172. Stereochemical Studies on Homoterpene Biosynthesis in Higher Plants; Mechanistic, Phylogenetic, and Ecological Aspects

by **Andreas Gäbler** and **Wilhelm Boland***

Institut für Organische Chemie der Universität, Richard-Willstätter-Allee 2, DW-7500 Karlsruhe

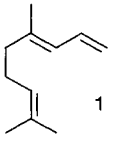
and **Ute Preiss** and **Helmut Simon**

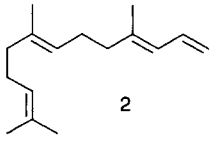
Lehrstuhl für Organische Chemie und Biochemie der Technischen Universität München, Lichtenbergstr. 4, DW-8046 Garching

(30.VIII.91)

In higher plants, the two homoterpenes 4,8-dimethylnona-1,3,7-triene (**1**) and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (**2**) originate from nerolidol (**3**) or geranyllinalool (**4**) by an oxidative cleavage of their C-skeletons. The reaction proceeds with exclusive loss of H_5–C(5) of **3** and formal production of a C_4 fragment. The site specificity of the enzyme(s) is identical for all of the hitherto examined plant families (Agavaceae, Asclepiadaceae, Asteraceae, Leguminosae, Magnoliaceae, and Saxifragaceae). The enzyme tolerates a wide range of structural modifications at the polar head of **3**. Instead of **3**, also geranylacetone **12** and the secondary alcohol **13** can be cleaved to the homoterpene **1** and as yet unidentified carbonyl fragments. The C=C bonds within the aliphatic chain of **3** seem to be essential for the oxidative bond cleavage as well as for recognition and embedding of the substrate into the active center of the enzyme(s). The feed-induced biosynthesis of **1** and **2** in leaves of the Lima bean *Phaseolus lunatus* infested with the spider mite *Tetranychus urticae* probably requires a preceding release of nerolidol (**3**) or geranyllinalool (**4**) from phytogenic glycosides prior to the fragmentation reaction. The microbial reduction of the trienoic acids **6** and **6a** is the key step for the synthesis of deuterium labelled nerolidol (3*RS*,5*R*)- and (3*RS*,5*S*)-**9**.

Introduction. – The two homoterpenes 4,8-dimethylnona-1,3,7-triene (**1**) and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (**2**) are widespread flower fragrances or volatiles of leaves of higher plants. Although they were first discovered just five years ago as minor constituents of the Cardamom oil [1], in the meantime from many botanical plant-families members are known which produce **1** and/or **2** as minor or even major volatiles [2] (*cf.* also the *Table*).

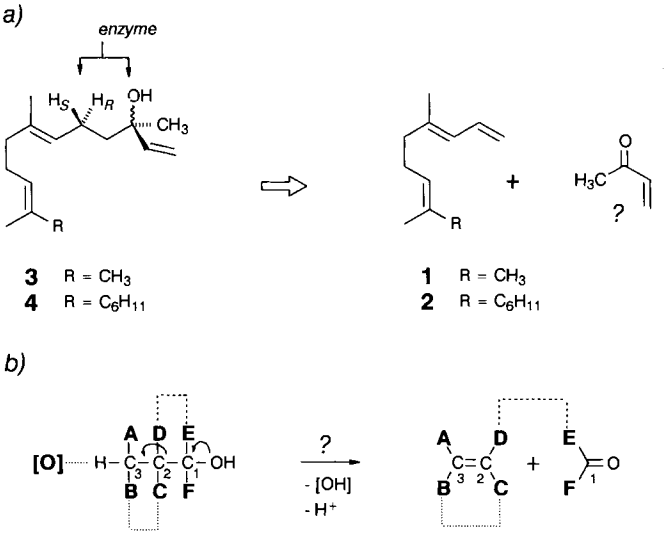




Recently, *Dicke et al.* demonstrated, that **1** and **2** also act as mediators in tritrophic plant/insect interactions [3]. For example, soon after the infestation of the Lima bean *Phaseolus lunatus* by the spider mite *Tetranychus urticae,* the two hydrocarbons **1** and **2** and several other volatiles are released into the air, and the females of one of the natural predators of the spider mite, namely *Phytoseiulus persimilis,* become attracted.

This high ecological impact, their broad occurrence, and their unusual C_{11} or C_{16} C-skeletons prompted us, to investigate their biosynthesis in more detail. We have already shown that **1** and **2** are formed from the two regular terpene alcohols nerolidol (**3**) and geranyllinalool (**4**) by oxidative bond cleavage as depicted in *Scheme 1a* [4]. The degradation proceeds with loss of one of the two enantiotopic H-atoms from C(5) of the terpene alcohols and is accompanied by the formation of a new double bond. The formal C_4 fragment, namely but-3-en-2-on has as yet not been found.

Scheme 1

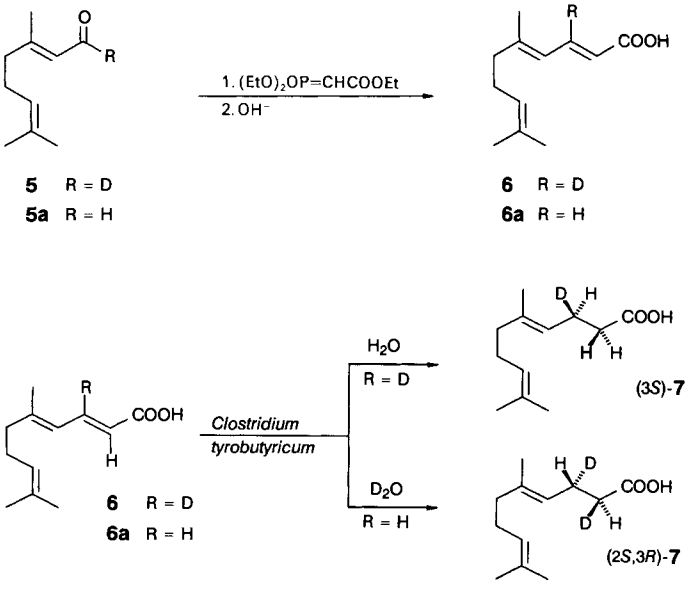


The actual mechanism of this biological oxidative bond cleavage, which might be rationalized as an 'oxidative *Grob* fragmentation', is not well understood. According to the very general description outlines in *Scheme 1b,* this reaction may underly a great number of known biotransformations. In principal, the corresponding enzymes could be used to cleave acyclic precursors, they may 'dealkylate' alicyclic and/or aliheterocyclic structures, or they may simply open ring systems (*Scheme 1b,* dotted and dashed lines). However, irrespective of the large diversity of substrates and transformations, there are several features in common. All precursors carry an O-atom, either as an OH group (acids, secondary, or tertiary alcohols) or as a C=O moiety (aldehydes, ketones, acids). In all biotransformations of this kind, the original C–O bond of the substrate is oxidized to a C=O bond, the central C(1)–C(2) σ-bond is broken, and the π-bond is introduced with removal of a single H-atom from C(3). H-Atoms at C(2), so far as present, are always retained. Primary or secondary alcohols might be oxidized to carbonyl compounds prior to their fragmentation [5], but at least tertiary alcohols should be formally cleaved as such. Besides of the olefinic substructure, aldehydes, ketones, CO_2, or acids are released as the C=O fragments. So far as well studied examples are concerned, the reactions are not only framed by the above conditions, but, in addition, by a highly enantioselective removal of the single H-atom from a prochiral center at C(3) (*cf. Fig. 1b*) and by a highly

ordered geometry of the transition state. Some particularly interesting and probably mechanistically related transformations matching all of the above formal criteria are the following: *i*) the oxidative decarboxylation of (un)saturated fatty acids yielding alk-1-enes in plants [6] and insects [7], *ii*) the oxidative decarboxylation of propionate substituents of coproporphyrinogens [8], *iii*) the biosynthesis of algal pheromones from (3*Z*,6*Z*,9*Z*)-dodeca-3,6,9-trienoic acid [9], *iv*) the conversion of loganin into secologanin [10] and related seco-ring biosyntheses, *v*) certain dealkylations in the field of steroids [11–13]. Another fatty-acid-derived example is the formation of the lachrymatory propanethial *S*-oxide in *Allium cepa* [14] and, last but not least, the biosynthesis of certain furoanocoumarins, like *e.g.* psoralene from (+)-marmesine [15], reflects all the features indicated in *Scheme 1b*. Up to now, only very few enzymes have been isolated and characterized, *e.g.* [16] [17], but common to all is the presence of an Fe-atom and the requirement for molecular oxygen (*cf. Scheme 1b*). To clarify, whether or not the biosynthesis of the two homoterpenes **1** and **2** belongs in fact to the same category of oxidative bond-cleavage reactions as outlined in *Scheme 1b*, we now describe the synthesis of deuterium-labelled 'nerolidols' (3*RS*,5*R*)- and (3*RS*,5*S*)-**9** as metabolic probes and their successful administration to and conversion by selected flowering plants.

Results and Discussion. – 1. *Synthesis of (3*RS,5R*)- and (3*RS,5S*)-Nerolidol* **9**. As the key step for the introduction of a chiral center at C(5) of nerolidol (**3**), the microbial reduction of the trienoic acids **6** or **6a** with *Clostridium tyrobutyricum* (strain *C. La 1*, DSM 1460) seemed to us most promising (*Scheme 2*). The enoate reductase of this microorganism catalyzes the transfer of two H-atoms to the *Si*-faces of the trigonal C(2) and C(3) atoms of a wide range of α,β-unsaturated acids [18]. Since only protons from the solvent together with electrons are incorporated into the product, a simple change from a

Scheme 2

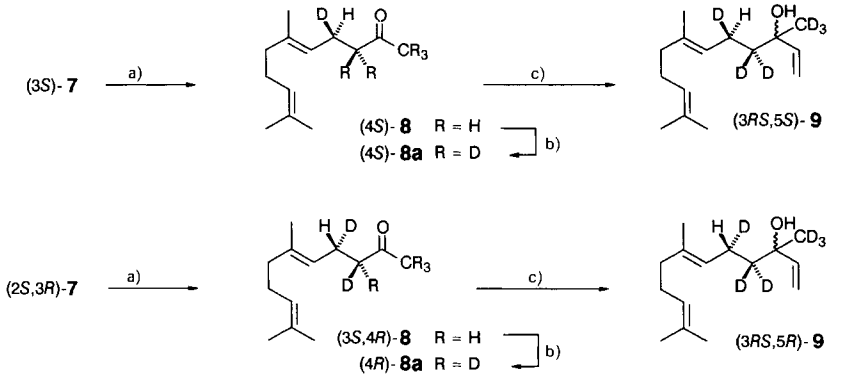


H_2O to a ^2H_2O buffer in conjunction with appropriately labelled (^1H or ^2H) α,β-unsaturated precursors, like *e.g.* **6** or **6a**, should allow a convenient synthesis of either (3*S*)-**7** or (2*S*,3*R*)-**7** in very high optical purity. Further elaboration of these intermediates lets expect a straightforward approach to either (3*RS*,5*S*)- or (3*RS*,5*R*)-**9**, respectively (*cf. Scheme 3*).

