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Oxidoreduction between Cycloalkanols and Cycloalkanones in the Cultured Cells of Nicotiana tabacum. Simulation of the Time-courses in the Oxidation of (+)-Borneol and the Reduction of (-)-Carvomenthone

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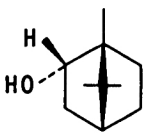
The time-courses in the oxidation of (+)-borneol and the reduction of (-)-carvomenthone in the cultured cells of Nicotiana tabacum were simulated on the basis of the permeability of 5- to 7-membered cycloalkanols and their corresponding cycloalkanones into the cultured cells and the ^{13}C NMR chemical shift of the carbonyl carbon of (+)-camphor which is the oxidation product of (+)-borneol and (-)-carvomenthone which is a substrate for the reduction, respectively.

Recently, we reported that the equilibrium of the oxidoreduction between 5- to 8-membered cycloalkanols and their corresponding cycloalkanones in the cultured cells of Nicotiana tabacum tends to lie toward the side of the alcohol in the case of cyclohexanol, but the side of the ketone in the cases of cyclopentanol, cycloheptanol, and cyclooctanol.[1,2] Further, the oxidoreduction between cycloalkanols and their corresponding cycloalkanones in the cultured cells was found to be governed by an NAD^+-dependent alcohol dehydrogenase which is similar to the dehydrogenase from tea seeds and horse liver.[2,3] It was furthermore established that the rate constants and the equilibrium constants of the enzymatic oxidoreduction can be predicted by the ^{13}C NMR chemical shift of the carbonyl carbon of the cycloalkanones.[3] We have developed a method for simulation of the time-courses in the oxidation of 5- to 7-membered cycloalkanols and the reduction of their corresponding cycloalkanones in the cultured cells on the basis of the reaction rates of the enzymatic oxidation and reduction and the permeability of the cycloalkanols and cycloalkanones into the cultured cells. Furthermore, the method has been applied to the oxidation of (+)-borneol (1) to (+)-camphor (2) and the reduction of (-)-carvomenthone (3) to (+)-carvomenthol (4) in the cultured cells.



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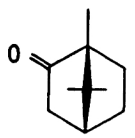

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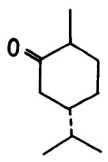

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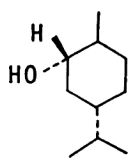

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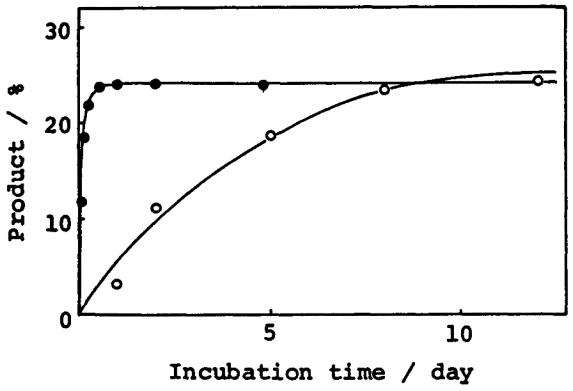

Fig. 1. Time-courses of the bioconversion of cyclohexanol into cyclohexanone with the enzyme system (—●—) and the cultured cells (—○—).

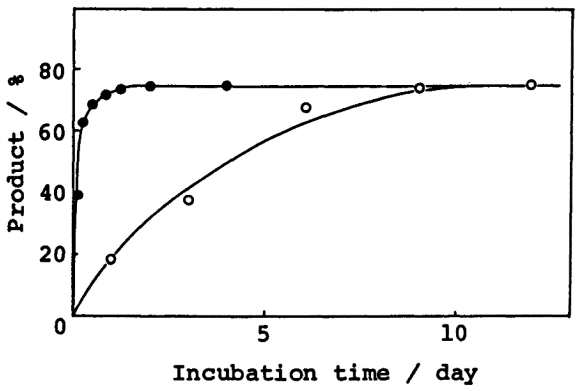

Fig. 2. Time-courses of the bioconversion of cyclohexanone into cyclohexanol with the enzyme system (—●—) and the cultured cells (—○—).

