Pergamon

0031-9422(95)00082-8

Phytochemistry, Vol. 39, No. 4, pp. 785–790, 1995

0031-9422/95 $9.50 + 0.00

BIOSYNTHESIS OF ACYCLIC HOMOTERPENES: ENZYME SELECTIVITY AND ABSOLUTE CONFIGURATION OF THE NEROLIDOL PRECURSOR

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(*Received in revised form* 19 *December* 1994)

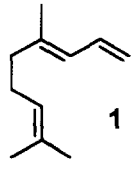
Key Word Index—*Fragaria* × *magna*; *Gerbera jamesonii*; *Gossypium herbaceum*; *Humulus lupulus*; *Lycopersicon esculentum*; *Phaseolus lunatus*; 4,8-dimethylnona-1,3,7,11-triene; (3*S*)-[12-$^2H_3$, 13-$^3H_3$]nerolidol; (3*R*)-[4-$^2H_2$, 15-$^2H_3$]nerolidol; biosynthesis.

Abstract—The acyclic homoterpene 4,8-dimethylnona-1,3,7-triene is a metabolite of (3*S*)-nerolidol. The absolute configuration of the precursor was established by GC–MS analysis of the molecular ion of the homoterpene produced after feeding a mixture consisting of equal amounts of (3*S*)-[12-$^2H_3$, 13-$^3H_3$]- and (3*R*)-[4-$^2H_2$, 15-$^2H_3$]nerolidol to various plants. The degree of the enantioselectivity of the converting enzyme was found to be characteristic for the selected plant or the plant cultivar. Thus leaves of *Phaseolus lunatus* and leaves of *Spatiphyllum wallisii* convert specifically (3*S*)-nerolidol, whereas leaves of *Fragaria* × *magna* and leaves of *Gossypium herbaceum* exhibit only a moderate degree of enantioselectivity (3*S*:3*R*, 66:34). The isotope distribution of the homoterpene emitted from leaves of *G. herbaceum* (herbivore inducible biosynthesis) and that of the homoterpene released from the blossoms (endogeneously controlled biosynthesis) of the plant is identical (*S*:*R*, 66:34) suggesting that the same enzyme is active within the different tissues or organs of the plant. A highly enantioselective synthesis of (3*S*)-[12-$^2H_3$, 13-$^3H_3$]- and (3*R*)-[4-$^2H_2$, 15-$^2H_3$]-nerolidol is described.

INTRODUCTION

The two homoterpenes **1** (4,8-dimethylnona-1,3,7-triene) and **2** (4,8,12-trimethyl-1,3,7,11-tridecatetraene) are typical constituents of the so called 'white-floral image' of night-scented flowers of the Orchidaceae, Cactaceae, Magnoliaceae and Liliaceae. A possible role in the pollination biology of these plants has been suggested [1]. More recently, the same compounds were identified by several authors among the volatiles emitted from leaves of higher plants after damage by herbivores [2, 3].

Dicke [2] identified **1** and **2** together with several other volatiles as synomones emitted from leaves of Lima beans (*Phaseolus lunatus*) after infestation with the two-spotted spider mite *Tetranychus urticae*. As shown by choice experiments, carnivorous mites (e.g. *Phytoseiulus persimilis*) strongly respond to **1** and move towards the odour source [4]. Obviously they associate the odour of **1** with their prey and find their way, chemically guided, to the infested plant. Turlings and Tumlinson [3] observed **1** and **2** among the volatiles released from corn plants (*Zea mays*) in response to damage by the beet army worm *Spodoptera exigua*. In this case, females of the parasitic wasp *Cotesia marginiventris* use the pattern of the induced volatiles to locate their prey [3]. In both examples, the two homoterpenes **1** and **2** are clearly absent in the headspace of healthy, undamaged plants. As shown recently, they are synthesized within several hours in response to certain lytic enzymes (e.g. β-glucosidase) of the feeding herbivore [5–7]. The C_{11} hydrocarbon **1** is produced from the sesquiterpenoid alcohol nerolidol; **2** is synthesized from the diterpenoid geranyllinalool by an analogous sequence [8]. Both routes were confirmed by experiments in which deuterium labelled precursors were fed to blossoms and/or leaves of higher plants. The experiments also showed that the ability to synthesize **1** and **2** is exceedingly widespread among angiosperms [5].



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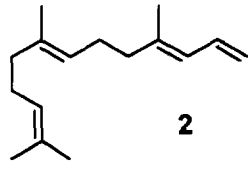

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Irrespective of this more or less general ability of higher plants to convert nerolidol into **1**, the precursor itself is only very scarcely emitted from the flower heads or plant leaves and, hence, chromatographic methods using chiral stationary phases can not be used for the determination of the absolute configuration and enantiomeric excess (e.e.) of the precursors. On the other hand, (3*S*)- and (3*R*)-nerolidol, as well as mixtures of both, are

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known to occur in plants [9, 10], and it is of interest to see, how the phytogenic enzymes handle the different enantiomers and enantiomeric mixtures. This question is particular important with respect to the high ecological impact of the derived volatiles as possible clues in the plants defense strategy. Here we describe a synthetic/analytic approach which simultaneously addresses the questions concerning the configuration of the nerolidol precursors and substrate tolerance of the converting enzymes. It is shown that the hitherto studied plants convert (3*S*)-nerolidol with high to moderate preference.

