생 약 학 회 지
Kor. J. Pharmacogn.
26(3) : 227~238 (1995)

Biotransformation of Exogenous Monoterpenoids by Plant Cell Culture

Seung-Won Shin
College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea

Abstract—Recent reports on biotransformation of monoterpene alchols, aldehydes, acetates and epoxides are summerized. The studies have focused on stereospecific reaction of the functional groups of exogenous foreign substrates by foreign plant cells and micro-organisms. An another important aspect of research is the development of the immobilization technique for cells or related enzymes.

Keywords—Biotransformation · monoterpene · cell culture

The production of useful secondary metabolites is of considerable interest in plant cell cuture research. However, with few exceptions, callus and cell suspension cultures tend to produce the desired compounds in much lower quantities than mother plants or in many cases, to produce the particular compounds not at all.[1-3] An another problem is the compositions or structures of the produced compounds are not coincident with the purposed useful compounds.[4,5]

It has been studied for improving yields of secondary metabolites by altering the composition of culture medium, varying the physical conditions, induction of polyploid cells, induction of morphological differentiation (organ culture, hairy root, etc.), creation of artificial accumulation sites and high-producing cell line selection. With rapid advances in molecular biology, it become possible to apply the biotechnological methods in this fields by increasing the the rate-limiting enzyme, creating a new branch for preexisting pathway, reducing the rate of an existing side reaction, manipulation of regulatory genes and selection of regulatory mutants.[6-9]

Biotransformation is the important research area to use the biochemical potential of the plant cell, micro-organisms and animal cells for bioconversion of substances employing the the biotechnological methods generally. With the development of the methods using the ability of cultured plant cells and micro-organism to metabolize or convert the substrate to desired structures will be able to support considerable advances in establishing high-yield and low-cost culturing system for industrial mass-production of the useful compounds.[10-13]

Recent studies on biotransformation have focused on stereospecific reaction of the functional groups of exogenous foreign substrates by foreign plant cells and micro-organisms. An another important aspect of research is the develoment of the immobilization technique for cells or related enzymes.[15-17]

Biotransformation of monoterpenoids is one of the attracive subjects of recent studies and because they are economically important compounds used as pharamceuticals, perfumes and flavouring agents. In production of essence, they are more important than sesquiterpenoids due to their high volatility. In most cases, the cultured cells tend to produce these compounds in much lower quantities than mother

Table I. Biotransformation of monoterpene alcohols

Substrates	Plants or Micro-organism	Products	ref.
geraniol	*Euphorbia characias*	nerol, geranial, neral	10
〃	*Nicotiana tabacum*	〃	10
〃	*Catharanthus roseus*	〃	10
〃	*Glycine max*	〃	10
〃	*Mellissa officinalis*	nerol	48
geraniol, citronellol (callus)	*Rosa damascena*	C_5. C_{10} oxidation product	19
geraniol (suspension)	〃	citral	19
(-)-borneol	*Eucalyptus perriniana*	(-)-borneol 2-O-β-gentibioside	38
〃	〃	(-)-borneol 2-O-β-sophoroside	38
〃	〃	(-)-borneol 2',6'-di-(4-β-glucopyranosyl)-D-glucopyranoside	38
〃	〃	6-exo-hydroxyborneol 2-O-β-D-glucopyranoside	38
〃	〃	4-hyroxyborneol 2-O-β-D-glucopyranoside	38
〃	〃	5-exo hydroxyborneol 2-O-β-gentibioside	38
〃	〃	5-exo-hydroxyborneol 2-O-β-glucopyranoside	38
(-)-menthol	*Asperigillus niger*	1-,2-,6-,7-,8-, and 9-hydroxymenthol	35
〃	*A. cellulosae*	4-hydroxymenthol	35
(+)-menthol	*A. niger*	7-hydroxymenthol	35
(1R,2R,4R)-(-)-carbomenthol	*Nicotiana tabacum*	(+)-carbomenthone (+)-neocarbomenthol	34
(1S,2S,4S)-(+)-carbomenthol	〃	(-)-carbomenthone	34
(1R,2S,4R)-(+)-neocarbomenthol	〃	(+)-carbomenthone	34
(1S,2R,4S)-(-)-neocarbomenthol	〃	(-)-carbomenthone	34
(1R,3R,4S)-(-)-menthol	〃	(-)-menthone (sc)	34
(1S,3S,4R)-(+)-menthol	〃	(+)-menthone (sc)	34
(1R,3S,4S)-(+)-neomenthol	〃	(-)-menthone (sc)	34
(1S,3R,4R)-(-)-neomenthol	〃	(+)-menthone (sc)	34
(1R,2S,4R)-(+)-borneol	〃	(+)-camphor	31, 34
(1S,2R,4S)-(-)-borneol	〃	(-)-camphor	31, 34
(1R,2R,4R)-(-)-isoborneol	〃	(+)-camphor	31, 34
(1S,2S,4S)-(+)-isoborneol	〃	(-)-camphor	31, 34
(1R,2R,3R,5S)-(-)-isopinocamphenol	〃	(+)-isopinocamphone	31, 34
(1S,2S,3S,5R)-(+)-isopinocamphenol	〃	(-)-isopinocamphenol	31, 34
(1R,2R,3S,5S)-(+)-neoisopinocamphenol	〃	(+)-neoisopinocamphenol	31, 34
(1S,2S,3R,5R)-(-)-neoisopinocamphenol	〃	(-)-neoisopinocamphenol	31, 34
(1R,2S,4R,5R)-(+)-neoisoverbanol	〃	(+)-cis-verbanone	31, 34
(1S,2R,4S,5S)-(-)-neoisoverbanol	〃	(+)-cis-verbanone	31, 34
(2R,4R)-(-)-cis-carveol	〃	(-)-carvone	31, 34
(2S,4S)-(+)-cis-carveol	〃	(-)-carrvone	31, 34
(2S,4R)-(-)-trans-carveol	〃	(-)-carvone	31, 34

