

Elucidating the Biosynthesis of the Aphid Sex Pheromone

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BBSRC Doctoral
Training Partnerships

**ROTHAMSTED
RESEARCH**

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DTP Statement for Examiners



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BBSRC Doctoral
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The University of Nottingham BBSRC Doctoral Training Partnership (DTP) is undertaken in partnership with Rothamsted Research. Its aim is to develop an innovative, integrated, student-led programme of individualised research training in the Biosciences through an effective cohort-based programme. Students are not recruited to an individual project but, through a series of lab rotations, identify a PhD programme that they wish to pursue in one of three BBSRC priority research areas (Food Security; Industrial Biotechnology and Bioenergy; World Class Biosciences)

Research Training Structure

The research training structure is framed by individual development needs analysis and has the following core components:

- Generic and research skills development
- High-level modular training
- Laboratory rotations
- Experiential learning through public engagement and outreach activity

Generic and research skills development comprises the following: induction, introductory training programme, annual 'Spring School' conference-style training event, participation in Sysmic (online systems and mathematical biology course), specialist training days at partner organisations, twilight (after-lab) training which is arranged on an *ad hoc* basis to meet identified needs, and seminar series.

In Year One there is an initial period of intensive training which takes place over the course of the first six months. This comprises a two-week introductory training programme, 24 weeks of laboratory rotations, followed by a week-long 'Spring School' (conference-style training event for all DTP cohorts) and ending in a science outreach event. Each lab rotation is assessed focussing on a different form of scientific communication (Report, Poster, Presentation) and students receive feedback from supervisors. The PhD project begins in Month 7. Students are also encouraged to choose from a portfolio of relevant Master Courses which they can attend alongside their lab rotation projects.

In Years Two to Four training is mainly concentrated in the week long annual Spring School, although additional training and seminars are delivered where required throughout the year. A three month Professional Internship for PhD Students (PIPS) placement is usually taken in Year Three and, as BBSRC specifies, this is not in an area related to the students' PhD research programme.

The integrated programme of training is mapped to BBSRC priority areas, BBSRC Skills Statement: Scientific and Core Skills for UK Bioscience and the Researcher Development Framework (RDF) to ensure breadth and depth of provision, and to encompass vulnerable skills and strategic competencies. It builds on the best practice principles outlined in the Statement of Expectations for Doctoral Training, RCUK (2013) and the 'Good Practice Elements in Doctoral Training', LERU (2014).

It offers a wide range of skills development opportunities to prepare students for a successful bioscience-related career and supports the exchange of ideas and experiences within and across cohorts. It also generates a strong community of postgraduates and supervisors to stimulate the formation of cross-disciplinary links through cohort development, which prepares students for a career in an increasingly multidisciplinary environment.

Professional Internships for PhD Students

Reflection Form

Name of Organization

Allen & Overy LLP, London

Details of Placement

During my 3-month placement at A&O, I gained hands-on experience of the legal framework surrounding intellectual property and assisted on areas of IP litigation. I was involved in numerous projects, focusing primarily on patent litigation, but I was also exposed to various 'Soft IP' matters.

Despite my initial lack of working knowledge of Law, I was entrusted early on with a variety of tasks. I was involved in preparing, cross-referencing and copy editing draft expert reports and declaration documents, which often required my attendance and note-taking at meetings with experts. Once briefed on a new subject, I would need to familiarise myself with the relevant literature, past and present, and use it to create written and/or pictorial arguments and/or reports, as required. On many occasions, I was asked to locate, review and summarise, at team meetings, case-relevant journal articles for matter use or compile lists of potential experts for up-and-coming pitches. I was also given the opportunity to attend a number of court case hearings for which I had gathered evidence for.

Placement Achievements

My knowledge of patent litigation, and intellectual property as a whole, has vastly improved through first-hand experience. During my placement, I gathered evidence for, and composed large sections of, reports to be used in court case hearings. Much of the information I had collated through literature searching was used either in pitches to potential clients or in existing cases to provide incontestable evidence in support of our clients. During my time at A&O, I was successful in finding key evidence in defence of our clients on a patent litigation case.

Skill development

Through conducting thorough literature searches, I refined skills requiring fast-paced, summarisation of large amounts of data. I was also required to communicate my literature review findings clearly and concisely to the team. Through much practice, my copy editing and proof-reading skills improved considerably. During meetings with expert witnesses, I was responsible for deciphering experts' messages, which were often worded unclearly, and therefore needed to grasp new concepts quickly. I was given the opportunity to work independently on a wide variety of tasks requiring variable levels of skill. Most of the work I conducted was under considerable time pressure and therefore required honed organisational skills.

Through invitation to team-led presentations and regular lunchtime talks, my knowledge of general IP topics, through to more specific subjects such as quotation and parody-related copyright cases and client-driven investigative work, greatly improved. I also now have a more thorough understanding of the patent application process.

Future Work

My reflections on the PIP at A&O are thoroughly positive and the experience has left me keen to pursue a career in the field of Patent Law. I found the placement to be fast-paced, intellectually-challenging and overall rewarding which has fuelled my enthusiasm towards an IP-related career. Although the initial learning curve was steep, I found that my practical laboratory experience and familiarity with scientific literature aided my progress. Exposure to such a wide range of scientific disciplines makes this career very attractive.

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Abbreviations

2,4-DNP	2,4-Dinitrophenylhydrazine
4-DMAP	4-Dimethylaminopyridine
8-HGO	8-Hydroxygeraniol oxidoreductase
Anh.	Anhydrous
Aq.	Aqueous
ATP	Adenosine triphosphate
Bu	Butyl
Cat.	Catalytic
COC	Cool-on-column
CYP	Cytochrome P450 enzyme
Cys.	Cysteine
D or ² H *	Deuterium atom
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DLGT	Deoxyloganetic acid glucosyltransferase
DLH	Deoxyloganic acid hydroxylase
DLS	Deoxyloganetic acid synthase
DMADP	Dimethylallyl diphosphate
DMNT	(<i>E</i>)-4,8-Dimethyl-1,3,7-nonatriene
DMSO	Dimethyl sulfoxide
DPAA	Diphenylacetic acid
E _{1cB}	Elimination unimolecular conjugate base
EBF	(<i>E</i>)-β-Farnesene
EDTA	Ethylenediamine tetra-acetic acid
Enz.	Enzyme
Eq.	Equivalents
Et	Ethyl
eV	Electron volts
FAD	Flavin adenine dinucleotide
FDP	Farnesyl diphosphate
FID	Flame ionisation detector

* D used in chemical structures and solvent names, otherwise ²H used throughout.

G8O	Geraniol 8-oxidase
GC	Gas chromatography
GDP	Geranyl diphosphate
GES	Geraniol synthase
GH1	Glycosyl hydrolase family 1
Glu.	Glutamate
Gluc.	Glucosyl
Gly.	Glycine
GM	Genetically-modified
GMC	Glucose methanol choline
HMBC	Heteronuclear multiple-bond correlation
HMG-CoA	3-Hydroxy-3-methylglutaryl-SCoA
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
Hrs	Hours
HSQC	Heteronuclear single quantum coherence spectrum
IBX	<i>o</i> -Iodoxybenzoic acid
IDP	Isopentenyl diphosphate
IPM	Integrated pest management
IP	Ion pairing
ISY	Iridoid synthase
<i>J</i>	Coupling constant
JHBP	Juvenile hormone-binding protein
KIE	Kinetic isotope effect
LAMT	Loganic acid <i>O</i> -methyltransferase
LC	Liquid chromatography
<i>m/z</i>	Mass to charge ratio
MCP	Modular circular polarimeter
<i>m</i> -CPBA	<i>meta</i> -3-Chloroperbenzoic acid
MEP	Methylerythritol phosphate
Me	Methyl
MHz	Mega Hertz
MIA	Monoterpene indole alkaloid
mins	Minutes

mp	Melting point
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MVA	Mevalonate
NAD(P)H	Nicotinamide adenine dinucleotide (phosphate)
NMR	Nuclear magnetic resonance
<i>o</i> -	Ortho-
OBP	Odorant binding protein
OP	Phosphate group
OPP	Diphosphate group
OR	Olfactory receptor
Oxone	Potassium peroxymonosulfate
<i>p</i> -	Para-
P5 β R	Progesterone 5 β -reductase
PDA	Photodiode array
pH	Potential of hydrogen
PPh ₃	Triphenylphosphine
Q-ToF	Quadrupole time of flight
RedAl [®]	Sodium bis(2-methoxyethoxy)aluminum hydride
RNAi	Ribonucleic acid interference
RP	Reverse phase
rt.	Room temperature
SCoA	Sulfur-Coenzyme A
SCR	Single cell recordings
SLM	Small lipophilic molecule
SLS	Secologanin synthase
STR	Strictosidine synthase
<i>t</i> -	Tertiary
TBAF	Tetrabutylammonium fluoride
TBDPS	<i>tertiary</i> -Butyl diphenyl silyl [chloride]
TEA	Triethylamine
THF	Tetrahydrofuran
TIC	Total ion chromatogram
TLC	Thin layer chromatography

TMTT	(<i>E,E</i>)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene
UPLC	Ultra performance liquid chromatography
UV	Ultra-violet
Vit.	Vitamin
VOC	Volatile organic compound

Abstract

The female-produced sex pheromone for many aphid species comprises a mixture of the iridoids (4a*S*,7*S*,7a*R*)-nepetalactone (**I**) and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**II**). Plant-derived biosynthesis of **I** and **II**, in plant species such as *Nepeta spp.* (Lamiaceae) and *Catharanthus spp.* (Apocynaceae), respectively, has received considerable attention due to their role as precursors to many pharmacologically active compounds, including the indole alkaloids. To test the hypothesis that aphids biosynthesize **I** and **II** *de novo* similarly to that elucidated for plants, isotopic labelling techniques have been deployed with oviparous pea aphids, *Acyrtosiphon pisum* (Homoptera: Aphididae).

A range of unlabelled and deuterium-labelled putative sex pheromone precursors were synthesised and applied to aphids *via* one of two methods; dietary incorporation or topical application. For feeding experiments, *A. pisum* oviparae were artificially fed using diet sachets. Under autumnal conditions, a simple aqueous 200 g/L D-sucrose solution, into which the compounds were dissolved, was found to sufficiently maintain oviparae. Topically-treated aphids were exposed to methanolic solutions of each compound by application of 0.2 µL onto their dorsal surface. Following treatment, the volatile organic compounds (VOCs) released by oviparae were collected by dynamic headspace collection and analysed by gas chromatography coupled to mass spectrometry (GC-MS) to confirm the presence and position of labelling.

Using unlabelled derivatives to establish optimal VOC collection parameters, dietary incorporation of all deuterated putative precursors evaluated failed to achieve deuterium incorporation into lactol **I** and lactone **II**. Successful deuterium incorporation, as evidenced by the presence of molecular ions at *m/z* 172 and 170 (corresponding to

$^2\text{H}_4$ -lactol **I** and $^2\text{H}_4$ -lactone **II**, respectively), was demonstrated following the topical application of [C-8,C-10]-deuterated C-8-oxidised geranyl and neryl derivatives. Conversely, deuterium incorporation was not evident using the citronellyl derivatives, highlighting the importance of 2,3-unsaturation during biosynthesis. Through the synthesis and application of [C-4,C-9- $^2\text{H}_5$]-labelled monoterpenyl derivatives, it was demonstrated that a kinetic isotope effect was not responsible for the lack of deuterium incorporation following application of deuterated monoterpenols and monoterpenals. To address unclarified aspects of lactol **I** and lactone **II** biosynthesis in aphids, such as precursor identity and cyclisation mechanism, the synthesis and purification of 8-hydroxymonoterpenol-8- β -D-glucoside and NADP ^2H probes was attempted but was unsuccessful.

These results suggest that aphids follow a route to lactol **I** and lactone **II** that is also utilised by plants such as *Catharanthus roseus* (Apocynaceae). This work is expected to support future research into fully elucidating aphid sex pheromone biosynthesis, including identifying the genes, and therefore enzymes, involved. Future implications in the field of biotechnology-based iridoid synthesis and pest management are anticipated.

1. Introduction

1.1 Aphids as Crop Pests

Aphids (Homoptera: Aphididae) contribute profoundly to the increasing pressures currently placed on agricultural systems in temperate regions of the world by reducing both the yield and quality of many economically important crops. Many aphid species are polyphagous and have devastated crops such as cereals, oilseeds, vegetable brassicas, potatoes, carrots, lettuce, sugar beet, peas and beans ¹. Host plant impairment is a direct consequence of the aphid feeding process, during which they remove essential nutrients from plant tissue by sucking on the phloem sap and injecting toxic saliva ²⁻⁴. Indirect damage also arises through aphid-vectored plant diseases, including luteoviruses and enamoviruses of the Luteoviridae family, and *via* the colonisation of photosynthesis-limiting saprophytic Ascomycete fungi on leaf surfaces, promoted by aphid honeydew secretions ⁵⁻⁶.

1.2 Aphid Life Cycle

Aphids are hugely successful crop pests, owing to their rapid reproductive rate, their exploitation of wide-ranging environments and their airborne dispersal mechanisms as a result of polymorphism ⁷. For many species within the Aphididae family, both alate (winged) and apterous (wingless) adult female morphs (virginoparae; Figure 1.1A) reproduce parthenogenetically throughout the summer, colonising summer (secondary) host plants. At the onset of autumn, alate gynoparae develop, which give birth to apterous oviparae (sexual females; Figure 1.1B) either on surviving secondary or winter (primary) host plants ⁵. The colony also produces alate males

(Figure 1.1C) whose primary role is to locate and mate with conspecific oviparae. Subsequent to mating, oviparae lay overwintering eggs (Figure 1.1D) which lie dormant until spring when the nymphs finally hatch and the cycle is repeated ².

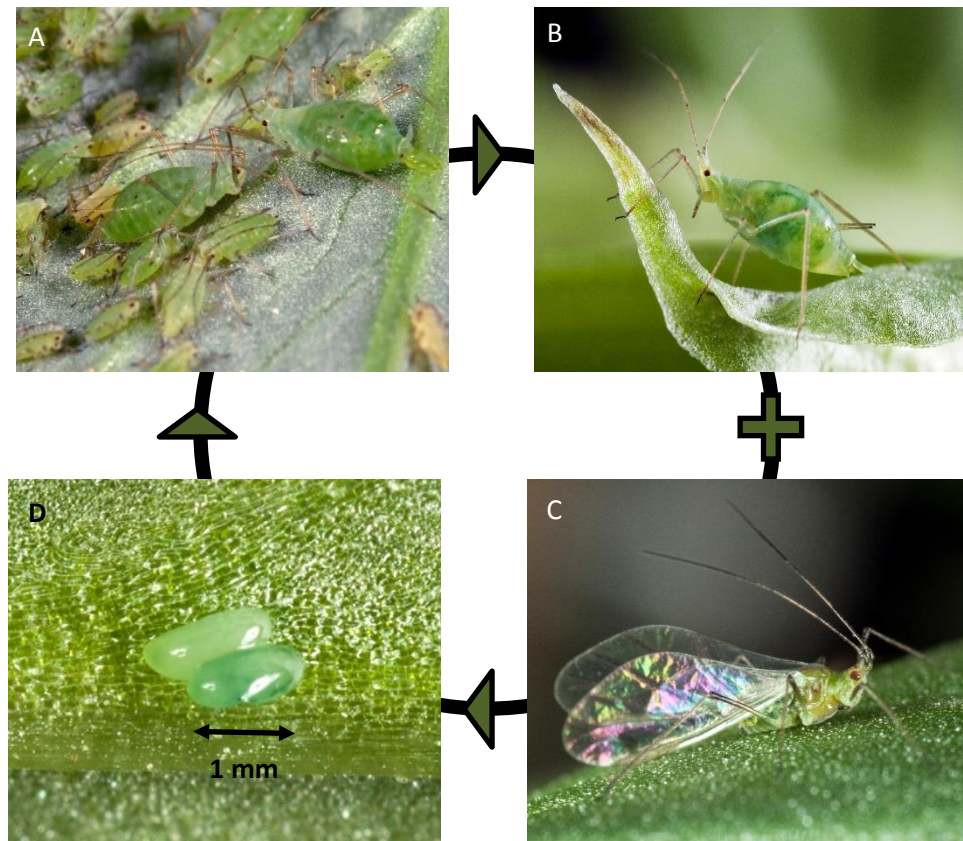


Figure 1.1. Images of (A) nymph and apterous virginoparae, (B) oviparae and (C) male pea aphids, *Acyrtosiphon pisum* (Homoptera: Aphididae), and (D) the overwintering eggs that the oviparae produce within the aphid life cycle.

1.3 Aphid Chemical Ecology

Inherent to all insect species, a collection of small, lipophilic molecules (SLMs), termed semiochemicals, are known to facilitate the aphid life cycle. These secondary metabolites form a critical part of the aphid life cycle by ensuring reproductive success, host location and defence against natural enemies. They act as chemical messengers between members of the same or different species (known as pheromones and

allelochemicals, respectively) by inducing a change in the behaviour or physiology of the receiving individual ⁸. They may also be used as synomones (interspecific allelochemicals of benefit to both the emitting and receiving species) or kairomones (allelochemicals detrimental to the emitting species but of benefit to the receiving species).

1.3.1 Aphid Sex Pheromone

The success of males in locating conspecific oviparae was found to be dependent on the release of a long-range sex pheromone emitted from scent plaques on the hind tibiae of oviparae. Whilst signalling, oviparae often raise their hind legs to aid dispersal of the pheromone (Figure 1.2A). Olfaction in aphids is yet to be fully elucidated, however male aphids detect the sex pheromone using olfactory receptors (ORs) associated with the secondary rhinaria of their antennae ⁹. Odorant binding proteins (OBPs) are then considered responsible for transporting these airborne lipophilic semiochemicals to chemoreceptor proteins within the male antennae, where a neuroelectrophysiological response ensues ¹⁰⁻¹².

The aphid sex pheromone was first identified by Pickett and colleagues using pentane washings of the excised hind legs of oviparous vetch aphids, *Megoura viciae* (Homoptera: Aphididae) ¹³⁻¹⁴, and found to comprise two bicyclic monoterpenoids of the iridoid class, namely (1*R*,4*aS*,7*S*,7*aR*)-nepetalactol (**1**) and (4*aS*,7*S*,7*aR*)-nepetalactone (**2**) (Figure 1.2B).

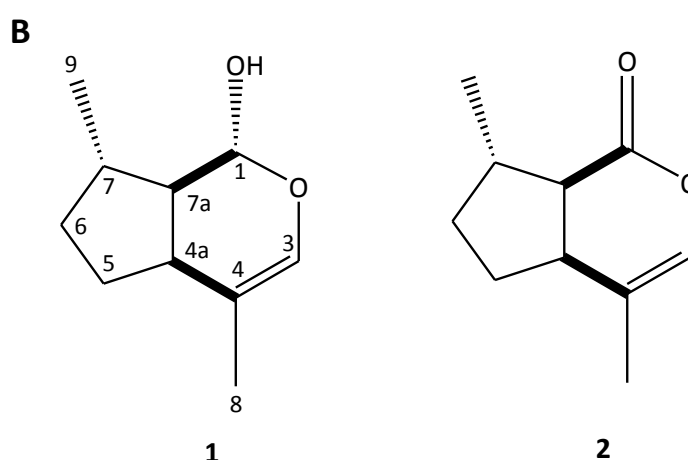


Figure 1.2. (A) Image of a ‘calling’ pea aphid, *Acyrtosiphon pisum* (Homoptera: Aphididae), ovipara releasing sex pheromone from its hind tibiae and (B) the chemical structures of the two iridoid aphid sex pheromone components, (1*R*,4*aS*,7*S*,7*aR*)-nepetalactol (**1**) and (4*aS*,7*S*,7*aR*)-nepetalactone (**2**).

The identification of lactol **1** and lactone **2**, and an understanding of the response they induced in aphids, was achieved *via* the coupling of gas chromatography (GC) and single cell electrophysiological recording techniques. Finely sharpened tungsten microelectrodes were placed at single olfactory cells (neurons) within the secondary rhinaria of male antennae and the aphid subjected to sample odours from the GC eluent to obtain single cell recordings (SCR) (Figure 1.3) ^{13, 15-16}. Structures of the electrophysiologically active compounds were then tentatively identified by GC-mass spectrometry (GC-MS) and confirmed using authentic standards, obtained through

chemical synthesis or isolation of natural products and authenticated by ^1H - and ^{13}C -nuclear magnetic resonance (NMR) spectroscopy ¹³. X-ray crystallography of the 3,5-dinitrobenzoate derivative of lactol **1** later enabled the stereochemical assignment at C-1 ¹⁴.

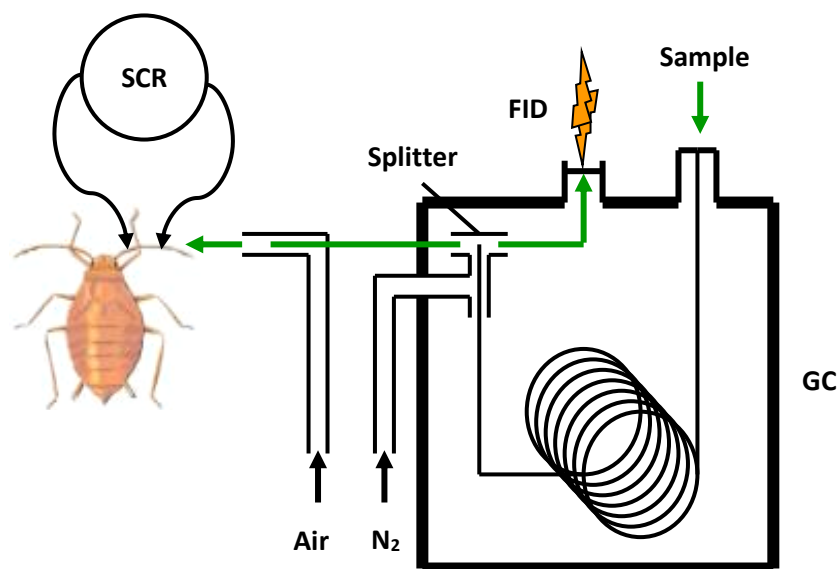


Figure 1.3. The coupled GC-electrophysiology system employed in identifying the aphid sex pheromone components ¹⁷.

Since their initial identification from *M. viciae* ¹³, a comparison of dynamic headspace collections from various Aphididae species has shown a variation in the ratio of lactol **1** and lactone **2** released, hypothesised to allow for interspecies differentiation ¹⁸. Intraspecies variation has also been observed, and found to be age-related, with both ratio and total secretion differences ¹⁹⁻²⁰.

As with the majority of chiral semiochemicals, the stereochemistry of lactol **1** and lactone **2**, especially at C-7, was found to be critical to the semiochemical activity and behavioural responses of the recipient aphid ²¹. In the case of lactone **2**, only the (4a*S*,7*S*,7a*R*)-diastereoisomer had attractant properties towards males of most aphid

species^{18, 22-23}. In addition to absolute and relative configuration, it was demonstrated that, for most species, both sex pheromone components are essential for achieving a behavioural response¹³.

The damson-hop aphid, *Phorodon humuli* (Homoptera: Aphididae), remains an exception to most aphids, instead emitting a sex pheromone that comprises a diastereotopic mixture of 1*S*- and 1*R*-(4*aR*,7*S*,7*aS*)-nepetalactol (**3**) (Figure 1.4)²⁴. Additionally, the presence of (1*R*,2*S*,5*S*)-dolichodial (**4**) (Figure 1.4) in the dynamic headspace of the rosy apple aphid, *Dysaphis plantaginea* (Homoptera: Aphididae) suggests that it may act as a third additional sex pheromone component, but further work is required to substantiate this for other Aphididae species²⁵.

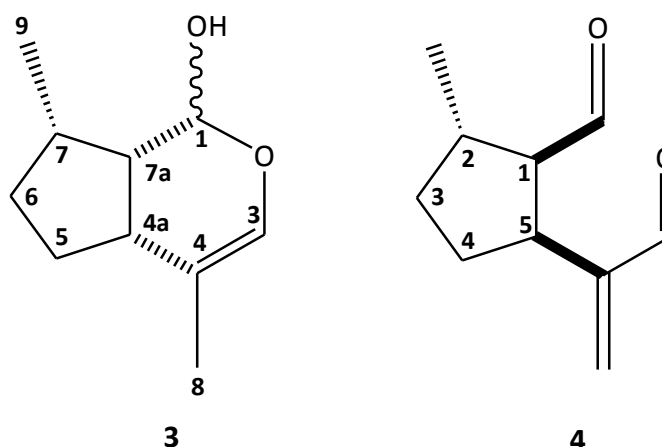


Figure 1.4. The chemical structures of 1*S*,1*R*-(4*aR*,7*S*,7*aS*)-nepetalactol (**3**) and putative sex pheromone component of the rosy apple aphid, *Dysaphis plantaginea* (Homoptera: Aphididae), (1*R*,2*S*,5*S*)-dolichodial (**4**).

Lactol **1** and lactone **2** have been isolated from other natural sources including the Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae), and catmint, *Nepeta cataria* (Lamiaceae), respectively (described in detail in Section 1.4.2.2)²⁶⁻²⁷. *N. cataria* could be using lactone **2** as a synomone by attracting aphid predators and parasitoids,

such as ladybird beetles (Coleoptera: Coccinellidae) and parasitic wasps (Hymenoptera: Apocrita), as these insects have adapted to utilising aphid sex pheromone components as kairomones to locate aphid colonisation ²⁸⁻²⁹. Whether of synomonal or kairomonal origin, a positive relationship between the arrival of aphid natural enemies and the release of lactone **2**, alone or in combination with lactol **1**, has been demonstrated in the field using trap catches ^{28, 30}.

1.3.2 Other Aphid Semiochemicals

Critical to the survival of all aphid species is their ability to communicate threats. In situations where the welfare of an aphid and its colony is threatened, such as during predatorial or parasitic attack, the targeted individual emits an alarm pheromone, prompting the recipients to cease feeding and escape the area ³¹. For most aphid species, (*E*)- β -farnesene (EBF) (**5**) is the primary component of the alarm pheromone ³²⁻³³. However, (1*E*,5*E*,8*S*)-germacrene A (**6**) ³⁴ and (1*S*,5*S*)- α -pinene (**7**) ³⁵ serve as additional components in alfalfa aphids, *Therioaphis maculata* and *Therioaphis riehmi* (Homoptera: Aphididae), and the vetch aphid, *Megoura viciae* (Homoptera: Aphididae), respectively (Figure 1.5) ³⁶. EBF **5** is also implicated in *A. pisum* wing polyphenism ³⁷ and acts, inadvertently, as a foraging cue, not only for ants, with whom a mutualistic relationship exists ³⁸, but also for parasitoids and predators.

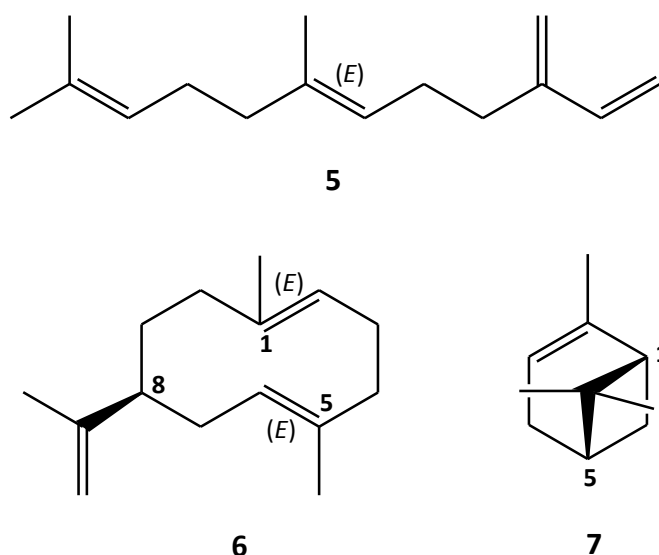


Figure 1.5. The chemical structures of aphid alarm pheromone components; (E)-β-farnesene (**5**), (1E,5E,8S)-germacrene A (**6**) and (1S,5S)-α-pinene (**7**).

This multitrophic network of communication between plant, herbivorous prey and natural enemy is further complicated by the plant's own system of defence. Defensive synomones are released from many host plants in response to aphid damage and often aid natural enemies in locating the host plant and pest aphid. Many plants suffering from aphid herbivory release (Z)-jasmones (**8**) (Figure 1.6), whose deterrent properties repel certain aphid species, thereby preventing further colonisation. (Z)-Jasmones **8** can also generate a defensive response, *via* aerial transmission, in neighbouring plants by stimulating the production of volatile organic compounds (VOCs), such as (E)-ocimene (**9**), (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (**10**) and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) (**11**) (Figure 1.6), which are attractive to the natural enemies of aphids³⁹⁻⁴⁰. 6-Methyl-5-hepten-2-one (**12**) and (Z)-3-hexen-1-yl acetate (**13**) (Figure 1.6) are amongst the VOCs released from aphid-infested broad bean, *Vicia faba* (Fabaceae), plants and were found to elicit the arrival of parasitic wasps, *Aphidius ervi* (Hymenoptera: Braconidae) that parasitise *A. pisum*⁴¹⁻⁴².

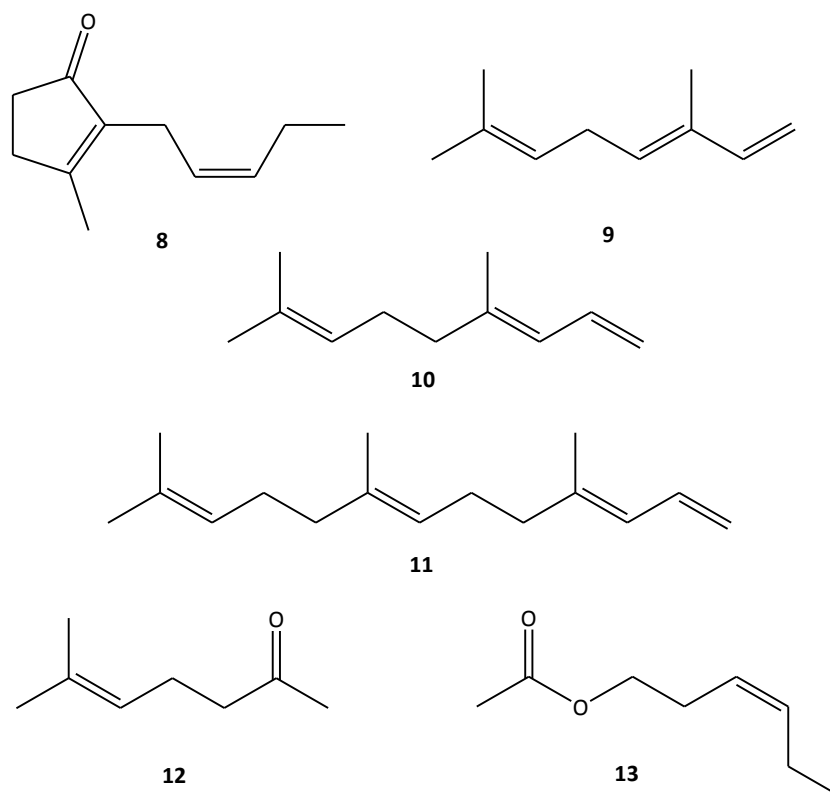


Figure 1.6. Plant synomones released upon aphid attack, namely (*Z*)-jasmone (**8**), (*E*)-ocimene (**9**), DMNT (**10**), TMTT (**11**), 6-methyl-5-hepten-2-one (**12**) and (*Z*)-3-hexen-1-yl acetate (**13**).

Conversely, certain compounds contributing to the volatile profiles of plants can serve as kairomones to host-specific aphids⁴³. Despite their ubiquity in the plant kingdom, the composition and ratio of these emitted compounds can be unique to a plant species and provide the specificity required by aphids to successfully target or avoid a given plant. Due to the finely tuned olfactory system of aphids, even minor changes in a plant profile can render the host plant unsuitable⁴⁴. Aphid proficiency in host differentiation helps them avoid colonising poor quality hosts and the associated risk of colony collapse⁴⁵⁻⁴⁶. Isothiocyanates, such as allyl isothiocyanate (**14**), 3-butenyl isothiocyanate (**15**) and 4-pentenyl isothiocyanate (**16**) (Figure 1.7), are emitted by plants belonging to the Brassicaceae family under aphid-induced stress and are used by certain Aphididae

species, such as the cabbage aphid, *Brevicoryne brassicae* (Homoptera), for host location purposes ³⁶.

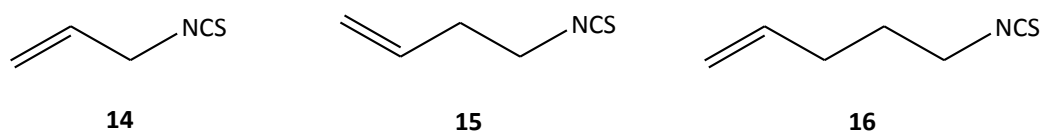


Figure 1.7. Isothiocyanates, allyl isothiocyanate (**14**), 3-butenyl isothiocyanate (**15**) and 4-pentenyl isothiocyanate (**16**), emitted by members of the Brassicaceae family and which aid host location for the cabbage aphid, *Brevicoryne brassicae* (Homoptera: Aphididae).

1.3.3 Management of Aphid Populations

Current management of aphid populations incorporates the heavy use of synthetic broad-spectrum pesticides. Examples for each common pesticide group, including organophosphate (*R*)-malathion (**17**), carbamate aldicarb (**18**), pyrethroid permethrin (**19**) and neonicotinoid imidacloprid (**20**), are shown in Figure 1.8 ⁴⁷⁻⁵¹. Their use, however, is plagued with rising levels of pest resistance and non-target effects ⁵².

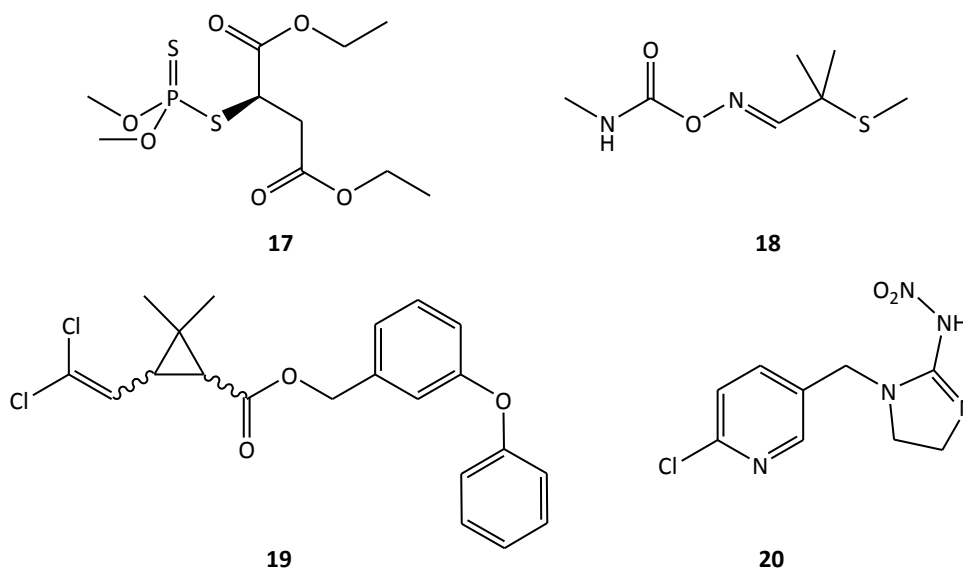


Figure 1.8. Examples of insecticides belonging to the four main insecticide groups, including organophosphate (*R*)-malathion (**17**), carbamate aldicarb (**18**), pyrethroid permethrin (**19**) and neonicotinoid imidacloprid (**20**).

The effective use of antifeedants, such as (1*R*,4*aS*,8*aS*)-polygodial (**21**) (Figure 1.9) ⁵³, and repellents, such as methyl salicylate (**22**) (Figure 1.9) ⁵⁴, against aphids have been described. The treatment of attractant-containing traps with biological control agents, such as the *Hyphomycetes* fungal pathogen, *Verticillium lecanii*, has also been used effectively ^{7, 55-56}. However, their effect is more pronounced when combined with other control methods in an integrated pest management (IPM) system ⁵⁷.

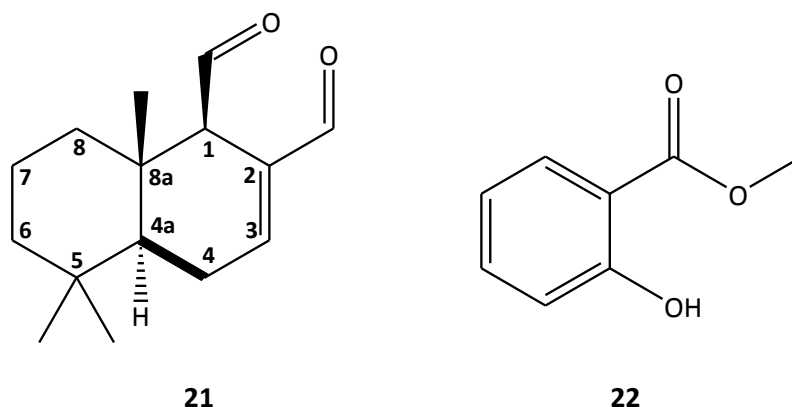


Figure 1.9. Insect antifeedant, (1*R*,4*aS*,8*aS*)-polygodial (**21**), and repellent, methyl salicylate (**22**).

With continuing climate and environmental changes threatening to exacerbate aphid-induced problems (where milder temperatures have already incited earlier nymph hatching and a consequent proliferation in aphid numbers) ⁵⁸, research into alternative, sustainable management strategies is ongoing.

1.3.4 Applications of Aphid Pheromones

In recent years, chemical ecologists have investigated inter- and intra-species chemical communication between aphids, their host plants and their natural enemies, to underpin new pest management strategies in agriculture. By disrupting the olfactory signalling between aphids and other organisms, it is possible to alter aphid behaviour

and affect activities essential to the continuation of the species, such as their ability to locate mating partners ⁹. As naturally occurring compounds, insect semiochemicals are viewed as a relatively non-toxic and species-specific alternative to pest management which are sufficiently detached from the negative connotations linked to insecticide usage ^{52, 59}.

Some success has been achieved in utilising aphid sex pheromone components, lactol **1** and lactone **2**, in mating disruption and mass trapping ⁶⁰⁻⁶². In the former, male aphids are prevented from locating oviparae by spraying synthetic sex pheromone at the site of the crops. With mass trapping methods, incoming males are lured into containment prior to reaching the females. Through this method, high numbers of gynoparae were caught in addition to males, indicating that the aphid sex pheromone also acts as an aggregation pheromone ⁶⁰. The use of the aphid sex pheromone in the recruitment of further biological control to the site of aphid colonisation, as discussed previously, highlights a potential avenue for reducing aphid numbers through the influence of organisms that feed on, infect or parasitise aphids and has proven the most successful of the direct aphid management programs ^{28, 63-64}.

The combination of these strategies with other methods in an IPM system is likely to receive further success ^{63, 65-66}. The use of semiochemicals could be incorporated into a more comprehensive stimulo-deterrent diversionary, or 'push-pull' strategy, whereby crop plants are either masked from, or made unattractive or unsuitable for, the aphids (push) whilst simultaneously luring the pests to a source of attraction such as traps or trap crops (pull). For example, crop plants could be treated with lactol **1**, lactone **2**, and an antifeedant or repellent to signal the presence of aphids to natural enemies and

deter feeding and then planted alongside trap crops which have been selected for their kairomonal properties and treated with biological control agents ⁶⁷.

Research into the exploitation, and subsequent employment in the field, of lactol **1** and lactone **2** for aphid management purposes remains material-intensive. Mass cultivation and subsequent harvest of *N. cataria* permits mass production of lactone **2** with 85-98% purity by steam distillation at a vastly reduced cost compared to preceding methods ⁶⁸. Sodium borohydride reduction of the distillate can then be used to produce nepetalactol, with 92% of the yield comprising a mixture of the required (1*R*,4*aS*,7*S*,7*aR*)-lactol **1** and the remaining 8% (1*S*,4*aS*,7*S*,7*aR*)-lactol **1**. This process has provided the materials for numerous studies into the application and use of the aphid sex pheromone as a field-based modifier of aphid behaviour. Prior to this, the pheromonal components were chemically synthesised with limited enantiomeric purity and on a smaller scale using (*S*)-citronellal, in a synthetic procedure described initially by Schreiber and co-workers ⁶⁹.

Strategies for aphid pest management based on mass trapping and mating disruption, however, are limited to Aphididae species that enter a sexual phase. Aphids are predominantly an agricultural pest on their secondary summer hosts where reproduction is parthenogenetic (i.e. asexual). The spring population could be reduced as a direct consequence of reducing the autumn population, however, most management tactics utilising the aphid sex pheromone have only a 2-month autumnal exploitation window. The recruitment of beneficial natural enemies of aphids, which respond to aphid sex pheromone lures all-year-round, overcomes this issue and remains the most successful practical application of lactol **1** and lactone **2** in managing aphid numbers in the field. Numerous studies have demonstrated the attraction of aphid parasitoids, such as parasitic wasps, *A. ervi* and *Praon barbatum* (Braconidae:

Praon), to aphid sex pheromone lures ^{28, 64} and a definitive correlation between aphid sex pheromone-induced recruitment of primary aphid parasitoids and decreased aphid abundance in the field has recently been confirmed ⁷⁰. The attraction of aphid predator lacewings, such as *Chrysoperla occulta* (Chrysopidae: Chrysoperla), to formulations of lactol **1** and lactone **2** has also been demonstrated ²⁹.

Recent advances in genetic engineering of biosynthetic pathways in plants, capable of producing SLMs such as insect pheromones, provides a promising avenue for more sustainable pest management methods. Developing genetically modified (GM) crop plants that are resistant to, or deter, pest inhabitants, or that produce synomones as secondary metabolites in parasitoid/predator-inducing quantities, would overcome issues relating to the deployment of highly volatile and chemically unstable semiochemicals by releasing the SLMs on formation ⁷¹. In plants, expressing genes for aphid secondary metabolites, such as pheromones, complicates the resistance response in the aphids, as selection for resistance would involve a physiologically demanding alteration in their response to inherent molecules rather than unrelated toxicants.

Aphid alarm pheromone EBF **5** is a common plant secondary metabolite, making it possible to heterologously express the required genes in crop plants. The model plant thale cress, *Arabidopsis thaliana* (Brassicales), and more recently wheat crops have been genetically modified to produce pure **5** in order to recruit parasitic wasps to the site, induce aphid dispersal and mediate wing induction ^{36-37, 72-74}. Despite successful laboratory-based behavioural assays, field trials were not effective in reducing aphid numbers or increasing parasitism, however further investigation into inclusive

production is required. There is also potential for this technology to focus on other semiochemicals.

To take advantage of the aphid sex pheromone for biotechnology-based pest management purposes, the biosynthetic pathway involved must be understood. Ongoing technological advances provide an excellent opportunity to finally tap into the aphids' inherent, but poorly understood, route to secondary metabolites such as lactol **1** and lactone **2** and the complete published *A. pisum* genome sequence should aid the characterisation of enzymes involved. To determine the biosynthetic pathways to natural products such as lactol **1** and lactone **2**, it is essential to understand their route of origin.

1.4 Biosynthetic Pathways

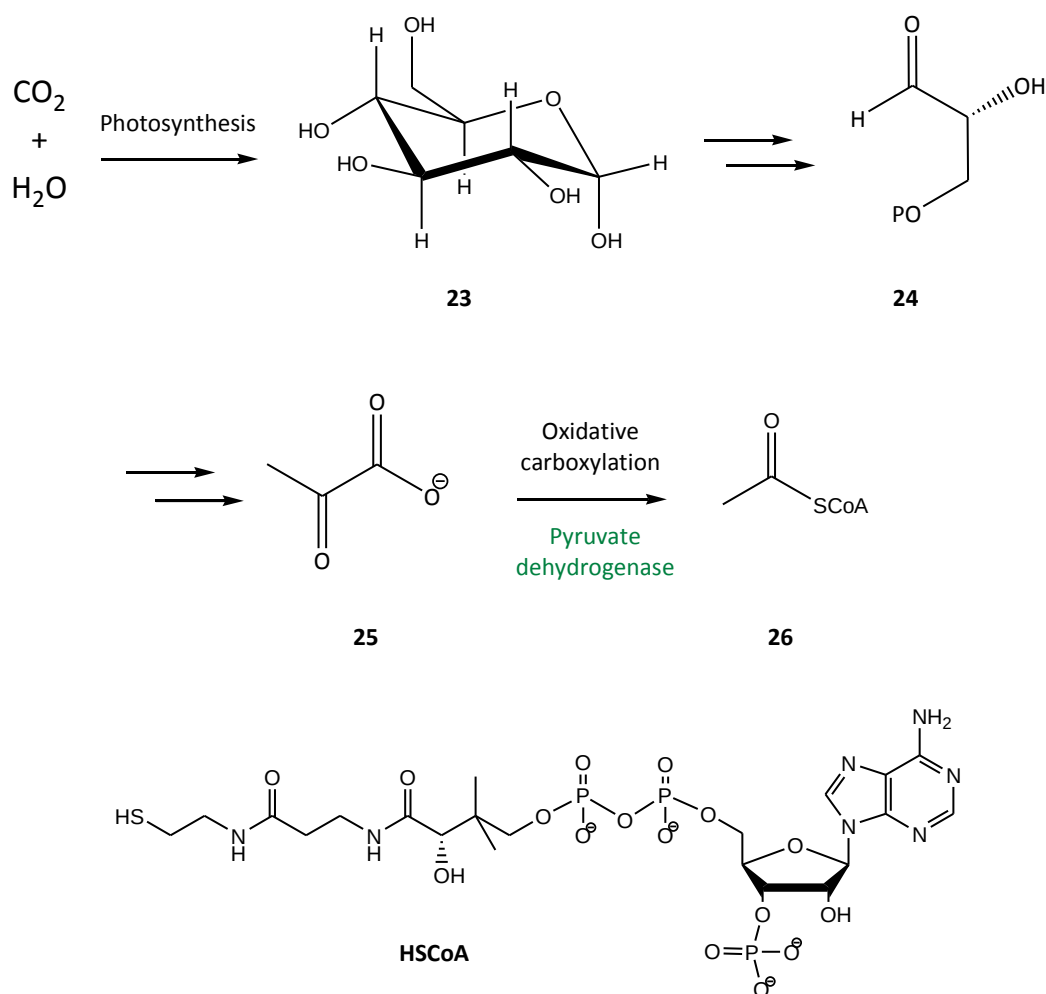
The majority of primary metabolic processes are common to all cellular organisms and utilise the sugar products of photosynthesis to provide the starting materials for secondary metabolic processes⁷⁵. The structural identity of secondary metabolites, as well as the processes by which they are biosynthesised, are often unique to members of a narrower taxonomic ranking⁷⁶. Insects can sequester many precursors from their food source and use the requisitioned compounds either directly or following structural modification. Alternatively, they can synthesise precursors *de novo* using products of primary metabolic processes *via* a variety of either fully elucidated or hypothesised biosynthetic pathways.

1.4.1 Isoprenoid Biosynthesis

Isoprenoids are chemically modified terpenes produced by both eukaryotes and prokaryotes. They are composed of five-carbon isoprene units and named according to the number of isoprene multiples incorporated; hemiterpenes (C₅), monoterpenes (C₁₀), sesquiterpenes (C₁₅), diterpenes (C₂₀) etc.⁷⁷ Owing to their low molecular weight, high lipophilicity, often volatile nature and high degree of structural and functional diversity, many isoprenoids derived from monoterpenes and sesquiterpenes are utilised in nature as semiochemicals which often require rapid gas-phase dispersal. Two biosynthetic routes to terpene precursors have been established, namely the mevalonate (MVA) and the methylerythritol phosphate (MEP) pathways. Both pathways utilise products of glycolysis.

1.4.1.1 Glycolysis

Glycolytic conversion of D-glucose (**23**) and other sugars, proceeding *via* D-glyceraldehyde 3-phosphate (**24**), gives pyruvate (**25**) (Scheme 1.1). Both **24** and **25** are used in the MEP pathway, whereas subsequent oxidative decarboxylation, catalysed by pyruvate dehydrogenase, is required to form the building block for the MVA pathway, acetyl-S-CoA (**26**) (Scheme 1.1)⁷⁸. Despite the variation in starting materials, both pathways produce the same universal terpene precursors, isopentenyl diphosphate (IDP) (**27**) and dimethylallyl diphosphate (DMADP) (**28**) (Section 1.4.1.2).



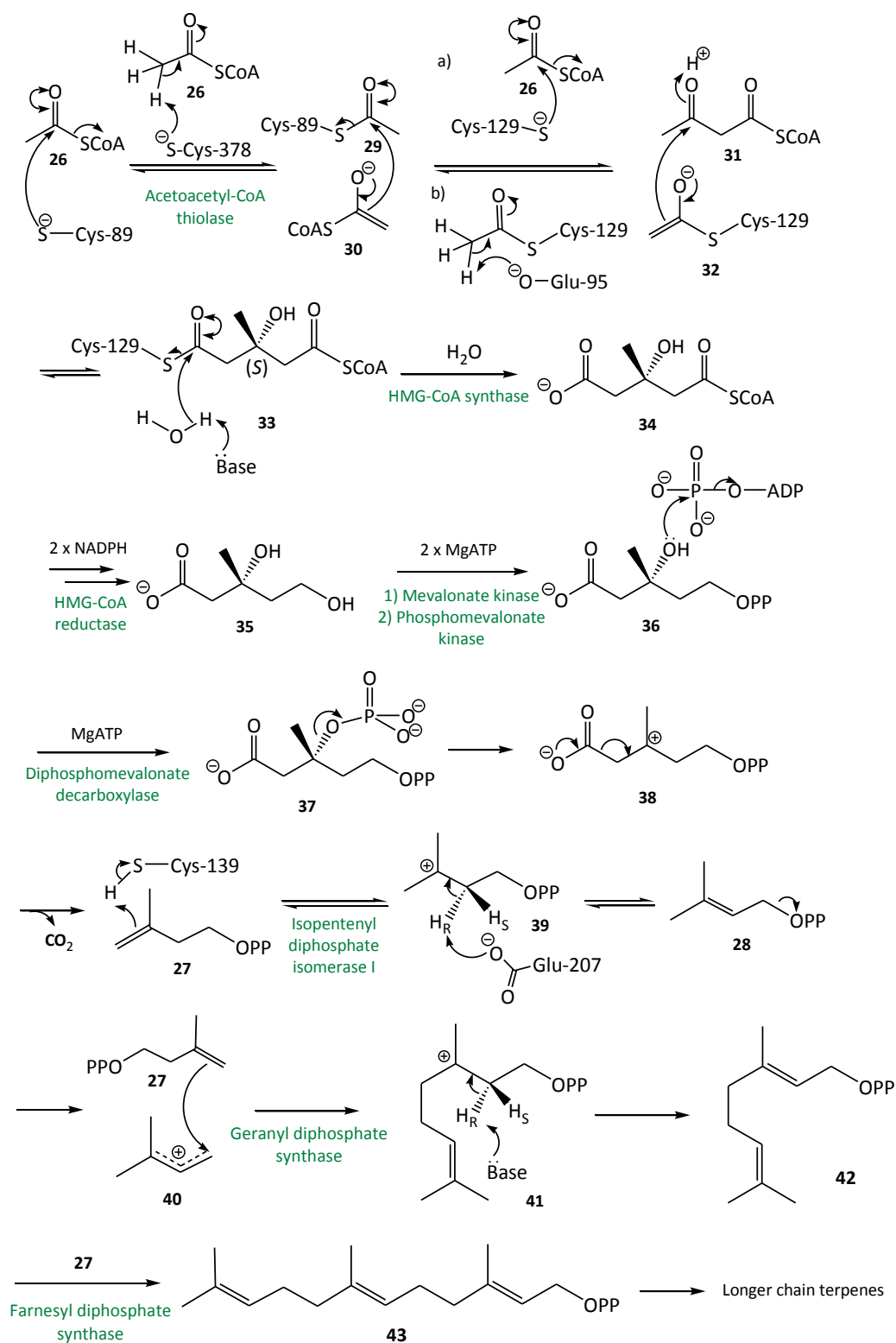
Scheme 1.1. Glycolysis leading to the formation of acetyl-SCoA (**26**). OP: phosphate group; HSCoA: Coenzyme A.

1.4.1.2 The Mevalonate Pathway

Fully characterised in 1967 by Wieckowski and Goodwin, the mevalonate (MVA) pathway was found to generate terpenes in the cytosol, mitochondria, endoplasmic reticulum and peroxisomes of most eukaryotes, including insects⁷⁹⁻⁸¹. This biosynthetic pathway, shown in Scheme 1.2, was first realised during isotopic labelling studies to determine the biosynthesis of cholesterol⁸². An initial thiolase-catalysed Claisen condensation between acetyl-SCoA (**26**)-derived acetyl-S-Cys-89 (**29**) and its Cys-378-deprotonated equivalent (**30**) gives acetoacetyl-SCoA (**31**). This reacts *via* a stereospecific aldol condensation with another **26**-derived unit, Glu-95-deprotonated

acetyl-S-Cys-129 (**32**), to give, through dithioester intermediate (**33**), 3-hydroxy-3-methylglutaryl-S-CoA (HMG-CoA; **34**)⁸³. The latter reaction requires water and HMG-CoA synthase.

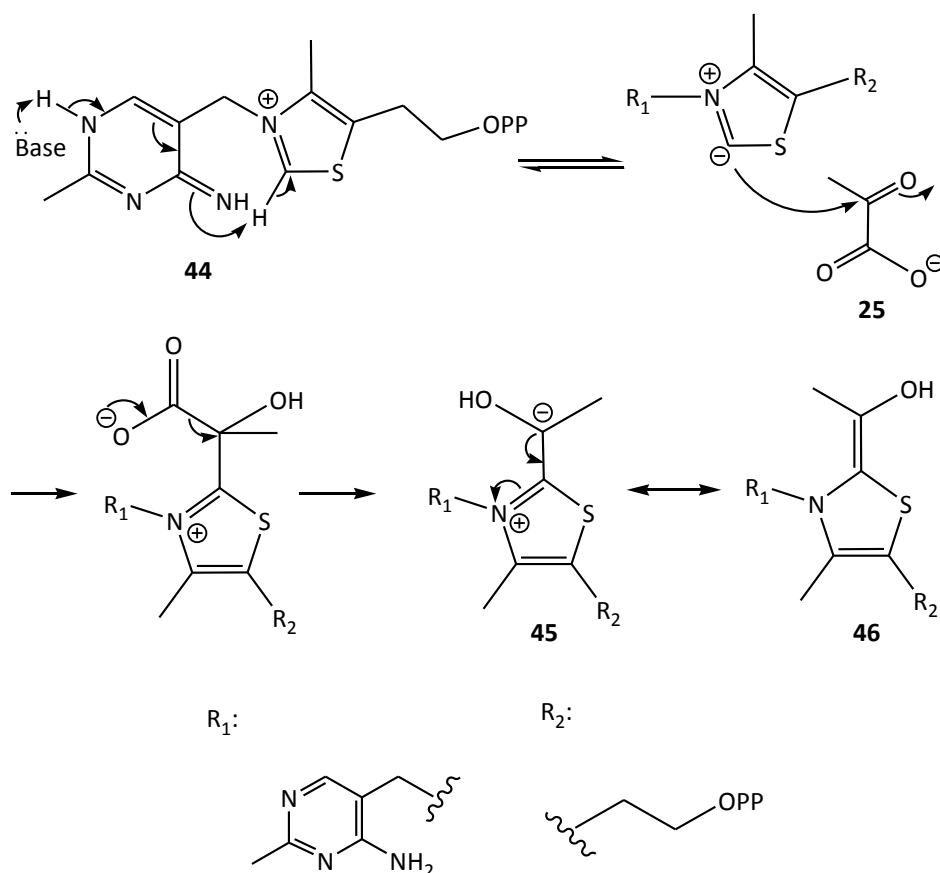
Reduction of the thioester moiety of **34** to (3*R*)-mevalonate (**35**) proceeds *via* a rate-limiting, NADPH-dependent step catalysed by HMG-CoA reductase⁸⁴, before ATP-driven phosphorylation to (3*R*)-mevalonate-5-diphosphate (**36**) with mevalonate kinase and phosphomevalonate kinase. Subsequent diphosphomevalonate decarboxylase-driven, ATP-dependent decarboxylation-elimination of **36** gives isopentenyl diphosphate (**27**). Isomerisation of IDP **27** to dimethylallyl diphosphate (**28**) is catalysed by isopentenyl diphosphate isomerase I and proceeds *via* protonation of the carbon-carbon double bond and deprotonation of the *pro-R* hydrogen of the resulting carbocation (**39**)⁸⁵⁻⁸⁶. Geranyl diphosphate synthase, a divalent metal ion-dependent prenyl transferase, catalyses the combination of 5-carbon units IDP **27** and its dephosphorylated isomer **40**, to generate carbocation intermediate **41**. Subsequent deprotonation of the *pro-R* hydrogen of **41** gives 10-carbon geranyl diphosphate (GDP) (**42**), the precursor to monoterpenes^{78, 80-81, 87}. Longer chain terpenyl diphosphate compounds are formed upon further addition of IDP (**27**) units and other prenyl transferases such as farnesyl diphosphate synthase, leading firstly to 15-carbon farnesyl diphosphate (FDP) (**43**) from which sesquiterpenes, such as aphid alarm pheromone component **5**, are derived⁸⁸.



Scheme 1.2. The MVA pathway leading to isoprenoids. CoA: Coenzyme A; OPP: diphosphate group; Cys: cysteine; Glu: glutamate.

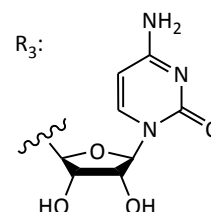
1.4.1.3 The Methylerythritol Pathway

In 1993, Rohmer and colleagues discovered an alternative route to IDP (**27**) and DMADP (**28**) through the combination of glycolysis products **24** and **25** ⁸⁹. Named the methylerythritol phosphate (MEP) pathway, it was found to occur exclusively in the plastids of plants, bacteria and algae ⁹⁰, and synergistically with the MVA pathway in plants ⁹¹. It is now recognised that, *in planta*, sesquiterpenes and triterpenes originate largely from the cytosolic MVA pathway, while hemiterpenes, monoterpenes, diterpenes and tetraterpenes from the plastidial MEP pathway ⁹². Thiamine diphosphate (**44**)-assisted activation of pyruvate (**25**) is initially required, involving decarboxylation and the subsequent formation of activated intermediate **45** in equilibrium with its enolamine equivalent (**46**) (Scheme 1.3) ⁷⁸.



Scheme 1.3. Thiamine diphosphate (**44**)-assisted activation of pyruvate (**25**). OPP: diphosphate group.

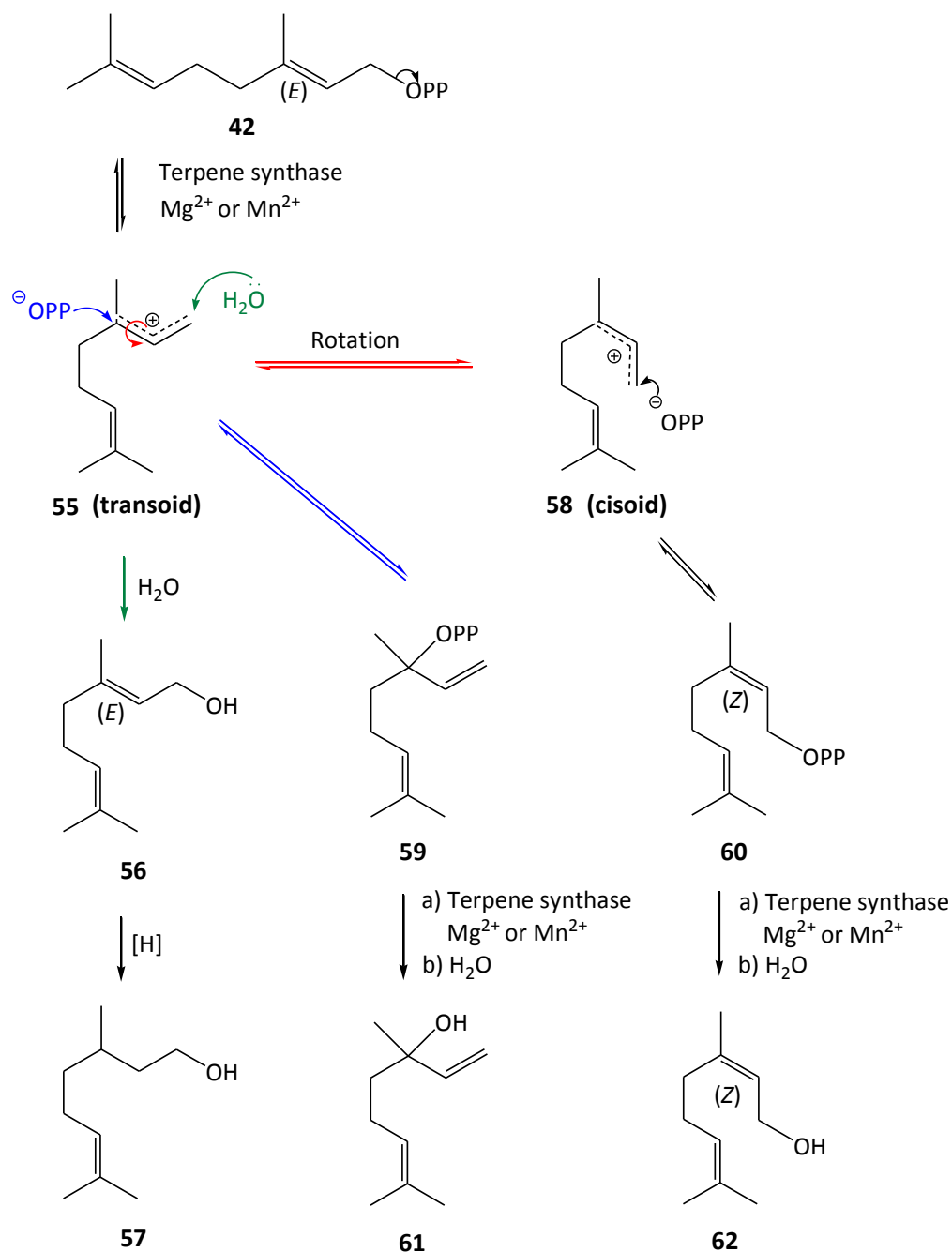
Nucleophilic attack of carbanion **45** on the carbonyl of D-glyceraldehyde 3-phosphate (**24**) and deprotonation of the resulting intermediate gives 1-deoxy-D-xylulose 5-phosphate (**47**) (Scheme 1.4). Either a reverse aldol-aldol reaction (as shown) or α -ketol rearrangement of **47** forms aldehyde **48** which undergoes NADPH reduction to 2-C-methyl-D-erythritol 4-phosphate (**49**). Triol **49** reacts with cytidine 5-triphosphate (**50**) to give 4-diphosphocytidyl-2-C-methyl-D-erythritol (**51**) which, after deprotonation and nucleophilic attack of the resulting alkoxide on ATP, is phosphorylated to 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate (**52**). Enzyme-catalysed intramolecular cyclisation of **52** gives 2-C-methyl-D-erythritol 2,4-cyclophosphate (**53**) *via* nucleophilic attack of the C-2 phosphate hydroxyl group on the cytidyl β -phosphate group⁹³. Phosphoanhydride **53** is stabilised in a positively charged enzyme pocket and bound to a [4Fe4S] cluster protein catalyst, facilitating ring-opening, reduction and final release of 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate (**54**) *via* an E_{1cB} elimination. The reduction and associated dehydroxylation of **54** to 5-carbon units **27** and **28** proceeds *via* a similar catalytic mechanism using an iron-sulfur cluster⁹³.



OP: phosphate group; OPP: diphosphate group.

1.4.1.4 Cationic Terpene Biosynthesis

Multi-product terpene synthases act on terpene diphosphate-derived carbocation precursors to produce terpenes (Scheme 1.5). As with prenyl transferases, terpene synthases are considered dependent on divalent metal ions, such as Mg^{2+} or Mn^{2+} , which bind the diphosphate moiety cleaved during GDP **42** ionisation⁹⁴. Through this, a resonance-stabilised geranyl carbocation (**55**) is generated which is hydrated to geraniol (**56**). Subsequent reduction of **56** provides 2,3-saturated citronellol (**57**) in the presence of an oxidoreductase. Alternatively, isomerisation of transoid cation **55** provides its cisoid neryl cation form (**58**) and subsequent phosphorylation of either **55** or **58** generates linalyl diphosphate (**59**) or neryl diphosphate (**60**), respectively. Terpene synthase-mediated dephosphorylation of **59** and **60**, followed by nucleophilic attack of water, yields linalool (**61**) and nerol (**62**), respectively (Scheme 1.5).

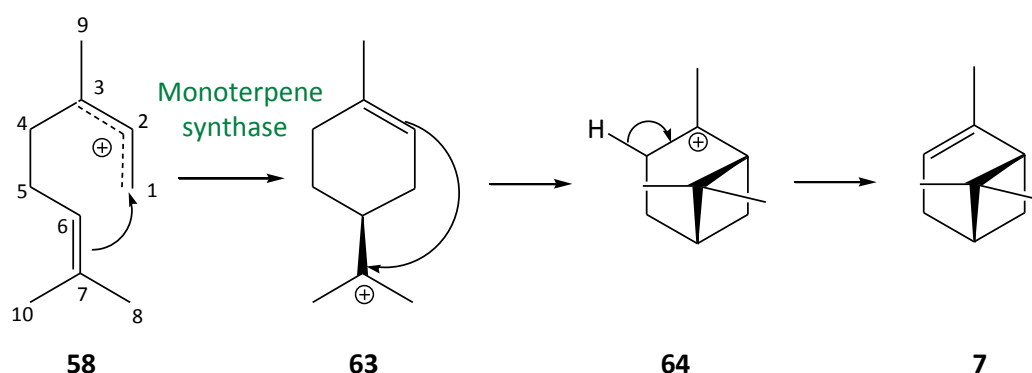


Scheme 1.5. The action of terpene synthases on monoterpene diphosphates.

OPP: diphosphate group.

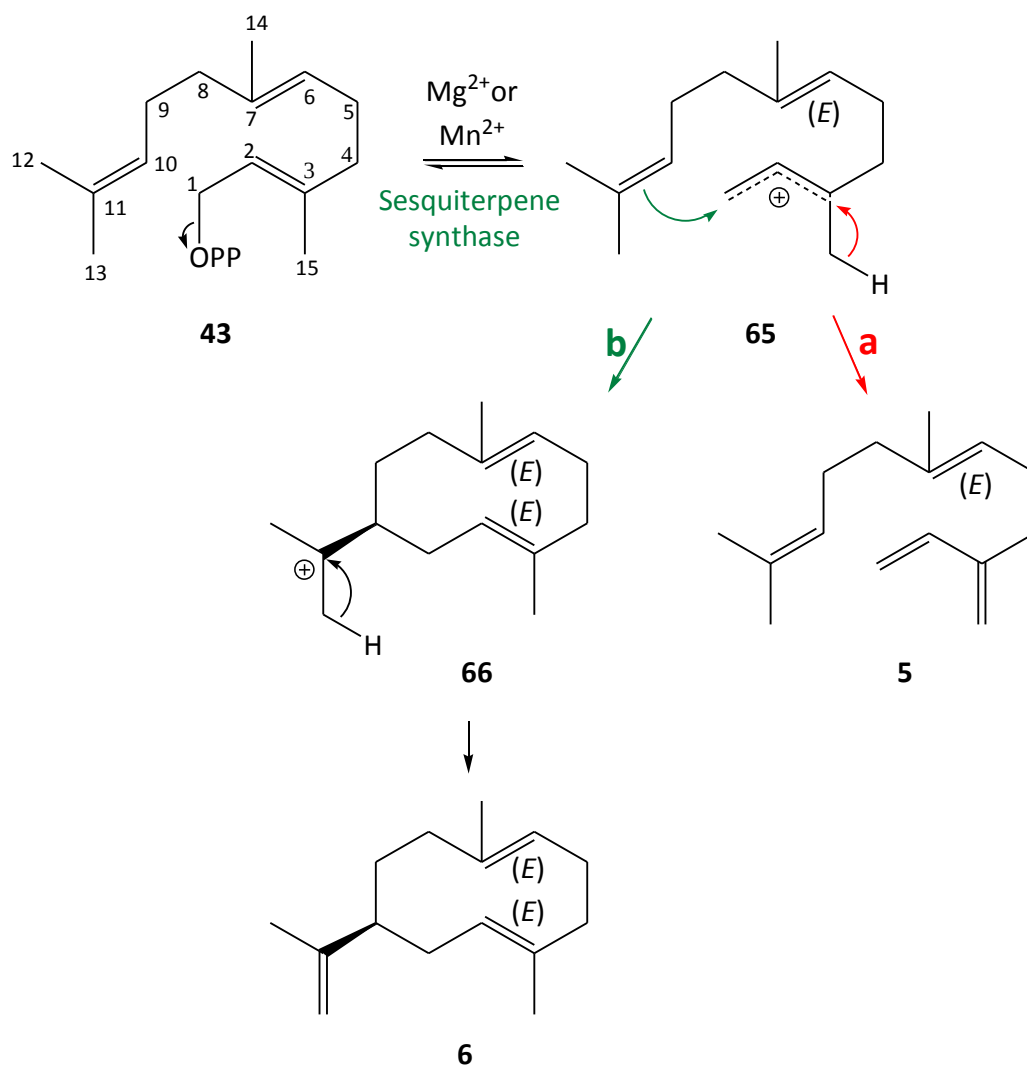
Carbocations **55** and **58** act as substrates for a variety of terpene synthases, resulting in various rearrangements, cyclisations and hydride shifts, prior to termination by deprotonation or hydration⁹⁵. Through these secondary transformations, a vast range of isoprenoid structures have been realised⁹⁶. Although no aphid terpene synthases

have been reported to date, putative biosynthetic pathways to aphid alarm pheromone components, **5**, **6** and **7** (Section 1.3.2), have been proposed (Schemes 1.6 and 1.7) ⁸⁸. Neryl cation **58** is thought to undergo a monoterpene synthase-catalysed double intramolecular cyclisation (Scheme 1.6). A 1,6- and subsequent 2,7-cyclisation gives cationic intermediate **64**, before termination by deprotonation to aphid alarm pheromone component, (1*S*,5*S*)- α -pinene **7**.



Scheme 1.6. Putative biosynthetic pathway to (1*S*,5*S*)- α -pinene (**7**) from carbocation **58**.

Similarly, sesquiterpene synthase-mediated biocatalysis of farnesyl diphosphate **43** produces farnesyl cation **65**. This can undergo either deprotonation to form principal aphid alarm pheromone component EBF **5** (Scheme 1.7; route a) or isomerise to (*E,E*)-germacradienyl cation **66** *via* an intramolecular 1,10-ring closure before deprotonation at C-13 to yield alfalfa aphid alarm pheromone component (1*E*,5*E*,8*S*)-germacrene A **6** (Scheme 1.7; route b).



Scheme 1.7. Putative biosynthetic pathway to EBF (**5**) (route **a**) and (1E,5E,8S)-germacrene A (**6**) (route **b**) via a common intermediate, farnesyl cation **65**.

1.4.2 Iridoid Biosynthesis

In comparison to the well-established route of cationic terpene biosynthesis discussed (Section 1.4.1.4), the biosynthesis of iridoids in plants and animals follows a different mechanism. It is widely accepted that the formation of the 5,6-membered bicyclic iridoid structure, such as that seen in lactol **1** and lactone **2**, does not proceed via the cyclisation of cationic terpene species using terpenyl diphosphates as the direct

cyclisation precursors but, instead, *via* the sequential oxidation of monoterpenols to their corresponding dialcohols and dialdehydes prior to cyclisation⁹⁷⁻⁹⁸.

The iridoid family represents a highly prevalent class of secondary metabolites found in both insects and plants. Since their first structural identification as methylcyclopentapyran monoterpenoids in 1958 (Figure 1.10A)⁹⁹, over 2500 iridoids have been characterised. Iridoids typically exist as aglucones or, as is common in plants, as water-soluble glucoside derivatives. Upon attack by pathogens or herbivores, plants release iridoids following the action of β -glucosidases on the plant's iridoid glucosides. For generalist insects, iridoids can have a toxic effect as a result of their aglucone activity, ultimately reducing survival rates and acting as a deterrent. However, for adapted aphids, they can be a phagostimulant¹⁰⁰. A subclass, known as the secoiridoids, arise *via* cleavage of the 6,7-bond in the iridoid cyclopentane ring (Figure 1.10B) and these, along with iridoids themselves, not only act as many insect semiochemicals but also possess a range of pharmacological activities¹⁰¹.

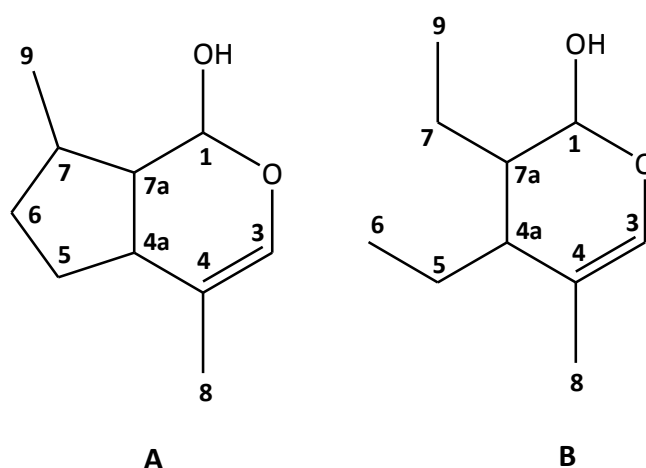
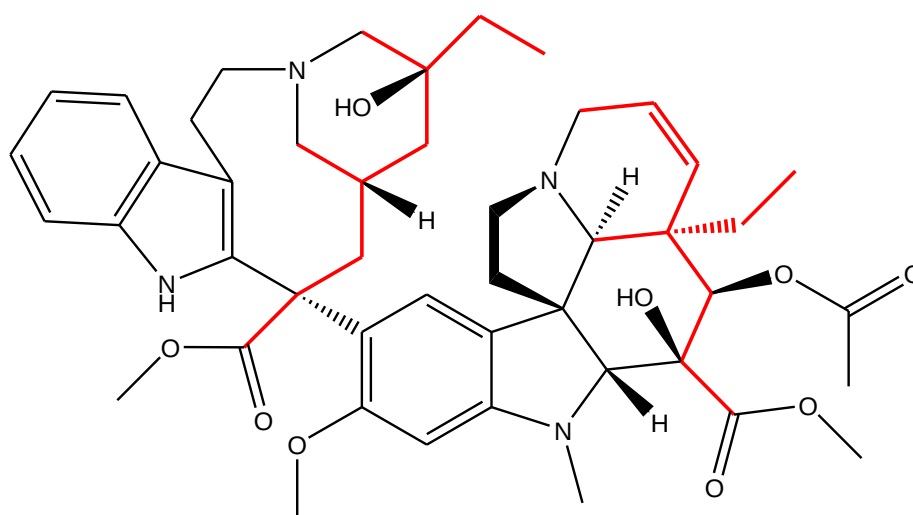


Figure 1.10. General structures of the (A) iridoid and (B) secoiridoid skeletons.

Iridoids and secoiridoids have received a great amount of attention over recent years due to their role as precursors to pharmacologically active compounds, including the

monoterpene indole alkaloids (MIAs) such as anti-cancer drug, vinblastine (**67**) (Figure 1.11) ¹⁰¹⁻¹⁰³. Sourced solely from *C. roseus* (Apocynaceae), vinblastine represents less than 6 µg/g fresh weight of the plant and is therefore associated with expensive isolation and purification procedures for little produce ¹⁰⁴.



67

Figure 1.11. The chemical structure of vinblastine (**67**) with geraniol (red) incorporated.

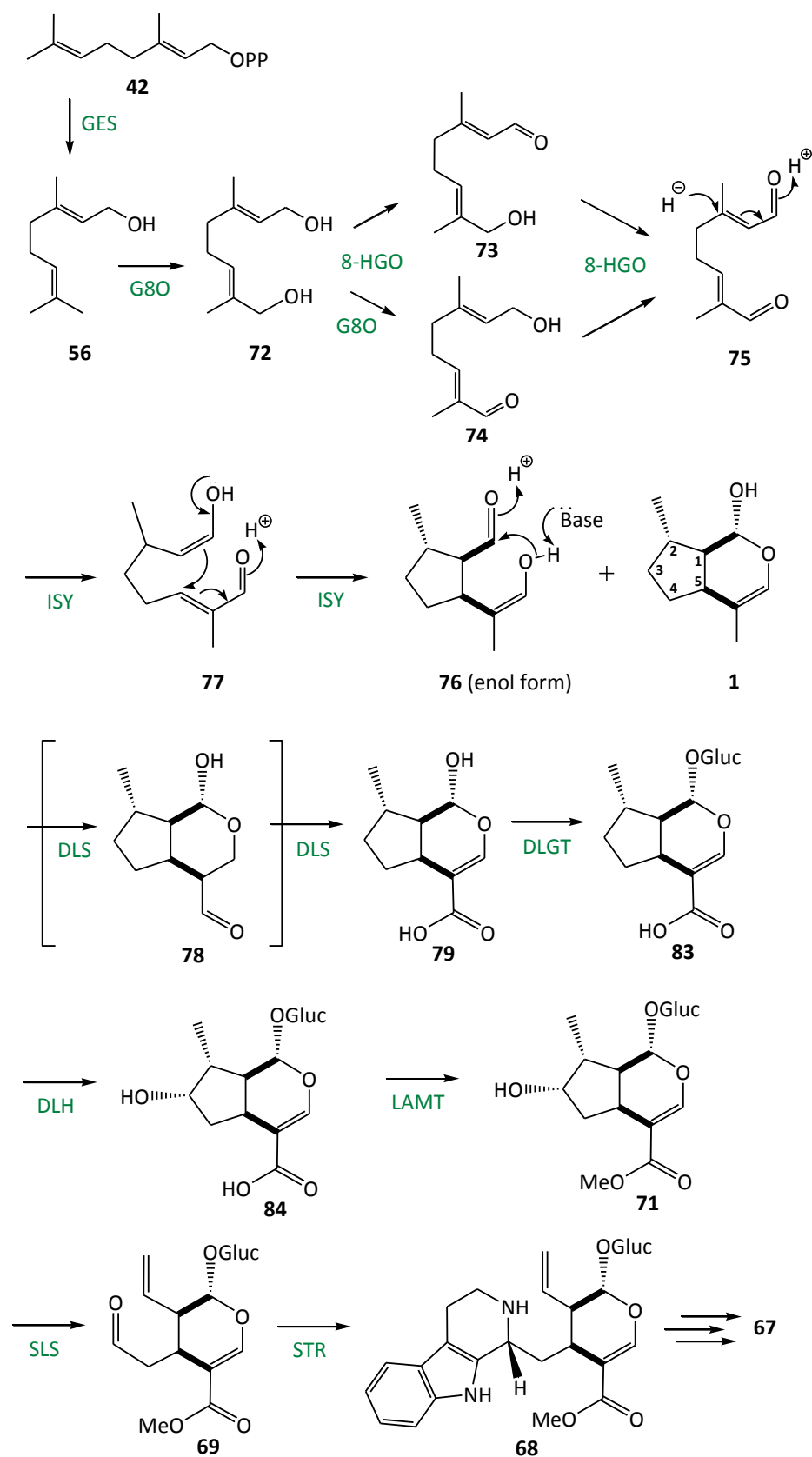
A simpler and more efficient route to their bicyclic iridane skeleton remains an attractive prospect as current synthetic procedures can be complex, expensive and time-consuming. The discovery and characterisation of enzymes involved in the biosynthetic routes to iridoids could improve the current synthetic approach to producing iridoid-based drugs and pesticidal compounds for integrated pest management (IPM) strategies. Plant-based research has been far more extensively conducted, however the discovery of insect-based enzymes may provide another valuable resource of, and potentially more commercially exploitable alternative to, biocatalysts.

1.4.2.1 Plant-Based Biosynthesis of Iridoids

Plants produce more diverse iridoids than insects, the majority present as their glucoside derivatives. On discovery of the bioactive nature of these compounds and their role as precursors to the indole alkaloids, elucidation of the biosynthetic pathways to these compounds became an active area of research in the 1970s. Owing to the diverse range of iridoids and monoterpene indole alkaloids (MIAs) that *C. roseus* produces, including vinblastine (**67**) (Section 1.4.2), the plant remains a prominent plant model for research into their biosynthesis ¹⁰⁵.

Common to the biosynthesis of all MIAs, vinblastine **67** is derived from precursor strictosidine (**68**), formed *via* the condensation of secoiridoid secologanin (**69**) and tryptophan-derived tryptamine (**70**) in the presence of strictosidine synthase (STR) (Scheme 1.8) ¹⁰⁴. Tryptamine biosynthesis was elucidated very early on but the pathway to secoiridoid **69** remained unsubstantiated. Developments in molecular biology have allowed for the complete structural and enzymatic elucidation of the iridoid pathway in *C. roseus* (Scheme 1.8), and the possibility of designing engineering platforms for the large-scale production of these commercially important plant products in heterologous hosts ¹⁰⁶. Gaining access to the natural biosynthetic pathways of valuable plant-derived compounds also enables genetically engineered enhancement of these pathways for producing crops of added value, relating to nutrition and pest resistance, for example

¹⁰⁷.



Scheme 1.8. Biosynthetic pathway to vinblastine **67** in Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae), via secologanin **69**, precursor to MIAs.

Gluc: glucosyl group.

