

## Caffeic Acid Esters in Tissue Cultures of *Heliotropium pervianum*

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*Heliotropium pervianum* L. (Boraginaceae), originated in South America (Peru or Ecuador), is one of the most popular perennial herbs for potted plants and garden ornamentals, presenting sweet fragrance from the flowers. The flavors, resembling vanilla essence, were once used as "heliotrope perfume". Recently, Hisano *et al.*<sup>1)</sup> analyzed the major volatiles and three compounds, benzaldehyde, benzyl acetate and *p*-anisaldehyde were identified. In comparison with several studies on flavor constituents, detailed chemical studies on the other secondary metabolites of this plant have not been done. In the present experiment, some tissue cultures (shoot and adventitious root) of this plant were established and the production of three antioxidant phenols, rosmarinic acid (RA)<sup>2)</sup>, lithospermic acid (LA)<sup>3)</sup> and lithospermic acid B (LAB)<sup>4)</sup>, the most common caffeic acid esters occurring in Labiatae and Boraginaceae, in these cultures and the intact plant was investigated.

The seeds (purchased at a market) were sterilized by the usual method (with 2% NaOCl, 8 min.) and germinated aseptically on 1/2 Murashige-Skoog (MS) solid medium<sup>5)</sup> (2.5 g/l gelrite) in the light (16 hr photoperiod/day, 5000 lux) at 25°C. The axenic plants were transferred and maintained on MS solid medium in the same culture conditions above. The roots of the plantlets *in vitro* were also cut, transferred into MS liquid medium (50 ml in 100 ml flask) containing 3 mg/l IAA to establish the adventitious root cultures. The root cultures were subcultured at 2 months intervals on a rotary shaker (100 rpm) in the dark (25°C) for over a year.

Caffeic acid esters (RA, LA and LAB) contents in *H. pervianum* tissue cultures (shoots and adventitious roots) and the intact plants were analyzed by HPLC. The lyophilized samples (mixture of three populations, *ca.* 20 mg) were mashed and extracted with EtOH (2 ml) for 16 hr at room temperature. Each extract, after filtration through a Millipore filter (0.5 µm), was subjected to HPLC analysis; column: Shim-pack CLC-ODS (6.0 mm × 150 mm), mobile phase: 85 mM H<sub>3</sub>PO<sub>4</sub>-CH<sub>3</sub>CN (5 : 1 → 1 : 4, in 25 min.), flow rate: 1.0 ml/min., column temperature: 35°C, detection: A 285 nm, R<sub>t</sub> (min.): RA (16.0), LA (16.6) and LAB (18.7).

RA, LA and LAB were produced in all tissues examined, *i. e.*, the intact plant (leaves and roots collected in July), *in vitro* plants and the adventitious roots (cultured for 4 weeks) (Table 1). In most herbs in Labiatae and Boraginaceae, the contents of LA and LAB are generally lower

**Table 1.** Contents of rosmarinic acid(RA), and lithospermic acid (LA) and lithospermic acid B(LAB) in *Heliotropium pervianum* (% as dry weight).

Materials	RA	LA	LAB
intact plant (leaves)	0.60	1.06	0.34
(roots)	0.77	1.39	0.04
<i>in vitro</i> plant (leaves)	3.60	0.57	0.42
adventitious root	0.04	0.42	0.42

(sometimes not detected) than that of RA (data not shown here). Therefore, it is noteworthy that *H. pervianum* is relatively rich in high molecular phenolics such as trimeric (LA) and tetrameric (LAB) derivatives of caffeic acid, being expected to be as important natural antioxidants.

Although in *H. pervianum* only the flavor constituents have attracted attention at this time, this finding clearly demonstrates the necessity (with goodly expectation) of the reevaluation of the aromatic and medicinal use of this horticulturally popular herb.

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### 《和文要約》

ヘリオトロープの組織培養とコーヒー酸エステル類の生産

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ヘリオトロープ (*Heliotropium pervianum* L.) の植物体, または茎葉および不定根培養組織において, コーヒー酸エステル類である rosmarinic acid, lithospermic acid および lithospermic acid B の生産を確認した。これらのフェノール類は, 重要な天然抗酸化化合物であり, 観賞用ハーブとして人気の高い本植物の新しい利用の可能性が考えられる。