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INTRODUCTION OF OXYGENATED FUNCTIONAL GROUPS INTO 3-CARENE AND 2-PINENE BY CULTURED CELLS

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Key Word Index—*Nicotiana tabacum*; Solanaceae; *Catharanthus roseus*; Apocyanaceae; cultured cells; biotransformation; epoxidation; hydroxylation; carene; pinene.

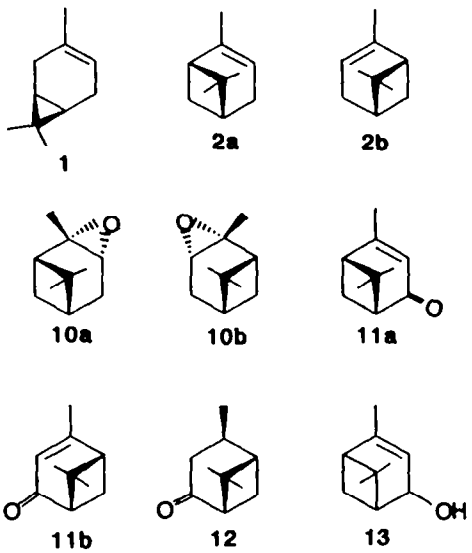
Abstract—The biotransformation of the monoterpene hydrocarbons 3-carene and 2-pinene by cell suspension cultures of *Nicotiana tabacum* and *Catharanthus roseus* was investigated. The cultures have the ability to regio- and enantioselectively introduce the oxygenated functional groups into the C=C double bond and the allylic positions of the substrates.

INTRODUCTION

Stereospecific introduction of an oxygenated functional group into unfunctionalized alkenes is one of the most important reactions in synthetic chemistry. Many studies have been reported on the specific oxidation of alkenes with chemical reagents [1–7] and microorganisms [8–11]. In connection with the development of a suitable methodology for the introduction of chirality into achiral compounds [12], we have now investigated the introduction of an oxygenated functional group into terpene hydrocarbons by use of cell suspension cultures of higher plants.

RESULTS AND DISCUSSION

Incubations of the monoterpene hydrocarbons (+)-(1*S*,6*R*)-3-carene (**1**), (+)-(1*S*,5*R*)-2-pinene (**2a**) and (−)-(1*R*,5*S*)-2-pinene (**2b**) with cell suspension cultures of *Nicotiana tabacum* and *Catharanthus roseus* were carried out at 25° for 24 hr in a similar manner to that described in ref. [13]. 3-Carene (**1**) was converted to (1*S*,3*S*,4*R*,6*R*)-3,4-epoxycarane (**3**), (1*S*,3*R*,4*R*,6*R*)-3,4-caranediol (**4**), (1*S*,6*R*)-3-caren-5-one (**5**), 3,6,6-trimethylcyclohepta-2,4-dien-1-one (**6**), 2-(5′-methylcyclohexa-2′,4′-dienyl) propan-2-ol (**7**) and 8-hydroxy-*m*-cymene (**8**) (Table 1). The structures of the products **5**–**8** were determined by interpretation of their mass and ^{1}H NMR data and by comparison with their reported data [1, 11]. Also, the structures of the products **3** and **4** were determined by comparison of their spectral data with those of synthetic specimens. In the light of our previous studies [14, 15] and based on literature data [16], the diol **4** may be produced by hydrolysis of the resultant epoxide **3**, and also 3-caren-5-one (**5**) may be formed by further oxidation of the resultant allyl alcohol **9** (Scheme 1). These observations indicate that 3-carene was first oxidized at the C=C double bond and its allylic position to give an epoxide and an allyl alcohol, respectively.



