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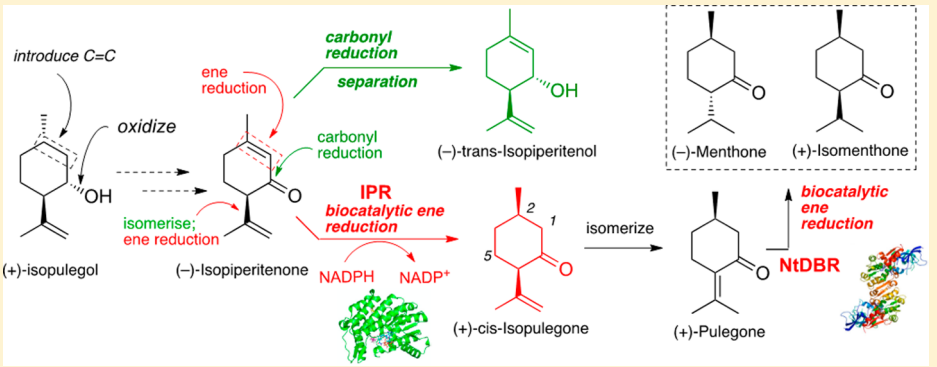
Chemoenzymatic Synthesis of the Intermediates in the Peppermint Monoterpenoid Biosynthetic Pathway

Aisling Ní Cheallaigh, David J. Mansell, Helen S. Toogood, Shirley Tait, Antonios Lygidakis, Nigel S. Scrutton,* and John M. Gardiner*

BBSRC/EPSRC Manchester Synthetic Biology Research Centre for Fine and Speciality Chemicals (SYNBIOCHEM), Manchester Institute of Biotechnology and School of Chemistry, The University of Manchester, 131 Princess Street, Manchester M1 7DN, U.K.

Supporting Information



ABSTRACT: A chemoenzymatic approach providing access to all four intermediates in the peppermint biosynthetic pathway between limonene and menthone/isomenthone, including noncommercially available intermediates (−)-*trans*-isopiperitenol (**2**), (−)-isopiperitenone (**3**), and (+)-*cis*-isopulegone (**4**), is described. Oxidation of (+)-isopulegol (**13**) followed by enolate selenation and oxidative elimination steps provides (−)-isopiperitenone (**3**). A chemical reduction and separation route from (**3**) provides both native (−)-*trans*-isopiperitenol (**2**) and isomer (−)-*cis*-isopiperitenol (**18**), while enzymatic conjugate reduction of (−)-isopiperitenone (**3**) with IPR [(−)-isopiperitenone reductase)] provides (+)-*cis*-isopulegone (**4**). This undergoes facile base-mediated chemical epimerization to (+)-pulegone (**5**), which is subsequently shown to be a substrate for *Nt*DBR (*Nicotiana tabacum* double-bond reductase) to afford (−)-menthone (**7**) and (+)-isomenthone (**8**).

Monoterpenoids are secondary metabolites found in higher order plants. They seemingly play no major role in the metabolic functioning of the plant but serve as deterrents or toxins to predators. Monoterpenoids are renowned for their characteristic fragrant odors and flavors and, as a result, have been extensively exploited by the agricultural, pharmaceutical, and food industries as insecticides, antiallergenic agents, perfumes, and food additives.[1] Most of the commonly used monoterpenoids, such as menthol, camphor, limonene, and carvone, remain largely obtained from natural sources. This requires large portions of agricultural land, and the extraction processes are environmentally costly and low-yielding. Several industrial synthetic routes to (−)-menthol (**10**) have been developed, which has increased worldwide capacity, notably the Symrise process from *m*-cresol (involving a resolution crystallization),[2] the Takasago process (using an asymmetric hydrogenation of a myrcene derivative and a diastereoselective cyclization),[3] and the BASF process [exploiting a catalytic ene-cyclization of (+)-citronellal to isopulegol, then hydrogenation to (−)-menthol].[4] Synthesis of (+)-neoisomenthol has been reported by reduction of (+)-isomenthone using $LiAlH_4$.[5]

The use of biosynthetically engineered microorganisms potentially offers an attractive alternative "green" approach to (−)-menthol (**10**, Scheme 1) and to a diverse range of other monoterpenoids, including those less available from harvested biomass. The peppermint biosynthetic pathway of *Mentha piperita* (Scheme 1) converts limonene (**1**) ultimately into the menthol systems (**9**−**12**). We have recently reported use of an engineered organism containing genes for the ene-reductase and dehydrogenase enzymes required to effect the synthesis of (−)-menthol (**10**) and (+)-neomenthol (**9**) from (+)-pulegone (**5**) (the latter steps of the biosynthetic pathway).[6] This provided a proof-of-principle for a new bioengineered synthesis approach to high-value menthols. The native biosynthetic pathway (Scheme 1) produces several potentially useful intermediates en route from limonene (**1**) through to (+)-pulegone (**5**), which are found in significantly lower abundance in peppermint essential oil than the menthols. The oil contains a high level of (−)-menthol (**10**), a moderate level of menthone (**7**), and low levels of (+)-pulegone (**5**) and