Table I. Continued

Substrates	Plants or Micro-organism	Products	ref.
(2R,4S)-(+)-trans-carveol	〃	(+)-carvone	31, 34
(1R,4R,5R)-(+)-cis-verbenol	〃	(+)-cis-verbanone	31, 34
	〃	(+)-verbenone	31, 34
(1S,4S,5S)-(-)-cis-verbenol	〃	(-)-cis-verbanone	31, 34
〃	〃	(-)-verbenone	31, 34
(+)-isopinocamphenol	*Rhizophus arrhizus*	(1S,2S,3S,5S)-pinane-3,5-diol	36, 37
〃	〃	Trans, trans-4(8)-menthene-2,6-diol	36, 37
〃	〃	(1S,2S,3S)-1-(1'-hydroxy-1'-methyl-propyl)-	36, 37
		2-methyl-bicyclo[3,1,0]hexane-3-ol	36, 37
〃	*Bacillus sphaericus*	(1S,2S,3S,5S)-pinane-3,5-dio	36, 37
〃	〃	(1S,2S,3S)-1-(1'-hydroxy-1'-methyl-propyl)-	36, 37
〃	〃	(1R,2S,3S,5R,6R)-pinane-3,9-diol	36, 37
〃	〃	(1R,2S,5S)-5-hydroxy-pinane-3-one	36, 37
〃	〃	2-methyl-bicyclo[3,1,0]hexane-3-ol	36, 37
〃	*Bacillus megaterium*	(1S,2S,3S,5S)-pinane-3,5-diol	36, 37
〃	〃	Trans, trans-4(8)-menthene-2,6-diol	36, 37
〃	*Botryphaeria rhodina*	(1R,2S,3S,5R)-pinane-1,3-diol	36, 37
〃	〃	(1S,2S,3S,5S)-pinane-3,5-diol	36, 37
〃	〃	(1R,2S,3S,5R,6R)-pinane-3,9-diol	36, 37
〃	〃	Trans, trans-4(8)-menthene-2,6-diol	36, 37
〃	〃	(1S,2S,3S)-1-(1'-hydroxy-1'-methyl-propyl)-	36, 37
〃	〃	2-methyl-bicyclo[3,1,0]hexane-3-ol	36, 37
〃	〃	(1R,2S,3S,5R)-pinane-1,3-diol	36, 37
〃	〃	(1R,2R,3S,5R)-pinane-2,3-diol	36, 37
〃	〃	(1S,2S,3S,5S)-pinane-3,5-diol	36, 37
〃	〃	(1R,2S,3S,5R)-3-acetoxy-pinane-5-ol	36, 37
〃	〃	(1S,2S,3S,5R,7R)-pinane-3,7-diol	36, 37
〃	〃	(1S,2S,3S,5R,6S)-pinane-3,8-diol	
〃	〃	(1R,2S,3S,5R,6R)-8-acetoxy-pinane-3-ol	36, 37
〃	〃	trans-4(8)-menthene-2,6-diol	36, 37
〃	〃	(1S,2S,3S)-1-(1'-hydroxy-1'-methyl-propyl)-2-methyl-bicyclo [3,1,0]hexane-3-ol	36, 37

