Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 17 (2006) 2287–2291

Plants-mediated reduction in the synthesis of homochiral secondary alcohols

Renato Bruni,[a] Giancarlo Fantin,[b] Silvia Maietti,[c] Alessandro Medici,[c] Paola Pedrini[c,*] and Gianni Sacchetti[c]

[a]*Dipartimento di Biologia evolutiva e funzionale (Sez. Biologia vegetale ed Orto botanico), Università di Parma, Via delle Scienze 5, I-43100 Parma, Italy*
[b]*Dipartimento di Chimica, Università di Ferrara, Via L. Borsari 46, I-44100 Ferrara, Italy*
[c]*Dipartimento delle Risorse Naturali e Culturali, Università di Ferrara, C.so Ercole I d'Este 32, I-44100 Ferrara, Italy*

Received 18 July 2006; accepted 21 July 2006
Available online 22 August 2006

Abstract—The reduction of 5-hexen-2-one **1**, 6-methyl-5-hepten-2-one **2**, acetophenone **3**, *cis*-bicyclo[3.2.0]hept-2-en-6-one **4** and 2-methylcyclohexanone **5** with various commercially available plants (i.e., *Brassica oleracea botrytis*, *Cucurbita maxima*, *Cucurbita pepo*, *Cynara scolimus*, *Daucus carota*, *Foeniculum vulgare* and *Musa sapientum*) is reported. In the reduction of ketones **1**–**3**, both (*S*)- and (*R*)-enantiomers **6**–**8** were obtained in good yields and with appreciable enantiomeric excesses. With racemic ketones **4** and **5**, both the diastereomeric *endo*/*exo* **9** and **10** and *cis*/*trans* **11** and **12** are produced with variable yields and enantiomeric excesses depending on the various plants used.

1. Introduction

Enantiomerically pure secondary alcohols are important as intermediates and chiral auxiliaries in organic synthesis[1,2] and common as pheromones,[3] aroma and flavour enhancing compounds. Among the current methodologies to obtain these chiral compounds, are the microbial or enzymatic reduction of the corresponding ketones and the kinetic resolution with lipases of the racemic alcohols via esterification or hydrolysis of the appropriate esters. More recently our work described the kinetic resolution of racemic secondary alcohols via oxidation with BY,[4] *Bacillus stearothermophilus*,[5,6] and *Yarrowia lipolytica*,[7] and a combination of this methodology with the complementary microbial reduction afforded excellent results.[8,9] In this field, an alternative approach is given by the use of in vitro plant cells.

Plant cell cultures have an important potential to perform biochemical reactions on organic compounds.[10] Most of these reactions, so far, have been confined to the biotransformation of secondary metabolites produced by plant cells.[11] Moreover, reductions of ketones and aldehydes of secondary metabolites using plant cell cultures occurred stereospecifically.[12–17] Over the last decade, various examples of the reduction of prochiral ketones to chiral alcohols are reported using growing[18–21] or immobilized[22,23] plant cell cultures. Only recently has the possibility of directly using parts of plants as biocatalysts been investigated using freshly cut carrot root in the reduction of 2-methyl- and 2-hydroxycyclohexanone.[24] In our recent and preliminary work, we reported the reduction of acetophenone with various uncultured cell plants[25] as a further tool for the organic chemist instead of BY in the synthesis of homochiral secondary alcohols.

Due to the importance of these compounds, we herein report the enantioselectivity observed in the reduction of ketones **1**–**5** (Scheme 1) to the corresponding homochiral alcohols using a series of plants both as differentiated and as undifferentiated cells.

