  Bull. Chem. Soc. Jpn., 59, 2865—2867 (1986)

The Stereochemistry of the Reduction of Carbon-Carbon Double Bond with the Cultured Cells of *Nicotiana tabacum*

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(Received April 19, 1986)

The stereochemistry in the reduction of the C–C double bond adjacent to the carbonyl group with the cultured cells of *Nicotiana tabacum* was investigated by feeding (4*R*)-[6-^{2}H]-(−)-carvone to the cultured cells. It was found that the hydrogen attack to the C–C double bond takes place stereospecifically from the *si*-face at C-1 and the *re*-face at C-6, being trans-addition.

A general mechanism for the biological reduction of a C–C double bond is briefly reviewed.[1] On the other hand, the reduction of a C–C double bond in living cells has been described for the biotransformation of steroids with plant cultured cells,[2–4] but little is reported on the stereochemical aspect of the reduction in the plant tissue cultures. We recently found that the cultured cells of *Nicotiana tabacum* reduce the C–C double bond adjacent to the carbonyl group of carvone (**1**) and then the carbonyl group, whereas the cells do not attack the C–C double bond in the 1-methylethenyl group.[5] It was found that the hydrogen attack to the conjugated C–C double bond takes place stereospecifically from the *si*-face at C-1.[5] However, the stereochemistry of the hydrogen attack at C-6 of **1** has not been elucidated yet. To complete the stereochemical studies on the reduction of the conjugated C–C double bond with the cultured cells of *N. tabacum,* we have now investigated the stereochemistry of the hydrogen addition to the 6-position of carvone by examining the orientation of deuterium in a resultant product in the biotransformation of ^{2}H-labeled carvone with the cultured cells.

Results and Discussion

Incubation of (4*R*)-[6-^{2}H]-(−)-carvone (**2**) (deuterated by 97.6 atom %) with the cultured cells of *N. tabacum* was carried out in a manner similar to that described in our previous paper[5] to yield neodihydrocarveol (**3**) as a main product. The mass spectrum of **3** showed a molecular ion peak at *m/z* 155 and $[M-H_2O]^+$ ion peak at *m/z* 137. The relative intensities of the peaks indicated that 97% of the molecules of **3** were labeled with deuterium. The proton noise decoupled ^{13}C NMR spectrum of the product **3** closely resembled to the spectrum of the nonlabeled neodihydrocarveol (**4**), except that the C-6 signal of **3** at δ_c 27.8 was a somewhat weak triplet ($J_{^{13}C-^2H}$=20 Hz), whereas the corresponding signal of the nonlabeled compound **4** was a singlet. This indicates that one of the hydrogen atoms at C-6 is deuterated.

The orientation of deuterium in the product **3** was determined by comparison of its ^{1}H NMR spectrum with that of the nonlabeled compound **4**, as given in Fig. 1. Complete assignments of the ^{1}H NMR signals of **3** and **4** were made on a 500 MHz NMR spectrum and are shown in Table 1. The assignments were confirmed by a two-dimensional H/H shift correlation NMR spectrum of the nonlabeled compound **4**; all correlations expected were found in the spectrum. The disappearance of the 6α-H signal in the ^{1}H NMR spectrum of **3** indicates the 6α-atom to be deuterium. Thus, it was demonstrated that the *R* hydrogen atom at the 6-position of **3** was added when the carbocyclic double bond of carvone (**2**) was reduced in the cultured cells of *N. tabacum.*

As has been described in our previous paper,[5] the cultured cells of *N. tabacum* have the ability to reduce (4*R*)- and (4*S*)-carvones; (4*R*)-carvone was transformed to (1*R*,2*S*,4*R*)-neodihydrocarveol and (4*S*)-carvone to (1*R*,4*S*)-isodihydrocarvone and (1*R*,2*S*,4*S*)-neoisodihydrocarveol. The formation of these compounds indicates that carvone is stereospecifically reduced to the compound with the chirality of *R* at the C-1 and that of *S* at the C-2 in its biotransformation with the cultured cells. Putting the previous and present results together, thus, the mode of the hydrogen

