Biotransformation by Plant Tissue Cultures; Oxidation-Reduction Relationship Between Cycloalkanones and the Corresponding Cycloalkanols in a Cell Suspension Culture of *Nicotiana tabacum*

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Abstract

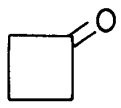
The interconversion between 4 to 8-membered cycloalkanones and the corresponding cycloalkanols by a cell suspension culture of *Nicotiana tabacum* was studied. It was found that the interconversion is at equilibrium and the balance in the equilibrium depends on the number of carbon atoms in the carbocyclic ring of the substrates; the equilibrium in the interconversion of 6-membered compound lies toward the side of the cycloalkanol, while in the cases of the 4, 5, 7, and 8-membered compounds the cycloalkanones are favoured.

Introduction

The ability of cultured cells of plants to metabolize foreign substrates and/or convert them into more useful substances is of considerable interest because of the specificity of the transformation which may be effected by such cells[1,2]. In such a status, we recently investigated the transformation of monoterpenoid alcohols and ketones with the cultured cells of *Nicotiana tabacum* "Bright Yellow", and found that the tobacco cells have the ability not only to reduce stereoselectively the carbon-carbon double bond adjacent to the carbonyl group of carvone as well as the carbonyl group[3], but also to hydroxylate regioselectively the allylic position of the carbon-carbon double bond of linalool[4]. Also, the cultured cells were found to have the ability to hydrolyze the acetoxyl group of linalyl acetate, dihydrolinalyl acetate, and α-terpinyl acetate, but the cells scarcely show such an ability

to γ-terpinyl acetate[5]. Furthermore, it was found that the cells have the ability to discriminate between the enantiomers of the bicyclic terpene alcohols[6].

To elucidate the ability of the cultured suspension cells of *Nicotiana tabacum*, we have now examined the transformation of 4 to 8-membered cycloalkanones and their corresponding cycloalkanols with the cultured suspension cells of *Nicotiana tabacum*.



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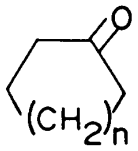
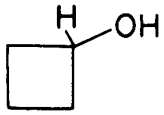


2: n=1
3: n=2
4: n=3
5: n=4



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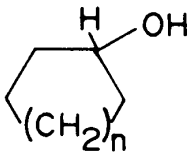

7: n=1
8: n=2
9: n=3
10: n=4

Experimental

Analytical (0.25 mm thick) and preparative TLC (0.75 mm thick) were carried out on a silica-gel plate (Merck, Type 60, GF_{254}). GLC analysis were performed on an instrument equipped with an FID and a glass column (3 mm × 2 m) packed with 15 % DEGS, 2 % OV-17, or 2 % OV-101 on chromosorb W (AW-DMCS; 80—100 mesh) by programming the column temperature at 50—150 ℃ with a rate of 2 ℃/min for DEGS and at 50—200 ℃, 2 ℃/min for OV-17 and OV-101. The areas of the peaks on the gas-liquid chromatogram were calculated by use of a Shimadzu C-R1A Chromatopac recording data processor for chromatography. ^1H NMR spectra were taken at 60 or 90 MHz for $CDCl_3$ solution with TMS as an internal referene. IR spectra were obtained on a JASCO 102 spectrophotometer.

Substrates.

Cyclobutanone [n_D^{25} 1.4223 (lit[7]., n_D^{16} 1.4220)] (**1**), cyclopentanone [n_D^{25} 1.4340 (lit[8]., n_D^{20} 1.43680)] (**2**), cyclohexanone [n_D^{25} 1.4475 (lit[8]., n_D^{20} 1.44977)] (**3**), cycloheptanone [n_D^{25} 1.4581 (lit[8]., n_D^{20} 1.45976)] (**4**), cyclobutanol [n_D^{25} 1.4340 (lit[7]., n_D^{19} 1.4339)] (**6**), cyclohexanol [n_D^{25} 1.4360 (lit[8]., n_D^{20} 1.46600)] (**8**), and cyclooctanol [n_D^{25} 1.4892 (lit[9]., n_D 1.487)] (**10**) were analytical grade commercial materials and used without further purification. Cyclooctanone [mp 40° (lit[10]., mp 40—42°)] (**5**) was prepared from **10** according to the method given in the literature[10]. Cyclopentanol [n_D^{25} 1.4585 (lit[8]., 1.45317)] (**7**) and cycloheptanol [n_D^{25} 1.4720 (lit[8]., n_D^{20} 1.47470)] (**9**) were prepared from **2** and **4** by reduction with $NaBH_4$ in MeOH

solution, respectively. All the samples were >99% pure by GLC.

Feeding of the substrates to the tobacco suspension cells.

