PREPARATION OF (10R,S)-[10-^{3}H]JUVENILE HORMONE III AND (10R,S,11S,R)-[10-^{3}H]JUVENILE HORMONE 0; CONVERSION OF [10-^{3}H] JUVENILE HORMONE III TO METHYL (2E,6E)-[10-^{3}H]FARNESOATE AND (2E,6E)-[10-^{3}H]FARNESOL

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SUMMARY

Samples of racemic [10-^{3}H]juvenile hormone III (methyl (2E,6E)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate) and [10-^{3}H]juvenile hormone 0 (methyl (2E,6E,10cis)-3,7-diethyl-10,11-epoxy-11-methyl-2,6-tridecadienoate) were prepared by NaB^3H_4 reduction of the corresponding 11-chloro-10-oxo ("chloroketone") precursors to their respective [10-^{3}H]chlorohydrins, followed by treatment of the latter with potassium carbonate in methanol. [10-^{3}H]Juvenile hormone III was converted to methyl [10-^{3}H]farnesoate (methyl (2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrienoate) by reduction with sodium iodide and zinc in acetic acid-sodium acetate. Further reduction of methyl [10-^{3}H]farnesoate with diisobutyl aluminum hydride afforded a sample of [10-^{3}H]farnesol ((2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol).

Key Words: [10-^{3}H]Juvenile Hormone III, [10-^{3}H]Juvenile Hormone 0, Methyl [10-^{3}H]Farnesoate, [10-^{3}H]Farnesol, Insect Juvenile Hormones, HPLC.

INTRODUCTION

The insect juvenile hormones (JHs) play a significant role in the develop-

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mental and reproductive endocrinology of insects (1). Together with the unique, homosesquiterpenoid structures of juvenile hormones JH I (2,3), JH II (3), JH 0 (4), and 4-methyl JH I (5), this has ensured a continued interest in these compounds by chemists and biologists alike. In our ongoing studies of the qualitative and quantitative titer determination of JH(s) in miscellaneous insect species (see 6) and the elucidation of individual enzymatic steps of the biosynthesis of the JHs (e.g. 7), we have relied heavily on the use of radiotracers. This in turn has often required the synthesis of radiolabeled precursors or products due to unavailability of suitable commercial products (e.g. 8,9). Recently, we studied the conversion of farnesol to farnesal and farnesoic acid by alcohol dehydrogenase in homogenates of corpora allata (the organ of JH biosynthesis) from the tobacco hornworm moth, Manduca sexta (10). These studies required a source of radiolabeled farnesol which was obtained by alkaline phosphatase hydrolysis of [1,5,9-^{3}H]farnesyl pyrophosphate (NEN). While adequate, this substrate was not totally satisfactory since the C-1 tritium moiety is lost following enzymatic oxidation and complicates analysis of reaction products. Furthermore, the [^{3}H]farnesyl pyrophosphate precursor is known to be unstable and has recently been discontinued by its sole supplier. Thus the need for an alternative source of radiolabeled farnesol is apparent. Cornforth et al. (11) had reported the one-stage reduction of di- and trisubstituted epoxides to olefins via the iodohydrins as intermediates. A similar reduction of the 10,11-epoxide of JH III should afford methyl (2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrienoate (methyl farnesoate), which in turn could be reduced to farnesol.

We report here on the preparation of radiolabeled JH III and its subsequent conversion to methyl (2E,6E)-farnesoate and (2E,6E)-farnesol. In addition, we have prepared samples of radiolabeled 10trans (unnatural) and 10cis (natural) JH 0.