The required dienoic acids **6** and **6a** are readily available from (1-^{2}H)geranial (**5**) or geranial **5a** as outlined in *Scheme 2*.

Olefination of **5** or **5a** with the anion of ethyl (diethoxyphosphoryl)acetate and saponification of the resulting esters ((*E*)/(*Z*) = 9:1) affords the acids **6** or **6a** ($\geq$ 98% (*E*) after crystallization from heptane). Although both acids are only sluggishly reduced by the cell fragments of *C. tyrobutyricum* cells, they are nevertheless cleanly converted to the acids (3*S*)-**7** and (2*S*,3*R*)-**7**, respectively. Overreduction or complications by the conjugated double bond (*e.g.* 1,4-addition of hydrogen) are not observed. In accord with the well established stereochemical course of the enzyme, the acid (3*S*)-**7** is obtained in H_2O buffer by *anti*-addition of two H-atoms to C(2) and C(3) of **6** (R = D). In a ^2H_2O buffer, two ^{2}H-atoms are delivered from the solvent in the same fashion to C(2) and C(3) of the unlabelled precursor **6a** (R = H), and the acid (2*S*,3*R*)-**7** is obtained with $\geq$ 97% e.e. as can be shown by the mandelate diester approach and ^{1}H-NMR (*vide infra, cf. Fig. 1*).

Scheme 3



a) MeLi, Me_3SiCl. b) MeOD/MeO^-. c) CH_2=CHMgBr.

Sequential treatment of (3*S*)-**7** or (2*S*,3*R*)-**7** with MeLi and Me_3SiCl [19] yields the two ketones (4*S*)-**8** and (3*R*,4*R*)-**8** (*Scheme 3*). Exchange of their α-protons in MeO^-/MeOD proceeds smoothly and leads to the two highly deuterated ketones (4*S*)-**8a** and (4*R*)-**8a** ($\geq$ 97% ^{2}H at C(1) and C(3)). Final addition of CH_2=CHMgBr furnishes the diastereoisomeric pairs (3*RS*,5*S*)- and (3*RS*,5*R*)-**9** in 66% overall yield from **7**. Due to the very high degree of ^{2}H-labelling, the metabolites of these precursors do not interfer with the natural homoterpenes of the plants upon MS analysis.

2. *Product Chirality by the Mandelate-Diester Approach and Ozonolytic Degradation.* Because of the presence of a chiral center at C(2) of (2*S*,3*R*)-**7**, this acid can be immedi-

ately assayed for its e.e. by ^{1}H-NMR (see *Fig. 1*). After esterification of (2*S*,3*R*)-**7** with methyl (+)-(*S*)-mandelate [20], the resulting diester **10b** is analyzed with simultaneous irradiation at 2.35 ppm (H–C(3)). The mandelate diester **10a** from (3*S*)-**7** can be used to monitor the chemical-shift differences of the diastereoisotopic, geminal 2 H–C(2). H_R and H_S of **10a** resonate as a well resolved *AB* system at 2.518 and 2.464 ppm, respectively. In contrast, only H_R of the (*S*)-mandelate diester **10b** from (2*S*,3*R*)-**7** appears as a broad *s* at 2.492 ppm, while the H_S (2.445 ppm) is completely absent due to the isotopic substitution with H. The chemical-shift difference of 0.026 ppm for H_R–C(2) of the two mandelate diesters results from the high-field shift caused by the additional ^{2}H-atom in **10b**. Thus, within the limits of error of the ^{1}H-NMR method, the optical purity of the acid (2*S*,3*R*)-**7** is $\geq 97\%$ e.e.

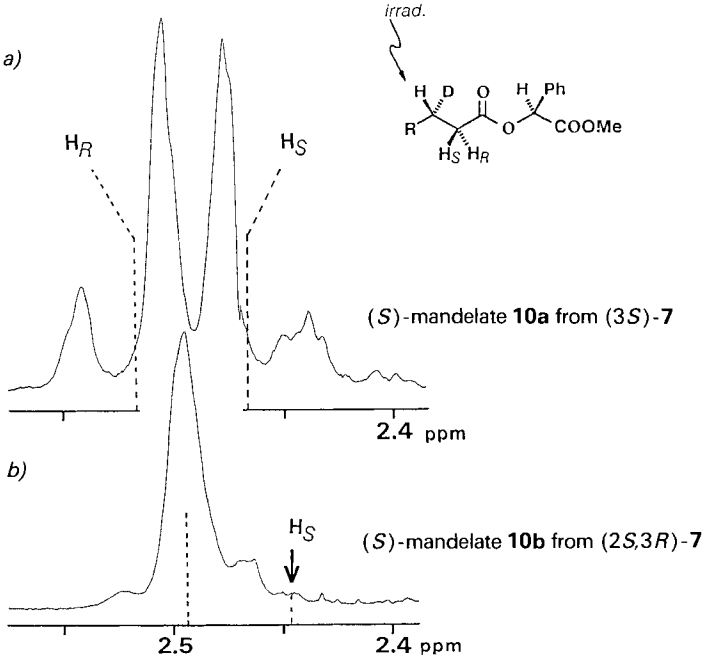
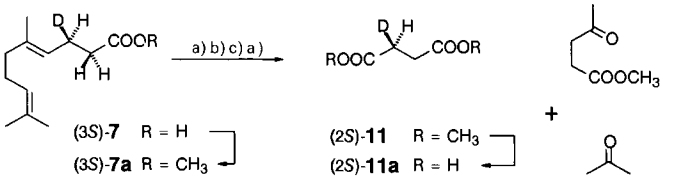


Fig. 1. *400-MHz ^{1}H-NMR spectra* ($CDCl_3$) *of mandelate diesters:* a) *mandelate diester* **10a** *prepared from (3S)-***7**; b) *mandelate diester* **10b** *prepared from (2S,3R)-***7**. Spectra are recorded with irradiation at 2.35 ppm of the adjacent methylene group (H–C(3)). The highfield shift of 0.026 ppm for the anisochronous H–C(α) of **10b** as compared to **10a** is due to the presence of the additional ^{2}H-atom at C(2) in **10b**.

Due to the *anti*-relationship between the two ^{2}H-atoms delivered by the enzyme, the absolute configuration at C(3) of (2*S*,3*R*)-**7** is *eo ipso* defined and has to be of comparable high e.e. ($\geq 97\%$). Compound (3*S*)-**7** is prepared by the same method and, hence, this acid should be also optically pure but with opposite configuration at C(3). On the other hand, since the precise knowledge of the e.e., as well as the absolute configuration at C(3) is essential for the metabolic study, this question is independently addressed by ozonolytic degradation of the acid (3*S*)-**7** (*cf. Scheme 4*).

Following esterification of (3*S*)-**7** with CH_2N_2, the ester (3*S*)-**7a** is treated at −78° with excess O_3, and the resulting ozonides are reduced with Me_2S. Oxidation of the intermediates with $RuCl_3/NaIO_4$ [21] and esterification yields (2*S*)-**11** with complete retention of the ^{2}H-label ($\geqslant$ 97% ^{2}H). The separation of the various (by-)products *via* chromatography is straightforward at the ester level, but due to an extremely small *Cotton* effect, the absolute configuration of the ester (2*S*)-**11** can not be determined by CD spectroscopy [22]. Since (2-^{2}H)succinic acid exhibits a stronger *Cotton* effect, the ester

Scheme 4



a) CH_2N_2. b) O_3, Me_2S. c) $RuCl_3$, $NaIO_4$.

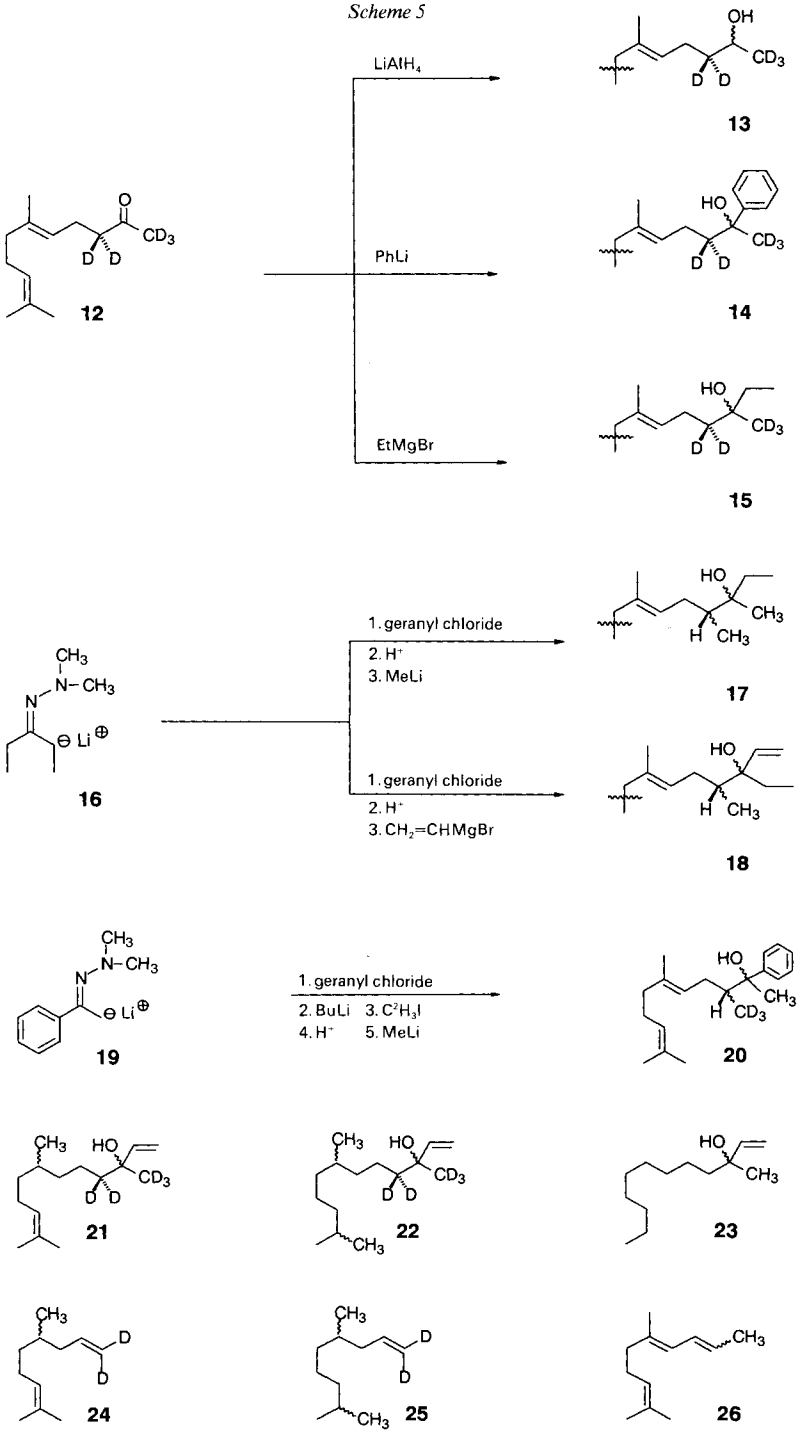
(2*S*)-**11** is saponified according to the protocol of *Cornforth* (3N HCl, 80°) [22] and yields (2*S*)-**11a** with complete conservation of the ^{2}H-label ($\geqslant$ 97% ^{2}H). As expected, (2*S*)-**11a** shows a positive *Cotton* effect [23] and, hence, the chiral center at C(3) of the acid (3*S*)-**7** is unambiguously identified as (3*S*).