Table I. Continued

Substrates	Plants or Micro-organism	Products	ref.
〃	*Norcardia sp.*	(1R,2S,3S,5R)-pinane-1,3-diol	36, 37
〃	〃	(1R,2R,3S,5R)-pinane-2,3-diol	36, 37
〃	〃	(1S,2S,3S,5S)-pinane-3,5-diol	36, 37
〃	〃	(1S,2S,3S,5R,7R)-pinane-3,7-diol	36, 37
〃	〃	(1S,2S,3S,5R,6S)-pinane-3,8-diol	36, 37
〃	〃	(1S,2S,3S)-1-(1'-hydroxy-1'-methyl-propyl)-2-methyl-bicyclo[3,1,0]hexane-3-ol	36, 37
(-)-isopinocamphenol	*Botryosphaeria rhodina*	(1R,2R,3S,5R)-pinane-2,3-diol	36, 37
〃	〃	(1S,2S,3S,5S)-pinane-3,5-diol	36, 37
〃	〃	(1R,2S,3S,5R,6R)-pinane-3,9-diol	36, 37
〃	〃	(1S,2S,3S)-1-(1'-hydroxy-1'-methyl-propyl)-2-methyl-bicyclo[3,1,0]hexane-3-ol	36, 37
〃	〃	(-)-isopinocamphenol acetate	36, 37
〃	〃	(1R,2R,3S,4R,5R)-pinane-3,4-diol	36, 37
〃	*Mortierella isabellina*	(1R,2S,3S,5R)-pinane-1,3-diol	36, 37
〃	〃	(1R,2R,3S,5R)-pinane-2,3-diol	36, 37
〃	〃	(1S,2S,3S,5S)-pinane-3,5-diol	36, 37
〃	〃	(1S,2S,3S)-1-(1'-hydroxy-1'-methyl-propyl)-2-methyl-bicyclo[3,1,0]hexane-3-ol	36, 37
〃	〃	(3R,4R)-2-(2'-hydroxy-1'-methyl-propyl)-3-methyl-cyclopentene-4-ol	36, 37
〃	*Norcardia sp.*	(1S,2S,3S,5S)-pinane-3,5-diol	36, 37
〃	〃	(1S,2S,3S)-1-(1'-hydroxy-1'-methyl-propyl)-2-methyl-bicyclo[3,1,0]hexane-3-ol	36, 37

plants and, in many cases, to produce the useful compounds not at all. In such a status, the ability of cells to convert the metabolites produced in cells or foreign substrates to useful compounds is of considerable interest.[18-20]

The reaction types and stereochemistry in the biotransformation depends on the functional group in the substrates administered and the structural moieties in the vicinity of the functional group.[21,22]

This article is an update of the previous reviews by Mulder-Krieger(1988)[23] and Mahato et al.(1990)[24] and a comprehensive summerize of the reports during the last ten years.

Hier are summerized the reports on biotransformation of monoterpenoids according to the chemical groups of substrates: 1. alcohols (Table I), 2. acetates and hydrocarbons (Table II), 3. aldehydes and ketones (Table III), 4. oxides (Table IV).

Alcohols

Since the conversion of geraniol was confirmed in cell suspension culture, the biotransformation of monoterpene alcohol has been

Table II. Biotransformation of monoterpene acetates and hydrocarbons

Substrates	Plants or Micro-organism	Products	ref.
1. Acetates			
(±)-menthyl acetate	*Spirodella oligorrhiza*	(-)-menthol	39, 40, 41
(±)-bornyl acetate	〃	(±)-borneol	39, 40, 41
(±)-trans-2-acetoxy-trans-dihydropinol	〃	(-)-trans-2-hydroxy-dihydropinol	39, 40, 41
(±)-cis-2-acetoxy-trans-dihydropinol	〃	(-)-cis-2-hydroxy-trans-dihydropinol	39, 40, 41
(±)-trans-2-acetoxy-cis-dihydropinol	〃	(±)-trans-2-hydroxy-cis-dihydropinol	39, 40, 41
linalyl acetate	*Papaver bracteatum*	linalool, geraniol, α-terpineol	39, 40, 41
(-)-bornyl acetate	*Nicotiana tabacum*	(-)-borneol	28
(+)-bornyl acetate	〃	(+)-borneol, camphor	28
(-)-isobornyl acetate	〃	(-)-iaoborneol, camphor	28
(+)-isobornyl acetate	〃	(+)-isoborneol	28
(-)-isopinocampheyl acetate	〃	(-)-isopinocamphenol	28
(+)-isopinocampheyl acetate	〃	(+)-isopinocamphenol	28
1-acetoxy-p-menth-4(8)-ene	*Nicotiana tabacum*	r-1-acetoxy-t-4,8-epoxy-p-menthane	32
	〃	r-1-acetoxy-c-4,8-epoxy-p-menthane	
(±)-8-acetoxy-p-menth-1-ene (α-terpinylacetate)	〃	8-acetoxy-c-4-p-menthane-r-1,t-2-diol	30, 32
2. hydrocarbons			
terpinolene	*Asperigillus niger*	fenchan-1.4-diol	35

Table III. Biotransformation of monoterpene aldehydes and ketones

Substrates	Plants or Micro-organism	Products	ref.
perillaldehyde	*Lavandula angustifolia*	perillyl alcohol	45
neral	〃	nerol	45
geranial	〃	geraniol	45
citronellal	〃	citronellol	45
〃	*Mellisa officinalis*	〃	48
〃	*Petroselinum crispum*	〃	46, 47, 16, 17
(+)-citronellal	*Dunaliella tertiolecta*	(+)-citronellol	49, 50, 51
(-)-citronellal	〃	(-)-citronellol	49, 50, 51
(±)-citronellal	〃	(±)-citronellol	49, 50, 51
citral	*Mellisa officinalis*	nerol, geraniol	48
〃	*Petroselinum crispum*	〃, 〃	16, 17, 46, 47
〃	*Euglena gracilis*	(-)-citronellal, (+)-citronellal	50
〃	〃	geraniol, nerol	50
〃	〃	(-)-citronellol, (+)-citronellol	50
〃	〃	(-)-citronellic acid, (+)-citronellic acid	50
〃	〃	geranic acid, neric acid	50
citral (geranial:neral=56:44)	*Dunaliella tertiolecta*	geraniol	49, 50, 51

