ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 5 (1998) 143–147

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Asymmetric hydrolysis of enol acetates with the cultured cells of *Marchantia polymorpha*

Toshifumi Hirata *, Kei Shimoda, Daisuke Ohba, Noriko Furuya, Shunsuke Izumi

Department of Chemistry, Faculty of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan

Received 26 September 1997; accepted 5 December 1997

Abstract

Asymmetric hydrolysis of enol acetates with enantiofacial protonation was investigated using plant cell cultures of *Marchantia polymorpha*. The cells hydrolyzed cyclohexanone enol acetates with alkyl groups at α-position to the acetoxyl group to give optically active ketones. Bulky substituent at α-position reduced optical yield of the product, and long side chain ($C \geq 3$) or a bulky *gem*-dimethyl bridge reversed the stereoselectivity of protonation at enantiotopic face of the enol intermediate.

Keywords: Asymmetric hydrolysis; Enantiofacial protonation; *Marchantia polymorpha*; Optically active ketone; Biotransformation

1. Introduction

Optically active α-substituted ketones are important chiral synthons in chemical synthesis. Strategies for efficient generation of optically enriched α-substituted cycloalkanone were developed in conception of asymmetric protonation of enolate compounds by enantioselective protonation of metal enolates using chiral proton sources [1–4]; as an attack of a proton occurs from one specified side of the double bond of an enol intermediate, the resulting ketone is optically active. Hydrolysis of enol esters accompanied with enantiofacial protonation of enol intermediates by use of microorganisms [5–7] and abzyme [8] as biocatalysts are very attractive methods of achieving optically active ketones.

In our recent studies, we observed a high enantioselective hydrolysis of *sec*-acetates by use of plant cell cultures of *Marchantia polymorpha* [9–11]. In connection with these studies, we have now applied the cultured cells of *M. polymorpha* for the enantioface differentiating hydrolysis of α-alkylated cyclohexanone enol acetates to produce optically active ketones. This paper describes the specificity of the hydrolysis using a number of substrates and the effects of the introduction of bulky substituent at the α-position of the acetoxyl group on the stereoselectivity of the protonation of the enol intermediate.

* Corresponding author.

1381-1177/98/$19.00
PII: S1381-1177(98)00023-X

2. Experimental

2.1. Analysis

Analytical and prep. TLC (0.25-mm thick) were carried out on silica-gel plates (Merck, Type 60, GF_{254}). GC analysis was performed by using a capillary column (0.25 mm × 25 m) coated by CP cyclodextrin β 236M-19 with N_2 as carrier gas (column temp., 100°C; split ratio of 50:1; flow late of N_2, 50 ml min^{-1}). ^1H NMR spectra were obtained on a JEOL GSX-270 spectrometer using tetramethylsilane as an internal standard in $CDCl_3$.

2.2. Substrates

Cyclohexanone enol acetates, **1**–**13**, were prepared by treatment of the corresponding racemic α-alkylated ketones with perchloric acid and acetic anhydride at room temperature [12].