3. *Synthesis of Substrate Analogues and References.* The questions of substrate tolerance and specificity of the enzyme(s) are addressed by three different series of modified 'nerolidols'. The first series (*Scheme 6*) contains substrates with modifications at the polar head of the molecule. The second series comprises molecules with an additional Me branch at C(4), while the third series consists of dihydro- and tetrahydronerolidol analogues.

The synthesis of the first series, *i.e.* **13**–**15**, is readily achieved according to *Scheme 5* using the highly deuterated ketone **12** as the common intermediate [4]. The second series, *i.e.* **17** and **18**, is obtained by analogy. Alkylation of the anion **16**, derived from pentan-3-one *N*,*N*-dimethylhydrazone, with geranyl chloride [24], hydrolysis, and alkylation of the resulting ketone with MeLi or CH_2=CHMgBr yields the alcohols **17** and **18** as mixtures of diastereoisomers. An additional ^{2}H-label can be omitted, since the expected olefin (*E*/*Z*)-**26** does not occur in the examined plant species. The deuterated phenyl derivative **20** is obtained by alkylation of the anion **19** of acetophenone *N*,*N*-dimethylhydrazone with, followed by remetallation and alkylation with C^2H_3I in a single operation (*Scheme 5*). Hydrolysis and final treatment of the resulting ketone with MeLi yields **20**.

The third series, namely compounds **21**–**23**, is obtained from the anion of [1,1,1,3,3,3-$^2H_6$]-acetone, *N*,*N*-dimethylhydrazone [4] by alkylation with *rac*-citronellyl iodide or *rac*-3,7-dimethyloctyl iodide, followed by hydrolysis and addition of CH_2=CHMgBr to the resulting ketone (→ **21** and **22**, resp.), and from undecan-2-one and CH_2=CHMgBr (→ **23**). The three references **24**–**26** are available from the corresponding aldehydes by *Wittig* reaction with appropriate phosphoranes.

Scheme 5


LiAlH4
PhLi
EtMgBr
12
13
14
15
1. geranyl chloride
2. H+
3. MeLi
16
17
1. geranyl chloride
2. H+
3. CH2=CHMgBr
18
1. geranyl chloride
2. BuLi 3. C2H3I
4. H+ 5. MeLi
19
20
21
22
23
24
25
26

4. *Administration Experiments with Selected Flowering Plants. Steric Course at C(5).* To evaluate the site specificity of the phytogenic enzymes which convert nerolidol into **1**, (3*RS*,5*R*)-**9** and (3*RS*,5*S*)-**9** were administered to selected flowering plants. Best results are obtained, if freshly disconnected flower heads are immediately placed into an aq. emulsion of (3*RS*,5*S*)- or (3*RS*,5*R*)-**9**. After 24–36 h incubation time, the odor of the flowers is entrapped on charcoal by air circulation in a closed system [25]. Following desorbtion of the carbon filters with CH_2Cl_2 the enriched volatiles were analyzed by GLC/MS. Because of the high ^{2}H-content, the artificial metabolites possess slightly shorter retention times than unlabelled **1**, and, hence, mass spectra of almost pure compounds can be obtained from the front area of an eluting compound. Furthermore, prolonged incubation times (> 36 h) lead to an extensive displacement of the natural (^{1}H)nerolidol by the externally added (^{2}H)precursor **9**. In these cases, the (^{2}H)metabolites are often found as the major or even sole products. *Fig. 2* shows a GLC of a typical incubation experiment with *Yucca filamentosa* and (3*RS*,5*S*)-**9**.

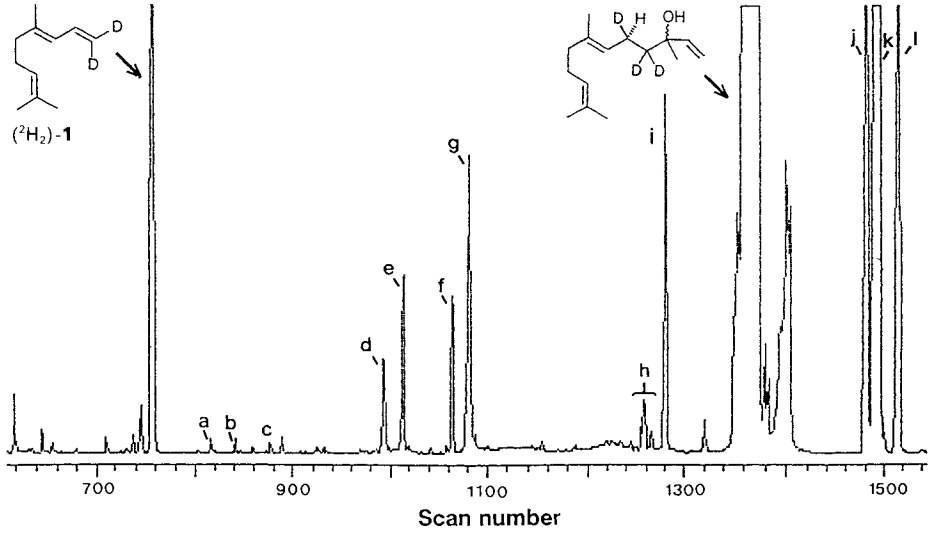


Fig. 2. *GLC Separation of the trapped volatiles of* Yucca filamentosa *after incubation with ($^2H_6$)nerolidol (3*RS,5S*)-***9***. Conditions: fused silica column *BP 5* (25 m × 0.32 mm). Temp. program: 45(5)–200° at 5°/min, then 200–280° at 20°/min. Sample size: 0.5 µl, splitless injection. According to MS, the artificial metabolite consists of *ca.* 80% ($^2H_2$)-**1** and 20% of the natural (^{1}H)-**1**. Identified compounds. (a) pentylbenzene, (b) ethyl benzoate, (c) methyl 2-hydroxybenzoate, (d) linalool, (e) geraniol, (f) impurity, (g) terpene alcohol; $M^{+\cdot} = 166$, (h) pentadecene isomers, (i) pentadecane, (j) heptadecadiene, (k) heptadecene, (l) heptadecane. The positions of the double bond of the alkenes were not determined.

The spectra of the artificial metabolites from administration of (3*RS*,5*R*)- or (3*RS*,5*S*)-**9** to *Y. filamentosa* are representative for the feeding experiments with other plants and will be discussed in the following. They demonstrate very clearly, that the fragmentation of nerolidol into the homoterpene **1** and the still unknown C_4-fragment involves an enantiospecific removal of a H-atom from C(5) of the precursor. After administration of (3*RS*,5*R*)-**9** to *Y. filamentosa*, the resulting metabolite displays a M^+ at

