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A SHORT RADIOSYNTHESIS OF NATURAL JUVENILE HORMONE III, METHYL [12-^{3}H]-(10*R*)-10,11-EPOXYFARNESOATE

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SUMMARY

A new asymmetric synthesis of natural JH III from methyl farnesoate allows introduction of tritium from sodium borotritide into the 12-methyl group. Selective allylic oxidation followed by Sharpless epoxidation of the (*E*)-allylic alcohol gives an epoxy alcohol (>97% e.e.), which is oxidized to the aldehyde. Sodium borotritide (65 Ci/mmol) reduction of the aldehyde is followed by tosylation, iodide displacement, and cyanoborohydride displacement to give [^{3}H]-(10*R*)-JH III, specific activity 14 Ci/mmol.

Key words: JH III, tritium-labelling, asymmetric synthesis, insect juvenile hormone

INTRODUCTION

JH III is the only naturally occurring insect juvenile hormone outside the order Lepidoptera (1). It has been synthesized in optically active form by resolution of diastereomeric esters of the 10,11-diol (2,3) in order to demonstrate enantioselective interaction with juvenile hormone binding proteins. The radiolabelled natural enantiomer has been prepared bearing a high specific activity tritium label in the ester methyl group by enzymatic methods (for JH I) (4) and by *in vitro* organ culture of cockroach (*Diploptera*) corpora allata (5). Labelled racemic JH III is prepared by reduction of the appropriate 11-chloro-10-oxo precursor (the Zoecon "chloroketone") with sodium borotritide (6); this material can be de-oxygenated to [10-^{3}H]-methyl farnesoate, a known JH-like compound from crustaceans (7). None of these procedures were adequate for the preparation of tens of millicuries of the natural enantiomer of JH III required by us for tritium-NMR studies and for countless experiments by insect biochemists.

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Our goal in the past five years has been the preparation of high specific activity radioligands for studying pheromone and hormone metabolism of insects (8,9). To complete a logical series which includes high specific activity [^{3}H]-(10*R*,11*S*)-JH I and JH II (10), we now report an asymmetric total synthesis of [^{3}H]-(10*R*)-JH III.

RESULTS AND DISCUSSION

The synthetic scheme is shown in Figure 1. Oxidation of farnesol to methyl farnesoate (2) was achieved in 56% overall yield via a two-step procedure using first MnO_2 in hexane to give farnesal, and then further oxidation with MnO_2 in acetic acid-sodium cyanide-methanol (11). Selective allylic oxidation of the terminal (*E*)-methyl group to hydroxyester **3** was accomplished in 36% yield using selenium dioxide and *t*-butyl hydroperoxide in methylene chloride (12). The Sharpless asymmetric epoxidation (13) was conducted at -50 °C using (-)-di-isopropyl tartrate to obtain the correct (10*R*) absolute configuration in the product epoxy alcohol **4**, which was obtained in >97% e.e. as determined by NMR analysis of the (+)-MTPA ester. Oxidation of alcohol **4** with 10 equiv. of chromium trioxide-pyridine complex afforded aldehyde **5** in 60% yield, which was purified to homogeneity by flash chromatography prior to the radiochemical steps.

