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Convenient synthesis of deuterium labelled sesquiterpenes

Nina Duhamel [a], Damian Martin [b], Roberto Larcher [c], Bruno Fedrizzi [a,*], David Barker [a,*]

[a] School of Chemical Sciences, University of Auckland, 23 Symonds St, Auckland, New Zealand
[b] The New Zealand Institute for Plant & Food Research Limited, 85 Budge Street, Blenheim 7240, Marlborough, New Zealand
[c] FEM-IASMA Fondazione Edmund Mach – Istituto Agrario di San Michele all'Adige, via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

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ABSTRACT

Sesquiterpenes are an important class of molecules, with roles ranging from pollination and signalling to defense mechanisms. Despite their apparent importance, the limited number of commercial standards has hindered their study and precise quantification. Herein, we report the syntheses of fourteen labelled sesquiterpenes with a high level of deuterium incorporation (>95%) for applications in MS-based studies.

Sesquiterpenes are an important member of the terpenoid family. Like other members of this family they are made of isoprene units, three in this case, and have an empirical formula of $C_{15}H_{24}$. More than 5000 sesquiterpenes have been identified and their roles range from plant growth regulators[1] to plant signalling species,[2] to herbivore-induced plant defences.[3] Some plant-derived sesquiterpenoids have also been identified as anti-inflammatory and anti-carcinogenic species.[4]

Their biogenesis is strictly linked to that of monoterpenes for which the current knowledge is much more extended.[5] In reality, sesquiterpene biogenesis is less understood, even if some crucial steps, such as the importance of the DOXP pathway and of the mevalonic acid pathway have been pinpointed.[6,7] These molecules possess a hydrocarbon backbone and have been grouped into a number of skeletal types according to their structure.[8] The highly diverse and complex skeletons have made the unique identification and quantification extremely challenging. Even in the most advanced and recent studies[9–11] significant limitations on their chemical detections are encountered, with several sesquiterpenes only tentatively identified and with almost no quantitative detection due to the lack of suitable standards.

Isotopic labelled precursors have been previously used to study the biogenesis of sesquiterpenes as well as the enzymes responsible for the huge range of cyclised products.[7,12,13] However, these studies fall short in the quantification and identification of other labelled species that could have potentially been formed. Similarly, the total syntheses of sesquiterpenes have been widely reported in the literature,[14] but these have not proven useful in terms of identifying and especially quantifying these species in plant tissues as no labelled sesquiterpenes were also synthesised.

The availability of pure, isotopically labelled, sesquiterpene analogues could prove pivotal to study the formation and evolution of these molecules in complex biological matrices, as vastly proven in other fields.[15] Therefore instead of introducing labelled sesquiterpene building blocks, which can lead to the formation of a number of sesquiterpenes without the possibility of resolving them, we have proposed synthetic strategies that allowed us to obtain new isotopically labelled sesquiterpenes. These molecules could then be used for analytical purposes in MS-based Standard Isotopic Dilution Analysis (SIDA) approaches.

The general approach was to obtain commercially available sesquiterpenes or sesquiterpenoids, and carry out efficient synthetic chemistry on these, in order to introduce deuterium atoms. The introduced deuterium atoms are required to be sufficiently stable to be retained in some of the fragments formed during ionisation in MS or MS/MS analyses. The use of deuterium labelling was chosen over the use of ^{13}C labelled compounds as this allowed commercially available non-labelled compounds to be utilised as starting materials. ^{13}C labelled sesquiterpenes have recently been reported for use in mechanistic studies on this class of compounds, however, they require *de novo* synthesis from more expensive labelled precursors.[16]

The first set of standards were created using hydroxyl containing sesquiterpenoids. The first structure used was the

* Corresponding authors. Tel.: +64 9 373 7599; fax: +64 64 373 7422.
E-mail addresses: b.fedrizzi@auckland.ac.nz (B. Fedrizzi), d.barker@auckland.ac.nz (D. Barker).

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commercially available sesquiterpene (*E*,*E*)-farnesol **1**. (*E*,*E*)-Farnesol **1** and its derivatives (*E*,*E*)-farnesyl acetate and (*E*,*E*)-methyl farnesoate are all known volatile compounds, though their contribution to wine aroma has not been explored. Addition of an acetate group via reaction of the primary alcohol with acetyl chloride proceeded in a straight forward manner to give (*E*,*E*)-farnesyl acetate. Replacing acetyl chloride with (D_3)-acetyl chloride provided a simple method for deuterated methyl group introduction. However, though easy to introduce, these acetyl deuterium labels can be easily lost to fragmentation during mass spectrometry resulting in the majority of the standards' fragments being identical to the target compounds'. In a revised synthesis, (*E*,*E*)-farnesol was oxidised to farnesal, then to (*E*,*E*)-farnesoic acid **2** using a Pinnick oxidation.[17] Reduction of the acid using lithium aluminium deuteride, gave (D_2)-farnesol **3**. Acetylation as above, using (D_3)-acetyl chloride, was then carried out, giving (D_5)-farnesyl acetate **4**.

