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ACTAS

Effect of phenylalanine addition to *Pimpinella anisum* hairy root cultures

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1. INTRODUCTION

Hairy root cultures of *P. anisum* (SANTOS *et al.* 1998) produce an essential oil that is mainly characterized by the presence of large amounts of *trans*-epoxypseudoisoeugenyl 2-methylbutyrate. This compound belongs to a rare group of phenylpropanoids that have been detected only in *Pimpinella* species (KUBECZKA *et al.* 1976; SALEM & CHARLWOOD 1995). The proposed biosynthetic pathway of epoxypseudoisoeugenyl 2-methylbutyrate involves L-phenylalanine as starting precursor (REICHLING *et al.* 1995). Therefore, the effect of L-phenylalanine addition on the growth and essential oil production of *P. anisum* hairy root cultures was investigated.

2. EXPERIMENTAL

2.1 Hairy Roots

Hairy root cultures of *P. anisum* were maintained in darkness in SH medium (SCHENK & HILDEBRANDT, 1972) at 24°C on orbital shakers (80 r.p.m.), as previously reported (SANTOS *et al.* 1998). L-Phenylalanine in aqueous solution (0.2M) was added to each culture flask, 16 days following subculture, to obtain a final substrate concentration of 1 mM or 3 mM. Cultures without substrate addition were used as control. The hairy roots growth was measured by the dissimilation method (SCHIRPISIMA *et al.* 1990). The results are the average of three separate experiments.

2.2 Essential Oil Isolation, Gas Chromatography and Gas Chromatography-Mass Spectrometry

The hairy root cultures were harvested periodically during the growth cycle (8, 24, 48, 72 and 96 hours after L-phenylalanine addition) and an internal standard (menthol-dichloromethane, 0.08% w/v) was added, prior to essential oil isolation, in a concentration of 16 mg/l. The essential oils were isolated by distillation-extraction, for 3h, using a Likens-Nickerson-type apparatus with *n*-pentane as solvent, and analysed by GC and GC-MS as previously reported (SANTOS *et al.* 1998). The results are the average of two injections from each sample of the three separate experiments.

3. RESULTS AND DISCUSSION

The addition of 1 mM L-phenylalanine to the hairy root cultures of *P. anisum*, Fig. 1, did not affect significantly the hairy root growth, but the addition of 3 mM L-phenylalanine appeared to induce a slight dissimilation increase, Fig. 2.

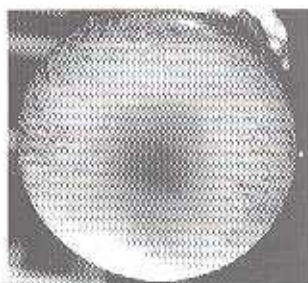


Fig. 1

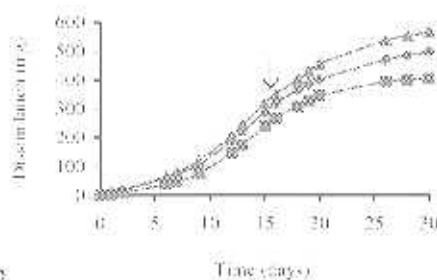


Fig. 2

Figures 1 and 2: Figure 1 – Hairy root culture of *Pimpinella anisum*, Figure 2 - Dissimilation growth curves of *Pimpinella anisum* hairy root cultures without (n- control) and with the addition of L-phenylalanine (n- 1mM, s- 3mM) at day 16 (arrow).

No direct relationship was seen between the addition of L-phenylalanine and the concentration of the main component of the oil, *trans*-epoxypseudoisoeugenyl 2-methylbutyrate, Fig. 3.

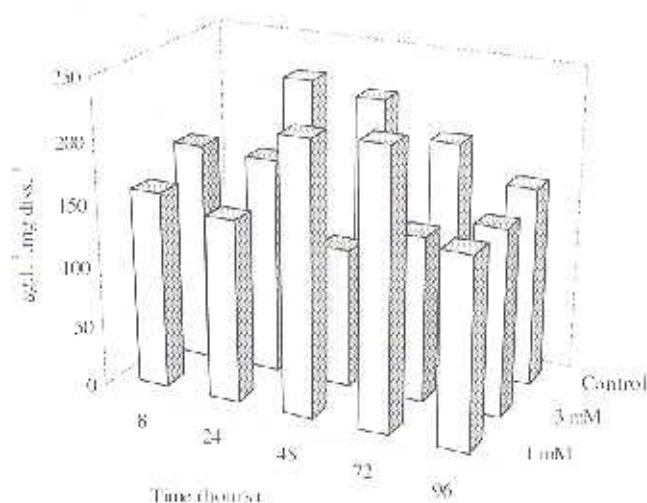


Figure 3: Time-course variation of the main constituent of the oil, *trans*-epoxypseudoisoeugenyl 2-methylbutyrate, in the control hairy root cultures and with the addition of L-phenylalanine 1mM and 3mM.

