Enantioselective synthesis of tri-deuterated (−)-geosmin to be used as internal standard in quantitation assays

Caterina Porcelli | Johanna Kreissl | Martin Steinhaus

Leibniz-Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), Freising, Germany

Correspondence
Martin Steinhaus, Leibniz-Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), Lise-Meitner-Straße 34, Freising 85354, Germany.
Email: martin.steinhaus@tum.de

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For the accurate and sensitive quantitation of the off-flavor compound geosmin, particularly in complex matrices, a stable isotopologue as internal standard is highly advantageous. In this work, we present a versatile synthetic strategy leading from (4aR)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one to tri-deuterated (−)-geosmin ((4S,4aS,8aR)-4,8a-dimethyl(3,3,4-2H3)octahydronaphthalen-4a(2H)-ol). The starting material was readily accessible from inexpensive 2-methylcyclohexan-1-one using previously published procedures.

KEYWORDS
(−)(2H3)geosmin, (−)-geosmin, (4S,4aS,8aR)-4,8a-dimethyl(3,3,4-2H3)octahydronaphthalen-4a(2H)-ol, (4S,4aS,8aR)-4,8a-dimethyloctahydronaphthalen-4a(2H)-ol, deuteration, internal standard, musty and earthy off-flavor

1 | INTRODUCTION

(−)-Geosmin (Figure 1) is a highly odorous molecule with a characteristic musty and earthy smell and a low odor detection threshold value in the range of 1–20 ng/kg.1,2 Its name is derived from the ancient Greek words “geo” meaning earth and “osme” meaning odor.

In nature, geosmin is produced as secondary metabolite by several types of microorganisms, including actinomycetes, cyanobacteria, myxobacteria, and fungi.3–5 The biosynthesis involves a Mg2+-dependent sesquiterpene synthase, which converts farnesyl diphosphate (FPP) to a mixture of sesquiterpenoids including geosmin.3 The compound can cause a musty and earthy off-flavor in foods and beverages such as drinking water, wine, fish, and cereals.6–8 In the worst case, the off-flavor may lead to consumers’ rejection and significant economic loss. Recently, we identified geosmin in fermented cocoa and demonstrated that it may be transferred in odor-active amounts to chocolate. To avoid this, an accurate and sensitive method for its detection and quantitation in fermented cocoa is essential. In gas chromatography–mass spectrometry (GC–MS) and in liquid chromatography–mass spectrometry (LC–MS), the use of a stable isotopically substituted analog of the target compound as internal standard is currently considered the best approach.9,10 Although racemic deuterated geosmin is available from chemical companies, it is highly expensive (~20 000 € for 150 mg). Therefore, we attempted to find a convenient synthetic route to deuterated geosmin as an alternative to the commercial product.

Most strategies reported in the literature on the synthesis of isotopically unmodified geosmin proceeded through 1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one.11–13 This dimethyloctalone intermediate was obtained from 2-methylcyclohexan-1-one via a Robinson annulation with pent-1-en-3-one, the latter generated in situ from 1-chloropentan-3-one by an acid-catalyzed dehydrochlorination as detailed by Zoretic et al. in 1975.14 This approach was also adopted in a paper on the synthesis of deuterated geosmin published in 1991, which to our knowledge is the only one reporting the preparation of an isotopically modified geosmin so far.15 In this work, the deuteration was accomplished by reacting cyclohexene oxide with (2H3)methylmagnesium iodide followed by oxidation of the obtained 2-(2H3)methylcyclohexan-1-ol to 2-(2H3)methylcyclohexan-1-one. The above mentioned
Robinson annulation then yielded the trideuterated dimethyloctalone. However, this approach would lead to a racemic product as well as impurities due to the lack of stereo- and regiocontrol. Therefore, we decided to synthesize deuterated geosmin from the isotopically unmodified dimethyloctalone obtained in a stereoselective approach previously reported by Revial et al. They used the auxiliary chiral amine (1S)-1-phenylethan-1-amine to convert commercially available rac-2-methylcyclohexan-1-one via (2R)-2-methyl-2-(3-oxopentyl)cyclohexan-1-one to the enantiopure dimethyloctalone (4aR)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydropyronaphthalen-2(3H)-one. Our aim was to use the latter compound and convert it to deuterated geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent. For the synthesis of isotopically unmodified chloroperbenzoic acid and hydrogen peroxide for the epoxidation of geosmin, they compared the suitability of oxidizing agent.