At first, the rate of the oxidoreduction between 5- to 7-membered cycloalkanols and their corresponding cycloalkanones in the cultured cells was compared with that of the oxidoreduction in the enzyme system prepared from the cultured cells. The time-course experiments with the cultured cells were carried out in a manner similar to that reported in ref. 2 and 4.[5] The time-courses in the enzymatic oxidation and reduction[7] were followed by measuring a change in the UV absorption at 340 nm due to a reduced form of nicotinamide adenine dinucleotide. The time-courses in the oxidation of cyclohexanol to cyclohexanone and the reduction of cyclohexanone to cyclohexanol are shown in Figs. 1 and 2, respectively. The half-lives of these substrates in the oxidation and reduction in the cells were about 3 days in both cases, whereas the half-lives in the case of the enzymatic oxidation and reduction were 10 min and 40 min, respectively. The difference in the half-lives was also observed in the oxidation and reduction between other cycloalkanols and their corresponding cycloalkanones.[1—3] These observations indicate that the rates of the oxidation and the reduction in the cultured cells were much slower than those of the oxidation and the reduction in the enzyme system prepared from the cultured cells. Such a time-lag is probably caused by permeation of cycloalkanols and cycloalkanones into the cells. The rate of permeation is known to depend on a gradient between intra- and extracellular concentrations of cycloalkanols;[8—10] the permeability constant can be estimated by using Hill's equation.[9] The permeability constants in the permeation of 5- to 7-membered cycloalkanols and their corresponding cycloalkanones into the cells were determined by substituting the concentration of the products in the time-courses with the cultured cells[5] in Hill's equation; the average of the permeability constants in the oxidation of 5- to 7-membered cycloalkanols to their corresponding cycloalkanones was 4.82×10^{-6} s^{-1}.

On the basis of the permeability constants and the rate constants predicted[3] by the ^{13}C NMR chemical shift of the carbonyl carbon of the

oxidation products, the time-courses in the oxidation of cycloalkanols in the cells were simulated as follows. Temporal infinitesimal change in the concentration of the product [P] at the time "t" is given by

$$d[P] = \{[S]_0 \cdot p \cdot k_{+1}/(k_{+1}+k_{-1}) - [P] \cdot p\}\, dt, \quad (1)$$

where $[S]_0$, p, k_{+1}, and k_{-1} denote the initial concentration of the substrate (cycloalkanols), the permeability constant, the rate constant of oxidation of cycloalkanols, and the rate constant of reduction of cycloalkanones, respectively. The initial term shows the infinitesimal change in the concentration of the product which was formed by oxidation of the substrate and the last term shows the infinitesimal change in the concentration of the product permeated into the cells. The solution of this differential Eq. 1 is

$$[P] = \{k_{+1} \cdot [S]_0/(k_{+1}+k_{-1})\}(1-e^{-p \cdot t}). \quad (2)$$

It has been recently found that the rate constants of the enzymatic oxidation of cycloalkanols (k_{+1}) and the enzymatic reduction of their corresponding cycloalkanones (k_{-1}) can be predicted by the ^{13}C NMR chemical shift of the carbonyl carbon of cycloalkanones.[3] If we use this relation, we can combine Eq. 2 to the ^{13}C NMR chemical shift of the carbonyl carbon of cycloalkanones, and the proportion of the concentration of product (P) is found

$$P = [P]/[S]_0 = \{1-\exp(-4.82\times10^{-6}\ t)\}/\{1+\exp(-0.284\delta_{C=O}+60.5)\}. \quad (3)$$

Therefore, the time-courses in the oxidation of cycloalkanols and their related compounds in the cells may be simulated on the basis of the permeability of cycloalkanols and their corresponding cycloalkanones into the cells and the ^{13}C NMR chemical shift of the carbonyl carbon of cycloalkanones.

This equation for simulation of the time-courses in the oxidation of cycloalkanols was applied to the oxidation of (+)-borneol (1) to (+)-camphor (2) in the cultured cells. Fig. 3 shows the time-courses in the oxidation of the borneol to camphor in the cultured cells.[5] The time-courses were simulated by substituting the carbonyl carbon ^{13}C NMR chemical shift of camphor ($\delta_{C=O}$ 218.9) in the Eq. 3 and are shown in Fig. 3. This simulation curve well fits with the bioconversion curve.