It was established that plant iridoids and their glucosides began with acyclic monoterpenol precursors, derived from the MVA and/or MEP pathway (sections 1.4.1.2 and 1.4.1.3) ¹⁰⁸⁻¹¹¹. Involvement of the MEP pathway was discovered after initial evidence, supporting the anticipated involvement of the MVA pathway, showed the incorporation of ¹⁴C-mevalonate **35** into *Nepeta cataria* (Lamiaceae)-derived lactone **2** ¹¹². Cross-talk between the two isoprenoid pathways has since been described and both pathways were found to contribute, although the MVA pathway to a lesser extent, to the biosynthesis of plant (seco)iridoid glucosides secologanin **69** and loganin **71** in plants such as *C. roseus* ¹¹³. In contrast to this, the biosynthesis of iridoid glucosides in the woodland strawberry, *Fragaria vesca* (Rosaceae) ¹¹⁴, and the nettle, *Lamium barbatum* (Lamiaceae) ¹¹⁵, has been found to involve solely the MVA or MEP pathway, respectively.

Through early radiolabelling studies, it was found that the acyclic dienyl monoterpenols, geraniol (**56**) and/or nerol (**62**), were accepted as precursors for a wide range of iridoid glucosides in the majority of plants ¹¹⁶. A common route in plants, and the one now fully established in *C. roseus* (Scheme 1.8), involves the sequential oxidation of monoterpenols **56** and/or **62** to their corresponding diols and diols, whereas 2,3-saturated monoterpenols, such as citronellol (**57**), and their oxidised derivatives, are often rejected as intermediates ¹¹⁷⁻¹¹⁸. Recently, the enzyme responsible for the dephosphorylation and hydroxylation of GDP **42** to geraniol **56**, named geraniol synthase (GES), was identified in *C. roseus* ¹¹⁹.

An oxidoreductase responsible for the dehydrogenation of allylic primary alcohols, including **56** and **62**, in the presence of NADP⁺ ¹⁰⁹ was originally characterised in the Indian shrub, *Rauwolfia serpentina* (Apocynaceae). The same enzyme was found to

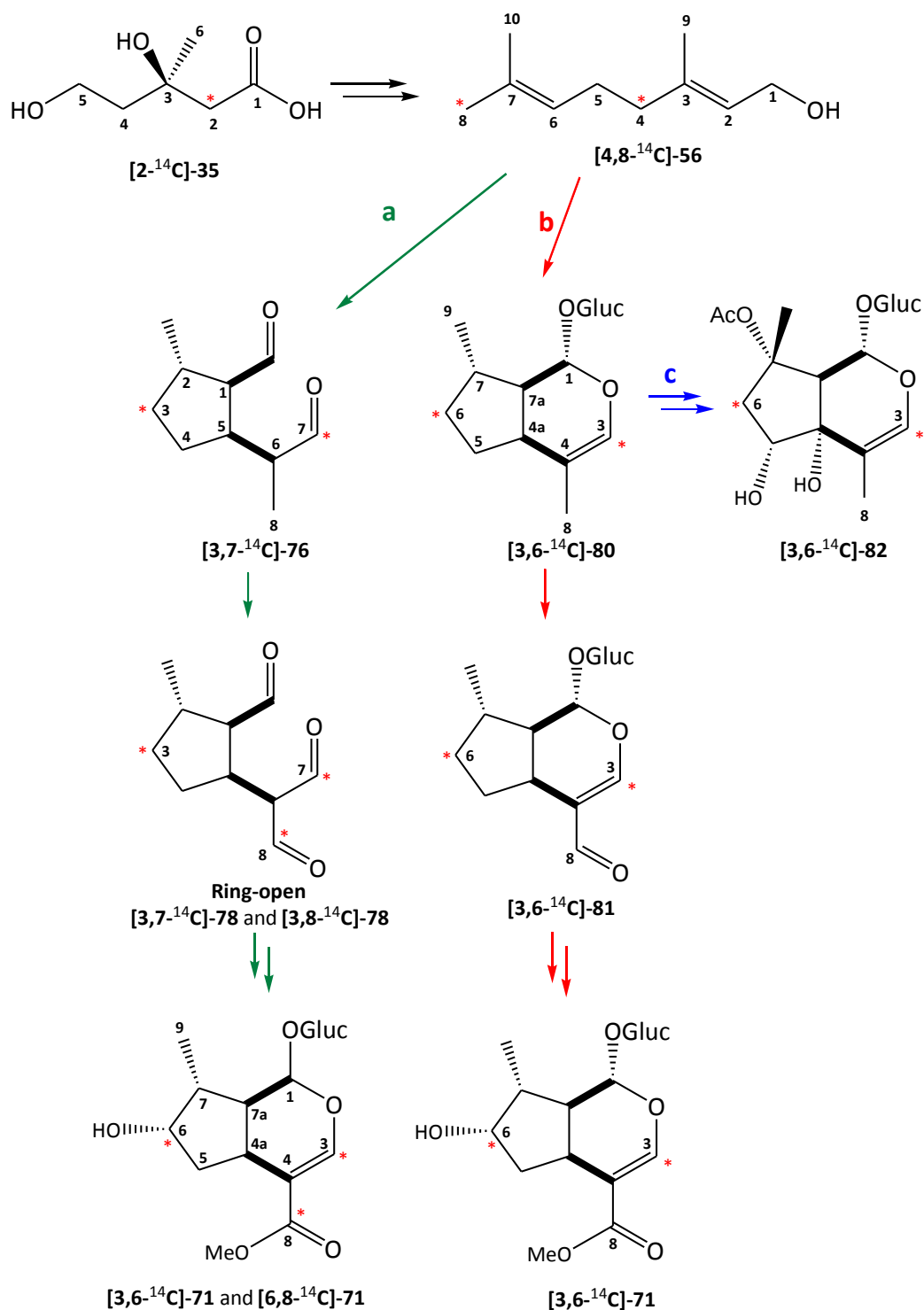
mediate the reversible dehydrogenation of diol **72** to hydroxyaldehyde **73**, or oxoalcohol **74**, and then dialdehyde **75** via the exclusive transferral of the *pro-R* hydrogen ¹²⁰. Subsequent cyclisation of substrate **75** to monocyclic (1*R*,2*S*,5*S*)-iridodial **76** also arose using crude enzyme extracts from *R. serpentina* ¹²¹. In the cytosol of *C. roseus*, the initial allylic oxidation at C-8, giving rise to 8-hydroxygeraniol **72**, was catalysed by cytochrome P450 enzyme CYP76B6, or geraniol 8-oxidase (G8O), and by other CYP76 enzymes in other plant species ¹²²⁻¹²³. G8O was also found to act as an hydroxylase towards 8-oxogeraniol **74** but was unable to metabolise 8-hydroxygeraniol **73** or further oxidised derivatives ¹²³. In *C. roseus*, further oxidation to intermediates **73** and/or **74**, and finally 8-oxogeraniol (**75**) was catalysed by NADP⁺-dependent 8-hydroxygeraniol oxidoreductase (8-HGO) ¹⁰⁴.

Dialdehyde **75** was recently confirmed as the cyclisation precursor to bicyclic lactol **1** and monocyclic iridodial **76**, co-synthesised in the presence of *C. roseus*-derived iridoid synthase (ISY) via a stereoselective, 1,4-reductive cyclisation reaction (shown in Scheme 1.8 as a 2-step process) ²⁷. Discovered through co-expression analysis, ISY is a homologue of the short-chain NADPH-dependent dehydrogenase, progesterone 5β-reductase (P5βR). Comparable to the P5βR-mediated reduction of progesterone's α,β-unsaturated carbonyl group, ISY was found to convert acyclic monoterpene **75** into enol (or enolate) intermediate **77** in the presence of hydride donor NAD(P)H ²⁷. A suspected Michael addition cyclisation of **77**, also mediated by ISY, was found to produce a mixture of closed-form lactol **1** and open-form iridodial **76** ¹⁰². This was found to be preferential over a Diels-Alder cyclisation step, demonstrated by incubating ISY with halogenated substrates, each primed to undergo only one of the two aforementioned mechanisms, and monitoring the enzymatic products ¹⁰². It is unlikely that *cis,trans*-fused lactol **1** and iridodial **76** interconvert under physiological conditions, as was originally suspected, but

are, in fact, co-produced ^{14, 102, 124}. Having obtained the crystal structure of ISY, the absence of certain lysine, cysteine and histidine residues in the active site made the involvement of a covalently-assisted cyclisation mechanism, prior to reduction, highly unlikely ¹²⁵. The isolation of reduced side products, such as 8-oxo-(*S*)-citronellal, in minor quantities provided evidence for an enzymatic mechanism involving a 'stand-alone' reduction preceding a cyclisation step ²⁷.

The oxidative reactions from 8-oxogeranial to monocyclic **76** and putative intermediate iridotrial (**78**) were found to be precursory to glucosylation in the production of many plant iridoid glucosides ^{104, 126-127}. From MVA-[2-¹⁴C] feeding experiments, the incorporation of [4,8-¹⁴C]-8-hydroxygeraniol (or its neryl equivalent) consistently led to a distribution of radioactive labelling between the C-3 and C-8 positions in iridoid glucosides from further down the iridoid pathway, such as loganin (**71**) in *C. roseus* ¹¹⁸. This scrambling of labels indicated that the C-8 and C-10 positions in the acyclic monoterpene precursors were oxidised to equivalency before the dihydropyran ring is fixed by glucosylation during the biosynthesis and, in turn, supported a mechanism involving oxidation followed by cyclisation (Scheme 1.9; route a) ¹²⁶. This phenomenon was also evident during the biosynthesis of other 8-oxygenated iridoid glucosides in plants such as *R. serpentina* and honeysuckles of the Lonicera genus (Caprifoliaceae) ¹⁰⁹⁻¹¹¹. Radiolabelled diol **72** and monocyclised dial **76** were incorporated into iridoid **71** to a much higher extent than acyclic 10-oxygenated compounds such as 8,10-dihydroxygeraniol and 8-oxo-10-hydroxygeranial, thereby supporting the conclusion that loganin **71** and the indole alkaloids of *C. roseus* are biosynthesised *via* direct cyclisation of acyclic diene **75** to iridodial **76**, followed by a successive three-step oxidation to deoxyloganetic acid (**79**) ¹¹¹.

Unexpectedly, the randomisation of labelling was not universally evident amongst plants of the same species and appeared to vary with the age of the plant ^{126, 128}. It was hypothesised that both the mechanism described and one where glucosylation takes place before the C-8 and C-10 methyl groups of the acyclic compounds (equivalent to the C-7 and C-8 methyl groups of monocyclic compounds **76** and **78**) become oxidised equivalently co-exist within certain plants, often taking place in young and mature plants, respectively ¹²⁹. Despite leading to identical products such as loganin **71**, this phenomenon is thought to benefit the plant by allowing for age-related differences in pool sizes of biosynthetic intermediates and biosynthesis. The dual system also allows for continued biosynthesis at different sites within the same plant ¹²⁸. The latter mechanism, common in older plants, maintains the non-equivalency of C-8 and C-10 and involves the intermediacy of iridodial glucoside (**80**) and iridotrial glucoside (**81**), therefore does not result in a scrambling of the isotopic labels (Scheme 1.9; route b). The same results were obtained with the C-8-methyl-bearing iridoid glucoside, lamioside (**82**), isolated from henbit deadnettle, *Lamium amplexicaule* (Lamiaceae) (Scheme 1.9; route c) ¹²⁶.



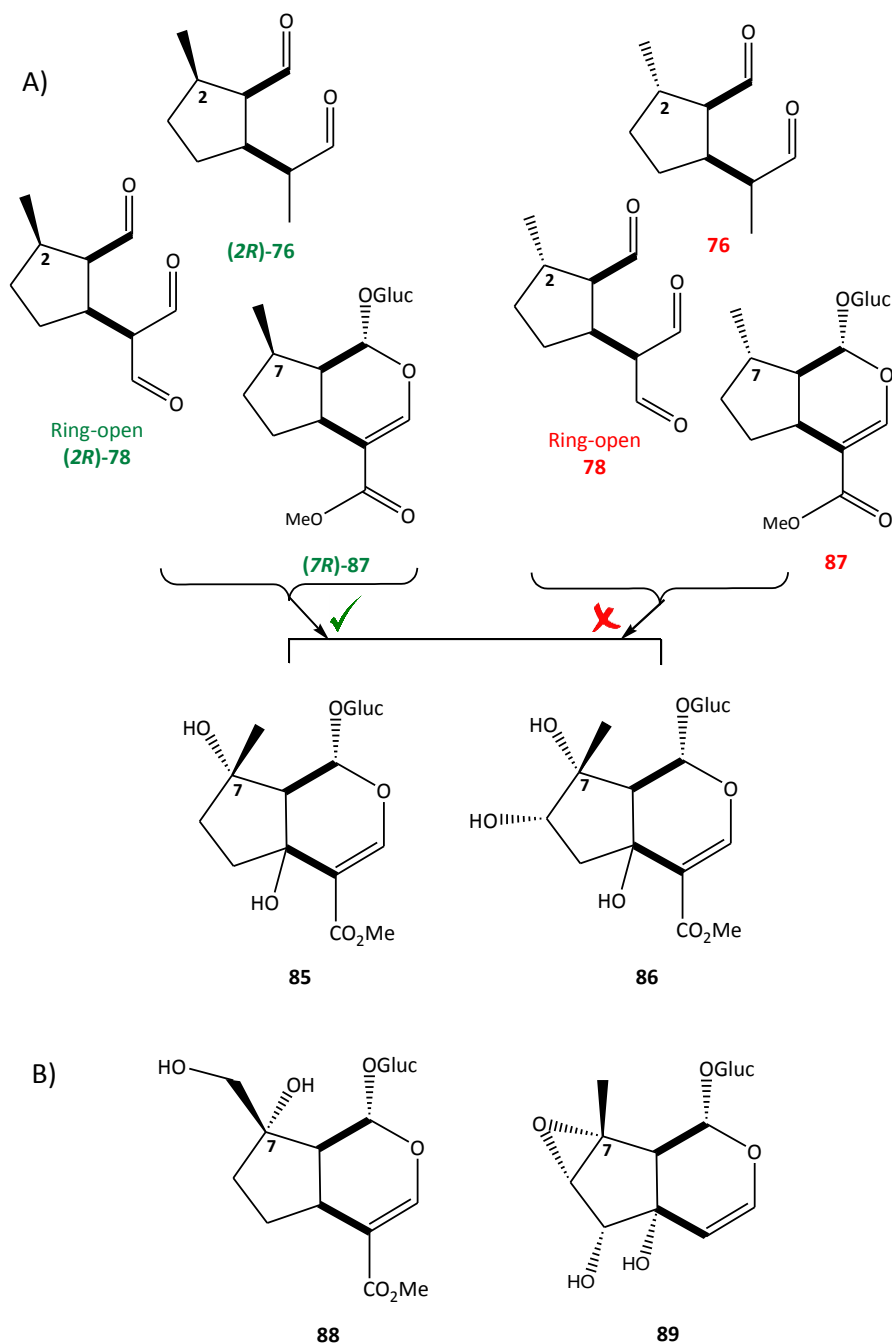
Scheme 1.9. Co-existing iridoid biosynthetic pathways in (route **a**) young and (route **b**) mature plants leading to variations in radiolabel scrambling in loganin (**71**) and (route **c**) lamioside (**82**) from Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae), and henbit deadnettle, *Lamium amplexicaule* (Lamiaceae), respectively. (* Indicates ¹⁴C-labelling).

Iridotrial **78** has never been isolated but was highly incorporated, alongside iridodial **76**, into iridoids such as loganin **71** and verbenalin in the common vervain, *Verbena Officinalis* (Verbenaceae), during isotopic labelling studies ¹²⁹⁻¹³⁰. Recently, cytochrome P450 enzyme CYP76A26, or deoxyloganetic acid synthase (DLS), was identified as the enzyme responsible for converting nepetalactol **1** to deoxyloganetic acid **79**, likely *via* trialdehyde **78** ¹³¹. Subsequent glucosylation of iridoid **79**, catalysed by deoxyloganetic acid glucosyltransferase (DLGT), gave deoxyloganic acid (**83**) ¹³² which, in turn, underwent deoxyloganic acid hydroxylase (CYP72A224; DLH)-mediated hydroxylation to loganic acid (**84**) ¹³³. Formation of the methylated product loganin **71** required loganic acid *O*-methyltransferase (LAMT)-driven catalysis ¹³⁴ before conversion to secologanin **69** in the presence of CYP72A1, or secologanin synthase (SLS) ¹³⁵. The conjunction of iridoid and amino acid-derived products, culminating in the biosynthesis of MIA precursor strictosidine (**68**), is catalysed by strictosidine synthase (STR) in a stereoselective Pictet-Spengler-type reaction ¹³⁶.

There are some known exceptions to the plant iridoid biosynthetic pathway outlined in Scheme 1.8. When examined, geranyl substrates could often be exchanged for their neryl equivalents without apparent significant effect, suggesting that the 2,3-double bond geometry has little effect on enzyme selectivity. Often, the (*E*)- and (*Z*)-derivatives are assumed interchangeable but examples exist where the neryl derivatives are not an active enzyme substrate, but the geranyl equivalents are (Section 1.4.2.2) ¹³⁷.

A similar biosynthetic pathway to the iridoid glucosides, ipolamiide (**85**) and lamiide (**86**), was also determined in a South African figwort, *Hebenstretia dentata* (Scrophulariaceae) ¹³⁸ but deuterium-labelled (2*R*)-iridodial ((**2R**)-**76**), (2*R*)-iridotrial ((**2R**)-**78**) and (7*R*)-deoxyloganin ((**7R**)-**87**) were far better incorporated into both

glucosides than their (2*S*)/(7*S*)-equivalents, **76**, **78** and **87** (Scheme 1.10A). This better explains the (7*R*)-stereochemistry of the glucoside products ¹³⁸⁻¹³⁹. Similar isotopic labelling results were obtained using cape jasmine, *Gardenia jasminoides* (Rubiaceae), for the biosynthesis of iridoid glucoside gardenoside (**88**) (Scheme 1.10B) ¹³⁰. Additionally, the (7*R*)-methyl group present in the iridoid antirrhinoside (**89**) (Scheme 1.10B) produced by snapdragon, *Antirrhinum majus* (Plantaginaceae), was recently found to arise *via* an inverted reductive cyclisation using the first-identified *epi*-ISY ¹⁴⁰.



Scheme 1.10. Preferential incorporation of the (2R)/(7R)-epimers over the corresponding (S)-equivalents of (A) iridodial (**76**), iridotrial (**78**) and deoxyloganin (**87**) into ipolamiide (**85**) and lamiide (**86**) of the South African figwort, *Hebenstretia dentata* (Scrophulariaceae), and (B) gardenoside (**88**) and antirrhinoside (**89**) of cape jasmine, *Gardenia jasminoides* (Rubiaceae) and snapdragon, *Antirrhinum majus* (Plantaginaceae), respectively.

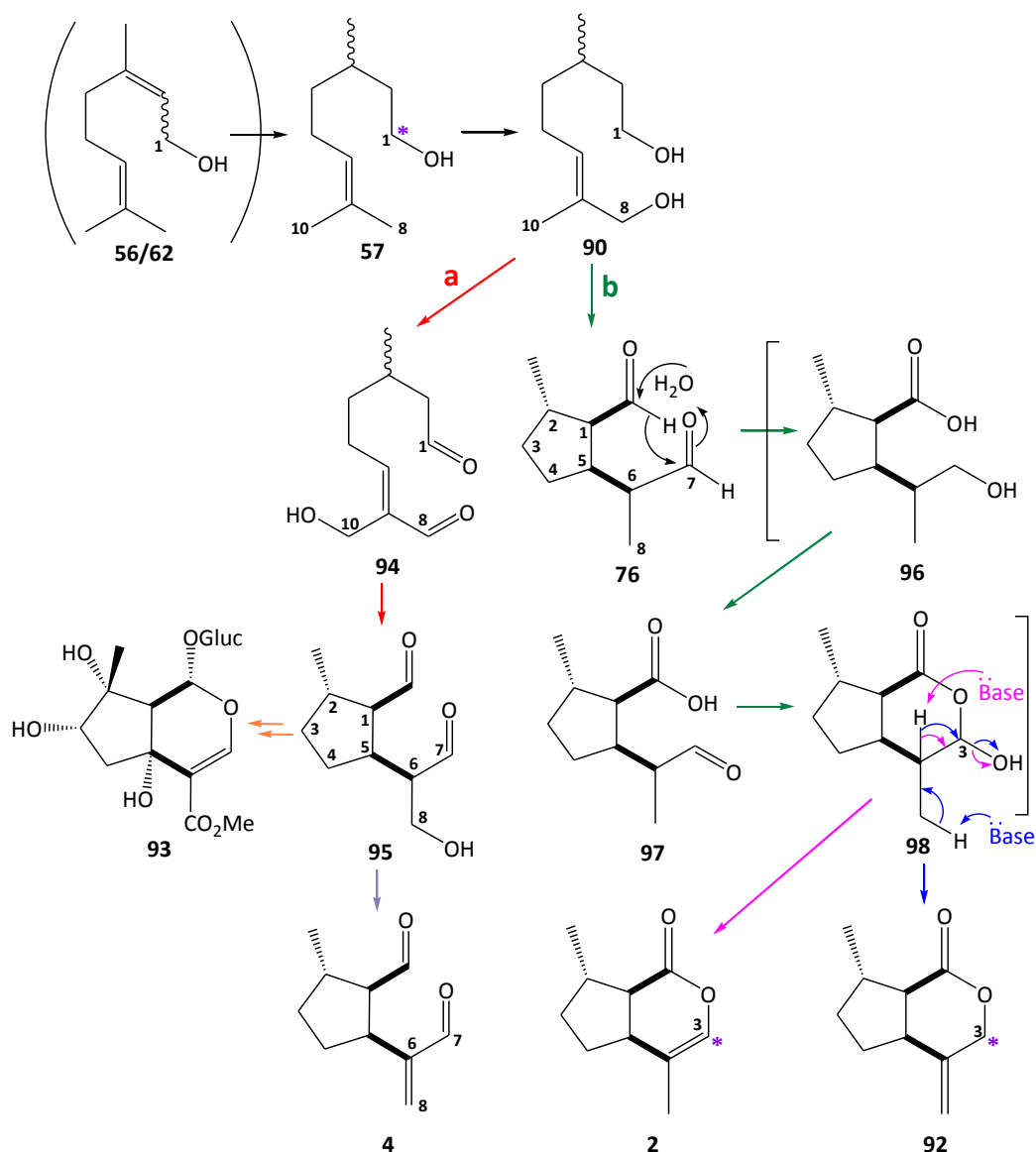
Despite the prevalent use of 2,3-unsaturated monoterpenol precursors for the biosynthesis of plant iridoid glucosides, a number of plant species were shown to favour

2,3-saturated monoterpenols as precursors, often to form structurally simpler or non-glucosidic iridoids. For the production of C-1-epimeric dolichodial **4** in cat thyme, *Teucrium marum* (Lamiaceae) ¹⁴¹, radiolabelling studies revealed that citronellol (**57**) and its related diol, 8-hydroxycitronellol (**90**), were far more efficient precursors than nerol **62** and its 8-hydroxylated derivative, 8-hydroxynерol (**91**) ¹⁴². The origin of monoterpenols **57** and **90**, however, remained uncertain and some considered them to be the putative products of geraniol **56** or nerol **62** reduction. Retained non-equivalency of positions C-7 and C-8 throughout biosynthesis (equivalent to positions C-8 and C-10, respectively, in acyclic **57** and **90**) was evidenced by a lack of label scrambling in iridoid **4**. The same results were obtained for the biosynthesis of dolicholactone (**92**), an iridoid also produced in *T. marum*, but in lower abundance (6-7% of **4**) ¹⁴³.

On feeding *T. marum* with radiolabelled iridodial ([**7-³H**]-**76**), poor incorporation was seen in iridoid **4**, suggesting that the 6,8-olefinic bond is preferentially formed in an oxidative process prior to ring closure rather than after closure by enzymatic dehydrogenation (Scheme 1.11; route a) ¹⁴². This was also witnessed during production of a third iridoid in *T. marum*, called teucrein (**93**) ¹⁴⁴. In contrast, iridodial **76** was a highly effective precursor for lactone **92**. From these results, it was hypothesised that dolichodial **4** and teucrein **93** follow a biosynthetic mechanism in *T. marum* that's similar to the route by which 8-oxygenated iridoid loganin **71** is produced in mature *C. roseus* plants. The intermediacy of 10-hydroxy-8-oxocitronellal (**94**) and 8-hydroxyiridodial (**95**) were proposed, in order to maintain the non-equivalency of C-7 and C-8 through to ring-closure (Scheme 1.11; route a).

During the production of **92**, a hydride shift from C-1 to C-3 was observed in the resulting iridoid on application of radiolabelled citronellol, [**1-³H**]-**57**, ¹⁴³. From this, it

was suggested that the heterocyclic ring of lactone **92** was formed *via* an intramolecular Cannizzaro-like reaction, possibly *via* intermediates 7-hydroxyiridoacid (**96**), 7-oxoiridoacid (**97**) and hydroxydiidronepetalactone (**98**) arising from nucleophilic attack of water at the C-1-aldehyde and a subsequent displacement of a hydride ion to C-7 in iridodial **76** (corresponding to C-3 in **92**) (Scheme 1.11; route b) ¹⁴³. The isotopic labelling results for dolicholactone **92**, including a preference for 2,3-saturated precursor **57**, mirrored those observed for (4a*S*,7*S*,7a*R*)-nepetalactone **2**, the major iridoid product of *N. cataria* and a minor product of dwarf catmint, *Nepeta racemosa* (Lamiaceae) ^{26, 145-146}. As a result, the mechanism proposed for the biosynthesis of lactone **92** was also deduced for lactone **2** (Scheme 1.11; route b). In support of this, 3-hydroxylated intermediate **98** was isolated in some *N. cataria* specimens ²⁶.



Scheme 1.11. Putative biosynthetic pathways to citronellol (**57**)-derived iridoids dolichodial **4** and teucrein **93** in cat thyme, *Teucrium marum* (Lamiaceae) (route **a**), and, *via* a hydride shift in iridodial **76**, to nepetalactone **2** and dolicholactone **92** in catmint, *Nepeta cataria* (Lamiaceae), and *T. marum*, respectively (route **b**).

* ^3H -labelling from C-1 in **57** to C-3 in **2** and **92**.

Despite the reported preference for 2,3-saturated monoterpenyl derivatives as precursors to lactone **2** in *N. cataria*, 8-oxogeranial (**75**) was recently successfully used to identify the ISY-equivalents in *N. cataria* and *N. mussinii* (Lamiaceae), responsible for cyclisation to the predominant products, *cis,trans* and *trans,cis*-lactone **2**, respectively¹⁴⁷. It was found that both species possess two ISY homologues, one more dominant

than the other and each with mutated lysine and glycine residues. On incubation with dial **75**, the *Nepeta* ISY product profile matched that observed using *C. roseus*, generating primarily *cis,trans*-nepetalactol **1** and its associated iridodial **76**. This suggests that the stereochemistry at the C-4a and C-7a positions in lactone **2** is established during a later epimerisation step rather than during cyclisation.

Common to all reaction mechanisms discussed, it was evident from the outcome of isotopic labelling studies that, despite a preference for specific precursors and intermediates, many plants have a certain degree of flexibility in the iridoid biosynthetic route that they use. This, seemingly, allows them to adapt to variable physiological conditions, both external and internal to the plant (such as precursor availability and site of synthesis, respectively) without hindering production of the iridoid.

1.4.2.2 Insect-Based Biosynthesis of Iridoids

Semiochemicals produced by insects often comprise iridoids and the characterisation of such compounds has been well researched¹⁴⁸⁻¹⁴⁹. The first insect-derived iridoids were discovered in the defensive secretion of the Argentinian ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae), from which the term 'iridoid' originates¹⁵⁰. The secretion was found to comprise *cis*-fused compounds, (4*S*,4*aS*,7*S*,7*aR*)-iridomyrecin (**99**) (Figure 1.12)¹⁵¹ and (1*R*,2*S*,5*S*)-dolichodial (**4**)¹⁵². The latter, alongside (1*R*,4*aS*,7*S*,7*aR*)-nepetalactol **1**, is a component of the defensive secretions for many ants of the dolichoderinae subfamily. The emission of iridoid **4** from ants also serves a mutually beneficial relationship with certain Australian butterflies which require ant guards to ensure the survival of their larvae¹⁵³.

A diastereoisomer of **4**, known as anisomorphal (**100**) (Figure 1.12), was identified as a component of the defensive secretion of the Southern walking stick insect, *Anisomorpha buprestoides* (Phasmida: Phasmatidae), and was used to confirm, for the first time, the involvement of the MVA pathway in insects through the incorporation of ^{14}C -mevalonolactone into **100**¹⁵⁴. The structurally related iridoid, (1*R*,2*S*,5*S*)-iridodial (**76**), and/or its related stereoisomers, are found as constituents of defensive secretions for many insects, including several ant species of the *Iridomyrmex* genus¹⁵⁵⁻¹⁵⁷ and also the coconut stick insect, *Graeffea crouani* (Phasmida: Phasmatidae), alongside aphid sex pheromone component **2**¹⁵⁸. Iridoid **76** was also identified as a male aggregation pheromone for the Golden Eye Lacewing, *Chrysopa oculata* (Neuroptera: Chrysopidae), a known aphid predator¹⁵⁹⁻¹⁶⁰.

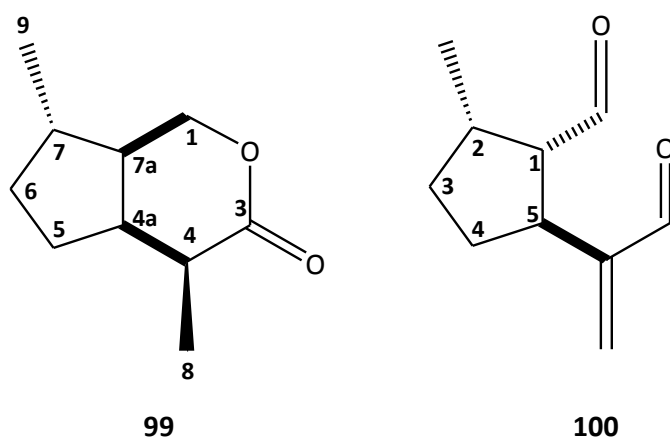
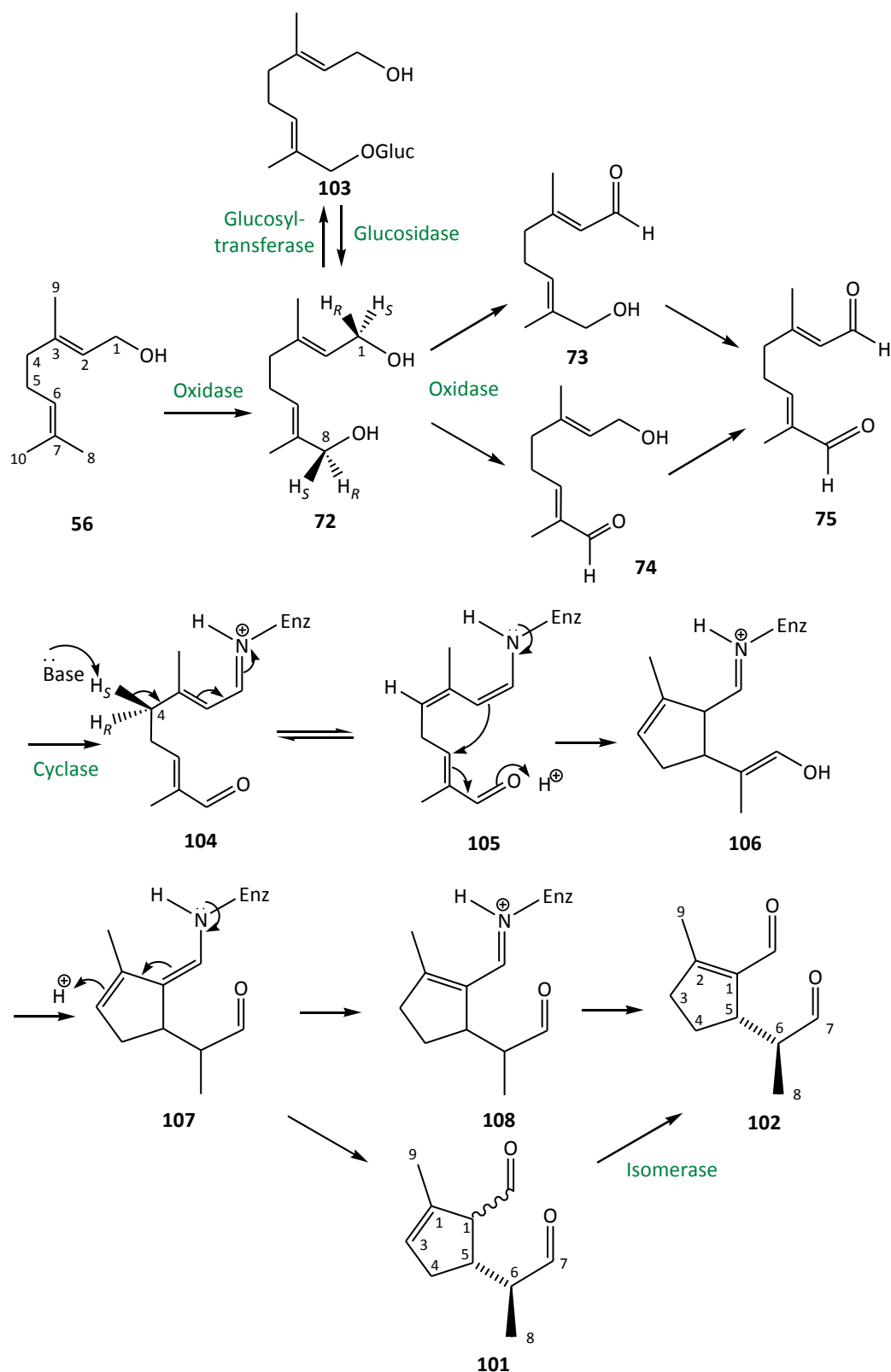


Figure 1.12. The chemical structures of the insect-derived iridoid compounds, (4*S*,4*aS*,7*S*,7*aR*)-iridomyrecin (**99**) and (1*S*,2*S*,5*S*)-dolichodial (anisomorphal; **100**).

Research into the biosynthesis of insect-derived iridoids is less advanced compared to plants. This is partially attributable to the lower prevalence of iridoids in insects and the more complex nature of achieving isotopic label incorporation. Studies conducted so far have revealed distinct similarities between plants and insects⁹⁷. The most extensive research into insect-based iridoid biosynthesis has centred around species belonging to the order Coleoptera. Larvae of several Coleopteran beetles, including herbivorous leaf

beetles of the Chrysomelidae family ^{148, 161} and carnivorous rove beetles of the Staphylinidae family ¹⁶², produce *de novo* defensive secretions comprising the iridoid compounds, plagiodial (**101**) and chrysomelidial (**102**) (Scheme 1.12). Upon disturbance, both are released from glandular reservoirs, everted by haemolymph (insect circulation) pressure ¹⁶³.



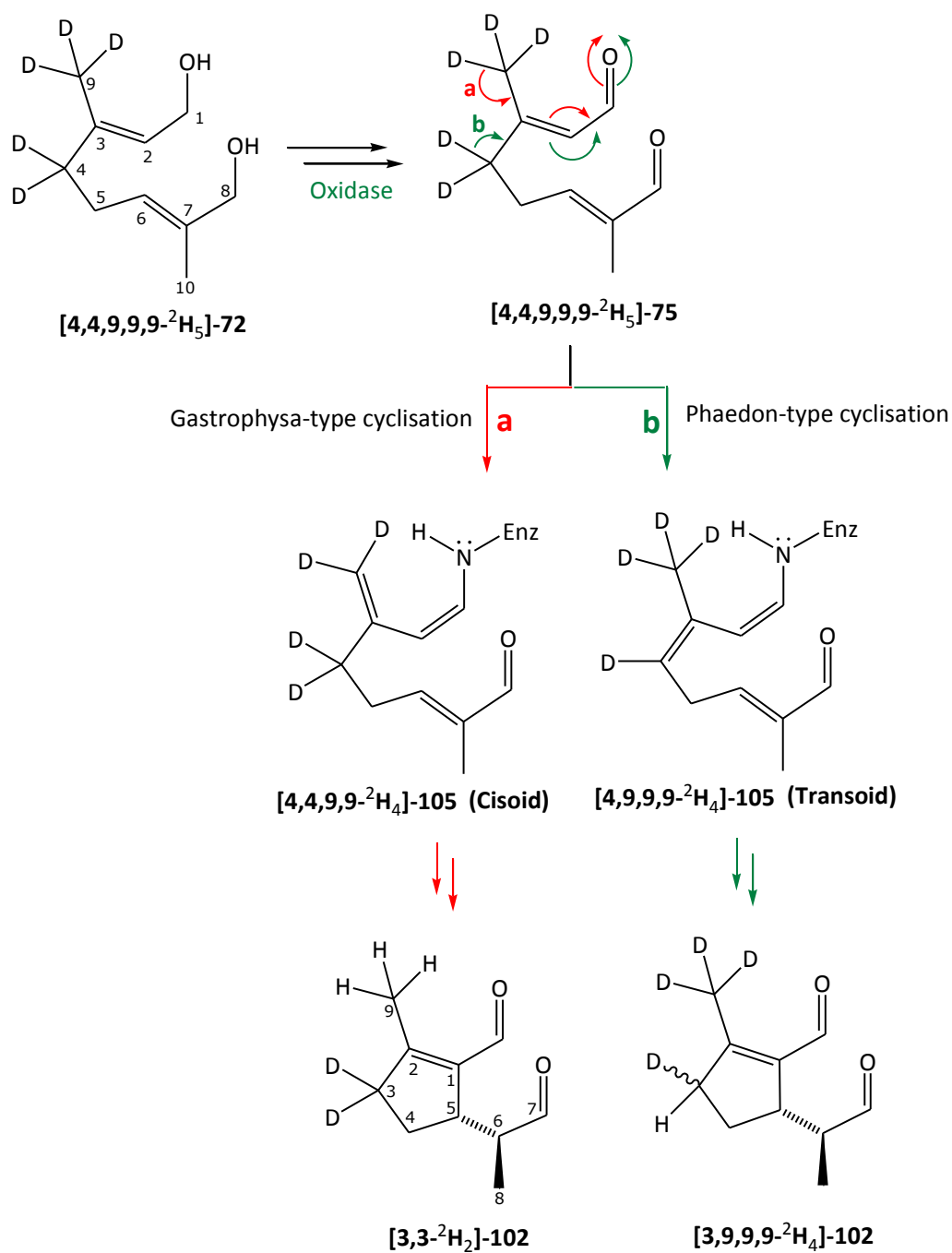
Scheme 1.12. Putative biosynthesis of plagiodial (**101**) and chrysolimelial (**102**) in Chrysomelidae: Phaeton beetle larvae. Gluc: Glucosyl group; Enz: Enzyme.

Taxonomic differences between these two species, however, are seen in their use of biosynthetic precursors; members of the Chrysomelidae family utilise the geraniol-based pathway solely (Scheme 1.12) ¹⁶⁴ whereas Staphylinids employ enzymes capable of oxidising citronellyl-derivatives ¹⁶². The monoterpenols were confirmed to be MVA-derived, thus verifying *de novo* biosynthesis in these beetles ^{137, 148}. Early studies revealed that 8-hydroxygeraniol (**72**), formed through the allylic oxidation of geraniol (**56**), becomes glucosylated in the fat body of Chrysomelids ⁸⁴ yielding hydrophilic 8-hydroxygeraniol-8-*O*- β -D-glucoside (**103**). From the gut, **103** is transported *via* the haemolymph to the glandular reservoirs where it is hydrolysed to recover diol **72** ^{137, 161}. The sugar moiety is thought to enable the transport of sparingly soluble aglucon **72** in the haemolymph ^{84, 163}. It was later discovered that both Chrysomelidae and Staphylinidae larvae could sequester glucoside precursors from plants using the same transportation and metabolic mechanisms as with, and in addition to, *de novo* production ¹⁶³. This dual flexibility provides the beetles with an alternative, less metabolically expensive route to the iridoids whilst maintaining their ability to adapt to different host plants without losing their defensive barrier ^{163, 165}.

Continued oxidation of diol **72**, *via* the stereoselective removal of the *pro-R* protons (C-1-H_R and C-8-H_R) and the resulting monoaldehydes 8-hydroxygeranial (**73**) and/or 8-oxogeraniol (**74**), gave 8-oxogeranial (**75**) in both Chrysomelidae and Staphylinidae beetles ^{162, 166}. This was found to take place within the glandular reservoir and involve an oxygen-dependent oxidase which uses molecular oxygen as the 2-electron acceptor (in contrast to plant-based enzymes which use NAD⁺) ^{120, 162, 167}. High substrate specificity for the enzymes involved was highlighted when the isomeric diol, 8-hydroxynerol (**91**), failed to metabolise ¹³⁷.

Following a Rauhut Currier-type reaction ¹⁶⁸, proline-assisted conversion of dialdehyde **75** to imine intermediate **104** is thought to precede base-assisted abstraction of the C-4 *pro-S* hydrogen to yield dienamine intermediate **105** ¹⁶⁹. Dienamine **105** is primed for a Michael addition cyclisation to give, *via* intermediates **106**, **107** and **108**, the iridoid chrysomelidial (**102**). Depending on the species, this occurs either directly or through the transitional iridoid plagiodial (**101**), where transpositioning of the olefinic bond is required to give **102** (Scheme 1.12) ^{162, 169}. The stereochemistry at C-5 and C-6 of iridoid **102** was found to vary between species ¹⁶⁹.

To obtain further clarity regarding the reductive cyclisation mechanism, *in vitro* incubation studies were conducted using [4*S*,5*R*-²H₂]-3-nor-8-hydroxygeraniol and gland extracts from larvae of Phaedon leaf beetles and Philonthus rove beetles. The results confirmed that C-4 proton removal was stereospecific and species-dependent; C-4-H_R abstraction was observed in members of the Philonthus genus whereas the C-4-H_S was removed in species of the Phaedon genus ¹⁶². Further isotopic labelling studies, using [4,4,9,9,9-²H₅]-8-hydroxygeraniol (**²H₅-72**), revealed an interspecies disparity in the mode of dialdehyde **75** cyclisation between the Gastrophysa and Phaedon leaf beetle genres (Scheme 1.13) ¹⁶⁹. Rather than losing C-4-H_S, deuterium-hydrogen exchange was only observed in iridoid **102** at the C-9 methyl position for Gastrophysa *spp.* It was suggested that the cyclase present in Gastrophysa beetles deprotonates the C-9 protons in the process of forming chrysomelidial **102** by passing through a reversibly formed cisoid dienamine intermediate during slow ring closure. This was confirmed experimentally by isotopic exchange using ²H₂O ¹⁶⁹. In contrast, the formation of **102** in Phaedon beetles proceeds *via* a transoid dienamine orientation, thus explaining the presence of **²H₄-102** in their defensive secretions (Scheme 1.13) ¹⁶⁹.



Scheme 1.13. Mechanistic differences between *Gastrophysa* (route **a**) and *Phaedon* (route **b**) leaf beetle species in the cyclisation of 8-oxogeranial (**75**) to chrysomelidial (**102**).

An understanding of the biosynthetic pathways to iridoids in insects, other than Coleopteran beetles, remains very limited. The biosynthetic route to the sex pheromone components of many Aphididae species, (1*R*,4*aS*,7*S*,7*aR*)-nepetalactol (**1**)

and (4a*S*,7*S*,7a*R*)-nepetalactone (**2**), has not yet been elucidated. *V. faba* (Fabaceae), the common host plant for *A. pisum*, *M. vicae* and other aphid species (Homoptera: Aphididae), is not known to produce either **1** or **2**, suggesting that *de novo* biosynthesis is taking place ¹⁴⁹. (*S*)-Citronellol (**(S)-57**) has been previously implicated in their production. Although behaviourally and electrophysiologically inactive, **(S)-57** was detected in oviparae volatile collections of several aphid species along with the sex pheromone components, raising a question over its role in the biosynthetic pathway ¹⁵. Further support of a mechanism incorporating 2,3-saturated monoterpenol **57** comes directly from plant studies, whereby *N. cataria* (Lamiaceae) biosynthesises lactone **2** using racemic citronellol **57** (Section 1.4.2.1). Alternatively, it is possible that the biosynthesis of lactol **1** in aphids follows the route taken by *C. roseus* to produce the same compound, proceeding *via* geraniol **56** or its *Z*-isomer, nerol **62**. With support for either route, or indeed the involvement of a different route entirely, the biosynthetic pathway to lactol **1** and lactone **2** in aphids remains elusive.

Currently, the repertoire of insect enzymes involved in iridoid biosynthesis continues to elude chemical ecologists. Complicating gene discovery in plants and insects is the near absence of gene clustering. However, functional clustering has been reported in microorganisms ¹⁷⁰. So far, multifunctional prenyl transferases have been determined in a few insects, including various aphid species, bark beetles and the mustard leaf beetle, *Phaedon cochleariae* ¹⁷¹⁻¹⁷³. These enzymes have been found capable of producing both GDP **42** and FDP **43** simultaneously. For aphids, this could suggest that a single enzyme is responsible for initiation of both the monoterpene and sesquiterpene biosynthetic routes in the production of sex pheromone monoterpene iridoid components **1** and **2** and alarm pheromone sesquiterpene, EBF **5** ¹⁶⁷. Enzyme amino acid residues and metal cofactors have been attributed to the product ratio observed ¹⁷⁴⁻¹⁷⁵.

Therefore, through minor co-factor-controlled adjustments of enzymes, insects appear capable of producing a manifold of products without the multitude of enzymes that plants possess.

Insect-based terpene synthases, involved in the dephosphorylation and subsequent oxidation of monoterpenyl diphosphates, have yet to be highlighted. Several prenyl transferases have been noted to possess additional terpene synthase activity ¹⁷¹. Enzymes responsible for the successive oxidation of monoterpenols to their corresponding dialdehydes, *via* their related dialcohols and transitional glucoside derivatives, have not yet been identified, however, it is hypothesised that an initial cytochrome P450-mediated hydroxylation could take place, in analogy to plant biosynthesis ¹⁶⁷. It is also anticipated that glucoside hydrolysis is catalysed by an enzyme belonging to the glucosyl hydrolase family 1 (GH1), which are often linked to defence activation or digestive roles in plants and insects, respectively ¹⁷⁶⁻¹⁷⁷. A glucose-methanol-choline (GMC) oxidase, named *Pc8HGO*, was recently found to catalyse 8-hydroxygeraniol **72** oxidation in *P. cochleariae* larvae in a flavin adenine dinucleotide (FAD)- and oxygen-dependent reaction ¹⁷⁸. An enzyme equivalent to the iridoid synthase found in *C. roseus* has not yet materialised in insects. Proteomic analysis using Chrysomeline larvae secretions failed to reveal any proteins similar to that of ISY ¹⁶⁷, however, recent larval RNA interference (RNAi) experiments were carried out to probe the mechanism and exposed a possible cyclase; a take-out protein belonging to the juvenile hormone-binding protein (JHBP) superfamily ¹⁷⁹.

In comparison to plants, the biosynthetic pathway(s) to iridoids in insects remains a largely undeveloped area of research with great scope. Knowledge of the intermediates and biocatalysts involved may offer short-cuts to the large-scale synthetic production

of important molecules, on exploitation. Potential agricultural applications towards innovative IPM strategies may be realised through the development of genetically modified crops, bioengineered to produce and emit SLMs inherent to pests such as aphids for pest management purposes. New routes to stereochemically pure and highly valuable compounds may also emerge. The application of biotechnology-based synthesis could improve on traditional synthetic methods where the production of target compounds is currently too difficult or expensive to achieve through mainstream synthetic chemistry, or where the compounds obtained through extraction are quantitatively limited ¹⁸⁰. Results so far have indicated that a range of structurally diverse iridoids, both naturally occurring and otherwise, can be formed from a few simple precursors by refinement of the enzymes discovered to date ¹⁸¹. The catalytic alternatives present in insects may provide such refinements. To take advantage of these synthetic biology tools in waiting, full elucidation of the biosynthesis of iridoid compounds in insects is required.

1.4.3 Biosynthetic Pathway Elucidation Procedures

To gain a thorough understanding of the biosynthetic routes to secondary metabolites such as lactol **1** and lactone **2**, the organism under scrutiny is commonly subjected to isotopic-labelling studies using hypothetically related labelled compounds whose metabolic fate can be monitored using mass spectrometry (MS). MS fragmentation patterns can be used to determine the localisation of deuterium atoms within the labelled compound, thereby providing invaluable mechanistic insight relating to inherent biosynthetic pathways and consequently aiding biomimetic synthetic research. Crucially, the isotopic label must not interfere with the organism's inherent metabolism or result in atypical toxicity, otherwise the results obtained could be

ambiguous or false. To avoid this, the label is often positioned in an easily identifiable location where loss due to standard metabolic turnover is unlikely ¹⁸²⁻¹⁸³.

Isotopic labelling studies in plants are often less problematic than those in animals and can be carried out *via* stem injection, chamber incubation or by immersing the plant in an aqueous solution containing the appropriate label ¹⁸². To conduct similar investigations using insects, dietary incorporation, direct injection and topical application methods have all seen past successes but require some forethought before their application.

The current understanding of chrysomelidial **102** biosynthesis in leaf beetle larvae owes itself to isotopic labelling and diet manipulation methods, whereby deuterium-labelled precursors were ingested, metabolised and secreted as labelled metabolites after feeding the larvae on impregnated leaves (Section 1.4.2.2). Mass spectrometry revealed a specific labelling pattern of the administered precursors and consequently provided an indication of the number and position of deuterium atoms in the product. A difference in resultant labelling positions exposed the interspecies variation in the mode of dialdehyde **75** cyclisation between the Gastrophysa and Phaedon genera (Scheme 1.13) ¹⁶⁹.

Through dietary incorporation procedures, information can also be gathered regarding the sequestration of precursors ¹⁶¹. Leaf impregnation techniques are common but rely on the use of leaf-ingesting species ¹⁷⁸. Sap-sucking aphids do not gnaw at leaves and therefore do not lend themselves to this method. Diet sachets can be used as an alternative dietary apparatus for studies involving aphids ¹⁸⁴.

Injection methods can provide data within a vastly shorter timescale than other methods. Deuterium incorporation was evident in iridoid **102** only an hour after aqueous solutions of deuterium-labelled putative precursors were directly injected into the haemolymph of beetle larvae, whereas a 3-day wait was required for evidence of significant isotope incorporation using impregnated leaves ¹⁶¹. Direct injection is also advantageous when the incorporation of compounds with feeding deterrent properties is required but can kill the subject if performed incorrectly or on an insect unable to withstand it ¹⁸⁵.

Topical application of deuterated compounds, or otherwise, is a less well-documented technique for use in insects but has been applied successfully to research into the juvenile hormone-like activity of EBF **5** derivatives ¹⁸⁶ and the biosynthetic pathway in bark beetles towards their aggregation pheromone component, frontalin ¹⁸⁷.

During isotopic labelling studies, label incorporation can be minimal and labelled product signals can be overwhelmed by those of the natural product. To overcome this, minor differences between the chemical structures of the natural and synthetic dietary compounds can be introduced to allow for the provision of analytical data (e.g. mass spectra) without superposition of the natural compound.

Deuterium labelled nor-precursors have been used as alternative metabolic probes for investigating the biosynthesis of natural and nor-chrysomelidial (nor-**102**) ¹⁶¹. Sufficient separation of **102** and nor-**102** by GC-MS was achieved, owing to the lower boiling point, and therefore earlier elution, of the nor derivative. This method, however, assumes that an absent methyl group has no effect on biosynthetic processes and that the nor- and naturally occurring compounds act in an analogous fashion. The absence of the methyl

group using rove beetles was found to heavily influence enzymatic stereochemical control ¹⁶², however the collection of labelled product strongly indicated successful ingestion, transportation and metabolism *in vivo* ¹³⁷.

Similarly, the sequestration of 8-hydroxygeraniol-8-*O*- β -D-glucoside (**103**) by leaf beetle larvae from host plants was demonstrated following the successful incorporation of artificially prepared thioglucosides into their defensive secretions ^{84, 163, 188}. Despite the thioglucosides showing distinct structural similarity to the natural compounds, with improved stability against hydrolytic enzymes, a degree of uncertainty remains when using fundamentally different substrates for direct comparisons in biosynthesis ¹⁶³.

An integrated approach of bioinformatics, biochemistry and genetics will likely be required to determine, unequivocally, the biosynthetic pathway to the aphid sex pheromone and the enzymes involved. The complete *A. pisum* genome sequence has been published, making it the first hemimetabolous insect for which a genome sequence has been fully established ^{12, 189}. From this, it has already been demonstrated that *A. pisum* contains all the essential genes for polyphenism (relating directly to the circadian clock, namely *Clock*, *Vrille*, *Pdp1*, *Cry1* and *Cry2*) and epigenetic regulation (including methylated developmental genes associated with the synthesis and degradation of juvenile hormone, namely *JHBP*, *JHEH* and *JHEBP*) ¹⁸⁹. Genetic factors encoding the transport proteins necessary for viral transmission were identified (including those with a known linkage to *Luteoviridae* trafficking and 12 genes for GTPase dynamin production) and also those for the production of chemosensory proteins and chemoreceptors (77 gustatory receptor and 79 odorant receptor genes were found to be involved, of which *ApOr1* is one) ^{12, 189}.

Many genes required for immune system components, including *IMD*, *dFADD*, *Dredd* and *Relish*, and for the biosynthesis of 11 amino acids, including those relating to the urea cycle (and the production of arginine) and those encoding for selenocysteine (*SBP2*, *Efsec*, *Secp43*, *pstk*, *SecS* and *SPS2*), were found to be lacking in the *A. pisum* genome ^{12, 189}. Interestingly, many genes, encoding amino acids that are lacking in *A. pisum*, were found present in the genome of the intracellular, mutualistic symbiotic bacterium, *Buchnera aphidicola* (Enterobacteriaceae), thereby enabling *A. pisum* to access a balanced diet ¹⁸⁹. The aphids, alongside their primary symbiont and various secondary symbionts, were found to compliment the nutritional uptake and synthetic capacity innate to *A. pisum* ¹⁸⁹.

By capitalising on the knowledge gained from interpretation of this genome, it may be possible to ascertain the genes involved in the biosynthesis of the aphid sex pheromone components, lactol **1** and lactone **2**. Subsequent identification of the enzymes involved may help to explain the stereochemistry of the reactions and the chirality of the products. *In vivo* and *in vitro* assays, employing techniques such as gene knockout (e.g. RNAi), heterologous reconstitution and substrate bioconversion using recombinantly produced candidate enzymes ¹⁹⁰, are often used for characterising the biocatalysts involved in biosynthesis, and can be applied to aphid sex pheromone studies.

Successful identification and a thorough understanding of the biochemical processes involved in aphid sex pheromone production could lead to novel applications in the field of biotechnology and pest management, and could also provide new synthetic biology tools for the elaboration of highly sought after compounds whose production *via* mainstream organic synthetic methods is currently problematic. It may eventually be possible to culture insect tissues or cells, or instead produce transgenic microorganisms

or plants, which can produce lactol **1**, lactone **2** and other iridoids with high stereochemical purity, either on a natural scale (for agricultural use) or in bulk (for large-scale production)^{170, 190}.

1.5 Project Aim and Objectives

This project is aimed at elucidating the biosynthesis of the aphid sex pheromone components, (1*R*,4*aS*,7*S*,7*aR*)-nepetalactol (**1**) and (4*aS*,7*S*,7*aR*)-nepetalactone (**2**), to provide the first full biosynthetic pathway to an hemimetabolous insect secondary metabolite. The results could deliver new synthetic biology tools and lead to new methods for aphid population management.

In order to achieve this, the following objectives must be fulfilled:

- Synthesise deuterium-labelled putative stable isotope precursors, namely geraniol (**56**), nerol (**62**) and citronellol (**57**) and proposed oxidised intermediates including the corresponding dialcohols and dialdehydes.
- Incubate *Acyrtosiphon pisum* (Homoptera: Aphididae) oviparae with and without labelled and unlabelled putative biosynthetic precursors, by dietary incorporation (using a simple, artificial diet) and/or topical application.
- Collect the headspace from *A. pisum* populations, treated with labelled and unlabelled materials, onto Porapak™ Q porous polymer using dynamic headspace collection (air entrainment).
- Analyse the collected volatile organic compounds by gas chromatography using non-polar and polar columns and confirm the incorporation of labelled materials by GC-MS.
- Provide mechanistic data on the production of lactol **1** and lactone **2** in aphids to support forthcoming research into intrinsic aphid genes involved in their biosynthesis.

2. Synthesis and Incorporation of Unlabelled Putative Biosynthetic Precursors and Intermediates

2.1 Identification of Sex Pheromone Components

The pea aphid, *Acyrtosiphon pisum* (Homoptera: Aphididae), was selected as the model species throughout this project. With a fully characterised genome ¹⁸⁹, *A. pisum* is well positioned for investigations into biosynthetic pathways inherent to the species. *A. pisum* are large, green or pink aphids of the Aphididae subfamily with a life cycle that resembles that of many aphid species. They reside mainly on leguminous host plants including broad beans, *Vicia faba* (Fabaceae), and peas, *Pisum sativum* (Fabaceae), and are a vector for over 30 plant viruses ¹⁹¹. *A. pisum* oviparae have been reported to release lactol **1** and lactone **2** in a 1:1 ratio ¹⁹², however deviations from this were observed consistently throughout this work, with lactol **1** production often exceeding that of lactone **2**. Age-related differences in the emission of lactol **1** and lactone **2** have been reported with other aphid species ¹⁹³ and may partially explain this disparity, as the age of oviparae used for experimentation was not controlled. Oviparae are distinguishable from other polymorphs by their darkened, thicker and often raised hind tibiae and their dark, mottled and rounder body, observable by optical microscopy. For this project, oviparae production was instigated by introducing a colony of *A. pisum*, reared on *V. faba*, to a controlled autumnal environment unit (L:D 12:12, 14 °C).

Dynamic headspace collection and solvent washing methods were initially evaluated for the collection of aphid sex pheromone ¹⁹⁴. The former allows for the collection of volatile organic compounds (VOCs) released by living aphids over time in a natural

environment whereas the latter only collects compounds being released at the time of sampling and those stored within the aphid body. During solvent washing procedures, *A. pisum* oviparae were crushed in diethyl ether, with the solution passed through dried magnesium sulfate and silanized glass wool to remove water and debris. Conversely, dynamic headspace collection methods involved passing purified air into a vessel containing *A. pisum* oviparae feeding on *V. faba* to create a positive pressure that reduces contamination from the external environment. The headspace, containing VOCs such as lactol **1** and lactone **2**, is then pumped out of the vessel *via* a Porapak™ Q porous polymer onto which the VOCs are adsorbed. The volatiles are subsequently eluted from the polymer using diethyl ether (Figure 2.1). For each technique, samples were performed in 4-6 replicates and 20 oviparae were used per replicate.

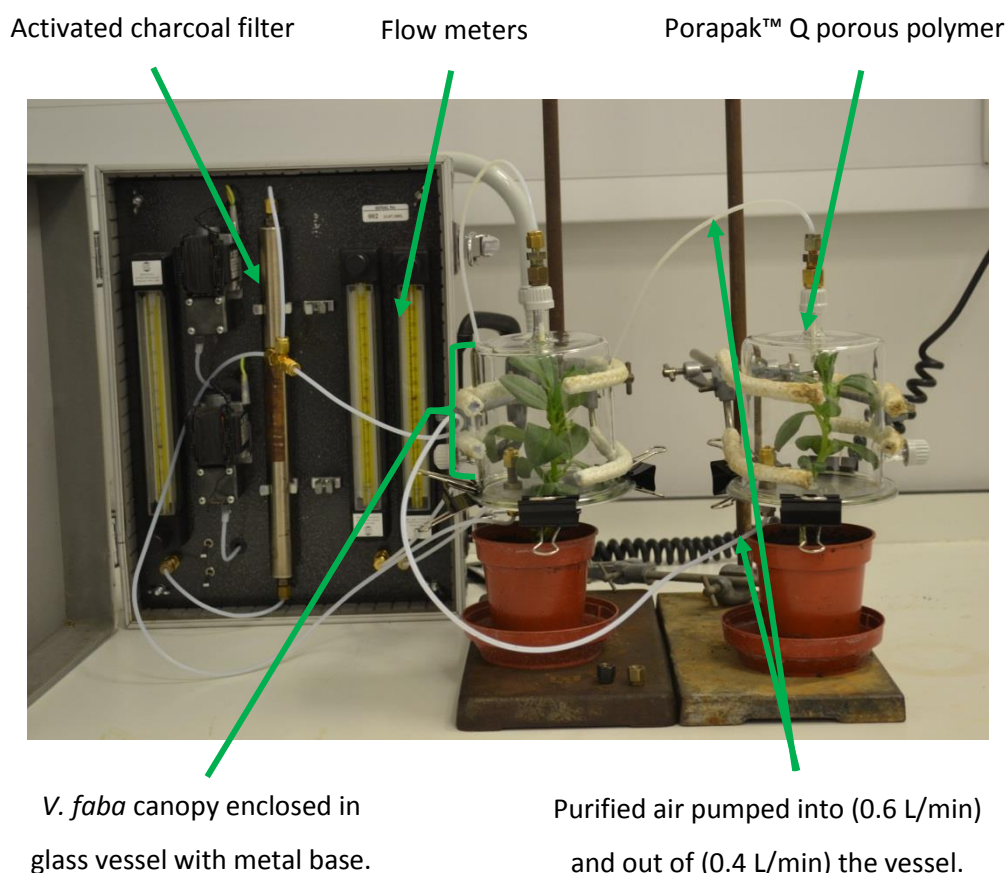


Figure 2.1. Dynamic headspace collection apparatus.

Successful aphid sex pheromone isolation was achieved by collecting VOCs from *A. pisum* oviparae. The gas chromatograms of *Nepeta cataria* (Lamiaceae)-derived lactone **2** and its reduction product, lactol **1**, in authentic standards (Figure 2.2A)⁶⁸ were compared with VOCs collected by dynamic headspace methods (Figure 2.2B) to confirm their presence. Neither sex pheromone component was evident in the GC chromatogram of VOCs collected from uninfested *V. faba* (Figure 2.2C).

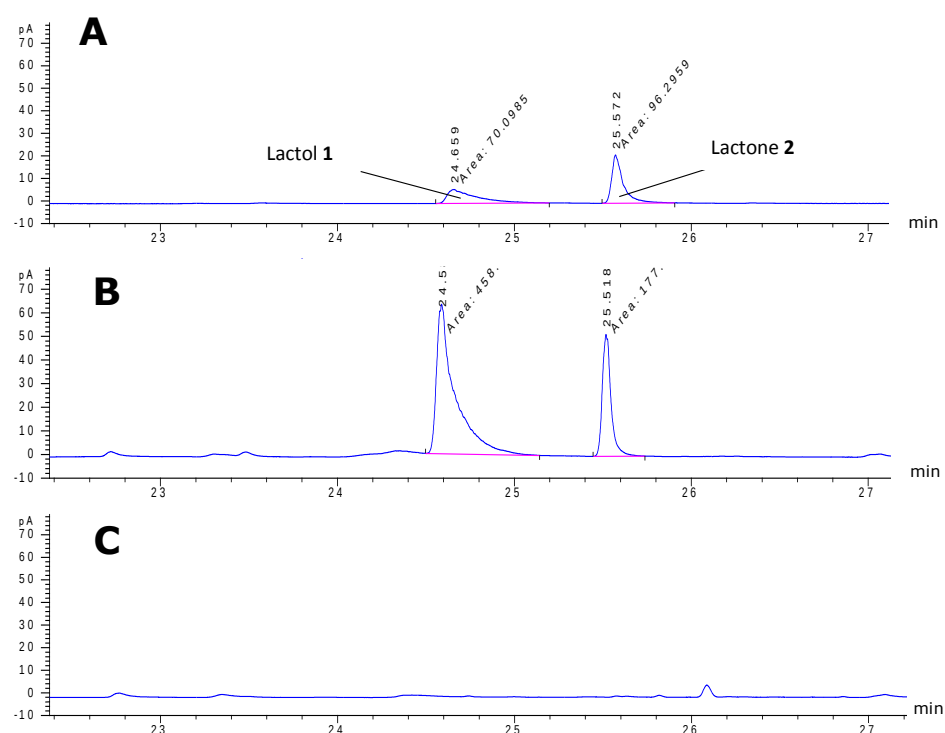


Figure 2.2. Electron impact ionisation GC chromatograms using a HP-1 column of (A) authentic lactol **1** and lactone **2** in a 10 ng/ μ L standard, and the dynamic headspace collections of (B) *Vicia faba* (Fabaceae) infested with *Acyrtosiphon pisum* (Homoptera: Aphididae) oviparae and (C) *V. faba*, uninfested.

Despite its higher molecular weight and polarity, GC traces show that lactol **1** elutes before lactone **2**, as has been described¹⁵. Also, as previously observed¹⁵, iridodial **76** was not evident in any dynamic headspace samples, suggesting that lactol **1** and its ring-opened equivalent **76** do not exist in an equilibrium as formerly reported^{27, 195}.

Mass spectra corresponding to the earlier (Figure 2.3A) and later (Figure 2.3B) eluting GC peaks in VOC collections from oviparae matched those of authentic standards of lactol **1** and lactone **2**, respectively.

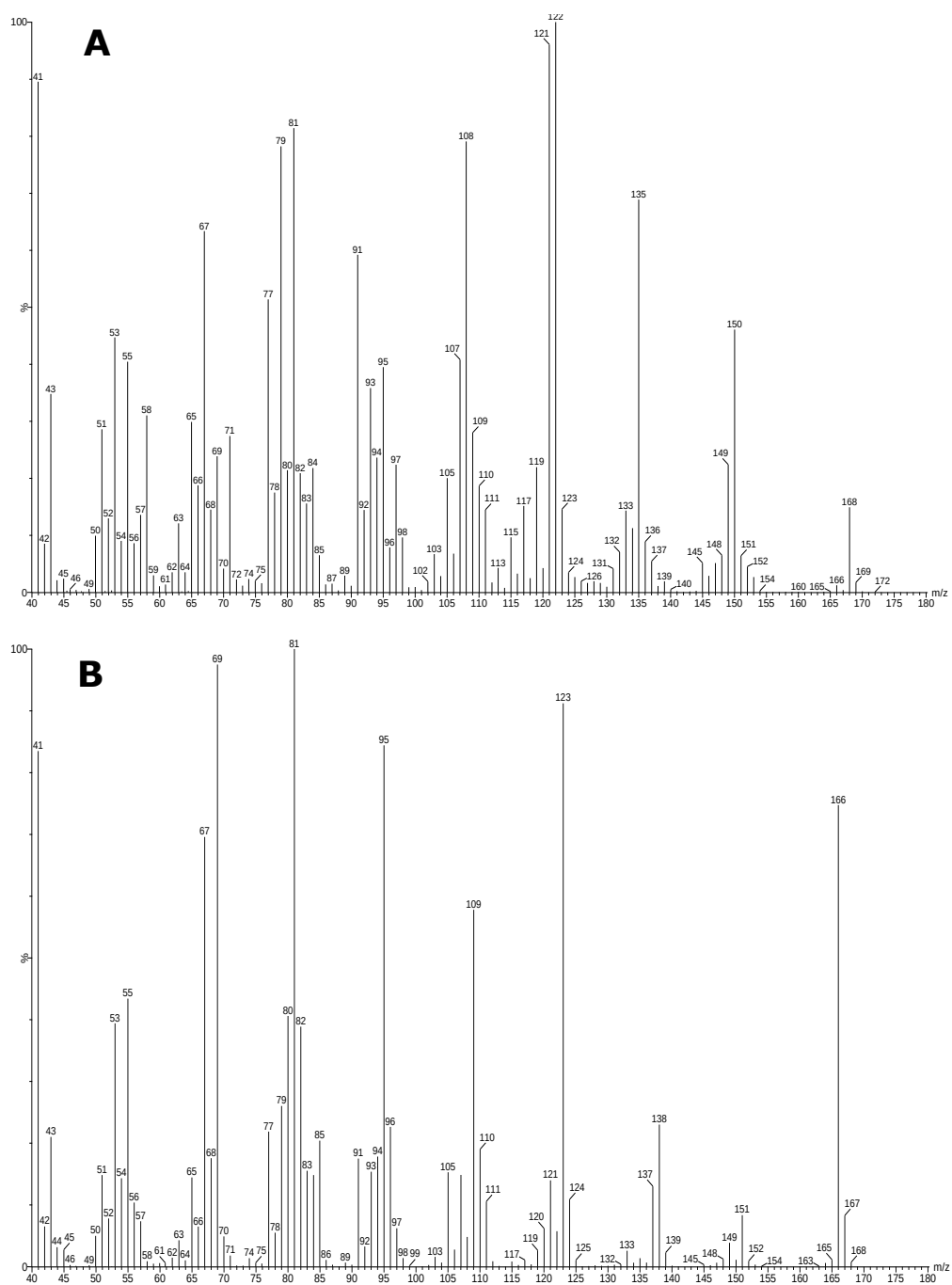


Figure 2.3. Electron ionisation mass spectra for **(A)** lactol **1** and **(B)** lactone **2** in VOC collections from *A. pisum* oviparae, which match those of the authentic standards ¹⁵.

Peak identification was further confirmed by GC peak enhancement using non-polar HP-1 and polar DB-Wax GC columns (Table 2.1).

Table 2.1. Lactol **1** and lactone **2** GC peak areas using HP-1 and DB-Wax columns.

Sample	Peak Area			
	HP-1		DB-Wax	
	Lactol 1	Lactone 2	Lactol 1	Lactone 2
Authentic Standard (10 ng/ μ L)	70.10	96.30	47.68	92.15
Dynamic headspace collection	173.51	176.93	145.70	214.62
Co-injection	223.81	275.22	308.83	376.40

Save for experimental error associated with manual injections, the co-injection peak areas for both compounds were consistent with the sum of the dynamic headspace collection and authentic standard peaks. Peak broadening, indicative of column degradation, was not observed. It was therefore concluded that the GC peaks in the dynamic headspace collections had been correctly identified as lactol **1** and lactone **2**.

Neither lactol **1** nor lactone **2** were evident in solvent washing samples, even after combining all 4 replicates (Figure 2.4). This is comparable to the results of a previous study investigating solvent washings of the greenbug, *Schizaphis graminum* (Homoptera: Aphididae) ¹⁹⁶. This may suggest that, rather than possessing pools of lactol **1** and lactone **2** within their body, *A. pisum* oviparae release the sex pheromone components immediately upon formation. Disruption may also temporarily halt the production of sex pheromone which could explain the absence of lactol **1** and lactone **2** in solvent washing samples. Despite filtering prior to GC injection, solvent washing samples remained a complex mixture of polar and non-polar compounds (evident in Figure 2.4B and likely originating from the contents of aphid organelles). Consequently, it was concluded that solvent washing was insufficient for *A. pisum* sex pheromone

collection. Dynamic headspace collection, preferable for the natural collection environment the method uses, was therefore used throughout the project.

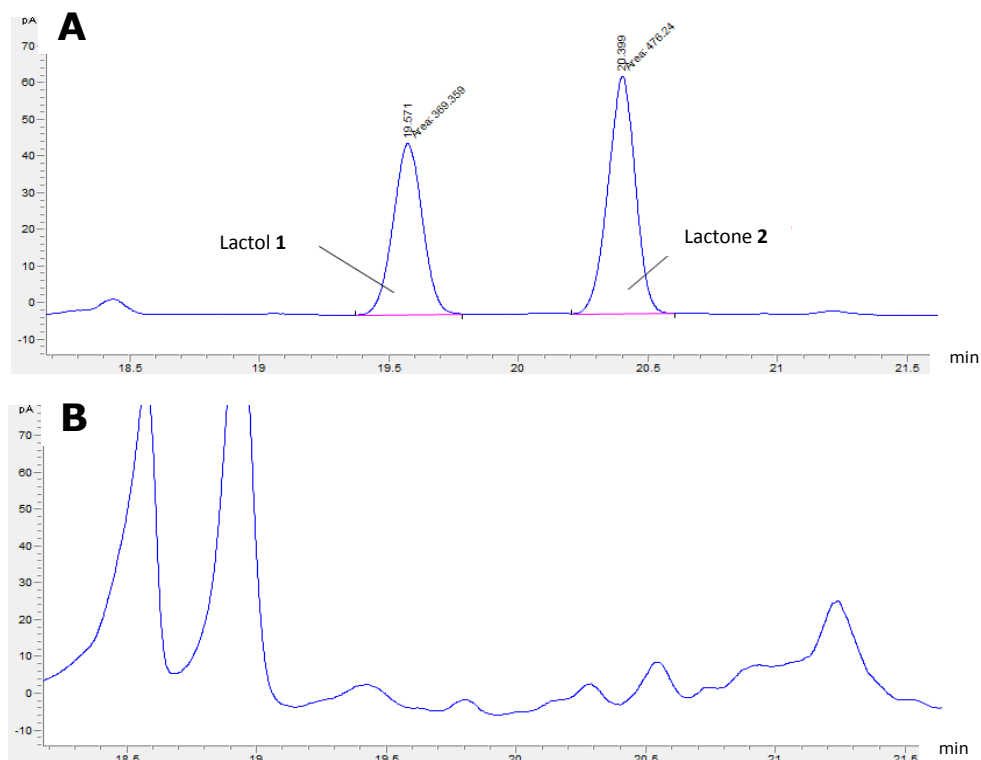
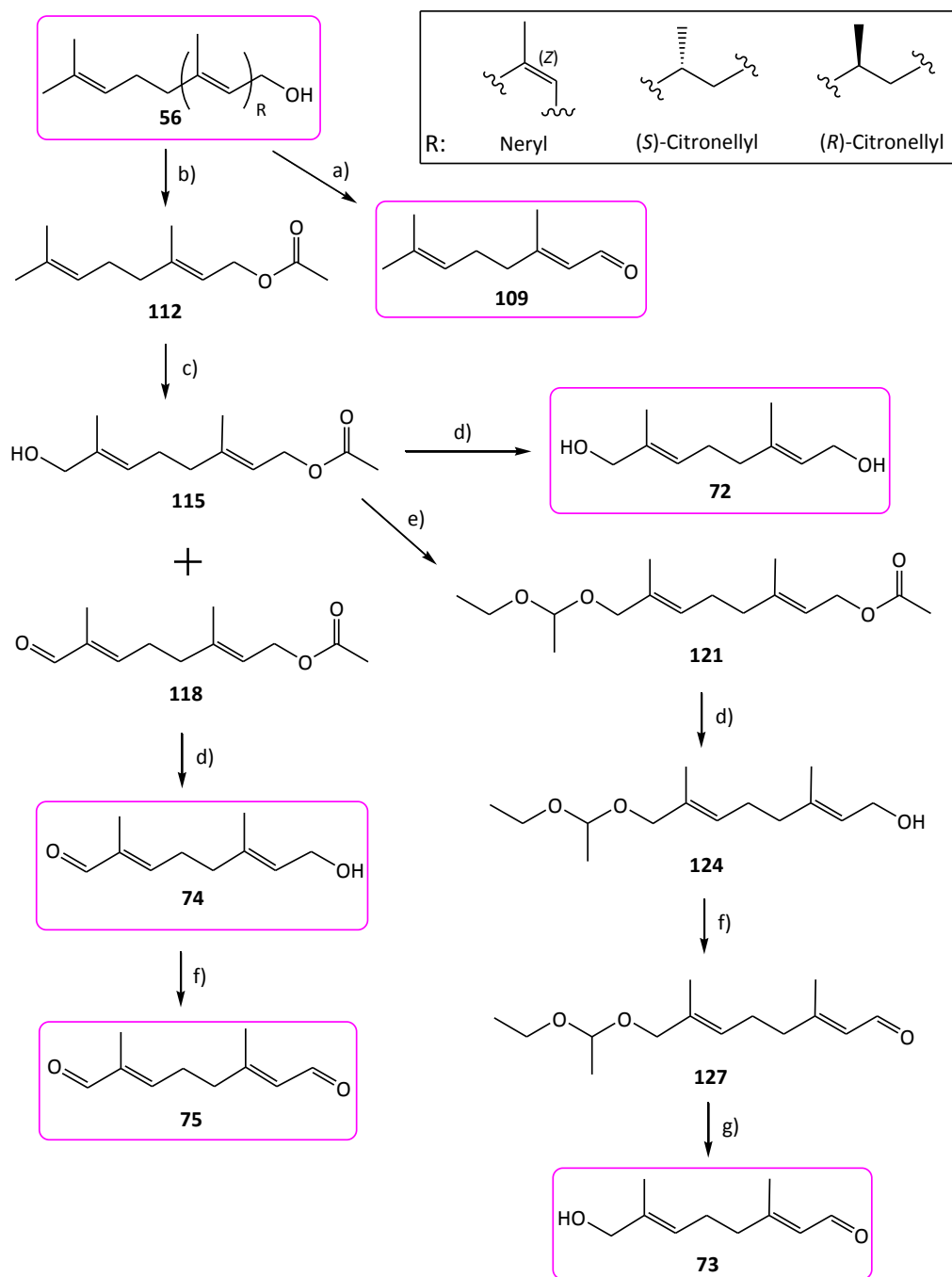


Figure 2.4. Comparison of the GC chromatograms of (A) authentic lactol **1** and lactone **2** in a 10 ng/ μ L standard and (B) a solvent washing sample of *A. pisum* oviparae.

2.2 Synthesis of Unlabelled Putative Precursors and Intermediates

Precursory to the application of isotopically labelled putative precursors and intermediates in biosynthetic elucidation procedures, the corresponding unlabelled compounds were investigated. This allowed for a direct comparison between results obtained on application of unlabelled and deuterium-labelled compounds and therefore renders substrate-specific effects on aphid survival and sex pheromone production distinguishable from potential kinetic isotope effects (KIEs).

Based on current knowledge of insect- and plant-based iridoid biosynthesis, monoterpenols geraniol (**56**), nerol (**62**) (*S*)-citronellol (**(S)-57**) and (*R*)-citronellol (**(R)-57**), and their C-1- and C-8-oxidised intermediates, were chosen for investigation. The synthetic route employed and compounds synthesised are shown in Scheme 2.1 and Figure 2.5. Throughout the thesis, only geranyl derivatives are depicted in schemes, whilst neryl, (*S*)-citronellyl and (*R*)-citronellyl derivatives are shown in figures below. Due to time limitations and the expense of the (*R*)-citronellol enantiomer, limited data was collected using (*R*)-citronellyl derivatives.



Scheme 2.1. Synthetic scheme for unlabelled geranyl derivatives of putative precursors and intermediates for the aphid sex pheromone (neryl, (S)-citronellyl and (R)-citronellyl derivatives contain different R groups as shown in black box, see Figure 2.5); a) MnO_2 , DCM, 40 hrs, rt; b) Ac_2O , pyridine, 4-DMAP, 2 hrs, rt; c) SeO_2 , *t*-BuO₂H, salicylic acid, DCM, 72 hrs, rt; d) K_2CO_3 , MeOH, 4 hrs, rt; e) Ethyl vinyl ether, anh. DCM, cat. pyridinium *p*-toluenesulfonate, 4hrs, rt; f) IBX, DMSO, 2 hrs, rt; g) Acetic acid:H₂O:THF (8:1:1), 3 hrs, rt. Pink boxes indicate compounds intended for aphid application.