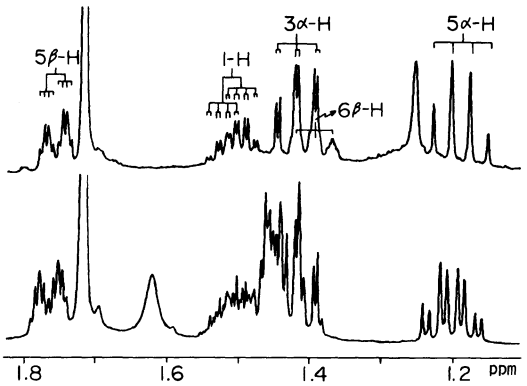

Fig. 1. Comparison of the ^{1}H NMR spectrum of ^{2}H-labeled product **3** (upper) with that of nonlabeled (+)-neodihyrocarveol (**4**) (lower).

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Table 1. ^{1}H NMR Spectral Data[a] of ^{2}H-Labeled Product **3** and Nonlabeled (+)-Neodihydrocarveol (**4**)

Protons	**3**		**4**	
	δ	J/Hz	δ	J/Hz
1-H	1.52 dqd	12.7, 6.5, 2.4	1.53 dqt	12.6, 6.6, 2.5
2-H	3.89 dt	3.2, 2.4	3.89 dtd	3.2, 2.5, 0.3
3α-H	1.42 ddd	13.2, 12.4, 2.4	1.42 ddd	13.3, 12.4, 2.5
3β-H	1.92 dddd	13.2, 3.3, 3.2, 3.1	1.92 dddd	13.3, 3.3, 3.2, 3.1
4-H	2.28 tt	12.4, 3.3	2.28 tt	12.4, 3.3
5α-H	1.19 dt	12.5, 12.4	1.20 dtd	12.5, 12.4, 4.5
5β-H	1.76 dtd	12.5, 3.3, 3.2	1.77 ddtd	12.5, 3.5, 3.3, 3.2
6α-H	Not detected		1.44 ddddd	12.7, 4.5, 3.5, 2.5, 0.3
6β-H	1.40 br t	12.7	1.42 dddd	12.7, 12.6, 12.4, 3.3
9-H$_2$	4.69 q	1.1	4.69 q	1.0
1-Me	0.97 d	6.5	0.97 d	6.6
8-Me	1.72 t	1.1	1.72 t	1.0

a) Measured at 500 MHz in $CDCl_3$.

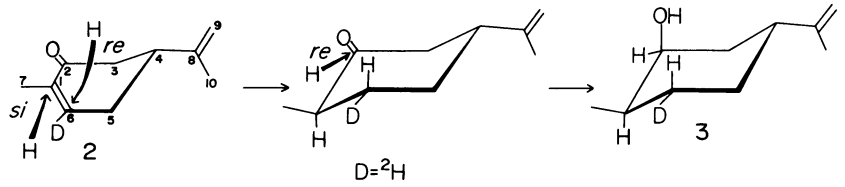
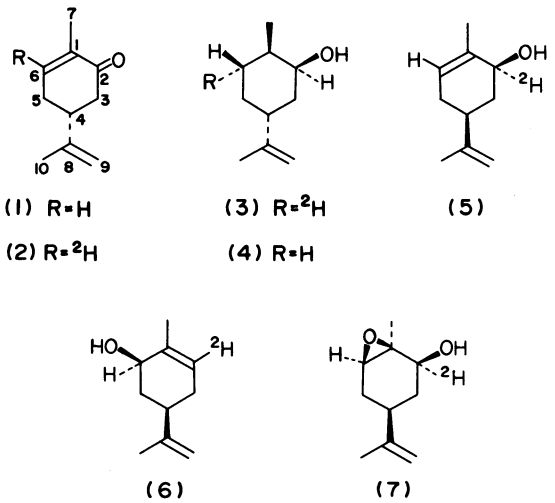


Fig. 2. Stereospecific hydrogen attack in the reduction of the C–C double bond and the carbonyl group of (−)-carvone (**2**) by the suspension cells of *N. tabacum*.

addition in the reduction of carvone with the cultured cells of *N. tabacum* was perfectly established as shown in Fig. 2; at first, the hydrogen attack to the carbocyclic double bond takes place stereospecifically from the *si*-face at C-1 and the *re*-face at C-6. Accordingly, the mode of the hydrogen addition to the C–C double bond conjugated with the carbonyl group is trans. After occurrence of this C–C double bond reduction, the hydrogen attack to the carbonyl group at the 2-position takes place stereospecifically from the *re*-face.