Table III. Continued

Substrates	Plants or Micro-organism	Products	ref.
myrtenal	*Euglena gracilis*	myrtenol, myrtenoic acid	50
(-)-perillaldehyde	*Euglena gracilis*	(-)-perillic acid (-)-perillyl alcohol	50
〃	〃	trans-shisool, cis-shisool,	50
〃	〃	trans-shisoic acid, cis-shisoic acid	50
〃	〃	trans-1,2-dihydroperilladehyde	50
〃	〃	cis-1,2-dihroperilladehyde	50
(-)-perillaldehyde	*Dunaliella tertiolecta*	(-)-perillyl alcohol, trans-shisool	49, 50, 51
(±)-perillaldehyde	〃	(±)-perillyl alcohol, trans-shisool	49, 50, 51
(-)-phellandral	*Euglena gracillis*	(-)-phellandrol	50
〃	〃	trans-tetrahydroperillyl alcohol	50
〃	〃	phellandric acid	50
〃	〃	cis-tetrahydroperillyl alcohol	50
(-)-phellandral	*Dunaliella tertiolecta*	(-)-phellandrol	49, 50, 51
trans-dihydroperillaldehyde	*Euglena gracillis*	trans-shisool, trans-shisoic acid	50
cis-dihydroperillaldehyde	〃	cis-shisool, cis-shisoic acid	50
1,2-dihydroperillaldehyde (trans:cis=87:17)	*Dunaliella tertiolecta*	trans-shisool, cis-shisool	49 ,50, 51
cumin aldehyde	〃	cumin alcohol	49, 50, 51
(+)-citronellal	*Euglena gracillis*	(+)-citronellol, d-citronellic acid	50
(-)-citronellal	〃	(-)-citronellol, l-citronellic acid	50
(-)-carvone	*Nicotiana tabacum*	(1R,4R)-dihydrocarvone	22
(1R)-(+)-p-menth-4(8)-en-3-one	*Nicotiana tabacum*	(1R,4S)-(-)-p-menth-3-one	21
〃	〃	(1R,4R)-(+)-p-menth-3-one	21
〃	〃	(1R,3R,4S)-p-menth-3-ol	21
〃	〃	(1R,4R)-(-)-4-hydroxy-p-menth-3-one	21
〃	〃	(1R,4S)-(+)-4-hydroxy-p-menth-3-one	21
(1R,4S)-(-)-p-menth-3-one	*Nicotiana tabacum*	(1R,3R,4S)-p-menth-3-ol	21
〃	〃	(1R,4R)-(-)-4-hydroxy-p-menth-3-one	21
〃	〃	(1R,4S)-(+)-4-hydroxy-p-menth-3-one	21
(1R,4R)-(+)-p-menth-3-one	*Nicotiana tabacum*	(1R,3R,4S)-p-menth-3-ol	21
〃	〃	(1R,4R)-(-)-4-hydroxy-p-menth-3-one	21
〃	〃	(1R,4S)-(+)-4-hydroxy-p-menth-3-one	21

Table III. Continued

Substrates	Plants or Micro-organism	Products	ref.
(1R,4R)-(+)-carbomenthone	*Nicotiana tabacum*	(1R,2R,4R)-(-)-carbomenthol	27, 33, 34
〃	〃	(1R,2S,4R)-(+)-neocarbomenthol	27, 33, 34
(1S,4S)-(-)-carbomenthone	〃	(1S,2S,4S)-(+)-carbomenthol	27, 33, 34
〃	〃	(1S,2R,4S)-(-)-neocarbomenthol	27, 33, 34
(1R,4S)-(-)-menthone	〃	(1R,4R)-4-hydroxy-p-menthan-3-one	27, 33, 34
〃	〃	(1R,3S,4S)-(+)-neomenthol	27, 33, 34
(1S,4R)-(+)-menthone	〃	(1S,4S)-4-hydroxy-p-menthan-3-one	27, 33, 34
〃	〃	(1S,3R,4R)-(+)-neomenthol	27, 33, 34
〃	〃	(1S,3S,4R)-(+)-menthol	27, 33, 34
(1R,4R)-(+)-camphor	〃	(1R,2S,5R)-(+)-cis-verbanone	27, 33, 34
(1S,4S)-(-)-camphor	〃	(1S,2R,5S)-(+)-cis-verbanone	27, 33, 34
carbotanacetone	*Aspergillus sp.*	p-menthane-2,9-diol, 8-hydroxycarveol,	35
〃	〃	p-menthane-2,9-diol acetate	35
(+)-fenchone	*Eucalyptus perriniana*	(1R,4R,5S)-5-hydroxyfenchan-2-one 5-O-β-D-glucopyranoside	51
〃	〃	(1R,4R,5S)-5-hydroxyfenchan-2-one 5-O-β-D-gentiobioside	51
〃	〃	(1R,4S,6R)-6-hydroxyfenchan-2-one 6-O-β-D-glucopyranoside	51
〃	〃	(1R,4S,6R)-6-hydroxyfenchan-2-one 6-O-β-D-gentiobioside	51
〃	〃	(1S,4S,7S)-7-hydroxyfenchan-2-one 7-O-β-D-glucopyranoside	51
〃	〃	(1S,4S,7S)-7-hydroxyfenchan-2-one 7-O-β-D-gentiobioside	51
(-)-fenchone	〃	(1S,4S,5R)-5-hydroxyfenchan-2-one 5-O-β-D-glucopyranoside	51
〃	〃	(1S,4R,6S)-6-hydroxyfenchan-2-one 6-O-β-D-glucopyranoside	51
〃	〃	(1S,4R,6S)-6-hydroxyfenchan-2-one 6-O-β-D-gentiobioside	51