RESULTS

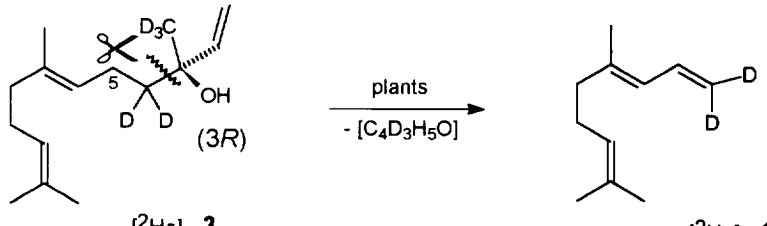
Synthesis of deuterium labelled (3R)- *and* (3S)-*nerolidol*

To address the absolute configuration of the precursor together with the degree of the enantioselectivity of the metabolizing enzymes in a single experiment, we planned to administer a 'pseudoracemate', consisting of equal amounts of differently labelled (3*S*)- and (3*R*)-nerolidol precursors as illustrated in Fig. 1. According to the degree of the enantioselectivity of the involved enzymes, one or the other of the two enantiomeric nerolidols (3*S*)-[12-$^2H_3$, 13-$^3H_3$]**3** and (3*R*)-[4-$^2H_2$, 15-$^2H_3$]**3** would be metabolized preferentially, releasing the correspondingly labelled homoterpene [9-$^2H_3$, 10-$^2H_3$]**1** or [4-$^2H_2$]**1** to the gas phase. GC–MS analysis of the collected volatiles would then be used to determine and to quantify the isotopic labelling.

If the isotopes are placed in remote parts of the molecule which are not involved in the oxidative degradation, primary isotope effects which could alter the isotopic substitution of the products are ruled out. The final bond cleaving step involves a *syn*-elimination of the H_s-5 hydrogen atom of nerolidol together with the polar head of the molecule [11]. For this reason no deuterium was introduced on to C-5 of the substrates [$^2H_5$]**3** and [$^2H_6$]**3**, respectively. The two deuterium atoms at C-4 of [$^2H_5$]**3** may cause a secondary isotope effect (change of the hybridization at C-4 from $sp^3 \rightarrow sp^2$), but the influence will generally be low and can be ignored in the first instance. Hence, mass spectral analysis of the molecular ion(s) will give a direct and reliable measure of the degree of enantioselectivity of the degrading enzymes.

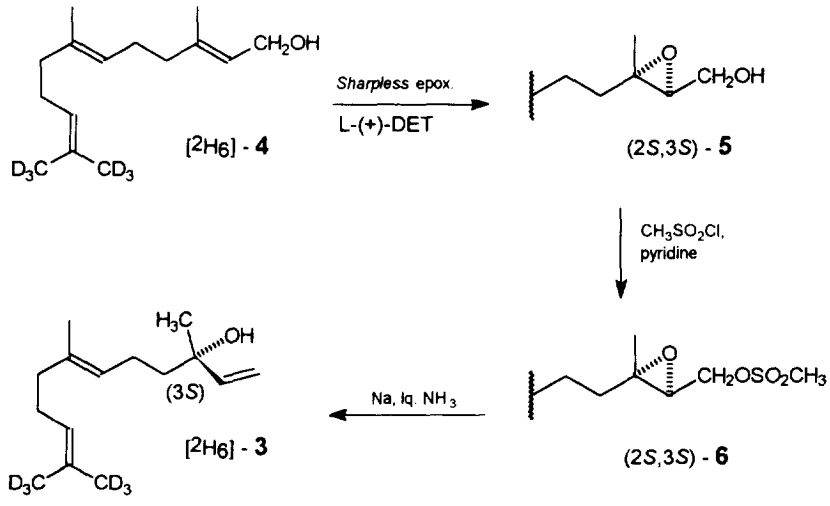
The synthesis of deuterium labelled (3*S*)-nerolidol was readily achieved starting from the known [$^2H_6$]farnesol (**4**) [12], Scheme 1. Chirality was introduced using the catalytic variant of the Sharpless epoxidation in the presence of L-(+)-diethyltartrate (L-(+)-DET) as the chiral auxiliary [13]. Based on the optical rotation of the resulting epoxifarnesol (2*S*, 3*S*)**5**, the epoxidation was achieved with *ca* 92 ± 3% e.e. [14]. The ^{1}H NMR spectral analysis of the corresponding Mosher-ester of (2*S*, 3*S*)**5** confirmed this e.e. [15]. Following conversion of the epoxyalcohol into the methanesulphonate (2*S*, 3*S*)**6**, reductive elimination of the mesylate with sodium in liquid ammonia provided (3*S*)-[12-$^2H_3$, 13-$^3H_3$]nerolidol (**3**) with retention of the configuration at C-3 [16]. Gas chromatography on a WCOT capillary column coated with 2,6-dimethyl-3-pentyl-β-cyclodextrin as the stationary phase established the optical purity of both enantiomers as ⩾ 87% e.e. after all chemical transformations [17]. Other elimination procedures, e.g. via epoxyhalides and zinc metal/sonication for reduction, gave lower yields [18].

