Pergamon

PII: S0031-9422(97)00688-2

Phytochemistry, Vol. 47, No. 6, pp. 1029–1036, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

BIOTRANSFORMATION OF LINALOOL TO FURANOID AND PYRANOID LINALOOL OXIDES BY *ASPERGILLUS NIGER*

JAN C. R. DEMYTTENAERE* and HENDRA M. WILLEMEN

Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Coupure Links 653, B-9000 Gent, Belgium. E-mail: Jan.Demyttenaere@rug.ac.be

(*Received in revised form* 7 *July* 1997)

Key Word Index—*Aspergillus niger*; *Penicillium digitatum*; fungus; spores; biotransformation; bioconversion; epoxidation; linalool; furanoid linalool oxide; pyranoid linalool oxide.

Abstract—Biotransformation of (±)-linalool with submerged shaking cultures of *Aspergillus niger*, particularly *A. niger* ATCC 9142, yielded a mixture of *cis*- and *trans*-furanoid linalool oxide (yield 15–24%) and *cis*- and *trans*-pyranoid linalool oxide (yield 5–9%). Biotransformation of (*R*)-(−)-linalool with the same strain yielded almost pure *trans*-furanoid and *trans*-pyranoid linalool oxide (ee > 95). These conversions were purely biocatalytic, since in acidified water (pH < 3.5) almost 50% linalool was recovered unchanged, the rest was lost by evaporation. The biotransformation was also carried out with growing surface cultures.

INTRODUCTION

In the course of our work related to the bioconversion of monoterpene alcohols by fungi, we investigated the biotransformation of linalool by *Aspergillus niger*. Linalool is a very wide-spread terpenol and is found in Oolong and Black Tea, together with the four linalool oxides and other flavour compounds [1–4]. The biotransformation of linalool (**1**) (Fig. 1) has been extensively studied in the past. The main reactions in the degradation of linalool by *Pseudomonas incognita* are oxidations and hydroxylations, leading to oleuropeic acid (**2**) and perillic acid (**3**) [5]. The degradation of linalool by a soil Pseudomonad [6, 7] led to a mixture of oxidation and hydroxylation compounds, such as furanoid linalool oxide (**4**), 2-vinyl-2-methyltetrahydrofuran-5-one (**5**), 8-hydroxylinalool (**6**), 8-carboxylinalool (**7**), oleuropeic acid (**2**) and α-terpineol (**8**). The regiospecific hydroxylation of linalool and its acetate by the fungus *A. niger* (isolated from garden soil) led to a mixture of 8-hydroxylinalool (**6**), geraniol (**9**) and α-terpineol (**8**) [8, 9]. More recently, the bioconversion of linalool by *B. cinerea*, a fungus of high interest in winemaking, causing the famous "noble rot" was described [10, 11]. This fungus was able to metabolise linalool to a mixture of oxidation and hydroxylation compounds: the diols 2,6-dimethyl-3,7-octadiene-2,6-diol (**10**), 2,6-dimethyl-1,7-octadiene-3,6-diol (**11**) and 8-hydroxylinalool (**6**) as well as α-terpineol (**8**). Besides 2-vinyl-2-methyltetrahydrofuran-5-one (**5**), the furanoid (**4**) and pyranoid (**12**) linalool oxides as well as 6-methyl-5-hepten-2-one (**13**) were reported. One of the last publications found dealt with the chemo-enzymatic synthesis of *cis*- and *trans*-furanoid linalool oxide [12], starting from (3*RS*,6*R*)-2,3-epoxylinalyl acetate, which was prepared chemically from (*R*)-linalool.

This paper reports the bioconversion of linalool to the pure linalool oxides, in their furanoid and pyranoid form, by *Aspergillus niger* and particularly *A. niger* ATCC 9142.

RESULTS AND DISCUSSION

Biotransformation of linalool by surface cultures

In a first experiment, the biotransformation of linalool by surface cultures of *Aspergillus niger* ATCC 9142 (ANT) was monitored over a period of 1 to 2 weeks.

Cultures were grown in Petri dishes on solid medium containing 0.05% linalool or 0.1% linalool. After incubation, the solid cultures were extracted at different time intervals: see Experimental section.

It was noticed that after 3 days the cultures with 0.05% linalool were fully grown and sporulation had occurred. The cultures with 0.1% linalool covered only part of the surface.

Most of the extracts contained both the substrate linalool and as main products furanoid linalool oxides

* Author to whom correspondence should be addressed.