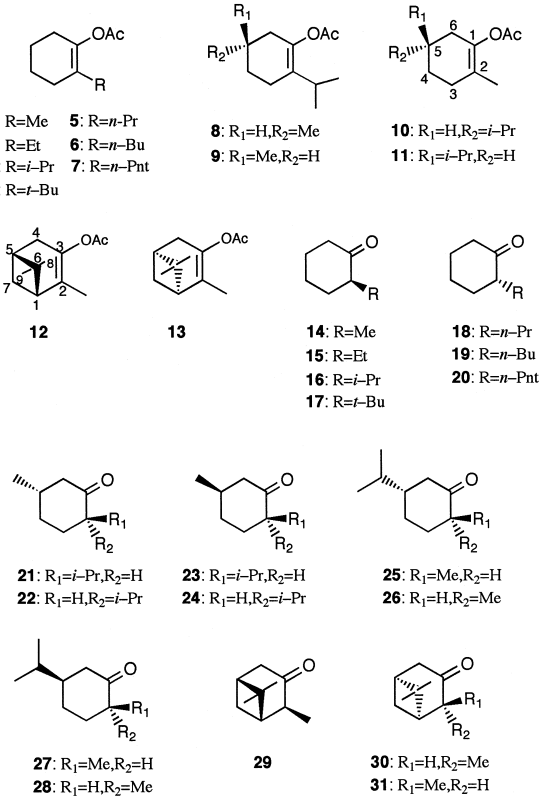
1-Acetoxy-2-methylcyclohexene (**1**): ^1H NMR ($CDCl_3$) δ 1.51 (s, 3H, Me), 2.13 (s, 3H, Ac); IR (neat) 1640 (C=C), 1750 (C=O) cm^{-1}. 1-Acetoxy-2-ethylcyclohexene (**2**): ^1H NMR ($CDCl_3$) δ 0.93 (t, 3H, Me), 1.92 (q, 2H, $-CH_2-$), 2.12 (s, 3H, Ac); IR (neat) 1690 (C=C), 1745 (C=O) cm^{-1}. 1-Acetoxy-2-isopropylcyclohexene (**3**): ^1H NMR ($CDCl_3$) δ 0.92 (d, 6H, isopropyl Me), 2.13 (s, 3H, Ac), 2.70 (m, 1H, >CH–); IR (neat) 1685 (C=C), 1750 (C=O) cm^{-1}. 1-Acetoxy-2-*t*-butylcyclohexene (**4**): ^1H NMR ($CDCl_3$) δ 1.03 (s, 9H, *t*-butyl Me), 2.06 (s, 3H, Ac); IR (neat) 1670 (C=C), 1750 (C=O) cm^{-1}. 1-Acetoxy-2-propylcyclohexene (**5**): ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H, Me), 1.37 (m, 2H, $-CH_2-$), 1.89 (t, 2H, $-CH_2-$), 2.13 (s, 3H, Ac); IR (neat) 1690 (C=C), 1750 (C=O) cm^{-1}. 1-Acetoxy-2-butylcyclohexene (**6**): ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H, Me), 2.13 (s, 3H, Ac); IR (neat) 1695 (C=C), 1745 (C=O) cm^{-1}. 1-Acetoxy-2-pentylcyclohexene (**7**): ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, Me), 2.12 (s, 3H, Ac); IR (neat) 1690 (C=C), 1740 (C=O) cm^{-1}. (S)-1-Acetoxy-2-isopropyl-5-methylcyclohexene (**8**): ^1H NMR ($CDCl_3$) δ 0.92 (d, 6H, isopropyl Me), 0.97 (d, 3H, 5-Me), 2.12 (s, 3H, Ac), 2.70 (m, ^1H, >CH–); IR (neat) 1690 (C=C), 1745 (C=O) cm^{-1}. (*R*)-1-Acetoxy-2-isopropyl-5-methylcyclohexene (**9**): ^1H NMR ($CDCl_3$) δ 0.91 (d, 3H, 5-Me), 2.11 (s, 3H, Ac); IR (neat) 1690 (C=C), 1750 (C=O) cm^{-1}. (*S*)-1-Acetoxy-2-methyl-5-isopropylcyclohexene (**10**): ^1H NMR ($CDCl_3$) δ 0.89 (m, 6H, isopropyl Me), 1.24 (m, ^1H, >CH–), 2.14 (s, 3H, Ac); IR (neat) 1650 (C=C), 1760 (C=O) cm^{-1}. (*R*)-1-Acetoxy-2-methyl-5-isopropylcyclohexene (**11**): ^1H NMR ($CDCl_3$) δ 0.89 (m, 6H, isopropyl Me), 1.24 (m, 1H, >CH–), 2.14 (s, 3H, Ac); IR (neat) 1650 (C=C), 1760 (C=O) cm^{-1}. (1*S*, 5*R*)-3-Acetoxy-2,6,6-trimethylbicyclo[3.1.1]-hept-2-ene (**12**): ^1H NMR ($CDCl_3$) δ 0.99 (s, 3H, *gem*-Me), 1.28 (s, 3H, 2-Me), 1.53 (s, 3H, *gem*-Me), 2.14 (s, 3H, Ac); IR (neat) 1695 (C=C), 1750 (C=O) cm^{-1}. (1*R*,5*S*)-3-Acetoxy-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (**13**): ^1H NMR ($CDCl_3$) δ 0.99 (s, 3H, *gem*-Me), 1.28 (s, 3H, 2-Me), 1.53 (s, 3H, *gem*-Me), 2.14 (s, 3H, Ac); IR (neat) 1695 (C=C), 1750 (C=O) cm^{-1}.