The synthesis of the pentadeuterated (3*R*)-[4-$^2H_2$, 15-$^2H_3$]nerolidol (**3**) is outlined in Scheme 2. Witting–Horner olefination of [$^2H_5$] geranylacetone [8] with triethylphosphono acetate yielded the labelled ethyl farnesoate, [$^2H_5$]**7**, as a mixture of isomers (*E*/*Z*, 88:12) [19]. The isomers were separated by chromatography on silica gel using pentane–ether (9:1). Reduction of the (*E*)-isomer of [$^2H_5$]**7** with aluminium hydride afforded the farnesol [$^2H_5$]**4**. Repetition of the sequence of Scheme 1 with [$^2H_5$]**4** and D-(−)-diethyltartrate [D-(−)-DET] as the chiral auxiliary yielded the required (3*R*)-nerolidol, [4-$^2H_2$, 15-$^2H_3$]**3** (⩾ 87% e.e. according to GC).

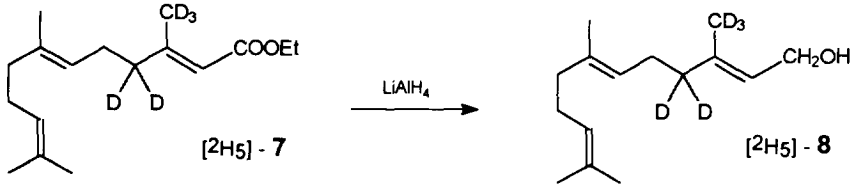


Precursor	Config.	Product	$[M]^+$ (*m/z*)
[$^2H_6$]- **3**	3*S*	[$^2H_6$]- **1**	156
[$^2H_5$]- **3**	3*R*	[$^2H_2$]- **1**	152

Fig. 1.



Scheme 1.



Scheme 2.

Administration experiments with selected plants

The 'pseudoracemate', consisting of equal amounts of (3*S*)-[12-$^2H_3$, 13-$^3H_3$]**3** and (3*R*)-[4-$^2H_2$, 15-$^2H_3$]**3** was fed through the petiole to freshly disconnected flower heads or leaves of selected plants by placing them into an emulsion of the 'pseudoracemate' in water. The precursors were emulsified in water (0.8–1.0 mg ml^{-1}) by sonication for 2 min using a cleaning bath (2 × 320 W). The resulting emulsions were stable for the period of the feeding experiment (up to 2 days). The immersed leaves or blossoms were placed in a small desiccator connected to a trapping device consisting of a miniature circulation pump and a charcoal trap. Flask, pump and filter holder, containing the carbon trap, were joined together forming a closed system (enclosed air volume: *ca* 850 ml) (Fig. 2).

The air circulation was maintained for 20 hr, while the produced volatiles were continuously adsorbed on to the charcoal trap [20]. Following desorbtion [21] from the carbon with methylene chloride (2 × 15 μl) the volatiles were directly analysed by GC–MS. Owing to the difference of four mass units between the molecular ions of the two differently labelled homoterpene products [$^2H_2$]**1** and [$^2H_6$]**1**, no mutual overlap of their molecular ions and/or their ^{13}C satellites occurs. Thus, the degree of the enantioselectivity of the involved enzyme(s) can be directly calculated from the peak areas of the two ions at *m/z* 152 and 156, respectively. The data obtained for the various plants tested are compiled in Table 1.

