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In Vivo ESR Spin Trapping Detection of Carbon-Centered α-Farnesene Radicals

ULRICH KRINGS,*,† MOGENS L. ANDERSEN,‡ AND RALF G. BERGER†

Institut für Lebensmittelchemie, Zentrum für Angewandte Chemie der Leibniz Universität Hannover, Wunstorfer Strasse 14, D-30453 Hannover, Germany, and Food Chemistry, Department of Food Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Federiksberg C, Denmark

Lyophilisates of the ascomycetes *Penicillium solitum* and *Aspergillus niger* converted α-farnesene to 7-hydroxyfarnesene as the major product. The radical mechanism of this bioconversion was proven by electron spin resonance (ESR) and GC-MS using the spin trapping technique. Intermediate carbon-centered radicals of α-farnesene were captured using two spin traps, 2-methyl-2-nitrosopropane and α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone, respectively. The evaluation of the coupling constants and hyperfine couplings of the ESR spectra showed that tertiary carbon radicals were trapped. The radical position at C7 of α-farnesene was derived from EI and CI mass spectra of the corresponding MNP spin adduct. The present study demonstrates that the complementary application of ESR and MS spectrometric data allows the detailed evaluation of a radical mechanism of a fungal terpene transformation reaction.

KEYWORDS: Carbon-centered radicals; ESR; α-farnesene; free radicals; spin trapping

INTRODUCTION

Terpene hydrocarbons and their oxyfunctionalized derivatives, the terpenoids, are among the most frequently investigated substrates of microbial biotransformations. They are used, for example, as potent flavors, fragrances, pharmaceuticals, fungicides, and enzyme inhibitors (*1–4*). Some terpene hydrocarbons are used as parent substances for syntheses, and they have also been suggested as substrates for selective biotransformations by micro-organisms and, therefore, yield natural flavor substances, for example, for food flavoring. The enzymatic introduction of oxygen, preferably at the allylic position of the terpene hydrocarbon, yields flavor alcohols, which can be further oxidized to the corresponding carbonyl compounds, for example, verbenone from α-pinene, carvone from limonene, and nootkatone from valencene (*3, 5, 6*). α-Farnesene (**1**), a minor constituent (up to 1%) of several essential oils such as apple, grapefruit, lime peel, orange, mandarin, or pepper, has not been extensively investigated as a substrate in microbial biotransformations. A recent paper described a screening of terpene-converting fungi for their potential to oxyfunctionalize α-farnesene and the identification and characterization of major odorous fungal oxidation products (*7*). In general, the reaction mechanism of terpene biotransformations is often deduced from the structure of the products and their respective regio- and stereochemical preferences (*6*). Thus, the initial introduction of oxygen was proposed to occur via a hydrogen abstraction at the diallylic position of α-farnesene, resulting in a radical, which was resonance-stabilized by the three double bonds between the C1 and C7 atoms (**Figure 1**). Direct in vivo detection of the reactive intermediates of microbial terpene bioconversion has been achieved in very few cases (*3, 6*). Because of the low steady state concentration of most radicals, the spin trapping technique was used for the detection and identification of short-lived free radicals. The addition of diamagnetic molecules (spin traps) to the reaction system may transform reactive free radicals into more stable paramagnetic species (spin adducts), which can be detected by electron spin resonance spectroscopy (ESR) (*8, 9*). This investigation undertook ESR and GC-MS experiments involving spin trapping of putative α-farnesene radicals derived from the initial attack of fungal enzymes on α-farnesene. This should prove if the initial reaction step in the formation of 3,7,11-trimethyldodeca-1,3(*E*),5(*E*),10-tetraen-7-ol (7-hydroxyfarnesene) (**2**) was radical mediated.

MATERIALS AND METHODS

Microorganisms. Two fungal strains, *Aspergillus niger* and *Penicillium solitum* were isolated from natural habitats and identified as described elsewhere (*7, 10*). The strains were maintained on yeast malt agar slants.

Medium and Culture Conditions. *Submerged Cultivation.* The fungal cultures were inoculated into a glucose/asparagine/yeast extract medium described elsewhere (*11*) and grown aerobically at 24 °C and 150 rpm on an orbital shaker. For biomass harvesting cultures (500 mL shake flasks, 200 mL medium volume) were inoculated with 10 mL 3-day-old precultures grown on the same medium and homogenized using an Ultraturrax homogenizer (Jahnke & Kunkel, Staufen Germany) prior to inoculation.

* Corresponding author (telephone +49 511 762 4583; fax +49 511 762 4547; e-mail krings@lci.uni-hannover.de).
† Zentrum für Angewandte Chemie der Leibniz Universität Hannover.
‡ University of Copenhagen.

