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Oxidative biotransformation of farnesol and 10,11-epoxyfarnesol by fungal strains

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Abstract

The biotransformation of farnesol (**1**), 10,11-epoxyfarnesol (**2**), geranylacetone (**3**) and 9,10-epoxygeranylacetone (**4**) was investigated using four strains of microorganisms selected in the screening procedure. The substrates were transformed by *Fusarium culmorum*, *Botrytis cinerea*, *Rhodotorula rubra*, *Rhodotorula marina* into oxyderivatives of geranylacetone. The biotransformation of farnesol after 2 days incubation with *F. culmorum* afforded mainly 8-hydroxygeranylacetone (**5**) and 9,10-epoxygeranylacetone (**3**) with high optical purities. The strains *R. rubra*, *R. marina* gave the 2,6-dimethyl-2,6-nonadien-1,9-diol (**6**) as the only product. *B. cinerea* transformed all substrates to 9,10-dihydroxygeranylacetone (**7**).

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Keywords: Biotransformation; Sesquiterpenes; Farnesol; *Fusarium culmorum*; *Rhodotorula rubra*; *Rhodotorula marina*; *Botrytis cinerea*

1. Introduction

Isoprenoids are widespread in the nature [1,2]. They occur in plants, fungi and animals. Many of them exhibit specific biological activity. They are known as a defensive compounds [3–5], antifungal and antibacterial compounds [6,7], plant hormones [8], insect pheromones [9] or anticancer agents [10]. For example, farnesol preferentially inhibits proliferation and induces apoptosis of cancer cells [11–14]. Moreover, as the carriers of specific odours some of them are of considerable industrial value in the flavors and perfumery industries as well as for pharmaceutical applications.

Total synthesis of these compounds and their derivatives is often difficult, so alternative way to produce them could be biotransformation of natural compound. This technique makes it possible to convert substrate regioselectively in a single step which may be carried out by microorganisms and products of this reaction are defined as natural. This is important because in recent years there has been an increasing tendency to replace "synthetic" flavoring substances by the "natural" ones.

One of the tasks of our research project is the synthesis of odoriferous derivatives of natural isoprenoids. Recently, we have published the synthesis of isoprenoid bicyclic lactones, which are characterized by interesting and valuable odors [15]. Here, we report the results of biotransformation of farnesol and its epoxide by fungal strains *Fusarium culmorum*, *Botrytis cinerea*, *Rhodotorula rubra*, *Rhodotorula marina*. The aim of this study was to obtain the cyclic and acyclic odoriferous oxyderivatives of this sesquiterpene alcohol. Our expectations arises from fact that farnesol itself possesses linden blossom odor and is also particularly suited for a use in flower composition. Moreover the *trans–trans* isomer of this compound is the most common in nature and occurs, for example, in ambrette seed oil [16]. We performed biotransformations of epoxyderivatives of farnesol and geranylacetone in order to check if the tested microorganisms posses the ability of cyclization this substrates. In literature there are describe only chemical methods of epoxyfarnesol cyclization [17]. The formation of carbon–carbon bonds in sesquiterpenoids by microorganisms are rather rare [18].

2. Materials and methods

2.1. Analysis

The progress of transformations, as well as the purity of isolated products, were checked by TLC technique on silica gel DC-Alufolien Kieselgel 60 F_{254} silica gel (0,2 mm; Merck), with hexane/acetone (3:1) as the developing solvent. Visualisation

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was effected with a solution of 10 g $Ce(SO_4)_2$ and 20 g phosphoromolybdenic acid in 1 dm^3 H_2SO_4, followed by heating. The same eluent was also used for preparative column chromatography performed on silica gel (Kieselgel 60, 230–400 mesh).

Gas chromatography (GC) analysis was carried out on a Varian CP-3380 instrument with HP-1 column (cross-linked methyl silicone, 30 m × 0.53 mm × 1.5 μm) and THERMO TR-5 (cross-linked 5% phenyl polisiloxane) capillary column (30 m × 0.32 mm × 1.0 μm). The enantiomeric compositions of the obtained products were determined by GC analysis using a (CP-cyclodextrin-B-2,3,6-M-19, 25 m × 0.25 mm × 0.25 μm) chiral column. The following temperature programs were applied:

(1) 110 °C for 1 min, 0.5 °C/min to 180 °C, 50 °C/min to 200 °C, for (±)-*9,10-epoxygeranylacetone* **(4)** R_t: (−) 12.4 and (+) 12.9 min;
(2) 120 °C for 1 min, 0.2 °C/min to 180 °C, 50 °C/min to 200 °C, for (±)-*8-hydroxygeranylacetone* **(5)** R_t: (−) 16.6 and (+) 17.3 min;
(3) 150 °C for 1 min, 2 °C/min to 180 °C, 50 °C/min to 200 °C, for (±)-*9,10-dihydroxy-6,10-dimethylundec-5-en-2-one* **(7)** R_t: (+) 6.7 and (−) 7.06 min;
(4) 100 °C for 1 min, 2 °C/min to 180 °C, 50 °C/min to 200 °C, for (±)-*7,8-epoxy-4,8-dimethylnon-3-en-1-ol* **(11)** R_t: (−) 34.5 and (+) 35.1 min.

^1H NMR and ^{13}C NMR spectra were recorded for $CDCl_3$ solutions on a Bruker Avance DRX 300 spectrometer. IR spectra were recorded on FTIR Thermo-Mattson IR 300 Spectrometer. Optical rotations were measured on a Autopol IV automatic polarimeter (Rudolph).

2.2. Substrates for biotransformation

The substrates used for the biotransformation experiments were: farnesol **(1)**, 10,11-epoxyfarnesol **(2)**, geranylacetone **(3)** and epoxygeranylacetone **(4)**. Farnesol and geranylacetone were purchased from Sigma–Aldrich and Fluka.

