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## Optical Analysis of Reduction Products of 2-Methylcyclohexanone by Aspergillus repens MA0197

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To cite this article: Hironobu Onishi, Mikiharu Doi, Yoshihiro Shuto & Yoshiro Kinoshita (1996) Optical Analysis of Reduction Products of 2-Methylcyclohexanone by Aspergillus repens MA0197, Bioscience, Biotechnology, and Biochemistry, 60:3, 486-487, DOI: <u>10.1271/bbb.60.486</u>

To link to this article: <u>http://dx.doi.org/10.1271/bbb.60.486</u>

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#### Note

# **Optical Analysis of Reduction Products of 2-Methylcyclohexanone by** *Aspergillus repens* MA0197

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The products of reduction of 2-methylcyclohexanone by *Aspergillus repens* MA0197 were analyzed quantitatively by GC after conversion to corresponding diastereoisomeric (-)-menthyl carbonate derivatives. Although the contents varied considerably with time, the predominant production of *S*-alcohol was observed, that is, Prelog's rule was obeyed.

### Key words: Katsuobushi; asymmetric reduction; molding; Aspergillus species; 2-methylcyclohexanone

Microbial and enzymatic conversions of organic compounds are interesting in terms of the stereoselectivity of the reactions.<sup>1)</sup> We have reported the reduction of some substituted cyclohexanones by *Aspergillus* species.<sup>2)</sup> In the case of 2-methylcyclohexanone, geometric *cis*- and *trans*-alcohols were produced by *A. repens* MA0197 and their absolute contents varied with time, suggesting that the isomers were produced at different ratios and then decomposed at different rates. Although the reduction of the substrate might yield four optically isomeric alcohols, the stereochemistry of the reaction has not been described. In this article, the GC chiral analyses of the reduction products of 2-methylcyclohexanone to clarify their absolute stereochemistry is described.

Among several methods for optical analysis of asymmetric alcohols,<sup>3,4</sup> we adopted the following gas chromatographic method in this experiment.

GC was done with a Shimadzu model GC-14A with a flame ionization detector on a silica capillary column (Shimadzu Techno Research CBP-20-M-25; 0.25 mm i.d. × 25 m). The column temperature was programed from 100 °C to 230 °C at the rate of 5 C/min and then held at 230 C. The injector and detector temperatures were 250 C. The flow rate of carrier gas  $(N_2)$  was 50 ml/min. Under these conditions, four optical isomers of 2-methylcyclohexanol were separated as their corresponding diastereomeric carbonate derivatives which were obtained by the use of (-)-menthyl chloroformate (III) (Fig. 1).5) The assignment of each peak was made by comparison with authentic specimens of the corresponding (-)-menthoxycarbonyl derivative of each optical isomer, which was obtained from  $(\pm)$ -tran- or  $(\pm)$ -cis-alcohol (Aldrich Chem. Co.) by the resolution based on the enantioselective esterification catalyzed by lipase (Candida cylindracea, Sigma Chem. Co.).<sup>6)</sup> The retention time and assignment of each peak was: a, 16.2 min (derived from 1); b, 16.6 min (derived from 2); c, 16.9 min (derived from 3); d, 17.1 min (derived from 4) (Fig. 2).

The metabolic experiments were done by the procedure described in our previous paper.<sup>2)</sup> Aspergillus repens MA0197 was incubated with 0.02% of 2-methylcyclohexanone (Wako Pure Chem. Co.) at 25 C for 60 days. At predetermined intervals, the products were extracted and analyzed by GC. GC conditions for quantitative analysis of the geometrical isomers of 2-methylcyclohexanol were the same as described in our previous paper.<sup>2)</sup> A

part of the mixture was analyzed after removing the solvent, ascording to the procedure mentioned above. Calculation of each optical isomer was done on the basis of both the measurement of geometrical isomers using hexadecane as an internal standard and the chiral analysis of (-)-methyl carbonate derivatives of the optical isomers.

