

Stereoselectivity in Oxidative and Reductive Transformations of p-Menthane Derivatives with the Cultured Cells of Nicotiana tabacum

Takayuki SUGA,\* Hiroki HAMADA,<sup>†</sup> Toshifumi HIRATA, and Shunsuke IZUMI  
Department of Chemistry, Faculty of Science, Hiroshima University,  
Higashisenda-machi, Naka-ku, Hiroshima 730

<sup>†</sup>Department of Fundamental Natural Science, Okayama University of Science,  
Ridai-cho, Okayama 700

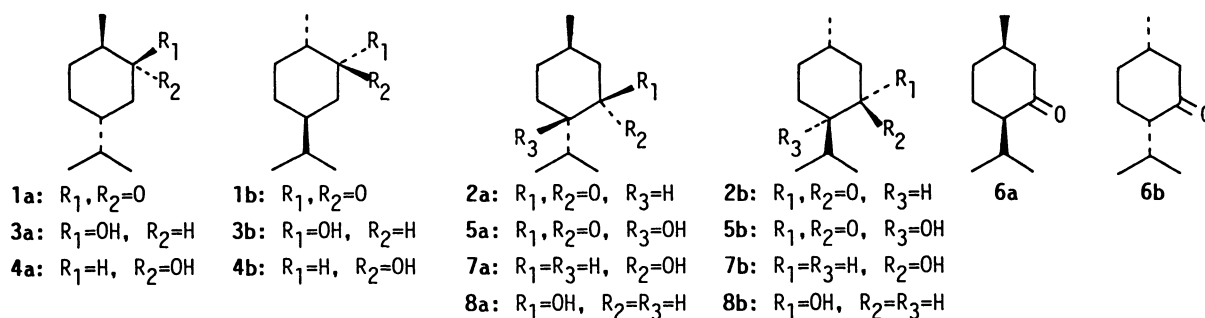
The biotransformation of the enantiomeric pairs of 2- and 3-oxygenated p-menthane derivatives with the cultured cells of Nicotiana tabacum was investigated. It was found that (i) the cultured cells transform only 2-oxygenated p-menthane derivatives to a great extent, (ii) the cultured cells cause the highly stereospecific reduction for (1R,4R)-2-oxo-p-menthane, whereas this is not the case for its enantiomer, and (iii) the cultured cells enantioselectively oxidize the hydroxyl group of 2-hydroxy-p-menthanes.

The oxidoreduction between cycloalkanols and their corresponding cycloalkanones in the cultured cells of Nicotiana tabacum was governed by an NAD<sup>+</sup>-dependent alcohol dehydrogenase<sup>1,2)</sup> and the balance in the equilibrium of the oxidoreduction depended on the carbon number in the carbocyclic ring of the cyclic compounds;<sup>2,3)</sup> the equilibrium tends to lie toward the side of the alcohol in the case of six-membered cycloalkanols. The cultured cells of N. tabacum discriminated the enantiomers of bicyclo[2.2.1]- and bicyclo[3.1.1]heptanols in the oxidation of their hydroxyl group.<sup>1,4,5)</sup> We have investigated the stereoselectivity in the reduction and oxidation of 2- and 3-oxygenated p-menthane derivatives with the cultured cells of N. tabacum, and here wish to communicate the new findings.

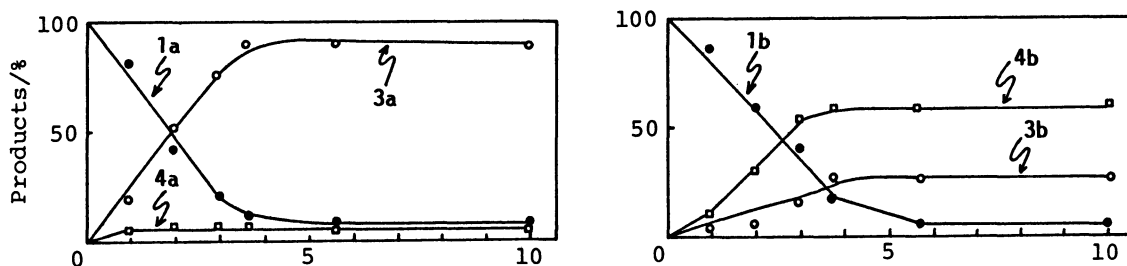
The feeding and time-course experiments were carried out in a manner similar to that described in Refs. 5 to 7. The time-courses in the reductive transformation of the enantiomeric pairs of carvomenthone (**1a** and **1b**)<sup>8)</sup> and menthone (**2a** and **2b**)<sup>9)</sup> are shown in Fig. 1. (1R,4R)-(+)-Carvomenthone (**1a**) was quantitatively converted to (1R,2S,4R)-(+)-neocarvomenthol (**3a**), whereas its (1S,4S)-(-)-enantiomer (**1b**) was converted to (1S,2S,4S)-(+)-carvomenthol (**4b**) and (1S,2R,4S)-(-)-neocarvomenthol (**3b**) in a ratio of 2:1. The cultured cells reduced both the enantiomers of carvomenthone (**1a** and **1b**) to a high extent, while the stereospecificity in the reduction of the enantiomers was different; the stereospecificity was extremely high in the reduction of (1R,4R)-carvomenthone (**1a**), but low in the reduction of its enantiomer **1b**. The preferential

