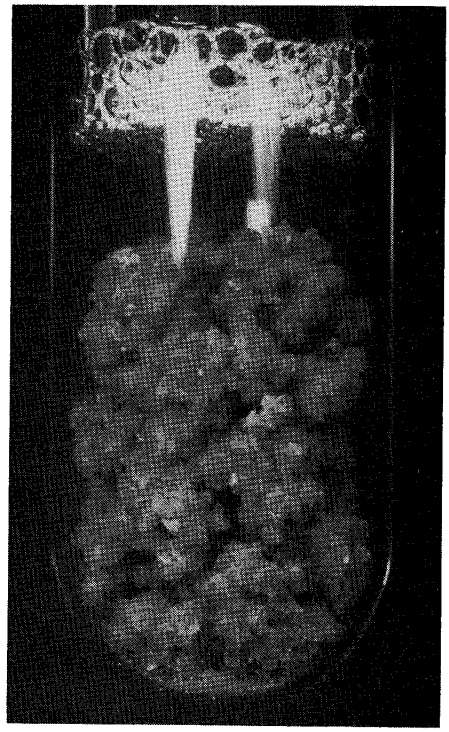
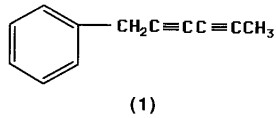


Fig. 1 The shoot primordia derived from shoot apex of *Artemisia capillaris*.

mg) was obtained by the extraction of the distillate with Et_2O and by the evaporation of the solvent. Capillen (1) and the remaining minor components were identified by comparison of those GC-MS with those of a previous paper[10].

Results and Discussion

The shoot primordia of *Artemisia capillaris* was obtained from the shoot apex of the plant according to the method of Tanaka and Ikeda[4]. (**Fig. 1**). The domes were cultured in sterilized Murashige-Skoog (MS) medium[11]with 6-benzylaminopurine (BAP) and α-naphthaleneacetic acid (NAA) added in various combinations to make 12 different media except for BAP 4 mg/*l* and NAA 4 mg/*l*. The optimum growth for the shoot primordia was found to be MS medium containing 6-benzylaminopurine (BAP) 2 mg/*l* without 1-naphthaleneacetic acid (NAA). The cultures were maintained at about 22-25°C under illumination by a fluorescent lamp (2, 000-8, 000 lux) for two weeks. The growth rate of the shoot primordia is about doubled with 2 weeks incubation. Steam distillation of the shoot primordia (BAP 2 mg/*l* without NAA) produced the essential oil. Analyses of the oil by capilary GC-MS revealed that the main component was capillen (**1**) and the remaining minor components were present in the same composition reproted in an earlier paper[10]. The mass



(1)

spectrum of (**1**) exhibited an intense peak at m/z 154 [M^+] and a fragment peak at 139 due to the ion peak losing a methyl group from the molecular ion.

In this work, we also induced the callus from the stem of the plant and analyed the resulting oil by GC-MS. To our surprise no capillen was detected in the callus and this result suggested that the

essential oil pattern of the callus is different from that of the whole plant. In this respect it may be pointed out that shoot primordia gave the same pattern of essential oils as the plant leaves while the stem callus did not produce any essential oils.

In conclusion, we showed that shoot primordia is very useful for the study and isolation of secondary metabolites from plants.

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《和文要約》

カワラヨモギの苗条原基でのキャピレンの生産

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カワラヨモギの成長点を培養して苗条原基を作成し，その苗条原基でのキャピレンの生産を調べた．