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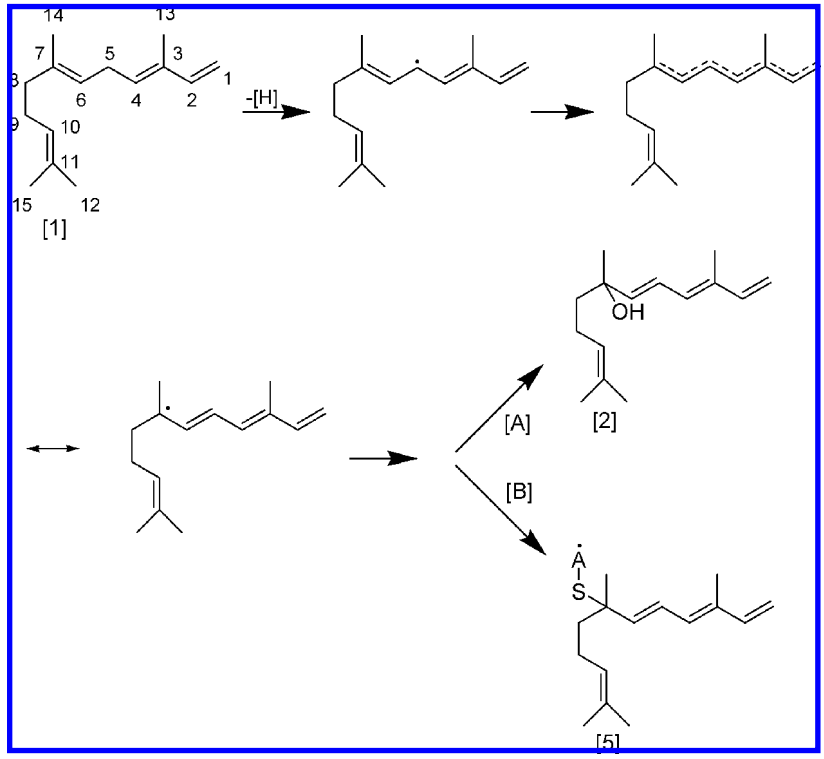

Figure 1. Proposed initial radical mechanism of fungal α-farnesene bioconversion (A, fungal lyophilisate; B, fungal lyophilisate + **3** or **4**; SA, spin trap adduct). For the complete bioconversion scheme see ref *7*.

Mycelium lyophilisates. After 4 days of fungal growth the active biomass of five cultures was harvested by centrifugation at 10,000 g and washed with 0.9% sodium chloride solution. The fungal biomass was transferred into a 400 mL beaker and shock frozen using liquid nitrogen and lyophilized at room temperature, 2×10^{-5} Pa and sterile conditions. 50 mg of each fungal lyophilisate was filled into a 4 mL glass reaction vial and rehydrated with 1.5 mL of MOPS buffer [0.1 M 4-(*N*-morpholino)butanesulfonic acid in H_2O, pH 7.0, adjusted with 0.1 M NaOH] for 1 h.

Mycelium Concentrates. Mycelium concentrates were prepared as described elsewhere (*7*). Ten milliliters of fresh liquid medium was added to approximately 20 g of fungal biomass (wet weight).

Conversion Experiments. The reaction was started by adding of 10 μL (to 50 mg of lyophilisate) or 50 μL (to 20 g of mycelium concentrate) of freshly distilled α-farnesene (Treatt, Bury St. Edmunds, Suffolk, U.K.), respectively. The mixture was extensively mixed using a vortexer, and the sealed vessels were further agitated at 24 °C and 150 rpm on an orbital shaker. In terms of ESR measurements, the respective spin traps 2-methyl-2-nitroso-propane (**3**) and α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (**4**) (both 99%, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), carefully dissolved and stored in H_2O protected from light, were added (20 mM final concentration) instantly to the rehydrated lyophilisate prior to α-farnesene addition. Prior to use, the spin trap solutions were measured and found free of any ESR signals.

Isolation and Identification of Transformation Products and Spin Adducts. After the addition of α-farnesene and the respective spin trap, the reaction solution (medium or buffer after different incubation times up to 24 h) was completely transferred into a separatory funnel, made up to 20 mL with saturated sodium chloride solution, and extracted three times with 20 mL of azeotropic pentane/diethyl ether (1:1.12 v/v). The combined extracts were dried over dry sodium sulfate and concentrated (42 °C) to a volume of 1 mL using a Vigreux column. Thymol (60 μg) (99%, Sigma-Aldrich Chemie GmbH) was added as an external standard.

