Synthesis of a New Deuterium-Labeled Phytol as a Tool for Biosynthetic Studies

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Abstract: An efficient and stereoselective synthesis of a new deuterium-labeled phytol as a useful tracer in biosynthetic processes is presented.

Key words: deuterium-labeled compounds, phytol, terpenoids, conjugate addition, natural products, biosynthesis

Phytol [(*E*)-3,7,11,15-tetramethylhexadec-2-en-1-ol] is an acyclic diterpene alcohol that occurs as a side chain of chlorophyll and is therefore abundantly present in nature, not only in plants but also in the animal kingdom. On being digested, the phytol moiety is released and can then be converted into other important metabolites, such as phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), whose accumulation in human blood and tissues has been linked to neurological disorders, peroxisomal biogenesis diseases, and apoptosis.[1] In addition, phytol can be used as a precursor of vitamins E[2] and K_1[3] and its balsamic properties are greatly appreciated in the fragrance industry, where it is used in approximately 0.1–1.0 metric tons per year.[4] Therefore, the preparation of new isotopically labeled phytol derivatives is important because they could be potentially useful in a number of relevant biological processes.[5]

In the course of our investigations aimed at the structural characterization of a bioactive natural compound (to be published), phytol appeared to be the most plausible biosynthetic precursor. Therefore, synthesis of a labeled analogue of phytol with the labeling far enough from the oxygenated function to avoid it being affected in the biosynthetic process was required. As is well known, isotopic labeling is a very useful technique for tracking the transformations undergone by a certain molecule or the mechanisms involved in many biochemical processes.[6]

Few labeled phytols (**1**–**4**) have been described so far in the literature (Figure 1).[5,7] Most of these derivatives, particularly **1** and **2**, contained the label at positions that can be also affected in the biosynthetic processes, and compound **4** was part of a mixture of differently labeled compounds.[7c] For these reasons, a more suitably labeled phytol, compound **5** with two deuterium atoms at C-5, away from the functionalized end of the molecule, was considered for our study.

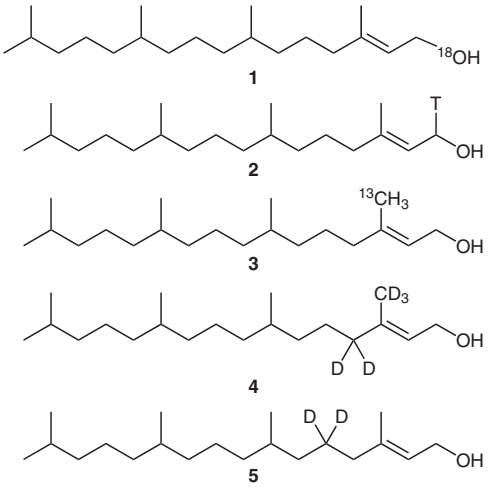

Figure 1

The strategy for the preparation of **5** was based on a straightforward approach by the regioselective opening of 3,4-epoxyisoprene with the appropriate labeled Grignard reagent (Scheme 1). 3,4-Epoxyisoprene can be obtained in a single step and with reasonable yield from methacrolein by selective 1,2-addition of dimethylsulfonium methylide.[8] For preparation of the other labeled building block **10**, farnesol (**6**) was initially treated with hydrogen and 10% palladium-on-carbon to give the corresponding hexahydrofarnesol (**7**), but the hydrogenolysis product farnesane was recovered almost exclusively. However, successful hydrogenation was achieved by using platinum-on-carbon as catalyst to provide the fully hydrogenated compound in quantitative yield.[9] Oxidation of compound **7** under standard conditions (CrO_3, H_2SO_4) furnished acid **8**, which was quantitatively reduced to the corresponding deuterated alcohol **9** with lithium aluminum deuteride ($LiAlD_4$). In this process, no trace of the mono- or non-labeled alcohol was detected, as demonstrated by the absence of the characteristic signal at δ = 3.67 ppm in the ^{1}H NMR spectrum. The alcohol **9** was transformed into the corresponding bromide **10** in 95% yield by treatment with triphenylphosphine–*N*-bromosuccinimide[10a] in dichloromethane. Reaction of **10** with freshly activated magnesium turnings in tetrahydrofuran,[10a,b] followed by treatment with a catalytic amount of copper(I) iodide, and finally with 3,4-epoxyisoprene at –78 °C yielded the expected compound **5** stereoselectively as the *E* isomer (*E*/*Z* = 96:4), the isomer corresponding to natural phytol, in a reasonable 45% yield. We have no-

