Pig Liver Alcohol Dehydrogenase Catalysed Stereoselective Reduction of Cage-shaped Ketones. Preparation of Axially Chiral (−)-(*R*)-Adamantane-2,6-diol with High Enantiomeric Purity

Yoshiki Hirose,* Munehisa Okutsu, Mika Anzai, Koichiro Naemura and Hiroaki Chikamatsu
Department of Chemistry, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560, Japan

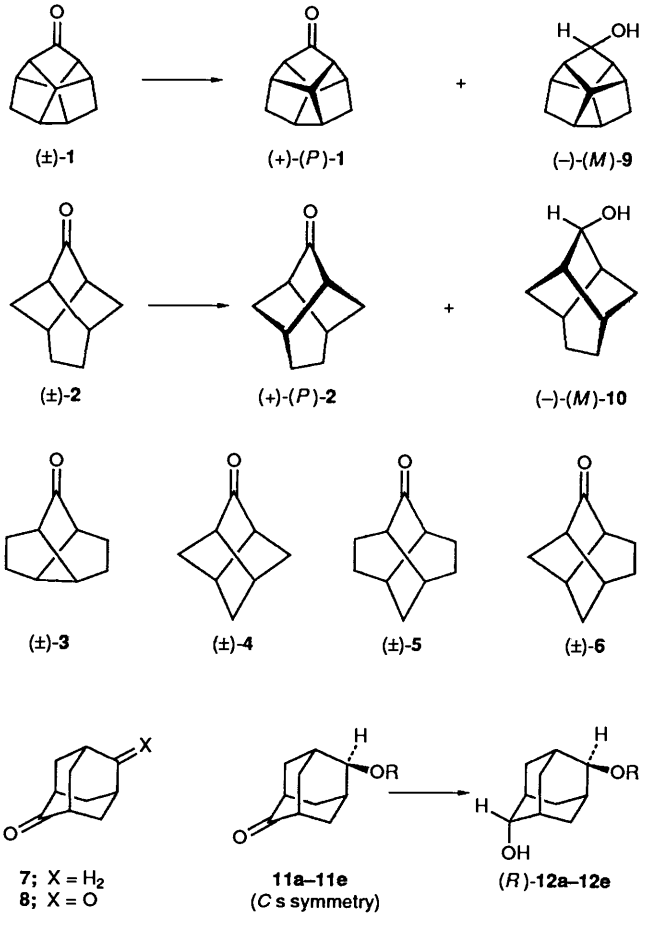
In a study of the stereoselective reductions of various cage-shaped ketones catalysed by pig liver alcohol dehydrogenase (PLADH), a typical axially chiral diol, (−)-(*R*)-adamantane-2,6-diol **12a** with high enantiomeric purity was prepared by asymmetric reduction of keto ester **11d**; based on this a new, remarkable function of the active site of the enzyme, which serves to enhance the stereoselectivity of the reaction is suggested.

Among the various versatile biocatalysts which have been used in organic synthesis to prepare optically active compounds of synthetic value, alcohol dehydrogenases together with hydrolases such as lipases are those which have been studied in the most detail.[1] We have been engaged in investigating the stereoselectivity of the oxido-reductions of compounds with rigid carbon frameworks, *e.g.*, **1**–**6**[2] and **8**[3] catalysed by horse liver alcohol dehydrogenase (HLADH) and microbes having high alcohol dehydrogenase activity. Ketones **7** and **8** with an adamantane skeleton are known to be very poor substrates for the HLADH catalysed reduction.[4] In the present paper we describe the results of PLADH[5] catalysed stereoselective reductions of the cage-shaped ketones, *i.e.* enantioselective reductions of racemic ketones having C_2 symmetry [(±)-D_3-trishomocubanone **1**, (±)-9-*twist*-brendanone **2**, (±)-2-brexanone **3**, (±)-D_{2d}-bisnoradamantanone **4**, (±)-2-twistanone **5**] and a ketone having C_1 symmetry [(±)-2-*twist*-brendanone **6**] and asymmetric reductions of adamantanone derivatives **11a**–**11e**. Stereoselectivities of PLADH catalysed reductions with respect to various types of substrates have not been widely investigated so far in contrast to those with HLADH, although its substrate specificity is regarded as quite broad.[6]

The NADPH-dependent enzyme used in the experiments was extracted from pig liver and purified 55–70 fold based on the protein contained in the crude extract partially according to the procedure reported by Kim *et al.*[7] with a slight modification.

