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Chirality and biosynthesis of lilac compounds in *Actinidia arguta* flowers

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Abstract

Biosynthesis of lilac compounds in 'Hortgem Tahi' kiwifruit (*Actinidia arguta*) flowers was investigated by treating inflorescences with d_5-linalool. The incorporation of the deuterium label into 8-hydroxylinalool, 8-oxolinalool, the lilac aldehydes, alcohols, and alcohol epoxides was followed by GC-MS and enantioselective GC-MS. Both (*R*)- and (*S*)-linalool were produced naturally by the flowers, but 8-hydroxylinalool, 8-oxolinalool, and the lilac aldehydes and alcohols occurred predominantly as the (*S*) and 5′(*S*)-diastereoisomers, respectively. The enantioselective step in the biosynthesis of the lilac aldehydes and alcohols was concluded to be the oxidation of linalool to (*S*)-8-hydroxylinalool. In contrast, the lilac alcohol epoxides had a 5′(*R*):(*S*) ratio, the same as for linalool, which suggests that either these compounds are not synthesised from the 5′(*S*)-configured lilac aldehydes and alcohols, or that if indeed they are, then it is by an enantioselective step that favours utilisation of the 5′(*R*)-configured compounds.

Keywords: *Actinidia arguta*; Monoterpenes; Linalool; 8-Hydroxylinalool; 8-Oxolinalool; Lilac aldehydes; Lilac alcohols; Lilac alcohol epoxides; Deuterium labelling; Enantioselective GC-MS

1. Introduction

Actinidia arguta (Sieb. Et Zucc.) Planch. ex Miq. var. *arguta* is a smooth-skinned, grape-sized kiwifruit species native to northern China, Korea, Siberia and Japan (Matich et al., 2003). Recently, we reported the isolation and synthesis of the previously unreported lilac alcohol epoxide (2-(5-methyl-5-(oxiran-2-yl)-tetrahydrofuran-2-yl)propan-1-ol) (**6**, Fig. 1), found in the flowers of *A. arguta* and only one other *Actinidia* species (*A. melanandra*). Lilac alcohol epoxide (**6**) was proposed to be a biosynthetic product of lilac aldehyde (**4**) and lilac alcohol (**5**).

The lilac compounds (**4**) and (**5**) were originally isolated from lilac (*Syringa vulgaris*) flower oil (Wakayama and Namba, 1970, 1974; Wakayama et al., 1971). Deuterium labelling experiments determined that the lilac alcohols (**5**) were derived from linalool (**1**) via the lilac aldehydes (**4**), and confirmed that both the lilac alcohols and aldehydes had the 5′(*S*) configuration (Burkhardt and Mosandl, 2003; Kreck et al., 2003). While lilac flowers produce exclusively (*S*)-linalool, incubation with racemic linalool gave both the 5′(*S*)- and 5′(*R*)-lilac aldehydes and alcohols. Thus, lilac flowers produce exclusively 5′(*S*)-configured lilac aldehydes and alcohols because the precursor, linalool, is synthesised entirely (>99%) in the (*S*) configuration.

In contrast to lilac flowers, and the flowers of many other *Actinidia* species (*deliciosa*, *setosa*, *rufa*, *hemsleyana*, *chrysantha*, *polygama*, and *macrosperma*) which produce >95% either (*S*)- or (*R*)-linalool (Matich, 2005), *A. arguta* produces both enantiomers of linalool. In 15 *arguta* genotypes (Matich, 2005), the (*R*):(*S*) ratio ranged from 93:7 to 2:98. Analysis of the flowers of eight *A. arguta* genotypes by Dötterl et al. (Dötterl et al., 2006), and by ourselves during a visit to their laboratory, determined that the lilac alcohols and aldehydes had predominantly the 5′(*S*) configuration, even though both (*R*)- and (*S*)-linalool were present in significant amounts. This suggested a highly enantioselective production of the lilac compounds from linalool.