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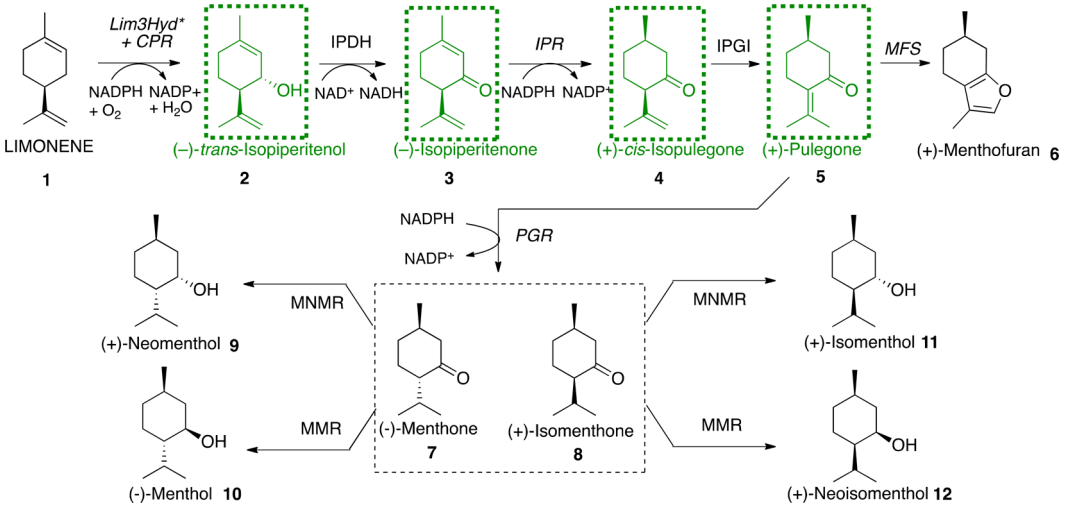
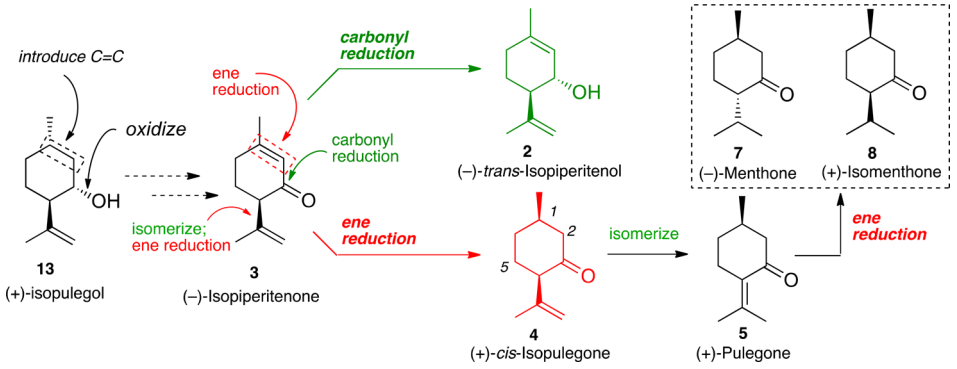
Scheme 1. Peppermint Biosynthetic Pathway[a]



[a]Enzymes: Lim3Hyd = (−)-limonene-3-hydroxylase; IPDH = (−)-*trans*-isopiperitenol dehydrogenase; IPR = (−)-isopiperitenone reductase; IPGI = (+)-*cis*-isopulegone isomerase; PGR = (+)-pulegone reductase; MMR = (−)-menthone:(−)-menthol reductase; MNMR = menthone:(+)-neomenthol reductase; MFS = (+)-menthofuran synthase.

Scheme 2. Proposed Chemical/Chemoenzymatic Synthesis of Peppermint Biosynthetic Pathway Intermediates 2−5 and Menthones 8 and 9 from (+)-Isopulegol (13)



(+)-menthofuran (**6**), making these the commercially available substrates from the extraction process. This leaves half of the compounds [(−)-*trans*-isopiperitenol (**2**), (−)-isopiperitenone (**3**), (+)-*cis*-isopulegone (**4**), (+)-isomenthone (**8**), and (+)-neoisomenthol (**12**)] in the biosynthetic pathway unobtainable in reasonable yields from natural sources.

Synthetic and/or biocatalytic routes to such intermediates would thus be advantageous, giving access to potentially valuable, currently noncommercially available, compounds.

Chemical routes toward these intermediates have been reported. However, these generally suffer from lack of regioselectivity and the formation of unwanted byproducts. Previous chemical approaches to (−)-isopiperitenone (**3**) have involved the allylic oxidation of (*R*)-limonene (**1**) using a range of different oxidants. Several variations employing chromium-based reagents have also been described, including oxidation with PCC,[7] $Cr(CO)_6$,[8] and CrO_3,[9] though often with poor yields. Other oxidative methods include using a combination of *tert*-butylhydroperoxide and either catalytic $Cr(CO)_6$,[10] catalytic dirhodium(II) caprolactamate,[11] or (trifluoroacetoxyiodo)benzene.[12] However, the usefulness of this approach is limited by a lack of regiocontrol, with oxidation occurring at both the 3- and 6-positions. Alternative approaches employing cyclization of linear starting materials are generally limited by the production of racemic products. For example, a [2 + 2] cycloaddition of a vinyl ketone in the presence of Et_3N, followed by ring-opening with $BF_3{\cdot}OEt_2$, has been described.[13] The cyclization of geraniol via oxidation using a polymer-bound oxoammonium reagent has also been used to generate isopiperitenone (**3**).[14] Oxidation of (−)-*cis*-isopiperitenol with MnO_2 has also been reported. The latter work also included enantioselective synthesis of (−)-*cis*-isopiperitenol (**18**). Formation of **18** as a diastereomeric mixture along with **2** has been reported via a transannular [2,3]-Wittig rearrangement of a nine-membered diallylic ether[15] and via a lipase-mediated resolution approach.[16] Mixtures of *cis*- and *trans*-isopiperitenol have been produced by $LiAlH_4$ reduction[8] and fluoride ion-induced cyclization of the corresponding formyl-bearing regioisomeric allylsilane.[17]

The only reported synthesis of pure (+)-*cis*-isopulegone (**4**) involves the isomerization of pulegone at high temperature and pressure.[18] A synthesis of a mixture of isopulegone diastereomers has been described starting from (−)-citronellal[19] and a fully racemic and isomeric mixture synthesis reported in modest yield via iron-complex-mediated vinyl ether addition to a 3-methylcyclohexanone enolate.[20]

Our prior work[2] demonstrated the application of metabolically engineered cellular biotransformations of (+)-pulegone

