

30. Biosynthesis of Homoterpenes in Higher Plants

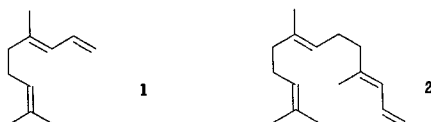
by Wilhelm Boland* and Andreas Gäbler

Institut für Organische Chemie, Richard-Willstätter-Allee, Postf. 6380, D-7500 Karlsruhe

(26.X.88)

In higher plants, the two homoterpenes 4,8-dimethylnona-1,3,7-triene (**1**) and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (**2**) are synthesized from the regular terpene alcohols nerolidol and geranyllinalool by fragmentation into the homoterpene and butenone. The biosynthetic pathway is evidenced by conversion of (²H)nerolidol in *Hoya purpureo-fusca*, *Magnolia liliiflora* nigra, *Robinia pseudoacacia*, and *Philadelphus coronarius*.

1. Introduction. – The two homoterpenes **1** and **2** have long been overlooked but are nonetheless widespread constituents of flower fragrances. Up to now, both compounds have been found in *ca.* 20 different flavour sources. Among them are some of the very important perfume plants like *e.g.* *Rosa damascena* or *Rosa centifolia* where they do occur on a trace level [1].



Although **1** and **2** have already been synthesized some 20 years ago [2], they were first recognized as natural products in 1986 by Maurer *et al.* as minor constituents of the Cardamom oil [3]. Recently, it was shown by Kaiser that both homoterpenes are even major constituents of the volatiles emitted from *Magnolia liliiflora* (Magnoliaceae), *Hoya carnosa* (Asclepiadaceae), *Robinia pseudoacacia* (Leguminosae), from Cactaceae like *Selenicereus hamatus* ('Queen of the night') or orchidaceae like *Aerangis friesiorum* [4]. Due to their widespread occurrence and their very recently discovered function as mediators in insect-plant interactions [5], the biosynthesis of these homoterpenes is of particular interest. According to a tentative biosynthetic scheme proposed by Kaiser, **1** and **2** could originate from the two regular terpene alcohols nerolidol and geranyllinalool by oxidative fragmentation into the homoterpene and but-3-en-2-one [4] (see below, Scheme 3).

We now report the synthesis of appropriately labelled precursors, namely (²H)nerolidol and (²H)geranyllinalool, and their successful administration to and metabolism by *Magnolia liliiflora* nigra, *Hoya purpureo-fusca*, *Robinia pseudoacacia*, and *Philadelphus coronarius*.

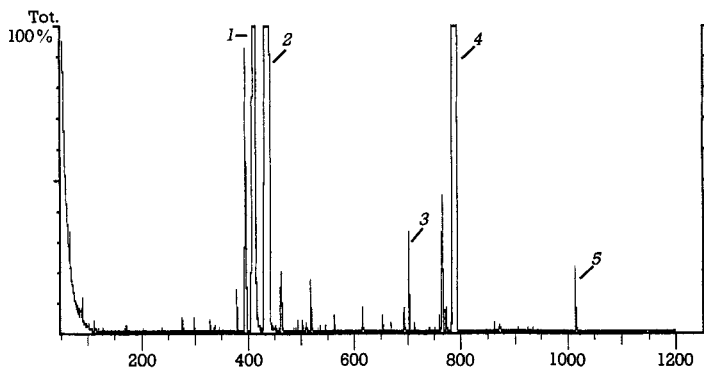


Fig. 1. GLC analysis of volatiles of *Magnolia liliiflora nigra*. Major compounds: 1 = linalool, 2 = (3*E*)-4,8-dimethylnona-1,3,7-triene (**1**), 3 = tetradecane, 4 = pentadecane, 5 = farnesol. The (^2H)homoterpene (^2H)-**1** elutes within the first front of the natural homoterpene.