Methods

Normal phase liquid chromatography (LC) was conducted using a modular liquid chromatograph consisting of a Haskel pneumatic amplifier pump, and a

Valco loop injector. Detection was at 254 nm with either a Chromatronix model 230 (semipreparative LC) or Spectra-Physics 8200 unit (analytical LC). Normal phase columns used were a 25 x 0.78 cm LiChrosorb SI-60 (semipreparative, 10 μm, slurry packed in these labs) or a 22 x 0.46 cm Zorbax Sil (analytical, DuPont). Several solvent systems were used and are described in the text where appropriate; all normal phase solvents were 50% saturated with water. Analytical reversed-phase LC was conducted using a 13 x 0.46 cm Spheri-5 RP-8 column (Brownlee, MPLC cartridge, precolumn 3 cm, column 10 cm) a Spectra-Physics Model 8700 solvent delivery system and an LDC UV Monitor III (214 nm). Solvent compositions are described in the text. Mass of product for determination of specific activity was determined by UV absorbance (on LC) relative to UV absorbance of known levels of injected unlabeled standards. The level of radioactivity was determined by collecting the whole of the zone corresponding to radiolabeled product from LC, for liquid scintillation counting (LSC) in a Packard 2425 or 4430 spectrometer. Detection of radiolabel on TLC plates was via a Packard Model 7201 Radiochromatogram Scanner.

EXPERIMENTAL

Materials

A sample of the JH 0 precursor (6, methyl (2E,6E)-11-chloro-3,7-diethyl-11-methyl-10-oxo-2,6-tridecadienoate) was obtained from a batch prepared by Anderson et al. (12); the JH III precursor (1, methyl (2E,6E)-11-chloro-10-oxo-3,7,11-trimethyl-2,6-dodecadienoate) was prepared by Dr. R. J. Anderson using the same method, from a Claisen reaction of methyl (2E)-6-hydroxy-3-methyl-7-methylene-2-octenoate with 2,2-dimethyoxy-3-chloro-3-methylbutane (13). Sodium [^{3}H]borohydride (NaB^3H_4, >200 mCi; specific activity 4.5 Ci/mmol) was obtained from Amersham Corporation. Diisobutyl aluminum hydride (DIBAH) was from Texas Alkyls Inc.; other chemicals were reagent grade. Diethyl ether (Mallinckrodt) was distilled before use. Thin-layer chromatography (TLC) plates were obtained from Analtech. Silica Sep-Pak cartridges (Waters Associates) were prewashed with ether followed by pentane.

Synthesis of racemic [10-^{3}H]JH III (3, see Fig. 1)

Six milligrams (20 μmol) of JH III precursor (1) was dissolved in 50 μl of

a mixture comprised of 9 volumes of methanol and 1 volume 0.1M potassium phosphate, pH 7.0. The latter solution was injected through a serum stopper, provided with a venting needle, into an ampoule (chilled on ice) containing an excess of NaB^3H_4. The reaction was allowed to proceed at 0°C following vigorous agitation. After 2.5 h, conversion to the chlorohydrin intermediate (2) was complete (monitored by TLC on 250 μm silica GF, hexane:Et_2O, 7:3 v/v and radiochromatogram scanning). Reactions were diluted with 100 μl 0.2M sodium acetate, pH 4.0, and extracted with ether. The ether extract was dried, concentrated, and purified by semipreparative normal phase LC using 15% ether in pentane. The desired product (k' 3.5) was collected and the solvent removed.

Chlorohydrin 2 was dissolved in 300 μl of methanol and ~5 mg of anhydrous K_2CO_3 was added with stirring (1 h at room temperature). Brine was added and the desired product (racemic [10-^{3}H]JH III, 3) was extracted with pentane containing 10% ether, then dried over anhydrous $MgSO_4$. The [10-^{3}H]JH III was purified by passage through a silica Sep-Pak, which was eluted with pentane:ether (2:1, v/v). The concentrated eluate was purified further by semipreparative normal phase liquid chromatography using 6% ether in pentane. The yield was 2.84 mg (20.7 μmol, ~54%) and 15.2 mCi; therefore, specific activity was 1.4 Ci/mmol.