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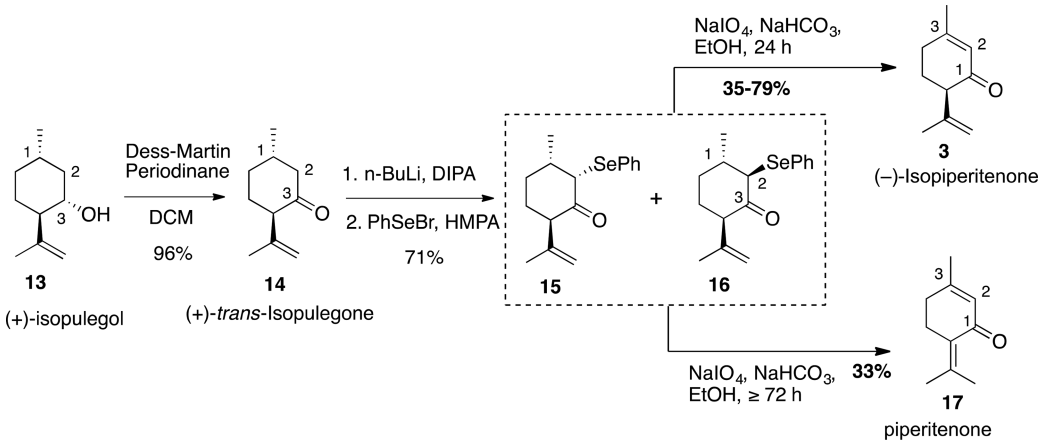
Scheme 3. Oxidation, Selenation, and Oxidative Elimination Synthesis of (−)-Isopiperitenone (3)



(**5**) through to the menthols, using bioengineered cells combining ene-reductase and menthone dehydrogenase-catalyzed processes. Here, we report chemical/chemoenzymatic syntheses of all the peppermint pathway intermediates **2**−**5** and of menthone (**7**) and isomenthone (**8**), on hundreds of milligram to gram scales. The synthetic plan (Scheme 2) envisaged starting from chemical oxidation of (+)-isopulegol (**13**) to the ketone, installation of α,β-unsaturation to give (−)-isopiperitenone (**3**), then either carbonyl or enone conjugate reductions of **3** to provide (−)-*trans*-isopiperitenol (**2**) or (+)-*cis*-isopulegone (**4**), respectively. We envisaged an isomerization strategy would deliver the thermodynamically more stable (+)-pulegone (**5**), from which a further ene reduction (via chemical or chemoenzymatic methods using isolated enzymes) could afford methone (**7**) and/or isomenthone (**8**). This paper describes completion of chemoenzymatic syntheses of all the biosynthetic intermediates in the peppermint pathway. Important elements of the current report are exploiting an efficient chemoenzymatic ene reduction of (−)-isopiperitinone (**3**) to (−)-*cis*-isopulgeone (**4**) and demonstrating a preparative scale chemoenzymatic conversion of (+)-pulegone (**5**) (from chemical isomerization of **4**) to the menthones.

This approach would provide practicable access to a number of monoterpenoid products, completing the chemoenzymatic route to the menthols[6] and providing access to a number of synthesized monoterpenoids.

RESULTS AND DISCUSSION

Oxidation of the secondary hydroxy group of (+)-isopulegol (**13**) to the ketone **14** was effected by Dess−Martin periodane oxidation[21] in 96% yield (Scheme 3). We reasoned that selenoxide elimination[22] may be a suitable approach to introduce the α,β-unsaturated carbonyl functionality of (−)-isopiperitenone (**3**) via selenation of the enolate derived from **14**.[23] Thus, treatment of the lithium enolate of **14** with PhSeBr afforded the 2-phenylselenylisopulegone derivatives **15** and **16** in good yields and as a 7:1 mixture of diastereomers, respectively, favoring the (2*S*)-isomer **15**. The high selectivity in favor of **15** could be anticipated from preferences for an axial-type enolate addition reaction in the chair for the major product pathway or via the twist-boat in the minor product pathway.

Each of the diastereomers **15** and **16** is anticipated to adopt one of the possible chair-like conformers. Assignment of the diastereoisomers was provided by ^{1}H NMR scalar coupling constants, specifically, that $J_{1,2}$ = 11.2 Hz for the minor isomer is consistent only with the all-equatorial conformer of the (2*R*)-isomer **16** (Figure 1), as the only possible conformer of either diastereomer which could have a large $J_{1,2}$.

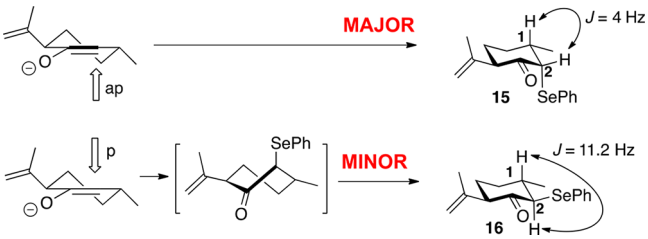


Figure 1. Confirmation of assignment of diastereomers of α-selenoterpenones **15** and **16** from $J_{1,2}$ coupling constants (*p* = parallel; ap = antiparallel, with respect to H-1).

The $J_{1,2}$ = 4 Hz for the major (2*S*)-isomer **15** combined with the significant shift of H-4 (from 3.08 ppm for isomer **16** to 3.85 ppm for isomer **15**) but comparable H-4 coupling constants (12 and 5 Hz) supports confirmation of the diastereostructure shown in the anticipated conformation.

Several oxidants (H_2O_2, $NaIO_4$, or *m*CPBA) were evaluated for oxidative elimination from **15**/**16** to provide (−)-isopiperitenone (**3**) on a small scale (up to 100 mg) and on larger scales (0.50−5 g). A $NaIO_4/NaHCO_3$ mixture was employed as the reagent of choice,[24] and reactions were quenched after 24 h. Extending the reaction (3 days) led to concurrent isomerization of the terminal double bond of the (−)-isopiperitenone (**3**) product to give piperitenone (**17**) in moderate recovery. Oxidative elimination provided (−)-isopiperitenone (**3**) in ca. 80% yields on the smallest scales (where preparative HPLC could be employed), but with a lower recovery (35−40%) on gram scales. Column chromatography followed by Kuglerohr distillation at reduced pressure proved an effective method for the removal of selenium contaminants on a gram scale. This successive purification method, in combination with incomplete oxidation, wherein starting materials were isolated in 10−40% yield on different gram scales, may explain the moderate recovery of **3**. There was no indication of any significant kinetic difference between oxidative elimination of the two diastereomers **15** and **16**. The isomers were separated and individually subjected to the oxidative elimination conditions, where TLC analysis (1 min, 5