2.3. Preparation of α-alkylated ketones

(±)-2-Methylcyclohexanone (**14**), (±)-2-ethylcyclohexanone (**15**), (±)-2-*t*-butylcyclohexanone (**17**) and (±)-2-propylcyclohexanone (**18**) were purchased from Wako Chemical (±)-2-Isopropylcyclohexanone (**16**) was prepared from 1-isopropylcyclohexene by hydroboration [13] and then by oxidation of resulting alcohol with pyridinium chlorochromate (PCC). (±)-2-Butylcyclohexanone (**19**) and (±)-2-pentylcyclohexanone (**20**) were prepared from 2-(ethoxycarbonyl)cyclohexanone by alkylation with corresponding alkyl iodide, followed by decarboxylation with conc. H_2SO_4, according to the reported method [7]. (2*R*,5*S*)-2-Isopropyl-5-methylcyclohexanone (**21**), $[\alpha]_D^{25} + 28.0$ (c 1.5, EtOH) {lit. [14]: $[\alpha]_D^{25}$ −29.9 for (2*S*,5*R*)-isomer} was prepared by oxidation of (1*S*,2*R*,5*S*)-2-isopropyl-5-methylcyclohexanol

with PCC, and (2*R*,5*R*)-2-isopropyl-5-methylcyclohexanone (**23**), $[\alpha]_D^{25}$ +94.8 (c 1.0, EtOH) {lit. [14]: $[\alpha]_D^{25}$ +95.0} was purchased from Aldrich Chemical. (2*S*,5*S*)-2-Methyl-5-isopropylcyclohexanone (**25**), $[\alpha]_D^{25}$ −5.3 (c 2.3, EtOH) {lit. [15]: $[\alpha]_D^{25}$ −6.0} and (2*S*,5*R*)-2-methyl-5-isopropylcyclohexanone (**27**), $[\alpha]_D^{25}$ −55.3 (c 1.0, EtOH) {lit. [16]: $[\alpha]_D^{25}$ −56.5} were prepared from (*S*)-2-methyl-5-(1-methylethenyl)cyclohex-2-en-1-one, $[\alpha]_D^{25}$ +57.1 (neat) and (*R*)-2-methyl-5-(1-methylethenyl) cyclohex-2-en-1-one, $[\alpha]_D^{25}$ −60.1 (neat), respectively, by reduction with zinc powder [17].

(1*S*,2*S*,5*R*)-2,6,6-Trimethylbicyclo[3.1.1]-heptan-3-one (**29**), $[\alpha]_D^{25}$ +11.0 (c 1.0, $CHCl_3$) {lit. [18]: $[\alpha]_D^{25}$ +8.8}, and (1*R*, 2*R*, 5*S*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-one (**30**), $[\alpha]_D^{25}$ −8.0 (c 1.0, $CHCl_3$) were prepared by oxidation of (1*S*,2*S*,3*S*,5*R*)- and (1*R*,2*R*,3*R*,5*S*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ol with PCC, respectively.



2.4. Cultures and biotransformation conditions

The suspension cells of *M. polymorpha* were prepared by the culture of *M. polymorpha* cells [19] in MSK-II medium with continuous shaking for 2 weeks. The cultured cells (30 g) were added to a 300 ml conical flask containing 100 ml of MSK-II starvation medium (glucose 0.2%). To the flask containing the suspension cells, the substrate (30 mg) was added. The transformation was performed by incubating the mixture at 25°C for 5 h on a rotary shaker (70 rpm) under illumination (1000 lux).

2.5. Identification of the products

The incubation product was extracted with ether and was subjected to chromatography on silica gel with pentane-ethyl acetate (95:5, v/v) to separate the products. The products were identified by comparison of their TLC, GC, and spectral data with those of authentic samples. The absolute configurations and enantiomeric purities of **14**–**20** were determined by CD-spectra of the products and the peak area of GLC on CP cyclodextrin β 236M-19 column (Chrompack). Diastereomeric excesses of **21**–**31** were also determined from the peak area of the corresponding diastereomers by GLC analyses on CP cyclodextrin β 236M-19 column.

The CD data of the ketones, **14**–**20**, were as follows. **14**: $[\theta]_{288}$ + 946 (c 0.10, MeOH) {lit. [20]: $[\theta]_{288}$ −987 for *R* enantiomer}; **15**: $[\theta]_{288}$ + 307 (c 0.04, MeOH) {lit. [21]: $[\theta]_{288}$ +2200}; **16**: $[\theta]_{288}$ +549 (c 0.4, MeOH) {lit. [22]: $[\theta]_{288}$ +2126}; **17**: $[\theta]_{288}$ +152 (c 0.03, MeOH) {lit. [23]: $[\theta]_{288}$ + 1690};**18**: $[\theta]_{288}$ −775 (c 0.15, MeOH) {lit. [20]: $[\theta]_{288}$ +2480 for *S* enantiomer}; **19**: $[\theta]_{288}$ −1942 (c 0.24, MeOH); **20**: $[\theta]_{288}$ −920 (c 0.04, MeOH).

