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Biosynthesis of C_{11} and C_{16} Homoterpenes in Higher Plants; Stereochemistry of the C-C-Bond Cleavage Reaction

Wilhelm Boland*, Andreas Gäbler, Matthias Gilbert and Zhuofu Feng

Max-Planck-Institut für Chemische Ökologie, Tatzendpromenade 1a, D-07745 Jena, Germany

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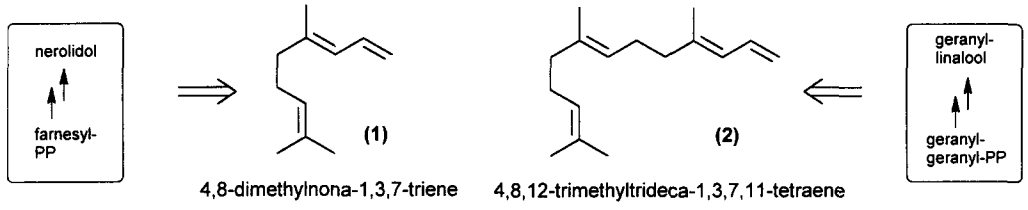
Abstract: In higher plants the C_{11} homoterpene 4,8-dimethylnona-1,3,7-triene **(1)** originates from oxidative degradation of nerolidol **(3)** or geranylacetone **(4)**. The geometry of the transition state of bond cleavage has been shown to be *syn*-periplanar, for both **3** and **4** by application of chirally labelled, deuterated precursors to flowers of *Magnolia liliiflora nigra* or leaves of the Lima bean *Phaseolus lunatus*. The synthesis of the deuterated, chiral metabolic probes **3** and **8** from nor-farnesene **(5)**, *via* the enantiomeric epoxides (2*S*,3*R*)-**6** and *ent*-**6**, is described.

Introduction.- In higher plants the C_{11} homoterpene **1**, and its higher homologue 4,8,12-trimethyltrideca-1,3,7,11-tetraene **(2)**, originate from nerolidol and geranyllinalool respectively.[1,2] Both hydrocarbons often contribute to the so called "White-Floral Image" of night-scented flowers (e.g. Orchidaceae, Cactaceae, Magnoliaceae, Liliaceae), and it has been suggested that they play a role in the pollination biology of these plants.[3] In addition, **1** and/or **2** are released from leaves of angiosperms that have been injured by herbivores.[4,5] Together with other volatiles they act as airborne „alarm calls", attracting insectivores to the damaged plant, leading to a reduction in the population of the attacking herbivore.[6,7] Due to the high ecological impact of **1** and **2**, a detailed knowledge of their biosynthesis is essential to understand the sequence of events between primary leaf damage and the later emission of the *de novo* synthesised volatiles.[8,9] According to previous studies, the C_{11} homoterpene **1** originates from oxidative degradation of the sesquiterpenoid alcohol nerolidol **(3)**, while the C_{16} compound **2** is a metabolite of the diterpenoid geranyllinalool.[1,2,10]



The oxidative degradation of nerolidol to **1** can be formally rationalised as single step fragmentation yielding **1** and but-1-en-2-one as the second fragment. However, but-1-en-2-one has yet to be detected. In addition, a degradation sequence *via* geranylacetone **(4)** *en route* to **1** seems to occur, since small amounts of **4** sometimes accompany **1** in flower and leaf volatiles.[11] **4** is expected to be cleaved to yield **1** and acetic acid. According to previous studies both compounds can, in fact, serve as precursors and their transformation into **1** proceeds with exclusive loss of the same allylic hydrogen atom, namely the C(4)-H_S from geranylacetone and the C(5)-H_S from nerolidol.[2] The geometry of the transition state and the actual mechanism of the bond cleavage reaction are, as yet, unknown. On the other hand, the type of the oxidative C-C-bond cleavage involved here is widespread in nature and resembles, for example, certain dealkylations of steroids[12] or the oxidative degradation of

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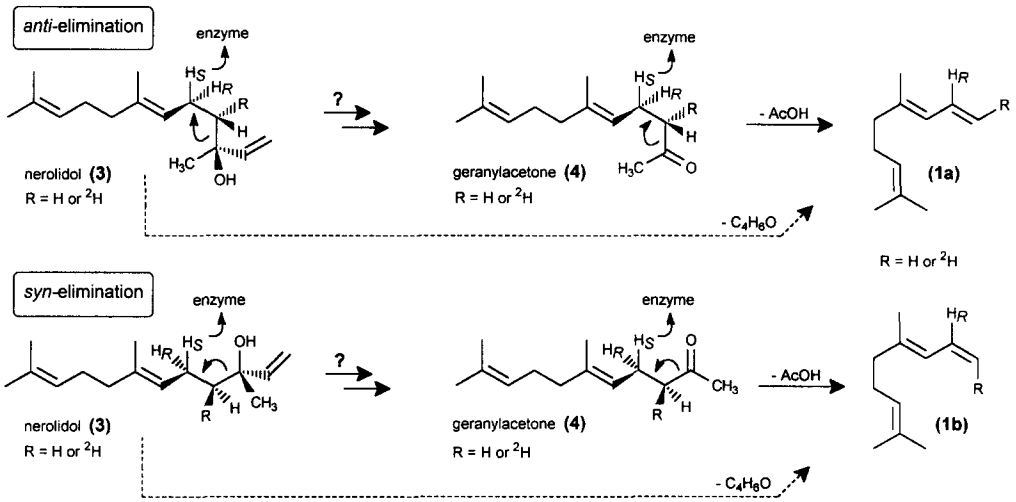
(+)-marmesin into the furanocoumarin psoralene,[13] all catalysed by enzymes from the family of cytochrome P450. With only one exception,[14] these reactions proceed as *syn*-eliminations of an oxygen-carrying functional group together with a hydrogen atom from a neighboured β-carbon atom (β-elimination). In this context, determination of the stereochemical course of the bond cleavage reaction leading from nerolidol **(3)** or geranylacetone **(4)** to the homoterpene **1** is of particular interest, since in this case an acyclic precursor is the substrate. Unlike the dealkylations of steroids or furanocoumarins, no severe constraints of the *C*-skeleton of **3** or **4** on the geometry of the transition state exist and, hence, the observed stereochemical course may be indicative of mechanistically inherent features of the bond cleavage reaction.



Scheme 1. Evaluation of the stereochemical course of oxidative degradation of nerolidol or geranylacetone to the homoterpene **1** using chiral metabolic probes.

