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SYNTHESIS OF (2*E*,6*E*)-[10-^{3}H]FARNESOL AND (2*E*,6*E*)-[10-^{3}H]FARNESAL FOR INSECT DEHYDROGENASE STUDIES

Stephanie E. Sen* and Gail M. Garvin

Department of Chemistry, Indiana University-Purdue University at Indianapolis (IUPUI)
402 North Blackford Street, Indianapolis, IN 46202

SUMMARY

[10-^{3}H]Farnesol ((2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol) and [10-^{3}H]farnesal ((2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-1-al) were synthesized by sequential reduction and oxidation of the corresponding *tert*-butyldiphenylsilyl protected 11,12,13-trisnoraldehyde, followed by Wittig homologation using isopropyltriphenylphosphorane. Direct reduction of the C-12 allylic bromide or allylic chloride proved to be an nonviable method, resulting in either multiple decomposition products or significant double bond transposition. Oxidation of the [10-^{3}H]labelled trisnoralcohol with pyridinium chlorochromate resulted in essentially complete retention of the radiolabel as was established for the corresponding deuterated material. [^{3}H]labelled farnesol and farnesal were obtained in 78% and 74% yields, respectively, from the trisnoraldehyde. These materials were used as radiotracers for examining the enzymatic activity of insect farnesol and farnesal dehydrogenase, key enzymes in the biosynthesis of juvenile hormone.

Key words: [^{3}H-]labelled farnesol, insect dehydrogenase, juvenile hormone biosynthesis.

INTRODUCTION

The final steps of juvenile hormone (JH) biosynthesis involve functionalization of the isoprenoid skeleton obtained by farnesyl synthase (Scheme 1). Among these transformations is the sequential oxidation of farnesol (**1**) to farnesal (**2**) and farnesoic acid (**3**), mediated by an alcohol and aldehyde dehydrogenase (1). To date, little structural or kinetic data is available on either of these two enzymes. Initial studies on insect farnesol and farnesal dehydrogenase used [1,5,9-^{3}H]farnesol (obtained by alkaline phosphatase hydrolysis of the commercially available diphosphate ester) to monitor enzymatic conversion in corpora allata homogenates of the lepidopteran insect, *Manduca sexta* (2). Unfortunately, oxidation of this material lead to significant amounts of radiolabelled polar material(s), resulting in dilution of counts throughout the reaction medium. In our studies on lepidopteran farnesol and farnesal dehydrogenase (3), we desired a less labile radiotracer to allow for a simpler and more efficient method of product analysis. Herein, we report the efficient synthesis of [10-^{3}H]farnesol and [10-^{3}H]farnesal, involving sequential reduction and oxidation of the C-10 position of the corresponding 11,12,13-trisnoraldehyde with NaB^3H_4 and pyridinium chlorochromate (PCC).

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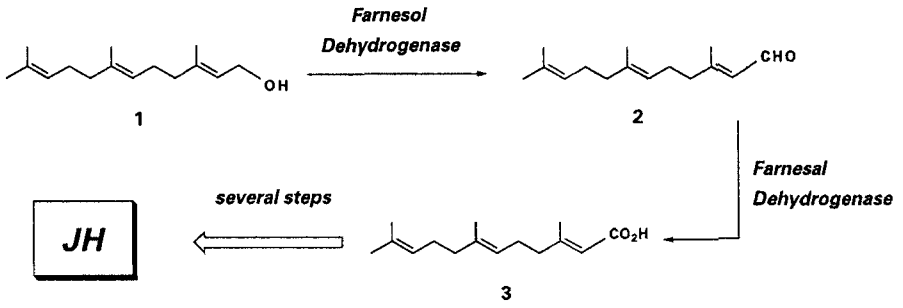
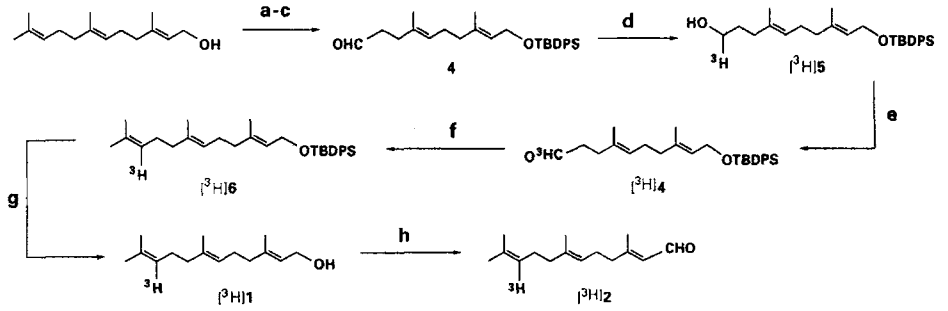
SCHEME 1