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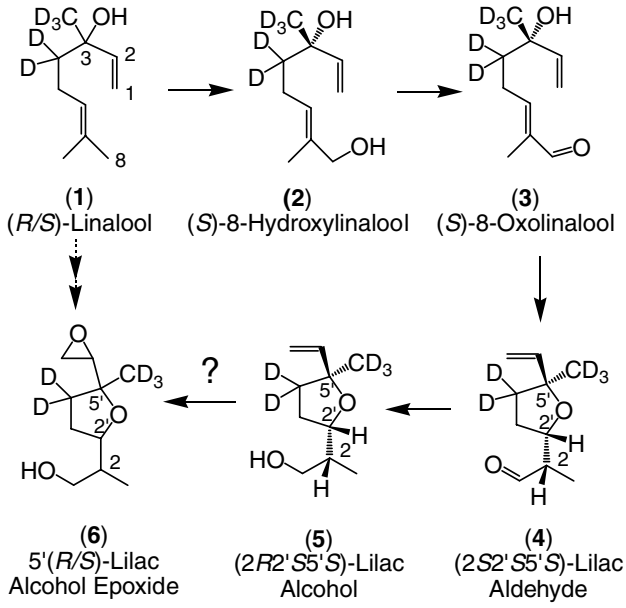

Fig. 1. Structures of deuterium labelled linalool metabolites identified in extracts of *Actinidia arguta* flowers and the proposed mechanism of their biosynthesis from linalool based on Kreck et al. (Kreck et al., 2003) and observations herein. The predominant configurations of compounds (**2**)–(**5**) are shown.

The aim of this work was to determine the biosynthetic origins of the lilac alcohol epoxides in *A. arguta*, using deuterium labelling, and to perform enantiomeric determinations on the compounds in the biosynthetic pathway from linalool through to the lilac alcohol epoxides, to determine the biochemical origin of the observed enantioselectivities.

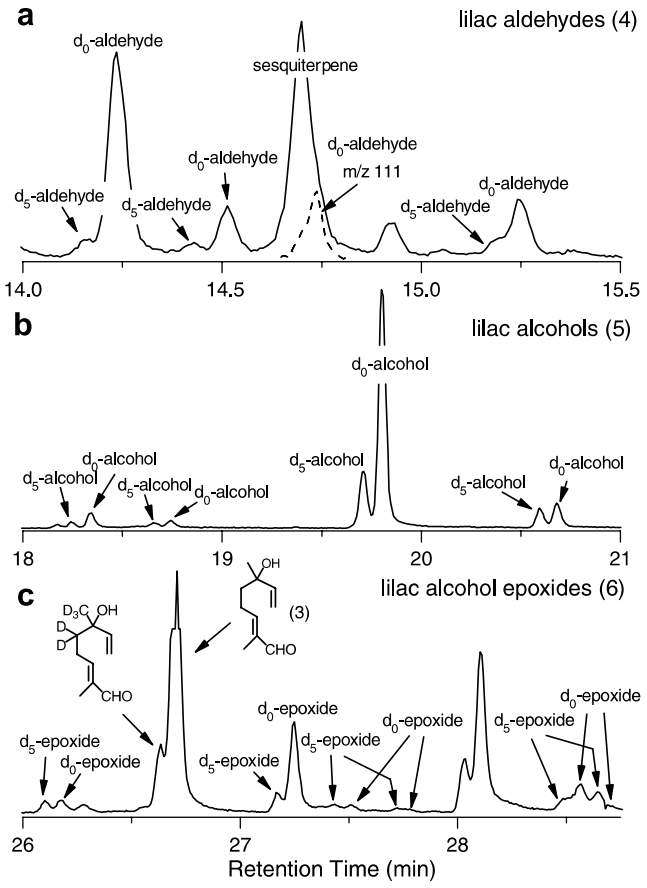

Fig. 2. TIC GC-MS chromatograms of deuterated and non-deuterated lilac aldehydes (a), alcohols (b), and alcohol epoxides and 8-oxolinalool (c) found in the solvent extracts of whole *Actinidia arguta* 'Hortgem Tahi' flowers floated in aqueous solutions of d_5-linalool.

2. Results and discussion

Treatment of 'Hortgem Tahi' flowers with d_5-linalool (**1**) showed that the d_5-label was incorporated into all the lilac compounds (**2**–**6**) (Fig. 2). The deuterated compounds eluted 5–6 s before the natural compounds, and only the d_5-lilac alcohol epoxides (**6**) have not previously been reported, but deuterated compounds (**1**–**5**) have been synthesised by Kreck et al. (Kreck et al., 2003) in their study of the biosynthesis of the lilac compounds in *S. vulgaris*. In the present study, ca. 20% of the lilac compounds present were deuterated. The percentages of the d_0 (and d_5) isotopomers were linalool (**1**); 1.4 (0.01), 8-hydroxylinalool ((**2**), not shown in Fig. 2); 5.7 (0.3), 8-oxolinalool (**3**); 9.3 (1.3), the lilac aldehydes (**4**); 2 (0.2), the lilac alcohols (**5**); 59 (17), and the lilac alcohol epoxides (**6**); 2.8 (1.1). One of the lilac aldehyde peaks (shown as *m*/*z* 111 in Trace A) co-eluted with an unidentified sesquiterpene. There was only a trace amount of d_5-linalool present (0.7% of the d_0-linalool). The d_5-labelling was considerably higher in the lilac compounds, with the d_5-alcohols being the major labelled compounds, just as the d_0-alcohols were the major unlabelled compounds. Thus this pathway favoured the production of the lilac alcohols and not the presumed end products, the lilac alcohol epoxides. This suggested some constriction in the pathway between the lilac alcohols and the lilac alcohol epoxides or that the epoxides might be derived from linalool via an alternate route. If so, their concentrations in the flower might show little correlation with those of the alcohols.

