28. The Bioconversion of (3*RS*,*E*)- and (3*RS*,*Z*)-Nerolidol into Oxygenated Products by *Streptomyces cinnamonensis*. Possible Implications for the Biosynthesis of the Polyether Antibiotic Monensin A?

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The ability of the monensin-producing organism *Streptomyces cinnamonensis* to bioconvert the (*E*)-and (*Z*)-isomers of nerolidol (= 3,7,11-trimethyldodeca-1,6,10-trien-3-ol) into new oxygenated products has been investigated. When a ^{3}H-labelled racemic form of each sesquiterpene was added to fermentations of *S. cinnamonensis*, several new ^{3}H-labelled products could be detected. Two products were isolated from bioconversion of (*E*)-nerolidol, the amide **8** and the diol **9** *(Scheme 2)*, whereas four products were isolated from bioconversion of (*Z*)-nerolidol, the epoxydiol **10,** triols **11** and **12,** and the tetrahydrofuryl alcohol **13** *(Scheme 4)*. Products **9**–**13** were obtained as a 1:1 mixture of diastereoisomers, and **12** was shown to arise by the overall *anti* addition of two OH groups to the trisubstituted (*Z*)-double bond of (*Z*)-nerolidol. Both isomers of nerolidol as well as the acetylene **7** are inhibitors of monensin production in shake cultures of *S. cinnamonensis*.

Introduction. – Microorganisms of the genus *Streptomyces* are well known for their capacity to produce secondary metabolites of diverse structural types, and they are by far the richest source of natural products with antibiotic or other useful biological activities. More recently, *Streptomycetes* have also attracted interest as a source of enzymes for the chemical transformation of various substrates and xenobiotics. A notable example is the ability of *Streptomyces griseus* ATCC 13273 to catalyze the stereo- and regiospecific oxidation of a diverse array of organic chemicals, including the hydroxylation of aromatic, cyclic, and aliphatic molecules, O-, S-, and N-oxidations, a C–C fission, epoxidation, and O- and N-dealkylation, upon compounds as diverse in structure as alkaloids, coumarins, rotenoids, and chromenes [1]. Crude extracts of *S. griseus* contain high levels [2] [3] of cytochrome P450, and recently, the purification and characterisation of a unique cytochrome P450 from this *S. griseus* strain was reported [1]. Cytochrome P450 dependent monooxygenases are also implicated in the biosynthesis of various antibiotics, and one active in erythromycin production has been purified [4] [5] from *S. erythraea*. In other systems, non-heme iron and α-ketoglutarate dependent oxygenases and desaturases are also known to be active in key steps in antibiotic biosynthesis, especially within the

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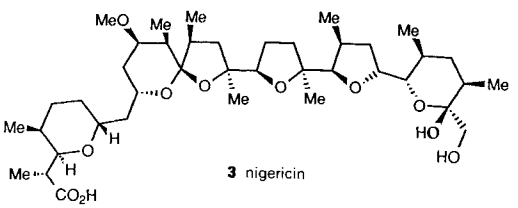
pathways to β-lactams [6]. Collectively, these studies may serve to herald the emergence from this genus of many other related oxygenases and desaturases, possibly sharing primary sequence homologies and having the ability to oxidise a wide spectrum of acceptable substrates.

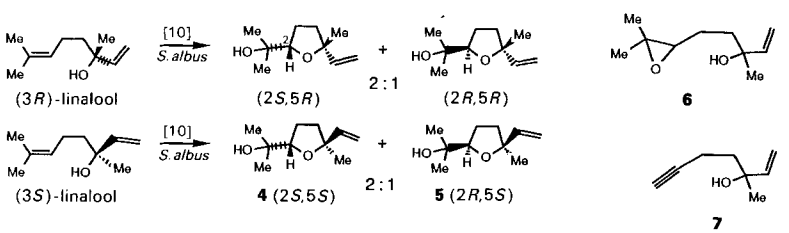
The biosynthesis of the polyether ionophore antibiotic monensin A is currently of interest because of the putative role [7] [8] of one or more epoxidases and a hydroxylase in the stereospecific transformation of the triene intermediate **2** along the pathway shown in Scheme 1 in [9]. As discussed in the preceding paper [9], direct support for the involvement of the triene **2** and triepoxide **1** in monensin biosynthesis has, so far, not been forthcoming. As an extension of our interest in this problem, we set out to determine whether epoxidases could be detected in the monensin-producing organism, using a whole-cell assay to monitor the bioconversion of the monoterpenoid nerolidol into



3 nigericin

oxygenated products. Nerolidol contains one trisubstituted double bond, so it was also of interest to establish whether any selectivity could be observed in the oxidation of (*E*)- *vs.* (*Z*)-nerolidol. This work was prompted by the observations of *David* and *Verschambre* [10] that whole-cell cultures of the nigericin(**3**)-producer *Streptomyces albus* catalysed the bioconversion of linalool into linalool oxides **4** and **5**, as shown in *Scheme 1*, ostensibly a simple model for the chemistry implicated in the monensin pathway. As a first step, the ability of *S. cinnamonensis* to catalyse this bioconversion of nerolidol was examined.

Scheme 1



Results. – *Bioconversion of Linalool.* Initial trial experiments involved the addition of racemic linalool to shake flask cultures of *S. cinnamonensis* grown in a complex production medium [11]. After extraction of the culture supernatant and chromatography, both diastereoisomeric linalool oxides *rac*-**4** and *rac*-**5** were isolated in a 1:1 ratio, their identities being confirmed by GC/MS and by NMR spectroscopy, after comparison to reference spectra derived from material of synthetic origin. The turnover yield of the oxides was *ca.* 1%, while most of the linalool could be recovered intact from the extract.

Prolonged fermentation runs in the presence of linalool did not significantly improve the yield of the oxides, which are low presumably because linalool is a poor substrate for the relevant oxygenases or because these enzymes are expressed at only very low levels. Alternatively, permeability to the cell membrane could again be a problem. It seems likely that epoxide **6** is an intermediate in this bioconversion although this was not detected as a component of the fermentation extract.

In a separate experiment, the acetylenic analogue **7** was synthesised as a potential suicide inhibitor of oxidases catalysing this bioconversion since acetylenes have been shown to cause the specific inhibition of both purified cytochrome P450 and iron-α-keto-glutarate dependent enzymes [12] [13]. The aim in synthesising this material was to determine its effect if any upon monensin production, since it seemed possible that the bioconversion of linalool might be catalysed by enzymes that are part of the monensin biosynthetic pathway. As anticipated, the addition of **7** to cultures of *S. cinnamonensis*, immediately after the normal onset of antibiotic production, caused the complete cessation of monensin biosynthesis. A careful analysis of the extract from such fermentations, however, failed to reveal the presence of any other interesting metabolites such as the putative triene intermediate **2**. It is possible that a very efficient pathway of β-oxidation in whole cells prevents the accumulation of intermediates in this pathway when these are closely related in structure to long-chain fatty acids.

*Bioconversion of (*E*)- and (*Z*)- Nerolidol.* For these bioconversion experiments ^{3}H-labelled forms of (*E*)- and (*Z*)-nerolidol were first synthesised starting from labelled- and geranyl- and neryl-acetone, respectively (two steps: *i*) lithium diisopropylamide (LDA), then CF_3COO^3H; *ii*) $CH_2CHMgBr$, THF; see *Exper. Part*). Initial bioconversion experiments using shake flask cultures indicated that the turnover yields were again low (< 1%), with most of the nerolidol being recoverable in high yield (> 95%). However, in each case, several new ^{3}H-labelled products were clearly evident by radio-TLC analysis of the fermentation extract. Each experiment was repeated on a larger scale by the addition of racemic (*E*)- or (*Z*)-[3-*methyl*-^{3}H]nerolidol (10 g) in two equal portions to a 5-l-batch fermentation of *S. cinnamonensis*. At the end of each fermentation, the cells were separated by centrifugation and both the supernatant and the cell paste were extracted with CH_2Cl_2.

