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Enantioselectivity in the Biotransformation of Bicyclo[3.1.1]heptanes with the Cultured Cells of Nicotiana tabacum

Takayuki SUGA,* Hiroki HAMADA,† and Toshifumi HIRATA

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730

†Department of Fundamental Natural Science, Okayama Univesity of Science, Ridai-cho, Okayama 700

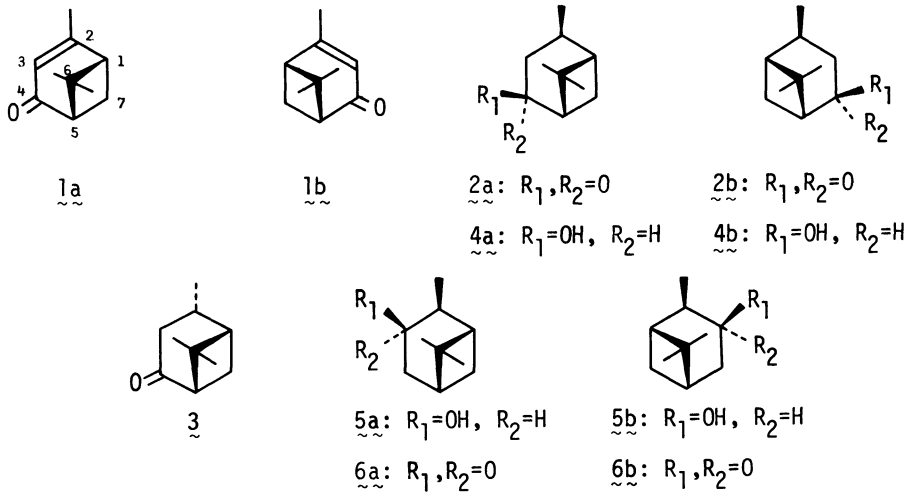
The biotransformation of the enantiomeric pairs of bicyclo-[3.1.1]heptane derivatives with the cultured cells of Nicotiana tabacum was investigated. The hydrogenation of the C-C double bond of verbenone took place enantioselectively in preference of the (1S,5S)-enantiomer and the hydrogen attack occurred stereospecifically from the re-face at its C-2. The oxidation of the hydroxyl group of neoisopinocampheol occurred enantioselectively in preference of the (1S,2S,3R,5R)-enantiomer, whereas such an enantioselective oxidation was not the case for neoisoverbanol.

Recent studies on the biotransformation of foreign substrates with the cultured cells of Nicotiana tabacum showed that the cultured cells have an ability to reduce the C-C double bond adjacent to the carbonyl group of carvone and then the carbonyl group of the resultant ketone.[1,2] Furthermore, it was found that the acetoxyl group of monoterpene acetates tends to suffer the enantioselective hydrolysis with the cultured cells.[3,4] We have investigated enantioselectivities in the hydrogenation of the C-C double bond and the oxidation of the hydroxyl group of bicyclo[3.1.1]heptane derivatives with the cultured cells of N. tabacum by use of their enantiomeric pairs as foreign substrates, and here wish to communicate the new findings.

A suspension of the cultured cells used in this investigation was prepared as described in our previous paper;[5] the callus tissues induced from the stem of Nicotiana tabacum "Bright Yellow" were transplanted to a 300 cm^3 conical flask containing 100 cm^3 of Murashige and Skoog's medium (pH 5.8)[6] and were cultured with continuous shaking at 25 °C for 3-4 weeks in the dark. The feeding and time-course experiments were carried out in a manner similar to that reported in Refs. 5 and 7; the substrate (10 mg) was administered to the above flask containing the suspension of the cultured cells under a sterile condition. The cultures were incubated under continuous shaking at 25 °C for 10 days in the dark. At a regular time

interval, a part (10 cm^3) of the incubated mixture was pipetted out under a sterile condition 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 of GLC. The products were identified by comparing their TLC, GLC, and/or GC-MS with those of authentic samples.

First, the enantioselectivity in the hydrogenation of the C-C double bond adjacent to the carbonyl group was examined. The time-courses in the biotransformation of (1S,5S)-(-)-verbenone (1a) and its enantiomer (1b)[8] were followed and are shown in Fig. 1. The (1S,5S)-enantiomer (1a) was quantitatively converted to (1S,2R,5S)-(-)-cis-verbanone (2a) after 10 days incubation, whereas the conversion of (1R,5R)-enantiomer (1b) to the corresponding ketone (2b) occurred to a slight extent. These facts indicate that the cultured cells discriminate the enantiomers and reduce the C-C double bond of the only (1S,5S)-enantiomer (1a). In addition, the hydrogenation of 1a with the cultured cells gave only cis-verbanone (2a), but not even a trace of