The callus tissues used in this study were induced from the stem of *Nicotiana tabacum* "Bright Yellow" and have been maintained for about 10 years. Just prior to use for this work, a part of the callus tissue was transferred to freshly prepared Murashige and Skoog's medium[11] (100 ml/300 ml conical flask) containing 2 ppm 2,4-dichlorophenoxyacetic acid and 3 % sucrose and grown with continuous shaking for 3—4 weeks at 25 ℃ in the dark. The substrate (10 mg/flask) was then added to the suspension cultures (about 50-70 g cells/flask) and the cultures incubated at 25 ℃ for 7—10 days on a rotary shaker (70 rpm) in the dark.

Identification of the products.

The suspension cells were filtered off and triturated with MeOH. The MeOH extract was concentrated and the residue was extracted with ether. The culture medium from the cells was extracted with ether. The two ether extracts were bulked, since they exhibited the same behaviour on TLC and GLC, after removal of the solvent examined by TLC and GLC. The transformation products were identified by direct comparison of TLC and GLC with those of authentic samples.

The time-courses on the biotransformation of the substrates with the tobacco suspension cells.

Time-course experiments in the biotransformation of the substrates were carried out as followes : the substrate (10 mg) was administered to each flask containing the precultured suspension cells, and then the mixture was incubated in the dark at 25 ℃ for 10 days with shaking (70 rpm). At a regular time interval, a part (10 ml) of the incubated cultured mixture was pipetted out under sterile conditions and then the pipetted-out mixture was extracted with ether to afford an ether extract. Each ether extract was subjected to GLC with a 15 % DEGS column at 100 ℃. The yields of the products were determined on the basis of the peak area on the chromatogram of GLC and are expressed as a relative percentage to the whole reaction mixture obtained.

Results and Discussion

Callus tissues induced from the stem of *Nicotiana tabacum*" Bright Yellow" were used in this work. The callus tissues were cultured in Murashige and Skoog's medium[11] with continuous for 3—4 weeks and then the substrates were administered. The cultures were then incubated at 25 ℃ for 7—10 days with shaking in

the dark. The time-courses of the biotransformation were followed, and it's result is shown in Fig. 1. After incubation for 8 days, cyclohexanone was converted to cyclohexanol in 80 % yield, but the other cycloalkanones, such as cyclobutanone, cyclopentanone, cycloheptanone, and cyclooctanone, were relatively unchanged as shown in Fig. 1 (a). On the contrary, cyclohexanol was converted into cyclohexanone in only 20 % yield, whereas the other cycloalkanols, such as cyclobutanol,