2.3. Synthesis of 10,11-epoxyfarnesol (2)

The synthesis was carried out according to the three-step method [19]. To a solution of alcohol (2 g, 0.009 mol) and pyridine (0.86 ml, 0.0108 mol) in anhydrous ether (15 ml) acetic anhydride (1 ml, 0.0108 mol) was added and the mixture was stirred at room temperature for 2 days. Then the reaction mixture was washed with 10% Na_2CO_3, brine and dried ($MgSO_4$). Crude product was purified by column chromatography (silica gel, hexane–acetone, 50:1). In the next step, obtained acetate (2.2 g, 0.0083 mol) was stirred with NBS (1.69 g, 0.0012 mol) in THF-H_2O solution (60:25 ml) at 0 °C for 4 h. Then the reaction mixture was diluted with ether (50 ml) and the organic solution was washed with brine and dried ($MgSO_4$). Product was purified by column chromatography (silica gel, hexane-ethyl acetate, 95:5 at the beginning and then hexane-ethyl acetate 1:1). In final step K_2CO_3 (2.8 g, 0.019 mol) was added to bromohydrin (1.8 g, 0.0049 mol) dissolved in absolute MeOH (20 ml). The reaction mixture was stirred at room temperature for 1 h. Then it was concentrated in vacuo, dissolved in ether, washed with brine and dried ($MgSO_4$). Column chromatography (silica gel, hexane–acetone, 4:1 at the beginning and then hexane–acetone 2:1) afforded 1.03 g of product. The yield of this three-step synthesis is 49%. The spectral data of bromohydrine **(1a)** and epoxide **(2)** are given below.

2.3.1. Bromohydrine of farnesol acetate (1a)

^1H NMR ($CDCl_3$), δ: 1.33 and 1.34 (two s, 6H, >$C(CH_3)_2$), 1.59 (s, 3H, CH_3-7), 1.70 (s, 3H, CH_3-3), 2.05 (s, 3H, CH_3–CO–O–), 2.05–2.20 (m, 8H, CH_2-9, CH_2-8, CH_2-5, CH_2-4), 3.96 (dd 1H, J = 11.4 and 1.6 Hz, H-10), 4.58 (d, 2H, J = 7.0 Hz, CH_2-1), 5.18 (m, 1H, H-2) 5.34 (m, 1H, H-6); IR (film, cm^{-1}): 3448(m), 2925(s), 1738(s), 1233(s).

2.3.2. (±)-10,11-Epoxyfarnesol (2)

^1H NMR ($CDCl_3$), δ: 1.25 and 1.29 (two s, 6H, >$C(CH_3)_2$), 1.59 (s, 3H, CH_3-7), 1.61 (two m, 2H, CH_2-9), 1.67 (s, 3H, CH_3-3), 2.02–2.13 (six m, 6H, CH_2-8, CH_2-5, CH_2-4), 2.69 (t, J = 6.2 Hz, 1H, H-10), 4.13 (d, 2H, J = 6.8 Hz, CH_2–OH), 5.15 (m, 1H, H-6), 5.40 (m, 1H, J = 6.8, H-2); ^{13}C NMR ($CDCl_3$), δ: 15.99, 16.24, 18.79, 24.87, 26.16, 27.29, 36.35, 39.41, 58.41, 59.37, 64.19, 123.64, 124.53, 134.37, 139.38; IR (film, cm^{-1}): 3426(s), 2961(s), 2924(s).

2.4. Synthesis of 9,10-epoxygeranylacetone (4)

The solution of *m*-CPBA (1.78 g, 0.01 mol) in CH_2Cl_2 (30 ml) was added drop wise to geranylacetone (2 g, 0.01 mol) in CH_2Cl_2 (20 ml) [20]. The reaction mixture was stirred at 0 °C for 3 h and then was washed with Na_2SO_3, $NaHCO_3$ and brine. The organic solution was dried over $MgSO_4$, then column chromatography (silica gel, hexane–acetone, 10:1) was performed. In this way pure (±)-9,10-epoxygeranylacetone (1.8 g, 86% yield) was obtained. Spectral data of product are as follows.

2.4.1. (±)-9,10-Epoxygeranylacetone (4)

^1H NMR ($CDCl_3$), δ: 1.23 and 1.27 (two s, 6H, >$C(CH_3)_2$), 1.55–1.63 (two m, 2H, CH_2-8), 1.61 (s, 3H, CH_3-6), 2.00–2.08 (two m, 2H, CH_2-7), 2.11 (s, 3H, CH_3 C(O)–), 2.24 (two m, 2H, CH_2-4), 2.44 (t, J = 7.4 Hz, 2H, CH_2-3), 2.66 (t, J = 6.3 Hz, 1H, H-9), 5.10 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$), δ: 15.99, 18.76, 22.41, 24.89, 27.34, 29.97, 36.31, 43.64, 58.34, 64.12, 123.21, 135.52, 208.69; IR (film, cm^{-1}): 2925(s), 1719(m).

2.5. Biotransformations

2.5.1. Microorganisms

The chemicals used for the preparation of the growing media were purchased from POCh (Poland).

The microorganisms were cultivated on a Sabouraud agar consisting of: aminobac (catalogue no. S-0002) 5 g, peptone K (S-0011) 5 g, glucose (549560117) 40 g and agar (S-0001) 15 g in distilled water 1 l at 28 °C and pH 5.5 and stored in refrigerator at 4 °C.

2.5.2. Screening procedure

The strains were cultivated at 25 °C in 300-ml Erlenmeyer flasks containing 100 ml of medium. After 4–6 days chemical substrates (10 mg) in 1 ml of acetone were added to the grown cultures and the mixtures were shaken. For the time-course analysis after 1, 2, 4 and 6 days 10 ml of the incubation mixtures was saturated with NaCl and then the products were extracted with dichloromethane. The extracts were dried over anhydrous $MgSO_4$ and the solvent was evaporated in vacuo. Residues were dissolved in 1 ml of acetone and analyzed by TLC and GC. In control experiments, the substrates were incubated in the medium without fungi.

Screening procedure was carried out for 20 fungal strains which were from the Institute of Biology and Botany, Medical Academy of Wrocław: *B. cinerea* (AM235), *Penicillium notatum* (904), *R. rubra* (AM4), *R. marina* (AM77), *Acremonium roseum* (AM346), *Saccharomyces cerevisiae* (AM464), *Mucor hiemalis* (AM450), *Piptoporus betulinus* (AM475), *Laetiporus sulphurens* (AM525), *Aspergillus nidulans* (AM243), *Candida viswanathi* (AM120), *Penicillium urticae* (AM84), *F. culmorum* (AM10), *Aspergillus glaucus* (AM211), *Penicillium vermiculatum* (AM30), *Penicillium hrysgenium* (AM112), *Cunninghamella japonica* (AM472), *Absidia cylindrospora* (AM336), from the Department of Biotechnology and Food Microbiology, University of Environmental and Life Sciences of Wrocław *Yarrowia lipolitica* (AR72) and from Agricultural University of Kraków *Pycinidiella resinae* (AR50). The screening of fungal strains led to the selection of four microorganisms that had ability to biotransformation of farnesol **(1)**, its epoxide **(2)**, geranylacetone **(3)** and 9,10-epoxygeranylacetone **(4)**.

