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STEREOSELECTIVITY OF THE REDUCTION OF CARVONE AND DIHYDROCARVONE BY SUSPENSION CELLS OF *NICOTIANA TABACUM**

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tissue culture; biotransformation; stereoselective reduction; carvone; dihydrocarvone; isodihydrocarvone.

Abstract—The biotransformation of foreign substrates with suspension cells of *Nicotiana tabacum* was tested with (4*R*)-(−)- and (4*S*)-(+)-carvones, (1*R*, 4*R*)-(+)- and (1*S*, 4*S*)-(−)-dihydrocarvones and (1*S*, 4*R*)-(+)- and (1*R*, 4*S*)-(−)-isodihydrocarvones. It was found that the cells reduced regio- and stereoselectively the C–C double bond adjacent to the carbonyl group from the *si*-face and then the carbonyl group from the *re*-face, leaving the C–C double bond in the 1-methylethenyl group unattacked.

INTRODUCTION

The ability of cultured cells of plants to metabolize foreign substrates is of considerable interest because of the specificity of the transformations which may be effected by such cells [1]. Previously, Furuya *et al.* had reported that tobacco suspension cells reduced the carbonyl group of progesterone, pregnenolone and testosterone [2, 3] and we had demonstrated the regio- and stereo-selective hydroxylation at not only the allylic positions of the C–C double bond, but also the double bond itself in the biotransformation of monoterpenoid alcohols, such as linalool and its dihydro derivatives [4, 5] and terpineols [6, 7], by cultured suspension cells of *Nicotiana tabacum*.

To clarify the metabolic behaviour of suspension cells of *N. tabacum* toward a molecule having an α,β-unsaturated carbonyl group and an isolated C–C double bond, we have studied the biotransformation of (4*R*)-(−)- and (4*S*)-(+)-carvones (**1a** and **1b**), (1*R*, 4*R*)-(+)- and (1*S*, 4*S*)-(−)-dihydrocarvones (**2a** and **2b**) and (1*S*, 4*R*)-(+)- and (1*R*, 4*S*)-(−)-isodihydrocarvones (**3a** and **3b**) with tobacco suspension cells.

RESULTS AND DISCUSSION

Callus tissues induced from the stem of *N. tabacum* Bright Yellow were used in this work. The callus tissues were cultured in Murashige and Skoog's medium [8] with continuous shaking for 3–4 weeks and then the monoterpenoids were administered. The cultures were then incubated at 25° for 7–10 days with shaking in the dark. The transformation products and their yields are given in Table 1.

It was found that (4*R*)-(−)- and (4*S*)-(+)-carvones (**1a** and **1b**) were transformed into dihydrocarvone (**2**), isodihydrocarvone (**3**), neodihydrocarveol (**4**), dihydrocarveol (**5**), isodihydrocarveol (**6**) and neoisodihydrocarveol (**7**) (Table 1). No products other than these transformation products were found even by careful and repeated TLC and GLC analyses. This indicates that no hydroxylation at the C–C double bonds and/or its allylic positions took place, although such hydroxylations were observed in the biotransformation of linalool and terpineols [4–7]. (4*R*)-(+)-Carvone (**1a**) gave (1*R*, 2*S*, 4*R*)-(+)-neodihydrocarveol (**4a**) as a major product. Its 4*S* isomer (**1b**) afforded (1*R*, 4*S*)-(−)-isodihydrocarvones (**3b**) and (1*R*, 2*S*, 4*S*)-(−)-neoisodihydrocarveol (**7b**) as main products. The preferential formation of these compounds indicates that carvone (**1**) is stereoselectively reduced to compounds with the configuration of *R* at C-1 and *S* at C-2.

Table 1. Biotransformation of carvone (**1**), dihydrocarvone (**2**) and isodihydrocarvone (**3**) by suspension cells of *N. tabacum*

Substrates	Transformation products (% substrate)*					
	2	**3**	**4**	**5**	**6**	**7**
(4*R*)-**1a**	1.6	<0.01	10.7	1.1	<0.01	<0.01
(4*S*)-**1b**	0.30	13.7	0.60	1.3	<0.01	7.9
(1*R*, 4*R*)-**2a**	—	<0.01	4.5	1.1	0.04	0.05
(1*S*, 4*S*)-**2b**	—	<0.01	2.7	4.9	<0.01	0.05
(1*S*, 4*R*)-**3a**	0.32	—	4.9	0.48	0.08	0.08
(1*R*, 4*S*)-**3b**	1.0	—	1.25	8.13	<0.01	4.38

*The characterizations of these compounds are described in the Experimental.