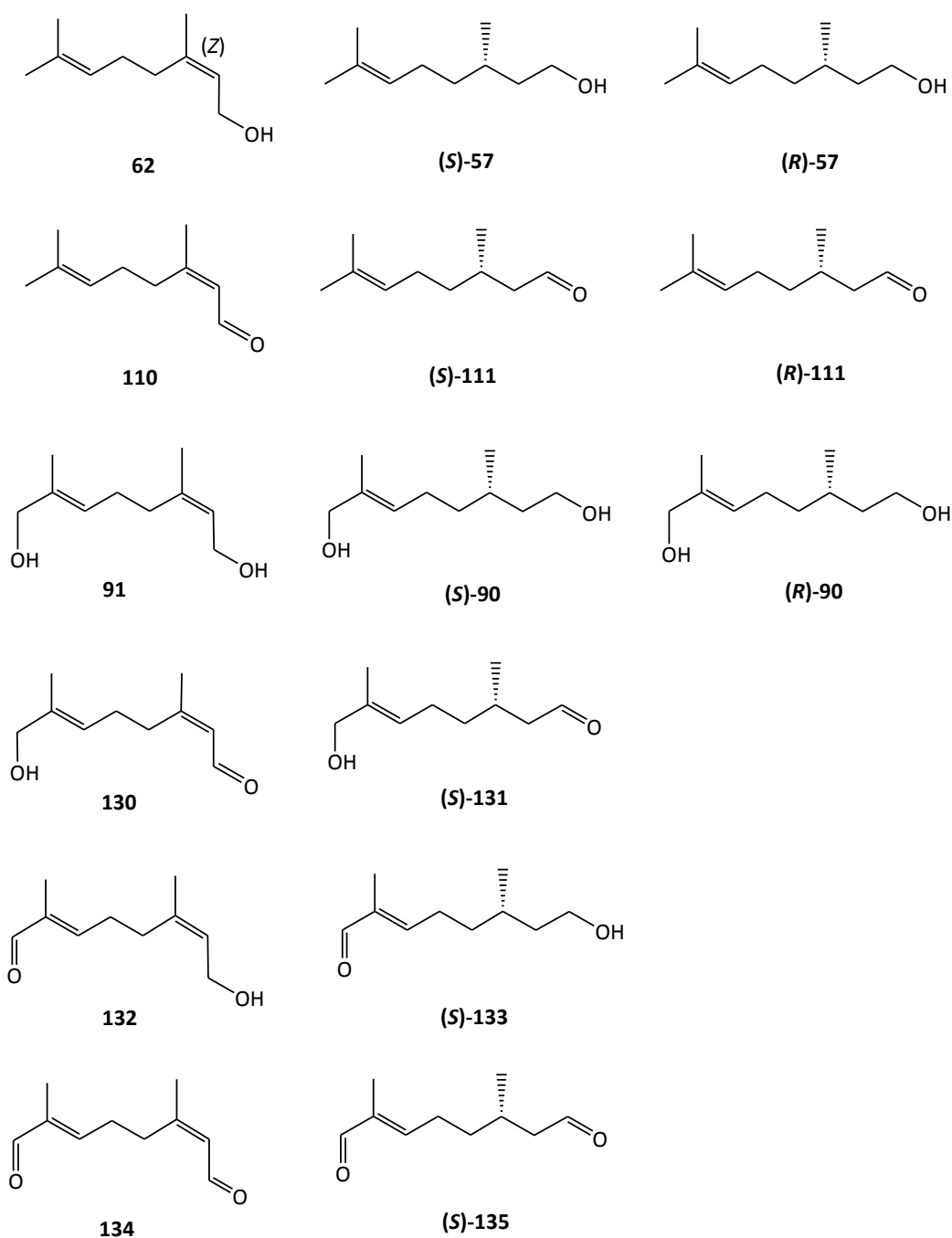
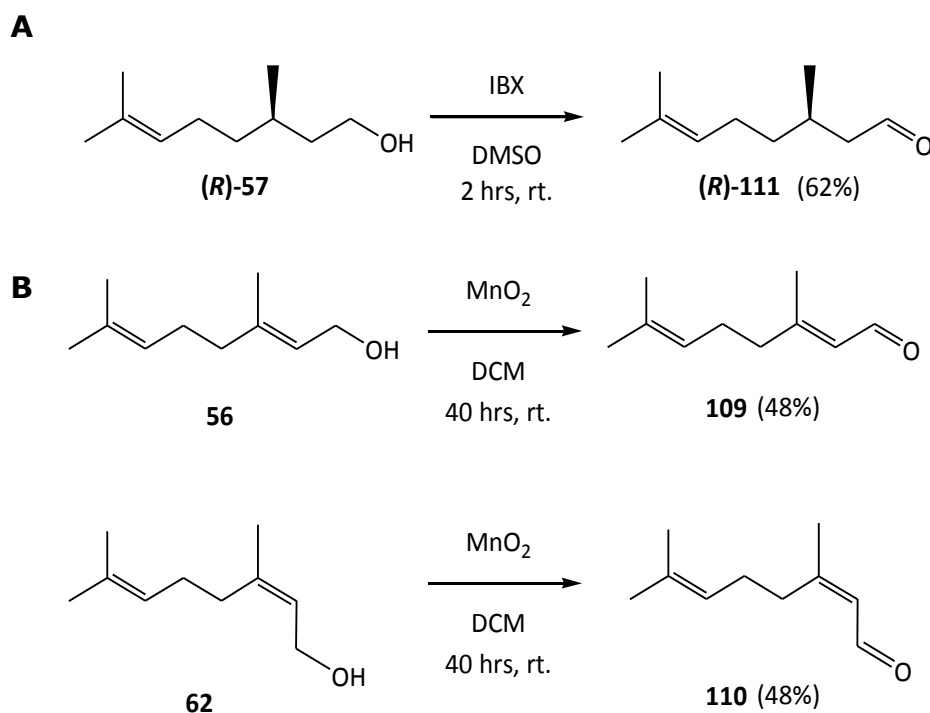


Figure 2.5. Unlabelled neryl, (*S*)-citronellyl and (*R*)-citronellyl derivatives of putative precursors and intermediates for the aphid sex pheromone intended for aphid application.

2.2.1 Synthesis of Monoterpenals

All monoaldehydes were synthesised except for (*S*)-citronellal (**(S)-111**) which was commercially available. Hypervalent periodinane reagent, 2-iodoxybenzoic acid (IBX), was used to oxidise (*R*)-citronellol (**(R)-111**) (Scheme 2.2A) but was not suitable for the oxidation of geraniol **56** or nerol **62** to their respective aldehydes, during which isomerisation of the conjugated alkene resulted, possibly due to the mild acidic properties of IBX. For the 2,3-unsaturated allylic monoterpenols, manganese dioxide was used as the oxidant (Scheme 2.2B) ¹⁹⁷. IBX was prepared by heating 2-iodobenzoic acid in the presence of potassium peroxymonosulfate, while MnO₂ was synthesised by adding potassium permanganate to manganese sulfate. Both oxidants were dried *via* vacuum desiccation for 48 hours prior to use.



Scheme 2.2. The synthesis of (*R*)-citronellal (**(R)-111**), geranial (**109**) and neral (**110**) from their corresponding monoterpenols *via* (A) IBX or (B) MnO₂ oxidation.

2.2.2 Synthesis of 8-Hydroxymonoterpenols

To synthesise the monoterpenyl dialcohols, commercially available monoterpenols were first protected as the corresponding acetates by treatment with acetic anhydride and 4-DMAP. Allylic oxidation at C-8 was achieved using selenium dioxide^{15, 69}. The initial formation of an allylic selenic acid (**136**) by an Alder-ene-like reaction is followed by a [2,3]-sigmatropic rearrangement to yield the allylic alcohol (**137**). The pseudoequatorial positioning of the alkyl group within the cyclic transition state of the rearrangement favours the oxidation of the *trans*-methyl group to yield (*E*)-products¹⁹⁸, thus providing stereoselective, as well as regioselective, control (Figure 2.6).

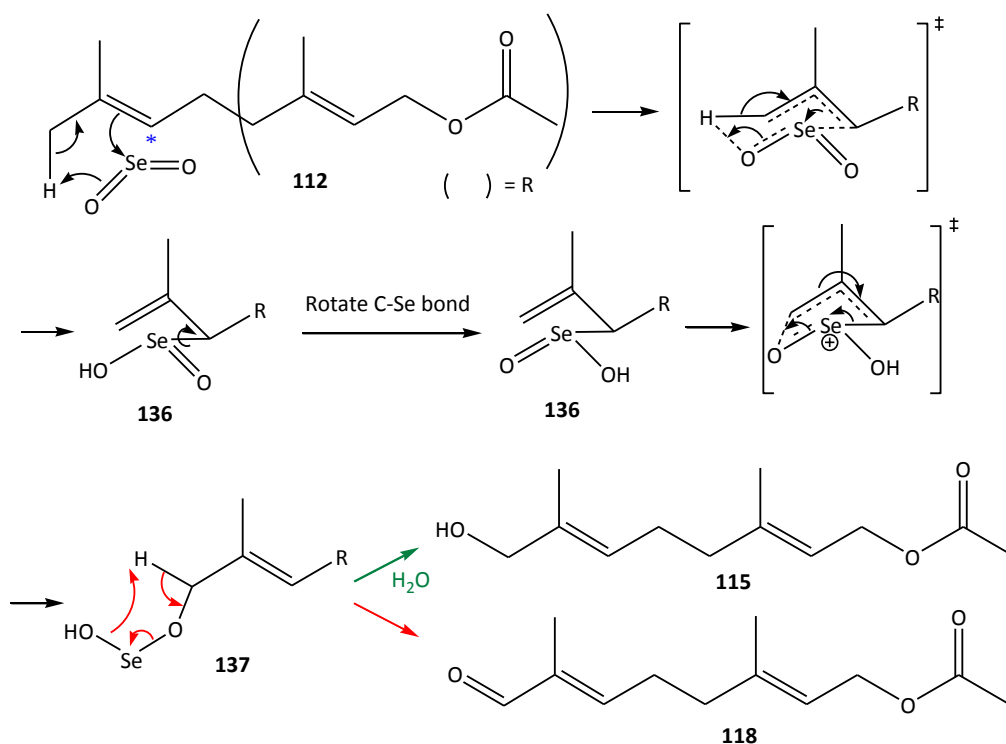
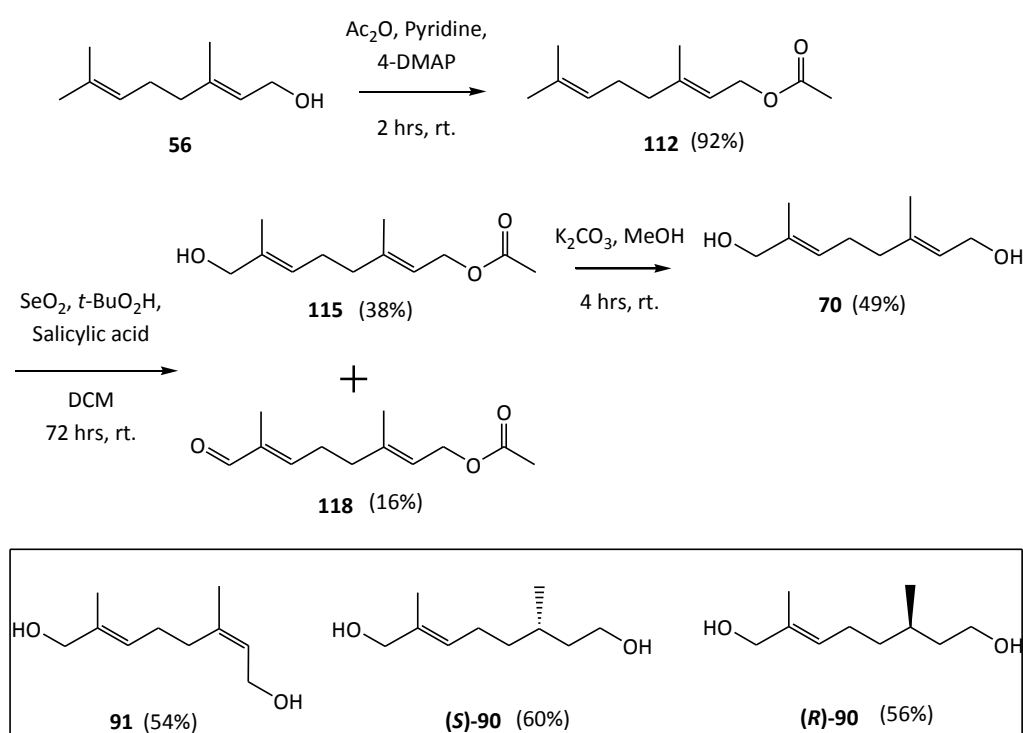


Figure 2.6. The mechanism for SeO₂ allylic oxidation of geranyl acetate **112**, via allylic selenic acid **136** and selenic alcohol **137**, yielding 8-hydroxygeranyl acetate **115** and 8-oxogeranyl acetate **118**.

Salicylic acid was introduced to the reaction mixture following reports that carboxylic acid catalysis increases yields, while *t*-butyl hydroperoxide was used to re-oxidise the

reduced Se (II) by-product, thereby requiring substoichiometric quantities of SeO_2 ¹⁹⁹. Unreacted starting material was present even after 7 days and aldehyde formation at C-8 varied with reaction duration. After 72 hours, the aldehyde product was produced in quantities approximately a third that of the dialcohol. Removal of the acetate group from the isolated 8-hydroxyacetates using potassium carbonate in methanol afforded the 8-hydroxymonoterpenols **72**, **91**, **(S)-90** and **(R)-90**. The synthetic route to the monoterpenyl dialcohols is summarised in Scheme 2.4.

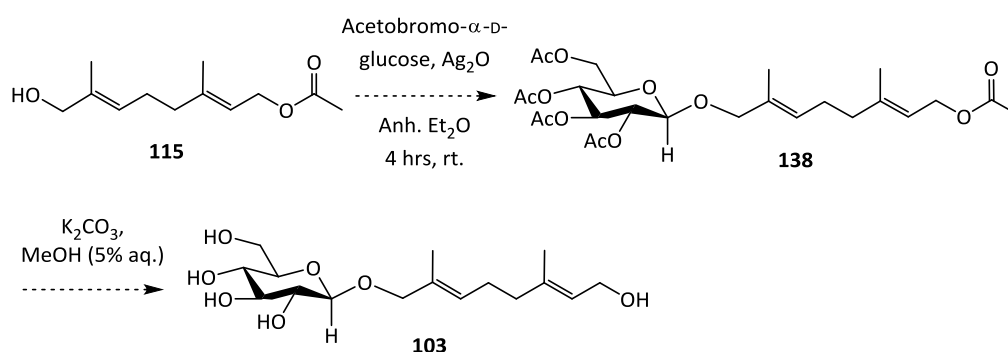


Scheme 2.4. The synthesis of 8-hydroxygeraniol (**72**), 8-hydroxynerol (**91**), 8-hydroxy-(*S*)-citronellol (**(S)-90**) and 8-hydroxy-(*R*)-citronellol (**(R)-90**) from their corresponding monoterpenols *via* SeO_2 -driven allylic oxidation.

To shed light on the possible sequestration of glucosides in *A. pisum*, as has been demonstrated in leaf beetles using synthetic thioglucosides ^{163, 200}, glucosylation of the 8-hydroxymonoterpenyl acetates to their corresponding 8-hydroxymonoterpenyl-8-*O*- β -D-glucosides was attempted. Leaf beetle larvae displayed a high degree of substrate

selectivity, with a 10-fold preference for the uptake of hydroxygeraniol thioglucosides over geraniol thioglucosides¹⁶³ and for the importation of hydroxygeraniol-8-thioglucosides over hydroxygeraniol-1-thioglucosides²⁰⁰. Despite their vulnerability towards hydrolytic enzymes, 8-hydroxygeraniol-8-O- β -D-glucosides were chosen for investigation rather than their 8-hydroxygeraniol-8-S- β -D-glucoside equivalents to prevent any deviations from normal sex pheromone production taking place owing to structural disparities.

Installation of the glucoside moiety was initially attempted using acetobromo- α -D-glucose and silver (I) oxide in a Koenigs–Knorr reaction, as reported in literature²⁰¹⁻²⁰². Reaction of the glucosyl bromide with a silver salt gives, on elimination of silver bromide, an oxocarbenium ion which is stabilised by neighbouring glucosyl acetate group. This ion is attacked by primary alcohols **115**, **116**, (*S*)-**117** or (*R*)-**117** via an S_N2 mechanism to give the acetylated glucosides with inversion at the joining glucosyl carbon. Global deacetylation with aqueous methanolic potassium carbonate was expected to yield the desired glucosides (Scheme 2.5).



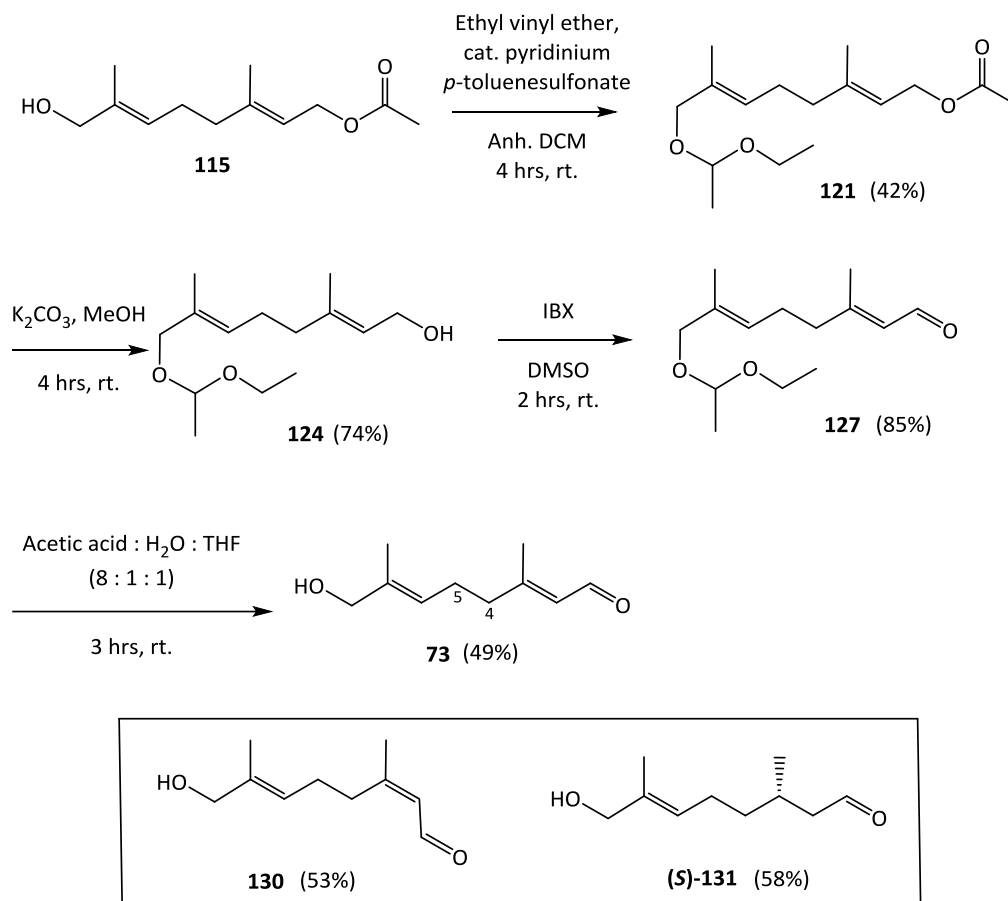
Scheme 2.5. The proposed synthesis of 8-hydroxygeranyl-8-O- β -D-glucoside from 8-hydroxygeranyl acetate via a Koenigs-Knorr reaction.

The Koenigs–Knorr reaction failed to yield the desired glucosides, instead producing a multi-product mixture. Repetition with newly purchased and dried Ag₂O under maintained dark reaction conditions gave the same results, as judged by continued TLC reaction monitoring over a longer period. Alternative sources of dried silver salt catalysts, including silver triflate ²⁰³ and silver carbonate ²⁰⁴, were investigated but without success. The addition of neither iodine nor molecular sieves improved the results for geranyl, neryl or citronellyl derivatives. Examples for glucosylation of 8-hydroxymonoterpenyl acetates in the literature were extremely limited. Methods usually described the C-1-glucosylation of monoterpenols or the synthesis of monoterpenyl thioglucosides. Synthesis of the pentaacetate of 8-hydroxygeraniol-8-*O*-β-D-glucoside (**138**) in 3% yield was reported using 8-hydroxygeraniol **72** ²⁰⁴, however using acetate-protected starting material was unlikely to inhibit the glucosylation procedure as structurally similar (2*E*)-hydroxy-2-methylbut-2-enyl-4-acetate was glucosylated successfully in 6% yield ²⁰⁵. Due to time constraints, glucosylation efforts were abandoned.

2.2.3 Synthesis of 8-Hydroxymonoterpenals

The synthesis of 8-hydroxymonoterpenals, **73**, **130** and (**5**)-**131**, followed the route shown in Scheme 2.6. Acetal protection of the 8-hydroxymonoterpenyl acetates **115**, **116** and (**5**)-**117** at C-8 was achieved using ethyl vinyl ether and catalytic pyridinium *p*-toluenesulfonate in anhydrous dichloromethane. Deacetylation utilising methanolic potassium carbonate gave the C-1-alcohols which were subsequently oxidised to their corresponding aldehydes using IBX. Final hydrolysis of the acetal protecting group was effected by treatment with 8:1:1 acetic acid:H₂O:THF to give the desired 8-hydroxy-aldehyde products. A minor amount of 2,3-alkene acid-promoted isomerisation was

observed for 8-hydroxygeranial **115** and 8-hydroxyneral **116**, with yields of the undesired stereoisomer amounting to approximately 6%.



Scheme 2.6. The synthesis of 8-hydroxygeranial (**73**), 8-hydroxyneral (**130**) and 8-hydroxy-(*S*)-citronellal (**(S)-131**) from their corresponding 8-hydroxymonoterpenyl acetates *via* acetal protection at C-8, acetate deprotection and IBX oxidation at C-1, and final acetal hydrolysis at C-8.

Interestingly, the ^1H -NMR spectrum for 8-hydroxygeranial was initially deceiving, showing what appeared to be a singlet at 2.30 ppm but no multiplets typical of the C-4- H_2 or C-5- H_2 protons. On further investigation, the singlet integrated to 4 protons and a heteronuclear single quantum coherence spectrum (HSQC) (Figure 2.7) revealed that its protons correlate to two separate carbon atoms (Figure 2.7 inset). It was

therefore concluded that, unlike that documented in the literature, the singlet was a signal for both the C-4-H₂ and C-5-H₂ protons ¹⁵.

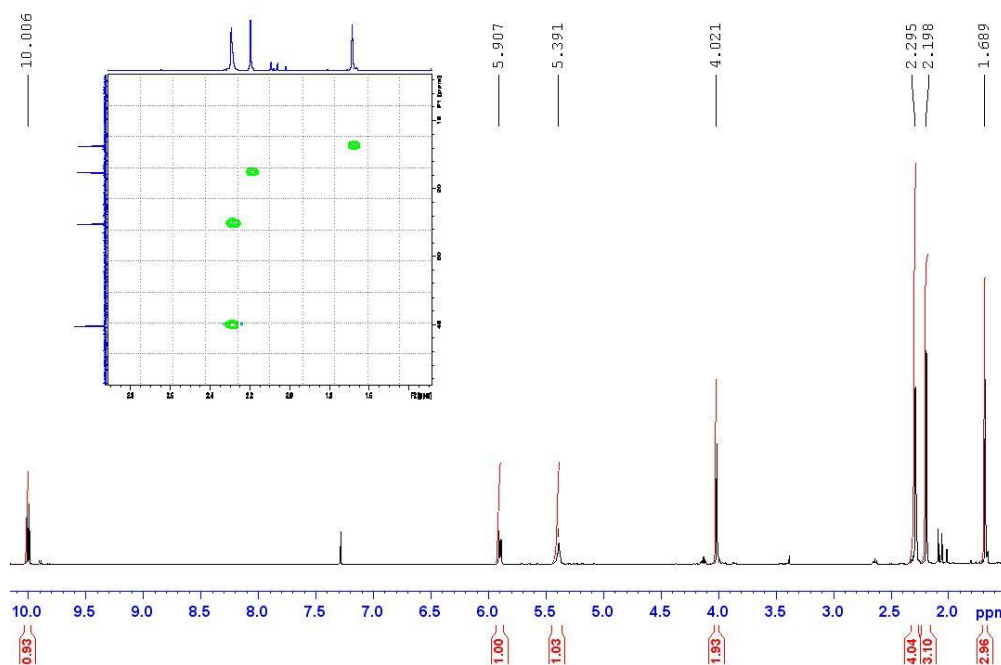
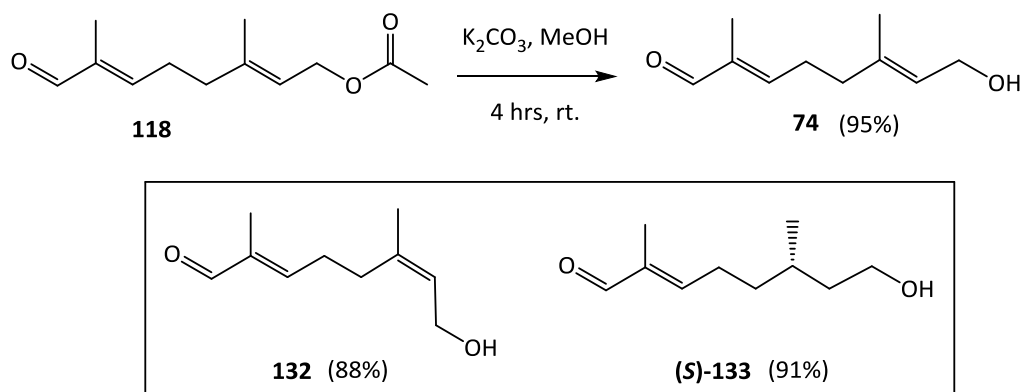


Figure 2.7. The ¹H-NMR (and inset, HSQC) spectra for 8-hydroxygeranial **73** in CDCl₃ showing a 4-proton ‘singlet’ at 2.30 ppm, with the protons attached to two different carbon atoms.

2.2.4 Synthesis of 8-Oxomonoterpenols

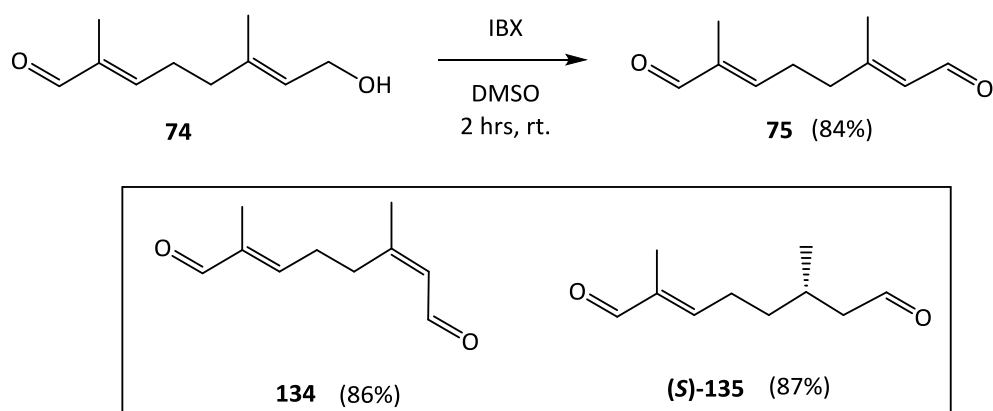
The 8-oxomonoterpenyl acetate over-oxidation products obtained during SeO₂-based allylic oxidation of the monoterpenyl acetates were isolated by flash chromatography and deprotected using methanolic potassium carbonate to afford the 8-oxomonoterpenols (Scheme 2.7).



Scheme 2.7. The synthesis of 8-oxogeraniol (**74**), 8-oxonerol (**132**) and 8-oxo-(*S*)-citronellol (**(S)-133**) from their corresponding 8-oxomonoterpenyl acetates *via* acetate deprotection using methanolic K_2CO_3 .

2.2.5 Synthesis of 8-Oxomonoterpenals

Oxidation of the 8-oxomonoterpenols to their corresponding dialdehydes was performed using IBX in DMSO (Scheme 2.8). Whilst 8-oxo-(*S*)-citronellal (**(S)-135**) was amenable to purification on a silica column, the less stable 2,3-unsaturated di-conjugated dialdehydes, **75** and **134**, degraded during flash chromatography purification. Subsequently, they were purified only by filtration through celite to remove the IBX by-products.



Scheme 2.8. The synthesis of 8-oxogeraniol (**75**), 8-oxonerol (**134**) and 8-oxo-(*S*)-citronellal (**(S)-135**) from their corresponding 8-oxomonoterpenols *via* IBX oxidation.

2.3 Incorporation Methods

To determine the biosynthetic pathway to the aphid sex pheromone, *A. pisum* oviparae were subjected to dietary incorporation and topical application of the unlabelled putative biosynthetic precursors and intermediates, as is common procedure in both plants and insects whose biosynthetic pathway to a secondary metabolite is under investigation.

2.3.1 Dietary Incorporation

By rearing *A. pisum* on an artificial diet, it is possible to incorporate synthetic compounds relating to the putative biosynthetic pathways to the aphid sex pheromone into a basal diet solution and study the compounds that they are converted into. Due to the sap-sucking nature of aphids, diet sachets (Figure 2.8A) were used rather than the leaf coating procedures described for leaf-chewing species. Aphids access the diet solution by piercing their stylet through the first Parafilm® layer of the sachet and consuming the solution contained within (Figure 2.8B).

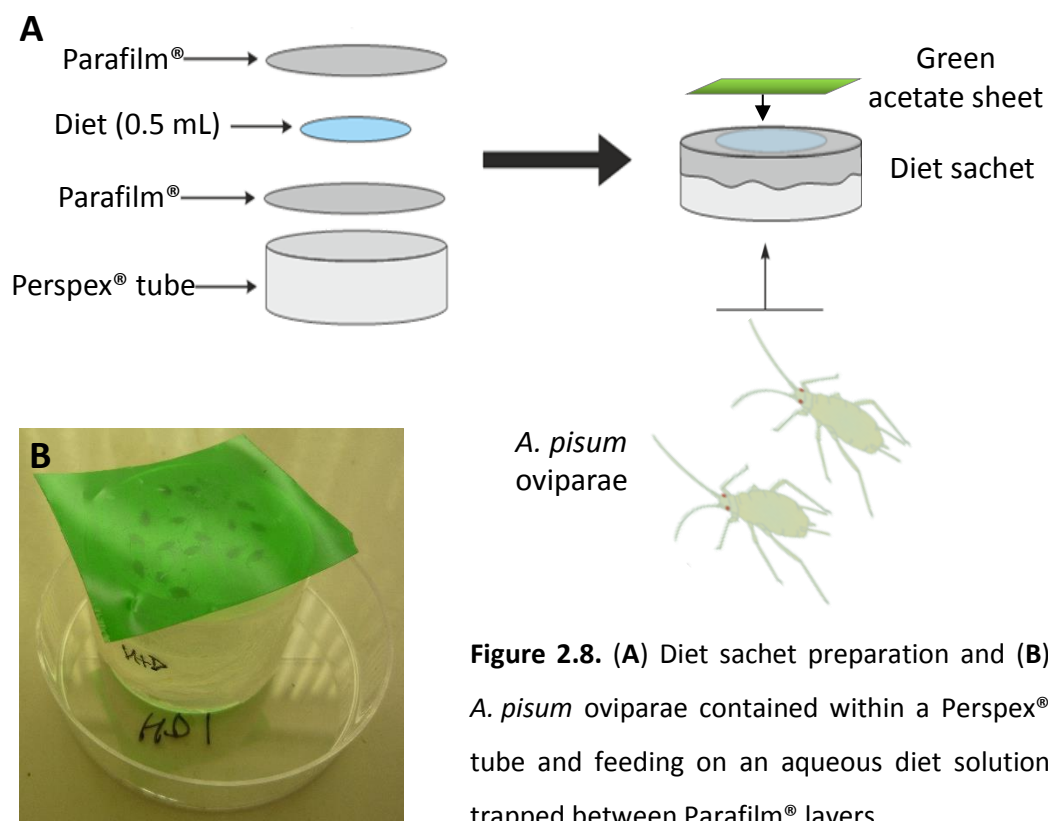


Figure 2.8. (A) Diet sachet preparation and **(B)** *A. pisum* oviparae contained within a Perspex® tube and feeding on an aqueous diet solution trapped between Parafilm® layers.

Critical to the success of both dietary incorporation and topical application studies is the absence of detrimental effects on aphid survival rate and sex pheromone production. Additionally, it is important that the basal diet solution, into which the investigated compounds will be dissolved, does not inhibit feeding or alter the aphids' natural metabolic processes. A diet designed for *M. viciae* aphids has been used successfully in maintaining a culture of the species for over thirty years¹⁸⁴. Artificial diets have also been reported in simplifying the maintenance of aphid colonies, including *A. pisum*²⁰⁶. However, despite the documented existence of several artificial diets optimised for maintenance of *A. pisum* and other Aphididae species, studies have not been conducted to date for the specific maintenance of *A. pisum* oviparae which may require either different quantities of, or alternative, ingredients for sex pheromone production.

A range of aqueous basal dietary solutions, differing in D-(+)-sucrose and supplement concentrations, were tested for optimal aphid survival rate and sex pheromone production (Table 2.2). The effect of two feeding environments, one aestival (L:D 16:8, 25 °C) and one autumnal (L:D 12:12, 14 °C), on survival rates and sex pheromone release were also assessed. Each diet sachet comfortably supported twenty feeding *A. pisum* oviparae and a 48-hour incubation period was introduced to allow for sufficient feeding before transferral to *V. faba* plants for dynamic headspace collection.

Table 2.2. Artificial diet compositions for *A. pisum* oviparae.

Ingredient (Amounts in mg/100 mL)	Diet					
	100 g/L	150 g/L	200 g/L	Woodcock	Hoang & Dung	Van Emden
D-Sucrose	10,000	15,000	20,000	10,000	17,000	15,000
K ₂ HPO ₄					750	750
MgSO ₄					110	123
L-Tyrosine				40	11	40
L-Asparagine					214	550
L-Aspartic acid					190	140
L-Tryptophan					58	80
L-Alanine					51	100
L-Arginine					300	270
L-Cysteine					43	40
L-Glutamic acid					124	140
L-Glutamine					241	150
Glycine					9	80
L-Histidine					182	80
L-Isoleucine				120	114	80
L-Leucine				160	114	80
L-Lysine				120	159	120
L-Methionine					43	40
L-Phenylalanine				80	47	40
L-Proline					66	80
L-Serine					60	80
L-Threonine				140	103	140
L-Valine					102	80
L-Ascorbic acid					100	100
Nicotinic acid					10	10
Folic acid					2	0.5
(+)-Pantothenic acid					5	5
Thiamine					2.5	
Citric acid					10	
Inositol					50	50
Choline chloride					50	50
EDTA Fe(III)-Na ₂ chelate					1.5	1.5
EDTA Zn-Na ₂ chelate					0.8	0.8
EDTA Mn-Na ₂ chelate					0.8	0.8
EDTA Cu-Na ₂ chelate					0.4	0.4
Pyridoxine hydrochloride (Vit. B6)					2.5	2.5
D-Biotin					0.1	0.1
Aneurine hydrochloride (Vit. B1)						2.5
Riboflavin (Vit. B2)						0.5

The number of surviving *A. pisum* oviparae on each artificial diet was recorded over 7 days and the results compared to those feeding on a natural food source, *V. faba*, contained within clip cages. On comparison with the expected death rate, estimated

from *V. faba*-fed aphids, a significant increase in aphid mortality was observed for all diets under aestival feeding conditions (Figure 2.9A).

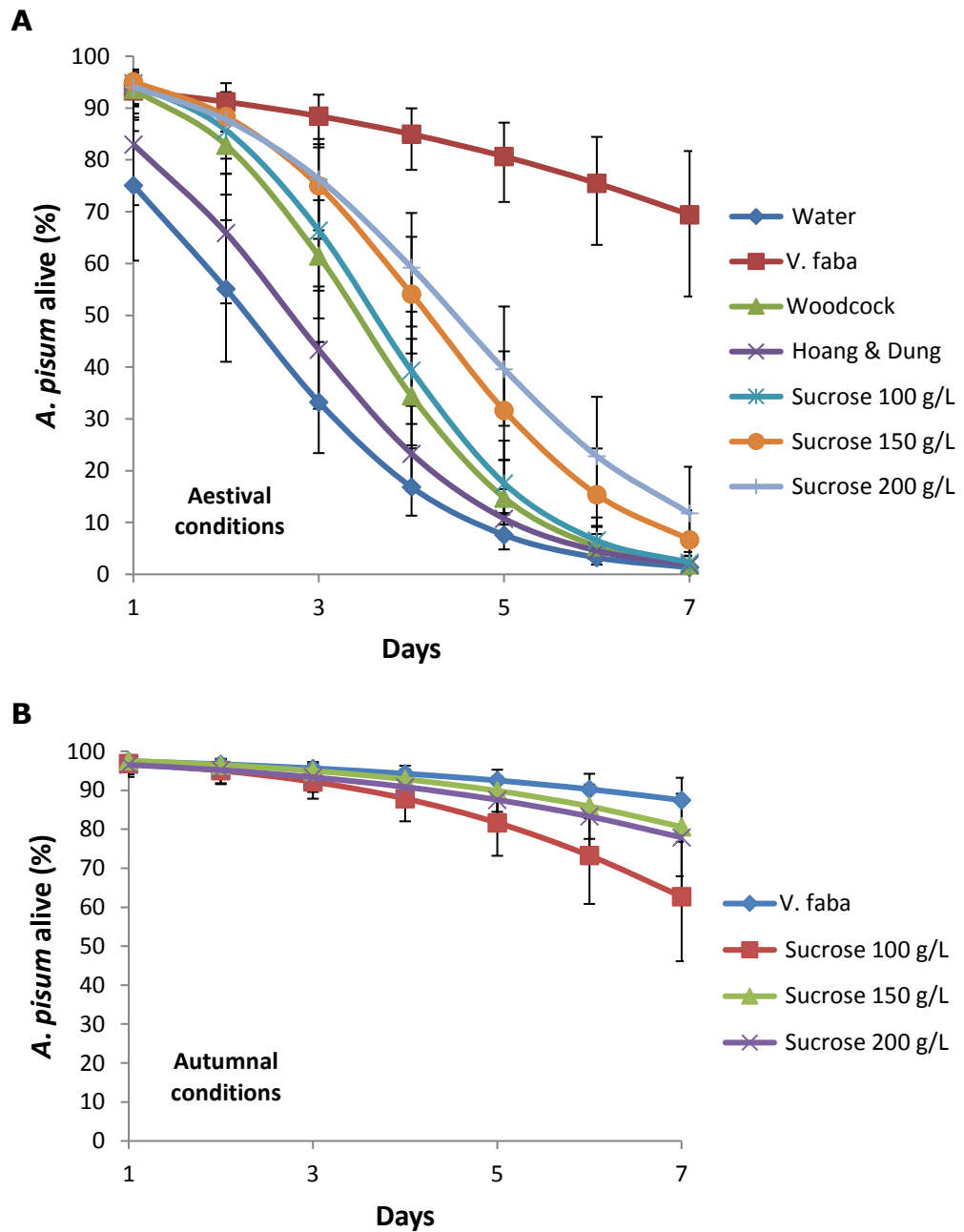


Figure 2.9. Seven-day survival rates (mean $\pm$ confidence levels) of *A. pisum* oviparae feeding on a range of diets in (A) aestival and (B) autumnal environments.

It was found, unsurprisingly, that water alone was not enough to sustain life. However, aphid response over time was statistically significantly different between the individual

diets ($F_{6,134}=11.37$, $p<0.001$ with logit-transformed data). A marginally improved response was apparent using the simpler sucrose-based diets, suggesting that the complex dietary components were present in toxic quantities. Despite reported success²⁰⁶, the inclusion of glutamine in the Hoang & Dung diet may explain its limited success as previous studies have suggested that its presence is detrimental to the feeding activities of this species²⁰⁷. An additional diet, designed by Van Emden for the maintenance of *M. persicae* colonies and comprising 37 components including amino acids, vitamins and minerals¹⁸⁴, was found to be lethal within 24 hours of feeding commencement and was therefore not included in the data. This highlights the potential differences in requirements between aphid species.

Due to their simplicity, ease of preparation and narrowly enhanced performance over the more complex diets under aestival conditions, sucrose-only diets were selected for further investigation into the effect of feeding environments. Considerable improvements in aphid life expectancy were observed upon removal of the transfer and containment stages associated with the aestival environment (Figure 2.9B). The reduction in handling- and temperature change-related stress could be responsible for these observations.

The survival rate of *A. pisum* oviparae on all diet solutions, independent of the diet content and the environment to which they were exposed, was reduced on comparison with that of *V. faba*-fed aphids. For the complex diets, this phenomenon could be explained by steady degradation of the diet solutions and subsequent ingestion of potentially toxic compounds. To minimise this risk, diet sachets were changed every 2-3 days. It is also possible that a host-specific species, such as *A. pisum*, requires a

phagostimulatory compound, present in *V. faba*, for optimal feeding, or that oviparae have more sensitive dietary requirements compared to asexual individuals.

In addition to achieving sufficient aphid survival, it is imperative that the basal diet solution does not affect sex pheromone production in oviparae and that substantial quantities of both sex pheromone components are still released for collection and analysis. To evaluate the diets' ability to maintain sufficient production of lactol **1** and lactone **2**, VOCs released from artificially-fed oviparae were collected by dynamic headspace collection after 2- and 7-day diet incubation periods and quantified against calibrated solutions for each analyte by GC (Figure 2.10). The positive control represents the VOCs collected from oviparae taken directly from the colony immediately prior to dynamic headspace collection. The negative control represents VOCs collected from the uninfested *V. faba* canopy.

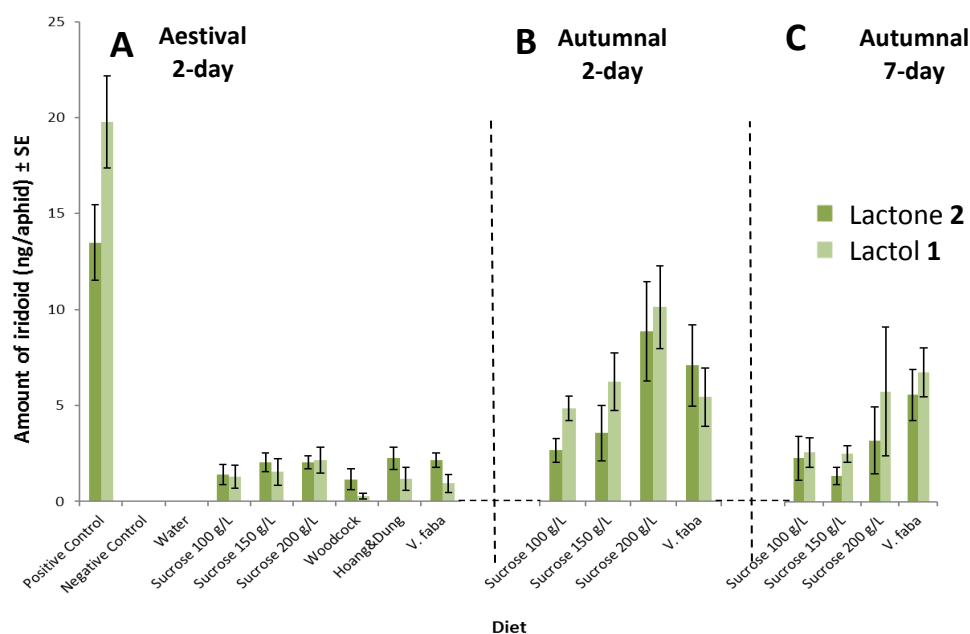


Figure 2.10. Sex pheromone production (mean ± confidence levels) in (A) aestival and (B & C) autumnal feeding environments, after (A & B) 2-day and (C) 7-day diet incubation periods.

Sex pheromone production was statistically significantly reduced after 48 hours of feeding on all artificial diets under aestival conditions (Figure 2.10A) when compared to the positive control (two-way ANOVA (d.f.=7,75): $p < 0.001$; 5% level LSD (3.23)). In contrast, significantly higher amounts of each component were obtained under autumnal conditions for each diet after an equal length of time (Figure 2.10B). Independent of diet make-up and environmental conditions, the production of both sex pheromone components did continue but the stress induced from enforced exposure to unnatural aestival conditions may be responsible for the reductive effect observed on their production. The emission of lactol **1** from aphids exposed to aestival conditions was noticeably reduced in comparison to lactone **2** production and could indicate an additional stress response to temperature.

The potential to employ longer diet incubation periods was also indicated by sufficient sex pheromone collections after 7 days (Figure 2.10C), despite the expected age-related decrease in lactol **1** and lactone **2** observed as seen with other aphid species^{20, 193}. This may be of use should the degree of incorporation of isotopically labelled compounds in the collected sex pheromone components be negligible after only 48 hours of feeding.

On combining results, *A. pisum* oviparae survival rate and sex pheromone production were found to be least affected whilst feeding on a simple 200 g/L D-sucrose-composed diet in an autumnal environment. This basal diet composition and feeding environment was therefore utilised for all subsequent incorporation studies.

2.3.2 Topical Application

Following several successes using topically applied compounds for insect biosynthetic pathway elucidation purposes¹⁸⁶⁻¹⁸⁷, a suitable topical application procedure for use with aphids was designed. To test the viability of using methanolic solutions of the compounds under investigation for topical application purposes, *A. pisum* oviparae were treated with 0.2 µL methanol, chosen for ease of handling small volumes¹⁸⁶, by a microsyringe directly onto the dorsal surface of the aphid (Figure 2.11A). After a 48-hour incubation period, during which the aphids were contained within clip cages and fed on *V. faba* leaves (Figure 2.11B), the VOCs were collected by dynamic headspace collection and compared to collections from a) untreated oviparae held within clip cages, b) oviparae taken directly from the colony immediately prior to dynamic headspace collection (positive control) and c) uninfested *V. faba* leaves (negative control). The incubation period was used to avoid contamination of aphid-produced VOCs with those of compounds applied to the aphid. The effect on sex pheromone production is shown in Figure 2.12.

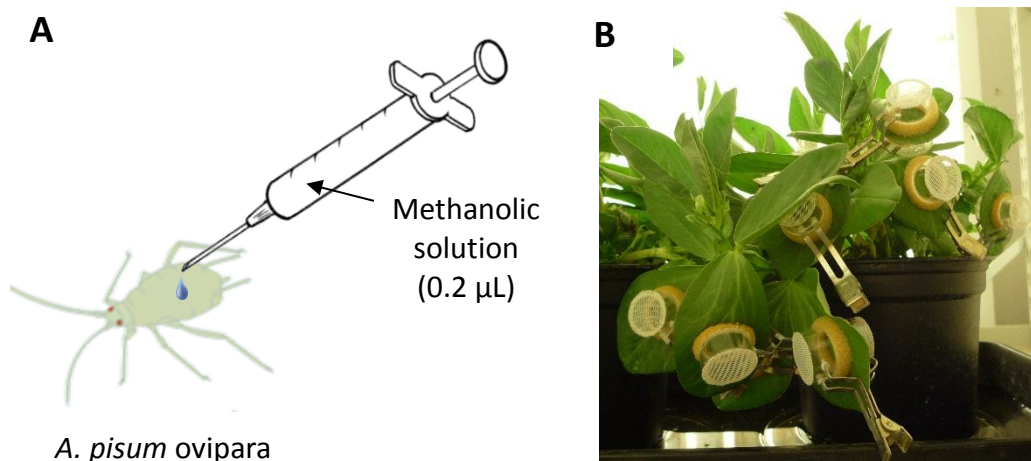


Figure 2.11. Topical application procedure whereby (A) a methanolic solution (0.2 µL) is applied to the dorsal surface of an *A. pisum* ovipara. (B) A 48-hour incubation period follows, during which oviparae are transferred to a *V. faba* plant and contained within a clip-cage, awaiting subsequent volatile collection by dynamic headspace collection.

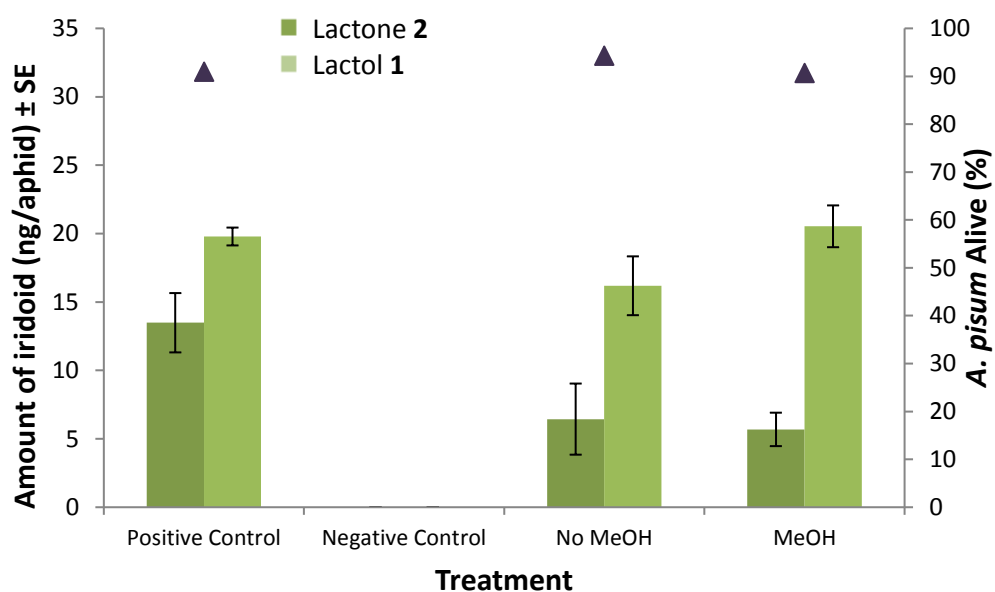


Figure 2.12. Aphid sex pheromone production (mean values ± confidence levels) following a 48-hour incubation period, with and without the topical application of methanol. ▲ Represents average *A. pisum* survival 48 hours post-topical application of methanol.

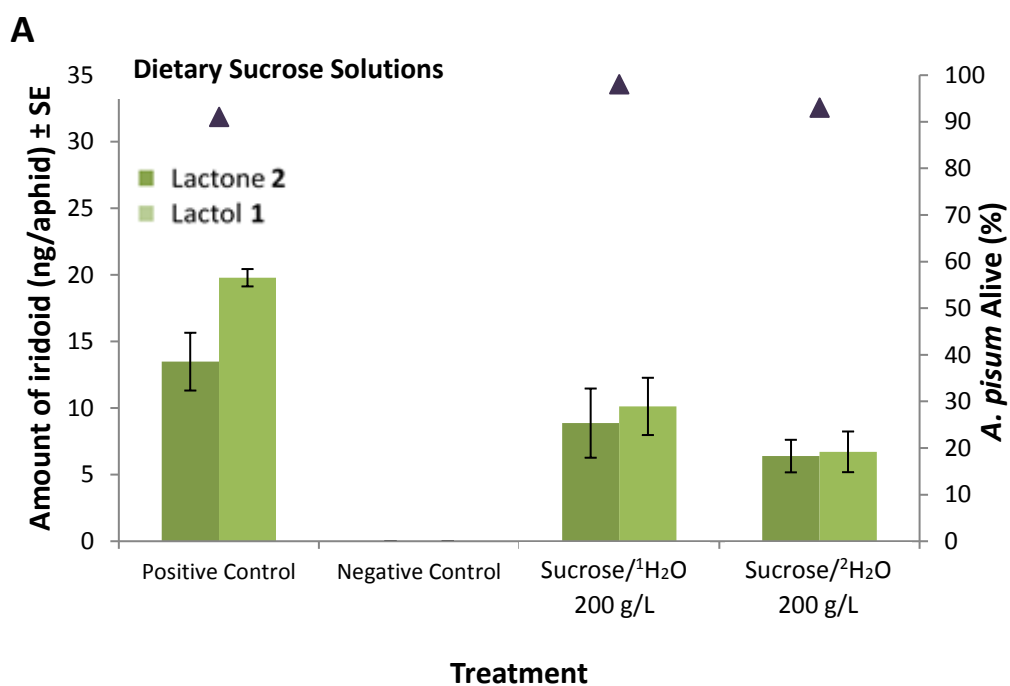
Despite the anticipated toxic effects of methanol, whereby aphids were momentarily paralysed after initial application, aphid survival rates of methanol-treated and untreated aphids were comparable, and survival was sufficient to allow for adequate sex pheromone collection and to illustrate their recovery. A decrease in the production of lactone **2**, when compared to the positive control, was evident in samples from both methanol-treated and untreated oviparae trapped in clip-cages, implying that its reduction may be attributable to clip-cage-induced stress rather than the topical application procedure. This contrasts with lactol **1** reduction as a possible result of temperature-related stress.

2.4 Incorporation of Unlabelled Putative Precursors and Intermediates: Results and Discussion

Having established working methods for the dietary incorporation and topical application of biosynthetically relevant compounds to *A. pisum*, biosynthetic pathway elucidation studies were commenced. The application of unlabelled compounds to aphids will provide a method of distinguishing between compound-specific and heavy isotope-induced effects in later isotopic labelling studies and will help to determine a suitable concentration at which to apply the deuterated monoterpenyl derivatives. Compounds were incorporated at two concentrations per application procedure; 125 µg/mL and 1 mg/mL solutions for dietary inclusion and 5 mg/mL and 100 mg/mL solutions for topical application. The lower dietary concentration was based on commonly reported diet analyte concentrations, the average 0.3-0.4 µL liquid uptake per day by aphids feeding on artificial diets and the number of aphids used per sachet.

2.4.1 Incorporation of Deuterium Oxide

To monitor the effect of heavy isotope ingestion on *A. pisum* survival rates and sex pheromone production, distilled water was substituted entirely for deuterium oxide ($^2\text{H}_2\text{O}$) in the 200 g/L sucrose diet solution (Figure 2.13A). Only 2-day feeding studies were conducted due to the expense of $^2\text{H}_2\text{O}$. Topical application of $^2\text{H}_2\text{O}$, present in a 50:50 methanolic mixture to aid absorption, was also performed (Figure 2.13B). Due to the high abundance and versatility of water in organisms, the presence, and indeed distribution, of deuterium atoms incorporated into lactol **1** and lactone **2** was not expected to reveal details of the mechanism.



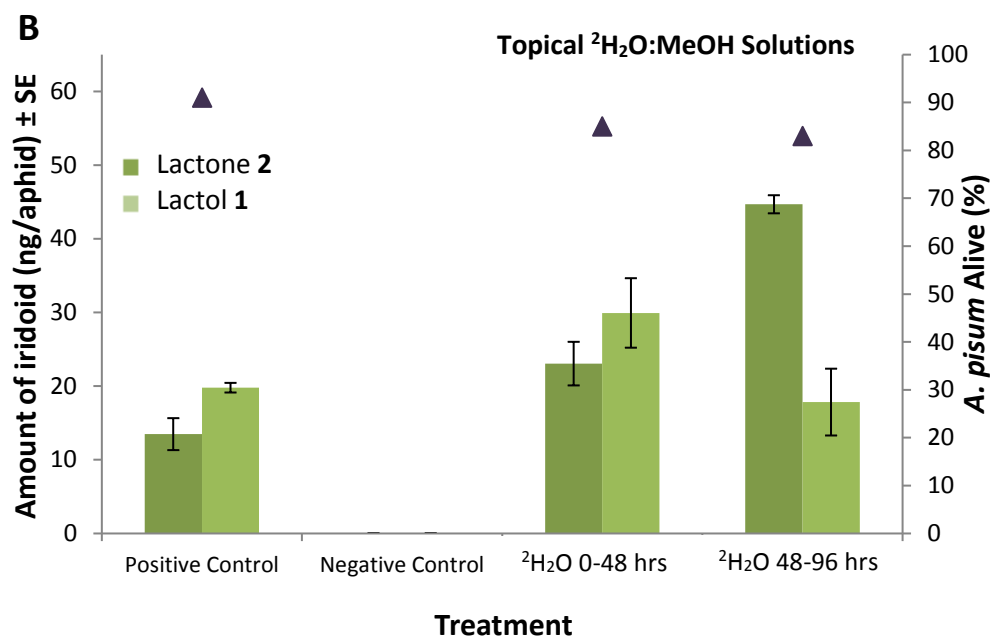


Figure 2.13. Sex pheromone production collections (mean $\pm$ confidence levels) (A) following a 48-hour diet incubation period with 200 g/L sucrose solution in $^2\text{H}_2\text{O}$ and (B) after topical application of 50:50 $^2\text{H}_2\text{O}:\text{MeOH}$, with VOC collections taken immediately (0-48-hour) or post-incubation period (48-96 hours). ▲ Represents average *A. pisum* survival 48 hours post-topical application.

The use of $^2\text{H}_2\text{O}$ as diet diluent did not significantly affect the survival rate or restrict the degree to which sex pheromone was produced after 2 days of feeding. Similarly, following topical application of 50:50 $^2\text{H}_2\text{O}:\text{MeOH}$, the survival rate and sex pheromone production were unaffected after 2 days. This data suggests that *A. pisum* can withstand considerable deuterium uptake and that the introduction of deuterium-labelled biosynthetic precursors and intermediates into live aphids will be feasible.

The total ion chromatogram (TIC) and individual ion chromatograms for m/z 166 and 167 (corresponding to unlabelled and $^2\text{H}_1$ -lactone **2**, respectively) and m/z 168 and 169 (corresponding to unlabelled and $^2\text{H}_1$ -lactol **1**, respectively), obtained using a 10 ng/ μL authentic standard, are represented in Figure 2.14A. On comparison with gas chromatograms obtained following $^2\text{H}_2\text{O}$ -based diet incubation (Figure 2.14B), a small

degree of separation is evident between the unlabelled and labelled iridoids. Surprisingly, there was little evidence of multi-deuterated lactol **1** or lactone **2** in dynamic headspace samples from $^2\text{H}_2\text{O}$ -fed *A. pisum* oviparae. Deuterated lactone **2** was found to elute earlier than its non-deuterated equivalent owing to the inverse isotope effect; a phenomenon regularly observed in GC, arising from differences in the non-covalent interactions between the stationary phase and the isotopomers²⁰⁸.

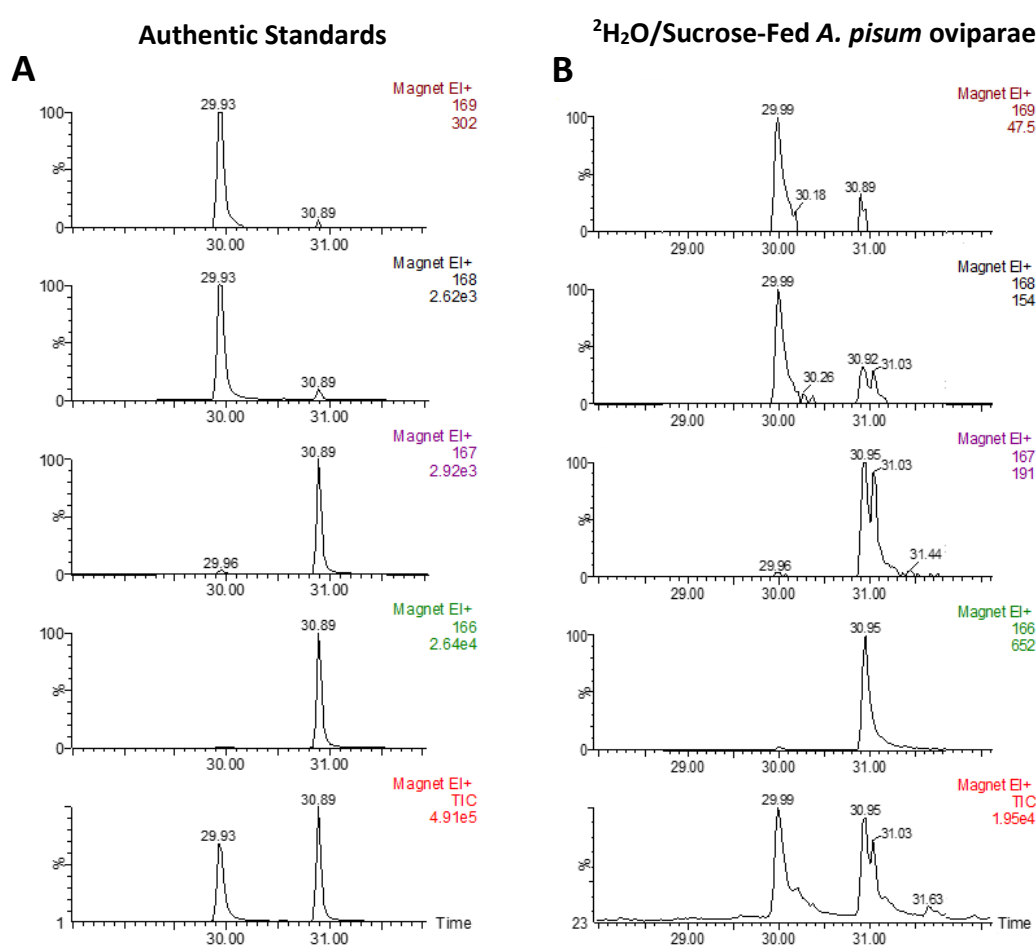


Figure 2.14. The TIC and individual ion chromatograms for m/z 166 (lactone **2**), 167, 168 (lactol **1**) and 169 using (A) a 10 ng/ μL authentic standard and (B) a dynamic headspace sample following VOC collection from $^2\text{H}_2\text{O}$ -fed *A. pisum* oviparae.

On comparison of lactol **1** and lactone **2** in the mass spectra of positive control samples (Figure 2.15A), where natural ^{13}C and ^2H abundance is observed at 1.1% and 0.015%, respectively, a higher molecular ion m/z 167:166 ratio was detected in the mass spectra of dynamic headspace collections following $^2\text{H}_2\text{O}$ -based diet incubation, suggesting that deuterium from the diet solution was incorporated into lactone **2** (Figure 2.15B). Despite the presence of an exchangeable hydroxyl proton, results were less conclusive for lactol **1**, with little evidence of deuterium incorporation observed. The reasons for this remain unknown and were not pursued further.

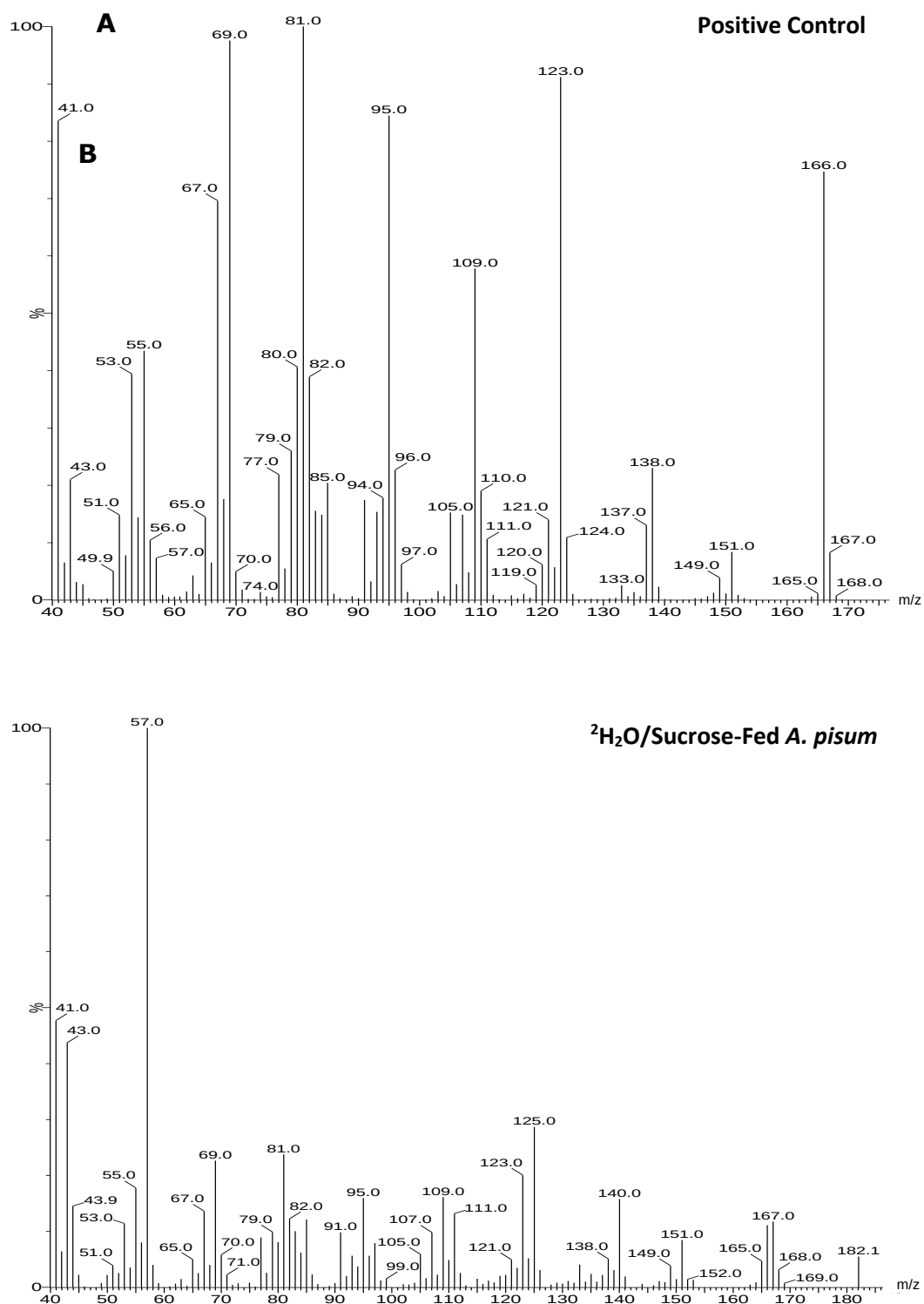


Figure 2.15. Mass spectra of lactone **2** collected from *A. pisum* feeding on
(A) *V. faba* (positive control) and **(B)** a 200 g/L sucrose solution in $^2\text{H}_2\text{O}$.

2.4.2 Incorporation of Monoterpenols

Unlabelled monoterpenols (**56**, **62** and (**S**)-**57**) were applied *via* dietary and topical methods to *A. pisum* oviparae to assess their impact on aphid survival and sex pheromone production. Addition to the diet at 125 µg/mL did not significantly affect *A. pisum* 7-day survival rate, however survival was impeded at 1 mg/mL for each monoterpenol (Figure 2.16).

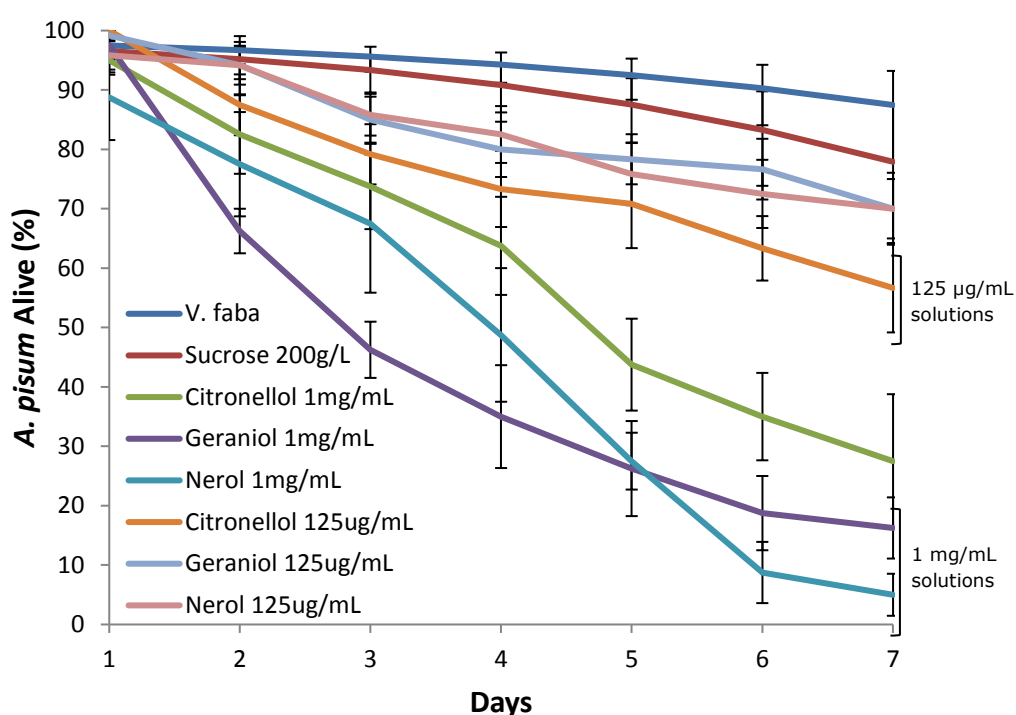


Figure 2.16. Seven-day survival rates (mean $\pm$ confidence levels) of *A. pisum* oviparae feeding on monoterpenols diets.

In contrast, the sex pheromone released was significantly inhibited at both concentrations, an effect that was most pronounced using (*S*)-citronellol (**(S)**-**57**) (Figure 2.17A). Topical application of the same compounds did not significantly inhibit sex pheromone production when 0.2 µL of each of the 5 mg/mL methanolic monoterpenol solutions were applied (Figure 2.17B). Conversely, the number of live aphids was largely depleted when 100 mg/mL solutions were applied, and neither lactol **1** nor lactone **2**

were observed in dynamic headspace samples from the surviving aphids (data not shown).

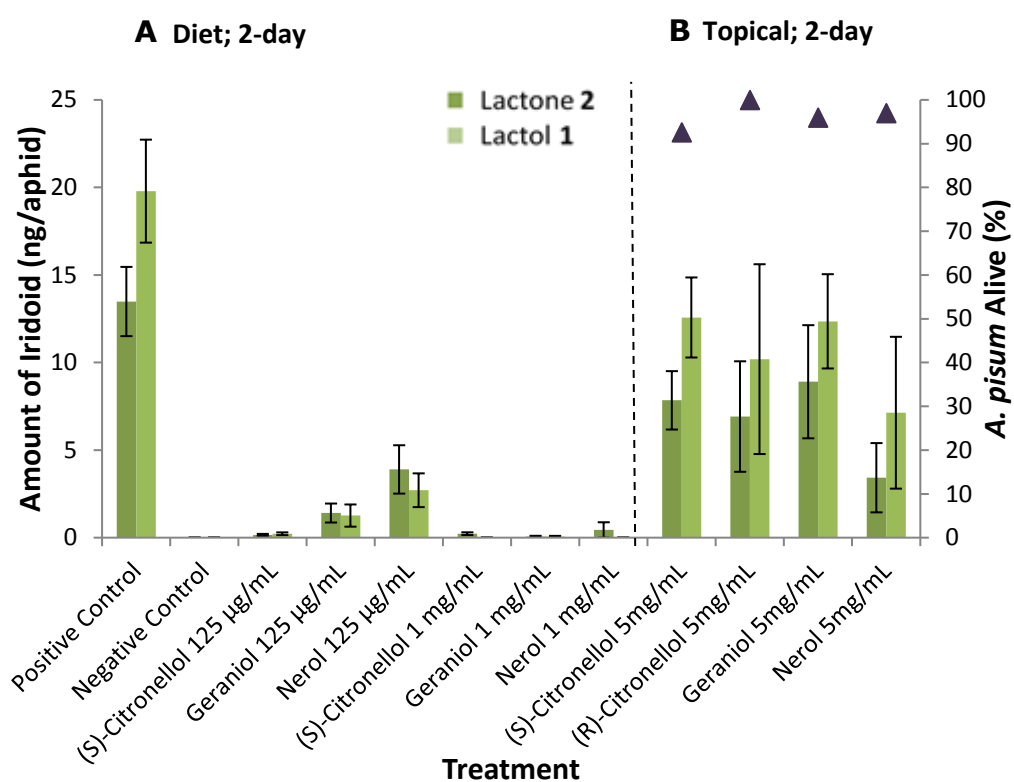


Figure 2.17. *A. pisum* sex pheromone production (mean $\pm$ confidence levels) following a 48-hour incubation period with (A) dietary and (B) topically applied monoterpenes.

▲ Represents average *A. pisum* survival 48 hours post-topical application of monoterpenes.

Unsurprisingly, only negligible amounts of both lactol 1 and lactone 2 were collected following a 7-day diet incubation period with 125 µg/mL monoterpene solutions (Figure 2.18), suggesting that a longer incubation will not be feasible when using the labelled monoterpenes with the current number of aphids used.

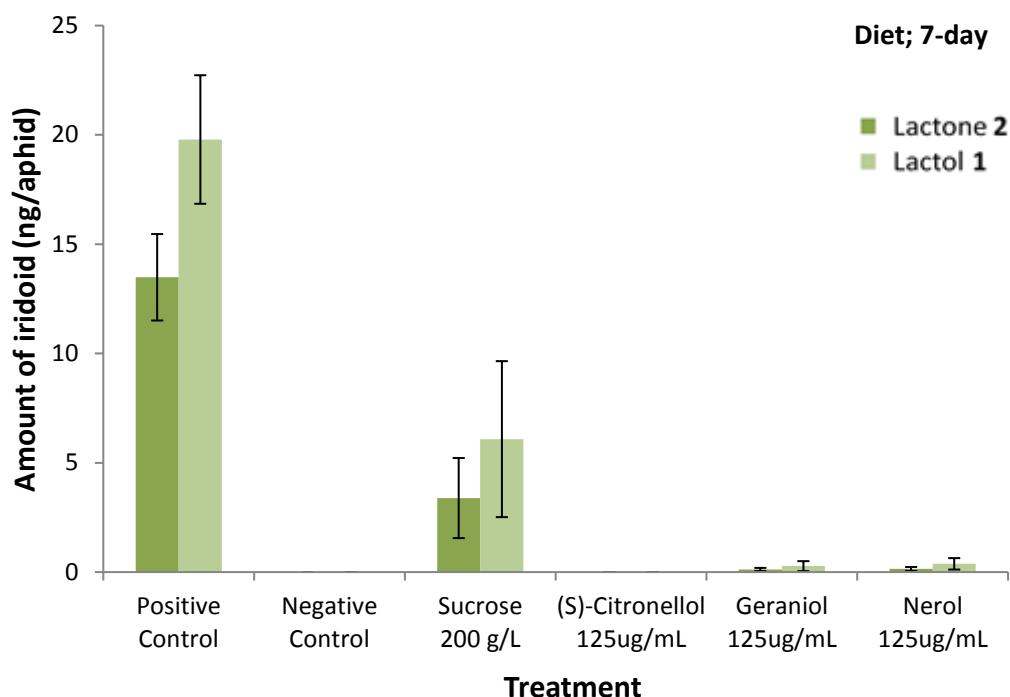


Figure 2.18. *A. pisum* sex pheromone production (mean $\pm$ confidence levels) after a 7-day diet incubation period using monoterpenols.

Satisfactory aphid survival rates and relatively unimpeded sex pheromone production rates obtained using topical application of 5 mg/mL monoterpenol solutions led to further experiments using this method for the $^2\text{H}_6$ -monoterpenols. However, the non-dietary nature of this procedure will provide no insight into the potential sequestration of compounds.

Significant differences in sex pheromone production observed using dietary and topical methods could imply that the ingestion and/or internal transport of these compounds is limited, likely resulting from restricted solubility in the haemolymph. As discussed, aphids may biosynthesise naturally occurring, water-soluble hydroxymonoterpenol glucosides in the fat body or sequester them from plants and use these as the initial precursors to lactol **1** and lactone **2**. Alternatively, *A. pisum* may biosynthesise

precursors to the sex pheromone components from entirely different compounds in the fat body.

The strong feeding deterrent effects associated with monoterpenol volatiles further explains the improved aphid sex pheromone collections through topical application¹³⁷. The incorporation of unlabelled and deuterated geraniol into Chrysomelid beetles was unsuccessful *via* dietary application but successful upon injection directly into the haemolymph of an aqueous solution of geraniol^{137, 163}. Despite the toxic effects and high mortality rates reportedly seen in *M. persicae* feeding on citronella grass essential oil (comprising terpenes such as (*S*)-citronellol (**57**), geraniol (**56**) and nerol (**62**))²⁰⁹, the survival rate of aphids fed on an aqueous sucrose-only solution and those fed on 125 µg/mL monoterpenol-containing sucrose solutions were comparable, indicating that feeding was not inhibited. The reduction in sex pheromone production in the monoterpenol-fed aphids would therefore signify an inhibitory effect on sex pheromone biosynthesis by these compounds, suggesting they may interfere with the inherent biosynthetic pathway.

2.4.3 Incorporation of Monoterpenals

Following the topical application of methanolic solutions of monoterpenals **109**, **110**, (*S*)-**111** and (*R*)-**111** to *A. pisum* oviparae, the release of lactol **1** and lactone **2** initially decreased but recovered after 48 hours, most notably for (*R*)-citronellal (*R*)-**111**-treated aphids (Figure 2.19). The prolonged immobilisation of aphids that was observed on application of the monoterpenals is likely responsible, for the aphids were unable to move or feed for several hours. A moderate decrease in survival rate was observed only for geraniol **109**-treated aphids.

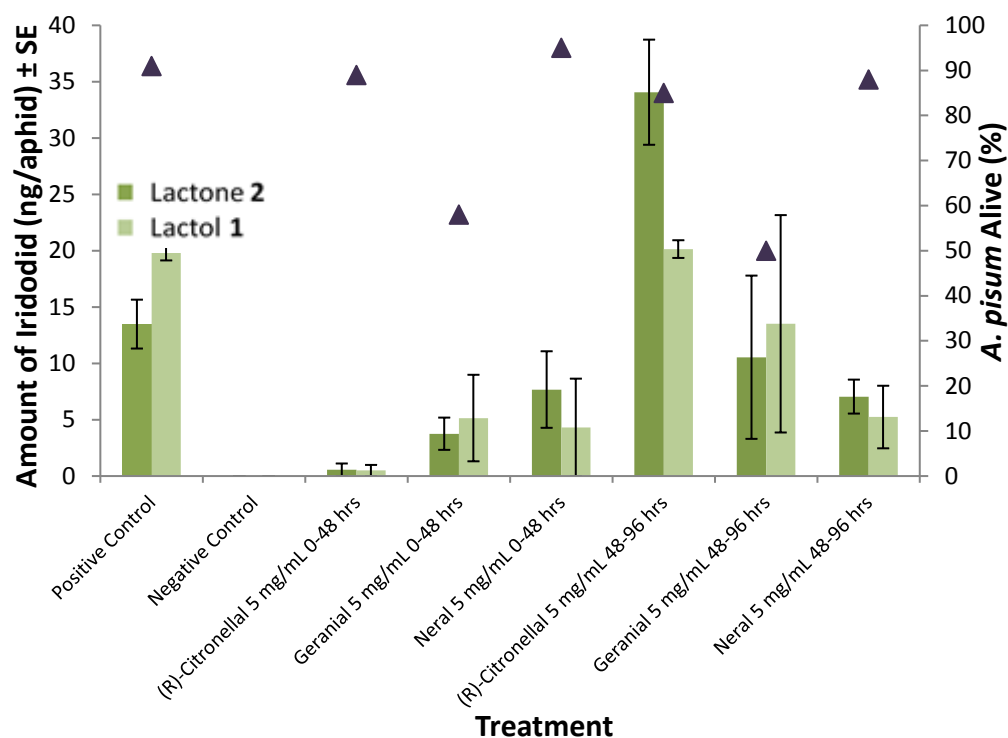


Figure 2.19. *A. pisum* sex pheromone production (mean $\pm$ confidence levels) after topical application of monoterpenals, with VOC collections taken immediately (0-48 hours) or post-incubation period (48-96 hours). $\blacktriangle$ Represents average *A. pisum* survival.

Aphids did not survive the topical application of any 100 mg/mL monoterpene solution and appeared paralysed within seconds of application, indicating that this concentration cannot be used in future investigations.

2.4.4 Incorporation of 8-Hydroxymonoterpenols

The effect of topically applied unlabelled 8-hydroxymonoterpenols, **72**, **91**, **(S)-90** and **(R)-90**, to *A. pisum* oviparae is shown in Figure 2.20.

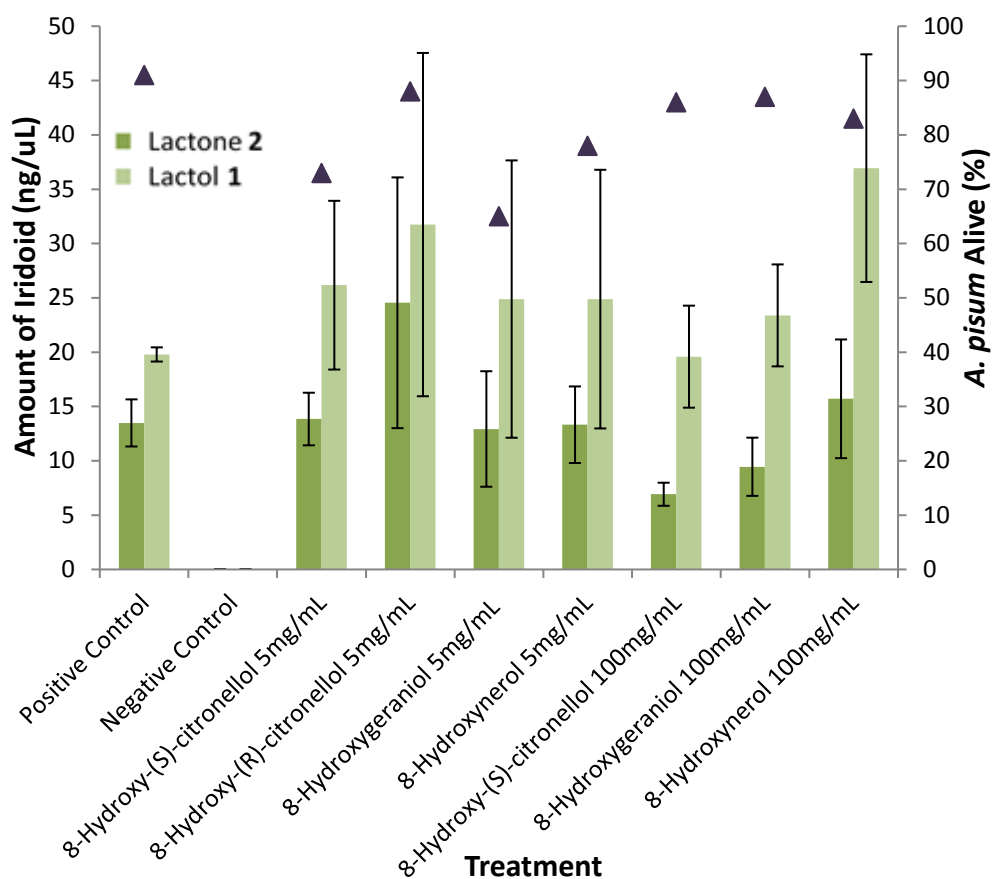


Figure 2.20. *A. pisum* sex pheromone production (mean $\pm$ confidence levels) after topical application of 8-hydroxymonoterpenols after a 48-hour incubation period.

▲ Represents average *A. pisum* survival.

Again, inconsistencies in oviparae age may explain the broad range in lactone **2** and, especially, lactol **1** collections observed for each data set. Despite this variance, the topical application procedure did not appear to negatively affect sex pheromone production or aphid survival, even at the higher concentration of 100 mg/mL, and can therefore be utilised in the isotopic labelling studies.

2.4.5 Incorporation of 8-Hydroxymonoterpenals

Topical application of unlabelled 8-hydroxymonoterpenals, **73**, **130** and **(S)-131**, to *A. pisum* oviparae was conducted using only the 5 mg/mL methanolic solutions (Figure

2.21), as the application of 100 mg/mL solutions of previously investigated monoterpenyl derivatives has resulted in equivalent, if not poorer, aphid survival rates and sex pheromone production.