DISCUSSION

As shown previously, the ability to synthesize and to release **1** and/or **2** to the environment is exceedingly widespread among angiosperms [5]. A number of plants are known which produce **1** and/or **2** as flower fragrances, others do not emit these volatiles until they are injured by a herbivore. According to the data shown in Table 1 all of the hitherto examined plants show a distinct preference for (3*S*)-nerolidol, but only two of them exhibit a very high degree of enantioselectivity (3*S*/3*R*, 96:4 and 94:6, respectively, Table 1). Taking into account the final enantiomeric excess (e.e.) of ⩾ 87% for each of the administered nerolidol precursors [$^2H_6$]-**3** and [$^2H_5$]-**3**, this corresponds, within the limits of error, to a virtually complete stereospecificity of the enzymes involved. The perfect coincidence of the isotopic labelling pattern of the homoterpene [$^2H_2$]/[$^2H_6$]-**1** which is released from leaves and blossoms of *Gossypium herbaceum* strongly suggests that the plant uses the same enzymes in different tissues or organs. Since untreated leaves of *G. herbaceum* do not emit **1** or **2**, this also indicates an independently controlled activation of the enzymes in the different organs. In leaves, jasmonic acid has been recently recognized as one of such signal molecules [6]. Most of the plants listed in Table 1 exhibit a broader substrate tolerance, on average between 3:1 and 2:1 in favour of the (3*S*)-nerolidol. Owing to this broad substrate tolerance an attacked and elicited plant

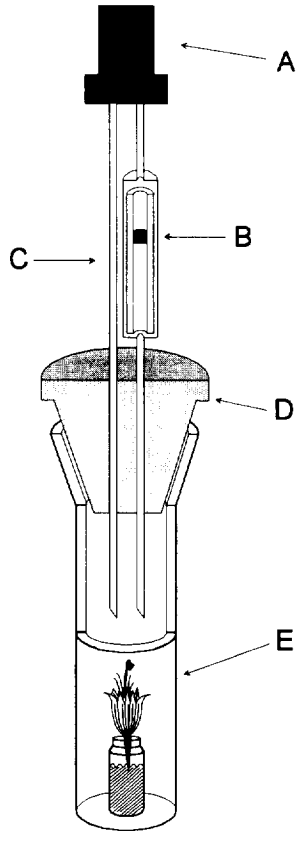


Fig. 2. Schematic drawing of the odour-collecting device. (A) circulation pump (*E. Fürgut*, D-88319 Aitrach, F.R.G.). (B) home-made precision stainless steel housing containing the charcoal trap (1.5 mg carbon; CLSA-Filter, CH-8405 Winterthur, Switzerland). The stainless steel housing was precisely polished to fit the precision glass liner containing a pad of charcoal. (C) Stainless steel tubes (i.d. 1.0 mm). (D) Teflon stopper. (E) Glass container, adequate to cover the biological object. The glass liner was removable from the stainless steel housing. The volatiles were desorbed from the carbon traps with small amounts of a suitable solvent (e.g. 2×20 μl of CH_2Cl_2, CS_2 or others) as described previously [21].

is, thus, able to convert enantiomeric mixtures of nerolidol with high efficacy into an airborne synomome that may attract useful insects. This ability to exploit enantiomeric mixtures might be particularly important for the conversion of a nerolidol containing blend emitted from an infested, neighbouring plant (e.g. a corn plant after damage by the beet army worm [3]). The compounds may be adsorbed to the surface of the leaf, internalized and eventually re-emitted after conversion into the synomones **1** and **2** or other compounds. Preliminary experiments with Lima beans and deuterium-labelled nerolidol clearly demonstrated this type of metabolism of airborne volatiles to be possible.

EXPERIMENTAL

Plant material. Leaves of *Spatiphyllum wallisii*, *Lycopersicon esculentum*, *Humulus lupulus* and *Gossypium herbaceum* were taken from fully developed plants from the Botanical Garden of Karlsruhe. Specimens of *Gerbera jamesonii*, cv *Sirtaki* were kindly provided by Prof. M. Dicke (Agricultural University, Wageningen, The Netherlands). Lima beans (*Phaseolus lunatus*, 'Ferry Morse' var. Jackson Wonder Bush, obtained from BASF AG Ludwigshafen) were grown from seeds in unsterilized garden soil. Individual plants were grown in pots (diam. 5.5 cm) at 22–25° using daylight fluorescent tubes at 800 lux and a photoperiod of 12 hr. Experiments were conducted with 11- to 15-day-old seedlings with two fully developed leaves. The cultivar *P. lunatus* (L.) Irak-PHA 8109/83 was obtained in 1990 from the Zentralinstitut für Genetik and Kulturpflanzenfoschung (D-06466 Gatersleben, Germany).

Table 1. Enantioselectivity of the nerolidol converting enzymes in selected plants

Plant (organ)	$[^2H_6]$**1** (%)	$[^2H_2]$**1** (%)
Phaseolus lunatus L.* (leaf)	96	4
Spatiphyllum wallisii (leaf)	94	6
Gerbera jamesonii cv Sirtaki (leaf)	82	18
Lycopersicon esculentum L. Karst. (leaf)	77	23
Humulus lupulus (leaf)	77	23
Phaseolus lunatus L., cv. Ferry-Morse (leaf)	76	24
Gossypium herbaceum (blossom)	66	34
G. herbaceum (leaf)	66	34
Fragaria × *magna* (leaf)	66	34

*See Experimental.

