當歸組培地上部的光暗生長效應

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摘要

組織培養基的調控，可以讓體胚長成的當歸植物只具備莖上部而不長出根部，此現象與正常狀態下植物的生長和消費者所使用的地下根部不同。吾人利用此現象，將已分化成植株地上部的當歸，分別置於光與暗的培養下，調查光於生成當歸醫藥成分 ligustilide 和 butylidene phthalide 的消長關係。實驗結果發現，地上莖部可於暗中合成所有的成分，唯其含量遠低於在光中生長的地上部。由此證明光於當歸植物地上部合成重要醫藥成分中 ligustilide 和 butylidene phthalide 所扮演的角色。

關鍵詞：當歸、體胚、組織培養、Ligustilide、Butylidene phthalide

Effect of Light on Ligustilide and Butylidene Phthalide Production by *Angelica acutiloba* K. Rootless Plants

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Abstract

Effect of light during the culture of regenerated *Angelica acutiloba* K. rootless plants from somatic embryos were studied. The contents of medicinally important chemical constituents –ligustilide and butylidene phthalide by means of gas chromatography (GC) were reported. Rootless plants cultured under dark condition (DRP) could synthesize almost the whole profile of compounds as the rootless plants cultured in photoperiod condition (PRP). However, the contents of ligustilide and butylidene phthalide in DRP are much lower than that in PRP.

Key Words：*Angelica acutiloba*, Somatic embryo, tissue culture, ligustilide, butylidene phthalide

I. INTRODUCTION

The root of *Angelica acutiloba* K. (Chinese name = Duggei), a perennial herb, are well-known crude drug used in Chinese traditional medicine as an analgesic, emmenagague, and tonic. For a long time, people take only roots as folk medicine or experimental materials to examine the pharmacologically active constituents (1,2,3) and prepare the essential oil (4). No attempts have ever been made to investigate the effects of light and aerial parts on the medicinally important constituents such as ligustilide and butylidene phthalide. The difficulties in examining the effect of light and the aerial are that one can't grow the plants without light and the aerial parts of plants can't grow independently without the roots. That is, plants or the aerial parts can't grow without light and roots. In the case of *A. acutiloba* we have shown that numerous somatic embryoids capable of developing into plantlets (5). Also we found that the embryoids would not protrude their roots in the medium inducing callus; nevertheless aerial parts (stems and leaves) grow vigorously in both dark and photoperiod condition. These rootless plants grown in darkness (DRP) or photoperiod condition (PRP) are now used in this report to investigate the role of aerial parts and light on the pharmacologically active constituents of *A. acutiloba*, namely: ligustilide and butylidene phthalide.

II. MATERIALS AND METHODS

Plant material and culture method

Plants of *A. acutiloba* K. were kindly given by Taiwan Agricultural Research Institute and grown in the glasshouse under natural conditions. The leaves, stems and roots were dissected from the healthy plants (ca. 30cm height), washed in running water thoroughly, surface sterilized with 2.5% sodium hypochlorite for 7 min then rinsed in sterile distilled water, thrice. These explants (leaf, stem and root) were cut into pieces (ca. 0.5cm long) and cultured on Murashige and Skoog's (MS) medium (6) containing 0.6 μM 2.4-dichlorophenoxyacetic acid (2,4-D), 0.05 g / l ascorbic acid, 1.05 g / l citric acid and 30 g / l sucrose. The medium pH was adjusted to 6.0 prior to addition of 2 g / l gelrite and sterilized by autoclaving at 121℃ for 15 min. These cultures were maintained in such medium and incubated at 25±1℃ in the dark. Callus tissue formed on stem explants resulted in numerous multiple shoots. With no root protruding, the multiple shoots would grow well in the same medium under 16 / 8 h, 2000 lux, photoperiod (PRP) or in darkness (DRP).

