

Enzymatic Acyloin Condensation of Acyclic Aldehydes*

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Corynespora cassiicola DSM 62474 and *Diplodia gossypina* ATCC 10936 were found to cleave some acyclic terminal terpendiols and prolongate them later by addition of a C₂-unit to afford 1,2-dihydroxy-propyl-compounds. The enzymes involved in this reaction from both microorganisms displayed a strong stereoselectivity which is completely different in both strains. The stereochemical requirements of the substrates for both strains and the mechanism were elucidated by using closely related substrates. The absolute configuration of the diols were solved by correlation with compounds of known absolute configuration.

Introduction

In 1921 Neuberg and Hirsch [1] found that fermenting baker's yeast prolongates benzaldehyde to (1*R*,2*S*)-1-phenyl-1,2-propandiol. This reaction was later intensively used for the synthesis of natural products with different aldehydes by Fuganti and co-workers [2]. In 1984 we reported on the acyloin condensation of citral [3] and activated acetate by strains of the genus *Mucor*. To date such a reaction has not been found for saturated aldehydes.

In a screen for strains capable of forming diols out of the 1-isobutenyl moiety of acyclic terpenoids [4] we found two organisms producing compounds with the 1,2-propandiol moiety. In this publication we will report on the substrate specificity of the strains, the configuration of these diols, and the mechanism of their formation.

Experimental

The fungi were precultivated for 72 h at 27 °C and 100 r.p.m. in 100 ml EM flasks containing 20 ml of the following medium: 1% of glucose, 1% of universalpeptone (Merck), 2% of malt extract and 0.3% of yeast extract. Then the substrate was added aseptically. After 16, 48, and 72 h samples were taken and analyzed as follows: To 1 ml of culture broth 0.2 ml ethylacetate was added and shaken for 2 min prior to

centrifugation. 10 µl of the extract were developed on HPTLC plates with dichloromethane-acetone 7:3 [5]. The spots were detected by spraying with anisaldehyde-sulfuric acid in acetic acid and heating to 110 °C for 1 min. For biotransformation in preparative scale the microorganisms were precultivated as described above. After 72 h the cultures were passed into five 2 liter flasks filled with 400 ml of sterilized medium and incubated for another period of 48 h. After this time the substrate was added and samples were taken using the procedure described above.

Extraction and purification: Culture medium and mycelia were separated by filtration and both extracted three times with ethyl acetate. The solvent was evaporated and the crude extract separated on Si-60 columns with a *n*-hexane/ethyl acetate gradient (changing from 19:1 to 1:1). When necessary the collected fractions were further purified by preparative TLC.

Instruments used: NMR: The ¹H NMR spectra were obtained at 400 MHz on a Bruker WM 400 spectrometer and the ¹³C NMR spectra at 75.5 MHz on a Bruker AM 300 spectrometer. If not stated otherwise CDCl₃ was used as solvent and TMS as internal standard. IR: spectra were measured on a IR Spectral-Photometer 297, Perkin Elmer, in chloroform. Mass spectra were recorded on a AEI 902S mass spectrometer with 70 eV. Optical rotation: Perkin-Elmer Polarimeter 241, in chloroform.

Biotransformation of 1.8 g myrcene **1** with *Diplodia gossypina* ATCC 10936 yielded after 24 h 600 mg **2**, 40 mg **3**, and 9 mg **4**. No erythro-nordiol was detected (amount ≤ 1 mg).

Threo-8-normyrcen-6,7-diol (2*S*,3*S*-6-methyliden-7-octen-2,3-diol) (**3**): Colorless oil, ¹H NMR: dd 5.26

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($J = 17.6, 0.9$ Hz) (1-H), dq 5.08 ($J = 10.8, 1$ Hz) (1'-H), ddd 6.37 ($J = 17.6, 10.8, 0.5$ Hz) (2-H), dddd 2.46 ($J = 16, 9.9, 5.3, 1.3$ Hz) (4-H), dddd 2.29 ($J = 16, 9.8, 6.4, 1.1$ Hz) (4'-H), dddd 1.69 ($J = 13.8, 9.9, 6.4, 3.3$ Hz) (5-H), dddd 1.59 ($J = 13.8, 9.8, 9.4, 5.3$ Hz) (5'-H), ddd 3.36 ($J = 9.4, 6.3, 3.3$ Hz) (6-H), dq 3.61 ($J = 6.3, 6.3$ Hz) (7-H), d 1.19 ($J = 6.3$) (8-H), d 5.03 ($J = 0.5$ Hz) (10-H), t 5.02 ($J = 1.4$ Hz) (10'-H).

$$\alpha = \frac{589\text{nm } 578\text{nm } 546\text{nm } 436\text{nm } 365\text{nm}}{-15.3^\circ - 16.1^\circ - 18.0^\circ - 30.2^\circ - 44.3^\circ} (c = 1.00).$$

20 mg of **3** was solved in 1 ml dry acetone and 1 mg *p*-toluenesulfonic acid was added. The solution was allowed to stand for 2 h, then sodiumhydrogen-carbonate was added, the solvent evaporated, the residue washed with water, dried and chromatographed giving 20 mg of the 1,3-dioxolane of **3**. ^1H NMR: dd 5.28 ($J = 17, 1$ Hz) (1-H), dd 5.09 ($J = 11, 1$ Hz) (1'-H), dd 6.40 ($J = 17, 11$ Hz) (2-H), dddd 2.46 ($J = 16, 10, 5, 1$ Hz) (4-H), dddd 2.28 ($J = 16, 10, 6, 1$ Hz) (4'-H), m 1.72 (5-H), ddd 3.58 ($J = 9, 8$ Hz) (6-H), dq 3.76 ($J = 8, 6$ Hz) (7-H), d 1.27 ($J = 6$ Hz) (8-H), s 5.06 (10-H), s 5.03 (10'-H), s 1.42 and s 1.40 ($=\text{C}(\text{CH}_3)_2$).

