SYNTHESIS OF A HIGHLY TRITIATED PHOTOAFFINITY LABELLED PHEROMONE ANALOG FOR THE MOTH ANTHERAEA POLYPHEMUS

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SUMMARY

The synthesis of the tritiated sex pheromone of the saturniid moth Antheraea polyphemus was accomplished by stereospecific T_2 reduction of (E)-hexadec-6-en-11-yn-1-yl acetate. The [11,12-$^3H_2$]-(E,Z)-6,11-hexadecadienyl acetate was then converted to the corresponding diazoacetate, a behaviorally active photoaffinity label for the A. polyphemus pheromone binding and receptor proteins. Synthesis of the enyne precursors is also described.

Key Words: pheromone analog, photoaffinity label, moth antennal proteins, Lepidoptera

INTRODUCTION

The existence of pheromone receptor molecules on the dendritic membranes of sensillar hairs of male moth antennae has not yet been demonstrated. Nonetheless, substantial progress in understanding the biochemical design of pheromone perception has been achieved for Antheraea polyphemus using [11,12-$^3H_2$]-(E,Z)-6,11-hexadecadienyl acetate, [^{3}H]-6E,11Z-16:OAc (1) (1-3). In addition, the diazoacetate (2) corresponding to this pheromone component has been prepared and shown to be behaviorally and electrophysiologically active (4,5). We describe here the details of the synthesis of (E)-hexadec-6-en-11-yn-1-yl acetate and its conversion into the [11,12-$^3H_2$] pheromone, (1). This material is then converted into [11,12-$^3H_2$] diazoacetate, [^{3}H]-6E,11Z-16:DZA (2).

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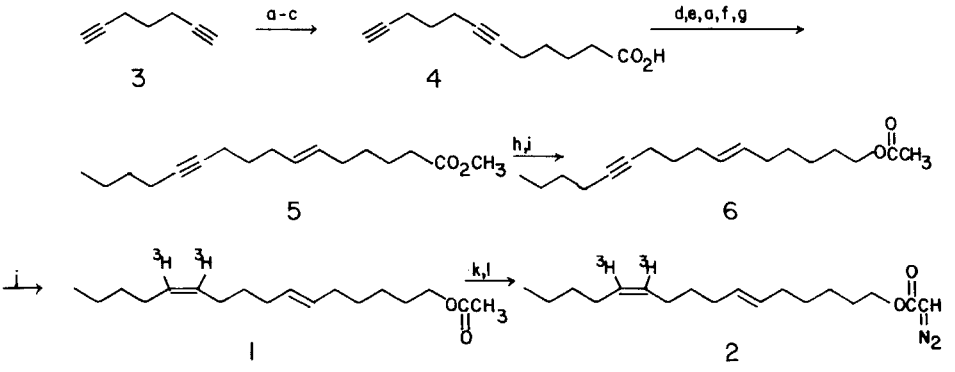
RESULTS AND DISCUSSION

The first published synthetic schemes (6) for (E,Z)-6,11-hexadecadienyl acetate lack stereospecificity in the generation of the alkene bonds and would not allow introduction of two tritiums per molecule in the final step. Bestmann and Li (7) have provided a stereospecific synthesis of 1, but no opportunity for reductive tritiation is available by their route. In our approach (Synthetic Scheme), the symmetrical lithium dianion of diyne 3 was monoalkylated with the lithium anion of 5-bromopentanoic acid (8). Selective reduction of the internal alkyne of 4 to the (E)-double bond was achieved by protection of the terminal alkyne as the sodium acetylide (9). The carbon skelton was completed by further alkylation of the acetylide carboxylate dianion with n-butyl bromide and isolation of the product as the methyl ester 5. Reduction and acetylation provided the desired enyne acetate 6 in an unoptimized 8% yield from 5-bromopentanoic acid.

The partial hydrogenation of enyne 6 in methanol with 5% $Pd/BaSO_4$ poisoned with quinoline afforded the A. polyphemus pheromone 1, identical by spectral and capillary GC criteria with authentic samples. Removal of unreacted 11-yne



Synthetic Scheme. Reagents: (a) $LiNH_2$, $NH_3(l)$; (b) $Br(CH_2)_4CO_2H$, THF; (c) H_3O^+; (d) $NaNH_2$, $NH_3(l)$; (e) Na, $NH_3(l)$; (f) $CH_3(CH_2)_3Br$, THF; (g) CH_2N_2; (h) $LiAlH_4$; (i) Ac_2O, Py; (j) H_2 ($^3H_2$), 5% $Pd/Ba/SO_4$, CH_3OH, quinoline; (k) CH_3OH, aq. KOH; (l) $ClCOCHNNHTs$, $(C_2H_5)_3N$, CH_2Cl_2.

material was readily accomplished at this point by chromatography on 20% $AgNO_3$ on silica gel with 2.5% ethyl acetate in hexane, and the isomeric purity was verified by capillary GC (30-m DB-5, 160° isothermal). The (6E,11Z) and (6Z,11Z) isomers prepared by another route (Golec, unpublished data) were clearly resolved under these conditions. Hydrolysis of the acetate and reesterification with the acid chloride of glyoxylic acid tosylhydrazone (10) gave, after addition of excess triethylamine, the diazoacetate 2.