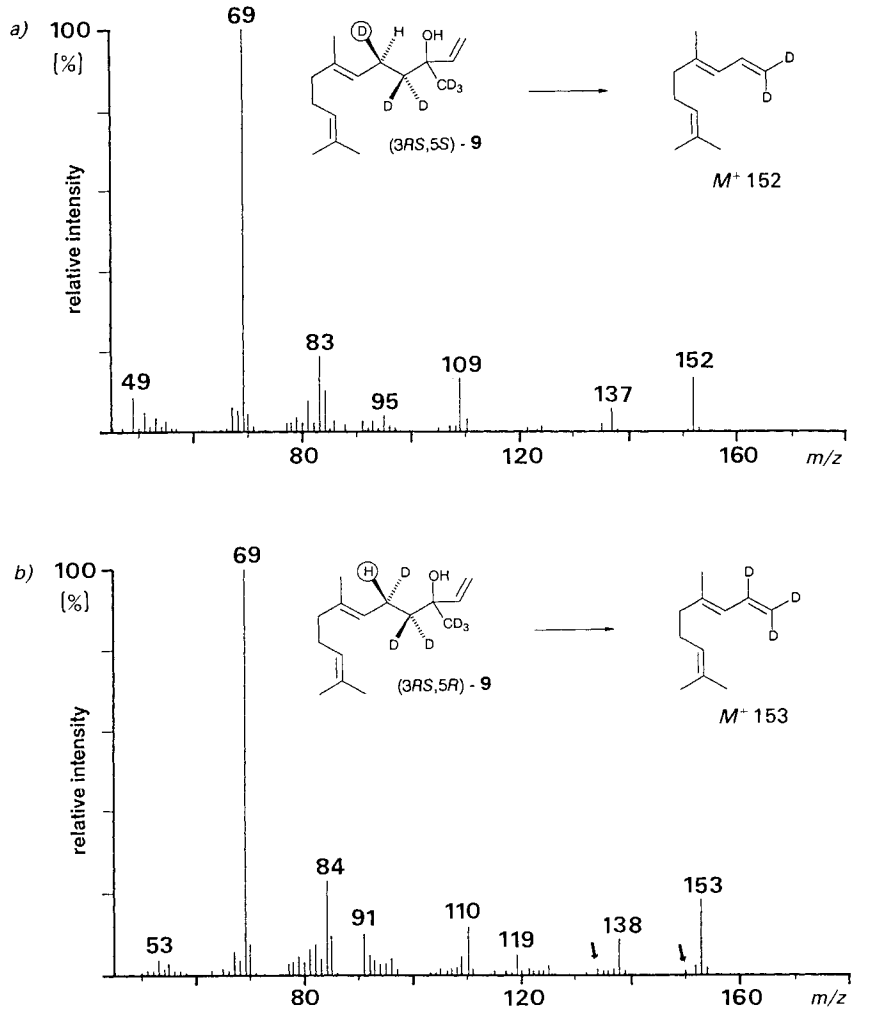


Fig. 3. *EI-MS* (70 eV) *Analysis of artificial metabolites from* Yucca filamentosa. Spectra are taken out of the front area of the eluting homoterpenes. a) *MS of (1,1-$^2H_2$)-***1** *from incubation with (3*RS,5S*)-***9**; b) *MS of (1,1,2-$^2H_3$)-***1** *from incubation with (3*RS,5R*)-***9**. Arrows indicate the small contamination by natural (^{1}H)-**1**. The H_S–C(5) is enantiospecifically removed upon the oxidative bond cleavage. Conditions: *Finnigan MAT 90 GC/MS*. Separation of the compounds was achieved on a *BP 5* fused silica column (25 m × 0.32 mm) under programmed conditions (70° for 5 min, then at 12°/min to 250°). Scan range: 45–300 Da/s.

153 Da (*Fig. 3b*). This is in accord with a complete conservation of all three ^{2}H-atoms of the C(4)–C(5) segment of (3*RS*,5*R*)-**9** in the homoterpene **1**.

In contrast, if (3*RS*,5*S*)-**9** is fed to *Y. filamentosa*, the M^+ of the corresponding homoterpene is found at 152 Da (*Fig. 3a*), and, thus, the fragmentation of (3*RS*,5*S*)-**9** into **1** and the formal C_4-fragment proceeds with exclusive loss of H_S–C(5) of the precursor. The fragments at lower *m/z* (compare *m/z* 137 and 138 or 109 and 110 Da; *Fig. 3a* and *3b*, resp.) corroborate this result. The same information is gained from

administration of (3*RS*,5*RS*)-**9** to *Y. filamentosa*. In this case, the artificial metabolite(s) display two M^+ of equal intensity at 152 and 153 Da, respectively. This, and the uniform 1:1 distribution of the fragment ions at lower *m/z* is important and exclude an intramolecular isotope effect. The substrate has to be embedded into the active center of the enzyme in such a precisely controlled manner, that only H_S–C(5), independent of its isotopic labelling, can be lost. The unequivocal site specificity of the enzyme(s) also implies, that the configuration at C(3) of **9** is either unimportant, or that only one of the two diastereoisomers is accepted as a substrate.

Besides of *Y. filamentosa*, several other homoterpene producing plants from different botanical families (*Table*) were collected over the year and incubated with (3*RS*,5*R*)- and (3*RS*,5*S*)-**9** as described above. The first result is that, in all cases, the administered **9** is readily incorporated and converted into the ^{2}H-labelled homoterpene **1**. Thus, irrespective of distant or close phylogenetic relationships, there is probably only one general mechanism operative in higher plants according to which **1** is formed. The second and even more important observation is made by looking onto the site specificity of the enzymes. Without exception, the stereochemical course is identical for all the plants listed in the *Table*.

Table. *Site Specificity of Homoterpene Biosynthesis in Various Plants*

Plant species	Family	($^2H_6$)Nerolidol **9**[a])		Plant species	Family	($^2H_6$)Nerolidol **9**[a])	
		(5*S*)	(5*R*)			(5*S*)	(5*R*)
Yucca filamentosa	Agavaceae	●	○	*Phaseolus lunatus*	Leguminosae	●	○
Hoya purpureo-fusca	Asclepiadaceae	●	○	*Robinia pseudoacacia*	Leguminosae	●	○
Erigeron annuus	Asteraceae	–	○	*Magnolia liliiflora nigra*	Magnoliaceae	●	○
Helianthus annuus	Asteraceae	●	○	*Philadelphus coronarius*	Saxifragaceae	●	○
Helianthus decapetales	Asteraceae	●	○				

[a]) ● = abstracted H-atom; ○ = remaining H-atom; – = not administered.

The enzyme of the primitive monocotyledon *Y. filamentosa* (Yuccaceae) abstracts H_S–C(5) from nerolidol as do the highly evolved dicotyledons (*e.g. Helianthus sp.* (Asteraceae)). Hence it follows, that the family of angiosperms must have aquired the ability to fragment nerolidol or geranyllinalool at a very early stage of their evolution. From then on, this enzyme seems to have survived the emergence of new plant families more or less unchanged.

5. *Biosynthesis of Homoterpenes in the Lima Bean (Phaseolus lunatus).* One of the most intriguing aspects of homoterpene production in higher plants is the tritrophic interaction between the Lima bean (*Phaseolus lunatus*) infested with the spider mite *Tetranychus urticae* and the predatory mite *Phytoseiulus persimilis*. *Dicke* and coworkers [3] showed recently, that females of the predatory mite *P. persimilis* are attracted to infested Lima beans *via* plant volatiles released from the damaged leaves. In contrast to the compounds which simply result from the mechanical damage of the leave surface by the spider mite, the two homoterpenes **1** and **2** originate from a more complex interaction, since damaged, but noninfested plants do not emanate **1** or **2** at any appreciable extend. As the spider mite *T. urticae* also lacks the homoterpenes, their enhanced formation has

to be the result of a still unknown chemo-enzymatic interaction between the plant and the spider mite. To clarify this particular question of the biosynthesis of **1** and **2** in *P. lunatus*, (5*RS*,3*RS*)-**9** or ($^2H_5$)geranyllinalool [4] were administered to infested and noninfested specimens of this plant. The result is, that after 24 h both, the infested plant and the noninfested control, release ^2H-labelled **1** or **2** as major volatiles. First traces of labelled **1** can be found as soon as 4 h after the beginning of the feeding experiment. In the case of **2**, this is the first successful experimental evidence for its biosynthesis from geranyllinalool *via* fragmentation of the latter [4]. Moreover, careful analysis of the mass spectra reveals, that the emanated homoterpenes from the noninfested plant are almost exclusively formed from the externally added deuterated nerolidol or geranyllinalool, respectively. In the case of the infested plant, the M^+ of **1** comprises two signals at 150 and 152 Da ($^1H/^2H$ ratio *ca.* 1:9). This suggests, that under the influence of the spider mite the formation of the 'natural' (^1H)homoterpene is probably due to an enhanced availability of the natural (^1H)precursor *via* an enzymatic process caused by the secretions of the phytophagous spider mite. Similar observations and conclusions were recently reported by *Turlings et al.* for the feed induced release of **1** and **2** from zea mays seedlings infested with caterpillars [26]. Since terpene alcohols are often bound as glycosides, we assume that the biochemical basis has to be seen in a preceding hydrolysis of the plant derived nerolidol- or geranyllinaloolglycosides by the secretions of the phytophagous mites and insects. A preliminary confirmation might be seen in the observation, that the treatment of artificially damaged leaves with human saliva also results in a pronounced emanation of **1** and **2** (*ca.* 6-fold enhancement). Certain α- and β-glycosidases have the same effect [27]. In conclusion of these experiments, we have to assume that the major difference between infested and noninfested plants is the low level of *free* nerolidol or geranyllinalool in the latter. Further work on the mobilization of nerolidol in infested plants is necessary to understand and corroborate this interesting aspects of the chemo-enzymatic interaction between the plant and the spider mite. Administration of the precursors (3*RS*,5*R*)- and (3*RS*,5*S*)-**9** shows that the site specificity of the enzyme(s) of the Lima bean is in agreement with the other examples of the *Table*.

6. *Fate of the Formal C_4-Fragment and Enzymatic Substrate Tolerance.* According to *Scheme 1*, nerolidol (**3**) is formally cleaved into the homoterpene **1** and but-3-en-2-one. While the olefin is easily detected in all incubation experiments, the unsaturated ketone is as yet not found. One might suspect, that but-3-en-2-one escapes the analysis due to its high chemical reactivity as a *Michael* acceptor in the cell or due to its high volatility. To cope with these arguments, the two nerolidol analogues **14** and **15** were fed to *Magnolia liliiflora nigra* or *Phaseolus lunatus*. After 2 d, the odor from the flower heads or leave surfaces was collected and analyzed as described before. While the aromatic precursor **14** is not cleaved, the saturated analogue **15** is smoothly converted to the (1,1-$^2H_2$)-homoterpene **1**, but the expected (1,1,1-$^2H_3$)butan-2-one is missing. Since the argument of high chemical reactivity does not apply to butan-2-one, its complete lack can be also not explained by the usual detoxification reactions for α,β-unsaturated ketones, namely their reduction to allylic alcohols or saturated ketones. Neither deuterated butan-2-one nor butan-2-ol are found among the collected volatiles.

The above experiments also demonstrate, that the vinyl group of nerolidol is clearly not essential for the enzymatic activity. It can be succesfully replaced by an Et moiety. This finding rules out rearrangements of the C-skeleton (nerolidol $\rightleftharpoons$ farnesol equi-

librium) prior to the fragmentation. Moreover, the additional methyl branch at C(4) of **17** and **18** does not hamper the oxidative bond cleavage. Administration of these metabolic probes to freshly cut plantlets of *P. lunatus* results in a *ca.* 1:1 mixture of (*E*)- and (*Z*)-**26**. Besides **14**, also the second aromatic analog **20** withstands the cleavage reaction; it seems to have inhibitory properties, since the formation of natural **1** is diminished or even suppressed.

