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Quorum Sensing in *Candida albicans*: Probing Farnesol's Mode of Action with 40 Natural and Synthetic Farnesol Analogs

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Abstract

The dimorphic fungus *Candida albicans* produces extracellular farnesol (3,7, 11-trimethyl-2,6,10-dodecatriene- 1-ol) which acts as a quorum-sensing molecule (QSM) to suppress filamentation. Of four possible geometric isomers of farnesol, only the *E,E* isomer possesses QSM activity. We tested 40 natural and synthetic analogs of farnesol for their activity in an *N*-acetylglucosamine-induced differentiation assay for germ tube formation (GTF). Modified structural features include the head group, chain length, presence or absence of the three double bonds, substitution of a backbone carbon by S, O, N, and Se heteroatoms, presence or absence of a 3-methyl branch, and the bulkiness of the hydrophobic tail. Of the 40 compounds, 22 showed QSM activity by their ability to reduce GTF by 50%. However, even the most active of the analogs tested had only 7.3% of the activity of *E,E-farnesol*. Structure-activity relationships were examined in terms of the likely presence in C. *albicans* of a farnesol binding receptor protein.

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Introduction

The dimorphic fungus *Candida albicans* is the first eukaryotic system shown to possess quorum-sensing behavior [1]. Quorum sensing has been thoroughly described in prokaryotes but had not been demonstrated in eukaryotes until recently. With this phenomenon, a secreted compound accumulates to a critical concentration in the medium and then acts back on the producing cells to elicit a physiological response. For Gram-negative bacteria, the quorum-sensing molecules are all members of a family of compounds known as acyl homoserine lactones [2]. In the case of *C. albicans,* the quorum-sensing molecule (QSM) is the sesquiterpene farnesol (3,7,11-trimethyl-2,6,10-dodecatriene-1-ol). Accumulation of farnesol blocks the morphological shift from yeasts to mycelia [1]. Farnesol blocks germ tube formation as triggered by serum, proline, or *N*-acetylglucosamine, and it is produced by and active on all (five) strains of *C. albicans* tested [1].

The effect of farnesol concerned morphological choice, not growth rate. At concentrations of up to 250 μM, farnesol did not alter the growth rate for C. *albicans;* actively budding yeasts were observed in all cases [1]. This finding was confirmed by Ramage et al. [3] who observed unimpaired growth rates up to 300 μM farnesol. The yeast-mycelia transition is critical for pathogenicity, and *C. albicans* mutants limited to the yeast morphology are avirulent [4]. A follow-up question based on this discovery is to identify farnesol's mode of action in blocking germ-tube formation in *C. albicans.* One way of approaching this question is to design, prepare, and assay analogs of farnesol with the expectation that comparison of the activity profiles would identify essential and extraneous structural features. There is also the possibility of synthesizing a compound with greater activity than that of farnesol. Analogs with enhanced activity could prove useful in a clinical setting for prevention or prophylaxis of Candidiasis. This study looks at a series of first generation analogs of farnesol and begins to uncover the biology of the quorum-sensing response in *Candida albicans* as mediated by farnesol. To date, we have analyzed 40 analogs of farnesol. Of these, 22 show activity as determined by their ability to reduce germ tube formation by at least 50% at concentrations up to 100 μM.

As well as gaining a better understanding of the biology of fungal quorum sensing, there are at least four practical advantages to

be gained from farnesol analogs. (1) Preliminary experiments with a mouse model show no obvious toxicity from farnesol. However, if farnesol toxicity were to become a problem at higher dosages or with prolonged treatment, it would be desirable to develop less toxic analogs that still maintain QSM activity. (2) Farnesol has limited water solubility. Modifications of the structure of farnesol might lead to active compounds that were more soluble and thus more easily deliverable in animal systems. (3) Analogs of farnesol might also possess improved pharmacokinetics, such as an enhanced ability to enter the blood stream via the gastrointestinal tract or peritoneum. Alternatively, since farnesol is usually excreted from animals after its conversion to farnesoic acid and dicarboxylic acids [5], specific structural changes may improve retention. Both scenarios could help maintain the analog in an available form in the host. (4) While our in vitro studies suggest a possible prophylactic use for farnesol, it is still unclear whether this will be seen in an animal model. Farnesol could prove to act as a virulence factor for C. *albicans*. This question was posed by Hornby et al. [1] based upon anticipated differences between in vitro studies performed in a glass vessel and in vivo studies, where cellular membranes might act as a sink for the lipophilic farnesol. If farnesol functions as a virulence factor for *C. albicans,* it would be important to develop farnesol analogs that act as antagonists of virulence.

Results

Our previous work [1] reported that both commercial mixed isomers farnesol and 96% *E,E*-farnesol exhibited QSM activity. However, subsequent comparisons with freshly opened bottles of farnesol showed significantly greater activity with the *E,E* isomer (**Table 1**) and differing activities with mixed isomers farnesol from different suppliers (Table 1). Ninety-six percent *E,E*-farnesol reduced GTF to fifty percent at 1.2 μM, with complete inhibition at ca. 7.5 μM (Table 1) while mixed isomers farnesol from Sigma (St Louis, MO) and Acros Organics (Pittsburgh, PAI reduced germ tube formation (GTF) to 50% at ca. 3.5 and 4.4 μM, respectively (Table 1). The three farnesol samples were therefore analyzed by *GCI* MS. The lower activity Acros mixed isomers farnesol contained only 33%-36% E,E-farnesol, whereas the higher activity Sigma mixed isomers farnesol contained 56% E,E-farnesol (Table

Table 1. QSM Activity of Three Commercial Samples of Farnesol

Farnesol Source	Percent *E,E*-Farnesol[a]	Concentration (μM) for 0% GTF[b]	Concentration (μM) for 50% GTF[b]	Calculated Concentration (μM) for 50% GTF Based on Percent *E,E*-Farnesol[c]
Sigma *E,E*-farnesol	96	7.5	1.2	1.2
Sigma mixed isomers	56	25	3.5	2.1
Across mixed isomers	36	30	4.4	3.2

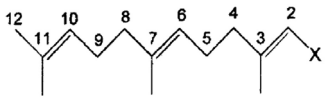
a. Determined by GC/MS as previously described [1].
b. Based on the regression analysis as described in Experimental Procedures.
c. Calculated values of concentration that would be needed if all observed activity were due to *E,E*-farnesol only, based on the known *E,E*-farnesol content of each sample.

1). There was sufficient *E,E*-farnesol in the two mixed isomers samples to account for their QSM activities (Table 1). Based upon the percentage of *E,E*-farnesol in the Sigma and Acros Organics samples, reduction to 50% GTF would have been expected to occur at concentrations of 2.1 and 3.2 μM, respectively (Table 1). These concentrations are lower than those determined experimentally for the mixed isomers samples. Thus, it appears that only *E,E*-farnesol possesses QSM activity, and furthermore, a comparison of observed QSM activity (Table 1) with that predicted by the *E,E*-farnesol content suggests the other isomers may even act to inhibit QSM activity.

Head Group Modifications

We tested six farnesol analogs with altered head groups (**Table 2**). These modifications were introduced to learn whether farnesol's C-1 hydroxyl was essential for QSM activity. An analog was considered to be active if it was capable of reducing GTF by 50% at concentrations of ≤100 μM. In practice, this definition meant that analogs were considered active if they had ≥1 % of the activity of *E,E*-farnesol. Farnesoic acid (analog 2, 3.3% relative activity) and the corresponding amide (3, 1.9%) maintained biological activity, whereas the aldehyde (4, 0.4%), bromide (5, 0.7%), methyl ester (6, 0.1 %), and amine (7, 0.1%) were inactive (Table 2). The sulfhydryl analog was too insoluble to be bioassayed. In particular, farnesoic acid, which has also been identified as a QSM produced by *C. albicans* [6], displayed only 3.3% of the

Table 2. Biological Activity of Farnesol Analogs Based on Modified Head Groups



Analog Number	X =	IC_{50} (μM)[a]	Relative Activity
1	CH_2OH (Farnesol)	1.2	100.0
2	COOH (Famesoic Acid)	36.2	3.26
3	$CONH_2$	62.3	1.89
4	CHO (Farnesal)		0.38
5	CH_2Br (Commercial *E*,*E*)		0.75
6	$COOCH_3$		0.10
7	CH_2NH_2		0.14

a. Inhibitory concentration (μM) at which germ tube formation is reduced to 50%.

activity exhibited by farnesol (Table 2). With regard to farnesol's mode of action, it is significant that the hydroxyl head group is not essential for QSM activity (Table 2). This conclusion is supported by the observation of Matsuoka and Oh that β-farnesene, with no functional groups whatsoever, retained QSM activity [7].

