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Stereospecific Biotransformation of (±) Phenylethyl Propionate by Cell Cultures of *Peganum harmala L.*

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ABSTRACT

The enzymatic potential of the cultured plant cells can be employed for bioconversion purposes. Plant enzymes are able to catalyze regio- and stereo-specific reactions, and therefore can be applied for the production of desired substances. The biotransformation of foreign substrates with suspension cells of *Peganum harmala* was tested with (±) phenylethyl propionate. The callus cultures of *Peganum harmala* were established from cotyledons, and healthy suspensions grown using Murashige and Skoog medium. In order to investigate the specificities of the hydrolysis, (-) and (+) phenylethyl propionate isomers were added to the cultures. The phenylethyl propionate isomers were converted to their corresponding alcohols. The two isomers showed different rates of conversion during the first 24 hours after feeding. These cultures were able to hydrolyse specifically the propionate group in (±) phenylethyl propionate. It was found that the cultured cells of *P. harmala* have the ability to hydrolyse the racemic phenylethyl propionate stereoselectively. *Iran. Biomed. J. 6 (1): 43-46, 2002*

Keywords: Stereoselectively, Cell cultures, *Peganum harmala*, Biotransformation, phenylethyl propionate

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INTRODUCTION

Peganums are 30-90-cm-high bushy herbs and widely distributed in the Irano-Turanion with extensions into the dry Mediterranean regions of Europe and Africa. The seeds and roots of *Peganum harmala* L. (*Zygophyllaceae*) contain alkaloids such as harmine, harmaline, harmol, and harmalol [1]. The seed extract has antispasmodic, antihistaminic [2], and vasorelaxant effects [3].

Cell cultures of *Peganum harmala L.* have been widely studied with a view to investigate their ability to produce amines and β-carboline alkaloids [4, 5]. Phenylethyl alcohol can be found in a number of natural essential oils, such as rose and orange balsams. It is used in flavor and perfumery industries [6] and has antibacterial and sedative effects [7].

Production of the essential oils as secondary metabolites in plant tissue cultures is difficult, possibly because the most volatile oils in intact plants are accumulated in specialized secretary organs, such as glandular hairs, resin ducts or schizogenous glands. These secretary organs are not normally formed in callus or suspension cultures. There are numerous reports on the non-accumulation of lower isoprenoids in undifferentiated, friable, fast growing callus and suspension cultures [8]. Improved metabolite production may be achieved by the addition of precursors to the culture medium. There are several reports on the biotransformation of terpene and non-terpene constituents by cultured cell of *P. harmala* [9-11]. The biochemical capability of the cultivated plant cells to transform exogenously supplied compounds offers a broad potential and an interesting contribution toward the modification of natural and synthetic chemicals [12]. To learn more about these potentials, we have studied the biotransformation of phenylethyl propionate isomers by *P. harmala* cultured suspension cells.

MATERIALS AND METHODS

Cell cultures. Surface of *Peganum harmala* seeds was sterilized in 30% (w/v) hydrogen peroxide containing 1% Tween 80 for 2 min, then germinated on wet filter paper in Petri dish in the dark at 25°C. The cotyledons were then transferred to Murashige and Skoog media [13] containing 5 ppm ascorbic acid, 5% coconut water, 2 ppm 2,4-dichlorophenoxyacetic acid and 0.1 ppm kinetin. The callus and suspensions were maintained in a

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12-h light/dark cycle at 27°C and subcultured every 4 weeks [14].