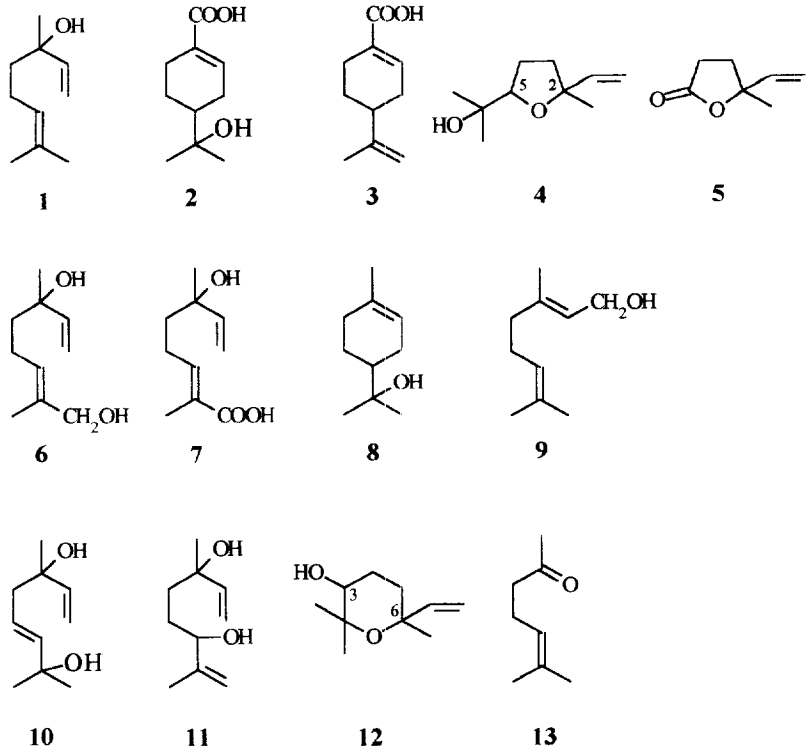

Fig. 1. Linalool (1) and metabolites thereof.

Table 1. Percentages of remaining linalool and yields of produced furanoid linalool oxides in SDSE-extracts of surface cultures grown in the presence of 0.05% and 0.1% linalool

Samples a: cultures with 0.05% linalool			Samples b: cultures with 0.1% linalool		
Sample	lin (%)	fur lin ox (%)	Sample	lin (%)	fur lin ox (%)
1 > 3 days	9.0	23.9	1 > 3 days	5.8	29.5
2 > 4 days	19.0	37.5	2 > 5 days	7.8	28.2
3 > 5 days	5.5	22.9	3 > 7 days	2.0	18.2
4 > 6 days	1.0	14.7	4 > 10 days	0.1	13.6
5 > 7 days	0.5	20.1	5 > 13 days	0.1	6.4
6 > 10 days	0.5	15.9	6 > 14 days	0.3	11.5
7 > 12 days	0.1	8.2	7 > 17 days	0.1	13.0
8 > 14 days	1.0	9.7	8 > 18 days	0.1	9.7

lin = linalool, fur lin ox = furanoid linalool oxide (mixt. of *cis* and *trans*)

(Table 1: the amounts of the *cis*- and *trans*-furanoid linalool oxides are combined); small amounts of pyranoid linalool oxides were also detected. In some cases small amounts of 2,6,6-trimethyl-2-vinyltetrahydropyran (**20**) (Fig. 2) were noticed: this compound is probably formed chemically.

When the substrate, linalool (99%) was analysed however, it was found that it contained small amounts of *trans*- and *cis*-furanoid linalool oxide (6.4% and 5.8% resp.). This means that auto-oxidation had occurred in the bottle.

Therefore, a second experiment was carried out with a fresh bottle of substrate, linalool (97%) almost free of oxides (< 0.5%). Cultures of *P. digitatum* and *A. niger* ATCC 9142 were grown in Petri dishes on solid media containing 0.1% linalool. A comparison was made with two control dishes with solid media also containing 0.1% linalool but not inoculated.

It was noticed that the linalool was metabolised completely by the cultures. The highest yield of furanoid linalool oxides was obtained with *A. niger* after 7 days (12.3%). In the control dishes, a large amount of unused linalool was present and only 0.5% of the oxides.