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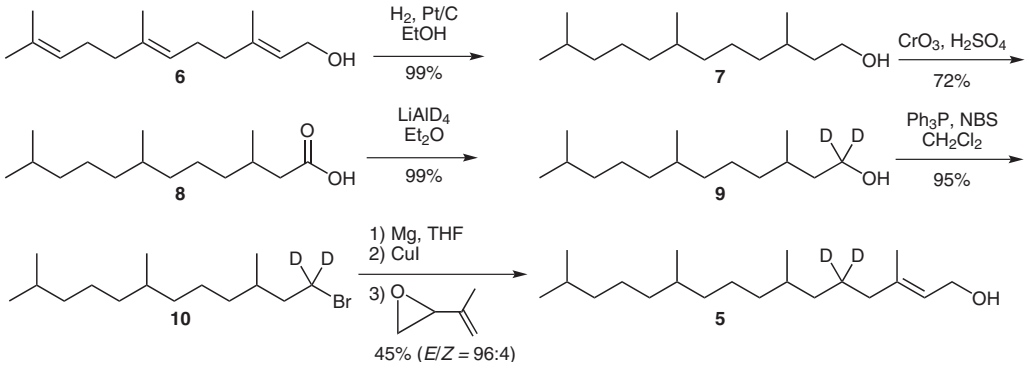

Scheme 1

ticed that an excess of magnesium is required in this reaction to avoid the homocoupling reaction. It is worthy of note that this synthetic strategy ensures the introduction of two deuterium atoms in a predefined position of the molecule, avoiding problematic mixtures of differently labeled compounds.

In conclusion, we have accomplished the synthesis of a new labeled phytol with two deuterium atoms at C-5, far enough from the functionalized end of the molecule, which makes it an attractive candidate for a variety of biosynthetic studies. The synthesis is short, stereoselective, and with a good overall yield (16.6%) from readily available starting materials.

All reactions involving air- or moisture-sensitive materials were carried out under argon. All solvents were dried and distilled according to standard procedures. Locations of deuterium atoms and purity of the labeled compounds were determined by elemental analysis, MS, and NMR. NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Varian Mercury 400 MHz spectrometer. IR spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. MS was carried out in EI mode (70 eV) on a Fisons MD 800 instrument. Elemental analyses were determined on a Carlo Erba 1108 and on a Metrohm Titrando 808 analyzer. HRMS was run on a UPLC Acquity (Waters, USA) coupled to a mass spectrometer LCT Premier XE (Waters, USA).

3,4-Epoxy-2-methylbut-1-ene[8]

Methacrolein (2.4 mL, 28.5 mmol) was added in 4 portions over 20 min to a stirred suspension of methyl trimethylsulfonium sulfate (6.14 g, 32.63 mmol) and NaOH (7.10 g, 0.18 mol) in anhyd CH_2Cl_2 (70 mL). After the mixture had stirred for 4 h at r.t., H_2O (100 mL) was added and the organic layer was separated and dried ($MgSO_4$). After filtration, the crude product was purified by distillation at atmospheric pressure; the material with bp 90 °C was collected as pure 3,4-epoxy-2-methylbut-1-ene.

Yield: 1.32 g (55%); colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.16 (m, 1 H), 5.01 (m, 1 H), 3.36 (m, 1 H), 2.85 (m, 1 H), 2.71 (m, 1 H), 1.61 (m, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 141.3 (C), 114.5 (CH_2), 54.4 (CH), 46.6 (CH_2), 16.0 (CH_3).

3,7,11-Trimethyldodecan-1-ol (7)[11]

A soln of farnesol (**6**; 5.00 g, 22.52 mmol) in EtOH (25 mL) was subjected to hydrogenation at atmospheric pressure in the presence of 10% Pt/C (200 mg) as catalyst.[9] After the mixture had stirred at r.t. for 12 h, the reaction was complete. The crude product was filtered on Celite and the solvent evaporated to yield the saturated alcohol **7** as a colorless oil, pure enough for the next step of the synthesis without need of further purification.

Yield: 5.13 g (99%).