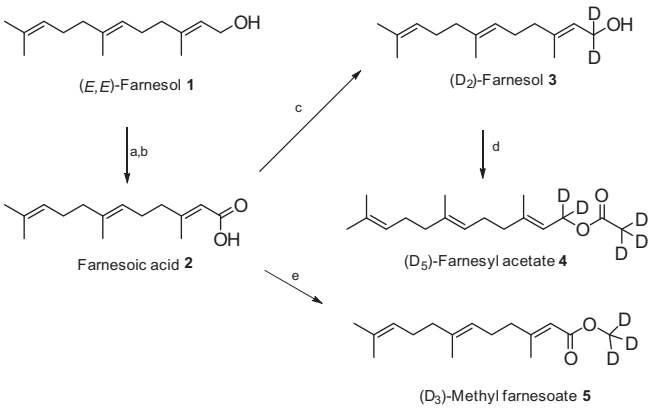
The second standard was made from (*E*,*E*)-farnesoic acid **2** via esterification using (D_3)-methanol and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to form (D_3)-methyl farnesoate **5** in 63% yield (Scheme 1).

The next set of standards were made from two commercially available cyclic sesquiterpenes, (+)-aromadendrene **6** and (−)-caryophyllene oxide **7**. These sesquiterpenes contain a single terminal alkene, and no other functional groups, other than the epoxide on the (−)-caryophyllene oxide **7**. The designed approach to deuterium labelling was the oxidation of the terminal alkene groups to the ketone derivatives, which would then undergo reaction with a deuterium labelled Wittig reagent, resulting in the original sesquiterpene where the terminal protons are replaced with deuteriums.

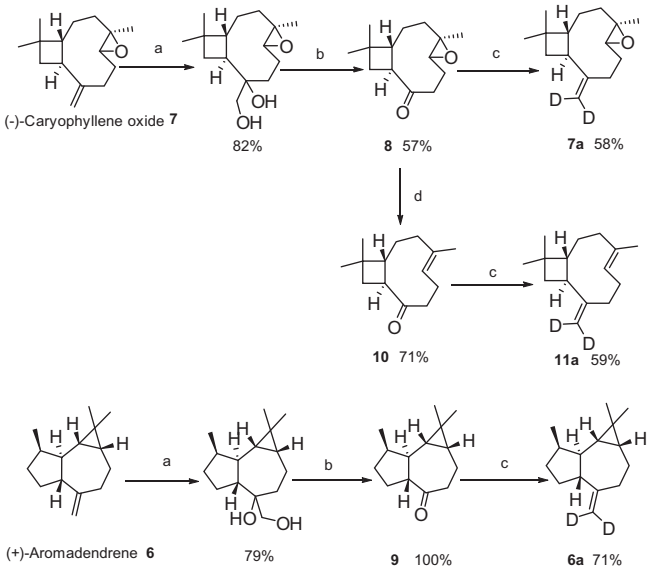
Ozone was initially explored for the synthesis of the ketone derivatives, but this resulted in significant amounts of side-products. However, use of a two-step procedure of dihydroxylation followed by diol cleavage gave the desired norsesquiterpene ketones **8** and **9** in good yield with little by-product formation. Another sesquiterpene was prepared via a de-epoxidation reaction, using an iodohydrin intermediate, of (−)-caryophyllene oxide ketone derivative (kobusone) **8** to form the β-caryophyllene ketone derivative **10** (Scheme 2). The de-epoxidation reaction gave a 5.5:1 ratio of the *trans* isomer, over the *cis* isomer (isocaryophyllene).

The deuterated Wittig reagent was freshly prepared by stirring methyl triphenylphosphonium bromide in excess deuterium oxide with sodium deuteroxide for 4 h.[18] The reaction time and the amount of sodium deuteroxide was optimised to maximise the percentage of deuterium exchange, while minimising the formation of the ylide. Although ultimately ylide formation is desired for the Wittig reaction, its formation during the deuterium exchange step is undesired, as the reactive ylide degrades with prolonged storage. The optimum stirring time, for the preparation of 1 g of deuterated reagent was 4 h, using 0.50 mL of sodium deuteroxide (30 wt.% in D_2O) in 10 mL of D_2O. Standard Wittig reaction conditions were then successfully employed with ketones **8**, **9** and **10** using the labelled Wittig reagent to give D_2-labelled products **6a**, **7a**, **11a** in 48–79% yields with approximately 95% deuterium incorporation.[19]

