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Synthesis of d_6-deuterated analogues of aroma molecules-β-damascenone, β-damascone and safranal

Shabnam Mosaferi [a], Rebecca E. Jelley [a], Bruno Fedrizzi [a,b,*], David Barker [a,b,c,*]

[a] School of Chemical Sciences, University of Auckland, 23 Symonds St, Auckland, New Zealand
[b] Centre for Green Chemical Science, School of Chemical Sciences, University of Auckland, New Zealand
[c] MacDiarmid Institute for Advanced Materials and Nanotechnology, Wellington, Private Bag 92019, Auckland, New Zealand

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ABSTRACT

β-Damascenone, a C_{13}-norisoprenoid, is an important aroma compound not only in wine but also in the perfume and flavouring industries. Its key contribution to the floral aromas of many wine varieties, and impact on the overall wine aroma profile, make its quantification in various matrices of increasing interest and demand. The stable isotope dilution assay (SIDA) is one of the most precise analytical methods based on using isotopically labelled standards. Herein, we describe the synthesis of d_6-β-damascenone, d_6-β-damascone, and d_6-safranal, other aroma compounds in nature, starting from the readily available deuterated starting material d_6-acetone.

Introduction

Aroma is one of the main factors in determining the quality of grapes and the wine made from them. The compounds which affect grape and wine aroma are the product of complex chemistry, and many factors in different biosynthetic pathways are involved in establishing their final concentration [1-3]. Aroma compounds can accumulate in grapes in free form or bound to a sugar, and these compounds contribute to wine quality and are responsible for the varietal aroma of wines [4,5]. The major groups of aroma compounds are terpenoids, norisoprenopids, aromatics, aliphatics, organo-sulfur compounds and methoxypyrazines [6]. The norisoprenoids, also known as the apocarotenoids, are compounds derived from carotenoid degradation (C_{40}) and most norisoprenoids contain 13 carbon atoms [6]. Norisoprenoids tend to accumulate in grapes as non-volatile, odourless, bound glycosylated conjugates. Free volatile forms are released by enzymatic or acid hydrolysis [6].

β-Damascenone, an example of a C_{13}-norisoprenoid, is one of the most important natural flavours and has been detected in a variety of foods (honey, raspberry, apple, grape, mandarin, purple passion fruit, elderberry, starfruit, tomato, peach, and lychee) and beverages (black tea, coffee, beer, and wine) [7]. It is produced commercially as one of the mainstays of the perfume and flavouring industries. It is formed naturally by the hydrolytic breakdown of complex secondary metabolites derived from carotenoids such as neoxanthin (Fig. 1) [8].

β-Damascenone was first identified and isolated from Bulgarian rose (*Rosa damascena*) oil by Demole *et al.* in 1970 [9]. In grapes and wines, it was first identified in 1974 by Schreier and Drawert [10]. Following this, its presence in numerous wines of different cultivars and other alcoholic beverages (whiskey, brandy, and rum) has been reported [11]. β-Damascenone is associated with aroma descriptors such as honey-like, woody, fruity-flowery, quince-like, especially apple, and baked apple. Buttery *et al.* reported its odour threshold in water as low as 2 ng/L [11]. In wines, the detection threshold of β-damascenone varies significantly. In sweet white wines, Etievant *et al.* estimated this value at 4.5 μg/L whilst the odour threshold for β-damascenone can reach 4 μg/L, depending on the wine matrix, in red wines [12].

Due to its importance, several analytical methods for quantifying β-damascenone have been developed. With a focus on the wine matrix, these methods have employed a number of gas chromatography-based techniques including GC × GC-TOFMS [13], GC–MS [14] and GC-O [15-18], preceded by various extraction techniques such as solid-phase extraction (SPE) [19-22], stir bar sorptive extraction (SBSE) [14], headspace solid-phase microextraction (HS-SPME) [23-27], solid-phase microextraction (SPME) [28,29], and headspace solid phase dynamic extraction (HS-SPDE) [30].

Stable isotope dilution assay (SIDA) is a technique that facilitates the accurate quantification of trace concentrations using isotopically labelled compounds as analytical standards [11,31,32]. Unfortunately, to date, the only reported synthesis of a deuterated β-damascenone

* Corresponding authors at: School of Chemical Sciences, University of Auckland, 23 Symonds St, Auckland, New Zealand.
E-mail addresses: b.fedrizzi@auckland.ac.nz (B. Fedrizzi), d.barker@auckland.ac.nz (D. Barker).