Portions of the extract from the bioconversion of (*E*)-[3-*methyl*-^{3}H]nerolidol were first fractionated by elution from *LH-20 Sephadex* with CH_2Cl_2. Two new ^{3}H-labelled products **8**[2]) and **9**[2]) *(Scheme 2)* could be detected in the eluate by TLC analysis (silica gel, AcOEt; detection by vanillin spray), one giving rise to a blue spot with R_f 0.23 (**8**) and the second a green spot with R_f 0.55 (**9**; *cf.* monensin A: bright red spot at R_f 0.48). Both **8** and **9** were purified by prep. TLC. Amide **8** was optically active $[\alpha]_D^{22} = -4.2$ ($c = 0.34$, CH_2Cl_2), although its optical purity was not determined. The compound did not react with diazomethane and showed an absorbance at 1680 cm^{-1} in the IR spectrum. In the ^{1}H-NMR spectrum ($CDCl_3$), two broad signals at 5.60 and 5.65 ppm slowly disappeared after a D_2O shake. Other data from the ^{1}H 2D-COSY and ^{13}C-NMR and mass spectra of **8** were entirely in accord with the proposed structure (see *Exper. Part*).

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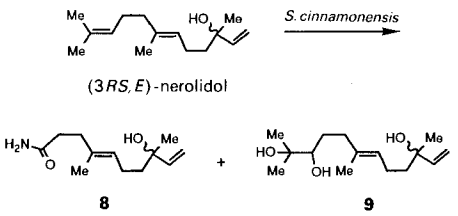
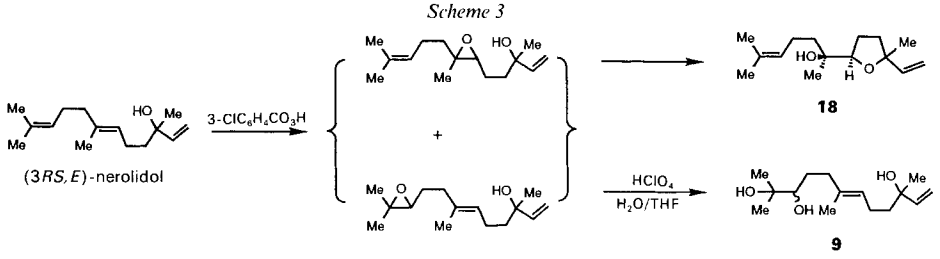
[2]) For convenience, we use the key numbers of the unlabelled compounds for discussions of the bioconversions of [^{3}H]-labelled precursors (for details, see *Exper. Part*).

Scheme 2



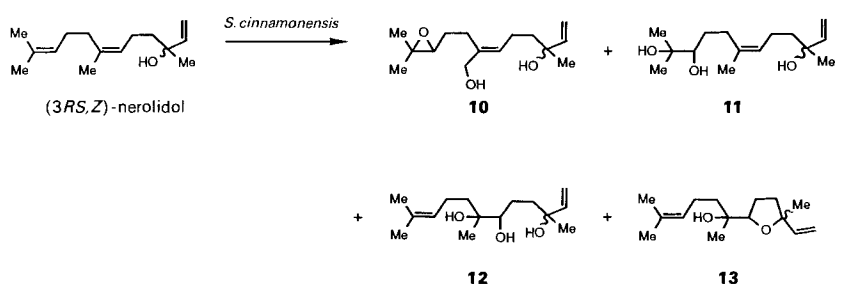
The bioconversion product **9** appeared to be a 1:1 mixture of diastereoisomers (NMR). This was confirmed after comparison to authentic material generated from (*E*)-nerolidol by synthesis (2 steps: *i*) 3-chloroperbenzoic acid (3-$ClC_6H_4CO_3H$), THF 0°; *ii*) $HClO_4$, THF/H_2O; *Scheme 3*). The synthetic mixture of diastereoisomers arising from oxidation of the terminal double bond showed NMR properties identical to those of the bioconversion product. No attempt was made to determine the enantioselectivity of the bioconversion process. No products arising from oxidation of the central trisubstituted (*E*)-double bond were detected.

Scheme 3



As before, the organic extract from the experiment using (*Z*)-[3-*methyl*-^{3}H]nerolidol was first chromatographed by elution from *LH-20 Sephadex* with CH_2Cl_2. This resulted in the isolation of unreacted (*Z*)-[3-*methyl*-^{3}H]nerolidol (> 95% recovery) and the partial purification of several new radiolabelled products (*Scheme 4*)[2]). This partially purified extract was then examined by TLC (see *Exper. Part*) and liquid scintillation counting. Each new product **10**–**13**[2]) (*Scheme 4*) was obtained homogeneous in a turnover yield of *ca.* 0.1%.

Scheme 4

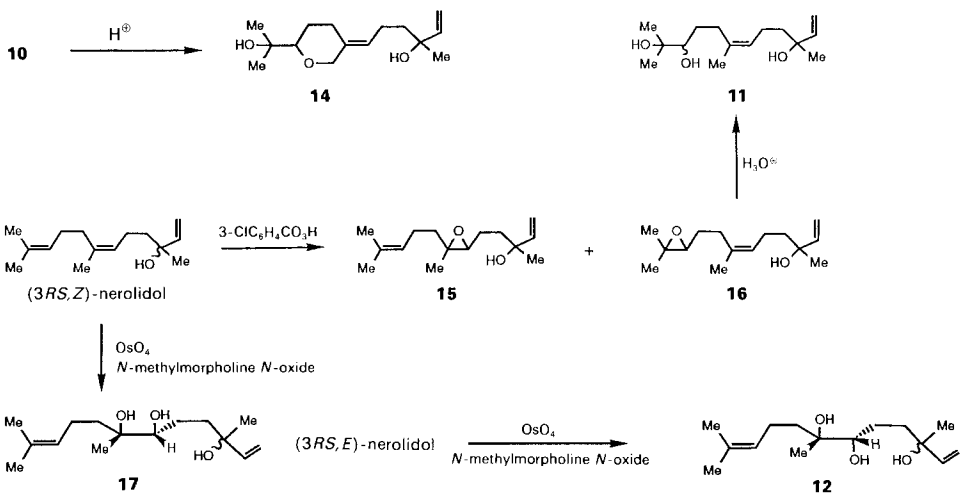


The structure of **10** (1:1 mixture of diastereoisomers) was established by spectroscopic analysis. Upon treatment with acid (trace HCl, MeCN), **10** was transformed cleanly into a new diol **14** *(Scheme 5)*.

The high-resolution Cl-MS (NH_3) of **10** showed a peak at *m/z* 272 consistent with the molecular formulae $C_{15}H_{30}NO_3$ [$(M + NH_4)^+$]. In the ^{1}H-NMR spectrum, there were no *s* in the region 1.5–1.8 ppm assignable to allylic Me groups, although signals in the region 4.9–6.1 ppm integrated to 4H, suggesting that one trisubstituted olefin remained intact. The epoxide proton resonated characteristically at 2.75 ppm as a *dd*, while the allylic CH_2OH group appeared as a slightly broadened *s* at 4.05 ppm. The observation of an NOE between this CH_2OH group and the olefinic proton at 5.49 ppm confirmed the (*E*)-configuration (unchanged!) of the central trisubstituted double bond. All assignments were supported also by coupling patterns determined by 2D-COSY NMR. ^{1}H 2D-COSY NMR, low-resolution FAB and high-resolution CI-MS (NH_3) established the structure of **14**. Notable in the ^{1}H-NMR spectrum of **14** was the absence of the epoxide proton at 2.75 ppm, the presence of a new signal at 3.45 ppm, and the appearance now of an *AB* system at 4.15 ppm for the allylic CH_2O group.

