Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 3, pp 351–361, 1996

0968-0896/96 $15.00+0.00

S0968-0896(96)00012-0

The Aphid Sex Pheromone Cyclopentanoids: Synthesis in the Elucidation of Structure and Biosynthetic Pathways

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Abstract—Identification of a range of aphid sex pheromones as comprising the cyclopentanoids (4a*S*,7*S*,7a*R*)-nepetalactone, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol and the (1*S*)- and (1*R*,4a*R*,7*S*,7a*S*)-nepetalactols required samples authenticated by ^{1}H and ^{13}C NMR. These and related compounds were provided by small scale synthesis and extraction from plants in the genus *Nepeta* (Lamiaceae). The subsequent discovery that the synthetic sex pheromones could attract males, and also parasitic wasps that attack aphids, has created a need for large scale syntheses of the cyclopentanoids. This is afforded by cyclisation of the 8-oxo-1-enamine of citronellal as originally developed by Schreiber and co-workers (1986). Investigation into the biosynthesis of the cyclopentanoids by plants for exploiting aphid sex pheromones in crop protection by means of molecular biology required synthesis of putative biosynthetic intermediates, some with radioactive isotopic labelling, particularly 8-oxidised monoterpene alcohols and aldehydes. Copyright © 1996 Elsevier Science Ltd

Introduction

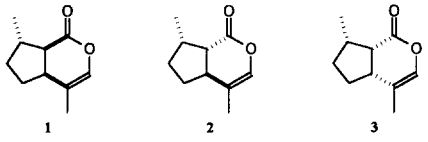
Aphids (Homoptera: Aphididae) are important pests of arable and horticultural crops, particularly in temperate regions, and can devastate protected crops. Aphids also occupy important positions within natural and semi-natural ecosystems. Sexual reproduction is on the winter (primary) host, usually a woody species upon which they originally evolved. However, their main role as pests is on the summer (secondary) host, usually an herbaceous plant which they subsequently evolved to colonise, thereby making use of the rapid growth in the spring and summer of annual or biennial plants. As autumn approaches, the asexually reproducing aphids on the summer host respond to the reduced daylight hours by producing winged sexual female precursors, termed 'gynoparae', which migrate to the winter host, facilitated by volatile semiochemicals (behaviour-controlling chemicals) released by these plants.[1,2] Wingless female aphids, 'oviparae', are then produced on the winter host and release sex pheromones from their hind legs.[3–6] The pheromone is detected by male aphids using the secondary rhinaria, which are organs on the third segment of the six-segmented antenna. The role of the aphid sex pheromone was considered to be no more than a close range aphrodisiac by various entomologists, including Steffan.[7] Nonetheless, Nault and Montgomery[8] suggested that aphid sex pheromones should be identified and synthesised, in order to investigate their true role in aphid chemical ecology. In the journal *Nature*[9] the first chemical characterisation of an aphid sex pheromone was published for the vetch aphid, *Megoura viciae*, as comprising two cyclopentanoids, a nepetalactone and a nepetalactol, with three and four asymmetric carbons, respectively.[9] This species of aphid was chosen for its relatively large size and for the ease with which the sexual forms could be created by artificial regulation of the day/night cycle. The characterisation of the sex pheromone was facilitated by the development of electrophysiological recording techniques so that individual olfactory nerve cells within the secondary rhinaria of the male insects could be located and nerve impulses recorded by the insertion of tungsten electrodes, finely sharpened by electrolysis.[10] The biologically active components of the pheromone, obtained from the hind legs of the females, were located on capillary column GC by coupling the effluent to the electrophysiological preparation and, simultaneously, to a flame ionisation detector.[11,12] After subsequent GC–MS, tentative identifications were made. However, since the aphids produced the pheromone at sub-nanogram levels, confirmation of structures was by comparison with materials obtained from plant sources and partial synthesis with authentication of structure by nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy. The availability of synthetic cyclopentanoid aphid sex pheromone components, and related compounds, has allowed the sex pheromones of a range of pest, and ecologically significant, aphids to be identified.[13–15] In-depth behavioural studies conducted in the laboratory and in the field then demonstrated relatively long-range attraction by the synthetic pheromones.[16–18] Furthermore, the availability of synthetic sex pheromone components allowed the discovery that certain wasps (Hymenoptera: Braconidae) parasitising aphids employ the sex pheromones in locating their aphid hosts.[1,19,20] The promise of the aphid sex pheromones for alternative methods of pest control not exclusively reliant upon conventional aphicides, also incorporating the ability to attract beneficial parasitic wasps by means

of the sex pheromones, has created a need for large-scale stereochemically controlled synthesis of the sex pheromone cyclopentanoids in extension of the methods employed for analytical purposes.

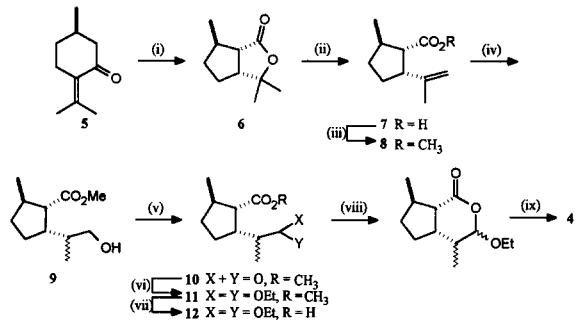
Since the aphid sex pheromone cyclopentanoids, or precursors, can be biosynthesised in plants, the associated genetics could be exploited by creating transgenic microorganisms which would produce the cyclopentanoids in fermentors. It may also be possible to produce new higher plant cultivars, producing cyclopentanoids, by genetic modification, which could be grown as companion crops to attract aphids from the harvestable crop or to attract the parasitic wasps into the crop plant canopy. The synthesis of putative cyclopentanoid biosynthetic precursors is playing an important role in elucidating the pathways to these compounds in certain plants for isolation of the associated genes.[21] Furthermore, synthesis has played an important role in confirming tentatively identified products from enzymes generated by molecular biological techniques based on gene sequence homology.[22] Here, the synthesis of cyclopentanoids, related compounds and putative biosynthetic precursors in the elucidation of aphid sex pheromone component structure and associated biosynthetic pathways is described.

Results and Discussion

GC single-cell recording (SCR) and GC–MS studies with the vetch aphid, *M. viciae*, showed the sex pheromone to comprise two components. The first identified was the (4a*S*,7*S*,7a*R*)-nepetalactone (*cis,trans*) (**1**). This compound was isolated from the catmint *Nepeta cataria* (Labiatae = Lamiaceae) by HPLC and characterised by high-field ^1H and ^{13}C NMR.[9] This compound was as active electrophysiologically as aphid-derived material, the 7*S-trans,cis* isomer **2**, also from *N. cataria*, was only weakly active and the 7*S-cis,cis* isomer **3** from *Nepeta racemosa* (=*mussinii*) inactive. However, whereas the total aphid leg extract attracted male aphids in the Pettersson olfactometer, used in assessment of behaviour in the laboratory, the electrophysiologically active *cis,trans* compound **1** did not. Since all of these structures had the same (*S*) stereochemistry at carbon 7 it was possible that behavioural activity was absent because the aphid was employing the (7*R*) stereochemistry.

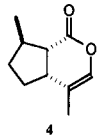


The compound with the (7*R*) stereochemistry, (4a*R*,7*R*,7a*S*)-nepetalactone (*cis,trans*) (**4**), was synthesised from (*R*)-pulegone (**5**). The procedure (Scheme 1) was based on that of Wolinsky[23] and employed the



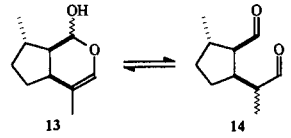
Scheme 1. (i) (a) Br_2; (b) NaOMe, MeOH; (c) aq KOH, heat; (ii) Bu^{t}OK, DMF, 140 °C, 3 h; (iii) CH_2N_2; (iv) (a) diisoamylborane; (b) NaOH, H_2O_2; (v) PCC; (vi) $HC(OEt)_3$; TsOH, EtOH; (vii) KOH, aq EtOH; (viii) 100 °C; (ix) 300 °C.

Favorsky rearrangement to give puleganolide (**6**). The butoxide-promoted ring opening giving **7**, esterification to **8** with diazomethane, then hydroboration of the exocyclic olefin **8** to give **9** after oxidation, preceded oxidation to the aldehyde **10**. Protection of the aldehyde as the diethyl acetal **11** allowed hydrolysis of the ester to give the acetal acid **12**. Stepwise elimination of ethanol at 100 °C and then at 300 °C under vacuum gave the required (4a*R*,7*R*,7a*S*)-nepetalactone (**4**), and the structure confirmed by NMR, MS and GC retention data. Although this synthetic route played an important role in the initial structure determination studies, the procedure remained unoptimised as the methods discussed later were favoured. Compound **4** gave no SCR response at physiologically relevant levels where a strong response was obtained for the (4a*S*,7*S*,7a*R*)-nepetalactone (**1**). Thus, it was concluded that, although the enantiomer **1** was indeed a component of the sex pheromone, for behavioural activity the other component detected by GC–SCR was required.