Biotransformation of 1.8 g *cis*-nerolidol **15** with *Diplodia gossypina* ATCC 10936 or *Corynespora cassicola* DSM 62474 yielded after 24 h no nordiols in amounts exceeding 2 mg.

Biotransformation of 375 mg **16** with *Diplodia gossypina* ATCC 10936 yielded after 116 h 100 mg **16**, 45 mg **19**, 20 mg **17**, 20 mg **18**, and 14 mg 4,8-dimethyl-8-hydroxy-4Z,9-decadienic acid.

Threo-10,11-dihydroxy-*cis*-12-nornerolidol (**17**): Epimeric at C-3, ^1H NMR: d 5.04 + 5.05 ($J = 10$ Hz) (1-H), d 5.21 ($J = 17$ Hz) (1'-H), dd 5.98 + 5.91 ($J = 17, 10$ Hz) (2-H), m 1.4–1.6 (4-H, 9-H), m 2.1 (5-H), t 5.18 ($J = 7$ Hz) (6-H), m 2.25 (8-H), m 2.1 (8'-H), ddd 3.28 ($J = 9, 7, 3$ Hz) (10-H), dq 3.60 ($J = 7, 7$ Hz) (11-H), d 1.18 ($J = 7$ Hz) (12-H), s(br) 1.68 (14-H), s 1.28 (15-H). MS (m/e): 224.1773 (M^+)(224.1776 calc. for $\text{C}_{14}\text{H}_{24}\text{O}_2$).

Erythro-10,11-dihydroxy-*cis*-12-nornerolidol (**18**): ^1H NMR: d 5.04 ($J = 10$ Hz) (1-H), d 5.20 ($J = 17$ Hz) (1'-H), dd 5.90 ($J = 17, 10$ Hz) (2-H), m 1.5 (4-H), m 2.08 (5-H), t 5.18 ($J = 7$ Hz) (6-H), m 2.2 (8-H), m 1.51 (9-H), ddd 3.54 ($J = 10, 4, 4$ Hz) (10-H), qd 3.77 ($J = 7, 4$ Hz) (11-H), d 1.14 ($J = 7$ Hz) (12-H), s(br) 1.68 (14-H), s 1.28 (15-H).

MS (m/e): 224.1769 (M^+)(224.1776 calc. for $\text{C}_{14}\text{H}_{24}\text{O}_2$).

$$\alpha = \frac{589\text{nm } 578\text{nm } 546\text{nm } 436\text{nm}}{+5.2^\circ + 5.4^\circ + 6.2^\circ + 9.9^\circ} (c = 1.00).$$

Biotransformation of 4.5 g *S*-citronellene **25** with *Diplodia gossypina* ATCC 10936 yielded after 24 h 1160 mg **26**, 200 mg **27**, 80 mg **28**, and 20 mg **29**.

3*S*,6*S*,7*S*-*Threo*-6,7-dihydroxy-8-norcitronellene (**27**): Colorless oil, ^1H NMR: dd 4.94 ($J = 17, 1$ Hz) (1-H), dd 4.90 ($J = 10.2, 1$ Hz) (1'-H), ddd 5.66 ($J = 17, 10.2, 7.5$ Hz) (2-H), m 2.10 (3-H), m 1.52 (4-H), m 1.35 (4'-H), m 1.52 (5-H), m 1.35 (5'-H), m 3.30 (6-H), dq 3.57 ($J = 6.3, 6.3$ Hz) (7-H), d 1.19 ($J = 6.3$ Hz) (8-H), d 1.00 ($J = 6.5$ Hz) (10-H). MS (m/e): 158.1308 (M^+)(158.1307 calc. for $\text{C}_9\text{H}_{18}\text{O}_2$).

$$\alpha = \frac{589\text{nm } 578\text{nm } 546\text{nm } 436\text{nm } 365\text{nm}}{-9.9^\circ - 10.3^\circ - 11.5^\circ - 18.1^\circ - 26.0^\circ} (c = 1.00).$$

3*S*,6*S*,7*R*-*Erythro*-6,7-dihydroxy-8-norcitronellene (**28**): ^1H NMR: d 4.89 ($J = 10$ Hz) (1-H), d 4.94 ($J = 17$ Hz), (1'-H), ddd 5.64 ($J = 17, 10, 8$ Hz) (2-H), m 2.12 (3-H), m 1.5–1.3 (4- and 5-H), m 3.57 (6-H), m 3.76 (7-H), d 1.14 ($J = 7$ Hz), (8-H), d 1.00 ($J = 7$ Hz) (10-H).

$$\alpha = \frac{589\text{nm } 578\text{nm } 546\text{nm } 436\text{nm}}{-2.3^\circ - 2.4^\circ - 2.7^\circ - 3.9^\circ} (c = 1.00).$$

Biotransformation of 1.45 g **38** with *Diplodia gossypina* ATCC 10936 yielded after 156 h 22 mg **38**, 185 mg **39**, 80 mg **40**, 32 mg **41**, and 5 mg **53**.