The constitution of triols **11** and **12** were assigned from spectroscopic data and by comparison to authentic materials of synthetic origin. Thus, oxidation of (*Z*)-nerolidol with 3-chloroperbenzoic acid afforded a 1:1 mixture of the two epoxy alcohols **15** and **16** which could be separated by flash chromatography *(Scheme 5)*. Upon treatment with aqueous acid, epoxy derivative **16** was converted into the vicinal diol **11** (1:1 mixture of

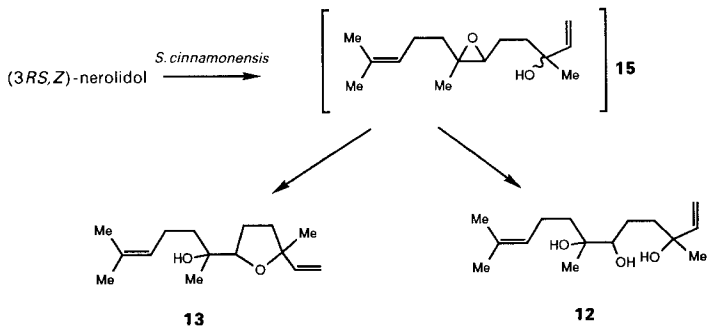
Scheme 5



diastereoisomers). This material proved to be identical (except for its optical activity) to the bioconversion product. The relative configuration of the vicinal diol moiety of **12** was established straightforwardly through comparison to the vicinal diols **17** and **12** obtained by oxidation of (*Z*)- and (*E*)-nerolidol, respectively, with OsO_4 and *N*-methylmorpholine *N*-oxide *(Scheme 5)*. Thus, during the bioconversion of (*Z*)-[3-*methyl*-^{3}H]nerolidol to **12**, the two new OH groups were added in an overall *anti*-fashion to the trisubstituted (*Z*)-double bond, most plausibly through the opening by H_2O of an epoxide intermediate.

Alcohol **13** proved more difficult to purify than the other bioconversion products, because it possessed chromatographic properties similar to those of (*Z*)-nerolidol. As a starting point, we reasoned that if the 6,7-epoxynerolidol **15** was an intermediate in the bioconversion to **12**, then **15** might also undergo cyclisation to form the tetrahydrofuryl alcohol **13** *(Scheme 6)*. Upon treatment of synthetic 6,7-epoxynerolidol **15** with acid or

Scheme 6



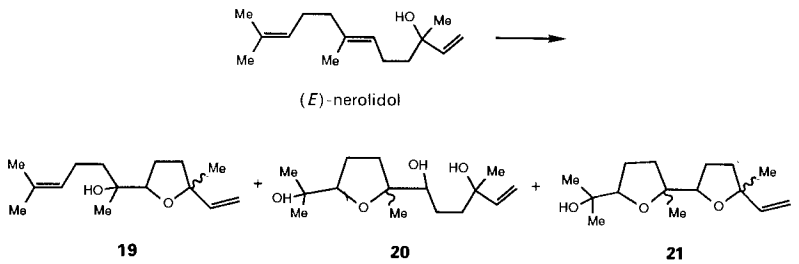
base, a single constitutional isomer was produced as a 1:1 mixture of diastereoisomers which was shown to be **13** by NMR analysis. This material also migrated with the corresponding bioconversion product upon TLC analysis, and its addition to the post *LH-20* fermentation extract and repurification gave material with a constant specific activity of ^3H corresponding to a 0.1% turnover yield from (*Z*)-[3-*methyl*-^3H]nerolidol. Finally, the crude extract was fractionated by TLC in order to remove as much (*Z*)-[3-*methyl*-^3H]nerolidol as possible, and fractions containing **13** were examined by GC/MS, using the synthetically derived material **13** as a standard. The chromatographic and MS properties of the two samples were identical, proving conclusively that bioconversion product **13** was indeed a 1:1 mixture of diastereoisomers. A similar analysis of the bioconversion products from (*E*)-[3-*methyl*-^3H]nerolidol confirmed that the corresponding cyclic material **18** (see *Scheme 3*) was not present in this extract. Thus, after addition of synthetic **18** to the crude extract, the level of specific radioactivity declined steadily upon repeated rounds of purification, dilution with (*E*)-nerolidol, and repurification. After six cycles, the specific activity of the product indicated that at best only a 0.02% bioconversion of (*E*)-[-*methyl*-^3H]nerolidol into **18** would have been possible. In practice, the presence of **18** could not be detected directly neither by TLC nor by GC/MS analysis of this extract.

Discussion. – The bioconversion of (*E*)-nerolidol into **8** and **9** *(Scheme 2)* and of (*Z*)-nerolidol into **10**–**13** *(Scheme 4)* by whole cells of *S. cinnamonensis* demonstrates for the first time the presence of epoxidases and a hydroxylase in the monensin-producing strain.

The very low efficiency of these biotransformations made the isolation and characterisation of new products an extremely difficult task. Except for **8**, each was obtained as a 1:1 mixture of diastereoisomers, and due to the small quantities isolated, no attempt was made to determine their enantiomeric purities. Analogous biotransformations of (*E*)- and (*Z*)-nerolidols into trihydroxylated and tetrahydrofuranoid structures, catalysed by

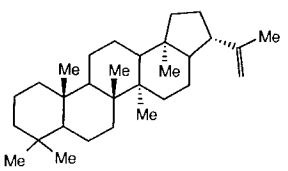
fungal strains, have been reported [14] [15] previously, and the yields of these biotransformation products were typically much higher than those seen in our work. *E.g.* (*E*)- and (*Z*)-nerolidols are transformed by *Diplodia gossypina* and *Corynespora* to the vicinal-diol derivatives **9** and **11**, respectively, amongst other products. Whereas, *Gibberella cyanea* and *Rhizopus arrhizus* are able to oxidise the central double bond of (*E*)-nerolidol giving tetrahydrofuran ethers [14], including **19**–**21** *(Scheme 7)*, the oxidation of the central double bond of (*Z*)-nerolidol in a similar manner has not been reported.

Scheme 7



The appearance in our experiments of the amide **8** from (*E*)-nerolidol is interesting. It is conceivable that the triol **9** is an intermediate in this catabolism, but the origin of the N-atom in **8** is currently unknown. A related transformation is known [16] during the biosynthesis of mycophenolic acid in fungi where oxidative cleavage of the central (*E*)-double bond of a farnesyl moiety occurs *via* a diol and an α-hydroxy ketone to give a carboxylic acid.

An unexpected feature of our work is the apparent preference shown for oxidation of the (*Z*)-double bond in (*Z*)-nerolidol, but not the (*E*)-double bond in (*E*)-nerolidol. It is unlikely that other oxidation products from (*E*)-nerolidol remained undetected because they were more rapidly degraded by the organism.



22 diploptene

Streptomyces cinnamonensis does have the capacity to produce terpenoid compounds since we have isolated and characterised both squalene and diploptene [17] (**22**) from whole cell extracts of this organism.

The normal *in-vivo* function of the enzymes catalysing these oxidative reactions is at present unknown. One possibility is that they are involved in the catabolism of aromatic materials normally present in complex constituents of the fermentation medium. Thus, the cytochrome P450 purified from *S. griseus* mentioned above [1–3], appears to be induced by an isoflavonoid constituent of soybean flour. If a related activity is involved here, it might explain the preference seen for oxidation of a (*Z*)-olefin, but not an (*E*)-olefin. More intriguing is the possibility that these oxidative enzymes might comprise

part of the secondary metabolism of the cell, and hence are active normally in the biosynthesis of monensin A. Very indirect evidence in support of this comes from the observation that linalool, nerolidol, and especially the acetylene **7** are effective inhibitors of monensin production when added to fermentations of the producing organism. If correct, this interpretation suggests that the intermediates acted upon by these enzymes might also possess one or more double bonds with the (*Z*)-configuration which is clearly inconsistent with the currently accepted biogenetic route to monensin A. At the least, therefore, these observations suggest that it may no longer be acceptable to assume that a triene with the (all-*E*)-configuration is involved in monensin A biosynthesis. Further experiments are now underway in an attempt to illuminate new facets of this interesting problem.