2.1. Chiral determination of the lilac compounds

The absolute configuration of the chiral centres of the compounds in a putative biosynthetic pathway can indicate if these compounds are biosynthetically related to one another, and can indicate points in the pathway where there is enzymatic control of the chirality of the products (Kreck et al., 2003; Schwab et al., 2001). Biosynthetic products of a chiral compound will usually have the same chirality as their parent, and if the parent compound is a mixture of enantiomers, the daughter compounds may also be a mixture of enantiomers with the same ratio of *R* and *S* enantiomers as their parent. Conversely, if the parent compound is a mixture of enantiomers, but its daughter compounds are chiral, then this might indicate that a biosynthetic enzyme was only capable of processing one particular enantiomer of the parent compound.

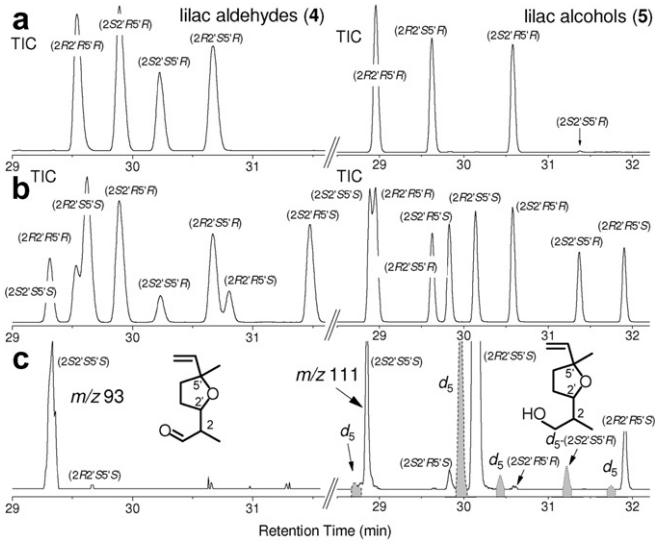

Fig. 3. Chromatograms of the enantioselective GC-MS separation of: (a) the four 5′(*R*) lilac aldehyde (left, TIC) and alcohol isomers (right, TIC) synthesised from (*R*)-linalool, (b) the eight 5′(*R* and *S*) isomers from racemic linalool (TIC), and (c) the two 5′(*S*) lilac aldehyde (*m*/*z* 93, left), the four 5′(*S*) and one 5′(*R*) alcohol (*m*/*z* 111, right), and the five deuterated (dotted lines, shaded peaks, *m*/*z* 116, right) alcohol isomers found in the solvent extracts of *Actinidia arguta* 'Hortgem Tahi' flowers.

The lilac compounds were resolved on an enantioselective GC stationary phase similar to that used by Kreck et al. and Dötterl et al. (Kreck et al., 2003; Dötterl et al., 2006), who determined the chiral assignments for all four pairs of lilac aldehyde and lilac alcohol enantiomers. Authentic compounds were synthesised from both racemic and (*R*)-linalool so that the absolute configurations at C-5′ were known. The C-2 and C-2′ configurations for each of the peaks resolved in our analytical system were assigned based upon the elution order obtained by Kreck et al. (2003), the relative peak heights of the four enantiomeric pairs, and the assignment of the peaks in a 'Hortgem Tahi' extract sent from this laboratory to that of Dötterl et al. (2006) for analysis. The isomeric lilac alcohol epoxides have not been fully characterised, and thus only the absolute configuration on C-5′ was identifiable. However, this information was sufficient to determine which linalool enantiomers had been metabolised through to the lilac alcohol epoxides.

