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Biotransformation of Foreign Substrates by Plant Cell Cultures —— For the Synthesis of Chiral Alcohols

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Biotransformation of foreign substrates by cultured cells can achieve the degree of stereochemical and regiochemical control needed in organic synthesis. The cultured cells catalyse transformations that may be difficult to accomplish by traditional organic synthesis. Furthermore, biotransformation with the cultured cells is useful, both on a small scale in the laboratory and on a larger scale in industry, as for example in the production of drugs, flavors, pigments and agrochemicals[1-8]. This article summarizes how to prepare chiral alcohols by using plant tissue cultures. A useful process for the preparation of a chiral alcohol is enantioselective hydrolysis of acetate, and there have been many investigations on the enantioselective hydrolysis of acetates by microorganisms and yeast[9,10]. However the enantioselective hydrolysis of acetates with the cultured cells of higher plants has not been systematically investigated. In the first section of this report, we focus on the similarities and the differences in enantioselectivity displayed during the hydrolysis of acetates by various plant species, especially *Nicotiana tabacum* and *Marchantia polymorpha*.

In the second section, we discuss the chiral reduction of the carbonyl group for the preparation of chiral alcohols. The reduction of ketones is usually in equilibrium with the oxidation of the corresponding alcohols and we focus on the equilibrium in the oxido-reduction reaction between ketones and alcohols.

(I) Hydrolysis of Acetates.

(i) *Enantioselective hydrolysis of monoterpene acetates with the cultured cells of* N. tabacum. The ability of the cultured cells for enantioselective hydrolysis was examined using the biotransformations of bornyl acetates (**1a** and **1b**), isobornyl acetate (**2a** and **2b**) and isopinocampheyl acetates (**3a** and **3b**)[11]. **Table 1** shows the rate constants for the hydrolyses of the acetates with the cultured cells of *N. tabacum*. The acetates were hydrolyzed to their corresponding alcohols by the cultured cells, but the extent of hydrolysis was quite different between their enantiomers. The hydrolysis of (1*S*, 2*R*, 4*S*)-bornyl acetate (**1a**) predominated over that of the (1*R*, 2*S*, 4*R*)-enantiomer (**1b**). Similarly, the hydrolysis of (1*R*, 2*R*, 4*R*)-isobornyl acetate (**2a**) was more predominant when compared with that of its enantiomer (**2b**). In the case of isopinocampheyl acetate, the (1*R*, 2*R*, 3*R*, 5*S*)-enantiomer (**3a**) was hydrolyzed in preference to the (1*S*, 2*S*, 3*S*, 5*R*)-enantiomer (**3b**). In the biotransformation of (1*R*, 2*S*, 4*R*)-bornyl acetate (**1b**) and (1*R*, 2*R*, 4*R*)-isobornyl acetate (**2a**), the yield of the respective hydrolyzed products decreased gradually and there was an increase in the yield of (1*R*, 4*R*)-camphor (**4**) with the lapse of the incubation time. This indicates that the

平田敏文・泉　俊輔
植物培養細胞による外来基質の生物変換—キラルなアルコール類の合成のために
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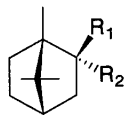
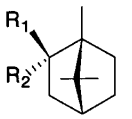
Table 1. Rate constants in the hydrolyses of acetates with the cultured cells of *N. tabacum*.

Substrates	Rate constant k (h^{-1})	
	R^{*1}	S^{*1}
Bornyl acetates (**1a** and **1b**)	4.1×10^{-3}	2.5×10^{-3}
Isobornyl acetates (**2a** and **2b**)	3.3×10^{-3}	2.1×10^{-3}
Isopinocampheyl acetates (**3a** and **3b**)	5.8×10^{-3}	4.1×10^{-4}

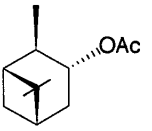
*1 *R* and *S* denote the configuration of the carbon atom bearing an acetoxyl group.