Similar oxidations at the C=C double bond and the allylic position were also observed in the biotransformation of the 2-pinenes, **2a** and **b**, by cell suspension cultures

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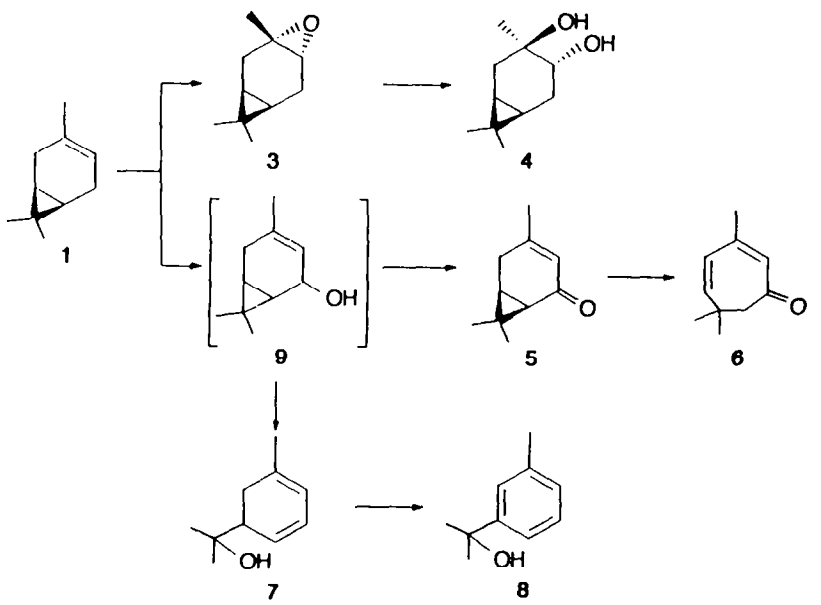

Scheme 1. Possible pathway for the biotransformation of 3-carene by cell suspension cultures of *N. tabacum* and *C. roseus*.

Table 1. Biotransformation of (1*S*, 6*R*)-3-carene (**1**) by cell suspension cultures of *N. tabacum* and *C. roseus*

Products	Yield (%)*	
	N. tabacum	*C. roseus*
3,4-Epoxycarane (**3**)	4.8	2.8
3,4-Caranediol (**4**)	1.4	0.7
3-Caren-5-one (**5**)	6.0	7.6
3,6,6-Trimethylcyclohepta-2,4-dien-1-one (**6**)	5.4	tr.†
2-(5'-methylcyclohexa-2',4'-dienyl)Propan-2-ol (**7**)	0.4	tr.
8-Hydroxy-*m*-cymene (**8**)	2.2	5.8

*Weight (%) of product relative to the substrate administered.
†tr.: Trace amount (< 0.1%).

Table 2. Biotransformation of (1*S*,5*R*)- and (1*R*,5*S*)-pinenes (**2a** and **b**) by a cell suspension culture of *N. tabacum*

Substrates	Products	Yield (%)*
(1*S*,5*R*)-2-Pinene (**2a**)	2,3-Epoxypinane (**10a**)	5.8
	2-Pinen-4-one (**11a**)	5.0
(1*R*,5*S*)-2-Pinene (**2b**)	2,3-Epoxypinane (**10b**)	tr.†
	2-Pinen-4-one (**11b**)	6.8
	Pinan-4-one (**12**)	2.8

*Weight (%) of product relative to the substrate administered.
†tr.: Trace amount (< 0.1 %).

of *N. tabacum*. (1*S*,5*R*)-2-Pinene (**2a**) was mainly converted into (1*S*,2*S*,3*R*,5*S*)-2,3-epoxypinane (**10a**) and (1*S*,5*R*)-2-pinen-4-one (**11a**), whereas (1*R*,5*S*)-2-pinene (**2b**) was transformed to (1*R*,5*S*)-2-pinen-4-one (**11b**) and (1*S*,2*R*, 5*S*)-pinan-4-one (**12**) with only very small amounts of the epoxide **10b** being obtained (Table 2). 2-Pinen-4-ones (**11a** and **b**) may be formed by further oxidation of the corresponding allyl alcohol (**13**) [17], which may be produced by oxidation at the allylic position of the C=C double bond of 2-pinenes (**2a** and **b**). Pinan-4-one (**12**) may be obtained from 2-pinen-4-one (**11b**) as a result of enantioselective hydrogenation by the cultured cells, as described in our previous paper [18].