Prior to GC-FID and GC-MS measurements, major transformation products were isolated by column chromatography using a silica gel column and gradient elution with pentane/Et_2O as described elsewhere (*7*). The α-farnesene-containing nonpolar fraction was discarded, whereas the fractions containing conversion products were pooled and reconcentrated by distillation using a Vigreux column.

One microliter was injected into a Fisons GC 8360 GC equipped with a cool on-column injector, a J&W (Folsom, CA) CW 20 M fused silica capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness), hydrogen as the carrier gas (52 cm/s), and a FID (230 °C) using a temperature program from 100 °C (2 min) to 160 °C at a rate of 5 °C/min to 230 °C with 3 °C/min hold for 5 min. Quantification was performed according to the external standard thymol. All experimental points are the means of at least two independent quantifications with a standard deviation of <10%.

GC-MS analysis was carried out using the same chromatographic conditions as for GC-FID analysis and helium as the carrier gas (38 cm/s). Identification of transformation products was achieved by comparison of electron impact ionization (EI) mass spectra with data from the literature (*7*) using a Fisons GC 8000 gas chromatograph and a Fisons MD 800 mass selective detector [interface, 230 °C; ion source, 200 °C; quadrupole, 100 °C; EI ionization (70 eV); scan range, *m*/*z* 33−400]. Chemical ionization (CI) was carried out with methane as reactant gas at 560 Pa.

ESR Spectrometry. Alternatively to solvent extraction, the buffered and suspended lyophilisate was directly analyzed by means of ESR spectroscopy. An aliquot of the reaction buffer containing the respective spin trap was transferred (immediately after mixing using a vortexer) directly into an ESR quartz capillary tube with an internal diameter of 0.75 mm (Wilmad, Buena, NJ), and the ESR spectrum was recorded for 60 s on a Miniscope MS200 spectrometer (Magnetech GmbH, Berlin, Germany). The measurements were carried out at room temperature with a microwave power of 20 mW, gain 9, and a modulation width of 0.1 mT. The ESR spectrometer was calibrated prior to measurement using the stable radical 2,2,6,6-tetramethylpiperidine-1-oxyl (98%, Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Simulation of ESR Spectra. Computer simulation of experimental ESR spectra was used for the calculation of hyperfine coupling constants. Simulation was performed by the Public EPR Software Tools (PEST) from the National Institute of Environmental Health Sciences available on the Internet (*12*).

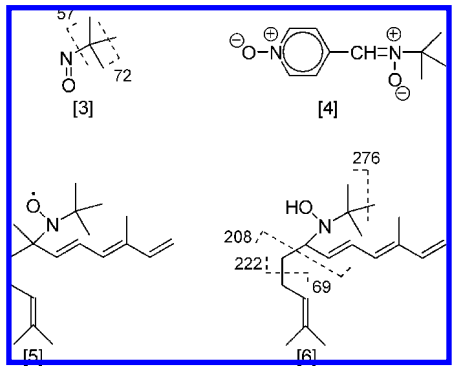

Figure 2. Spin traps 2-methyl-2-nitrosopropane (**3**), α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (**4**), and corresponding assumed spin trap adducts (radical **5** and reduced radical **6** of **3**) and EI fragmentation pattern.

Table 1. Peak Concentrations of 7-Hydroxyfarnesene (*E*/*Z*) (**2**) 24 h after α-Farnesene (**1**) Supplementation

	E/*Z*	
	mycelium concentrate (mg/L)	lyophilisate (mg/L)
P. solitum	20.8/3.8	31.6/1.0
A. niger	52.1/6.5	15.4/1.3
chemical blank	2.6/1.1	2.8/1.3
$FeSO_4$	15.6/1.5	12.3/0.9

RESULTS AND DISCUSSION

Mycelium concentrates of two fungal strains, *A. niger* and *P. solitum*, were shown to oxidize the sesquiterpene α-farnesene (**1**). The main product of the bioconversion (up to more than 100 mg/L in some experiments) was 7-hydroxyfarnesene (**2**), which was also generated in chemical blanks, but to a much lower amount (<10 mg/L) (*7*). The generation of the tertiary alcohol at C7 requires the enzymatic attack of the diallylic position at C5 of the α-farnesene followed by a rearrangement of the double bonds, yielding the hydroxyl group in conjugation to three double bonds (**Figure 1**, pathway A). Two different mechanisms, one through an intermediate radical and one through a carbocation, have been proposed (*7*). It is current academic opinion that, for instance, the cyclization and isomerization of mono- and bicylic sesquiterpene skeletons occur through intermediate carbocations (*13*). The initial introduction of oxygen to terpene hydrocarbons could be achieved by more stereospecific enzymes, such as P450 mono-oxygenases. However, the location of the hydroxyl group at C7 of α-farnesene (vinylic position) without any stereochemical preference (racemic mixture of **2**) excludes the contribution of more regio- and stereospecific mono-oxygenases (*7*). Nonspecific oxygenations of terpene hydrocarbons by fungi are not unlikely, as demonstrated for limonene, α-farnesene, and valencene biotransformations (*3, 5, 6*).