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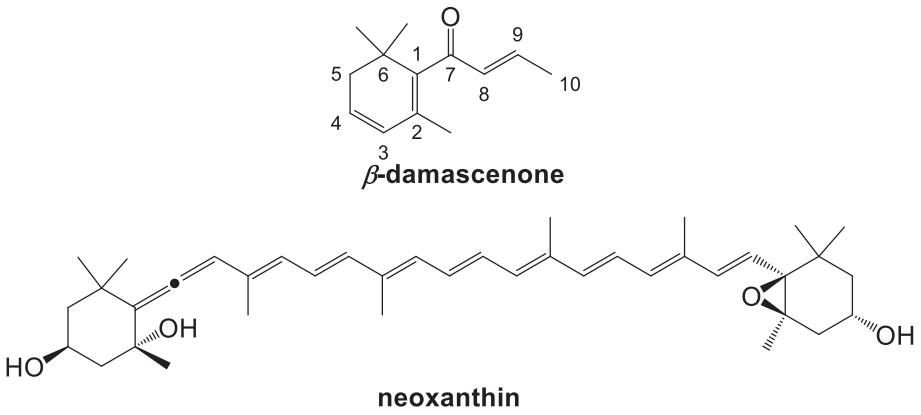
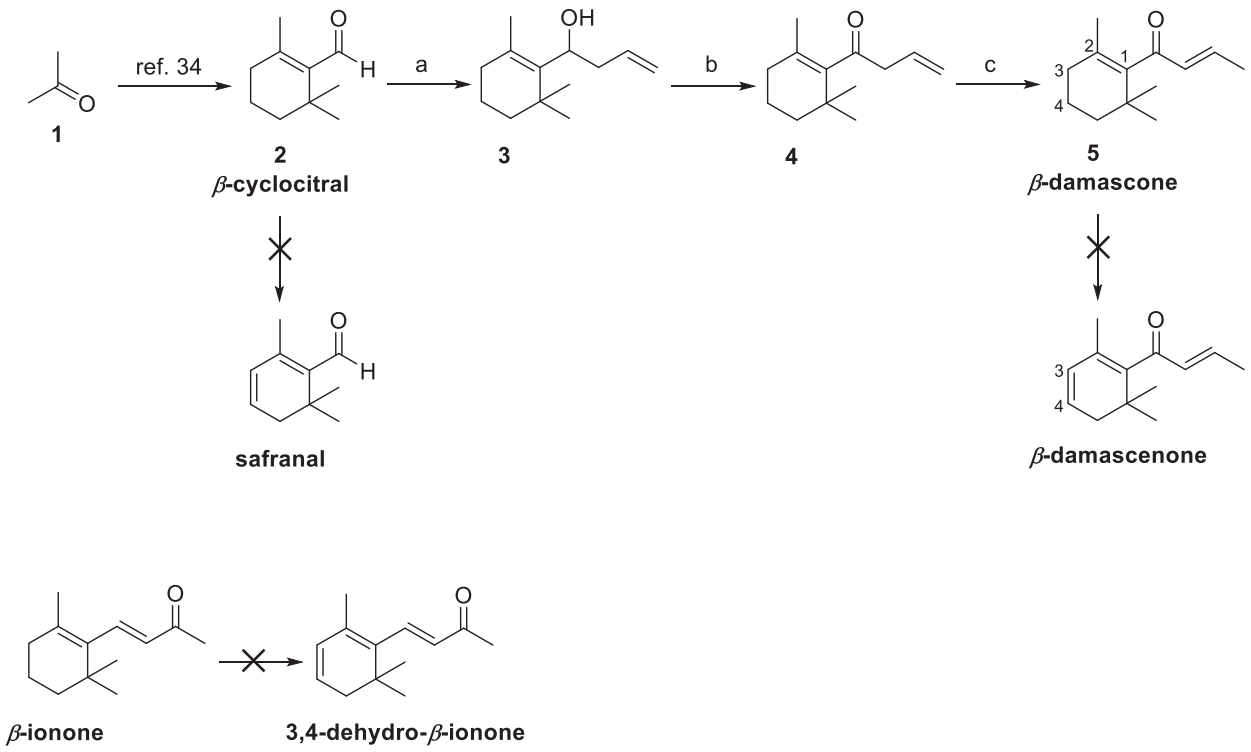


Fig. 1. β-damascenone (numbered carbon atoms) and natural precursor neoxanthin.