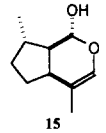
The mass spectrum of the second pheromonal component showed a parent ion higher by two atomic mass units. The corresponding (4a*S*,7*S*,7a*R*)-lactol (**13**) was initially discounted, since the GC retention time was less than for the lactone **1**, in spite of a higher molecular weight and increased polarity. Also, it was reported in the literature[24,25] that such a lactol would be in equilibrium with the ring-opened dialdehyde, an iridodial **14**, and no trace of such could be detected at the considerably shorter retention times found for authentic iridodials.[9] Prominently abundant ions at *m/z* 58, arising from McLafferty rearrangement of the α-methylaldehyde system, typify the iridodials, whereas in the spectrum of the compound from the aphid, although present, this ion was relatively much less abundant.[9] Since this aspect of the spectrum could be explained by conversion to an iridodial during ionisa-

tion, the lactol structure was reconsidered. Accordingly, the nepetalactone **1** was reduced by DIBAL-H to give the nepetalactol **13** and comprised a 9:1 mixture of the diastereomers arising from the asymmetry at carbon 1, but which could only be separated on GC as the acetates. Since only one biosynthetic pathway was expected in the aphid, then the stereochemistry for the aphid lactol would be as for structure **13**, i.e. 4a*S*,7*S*,7a*R* (*cis,trans*). The stereochemistry of the main lactol diastereomer was established, by X-ray crystallography of the 3,5-dinitrobenzoate, as (1*R*) (**15**).[14]



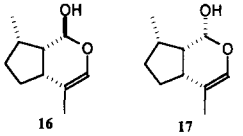
13 14

The (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**15**) was shown by GC, GC–MS, electrophysiological and behavioural studies to comprise the other component of the pheromone, although again the nepetalactol **13** was not behaviourally active alone. When the two compounds **1** and **13** were combined and tested in the Pettersson olfactometer at the same level as the natural pheromone components, there was no statistically significant difference between the attractiveness of the natural and synthetic mixtures. In accordance with studies on the natural pheromone, no iridodials could be detected in samples of synthetic **13**. In addition, the lactol derived from the *cis,cis* nepetalactone **3** by DIBAL-H reduction was stable against conversion to iridodials and was shown to comprise the (1*S*) (**16**) and the (1*R*) (**17**) isomers in 7:3 ratio. Similar reduction of the *trans*-fused lactone **2** gave the ring-opened iridodial products. This was in agreement with the literature, but where presumably the high stability of the *cis*-fused lactols against ring opening had not been encountered.



15

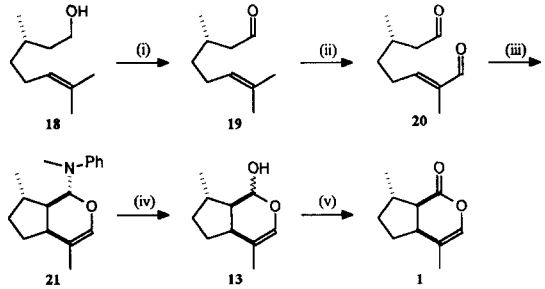
The elucidation of the various cyclopentanoid structures by synthetic and semi-synthetic approaches based on natural product availability has so far allowed the identification of the sex pheromones for a further 15 species as principally comprising the lactone **1** and the lactol **15**. However, aphids occupying similar ecosystems, for example the black bean aphid *Aphis fabae* and the pea aphid *Acyrthosiphon pisum* both feeding on legumes (Fabaceae = Leguminosae), have different proportions, with *A. fabae* having a substantially higher proportion of lactone **1** and *A. pisum* a higher proportion of the lactol **15**.[15] Other aphids showing, like *A. fabae*, a higher proportion of the lactone are the grain aphid *Sitobion avenae*,[18] the blackberry-cereal aphid *Sitobian fragariae*,[17] the raspberry aphid *Amphorophora idaei* (personal communication, L. J. Wadhams), the cabbage aphid *Brevicoryne brassicae*[20] and the *Tuberocephalus momonis* (personal communication, L. J. Wadhams). Aphids showing a higher proportion of lactol **15**, as for *A. pisum*, are the rosy apple aphid *Dysaphis plantaginea* (personal communication, L. J. Wadhams), the bird-cherry-oat aphid *Rhopalosiphum padi* (personal communication, L. J. Wadhams), two species of *Cryptomyzus*,[26] the potato aphid *Macrosiphum euphorbiae* (personal communication, L.J. Wadhams) and the greenbug *Schizaphis graminum*, in which there was evidence that lactone **1** could inhibit the attractiveness of the lactol **15**.[13] The major aphid pest as a virus vector, the peach-potato aphid *Myzus persicae*, contained very low but similar amounts of the two cyclopentanoids **1** and **15**. The damson-hop aphid *Phorodon humuli* contained neither compound **1** nor **13**, but a mixture of the two diastereomeric nepetalactols, **16** and **17**.[16]



16 17

Field studies using water traps with lures comprising **16** and **17** at the ratio obtained directly by DIBAL-H reduction of the lactone **3** caught thousands of male *P. humuli* against only tens in the control traps, and far more than conventional suction traps which process large volumes of air.[16] Thus, it was demonstrated for the first time that aphid sex pheromones could be involved in long-range attraction. Since then, field studies have been successful in attracting other aphid species, e.g. *S. fragariae*,[17] *B. brassicae*[20] and *R. padi* (personal communication, J. Hardie).

These discoveries and the further important development involving the attraction of the parasitic wasps that attack aphids has created a demand for larger scale synthesis of the aphid sex pheromone cyclopentanoids.[1,19,20] In order to make the cyclopentanoids on a larger scale, new synthetic approaches were sought and a method based on that of Schreiber (Scheme 2)



18 19 20

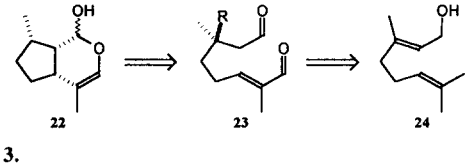
21 13 1

Scheme 2. (i) pyr.SO_3 complex, DMSO, Et_3N; (ii) SeO_2, ^tBuOOH, (iii) MeNHPh, 4 Å sieves; (iv) TsOH, THF: H_2O (1:1); (v) $AgNO_3$, Celite, refluxing benzene.

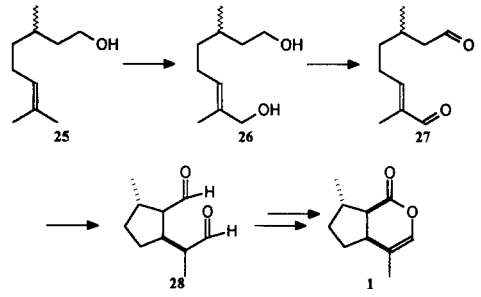
adapted to the needs of this programme.[27] By analogy with the reaction of vinyl ketones with enol ethers, Stork[28] has shown that addition of aldehyde enamines to α,β-unsaturated ketones by a Diels–Alder type reaction gives α-substituted α,β-unsaturated cyclohexenones. This method has been adapted by Schreiber for [4+2] cycloaddition of aldehyde enamines with α,β-unsaturated aldehydes to afford dihydropyrans. Here, (*S*)-citronellol (**18**) was oxidised to the aldehyde **19**, and further oxidised by selenium dioxide to yield 8-oxocitronellal (**20**). Treatment with *N*-methylaniline produced the enamine, which underwent [4+2] cycloaddition to **21**. Hydrolysis of the amine produced the *cis,trans* nepetalactol **13**, which was oxidised to the *cis,trans* nepetalactone **1**. The synthesis was conducted on a relatively large scale, i.e. 30 g of the starting material **18**, to produce quantities required for field trials, but it was noted that yields for individual reactions were lower than those quoted by Schreiber on a smaller scale, i.e. 60 mg of **18**.

The necessary (*S*) stereochemistry is provided by commercially available (*S*)-citronellol (**18**). However, various levels of enantiomeric purity are available: 100, 95 and 50% (*S*), at reducing costs. Field studies (personal communication, J. Hardie) on synthetic (4a*S*,7*S*,7a*R*)-nepetalactone (**1**), obtained from the three grades of (*S*)-citronellol (**18**), showed that there was a significant diminution in activity from 100% enantiomeric purity to approximately half at 95% and less than a third at 50%. However, the synthesised lactone **1** with the highest purity was significantly more active than the plant-derived lactone **1**, possibly because the synthetic material was completely free from other plant components, which can themselves have some repellent activity (personal communication, L. J. Wadhams).