studied in many aspect.[25,26]

One of the important subjects of the advanced research is the regio- and stereospecificity of the reaction.[27-29] In cell suspension culture of *Nicotiana tabacum*, the selective transformation of methyl group of linallol into the hydroxy methly group and the ability of the cells to discriminate between the enantiomers were observed.[30,31] The cultured cells favour (1R,2S,4R)-9-(+)-borneol in preference to its enantiomer. Such an ability of the cultured cells was also investigated with the monoterpenoids such as terpineol derivatives, having terminal, endocyclic and exocyclic C-C double bonds.[32,33]

Hamata[34] has carried out experiments with

Table VI. Biotransformation of monoterpene oxides

Substrates	Plants or Micro-organism	Products	ref.
1,8-cineol	*Glomerella cingulata*	2-exo-hydroxy-1,8-cineol	52
〃	〃	2-endo-hydroxy-1,8-cineol	52
〃	〃	3-endo-hydroxy-1,8-cineol	52
〃	〃	3-exo-hydroxy-1,8-cineol	52
〃	〃	(1R.2R.4S)-2-endo-hydroxy-1,8-cineolyl malonate	52
1,8-cineol	*Eucalyptus perriniana*	(1R,2R,4S)-1.8-epoxy-p-menthan-2yl-O-β-D-glycoayranoside	53
〃	〃	(1S,3R,4R)-1.8-epoxy-p-menthan-2yl-O-β-D-glycoayranoside	53
〃	〃	(1R,3S,4S)-1.8-epoxy-p-menthan-2yl-O-β-D-glycoayranoside	53
〃	〃	(1S,2S,4R)-1.8-epoxy-p-menthan-2yl-O-β-D-glycoayranosyl-β-D-glycoayranoside	53
〃	〃	(1S,2S,4R)-1.8-epoxy-p-menthan-2yl-O-β-D-glycoayranoside	53
(-)-cis-rose oxide	*Aspergillus niger*	(-)-cis-9-hydroxy-7E-rose oxide	54
		(-)-cis-7E-rose oxide-8-carboxylic acid	54
(-)-trans-rose oxide	〃	(-)-trans-9-hydroxy-7E-rose oxide	54
		(-)-trans-7E-rose oxide-8-carboxylic acid	54

enantiomers of menthol, borneol and carveols. Hier was confirmed the enantioselectivity in the oxidation of these secondary alcohols.

The introduction of the functional group at nonactivated carbon atom by micro-organisms were studied in cultures of *Asperigillus niger*, its strains and related species.[35] (+)- and (-)-menthol, terpinolene and (-)-carvotalacetone were converted to obtain various hydroxylated and reduced products. It was shown (-)-menthol was nonspecifically transformed to give six hydroxylated products, mainly 3-hydroxy and 9-hydroxymethanol. On the other hand, the main product from conversion of (+)-menthol was 7-hydroxymenthol. The products of biotransformations were different according to the various species of *Aspergillus*.

The hydroxylation of isopinocamphenol by bacterial and fungal strains was experimented by Abraham.[36,37] As results, 14 hydroxylated compounds could be characterized. The sites of hydroxylation were similar at (+)- and (-)-isopinocamphenol, but the significantly different yields indicated that there must be a pronounced enantioselectivity of the enzymes related to the reaction.

The glycosylation of (-)-borneol was experimented in cell suspension of *Eucalyptus perriniana*.[38] Six compounds, (-)-borneol-2-O-b-gentio-bioside, (-)-borneol-2-O-b-sophoroside and etc. were isolated and identified as products. It is considered that the hydroxylation of C-9 was more preferable than the hydroxylation of C-1 at C-5.