To investigate a possible enzyme-initiated radical mechanism as the initial step of α-farnesene oxidation, the assumed and most stable intermediate carbon radical at C7 had to be trapped in vivo using adequate spin traps (**Figures 1** and **2**). To ascertain that the slow autoxidation of α-farnesene occurred at least in part along the same radical mechanism as the fungal bioconversion, the autoxidation rate was increased by the addition of Fe^{2+} to the liquid medium; this gave observable ESR signals. For the detection of carbon-centered α-farnesene radicals, the following spin traps were used (**Figure 2**): (i) 2-methyl-2-nitrosopropane (**3**) and (ii) α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (**4**). Both **3** and **4** were shown to trap carbon-centered radicals. **3** was used to detect carbon-centered radicals of carotenoids and β-ionone ions (*14*) and derivatives of isohumulones (*15*). **4** was used to confirm carbon-centered radicals in Fenton reaction model systems to evaluate the antioxidative and pro-oxidative effects of extracts made from cherry liquor pomace (*16*) and intermediates in the degradation of furfuryl mercaptan (*17*).

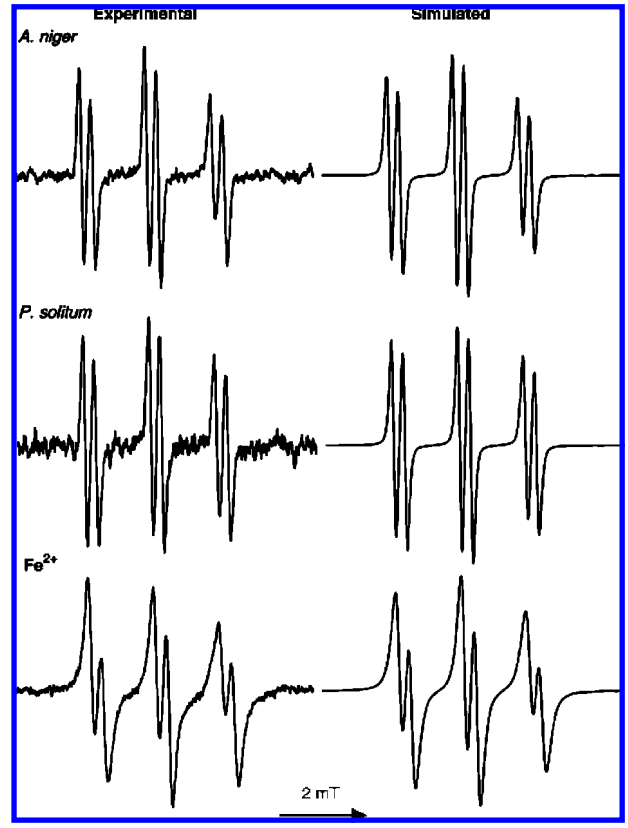

Figure 3. ESR spectra of α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (**4**) spin adducts generated by treating α-farnesene (10 μL) in 1.5 mL of MOPS buffer (pH 7.0) for 24 h with *A. niger* lyophilisate (50 mg), *P. solitum* lyophilisate (50 mg), or $FeSO_4$ (0.03 mmol). Digitally simulated ESR spectra were all based on a single nitroxyl radical with hyperfine coupling constants $a_N = 1.53$ mT and $a_H = 0.26$ mT, but with various line widths.

ESR Analyses of Fungal Lyophilisates. The mycelium concentrate from submerged culture was incompatible with direct online ESR measurements in ongoing bioconversion experiments. Recently, it was demonstrated that fungal lyophilisates were superior to submerged cultivation in valencene conversion to yield nootkatone (*18*). Therefore, the active biomass of *P. solitum* and *A. niger* was freeze-dried and resuspended in a MOPS buffer (pH 7). The bioconversion was carried out in 1.5 mL microassays for 24 h. The conversion products of α-farnesene were the same for mycelium concentrate and for the lyophilisate (data not shown). **Table 1** gives the peak concentration of the main α-farnesene bioconversion products using either mycelium concentrates or lyophilisates.