formation of (+)-neocarvomenthols (**3a**) from **1a** and (+)-carvomenthols (**4b**) from **1b** indicates that the cultured cells stereoselectively convert the carvomenthones (**1a** and **1b**) to the corresponding hydroxyl compounds with the chirality of  $\underline{S}$  at the carbon atom bearing the hydroxyl group.

The reductive transformation of (1 $\underline{R}$ ,4 $\underline{S}$ )-(-)- and (1 $\underline{S}$ ,4 $\underline{R}$ )-(+)-menthones (**2a** and **2b**) gave (1 $\underline{R}$ ,4 $\underline{R}$ )- and (1 $\underline{S}$ ,4 $\underline{S}$ )-4-hydroxy-p-menth-3-ones (**5a** and **5b**), respectively, as a main product, in addition to isomenthones (**6a** and **6b**) and 3-hydroxy-p-menthanes (**7a**, **7b**, and **8b**). Details for the structure determinations of **5a** and **5b** will be reported elsewhere in the near future. The time-course experiments show that the reduction of the carbonyl group of **2a** and **2b** slightly occurred, though the balance of the equilibrium in the oxidoreduction between the menthones (**2a** and **2b**) and their corresponding alcohols in the cultured cells would be expected to lie toward the side of the alcohols.<sup>2,3)</sup> This low conversion, as compared with the cases of 2-oxo-p-menthanes, may be caused by the steric hindrance owing to the methylethyl group adjacent to the carbonyl



(a) (1 $\underline{R}$ ,4 $\underline{R}$ )- and (1 $\underline{S}$ ,4 $\underline{S}$ )-Carvomenthones (**1a** and **1b**)



(b) (1 $\underline{R}$ ,4 $\underline{S}$ )- and (1 $\underline{S}$ ,4 $\underline{R}$ )-Menthones (**2a** and **2b**)

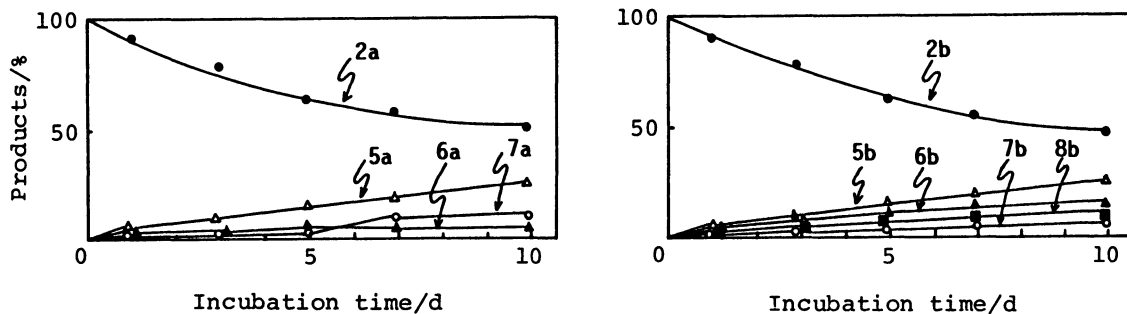
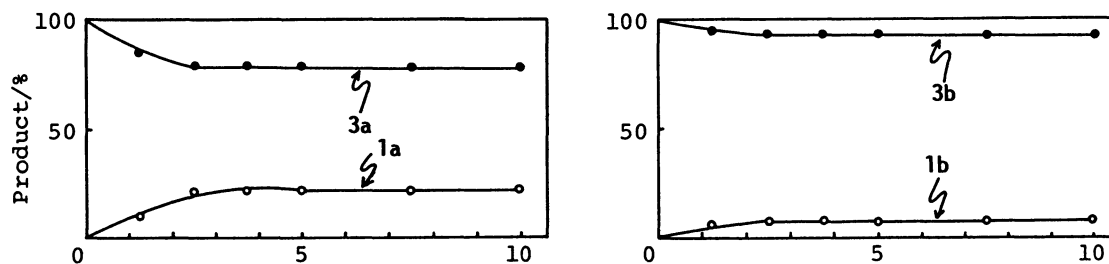