RESULTS AND DISCUSSION

[10-^{3}H]Farnesol was prepared in 7 steps from farnesol, as outlined in Scheme 2. Key to this sequence is the sequential reduction and oxidation of the *tert*-butyldiphenylsilyl (TBDPS) protected trisnoraldehyde **4**, obtained by selective epoxidation of the Δ-10,11 sesquiterpene olefin using previously established methodology (4). Reduction of the aldehyde moiety with NaB^3H_4 (5 mCi, 0.36 Ci/mmol), followed by oxidation with 3 equiv of pyridinium chlorochromate (PCC) at room temperature (rt) overnight, provided [10-^{3}H]**4** (after purification by silica gel chromatography) in good radiochemical yield (90%). Integration of the ^{1}H NMR of the aldehyde obtained from the analogous synthetic sequence using NaB^2H_4 (98% atom D) revealed a 5% loss of isotopic labelling, as expected for the weaker C-^{2}H versus C-^{3}H bond. The tritiated trisnoraldehyde was reacted with 2.5 equiv isopropyl triphenylphosphorane at -78°C to regenerate the farnesol carbon skeleton, then deprotected with 4 equiv of tetrabutylammonium fluoride (TBAF) (5) to yield [10-^{3}H]farnesol in a 78% overall isolated yield from **4**. A portion of this material was converted in essentially quantitative yield to [10-^{3}H]farnesal, by overnight reaction with activated MnO_2 at 4 °C (6).

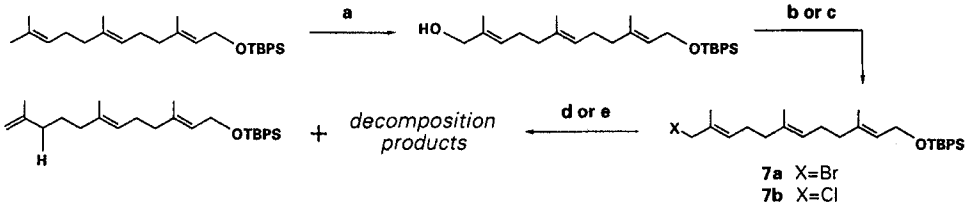
SCHEME 2



a) *tert*-Butyldiphenylsilyl chloride, Et_3N, DMAP, CH_2Cl_2; b) *N*-bromosuccinimide, THF:H_2O; c) H_5IO_6, THF:H_2O; d) $NaBH^3H_4$, EtOH; e) PCC, CH_2Cl_2; f) $(CH_3)_2CH{=}PPh_3$, THF; g) TBAF, THF; MnO_2, $NaHCO_3$, hexane, 4 °C.

Despite the simplicity of the above reaction sequence, an alternate route was examined which would allow for radiolabel incorporation as one of the last synthetic transformations. Reaction of a variety of substituted allylic halides with $NaBH_4$ in polar aprotic solvents such as HMPA or DMSO has been shown to be an efficient displacement method (7). We examined the utility of this procedure for the preparation of [12-^{3}H]farnesol, by $NaBH_4$ reduction of silyl protected allylic bromide **7a** and allylic chloride **7b**, obtained by reaction of the corresponding C-12 allylic alcohol with PPh_3 and either CBr_4 or CCl_4 in CH_2CH_2 (Scheme 3). To our surprise, reduction of either material with $NaBH_4$ in HMPA or DMSO, at several different temperatures (5 °C, rt, and 50 °C), resulted in either significant decomposition or transposition of the Δ-10,11 olefin by hydride addition to the C-10 center. This observation has been noted in related trisubstituted allylic systems (8) and appears to result from unfavorable steric and electronic interactions between the incoming nucleophile and the C-12 allylic center.

SCHEME 3



a) SeO_2, tBuOOH, CH_2Cl_2; c) CCl_4, PPh_3,CH_2Cl_2; c) CBr_4, PPh_3, CH_2Cl_2; d) $NaBH_4$, HMPA; e) $NaBH_4$, DMSO.

Several synthetic methods have been developed for the preparation of isotopically labelled farnesol and farnesyl derivatives (9-16). Despite the wealth of information available, there are few convenient non-enzymatic procedures for tritium incorporation into the farnesol skeleton other than the C-1 position where loss of label occurs upon enzymatic oxidation by insect farnesol and farnesal dehydrogenase. The described procedure offers an easy synthetic route to [10-^{3}H]farnesol and [10-^{3}H]farnesal, in high chemical yield and radiochemical recovery.