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Experimental Part

1. *General.* See [9].

2. *(3RS)-3-Methylhept-1-en-6-yn-3-ol* (**7**). Hex-5-yn-2-one was prepared from prop-2-ynyl bromide and ethyl acetoacetate according to [18]. Hex-5-yn-2-one (10.0 g, 104 mmol) in THF (10 ml) was added dropwise to vinylmagnesium bromide (2.70 g, 208 mmol) in THF (10 ml) at r.t. and stirred for 22 h. After addition of sat. NH_4Cl soln. (1 l), the aq. phase was extracted with Et_2O, the combined org. phase washed with dil. HCl soln. and brine, dried ($MgSO_4$), and evaporated, and the residue distilled under reduced pressure: **7** (5.4 g, 42%). B.p. 62°/20 Torr. IR ($CHCl_3$): 3400*s*, 3300*m*, 2120. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.16 (*s*, Me–C(3)); 1.65 (*m*, CH_2(4)); 1.88 (*t*, J = 3, H–C(7)); 2.10 (*m*, CH_2(5)); 2.81 (br. *s*, OH); 4.95 (*dd*, J = 2, 10, 1 H–C(1)); 5.11 (*dd*, J = 2, 16, 1 H–C(1)); 5.73 (*dd*, J = 10, 16, H–C(2)).

3. *(3RS,6Z)-3,7,11-[3-*methyl-^{3}*H]Trimethyldodeca-1,6,10-trien-3-ol* (= *(3RS,Z)-[3-*methyl-^{3}*H]Nerolidol*). ^{3}[H]H_2O (16 µl, 80 µCi) was added to $(CF_3CO)_2O$ (0.185 g, 0.88 mmol) in THF (2 ml) to form a soln. of $CF_3COO[^3H]$. Lithium diisopropylamide (LDA; 2.0 ml, 1.69 mmol) was added dropwise to nerylacetone (= (5*Z*)-6,10-dimethylundeca-5,9-dien-2-one; 0.31 g, 1.60 mmol) in THF (3 ml) cooled to −70° and stirred for 5 min. The $CF_3COO[^3H]$ in THF was then added and stirred for a further 5 min before more nerylacetone (3.44 g, 17.75 mmol) in Et_2O was added. After warming to r.t., the Et_2O layer was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. The residue was distilled (78°/0.5 Torr): [1-^{3}H]nerylacetone (2.93 g).

Vinylbromide (3.5 ml) in THF (30 ml) was added to Mg (0.7 g, 29.3 mmol) and I_2 (1 mg, cat.) in THF (5 ml). Once a clear soln. had formed, [1-^{3}H]nerylacetone (2.93 g, 15.1 mmol) in THF (5 ml) was added and the soln. stirred overnight. Sat. aq. NH_4Cl soln. (5 ml) was added and the mixture extracted with Et_2O. The combined org. phase was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. The residue was distilled: *(3RS,Z)-[3-*methyl-^{3}*H]nerolidol* (2.58 g, 77.0%). B.p. 106°/0.5 Torr. ^{1}H-NMR (270 MHz, $CDCl_3$): 1.28 (*s*, Me–C(3)); 1.60 (*s*, H–C(11)); 1.68 (*s*, Me–C(7), CH_3(12)); 1.90 (br., OH); 2.05 (CH_2(4), CH_2(5), CH_2(8), CH_2(9)); 5.20 (*d*, J = 10, ^{1}H–C(1)); 5.10 (*m*, H–C(6), H–C(10)); 5.20 (*d*, J = 16, 1 H–C(1)); 5.91 (*dd*, J = 16, 10, H–C(2)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 17.7, 22.6 (2 *q*); 23.5 (*t*); 25.8 (*q*); 26.6 (*t*); 27.8 (*q*); 32.0, 42.5 (2 *t*); 73.4 (*s*); 111.8 (*t*); 124.4, 125.15 (2 *d*); 131.6, 135.6 (2 *s*); 145.15 (*d*).

The specific activity was determined as follows: Et_3N (800 µl, 5.8 mmol), 4-(dimethylamino)pyridine (74 mg, 0.61 mmol), and 4-nitrobenzoyl chloride (170 mg, 0.92 mmol) were added to (3*RS*, *Z*)-[3-*methyl*-^{3}H]nerolidol (154 µl, 134.9 mg, 0.608 mmol) in pyridine (4 ml). The org. phase was washed with H_2O, sat. $CuSO_4$ soln. and brine, dried ($MgSO_4$), and evaporated and the residue purified by FC ($CHCl_3$): benzoate derivative ($8.94 \cdot 10^7$ dpm mmol^{-1}). ^{1}H-NMR (270 MHz, $CDCl_3$): 1.55 (*s*, Me–C(3)); 1.65 (*s*, Me–C(7), Me–C(11)); 1.74 (*s*, CH_3(12)); 2.02 (*m*, CH_2(4), CH_2(5), CH_2(8), CH_2(9)); 5.11 (*m*, H–C(6), H–C(10)); 5.18 (*d*, J = 17, 1 H–C(1)); 5.27 (*d*, J = 13, 1 H–C(1)); 6.09 (*dd*, J = 17, 13, H–C(2)); 7.84 (*m*, 4 arom. H).

4. *(3RS,6E)-3,7,11-[3-*methyl-^{3}H*]Trimethyldodeca-1,6,10-trien-3-ol* (= *(3RS,E)-[3-*methyl-^{3}H*]Nerolidol*) was synthesised from (= (5*E*)-6,10-dimethylundeca-5,9-dien-2-one) geranylacetone by the method used for the (*Z*)-isomer. B.p. 102°/0.4 Torr. ^{1}H-NMR (270 MHz, $CDCl_3$): 1.28 (*s*, Me–C(3)); 1.62 (*s*, Me–C(11), CH_3(12)); 1.70 (*s*, Me–C(3)); 2.05 (*m*, CH_2(4), CH_2(5), CH_2(8), CH_2(9), OH); 5.04 (*d*, *J* = 10, 1 H–C(1)); 5.02–5.20 (*m*, H–C(6), H–C(10)); 5.22 (*d*, *J* = 17, 1 H–C(1)); 5.97 (*dd*, *J* = 17, 12, H–C(2)).

The specific radioactivity was determined by the method used for the (*Z*)-isomer: $3.73 \cdot 10^7$ dpm mmol^{-1}.

5. *Growth of* S. cinnamonensis *and Feeding of Labelled (*E*)- and (*Z*)-Nerolidol.* A spore suspension of *S. cinnamonensis* strain A3823.5 was added to four sterile vegetative media (60 ml) each in a wide-necked *Erlenmeyer* flask (500 ml), and the cultures were placed on an orbital shaker at 30° for 18 h. These cultures were used to inoculate a 5-l-batch fermentation grown with stirring at 1000 rpm, 30°, with an air flow of 5 l min^{-1}. After 38 h, monensin production had commenced, and so (3*RS,E*)-[3-*methyl*-^{3}H]nerolidol (5.0 g, $1.34 \cdot 10^8$ dpm) was added, with a second batch (5.0 g, $1.34 \cdot 10^8$ dpm) added after 49 h. The fermentation was stopped after 102 h and the broth centrifuged (10000 rpm, 10 min, *Beckman JA14* rotor). The supernatant and cell paste were extracted (CH_2Cl_2) and the extracts dried (Na_2SO_4) and evaporated: crude extract (32 g) which was stored at −20°. Crude extract (3 g) was fractionated by eluting from an *LH-20 Sephadex* column (75 × 2.5 cm) in CH_2Cl_2 (flow rate 0.15 ml min^{-1}, 20 min fractions). *Fr. 70–90* and *95–105*, resp., were combined and evaporated. Both residues were further fractionated by TLC (AcOEt): 1 mg of [^{3}H]-**9** (R_f 0.44) and 4 mg of [^{3}H]-**8** (R_f 0.31), resp. The TLC colorimetric assay used to detect metabolites involved spraying the developed and dried TLC plate with vanillin (3 g) in MeOH (97 ml) and conc. H_2SO_4 soln. (2.4 ml). The plate was then dried with heating at 80°.