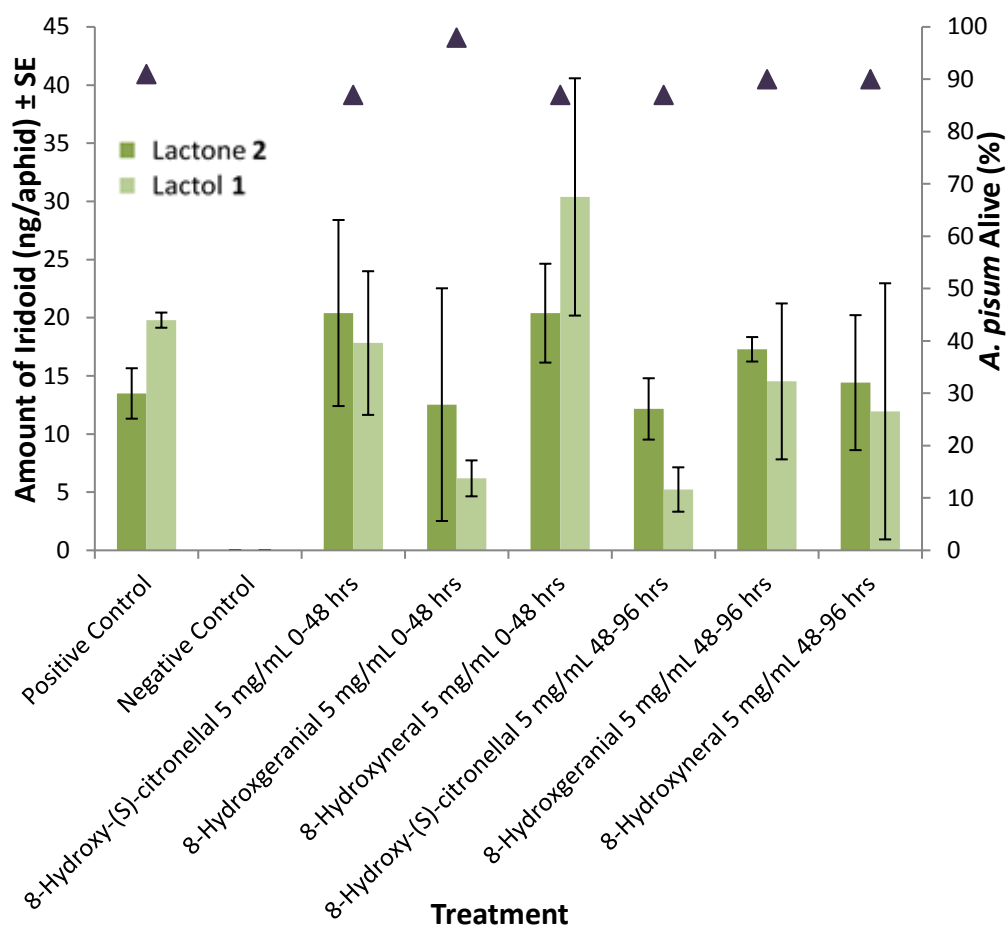


Figure 2.21. *A. pisum* sex pheromone production (mean $\pm$ confidence levels) after topical application of 8-hydroxymonoterpenals, with VOC collections taken immediately (0-48 hours) or post-incubation period (48-96 hours).

▲ Represents average *A. pisum* survival.

Although neither the release of lactone **2** nor aphid survival were substantially altered, lactol **1** production was often reduced in comparison to lactone **2**.

2.4.6 Incorporation of 8-Oxomonoterpenols

Unlabelled 8-oxomonoterpenols, **74**, **132** and **(S)-133**, were topically applied to *A. pisum* oviparae and the VOCs collected immediately after application, without the 48-hour incubation period (Figure 2.22). The incubation period had primarily been incorporated into the method in order to avoid contamination of aphid-produced VOCs with those of compounds applied to the aphid, but signal interference was not observed. In most cases, the incubation period had not served to improve sex pheromone production and was, as expected, associated with a reduction in the number of surviving aphids.

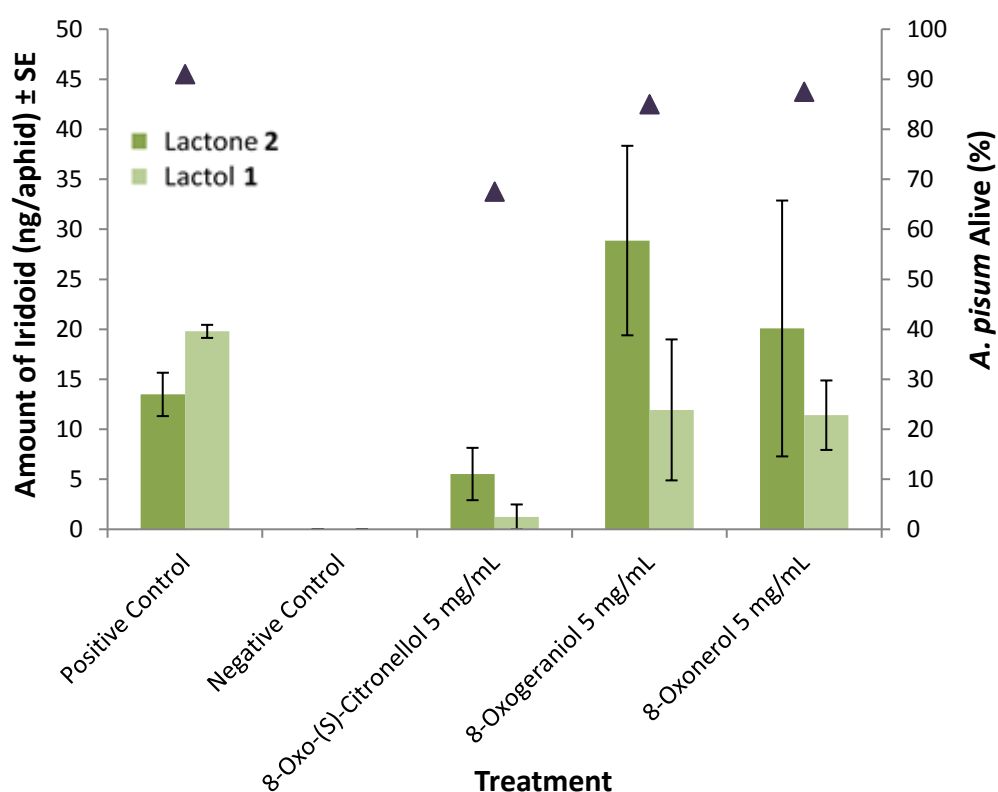


Figure 2.22. *A. pisum* sex pheromone production (mean ± confidence levels) immediately after topical application of 8-oxomonoterpenols. ▲ Represents average *A. pisum* survival.

Similar to the results observed using 8-hydroxymonoterpenals, the production of lactol **1** was suppressed in relation to lactone **2** on application of each 8-oxomonoterpenyl derivative.

2.4.7 Incorporation of 8-Oxomonoterpenals

As with 8-oxomonoterpenol incorporation, the topical application of 5 mg/mL methanolic solutions of unlabelled 8-oxomonoterpenals, **75**, **137** and **(S)-138**, instigated a reduction in lactol **1** production in relation to lactone **2** formation and showed an increase in lactone **2** production compared to the positive control (Figure 2.23). Despite aphid survival significantly decreasing in the 48 hours following topical application of both 8-oxo-(*S*)-citronellal and 8-oxoneral, the survival rates of 8-oxogeranial-treated aphids were comparable to those of untreated aphids, thereby representing a potential intermediate in lactone **2** biosynthesis. These unstable dialdehydes were either applied immediately or stored at -20 °C under nitrogen prior to application to avoid degradation and were assumed pure when applied.

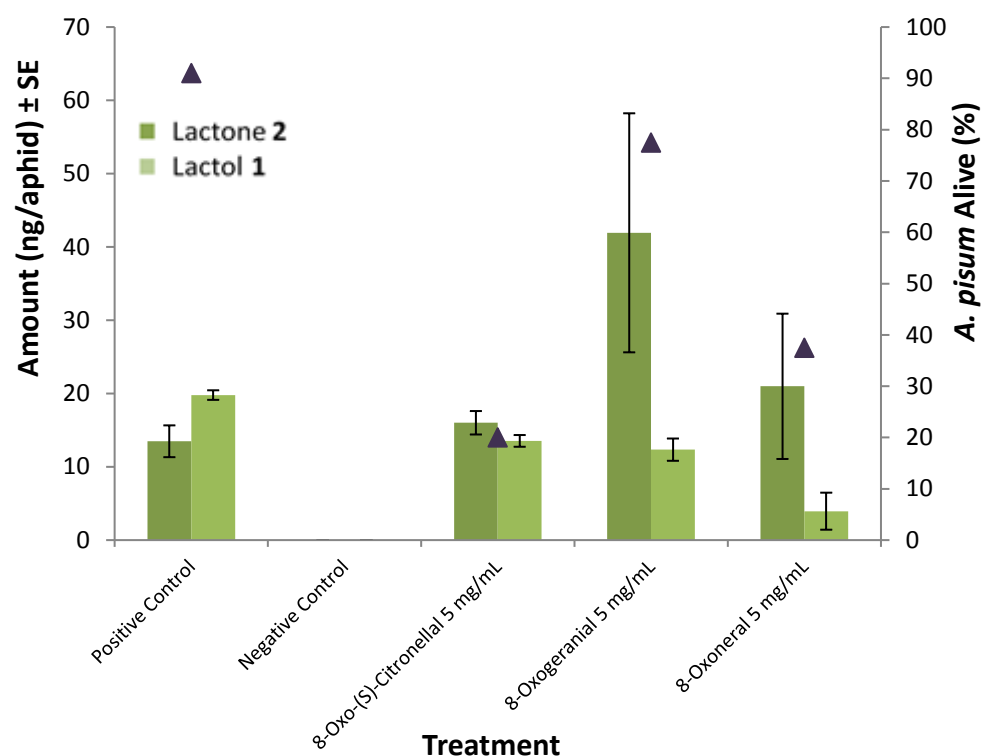


Figure 2.23. *A. pisum* sex pheromone production (mean $\pm$ confidence levels) after topical application of 8-oxomonoterpenals. $\blacktriangle$ Represents average *A. pisum* survival.

The results obtained from the application of unlabelled putative precursors and intermediates to *A. pisum* oviparae can be applied to forthcoming deuterium incorporation studies (Chapter 3). Topical application of all compounds gave improved results when compared to dietary incorporation methods and, from the conditions tested, optimum results were often achieved using 5 mg/mL methanolic solutions and when collecting VOCs immediately following exposure. This method will therefore be primarily focussed on during isotopic labelling studies.

3. Synthesis and Incorporation of [C-8,C-10-²H_n]-Labelled Putative Precursors and Intermediates

Heavy isotope labelling, specifically deuterium labelling, was used to probe the biosynthetic pathway to the aphid sex pheromone. This method was chosen over radioisotope labelling which requires a hazardous and often lengthy degradation process. Due to the higher natural abundance of heavy isotopes, however, greater label incorporation is required to achieve convincing isotopic enrichment ⁷⁶. Additionally, the incorporation of isotopically labelled compounds into living insects is often minimal ($\leq 1\%$) due to the numerous metabolic processes potentially competing for use of the labelled intermediates ⁷⁶, a problem often confounded by the labelled product being obscured by the natural isotopomer.

Mass spectrometry (MS) was used to trace the metabolic fate of the label in collected sex pheromone samples, as dynamic headspace collections provided an insufficient amount of lactol **1** and lactone **2** ($< \mu\text{g}$) for analysis by NMR spectroscopy. The intensity of the molecular (M+1) ion in mass spectra, arising from the presence of the naturally abundant (1.1%) ¹³C-isotope, makes it hard to distinguish between natural sex pheromone and that of ²H₁-lactol **1** and ²H₁-lactone **2** arising from singular deuterium incorporation ⁷⁶. To avoid superposition of the natural isotopomer, multi-labelled putative biosynthetic precursors and intermediates for the aphid sex pheromone were designed initially, containing labels in locations not critically related to the hypothesised mechanism of biosynthesis and where normal metabolic processes are unlikely to remove them. From these labelled compounds, label incorporation, or lack of, into the

aphid sex pheromone can confirm or rebut their involvement in the biosynthetic pathway. Additional labels or alterations to these compounds may then provide data to aid biosynthetic mechanism considerations.

3.1 Synthesis of [C-8,C-10- $^2\text{H}_n$]-Labelled Putative Precursors and Intermediates

The initial synthesis of [8,8,8,10,10,10- $^2\text{H}_6$]-monoterpenols (Figure 3.1) was conducted to ensure that the mass-to-charge ratio (m/z) resulting from isotopic label incorporation is distinguishable from those resulting from natural isotopomers.

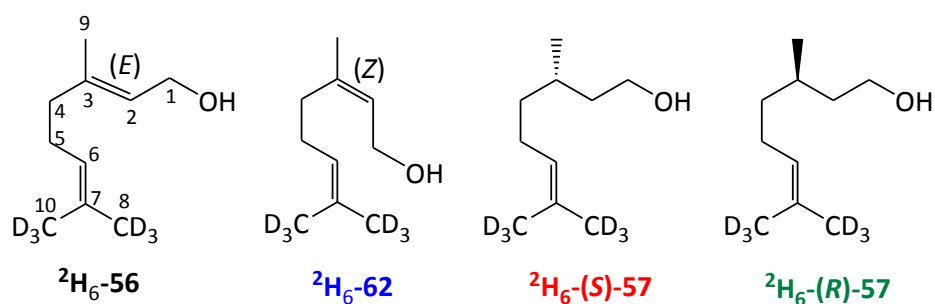
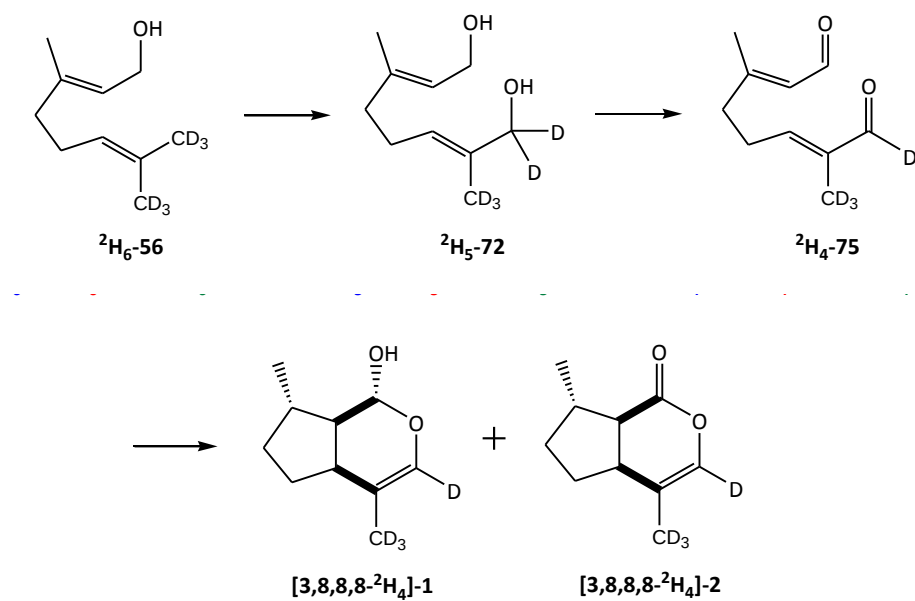


Figure 3.1. Structures of the [8,8,8,10,10,10- $^2\text{H}_6$]-monoterpenols showing the position of deuterium incorporation.

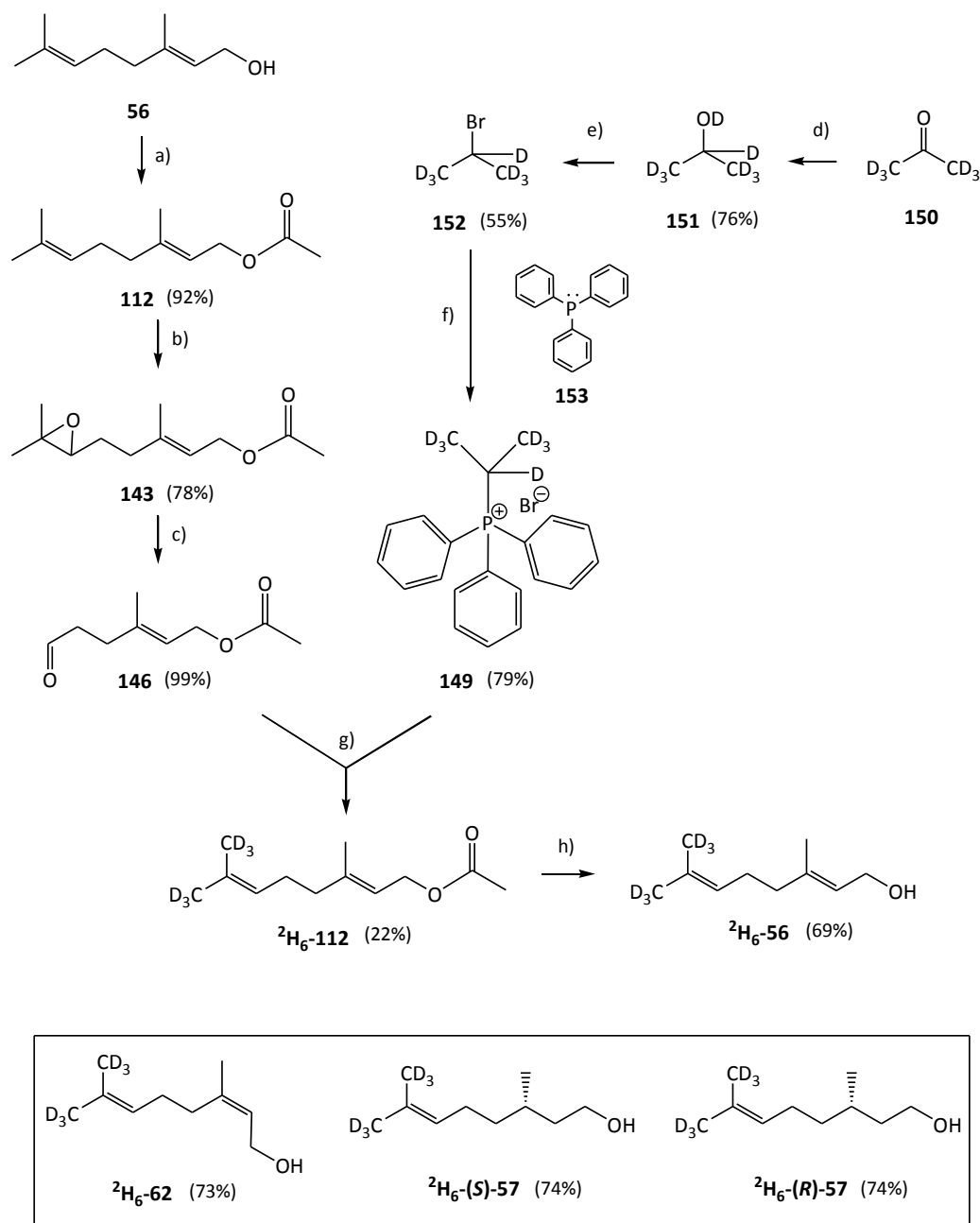
Independent of the biosynthetic pathways currently hypothesised (Section 1.3.2.1), incorporation of the $^2\text{H}_6$ -monoterpenols and their oxidised derivatives into lactol **1** and lactone **2** was expected to follow the labelling route shown in Scheme 3.1. Consequently, molecular ions of m/z 170 and 172 in the mass spectra of dynamic headspace collections, representing $^2\text{H}_4$ -lactone **2** and $^2\text{H}_4$ -lactol **1** respectively, would indicate deuterium incorporation, suggestive of their involvement in the biosynthesis.



Scheme 3.1. Putative biosynthetic labelling route from ${}^2\text{H}_6$ -monoterpenols to [3,8,8,8- ${}^2\text{H}_4$]-lactol **1** and [3,8,8,8- ${}^2\text{H}_4$]-lactone **2** (shown only for ${}^2\text{H}_6$ -geraniol) in aphids.

3.1.1 Synthesis of [8,8,8,10,10,10- ${}^2\text{H}_6$]-Monoterpenols

The synthetic procedure to the [8,8,8,10,10,10- ${}^2\text{H}_6$]-monoterpenols is outlined in Scheme 3.2, following a similar procedure to that described by Luan and colleagues²¹⁰.



Scheme 3.2. Synthetic scheme for $^2\text{H}_6$ -labelled geraniol ($^2\text{H}_6$ -56), nerol ($^2\text{H}_6$ -62), (*S*)-citronellol ($^2\text{H}_6$ -(*S*)-57) and (*R*)-citronellol ($^2\text{H}_6$ -(*R*)-57); a) Ac_2O , pyridine, 4-DMAP, 2 hrs, rt; b) *m*-CPBA, DCM, 2 hrs, 0 °C; c) periodic acid, sodium periodate, THF:H₂O (9:1), 1 hr, 0 °C to rt; d) LiAlD_4 , diethylene glycol dimethyl ether, 1 hr, 0 °C; e) PBr_3 , 24 hrs, -10 °C; f) PPh_3 , 24 hrs, 150 °C; g) *n*-BuLi, THF, 4 hrs, -78 °C to rt; K_2CO_3 , MeOH, 4 hrs, rt.

Following acetyl protection of commercially available monoterpenols, **56**, **62**, (*S*)-**57** and (*R*)-**57**, using acetic anhydride, epoxidation of the 6,7-alkene was effected using *m*-CPBA

in a classic Prilezhaev reaction. Low initial yields of the geranyl and neryl epoxide derivatives, 24% and 32% respectively, were found to result from the epoxidation of the 2,3-alkene (as determined by NMR spectroscopy; Figure 3.2). To limit over-oxidation, the *m*-CPBA was added gradually, over a shorter amount of time (<30 minutes) whilst maintaining a temperature of 0 °C. The extent of epoxidation was monitored by TLC throughout to maximise yields of the desired products. This resulted in an increase in yield of the desired monoepoxide (76-78%), although the diepoxide was detected prior to full consumption of the monoterpenyl acetate starting materials.

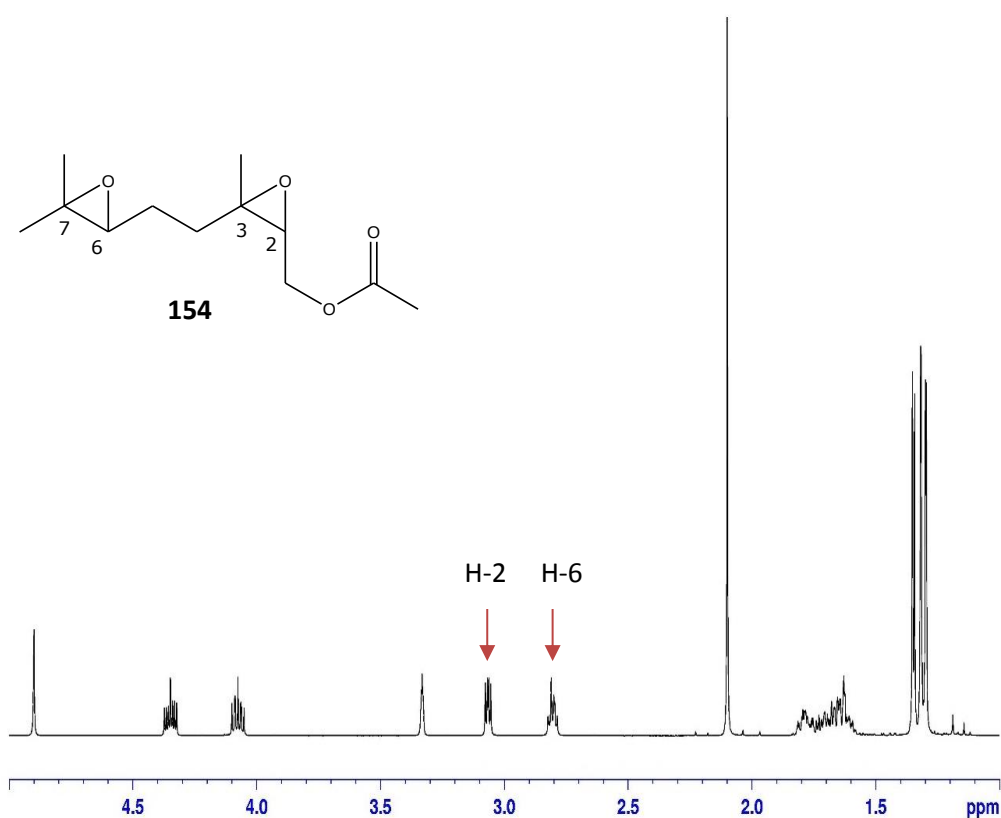
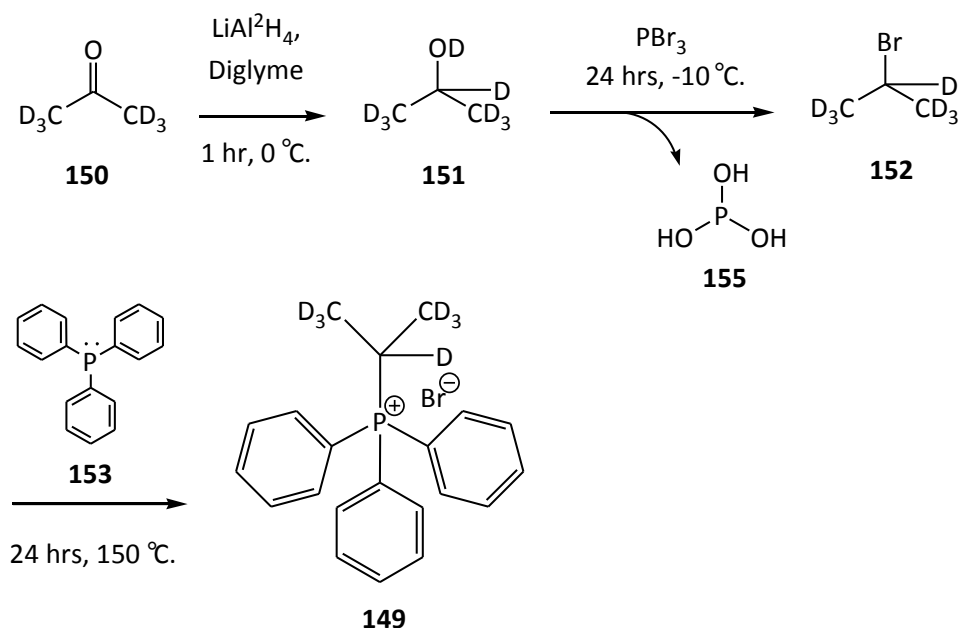


Figure 3.2. ^1H -NMR spectrum (and, inset, the structure) of the 2,6-diepoxyneryl acetate (**154**) by-product.

Oxidative cleavage of the epoxides to the corresponding aldehydes was initially attempted using 1.1 eq. of periodic acid under anhydrous conditions, with a reaction time of 90 minutes. In addition to the desired aldehyde product, multiple by-products were observed resulting in relatively low yields (40-60%). A literature search revealed

an optimised method using both sodium periodate (0.6 eq.) and periodic acid (1.1 eq.), each introduced at 0 °C using a 9:1 THF:H₂O solvent mixture ²¹¹. This new method vastly improved the yield (75-99%) of the desired aldehyde. This could be attributable to the presence of water, which acts to partially dissolve the sodium periodate salt and promotes epoxide ring-opening. The latter is the rate-limiting step in a two-step process, involving initial hydrolysis of the epoxide to yield a diol intermediate followed by subsequent cleavage of the C-C bond to deliver the aldehyde ²¹²⁻²¹³.

Conversion of the aldehydes to the corresponding ²H₆-monoterpenyl acetates *via* a Wittig reaction utilised a [1,1,1,2,3,3,3-²H₇]-isopropyltriphenylphosphonium bromide salt (**149**). This salt was prepared by heating commercially available triphenylphosphine (**153**) and ²H₇-2-bromopropane (**152**) at 150 °C and stirring for 40 hours prior to recrystallisation with ethanol:diethyl ether (1:10). To avoid purchasing large quantities of expensive ²H₇-2-bromopropane **152**, the bromoalkane was partially synthesised in-house (Scheme 3.3). ²H₆-Acetone (**150**) was reduced to ²H₇-isopropanol (**151**) using LiAl²H₄ and separated from the reaction mixture by distillation, collecting the distillate at 115-127 °C ²¹⁴. Diethylene glycol dimethyl ether (diglyme) was chosen as the solvent due to its high boiling point (162 °C), and therefore ease of separation from product **151**, and for its ability to efficiently solvate the lithium cation. Once isolated, deuterated alcohol **151** was brominated to ²H₇-2-bromopropane **152** using phosphorus tribromide and separated from a phosphonic acid tautomer by-product (**155**) by routine distillation at 85-105 °C.



Scheme 3.3. Synthetic route to [1,1,1,2,3,3,3- ^7H]-isopropyltriphenylphosphonium bromide salt **149**.

The Wittig reaction produced the four desired $^2\text{H}_6$ -monoterpenyl acetates in yields not exceeding 31%. It was imperative to improve on these low initial yields due to the expense of, and lengthy synthetic procedure for, the deuterated phosphonium salt. The reaction was repeated using the corresponding non-deuterated phosphonium salt to determine whether the chemical structures of the aldehydes themselves could be causing the depleted yields. No improvement was seen, suggesting that a structural effect was indeed being observed.

Two unidentified by-products of higher polarity, one found in equal quantities to that of the desired product and the other representing approximately 50% of the total yield, were consistently observed and the higher-yielding impurity isolated for NMR spectroscopy analysis. Following a Wittig reaction using acetate-protected aldehyde **146**, the ^1H -NMR spectra of the expected product, $^2\text{H}_6$ -**56**, (Figure 3.3A) and the impurity (Figure 3.3B) were compared. The impurity gave a similar spectrum but with evidence

of a downfield shift in the chemical shift of the acetate methyl group (H-12) and a new peak at 3.5 ppm.

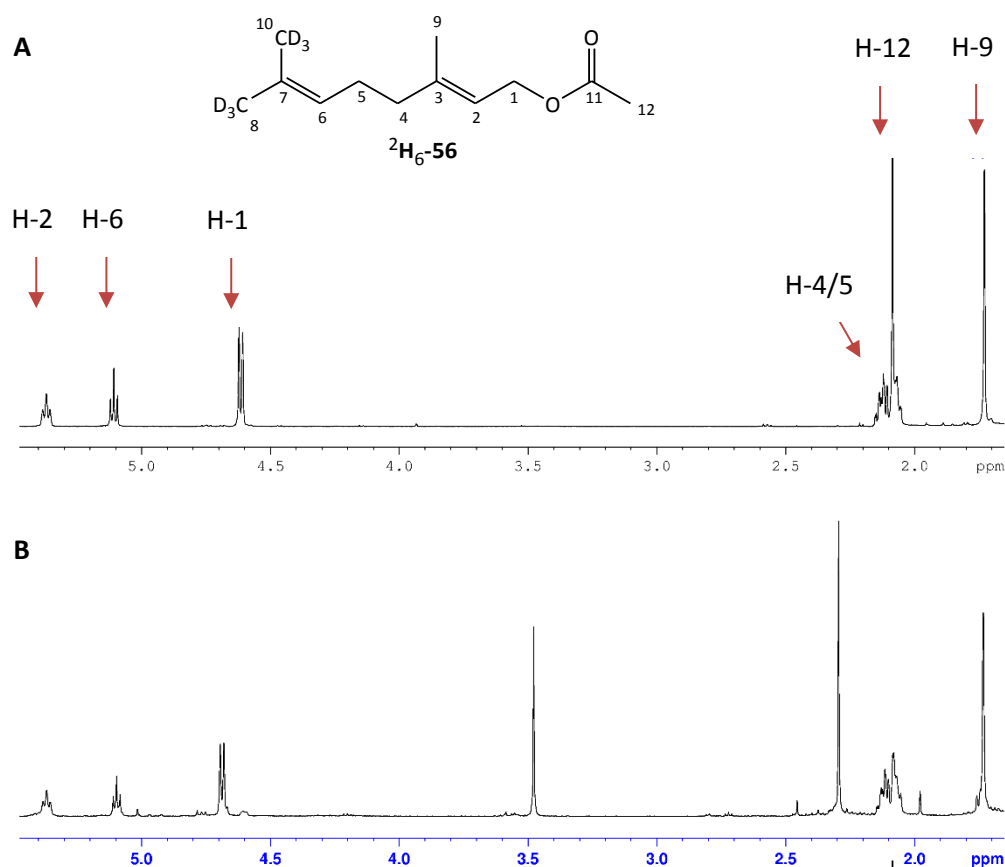


Figure 3.3. ^1H -NMR spectral comparison of (A) the desired product, $^2\text{H}_6$ -geraniol ($^2\text{H}_6$ -56), and (B) the impurity formed during a Wittig reaction using acetylated aldehyde **146**. Inset, the structure of $^2\text{H}_6$ -56.

From examining the ^1H - and ^{13}C -NMR spectra of the impurity (Figure 3.4, A and B respectively), the identity of the impurity was hypothesised to be $^2\text{H}_6$ -geranyl acetoacetate (**165**). This explained the singlet peak at 3.5 ppm integrating to 2 protons (H-12), the deshielding of the acetate methyl protons corresponding to the singlet integrating to 3 protons at 2.3 ppm (H-14), and a downfield carbon peak at 201 ppm arising from the presence of a ketone (C-13).

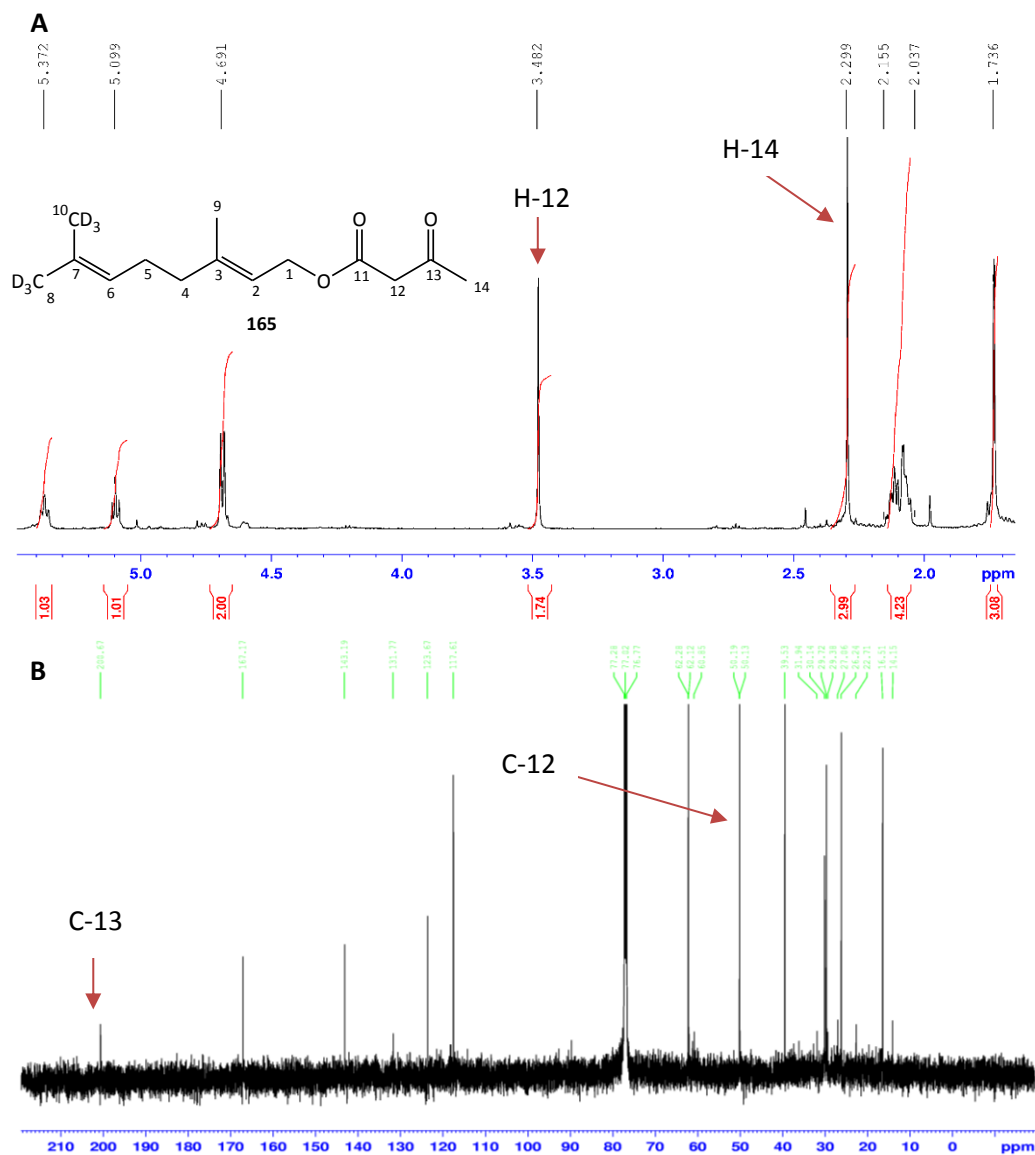


Figure 3.4. The (A) ^1H -NMR spectrum and (B) ^{13}C -NMR spectrum of the Wittig impurity with peaks present in the impurity, but not in the desired product $^2\text{H}_6$ -**56**, annotated.

Inset, the hypothesised structural identity of the impurity,
 $^2\text{H}_6$ -geranyl acetoacetate (**165**).

A heteronuclear multiple-bond correlation (HMBC) spectrum of the impurity generated during the Wittig reaction is shown in Figure 3.5. Coupling was evident between the ketone carbon (C-13) and a) the shifted acetate methyl protons (H-14) and b) the protons α to both carbonyl groups (H-12) (green arrows). Coupling was also observed between the acetoxy carbon (C-11) and a) the alkoxy protons (H-1) and b) H-12, as well

as between C-12 and H-14. All spectra were found to match those reported in the literature for geranyl acetoacetate after correcting for the deuterium labelling²¹⁵. These results also matched those of the impurity formed using the (*S*)-citronellyl derivative.

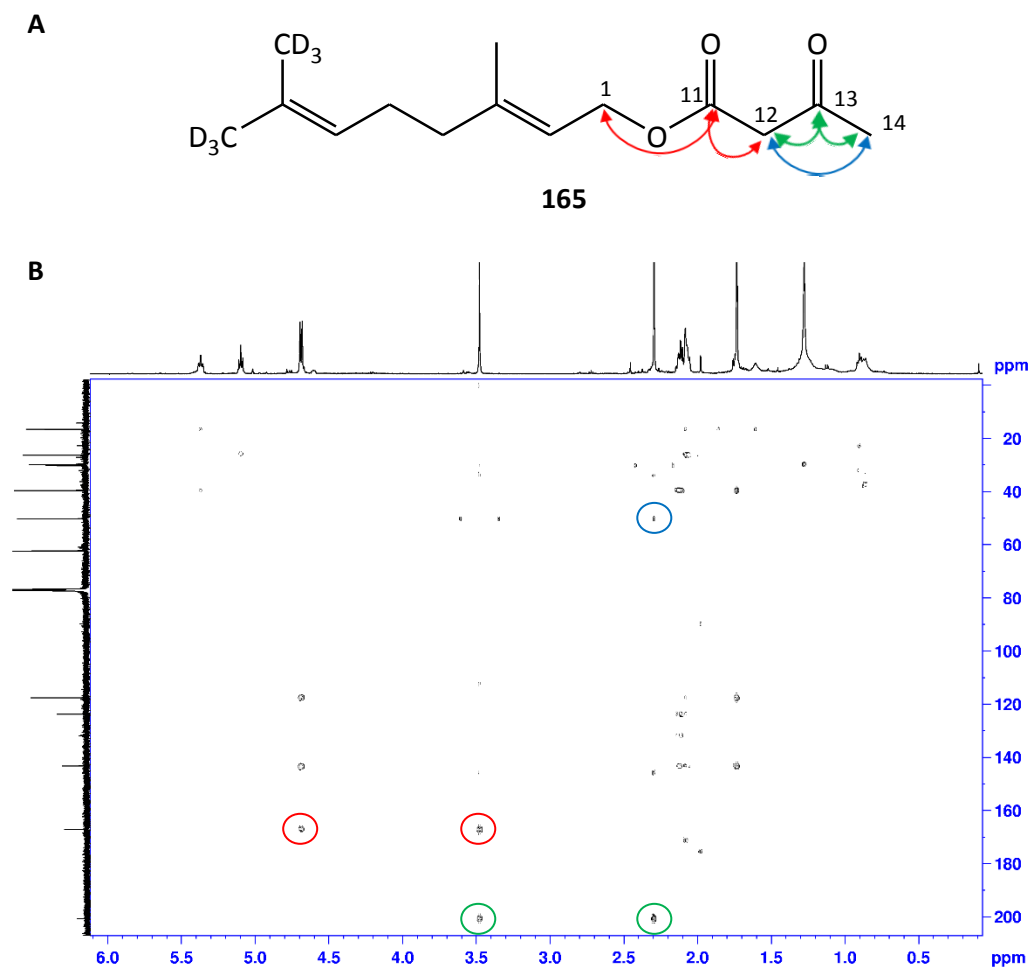
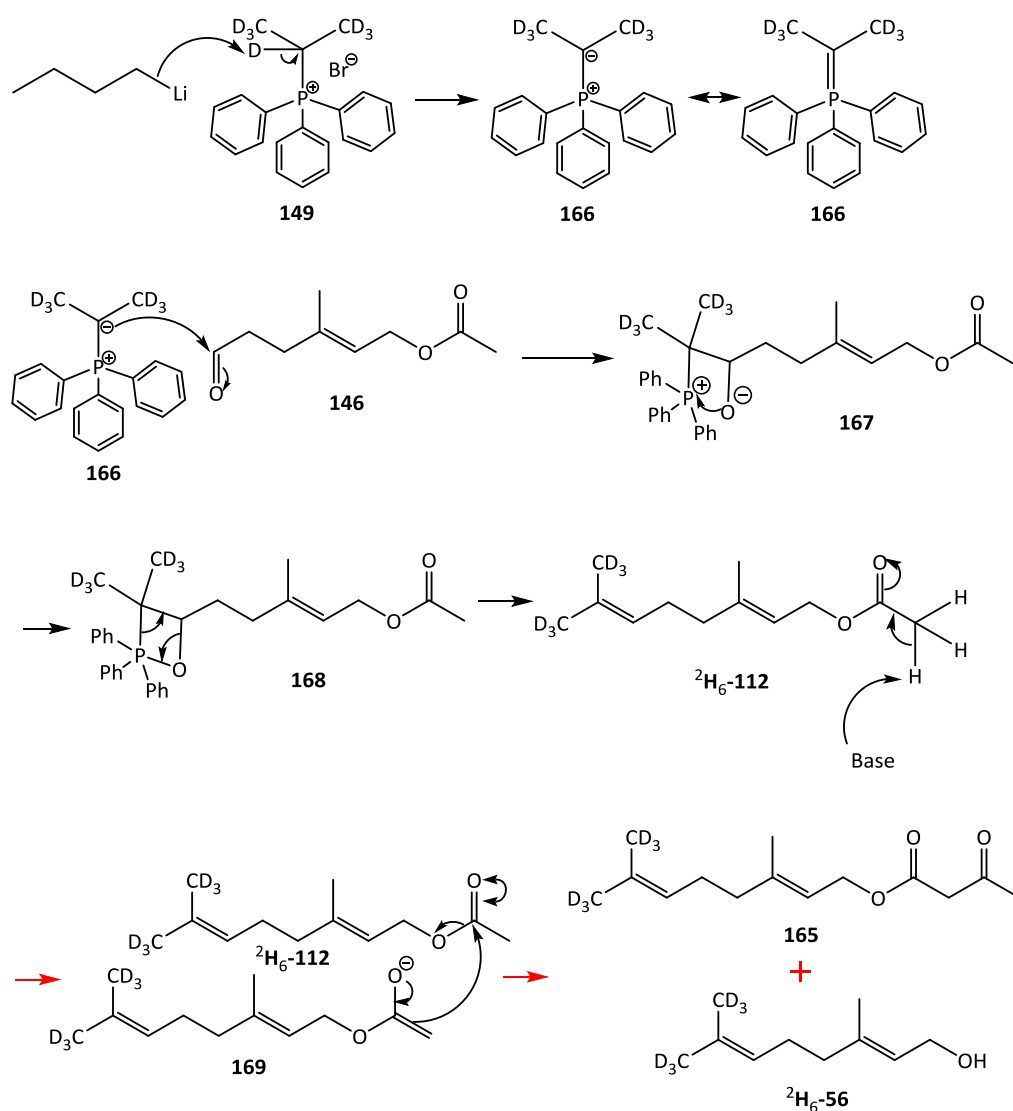


Figure 3.5. (A) The ¹³C-¹H coupling evident in Wittig impurity **165** and (B) the corresponding HMBC spectrum of **165** with highlighted coupling circled.

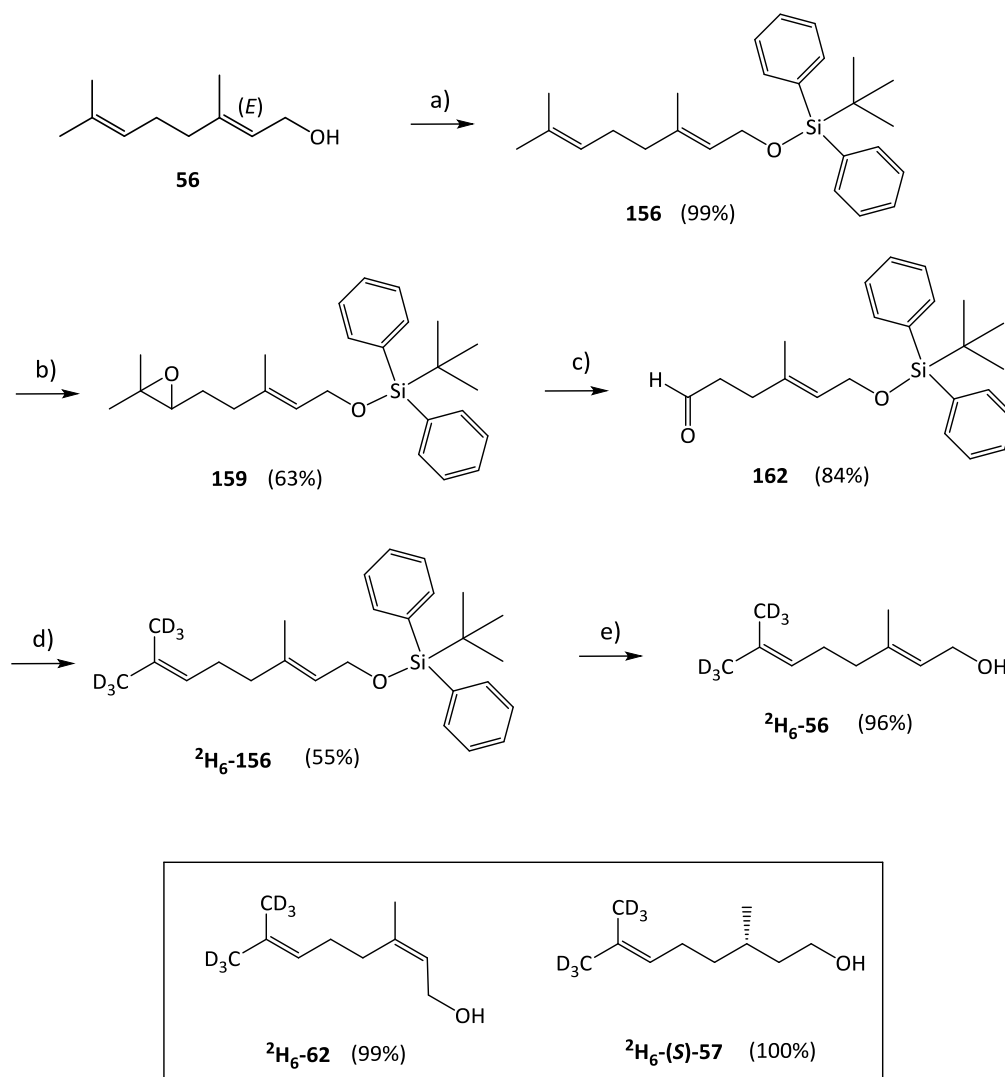
The lack of examples in the literature of using an acetyl protecting group during a Wittig reaction is likely a result of the low yields arising from its use, however an explanation for the poor yields has not been given to date. The formation of polar by-products, ²H₆-geranyl acetoacetate (**165**) and ²H₆-geraniol (**²H₆-56**), during the Wittig reaction of aldehyde **146** seems a likely cause of the low yields observed here, and may proceed *via* the mechanism shown in Scheme 3.4. Deprotonation of phosphonium salt **149** with

n-BuLi to give ylide **166**, and subsequent attack of the ylide on aldehyde **146**, would proceed as normal. The formation of betaine intermediate **167** would give, on cyclisation, its more stable form, oxaphosphetane **168**, which then collapses to give the desired product, $^2\text{H}_6$ -geranyl acetate ($^2\text{H}_6$ -**112**). However, further deprotonation of the acetate group, assisted by basic ylide **166**, could follow to give enolate **169**. An aldol reaction between **169** and $^2\text{H}_6$ -**112** would then yield both **165** and $^2\text{H}_6$ -**56**.



Scheme 3.4. Putative synthetic route to the Wittig reaction impurity, $^2\text{H}_6$ -geranyl acetoacetate **165**, and side-product, $^2\text{H}_6$ -geraniol $^2\text{H}_6$ -**56**.

For this project, the acetate group had originally been selected to facilitate a global deprotection of a multi-acetylated glucoside probe. This was no longer required and the consistently low yields incited a change in protecting group. Sen and Ewing²¹⁶ documented a 92% yield using a structurally similar aldehyde utilising a TBDPS-protecting group. To test the viability of using TBDPS-protected monoterpenols as an alternative, geraniol **56**, nerol **62** and (S)-citronellol (**S**)-**57** were protected (*via* the addition of *tert*-butyldiphenylsilyl chloride and imidazole), epoxidised, cleaved and deuterated as detailed before, resulting in yields of 55%, 52% and 44%, respectively, for the Wittig reaction. Final deprotection using TBAF was carried out successfully, thus completing an improved method to the ²H₆-monoterpenols (Scheme 3.5).

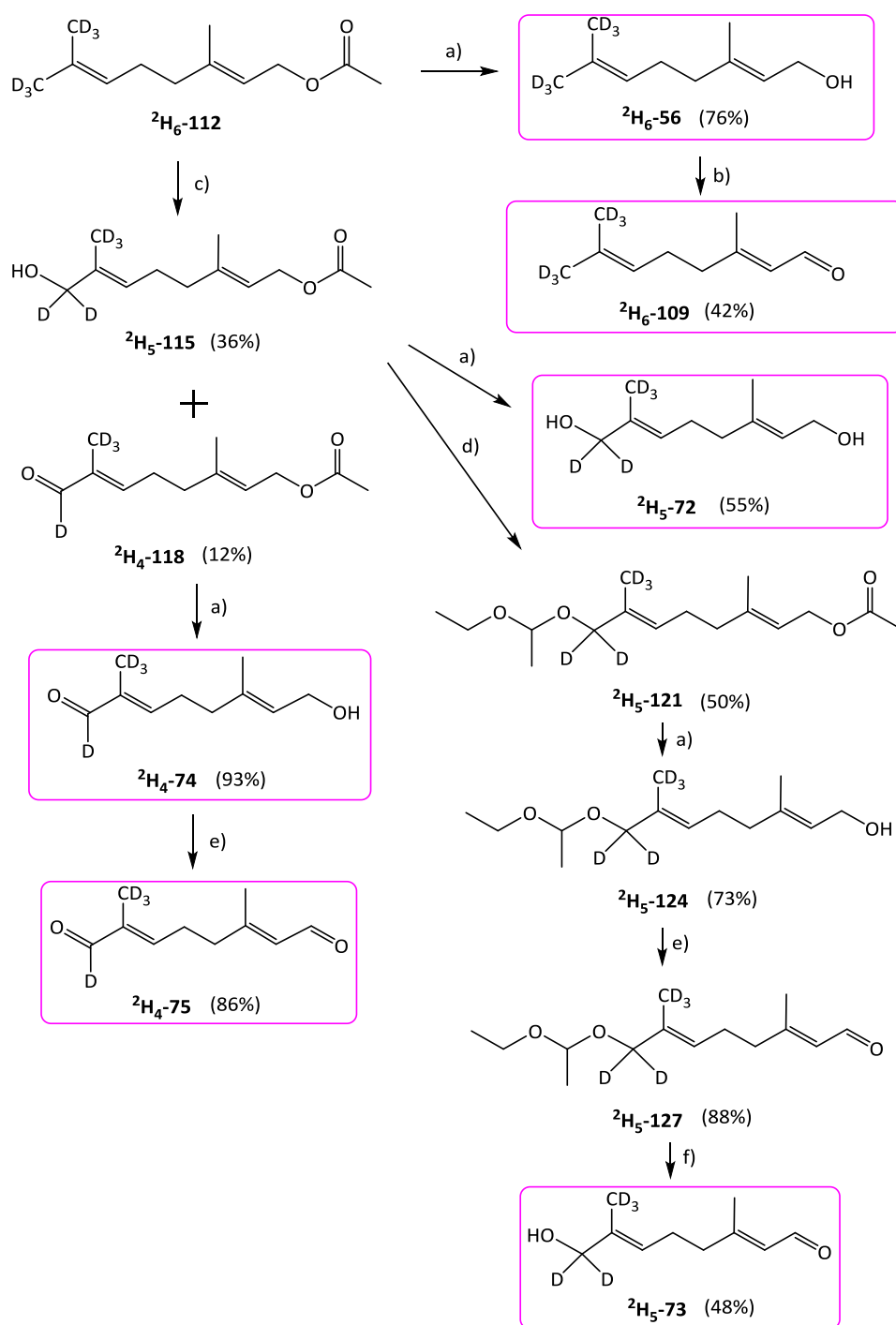


Scheme 3.5. Synthetic scheme for $^2\text{H}_6$ -labelled monoterpenols, geraniol, nerol and (S)-citronellol, via TBDPS ether-protected intermediates with the corresponding yields (%); a) *t*-butyldiphenyl silyl chloride, imidazole, DCM, 19 hrs, 0 °C to rt; b) *m*-CPBA, DCM, 2 hrs, 0 °C; c) periodic acid, sodium periodate, THF:H₂O (9:1), 1 hr, 0 °C to rt; d) *n*-BuLi, THF, 4 hrs, -78 °C to rt; e) TBAF, THF, 1 hr at 0 °C then 3 hrs at rt.

3.1.2 Synthesis of Oxidised Derivatives of the [8,8,8,10,10,10- $^2\text{H}_6$]-monoterpenols

To probe the biosynthetic pathway of the aphid sex pheromone using isotopically-labelled compounds, it was imperative to synthesise putative intermediates along the

route of oxidation towards lactol **1** and lactone **2**. The synthetic procedure to these compounds using the acetate-protected [8,8,8,10,10,10- $^2\text{H}_6$]-monoterpenols initially synthesised is shown in Scheme 3.6 and Figure 3.6, based on the synthetic scheme described by Dawson and colleagues ¹⁵ and described in Section 2.2 for the synthesis of the unlabelled oxidised monoterpenol derivatives. Except for TBAF-deprotection steps, no other alterations to this procedure were required when using TBDPS ether-protected monoterpenols, however the 8-hydroxymonoterpenals were not synthesised from TBDPS ether-protected materials due to sufficiently available $^2\text{H}_5$ -8-hydroxymonoterpenyl acetates.



Scheme 3.6. Synthetic scheme for deuterium-labelled geranyl derivatives of putative precursors and intermediates for the aphid sex pheromone with the corresponding yields (%) (neryl, (*S*)-citronellyl and (*R*)-citronellyl derivatives contain different R groups as shown in black box, see Figure 2.5); a) K_2CO_3 , MeOH, 4 hrs, rt; b) MnO_2 , DCM, 40 hrs, rt; c) SeO_2 , *t*-BuO₂H, salicylic acid, DCM, 72 hrs, rt; d) Ethyl vinyl ether, anh. DCM, cat. pyridinium *p*-toluenesulfonate, 4 hrs, rt; e) IBX, DMSO, 2 hrs, rt; f) Acetic acid:H₂O:THF (8:1:1), 3 hrs, rt. Pink boxes indicate compounds intended for aphid application.

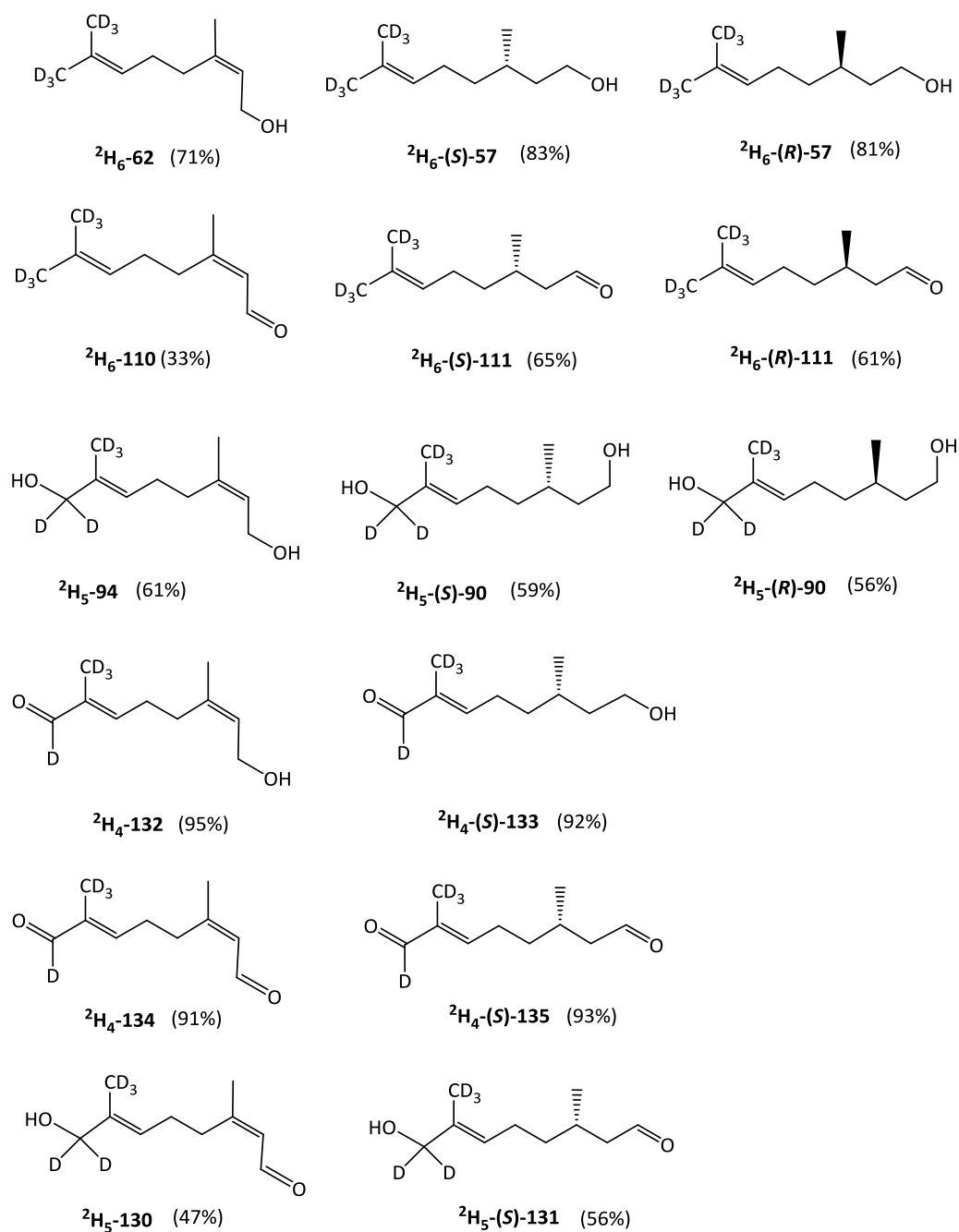


Figure 3.6. Deuterium-labelled neryl, (*S*)-citronellyl and (*R*)-citronellyl derivatives of putative precursors and intermediates for the aphid sex pheromone intended for aphid application.

Despite improved yields using TBDPS-protected monoterpenols, combining the low-yielding Wittig reaction and allylic oxidation afforded only small quantities of the deuterated precursors and intermediates. Regardless of the protecting group used,

neither the epoxidation, epoxide cleavage nor the Wittig reaction were amenable to scaling up by a factor of 5, perhaps due to non-uniform cooling/heating of the reaction mixtures.

3.2 Incorporation of [8,8,8,10,10,10-²H₆]-Monoterpenols and their Oxidised Derivatives

3.2.1 Incorporation of [8,8,8,10,10,10-²H₆]-Monoterpenols

Drawing from the success seen using topically applied 5 mg/mL monoterpenol solutions, the [8,8,8,10,10,10-²H₆]-monoterpenols (shown in Figure 3.7) were applied to *Acyrtosiphon pisum* (Homoptera: Aphididae) oviparae *via* this route, and also *via* 125 µg/mL dietary solutions to check for evidence to support the sequestration of precursors. Dynamic headspace collections using oviparae exposed to the hexadeuterated monoterpenols were initially collected following a 48-hour incubation period but deuterium incorporation was not evident following the topical application or dietary incorporation of either ²H₆-geraniol (**²H₆-56**), ²H₆-nerol (**²H₆-62**), ²H₆-(*S*)-citronellol (**²H₆-(*S*)-57**) or ²H₆-(*R*)-citronellol (**²H₆-(*R*)-57**). On elimination of this incubation step, immediate volatile collection for 48 hours post-treatment was attempted, involving adhesively attached diet sachets for the dietary method (Figure 3.8). The adhesive tape was subjected to dynamic headspace collection separately to check for interfering GC peaks however none were present. Using both topical and dietary methods, deuterium incorporation was still not achieved and the mass spectra obtained resembled those of the unlabelled products (Section 2.1). Although free to roam around the glass vessel, with the absence of a plant canopy most aphids continued to feed on the diet sachet contents, so it can be assumed that the uptake of deuterated

compounds into the aphids was successful and that the lack of deuterium incorporation is compound-specific.

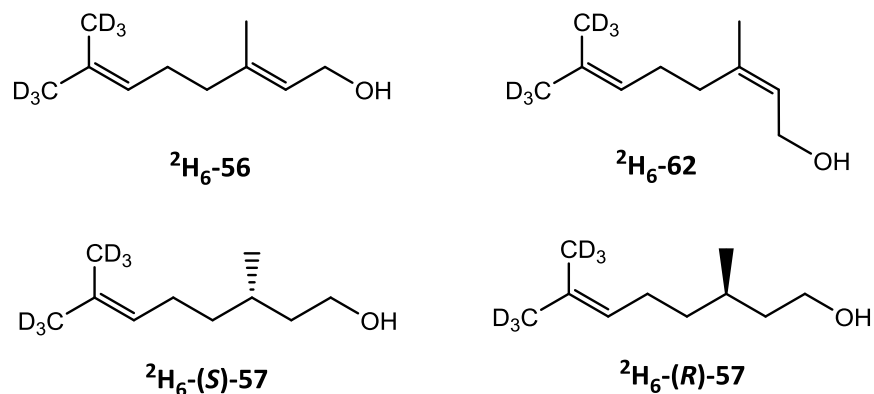


Figure 3.7. Structures of the [8,8,8,10,10,10- $^2\text{H}_6$]-monoterpenols applied to *Acyrtosiphon pisum* oviparae by dietary incorporation.

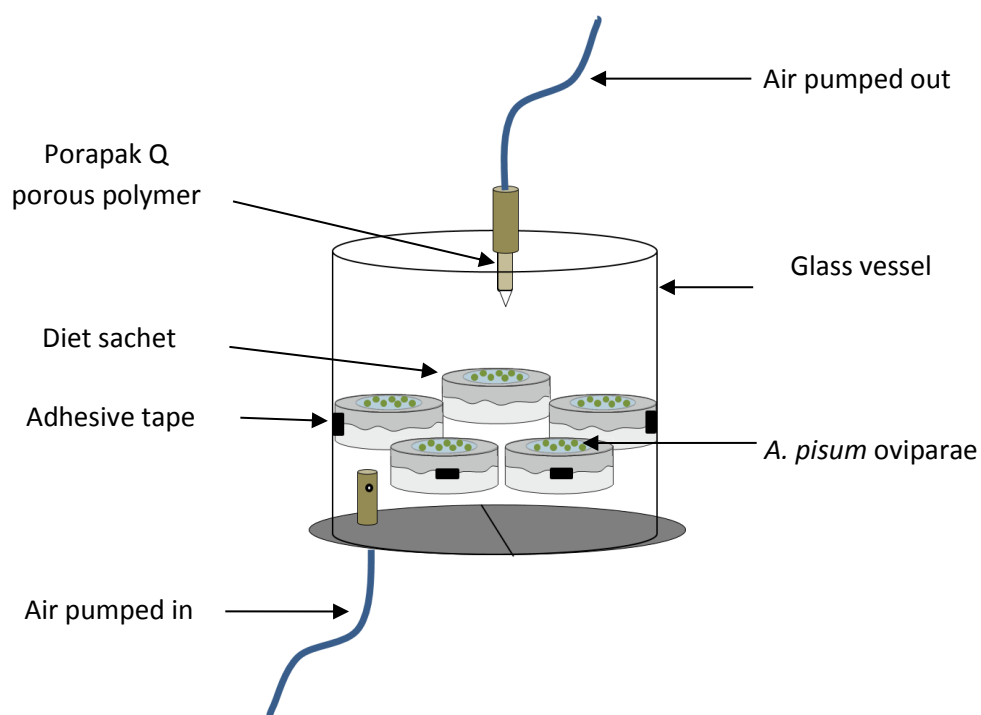


Figure 3.8. Apparatus for immediate dynamic headspace collection using diet-fed *Acyrtosiphon pisum* (Homoptera: Aphididae) oviparae.

As the incorporation rate of isotopic labels in insects was expected to be small, the total number of *A. pisum* oviparae used was increased from 20 to 100 (for dietary replicates)

and from 20 to 50 (for topical replicates) in case the amount of $^2\text{H}_4$ -lactol **1** and $^2\text{H}_4$ -lactone **2** produced was below the detection threshold. An extension of the volatile collection period to 7 days was also introduced to help maximise the quantity of sex pheromone collected and to capture evidence of deuterium incorporation should the time required for oviparae to process the deuterated precursors be longer than 4 days (if a pool of lactol **1** and lactone **2** exists within the aphid, for instance). Finally, the application of deuterated putative precursors at concentrations higher than those investigated was attempted to ensure a sufficient amount was reaching the site of biosynthesis within the aphids. Deuterated compounds were introduced at concentrations of 500 $\mu\text{g}/\text{mL}$ (in diet solutions) and 50 mg/mL (in topical solutions), representing a 4-fold and 10-fold increase, respectively. These concentration values were selected so that the prospect of observing deuterium incorporation was significantly improved, but not to the detriment of aphid survival or sex pheromone production rates, as determined in Chapter 2.

Following these alterations, dietary and topical application of the $^2\text{H}_6$ -monoterpenols was repeated but $^2\text{H}_4$ -lactol **1** and $^2\text{H}_4$ -lactone **2** were still not observed. The administered $^2\text{H}_6$ -monoterpenols were not detected in dynamic headspace collections suggesting that they had been fully absorbed after topical application. Moreover, the presence of surviving aphids after 7 days feeding on $^2\text{H}_6$ -monoterpenols indicates successful ingestion of the diet solution, for the life expectancy of aphids feeding on water does not exceed 2 days. Therefore, dietary consumption of the labelled compounds, in addition to the D-sucrose, seems highly likely. The fate of the $^2\text{H}_6$ -monoterpenols was further investigated using diethyl ether washes of the treated aphids' legs and body surfaces, but still they were not observed. Further examination

may have revealed residual traces of the $^2\text{H}_6$ -monoterpenols inside the aphid body or in their excreted honeydew.

A possible explanation for the continued lack of deuterium incorporation is that the monoterpenols are not used as precursors in the biosynthesis of the aphid sex pheromone. Although an entirely separate route to lactol **1** and lactone **2** is unlikely, it remains possible that the precursor used by aphids is different to the monoterpenols that are classically used in iridoid biosynthesis in other organisms. Successful deuterium incorporation following the application of intermediates from further down the putative biosynthetic routes may imply that the sex pheromone originates from a different source.

Alternatively, a kinetic isotope effect (KIE) may be responsible for the lack of deuterium incorporation observed. The enzymes responsible for the initial C-8-oxidation of monoterpenols in aphids may not be able to break the stronger C-D bond, as would be required for the proposed allylic oxidation to the 8-hydroxymonoterpenols. This hypothesis is further tested in Chapter 4.

3.2.2 Incorporation of [8,8,8,10,10,10- $^2\text{H}_6$]-Monoterpenals

The dietary and topical application of the $^2\text{H}_6$ -monoterpenals, $^2\text{H}_6$ -**56**, $^2\text{H}_6$ -**62**, $^2\text{H}_6$ -(**S**)-**57** and $^2\text{H}_6$ -(**R**)-**57**, was similarly unsuccessful, with mass spectra resulting from dynamic headspace collections of treated aphids resembling those of unlabelled lactol **1** and lactone **2**. As with the $^2\text{H}_6$ -monoterpenols, it is possible that either a KIE is responsible for the absence of deuterium incorporation or that monoterpenals are not involved in the biosynthetic pathway to lactol **1** and lactone **2**.

3.2.3 Incorporation of [8,8,10,10,10-²H₅]-8-Hydroxymonoterpenols

Methanolic solutions of the [8,8,10,10,10-²H₅]-8-hydroxymonoterpenols, ²H₅-**72**, ²H₅-**91**, ²H₅-(*S*)-**90** and ²H₅-(*R*)-**90**, were topically applied to aphids. Fifty aphids were used per replicate and each had 0.2 μL of either a 5 mg/mL or a 50 mg/mL solution applied to their dorsal surface. VOCs were collected over 7 days immediately following exposure.

The presence of *m/z* 172, 170 and deuterium-labelled fragments in GC-mass spectra of dynamic headspace collections using both ²H₅-**72**- and ²H₅-**91**-treated aphids provided conclusive evidence of the formation of ²H₄-lactol **1** and ²H₄-lactone **2** and therefore of successful deuterium incorporation using these compounds. Deuterium incorporation was observed using both 5 mg/mL and 50 mg/mL ²H₅-**72**- and ²H₅-**91** concentrations. The procedure was repeated after 1, 3 and 5 days to evaluate the duration of incorporation. Significant quantities of both ²H₄-**1** and ²H₄-**2** were collected at each of these time points, suggesting that the aphid sex pheromone is biosynthesised rapidly with a high turnover (as evidenced from the 24-hour collections) but also that it may be stored *in vivo* (as evidenced from the later collections). The emission of deuterated sex pheromone 7 days after the application of ²H₅-**72** and ²H₅-**91** could, however, indicate gradual absorption of the compounds through the dorsal surface.

GC analysis of a VOC extract obtained 24 hours post-treatment with ²H₅-**72** is shown in Figure 3.9. A small degree of GC separation between the natural and ²H₄-isotopomers was evident for both lactol **1** and lactone **2**, with the tetra-deuterated component eluting slightly earlier, as expected²⁰⁸.

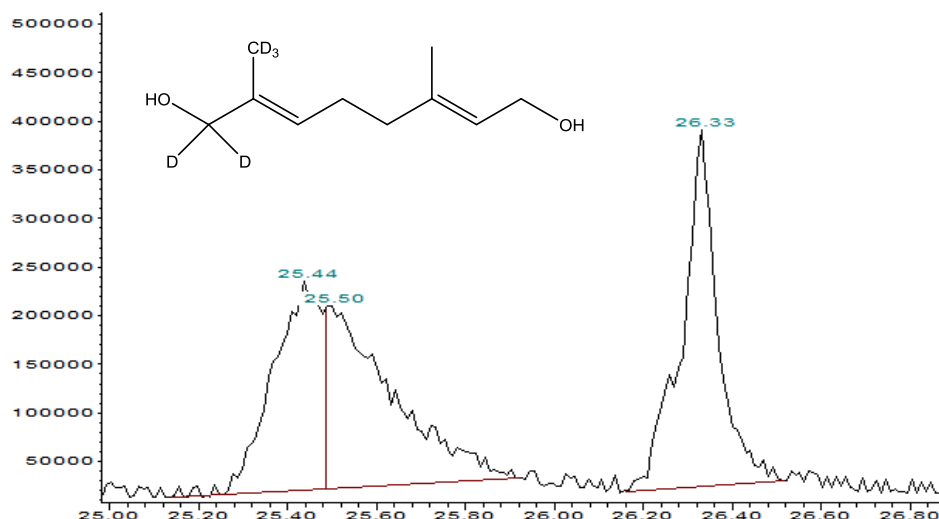


Figure 3.9. GC separation of lactol **1**, lactone **2** and their $^2\text{H}_4$ -isotopomers following the topical treatment of *A. pisum* oviparae with $^2\text{H}_5$ -8-hydroxygeraniol ($^2\text{H}_5$ -**72**, shown inset).

Mass spectra for unlabelled and $^2\text{H}_4$ -labelled lactol **1** and lactone **2**, are shown in Figures 3.10 and 3.11, respectively. In addition to the presence of molecular ions m/z 172 and 170, representing $^2\text{H}_4$ -**1** and $^2\text{H}_4$ -**2** respectively, a loss of H_2O (m/z 18) from unlabelled lactol **1** and $^2\text{H}_4$ -**1** and, represented by the fragments at m/z 150 and 154 in Figure 3.10A and 3.10B respectively, maintains the pattern of deuterium incorporation as expected. Similarly, the loss of CO (m/z 28) from unlabelled lactone **2** and $^2\text{H}_4$ -**2**, represented by the fragments at m/z 138 and 142 in Figure 3.11A and 3.11B respectively, provides the predicted m/z values when incorporating the absence of carbon monoxide. Additional fragment ions delivered evidence of deuterium incorporation, with many fragments in the mass spectra for the unlabelled components increasing in m/z value by 3 or 4 units in the mass spectra for the deuterium-labelled iridoids. As expected, deuterium incorporation was not evident in the fragment ions typifying a methylcyclopentane ring, such as m/z 81, thereby confirming that the deuterium atoms within $^2\text{H}_4$ -lactol **1** and $^2\text{H}_4$ -lactone **2** are not present in the 5-membered ring.

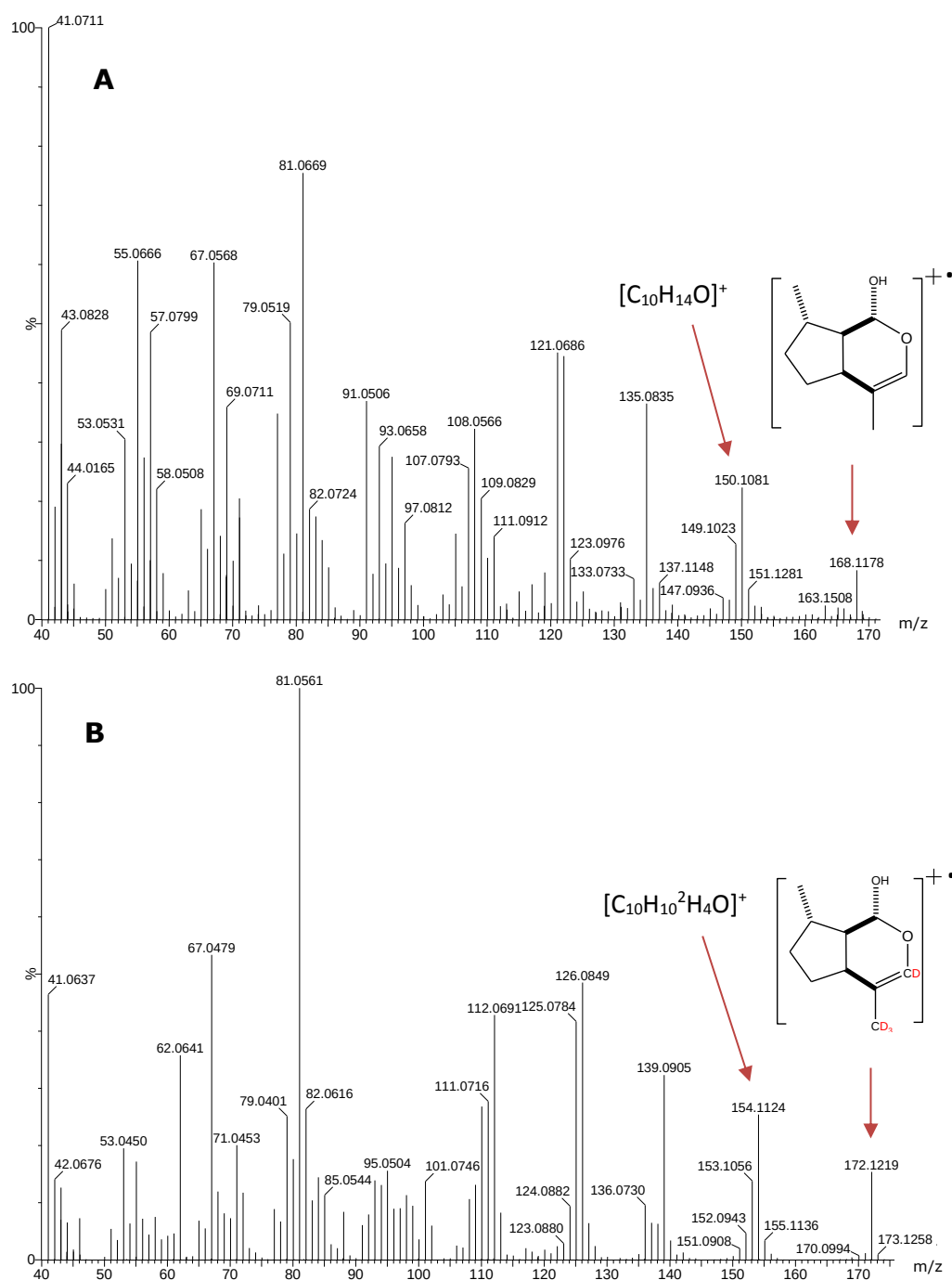


Figure 3.10. Mass spectra for (A) unlabelled and (B) $^2H_4$ -labelled lactol **1**, showing molecular ions m/z 168 and 172, respectively, and fragment ions with a loss of H_2O at m/z 150 and 154, respectively. **B** was obtained following the topical application of $^2H_5$ -72.

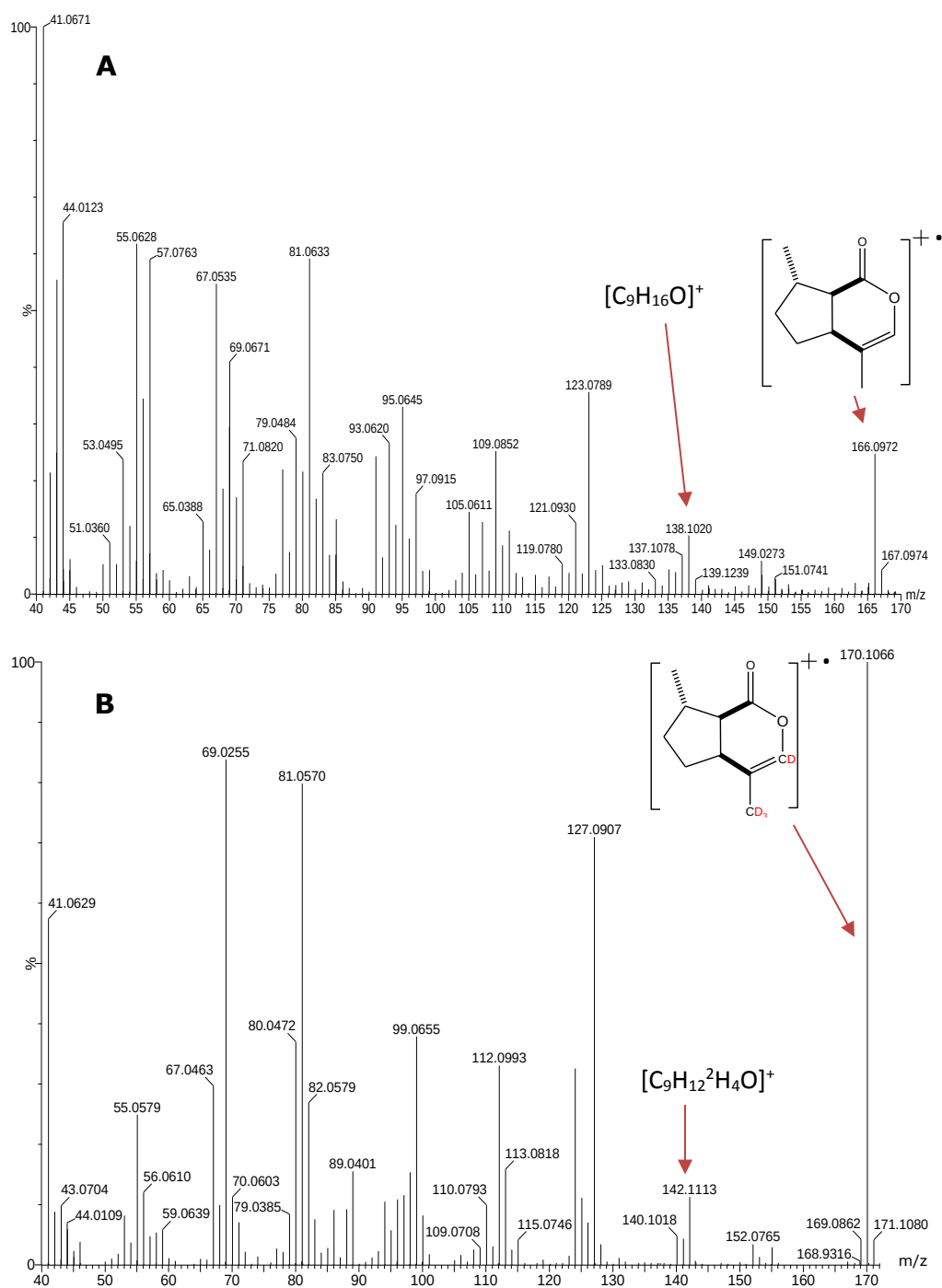


Figure 3.11. Mass spectra for **(A)** unlabelled and **(B)** $^2H_4$ -labelled lactone **2**, showing molecular ions m/z 166 and 170, respectively, and fragment ions with a loss of CO at m/z 138 and 172, respectively. **B** was obtained following the topical application of $^2H_5$ -**72**.