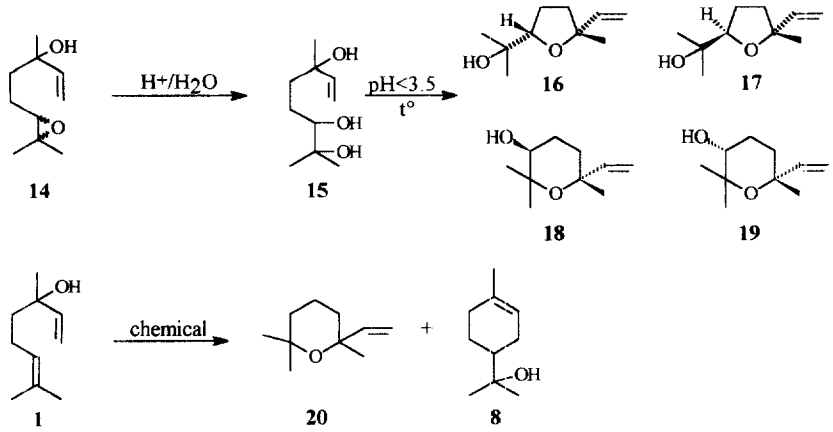

Fig. 2. Chemical decomposition of linalool (**1**) (after [19]).

Biotransformation of linalool by liquid cultures

The bioconversion of linalool by liquid cultures of five different strains of *A. niger* and one strain of *P. digitatum* was compared. The cultures were incubated on a rotary shaker and samples were taken as outlined in the Experimental section.

All samples contained linalool and both *cis*- and *trans*-furanoid linalool oxide. It was also found that *A. niger* DSM 821 (ANA) and *A. niger* ATCC 9142 (ANT) were the best producers of linalool oxides.

The biotransformation of linalool by *A. niger* was repeated in six-fold with 1 strain: *A. niger* ATCC 9142 (ANT). Six parallel cultures were grown, one spore suspension was prepared and one control experiment was run. All culture flasks were incubated as in the previous experiment, samples were taken and extracted as outlined in the experimental section. The results of all extracts for each flask were combined and are listed in Table 2 (ANT 1–6 are the liquid cultures 1–6, Sp. susp. is the spore suspension).

From these data it is clear that liquid cultures of *A. niger* are able to transform linalool to a mixture of *cis*- and *trans*-furanoid linalool oxide (yield 15–24%) and *cis*- and *trans*-pyranoid linalool oxide (yield 5–9%), a transformation that does not take place in acidified water in the absence of fungal cells, or in the presence of fungal spores. However spores are able to convert linalool to α-terpineol with a yield of 14%, a transformation that was not found with whole fungal cells, but which occurs slightly in acidified water. It is also clear that almost 50% of the added linalool is recovered unchanged in the control flask, whilst most of the added substrate is consumed or metabolised by both the fungal cells and the spores. The differences between the linalool contents are believed to be due to evaporation losses.

From the GC-analyses on an apolar DB-5 column it could not be seen whether each *cis*- and *trans*-linalool oxide consisted of 1 or 2 enantiomers. Some samples were also injected on a chiral column (CYDEX-B). From these analyses, it was found that only the 1 *cis*- or the 1 *trans*-enantiomer for each linalool oxide was formed and hence that the biotransformation reaction was enantioselective.

The average cell dry weight for the six cultures was 0.57 g biomass/100 ml culture. No correlation was found between the cell dry weight and the biotransformation efficiency.

Control experiments with acidified water

To check if linalool was oxidised in the absence of fungi due to acid or auto-oxidation a control experi-

Table 2. Total amount of recovered linalool, linalool oxides and α-terpineol in six cultures of *A. niger* ATCC 9142 (ANT 1–6), the spore suspension (Sp. susp.) and the control flask (μl)

Compound	ANT 1	ANT 2	ANT 3	ANT 4	ANT 5	ANT 6	Sp. susp.	Control
trans fur ox	18.37	15.27	12.59	12.99	11.09	11.57	0.74	0.52
cis fur ox	18.99	15.82	12.76	12.96	11.08	11.63	0.70	0.27
linalool	2.12	13.44	29.56	29.38	25.74	28.62	26.35	65.64
trans pyr ox	4.26	3.06	2.82	2.90	2.46	2.31	0.14	<0.01
cis pyr ox	9.80	6.73	5.74	5.72	4.79	5.11	0.27	0.01
a-terpineol	0.10	0.35	0.23	0.03	0.04	0.13	21.46	1.58

trans fur ox = *trans*-furanoid linalool oxide, *cis* fur ox = *cis*-furanoid linalool oxide.
trans pyr ox = *trans*-pyranoid linalool oxide, *cis* pyr ox = *cis*-pyranoid linalool oxide.