The feeding experiment involving (3*RS,Z*)-[3-*methyl*-^{3}H]nerolidol was carried out in the same way. After 28 and 37 h, (3*RS,Z*)-[3-*methyl*-^{3}H]nerolidol (5.0 g, $2.11 \cdot 10^8$ dpm) was added. The culture was worked up after 101 h. Crude extract (3 g) was fractionated by elution from an *LH-20 Sephadex* column (75 × 2.5 cm) in CH_2Cl_2 (flow rate 0.15 ml min^{-1}, 20 min fractions). *Fr. 95–105* were combined and evaporated. The residue was purified further by TLC (AcOEt): 0.5–1 mg of [^{3}H]-**10** (R_f 0.45). Similarly, *Fr. 100–115* yielded, after TLC (AcOEt/CH_2Cl_2 65:35), 0.5–1 mg each of [^{3}H]-**11** (R_f 0.32) and [^{3}H]-**12** (R_f 0.35).

*(4E,8RS)-8-Hydroxy-4,8-[8-*methyl-^{3}H*]dimethyldeca-4,9-dieneamide* ([^{3}H]-**8**). $[\alpha]_D^{23} = -4.2$ (*c* = 0.34, CH_2Cl_2). IR ($CHCl_3$): 3600*s*, 3510*m*, 3420*m*, 3600–3300*m*, 1680*s*, 1595*m*. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.31 *s*, Me–C(8)); 1.58 (*m*, CH_2(7)); 1.62 *s*, Me–C(4)); 2.06 (*m*, CH_2(6)); 2.34 (br. *s*, CH_2(2), CH_2(3)); 5.07 (*dd*, *J* = 9.8, 2.0, 1 H–C(10)); 5.25 (*dd*, *J* = 16, 2.0, 1 H–C(10)); 5.26 (*m*, H–C(5)); 5.48 (br. *m*, NH_2); 5.94 (*dd*, *J* = 9.8, 16.0, H–C(9)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 16.1, 22.9, 28.1 (3 *q*); 34.5, 35.2, 42.0 (3 *t*); 73.6 (*s*); 112.0 (*t*); 125.7 (*d*); 134.0 (*s*); 145.1 (*d*); 175.5 (*s*). CI-MS (NH_3): 229 (21, *M* + NH_4), 212 (21), 194 (100). FAB-MS: 234 (5, *M* + Na), 212 (31, *M* + H), 194 (100), 177 (27), 149 (38), 126 (32). HR-FAB-MS: 212.16450 (*M* + H^+, $C_{12}H_{21}NO_2$ + H, calc. 212.16506).

*(3RS,10RS,6E)-2,6,10-[10-*methyl-^{3}H*]Trimethyldodeca-6,11-diene-2,3,10-triol* ([^{3}H]-**9**). $[\alpha]_D^{23} = +12.1$ (*c* = 0.19, CH_2Cl_2). IR ($CHCl_3$): 3300*m* (br.), 1492*w*. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.17 (*s*, Me); 1.22 (*s*, Me); 1.27 (*s*, Me); 1.42 (*m*, 1 H–C(4)); 1.58 (*m*, CH_2(9), 1 H–C(4)); 1.60 (*s*, Me–C(6)); 2.08 (*m*, CH_2(8), 1 H–C(5)); 2.24 (*m*, 1 H–C(5)); 3.36 (*dd*, *J* = 10, 3, H–C(3)); 5.08 (*dd*, *J* = 10, 2, 1 H–C(12)); 5.23 (*m*, H–C(7)); 5.23 (*dd*, *J* = 16, 2, 1 H–C(12)); 5.92 (*dd*, *J* = 16, 10, H–C(11)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 16.0 (*q*); 22.8 (*t*); 23.5, 26.6, 28.0 (3 *q*); 29.7, 36.9, 42.1 (3 *t*); 73.1, 73.6 (2 *s*); 78.4 (*d*); 111.8 (*t*); 125.2 (*d*); 135.4 (*s*); 145.2 (*d*). CI-MS (NH_3): 274 (18, *M* + NH_4), 256 (9), 239 (11), 221 (100). HR-CI-MS: 274.23564 (*M* + NH_4, $C_{15}H_{28}O_3$ + NH_4, calc. 274.23822).

(3′RS,6RS,2E)-2-(3′,4′-Epoxy-4′-methylpentyl)-6-[^{3}*H]methylocta-2,7-diene-1,6-diol* ([^{3}H]-**10**). $[\alpha]_D^{23} = +10.1$ (*c* = 0.035, CH_2Cl_2). ^{1}H-NMR (360 MHz, $CDCl_3$ with D_2O): 1.27 (*s*, Me); 1.29 (*s*, Me); 1.33 (2*s*, Me); 1.61 (*m*, CH_2(5)); 1.68 (*m*, CH_2(2′)); 2.13 (*m*, CH_2(4)); 2.30 (*m*, CH_2(1′)); 2.75 (*dd*, *J* = 9.3, 7.0, H–C(3′)); 4.05 (br. *s*, CH_2(1) OD); 5.17 (*dd*, *J* = 10.5, 3.5, 1 H–C(8)); 5.24 (*dd*, *J* = 15, 3.5, 1 H–C(8)); 5.49 (*t*, *J* = 7.0, H–C(3)); 5.92 (*dd*, *J* = 15, 10.5, H–C(7)). ^{13}C-NMR (67.9 MHz, $CDCl_3$); 18.9 (*q*); 22.3, 25.0 (2 *t*); 25.1 (*q*); 27.8 (*t*); 27.9, 28.3 (2 *q*); 29.8, 42.0, 42.1 (3 *t*); 64.2 (*d*); 67.4, 111.9, 112.1 (3 *t*); 128.2, 144.9, 145.1 (3 *d*). CI-MS (NH_3): 253 (9.5), 237 (31.3), 219 (46.3), 74 (73.2), 58 (100). HR-MS: 272.2222 (*M* + NH_4), $C_{15}H_{26}O_3$ + NH_4, calc. 272.22225).

(3RS,6R,7R*)-3,7,11-[3-*methyl-^{3}H*]Trimethyldodeca-1,10-diene-3,6,7-triol* ([^{3}H]-**12**). $[\alpha]_D^{22} = +22.9$ (*c* = 0.035, CH_2Cl_2). IR (CH_2Cl_2): 3600*s*, 3420*s*, 1610*w*, 1090*m*. ^{1}H-NMR (360 MHz, $CDCl_3$ with D_2O): 1.15 (2 *s*, Me–C(7)); 1.32 (*s*, Me–C(3)); 1.41 (*m*, 1 H–C(5)); 1.45 (*m*, 1 H–C(8)); 1.48–1.62 (*m*, 1 H–C(5), 1 H–C(8), 1 H–C(4)); 1.63 (*s*, CH_3(12)); 1.69 (*s*, Me–C(11)); 1.80 (*m*, 1 H–C(4)); 2.08 (*m*, CH_2(9)); 3.43 (*m*, H–C(6)); 5.10 (*m*, 1 H–C(1)); 5.13 (*m*, H–C(10)); 5.24 (*m*, 1 H–C(1)); 5.90 (*m*, H–C(2)). CI-MS (CH_4): 257 (2.1, *M* + H), 239 (15.0), 221 (100), 203 (17.1). HR-MS: 256.2285 (*M* + NH_4 − H_2O, $C_{15}H_{28}O_3$ + NH_4 − H_2O, calc. 256.2276).