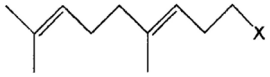
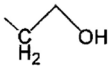
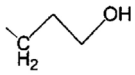
Double Bond Modifications

We next examined five analogs based upon alterations of the 2,3 double bond in farnesol (**Table 3**). The 2,3 cyclopropane ring analog (8, 3.3%) maintained biological activity, whereas the 2,3 epoxide (9, 0.4%) and 2,3 hydrogenated (10, 0.4%) analogs were inactive (Table 3). The terminal 10,11 double bond was also essential for activity in that the 10,11 hydrogenated (11, 0.3%) and fully hydrogenated (12, 0.3%) analogs were inactive (Table 3). Not unexpectedly, all-trans retinol (vitamin A), a farnesol analog with five conjugated double bonds and a bulky, cyclic tail group was also inactive (data not shown).

Sulfur-Containing Analogs

We next tested a series of 14 analogs that incorporated sulfur atoms in place of a main chain CH_2 unit (**Tables 4 & 5**). Seven of the eight analogs with a sulfur in the 4 position retained QSM activity (Table 4) including both the *E*- and Z- isomers of 4-thia farnesol (13, 3.7% and 14, 3.4%, respectively). Surprisingly, the 2,3 hydrogenated 4-thia analog

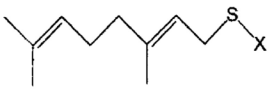
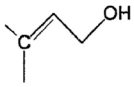
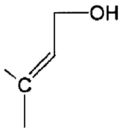
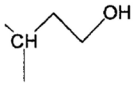
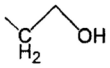
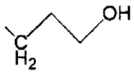
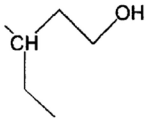
Table 3. Biological Activity of Farnesol Analogs Based on Modifications to Their 2,3 Double Bond

Analog Number	Double Bond Modification	IC_{50} (μM)	Relative Activity
1	None	1.2	100.0
8	2,3-cyclopropane	35.9	3.26
9	2,3-epoxide		0.38
10	2,3-hydrogenated		0.45
11	10,11-hydrogenated		0.31
12	Hydrogenate all three double bonds		0.31
	 X =		
27		68.7	1.72
28		23.2	5.08
29		24.4	4.80

(15, 7.3%) retained activity (Table 4), to the extent that it was ca. 16-fold more active than the 2,3 hydrogenated analog (10) offarnesol itself (Table 3). The importance of the proximal 3-methyl side chain in the 4-thia series was examined (Table 4) via comparison of the methylated compound 15 with the demethylated series represented by compounds 16 (3.7%), 17 (1.5%), and 18 (1.7%). All three demethyl analogs retained activity, as did the 3,4 disulfide (19, 1.6%). We can then make the comparison among three compounds (17, 15, and 20 [0.5%]) with increasingly bulky 3-side chains (Table 4); activity increases 5-fold with insertion of the 3-methyl (15 versus 17) and then decreases 16-fold with the bulkier 3-ethyl (20 versus 15).

Similarly, five of the six analogs with the sulfur atom in the 9 position also retained QSM activity (Table 5). The 9-thia series with n-butyl (21, 4.9%), isobutyl (22, 2.6%), and t-butyl (23, 0.9%) showed decreasing activity with increased branching (Table 5). The relative inactivity of the t-butyl9-thia analog (23) should not be due merely to greater hydrophobicity because the n-pentyl (24, 1.8%), benzyl (25, 1.7%), and

Table 4. Biological Activity of Farnesol Analogs with Sulfur Atoms at the 4 Position

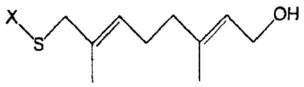
Analog Number	X = 		IC_{50} (μM)	Relative Activity
13		(*trans*)	32.1	3.67
14		(*cis*)	35.1	3.36
15			16.0	7.34
16			31.6	3.70
17			79.1	1.47
18			67.0	1.75
19		(disulfide)	72.4	1.61
20				0.48

phenyl (26, 1.5%) 9-thia analogs still had QSM activity (Table 5). However, it could reflect the bulkier cross-section of the t-butyl being unable to fit into the narrow cleft of a farnesol receptor.

Chain Length Modifications

Farnesol is a 15 carbon sesquiterpene; it has a 12 carbon chain with three methyl branches. We next examined whether altering the chain length alters QSM activity. Three demethylated, 2,3-hydrogenated analogs (27, 1.7%; 28, 5.1%; and 29, 4.8%) have chain lengths of 10-12 carbons (Table 3) and lack the 2,3 double bond and 3-methyl branch

Table 5. Biological Activity of Farnesol Analogs with Sulfur Atoms at the 9 Position



Analog Number	X =	IC_{50} (μM)	Relative Activity
21	n-butyl	23.8	4.94
22	Isobutyl	44.3	2.64
23	*tert*-butyl		0.86
24	n-pentyl	66.8	1.75
25	Benzyl	68.9	1.72
26	Phenyl	79.2	1.47

found in farnesol. Compounds 27-29 all exhibited activity, suggesting some flexibility with regard to chain lengths. This flexibility with regard to chain length was also shown by the 4-thia (Table 4) and 9-thia (Table 5) analogs. Counting S and $–CH_2–$ as equivalent, compounds 16-18 in the 4-thia series (with chain lengths of 11-13, respectively) all had QSM activity (Table 4) as did compounds 21 and 24 in the 9-thia series (with chain lengths of 13-14, Table 5).

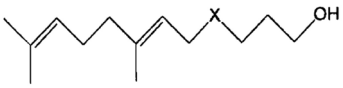
Heteroatoms in the Carbon Chain

Based on our success with the sulfur-containing analogs (Tables 4 and 5), we tried a series of other heteroatoms at the 4 and 9 positions (**Table 6**). The 4-Se analog (30, 2.6%) was the only one which retained QSM activity; all the O-containing analogs and the one N-containing analog (31,0.03%) were inactive (Table 6). In each case, the oxygen-containing analog was less active than the corresponding sulfur-containing analog. Compound 32 (0.1%) was 21-fold less active than 17, compound 33 (0.5%) was 14-fold less active than 15, compound 34 (0.4%) was 4.6-fold less active than 18, and compound 35 (1.0%) was 2.8-fold less active than 22.

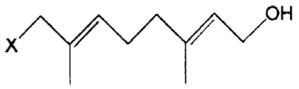
Hydroxyl and Epoxide Analogs of Farnesol

As seen in Table 3, the epoxide at the 2,3 position (9) was ca. 260-fold less active than *E,E*-farnesol. However, the epoxide at the 10,11 position (36, 1.7%) was still active, as were the 10-hydroxyl (37, 1.7%) and

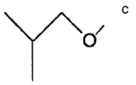
Table 6. Biological Activity of Farnesol Analogs Containing Heteroatoms at the 4 or 9 Positions



Analog Number	X =	IC_{50} (μM)	Relative Activity
29	CH_2	24.4	4.80
17	S	79.1	1.47
30	Se	45.7	2.57[a]
32	O		0.07
33	O (with methyl branch added back at 3-position)		0.51
18	SCH_2–[b]		
34	OCH_2–[b]		
31	NCH_2–[b]		



X =

35			0.96

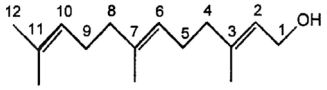
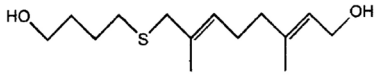
a. Compound 30 was 33% *E* and 67% *Z* at the 6,7-double bond. If only the *E* isomer was active, it would have a relative activity of 7.72.

b. 18, 31, and 34 have four carbons instead of three, between the head group hydroxyl and the heteroatom.

c. Compounds 35 and 22 are identical except that 35 has 9-O while 22 has 9-S.