*Part 2 in the series "The Biotransformation of Foreign Substrates by Tissue Cultures". For Part 1, see ref. [5].

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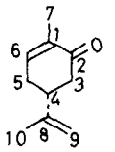
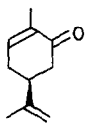


1a



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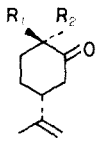
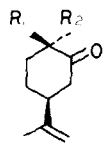


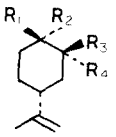
2a R_1 = Me, R_2 = H



2b R_1 = H, R_2 = Me

3a R_1 = H, R_2 = Me

3b R_1 = Me, R_2 = H

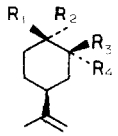


4a R_1 = Me, R_2 = H, R_3 = OH, R_4 = H

5a R_1 = Me, R_2 = H, R_3 = H, R_4 = OH

6a R_1 = H, R_2 = Me, R_3 = OH, R_4 = H

7a R_1 = H, R_2 = Me, R_3 = H, R_4 = OH



4b R_1 = H, R_2 = Me, R_3 = H, R_4 = OH

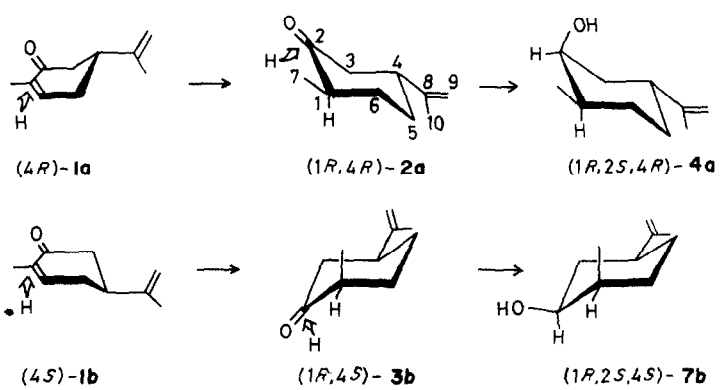
5b R_1 = H, R_2 = Me, R_3 = OH, R_4 = H

6b R_1 = Me, R_2 = H, R_3 = H, R_4 = OH

7b R_1 = Me, R_2 = H, R_3 = OH, R_4 = H

To elucidate the reduction sequence of the C–C double bond and the carbonyl group in the α,β-unsaturated carbonyl grouping and also to establish the stereoselectivity observed in the reduction of the carbonyl group of carvone (**1**), we examined the biotransformation of (1*R*, 4*R*)-(+)- and (1*S*, 4*S*)-(−)-dihydrocarvones (**2a** and **2b**) and (1*S*, 4*R*)-(+)- and (1*R*, 4*S*)-(−)-isodihydrocarvones (**3a** and **3b**) by tobacco suspension cells. The products obtained were similar to those in the biotransformation of carvone (**1**) (Table 1). The major product from **2a** and **2b** was (1*R*, 2*S*, 4*R*)-(+)-neodihydrocarveol (**4a**) and (1*S*, 2*S*, 4*S*)-(+)-dihydrocarveol (**5b**), respectively. On the other hand, **3a** gave **4a** as a major product and **3b** was mainly transformed to **5b** and **7b**. These results indicate that the carbonyl group in dihydrocarvones (**2** and **3**) is subject to selective hydrogen attack, as a result of which all the transformation products have the *S* configuration at C-2.

Thus, it was clarified that the cultured suspension cells of *N. tabacum* reduced the C–C double bond adjacent to the carbonyl group and then the carbonyl group itself, leaving the C–C double bond in the 1-methylethenyl group unattacked. The hydrogen attack to the C–C double bond adjacent to the carbonyl group and then the carbonyl group takes place stereoselectively from the *si*-face at C-1 and the *re*-face at C-2, respectively (Scheme 1). The stereospecific reduction of the carbonyl group was further demonstrated in the biotransformation of dihydrocarvones (**2** and **3**). Although the hydroxylations at the C–C double bond and its allylic positions have been observed in the biotransformation of the monoterpenoid alcohols, such as linalool and terpineols [4–7], with tobacco suspension cells, no hydroxylation took place in the biotransformation of the monoterpenoid ketones **1–3**. It is fascinating to note that, in the biotransformation of foreign substrates with cultured suspension cells of *N. tabacum*, the cells show different metabolic behaviours resulting in reduction and hydroxylation dependent on the structure of the substrates administered.