Fig. 1. The time-courses in the biotransformation of the enantiomeric pairs of (a) carvomenthones and (b) menthones with the cultured cells of *N. tabacum*.

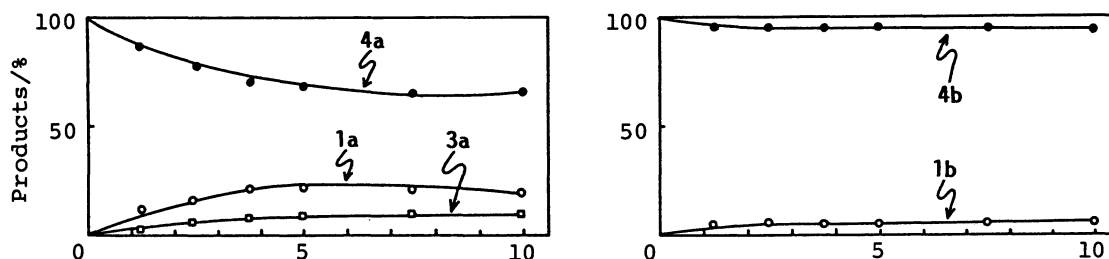
group. In contrast with the reductive conversion, the cultured cells regio- and stereoselectively hydroxylated the  $\alpha$ -position of the carbonyl group of 3-oxo-p-menthanes; the occurrence of such a hydroxylation is a first example in the biotransformation with the cultured cells.

The stereoselectivity in the oxidation of the enantiomeric pairs of 2- and 3-hydroxy-p-menthanes, such as (1R,2S,4R)-(+)- and (1S,2R,4S)-(-)-neocarvomenthols (3a and 3b),<sup>8)</sup> (1R,2R,4R)-(-)- and (1S,2S,4S)-(+)-carvomenthols (4a and 4b),<sup>8)</sup> and (1R,3R,4S)-(-)- and (1S,3S,4R)-(+)-menthols (8a and 8b),<sup>10)</sup> was investigated. Fig. 2 shows the time-courses in the oxidative transformation of these compounds. The 2-hydroxy-p-menthanes (3a and 4a) were oxidized to (1R,4R)-(+)-carvomenthone (1a) to a small extent, whereas the oxidation of their enantiomers (3b and 4b) scarcely occurred. On the other hand, both the enantiomers of 3-hydroxy-p-menthanes (8a and 8b) were barely converted to their corresponding ketones. These facts indicate that the cultured cells enantioselectively oxidize the hydroxyl group of 2-hydroxy-p-menthanes, but the cells accept

(a) (1R,2S,4R)- and (1S,2R,4S)-Neocarvomenthols (3a and 3b)



(b) (1R,2R,4R)- and (1S,2S,4S)-Carvomenthols (4a and 4b)



(c) (1R,3R,4S)- and (1S,3S,4R)-Menthols (8a and 8b)

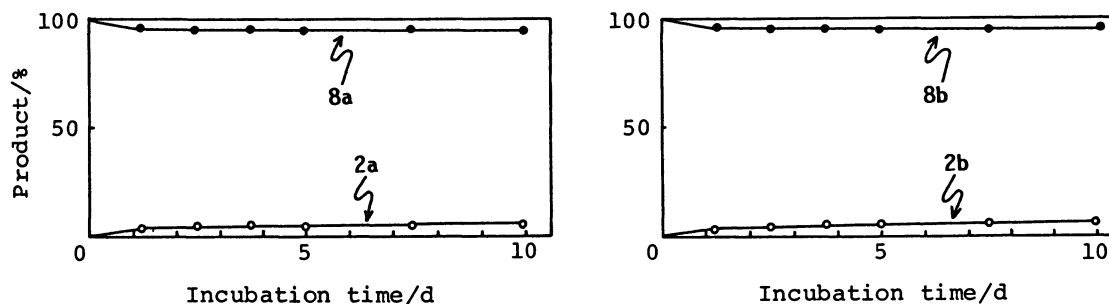


Fig. 2. The time-courses in the biotransformation of the enantiomeric pairs of (a) neocarvomenthols, (b) carvomenthols, and (c) menthols with the the cultured cells of *N. tabacum*.