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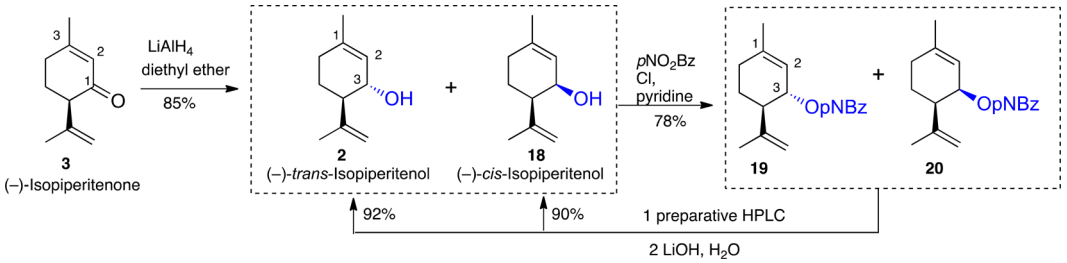
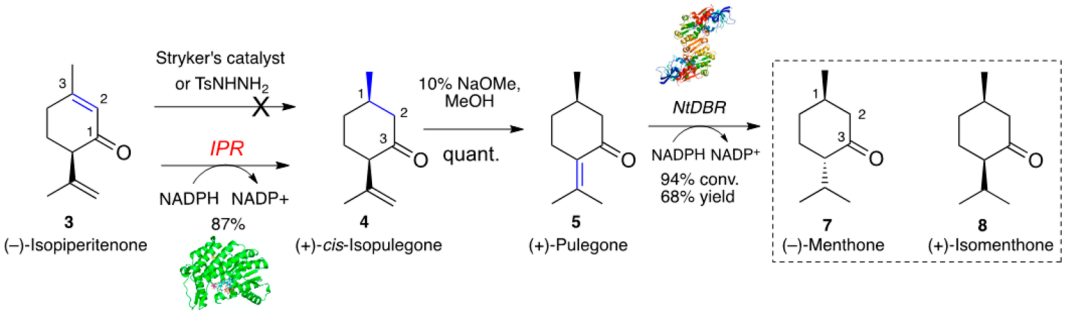
Scheme 4. Synthesis of Pure *cis* and *trans* (−)-Isopiperitinols from (−)-Isopiperitenone (3)



Scheme 5. Sequential Chemoenzymatic Conjugate Reductions for Synthesis of *cis*-Isopulegone (4) and Menthone/Isomenthone (7/8)



min, 15 min, 30 min, 1 h, 4 h, 16 h, 24 h) indicated similar reaction progress for both diastereomers. Overall, Scheme 3 illustrates a convenient gram-scale route to pure (−)-isopiperitenone (**3**) in yields of 20−25% over the three steps.

With this gram-scale route to pure (+)-isopiperitenone (**3**) in place, conversion to isopiperitenol was evaluated.

Reduction of **3** with $LiAlH_4$ afforded *trans*/*cis*-isopiperitenols (**2** and **18**) in 85% yield in a ratio of 3:2, with the major product, *trans*-isopiperitenol (**2**) being the naturally observed isomer in the peppermint pathway (Scheme 4). These diastereomers proved inseparable by column chromatography, but their *p*-nitrobenzoyl ester derivatives, **19** and **20**, were separable by preparative HPLC (Figure S11, Supporting Information) (separation by crystallization proved ineffective). Hydrolysis of the *p*-nitrobenzoyl group with LiOH in H_2O/THF afforded the separate stocks of pure *trans*- and *cis*-isopiperitenol (**2** and **18**), respectively.

Stereoselective conjugate reduction of α,β-unsaturated ketones typically involves the use of transition metal catalysts. Stryker's reagent is a Cu catalyst for the conjugate reduction of a range of enones and enoates.[25] Attempted conjugate reduction of isopiperitenone (**3**) to *cis*-isopulegone (**4**) employing Stryker's catalyst evidenced no reduction to *cis*-isopulegone (**4**), even with prolonged reaction time. The same outcome was observed either with $(Ph_3P)_3RhCl/Et_3SiH$ or when tosyl hydrazone was employed[26] (Scheme 5). The native peppermint enzyme, which acts on the (−)-isopiperitenone (**3**) to produce (+)-*cis*-isopulegone (**4**), is known, and thus a preparative scale biotransformation using isopiperitenone reductase (IPR, Uniprot: Q6WAU1)[27] was evaluated. Monitoring by GC showed full conversion in 1 h with no evidence of degradation. Biotransformation was thus performed on preparative scale to give *cis*-isopulegone (**4**) on a ∼600 mg scale.

With a preparative scale chemoenzymatic synthesis of (+)-*cis*-isopulegone (**4**) established, treatment of **4** with NaOMe/MeOH effected isomerization of the terminal double bond to give the more stable conjugated, tetrasubstituted C-2 exocyclic olefinic bond of (+)-pulegone (**5**) in quantitative yield, suitable for subsequent use to take directly on without further purification. A second conjugate reduction, involving the exocyclic double bond of (+)-pulegone (**5**), was needed to give menthone (**7**) and/or isomenthone (**8**). Conjugate reduction using Stryker's catalyst, $Na_2S_2O_6$, and tosyl hydrazine all produced some menthone and isomenthone products, but these reductions did not go to completion. As with the conjugate reduction of (−)-isopiperitenone (**3**) (vide supra), the poor efficacy of chemical methods for such conjugate reductions led to assessment of biocatalytic reduction. The enzyme double-bond reductase (DBR) from *Nicotiana tabacum* (*Nt*DBR-C-His6 in pET21b; Uniprot: Q9SLN8) was prepared as described[28] and used for a preparative biotransformation. The in vivo capacity of *Nt*DBR has already been demonstrated in our prior report on bioengineered menthol synthesis.[6] Here, the isolated *Nt*DBR effected conjugate reduction of (+)-pulegone (**5**) to give both menthone (**7**) and isomenthone (**8**) in 94% conversion (HPLC, 55:45 menthone:isomenthone) isolated in 68% yield (37% menthone, 31% isomenthone). The reaction was devoid of side-product formation, and the products could readily be separated by column chromatography.

In conclusion, we have developed a chemoenzymatic route to the four intermediates (**2**−**5**) in the peppermint pathway from limonene to (+)-pulegone (**5**) and subsequently to menthone (**7**) and isomenthone (**8**), and, additionally, this provides a route to pure (−)-*cis*-isoiperitenol (**18**). This exploits the efficacy of two sequential biocatalytic enone conjugate reductions with IPR and *Nt*DBR and also completes synthetic−biocatalytic access to all intermediates in this pathway. Multihundred milligram syntheses of noncommercially available biosynthesis intermediates (−)-isopiperitenone (**3**) and (+)-*cis*-isopulegone (**4**) are now readily accessible, along with a convenient conversion of **4** into (+)-pulegone (**5**).