6-hydroxyl (38, 1.5%) analogs (**Table 7**). The 10,11 epoxide of farnesol is, of course, the reduced form of insect juvenile hormone III (39) which has a methyl ester at the C-1 position instead of the hydroxyl in farnesol. Thus, the QSM inactivity of juvenile hormone III (39) is expected from the inactivity of the corresponding methyl ester of farnesol (6, Table 2). Finally, in terms of designing a potential affinity column for farnesol binding proteins, the omega-hydroxy 9-thia compound (40, 0.1 %) was 49-fold less active than its nonhydroxylated parent compound (21, Table 5). Similarly, all other farnesol analogs with a terminal/ω-hydroxyl group were inactive (data not shown). Future studies will be directed toward the design of an affinity column for farnesol binding proteins.

Table 7. Biological Activity of Hydroxy and Epoxide Analogs of Farnesol

Analog Number		IC_{50} (μM)	Relative Activity
1		1.2	100
9	2,3-epoxide		0.38
36	10,11-epoxide[a]	68.8	1.72
37	10-hydroxyl[b]	68.5	1.72
38	6-hydroxyl[b]	79.2	1.47
39	Juvenile Hormone III 0.16		
40			0.10

a. Compound 36 is a modified (reduced) form of juvenile hormone III in which the carboxyl group has been reduced to a primary alcohol.

b. Hydroxyls were introduced as racemic mixtures at the 10 (37) and 6 position (38) by hydroboration of the 10–11 and 6–7 double bonds, respectively.

Discussion

We have examined 40 natural and synthetic analogs of farnesol with regard to their ability to block germ tube formation in *Candida albicans*. Twenty-two of the forty analogs exhibited QSM activity. Thus, farnesol itself is not a requisite for QSM activity; there is flexibility with regard to the head group (Table 2), double bonds (Table 3), chain length (Tables 3 and 4), and the presence of sulfur and selenium heteroatoms in the farnesyl backbone (Tables 4-6). All of our assays involve adding analogs to cells programmed for GlcNAc-induced germ tube formation. Thus, if we assume that there is an intracellular target/receptor for farnesol, the exogenous farnesol must cross the cytoplasmic membrane and then bind to the target. Therefore, the administered level of farnesol or farnesol analog may not reflect the actual intracellular concentration available to the receptor or target. As a corollary, a farnesol analog could be inactive either because it has difficulty crossing the membrane or difficulty binding to the target. For instance, it is reasonable to suppose that the dihydroxy analog (40) and the analogs containing a basic nitrogen (7 and 31) might have difficulty crossing the cytoplasmic membrane. Our focus on a

putative farnesol binding protein is made with full realization that existing data do not yet prove the existence of farnesol binding proteins in *C. albicans*.

At present, virtually nothing is known about farnesol's mode of action in regulating fungal cell morphology. One of the purposes in examining this collection of farnesol analogs was to put constraints on the possible modes of action for farnesol. As a starting point, farnesol could bind to a specific receptor. This receptor could be either a fungal analog of the nuclear FXR receptor found in most higher eukaryotes [8, 9] or a different farnesolspecific receptor. However, a genomic search of *S. cerevisiae* and *C. albicans* failed to identify sequences similar to mammalian FXR receptors. Thus, if activity is mediated through a farnesol receptor, it is likely to be of a type not previously observed.

What can we infer about the farnesol binding pocket of the putative receptor? How are conformational space and topographical space related to molecular recognition? One approach to our structure-activity results is the linear approach based upon the three regions of farnesol which are altered: (i) The C, terminal hydroxyl group, (ii) the C_1-C_4 "head group" region, and (iii) the C_9-C_{12} "tail" region. We have not yet analyzed enough analogs of the C_5-C_8 "midchain" region to draw any useful conclusions. In this regard, with the exception of 30, the central double bond of all the analogs (equivalent to the 6,7 double bond of farnesol) retained the *E* configuration of the starting material (usually geranyl bromide or chloride). It is tempting to suggest that the 6,7 double bond is critical for activity because compounds 13 (*E*) and 14 (*Z*), differing at the 2,3 double bond, had equivalent activities (Table 4).

The terminal hydroxyl group proved almost invariant. Although farnesoic acid and the amide (2 and 3) displayed activity, functional groups of similar size (aldehyde, methyl ester) or hydrogen-bonding ability (amine) possessed no QSM activity. In this regard, the reduced activity observed [1] for nerolidol (3,7, 11-trimethyl-1,6,10-dodecatriene-3-ol) could reflect either the altered regiochemistry or increased steric bulk of the head group relative to the C_1 primary hydroxyl of farnesol. On the other hand, nerolidol differs from farnesol only in a 1,3-allylic transposition of the hydroxyl and alkene groups, and it is possible that allylic isomerization to farnesol may be the source of the "activity" of nerolidol.

The rest of the head group region, however, proved fertile ground for analog development. A primary alcohol was retained as a fixed element in all designs. It is interesting to analyze the alkanol analogs based on the assumption that the role of the farnesol C_1-C_4 region is to hold the alcohol in a particular register relative to the remainder of the molecule. The three-carbon span in 27 may be too short to allow the alcohol to occupy the same space as in farnesol, whereas the four-carbon span in 28 can achieve a farnesol-like disposition through a low-energy extended conformation and the longer five-carbon span in 29 can adopt a similar overall shape through introduction of a single gauche kink. As a corollary, the inactivity of 10 suggests that the presence of a C-3 methyl on the same five-carbon span prevents the assumption of a farnesol-like conformation. The subtlety of these interactions is further illustrated by comparing removal of the 3-methyl group for the all-carbon molecules (10 versus 29, 11-fold increase in activity), the sulfur-containing analogs (15 versus 17, 5-fold decrease in activity), and oxygen-containing analogs (33 versus 32, 7-fold decrease in activity).

The need for a tail region was clear from the complete lack of activity of the shorter (C_{10}) geraniol [1]. Similarly, the lack of activity [1] of the diterpene (C_{20}) geranylgeraniol could represent "too much" tail in terms of poor fit into a receptor or excess hydrophobicity. Replacement of the terminal 4-methyl-3-pentenyl unit of farnesol with an isobutyl ether (35) resulted in loss of activity, whereas the 10,11-epoxide (36) and the 10-hydroxyl (37) analogs retained activity. Similarly, the n-butyl and isobutyl thioethers (21 and 22) retained activity while the inactivity of a *tert*-butyl thioether (23) suggested intolerance for steric bulk in the C_9-C_{10} region.

A second approach to our structure-activity results is based on the implications of substituting heteroatoms into the farnesol backbone. Most of the analogs containing a thioether retained QSM activity (Tables 4 and 5). The exact chain length did not appear to be a critical variable. Compare the activities of 27-29 (Table 3) and 16-18 (Table 4). In the latter series, 16, 17, and 18 had two-, three-, and four-carbon spacers between the sulfur and the hydroxyl, respectively. We note three points of interest in activity comparisons among four pairs of molecules, 28 and 16, 29 and 17, 10 and 15, and 1 and 13/14, which are identical except for the replacement of CH_2 by sulfur. First, for four compounds (13/14, 16, and 17) introduction of the sulfur

atom decreases QSM activity relative to the carbon-containing analog, whereas 15 displays 16-fold greater activity than the nonsulfur analog. Second, the mixed disulfide (19) also retains activity (Table 4). Thus, we have a sequence of three active compounds (29, 17, and 19) with nearly equivalent chain lengths but containing zero, one, and two sulfurs, respectively. The final point of note is that compound 15 was one of only nine analogs tested (8-10, 20, and 36-39 were the others) that possessed a chiral center. Compound 15 was prepared and assayed as a racemic mixture; it is likely that one of the two enantiomers would possess even greater activity.