IR (film): 3349, 2955, 2927, 2869, 1463, 1378, 1056, 908, 735 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.67 (m, 2 H), 1.55 (b, 3 H), 1.01–1.38 (b, 14 H), 0.86 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 61.2 (CH_2), 40.0 (CH_2), 39.3 (CH_2), 37.5 (CH_2), 37.4 (CH_2), 37.3 (CH_2), 32.8 (CH), 29.5 (CH), 27.9 (CH), 24.8 (CH_2), 24.4 (CH_2), 22.7 (CH_3), 22.6 (CH_3), 19.7 (CH_3), 19.6 (CH_3).

MS (EI, 70 eV): *m*/*z* (%) = 210 [M – 18]$^+$ (1), 182 (13), 140 (28), 125 (88), 111 (84), 97 (91), 83 (91), 69 (100), 57 (97), 43 (82).

3,7,11-Trimethyldodecanoic acid (8)[12]

A soln of Jones reagent, prepared from CrO_3 (3.19 g), concd H_2SO_4 (2.6 mL), and H_2O (9 mL) until the reaction mixture turned red, was added to a soln of alcohol **7** (3.00 g, 13.0 mmol) in acetone (75 mL) at 0 °C. After the mixture had stirred at 0 °C for 4 h, propan-2-ol (10 mL) was added to stop the reaction, the solvent was evaporated, and the residue was purified by column chromatography (silica gel, hexane–Et_2O, 99:1); this gave acid **8**.

Yield: 2.28 g (72%); pale yellow oil.

IR (film): 3084, 2955, 2927, 2678, 1709, 1463, 1298, 937 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.37 (m, 1 H), 2.14 (m, 1 H), 1.96 (m, 1 H), 1.52 (m, 1 H), 1.05–1.38 (br, 13 H), 0.97 (d, *J* = 8.0 Hz, 3 H), 0.86 (d, *J* = 8.0 Hz, 6 H), 0.84 (d, *J* = 8.0 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.7 (C), 41.6 (CH_2), 39.3 (CH_2), 37.3 (CH_2), 37.1 (CH_2), 37.0 (CH_2), 32.7 (CH), 30.2 (CH), 28.0 (CH), 24.8 (CH_2), 24.3 (CH_2), 22.7 (CH_3), 22.6 (CH_3), 19.7 (CH_3), 19.6 (CH_3).

MS (EI, 70 eV): *m*/*z* (%) = 242 [M$^+$] (3), 227 (2), 180 (30), 157 (57), 139 (39), 111 (45), 97 (79), 87 (100), 71 (84), 57 (87), 43 (68).

[1,1-$^2H_2$]-3,7,11-Trimethyldodecan-1-ol (9)

A soln of **8** (1.58 g, 6.5 mmol) in anhyd Et_2O (10 mL) was slowly added to a suspension of $LiAlD_4$ (0.41 g, 9.8 mmol) in anhyd Et_2O (10 mL) at 0 °C. The reaction mixture was then refluxed until complete consumption of **10** (3 h). The mixture was cooled to 0 °C and quenched with H_2O (0.4 mL), 15% aq NaOH (0.4 mL), and H_2O (1.2 mL). The salts were filtered and the solvent was removed under vacuum to provide alcohol **9** as a pale yellow oil, pure enough for the next step of the synthesis without need of further purification.

Yield: 1.5 g (99%).

IR (film): 3335, 2955, 2926, 2869, 1463, 1378, 969, 908, 736 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.47–1.61 (br, 3 H), 1.01–1.41 (br, 14 H), 0.87 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 60.51 (quin, *J* = 21.0 Hz, CD_2), 39.8 (CH_2), 39.3 (CH_2), 37.4 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 32.8 (CH), 29.4 (CH), 27.9 (CH), 24.8 (CH_2), 24.3 (CH_2), 22.7 (CH_3), 22.6 (CH_3), 19.7 (CH_3), 19.6 (CH_3).

MS (EI, 70 eV): *m*/*z* (%) = 230 [M^+] (1), 212 (2), 182 (12), 142 (27), 127 (87), 111 (78), 99 (77), 85 (84), 71 (100), 57 (98), 43 (82).

Anal. Calcd for $C_{15}H_{30}D_2O$: C, 78.19; H, 14.87. Found: C, 78.34; H, 14.50.

