Enzymatic Acyloin Condensation of Acyclic Aldehydes*

Wolfgang R. Abraham and Burghard Stumpf

GBF-Gesellschaft für Biotechnologische Forschung mbH, AG, Mikrobielle Stoffumwandlung, Mascheroder Weg 1, D-3300 Braunschweig, Bundesrepublik Deutschland

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Corynespora cassicola DSM 62474 and Diplodia gossypina ATCC 10936 were found to cleave some acyclic terminal terpenoids 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 (1R,2S)-1-phenyl-1,2-propanediol. 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-propanediol 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 universal-salpeptone (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-Eimer 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 (2S,3S-6-methyliden-7-octen-2,3-diol) (3): Colorless oil, 1H NMR: dd 5.26...
$J = 17.6, 0.9$ 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 ($J = 16, 9.8, 6.4, 1$ 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), dddd 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. $^1$H 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), dddd 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 ($= (CH_3)_2$).

Biotransformation of 1.8 g cis-nerolidol 15 with *Diplodia gossypina* ATCC 10936 or *Corynespora cassiicola* 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-4,2,9-decadienic acid.

*Threeo-10,11-dihydroxy-cis-12-nornerolidol (17)*: Epimeric at C-3. $^1$H 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), dddd 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): 224.1776 (M+) (224.1776 calc. for $C_{16}H_{23}O_5$).

\[ \alpha = \frac{589 \text{ nm} 578 \text{ nm} 546 \text{ nm} 436 \text{ nm} 365 \text{ nm}}{+5.2^\circ +5.4^\circ +6.2^\circ +9.9^\circ} (c = 1.00). \]

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.

*3S,6S,7S-Threeo-6,7-dihydroxy-8-norcitronellene (27)*: Colorless oil, $^1$H NMR: d 4.94 ($J = 17, 1$ Hz) (1-H), dd 4.90 ($J = 10.2, 1$ Hz) (1'-H), dddd 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 $C_{8}H_{13}O_2$).

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

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.

*3R,6S-6-hydroxy-7-oxo-8-norcitronellene (53)*: $^1$H NMR: dddd 4.97 ($J = 17.2, 1.8, 12$ Hz) (1-H), dddd 4.94 ($J = 10.5, 1.8, 7$ Hz) (1'-H), dddd 5.68 ($J = 17.2, 10.5, 7.8$ 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 1.8 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 3S-2,6-dimethyl-5-hepten-2,3-diol. The erythro-nordiol was not detected (amount ≤ 1 mg).
Threeo-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 (= C(CH₃)₂). αD = -2.2° (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.

Threeo-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 C₁₀H₁₉O₂).

Biotransformation of 1.2 g nerylacetone 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).

α = 589 nm 578 nm 546 nm 436 nm
-3.3° -3.7° -4.3° -7.6° (c = 0.80).

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-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), dq 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 C₁₀H₁₇O₂).

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.

Threeo-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 C₁₀H₁₅O₂).

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 (= C(CH₃)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 C₁₀H₁₅O₂).

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 (= C(CH₃)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, 6, 10, 11-epoxy-farnesol, and 15 mg 2Z, 6Z-10, 11-epoxy-farnesol.

Threo-10,11-di hydroxy-2E, 6E-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), αD +0.8° (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).

Erythro-10,11-di hydroxy-2Z, 6E-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-di hydroxy-2E, 6E-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 3.68 (J = 10, 7, 7 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 C18H30O3.

\[
\begin{align*}
\alpha &= 589 \text{ nm } 578 \text{ nm } 546 \text{ nm } 365 \text{ nm } 359 \text{ nm} \\
&= +3.5^\circ +3.7^\circ +4.4^\circ +5.2^\circ +6.0^\circ 
\end{align*}
\]

Erythro-10,11-di hydroxy-2Z, 6E-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-di hydroxy-11-norgeranylacetone (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), dq 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-di hydroxy-11-norgeranylacetone (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.

Fig. 2. Substrates and their biotransformation products.
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 erythrodiois 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 configuration of the nordiols formed by Diplodia gossypina ATCC 10936 could be deduced. The threo-nordiol (3) gave $\alpha_D - 15.3^\circ$ while 2S,3S-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, 3-citronellene (25), 2,6-dimethyl-1,5-heptadiene (30), nerylacetone (33), and $R$-citronellen (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).

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<td>t 112.8</td>
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<td>q 16.6</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>q 20.1</td>
<td>q 20.2</td>
<td>-</td>
<td>q 20.5</td>
<td>q 20.3</td>
<td>q 20.1</td>
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$^a$ Assignments may be interchanged.
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 cassiicola* DSM 62474 revealed
almost opposite selectivity compared to *Diplodia
gossypina*. *Corynespora cassiicola* DSM 62474
formed the norcompounds with trans-nerolidol (5),
2,6-E,6-E- and 2,2,6-E-farnesol (10) and geranylacetone
(20), while the 6-E,farnesols, myrcene (1), citronel-
lene (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 noridiols 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
noridiols and the trinoralcohols so the formation of
norcompounds out of the diols seemed to be very
likely. Extended fermentation of the diol (38) afforded
indeed the noridiols (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 noridiols can be pro-
posed: 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 trinorcompound
or prolonged to the ketol. The final step in the
formation of noridiols 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 cassiicola* DSM 62474. Besides the
different substrate selectivity this behavior is the
main difference of the biotransformation of these
substances between these two microorganisms.

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

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**Table I b.** $^1$C NMR data of 8, 9, 11–12a, 15, and 16 (75.5 MHz, CDCl$_3$, TMS as internal standard).

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<th>11</th>
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$^a$ Compounds epimeric at C-3.
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 reduc­
tion of the intermediary formed ketol.

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