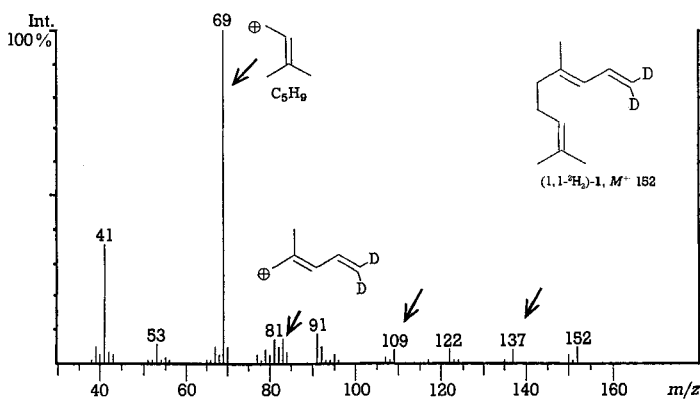


Fig. 2. MS of (1,1,2- H_2)-**1** obtained after incubation of *Magnolia liliiflora nigra* with ($^2\text{H}_5$)nerolidol **6a**. Due to insufficient separation of the (^1H)- and the (^2H)compound, some fragments of the natural (^1H)homoterpene **1** are also present. Typical fragments of the (^2H)compound (1,1,2- H_2)-**1** are indicated by arrows.

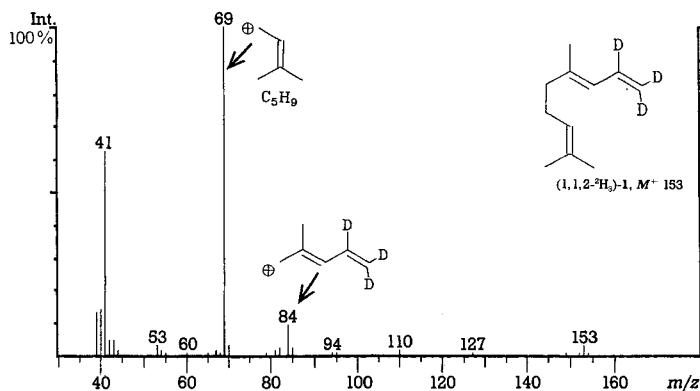


Fig. 3. MS of (1,1,2,2- H_3)-**1** obtained after incubation of *Magnolia liliiflora nigra* with ($^2\text{H}_7$)nerolidol **6b**. The spectrum is fully consistent with the synthetic reference **12b** (no contamination with the natural (^1H)homoterpene **1**).

The molecular ion of (1,1- $^2\text{H}_2$)-**1** at m/z 152 (150 for **1**), together with the 2-methylpentadienyl ion at m/z 83 (81 for **1**) and the dimethylallyl fragment at m/z 69 (69 for **1**) unequivocally verify the administered ($^2\text{H}_3$)nerolidol **6a** as the precursor of the new metabolite. Furthermore, GLC and MS data are fully consistent with those of the synthetic reference **12a**.

If ($^2\text{H}_3$)nerolidol **6b** is administered to *Magnolia liliiflora nigra*, the molecular ion of the resulting ($^2\text{H}_3$)homoterpene (1,1,2- $^2\text{H}_3$)-**1** at m/z 153 indicates that one and only one of the originally two ^2H -labels at C(5) of **6b** has been lost during double-bond formation (see Fig. 3). Also the 2-methylpentadienyl fragment shows the expected shift to m/z 84, while the terminal dimethylallyl fragment remains unchanged (m/z 69), as is the case for the reference compound **12b**.

It is interesting to note, that the used specimens of *Philadelphus coronarius* and *Robinia pseudoacacia* do not produce the natural (^1H)homoterpenes [4], at least not at a level which can be monitored by GLC or GLC/MS. However, the required enzyme(s) have to be present, since both plants produce the ($^2\text{H}_2$)homoterpene (1,1- $^2\text{H}_2$)-**1** after administration of ($^2\text{H}_3$)nerolidol **6a**.