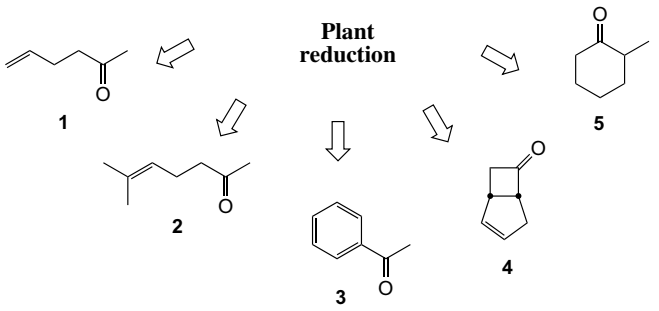
2. Results and discussion

2.1. Reduction with plants (differentiated cells)

All the substrates are incubated with differentiated cells of various plants, selected on the basis of the previous

* Corresponding author. Tel.: +39 0532 293776; e-mail: pdp@unife.it

0957-4166/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2006.07.029



Scheme 1.

screening carried out on acetophenone.[25] Table 1 reports the parts (differentiated cells) of the plants utilized in the reduction of ketones **1**–**5**.

All the listed plants gave reduction products even if not with all substrates. The biotransformation procedure is very simple owing to the easy availability of plants, the use of water without a carbon source and the simple work up, because no emulsion is formed. In Table 2 the most interesting results in the synthesis of homochiral alcohols **6**–**12** with a selected number of plants that reduce all the substrates, are reported.

5-Hexen-2-one **1** was quantitatively reduced to (*S*)-hexenol **6** (ee 60%) by *Daucus carota* (carrot) in 4–6 days depending on the concentration of substrate. Furthermore *Brassica oleraceae bothrytis* (cauliflower), *Cucurbita maxima* (pumpkin), *Foeniculum vulgare* (fennel) and *Musa sapientum* (banana) afforded the same enantiomer with variable yields (23–59%) and enantiomeric excesses (11–80%). The (*R*)-hexenol, on the other hand, was obtained by reduction with *Cucurbita pepo* (marrow) in 63% yield (ee 74%) while *Cynara scolimus* (artichoke) initially produces the (*S*)-enantiomer in 63% yield with low ee (23%). After 4 days, the yield decreases with the presence of a greater quantity of the (*R*)-alcohol **6**. The lower yield and the inversion of configuration are probably due to the oxidation of the (*S*)-alcohol, carried out by the same enzyme.

(*S*)-6-Methyl-5-hepten-2-ol **7** [(*S*)-sulcatol] is obtained with excellent yields (92–97%) by reduction with *D. carota* (ee 62%) and *F. vulgare* (ee 90%). All the other plants gave worse yields (7–38%) but with valuable ees (40–72%). On the other hand, the (*R*)-sulcatol (ee 56%) **7** was only obtained in poor yield (13%) by *C. scolimus* reduction.

D. carrot shows the best activity in the reduction of acetophenone **3** giving pure (*S*)-1-phenylethanol **8** in quantitative yield. Excellent enantiomeric excesses (89–100%) but lower yields of the same (*S*)-enantiomer (20–80%) were obtained with *F. vulgare*, *C. maxima*, *M. sapientum* and *C. pepo* while *B. oleracea botrytis* afforded good yields (52%) of the (*R*)-1-phenylethanol (ee 78%).

In the reduction of *cis*-bicyclo[3.2.0]hept-2-en-6-one **4**, all plants produced the diastereomeric *endo*-(1*S*,5*R*,6*S*)-bicycloheptenol **9** and the *exo*-(1*R*,5*S*,6*S*)-bicycloheptenol **10** in variable yields and enantiomeric excesses. The best result regarding the yields was obtained after 6 days with *D. carota* (30 g) that reduces ketone **4** (1 g/L) to afford altogether 77% of the alcohols **9** and **10** (50% and 27%, respectively) with quite good ees (72% and 54%, respectively). Interesting results were also achieved with *B. oleracea botrytis* (for **9**: 27% with ee 70%; for **10**: 10% with ee 85%) and *C. scolimus* (for **9**: 17% with ee 85%; for **10**: 17% with ee 72%) after 2 days of incubation. Only the *exo*-alcohol **10**, however, was obtained enantiomerically pure both with *D. carota* (15 g) after 4 days starting from 0.1 g/L of ketone and with *M. sapientum* (30 g), after 6 days starting from 1 g/L of **4**.