*(3RS,10RS,6Z)-2,6,10-[10-*methyl-^{3}H*]Trimethyldodeca-6,11-diene-2,3,10-triol* ([^{3}H]-**11**). $[\alpha]_D^{22} = +28.2$ (*c* = 0.022, CH_2Cl_2). IR ($CHCl_3$): 3410*m*, 1380*s*, 840*s*. ^{1}H-NMR (360 MHz, $CDCl_3$ with D_2O): 1.16 (*s*, Me); 1.20

(*s*, Me); 1.28 (2 *s*, Me); 1.40 (*m*, 1 H–C(4)); 1.60 (*m*, 1 H–C(4), CH_2(9); 1.70 (*s*, Me); 2.04 (*m*, 1 H–C(5)); 2.17 (*m*, 1 H–C(5), 1 H–C(8)); 2.30 (*m*, 1 H–C(8)); 3.31 (*d*, *J* = 11, H–C(3)); 5.07 (*dd*, *J* = 10, 6, 1 H–C(12)); 5.24 (*m*, H–C(7), 1 H–C(12)); 5.92 (2*dd*, *J* = 16, 10, H–C(11)). CI-MS (NH_3): 274 (3.7, *M* + NH_4), 256 (7.3), 239 (8.6), 221 (100), 203 (8.1). HR-MS: 274.2368 (*M* + NH_4, $C_{15}H_{28}O_3$ + NH_4, calc. 274.2382).

6. *(3*RS,6R*,7R*)-6,7-Epoxy-3,7,11-trimethyldodeca-1,10-dien-3-ol* (= *6,7-Epoxy-(*E*)-nerolidol*) *and (3*RS,*10*RS,6E*)-10,11-Epoxy-3,7,11-trimethyldodeca-1,6-dien-3-ol* (= *10,11-Epoxy-(*E*)-nerolidol*). At 0°, 3-$ClC_6H_4CO_3H$ (85%; 654 mg, 3.24 mmol) was added over 3 h to (*E*)-nerolidol (600 mg, 2.70 mmol) in CH_2Cl_2 (20 ml). The soln. was stirred for 1 h at 0° before sat. aq. $Na_2S_2O_3$ soln. (5 ml) was added. After 10 min, the mixture was extracted with CH_2Cl_2, the combined org. phase washed with H_2O and brine, dried ($MgSO_4$), and evaporated, and the residue purified by FC (Et_2O/petroleum ether 1:2): title compounds.

*6,7-Epoxy-(*E*)-nerolidol:* IR ($CHCl_3$): 3620*m*, 3460*m*, 1135*s*. ^{1}H-NMR (360 MHz, $CDCl_3$ with D_2O): 1.28 (*s*, Me–C(3), Me–C(7)); 1.52 (*m*, 1 H–C(8)); 1.55 (*m*, 1 H–C(5)); 1.6 (*m*, 1 H–C(4), 1 H–C(8)); 1.62 (*s*, CH_3(12)); 1.67 (*m*, 1 H–C(5)); 1.69 (*s*, Me–C(11)); 1.73 (*m*, 1 H–C(4)); 2.08 (OH, CH_2(9)); 2.72 (*m*, H–C(6)); 5.07 (*m*, 1 H–C(1)); 5.12 (*m*, H–C(10)); 5.22 (2*dd*, *J* = 13, 3.4, 1 H–C(1)); 5.90 (*m*, H–C(2)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 17.9, 22.4 (2 *q*); 23.6, 24.3 (2 *t*); 26.1, 28.1, 28.5, 28.9 (4 *q*); 33.1 (*t*); 62.1 (*s*); 65.1, 65.3 (2 *d*); 73.1 (*s*); 112.2, 112.3 (2 *t*); 123.8, 123.9 (2 *d*); 132.2 (*s*); 145.0 (*d*). CI-MS (NH_3): 256 (11.6, *M* + NH_4), 239 (43.7, *M* + H), 237 (54.5), 221 (100), 203 (32.6). HR-MS: 239.2022 (*M* + H, $C_{15}H_{26}O_2$ + H, calc. 239.20111).

*10,11-Epoxy-(*E*)-nerolidol:* IR ($CHCl_3$): 3600*m*, 3480*m*, 1120*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 1.22 (*s*, Me); 1.24 (*s*, Me); 1.26 (*s*, Me); 1.49–1.66 (*m*, CH_2(9), CH_2(4); 1.60 (*s*, Me–C(7); 1.90 (br., OH); 2.04 (*m*, CH_2(5), CH_2(8); 2.68 (*t*, *J* = 6.8, H–C(10); 5.04 (*dd*, *J* = 10, 2, 1 H–C(1)); 5.17 (*m*, H–C(6)); 5.19 (*dd*, *J* = 16, 2 H–C(1)); 5.89 (*dd*, *J* = 16, 10 H–C(2)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 16.1, 18.9 (2 *q*); 22.8 (*t*); 25.0 (*q*); 27.5 (*t*); 27.9 (*q*), 36.4 (*t*); 42.1 (2 *t*), 58.5 (*s*), 64.3 (*d*); 73.5 (*s*); 111.8 (*t*); 125.0 (*d*); 134.6 (*s*); 145.1 (*d*). CI-MS (NH_3): 256 (16.1, *M* + NH_4), 239 (9.5, *M* + H), 238 (7.9), 237 (28.7), 221 (55.5), 135 (32.1). HR-MS: 239.20132 (*M* + H, $C_{15}H_{26}O_2$ + H, calc. 239.20111).

7. *(3*RS,*10*RS,6E*)-2,6,10-Trimethyldodeca-6,11-diene-2,3,10-triol* (**9**). To a soln. of 10,11-epoxy-(*E*)-nerolidol (20 mg, 42 µmol) in THF/H_2O 1:1 (1 ml), $HClO_4$ (3%; 100 µl) was added and the mixture stirred for 1.5 h before sat. aq. NaCl soln. was added. The mixture was extracted with Et_2O, the combined org. phase washed with sat. aq. $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated, and the residue purified by FC (Et_2O): **9**. IR (CH_2Cl_2): 3600*s*, 1605*w*, 1492*m*. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.17 (*s*, Me); 1.21 (*s*, Me); 1.27 (*s*, Me–C(10)); 1.42 (*m*, 1 H–C(4)); 1.58 (*m*, CH_2(9), 1 H–C(4)); 1.60 (*s*, Me–C(6)); 1.85 (br., OH); 2.08 (*m*, 1 H–C(5), CH_2(8)); 2.23 (*m*, 1 H–C(5); 2.35 (br., OH); 2.51 (br., OH); 3.36 (*dd*, *J* = 10, 3 H–C(3)); 5.08 (*dd*, *J* = 10, 2, 1 H–C(12)); 5.23 (*m*, H–C(7)); 5.23 (*dd*, *J* = 16, 2, 1 H–C(12)); 5.92 (*dd*, *J* = 10, 16, H–C(11)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 16.1 (*q*); 22.9 (*t*); 23.4, 26.6, 28.0 (3 *q*); 29.7, 36.9, 42.1 (3 *t*); 73.3, 73.8 (2 *s*); 78.3 (*d*); 112.0 (*t*); 125.2 (*d*); 135.4 (*s*); 145.1 (*d*). CI-MS (NH_3): 274 (33.2, *M* + NH_4), 256 (19.6), 239 (41.9), 221 (100), 203 (30.2), 153 (23.0). HR-MS: 274.2362 (*M* + NH_4, $C_{15}H_{28}O_3$ + NH_4, calc. 274.23822).