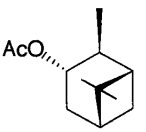
1a: R_1=H, R_2=OAc
2b: R_1=OAc, R_2=H



1b: R_1=H, R_2=OAc
2a: R_1=OAc, R_2=H
4: R_1,R_2=O



3a



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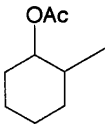

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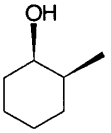

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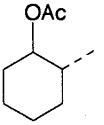

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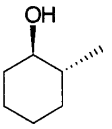

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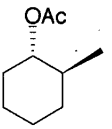

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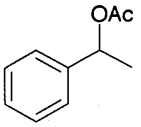

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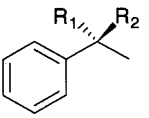
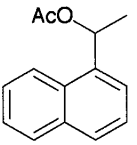


10a: R_1=OH, R_2=H
10b: R_1=H, R_2=OH



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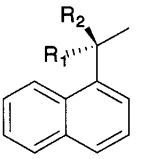
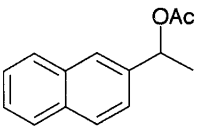


12a: R_1=OH, R_2=H
12b: R_1=H, R_2=OH



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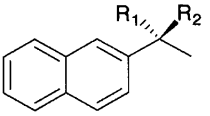
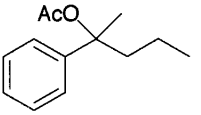


14a: R_1=OH, R_2=H
14b: R_1=H, R_2=OH



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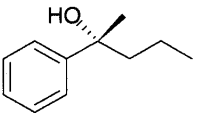

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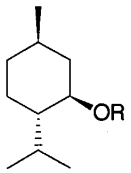
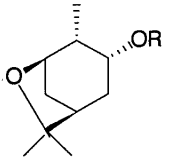
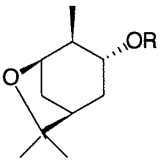


17: R=Ac
18: R=H



19: R=Ac
20: R=H



21: R=Ac
22: R=H

Table 2. Comparison of k_R/k_S in the hydrolyses of acetates with the cultured cells of *M. polymorpha* and *N. tabacum*.

Substrates	*M. polymorpha*	*N. tabacum*
	k_R/k_S	k_R/k_S
Bornyl acetates (**1a** and **1b**)	1.4	1.6
Isobornyl acetates (**2a** and **2b**)	>260	1.6
Isopinocampheyl acetates (**3a** and **3b**)	9.1	14

Table 3. Hydrolyses of the (±)-*cis*- and (±)-*trans*-2-methylcyclohexanyl acetates (**5** and **7**) by the cultured cells of *M. polymorpha*.

Substrates	Time	Conversion	Alcohols		Acetates (recovered)		E-value
	h	%	e.e.(%)	config.	e.e.(%)	config.	
5	4	18	90	*R*	12	*S*	23
7	2	53	80	*R*	>99	*S*	27

formation of camphor was due to the further oxidation of the corresponding alcohol by the cultured cells.

(ii) *Enantioselective hydrolyses of monoterpene acetates with the cultured cells of* M. polymorpha. Enantioselective hydrolysis was observed in the biotransformation of bicyclic monoterpene acetates, such as bornyl acetates (**1a** and **1b**), isobornyl acetates (**2a** and **2b**) and isopinocampheyl acetates (**3a** and **3c**), with the cultured cells of *M. polymorpha*. **Table 2** shows the ratio of the rate constants for the hydrolysis of the acetates having *R*- and *S*-configuration, respectively, by the cultured cells of *M. polymorpha* and *N. tabacum*. These acetates were hydrolyzed to their corresponding alcohols by both species of cultured cells, but the enantioselectivity of hydrolysis was quite different between the cultured cells of *N. tabacum* and those of *M. polymorpha*. This was especially so in the case of isobornyl acetate ; enantioselectivity in the hydrolysis with the cultured cells of *M. polymorpha* was higher than that with the cultured cells of *N. tabacum* (**Table 2**). However, the cultured cells of both plants, *N. tabacum* and *M. polymorpha*, were found to hydrolyze preferentially the acetate having the *R*-configuration.