3*R*,6*S*-6-hydroxy-7-oxo-8-norcitronellene (**53**): ^1H NMR: ddd 4.97 ($J = 17.2, 1.8, 1.2$ Hz) (1-H), ddd 4.94 ($J = 10.5, 1.8, 1$ Hz) (1'-H), ddd 5.68 ($J = 17.2, 10.5, 7.8$ Hz) (2-H), m 2.12 (3-H), m 1.4 (4-H and 5-H), m 1.87 (5'-H), m 4.17 (6-H), s 2.20 (8-H), d 1.01 ($J = 6.7$ Hz) (10-H). In C_6D_6 : dddd 1.54 ($J = 13, 11, 6, 3.7$ Hz) (5-H), dddd 1.13 ($J = 13, 10, 7.7, 5.7$ Hz) (5'-H), dd 3.71 ($J = 7.7, 3.7$ Hz) (6-H), s 1.50 (8-H), d 0.87 ($J = 6.7$ Hz) (10-H).

$$\alpha = \frac{589\text{nm } 578\text{nm } 546\text{nm } 436\text{nm}}{+7.7^\circ + 8.1^\circ + 9.9^\circ + 35.4^\circ} (c = 0.34).$$

Biotransformation of 1.8 g of a 1:1 mixture of 2,6-dimethyl-1,5-heptadiene **30** and 2,6-dimethyl-2,5-heptadiene **48** with *Diplodia gossypina* ATCC 10936 yielded after 24 h 210 mg **31**, 60 mg **32**, 50 mg 2,2-dimethyl-3-hydroxy-5-(2'-hydroxy-2'-propyl)-tetrahydrofuran, and 30 mg 3*S*-2,6-dimethyl-5-hepten-2,3-diol. The erythro-nordiol was not detected (amount ≤ 1 mg).

Threo-2-methyl-hepten-5,6-diol (32): ^1H NMR: s(br) 4.78 (1-H), s(br) 4.76 (1'-H), m 2.24 (3-H), m 2.14 (3'-H), m 1.66 (4-H), m 1.56 (4'-H), m 3.38 (5-H), dq 3.65 ($J=7, 7$ Hz) (6-H), d 1.22 ($J=7$ Hz) (7-H), s(br) 1.76 (9-H).

32 reacted with acidic acetone to the 1,3-dioxolane: ^1H NMR: s(br) 4.74 (1-H), s(br) 4.71 (1'-H), m 2.22 (3-H), m 2.08 (3'-H), m 1.68 (4-H), m 3.55 (5-H), dq 3.75 ($J=8, 6$ Hz) (6-H), d 1.27 ($J=6$ Hz) (7-H), s(br) 1.74 (9-H), s 1.40 ($=\text{C}(\text{CH}_3)_2$). $\alpha_{\text{D}} -2.2^\circ$ ($c=0.22$).

Biotransformation of 1.8 g nerylacetone **33** with *Diplodia gossypina* ATCC 10936 yielded after 24 h 4 mg **33**, 1000 mg **34**, 110 mg alcohol of **34**, and 12 mg **35**.

Threo-9,10-dihydroxy-11-nornerylacetone (35): ^1H NMR: s 2.14 (1-H), t 2.50 ($J=7$ Hz) (3-H), m 2.26 (4-H), t 5.07 ($J=7$ Hz) (5-H), m 2.15 (7-H), m 2.36 (7'-H), m 1.60 (8-H), m 1.46 (8'-H), ddd 3.30 ($J=9, 7, 3$ Hz) (9-H), dq 3.62 ($J=7, 7$ Hz) (10-H), d 1.20 ($J=7$ Hz) (11-H), s(br) 1.67 (13-H). MS (m/e): 214.1565 (M^+)(214.1569 calc. for $\text{C}_{12}\text{H}_{22}\text{O}_3$).

$$\alpha = \frac{589 \text{ nm}}{-3.3^\circ} \frac{578 \text{ nm}}{-3.7^\circ} \frac{546 \text{ nm}}{-4.3^\circ} \frac{436 \text{ nm}}{-7.6^\circ} \quad (c=0.80).$$

Biotransformation of 1.2 g **34** with *Diplodia gossypina* ATCC 10936 yielded after 120 h 15 mg **35** and 13 mg **36**.

Erythro-9,10-dihydroxy-11-nornerylacetone (36): ^1H NMR: s 2.14 (1-H), t 2.49 ($J=7$ Hz) (3-H), m 2.19 (4-H), t 5.08 ($J=7$ Hz) (5-H), m 2.30 (7-H), m 1.51 (8-H), dt 3.56 ($J=10, 4$ Hz) (9-H), qd 3.79 ($J=7, 4$ Hz) (10-H), d 1.16 ($J=7$ Hz) (11-H), s(br) 1.67 (13-H).

$$\alpha = \frac{589 \text{ nm}}{+5.8^\circ} \frac{578 \text{ nm}}{+6.8^\circ} \frac{546 \text{ nm}}{+7.4^\circ} \frac{436 \text{ nm}}{+12.4^\circ} \quad (c=0.50).$$

Biotransformation of 1.8 g *R*-citronellene **37** with *Diplodia gossypina* ATCC 10936 yielded after 24 h 375 mg **38**, 30 mg **39**, and 12 mg **40**.