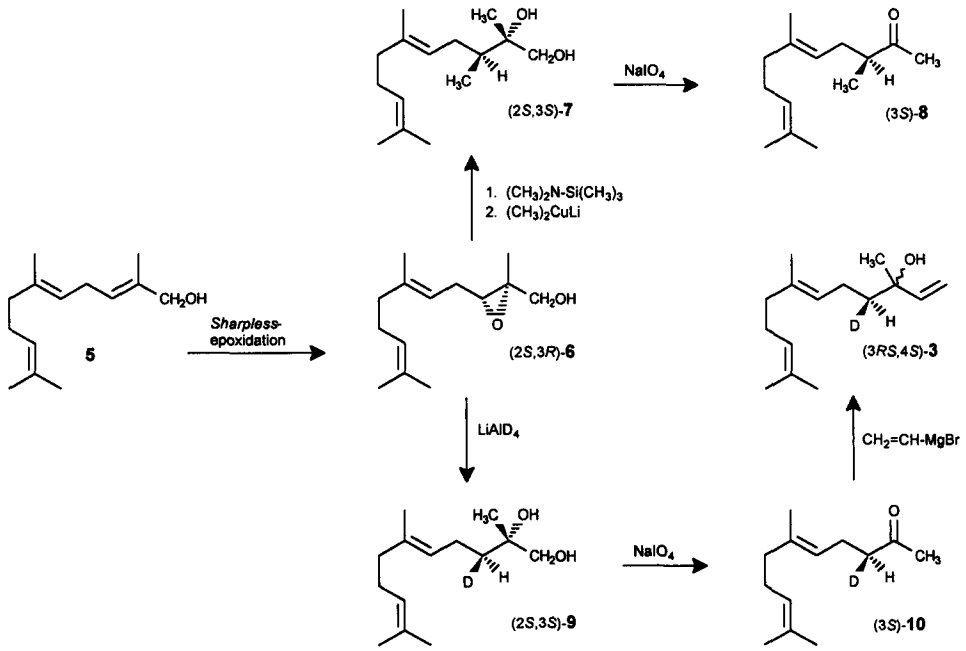
As depicted in Scheme 1, the stereochemical course of the bond cleavage reaction can be determined by using either labeled nerolidol (at C(4)) or labeled geranylacetone (at C(3)). If the degradative pathway proceeds, indeed, from nerolidol *via* geranylacetone, both precursors are expected to yield the same stereoisomer of labelled **1**. If tolerated by the enzyme, a methyl group at C(3)/(4) of the precursors would be of special value, since in this case the stereochemistry of the metabolites can be readily determined by GLC (separation of *E*- or *Z*-**14**), while the configuration of the deuterated metabolites is only available from less sensitive IR- or NMR approaches. Table 1 summarises the relation between the configuration of the metabolic probes and the stereochemistry of the resulting olefin with respect to the stereochemical mode of the bond cleavage reaction.

precursor	R	*syn*-elimination	*anti*-elimination
(*3RS,4S*)-**3**	^2H	*cis*-**1**	*trans*-**1**
ent-**3**	^2H	*trans*-**1**	*cis*-**1**
(*3S*)-**4**	CH_3	*cis*-**14**	*trans*-**14**
ent-**4**	CH_3	*trans*-**14**	*cis*-**14**

Table 1. Stereochemistry of olefinic metabolites with respect to the configuration of the precursors and the stereochemical mode of the bond cleavage.

Here we report that the oxidative bond cleavage reaction proceeds by *syn*-elimination of the polar head together with the allylic C(4/5)-H_S hydrogen atom (β-cleavage), for both the nerolidol- and geranylacetone precursors. For acyclic substrates this is the first report on the stereochemical course of such an oxidative bond cleavage reaction. Highly enantioselective syntheses of deuterium labelled or structurally modified metabolic probes related to nerolidol **(3)** and geranylacetone **(4)** are described.

Synthesis of Metabolic Probes and References.- To evaluate the transition state geometry of the bond cleavage reaction, depicted in Scheme 1, it is essential to have a reporter substituent at C(4) of **3** or **4**. Particularly well suited for the introduction of such a group is a chiral epoxide like (2*S*,3*R*)-**6** (cf. Scheme 2), since the introduction of a deuterium atom and of a methyl group can be readily achieved from the same precursor. Moreover, the resulting intermediates can be elaborated further to nerolidol- and geranylacetone-type of metabolic probes as outlined in Scheme 2. The required nor-farnesol **(5)** is available in large quantities from geranylbromide[15] and directly furnishes the enantiomeric epoxyalcohols (2*S*,3*R*)-**6** and *ent*-**6** by a Sharpless epoxidation.[16]

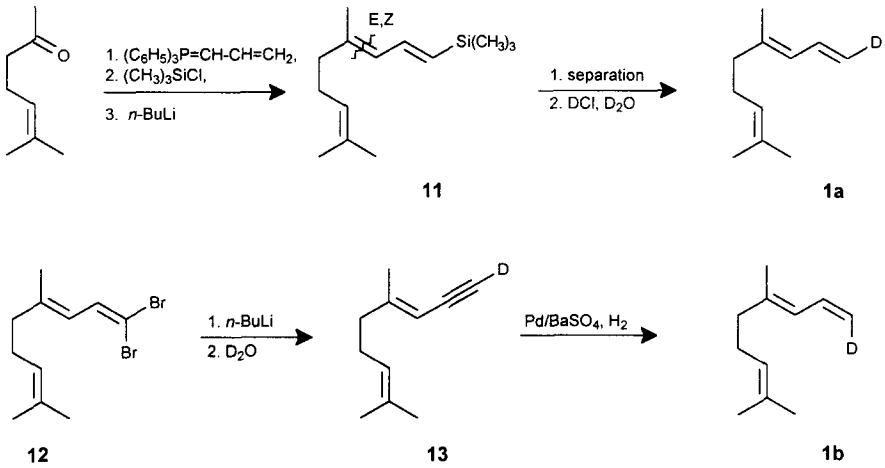


Scheme 2. Synthesis of metabolic probes. The same sequence was followed to synthesise the enantiomers *ent*-**3** and *ent*-**6** → *ent*-**10**.

The enantiomeric epoxides were of high optical purity (> 96% ee) as could be shown by ^{1}H NMR of the corresponding Mosher esters.[17] If the O-TMS ether of the 2,3-epoxyalcohol (2*S*,3*R*)-**6**, prepared in situ upon treatment of **6** with $(CH_3)_2N$-$Si(CH_3)_3$, was reacted with an excess $(CH_3)_2CuLi$, the oxirane was alkylated exclusively at the less hindered carbon atom yielding (2*S*,3*S*)-**7**.[18] The same regioselectivity was observed upon $LiAlD_4$ reduction of the unprotected epoxyalcohol (2*S*,3*R*)-**6** to the diol (2*S*,3*S*)-**9**. Cleavage of the diols (2*S*,3*S*)-**7** or (2*S*,3*S*)-**9** with $NaIO_4$ proceeded under neutral conditions and furnished the two ketones (3*S*)-**8** and (3*S*)-**10**. Addition of vinylmagnesium bromide to (3*S*)-**10** yielded the labelled nerolidol ((3*RS*,4*S*)-**3**). Chi-

rality at C(3) was not essential, since out of a racemic mixture the enantiomer (3*S*)-**3** was selectively metabolised without inhibition by the other enantiomer.[19] Repetition of the sequence with *ent*-**6** yielded the enantiomeric metabolic probes *ent*-**3**, *ent*-**8** and *ent*-**10**.