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In combination with our previous work[6] describing the conversions of menthone and isomenthone to all four menthol-type products, the chemoenzymatic access to menthone and isomenthone reported herein completes the formal chemoenzymatic syntheses of all materials in the peppermint biosynthetic pathway from (+)-limonene to the menthols.

EXPERIMENTAL SECTION

General Experimental Procedures. Unless otherwise stated, all reactions were carried out in oven-dried glassware. Reactions were monitored by TLC on silica gel 60 F_{254} plates and visualized by ultraviolet or staining with the agents phosphomolybdic acid, vanillin, or $KMnO_4$, as specified in the specific procedures. Column chromatography was performed on Merck silica gel 60 (particle size 40−63 μm). ^{1}H NMR and ^{13}C NMR spectra were obtained using a 400 MHz and an 800 MHz spectrometer and are reported as chemical shift on the parts per million scale. Multiplicity is abbreviated (br = broad, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, etc.), and coupling constants were obtained in hertz. Assignments were aided by DEPT 90, DEPT 135, COSY, and HMQC. Compound numbering adopts the *p*-menthane convention. Infrared spectra were recorded on an FTIR spectrometer. High-resolution mass spectra were obtained at the University of Manchester. Gas chromatography used an Agilent Technologies 7890A GC system equipped with an FID detector and a 7693 autosampler. A DB-WAX column (30 m; 0.32 mm; 0.25 μm film thickness; JW Scientific) was used with an injector temperature at 220 °C with a split ratio of 20:1 (1 μL injection). The carrier gas was He with a flow rate of 1 mL min^{-1} and a pressure of 5.1 psi. The program began at 40 °C with a hold for 1 min followed by an increase of temperature to 210 °C at a rate of 10 °C/min, with a hold at 210 °C for 1 min. The FID detector was maintained at a temperature of 250 °C with a flow of H_2 at 30 mL/min. GC-MS was performed on an Agilent Technologies 7890A GC with an Agilent Technologies 5975C inert XL-EI/CI MSD with triple axis detector. A Zebron ZB-Semi Volatiles column (15 m × 0.25 mm × 0.25 μm film thickness, Phenomenex) was used with an injector temperature of 220 °C with a split ratio of 10:1 (1 μL injection). The carrier gas was He with a flow rate of 1 mL min^{-1} and a pressure of 5.1 psi. The program began at 40 °C with a hold for 3 min followed by an increase of temperature to 210 °C at a rate of 10 °C/min, with a hold at 210 °C for 3 min. The MS fragmentation patterns were entered into the NIST/EPA/NIH 11 (mass spectral library for identification of a potential match). NMR spectra were reprocessed using Nucleomatica iNMR 4.0, MestreNova, or Bruker TopSpin.

(+)-*trans*-Isopulegone (14). A solution of (+)-isopulegol (**13**) (0.90 g, 5.83 mmol) in anhydrous DCM (50 mL) was added to a suspension of Dess−Martin periodinane (2.97 g, 7.00 mmol) in dichloromethane (DCM) (50 mL) at 0 °C, and the reaction mixture stirred overnight. A 1 M solution of NaOH (25 mL) was added at 0 °C, and the reaction mixture stirred for 10 min. The organic layer was washed with H_2O (3 × 20 mL) and brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexanes/EtOAc, 95:5) to give **14** as a colorless oil (0.85 mg, 96% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (1H, app. pent., J 1.5 Hz, $\underline{H_A}H_B$-10), 4.72 (1H, dq, J 2.4, 0.8 Hz, $H_A\underline{H_B}$-10), 2.95 (1H, dd, J 13.0, 5.4 Hz, H-4), 2.40 (1H, ddd, J 13.3, 3.6, 2.4 Hz, H_{eq}-2), 2.09−2.00 (2H, m, H_{eq}-5 + H_{ax}-2), 1.96−1.85 (2H, m, H_{eq}-6 + H-1), 1.79 (1H, qd, J 13.1, 3.3 Hz, H_{ax}-5), 1.75 (3H, app. dd, J 1.6, 0.8 Hz, CH_3-9), 1.49−1.36 (1H, m, H_{ax}-6), 1.03 (3H, d, J 6.4 Hz, CH_3-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.5 (C═O), 143.6 (C-8), 113.0 (C-10), 57.8 (C-4), 50.7 (C-2), 35.5 (C-1), 34.0 (C-6), 31.3 (C-5), 22.5 (C-7), 21.5 (C-9).

(2*S*)-Phenylselenylisopulegone (15) and (2*R*)-Phenylselenylisopulegone (16). *n*-BuLi (6.3 mL of 1.6 M in hexanes, 10.1 mmol) was added dropwise to a solution of diisopropylamine (1.25 g, 12.3 mmol) in anhydrous tetrahydrofuran (THF) (50 mL) at −78 °C. After 5 min the reaction mixture was removed from the bath and stirred for a further 30 min. The reaction mixture was again cooled to −78 °C, and a solution of *trans*-isopulegone (0.85 g, 5.58 mmol) dissolved in anhydrous THF (50 mL) was added dropwise and stirred at −78 °C. After 1 h a solution of PhSeBr (1.98 g, 8.38 mmol) and hexamethylphosphoramide (1.50 g, 8.38 mmol) in anhydrous THF (50 mL) was added rapidly. The reaction mixture was stirred for 5 min at −78 °C, removed from the bath, and stirred for a further 2 h. The mixture was diluted with DCM (200 mL) and washed with saturated NH_4Cl (3 × 50 mL), H_2O (2 × 50 mL), and brine (1 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexanes/Et_2O, 95:5, v/v), and the product isolated as an orange oil that was a mixture of the (2*S*, **15**) and (2*R*, **16**) diastereoisomers (1.20 g, 6:1 71% yield). Major (2*S*, **15**): ^{1}H NMR (800 MHz, $CDCl_3$) δ 7.46−7.45 (2H, m, ArH), 7.21−7.17 (3H, m, ArH), 4.87 (1H, app. pent., J 1.6 Hz, $\underline{H_A}H_B$-10), 4.66 (1H, s, $H_A\underline{H_B}$-10), 3.77 (1H, dd, J 12, 4.8 Hz, H-4), 3.74 (1H, dd, J 4, 0.8 Hz, H-2), 2.11−2.07 (1H, m, H-1), 1.95−1.92 (1H, m, H_{eq}-5), 1.71−1.6 (3H, m, H_{ax}-5 + H-6), 1.63−1.53 (1H, m, H_{ax}-6), 1.19 (3H, d, J 6.4 Hz, CH_3-7); ^{13}C NMR (200 MHz, $CDCl_3$) δ 207.2 (C═O), 143.4 (C-8), 134.8, 129.3, 128.7, 128.2, 113.2 (C-10), 60.8 (C-2), 52.1 (C-4), 38.2 (C-1), 30.8 (C-5), 30.1 (C-6), 21.5 (C-9), 20.2 (C-7); HRMS $C_{16}H_{21}OSe$ [M + H$^+$] calcd 309.0758, found 309.0752.

