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Oxidoreduction between Cycloalkanols and Cycloalkanones in the Cultured Cells of Nicotiana tabacum. Correlation of the Reaction Rate with the ^{13}C NMR Chemical Shift of the Carbonyl Carbon

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The enzyme system responsible for the oxidoreduction between cycloalkanols and their corresponding cycloalkanones in the cultured cells of Nicotiana tabacum was found to be alcohol dehydrogenase which is similar to the dehydrogenase from tea seeds and horse liver. The rate constants and the equilibrium constants of the oxidoreduction between the cycloalkanols and their corresponding cycloalkanones with this enzyme system were well correlated with the ^{13}C NMR chemical shift of the carbonyl carbon of the oxidation products, i.e., the cycloalkanones.

The balance of the equilibrium in the oxidoreduction between cycloalkanols and their corresponding cycloalkanones was recently found to be determined by the number of carbon atoms in the carbocyclic ring of these substrates.[1,2] On the other hand, the ring-size effect in the reduction of cycloalkanones with $NaBH_4$ was interpreted on the basis of the difference in internal strain in the carbocyclic ring.[3] Generally, the reactivity depends on the electron density of the reacting species and the ^{13}C NMR chemical shift is used as a parameter of the electron density.[4] We have investigated correlations of the reaction rates of the enzymatic oxidoreduction with the ^{13}C NMR chemical shifts of the carbon atom bearing the hydroxyl group of cycloalkanols and the carbonyl carbon of cycloalkanones.

Firstly, the enzyme system responsible for the oxidoreduction of the cycloalkanols and their corresponding cycloalkanones in the cultured cells of N. tabacum was characterized. The enzyme system was prepared from the cultured cells of N. tabacum by treating a cell-free extract of the cells with cold acetone and then ammonium sulfate. Substrate specificity of this system was tested for the oxidation of alkanols; ethanol was found to be the best substrate of the alkanols. Thermal stability was relatively high; the activity was retained even when the enzyme system was maintained at 30 °C for 15 min, but it was completely lost at 60 °C. Optimum pH was 8.6 for an NAD-dependent reaction and 6.8 for an NADH-dependent reaction. The properties, such as the substrate specificity, the thermal stability, and the optimum pH, were quite similar to those of alcohol dehydrogenase isolated from tea seeds[5] and horse liver.[6,7] The enzyme system catalyzed the oxidation of

the cycloalkanols, such as cyclopentanol (1), cyclohexanol (2), 2-methylcyclohexanol (3), 3-methylcyclohexanol (4), and cycloheptanol (5). Thus, the enzyme system responsible for the oxidoreduction between the cycloalkanols and their corresponding cycloalkanones in the cultured cells of N. tabacum was found to be alcohol dehydrogenase.

The reaction rates in the oxidation of cycloalkanols and the reduction of the corresponding cycloalkanones with the enzyme system were determined as follows. In the case of oxidation, the enzyme system (0.6 μg protein) was added to a solution of the substrate (0.01—4.0 mM) in 0.1 M glycine-NaOH buffer (pH 9.0). In the case of reduction, 0.1 M potassium phosphate buffer (pH 7.0) was used as the buffer solution. The mixture was incubated at 25 °C for 2 h and the reaction was monitored by means of UV absorption measurements. The initial rates of the oxidoreduction were determined by measuring the change in the UV absorption at 340 nm due to a reduced form of nicotinamide adenine dinucleotide and then by the least square method with the Lineweaver-Burk's equation.[8] The cycloalkanols used as substrate for the oxidation were cyclopentanol (1), cyclohexanol (2), 2-methylcyclohexanol (3), 3-methylcyclohexanol (4), and cycloheptanol (5), and the cycloalkanones used for the reduction were cyclopentanone (6), cyclohexanone (7), 2-methylcyclohexanone (8), 3-methylcyclohexanone (9), and cycloheptanone (10). The rate constants of the oxidation of the cycloalkanols (k_{+1}), the rate constants of the reduction of the cycloalkanones (k_{-1}), and the equilibrium constants of the oxidoreduction (K) between the cycloalkanols and cycloalkanones were correlated with the ^{13}C NMR chemical shifts of the carbon atom bearing the hydroxyl group of cycloalkanols and the carbonyl carbon of the cycloalkanones, because the ^{13}C NMR chemical shift reflects the electron density on the carbon atom. The correlations of ln k_{+1}, ln k_{-1}, and ln K against the ^{13}C NMR chemical shifts of the cycloalkanols were represented by the following empirical formulas with r (the correlation coefficient) = 0.75, 0.71, and 0.68, respectively, for Eq. 1—3.