Enantioselective GC-MS of the lilac aldehydes present in the 'Hortgem Tahi' flowers revealed two 5′(*S*)-configured isomers tentatively identified as 2*S*2′*S*5′*S* (major peak, 96%) and 2*R*2′*S*5′*S*, respectively (Fig. 3c). The 'Hortgem Tahi' flowers also contained all four of the 5′(*S*)-configured lilac alcohols in the elution order 2*S*2′*S*5′*S*, 2*S*2′*R*5′*S*, 2*R*2′*S*5′*S*, and 2*R*2′*R*5′*S*. The most abundant isomer (2*R*2′*S*5′*S*, 90%), corresponded to the reduction product of the most abundant lilac aldehyde (2*S*2′*S*5′*S*)[1] isomer. The second most abundant (6.6%) lilac alcohol isomer (2*S*2′*S*5′*S*) was the product of the second, and smaller, of the two aldehyde peaks (2*R*2′*S*5′*S*). A trace of a 5′(*R*)-configured lilac alcohol isomer (2*S*2′*R*5′*R*) was detected at RT = 30.6 min (Fig. 3c) with a TIC peak area of approximately 0.28% of its enantiomer-pair (2*R*2′*S*5′*S*). The d_5-lilac alcohols were the only deuterated products of the racemic d_5-linalool that were concentrated enough for an enantioselective analysis (Fig. 3c). The majority (85%) of the d_5-lilac alcohol peaks in Fig. 3c correspond to the 5′*S* conformation. This is less than the 99.7% 5′*S* majority observed for the non-deuterated compounds, and the origin of this disparity is not clear to us. However, this still does confirm a selectivity in favour of the 5′*S* conformation.

The predominantly 5′(*S*) configuration of the lilac aldehydes and alcohols in 'Hortgem Tahi' flowers did not originate from their biosynthetic precursor, linalool, which had a (*R*):(*S*) ratio of 79:21 (Fig. 4). Analysis of a flower extract from a second vine, grown in the same orchard, produced 79.5:20.5, whereas a determination from a sample from the previous year (Matich, 2005) gave a ratio of 59:41. The highly enantioselective processing of (*S*)-linalool to the lilac aldehydes and alcohols is in contrast to the findings of previous workers (Burkhardt and Mosandl, 2003; Kreck et al., 2003; Kreck and Mosandl, 2003) who found that the exclusively 5′(*S*)-configured lilac diastereoisomers in lilac flowers resulted from their biosynthesis from an exclusively (*S*)-linalool precursor. These workers incubated lilac flowers with racemic linalool and identified both 5′(*R*) and 5′(*S*)-configured lilac compounds in extracts of the flowers. Previous

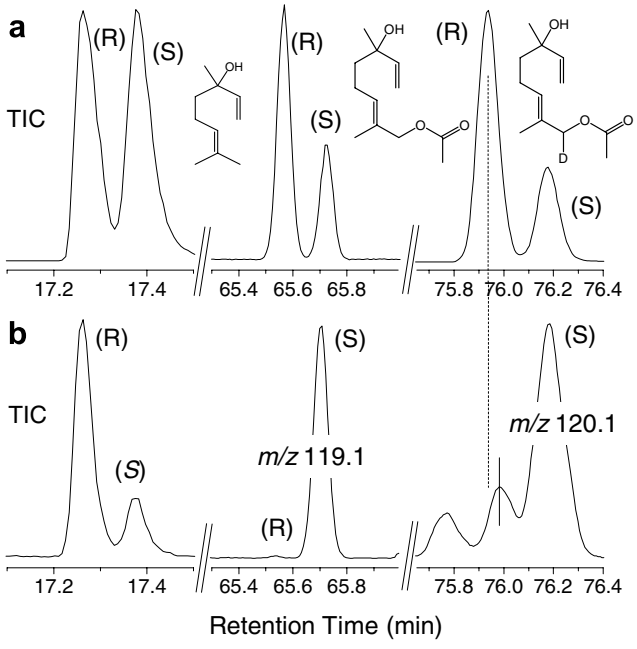

Fig. 4. Chromatograms of the enantioselective GC-MS separation of: (a) racemic linalool (TIC, left), of co-injections of racemic 8-acetoxylinalool with (*R*)-8-acetoxylinalool (TIC, middle) and racemic d_1-8-acetoxylinalool with (*R*)-d_1-8-acetoxylinalool (TIC, right); and (b) the enantiomers of linalool (TIC, left), of 8-acetoxylinalool (*m*/*z* 119.1, middle) and 8-d_1-8-acetoxylinalool (*m*/*z* 120.1, right) produced by derivatisation of the solvent extracts of *Actinidia arguta* 'Hortgem Tahi' flowers.