In contrast, minimal deuterium incorporation was evident following topical application of either $^2H_5$ -(*S*)-**90** or $^2H_5$ -(*R*)-**90** at 5 mg/mL and sex pheromone production ceased

entirely using the 50 mg/mL solutions, suggesting that the citronellyl derivatives are unlikely to be involved in the biosynthetic pathway and that the 2,3-unsaturated moiety is required for the biosynthesis to proceed. The deuterated 2,3-saturated substrates may sufficiently block the active sites of the enzymes responsible for further oxidative metabolism, thereby preventing the oxidation of the natural substrates and leading to inhibited lactol **1** and lactone **2** production at higher concentrations. The production of a small amount of $^2\text{H}_4\text{-1}$ and $^2\text{H}_4\text{-2}$ using 5 mg/mL citronellyl solutions may indicate partial oxidative success as a substrate of the biosynthetic enzymes downstream or may arise from prior oxidation of the 2,3-saturated moiety to their geranyl or neryl counterpart by oxidases present ²¹⁷.

3.2.4 Incorporation of [8,8,10,10,10- $^2\text{H}_5$]-8-Hydroxymonoterpenals

Methanolic solutions (5 mg/mL) of the [8,8,10,10,10- $^2\text{H}_5$]-8-hydroxymonoterpenals, $^2\text{H}_5\text{-73}$, $^2\text{H}_5\text{-130}$, and $^2\text{H}_5\text{-(S)-131}$, were topically applied to aphids and VOCs collected over 24 hours immediately following exposure. Similar to the results using topically-applied $^2\text{H}_5$ -8-hydroxymonoterpenols, the presence of m/z 172 (Figure 3.12A), m/z 170 (Figure 3.12B) and deuterium-labelled fragments using both $^2\text{H}_5\text{-73}$ - and $^2\text{H}_5\text{-130}$ -treated aphids provided evidence of the formation of both $^2\text{H}_4\text{-1}$ and $^2\text{H}_4\text{-2}$, and with this the suggested involvement of either 8-hydroxygeranial or 8-hydroxyneral in the biosynthetic pathway of the aphid sex pheromone components. The fragmentation pattern and associated deuterium incorporation was equivalent to that observed using the $^2\text{H}_5$ -2,3-unsaturated diols, $^2\text{H}_5\text{-72}$ - and $^2\text{H}_5\text{-94}$.

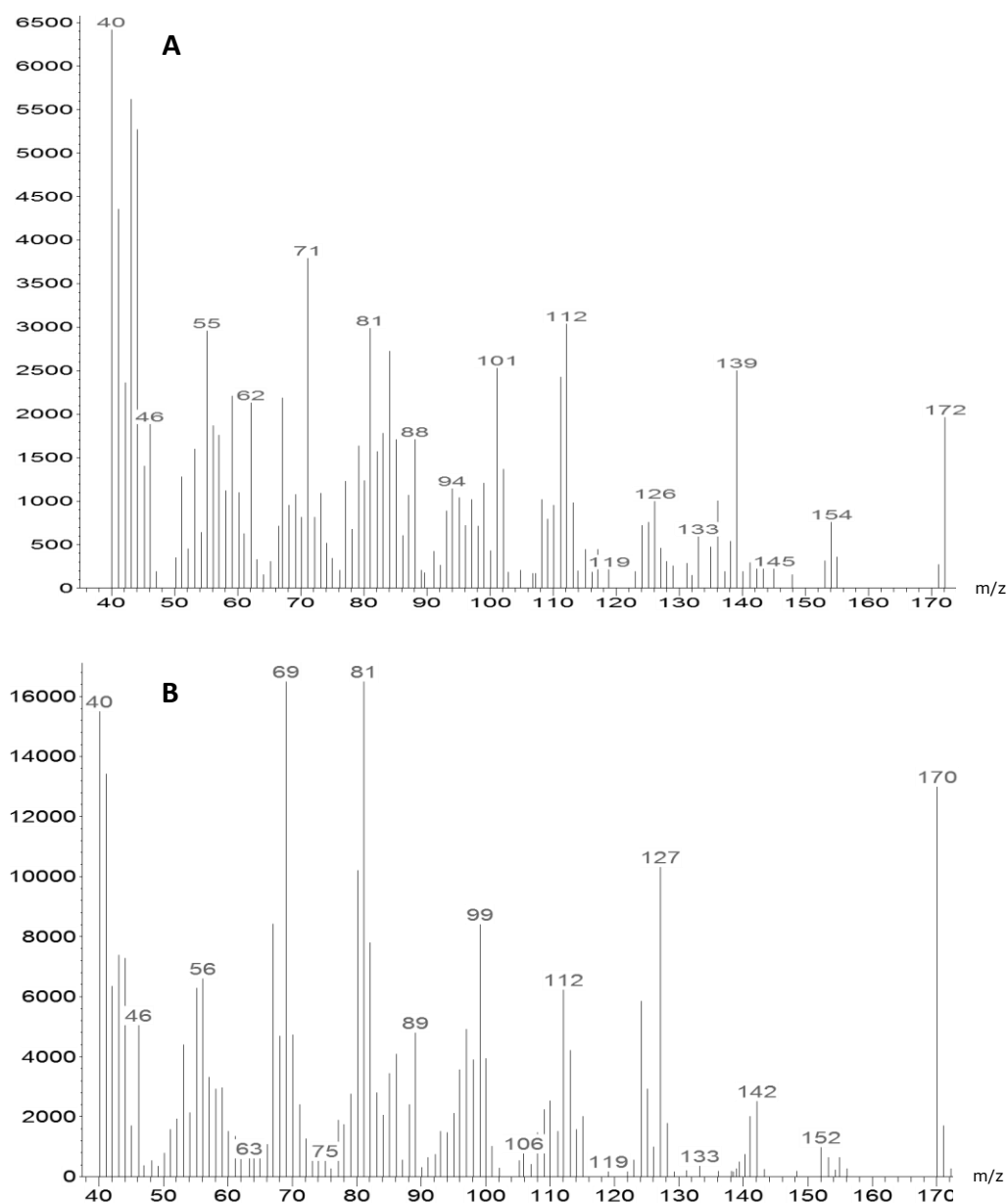


Figure 3.12. Mass spectra for (A) $^2\text{H}_4$ -labelled lactol **1** and (B) $^2\text{H}_4$ -labelled lactone **2** following the topical application of $^2\text{H}_5$ -**73**.

In contrast to this, and in agreement with the results using topically-applied $^2\text{H}_5$ -(S)-**90**, deuterium incorporation was not observed following the topical application of $^2\text{H}_5$ -(S)-**131**. This again negates the involvement of citronellyl intermediates in the biosynthetic pathway and suggests an involvement of the 2,3-alkene moiety during the mechanism.

3.2.5 Incorporation of [8,10,10,10-²H₄]-8-Oxomonoterpenols

The formation of both ²H₄-**1** (Figure 3.13A) and ²H₄-**2** (Figure 3.13B) was observed following the topical application of 5 mg/mL methanolic [8,10,10,10-²H₄]-8-oxomonoterpenols, ²H₄-**74** and ²H₄-**132**, but not ²H₄-(**S**)-**133**, after 24-hour volatile collection. This, again, emphasises the necessity of the 2,3-unsaturation of monoterpenyl intermediates during the biosynthetic pathway of lactol **1** and lactone **2**. The deuterated fragments observed showed no deviation from those seen previously.

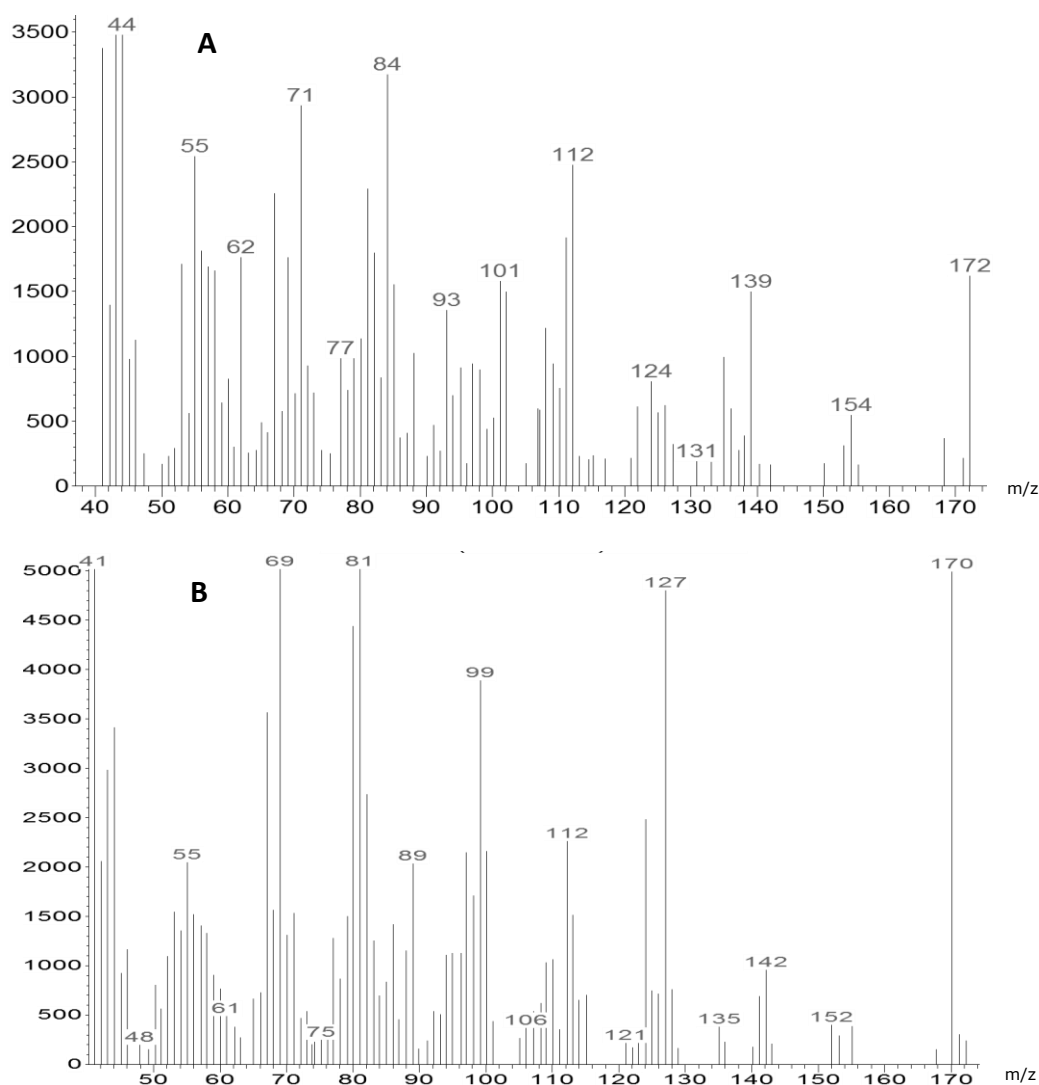


Figure 3.13. Mass spectra for (A) ²H₄-labelled lactol **1** and (B) ²H₄-labelled lactone **2** following the topical application of ²H₄-**74**.

3.2.6 Incorporation of [8,10,10,10-²H₄]-8-Oxomonoterpenals

Both ²H₄-**1** (Figure 3.14A) and ²H₄-**2** (Figure 3.14B) were emitted from *A. pisum* oviparae upon topical application of 5 mg/mL methanolic [8,10,10,10-²H₄]-8-oxomonoterpenals, ²H₄-**75** and ²H₄-**134**, whereas only unlabelled lactol **1** and lactone **2** were detected following application of the 2,3-saturated dial, ²H₄-(**S**)-**135**. Deuterium incorporation and fragmentation patterns were comparable to those observed using oxidised derivatives ²H₅-**72**, ²H₅-**73**, ²H₄-**74**, ²H₅-**91**, ²H₅-**130** and ²H₄-**132**.

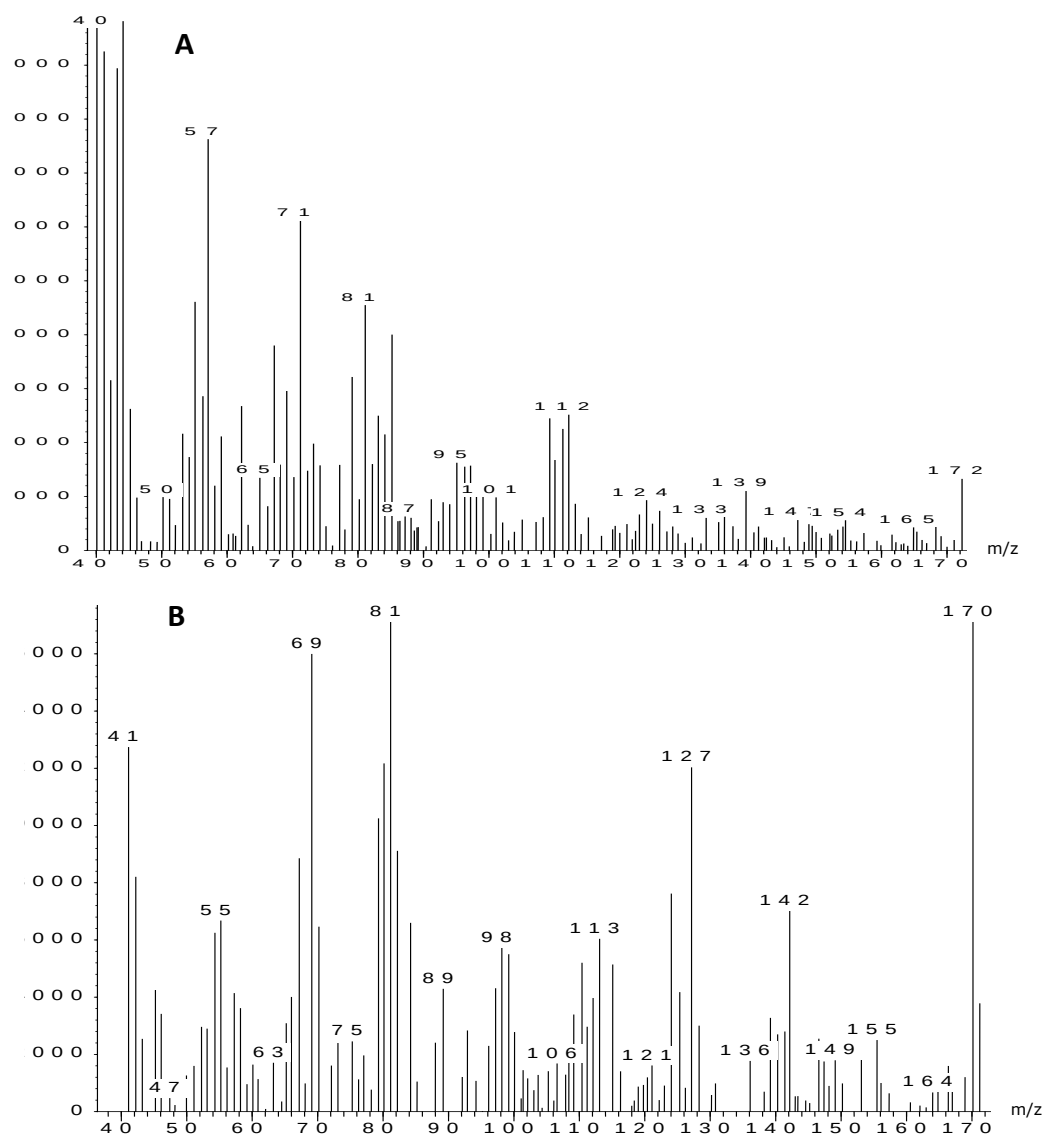


Figure 3.14. Mass spectra for (A) ²H₄-labelled lactol **1** and (B) ²H₄-labelled lactone **2** following the topical application of ²H₄-**75**.

3.3 Implications of the Observed Deuterium Incorporation using Oxidised Derivatives of the [8,8,8,10,10,10-²H₆]-Monoterpenols

Deuterium incorporation was consistently observed following the topical application of both neryl and geranyl oxidised derivatives of the monoterpenols, but not with the corresponding 2,3-saturated citronellyl derivatives (Figure 3.7), indicating that 2,3-unsaturation is essential for use in the biosynthetic pathway to lactol **1** and lactone **2** and may involve a reductive cyclisation, as demonstrated in *Catharanthus roseus* (Apocynaceae) ²⁷. It also indicates that the geometric configuration at C-2 may be irrelevant during each of the oxidation steps. ISY, the iridoid synthase responsible for reductive cyclisation during lactol **1** biosynthesis in *C. roseus*, has been reported to use either 8-oxogeranial **75** or 8-oxoneral **134** as substrate ²⁷.

Qin et al ²¹⁸ performed X-ray crystallography using *C. roseus*-derived iridoid synthase co-crystallised with NADP⁺ and natural substrate 8-oxogeranial, demonstrating the proximity of C-4 of NADP⁺ to the 8-oxogeranial C-3 methyl group (Figure 3.15A). During cyclisation of 8-oxogeranial, the C-3 methyl group would adopt a pseudo-equatorial position in the cyclopentane envelope conformation whereas, with 8-oxoneral, a pseudo-axial position would be adopted (Figure 3.15B). With the latter, the positioning of the methyl group may inhibit hydride transfer or hydrogen bonding to the co-factor and therefore interfere with the reductive cyclisation, especially if the mechanism is concerted. If so, to maintain the (7S)-stereochemistry in lactol **1** and lactone **2**, the co-factor may require re-positioning depending on which of the (*E*)- or (*Z*)-isomers is present, should the active site allow. Further investigation is required to ascertain whether *A. pisum* oviparae use 8-oxogeranial alone, or in combination with 8-oxoneral, as cyclisation precursor during the biosynthetic pathway and whether the

stereochemistry of lactol **1** and lactone **2** differ at C-7 depending on the (*E*)- or (*Z*)- isomer used. If their application results in (*7S*)- and (*7R*)-lactol **1** and lactone **2**, respectively, this could suggest that *A. pisum* only use the (*E*)-isomer in nature, or that an isomerase is later able to correct the C-7 stereoconfiguration.

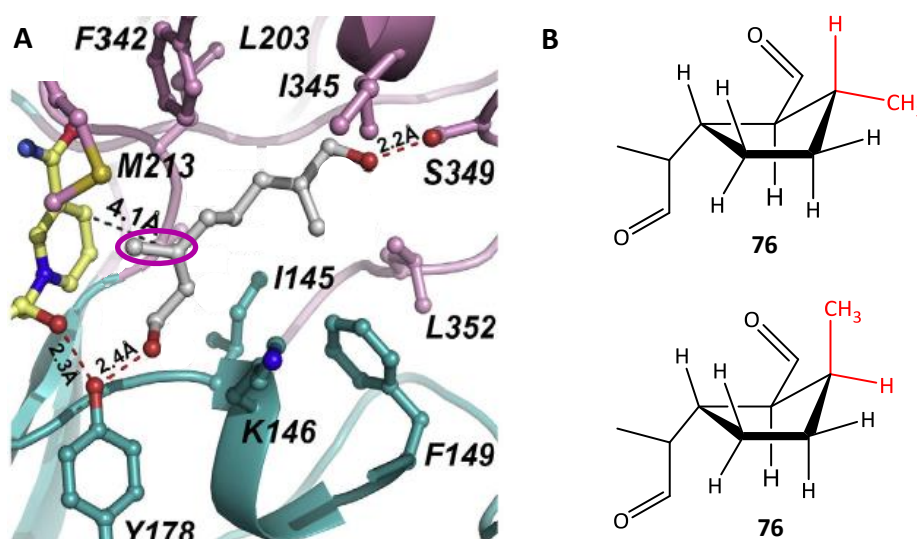
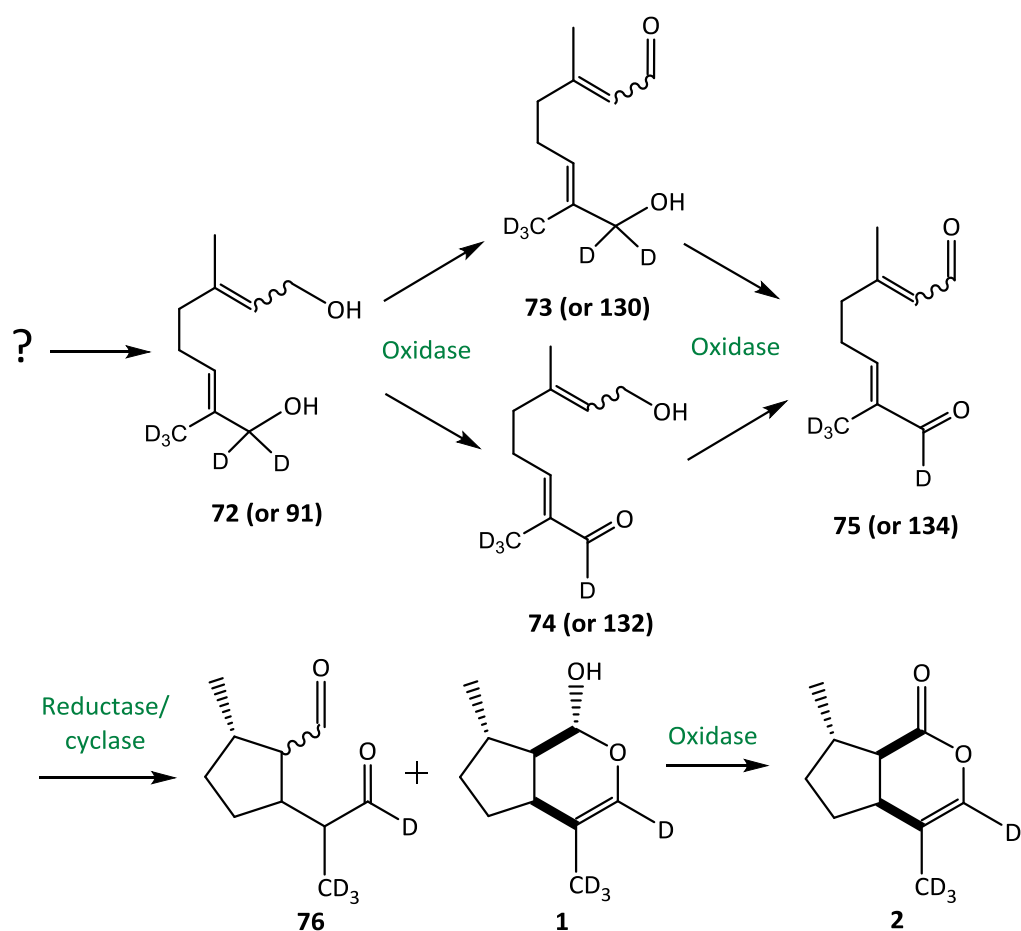


Figure 3.14. (A) X-ray crystallography of the ISY active site co-crystallised with substrate 8-oxogeranial and NADP⁺, taken directly from Qin *et al*²¹⁸. (B) Iridodial formed from 8-oxogeranial (above) and from 8-oxoneral (below), leading to pseudo-equatorial and pseudo-axial C-3 methyl group positions, respectively.

Deuterated diols, ²H₅-**72** and ²H₅-**91**, represent the earliest-occurring intermediates in the proposed biosynthetic pathway to result in ²H₄-**1** and ²H₄-**2** biosynthesis, with the natural precursor(s) remaining unidentified. Citronellyl derivatives are presumed unlikely precursors to lactol **1** and lactone **2**, however both geranyl and neryl derivatives are thought to be involved. A putative biosynthetic pathway to the aphid sex pheromone components in *A. pisum* oviparae is shown in Scheme 3.7.



Scheme 3.7. Putative biosynthetic pathway to lactol **1** and lactone **2** in *A. pisum* oviparae.

During Chapter 4, the biosynthetic mechanism towards lactol **1** and lactone **2** in aphids is further probed, using topical application and isotopic labelling methods that have been fully established within Chapters 2 and 3.

4. Synthesis and Incorporation of Additional Deuterated Probes

To obtain further evidence for the mechanism underlying the biosynthesis of lactol **1** and lactone **2**, additional deuterated probes were synthesised and applied to *A. pisum* oviparae. In this chapter, the application of [C-4,C-8- $^2\text{H}_5$]-monoterpenols and deuterated co-factor, NADP ^2H , are considered to address currently undefined details of the biosynthetic route, such as precursor identification and cyclisation mechanism.

4.1 Synthesis and Incorporation of [C-4,C-9- $^2\text{H}_5$]-Labelled Putative Precursors and Intermediates

To investigate the possibility that a kinetic isotope effect (KIE) was responsible for the lack of deuterium incorporation observed following topical application of the [8,8,8,10,10,10- $^2\text{H}_6$]-monoterpenols, monoterpenols with deuterium labelling in a position not expected to undergo oxidation were topically applied. The [4,4,9,9,9- $^2\text{H}_5$]-monoterpenols (Figure 4.1) fit this criterion.

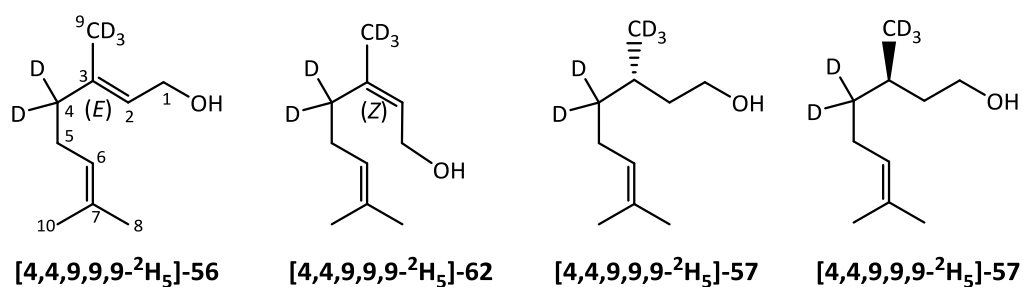
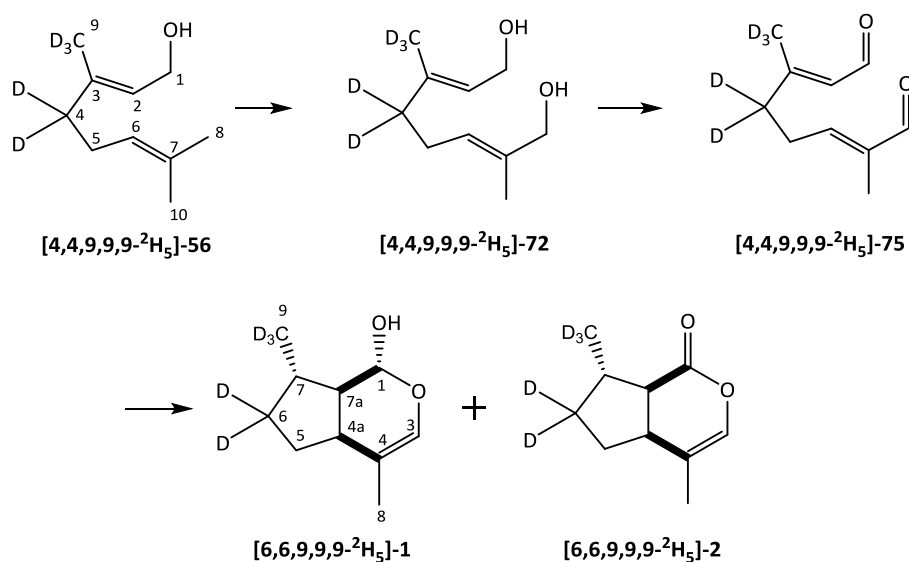


Figure 4.1. Structures of the [4,4,9,9,9- $^2\text{H}_5$]-monoterpenols showing the position of deuterium incorporation.

Independent of the biosynthetic pathways hypothesised, deuterium incorporation into lactol **1** and lactone **2** on application of the $^2\text{H}_5$ -monoterpenols would be expected to

follow the labelling route shown in Scheme 4.1. Accordingly, molecular ions of m/z 171 and 173 in the mass spectra of volatile extracts collected by dynamic headspace collection, representing $^2\text{H}_5$ -lactone **2** ($^2\text{H}_5$ -**2**) and $^2\text{H}_5$ -lacto1 **1** ($^2\text{H}_5$ -**1**) respectively, would indicate their involvement in the biosynthesis.

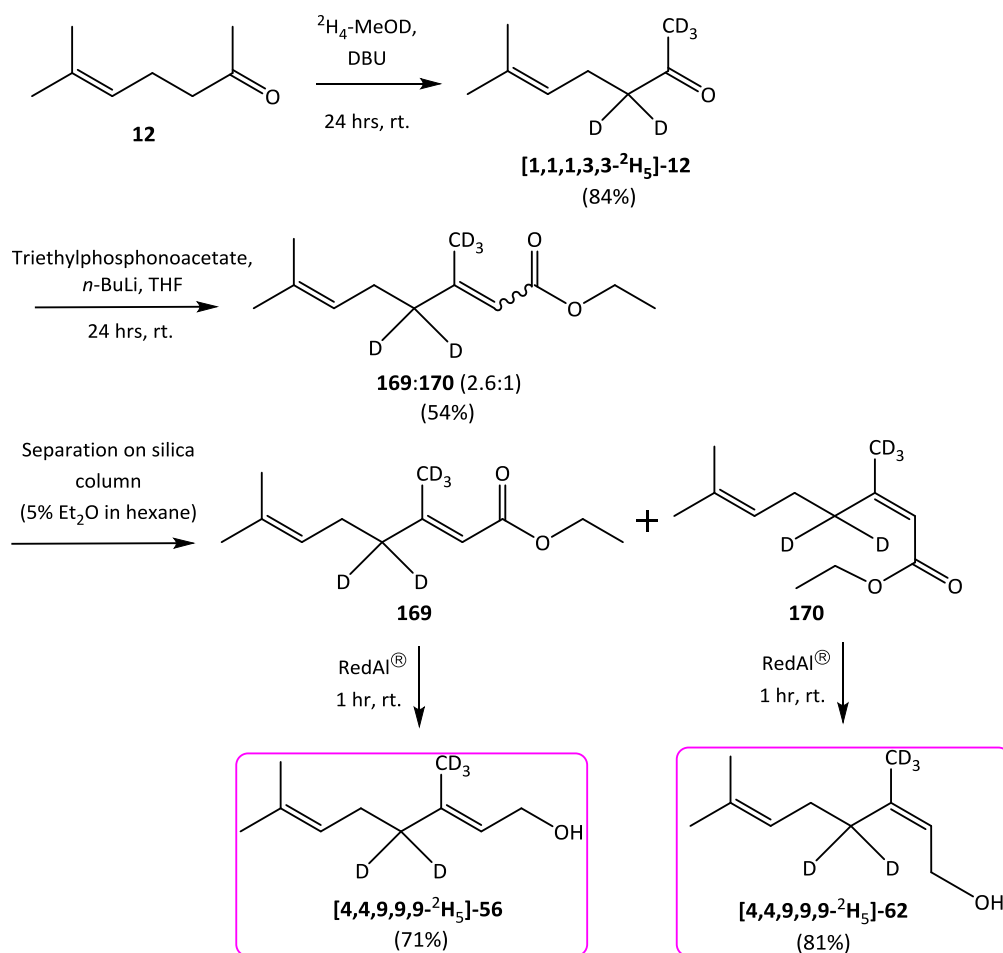


Scheme 4.1. Putative biosynthetic labelling route from the [4,4,9,9,9- $^2\text{H}_5$]-monoterpenols to [6,6,9,9,9- $^2\text{H}_5$]-lactol **1** and [6,6,9,9,9- $^2\text{H}_5$]-lactone **2** (shown only for $^2\text{H}_6$ -geraniol) in aphids.

4.1.1 Synthesis of [C-4,C-9- $^2\text{H}_5$]-Labelled Putative Precursors and Intermediates

4.1.1.1 Synthesis of [4,4,9,9,9- $^2\text{H}_5$]-Monoterpenols

The synthetic route to [4,4,9,9,9- $^2\text{H}_5$]-geraniol ($^2\text{H}_5$ -**56**) and [4,4,9,9,9- $^2\text{H}_5$]-nerol ($^2\text{H}_5$ -**62**) is shown in Scheme 4.2.



Scheme 4.2. Synthetic scheme for [4,4,9,9,9- $^2\text{H}_5$]-geraniol ($^2\text{H}_5$ -**56**) and [4,4,9,9,9- $^2\text{H}_5$]-nerol ($^2\text{H}_5$ -**62**), from 6-methyl-5-hepten-2-one **12**. Pink boxes indicate compounds intended for aphid application.

Penta-deuterated 6-methyl-5-hepten-2-one (**[1,1,1,3,3- $^2\text{H}_5$]-12**) was synthesised from unlabelled ketone **12** via hydrogen-deuterium exchange using MeOD in the presence of a base, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). A Horner-Wadsworth-Emmons reaction using **[1,1,1,3,3- $^2\text{H}_5$]-12**, triethylphosphonoacetate and *n*-BuLi afforded a 2.6:1 mixture of $^2\text{H}_5$ -ethyl geranate (**169**) and $^2\text{H}_5$ -ethyl nerate (**170**)²¹⁹. Separation of the isomers was initially attempted using a silver nitrate-impregnated silica column and a range of ethyl acetate:petroleum ether 40-60 °C-based mobile phases, but without success. Successful separation arose using silica column chromatography and a mobile phase comprising 5% Et₂O in hexane. Figure 4.2 shows the isotopic purity of $^2\text{H}_5$ -ethyl

geranate **169**, originating from the partial hydrogen-deuterium exchange of ketone **12** initially ¹⁶⁹.

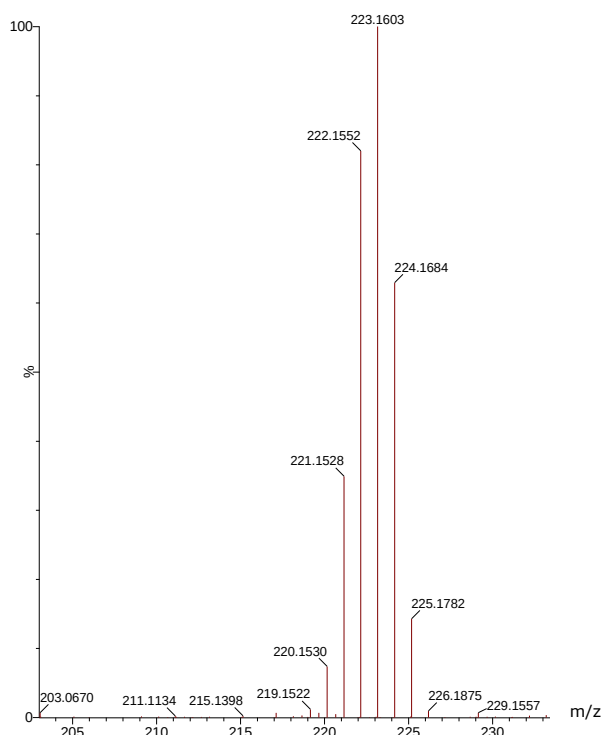
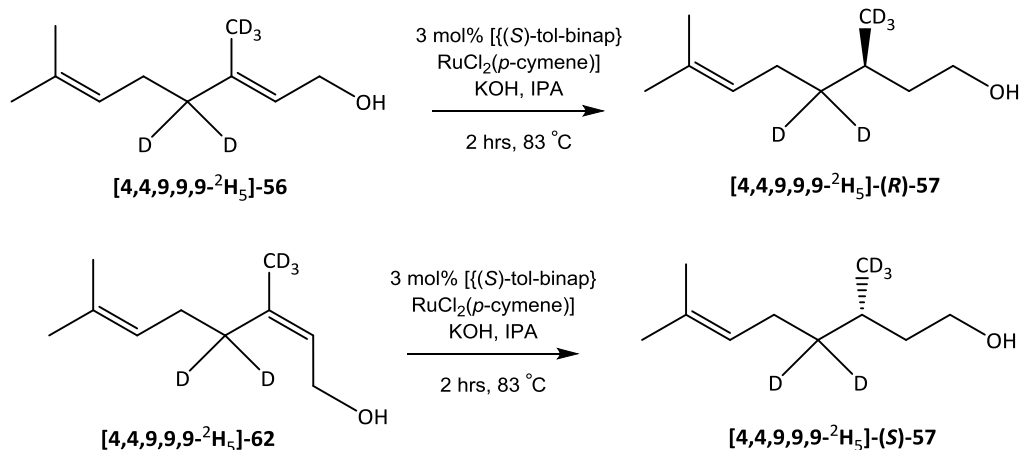


Figure 4.2. Isotopic purity of ²H₅-ethyl geranate (**169**) at *m/z* 224 ([M+Na⁺]). The presence of [M+Na⁺] peaks at *m/z* 219, 220, 221, 222 and 223, representing ²H₀-, ²H₁-, ²H₂-, ²H₃- and ²H₄-**169**, respectively, illustrates the extent of incomplete hydrogen-deuterium exchange.

Reduction of the individual 2,3-unsaturated ²H₅-ethyl monoterpenates was conducted using RedAl® ²²⁰ to give ²H₅-geraniol (²H₅-**56**) and ²H₅-nerol (²H₅-**62**). The synthesis of their 2,3-saturated equivalents, ²H₅-(*S*)-citronellol (²H₅-(*S*)-**57**) and ²H₅-(*R*)-citronellol (²H₅-(*R*)-**57**) (Scheme 4.3), was attempted using a ruthenium catalyst, [(*S*)-tol-binap]RuCl₂(*p*-cymene) (**171**) (Figure 4.3), which was reported to catalyse asymmetric transfer hydrogenation of allylic alcohols ²²¹.



Scheme 4.3. The proposed synthesis of [4,4,9,9,9-²H₅]-(*S*)-citronellol (**2H₅-(*S*)-57**) and [4,4,9,9,9-²H₅]-(*R*)-citronellol (**2H₅-(*R*)-57**) from their corresponding 2,3-unsaturated ²H₅-monoterpenols.

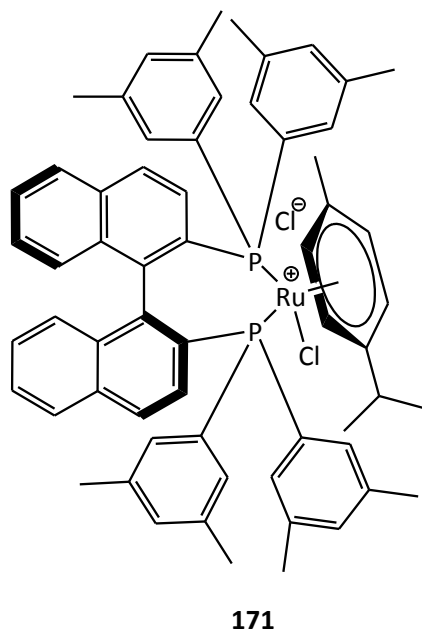


Figure 4.3. The ruthenium catalyst [(*S*)-tol-binap]RuCl₂(*p*-cymene) (**171**) used in the reduction of ²H₅-geraniol (**2H₅-56**) and ²H₅-nerol (**2H₅-62**).

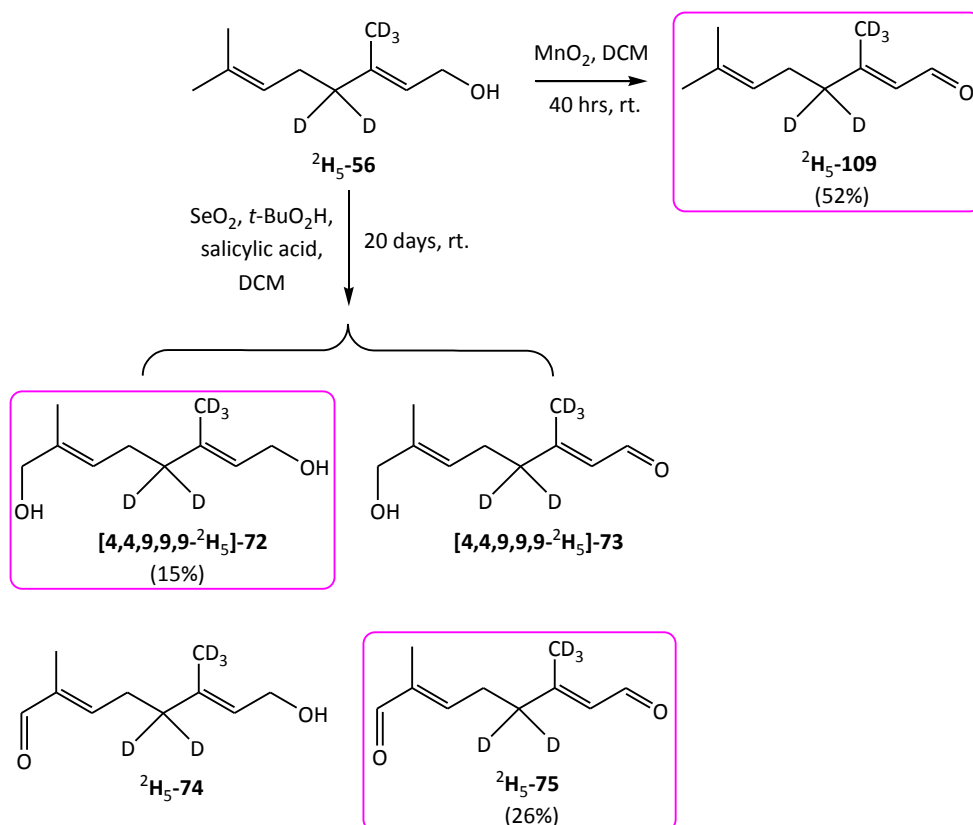
The first attempt at stereoselective hydrogenation using catalyst **171** was unsuccessful and the reaction was not repeated due to the high cost of purchasing the catalyst. As an oxygen-sensitive catalyst, it is possible that the solvents and reagents were not

degassed sufficiently prior to the reaction which may be accountable for the lack of success observed.

4.1.1.2 Synthesis of the Oxidised Derivatives of [4,4,9,9,9-²H₅]-Geraniol

Oxidation of ²H₅-geraniol (**²H₅-56**) was carried out at C-1 and C-8 to potentially shed light on the identification of the precursor for the aphid sex pheromone components and to aid the interpretation of lactol **1** and lactone **2** MS fragmentation. The synthetic procedure to these compounds, based on the synthetic scheme described by Dawson and colleagues¹⁵ and described in Sections 2.2 and 3.1.2, is shown in Scheme 4.4. Due to limited material, ²H₅-nerol was not oxidised further.

Allylic oxidation of unprotected alcohol ²H₅-**56** resulted in a mixture of diol [4,4,9,9,9-²H₅]-**72**, hydroxymonoterpenal [4,4,9,9,9-²H₅]-**73**, oxomonotepenal ²H₅-**74** and dial ²H₅-**75**. Diol [4,4,9,9,9-²H₅]-**72** and dial ²H₅-**75** were separated from the four-product mixture by column chromatography, however [4,4,9,9,9-²H₅]-**73** and ²H₅-**74** co-eluted and were consequently not used.



Scheme 4.4. Synthetic scheme for the oxidised derivatives of $^{2}\text{H}_5$ -geraniol with the corresponding yields (%). Pink boxes indicate products applied to *A. pisum* oviparae.

4.1.2 Incorporation of [C-4,C-9- $^{2}\text{H}_5$]-Labelled Putative Precursors and Intermediates

4.1.2.1 Incorporation of [4,4,9,9,9- $^{2}\text{H}_5$]-Monoterpenols

$^{2}\text{H}_5$ -Geraniol ($^{2}\text{H}_5\text{-56}$) and $^{2}\text{H}_5$ -nerol ($^{2}\text{H}_5\text{-62}$) were topically applied to *A. pisum* oviparae and the VOCs emitted from treated aphids were collected for 48 hours. Deuterium incorporation was not evident following treatment with either monoterpenol. This observation likely rules out a KIE being responsible. It remains possible that the monoterpenols are not used as precursors in the biosynthesis of the aphid sex pheromone and that an entirely alternative precursor is utilised, or that the

glucosylated derivatives of monoterpenols are required for their transportation within the aphid body to the site of biosynthesis, as has been demonstrated in leaf beetle larvae ¹⁶³. Similarly, it is possible that monoterpenol hydroxylation is unable to take place at the site of application and that aphids lack the ability to transport the precursors to the site of bioconversion.

4.1.2.2 Incorporation of [4,4,9,9,9-²H₅]-Geranial

As with ²H₅-geraniol (**²H₅-56**) and ²H₅-nerol (**²H₅-62**), the topical application of ²H₅-geranial (**²H₅-109**) did not result in any deuterium incorporation in lactol **1** or lactone **2** for reasons likely similar to those for the corresponding monoterpenols (Section 4.1.2.1).

4.1.2.3 Incorporation of [4,4,9,9,9-²H₅]-8-Hydroxygeraniol

Despite a large presence of unlabelled sex pheromone in 48-hour VOC collections, deuterium incorporation was observed following the topical application of 5 mg/mL methanolic [4,4,9,9,9-²H₅]-8-hydroxygeraniol to *A. pisum* oviparae. The presence of *m/z* 171 and 173 molecular ions in mass spectra of dynamic headspace collections provides evidence of the formation of [6,6,9,9,9-²H₅]-lactone **2** and [6,6,9,9,9-²H₅]-lactol **1**, respectively. There is also evidence of molecular ions representing ²H_{0→4}-lactol **1** and ²H_{0→4}-lactone **2**, as expected using deuterated precursors of variable isotopic purity. This supports the results observed previously using topically applied [8,8,10,10,10-²H₅]-8-hydroxygeraniol (**²H₅-72**) (Section 3.2.3) and confirms the involvement of diol **72** in the biosynthesis of lactol **1** and lactone **2**. Mass spectra for [6,6,9,9,9-²H₅]-lactol **1** (**A**) and [6,6,9,9,9-²H₅]-lactone **2** (**B**) are shown in Figure 4.4.

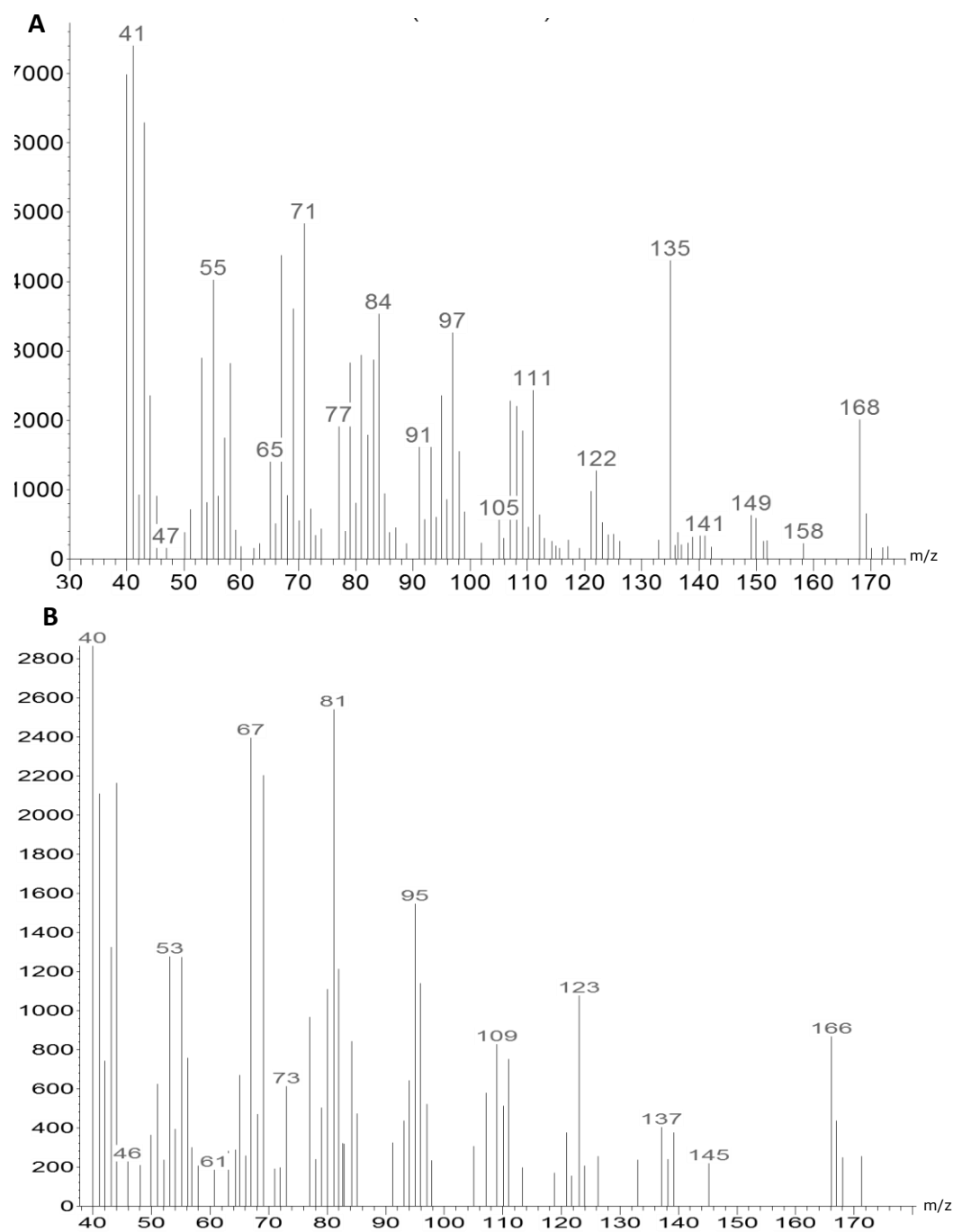


Figure 4.4. Mass spectra for **(A)** [6,6,9,9,9- $^2\text{H}_5$]-lactol **1** and **(B)** [6,6,9,9,9- $^2\text{H}_5$]-lactone **2**, along with their $^2\text{H}_{0\rightarrow 4}$ -derivatives, following the topical application of [4,4,9,9,9- $^2\text{H}_5$]-**72**.

In addition to the presence of molecular ions m/z 171 and 173, other fragment ions delivered evidence of deuterium incorporation, with many fragments in the mass spectra of the unlabelled components increasing in m/z value by 1, 2, 3, 4 or 5 units in

the mass spectra for the deuterium-labelled iridoids. There is evidence of deuterium incorporation in the fragment ions typifying a methylcyclopentane ring, such as m/z 81, thereby confirming that the deuterium atoms within $^2\text{H}_5$ -lactol **1** and $^2\text{H}_5$ -lactone **2** are present in the 5-membered ring, as expected.

4.1.2.4 Incorporation of [4,4,9,9,9- $^2\text{H}_5$]-8-Oxogeranial

A methanolic solution of 5 mg/mL [4,4,9,9,9- $^2\text{H}_5$]-8-oxogeranial, $^2\text{H}_5$ -**75**, was topically applied to aphids and the VOCs from treated aphids collected over 48 hours immediately following exposure. Conclusive deuterium incorporation was evident, owing to the presence of [6,6,9,9,9- $^2\text{H}_5$]-lactol **1** at m/z 173 (Figure 4.5A) and [6,6,9,9,9- $^2\text{H}_5$]-lactone **2** at m/z 171 (Figure 4.5B). Deuterium-labelled fragments were also observed, as observed following [4,4,9,9,9- $^2\text{H}_5$]-8-hydroxygeraniol treatment. This supports previous results following the topical application of [8,10,10,10- $^2\text{H}_4$]-8-oxogeranial ($^2\text{H}_4$ -**75**) (Section 3.2.6) and confirms the involvement of dial **75** in the biosynthesis of lactol **1** and lactone **2**.

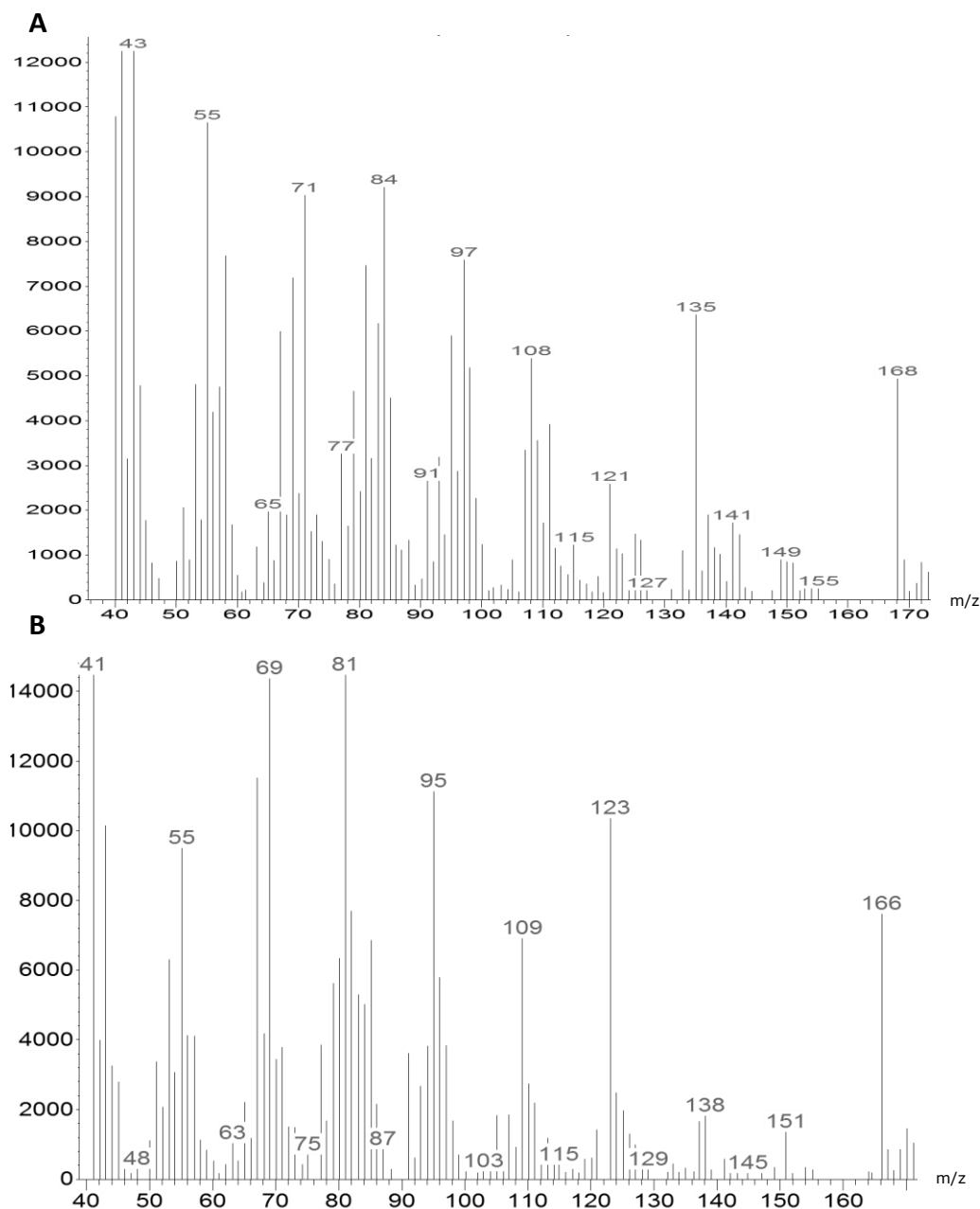


Figure 4.5. Mass spectra for (A) [6,6,9,9,9-²H₅]-lactol **1** and (B) [6,6,9,9,9-²H₅]-lactone **2**, along with their ²H_{0→4}-derivatives, following the topical application of [4,4,9,9,9-²H₅]-**75**.

4.2 Synthesis and Incorporation of NADP²H

The stereospecific, NADPH-dependent reductive cyclisation of dial **75** has been confirmed in plants, such as *Catharanthus roseus*, in the biosynthesis of lactol **1**²⁷. The

potential involvement of this enzymatic transformation in aphids during the biosynthesis of lactol **1** and lactone **2** may be probed *via* the synthesis, and subsequent application, of NADP²H (**172**) (Figure 4.6) to *A. pisum* oviparae. With stereoselective deuterium-labelling at the position of hydride donation (C-4) in NADP²H (**172**), it may be possible to confirm the involvement of NADPH and site of hydride addition, as well as clarify the level of stereocontrol during the reductive cyclisation mechanism. It was acknowledged that, due to the involvement of NADP²H (**172**) in numerous metabolic processes, not least the mevalonic acid pathway, unpredictable patterns of label incorporation may arise and therefore the data would need to be interpreted with caution. Successful synthesis of NADP²H (**172**) may also allow for future *in vitro* enzymatic studies during which the rate of NADP²H (**172**) consumption could be measured and the hydride donation process monitored in the presence of biosynthetic precursors and intermediates through kinetic methods and NMR spectroscopy¹⁰².

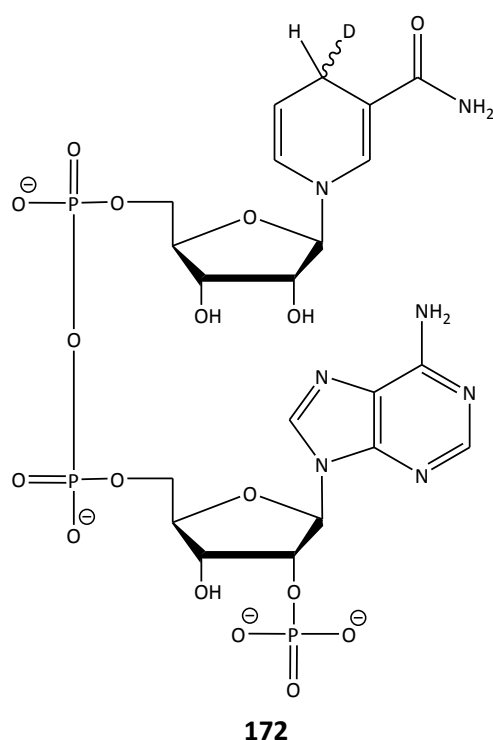


Figure 4.6. The structure of NADP²H (**172**).

The effect of NADPH consumption on *A. pisum* survival rate and sex pheromone production over 48 hours and 7 days was initially investigated using commercially available NADPH. Its addition to the 200 g/L D-sucrose diet, at both 125 µg/mL and 1 mg/mL, was not found to significantly affect *A. pisum* survival rate (Figure 4.7).

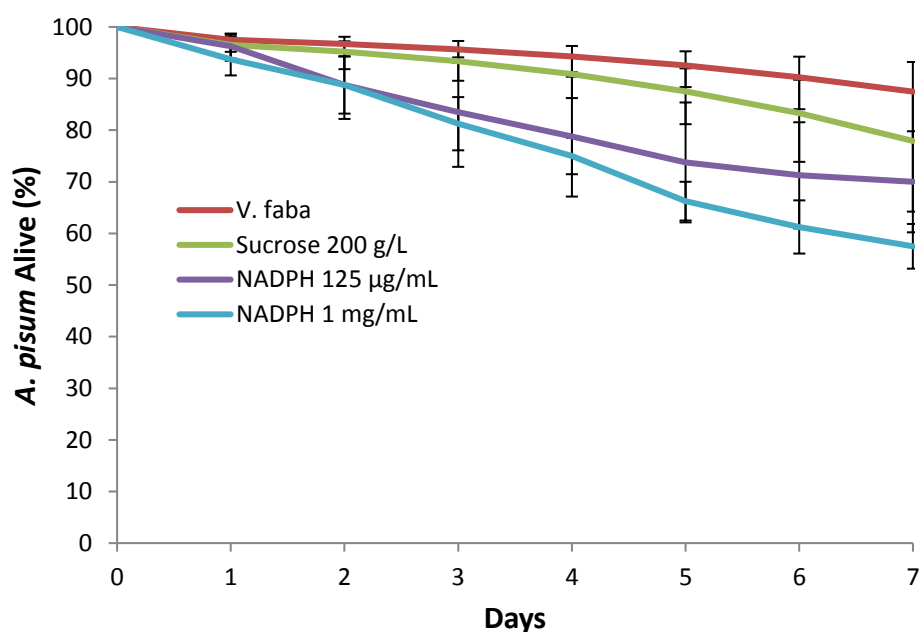


Figure 4.7. *A. pisum* oviparae 7-day survival rate on an NADPH-inclusive diet.

Similarly, sex pheromone production was not hindered by the presence of NADPH in the artificial diet after a 48-hour feeding period (Figure 4.8). Reductions in the production of both lactol **1** and lactol **2** were evident after a 7-day incubation period, and were likely an age-related effect, as has been described in other aphid species^{20, 193}. Reductions in their production, however, was not substantial enough to decrease the viability of prolonged NADPH incubation studies should they be required for sufficient collection of labelled product (Figure 4.9).

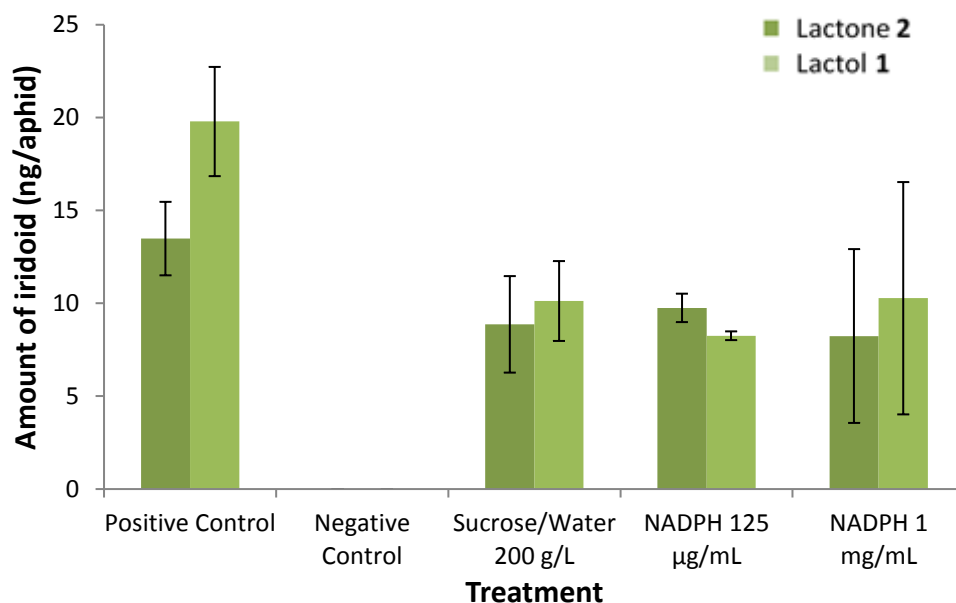


Figure 4.8. *A. pisum* sex pheromone production (mean $\pm$ confidence levels) following a 48-hour incubation period with dietary NADPH.

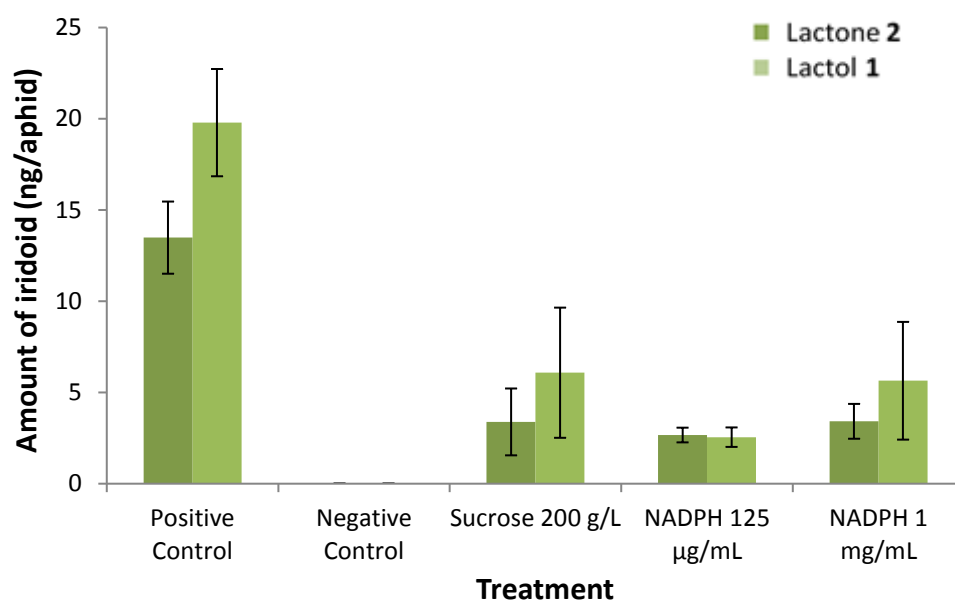


Figure 4.9. *A. pisum* sex pheromone production (mean $\pm$ confidence levels) following a 7-day incubation period with dietary NADPH.

Despite being sparingly soluble in methanol, NADPH was topically applied to *A. pisum* oviparae as a 5 mg/mL methanolic solution and the VOCs collected from the treated

aphids (Figure 4.10). Neither survival rate nor sex pheromone production were significantly affected.

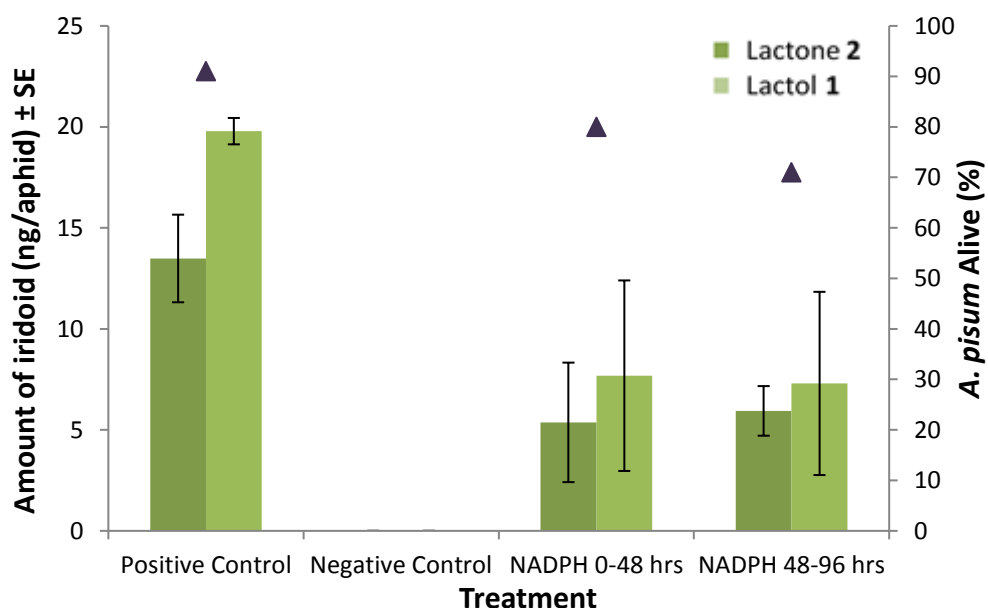
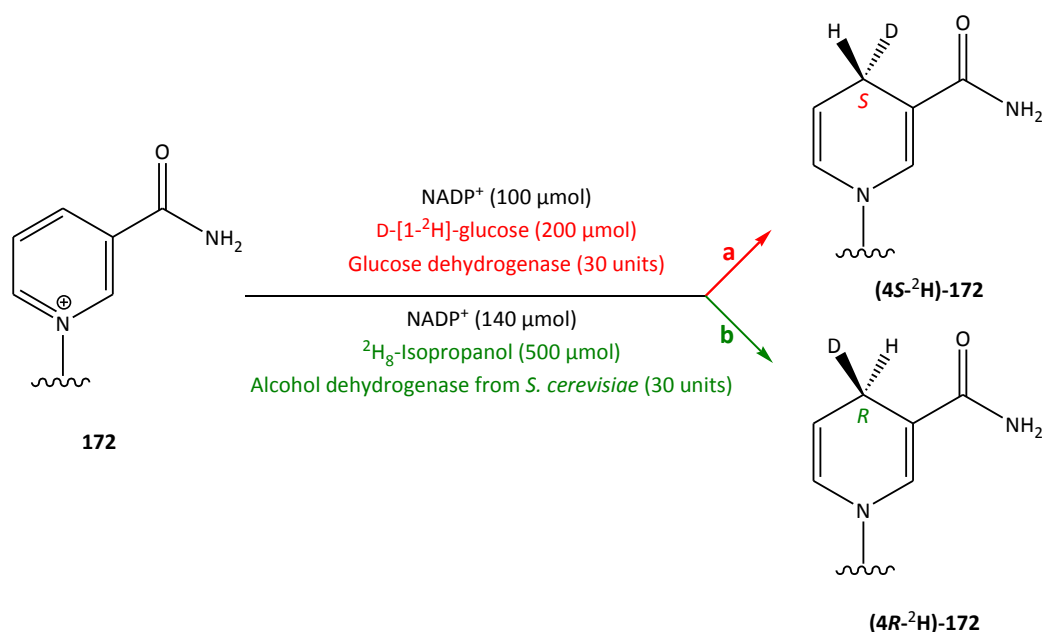


Figure 4.10. Sex pheromone production (mean $\pm$ confidence levels) following topical application of a 5 mg/mL methanolic NADPH solution, with VOC collections taken immediately (0-48 hours) or post-incubation period (48-96 hours). $\blacktriangle$ Represents average *A. pisum* survival post-topical application.

The synthesis of (4*S*- ^2H)- and (4*R*- ^2H)-NADP ^2H ((**4*S*- ^2H**)-172 and (**4*R*- ^2H**)-172, respectively) followed published procedures ²²²⁻²²³, as illustrated in Scheme 4.5. The deuterated (4*S*)- and (4*R*)-diastereomers were synthesised enzymatically using NADP $^+$ and either glucose dehydrogenase or *S. cerevisiae*-derived alcohol dehydrogenase, respectively. D-[1- ^2H]-Glucose and $^2\text{H}_8$ -isopropanol served as deuterium sources for the (4*S*)- and (4*R*)-diastereomers, respectively.



Scheme 4.5. Synthetic route to the (4S-²H)- and (4R-²H)-NADP²H diastereomers ((4S-²H)-172 and (4R-²H)-172, respectively).

The NADP²H (172) diastereomers were purified by semi-preparative reverse phase liquid chromatography. The method was developed using NADP⁺, purchased in its reduced (NADPH) and oxidised (NADP⁺) forms, to allow for the separation of the product and any unreacted starting material. UV detection at 258 nm (at which both products absorb) and 340 nm (at which only NADPH absorbs) aided the process of peak identification (Figure 4.11).

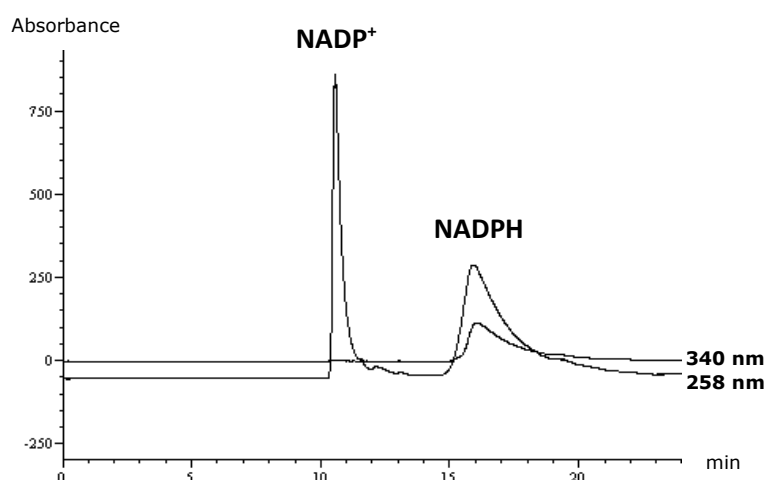


Figure 4.11. LC separation of NADP⁺ and NADPH, showing mutual UV absorbance at 258 nm but NADPH-specific UV absorbance at 340 nm.

A phosphate-based mobile phase (pH 6.0), as described by McCracken et al ²²³, provided good peak resolution of NADP⁺ and NADPH but was incompatible with prolonged stability, which was likely necessary for dietary work ²²⁴. A NaCl:Tris buffered mobile phase, reportedly used by Markham et al ²²⁵, provided reasonable separation of the analytes, but excess NaCl in the resulting solution made it unsuitable for diet incorporation. Desalting methods proved futile and mass spectral identification of the product was hindered due to NaCl-induced signal suppression. Sporty et al ²²⁶ described a method using an ammonium acetate:acetonitrile gradient but the minimal peak separation achieved did not lend itself to semi-preparative work.

Ion-pairing chromatography delivered good separation of NADP⁺ and NADPH. Highly volatile triethylamine (TEA) was employed as ion pairing reagent due to its compatibility with MS detection methods and its easy removal. Using a TEA-based eluent, adjusted to pH 4.5 as detailed by Knee et al ²²⁷, and a methanol gradient, a retention time difference of approximately 5 minutes was achieved between NADP⁺ and NADPH. Following semi-preparative collection, the pH of fraction solutions was adjusted to pH 8.5 and the methanol was removed by rotary evaporation prior to freeze-drying. Surprisingly, neither **(4S-²H)-172** nor **(4R-²H)-172** were recovered from the fractions, despite their presence during semi-preparative collection. The limited stability of the diastereomers at the low eluent pH during chromatographic separation may be accountable. Alternatively, the temperature-sensitive products may have degraded during the evaporation of methanol which was carried out using a 30 °C heat bath. Due to time constraints, efforts to isolate **(4S-²H)-172** and **(4R-²H)-172** were abandoned.

5. Conclusions

In the course of this research, isotopic labelling studies have been conducted using *Acyrtosiphon pisum* (Homoptera: Aphididae) oviparae to aid investigations into the biosynthesis of aphid sex pheromone components, (1*R*,4*aS*,7*S*,7*aR*)-nepetalactol (**1**) and (4*aS*,7*S*,7*aR*)-nepetalactone (**2**). For this, a range of unlabelled and deuterium-labelled putative precursors and intermediates, namely monoterpenols and their C-1 and/or C-8-oxidised derivatives, have been synthesised and applied to aphids. Analysis of the volatile organic compounds (VOCs) emitted from oviparae was conducted by mass spectrometry (MS) to monitor the prospective incorporation of isotopic labels. Unlike solvent washing methods, dynamic headspace collection provided VOCs in sufficient quantity for MS analysis, during which the volatiles emitted from treated aphid oviparae, feeding on their host plant, *Vicia faba* (Fabaceae), were absorbed onto a Porapak™ Q porous polymer and subsequently eluted for analysis.

Initially, unlabelled putative precursors and intermediates to the aphid sex pheromone were synthesised and used in the evaluation of two methods of incorporation; dietary inclusion and topical application. Dietary incorporation was achieved using diet sachets, whereby aphids accessed a solution of the unlabelled compounds trapped between two layers of Parafilm®. A range of feeding environments and aqueous D-sucrose-based solutions, into which the compounds were dissolved, were evaluated for their ability to maintain *A. pisum* oviparae. Following dynamic headspace collection from artificially-fed oviparae, it was found that aphid survival rate and aphid sex pheromone production were least effected using a simple 200 g/L aqueous D-sucrose solution under autumnal conditions (L:D 12:12, 14 °C). An incubation period of 48 hours, prior to 48-hour VOC collection, was introduced to allow for sufficient dietary uptake. Dietary uptake was

confirmed by comparing the moderate survival rate of D-sucrose-fed oviparae to the drastically reduced survival of oviparae on a water-only diet over a 7-day period. In permitting the simple and controlled maintenance of an *A. pisum* oviparae colony without excessive detrimental effect, this diet could be implicated in other research programs involving aphid oviparae ²⁰⁶.

Subsequent inclusion of unlabelled monoterpenols and their respective monoaldehydes into the dietary solution was optimised in terms of analyte concentration and VOC collection duration, and the results were used to guide the later addition of deuterium-labelled equivalents. From the parameters investigated for each compound, 125 µg/mL dietary solutions and a VOC collection duration of 48 hours offered improved results regarding aphid survival rate and sex pheromone production when compared to using 1 mg/mL solutions or longer collection periods.

A method for the topical application of putative precursors and intermediates was also investigated, whereby methanolic solutions of each compound were applied in 0.2 µL quantities to the dorsal surface of the aphid. This method of incorporation was expected to overcome potential issues regarding the internal transportation of hydrophilic oxidised derivatives within aphid haemolymph. Methanolic solutions of unlabelled compounds were found to be detrimental to aphid survival when applied at 100 mg/mL, however sufficient aphid survival rates and sex pheromone production were achieved using 5 mg/mL solutions and a VOC collection duration of 48 hours. An incubation period, initially introduced to avoid contamination of aphid-produced VOCs with those of the compounds applied, was found to be unnecessary.

To commence isotopic labelling studies, [8,8,8,10,10,10- $^2\text{H}_6$]-monoterpenols and their oxidised derivatives were synthesised so that labelled material contained deuterium atoms in a position where normal metabolic turnover is unlikely to remove them and where superpositioning of the natural lactol **1** and lactone **2** isotopomers is avoided. The synthetic route to the $^2\text{H}_6$ -monoterpenols was based on a procedure described by Luan *et al*²¹⁰, with modifications employed to maximise yields. Most notably, the low-yielding Wittig reaction utilised in the synthesis of $^2\text{H}_6$ -monoterpenyl acetates was improved through use of a TBDPS ether-protected Wittig precursor, resulting in a two-fold increase in yield. The low yields observed with acetate-protected compounds was confirmed to result from an undesired aldol reaction producing $^2\text{H}_6$ -monoterpenyl acetoacetate and $^2\text{H}_6$ -monoterpenol by-products. Oxidised derivatives of the protected $^2\text{H}_6$ -monoterpenols were subsequently synthesised using a similar procedure to that described by Dawson *et al*¹⁵, involving SeO_2 -based allylic oxidation.

Neither dietary incorporation nor topical application of the $^2\text{H}_6$ -monoterpenols or $^2\text{H}_6$ -monoterpenals resulted in deuterium incorporation into lactol **1** and lactone **2**, as would be evidenced by the presence of molecular ions at m/z 172 and 170 (for lactol $^2\text{H}_4$ -**1** and lactone $^2\text{H}_4$ -**2**, respectively). Procedural alterations were effected to promote the detection of isotopic incorporation; VOC collection time, analyte concentration and the number of oviparae used per replicate were all increased. *Via* dynamic headspace collection of *A. pisum* oviparae continuing to feed from diet sachets, the preliminary 48-hour incubation period employed during feeding studies was also eliminated so that deuterium incorporation would be captured should the aphids synthesise their sex pheromone immediately upon uptake. Additionally, in the likelihood that oviparae require longer to process the precursors or, instead, possess a pool of lactol **1**, lactone **2** or biosynthetic intermediates, VOC collection time was extended to 7 days.

Despite these changes, deuterium incorporation into lactol **1** and lactone **2** was still not observed. These results indicate that either *A. pisum* use alternative precursors for the sex pheromone components or that transportation of the aglucones through the haemolymph *via* either method of application is not possible. The latter may advocate the sequestration of hydrophilic precursors, such as 8-hydroxymonoterpenol-8- β -D-glucosides, in aphids, as is evident with various Coleopteran beetles⁸⁴. Glycosylation of the 8-hydroxymonoterpenyl acetates *via* a Koenigs-Knorr reaction proved unsuccessful and requires further investigation. The synthesis and application of monoterpenyl diphosphates, representing precursors derived from the mevalonate (MVA) pathway, was not carried out due to the anticipated action of highly prevalent phosphatases on these precursors prior to incorporation.

A kinetic isotope effect (KIE) was considered as an alternative explanation for the lack of observed deuterium incorporation into lactol **1** and lactone **2**. This prompted the synthesis of putative precursors and intermediates, deuterium-labelled in a position not expected to undergo oxidation. The synthesis of 2,3-unsaturated [4,4,9,9,9-²H₅]-monoterpenols was achieved by initial hydrogen-deuterium exchange between 6-methyl-5-hepten-2-one and CD₃OD. The deuterated product, revealing a characteristic pattern of isotopic purity, was subjected to a Horner-Wadsworth-Emmons reaction before chromatographic separation of the resulting ²H₅-ethyl geranate/nerate mixture and subsequent reduction to ²H₅-geraniol and ²H₅-nerol. Subsequent reduction of these 2,3-unsaturated alcohols to their corresponding 2,3-saturated counterparts using ruthenium catalyst [(*S*)-tol-binap]RuCl₂(*p*-cymene) was unsuccessful. Following the topical application of 5 mg/mL methanolic solutions of ²H₅-geraniol, ²H₅-nerol and their respective aldehydes, deuterium incorporation was still

not observed, as would be evidenced by the presence of molecular ions at m/z 173 and 171 (for lactol $^2\text{H}_5\text{-1}$ and lactone $^2\text{H}_5\text{-2}$, respectively). A search for molecular ions showing a partial degree of deuterium incorporation also proved futile. Instead, dynamic headspace collections from treated oviparae revealed only the natural lactol **1** and lactone **2** isotopomers. These results provide evidence that a KIE is not responsible for the lack of isotopic labelling in the aphid sex pheromone following deuterated monoterpenol and monoterpene treatment.