Thus, both the all-carbon alkanols (27-29) and the thiaalkanols (16-18) retain significant activity. In theory, the corresponding *O*-ethers should combine the best features of the alkanols (similar C-O and C-C bond lengths) and the thiaalkanols (similar conformational preferences). However, none of the *O*-ethers had activity (Table 6). This outcome may be due to the greater polarity of the ethers as well as to an intramolecular H bond between the *O*-ether and the C-1 alcohol. This explanation is supported by a comparison between the inactive *O*-ether (32) and the highly active *Se*-ether (30). The results could also reflect the relative C-X bond lengths: C-Se > C-S > C-C > C-O.

A third approach to our structure-activity results is based on the role of alkenes in providing conformational constraints on the farnesol backbone. The three trisubstituted alkene subunits of farnesol each impart significant conformational constraints on neighboring linkages. In particular, allylic strain from the methyl groups at $C_{3,}$ $C_{7,}$ and C_{11} precludes conformations which place the C_1-O, C_4-$C_{5,}$ or C_8-C_9 bonds into juxtaposition with the methyl branches. Similarly, 1,2-strain disfavors conformations which juxtapose the C_4-C_5 or C_8-C_9 bonds with the C_3 or C_7 methyl branches, respectively. The importance of these alkene-induced conformational constraints may be reflected in the lack of activity of the 2,3-dihydrofarnesol (10) and the 2,3,6,7,10,11-hexahydro (saturated) farnesol (12). At the same time, the results from the head and tail analogs demonstrate that neither the C_2-C_3 nor C_{10}-C_{11} double bonds are required for activity; structurally related thioethers retain activity (Tables 4 and 5). Given the juxtaposition of the activity retained by the thioethers versus that lost by removal of the double bonds in compounds 10 and 12, one can imagine three possible roles for the alkene units of farnesol: (i) fitting into a narrow cleft, (ii) precluding particular conformations and

thereby organizing the main chain into the proper conformation for binding, or (iii) providing electron density or van der Waals surface for a particular interaction with the receptor.

Each of the ideas has attractive features, and of course, they are not mutually exclusive. The first idea of a narrow cleft cannot be endorsed or completely ruled out from our results. The epoxy (9) and cyclopropane (8) analogs preserve much of the conformational constraints of the 2,3-alkene but significantly enlarge the cross-sectional area of the alkene. The 2,3 cyclopropane analog maintains activity, but the more polar 2,3 epoxide has lost activity (Table 3). The second idea in which each of three trisubstituted alkenes exerts significant conformational constraints on the surrounding region suggests an obvious role in providing bias toward particular conformers. This hypothesis is supported by our discovery that the QSM activity of commercial farnesols correlates closely with the fraction of the natural *E,E*-isomer (Table 1). The third idea in which the alkene units provide needed electron density fits with the strong activity of the thioethers. Replacement of either the head (Table 4) or tail (Table 5) alkene with a straight chain thioether maintains QSM activity. However, the activity of analogs containing linear methylene head groups (27–29) suggests that electron density in the form of an alkene or a thioether is not essential. Thus, in summary, the factors which seem necessary for farnesol's QSM activity and binding to a presumptive farnesol binding protein include: (i) a C-1 hydroxyl; (ii) a C-3 methyl group (but no larger); (iii) a hydrophobic tail; and (iv) the appropriate conformational constraints on backbone conformers as provided by the three, trisubstituted alkenes of farnesol. Factors which do not seem as critical include: (i) absolute chain length; (ii) the chemical structure of the C_9-C_{12} hydrophobic tail; and (iii) the origin of the needed conformational constraints in the backbone. S and Se heteroatoms are permitted.

Significance

Farnesol is a quorum-sensing molecule in *Candida albicans* that acts to block the transition from yeasts to mycelia. It is widely agreed that this morphological transition is a critical feature of this organism's pathogenicity. A further understanding of the action of farnesol on a

molecular level could lead to better control of this common human pathogen. To address this topic, we created a series of farnesol analogs and examined their ability to inhibit mycelial development. Taken together, our results provide four major advancements to the understanding of the molecular action of farnesol. (1) Of the four possible geometric isomers of farnesol, only the *E,E* isomer possesses QSM activity. (2) Structural analogs of farnesol that retain biological activity can be synthesized. In other words, farnesol itself is not essential for the activity seen in *C. albicans*. (3) Subtle changes in the structure of farnesol lead to significant changes in the activity. (4) These analogs indicate which parts of the molecule can be altered and still retain activity and which are essential for activity. This information may help in the development of second generation farnesol analogs with useful pharmacokinetic properties. For instance, studies using cyclization to achieve conformational constraint seem indicated. However, even the most active of the analogs tested had only 7.3% of the activity of *E,E*-farnesol, and thus they are unlikely to have any therapeutic potential as farnesol agonists in hosts infected by *C. albicans*. However, if farnesol does prove to be a virulence factor for C. *albicans,* these analogs may prove to be effective antagonists for farnesol.

Experimental Procedures

Strain and Chemicals

Candida albicans A72 was obtained from Patrick Sullivan, University of Otago, Dunedin, New Zealand. A stock culture was grown in modified glucose-salts-biotin medium, washed in potassium phosphate buffer, and stored in the same buffer as previously described [1]. Commercial mixed isomers farnesol (Acros Organics and Sigma), *E,E*-farnesol (Sigma), and farnesal (Pfaltz and Bauer, Waterbury, CT) were stored at –20°C with desiccant. Juvenile Hormone III, *E,E*-farnesyl bromide, and all-trans retinol (Sigma) were stored at –20°C. Our fresh farnesol samples were roughly ten times more active than those we had reported previously [1], probably because this time we were scrupulous to exclude oxygen by resealing the pure farnesol oils under nitrogen and storing them with desiccation.

General Synthetic and Analytical Procedures

General procedures for synthesis and characterization of substrates follow. Experimental procedures and characterization data for individual compounds are found in the accompanying appendix. All reagents and solvents were used as supplied commercially, except tetrahydrofuran (THF; distilled from sodium/benzophenone), CH_2Cl_2 (distilled from CaH_2), hexamethylphosphoric triamide (HMPA; distilled from CaH_2 and stored over 4 Å mol sieves), and *N,N*-dimethylformamide (DMF; stored over 4 Å sieves). Unless otherwise noted, reactions were run under a blanket of N_2 in a round-bottom flask equipped with a magnetic stirrer. Except where noted, NMR spectra were taken as $CDCl_3$ solutions at 300 MHz (^1H) or 75 MHz (^{13}C). Infrared spectra were acquired on neat films on a ZrSe crystal. Purification of most compounds was based upon air-driven (2-5 psi) flash chromatography on 230-400 mesh silica; the solvent system employed is listed for each compound. Thin-layer chromatography (TLC) employed silica (0.25 mm layer thickness) on glass plates; detection was accomplished with a hand-held UV lamp, iodine vapor, 1% aqueous $KMnO_4$ (alkenes), or charring with a solution of ceric sulfate and ammonium molybdate in 10% H_2SO_4 (most compounds). Analytical and semipreparative HPLC employed 0.5 × 25 cm and 2.1 × 25 cm silica columns, with detection by refractive index. Mass spectra were obtained at the Nebraska Center for Mass Spectrometry (Lincoln, NE). All prepared compounds were homogeneous by TLC, ^1H NMR, and ^{13}C NMR except for compound 30, which was 33% *trans* and 67% *cis* at the 6,7 double bond.