Thus, cultured suspension cells of *N. tabacum* and *C. roseus* were found to have the ability to introduce oxygenated functional groups, such as epoxy and hydroxy groups, into 3-carene and 2-pinene; the epoxidation of the C=C double bond occurred stereo- and enantioselectively, while the hydroxylation occurred regioselectively at the allylic position of the C=C double bond.

EXPERIMENTAL

Analytical and prep. TLC: 0.25 mm thick silica gel plates (Merck silica gel 60; GF_{254}); FID-GC: glass column (3 mm × 2 m) packed with 15% DEGS and 2% OV-17 on Chromosorb W (AW-DMCS; 80–100 mesh) at 60–160° (2° min^{-1}), respectively; GC-EI-MS (70 eV): mass spectrometer coupled with a GC fitted with a CBP-10 column (0.53 mm × 12 m) and an Ultra-1 column (0.2 mm × 12 m); ^1H NMR: 270 and 500 MHz, $CDCl_3$ with TMS as int. standard.

Substrates. (+)-(1*S*,6*R*)-3-Carene (**1**) (Aldrich) was purified by CC on silica gel with pentane: >99.5% pure on GC; $[\alpha]_D^{25}$ + 23.9 (MeOH; *c* 3.9); ^1H NMR: δ0.58–0.72 (2H, *m*, H-1 and H-6), 0.76 and 1.02 (6H, *s*, *gem*-dimethyl), 1.60 (3H, *s*, Me-7), 1.74–1.97 (2H, *m*, H-2), 2.12–2.37 (2H, *m*, H-5) and 5.23 (1H, *t*, H-4). (+)-(1*S*, 5*R*)-2-Pinene (**2a**)

[>99.1% pure on GC; $[\alpha]_D^{25}$ +49.0 (EtOH; *c* 10.2)] and (−)-(1*R*,5*S*)-2-pinene (**2b**) [>99.5% pure on GC; $[\alpha]_D^{25}$ −50.1 (EtOH; *c* 5.5)] were obtained from Aldrich.

Biotransformation of the substrates. The suspension cells of *N. tabacum* and *C. roseus* were prepared as described in refs [13, 19]. The cultured cells were added to a 1 l conical flask containing 500 ml of Murashige and Skoog's medium [20]. To the flask containing the suspension cells, the substrate (100 mg), either 3-carene or 2-pinene, was administered; the total amount of substrate used was 1.0 g. The transformation of the substrate was brought about by incubating the cultured mixture at 25° for 24 hr on a rotary shaker (70 rpm) in the dark.

Isolation of products. After incubation, the culture medium was filtered from the cells and extracted with Et_2O. The products were isolated from the Et_2O-soluble fraction by chromatography on silica gel with pentane–EtOAc (50:1) and prep. TLC on silica gel (hexane–EtOAc, 7:3). The products were identified as follows.

3,4-*Epoxycarane* (**3**). MS *m/z* (rel. int.): 152 $[M]^+$ (21), 137 (55), 123 (29), 119 (72), 109 (100), 91 (50), 81 (71) and 67 (66); ^{1}H NMR: δ0.45 and 0.53 (each 1H, *dt*, *J* = 1.9 and 8.9 Hz, H-1 and H-6), 0.73 and 1.01 (each 3H, *s*, 7-dimethyl), 1.26 (3H, *s*, H_3-3), 1.49 (1H, *dd*, *J* = 2.2 and 16.1 Hz, Ha-2), 1.64 (1H, *dt*, *J* = 2.2 and 16.5 Hz, Ha-5), 2.15 (1H, *dd*, *J* = 8.9 and 16.1 Hz, Hb-2), 2.30 (1H, *ddd*, *J* = 1.9, 8.9 and 16.5 Hz, Hb-5) and 2.83 (1H, *br t*, *J* = 1.9 Hz, H-4).