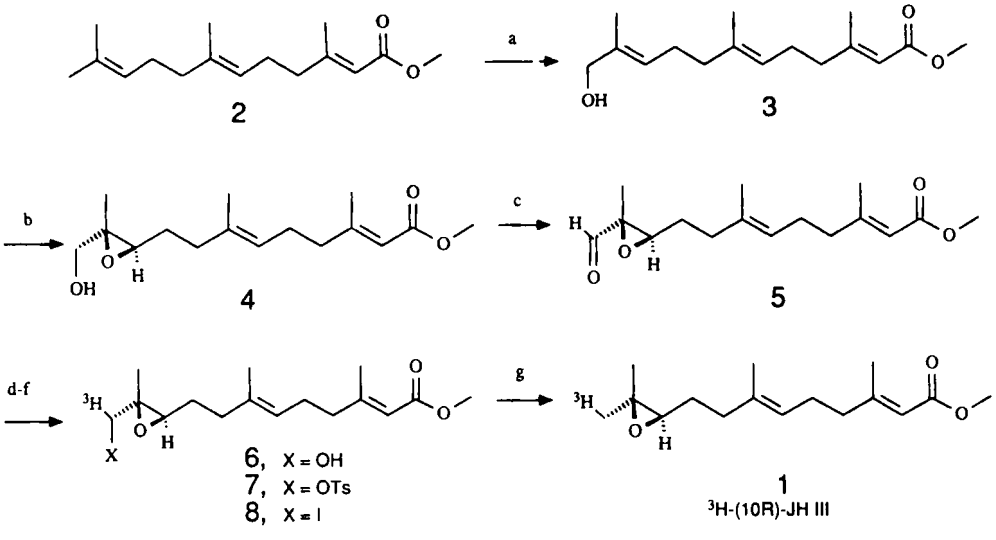

Fig.1 Radiosynthesis of (10*R*)-JH III. Reagents: (a) SeO_2, *t*-BuOOH, CH_2Cl_2; $Ti(Oi\text{-}Pr)_4$, (-)-DET, *t*-BuOOH, CH_2Cl_2; (c) CrO_3-Py, CH_2Cl_2; (d) [^{3}H]-$NaBH_4$, EtOH; (e) TsCl, Py; (f) NaI, acetone; (g) $NaBH_3CN$, HMPA.

Preliminary runs were performed with unlabelled materials, and we determined that the use of labelled $NaCNBH_3$ would be an expensive and inefficient route for incorporation of the tritium label. For the radiosynthesis, we reduced aldehyde **5** with 500 mCi of sodium borotritide (NEN, 65 Ci/mmol) in ethanol for 1 h at 0 °C and quenching with 0.1 M NaH_2PO_4 to prevent ester hydrolysis. Chromatography afforded the labelled epoxy alcohol **6** in >85% radiochemical yield. The alcohol was converted to the tosylate with *p*-toluenesulfonyl chloride in pyridine at 0 °C, and the chromatographed tosylate **7** was converted to the labelled iodide **8** in 80% yield using excess sodium iodide in acetone at room temperature. The epoxy iodide **8** was dissolved in distilled hexamethylphosphoric triamide (HMPA) and stirred 4 days with a 10-fold excess of sodium cyanoborohydride. Flash chromatography afforded analytically pure [^{3}H]-(10*R*)-JH III in 40% overall yield from epoxy alcohol **6**, specific activity 14 Ci/mmol. We and others have employed this material for JH binding protein assays (14), and we have converted some of this material to [^{3}H]-methyl farnesoate for studies of binding and of farnesoic acid metabolism.

EXPERIMENTAL

Methyl (*E,E*)-12-hydroxy-3,7,11-trimethyl-2,6,10-dodecatrienoate (**3**). To 10 mL of CH_2Cl_2 containing 90% *t*-butyl hydroperoxide (120 µL, 0.8 mmol), 24 mg of selenium dioxide (0.22 mmol) was added. The mixture was stirred for 30 min at room temperature in the dark, chilled in an ice bath, and a solution of methyl farnesoate (**2**) (100 mg, 0.39 mmol) in 3 mL of CH_2Cl_2 solution was added slowly. After stirring for 1 - 1.5 h at 0 °C (monitored by TLC), the reaction mixture was poured into ether (20 mL) and the ether layer was washed (H_20), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dissolved in 3 mL of EtOH, $NaBH_4$ (4 mg, 0.1 mmol) was added, and the mixture was stirred for 1 h at 0 °C and the product was extracted with ether. Flash chromatography (5 - 20% ethyl acetate/hexane, EA/H) of the residue yielded 38 mg of unreacted **2**, 25 mg of the desired alcohol **3** (0.09 mmol, 36% yield) and 7 mg of the undesired secondary alcohol. ^{1}H NMR δ 1.60 (s, 3H), 1.65 (s, 3H), 2.16 (s, 3H), 3.68 (s, 3H), 3.97 (s, 2H), 5.08 (bs, 1H), 5.36 (t,1H, J = 6.9 Hz), 5.66 (s, 1H).