Bioassays of Farnesol and Related Compounds

The bioassays were performed in 25 ml Erlenmeyer flasks in a differentiation medium consisting of 11 mM imidazole, pH 6.5, 3 mM $MgSO_4$, 2.6 mM *N*-acetyl-*D*-glucosamine (GlcNAc) as the germ tube inducer, and either methanol (for a control) or a methanolic solution of the compound being tested. All solutions of farnesol in methanol were used immediately after preparation. Despite the fact that dilute aqueous solutions of farnesol can be stable for years [1], pure farnesol oils are highly susceptible to air oxidation resulting in modification of the 10,11-alkene unit. Our assays of the 10,11-epoxide and the 10-hydroxyl compounds (Table 7) confirm that oxidative modification

of the 10,11-alkene is accompanied by a dramatic decrease in QSM activity. Prior to analysis, all compounds were dried, stored at 4°C for no more than 24 hr, and resuspended as a 25 mM stock solution in 100% methanol immediately before use. The final concentration of methanol in the bioassays was ≤1 %. All compounds were tested at 10, 50, and 100 μM for their ability to block germ tube formation. These concentrations are well below the 250-300 μM levels at which farnesol itself does not impact the growth rate of *C. albicans* [1, 3]. Flasks were preincubated at 37°C for 20 min and then inoculated to 5×10^6 cells/ml of *C. albicans* A72. Flasks were incubated at 37°C for 4 hr with shaking at 250 rpm on a New Brunswick Scientific Co. G2 shaker. After 4 hr, cells were examined by phase-contrast microscopy. At least 100 cells were counted for every flask and analyzed for percent germ tube formation. Compounds which exhibit farnesol-like activity cause a shift from germ tubes to actively budding yeasts. Previous studies indicated that any lethal compounds or lethal concentrations instead gave phase dark, undifferentiated cells. None of analogs tested exhibited toxicity at concentrations up to 100 μM.

Activity Calculations

Two measures of analog activity were employed. In the first, we compared the concentrations of farnesol and the analog necessary to lower the percent germ tubes formed to 50%. An analog is considered to be active if it was capable of reducing germ tube formation by 50% at the highest concentration tested. For inactive analogs, we used an exponential decay function to model the activities of the farnesol standard and the farnesol analogs according to the function $G = G_0 \times e^{(b \times c)}$, where G represents the percentage of germ tubes, C, the concentration of sample in the assay, G_0, the percentage of germ tubes when C = 0, and b, the steepness of the decay. At zero concentration, 95%-99% mycelia were observed for all samples, and therefore, G_0 was fixed at 95. This allowed a single parameter, b, to be determined by the least-squares method using the Maple V program (Waterloo Maple, Waterloo, CAl. A commercial sample of *E,E*-farnesol (Sigma) was chosen as the standard. The percentage activity for each analog was then determined by the ratio of the calculated b values from the regression algorithm. Results are presented in the tables as "relative activity."

Supplemental Data — Experimental procedures and characterization (TLC, ^{1}H and ^{13}C NMR, and IR) for compounds 7, 11, 13-19, 21-23, 25, 27-35, and 37-38 can be found at http://www.chembiol.com/cgi/content/full/10/8/743/DC1 ; references to preparations of known compounds (3, 6, 8-10, 12, 26, and 36) are also provided.

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APPENDIX/SUPPORTING INFORMATION

Known Farnesol analogs: Eight of the farnesol analogs used in this work have been synthesized and characterized previously. They are: compound 3, the farnesoyl amide, see appendix reference #1 below; compound 6, the methyl ester of farnesoic acid, ref #2; compound 8, 2,3-cyclopropane, ref #3; compound 9, 2,3-epoxide, ref #4; compound 10, 2,3-dihydro, ref #5; compound 12, perhydro, ref #6; compound 26, 8-phenylthiogeraniol, ref #7; and compound 36, 10,11-epoxide, ref #8. In each case, the identities and purities of the previously reported compounds were determined by 1H and 13C NMR. The other farnesol analogs described below had not been previously reported. Compounds 1,2,4,5, and 39 were purchased commercially.

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Previously unreported farnesol analogs

Farnesyl amine [(2*E*,6*E*)-3,7,11-trimethyldodecadienylamine] (compound 7): To the solution of *N*-farnesyl trifluoroacetamide (0.7mmol) in THF (5mL) was added 6N NaOH (3mmol). The solution was refluxed for 5 h. and then solvent was removed under reduced pressure. The residue was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4 and evaporated *in vacuo* to give farnesylamine (95%): R_f=0.5 (10% NH_3-saturated MeOH in CH_2Cl_2); ^{1}H δ 1.33(s, 2H), 1.59(s, 6H), 1.62(s, 3H), 1.67(s, 3H), 1.96-2.10(m, 8H), 3.26(d, 2H, 6.9Hz), 5.09(m, 2H),

5.25(m, 1H); ^{13}C NMR δ 15.96, 16.05, 17.63, 25.64, 26.40, 39.51, 39.61, 39.67, 124.00, 124.32, 125.87, 131.26, 135.13, 136.35; IR 2920, 1742,1563, 1440, 1291, 815 cm^{-1}.

(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6-dienol (compound 11): To a solution of 1-bromo-3-methylbutane (10mmol) in dry THF (25mL) was added Mg (9mmol, turnings) and a crystal of I_2. The reaction was heated to reflux for 2 h (complete consumption of Mg), cooled to -78°C and diluted with HMPA (3.5mL). A solution of (*E*)-8-bromogeranyl acetate (1mmol) in THF (1mL) was added and the reaction was kept at -78°C for 2 h. The reaction was then brought to room temperature and stirred for 10 h. Work-up as before**,** followed by flash chromatography (20% EA/hex) afforded 10,11-dihydrofarnesol in 60% yield: R_f=0.3 (20% EA/hex); ^{1}H NMR δ 0.87(d, 6H, 7.2Hz), 1.12(m, 2H), 1.37(m, 2H), 1.52(m, 1H), 1.57(s, 3H), 1.67(s, 3H), 1.93(t, 2H, 7.5Hz), 2.05(m, 4H), 4.14(d, 2H, 6.9Hz), 5.08(m, 1H), 5.41(m, 1H); ^{13}C NMR δ 15.84, 16.22, 22.60, 25.70, 26.24, 27.84, 38.56, 39.55, 39.86, 59.33, 123.33, 123.45, 135.71, 139.71.

(2*E*)-4-thiafarnesol[(2*E*,6*E*)-3,7,11-Trimethyl-4-thia-2,6,10-dodecatrienol] (compound 13) and (2*Z*)-4-thiafarnesol) [(2*Z*,6*E*)-3,7,11-Trimethyl-4-thia-2,6,10-dodecatrienol] (compound 14): To a solution of (*E*)-geranyl thioacetate (3.7mmol) in THF (15mL) was added MeONa (11.1mmol in 3mL of MeOH). After 30min, the reaction was cooled to -78°C and ethyl 2-butynoate (4.44mol) was added drop wise via syringe. After 10 h, the reaction was worked up as for the thioethers (e.g. compound 15) and concentrated to furnish the alkenoate thioether as a crude oil, which was used without further purification.

The oil was dissolved in THF (2ml), cooled down to -78°C and subjected to the diisobutyl aluminum hydride (DIBAL) reduction (10mmol of 1.5M solution in toluene).

Following a standard work-up, the *Z* and *E* thioenol isomers were separated by flash chromatography with 20% EA/hex to furnish 0.511g (50%) of the *E*-isomer (compound 13) and 0.128g (12%) of the *Z* isomer (compound 14).

(2*E*,6*E*)-3,7,11-trimethyl-4-thiadodeca-2,6,10-trienol (compound 13): R_f=0.3 (20% EA/hex); ^{1}H δ 1.42(s, 1H), 1.59(s, 3H), 1.68(s, 6H), 1.93(s, 3H), 2.03(m, 4H), 3.36(d, 2H, 7.2Hz), 4.19(d, 2H, 7.2), 5.07(m, 1H), 5.25(m, 1H), 5.43(m, 1H); ^{13}C δ 16.11, 17.63, 17.88, 25.61, 26.37, 29.39, 39.47, 59.22, 118.13, 120.72, 123.81, 131.67, 136.25, 140.17; IR 3347, 2952, 2929, 2871, 1454, 1060 cm^{-1}.