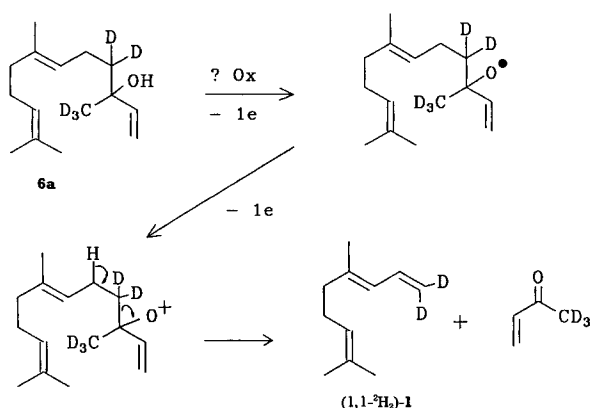
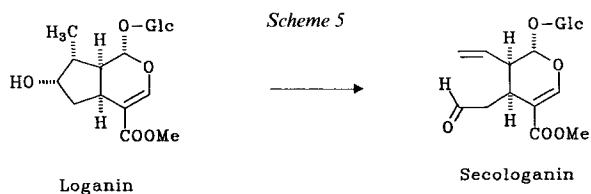
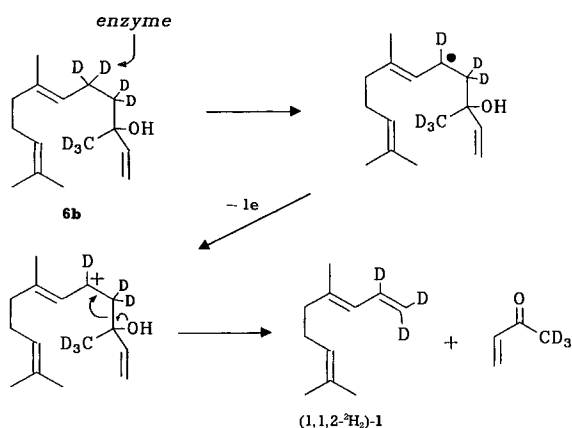
The second fragment, namely but-3-en-2-one, is not observed in any of the above incubation experiments. Due to its high chemical activity in addition reactions ([4 + 2] cycloaddition [6] and [1,4]addition), it might be trapped in further reactions prior to analysis.

Incubation experiments with geranyl($^2\text{H}_3$)linalool **9** are as yet less conclusive. Only with *Philadelphus coronarius* and **9** the formation of a labelled (H_2)-**2** seems to occur. However, due to its very low abundance, no conclusive information about the molecular ion can be obtained. Although most of the typical fragments of the ($^2\text{H}_2$)reference **13** at m/z 69, 81/83, 97, and 136 versus m/z 69, 81, 95, 134 for the (^1H)compound are matched by the metabolite, further experiments with improved administration and recovery techniques are required to confirm that the same fragmentative degradation is also valid for the formation of **2** from the higher homologue geranyllinalool.

4. Discussion. – The present work provides the first experimental evidence for the biosynthesis of the homoterpenes **1** and, with restrictions, **2** from the regular terpene alcohols nerolidol or geranyllinalool, respectively. It is in agreement with the tentative biosynthetic scheme proposed by Kaiser [4] (see Scheme 3). According to this, nerolidol or geranyllinalool are enzymatically attacked at the O-atom and after electron transfer cleavage into the corresponding homoterpenes **1** or **2**, respectively, and butenone occurs.

Alternatively, the enzymatic attack could also occur on C(5), leaving a radical at this position which, after a second electron transfer, could fragment into the same products (Scheme 4). Both mechanisms are framed by the experimental results and can as yet not be distinguished by the hitherto performed incubation experiments.

The new fragmentation of nerolidol or geranyllinalool exhibits striking similarities to the bioconversion of loganin into secologanin [10] (Scheme 5). In this example of a secondary alicyclic alcohol, a central σ bond between a Me group and an adjacent OH moiety is enzymatically cleaved and leads to a vinyl and aldehyde fragment within the same molecule. Intermediates which could provide evidence for the enzymatic attack at either the O-atom or the exocyclic Me group have not been observed as yet.

Scheme 3. Biosynthesis of the Homoterpenes **1** or **2** According to Kaiser [4], Exemplified with (2H_5)Nerolidol **6a**Scheme 4. Biosynthesis of Homoterpenes **1** or **2**. Exemplified with (2H_7)Nerolidol **6b**

In the present case, determination of $K_{H/D}$ for removal of an H-atom from C(5) is expected to give more information about this principal question, and it is the subject of further investigations.

Financial support by the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie* (Frankfurt am Main) is gratefully acknowledged. We thank Dr. R. Kaiser (Givaudan AG, Dübendorf, Switzerland) for helpful discussions and valuable advice. We are deeply indebted to Dr. F.-J. Marner, University of Cologne, for most of the mass spectra.