The labelling pattern (%) of the molecular ions of $[^2H_6]$**1** and $[^2H_2]$**1** reflects the degree of enantioselectivity of the degrading enzymes for (3*S*)-**3** and (3*R*)-**3**. $[^2H_6]$-**1** originates from (3*S*)-[12-$^2H_3$, 13-$^3H_3$]**3**; $[^2H_2]$**1** is the product of (3*R*)-[4-$^2H_2$, 15-$^2H_3$]**3**.

Synthetic procedures. Reactions were performed under Ar. Solvents and reagents were purified and dried prior to use. Solns were concd by flash evapn under red. pres. Dry Na_2SO_4 was used for drying. Optical rotation: Perkin-Elmer 241 Polarimeter. ^1H and ^{13}C NMR: 250 and 400 MHz, $CDCl_3$, TMS as int. standard; MS: Finnigan MAT 90 GLC/MS system and Finnigan ITD 800 combined with a Carlo-Erba gas chromatograph, model Vega, equipped with a fused-silica capillary SE 30, (10 m × 0.32 mm); carrier gas, He at 30 cm sec^{-1}; scan range: 35–350 amu. Analytical GLC: Carlo-Erba gas chromatograph, GC 6000, Vega Series 2, equipped with a fused silica capillary SE 30 (10 m × 0.32 mm); N_2 at 30 cm sec^{-1} as carrier. CC: silica gel, Si 60, (0.040–0.063 mm, E. Merck, Darmstadt, Germany).

(2E,6E,10E)-3,7,11-*Trimethyl*-[4-$^2H_2$,15-$^2H_3$]-*dodeca*-2,6,10-*trienoic acid ethyl ester* ($[^2H_5]$**8**). A soln of triethylphosphono acetate (8.96 g, 40 mmol) in THF (35 ml) was added within 15 min to a chilled and well stirred suspension of NaH (1.57 g, 55, 7 mmol as an 80% suspension in mineral oil) in THF (100 ml). Stirring was continued, and after 1 hr geranylacetone $[^2H_5]$**7** (5.0 g,

25 mmol) [8] was added slowly. The mixture was allowed to come to room temp. (2 hr). The reaction was complete after 2 hr at reflux. After hydrolysis with aq. Na_2CO_3 (80 ml, 10%), the product (3*E*/3*Z* = 88:12) was extracted with Et_2O (3 × 50 ml) and purified by chromatography on silica gel with pentane–Et_2O (9:1). Yield of (3*E*)-[$^2H_5$]**8**: 4.59 g (68%, > 95% according to GLC). IR ν_{max}^{KBr} cm^{-1}: 2978, 2926, 2856, 2203, 1717, 1642, 1446, 1375, 1352, 1269, 1215, 1149, 1097, 1044, 975, 868, 834; ^1H NMR ($CDCl_3$): δ1.27 (3H, *t*, Me-CH_2-O), 1.60 (6H, *s*, H-13, H-14), 1.68 (3H, *s*, H-12), 1.97–2.07 (4H, *m*, H-8/9), 2.15 (2H, *d*, *J* = 3.3 Hz, H-5), 4.14 (2H, *q*, Me-CH_2-O), 5.06 (2H, *t*, H-6, H-10), 5.66 (1H, *s*, H-4); EI-MS 70 eV, *m/z* (rel. int.): 269 $[M]^+$ (46), 226 (19), 166 (7), 153 (25), 137 (25), 133 (45), 109 (30), 81 (34), 69 (100), 55 (17), 41 (43); HR-MS *m/z*: $[M]^+$ 269.2392 (calcd. 269.2403 for $C_{17}H_{23}D_5O_2$).

(2E,6E,10E)-3,7,11-*Trimethyl*-[4-$^2H_2$,15-$^2H_3$]-*dodeca*-2,6,10-*trien*-1-*ol* [$^2H_5$] (**4**). A chilled and well stirred suspension of $LiAlH_4$ (1,2 g, 31 mmol) in dry Et_2O (100 ml) was gradually treated with $AlCl_3$ (1.47 g, 11 mmol). After 1 hr an ethereal soln (50 ml) of the ester [$^2H_5$]**7** (4.48 g, 16.6 mmol) was added. Stirring was maintained for 1 hr prior to hydrolysis. Aq. NaOH (2 N) was added, until a white, granular ppt. was formed. Removal of solids and extractive work-up with Et_2O yielded the alcohol [$^2H_5$]**4** after chromatography on silica gel with pentane–Et_2O (4:1). Yield: 3.31 g (88%). IR ν_{max}^{KBr} cm^{-1}: 3339, 2967, 2922, 2856, 2234, 2196, 2091, 1661, 1445, 1376, 1238, 1152, 1109, 1070, 1031, 988, 831; ^1H NMR ($CDCl_3$): δ1.19 (1H, *s*, HO), 1.60 (6H, *s*, H-13, H-14), 1.68 (3H, *s*, H-12), 2.00–2.11 (6H, *m*, H-5, H-8, H-9), 4.15 (2H, *d*, *J* = 6.2 Hz, H-1), 5.11 (2H, *m*, H-6, H-10), 5.42 (1H, *t*, H-2); EI-MS 70 eV, *m/z* (rel. int.): 227 $[M]^+$ (5), 209 (3), 184 (3), 166 (4), 136 (13), 127 (9), 93 (11), 81 (26), 69 (100), 41 (25); HR-MS *m/z*: $[M]^+$ 227.2292 (calcd. 227.2297 for $C_{15}H_{21}D_5O$).

