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Enantioselective Hydrolysis of 2-Methylcyclohexanyl Acetates with the Cultured Cells of *Marchantia polymorpha*

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Abstract: Enantioselectivity in the hydrolysis of *trans*- and *cis*-2-methylcyclohexanyl acetates with the cultured cells of *Marchantia polymorpha* was investigated. The cultured cells were found to hydrolyze preferentially the acetates having the *R*-configuration.

Enantioselective hydrolysis of acetates with biocatalysts, i.e. microorganisms, is a useful process for the preparation of chiral alcohols.[1-4] However, there have been very few reports on the hydrolysis of acetates with the cultured cells of higher plants.[5] In connection with the development of suitable methods for the preparation of chiral compounds, we have now investigated the enantioselectivity in the hydrolysis of *sec*-acetates, such as *trans*- and *cis*-2-methylcyclohexanyl acetates, with cultured cells of *Marchantia polymorpha*.

The time-courses were determined for the hydrolysis of the acetates with the cultured suspension cells.[6] The substrate (20 mg) was administered to the flask containing the cell cultures (20 g) in MSK-2 medium[8] (80 ml), and the cultures were incubated at 25 ℃. Samples were taken at regular intervals and the incubation mixture was extracted with diethyl ether. The extract was then subjected to GLC and the yields of products were determined from the GLC trace by use of a standard curve. In each case of the hydrolysis of racemic *trans*- and *cis*-2-methylcyclohexanyl acetates, the rate of hydrolysis was fast and half of the acetate was hydrolyzed in a first 6~10 hr period.

The absolute configuration and optical purity of the product at several stages of the incubation were determined by analyses of the ^{1}H NMR spectrum of the MTPA derivative.[9-11] The configuration at the carbon atom bearing the hydroxyl group of *trans*- and *cis*-2-methylcyclohexanols obtained was mainly *R* in each case. Figure 1 shows the correlation between the yield of the alcohol and its optical purity. 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.

A large scale incubation of racemic *trans*-2-methylcyclohexanyl acetate (900 mg) with the cultured cells of *M. polymorpha* was monitored by GLC and the incubation was stopped when it had reached about 50% yield of the alcohol. (1*R*,2*R*)-(-)-*trans*-2-methylcyclohexanol[12] was obtained in a 53% yield: $[\alpha]_D^{25}$ -38.2 (*c* 9.6, EtOH); 80% e.e. by ^{1}H NMR analysis of the corresponding MTPA ester. The acetate was recovered unchanged in a 45% yield, $[\alpha]_D^{25}$ +69.9 (*c* 0.64, EtOH), and identified as (1*S*,2*S*)-(+)-*trans*-2-

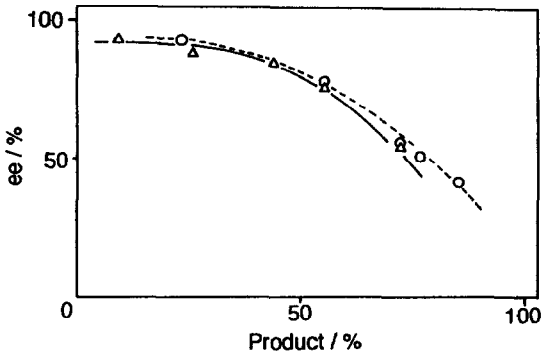

Fig. 1. Optical purities of the products at several stages of the hydrolysis of racemic *trans*-2-methylcyclohexanyl acetate (---o---) and *cis*-2-methylcyclohexanyl acetate (—Δ—) with the cultured cells of *M. polymorpha*.

methylcyclohexanyl acetate (>99% e.e.)[13] by ^{1}H NMR measurement of the corresponding MTPA ester.

Thus, highly enantioselective hydrolyses of *trans*- and *cis*-2-methylcyclohexanyl acetates have been achieved by the cultured suspension cells of *M. polymorpha*.

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6. The cultured cells of *M. polymorpha*[7] has routinely been subcultured every two weeks using MSK-2 medium[8] for more than two years; the cultures were maintained by incubating about 10 g of the suspension cells in a 300-ml conical flask containing 100 ml of fresh medium at 25 °C on a rotary shaker at 70 rpm under 3000 lux illumination.
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9. Δδ values [Δδ(ppm) = δ{(*R*)-MTPA ester of (+)-alcohol} − δ{(*R*)-MTPA ester of (-)-alcohol}][10,11] of their MTPA ester were +0.0001 (1-H), -0.070 (2-H), +0.161 (2-Me) for the *trans*-alcohol and -0.006 (1-H), -0.051 (2-H), +0.110 (2-Me) for the *cis*-alcohol.
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