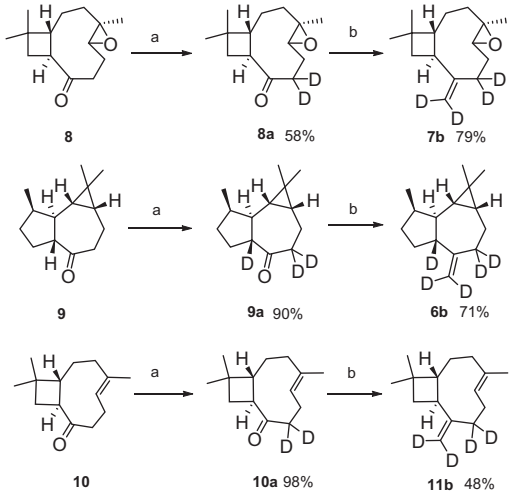
Considering the possible EI-MS structural lability of the deuterated methylene groups from these terminal alkenes, it was decided to introduce additional deuterium labels to the carbon adjacent to



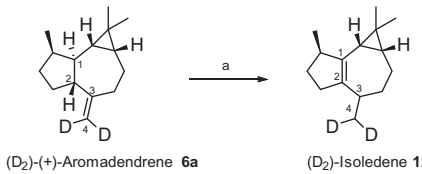
Scheme 1. Synthesis of farnesol based standards. (a) 1. DMP (1.5 equiv), CH_2Cl_2, rt, 4 h; (b) $NaClO_2$ (9 equiv), NaH_2PO_4 (7 equiv), *t*-BuOH/2-methyl-2-butene 4:1, rt, 48 h. 92% (over two steps); (c) $LiAlD_4$ (2 equiv), Et_2O, 1 h, 94%; (d) (D_3)-acetyl chloride (1.2 equiv), pyridine (1.2 equiv), CH_2Cl_2, 23 h, 55%; (e) EDC (10 equiv), DMAP (16 equiv), CH_2Cl_2, CD_3OH (50 equiv), 63%.



Scheme 2. Synthesis of deuterated alkenes. (a) OsO_4 (0.01 equiv), NMO (3 equiv), *t*-BuOH/H_2O 3:1, 70 h; (b) sodium metaperiodate (1.2 equiv), MeOH/H_2O 3:1, 4 h; (c) CD_3PPh_3Br (2 equiv), *n*-BuLi (1.5 equiv), THF, −78 °C to rt, 24 h; (d) Zn (5.7 equiv), NaI (1.7 equiv), NaOAc (1.03 equiv), AcOH, 48 h, rt.



Scheme 3. Synthesis of α-deuterated ketones and highly substituted sesquiterpenes. (a) D_2O (16 equiv), NaOD (1 equiv), dioxane, reflux 100 °C, 40 h; (b) CD_3PPh_3Br (2 equiv), *n*-BuLi (1.5 equiv), THF, −78 °C to rt, 24 h.



Scheme 4. Rearrangement of labelled aromadendrene **6a** to isoledene **12a**. (a) K/Al_2O_3, hexanes, 12 h, rt, 50%.

the terminal alkene. This was possible by exchange of the acidic α-protons of the previously prepared ketone intermediates. Using a method reported by Tkachov et al.,[20] deuteration of ketones **8**–**10** was found to be efficient giving deuterated ketones **8a**–**10a**.[21] Deuteration of ketones **8** and **10** resulted in exchange of the α-protons on the 9 membered ring, but not of the proton at the bridging carbon with the cyclobutane ring. However, with ketone **9** deuterium substitution occurred at both the α-carbons, resulting in (D_3)-ketone **9a** (Scheme 3). It should be noted that the labelled ketones **8a**–**10a** are also useful SIDA MS/MS analysis standards, as these norsesquiterpenes have all been identified as either natural products or, for **9a**, of interest as a synthetic starting material.[22]

With these (D_2) and (D_3) labelled ketones in hand, the Wittig reaction was undertaken on ketones **8a**–**10a** to give (D_4) and (D_5) labelled sesquiterpenes **6b**, **7b** and **11b**, now containing deuterium labels on both cyclic and exocyclic carbons (Scheme 3).

Finally an additional deuterated sesquiterpene was prepared via the rearrangement of (D_2)-aromadendrene **6a** to (D_2)-isoledene **12a**. The rearrangement occurs under harsh conditions upon reaction of aromadendrene **6** with potassium metal on Al_2O_3 in hexanes, but no mechanistic information has been reported.[23] It was discovered by undertaking the rearrangement on (D_2)-labelled aromadendrene **6a**, that the deuteriums remain on the exocyclic methyl (C-4), with no loss of deuterium at this position; thus suggesting that proton transfer from C-1 and C-2 to C-3 and C-4 occurs without scrambling of the protons at the terminal C-4 position (Scheme 4).