Table 3. Total amount of recovered linalool and linalool oxides in six cultures of *A. niger* ATCC 9142 (ANT 1–4 with (±)-linalool, ANT 5R–6R with (*R*)-(−)-linalool), the spore suspension (Sp. susp.) and the control flask (μl)

Compound	ANT 1	ANT 2	ANT 3	ANT 4	ANT 5R	ANT 6R	Sp. susp	Control
fur ox 1 > *R*	9.80	7.44	10.40	9.18	14.94	14.52	0.56	0.17
fur ox 2 > *S*	0.30	1.10	0.89	0.90	0.26	0.35	0.13	0.12
fur ox 3 > *R*	0.22	0.62	0.50	0.50	0.63	0.63	0.11	0.11
fur ox 4 > *S*	10.38	8.17	10.98	9.77	0.26	0.49	0.59	0.13
linalool	3.62	2.87	2.98	2.85	2.31	2.45	59.38	51.89
pyr ox 1 > *R*	4.79	4.40	6.02	5.28	7.86	7.21	0.15	0.02
pyr ox 2 > *S*	4.91	3.44	4.92	4.23	0.01	0.32	0.18	0.08
pyr ox 3 = ?	0.27	0.14	0.00	0.00	0.14	0.27	0.49	1.12
pyr ox 4 = ?	0.02	0.00	0.00	0.00	0.00	0.28	0.53	1.23
8-HO-linalool	3.86	1.44	3.49	2.93	10.85	7.30	3.69	0.07
ee for fur > *R*	97.84	92.26	95.40	94.81	95.96	95.87	—	—
ee for fur > *S*	97.22	88.17	92.49	91.54	—	—	—	—

fur lin ox: fur ox 1 = 2*R*,5*R*; fur ox 2 = 2*S*,5*S*; fur ox 3 = 2*R*,5*S*; fur ox 4 = 2*S*,5*R*.
pyr lin ox: pyr ox 1 = 3*S*,6*R*; pyr ox 2 = 3*S*,6*S*; pyr ox 3 & 4 = ?
ee for fur > *R* = ee for *trans*-furanoid linalool oxide > (*R*)-linalool.
ee for fur > *S* = ee for *cis*-furanoid linalool oxide > (*S*)-linalool.

ment was run with two flasks of 100 ml acidified water (pH 3.3) containing 0.1% (v/v) linalool. The possible transformation of linalool in this acid medium was monitored over a 12-day period.

From the samples taken from the control flasks, it was noticed that the linalool (97%) contained small amounts of linalool oxides (0.4%) as impurities but no auto-oxidation occurred. Therefore, it can be concluded that the transformation of linalool to linalool oxides is not acid catalysed but is a microbial transformation.

Biotransformation of (R)-(−)-linalool by liquid cultures

The biotransformation of (±)-linalool and (*R*)-(−)-linalool by *A. niger* ATCC 9142 (ANT) was compared. Four parallel cultures of ANT were grown, one spore suspension was prepared and one control experiment was run. At the same time, two parallel cultures of ANT were grown and treated with (*R*)-(−)-linalool as substrate: see Experimental section. The results for the analysis of the extracts are listed in Table 3.

From these data it is clear that (*R*)-(−)-linalool is converted mainly to *trans*-furanoid linalool oxide (ee 95.9–96.0) and *trans*-pyranoid linalool oxide. The mixture (±)-linalool is converted to a mixture of *cis*- and *trans*-furanoid and pyranoid linalool oxide. As side-product, a hydroxylated compound was identified: from the mass spectra available 8-hydroxylinalool (2,6-dimethyl-2,7-octadiene-1,6-diol) was suggested (Table 3). From the analyses on chiral GC, it was also clear that only one *cis*- and one *trans*-form was present for each linalool oxide. This confirms the enantioselective biotransformation of linalool to furanoid and pyranoid linalool oxide. As a control the enantiomeric composition of the commercial sample of furanoid linalool oxide and the furanoid linalool oxide formed in the old bottle of linalool (99%) was checked with chiral GC: in both cases the four enantiomers of furanoid linalool oxide were noticed in equal concentration.