The *cis,cis* nepetalactone **3** necessary for further development of sex pheromone-based control of *P. humuli* is not directly available from the Schreiber synthetic approach since the (*S*) stereochemistry of the pro-7-methyl group, in the case of the route described from (*S*)-citronellol (**18**), directs the stereochemistry at the pro-4a and -7a positions, relatively *trans* [i.e. in the case of (7*S*) stereochemistry, (4a*S*) and (7a*R*)]. However, if a more bulky group could be placed at pro-7-carbon in place of the hydrogen, this could direct the stereochemistry *cis* relative to the methyl group, i.e. (4a*R*,7a*S*). Accordingly, a disconnection scheme is proposed to the diastereomeric mixture **22** of the lactols **16** and **17** (Scheme 3). Here, sharpless asymmetric epoxidation of an appropriate derivative of geraniol (**24**), followed by selective ring opening would allow introduction of functionality at the pro-7



Scheme 3.

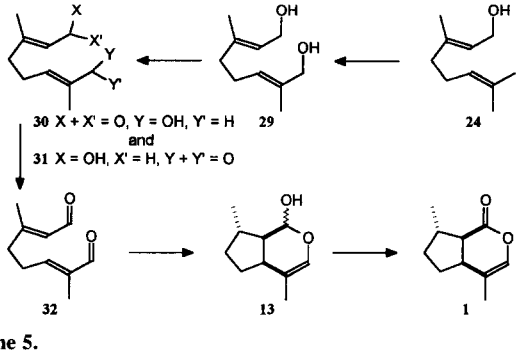


Scheme 4.

position. Conversion of this to the dialdehyde (**23**) (R = OH) would provide a suitable substrate for cyclisation using Schreiber's approach. Later removal of the R functionality, i.e. —OH or derivative, would yield the desired lactol **22**.

The biosynthetic pathways in plants to the cyclopentanoids studied here have not been fully elucidated, but two routes have been proposed. For *N. cataria*, Bellesia et al.[29] suggested citronellol (**25**), without definition of stereochemistry, as the initial substrate (Scheme 4). Oxidation of the citronellol (**25**) to the 8-hydroxy compound **26** followed by further oxidation to the dialdehyde **27** and subsequent generation of the intermediate anionic in the pro-7a position would then allow cyclisation to the iridodial **28**. This could be oxidised and cyclised further to the lactone **1** found predominantly in this plant. Radiolabeled citronellol (**25**) was incorporated in these studies, whereas geraniol (**24**) and its (*Z*) isomer, nerol, were not. However, work on *Rauwolfia serpentina* (Apocynaceae) suggested both geraniol and nerol as substrates for the cyclopentanoid system in this plant (Scheme 5).[30] In this work, geraniol (**24**) is also oxidised to the 8-hydroxy compound **29**, which is then sequentially oxidised via either the 8-hydroxygeranial (**30**) or 8-oxogeraniol (**31**) to the dialdehyde **32**. The dialdehyde **32** is then required to be reductively cyclised to the lactol **13** before oxidation to the lactone **1**, although this is unlikely to involve a concerted mechanism.

In the studies described here, *N. racemosa* was chosen, since the predominant lactone has the *cis,cis* structure **3** of the *P. humuli* sex pheromone but which has so far

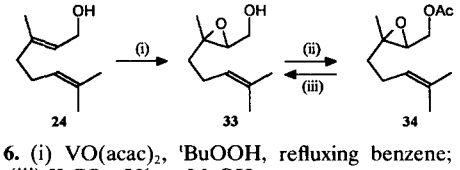


Scheme 5.

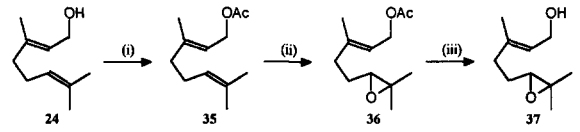
eluded convenient synthesis. Putative starting materials based on both geraniol **24** and nerol, i.e. (*E*) and (*Z*) stereochemistry, have been used and the proposed route is exemplified for the former. Synthesis of 1-[^{3}H]-geraniol facilitated isolation of a microsomal fraction that contained a specific cytochrome *P*450, which catalysed oxidation of this substrate. This was achieved by oxidation of geraniol (**24**) to geranial using active manganese dioxide followed by sodium boro-[^{3}H]hydride reduction.[31] The product was confirmed as 8-hydroxygeraniol (**29**) by comparison with authentic material.[31]

The next step was to attempt isolation of the gene coding for the cytochrome *P*450 causing specific oxidation to the 8-hydroxygeraniol (**29**). In order to further this, cytochromes *P*450 from other plant systems were investigated and one, isolated from avocado, *Persea americana* (Lauraceae), showed activity with both nerol and geraniol.[32] A preparation of this enzyme was therefore obtained and the products investigated by GC–MS. Although these products had structural features giving rise to similarity with the spectra for 8-hydroxy monoterpene alcohols, there were substantial differences, suggesting that they were monoterpene alcohol epoxides, as encountered previously.[33] However, for complete characterisation, the full range of monoepoxides was required as synthetic authenticated samples, i.e. the 2,3-epoxygeraniol (**33**), the 2,3-epoxynerol, the 6,7-epoxygeraniol (**37**) and the 6,7-epoxynerol.

The preparation of 2,3-epoxygeraniol (**33**) is shown (Scheme 6) with the same approach for 2,3-epoxynerol. Geraniol (**24**) was selectively epoxidised to its 2,3-epoxide by treatment with vanadyl acetylacetonate and *t*-butyl hydroperoxide but purification of epoxide **33** by distillation of the crude mixture was not possible because of thermal decomposition. However, the mixture was acetylated under standard conditions to afford acetate **34**, which could be purified without decomposition. Subsequent deacetylation provided the desired epoxide **33** in good purity for GC–MS analysis. The two 2,3-epoxy compounds, i.e. **33** and the isomer, typically contained abundant ions at *m/z* 69 from the allylic cleavage of the parent ion to give 3,3-dimethylallyl radical ion, and abundant ions at *m/z* 82, possibly from a McLafferty type rearrangement involving the epoxide.



Scheme 6. (i) $VO(acac)_2$, ^tBuOOH, refluxing benzene; (ii) Ac_2O, pyridine; (iii) K_2CO_3, 5% aq MeOH.

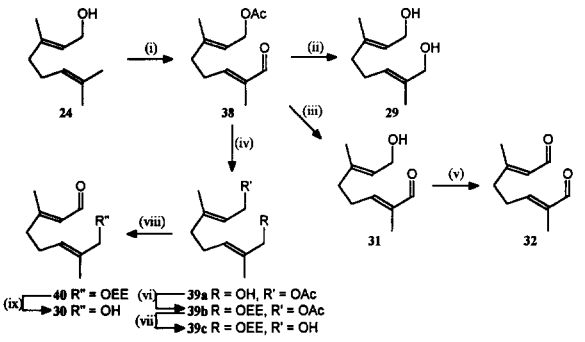


Scheme 7. (i) Ac_2O, pyridine, 4-DMAP; (ii) mCPBA, $NaHJCO_3$; (iii) K_2CO_3, 5% aq MeOH.

The preparation of 6,7-epoxygeraniol (**37**) is shown (Scheme 7) with the same approach for 6,7-epoxynerol. Deactivation of the 2,3-double bond of geraniol was achieved through acetylation of the alcohol to afford the acetate **35**. Treatment of **35** with 3-chloroperoxybenzoic acid and sodium bicarbonate allowed exclusive epoxidation of the 6,7-double bond to generate epoxide **36**. Subsequent deacetylation provided the desired epoxide **37** in good purity for GC–MS analysis. The two 6,7-epoxy compounds, i.e. **37** and the isomer, showed abundant ions at *m/z* 85, arising also from allylic cleavage of the fragment incorporating the hydroxy group and abundant ions at *m/z* 59 resulting, presumably, from rearrangement of the epoxide to give the tertiary alcohol at the *gem*-dimethyl group.

The two epoxides **33** and **37** derived from geraniol (**24**) and the corresponding two from nerol were confirmed as being the main oxidation products from the *P. americana* *P*450 system treated with geraniol and nerol respectively, thereby demonstrating the value of synthesis in elucidating the structures of products from enzymic systems. Although significant sequence homology[22] was found between the cytochromes *P*450 from *P. americana* and the *N. racemosa*, oxidation by the *P. americana* cytochrome *P*450 to the 2,3- and 6,7-epoxides (**33** and **37**), and isomers is a relatively trivial reaction similar to that obtained by aerial oxidation,[33] whereas the *N. racemosa* cytochrome *P*450 caused highly specific oxidation to the 8-hydroxygeraniol (**29**), -nerol and -citronellol (**26**).