C-8-oxidised intermediates from further down the putative biosynthetic pathway were investigated to further explore the route taken to lactol **1** and lactone **2** in aphids. MS analysis of dynamic headspace collections using *A. pisum* oviparae, topically treated with 5 mg/mL methanolic solutions of [8,8,10,10,10- $^2\text{H}_5$]-8-hydroxygeraniol and [8,8,10,10,10- $^2\text{H}_5$]-8-hydroxyneryl, revealed the presence of lactol $^2\text{H}_4\text{-1}$ and lactone $^2\text{H}_4\text{-2}$ at m/z 172 and 170, respectively. Associated deuterated fragment ions were also evident. In contrast, deuterium incorporation was minimal following the topical application of [8,8,10,10,10- $^2\text{H}_5$]-8-hydroxy-(*S*)-citronellol and [8,8,10,10,10- $^2\text{H}_5$]-8-hydroxy-(*R*)-citronellol. Conclusive evidence of deuterium incorporation using the 2,3-unsaturated diols but not their 2,3-saturated counterparts provides evidence that the (*E*) and/or (*Z*)-diols are involved in the biosynthetic pathway of both aphid sex pheromone components and that unsaturation at C-2 is a requirement for successful biosynthesis. Conversely, these results suggest that 8-hydroxycitronellol is unlikely to play a role in the biosynthesis. Following the topical treatment of aphids, lactol $^2\text{H}_4\text{-1}$ and lactone $^2\text{H}_4\text{-2}$ were evident after just 24 hours, indicating rapid biosynthesis of the aphid sex pheromone, as well as after 7 days, suggesting that a storage mechanism may exist within aphids.

The topical application of [8,8,10,10,10-²H₅]-8-hydroxygeranial, [8,10,10,10-²H₄]-8-oxogeraniol, [8,10,10,10-²H₄]-8-oxogeranial and their neryl equivalents each resulted in the formation of lactol **²H₄-1** and lactone **²H₄-2**, indicating their involvement in the biosynthesis. Equally, and in agreement with previous results using the citronellyl diols, no deuterium incorporation was observed following the application of the (*S*)-citronellyl equivalents, further confirming the importance of the olefinic bond at C-2 to the biosynthetic mechanism. Topical treatment of *A. pisum* oviparae with [4,4,9,9,9-²H₅]-geraniol oxidation products, namely [4,4,9,9,9-²H₅]-8-hydroxygeraniol and [4,4,9,9,9-²H₅]-8-oxogeranial, revealed molecular ions at *m/z* 173 and 171, representing the formation of lactol **²H₅-1** and lactone **²H₅-2**, respectively. This confirmed the involvement of the geranyl diol and dial in the biosynthetic route and aided MS fragment elucidation. Investigations into the cyclisation mechanism and potential involvement of NADPH during the biosynthesis of aphid-derived iridoids, lactol **1** and lactone **2**, were commenced using deuterated co-factor, (*4S*)- and (*4R*)-NADPD, however, following their enzymatic synthesis, the purification of these compounds by semi-preparative chromatographic separation was unsuccessful and work to rectify this is ongoing.

Isotopic labelling results so far indicate that *A. pisum* oviparae, and potentially oviparae of other Aphididae species, biosynthesise their sex pheromone *via* the route shown in Figure 5.1. This correlates with the results of biosynthetic studies performed in certain lactol **1**-producing plants, including *C. roseus* ²⁷, involving the reductive cyclisation of 8-oxogeranial. Further investigation is required to address unclarified aspects of lactol **1** and lactone **2** biosynthesis in aphids, including precursor origin and identity, cyclisation mechanism and, crucially, the enzymes involved.

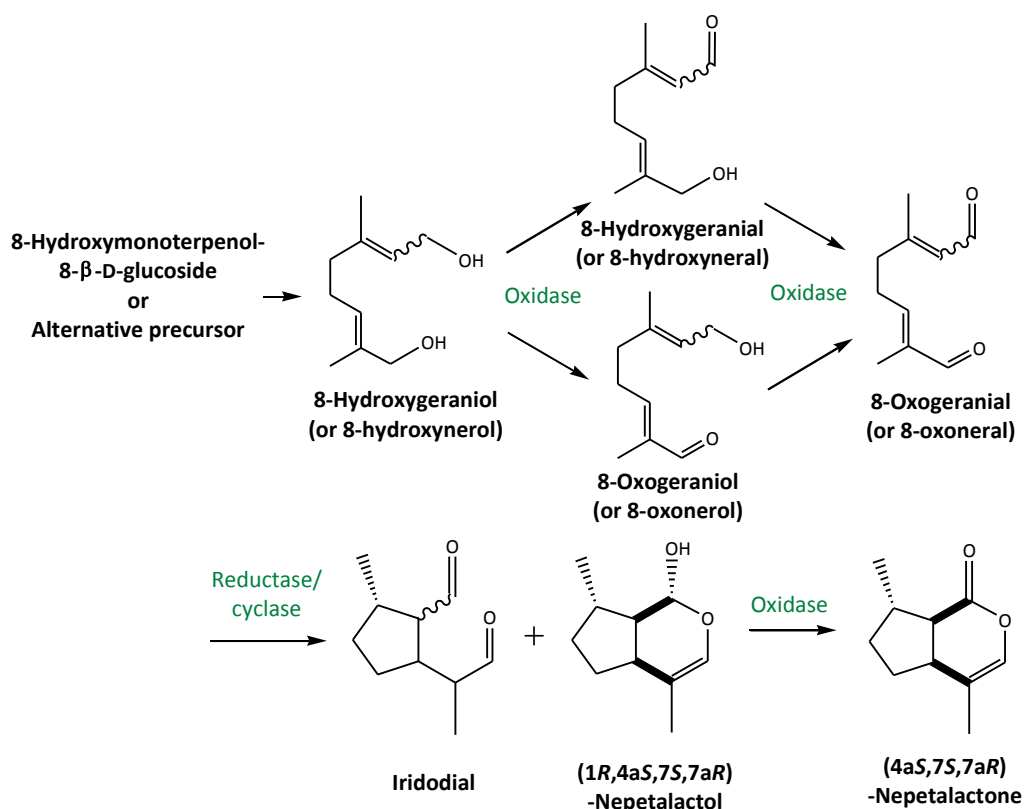


Figure 5.1. Putative biosynthetic pathway to lactol **1** and lactone **2** in *A. pisum* oviparae.

The work conducted during this project forms a platform for continued research into elucidating the biosynthetic pathway to the aphid sex pheromone and will support forthcoming research into identifying the genes linked to the biosynthesis of lactol **1** and lactone **2** in aphids. Armed with a completed genome sequence for *A. pisum*, *in vivo* and *in vitro* assays, employing techniques such as gene knockout, heterologous reconstitution and substrate bioconversion assays using recombinantly produced enzymes, will help to characterise the biocatalysts involved. In turn, this may offer improved biotechnology-based routes to stereochemically pure, highly valuable compounds¹⁰⁷.

In terms of agriculture, the genes identified may be heterologously expressed in crop plants that suffer from aphid herbivory and these transgenic plants used in innovative

IPM strategies. Through genetic engineering, the production and emission of lactol **1** and lactone **2** by the plants themselves could artificially increase aphid parasitoid numbers to areas requiring pest management. This strategy of enhancing plant defence could provide a method of aphid management that is less susceptible to mechanisms of aphid resistance.

6. Future Work

To provide a complete understanding of the biosynthetic route to the aphid sex pheromone, several aspects of the biosynthesis need to be addressed. The identity of the precursor remains obscure and the involvement of monoterpenyl glucoside derivatives, as reported for some species of Coleopteran beetle larvae⁸⁴, requires further investigation. Following unsuccessful synthesis of the acetylated 8-hydroxymonoterpenol-8-*O*- β -D-glucosides, the synthesis and application of thioglucosides, as previously described by Boland and colleagues^{84, 163, 188}, may shed light on the origin of sex pheromone components, lactol **1** and lactone **2**.

To ascertain whether aphids are capable of sequestering precursors and/or intermediates to their sex pheromone through phloem-feeding, in addition to *de novo* methods of biosynthesis, incorporation of the deuterium-labelled precursors into phloem sap *via* hydroponic feeding of the host plant may be possible. If successful, dynamic headspace collection of aphids feeding on these treated plants may show evidence of deuterium incorporation into lactol **1** and lactone **2**, should sequestration be a method they use.

Currently, the site of biosynthesis for each step of the biosynthetic pathway remains undetermined. Successful isotopic labeling following the topical application of deuterated intermediates onto the dorsal surface of the aphid may indicate that the site of biosynthesis is close to the site of absorption (i.e. within the fat body). Alternatively, the site of topical application may be irrelevant if the intermediates are sufficiently transported in the haemolymph from point of contact. To identify the site of biosynthesis, *in vitro* incubation of the deuterated compounds with individual

anatomical segments of the dissected aphid body (such as the head, legs, and fat body), each containing enzymes native to the segment used, could be used to highlight site-specific biosynthetic steps by analysing the products of incubation.

Furthermore, the confirmed release of the aphid sex pheromone from the hind tibiae of oviparae ¹⁹ may indicate the occurrence of a latter biosynthetic step or steps at the site of emission. To investigate this possibility, deuterium-labelled intermediates from further down the biosynthetic route, including iridodial and lactol **1**, could be synthesised and directly applied to the hind legs, rather than the dorsal surface. Evidence of deuterium incorporation in lactol **1** and lactone **2** of dynamic headspace collections would imply a biosynthetic role of the hind-legs, in addition to their role in emission.

The intended synthesis and application of NADP²H to investigate the involvement and C-7-stereochemistry of a reductive cyclisation step was not successful, largely because a solution to NADP²H purification complications was not found within the time frame of this project. An improved method for the synthesis and isolation of NADP²H would, upon application, provide insight into the reductive nature of the cyclisation method, despite the multiple labelling that would result *via* the mevalonate (MVA) pathway. The mechanism of reductive cyclisation, if applicable, is anticipated to proceed similarly to the mechanism confirmed in *C. roseus*, *via* the coupling of a reduction step with a Michael addition, rather than Diels Alder, cyclisation. Evidence to support this mechanism could be obtained through use of the halogenated probes described by Lindner et al ¹⁰², each primed to undergo only one of the possible cyclisation routes.

Isotopic labelling studies throughout the project indicated that the (*E*)- and (*Z*)-isomers of oxidised monoterpenyl derivatives were interchangeable regarding the biosynthetic pathway. However, it remains possible that aphids use only one isomer and that the other, although an efficient substrate for the biosynthetic enzymes involved, results in opposite stereochemistry at C-7. If true, this would likely arise from the altered conformation of neryl substrates in the enzyme's active site compared to their geranyl counterparts. To date, reports have suggested that both geranyl and neryl derivatives are successfully metabolised in the synthesis of lactol **1**, but the specific stereochemistry at C-7 resulting from incorporation of the neryl derivatives has, to the best of my knowledge, not been explicitly confirmed ¹²⁵. By determining the stereochemistry of lactol **1** produced following the application of deuterated 8-oxogeranial and 8-oxoneral separately, it may be possible to confirm or invalidate the reported equivalence of the (*E*)- and (*Z*)-derivatives.

Further probing of the biosynthetic mechanism could also confirm the suspected intermediacy of lactol **1** in the biosynthesis of lactone **2** *via* the action of an oxidase. This has, so far, been determined in plants such as *Nepeta racemosa* (Lamiaceae) ²²⁸. The synthesis of deuterium-labelled lactol **1**, *via* LiAlH₄-reduction of lactone **2** to iridodial ¹²⁴ and subsequent oxidation, would afford the deuterated material to facilitate this investigation.

Finally, the key enzymes utilised in the biosynthetic pathway, including terpene synthases, oxidases (such as cytochrome P450 enzymes) and the aphid equivalent of iridoid synthase (ISY), require characterization to help complete the understanding of the biosynthetic pathway to the aphid sex pheromone. This remains a multidisciplinary

task, encompassing molecular biology, bioinformatics and investigative chemistry methods.

Using the published genome sequence for *Acyrtosiphon pisum* (Homoptera: Aphididae), it is possible to check for gene sequence homology between aphids and plants and, in doing so, potentially identify the genes involved in the biosynthesis of aphid sex pheromone components, lactol **1** and lactone **2**. Once known, heterologous reconstitution of these genes in crop plant hosts could provide valuable crops with bioengineered protection against target aphid pests. Continuing advancements in molecular biology, including the advent of genome editing technology CRISPR/Cas 9, may provide an opportunity to achieve this ¹⁰⁷. If successful, these transgenic crops could exploit the role of semiochemical communication between aphid and parasitoid by promoting parasitism at the site of aphid aggregation.

In identifying the genes involved, unique biocatalysts will likely be encountered. Full characterisation of these enzymes may help to explain the stereochemistry of the reactions and the chirality of the products involved in the biosynthesis of lactol **1** and lactone **2** in aphids. Their discovery may also provide new tools for synthetic biologists, and for the wider field of biotechnology as a whole.

7. Experimental

7.1 Biology Experimental

7.1.1 *Acyrtosiphon pisum* Colony

A colony of *Acyrtosiphon pisum* (Homoptera: Aphididae), specifically the green morph, were introduced to a controlled environment unit and reared on broad bean, *Vicia faba*, plants. The production of oviparae was encouraged using autumnal conditions (L:D 12:12, 14 °C). Oviparae were identified under a microscope by their darkened, thicker hind tibiae and their darker, mottled and rounder body.

7.1.2 *Vicia faba* Host Plants

Non-flowering *V. faba* plants, seeded 3 weeks prior to use and grown in a glasshouse (L:D 16:8, 20 °C), were used to maintain the *A. pisum* colony and were potted individually 24 hours prior to experimental use during dynamic headspace collections.

7.1.3 Incorporation of Compounds into Aphids

7.1.3.1 Dietary Incorporation

Two controlled feeding environments were investigated, varying in temperature and photoperiod to mimic both aestival (L:D 16:8, 25 °C) and autumnal conditions (L:D 12:12, 14 °C). A series of D-(+)-sucrose composed artificial diets (0, 100, 150 and 200 g/L) solutions were prepared in HPLC-grade water, along with additional simple ¹⁷

and complex ^{184, 206}, literature-based diets, containing amino acids, vitamins and minerals (Section 2.3.1). These solutions were contained in diet sachets, from which *A. pisum* fed.

Diet sachets were prepared by enclosing 500 µL of the appropriate diet solution between two sheets of Parafilm M[®], each pre-treated by soaking in distilled water and drying before use, and stretched over one end of a Perspex[®] ring. Aphids (n = 20) were transferred to the inner part of the ring and trapped within the sachet on a plastic petri dish, allowing them to feed on the diet solution through the first Parafilm M[®] layer. Green acetate sheets were placed on top of each sachet to mimic the plant's hue and provide a more realistic, natural environment for the aphids. Excess diet solution was stored at -22 °C. Diet sachets were produced as and when required and were changed every 2-3 days to prevent the loss of vitamins and to avoid microbial contamination of the solutions ¹⁸⁴. Deceased aphids were removed daily.

7.1.3.2 Topical Incorporation

Putative biosynthetic precursors and intermediates were dissolved in methanol and a microsyringe was used to apply 0.2 µL of these methanolic solutions directly onto the dorsal surface of *A. pisum* oviparae (Section 2.3.2). For experiments requiring a 48-hour incubation period, the topically treated aphids were transferred to clip cages in groups of five and fed on *V. faba* leaves in the interim. All topical application studies were conducted under autumnal conditions (L:D 12:12, 14 °C).

7.1.4 Sex Pheromone Collection by Dynamic Headspace Collection

Volatile organic compounds (VOCs) released by *A. pisum* oviparae, including aphid sex pheromone components lactol **1** and lactone **2**, were collected by dynamic headspace collection (Section 2.1). During this process, air was purified using an activated charcoal filter and pumped through PTFE tubing into a 1 L glass vessel containing *A. pisum* oviparae at 0.6 L/min. *A. pisum* were added to the vessels through an opening in the ceiling of the vessel and were fed on *V. faba* plants whose canopies were enclosed in the vessel, with a metal plate, containing a hole for the stem, clipped underneath to form the base. To maintain a positive pressure, the headspace was pumped out of the vessel at 0.4 L/min via a Porapak™ Q porous polymer, onto which the VOCs were adsorbed. Porapak™ tubes were prepared by adding 50 mg of Porapak™ Q 50/80 mesh absorbent polymer to a narrow glass tube contained between two plugs of glass wool. Prior to use, each tube was conditioned by washing with re-distilled solvent (2 mL dichloromethane (DCM) then 2 mL diethyl ether (Et₂O)) and then heating to 132 °C under a stream of nitrogen for 2 hours. VOCs collected on Porapak™ tubes were subsequently eluted from the polymer using 750 µL of re-distilled Et₂O and concentrated under a stream of nitrogen to a final volume of 100 µL. This afforded GC-MS samples which were stored in screw cap glass vials at -22 °C awaiting analysis.

7.2 Analysis Experimental

7.2.1 Reference Materials and Calibration Solutions

Calibration solutions were prepared in re-distilled hexane using material obtained prior to this project; lactone **2** (98%) was extracted from *Nepeta cataria* (Lamiaceae)

and a portion reduced to lactol **1** (95%) using NaBH₄⁶⁸. The concentration range used was based on expected sex pheromone emissions by *A. pisum* oviparae (Table 5.1). Example GC calibration curves for each iridoid are shown in Figure 5.1. These were used to quantify lactol **1** and lactone **2**. Fresh calibration solutions were prepared each month.

Table 5.1. Calibration solution concentrations.

Analyte (ng/μL)	Calibration Solution				
	1	2	3	4	5
Lactol 1	1	5	10	50	100
Lactone 2	1	5	10	50	100

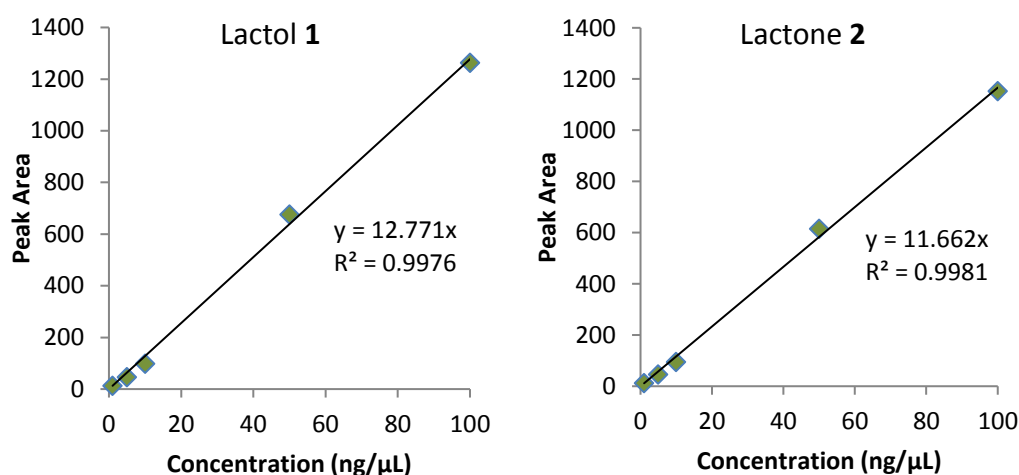


Figure 5.1. GC calibration curves for lactol **1** and lactone **2**.

7.2.2 GC Analysis

Sex pheromone samples were analysed using an Agilent 6890N GC fitted with dual cool-on-column (COC) injector inlets, a non-polar HP-1 column (50 m, 0.32 mm ID, 0.52 μm film thickness; J & W Scientific), a polar DB-Wax column (30 m, 0.32 mm ID, 0.5 μm film thickness; J & W Scientific) and a flame ionisation detector (FID). Hydrogen and

nitrogen were used as the carrier gas and make-up gas, respectively. Samples were manually injected, each 4 μL in volume, and the results quantified using a set of calibration standards (Section 5.2.1). The quantity (in $\text{ng}/\mu\text{L}$) of lactol **1** and lactone **2** released, per aphid, was recorded. The temperature gradient used for both columns is shown in Table 5.2.

Table 5.2. GC temperature gradient used for headspace collection samples.

Temperature ($^{\circ}\text{C}$)	Rate ($^{\circ}\text{C}/\text{min}$)	Hold (mins)	Runtime (mins)
30	0	1	1
150	5	0.1	25.1
230	10	20	53.1

GC-MS analysis was undertaken using a Micromass Autospec Ultima magnetic sector mass spectrometer coupled to an Agilent 6890N GC, fitted with the same injector inlet and HP-1 column as the GC-FID. Ionisation was by electron impact (70 eV, 220 $^{\circ}\text{C}$). The oven temperature programme is shown in Table 5.3.

Table 5.3. GC-MS temperature gradient used for headspace collection samples.

Temperature ($^{\circ}\text{C}$)	Rate ($^{\circ}\text{C}/\text{min}$)	Hold (mins)	Runtime (mins)
30	0	5	5
250	5	21	70

7.2.3 LC Analysis

Semi-preparative HPLC purification of the NADP^2H diastereomers was performed by ion pairing (IP) high performance liquid chromatography (HPLC). Separation was provided using a semi-preparative (250 x 10 mm) C18 reverse phase (RP) column and elution solvent gradient, utilising A) 10 mM triethylamine at pH 4.5 (IP reagent) in

distilled water and B) methanol. UV detection at 258 nm and 340 nm aided the identification of NADP²H and unreacted NADP⁺. Coupled ultra-performance LC (UPLC) tandem mass spectrometry (LC-MS/MS) provided further product identification. This comprised a Micromass Quattro Ultima triple quadrupole mass spectrometer attached to a Waters Acquity UPLC system. Samples were injected onto an Acquity UPLC BEC C18 column (1.7 μ m, 2.1 x 150 mm) and ionisation was operated in positive ion mode at atmospheric pressure, with a capillary voltage of 3 kV and cone voltage of 30. The PDA detection scan range was set at 210 to 400 nm.

7.2.4 High Resolution Mass Spectrometry (HRMS)

High resolution mass spectra were obtained for all synthesized compounds. HRMS was conducted using a hybrid quadrupole time of flight (Q-ToF) Ultima API mass spectrometer with MS/MS capability and high resolving power (7000-10,000 FWHM). Samples were introduced to the ESI interface, comprising an electrospray probe and a Z-spray source, through an infusion pump (10 μ L/min). Source temperature and desolvation temperature were 90 °C and 190 °C, respectively.

7.2.5 NMR Spectroscopy

Spectroscopic analysis was carried out for all synthesised compounds. NMR spectra were recorded using a Bruker spectrometer with a 5 mm BBO BB-1H probe, and set at 500 MHz for ¹H spectra and 125 MHz for ¹³C spectra. Deuterated chloroform (CDCl₃) and methanol (CD₃OD) were stored over 4 Å molecular sieves and used as both sample solvents and internal standards. Additional 2D-NMR spectroscopy experiments were performed when necessary to aid in assignments.

7.2.6 Polarimetry

The specific rotation ($[\alpha]_D^{20}$) of all optically active compounds synthesised was measured using an Anton Paar Modular Circular Polarimeter (MCP 100). Measurements were made using methanolic solutions of each compound (approximately 20 mg/mL), and the temperature (T) and light wavelength (λ) used were set to 20 °C and 589 nm, respectively. Specific rotations were calculated using Biot's Formula (Formula 7.1), incorporating the measured optical rotation in degrees (α), the path length in decimeters (l) and sample concentrations in g/100 mL.

$$[\alpha]_{\lambda}^T = \frac{100 \cdot \alpha}{l \cdot c}$$

Formula 7.1. Biot's Formula

7.2.7 Melting Points

Melting points were measured using a Gallenkamp melting point apparatus with coarse and fine temperature control.

7.2.8 Statistical Analysis

Statistical analysis was carried out using GenStat (16th Edition). Survival data was logit-transformed prior to performing further statistical tests. Two-way analysis of variance (ANOVA) was applied to initial sex pheromone production data using treated aphids.

7.3 Chemistry Experimental

All reagents and solvents used, deuterated or otherwise, were purchased from Sigma Aldrich, Apollo Scientific, Fisher Scientific, QMX Laboratories or Goss Scientific and used without further purification, unless otherwise stated. With the exception of solvents and chemical structures where deuterium atoms are denoted as D, they will be referred to as ^2H .

Flash chromatography was conducted using Fluka silica gel (40-63 μm , 60 Å). TLC was carried out using aluminium-supported sheets pre-coated with Merck silica gel 60 F₂₅₄. TLC staining solutions, used to aid visualisation, were limited to 1) 2,4-dinitrophenylhydrazine (2,4-DNP), prepared by dissolving 2,4-DNP (12 g), H_2SO_4 (60 mL) and distilled water (80 mL) in 95% ethanol (200 mL) and 2) potassium permanganate (KMnO_4), obtained by dissolving KMnO_4 (1.5 g), K_2CO_3 (10 g) and 10% NaOH (1.25 mL) in distilled water (200 mL).

7.3.1 General Synthetic Procedures

Quantities given represent those used in the synthesis of geranyl derivatives and will vary between the compounds synthesised (described in section 7.3.15).

7.3.2 *n*-BuLi titration

Prior to use, *n*-BuLi was titrated under nitrogen using a solution of diphenylacetic acid (DPAA; 100 mg) in anhydrous THF (2 mL). A persistent yellow colour indicated the formation of the lithiated dianion and therefore signalled the end-point.

7.3.3 General Synthesis for Acetate Protection

To a solution of alcohol (2.25 g, 1 eq.) in acetic anhydride (2.66 mL, 2 eq.) and anhydrous pyridine (5.46 mL, 5 eq.), under nitrogen, was added a substoichiometric amount of 4-dimethylaminopyridine (1.4 mg) and the solution stirred at room temperature for 2 hours. The reaction mixture was then poured into ice-cold distilled water (20 mL) and the aqueous layer extracted using Et₂O (3 x 20 mL). The combined organic extracts were washed with distilled water (2 x 20 mL), 2 M HCl (20 mL) and saturated sodium hydrogen carbonate solution (20 mL), and dried using anhydrous magnesium sulfate (MgSO₄). The solvent was removed under vacuum.

7.3.4 General Synthesis for Acetal Protection

To a stirred solution of ethyl vinyl ether (404 µL, 1 eq.) in anhydrous DCM (20 mL) was added 8-hydroxymonoterpenyl acetate (889 mg, 1 eq.) and substoichiometric pyridinium *p*-toluenesulfonate (12 mg). After 4 hours, the reaction mixture was diluted with Et₂O (20 mL) and washed with brine (2 x 20 mL). The organic phase was dried with anhydrous MgSO₄ and the solvent removed under vacuum.

7.3.5 General Synthesis for TBDPS Ether Protection

To a solution of alcohol (6 g, 1 eq.) in DCM (100 mL) was added *t*-butyldiphenylsilyl chloride (21.3 g, 1.4 eq.). The solution was cooled to 0 °C and placed under nitrogen before adding imidazole (5.27 g, 1.4 eq.). After stirring for 12 hours, distilled water

(150 mL) was added and the aqueous phase extracted with DCM (2x 100 mL). The combined organic layers were washed with brine and dried with anhydrous MgSO_4 before concentrating under vacuum.

7.3.6 General Synthesis for Acetate Deprotection

To a solution of acetate-protected alcohol (160 mg, 1 eq.) in 5% aqueous MeOH (10 mL) was added potassium carbonate (85 mg, 2.6 eq.). After stirring for 24 hours at room temperature, the methanol was removed under vacuum and the residue taken up in distilled water (50 mL) before extracting with ethyl acetate (EtOAc) (3 x 20 mL). The combined organic layers were dried with anhydrous MgSO_4 and concentrated under vacuum.

7.3.7 General Synthesis for Acetal Deprotection

To a solution of acetal-protected monoterpene (335 mg, 1 eq.) was added a solution of acetic acid, distilled water and THF (8:1:1) (10 mL) and the reaction mixture stirred for 3 hours at room temperature. The mixture was then shaken with Et_2O (30 mL) and the organic layer separated, dried with anhydrous MgSO_4 and concentrated under vacuum.

7.3.8 General Synthesis for TBDPS Ether Deprotection

To a solution of TBDPS ether-protected alcohol (241 mg, 1 eq.) in THF (10 mL) at 0 °C was added tetrabutylammonium fluoride hydrate (229 mg, 2 eq.). The solution was stirred for 1 hour at 0 °C and then for another 2 hours at room temperature. The

reaction was quenched by adding 2 M HCl (20 mL) and the solution extracted using Et₂O (3 x 20 mL). The organic fractions were combined and dried with anhydrous MgSO₄ before concentrating under vacuum.

7.3.9 General Synthesis for Allylic Oxidation

To a solution of selenium dioxide (21 mg, 0.03 eq.) and salicylic acid (86 mg, 0.01 eq.) in DCM (10 mL) under nitrogen was added *t*-butyl hydroperoxide (1.8 mL, 3 eq.). The solution was stirred for 15 minutes before adding a solution of monoterpenol (1.24 g, 1 eq.), protected or otherwise, in DCM (10 mL). The reaction mixture was stirred for a further 72 hours before diluting with DCM (20 mL) and adding aqueous sodium thiosulfate (40 mL) and saturated sodium hydrogen carbonate solution (40 mL) to quench the reaction. After stirring for an additional 30 minutes, the organic phase was separated and the aqueous fraction extracted with DCM (3 x 50 mL). The organic layers were combined and washed with brine (100 mL), dried with anhydrous MgSO₄ and concentrated under vacuum.

7.3.10 General Synthesis for IBX Oxidation

To a solution of alcohol (0.52 g, 1 eq.) in DMSO (20 mL) was added IBX (0.9 g, 1.5 eq. per alcohol group). The reaction mixture was stirred for 2 hours at room temperature before diluting with Et₂O (20 mL). After a further 5 minutes of stirring, the mixture was filtered over celite and the filtrate diluted with distilled water (20 mL) and the solution was shaken and the organic layer separated and washed with saturated sodium hydrogen carbonate solution (3 x 50 mL) and brine (50 mL) before drying with anhydrous MgSO₄ and concentrating under vacuum.

7.3.11 General Synthesis for MnO₂ Oxidation

To a solution of alcohol (1 g, 1 eq.) in DCM (20 mL) was added MnO₂ (2.8 g, 5 eq.). The solution was stirred for 24 hours at room temperature before filtering over celite and evaporating the solvent under vacuum.

7.3.12 General Synthesis for Epoxidation

A solution of acetate- or TBDPS ether-protected monoterpenol (4.43 g, 1 eq.) in DCM (20 mL) was cooled to 0°C and a suspension of *meta*-chloroperoxybenzoic acid (5.06 g, 1 eq.) in DCM (30 mL) added slowly whilst stirring. After 30 minutes of stirring at room temperature, further *m*-CPBA was added as required to complete the reaction (monitored by TLC). The solution was then filtered and the filtrate washed with saturated sodium hydrogen carbonate (50 mL), distilled water (50 mL) and brine (50 mL) before drying with anhydrous MgSO₄ and concentrating under vacuum.

7.3.13 General Synthesis for Epoxide Cleavage

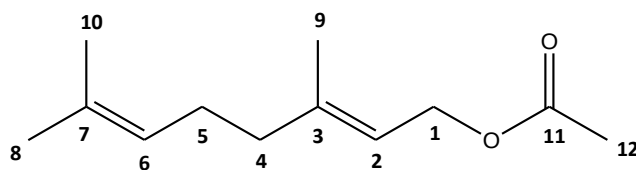
To a solution of epoxide (6.53 g, 1 eq.) in THF:distilled water (9:1) (40 mL) was added sodium periodate (3.95 g, 0.6 eq.) and periodic acid (7.71 g, 1.1 eq.), sequentially, at 0 °C. The reaction mixture was stirred at 0 °C for 10 minutes and then warmed to room temperature and stirred for 1 hour. The reaction mixture was quenched with saturated sodium hydrogen carbonate solution (40 mL) before pouring into distilled water (40 mL) and extracting with EtOAc (3 x 40 mL). The organic layers were combined and washed with brine (100 mL) before drying with anhydrous MgSO₄ and concentrating under vacuum.

7.3.14 General Synthesis for Wittig Reaction

[1,1,1,2,3,3,3- $^2\text{H}_7$]-*iso*-Propyltriphenylphosphonium bromide (8.11 g, 1 eq.) was dissolved in THF (50 mL) under nitrogen and cooled to $-78\text{ }^\circ\text{C}$. A solution of *n*-butyl lithium in hexane (16.33 mL, 1 eq.) was added, dropwise, and the reaction mixture was stirred for 30 minutes whilst allowing to warm to room temperature. Aldehyde (3.52 g, 1 eq.) was then added at $-78\text{ }^\circ\text{C}$ and the reaction mixture stirred for 4 hours, again whilst allowing to warm to room temperature. Saturated ammonium chloride solution (50 mL) was added and the solution stirred for 5 minutes to quench the reaction. The solution was then diluted with distilled water (500 mL) and extracted with ethyl acetate (3 x 100 mL). The combined extracts were dried with anhydrous MgSO_4 and concentrated under vacuum.

7.3.15 Chapter 2 Synthesis

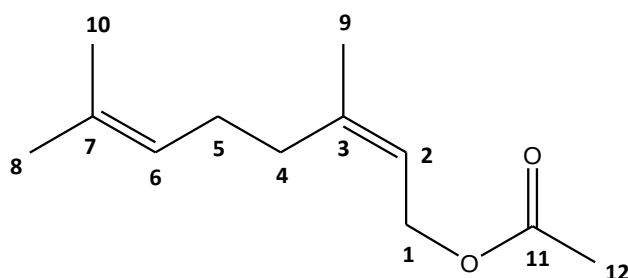
(2*E*)-3,7-Dimethyl-2,6-octadien-1-yl acetate (**112**) (Geranyl acetate)



Following the general acetate protection procedure using geraniol (**56**) (2.25 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 $^\circ\text{C}$) to give (2*E*)-3,7-dimethyl-2,6-octadien-1-yl acetate (**112**) (2.41 g, 84%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 2965, 2933, 2874, 1742, 1671, 1429, 1381, 1236, 1045, 1023, 962, 611; δ_{H} (CDCl_3 , 500 MHz)¹⁵ 5.37 (1H, t, *J* 7.3, H-2), 5.11 (1H, t, *J* 6.8, H-6), 4.61 (2H, d, *J* 7.3, H-1), 2.16-2.10 (2H, m, H-5), 2.08 (3H, s, H-12), 2.08-2.04 (2H, m, H-4), 1.73 (3H, s, H-8/10), 1.71 (3H, s, H-8/10), 1.63 (3H, s, H-9); δ_{C} (CDCl_3 , 125 MHz) 171.5 (C-11), 142.6

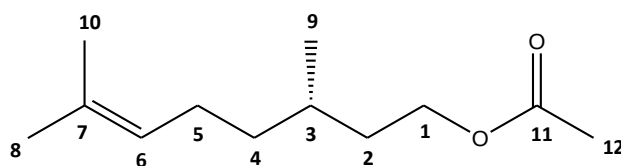
(C-3), 131.8 (C-7), 123.0 (C-6), 122.6 (C-2), 61.1 (C-1), 32.9 (C-5), 26.9 (C-4), 25.3 (C-9), 23.4 (C-8/10), 21.2 (C-12), 17.6 (C-8/10). HRMS: Found: 219.1346. $C_{12}H_{20}O_2$ (M+Na⁺) Calculated 219.1356.

(2Z)-3,7-Dimethyl-2,6-octadien-1-yl acetate (113**) (Neryl acetate)**



Following the general acetate protection procedure using nerol (**62**) (2.24 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2Z)-3,7-dimethyl-2,6-octadien-1-yl acetate (**113**) (2.45 g, 86%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2970, 2934, 2874, 1737, 1672, 1445, 1375, 1230, 1045, 1022, 955, 607; δ_H (CDCl₃, 500 MHz)¹⁵ 5.38 (1H, t, *J* 7.3 Hz, H-2), 5.11 (1H, t, *J* 6.9 Hz, H-6), 4.58 (2H, d, *J* 7.3 Hz, H-1), 2.16-2.11 (2H, m, H-5), 2.12-2.09 (2H, m, H-4), 2.08 (3H, s, H-12), 1.79 (3H, s, H-9), 1.71 (3H, s, H-8/10), 1.63 (3H, s, H-8/10); δ_C (CDCl₃, 125 MHz) 171.2 (C-11), 142.7 (C-3), 132.2 (C-7), 123.6 (C-6), 119.1 (C-2), 61.1 (C-1), 32.2 (C-5), 26.7 (C-4), 25.7 (C-9), 23.5 (C-8/10), 21.1 (C-12), 17.7 (C-8/10). HRMS: Found: 219.1367. $C_{12}H_{20}O_2$ (M+Na⁺) Calculated 219.1356.

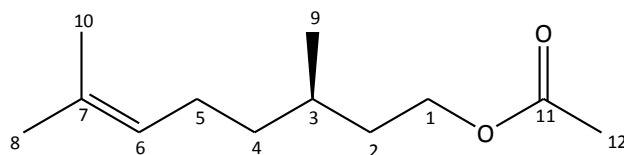
(3S)-3,7-Dimethyl-6-octen-1-yl acetate ((S)-114**) ((S)-Citronellyl acetate)**



Following the general acetate protection procedure using (S)-citronellol ((S)-**57**) (1.74 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether

40-60 °C) to give (3*S*)-3,7-dimethyl-6-octen-1-yl acetate (**(S)-114**) (2.08 g, 94%) as a colourless oil: $[\alpha]_D^{20}$ -2.2 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2969, 2934, 2878, 1736, 1455, 1365, 1234, 1047, 974, 631, 607; δ_H (CDCl₃, 500 MHz)¹⁵ 5.11 (1H, t, *J* 6.7, H-6), 4.13-4.11 (2H, m, H-1), 2.07 (3H, s, H-12), 2.01-1.99 (2H, m, H-5), 1.71 (3H, s, H-8/10), 1.63-1.68 (1H, m, H-2a), 1.57 (3H, s, H-8/10), 1.57-1.54 (1H, m, H-3), 1.48-1.45 (1H, m, H-2b), 1.37-1.33 (1H, m, H-4a), 1.22-1.20 (1H, m, H-4b), 0.94 (3H, d, *J* 6.5, H-9); δ_C (CDCl₃, 125 MHz) 171.3 (C-11), 131.4 (C-7), 124.6 (C-6), 63.1 (C-1), 37.0 (C-4), 35.4 (C-2), 29.5 (C-3), 25.8 (C-8/10), 25.4 (C-5), 21.1 (C-12), 19.4 (C-9), 17.7 (C-8/10). HRMS: Found: 221.1512. C₁₂H₂₂O₂ (M+Na⁺) Calculated 221.1521.

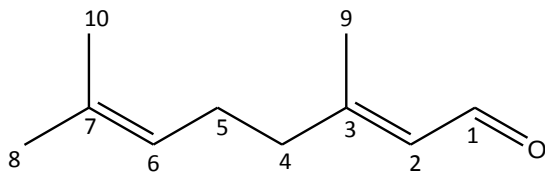
(3*R*)-3,7-Dimethyl-6-octen-1-yl acetate ((*R*)-114) ((*R*)-Citronellyl acetate)



Following the general acetate protection procedure using (*R*)-citronellol (**(R)-57**) (1.84 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3*S*)-3,7-dimethyl-6-octen-1-yl acetate (**(R)-114**) (2.22 g, 95%) as a colourless oil: $[\alpha]_D^{20}$ +2.6 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2970, 2934, 2875, 1740, 1455, 1365, 1233, 1048, 974, 629, 610; δ_H (CDCl₃, 500 MHz) 5.12 (1H, t, *J* 6.7, H-6), 4.15-4.11 (2H, m, H-1), 2.06 (3H, s, H-12), 2.02-1.97 (2H, m, H-5), 1.71 (3H, s, H-8/10), 1.68-1.64 (1H, m, H-2a), 1.53 (3H, s, H-8/10), 1.57-1.54 (1H, m, H-3), 1.47-1.44 (1H, m, H-2b), 1.40-1.36 (1H, m, H-4a), 1.24-1.21 (1H, m, H-4b), 0.94 (3H, d, *J* 6.5, H-9); δ_C (CDCl₃, 125 MHz) 171.8 (C-11), 132.0 (C-7), 125.3 (C-6), 63.0 (C-1), 37.3 (C-4), 35.7 (C-2), 29.5 (C-3), 25.7 (C-8/10), 25.0 (C-5), 21.0 (C-12), 19.5 (C-9), 17.4 (C-8/10). HRMS: Found: 221.1514. C₁₂H₂₂O₂ (M+Na⁺) Calculated 221.1521.

(2E)-3,7-Dimethyl-2,6-octadien-1-al (109)

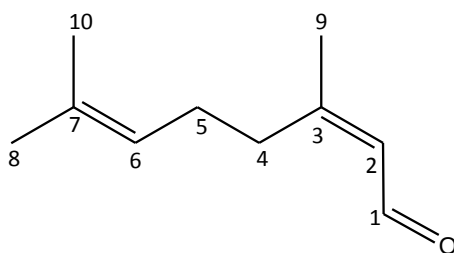
(Geranial)



Following the general MnO_2 oxidation procedure using geraniol (**56**) (1 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2E)-3,7-dimethyl-2,6-octadien-1-al (**109**) (477 mg, 48%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3071, 2959, 2930, 2853, 1736, 1674, 1475, 1442, 1428, 1396, 1375, 1302, 1240, 1187, 1158, 1110, 1045, 844, 821, 743, 706, 634, 508, 492, 431; δ_{H} (CDCl_3 , 500 MHz) 10.01 (1H, d, J 7.5, H-1), 5.90 (1H, d, J 7.5, H-2), 5.07 (1H, t, J 6.4, H-6), 2.28-2.20 (4H, m, H-4 and H-5), 2.19 (3H, s, H-9), 1.70 (3H, s, H-8), 1.63 (3H, s, H-10); δ_{C} (CDCl_3 , 125 MHz) 191.4 (C-1), 163.9 (C-3), 133.0 (C-7), 127.4 (C-6), 122.6 (C-2), 40.6 (C-8), 25.7 (C-5), 17.7 (C-4), 15.2 (C-9), 12.9 (C-10). HRMS: Found: 175.1103. $\text{C}_{10}\text{H}_{16}\text{O}$ ($\text{M}+\text{Na}^+$) Calculated 175.1093.

(2Z)-3,7-Dimethyl-2,6-octadien-1-al (110)

(Neral)

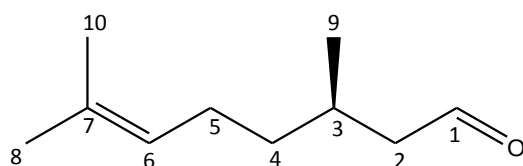


Following the general MnO_2 oxidation procedure using nerol (**62**) (1 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2Z)-3,7-dimethyl-2,6-octadien-1-al (**110**) (318 mg, 32%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3074, 2961, 2931, 2851, 1740, 1667, 1473, 1441, 1428, 1397, 1376, 1302,

1241, 1188, 1155, 1109, 1045, 839, 820, 743, 707, 634, 511, 492, 434; δ_{H} (CDCl_3 , 500 MHz)¹⁵ 9.91 (1H, d, J 7.5, H-1), 5.90 (1H, d, J 7.5, H-2), 5.12 (1H, t, J 6.4, H-6), 2.61 (2H, t, J 6.4, H-4), 2.25-2.15 (2H, m, H-5), 2.00 (3H, s, H-9), 1.71 (3H, s, H-8), 1.62 (3H, s, H-10); δ_{C} (CDCl_3 , 125 MHz) 190.9 (C-1), 163.9 (C-3), 133.8 (C-7), 128.7 (C-6), 122.3 (C-2), 32.6 (C-8), 27.1 (C-5), 25.7 (C-4), 25.1 (C-9), 17.8 (C-10). HRMS: Found: 175.1102. $\text{C}_{10}\text{H}_{16}\text{O}$ ($\text{M}+\text{Na}^+$) Calculated 175.1093.

((3R)-3,7-Dimethyl-6-octen-1-al ((R)-111)

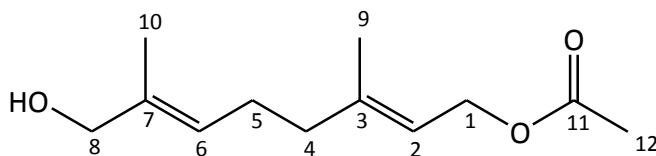
((R)-Citronellal)



Following the general IBX oxidation procedure using (*R*)-citronellol (**(R)-57**) (0.52 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (*3R*)-3,7-dimethyl-6-octen-1-al (**(R)-111**) (316 mg, 62%) as a colourless oil: $[\alpha]_{\text{D}}^{20} +8.6$ (c 1.0, MeOH); ν_{max} (thin film)/ cm^{-1} 2991, 2951, 2921, 2873, 2710, 1726, 1644, 1455, 1406, 1375, 1283, 1240, 1187, 1168, 1115, 1015, 981, 902, 894, 821, 743, 684, 528, 510, 431; δ_{H} (CDCl_3 , 500 MHz) 9.78 (1H, s, H-1), 5.10 (1H, t, J 6.9, H-6), 2.52 (1H, dd, J 6.9 and 1.2, H-2a), 2.25 (1H, dd, J 6.9 and 1.2, H-2b), 2.12-2.07 (1H, m, H-3), 2.08-1.97 (2H, m, H-5), 1.70 (3H, s, H-8), 1.64 (3H, s, H-10), 1.41-1.34 (1H, m, H-4a), 1.34-1.25 (1H, m, H-4b), 0.98 (3H, d, J 6.9, H-9); δ_{C} (CDCl_3 , 125 MHz) 191.0 (C-1), 134.4 (C-7), 129.6 (C-6), 57.8 (C-8), 38.3 (C-5), 29.6 (C-2), 26.8 (C-3), 25.2 (C-4), 15.2 (C-9), 12.9 (C-10). HRMS: Found: 177.1243. $\text{C}_{10}\text{H}_{16}\text{O}$ ($\text{M}+\text{Na}^+$) Calculated 177.1255.

(2E,6E)-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-yl acetate (115)

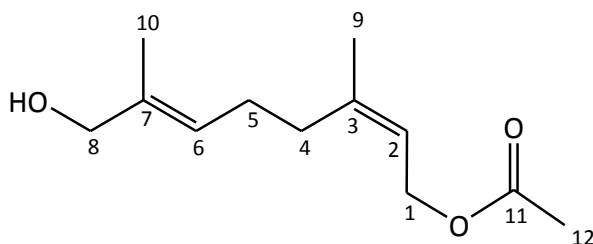
(8-Hydroxygeranyl acetate)



Following the general allylic oxidation procedure using geranyl acetate (**112**) (1.24 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2E,6E)-3,7-dimethyl-8-hydroxy-2,6-octadien-1-yl acetate (**115**) (511 mg, 38%) as a colourless oil: $\nu_{\max}$ (thin film)/ cm^{-1} 3409, 2965, 2919, 2859, 1736, 1671, 1442, 1381, 1366, 1229, 1019, 851, 644, 608; δ_{H} (CDCl_3 , 500 MHz)¹⁵ 5.39 (1H, t, J 6.8, H-6), 5.36 (1H, t, J 7.1, H-2), 4.61 (2H, d, J 7.1, H-1), 4.02 (2H, d, J 5.3, H-8), 2.23-2.16 (2H, m, H-5), 2.14-2.08 (2H, m, H-4), 2.09 (3H, s, H-12), 1.73 (3H, s, H-9), 1.69 (3H, s, H-10); δ_{C} (CDCl_3 , 125 MHz) 171.2 (C-11), 141.7 (C-3), 135.3 (C-7), 125.4 (C-6), 118.7 (C-2), 69.0 (C-8), 61.5 (C-1), 39.1 (C-4), 25.7 (C-5), 21.1 (C-12), 16.4 (C-9), 13.7 (C-10). HRMS: Found: 235.1311. $\text{C}_{12}\text{H}_{20}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 235.1305.

(2Z,6E)-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-yl acetate (116)

(8-Hydroxyneryl acetate)

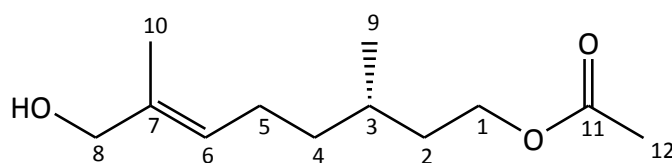


Following the general allylic oxidation procedure using neryl acetate (**113**) (1 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-3,7-dimethyl-8-hydroxy-2,6-octadien-1-yl acetate (**116**) (497 mg, 46%) as a colourless oil: $\nu_{\max}$ (thin film)/ cm^{-1} 3397, 2967, 2936, 2862, 1735, 1673, 1444, 1378,

1366, 1230, 1019, 953, 852, 645, 608; δ_{H} (CDCl_3 , 500 MHz) 5.43-5.39 (2H, t, J 7.1, H-6), 5.40-5.35 (2H, t, J 7.1, H-2), 4.59 (2H, d, J 7.1, H-1), 4.02 (2H, d, J 5.5, H-8), 2.19-2.17 (4H, m, H-4 and H-5), 2.08 (3H, s, H-12), 1.80 (3H, s, H-9), 1.69 (3H, s, H-10); δ_{C} (CDCl_3 , 125 MHz) 171.2 (C-11), 141.9 (C-3), 135.6 (C-7), 124.8 (C-6), 119.6 (C-2), 68.8 (C-8), 61.2 (C-1), 31.8 (C-4), 25.9 (C-5), 23.4 (C-9), 21.1 (C-12), 13.7 (C-10). HRMS: Found: 235.1309. $\text{C}_{12}\text{H}_{20}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 235.1305.

(3*S*,6*E*)-3,7-Dimethyl-8-hydroxy-6-octen-1-yl acetate ((*S*)-117**)**

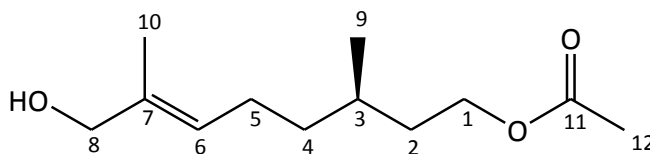
(8-Hydroxy-(*S*)-citronellyl acetate)



Following the general acetate protection procedure using (*S*)-citronellyl acetate ((*S*)-**114**) (1 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-3,7-dimethyl-8-hydroxy-6-octen-1-yl acetate ((*S*)-**117**) (421 mg, 39%) as a colourless oil: $[\alpha]_{\text{D}}^{20}$ -3.9 (c 1.0, MeOH); ν_{max} (thin film)/ cm^{-1} 3427, 2959, 2918, 2870, 1737, 1724, 1459, 1435, 1366, 1390, 1049, 849, 607; δ_{H} (CDCl_3 , 500 MHz) 5.43 (1H, t, J 6.7, H-6), 4.19-4.09 (2H, m, H-1), 4.03 (2H, m, H-8), 2.17-2.02 (2H, m, H-5), 2.03 (3H, s, H-12), 1.74-1.68 (1H, m, H-2a), 1.71 (3H, s, H-10), 1.64-1.57 (1H, m, H-3), 1.52-1.45 (1H, m, H-2b), 1.46-1.39 (1H, m, H-4a), 1.32-1.23 (1H, m, H-4b), 0.92 (3H, d, J 6.5, H-9); δ_{C} (CDCl_3 , 125 MHz) 171.3 (C-11), 134.8 (C-7), 126.2 (C-6), 69.4 (C-8), 63.0 (C-1), 36.6 (C-4), 35.4 (C-2), 29.5 (C-3), 25.0 (C-5), 19.4 (C-12), 17.5 (C-9), 13.6 (C-10). HRMS: Found: 237.1463. $\text{C}_{12}\text{H}_{22}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 237.1467.

(3*R*,6*E*)-3,7-Dimethyl-8-hydroxy-6-octen-1-yl acetate ((*R*)-117)

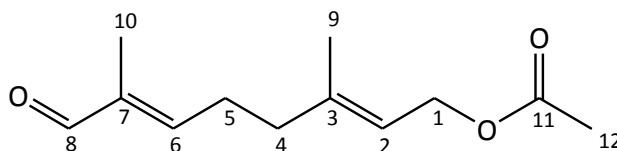
(8-Hydroxy-(*R*)-citronellyl acetate)



Following the general acetate protection procedure using (*R*)-citronellyl acetate ((*R*)-114) (712 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3*R*,6*E*)-3,7-dimethyl-8-hydroxy-6-octen-1-yl acetate ((*R*)-117) (323 mg, 42%) as a colourless oil [α]_D²⁰ +3.6 (c 1.0, MeOH); ν_{max} (thin film)/cm⁻¹ 3430, 2957, 2911, 2875, 1731, 1723, 1459, 1436, 1370, 1390, 1049, 848, 603; δ_{H} (CDCl₃, 500 MHz) 5.41 (1H, t, *J* 6.7, H-6), 4.17-4.07 (2H, m, H-1), 4.02 (2H, m, H-8), 2.14-2.00 (2H, m, H-5), 2.06 (3H, s, H-12), 1.73-1.64 (1H, m, H-2a), 1.70 (3H, s, H-10), 1.62-1.52 (1H, m, H-3), 1.50-1.42 (1H, m, H-2b), 1.43-1.35 (1H, m, H-4a), 1.28-1.21 (1H, m, H-4b), 0.94 (3H, d, *J* 6.5, H-9); δ_{C} (CDCl₃, 125 MHz) 171.3 (C-11), 134.6 (C-7), 126.2 (C-6), 69.0 (C-8), 63.0 (C-1), 36.1 (C-4), 35.4 (C-2), 29.5 (C-3), 25.0 (C-5), 21.1 (C-12), 19.4 (C-9), 13.7 (C-10). HRMS: Found: 237.1469. C₁₂H₂₂O₃ (M+Na⁺) Calculated 237.1461.

(2*E*,6*E*)-3,7-Dimethyl-8-oxo-2,6-octadien-1-yl acetate (118)

(8-Oxogeranyl acetate)

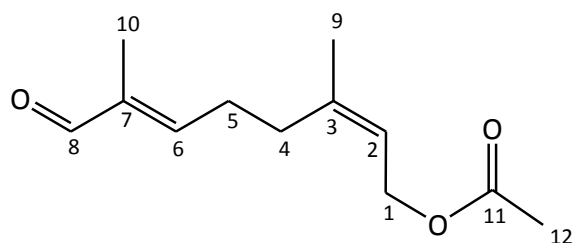


Following the general allylic oxidation procedure using geranyl acetate (112) (1.24 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-3,7-dimethyl-8-oxo-2,6-octadien-1-yl acetate (118) (210 mg, 16%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 3021, 2962, 2862, 1738, 1666, 1459, 1446, 1378, 1365, 1228, 1047, 955, 860, 608; δ_{H} (CDCl₃, 500 MHz) 9.37 (1H, s, H-8), 6.44 (1H, t, *J* 7.3,

H-6), 5.37 (2H, d, *J* 7.0, H-2), 4.58 (2H, d, *J* 7.0, H-1), 2.52-2.45 (2H, m, H-5), 2.22 (2H, t, *J* 7.5, H-4), 2.04 (3H, s, H-12), 1.73 (6H, s, H-9 and H-10); δ_c (CDCl₃, 125 MHz) 195.5 (C-8), 171.1 (C-11), 153.5 (C-3), 140.5 (C-7), 139.8 (C-6), 119.6 (C-2), 61.1 (C-1), 37.8 (C-4), 27.0 (C-5), 21.0 (C-12), 16.4 (C-9), 9.3 (C-10). HRMS: Found: 233.1141. C₁₂H₁₈O₃ (M+Na⁺) Calculated 233.1148.

(2Z,6E)-3,7-Dimethyl-8-oxo-2,6-octadien-1-yl acetate (119**)**

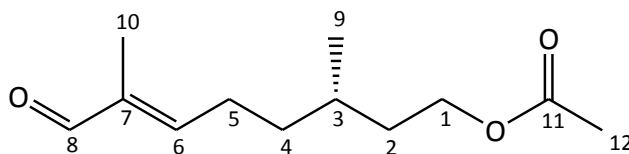
(8-Oxoneryl acetate)



Following the general allylic oxidation procedure using neryl acetate (**113**) (1 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-3,7-dimethyl-8-oxo-2,6-octadien-1-yl acetate (**119**) (160 mg, 15%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3025, 2960, 2864, 1738, 1667, 1461, 1445, 1379, 1365, 1228, 1044, 957, 861, 608; δ_H (CDCl₃, 500 MHz) 9.41 (1H, s, H-8), 6.49 (1H, t, *J* 7.3, H-6), 5.45 (1H, t, *J* 7.3, H-2), 4.57 (2H, d, *J* 7.3, H-1), 2.50-2.45 (2H, m, H-5), 2.33 (2H, t, *J* 7.5, H-4), 2.06 (3H, s, H-12), 1.80 (3H, s, H-9), 1.77 (3H, s, H-10); δ_c (CDCl₃, 125 MHz) 195.2 (C-8), 171.0 (C-11), 153.1 (C-3), 140.9 (C-7), 139.9 (C-6), 120.5 (C-2), 60.7 (C-1), 30.5 (C-4), 27.4 (C-5), 23.3 (C-9), 21.1 (C-12), 9.7 (C-10). HRMS: Found: 233.1157. C₁₂H₁₈O₃ (M+Na⁺) Calculated 233.1148.

(3*S*,6*E*)-3,7-Dimethyl-8-oxo-6-octen-1-yl acetate ((S**)-120)**

(8-Oxo-(*S*)-citronellyl acetate)

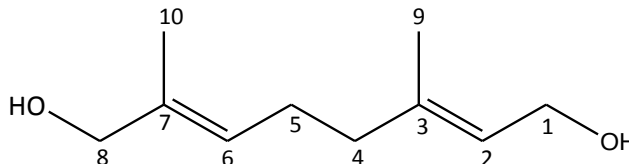


Following the general allylic oxidation procedure using (*S*)-citronellyl acetate ((**S**)-114)

(1 g), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-3,7-dimethyl-8-oxo-6-octen-1-yl acetate ((**S**)-120) (153 mg, 14%) as a colourless oil: $[\alpha]_D^{20}$ -6.2 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3023, 2960, 2862, 1741, 1664, 1459, 1444, 1375, 1360, 1228, 1047, 958, 861, 607; δ_{H} (CDCl₃, 500 MHz) 9.89 (1H, s, H-8), 6.49 (1H, t, *J* 6.7, H-6), 4.17-4.05 (2H, m, H-1), 2.43-2.30 (2H, m, H-5), 2.07 (3H, s, H-12), 1.74 (3H, s, H-10), 1.73-1.68 (1H, m, H-2a), 1.68-1.60 (1H, m, H-3), 1.60-1.55 (1H, m, H-2b), 1.43-1.32 (2H, m, H-4), 0.98 (3H, d, *J* 6.5, H-9); δ_{C} (CDCl₃, 125 MHz) 195.4 (C-8), 171.2 (C-11), 154.6 (C-7), 139.5 (C-6), 62.7 (C-1), 35.4 (C-2 and C-4), 29.7 (C-3), 26.5 (C-5), 21.1 (C-12), 19.3 (C-9), 9.3 (C-10). HRMS: Found: 235.1311. C₁₂H₂₀O₃ (M+Na⁺) Calculated 235.1305.

(2*E*,6*E*)-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-ol (72**)**

(8-Hydroxygeraniol)

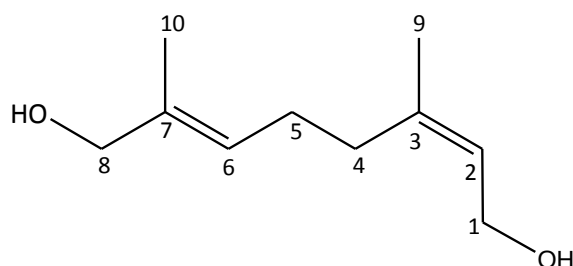


Following the general acetate deprotection procedure using 8-hydroxygeranyl acetate (**115**) (160 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-3,7-dimethyl-8-hydroxy-2,6-octadien-1-ol (**72**) (63 mg, 49%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3320, 2917, 2859, 1668, 1439, 1383, 1235, 1064, 995, 840; δ_{H} (CDCl₃, 500 MHz)¹⁵ 5.28 (2H, dt, *J* 6.9 and 1.4, H-2 and H-6), 3.97 (2H,

d, J 6.9, H-1), 3.82 (2H, s, H-8), 2.11-2.03 (2H, m, H-5), 2.02-1.95 (2H, m, H-4), 1.55 (3H, s, H-9), 1.51 (3H, s, H-10); δ_{C} (CDCl₃, 125 MHz) 141.1 (C-3), 134.4 (C-7), 126.6 (C-6), 121.8 (C-2), 67.6 (C-1), 57.8 (C-8), 38.3 (C-5), 25.2 (C-4), 15.2 (C-9), 12.8 (C-10). HRMS: Found: 193.1185. C₁₀H₁₈O₂ (M+Na⁺) Calculated 193.1199.

(2Z,6E)-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-ol (91)

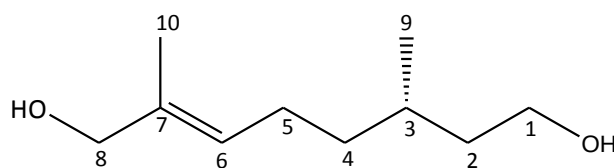
(8-Hydroxyneryl)



Following the general acetate deprotection procedure using 8-hydroxyneryl acetate (**116**) (277 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-3,7-dimethyl-8-hydroxy-2,6-octadien-1-ol (**91**) (120 mg, 54%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 3313, 2964, 2916, 2859, 1668, 1444, 1377, 1303, 1234, 1061, 995, 837, 600; δ_{H} (CDCl₃, 500 MHz) 5.43-5.33 (2H, m, H-2/6), 4.05 (2H, d, J 7.2, H-1), 3.92 (2H, s, H-8), 2.18-2.08 (4H, m, H-4 and H-5), 1.72 (3H, s, H-9), 1.60 (3H, s, H-10); δ_{C} (CDCl₃, 125 MHz) 141.1 (C-3), 134.8 (C-7), 126.2 (C-6), 122.7 (C-2), 67.5 (C-1), 57.9 (C-8), 30.7 (C-5), 25.6 (C-4), 22.5 (C-9), 12.9 (C-10). HRMS: Found: 193.1198. C₁₀H₁₈O₂ (M+Na⁺) Calculated 193.1199.

(3S,6E)-3,7-Dimethyl-8-hydroxy-6-octen-1-ol ((S)-90)

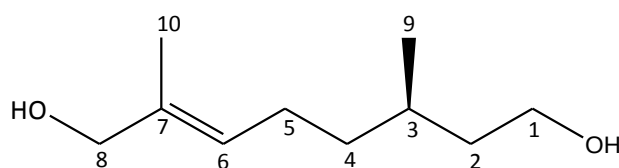
(8-Hydroxy-(S)-citronellol)



Following the general acetate deprotection procedure using 8-hydroxy-(*S*)-citronellyl acetate (**(*S*)-117**) (178 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-3,7-dimethyl-8-hydroxy-6-octen-1-ol (**(*S*)-90**) (86 mg, 60%) as a colourless oil: $[\alpha]_D^{20}$ -6.3 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3307, 2956, 2917, 2856, 1672, 1454, 1377, 1261, 1223, 1055, 1009, 847, 802; δ_{H} (CDCl₃, 500 MHz) 5.42 (1H, t, *J* 7.2, H-6), 3.92 (2H, s, H-8), 3.57-3.45 (2H, m, H-1), 2.09-1.97 (2H, m, H-5), 1.61 (3H, s, H-10), 1.50-1.36 (2H, m, H-2), 1.30-1.18 (2H, m, H-4), 1.16-1.05 (1H, m, H-3), 0.86 (3H, d, *J* 6.4, H-9); δ_{C} (CDCl₃, 125 MHz) 133.9 (C-7), 127.7 (C-6), 67.7 (C-8), 59.9 (C-1), 38.3 (C-4), 35.9 (C-3), 28.3 (C-2), 24.4 (C-5), 18.6 (C-9), 12.9 (C-10). HRMS: Found: 195.1349. C₁₀H₂₀O₂ (M+Na⁺) Calculated 195.1356.

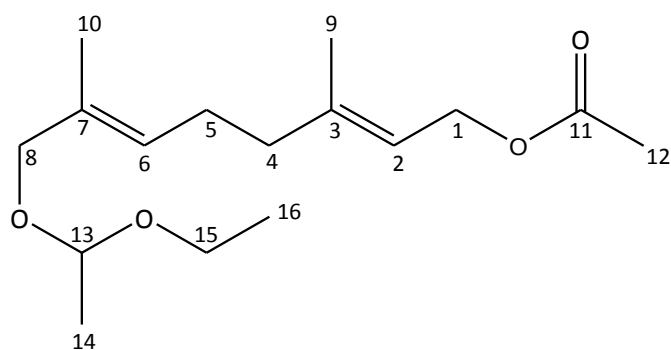
(3*R*,6*E*)-3,7-Dimethyl-8-hydroxy-6-octen-1-ol ((*R*)-90)

(8-Hydroxy-(*R*)-citronellol)



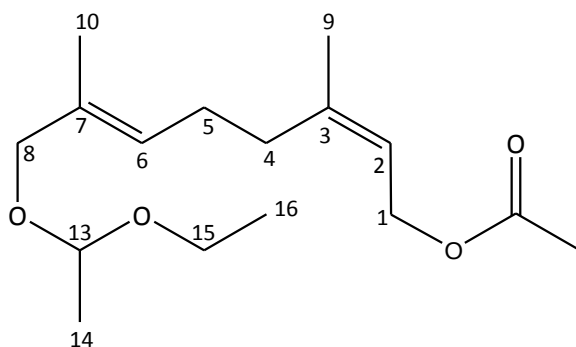
Following the general acetate deprotection procedure using 8-hydroxy-(*R*)-citronellyl acetate (**(*R*)-117**) (107 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3*R*,6*E*)-3,7-dimethyl-8-hydroxy-6-octen-1-ol (**(*R*)-90**) (48 mg, 56%) as a colourless oil: $[\alpha]_D^{20}$ +7.6 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3327, 2955, 2918, 2867, 1707, 1455, 1362, 1227, 1055, 1008, 848, 600, 531; δ_{H} (CDCl₃, 500 MHz) 5.39 (1H, t, *J* 7.2, H-6), 3.98 (2H, s, H-8), 3.71-3.50 (2H, m, H-1), 2.12-1.99 (2H, m, H-5), 1.66 (3H, s, H-10), 1.65-1.53 (2H, m, H-2), 1.45-1.33 (2H, m, H-4), 1.26-1.18 (1H, m, H-3), 0.90 (3H, d, *J* 6.4, H-9); δ_{C} (CDCl₃, 125 MHz) 134.6 (C-7), 126.3 (C-6), 68.9 (C-8), 60.9 (C-1), 39.7 (C-4), 36.7 (C-3), 29.0 (C-2), 25.0 (C-5), 19.5 (C-9), 13.7 (C-10). HRMS: Found: 195.1360. C₁₀H₂₀O₂ (M+Na⁺) Calculated 195.1356.

(2*E*,6*E*)-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (121**)**



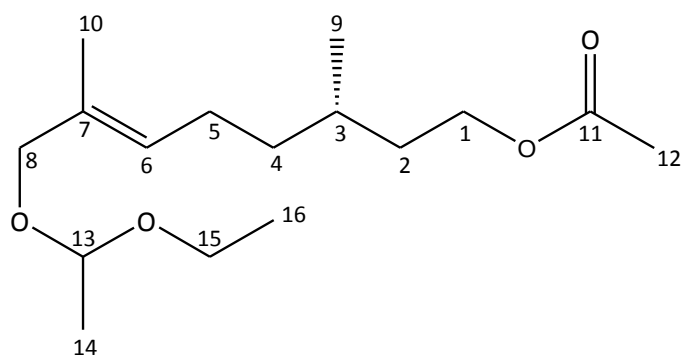
Following the general acetal protection procedure using 8-hydroxygeranyl acetate (**115**) (889 mg), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (**121**) (500 mg, 42%) as a pale yellow oil: ν_{max} (thin film)/ cm^{-1} 2978, 2921, 2860, 1736, 1671, 1444, 1381, 1366, 1229, 1130, 1085, 1019, 952, 852, 802, 608; δ_{H} (CDCl_3 , 500 MHz) 5.43 (1H, t, J 7.0, H-2), 5.38 (1H, t, J 6.9, H-6), 4.72 (1H, q, J 5.5, H-13), 4.60 (2H, d, J 7.0, H-1), 3.98 (1H, d, J 11.3, H-8a), 3.86 (1H, d, J 11.3, H-8b), 3.58 (1H, dq, J 9.3 and 7.0, H-15a), 3.51 (1H, dq, J 9.3 and 7.0, H-15b), 2.23-2.16 (2H, m, H-5), 2.12-2.08 (2H, m, H-4), 2.08 (3H, s, H-12), 1.72 (3H, s, H-9), 1.59 (3H, s, H-10), 1.34 (3H, d, J 5.5, H-14), 1.22 (3H, t, J 7.0, H-16); δ_{C} (CDCl_3 , 125 MHz) 171.5 (C-11), 141.1 (C-3), 132.4 (C-7), 127.6 (C-6), 119.8 (C-2), 98.6 (C-13), 71.8 (C-8), 62.6 (C-1), 59.8 (C-15), 39.3 (C-4), 25.8 (C-5), 22.2 (C-12), 20.01 (C-14), 16.5 (C-9), 15.5 (C-16), 13.7 (C-10). HRMS: Found: 307.1876. $\text{C}_{16}\text{H}_{28}\text{O}_4$ ($\text{M}+\text{Na}^+$) Calculated 307.1885.

(2Z,6E)-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (122)



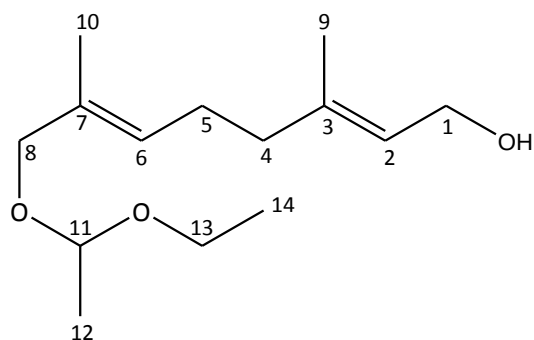
Following the general acetal protection procedure using 8-hydroxyneryl acetate (**116**) (897 mg), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (**122**) (673 mg, 56%) as a pale yellow oil: ν_{max} (thin film)/ cm^{-1} 2988, 2926, 281, 1736, 1670, 1443, 1381, 1369, 1230, 1130, 1086, 1019, 952, 8514, 802, 608; δ_{H} (CDCl_3 , 500 MHz) 5.43-5.37 (2H, m, H-2 and H-6), 4.76 (1H, q, J 5.5, H-13), 4.61 (2H, d, J 6.9, H-1), 3.99 (1H, d, J 11.3, H-8a), 3.88 (1H, d, J 11.3, H-8b), 3.63 (1H, dq, J 9.3 and 7.1, H-15a), 3.46 (1H, dq, J 9.3 and 7.1, H-15b), 2.20-2.14 (4H, m, H-4 and H-5), 2.05 (3H, s, H-12), 1.73 (3H, d, J 0.9, H-9), 1.61 (3H, s, H-10), 1.30 (3H, d, J 5.5, H-14), 1.19 (3H, t, J 7.1, H-16); δ_{C} (CDCl_3 , 125 MHz) 171.5 (C-11), 141.1 (C-3), 132.4 (C-7), 127.6 (C-6), 119.8 (C-2), 98.6 (C-13), 71.8 (C-8), 62.6 (C-1), 59.8 (C-15), 31.3 (C-4), 25.8 (C-5), 22.2 (C-12), 20.0 (C-14), 16.5 (C-9), 15.5 (C-16), 13.9 (C-10). HRMS: Found: 307.1891. $\text{C}_{16}\text{H}_{28}\text{O}_4$ ($\text{M}+\text{Na}^+$) Calculated 307.1885.

(3*S*,6*E*)-3,7-Dimethyl-8-(1-ethoxyethoxy)-6-octen-1-yl acetate ((*S*)-123)



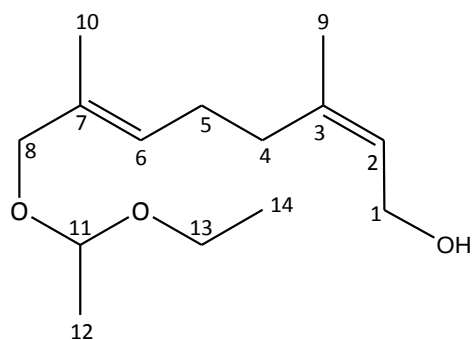
Following the general acetal protection procedure using 8-hydroxy-(*S*)-citronellyl acetate ((*S*)-117) (876 mg), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-yl acetate (**123**) (691 mg, 59%) as a pale yellow oil: $[\alpha]_D^{20}$ -3.2 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2974, 2929, 2862, 1741, 1671, 1444, 1381, 1360, 1230, 1131, 1087, 1019, 951, 852, 801, 608; δ_{H} (CDCl₃, 500 MHz) 5.42 (1H, t, *J* 7.0, H-6), 4.72 (1H, q, *J* 5.5, H-13), 4.16-4.07 (2H, m, H-1), 3.99 (1H, d, *J* 11.2, H-8a), 3.87 (1H, d, *J* 11.2, H-8b), 3.67 (1H, dq, *J* 9.2 and 7.1, H-15a), 3.51 (1H, dq, *J* 9.2 and 7.1, H-15b), 2.13-2.00 (2H, m, H-5), 2.06 (3H, s, H-12), 1.73-1.65 (1H, m, H-2a), 1.68 (3H, s, H-10), 1.61-1.54 (1H, m, H-3), 1.49-1.44 (1H, m, H-2b), 1.44-1.37 (1H, m, H-4a), 1.33 (3H, d, *J* 5.5, H-14), 1.28-1.20 (1H, m, H-4b), 1.22 (3H, t, *J* 7.1, H-16), 0.94 (3H, d, *J* 6.4, H-9); δ_{C} (CDCl₃, 125 MHz) 171.9 (C-11), 134.7 (C-7), 127.8 (C-6), 98.3 (C-13), 72.2 (C-8), 62.4 (C-1), 59.4 (C-15), 38.2 (C-4), 35.7 (C-3), 27.9 (C-2), 25.5 (C-5), 22.1 (C-12), 20.4 (C-14), 19.5 (C-9), 15.5 (C-16), 13.6 (C-10). HRMS: Found: 309.2029. C₁₆H₃₀O₄ (M+Na⁺) Calculated 309.2042.

(2*E*,6*E*)-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (124)



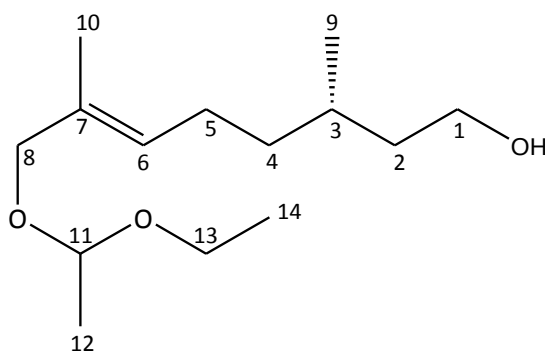
Following the general acetate deprotection procedure using (2*E*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (**121**) (504 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (**124**) (318 mg, 74%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3398, 2973, 2924, 2874, 1667, 1444, 1382, 1291, 1104, 1081, 1056, 1016, 844; δ_{H} (CDCl_3 , 500 MHz) 5.45-5.41 (2H, m, H-2 and H-6), 4.65 (1H, q, J 5.5, H-11), 4.11 (2H, d, J 7.1, H-1), 3.99 (1H, d, J 11.2, H-8a), 3.86 (1H, d, J 11.2, H-8b), 3.66 (1H, dq, J 9.5 and 7.1, H-13a), 3.51 (1H, dq, J 9.5 and 7.1, H-13b), 2.50 (1H, s, OH), 2.22-2.18 (2H, m, H-5), 2.10-2.03 (2H, m, H-4), 1.70 (6H, s, H-9 and H-10), 1.35 (3H, d, J 5.5, C-12), 1.22 (3H, t, J 7.1, C-14); δ_{C} (CDCl_3 , 125 MHz) 140.1 (C-3), 133.4 (C-7), 127.6 (C-6), 123.8 (C-2), 99.2 (C-11), 72.5 (C-8), 61.6 (C-13), 59.8 (C-1), 39.3 (C-4), 26.2 (C-5), 19.9 (C-12), 16.2 (C-9), 15.6 (C-14), 14.3 (C-10). HRMS: Found: 265.1789. $\text{C}_{14}\text{H}_{26}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 265.1780.

(2*Z*,6*E*)-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (125)



Following the general acetate deprotection procedure using (2Z,6E)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (**122**) (674 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (**125**) (425 mg, 74%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3395, 2974, 2933, 2874, 1667, 1446, 1382, 1228, 1105, 1079, 1057, 1016, 842; δ_{H} (CDCl_3 , 500 MHz) 5.46-5.40 (2H, m, H-2/6), 4.74 (1H, q, J 5.5, H-11), 4.10-4.08 (2H, m, H-1), 3.98 (1H, d, J 11.1, H-8a), 3.84 (1H, d, J 11.1, H-8b), 3.66 (1H, dq, J 9.4 and 7.1, H-13a), 3.51 (1H, dq, J 9.4 and 7.1, H-13b), 2.21-2.11 (4H, m, H-4 and H-5), 1.85 (1H, s, OH), 1.76 (3H, s, C-9), 1.66 (3H, s, C-10), 1.34 (3H, d, J 5.5, H-12), 1.22 (3H, t, J 7.1, C-14); δ_{C} (CDCl_3 , 125 MHz) 138.1 (C-3), 133.4 (C-7), 127.4 (C-6), 125.8 (C-2), 99.2 (C-11), 71.5 (C-8), 60.6 (C-13), 59.6 (C-1), 32.3 (C-4), 26.2 (C-5), 23.5 (C-9), 19.9 (C-12), 15.6 (C-14), 14.5 (C-10) HRMS: Found: 265.1764. $\text{C}_{14}\text{H}_{26}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 265.1780.

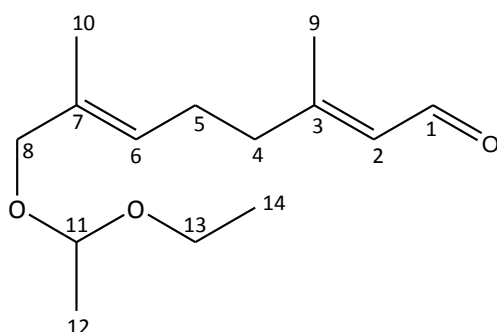
(3S,6E)-3,7-Dimethyl-8-(1-ethoxyethoxy)-6-octen-1-ol ((S)-126)



Following the general acetate deprotection procedure using (3S,6E)-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-yl acetate (**(S)-123**) (274 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3S,6E)-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-ol (**(S)-126**) (213 mg, 91%) as a pale yellow oil: $[\alpha]_D^{20}$ -2.8 (c 1.0, MeOH); ν_{max} (thin film)/ cm^{-1} 3400, 2961, 2930, 2874, 1664, 1455, 1381, 1345, 1228, 1105, 1056, 1009, 956, 851; δ_{H} (CDCl_3 , 500 MHz) 5.42 (1H, t, J 7.1, H-6), 4.72 (1H, q, J 5.5, H-11), 3.98 (1H, d, J 11.0, H-8a), 3.88 (1H, d, J 11.0, H-8b), 3.75-3.64 (2H, m, H-1),

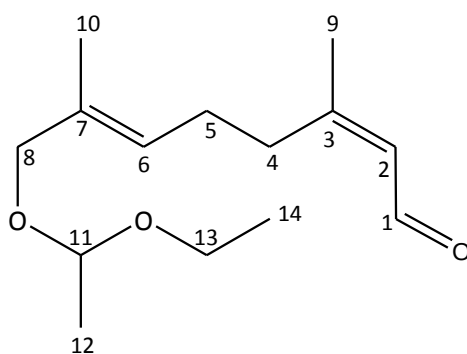
3.53-3.48 (2H, m, H-13), 2.13-2.00 (2H, m, H-5), 1.72-1.54 (2H, m, H-2), 1.68 (3H, s, H-10), 1.45-1.35 (2H, m, H-3 and H-4a), 1.33 (3H, d, J 5.5, H-12); 1.29-1.20 (1H, m, H-4b), 1.23 (3H, t, J 7.0, H-14), 0.93 (3H, d, J 6.4, H-9); δ_c (CDCl₃, 125 MHz) 134.4 (C-7), 126.6 (C-6), 97.6 (C-11), 73.8 (C-8), 62.6 (C-1), 58.8 (C-13), 38.3 (C-4), 35.9 (C-3), 28.1 (C-2), 25.2 (C-5), 20.6 (C-12), 16.5 (C-9), 15.8 (C-14), 12.6 (C-10) HRMS: Found: 267.1944. C₁₄H₂₆O₃ (M+Na⁺) Calculated 267.1936.