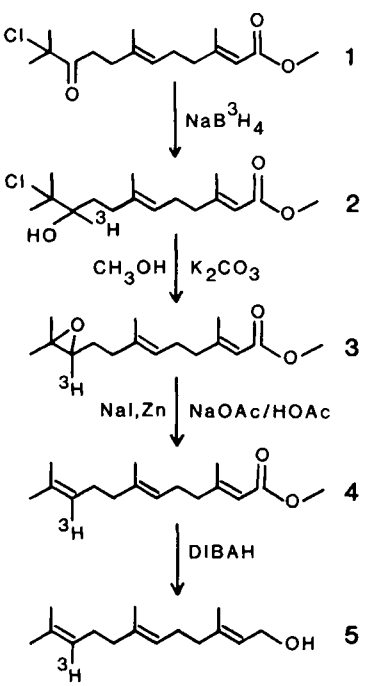

Figure 1. Scheme for the preparation of [10-^{3}H]JH III (3) and its subsequent conversion to methyl [10-^{3}H]farnesoate (4) and [10-^{3}H]farnesol (5).

Conversion of [10-^{3}H]JH III to [10-^{3}H]Methyl Farnesoate (4, Fig. 1)

Zinc dust (7.5 mg, 115 μmol) was added to a stirred solution of sodium iodide (4.5 mg, 30 μmol) and sodium acetate (0.75 mg, 9.2 μmol) in 20 μl of glacial acetic acid in a 1 ml Microflex vial (Kontes). The vial was fitted with a Microflex valve-cap assembly and chilled on an ice bath. Ten millicuries (1.9 mg, 7.1 μmol) of [10-^{3}H]JH III (3) dissolved in 15 μl of THF was injected slowly into the stirred solution. Small aliquots were removed for TLC analysis and radiochromatogram scanning to monitor the progress of the reaction. After 4 h, an additional 1.1 mg of sodium iodide and 0.2 mg of sodium acetate in 5 μl HOAc was injected into the vial. The reaction was allowed to proceed at ~0°C for a total of 21 h at which time methyl farnesoate (4) appeared to be the major radiolabeled product with lesser amounts of starting material and a polar unknown (by TLC). After dilution with brine, the reaction mixture was extracted with ether. Ether extracts were washed with $KHCO_3$/brine and then dried ($MgSO_4$). Following removal of ether, the sample was redissolved in pentane and applied to a silica Sep-Pak. The latter was eluted with 5 ml of pentane, 5 ml of 1% ether in pentane, and finally 10 ml of 2% ether in pentane. Most of the desired product was present in the combined 1% and 2% ether eluents and was essentially radiochemically pure (TLC). The methyl [10-^{3}H]farnesoate was further purified by preparative LC using 1% ether in pentane (k' 4). Radiochemical purity of the sample was investigated by analytical normal phase LC of an aliquot using 1% ether in pentane and liquid scintillation counting of collected 1 min fractions. The sample was >99% methyl (2E,6E)-[10-^{3}H]farnesoate (k' 6.1) and contained less than 0.03% of the corresponding labeled cis isomer (k' 4.3). Specific activity was determined by reversed-phase LC using 60% acetonitrile; we obtained a value of 1.7 Ci/mmol. Yield was about 4 mCi (40%, 1.9 μmol). By reversed-phase LC analysis the radiochemical purity of the product was ~98%.

Conversion of Methyl [10-^{3}H]Farnesoate to [10-^{3}H]Farnesol (5, Fig. 1)

One mCi (~0.5 μmol) of methyl [10-^{3}H]farnesoate (4) in 10 μl of hexane was injected slowly with a syringe into 10 μl of an ice cold 1M solution of DIBAH in hexane (in a 1 ml Microflex vial with Microflex valve cap and under N_2). The

contents of the vial were vortexed frequently during the addition; the syringe was washed 3X with 10 µl hexane and the washings were also injected into the vial. After 20 min, the reaction was ended by addition of hexane and water; the hexane layer was removed and the aqueous layer further extracted with ether. [An aliquot of the combined solvent extracts was removed for TLC (hexane:ether, 50:50 v/v) followed by radiochromatogram scanning, which showed that farnesol (5) was the major product along with substantial amounts of unreacted starting material and a polar unknown product.] The crude product was dissolved in pentane/ether (98:2 v/v) and applied to a silica Sep-Pak. The bulk of the unreacted methyl farnesoate was removed by elution with 6 ml of 2% ether in pentane and saved; [10-^{3}H]farnesol and polar unknowns were eluted with ether. The ether eluent was purified by semipreparative normal phase LC using 8% ether in pentane. Under these conditions the k' for farnesol was ~17. The fractions containing [10-^{3}H]farnesol were pooled, evaporated, and redissolved in hexane:toluene (1:1, v/v). Analysis of an aliquot by normal phase LC using 12% ether in pentane and collection of 1 min fractions for LSC showed the radiochemical purity to be ~94% (2E,6E)-[10-^{3}H]farnesol and ~2.5% (2Z,6E)-[10-^{3}H]farnesol. Specific activity of the product (1.4 Ci/mmol) was determined by reversed-phase LC using 55% CH_3CN as solvent. Yield of product was ~322 µCi (~30%, 0.15 µmol).

