SYNTHESIS OF [8,9-$^3H_2$]-(7*S*)-METHOPRENE, A JUVENILE HORMONE ANALOG, BY SELECTIVE REDUCTION OF A PROTECTED TRIENOATE

Marcus F. Boehm and Glenn D. Prestwich*
Department of Chemistry
State University of New York
Stony Brook, New York 11794-3400

SUMMARY

High specific activity ^3H-(7*S*)-methoprene is prepared in seven steps from (3*S*)-citronellol by the use of iron tricarbonyl to protect the dienoate moiety of isopropyl (2*E*,4*E*)-3,7,11-trimethyl-2,4,8-dodecatrienoate during heterogeneous tritiation of remote olefinic bond.

Key words: selective tritiation, dienoate complex, iron carbonyl protecting group, insect growth regulator, juvenile analog

INTRODUCTION

The molecular mechanisms of action of the insect juvenile hormones (JH) and the juvenile hormone analogs (JHA) are currently unknown. One reason for the lack of molecular details has been the paucity of sufficiently high specific activity radioligands for detecting putative receptor and binding proteins with nanomolar binding constants (1). Convenient radiosyntheses have been described for the primary insect juvenile hormones (JH I, II, and III) (2,3) and for commercially important insect growth regulators (e.g., the Zoecon dodecadienoates) (4,5,6). Selected biologically active compounds with tritium- and iodine-labels are shown below. The separate (10*R*,11*S*) and (10*S*,11*R*) enantiomers of both JH I and JH II have been prepared with >95% e.e. in radioinert form and labelled with tritium at 58 Ci/mmol (2).

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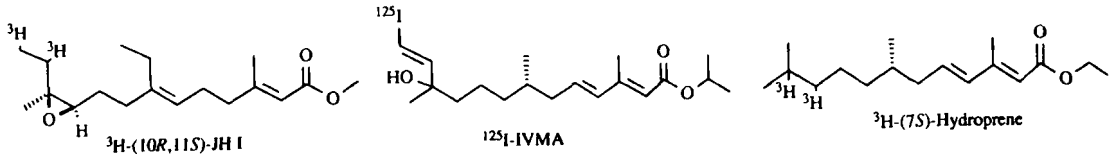
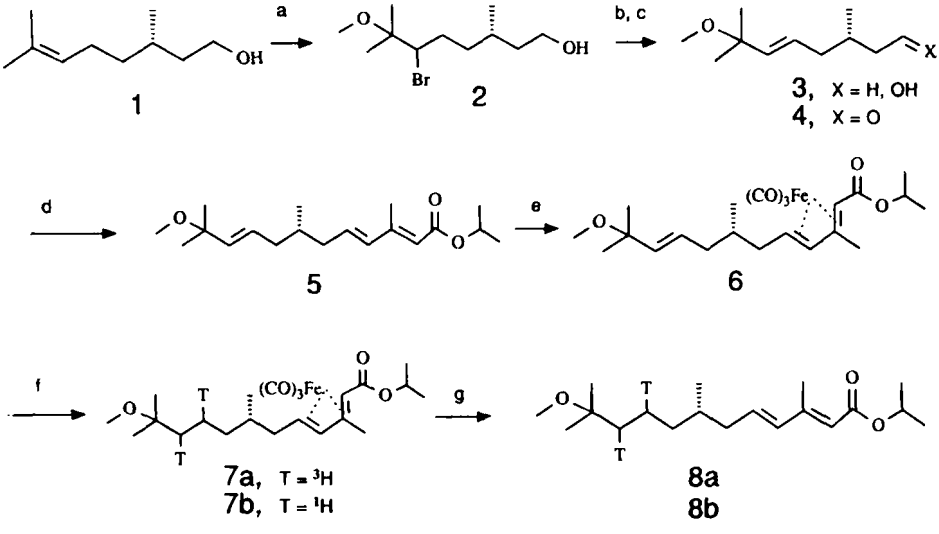


IVMA, an iodovinyl analog of methoprene alcohol (5), is as effective as methoprene in both *in vitro* and *in vivo* experiments with *Manduca sexta* epidermis. Moreover, ^{125}I-IVMA shows saturable, specific binding to epidermal nuclei (7). Hydroprene, a commercially available insecticide, was synthesized and labelled with tritium at >58 Ci/mmol using an iron tricarbonyl adduct for protection of the dienoate moiety. The synthesis described herein employs the dienoate protection strategy and now offers a useful route to high specific activity tritium-labelled methoprene (8), which is now being used to characterize methoprene receptor proteins in insects.



