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Enantioselective hydrolyses of α-methylated cyclohexyl acetates by the cultured cells of *Marchantia polymorpha*

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Abstract

A high enantioselectivity was observed in the hydrolyses of racemic α-methylated cyclohexyl acetates with the cultured cells of *Marchantia polymorpha*. The enantiomeric excesses of alcohols obtained in the hydrolyses were correlated with the torsional angles between the acetoxyl and α-methyl groups. They have made it possible to predict the optical purity as well as the absolute stereochemistry of alcohols hydrolyzed by using the cultured cells of *M. polymorpha*.

Keywords: Enantioselective hydrolysis; Cyclohexyl acetate; Cultured cells; *Marchantia polymorpha*

1. Introduction

The enantioselective hydrolyses of acetates with biocatalysts is a useful process for the preparation of chiral synthons for organic synthesis of natural products. Therefore, many works have been extensively investigated. For example, Ziffer et al. [1] proposed a rule based on the sizes of the substituents to predict which enantiomer of secondary acetates was hydrolyzed faster by cultures of *Rhizopus nigricans*. Kazlauskas et al. [2] reported that this rule was applicable to hydrolysis of a number of secondary acetates for three hydrolases, i.e. bovine pancreatic cholesterol esterase, esterase from *Candida rugosa*, and lipase from *Pseudomonas cepacia*. Toone et al. [3] proposed a model of the active site of porcine liver esterase. Concerning with hydrolysis of acetates of cyclic terpene alcohols, Oritani and Yamashita showed that (*R*)-acetates were preferentially hydrolyzed by employing *Bacillus subtilis* [4].

Recently, we have shown that the cultured cells of *Marchantia polymorpha* hydrolyzed enantioselectively the acetoxyl groups at the stereogenic centers of (*R*)-configuration of acetates [5,6], and asymmetry was also induced in the hydrolyses of *meso*-diacetates [7]. In an effort to develop a method for preparing chiral alcohols of a predictable configuration, we have investigated the steric effect of the α-substituents in the asymmetric hydrolyses of racemic α-methylated secondary acetates, i.e. cyclohexyl acetates (**1** and **2**) and bicyclo[2.2.1]heptyl

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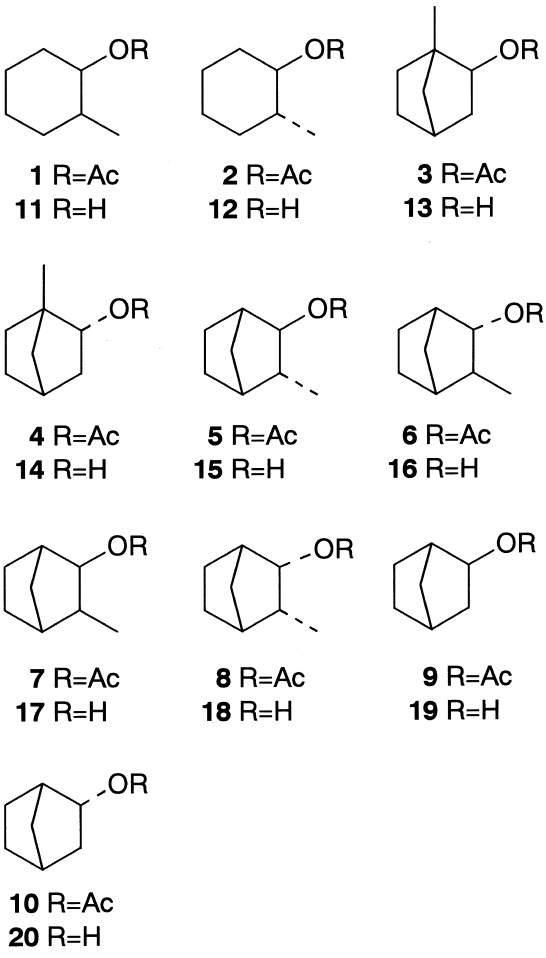
acetates (**3**–**8**, **9** and **10**) with conformationally rigid carbon skeleton.