The synthesis of the stereoisomeric metabolites (*E*)-**1a** and (*Z*)-**1b** (R = ^{2}H, cf. Scheme 1) is outlined in Scheme 3. Wittig olefination of 6-methylhept-5-en-2-one with (3-trimethylsilylpropenylidene)triphenylphosphorane, prepared in situ from propenylidenetriphenylphosphorane and TMSCl,[20] yielded **11** as a mixture of stereoisomers with the (*E*)-isomer prevailing (*E*:*Z* ≈ 85:15). Separation of the two isomers was achieved by preparative GLC. Protodesilylation of (*E*)-**11** with DCl proceeded without side reactions and yielded configurationally pure (*E*)-**1a**.[21] The corresponding (*Z*)-isomer **1b** was obtained from geranial via the dibromide **12** by dehydrohalogenation and hydrolysis of the resulting lithium acetylide with D_2O.[22]



Scheme 3. Synthesis of (*E*)- and (*Z*)-[1-^{2}H]-homoterpenes

Hydrogenation of the acetylene [1-^{2}H]-**13** with Lindlar's catalyst under carefully optimised conditions afforded (*Z*)-[1-^{2}H]-**1b** in good yield. The reduction of **13** with Zn(*Cu,Ag*),[23] activated by successive treatment with Cu(II) and Ag(I) salts, was principally possible, but led to a considerable ^{2}H/^{1}H- exchange. 5,9-Dimethyl-deca-2*E*,4,8-triene ((*E*)-**14**) and 5,9-dimethyl-deca-2*Z*,4,8-triene ((*Z*)-**14**) were obtained from geranial by Wittig reaction with ethylidenetriphenylphosphorane and separation of the isomers by preparative GLC.[24]

Evaluation of the Stereochemistry of Homoterpene Biosynthesis.- If freshly cut plantlets of the Lima bean (*Phaseolus lunatus*) were placed into aq. emulsions of (3*RS*,4*S*)-**3** or *ent*-**3**, the administered precursor was rapidly metabolised, and the resulting homoterpene was released to the atmosphere. Untreated leaves, kept under the same conditions, did not produce **1**. The volatiles were collected by air circulation on activated carbon as described.[19] Subsequent GLC/MS analysis secured that the released homoterpenes **1a** or **1b** were produced exclusively from the externally offered precursor **3**, since only a molecular ion at m/z 151 *Da* was observed indicating that the metabolite was not contaminated with unlabeled natural material (*m/z* = 150 Da).[2] A sensitive on-line method like GLC-FTIR is particularly well suited for the evaluation of the configuration of the very low amounts of the deuterated metabolites, using the synthetic references of (*E*)-**1a** and (*Z*)-**1b** for an unambiguous deduction of the stereochemistry. As shown in Figure 1, the synthetic references (*E*)-**1a** and (*Z*)-**1b** (dotted lines) exhibited, indeed, characteristic absorbtions. The (*E*)-isomer (Figure 1b) showed a vinyl twist band at 976 cm^{-1}, while the (*Z*)-isomer (Figure 1a) displayed a typical vinyl wagging band at 798 cm^{-1}. The absorbances at 1384, 1447 and 1647 cm^{-1} were common to both isomers. Considering now that (3*RS*,4*S*)-**3** is

converted into the (*Z*)-isomer **1b** and that (*3RS,4R*)-**3** yields the (*E*)-isomer **1a**, the bond cleavage reaction, depicted in Scheme 1, has to proceed as a formal *syn*- elimination (cf. Table 1 and Scheme 4). Due to the almost perfect coincidence between the gasphase infrared spectra of the synthetic references **1a** and **1b** and those of the corresponding metabolites (Figure 1a and 1b), a defined geometry of the transition state is proven. Analogous results were obtained with the same precursors administered to inflorescences of *Magnolia liliiflora nigra*.[1,2] Again, the emitted homoterpenes proved to be homogeneous products of a formal *syn*-elimination of the polar head, together with the C(5)-H_S hydrogen atom.

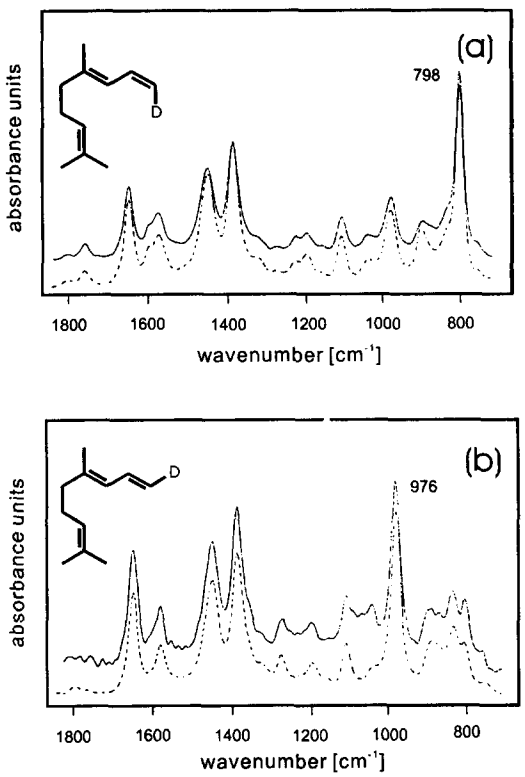
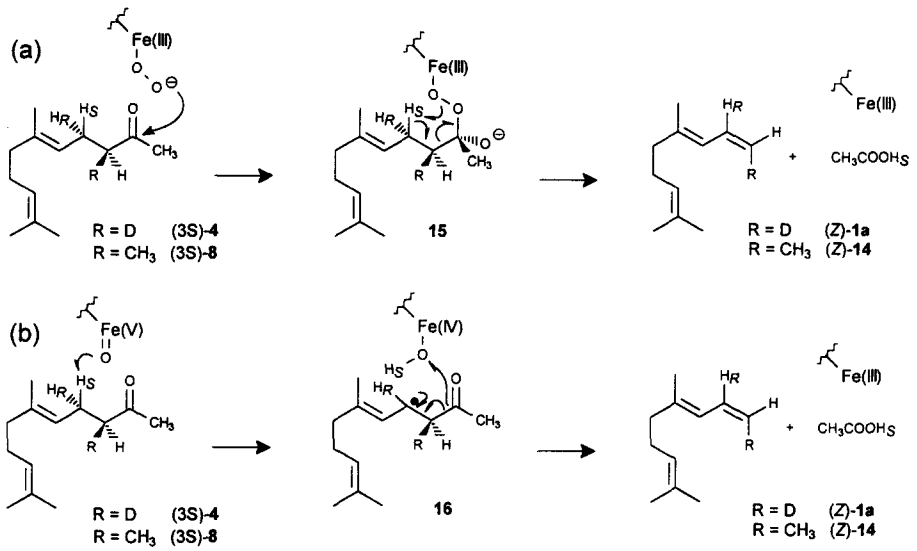


