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ROSMARINIC ACID FROM *LAVANDULA VERA MM* CELL CULTURE

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Key Word Index—*Lavandula vera*; Lamiaceae; plant cell culture; rosmarinic acid; caffeic acid.

Abstract—Rosmarinic acid was isolated as the main phenolic component of *Lavandula vera MM* cell culture. It was identified by means of TLC, HPLC, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In vitro cultured cells of *Lavandula vera*, which were being examined as a potential source of biotin [1, 2], were found out to synthesize and excrete a blue pigment into the cultural medium [3–5]. This was identified by Banthorpe *et al.* [6] as an enol ester of caffeic acid. We also obtained cell cultures of this species, using *L. vera* plants that produce phenolic compounds with antimicrobial and antioxidant activities [7]. This paper deals with the identification and quantitative determination of the main phenolics found in this cell culture.

RESULTS AND DISCUSSION

A methanolic extract from fresh cells of *L. vera MM* grown in LS medium [8] for nine days was used. The UV spectrum showed maxima between 280 and 340 nm, indicative of the presence of phenolic acids. Rosmarinic (RA), caffeic (CA), *p*-coumaric and ferulic acids were identified in the extracts by TLC. The extracts contained a comparatively large amount of RA, smaller amounts of CA and traces of *p*-coumaric and ferulic acids (Table 1).

Table 1. Content of phenolic acids in cell biomass of *Lavandula vera MM*

Compound	Content* (mg g^{-1} dry wt)
Rosmarinic acid	5.15±0.73
Caffeic acid	0.37±0.02
p-Coumaric acid	Trace
Ferulic acid	Trace

*Data represent the mean values of five different flasks and standard deviation.

Banthorpe and co-workers [6, 9] reported that the main phenolic compound synthesized by *L. vera* callus culture was a blue pigment, which they identified as a complex of Fe(II) with the isomers of an enol ester formed by condensation of dopaldehyde with caffeic acid. Our *L. vera MM* strain, however, produced mainly RA. The latter was isolated from ethylacetate extracts of cell biomass as a yellowish powder (see Experimental) and its identity was confirmed by means of HPLC with a photodiode array detector, ^{1}H NMR, ^{13}C NMR and mass spectroscopy [10–12]. This is the first report on the synthesis of RA by a *L. vera* cell culture, although RA is known to be synthesized by cultures of other species of the Lamiaceae [13–15].

EXPERIMENTAL

Culture method. Callus culture strain *L. vera MM* was originally derived from the leaves of *L. vera* plants in LS basal agar medium [8] containing 0.2 mg l^{-1} 2,4-dichlorophenoxyacetic acid and 3% sucrose. It was subcultured on the same medium at 25–28° in the dark for more than 3 years at 2 week intervals. *L. vera MM* cell suspension cultures were grown using the same liquid medium [8] in Erlenmeyer flasks with 1/5 net vol. on the shaker (11.6 rad s^{-1}). It was subcultured at intervals of 1 week for 1 year. Cell biomass was harvested for chemical analyses 9 days after inoculation.

Extraction and isolation. Cells (20 g fr. wt) were extracted with MeOH (3 × 100 ml). The combined extracts were evapd *in vacuo* and the dry residue was extracted again with EtOAc (2 × 50 ml). The EtOAc extracts were evd to dryness. The residue was dissolved

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in a minimum vol. of MeOH and subjected to chromatography.

TLC and HPLC. MeOH and EtOAc extracts were fractionated by TLC (silica gel GF 254) using the following systems: (1) toluene–EtOAc–HOAc–MeOH–H_2O (20:60:10:20:3); (2) toluene–EtOAc–HOAc (4:5:2) and (3) $CHCl_3$–MeOH–H_2O (30:11:2).

RA was isolated from the EtOAc extract by prep. TLC on silica gel GF_{254} using solvent system 1.

HPLC analyses were carried out on a Perkin Elmer liquid chromatograph with UV detection. The conditions were: analyt. column PEC_{18} (10 μm, 250 × 4.6 mm i.d.); mobile phase: 2% HOAc (A) and 2% HOAc–MeCN (7:3) were used and a linear gradient 70–30% A over 40 min was applied; flow rate: 1 ml min^{-1}; pressure: 10 MPa; detection at 280 nm. Authentic samples of RA, CA and *p*-coumaric and ferulic acids were used as markers. The external standard method was used for quantitative determination of the analysed compounds.

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