2 | RESULTS AND DISCUSSION

Enantiopure (4aR)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydropyronaphthalen-2(3H)-one 1 was converted to trideuterated geosmin by a sequence of four synthetic steps as depicted in Scheme 1. The first step was the epoxidation of the double bond in 1. Based on the work of Gosselin et al., mCPBA was chosen as oxidizing agent. For the synthesis of isotopically unmodified geosmin, they compared the suitability of m-chloroperbenzoic acid and hydrogen peroxide for the epoxidation of 1. The use of the peroxy acid afforded a 96:4 mixture of the α- and β-epoxyketones and an overall yield of 80% of the α-epimer, whereas such a high stereoselectivity could not be achieved with hydrogen peroxide. Gosselin et al. concluded that the steric hindrance induced by the angular methyl group over the β-face in 1 accounts for the preferential attack of the bulky aromatic peroxy acid molecule on the α-face. We adopted the approach of Gosselin et al. for the epoxidation of 1 but applied NaHCO₃ as an additional base because preliminary experiments had revealed that this slightly increased the yield (data not shown). This was to be expected as the acidity of m-chloroperbenzoic acid can lead to side products. Protonation of the double bond in the educt could lead to the formation of an alcohol whereas protonation of the epoxide would lead to the formation of a diol. The epoxidation step proceeded with a yield of 78%. The epoxide was then subjected to reduction with LiAlD₄, which led to the incorporation of two deuterium atoms and finally resulted in diol 3.

By selective tosylation, the secondary hydroxy group of 2 was converted into a good leaving group, affording 4. Without isolation of 4, a second reduction step with LiAlD₄ replaced the tosyl group by deuterium, finally leading to the trideuterated target molecule (4S,4aS,8aR)-4,8a-dimethyl(3,3,4-H₃)octahydropyronaphthalen-4a(2H)-ol 5, that is, (2H₃)geosmin. The compound was purified by flash chromatography. The overall yield from 1 was 24%. The enantiomeric distribution of (2H₃)geosmin was determined by GC–MS using a β-cyclodextrin-based chiral column. The elution order was taken from a previous report on the enantioseparation of geosmin in wine. Results indicated an enantiomeric purity of 91%, which confirmed the proposed enantioselectivity of the synthetic approach.

The incorporation of three deuterium atoms was confirmed by GC–MS. The EI mass spectrum of (2H₃)geosmin (Figure 2A) showed a molecular ion of m/z 185, whereas the spectrum of the isotopically unmodified geosmin showed a molecular ion of m/z 182 (Figure 2B). No signals of m/z 182, 183, and 184 were present in the spectrum of the synthesized molecule, showing that no undeuterated, monodeuterated, and dideuterated geosmin isotopologues were present. Thus, the approach resulted in a uniformly trideuterated product. Further evidence was achieved by NMR. ¹H and ¹³C NMR spectra allowed to unambiguously assign the positions of the three deuterium atoms. The singlet obtained in the ¹H NMR spectrum confirmed the presence of the deuterium atom at C4. Moreover, the multiplicity of the signals obtained in the ¹³C NMR spectrum for carbons C3 and C4 indicated the coupling with two and one deuterium atoms, respectively.
3 | CONCLUSIONS

By using LiAlD₄ for the incorporation of deuterium atoms, we developed a quick and convenient approach for the preparation of uniformly trideuterated geosmin from the dimethyloctalone (4aR)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one with 24% yield and 91% enantiomeric purity. The synthesis proceeded in four steps among which two were performed one-pot. In combination with the stereoselective preparation of the dimethyloctalone from inexpensive racemic 2-methylcyclohexanone according to Revial et al., we achieved an overall yield of 16%. (²H₃)Geosmin can be used as an internal standard for the sensitive quantitation of geosmin in complex matrices by GC–MS or LC–MS.