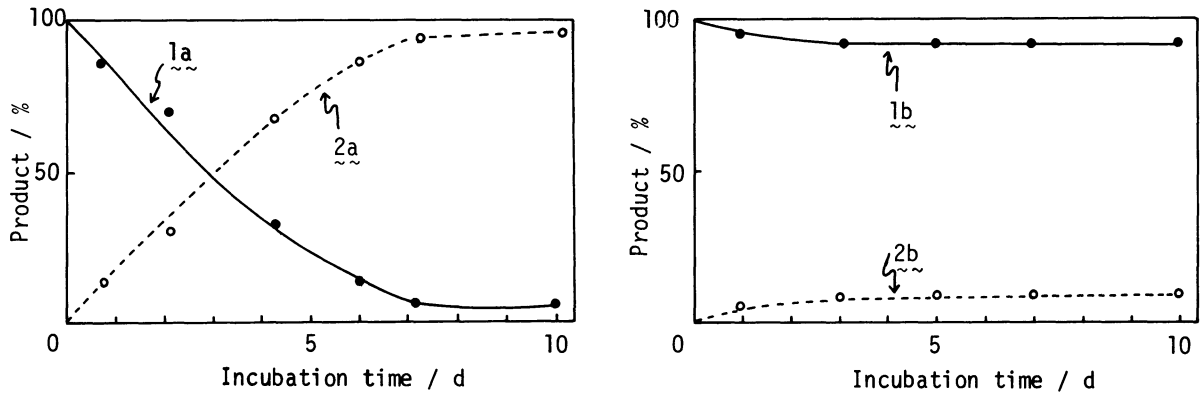


Fig. 1. The time-courses in the biotransformation of (1S,5S)- and (1R,5R)-verbenones (1a and 1b) with the cultured cells of N. tabacum.

trans-isomer 3; no trans-isomer 3 was found in spite of careful analyses by TLC, GLC, and GC-MS. This indicates occurrence of the stereospecific hydrogen attack to the C-C double bond of 1a from the re-face at its C-2. No further conversion of the cis-verbanone (2a), which was produced by hydrogenation of 1a with the cultured cells, into neoisoverbanol (4a) was observed. This may be on the ground that the balance of the equilibrium between 2a and its corresponding alcohol (4a) in the oxidoreduction in the cultured cells is predicted to lie toward the side of the cis-verbanone (2a), because the equilibrium constant in the oxidoreduction of 4a ⇄ 2a is estimated to be about 1.4 on the basis of the ^{13}C NMR chemical shift (δ 214.1) of the carbonyl carbon of 2a.[11]

Next, the enantioselectivity in the oxidation of the hydroxyl group was examined with the enantiomeric pairs of 3- and 4-hydroxylated bicyclo[3.1.1]-heptane derivatives, such as (1S,2R,4S,5S)-(-)- and (1R,2S,4R,5R)-(+)-neoisoverbanols (4a and 4b)[12] and (1S,2S,3R,5R)-(-)- and (1R,2R,3S,5S)-(+)-neoisopinocampheol (5a and 5b).[14] The time-courses in the biotransformation of these enantiomeric pairs are shown in Figs. 2 and 3, respectively. The

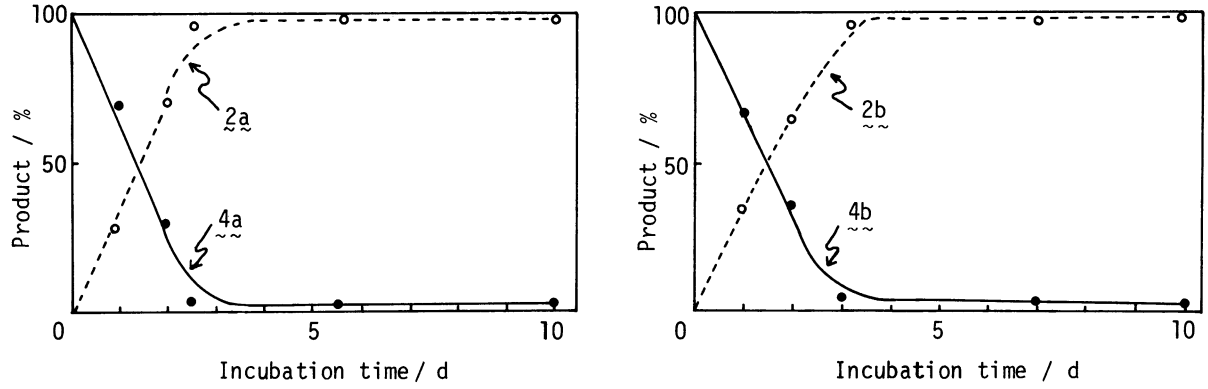


Fig. 2. The time-courses in the biotransformation of (1S,2R,4S,5S)- and (1R,2S,4R,5R)-neoisoverbanols (4a and 4b) with the cultured cells of N. tabacum.