It has to be pointed out that the literature concerning the elution order of both the furanoid and the pyranoid linalool oxides is contradictory and not consistent. Only very recently however, it was noticed and published by Mosandl and his co-workers [13–15] that a mistake had occurred in the early stage of the research concerning linalool oxides. The correct elution order for the four furanoid linalool oxides is: (+)-*trans*-(2*R*, 5*R*), (−)-*trans*-(2*S*, 5*S*), (−)-*cis*-(2*R*, 5*S*), (+)-*cis*-(2*S*, 5*R*) for PME-β-CD (Permethylated β-cyclodextrin chiral stationary phase) (comparable to the CYDEX-B column as used in this work). The elution order for the oxides on normal non-chiral stationary phases is: *trans*, *cis* furanoid linalool oxide for both non-polar and polar stationary phases. These findings were based on NMR-data and application of the NOE on the furanoid linalool oxides. This means that most data about the elution order given in literature until now are incorrect

The elution order of pyranoid linalool oxides on non-chiral stationary phase is: *trans*, *cis* for both non-polar and polar columns [2, 16]. When we analysed samples of cultures treated with (±)-linalool on a non-chiral stationary phase, we obtained two sharp peaks. When the same sample was analysed on chiral phase (CYDEX-B) we obtained two sharp main peaks followed by two smaller ones. When we analysed samples of cultures treated with (*R*)-linalool on a non-chiral stationary phase, we obtained one sharp peak, corresponding with the first peak of samples containing the two oxides, thus corresponding to *trans*-

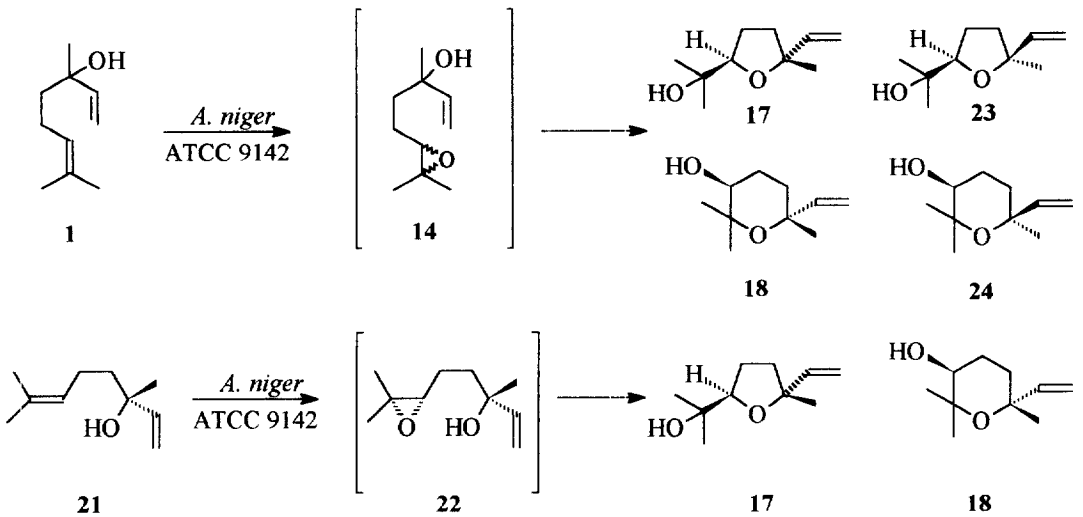

Fig. 3. Biotransformation of linalool by *A. niger* ATCC 9142.

pyranoid linalool oxide, which can only be the cyclisation product of (*R*)-linalool (**21**), thus *trans*-(3*S*, 6*R*)-pyranoid linalool oxide (**18**) (Fig. 3). When this sample was analysed on the chiral phase, only one sharp peak was obtained, corresponding with the first peak of samples containing the two oxides, followed by three very small peaks. Therefore, we can conclude that the order of appearance on a chiral column is: *trans*-(3*S*, 6*R*)-, *cis*-(3*S*, 6*S*)-pyranoid linalool oxide followed by the two remaining enantiomers (order of these two not known).

In the literature, only two articles with a comparable biotransformation of linalool to pure linalool oxides were found: the microbial cyclisation of linalool to furanoid linalool oxides by a nigericin-producing *Streptomyces albus* [17] and the biotransformation of (−)- and (+)-linalool to furanoid and pyranoid linalool oxides by *Diplodia gossypina* ATCC 10936 [18].

The chemical formation of linalool oxides from linalool in aqueous solutions at low pH (<3.5) was not noticed in our experiments. However, the acid catalysed formation of 6,7-epoxylinalool (**14**) to the triol 2,6-dimethyloct-7-ene-2,3,6-triol (**15**), which is then further converted chemically to *cis*- (**16**) and *trans*-furanoid (**17**) linalool oxides has been described [19, 20] (Fig. 2). The partial decomposition of linalool (**1**) to 2,6,6-trimethyl-2-vinyltetrahydropyran (**20**) and α-terpineol (**8**) is also subscribed to a chemical reaction at low pH [20].