In contrast to **13**, **15**, **17**, and **18**, the dihydro- and tetrahydro analogues **21**, **22**, and **23** are not converted to **1**, indicating that the allylic position of the removed hydrogen next to the C(6)=C(7) bond of (3*RS*,5*R*)- or (3*RS*,5*S*)-**9** is essential. Moreover, the aliphatic terminus of nerolidol is responsible for the recognition and embedding of the precursor into the active center of the enzyme. This is supported by the observation, that leaves of *P. lunatus* emanate fairly large amounts of linalool after infestation with *T. urticae* [3] (released from glycosides?). The compound accumulates, since it is not cleaved into the corresponding 4-methylpenta-1,3-diene and a C(4)-fragment. Except of the shorter aliphatic terminus the arrangement of functional groups of linalool is identical with **3**, but incubation experiments with labelled (^{2}H)-linalool confirm that this compound is certainly not a substrate.

The most striking observations are made upon administration of the secondary alcohol **13** or (1,1,1,3,3-$^2H_5$)geranylacetone to plantlets of *P. lunatus* or flower heads of *Magnolia liliiflora nigra*. In fact, both substrates are smoothly converted into labelled **1**.

Besides of this very broad substrate tolerance of the enzymes, the experiments unambiguously demonstrate, that *the catalytical equipment of the nerolidol cleaving enzyme(s) is also capable to convert isostructural secondary alcohols and ketones* into the C_{11} hydrocarbon **1**.

In conclusion of the above experiments, there are surprising parallels to the dealkylation reactions in the field of steroids [5] [12] which are carried out by cytochrome P-450. Together with the complete lack of the expected carbonyl fragments like but-3-en-2-one and butan-2-one (*vide supra*), it is reasonable to assume, that nerolidol **3** and its analogues might be first processed to geranylacetone prior to the final oxidative bond cleavage leading to **1** or **2**. In this case, of course, two moles of acetate would be formed instead of an intact C_4-fragment.

For the biosynthesis of the two homoterpenes **1** and **2**, as discussed here, a number of problems is left open. Most important are the questions regarding the intermediates or sequence of reactions, as well as the geometry of the transition state of the oxidative bond cleavage (synperiplanar) [28]. These problems, the still unknown absolute configurations and enantiomeric purity of nerolidol in the various plant species (*cf.* the *Table*) are currently evaluated and will be presented together with a detailed mechanistic discussion in due course.

Financial support by the *Deutsche Forschungsgemeinschaft* (Bonn) and the *Fonds der Chemischen Industrie* (Frankfurt am Main) is gratefully acknowledged. We also thank the *Bayer AG* (Leverkusen) and the *BASF* (Ludwigshafen) for generous supply with chemicals and solvents. Special thanks are due to Prof. Dr. *G. Snatzke*, Ruhr-Universität, Bochum, for recording the CD spectra. We thank Dr. *H. Röttele* and Dr. *I. Zahorsky* for the NMR and some of the mass spectra. Practical assistance of Mr. *Fritz Keller* is gratefully acknowledged.

Experimental Part

General. Reactions are performed under Ar. Solvents and reagents were purified and dried prior to use. Anh. $MgSO_4$ was used for drying operations. Solns. were usually concentrated by flash evaporation under reduced pressure. Anal. TLC: 20 × 20 cm TLC plates, SiO_2 *60* F_{254}, layer thickness 0.2 mm (*E. Merck & Co.*, D-61 Darmstadt). Anal. GLC: *Carlo Erba* gas chromatograph, *HRGC 5300, Mega* series, equipped with fused-silica capillaries, *SE 30* (10 m × 0.31 mm); H_2 at 30 cm^3/s served as carrier gas. CD (ε): *ISA-Mark 6, Jobin-Yvon.* IR (cm^{-1}): *Perkin-Elmer 882* IR spectrophotometer. ^{1}H-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^3/s as carrier gas.

(2E,4E)-5,9-Dimethyl(3-^{2}H)deca-2,4,8-trienoate (**6**). (1-$^2H_1$)Geranial (4.0 g, 26.1 mmol) is slowly added at r.t. to a stirred soln. of the anion derived from ethyl (diethoxyphosphoryl)acetate (5.60 g, 29.0 mmol; NaH as base) in dry THF (60 ml). After 1 h at r.t., the mixture is hydrolyzed with a 10% aq. Na_2CO_3 soln. (50 ml), and the ester is extracted with Et_2O (3 × 50 ml). The combined org. layers are washed with H_2O (3 × 20 ml), dried, and evaporated. CC on SiO_2 affords a mixture of stereoisomers ((*E*)/(*Z*) = 9:1; GLC): 4.5 g (77%). Colorless oil. ^{1}H-NMR (250 MHz): 6.00 (*s*, H–C(4)); 5.79 (*s*, H–C(2)); 5.08 (br. *m*, H–C(8)); 4.21 (*q*, CH_3CH_2O); 2.15 (br. *d*, 2 H–C(6), 2 H–C(7)); 1.90 (*s*, CH_3–C(5)); 1.69 (*s*, 3 H–C(10)); 1.61 (*s*, CH_3–C(9)); 1.30 (*t*, CH_3CH_2O).

The ester (4.5 g, 20.0 mmol) is saponified with KOH (1.68 g, 30.0 mmol) in MeOH/H_2O (1.2:1; 220 ml) at reflux for 3 h. After cooling to r.t., the mixture is acidified (15 ml of 2N HCl), the bulk of solvents evaporated, and the crude acid is extracted with Et_2O (3 × 30 ml). Drying and evaporation give a solid which is recrystallized from heptane to yield 2.32 g (64%) of pure **6** (≥ 99% (*E*) according to GLC of the Me_3Si ester). IR (KBr): 3650–2350 (br.), 2969*s*, 2928*s*, 2855*s*, 1693*s*, 1625*s*, 1596*s*, 1419*m*, 1305*s*, 1238*m*, 1180*m*, 890*m*, 707*m*. ^{1}H-NMR (250 MHz): 6.05 (*s*, H–C(4)); 5.80 (*s*, H–C(2)); 5.09 (br. *m*, H–C(8)); 2.18 (br. *s*, 2 H–C(6), 2 H–C(7)); 1.93 (*s*, CH_3–C(5)); 1.69 (*s*, 3 H–C(10)); 1.62 (*s*, CH_3–C(9)). MS (70 eV): 195 (2, M^+), 127 (8), 112 (10), 92 (3), 82 (16), 80 (13), 69 (100), 53 (14). HR-MS (free acid): 195.1361 ($C_{12}H_{17}{}^2H_1O_2$, M^+, calc. 95.1370).

(2E,4E)-5,9-Dimethyldeca-2,4,8-trienoic Acid (**6a**). From geranial (10.0 g, 65.4 mmol) as described for **6**. Yield: 5.3 g (59%, overall). HR-MS (free acid): 194.1312 ($C_{12}H_{18}O_2$, M^+, calc. 194.1307).

Microbial Reduction of **6** *or* **6a**: *General Procedure:* Clostridium tyrobutyricum (Strain: *C. La 1*, DSM 1460) was grown, stored, and manipulated as described [18] [29]. For the experiment in ^{2}H-buffer, wet packed cells were freeze dried (under exclusion of O_2) and resuspended in ^2H_2O. *Reduction of* **6**: A total volume of 116 ml containing the Na salt of **6** (2.3 g, 11.6 mmol), 3.37 g of wet packed cells, 11.6 mg of tetracycline·HCl, 1.0 mM methylviologen, and 0.1M potassium-phosphate buffer at pH 7.0. Under an atmosphere of 1 bar H_2 gas, the vessel, equipped with a Hg-filled '*Warburg* manometer', was shaken at 35°. After *ca.* 50% conversion (13 h), the uptake of H_2 untypically ceased. Addition of a second portion of the bacterial-cells mass (5.11 g) restarted the H_2 uptake, and complete reduction of the substrate was achieved within 3 h. The suspension was acidified to pH 1.5 with dil. H_2SO_4 and extracted with Et_2O. Removal of solvents i.v. afforded 2.2 g of crude (3*S*)-**7** which was purified by CC (silica gel; hexane/Et_2O 3:2 (*v/v*)): 2.1 g (90.3%).

For the preparation of (2*S*,3*R*)-**7**, the Na salt of **6a** (4.88 g, 25.0 mmol) in a total volume of 250 ml of 0.1M ^2H_2O buffer, p^{2}H 7.0, containing 1 mM methylviologen, 25.0 mg of tetracycline·HCl, 0.6 mM $K_3[Fe(CN)_6]$, and 27.7 g of freeze-dried cells was hydrogenated under H_2 (not $^2H_2$) as described. Isolation and purification as above: 4.26 g (87%) of (2*S*,3*R*)-**7**. The hexacyanoferrate(III) was present, since it was added (0.5 ml, 10 mM per 1 g of wet packed cells) before drying of the *C. tyrobutyricum* cells.

(3S,4E)-5,9-Dimethyl(3-^{2}H)deca-4,8-dienoic Acid ((3*S*)-**7**). IR (film): 3600–2400*s*, 2971*s*, 2920*s*, 2880*s*, 2165*w* (br.), 1713*s*, 1439*s*, 1287*s*, 1224*s*, 935*m* (br.). ^{1}H-NMR (400 MHz): 5.15–5.05 (*m*, H–C(8), H–C(4)); 2.40–2.27 (*m*, 2 H–C(2), H–C(3)); 2.10–1.95 (*m*, 2 H–C(7), 2 H–C(6)); 1.68 (*s*, CH_3–C(5)); 1.66 (*s*, 3 H–C(10)); 1.61 (*s*, CH_3–C(9)). MS (70 eV; as methyl ester (3*S*)-**7a**): 168 (26, $[M - C_2H_3O]^+$), 136 (13), 123 (4), 100 (31), 94 (5), 82 (35), 69 (66), 53 (12), 41 (100). HR-MS ((3*S*)-**7a**): 211.1675 ($C_{13}H_{21}{}^2HO_2$, M^+, calc. 211.1682).

(2S,3R,4E)-5,9-Dimethyl(2,3-$^2H_2$)deca-4,8-dienoic Acid ((2*S*,3*R*)-**7**). IR (film): identical with (3*S*)-**7**. ^{1}H-NMR (400 MHz): 5.15–5.04 (*m*, H–C(8), H–C(4)); 2.37 (br. *d*, H–C(2)); 2.32 (br. *q*, H–C(3)); 2.06 (br. *t*, 2 H–C(6)); 1.98 (*m*, 2 H–C(7)); 1.68 (*d*, CH_3–C(5)); 1.62 (*s*, 3 H–C(10)); 1.60 (*s*, CH_3–C(9)). MS (70 eV): 212 (4, M^+), 181 (7), 137 (22), 122 (12), 109 (50), 94 (18), 82 (40), 69 (57), 55 (21), 41 (100).