Scheme 1. Synthesis of β-damascone. Reagents and conditions: (a) 1 M allylmagnesium bromide (2.2 eq.), THF, rt, 4 h, 57%; (b) DMP (1.1 eq.), CH_2Cl_2, rt, 2 h, 96%; (c) DBU (3.0 eq.), CH_2Cl_2, 0 ℃, overnight, 92%.

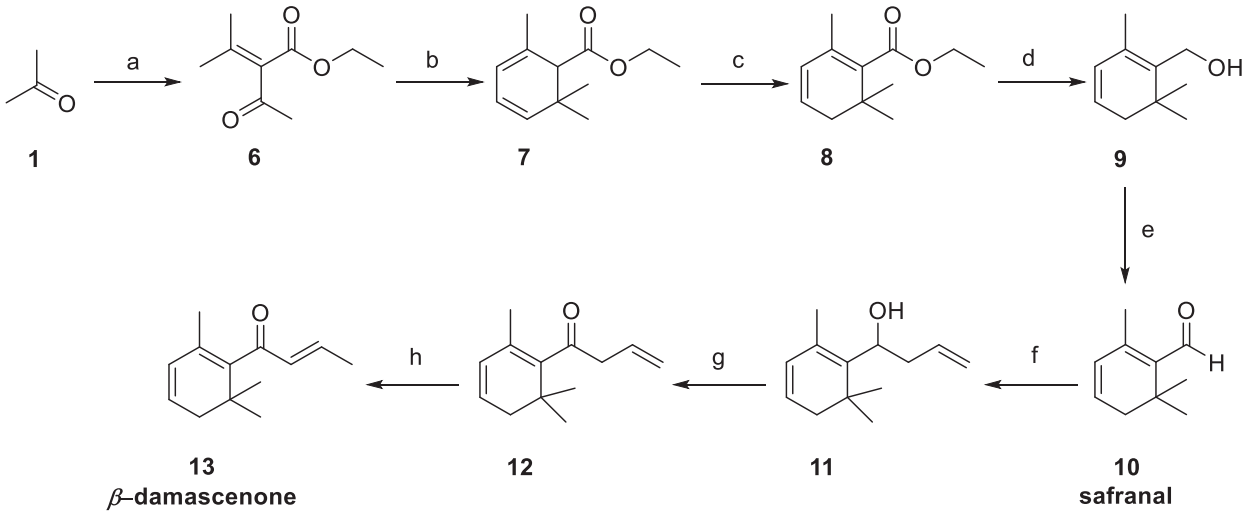
introduces four deuterium atoms at C-9 and C-10 [31] and these deuterium atoms are readily lost during mass spectrometry fragmentation limiting these derivatives use for analysis. These deuteriums are also susceptible to exchange in acidic or basic conditions making its use in non-neutral aqueous approaches, such as wine, limited.

Recently we reported the synthesis of another deuterated norisoprenoid, β-ionone, from inexpensive, and readily available, deuterated solvent d_6-acetone [33]. Due to the structural similarity of β-ionone, it was considered that deuterated d_6-β-damascenone could be prepared using the same starting material. This would result in the inclusion of six deuterium atoms to the non-labile methyl groups on the cyclohexene ring to yield a more suitable and robust analytical standard for the analysis of β-damascenone.

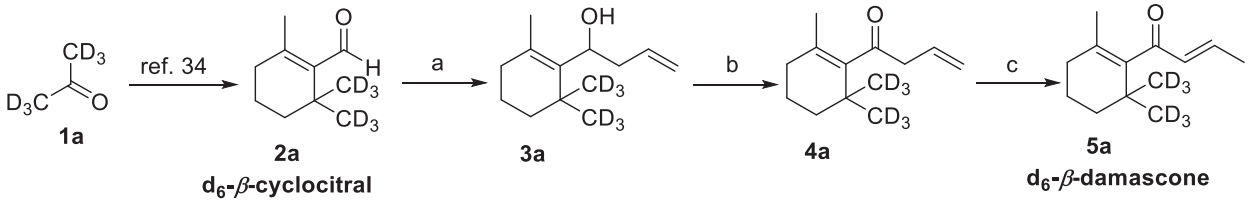
Result and discussion

As β-cyclocitral (**2**) was previously synthesised from acetone (**1**) [33], the proposed synthesis was first examined using unlabelled β-cyclocitral to prove the methodology. Addition of allylmagnesium bromide to β-cyclocitral (**2**) gave allylic alcohol **3** in 57% yield (Scheme 1) [34], which was oxidised using Dess-Martin periodinane (DMP) to provide ketone **4** in 96% yield. Rearrangement of the allyl group in **4** using DBU afforded the conjugated ketone **5** in 92% yield, which itself is a well-known aroma compound, ***β*-damascone**.