[1] The CIP sequence rules (Hellwinkel, 2001) dictate that reduction of the lilac aldehyde to the lilac alcohol changes the assigned conformation from 2(*S*) for the aldehyde to 2(*R*) for the alcohol.

enantiomeric determinations of *A. arguta* flowers by us (Matich, 2005) and previous workers (Dötterl et al., 2006) failed to identify any 5′(*R*)-configured lilac compounds. In the present study it was only possible to identify a single 5′(*R*)-configured alcohol after greatly concentrating the sample. The origin of the trace of (2*S*2′*R*5′*R*)-lilac alcohol found in the 'Hortgem Tahi' flowers may arise from a minor non-selectivity in the enzyme, or from non-enzymatic allylic oxidation and cyclisation in the flower.

In contrast to lilac (*S. vulgaris*), there are other plants that produce both enantiomers of linalool, but are enantioselective in their use of it. We have found an *A. melanandra* genotype whose flowers contain 18:82 (*R*):(*S*) linalool and yet it produces exclusively the 5′(*S*)-configured lilac alcohols (Matich, 2005). Linalool in *Prunus laurocerasus* is 55:45 (*R*):(*S*), and *Viburnum opulus* is 19:81, yet both plants produce only the 5′(*S*)-configured lilac aldehydes and alcohols (Dötterl et al., 2006).

The origin of the enantioselectivity in the biosynthesis of the lilac compounds in *A. arguta* was determined by examining the chirality of the biosynthetic intermediates, 8-hydroxy- and 8-oxolinalool. Previous workers have managed to separate the enantiomers of 8-hydroxylinalool (Luan et al., 2004), but neither of these two compounds would resolve on our enantioselective GC columns (β-Dex™ 325 and 225; Supelco, and Chiraldex GTA; Alltech), possibly because of their high polarity. To ameliorate this problem, 8-hydroxylinalool was acetylated with lipase (Pàmies and Bäckvall, 2001) to 8-acetoxylinalool, a compound previously reported in wheat seeds (Dudai et al., 2004). The 8-acetoxylinalool enantiomers were resolved on the β-Dex™ 325 capillary column, where the (*R*) enantiomer eluted before the (*S*) (Fig. 4). 8-Hydroxylinalool in the flower extract was predominantly the (*S*)-enantiomer with only a trace (<1%) of (*R*)-8-hydroxylinalool detectable (Fig. 4b).

Oxidation of linalool to 8-hydroxylinalool appears to be highly enantioselective, and the compounds further down the biosynthetic chain should have the same chirality. The chirality of 8-oxolinalool was determined after reduction to 8-d_1-8-hydroxylinalool, which was in turn acetylated, with lipase, to produce 8-d_1-8-acetoxylinalool. The deuterated compound, in the derivatised flower extract, eluted 3 s before the natural, non-deuterated compound and this, combined with the d_1/d_0 mass difference, allowed resolution of d_0-8-acetoxylinalool (derived from 8-hydroxylinalool) from 8-d_1-8-acetoxylinalool (derived from 8-oxolinalool). Even though a slower GC oven ramp was used to increase the elution time, one interfering peak in the chromatogram of the derivatised flower extract (marked by the solid vertical line in Fig. 4b) could not be completely separated from the (*R*)-d_1-8-acetoxylinalool peak in the standard (marked by the dashed vertical line in Fig. 4a). Integration of the co-eluting section of the interfering peak determined that if present, (*R*)-d_1-8-acetoxylinalool constituted less than 5% of the amount of the (*S*)-enantiomer. The predominance of (*S*)-8-oxolinalool in the flower is in agreement with 8-oxolinalool being within the biosynthetic pathway to the 5′(*S*)-configured lilac compounds. To our knowledge the enantiomeric composition of 8-oxolinalool has not previously been determined.

Although the enantioselective oxidation of linalool produces predominantly (*S*)-8-hydroxylinalool, and subsequently (*S*)-8-oxolinalool, it is not clear whether the cyclisation of this later compound to the lilac aldehyde is enzymatic or spontaneous. If the cyclisation is enantioselective, then it is most probably enzymatic. The lilac aldehydes in the 'Hortgem Tahi' flowers had a 2(*R*):(*S*) ratio of 4:96 and a 2′(*R*):(*S*) ratio of 0:100 (Fig. 3c). This suggests that the cyclisation is enzyme catalysed in these flowers and favours the (*S*) configuration on these two carbon atoms, because a non-enzymatic cyclisation would be expected to be non-enantioselective. These C2 and C2′ configurations were conserved in the lilac alcohols (with ratios of 8:92 and 4:96, respectively (Fig. 3c)).