4 | EXPERIMENTAL

4.1 | Chemicals and materials

The chemicals used were obtained from commercial sources: m-chloroperbenzoic acid (77%), p-toluenesulfonyl chloride, pyridine, sodium sulfate, and lithium aluminum deuteride were purchased from Merck (Darmstadt, Germany); sodium bicarbonate from Alfa Aesar (Karlsruhe, Germany); tetrahydrofuran from Santa Cruz Biotechnology (Heidelberg, Germany). Diethyl ether and dichloromethane were purchased in technical grade from Fisher Scientific (Loughborough, UK) and VWR (Darmstadt, Germany), respectively, and they were freshly distilled before use. Hexane, tetrahydrofuran, and chloroform were purchased in technical grade and stored over molecular sieves (4 Å). Chloroform was filtered through alumina before use to eliminate traces of ethanol present as stabilizer. Silica gel 60 (particle size: 0.035–0.070 mm) and LiChroprep® DIOL (particle size: 0.040–0.063 mm) used for purification as well as precoated silica gel thin-layer chromatography (TLC) plates (layer thickness 750 μm, no fluorescence indicator) used for reaction monitoring were purchased from Merck. Hexane and diethyl ether mixtures in different proportions were used as mobile phase. Cerium ammonium molybdate or potassium permanganate solutions were employed in TLC as stains for substance detection, followed by heat treatment (200°C).

4.2 | Gas chromatography–mass spectrometry

El mass spectra were recorded using a GC–MS system consisting of a Trace GC Ultra gas chromatograph coupled to a single quadrupole ISQ mass spectrometer (Thermo Fisher Scientific, Dreieich, Germany). Compounds were dissolved in dichloromethane at a concentration of ~20 μg/mL. An aliquot (1 μL) was introduced by an autosampler GC PAL, PAL Firmware 2.5.2 (Chromtech, Bad Camberg, Germany), into a PTV injector (Thermo Fisher Scientific) at 40°C. The injector temperature was raised at 12°C/s to 60°C (held for 0.5 min) and then by 10°C/s to 240°C (held for 1 min). The carrier gas was helium at a flow rate of 2 mL/min. The splitflow...
was 24 mL/min. The column was a DB-1701 coated fused silica capillary, 30 m × 0.25 mm i.d., 0.25-µm film thickness (Agilent, Waldbronn, Germany). The initial oven temperature was 40°C. After 2 min, it was raised at 6°C/min to 230°C (held for 5 min). Mass spectra were acquired at an ionization energy of 70 eV and a scan range of 40–300 m/z. The mass spectra were evaluated using Xcalibur 2.0 software (Thermo Fisher Scientific). Chemical ionization (CI) mass spectra were recorded using an enantioGC–MS system consisting of a Trace 1310 gas chromatograph coupled to a Q Exactive mass spectrometer (Thermo Fisher Scientific). Compounds were dissolved in dichloromethane at a concentration of ~10 µg/mL. An aliquot (1 µL) was introduced by a TRI Plus RSH autosampler (Thermo Fisher Scientific) into a PTV injector (Thermo Fisher Scientific) used in on-column mode. The carrier gas was helium at a flow rate of 50 mL/min. Mass spectra were acquired with targeted SIM range of 40–300 m/z. The mass spectra were evaluated using Xcalibur 2.0 software (Thermo Fisher Scientific). The mass spectra were evaluated using Xcalibur 2.0 software (Thermo Fisher Scientific).

4.3 | NMR spectroscopy

One-dimensional and 2-D NMR data (1H, 13C, gs-COSY, gs-HSQC, and gs-HMBC) were acquired with an Avance III 400 MHz system (Bruker, Rheinstetten, Germany) equipped with a Z-gradient 5-mm multinuclear observe probe (BBFO plus) at 298 K. The compounds were dissolved in CDCl3 containing 0.03% (v/v) TMS (Eurisotop, Saint-Aubin, France). All spectra were referenced to TMS (0.0 ppm). For data processing and analysis, Topspin 3.2 (Bruker) and MestReNova 12.0.4 (Mestrelab Research, Santiago de Compostela, Spain) were used.