D. carota is also especially active in the reduction of 2-methylcyclohexanone **5** giving, after 2 days, the (1*S*,2*R*)-*cis*-methylcyclohexanol **11** (46%, ee 20%) and (1*S*,2*S*)-*trans*-methylcyclohexanol **12** (54%, ee 90%). Good yields (26–52%) and enantiomeric excesses (70–92%) of the *trans*-alcohols **12** were also achieved with *C. maxima*, *C. scolimus* and *F. vulgare* while, with yields as good (26–63%) as for **12**, the *cis*-alcohol **11** was produced with lower enantiomeric excesses (10–30%). On the other hand, low yields and ees both of *cis*-**11** and *trans*-**12** were obtained by the reductions with *B. oleracea botrytis* and *M. sapientum*.

2.2. Reduction with plants (undifferentiated cells)

Even if the reduction of ketones with differentiated cells was very simple, this method did have some drawbacks, regarding the possible use of various cultivars for the same species and the different activity of the plant cells due to the different season of harvesting them.

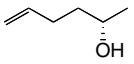
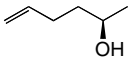
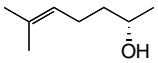
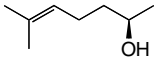
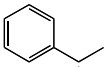
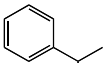
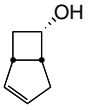
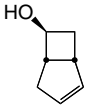
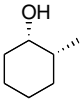
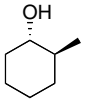
In an attempt to avoid the potential irreproducibility of the biotransformations, undifferentiated cells of some plants were produced (Table 3) and tested in the reduction of ketones **3** and **5** that have presented great affinity for most of the plants.

The choice of the plants was based firstly on their wide diffusion as agro-industrial and by-products sources. In fact,

Table 1. Plants (differentiated cells) utilized in reduction

Plants	Common name	Family	Part
Brassica oleracea botrytis	Cauliflower	Brassicaceae	Inflorescence
Cucurbita maxima	Pumpkin	Cucurbitaceae	Fruit
Cucurbita pepo	Marrow, field pumpkin	Cucurbitaceae	Fruit
Cynara scolymus	Artichoke	Asteraceae	Floral stalk
Daucus carota	Carrot	Apiaceae	Root
Foeniculum vulgare	Fennel	Apiaceae	Stalk
Musa sapientum	Banana	Musaceae	Fruit

Table 2. Reduction of ketones **1**–**5** with plants (differentiated cells)

Ketone	Plants (g/100 mL H_2O)	Time (days)	Products	Yield (%) (ee, %)
1[a]	*Daucus carota* (15)	4		**100** (60)
1[b]	*Daucus carota* (18)	6		**95** (60)
1[a]	*Cucurbita maxima* (14)	4	 (*S*)-**6**	**59** (77)
1[a]	*Foeniculum vulgare* (25)	4		**54** (11)
1[b]	*Musa sapientum* (30)	6		**35** (80)
1[b]	*Brassica oleracea botrytis* (30)	6		**23** (36)
1[a]	*Cucurbita pepo* (11)	4	 (*R*)-**6**	**63** (74)
1[a]	*Cynara scolimus* (11)	1	(*S*)-**6**	**68** (23)
		4	(*R*)-**6**	**51** (16)
2[b]	*Daucus carota* (21)	6		**97** (62)
2[b]	*Foeniculum vulgare* (30)	3		**92** (90)
2[b]	*Cucurbita maxima* (30)	6	 (*S*)-**7**	**38** (40)
2[b]	*Cucurbita pepo* (30)	6		**12** (72)
2[b]	*Musa sapientum* (30)	6		**13** (66)
2[b]	*Brassica oleracea botrytis* (30)	6		**7** (49)
2[b]	*Cynara scolimus* (30)	6	 (*R*)-**7**	**13** (56)
3[a]	*Daucus carota* (15)	1		**100** (100)
3[a]	*Foeniculum vulgare* (18)	2		**80** (100)
3[a]	*Cucurbita maxima* (16)	2		**79** (90)
3[a]	*Musa sapientum* (15)	1		**55** (100)
3[a]	*Cucurbita pepo* (16)	2	 (*S*)- **8**	**20** (89)
3[a]	*Cynara scolimus* (12)	1		**15** (48)
3[a]	*Brassica oleracea botrytis* (17)	1	 (*R*)-**8**	**52** (78)
4[a]	*Brassica oleracea botrytis* (20)	2		**27** (70)
				10 (85)
4[a]	*Daucus carota* (15)	4		**8** (80)
				22 (100)
4[b]	*Daucus carota* (30)	6	 (1*S*,5*R*,6*S*)-**9**	**50** (72)
				27 (54)
4[a]	*Cucurbita maxima* (19)	2	and	**10** (30)
				4 (12)
4[a]	*Cynara scolimus* (10)	2		**17** (85)
				17 (72)
4[a]	*Foeniculum vulgare* (17)	2	(1*R*,5*S*,6*S*)-**10**	**5** (75)
				8 (65)
4[b]	*Musa sapientum* (30)	6		**12** (25)
				9 (100)
5[b]	*Brassica oleracea botrytis* (30)	6		**11** (20)
				6 (92)
5[b]	*Daucus carota* (30)	2	(1*S*,2*R*)-**11**	**46** (20)
				54 (90)
5[b]	*Cucurbita maxima* (30)	6		**34** (24)
			and	**26** (92)
5[b]	*Cynara scolimus* (30)	6		**63** (10)
				30 (70)
5[b]	*Foeniculum volgare* (30)	4	(1*S*,2*S*)-**12**	**26** (30)
				52 (92)
5[b]	*Musa sapientum* (30)	6		**15** (20)
				12 (20)

