Biotransformation of Citronellal by *Solanum aviculare* Suspension Cultures: Preparation of *p*-Menthane-3,8-diols and Determination of Their Absolute Configurations

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Citronellal was transformed by *Solanum aviculare* suspension cultures to menthane-3,8-diols. *cis*-Menthane-3,8-diol dominated over the *trans*-isomer (39% and 15%, respectively). Absolute configurations of menthane-3,8-diols were assigned by critical analysis of ^{1}H and ^{19}F NMR spectra of prepared esters with 2-methoxy-2-phenyl-3,3,3-trifluoropropanoic acid. Citronellol and isopulegol were other products of the transformation (23% and 17%, respectively). The reaction course was identical for both citronellal enantiomers.

Biotransformations by plant cells have been studied with the aim to achieve the formation of natural secondary products, which can be potentially used in industrial production or, for environmental purposes, to degrade undesired xenobiotic chemicals.

Cell culture research contains a wealth of information about how numerous natural and synthetic organic chemicals are produced and/or transformed by a number of plants.[1,2] The most important transformation reactions discovered to date include glycosylation, hydroxylation, reduction, esterification, epoxidation, isomerization, oxidation, and transfer of a methoxy group. Depending on the chosen conditions, an individual compound is converted to different products. For example, Stohs[3] achieved conversion of progesterone in nine different chemical reactions using 14 different plant cell cultures. On the other hand, one specific cell line is able to express enzymes necessary to transform different substances added. In the case of transformation of cyclic alcohols and monoterpenes using tobacco (*Nicotiana tabacum*) cells, the following reactions occur: regioselective hydroxylation of C=C double bonds, enantioselective hydroxylation, stereospecific reduction of keto groups, and reciprocal conversion of cyclic alcohols and ketones.[4] Transformations of different monoterpenes using plant cells as biocatalysts have been studied earlier in our laboratory. These studies included oxidation of verbenol by free and immobilized *Solanum aviculare* cells,[5] transformation of limonene to carvone via carveol (hydroxylation and oxidation) by *S. aviculare* and *Dioscorea deltoidea* cells,[6] and production of verbenols and verbenone from α-pinene catalyzed by *Picea abies* suspension cultures.[7]

Citronellal as a substrate was transformed earlier by microorganisms[8−11] or by germinating wheat seeds.[12] Tissue cultures of higher plants (*Petroselinum crispum*[13] and several *Mentha* species[14]) were also studied for their abilities to transform citronellal. In all reported cases, the transformation led to the formation of citronellol. In this contribution, we describe transformations of citronellal to menthane-3,8-diols by *S. aviculare* suspension cultures and the determination of the absolute configurations of main products.

Initial experiments were done with citronellol as substrate and *S. aviculare* cells as a biocatalyst. Biotransformation of citronellol by means of horseradish peroxidase with the aim to prepare rose oxide was described earlier by Kaminska and co-workers.[15] Our experiments however showed that *S. aviculare* cells were unable to oxidize citronellol as opposed to *cis*-verbenol.[5] On the other hand, the cells transformed citronellal to a mixture of alcohols. After 14 days of transformation, we were able to isolate and identify citronellol (23%), isopulegol (15%), and two isomers of *p*-menthane-3,8-diol (*cis*, 39% and *trans*, 17%), as well as the rest of the starting citronellal (6%) (Scheme 1). The kinetics of their formation was a typical curve of biotransformation without formation of intermediates. The proportion of citronellol became constant after 3 days, while amounts of the other products formed were still increasing with time. After 10 days, the mixture had no longer changed. Both enantiomers of citronellal showed the same pattern in the course of transformation.

There exist eight stereoisomers of menthane-3,8-diols in principle, due to the presence of three chiral centers in positions 1, 3, and 4. Starting from citronellals with known absolute configurations in position 1, only four isomers were isolated, two from each enantiomer of citronellal (Scheme 1). The absolute configurations on chiral centers were assigned by critical analysis of ^{1}H and ^{19}F NMR spectra of prepared esters with 2-methoxy-2-phenyl-3,3,3-trifluoropropanoic acid [Mosher acid, 2-methoxy-2-(trifluoromethyl)phenylacetic acid, MTPA]. This method is generally used in NMR spectroscopy of various alcohols and amines.[16] The method is based on comparison of relative changes of ^{1}H NMR chemical shifts of protons in α- and α′-positions to the carbon bearing the esterified hydroxyl group and relative changes of ^{19}F NMR chemical shifts of CF_3 groups in both *R*-(+)- and *S*-(−)-MTPA esters. The model assumes[17] that the CF_3 group and carbonyl oxygen are eclipsed so that the preferred conformation of the system displays the carbinyl hydrogen eclipsed with the carbonyl group (this assumption was confirmed by chiroptical measurements). An extended *trans* ester conformation places one of the groups close to the phenyl ring. This group is, therefore, more shielded and is shifted to higher field in one diastereoisomer compared to the second one. Theoretical background for an explanation of changes in ^{19}F NMR spectra is based on differences in shielding of the CF_3 moiety on the same side of the $CF_3-C-CO-CH$ plane, producing the deviation of the CF_3 group from this plane