Figure 1. Gasphase infrared spectra of the labeled metabolites **1a** and **1b** and of the corresponding synthetic references. References: --------. Metabolites: ——— . (a) homoterpene metabolite from administration of (*3RS,4S*)-**3**. (b) homoterpene metabolite from administration of (*3RS,4R*)-**3** (= *ent*-**3**).

The enantiomeric 3-methylgeranylacetones (3*S*)-**8** and *ent*-**8** also proved to be substrates, and both compounds were smoothly metabolised to the corresponding conjugated dienes (*E*)-**14** and (*Z*)-**14** albeit with lower yields than the labelled nerolidols. Again, in line with a *syn*-elimination (3*S*)-**8** (cf. Scheme 1, R = CH_3, 89% ee according to GLC on a chiral stationary phase) was metabolised to a mixture of (2*Z*)-**14** (66%) and (2*E*)-**14** (34%). From the enantiomer (3*R*)-**8** (88% ee) the isomer (2*E*)-**14** (75%) was formed in excess and (2*Z*)-**14** (25%) became the minor isomer (cf. Figure 1 and Table 1). As a racemic precursor of the type **8** yielded (*E/Z*)-**14** in equal amounts, a notable influence of the methyl group on the geometry of the transition state can be ignored. The low configurational purity of the metabolites (*E*)-**14** and (*Z*)-**14** was in contrast to the findings with deuterium labelled nerolidol precursors **3**. It can be attributed to the facile racemization of the two ketones (3*S*)-**8** and *ent*-**8**, upon contact with acidic or basic plant constituents, prior to the fragmentation. The rather different configurational purity of the metabolites (*E*)-**14** and (*Z*)-**14**, produced from (3*S*)- or *ent*-**8**, of virtually the same *ee* (89%) strongly supports this assumption.

Mechanistic Aspects of Homoterpene Biosynthesis.- Oxidative bond cleavage reactions leading to olefins, as discussed here, appear to be generally catalysed by enzymes from the family cytochrome P450. Examples are (*vide supra*) dealkylations of steroids,[12] the biosynthesis of furanocoumarins[13] as well as a number of ring opening reactions leading to *seco*-compounds, e.g. *seco*-loganin. Typical for most of these transformations is a sequential oxidation of the substituent to a carbonyl group (-CH_3 → -CH_2OH → -CHO) prior the final bond cleavage reaction, which proceeds as a *syn*-elimination of the carbonyl group together with a hydrogen atom attached to a β-carbon atom (β-cleavage). Owing to the finding that both, nerolidol **(3)** and geranylacetone **(4)**, are metabolised to the homoterpene **1** by loss of the same hydrogen atom, from the allylic methylene group, *via*

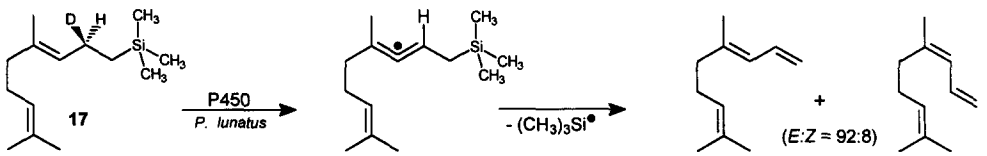
a *syn*-periplanar transition state geometry, it is reasonable to assume that the degradation of nerolidol **(3)** can proceed in a sequential fashion with geranylacetone **(4)** as an intermediate. To cope with the peculiar stereochemistry of such bond cleavage reactions, Akhtar et al. have proposed an alternate mode of action of the P450 enzymes, by assuming a nucleophilic attack of an intermediate Fe(III)-O-O$^-$ onto the carbonyl carbon atom of the substrate, followed by a homolytic cleavage of the labile O-O-bond of the ensuing peroxyhemiketal (Scheme 4a).[12] According to the „cyclic character" of the transition state, this mechanism readily accounts for the observed *syn*-elimination. Such a mechanism does not, however, follow the well established mode of action of P450 enzymes, and, up to now, no convincing experimental evidence has been published. Moreover, as shown recently, the formally related transformation of (+)-marmesin into psoralene clearly does not proceed through a peroxyhemiketal, since no carbonyl intermediate is involved.[13] While in all previous examples, the rather bulky *C*-skeleton of the substrates may influence the geometry of the transition state, this is different in the present case. The two acylic substrates nerolidol **(3)** or geranylacetone **(4)** can adopt conformations which allow the formation of an olefin *via* a *syn*- or an *anti*-periplanar orientation of the relevant groups with equal ease (cf. Figure 1). Therefore, an exclusive *syn*-elimination described here, should be inherently linked to the mechanism of this type of oxidative bond cleavage reactions.



Scheme 4. Mechanistic alternatives accounting for a *syn*-elimination. (a) Involvement of a cyclic peroxyhemiketal according to Ahktar.[12] (b) Conventional P450 radical mechanism; *syn*-elimination as a consequence for the need of a rapid acyl radical transfer. Analogous considerations are valid for a direct transformation **3** → **1**. Only formal charges of the Fe-centre are given.

Although the assumption of a cyclic peroxyhemiketal intermediate like **15** could account for the observed stereochemistry, a typical P450 type mechanism involving an allylic radical **16**, as outlined in Scheme 4b, is much more likely. **16** immediately stabilises by β-cleavage yielding the homoterpenes **1a** (R = ^{2}H) or (*Z*)-**14** (R = CH_3) and an acyl radical which, in turn, immediately attacks the neighboured Fe-centred hydroxyl group, generating acetic acid and a reduced cytochrome catalyst. The *syn*-periplanar orientation of the β-hydrogen atom and the acyl moiety is, thus, nothing but the direct consequence of the need for a rapid transfer of the Fe-bound hydroxyl group to the reactive acyl radical. The mechanism, as outlined in Scheme 4b, also matches the observation that the chiral silicon analogue (2*S*)-**17** of nerolidol (cf. Scheme 5) is metabolised by leaves of *P.*

lunatus to the homoterpene **1**. Again, the allylic hydrogen atom C(4)-H_S is lost preferentially indicating that the compound may be converted by the same cytochrome P450 enzyme as the genuine precursors **3** and **4**. Moreover, the metabolite was obtained as a mixture of 3*E*,*Z*-isomers (*E*:*Z* =92:8) strongly suggesting an isomerisation process of an intermediate allylic radical prior to fragmentation.