[a] Concentration of ketone 0.1 g/L.
[b] Concentration of ketone 1 g/L.

their ability to act as enantioselective biotransformation tools could open new perspectives of agro-industrial exploitation of the biomasses mainly towards the production of functional compounds (e.g., pharmaceuticals). Some of the plants are instead known for their importance (i.e., *Zanthoxylum stenophyllum*) and their possible biotransformative capacity could widen their biotechnological uses, both for pharmaceutical compounds produced

Table 3. Plants (undifferentiated cells) utilized in reduction

Plants	Common name	Family	Part
Daucus carota	Carrot	Apiacee	Callus
Ficus benjamina	Ficus	Moraceae	Callus
Oryza sativa (Roma)	Rice	Graminaceae	Callus
Oryza sativa (vialone)	Rice	Graminaceae	Callus
Papaver rhoeas	Poppy	Papaveraceae	Callus
Zanthoxylum stenophyllum (white)	Narrow-leaf prickly ash	Rutaceae	Callus
Zanthoxylum stenophyllum (yellow)	Narrow-leaf prickly ash	Rutaceae	Callus

Table 4. Reduction of ketones **3** and **5** with plants (undifferentiated cells)

Ketone[a]	Plants	Time (days)	Reaction product	Yield (%) (ee, %)
3	*Daucus carota*	10	(*S*)-**8**	**46** (100)
	Oryza sativa (*Roma*)	14		**86** (100)
	Zanthoxylum stenophyllum (*yellow*)	14		**11** (100)
5	*Daucus carota*	12	(1*S*,2*R*)-**11** and (1*S*,2*S*)-**12**	**49** (25) **51** (100)
	Ficus benjamina	8		**35** (33) **65** (100)

[a] Concentration of ketone 0.1 g/L.

in vitro[26] and for the enantioselective transformation of functional compounds. Secondly, some of the plants checked were chosen because of the very good results previously shown in the reduction of all the substrates (i.e., *D. carota*). The results of the reduction of ketones **3** and **5** are summarized in Table 4.

The undifferentiated cells of *D. carota* show a lower activity towards acetophenone **3** than the differentiated cells. In fact after 10 days, (*S*)-alcohol **8** is only produced in 46% yield, while the enantiomeric excess remains excellent (100%). Conversely with 2-methylcyclohexanone **5**, both yields and enantiomeric excesses of the diastereomeric alcohols **11** and **12** are practically unaltered although the biotransformation time is longer (12 days).