As the resuls of GC analyses, four peaks corresponding to the isomers of 2-methylcyclohexanol were observed throughout the cultivation. However the proportions of each isomer varied markedly with time (Fig. 3). During the earlier stage of cultivation, (1S,2R)-isomer, **2**, was a major product and then **1**, **4**, and **3**, in that order. As mentioned in our last paper,<sup>21</sup> the former two isomers, **1** and **2**, had a *cis*-geometry, which were supposed to be produced by the reduction of the carbonyl group of 2-methylcyclohexanone from the less hindered equatorial direction of the preferred conformer (**Ie** and **He**) with an equatorial methyl group (Fig. 4). On the other hand, the other two isomers, **3** and **4**, had a *trans*-geometry, which were produced by the reduction of the less predominant axial methyl conformers (**Ia** and **IIa**) also from the equatorial direction.

Stereoselective reduction of a prochiral ketone mediated by



Fig. 1. Derivatization of 2-Methylcyclohexanols with (-)-Menthyl Chloroformate.



**Fig. 2.** GC Separation of Stereoisomers of 2-Methylcyclohexanols as (-)-Menthoxycarbonyl Derivatives.

**a**, *cis*-isomer (1R,2S); **b**, *cis*-isomer (1S,2R); **c**, *trans*-isomer (1R,2R); **d**, *trans*-isomer (1S,2S).



Fig. 3. Course of Production of 2-Methylcyclohexanol Isomers.

(**(**), *cis*-isomer **1** (1*R*.2*S*): (**(**), *cis*-isomer **2** (1*S*.2*R*): (**(**), *trans*-isomer **3** (1*R*.2*R*): (**(**), *trans*-isomer **4** (1*S*.2*S*).



Fig. 4. Proposed Mechanism of Reduction of 2-Methylcyclohexanones.

backer's yeast is known to afford an (S)-alcohol (Prelog's rule).<sup>7)</sup> In these experiments Prelog's rule was fundamentally obeyed. Figure 3 shows that the (2R)-ketone, II, always afforded mainly the *cis*-alcohol, **2** (1*S*,2*R*), *i.e.*, the Prelog's product. In the reduction of the (2S)-ketone, I, the formation of the *cis*-alcohol, **1** (1*R*,2*S*) (anti-Prelog's product) was depressed and accordingly, the ratio of the *trans*-alcohol **4** (1*S*,2*S*) (Prelog's product) increased and became the main product with time.

As mentioned in our last paper,<sup>2)</sup> the change of the ratios of geometrical isomers with time reflects the different rates not only of their productions but also of their decompositions and isomerizations. However, the ratios in the earlier part of the culture were supposed to reflect the rate of the reduction by the mold. It was of interest to clarify the effect of the methyl group of the substrate at C-2 upon the reduction. As shown in Fig. 5, the



Fig. 5. Diastereoselectivity of Reduction of 2-Methylcyclohexanones.

stereochemistry of the substituent influenced the course of the reaction. In the substrate with (2R)-2-methyl group (IIe and IIa), the reduction proceeded to yield the ordinary (1S)-cis-alcohol (2) in 90% diastereomeric excess; while the (2S)-2-methylketone (Ie and Ia) afforded the (1R)-cis-alcohol (1) in only 20% diastereomeric excess. In both cases the ratio varied with time and the (1S)-isomers (2 and 4) became predominant. In the last paper,<sup>2)</sup> we indicated that the product ratio in the reduction of 2-methylcyclohexanone was not explicable by conformational equilibrium alone, possibly because the vicinal equatorial methyl substituent might somewhat interfere with the approach of the bulky reducing agents. The results obtained in this study suggested that such interference seemed to be exerted more severely by the (2S)-2-methyl group than by the (2R)-2-methyl group. With the former, some parts of the stable conformer (Ie) with an equatorial methyl group should, in advance of reduction, undergo ring conversion into the unstable conformer (Ia) with an axial methyl group. In the latter case, with little steric interference, the initial ratio of the reduction product might largely reflect the conformational equilibrium (Fig. 4).

Although the diastereo- and enantio-selectivities of the biochemical reduction using *A. repens* MA0197 were not satisfactory in view of synthetic organic chemistry, they might be improved by modification of the culture conditions and/or substrate structures.<sup>8)</sup>

Since the mold *A. repens* MA0197, used in this study, is one of the fungal strains used during the molding process in commercial *Katsuobushi* production,<sup>9)</sup> the reaction might have some role in the creation of the *Katsuobushi* flavor.

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