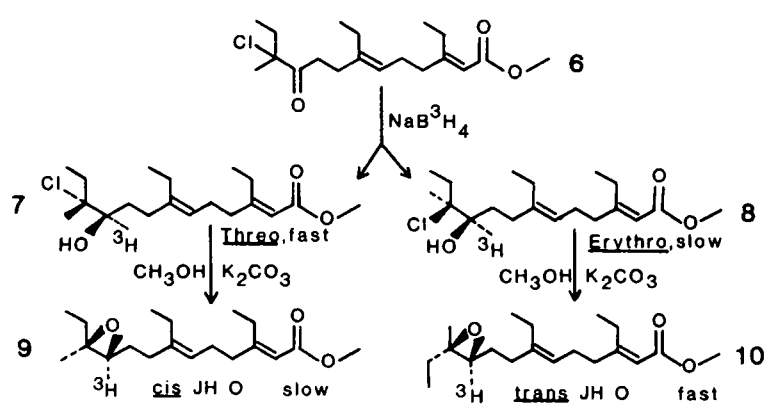

Figure 2. Scheme for the preparation of racemic 10,11cis and trans-[10-^{3}H]JH 0 (9,10).

Synthesis of racemic 10,11cis and trans-[10-^{3}H]JH 0 (9,10, Fig. 2)

Methodology was similar to that described for synthesis of [10-^{3}H]JH III except that a smaller quantity (0.7 mg of JH 0 precursor, 2 μmol, dissolved in 25 μl of methanol/phosphate buffer) was used. The diastereomeric chlorohydrin products (7,8) were extracted with ether and subjected to TLC on two 500 μm thick 5 x 20 cm silica GF plates (hexane:ether, 7:3, v/v). The barely resolved upper (7, threo) and lower (8, erythro) isomers were scraped from the plate as one zone, eluted with acetonitrile, and filtered through a cotton plug. The threo (k' 4) and erythro (k' 6.7) diastereomers were separated by normal phase semipreparative LC; solvent was 10% ether in pentane.

Conversion of the threo chlorohydrin to cis-JH 0 (9) and the erythro chlorohydrin to trans-JH 0 (10) was effected by stirring with powdered anhydrous K_2CO_3 in 100 μl of methanol for 1 h. After dilution with brine, and solvent extraction, the products were dried ($MgSO_4$). Polar impurities were removed by passage of the samples through a silica Sep-Pak (solvent used to elute the desired products was pentane:ether, 2:1, v/v). The products were finally purified by normal phase semipreparative LC (5% ether in pentane). K' values for the 10,11cis and trans racemates were 5.0 and 4.75 respectively. Specific activity was found to be 1.4 Ci/mmol for each isomer. Yields were 230 μCi (0.16 μmol) of 10,11cis isomer, and 204 μCi (0.15 μmol) of 10,11trans isomer.

RESULTS AND DISCUSSION

Three of the natural juvenile hormones (JH I, II, and III) are commercially available (NEN) labeled with tritium at C-10; these were prepared by the same general method as described here, but using NaB^3H_4 of higher specific activity for reduction (D. Ahern and D. A. Schooley, unpublished). However, JH 0 is not available from commercial sources in labeled (or nonlabeled) form. Because of our need for a large quantity of [^{3}H]JH III, along with some [^{3}H]JH 0, we chose to radiosynthesize JH III, rather than to purchase it. The chloroketone precursors for radiosynthesis of JH 0 and JH III were purified by semipreparative normal phase LC to minimize the need for radiochemical purification. The reduction with NaB^3H_4 to the chlorohydrins proceeds