Experimental Part

General. Solvents and reagents were purified and dried prior to use. Anhydrous MgSO_4 was used for drying operations. Solutions were generally concentrated by flash evaporation under reduced pressure. Anal. GLC: Carlo Erba gas chromatograph, series 4200, equipped with fused-silica capillaries, SE 30 (40 m $\times$ 0.31 mm); carrier gas, H_2 at 30 cm/s. IR (cm^{-1}): Beckmann Acculab 8. ^1H -NMR (250 MHz, TMS as internal standard): Bruker Cryospec WM 250. MS (m/z): Finnigan 4510 GLC/MS system and Finnigan ITD combined with a Carlo Erba gas chromatograph, model Vega; carrier gas, He at 30 cm/s.

(1,1,1,3,3,3- $^2\text{H}_6$) Acetone N,N-Dimethylhydrazone (**3**). To a well stirred solution of ($^2\text{H}_6$)acetone (10.0 g, 0.16 mol) in CH_2Cl_2 (100 ml) were added dry, finely grounded molecular sieves (3 Å; 10.0 g) and ($^2\text{H}_4$)acetic acid (1 ml). After a brief exothermic reaction, stirring was continued for 2 h, the solids were removed by suction, the filtrate was washed with Na_2CO_3 solution (10%) and dried (NaOH pellets), and most of the solvent was evaporated. Distillation gave 5.4 g (36%) of **3** as a colorless liquid. B.p. $93^\circ/760$ Torr. IR: 3000s, 2950s, 2860s, 2825s, 2780m, 2210w, 1635s, 1470s, 1450m, 1265m, 1020s, 985m, 725w. ^1H -NMR: 1.92 (m, ca. 0.8 H, CH_3 , corresponding to ca. 92% ^2H); 2.44 (s, $(\text{CH}_3)_2\text{N}$). MS: 106 (100, M^+ , $\text{C}_5^1\text{H}_6^2\text{H}_6\text{N}_2$), 105 (22), 104 (10), 62 (8), 61 (15), 60 (14), 58 (2), 48 (4), 47 (13), 46 (61), 45 (32), 44 (63), 43 (9), 42 (36), 40 (5).

1-Chloro-3,7-dimethyl(1,1- $^2\text{H}_2$)octa-2,6-diene (**4b**). According to [7], **4b** was prepared in 71% yield from (1,1- $^2\text{H}_2$)geraniol [6]. IR: 2980s, 2940s, 2870s, 2180w, 1660w, 1600w, 1450m, 1385s, 1195m, 1180s, 950m, 820m. ^1H -NMR: 1.60 (s, CH_3); 1.68 (s, CH_3); 1.72 (d, $J = 2$, CH_3); 2.08 (br. m, 2 CH_2); 5.08 (br. t, $=\text{CH}$); 5.43 (s, $=\text{CHC}^2\text{H}_2$); no d at 4.10 (CH_2Cl) indicates $\gg 98\%$ ^2H . MS: 174, 176 (1, 0.5, M^+ , $\text{C}_{10}\text{H}_{17}^2\text{H}_2\text{Cl}$); 159 (1), 139 (4), 131 (11), 123 (8), 109 (9), 97 (4), 95 (6), 82 (7), 70 (23), 69 (100), 55 (9), 53 (11), 43 (12), 41 (75).

(^2H)Geranylacetone **5** and Farnesyl(^2H)acetone **8**: General Procedure. To a solution of **3** (4.4 g, 0.041 mol) in dry THF (40 ml) at -5° , BuLi (1.0 equiv.) in hexane was slowly added with stirring. The solution turned dark. After 30 min, a solution of the corresponding geranyl or farnesyl chloride (0.041 mol) in the same solvent (50 ml) was added, and stirring was continued for 4 h. Following hydrolysis with 2N HCl (20 min) and workup (Na_2CO_3 solution, brine, H_2O), the crude products were purified by CC on silica gel (pentane/ Et_2O 4:1).