Scheme 5. Oxidative degradation of heteroatom analogues of geranylacetone **4** by enzymes from *Phaseolus lunatus*.

The β-silicon, next to the radical, on one hand stabilises the radical and on the other hand allows a rapid fragmentation of the reactive intermediate into an olefin and a silyl radical which finally attacks the Fe-centred hydroxy group.[25] Clearly, the silicon analogue **17** can not be degraded to a geranylacetone analogue prior to the final bond cleavage reaction. Unlike a previous example from the field of steroids[26] in this case no inhibitory effects were noticed. In a similar fashion, analogues containing a thioether (S-CH_3) or a sulfoxide (SO-CH_3) moiety instead of the trimethylsilyl group, were smoothly converted into **1**. Together with the observation that the neighbouring double bond proved to be essential (6,7-dihydronerolidol was not converted), especially the transformations of the artificial analogues like **17** and the related sulfur compounds, provide strong arguments for an initial attack of the enzyme at the allylic hydrogen atom, as outlined in Scheme 4b. Determination of whether nerolidol is generally degraded to **1** *via* geranylacetone, or whether direct conversion of **3** into **1** and but-1-en-2-one also occurs, and to which extent both pathways may be utilised, has to await the results of ongoing studies with isolated enzymes.

Experimental

General. Reactions were performed under Ar. Solvents and reagents were purified and dried prior to use. Anh. $MgSO_4$ was used for drying operations. Solutions were usually concentrated by flash evaporation under reduced pressure. Anal. GLC: Carlo Erba gas chromatograph, HRGC 5300, Mega series, equipped with fused-silica capillaries, SE 30 (10m x 0.31mm); H_2 at 30 cm/sec served as carrier gas. Polarimetry: Perkin-Elmer 241 polarimeter. IR (cm^{-1}): Perkin-Elmer 882 Infrared-Spectrophotometer. GLC/FTIR (cm^{-1}): Bruker IFS 48 GC/FTIR instrument, combined with a Carlo Erba gas chromatograph, model Mega, HRGC 5300. Compounds were separated on a BP 1 fused silica column (25 m x 0.32 mm) under programmed conditions (50° for 2 min, then at 10° /min to 250°). Light pipe: 250°. A sampling rate of 8 scans/sec, averaged to a single scan, was used. ^1H NMR (250 MHz or 400 MHz, $CDCl_3$, TMS as internal standard): Bruker Cryospec WM 250 and Bruker WM 400. MS (m/z): Finnigan MAT 90 and Finnigan ITD 800 combined with a Carlo Erba gas chromatograph, model Vega; He at 30 cm/sec as carrier gas. Anal. TLC: 20 x 20 cm TLC plates, SiO_2 60 F254, layer thickness 0.2 mm (Macherey & Nagel, Düren, FRG).

(2*E*,5*E*)-2,6,10-Trimethylundeca-2,5,9-trien-1-ol (5).

2-Methyl-3-(phenylsufinyl)prop-2-ene[15] (8.5 g, 47.2 mmol) was added with stirring to a cold solution (-60°) of Li-N(*i*-$C_3H_7)_2$ (52 mmol) in THF (130 ml). After 15 min the resulting anion was alkylated by addition of geranyl bromide (12.2 g, 56.67 mmol). The temp. was maintained, with stirring, at -30° until the yellow colour

faded (1 h). Then, a sat. aq. NH_4Cl (30 ml) was added, and the product was extracted with CH_2Cl_2 (3 x 50 ml). Following removal of the solvents, the crude residue was redissolved in MeOH (50 ml) and $(CH_3O)_3P$ (11.65 g, 94.4 mmol, freshly distilled from Na) was added. Stirring was continued for 12 h and after extractive work-up and removal of solvents, **5** was purified by distillation. Yield: 3.7 g (38%). Bp.: 88° at 0.1 Torr. IR (KBr, film) ν: 3341s (br.), 2972s, 2919s, 2860s, 1669w, 1448m, 1379m, 1013m, 833w cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ: 5.40 (t, H-C(9)); 5.15-5.08 (m, H-C(3) and H-C(5)); 4.01 (s, 2 H-C(1)); 2.74 (t, 2 H-C(4)); 2.09-1.08 (m, 2 H-C(7) and 2 H-C(8)); 1.70 (s, CH_3-C(2)); 1.67 (s, 3 H-C(11)); 1.63 (s, CH_3-C(10)); 1.60 (s, CH_3-C(6); 1.48 (s, OH). MS (70 eV) *m/z*: 165 (2,$M^{+\bullet}$-$COCH_3$), 147(2), 135(3), 123(23), 107(9), 93(20), 91(13), 81 (41), 69(52), 67(22), 55(22), 41(100). HR-MS: calcd. for $C_{14}H_{24}O$ ($M^{+\bullet}$): 208.1827, found: 208.1789.

(2*S*,3*R*)-2,3-Epoxy-2,6,11-trimethylundeca-5,9-dienol ((2*S*,3*R*)-6).