Flavor isolation of PRP and DRP

131.90g PRP and 159.33g DRP were separately blended with 1000 ml distilled water for 2 min in a Waring Blender. Volatile constituents were extracted by using 50 ml petane ether for 3 hr in a Likens-Nickerson apparatus using steam as heating sourse. 5 ml octadecane stock solution (0.1558 g / 1000 ml petane) was added as internal standard. Water in extracts were removed with anhydrous Na_2SO_4 and concentrated to minimal volume by using a spinning band distillation apparatus.

Gas chromatography

Gas chromatography was conducted on a Varian 3400 equipped with a flame ionization detector (FID). A 30 m × 0.25 mm fused silica column (DB- WAX J & W scientific) was used. The over temperature was programmed from 50 to 210℃ at 2 ℃/ min. The carrier gas was hydrogen at a flow rate of 1.069 ml / min and the method of injection is split injection 10 : 1. Values reported were from the average of two analyses. The linear retention indices of the volatile compounds were calculated with n-paraffin (C8-C25).

Gas chromatography-Mass spectrometry

GC-MS was conducted with a Finnigan mat ITD 700 Series system. The gas chromatography was installed with a fused silica capillary column (DB-WAX J&W scientific 30 m × 0.25 mm). Operational parameters were as followed: carrier gas, hydrogen; ionization voltage, 70 eV; ion source temperature, 210℃.

III. RESULTS AND DISCUSSION

Callus was induced from stem explants of *A. acutiloba* K. 2 to 3 weeks after inoculating on MS medium supplemented with 0.6 μM 2,4-D, 0.05 g/l ascorbic acid and 1.05 g/l citric acid. The cultured stem segments produced off-white to pale

yellow, compact callus. Prolonged culturing of the callus in the same medium resulted in numerous multiple shoots in the darkness. When these multiple shoots were transferred to a growth chamber with 16/8 h photoperiod, they turned green within 2 days. With no root protruding, the multiple shoots would grow well in the same medium under photoperiod (PRP) or in darkness (DRP). The PRP (Fig. 1) would grow vigorously with the green stems and leaves similar to the intact plant with roots. The DRP (Fig. 2) are off-white to pale yellow in color and much thinner than PRP. Also the growth rate of DRP is much slower than of PRP. Figure 3 and 4 show the capillary gas chromatograms of volatile components of PRP and DRP respectively. The ligustilide and butylidene phthalide in PRP and DRP are show Table É. To compare these figures, the amount of ligustilide and butylidene phthalide in PRP are in turn 39 and 2.2 times higher than that in DRP. The ratio of ligustilide to butylidene phthalide varies from 0.25 in DRP to 4.50 in PRP. These results indicate that light play an important role in the synthesis of ligustilide and butylidene phthalide by the aerial parts of *A. acutiloba*, although they also can be synthesized in darkness. It had been reported that the chemical structures of ligustilide and butylidene phthalide are different only at one double as shown in Figure 5 (4). And the ratio between these two compounds in our experiment shifted from 0.25 in DRP to 4.50 in PRP. It is strongly suggested that light may provide an environment, which favor the conversion of ligustilide from butylidene phthalide.

There have been many papers deal with the effect of light on the production of secondary metabolites by plant cell cultures (7, 8, 9). These results are not apparent and variable. It is the first time to use tissue culture rootless plant (stems and leaves) to investigate the influence of light on pharmacologically active compounds. Taken for granted that the ligustilide and butylidene phthalide are root derived pharmacologically active compounds. No one has reported these principles are synthesized by roots itselves or translocated from aerial parts. Taking into consideration that under influence of light the content at ligustilide and butylidene phthalide in PRP are 39 and 2.2 times that in DRP, it is suggested that of least the major synthesis site of ligustilide should take place in the aerial parts of *A. acutiloba*. Therefore, on studying the secondary metabolites production by plant cell cultures, we should try to replace the light stimulation with application of different plant hormones in the cell cultures.