Substrate feeding and product extraction. The substrates were obtained from Aldrich, UK. Chemical purity (greater than 98%) was determined by capillary gas chromatography (GC). Solutions of substrates were dissolved in a water-miscible solvent (ethanol 70%), which resulted in good mixing of the substrate upon addition to the aqueous medium. The substrates were added to suspension cultures to make a final concentration of 100 ppm, cell volume (50% p.v). Control readings were made without addition of substrate to cultures and with addition of substrate to cell-free medium. The cultures were incubated under the conditions mentioned above. After the incubation period, the flask was swirled to ensure good mixing and two samples were removed with a 10-ml pre-sterilized, glass-tippless pipette. A new pipette was used for each sample. The cultures were homogenized and analyzed by identical protocols at different time periods. *P. harmala* cultures, to which phenylethyl propionate had been added, were sampled on 0, 2, 4, 6, 8, and every twelve hours [15, 16]. Also, the conversions of the substrates, (±) phenylethyl propionate were tested using crude extracts of *P. harmala* cultured cells and quantitative and qualitative data were obtained. Substrates and products were extracted with an equal volume of dichloromethane, followed by centrifugation (1000 × g for 5 min) and the volume reduction of the extract [17]. The time course for hydrolysis of phenylethyl propionate was constructed from data points calculated from four results (two experiments, using duplicate samples).

Gas chromatography analysis of bioproducts. The bioproduction components and added substrates were analyzed as follows: 1 µl of the reduced extract prepared above was subjected to capillary gas chromatography (GC). A Hewlett Packard 5890 GC, fitted with a flame ionization detector, was used for the analysis of volatiles. The capillary column (30 × 0.31 mm) was a high performance fused silica cross-linked methyl silicon megabore. The film thickness was 3.0 m. The results were recorded on a Hewlett Packard 3392A integrator. The operating conditions were as follows: The temperature program used was 70 to 280°C at 10°C/min, injector and detector temperature 270°C. The identity of products and substrates were confirmed by co-chromatography and gas chromatography-mass spectrometry (GC-MS). When GC was used, quantitative estimation of the conversion of the substrates was performed using pentadecane as the internal standard [18].

***Identification of components by* GC-MS**. Determination of mass spectra was carried out using a Kratos Concept 25 spectrometer at 70 eV equipped with a Sun Mash 3 computer data output. The operating conditions were similar to those of GC analysis, but with Helium as the carrier gas. Mass spectrometer conditions were: ionization current, 1A; ionization potential, 70 eV; source temperature, 150; resolution, 1000; scan speed, 1 sec/decade. The identification was based on retention data, electron-impact-mass spectra and comparison of chemical ionization spectral data with those relevant reference samples and the literature [19, 20].

RESULTS AND DISCUSSION

The GC analysis of the cultures fed with (±) phenylethyl propionate produced two more major peaks. Their fragmentation patterns of the electron-impact-mass spectra were 91, 92, 65, 122, 77, 104. These peaks were identified as (+) and (-) phenylethyl alcohols. The time course of the conversions was presented in Figure 1. (+) and (-) phenylethyl propionates were converted to their corresponding alcohols, (+) and (-) phenylethyl alcohols. The biochemical potential of *P. harmala* cell cultures to perform selective hydrolysis is of considerable interest in connection with their biotechnological utilization. Enantio-selective hydrolysis is useful for the optical resolution of racemic acetates and has been observed in biotransformation of (*RS*)-1-phenylethyl acetate and its derivatives with cultured cells of *Spirodela oligorrhiz* [21]. The hydrolysis of (+) and (-) phenylethyl acetates and (+) and (-) menthyl acetates using potato and artichoke tubers for the synthesis of alcohols were also investigated [22].

As shown in Figure 1 the maximum concentration of both products, (+) and (-) phenylethyl alcohols were reached after 48 hours. After 24 hour, 90% of one pair of optical isomers disappeared from the culture, whereas in the same time 70% of another pair were converted to the product. In addition, it was evident that the biotransformation rate of one pair was higher than another one in the first 24 hours, but the amounts of the products were approximately equivalent after 48 hours. Similarly,