An excellent result was obtained in the reduction of ketone **3** with *Oryza sativa* that after 14 days afforded the pure alcohol (*S*)-**8** in 86% yield. Lower yields (11%) of the same product, however, were obtained with *Zanthoxylum stenophyllum* (*yellow*) but always with high ees.

Regarding the reduction of ketone **5**, among the plant undifferentiated cells listed in Table 4, only *Ficus benjamina* gave the reduction products **11** and **12** (35% and 65% yield, respectively) with ees comparable with those obtained with *D. carota*. No reduction products, starting both from the ketones **3** and **5**, are obtained with the other plants listed in Table 4.

3. Conclusions

In conclusion we can see that differentiated cells are more efficient in the reduction of the selected ketones than the undifferentiated cells. These exhibit lower yields and longer time against greater reproducibility. These drawbacks can be overcome by extracting the enzyme responsible for the reduction. Work is currently in progress in our laboratory focussing on *D. carota*.

4. Experimental

5-Hexen-2-one **1**, 6-methyl-5-hepten-2-one **2**, acetophenone **3**, *cis*-bicyclo[3.2.0]hept-2-en-6-one **4** and 2-methylcyclohexanone **5**, 5-hexen-2-ol **6**, 1-phenylethanol **8**, *cis*-2-methylcyclohexanol **11**, *trans*-2-methylcyclohexanol **12**, biotin, 2,4-dichlophenoxyacetic acid, 1-naphthalenacetic acid, kinetin, 6-benzylaminopurine and Gamborg's B-5 basal salt mixture (Sigma) are commercially available. 6-Methyl-5-hepten-2-ol **7**, *endo*-bicyclo[3.2.0]hept-2-en-6-ol **9** and *exo*-bicyclo[3.2.0]hept-2-en-6-ol **10** were prepared from the corresponding available ketones (Merck and Fluka) by reduction with $NaBH_4$.

4.1. Enantiomer separation

Gas chromatographic analyses were performed on a Carlo Erba GC 6000 Vega series 2.

Enantiomer separation on Megadex 5 column (25 m × 0.25 mm) containing dimethyl-*n*-pentyl β-cyclodextrin in OV 1701 from Mega. For the reduction of **1**, carrier gas: helium 82 kPa; temp 80–150 °C (0.5 °C/min), retention time (min): **1**, 4.41; (*S*)-**7**, 6.95; (*R*)-**7**, 7.08. For the reduction of **2**, carrier gas: helium 82 kPa; temp 80–200 °C (1.5 °C/min), retention time (min): **2**, 9.45; (*S*)-**7**, 13.27; (*R*)-**7**, 13.85. For reduction of **3**, carrier gas: helium 70 kPa; temp 70–200 °C (1.5 °C/min), retention time (min): **3**, 9.39; (*R*)-**8**, 14.59; (*S*)-**8**, 15.43. For the reduction of **4**, carrier gas: helium 80 kPa; temp 100–200 °C (1.5 °C/min), retention time (min): (1*S*,5*R*)-**4**, 5.74; (1*R*,5*S*)-**4**, 5.97; (1*S*,5*R*,6*R*)-**10** (as acetyl derivative), 9.29; (1*R*,5*S*,6*S*)-**10** (as acetyl derivative), 9.40; (1*S*,5*R*,6*S*)-**9** (as acetyl derivative), 10.99; (1*R*,5*S*,6*R*)-**9** (as acetyl derivative), 11.73. For reduction of **5**, the separation was achieved on a Megadex DETTBSβ column (25 m × 0.25 mm) containing diethyl-*tert*-butylsilyl β-cyclodextrin in OV 1701 from Mega, carrier gas: helium 100 kPa; temp 70–200 °C (1.5 °C/min), retention time (min): (*S*)-**5**, 8.27; (*R*)-**5**, 8.53; (1*S*,2*S*)-**12**, 9.60; (1*R*,2*R*)-**12**, 9.78; (1*R*,2*S*)-**11** (as acetyl derivative), 10.77; (1*S*,2*R*)-**11** (as acetyl derivative), 12.01.