Methyl (*E,E*)-(10*R*,11*R*)-10,11-epoxy-12-hydroxy-3,7,11-trimethyl-2,6-dodecadienoate (**4**). To a solution of titanium tetraisopropoxide Ti(O*i*-Pr)$_4$ (0.59 mL, 2 mmol) in 10 mL of dry CH_2Cl_2 was added (-)-di-isopropyl tartrate (490 mg, 2 mmol) at -50 °C. The mixture was stirred for 10 min at that temperature and a 0.7 mL aliquot (0.14 mmol) was transferred into a solution of the alcohol

(3, 35 mg, 0.13 mmol) in 2 mL of dry CH_2Cl_2 at -50 °C. After stirring for 10 min, 0.35 mL of 1 M CH_2Cl_2 solution of dried *t*-butyl hydroperoxide (2.5 mL 90% *t*-BuOOH mixed with 22.5 mL of CH_2Cl_2, dried over $MgSO_4$ for 24 h and kept at -20 °C prior to use) was injected into the flask and the mixture was stirred in a thermostat-controlled -50 °C bath for 15 h. Then, 0.5 mL of 10% tartaric acid was added in and the solution (with flakes of ice) was stirred for 1 h at -23 °C and then for 0.5 h at room temperature before the mixture was diluted with 150 mL of 20% EA/H. The aqueous layer was back-extracted with ether, and the combined organic layers were washed, dried, and flash chromatographed to give 45 mg of crude epoxy alcohol 4 (contaminated with di-isopropyl tartrate). ^1H NMR: δ 1.25 (s, 3H), 1.60 (s, 3H), 2.13 (s, 3H), 3.65 (s, 3H), 4.45 (s, 2H), 5.10 (m, 1H), 5.63 (s, 1H).

The (+)-MTPA ester of epoxy alcohol 4 (1 mg) was prepared in CCl_4 solution with (+)–α-methoxy-α-trifluoromethyl phenylacetyl chloride (0.02 mmol/100 μl) and 3.7 mg (0.04 mmol) of pyridine (20 °C, 16 h). The reaction mixture was loaded directly onto a flash silica column and chromatographed (5% EA/H). ^1H NMR (300 MHz, $CDCl_3$) showed diagnostic peaks for the -CH_2-OMTPA protons as an AB quartet, δ 4.21 ppm (d, J = 11.6 Hz) and δ 4.37 (d, J = 11.6 Hz). Other peaks in the δ 4.1 to 4.5 ppm region can account for less than 2% of the total integrated methylene signal, indicating a minimum of 97% e.e.

Methyl (*E*,*E*)-(10*R*,11*R*)-10,11-epoxy-12-oxo-3,7,11-trimethyl-2,6-dodecadienoate (5). To 0.5 mL of dry CH_2Cl_2 in a round bottom flask was added 100 μL (1.3 mmol) of dry pyridine followed by 110 mg (1.1 mmol) of dry CrO_3. The mixture was stirred at room temperature for 1 h until a burgundy-colored slurry was formed. A solution of tartrate-containing alcohol 4 (19 mg, 0.07 mmol) in 2 mL of CH_2Cl_2 was added and the black mixture was stirred for 1 h. The reaction was diluted with 5 mL of 20% EA/H and the organic layer was transferred to a separatory funnel; the solid residue was dissolved in saturated K_2CO_3 solution and transferred to the same funnel where the aqueous layer was extracted (3 x 50 mL) with 20% E/H and the organic phases were combined, washed (brine), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by pipet flash chromatography (5% EA/H) to yield 11 mg of pure epoxyaldehyde (5, 0.038 mmol, 60% yield), free from tartrate contamination. ^1H NMR: δ 1.40 (s,3H), 1.64 (s, 3H), 1.74 (q, 2H, J = 6.1 Hz), 2.16 (s, 3H), 3.11 (t, 1H, J = 6.1 Hz), 3.67 (s, 3H), 5.15 (m, 1H), 5.66 (s, 1H), 8.84 (s, 1H).

Methyl [12-^{3}H]-(*E*,*E*)-(10*R*,11*R*)-10,11-epoxy-12-hydroxy-3,7,11-trimethyl-2,6-dodecadienoate (**6**). To an ice-cooled ampoule containing NaB^3H_4 (Dupont-NEN, 500 mCi, 65 Ci/mmol, 0.0076 mmol), aldehyde **5** (11 mg, 0.038 mmol, 2-fold excess based on available hydride equivalents) was added as 1 mL ethanolic solution and the mixture was stirred at 0 °C for 1 h before 0.2 mL of 0.1 M NaH_2PO_4 solution was added. The resulting mixture was diluted with ether (2 x 3 mL) and dried over $MgSO_4$. The solvent was then concentrated under a stream of N_2, and the residue was dissolved in hexane and loaded onto a short silica gel pipet column. Unreacted aldehyde was eluted with 5% EA/H (2 mL) and then the epoxy alcohol was eluted with 30% EA/H (4 mL) to give 420 mCi (>85% radiochemical yield) of tritiated epoxy alcohol **6**.