A cold (-23°) and well stirred solution of Ti(*i*-PrO)$_4$ (7.1 g, 25.0 mmol) in dry CH_2Cl_2 (200 ml) was treated gradually with L-(+)-diethyl tartrate (4.46 ml, 26.0 mmol). The complex was "aged" for 5 min, prior to addition of the nor-farnesol **5** (5.0 g, 24.04 mmol). Then, *t*-BuOOH (22.2 ml, 48.1 mmol, 3M solution in *i*-octane) was injected, and stirring was continued for 8 h. The mixture was hydrolysed with aq. tartaric acid (50 ml of a 10% solution) for 30 min at -23° and further at rt. for 1 h. Then, the organic layer was separated, washed with H_2O (20 ml) and Et_2O was added (150 ml). The ethereal layer was stirred with 1N NaOH (70 ml) for 30 min at 0°. Usual workup and chromatography on silica gel using pentane/Et_2O (70:30, v:v) for elution furnished (2*S*,-3*R*)-**6** as a colourless viscous oil. Yield: 4.5 g (84%). $[\alpha]^{589}_{21}$= -10.8 (c = 9.4, $CHCl_3$). IR (KBr, film) ν: 3438s (br.), 2970s, 2928s, 2860s, 1667w, 1449m, 1382m, 1074m, 1037s, 890w, 858w cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ: 5.17 (t, H-C(9)); 5.09 (t, H-C(5)); 3.73-3.53 (m, 2 H-C(1)); 3.05 (t, H-C(3)); 2.38 (m, H-C(4)); 2.21 (m, H-C(4)); 2.05 (m, 2 H-C(7), 2 H-C(8) and OH); 1.68 (s, 3 H-C(11)); 1.64 (s, CH_3-C(10)); 1.60 (s, CH_3-C(6)); 1.32 (s, CH_3-C(2)). MS (70 eV) *m/z*: 137 (3,$M^{+\bullet}$-C_3H_6O), 123(5), 107(10), 95(8), 81(11), 69(68), 67(19), 53(12), 41(100). HR-MS: m/z calcd. for $C_{14}H_{22}O$ ($M^{+\bullet}$-H_2O): 206.1655, found: 206.1653.

(2*R*,3*S*)-2,3-Epoxy-2,6,11-trimethylundeca-5,9-dienol (*ent*-6).

Prepared from **5** (5.0 g 20.04 mmol) and D-(-)-diethyl tartrate as described for (2*S*,3*R*)-**6**. Yield: 4.5 g (84%). $[\alpha]^{589}_{21}$= 9.66 (c = 1.14, $CHCl_3$). Spectroscopic data identical with (2*S*,3*R*)-**6**. HR-MS: m/z calcd. for $C_{14}H_{22}O$ ($M^{+\bullet}$-H_2O): 206.1655, found: 206.1661.

(2*S*,3*S*)-2,3,6,10-Tetramethylundeca-5,9-diene-1,2-diol ((2*S*,3*S*)-7).

A chilled solution of (2*S*,3*R*)-**6** (0.10 g, 0.45 mmol) in CH_2Cl_2 (10 ml) was stirred for 10 min with $(CH_3)_2N$-$Si(CH_3)_3$ (52.4 mg, 0.45 mmol). Then, the solvent was removed in vacuo, and the silylated epoxyalcohol was injected into a cooled (-20°) and stirred suspension of $(CH_3)_2CuLi$ (3.90 mmol) in Et_2O (15 ml). The temp. was maintained with stirring for 12 h, followed by hydrolysis with a sat. aq. NH_4Cl (15 ml). The product was extracted with Et_2O (3 x 15 ml). The combined extracts were washed with aq. NH_4OH (10 ml, 17%-soln.) and H_2O (10 ml). After drying ($MgSO_4$) and removal of solvents the crude product was redissolved in 0.5 N MeOH/HCl (25 ml) and stirred for 20 min. Evaporation of the solvents i.v. and chromatography on silica gel using pentane/Et_2O (70:30, v:v) for elution afforded the diol as a colourless viscous oil. Yield: 0.90 g (83%). $[\alpha]^{589}_{21}$ = -7.27 (c = 3.0 $CHCl_3$). IR (KBr, film) ν: 3406s, (br.), 2972s, 2924s, 1669w, 1449s, 1377s, 1121m, 1044s, 941w, 909m, 871w, 827w, 734s cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ: 5.17 (t, H-C(9)), 5.08 (t, H-C(5)), 3.56 (d, 1 H-C(1)), 3.42 (d 1 H-C(1)); 2.38-2.26 (m, H-C(3)), 2.14-1.90 (m, 2 H-C(8), 2 H-C(7) and 2 H-C(4)),

1.88-1.70 (m,br. 2 OH), 1.68 (s, 3 H-C(11)); 1.63 (s, CH_3-C(10)), 1.60 (s, CH_3-C(6)); 1.09 (s, CH_3-C(2)), 0.84 (d, CH_3-C(3)). MS (70 eV) *m/z*: 191(10,$M^{+\bullet}$-H_2O,-CH_2OH), 179(10), 161(3), 149(2), 135(9), 123(9), 107(16), 95(62), 81(29), 69(70), 67(32), 57(40), 43(65), 41(100). HR-MS: m/z calcd. for $C_{15}H_{26}O$ ($M^{+\bullet}$-H_2O): 222.1962, found: 222.1984.

(2*R*,3*R*)-2,3,6,10-Tetramethylundeca-5,9-diene-1,2-diol (*ent*-7).

Prepared from *ent*-**6** (0.10 g, 0.45 mmol) as described for (2*S*,3*S*)-**7**. Yield: 0.90 g (83%). $[\alpha]^{589}_{22}$ = 7.1 (c = 2.60, $CHCl_3$). Spectroscopic data identical with (2*S*,3*S*)-**7**. HR-MS: m/z calcd. for $C_{15}H_{26}O$ ($M^{+\bullet}$-H_2O): 222.1984; found: 222.2024.

(3*S*,5*E*)-3,6,10-Trimethylundeca-5,9-dien-2-one ((3*S*)-8).

To a solution of (2*S*,3*S*)-**7** (0.16 g, 0.72 mmol) in THF (5 ml) aq. $NaIO_4$ (0.65 g, 3.0 mmol) in H_2O (2.25 ml) was added with rapid stirring. Stirring was continued for 30 min at rt. Extractive workup with Et_2O and removal of solvents yielded (3*S*)-**8** which was purified by chromatography on silica gel using pentane/Et_2O (90:10, v:v) for elution. Yield: 0.12 g (86%). 88% *ee* according to GLC on 2-*O*-methyl-3,6-*O*-pentyl-β-cyclodextrin as the chiral stationary phase.[27] IR (KBr, film) ν: 2969s, 2928s, 1709s, 1450s, 1375s, 1355s, 1260s, 1160s, 1107s, 881m, 806s cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ: 5.07 (t, H-C(9)) and H-C(5)); 2.60-2.45 (m, H-C(3)); 2.38-2.21 (m, H-C(4)); 2.13 (s, 3 H-C(1)); 2.10-1.96 (m, H-C(4), 2 H-C(8), 2 H-C(7)); 1.67 (s, 3 H-C(11)); 1.60 (s, CH_3-C(10) and CH_3-C(6)), 1.07 (d, CH_3-C(3)). MS (70 eV) *m/z*: 165 (2,$M^{+\bullet}$-CH_3CO), 147(1), 136(5), 121(8), 109(3), 95(8), 81(7), 69(21), 53(6), 43(100), 41(40). HR-MS: m/z calcd. for $C_{14}H_{24}O$ ($M^{+\bullet}$): 208.1827; found: 208.1826.