smoothly. However, both unlabeled and labeled preparations of borohydride are frequently contaminated with base, which may convert the chlorohydrins directly to the epoxide in the reduction step. This is not a liability in the synthesis of [10-^{3}H]JH III, but it is very undesirable in synthesis of [10-^{3}H]JH 0. Because JH 0 and its chlorohydrin precursors contain asymmetric carbon atoms at both C-10 and C-11, four isomeric chlorohydrins result from reduction of the racemic chloroketone. The threo and erythro chlorohydrins (each a racemate) separate slightly on TLC or readily on LC. However, the racemic cis and trans epoxides formed upon cyclization of the isomeric chlorohydrins are separable only with difficulty on LC, and are quite inseparable on TLC.

Accordingly, we used 90% methanol containing 0.01M phosphate buffer, pH 7, for the borohydride reduction. This precaution resulted in formation of the chlorohydrins uncontaminated by the epoxides.

While the procedure of Cornforth et al. (11) for deoxygenation of epoxides is a well known reaction, we were pleasantly surprised that it works well with JH III. JH III is very sensitive to rearrangement under acidic conditions. In fact, van Tamelen's group (14) studied the cyclization of racemic methyl epoxy-farnesoate [(10R,S)JH III] to mixtures of monocyclic and bicyclic sesquiter-penoids, catalyzed by 85% phosphoric acid or by boron trifluoride etherate in benzene. These studies were carried out before this material was isolated as an authentic juvenile hormone of insects (15). It is of interest that we could not detect appreciable amounts of such cyclization reactions in glacial acetic acid, the solvent for the Cornforth deoxygenation, although some unknown products were formed.

Cornforth et al. (11) have shown that although formation of an intermdiate iodohydrin appears to be stereoselective, the reduction of the iodohydrin with zinc is almost completely non-stereoselective, in the case of disubstituted olefins. Thus, we have not attempted to apply this deoxygenation reaction to labeled JH 0 (or to commercially available JH I or JH II), as formation of C-10,11 isomeric mixtures of olefins is expected.

All radiolabeled products were found to co-chromatograph with corresponding unlabeled standards via normal phase and/or reversed-phase LC. Analysis by gas

chromatography/mass spectrometry further confirmed that the radiolabeled products were identical with authentic standards. Portions of the samples of [10-^{3}H]JH III and 10,11cis-[10-^{3}H]JH 0 were converted to the corresponding 11-methoxy-d_3-10-hydroxy (MH) derivatives (6). We utilize these routinely as LC tracers to ensure that liquid chromatographic conditions used during our JH titer determination studies result in satisfactory resolution of JH 0 and JH III d_3 MHs from the appropriate phenylurea marker compounds (6). In this way we avoid having to use UV absorption detection of synthetic, unlabeled standards at relatively high levels of mass, which can contribute to considerable contamination of samples (see comments in 16).

Neither farnesol nor methyl farnesoate are commercially available in isotopically labeled form. There is currently much interest in juvenile hormone biosynthesis and its control, e.g. by farnesol and farnesoic acid (17). Additionally, methyl farnesoate may play a hormonal role in insects (18) and other invertebrates (19, and Laufer et al., in preparation). We have recently utilized [10-^{3}H]farnesol in studies on farnesol dehydrogenase in Manduca sexta corpora allata (F. C. Baker et al., unpublished results). Both radiolabeled farnesol and methyl farnesoate have been used as internal standards during development of an isotope dilution method for quantification of these compounds in biological samples (Baker et al., in preparation). Radiolabeled methyl farnesoate has also been invaluable for monitoring the stability of this compound during various analytical chemical manipulations. We have observed that nanogram levels of methyl [10-^{3}H]farnesoate undergo considerable decomposition when applied to silica TLC plates if the latter are not developed immediately. One of the products co-migrates with JH III, an observation also reported by Dr. G. E. Pratt and co-workers (at the Second International CNRS Symposium on Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, Strasbourg, France, September 1983). We have also found that methyl farnesoate can be converted (0.1% or less) to JH III during processing of biological samples for JH titer determinations (19).

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