Two spin traps (**3** and **4**) suitable to detect carbon-centered radicals (**Figure 2**) were added to the respective conversion buffer, and the reaction was started by adding the required amount of α-farnesene. After 1, 2, 5, 10, and 24 h, aliquots of the bioconversion buffer were withdrawn directly into an ESR quartz capillary tube and ESR spectra were recorded. Using **4** for spin trapping, the first weak ESR signals appeared after 1 h and increased without alteration of the signal shape over time. The ESR spectra observed after 24 h of reaction time consisted

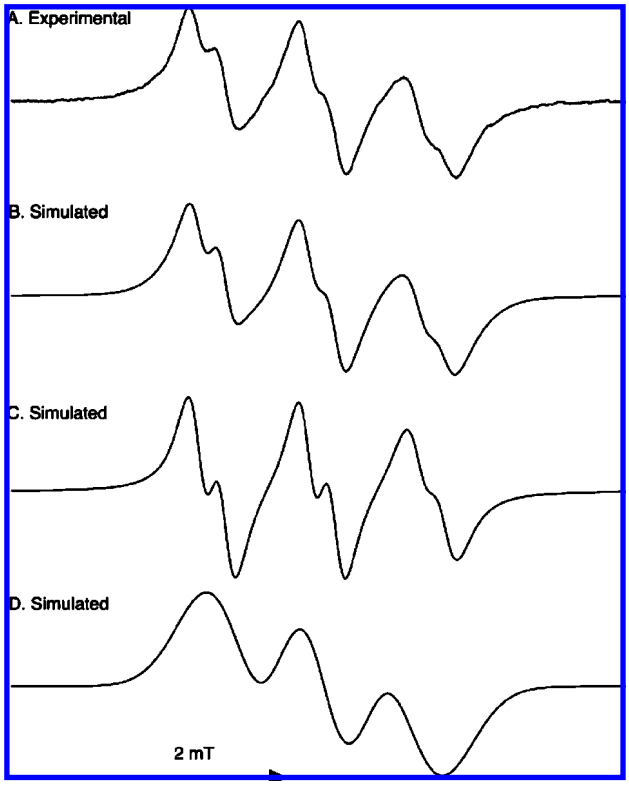

Figure 4. ESR spectra of 2-methyl-2-nitrosopropane (**3**) spin adducts formed in 1.5 mL of MOPS buffer (pH 7.0) for 24 h by a mixture of α-farnesene (10 μL) and *A. niger* (50 mg): (**A**) experimental spectrum; (**B**) digitally simulated ESR spectrum caused by a mixture (35:65 mol %) of two spin adducts [**C**, **D** (**C**, digitally simulated ESR spectrum of a spin adduct having hyperfine coupling constants a_N = 1.41 mT and a_H = 0.34 mT; **D**, digitally simulated ESR spectrum of a spin adduct having hyperfine coupling constant a_N = 1.03 mT)].

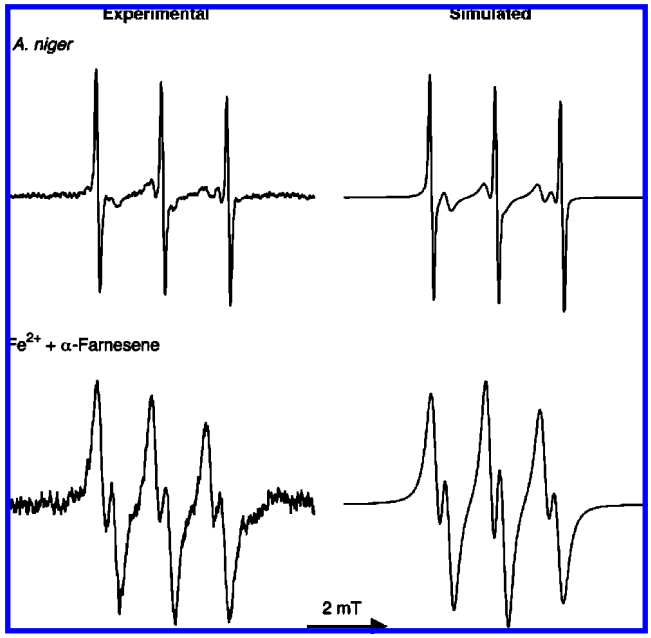

Figure 5. Experimental and digitally simulated ESR spectra of 2-methyl-2-nitrosopropane (**3**) spin adducts formed in 1.5 mL of MOPS buffer (pH 7.0) for 24 h by (**A**) *A. niger* (50 mg) alone and (**B**) a mixture of $FeSO_4$ and α-farnesene (0.03 mmol).