Kreck and Burkhardt (Burkhardt and Mosandl, 2003; Kreck and Mosandl, 2003) found that the lilac compounds in lilac flowers were of the 5′(*S*) configuration because the flowers produced exclusively (*S*)-linalool. Lilac flowers treated with racemic linalool produced more of the 5′(*R*)-configured isomers than of their 5′(*S*) counterparts. Further examination of their results revealed 2′(*R*):(*S*) ratios in the lilac aldehydes and alcohols of 19:81 and 19.5:80.5, respectively (Kreck and Mosandl, 2003). Lilac flowers treated with racemic d_5-linalool produced lilac compounds that were also predominantly of the 2′(*S*) configuration. Treatment with the unnatural linalool precursor, (*R*)-d_6-linalool, gave more modest 2′(*R*):(*S*) ratios (41:59 and 38:62, respectively) in the lilac aldehydes and alcohols. Finally, [5,5-$^2H_2$]-deoxy-D-xylose produced the 5′(*S*)-configured deuterated lilac aldehydes and alcohols with 2′(*R*):(*S*) ratios of 38:62 and 18:82, respectively, and the non deuterated compounds with ratios of 34:66 and 21:79, respectively (Burkhardt and Mosandl, 2003). Therefore, in lilac flowers, cylisation of 8-oxolinalool to the lilac aldehydes slightly favours formation of the 2′(*S*) configuration, which implies some enzymatic control of the cylisation step.

The 5′(*R*) and the 5′(*S*) isomers of the synthetic lilac alcohol epoxides were partially separated on the β-Dex 325 enantioselective GC column (Figs. 5a and b). The chromatogram of the 'Hortgem Tahi' flower extract contained nine peaks (five 5′(*R*) and four 5′(*S*) (Fig. 5c)). The large 5′(*S*) and 5′(*R*) peaks at around 41.6 min were probably due to co-elution of several C-2 and C-2′ isomers. In the flower extracts (Fig. 5c) the 5′(*R*) isomers constituted 81% of the lilac alcohol epoxides present, with the major 5′(*R*) peak constituting 62.5% of the total epoxides. The 5′(*R*):(*S*) ratio in the epoxides (81:19) reflects the (*R*):(*S*) ratio of linalool (79:21). The predominant 5′(*S*) configuration of the lilac alcohols and aldehydes suggests either that the lilac alcohol epoxides are not synthesised by epoxidation of any of these compounds, or that if they are then it is necessary to invoke an enantioselectivity in favour of processing the 5′(*R*)-configured isomers. An alternative reaction sequence might first involve epoxidation of linal-

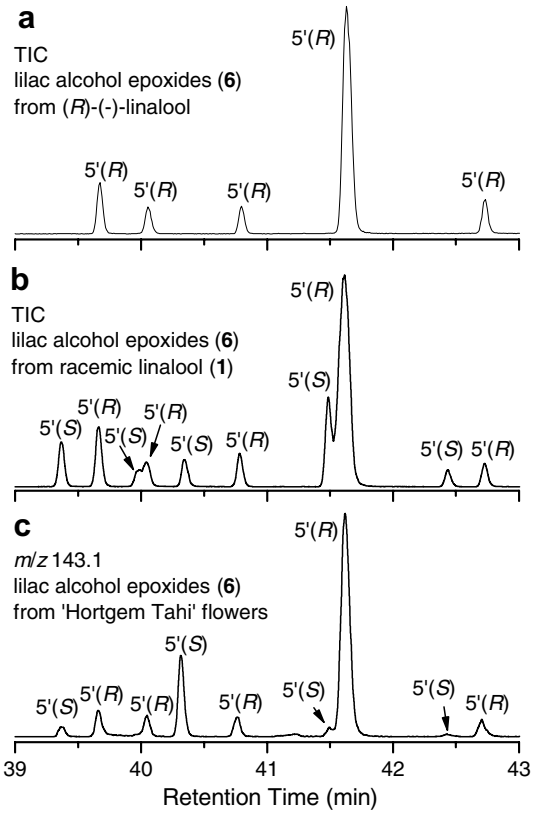

Fig. 5. Enantioselective GC-MS analysis (β-Dex 325) of: (a) 5′(*R*)-lilac alcohol epoxides chemically synthesised from (*R*)-linalool; (b) 5′ (*R*/*S*)-lilac alcohol epoxides synthesised from (*R*/*S*)-linalool; and (c) separation of the lilac alcohol epoxides found in the solvent extracts of *Actinidia arguta* 'Hortgem Tahi' flowers.

ool, and then C-8 oxidation and cyclisation to produce the lilac epoxide compounds. Epoxidation of olefins is known to be mediated by cytochrome P450 enzymes (Martinez and Stewart, 2000).