EXPERIMENTAL

Solvents (with the exception of DMSO) were obtained from Fisher Scientific Co. NaB^3H_4 was obtained from New England Nuclear. Unless otherwise stated, all other chemicals were obtained from Aldrich Chemical Co. THF was dried from benzophenone sodium ketyl. Methylene chloride was distilled over CaH_2. ^{1}H NMR were obtained using a General Electric QE-300 with $CDCl_3$ as solvent. Thin layer chromatography (TLC) was performed on MN Polygram Sil G/UV 254 (4 cm x 8 cm) and the plates were visualized with vanillin/H_2SO_4 unless otherwise noted. Flash chromatography was performed on EM Science silica gel 60 (40-60 μm). Radiochemical samples were counted in a Beckman LS-1801 liquid scintillation counter using Scintiverse BD as scintillation cocktail. *tert*-Butyldiphenylsilyl protected farnesol ((2*E*,6*E*)-1-((*tert*-butyldiphenylsilyl)oxy)-3,7,11-trimethyl-2,6,10-dodecatriene) was prepared as previously described (4).

(2*E*,6*E*)-10-Bromo-1-((*tert*-butyldiphenylsilyl)oxy)-11-hydroxy-3,7,11-trimethyl-2,6-dodecadiene (5). The bromohydrin was prepared using a modified procedure of van Tamelen and Curphey (17). To a stirred solution of silyl protected farnesol (500 mg, 1.1 mmol), in a 3:1 mixture of THF and water (9 mL of THF), was added *N*-bromosuccinimide (1.05 equiv). After allowing the solution to stir at 0 °C for 3 h in darkness, water was added. The mixture was extracted with hexane and the combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give a colorless oil. Flash chromatography of this material with 5% ethyl acetate-hexane (EA/H) yielded the bromohydrin (230 mg, 38%): ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 9H, C(C*H*$_3$)$_3$), 1.38 (s, 6H, C-12, C-13 C*H*$_3$), 1.44 (s, 3H, C-14 C*H*$_3$), 1.45 (s, 3H, C-15 C*H*$_3$), 2.18 (m, 8H, C=CC*H*$_2$), 4.08 (m, 1H, C*H*Br), 4.35 (d, J= 6.3 Hz, 2H, C*H*$_2$OSi), 5.32 (t, 1H, J= 6.5 Hz, C-6 C=C*H*), 5.51 (t, J=6.3, 1H, C-2 C=C*H*), 7.50 (m, 6H, meta, para Ar*H*), 7.81 (m, 4H, ortho Ar*H*).

(2*E*,6*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadiene (5). The epoxide was formed by the addition of anhydrous potassium carbonate (350 mg, 7 equiv) to a stirred solution of the bromohydrin (200 mg, 0.36 mmol) in methanol (15 mL). After the mixture was left to stir at rt for 10 min, water was added and the mixture was extracted with hexane. The organic extract was dried with $MgSO_4$ and concentrated to yield the epoxide as a pale yellow oil (170 mg, quantitative): ^{1}H NMR ($CDCl_3$) δ 1.10 (s, 9H, C(C*H*$_3$)$_3$), 1.31 (s, 6H, C-12, C,13 C*H*$_3$), 1.35 (s, 3H, C-14 C*H*$_3$), 1.50 (s, 3H, C-15 C*H*$_3$), 2.11 (m, 8H, C=CC*H*$_2$), 2.76 (t, 1H, J= 6.2 Hz, C-10 C*HO*), 4.27 (d, 2H, J= 6.2 Hz, C*H*$_2$OSi), 5.22 (t, 1H, J= 6.4 Hz, C-6 C=C*H*), 5.44 (t, 1H, J= 6.2 Hz, C-2 C=C*H*), 7.75 (m, 6H, meta, para Ar*H*), 7.45 (m, 4H, ortho Ar*H*).