Retention time for the ketones in the GLC was the following: (*S*)- and (*R*)-**14**, 11.8 and 12.8 min; (*S*)- and (*R*)-**15**, 12.7 and 12.9 min; (*R*)- and (*S*)-**16**, 23.8 and 24.0 min; (*R*)- and (*S*)-**17**, 18.1 and 18.4 min; (*S*)- and (*R*)-**18**, 27.7 and 27.9 min; (*S*)- and (*R*)-**19**, 59.8 and 60.2 min; (*S*)- and (*R*)-**20**, 72.1 and 72.8 min.

3. Results and discussion

Cyclohexanone enol acetates with various substituents at the α-position to the acetoxyl group were subjected to the hydrolysis by a suspension culture of *M. polymorpha*. When 1-acetoxy-2-methylcyclohexene (**1**) was used as substrate, hydrolysis effectively occurred to give (*S*)-2-methylcyclohexanone (**14**) in over 99% yield with high enantioselectivity (> 99% e.e.), as shown in Table 1. On the other hand, with the enol acetate (**2**) having ethyl group at the α-position to the acetoxyl group, the enantiomeric purity of resulting ketone was drastically reduced (25% e.e.). Similarly, the hydrolysis of 1-acetoxy-2-isopropylcyclohexene (**3**) resulted in low enantioselectivity (26% e.e.). The substrate (**4**) with *t*-butyl group as the α-substituent was hydrolyzed in 25% yield, and the protonation at α-position showed only poor enantioselectivity (9% e.e.). These results indicate that bulkiness of the alkyl group at the α-position to the acetoxyl group of the substrates lowers the optical yield of the resulting ketones, though the protonation of the enol intermediates from these substrates occurred preferentially from the same enantiotopic face of the C=C bond.

Table 1
Hydrolyses of enol acetates by the cultured cells of *M. polymorpha*

Substrates	Products	Conv. (%)	e.e. (%)	Configuration[a]
1	**14**	> 99	> 99	*S*
2	**15**	> 99	25	*S*
3	**16**	> 99	26	*R*
4	**17**	25	9	*R*
5	**18**	> 99	30	*R*
6	**19**	87	38	*R*
7	**20**	73	43	*R*
8	**21**	99	44[b]	*R*
9	**23**	97	63[b]	*R*
10	**25**	97	72[b]	*S*
11	**27**	> 99	36[b]	*S*
12	**29**	> 99	> 99[b]	*S*
13	**30**	> 99	55[b]	*R*

[a] Preferred configuration at the α-position to the carbonyl group of the products.
[b] Diastereomeric excess.

Effect of the chain length of a normal alkyl substituent at the α-position to the acetoxyl group on the enantioselectivity of the protonation was investigated. In the case of the substrate (**5**) having propyl group as the α-substituent, the hydrolysis effectively proceeded in 99% yield, and interestingly, the preferred configuration of the resulting ketone was *R* (30% e.e.). In the hydrolysis of the enol acetates (**6** and **7**) having butyl and pentyl groups, the configurations of the resulting ketones were *R*. These results demonstrate that the stereoselectivity of the protonation of these enol intermediates is reversed by the chain elongation ($C \geq 3$) of *n*-alkyl group at the α-position to the acetoxyl group.

To clarify the effect of the alkyl substituents at other positions on the stereoselectivity, hydrolysis of monoterpene ketone enol acetates, **8**–**13**, were examined. The preferred configuration at the α-position to the carbonyl group in the pair of diastereomers obtained in the hydrolyses of **8** and **9** was *R*, as shown in Table 1; stereoselectivities of the protonation of the corresponding enol intermediate were identical to the case of the hydrolysis of **1**. In the hydrolyses of **10**–**12**, the preferred configuration at the α-position to the carbonyl group in the corresponding diastereomeric pairs obtained was *S*. These results also show that the proton attack of the enol intermediates occurs stereoselectively at the same enantiotopic face as in the hydrolysis of **1**. On the other hand, **13** was hydrolyzed to (1*R*, 2*R*, 5*S*)-2,6,6-trimethylbicyclo[3.1.1]-heptan-3-one (**30**) and (1*R*, 2*S*, 5*S*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-one (**31**) (77: 22); in this case the preferred configuration at the α-position to the carbonyl group was *R*. This result indicates that the protonation of the enol intermediate occurred preferentially from the reverse side of the C=C bond, compared with the case of hydrolysis of **1**.