of a triplet of doublets, which are typical for **4** spin adducts (**Figure 3**). The hyperfine coupling constants of the spin adducts (a_N = 1.53 mT and a_H = 0.26 mT) indicate that carbon-centered radicals were trapped (*19*). Control ESR experiments of reaction mixtures (buffer + α-farnesene + **4**) without fungal lyophilisate or solutions (buffer + α-farnesene without **4**) with Fe^{2+} only gave no ESR signals. A buffer solution containing α-farnesene and **4** but treated with Fe^{2+} instead of fungal lyophilisate gave an ESR spectrum with coupling constants similar to those observed in the other experiments; however, the line widths were broader (**Figure 3**). The similarity of ESR spectra obtained with Fe^{2+} indicates that the autoxidation of α-farnesene occurs at least partly along the same reaction (radical) mechanism. Willershausen and Graf (*20*) reported that the supplementation of a valencene-containing phosphate buffer with $FeSO_4$ at different pH values yielded 4 times more nootkatone compared to Fenton's reagent and hydrogen peroxide alone. A detailed mechanism of the reaction was not described.

Table 2. Mass Spectrometric Data (*m*/*z*, Relative Abundance)

3, EI	**5**, EI	**5**, CI	**2**, EI
87 [$M^{\bullet+}$] nd	291 [$M^{\bullet+}$] (0.1)	292 [MH^+] (28)	220 [$M^{\bullet+}$] (1.0)
72 [$M^+ - CH_3$] (3.6)	276 [$M^+ - CH_3$] (3.1)	291 (63)	205 ($M^+ - CH_3$, 4.3)
57 [$M^+ - NO$] (39)	222 (9.6)	290 (45)	202 ($M^+ - H_2O$, 9.1)
41 (100)	204 (10.9)	277 (10)	187 (4.7)
39 (47)	166 (4.0)	276 (46)	175 (8.5)
	148 (9.0)	265 (3.5)	162 (45)
	119 (15)	236 (53)	159 (30)
	107 (24)	**208 (4)**[a]	119 (18)
	93 (43)	204 (28)	105 (27)
	82 (55)	189 (11)	95 (30)
	72 (71)	166 (18)	93 (39)
	69 (100)	154 (39)	69 (41)
	57 (69)	82 (100)	43 (100)
		72 (83)	

[a] Indicating the fragmentation in α-position to the spin trap attached at C7 of α-farnesene.

The ESR spectra of α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone spin adducts characterized the fungal bioconversion of α-farnesene as a radical-mediated mechanism, but did not allow the localization of the carbon-centered radical position within the α-farnesene molecule. Experiments were therefore pursued using spin trap **3**, because ESR spectra of 2-methyl-2-nitrosopropane spin adducts can give information about the structure of the trapped radicals. The first ESR signals appeared already after 1 h and increased without alteration of the signal shape over time. A complex ESR spectrum was observed when α-farnesene was contacted with an *A. niger* lyophilisate in the presence of **3** (**Figure 4**). The experimental spectrum could be satisfactorily fitted to a digitally simulated spectrum based on a mixture (**Figure 4C**,**D**) of a spin adduct giving a triplet of lines (a_N = 1.03 mT, relative integrated intensity = 65%) and a spin adduct giving a triplet of doublet lines (a_N = 1.41 mT and a_H = 0.34 mT, relative integrated intensity = 35%). The hyperfine coupling patterns indicate that the two types of trapped radicals were a tertiary carbon-centered radical and a secondary carbon-centered radical, respectively.

Control experiments with spin trap **3** in the buffer solution did not result in ESR signals, whereas **3** added to *A. niger* lyophilisate (MOPS buffer) gave a strong ESR signal (**Figure 5**). However, the shape and coupling constants of these ESR signals were quite different from those obtained with α-farnesene. Digitally simulated spectra based on a mixture of a radical with a triplet signal (a_N = 1.69 mT) and a radical giving a triplet of doublet lines (a_N = 1.39 mT and a_H = 0.33 mT) showed a good fit to the experimental ESR spectrum. The first of the two radicals was assigned to the di-*tert*-butylnitroxyl radical, which