(2*E*,6*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-decadien-10-al (4) (4). The epoxide (100 mg, 0.2 mmol) was dissolved in a 10:1 mixture of THF and water (10 mL of THF). Periodic acid (48 mg, 1 equiv) was added and the solution was stirred for 4 h at rt. The reaction was quenched by addition of water and the resulting mixture was extracted with ether. The ether extracts were washed with 10% $NaHCO_3$ and brine, dried with $MgSO_4$ and concentrated. Flash chromatography (5% EA/H) afforded the trisnoraldehyde derivative of farnesol as a pale yellow oil (50 mg, 57%): ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 9H, C(C*H*$_3$)$_3$), 1.2 (s, 3H, C-11 C*H*$_3$), 1.6 (s, 3H, C-12 C*H*$_3$), 2.3 (t, 4H, J= 7.4 Hz, C-4, C-8 C*H*$_2$), 2.5 (t, 4H, J= 7.4 Hz, C-5, C-9 C*H*$_2$), 4.21 (d, J=6.4 Hz, C*H*$_2$OSi), 5.13 (t, J= 6.2, 1H, C-6 C=C*H*), 5.36 (t, J= 6.2 Hz, 1H, C-2 C=C*H*), 7.38 (m, 6H, meta, para Ar*H*), 7.69 (m, 4H, ortho Ar*H*), 9.72 (t, 1H, J= 7.4 Hz, C*H*O).

(2*E*,6*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-decadien-10-ol (5). Aldehyde **4** (43 mg, 0.1 mmol) was dissolved in stock solution of $NaBH_4$ (1 mg/mL, 940 μL, in EtOH) and was stirred at rt for 30 min. The mixture was then quenched by the addition of 3 drops of 2N acetic acid. The crude product was purified by flash chromatography (1-10% EA/H gradient) to provide alcohol **5** (38 mg, 90%): ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 9H, C(C*H*$_3$)$_3$), 1.44 (s, 3H, C-11 C*H*$_3$), 1.62 (s, 3H, C-12 C*H*$_3$), 2.05 (m, 6H, C=CC*H*$_2$), 3.61 (t, 2H, J= 6.4 Hz, C*H*$_2$OH), 4.22 (d, 2H, J= 6.1 Hz, C*H*$_2$OSi), 5.15 (t, 1H, J= 6.2 Hz, C-6 C=C*H*), 5.38 (t, 1H, J= 6.1 Hz, C-2 C=C*H*), 7.39 (m, 6H, meta, para Ar*H*), 7.69 (m, 4H, ortho Ar*H*).

(2*E*,6*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-decadien-10-al. The trisnoraldehyde was regenerated by PCC oxidation of the corresponding alcohol. To a solution of alcohol **5** (20 mg, 0.05 mmol) and methylene chloride (10 mL), PCC (30 mg, 3 equiv) was added. After this solution was left to stir for 2 h at rt, ether (15 mL) was added. The resulting solution was then filtered through a Florisil column and concentrated to yield aldehyde **4** (15 mg, 76%).

(2*E*,6*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7,11-trimethyl-2,6,10-dodecatriene (6). Isopropyltriphenyl phosphonium iodide (270 mg, 0.63 mmol, dried overnight under vacuum) was suspended in THF (10 mL) and the mixture was cooled to -78 °C, under an argon atmosphere. *n*-Butyllithium (1.6 M, 0.4 mL) was added and the solution was warmed to 0 °C to allow for complete formation of the blood red ylide. A portion of the ylide (1 mL, 2.3 equiv), was added dropwise to a cooled solution of the silyl protected aldehyde (11 mg, 0.026 mmol) in THF (5 mL) at 0 °C. After stirring for 4 h at 0 °C, pentane (10 mL) was added to the reaction mixture and the solution was filtered through a cotton plug to remove the precipitated triphenylphosphine oxide and unreacted phosphonium salt. Flash chromatography of the concentrated eluent (hexane to 10 %EA/H gradient) yielded pure silyl protected farnesol (9 mg, 75%).

(2*E*,6*E*)-3,7,11-Trimethyl-2,6,10-dodecatriene-1-ol (1). To a solution of silyl protected alcohol **6** (50 mg, 0.1 mmol) in dry THF (10 mL) at 0 °C, TBAF (1M, 4 equiv) was added. The solution was then warmed to rt and left to stir for an additional hour. Purification by flash chromatography (5%-20% EA/H gradient) provided farnesol (24 mg, quantitative). Both the ^{1}H NMR spectrum and GLC retention time were identical to those of commercially available farnesol.

(2*E*,6*E*)-[10-^{3}H]-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-decadien-10-ol ([^{3}H]5). Aldehyde **4** (35 mg, 0.08 mmol, 50% molar excess) was diluted in ethanol (1 mL) and added dropwise to a vial containing a cold solution of NaB^3H_4 (360 mCi/mmol, 5 mCi) in ethanol (0.5 mL). After stirring at 0 °C for 1 h, the mixture was warmed to rt then quenched by the addition of 3 drops of 2N acetic acid. The material was extracted with hexane/ether (1:1), dried over $MgSO_4$, and concentrated by a gentle stream of argon. Flash chromatography of the resulting oil using a Pasteur pipette column (hexane to 1% EA/H gradient) afforded alcohol **5** (21 mg, 89%, 80 mCi/mmol, 88% radiochemical yield).