The 79:21 (*R*):(*S*) ratio of linalool observed in the flower extract may result from the high enantioselectivity of the biosynthetic pathways that utilise it rather than the enantioselectivity (or lack of) of its biosynthesis. The predominantly (*S*)-configured lilac compounds (Fig. 1) were 95% of all the lilac compounds in the extract compared with linalool, which was 1.4%. Thus it is possible that the pool of (*S*)-linalool was depleted by the synthesis of these (*S*)-configured compounds, and consequently the (*R*):(*S*) ratio of linalool on synthesis might be quite different from the measured (*R*):(*S*) ratio. The lilac alcohol epoxides constituted only 3.6% of the lilac compounds in this sample, had the same 5′(R):(*S*) ratio as for linalool, and would therefore seem to have less impact on linalool enantiomer ratios.

3. Experimental

3.1. Plant material

Canes of *A. arguta* (Sieb. Et Zucc.) Planch. ex Miq. var *arguta* ('Hortgem Tahi') were collected from a commercial block at the HortResearch Research Orchard, Te Puke, New Zealand, on 11th November 2005 and were transported to the Palmerston North Research Centre with the cut stems in water. Whole flowers (7.2 g) were removed from their canes and floated in a 10 cm dia. Petri dish containing 0.1 mg ml^{-1} each of Tween 80 (Difco, Detroit) and d_5-(*R*/*S*)-linalool in water (15 ml). The Petri dish was placed in a sealed glass jar at room temperature for 48 h. The flowers and the d_5-linalool solution were extracted for 60 h at 5 °C with 1:1 pentane:Et_2O (25 ml, Ajax) which had been purified by distillation and passage through a column of activated alumina (Perrin and Armarego, 1988). The extract was filtered, dried over $MgSO_4$ (Ajax), and the sample volume reduced to ca. 1.5 ml for GC-MS analysis.

3.2. Chemical syntheses

Non-deuterated 8-hydroxylinalool, 8-oxolinalool, the lilac aldehydes, alcohols and alcohol epoxides were synthesised from both racemic (Aldrich) and *R*-(−)-linalool (Fluka) (Matich et al., 2006). Racemic d_5-linalool ([4,4,10,10,10-$^2H_5$]-linalool) was synthesised from 6-methylhept-5-en-2-one (Aldrich, 99%) (Kreck et al., 2003). This d_5-linalool was used to synthesise the deuterated GC-MS standards, 8-oxolinalool, the lilac aldehydes and alcohols (Matich et al., 2003), and the lilac alcohol epoxides (**6**) (Matich et al., 2006) EI-GC-MS: *m*/*z* [fragment] (rel. int.): 149 (3), 148 $[M-C_2H_3O]^+$ (89), 132 $[M\text{-}C_3H_7O]^+$ (75), 130 $[M-C_2H_3O-H_2O]^+$ (24), 118 (7), 87 (17), 86 (20), 85 $[CH_3C(CD_3)CDCHCH]^+$ (76), 84 (16), 46 (100). d_5-8-Hydroxylinalool was identified based on its elution just before d_0-8-hydroxylinalool and its mass spectrum (Kreck et al., 2003).

8-Acetoxylinalool ((*E*)-6-hydroxy-2,6-dimethylocta-2,7-dienyl acetate) was synthesised enzymatically from (*R*)- and racemic 8-hydroxylinalool. To a solution of racemic or (*R*)-diol (10 μl) and vinyl acetate (acetate donor, 50 μl, Aldrich) in 1 ml of Et_2O was added Lipase PS-D "Amano" I (6 mg, Amano Enzyme Inc., Nagoya, Japan) and the mixture was stirred at room temperature for 30 min. The powdered enzyme was filtered off, the solution evaporated to dryness to remove the solvent, the remaining vinyl acetate and the ethanal produced, and the acetate was taken up in Et_2O (yield >99.5% by GC-MS). GCMS analysis of 8-acetoxylinalool produced from racemic 8-hydroxylinalool determined that it was racemic (50.1:49.9, (*R*):(*S*)). EI-GC-MS, *m*/*z* [fragment] (rel. int.): 152 $[M-60]^+$ (4), 137 (23), 119 (37), 110 (25), 84 (31), 82 (53), 81 (31), 71 (93), 68 (53), 67 (100) 55 (25), 43 (83). ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.89 (1H, *dd*, $J = 17.3$, 10.8 Hz, H-7), 5.44 (1H, *td*, $J = 7.2$, 1.2 Hz, H-3), 5.20 (1H, *dd*, $J = 17.3$, 1.2 Hz, H-8_{trans}), 5.05 (1H, *dd*, $J = 10.8$, 1.2 Hz, H-8_{cis}) 4.42 (2H, *brs*, H-1), 2.14–1.99 (2H, *m*, H-4), 2.04 (3H, *s*, OAc), 1.62 (3H, *brs*, 2-Me), 1.60–1.53 (2H, *m*, H-5), 1.27 (3H, *s*, 6-Me). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 172.0 (OAc), 145.8 (C7), 131.3 (C2), 130.4 (C3),