Experimental

Analytical and preparative TLC were carried out on 0.25-mm and 0.5-mm thick silica-gel plates (Merck silica gel 60, GF_{254}). GLC analyses were performed on an instrument equipped with FID and a glass column (3 mm×2 m) packed with 15% DEGS, 15% PEG-20M, and 2% OV-17 on Chromosorb W (AW-DMCS; 80—100 mesh) at 100, 120, and 90—200 °C (3 °C min^{-1}), respectively. ^{1}H NMR spectra were obtained at 90 and 500 MHz in $CDCl_3$ with TMS as an internal standard. GC–MS spectra were recorded on a mass spectrometer equipped with a gas chromatograph with 15% DEGS column (3 mm×2 m) by EI mode at 70 eV.

Substrate. (4*R*)-[6-^{2}H]-(−)-Carvone (**2**) was prepared by reduction of (+)-carvone with NaB^2H_4, isomerization of the resultant (+)-*cis*-carveol (**5**) to (−)-enantiomer **6**,[6] and then oxidation of the enantiomer **6**, as described below.

(i) (4*S*)-[2-^{2}H]-(+)-*cis*-Carveol (5). To a soln of (+)-carvone (3.8 g) ($[\alpha]_D^{25}$ +59.0° (neat); n_D^{25} 1.4990) in MeOH (20 cm^3), NaB^2H_4 (3.0 g) was added by portions at 0 °C. After standing for 30 min at room temp, the reaction mixture was extracted with ether and then subjected to preparative TLC with hexane–EtOAc (7:3, v/v) to give (4*S*)-[2-^{2}H]-(+)-*cis*-carveol (**5**) (3.0 g): *m/z* (rel intensity) 153 (M^+, 2), 135 (M^+-H_2O, 38), 120 (23), and 85 (100).

(ii) 1,3-Transposition of 5. According to the reported procedure,[6] the ^{2}H-labeled (+)-*cis*-carveol (**5**) (3.0 g) was oxidized with *t*-butyl hydroperoxide and oxobis(2,4-pentanedionato-*O*,*O*′)vanadium(IV) to give a ^{2}H-labeled epoxy-alcohol **7** (1.3 g). This epoxy-alcohol **7** was converted to the mesylate derivative (1.8 g) with mesyl chloride–triethylamine. Treatment of the mesylate (1.8 g) with a

sodium-naphthalene reagent[6] dissolved in THF afforded (4*R*)-[6-^{2}H]-(−)-*cis*-carveol (**6**) (0.48 g): ^{1}H NMR (90 MHz) δ=1.64 (s, 6H, 1- and 8-Me), 4.09 (m, 1H, 2-H), and 4.60 (m, 2H, 9-H_2); *m/z* (rel intensity) 153 (M^+, 1), 135 (M^+-H_2O, 35), 120 (23), and 85 (100).

(iii) Oxidation of 6. To a soln of the ^{2}H-labeled (−)-*cis*-carveol (**6**) (0.41 g) in CH_2Cl_2 (50 cm^3), pyridinium-chromium trioxide[7] (2.2 g) was added drop by drop at 5 °C. The mixture was stirred for 1 h at room temp. After filtration, the product was extracted with ether, dried (Na_2SO_4), and freed of the solvent. The residue was subjected to preparative TLC with hexane-EtOAc (95:5, v/v) to give (4*R*)-[6-^{2}H]-(−)-carvone (**2**) (0.28 g): $[\alpha]_D^{25}$ −59.3° (*c* 1.5, EtOH); n_D^{25} 1.4997 (lit,[5] $[\alpha]_D^{25}$ −60.4°, n_D^{25} 1.5000); >99% pure on GLC; *m/z* (rel intensity) 151 (M^+, 6), 136 (M^+-CH_3, 3), and 83 (100); ^{1}H NMR (90 MHz) δ=1.76 (bs, 6H, 1- and 8-Me) and 4.78 (bs, 2H, 9-H_2). The signal at δ 6.78 for C(6)-H, which is observed in the ^{1}H NMR spectrum of nonlabeled carvone, disappeared in the NMR spectrum of **2**. The relative intensities of the molecular ion peaks in the mass spectra of **2** and nonlabeled carvone indicated that 97.6% of the molecules of carvone were labeled with deuterium.