(2*E*,6*E*)-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (127)



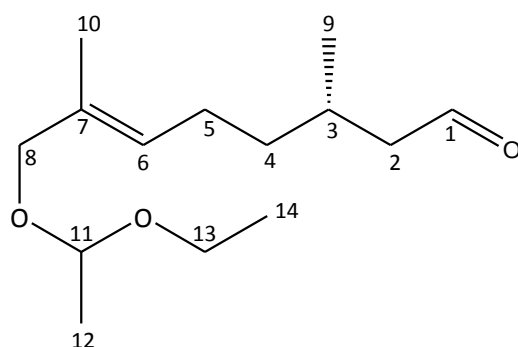
Following the general IBX oxidation procedure using (2*E*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (**124**) (317 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (**127**) (267 mg, 85%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2973, 2926, 2874, 1745, 1667, 1444, 1380, 1296, 1104, 1085, 1055, 1016, 843; δ_H (CDCl₃, 500 MHz) 10.00 (1H, d, J 8.0, H-1), 5.89-5.87 (1H, m, H-2), 5.40 (1H, t, J 7.9, H-6), 4.71 (1H, q, J 5.5, H-11), 3.98 (1H, d, J 11.4, H-8a), 3.87 (1H, d, J 11.4, H-8b), 3.66 (1H, dq, J 9.3 and 7.0, H-13a), 3.50 (1H, dq, J 9.3 and 7.0, H-13b), 2.32-2.27 (4H, m, H-4 and H-5), 2.30 (3H, s, H-9), 1.68 (3H, s, H-10), 1.32 (3H, d, J 5.5, H-12), 1.22 (3H, t, J 7.0, H-14); δ_c (CDCl₃, 125 MHz) 191.8 (C-1), 162.1 (C-3), 133.4 (C-7), 127.6 (C-2), 126.8 (C-6), 99.1 (C-11), 70.8 (C-8), 61.0 (C-13), 40.3 (C-4), 25.4 (C-5), 19.7 (C-12), 17.2 (C-9), 15.2 (C-14), 13.9 (C-10). HRMS: Found: 263.1602. C₁₄H₂₄O₃ (M+Na⁺) Calculated 263.1623.

(2Z,6E)-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (128)



Following the general IBX oxidation procedure using (2Z,6E)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (**125**) (423 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (**128**) (403 mg, 96%) as a colourless oil: $\nu_{\max}$ (thin film)/ cm^{-1} 2978, 2935, 2870, 1745, 1667, 14414, 1380, 1296, 1102, 1085, 1052, 1013, 844; δ_{H} (CDCl_3 , 500 MHz) 9.95 (1H, d, J 7.0, H-1), 5.89 (1H, d, J 7.0, H-2), 5.44 (1H, t, J 7.4, H-6), 4.71 (1H, q, J 5.5, C-11), 3.98 (1H, d, J 11.5, H-8a), 3.86 (1H, d, J 11.5, H-8b), 3.66-3.63 (1H, m, H-13a), 3.54-3.51 (1H, m, H-13b), 2.34-2.28 (2H, m, H-4), 2.28-2.25 (2H, m, H-5), 2.01 (3H, s, H-9), 1.68 (3H, s, H-10), 1.34 (3H, d, J 5.5, H-12), 1.22 (3H, t, J 7.0, H-14); δ_{C} (CDCl_3 , 125 MHz) 190.8 (C-1), 162.8 (C-3), 133.4 (C-7), 127.9 (C-2), 126.1 (C-6), 99.1 (C-11), 70.6 (C-8), 61.0 (C-13), 33.3 (C-4), 26.4 (C-5), 25.2 (C-9), 19.7 (C-12), 15.6 (C-14), 13.7 (C-10). HRMS: Found: 263.1641. $\text{C}_{14}\text{H}_{24}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 263.1623.

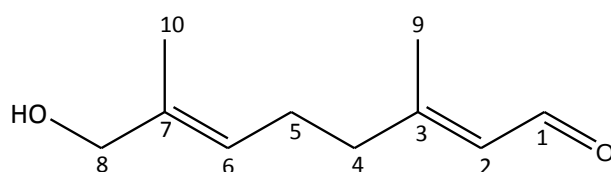
(3*S*,6*E*)-3,7-Dimethyl-8-(1-ethoxyethoxy)-6-octen-1-al ((*S*)-129)



Following the general IBX oxidation procedure using (3*S*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-ol ((*S*)-126) (214 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-al ((*S*)-129) (180 mg, 85%) as a pale yellow oil: $[\alpha]_D^{20}$ -2.1 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2981, 2928, 2870, 17445, 1667, 1443, 1380, 1292, 1104, 1088, 1053, 1016, 844; δ_{H} (CDCl₃, 500 MHz) 9.78 (1H, m, H-1), 5.42 (1H, t, *J* 7.4, H-6), 4.73 (1H, q, *J* 5.5, H-11), 3.99 (1H, d, *J* 11.2, H-8a), 3.86 (1H, d, *J* 11.2, H-8b), 3.66 (1H, dq, *J* 9.1 and 7.0, H-13a), 3.50 (1H, dq, *J* 9.1 and 7.0, H-13b), 2.46-2.40 (1H, m, H-2a), 2.30-2.26 (1H, m, H-2b), 2.13-2.00 (2H, m, H-5), 1.70 (3H, s, H-10), 1.45-1.38 (2H, m, H-3 and H-4a), 1.34 (3H, d, *J* 5.5, H-12); 1.31-1.27 (1H, m, H-4b), 1.23 (3H, t, *J* 7.0, H-14), 0.99 (3H, d, *J* 6.2, H-9); δ_{C} (CDCl₃, 125 MHz) 191.5 (C-1), 135.6 (C-7), 127.5 (C-6), 94.6 (C-11), 74.2 (C-8), 58.5 (C-13), 38.2 (C-4), 35.4 (C-3), 29.1 (C-2), 25.8 (C-5), 20.8 (C-12), 16.5 (C-9), 15.9 (C-14), 12.3 (C-10) HRMS: Found: 265.1799. C₁₆H₃₀O₄ (M+Na⁺) Calculated 265.1780.

(2*E*,6*E*)-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-al (73)

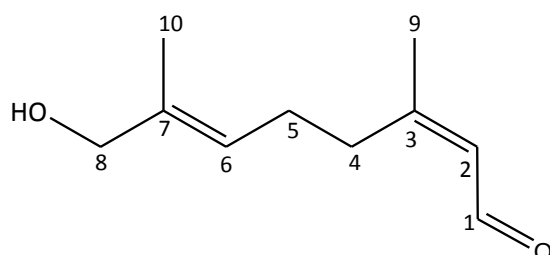
(8-Hydroxygeranial)



Following the general acetal deprotection procedure using (2*E*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (**127**) (335 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-3,7-dimethyl-8-hydroxy-2,6-octadien-1-al (**73**) (115 mg, 49%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3421, 2969, 2933, 2873, 1716, 1667, 1443, 1379, 1141, 1015, 852; δ_{H} (CDCl₃, 500 MHz) 10.01 (1H, d, *J* 8.2, H-1), 5.90 (1H, d, *J* 8.2, H-2), 4.39 (1H, t, *J* 8.2, H-6), 4.01 (2H, s, H-8), 2.31-2.30 (4H, m, H-4 and H-5), 2.00 (3H, s, H-9), 1.69 (3H, s, H-10); δ_{C} (CDCl₃, 125 MHz) 191.4 (C-1), 163.5 (C-3), 136.2 (C-7), 127.5 (C-2), 123.8 (C-6), 68.5 (C-8), 40.2 (C-4), 25.2 (C-5), 17.6 (C-9), 13.7 (C-10). HRMS: Found: 191.1050. C₁₀H₁₆O₂ (M+Na⁺) Calculated 191.1043.

(2*Z*,6*E*)-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-al (130)

(8-Hydroxyneral)



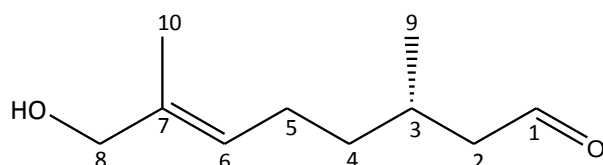
Following the general acetal deprotection procedure using (2*Z*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (**128**) (420 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*Z*,6*E*)-3,7-dimethyl-8-hydroxy-2,6-octadien-1-al (**130**) (156 mg, 53%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3431, 2971, 2930, 2872, 1710, 1668, 1440, 1376, 1221, 1157, 1125, 1445, 852, 530; δ_{H} (CDCl₃, 500 MHz) 9.88 (1H, d, *J* 8.2, H-1), 5.89 (1H, d, *J* 8.2, H-2), 5.40 (1H, t, *J* 8.2, H-6), 3.99 (2H, s, H-8), 2.63 (2H, t, *J* 7.3, H-4), 2.32-2.27 (2H, m, H-5), 2.01 (3H, s, H-9), 1.66 (3H, s, H-10); δ_{C} (CDCl₃, 125 MHz) 191.5 (C-1), 163.6 (C-3), 137.3 (C-7), 129.0 (C-2), 124.0

(C-6), 68.5 (C-8), 32.2 (C-4), 26.5 (C-5), 25.1 (C-9), 13.7 (C-10). HRMS: Found: 191.1049.

C₁₀H₁₆O₂ (M+Na⁺) Calculated 191.1043.

(3*S*,6*E*)-3,7-Dimethyl-8-hydroxy-6-octen-1-al ((*S*)-131)

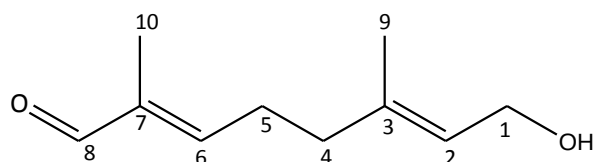
(8-Hydroxy-(*S*)-citronellal)



Following the general acetal deprotection procedure using (3*S*,6*E*)-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-al ((*S*)-129) (194 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-3,7-dimethyl-8-hydroxy-6-octen-1-al ((*S*)-131) (79 mg, 58%) as a pale yellow oil: $[\alpha]_D^{20}$ -6.6 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3393, 2928, 2933, 2872, 1710, 1457, 1379, 1170, 1042, 1009, 789; δ_{H} (CDCl₃, 500 MHz) 9.75 (1H, m, H-1), 5.49 (1H, t, *J* 6.9, H-6), 4.00 (2H, s, H-8), 2.43 (1H, m, H-2a), 2.36 (1H, m, H-2b), 2.13-2.01 (3H, m, H-3 and H-5), 1.66 (3H, s, H-10), 1.46-1.37 (1H, m, H-4a), 1.36-1.28 (1H, m, H-4b), 0.99 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 203.0 (C-1), 135.2 (C-7), 125.5 (C-6), 68.8 (C-8), 51.0 (C-2), 36.6 (C-4), 27.7 (C-5), 21.1 (C-3), 19.8 (C-9), 13.8 (C-10). HRMS: Found: 193.1199. C₁₀H₁₈O₂ (M+Na⁺) Calculated 193.1197.

(2*E*,6*E*)-3,7-Dimethyl-8-oxo-2,6-octadien-1-ol (74)

(8-Oxogeraniol)

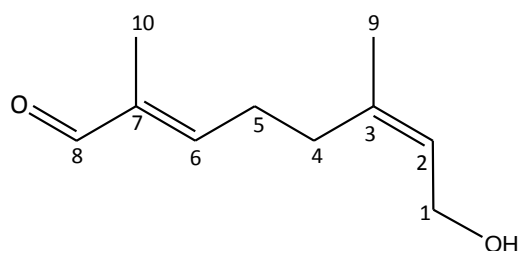


Following the general acetate deprotection procedure using 8-oxogeranyl acetate (**118**) (228 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether

40-60 °C) to give (2*E*,6*E*)-3,7-dimethyl-8-oxo-2,6-octadien-1-ol (**74**) (184 mg, 95%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 3327, 2958, 2858, 1709, 1681, 1462, 1389, 1266, 1055, 800; δ_{H} (CDCl₃, 500 MHz) 9.36 (1H, s, H-8), 6.51 (1H, t, *J* 7.1, H-6), 5.44 (1H, t, *J* 7.0, H-2), 4.20 (2H, d, *J* 7.0, H-1), 2.53 (2H, td, *J* 7.1 and 1.3, H-5), 2.21 (2H, t, *J* 7.1, H-4), 1.75 (3H, s, H-10), 1.71 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 195.4 (C-8), 154.1 (C-7), 140.2 (C-3), 137.2 (C-6), 124.8 (C-2), 57.6 (C-1), 37.3 (C-5), 27.2 (C-4), 16.2 (C-10), 9.4 (C-9). HRMS: Found: 191.1050. C₁₀H₁₆O₂ (M+Na⁺) Calculated 191.1043.

(2*Z*,6*E*)-3,7-Dimethyl-8-oxo-2,6-octadien-1-ol (132)

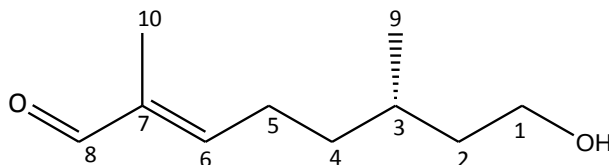
(8-Oxonerol)



Following the general acetate deprotection procedure using 8-oxoneryl acetate (**119**) (177 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*Z*,6*E*)-3,7-dimethyl-8-oxo-2,6-octadien-1-ol (**132**) (132 mg, 88%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 3338, 2955, 2856, 1704, 1682, 1463, 1389, 1262, 1055, 804; δ_{H} (CDCl₃, 500 MHz) 9.39 (1H, s, H-8), 6.51 (1H, t, *J* 7.1, H-6), 5.46 (1H, t, *J* 7.0, H-2), 4.16 (2H, d, *J* 7.0, H-1), 2.50 (2H, td, *J* 7.1 and 1.4, H-5), 2.25 (2H, t, *J* 7.1, H-4), 1.75 (3H, s, H-10), 1.73 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 195.6 (C-8), 154.1 (C-7), 140.1 (C-3), 137.5 (C-6), 124.9 (C-2), 58.6 (C-1), 31.3 (C-5), 27.2 (C-4), 23.2 (C-10), 9.4 (C-9). HRMS: Found: 191.1049. C₁₀H₁₆O₂ (M+Na⁺) Calculated 191.1043.

(3*S*,6*E*)-3,7-Dimethyl-8-oxo-6-octen-1-ol ((*S*)-133)

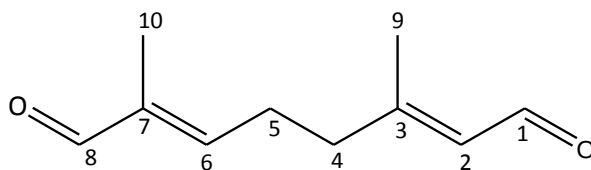
(8-Oxo-(*S*)-citronellol)



Following the general acetate deprotection procedure using 8-oxo-(*S*)-citronellyl acetate ((*S*)-120) (164 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-3,7-dimethyl-8-oxo-6-octen-1-ol ((*S*)-133) (127 mg, 91%) as a pale yellow oil: $[\alpha]_D^{20}$ -7.1 (*c* 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3387, 2958, 2926, 2870, 1670, 1636, 1464, 1379, 1256, 806; δ_{H} (CDCl₃, 500 MHz) 9.37 (1H, s, H-8), 6.55 (1H, t, *J* 6.9, H-6), 3.73-3.68 (2H, m, H-1), 2.40-2.32 (1H, m, H-3), 1.75 (3H, s, H-10), 1.65-0.96 (6H, m, H-2, H-4 and H-5), 0.99 (3H, d, *J* 6.5, H-9); δ_{C} (CDCl₃, 125 MHz) 195.6 (C-8), 155.5 (C-7), 140.3 (C-6), 60.2 (C-1), 39.4 (C-4), 35.3 (C-2), 29.5 (C-3), 26.2 (C-5), 19.8 (C-10), 9.6 (C-9). HRMS: Found: 193.1199. C₁₀H₁₈O₂ (M+Na⁺) Calculated 193.1197.

(2*E*,6*E*)-3,7-Dimethyl-8-oxo-2,6-octadien-1-al (75)

(8-Oxogeranial)

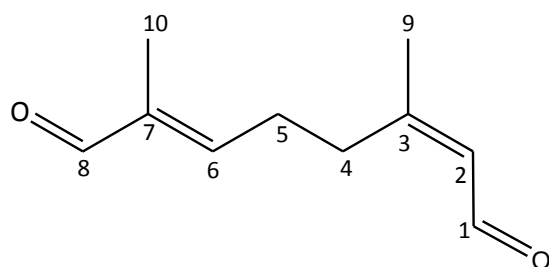


Following the general IBX oxidation procedure using 8-oxogeraniol (**74**) (161 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-3,7-dimethyl-8-oxo-2,6-octadien-1-al (**75**) (134 mg, 84%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2925, 2855, 2717, 1759, 1667, 1440, 1406, 1382, 1360, 1239, 1193, 1030, 1005, 822; δ_{H} (CDCl₃, 500 MHz) 10.03 (1H, d, *J* 7.6, H-1), 9.45 (1H, s, H-8), 6.44

(1H, td, *J* 7.2 and 1.3, H-6), 5.94 (1H, dq, *J* 7.6 and 1.1, H-2), 2.58 (2H, td, *J* 7.2 and 7.2, H-5), 2.48 (2H, t, *J* 7.2, H-4), 2.21 (3H, d, *J* 1.3, H-9), 1.81 (3H, d, *J* 1.6, H-10); δ_c (CDCl₃, 125 MHz) 195.1 (C-8), 191.4 (C-1), 161.1 (C-3), 152.4 (C-6), 140.6 (C-7), 127.7 (C-2), 38.3 (C-4), 26.2 (C-5), 17.6 (C-9), 9.4 (C-10). HRMS: Found: 189.0874. C₁₀H₁₆O₂ (M+Na⁺) Calculated 189.0892.

(2Z,6E)-3,7-Dimethyl-8-oxo-2,6-octadien-1-al (134**)**

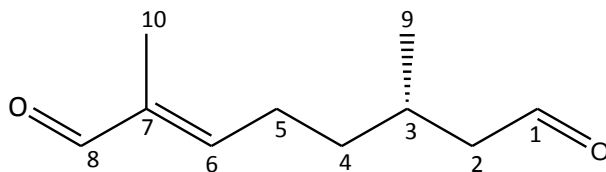
(8-Oxoneral)



Following the general IBX oxidation procedure using 8-oxonerol (**132**) (100 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-3,7-dimethyl-8-oxo-2,6-octadien-1-al (**134**) (85 mg, 86%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2924, 2855, 2763, 1759, 1671, 1443, 1400, 1378, 1246, 1201, 1158, 1109, 1038, 856; δ_H (CDCl₃, 500 MHz) 9.87 (1H, d, *J* 7.6, H-1), 9.35 (1H, s, H-8), 6.44-6.40 (1H, m, H-6), 5.90 (1H, d, *J* 7.6, H-2), 2.70-2.67 (2H, m, H-5), 2.56-2.51 (2H, m, H-4), 1.94 (3H, s, H-9), 1.73 (3H, s, H-10); δ_c (CDCl₃, 125 MHz) 195.0 (C-8), 190.4 (C-1), 161.3 (C-3), 150.4 (C-6), 140.6 (C-7), 128.7 (C-2), 31.3 (C-4), 27.2 (C-5), 23.9 (C-9), 9.4 (C-10). HRMS: Found: 189.0905. C₁₀H₁₆O₂ (M+Na⁺) Calculated 189.0892.

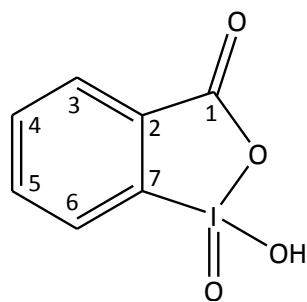
(3*S*,6*E*)-3,7-Dimethyl-8-oxo-6-octen-1-al ((S**)-135)**

(8-Oxo-(*S*)-citronellal)



Following the general IBX oxidation procedure using 8-oxo-(*S*)-citronellol ((**S**)-133) (92 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-3,7-dimethyl-8-oxo-6-octen-1-al ((**S**)-135) (79 mg, 87%) as a pale yellow oil: $[\alpha]_D^{20}$ -9.2 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2959, 2928, 2829, 1721, 1667, 1637, 1460, 1380, 1253, 1050, 967, 875; δ_{H} (CDCl₃, 500 MHz) 9.82 (1H, t, *J* 2.2, H-1), 9.44 (1H, s, H-8), 6.52 (1H, td, *J* 7.0 and 1.4, H-6), 2.51-2.37 (4H, m, H-2 and H-5), 2.17-2.11 (1H, m, H-3), 1.74 (3H, d, *J* 1.0, H-10), 1.65-1.54 (1H, m, H-4a), 1.50-1.38 (1H, m, H-4b), 1.00 (3H, d, *J* 7.0, H-9); δ_{C} (CDCl₃, 125 MHz) 202.7 (C-1), 196.1 (C-8), 154.4 (C-6), 140.3 (C-7), 51.3 (C-2), 35.9 (C-4), 28.4 (C-3), 27.0 (C-5), 20.5 (C-9), 9.8 (C-10). HRMS: Found: 191.1058. C₁₀H₁₈O₂ (M+Na⁺) Calculated 191.1048.

Iodoxybenzoic acid (IBX)



To a solution of potassium peroxymonosulfate (Oxone®) (37.2 g) in distilled water (200 mL) was added 2-iodobenzoic acid (5 g). The reaction mixture was stirred at 70 °C for 3 hours before cooling to 0 °C and continuing to stir for another 2 hours. The precipitate was collected *via* filtration and the solid rinsed with cold distilled water (200 mL) and cold acetone (200 mL). The solid was dried for 48 hours in a vacuum

desiccator, giving IBX (4.81 g, 85%) as a white powder. δ_{H} ($^2\text{H}_6$ -DMSO, 500 MHz) 8.14 (1H, d, H-3/6), 8.04 (1H, d, H-3/6), 8.01 (1H, t, H-4/5), 7.84 (1H, t, H-4/5).

Manganese Dioxide

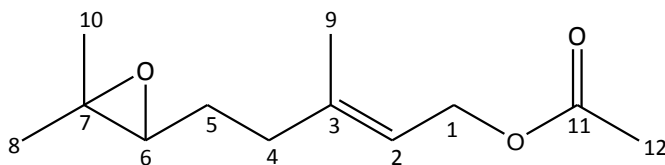


To a solution of pentahydrate manganese sulfate (30 g, 1 eq.) in distilled water (900 mL) was slowly added a solution of potassium permanganate (29.7 g, 1.4 eq.) in distilled water (900 mL) and the reaction mixture stirred for 24 hours at room temperature. The precipitate was collected by vacuum distillation and the solid washed with distilled water until there was minimal colour. The powder was vacuum desiccated for 48 hours.

7.3.16 Chapter 3 Synthesis

(2E)-3,7-Dimethyl-6,7-epoxy-2-octen-1-yl acetate (**143**)

(6,7-Epoxygeranyl acetate)

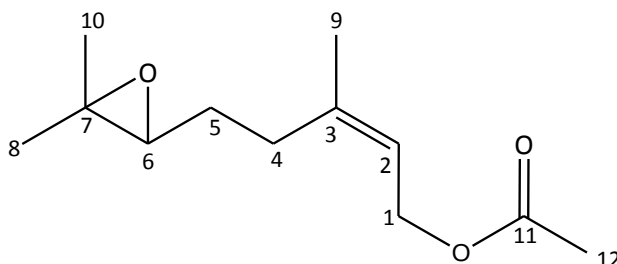


Following the general epoxidation procedure using geranyl acetate (**112**) (4.43 g), the crude product was purified on silica gel (25% EtOAc in petroleum ether 40-60 °C) to give (2E)-3,7-dimethyl-6,7-epoxy-2-octen-1-yl acetate (**143**) (3.74 g, 78%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 2959, 2930, 2857, 1740, 1671, 1590, 1472, 1462, 1378, 1245, 1189, 1110, 1047, 863, 822, 739, 700, 688, 612, 503, 488; δ_{H} (CDCl_3 , 500 MHz) 5.42 (1H, tq, *J* 7.0 and 1.2, H-2), 4.62 (2H, d, *J* 7.0, H-1), 2.78 (1H, t, *J* 6.4, H-6), 2.31-2.15 (2H, m, H-4), 2.04 (3H, s, H-12), 1.77 (3H, s, H-9), 1.74-1.64 (2H, m, H-5), 1.31 (3H, s, H-8/10), 1.28 (3H, s, H-8/10); δ_{C} (CDCl_3 , 125 MHz) 172.8 (C-11), 142.4 (C-3), 120.5 (C-2), 65.6 (C-6),

62.2 (C-1), 60.3 (C-7), 37.2 (C-4), 28.1 (C-5), 25.0 (C-8/10), 20.9 (C-12), 18.9 (C-8/10), 16.4 (C-9). HRMS: Found: 235.1310. $C_{12}H_{20}O_3$ ($M+Na^+$) Calculated 235.1306.

(2Z)-3,7-Dimethyl-6,7-epoxy-2-octen-1-yl acetate (144**)**

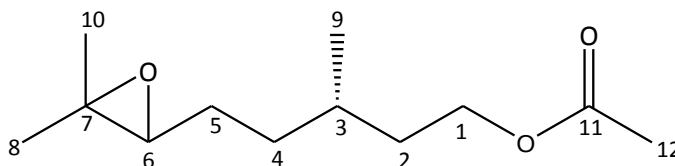
(6,7-Epoxyneryl acetate)



Following the general epoxidation procedure using neryl acetate (**113**) (4.68 g), the crude product was purified on silica gel (25% EtOAc in petroleum ether 40-60 °C) to give (2Z)-3,7-dimethyl-6,7-epoxy-2-octen-1-yl acetate (**143**) (3.85 g, 76%) as a colourless oil: $\nu_{\max}$ (thin film)/ cm^{-1} 2964, 2929, 2854, 1741, 1678, 1594, 1472, 1460, 1375, 1245, 1188, 1110, 1048, 864, 740, 700, 688, 611, 504, 486; δ_{H} (CDCl_3 , 500 MHz) 5.42 (1H, t, J 7.3, H-2), 4.60 (2H, d, J 7.3, H-1), 2.73 (1H, t, J 6.3, H-6), 2.37-2.22 (2H, m, H-4), 2.20 (3H, s, H-12), 1.81 (3H, s, H-9), 1.74-1.58 (2H, m, H-5), 1.33 (3H, s, H-8/10), 1.29 (3H, s, H-8/10); δ_{C} (CDCl_3 , 125 MHz) 173.6 (C-11), 142.1 (C-3), 119.4 (C-2), 66.6 (C-6), 61.0 (C-1), 60.3 (C-7), 37.0 (C-4), 28.4 (C-5), 25.1 (C-8/10), 20.9 (C-12), 18.3 (C-8/10), 16.3 (C-9). HRMS: Found: 235.1296. $C_{12}H_{20}O_3$ ($M+Na^+$) Calculated 235.1306.

(3S)-3,7-Dimethyl-6,7-epoxyoctan-1-yl acetate ((S)-145**)**

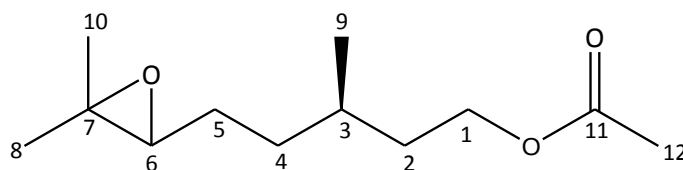
(6,7-Epoxy-(S)-citronellyl acetate)



Following the general epoxidation procedure using (*S*)-citronellyl acetate (**(*S*)-114**) (5.65 g), the crude product was purified on silica gel (25% EtOAc in petroleum ether 40-60 °C) to give (3*S*)-3,7-dimethyl-6,7-epoxyoctan-1-yl acetate (**(*S*)-145**) (4.21 g, 69%) as a colourless oil: $[\alpha]_D^{20}$ -4.9 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3406, 2957, 2929, 2870, 1720, 1571, 1461, 1428, 1381, 1255, 1160, 1119, 1071, 1041, 903, 751, 736; δ_{H} (CDCl₃, 500 MHz) 4.19-4.07 (2H, m, H-1), 2.73 (1H, t, *J* 6.1, H-6), 2.07 (3H, s, H-12), 1.76-1.37 (6H, m, H-2, H-3, H-4 and H-5), 1.33 (3H, s, H-8/10), 1.30 (3H, s, H-8/10), 0.96 (3H, d, *J* 6.7, H-9); δ_{C} (CDCl₃, 125 MHz) 171.5 (C-11), 78.9 (C-7), 77.9 (C-6), 67.5 (C-1), 63.0 (C-2), 60.1 (C-3), 57.7 (C-8), 30.7 (C-5), 25.6 (C-4), 22.5 (C-9), 21.2 (C-12), 12.9 (C-10). HRMS: Found: 237.1470. C₁₂H₂₂O₃ (M+Na⁺) Calculated 237.1461.

(3*R*)-3,7-Dimethyl-6,7-epoxyoctan-1-yl acetate ((*R*)-145**)**

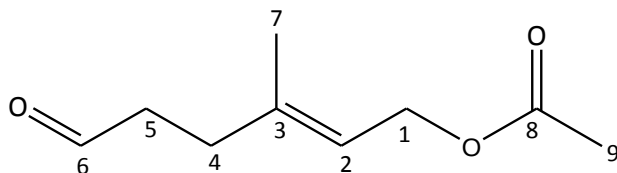
(6,7-Epoxy-(*R*)-citronellyl acetate)



Following the general epoxidation procedure using (*R*)-citronellyl acetate (**(*R*)-114**) (1.76 g), the crude product was purified on silica gel (25% EtOAc in petroleum ether 40-60 °C) to give (3*R*)-3,7-dimethyl-6,7-epoxyoctan-1-yl acetate (**(*R*)-145**) (1.35 g, 71%) as a colourless oil: $[\alpha]_D^{20}$ +3.3 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3401, 2956, 2929, 2873, 1720, 1575, 1462, 1429, 1381, 1251, 1160, 1120, 1071, 1039, 901, 751, 732; δ_{H} (CDCl₃, 500 MHz) 4.17-4.09 (2H, m, H-1), 2.72 (1H, t, *J* 6.2, H-6), 2.08 (3H, s, H-12), 1.73-1.40 (6H, m, H-2, H-3, H-4 and H-5), 1.33 (3H, s, H-8/10), 1.290 (3H, s, H-8/10), 0.95 (3H, d, *J* 6.7, H-9); δ_{C} (CDCl₃, 125 MHz) 171.4 (C-11), 79.0 (C-7), 77.9 (C-6), 67.3 (C-1), 63.1 (C-2), 60.0 (C-3), 57.7 (C-8), 30.7 (C-5), 25.5 (C-4), 22.5 (C-9), 21.0 (C-12), 13.0 (C-10). HRMS: Found: 237.1470. C₁₂H₂₂O₃ (M+Na⁺) Calculated 237.1461.

(2E)-3-Methyl-6-oxohex-2-en-1-yl acetate (146)

(6-Oxogeranyl acetate)

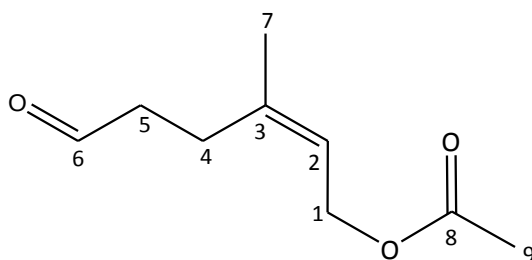


Following the general epoxide cleavage procedure using 6,7-epoxygeranyl acetate (**143**)

(6.53 g), the crude product was purified on silica gel (20% EtOAc in petroleum ether 40-60 °C) to give (2E)-3-methyl-6-oxohex-2-en-1-yl acetate (**146**) (5.18 g, 99%) as a colourless oil: $\nu_{\max}$ (thin film)/ cm^{-1} 2939, 1727, 1673, 1444, 1368, 1228, 1022, 954, 916, 733, 644, 608; δ_{H} (CDCl_3 , 500 MHz) 9.81 (1H, t, J 1.6, H-6), 5.38 (1H, t, J 7.0, H-2), 4.60 (2H, d, J 7.0, H-1), 2.60 (2H, td, J 7.2 and 1.6, H-5), 2.39 (2H, t, J 7.2, H-4), 2.07 (3H, s, H-9), 1.74 (3H, s, H-7); δ_{C} (CDCl_3 , 125 MHz) 201.8 (C-6), 171.1 (C-8), 140.1 (C-3), 119.4 (C-2), 61.1 (C-1), 41.8 (C-4), 31.5 (C-5), 21.1 (C-9), 16.6 (C-7). HRMS: Found: 193.0841. $\text{C}_9\text{H}_{14}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 193.0835.

(2Z)-3-Methyl-6-oxohex-2-en-1-yl acetate (147)

(6-Oxoneryl acetate)



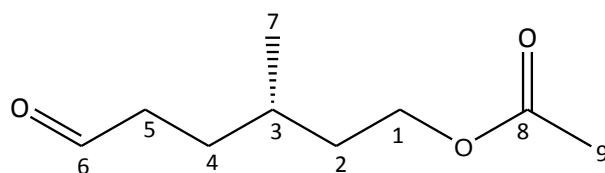
Following the general epoxide cleavage procedure using 6,7-epoxyneryl acetate (**144**)

(6.12 g), the crude product was purified on silica gel (20% EtOAc in petroleum ether 40-60 °C) to give (2Z)-3-methyl-6-oxohex-2-en-1-yl acetate (**147**) (4.27 g, 87%) as a colourless oil: $\nu_{\max}$ (thin film)/ cm^{-1} 2973, 1731, 1673, 1445, 1378, 1229, 1022, 954, 917,

733, 646, 608; δ_{H} (CDCl_3 , 500 MHz) 9.82 (1H, t, J 1.3, H-6), 5.43 (1H, t, J 7.2, H-2), 4.60 (2H, d, J 7.2, H-1), 2.57 (2H, t, J 7.5, H-5), 2.47 (2H, t, J 7.5, H-4), 2.07 (3H, s, H-9), 1.78 (3H, s, H-7); δ_{C} (CDCl_3 , 125 MHz) 201.4 (C-6), 171.4 (C-8), 140.5 (C-3), 120.7 (C-2), 60.7 (C-1), 42.1 (C-4), 24.5 (C-5), 23.1 (C-9), 21.2 (C-7). HRMS: Found: 193.0843. $\text{C}_9\text{H}_{14}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 193.0835.

(3S)-3-Methyl-6-oxohexan-1-yl acetate ((S)-148)

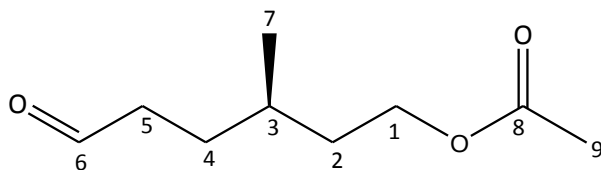
(6-Oxo-(S)-citronellyl acetate)



Following the general epoxide cleavage procedure using 6,7-epoxy-(S)-citronellyl acetate ((S)-145) (7.80 g), the crude product was purified on silica gel (20% EtOAc in petroleum ether 40-60 °C) to give (3S)-3-methyl-6-oxohexan-1-yl acetate ((S)-148) (4.70 g, 75%) as a colourless oil: $[\alpha]_{\text{D}}^{20}$ -5.2 (c 1.0, MeOH); ν_{max} (thin film)/ cm^{-1} 2960, 2932, 1732, 1432, 1386, 1367, 1231, 1173, 1050, 1033, 959, 643, 607; δ_{H} (CDCl_3 , 500 MHz) 9.81 (1H, t, J 1.8, H-6), 4.20-4.06 (2H, m, H-1), 2.58-2.38 (2H, m, H-5), 2.07 (3H, s, H-9), 1.78-1.67 (2H, m, H-2/4), 1.66-1.57 (1H, m, H-3), 1.56-1.44 (2H, m, H-2/4), 0.96 (3H, d, J 6.5, H-7); δ_{C} (CDCl_3 , 125 MHz) 204.5 (C-6), 173.0 (C-8), 64.0 (C-1), 32.9 (C-4), 31.4 (C-5), 27.6 (C-7), 26.2 (C-9). HRMS: Found: 195.0113. $\text{C}_9\text{H}_{16}\text{O}_3$ ($\text{M}+\text{Na}^+$) Calculated 195.0997.

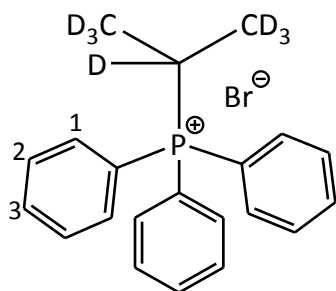
(3*R*)-3-Methyl-6-oxohexan-1-yl acetate ((*R*)-148)

(6-Oxo-(*R*)-citronellyl acetate)



Following the general epoxide cleavage procedure using 6,7-epoxy-(*R*)-citronellyl acetate ((*R*)-145) (1.52 g), the crude product was purified on silica gel (20% EtOAc in petroleum ether 40-60 °C) to give (3*R*)-3-methyl-6-oxohexan-1-yl acetate ((*R*)-148) (989 mg, 81%) as a colourless oil: $[\alpha]_D^{20} +4.7$ (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2961, 2931, 1730, 1431, 1386, 1367, 1230, 1173, 1052, 1033, 956, 644, 607; δ_H (CDCl₃, 500 MHz) 9.80 (1H, t, *J* 1.7, H-6), 4.19-4.06 (2H, m, H-1), 2.55-2.43 (2H, m, H-5), 2.08 (3H, s, H-9), 1.77-1.67 (2H, m, H-2/4), 1.66-1.58 (1H, m, H-3), 1.57-1.45 (2H, m, H-2/4), 0.95 (3H, d, *J* 6.6, H-7); δ_C (CDCl₃, 125 MHz) 204.2 (C-6), 173.2 (C-8), 63.3 (C-1), 32.6 (C-4), 31.3 (C-5), 27.6 (C-7), 26.1 (C-9). HRMS: Found: 195.0982. C₉H₁₆O₃ (M+Na⁺) Calculated 195.0997.

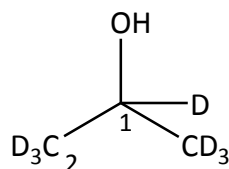
[1,1,1,2,3,3,3-²H₇]-*i*-Propyltriphenylphosphonium bromide (149)



Triphenylphosphine (**153**) (18.9 g, 1 eq.) and 2-bromo-[1,1,1,2,3,3,3-²H₇]-propane (**152**) (9.0 g, 1 eq.) were stirred together at 150 °C for 40 hours in a high-pressure, thickened, round-bottomed flask with a sealed cap. The product was recrystallized using EtOH:Et₂O (1:3 v/v) and dried by vacuum desiccation for 48 hours to give [1,1,1,2,3,3,3-²H₇]-*i*-propyltriphenylphosphonium bromide (**149**) (13.4 g, 79%) as a white, crystalline solid:

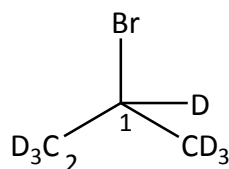
mp 228-229 °C (literature 238-239 °C); δ_{H} (CDCl_3 , 500 MHz) 8.05-7.92 (6H, m, H-1), 7.82 (3H, t, J 8.1, H-3), 7.74-7.63 (6H, m, H-2).

[1,1,1,2,3,3,3- $^2\text{H}_7$]-Propan-2-ol (151**)**



To a stirred solution of LiAl^2H_4 (1.05 g, 1 eq.) in diethylene glycol dimethyl ether (diglyme) (20 mL) under nitrogen at 0 °C was slowly added $^2\text{H}_6$ -acetone (4.45 g, 1 eq.). The suspension was stirred at 0 °C for 1 hour before slowly adding diethylene glycol (20 mL). The product was distilled from the reaction mixture and the fraction boiling at 115-127 °C collected to give $^2\text{H}_7$ -propan-2-ol (**151**) (3.17 g, 76%) as a colourless liquid. $^{214}\delta_{\text{C}}$ (CDCl_3 , 125 MHz) 63.5 (C-1*), 24.1 (C-2*). * Multiplet

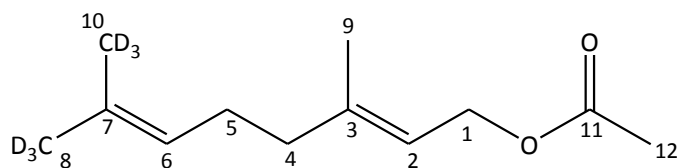
2-Bromo-[1,1,1,2,3,3,3- $^2\text{H}_7$]-propane (152**)**



To a stirred solution of $^2\text{H}_7$ -propan-2-ol (**151**) (3.17 g, 1 eq.) at -10 °C (using an ethylene glycol-dry ice bath) was added dropwise phosphorous tribromide (PBr_3) (12.96 g, 1 eq.). The reaction mixture was stirred at room temperature for 12 hours and the product isolated by distillation. Collection of the fraction boiling at 85-105 °C gave 2-bromo-[1,1,1,2,3,3,3- $^2\text{H}_7$]-propane (**152**) (1.25 g, 55%) as a colourless liquid. $^{214}\delta_{\text{C}}$ (CDCl_3 , 125 MHz) 46.7 (C-1*), 28.6 (C-2*). * Multiplet

(2E)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-2,6-octadien-1-yl acetate (²H₆-112)

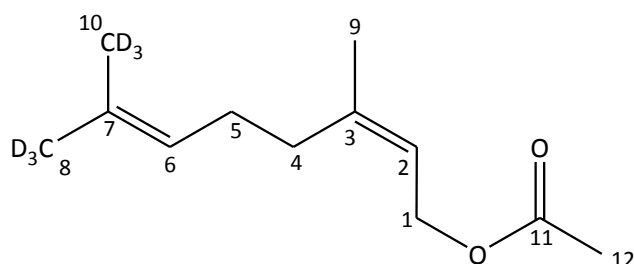
(²H₆-Geranyl acetate)



Following the general Wittig reaction procedure using 6-oxogeranyl acetate (**146**) (3.52 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2E)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-2,6-octadien-1-yl acetate (²H₆-**112**) (892 mg, 22%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2958, 2935, 1738, 1443, 1380, 1229, 1046, 1022, 954, 864, 608; δ_{H} (CDCl₃, 500 MHz)²¹⁰ 5.37 (1H, t, *J* 7.0, H-2), 5.11 (1H, t, *J* 7.0, H-6), 4.62 (2H, d, *J* 7.0, H-1), 2.16-2.10 (2H, m, H-5), 2.09 (3H, s, H-12), 2.09-2.04 (2H, m, H-4), 1.74 (3H, s, H-9), δ_{C} (CDCl₃, 125 MHz) 167.1 (C-11), 143.1 (C-3), 132.0 (C-7), 123.7 (C-6), 117.7 (C-2), 62.3 (C-1), 50.2 (C-5), 39.5 (C-4), 26.3 (C-9), 23.1 (C-8/10*), 17.8 (C-8/10*), 16.5 (C-12). HRMS: Found: 225.1731. C₁₂H₁₄²H₆O₂ (M+Na⁺) Calculated 225.1726. * Multiplet

(2Z)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-2,6-octadien-1-yl acetate (²H₆-113)

(²H₆-Neryl acetate)

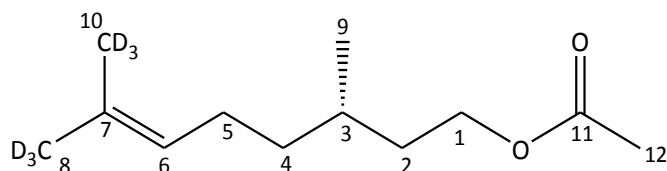


Following the general Wittig reaction procedure using 6-oxoneryl acetate (**147**) (565 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2Z)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-2,6-octadien-1-yl acetate (²H₆-**113**) (202 mg, 31%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3021, 2962, 1738, 1666,

1446, 1378, 1365, 1274, 1228, 1046, 1021, 955, 860, 608; δ_{H} (CDCl_3 , 500 MHz) 5.36 (1H, t, J 7.2, H-2), 5.10 (1H, t, J 6.9, H-6), 4.55 (2H, d, J 7.2, H-1), 2.15-2.11 (2H, m, H-5), 2.10-2.07 (2H, m, H-4), 2.06 (3H, s, H-12), 1.80 (3H, s, H-9); δ_{C} (CDCl_3 , 125 MHz) 171.5 (C-11), 143.4 (C-3/7), 132.6 (C-3/7), 123.4 (C-6), 119.6 (C-2), 61.0 (C-1), 32.2 (C-5), 26.5 (C-4), 25.5 (C-9), 23.5 (C-8/10*), 18.3 (C-8/10*), 18.1 (C-12). HRMS: Found: 225.1741. $\text{C}_{12}\text{H}_{14}^2\text{H}_6\text{O}_2$ ($\text{M}+\text{Na}^+$) Calculated 225.1726. * Multiplet

(3S)-[8,8,8,10,10,10- $^2\text{H}_6$]-3,7-Dimethyl-6-octen-1-yl acetate ($^2\text{H}_6$ -(S)-114)

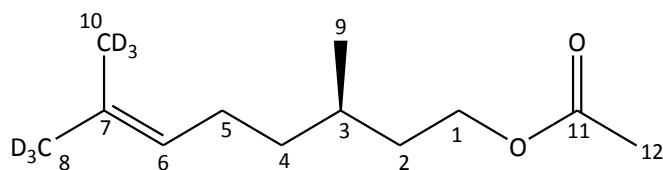
($^2\text{H}_6$ -(S)-Citronellyl acetate)



Following the general Wittig reaction procedure using 6-oxo-(S)-citronellyl acetate ((S)-**148**) (4.19 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3S)-[8,8,8,10,10,10- $^2\text{H}_6$]-3,7-dimethyl-6-octen-1-yl acetate ($^2\text{H}_6$ -(S)-**114**) (1.06 g, 22%) as a colourless oil: $[\alpha]_D^{20}$ -3.9 (c 1.0, MeOH); ν_{max} (thin film)/ cm^{-1} 2960, 2929, 1737, 1460, 1437, 1367, 1234, 1047, 1022, 972, 607; δ_{H} (CDCl_3 , 500 MHz) 5.11 (1H, t, J 7.1, H-6), 4.18-4.07 (2H, m, H-1), 2.08 (3H, s, H-12), 2.05-1.93 (2H, m, H-5), 1.74-1.65 (1H, m, H-2a), 1.59-1.50 (1H, m, H-3), 1.49-1.41 (1H, m, H-2b), 1.39-1.32 (1H, m, H-4a), 1.25-1.16 (1H, m, H-4b), 0.93 (3H, d, J 6.6, H-9); δ_{C} (CDCl_3 , 125 MHz) 171.1 (C-11), 131.8 (C-7), 125.3 (C-6), 63.8 (C-1), 36.0 (C-4), 33.9 (C-2), 29.0 (C-3), 25.8 (C-8/10*), 25.3 (C-5), 21.6 (C-12), 19.2 (C-9), 17.9 (C-8/10*). HRMS: Found: 227.1899. $\text{C}_{12}\text{H}_{16}^2\text{H}_6\text{O}_2$ ($\text{M}+\text{Na}^+$) Calculated 227.1889. * Multiplet

(3*R*)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-6-octen-1-yl acetate (²H₆-(*R*)-114)

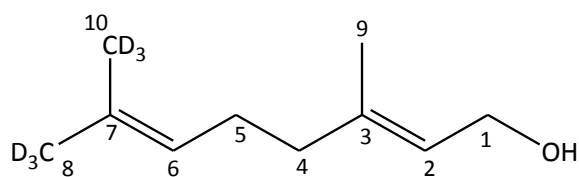
(²H₆-(*R*)-Citronellyl acetate)



Following the general Wittig reaction procedure using 6-oxo-(*R*)-citronellyl acetate (**(*R*)-148**) (764 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3*R*)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-6-octen-1-yl acetate (²H₆-(*R*)-**114**) (220 mg, 25%) as a colourless oil: $[\alpha]_D^{20} +3.6$ (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2959, 1736, 1460, 1436, 1367, 1234, 1046, 1022, 975, 608; δ_{H} (CDCl₃, 500 MHz) 5.12 (1H, t, *J* 6.6, H-6), 4.16-4.11 (2H, m, H-1), 2.06 (3H, s, H-11), 2.03-1.97 (2H, m, H-5), 1.68-1.63 (1H, m, H-2a), 1.54 (1H, m, H-3), 1.44 (1H, m, H-2b), 1.37 (1H, m, H-4a), 1.22 (1H, m, H-4b); δ_{C} (CDCl₃, 125 MHz) 171.9 (C-11), 132.3 (C-7), 125.3 (C-6), 63.4 (C-1), 37.2 (C-4), 35.2 (C-2), 29.4 (C-3), 26.1 (C-8/10*), 25.1 (C-5), 21.0 (C-12), 18.8 (C-9), 17.4 (C-8/10*). HRMS: Found: 221.1514. C₁₂H₁₆²H₆O₂ (M+Na⁺) Calculated 227.1889. * Multiplet

(2*E*)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-2,6-octadien-1-ol (²H₆-56)

(²H₆-Geraniol)

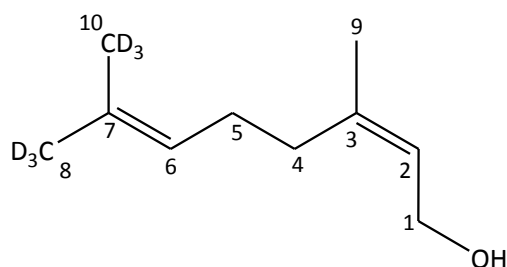


Following the general acetate deprotection procedure using ²H₆-geranyl acetate (²H₆-**112**) (147 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*E*)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-2,6-octadien-1-ol (²H₆-**56**) (91 mg, 76%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3313, 2958, 2927, 2858, 2225, 2190, 1667, 1441, 1381, 1239, 1097, 1048, 999, 862; δ_{H} (CDCl₃, 500 MHz) 5.44 (1H, t, *J* 7.0, H-

2), 5.12 (1H, t, J 7.0, H-6), 4.18 (2H, d, J 7.0, H-1), 2.15-2.09 (2H, m, H-5), 2.09-2.03 (2H, m, H-4), 1.71 (3H, s, H-9), δ_c (CDCl₃, 125 MHz) 140.0 (C-3), 131.7 (C-7), 124.0 (C-6), 123.3 (C-2), 59.6 (C-1), 39.8 (C-5), 26.5 (C-4), 24.6 (C-8*), 16.9 (C-10*), 16.4 (C-9),. HRMS: Found: 183.1658. C₁₀H₁₂²H₆O (M+Na⁺) Calculated 183.1626. * Multiplet

(2Z)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-2,6-octadien-1-ol (²H₆-62)

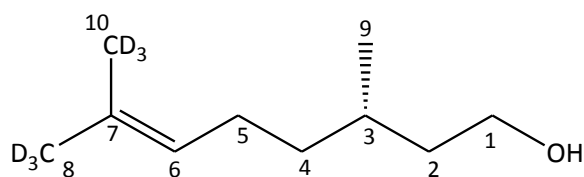
(²H₆-Nerol)



Following the general acetate deprotection procedure using ²H₆-neryl acetate (²H₆-113) (83 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2Z)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-2,6-octadien-1-ol (²H₆-62) (48 mg, 71%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3356, 2960, 2932, 2858, 2225, 2192, 1741, 1726, 1428, 1242, 1112, 1047, 998, 861, 700; δ_H (CDCl₃, 500 MHz) 5.45 (1H, t, J 7.2, H-2), 5.11 (1H, t, J 7.2, H-6), 4.09 (2H, d, J 7.2, H-1), 2.14-2.05 (4H, m, H-4/5), 1.61 (3H, s, H-9), δ_c (CDCl₃, 125 MHz) 139.8 (C-3), 132.4 (C-7), 124.5 (C-6), 123.8 (C-2), 58.9 (C-1), 32.0 (C-5), 25.7 (C-8*), 23.4 (C-4), 17.7 (C-9), 17.7 (C-10*). HRMS: Found: 183.1678. C₁₀H₁₂²H₆O (M+Na⁺) Calculated 183.1626. * Multiplet

(3S)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-6-octen-1-ol (²H₆-(S)-57)

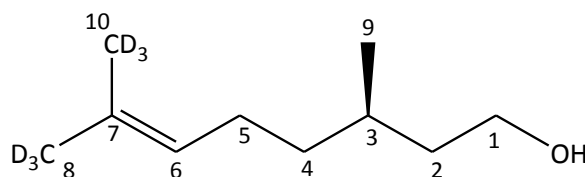
(²H₆-(S)-Citronellol)



Following the general acetate deprotection procedure using $^2\text{H}_6$ -(S)-citronellyl acetate ($^2\text{H}_6$ -(S)-**114**) (165 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3S)-[8,8,8,10,10,10- $^2\text{H}_6$]-3,7-dimethyl-6-octen-1-ol ($^2\text{H}_6$ -(S)-**57**) (112 mg, 83%) as a colourless oil: $[\alpha]_D^{20}$ -4.6 (c 1.0, MeOH); ν_{max} (thin film)/ cm^{-1} 3327, 2956, 2925, 2872, 2224, 2111, 1458, 1377, 1050, 1008, 965, 857; δ_{H} (CDCl_3 , 500 MHz) 5.12 (1H, t, J 7.6, H-6), 3.77-3.65 (2H, m, H-1), 2.00-1.86 (2H, m, H-5), 1.68-1.46 (3H, m, H-2/3), 1.28-1.09 (2H, m, H-4), 0.91 (3H, d, J 6.5, H-9); δ_{C} (CDCl_3 , 125 MHz) 131.1 (C-7), 125.0 (C-6), 63.9 (C-1), 39.9 (C-4), 35.1 (C-2), 29.4 (C-3), 25.2 (C-5), 24.9 (C-8*), 19.8 (C-9), 18.3 (C-10*). HRMS: Found: 185.1770. $\text{C}_{10}\text{H}_{14}^2\text{H}_6\text{O}$ ($\text{M}+\text{Na}^+$) Calculated 185.1782. * Multiplet

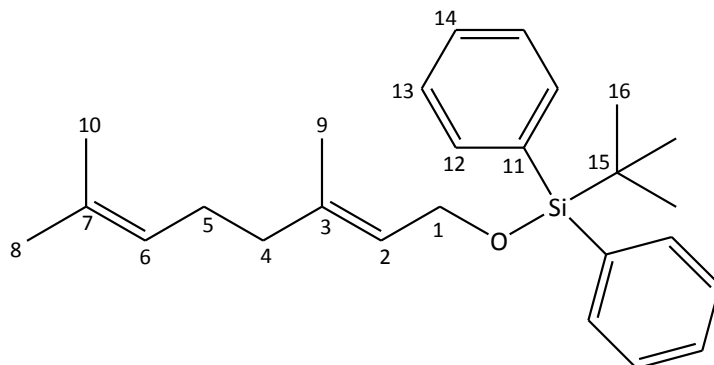
(3R)-[8,8,8,10,10,10- $^2\text{H}_6$]-3,7-Dimethyl-6-octen-1-ol ($^2\text{H}_6$ -(R)-57**)**

($^2\text{H}_6$ -(R)-Citronellol)



Following the general acetate deprotection procedure using $^2\text{H}_6$ -(R)-citronellyl acetate ($^2\text{H}_6$ -(R)-**114**) (42 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3R)-[8,8,8,10,10,10- $^2\text{H}_6$]-3,7-dimethyl-6-octen-1-ol ($^2\text{H}_6$ -(R)-**57**) (28 mg, 81%) as a colourless oil: $[\alpha]_D^{20}$ +4.8 (c 1.0, MeOH); ν_{max} (thin film)/ cm^{-1} 3331, 2957, 2925, 2872, 2220, 2110, 147, 1377, 1053, 1008, 968, 857; δ_{H} (CDCl_3 , 500 MHz) 5.10 (1H, t, J 7.6, H-6), 3.79-3.65 (2H, m, H-1), 2.01-1.88 (2H, m, H-5), 1.68-1.47 (3H, m, H-2/3), 1.27-1.11 (2H, m, H-4), 0.90 (3H, d, J 6.6, H-9); δ_{C} (CDCl_3 , 125 MHz) 130.9 (C-7), 125.2 (C-6), 63.9 (C-1), 39.3 (C-4), 35.6 (C-2), 29.3 (C-3), 25.2 (C-5), 24.6 (C-8*), 19.8 (C-9), 18.2 (C-10*). HRMS: Found: 185.1802. $\text{C}_{10}\text{H}_{14}^2\text{H}_6\text{O}$ ($\text{M}+\text{Na}^+$) Calculated 185.1782. * Multiplet

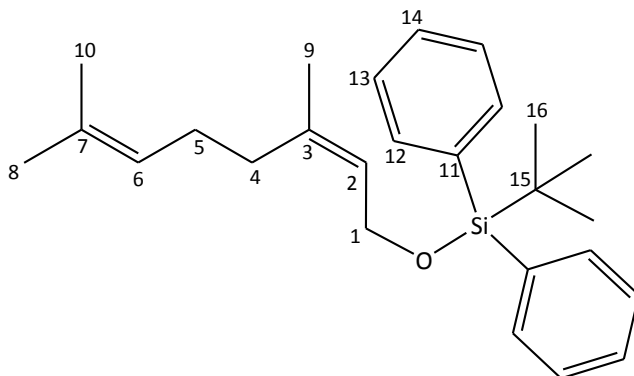
(Geranyl TBDPS)



212

(2Z)-3,7-Dimethyl-1-(*t*-butyldiphenylsilyl)oxy-2,6-octadiene (157)

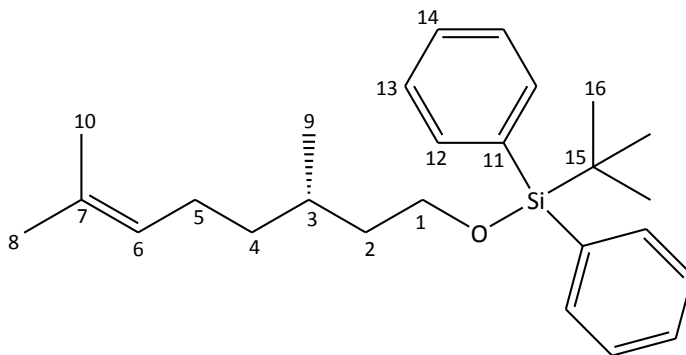
(Neryl TBDPS)



Following the general *t*-butyldiphenyl silyl ether (TBDPS) protection procedure using nerol (**62**) (3.08 g), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (2Z)-3,7-dimethyl-1-(*t*-butyldiphenylsilyl)oxy-2,6-octadiene (**156**) (7.85 g, 100%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 2961, 2931, 2857, 1742, 1428, 1375, 1239, 1110, 1049, 822, 782, 738, 700, 612, 502; δ_{H} (CDCl_3 , 500 MHz) 7.73 (4H, d, J 8.3, H-12), 7.48-7.37 (6H, m, H-13 and H-14), 5.43 (1H, dt, J 6.4 and 1.0, H-2), 5.12 (1H, dt, J 6.9 and 1.2, H-6), 4.27 (2H, d, J 6.4, H-1), 2.11-1.94 (2H, m, H-5), 1.94-1.88 (2H, m, H-4), 1.73 (3H, s, H-8/10), 1.63 (3H, s, H-9), 1.53 (3H, s, H-8/10), 1.07 (9H, s, H-16). δ_{C} (CDCl_3 , 125 MHz) 137.5 (C-11), 135.8 (C-12), 134.0 (C-3), 131.8 (C-7), 129.5 (C-14), 127.6 (C-13), 124.9 (C-6), 123.9 (C-2), 60.8 (C-1), 32.2 (C-4), 26.9 (C-16), 26.6 (C-5), 25.7 (C-8/10), 23.4 (C-15), 19.2 (C-8/10), 17.6 (C-9). HRMS: Found: 415.2446. $\text{C}_{26}\text{H}_{36}\text{OSi}$ ($\text{M}+\text{Na}^+$) Calculated 415.2427.

(3S)-3,7-Dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6-octene ((S)-158)

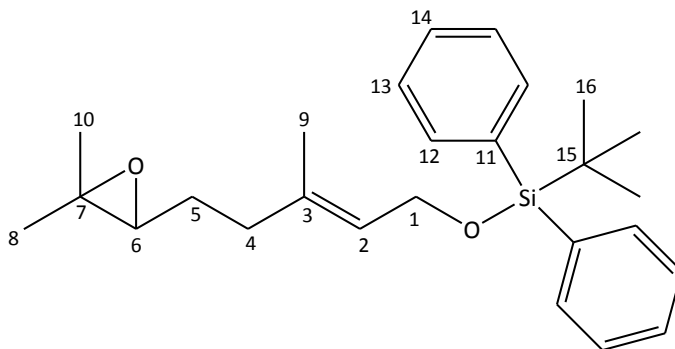
((S)-Citronellyl TBDPS)



Following the general *t*-butyldiphenyl silyl ether (TBDPS) protection procedure using (*S*)-citronellol ((**S**)-57) (7.1 g), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (3*S*)-3,7-dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6-octene ((**S**)-158) (15.3 g, 85%) as a colourless oil: $[\alpha]_D^{20} +1.0$ (*c* 1.0, MeOH) (cf. (**R**)-158: -1.4); $\nu_{\max}$ (thin film)/cm⁻¹ 3071, 2958, 2929, 2857, 1472, 1462, 1378, 1107, 1086, 998, 822, 736, 700, 613, 503; δ_{H} (CDCl₃, 500 MHz) 7.70 (4H, d, *J* 8.2, H-12), 7.48-7.38 (6H, m, H-13 and H-14), 5.12 (1H, dt, *J* 7.2 and 1.4, H-6), 3.77-3.67 (2H, m, H-1), 2.06-1.93 (2H, m, H-5), 1.71 (3H, s, H-8/10), 1.70-1.61 (2H, m, H-2a and H-3), 1.64 (3H, s, H-8/10), 1.42-1.38 (1H, m, H-2b), 1.38-1.29 (1H, m, H-4a), 1.20-1.12 (1H, m, H-4b), 1.08 (9H, s, H-16), 0.87 (3H, d, *J* 6.8, H-9). δ_{C} (CDCl₃, 125 MHz) 135.6 (C-12), 134.2 (C-11), 131.1 (C-7), 129.5 (C-14), 127.6 (C-13), 125.0 (C-6), 62.2 (C-1), 39.7 (C-2), 37.2 (C-4), 29.1 (C-3), 26.9 (C-16), 25.8 (C-8/10), 25.5 (C-5), 19.6 (C-9), 19.3 (C-15), 17.7 (C-8/10). HRMS: Found: 417.2595. C₂₆H₃₈OSi (M+Na⁺) Calculated 417.2590.

(2E)-3,7-Dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6,7-epoxy-2-octene (159)

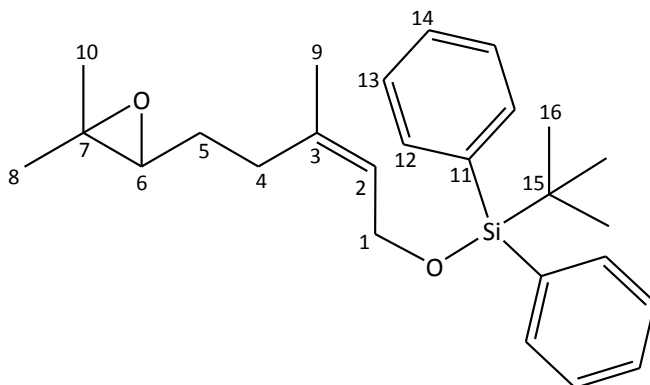
(6,7-Epoxygeranyl TBDPS)



Following the general epoxidation procedure using geranyl TBDPS (**156**) (175 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40–60 °C) to give (2E)-3,7-dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6,7-epoxy-2-octene (**159**) (115 mg, 63%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3071, 2960, 2931, 2857, 1740, 1428, 1377, 1240, 1110, 1045, 822, 782, 739, 700, 612, 488; δ_{H} (CDCl_3 , 500 MHz) 7.75 (4H, d, J 7.2, H-12), 7.50–7.41 (6H, m, H-13 and H-14), 5.49 (1H, t, J 6.0, H-2), 4.29 (2H, d, J 6.0, H-1), 2.76 (1H, t, J 6.3, H-6), 2.25–2.18 (1H, m, H-5), 2.18–2.10 (1H, m, H-4), 1.53 (3H, s, H-8/10), 1.37 (3H, s, H-9), 1.32 (3H, s, H-8/10), 1.11 (9H, s, H-16). δ_{C} (CDCl_3 , 125 MHz) 136.2 (C-11), 135.7 (C-12), 134.0 (C-3), 129.6 (C-14), 127.7 (C-13), 124.7 (C-2), 64.1 (C-6), 61.1 (C-1), 58.4 (C-7), 36.2 (C-5), 27.2 (C-4), 26.9 (C-16), 25.0 (C-8/10), 19.2 (C-15), 18.8 (C-8/10), 16.4 (C-9). HRMS: Found: 431.2379. $\text{C}_{26}\text{H}_{36}\text{O}_2\text{Si}$ ($\text{M}+\text{Na}^+$) Calculated 431.2382.

(2Z)-3,7-Dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6,7-epoxy-2-octene (160)

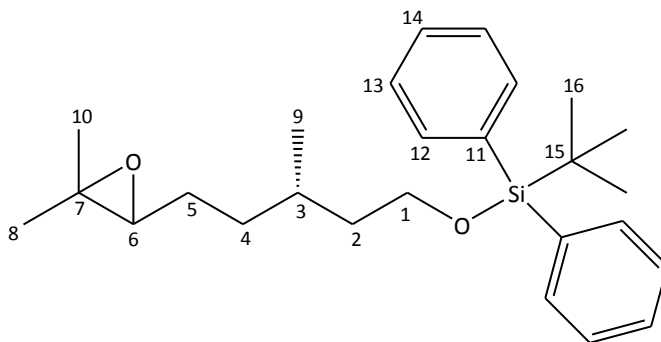
(6,7-Epoxyneryl TBDPS)



Following the general epoxidation procedure using neryl TBDPS (**157**) (8.7 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2Z)-3,7-dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6,7-epoxy-2-octene (**160**) (5.65 g, 62%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3078, 2961, 2931, 2855, 1740, 1428, 1376, 1240, 1113, 1044, 821, 782, 740, 700, 612, 478; δ_{H} (CDCl_3 , 500 MHz) 7.76 (4H, d, J 7.0, H-12), 7.50-7.40 (6H, m, H-13 and H-14), 5.51 (1H, t, J 6.0, H-2), 4.28 (2H, d, J 6.0, H-1), 2.62 (1H, t, J 6.1, H-6), 2.11-2.01 (2H, m, H-5), 1.78 (3H, s, H-9), 1.70-1.59 (1H, m, H-4a), 1.54-1.45 (1H, m, H-4b), 1.28 (3H, s, H-8/10), 1.22 (3H, s, H-8/10), 1.11 (9H, s, H-16). δ_{C} (CDCl_3 , 125 MHz) 136.0 (C-11), 135.1 (C-12), 134.2 (C-3), 129.5 (C-14), 127.7 (C-13), 123.7 (C-2), 64.3 (C-6), 61.5 (C-1), 58.8 (C-7), 36.1 (C-5), 25.2 (C-4), 26.8 (C-16), 24.6 (C-8/10), 19.9 (C-15), 18.8 (C-8/10), 16.5 (C-9). HRMS: Found: 431.2379. $\text{C}_{26}\text{H}_{36}\text{O}_2\text{Si}$ ($\text{M}+\text{Na}^+$) Calculated 431.2382.

(3*S*)-3,7-Dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6,7-epoxyoctane ((*S*)-161)

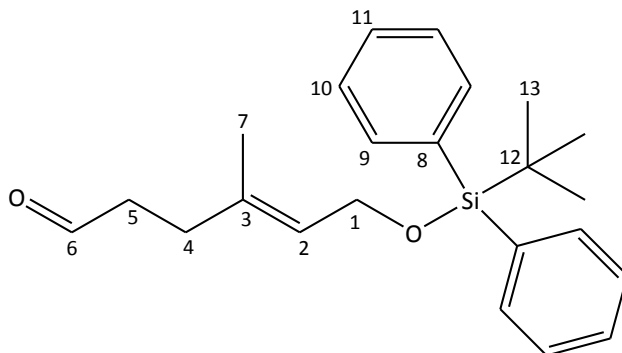
(6,7-Epoxy-(*S*)-citronellyl TBDPS)



Following the general epoxidation procedure using (*S*)-citronellyl TBDPS ((*S*)-158) (7.5 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3*S*)-3,7-dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6,7-epoxyoctane ((*S*)-161) (4.46 g, 57%) as a colourless oil: $[\alpha]_D^{20} -1.4$ (*c* 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3071, 2958, 2929, 2857, 1428, 1107, 1087, 822, 736, 700, 613, 503; δ_{H} (CDCl₃, 500 MHz) 7.69 (4H, d, *J* 7.1, H-12), 7.47-7.38 (6H, m, H-13 and H-14), 3.78-3.67 (2H, d, *J* 6.5, H-1), 2.70 (1H, m, H-6), 1.71-1.61 (2H, m, H-2a and H-3), 1.60-1.45 (2H, m, H-4a and H-5), 1.45-1.36 (2H, m, H-2b and H-4b), 1.33 (3H, s, H-8/10), 1.29 (3H, s, H-8/10), 1.08 (9H, s, H-16), 0.89 (3H, d, *J* 6.8, H-9). δ_{C} (CDCl₃, 125 MHz) 135.6 (C-12), 134.1 (C-11), 129.6 (C-14), 127.6 (C-13), 64.7 (C-6), 62.1 (C-1), 58.3 (C-7), 39.5 (C-2), 33.6 (C-4), 29.4 (C-3), 26.9 (C-16), 26.4 (C-5), 25.0 (C-8/10), 19.6 (C-15), 19.2 (C-9), 18.7 (C-8/10). HRMS: Found: 433.2556. C₂₆H₃₈O₂Si (M+Na⁺) Calculated 433.2539.

(2E)-3-Methyl-1-(*t*-butyldiphenylsilyl)oxy-6-oxo-2-hexene (162)

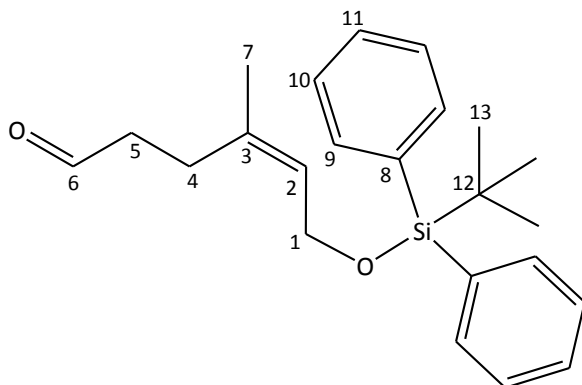
(6-Oxogeranyl TBDPS)



Following the general procedure for epoxide cleavage using 6,7-epoxygeranyl TBDPS (**159**) (92 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2E)-3-methyl-1-(*t*-butyldiphenylsilyl)oxy-6-oxo-2-hexene (**162**) (86 mg, 84%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3071, 2958, 2891, 2715, 1725, 1472, 1428, 1387, 1261, 1188, 1110, 1054, 853, 779, 739, 700, 609, 503; δ_{H} (CDCl_3 , 500 MHz) 9.79 (1H, t, J 1.8, H-6), 7.72 (4H, d, J 7.8, H-9), 7.50-7.38 (6H, m, H-10 and H-11), 5.42 (1H, t, J 6.2, H-2), 4.26 (2H, d, J 6.2, H-1), 2.54 (2H, t, J 7.9, H-4), 2.33 (1H, td, J 7.9 and 1.8, H-5), 1.48 (3H, s, H-7), 1.07 (9H, s, H-13). δ_{C} (CDCl_3 , 125 MHz) 202.0 (C-6), 135.6 (C-9), 135.0 (C-8), 133.9 (C-3), 129.6 (C-11), 127.7 (C-10), 125.0 (C-2), 61.0 (C-1), 41.9 (C-5), 31.5 (C-4), 26.9 (C-13), 19.2 (C-12), 16.5 (C-7). HRMS: Found: 389.1920. $\text{C}_{23}\text{H}_{30}\text{O}_2\text{Si}$ ($\text{M}+\text{Na}^+$) Calculated 389.1913.

(2Z)-3-Methyl-1-(*t*-butyldiphenylsilyl)oxy-6-oxo-2-hexene (163)

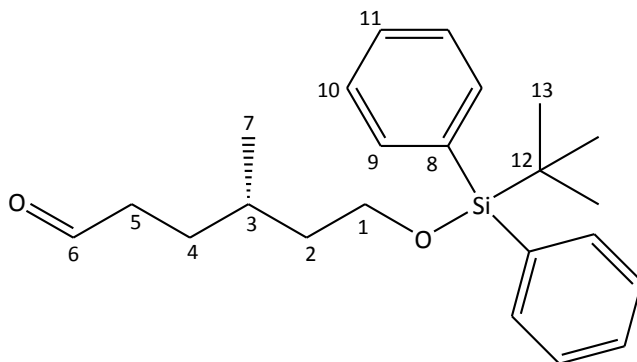
(6-Oxoneryl TBDPS)



Following the general procedure for epoxide cleavage using 6,7-epoxyneryl TBDPS (**160**) (3.35 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2Z)-3-methyl-1-(*t*-butyldiphenylsilyl)oxy-6-oxo-2-hexene (**163**) (2.84 g, 76%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3073, 2958, 2881, 2712, 1727, 1476, 1428, 1387, 1266, 1174, 1110, 1055, 853, 780, 739, 701, 608, 503; δ_{H} (CDCl_3 , 500 MHz) 9.68 (1H, t, J 1.3, H-6), 7.73 (4H, d, J 7.8, H-9), 7.46-7.40 (6H, m, H-10 and H-11), 5.45 (1H, t, J 6.2, H-2), 4.23 (2H, d, J 6.2, H-1), 2.39 (2H, t, J 7.9, H-4), 2.20 (1H, t, J 7.9, H-5), 1.13 (3H, s, H-7), 1.10 (9H, s, H-13). δ_{C} (CDCl_3 , 125 MHz) 201.80 (C-6), 135.3 (C-9), 135.3 (C-8), 133.8 (C-3), 129.2 (C-11), 127.5 (C-10), 125.4 (C-2), 61.5 (C-1), 41.8 (C-5), 30.2 (C-4), 27.1 (C-13), 21.2 (C-12), 16.6 (C-7). HRMS: Found: 389.1917. $\text{C}_{23}\text{H}_{30}\text{O}_2\text{Si}$ ($\text{M}+\text{Na}^+$) Calculated 389.1913.

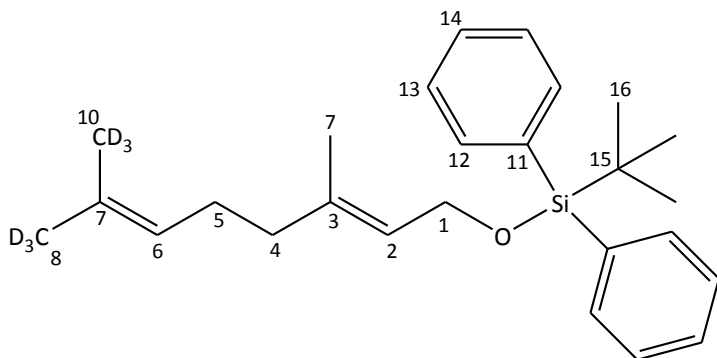
(3*S*)-3-Methyl-1-(*t*-butyldiphenylsilyl)oxy-6-oxohexane ((*S*)-164)

(6-Oxo-(*S*)-citronellyl TBDPS)



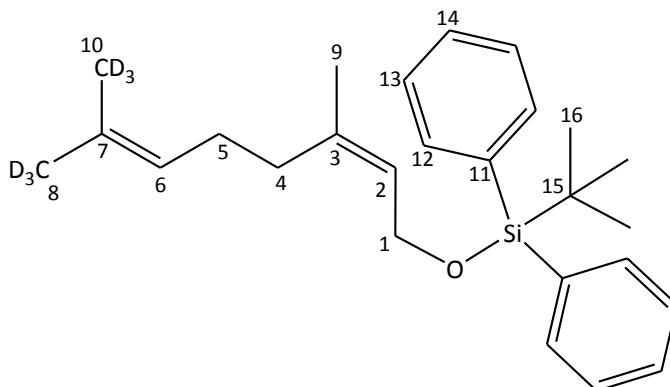
Following the general procedure for epoxide cleavage using 6,7-epoxy-(*S*)-citronellyl TBDPS ((*S*)-161) (4.1 g), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3*S*)-3-methyl-1-(*t*-butyldiphenylsilyl)oxy-6-oxohexane ((*S*)-164) (3.14 g, 69%) as a colourless oil: $[\alpha]_D^{20} +0.68$ (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3074, 2953, 2890, 2714, 1472, 1427, 1387, 1265, 1186, 1110, 1054, 851, 779, 732, 700, 609, 504; δ_{H} (CDCl₃, 500 MHz) 9.75 (1H, t, *J* 1.9, H-6), 7.70 (4H, d, *J* 7.6, H-9), 7.49-7.38 (6H, m, H-10 and H-11), 3.77-3.67 (2H, m, H-1), 2.49-2.37 (2H, m, H-5), 1.71-1.56 (3H, m, H-2 and H-3), 1.50-1.38 (2H, m, H-4), 1.09 (9H, s, H-13), 0.88 (3H, d, *J* 6.7, H-7). δ_{C} (CDCl₃, 125 MHz) 200.4 (C-6), 135.7 (C-9), 134.6 (C-8), 129.5 (C-11), 127.5 (C-10), 62.5 (C-1), 36.8 (C-5), 35.9 (C-2), 33.2 (C-4), 31.0 (C-3), 27.0 (C-13), 21.3 (C-12), 16.4 (C-7). HRMS: Found: 391.2074. C₂₃H₃₂O₂Si (M+Na⁺) Calculated 391.2070.