2.6. Preparative biotransformation

2.6.1. General procedure

In order to isolate and identify the products, preparative-scale biotransformation were performed in 16–18 flasks (300-ml Erlenmeyer) containing 100 ml of medium in each flask. After 2–4 days of shaking the medium was saturated with NaCl and the products were extracted three times with dichloromethane after centrifugation (8000 rpm, 10 min) of biomass. The organic extracts were pooled, dried ($MgSO_4$) and evaporated in vacuo. The crude product mixtures were separated by column chromatography (silica gel, hexane:acetone 3:1) to obtain pure biotransformation products.

*2.6.2. Preparative biotransformation of farnesol **(1)***

The preparative biotransformation of farnesol (160 mg) gave 25 mg (13% isolated yield) of (−)-8-hydroxygeranylacetone **(5)** and 16 mg (11% isolated yield) of (+)-9,10-epoxygeranylacetone **(3)**. Obtained spectral data of (+)-9,10-epoxygeranylacetone were the same as the racemic sample; $[\alpha]_D^{20} = +22.8°$ ($c = 0.8$, $CHCl_3$), ee = 94%. The physical and spectral data of second product are as follows.

*2.6.2.1. (−)-8-Hydroxygeranylacetone **(5)**.* $[\alpha]_D^{20} = -21.2°$ ($c = 0.75$, $CHCl_3$, ee = 92%);

^1H NMR ($CDCl_3$), δ: 1.66 and 1.67 (two s, 6H, =C(CH_3)$_2$), 1.71 (s, 3H, CH_3-6), 2.11–2.13 (two m, 2H, CH_2-7), 2.13 (s, 3H, CH_3C(O)–), 2.29 (two m, 2H, CH_2-4), 2.48 (t, $J = 7.5$ Hz, 2H, CH_2-3), 4.43 (m, 1H, H-8), 5.12–5.20 (two m, 2H, H-5 and H-9); ^{13}C NMR ($CDCl_3$), δ: 16.15, 18.15, 22.43, 25.70, 29.92, 43.37, 48.02, 65.90, 126.59, 127.40, 132.91, 135.90, 208.58; IR (film, cm^{-1}): 3418(m), 2927(s), 1714(s).

The biotransformation of farnesol **(1)** with *R. rubra*, *R. marina* gave the same product: (*E*)-2,6-dimethylnona-2,6-dien-1,9-diol **(6)**. In the preparative scale from 160 mg of farnesol 24 mg (18% isolated yield) and 16 mg (12% isolated yield) of product **(6)** was obtained in biotransformation with *R. rubra* and *R. marina*, respectively. The method of the extraction was the same as described previously. The spectral data of the product are as follows.

*2.6.2.2. 2,6-Dimethylnona-2,6-dien-1,9-diol **(6)**.* ^1H NMR ($CDCl_3$), δ: 1.64 (s, 3H, CH_3-2), 1.66 (s, 3H, CH_3-6), 2.08–2.18 (m, 6H, CH_2-5, CH_2-4 and two OH), 2.28 (m, 2H, CH_2-8), 3.61 (t, $J = 6.1$ Hz, 2H, CH_2-9), 3.98 (s, 2H, CH_2-1), 5.13 (m, 1H, H-7), 5.34 (m, 1H, H-3); ^{13}C NMR ($CDCl_3$), δ: 13.67, 16.00, 25.69, 31.27, 39.33, 62.22, 68.94, 120.71, 125.81, 135.27,138.29; IR (film, cm^{-1}): 3350(m), 2925(s).

The biotransformation of farnesol **(1)** (180 mg) with *B. cinerea* resulted in the one product (−)-9,10-dihydroxy-6,10-dimethylundec-5-en-2-one **(7)** (49 mg, 29% isolated yield). The physical and spectral data of the product are as follows.

*2.6.2.3. (−)-9,10-Dihydroxy-6,10-dimethylundec-5-en-2-one **(7)**.* $[\alpha]_D^{20} = -31.4°$ ($c = 1.75$, $CHCl_3$, ee = 41%), Lit. [21] $[\alpha]_D = -19.3°$ ($c = 0.78$, CH_3OH),

^1H NMR ($CDCl_3$), δ: 1.16 and 1.20 (two s, 6H, –C(OH)(CH_3)$_2$), 1.37–1.43 (two m, 2H, CH_2-8), 1.55 (two m, 2H, CH_2-7), 1.60 (s, 3H, CH_3-6), 2.03–2.09 (two m, 2H, CH_2-3), 2.14 (s, 3H, –C(O)CH_3), 2.23–2.50 (two m, 2H, CH_2-4), 2.47 (t, $J = 7.4$ Hz, 2H, CH_2-3), 3.33 (dd, $J = 10.4$ and 1.2 Hz, 1H, H-9), 5.15 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$), δ: 15.93, 22.44, 23.29, 26.43, 29.61, 29.95, 36.76, 43.64, 73.04, 78.16, 123.33, 136.23, 208.91; IR (film, cm^{-1}): 3421(s), 2927(s), 1710(s).

*2.6.3. Preparative biotransformation of (±)-10,11-epoxyfarnesol **(2)***

The preparative-scale transformations of (±)-10,11-epoxyfarnesol **(2)** (180 mg) with *F. culmorum* was carried out for 4 days and gave three products. The mixture was extracted and then the products were separated by means of the column chromatography (silica gel, hexan: acetone 3:1) to obtain pure biotransformation products: (−)-9,10-epoxygeranylacetone **(4)** (31 mg, 19% isolated yield, $[\alpha]_D^{20} = -20.3°$ ($c = 1.25$, $CHCl_3$, ee = 80%) (−)-9,10-dihydroxy-6,10-dimethylundec-5-en-2-one **(7)** (15 mg, 9% isolated yield, $[\alpha]_D^{20} = -2.94°$ ($c = 0.75$, $CHCl_3$), ee = 4%), and 10-hydroxy-6,10-dimethylundec-5-en-2,9-dione **(8)** (22 mg, 13% isolated yield). The spectral data of the product **(8)** are given below.