Acetates and hydrocarbons - The enantiospecific hydrolysis of the acetates with clone plants were experimented by Pawlowicz et al.[39-41] It seemed that R alchols are formed faster than S. in culture of clone *Spirodela oligorrhiza*. Menthyl acetate hydrolyzed enantiospecifically converted to (-)-menthol (R-configuration) mainly. Under the same conditions,

(±)-borneol was formed from bornyl acetate. The three racemic 2-hydroxy-dihydropinol acetates showed different hydrolysis in enantiospecifity with one another. The degree of hydrolysis was affected by the concentration of the substrate.

Hook et al.[42-44] found that the acyclic monoterpene, linallyl acetate was transformed into linallol(24%), geraniol(14%), and α-terpineol(4.5%) in suspension culture of *Papaver bracteatum* over 36 hr period. The same pattern of transformation resisted in cultivation over 14 days.

Aldehydes and ketones

The reductive capability of biotransformation of exogenous monoterpene aldehyde were tested by cell suspension culture of *Lavandula angustifolia*. Monoterpene aldehydes and related compounds were reduced to corresponding alcohols. The different rates of reduction at acyclic, cyclic and aromatic aldehydes suggested the presents of semi-specific reductase.[45]

Gbolade and Lochwood observed differences between the cultivars of *Petroselinum crispum cv.* in bioconversion of citral and citronellal into geraniol, nerol and citronellol.[46] They compared the conversion of monoterpenes by freely suspended and polyurethane foam-immobilized cells of *Petroselinum crispum*. The efficacy of the reduction of citral and citronellal by immobilization of cells was lower than that by suspended cells. It seemed that the immobilization of cells may not necessarily lead to higher capability of bioconversion.[47]

To approach to the problem that cultured cells were not able to accumulate the typical flavour of the mother plant, the conversion of citral, citronellal and geraniol into nerol and geraniol, and citronellol was investigated in cellsuspension cultures of *Melissa officinalis*.[48]

Noma et al. have used photosynthetic microorganisms to experiment the biotransformation of monoterpene aldehydes. The cultured *Dunaliella tertiolecta* reduced all saturated and unsaturated terpene aldehydes to corresponding alchols.[49]

In the experiment using *Euglena gracilis*, α-, β-unsaturated aldehydes were reduced to the corresponding alchol and then hydrogenated at the double bond. The saturated terpene aldehydes and aromatic aldehydes were transformed to the corresponding alcohol.[50]

Regio- and stereoselectivity on hydroxylation of bicyclic monoterpene,(+)- and (-)-fenchone were observed in suspension culture of *Eucalyptus perriniana*.[51] Six new glycosides were identified as products of biotransformation.

Oxides

1,8-cineol is the most useful monoterpene oxide at present. The hydroxylation of 1,8-cineol by *Glomerella cingulata*, a micro-organism was studied.[52] C-2 or C-3 carbon was hydroxylated and both of exo- and endo-hydroxy compounds were produced by transformation.

Ohihara and Furuya have experimented the hydroxylation and glucosylation of exogenous 1,8-cineol in cell culture of *Eucalyptus perriniana*.[53]

Rose oxide, one of the component in rose oil, is an another important and useful compound of this group. The biotransformation of two diastereoisomeric rose oxides were observed in experiments using *Asperigillus niger*.[54] The fungus oxidized C-9 of rose oxide diastereoselectively and produced the corresponding primary alcohol having *E*-configuration at C-7/C-8 double bond.