6,10-Dimethyl(1,1,1,3,3,3- $^2\text{H}_5$)undeca-5,9-dien-2-one (**5a**). From **4a** and **3** in 64% yield. IR: 2970s, 2920s, 2860s, 2180w, 1713s, 1445m, 1380m, 1250m, 1110m, 1035m, 830w. ^1H -NMR: 1.62 (s, CH_3); 1.64 (s, CH_3); 1.69 (s, CH_3); 1.93–2.17 (m, 2 CH_2); 2.28 (d, $J = 8$, $\text{CH}_2\text{C}^2\text{H}_2$); 2.43 (m, ca. 0.4 H, CH_2 , corresponding to $\approx 92\%$ ^2H); 5.07 (t, $J = 8$, 2 $\text{CH}=\text{CH}$). MS: 198 (0.5, M^+ , $\text{C}_{13}\text{H}_{17}^2\text{H}_5\text{O}$), 180 (5), 155 (5), 136 (12), 121 (4), 109 (8), 93 (8), 82 (3), 69 (68), 53 (8), 46 (70), 45 (100), 44 (51), 41 (72).

6,10-Dimethyl(1,1,1,3,3,4,4- $^2\text{H}_7$)undeca-5,9-dien-2-one (**5b**). From **4b** and **3** in 23% yield. IR: 2970s, 2920s, 2860s, 2215w, 1710s, 1450m, 1380m, 1260s, 1105m, 1054m, 825w. ^1H -NMR: 1.59 (s, CH_3); 1.62 (s, CH_3); 1.68 (s, CH_3); 1.90–2.15 (m, 2 CH_2); 2.41 (m, ca. 0.2 H, CH_2 , corresponding to $\approx 97\%$ ^2H); 5.05 (br. t, 2 $\text{CH}=\text{CH}$). MS: 201 (0.5, M^+ , $\text{C}_{13}\text{H}_{15}^2\text{H}_7\text{O}$), 158 (4), 138 (8), 123 (3), 113 (4), 95 (5), 69 (48), 46 (100), 45 (48), 41 (48).

6,10,14-Trimethyl(1,1,1,3,3,3- $^2\text{H}_5$)pentadeca-5,9,13-trien-2-one (**8**). From **7** and **3** in 29% yield. IR: 2970s, 2920s, 2860s, 1715s, 1450m, 1375m, 1250m, 1030m, 990m, 830w. ^1H -NMR: 1.62 (s, 2 CH_3); 1.72 (s, 2 CH_3); 1.95–2.17 (m, 4 CH_2); 2.20 (d, $J = 8$, $\text{CH}_2\text{C}^2\text{H}_2$); 5.08 (m, 3 $\text{CH}=\text{CH}$). MS: 267 (0.7, M^+ , $\text{C}_{18}\text{H}_{25}^2\text{H}_5\text{O}$), 224 (0.5), 204 (0.6), 183 (0.9), 161 (1), 136 (21), 112 (4), 107 (4), 95 (7), 93 (11), 81 (28), 69 (100), 55 (8), 53 (8), 46 (95), 45 (42), 41 (63).

(^2H)Nerolidol **6** and Geranyl(^2H)linalool **9**: General Procedure. To a chilled solution of vinylmagnesium bromide (5.5 mmol) in dry THF (5.0 ml) were added, with stirring, **5** or **8** (5.0 mmol) in THF (5 ml). After 1 h, the mixture was hydrolyzed with sat. NH_4Cl solution, extracted with Et_2O , washed with H_2O , dried, and purified by CC on silica gel (pentane/ Et_2O 9:1).

3,7,11-(3-methyl- $^2\text{H}_3$)-Trimethyl(4,4- $^2\text{H}_2$)dodeca-1,6,10-trien-3-ol (**6a**). From **5a** in 53% yield. IR: 3420 (br.), 3090w, 2970s, 2930s, 2860s, 2230w, 1450m, 1375m, 1110m, 995m, 920m, 830m. ^1H -NMR: 1.58 (s, 2 CH_3); 1.68 (s, CH_3); 1.90–2.12 (m, 3 CH_2); 5.05 (dd, $J = 10.4$, 1, 1 H, $=\text{CH}_2$); 5.12 (t, $J = 7$, 2 $\text{CH}=\text{CH}$); 5.20 (dd, $J = 17.1$, 1, 1 H, $=\text{CH}_2$); 5.92 (dd, $J = 17.1$, 10.4, $\text{CH}=\text{CH}_2$). MS: 209 (2, $M^+ - \text{H}_2\text{O}$, $\text{C}_{15}\text{H}_{21}^2\text{H}_5\text{O}$), 165 (9), 152 (3), 136 (12), 123 (31), 109 (18), 97 (34), 93 (40), 81 (31), 69 (100), 67 (39), 55 (28), 53 (30).