*2.6.3.1. 10-Hydroxy-6,10-dimethylundec-5-en-2,9-dione **(8)**.* ^1H NMR ($CDCl_3$), δ: 1.37 (s, 6H, –C(OH)(CH_3)$_2$), 1.63 (s,

3H, CH_3-6), 2.13 (s, 3H, –C(O)CH_3), 2.24–2.30 (four m, 4H, CH_2-5 and CH_2-8), 2.46 (t, J = 7.3 Hz, 2H, CH_2-4), 2.62 (t, J = 7.3 Hz, 2H, CH_2-9), 3.78 (s, 1H, OH), 5.10 (m, 1H, H-7); ^{13}C NMR ($CDCl_3$), δ: 16.10, 22.32, 26.47, 29.97, 30.94, 33.29, 34.17, 43.47, 76.2, 123.59, 134.73, 208.60, 213.97; IR (film, cm^{-1}): 3425(m), 2927(s), 1711(s).

In the case of three other strains—*B. cinerea*, *R. rubra* and *R. marina* the transformation of 10,11-epoxyfarnesol **(2)** (180 mg) gave the same product 9,10-dihydroxy-6,10-dimethyl-undec-5-en-2-one **(7)**. In the experiment with *B. cinerea* 31 mg of (−)-9,10-dihydroxygeranylacetone **(7)** with predominant of isomer (−) was obtained (18% isolated yield, $[\alpha]_D^{20} = -21.7°$, ee = 28%). In the transformation with *R. rubra* 20 mg of product **(7)** with predominant of (+)-enantiomer (12% isolated yield) $[\alpha]_D^{20} = +1.54°$, ee = 2% was obtained and in the process with *R. marina* 16 mg of the same product (9% isolated yield, $[\alpha]_D^{20} = +3.27°$, ee = 4%) we obtained.

*2.6.4. Preparative biotransformation of geranylacetone **(3)***

The microbial transformation of geranylacetone **(3)** (180 mg) with *F. culmorum* gave the mixture of three products: 5 mg of 8-hydroxygeranylacetone **(5)** (3% isolated yield, $[\alpha]_D^{20} = -10.1°$ (c = 0.25, $CHCl_3$), ee = 42%) 51 mg of 10-hydroxy-6,10-dimethylundec-5-en-2,9-dione **(8)** (24% yield) and 40 mg of 9,10-dihydroxy-6,10-dimethylundecen-5-en-2-one **(7)** (19% yield, $[\alpha]_D^{20} = +1.57°$ (c = 1.15, $CHCl_3$), ee = 2%).

The biotransformation of geranylacetone **(3)** (180 mg) with *R. rubra* and *R. marina* afforded the same product, 4,8-dimethylnonan-3,7-dien-1-ol **(9)**. 35 mg and 29 mg of this product (22% and 19% yield after 2 and 4 days of incubation, respectively) were isolated. Its spectral data are given below.

*2.6.4.1. 4,8-Dimethylnona-3,7-dien-1-ol **(9)**.* ^{1}H NMR ($CDCl_3$), δ: 1.60 and 1.64 (two s, 6H, =C$(CH_3)_2$), 1.68 (s, 3H, CH_3-4), 1.99–2.08 (m, 5H, CH_2-5, CH_2-6 and OH), 2.28 (two m, 2H, CH_2-2), 3.61 (t, J = 6.6 Hz, 2H, CH_2-1), 5.05–5.14 (two m, 2H, H-3 and H-7); ^{13}C NMR ($CDCl_3$), δ: 16.12, 17.63, 25.63, 26.49, 31.40, 39.75, 62.34, 119.82, 124.08, 131.64, 138.87; IR (film, cm^{-1}): 3342(m), 2925(s).

The preparative transformations of geranylacetone **(3)** (180 mg) by means of *B. cinerea* gave 61 mg (29% isolated yield) of (+)-9,10-dihydroxy-6,10-dimethyl-undecen-5-en-2-one **(7)** ($[\alpha]_D^{20} = +31.8°$ (c = 2.3, $CHCl_3$) ee = 45%) after 4 days.

*2.6.5. Preparative biotransformation of (±)-9,10-epoxygeranylacetone **(4)***

The biotransformation of (±)-10,11-epoxygeranylacetone **(4)** (160 mg) with *R. marina* led to the mixture of (±)-9,10-epoxy-6,10-dimethylundec-5-en-2-ol **(10)** (36 mg, 22% isolated yield) and (+)-7,8-epoxy-4,8-dimethylnon-3-en-1-ol **(11)** (32 mg, 23% isolated yield). The products were separated by column chromatography. The spectral data of pure products are given below.

*2.6.5.1. 9,10-Epoxy-6,10-dimethylundec-5-en-2-ol **(10)**.* ^{1}H NMR ($CDCl_3$), δ: 1.19 (d, 3H, J = 6.2 Hz, CH_3-2), 1.26 and 1.30 (two s, 6H, >C$(CH_3)_2$), 1.45–1.53 (two m, 2H, CH_2-8), 1.55–1.64 (two m, 2H, CH_2-3), 1.64 (s, 3H, CH_3-6), 2.07–2.19 (m, 5H, CH_2-4, CH_2-7 i OH), 2.70 (t, J = 6.2 Hz, 1H, CH_2-9), 3.80 (d, J = 6.2 Hz, 1H, H-2), 5.20 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$), δ: 15.95, 18.72, 23.49, 24.33, 24.85, 27.37, 36.37, 39.09, 58.29, 64.16, 67.88, 124.55, 134.68; IR (film, cm^{-1}): 3425(s), 2963(s), 2926(s).

*2.6.5.2. 7,8-Epoxy-4,8-dimethylnon-3-en-1-ol **(11)**.* $[\alpha]_D^{20} = +31.2°$ (c = 0.96, $CHCl_3$), ee = 92%;

^{1}H NMR ($CDCl_3$), δ: 1.25 and 1.29 (two s, 6H, >C$(CH_3)_2$), 1.61–1.64 (two m, 2H, CH_2-6), 1.66 (s, 3H, CH_3-4), 2.12–2.35 (m, 5H, CH_2-2, CH_2-5 and OH), 2.69 (dd, J = 5.5 and 1.3 Hz, 1H, H-7), 3.62 (td, J = 6.3 and 1.0 Hz, 2H, CH_2–OH), 5.18–5.22 (m, 1H, H-3); ^{13}C NMR ($CDCl_3$), δ: 16.14, 18.73, 24.79, 27.15, 31.48, 36.65, 62.35, 64.26, 120.83, 137.66; IR (film, cm^{-1}): 3420(s), 2962(s), 2927(s), 1379(m).