(2*Z*,6*E*)-3,7,11-Trimethyl-4-thiadodeca-2,6,10-trienol (compound 14): R_f=0.4 (20% EA/hex); ^{1}H NMR δ 1.25(s, 1H), 1.59(s, 3H), 1.66(s, 6H), 2.04(m, 7H), 3.35(d, 2H, 7.5Hz), 4.27(d, 2H, 6.9Hz), 5.06(m, 1H), 5.21(m, 1H), 5.75(m, 1H); ^{13}C δ 16.06, 17.65, 23.83, 25.62, 26.37, 29.04, 39.47, 59.97, 119.89, 123.79, 129.34, 131.68, 134.08, 139.34; IR 3321, 2917, 1629, 1438, 1375, 1224, 1079, 1001, 836 cm^{-1}.

Preparation of thioethers (compounds 15, 16, 17 and 18): Into a solution of mercaptoalcohol (2mmol) in dry tetrahydrofuran (THF)(20mL) was added NaOH (2mmol). The stirred solution was cooled to -78°C and (*E*)-geranyl chloride (1mmol) was added via syringe. The reaction was allowed to warm to room temperature, and, after 12 h, concentrated under reduced pressure. The residue was diluted with water and extracted with 20% ethyl acetate/hexane (EA/hex). The organic layer was washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (10% EA/hex).

(*E*)-3,7,11-Trimethyl-4-thiododeca-6,10-dienol (compound 15): (95% yield); R_f=0.4 (20% EA/hex); ^{1}H δ 1.30(d, 3H, 6.9Hz), 1.58(s, 3H), 1.66(s, 6H), 2.04(m, 5H), 2.87(m,

1H), 3.18(d, 2H, 7.5Hz), 3.76(m, 2H), 5.07(m, 1H), 5.24(m, 1H); ^{13}C δ 15.99, 17.62, 21.93, 25.61, 26.40, 28.01, 36.90, 39.10, 39.52, 60.86, 120.42, 123.89, 131.61, 138.72; IR 3365, 2920, 1450, 1375, 1045, 994, 846 cm^{-1}.

(*E*)-6,10-Dimethyl-3-undeca-5,9-dienol (compound 16): (93% yield); R_f=0.4 (20% EA/hex); ^{1}H δ 1.59(s, 3H), 1.64(s, 3H), 1.67(s, 3H), 2.05(m, 4H), 2.24(s, 1H), 2.67(t, 2H, 6Hz), 3.15(d, 2H, 7.8Hz), 3.69(q, 2H, 5.7Hz), 5.06(m, 1H), 5.22(m, 1H); ^{13}C δ 15.99, 17.65, 25.64, 26.37, 28.72, 34.08, 39.54, 60.28, 120.20, 123.84, 131.71, 139.28; IR 3375, 2914, 1742, 1440, 1378, 1225, 1045, 830 cm^{-1}.

(*E*)-7,11-Dimethyl-4-thiadodeca-6,10-dienol (compound 17): (84% yield); R_f=0.4 (20% EA/hex); ^{1}H δ 1.60(s, 3H), 1.65(s, 3H), 1.67(s, 3H), 1.76(s, 1H), 1.83(m, 2H), 2.05(m, 4H), 2.58(t, 2H, 7.2Hz), 3.16(d, 2H, 7.8Hz), 3.74(t, 2H, 5.7Hz), 5.08(m, 1H), 5.23(m, 1H); ^{13}C δ 16.02, 17.65, 25.64, 26.42, 27.73, 29.26, 31.87, 39.55, 61.98, 120.34, 123.89, 131.65, 138.88; IR 3358, 2919, 1739, 1442, 1375, 1217, 1042 cm^{-1}.

(*E*)-8,12-Dimethyl-5-thiotrideca-7,11-dienol (compound 18): (85% yield); R_f=0.4 (20% EA/hex); ^{1}H δ 1.51(s, 1H), 1.60-1.67(m, 13H), 2.04(m, 4H), 2.49(m, 2H), 3.14(d, 2H, 7.8Hz), 3.65(s, 2H), 5.07(m, 1H), 5.22(m, 1H); ^{13}C δ 15.97, 17.65, 25.64, 25.84, 26.42, 29.22, 30.67, 31.93, 39.55, 62.37, 120.53, 123.91, 131.59, 138.59; IR 3357, 2923, 1733, 1443, 1374, 1224, 1053 cm^{-1}.

(*E*)-7,11-dimethyl-3,4-dithiododeca-6,10-dienol (compound 19): To a solution of geranyl thio acetate (0.5mmol) in 50:50 THF/MeOH was added NaOH (10mmol) and the mixture was stirred for 30 min. Following addition of 2-mercaptoethanol (5mmol), the solution was exposed to air for 10 h. Standard work-up followed by purification by flash chromatography with 20% EA/hex afforded the mixed disulfide in 28mg (6%): R_f=0.5

(hexane); ^{1}H NMR δ 1.60(s, 3H), 1.68(s, 3H), 1.70(s, 3H), 2.06(m, 5H), 2.85(t, 2H, 5.7Hz), 3.42(d, 2H, 8.1Hz), 3.88(quartet, 2H, 5.7Hz), 5.08(m, 1H), 5.28(m, 1H); ^{13}C NMR δ 16.31, 17.66, 25.65, 26.31, 37.09, 39.61, 41.30, 60.28, 118.63, 123.75, 131.80, 141.48.

9-thia analogs (compounds 21-23 and 35)

(*E*)-8-hydroxygeranyl acetate: A solution of (*E*)-geranyl acetate (46mmol) and SeO_2 (41mmol) in ethanol (70mL) was refluxed for 3h and then allowed to cool after which $NaBH_4$ (26mmol) was added. After 30 min, the reaction was filtered through a paper filter and solvent was removed under reduced pressure. Work-up and chromatography (20% EA/Hex) as above furnished 41% of (*E*)-8-hydroxygeranyl acetate along with 20% of recovered starting material: R_f=0.4 (50% EA/hex); ^{1}H NMR δ 1.61(s, 3H), 1.66(s, 3H), 2.00-2.11(m, 8H), 3.93(s, 2H), 4.53(d, 2H, 6.9Hz), 5.30(m, 2H); ^{13}C δ 13.5, 16.3, 20.9, 25.5, 38.9, 61.3, 68.3, 118.5, 124.9, 135.1, 141.6, 171.1.

(*E)*-8-bromogeranyl acetate: Into a -78°C solution of (*E*)-8-hydroxygeranyl acetate (2mmol) and pyridine (2mmol) in THF (15mL) was added PBr_3 (1mmol). The reaction was allowed to warm to room temperature and stirred for 3 h. The reaction was then quenched with 0°C sat. aq. $NaHCO_3$ and extracted with 20% EA/hex. The crude allyl bromide (estimated yield of 90%) was used directly for subsequent reactions.

9-thiafarnesol analogs (compounds 21, 22, and 23): To a solution of thiol (2.5mmol) in THF (7mL) was added NaOH (1mmol) followed by (*E*)-8-bromogeranyl acetate (0.5mmol in 0.5mL of THF). The mixture was stirred for 10 h whereupon 6N NaOH (0.5mL) and H_2O (3mL) were added. After stirring for 30 min., the reaction was worked

up as for other thioethers (e.g. compound 15). The oil was purified by flash chromatography with 20% EA/hex to furnish the thioether.

(2*E*,6*E*)-3,7-Dimethyl-9-thiatridecadienol (compound 21): (89% yield); R_f=0.3 (20% EA/hex); ^{1}H NMR (500Mz) δ 0.90(t, 3H, 7.5Hz), 1.27(s, 1H), 1.38(m, 2H), 1.52(m, 2H,), 1.67(s, 3H), 1.70(s, 3H), 2.06(m, 2H), 2.16(m, 2H), 2.37(t, 2H, 7.5Hz), 3.07(s, 2H), 4.14(d, 2H, 6.5Hz), 5.22(m, 1H), 5.41(m, 1H); ^{13}C NMR (125Mz) δ 13.70, 14.92, 16.17, 22.02, 26.28, 30.40, 30.89, 31.43, 39.28, 41.27, 59.31, 123.64, 127.26, 131.48, 139.20; IR 3336, 2920, 1445, 1219, 1004 cm^{-1}.

