SYNTHESES OF HOMOLOGOUS, TRITIUM-LABELLED PHOTOAFFINITY ANALOGS OF THE NATURAL JUVENILE HORMONES JH I, JH II, AND JH III

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SUMMARY

Enantiomerically enriched (>92% e.e.) tritium-labelled diazoacetate photoaffinity analogs EBDA and EHDA (each 58 Ci/mmol) and EFDA (14 Ci/mmol) were prepared from the corresponding tritiated juvenile hormone (JH) homologs JH I, JH II, and JH III in two steps. Selective reduction of the ester group and subsequent acylation gave the target compounds.

Key words: Insect juvenile hormone, binding protein, photoaffinity label, diazoacetate, selective reduction, tritium, natural enantiomer

INTRODUCTION

Insect juvenile hormones (JH I, JH II, and JH III) are homologous sesquiterpenoids containing an epoxide ring with one or two stereogenic centers (Figure 1). The different biological properties of the enantiomers have been demonstrated. For example, binding studies as well as studies of the biological activities of the natural (10*R*) and "unnatural" (10*S*) isomers of JH III indicate that the (10*R*) enantiomer displays a high degree of stereoselectivity for both hemolymph binding proteins and receptors at the target site (1,2). Similarly, natural (10*R*,11*S*)-JH I and JH II enantiomers show different binding affinities (3) and esterase-catalyzed hydrolysis rates (4) with a variety of lepidopteran proteins when compared with the unnatural (10*S*,11*R*) enantiomers.

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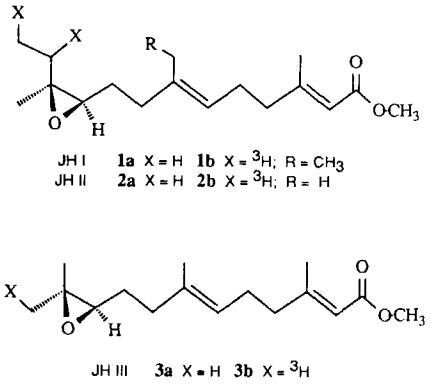


Figure 1. Structures of insect hormones JH I, JH II, and JH III.

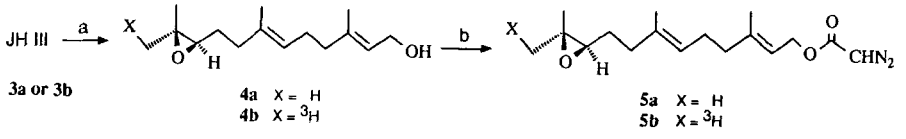
We anticipated that the homologous series of photoaffinity labels would also show different binding affinities. Thus, we prepared [3H]-EBDA (an analog of JH I), [3H]-EHDA (an analog of JH II), and [3H]-EFDA (an analog of JH III) (5,6,7,8) by a variety of synthetic schemes. Photoaffinity labelling of extracellular and cellular JH binding proteins has been reported briefly for the higher homologs [3H]-EHDA and [3H]-EBDA (7), and the first characterization of a nuclear JH receptor employs these same two ligands (9). We now report the experimental details for the syntheses of three enantiomerically enriched photoaffinity labels from the three high specific activity, naturally-occurring, JH homologs.

RESULTS AND DISCUSSION

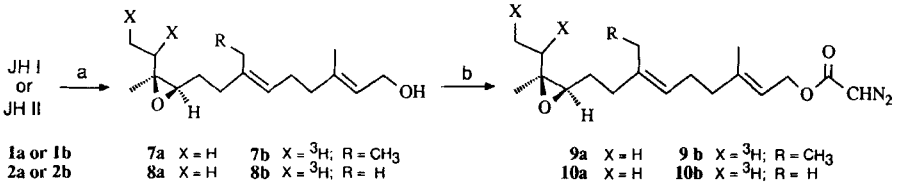
Ready access to all three JHs (**1b**, **2b**, and **3b**) in high enantiomeric purity (>92% e.e.) and high specific activity (10,11) enabled us to use them as starting materials for the syntheses of their diazoacetate analogs **5b**, **9b**, and **10b** *via* their alcohol derivatives. (10*R*)-10,11-Epoxyfarnesol (**4a**) has been previously obtained from L-glutamic acid (12) or by selective fungal metabolism of racemic epoxyfarnesol (13). Earlier, racemic JH II had been converted into its farnesol derivative by reduction of the ester group by $LiAlH_4$, although no experimental details were reported for this procedure (14).