8. *(3*RS,1″RS,6E*)-3-Methyl-6-[tetrahydro-6′-(1″-hydroxy-1″-methylethyl)-2′*H*-pyran-3′-ylidene]hex-1-en-3-ol* (**14**). A soln. of **10** (1 mg) in MeCN (500 µl) was treated with HCl (300 µl of soln. containing 50 µl of conc. HCl in 4 ml of MeCN). The soln. was stirred at r.t. (TLC monitoring, detection of epoxides [19] by 1% 4-(nitrobenzyl)pyridine in CCl_4, then 10% tetraethylenepentamine in CCl_4 and heat). After 4 h, the solvent was evaporated and the product purified by elution from an *LH-20 Sephadex* column (CH_2Cl_2), followed by TLC (AcOEt): **14**. ^{1}H-NMR (360 MHz, $CDCl_3$ with D_2O): 1.26 (2*s*, Me); 1.34 (*s*, Me); 1.36 (*s*, Me); 1.63 (*m*, CH_2(4)); 1.82 (*m*, CH_2(5′)); 2.13 (*m*, CH_2(5)); 2.32 (*m*, CH_2(4′)); 3.54 (*m*, H–C(6′)); 4.15 (*AB*, CH_2(2′)); 5.09 (*dd*, *J* = 10.5, 3, 1 H–C(1)); 5.27 (*m*, 1 H–C(1), H–C(6)); 5.93 (*dd*, *J* = 15, 10.5, H–C(2)). FAB-MS (thioglycerol): 471 (29, *M* + 2 ($C_3H_8O_2S$) + H), 453 (29), 363 (29), 345 (41), 327 (19), 255 (22), 237 (25), 219 (21), 147 (58). HR-CI-MS: 272.2215 (*M* + NH_4, $C_{15}H_{26}O_3$ + NH_4, calc. 272.22225).

9. *(3*RS,6R*,7S*)-6,7-Epoxy-3,7,11-trimethyldodeca-1,10-dien-3-ol* (= *6,7-Epoxy-(*Z*)-nerolidol;* **15**) *and (3*RS,*10*RS,6Z*)-10,11-Epoxy-3,7,11-trimethyldodeca-1,6-dien-3-ol* (= *10,11-Epoxy-(*Z*)-nerolidol;* **16**). From (*Z*)-nerolidol as above for 6,7-epoxy-(*E*)-nerolidol and 10,11-epoxy-(*E*)-nerolidol. The 1:1 mixture **15**/**16** was separated by FC Et_2O/petroleum ether 1:2.

15: R_f 0.39. IR ($CHCl_3$): 3620*m*, 3460*m*, 1135*s*. ^{1}H-NMR (360 MHz, $CDCl_3$ with D_2O): 1.29 (*s*, 6 H, 2 Me); 1.5 (*m*, 1 H–C(8)); 1.55 (*m*, 1 H–C(5)); 1.6 (*m*, 1 H–C(4), 1 H–C(8)); 1.62 (*s*, (CH_3(12)); 1.67 (*m*, 1 H–C(5)); 1.68 (*s*, Me–C(11)); 1.72 (*m*, 1 H–C(4)); 2.08 (OH, CH_2(9)); 2.72 (*m*, H–C(6)); 5.07 (*m*, 1 H–C(1)); 5.11 (*m*, H–C(10)); 5.22 (*m*, 1 H–C(1)); 5.90 (*m*, H–C(2)). CI-MS (NH_3): 256 (13.0, *M* + NH_4), 239 (46), 237 (54), 221 (100), 203 (32). HR-MS: 239.2032 (*M* + H, $C_{15}H_{26}O_2$ + H, calc. 239.20111).

16: R_f 0.42. IR ($CHCl_3$): 3600*m*, 3460*m*, 1135*s*. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.26 (*s*, 2 Me–C(11)); 1.31 (2*s*, CH_3(3)); 1.54 (*m*, CH_2(9)); 1.59 (*m*, CH_2(4)); 1.68 (*d*, *J* = 1.5, Me–C(7)); 1.82 (br. *s*, OH); 2.05 (*m*, CH_2(8)); 2.16 (*m*, CH_2(5)); 2.70 (2*t*, *J* = 6.5, H–C(10)); 5.02 (2*dd*, *J* = 10, 3, 1 H–C(1)); 5.17 (*m*, H–C(6)); 5.21 (2*dd*, *J* = 15, 3, 1 H–C(1)); 5.98 (*dd*, *J* = 15, 10, H–C(3)). CI-MS (NH_3): 256 (23.4, *M* + NH_4), 239 (34.5), 238 (16.7), 221 (100), 203 (89.3), 127 (47.2). HR-MS: 239.2010 (*M* + H, $C_{15}H_{26}O_2$ + H, calc. 239.20111).

10. *(2*RS,*10*RS,*6Z)-2,6,10-Trimethyldodeca-6,11-diene-2,3,10-triol* (**11**). A soln. of **16** in THF/H_2O 1:1 (1 ml) was treated with 3% $HClO_4$ soln. (70 µl). The soln. was stirred for 1.5 h, then NaCl was added and the mixture extracted with Et_2O. The combined org. layers were washed with sat. aq. $NaHCO_3$ soln. and brine, dried ($MgSO_4$), and evaporated. The product was purified by FC (Et_2O). IR ($CHCl_3$): 3410*s*, 1390*s*. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.15 (*s*, Me); 1.19 (*s*, Me); 1.28 (2*s*, Me–C(10)); 1.39 (*m*, 1 H–C(4)); 1.58 (*m*, 1 H–C(4), CH_2(9)); 1.70 (*s*, Me–C(6)); 2.03–2.30 (*m*, CH_2(5), CH_2(8), OH); 3.30 (*d*, *J* = 11, H–C(3)); 5.05 (2*dd*, *J* = 10, 6, ^{1}H–C(12)); 5.22 (*m*, 1 H–C(12), H–C(7)); 5.91 (2*dd*, *J* = 16, 10, H–C(11)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 22.6 (*t*); 23.4, 23.5, 26.6, 27.7, 28.5 (5 *q*); 28.58, 28.63, 29.5, 29.6, 42.4, 42.5 (6 *t*); 73.2, 74.1 (2 *s*); 77.8, 77.9 (2 *d*); 111.8, 112.0 (2 *t*); 126.1 (*d*); 135.1 (*s*); 144.9, 145.6 (6 *d*). CI-MS (NH_3): 274 (34.5, *M* + NH_4), 256 (100), 203 (34), 127 (36). HR-MS: 274.2389 (*M* + NH_4, $C_{15}H_{28}O_3$ + NH_4, calc. 274.2382).

11. *(3*RS,*6*R*,*7*S*)-3,7,11-Trimethyldodeca-1,10-diene-3,6,7-triol* (**17**). *N*-Methylmorpholine *N*-oxide (0.32 g, 2.39 mmol) was added to (*Z*)-nerolidol (0.5 g, 2.25 mmol) in acetone/H_2O 1:1 (5 ml) at 0°. OsO_4 (88.8 ml, 2.5 wt-% soln.) was added and the mixture allowed to warm to r.t. After stirring for 48 h, a slurry of sodium dithionate (50 mg), magnesium silicate (600 mg), and H_2O (4 ml) was added. The mixture was stirred for 10 min before the magnesium silicate was filtered off. The pH of the filtrate was adjusted to pH 7.0 with 1M H_2SO_4 and the acetone evaporated. The aq. layer was then acidified to pH 2, before solid NaCl was added prior to extraction with AcOEt. The combined org. phase was washed with brine, dried ($MgSO_4$), and evaporated and the residue purified by FC (AcOEt/CH_2Cl_2 65:35): **17**. IR ($CHCl_3$): 3410*s*, 1650*s*, 1390*m*. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.15 (2*s*, Me–C(7)); 1.29 (2*s*, Me–C(3)); 1.4 (*m*, 1 H–C(8), 1 H–C(5)); 1.61 (*s*, Me); 1.67 (*s*, Me); 1.5–1.66 (*m*, 1 H–C(8), 1 H–C(5)); 1.80 (*m*, CH_2(4)); 2.0–2.2 (*m*, CH_2(9)); 3.0 (v. br., 3 OH); 3.35 (*m*, H–C(6)); 5.04 (2*dd*, 1 H–C(1)); 5.12 (*m*, H–C(10)); 5.21 (*m*, 1 H–C(1)); 5.88 (*m*, H–C(2)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 17.8 (*q*); 22.2 (*t*); 23.5 (*q*); 25.6 (*t*); 25.9, 27.7, 29.0 (3 *q*); 29.8, 35.9, 36.0, 39.1, 39.7 (5 *t*); 73.3, 75.0 (2 *s*); 78.8, 79.3 (2 *d*); 111.8, 112.4 (2 *t*), 124.8 (*d*); 131.9 (*s*); 144.8, 145.4 (2 *d*). CI-MS (NH_3): 274 (29.0, *M* + NH_4), 257 (10.2), 256 (8.1), 239 (77), 221 (100), 203 (35), 127 (32). HR-MS: 274.2369 (*M* + NH_4, $C_{15}H_{28}O_3$ + NH_4, calc. 274.2382).

