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

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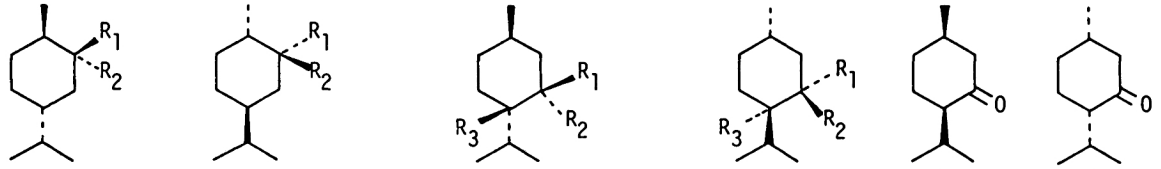
The biotransformation of the enantiomeric pairs of 2- and 3-oxygenated p-menthane derivatives with the cultured cells of Nicotiana tabacum was investigated. It was found that (i) the cultured cells transform only 2-oxygenated p-menthane derivatives to a great extent, (ii) the cultured cells cause the 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^+-dependent alcohol dehydrogenase[1,2] and the balance in the equilibrium of the oxidoreduction depended on the carbon number in the carbocyclic ring of the cyclic compounds;[2,3] 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.[1,4,5] 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**)[8] and menthone (**2a** and **2b**)[9] 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 (+)-neocarvomenthol (**3a**) from **1a** and (+)-carvomenthol (**4b**) from **1b** indicates that the cultured cells stereoselectively convert the carvomenthones (**1a** and **1b**) to the corresponding hydroxyl compounds with the chirality of S at the carbon atom bearing the hydroxyl group.

The reductive transformation of (1R,4S)-(-)- and (1S,4R)-(+)-menthones (**2a** and **2b**) gave (1R,4R)- and (1S,4S)-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.[2,3] 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



1a: R_1, R_2=O **1b**: R_1, R_2=O **2a**: R_1, R_2=O, R_3=H **2b**: R_1, R_2=O, R_3=H **6a** **6b**

3a: R_1=OH, R_2=H **3b**: R_1=OH, R_2=H **5a**: R_1, R_2=O, R_3=OH **5b**: R_1, R_2=O, R_3=OH

4a: R_1=H, R_2=OH **4b**: R_1=H, R_2=OH **7a**: R_1=R_3=H, R_2=OH **7b**: R_1=R_3=H, R_2=OH

8a: R_1=OH, R_2=R_3=H **8b**: R_1=OH, R_2=R_3=H

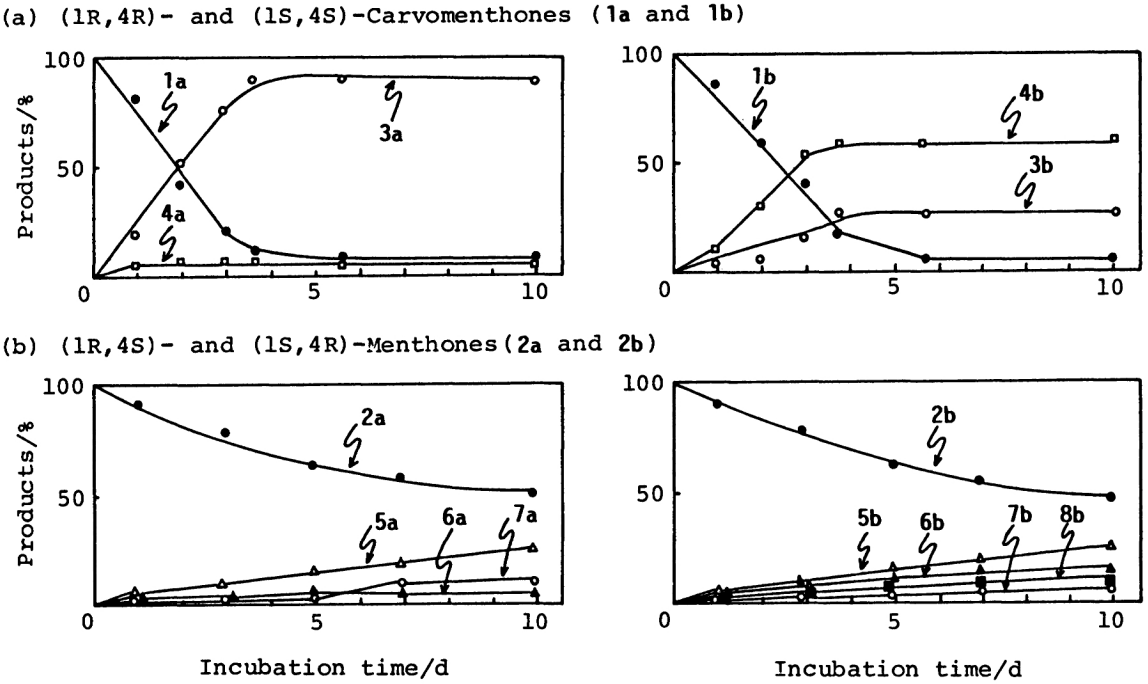


Fig. 1. The time-courses in the biotransformation of the enantiomeric pairs of (a) carvomenthones and (b) menthones with the cultured cells of N. tabacum.