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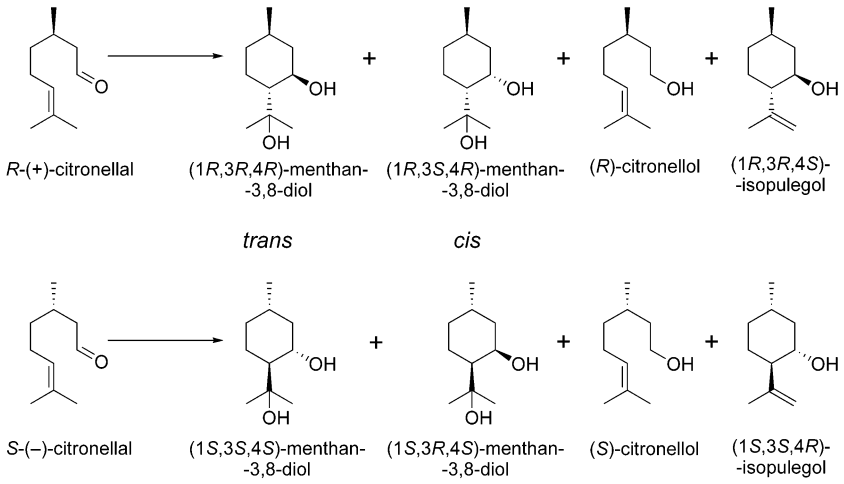
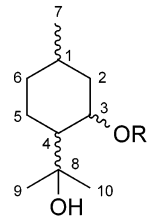
Scheme 1



and resulting in increasing of shielding of the appropriate signal. Due to this mechanism, the final effect results in the different signs of the chemical shifts in the ^{1}H and ^{19}F NMR spectra. On the basis of the known absolute configuration on the carbon atom of the MTPA moiety, the absolute configuration on the other centers can be assigned in this way.

By esterification of menthane-3,8-diols, only the secondary hydroxyl group in position 3 reacts. We analyzed the resulting monoesters with both *R*-(+)- and *S*-(−)-MTPA. Only four pairs of different NMR spectra from the resulting eight esters were found. Spectra of the *R*-(+)-MTPA ester of 3,4-*trans*-1*R*-menthane-3,8-diol (**1b**) and *S*-(−)-MTPA-3,4-*trans*-1*S*-menthane-3,8-diol (**2c**) were identical, as well as spectra of the following pairs: *R*-(+)-MTPA ester of 3,4-*trans*-1*S*-menthane-3,8-diol (**2b**) and *S*-(−)-MTPA-3,4-*trans*-1*R*-menthane-3,8-diol (**1c**), *R*-(+)-MTPA ester of 3,4-*cis*-1*R*-menthane-3,8-diol (**3b**) and *S*-(−)-MTPA-3,4-*cis*-1*S*-menthane-3,8-diol (**4c**), and finally *R*-(+)-MTPA ester of 3,4-*cis*-1*S*-menthane-3,8-diol (**4b**) and *S*-(−)-MTPA-3,4-*cis*-1*R*-menthane-3,8-diol (**3c**).



1a-4c

1-4	R
a	H
b	*R*-(+)-MTPA
c	*S*-(−)-MTPA

Compound	1	2	3	4
Configuration	1*R*,3*R*,4*R*	1*S*,3*S*,4*S*	1*R*,3*S*,4*R*	1*S*,3*R*,4*S*

Final assignments of absolute configuration on chiral centers C-3 and C-4 were based on the following assumptions: (i) the cyclohexane ring is in the chair form; (ii) the bulky 2-hydroxy-2-propyl moiety is in the thermodynamically more stable equatorial position. ^{1}H and ^{19}F NMR data of measured MTPA esters are shown in Table 1 (Supporting Information). Comparing the **1b** and **1c** chemical shifts of protons H-2 and H-7 demonstrated an upfield shift ($\Delta\delta = \delta_{(R)-MTPA} - \delta_{(S)-MTPA}$), in the case of protons H-4, H-9, and H-10 and then a downfield shift. On the basis of the above-mentioned theory,[17] a transparent 3,4-*trans* configuration of the hydroxyl and 2-hydroxy-2-propyl group (CH−O proton with two diaxial and one axial−equatorial coupling constants), and a known absolute configuration of the starting material, we assigned the absolute configurations on all remaining chiral centers in the molecule (Table 2, Supporting Information). For assignment of the remaining isolated compounds, a similar procedure was used. Based on the above-mentioned results, the stereochemistry of biotransformation of both enantiomers of citronellal is shown in Scheme 1.