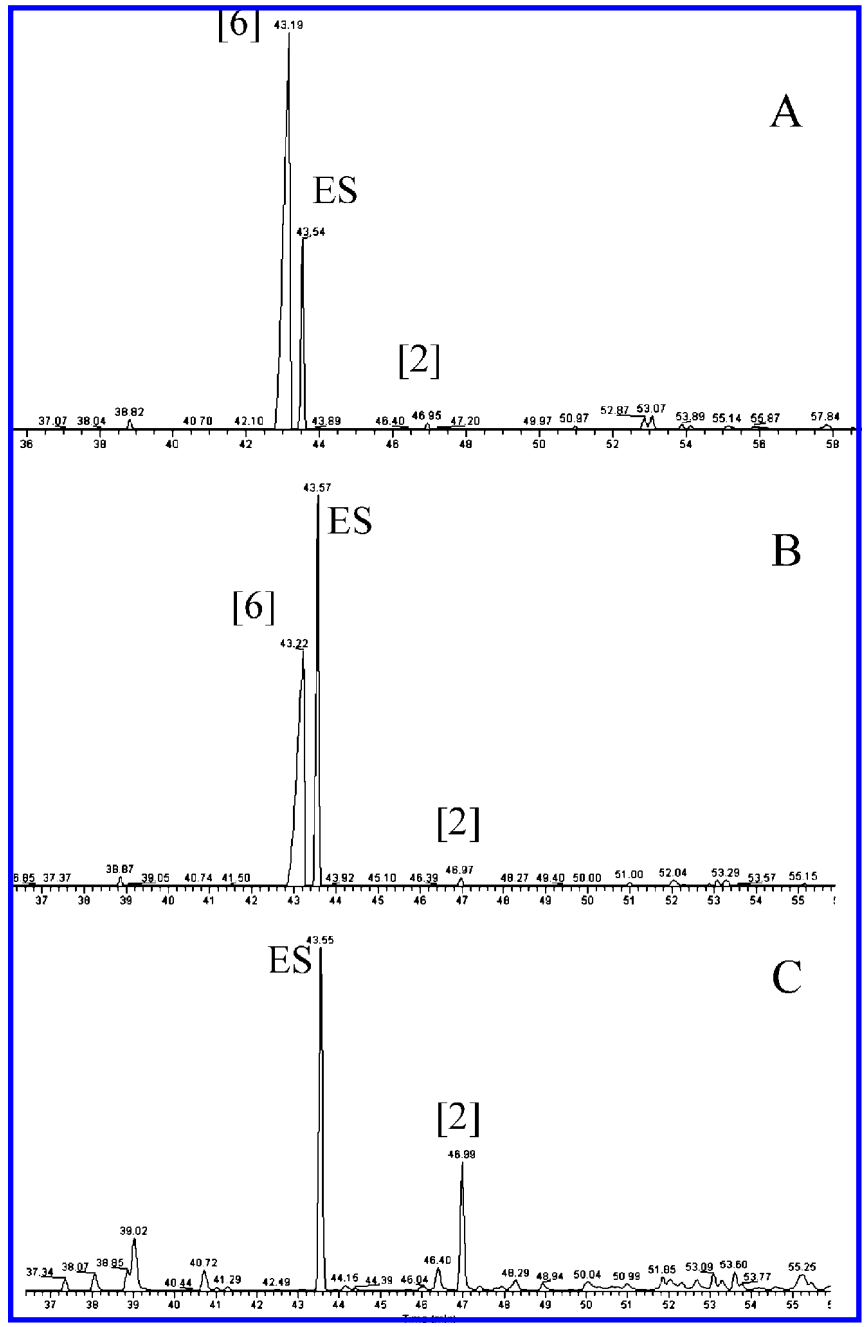

Figure 6. GC-MS chromatograms of α-farnesene (10 μL) bioconversion in 1.5 mL of MOPS buffer (pH 7.0) for 24 h using 2-methyl-2-nitrosopropane (**3**) for spin trapping: (**A**) *A. niger*; (**B**) Fe^{2+}; (**C**) *A. niger* (50 mg) without the addition of **3**. ES, external standard; **2**, 7-hydroxyfarnesene; **6**, reduced spin adduct of **3** of the C7 radical of α-farnesene.

is a common degradation product of **3** (*19*). A second control experiment, wherein α-farnesene in the presence of **3** was mixed with Fe^{2+} instead of *A. niger*, gave an ESR spectrum that could be fitted by digital simulation to the ESR spectrum arising from a single radical with $a_N = 1.41$ mT and $a_H = 0.34$ mT (**Figure 5**). The similarity of the coupling constants suggested that the latter radical was formed in all three experiments. It was also formed in the control experiment without α-farnesene, and it must therefore be assigned to a spin adduct formed by trapping of secondary radicals that are generated from the oxidation of the MOPS buffer or **3** itself.