<Received 7 August, 1995>

References

1. Verpoorte, R., Harkes, P.A.A. and Ten Hoopen, H.J.G.: Plant cell culture as a tool in the production of secondary metabolites. prospects and problems. Topics in pharmaceutical Sciences, pp.263-281. Elsevier Science Publisher B.V, Amsterdam (1987)
2. Gbolade, A.A. and Lockwood, G.B.: Volatile constituents from parseley cultures. *Flavour Frag. J.* 4. 69 (1989)
3. Banthorpe, D.V., Branch, S.A., Poots, I. and Fordham, W.D.: Accumulation of 2-phenylethanol by callus derived from leaf-bud of *Rosa damascena, Phytochemistry* **27**, 795 (1988).
4. Bohm, H.: The inability of plant cell cultures to procuced secondary substances.,Proc.5th Intl.Cong. Plant Tissue and Cell Culture, pp.325-328, Japanese Assoc.Plant Tissue Culture, Tokyo (1982)
5 Dix, P.J.: Plant cell line selection, VCH, Weinheim (1990)
6. Bruns, B.K., Hahlbrock, K. and Schafer, E.: Fluence dependence of the ultraviolet-light-induced accumulation of chalcone synthase mRna and effects of blue and far-red light in cultured parseley cells, Planta **169**, 393 (1986)
7. Yamamda Y. and Hashimoto, T.: Possibilities for improving of secondary metabolites in plant cell cultures, Progress in plant cellular and molecular biology, Kluwer Academic Publishers, p. 547 (1990).
8. Evans, D.A., Sharp, W.R. and Ammirato, P.V.: Handbook of plant cell culture, Macmillan Publishing Company, New York P.264 (1987).
9. Christen, P., Roberts, M.F., Phillipson, J.D. and Evans, W.C.: High-yield production of tropane alkaloids by hairy-root cultures of a *Datura candida* hybrid, *Plant Cell Rep.* **8**, 75 (1989).
10. Carriere, F., Gil, G., Tapie, P. and Chagvardieff, P.: Biotransformation of geraniol by photoautotrophic, photomixotrophic and heterotrophic plant cell suspensions, *Phytochemistry* **28**, 1087 (1989).
11. Dicosmo, F.: Stragegies to improve yields of secondary metabolites to industrially interesting levels, Progress in Plant cellular and molecular biology, Kluwer Academic Publishers, p. 717 (1990).
12. Robins, R.J. and Rhodes, M.J.C.: Manipulating secondary metabolism in culture, Cambridge University Press, p.1521 (1988).
13. Charlwood, B. Hegarty, P.K. and Charlwood, K.A.: The synthesis and biotransformation of monoterpenes by plant cells in culture, Secondary metabolism in plant cell cultures, Cambridge University Press , pp.1503 (1986).
14. Mahato, S.B. and Majumdar, I.: Current Trends in microbial steroid biotransformation, *Phytochemistry* **34**, 883 (1993).
15. Noma, Y., Okajima, Y., Takahashi, H, Asakawa, Y.: Biotransformation of aromatic aldehydes and related compounds by *Euglena gracilis Z., Phytochemistry* **30**, 2969 (1991).
16. Corchete, P. and Yeoman, M.M.: Biotransformation of (-)-codeinone to (-)-codeine by *Papaver somniferum* cells immobilized in reticulated polyurethane foam., *Plant Cell Reports* **8**, 128 (1989).
17. Ishida, B.K.: Improved diosgenin production in *Dioscorea deltoidea* cell cultures by immobilization in polyurethane foam, *Plant Cell Reports* **7**, 270 (1988).
18. Shin, S., Kim, G.S. and Chi, H.J.: Production of essential oils by tissue culture of *Schizonepeta tenuifolia, Kor. J. Pharmacogn.* **25**, 31 (1994).
19. Banthorpe, D.V., Gray, T.J., Poots, I. and Fordaham, W.D.: Monoterpene metabolism in cultures of Rosa species, *Phytochemistry* **25**, 2321 (1986).
20. Shin, S., Kim, H.K. and Chi, H.J.: Production of giant hyssop oil by plant tissue culture, *Kor. J. Pharmacogn.* **22**, 91 (1991)
21. Suga, T., Toshifumi, H., Hamada, H. and Murakami, S.: Biotransformation of 3-oxo-p-menthane derivatives by cultured cells of *Nicotiana tabacum, Phytochemistry* **27**, 1041 (1988).
22. Hirata, T., Tang, Y.,Okano, K. and Suga, T.: Stereochemistry of reduction of the endocyclic double bond of (-)-carvone with the enzyme preparation from cultured cells of *Nicotiana tabacum, Phytochemistry* **28**, 3331 (1989).
23. Mulder-Krieger, Th., Verpoorte, R., Svendsen, A.B. and Scheffer J.J.C.: Production of essential oils and flavours in plant cell and tissue cultures. A review, *Plant cell, Tissue and*

Organ Culture **13**, 85 (1988).