Based on our findings and the literature data available, we suggest that the biotransformation of linalool by *A. niger* occurs as outlined in Fig. 3.

When a mixture of (±)-linalool (**1**) is added to submerged growing fungal cultures, it is transformed mainly to *trans*- (**17**) and *cis*- (**23**) furanoid linalool oxide and *trans*- (**18**) and *cis*- (**24**) pyranoid linalool oxide.

When pure (*R*)-(−)-linalool (**21**) is added to the cultures, it is transformed to *trans*-(2*R*,5*R*)-furanoid linalool oxide (**17**) and *trans*-pyranoid linalool oxide (**18**). Since the biotransformation of (*R*)-(−)-linalool by *A. niger* is carried out stereospecifically to *trans*-furanoid linalool oxide, and since the enzymatic formation of *trans*-(2*R*,5*R*)-furanoid linalool oxide (**17**) from (3*S*,6*R*)-2,3-epoxylinalyl acetate is described in literature [16], it is assumed that (3*S*,6*R*)-2,3-epoxylinalool (**22**) is the key intermediate in this fungal bioconversion.

EXPERIMENTAL

Microorganisms. One strain of *Penicillium digitatum* was used and five strains of *Aspergillus niger*. The *P. digitatum* strain, earlier misidentified as *P. italicum*, was re-identified by the MUCL (Mycothèque de l'Université Catholique de Louvain, Laboratoire de Mycologie Systématique et Appliquée, Belgium) as *P. digitatum* (Persoon: Fries) Saccardo [21]. Earlier research showed that this strain was able to transform geraniol, nerol and citral to 6-methyl-5-hepten-2-one [22–24]. During the experiments, this strain was designated as CLE. The *A. niger* strains were designated as follows: ANG (*A. niger* isolated from a Cypress branch), ANA (*A. niger* DSM 821, obtained from Deutsche Sammlung von Mikroorganismen), WEL (isolated from an African *Welwitschia* plant), COR (isolated from a coriander branch), ANT (*A. niger* ATCC 9142 obtained from the American Type Culture Collection). The cultures were maintained by periodic subculture (every 2 weeks) on malt extract agar (MEA).

Growth medium. For the isolation, growth and maintenance of the fungi as surface cultures, two media were used: malt extract agar (MEA) (malt extract 2%, bacteriological peptone 0.1%, glucose 2% and agar 2%) and Sabouraud Dextrose Agar (SAB) (Oxoid) (Mycological peptone 1.0%, glucose 4.0%

and agar 1.5%). Fungi were stored in Petri dishes, filled with 20 ml sterile medium. For cultivation as submerged liquid cultures, a liquid medium was used: YMPG (yeast extract 0.5%, malt extract 1%, bacteriological peptone 0.5%, glucose 1%).

Preparation of a spore suspension. From 2- to 4-week-old surface cultures on Petri dishes, a spore suspension was prepared. Onto the sporulated surface culture, 15 ml of a sterile physiological Tween 80 soln (0.85% NaCl, 0.1% Tween 80 in distilled water) was poured. The spores were brought into suspension with a sterile glass bar. It was found that the spore suspension thus obtained contained *ca* 10^8 spores ml^{-1}. This spore suspension was used to inoculate conical flasks filled with liquid medium but also for biotransformation experiments. 100 ml spore suspension was prepared from 10 Petri dish surface cultures. To this suspension 0.5 ml of a 10% (v/v) linalool/EtOH soln was added. At different time intervals 5-ml samples were taken with a sterile pipette and extracted with 2×1.5 ml Et_2O. After addition of 1 ml of a standard soln of 0.1% (v/v) *n*-decane in Et_2O, the sample was directly analysed by GC and GC-MS.

Cultivation of liquid submerged cultures in conical flasks. The fungi were cultivated in 500-ml conical flasks filled with 100 ml liquid medium (YMPG). After sterilisation of the media (121°, 1.2 bar, 15 min), the media were inoculated with 1 ml of a spore suspension.