Substrate specificities of the enzyme were first surveyed by the preliminary small scale experiments carried out using coupled enzyme coenzyme recycling systems.[5],† Ketones (±)-**1**, **7** and **8** were found to be reduced very fast. Times necessary to reduce half the amount of the substrates ($t_{\frac{1}{2}}$) were 5.5, 4.0 and 1.3 h for (±)-**1**, **7** and **8**, respectively. Reduction of (±)-**2** proceeded slowly (36 h for 6% conversion). Reduction of (±)-**3** ceased at 1.1% conversion after 2 days incubation, which was too slow to carry out a preparative scale experiment. The other three substrates were immune to the enzyme. These general aspects of the reductions of the chiral ketones **1**–**6** were similar to those with HLADH, *i.e.*, i) the order of the reduction rates of **1**–**3** was **1** > **2** > **3**, ii) (±)-**4** (±)-**5** and (±)-**6** were immune toward PLADH and iii) stereoselectivities described below.

Table 1 shows the results of the preparative scale enantioselective reduction of (±)-**1** and (±)-**2** catalysed by PLADH. The reaction shown in entry 2 was carried out with a molar ratio of G–6–P/(±)-**1** of 0.5 under a supposition of an idealized kinetic resolution, and was found to be more advantageous than that in entry 1 due to the spontaneous cessation of the reaction at the stage of 50% conversion, as well as the slightly greater enantiomeric purity of the products, although it took a slightly longer period. Reduction of (±)-**2** was actually slow as above. In the preparative scale experiment, it took 8 days incubation to reach the 40% conversion, even though a large amount of the enzyme was added once every 2 days. In every case, extraction with ether followed by preparative TLC [silica gel, pentane–ether (4:1)] afforded the recovered ketone enriched with (+)-(*P*) enantiomer and the (−)-(*M*) alcohol, from which the stereoselectivity of PLADH seems to obey the (*M*)-C_2-ketone rule (Scheme 1).[2b]



Scheme 1

† All preliminary experiments were performed under the following conditions: substrate (0.3 mmol), PLADH (5 units)[7], $NADP^+$ (0.01 mmol) G–6–P–DH (35 units), G–6–P (0.36 mmol) and $MgCl_2$ (0.22 mmol) in phosphate buffer (pH 7.2, 60 cm^3) at 30 °C (GLC monitoring).

Table 1 PLADH catalysed kinetic resolution of (±)-**1** and (±)-**2**[a]

Entry	Substrate	Time (h)	Conversion (%)	Products			
				Ketone	ee (%) [Yield (%)]	Alcohol	ee (%) [Yield (%)]
1	(±)-**1**	10	43	(+)-(*P*)-**1**	33 (53)	(−)-(*M*)-**9**	32 (37)
2	(±)-**1**	24	51	(+)-(*P*)-**1**	44 (41)	(−)-(*M*)-**9**	36 (39)
3	(±)-**2**	190	40	(+)-(*P*)-**2**	29 (54)	(−)-(*M*)-**10**	68 (34)

[a] Conditions: substrate (1.0 mmol), PLADH [7 units for (±)-**1** and *ca.* 60 units for (±)-**2**], $NADP^+$ (0.03 mmol), G–6–P–DH [70 units for (±)-**1** and 140 units for (±)-**2**] and G–6–P (1.0 mmol for entry 1, 0.5 mmol for entry 2 and 2.5 mmol for entry 3) in phosphate buffer (pH 7.2, 180 cm^3) at 27 °C.

Table 2 PLADH catalysed asymmetric reduction of 6-acyloxyadamantan-2-ones **11a–11e**[a]

Substrate			Alcohol **12**	
11	R	$t_{\frac{1}{2}}$(h)	Configuration	ee (%)[b]
a	H	10.6	*R*	22
b	COMe	1.2	*R*	53
c	$COCHMe_2$	0.4	*R*	85
d	$COCMe_3$	0.2	*R*	95
e	COPh	2.3[c]	*R*	63

[a] All the experiments were performed under the same conditions as described in the Experimental section. [b] Because of the small absolute rotation value of the diol **12a**,[3] the enantiomeric purity of each product was determined by an HPLC analysis of the bis(phenylcarbamate) derivative of the diol **12a** prepared by respective reduction of **12b–12e** with $LiAlH_4$; Column; Amylose tris(3,5-dimethylphenylcarbamate) on silica gel,[8] eluent; hexane–ethanol (95:5). [c] The relatively smaller reaction rate of **11e** may be caused by the lower solubility of the substrate in the buffer.

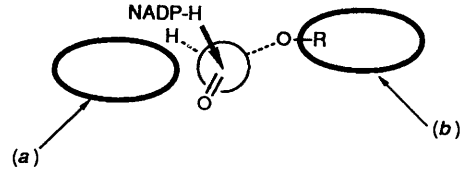


Fig. 1 Cubic-Space-Section-Model like orientation of 6-acyloxyadamantan-2-ones. Circle: Adamantane skeleton. (*a*), (*b*) (Ovals): Hydrophobic sections suggested by Prelog *et al.* and from this study, respectively.