2. Experimental

2.1. General procedure

Analytical and prep. TLC were carried out on 0.25-mm thick silica gel plates (Merck silica gel 60; GF_{254}). GLC analyses were performed on an instrument equipped with FID and a 15% DEGS column (3 mm × 2 m) at 60–160°C (2°C/min) and a CP cyclodextrin β 236-M 19 column (0.5 mm × 25 m) at 200°C. ^{1}H NMR spectra was obtained at 270 MHz in $CDCl_3$ with tetramethylsilane as an internal standard.

The absolute configurations and enantiomeric purities of products were determined by analyses of the ^{1}H NMR spectra of the corresponding MTPA derivatives [8]. Preferred conformations of acetates (**3**–**8**) and the torsional angles between the acetoxyl group and the methyl group of their acetates were estimated by the calculation on the two molecular mechanics programs, MM2 (QCPE395) [9] and Chem3D Pro [10].

2.2. Materials

(±)-Bicyclo[2.2.1]heptan-2-one, (±)-3-methylenebicyclo[2.2.1]heptan-2-one, (±)-*cis*-2-methylcyclohexanol (**11**), (±)-*trans*-2-methylcyclohexanol (**12**), (±)-2β-hydroxy-8,9,10-trinorbornane (**19**) and (±)-2α-hydroxy-8,9,10-trinorbornane (**20**) were commercial material of Tokyo-kasei Chem., Tokyo, Japan.

According to the reported procedure [11], acetate (**3**) was prepared from bicyclo[2.2.1]heptan-2-one via Wagner–Meerwein rearrangement of 2-methylbicyclo[2.2.1]heptan-2-ol. Acetates (**1**, **2** and **4**–**8**, **9** and **10**) were prepared from their corresponding alcohols (**11**,**12**,**14**–**20**) by acetylation with acetic anhydride.

(±)-2β-acetoxy-8,9-dinorbornane (**3**): 98% pure on GLC; IR (neat) 1735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.54 (1H, d, $J = 7.3$, >C*H*-OAc), 2.03 (3H, s, OAc), 1.12 (3H, s).

(±)-2α-acetoxy-8,9-dinorbornane (**4**): 98% pure on GLC; IR (neat) 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.70 (1H, m, >C*H*-OAc), 2.05 (3H, s, OAc), 1.10 (3H, s).

(±)-2β-acetoxy-3α-methyl-8,9,10-trinorbornane (**5**): 95.8% pure on GLC; IR (neat) 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.04 (1H, brs, >C*H*-OAc), 2.02 ± 3H, s, OAc), 1.13(3H, d, $J = 7.3$).

(±)-2α-acetoxy-3β-methyl-8,9,10-trinorbornane (**6**): 99% pure on GLC; IR (neat) 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.47 (1H, m, >C*H*-OAc), 2.04 (3H, s, OAc), 1.02 (3H, d, $J = 7.3$).

(±)-2β-acetoxy-3β-methyl-8,9,10-trinorbornane (**7**): 98.2% pure on GLC; IR (neat) 1734 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.70 (1H, m, >C*H*-OAc), 2.03 (3H, s, OAc), 0.83 (3H, d, $J = 7.3$).

(±)-2α-acetoxy-3α-methyl-8,9,10-trinorbornane (**8**): 95.4% pure on GLC; IR (neat) 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.96 (1H, dd, $J = 9.3, 4.4$, >C*H*-OAc), 2.06 (3H, s, OAc), 0.81 (3H, d, $J = 6.4$).