We synthesized diazoacetates **5a**, **9a**, and **10a** and their tritium-labelled derivatives **5b**, **9b**, and **10b** by a newly optimized reduction - acylation procedure as illustrated in Schemes 1 and 2. When JH III (**3a**) was reduced with three molar equivalents of $LiAlH_4$ at 0-5 °C, epoxyfarnesol **4a** was obtained in 60% yield. Reduction of JH II (**2a**) with 2.2 equivalents of

di-isobutylaluminum hydride (DIBAL-H) in hexane at -20 °C gave the corresponding alcohol **8a** in 58% yield. Some starting ester could also be recovered. An analogous reduction of JH I (**1a**) with four equivalents of DIBAL-H resulted in an improved yield (75%) of alcohol **7a**. The alcohols were then acylated with glyoxylic acid chloride tosylhydrazone (**6**) and converted into diazoacetates **5a**, **9a**, **10a** using N,N-dimethylaniline followed by triethylamine as reported by Corey and Myers (15). The repetition of the procedures using labelled starting materials gave the tritium-labelled diazoacetates **5b**, **9b**, and **10b**.



Scheme 1. Synthesis of (10*R*)-EFDA (**5**) from (10*R*)-JH III (**3**). Reagents and conditions: (a) $LiAlH_4$, ether, 0-5 °C; (b) acid chloride **6**, N,N-dimethylaniline, CH_2Cl_2, 0-5 °C; then Et_3N, room temperature.



Scheme 2. Synthesis of (10*R*,11*S*)-EBDA (**9**) and (10*R*,11*S*)-EHDA (**10**) from (10*R*,11*S*)-JH I (**1**) and (10*R*,11*S*)-JH II (**2**).Reagents and conditions: (a) DIBAL-H, hexane, -20 °C; (b) acid chloride **6**, N,N-dimethylaniline, CH_2Cl_2, 0-5 °C, then Et_3N, room temperature.

MATERIALS AND METHODS

All reactions were performed under dry N_2. [^{3}H]-Sodium borohydride (64.5 Ci/mmol), used for the preparation of [12-^{3}H]-JH III (specific activity: 15 Ci/mmol), was purchased from Amersham. Tritiations of the unsaturated JH I and JH II precursors were carried out at the National Tritium Labeling Facility, Berkeley, CA, using carrier-free tritium gas giving enantiomerically enriched (>92% e.e.) [12,13-$^3H_2$]-(10*R*,11*S*)-JH I and [12,13-$^3H_2$]-(10*R*,11*S*)-JH II (specific activities for each were 58 Ci/mmol) as described earlier (10).

CH_2Cl_2 was distilled over CaH_2. Diethyl ether and benzene were distilled over sodium benzophenone ketyl. Hexane was distilled and dried over sodium. Flash chromatography purifications were performed on Woelm Silica (32-63 μm) in disposable Pasteur pipets.

^{1}H-NMR spectra were determined on a General Electric QE-300 spectrometer. IR spectra were recorded on a Perkin Elmer 1600 Series FTIR spectrometer. UV spectra were measured in hexane on a Perkin Elmer Lambda 5 UV/VIS spectrophotometer. The HPLC analyses and preparative separations were carried out on a Waters Associates chromatograph, fitted with a Kratos-Schoeffel SF 770 Spectroflow detector set to 245 nm, using a Fisher Resolvex Sil (4.6 mm x 25 cm) column. The eluent used was 10% EtOAc in hexane. The radiochemical homogeneity of the labelled compounds was assessed by TLC using EN^{3}HANCE^{TM} spray and fluorography using Kodak XAR-5 film. Radioactive samples were counted in an LKB 1218 RackBeta liquid scintillation counter using ScintiVerse II (Fisher Scientific) scintillation cocktail. The tritiated compounds were stored in toluene-heptane solution below -15 °C in sealed ampoules.