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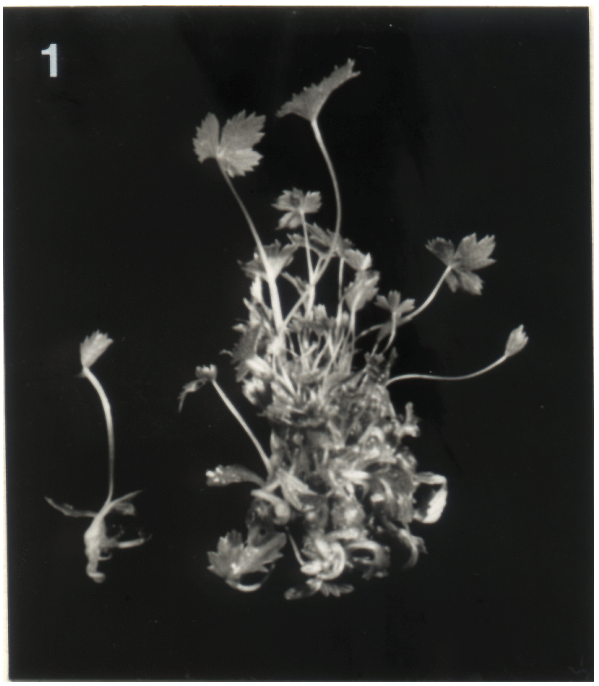


Fig. 1. Tissue culture rootless plants cultured in photoperiod condition (PRP)

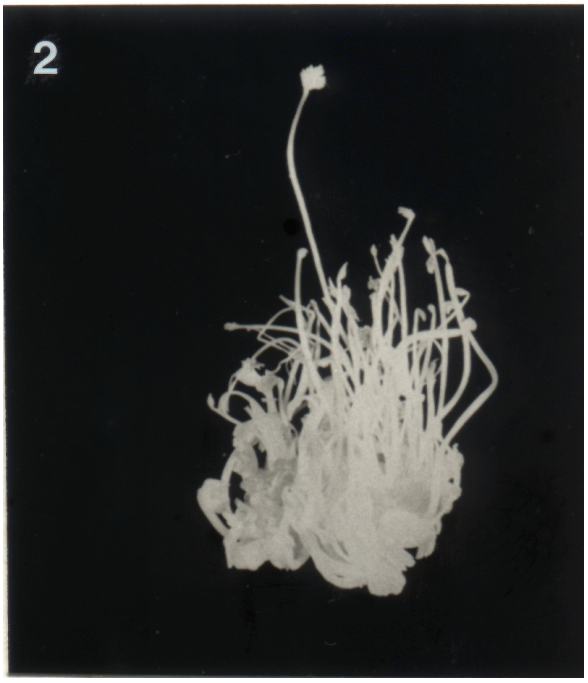


Fig. 2. Tissue culture rootless plants cultured in darkness (DRP).