(3*R*,5*E*)-3,6,10-Trimethylundeca-5,9-dien-2-one (*ent*-8).

Prepared from *ent*-7 (90.0 mg, 0,4 mmol) as described for (3*S*)-**8**. Yield: 50.0 mg (64%). 89% *ee* according to GLC on 2-*O*-methyl-3,6-*O*-pentyl-β-cyclodextrin as the chiral stationary phase.[27] Spectroscopic data identical with (3*S*)-**8**. HR-MS: m/z calcd. for $C_{14}H_{24}O$ ($M^{+\bullet}$): 208.1827; found: 208.1810.

[3-^{2}H]-(2*S*,3*S*)-2,6,10-Trimethylundeca-5,9-diene-1,2-diol ((2*S*,3*S*)-9).

Epoxyalcohol (2*S*,3*R*)-**6** (3.0 g, 13.4 mmol) and $LiAlD_4$ (0.67 g, 27.9 mmol) were refluxed in Et_2O (60 ml) for ca. 17 h. After cooling (0°), water was added and following extractive workup with ether, the crude product was purified by chromatography on silica gel using pentane/Et_2O (1:1, v:v) for elution. Yield: 2.67 g (88%). $[\alpha]^{589}_{22}$ = 1.80 (c = 22.8, $CHCl_3$). IR (KBr, film) ν: 3406br.,s, 2972s, 2918s, 1666w, 1449s, 1376s, 1276w, 1121m, 1049s, 941w, 907m, 863w, 734s cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ: 5.17 (t, 1 H-C(9)); 5.11 (t, 1 H-C(5)); 3.44 (AB-syst., 2 H-C(1)); 2.10-1.95 (m, 2 H-C(8), 2 H-C(7), 2 H-C(4) and 1 H-C(3)); 1.88 (s, 2 OH); 1.68 (s, 3 H-C(11)); 1.63 (s, CH_3-C(10)); 1.60 (s, CH_3-C(6)); 1.19 (s, CH_3-C(2)). MS (70 eV) *m/z*: 178(5,$M^{+\bullet}$-H_2O, -CH_2OH), 166(11), 148(6), 136(7), 122(11), 108(8), 95(11), 82(36), 69(77), 57(15), 41(100). HR-MS: calcd. for $C_{14}H_{23}{}^2HO$ ($M^{+\bullet}$-H_2O): 209.1890; found: 209.1893.

[3-^{2}H]-(2*R*,3*R*)-2,6,10-Trimethylundeca-5,9-diene-1,2-diol (*ent*-9).

Prepared from *ent*-**6** (3.00 g, 13.4 mmol) as described for (2*S*,3*S*)-**9**. Yield: 2.63 g (87%). $[\alpha]^{589}_{22}$ = -0,5 (c = 9.4, $CHCl_3$). HR-MS: m/z calcd. for $C_{14}H_{25}{}^2HO_2$ ($M^{+\bullet}$): 227.1996; found: 227.1980.

[4-^{2}H]-(3*RS*,4*S*)-3,7,11-Trimethyldodeca-1,6,10-trien-3-ol ((3*RS*,4*S*)-3).

A solution of the diol (2*S*,3*S*)-**9** (2.57 g, 11.32 mmol) in THF (50.0 ml) was cleaved by stirring for 30 min at rt. with an aq. solution of $NaIO_4$ (7.4 g, 34.6 mmol) in water (25 ml). Following extractive workup with Et_2O and drying ($MgSO_4$), the solution was slowly concentrated in vacuo to ca. 3.0 ml and gradually added to a cold (-78°) solution of vinylmagnesium bromide (12.5 mmol) in THF (15 ml). Stirring was continued for 3 h, and the solution was allowed to come to rt. Following hydrolysis with aq. NH_4Cl (5.0 ml, 10%-soln.) and extractive workup with Et_2O, the product was purified by chromatography on silica gel using pentane/Et_2O (9:1, v:v) for elution. Yield: 1.3 g (56%). IR (KBr, film) ν: 3420s (br.), 2974s, 2929s, 2861s, 2156w, 1699w, 1662w, 1643w, 1451s, 1412m, 1375s, 1108s, 995s, 919s, 830w cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 5.92 (dd, J=17.3, 10.7, 1 H-C(2)); 5.21 (d, J=17.3, 1 H-C(1)); 5.17-5.08 (m, 1 H-C(6) and 1 H-C(10)); 5.06 (d, J=10.7, 1 H-C(1)); 2.07-1.97 (m, 2 H-C(9), 2 H-C(8), 2 H-C(5) and 1 H-C(4)); 1.68 (s. 3 H-C(12)); 1.60 (s, CH_3-C(11) and CH_3-C(7)); 1.55 (s, 1 OH); 1.28 (s, CH_3-C(3)). MS (70 eV) *m/z*: 205 (1, $M^{+\bullet}$-H_2O), 190(2), 178(1), 162(7), 149(3), 136(8), 121(9), 108(14), 107(14), 93(23), 81(16), 69(55), 55(21), 41(100). HR-MS: m/z calcd. for $C_{15}H_{25}{}^2HO$ ($M^{+\bullet}$-H_2O): 205.1941; found: 205.1938.

(3*RS*,4*R*)-3,7,11-Trimethyl[4-^{2}H]dodeca-1,6,10-trien-3-ol (*ent*-3).

Prepared from the diol *ent*-**9** (2.57 g, 11.32 mmol) as described for (3*RS*,4*S*)-**3**. Yield: 1.90 (82%) Spectroscopic data identical with (3*RS*,4*S*)-**3**. HR-MS: m/z calcd. for $C_{15}H_{23}{}^2H$ ($M^{+\bullet}$-H_2O): 205.1941, found: 205.1938.

(1*E*,3*E*)-(4,8-Dimethyl-nona-1,3,7-trienyl)-trimethyl-silane (11).