Epoxidation of farnesol. A dry, thermostated reaction flask (− 20°) was charged with powdered molecular sieves (0.43 g, 4 Å) and CH_2Cl_2 (150 ml). To the stirred suspension is added sequentially, with stirring, L-(+)-diethyltartrate (0.23 g, 1,12 mmol), Ti(O-*i*-Pr)$_4$ (0.21 g, 0,74 mmol) and t-butyl hydroperoxide (7,5 ml, 22.5 mmol of a 3 M soln in *i*-octane). Following an ageing period of 20 min, a soln of the labelled farnesol [$^2H_6$]**4** (3.4 g, 15 mmol) in CH_2Cl_2 (10 ml) was added. The reaction was completed within 2 hr and hydrolysed with H_2O (4.2 ml). The mixture was allowed to come to room temp. (30–60 min) and then the tartrate esters were hydrolysed by addition of NaOH (6.0 ml of a 5 M, NaCl satd soln), the emulsion separated after about 20 min. Extractive work-up with CH_2Cl_2 (2 × 5 ml) and chromatography on silica gel with pentane–Et_2O (70:30) yielded the epoxyfarnesol.

(2S,3S,6E)-2,3-*Epoxy*-3,7,11-*trimethyl*-[12-$^2H_3$, 13-$^2H_3$]-*dodeca*-6,10-*dien*-1-*ol* [(2S,3S)-[$^2H_6$]**5**]. Prepared from [$^2H_6$]**4** (3,4 g, 15 mmol) and L-(+)-DET. Yield: 3.1 g (87%). $[\alpha]_{546}^{20}$ − 7.9 (CH_2Cl_2; *c* 9.10). IR ν_{max}^{KBr} cm^{-1}: 3431, 2959, 2929, 2857, 2223, 2190, 2110, 2064, 1728, 1663, 1453, 1384, 1250, 1158, 1076, 1035, 956, 864, 808; ^1H NMR ($CDCl_3$): δ1.30 (3H, *s*, H-15), 1.41–1.75 (2H, *m*, H-4), 1.60 (3H, *s*, H-14), 1.98–2.11 (6H, *m*, H-5, H-8, H-9), 2.98 (1H, *dd*, H-2, $J_{1,2trans}$ = 6.7 Hz, $J_{1,2cis}$ = 4.1 Hz), 3.68 (1H, *m*, H_A-1), 3.80 (1H, *m*, H_B-1), 5.10 (2H, *m*, H-6, H-10); EI-MS 70 eV, *m/z* (rel. int.): 244 $[M]^+$ (1), 226 (3), 213 (5), 195 (6), 156 (5), 138 (9), 109 (44), 95 (15), 81 (68), 75 (100), 55 (16), 43 (46); HR-MS *m/z*: $[M]^+$ 244.2308 (calcd. 244.2309 for $C_{15}H_{20}D_6O_2$).

(2R,3R,6E)-2,3-*Epoxy*-3,7,11-*trimethyl*-[4-$^2H_2$, 15-$^2H_3$]-*dodeca*-6,10-*dien*-1-*ol* [(2R,3R)-[$^2H_5$]**5**]. Prepared from [$^2H_5$]**4** (3.14 g, 15 mmol) and D-(−)-DET. Yield: 3.1 g (87%). $[\alpha]_{546}^{22}$ 7.7 (CH_2Cl_2; *c* 9.20). IR ν_{max}^{KBr} cm^{-1}: 3431, 2968, 2919, 2856, 2219, 2115, 1669, 1449, 1376, 1260, 1224, 1107, 1042, 997, 949, 805; ^1H NMR ($CDCl_3$): δ1.60 (6H, *s*, H-13, H-14), 1.68 (3H, *s*, H-12), 1.38 (1H, *s*, OH), 1.90–2.15 (6H, *m*, H-5, H-8, H-9), 2.96 (1H, *dd*, $J_{1,2trans}$ = 6.7 Hz, $J_{1,2cis}$ = 4.1 Hz, H-2), 3.68 (1H, *m*, H_A-1), 3.82 (1H, *m*, H_B-1), 5.1 (2H, *m*, H-6, H-10); EI-MS 70 eV, *m/z* (rel. int.): 243 $[M]^+$ (0.3), 225 (0.4), 212 (1.3), 199 (1), 182 (9), 136 (23), 114 (27), 93 (13), 83 (40), 69 (100), 46 (20), 41 (34); HR-MS *m/z*: 243.2258 (calcd. 243.2246 for $C_{15}H_{21}D_5O_2$).