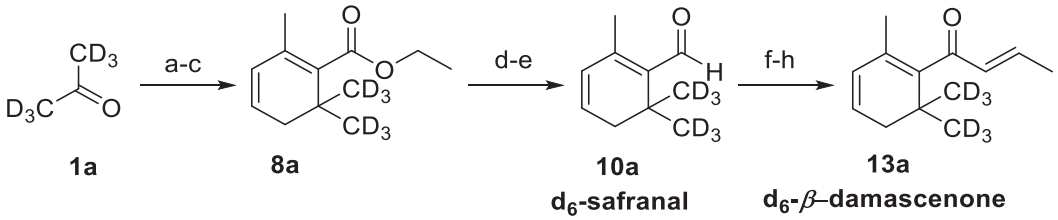
With β-damascone prepared, conversion to β-damascenone required dehydrogenation between C-3 and C-4 to form the cyclohexadiene found in β-damascenone. Limited methods for such a transformation have been



Scheme 2. Synthesis of safranal and *β*-damascenone. Reagents and conditions: (a) ethyl acetoacetate (0.7 eq.), $ZnBr_2$ (0.1 eq.), Ac_2O (0.9 eq.), 60 ℃, 72 h, 20%; (b) allyltriphenylphosphonium bromide (1.1 eq.), 1.6 M *n*-BuLi (1.1 eq.), THF, 0 ℃, overnight, 48%; (c) *p*-TsOH (0.2 eq.), toluene, 110 ℃, overnight, 51%, plus 15% **7**, partially seperable; (d) 1 M DIBAL-H (2.5 eq.), CH_2Cl_2, −78 ℃, 2 h, 46%; (e) DMP (1.1 eq.), CH_2Cl_2, rt, 2 h, 58%; (f) 1 M allylmagnesium bromide (2.2 eq.), THF, rt, 4 h, 51%; (g) DMP (1.1 eq.), CH_2Cl_2, rt, 2 h, 66%; (h) DBU (3.0 eq.), CH_2Cl_2, 0 ℃, overnight, 43%.



Scheme 3. Synthesis of d_6-*β*-damascone. Reagents and conditions: (a) 1 M allylmagnesium bromide (2.2 eq.), THF, rt, 4 h, 50%; (b) DMP (1.1 eq.), CH_2Cl_2, rt, 2 h, 93%; (c) DBU (3.0 eq.), CH_2Cl_2, 0 ℃, overnight, 94%.



Scheme 4. Synthesis of d_6-safranal and d_6-*β*-damascenone. Reagents and conditions; (a) ethyl acetoacetate (0.7 eq.), $ZnBr_2$ (0.1 eq.), Ac_2O (0.9 eq.), 60 ℃, 72 h, 25%; (b) allyltriphenylphosphonium bromide (1.1 eq.), 1.6 M *n*-BuLi (1.1 eq.), THF, 0 ℃, overnight, 46%; (c) *p*-TsOH (0.2 eq.), toluene, 110 ℃, overnight, 52%; (d) 1 M DIBAL-H (2.5 eq.), CH_2Cl_2, −78 ℃, 2 h, 44%; (e) DMP (1.1 eq.), CH_2Cl_2, rt, 2 h, 60%; (f) 1 M allylmagnesium bromide (2.2 eq.), THF, rt, 4 h, 59%; (g) DMP (1.1 eq.), CH_2Cl_2, rt, 2 h, 45%; (h) DBU (3.0 eq.), CH_2Cl_2, 0 ℃, overnight, 47%.

reported [35-38], and all use allylic functionalisation of C-3 before elimination to form the C-3/4 alkene.

Different methods (see Table 1 in supplementary materials) were examined for the synthesis of *β*-damascenone from *β*-damascone. In most of the attempts, at first, *N*-bromosuccinimide (NBS) in the presence of benzoyl peroxide (BPO), azobisisobutyronitrile (AIBN), or visible light (hν) was added to *β*-damascone to prepare the allyl bromide intermediate [36,37]. In the second step, different bases such as sodium carbonate (Na_2CO_3), 1,4-diazabicyclo[2.2.2]octane (DABCO), or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were used to facilitate the dehydrobromination reaction. In another attempt, benzoic acid (BzOH), cobalt (II) chloride ($CoCl_2$) and di-tert-butyl peroxide (DTBP) were added to *β*-damascone in an attempt to perform an allylic cobalt-catalyzed oxidative esterification reaction; p-toluenesulfonic acid (p-TsOH) was added with the hope of dehydrocarboxylating the ester to the alkene [38].