In the first experiment, the bioconversion of linalool by liquid cultures of five different *A. niger* strains and one *P. digitatum* strain was compared. To each flask 0.25 ml of a 20% linalool (97%)/EtOH-soln was added (*i.e.* 50 μl linalool per flask). The flasks were inoculated with the following strains: ANG, ANA, WEL, COR, ANT, CLE. The cultures were placed on a rotary shaker at 24° and 100 rpm. After 3 days, 5-ml samples were taken and extracted and analysed in the same way as the spore suspension cultures. During an additional period of 10 days 7 more samples were taken and two more substrate additions took place: after 4 and 7 days. The ninth and last sample was taken 1 week after the last substrate addition.

The bioconversion of linalool was repeated in six-fold with *A. niger* ATCC 9142 and compared with spore suspension and control flask (distilled water acidified with HOAc to pH 3.5). At the time of inoculation, 0.5 ml of a 10% linalool (97%) soln in EtOH (50 μl linalool) was added to the liquid cultures, the spore suspension and the control flask. During an additional period of two weeks, 8 samples were taken: after 4, 5, 6, 7, 8, 11, 13 and 15 days. After 5 and 8 days, two more linalool additions were carried out (0.5 ml of the 10% soln) to give an overall substrate addition of 150 μl linalool per 100 ml liquid culture. After 20 days the experiment was stopped and the contents of the cultures were divided in two parts: one part was filtered and dried to measure the biomass, and one part was extracted to analyse the concentration of volatile components. The amount of volatiles in the filtered part was calculated from the extracted part.

In a final experiment, the bioconversion of (±)-linalool and (*R*)-(−)-linalool was compared. Four cultures of ANT, one spore suspension and one control flask (distilled water at pH 3.1) were used. At the time of inoculation, 1 ml of a 5% linalool (97%) soln in EtOH (50 μl linalool) was added to the liquid cultures, the spore suspension and the control flask. During an additional period of two weeks, 10 samples were taken and four more linalool additions were carried out (0.5 ml of the 5% soln) to give an overall substrate addition of 150 μl linalool per 100 ml liquid culture. After 20 days the experiment was stopped. The bioconversion of (*R*)-(−)-linalool was monitored with 2 parallel cultures of ANT: during a ten-day period 3 times 50 μl (*R*)-(−)-linalool was added to the cultures and 8 samples were taken. All samples were analysed by GC with a non polar DB-5 column and with a chiral CYDEX-B column.

Cultivation of fungi as surface cultures in Petri dishes for bioconversion. The bioconversion of linalool by surface cultures grown in Petri dishes was studied.

In the first experiment, an old bottle of linalool (99%) was used. Fifteen Petri dishes were filled with 20 ml medium (SAB) containing 0.05% linalool and 0.2% EtOH, and 15 Petri dishes were filled with 20 ml SAB containing 0.1% linalool and 0.4% EtOH. All Petri dishes were inoculated with 0.1 ml of a spore suspension of ANT. After inoculation the cultures were incubated at 30° during 24 hr and kept at room temp until the time of extraction. During this period, 2 surface cultures (except for the last culture: only one) with the same substrate conc were extracted every (other) day: for the cultures with 0.05% linalool this was done after 3, 4, 5, 6, 7, 10, 12 and 14 days (samples 1a–8a); for the cultures with 0.1% linalool this was done after 3, 5, 7, 10, 13, 14, 17 and 18 days (samples 1b–8b).

In the second experiment, a fresh bottle of linalool (97%) was used. Ten Petri dishes were filled with 20 ml medium (SAB) containing 0.1% linalool (97%). Four of them were inoculated with *Aspergillus niger* ATCC 9142, two with *Penicillium digitatum* and four were not inoculated but kept as control dishes. They were incubated at 30° during 24 hr and stored at room temp until the time of extraction, which was done after 7 and 10 days.

Extraction of surface cultures. Since linalool oxides are less volatile than linalool, no headspace samples of the surface cultures could be taken, in contrast to the biotransformation of citral and citrol to 6-methyl-5-hepten-2-one, which is much more volatile than the substrate [22, 24]. Therefore the bioconversion was monitored by simultaneous distillation solvent extraction (SDSE) according to Likens-Nickerson. The whole contents of two Petri dishes were cut in small pieces (1 cm^2) and put in a 250 ml-flask. Distilled water was added to adjust the volume to 100 ml. The extraction was carried out with 12 ml CH_2Cl_2 during

1.5 hr. After addition of 1 ml of a standard soln of 0.1% (v/v) *n*-decane in Et_2O, the sample was directly analysed by GC and GC-MS.