(4S,5E)-6,10-Dimethyl(1,1,1,3,3,4-$^2H_6$)undeca-5,9-dien-2-one ((4*S*)-**8a**). A soln. of (3*S*)-**7** (0.36 g, 1.77 mmol) in THF (15 ml) is gradually treated with stirring at 0° with MeLi (4.44 ml of a 1.6M soln. in Et_2O). Stirring is continued for 2 h, and Me_3SiCl (4.22 ml, 35.6 mmol) is rapidly added. The mixture is allowed to come to r.t. and hydrolyzed by addition of 2N HCl (8.0 ml). After usual workup, the ketone, contaminated with a small amount of the corresponding tertiary alcohol (*ca.* 5%), is purified by CC (SiO_2, hexane/Et_2O 9:1 (*v/v*)): 0.3 g (83%) of (4*S*)-**8**. For the introduction of additional ^{2}H-atoms, (4*S*)-**8** (0.27 g, 1.38 mmol) is dissolved in MeOD/MeO^- (0.7 mmol of

MeO^- in 4.2 ml of MeOD) and stirred overnight at r.t. H_2O (10 ml) is added, and the product is extracted with Et_2O (3 × 25 ml). Purification as above yields 0.24 g (88%) of (4*S*)-**8a** as a colorless liquid. IR (film): 2969*s*, 2920*s*, 2858*s*, 2171 (br.), 1712*s*, 1449*m*, 1376*m*, 1286*m*, 1246*s*, 1108*w*, 824*w*. ^{1}H-NMR (400 MHz): 5.07 (*m*, H–C(9), H–C(5)); 2.24 (*d*, 1 H–C(4)); 2.08–1.95 (*m*, 2 H–C(8), 2 H–C(7)); 1.68 (*s*, CH_3–C(6)); 1.61 (*s*, 3 H–C(11)); 1.60 (*s*, CH_3–C(10)). MS (70 eV): 156 (5), 137 (13), 131 (1), 122 (6), 111 (8), 94 (9), 81 (7), 69 (50), 67 (13), 53 (11), 46 (100), 41 (81). HR-MS: 200.2022 ($C_{13}H_{16}{}^2H_6O$, M^+, calc. 200.2047).

*(4*R*,5*E*)-6,10-Dimethyl(1,1,1,3,3,4-$^2H_6$)undeca-5,9-dien-2-one* ((4*R*)-**8a**). Prepared from (2*S*,3*R*)-**7** (1.50 g, 10.2 mmol) as described for (4*S*)-**8a**: 1.2 g (59%, overall). Spectroscopic data identical with (4*S*)-**8**. HR-MS: 200.2037 ($C_{13}H_{16}{}^2H_6O$, M^+, calc. 200.2047).

*(3*RS,5*S,6*E*)-3-($^2H_3$)Methyl-7,11-dimethyl(4,4,5-$^2H_6$)dodeca-1,6,10-trien-3-ol* ((5*S*)-**9**). A soln. of (4*S*)-**8a** (0.1 g, 0.51 mmol) in dry THF (1 ml) is added at r.t. to a stirred soln. of CH_2=CHMgBr (0.56 mmol) in THF (1 ml). Stirring is continued for 1 h, followed by hydrolysis with a chilled 10% soln. of NH_4Cl (5 ml). Purification is achieved by CC (silica gel, pentane/Et_2O 9:1 (*v*/*v*)): 0.95 g (83%). IR (film: 3391 (br.), 3090*w*, 2971*s*, 2922*s*, 2858*s*, 2227*w* (br.), 1642*w*, 1447*m*, 1376*m*, 1108*m*, 996*s*, 920*s*. ^{1}H-NMR (250 MHz): 5.92 (*dd*, *J* = 17.5, 11.6, H–C(2)); 5.21 (*dd*, *J* = 17.5, 1.5, 1 H–C(1)); 5.12 (*m*, H–C(10), H–C(6)); 5.06 (*dd*, *J* = 11.6, 1.5, 1 H–C(1)); 2.13–1.93 (*m*, 2 H–C(9), 2 H–C(8), OH); 1.68 (*s*, 3 H–C(12)); 1.60 (*s*, CH_3–C(7), CH_3–C(11)). MS (70 eV): 195 ([$M^+ - H_2O, - CH_3$]$^+$), 167 (4), 166 (5), 137 (9), 122 (9), 111 (11), 98 (13), 94 (14), 81 (13), 74 (25), 69 (64), 55 (16), 46 (47), 41 (100). HR-MS: 210.2237 ($C_{15}H_{18}{}^2H_6$, [$M - H_2O$]$^+$, calc. 210.2255).

*(3*RS,5*R,6*E*)-3-($^2H_3$)Methyl-7,11-dimethyl(4,4,5-$^2H_3$)dodeca-1,6,10-trien-3-ol* ((5*R*)-**9**). Prepared from (4*R*)-**8a** (0.13 g, 0.65 mmol) as described for (5*S*)-**9**. Yield: 0.15 g (88%). Spectroscopic data identical with those of (3*RS*,5*S*)-**9**.

Mandelate Diesters: General Procedure. To a cold soln. (−10°) of (3*S*)-**7** (71.0 mg, 0.36 mmol) and 4-(dimethylamino)pyridine (1 mg, 8.1 μmol) in CH_2Cl_2 (3 ml) is added with stirring (+)-(*S*)-methyl 2-hydroxy-2-phenylethanoate (60 mg, 0.36 mmol) and dicyclohexylcarbodiimid (74.6 mg, 0.36 mmol). Stirring is continued for 4 h at −10°. After filtration of the precipitated urea and evaporation of the solvents i.v., the mandelate diester **10a** is purified by CC (silica gel, pentane/Et_2O, 9:1 (*v*/*v*)): 76.3 mg (60%).

Mandelate Diester **10a** *of (3*S*)-***7***.* IR (film): 3038*w*, 2959*s*, 2926*s*, 2859*m*, 2256*w* 1746*s*, 1437*s*, 1271*s*, 1217*s*, 1152*s*, 910*s*, 732*s*. ^{1}H-NMR (400 MHz): 7.48–7.38 (*m*, 5 arom. H); 5.93 (*s*, PhC*H*); 5.13–5.05 (*m*, H–C(8), H–C(4)); 3.72 (*s*, CH_3O); 2.56–2.41 (*m*, 2 H–C(2)); 2.40–2.32 (*q*, 1 H–C(3)); 2.04 (*m*, 2 H–C(7)); 1.96 (*m*, 2 H–C(6)); 1.68 (*s*, 3 H–C(10)); 1.62–1.59 (2 *s*, CH_3–C(5), CH_3–C(9)). MS (70 eV): 302 (8, [$M - CH_3CO$]$^+$), 242 (2), 196 (23), 178 (12), 149 (42), 136 (26), 121 (65), 105 (15), 91 (18), 77 (29), 69 (92), 41 (100). HR-MS: 345.2021 ($C_{21}H_{27}{}^2HO_4$, M^+, calc. 345.2050).

Mandelate Diester **10b** *of (2*S*,3*R*)-***7***.* From (2*S*,3*R*)-**7** (72 mg, 0.36 mmol) in 60% yield. IR (film): identical with that of **10a**. ^{1}H-NMR (400 MHz): identical with that of **10a**, except for: 2.495 (*d*, 1 H–C(2)); 2.345 (*t*, 1 H–C(3)). MS (70 eV): 303 (7, [$M - CH_3CO$]$^+$), 243 (3), 197 (21), 179 (11), 149 (52), 121 (66), 105 (15), 91 (18), 77 (39), 69 (100), 41 (98). HR-MS: 346.2149 ($C_{21}H_{26}{}^2H_2O_4$, M^+, calc. 346.2113).

*Ozonolytic Degradation of (3*S*)-***7a***.* O_3 is passed at −78° into a soln. of (3*S*)-**7a** (0.210 g, 1.00 mmol; ⩾ 97% ^{2}H at C(3)) in dry AcOEt (10 ml), until the blue color just persists. Excess of the oxidant is expelled with N_2, and Me_2S (7.5 ml, 10.2 mmol) is added. The soln. is allowed to come to r.t. and evaporated. The crude carbonyl compounds are immediately oxidized with $RuCl_3/NaIO_4$ in $MeCN/CCl_4/H_2O$ [21]. After extractive workup, the resulting acids are esterified with CH_2N_2 and separated by CC (silica gel, hexane/Et_2O 85:15, (*v*/*v*)): 100 mg (69%) of (2*S*)-**11**. Saponification of (2*S*)-**11** is achieved by heating (2*S*)-**11** (100.0 mg, 0.87 mmol) in 3N HCl (1 ml) for 1 h at 80° [22]. Evaporation gives the free acid (2*S*)-**11a** which is recrystallized from H_2O: 66.0 mg (88%). Re-esterification with CH_2N_2 and isotope analysis by MS indicates the complete retention of the ^{2}H-label (⩾ 97% ^{2}H at C(2)) throughout the degradation procedure. $[\Theta]_{218.8} = 82.5$ (*c* = 0.173M in H_2O, *d* = 0.1 cm).

*(2*RS,5*E)-6,10-Dimethyl(1,1,1,3,3-$^2H_5$)undeca-5,9-dien-2-ol* (**13**). From **12** (0.5 g, 2.5 mmol) by reduction with $LiAlH_4$ in dry THF (15 ml): 0.45 g (89.5%). IR (film): 3396*s* (br.), 2970*s*, 2920*s*, 2221*w*, 1440*m*, 1377*w*, 1069*m* (br.). ^{1}H-NMR (250 MHz): 5.13 (*t*, 1 H–C(5)); 5.08 (*t*, 1 H–C(9)); 3.78 (br. *s*, 1 H–C(2)); 2.17–1.92 (*m*, 2 H–C(8), 2 H–C(7), 2 H–C(4)); 1.68 (*s*, 3 H–C(11)); 1.62, 1.60 (*s*, CH_3–C(6), CH_3–C(10)); 1.53 (*s*, OH). MS (70 eV): 155 (1, [$M - 46$]$^+$), 138 (11), 123 (6), 113 (10), 95 (9), 81 (11), 69 (93), 41 (100). HR-MS 201.2163 ($C_{13}H_{19}{}^2H_5O$, M^+, calc. 201.2141).