Scheme I. Radiosynthesis of [8,9-$^3H_2$]-(7*S*)-methoprene.

Reagents and conditions: (a) NBS, CH_3OH, 0 °C;
(b) Ph_3CK, $CH_3OCH_2CH_2OCH_3$, 0 °C; (c) PCC, CH_2Cl_2;
(d) $(iPrO)_2POCH_2CH{=}C(CH_3)CO_2iPr$, NaH, DMF;
(e) $Fe_3(CO)_{12}$, C_6H_6, reflux; (f) $^3H_2$, 5% Rh/C, $CH_3CO_2C_2H_5$;
(g) $Ce(NH_4)_2(NO_3)_2$, CH_3CN.

RESULTS AND DISCUSSION

The synthesis is illustrated in Scheme I. Citronellol **1** was treated with 1.5 equivalents of N-bromosuccinimide in methanol at 0° C to give the 6-bromo-7-methoxy compound **2** (94% yield) as a mixture of two diastereomers. Addition of excess tritylpotassium (9) to bromohydrin **2** in glyme at 0° C resulted in rapid dehydrobromination to give the alkenol **3** (68% yield). The oxidation of alkenol **3** with PCC resulted in the formation of the aldehyde **4** (76% yield). Condensation of the aldehyde **4** with the anion of diisopropyl 3-isopropoxycarbonyl-2-methyl-2-propenyl phosphonate in DMF gave a 3:1 mixture of (2*E*,4*E*) and (2*Z*,4*E*) isomers **5** (8) which was used without further purification (68% total yield). Reaction of the dodecatrienoate with excess triiron dodecacarbonyl in benzene under reflux (6 h) resulted in the iron tricarbonyl-dienoate complex **6** (74% yield) (4). The reaction was monitored by observing the disappearance of the 1625 cm^{-1} (C=C) IR absorption band from the uncomplexed diene. Heterogeneous tritiation using 5% Rh/C in EtOAc and carrier-free tritium gas at room temperature for 3 h afforded the tritiated complex **7a** (88% yield). It is noteworthy that in trial hydrogenations, homogeneous catalysts (e.g., $(Ph_3P)_3RhCl$ in benzene, $Ir(cod)py(PCy_3)PF_6$ in CH_2Cl_2) failed to effect the hydrogenation of the α-alkoxy substituted alkene. Oxidative removal of the iron tricarbonyl moiety with 10 eq. ceric ammonium nitrate in acetonitrile and purification by HPLC (2% EtOAc-hexane) gave methoprene **8** with a specific activity of 84 Ci/mmol (55% yield, purity >92% by HPLC and Bioscan) UV λ_{max} = 261 nm (ε = 28,400).

EXPERIMENTAL METHODS

(S)-(-)-Bromo-7-methoxy-3,7-dimethyloctan-1-ol (**2**). To 2 g (12.82 mmol) of citronellol **1** in 80 mL of methanol at 0° C was added 3.42 g (19.23 mmol) of NBS, and the mixture was stirred at 0° C for 1 h. The methanol was removed, and the product was redissolved in 5% EtOAc-hexane. After washing (H_2O and brine), the solution was dried *in vacuo* and chromatographed (SiO_2, 15% EtOAc-hexane) to give 3.2 g (12.0 mmol, 94% yield) of bromoether **2**. TLC (20% EtOAc-hexane): R_f = 0.13; ^{1}H-NMR ($CDCl_3$, 300 MHz), δ 0.92 (d, *J* = 6.0 Hz, C-3 CH_3, [3*S*,6*RS*]), 0.93 (d, *J* = 6.0 Hz, C-3 CH_3, [3*S*,6*RS*]), 1.29 (s, C-7 CH_3, [3*S*,6*RS*]), 1.33 (s, C-7 CH_3, [3*S*,6*RS*]), 3.70 (br m, H-1), 3.91 (dd, *J* = 5.4, 2.0 Hz, H-6, [3*S*,6*RS*], 3.95 (dd, *J* = 5.4, 2.0 Hz, H-6, [3*S*,6*RS*]).