3R,6S,7S-Threo-6,7-dihydroxy-8-norcitronellene (39): ^1H NMR: d(br) 4.93 ($J=17$ Hz) (1-H), d(br) 4.90 ($J=10$ Hz) (1'-H), ddd 5.62 ($J=17, 10, 8$ Hz) (2-H), m 2.12 (3-H), m 1.39 (4-H), m 1.47 (5-H), m 3.30 (6-H), dq 3.56 ($J=7, 7$ Hz) (7-H), d 1.18 ($J=7$ Hz) (8-H), d 1.01 ($J=7$ Hz) (10-H). MS (m/e): 158.1309 (M^+)(158.1307 calc. for $\text{C}_9\text{H}_{18}\text{O}_2$).

$$\alpha = \frac{589 \text{ nm}}{+6.6^\circ} \frac{578 \text{ nm}}{+6.9^\circ} \frac{546 \text{ nm}}{+7.7^\circ} \frac{436 \text{ nm}}{+12.6^\circ} \frac{365 \text{ nm}}{+18.9^\circ} \quad (c=1.00).$$

3R,6S,7S-Erythro-6,7-dihydroxy-8-norcitronellene (40): ^1H NMR: d(br) 4.95 ($J=17$ Hz) (1-H), d(br) 4.92 ($J=11$ Hz) (1'-H), ddd 5.67 ($J=17, 11, 8$ Hz) (2-H), m 2.13 (3-H), m 1.55–1.3 (4- and 5-H), m 3.60 (6-H), qd 3.80 ($J=7, 4$ Hz) (7-H), d 1.14 ($J=7$ Hz) (8-H), d 1.01 ($J=7$ Hz) (10-H). MS (m/e): 158.1308 (M^+)(158.1307 calc. for $\text{C}_9\text{H}_{18}\text{O}_2$).

Biotransformation of 1.9 g of racemic trans-nornerolidol **5** with *Corynespora cassicola* DSM 62474 yielded after 24 h 45 mg **5**, 1400 mg **6**, 40 mg **7**, 20 mg **9**, and 15 mg **8**, beside two other products not mentioned here.

Threo-10,11-dihydroxy-trans-12-nornerolidol (8): ^1H NMR: d 5.05 ($J=10$ Hz) (1-H), d 5.19 ($J=17$ Hz) (1'-H), dd 5.88 ($J=17, 10$ Hz) (2-H), m 1.6 (4-H), m 2.1 (5-H), t 5.18 ($J=7$ Hz) (6-H), m 2.1 (8-H), m 1.6 (9-H), ddd 3.30 ($J=9, 7, 4$ Hz) (10-H), dq 3.59 ($J=7, 7$ Hz) (11-H), d 1.19 ($J=7$ Hz) (12-H), s(br) 1.61 (14-H), s 1.28 (15-H). MS (m/e): 224.1769 (M^+)(224.1776 calc. for $\text{C}_{14}\text{H}_{24}\text{O}_2$).

8 reacted with acidic acetone to the 1,3-dioxolane: ^1H NMR: d 5.05 ($J=10$ Hz) (1-H), d 5.20 ($J=17$ Hz) (1'-H), dd 5.90 ($J=17, 10$ Hz) (2-H), m 1.6 (4-H), m 2.04 (5-H), t 5.16 ($J=7$ Hz) (6-H), m 2.15 (8-H), m 2.04 (8'-H), m 1.6 (9-H), ddd 3.49 ($J=7, 6, 6$ Hz) (10-H), dq 3.72 ($J=8, 6$ Hz) (11-H), d 1.25 ($J=6$ Hz) (12-H), s(br) 1.61 (14-H), s 1.29 (15-H), s 1.39 and s 1.38 ($=\text{C}(\text{CH}_3)_2$). Irradiation at 1.25 gave a NOE enhancement at 3.49 and 3.72.

Erythro-10,11-dihydroxy-trans-12-nornerolidol (9): ^1H NMR: d 5.04 ($J=10$ Hz) (1-H), d 5.19 ($J=17$ Hz) (1'-H), dd 5.87 ($J=17, 10$ Hz) (2-H), m 1.55 (4-H), m 2.04 (5-H), t 5.19 ($J=7$ Hz) (6-H), m 2.16 (8-H), m 2.05 (8'-H), m 1.55 (9-H), ddd 3.57 ($J=8, 5, 5$ Hz) (10-H), dq 3.76 ($J=7, 5$ Hz) (11-H), d 1.15 ($J=7$ Hz) (12-H), s(br) 1.61 (14-H), s 1.28 (15-H). MS (m/e): 224.1773 (M^+)(224.1776 calc. for $\text{C}_{14}\text{H}_{24}\text{O}_2$).

9 reacted with acidic acetone to the 1,3-dioxolane: ^1H NMR: d 5.04 ($J=10$ Hz) (1-H), d 5.12 ($J=17$ Hz) (1'-H), dd 5.89 ($J=17, 10$ Hz) (2-H), m 1.6 (4-H), m 2.0 (5-H), t 5.16 ($J=7$ Hz) (6-H), m 2.16 (8-H), m 2.0 (8'-H), m 1.6 (9-H), ddd 3.98 ($J=10, 6, 6$ Hz) (10-H), dq 4.21 ($J=6, 6$ Hz) (11-H), d 1.15 ($J=6$ Hz) (12-H), s(br) 1.61 (14-H), s 1.28 (15-H), s 1.44 and s 1.33 ($=\text{C}(\text{CH}_3)_2$).