The absolute configurations of the compounds were determined by comparing the sign of their specific rotation with those of the literature: for (*R*)-**6**[8] $[\alpha]_D = -12.1$ (*c* 4.6, $CHCl_3$); for (*R*)-**7**[8] $[\alpha]_D = -14.5$ (*c* 1.3, EtOH); for (*R*)-**8**[5]

$[\alpha]_D = +41$ (*c* 5.1, $CHCl_3$); for (1*R*,5*S*,6*R*)-**9**[6] $[\alpha]_D = -68$ (*c* 1.1, $CHCl_3$); for (1*R*,5*S*,6*S*)-**10**[27] $[\alpha]_D = -91$ (*c* 2.6, $CHCl_3$); for (1*S*,2*R*)-**11**[28] $[\alpha]_D = +18$ (*c* 1.0, MeOH); for (1*R*,2*R*)-**12**[29] $[\alpha]_D = -38.2$ (*c* 9.6, EtOH).

4.2. Reduction of ketones 1–5 with plants (differentiated cells)

The plants (Table 1) were washed with 5% sodium hypochlorite and then with ethanol, peeled with a sterilized cutter and cut under a sterile hood. To a stirred suspension of the selected plant prepared as described (20–30 g, see Table 3) in water (100 mL), chloramphenicol (60 mg) and the proper substrate (0.1 g in 1 ml of DMSO) were added. The suspension was maintained at 25 °C in the dark and aliquots were periodically withdrawn and monitored by GLC using biphenyl as the internal standard. The best results are reported in Table 2.

4.3. Preparation of plant undifferentiated cells. General procedure

The agarized culture medium was prepared dissolving in demineralized water (1 L), Gamborg's B-5 basal salt mixture (Sigma) (3.69 g/L), saccharose (30 g/L) and Agar (10 g/L). For *D. carota* and *Zanthoxylum stenophyllum* biotin (1×10^{-4} g/L), 2,4-dichlophenoxyacetic acid (2,4-DA, 2×10^{-4} g/L), 1-naphthalenacetic acid (NNA, 1×10^{-3} g/L) and kinetin (1×10^{-3} g/L) were added. For *Oriza sativa*, 2,4-DA (0.5×10^{-3} g/L) and 6-benzylaminopurine (6-BAP, 0.25×10^{-3} g/L) were added. For *Papaver rhoeas*, 2,4-DA (0.5×10^{-3} g/L) and 6-BAP (0.125×10^{-3} g/L) were added. The plants are washed with demineralized sterile water, poured in 5% sodium hypochlorite for 30 min, washed again with water and then treated with ethanol (20 min). The superficial layer (1–2 cm) was removed and a piece of the selected plant (5 mm) obtained with a sterilized cutter under a sterile hood was poured in Petri dishes with the appropriate agarized medium. After 2 weeks at 25 °C in the dark, an appreciable amount of undifferentiated cells was produced and used for the reduction process.

4.4. Reduction of ketones 3 and 5 with plants (undifferentiated cells)

The culture medium was prepared by dissolving in demineralized water (1 L), a Gamborg's B-5 basal salt mixture (Sigma) (3.69 g/L), saccharose (30 g/L), biotin (1×10^{-4} g/L), 2,4-dichlophenoxyacetic acid (2,4-DA, 2×10^{-4} g/L), 1-naphthalenacetic acid (NNA, 1×10^{-3} g/L) and kinetin (1×10^{-3} g/L).

The culture medium (100 mL), previously sterilized at 120 °C for 20 min, was inoculated with the undifferentiated cells (1 g) of the selected plant (Table 3), and chloramphenicol (40 mg) was added. The suspension was stirred (120 rpm) for 24 h in the dark at room temperature. A grown culture was added followed by the proper substrate (100 μL from a solution containing 0.1 g in 1 ml of EtOH). Aliquots were withdrawn periodically and monitored by GLC using biphenyl as the internal standard. The best results are reported in Table 4.