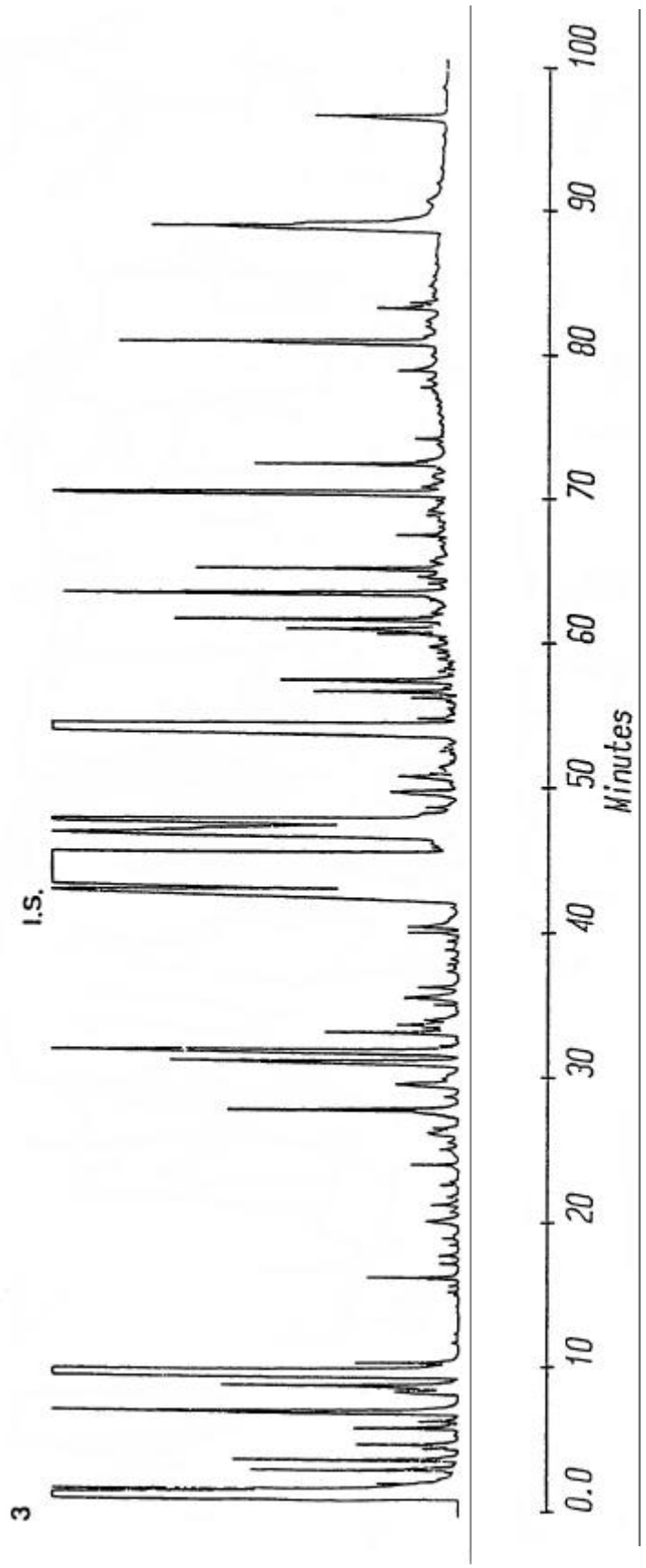


Fig. 3. Capillary gas chromatogram of volatiles of DRP isolated in a Likens-Nickerson distillation apparatus.