Biotransformation of 1.4 g of **6** with *Corynespora cassicola* DSM 62474 yielded after 120 h 144 mg **9**, 116 mg **8**, and 67 mg **7**.

Biotransformation of 1.9 g of a mixture of stereoisomers of farnesol **10** with *Corynespora cassicola* DSM 62474 yielded after 48 h 800 mg of the stereoisomers of **11**, 50 mg **14**, 50 mg **14a**, 25 mg **12**, 25 mg **12a**, 25 mg **13**, 25 mg **13a**, 17 mg 2*E*,6*Z*-10,11-epoxy-farnesol, and 15 mg 2*Z*,6*Z*-10,11-epoxy-farnesol.

Threo-10,11-dihydroxy-2*E*,6*E*-12-norfarnesol (**12**): Not completely free of **12a**, ¹H NMR: d 4.12 (*J* = 7 Hz) (1-H), t 5.34 (*J* = 7 Hz) (2-H), m 2.1 (4-H, 5-H, 8-H), t 5.11 (*J* = 7 Hz) (6-H), m 3.28 (10-H), m 3.56 (11-H), d 1.16 (*J* = 7 Hz) (12-H), s(br) 1.60 (14-H), s(br) 1.64 (15-H). $\alpha_D +0.8^\circ$ (*c* = 1.00).

12 reacted with acidic acetone to the 1,3-dioxolane: ¹H NMR: d 4.13 (*J* = 7 Hz) (1-H), t 5.38 (*J* = 7 Hz) (2-H), m 2.1 (4-H, 5-H, 8-H), t 5.12 (*J* = 7 Hz) (6-H), m 3.49 (10-H), m 3.70 (11-H), d 1.25 (*J* = 7 Hz) (12-H), s(br) 1.61 (14-H), s(br) 1.67 (15-H), s 1.39 and s 1.38 (=C(CH₃)₂).

Threo-10,11-dihydroxy-2*Z*,6*E*-12-norfarnesol (**12a**): Not completely free of **12**, ¹H NMR: d 4.08 (*J* = 7 Hz) (1-H), t 5.38 (*J* = 7 Hz) (2-H), m 2.1 (4-H, 5-H, 8-H), t 5.11 (*J* = 7 Hz) (6-H), m 3.30 (10-H), m 3.56 (11-H), d 1.16 (*J* = 7 Hz) (12-H), s(br) 1.60 (14-H), s(br) 1.72 (15-H).

Erythro-10,11-dihydroxy-2*E*,6*E*-12-norfarnesol (**13**): Not completely free of **13a**, ¹H NMR: d 4.12 (*J* = 7 Hz) (1-H), t 5.34 (*J* = 7 Hz) (2-H), m 2.1 (4-H, 5-H, 8-H), t 5.11 (*J* = 7 Hz) (6-H), m 3.56 (10-H), m 3.72 (11-H), d 1.13 (*J* = 6 Hz) (12-H), s(br) 1.60 (14-H), s(br) 1.64 (15-H).

13 reacted with acidic acetone to the 1,3-dioxolane: ¹H NMR: d 4.13 (*J* = 7 Hz) (1-H), t 5.38 (*J* = 7 Hz) (2-H), m 2.1 (4-H, 5-H, 8-H), t 5.12 (*J* = 7 Hz) (6-H), ddd 3.98 (*J* = 10, 5, 5 Hz) (10-H), dq 4.21 (*J* = 6, 6 Hz) (11-H), d 1.15 (*J* = 6 Hz) (12-H), s(br) 1.61 (14-H), s(br) 1.67 (15-H), s 1.44 and s 1.33 (=C(CH₃)₂). MS (*m/e*): 282.2193 (*M*⁺) (282.2195 calc. for C₁₇H₃₀O₃).

$$\alpha = \frac{589\text{nm } 578\text{nm } 546\text{nm } 436\text{nm } 365\text{nm}}{+3.5^\circ +3.7^\circ +4.4^\circ +5.2^\circ +6.0^\circ} (c = 0.26).$$

Erythro-10,11-dihydroxy-2*Z*,6*E*-12-norfarnesol (**13a**): Not completely free of **13**, ¹H NMR: d 4.09 (*J* = 7 Hz) (1-H), t 5.39 (*J* = 7 Hz) (2-H), m 2.1 (4-H, 5-H, 8-H), t 5.14 (*J* = 7 Hz) (6-H), m 3.56 (10-H), m 3.75 (11-H), d 1.14 (*J* = 7 Hz) (12-H), s(br) 1.60 (14-H), s(br) 1.72 (15-H).

13a reacted with acidic acetone to the 1,3-dioxolane: ¹H NMR: d 4.09 (*J* = 7 Hz) (1-H), t 5.42 (*J* =

7 Hz) (2-H), m 2.1 (4-H, 5-H, 8-H), t 5.13 (*J* = 7 Hz) (6-H), ddd 3.98 (*J* = 10, 5, 5 Hz) (10-H), dq 4.22 (*J* = 6, 6 Hz) (11-H), d 1.16 (*J* = 6 Hz) (12-H), s(br) 1.61 (14-H), s(br) 1.72 (15-H), s 1.44 and s 1.33 (=C(CH₃)₂).