The next step, involving the oxidation of the 8-hydroxy terpene alcohols, was investigated initially by preparing 8-[^{3}H]-8-hydroxygeraniol and the corresponding nerol and citronellol. This work facilitated purification to homogeneity of an $NADP^+$ oxidoreductase from *N. racemosa*, responsible for oxidising these substrates at both carbons 1 and 8 to the corresponding aldehydes. In order to characterise the products, authentic 8-oxogeraniol (**31**), 8-hydroxygeranial (**30**) and 8-oxogeranial (**32**) were prepared, together with the analogous products relating to nerol and citronellol (Scheme 8). However, no substrate specificity could be clearly determined between these three substrate types, nor was it possible to determine the exact route, whether 8 or 1 oxidation took place first.[34] Nonetheless, the partial enzyme characterisations ensuing from the synthesis studies reported here are providing sufficient information for direct molecular genetic studies and



Scheme 8. (i) (a) Ac_2O, pyridine, 4-DMAP; (b) SeO_2, ^tBuOOH; (ii) $LiAlH_4$, (iii) K_2CO_3, 5% aq MeOH; (iv) $NaBH_4$; (v) MnO_2 (active); (vi) ethyl vinyl ether, TsOH; (vii) K_2CO_3, 5% aq MeOH; (viii) MnO_2 (active); (ix) MeOH, TsOH.

subsequent cloning and over-expression of the genes associated with these enzymes.

Studies with the 1,8-monoterpene dialdehydes have so far not allowed isolation of any cyclase activity from various of the target plant species. However, further studies on the composition of aphid sex pheromones has indicated that, for the species *S. fragariae*, *D. plantaginea*, *R. padi*, *P. humuli*, *Cryptomyzus maudamantia*, *A. pisum* and *M. persicae*, citronellol (**25**) (absolute stereochemistry not yet established), although electrophysiologically and behaviourally inactive, accompanies the cyclopentanoid sex pheromone components. Thus, it is suggested that citronellol (**25**) may be the precursor for the cyclopentanoids biosynthesised in aphid sex pheromones. Current feeding studies and direct injection and topical treatment with ^{13}C labeled substrates is facilitating investigation of this putative biosynthetic route by direct application of GC–MS.

Experimental

General

Spectroscopic techniques. NMR spectra were recorded in deuterochloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal standard (δ 0.0 ppm) using a JEOL GNX 400 spectrometer at 400 MHz for ^1H spectra and at 100 MHz for ^{13}C spectra. Some ^1H spectra were recorded on a JEOL PMX 60 MHz spectrometer and are specified as ^1H NMR (60 MHz).

Mass spectra were obtained using a 70–250 (VG Analytical, Manchester) 70 eV mass spectrophotometer.

GC–MS was carried out using the following equipment and conditions. GC: Hewlett Packard 5890 GC, with a cold on-column injector. Column: 30 m, 320 μm i.d., DB-1 (J.&W. Scientific, Folsom). Temperature programme: 30 °C for 5 min, 5 °C/min to 250 °C, isothermal. MS: directly coupled 70–250 (VG Analytical, Manchester), 70 eV, 250 °C.

MS data are expressed as ion *m/z* [M^+ (molecular ion), % of base peak] followed by the eight most intense ions *m/z* (% base peak).

Polarimetry measurements were obtained using a Bendix-NPL polarimeter, Type 143. All measurements were made at 23 °C.

Chromatographic and purification techniques. TLC was carried out using prepared plates (Merck, D.C. Kieselgel 60 F254) of 0.2 mm thickness on plastic sheets.

'Flash' chromatography was carried out using Merck Kieselgel 60 with a variety of solvent systems.

Solvents and reagents. Solvent extracts were dried using anhydrous magnesium sulfate. All solvent evaporations were carried out using a Büchi rotary evaporator under water pump reduced pressure. The following solvents were dried and/or distilled before use: Tetrahydrofuran (THF) — heated at reflux and distilled from sodium and benzophenone. Dimethylformamide (DMF) — heated at reflux and distilled from sodium and benzophenone. Dichloromethane — stored over molecular sieves. Diethyl ether — stored over sodium wire. All other solvents were used as supplied except when specified.

(1*S*,2*S*,5*R*)-Methyl-2-isopropenyl-5-methylcyclopentane-1-carboxylate (8) (methyl isopulegenate). *trans*-Puleganolide (**6**) (5.0 g, 0.03 mol) in dimethyl formamide (10 mL) was added to a suspension of freshly sublimed potassium *t*-butoxide (3.75 g, 0.33 mol) in DMF (10 mL) at 125 °C under nitrogen. The reaction mixture was then kept at 140 °C for 3 h, then allowed to cool, treated with ice-water (100 mL) and extracted with diethyl ether (100 mL). The aqueous phase was acidified with acetic acid and extracted with diethyl ether (100 mL). The diethyl ether was washed with water, dried and concentrated to leave a yellow oil (4.4 g, 88%) which, when analysed by ^1H NMR spectroscopy, showed the required isomer **7** in 90% purity. ^1H NMR (60 MHz): δ 1.10 (d, 3H), 1.30–3.00 (m, 7H), 1.76 (s, 3H), 4.70 (s, 2H), 11.65 (s, 1H). Compound **7** was methylated quantitatively using diazomethane in ether to give **8** (4.8 g, 100%). ^1H NMR: δ 1.06 (d, 3H), 1.30–3.00 (m, 7H), 1.72 (s, 3H), 3.51 (s, 3H), 4.64 (s, 2H).

(1*S*,2*S*,5*R*)-Methyl 2-(1-methyl-2-hydroxyeth-1-yl)-5-methylcyclopentane-1-carboxylate (9). 2-Methyl-2-butene (4.1 g, 0.058 mol) was added dropwise to borane–methylsulfide (2.0 M) in diethyl ether (13.5 mL, 0.027 mol) at 0 °C and maintained at 0 °C for 2 h. Then methyl isopulegenate (5.0 g, 90%, 0.025 mol) was added and the reaction allowed to reach room temperature and left for 3 h. The borane was decomposed by simultaneous dropwise addition of solutions of sodium hydroxide (1.1 g) in 50% aqueous ethanol

(11 mL), and hydrogen peroxide (50%, 5.5 mL) in water (5.5 mL) maintaining the temperature below 25 °C. Stirring was continued for a further 3 h. Diethyl ether (50 mL) and water (50 mL) were added and the mixture partitioned. The organic phase was washed with water (2 × 50 mL), dried, concentrated and the residue chromatographed on Florisil (100 g) eluting with increasing proportions of diethyl ether in petroleum ether. The appropriate fractions were combined and concentrated to leave a clear gum (2.0 g, 40%). ^{1}H NMR (60 MHz): δ 1.00 (m, 6H), 1.30–2.60 (m, 8H), 3.06 (br s, 1H), 3.48 (m, 2H), 3.60 (s, 3H).

(1*S*,2*S*,5*R*)-Methyl 2-(1-methyl-2-oxoeth-1-yl)-5-methylcyclopentane-1-carboxylate (10). The ester–alcohol **9** (2.0 g, 0.01 mol) was added to a well-stirred suspension of pyridinium chlorochromate (3.0 g, 0.015 mol) in dichloromethane (40 mL). Stirring was continued for a further 2 h. Diethyl ether (40 mL) was added and the solvent decanted from the insoluble materials and passed through a short Florisil column. After concentration of the eluate, a colourless oily product remained (1.5 g, 74%). ^{1}H NMR (60 MHz): δ 1.10 (m, 6H), 1.30–2.60 (m, 8H), 3.64 (s, 3H), 9.58 (d, 1H).

(1*S*,2*S*,5*R*)-Methyl 2-(1-methyl-2,2-diethoxyeth-1-yl)-5-methylcyclopentane-1-carboxylate (11). The ester–aldehyde **10** (1.5 g, 0.0076 mol) was added to triethyl orthoformate (1.5 g, 0.01 mol) in ethanol (20 mL) with a trace of *p*-toluenesulfonic acid monohydrate (10 mg) and left at room temperature for 3 h. Saturated sodium bicarbonate solution (10 mL) was added and the mixture partitioned by the addition of hexane (50 mL) and water (100 mL). The organic phase was washed with water, dried and concentrated to leave a clear product as an oil (1.6 g, 77%). ^{1}H NMR (60 MHz): δ 0.80–1.40 (m, 12H), 1.50–2.60 (m, 8H), 3.20–3.70 (m, 4H), 3.60 (s, 3H), 4.21 (d, 1H).

(1*S*,2*S*,5*R*)-2-(1-Methyl-2,2-diethoxyeth-1-yl)-5-methylcyclopentane-1-carboxylic acid (12). The ester–diethyl acetal **11** (0.2 g, 0.73 mmol) was heated to reflux for 2 h in ethanol (5 mL), water (1 mL) and potassium hydroxide (0.2 g). The reaction mixture was diluted with water (20 mL) and extracted with hexane (20 mL). The aqueous phase was acidified with acetic acid (0.2 g) and extracted with diethyl ether (50 mL). The diethyl ether layer was washed with water (50 mL), dried and concentrated to leave a colourless gum (0.11 g, 58%). ^{1}H NMR (60 MHz): δ 0.80–1.20 (m, 12H), 1.40–2.60 (m, 8H), 3.20–3.70 (m, 4H), 4.22 (d, 1H), 11.50 (s, 1H).