References

1. Stinson, S. C. *Chem. Eng. News* **1992**, 46.
2. Morrison, J. D. *Asymmetric Synthesis*; Academic Press: New York, 1984.
3. Mori, K. *Tetrahedron* **1989**, *45*, 3233.
4. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Poli, S.; Sinigaglia, M. *Tetrahedron Lett.* **1993**, *34*, 883.
5. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Poli, S. *Tetrahedron: Asymmetry* **1993**, *4*, 1607.
6. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Rosini, G. *Tetrahedron: Asymmetry* **1994**, *5*, 1635.
7. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Fontana, S. *Tetrahedron: Asymmetry* **2000**, *11*, 2367.
8. Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Medici, A.; Pedrini, P. *Tetrahedron: Asymmetry* **1995**, *6*, 3047.
9. Fantin, G.; Fogagnolo, M.; Marotta, E.; Medici, A.; Pedrini, P.; Righi, P. *Chem. Lett.* **1996**, 511.
10. Reinhard, E.; Alfermann, A. W. *Adv. Biochem. Eng.* **1980**, *16*, 49.
11. Alfermann, A. W. In *Biocatalysis in Organic Synthesis*; Tramper, J., Van der Plas, H. C., Linko, P., Eds.; Elsevier: Amsterdam, 1985; p 25.
12. Ishihara, K.; Hamada, H.; Hirata, T.; Nakajima, N. *J. Mol. Catal. B: Enzym.* **2003**, *23*, 145.
13. Hirata, T.; Hamada, T.; Aoki, T.; Suga, T. *Phytochemistry* **1982**, *21*, 2209.
14. Lappin, G. J.; Stride, J. D.; Tampion, J. *Phytochemistry* **1987**, *26*, 995.
15. Hamada, H.; Nakamura, N.; Ito, S.; Kawabe, S.; Funamoto, T. *Phytochemistry* **1988**, *27*, 3807.
16. Hamada, H. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 869.
17. Gotoh, S.; Akoi, M.; Iwaeda, T.; Izumi, S.; Hirata, T. *Chem. Lett.* **1994**, 1519.
18. Villa, R.; Molinari, F.; Levati, M.; Aragozzini, F. *Biotechnol. Lett.* **1998**, *20*, 1105.
19. Nakamura, K.; Miyoshi, H.; Sugiyama, T.; Hamada, H. *Phytochemistry* **1995**, *40*, 1419.
20. Kergomard, A.; Renard, M. F.; Veschambre, H.; Courtois, D.; Petiard, V. *Phytochemistry* **1988**, *27*, 407.
21. Takemoto, M.; Yamamoto, Y.; Achiwa, K. *Chem. Pharm. Bull.* **1998**, *46*, 419.
22. Naoshima, Y.; Akakabe, Y. *J. Org. Chem.* **1989**, *54*, 4237.
23. Naoshima, Y.; Akakabe, Y. *Phytochemistry* **1991**, *30*, 3595.
24. Baldassarre, F.; Bertoni, G.; Chiappe, C.; Marioni, F. *J. Mol. Catal. B: Enzym.* **2000**, *11*, 55.
25. Bruni, R.; Fantin, G.; Medici, A.; Pedrini, P.; Sacchetti, G. *Tetrahedron Lett.* **2002**, *43*, 3377.
26. Biondi, S.; Antognoni, F.; Crespi Perellino, N.; Sacchetti, G.; Minghetti, A.; Poli, F. *Plant Biosyst.* **2004**, *138*, 117.
27. Dawson, M. J.; Lawrence, G. C.; Lilley, G.; Todd, M.; Noble, D.; Green, S. M.; Roberts, S. M.; Wallace, T. W.; Newton, R. F.; Carter, M. C.; Hallett, P.; Paton, J.; Reynolds, D. P.; Young, S. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2119.
28. Matsumoto, K.; Kawabata, Y.; Takahashi, J.; Fujita, Y.; Hatanaka, M. *Chem. Lett.* **1998**, 283.
29. Hirata, T.; Izumi, S.; Akita, K.; Yoshida, H.; Gotoh, S. *Tetrahedron: Asymmetry* **1993**, *4*, 1465.