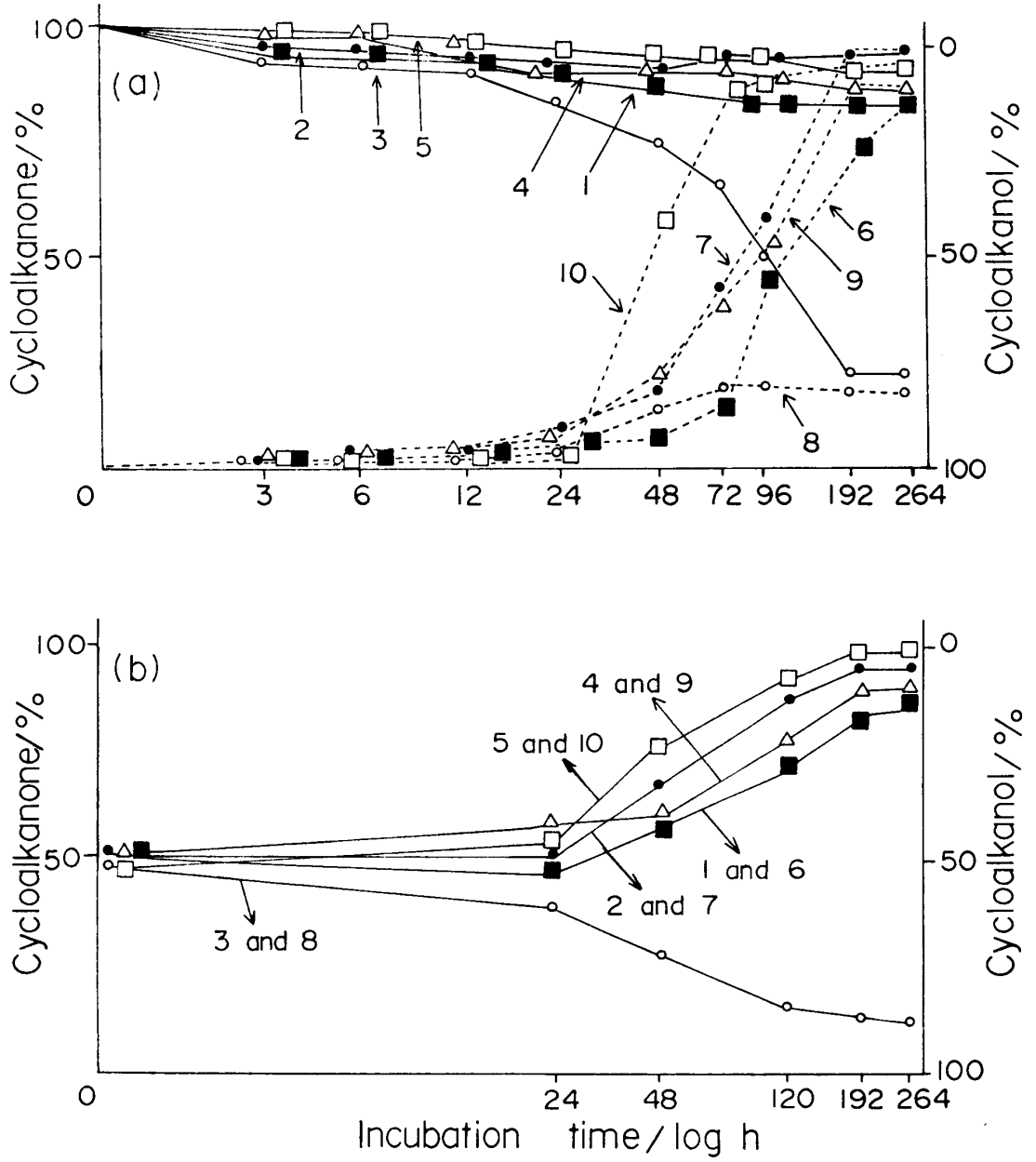

Fig. 1. The time-courses in the bioconversion of the following substrates with the suspension cells of *Nicotiana tabacum*; (a) cyclobutanone (**1**), cyclopentanone (**2**), cyclohexanone (**3**), cycloheptanone (**4**), cyclooctanone (**5**), cyclobutanol (**6**), cyclopentanol (**7**), cyclohexanol (**8**), cycloheptanol (**9**), and cyclooctanol (**10**); (b) the equimolar mixtures of cyclobutanone (**1**) and cyclobutanol (**6**), cyclopentanone (**2**) and cyclopentanol (**7**), cyclohexanone (**3**) and cyclohexanol (**8**), cycloheptanone (**4**) and cycloheptanol (**9**), and cyclooctanone (**5**) and cyclooctanol (**10**).

cyclopentanol, cycloheptanol, and cyclooctanol, were quantitatively converted into the corresponding cycloalkanones. The proportion of the cycloakanone to the cycloalkanol in the incubation mixture became constant with the lapse of the incubation time, depending on the member size of the carbocyclic ring of the substrates. These facts were confirmed by the time-course experiments which were performed by use of an equimolar mixture of the cycloalkanones and their corresponding cycloalkanols as a substrate. The results are shown in Fig. 1(b). In these cases the proportion of the cycloalkanone to the cycloalkanol also becames constant after incubation for 8 days and converges on the point of about 80 % of cyclohexanol in the case of the 6-membered carbocyclic ring compound. In the cases of the 4, 5, 7, and 8-membered carbocyclic ring compounds, on the contrary, the proportion converges on the point of about 90 % of the cycloalkanones and 10 % of the corresponding cycloalkanols.

Thus, it was established that the bioconversion of the 4 to 8-membered cyclic compounds with the suspension cells of *Nicotiana tabacum* is an interconvertible oxidoreduction between the cycloalkanones and their corresponding cycloalkanols, and the interconversion between the cycloalkanones and their corresponding cycloalkanols is at equilibrium. Further, the balance of the equilibrium in the interconversion was found to depend on the number of carbon atoms in the carbocyclic ring of these substrates; the equilibrium tends to shift toward the side of the cycloalkanol in the case of the 6-membered cyclic compound, while it is far in the direction of the cycloalkanones in the cases of the 4, 5, 7, and 8-membered cyclic compounds. Such an equilibrated interconversion between the cycloalkanones and the cycloalkanols suggests the participation of a dehydrogenase in the bioconversion of the cycloalkanones and the corresponding cycloalkanols with the suspension cells. There is a possibility that the enzyme participating in the bioconversion may be induced in the cultured cells by administrating the foreign substrates to them, because the transformation abilities of the cultured cells depend on the structural varieties of the foreign substrates administered[12]. The ring-size effect reminds us of the recent report[13] on the substrate specificity of horse liver alcohol dehydrogenase for the oxidoreduction of cyclic ketones and the corresponding alcohols. It would be interesting to investigate the reason why the equilibrium in the redox interconversion between the cycloalkanones and the corresponding cycloalkanols depends on the ring size of these substrates, and research on an enzymic level is now in progress.

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