(10*R*) (2*E*,4*E*)-(10,11)-Epoxyfarnesol (**4a**). To an ice-cooled solution of 26.0 mg (0.097 mmol) of (10*R*)-JH III (**3a**) (11) in 4 ml of dry ether was added 11.2 mg (0.294 mmol) of $LiAlH_4$. The suspension was stirred at 0-5 °C for 30 min and at room temperature for an additional 30 min. The reaction mixture was then cooled to 0 °C, and 10 μl of water, 10 μl of 20% aq. NaOH solution, and 20 μl of water were added subsequently. After stirring for 10 min, 3 ml of ether and $MgSO_4$ were added to the reaction mixture which was filtered and concentrated. The residue was purified by flash chromatography (15% EtOAc in hexane) to give 14.0 mg (60% yield) of pure **4a** IR (film): 3400, 1620, 1200 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.26 (3H, s), 1.30 (3H, s), 1.62 (3H, s), 1.67 (3H, s), 2.0-2.2 (8H, m), 2.70 (1H, t, J = 7.2 Hz), 4.14 (2H, d, J = 5.8 Hz), 5.16 (1H, t, J = 7 Hz), 5.40 (1H, t, J = 7.0 Hz).

(10*R*)-10,11-Epoxyfarnesyl diazoacetate (**5a**). To an ice-cold solution of 13.0 mg (0.054 mmol) of alcohol **4a**, 21.2 mg (0.081 mmol) of acid chloride derivative **6** (16) in 3.4 ml of dry CH_2Cl_2 was added 11 μl (0.086 mmol) of N,N-dimethylaniline. The solution was stirred at 0-5 °C for 30 min, and then 23 μl (0.165 mmol) of triethylamine was introduced and the solution was stirred at 0-5 °C for 30 min and at room temperature for 1 h. Then the reaction mixture was diluted with 10 ml of 10% EtOAc in hexane, washed with 10% aq. citric acid

solution and brine, dried ($MgSO_4$), and concentrated. Purification by flash chromatography (10% EtOAc in hexane) gave 11 mg (66% yield) of diazoacetate **5a** as a pale yellow oil. IR (neat) 2100, 1685, 1240, 1190 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.26 (3H, s), 1.30 (3H, s), 1.62 (3H, s), 1.71 (3H, s), 2.0-2.2 (8H, m), 2.70 (1H, t, J = 6.2 Hz), 4.68 (2H, d, J = 7.2 Hz), 4.76 (1H, s), 5.15 (1H, t, J = 6.2 Hz), 5.34 (1H, t, J = 6.6 Hz).

(2*E*,4*E*)-(10*R*,11*S*)-10,11-Epoxy-3,7,11-trimethyl-2,6-tridecadien-1-ol (8a). To a solution of 5.1 mg (0.018 mmol) of JH II **2a** in 5 ml of dry hexane was added 40 µl (0.040 mmol) of DIBAL-H at -20 °C. The reaction mixture was stirred at this temperature for 1 h then at 0 °C for 30 min, then quenched with 150 µl of methanol and 1 ml of water. The phases were separated, the aqueous phase extracted with hexane (2 x 2 ml); the organic extracts were combined, washed with brine, dried ($MgSO_4$), concentrated and purified by flash chromatography (15% EtOAc in hexane) to give 2.6 mg (58% yield) of pure product **8a**. IR (film) 3400, 1620, 1200 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.99 (3H, t, J = 6.8 Hz), 1.29 (3H, s), 1.61 (3H, s), 1.66 (3H, s), 2.70 (1H, t, J = 6.5 Hz), 4.12 (2H, d, J = 7.2 Hz), 5.10 (1H, m), 5.32 (1H, t, J = 6.5 Hz).

(2*E*,4*E*)-(10*R*,11*S*)-10,11-Epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadien-1-ol (7a). This compound was prepared in a similar manner as described for alcohol **8a** from 4.5 mg (0.015 mmol) of JH I (**1a**) using 0.060 mmol of DIBAL-H at -20 °C in 75% yield. IR (film): 3400, 1640, 1240 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.97 (3H, t, J = 7.5 Hz), 0.99 (3H, t, J = 6.8 Hz), 1.29 (3H, s), 1.66 (3H, s), 2.69 (1H, t, J = 6.5 Hz), 4.12 (2H, d, J = 7.2 Hz), 5.15 (1H, m), 5.36 (1H, t, J = 6.5 Hz).