Scheme 1. Stereoselective hydrogen attack in the reduction of the C–C double bond and the carbonyl group of carvone (**1**) by suspension cells of *N. tabacum*.

EXPERIMENTAL

TLC. Si gel (0.5 mm) developed with (i) EtOAc–hexane (3:7), (ii) EtOAc–petrol (3:7), (iii) C_6H_6–Me_2CO (7:3) and (iv) petrol–Me_2CO (4:1); GLC: FID, glass column (3 mm × 2 m) packed with 15% DEGS, 2% OV-17 and 2% OV-101 on Chromosorb W (AW-DMCS) (80–100 mesh) at 105°, 90° and 90–280° (5°/min), respectively; ^{1}H NMR: 60 MHz, $CDCl_3$, TMS as int. standard; EIMS: 70 eV, direct-inlet system; GC/MS: glass column (3 mm × 2 m) packed with 15% DEGS on Chromosorb W (AW-DMCS) (80–100 mesh), EI ion source 40 eV or a CI ion source at 150 eV with isobutane.

Substrates. (−)-Carvone (**1a**), donated from Takasago Perfumery Co., Ltd, was rectified to give a sample with bp 93.5–94°/1066 Pa (8 mm Hg), $[\alpha]_D^{25}$ −(60.4° (neat), n_D^{25} 1.5000 and d_4^{25} 0.9607 (lit. [9] $[\alpha]_D$ −(59.7°; n_D^{20} 1.4988; d_{15}^{15} 0.9652). Following the method reported in ref. [10], (+)-carvone (**1b**) isolated from caraway oil was purified via carvoxime, mp 71–72° (lit. [9] mp 72°) to give a specimen with bp 92–93°/1066 Pa (8 mm Hg), $[\alpha]_D^{25}$ + 57.1° (neat), n_D^{25} 1.4950 and d_4^{25} 0.9552 (lit. [9] $[\alpha]_D$ + 60.0°; n_D^{20} 1.49952; d_{15}^{15} 0.9645). (+)-Dihydrocarvone (**2a**) with $[\alpha]_D^{25}$ + 13.0° (EtOH; *c* 0.6) [lit. [11] $[\alpha]_D^{22}$ + 13.12° (EtOH; *c* 6)] and (+)-isodihydrocarvone (**3a**) with $[\alpha]_D^{25}$ + 20.7° (EtOH; *c* 0.4) were prepared from **1a** by reduction with Zn powder in 25% ethanolic KOH soln [11]. (−)-Dihydrocarvone (**2b**) with $[\alpha]_D^{25}$ − 13.6° (EtOH; *c* 1.0) (lit. [9] $[\alpha]_D$ − 16.3°) and (−)-isodihydrocarvone (**3b**) with $[\alpha]_D^{25}$ − 18.2° (EtOH; *c* 0.3) [lit. [12] $[\alpha]_D^{22}$ − 16.0° (EtOH; *c* 7.6)], were prepared from **1b** in the same manner as above. All the samples were >99% pure by GLC.

Feeding of the monoterpenoids to the tobacco suspension cells. The callus tissues used in this study were induced from the stem of *N. tabacum* Bright Yellow and have been maintained for about 8 years. Just prior to use for this work, a part of the callus tissue was transferred to freshly prepared Murashige and Skoog's medium [8] (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; total 200–300 mg) 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.

Isolation and identification of the products. The suspension cells were filtered off and triturated with MeOH. The MeOH extract was concd to small volume and the residue was extracted with $CHCl_3$. The culture medium from the cells was extracted with $CHCl_3$. The two $CHCl_3$ extracts were bulked, since they exhibited the same behaviour on TLC and GLC, and after removal of the solvent examined by TLC and GLC. The transformation products were isolated from the $CHCl_3$ extract by prep. TLC on Si gel (EtOAc–hexane, 1:4), and identified by direct comparison of physical constants, TLC, GLC and spectral data with those of authentic samples. The physical constants and spectral data are shown below. The products present in small amounts were identified by direct comparison of TLC, GLC and/or GC/MS with those of authentic samples. The authentic samples were prepared following the methods described in ref. [11]. The amounts of the products were determined from the GLC trace by use of a standard curve prepared with carvone. The yields of the products are given in Table 1.