GC-MS Analyses of Spin-Trapped Farnesene. The tertiary radical that gave rise to a spin adduct with a triplet ESR spectrum in the experiment with α-farnesene and *A. niger* must be formed by oxidation of α-farnesene because it was not observed in the control experiments. This could be either a C3- or a C7-centered α-farnesene radical; however, the exact localization of the radical center could not be derived from the ESR experiments. Detailed information of the molecular structure was derived using mass spectrometry. The molecular size can be obtained by soft ionization techniques such as chemical (CI), electrospray (ESI), or field (FI) ionization. Significant structural information is gathered by hard ionization techniques such as electron impact (EI) ionization or advanced MS/MS techniques. GC-MS analysis of experiments conducted with spin trap **3** demonstrated that the spin adduct was sufficiently stable to be analyzed by a GC equipped with cool on-column injection. The EI mass spectrum of **3** (**Table 2**) was characterized by a missing $M^{\bullet+}$ ion ($m/z = 87$) and intense ions at $M - CH_3$ (m/z 72) and M

− NO ion ($m/z = 57$). The GC-MS chromatograms of solvent extracts of bioconversion buffers after 24 h of lyophilisates of *A. niger* are shown in **Figure 6**. In the absence of **3** all bioconversion products including the main product 7-hydroxyfarnesene (**2**) were generated. However, in the presence of spin trap **3** the generation of α-farnesene transformation products was almost completely suppressed, but a large new GC peak emerged. At the same retention time a GC peak was detectable in the Fe^{2+} system. In both cases this new compound was assumed to be the corresponding 2-methyl-2-nitrosopropane adduct (**5**) of the same carbon-centered α-farnesene radical. The generation of α-farnesene oxidation products was suppressed with antioxidants such as butylated hydroxyanisole (BHT) and in the absence of oxygen as well, supporting the radical mechanism (data not shown). The mass spectrometric data of this new compound (EI, CI) are listed in **Table 2**. The molecular ion of the spin adduct radical was expected at 290 u. In the EI mass spectrum the highest *m/z* value was at *m/z* 276. The mass spectrum of **3** itself does not show a molecular ion either, and $M^+ - CH_3$ was the largest fragment ion. The corresponding fragment ion of the proposed α-farnesene spin adduct (**5**) of **3** should have occurred at *m/z* 275, but was found at *m/z* 276. However, this is not a contradiction in terms: Although the spin trap radicals are much more stable than the short-lived genuine radicals, they are still reactive compounds and can, for example, abstract hydrogen atoms from a neighbor compound (**Figure 2**). It is not likely that a reactive radical can be analyzed by means of GC without any alteration. The molecular mass of the hydrogenated α-farnesene spin adduct (**6**) was confirmed using methane as the CI reactant gas. Intense ions at *m/z* 292 (MH^+) and *m/z* 290 ($MH^+ - H_2$) were detected. Further intense fragment ions of the EI mass spectrum of **6** at *m/z* 222 and 204 were derived from the cleavage of the 2-methyl-2-nitrosopropane (**3**) moiety (−87) and the prenyl moiety (−69), respectively (**Figure 2**). An important fragment ion occurred at *m/z* 208, which gave evidence as to which position of α-farnesene (**3**) was attached. This ion can be explained by a cleavage of the C7,8 bond, which is in allylic position to the C5,6 double bound and in α-position to the nitrogen atom of the 2-methyl-2-nitrosopropane (**3**) moiety. The α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone farnesene adduct (**5**) was not amenable to GC and GC-MS analyses because of insufficient volatility.

Positional Localization of the Intermediary Farnesene Radical. The identification of α-farnesene metabolites obtained from feeding experiments using two ascomycetes, *A. niger* and *P. solitum*, respectively, allowed the derivation of a putative reaction mechanism. The isomerization of isolated double bonds into conjugated double bonds as well as the racemic mixture analyzed for the main bioconversion product 7-hydroxyfarnesene (*7*) indicated an intermediate carbocation or a carbon-centered radical at the C7 carbon atom of α-farnesene. ESR spin trapping offered the possibility to detect intermediate carbon-centered radicals (*21, 22*). The detection of both spin adducts using ESR (**3**, **4**) and GC-MS (**3**) in combination allowed for the determination of the exact radical position in the molecule.

The interplay of two independent methods confirmed that the generation of 7-hydroxyfarnesene by *A. niger* and *P. solitum* occurred along the radical mechanism as shown in **Figure 1**. The coupling constants of the 2-methyl-2-nitrosopropane (**3**) ESR signal and the mass spectrum of the spin adduct proved that the initial secondary carbon radical was delocalized along the double bonds. Therefore, the most intense ESR signal was caused by the more stable tertiary radical at C7. These results are in full accordance with the finding that the C7 position was preferred for the oxidation of α-farnesene, yielding 7-hydroxyfarnesene (*7*).

ABBREVIATIONS USED

MOPS, 4-(*N*-morpholino)butanesulfonic acid; ESR, electron spin resonance; [$M^{\bullet+}$], molecule radical cation; FID, flame ionization detector; EI, electron impact ionization; CI, chemical ionization.

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