3,4-*Caranediol* (**4**). $[\alpha]_D^{25}$ +3.63 (MeOH; *c* 1.10); MS *m/z* (rel. int.): 170 $[M]^+$ (2), 152 (22), 137 (30), 109 (50), 97 (30), 74 (64) and 43 (100); ^{1}H NMR: δ0.67–0.77 (2H, *m*, H-1 and H-6), 0.97 (3H, *s*, H_3-8), 0.99 (3H, *s*, H_3-9), 1.21 (3H, *s*, H_3-10), 1.60–1.72 (2H, *m*, H_2-2), 1.93–2.17 (2H, *m*, H_2-5) and 3.37 (1H, *t*, *J* = 2.7 and 10.8 Hz, H-4).

3-*Caren*-5-*one* (**5**). IR ν_{max} cm^{-1}: 1650 (C=O); MS *m/z* (rel. int.): 150 $[M]^+$ (100), 135 (34), 108 (32), 107 (64), 91 (40) and 79 (24); ^{1}H NMR: δ1.04 (3H, *s*, H_3-8), 1.19 (3H, *s*, H_3-9), 1.45 (1H, *t*, *J* = 8.0 Hz, H-1), 1.56 (1H, *d*, *J* = 8.1 Hz, H-6), 1.87 (3H, *bs*, H_3-10), 2.32 (1H, *d*, *J* = 20.8 Hz, Hb-2), 2.64 (1H, *dd*, *J* = 7.9 and 20.8 Hz, Ha-2) and 5.83 (1H, *bs*, H-4).

3,6,6-*Trimethylcyclohepta*-2,4-*dien*-1-*one* (**6**). IR ν_{max} cm^{-1}: 1640 (C=O); MS *m/z* (rel. int.): 150 $[M]^+$ (100), 135 (44), 108 (50), 107 (64), 91 (56) and 79 (42); ^{1}H NMR: δ1.11 (6H, *s*, *gem*-dimethyl), 2.01 (3H, *br s*, H_3-3), 2.60 (2H, *s*, H_2-7), 5.75 (1H, *d*, *J* = 5.5 Hz, H-4), 6.04 (1H, *brs*, H-2) and 6.07 (1H, *d*, *J* = 5.5 Hz, H-5).

2-(5′-*methylcyclohexa*-2′,4′-*dienyl*)*Propan*-2-*ol* (**7**). MS *m/z* (rel. int.): 152 $[M]^+$ (2), 134 (12), 119 (36), 94 (82), 91 (41), 79 (54) and 59 (100); ^{1}H NMR: δ1.21 (6H, *s*, H_3-1 and H_3-3), 1.79 (3H, *br s*, H_3-5′), 2.17 (2H, *br d*, *J* = 10.9 Hz, H_2-6′), 2.37 (1H, *m*, H-1′), 5.61 (1H, *br t*, *J* = 9.8 Hz, H-3′), 5.62 (1H, *dd*, *J* = 3.5 and 9.9 Hz, H-4′) and 5.94 (1H, *ddd*, *J* = 3.0, 5.4 and 9.9 Hz, H-2′).

8-*Hydroxy*-m-*cymene* (**8**). MS *m/z* (rel. int.): 150 $[M]^+$ (10), 135 (55), 132 (74), 107 (56) and 43 (100); ^{1}H NMR: δ1.58 (6H, *s*, *gem*-dimethyl), 2.37 (3H, *s*, H_3-1), 7.07 (1H, *dt*, *J* = 7.3 and 2.0 Hz, H-2), 7.24 (1H, *t*, *J* = 7.8 Hz, H-3), 7.32 (1H, *t*, *J* = 2.1 Hz, H-6) and 7.38 (1H, *dt*, *J* = 7.8 and 2.0 Hz, H-4).

Preparation of authentic samples. 3,4-Epoxycarane (**3**) and 2,3-epoxypinane (**10a**) were prepared from 3-carene (**1**) and 2-pinene (**2a**), respectively, by epoxidation with *m*-chloroperbenzoic acid. After working-up as usual, the reaction products were isolated and purified by CC on silica gel with $CHCl_3$–C_6H_6 (14:1). 3,4-Caranediol (**4**) was prepared from the epoxide **3** by hydrolysis with HCl (pH 4.0).

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