(4a*R*,7*R*,7a*S*)-Nepetalactone (4). The acid acetal **12** (0.11 g) was heated at 100 °C for 0.25 h under nitrogen. Analysis of the ^{1}H NMR spectrum indicated cyclisation of the starting material consistent with two diastereomeric products, confirmed by GC analysis in near equal ratio. ^{1}H NMR (60 MHz): δ 0.90–1.40 (m, 9H), 1.40–2.60 (m, 8H), 3.64 (m, 2H), 4.90 (m, 1H). The ethoxydihydronepetalactone (0.1 g) was sealed in a glass ampoule under vacuum of 1.0 torr. The ampoule was then heated at 300 °C for 1 h. The mixture was purified on Florisil (20 g) eluting with increasing proportions of diethyl ether in hexane and the fractions monitored by TLC. GC–MS revealed the required compound to be the major component. ^{1}H NMR data were in agreement with those of the natural material. MS: *m/z* 166 (M^+, 61%), 123 (70), 109 (41), 95 (59), 81 (81), 69 (100), 55 (35), 41 (74).

(3*S*)-3,7-Dimethyl-6-octenal (19) [(*S*)-citronellal]. Triethylamine (66.4 mL, 0.5 mol) was added dropwise to a cooled (ice bath), stirred solution of (*S*)-citronellol (**18**) (15.6 g, 0.1 mol) in dry dichloromethane (750 mL) under nitrogen atmosphere. A solution of sulfur trioxide–pyridine complex (47.7 g, 0.3 mol) in dimethyl sulfoxide (156 mL, 2.2 mol) was added dropwise to the citronellol solution and the resultant mixture was stirred for 15 h. Repeated dilution of the reaction mixture with petroleum ether (60–80 fraction) and concentration allowed careful removal of the dichloromethane solvent. The solution was further diluted with petroleum ether (250 mL) and washed with distilled water (6 × 350 mL), then dried and concentrated to give **19** (15.0 g, 97%) in high purity. ^{1}H NMR: δ 0.97 (d, 3H, J = 7 Hz), 1.19–1.26 (m, 1H), 1.26 (m, 1H), 1.60 (s, 3H), 1.68 (d, 3H, J = 1 Hz), 1.94–2.12 (m, 3H), 2.14–2.27 (m, 1H), 2.37–2.44 (m, 1H), 5.09 (m, 1H), 9.75 (t, 1H, J = 2 Hz). ^{13}C NMR: δ 17.5, 19.7, 25.3, 25.5, 27.6, 36.8, 50.8, 123.9, 131.5, 202.7.

(2*E*,6*S*)-2,6-Dimethyl-2-octenedial (20) [(*S*)-8-oxocitronellal]. (*S*)-Citronellal (**19**) (20 g, 0.13 mol), selenium dioxide (1.44 g, 0.013 mol) and dry *t*-butyl hydroperoxide (6.0 M in isooctane; 43.3 mL, 0.26 mol) were stirred in dry dichloromethane (350 mL) for 48 h. Saturated sodium thiosulfate solution (200 mL) was added and the reaction mixture stirred for 15 min. The organic phase was removed and washed with saturated sodium bicarbonate solution, brine, then dried and concentrated. The product was purified by flash column chromatography, eluting with a gradient of petroleum ether and diethyl ether to give **20** (9.4 g, 43%). ^{1}H NMR: δ 1.02 (d, 3H, J = 7 Hz), 1.40–1.49 (m, 1H), 1.54–1.62 (m, 1H), 1.74 (br s, 3H), 2.12–2.17 (m, 1H), 2.31–2.51 (m, 4H), 6.51 (t, 1H, J = 7 Hz), 9.40 (s, 1H), 9.78 (t, 1H, J = 2 Hz). ^{13}C NMR: δ 8.8, 19.3, 26.1, 27.3, 34.8, 50.4, 139.1, 153.7, 194.8, 201.8.

(2*S*,9*S*)-5,9-Dimethyl-2(*N*-methylphenylamino)-3-oxabicyclo[4.3.0]-4-nonene (21). To a solution of (*S*)-8-oxocitronellal (**20**) (3.7 g, 0.022 mol) in dry diethyl ether (125 mL) was added freshly activated 4A molecular sieves (8–12 mesh, 40 g). After stirring for 10 min, *N*-methylaniline (2.39 mL, 0.022 mol) was added and the reaction mixture was stirred for 10 h. Filtration of the mixture through a plug of Celite afforded a solution which was concentrated to give a yellow oil. The product was purified by flash column chromatography, eluting with a gradient of petroleum ether and diethyl ether to afford **21** (3.8 g, 67%). ^{1}H NMR: δ 1.07 (d, 1H, J = 7 Hz), 1.08–1.28 (m, 2H), 1.61 (t, 3H,

$J=1$ Hz), 1.83–1.90 (m, 2H), 2.05–2.13 (m, 2H), 2.37 (m, 1H), 2.96 (s, 3H), 4.77 (d, 1H, $J=11$ Hz), 6.22 (br s, 1H), 6.80–7.26 (m, 5H). ^{13}C NMR: δ 17.0, 21.5, 32.7, 33.2, 33.7, 36.5, 42.3, 45.5, 88.4, 113.0, 115.9, 119.2, 129.0, 137.7, 150.7.

(1*R,4a*S*,7*S*,7a*R*)-Nepetalactol (13).** To a solution of **21** (3.2 g, 0.0124 mol) in tetrahydrofuran/distilled water 1:1 (125 mL) was added *p*-toluenesulfonic acid monohydrate (2.5 g, 0.0132 mol). The mixture was stirred for 1 h and then quenched with saturated sodium bicarbonate solution (75 mL). The tetrahydrofuran solvent was removed under reduced pressure and the residue was diluted with distilled water and extracted with diethyl ether (3 × 75 mL). The organic extracts were dried and concentrated to give a yellow oil. The product was purified by flash column chromatography, eluting with a gradient of petroleum ether and diethyl ether to afford **13** as a 9:1 mixture of 1-OH isomers (1.8 g, 86%); $[\alpha]_D -41.5°$ (*c* 2.1, ethanol). ^{1}H NMR: δ 1.08 (d, 3H, $J=6$ Hz), 1.10–1.19 (m, 1H), 1.24–1.37 (m, 1H), 1.55 (br s, 3H), 1.58–1.77 (m, 1H), 1.80–2.00 (m, 3H), 2.41–2.47 (m, 1H), 4.31 (d, 1H, OH, $J=6$ Hz), 4.82 (t, 1H, $J=6$ Hz), 6.0 (br s, 1H). ^{13}C NMR (major isomer): δ 16.2, 20.5, 30.8, 33.2, 35.7, 38.8, 50.2, 94.3, 113.5, 134.1.

(4a*S*,7*S*,7a*R*)-Nepetalactone (1). The lactol **13** (1.8 g, 0.0107 mol) was added to a well-stirred suspension of silver carbonate (14 g, 0.0508 mol) and Celite (9.4 g) in dry benzene (100 mL). The mixture was heated at reflux for 2 h, then cooled and filtered over magnesium sulfate. The filtrate was concentrated to give a pale oil. The product was purified by flash column chromatography, eluting with a gradient of petroleum ether and diethyl ether to give **1** (1.1 g, 60%); $[\alpha]_D +18.3°$ (*c* 1.25, ethanol). The ^{1}H and ^{13}C NMR data for **1** were in agreement with those of the natural nepetalactone.

(2*R,3*R**)-Epoxy-3,7-dimethyl-6-octen-1-ol (33) (2,3-epoxygeraniol).** To a solution of geraniol (20 g, 0.129 mol) and vanadyl acetylacetonate (0.5 g, 0.0018 mol) in refluxing benzene (150 mL) was added, dropwise over 20 min, dry *t*-butyl hydroperoxide (12.8 g, 0.142 mol) dissolved in a small quantity of benzene (2–3 mL).[35] After 10 h, the mixture was allowed to cool and the organic phase washed with sodium metabisulfite solution, dried and concentrated. The resulting epoxide decomposed upon attempted distillation and was acetylated in situ to facilitate purification. Thus, the cooled organic phase was stirred with acetic anhydride (50 mL) and pyridine (75 mL) for 24 h. The mixture was then poured onto ice-water and washed with distilled water, 1 M hydrochloric acid, sodium bicarbonate, then dried and concentrated. The acetate **34** was distilled (23.8 g, 87%), bp 153–156 °C (2.0 mmHg), to give a pale oil. This was dissolved in aqueous methanol (10 mL distilled water in 200 mL methanol), potassium carbonate (6.9 g, 0.05 mol) was added and the mixture stirred for 1 h. The methanol was removed under reduced pressure and the residue was taken up in water and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried and concentrated to give exclusively **33** (17.9 g, 94%) in high purity. ^{1}H NMR: δ 1.29 (s, 3H), 1.43–1.64 (m, 2H), 1.61 (s, 3H), 1.68 (s, 3H), 2.07–2.17 (m, 2H), 2.98 (dd, 1H, $J=6.4$ Hz), 3.14 (br s, 1H), 3.66 (dd, 1H, $J=12.6$ Hz), 3.80 (dd, 1H, $J=12.4$ Hz), 5.08 (t, 1H, $J=7$ Hz). ^{13}C NMR: δ 16.7, 17.6, 23.7, 25.7, 38.6, 61.2, 61.3, 63.3, 123.4, 132.1. MS: *m/z* 170 (M^+, 1%), 109 (23), 82 (59), 69 (71), 67 (39), 55 (30), 43 (40), 41 (100), 39 (26).