In all cases the desired product was not obtained and only starting material was recovered. It was considered that the multiple allylic sites on *β*-damascone were problematic. Therefore, attempts were made to introduce the alkene on *β*-cyclocitral which does not contain the *α*,*β*-unsaturated ketone functionality [39] and also on *β*-ionone, which has previously been reported to have been converted to 3,4-dehydro-*β*-ionone [40,41]. In these cases, complex mixtures of products were obtained with few containing only trace amounts of the desired diene.

Being unable to introduce the C-3/4 alkene at the latter stage, a new synthetic strategy for the synthesis of *β*-damascenone (**13**) from acetone (**1**) was proposed (Scheme 2). The reaction of acetone (**1**) with ethyl acetoacetate in the presence of zinc bromide and acetic anhydride gave

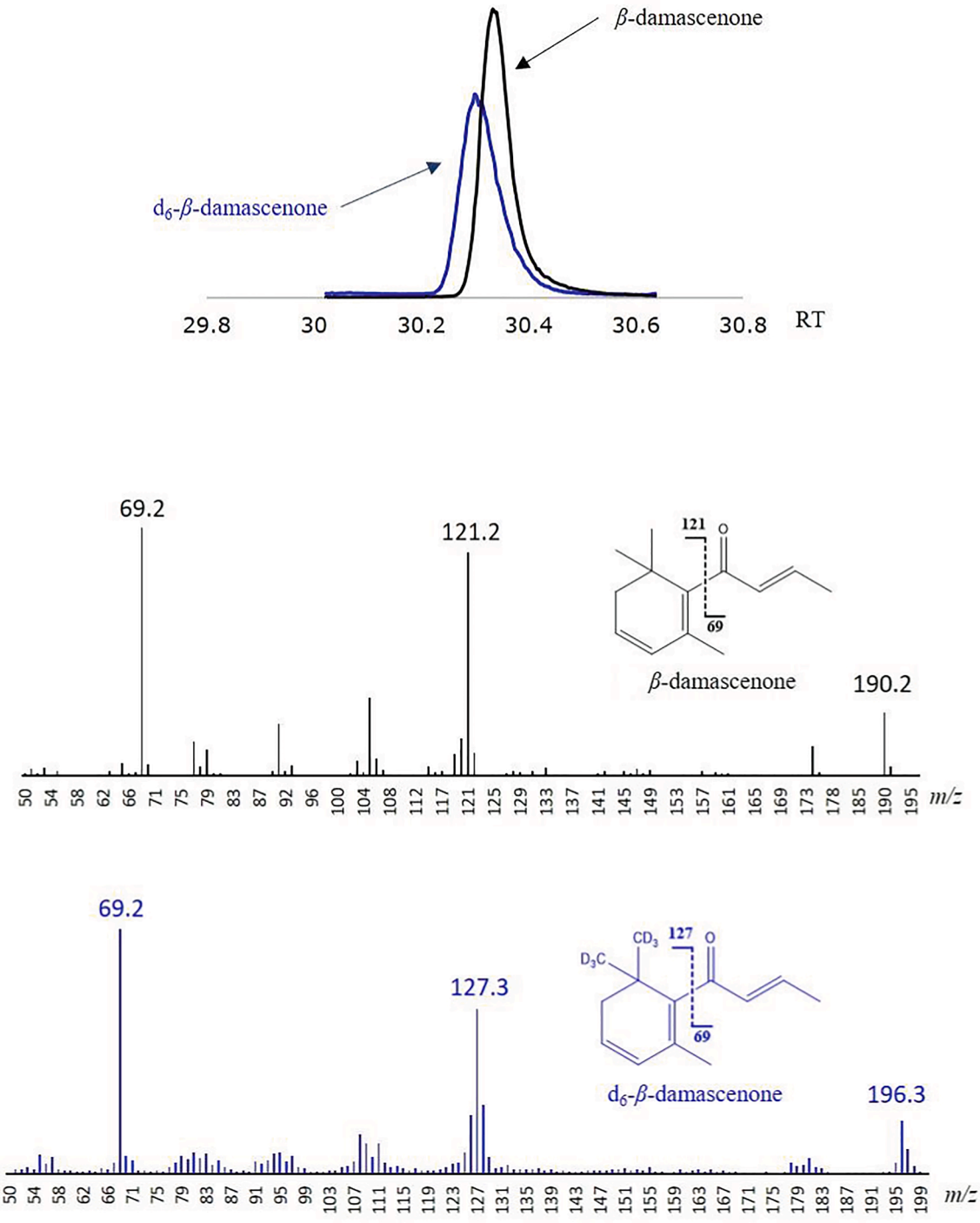


Fig. 2. GC–MS chromatograms and spectrums for unlabelled and labelled-β-damascenone.