Minor (2*R*, **16**): ^{1}H NMR (800 MHz, $CDCl_3$) δ 7.60−7.57 (2H, m, ArH), 7.26−7.21 (3H, m, ArH), 4.94 (1H, app. pent., J 1.4 Hz, $\underline{H_A}H_B$-10), 4.75 (1H, app. pent. J 0.8 Hz, $H_A\underline{H_B}$-10), 3.69 (1H, dd, J 11.2, 1.2 Hz, H-2), 3.08 (1H, dd, J 12.8, 5.2 Hz, H-4), 2.11−2.03 (2H, m, H-1, H_{eq}-6), 2.0−1.95 (1H, m, H-5), 1.86−1.79 (1H, app dq, J 4, 13.3 Hz, H_{ax}-5), 1.76 (3H, app. dd, J 1.2, 0.8 Hz, CH_3-9), 1.63−1.53 (1H, m, H_{ax}-6), 1.19 (3H, d, J 6.4 Hz, CH_3-7); ^{13}C NMR (200 MHz, $CDCl_3$) δ 205.4 (C═O), 143.0 (C-8), 134.7, 129.6, 129.0, 127.5, 113.0 (C-10), 63.2 (C-2), 57.9 (C-4), 42.1 (C-1), 34.1 (C-6), 30.4 (C-5), 22.8 (C-7), 21.5 (C-9).

(−)-Isopiperitenone (3). *Small Scale.* $NaIO_4$ (0.10 g, 0.48 mmol) was added to $NaHCO_3$ (28 mg, 0.32 mmol) in EtOH (8 mL) at 0 °C, and the reaction mixture allowed to warm to room temperature overnight with stirring. The mixture was diluted with DCM (20 mL) and washed with H_2O (3 × 5 mL) and brine (1 × 5 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (hexanes/EtOAc, 9:1, v/v), and the product was further purified by preparative HPLC to remove selenide traces (ACE silica gel column, hexanes/EtOAc, 95:5, 20 mg/mL concentration, injected 2.5 mL). This gave (+)-isopiperitenone (**3**; 0.04 g, 79%) as a pale yellow oil.

Large Scale. $NaIO_4$ (6.30 mmol) was added to a mixture of 2-phenylselenylisopulegone (**15**/**16**) (3.00 mmol) and $NaHCO_3$ (6.30 mmol) in EtOH (0.18 M) at 0 °C, and the reaction mixture allowed to warm to room temperature overnight with stirring. Since the reaction had not progressed to completion and prolonged reaction times lead to isomerization to piperitenone, the reaction was quenched after 24 h. The mixture was diluted with DCM (equivalent volume to EtOH) and washed with H_2O (3 × 0.25 volume equivalents to DCM) and brine (1 × 0.25 volume equivalents to DCM). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resultant residue was purified by flash column chromatography (hexanes/EtOAc, 9:1, v/v) to give the starting material (10−46% recovered) and the product as a pale yellow oil. Further purification by Kugelrohr distillation (160 °C under reduced pressure) was required to remove traces of selenium byproducts to give (+)-isopiperitenone (**3**) as a colorless oil (35−40% yield). Reactions were performed on 0.5 to 5.8 g of 2-phenylselenylisopulegone, yielding (+)-isopiperitenone (**3**) (85.0 mg to 1.13 g). Data matched those reported (reference with ^{1}H data only).[13] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90−5.88 (1H, m, H-2), 4.95 (1H, app. pent., J 1.5 Hz, $\underline{H_A}H_B$-10), 4.75 (1H, dq, J 2.5, 0.8 Hz, $H_A\underline{H_B}$-10), 2.95 (1H, ddd, J 10.7, 4.9, 0.7 Hz, H-4), 2.36−2.27 (2H, m, H-6), 2.14−2.06 (1H, m, H_{eq}-5), 2.06−1.98 (1H, m, H_{ax}-5), 1.95−1.94 (3H, m, CH_3-7), 1.74−1.73 (3H, app. dd, J 1.2, 0.8 Hz, CH_3-9); ^{13}C

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NMR (100 MHz, $CDCl_3$) δ 199.5 (C═O), 162.0 (C-1), 143.5 (C-8), 126.9 (C-2), 113.7 (C-10), 54.0 (C-4), 30.5 (C-6), 27.8 (C-5), 24.4 (C-7), 20.8 (C-9); HRMS $C_{10}H_{14}ONa$ [M + Na^+] calcd 173.0942, found 173.0936.

Piperitenone (17). $NaIO_4$ (2.09 g, 9.76 mmol) was added to a mixture of 2-phenylselenyl-*trans*-isopulegone (1.50 g, 4.88 mmol) and $NaHCO_3$ (0.41 g, 4.88 mmol) in EtOH (100 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 3 days. The mixture was diluted with DCM (200 mL) and washed with H_2O (3 × 50 mL) and brine (1 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resultant residue was purified by flash column chromatography (hexanes/EtOAc, 95:5) to give a pale yellow oil (0.24 g, 33%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (1H, app. sext., *J* 1.3 Hz, H-2), 2.64 (2H, br t, *J* 6.2 Hz, H-5), 2.28 (2H, tq, *J* 6.3, 0.7 Hz, H-6), 2.08−2.06 (3H, m, CH_3-10), 1.91 (3H, dt, *J* 1.6, 0.8 Hz, CH_3-7), 1.85−1.83 (3H, m CH_3-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.8 (C═O, C-1), 159.9 (C-6), 142.7 (C-3), 129.1 (C-8), 128.9 (C-2), 32.0 (C-4), 28.1 (C-5), 23.9 (C-7), 23.0 (C-10), 22.6 (C-9); ^{1}H and ^{13}C data match those reported;[29] HRMS $C_{10}H_{14}ONa$ [M + Na^+] calcd 173.0942, found 173.0942.