24. Suga, T. and Hirata, T.: Biotransformation of exogenous substrates by plant cell cultures, *Phytochemistry* **29**, 2393 (1990).
25. Itokawa, H., Takeya, K and Akasu, M.: Stereochemistry of primary allylic alcohols by cell-free system of callus induced from *Cannabis sativa Chem. Pharm. Bull.* **24**, 1681 (1976).
26. Itokawa, H., Takeya, K and Mihashi, S.: Biotransformation of Cannabinoid Precursors and related alcohols by suspension sultures of callus induced from *Cannabis sativa* L., *Chem. Pharm. Bull.* **25**, 1941 (1977).
27. Suga, T., Hirata, T. and Lee, Y.S.,: The enantioselective biotransformation of α-terpineol and its acetate with the cultured cells of *Nicotiana tabacum., Chem. Letters*, 1595 (1982).
28. Suga, T., Hirata, T. and Izumi, S.: Enatioselectivity in the hydrolysis of bicyclic monoterpene acetates with the cultured cells of Nicotiana tabacum, *Phytochemistry* **25**, 2791 (1986).
30. Hirata, T., Lee, Y.S. and Suga, T.: The stereospecific hydroxylation of endocyclic ethylenic linkage in the biotransformation of α-terpinyl acetate with cultured suspension cells of *Nicotiana tabacum, Chem. letters*, 671 (1982).
31. Suga, T., Hirata, T., Hamada, H. and Futatsugi, M.: Enantioselectivity in the biotransformation of bicyclic monoterpene alcohols with the cultured suspension cells of *Nicotiana tabacum, Plant Cell Reports* **2**, 186 (1983).
32. Hirata, T., Izumi, S., Ekida, T. and Suga, T.: Hydroxylation of acetoxy-p-menthenes in the cultured cells of *Nicotiana tabacum*. Epoxidation of C-C double bond., *Bull. Chem. Soc. Jpn.* **60**, 289 (1987)
33. Suga, T., Hirata, T. and Hamada, H.: The stereochemistry of the reduction of C-C double bond with the cultured cells of *Nicotiana tabacum, Bull. Chem. Soc. Jpn.* **59**, 2865 (1986).
34. Hamada, H.: Enantioselectivity in the biotransformation of mono- and bicyclic monoterpenoids with the cultured cells of *Nicotiana tabacum, Bull. Chem. Soc. Jpn.* **61**, 869 (1988).
35. Asakawa, Y., Takahashi, H., Toyota, M. and Noma, Y.: Boitransformation of monoterpenoids, (-)-and (+)-menthols, terpinolene and carvotanacetone by *Aspergillus* Species, *Phytochemistry* **30**, 3981 (1991).
36. Abraham W.R.: Phylogeny and Biotransformation. Part 5: Biotransformation of isopinocampheol, *Z. Naturforsch.* **49c**, 553 (1994).
37. Abraham W.-R., Arfmann H.-A: Microbial hydroxylation of activated acyclic monoterpene hydrocarbon, *Tetrahedron* **48**, 6681 (1992).
38. Orihara, Y. and Furuya, T.: Biotransformation of (-)-borneol by culrtured cells of *Eucalyptus perriniana, Phytochemistry* **34**, 1045 (1993).
39. Pawlowicz, P., Piatkowski, K. and Siewiski, A.: Enantiospecific hydrolysis of acetates of racemic monoterpenic alcohols by *Spirodela oligorrhiza, Phytochemistry* **27**, 2809 (1988).
40. Tlomak, E., Pawlowicz, P., Czerwi ski, W. and Siewiski, A.: Transformation of androstane derivatives by *Spirodella oligorrhiza, Phytochemistry* **25**, 61 (1986).
41. Pawlowicz, P. and Siewiski, A.: Enatioselective hydrolysis of esters and the oxidation of aromatic-aliphatic alcohols obtained therefrom by *Spirodela oligorrhiza, Phytochemisty* **26**, 1001 (1987).
42. Hook, I., Lecky, R., Mckenna, B. and Sheridan, H.: Biotransformation of linalyl acetate by suspension cultures of Papaver bracteatum, *Phytochemistry* **29**, 2143 (1990).
43. Hook, I., Sheridan, H. and Wilson, G.: Alkaloids of cell cultures derived from strains of *Papaver bracteatum, Phytochemistry* **27**, 2137 (1988).
44. Hennesy, D. Hook, I, McGee, A. and Sheridan, H.: Hydrocinnamic acid esters from cell suspension cultures and plant of *Leontopodium alpinum, Phytochemistry* **28**, 489 (1989).
45. Lappin, G.J., Stride, J.D. and Tampion, J.: Biotransformation of monoterpenoids by suspension cultures of *Lavandula angustifolia, Phytochemistry* **26**, 995 (1987).
46. Gbolade, A.A. and Lockwood, G.B.: Metabolic studies of volatile constituents in tissue cultures of *Petroselinum crispum* (Mill) Nyman, *J. Plant Physiol.* **136**, 198 (1990).

47. Gbolade, A.A. and Lockwood, G.B.: Biotransformation of monoterpenes by polyurethane foam-immobilized cells of *Petroselinum crispum* (Mill) Nyman, *Z., Naturforsch.* **45c**, 245 (1990).
48. Gbolade, A.A. and Lockwood, G.B.: Metabolic studies of volatile constituents in tissue cultures of *Melissa officinalis* L., *J. Plant Physiol.* **140**, 28 (1992).
49. Noma, Y., Akehi, E., Miki, N. and Asakawa, Y.: Biotransformation of terpene aldehydes, aromatic aldehydes and related compounds by *Dunaliella tertiolecta*, *Phytochemistry* **31**, 515 (1992).
50. Noma, Y., Takahashi, H. and Asakawa, Y.: Biotransformation of terpene aldehydes by *Euglena gracilis Z*, *Phytochemistry* **30**, 1147 (1991).
51. Orihara, Y. and Furuya, T.: Biotransformation of (+)- and (-) fenchone by cultured cells of *Eucalyptus perriniana*, *Phytochemistry* **36**, 55 (1994).
52. Gbolade, A.: Biotransformation of monoterpenes, *Zeitschrift für Naturforschung C.* **45**, 245 (1990).
53. Orihara, Y. and Furuya, T.: Biotransformation of 1,8-cineole by cultured cells of *Eucalyptus perriniana*, *Phytochemistry* **35**, 641 (1994).
54. Miyazawa, M. Yokote, K. and Kameoka, H.: Biotransformation of the monoterpenoid, rose oxide, by *Aspergillus niger*, *Phytochemistry* **39**, 85 (1995).