(2*R,3*S**)-epoxy-3,7-dimethyl-6-octen-1-ol (2,3-epoxynerol).** The equivalent nerol isomer was prepared from nerol as described for geraniol (81% yield for three steps). ^{1}H NMR: δ 1.28 (s, 3H), 1.44–1.67 (m, 2H), 1.61 (s, 3H), 1.69 (s, 3H), 2.06–2.17 (m, 2H), 2.97 (dd, 1H, $J=7.4$ Hz), 3.03 (br s, 1H), 3.64 (dd, 1H, $J=12.7$ Hz), 3.81 (dd, 1H, $J=12.4$ Hz), 5.09 (t, 1H, $J=7$ Hz). ^{13}C NMR: δ 17.6, 22.2, 24.1, 25.7, 33.2, 61.2, 61.6, 64.5, 123.3, 132.4. MS: *m/z* 170 (M^+, 1%), 109 (37), 82 (62), 69 (84), 67 (43), 55 (35), 43 (55), 41 (100), 39 (29).

(2*E*,6*R)-6,7-Epoxy-3,7-dimethyl-2-octen-1-ol (37) (6,7-epoxygeraniol).** Geraniol was converted to geranyl acetate (**35**) under standard conditions (acetic anhydride, pyridine, 4-dimethylaminopyridine). Geranyl acetate (**35**) (0.98 g, 0.005 mol) and 3-chloroperoxybenzoic acid (mCPBA) (0.86 g, 0.005 mol) were stirred in dichloromethane (100 mL) with sodium bicarbonate (0.42 g, 0.005 mol) for 48 h. The mixture was stirred with sodium metabisulfite solution to destroy unreacted mCPBA, washed with sodium bicarbonate solution, dried and concentrated to give **36**. Subsequent deacetylation as described previously gave exclusively **37** (0.69 g, 82% for two steps) in high purity. ^{1}H NMR: δ 1.27 (s, 3H), 1.31 (s, 3H), 1.69 (s, 3H), 1.63–1.71 (m, 2H), 2.09–2.24 (m, 2H), 2.71–2.75 (m, 1H), 2.81 (br s, 1H), 4.09–4.17 (m, 2H), 5.44 (td, 1H, $J=7.1$ Hz). ^{13}C NMR: δ 16.2, 18.7, 24.8, 27.1, 36.2, 58.6, 58.9, 64.1, 124.4, 137.7. MS: *m/z* 170 (M^+, 1%), 85 (67), 84 (67), 81 (75), 71 (66), 59 (85), 43 (84), 41 (100), 39 (49).

(2*Z*,6*R)-6,7-Epoxy-3,7-dimethyl-2-octen-1-ol (6,7-epoxynerol).** The equivalent nerol isomer was prepared from nerol as described for geraniol (80% yield for two steps). ^{1}H NMR: δ 1.28 (s, 3H), 1.31 (s, 3H), 1.55–1.74 (m, 2H), 1.76 (d, 3H, $J=1$ Hz), 2.18–2.30 (m, 2H), 2.73 (dd, 1H, $J=7.5$ Hz), 2.79 (br s, 1H), 4.09 (dd, 1H, $J=12.7$ Hz), 4.14 (dd, 1H, $J=12.6$ Hz), 5.44 (t, 1H, $J=7$ Hz). ^{13}C NMR: δ 18.8, 23.3, 24.8, 27.0, 28.5, 58.5, 58.9, 64.0, 125.4, 138.1. MS: *m/z* 170 (M^+, 1%), 85 (62), 81 (82), 71 (59), 67 (34), 59 (85), 43 (74), 41 (100), 39 (43).

(2*E*,6*E*)-8-Acetoxy-2,6-dimethyl-2,6-octadienal (38) (8-oxogeranyl acetate). Geranyl acetate (**35**) (prepared as described previously; 39.3 g, 0.2 mol), selenium dioxide (2.22 g, 0.02 mol) and dry *t*-butyl hydroperoxide (2.5 M in toluene; 240 mL, 0.6 mol) were stirred at room temperature for 5 days.[36] The reaction mixture was washed with saturated sodium thiosulfate solution, saturated sodium bicarbonate solution and

brine, then dried and concentrated. The product was purified by flash column chromatography, eluting with a gradient of petroleum ether and diethyl ether to produce **38** (16.8 g, 40%). ^{1}H NMR: δ 1.74 (s, 3H), 1.76 (d, 3H, J=1 Hz), 2.04 (s, 3H), 2.25 (m, 2H), 2.5 (m, 2H), 4.59 (d, 2H, J=7 Hz), 5.39 (m, 1H), 6.48 (td, 1H, J=7.1 Hz), 9.39 (s, 1H). ^{13}C NMR: δ 16.3, 20.9, 27.0, 37.7, 61.1, 119.6, 139.6, 140.4, 153.6, 171.0, 195.1.

(2*E*,6*E*)-2,6-Dimethyl-2,6-octadiene-1,8-diol (29) (8-hydroxygeraniol). 8-Oxogeranyl acetate (**38**) (4.2 g, 0.02 mol) was stirred in dry diethyl ether and the mixture cooled in an ice bath. Lithium aluminum hydride (1.5 g, 0.04 mol) was added slowly to the mixture, and the mixture stirred for 1 h.[32] The excess lithium aluminum hydride was destroyed by dropwise addition of distilled water (1.5 mL), 15% sodium hydroxide solution (1.5 mL), followed by distilled water (4.5 mL). The precipitate formed was removed by filtration and the filtrate concentrated. The product was purified by flash column chromatography, eluting with a gradient of petroleum ether and diethyl ether to yield **29** (2.55 g, 75%). ^{1}H NMR: δ 1.69 (s, 3H), 1.71 (s, 3H), 2.06–2.10 (m, 2H), 2.20–2.25 (m, 2H), 3.55 (br s, 2H), 3.95 (s, 2H), 4.15 (d, 2H, J=7 Hz), 5.42 (m, 2H). ^{13}C NMR: δ 13.7, 16.1, 25.5, 39.0, 59.0, 68.4, 123.9, 125.1, 135.0, 138.4. MS: *m/z* 170 (M^+, 0%), 68 (37), 67 (21), 57 (20), 55 (24), 53, (17), 43 (100), 41 (84), 39 (37).

(2*E*,6*E*)-2,6-Dimethyl-2,6-octadiene-1,8-[8-^{3}H]-diol ([8-^{3}H]-8-hydroxygeraniol). Sodium boro^3[H]hydride (500 mCi/mmol, 0.48 mg, 0.0125 mmol) in ethanol (100 μL) was dispensed into a 5 mL pear-shaped flask. A solution of 8-oxogeranyl acetate (**38**) (10.5 mg, 0.05 mmol) in ethanol (1 mL) was added slowly dropwise. The mixture was stirred for 60 min before being poured onto distilled water (10 mL) and extracted with diethyl ether (3 × 15 mL). The combined extracts were washed with saturated sodium bicarbonate solution, then dried and concentrated to yield [8-^{3}H] 8-hydroxygeranyl acetate (2.5 mCi, 40% radiochemical yield). This was deacetylated as described previously to give a pale oil. The crude product was dissolved in diethyl ether (1 mL) and loaded onto a silica TLC sheet (20 × 20 cm). The plate was developed once in 30% ethyl acetate in hexane, dried and developed once in 50% ethyl acetate in hexane. The product was not identifiable by UV, so a small strip was cut from the side of the plate and developed in an iodine tank. The position of the product was noted and marked on the plate. The product band was cut away and soaked in ethyl acetate overnight. Filtration and concentration yielded [8-^{3}H]-8-hydroxygeraniol (1.5 mCi, 60% radiochemical yield) which had properties identical (^{1}H NMR and ^{13}C NMR) to 8-hydroxygeraniol (**29**). The same procedure was applied to produce the equivalent nerol and citronellol isomers.