(2*E*,6*E*)-3,7,11-Trimethyl-9-thiadodecadienol (compound 22): (86% yield); R_f=0.3 (20% EA/hex); ^{1}H NMR(500Mz) δ 0.96(d, 6H, 8.5Hz), 1.27(s, 1H), 1.66-1.76(m, 7H), 2.07(m, 2H), 2.15(m, 2H), 2.25(d, 2H, 8.5Hz), 3.05(s, 2H), 4.15(d, 2H, 5Hz), 5.21(m, 1H), 5.41(m, 1H); ^{13}C NMR(125Mz) δ 14.92, 16.19, 22.05, 26.29, 28.28, 39.29, 39.90, 41.80, 59.33, 123.64, 127.34, 131.55, 139.23.

(2*E*,6*E*)-3,7,10,10-Tetramethyl-9-thiaundecadienol (compound 23): (91% yield); R_f=0.4 (20% EA/hex); ^{1}H NMR δ 1.32(s, 9H), 1.60(s, 1H), 1.66(s, 3H), 1.72(s, 3H), 2.06(m, 2H), 2.13(m, 2H), 3.17(s, 2H), 4.14(d, 2H, 9Hz), 5.54(m, 1H), 5.41(m, 1H); ^{13}C δ 15.52, 16.13, 26.24, 30.85, 38.27, 38.98, 42.35, 59.33, 123. 76, 127.13, 132.10, 139.16.

(*E*,*E*)-3,7,11-Trimethyl-9-oxadodeca-2.6-dienol (compound 35): (92% yield); To the suspension of NaH (3mmol, 60% dispersion in mineral oil) in THF (10mL) was added isobutyl alcohol (10mmol) followed by (*E*)-8-bromogeranyl acetate (0.5mmol in 0.5mL of THF). After 10 h, 6N NaOH (0.5mL) and H_2O (3mL) were added and the reaction was stirred for 30 min. Work-up as before furnished 0.104g (92%) of the ether as an oil: R_f=0.3 (20% EA/hex); ^{1}H NMR δ 0.91(d, 6H, 6Hz), 1.23(s, 1H), 1.64(s, 3H), 1.69(s,

3H), 1.86(m, 1H), 2.03-2.23(m, 4H), 3.12(d, 2H, 6Hz), 3.83(s, 2H), 4.15(d, 2H, 6.4Hz), 5.34(m, 2H); ^{13}C NMR δ 14.5, 17.0, 20.1, 26.7, 29.2, 39.8, 60.1, 77.4, 77.6, 124.4, 127.6, 133.8, 140.0. IR 3375, 2920, 1460, 1086, 1004 cm^{-1}.

Alkanol analogs (compounds 27-29).

(*E*)-5,9-Dimethyldeca-4,8-dienol (compound 27): Compound 27 was prepared from (*E*)-geranyl chloride and vinyl magnesium bromide by a two step procedure similar to that used for compound 19. The initial product was (*E*)-5,9-dimethyldeca-1,4,8-triene *(1-24)*: R_f=0.9 (hexane); ^{1}H NMR δ 1.62(s, 6H), 1.7(s, 3H), 2.76(t, 2H, 6.6), 4.94-5.18(m, 4H), 5.81(m, 1H). Hydroboration of the triene as described above furnished compound 20 in 57% (two steps): R_f=0.3 (20% EA/hex); ^{1}H δ 1.48(s, 1H), 1.57-1.67 (m, 11H), 2.06(m, 6H), 3.63(t, 2H, 6.6Hz), 5.12(m, 2H); ^{13}C NMR δ 15.91, 17.63, 24.23, 25.65, 26.61, 32.70, 39.67, 62.68, 123.73, 124.23, 131.40, 135.77; IR 3339, 2919,1736, 1450, 1214, 1050 cm^{-1}.

(*E*)-6,10-Dimethylundeca-5,9-dienol (compound 28): (*E*)-geranyl chloride (3mmol) was added drop-wise via syringe into a -78°C solution of allyl magnesium chloride (7.5mmol, 2M solution in THF) and hexamethylphosphoric triamide (HMPA)(1mL) in THF (6mL). The reaction was allowed to warm to room temperature, and after 12 h, quenched with water. The pentane extract was washed with water, dried over Na_2SO_4 and concentrated by evaporation at normal pressure. The resulting oil was purified by flash chromatography with pentane to furnish (*E*)-6,10-dimethylundeca-1,5,9-triene *(1-22)* in 67% yield: R_f=0.9 (hexane); ^{1}H 1.62(s, 6H), 1.70(s, 3H), 1.99-2.12(m, 8H), 4.94-5.18(m, 4H), 5.84(m, 1H).

9-BBN (9-borabicyclononane) (2mmol, 0.5M solution in THF) was added to a solution of the triene (estimated 2mmol) in THF (15mL). After 5 h, the reaction was quenched by the successive addition of ethanol (2mL), 6N aq. NaOH (3mmol), and 30% H_2O_2 (4mmol). The mixture was diluted with water and extracted with 20% EA/hex. Work-up as before and purification by flash chromatography with 20% EA/hex furnished the alcohol in 53% yield over two steps. R_f=0.4 (20% EA/hex); ^{1}H NMR δ 1.40(m, 3H), 1.59(m, 8H), 1.60(s, 6H), 1.68(s, 3H), 2.00(m, 6H), 3.64(t, 2H, 6.6Hz), 5.10(m, 2H); ^{13}C δ 15.96, 17.65, 25.67, 25.90, 26.67, 27.55, 32.32, 39.70, 64.97, 124.23, 124.32, 131.30, 135.29; IR 3331, 2922, 2922, 1443, 1373, 1058 cm^{-1}.

(*E*)-7,11-Dimethyldodeca-6,10-dienol (compound 29): A solution of 4-bromo-1-butene (30mmol) in THF (17mL) was refluxed for 3 h over Mg (30 mmol) until the turnings were consumed. The resulting solution was cooled to -78°C and diluted with HMPA (2.5mL). Addition of (*E*)-geranyl bromide (3mmol), followed by work-up and purification as above afforded (*E*)-6,10-dimethyldeca-1,6,10-triene *(1-30)*: R_f=0.9 (hexane); ^{1}H δ 1.43(m, 2H), 1.60(s, 6H), 1.69(s, 3H), 2.04(m, 6H), 4.92-5.13(m, 4H), 5.81(m, 1H).

Hydroboration as before furnished dienol compound 29 in 42% yield over two steps: R_f=0.5 (20% EA/hex); ^{1}H δ 1.31(s, 1H), 1.35(m, 4H), 1.59(m, 8H), 1.68(s, 3H), 2.00(m, 6H), 3.63(t, 2H, 6.3Hz), 5.11(m, 2H); ^{13}C δ 15.94, 17.65, 25.33, 25.67, 26.72, 27.79, 29.60, 32.71, 39.70, 63.03, 124.35, 124.43, 131.26, 135.0; IR 3335, 2926, 2852, 1447, 1377, 1054 cm^{-1}.