*(2*RS,5*E)-6,10-Dimethyl-2-phenyl(1,1,1,3,3-$^2H_5$)undeca-5,9-dien-2-ol* (**14**). From **12** (0.25 g, 1.25 mmol) with PhLi (1.2 ml of a 2M soln. in cyclohexane/Et_2O, 2.4 mmol) in dry THF (20 ml) at 0°: 0.251 (73%). IR (film): 3448*m* (br.), 3087*w*, 3063*w*, 3030*w*, 2971*s*, 2925*s*, 2859*s*, 2228*w* (br.), 1493*m*, 1446*s*, 1376*m*, 1046*m*, 758*m*, 700*s*. ^{1}H-NMR (250 MHz): 7.46–7.18 (*m*, 5 arom. H); 5.16–5.02 (*m*, H–C(5), H–C(9)); 2.10–1.80 (*m*, 2 H–C(8), 2 H–C(7),

2 H–C(4)); 1.69 (*s*, 3 H–C(11)); 1.60 (*s*, CH_3–C(10)); 1.57 (*s*, OH); 1.48 (*s*, CH_3–C(6)); ⩾ 97% ^2H at C(1) and C(3). MS (70 eV): 259 (3, $[M - H_2O]^+$), 215 (1), 190 (1), 147 (8), 136 (32), 124 (24), 121 (15), 105 (11), 93 (16), 77 (12), 69 (35), 46 (100), 41 (71). HR-MS: 259.2368 ($C_{19}H_{21}{}^2H_5$, $[M - H_2O]^+$, calc. 259.2348).

*(3*RS,6E*)-3-(*$^2H_3$*)Methyl-7,11-dimethyl(4,4-*$^2H_2$*)dodeca-6,10-dien-3-ol* (**15**). From **12** (0.3 g, 1.5 mmol) and EtMgBr (4.13 mmol) in dry Et_2O (5 ml): 0.27 g (78%). IR (film): 3385*m* (br.), 2970*s*, 2927*s*, 2860*s*, 2223*w* (br.), 1450*m*, 1377*m*, 1153*m*, 1112*m*, 1031*m*, 983*w*. ^{1}H-NMR (250 MHz): 5.15 (*t*, H–C(6)); 5.10 (*t*, H–C(10)); 2.13–1.94 (*m*, 2 H–C(9), 2 H–C(8), 2 H–C(5)); 1.68 (*s*, 3 H–C(12)); 1.63 (*s*, CH_3–C(7)); 1.61 (*s*, CH_3–C(11)); 1.51 (*q*, 2 H–C(2)); 1.25 (*s*, OH); 0.92 (*t*, 3 H–C(1)); ⩾ 97% ^2H at C(4) and CH_3–C(3). MS (70 eV): 211 (5, $[M - H_2O]^+$), 182 (3), 168 (10), 142 (12), 136 (14), 121 (12), 111 (6), 99 (28), 83 (34), 76 (22), 69 (67), 57 (22), 46 (34), 41 (100). HR-MS: 211.2319 ($C_{15}H_{23}{}^2H_5O$, $[M - H_2O]^+$, calc. 211.2348).

*(3*RS,4RS,6E*)-3,4,7,11-Tetramethyldodeca-6,10-dien-3-ol* (**17**). A soln. of pentan-3-one *N,N*-dimethylhydrazone (1.5 g, 11.72 mmol) in dry THF (10 ml) is gradually treated with stirring at −5° with BuLi (4.7 ml, 2.5M in hexane, 11.72 mmol). After 30 min, the anion **16** is alkylated by addition of a soln. of geranyl chloride (1.8 g, 11.72 mmol) in THF (10 ml). The mixture is allowed to come to r.t. over 2 h and hydrolyzed with 2N HCl (5.0 ml) for 30 min. Following extractive workup with Et_2O (4 × 10 ml), the resulting ketone is purified by CC (silica gel): 2.32 g (89%). Then, a soln. of the above ketone (0.7 g, 3.15 mmol) in dry THF (10 ml) is gradually treated with stirring at −10° with MeLi (2.3 ml of a 1.5M soln. in Et_2O, 3.46 mmol). Usual workup and CC (SiO_2, pentane/Et_2O 4:1) affords **17** as a mixture of diastereoisomers: 0.62 g (83%). IR (film): 3469*m* (br.), 2971*s*, 2930*s*, 2882*s*, 1452*m*, 1378*m*, 1121*m*, 979*m*, 905*m*. ^{1}H-NMR (250 MHz): 5.21–5.05 (*m*, 1 H–C(6), 1 H–C(9)); 2.35–1.95 (*m*, 2 H–C(9), 2 H–C(8), 2 H–C(5)); 1.87–1.70 (*m*, 1 H–C(4)); 1.68 (*s*, 3 H–C(12)); 1.60 (*s*, CH_3–C(11), CH_3–C(7)); 1.59–1.45 (*m*, 2 H–C(2)); 1.10 (*d*, CH_3–C(4)); 0.95–0.80 (*m*, 3 H–C(1), CH_3–C(3)). MS (70 eV): 220 (1, $[M - 18]^+$), 205 (1), 191 (2), 177 (3), 151 (7), 136 (8), 121 (7), 109 (19), 95 (35), 81 (45), 69 (73), 55 (60), 41 (100). HR-MS: 220.2194 ($C_{16}H_{30}O$, $[M - H_2O]^+$, calc. 220.2191).

*(3*RS,4RS,6E*)-3-Ethyl-4,7,11-trimethyldodeca-1,6,10-trien-3-ol* (**18**). Prepared from the same ketone (0.7 g, 3.15 mmol) as used for **17** by injection into a stirred soln. of vinylmagnesium bromide (3.5 mmol) in THF (5 ml) at r.t. Workup and purification as described for **17**: 0.48 g (61%) of **18**. IR (film): 3536*w* (br.), 2979*s*, 2935*s*, 2878*s*, 1453*m*, 1380*m*, 1118*s*, 922*m*. ^{1}H-NMR (250 MHz): 5.85 (*dd*, 1 H–C(2)); 5.24 (*d*, *J* = 17.5, 1 H–C(1)); 5.18 (*d*, *J* = 11.7, 1 H–C(1)); 5.23–5.04 (*m*, 1 H–C(6), 1 H–C(10)); 2.32–1.95 (*m*, 2 H–C(9), 2 H–C(8), 2 H–C(5)); 1.87–1.48 (*m*, 1 H–C(4), CH_3CH_2–C(3)); 1.69 (*s*, 3 H–C(12)); 1.60, 1.58 (2*s*, CH_3–C(11), CH_3–C(7)); 0.92–0.80 (*m*, CH_3CH_2–C(3), CH_3–C(4)). MS (70 eV): 203 (1, $[M - H_2O - C_2H_5]^+$), 189 (5), 163 (3), 147 (5), 136 (5), 121 (12), 109 (20), 93 (21), 81 (33), 69 (70), 55 (35), 41 (100). HR-MS: 232.2229 ($C_{17}H_{30}O$, $[M - H_2O]^+$, calc. 232.2191).

*(2*RS,3RS,5E*)-3-(*$^2H_3$*)Methyl-6,10-dimethyl-2-phenylundeca-5,9-dien-2-ol* (**20**). A stirred soln. of acetophenone *N,N*-dimethylhydrazone (0.4 g, 2.5 mmol) in dry THF (4 ml) is metallated at 0° with BuLi (1.0 ml of a 2.5M soln. in hexane, 2.5 mmol; → **19**). After 30 min, a soln. of geranyl chloride (0.435 g, 2.5 mmol) in dry THF (4 ml) is gradually added, followed by HMPA (1 ml). Stirring is continued overnight. Then, the hydrazone is remetallated with BuLi (0.9 ml, 2.25 mmol) at 0° for 30 min, and C^2H_3I (0.32 g, 2.25 mmol) is added. After 1 h at r.t., the mixture is hydrolyzed with 2N, HCl (2.0 ml). Extractive workup (Et_2O) and CC (silica gel, pentane/Et_2O) afford 0.5 g (73%) of a ketone. A soln. of this ketone (0.45 g, 1.72 mmol) in dry THF (5 ml) is alkylated at −10° by addition of MeLi (1.2 ml of an 1.5M soln. in Et_2O, 1.89 mmol). Usual workup and CC (silica gel, pentane/Et_2O, 4:1) gives **20** (0.41 g, 83%). IR (film): 3461*m* (br.), 2965*s*, 2931*s*, 2876*s*, 2221*w*, 1670*m*, 1492*m*, 1446*s*, 1376*s*, 1105*s*, 1068*s*, 760*m*, 701*s*. ^{1}H-NMR (250 MHz): 7.39–7.13 (*m*, 5 arom. H); 5.10 (*m*, 1 H–C(9), 1 H–C(5)); 2.15–1.75 (*m*, 2 H–C(8), 2 H–C(7), 2 H–C(4)); 1.60 (*s*, 3 H–C(11)); 1.52 (*s*, CH_3–C(10)); 1.48 (*s*, OH); 1.45 (*s*, CH_3–C(6)); 1.38 (*s*, 3 H–C(1)). MS (70 eV): 271 (5, $[M - H_2O]^+$), 228 (3), 200 (12), 187 (10), 172 (3), 160 (15), 136 (10), 121 (15), 105 (15), 91 (15), 81 (30), 69 (67), 41 (100). HR-MS: 271.2356 ($C_{20}H_{27}{}^2H_3O$, $[M - H_2O]^+$, calc. 271.2379).

*(3*RS,7RS*)-3-(*$^2H_3$*)Methyl-7,11-dimethyl(4,4-*$^2H_2$*)dodeca-1,10-dien-3-ol* (**21**). Prepared from citronellyl iodide (3.0 g, 11.3 mmol) by condensation with the anion of (1,1,1,3,3,3-$^2H_6$)acetone *N,N*-dimethylhydrazone [4] (11.5 mmol) and subsequent treatment of the resulting ketone with CH_2=CHMgBr: 1.90 g (71%, overall). IR (film): 3389*m*, 3088*w*, 2966*s*, 2929*s*, 2871*s*, 2227*w*, 1642*w*, 1459*s*, 1376*s*, 995*s*, 919*s*. ^{1}H-NMR (250 MHz): 5.93 (*dd*, *J* = 17.9, 11.7, 1 H–C(2)); 5.19 (*d*, *J* = 17.9, 1 H–C(1)); 5.09 (*t*, 1 H–C(10)); 5.03 (*d*, *J* = 11.7, 1 H–C(1)); 1.96 (*m*, 2 H–C(9)); 1.68 (*s*, 3 H–C(12)); 1.60 (*s*, CH_3–C(11)); 1.42 (*s*, OH); 1.39–1.01 (*m*, 2 H–C(8), 1 H–C(7), 2 H–C(6), 2 H–C(5)); 0.86 (*d*, CH_3–C(7)); ⩾ 97% ^2H at C(4) and CH_3–C(3). MS (70 eV): 211 (2, $[M - H_2O]^+$), 168 (1), 153 (2), 137 (3), 125 (7), 109 (28), 96 (31), 82 (31), 74 (62), 69 (45), 55 (38), 46 (64), 41 (100). HR-MS: 211.2302 ($C_{15}H_{23}{}^2H_5O$, $[M - H_2O]^+$, calc. 211.2348).