The enantioselective hydrolyses of 2-methylcyclohexanyl acetates with the cultured cells were investigated with a view to possible practical applications[12]. Transformation of racemic *cis*-2-methylcyclohexanyl acetates (**5**) with the cultured cells of *M. polymorpha* gave (1*R*, 2*S*)-*cis*-2-methylcyclohexanol (**6**) with 90% optical purity. Similarly, the hydrolysis of racemic *trans*-2-methylcyclohexanyl acetate (**7**) gave (1*R*, 2*R*)-*trans*-2-methylcyclohexanol (**8**) and the unchanged substrate, (1*S*, 2*S*)-*trans*-2-methylcyclohexanyl acetate (**7a**), for which the optical purities were about 80-99% enantiomer excess (**Table 3**). Such enantioselective hydrolyses would be useful for the preparation of chiral alcohols.

(iii) *Enantioselective hydrolyses of acetates with other cultured cells.* The hydrolyses of the acetates with the plant cell cultures are summarized in **Table 4**. The cultured cells of almost all species hydrolyzed preferentially the acetate having the *R*-configuration. We refer to this as the "*R* selective rule" and it might be a common feature for the hydrolysis of acetates with plant tissue cultures.

(II) Reduction of the Carbonyl Group.

(i) *Stereospecificity in the reduction of the carbonyl group.* Another useful process for the

Table 4. Hydrolyses of acetates with plant cell cultures.

Substrates	Products	Plant species	Config.*1	Ref.
Bornyl acetate (**1a**)	Borneol	*Nicotiana tabacum*	*R*	11
		Marchantia polymorpha	*R*	
Isobornyl acetate (**2a**)	Isoborneol	*Nicotiana tabacum*	*R*	11
		Marchantia polymorpha	*R*	
Isopinocampheyl acetate (**3a**)	Isopinocampheol	*Nicotiana tabacum*	*R*	11
		Marchantia polymorpha	*R*	
cis-2-Methylcyclohexanyl acetate (**5**)	*cis*-2-Methylcyclohexanol (**6**)	*Marchantia polymorpha*	*R*	12
trans-2-Methylcyclohexanyl acetate (**7**)	*trans*-2-Methylcyclohexanol (8)	*Marchantia polymorpha*	*R*	12
1-Phenylethyl acetate (**9**)	1-Phenylethanol (**10a**)	*Spirodela oligorrhiza*	*R*	13
1-(1-Naphthyl) ethyl acetate (**11**)	1-(1-Naphthyl) ethanol (**12a**)	*Spirodela oligorrhiza*	*R*	13
1-(2-Naphthyl) ethyl acetate (**13**)	1-(2-Naphthyl) ethanol (**14a**)	*Spirodela oligorrhiza*	*R*	13
2-Phenylbutanyl acetate (**15**)	2-Phenylbutanol (**16**)	*Spirodela oligorrhiza*	*R*	13
Menthyl acetate (**17**)	Menthol (**18**)	*Spirodela oligorrhiza*	*R*	14
		Epidendrum ochraceum	*R*	15
trans-2-Hydroxy-*trans*-*dihydropinanyl acetate* (**19**)	*trans*-2-Hydroxy-*trans*-dihydropinanol (**20**)	*Spirodela oligorrhiza*	*R*	14
trans-2-Hydroxy-*cis*-dihydropinanyl acetate (**21**)	*trans*-2-Hydroxy-*cis*-dihydropinanol (**22**)	*Spirodela oligorrhiza*	*R*	14

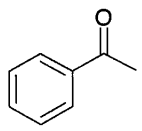
*1 Config. denotes the configuration of the carbon atom bearing a hydroxyl group of the alcohol which is mainly produced.