Methyl [12-^{3}H]-(*E*,*E*)-(10*R*,11*R*)-10,11-epoxy-12-tosyloxy-3,7,11-trimethyl-2,6-dodecadienoate (**7**). Following a successful "cold" run, several independent lots of the labelled epoxy alcohol **6** were tosylated. For example, 40 mCi of alcohol **6** was dissolved in 1 mL of dry pyridine treated with 2 mg of tosyl chloride, and stirred at 0 °C for 0.5 h and then at -10 °C for 18 h. The mixture was diluted with water, extracted with ether, worked up as usual, concentrated, and chromatographed on silica gel with 0 to 5% EA/H to obtain purified labelled epoxy tosylate **7**. The radiochemical and chemical purity were assessed by TLC, using EnHance spray and fluorography to verify co-chromatography of mass and radioactivity.

The unlabelled tosylate showed the following spectral properties. IR: 1740 (C=O), 1640 (C=C), 1580, 1540 (aromatic C=C), 1200 (C-O) cm^{-1}; ^{1}H NMR: δ 1.29 (s, 3H), 1.60 (s, 3H), 2.17 (s, 3H), 2.45 (s, 3H), 2.76 (t, 1H, J = 7.4 Hz), 3.68 (s, 3H), 3.92 (s, 2H), 5.08 (bs, 1H), 5.67 (s, 1H), 7.34 (d, 2H, J = 7.7 Hz), 7.82 (d, 2H, J = 7.7 Hz).

Methyl [12-^{3}H]-(*E*,*E*)-(10*R*,11*R*)-10,11-epoxy-12-iodo-3,7,11-trimethyl-2,6-dodecadienoate (**8**). Using the procedure below described for cold material, the labelled tosylate **7** was converted in several lots to the labelled iodide **8**. For example, to a dry acetone solution (10 mL) of pure unlabelled epoxytosylate (5.1 mg, 0.012 mmol) was added 40 mg of NaI (excess) and the resulting mixture was stirred at room temperature for 10 h. Ether (20 mL) was then added and the mixture washed (brine), dried ($MgSO_4$), and concentrated *in vacuo*, and purified by flash chromatography purification (5% EA/H) yielded 4.2 mg of epoxy iodide in 82% yield. For the labelled material, radio-TLC/autoradiography and a Bioscan Imaging Analyzer were employed to demonstrate chemical and radiochemical integrity. ^{1}H-NMR (unlabelled, diagnostic signals) δ 2.85 (t, 1H, J = 6.3 Hz), 3.08 (d, 1H, J = 10 Hz), 3.23 (d, 1H, J = 10 Hz).

Methyl [12-^{3}H]-(*E*,*E*)-(10*R*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate (**1**). Unlabelled (10*R*)-JH III was prepared from the unlabelled iodide as follows. To an HMPA solution (0.5 mL) of the unlabelled iodide (4 mg, 0.01 mmol) in a 3 mL vial, 5 mg of $NaBH_3CN$ (0.08 mmol) was added and the resulting mixture was stirred at room temperature for 100 h before 0.3 mL of 0.1 M NaH_2PO_4 was introduced. The mixture was then extracted with *n*-pentane (5 x 1.5 mL) and the combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified with flash chromatography (0 to 10% EA/H) to give 1.9 mg of JH III (0.0073 mmol, 73% yield). This material has spectral and chromatographic properties identical to the authentic material.

With a similar procedure, the labelled iodide **8** was converted in several lots to the labelled (10*R*)-JH III (**1**) and purified by repeated flash chromatography to remove the close-eluting 12-iodo component. A final specific activity of 14 Ci/mmol was calculated on the basis of injected radioactivity and calibrated HPLC peak height vs. a racemic JH III standard.

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