The other main components of the constitutive volatiles of the *P. anisum* hairy root cultures were always detected, in varying amounts, independently of the added amount of L-phenylalanine, Fig. 4-6. A slight increase in the concentration of *trans*-pseudoisoeugenyl 2-methylbutyrate seemed to be induced by the addition of increasing amounts of L-phenylalanine, Fig. 4-6.

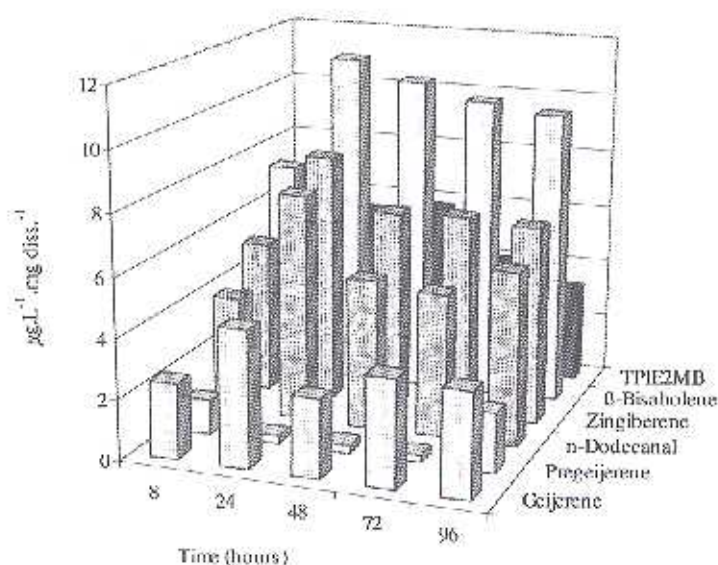


Figure 4: Time-course variation of some of the other constitutive volatiles of *Pimpinella anisum* hairy root control cultures (TPIE2MB – *trans*-pseudoisoeugenyl 2-methylbutyrate).

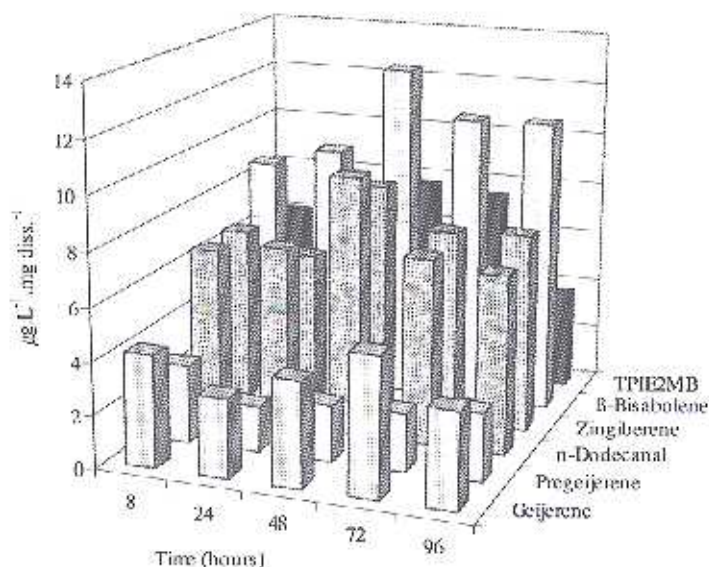


Figure 5: Time-course variation of some of the other constitutive volatiles of *Pimpinella anisum* hairy root cultures, after the addition of 1mM of L-phenylalanine at day 16 (TPIE2MB = *trans*-pseudoisoeugenyl 2-methylbutyrate).

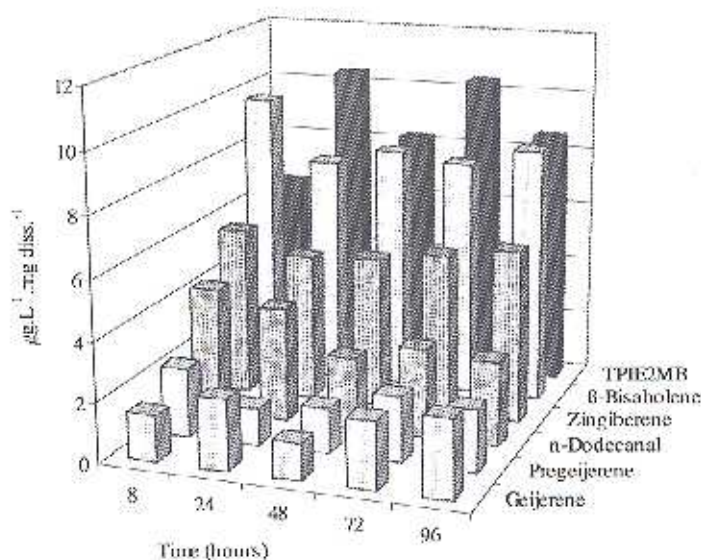


Figure 6: Time-course variation of some of the other constitutive volatiles of *Pimpinella anisum* hairy root cultures, after the addition of 3mM of L-phenylalanine at day 16 (TPIE2MB = *trans*-pseudoisoeugenyl 2-methylbutyrate).

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