3,7,11-(3-methyl- $^2\text{H}_3$)-Trimethyl(4,4,5,5- $^2\text{H}_4$)dodeca-1,6,10-trien-3-ol (**6b**). From **5b** in 98% yield. IR: 3405 (br.), 3090w, 2970s, 2920s, 2860s, 2230m, 1640m, 1450m, 1377s, 1190m, 1105m, 1070m, 995s, 920s, 825w. ^1H -NMR: 1.60 (s, 2 CH_3); 1.68 (s, CH_3); 1.90–2.15 (m, 2 CH_2); 5.05 (dd, $J = 10.4$, 1 H, $=\text{CH}_2$); 5.12 (s, CHC^2H_2); 5.12 (t, $=\text{CHCH}_2$); 5.20 (dd, $J = 17.1$, 1, 1 H, $=\text{CH}_2$); 5.92 (dd, $J = 17.1$, 10.4, $\text{CH}=\text{CH}_2$). MS: 211 (5, $M^+ - \text{H}_2\text{O}$, $\text{C}_{15}\text{H}_{19}^2\text{H}_7\text{O}$), 138 (18), 123 (12), 111 (7), 99 (8), 95 (14), 82 (11), 74 (25), 69 (100), 55 (15), 46 (38), 41 (95).

3,7,11,15-(3-methyl- $^2\text{H}_3$)-Tetramethyl(4,4- $^2\text{H}_2$)hexadeca-1,6,10,14-tetraen-3-ol (9). From **8** in 95% yield. IR: 3410 (br.), 3090w, 2970s, 2920s, 2860s, 2230w, 1450s, 1380s, 1190m, 1110m, 1050m, 995m, 920s, 830m, 790m. $^1\text{H-NMR}$: 1.60 (s, 2 CH_3); 1.68 (s, 2 CH_3); 1.90–2.15 (m, 5 CH_2); 5.05 (dd, $J = 11, 1, 1 \text{ H}, =\text{CH}_2$); 5.05–5.15 (m, 3= CH); 5.20 (dd, $J = 17.5, 1, 1 \text{ H}, =\text{CH}_2$); 5.92 (dd, $J = 17.5, 1, \text{CH}=\text{CH}_2$). MS: 277 (0.3, $M^+ - \text{H}_2\text{O}$, $\text{C}_{20}\text{H}_{29}^2\text{H}_5\text{O}$), 204 (1), 189 (1), 166 (1.5), 136 (5), 121 (4), 109 (8), 95 (11), 93 (10), 81 (32), 74 (12), 69 (100), 55 (12), 46 (17), 43 (15), 41 (55).

Reference Substances 12a, 12b, and 13: General Procedure. To a chilled soln. of ($^2\text{H}_2$)methylidene(triphenyl)phosphorane (13.0 mmol) in dry THF (15 ml) was added, with stirring, a soln. of geranial, ($1\text{-}^2\text{H}$)geranial, or farnesal (10 mmol in 5 ml of THF). Stirring was continued for 1 h, and after usual workup, the product was purified by CC on silica gel using pentane.

4,8-Dimethyl(1,1- $^2\text{H}_2$)nona-1,3,7-triene (12a). From geranial in 50% yield. IR: 3025w, 2970s, 2930s, 2860s, 2215w, 1650w, 1450m, 1380m, 1105m, 975m, 933m, 880m, 715m. $^1\text{H-NMR}$: 1.61 (s, CH_3); 1.69 (s, CH_3); 1.77 (s, CH_3); 2.00–2.20 (m, 2 CH_2); 5.1 (m, $=\text{CH}$); 5.87 (d, $J = 11.3, =\text{CH}-\text{CH}=\text{C}^2\text{H}_2$); 6.56 (d, $J = 11, \text{CH}=\text{C}^2\text{H}_2$). MS: 152 (7, M^+ , $\text{C}_{11}\text{H}_{16}^2\text{H}_2$), 137 (7), 124 (2), 109 (9), 95 (3), 83 (17), 69 (100), 53 (6), 41 (22).