Table 5. Reduction reactions of ketones with plant cell cultures.

Substrates	Products	Plant species	Config.*1	Ref.
Acetophenone (**23**)	1-Phenylethanol (**10b**)	*Daucus carota*	*S*	16
	1-Phenylethanol (**10b**)	*Nicotiana tabacum*	*S*	16
	1-Phenylethanol (**10a**)	*Gardenia jasminoides*	*R*	16
Propiophenone (**24**)	1-1-Phenyl-1-propanol (**25a**)	*Daucus carota*	*S*	16
	1-Phenyl-1-propanol (**25a**)	*Nicotiana tabacum*	*S*	16
	1-Phenyl-1-propanol (**25b**)	*Gardenia jasminoides*	*R*	16
1-Naphthyl methyl ketone (**26**)	1-(1-Naphthyl) ethanol (**12b**)	*Daucus carota*	*S*	16
	1-(1-Naphthyl) ethanol (**12b**)	*Nicotiana tabacum*	*S*	16
	1-(1-Naphthyl) ethanol (**12b**)	*Gardenia jasminoides*	*S*	16
2-Naphthyl methyl ketone (**27**)	1-(2-Naphthyl) ethanol (**14b**)	*Daucus carota*	*S*	16
	1-(2-Naphthyl) ethanol (**14b**)	*Nicotiana tabacum*	*S*	16
(1*R*, 4*R*)-Dihydrocarvone (**28**)	(1*R*, 2*S*, 4*R*)-Neodihydrocarveol (**29**)	*Nicotiana tabacum*	*S*	17
(1*S*, 4*S*)-Dihydrocarvone (**30**)	(1*S*, 2*R*, 4*S*)-Neodihydrocarveol (**31**)	*Nicotiana tabacum*	*R*	17
	(1*S*, 2*S*, 4*S*)-Dihydrocarveol (**32**)	*Nicotiana tabacum*	*S*	17
(1*S*,4*R*)-Isodihydrocarvone (**33**)	(1*S*, 2*S*, 4*R*)-Isodihydrocarveol (**34**)	*Nicotiana tabacum*	*S*	17
(1*R*,4*S*)-Isodihydrocarvone (**35**)	(1*S*,2*S*,4*R*)-Isodihydrocarveol (**36**)	*Nicotiana tabacum*	*S*	17
Menthone (**37**)	Neomenthol (**38**)	*Mentha* sp.	*R*	18
(1*S*, 4*S*)-Carvomenthone (**39**)	(1*S*, 2*S*,4*S*)-Carvomenthol (**40**)	*Nicotiana tabacum*	*S*	19, 20
(1*R*,4*R*)-Carvomenthone (**41**)	(1*R*, 2*R*,4*R*)-Carvomenthol (**42**)	*Nicotiana tabacum*	*S*	19. 20
α-Dicarvone (**43**)	6, 6′-Bis-*p*-menth-8-en-2-ol (**44**)	*Nicotiana tabacum*	*R*	21

*1 Config. denotes the configuration of the carbon atom bearing a hydroxyl group of the alcohol which is mainly produced.

preparation of a chiral alcohol is the chiral reduction of a ketone. There are many reports of the reduction of ketones and aldehydes to the corresponding alcohols with plant cell cultures. The reductions of ketones by plant tissue cultures are summarized in **Table 5**. In many cases, the



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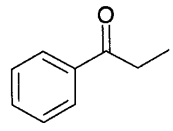

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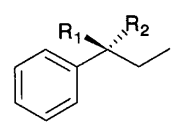
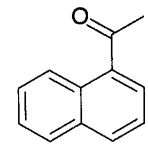


25a: R_1=OH, R_2=H
25b: R_1=H, R_2=OH



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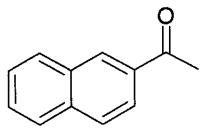