112.9 (C8), 74.3 (C6), 71.2 (C1), 42.5 (C5), 28.9 (6-Me), 23.5 (C4), 22.0 (OAc), 14.9 (2-Me).

8-d_1-8-Acetoxylinalool ([1-$^2H_1$]-(*E*)-6-hydroxy-2,6-dimethylocta-2,7-dienyl acetate) was synthesised from (*R*)- and racemic 8-oxolinalool. To a stirred solution containing 2 μl of racemic and 1 μl of (*R*)-8-oxolinalool in Et_2O (0.5 ml) was added $LiAlD_4$ (10 mg, Acros). After 5 min the hydride was quenched with satd. NH_4Cl (2 ml), the volume of the ethereal solution was increased to 2 ml and the solution was filtered through $MgSO_4$. Vinyl acetate (50 μl) and lipase (5 mg) were added and the mixture was stirred at room temperature for 30 min. The enzyme was filtered off, the solution was evaporated to dryness, and the acetate was taken up in 1.5 ml of Et_2O. EI-GC-MS, *m/z* [fragment] (rel. int.): 153 $[M-60]^+$ (10), 138 (29), 120 (44), 110 (49), 96 (34), 83 (33), 82 (67), 71 (94), 68 (100), 67 (56), 55 (36), 43 (86).

3.3. Derivatisation of flower extracts

Acetylation: Flower extract (0.5 ml) was acetylated by addition of vinyl acetate (60 μl) and lipase (5.3 mg). After stirring for 60 min the solution was filtered through $MgSO_4$ prior to GC-MS analysis.

Reduction and acetylation: The flower solvent extract (0.65 ml) was dried by filtering through $MgSO_4$ and Et_2O was added to increase the total volume to 1 ml. $LiAlD_4$ (10 mg) was added to the stirred solution and after 5 min the reaction mixture quenched with satd. NH_4Cl (2 ml), the volume of the ethereal solution was increased to 2.5 ml with Et_2O and the solution was filtered through $MgSO_4$. Vinyl acetate (50 μl) and the lipase (5 mg) were added and the mixture stirred for 30 min before filtration as above.

3.4. GC-MS analyses

GC-MS analyses used an Agilent 6890N GC coupled to a Waters GCT time of flight (TOF) mass spectrometer. Separations of the d_5-labelled compounds were carried out using a 20 m × 0.18 mm i.d. × 0.18 μm film thickness DB-Wax (Agilent) column at a helium flow of 1 ml min^{-1} after a 1 min splitless injection at 200 °C. The oven temperature ramp was 35 °C for 1 min, 5 °C min^{-1} to 240 °C, and hold for 15 min. The linalool enantiomers were separated using a Supelco β-Dex™ 225 capillary column, and all other chiral compounds using a β-Dex™ 325 capillary column (both 30 m × 0.25 mm i.d. × 0.25 μm film thickness). The 1 min splitless injections were at 220 °C and the oven temperature programmes were: for linalool; 35 °C for 1 min, 5 °C min^{-1} to 120 °C, 10 °C min^{-1} to 230 °C, and hold 15 min, for 8-acetoxylinalool; 35 °C for 1 min, 1.8 °C min^{-1} to 150 °C, and 8 °C min^{-1} to 230 °C, for 8-d_1-8-acetoxylinalool; 35 °C for 1 min, 1.5 °C min^{-1} to 150 °C, and 8 °C min^{-1} to 230 °C, for lilac aldehydes; 50 °C for 1 min, 2 °C min^{-1} to 120 °C, 10 °C min^{-1} to 230 °C, and hold 5 min, for lilac alcohols and lilac alcohol epoxides; 50 °C for 1 min, 2.5 °C min^{-1} to 230 °C.

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