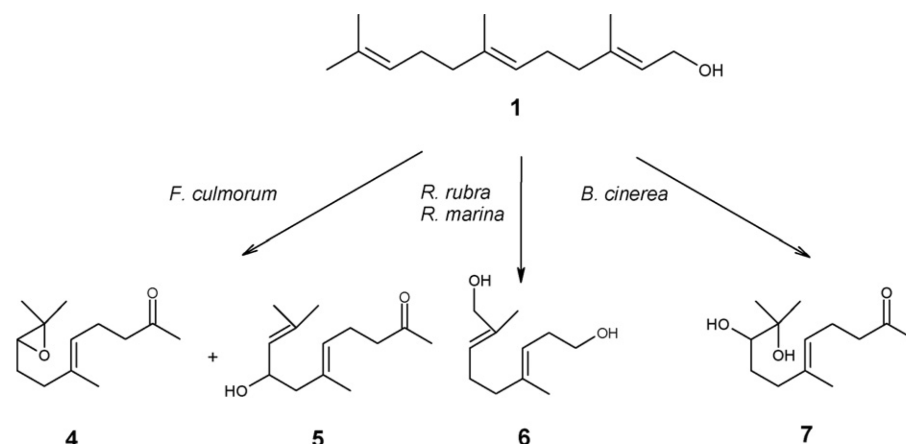
In the transformation of **(4)** with other strains: *R. rubra*, and *F. culmorum* the same product—9,10-dihydroxygeranylacetone **(7)** was obtained. In the experiment with *R. rubra* from 180 mg of 9,10-epoxygeranylacetone 64 mg (33% isolated yield) of product **(7)** were obtained ($[\alpha]_D^{20} = +13.1°$ (c = 2.2, $CHCl_3$), ee = 18%) while in the transformation with *B. cinerea* we obtained 62 mg of product **(7)** (32% isolated yield, $[\alpha]_D^{20} = +25.2°$ (c = 0.9, $CHCl_3$), ee = 24%). In the experiment with *F. culmorum* we obtained 65 mg of the same product (33% isolated yield, $[\alpha]_D^{20} = +1.6°$ (c = 2.5, $CHCl_3$), ee = 2%).

3. Results and discussion

The screening of 20 fungal strains led to the selection of four microorganisms: *F. culmorum*, *B. cinerea*, *R. rubra* and *R. marina*, that have ability to the biotransformation of farnesol **(1)**, its epoxide **(2)**, geranylacetone **(3)** and 9,10-epoxygeranylacetone **(4)**.

In the literature the biotransformation of a mixture of four farnesol isomers by *Rhodococcus rubropertinctus* (DSM 43197) was described. Two products with the carbon chain reduced by two carbon atoms were obtained [22]. Nankai et al. investigated the transformation (2*Z*, 6*Z*)-farnesol using the plant pathogenic fungus *Glomerella cingulata* as a biocatalyst. They observed the oxidation of the terminal double bond, isomerization of 2,3-double bond and further degradation [23,24].

In our research, first substrate—farnesol **(1)** was converted by *F. culmorum*, *B. cinerea*, *R. rubra* and *R. marina* to the oxyderivatives of geranylacetone (Scheme 1). The progress of biotransformation of farnesol **(1)** with these strains is shown in Fig. 1. The biotransformation of farnesol with *F. culmorum* proceeded very fast. Two days of incubation gave the mixture of products containing 2% of substrate, 40% of (+)-9,10-epoxygeranylacetone **(4)** and 58% of (−)-8-hydroxygeranylacetone **(5)**. After that time in the reaction mixture the substrate was not observed. The composition of the product mixture did not change during the next 4 days of transformation. *R. rubra* gave only one product—diol **(6)** (98%) after 2 days incubation whereas in experiment with *R. marina* after 4 days incubation 51% of this product were obtained. So the transformation of farnesol with *R. marina* proceeded



Scheme 1. Biotransformation of farnesol (**1**).

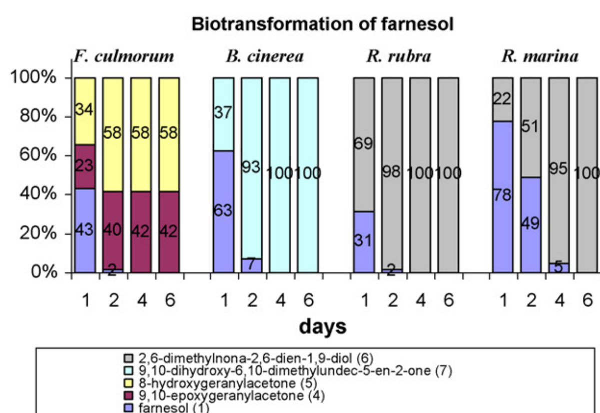


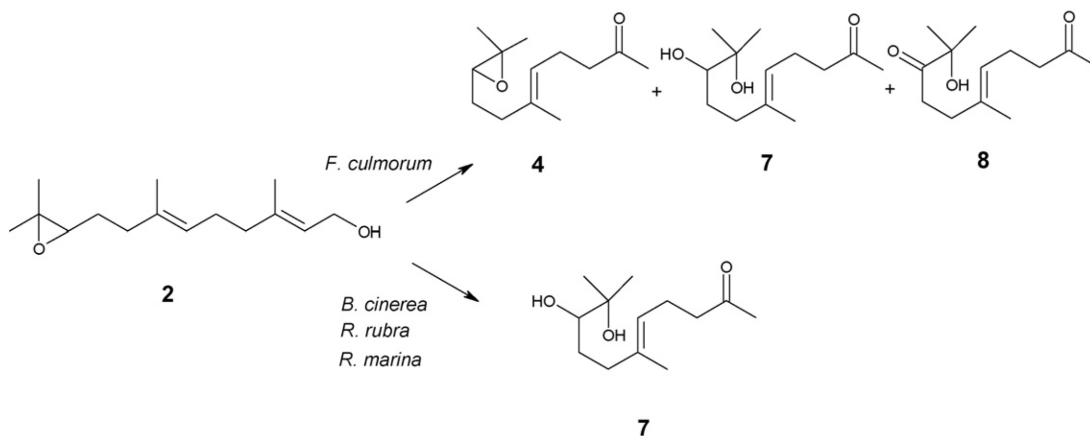
Fig. 1. The composition (in % according to GC) of product mixtures.

slower and in the result gave smaller amount of product (**6**). The last strain *B. cinerea* converted substrate (**1**) very fast giving 93% of 9,10-dihydroxygeranylacetone (**7**) after 2 days incubation.