(2E)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-1-(*t*-butyldiphenylsilyl)oxy-2,6-octadiene (²H₆-156) (²H₆-Geranyl TBDPS)



Following the general procedure for the Wittig reaction using 6-oxogeranyl TBDPS (**162**) (90 mg), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (2E)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-1-(*t*-butyldiphenylsilyl)oxy-2,6-octadiene (²H₆-**156**) (48 mg, 55%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 3344, 2959, 2857, 1668, 1472, 1428, 1110, 1050, 856, 823, 738, 700, 612, 503; δ_{H} (CDCl₃, 500 MHz) 7.75 (4H, d, *J* 7.9, H-12), 7.49-7.38 (6H, m, H-13 and H-14), 5.44 (1H, d, *J* 6.2, H-2), 5.15 (1H, d, *J* 7.0, H-6), 4.28 (2H, d, *J* 6.2, H-1), 2.16-2.07 (2H, m, H-5), 2.06-1.99 (2H, m, H-4), 1.48 (3H, s, H-9), 1.11 (9H, s, H-16). δ_{C} (CDCl₃, 125 MHz) 137.2 (C-11), 135.3 (C-12), 134.5 (C-3), 131.9 (C-7), 129.4 (C-14), 127.8 (C-13), 124.1 (C-6), 124.0 (C-2), 61.8 (C-1), 38.6 (C-4), 26.2 (C-16), 26.0 (C-5), 25.9 (C8/10*), 19.5 (C-15), 18.0 (C8/10*), 16.1 (C-9). HRMS: Found: 421.2816. C₂₆H₃₀²H₆OSi (M+Na⁺) Calculated 421.2804. * Multiplet

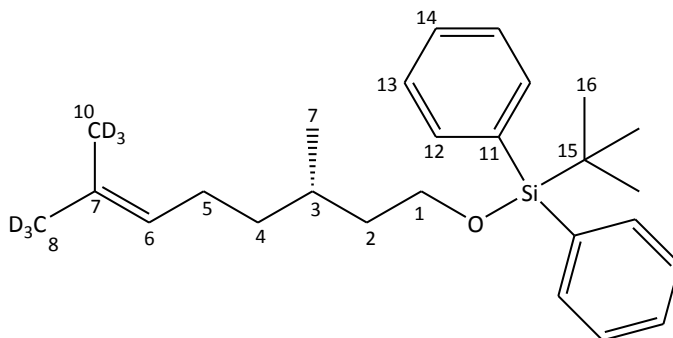
(2Z)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-1-(*t*-butyldiphenylsilyl)oxy-2,6-octadiene (²H₆-157) (²H₆-Neryl TBDPS)



Following the general procedure for the Wittig reaction using 6-oxoneryl TBDPS (**163**) (2.55 g), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (2Z)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-1-(*t*-butyldiphenylsilyl)oxy-2,6-octadiene (²H₆-**157**) (1.29 g, 52%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 3389, 2959, 2857, 1665, 1472, 1428, 1110, 1048, 857, 823, 738, 699, 612, 502; δ_{H} (CDCl₃, 500 MHz) 7.73 (4H, d, *J* 7.9, H-12), 7.47-7.35 (6H, m, H-13 and H-14), 5.41 (1H, t, *J* 6.2, H-2), 5.01 (1H, t, *J* 7.0, H-6), 4.21 (2H, d, *J* 6.2, H-1), 1.99-1.94 (2H, m, H-5), 1.94-1.89 (2H, m, H-4), 1.73 (3H, s, H-9), 1.07 (9H, s, H-16). δ_{C} (CDCl₃, 125 MHz) 137.5 (C-11), 135.6 (C-12), 134.1 (C-3), 131.6 (C-7), 129.5 (C-14), 127.7 (C-13), 125.0 (C-6), 124.0 (C-2), 60.8 (C-1), 32.3 (C-4), 27.0 (C-16), 26.6 (C-5), 25.8 (C-8/10*), 23.5 (C-15), 19.3 (C-9), 19.1 (C-8/10*). HRMS: Found: 421.2787. C₂₆H₃₀²H₆OSi (M+Na⁺) Calculated 421.2804. * Multiplet

(3S)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6-octene

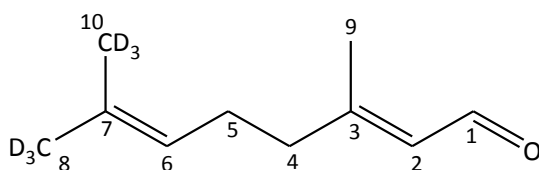
(²H₆-(S)-158) (²H₆-(S)-Citronellyl TBDPS)



Following the general procedure for the Wittig reaction using 6-oxo-(S)-citronellyl TBDPS ((S)-164) (4.37 g), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (3S)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-1-(*t*-butyldiphenylsilyl)oxy-6-octene (²H₆-(S)-158) (1.87 g, 44%) as a colourless oil: $[\alpha]_D^{20} +1.2$ (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3072, 2957, 2929, 2857, 1740, 1472, 1428, 1108, 1087, 909, 823, 734, 700, 613, 503; δ_{H} (CDCl₃, 500 MHz) 7.71 (4H, d, *J* 7.9, H-12), 7.48-7.38 (6H, m, H-13 and H-14), 5.11 (1H, t, *J* 7.0, H-6), 3.78-3.68 (2H, m, H-1), 2.07-1.93 (2H, m, H-5), 1.70-1.61 (2H, m, H-2a and H-3), 1.43-1.29 (3H, m, H-2b and H-4a), 1.21-1.10 (1H, m, H-4b), 1.08 (9H, s, H-16), 0.85 (3H, d, *J* 6.7, H-9). δ_{C} (CDCl₃, 125 MHz) 135.6 (C-12), 134.2 (C-11), 130.9 (C-7), 129.5 (C-14), 127.6 (C-13), 125.0 (C-6), 62.2 (C-1), 39.6 (C-2), 37.2 (C-4), 29.0 (C-3), 26.9 (C-16), 25.5 (C-5), 25.1 (C-8/10*), 19.7 (C-15), 19.3 (C-9), 17.6 (C-8/10*). HRMS: Found: 423.2674. C₂₆H₃₀²H₆OSi (M+Na⁺) Calculated 423.2690. * Multiplet

(2E)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-2,6-octadien-1-al (²H₆-109)

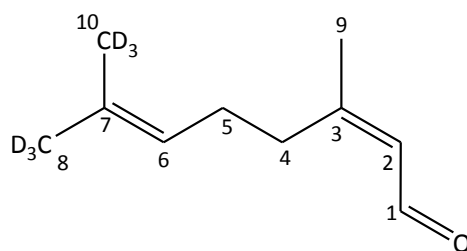
(²H₆-Geranial)



Following the general MnO_2 oxidation procedure using $^2\text{H}_6$ -geraniol ($^2\text{H}_6$ -56) (241 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2*E*)-[8,8,8,10,10,10- $^2\text{H}_6$]-3,7-dimethyl-2,6-octadien-1-al ($^2\text{H}_6$ -109) (40 mg, 42%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 2960, 2858, 1737, 1671, 1429, 1374, 1244, 1048, 841, 822, 742, 700, 633, 509; δ_{H} (CDCl_3 , 500 MHz) 10.02 (1H, d, J 6.9, H-1), 5.90 (1H, d, J 6.9, H-2), 5.08 (1H, t, J 6.3, H-6), 2.29-2.20 (4H, m, H-4 and H-5), 2.20 (3H, s, H-9); δ_{C} (CDCl_3 , 125 MHz) 191.3 (C-1), 163.8 (C-3), 133.4 (C-7), 127.4 (C-6), 122.8 (C-2), 40.7 (C-8*), 25.7 (C-5), 17.5 (C-4), 15.2 (C-9), 12.5 (C-10*). HRMS: Found: 181.1462. $\text{C}_{10}\text{H}_{10}^2\text{H}_6\text{O}$ ($\text{M}+\text{Na}^+$) Calculated 181.1470. * Multiplet

(2*Z*)-[8,8,8,10,10,10- $^2\text{H}_6$]-3,7-Dimethyl-2,6-octadien-1-al ($^2\text{H}_6$ -110)

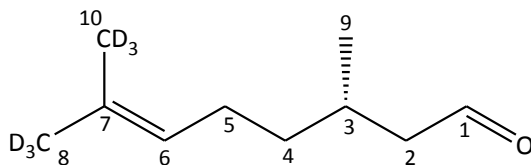
($^2\text{H}_6$ -Neral)



Following the general MnO_2 oxidation procedure using $^2\text{H}_6$ -nerol ($^2\text{H}_6$ -62) (77 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2*Z*)-[8,8,8,10,10,10- $^2\text{H}_6$]-3,7-dimethyl-2,6-octadien-1-al ($^2\text{H}_6$ -110) (10 mg, 33%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 2959, 2858, 1739, 1672, 1429, 1374, 1241, 1047, 841, 820, 742, 701, 633, 506; δ_{H} (CDCl_3 , 500 MHz) 9.90 (1H, d, J 7.4, H-1), 5.90 (1H, d, J 7.4, H-2), 5.14 (1H, t, J 6.4, H-6), 2.59 (2H, t, J 6.4, H-4), 2.31-2.20 (2H, m, H-5), 2.11-2.03 (2H, m, H-5), 2.00 (3H, s, H-9); δ_{C} (CDCl_3 , 125 MHz) 190.9 (C-1), 164.0 (C-3), 133.9 (C-7), 128.7 (C-6), 122.3 (C-2), 32.6 (C-5), 32.6 (C-8*), 27.1 (C-4), 25.2 (C-9), 17.4 (C-10*). HRMS: Found: 181.1464. $\text{C}_{10}\text{H}_{10}^2\text{H}_6\text{O}$ ($\text{M}+\text{Na}^+$) Calculated 181.1470. * Multiplet

(3S)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-6-octen-1-al (²H₆-(S)-111)

(²H₆-(S)-Citronellal)



Following the general IBX oxidation procedure using ²H₆-(S)-citronellol (²H₆-(S)-57) (201 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3S)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-6-octen-1-al (²H₆-(S)-111) (52 mg, 65%) as a colourless oil: $[\alpha]_D^{20}$ -7.9 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2954, 2929, 2871, 2230, 1722, 1667, 1457, 1363, 1290, 1246, 1109, 1047, 1017, 968, 861, 746, 702, 542; δ_H (CDCl₃, 500 MHz) 9.79 (1H, m, H-1), 5.10 (1H, t, *J* 6.9, H-6), 2.43 (1H, dd, *J* 6.9, H-2a), 2.25 (1H, dd, *J* 6.9, H-2b), 2.11-1.96 (3H, m, H-3 and H-5), 1.44-1.25 (2H, m, H-4), 0.98 (3H, d, *J* 6.9, H-9); δ_C (CDCl₃, 125 MHz) 191.2 (C-1), 134.5 (C-7), 129.6 (C-6), 69.3 (C-8*), 38.2 (C-5), 29.9 (C-2), 26.7 (C-3), 25.2 (C-4), 15.2 (C-9), 13.6 (C-10*). HRMS: Found: 183.1608. C₁₀H₁₂²H₆O (M+Na⁺) Calculated 183.1626. * Multiplet

(3R)-[8,8,8,10,10,10-²H₆]-3,7-Dimethyl-6-octen-1-al (²H₆-(R)-111)

(²H₆-(R)-Citronellal)

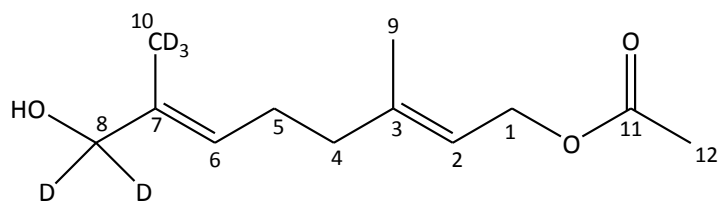


Following the general IBX oxidation procedure using ²H₆-(R)-citronellol (²H₆-(R)-57) (37 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3R)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-6-octen-1-al (²H₆-(R)-111) (9 mg, 61%) as a colourless oil: $[\alpha]_D^{20}$ +8.3 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2955, 2932, 2871, 2231, 1722, 1666, 1457, 1363, 1295, 1244, 1109, 1047, 1011, 968, 861, 745, 702, 542; δ_H (CDCl₃, 500 MHz) 9.76 (1H, m, H-1), 5.11 (1H, t, *J* 6.9, H-6), 2.41 (1H, dd, *J* 6.8, H-

2a),), 2.27 (1H, dd, J 6.8, H-2b), 2.15-1.99 (3H, m, H-3 and H-5), 1.44-1.29 (2H, m, H-4), 1.01 (3H, d, J 6.9, H-9), 1.51 (3H, s, H-10); δ_c (CDCl₃, 125 MHz) 190.9 (C-1), 134.4 (C-7), 129.6 (C-6), 57.7 (C-8*), 38.3 (C-5), 29.6 (C-2), 26.7 (C-3), 25.3 (C-4), 15.4 (C-9), 13.0 (C-10*). HRMS: Found: 183.1624. C₁₀H₁₂²H₆O (M+Na⁺) Calculated 183.1626. * Multiplet

(2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-yl acetate

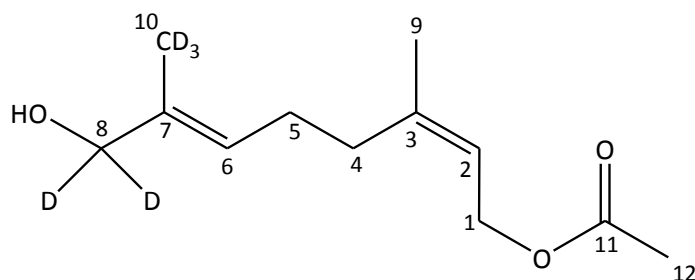
(²H₅-115) (²H₅)-8-Hydroxygeranyl acetate)



Following the general allylic oxidation procedure using ²H₆-geraniol (**²H₆-56**) (631 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-2,6-octadien-1-yl acetate (**²H₅-115**) (287mg, 36%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3395, 2937, 1736, 1716, 1670, 1441, 1366, 1229, 1021, 954, 870; δ_H (CDCl₃, 500 MHz) 5.31 (1H, t, J 6.8, H-6), 5.34 (1H, t, J 7.0, H-2), 4.69 (2H, d, J 7.0, H-1), 2.25-2.16 (2H, m, H-5), 2.15-2.11 (2H, m, H-4), 2.08 (3H, s, H-12), 1.73 (3H, s, H-9); δ_c (CDCl₃, 125 MHz) 171.7 (C-11), 142.7 (C-3), 135.1 (C-7), 124.4 (C-6), 118.6 (C-2), 69.1 (C-8*), 61.6 (C-1), 39.0 (C-4), 25.7 (C-5), 21.3 (C-12), 16.7 (C-9), 13.7 (C-10*). HRMS: Found: 240.1617. C₁₂H₁₅²H₅O₃ (M+Na⁺) Calculated 240.1613. * Multiplet

(2Z,6E)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-yl acetate

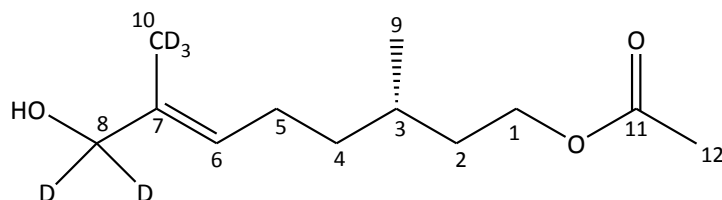
(²H₅)-116) (²H₅)-8-Hydroxyneryl acetate)



Following the general allylic oxidation procedure using ²H₆-nerol (²H₆-62) (402 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-2,6-octadien-1-yl acetate (²H₅-116) (213 mg, 42%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3385, 2935, 1732, 1711, 1675, 1441, 1368, 1229, 1021, 955, 873; δ_{H} (CDCl₃, 500 MHz) 5.40 (1H, t, *J* 6.9, H-6), 5.37 (1H, t, *J* 7.3, H-2), 4.57 (2H, d, *J* 7.3, H-1), 2.17-2.14 (2H, m, H-4 and H-5), 2.06 (3H, s, H-12), 1.78 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 171.2 (C-11), 142.0 (C-3), 135.5 (C-7), 125.0 (C-6), 119.5 (C-2), 68.5 (C-8*), 61.2 (C-1), 31.8 (C-4), 25.9 (C-5), 23.4 (C-12), 21.1 (C-9), 13.3 (C-10*). HRMS: Found: 240.1612. C₁₂H₁₅²H₅O₃ (M+Na⁺) Calculated 240.1613. * Multiplet

(3S,6E)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-6-octen-1-yl acetate

(²H₅-(S)-117) (²H₅)-8-Hydroxy-(S)-citronellyl acetate)

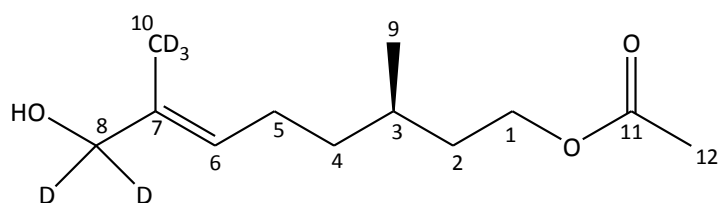


Following the general allylic oxidation procedure using ²H₆-(S)-citronellol (²H₆-(S)-57) (371 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3S,6E)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-6-octen-1-yl acetate (²H₅-(S)-117) (201 mg, 43%) as a colourless oil: $[\alpha]_{\text{D}}^{20}$ -4.1 (c 1.0, MeOH); $\nu_{\max}$

(thin film)/cm⁻¹ 3391, 2937, 1737, 1670, 1441, 1366, 1234, 1021, 956, 870; δ_{H} (CDCl₃, 500 MHz) 5.48 (1H, t, *J* 6.7, H-6), 4.13-4.11 (2H, m, H-1), 2.26-2.21 (2H, m, H-5), 2.02 (3H, s, H-12), 1.64-1.60 (1H, m, H-2a), 1.59-1.57 (1H, m, H-3), 1.53-1.50 (1H, m, H-2b), 1.37-1.33 (1H, m, H-4a), 1.25-1.22 (1H, m, H-4b), 0.91 (3H, d, *J* 6.5, H-9); δ_{C} (CDCl₃, 125 MHz) 171.5 (C-11), 134.8 (C-7), 126.9 (C-6), 69.8 (C-8*), 63.2 (C-1), 36.7 (C-4), 35.3 (C-2), 29.9 (C-3), 25.5 (C-5), 19.5 (C-12), 14.0 (C-9), 13.5 (C-10*). HRMS: Found: 242.1767. C₁₂H₁₇²H₅O₃ (M+Na⁺) Calculated 242.1775. * Multiplet

(3*R*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-6-octen-1-yl acetate

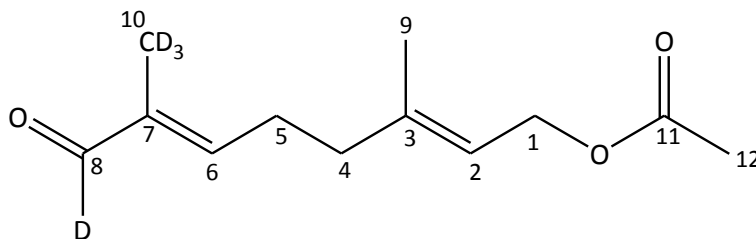
(²H₅-(*R*)-117) (²H₅)-8-Hydroxy-(*R*)-citronellyl acetate)



Following the general allylic oxidation procedure using ²H₆-(*R*)-nerol (²H₆-(*R*)-62) (163 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3*R*,6*E*)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-8-hydroxy-6-octen-1-yl acetate (²H₅-(*R*)-116) (78 mg, 38%) as a colourless oil: [α]_D²⁰ +4.0 (*c* 1.0, MeOH); ν_{max} (thin film)/cm⁻¹ 3396, 2936, 1737, 1672, 1441, 1367, 1237, 1021, 953, 874; δ_{H} (CDCl₃, 500 MHz) 5.45 (1H, t, *J* 6.7, H-6), 4.14-4.11 (2H, m, H-1), 2.24-2.21 (2H, m, H-5), 2.01 (3H, s, H-12), 1.70-1.69 (1H, m, H-2a), 1.57-1.54 (1H, m, H-3), 1.43-1.40 (1H, m, H-2b), 1.40-1.37 (1H, m, H-4a), 1.28-1.22 (1H, m, H-4b), 0.92 (3H, d, *J* 6.5, H-9); δ_{C} (CDCl₃, 125 MHz) 171.4 (C-11), 134.6 (C-7), 126.5 (C-6), 69.0 (C-8*), 63.2 (C-1), 36.4 (C-4), 35.2 (C-2), 29.9 (C-3), 25.3 (C-5), 19.5 (C-12), 13.9 (C-9), 13.7 (C-10*). HRMS: Found: 242.1767. C₁₂H₁₇²H₅O₃ (M+Na⁺) Calculated 242.1775. * Multiplet

(2*E*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-2,6-octadien-1-yl acetate (²H₄-118)

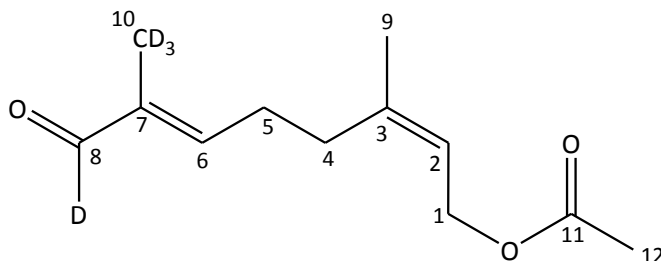
(²H₄-8-Oxogeranyl acetate)



Following the general allylic oxidation procedure using ²H₆-geranyl acetate (²H₄-112) (503 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-2,6-octadien-1-yl acetate (²H₄-118) (64 mg, 12%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2966, 1732, 1670, 1638, 1441, 1368, 1230, 1030, 953, 607; δ_{H} (CDCl₃, 500 MHz) 6.45 (1H, t, *J* 7.3, H-6), 5.39 (2H, t, *J* 7.0, H-2), 4.58 (2H, d, *J* 7.0, H-1), 2.52-2.45 (2H, m, H-5), 2.25-2.21 (2H, m, H-4), 2.04 (3H, s, H-12), 1.73 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 195.8 (C-8*), 171.3 (C-11), 141.7 (C-3), 135.0 (C-7), 125.5 (C-6), 118.7 (C-2), 61.5 (C-1), 39.1 (C-4), 25.7 (C-5), 21.1 (C-12), 16.4 (C-9), 9.4 (C-10*). HRMS: Found: 237.1406. C₁₂H₁₄²H₄O₃ (M+Na⁺) Calculated 237.1399. * Multiplet

(2*Z*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-2,6-octadien-1-yl acetate (²H₄-119)

(²H₄-8-Oxoneryl acetate)



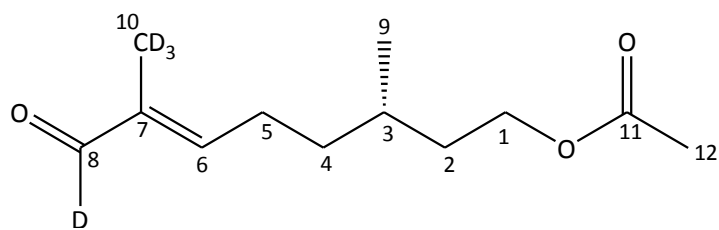
Following the general allylic oxidation procedure using ²H₆-neryl acetate (²H₄-113) (371 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2*Z*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-2,6-octadien-1-yl acetate

(²H₄-119) (59 mg, 15%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2986, 1738, 1671, 1637, 1441, 1369, 1230, 1035, 956, 608; δ_{H} (CDCl₃, 500 MHz) 6.48 (1H, t, *J* 7.2, H-6), 5.46 (1H, t, *J* 7.2, H-2), 4.60 (2H, d, *J* 7.2, H-1), 2.51-2.44 (2H, m, H-5), 2.33 (2H, t, *J* 7.2, H-4), 2.04 (3H, s, H-12), 1.78 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 195.5 (C-8*), 171.7 (C-11), 151.5 (C-3), 140.8 (C-7), 139.5 (C-6), 120.4 (C-2), 60.3 (C-1), 30.7 (C-4), 27.8 (C-5), 23.4 (C-9), 21.1 (C-12), 9.7 (C-8*). HRMS: Found: 237.1398. C₁₂H₁₄²H₄O₃ (M+Na⁺) Calculated 237.1399.

* Multiplet

(3*S*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-6-octen-1-yl acetate (²H₄-(*S*)-120)

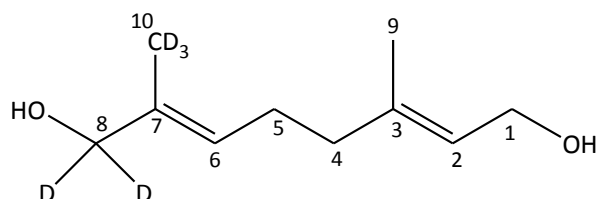
(²H₄-8-Oxo-(*S*)-citronellyl acetate)



Following the general allylic oxidation procedure using ²H₆-(*S*)-citronellyl acetate (²H₄-(*S*)-114) (480 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-6-octen-1-yl acetate (²H₄-(*S*)-120) (61 mg, 12%) as a colourless oil: $[\alpha]_{\text{D}}^{20}$ -5.2 (*c* 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2959, 2926, 1736, 1669, 1638, 1460, 1367, 1233, 1048, 928, 607; δ_{H} (CDCl₃, 500 MHz) 6.41 (1H, t, *J* 6.7, H-6), 4.11-4.00 (2H, m, H-1), 2.37-2.23 (2H, m, H-5), 1.98 (3H, s, H-12), 1.67-1.60 (1H, m, H-2a), 1.58-1.52 (1H, m, H-3), 1.52-1.44 (1H, m, H-4a), 1.44-1.38 (1H, m, H-2b), 1.34-1.25 (1H, m, H-4b), 0.88 (3H, d, *J* 6.5, H-9); δ_{C} (CDCl₃, 125 MHz) 195.6 (C-8*), 171.2 (C-11), 154.6 (C-7), 139.2 (C-6), 62.8 (C-1), 35.4 (C-4), 35.3 (C-2), 29.7 (C-3), 26.5 (C-5), 21.1 (C-12), 19.2 (C-9), 9.9 (C-10*). HRMS: Found: 239.1565. C₁₂H₂₂O₃ (M+Na⁺) Calculated 239.1556. * Multiplet

(2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-ol (²H₅-72)

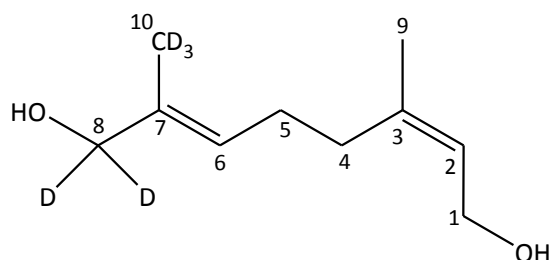
(²H₅-8-Hydroxygeraniol)



Following the general acetate deprotection procedure using ²H₅-8-hydroxygeranyl acetate (²H₅-115) (73 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-2,6-octadien-1-ol (²H₅-72) (35 mg, 55%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3308, 2961, 2920, 2851, 1668, 1441, 1380, 1260, 1088, 1011, 959, 868, 798; δ_{H} (CDCl₃, 500 MHz) 5.42 (2H, m, H-2 and H-6), 4.07 (2H, d, *J* 7.0, H-1), 2.18-2.12 (4H, m, H-4 and H-5), 1.53 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 141.3 (C-3), 134.1 (C-7), 126.5 (C-6), 121.9 (C-2), 66.9 (C-1), 57.5 (C-8*), 38.0 (C-5), 25.6 (C-4), 15.1 (C-9), 12.2 (C-10*). HRMS: Found: 198.1516. C₁₀H₁₃²H₅O₂ (M+Na⁺) Calculated 198.1513. * Multiplet

(2*Z*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-ol (²H₅-94)

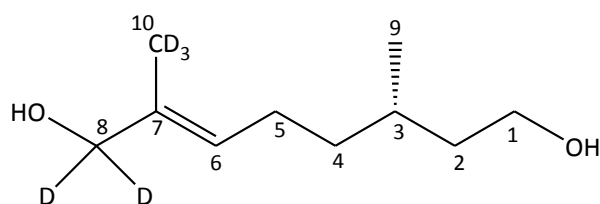
(²H₅-8-Hydroxyneryl)



Following the general acetate deprotection procedure using ²H₅-8-hydroxyneryl acetate (²H₅-116) (34 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (2*Z*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-2,6-octadien-1-ol (²H₅-94) (18 mg, 61%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3304, 2961, 2925, 2857, 1667, 1446, 1377, 1246, 1191, 1116, 1088, 997, 959, 869; δ_{H} (CDCl₃, 500 MHz)

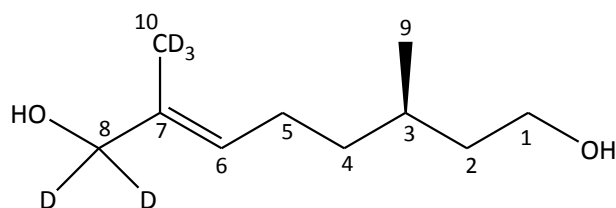
5.43-5.31 (2H, m, H-2 and H-6), 4.03 (2H, d, J 7.1, H-1), 2.20-2.12 (4H, m, H-4 and H-5), 1.71 (3H, s, H-9); δ_c (CDCl₃, 125 MHz) 141.3 (C-3), 133.9 (C-7), 126.2 (C-6), 122.9 (C-2), 68.0 (C-1), 57.7 (C-8*), 31.0 (C-5), 25.6 (C-4), 22.4 (C-9), 12.5 (C-10*). HRMS: Found: 198.1522. C₁₀H₁₃²H₅O₂ (M+Na⁺) Calculated 198.1513. * Multiplet

(3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-6-octen-1-ol (²H₅-(*S*)-90)



Following the general acetate deprotection procedure using ²H₅-8-hydroxy-(*S*)-citronellol (²H₅-(*S*)-117) (47 mg), the crude product was purified on silica gel (30% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-6-octen-1-yl acetate (²H₅-(*S*)-90) (24 mg, 59%) as a colourless oil: $[\alpha]_D^{20}$ -5.9 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3415, 2960, 2933, 2860, 1738, 1447, 1391, 1239, 1113, 1045, 840, 820, 743, 701, 610; δ_H (CDCl₃, 500 MHz) 5.44 (1H, t, J 7.0, H-6), 3.55-3.45 (2H, m, H-1), 2.11-1.97 (2H, m, H-5), 1.49-1.35 (1H, m, H-2), 1.30-1.19 (2H, m, H-4), 1.18-1.09 (1H, m, H-3), 0.86 (3H, d, J 6.5, H-9); δ_c (CDCl₃, 125 MHz) 134.0 (C-7), 127.5 (C-6), 67.9 (C-8*), 60.2 (C-1), 38.4 (C-4), 35.8 (C-3), 28.3 (C-2), 24.1 (C-5), 19.0 (C-9), 12.8 (C-10*). HRMS: Found: 200.1646. C₁₀H₁₅²H₅O₂ (M+Na⁺) Calculated 200.1670. * Multiplet

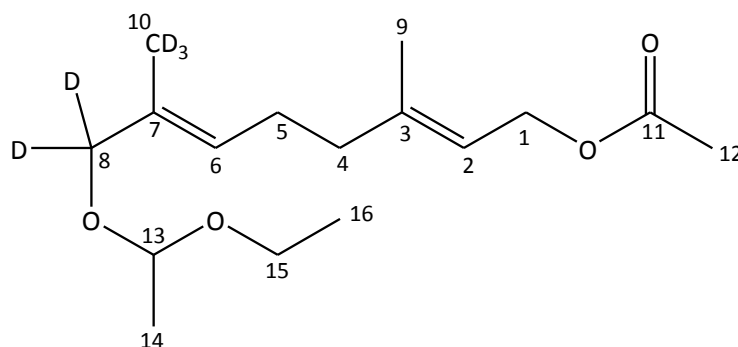
(3*R*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-6-octen-1-ol (²H₅-(*R*)-90)



Following the general acetate deprotection procedure using ²H₅-8-hydroxy-(*R*)-citronellol (²H₅-(*R*)-117) (23 mg), the crude product was purified on silica gel (30% EtOAc

in petroleum ether 40-60 °C) to give (3*R*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-6-octen-1-yl acetate (²H₅-(**R**)-**90**) (11 mg, 56%) as a colourless oil: $[\alpha]_D^{20} +7.2$ (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3420, 2963, 2933, 2864, 1734, 1448, 1391, 1238, 1111, 1045, 840, 822, 744, 701, 608; δ_H (CDCl₃, 500 MHz) 5.40 (1H, t, *J* 7.0, H-6), 3.69-3.50 (2H, m, H-1), 2.12-1.97 (2H, m, H-5), 1.66-1.53 (2H, m, H-2), 1.43-1.35 (2H, m, H-4), 1.28-1.19 (1H, m, H-3), 0.89 (3H, d, *J* 6.5, H-9); δ_C (CDCl₃, 125 MHz) 134.5 (C-7), 127.0 (C-6), 68.3 (C-8*), 60.8 (C-1), 39.4 (C-4), 36.8 (C-3), 28.7 (C-2), 25.1 (C-5), 19.6 (C-9), 13.4 (C-10*). HRMS: Found: 200.1658. C₁₀H₁₅²H₅O₂ (M+Na⁺) Calculated 200.1670. * Multiplet

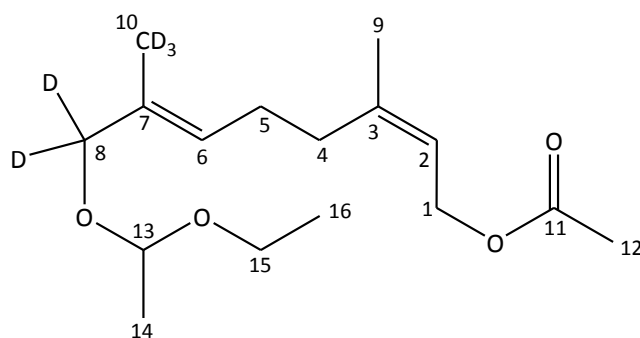
(2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (²H₅-121**)**



Following the general acetal protection procedure using ²H₅-8-hydroxygeranyl acetate (²H₅-**115**) (176 mg), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (²H₅-**121**) (126 mg, 50%) as a pale yellow oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2974, 2920, 2866, 1738, 1677, 1447, 1390, 1365, 1230, 1133, 1085, 1016, 955, 850, 800, 610; δ_H (CDCl₃, 500 MHz) 5.44 (1H, t, *J* 6.8, H-2/6), 5.44 (1H, t, *J* 6.8, H-2/6), 4.75 (1H, q, *J* 5.5, H-13), 4.62 (2H, d, *J* 6.8, H-1), 3.60 (1H, dq, *J* 9.2 and 7.0, H-15a), 3.52 (1H, dq, *J* 9.2 and 7.0, H-15b), 2.21-2.16 (2H, m, H-5), 2.12-2.08 (2H, m, H-4), 2.08 (3H, s, H-12), 1.75 (3H, s, H-9), 1.35 (3H, d, *J* 5.5, H-14), 1.22 (3H, t, *J* 7.0, H-16); δ_C (CDCl₃, 125 MHz) 171.1 (C-11), 141.1 (C-3), 132.4 (C-7), 126.9 (C-6), 120.2 (C-2), 98.7 (C-13), 71.1 (C-8*),

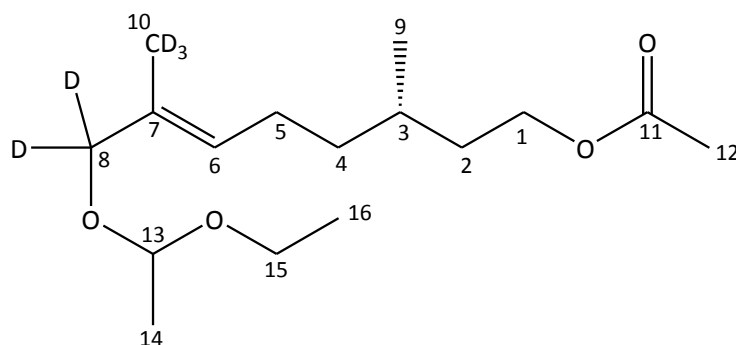
62.6 (C-1), 59.8 (C-15), 39.3 (C-4), 25.7 (C-5), 22.2 (C-12), 20.0 (C-14), 16.9 (C-9), 15.6 (C-16), 13.6 (C-10*). HRMS: Found: 312.2197. C₁₆H₂₃²H₅O₄ (M+Na⁺) Calculated 312.2194. * Multiplet

(2Z,6E)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (²H₅-122)



Following the general acetal protection procedure using ²H₅-8-hydroxyneryl acetate (²H₅-116) (120 mg), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (²H₅-122) (103 mg, 60%) as a pale yellow oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2979, 2921, 2865, 1738, 1675, 1447, 1393, 1363, 1230, 1134, 1085, 1016, 958, 851, 801, 610; δ_{H} (CDCl₃, 500 MHz) 5.44-5.38 (2H, m, H-2 and H-6), 4.75 (1H, q, *J* 5.6, H-13), 4.61 (2H, d, *J* 7.1, H-1), 3.62 (1H, dq, *J* 9.2 and 7.1, H-15a), 3.48 (1H, dq, *J* 9.2 and 7.1, H-15b), 2.21-2.15 (4H, m, H-4 and H-5), 2.06 (3H, s, H-12), 1.71 (3H, s, H-9), 1.32 (3H, d, *J* 5.6, H-14), 1.20 (3H, t, *J* 7.1, H-16); δ_{C} (CDCl₃, 125 MHz) 171.3 (C-11), 141.1 (C-3), 132.2 (C-7), 127.9 (C-6), 120.1 (C-2), 98.7 (C-13), 71.8 (C-8*), 62.4 (C-1), 59.8 (C-15), 39.3 (C-4), 25.7 (C-5), 22.4 (C-12), 20.3 (C-14), 16.6 (C-9), 15.6 (C-16), 13.9 (C-10*). HRMS: Found: 312.2206. C₁₆H₂₃²H₅O₄ (M+Na⁺) Calculated 312.2194. * Multiplet

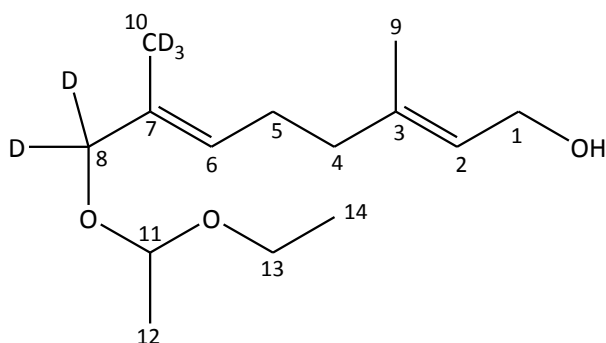
(3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-6-octen-1-yl acetate (²H₅-(*S*)-123)



Following the general acetal protection procedure using ²H₅-8-hydroxy-(*S*)-citronellyl acetate (²H₅-(*S*)-117) (119 mg), the crude product was purified on silica gel (10% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-yl acetate (²H₅-122) (105 mg, 62%) as a pale yellow oil: $[\alpha]_D^{20}$ -3.1 (*c* 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2979, 2928, 2867, 1677, 1445, 1393, 1365, 1230, 1133, 1088, 1016, 955, 852, 803, 611; δ_{H} (CDCl₃, 500 MHz) 5.42 (1H, t, *J* 7.0, H-6), 4.73 (1H, q, *J* 5.4, H-13), 4.18-4.06 (2H, m, H-1), 3.66 (1H, dq, *J* 9.0 and 7.0, H-15a), 3.52 (1H, dq, *J* 9.0 and 7.0, H-15b), 2.14-1.99 (2H, m, H-5), 2.07 (3H, s, H-12), 1.74-1.63 (1H, m, H-2a), 1.61-1.52 (1H, m, H-3), 1.50-1.44 (1H, m, H-2b), 1.44-1.37 (1H, m, H-4a), 1.34 (3H, d, *J* 5.4, H-14), 1.27-1.20 (1H, m, H-4b), 1.23 (3H, t, *J* 7.0, H-16), 0.94 (3H, d, *J* 6.5, H-9); δ_{C} (CDCl₃, 125 MHz) 172.0 (C-11), 134.5 (C-7), 127.6 (C-6), 98.3 (C-13), 72.5 (C-8*), 62.6 (C-1), 59.8 (C-15), 38.3 (C-4), 35.6 (C-3), 27.7 (C-2), 25.2 (C-5), 22.1 (C-12), 20.3 (C-14), 19.8 (C-9), 15.3 (C-16), 13.5 (C-10*). HRMS: Found: 314.2369. C₁₆H₂₅²H₅O₄ (M+Na⁺) Calculated 314.2351. * Multiplet

(2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol

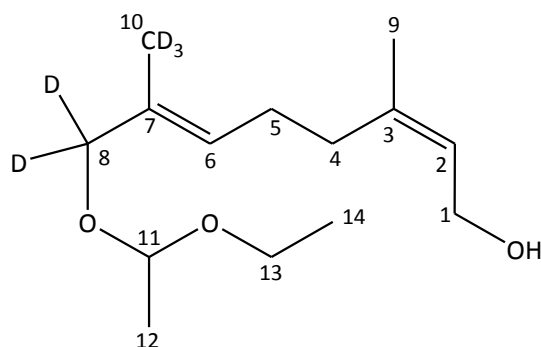
(²H₅-124)



Following the general acetate deprotection procedure using (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (²H₅-121) (154 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (²H₅-124) (96 mg, 73%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 3423, 2978, 2899, 1669, 1482, 1380, 1128, 1104, 1058, 998, 919, 730, 646; δ_{H} (CDCl₃, 500 MHz) 5.46-5.39 (2H, m, H-2 and H-6), 4.72 (1H, q, *J* 5.5, H-11), 4.16 (2H, d, *J* 7.0, H-1), 3.66 (1H, dq, *J* 9.2 and 7.0, H-13a), 3.51 (1H, dq, *J* 9.2 and 7.0, H-13b), 2.23-2.15 (2H, m, H-5), 2.13-2.05 (2H, m, H-4), 1.70 (3H, s, H-9), 1.46 (1H, s, OH), 1.34 (3H, d, *J* 5.5, H-12), 1.23 (3H, t, *J* 7.0, H-14); δ_{C} (CDCl₃, 125 MHz) 139.1 (C-3), 132.3 (C-7), 127.7 (C-6), 123.9 (C-2), 98.6 (C-11), 72.8 (C-8*), 60.3 (C-13), 59.4 (C-1), 39.1 (C-4), 25.9 (C-5), 19.9 (C-12), 16.2 (C-9), 15.3 (C-14), 14.1 (C-10*). HRMS: Found: 270.2094. C₁₄H₂₁²H₅O₃ (M+Na⁺) Calculated 270.2088. * Multiplet

(2Z,6E)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol

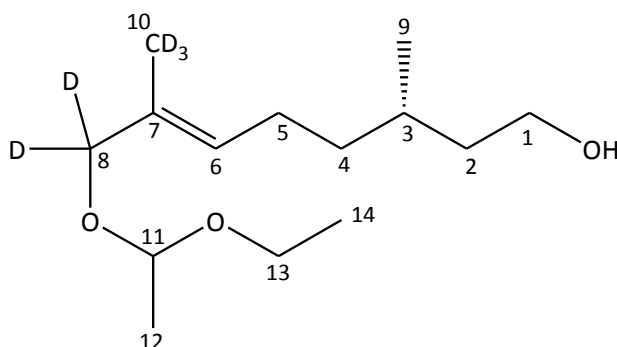
(²H₅-125)



Following the general acetate deprotection procedure using (2Z,6E)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-yl acetate (²H₅-122) (113 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (²H₅-125) (75 mg, 78%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3381, 2976, 2925, 2878, 1673, 1448, 1380, 1290, 1104, 1085, 1057, 1012, 82; δ_{H} (CDCl₃, 500 MHz) 5.46-5.44 (2H, m, H-2 and H-6), 4.74 (1H, q, *J* 5.5, H-11), 4.09 (2H, d, *J* 7.0, H-1), 3.66 (1H, dq, *J* 9.2 and 7.0, H-13a), 3.49 (1H, dq, *J* 9.2 and 7.0, H-13b), 2.22-2.12 (4H, m, H-4 and H-5), 1.88 (1H, s, OH), 1.76 (3H, s, H-9), 1.33 (3H, d, *J* 5.5, H-12), 1.22 (3H, t, *J* 7.0, H-14); δ_{C} (CDCl₃, 125 MHz) 138.4 (C-3), 132.7 (C-7), 127.4 (C-6), 125.3 (C-2), 98.7 (C-11), 70.9 (C-8*), 60.3 (C-13), 59.0 (C-1), 31.3 (C-4), 25.8 (C-5), 23.3 (C-12), 19.7 (C-9), 15.3 (C-14), 14.5 (C-10*). HRMS: Found: 270.2084. C₁₄H₂₁²H₅O₃ (M+Na⁺) Calculated 270.2088. * Multiplet

(3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-6-octen-1-ol

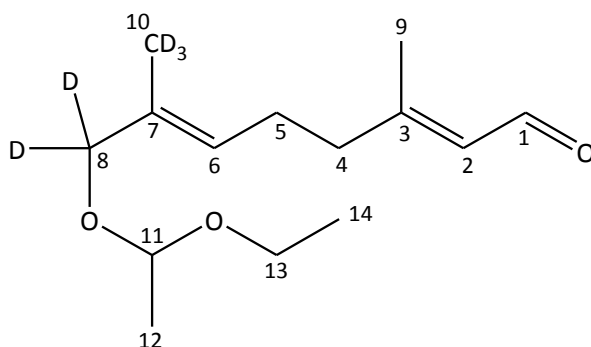
(²H₅-(*S*)-126)



Following the general acetate deprotection procedure using (3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-yl acetate (²H₅-(*S*)-123) (102 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-ol (²H₅-(*S*)-126) (74 mg, 85%) as a pale yellow oil: $[\alpha]_D^{20}$ -2.9 (*c* 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3402, 2959, 2927, 2872, 1454, 1381, 1128, 1105, 1055, 1007, 958, 850; δ_{H} (CDCl₃, 500 MHz) 5.43 (1H, t, *J* 7.0, H-6), 4.73 (1H, q, *J* 5.5, H-11), 3.76-3.62 (3H, m, H-1 and H-13a), 3.49 (1H, dq, *J* 9.0 and 7.0, H-13b), 2.14-1.99 (2H, m, H-5), 1.68-1.56 (3H, m, H-2), 1.46-1.36 (3H, m, H-3 and H-4), 1.35 (3H, d, *J* 5.5, H-12), 1.29-1.25 (1H, m, H-4b), 1.23 (3H, t, *J* 7.0, H-14), 0.93 (3H, d, *J* 6.5, H-9); δ_{C} (CDCl₃, 125 MHz) 131.9 (C-7), 128.5 (C-6), 98.8 (C-11), 73.8 (C-8*), 61.2 (C-1), 60.7 (C-13), 39.9 (C-4), 36.9 (C-3), 29.3 (C-2), 25.3 (C-5), 19.9 (C-12), 19.5 (C-9), 15.4 (C-14), 12.2 (C-10*). HRMS: Found: 272.2231. C₁₄H₂₃²H₅O₃ (M+Na⁺) Calculated 272.2245. * Multiplet

(2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al

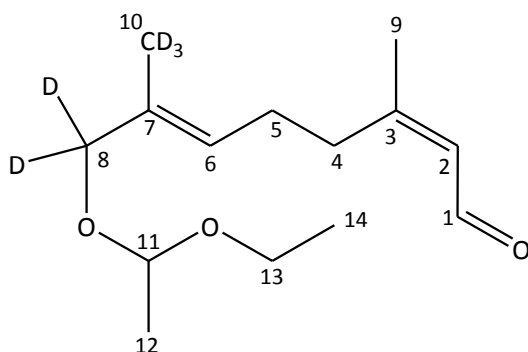
(²H₅-127)



Following the general IBX oxidation procedure using (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (²H₅-124) (81 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (²H₅-127) (71 mg, 88%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 2974, 2925, 2877, 1668, 1447, 1380, 1293, 1103, 1085, 1056, 1074, 841; δ_{H} (CDCl₃, 500 MHz) 10.01 (1H, d, *J* 8.0, H-1), 5.89-5.86 (1H, m, H-2), 5.42 (1H, t, *J* 8.0, H-6), 4.70 (1H, q, *J* 5.6, H-11), 3.65 (1H, dq, *J* 9.1 and 7.1, H-13a), 3.55 (1H, dq, *J* 9.1 and 7.1, H-13b), 2.32-2.28 (4H, m, H-4 and H-5), 2.29 (3H, s, H-9), 1.31 (3H, d, *J* 5.6, H-12), 1.20 (3H, t, *J* 7.1, H-14); δ_{C} (CDCl₃, 125 MHz) 191.5 (C-1), 162.5 (C-3), 133.4 (C-7), 127.8 (C-2), 126.6 (C-6), 99.0 (C-11), 70.5 (C-8*), 60.9 (C-13), 40.8 (C-4), 25.6 (C-5), 19.9 (C-12), 17.2 (C-9), 15.2 (C-14), 13.9 (C-10*). HRMS: Found: 268.1918. C₁₄H₁₉²H₅O₃ (M+Na⁺) Calculated 268.1932. * Multiplet

(2Z,6E)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al

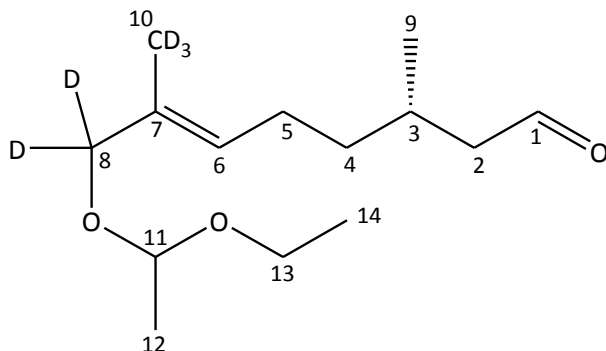
(²H₅-128)



Following the general IBX oxidation procedure using (2Z,6E)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-ol (²H₅-125) (66 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (2Z,6E)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (²H₅-128) (58 mg, 89%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 2981, 2926, 2877, 1669, 1450, 1380, 1294, 1103, 1087, 1066, 1072, 840; δ_{H} (CDCl₃, 500 MHz) 9.93 (1H, d, *J* 7.0, H-1), 5.89 (1H, d, *J* 7.0, H-2), 5.42 (1H, t, *J* 7.2, H-6), 4.70 (1H, q, *J* 5.5, H-11), 3.65-3.63 (1H, m, H-13a), 3.55-3.52 (1H, m, H-13b), 2.32-2.31 (2H, m, H-4), 2.31-2.29 (2H, m, H-5), 2.04 (3H, s, H-9), 1.37 (3H, d, *J* 5.5, H-12), 1.21 (3H, t, *J* 7.0, H-14); δ_{C} (CDCl₃, 125 MHz) 191.0 (C-1), 162.6 (C-3), 133.2 (C-7), 127.8 (C-2), 126.2 (C-6), 99.0 (C-11), 70.5 (C-8*), 60.7 (C-13), 33.2 (C-4), 26.1 (C-5), 25.2 (C-9), 19.8 (C-12), 15.7 (C-14), 13.4 (C-10*). HRMS: Found: 268.1911. C₁₄H₁₉²H₅O₃ (M+Na⁺) Calculated 268.1932. * Multiplet

(3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-(1-ethoxyethoxy)-6-octen-1-al

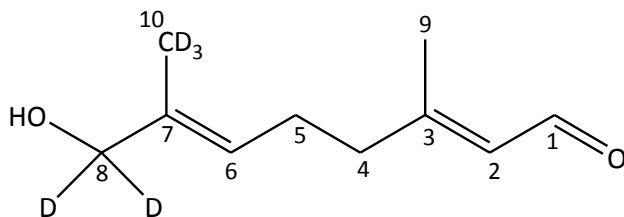
(²H₅-(*S*)-129)



Following the general IBX oxidation procedure using (3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-ol (²H₅-(*S*)-126) (58 mg), the crude product was purified on silica gel (15% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-al (²H₅-(*S*)-129) (48 mg, 84%) as a pale yellow oil: $[\alpha]_D^{20}$ -2.4 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2996, 2935, 2879, 1667, 1447, 1381, 1293, 1108, 1085, 1054, 1071, 845; δ_{H} (CDCl₃, 500 MHz) 9.80 (1H, s, H-1), 5.43 (1H, t, *J* 7.2, H-6), 4.73 (1H, q, *J* 5.5, H-11), 3.64 (1H, dq, *J* 9.2 and 7.0, H-13a), 3.52 (1H, dq, *J* 9.2 and 7.0, H-13b), 2.47-2.45 (1H, m, H-2a), 2.43-2.39 (1H, m, H-2b), 2.12-1.99 (2H, m, H-5), 1.46-1.39 (2H, m, H-3 and H-4a), 1.38 (3H, d, *J* 5.5, H-12), 1.36-1.29 (1H, m, H-4b), 1.23 (3H, t, *J* 7.0, H-14), 0.98 (3H, d, *J* 6.3, H-9); δ_{C} (CDCl₃, 125 MHz) 191.2 (C-1), 135.9 (C-7), 127.5 (C-6), 94.8 (C-11), 74.1 (C-8*), 58.8 (C-13), 37.9 (C-4), 35.4 (C-3), 29.3 (C-2), 25.7 (C-5), 20.9 (C-12), 16.5 (C-9), 15.9 (C-14), 12.3 (C-10*). HRMS: Found: 270.2102. C₁₄H₂₁²H₅O₃ (M+Na⁺) Calculated 270.2088. * Multiplet

(2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-al (²H₅-73)

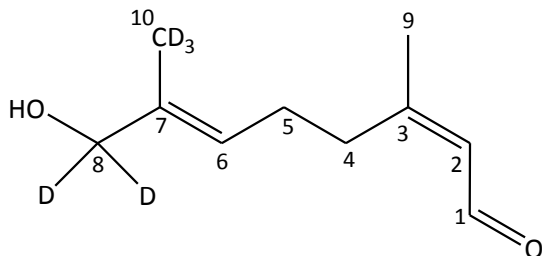
(²H₅-8-Hydroxygeranial)



Following the general acetal deprotection procedure using (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (²H₅-127) (44 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-2,6-octadien-1-al (²H₅-73) (15 mg, 48%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3425, 2968, 2933, 2872, 1716, 1664, 1443, 1379, 1146, 1011, 850; δ_{H} (CDCl₃, 500 MHz) 10.04 (1H, d, *J* 8.2, H-1), 5.86 (1H, d, *J* 8.2, H-2), 4.40 (1H, t, *J* 8.2, H-6), 2.33-2.30 (4H, m, H-4 and H-5), 2.00 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 191.6 (C-1), 163.5 (C-3), 136.4 (C-7), 127.6 (C-2), 123.9 (C-6), 68.6 (C-8*), 40.2 (C-4), 24.9 (C-5), 17.9 (C-9), 13.9 (C-10*). HRMS: Found: 196.1372. C₁₀H₁₁²H₅O₂ (M+Na⁺) Calculated 196.1357. * Multiplet

(2*Z*,6*E*)-[8,8,10,10,10-²H₅]-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-al (²H₅-130)

(²H₅-8-Hydroxyneral)

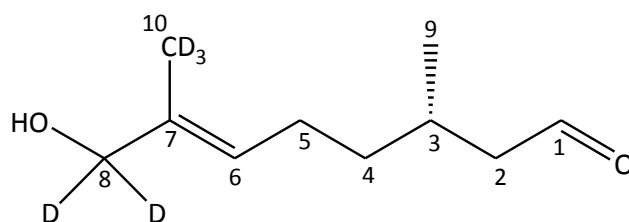


Following the general acetal deprotection procedure using (2*Z*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-(1-ethoxyethoxy)-2,6-octadien-1-al (²H₅-128) (42 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*Z*,6*E*)-[8,8,10,10,10-²H₅]-3,7-dimethyl-8-hydroxy-2,6-octadien-1-al (²H₅-130) (14 mg,

47%) as a colourless oil: $\nu_{\max}$ (thin film)/ cm^{-1} 3427, 2974, 2933, 2870, 1716, 1666, 1443, 1379, 1141, 1016, 852; δ_{H} (CDCl_3 , 500 MHz) 9.87 (1H, d, J 8.2, H-1), 5.86 (1H, d, J 8.2, H-2), 5.40 (1H, t, J 8.2, H-6), 2.63 (2H, t, J 7.1, H-4), 2.32-2.25 (2H, m, H-5), 2.00 (3H, s, H-9); δ_{C} (CDCl_3 , 125 MHz) 191.8 (C-1), 163.1 (C-3), 137.4 (C-7), 128.8 (C-2), 124.2 (C-6), 68.5 (C-8*), 32.2 (C-4), 26.9 (C-5), 25.2 (C-9), 13.8 (C-10*). HRMS: Found: 196.1380. $\text{C}_{10}\text{H}_{11}^2\text{H}_5\text{O}_2$ ($\text{M}+\text{Na}^+$) Calculated 196.1357. * Multiplet

(3*S*,6*E*)-[8,8,10,10,10- $^2\text{H}_5$]-3,7-Dimethyl-8-hydroxy-6-octen-1-al ($^2\text{H}_5$ -(*S*)-131)

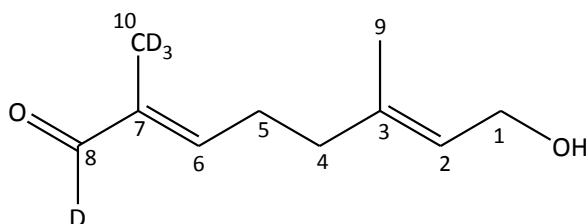
($^2\text{H}_5$ -8-Hydroxy-(*S*)-citronellal)



Following the general acetal deprotection procedure using (3*S*,6*E*)-[8,8,10,10,10- $^2\text{H}_5$]-3,7-dimethyl-8-(1-ethoxyethoxy)-6-octen-1-al ($^2\text{H}_5$ -(*S*)-129) (28 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-[8,8,10,10,10- $^2\text{H}_5$]-3,7-dimethyl-8-hydroxy-6-octen-1-al ($^2\text{H}_5$ -(*S*)-131) (11 mg, 56%) as a pale yellow oil: $[\alpha]_D^{20}$ -5.8 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/ cm^{-1} 3423, 2966, 2931, 2873, 1712, 1443, 1780, 1140, 1013, 852; δ_{H} (CDCl_3 , 500 MHz) 9.77 (1H, m, H-1), 5.46 (1H, t, J 7.0, H-6), 2.45-2.39 (1H, m, H-2a), 2.39-2.35 (1H, m, H-2b), 2.14-2.05 (3H, m, H-3 and H-5), 1.49-1.39 (1H, m, H-4a), 1.39-1.27 (1H, m, H-4b), 1.03 (3H, s, H-9); δ_{C} (CDCl_3 , 125 MHz) 200.8 (C-1), 135.4 (C-7), 125.3 (C-6), 68.9 (C-8*), 51.4 (C-2), 36.7 (C-4), 28.0 (C-5), 21.0 (C-3), 19.9 (C-9), 13.8 (C-10*). HRMS: Found: 198.1501. $\text{C}_{10}\text{H}_{13}^2\text{H}_5\text{O}_2$ ($\text{M}+\text{Na}^+$) Calculated 198.1513. * Multiplet

(2*E*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-2,6-octadien-1-ol (²H₄-74)

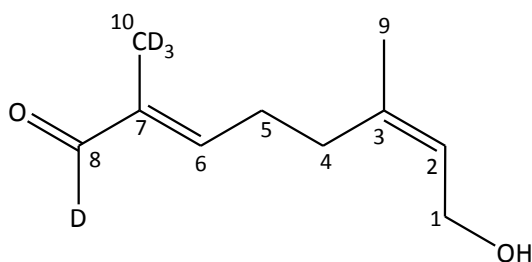
(²H₄-8-Oxogeraniol)



Following the general acetate deprotection procedure using ²H₄-8-oxogeranyl acetate (²H₄-118) (66 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-2,6-octadien-1-ol (²H₄-74) (49 mg, 93%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3333, 2961, 2870, 1710, 1681, 1464, 1389, 1268, 1055, 801; δ_{H} (CDCl₃, 500 MHz) 6.48 (1H, t, *J* 7.6, H-6), 5.28 (1H, t, *J* 7.0, H-2), 4.17 (2H, d, *J* 7.0, H-1), 2.48 (2H, td, *J* 7.6, H-5), 2.21 (2H, t, *J* 7.6, H-4), 1.83 (1H, s, OH), 1.70 (3H, s, H-9); δ_{C} (CDCl₃, 125 MHz) 195.5 (C-8*), 154.4 (C-7), 140.1 (C-3), 137.4 (C-6), 124.8 (C-2), 57.6 (C-1), 37.3 (C-5), 27.2 (C-4), 16.2 (C-10*), 9.5 (C-9). HRMS: Found: 195.1305. C₁₀H₁₂²H₄O₂ (M+Na⁺) Calculated 195.1295. * Multiplet

(2*Z*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-2,6-octadien-1-ol (²H₄-132)

(²H₄-8-Oxonerol)

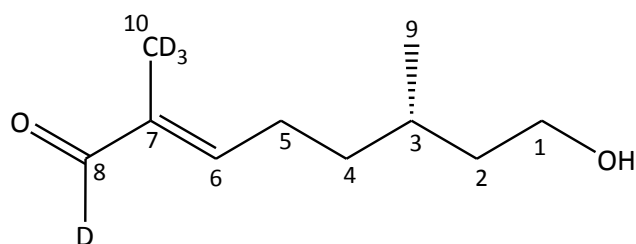


Following the general acetate deprotection procedure using ²H₄-8-oxoneryl acetate (²H₄-119) (58 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*Z*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-2,6-octadien-1-ol (²H₄-132) (44 mg, 95%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3341, 2965, 2854, 1708, 1685, 1463, 1389, 1266, 1056, 800; δ_{H} (CDCl₃, 500 MHz) 6.50 (1H, t, *J* 7.0 H-6), 6.50 (1H,

t, J 7.0 Hz), 4.15 (2H, d, J 7.0, H-1), 2.53 (2H, td, J 7.0, H-5), 2.28 (2H, t, J 7.0, H-4), 1.72 (3H, s, H-9); δ_c (CDCl₃, 125 MHz) 196.1 (C-8*), 154.4 (C-7), 140.1 (C-3), 137.6 (C-6), 124.8 (C-2), 58.6 (C-1), 31.3 (C-5), 27.2 (C-4), 23.3 (C-10*), 9.6 (C-9). HRMS: Found: 195.1288. C₁₀H₁₂²H₄O₂ (M+Na⁺) Calculated 195.1295. * Multiplet

(3*S*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-6-octen-1-ol (²H₄-(*S*)-133)

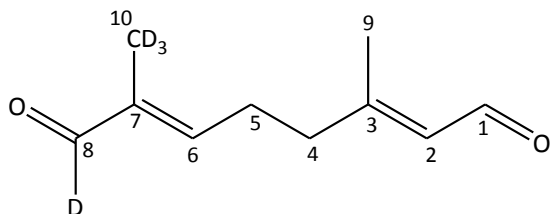
(²H₄-8-Oxo-(*S*)-citronellol)



Following the general acetate deprotection procedure using ²H₄-8-oxo-(*S*)-citronellyl acetate (²H₄-(*S*)-120) (57 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-6-octen-1-ol (²H₄-(*S*)-133) (42 mg, 92%) as a colourless oil: $[\alpha]_D^{20}$ -7.8 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 3386, 2956, 2924, 2871, 1669, 1637, 1460, 1379, 1260, 1096, 1058, 1013, 969, 799; δ_H (CDCl₃, 500 MHz) 6.50 (1H, t, J 6.9, H-6), 3.75-3.69 (2H, m, H-1), 2.40-2.33 (1H, m, H-3), 1.66-0.98 (6H, m, H-2, H-4 and H-5), 1.01 (3H, s, H-9); δ_c (CDCl₃, 125 MHz) 195.7 (C-8*), 155.2 (C-7), 140.4 (C-6), 60.2 (C-1), 39.8 (C-4), 35.3 (C-2), 29.6 (C-3), 26.6 (C-5), 19.9 (C-10*), 9.2 (C-9). HRMS: Found: 197.1466. C₁₀H₁₄²H₄O₂ (M+Na⁺) Calculated 197.1452. * Multiplet

(2*E*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-2,6-octadien-1-al (²H₄-75)

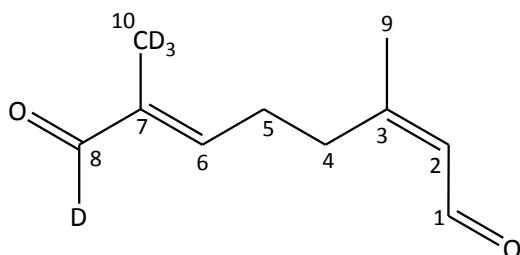
(²H₄-8-Oxogeranial)



Following the general IBX oxidation procedure using ²H₄-8-oxogeraniol (²H₄-74) (36 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*E*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-2,6-octadien-1-al (²H₄-75) (31 mg, 86%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 1708, 1673, 1421, 1359, 1221, 1092, 903, 529; δ_{H} (CDCl₃, 500 MHz) 10.00 (1H, d, *J* 7.6, H-1), 6.45 (1H, td, *J* 7.2 and 1.2, H-6), 5.99 (1H, dq, *J* 7.6 and 1.2, H-2), 2.61 (2H, td, *J* 7.2 and 7.2, H-5), 2.50 (2H, t, *J* 7.2, H-4), 2.20 (3H, d, *J* 1.3, H-9); δ_{C} (CDCl₃, 125 MHz) 195.3 (C-8*), 191.4 (C-1), 161.1 (C-3), 152.6 (C-6), 140.4 (C-7), 127.3 (C-2), 38.0 (C-4), 26.6 (C-5), 17.3 (C-9), 9.8 (C-10*). HRMS: Found: 193.1142. C₁₀H₁₀²H₄O₂ (M+Na⁺) Calculated 193.1139. * Multiplet

(2*Z*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-2,6-octadien-1-al (²H₄-134)

(²H₄-8-Oxoneral)

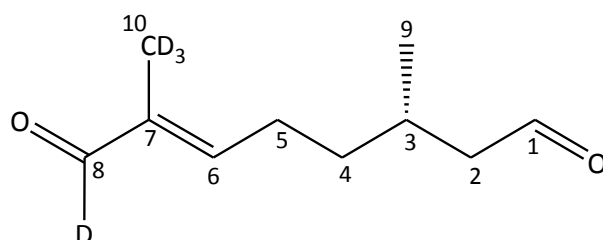


Following the general IBX oxidation procedure using ²H₄-8-oxonerol (²H₄-132) (27 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (2*Z*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-2,6-octadien-1-al (²H₄-134) (24 mg, 91%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 2924, 2855, 1733, 1668, 1638, 1440, 1380, 1234, 1162, 1040; δ_{H} (CDCl₃, 500 MHz) 9.89 (1H, d, *J* 7.6, H-1), 6.48-6.43 (1H, m, H-6),

5.91 (1H, d, *J* 7.6, H-2), 2.71-2.67 (2H, m, H-5), 2.56-1.52 (2H, m, H-4), 1.95 (3H, s, H-9); δ_c (CDCl₃, 125 MHz) 195.9 (C-8*), 190.2 (C-1), 161.1 (C-3), 150.6 (C-6), 140.4 (C-7), 128.8 (C-2), 38.3 (C-4), 27.2 (C-5), 23.6 (C-9), 9.7 (C-10*). HRMS: Found: 193.1130. C₁₀H₁₀²H₄O₂ (M+Na⁺) Calculated 193.1139. * Multiplet

(3*S*,6*E*)-[8,10,10,10-²H₄]-3,7-Dimethyl-8-oxo-6-octen-1-al (²H₄-(*S*)-135)

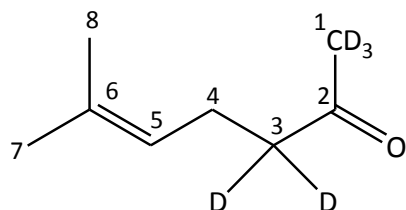
(²H₄-8-Oxo-(*S*)-citronellal)



Following the general IBX oxidation procedure using ²H₄-8-oxo-(*S*)-citronellol (²H₄-(*S*)-**133**) (16 mg), the crude product was purified on silica gel (50% EtOAc in petroleum ether 40-60 °C) to give (3*S*,6*E*)-[8,10,10,10-²H₄]-3,7-dimethyl-8-oxo-6-octen-1-al (²H₄-(*S*)-**135**) (15 mg, 93%) as a pale yellow oil: $[\alpha]_D^{20}$ -9.1 (c 1.0, MeOH); $\nu_{\max}$ (thin film)/cm⁻¹ 2957, 2927, 1721, 1667, 1637, 1460, 1380, 1253, 1050; δ_H (CDCl₃, 500 MHz) 9.81 (1H, t, *J* 2.2, H-1), 6.53 (1H, td, *J* 7.1 and 1.3, H-6), 2.51-2.39 (4H, m, H-2 and H-5), 2.20-2.14 (1H, m, H-3), 1.66-1.59 (1H, m, H-4a), 1.53-1.39 (1H, m, H-4b), 1.00 (3H, d, *J* 7.0, H-9); δ_c (CDCl₃, 125 MHz) 196.6 (C-8*), 203.0 (C-1), 154.6 (C-6), 140.4 (C-7), 51.2 (C-2), 36.0 (C-4), 28.8 (C-3), 26.7 (C-5), 20.3 (C-9), 9.3 (C-10*). HRMS: Found: 195.1306. C₁₀H₁₂²H₄O₂ (M+Na⁺) Calculated 195.1295. * Multiplet

7.3.17 Chapter 4 Synthesis

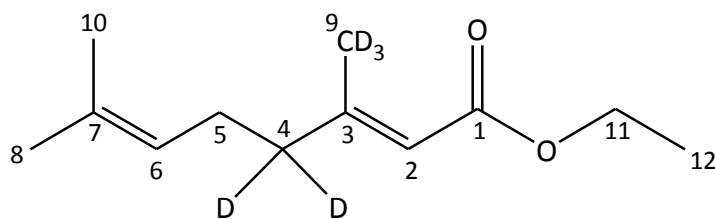
[1,1,1,3,3-²H₅]-6-Methyl-5-hepten-2-one (²H₅-**12**)



To a solution of 6-methyl-5-hepten-2-one (**12**) (5.29 g, 1 eq.) in CD₃OD (45 g, 30 eq.) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (638 mg, 0.1 eq.). The reaction mixture was stirred under nitrogen for 24 hours at room temperature. The solvent was removed and the crude product was purified on silica gel (80% EtOAc) to give [1,1,1,3,3-²H₅]-6-methyl-5-hepten-2-one (²H₅-**12**) (4.62 g, 84% (calculated as the ²H₅-product)) as a yellow oil: δ_{H} (CDCl₃, 500 MHz) 5.07 (1H, t, *J* 7.0, H-5), 2.49-2.41 (0.4H, m, H-3), 2.30-2.22 (2H, d, *J* 7.0, H-4), 2.16-2.10 (0.6H, m, H-1), 1.69 (3H, s, H-7/8), 1.63 (3H, s, H-7/8); δ_{C} (CDCl₃, 125 MHz) 209.2 (C-2), 132.6 (C-6), 122.7 (C-5), 43.4 (C-1*), 29.5 (C-3*), 25.7 (C-7/8), 22.4 (C-4), 17.7 (C-7/8). HRMS: Found: 154.1254. C₈H₉²H₅O (M+Na⁺) Calculated 154.1251. * Multiplet

(2E)-[4,4,9,9,9-²H₅]-3,7-Dimethyl-2,6-ethyloctadienoate (**169**)

(²H₅-Ethylgeranate)

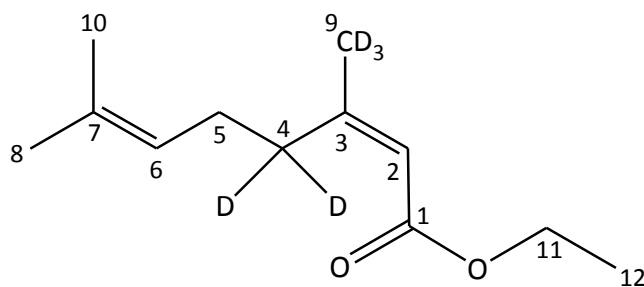


To triethylphosphonoacetate (4.0 g, 1 eq.) in THF (30 mL), stirred under nitrogen at -60 °C, was slowly added a slight excess of *n*-BuLi (14.7 mL, 1.05 eq.). The mixture was stirred at -60 °C for 20 minutes to give a yellow-tinted solution of lithiated

phosphonoacetate. A solution of [1,1,1,3,3- $^2\text{H}_5$]-6-methyl-5-hepten-2-one ($^2\text{H}_5$ -**12**) (2.34 g, 1 eq.) in THF was cooled to -20 °C and added to the phosphonoacetate anion. After stirring for 24 hours at room temperature, the mixture was quenched using saturated ammonium chloride (50 mL) and the aqueous phase extracted with Et₂O (3 x 50 mL). The combined organic layers were dried with anhydrous MgSO₄ and concentrated under vacuum to give a 2.6:1 mixture of $^2\text{H}_5$ -ethyl geranate (**169**) and $^2\text{H}_5$ -ethyl nerate (**170**). Separation of the (*E*) and (*Z*)-isomers was achieved on silica gel (5% Et₂O in hexane) to give pure (2*E*)-[4,4,9,9,9- $^2\text{H}_5$]-3,7-dimethyl-2,6-ethyloctadienoate (**169**) (1.41 g, 39%) as a colourless oil: δ_{H} (CDCl₃, 500 MHz) 5.68 (1H, s, H-2), 5.10-5.08 (1H, m, H-6), 4.16 (2H, q, H-11), 2.17 (2H, d, *J* 7.0, H-5), 1.70 (3H, s, H-8/10), 1.62 (3H, s, H-8/10), 1.30 (3H, t, *J* 5.6, H-12); δ_{C} (CDCl₃, 125 MHz) 166.9 (C-1), 159.6 (C-3), 132.5 (C-7), 123.0 (C-6), 115.6 (C-2), 59.4 (C-11), 40.6 (C-9*), 26.0 (C-8/10), 25.7 (C-5), 18.4 (C-4*), 17.7 (C-8/10), 14.4 (C-12). HRMS: Found: 224.1677. C₁₂H₁₅ $^2\text{H}_5$ O₂ (M+Na⁺) Calculated 224.1670. * Multiplet

(2*Z*)-[4,4,9,9,9- $^2\text{H}_5$]-3,7-Dimethyl-2,6-ethyloctadienoate (170**)**

($^2\text{H}_5$ -Ethylnerate)

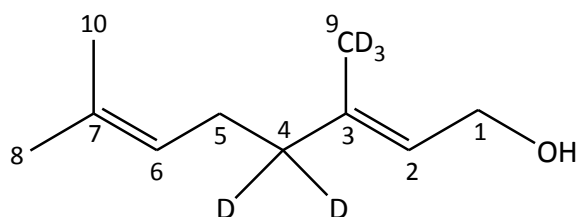


The Horner-Wadsworth-Emmons procedure described above, and subsequent separation of the (*E*) and (*Z*)-products on silica gel, also yielded pure (2*Z*)-[4,4,9,9,9- $^2\text{H}_5$]-3,7-dimethyl-2,6-ethyloctadienoate (**170**) (533 mg, 15%) as a colourless oil: δ_{H} (CDCl₃, 500 MHz) 5.67 (1H, s, H-2), 5.18 (1H, t, *J* 7.0, H-6), 4.16 (2H, q, H-11), 2.20-2.14 (2H, m, H-5), 1.69 (3H, s, H-8/10), 1.65 (3H, s, H-8/10), 1.29 (3H, t, *J* 5.8, H-12); δ_{C} (CDCl₃, 125

MHz) 167.1 (C-1), 160.0 (C-3), 132.1 (C-7), 123.7 (C-6), 116.3 (C-2), 59.4 (C-11), 33.2 (C-9*), 30.8 (C-8/10), 25.7 (C-5), 23.7 (C-4*), 17.7 (C-8/10), 14.3 (C-12). HRMS: Found: 224.1654. $C_{12}H_{15}^2H_5O_2$ ($M+Na^+$) Calculated 224.1670. * Multiplet

(2E)-[4,4,9,9,9- $^2H_5$]-3,7-Dimethyl-2,6-octadien-1-ol ($^2H_5$ -56)

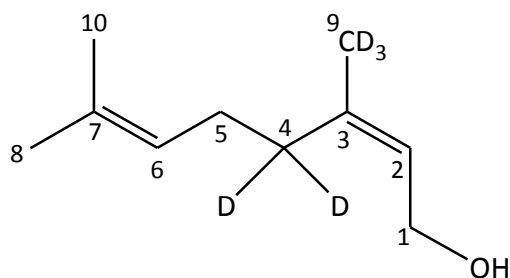
($^2H_5$ -Geraniol)



To a mixture of $^2H_5$ -ethylgeranate (**169**) (1.41 g, 1 eq.) in anhydrous Et_2O (10 mL), stirring at 0 °C under nitrogen, was added sodium bis(2-methoxyethoxy)aluminum hydride solution (RedAl®) (14.7 mL, 3.4 eq.). The reaction mixture was stirred for 2 hour at room temperature before slowly adding water (10 mL). The resulting suspension was diluted and extracted with Et_2O (2 x 20 mL) before drying the combined organic layers with anhydrous $MgSO_4$ and removing the solvent. The crude product was purified on silica gel (20% $EtOAc$ in petroleum ether 40-60 °C) to give (2E)-[4,4,9,9,9- $^2H_5$]-3,7-dimethyl-2,6-octadien-1-ol ($^2H_5$ -56) (788 mg, 71%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3341, 2967, 2916, 2879, 1660, 1441, 1377, 1290, 1110, 986, 824; δ_H ($CDCl_3$, 500 MHz) 5.43 (1H, t, J 7.2, H-2), 5.12 (1H, t, J 7.2, H-6), 4.18-4.15 (2H, m, H-1), 2.13-2.08 (2H, m, H-5), 1.70 (3H, s, H-8/10), 1.62 (3H, s, H-8/10); δ_C ($CDCl_3$, 125 MHz) 139.7 (C-3), 131.7 (C-7), 123.9 (C-6), 123.3 (C-2), 59.4 (C-1), 39.6 (C-5), 26.5 (C-4*), 25.7 (C-8/10), 17.7 (C-8/10), 16.9 (C-9*). HRMS: Found: 182.1571. $C_{10}H_{13}^2H_5O$ ($M+Na^+$) Calculated 182.1564. * Multiplet

(2Z)-[4,4,9,9,9-²H₅]-3,7-Dimethyl-2,6-octadien-1-ol (²H₅-62)

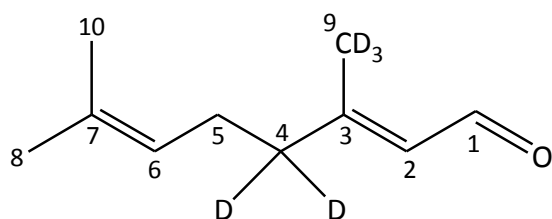
(²H₅-Nerol)



Following the procedure and stoichiometry described for the synthesis of ²H₅-geraniol (²H₅-56) (533 mg), reduction of ²H₅-ethylnerate (**170**) gave (2Z)-[4,4,9,9,9-²H₅]-3,7-dimethyl-2,6-octadien-1-ol (²H₅-62) (341 mg, 81%) as a colourless oil: $\nu_{\max}$ (thin film)/cm⁻¹ 3332, 2967, 2915, 2879, 1661, 1442, 1377, 1300, 1110, 989, 870; δ_{H} (CDCl₃, 500 MHz) 5.47 (1H, t, *J* 7.2, H-2), 5.11 (1H, t, *J* 7.2, H-6), 4.10 (2H, d, *J* 7.2, H-1), 2.15-2.07 (2H, m, H-5), 1.69 (3H, s, H-8/10), 1.62 (3H, s, H-8/10), 1.41 (1H, s, OH); δ_{C} (CDCl₃, 125 MHz) 139.7 (C-3), 132.4 (C-7), 123.9 (C-6), 123.4 (C-2), 59.0 (C-1), 26.4 (C-5), 25.7 (C-8/10), 17.8 (C-8/10), 17.7 (C-4*), 15.1 (C-9*). HRMS: Found: 182.1481. C₁₀H₁₃²H₅O (M+Na⁺) Calculated 182.1564. * Multiplet

(2E)-[4,4,9,9,9-²H₅]-3,7-Dimethyl-2,6-octadien-1-al (²H₅-109)

(²H₅-Geranial)

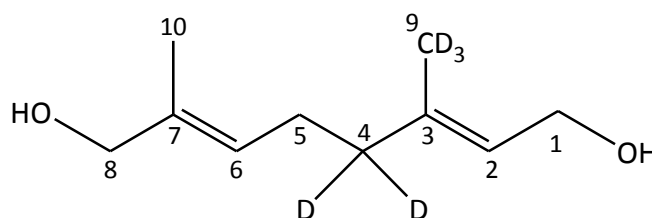


Following the general MnO₂ oxidation procedure using ²H₅-geraniol (²H₅-56) (191 mg), the crude product was purified on silica gel (30% ethyl acetate in petroleum ether 40-60 °C) to give (2E)-[4,4,9,9,9-²H₅]-3,7-dimethyl-2,6-octadien-1-al (**109**) (98 mg, 52%)

as a colourless oil: δ_{H} (CDCl_3 , 500 MHz) 10.03 (1H, d, J 6.9, H-1), 5.91 (1H, d, J 6.9, H-2), 5.09 (1H, t, J 6.4, H-6), 2.27-2.20 (2H, m, H-5), 1.70 (3H, s, H-8), 1.62 (3H, s, H-10); δ_{C} (CDCl_3 , 125 MHz) 191.1 (C-1), 163.7 (C-3), 133.1 (C-7), 127.4 (C-6), 122.5 (C-2), 40.4 (C-8), 25.7 (C-5), 17.5 (C-4*), 15.3 (C-9*), 12.8 (C-10). HRMS: Found: 180.1415. $\text{C}_{10}\text{H}_{11}^2\text{H}_5\text{O}$ ($\text{M}+\text{Na}^+$) Calculated 180.1408. * Multiplet

(2*E*)-[4,4,9,9- $^2\text{H}_5$]-3,7-Dimethyl-8-hydroxy-2,6-octadien-1-ol ([4,4,9,9- $^2\text{H}_5$]-72)

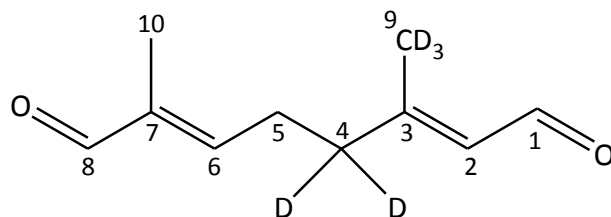
([4,4,9,9- $^2\text{H}_5$]-8-Hydroxygeraniol)



Following the general allylic oxidation procedure using $^2\text{H}_5$ -geraniol ($^2\text{H}_5$ -56) (351 mg), the crude product was purified on silica gel (30% ethyl acetate in petroleum ether 40-60 °C) to give (2*E*)-[4,4,9,9- $^2\text{H}_5$]-3,7-dimethyl-8-hydroxy-2,6-octadien-1-ol ($^2\text{H}_5$ -72) (58 mg, 15%) as a colourless oil: ν_{max} (thin film)/ cm^{-1} 3313, 2920, 2861, 1672, 1484, 1301, 1216, 1110, 992; δ_{H} (CDCl_3 , 500 MHz) 5.43-5.34 (2H, m, H-2 and H-6), 4.14 (2H, d, J 6.9, H-1), 3.98 (2H, s, H-8), 2.27 (2H, br s, OH), 2.16 (2H, d, J 7.0), 1.65 (3H, s, H-10); δ_{C} (CDCl_3 , 125 MHz) 141.0 (C-3), 134.2 (C-7), 126.6 (C-6), 121.8 (C-2), 67.3 (C-1), 57.7 (C-8), 38.5 (C-5), 25.0 (C-4*), 15.2 (C-9*), 12.4 (C-10). HRMS: Found: 198.1504. $\text{C}_{10}\text{H}_{13}^2\text{H}_5\text{O}_2$ ($\text{M}+\text{Na}^+$) Calculated 198.1513. * Multiplet

(2E)-[4,4,9,9,9-²H₅]-3,7-Dimethyl-8-oxo-2,6-octadien-1-al (²H₅-75)

(²H₅-8-Oxogeranial)



Following the general allylic oxidation procedure using ²H₅-geraniol (**²H₅-56**) (350 mg), the crude product was purified on silica gel (30% ethyl acetate in petroleum ether 40-60 °C) to give (3*R*)-[8,8,8,10,10,10-²H₆]-3,7-dimethyl-8-hydroxy-6-octen-1-yl acetate (**²H₅-(*R*)-116**) (98 mg, 26%) as a colourless oil: ν_{max} (thin film)/cm⁻¹ 2964, 2927, 2858, 1715, 1674, 1455, 1377, 1275, 1156, 1070; δ_{H} (CDCl₃, 500 MHz) 10.01 (1H, d, *J* 7.8, H-1), 9.45 (1H, s, H-8), 6.43 (1H, td, *J* 7.2 and 1.3, H-6), 5.91 (1H, dq, *J* 7.8 and 1.0, H-2), 2.60 (2H, td, *J* 7.2 and 7.2, H-5), 1.79 (3H, d, *J* 1.6, H-10); δ_{C} (CDCl₃, 125 MHz) 195.7 (C-8), 191.6 (C-1), 161.2 (C-3), 152.4 (C-6), 140.3 (C-7), 127.8 (C-2), 38.1 (C-4*), 26.2 (C-5), 17.8 (C-9*), 9.4 (C-10). HRMS: Found: 194.1209. C₁₀H₉²H₅O₂ (M+Na⁺) Calculated 194.1200.

* Multiplet

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