Selective Biotransformation of Monoterpenoids by Cell Suspensions of *Petroselinum crispum*

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Cell suspension cultures of *Petroselinum crispum* (Mill) Nyman cultivars "Paramount" and "Plain-leaved" were capable of biotransforming exogenously supplied geraniol largely into nerol and minor quantities of neral and geranial. Maximal conversions into nerol (32–36%); and neral: <1% (cult. "Plain-leaved"), <5% (cult. "Paramount") were usually recorded by 24 h. Over the incubation period a low proportion of the substrate, 32–40% was involved in biotransformation. Cyclic and other acyclic monoterpenoids were not biotransformed.

Introduction

Plant cell cultures offer a vital tool for biotransformation studies of substrates of widely divergent chemical nature, and this has been broadly documented [1, 2]. Ever since the initial reports [3, 4] of the ability of suspensions of certain *Mentha* cell lines and *Cannabis* cultures to bioconvert different chemicals, wide ranging studies in this area have been undertaken. However, despite the extensive work on cultures of parsley [5, 6] there is no information on the metabolism of the monoterpenoids by these cultures.

In pursuance of our investigations of the biosynthesis of volatile constituents by parsley cultures, we studied the metabolism of a variety of monoterpenes using parsley cell suspensions, and hereby present the details of the selective conversion of such compounds by cells of *P. crispum* cultivars "Paramount" and "Plain-leaved" cultured *in vitro*.

Materials and Methods

Callus induction

Seeds were surface sterilized with 10 ml aliquots of 100 ml, 30% H_2O_2 containing two drops of Tween 80 for 2 min, and germinated in sterile petri-dishes containing sterile distilled water, in continuous darkness at 25 °C. Callus was induced from germinated seedlings aseptically explanted onto 50 ml B5 [7] agar medium supplemented with 3% sucrose, 5% coconut water, 0.1 ppm kinetin, 1.0 ppm 2,4-dichlorophenoxyacetic acid; and incubated under a regime of 12 h light (Thorn white flourescent lamps, 6000 Lux) and 12 h darkness. Callus cultures were subcultured every six weeks (= one generation).

Suspension cultures

Cell suspensions were initiated from the fifth and tenth generations respectively of actively growing callus tissues of cult. "Plain-leaved" and "Paramount", by transferring several friable lumps into B5 liquid medium having the same composition as the above, but without agar. The cultures were maintained by 1:2 dilution every 6 weeks at a shaking speed of 80 rpm. The second generation of cell suspensions were maintained for three weeks prior to biotransformation experiments.

Precursor feeding and extraction

250 ml Erlenmeyer flasks containing 100 ml suspension cultures, PCV 53% were inoculated in duplicate with 0.25 ml 1.2% (= 30 ppm) of filter-sterilized ethanolic solutions of pulegone and menthol (Aldrich Chemical Co. Ltd., Wisconsin), menthone (Ralph N. Emanuel Ltd., England), geraniol (Koch-Light Laboratory Ltd., England) and citronellol (Sigma Chemical Co. Ltd., St. Louis, U.S.A.) using a Millipore filter (0.45 μm pore size). Incubation was carried out in continuous light for 24 h. Over the incubation period, cultures were sampled, prechilled for 30 min and vacuum filtered. Prechilled cells were freeze-dried for 12 h using Edwards Modulyo EF4 freeze dryer, and extracted in redistilled dichloromethane. The filtrates were similarly extracted for 2 h by shaking. The dichloromethane extracts for each analysis were reduced under nitrogen to 200 μl prior to GC analyses.

Analysis of components by GC & GC/MS

1 μl quantities of the concentrated extracts were analyzed on a Hewlett-Packard 5890 GC instrument equipped with an on-column injector and FID. Regular analyses were performed on a capillary column (fused silica carbowax 20 M; 25 m × 0.32 mm i.d.) under the following conditions: temp.prog. 50–180 °C at 5 °C/min; detector temp. 285 °C; carrier gas N_2 at 2 ml/min. Authentic samples of the

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different substrates, their conversion products and an appropriate internal standard, citronellal or pentadecane were distinctly separated under the conditions specified above. GC/MS analysis was performed on a Kratos MS25 instrument, equipped with a DS-55 computer data output under similar conditions, except with He as the carrier gas. The all-glass jet separator interface operated at 250 °C.

Quantitative estimations of the products were performed using the appropriate internal standards and an average of duplicate determinations gave each time point.

Results and Discussions

Following the cessation of secondary product synthesis after the fifth and tenth generations respectively by callus cultures of cult. "Plain-leaved" and "Paramount" (unpublished results), a variety of monoterpenoids were exogenously fed into cell suspensions of these two cultivars to ascertain the activity of the enzymes of the terpenoid pathway.