Suzuki et al. studied the transformation of racemic epoxyfarnesol by *Helminthosporium sativum*. They obtained three products. The results let the authors postulate the mechanisms of this bioconversion with the opening of the epoxy ring as the first step of this process. The product of this opening is (−)-10,11-dihydroxyfarnesol (12.4%). Then the hydroxy group in allylic position was oxidized giving (−)-10,11-dihydroxyfarnesic acid and finally the (−)-9,10-dihydroxygeranylacetone (6.6%) [21].

In our experiments the process of biotransformation of epoxyfarnesol proceeded in a different way. After 4 days of incubation with *F. culmorum* (Scheme 2) we obtained three products: 45% (−)-9,10-epoxygeranylacetone (**4**), 23% (−)-9,10-dihydroxygeranylacetone (**7**) and 32% 10-hydroxy-6,10-dimethyl-undec-5-en-2,9-dione (**8**). The progress of biotransformation of farnesol (**1**) is illustrated in Fig. 2. The analysis of composition of products mixture indicates that at first shortering of carbon chain takes place and epoxygeranylacetone is formed. Then the oxirane ring is opened and one of the hydroxy group is oxidized. The strains *B. cinerea*, *R. rubra* and *R. marina* gave only one product (**7**) and after 4 days we did not observed the substrate in the mixtures. No product of cyclization was observed.

The biotransformation of geranylacetone was described by Miyazawa at al. [25]. In their studies geranylacetone was transformed to (*E*)-10-hydroxy-6,10-dimethylundec-5-en-2-one as the major metabolite. (*E*)-9,10-Dihydroxy-6,10-dimethylundec-5-en-2-one, (*E*)-6,10-dimethylundeca-5,9-dien-2-ol (*E*)-6,10-dimethylundec-5-en-2,9,10-triol and (*E*)-6,10-dimethyl-



Scheme 2. Biotransformation of 10,11-epoxyfarnesol (**2**).

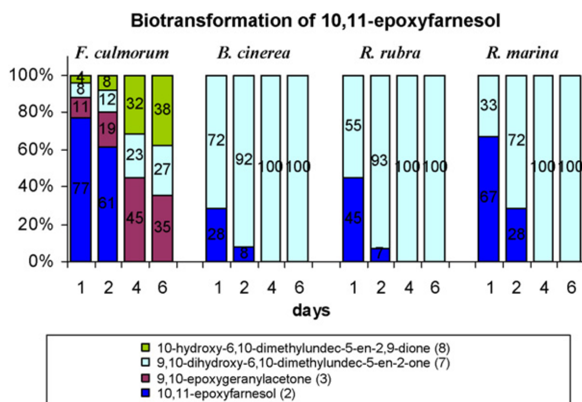


Fig. 2. The composition (in % according to GC) of product mixtures.

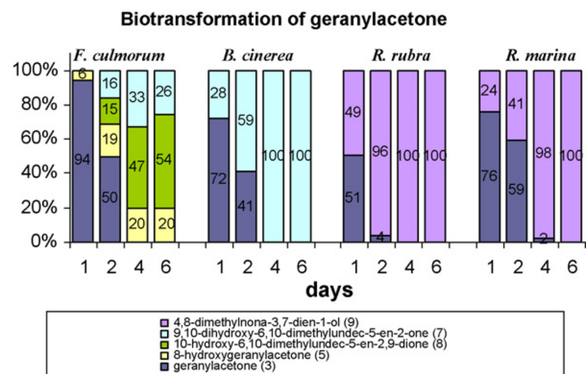


Fig. 3. The composition (in % according to GC) of product mixtures.

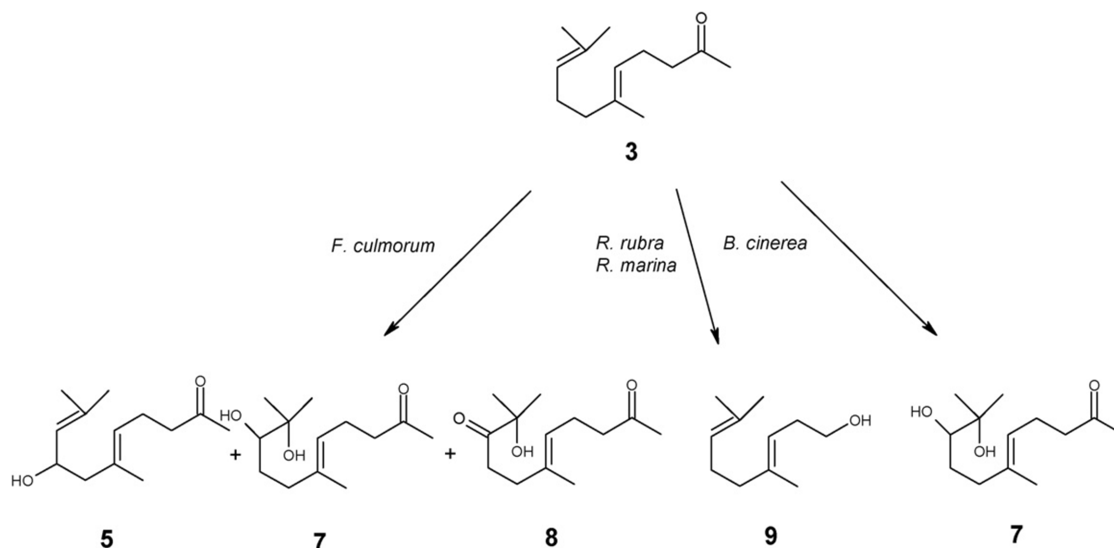
undec-5-en-2,10-diol were also identified in product mixture.

In our biotransformation the strain *F. culmorum* converted geranylacetone to three products (Scheme 3). After 2 days of biotransformation the level of conversion reached 50%. On the 4th day only products were detected in the mixture (Fig. 1). Analyzing the progress of biotransformation one can see that the dihydroxygeranylacetone **(7)** was oxidized to product **(8)**. *R. rubra* and *R. marina* gave only one product—alcohol **(9)**. The content of the substrate decreased to 4% after 2 days of biotransformation with the first strain. In the case of *R. marina* after 2 days the conversion of substrate to alcohol **(9)** reached about 40% and during next days of process it increased to 100%. So the geranylacetone in the culture of *R. rubra* was transformed faster. Formation of homogeraniol **(9)** was very interesting from the practical point of view because this compound was characterized by very intensive, fruity odour with well-marked citrus note (Fig. 3). The transformation of geranylacetone **(3)** with *B. cinerea* afforded compound **(7)** after 4 days and it was the only product detected.