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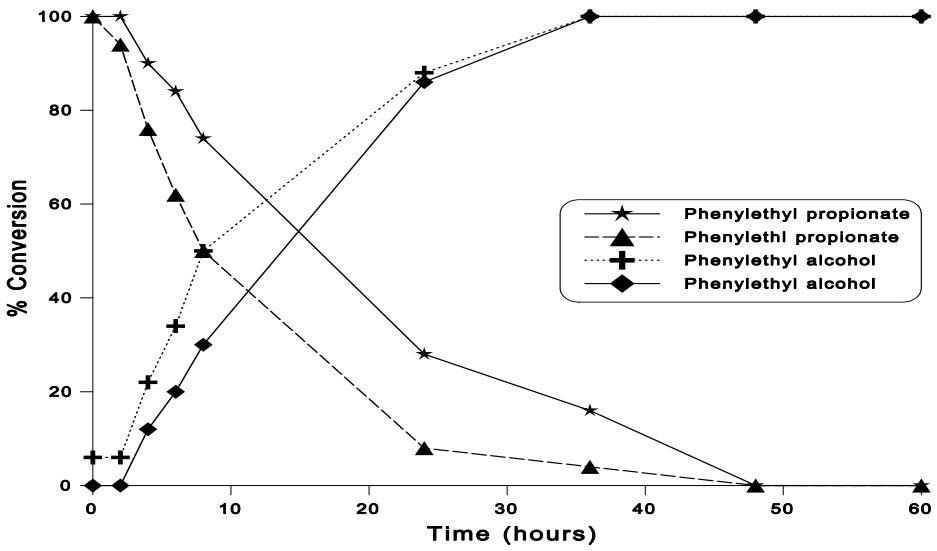

Fig.1. Time course of the conversion of (+) and (-) phenylethyl propionate to (+) and (-) phenylethyl alcohol by cell suspension of *P. harmala*, showing duplicate results at each data point.

Orihara and Furuya [23] reported the stereo-selectivity with biotransformation of (+)- and (-)-fenchone by cultured cells of *Eucalyptus perriniana* and discussed the regio- and stereo-selectivity of the hydroxylation of bicyclic monoterpenes.

The results obtained from the biotransformation experiments using cell cultures and crude cells extracts suggest that enzymes were present only in the cells, and the substrates and products had to be extracted from the entire culture. Balsevich [24] examined the biotransformation of 10-hydroxy geraniol by cultures of *Catharanthus roseus*. The reduced products were all found to be present in the culture medium. The medium itself was devoid of any dehydrogenase activity and it was suggested that this was evidence for the existence of a membrane-bound enzyme.

From the data presented in this study, it was found that *P. harmala* cell cultures possess biochemical ability to perform specific biotransformation reactions on (+) and (-) phenylethyl propionates administered exogenously. Berlin et al. [25] reported that tissue cultures of *P. harmala* were a suitable model system to study how to manipulate the formation of secondary metabolites. In our study, we demonstrated that *P. harmala* cultures had large capacities for biotransformation and great potentials for the selective structural modification on chiral molecules.

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REFERENCES

1. Jing, C. and Mitchelson, K.R. (1997) Improved separation of six harmane alkaloids by high-performance capillary electrophoresis. *J. Chromatoghr. 761: 297-305*.
2. Aqel, M. and Hadidi, M. (1991) Direct relaxant effect of *Peganum harmala* seed extract on smooth muscles of rabbit and guinea pig. *Int. J. Pharmacog. 2: 336-344*.
3. Shi, C.C., Chen, S.Y., Wang, G.J., Liao, J.F. and Chen, C.F. (2000) Vasorelaxant effect of harman. *Eur. J. Pharmacol. 390: 319-325*.
4. Berlin, J., Rugenhagen, C., Dietze, P., Fecker, L.F., Goddijn, O.J.M. and Hoge, H.C. (1993) Increased production of serotonin by suspension and root cultures of *Peganum harmala* transformed with a tryptophan decarboxylase cDNA clone from *Catharanthus roseus*. *Trans. Res. 2: 336-344*.
5. Sasse, F., Heckenberg, U. and Berlin, J. (1982) Accumulation of β-carbolin alkaloids and serotonin by cell cultures of *Peganum harmala L. Plant Physiol. 69: 400-402*.
6. Ewans, W.C. (1996) Trease and Evans' Pharmacognosy. WB Sunders Company Ltd, London, pp. 263-264.