2.3. Hydrolyses of the acetates

The cultured cells of *M. polymorpha* have been subcultured routinely every 2 weeks using MSK-2 medium [12] for more than 6 years [5]. The cultured cells (30 g) were added to a 300-ml conical flask containing 120 ml of MSK-2 starvation medium (glucose 0.2%). To the flask containing the suspension cells, the substrate (20 mg) was administered, and the cultures were incubated at 25°C on a rotary shaker (70 rpm) under illumination (1000 lx). After incubation, the culture medium filtered from the cells was extracted with ether. Products were isolated from the ether soluble fraction by chromatography on silica gel with hexane–ethyl acetate (50:1, v/v). The products were identified by comparison with the GLC and spectroscopic data of authentic samples.

2.4. Preparation of authentic samples

(±)-2β-hydroxy-8,9-dinorbornane (**13**). (±)-**3** was reduced with $LiAlH_4$ to give (±)-**13**: IR (neat) 3382 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.46 (1H, dd, $J = 4.9, 2.0$, >C*H*-OH), 1.78 (1H, ddd, $J = 13.2, 6.8, 2.4$), 1.14 (3H, s).

(±)-2β-hydroxy-8,9-dinorbornane (**14**). According to the reported procedure [13], CrO_3 dissolved in sulfuric acid was added to a solution of **13** (2.5 g, 19.7 mmol) in acetone (100 ml) under stirring. The mixture was stirred for 3 h at room temperature. The product was extracted with ether to give (±)-1-methylbicyclo[2.2.1]heptan-2-one. The ketone (600mg, 4.8 mmol) was reduced with $LiAlH_4$ and purified by column chromatography on silica gel with hexane-ethyl acetate (9:1, v/v) to give (±)-**14** (541 mg): IR (neat) 3320 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.84 (1H, dq, $J = 8.2, 1.8$, >C*H*-OH), 1.84 (1H, tdd, $J = 12.8, 4.6, 1.8$), 1.39 (1H, td, $J = 11.5, 4.6$), 1.66 (1H, m), 1.10 (3H, s).

(±)-2β-hydroxy-3α-methyl-8,9,10-trinorbornane (**15**) and (±)-2α-hydroxy-3α-methyl-8,9,10-trinorbornane (**18**). According to the reported procedure [14], (±)-3-methylenebicyclo[2.2.1]heptan-2-one was hydrogenated with Pd-C, and then reduced with $NaBH_4$ to give a crude alcohols. The alcohols were purified by column chromatography on silica gel with hexane-ethyl acetate (9:1, v/v) to (±)-**15** [IR (neat) 3320 cm^{-1}; ^1H NMR ($CDCl_3$)δ 3.12 (1H, brs, >C*H*-OH), 0.99 (3H, d, $J = 7.3$)] and (±)-**18** [IR (neat) 3370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.09 (1H, dd, $J = 10.4, 4.6$, >C*H*-OH), 0.87 (3H, d, $J = 7.3$)].

(±)-2α-hydroxy-3β-methyl-8,9,10-trinorbornane (**16**) and (±)-2β-hydroxy-3β-methyl-8,9,10-trinorbornane (**17**). According to the reported proce-

Table 1
Hydrolyses of acetates (**1**, **2**, **3**–**8**, **9** and **10**) with the cultured cells of *M. polymorpha*

Substrate	Reaction time (h)	Product	Yield (%)[a]	Preferred configuration	e.e. (%)[b]	E-value[c]	Torsional angle (°)[d]
1	4	11	18	*R*	90	23	60
2	2	12	53	*R*	80	27	61
3	12	13	48	*R*	83	26	44
4	12	14	30	*R*	56	4	80
5	12	15	66	*S*	49	9	113
6	6	16	68	*S*	34	4	108
7	12	17	54	*S*	86	106	5
8	12	18	9	*S*	40	2	2
9	6	19	43	–	0	1	–
10	12	20	31	–	0	1	–

[a] Relative percentage in the reaction mixture.
[b] Enantiomeric purities of products were determined by analyses of the ^1H NMR spectra of the corresponding MTPA derivatives [8].
[c] Values determined from the extent of conversion and the enantiomeric excess of the products [16].
[d] The torsional angles between the acetoxyl groups and methyl groups were estimated by MM2 method [9,10].