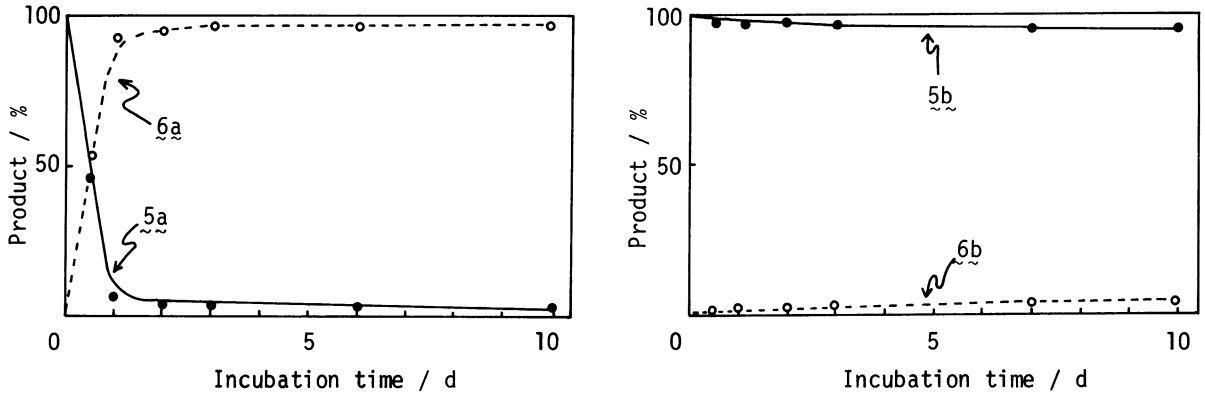


Fig. 3. The time-courses in the biotransformation of (1S,2S,3R,5R)- and (1R,2R,3S,5S)-neoisopinocampheols (5a and 5b) with the cultured cells of N. tabacum.

oxidation of neoisoverbanols (4a and 4b) took place for both the enantiomers to a comparable extent, giving (1S,2R,5S)-(-)- and (1R,2S,5R)-(+)-cis-verbanones (2a and 2b), respectively. On the contrary, the oxidation of neoisopinocampheols (5a and 5b) was preferential for 5a and yielded (1S,2S,5R)-(-)-isopinocamphone (6a).

Thus, enantioselectivities in the hydrogenation of the C-C double bond of bicyclo[3.1.1]heptane derivatives and in the oxidation of their hydroxyl group with the cultured cells of N. tabacum were established as follows: (i) The hydrogenation of the C-C double bond of verbenone (1) occurred enantioselectively in preference of the (1S,5S)-enantiomer (1a) and the hydrogen attack took place from the re-face of the C-C double bond. (ii) The oxidation of the hydroxyl group of neoisopinocampheol (5) occurred enantioselectively in preference of the (1S,2S,3R,5R)-enantiomer (5a), whereas both the enantiomers of neoisoverbanol (4) suffered the oxidation to a comparable extent; the cultured cells discriminate the enantiomeric pair of the 3-hydroxyl derivative, but this was not the case for the 4-hydroxyl derivative.

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8) (1S,5S)-(-)-Verbenone (1a) {$[\alpha]_D^{25}$ -209.3° (c 2.3, $CHCl_3$) [lit.[9] $[\alpha]_D^{25}$ -208° ($CHCl_3$)]} and (1R,5R)-(+)-verbenone (1b) {$[\alpha]_D^{25}$ +210.5° (c 1.5, $CHCl_3$)} were prepared from (+)- and (-)-α-pinene by t-butyl chromate oxidation,[10] respectively.
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12) Hydrogenation of (-)-verbenone (1a) in the presence of Pd-C, followed by reduction of the resultant (-)-cis-verbanone (2a) with $LiAlH_4$ afforded (1S,2R,4S,5S)-(-)-neoisoverbanol (4a) {$[\alpha]_D^{25}$ -5.0° (c 1.5, benzene)}. The (1R,2S,4R,5R)-(+)-enantiomer (4b) {$[\alpha]_D^{25}$ +5.2° (c 1.3, benzene) [lit.[13] $[\alpha]_D^{25}$ +5.3° (benzene)]} was prepared from (+)-verbenone (1b) in the same manner as used for the preparation of 4a.
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14) According to the reported procedure,[15] (1S,2S,3R,5R)-(-)-neoisopinocampheol (5a) {$[\alpha]_D^{25}$ -33.8° (c 1.7, benzene)} was prepared by hydroboration of (-)-α-pinene, oxidation of the resultant (-)-isopinocampheol to (-)-isopinocamphone (6a) with $Na_2Cr_2O_7$, and then reduction of 6a with $LiAlH_4$. (1R,2R,3S,5S)-(+)-Enantiomer (5b) {$[\alpha]_D^{25}$ +34.5° (c 1.6, benzene) [lit.[15] $[\alpha]_D^{25}$ -32.8° (benzene)]} was prepared from (+)-α-pinene in the same manner as above.
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