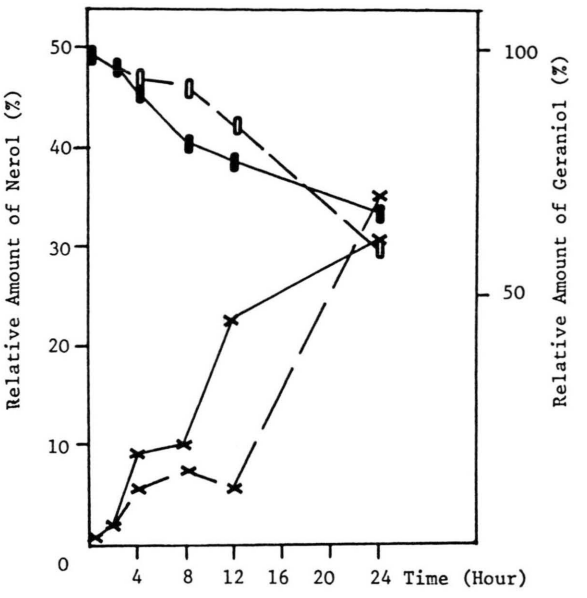

Fig. 1. Biotransformation of geraniol by parsley cultures. Production of monoterpenes by cult. "Plain-leaved": ×——× nerol, ●——● geraniol; cult. "Paramount": ×– – –× nerol, ○– – –○ geraniol.

Geraniol bioconversion

About 32–40% of geraniol was converted by cultures of both cultivars into nerol, neral and geranial during 24 h. Maximal amounts of nerol were extracted from both cultures at 24 h (Fig. 1). The yield of neral compared with its corresponding alcohol was generally low, <5% (Fig. 2). Although this aldehyde was undetected from cultures of cult. "Plain-leaved" at the onset of substrate feeding (Oh), its yield was about 14 and 10 times greater in cultures of cult. "Paramount" at 12 and 24 h respectively. The yield of geranial fluctuated throughout the incubation period, but maximal at 8 and 12 h for cult. "Plain-leaved" and "Paramount" respectively (data not shown). The higher transformation capacity into neral (14-fold at 12 h) and nerol (up to 12 h, 4-fold at 12 h) demonstrated by cultures of cult. "Paramount" and "Plain-leaved" respectively is in line with the reported differences [4, 8] in biotransformation abilities of *Mentha* cell lines. The kinetics of disappearance of geraniol from cultures and the concomitant formation of nerol in particular, followed a characteristic pattern that is frequently described for the bioconversion of monoterpenes [9, 10].

Metabolism of the substrate and the transformation products [8, 11] cannot be ruled out as contribut-

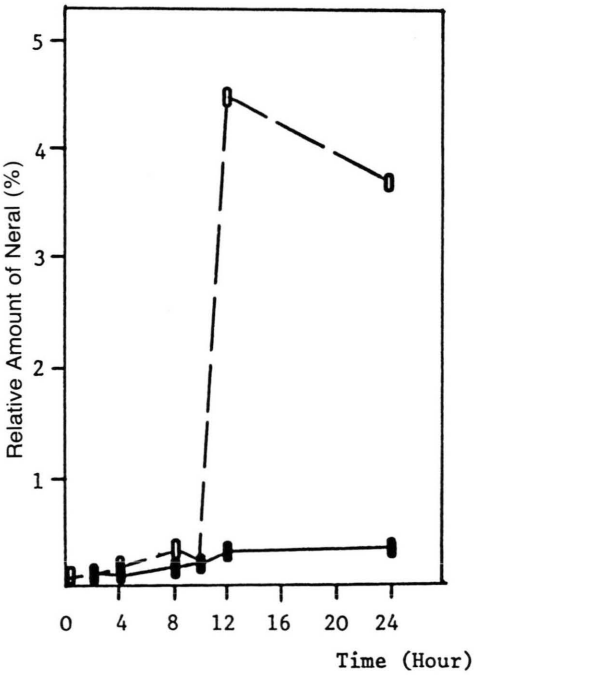

Fig. 2. Biotransformation of geraniol by parsley cultures. Production of neral by cult. "Plain-leaved": ●——●; cult. "Paramount": ○– – –○.

ing to the low yields of the neoformed terpenic substances in parsley cell suspensions.

Interconversion of geraniol into nerol and their oxidations has been attributed to an alcohol oxidase [3]. This, and the detection of the conversion products in both the cells and media suggest the presence of these enzymes in parsley cultures. Since the conversion products of geraniol could not be detected in the culture media without cells, it is reasonable to expect biotransformation to have occurred.