dure [15], (±)-bicyclo[2.2.1]heptan-2-one was methylated with LDA and methyl iodide, and then reduced with $NaBH_4$ to give a crude alcohols. The alcohols were purified by column chromatography on silica gel with hexane-ethyl acetate (9:1, v/v) to give (±)-**16** [IR (neat) 3330 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.65 (1H, d, $J = 3.7$, >C*H*-OH), 0.99 (3H, d, $J = 7.3$)] and (±)-**17** [IR (neat) 3375 cm^{-1}; ^1H NMR ($CDCl_3$) $\delta = 3.27$ (1H, brs, >C*H*-OH), 0.92 (3H, d, $J = 7.3$)].

3. Result and discussion

In the time-courses of the hydrolyses of racemic *cis*-2- and *trans*-2-methylcyclohexyl acetates (**1** and **2**) with the cultured cells of *M. polymorpha*, the rate of hydrolysis was fast and half of the acetate was hydrolyzed in a first 6–10 h period in each case. The absolute configuration at the carbon atom bearing the hydroxy group of alcohols obtained was mainly (*R*) in each case. The enantiomeric excesses of the alcohols in a 50% yield were more than 80%, whereas the enantiomeric excesses decreased markedly during further hydrolysis. These results indicate that the hydrolysis with the cultured cells of *M. polymorpha* is highly enantioselective.

The conversion yields and enantioselectivities in the hydrolyses of acetates were shown in Table 1. Concerning the hydrolyses of bicyclo[2.2.1]heptyl acetates (**3**–**8**, **9** and **10**), a very high enantioselectivity was observed in the hydrolyses of racemic **3** and **7**. The preferential configuration at C-2 is (*R*) in the hydrolysis of racemic **3** and **4**; however, *S*-configuration preference was observed in the case of **5**–**8**. These results could be accounted for by the above-mentioned rule [1,2]. On the other hand, enantioselectivity was not observed in the hydrolyses of racemic **9** and **10**, which do not have α-methyl substituent. These observations indicate that the cultured cells of *M. polymorpha* hydrolyzed enantioselectively the acetates, and that α-substituted methyl group exerted a strong influence on the enantioselectivity.

The E-values [16] in the hydrolyses of **1**, **2**, **3**–**7** were correlated hyperbolically with the torsional angles (φ) between the acetoxyl groups and α-methyl groups, as shown in Fig. 1. However, such a correlation was not observed for the hydrolysis of **8**. Considering that the rate of hydrolysis of **8** was very low compared with other compounds (Table 1), the enantioselectivity in the hydrolysis of **8** might be affected by uncertain factors.

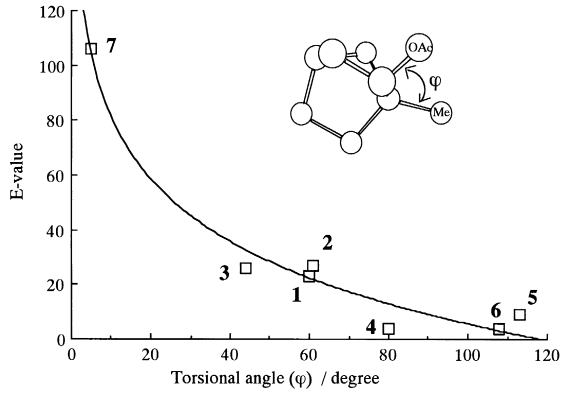


Fig. 1. The correlation between the torsional angles and the E-values.

Consequently, enantioselective hydrolyses with cultured plant cells are especially useful because they are efficient and convenient for preparation of chiral compounds, and it is possible to estimate the optical purity as well as the absolute stereochemistry of an alcohol prepared by using the cultured cells of *M. polymorpha*.

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