12. *(3*RS,*6*R*,*7*R*)-3,7,11-Trimethyldodeca-1,10-diene-3,6,7-triol* (**12**). From (*E*)-nerolidol as above for **17**. It was purified by FC (CH_2Cl_2/MeOH 96:4) followed by prep. TLC (CH_2Cl_2/MeOH 90:10). IR ($CHCl_3$): 3600*m*, 3420*m*, 1620*w*, 1080*s*. ^{1}H-NMR (270 MHz, $CDCl_3$): 1.15 (2 *s*, Me); 1.31 (*s*, Me); 1.42 (*m*, 1 H–C(5)); 1.45 (*m*, 1 H–C(8)); 1.48–1.62 (*m*, 1 H–C(5), 1H–C(8), 1 H–C(4)); 1.64 (*s*, Me); 1.70 (*s*, Me); 1.82 (*m*, 1 H–C(4)); 2.1 (*m*, CH_2(9)); 2.30–3.0 (br., 3 OH); 3.43 (*t*, *J* = 7, H–C(6)); 5.10 (*m*, 1 H–C(1)); 5.13 (*m*, H–C(10)); 5.24 (*dd*, *J* = 16, 4, 1 H–C(1)); 5.90 (*m*, H–C(2)). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 17.9 (*q*); 21.1, 22.2, 25.8 (3 *t*); 28.3, 29.0 (2 *q*); 39.0, 39.1, 39.6 (3 *t*); 73.3, 73.4, 75.3 (3 *s*); 77.6 (*d*); 77.9; 112.0, 112.3 (2 *t*); 124.5 (*d*), 132.3 (*s*); 145.0, 145.2 (2 *d*). CI-MS (NH_3): 274 (4, *M* + NH_4), 256 (3), 239 (17), 221 (100), 203 (7). HR-MS: 274.2375 (*M* + NH_4, $C_{15}H_{28}O_3$ + NH_4, calc. 274.2382).

13. *(2*R*,2′R*,5′*RS)-2-(5′-Ethenyltetrahydro-5′-methylfuran-2′-yl)-6-methylhept-5-en-2-ol* (**13**). A soln. of **15** (10 mg, 42 mmol) in CH_2Cl_2 and *Amberlyst 15* (4 mg) was stirred for 4 h. The mixture was filtered and evaporated and the product purified by FC (Et_2O/petroleum ether 1:9: **13**). IR (CH_2Cl_2): 3508*s*, 1100*m*. ^{1}H-NMR (270 MHz, $CDCl_3$); 1.11 (2*s*, Me(1)); 1.32 (*s*, Me–C(5′)); 1.52 (*m*, CH_2(3)); 1.60 (*s*, Me); 1.68 (*s*, Me); 1.7–1.9 (*m*, CH_2(3′), CH_2(4′)); 2.06 (*m*, CH_2(4)); 3.86 (*m*, H–C(2′)); 5.00 (*m*, 1 H, CH_2=CH); 5.14 (*m*, H–C(5)); 5.19 (*m*, 1 H, CH_2=CH); 5.91 (*m*, CH_2=CH). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 17.8, 21.5 (2 *q*); 22.5, 22.7 (2 *t*); 25.9 (*q*); 26.3, 26.5 (2 *t*); 27.1 (*q*); 37.6, 38.0, 40.4 (3 *t*); 72.7, 73.0 (2 *s*); 82.7; 83.1 (*s*); 84.4 (*d*); 111.4, 111.7 (2 *t*); 124.9 (*d*); 131.5 (*s*); 144.0; 144.5 (*d*); ^{13}C,^{1}H-NMR after a D_2O shake: shift of signal at 72.713 to 72.623 [20] [21]; all other signals not shifted significantly (< 0.01 ppm). CI-MS (NH_3): 256 (28.7, *M* + NH_4); 239 (17.8), 221 (69.4), 203 (8.6). HR-MS: 256.2277 (*M* + NH_4, $C_{15}H_{26}O_2$ + NH_4, calc. 256.2277).

14. *(2*R*,2′S*,5′*RS)-2-(5′-Ethenyltetrahydro-5′-methylfuran-2′-yl)-6-methylhept-5-en-2-ol* (**18**). From 6,7-epoxy-(*E*)-nerolidol (10 mg, 42 mmol) exactly as above (**13** from **15**). IR (CH_2Cl_2): 3580*s*, 1100*m*. ^{1}H-NMR (270 MHz, $CDCl_3$): 1.11 (2*s*, Me); 1.31 (*s*, Me); 1.50 (*m*, CH_2(3)); 1.60 (*s*, Me); 1.67 (*s*, Me); 1.7–1.9 (*m*, CH_2(3′), CH_2(4′)); 2.04 (*m*, CH_2(4)); 3.86 (*m*, H–C(2′)); 5.01 (*m*, CH_2=CH); 5.14 (*m*, H–C(5)); 5.19 (*m*, 1 H, CH_2=CH); 5.90 *m*, 1 H, CH_2=CH). ^{13}C-NMR (67.9 MHz, $CDCl_3$): 17.8, 21.7 (2 *q*); 22.5, 22.7 (2 *t*); 26.0 (*q*); 26.3, 26.5 (2 *t*); 27.1 (*q*); 37.5, 38.0, 40.4 (3 *t*); 72.8, 73.1 (2 *s*); 82.7; 83.1 (*s*); 84.5 (*d*); 111.4, 111.7 (2 *t*); 124.9, (*d*); 131.5 (*s*); 144.1;

144.5 (*d*); after D_2O shake, upfield shift only of the signal at 72.8. CI-MS (NH_3): 256 (26.7, $M + NH_4$), 239 (19.9), 221 (71.7), 203 (8.9). HR-MS: 256.2273 ($M + NH_4$, $C_{15}H_{26}O_2 + NH_4$, calc. 256.2277).

*Detection and Characterisation of (2*R**,2′R*,5′RS)-2-(5′-Ethenyltetrahydro-5′-[^{3}H]methylfuran-2′-yl)-6-methylhept-5-en-2-ol* ([^{3}H]-**13**). Compound **13** prepared above (197.5 mg) was added to the post *LH-20* fermentation extract (1.00 g) of *S. cinnamonensis* (3*R*S,Z)-[3-*methyl*-^{3}H]nerolidol feeding experiment. The mixture was repeatedly fractionated by FC (CH_2Cl_2/AcOEt 98:2) until constant radioactivity was attained in the reisolated ([^{3}H]-**13**). After each chromatographic step unlabelled (3*R*S,*Z*)-nerolidol (50 mg) was added to the purified [(^{3}H]-**13**). The specific radioactivtiy of [^{3}H]-**13** and (3*R*S,*Z*)-[3-methyl-^{3}H]nerolidol was monitored after each step and was constant for [^{3}H]-**13** after 7 steps, corresponding to a total of 0.13% of the radioactivity added to the fermentation. The fermentation extract (0.5 g) was chromatographed by TLC (AcOEt). A band at R_f 0.78 was removed and extracted, the solvent removed, and the residue analysed by GC/MS (column *OV1*); the retention times of unlabelled **13** and [^{3}H]-**13** were identical. GC/CI-MS (NH_3): 239 (4.1, $M + H$), 221 (44), 203 (3), 127 (100).

A similar protocol failed to reveal **18** in the fermentation extract from the (3*R*S,*E*)-[3-*methyl*-^{3}H]nerolidol feeding experiment.

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