A chilled and well stirred suspension of allyl(triphenyl)phosphonium bromide (0.77 g, 2.0 mmol) in THF (20 ml) was gradually treated with a solution of *n*-BuLi (2.0 mmol). After 30 min $(CH_3)_3SiCl$ (0.217 g, 2.0 mmol) was added, and stirring was continued for 1 h at 0°. Then, the solution was cooled again (-70°) and deprotonated with *n*-BuLi (2.0 mmol). The dark red solution was allowed to come to 0°, and 6-methylhept-5-en-2-one **11** (0.25 g, 2.0 mmol) was added. Following 12 h stirring at rt. the mixture was hydrolysed with aq. Na_2CO_3 (10 ml, 10%-soln). Extractive workup with Et_2O and chromatography on silica gel using pentane for elution afforded the silane **11** as a mixture of (1*E*,3*E*)-**11** (85%) and (1*E*,3*Z*)-**11** (15%). Configurationally pure (1*E*,-3*E*)-**11** was obtained by preparative GLC (2 m x 0.5 cm, 10% FFAP on Chromosorb P). Yield: 63.0 mg (15% overall). IR (KBr, film) ν: 3004s, 2958s, 2858s, 1638s, 1571s, 1440s, 1379s, 1349m, 1246s, 1161w, 1104w, 984s, 870s, 834s, 763m, 737m, 722m, 691m cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 6.76 (dd, J=18.2, 10.4, H-C(2)); 5.87 (d, J=10.4, H-C(3)); 5.73 (d, J=18.2, H-C(1)); 5.13-5.07 (m, H-C(7)); 2.16-2.03 (m, 2 H-C(6) and 2 H-C(5)); 1.80 (s, CH_3-C(4)); 1.69 (s, 3 H-C(9)); 1.61 (s, CH_3-C(8)); 0.08 (s, $(CH_3)_3Si$). MS (70 eV) *m/z*: 207(3,[$M^{+\bullet}$-CH_3]), 192(2), 154(4), 148(5), 133(3), 123(7), 107(5), 93(14), 81(22), 73(100), 69(30), 59(21), 41(57). HR-MS: m/z calcd. for $C_{14}H_{26}Si$: 222.1804 ($M^{+\bullet}$), found: 222.1809.

[1-^{2}H]-(1*E*,3*E*)-4,8-Dimethylnona-1,3,7-trien (1a).

A solution of (1*E*)-**11** (34 mg, 0.15 mmol) in CH_3CN (1.0 ml) was stirred with conc. DCl (0.3 ml) for 5 min. Extractive workup and chromatography on silica gel using pentane for elution yielded configurationally pure (*E*)-**1a**. Yield: 20.0 mg (86%). IR (gasphase) ν: 3044m, 2977s, 2928s, 2870s, 2276w, 1798w, 1647w, 1578w, 1446w, 1385w, 1273w, 1192w, 1107w, 976m, 883w, 825w cm^{-1}. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ: 6.57 (dd, J= 16.7 and 10.8, H-C(2)); 5.85 (d, J= 10.8, H-C(3)); 5.13-5.04 (m, H-C(7)); 5.07 (d, J=16.7, H-C(1)); 2.17-1.95

(m, 2 H-C(6) and 2 H-C(5)); 1.75 (s, CH_3-C(4)); 1.68 (s, 3 H-C(9)); 1.60 (s, CH_3-C(8)). MS (70 eV) *m/z*: 151(1,$M^{+\bullet}$), 136(7), 123(3), 108(6), 94(3), 82(10), 80(14), 69(80), 67(12), 53(11), 41(100). HR-MS: m/z calcd. for $C_{11}H_{17}{}^2H$: 151.1471 ($M^{+\bullet}$), found: 151.1451.

[1-^{2}H]-4,8-Dimethylnona-3,7-dien-1-yne (13).

A cold (-78°) and well stirred solution of the dibromide **12** (2.0 g, 6.5 mmol)[22] in THF (20 ml) was gradually treated with *n*-BuLi (5.2 ml of a 2.5 M solution in hexane, 12.9 mmol). Stirring was continued for 1 h and D_2O (1.0 g, 50.0 mmol) was slowly added. The alkyne was purified by chromatography on silica gel using pentane for elution. Yield: 0.5g (52%). IR (KBr, film) ν: 2972s, 2930s, 2858s, 2584s, 1626m, 1443s, 1379s, 1259w, 1108m, 1062m, 830m, 810m cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ: 5.27 (s, H-C(3)); 5.11-5.03 (t,br., H-C(7)); 2.12 (s,br., 2 H-C(6) and 2 H-C(5)); 1.91 (s, CH_3-C(4)); 1.69 (s, 3 H-C(9)); 1.60 (s, CH_3-C(8)). MS (70 eV) *m/z*: 134(25, $M^{+\bullet}$-CH_3), 120(2), 106(8), 92(10), 78(12), 69(71), 67(8), 53(10), 41(100). HR-MS: m/z calcd. for $C_{11}H_{15}{}^2H$: 149.1315 ($M^{+\bullet}$), found: 149.1293.

[1-^{2}H]-(1*Z*,3*E*)-4,8-Dimethylnona-1,3,7-triene (1b).

The alkyne **13** (0.15 g, 1.0 mmol), Lindlar's catalyst (5 mg; Fluka, Switzerland) and freshly distilled quinoline (13.0 mg) were dissolved in THF (5 ml). The hydrogenation was started by rapid stirring and the course of the reaction was monitored by GLC. Removal of the catalyst and chromatography on silica gel using pentane for elution afforded (*Z*)-**1b**. Yield: 0.12g, 80%. IR (gasphase) ν: 3040s, 2974s, 2927s, 2870s, 2257w, 1647w, 1447w, 1385w, 975w, 899w, 798m cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ: 6.56 (t, 1 H-C(2)); 5.85 (d, J=10.8, 1 H-C(3)); 5.13-5.04 (m, 1 H-C(7)); 4.95 (d, J=10.2, 1 H-C(1)); 2.17-1.95 (m, 2 H-C(6) and 2 H-C(5)); 1.75 (s, CH_3-C(4)); 1.68 (s, 3 H-C(9)); 1.60 (s, CH_3-C(8)). MS (70 eV) *m/z*: data identical with **1a**. HR-MS: m/z calcd. for $C_{11}H_{17}{}^2H$: 151.1471 ($M^{+\bullet}$), found: 151.1433.

Incubation Experiments and Collection of Volatiles.

Suspensions of the labelled substrates in H_2O (1.0 mg/ml tap water) were sonicated (130 W) for 2 min at 0°. Freshly cut plantlets of *Phaseolus lunatus* (ca. 3-5 g each) were immersed into the emulsion of the precursors. After 48 h, the incubated plants were placed into a closed system, and the released volatiles were adsorbed from the circulating air over a period of 24 h onto a charcoal trap (1.5 mg; CLSA Filter, Le Ruisseau de Montbrun, F-09350 Daumazan sur Arize, France).[19] The carbon traps were desorbed with 2 x 15 µl CH_2Cl_2. The volatiles from 15 samples were combined and carefully concentrated at 0° by a gentle stream of N_2. The compounds were prepurified by preparative TLC (silica gel, pentane), and after careful removal of the solvents as above, a concentrated solution of the reextracted olefin **1** (ca. 5-10 µg in 20 µl CH_2Cl_2) was used for GLC/FTIR. Incubation experiments with (3*S*)- and *ent*-**8** were carried out as above. After collection of the volatiles, the stereochemistry of the metabolites was determined by GLC-MS using authentic references.

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