Rate constants;

$$k_{+1} = \exp(-0.195\,\delta_{C\text{-}OH} + 6.4) \quad (1)$$

$$k_{-1} = \exp(-0.515\,\delta_{C\text{-}OH} + 29.2) \quad (2)$$

Equilibrium constant;

$$K = (k_{+1}/k_{-1}) = \exp(0.318\,\delta_{C\text{-}OH} - 22.6) \quad (3)$$

where $\delta_{C\text{-}OH}$ denotes the ^{13}C NMR chemical shift of the carbon atom bearing the hydroxyl group. The ^{13}C NMR chemical shifts were not well correlated with the rate constants and the equilibrium constants.

On the other hand, a plot of ln k_{+1}, ln k_{-1}, and ln K against the ^{13}C NMR chemical shifts of the carbonyl carbon yielded a straight line, as shown in Figs. 1 and 2. These correlations were represented by the following empirical formulas with r = 0.97, 0.95, and 0.94, respectively, for Eq. 4—6.

Rate constants;

$$k_{+1} = \exp(-0.162\,\delta_{C=O} + 27.1) \quad (4)$$

$$k_{-1} = \exp(-0.447\,\delta_{C=O} + 87.7) \quad (5)$$

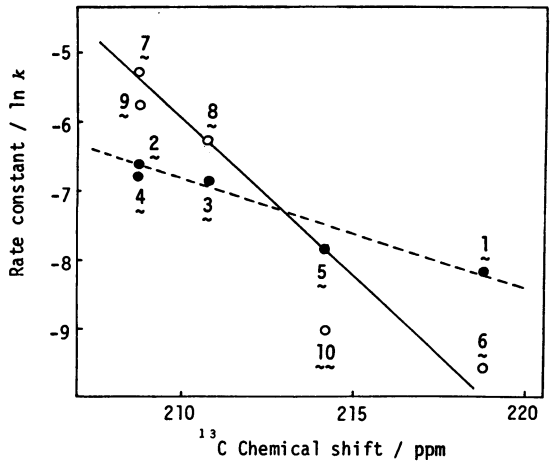

Fig. 1. Correlations of the rate constants of the oxidation of cycloalkanols (1—5) (k_{+1}; --●--) and the reduction of cycloalkanones (6—10) (k_{-1}; —○—) with the ^{13}C NMR chemical shift of the carbonyl carbon of the cycloalkanones involved in the reactions.

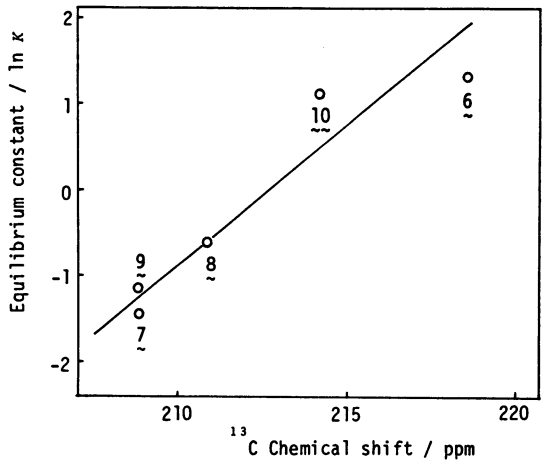

Fig. 2. Correlation of the equilibrium constant of the oxidoreduction between cycloalkanols (1—5) and cycloalkanones (6—10) with the ^{13}C NMR chemical shift of the carbonyl carbon of the cycloalkanones (6—10) involved in the equilibrium.

Equilibrium constant;

$$K = (k_{+1}/k_{-1}) = \exp(0.284\,\delta_{C=O} - 60.5) \qquad (6)$$

where $\delta_{C=O}$ denotes the ^{13}C NMR chemical shift of the carbonyl carbon of the cycloalkanones. The rate constants and equilibrium constants against the ^{13}C NMR chemical shifts of the carbonyl carbon were better correlated with those against the chemical shifts of the carbon atom bearing the hydroxyl group. Therefore, the rate constants and equilibrium constants of the enzymatic oxidoreduction between the cycloalkanols and their corresponding cycloalkanones were found to be predicted by the ^{13}C NMR chemical shifts of the carbonyl carbon of the cycloalkanones with the Eq. 4—6; this indicates a dependence of the rate of the enzymatic oxidoreduction on the electron density of the carbonyl carbon of the oxidation products.

Thus, it was established that (i) the enzyme system responsible for the oxidoreduction between the cycloalkanols and their corresponding cycloalkanones in the cultured cells of N. tabacum was alcohol dehydrogenase which is similar to the dehydrogenase from tea seeds and horse liver and (ii) the rate constants and equilibrium constants of the enzymatic oxidoreduction can be predicted by the ^{13}C NMR chemical shift, which reflects the electron density of the carbonyl carbon of the oxidation products, i.e., the cycloalkanones.

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