4.4 | (1S,4aR,8aS)-1,4a-dimethyl(1,2-2H2)-octahydronaphthalene-2,8a(1H)-diol (3)

Under an argon atmosphere, lithium aluminum deuteride (185 mg, 4.41 mmol) was suspended in dry THF (15 mL), and the flask was heated to gentle reflux. Epoxide 2 (342 mg, 1.76 mmol) was dissolved in dry THF (5 mL) and added dropwise. After 2 h, the flask was cooled on ice, and a saturated aqueous solution of sodium carbonate (5 mL) was slowly added. Hydrochloric acid (1%; 1 mL) was added, and the mixture was stirred. The aqueous layer was separated and extracted with diethyl ether (2 × 20 mL). The organic phase and the diethyl ether extracts were combined, washed with brine (2 × 20 mL), and dried over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel to afford 342 mg of 3 (78% yield). TLC: Rf 0.48 (hexane/diethyl ether, 4 + 1, v + v). MS (EI): m/z (%): 109 (100), 67 (41), 133 (30), 81 (26), 43 (25), 176 (18), 151 (18), 137 (15), 55 (13), 110 (11). 1H NMR (400 MHz, CDCl3, 298 K, gs-COSY): δ 2.43 (ddd, 2J = 19.2 Hz, 3J = 8.2, 1.9 Hz, 1H, H-3α), 2.31 (ddd, 2J = 19.2 Hz, 3J = 11.4, 7.6 Hz, 1H, H-3β), 2.04 (m, 1H, H-4α), 1.97 (m, 1H, H-7α), 1.83 (m, 1H, H-8α), 1.65 (m, 1H, H-6α), 1.58 (m, 1H, H-6β), 1.56 (m, 2H, H-5), 1.55 (m, 1H, H-8β), 1.50 (m, 1H, H-7β), 1.38 (s, 3H, H-9), 1.22 (m, 1H, H-4β), 1.05 (s, 3H, H-10). 13C NMR (100 MHz, CDCl3, 298 K, gs-HSQC, gs-HMBC): δ 208.2 (C-2), 72.0 (C-8a), 65.5 (C-1a), 38.3 (C-5), 34.4 (C-4a), 33.5 (C-3), 32.1 (C-4), 26.4 (C-7), 24.1 (C-8), 21.0 (C-6), 20.7 (C-10), 11.3 (C-9).

was brought to room temperature and left under magnetic stirring for additional 24 h. The suspension was washed with a mixture of a saturated aqueous sodium thiosulfate solution and a saturated aqueous sodium carbonate solution (1 + 1, v + v, 2 × 50 mL), followed by brine (50 mL) and finally dried over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel to afford 342 mg of 3 (78% yield). TLC: Rf 0.48 (hexane/diethyl ether, 4 + 1, v + v). MS (EI): m/z (%): 109 (100), 67 (41), 133 (30), 81 (26), 43 (25), 176 (18), 151 (18), 137 (15), 55 (13), 110 (11). 1H NMR (400 MHz, CDCl3, 298 K, gs-COSY): δ 2.43 (ddd, 2J = 19.2 Hz, 3J = 8.2, 1.9 Hz, 1H, H-3α), 2.31 (ddd, 2J = 19.2 Hz, 3J = 11.4, 7.6 Hz, 1H, H-3β), 2.04 (m, 1H, H-4α), 1.97 (m, 1H, H-7α), 1.83 (m, 1H, H-8α), 1.65 (m, 1H, H-6α), 1.58 (m, 1H, H-6β), 1.56 (m, 2H, H-5), 1.55 (m, 1H, H-8β), 1.50 (m, 1H, H-7β), 1.38 (s, 3H, H-9), 1.22 (m, 1H, H-4β), 1.05 (s, 3H, H-10). 13C NMR (100 MHz, CDCl3, 298 K, gs-HSQC, gs-HMBC): δ 208.2 (C-2), 72.0 (C-8a), 65.5 (C-1a), 38.3 (C-5), 34.4 (C-4a), 33.5 (C-3), 32.1 (C-4), 26.4 (C-7), 24.1 (C-8), 21.0 (C-6), 20.7 (C-10), 11.3 (C-9).