Bioconversion of ketones and other alcohols

Exogenous cyclic ketones and alcohol, and the acyclic citronellol were separately recovered from cultures of both cultivars over the 24 h period, without any detection of their possible conversion products. The time courses for the disappearance of pulegone and citronellol can be seen in Fig. 3. The inability of parsley cultures to metabolize the exogenous ketones and menthol characteristic of *Mentha* spp., and the acyclic citronellol is not unique. Apart from certain cell lines of *Mentha* spp. [4, 8], successful metabolism of these compounds has not been demonstrated in other plant cultures [12].

Geraniol and its conversion products, and the unbiotransformed monoterpenic substrates could not be extracted from the cultures after 24 h. A similar disappearance of certain substrates and products from cultures after 24 h has been described elsewhere [8].

These results indicate the presence of relevant biosynthesizing enzymes in cell suspensions of parsley which are capable of selective catalyzation of biotransformational processes. We have also demonstrated the influence of different cultivars of parsley cultures in the bioconversion of geraniol. As a first report on biotransformation studies using parsley cultures, our findings suggest the suitability of these cell suspensions for further metabolic studies and this possibility is currently being investigated.

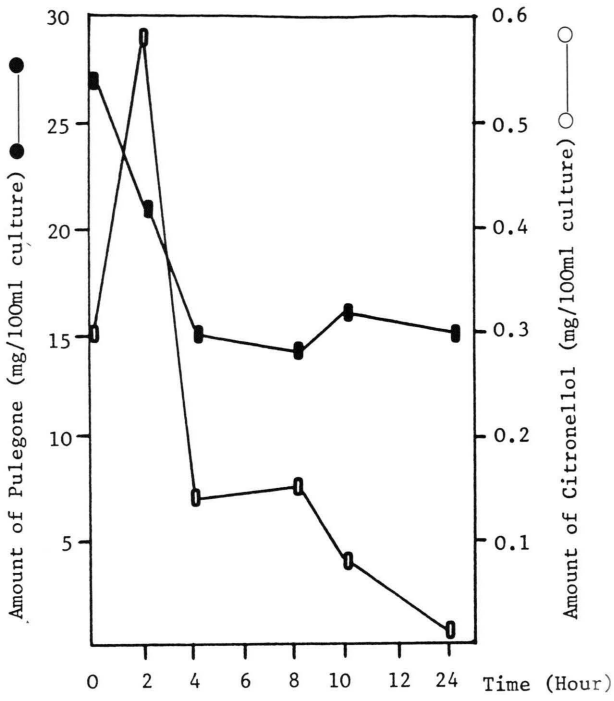

Fig. 3. Kinetics of disappearance of monoterpenes from *P. crispum* cult. "Plain-leaved" cultures.

[1] W. G. W. Kurz and F. Constabel, in: Adv. in Applied Microbiology (P. Perlman, ed.), **Vol. 25,** pp. 209–240, Academic Press, New York 1979.
[2] E. Reinhard and A. W. Alfermann, in: Adv. in Biochemical Engineering (A. Feichter, ed.), **Vol. 16** (Plant Cell Cultures), pp. 49–83, Springer Verlag, Berlin 1980.
[3] H. Itokawa, K. Takeya, and S. Mihashi, Chem. Pharm. Bull. **25,** 1941–1946 (1977).
[4] D. Aviv and E. Galun, Planta Medica **33,** 70–77 (1978).
[5] B. Bruns, K. Hahlbrock, and E. Schafer, Planta **169,** 393–398 (1986).
[6] K. R. Davis and K. Hahlbrock, Plant Physiol. **84,** 1286–1290 (1987).
[7] O. L. Gamborg, R. A. Miller, and K. Ojima, Exp. Cell Res. **50,** 151–158 (1968).
[8] D. Aviv, E. Krochmal, A. Dantes, and E. Galun, Planta Med. **42,** 236–243 (1981).
[9] C. Ambid, M. Moisseeff, and J. Fallot, Plant Cell Rep. **1,** 91–93 (1982).
[10] G. J. Lappin, J. D. Stride, and J. Tampion, Phytochemistry **26,** 995–997 (1987).
[11] R. G. Berger and F. Drawert, Z. Naturforsch. **43c,** 485–490 (1988).
[12] Th. Mulder-Krieger, R. Verpoorte, A. Baerheim-Svendsen, and J. J. C. Scheffer, Plant Cell, Tissue and Organ Culture **13,** 85–154 (1988, and references therein).

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