Biotransformation of 500 mg of **16** with *Corynespora cassicola* DSM 62474 yielded after 48 h 347 mg **16**, 40 mg **17**, 31 mg **18**, and 12 mg **19**.

Biotransformation of 1.9 g of geranylacetone **20** with *Corynespora cassicola* DSM 62474 yielded after 24 h 42 mg **20**, 630 mg **21**, 240 mg alcohol of **21**, 60 mg **24**, 30 mg alcohol of **24**, 8 mg **22**, 8 mg **23**, 18 mg 7,11-dihydroxy-geranylacetone, 8 mg 7-hydroxy-geranylacetone, and nine other minor products.

Threo-9,10-dihydroxy-11-norgeranylacetol (**22**): ¹H NMR: d 1.20 (*J* = 7 Hz) (1-H), m 3.79 (2-H), m 2.1 (4-H), t 5.20 (*J* = 7 Hz) (5-H), m 2.1 (7-H), m 3.31 (9-H), dq 3.59 (*J* = 6 Hz) (10-H), d 1.20 (*J* = 6 Hz) (11-H), s(br) 1.64 (13-H).

22 reacted with acidic acetone to the corresponding 1,3-dioxolane: ¹H NMR: d 1.19 (*J* = 7 Hz) (1-H), ddq 3.80 (*J* = 7, 7, 7 Hz) (2-H), t 5.17 (*J* = 7 Hz) (5-H), ddd 3.49 (*J* = 10, 7, 6 Hz) (9-H), dq 3.71 (*J* = 7, 6 Hz) (10-H), d 1.25 (*J* = 6 Hz) (11-H), s(br) 1.63 (13-H), s 1.39 and s 1.38 (=C(CH₃)₂).

Erythro-9,10-dihydroxy-11-norgeranylacetol (**23**): Isolated only as 2,2-dimethyl-1,3-dioxolane: ¹H NMR: d 1.19 (*J* = 7 Hz) (1-H), m 3.80 (2-H), m 5.18 (5-H), m 3.99 (9-H), m 4.22 (10-H), d 1.15 (*J* = 6 Hz) (11-H), s(br) 1.63 (13-H), s 1.44 and s 1.33 (=C(CH₃)₂).

Results

After a screen of microorganisms capable of forming the diol by the attack of the 1-isobutenyl moiety of acyclic terpenoids two strains were found to produce additional norterpenes. The biotransformation of myrcene (**1**) with *Diplodia gossypina* ATCC 10936 afforded 6,7-dihydroxy-myrcene (**2**), 4-methyliden-5-hexenol-1 (**4**) and 8-nor-6,7-dihydroxy-myrcene (**3**). *Corynespora cassicola* DSM 62474 afforded also the corresponding nordiols **8** and **9** with *trans*-nerolidol (**5**) lacking here the 12-methyl group.

The configuration of the dihydroxy-norterpenoids was solved by reacting them with acetone and catalytic amounts of acid to form the 5-substituted 2,2,4-trimethyl-1,3-dioxolane. In these compounds the conformation of the asymmetric centers of the vicinal diol is fixed and NOE experiments could be per-

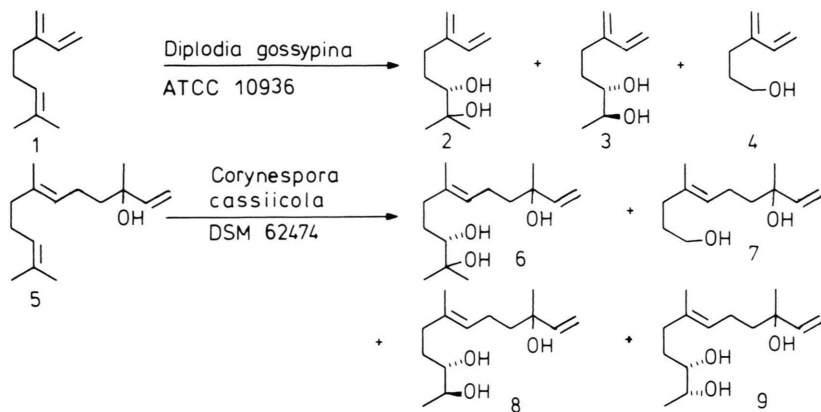


Fig. 1. Biotransformation of myrcene (**1**) with *Diplodia gossypina* ATCC 10936 and *trans*-nerolidol (**5**) with *Corynespora cassiicola* DSM 62474.

	R=				CH ₂ OH
	10	11	2E: 12 2Z: 12a	2E: 13 2Z: 13a	14
	15	16	17	18	19
	20 R'=O	21 R'=O	22 R'=H,OH	23 R'=H,OH	24 R'=O
	25	26	27	28	29
	30	31	32		
	33	34	35	36	
	37	38	39	40	41
	42	43	44	45	46
	47	48	49	50	

Fig. 2. Substrates and their biotransformation products.