group. In contrast with the reductive conversion, the cultured cells regio- and stereoselectively hydroxylated the α-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**),[8] (1R,2R,4R)-(-)- and (1S,2S,4S)-(+)-carvomenthols (**4a** and **4b**),[8] and (1R,3R,4S)-(-)- and (1S,3S,4R)-(+)-menthols (**8a** and **8b**),[10] 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

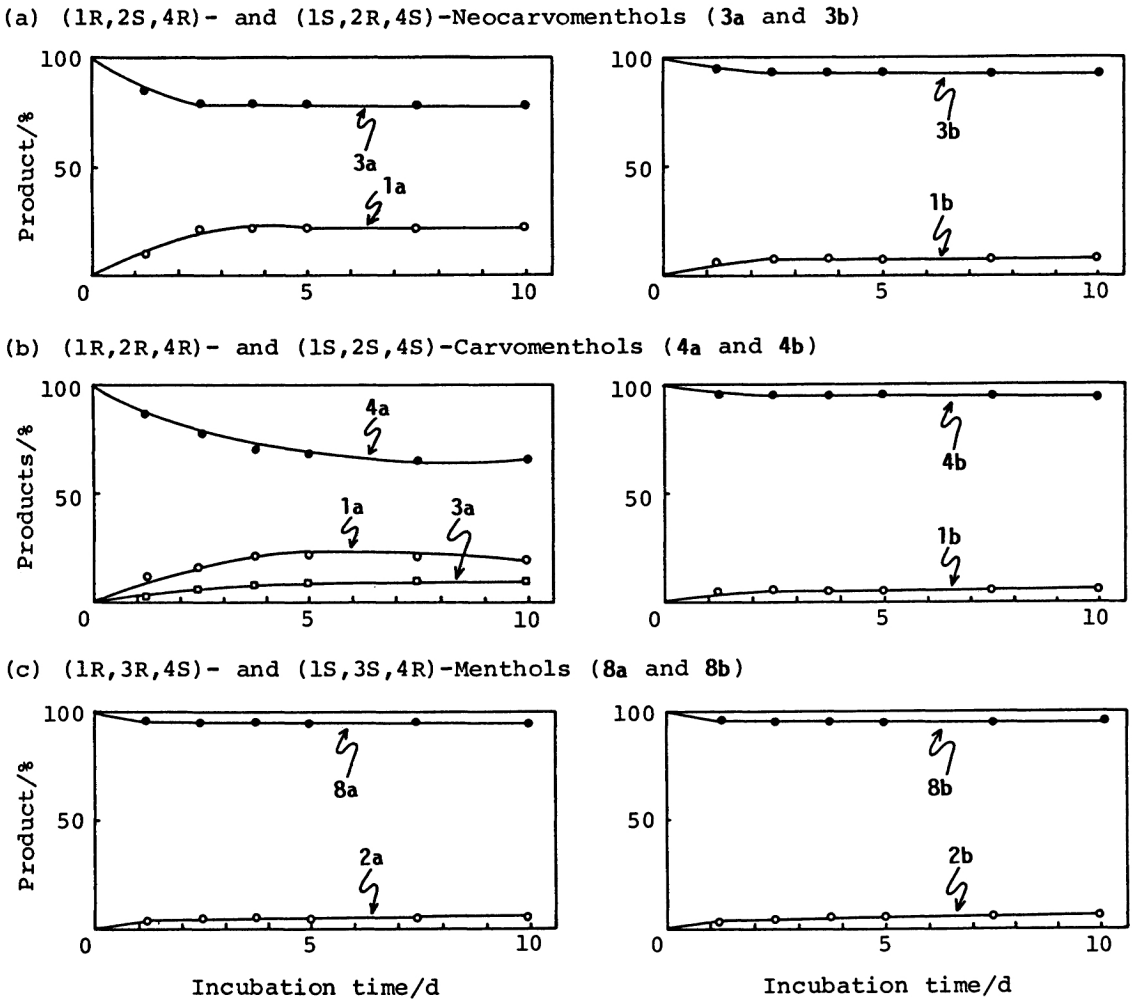


Fig. 2. The time-courses in the biotransformation of the enantiomeric pairs of (a) neocarvomenthols, (b) carvomenthols, and (c) menthols with the the cultured cells of N. tabacum.

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.[2,3] In addition, the biotransformation of **4a** gave neocarvomenthol (**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 (1R,4R)-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.

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8) **1a**: $[\alpha]_D^{25}$ +5.9°(c 1.0, EtOH) and **1b**: $[\alpha]_D^{25}$ -5.3°(c 2.3, EtOH), **3a**: $[\alpha]_D^{25}$ +40.3°(c 1.5, MeOH) and **3b**: $[\alpha]_D^{25}$ -40.6°(c 2.0, MeOH), and **4a**: $[\alpha]_D^{25}$ -21.0° (c 1.0, MeOH) and **4b**: $[\alpha]_D^{25}$ +21.7°(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° (c 1.0, EtOH) and **2b**: $[\alpha]_D^{25}$ +28.0° (c 1.5, EtOH) were prepared from (-)- and (+)-menthols by pyridinium dichromate oxidation, respectively.
10) **8a**: $[\alpha]_D^{25}$ -49.3° (c 2.0, EtOH) and **8b**: $[\alpha]_D^{25}$ +48.7° (c 1.5, EtOH) were commercial materials.

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