(*S*)-(-)-Methoxy-3,7-methyl-5-octen-1-ol (**3**) . To 2 g (7.52 mmol) of bromoether **2** in 8 mL of glyme at 0° C was added 22.6 mmol of tritylpotassium in glyme, and the mixture was stirred at 0° C for 10 min. The reaction was quenched with 20 mL of H_2O, the organics were extracted (5% EtOAc-hexane) and purified (SiO_2, 15% EtOAc-hexane) to give 952 mg (5.10 mmol, 68% yield) of hydroxy ether **3**. TLC: R_f = 0.12; ^{1}H-NMR ($CDCl_3$, 300 MHz), δ 0.92 (d, *J* = 6.6 Hz, C-3 CH_3), 1.26 (s, C-7 CH_3 + H-8), 3.15 (s, -OCH_3), 3.68 (br m, H-1), 5.41 (d, *J* = 15.6 Hz, H-6), 5.53 (d of t, *J* = 15.6, 6.9 Hz, H-5).

(*S*)-(-)-7-Methoxy-3,7-methyl-5-octen-1-al (**4**). To 680 mg (3.70 mmol) of hydroxy ether **3** in 20 mL of CH_2Cl_2 was added 1.2 gm (5.57 mmol) of pyridinium chlorochromate (PCC). The reaction was stirred for 1.5 h and then diluted with 100 mL of ether. After filtration of the mixture through Florisil and removal of the solvent, the crude product was chromatographed (SiO_2, 7% EtOAc-hexane) to give 520 mg (2.8 mmol, 76% yield) of pure aldehyde **4**. TLC: R_f = 0.40; ^{1}H-NMR ($CDCl_3$, 300 MHz), δ 0.98 (d, *J* = 6.6 Hz, C-3 CH_3), 1.26 (s, C-7 CH_3 + H-8), 3.13 (s, -OCH_3), 5.45 D, *J* = 15.9 Hz, H-6), 5.53 (d of t, *J* = 15.9, 6.3 yHz, H-7), 9.76 (t, *J* = 2.1 Hz, H-1).

Isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4,8-dodecatrienoate (**5**). To 337 mg (1.1 mmol) of diisopropyl 3-isopropoxycarbonyl-2-methyl-2-propenyl phosphonate in 3 mL of DMF was added 26 mg (1.1 mmol) of sodium hydride at 0° C. The mixture was stirred at 0° C for 15 min and warmed to ambient temperature for a further 30 min. After cooling the reaction mixture to 0° C, 200 mg (1.1 mmol) of the aldehyde **4** was added and the reaction was stirred for 1.5 h. The reaction was quenched with 5 mL of brine, extracted 3 times with ether, dried ($MgSO_4$), and chromatographed (1% ether-hexane) to give 230 mg (0.75 mmol, 68% yield) of a 3:1 mixture of (2*E*,4*E*):(2*Z*,4*E*) isomers of trienoate **5**. TLC: R_f = 0.65; ^{1}H-NMR ($CDCl_3$, 300 MHz), δ 0.93 (d, *J* = 6.6 Hz, C-7 CH_3), 1.26 (d, *J* = 6.3 Hz, -$OCH(CH_3)_2$,), 1.26 (s, C-H CH_3 + H-12), 1.98 (d, *J* = 1.2 Hz, C-3 CH_3 [2*Z*,4*E*]), 2.27 (d, *J* = 1.2 Hz, C-3 CH_3 [2*E*,4*E*]), 3.15 (s, -OCH3), 5.05 (sept., *J* = 6.3 Hz, -$OCH(CH_3)_2$), 5.40 (d, *J* = 15.9 Hz, H-10), 5.50 (d of t, *J* = 15.9, 6.3 Hz, H-9), 5.67 (d, *J* = 1 Hz, H-2), 6.08 (m, H-4 + H-5); HRMS (70 eV), *m/z* (relative intensity) 308.2352 (4%); calculated for $C_{19}H_{32}O_3$, 308.2351.

Isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4,8-dodecatrienoate-$FeCO_3$ (**6**). To 100 mg (0.32 mmol) of **5** in 25 mL of dry benzene was added 162 mg (0.32 mmol) of triiron

dodecacarbonyl and the reaction refluxed for 7 h at 85° C. Since the R_f values on silica TLC of starting material and product were identical, the reaction was monitored by observing the disappearance of the 1625 cm^{-1} (C=C) IR absorption band from the uncomplexed diene. The solvent was removed and the product was purified by chromatography (elution first with hexane to remove excess triiron dodecacarbonyl and then 6% EtOAc-hexane) to give 106 mg (0.24 mmol, 74% yield) of the iron carbonyl complex **6**. TLC: R_f = 0.59; ^{1}H-NMR ($CDCl_3$, 300 MHz), δ 0.94 (br d, *J* = 6.6 Hz, C-7 CH_3), 1.24 (br s, -OCH(CH_3)$_2$ + C-11 CH_3 + H-12), 2.51 (s, C-3 CH_3), 3.15 (s, -OCH_3), 5.05 (br m, -OCH(CH_3)$_2$ + H-2 + H-4 + H-5), 5.46 (br m, H-9 + H-10); HRMS (70 ev), *m/z* (relative intensity) 448.1557 (1%); calculated for $C_{22}H_{32}O_6Fe$, 448.1548.

Isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate-$FeCO_3$ (**7**). To 15 mg (0.033 mmol) of protected trienoate **6** in 2 mL EtOAc was added 55 mg (0.027 mmol) of 5% Rh/C and the mixture degassed 3 times and flushed with hydrogen gas. The suspension was vigorously stirred under a hydrogen atmosphere for 3 h and then filtered through a silica gel pipette column to give 13 mg (0.029 mmol 88% yield) of the dihydro product **7**. TLC: R_f = 0.62; ^{1}H-NMR ($CDCl_3$, 300 MHz), δ 0.94 (br m, C-7 CH_3), 1.14 (s, C-11 CH_3 + H-12), 1.24 (br s, -OCH(CH_3)$_2$), 2.51 (s, C-3 CH_3), 3.18 (s, -OCH_3), 5.02 (br m, -OCH(CH_3)$_2$ + H-2 + H-4 + H-5); HRMS (70 ev), *m/z* (relative intensity) 450.1708 (1%), calculated for $C_{22}H_{34}O_6Fe$, 450.1705.

Isopropyl (2*E*,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate (**8**) (Methoprene). To 13 mg (0.029 mmol) of protected dienoate **7** in 10 mL CH_3CN at 0° C was added 102 mg (0.202 mmol) of ceric ammonium nitrate. The reaction mixture was stirred at 0° C for 4 h and diluted with 100 mL of 20% EtOAc-hexane. The organics were washed (H_2O), concentrated and chromatographed (7% EtOAc-hexane) to give 7.5 mg (0.024 mmol, 83% yield) of methoprene **8**. TLC: R_f = 0.60; ^{1}H-NMR ($CDCl_3$, 300 MHz), δ 0.90 (d, *J* = 6.6 Hz, C-7 CH_3), 1.14 (s, C-11 CH_3 + H-12), 1.26 (d, ϑ = 6.2 Hz, -OCH(CH_3)$_2$) 2.28 (d, *J* = 1.2 Hz, C-3 CH_3), 3.15 (s, -OCH_3), 5.04 (sept., *J* = 6.2 Hz, -OCH(CH_3)$_2$), 5.64 (d, *J* = 1 Hz, H-2), 6.06 (m, H-4 + H-5). UV: λ_{max} = 261 nm (ε = 28,600).

Isopropyl [8,9-$^3H_2$]-(2*E*,4*E*)-3,7,11-trimethyl-dodecadienoate-$FeCO_3$ (**7a**). A suspension of 15 mg (0.033 mmol) of protected trienoate **6** and 55 mg of 5% Rh/C in 2 mL of

EtOAc was frozen, thawed, degassed and flushed with nitrogen 3 times followed by addition of carrier-free tritium gas. The suspension was vigorously stirred under the tritium atmosphere for 3 h followed by degassing and filtration through a glass fiber filter to remove the catalyst. Flash chromatography through a pipette column (10% EtOAc-hexane) gave 13 mg (0.029 mmol, 88% yield) of the ditritio product **7a**. The radioactivity coeluted (HPLC, TLC) with an authentic sample of dihydro product **7b**. The radiolabelled iron complex was stored in toluene-heptane (1:5) below -20° C.

Isopropyl [8,9-$^3H_2$]-(2*E*,4*E*)-3,7,11-trimethyl-dodecadienoate (**8a**) ([^{3}H-]Methoprene). To 2.5 mg (0.0056 mmol) of the 8,9-ditritio iron complex **7a** was added 30 mg (0.06 mmol) of ceric ammonium nitrate. The reaction mixture was stirred at 0° C for 4 h and diluted with 5 mL of 20% EtOAc-hexane. The organics were washed (H_2O), concentrated and purified by HPLC (3% EtOAc-hexane) to give 0.95mg (0.0031 mmol) of [^{3}H]-methoprene **8a** (55% yield, >92% pure by HPLC and Bioscan). The total radioactivity was 260 mCi giving a specific activity of 83.9 Ci/mmol; λ_{max} (hexane) = 261 nm (ε = 28,400). The product coeluted with authentic methoprene on HPLC and TLC.

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