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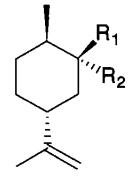
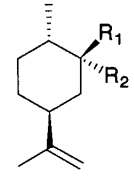
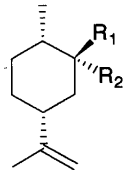


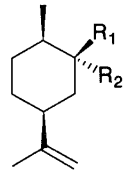
28: R_1,R_2=O
29: R_1=OH, R_2=H



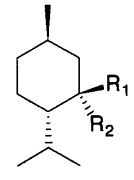
30: R_1,R_2=O
31: R_1=H, R_2=OH
32: R_1=OH, R_2=H



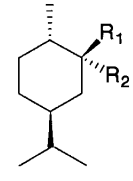
33: R_1,R_2=O
34: R_1=OH, R_2=H



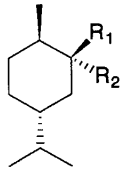
35: R_1,R_2=O
36: R_1=H, R_2=OH



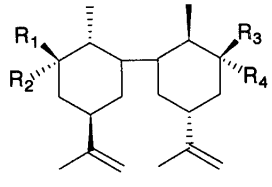
37: R_1,R_2=O
38: R_1=H, R_2=OH



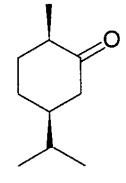
39: R_1,R_2=O
40: R_1=H, R_2=OH



41: R_1,R_2=O
42: R_1=OH, R_2=H



43: R_1,R_2=O, R_3,R_4=O
44: R_1=R_4=OH, R_2=R_3=H



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alcohols having the *S*-configuration were produced from the corresponding ketones by the cultured cells but there are a number of exceptions.

We found that the reduction of 2-oxygenated *p*-menthanes, such as (1*R*, 4*R*)- and (1*S*, 4*S*)-dihydrocarvones (**28** and **30**) and (1*S*, 4*R*)- and (1*R*, 4*S*)-isodihydrocarvones (**33** and **35**) with cultured cells of *N. tabacum* occurred stereospecifically. The hydrogen attack in the reduction takes place preferentially from *re*-face of the carbonyl group to give the hydroxyl compounds with the *S*-chirality at the position bearing the hydroxyl group[17,19,20].

Interestingly, the cultured cells discriminated between the 2- and 3-oxygenated *p*-menthanes in their reductive conversions; *p*-menthan-2-ones were converted to their corresponding alcohols in good yield, but this was not the case with the *p*-menthan-3-ones[22]. In addition, the cells stereospecifically reduced (1*R*, 4*R*)-2-oxo-*p*-menthane (**41**), whereas the specificity was low in the case of the (4*S*)-epimer (**45**)[19,20].

(ii) *Equilibrium in the oxido-reduction reaction.* Alcohols were converted to the corresponding aldehydes or ketones by the plant cell cultures. The conversion between a cycloalkanol and its

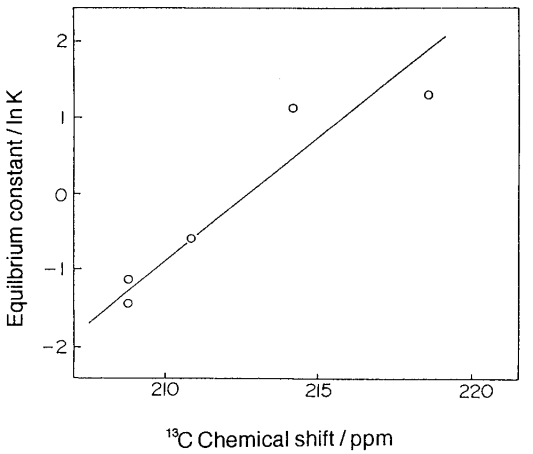

Fig. 1 Correlation of the equilibrium constant of the oxido-reduction reaction between cycloalkanols and cycloalkanones with the ^{13}C NMR chemical shift of the carbonyl carbon of the cycloalkanones involved in the equilibrium.

