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Plants in organic synthesis: an alternative to baker's yeast

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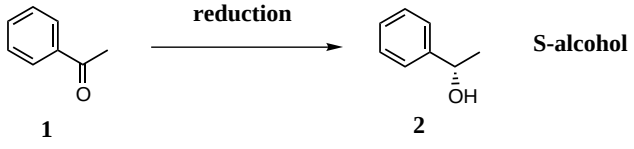
Abstract—The reduction of acetophenone **1** and the hydrolysis of 1-acetoxy-2-methylcyclohexene **3** with various commercially available plants to the corresponding *S*-carbinol **2** and *S*-ketone **4** are described. The further incubation of 2-methylcyclohexanone **4** with some plants affords the enantiomerically pure *trans*- and/or *cis*-alcohol **5** and **6**, respectively.

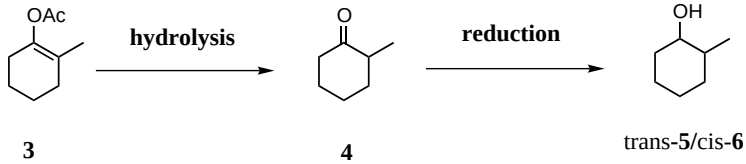
The biochemical potential of plant cell cultures to produce specific secondary metabolites such as drugs, flavours, pigments and agrochemicals is of considerable interest in connection with their biotechnological utilisation.[1] However, these cultures retain the ability to transform specifically exogenous substrates administered to the culture cells.[2,3] For the last 20 years plant cell cultures have been used for the transformation of important classes of compounds such as phenylpropanoids, terpenoids and alkaloids.[4] Moreover, reductions of ketones and aldehydes of secondary metabolites using plant cell cultures occurred stereospecifically.[5–9] In the last decade various examples of reduction of prochiral ketones to chiral alcohols are reported using growing[10–13] or immobilised[14,15] plant cells cultures. Only recently the possibility to directly use parts of plants as biocatalyst has been investigated using freshly cut carrot root in the reduction of 2-methyl- and 2-hydroxy cyclohexanone.[16]



Scheme 1.

In this paper the reduction of acetophenone **1** (Scheme 1) and the hydrolysis, followed, in some cases, by reduction, of 1-acetoxy-2-methylcyclohexene **3** (Scheme 2) with various plants[17] are described.

The biotransformation procedure is very simple and can be an alternative to baker's yeast owing the easy availability of plants, the use of water without carbon source and the simple work up because no emulsion is formed. The screening procedure is as follows: to a stirred suspension, in water (100 mL), of the selected plant (40 g), finely cut in sterile environment,[18] chloramphenicol (60 mg) and the proper substrate (0.1 g) in ethanol (1 mL) are added at 25°C. Aliquots were withdrawn periodically and monitored by GLC.[19] Simple filtration of the suspension and extraction with diethyl ether afford the reaction products. The most significant results obtained in the reduction of acetophenone **1** and in the hydrolysis of enol acetate **3** are summarised in Tables 1 and 2, respectively.



Scheme 2.

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Table 1. Reduction of acetophenone with plants

Plants	Time[a] (days)	Alcohol **2**	
		Yield (%)	Ee % (abs. conf.)
Carrot (*D. carota*)[b]	3	100	100 (*S*)
Fennel (*F. vulgare*)[c]	3	37	100 (*S*)
Marrow (*C. pepo*)[d]	3	10	100 (*S*)

[a] All biotransformations are monitored by GLC at 1, 3 and 5 days.
[b] Root.
[c] Stalk.
[d] Fruit.

Reduction of ketone 1

Carrot (*Daucus carota*, root) reduces quantitatively (after 3 days) the prochiral ketone **1** to the pure *S*-methylphenylcarbinol **2** (ee 100%). Lower yields are obtained with fennel (*Foeniculum vulgare*, stalk) and marrow (*Cucurbita pepo*, fruit), 37 and 10%, respectively, although with high enantioselectivity (ee 100%). Other plants as aubergine (*Solanum melongena*, fruit), cucumber (*Cucumis sativus*, fruit), white and red onion (*Allium cepa*, bulb), garlic (*Allium sativum*, bulb) and radish (*Raphanus sativus*, rhizome) do not give reduction either after 5 days incubation.

Hydrolysis and reduction of enol acetate 3

More interesting is the hydrolysis of 1-acetoxy-2-methylcyclohexene **3** with a very large variety of plants. All plants produce the 2-methylcyclohexanone **4** in high yields by hydrolysis of the enol acetate **3** in a few hours. In particular cucumber, mamey sapote (*Pouteria sapota*, fruit), banana passion fruit (*Passiflora tarminiana*, fruit), white and red onion, garlic and radish give the *S*-ketone **4** with fairly good enantiomeric excesses (33–44%) in 8–24 h, while fennel, marrow, aubergine and chayote squash (*Sechium edule*, fruit) afford the same product with lower enantioselectivity (ee 3–17%).