(+)-*cis*-Isopulegone (4) and (+)-Pulegone (5). (+)-*cis*-Isopulegone (**4**) was synthesized by preparative scale bioreduction of (−)-isopiperitenone (**3**) with IPR. The preparative scale biotransformation was performed in small multiples rather than as a single large batch. These 58 × 25 mL vials, each with a total volume of 10 mL, contained phosphate buffer (50 mM KH_2PO_4/K_2HPO_4 pH 6.4), (−)-isopiperitenone (**3**) (8 mM, 250 mM stock in EtOH; total substrate 700 mg), IPR (2.0 μM), dithiothreitol (1 mM), $NADP^+$ (20 μM), glucose (30 mM), and glucose dehydrogenase (GDH; 10 U). The vials were sealed and shaken at 25 °C and 130 rpm. After 4 h the biotransformation was terminated by extraction with Et_2O (2 × 100 mL), and the combined organic layer washed with brine (1 × 50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash column chromatography gave (+)-*cis*-isopulegone (**4**) as a colorless oil (617 mg, 87%). (+)-Pulegone (**5**) (data matched those reported)[30] was obtained quantitatively from (+)-isopulegone (**4**) by dissolving **4** (20 mg) in methanol (2 mL) and 10% aqueous NaOH w/v (0.2 mL), the crude isolate being of sufficient purity for direct biotransformation (vide infra). (+)-*cis*-Isopulegone (**4**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.97 (1H, app. sext., *J* 1.4 Hz, $\underline{H_A}H_B$-10), 4.80−4.78 (1H, m, $H_A\underline{H_B}$-10), 2.97 (1H, t, *J* 6.4 Hz, H-4), 2.42−2.36 (1H, m, H_{eq}-2), 2.22−2.11 (3H, m, H-1 + H_{eq}-5 + H_{ax}-2), 1.90−1.77 (2H, m, H_{ax}-5 + H_{eq}-6), 1.74−1.72 (3H, m, CH_3-9), 1.63−1.54 (1H, m, H_{ax}-6), 0.98 (3H, d, *J* 6.8 Hz, CH_3-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.1 (C═O), 142.9 (C-8), 112.7 (C-10), 57.0 (C-4), 48.3 (C-2), 33.5 (C-1), 30.2 (C-6), 27.7 (C-5), 21.8 (C-9), 20.7 (C-7).

***cis*/*trans*-Isopiperitenol (18 and 2).** A solution of (−)-isopiperitenone (**3**) (0.10 g, 0.66 mmol) in anhydrous Et_2O (5 mL) was added dropwise to a suspension of $LiAlH_4$ (0.03 g, 0.85 mmol) in anhydrous Et_2O (5 mL) at 0 °C under N_2 and stirred for 30 min. The mixture was quenched using the Fieser method: H_2O (0.04 mL) was added slowly at 0 °C, followed by 1 M NaOH (0.15 mL) and then H_2O again (0.15 mL). The mixture was stirred for 30 min, $MgSO_4$ was added, and the mixture was stirred for a further 15 min, filtered through Celite, and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexanes/EtOAc, 95:5, v/v) to give a mixture of *cis*/*trans*-isopiperitenol (**18** and **2**) (2:3, 85%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69−5.66 (0.4H, m, H-2 *cis*), 5.45−5.43 (0.6H, m, H-2 *trans*), 5.01−4.99 (0.4, m, $\underline{H_A}H_B$-10 *cis*), 4.90−4.88 (0.6H, m, $\underline{H_A}H_B$-10 *trans*), 4.85−4.84 (0.6H, m, $H_A\underline{H_B}$-10 *trans*), 4.81−4.80 (0.4H, m, $H_A\underline{H_B}$-10 *cis*), 4.15−4.09 (1H, m, H-*cis*/*trans*), 2.14−1.88 (3H, H-+ CH_2 *cis*/*trans*), 1.83−1.82 (1.2H, m, CH_3-9 *cis*), 1.78−1.54 (2H, m, CH_2 *cis*/*trans*), 1.73−1.71 (3H, m, CH_3-9 *trans* + CH_3-7 *cis*), 1.70−1.69 (1.8H, m, CH_3-7 *trans*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.7/146.6 (C-8 *cis*/*trans*), 139.9/136.9 (C-1 *cis*/*trans*), 124.4/122.5 (C-2 *trans*/*cis*), 112.5/111.9 (C-10 *trans*/*cis*), 68.8/64.0 (C-3 *trans*/*cis*), 51.1/46.3 (C-4 *trans*/*cis*), 31.3/30.4 (C-6 *cis*/*trans*), 26.3 (C-5 *trans*), 23.6/23.2 (C-7 *cis*/*trans*), 22.8 (C-9 *cis*), 21.0 (C-5 *cis*), 19.5 (C-9 *trans*). Data matched those reported.[8]