ester **6** [42], which was followed by a tandem Wittig/electrocyclisation reaction with allyltriphenylphosphoranyl ylide to afford cyclic ester **7** in 48% yield [39]. A rearrangement reaction, which introduces the desired diene functionality found in ***β*-damascenone** earlier in the synthesis was then conducted reacting ester **7** with *p*-toluenesulfonic acid and afforded the fully conjugated ethyl β-safranate (**8**) in 51% yield, along with recovered 15% of starting material **7** (as determined by ratios of products in the ^{1}H NMR) [42]. Due to the difficulties in the separation of product **8**, the mixture of starting material **7** and product **8** was used for the next step. Reduction of the mixture **7** and **8** using DIBAL-H provided two alcohols, and the required primary alcohol **9** could be easily isolated in 58% yield. Alcohol **9** was then oxidised using Dess-Martin periodinane (DMP) to give the well-known aroma compound **10**, commonly known as **safranal**. Safranal (**10**) was reacted with allylmagnesium bromide to give unstable allyl alcohol **11** which was oxidized immediately using DMP to provide ketone **12** in 66% yield. DBU was used for the rearrangement of **12** to afford the desired ***β*-damascenone** (**13**) in 43% yield.

With the synthetic routes to aroma compounds determined, the synthesis of d_6-β-damascone (**5a**) was then completed using d_6-acetone (**1a**) rather than acetone (**1**) (Scheme 3).

Finally, the synthesis of d_6-safranal (**10a**) and d_6-β-damascenone (**13a**) was achieved using the same procedure used to synthesise β-damascenone (**13**) from acetone (**1**) (Scheme 4).

When we compared the overall yields for the non-deuterated and deuterated derivates of β-damascone, safranal, and β-damascenone there were no major differences in the yields (e.g. **2** to **5**, overall yield 50% vs **2a** to **5a**, overall yield 43%). The overall yields for safranal, and thus β-damascenone from acetone are quite low, but are greatly affected by the poor yields on the first two steps. These steps can fortunately be undertaken at large scale using the readily available and comparatively cheap acetone or d_6-acetone.[33]

The NMR and HRMS data (shown in the supplementary file) are consistent with no loss of deuterium labelling during the synthesis from d_6-acetone. The ^{1}H NMR spectra of all deuterated compounds are missing the six protons assigned to the geminal methyl groups present in their unlabeled counterparts. The observation of broad signals in the ^{13}C NMR spectra is again consistent with complete deuteration of the geminal methyl groups. In addition, the HRMS data experimentally found for these deuterated compounds show the molecular ion at 6 *m/z* units greater than their unlabeled analogues.

Newly synthesised d_6-β-damascenone (**13a**) and unlabelled β-damascenone were subsequently injected on the GC–MS to confirm the anticipated fragmentation patterns for these compounds (Fig. 2). A key fragmentation pathway for β-damascenone is the loss of the β-unsaturated ketone side chain via the bond cleavage between C1 and C7 (121.2 *m/z*, Fig. 2). The peak at 175 *m/z* in the mass spectrum of unlabelled β-damascenone is indicative of the loss of a methyl group. Peaks at 178 and 181 *m/z* in the mass spectrum of d_6-β-damascenone suggests cleavage of CH_3 or CD_3, respectively.

In conclusion, syntheses of d_6-β-damascone (**5a**), d_6-safranal (**10a**), and d_6-β-damascenone (**13a**) has been achieved from the readily available deuterated starting material d_6-acetone. These isotopically labelled analogues could be used as analytical standards for precise quantitative analyses of these essential aroma compounds.

CRediT authorship contribution statement

Shabnam Mosaferi: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Rebecca E. Jelley:** Conceptualization, Supervision. **Bruno Fedrizzi:** Conceptualization, Supervision, Funding acquisition. **David Barker:** Conceptualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2021.100264.

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