(2*E*,4*E*)-(10*R*,11*S*)-10,11-Epoxy-3,7,11-trimethyl-2,6-tridecadienyl diazoacetate (10a). A similar procedure as described for **5a** afforded 1.9 mg (65% yield) of diazoacetate **10a** from 3.1 mg (0.011 mmol) of alcohol **8a**. IR (film) 3120, 2100, 1700, 1230 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.99 (3H, t, J = 7.0 Hz), 1.29 (3H, s), 1.60 (3H, s), 1.67 (3H, s), 2.69 (1H, t, J = 6.3 Hz), 4.70 (2H, d, J = 7.2 Hz), 5.13 (1H, m)., 5.33 (1H, t, J = 6.5 Hz). UV (hexane): 244 nm; ε = 14,900.

(2*E*,4*E*)-(10*R*,11*S*)-10,11-Epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienyl diazoacetate (9a). A similar procedure as described for **5a** afforded 2.1 mg (70% yield) of

diazoacetate **9a** from 2.9 mg (0.01 mmol) of alcohol **7a**. IR (film) 3120, 2100, 1690, 1240 cm-1. 1H-NMR ($CDCl_3$): δ 0.97 (3H, t, *J* = 7.5 Hz), 1.02 (3H, t, *J* = 6.3 Hz), 1.30 (3H, s), 1.69 (3H, s), 2.69 (1H, t, *J* = 6.2 Hz), 4.71 (2H, d, *J* = 7.0 Hz), 5.15 (1H, m), 5.34 (1H, t, *J* = 6.7 Hz).

[12-^{3}H]-(10*R*)-10,11-Epoxyfarnesol (4b). To an ice-cold solution of 57 mCi (3.8 μmol) of [12-^{3}H]-JH III (**3b**) in 100 μl of dry ether was added 60 μl (12 μmol) of a 0.2 M ethereal $LiAlH_4$ solution, prepared by diluting the commercially available (Aldrich) 1.0 M solution. After stirring the reaction mixture at 0-5 °C for 1 h, several drops of 20% aq. NaOH solution and water were added, the mixture was diluted with hexane (3 ml) followed by the introduction of $MgSO_4$. After stirring for 15 min, the solvent was evaporated, and the residue was loaded onto a pipet column for chromatography. Elution (10-20% EtOAc in hexane) gave 24.4 mCi of alcohol **4b** (43% radiochemical yield).

[12,13-$^3H_2$]-(2*E*,4*E*)-(10*R*,11*S*)-10,11-Epoxy-3,7,11-trimethyl-2,6-tridecadien-1-ol (8b) To a solution of 40.0 mCi (0.69 μmol) of [12,13-$^3H_2$]-JH II (**2b**) in 250 μl dry hexane at -20 °C (dry ice - CCl_4 cooling bath) was added 20 μl (2.0 μmol) of a 0.1M DIBAL-H solution in hexane, prepared by tenfold-dilution of a 1.0M DIBAL-H hexane solution (Aldrich). After stirring at -20 °C for 1 h then at 0 °C for 30 min, the reaction mixture was quenched by 10 μl of methanol and diluted with 2 ml of hexane. Small amounts of Celite and $MgSO_4$ were added to the mixture and, after stirring for 20 min, the solvent was evaporated with a slow stream of dry nitrogen. The residue was then loaded onto a pipet column for purification by flash chromatography (5-20% EtOAc in hexane). The radiochemical yield of alcohol **8b** was 25.0 mCi (62%). (About 4 mCi of the starting ester **2b** was also recovered.)

[12,13-$^3H_2$]-(2*E*,4*E*)-(10*R*,11*S*)-10,11-Epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadien-1-ol (7b). This compound was prepared from 58.0 mCi (1.0 μmol) of [12,13-$^3H_2$]-JH I (**1b**) in 250 μl at -20 °C 40 μl (4.0 μmol) of a 0.1M DIBAL-H solution in hexane. The radiochemical yield of alcohol **7b** was 41.7 mCi (72%).