Preliminary studies in grape and wine were based on gas chromatography tandem mass spectrometry (GC–MS/MS). Optimisation of the MS/MS conditions allowed us to focus on the molecular ion and exploit the 3–5 Da *m*/*z* shift caused by the stable isotopic labelling. The standards prepared were used as internal standards in stable isotopic dilution (SID) MS/MS studies, to accurately quantify sesquiterpene levels in biological samples. Figure 1 shows the EI-MS/MS scan spectra obtained for some labelled and unlabelled sesquiterpenes studied.

In conclusion the methods presented herein provide a robust route for the synthesis of deuterated sesquiterpenes with various amounts of deuteration as required. The use of these labelled sesquiterpenes in the quantitative analysis of plant and fruit matrices will be reported in due course.

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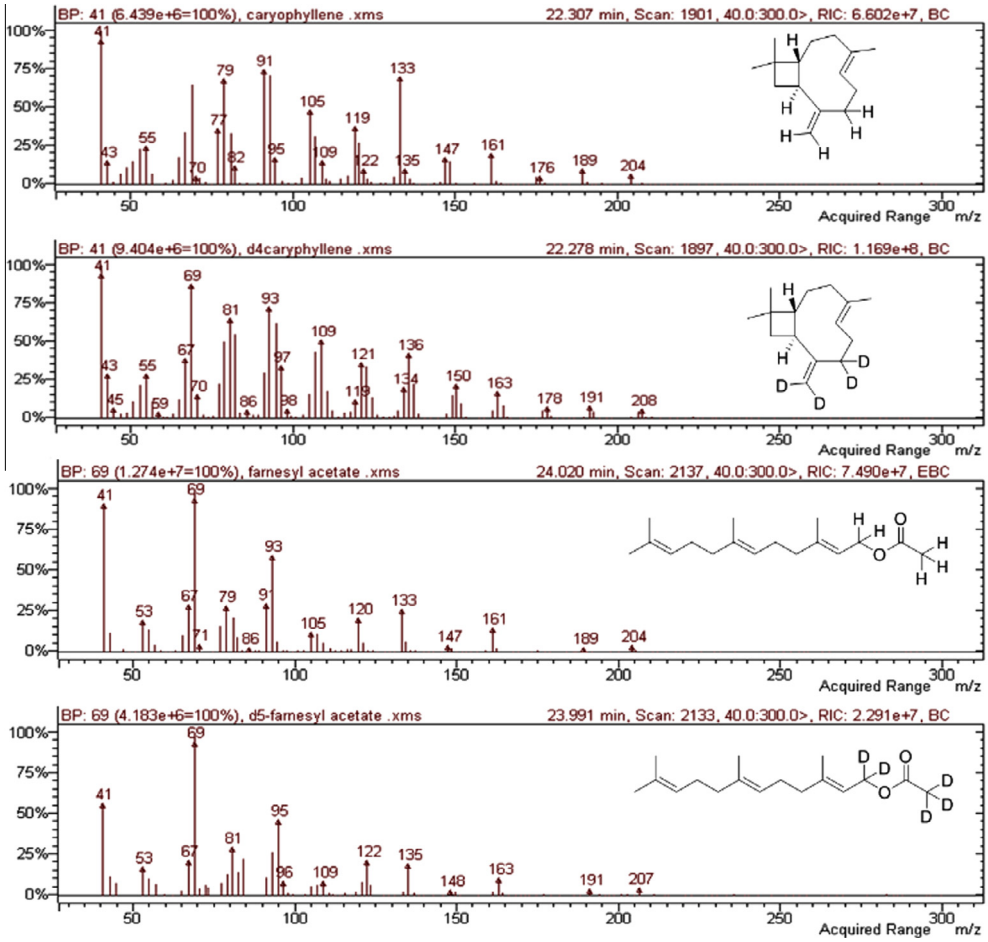

Figure 1. Examples of MS experiment with labelled and unlabelled sesquiterpenes fragmentation.

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Supplementary data

Supplementary data (full experimental details, characterisation data and NMR spectra for all compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.08.079.

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19. *General procedure for deuteration of sesquiterpene ketones:* To methyltriphenylphosphonium bromide or (D_3)-methyltriphenylphosphonium bromide (0.5 mmol) in THF (1.0 mL) under nitrogen at −78 °C was added *n*-BuLi (0.36 mmol) dropwise. The resulting mixture was stirred at 0 °C for 10 min, then cooled to −78 °C before adding a solution of ketone (0.24 mmol) in THF (1 mL) dropwise. The mixture was warmed to room temperature and stirred for a further 24 h. Water (5 mL) was slowly added, and the resulting mixture extracted with diethyl ether (25 mL). The organic layer was washed with 2 M HCl (5 mL), then 4 M NaOH (5 mL). The combined basic aqueous washes were further extracted with diethyl ether (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed in vacuo. The crude product was purified using flash chromatography (petroleum ether) to give the desired alkenes.
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