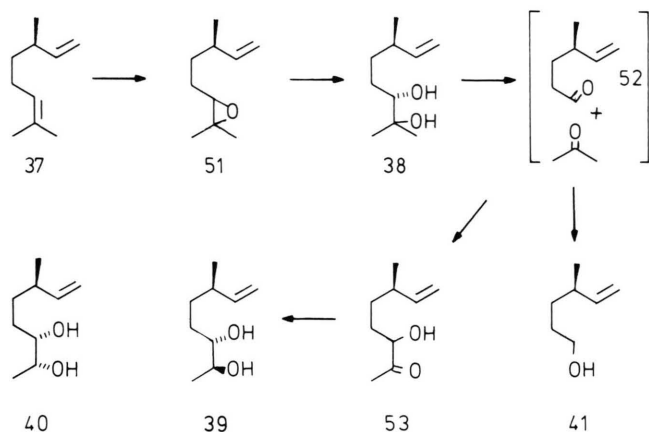


Fig. 3. Proposed reaction path to nordiols. Beside different substrate specificity this path differs in *Diplodia gossypina* ATCC 10936 and *Corynespora cassiicola* DSM 62474 only in the enantioselectivity of the last step.

formed. Irradiation at the 4-methyl group gave in the NOE experiment of the 1,3-dioxolane derivative of **3** an enhancement at 5-H so that the diol must have the threo-configuration. In the case of the 1,3-dioxolane of the nordiol (**9**) of *trans*-nerolidol an enhancement of the methylene-proton adjacent to C-5 was observed requiring the erythro-configuration of the diol. Threo- and erythrodiols can easily be discerned by their ^{13}C NMR resonances. The range of resonances of the methyl group adjacent to the diol part depends on the diol configuration. The range of resonances of these methyl groups is shifted to higher fields in the erythro-diols while the vicinal carbon is deshielded in relation to that one of the threo-diols (Table I).

These diols were further correlated with 2,3-diols of known absolute configuration so the absolute con-

figuration of the nordiols formed by *Diplodia gossypina* ATCC 10936 could be deduced. The threo-nordiol (**3**) gave $\alpha_D -15.3^\circ$ while 2*S*,3*S*-octan-2,3-diol [6] has $\alpha_D -18.5^\circ$. It is noteworthy that the absolute configuration of the diol **27** and **39** is independent from the configuration at C-3. This correlation was very uncertain with the nordiols formed by *Corynespora cassiicola* DSM 62474 because the strength of the optical rotation of these compounds were rather low. The data we obtained however pointed to the same absolute configuration as the nordiols of *Diplodia gossypina*.

Both strains revealed a pronounced substrate specificity. *Diplodia gossypina* ATCC 10936 afforded with myrcene **1**, *S*-citronellene (**25**), 2,6-dimethyl-1,5-heptadiene (**30**), nerylacetone (**33**), and *R*-citronellene (**37**) the norcompounds, whereas *trans*-

Table Ia. ^{13}C NMR data of **3**, **23**, **24**, **27**, **32**, **33** and **47** (75.5 MHz, CDCl_3 , TMS as internal standard).

	3	23	24	27	32	33	47
C-1	t 115.9 ^a	t 112.8	t 112.9	q 22.4	t 113.0	t 113.0	t 113.2
C-2	d 138.7	d 144.5	d 144.4	s 145.7	d 144.3	d 144.3	d 114.1
C-3	s 146.0	d 37.9	d 38.0	t 33.9	d 37.8	d 37.8	d 37.8
C-4	t 31.8	t 32.5	t 33.0	t 31.3	t 32.3	t 32.8	t 31.6
C-5	t 27.4	t 31.1	t 29.6	d 76.0	t 31.0	t 29.4	t 31.4
C-6	d 75.9	d 76.5	d 75.3	d 70.9	d 76.2	d 75.0	d 77.1
C-7	d 70.8	d 70.9	d 70.5	q 19.6	d 70.9	d 70.5	s 209.8
C-8	q 19.5	q 19.5	q 16.6	—	q 19.6	q 16.7	q 25.2
C-9	—	—	—	t 110.3	—	—	—
C-10	t 113.5 ^a	q 20.1	q 20.2	—	q 20.5	q 20.3	q 20.1

^a Assignments may be interchanged.

Table Ib. ^{13}C NMR data of **8**, **9**, **11**–**12a**, **15**, and **16** (75.5 MHz, CDCl_3 , TMS as internal standard).

	8	9	11	11a	12	12a	15^a	16^a
C-1	t 111.8	t 111.8	t 59.2	t 58.9	t 59.2	t 59.1	t 118.8/118.7	t 111.7
C-2	d 145.1	d 145.1	d 124.1	d 124.4	d 124.1	d 124.6	d 145.2/144.8	d 145.2/145.0
C-3	s 73.5	s 73.5	s 138.5	s 139.2	s 138.7	s 139.4	s 73.5	s 73.5
C-4	t 42.1	t 42.1	t 39.2	t 31.1	t 39.3	t 31.7	t 42.4/ 42.3	t 42.4
C-5	t 22.8	t 22.8	t 25.6	t 26.0	t 25.6	t 26.1	t 22.4	t 22.5
C-6	d 125.1	d 125.1	d 124.8	d 124.9	d 124.8	d 124.9	d 125.7	d 125.9
C-7	s 135.3	s 135.3	s 134.9	s 135.7	s 134.9	s 135.5	s 135.1/135.0	s 135.1
C-8	t 35.8	t 36.2	t 35.9	t 35.5	t 35.6	t 36.0	t 27.7/ 27.5	t 28.1
C-9	t 31.5	t 29.8	t 30.9	t 31.6	t 29.2	t 29.4	t 31.4	t 29.8
C-10	d 76.0	d 74.8	d 75.5	d 75.3	d 74.1	d 74.3	d 75.7/ 75.6	d 74.7/ 74.6
C-11	d 70.9	d 70.5	d 70.9	d 70.8	d 70.5	d 70.6	d 70.8	d 70.7
C-12	q 19.5	q 16.9	q 19.4	q 19.4	q 16.6	q 17.0	q 19.5	q 17.0
C-14	q 16.0	q 16.0	q 15.8	q 15.8	q 15.8	q 15.8	q 23.2	q 23.2
C-15	q 28.0	q 27.9	q 15.8	q 23.0	q 15.8	q 23.1	q 28.2	q 28.0