(2*Z*,6*E*)-2,6-Dimethyl-2,6-octadiene-1,8-diol (8-hydroxynerol). The equivalent nerol isomer was prepared from nerol as described for geraniol (73%). ^{1}H NMR: δ 1.63 (s, 3H), 1.74 (s, 3H), 2.02–2.20 (m, 4H), 3.94 (s, 2H), 4.04 (d, 2H, J=7 Hz), 5.40 (m, 2H). ^{13}C NMR: δ 13.6, 23.2, 25.1, 31.2, 58.5, 68.1, 124.7, 124.9, 135.5, 137.8. MS: *m/z* 170 (M^+, 1%), 84 (58), 68 (84), 67 (45), 55 (34), 43 (100), 41 (85), 39 (39), 29 (48).

(±)-, (6*R*)- and (6*S*)-2,6-Dimethyl-(2*E*)-octen-1,8-diol (26) (8-hydroxycitronellol). (±)-8-Hydroxycitronellol, (3*R*)-8-hydroxycitronellol and (3*S*)-8-hydroxycitronellol were prepared from (±)-citronellol, (*R*)-citronellol and (*S*)-citronellol, respectively, as described for geraniol (76%). ^{1}H NMR: δ 0.90 (d, 3H, J=6 Hz), 1.17–1.31 (m, 2H), 1.33–1.44 (m, 2H), 1.54–1.69 (m, 3H), 1.65 (s, 3H), 2.76 (br s, 1H, OH), 2.85 (br s, 1H, OH), 3.58–3.69 (m, 2H), 3.95 (br s, 2H), 5.38 (t, 1H, J=7 Hz). ^{13}C NMR: δ 13.7, 19.5, 24.9, 28.9, 36.7, 39.7, 60.7, 68.6, 126.1, 134.6. MS: *m/z* 172 (M^+, 1%), 81 (59), 71 (55), 69 (57), 68 (55), 67 (44), 55 (89), 43 (100), 41 (81).

(2*E*,6*E*)-8-Hydroxy-2,6-dimethyl-2,6-octadienal (31) (8-oxogeraniol). 8-Oxogeranyl acetate (**38**) (0.42 g, 0.002 mol) was deacetylated as described previously to give **31** (0.29 g, 87%) The product was purified by flash column chromatography, eluting with a gradient of petroleum ether and diethyl ether. ^{1}H NMR: δ 1.70 (s, 3H), 1.75 (s, 3H), 2.22 (t, 2H, J=7 Hz), 2.51 (td, 2H, J=7.7 Hz), 4.16 (d, 2H, J=7 Hz), 5.45 (t, 1H, J=7 Hz), 6.49 (t, 1H, J=7 Hz), 9.38 (s, 1H). ^{13}C NMR: δ 9.2, 16.2, 27.1, 37.8, 59.0, 124.8, 137.3, 139.5, 154.2, 195.4. MS: *m/z* 168 (M^+, 0.6%), 135 (39), 121 (34), 84 (100), 83 (39), 57 (33), 55 (46), 41 (86), 29 (55).

(2*Z*,6*E*)-8-Hydroxy-2,6-dimethyl-2,6-octadienal (8-oxonerol). The equivalent nerol isomer was prepared from nerol as described for geraniol (87%). ^{1}H NMR: δ 1.75 (s, 3H), 1.78 (s, 3H), 2.28 (t, 2H, J=7 Hz), 2.47 (td, 2H, J=7.7 Hz), 4.12 (d, 2H, J=7 Hz), 5.48 (t, 1H, J=7 Hz), 6.50 (t, 1H, J=7 Hz), 9.40 (s, 1H). ^{13}C NMR: δ 9.2, 23.2, 27.5, 30.4, 58.6, 125.8, 137.5, 139.6, 153.9, 195.5. MS: *m/z* 168 (M^+, 8%), 97 (38), 85 (25), 84 (100), 83 (33), 55 (44), 43 (29), 41 (88), 39 (41).

(2*E*,6*R)-8-Hydroxy-2,6-dimethyl-2-octenal [(±)-8-oxocitronellol].** (±)-8-Oxocitronellol was prepared from (±)-citronellol as described for geraniol (81%). ^{1}H NMR: δ 0.95 (d, 3H, J=6 Hz), 0.91–1.70 (m, 6H), 1.75 (s, 3H), 2.30–2.43 (m, 1H), 3.20 (br s, 1H, OH), 3.61–3.74 (m, 2H), 6.51 (t, 1H, J=7 Hz), 9.38 (s, 1H). ^{13}C NMR: δ 9.2, 19.3, 26.6, 29.3, 35.6, 39.4, 60.7, 139.2, 155.4, 195.7. MS: *m/z* 170 (M^+, 10%), 97 (73), 95 (46), 81 (37), 69 (40), 67 (47), 55 (70), 43 (57), 41 (100).

(2*E*,6*E*)-8-Hydroxy-3,7-dimethyl-2,6-octadienal (30) (8-hydroxygeranial). 8-Oxogeranyl acetate (**38**) (10.0 g, 0.048 mol) was stirred in ethanol (150 mL) and the mixture cooled in an ice bath. Sodium borohydride (3.8

g, 0.01 mol) was added gradually and then the mixture allowed to warm to room temperature. The mixture was poured onto ice-water and extracted with diethyl ether (3 × 100 mL). The combined extracts were dried and concentrated to give 8-hydroxygeranyl acetate (**39a**). Compound **39a** (10.0 g, 0.047 mol) was stirred in dry dichloromethane (150 mL) with ethyl vinyl ether (3.4 g, 0.047 mol) and catalytic pyridinium *p*-toluenesulfonate for 4 h. The reaction mixture was diluted with diethyl ether (150 mL) and washed with half-saturated brine to remove the catalyst. The organic phase was dried and concentrated to yield the C-8 protected alcohol **39b** (12.1 g, 88% from **38**). Compound **39b** (6.2 g, 0.022 mol) was deacetylated as described previously and the resulting C-1 alcohol **39c** (4.84 g, 0.02 mol) was stirred with manganese dioxide (active; 100 g)[37] in chloroform (200 mL) for 48 h. The reaction solution was filtered and the filtrate dried and concentrated to yield the C-1 aldehyde **40** (4.1 g, 0.017 mol). This was stirred in acetic acid/water/tetrahydrofuran 8:1:1 (50 mL) at room temperature for 3 h. The solvents were removed under reduced pressure to yield **30** (2.4 g, 66% from **39b**). ^{1}H NMR: δ 1.66 (s, 3H), 2.00–2.10 (m, 2H), 2.18 (d, 3H, J=1 Hz), 2.14–2.27 (m, 2H), 2.96 (br s, 1H), 3.98 (s, 2H), 5.38 (m, 1H), 5.88 (d, 1H, J=8 Hz), 9.97 (d, 1H, J=8 Hz). ^{13}C NMR: δ 13.7, 17.6, 25.2, 40.2, 68.3, 123.5, 127.4, 136.2, 163.9, 191.5. MS: *m/z* 168 (M^+, 0%), 85 (12), 84 (63), 83 (30), 55 (17), 53 (10), 43 (100), 41 (39), 39 (39).

(2*Z*,6*E*)-8-Hydroxy-3,7-dimethyl-2,6-octadienal (8-hydroxyneral). The equivalent nerol isomer was prepared from nerol as described for geraniol (58% from 8-oxoneryl acetate). ^{1}H NMR: δ 1.64 (s, 3H), 2.00 (d, 3H, J=1 Hz), 2.02–2.65 (m, 4H), 2.80 (br s, 1H), 3.99 (s, 2H), 5.39 (m, 1H), 5.90 (d, 1H, J=8 Hz), 9.87 (d, 1H, J=8 Hz). ^{13}C NMR: δ 13.7, 25.1, 26.5, 32.5, 68.1, 123.0, 128.6, 137.0, 164.0, 191.1. MS: *m/z* 168 (M^+, 4%), 84 (54), 83 (49), 82 (42), 80 (29), 55 (29), 43 (100), 41 (58), 39 (37).

(2*E*,6*E*)-3,7-Dimethyl-2,6-octadienedial (32) (8-oxogeranial). 8-Oxogeraniol (**31**) (1.68 g, 0.01 mol) was oxidized with manganese dioxide (active; 30 g) as described earlier to yield **32** (1.46 g, 88%). ^{1}H NMR: δ 1.77 (d, 3H, J=1 Hz), 2.23 (d, 3H, J=1 Hz), 2.45 (t, 2H, J=7 Hz), 2.60 (td, 2H, J=7.7 Hz), 5.91 (dq, 1H, J=7.1 Hz), 6.45 (tq, 1H, J=7.1 Hz), 9.40 (s, 1H), 10.01 (d, 1H, J=8 Hz). ^{13}C NMR: δ 9.3, 17.6, 26.3, 38.7, 127.7, 140.2, 151.7, 161.6, 191.0, 194.8. MS: *m/z* 166 (M^+, 1%), 109 (52), 84 (51), 83 (67), 82 (34), 55 (100), 53 (35), 41 (52), 39 (73).