neither enantiomers of 3-hydroxy-*p*-menthane for the oxidative transformation. The low conversion of 2-hydroxy-*p*-menthanes (**3a** and **4a**) to their corresponding ketone **1a** as shown in (a) and (b) of Fig. 2 may be on the ground that the balance of the equilibrium in the oxidoreduction between the alcohols, **3a** and **4a**, and the ketone **1a** in the cultured cells lies toward the side of the alcohols.<sup>2,3)</sup> In addition, the biotransformation of **4a** gave neocarvomenthyl (**3a**) besides the oxidation product **1a**, as shown in (b) of Fig. 2. The formation of **3a** may be caused by further conversion of the product **1a** with the cultured cells.

Thus, it was established as follows: (i) the cultured cells of *N. tabacum* discriminate 2- and 3-oxygenated *p*-menthanes in their reductive and oxidative conversions; 2-oxygenated *p*-menthanes were converted to their corresponding alcohols and ketones, but this is not the case for 3-oxygenated *p*-menthanes. (ii) The cells cause the highly stereospecific reduction of (1*R*,4*R*)-2-oxo-*p*-menthane, whereas the specificity is low in the case of its enantiomer. The hydrogen attack in the reduction takes place preferentially from the *re* face of the carbonyl group to give the hydroxy compounds with the chirality of *S* at the position bearing the hydroxyl group. (iii) The cells discriminate the enantiomers of 2-hydroxy-*p*-menthanes in their oxidative conversion to the corresponding 2-oxo-*p*-menthanes.

The present work was in part supported by Grant-in-Aids for Developmental Scientific Research No. 57840030 (1982-3; to T.S.) and 59840013 (1984-6; to T.H.) from the Ministry of Education, Science and Culture.

#### References

- 1) T. Suga and T. Hirata, *Nippon Kagaku Kaishi*, **1983**, 1352.
- 2) T. Suga, S. Izumi, and T. Hirata, *Chem. Lett.*, **1986**, 2053.
- 3) T. Suga, H. Hamada, and T. Hirata, *Plant Cell Rep.*, **2**, 66 (1983).
- 4) T. Suga, T. Hirata, H. Hamada, and M. Futatsugi, *Plant Cell Rep.*, **2**, 186 (1983).
- 5) T. Suga, H. Hamada, and T. Hirata, *Chem. Lett.*, **1987**, 471.
- 6) T. Hirata, T. Aoki, Y. Hirano, T. Ito, and T. Suga, *Bull. Chem. Soc. Jpn.* **54**, 3527 (1981).
- 7) Y. S. Lee, T. Hirata, and T. Suga, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 2475.
- 8) **1a**:  $[\alpha]_D^{25} +5.9^\circ$  ( $c$  1.0, EtOH) and **1b**:  $[\alpha]_D^{25} -5.3^\circ$  ( $c$  2.3, EtOH), **3a**:  $[\alpha]_D^{25} +40.3^\circ$  ( $c$  1.5, MeOH) and **3b**:  $[\alpha]_D^{25} -40.6^\circ$  ( $c$  2.0, MeOH), and **4a**:  $[\alpha]_D^{25} -21.0^\circ$  ( $c$  1.0, MeOH) and **4b**:  $[\alpha]_D^{25} +21.7^\circ$  ( $c$  1.8, MeOH) were prepared from (+)- and (-)-dihydrocarvones, (+)- and (-)-neodihydrocarveols, and (-)- and (+)-dihydrocarveols by hydrogenation with  $H_2/Pd-C$ , respectively.
- 9) **2a**:  $[\alpha]_D^{25} -27.3^\circ$  ( $c$  1.0, EtOH) and **2b**:  $[\alpha]_D^{25} +28.0^\circ$  ( $c$  1.5, EtOH) were prepared from (-)- and (+)-menthols by pyridinium dichromate oxidation, respectively.
- 10) **8a**:  $[\alpha]_D^{25} -49.3^\circ$  ( $c$  2.0, EtOH) and **8b**:  $[\alpha]_D^{25} +48.7^\circ$  ( $c$  1.5, EtOH) were commercial materials.

(Received February 18, 1987)