Control experiment with acidified water. Two flasks of 500 ml were filled with 100 ml distilled water, adjusted to pH 3.3 with HOAc. Substrate was added to both flasks: 1 ml of a 10% soln of linalool (97%) in ethanol (*i.e.* 100 μl linalool). The flasks were placed on a magnetic stirrer at room temp. After 3, 5, 7 and 11 days a 5 ml sample was taken from both flasks and extracted with 2 × 1 ml Et_2O. Samples were analysed by means of GC after addition of 1 ml a standard soln of 0.1% (v/v) *n*-decane in Et_2O.

Chemical compounds. Linalool (99%) (old sample, slightly decomposed during storage) and (±)-linalool (97%) (new sample: unchanged) were obtained from Aldrich, *R*-(−)-linalool (97%) was obtained from FLUKA, one sample of *cis*- and *trans*-furanoid linalool oxide (mixt. of isomers) was purchased from ROTH (Karlsruhe) and another from FLUKA. α-terpineol was obtained from Janssen Chimica (Belgium).

Analysis of the samples with GC and GC-MS. GC-analyses were performed with a Delsi-200 instrument, equipped with a DB-5 FSOT (fused silica open tubular) column (30 m × 0.32 mm i.d.; coating thickness 1 μm) and FID. Injector: cold on-column injector, detector temp. 230°; oven temp. programmed from 50–160° at 5° min^{-1} and from 160–220° at 10° min^{-1}; carrier gas (He) 1.9 ml min^{-1}; air 250 ml min^{-1}; H_2 25 ml min^{-1}. Peak areas were calculated by a computer equipped with Nelson-software. Chiral GC-analyses were performed with the same Delsi-200 instrument, same conditions, equipped with a CYDEX-B chiral FSOT column (SGE) (25 m × 0.33 mm i.d.; coating thickness 0.25 μm) and FID. GC-MS analyses were performed with an HP 5890 gas chromatograph, equipped with a DB-5 FSOT column (30 m × 0.25 mm i.d.; coating thickness 0.25 μm) and an HP 5970A mass selective detector (quadrupole type). Working conditions: injector: 220°; oven temp. programmed from 50–160° at 5° min^{-1} and from 160–220° at 10° min^{-1}; carrier gas (He) 1 ml min^{-1}; ionisation: EI 70 eV; acquisition parameters: scanned *m/z*: 40–180 (5′–20′), 40–250 (>20′). Substances were identified by comparison of their mass spectra and retention indexes (Kováts Indexes) [13–15, 25–27] with those of reference substances. Response Factors in relation to *n*-decane (Delsi): *cis*- and *trans*-furanoid linalool oxide: 1.23; linalool: 1.03; α-terpineol: 1.10. Retention indexes (for HP resp. Delsi): *trans*-furanolinalool oxide: 1073 resp. 1078; *cis*-furanolinalool oxide: 1089 resp. 1093; linalool: 1104 resp. 1100; *trans*-pyranolinalool oxide: 1172 resp. 1178; *cis*-pyranolinalool oxide: 1176 resp. 1181; α-terpineol: 1198 resp. 1197; 8-hydroxylinalool: 1361 resp. 1370.

Mass spectra. trans-Furanolinalool oxide *m/z*: (rel. int.): 59 (100), 43 (77), 55 (38), 41 (37), 94 (28), 93 (27), 67 (25), 68 (25), 111 (19), 81 (13). *cis*-Furanolinalool oxide *m/z*: (rel. int.): 59 (100), 43 (67), 55 (40), 41 (33), 94 (28), 68 (25), 93 (23), 67 (21), 111 (16), 81 (11). Linalool *m/z*: (rel. int.): 43 (100), 41 (87), 71 (87), 55 (62), 93 (47), 69 (33), 80 (23), 67 (17), 53 (13), 121 (12). *trans*-Pyranolinalool oxide *m/z*: (rel. int.): 68 (100), 59 (93), 43 (85), 67 (55), 94 (48), 41 (42), 55 (34), 53 (21), 79 (18), 57 (13). *cis*-Pyranolinalool oxide *m/z*: (rel. int.): 59 (100), 68 (96), 43 (79), 67 (52), 94 (50), 41 (47), 55 (31), 53 (23), 79 (23), 57 (13). α-Terpineol *m/z*: (rel. int.): 59 (100), 43 (54), 93 (40), 121 (28), 81 (27), 41 (25), 136 (22), 67 (19), 79 (14), 92 (14). 8-Hydroxylinalool *m/z*: (rel. int.): 43 (100), 71 (47), 67 (35), 41 (31), 55 (28), 68 (14), 79 (11), 82 (10), 53 (8), 81 (8), 93 (8).

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