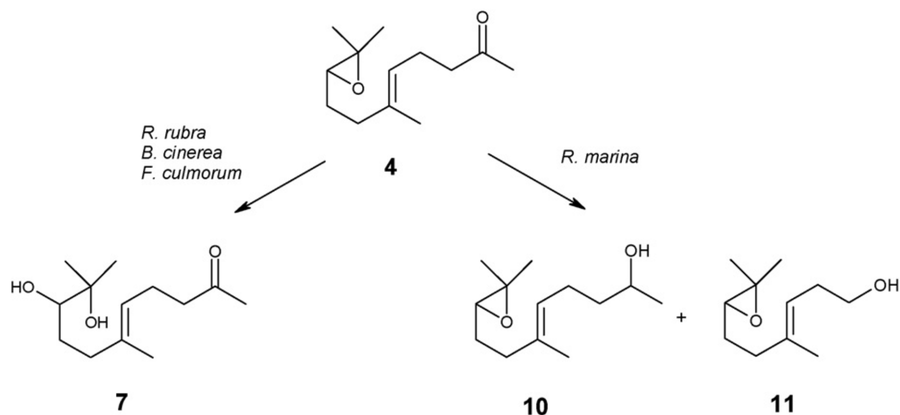
The last substrate, 9,10-epoxygeranylacetone **(4)**, was transformed by *R. marina* into (±)-9,10-epoxy-6,10-dimethylundec-5-en-2-ol **(10)** and (+)-7,8-epoxy-4,8-dimethylnona-3-en-1-ol **(11)**. After 2 days of the process the composition of the products mixture remained constant. *R. rubra*, *B. cinerea* and *F. culmorum* gave the same product 9,10-dihydroxygeranylacetone with the predominance of enantiomer (+) **(7)** (Scheme 4). The most effective strain was *B. cinerea* which converted the substrate in 90% after 2 days incubation (Fig. 4).

The results of biotransformation of compound **(1)**, **(2)**, **(3)** and **(4)** allowed us to propose their biodegradation pathways in the culture of fungal strains studied. The biodegradation pathway of farnesol and epoxyfarnesol with these strains is described in Scheme 5.

In the preparative biotransformation of farnesol **(1)** with *F. culmorum* we observed the degradation of carbon chain. This type of degradation was described for example in biotransformation of geraniol and geranial performed by the bacterial strain *Pseudomonas incognita* [26,27]. The authors proposed two pathways for the degradation of geraniol. First pathway leads to 6-methyl-5-hepten-2-one (MHO) while in the second pathway β-keto acid was formed. The first pathway of biodegradation



Scheme 3. Biotransformation of geranylacetone **(3)**.



Scheme 4. Biotransformation of 9,10-epoxygeranylacetone **(4)**.

was also observed by Demyttenaere and De Pooter [28] Demyttenaere and De Kimpe [29] in the transformation of geraniol, geranial, nerol and neral by *P. digitatum*.

It looks that similarly to geraniol, the first step of biodegradation of farnesol was the oxidation of the 2,3-double bond to epoxide **(A)** and the subsequent hydrolysis to triol **(B)** which was further oxidized to ketodiol **(C)**. The ketodiol was then converted into geranylacetone **(3)** by an oxidative process. Next step, was the enantio (ee = 92%) and regioselective hydroxylation at C-8. The same product (with ee = 42%) was obtained in biotransformation of geranylacetone with *F. culmorum* (Scheme 3). Geranylaceton in the culture of *F. culmorum* was also oxidized to the 9,10-epoxygeranylacetone. The enantio (ee = 94%) and regioselective epoxidation of terminal double bond in geranylacetone **(3)** had place The opening of the epoxy ring in 9,10-epoxygeranylacetone **(4)**, leading to the 9,10-dihydroxy-6,10-dimethylundec-5-en-2-one **(7)** and oxidation of the hydroxy group at C-9 to the carbonyl one, were the final steps. The last two compounds were also obtained by biotransformation of geranylacetone with *F. culmorum*. The biotransformation of epoxyfarnesol **(2)** and epoxygeranylacetone **(4)** gave also product **(7)**. So it should be noticed that *F. culmorum* transforms farnesol via the epoxidation of C2–C3 double bond and then the terminal double bond of geranylacetone is epoxidized. The opening of the epoxide **(4)** and oxidation of diol **(7)** afford the final product of transformation.

Yeast strain *R. rubra* also showed the activity in the oxidative biodegradation of the studied substrate. Farnesol was transformed by this microorganism to hydroxyhomogeraniol **(6)** (Scheme 1). The same product was observed by Müller et al. in biotransformation of geranylacetone with utilization other microorganisms: *Trichoderma koningii* (DSM 85068) and *Mucor griseocyanus* (DSM 1173) [30]. However we did not observed this compound in the product mixture obtained from biotransformation of geranylacetone with *R. rubra*. This result allow us to postulate the alternative pathway of formation of compound **(6)**. In the first step farnesol was hydroxylated to diol (*E*), like in biotransformation of farnesol with *Aspergillus niger* (DSM 63263) [22]. Then diol was oxidized via Baeyer–Villiger reaction to ester **(J)** which was subsequently hydrolyzed to hydroxyhomogeraniol **(6)**.

The biotransformation of 10,11-epoxyfarnesol **(2)** and epoxygeranylacetone **(4)** by *R. rubra* led to the same final product (+)-9,10-dihydroxygeranylacetone **(7)** which was a result of hydrolysis of oxirane ring.

The second yeast strain—*R. marina* transformed farnesol **(1)** and geranylacetone **(3)** in the same way as the *R. rubra* to the diol **(6)** and homogeraniol **(9)**. 10,11-Epoxyfarnesol **(2)** was converted by this microorganism into the epoxygeranylacetone **(4)** and then the epoxy ring was opened to diol **(7)**. However, the biotransformation of epoxygeranylacetone **(4)** with this strain afforded a mixture of two products. The first one was the result of enantioselective reduction of carbonyl group and the second one epoxyalcohol **(11)** was the result of the Baeyer–Villiger oxidation of compound **(4)** and further hydrolysis of acetate. These results showed that *R. marina* has redox activity towards epoxygeranylacetone. The compound **(11)** was obtained previously only as the product of chemical synthesis [19] (Scheme 5).