(2*Z*,6*E*)-3,7-Dimethyl-2,6-octadienedial (8-oxoneral). The equivalent nerol isomer was prepared from nerol as described for geraniol (88%). ^{1}H NMR: δ 1.70 (s, 3H), 1.95 (s, 3H), 2.56 (t, 2H, J=7 Hz), 2.72 (t, 2H, J=7 Hz), 5.89 (d, 1H, J=7 Hz), 6.40 (t, 1H, J=7 Hz), 9.30 (s, 1H), 9.88 (d, 1H, J=7 Hz). ^{13}C NMR: δ 9.2, 24.7, 27.6, 31.0, 128.9, 140.3, 151.0, 161.5, 190.0, 194.7. MS: *m/z* 166 (M^+, 1%), 95 (84), 84 (78), 83 (80), 82 (57), 55 (100), 41 (71), 39 (72), 29 (58).

(2*E*,6*R)-2,6-Dimethyl-2-octenedial (27) [(±)-8-oxocitronellal].** Dry dichloromethane (10 mL) and oxalyl chloride (0.19 mL, 2.2 mmol) were stirred under nitrogen at −78 °C. Dimethyl sulfoxide (0.44 mL, 4.8 mmol) in dichloromethane (1 mL) was added dropwise and the reaction stirred for 10 min before (±)-8-hydroxycitronellol (**26**) (0.172 g, 1 mmol) in dichloromethane (1 mL) was slowly added. After 15 min, triethylamine (1.39 mL, 10 mmol) was added and the mixture allowed to warm to room temperature before pouring onto distilled water (20 mL). The aqueous layer was separated and extracted further with dichloromethane (2 × 20 mL). The combined organic extracts were washed with saturated sodium bicarbonate solution, then dried and concentrated to give **27** (0.14 g, 85%) in high purity. ^{1}H NMR: δ 1.03 (d, 3H, J=7 Hz), 1.44 (m, 1H), 1.58 (m, 1H), 1.75 (d, 3H, J=1 Hz), 2.15 (octet, 1H, J=7 Hz), 2.34 (m, 1H), 2.46 (m, 1H), 2.40 (m, 2H), 6.49 (td, 1H, J=7.1 Hz), 9.78 (d, J=2 Hz). ^{13}C NMR: δ 9.9, 20.4, 27.2, 28.4, 35.9, 51.6, 140.2, 154.8, 195.9, 202.9. MS: *m/z* 170 (M^+, 1%), 124 (72), 109 (45), 97 (73), 95 (45), 55 (73), 43 (57), 41 (100), 29 (55).

Acknowledgements

IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. This work was in part supported by the United Kingdom Ministry of Agriculture, Fisheries and Food.

References

1. Pickett, J. A.; Wadhams, L. J.; Woodcock, C. M.; Hardie, J. *Annu. Rev. Entomol.* **1992**, *37*, 67.

2. Pickett, J. A.; Wadhams, L J.; Woodcock, C. M. *Brighton Crop Prot. Conf.-Pests Dis.* **1994**, 1239.

3. Pettersson, J. *Entomol. Scand.* **1970**, *1*, 63.

4. Pettersson, J. *Entomol. Scand.* **1971**, *2*, 81.

5. Marsh, D. *Nature New Biol.* **1972**, *238*, 31.

6. Marsh, D. *J. Entomol.* **1975**, *50*, 43.

7. Steffan, A. W. *Entomol. Gener.* **1987**, *12*, 235.

8. Nault, L. R.; Montgomery, M. E. *Aphids as Vectors*; Harris, K. F.; Maramorosch, K., Eds.; Academic: London, 1977; pp 527–545.

9. Dawson, G. W.; Griffiths, D. C.; Janes, N. F.; Mudd, A.; Pickett, J. A.; Wadhams, L. J.; Woodcock, C. M. *Nature* **1987**, *325*, 614.

10. Boeckh, J. *Z. Vergleichende Phys.* **1962**, *46*, 212.

11. Wadhams, L. J. *Chromatography and Isolation of Insect Hormones and Pheromones*; McCaffery, A. R.; Wilson, I. D., Eds.; Plenum: New York, 1990; pp 289–298.

12. Wadhams, L. J. *Insect Molecular Science*; Crampton, J. M.; Eggleston, P.; Academic: 1991; pp 152–162.

13. Dawson, G. W.; Griffiths, D C.; Merritt, L. A.; Mudd, A.; Pickett, J. A.; Wadhams, L. J.; Woodcock, C. M. *Entomol. Exp. Appl.* **1988**, *48*, 91.

14. Dawson, G. W.; Janes, N. F.; Mudd, A.; Pickett, J. A.; Slawin, A. M. Z.; Wadhams, L. J.; Williams, D. J. *Pure Appl. Chem.* **1989**, *61*, 555.

15. Dawson, G. W.; Griffiths, D. C.; Merritt, L. A.; Mudd, A.; Pickett, J. A.; Wadhams, L. J.; Woodcock, C. M. *J. Chem. Ecol.* **1990**, *16*, 3019.

16. Campbell, C. A. M.; Dawson, G. W.; Griffiths, D. C.; Pettersson, J.; Pickett, J. A.; Wadhams, L. J.; Woodcock, C. M. *J. Chem. Ecol.* **1990**, *16*, 3455.

17. Hardie, J.; Nottingham, S. F.; Dawson, G. W.; Harrington, R.; Pickett, J. A.; Wadhams, L. J. *Chemoecology* **1992**, *3*, 113.

18. Lilley, R.; Hardie, J.; Merritt, L. A.; Pickett, J. A.; Wadhams, L. J.; Woodcock, C. M. *Chemoecology* **1995**, *5/6, (1)*, 43.

19. Hardie, J.; Nottingham, S. F.; Powell, W.; Wadhams, L. J. *Entomol. Exp. Appl.* **1991**, *61*, 97.

20. Gabrys, B. J.; Gadomski, H. J.; Pickett, J. A.; Sobota, G. T.; Wadhams, L. J.; Woodcock, C. M. *J. Chem. Ecol.*, in press.

21. Hallahan, D. L.; Pickett, J. A.; Wadhams, L. J.; Wallsgrove, R. M.; Woodcock, C. M. *Plant Genetic Manipulation for Crop Protection*; Gatehouse, A. M. R.; Hilder, V. A.; Boulter, D., Eds.; C.A.B. International: Wallingford, 1992; pp 215–248.

22. Hallahan, D. L.; Lau, S.-M. C.; Harder, P. A.; Smiley, D. W. M.; Dawson, G. W.; Pickett, J. A.; Christoffersen, R. E.; O'Keefe, D. P. *Biochim. Biophys. Acta* **1994**, *1201*, 94.

23. Wolinsky, J.; Eustace, E. *J. Org. Chem.* **1972**, *37*, 3376.

24. Abou-Donia, S. A.; Fish, L. J.; Pattenden, G. *Tetrahedron Lett.* **1971**, *43*, 4037.

25. Cavill, G. W. K.; Ford, D. L. *Aust. J. Chem.* **1960**, *13*, 296.

26. Guldemond, J. A.; Dixon, A. F. G.; Pickett, J. A.; Wadhams, L. J.; Woodcock, C. M. *Physiol. Entomol.* **1993**, *18*, 137.

27. Schreiber, S. L.; Meyers, H. V.; Wiberg, K. B. *J. Am. Chem. Soc.* **1986**, *108*, 8274.

28. Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrerll, R. *J. Am Chem. Soc.* **1963**, *85*, 207.

29. Bellesia, F.; Grandi, R.; Pagnoni, U. M.; Pinetti, A.; Trave, R. *Phytochemistry* **1984**, *23*, 83.

30. Uesato, S.; Ikeda, H.; Fujita, T.; Inouye, H.; Zenk, M. H. *Tetrahedron Lett.* **1987**, *28*, 4431.

31. Hallahan, D. L.; Dawson, G. W.; West, J. M.; Wallsgrove, R. M. *Plant Physiol. Biochem.* **1992**, *30*, 435.

32. Hallahan, D. L.; Nugent, J. H. A.; Hallahan, B. J.; Dawson, G. W.; Smiley, D. W. M.; West, J. M.; Wallsgrove, R. M. *Plant Physiol.* **1992**, *98*, 1290.

33. Pickett, J. A.; Williams, I. H.; Smith, M. C.; Martin, A. P. *J. Chem. Ecol.* **1981**, *7*, 543.

34. Hallahan, D. L.; West, J. M.; Wallsgrove, R. M.; Smiley, D. W. M.; Dawson, G. W.; Pickett, J. A.; Hamilton, J. G. C. *Arch. Biochem. Biophys.* **1995**, *318*, 105.

35. Sharpless, K. B.; Michaelson, R. C. *J. Am. Chem. Soc.* **1973**, *95*, 6136.

36. Umbreit, M. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1977**, *99*, 5526.

37. Attenburrow J.; Cameron, A. F. B.; Chapman, J. H.; Evans, R. M.; Hems, B. A.; Jansen, A. B. A.; Walker, T. *J. Chem. Soc.* **1952**, 1094.

(*Received* 15 *September* 1995; *accepted* 19 *October* 1995)