Thus, asymmetric hydrolysis of enol esters has been realized with discrimination of the enantiotopic faces of C–C double bond of the enol intermediate by incubation with *M. poly-*

morpha, and optically active α-substituted ketones were prepared. It can be seen that in the hydrolysis of substrates with long side chain ($C \geq 3$) at α-position of the acetoxyl group, and in the case of **13** with bulky *gem*-dimethyl bridge at α-side of the enantioface, the protonation occurred from the other side of the C–C double bond, compared with the substrates having short side chain.

It has been reported that the stereoselectivity in the hydrolyses of α-substituted cyclohexanone enol acetates by yeast was not modified by chain elongation of the alkyl group at α-position to the acetoxyl group [7]. Therefore, the results obtained here apparently indicate that the esterase in the *M. polymorpha* cell cultures differs from that of yeast. Two explanations for the inversion of enantioselectivity due to chain elongation of α-substituent may be postulated: two different enzymes with opposite stereoselectivities may exist in the cultured cells of *M. polymorpha*, or the turn over of the substrate in the active site of the enzyme occurs due to steric hindrance by the substituent. Further investigations using the enzyme preparation from the cultured cells of *M. polymorpha* are in progress.

Acknowledgements

The authors thank the Instrument Center for Chemical Analysis of Hiroshima University for the measurement of NMR spectra.

References

[1] K. Matsumoto, H. Ohta, Tetrahedron Lett. 32 (1991) 4729.
[2] K. Fuji, K. Tanaka, H. Miyamoto, Tetrahedron Asymmetry 4 (1993) 247.
[3] K. Fuji, T. Kawabata, A. Kuroda, J. Org. Chem. 60 (1995) 1914.
[4] K. Fuji, T. Kawabata, A. Kuroda, T. Taga, ICR Annu. Rep. 2 (1996) 36.
[5] H. Ohta, K. Matsumoto, S. Tsutsumi, T. Iori, J. Chem. Soc., Chem. Commun. (1989) 485.
[6] K. Matsumoto, H. Ohta, Chem. Lett. (1989) 1589.
[7] K. Matsumoto, S. Tsutsumi, T. Ihori, H. Ohta, J. Am. Chem. Soc. 112 (1990) 9614.
[8] I. Fujii, R.A. Lerner, K.D. Janda, J. Am. Chem. Soc. 113 (1991) 8528.
[9] T. Hirata, S. Izumi, K. Akita, H. Yoshida, S. Gotoh, Tetrahedron Asymmetry 4 (1993) 1465.
[10] S. Gotoh, M. Aoki, T. Iwaeda, S. Izumi, T. Hirata, Chem. Lett. (1994) 1519.
[11] T. Hirata, S. Izumi, M. Aoki, S. Gotoh, R. Utsumi, Chirarity 9 (1997) 250.
[12] C. Petrier, J.L. Luche, Tetrahedron Lett. 28 (1987) 2351.
[13] G. Zweifel, H.C. Brown, J. Am. Chem. Soc. 86 (1964) 393.
[14] M. Gall, H.O. House, Org. Synth. Coll. VI (1988) 121.
[15] H.C. Brown, C.D. Gary, J. Am. Chem. Soc. 83 (1961) 2952.
[16] R.G. Johnston, J. Read, J. Chem. Soc. (1935) 1138.
[17] J.J. Barieux, J. Gore, Bull. Soc. Chim. Fr. (1971) 3878.
[18] Z. Chabudzinski, D. Sedzik, Z. Rykowski, Rocz. Chem. 41 (1967) 1751.
[19] K. Katoh, M. Ishikawa, K. Miyake, Y. Ohta, Y. Hirose, T. Iwamura, Physiol. Plant. 49 (1980) 241.
[20] C.J. Cheer, C. Djerassi, Tetrahedron Lett. 43 (1976) 3877.
[21] A.I. Meyers, D.R. Williams, G.W. Erickson, S. White, M. Druelinger, J. Am. Chem. Soc. 193 (1981) 3081.
[22] C. Djerassi, P.A. Hart, C. Beard, J. Am. Chem. Soc. 86 (1964) 85.
[23] C. Djerassi, P.A. Hart, E.J. Warawa, J. Am. Chem. Soc. 86 (1964) 78.