4.5 | (1S,4aR,8aS)-1,4a-dimethyl(1,2-2H2)-octahydronaphthalene-2,8a(1H)-diol (3)

Under an argon atmosphere, lithium aluminum deuteride (185 mg, 4.41 mmol) was suspended in dry THF (15 mL), and the flask was heated to gentle reflux. Epoxide 2 (342 mg, 1.76 mmol) was dissolved in dry THF (5 mL) and added dropwise. After 2 h, the flask was cooled on ice, and a saturated aqueous solution of sodium sulfate (5 mL) was slowly added. Hydrochloric acid (1%; 1 mL) was added, and the mixture was stirred. The aqueous layer was separated and extracted with diethyl ether (2 × 20 mL). The organic phase and the diethyl ether extracts were combined, washed with brine (2 × 20 mL), and dried over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure, and the crude product was purified by flash chromatography on silica gel to afford 198 mg of 1 (56% yield). TLC: Rf 0.28 (hexane/diethyl ether, 2 + 3, v + v). MS (EI): m/z (%): 112 (100), 125 (71), 126 (42), 97 (38), 182 (31), 200 (19), 111 (17), 43 (14), 55 (13), 113 (13). 1H NMR (400 MHz, CDCl3, 298 K, gs-COSY): δ 1.85 (ddd, 2J = 12.2 Hz, 3J = 3.4, 2.4 Hz, 1H, H-3α), 1.74 (dd, 2J = 13.6 Hz, 3J = 4.2, 1H, H-4α), 1.63-1.53 (m, 6H, H-3β, H-5α, H-6α, H-7, H-8α), 1.44 (m, 1H, H-6β), 1.42 (m, 1H, H-8β), 1.09 (m, 1H, H-5β), 1.07 (m, 1H, H-4β), 1.05 (s, 3H, H-10), 0.97 (s, 3H, H-9). 13C NMR (100 MHz, CDCl3, 298 K, gs-HSQC, gs-HMBC): δ 75.6 (C-8a), 72.1
(t, 2J_{D-C} = 22.2 Hz, C-2), 41.9 (t, 2J_{D-C} = 19.0 Hz, C-1), 36.9 (C-4a), 35.1 (C-5), 33.7 (C-4'), 30.5 (C-3), 30.2 (C-8), 20.7 (C-7), 20.4 (C-10), 20.2 (C-6), 9.8 (C-9).

4.6 | (4S,4aS,8aR)-4,8a-dimethyl(3,3,4,2H3) octahydronaphthalen-4a(2H)-ol (5)

Diole 3 (198 mg, 0.99 mmol) was dissolved in chloroform (40 mL). Under an argon atmosphere, pyridine (800 µL, 9.90 mmol) and subsequently p-toluenesulfonyl chloride (1.91 g, 10.0 mmol) were added slowly under stirring. The mixture was kept for 72 h at 10°C. A suspension of lithium aluminum deuteride (83.4 mg, 1.99 mmol) in dry THF (10 mL) was added dropwise, and the reaction mixture was heated at reflux. After 4 h, the mixture was cooled down to room temperature. Diethyl ether (20 mL) was added, followed by water (5 mL), and subsequently aqueous hydrochloric acid (1%; 20 mL). The organic phase was separated and washed with an aqueous sodium hydrogen carbonate solution (5%; 20 mL) and brine (20 mL). After drying over anhydrous sodium sulfate and filtration, the solvents were removed under reduced pressure, and the crude product was purified by flash chromatography on a diol phase to give 98.5 mg of 5 (55% yield) with an enantiomeric purity of 91% (enantioGC-MS, BGB-176 column). TLC: Rf 0.41 (hexane/diethyl ether, 2 + 3, v + v). MS (EI): m/z (%): 112 (100), 43 (23), 97 (19), 113 (18), 55 (18), 111 (16), 41 (15), 83 (14), 67 (12), 69 (12). The full MS (EI) is depicted in Figure 2. MS (CI): calculated mass for C_{48}H_{38}D_{10}O: 185.3214; found 185.3218.