The transformation of farnesol, epoxyfarnesol, as well as geranylacetone and its epoxide with the last fungal strain *B. cinerea* (Scheme 5) afforded the same product 9,10-dihydroxy-6,10-dimethylundec-5-en-2-one **(7)**—regardless on the initial substrate. The structures of this product allow suggesting that the

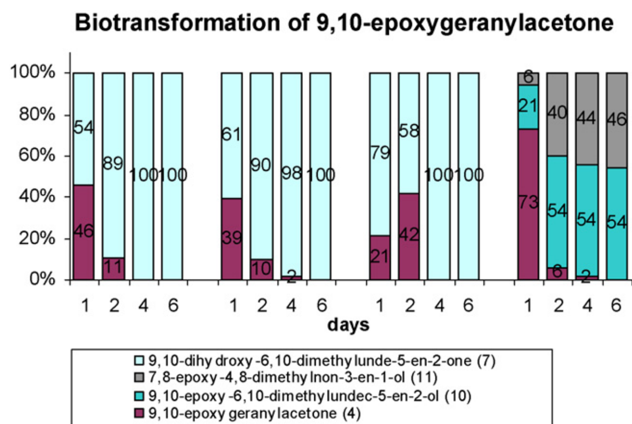
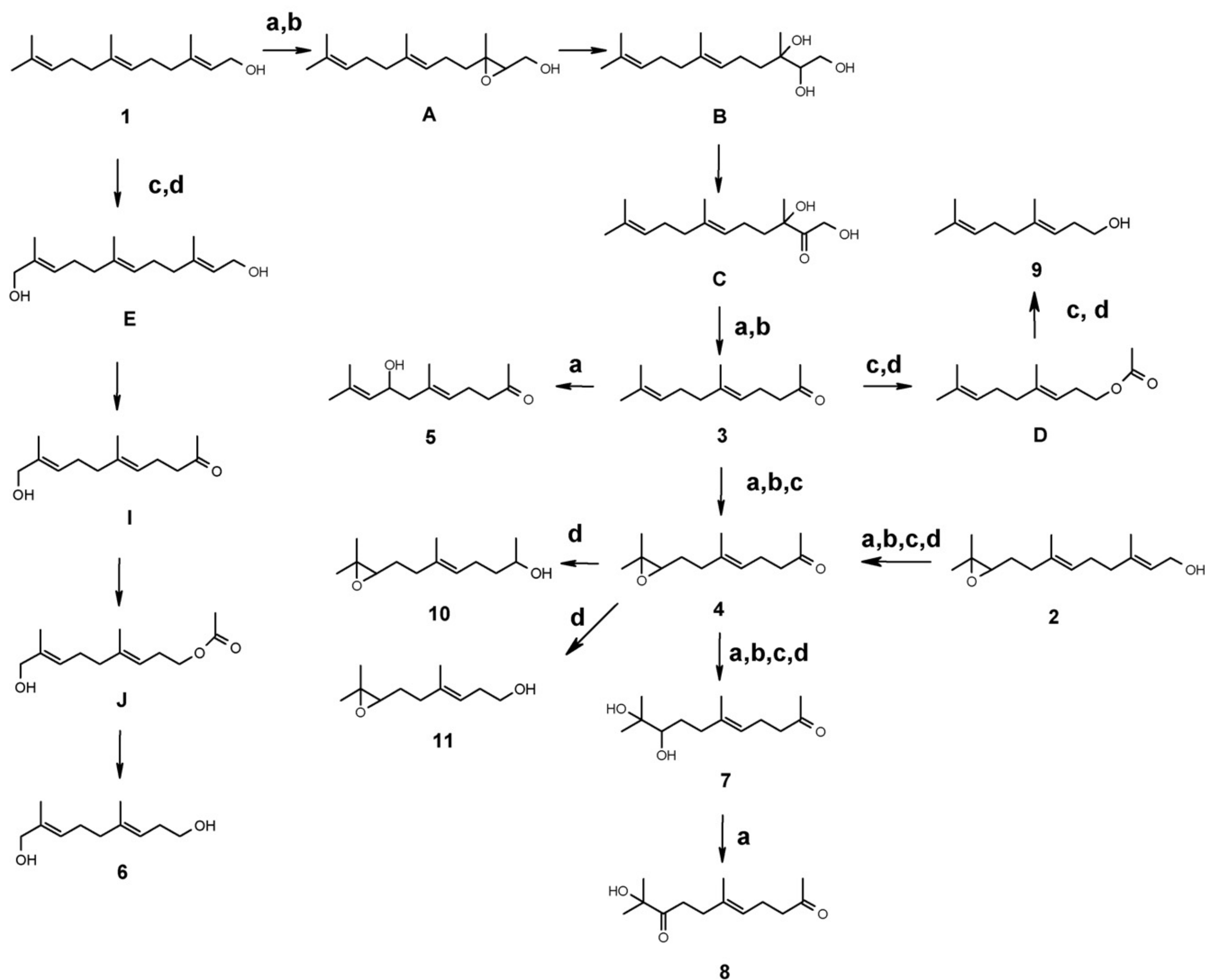


Fig. 4. The composition (in % according to GC) of product mixtures.



Scheme 5. Biodegradation pathway of farnesol **(1)** and 10,11-epoxyfarnesol **(2)** by *Fusarium culmorum* **(a)**, *Botrytis cinerea* **(b)**, *Rhodotorula rubra* **(c)**, *Rhodotorula marina* **(d)**.

transformation of the first two substrates proceeds via epoxygeranylacetone.

4. Conclusions

To sum up the results presented above the following can be pointed out:

(1) In the process of biotransformation of farnesol **(1)**, 10,11-epoxyfarnesol **(2)**, geranylacetone **(3)** and 9,10-epoxygeranylacetone **(4)** with selected strains of fungi *F. culmorum* and *B. cinerea* and yeast *R. rubra* and *R. marina* oxyderivatives of geranylacetone are formed.
(2) The strains *R. rubra* and *R. marina* have ability to the regioselective Baeyer–Villiger oxidation of geranylacetone.
(3) *F. culmorum* and *R. marina* transforme farnesol and epoxygeranylacetone with high enantioselectivity (94% and 92%, respectively).
(4) The strains *F. culmorum, B. cinerea*, *R. rubra* and *R. marina* do not have the ability to cyclization of 10,11-epoxyfarnesol **(2)** and 9,10-epoxygeranylacetone **(4)**.

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