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[DOR: 20.1001.1.1028852.2002.6.1.6.7]

7. Budavari, S., Oneil, M.J., Smith, A., Heckelman, P.E. and Kinneary, J.F. (1996) The Merk Index, Merk Research Laboratories, New Jersey. pp. 7370-7371.
8. Charlwood, B.V. and Brown, J.T. (1988) The accumulation of mono- and sesquiterpenes in tissue culture. *Biochem. Soc. Trans. 16: 61-66*.
9. Courtois, D., Yvernel, D. and Florin, B. (1988) Conversion of tryptamine to serotonin by cell suspension cultures of *Peganum harmala*. *Phytochem. 27: 3137-3142*.
10. Zhu, W., Asghari, G. and Lockwood, G.B. (2000) Factors affecting volatile terpene and non-terpene biotransformation products in plant cell cultures. *Fitoterapia 71: 501-506*.
11. Zhu, W. and Lockwood, G.B. (2000) Enhanced biotransformation of terpenes in plant cell suspension using controlled release polymer. *Biotechnol. Lett. 22: 659-662*.
12. Hamada, H., Miyamoto, Y., Nakajima, N. and Furuya, T. (1998) Highly selective transformation by plant catalysts. *J. Mol. Catal. B: Enz. 5: 187-189*.
13. Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant 15: 437-439*.
14. Dixon, R.A. and Gonzales, R.A. (1994) Plant cell culture- a practical approach. IRL Press Ltd., Oxford.
15. Suga, T. and Hirata, T. (1990) Biotransformation of exogenous substrates by plant cell cultures. *Phytochem. 29: 2393-2406*.
16. Lappin, G.H., Stride, J.D. and Tampion, J. (1987) Biotransformation of monoterpenoids by suspension cultures of *Lavandula angustifolia*. *Phytochem. 26: 995-997*.
17. Guardiola, J., Iborra, J.L., Rodenas, L. and Canovas, M. (1996) Biotransformation from geraniol to nerol by Immobilized grapevin cells. *Appl. Biochem. Biotechnol. 56: 169-180*.
18. Lockwood, G.B. (2001) Techniques for gas chromatography of volatile terpenoids from a range of matrices. *J. Chromatograph. 939: 23-31*.
19. Adams, R.P. (1989) Identification of essential oils by ion trop mass spectroscopy. Academic Press, California.
20. Davies, N.W. (1990) Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon, and Carbowax 20m phases. *J. Chromatogr. 503: 1-24*.
21. Pawlowicz, P. and Siewinski, A. (1987) Enanatioselective hydrolysis of esters and oxidation of aromatic-aliphatic alcohols obtained there from by *Spirodela oligorrhiza*. *Phytochem. 26: 1001-1004*.
22. Mironowicz, A. (1998) Biotransformation of racemic acetates by potato and topinambur tubers. *Phytochemistry 47: 1531-1534*.
23. Orihara, Y. and Furuya, T. (1990) Biotransformation of 18 β-glycyrrhetinic acid by cultured cells of *Eucalyptus perriniana* and *Coffe arabica*. *Phytochem. 29: 3123-3126*.
24. Balsevich, J. (1985) Biotransformation of 10-hydroxygeraniol and related compounds by a cell suspension culture of *Catharanthus roseus*; the formation of reduced products. *Plant Medica 2: 128-132*.
25. Berlin, J., Rugenhagen, C., Kuzovkin, I.N., Fecker, L.F. and Sasse, F. (1994) Are tissue cultures of *Peganum harmala* a useful model system for studying how to manipulate the formation of secondary metabolites? *Plant cell Tissue Cult. 38: 289-297*.

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