^a Compounds epimeric at C-3.

nerolidol (**5**), geranylacetone (**20**), linalool (**44**), alloocimene (**47**), 2,6-dimethyl-2,5-heptadiene (**48**), 2,5-dimethyl-2,4-hexadiene (**49**), and citronellol (**50**) all formed the diol at the 1-isobutenyl moiety but no norcompounds. This implies that this strain requires two methylene groups vicinal to the double bond and an additional double bond for nordiol formation. An oxygen function in this part of the molecule is not tolerated.

Corynespora cassicola DSM 62474 revealed almost opposite selectivity compared to *Diplodia gossypina*. *Corynespora cassicola* DSM 62474 formed the norcompounds with *trans*-nerolidol (**5**), 2*E*,6*E*- and 2*Z*,6*E*-farnesol (**10**) and geranylacetone (**20**), while the 6*Z*-farnesols, myrcene (**1**), citronellene (**25**) and (**37**), nerylacetone (**33**), geraniol (**43**), linalool (**44**), nerol (**45**), and 2,6-dimethyl-5-heptenol-2 (**46**) yielded the diols of the 1-isobutenyl moiety but not the norcompounds. Both strains formed the nordiols of *cis*-nerolidol (**15**) but in both cases these compounds were only formed if the diol (**16**) was used as substrate, so the yields were very low. From these results it can be deduced that this strain requires a geranyl moiety which must have an additional methylene group. While the double bond in the central part of the molecule has to have here the *E*-configuration to give the norcompounds, a *Z*-configuration is essential for the same reaction with *Diplodia gossypina* ATCC 10936.

During the course of the fermentation the diol occurred at first in the culture broth followed by the nordiols and the trinor alcohols so the formation of

norcompounds out of the diols seemed to be very likely. Extended fermentation of the diol (**38**) afforded indeed the nordiols (**39**) and (**40**), the trinor alcohol (**41**) and the ketol (**53**). This ketol is assumed to be the primary product of the prolongation of the intermediary formed aldehyde (**52**) which originates obviously from the diol cleavage of (**38**). Despite of the different selectivity of the two strains a common mechanism for the formation of nordiols can be proposed: First the double bond is epoxidated, then the epoxide hydrolyzed to the diol which is cleaved to the aldehyde and acetone. The aldehyde can either be reduced to the alcohol giving the trinor compound or prolonged to the ketol. The final step in the formation of nordiols is the reduction of this ketol in both strains. While this reduction leads preferably to the *S*-alcohol with *Diplodia gossypina* ATCC 10936, *S*- and *R*-alcohol are formed in equal amounts with *Corynespora cassicola* DSM 62474. Besides the different substrate selectivity this behavior is the main difference of the biotransformation of these substances between these two microorganisms.

Corynespora cassicola DSM 62474 afforded always a ratio of threo- and erythro-nordiols which was close to 1:1. *Diplodia gossypina* ATCC 10936 afforded preferentially the threo-nordiols. Here the ratio varies from 2.5:1 (*S*-citronellene) to $\geq 60:1$ (2,6-dimethyl-1,5-heptadien) depending on fermentation conditions and substrate. Using the diols as substrates to produce the nordiols the yields increase whereas the ratio decreases to about 1:1 to 7:1. The reasons are still not very well understood

but reduction of ketones with yeasts showed a strong dependence of optical purity of the alcohol from the concentration of substrate and glucose [7]. It seems to be plausible to apply these results for the reduction of the intermediary formed ketol.

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- [1] C. Neuberg and J. Hirsch, *Biochem. Z.* **115**, 282–310 (1921).
- [2] C. Fuganti, P. Grasselli, and G. Marinoni, *Tetrahedron Lett.* **1979**, 1161–1164; R. Bernardi, C. Fuganti, P. Grasselli, and M. Marinoni, *Synthesis* **1980**, 50–52; G. Bertolli, G. Fronza, C. Fuganti, P. Grasselli, L. Majori, and F. Spreafico, *Tetrahedron Lett.* **22**, 965–968 (1981), and ref. therein.
- [3] B. Stumpf, W.-R. Abraham, L. Witte, and K. Kieslich, 14th IUPAC-Symposium on Natural Products, Poznan, p. 278 (1984).
- [4] W.-R. Abraham, B. Stumpf, and K. Kieslich, *Chirale Verbindungen mit 1,2-dihydroxy-2-methyl-propyl-Struktur und Herstellungsverfahren*; Germ. Pat. P 3418054.0 (1984).
- [5] W.-R. Abraham, B. Stumpf, and K. Kieslich, *Appl. Microbiol. Biotechnol.* **24**, 24–30 (1986).
- [6] K. Mori and T. Otsuka, *Tetrahedron* **41**, 553–556 (1985).
- [7] B. Wipf, E. Kupfer, R. Bertazzi, and H. G. W. Leuenberger, *Helv. Chim. Acta* **66**, 485–488 (1983).