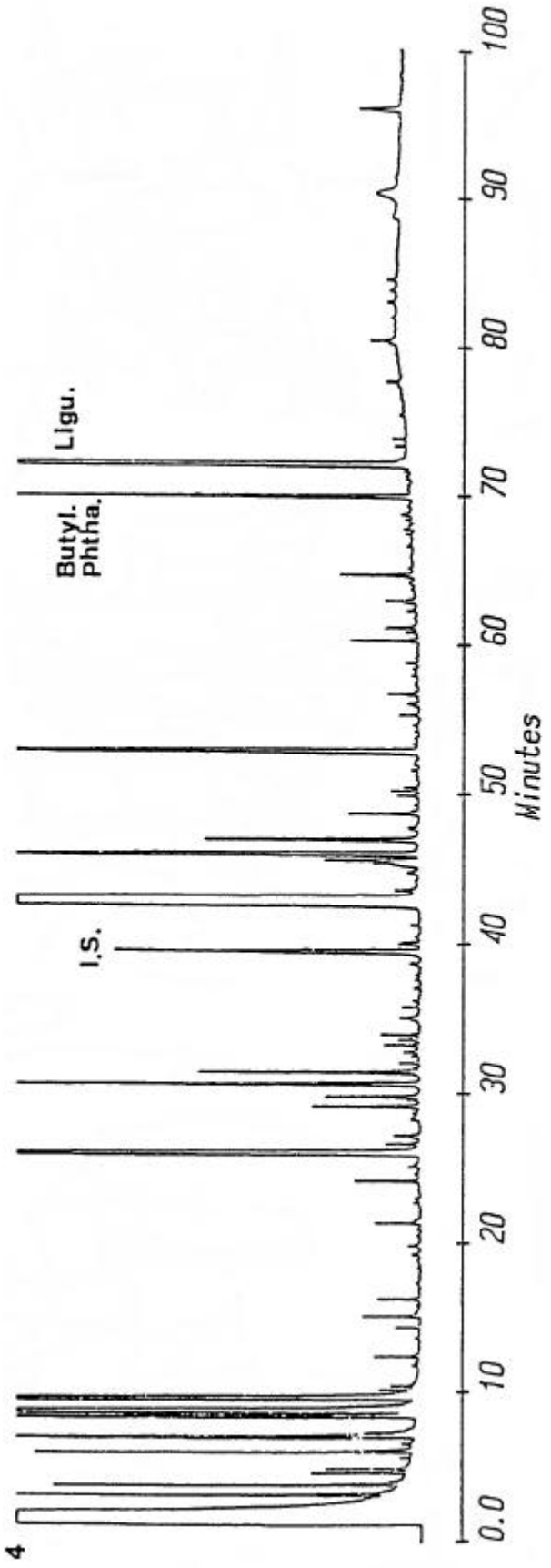
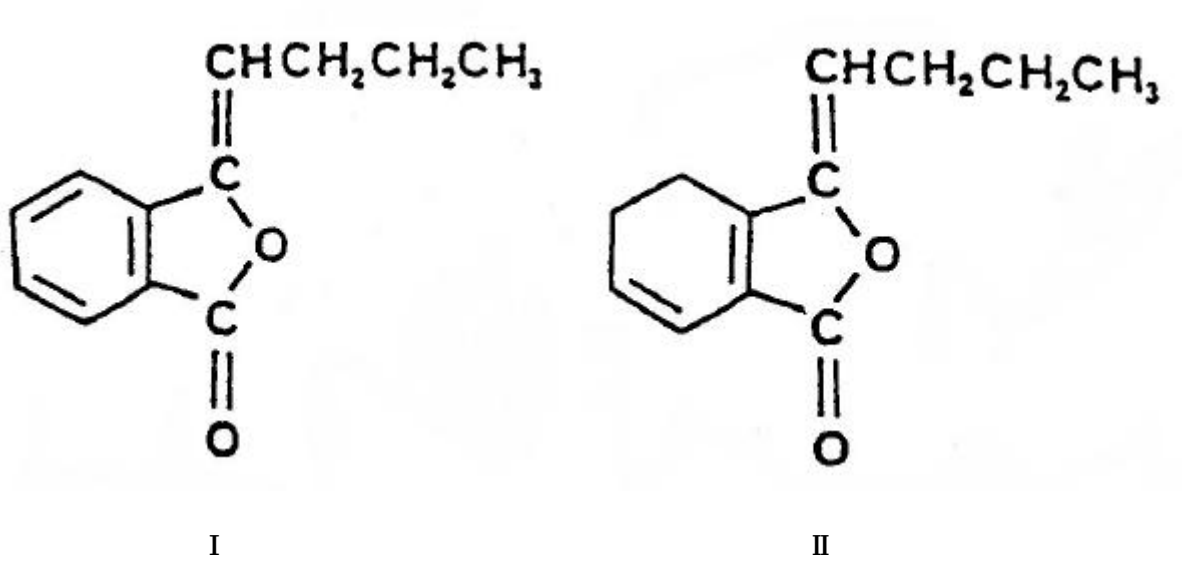


Fig. 4. Capillary gas chromatogram of volatiles of PRP isolated in a Likens-Nickerson distillation apparatus.



I Ⅱ

Fig. 5. Chemical structure of butylidene phthalide (I) and ligustilide (Ⅱ).

Table É. Amount of ligustilide and butylidene phthalide in rootless plant cultured under darkness (DRP) and photoperiod (PRP)

	rootless plants in photoperiod (PRP) (A)	rootless plants in darkness (DRP) (B)	ratio (A/B)
ligustilide*(C)	249.0**	6.4	39
butylide phthalide*(D)	55.3	25.2	2. 2
Ratio (C/D)	4.50	0.25	

* Average of two experiment.

* * All yields (μg/g fresh wt.) were calculated with octadecane as internal standard.