7,11-Dimethyl-4-selenadodeca-6,10-dienol (compound 30): To a -78°C solution of KSeCN (2.2mmol) in THF (10mL) was added the tetrahydropyranyl ether of 3-

bromopropanol (2mmol). After stirring for 10h, the reaction was diluted with water and extracted with pentane. The organic layer was washed with water, dried over sodium sulfate and evaporated *in vacuo* to give the selenocyanate which was used without further purification. Into a -78°C THF solution of the selenocyanate was successively added $NaBH_4$ (4mmol), ethanol (1mL) and (*E*)-geranyl chloride (1mmol). The reaction was warmed to room temperature and stirred for 10 h. Following work-up as before, the crude residue was treated with 100mg of pyridinium *p*-toluenesulfonate in 5ml MeOH for 7 h. Concentration, followed by flash chromatography with 20% EA/hex, afforded the selenoether (0.215 g, 78%) as a 32:67 mixture of *Z*:*E* isomers according to GC/MS (retention times 16.98:17.28 min.): R_f=0.4 (20% EA/hex); ^{1}H (mixture of *E* and *Z* isomers) δ 1.57-1.71(m, 9H), 1.89(p, 2H, 6.6Hz), 2.04(m, 5H), 2.58(m, 2H), 3.20(d, 2H, 8.4Hz), 3.69(t, 2H, 6.3Hz), 5.05(m, 1H), 5.31(m, 1H); ^{13}C NMR(mixture of E and Z isomers) δ 15.77, 17.59, 19.44, 19.83, 20.70, 23.25, 25.58, 26.41, 26.49, 31.61, 32.92, 39.41, 39.51, 62.37, 62.43, 120.93, 121.58, 123.79, 123.85, 131.55, 131.84, 138.29, 138.38: FAB-MS: M/Z$(M+Li)^+$=283.1

(*E*)-8,12-Dimethyl-5-azatrideca-7,11-dienol (compound 31): To a solution of 4-aminobutanol (2mmol) in THF (15mL) was added $NaHCO_3$ (5mmol) and (*E*)-geranyl bromide (1mmol). After stirring for 10 h., the reaction mixture was found to still contain unreacted geranyl bromide and was heated to reflux for 2 h. Work-up as before followed by flash chromatography with a 10% solution of sat. NH_3/MeOH in CH_2Cl_2 furnished 0.025g (11%) of the desired secondary amine along with 0.1516g (42%) of the tertiary amine byproduct. R_f=0.2 (10% of sat. NH_3/MeOH in CH_2Cl_2); ^{1}H δ 1.58-1.66(m, 13H), 2.02(m, 4H), 2.64(t, 2H, 5.7Hz), 3.23(d, 2H, 6.9Hz), 3.33(s, 3H), 3.57(t, 2H, 4.5Hz),

5.06(m, 1H), 5.24(m, 1H); ^{13}C δ 16.19, 17.65, 25.64, 26.42, 28.53, 32.39, 39.57, 46.49, 48.79, 62.46, 121.35, 123.95, 131.58, 138.94.

Preparation of ethers (compounds 32 and 34): The diol (7mmol) was added to a stirred suspension of NaH (3mmol as 60% dispersion in mineral oil) in distilled THF (20mL). The mixture was stirred for 20 minutes. (*E*)-geranyl bromide (1mmol) was added drop wise via syringe and the reaction was refluxed for 4 h. Work-up as above followed by flash chromatography (20% EA/hex) furnished the ether.

(*E*)-7,11-Dimethyl-4-oxadodeca-6,10-dienol (compound 32): (72% yield); R_f=0.3 (20% EA/hex); ^{1}H δ 1.59(s, 3H), 1.65(s, 3H), 1.67(s, 3H), 1.82(pentet, 2H, 5.7Hz), 2.04(m, 4H), 2.52(s, 1H), 3.60(t, 2H, 5.7Hz), 3.76(t, 2H, 5.4Hz), 3.98(d, 2H, 6.9Hz), 5.07(m, 1H), 5.32(m, 1H); ^{13}C δ 16.38, 17.62, 25.62, 26.28, 32.03, 39.51, 62.01, 67.54, 69.21, 120.59, 123.87, 131.64, 140.34.

(*E*)-8,12-Dimethyl-5-oxatrideca-7,11-dienol (compound 34): (76% yield); R_f=0.3 (20% EA/hex); ^{1}H δ 1.59(s, 3H), 1.67(m, 10H), 2.04(m, 4H), 2.58(s, 1H), 3.45(t, 2H, 6Hz), 3.62(d, 2H, 5.4Hz), 3.98(d, 2H, 6.6Hz), 5.08(m, 1H), 5.33(m, 1H); ^{13}C δ 16.37, 17.62, 25.62, 26.31, 26.93, 30.34, 39.54, 62.68, 6729, 70.00, 120.52, 123.91, 131.61, 140.33; IR 3396, 2920, 2858, 1445, 1373, 1102, 1061 cm^{-1}.

(*E*)-3,7,11-trimethyl-4-oxydodeca-6,10-dienol (compound 33): A suspension of NaH (4.5mmol, 60% dispersion in mineral oil) was washed with hexane (2 x 0.3mL each), after which was added 3-butene-2-ol (4.5mmol) in 15mL of dry THF followed by (*E*)-geranyl bromide (1.5mmol). The reaction was heated to reflux for 10h and then cooled. Solvent was removed at the reduced pressure, and the residue was diluted with water. The pentane extract was dried with Na_2SO_4 and evaporated at normal pressure to give a

crude oil. Without further purification the crude material was dissolved in THF (5mL) and 9-BBN (9-borabicyclononane, 1.5mmol) was added. After 10 h., the reaction was quenched by the successive addition of ethanol (2mL), 6N NaOH (3mmol), and 30% H_2O_2 (4mmol). Following work-up as for compound 26, chromatography (20% EA/Hex) furnished the ether in 84% yield over two steps: R_f=0.3 (20% EA/hex); ^{1}H NMR δ 1.18(d, 3H, 6Hz), 1.59(s, 3H), 1.66-1.77(m, 8H), 2.04(m, 4H), 2.81(s, 1H), 3.75(m, 3H), 3.93(m, 1H), 4.08(m, 1H), 5.07(m, 1H), 5.32(m, 1H); ^{13}C δ 16.37, 17.63, 19.45, 25.64, 26.29, 38.64, 39.52, 61.22, 64.74, 74.67, 120.84, 123.89, 131.65, 140.24; IR 3411, 2925, 1454, 1050 cm^{-1}.

(2*E*,6*E*)-3,7,11-trimethyldodecadiene-1,10-diol and (2*E*)-3,7,11-trimethyldodeca-2,10-diene-1,6-diol (compounds 37 and 38): Into a solution of farnesol (1mmol) in THF (10mL) was added 9-BBN (9-borabicyclononane, 2mmol). The reaction was stirred for 10 h and then quenched by the successive addition of ethanol (2mL), 6N NaOH (3mmol), and 30% H_2O_2 (4mmol). The resulting mixture was readily separated by flash chromatography (50% EA/hex) to afford 90% of recovered farnesol, 0.096g of 10-hydroxyfarnesol and 0.144g of 6-hydroxyfarnesol.

(2*E*,6*E*)-3,7,11-Trimethyldodecadiene-1,10-diol (compound 37): R_f=0.5 (50% EA/hex); ^{1}H NMR δ 0.89(d, 6H, 6.6Hz), 1.47-1..66(m, 9H), 2.03-2.19(m, 6H), 3.35(m, 1H), 4.10(d, 2H, 6.9Hz), 5.16(m, 1H), 5.39(m, 1H); ^{13}C NMR δ15.76, 17.34, 18.67, 25.64, 30.88, 31.23, 33.53, 36.69, 39.23, 59.20, 76.85, 124.24. 124.78, 135.96, 138.74; IR 3334, 2920, 1670, 1445, 1378, 999 cm^{-1}. FAB-MS: M/Z(M+Li)$^+$=247.2.

(2*E*)-3,7,11-Trimethyldodeca-2,10-diene-1,6-diol (compound 38): R_f=0.4 (20% EA/hex); ^{1}H NMR δ 0.89(d, 3H, 6.6Hz), 1.24(m, 2H), 1.51(m, 2H), 1.59(s, 3H), 1.68(m,

6H), 1.35-2.28(m, 5H), 3.44(m, 1H), 4.14(d, 2H, 6.9Hz), 5.09(m, 1H), 5.44(m, 1H); ^{13}C NMR δ 15.04, 16.24, 17.63, 25.64, 25.68, 31.14, 32.04, 36.16, 38.45, 59.26, 75.59, 123.59, 124.55, 131.51, 139.74; IR 3339, 2920, 2366, 2366, 1455, 1383, 999 cm^{-1}. FAB-MS: $M/Z(M+Li)^{+}$=247.2.