*(3*RS,7RS*)-3-(*$^2H_3$*)Methyl-7,11-dimethyl(4,4-*$^2H_2$*)dodec-1-en-3-ol* (**22**). Prepared from racemic 3,7-dimethyloctyl iodide (4.02 g, 15.0 mmol) by condensation with the anion of (1,1,1,3,3,3-$^2H_6$)acetone *N,N*-

dimethylhydrazone (15.2 mmol) and subsequent treatment of the resulting ketone with CH_2=CHMgBr: 1.78 g (62%, overall). IR (film): 3305*s* (br.), 3080*w*, 2957*s*, 2931*s*, 2871*s*, 2227*w* (br.), 1639*w*, 1465*s*, 1380*m*, 1365*m*, 995*m*, 919*s*. ^{1}H-NMR (250 MHz): 5.91 (*dd*, J = 17.5, 10.8, 1 H–C(2)); 5.18 (*d*, J = 17.5, 1 H–C(1)); 5.03 (*d*, J = 10.8, 1 H–C(1)); 1.62–1.00 (*m*, 12 aliph. H); 0.83 (*d*, 3 H–C(12), CH_3–C(11)); 0.82 (*d*, CH_3–C(7)); ⩾ 97% ^{2}H at C(4) and CH_3–C(3). MS (70 eV): 139 (0.5), 127 (6), 110 (4), 97 (10), 83 (18), 74 (100), 55 (30), 41 (68). HR-MS: 212.2463 ($C_{15}H_{25}{}^2H_5O$, [M – HDO]$^+$, calc. 212.2442).

(3RS)-2-Methyldodec-1-en-3-ol (**23**). Prepared from undecan-2-one (0.44 g, 2.6 mmol) on treatment with CH_2=CHMgBr (3.0 mmol): 0.47 g (97%). IR (film): 3384*s* (br.), 3089*w*, 2959*s*, 2931*s*, 2956*s*, 1639*w*, 1466*s*, 1411*m*, 1370*m*, 994*m*, 919*s*. ^{1}H-NMR (250 MHz): 5.92 (*dd*, J = 17.1, 10.4, 1 H–C(2)); 5.19 (*d*, J = 17.1, 1 H–C(1)); 5.03 (*d*, J = 10.4, 1 H–C(1)); 1.50 (br. *m*, 2 H–C(4)); 1.43 (*s*, OH); 1.28 (br. *s*, 14 aliph. H, CH_3–C(3)); 0.88 (*t*, 3 H–C(12)). MS (70 eV): 183 (1, [M – CH_3]$^+$), 125 (2), 111 (6), 97 (15), 81 (12), 71 (100), 55 (17), 43 (62). HR-MS: 180.1884 ($C_{13}H_{26}O$, [M – H_2O]$^+$, calc. 180.1878).

(4RS)-4,8-Dimethyl(1,1-$^2H_2$)nona-1,7-diene (**24**). Prepared by addition of citronellal (0.99 g, 6.33 mmol) to a chilled soln. of C^2H_2=PPh_3 (8.23 mmol) in dry THF (20 ml). Workup as usual: 0.52 g (54%). IR (film): 2967*s*, 2915*s*, 2875*s*, 2307*w*, 2214*w*, 1600*w*, 1454*m*, 1376*m*, 981*w*, 933*w*, 726*m*. ^{1}H-NMR (250 MHz): 5.79 (br. *s*, 1 H–C(2)); 5.12 (*t*, 1 H–C(7)); 2.13–1.82 (*m*, 2 H–C(3), 2 H–C(6)); 1.69 (*s*, 3 H–C(9)); 1.60 (*s*, CH_3–C(8)); 1.57–1.42 (*m*, 1 H–C(4)); 1.41–1.08 (*m*, 2 H–C(5)); 0.88 (*d*, CH_3–C(4)). MS (70 eV): 154 (1, M^+), 139 (3), 123 (2), 111 (14), 97 (20), 83 (25), 69 (88), 55 (28), 41 (100). HR-MS: 154.1687 ($C_{11}H_{18}{}^2H_2$, M^+, calc. 154.1690).

(4RS,8RS)-4,8-Dimethyl(1,1-$^2H_2$)non-1-ene (**25**). Prepared by addition of 3,7-dimethyloctanal (1.0 g, 6.33 mmol) to a chilled soln. of C^2H_2=PPh_3 (8.23 mmol) in dry THF (20 ml). Workup as usual: 0.5 g (50%). IR (film): 2958*s*, 2928*s*, 2872*s*, 2308*w*, 2215*w*, 1600*w*, 1465*m*, 1381*m*, 980*w*, 726*m*. ^{1}H-NMR (250 MHz): 5.85 (br. *s*, 1 H–C(2)); 2.18–1.80 (*m*, 2 H–C(3)); 1.60–1.40 (*m*, 1 H–C(4), 1 H–C(8)); 1.38–1.00 (*m*, 2 H–C(5), 2 H–C(6), 2 H–C(7)); 0.88 (*d*, CH_3–C(4), CH_3–C(8), 3 H–C(9)). MS (70 eV): 156 (0.3, M^+), 141 (0.4), 128 (0.6), 111 (4), 99 (4), 84 (9), 71 (67), 57 (95), 43 (92), 41 (100). HR-MS: 156.1828 ($C_{11}H_{20}{}^2H_2$, M^+, calc. 156.1847).

Incubation Experiments. Suspensions of labelled substrates in H_2O (0.1–1.0 mg/ml tap water) are sonicated (130 W) for 2 min. Freshly disconnected flower heads or plantlets of *Phaseolus lunatus* are directly immersed into the above emulsions of the precursors. After 24–48 h, the incubated plants are placed into an extraction vessel [25], and the released volatiles are collected on a charcoal filter (1.5 mg) over a period of 12–24 h. Following desorption of the filters with 2 × 15 µl CH_2Cl_2, the extracts are directly analyzed by GLC/MS.

REFERENCES

[1] B. Maurer, A. Hauser, J.-C. Froidevaux, *Tetrahedron Lett.* **1986,** *27,* 2111.
[2] R. Kaiser, '19th International Symposium on Essential Oils and other Natural Substrates', Greifensee, Switzerland, Sept. 7–10, 1988.
[3] M. Dicke, T. A. van Beek, M. A. Posthumus, N. Ben Dom, H. van Bokhoven, A. E. De Groot, *J. Chem. Ecol.* **1990,** *16,* 381.
[4] W. Boland, A. Gäbler, *Helv. Chim. Acta* **1989,** *72,* 247.
[5] J. N. Wright, M. Akhtar, *Steroids* **1990,** *55,* 142, and ref. cit. therein.
[6] G. Görgen, W. Boland, *Eur. J. Biochem.* **1989,** *185,* 237.
[7] G. Görgen, C. Frössl, W. Boland, K. Dettner, *Experientia* **1990,** *46,* 700.
[8] J. S. Seehra, P. M. Jordan, M. Akhtar, *Biochem. J.* **1983,** *209,* 709.
[9] C. Neumann, W. Boland, *Eur. J. Biochem.* **1990,** *191,* 453.
[10] L.-F. Tietze, *Angew. Chem.* **1983,** *95,* 840; *ibid. Int. Ed.* **1983,** *22,* 828, and ref. cit. therein.
[11] H. Kohara, K. Shimizu, *Biochem. Biophys. Acta* **1987,** *921,* 90.
[12] D. M. Harrison, *Nat. Prod. Rep.* **1990,** *7,* 459, and ref. cit. therein.
[13] P. A. Cole, C. H. Robinson, *J. Med. Chem.*, **1990,** *33,* 2933.
[14] R. J. Parry, G. R. Sood, *J. Am. Chem. Soc.* **1989,** *111,* 4514.
[15] D. Hamerski, U. Matern, *Eur. J. Biochem.* **1988,** *171,* 369.
[16] J.-M. Camadro, H. Chambon, J. Jolles, P. Labbe, *Eur. J. Biochem.* **1986,** *156,* 579, and ref. cit. therein.
[17] C. J. Corbin, S. Graham-Lorence, M. McPhaul, J. L. Mason, C. R. Mendelson, E. R. Simpson, *Proc. Natl. Acad. Sci. U.S.A.* **1988,** *85,* 8948.
[18] I. Thanos, J. Bader, H. Günther, S. Neumann, F. Krauss, H. Simon, *Methods Enzymol.* **1987,** *136,* 302.
[19] G. M. Rubottom, C. Kim, *J. Org. Chem.* **1983,** *48,* 1550.

[20] D. Parker, *J. Chem. Soc., Perkin Trans. 2* **1983,** 83.
[21] P. H. J. Carlsen, V. S. M. Katsuki, K. B. Sharpless, *J. Org. Chem.* **1981,** *46,* 3936.
[22] J. W. Cornforth, F. R. S. Rita, H. Cornforth, C. Donninger, G. Popjak, G. Rybak, G. J. Schroepfer, *Proc. R. Soc. London [Ser.] B* **1966,** *163,* 436.
[23] J. W. Cornforth, G. Rybak, *Biochem. Biophys. Res. Commun.* **1962,** *9,* 371.
[24] E. J. Corey, D. Enders, *Chem. Ber.* **1978,** *111,* 1337.
[25] W. Boland, P. Ney, L. Jaenicke, L. Gassmann, in 'Analysis of Volatiles', Ed. P. Schreier, Walter de Gruyter & Co., Berlin–New York, 1984, p. 371.
[26] T. C. J. Turlings, J. H. Tumlinson, W. J. Lewis, *Science* **1990,** *250,* 1251.
[27] J. Donat, W. Boland, in preparation.
[28] A. Gäbler, W. Boland, in preparation.
[29] J. Bader, H. Simon, *Arch. Microbiol.* **1980,** *127,* 279.