In contrast with the results obtained using HLADH; adamantan-2-one **7** and adamantane-2,6-dione **8** were reduced very fast by PLADH as above to yield adamantan-2-ol and the achiral ketol **11a**, respectively. In the latter case, however, the next step to give the axially chiral diol **12a** was found to be unsatisfactory. The reaction is slow and, moreover, the enantiomeric purity of the resulting **12a** is low as shown in Table 2, which was obtained by a separate experiment using **11a** as the substrate.

In practice enzymatic reductions of diketones usually cease at the ketol stage. The low rate of reduction of ketols to diols compared with that of diketones to ketols may be attributed to the hardness of binding of the ketol with a hydrophilic hydroxy group to the hydrophobic active site of the enzyme. With this consideration in mind, we next examined the PLADH catalysed asymmetric reduction of keto esters **11b–11e*** prepared by the respective acylation of **11a** (Table 2). To our surprise, not only the reaction rate but also the stereoselectivity increased drastically with increasing the bulkiness and/or the hydrophobicity of the side chain of the substrates. In particular, the asymmetric reduction of ketopivalate **11d**, whose $t_{\frac{1}{2}}$ value is much smaller than that of the intact diketone **8**, was completed within 1.5 h at room temperature, yielding the ester alcohol (+)-(*R*)-**12d** with 95% ee. The axially chiral diol (−)-(*R*)-**12a**[3] with the same enantiomeric purity as **12d** was obtained by reduction with $LiAlH_4$, Ester alcohols **12b–12e** enriched with (*R*)-enantiomer were afforded in all the cases, which may imply that the acyloxy group occupied the rear right region of the active site during the catalysis as shown in Fig. 1 which is drawn in a similar orientation as in the cubic space section model for HLADH.[9]

This *si*-face selectivity of the enzyme is obviously opposite to the stereoselectivity which has been hitherto expected on the basis of the work of Dutler. Prelog *et al.* who observed a *re*-face selectivity in studies of the enantioselective reduction of some decalin-1-ones.[5] The hydrophobic section [oval (*a*) in Fig. 1], whose close location to the catalytic site has been suggested by the ETH group to explain the *re*-face selectivity, however, seems not to be able to contribute to the asymmetric reduction of adamantanones **11b–11e** because these substrate molecules show no difference in hydrophobicity in either side of the α- and β-positions to the carbonyl group. We suggest here another hydrophobic section (pocket) which is located at the rear right end of the active site [shadowed oval (*b*) in Fig. 1] and to which the acyl group binds during the catalysis to result in the *si*-face selectivity. Thus, the two opposite selectivities are not inconsistent with each other, while it is not obvious whether these two hydrophobic sections are separated from each other or not. The stronger the side chain of the substrate binds to the pocket, the higher the rate and the stereoselectivity of the reaction become, owing to enhancement of fixing of the orientation of the substrate to the active site. The pivaloyloxy group seems to fit into the pocket best within the substrates investigated in this study.

The findings described here have prompted us to investigate the HLADH-catalysed reduction of these ketones with an adamantane skeleton which have so far been regarded as very poor substrates for HLADH catalysis.[6] Efforts on this aspect is now in progress and will be reported shortly.

Experimental

Asymmetric Reduction of 6-Pivaloyloxyadamantan-2-one **11d**.—PLADH used in the experiment was purified 70 fold based on the protein contained in the crude extract by the following steps: i) ammonium sulfate precipitation, ii) two successive anion exchange chromatography (Whatman, DE-23 and Pharmacia, DEAE Sepharose CL-6B), and iii) gel filtration (Pharmacia, Sepharose CL-6B). A solution of PLADH (8.2 units)[7] and a suspension of glucose-6-phosphate dehydrogenase (G–6–P–DH) (35 units, Boehringer, Mannheim) was added to a stirred mixture of powdered ketopivaloate **11d** (78 mg, 0.31 mmol), glucose-6-phosphate (G–6–P) (127 mg, 0.37 mmol), magnesium chloride (47 mg, 0.25 mmol) and $NADP^+$ (8.3 mg, 0.011 mmol) in 0.1 mol dm^{-1} phosphate buffer (pH 7.2, 70 cm^3)

* All new compounds gave satisfactory spectroscopic data.

and the mixture was incubated for 2h at 27 °C. The reaction proceeded to 50% conversion and to completion after 12 min and 1.5 h, respectively (GLC monitoring). After the usual work-up, the crude ethereal extract was then purified by preparative TLC [silica gel, $CHCl_3$–CH_3OH (98:2)] followed by sublimation (120 °C/12 mmHg) to afford colourless crystals of **12d** (66 mg, 84% yield); m.p. 110.5–112 °C (sealed tube) (Found: M^+ 252.1734. $C_{15}H_{24}O_3$ requires *M*, 252.1725); $[\alpha]_D$ + 0.89 ($[\alpha]_{435}$ + 1.50 in MeOH).*

* $[\alpha]_D$ Values are given in 10^{-1} deg cm^2 g^{-1}.

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