On the other hand, carrot, cherimoya (*Annona cherimola*, fruit), wild cucumber (*Cyclanthera pedata*, fruit), giant granadilla (*Passiflora quadrangularis*, fruit), and oca (*Oxalis tuberosa*, tuber), after the hydrolysis of the enol acetate **3** to 2-methylcyclohexanone **4**, reduce the latter to the homochiral *trans*- and/or *cis*-alcohols **5** and **6**, respectively. In particular, carrot, after 2 h

Table 2. Hydrolysis and reduction of 1-acetoxy-2-methylcyclohexene with plants

Plants	Time[a] (h)	Ketone **4**		*trans*-Alcohol **5**		*cis*-Alcohol **6**	
		Yield (%)	Ee (%)[b]	Yield (%)	Ee (%)[b]	Yield (%)	Ee (%)[b]
Cucumber (*C. sativus*)[c]	24	95	43 (*S*)	–		–	
Mamey sapote (*P. sapota*)[c]	24	100	42 (*S*)	–		–	
Banana passion fruit (*P. tarminiana*)[c]	24	100	33 (*S*)	–		–	
White onion (*A. cepa*)[d]	24	75	41 (*S*)	–		–	
Red onion (*A. cepa*)[d]	24	88	44 (*S*)	–		–	
Garlic (*A. sativum*)[d]	24	93	43 (*S*)	–		–	
Radish (*R. sativus*)[e]	8	100	41 (*S*)	–		–	
Chayote squash (*S. edule*)[c]	24	100	9 (*S*)	–		–	
Fennel (*F. vulgare*)[f]	24	94	12 (*S*)	–		–	
Marrow (*C. pepo*)[c]	24	100	3 (*S*)	–		–	
Aubergine (*S. melongena*)[c]	24	73	17 (*S*)	–		–	
Carrot (*D. carota*)[g]	2	89	45 (*S*)	5		–	
	24	14	100 (*R*)	75	100 (1*S*,2*S*)	11	
Cherimoya (*A. cherimola*)[c]	24	90	15 (*S*)	–		–	
	72	24	85 (*S*)	30	100 (1*S*,2*S*)	46	95 (1*S*,2*R*)
Wild cucumber (*C. pedata*)[c]	24	100	8 (*R*)	–		–	
	144	42	35 (*S*)	33	100 (1*S*,2*S*)	25	50 (1*S*,2*R*)
Giant granadilla (*P. quadrangularis*)[c]	24	52	37 (*S*)				
	72	41	82 (*S*)	26	100 (1*S*,2*S*)	28	65 (1*S*,2*R*)
Oca (*O. tuberosa*)[h]	24	95	7 (*R*)	–		5	
	72	44	99 (*S*)	–		56	100 (1*S*,2*R*)

[a] All biotransformations are monitored by GLC at 2 h, 4 h, 8 h, 24 h, 3 days and 6 days.
[b] Absolute configuration in parenthesis.
[c] Fruit.
[d] Bulb.
[e] Rhizome.
[f] Stalk.
[g] Root.
[h] Tuber.

incubation, totally hydrolyses the enol acetate producing the *S*-2-methylcyclohexanone in 89% yield (ee 45%) together with a small amount of the *trans*-alcohol **5** (5%). The reduction goes on and after 24 h the enantiomerically pure 1*S*,2*S*-*trans*-2-methylcyclohexanol **5** (75% yield, ee 100%) is obtained together with *cis*-alcohol (11%), leaving the pure *R*-ketone (14% yield, ee 100%).

Cherimoya, wild cucumber and giant granadilla, on the contrary, hydrolyse the enol acetate **3** more slowly (24 h) and with lower enantioselectivity (ee 8–37%), while after 3/6 days the reduction products **5** and **6** are detected in good yields and enantiomeric excesses. In particular, the enantiomerically pure *trans*-alcohols **5** (ee 100% of the 1*S*,2*S*-enantiomer) are obtained in 26–33% yield, while the *cis*–alcohol **6** (25–46% yield) is produced with lower ee (50–95% of the 1*S*,2*R*-enantiomer). In all cases the enantiomeric excesses of the unreacted ketone (24–42% yield) increases (35–85% of the *S*-enantiomer). Oca, on the other hand, acts similarly in the hydrolysis of **3** giving, in 24 h, the ketone **4** (95%, ee 7% of the *R*-enantiomer) with traces of *cis*-alcohol, while after 3 days the pure alcohol **6** (ee 100% of the 1*S*,2*R*-enantiomer) is obtained in good yield (56%) leaving the enantiomerically pure *S*-ketone (44%, ee 99%).

In conclusion the enantioselective reduction and hydrolysis with not-cultured cell plants can be viewed as further tool for the organic chemist, if compared with the use of baker's yeast, in virtue both of the easiness of execution and of the limited biochemical and microbiological skill needed.

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17. All the plants are commercially available.
18. The plants are washed with 5% sodium hypochlorite and then ethanol, peeled with a sterilised cutter and cut under a sterile hood.
19. For reduction of **1**, enantiomer separation is achieved on Megadex 5 column (25×0.25 mm) containing dimethyl-*n*-pentyl β-cyclodextrin in OV 1701; carrier gas: helium 70 kPa; temp. 70–200°C (1.5°C/min), retention time (min): **1**, 9.39 *R*-**2**, 14.59; *S*-**2**, 15.43. For hydrolysis of **3**, enantiomer separation was achieved on a Megadex DETTBSβ column (25×0.25 mm) containing diethyl-*tert*-butylsilyl β-cyclodextrin in OV 1701; carrier gas: helium 100 kPa, temp. 70–200°C (1.5°C/min), retention time (min): *S*-**4**, 8.27; *R*-**4**, 8.53; 1*S*,2*S*-**5**, 9.60; 1*R*,2*R*-**5**, 9.78; 1*R*,2*S*-**6**, 10.77 (as acetyl derivative); 1*S*,2*R*-**6**, 12.01 (as acetyl derivative).