Previous papers on the biotransformation of citronellal[8−14] report the formation of citronellol as a single product. Our results represent the first report on the formation of menthane-3,8-diols from citronellal by plant cell biotransformation. Only two diastereoisomers from the four possible isomeric products were formed. Both *cis*- and *trans*-menthane-3,8-diols were isolated earlier from *Eucalyptus citriodora*, and they exhibited an allelopathic effect.[18] It was also reported that these compounds have a potent repellent effect against *Anopheles* mosquitoes.[19−23] A synthetic approach to these biologically active compounds was reported recently.[24]

Experimental Section

Plant Cell Cultures. A suspension culture of *Solanum aviculare* Forst was obtained in 1980 from callus (strain KK1N) that was derived from the leaf of a plant cultivated aseptically from seed supplied by the Botanical Garden in Kew.[25] This culture was subcultivated in 5-day intervals in nutrient medium according to Murashige and Skoog in the modification of Linsmayer and Skoog, containing 1×10^{-6} mol·L^{-1} 2,4-dichlorophenoxyacetic acid and 1×10^{-6} mol·L^{-1} kinetine, at a temperature of 27 °C in the dark on a roller (5 rpm).

Biotransformation. During the biotransformation experiments, 100 mL of a 5-day-old suspension containing 15 g of cell fresh weight was incubated at standard conditions in 500 mL flasks with 15 mg of substrate. The degree of conversion was measured using gas chromatography.

After incubation time, the cells were separated from the nutrient medium by filtration. Then the cells were homogenized in acetone (50 mL), and after 24 h, they were mixed with water (200 mL) and extracted with light petroleum (3 × 50 mL). The organic extracts were combined and dried over sodium sulfate, filtered, and evaporated in vacuo. The nutrient medium was extracted in the same manner. The residues obtained were dissolved in 2 mL of hexane and used for GC analysis.

Kinetic studies and the biotransformation on a preparative scale were done with (*S*)-(−)- and (*R*)-(+)-citronellal (92% purity, Lachema Brno, Czechoslovakia) as a substrate.

Analysis. The resulting compounds were separated by GC. They were identified by comparison of their retention times with standards and using GC−MS. Both menthanediols as main products were isolated by preparative column chromatography on silica gel.

An HP 5890A gas chromatograph with FID was used with a fused silica DB-1 capillary column (methyl silicone), 30 m × 0.25 mm, film thickness 0.25 μm, carrier gas H_2, linear velocity 63 cm/s at 40 °C. The temperature program was 40 °C to 100 °C at 2 °C/min, then 300 °C at 20 °C/min; injector temperature 200 °C, split ratio 50:1, detector temperature 250 °C.

An integrated system consisting of a ZAB-EQ mass spectrometer and an HP-5890A gas chromatograph was used with a fused silica OV-1 capillary column (25 m × 0.25 mm, film thickness 0.25 μm), carrier gas helium, linear velocity 50 cm/s at 40 °C. The temperature program was similar to that mentioned above; injector temperature 150 °C, split ratio 50:1; EI ionization, electron energy 70 eV, temperature of ion source from 80 to 180 °C.

Preparation of Esters with *R*-(+)- and *S*-(−)-2-Methoxy-2-phenyl-3,3,3-trifluoropropanoic Acids (MTPA esters). A general procedure used for the preparation of the MTPA esters on a milligram scale starting from (*S*)-(+)- and (*R*)-(−)-2-methoxy-2-phenyl-3,3,3-trifluoropropanoyl chloride (MTPCl) and a chiral alcohol was already described.[16,17] A solution of MTPCl (0.023 mmol) in benzene (0.5 mL) and a solution of 4-(dimethylamino)pyridine (0.023 mmol) in pyridine (0.1 mL) are added to the corresponding menthanediol (0.023 mmol). The resulting mixture is allowed to stand in a sealed vial under argon atmosphere for 2−4 h. The solvent is then evaporated, and the residue is applied on the top of a small column filled with silica gel. A rapid purification afforded products **1b**, **1c**, **2b**, **2c**, **3b**, **3c**, **4b**, and **4c** in 95% yields.

NMR Measurements. ^{1}H and ^{19}F NMR spectra were recorded on a Varian Unity-500 spectrometer at 499.8 MHz for ^{1}H and 470.3 ^{19}F NMR in deuteriochloroform, using tetramethylsilane (^{1}H) $\delta = 0.0$ and hexafluorobenzene (^{19}F) $\delta = -162.9$ as internal standards.

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Supporting Information Available: Tables 1 and 2 showing NMR data of MTPA esters **1b**−**4c** are available free of charge via the Internet at http://pubs.acs.org.

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