Incubation of (4*R*)-[6-^{2}H]-(−)-Carvone (2) with the Tobacco Suspension Cells. The suspension cells of *N. tabacum* "Bright Yellow" were prepared as described in Ref. 5; the cells were cultured in a 300-ml conical flask containing 100 ml of Murashige and Skoog's medium.[8] To the flask containing the suspension cells (about 50—70 g fresh wt/flask), the ^{2}H-labeled carvone (**2**) (10 mg/flask; total 500 mg) was administered, and then the cultures were incubated at 25 °C for 10 d on a rotary shaker (70 rpm) in the dark.

Isolation and Identification of the Product. The cultured mixture was worked up in a manner similar to that described in Ref. 5; a product was extracted with ether and subjected to preparative TLC on silica gel with hexane-EtOAc (1:4, v/v). The product was identified by direct comparison of TLC, GLC, and spectral data with those of an authentic sample. The physical constants and spectral data of the product were as shown below.

(1*R*,2*S*,4*R*)-[6-^{2}H]-(+)-neodihydrocarveol (3): $[\alpha]_D^{25}$ +30.0° (*c* 0.15, EtOH); IR ν_{max} (film) 3435 (OH) and 1645 (C=C) cm^{-1}; ^{1}H NMR (90 MHz) δ=0.98 (3H, d, *J*=6 Hz, 1-Me), 1.71 (3H, s, 8-Me), 3.88 (1H, bs, >CH-OH), 4.68 (2H, bs, >C=CH_2), (500 MHz) see Table 1; ^{13}C NMR (22.6 MHz, in $CDCl_3$) δ_c=150.2 (s, C-8), 108.7 (t, C-9), 71.0 (d, C-2), 38.8 (t, C-3), 37.8 (d, C-4), 36.1 (d, C-1), 31.4 (t, C-5), 27.8 (bm, C-6), 21.0 (q, C-7), 18.3 (q, C-10); *m/z* (rel intensity) 155 (M^+, 4), 137 (M^+-H_2O, 77), 122 (79), 108 (100), 94 (66), and 79 (62).

Preparation of Authentic Neodihydrocarveol (4). Following the procedure described in the Ref. 9, (+)-neodihydrocarveol (**4**) was prepared from (−)-carvone by the reduction with zinc powder: $[\alpha]_D^{25}$ +29.9° (*c* 0.30, EtOH) (lit,[5] $[\alpha]_D^{25}$ +29.1°), IR ν_{max} (film) 3442 (OH) and 1645 (C=C) cm^{-1}; ^{1}H NMR (90 MHz) δ=0.98 (3H, d, *J*=6 Hz, 1-Me), 1.70 (3H, s, 8-Me), 3.90 (1H, br, >CH-OH), 4.71 (2H, bs, >C=CH_2), (500 MHz) see Table 1; *m/z* (rel intensity) 154 (M^+, 5), 136 ($M-H_2O$, 55), 121 (67), 79 (57), and 41 (100).

The authors thank Takasago Perfumery Co. Ltd. for a gift of the sample of (−)-carvone, Drs. Akio Yasui and Shigetoshi Amiya of Central Research Laboratories of Kuraray Co. Ltd. for the measurement of the 500 MHz ^{1}H NMR, and the JEOL Co. Ltd. for the measurement of the two-dimensional H/H shift correlation NMR. The present work was in part supported by Grant-in-Aids for Developmental Scientific Research No. 57840030 (1982) and 59840013 (1984) from the Ministry of Education, Science and Culture.

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