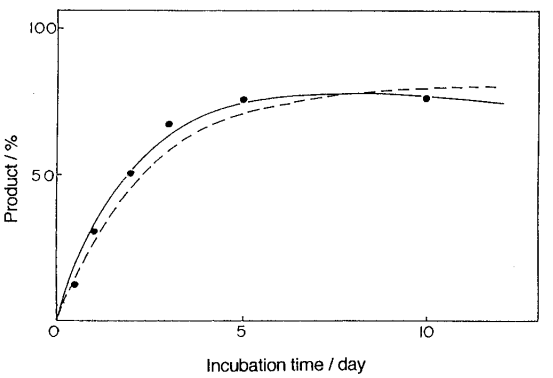

Fig. 2 Comparison of the bioconversion of (1*S*,4*S*)-carvomenthone (**39**) into (1*S*,2*S*,4*S*)-carvomenthol (**40**) in the cultured cells (—●—) with its simulation (·····).

corresponding ketone in the cultured cells of *N. tabacum* was reversible[23] and the oxido-reduction reactions were governed by an NAD^+-dependent alcohol dehydrogenase[24,25]. The balance of the equilibrium depends on the carbon number in the carbocyclic ring of the cyclic compound. The equilibrium tends to lie toward the side of the alcohol in the case of a six-membered cyclic compound, while it is mostly in the direction of the ketone for the five, seven, and eight-membered cyclic compounds[23].

Such a ring-size effect was found in the reduction of cycloalkanones with $NaBH_4$, and this effect was interpreted on the basis of the difference in the internal strain in the carbocyclic ring[26]. Generally, the reactivity depends on the electron density of the reacting species and the ^{13}C NMR chemical shift can be used as a parameter of the electron density. **Fig. 1** shows the correlations of the equilibrium constants of the oxido-reduction reactions between cycloalkanones and cycloalkanols with the ^{13}C NMR chemical shifts of the carbonyl carbon of the cycloalkanols. The equilibrium constants of the oxido-reduction reaction between the cycloalkanols and their corresponding cycloalkanones are correlated with the ^{13}C NMR chemical shift values of the carbonyl carbon of the oxidation products[25,27]. The data presented in **Fig. 1** reveals a rule for determining the direction of the reaction. If the ^{13}C NMR chemical shift of the carbonyl carbon of the substrate is more than 213 ppm, the reaction is oxidative. On the other hand, if the chemical shift is less than 213

ppm, the reduction reaction will proceed in preference to the oxidation.

(iii) *Simulation of the reduction of the carbonyl group with the cultured cells.* A method for simulating the time-course of the reduction of cycloalkanones by the cultured cells of *N. tabacum* was developed on the basis of the permeability constants of the substrates into the cultured cells and the ^{13}C NMR chemical shift of the carbonyl carbon of the cycloalkanones[25,27]. It was found that the incubation time (t), the proportion (P) of the amount of product to the initial amount of substrate supplied to the cultured cells can be predicted by the following equation:

$$P=\{1-\exp(-4.82\times10^{-6}t)\}/\{1+\exp(0.284\delta_{c=o}-60.5)\}$$

where $\delta_{c=o}$ denotes the ^{13}C NMR chemical shift of the carbonyl carbon. This equation was applied to the reduction of (1*S*, 4*S*)-carvomenthone (**39**) to (1*S*, 2*S*, 4*S*)-carvomenthol (**40**) in the cultured cells of *N. tabacum*; the time-course for the reduction of the compound **39** was simulated on the basis of the ^{13}C NMR chemical shift of the carbonyl carbon of carvomenthone ($\delta=211.8$), as shown in **Fig. 2**[19,20,25,27]. The simulation curve for the reduction fits nicely with the bioconversion curve.

This method can be widely applied to the simulation of the time-course of the oxido-reduction reaction of cyclic alcohols and ketones by cultured cells. This method is useful for predicting the equilibrium constants and the time-courses in biotransformation, prior to incubation.

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