4,8-Dimethyl(1,1,2- $^2\text{H}_3$)nona-1,3,7-triene (12b). From ($1\text{-}^2\text{H}$)geranial in 71% yield. IR: 3040m, 2970s, 2930s, 2860s, 2210w, 1655m, 1540w, 1445m, 1380m, 1105m, 1020m, 880m, 825m, 700s. $^1\text{H-NMR}$: 1.62 (s, CH_3); 1.68 (s, CH_3); 1.78 (s, CH_3); 2.00–2.20 (m, 2 CH_2); 5.10 (m, $=\text{CH}$); 5.87 (s, $=\text{CH}-\text{C}^2\text{H}=\text{C}^2\text{H}_2$). MS: 153 (2, M^+ , $\text{C}_{11}\text{H}_{15}^2\text{H}_3$), 138 (2), 110 (2), 96 (1), 84 (11), 69 (100), 55 (3), 53 (3), 41 (67).

4,8,12-Trimethyl(1,1,2- $^2\text{H}_2$)trideca-1,3,7,11-tetraene (13). From (*E*)-farnesol via oxidation (MnO_2) to farnesal and Wittig reaction in 65% yield. IR: 3020w, 2960s, 2920s, 2850s, 2360w, 2210w, 1640w, 1445m, 1375m, 1100w, 925m, 825m, 705s. $^1\text{H-NMR}$: 1.62 (s, 2 CH_3); 1.68 (s, CH_3); 1.78 (s, CH_3); 1.90–2.25 (m, 4 CH_2); 5.10 (m, 2 $\text{CH}=\text{}$); 5.84 (d, $J = 11.5, =\text{CHCH}=\text{C}^2\text{H}_2$); 6.55 (d, $J = 11, \text{CH}=\text{C}^2\text{H}_2$). MS: 205 (3, $M^+ - 15, \text{C}_{16}\text{H}_{24}^2\text{H}_2$), 177 (4), 162 (2), 136 (3), 134 (3), 121 (3), 109 (4), 95 (18), 83 (11), 81 (58), 69 (100), 67 (18), 55 (8), 53 (13), 41 (73).

Incubation Experiments. Suspensions of labelled nerolidol or geranylinalool in H_2O (0.1–1.0 mg/ml) were sonicated (130 W) for 2 min. Freshly disconnected flower heads of *Hoya purpureo-fusca*, *Magnolia liliiflora nigra*, *Robinia pseudoacacia*, and *Philadelphus coronarius* were immersed into the emulsions of the precursors **6a**, **6b**, or **9**. After 24 h, the incubated plants were placed into fresh H_2O , and the emitted volatiles were entrapped on a charcoal filter for another 24 h as described in [9]. Following desorption of the filters with $2 \times 15 \mu\text{l}$ CH_2Cl_2 , the extracts were directly analyzed by GLC/MS.

REFERENCES

- [1] R. Kaiser, 19th International Symposium on Essential Oils and Other Natural Substrates, Greifensee (Switzerland), 1988, Sept. 7–10.
- [2] G. Pattenden, B. C. L. Weedon, *J. Chem. Soc. (C)* **1968**, 1984.
- [3] B. Maurer, A. Hauser, J.-C. Froidevaux, *Tetrahedron Lett.* **1986**, 27, 2111.
- [4] R. Kaiser, EUCHEM 1987, 'Semiochemicals in the Plant and Animal Kingdoms', Angers, France, 1987.
- [5] T. A. van Beek, 19th International Symposium on Essential Oils and Other Natural Substrates, Greifensee (Switzerland), 1988, Sept. 7–10.
- [6] R. Kaiser, D. Lamparsky, *Helv. Chim. Acta* **1984**, 67, 1184.
- [7] D. Arigoni, E. L. Eliel, *Topics Stereochem.* **1969**, 4, 127.
- [8] G. Stork, P. A. Grieco, M. Gregson, *Org. Synth.* **1974**, 54, 68.
- [9] W. Boland, P. Ney, L. Jaenicke, G. Gassmann, in 'Analysis of Volatiles', Ed. P. Schreier, Walter de Gruyter & Co., Berlin–New York, 1984, pp. 371–380.
- [10] L. F. Tietze, *Angew. Chem.* **1983**, 95, 840; *ibid. Int. Ed.* **1983**, 22, 828.