[1,1-$^2H_2$]-1-Bromo-3,7,11-trimethyldodecane (10)

NBS (1.31 g, 7.4 mmol) was added portionwise over 30 min to a soln of **9** (1.50 g, 6.5 mmol) and Ph_3P (2.05 g, 7.8 mmol) in anhyd CH_2Cl_2 (10 mL) at 0 °C.[10a] The reaction mixture was stirred for 30 min and then concentrated under reduced pressure. Purification by column chromatography (silica gel, hexane) afforded **10**.

Yield: 1.82 g (95%); colorless oil.

IR (film): 3006, 2925, 2860, 1462, 1251, 908, 736 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.87 (m, 1 H), 1.50–1.67 (br, 3 H), 1.08–1.37 (br, 13 H), 0.87 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 39.8 (CH_2), 39.4 (CH_2), 37.4 (CH_2), 37.3 (CH_2), 36.8 (CH_2), 32.8 (CH), 31.7 (quin, *J* = 22.0 Hz, CD_2), 31.6 (CH), 28.0 (CH), 24.8 (CH_2), 24.2 (CH_2), 22.7 (CH_3), 22.6 (CH_3), 19.7 (CH_3), 19.0 (CH_3).

MS (EI, 70 eV): *m*/*z* (%) = 294 [M + 1]$^+$ (1), 292 [M – 1]$^+$ (1), 279 (3), 277 (3), 209 (36), 207 (38), 181 (28), 179 (29), 167 (27), 165 (28), 153 (44), 151 (46), 127 (62), 113 (79), 99 (42), 97 (44), 85 (72), 71 (100), 57 (99), 43 (43).

Anal. Calcd for $C_{15}H_{29}D_2Br$: C, 61.42; H, 11.34; Br, 27.24. Found: C, 61.36; H, 10.98; Br, 27.03.

[5,5-$^2H_2$]-(*E*)-3,7,11,15-tetramethylhexadec-2-en-1-ol (5)

1,2-Dibromoethane (32 μL, 0.37 mmol) was added to a suspension of Mg turnings (0.19 g, 7.8 mmol) in anhyd THF (1.5 mL). The mixture was stirred at 50 °C for 15 min, the solvent was removed by syringe, and the black activated turnings were suspended again in anhyd THF (1.5 mL). A soln of **10** (0.57 g, 1.9 mmol) in anhyd THF (1.5 mL) was slowly added and the mixture was stirred at r.t. for 2 h. The Grignard reagent soln of **10** was added at –78 °C via cannula to a second flask containing a suspension of CuI (15 mg, 97 μmol) in THF (1.5 mL). The reaction mixture was stirred at –78 °C for 5 min. Then, 3,4-epoxyisoprene (0.19 g, 2.3 mmol) was added and after additional stirring for 5 min at –78 °C, the mixture was allowed to warm to r.t. for 1 h. After this time, the solvent was removed under reduced pressure and the crude was directly purified by column chromatography (silica gel, hexane–Et_2O, 4:1); this afforded alcohol **5**.

Yield: 0.26 g (45%), *E*/*Z* = 96:4, colorless oil.

IR (film): 3329, 2954, 2925, 2869, 1463, 1378, 1002 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.40 (m, 1 H), 4.14 (d, *J* = 8.0 Hz, 2 H), 1.97 (s, 2 H), 1.66 (s, 3 H), 1.52 (m, 1 H), 1.00–1.39 (br, 17 H), 0.85 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.2 (C), 123.1 (CH), 59.4 (CH_2), 39.7 (CH_2), 39.4 (CH_2), 37.4 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 36.5 (CH_2), 32.8 (CH), 32.6 (CH), 28.0 (CH), 24.8 (CH_2), 24.5 (CH_2), 22.7 (CH_3), 22.6 (CH_3), 19.7 (CH_3), 19.6 (CH_3), 16.2 (CH_3).

MS (EI, 70 eV): *m*/*z* (%) = 298 [M]$^+$ (1), 280 (6), 139 (18), 125 (52), 111 (47), 97 (56), 84 (58), 71 (100), 57 (58), 43 (56).

HRMS (ESI): *m*/*z* calcd for $C_{20}H_{39}D_2O$ [M + 1]$^+$: 299.3283; found: 299.3283.

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