(2S,3S,6E)-2,3-*Epoxy*-3,7,11-*trimethyl*-[12-$^2H_3$, 13-$^2H_3$]-*dodeca*-6,10-*dien*-1-*methanesulphonate* [(2S,3S)-[$^2H_6$]**6**]. A dry, thermostatted reaction flask (− 20°) was charged with a soln of epoxyfarnesol (2S,3S)**5** (2.82 g, 11,6 mmol) in CH_2Cl_2 (100 ml). Triethylamine (1.60 g, 13.9 mmol) and CH_4-sulphonyl chloride (1.60 g, 13.9 mmol) added slowly. After 1 hr the mixture was allowed to come to room temp. and hydrolysed by addition of $NaHCO_3$ (30 ml of a saturated soln). Extractive work-up with Et_2O and chromatography on silica gel with pentane–Et_2O (70:30) afforded the ester as a viscous oil. Yield: 3.64 g (99%). $[\alpha]_{546}^{20}$ − 17.3 (CH_2Cl_2; *c* 9.00). IR ν_{max}^{KBr} cm^{-1}: 2938, 2857, 2224, 2190, 2111, 2064, 1730, 1663, 1453, 1357, 1268, 1177, 1091, 1049, 957, 726; ^1H NMR ($CDCl_3$): δ1.33 (3H, *s*, H-15), 1.40–1.75 (2H, *m*, H-4), 1.60 (3H, *s*, H-14), 1.98–2.14 (6H, *m*, H-5, H-8, H-9), 3.08 (4H, *m*, H-2, S-Me), 4.25 (1H, *dd*, $J_{1,1}$ = 11.7 Hz, $J_{1,2trans}$ = 7.05 Hz, H_a-1), 4.43 (1H, *dd*, $J_{1,1}$ = 11.7 Hz, $J_{1,2cis}$ = 4.10 Hz, H_b-1), 5.10 (2H, *m*, H-6, H-10); EIMS 70 eV, *m/z* (rel. int.): 322 $[M]^+$ (0.6), 273 (3), 226 (4), 208 (2), 177 (9), 142 (11), 133 (19), 129 (21), 109 (30), 81 (58), 75 (100), 43 (18); HR-MS *m/z*: $[M]^+$ 322.2036 (calcd. 322.2084 for $C_{16}H_{22}D_6O_4S$)

(2R,3R,6E)-2,3-*Epoxy*-3,7,11-*trimethyl*-[4-$^2H_2$, 15-$^2H_3$]-*dodeca*-6,10-*dien*-1-*methanesulphonate* [(2R,3R)-[$^2H_5$]**6**]. Prepared from (2*R*,3*R*)**5** as above. $[\alpha]_{546}^{20}$ 17.1 (CH_2Cl_2; *c* 7.45). IR ν_{max}^{KBr} cm^{-1}: 2967, 2917, 2856, 2222, 1732, 1668, 1451, 1359, 1265, 1178, 1092, 981, 959, 832, 728; ^1H NMR ($CDCl_3$): δ1.60 (6H, *s*, H-13, H-14), 1.67 (3H, *s*, H-12), 1.98–2.09 (6H, *m*, H-5, H-8, H-9), 3.09 (4H, *m*, H-2, S-Me), 4.24 (1H, *dd*, $J_{1,1}$ = 11.7 Hz, $J_{1,2trans}$ = 7.20 Hz, H_a-1), 4.43 (1H, *dd*, $J_{1,1}$ = 11.7 Hz, $J_{1,2cis}$ = 4.10 Hz, H_b-1), 5.10 (2H, *m*, H-6, H-10); EI-MS 70 eV, *m/z* (rel. int.): 321 $[M]^+$ (2), 278 (1), 225 (1), 182 (10), 136 (40), 123 (13), 109 (14), 83 (32), 69 (100), 41 (31); HR-MS *m/z*: $[M]^+$ 321.2012 (calcd. 321.2022 for $C_{16}H_{23}D_5O_4S$).