***cis*/*trans*-Isopiperitenol *p*-Nitrobenzoates (20/19).** Isopiperitenol (**18**/**2**) (70 mg, 0.45 mmol) was dissolved in pyridine (3.2 mL) and cooled to 0 °C. To the solution was added *p*-nitrobenzoyl chloride (150 mg, 0.808 mmol), and the solution was stirred at room temperature for 3 h. The mixture was poured into H_2O and extracted with DCM (10 mL). The organic layer was washed with 2 M HCl (3 × 5 mL), saturated bicarbonate solution (2 × 5 mL), and brine (5 mL). The organic phase was dried ($MgSO_4$) and filtered, and the solvents were removed in vacuo. Flash column chromatography (hexanes/Et_2O, 96:4, v/v) afforded **19**/**20** as a waxy solid (107 mg, 78%). The *cis* and *trans* isomers were separated by preparative HPLC (ACE silica column (250 × 4.5 mm id); 10 mg/mL; injected 100 to 500 μL; 0.5% to 3% EtOAc/hexane; *cis* 28.3 min and *trans* 29.7 min). *trans*-**19**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, 2H, *J* 9.2 Hz, ArH), 8.18 (d, 2H, *J* 9.2 Hz, ArH), 5.65 (d, 1H, *J* 8.8 Hz, H-3), 5.45 (br s, 1H, H-2), 4.76 (m, 2H, H-10a, H-10b), 2.54−2.48 (m, 1H, H-4), 2.2−2.11 (m, 1H, H-6a), 2.04−1.98 (m, 1H, H-6b), 1.86−1.76 (m, 2H, H-5a, H-5b), 1.85−1.78 (m, 3H, *J* 4.4 Hz, H-9), 1.57 (s, 3H, H-7). *cis*-**20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* 9.2 Hz, 2H, ArH), 8.14 (d, *J* 9.2 Hz, 2H, ArH), 5.75- 5.73 (br d, *J* 6.0 Hz, 1H H-3), 5.61−5.59 (m, 1H, H-2), 4.84−4.79 (m, 2H, H-10a, H-10b), 2.31−2.28 (m, 1H, H-4), 2.17−2.15 (m, 2H, H-6a, H-6b), 1.84−1.83 (m, 1H, H-5a), 1.79−1.79 (m, 1H, H-5b), 1.78 (s, 3H, H-9), 1.76 (s, 3H, H-7).

(−)-*trans*-Isopiperitenol (2). *trans*-Isopiperitenol *p*-nitrobenzoate (40 mg, 0.13 mmol) was dissolved in THF (1 mL) in a screw-top sample vial and to this were added $LiOH{\cdot}H_2O$ (13 mg, 0.33 mmol) and H_2O (160 μL). The vial was sealed, and the mixture was heated to 60 °C for 4 h. The mixture was placed on ice, diluted with Et_2O (40 mL), and washed with H_2O (3 × 1 mL). The organic phase was dried ($MgSO_4$) and filtered, and solvents were removed. Flash column chromatography (hexanes/EtOAc, 9:1, v/v) provided (−)-*trans*-isopiperitenol (**2**) (18 mg, 92% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45−5.44 (m, 1H, H-2), 4.89−4.88 (m, 1H, H-10a), 4.85−4.84 (1H, H-10b), 4.13−4.10 (m, 1H, H-3), 2.10−2.04 (m, 2H, H-4, H-6a), 1.95−1.93 (m, 1H, H-6b), 1.73−1.72 (m, 3H, H-9), 1.69−1.69 (m, 3H, H-7), 1.65−1.56 (m, 2H, H-5).

(−)-*cis*-Isopiperitenol (18). *cis*-Isopiperitenol *p*-nitrobenzoate (**20**) (40 mg, 0.13 mmol) was dissolved in THF (1 mL) in a screw-top sample vial and to this were added $LiOH{\cdot}H_2O$ (13 mg, 0.33 mmol) and H_2O (160 μL). The vial was sealed, and the mixture was heated to 60 °C for 4 h. The mixture was placed on ice, diluted with Et_2O (40 mL), and washed with H_2O (3 × 10 mL). The organic phase was dried over $MgSO_4$ and filtered, and the solvent was removed. Column chromatography (hexane s/EtOAc, 9:1, v/v) provided **18** (18 mg, 90% yield). Data matched those reported.[13] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.59−5.67 (m, 1H, H-2), 5.00−4.99 (m, 1H, H-10a), 4.81−4.81 (m, 1H, H-10b), 4.14−4.13 (m, 1H, H-3), 2.14−2.13 (m, 1H, H-4), 2.10−1.99 (m, 2H, H-6), 1.83 (s, 3H, H-9), 1.80−1.74 (m, 2H, H-5), 1.72 (s, 3H, H-7).

Menthone (7) and Isomenthone (8). A preparative scale bioreduction of (+)-pulegone (**5**) with DBR from *Nicotiana tabacum* (*Nt*DBR-C-His6 in pET21b; Uniprot: Q9SLN8) was performed. The preparative scale biotransformation was performed in three multiples rather than as a single batch. Each of three 25 mL vials, with a total volume of 10 mL, contained phosphate buffer (50 mM KH_2PO_4/K_2HPO_4 pH 6.4), (+)-pulegone (**5**, 8 mM, 250 mM stock in EtOH, total substrate 36 mg), DBR (2.0 μM), $NADP^+$ (10 μM), glucose (15 mM), and GDH (10 U). The vials were sealed and shaken at 30 °C and 130 rpm. After 4 h the biotransformation was terminated by extraction with Et_2O (2 × 100 mL), and the combined organic layer washed with brine (1 × 50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash column chromatography (hexanes/EtOAc, 95:5, v/v) gave menthone (**7**) and isomenthone (**8**) as clear oils (37% menthone, 31% isomenthone, total 68%). GC 10.4 and 10.6 min, respectively. Analytical data for both matched known data, and GC matched standards. Isomenthone (**8**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 (ddt, 1H, H-2a, *J* 13.1, 4.5, 1.2 Hz), 2.11 (dd,

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1H, H-2b, *J* 13.2, 10.1 Hz), 2.06−1.91 (m, 4H, H-1, H-4, H-5a, H-8), 1.76−1.66 (m, 2H, H-5b, H-6a), 1.52−1.43 (m, 1H, H-6b), 0.99 (d, 3H, H-7a-c, *J* 6.6 Hz), 0.93 (d, 3H, H-9a-c/H-10a-c, *J* 6.5 Hz), 0.84 (d, 3H, H-9a-c/H-10a-c, *J* 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 214.7 (C═O), 48.2, 34.5, 29.6, 27.1, 27.0, 21.6, 21.0, 20.0.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b01026.

Copies of NMR spectra and chromatograms (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: nigel.scrutton@manchester.ac.uk. Tel: +44 (0)161 306 5152.

*E-mail: gardiner@manchester.ac.uk. Tel: +44 (0)161 306 4530.

ORCID

Aisling Ní Cheallaigh: 0000-0003-2666-5089

Helen S. Toogood: 0000-0003-4797-0293

Nigel S. Scrutton: 0000-0002-4182-3500

John M. Gardiner: 0000-0003-1827-6817

Notes

The authors declare no competing financial interest.

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