REFERENCES

1. Maga JA. Musty, earthy aromas. Food Rev Int. 1987;3(3):269-284.
2. Darriet P, Pons M, Lamy S, Dubourdieu D. Identification and quantification of geosmin, an earthy odorant contaminating wines. J Agric Food Chem. 2000;48(10):4835-4838.
3. Cane DE, He X, Kobayashi S, Omura S, Ikeda H. Geosmin biosynthesis in Streptomyces avermitilis. Molecular cloning, expression, and mechanistic study of the germacradienol/geosmin synthase. J Antibiot. 2006;59(8):471-479.
4. Jiang J, He X, Cane DE. Biosynthesis of the earthy odorant geosmin by a bifunctional Streptomyces coelicolor enzyme. Nat Chem Biol. 2007;3(11):711-715.
5. Giglio S, Jiang J, Saint CP, Cane DE, Monis PT. Isolation and characterization of the gene associated with geosmin production in cyanobacteria. Environ Sci Technol. 2008;42(21):8027-8032.
6. Gerber NN, Lechevalier HA. Geosmin, an earthy-smelling substance isolated from actinomycetes. Biotechnol Bioeng. 1965;9(3):321-327.
7. Callejon RM, Ubeda C, Rios-Reina R, Morales ML, Troncoso AM. Recent developments in the analysis of musty odor compounds in water and wine: a review. J Chromatogr A. 2016;1428:72-85.
8. Borjeson TS, Stollman UM, Schnurer JL. Off-odorous compounds produced by molds on oatmeal agar - identification and relation to other growth characteristics. J Agric Food Chem. 1993;41(11):2104-2111.
9. Schieberle P, Grosch W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. J Agric Food Chem. 1987;35(2):252-257.
10. Steinhaus M. Gas chromatography-olfactometry: principles, practical aspects and applications in food analysis. In: Advanced Gas Chromatography in Food Analysis. Cambridge: The Royal Society of Chemistry; 2020:337-399.
11. Ayer WA, Browne LM, Fung S. Metabolites of birds nest fungi. J am Chem Soc. 1985;107(1):273-274.
12. Pfau M, Revial G, Guingant A, d’Angelo J. Enantioselective synthesis of quaternary carbon centers through Michael-type addition and subsequent hydrolysis of a beta-chloroketone in presence of an acid. Tetrahedron. 1989;30(21):2775-2778.
13. Zoretic PA, Branchaud B, Maestre T, Robinson annelations with a beta-chloroketone. J Label Compd Radiopharm. 1991;29(7):823-828.

ORCID

Caterina Porcelli https://orcid.org/0000-0003-2704-2787
Johanna Kreissl https://orcid.org/0000-0002-5252-0409
Martin Steinhaus https://orcid.org/0000-0002-9879-1474

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16. Revial G. Asymmetric Michael-type alkylation of chiral imines - enantioselective syntheses of (−)-geosmin and two other related natural terpenes, as well as enant-(+)-geosmin. *Tetrahedron Lett.* 1989;30(31):4121-4124.

17. Revial G, Pfau M. (R)-(−)-10-methyl-1(9)-octal-2-one. *Org Synth.* 1992;70:35-46.

18. Fringuelli F, Germani R, Pizzo F, Savelli G. Epoxidation reaction with m-chloroperoxybenzoic acid in water. *Tetrahedron Lett.* 1989;30(11):1427-1428.

19. Darriet P, Lamy S, La Guerche S, et al. Stereodifferentiation of geosmin in wine. *Eur Food Res Technol.* 2001;213(2):122-125.

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