(3S,6E)-3,7,11-*Trimethyl*-[12-$^2H_3$, 13-$^2H_3$]-*dodeca*-1,6,10-*trien*-3-*ol* ([$^2H_6$]**3**). Ammonia (*ca* 40 ml) was con-

densed to a cold (− 78°) soln of the mesyl ester (2*S*,3*S*)**6** (1,2 g, 72 mmol) in THF (100 ml). The mixture was allowed to come to − 40°, and Na was added in small portions until the blue colour persisted for at least 15 min. Et_2O (50 ml) and NH_4Cl (*ca* 3 g) were added, and the NH_3 evapd. Extractive work-up with Et_2O and chromatography on silica gel with pentane–Et_2O (9:1) afforded the labelled nerolidol. Yield: 0.52 g (61%). $[\alpha]_{578}^{20}$ 13.0 (CH_2Cl_2; *c* 9.53). IR ν_{max}^{KBr} cm^{-1}: 3393, 3086, 2964, 2926, 2854, 2224, 2190, 2110, 2064, 1663, 1642, 1452, 1411, 1371, 1158, 1110, 1049, 995, 920, 861, 692; ^1H NMR ($CDCl_3$): δ 1.28 (3H, *s*, H-15), 1.54–1.62 (2H, *m*, H-4), 1.59 (3H, *s*, H-14), 1.98–2.08 (6H, *m*, H-5, H-8, H-9), 5.05–5.14 (2H, *m*, H-6, H-10), 5.06 (1H, *dd*, $J_{1,1}$ = 1.2 Hz, $J_{1,2cis}$ = 10.6 Hz, H-1), 5.21 (1H, *dd*, $J_{1,1}$ = 1.3 Hz, $J_{1,2trans}$ = 17.4 Hz, H-1), 5.91 (1H, *dd*, $J_{1,2trans}$ = 17.4 Hz, $J_{1,2cis}$ = 10.7 Hz, H-2); EI-MS 70 eV, *m/z* (rel. int.): 210 $[M - H_2O]^+$ (4), 161 (20), 142 (28), 119 (11), 107 (35), 93 (78), 81 (32), 75 (100); HR-MS *m/z*: $[M]^+$ 228.2333 (calcd. 228.2360 for $C_{15}H_{20}D_6O$).

(3R,6E)-3,7,11-*Trimethyl*-[4-$^2H_2$,15-$^2H_3$]-*dodeca*-1,6,10-*trien*-3-*ol* ([$^2H_5$]**3**). From (2*R*,3*R*)**6** as above. $[\alpha]_{578}^{22}$ − 12.7 (CH_2Cl_2; *c* 8.90). IR ν_{max}^{KBr} cm^{-1}: 3403, 3085, 2967, 2924, 2855, 2228, 1842, 1667, 1627, 1445, 1408, 1376, 1278, 1190, 1108, 1049, 996, 920, 844, 665; ^1H NMR ($CDCl_3$): δ 1.59 (6H, *s*, H-13, H-14), 1.68 (3H, *s*, H-12), 1.97–2.04 (6H, *m*, H-5, H-8, H-9), 5.03–5.15 (2H, *m*, H-6, H-10), 5.06 (1H, *dd*, $J_{1,1}$ = 1.3 Hz, $J_{1,2trans}$ = 10.7 Hz, H-1), 5.21 (1H, *dd*, $J_{1,1}$ = 1.3 Hz, $J_{1,2trans}$ = 17.3 Hz, H-1), 5.91 (1H, *dd*, $J_{1,2trans}$ = 17.3 Hz, $J_{1,2cis}$ = 10.7 Hz, H-2); MS 70 eV, *m/z* (rel. int.): 227 $[M]^+$ (0.2), 209 (4), 184 (2), 166 (11), 136 (40), 123 (17), 97 (16), 69 (100), 57 (13), 41 (47); HR-MS *m/z*: $[M]^+$ 227.2278 (calcd. 227.2297 for $C_{15}H_{21}D_5O$).

Feeding experiments. Equal amounts of (3*S*)-[12-$^2H_3$, 13-$^3H_3$]**3** and (3*R*)-[4-$^2H_2$, 15-$^2H_3$]**3** (2.5 mg, each) were added to a small round bottomed flask with water (5 ml). Sonication of the flask for 2 min (cleaning bath, 2 × 320 W) gave an emulsion which was stable for several days. Freshly cut plants with *ca* 1–2 leaves were immediately placed into the emulsion. The immersed plants were enclosed in the odour collecting device shown in Fig. 1, and air circulation maintained for 20 hr. During circulation, the produced volatiles were adsorbed on to the charcoal trap [20]. After desorbtion [21] from the carbon traps with CH_2Cl_2 (2 × 15 μl) the volatiles were directly analysed by GC-MS. Separation of the compounds was achieved on a WCOT fused-silica column (BP 5, 25 m × 0.31 mm) under programmed conditions (60° for 3 min, then 12° min^{-1} up to 240° for 8 min). MS (Finnigan MAT 90): mass range 35–220 amu. Injection port and transfer line at 220°.

Acknowledgements—Financial support by the Deutsche Forschungsgemeinschaft (Bonn) and the Fonds der Chemischen Industrie (Frankfurt am Main) is gratefully acknowledged. We also thank Bayer AG (Leverkusen) and BASF (Ludwigshafen) for generously supplying chemicals and solvents.

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