(2*E*,6*E*)-[10-^{3}H]-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-decadien-10-al ([^{3}H]4). To the round bottom flask containing alcohol **5** in dry CH_2Cl_2 (3 mL) was added PCC (46 mg, 4 equiv). An balloon filled with argon was connected to the flask and the mixture was left to stir overnight. Unreacted oxidant was precipitated from solution by the addition of ether (6 mL) and the resulting suspension was filtered through a Florisil Pasteur pipette column. After several ether washings, the eluent was concentrated under an argon stream to yield [10-^{3}H]labelled aldehyde **4** (21 mg, quantitative).

(2*E*,6*E*)-[10-^{3}H]-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7,11-trimethyl-2,6,10-dodecatriene ([^{3}H]6). Isopropyl-triphenylphosphonium iodide (400 mg, 0.93 mmol) was dissolved in dry THF (13 mL) and cooled to -78 °C. *N*-butyllithium (1.6 M, 0.55 mL, 0.95 equiv) was added to yield an orange-colored solution. After warming the solution to 0 °C for 1 h, a portion of the ylide (1.9 mL, 2.5 equiv) was added dropwise to a solution of [10-^{3}H]aldehyde **4** (0.05 mmol) in dry THF (2 mL) at -78 °C. The reaction mixture was then left to warm to rt overnight. The resulting cloudy suspension was quenched by the addition of 4 drops of brine and the solvent was evaporated using argon gas. Pentane (5 mL) was added, and the solution was filtered through a cotton plug. Purification by flash chromatography using a Pasteur pipette column (hexane to 5% EA/H) afforded [10-^{3}H]TBDPS protected farnesol (20 mg, 87%).

(2*E*,6*E*)-[10-^{3}H]-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol ([^{3}H]1). TBDPS protected farnesol (0.04 mmol) was diluted in dry THF (3 mL) and cooled to 0 °C. TBAF (1 M, 4 equiv) was added and the solution was

stirred overnight. Solvent evaporation followed by flash chromatography of the resulting oil using a Pasteur pipette column (5%-20% EA/H gradient) yielded pure [10-^{3}H]labelled farnesol (10 mg, quantitative, 75 mCi/mmol, 83% radiochemical yield.).

(2*E*,6*E*)-[10-^{3}H]-3,7,11-Trimethyl-2,6,10-dodecatrien-1-al ([^{3}H]2). [10-^{3}H]Farnesal was prepared by allylic oxidation of the corresponding alcohol using a modified MnO_2 procedure that eliminates geometric isomerization of the Δ-2,3 olefin (6). To a solution of labelled farnesol (1.5 mg, 6.7 μmol) in dry hexane (1 mL) was added $NaHCO_3$ (2 mg) and activated MnO_2 (6 mg, 10 equiv). The solution was stirred at 4 °C overnight, then filtered through a short pad of Celite. Concentration of the eluent yielded [10-^{3}H]farnesal (1.4 mg, 94%).

Enzyme assays. Four corpora allata-corpora cardiaca pair from the lepidopteran *Manduca sexta* (18) were placed in 450 μL of 100 mM Tris-HCl buffer (pH 7.4) and homogenized on ice using a Duall glass homogenizer (Kontes). After removal of cellular debris by centrifugation at 3,000 X g for 10 minutes, 45 μL aliquots of supernatant were placed in microcentrifuge tubes. Tween 80 and NAD were added as stock solutions (5 μL each) to give final concentrations of 0.05% and 5 mM, respectively.

Radioactively labelled substrates were added in DMSO (1 μL) to give a final concentration of 4 μM. The solution was incubated at 28 °C on a rotary shaker for 60 minutes, then quenched by the addition of acetonitrile (60 μL) containing farnesol, farnesal, and farnesoic acid standards. The reaction mixture was extracted twice with CH_2Cl_2 (60 μL each), and the organic extract concentrated. TLC of this material by double elution with 10% EA/H, containing 5% triethylamine, gave clean separation of starting material and products (R_f farnesol = 0.43, R_f farnesal = 0.88, R_f farnesoic acid = 0.09). TLC plates were cut into several zones following visualization of the aldehyde and acid by UV fluorescence and of the alcohol by water staining, then analyzed by liquid scintillation counting. Under these assay conditions, further metabolism to epoxyfarnesoate, methyl farnesoate, or JH was not observed, as determined by TLC and HPLC analysis.

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18. Either 1-day old, 5th stadium larvae or 2-day old adult females were used.