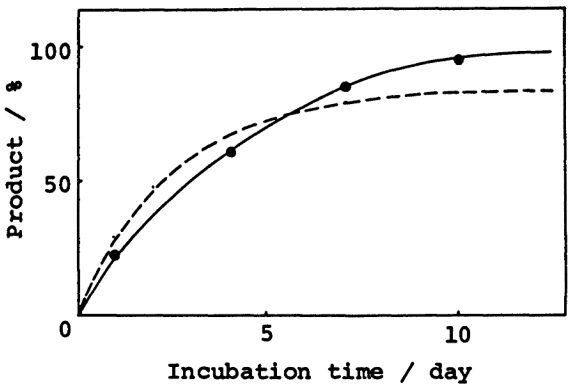

Fig. 3. Comparison of the bioconversion of (+)-borneol (1) into (+)-camphor (2) in the cultured cells (--●--) with its simulation (-----).

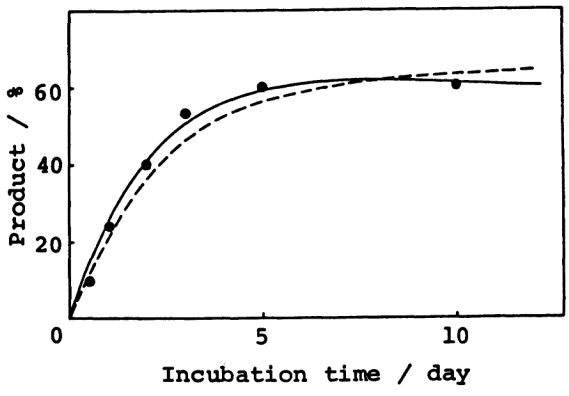

Fig. 4. Comparison of the bioconversion of (-)-carvomenthone (3) into (+)-carvomenthol (4) in the cultured cells (--●--) with its simulation (-----).

On the other hand, the time-courses in the reduction of cycloalkanones were simulated in a manner similar to that described above. The simulated equation is as follows:

$$P = \{1-\exp(-4.82\times10^{-6}\ t)\}/\{1+\exp(0.284\delta_{C=O}-60.5)\}. \quad (4)$$

This equation was applied to the reduction of (-)-carvomenthone (3) to (+)-carvomenthol (4). The time-courses in the reduction of carvomenthone to carvomenthol[5] were simulated on the basis of the ^{13}C NMR chemical shift of the carbonyl carbon of carvomenthone ($\delta_{C=O}$ 211.8) and are shown in Fig. 4. Simulation curve for the reduction well fits again with the bioconversion curve.

It was thus established that the time-courses in the oxidation of cycloalkanols and the reduction of cycloalkanones in the cultured cells can be simulated on the basis of the permeability of cycloalkanols and cycloalkanones into the cultured cells and the carbonyl carbon ^{13}C NMR chemical shift of cycloalkanones involved in the equilibrium of the oxidoreduction between cycloalkanols and the cycloalkanones.

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5) The time-course experiments were carried out in a manner similar to that reported in our previous papers;[2,4] the substrate (10 mg) was administered to the flask containing the precultured suspension cells (50 g fresh wt/ 100 cm^3 Murashige-Skoog's medium[6]), and the cultures were incubated at 25 °C for 10 days with shaking. A part (10 cm^3) of the incubated mixture was pipetted out at a regular time interval and treated with ether to extract a reaction mixture. The relative percentage for the total amount of the reaction mixture was determined on the basis of the peak areas on GLC. The time-courses thus obtained are as shown in Figs. 1—4.
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7) The enzymatic oxidation and reduction were carried out in a manner similar to that reported in our previous paper;[2] the suspension cells (50 g fresh wt) were macerated at 0 °C in a Waring Blender for 90 sec with 50 mM Tris-HCl buffer solution (100 cm^3, pH 7.5) containing 10 mmol of 2-mercaptoethanol and filtered through gauze to give a homogenate. The homogenate was centrifuged at 13 000 g for 30 min at 0 °C. Cold acetone (0 °C, 500 cm^3) was poured into the supernatant with vigorous stirring under ice-cooling and then the precipitate was treated with ammonium sulfate (25—60% saturated). The salting-out precipitate was used as the enzyme system. In the enzymatic oxidation of cycloalkanol, the enzyme system (0.6 μg protein) was added to a solution of cycloalkanol (1 mM) and NAD^+ (1.2 mM) in 0.1 M glycine-NaOH buffer (pH 9.0). In the case of the reduction, NADH (1.2 mM) in 0.1 M potassium phosphate buffer (pH 7.0) was used for the reductant. The mixture was incubated at 25 °C for 60 h.
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