[12-^{3}H]-(10*R*)-10,11-Epoxyfarnesyl diazoacetate (5b). To an ice-cold solution of 24 mCi (1.6 μmol) of alcohol **4b** in 100 μl of dry CH_2Cl_2 was added 35 μl (3.5 μmol) of 0.1 M acid chloride **6** solution in dry CH_2Cl_2 followed by the introduction of 35 μl (3.5 μmol) of

0.1 M N,N dimethylaniline in dry CH_2Cl_2. After stirring the reaction mixture for 30 min at 0 °C, 70 µl (7.0 µmol) of 0.1 M Et_3N in dry CH_2Cl_2 was added and the stirring was continued at 0 °C for 30 min then at room temperature for 45 min. The reaction mixture was diluted with 3 ml of hexane, 0.5 ml water and a few drops of a 10% aq. citric acid solution, the phases were separated, the aqueous layer extracted with hexane (1 ml), the organic extracts were combined, washed with brine, dried ($MgSO_4$), concentrated using a slow stream of dry nitrogen. The residue was purified by flash chromatography (5-15% EtOAc in hexane) to give 12.4 mCi (52% radiochemical yield) of pure EFDA **5b** which was further purified by HPLC before bioassay.

[12,13-$^3H_2$]-(2*E*,6*E*)-(10*R*,11*S*)-10,11-Epoxy-3,7,11-trimethyl-2,6-tridecadienyl diazoacetate (10b). This compound was prepared from 12.5 mCi (0.21 µmol) of alcohol **8b**, 0.35 µmol of acid chloride **6**, 0.35 µmol of N,N-dimethylaniline, and 1.0 µmol of Et_3N as described above for EFDA (**5b**). The radiochemical yield was 7.2 mCi (58%). The product was further purified by HPLC.

[12,13-$^3H_2$]-(2*E*,6*E*)-(10*R*,11*S*)-10,11-Epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienyl diazoacetate (9b). This compound was prepared from 8.5 mCi (0.15 µmol) of alcohol **7b**, 0.25 µmol of acid chloride **6**, 0.25 µmol of N,N-dimethylaniline, and 1.0 µmol of Et_3N as described above for EFDA **5b**. The radiochemical yield was 5.6 mCi (65%).

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REFERENCES

1. Kindle H., Winistorfer M., Lanzrein B. and Mori K. -- *Experientia* **45**:356 (1989)

2. Schooley D.A., Bergot B.J., Goodman W. and Gilbert L.I. -- *Biochem. Biophys. Res. Commun.* **81**: 743 (1978)

3. Prestwich G.D., Robles S, Wawrzenczyk C. and Bühler A. -- *Insect Biochem.* **17**:551 (1987)

4. Abdel-Aal Y.A.I., Hanzlik T.N., Hammock B.D., Harshman L. and Prestwich G.D. -- *Comp. Biochem. Physiol.* **90B**:117 (1988)

5. Eng W.-s., Ph.D. Dissertation, State University of New York at Stony Brook, Stony Brook, New York (1987)

6. Prestwich G.D., Singh A.K., Carvalho J.F., Koeppe J.K., Kovalick G.E. and Chang E.S. -- *Tetrahedron* **40**:529 (1984)

7. Prestwich G.D., Boehm M.F., Eng W.-s., Kulcsar P., Maldonado N., Robles S., Sinha U. and Wawrzehczyk C., In *Endocrinological Frontiers of Physiological Insect Ecology* (F. Sehnal, A. Zabża, and D.L. Denlinger, eds.), Wrocław Technical University Press, Wrocław, Poland, pp. 963-973 (1988)

8. Prestwich G.D., Eng W.-s., Boehm M.F. and Wawrzenczyk C. -- *Insect Biochem.*, **17**:1033 (1987)

9. Palli S.R., Osir E., Boehm M.F., Eng W.-s., Edwards M., Kulcsar P., Prestwich G.D. and Riddiford, L.M. -- *Proc. Natl. Acad. Sci. USA*, in press (1989).

10. Prestwich G.D. and Wawrzeńczyk C. -- *Proc. Natl. Acad. Sci. (USA)* **82**:5290 (1985)

11. Eng W.-s. and Prestwich G.D. -- *J. Labelled Cmpnd. Radiopharm.* **25**:627 (1988)

12. Yamada S.-i., Oh-hashi N. and Achiwa K. -- *Tetrahedron Lett.* 2561 (1976)

13. Suzuki Y. and Marumo S. -- *J. Chem. Soc., Chem. Commun.* 1199 (1971)

14. Imai K., Marumo S., Mori K. -- *J. Am. Chem. Soc.* **96**:5925 (1974)

15. Corey E.J. and Myers A.G. -- *Tetrahedron Lett.* **25**:3559 (1984)

16. House H.O. and Blankley C.J. -- *J. Org. Chem.* **33**:53 (1968)