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BIOTRANSFORMATION OF LINALYL ACETATE BY SUSPENSION CULTURES OF *PAPAVER BRACTEATUM*

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Key Word Index—*Papaver bracteatum*; Papaveraceae; cell suspension cultures; biotransformation; acyclic monoterpene; linalyl acetate.

Abstract—Suspension cultures of *Papaver bracteatum* biotransform the acyclic monoterpene, linalyl acetate, into linalool, geraniol and the monocyclic alcohol, α-terpineol. Total metabolism of linalyl acetate occurs within eight days of substrate addition, with 40% of the substrate being transformed within the first 36 hr. No regiospecific hydroxylation of the 3-methyl-2-butenyl group is observed.

INTRODUCTION

Selective oxidation of the terminal allylic methyl groups in acyclic monoterpenes by plant and fungal systems have been reported [1, 2]. The search for cell systems which can catalyse such regiospecific hydroxylations is ongoing. In accordance with our interest in secondary metabolite production by plant cell cultures [3, 4] and their potential use in the selective biotransformation of foreign substrates we have investigated the capacity of a suspension culture of *Papaver bracteatum* to metabolize the model compound linalyl acetate.

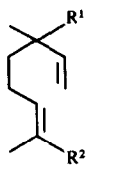
RESULTS AND DISCUSSION

Hirata *et al.* [1] have reported that cell cultures of *Nicotiana tabacum* hydrolyse the acetoxyl group of linalyl acetate (**1**) and also selectively hydroxylate the *trans* methyl group yielding two main products, 8-hydroxylinalool (**5**) and 8-hydroxylinalyl acetate (**6**), while Madyastha and Murty [2] have shown that **1** is converted to linalool (**2**), geraniol (**3**), α-terpineol (**4**) and **5** by *Aspergillus niger*.

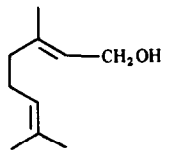
Fermentation of **1** with suspension cultures of *P. bracteatum* over a 36 hr period resulted in its biotransformation to **2** (24%), α-terpineol **3** (14%) and **4** (4.5%), with a large amount of the substrate (57.5%) remaining unchanged (Fig. 1). The same pattern of metabolite formation was seen when **1** was incubated over a 14 day period (Fig. 2). Total hydrolysis of acetate occurred after eight days with steady state concentrations of **2** (64%), **3** (29%) and **4** (7%) being maintained by 14 days. No additional products arising from selective oxidation were observed.

Incubation of *P. bracteatum* with the parent alcohol **2** resulted in a slight reduction of cell viability, probably due to the surface toxic effects of monoterpene alcohols [5]. No biotransformation products of **2** were detected after a 14 day incubation period. This result suggests that **2** is not an intermediate in the formation of **3** and **4** from **1**.

Controls in which the medium and substrate were incubated under identical conditions of pH to the test substances [3] and for similar time periods were analysed in parallel with the time course experiment. Medium catalysed transformation of **1** to **3** and **4** was significant by day eight of the 14 day experiment (26%). Transformation of **2** did not occur in the medium controls. Previous reports on the biotransformation of **1** by plant cell cultures [1, 6] do not take into account such medium reactivity. However, in the case of *P. bracteatum* deacylation activity is complete before medium effects become significant.



1 R^1 = OAc, R^2 = Me
2 R^1 = OH, R^2 = Me
5 R^1 = OAc, R^2 = CH_2OH
6 R^1 = OH, R^2 = CH_2OH



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*Authors to whom correspondence should be addressed.

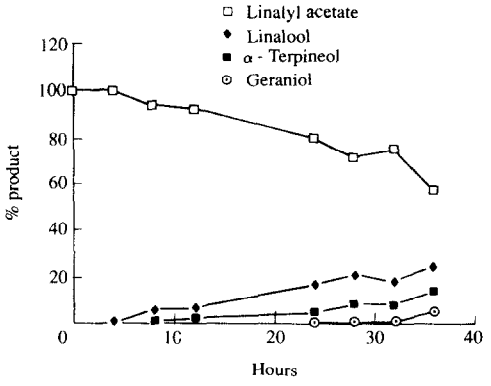

Fig. 1. Biotransformation of **1** by a suspension culture of *P. bracteatum* after 36 hr.

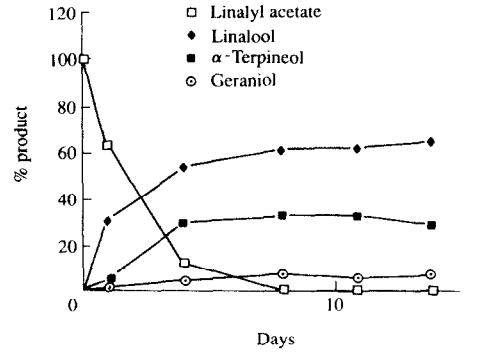

Fig. 2. Biotransformation of **1** by a suspension culture of *P. bracteatum* after 14 days.

Transformation products were detected by comparing the organic soluble constituents of the culture medium and controls, by means of TLC, GC and GC-MS. Products were separated by column chromatography and their structures confirmed by spectroscopic means and by comparison with authentic samples. Suspension cultures of *P. bracteatum* can hydrolyse the acetoxyl group of **1** in addition to catalysing its cyclization and hydroxylation. Selective hydroxylation of the 3-methyl butenyl group of **1** does not occur.

EXPERIMENTAL

^{1}H NMR were run at 60 and 270 MHz in $CDCl_3$ with TMS as int. std. GC-MS were recorded on a quadropole instrument fitted with a SE 54 (15 m) column (temp. range 50–280°). GC was conducted on a FID instrument fitted with a glass column (2 m × 0.3 m) packed with 15% Carbowax 20 m on Gas Chrom. W.A.W. (80–100 mesh) operated at 125–195° (7°/min) with a N_2 flow rate of 30 ml min^{-1}. Merck Kieselgel 60 (70–230 ASTM) was used for CC.

Incubation of substrate with suspension cultures of P. bracteatum. Suspension cultures were grown on a modified Murashige and Skoog medium under conditions described in ref. [3]. For the purposes of these expts cells were subcultured by transferring 5 ml of suspension culture into 45 ml of fr. liquid medium in 100 ml conical flasks. Cultures were grown for 10 days after which the substrate was added (30 mg per 50 ml suspension culture). For time-course determinations of the biotransformation of **1**, flasks were taken for analyses at the time points shown in Figs 1 and 2. Determinations were carried out in triplicate and mean values taken. Substrate, at the same concn, was also added to flasks containing the medium only (controls). The control medium was maintained at the same pH as the cell containing flasks. These were analysed at the same time points as the cultured cells.

Isolation of biotransformation products. The incubation mixt. was filtered to remove cells. The culture medium was then extd with EtOAc and dried *in vacuo*. The residue was taken up in Et_2O (1 ml) prior to analysis. The mixt. of biotransformation products was separated by CC on silica gel ($CHCl_3$–MeOH, 19:1). The compounds isolated were characterized by physical and spectral means.

Linalool (**2**). IR ν_{max}^{film} cm^{-1}: 3400. MS *m/z* (rel. int.): 136 (5) $[M-H_2O]^+$.

α-Terpineol (**3**). IR ν_{max}^{film} cm^{-1}: 1600. MS *m/z* (rel. int.): 139 (5) $[M-H_2O]^+$.

Geraniol (**4**). IR ν_{max}^{film} cm^{-1}: 3300. MS *m/z* (rel. int.): 154 (3) $[M]^+$.

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