Dihydrocarvone (**2**). $[\alpha]_D^{25}$ + 12.5° (EtOH; *c* 0.16) [lit. [11] $[\alpha]_D^{22}$ + 13.12° (EtOH; *c* 6)]; IR ν_{max}^{film} cm^{-1} 1710 (C=O) and 1642 (C=C): ^{1}H NMR: δ 1.03 (3H, *d*, *J* = 6 Hz, 1-Me), 1.75 (3H, *s*, 8-Me), 4.77 (2H, *brs*, $\rangle$C=CH$_2$); MS *m/z* (rel. int.): 152 [M]$^+$ (22), 137 [M − Me]$^+$ (16), 109 (35), 95 (82), 67 (100).

Isodihydrocarvone (**3**). $[\alpha]_D^{25}$ − 19.0° (EtOH; *c* 0.1) [lit. [12] $[\alpha]_D^{22}$ − 16.0° (EtOH; c 7.6)]; IR ν_{max}^{film} cm^{-1}: 1710 (C=O) and 1640 (C=C); ^{1}H NMR: δ 1.08 (3H, *d*, *J* = 6 Hz, 1-Me), 1.75 (3H, *s*, 8-Me), 4.81 (2H, *d*, *J* = 8 Hz, $\rangle$C=CH$_2$); MS *m/z* (rel. int.): 152 [M]$^+$ (37), 137 [M − Me]$^+$ (16), 109 (40), 95 (90), 67 (100).

Neodihydrocarveol (**4**), $[\alpha]_D^{25}$ + 29.1° (EtOH; *c* 0.30) [lit. [11] $[\alpha]_D^{22}$ + 32.30° (EtOH; *c* 1.5)]; IR ν_{max}^{film} cm^{-1}: 3442 (OH) and 1645 (C=C); ^{1}H NMR: δ 0.98 (3H, *d*, *J* = 6 Hz, 1-Me), 1.70 (3H, *s*, 8-Me), 3.92 (1H, *br*, $\rangle$CH–OH), 4.73 (2H, *brs*, $\rangle$C=CH$_2$); MS *m/z* (rel. int.): 154 [M]$^+$ (5), 136 [M − H_2O]$^+$ (55), 121 (68), 107 (73), 93 (50), 79 (57), 41 (100).

Dihydrocarveol (**5**), $[\alpha]_D^{25}$ − 33.3° ($CHCl_3$; *c* 0.18) [lit. [11] $[\alpha]_D^{20}$ − 30.30° ($CHCl_3$; *c* 2.0)]; IR ν_{max}^{film} cm^{-1}: 3372 (OH) and 1645 (C=C); ^{1}H NMR: δ 1.03 (3H, *d*, *J* = 6 Hz, 1-Me), 1.73 (3H, *s*, 8-Me), 3.17 (1H, *br*, $\rangle$CH–OH), 4.72 (2H, *bs*, $\rangle$C=CH$_2$); MS *m/z* (rel. int.): 154 [M]$^+$ (14), 136 [M − H_2O]$^+$ (65), 121 (59), 107 (54), 93 (68), 79 (47), 41 (100).

Isodihydrocarveol (**6**). GC/MS: EIMS *m/z* (rel. int.): 154 [M]$^+$ (20), 136 [M − H_2O]$^+$ (82), 121 [M − H_2O − Me]$^+$ (75), 107 (99), 93 (79), 82 (100), 79 (76), 41 (65); CIMS *m/z* (rel. int.): 155 [M + H]$^+$ (5), 137 [M + H − H_2O]$^+$ (100).

Neoisodihydrocarveol (**7**). $[\alpha]_D^{25}$ − 20.0° (EtOH; *c* 0.2) [lit. [12] $[\alpha]_D^{22}$ − 25.35° (EtOH; *c* 2.0)]; IR ν_{max}^{film} cm^{-1}: 3374 (OH) and 1646 (C=C); ^{1}H NMR: δ 0.94 (3H, *d*, *J* = 7 Hz, 1-Me), 1.72 (3H, *s*, 8-Me), 3.75 (1H, *br*, $\rangle$CH–OH), 4.70 (2H, *brs*, $\rangle$C=CH$_2$); MS *m/z* (rel. int.): 154 [M]$^+$ (7), 136 [M − H_2O]$^+$ (53), 121 (67), 107 (71), 93 (100), 79 (49), 41 (53).

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