The radiosynthesis was carried out at the new NIH Tritiation Facility at the Lawrence Berkeley Laboratory. A sample of 10 mg of enyne acetate was reduced under the above conditions to approximately 75% conversion under 740 torr of carrier-free T_2 gas in a glove box. The crude product was flash chromatographed (11) first on 45-60 μm silica gel and then on 20% $AgNO_3$ on 45-60 μm silica gel to give a clean separation of the 11-ene and 11-yne products. A portion of the tritiated pheromone was saved for metabolic experiments. The remainder was hydrolyzed and converted to the high specific activity diazoacetate (2) which was again purified in the glove box by flash chromatography using a disposable pipette column.

Labelled photoaffinity analogs of insect juvenile hormones (JH) have been synthesized (12) and shown to be powerful new tools for studying insect JH binding proteins (13). The use of this high specific activity pheromone photoaffinity label in attempts to characterize pheromone binding and receptor proteins of male A. polyphemus antennae will be described elsewhere (14). The unlabelled 6E,11Z-16:DZA (2) and analogs 6Z,11Z-16:DZA and 6E,11-yne-16:DZA have shown that the sensillar esterase of A. polyphemus prefers the correct geometry of the hydrophobic side chain (15).

EXPERIMENTAL METHODS

General. Elemental analyses were performed by Galbraith, Inc. Knoxville, Tennessee and high resolution mass spectra (HRMS) were obtained on an AEI-MS-9. Gas chromatographic analyses were performed on a Hewlett-Packard Model 5830A equipped with 8 m x 2.5 mm columns and a flame ionization detector. Later work ;as perfomed on a Varian 3700 equipped with 30 m x 0.25 mm fused silica

capillary columns. Preparative GC was accomplished on a Hewlett-Packard Model 700 instrument equipped with 3 m x 5 mm columns and dual thermal conductivity detectors. NMR spectra were obtained by using a Varian EM-360 instrument operating at 60 MHz or a Nicolet NT-300 at 300 MHZ. Chemical shifts (δ) are reported relative to TMS. Thin layer chromatography (TLC) was performed on silica gel plates containing a fluorescent indicator. Silver nitrate was incorporated by dipping the plates for 5 sec in 5% $AgNO_3$ in 1:1 ethanol-acetonitrile. Unlabelled materials were visualized by spraying with 2% $Cu(OAc)_2$-15% H_3PO_4 followed by heating at ca. 120°C; radiolabelled materials were visualized with I_2. Column chromatography was done using 80-100 mesh silica gel or on "flash" grade 230-400 mesh silica gel.

6,11-Dodecadiynoic acid (4). Lithium amide was prepared from lithium (2.53 g, 364 mmol) in 500 ml of anyhydrous liquid NH_3 with powdered $Fe(NO_3)_3.9\ H_2O$ (200 mg) as catalyst. After 45 min (blue color faded to grey), freshly distilled 1,6-heptadiyne 3 (18.64 g, 202 mmol) in 50 ml of dry THF was added dropwise. After stirring for 60 min, 5-bromopentanoic acid (7.324 g, 40.5 mmol) in 200 ml of dry THF was added, the solution was stirred under reflux for 8 hr, and the NH_3 was then evaporated. The residue was diluted with H_2O and acidified to pH 4.0 with oxalic acid. The product was isolated by extraction with ethyl acetate, the combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated in vacuo to give a yellow liquid. Vacuum distillation afforded a colorless liquid, bp 132-136°C/4.0 torr, 5.54 g (71%); IR(CCl_4) 3330 (C≡CH), 2975, 1725(CO, acid), 645 (C≡CH) cm^{-1}; NMR ($CDCl_3$) δ1.3-2.0 ($-CH_2-$) 2.0-3.9 ($HC\equiv C-CH_2, CH_2C\equiv CCH_2-$ and $-CH_2CO_2-$) and 12.70 (CO_2H, s).

(E)-6-Dodecen-11-ynoic acid. Sodamide was prepared from sodium (1.98 g, 86.4 mmol) in 300 ml of anhydrous liquid NH_3 with powdered $Fe(NO_3)_3.9\ H_2O$ (50 mg) as catalyst. The diyne acid 4 (5.54 g, 28.8 mmol) in 25 ml of dry ether was added slowly; after 30 min stirring, sodium (3.94 g, 171 mmol) was added chunkwise over a 30 min interval. Stirring was continued for an additional 2 hr

and the reaction was quenched with NH_4Cl. Workup as above followed by distillation yielded 5.00 g of a colorless liquid, bp 100-120°C/6.0 torr; NMR ($CDCl_3$) δ 5.42 (vinylic), 12.05 (CO_2H, s).

Methyl (E)-6-Hexadecen-11-ynoate (5). Lithium amide was prepared as above from lithium (500 mg, 72.0 mmol) in 300 ml of anhydrous liquid NH_3. After 30 min, the enyne acid (5.00 g, 25.7 mmol) in 25 ml of dry THF was added slowly, followed in 30 min by 1-bromobutane (21.00 g, 154.44 mmol) in 25 ml of dry THF. The solution was stirred for 8 hr under reflux, the NH_3 was then allowed to evaporate, and the residue was acidified and worked up as above to give 5.00 g of a colorless liquid, bp 111-162°C/3.0 torr,: NMR ($CDCl_3$) δ0.93 (CH_3), 5.48 (vinylic), 10.48 (CO_2H, s). The distilled enyne acid was then esterified with excess etheral diazomethane and purified by vacuum distillation to give 4.54 g of 5, bp 90-115°C/0.1 torr. Column chromatography on SiO_2 of the distilled ester 5 with ethyl acetate:hexane (1:9) to remove unreduced 6-yne material (1.65 g) gave the purified enyne ester 5 (1.51 g), which was homogeneous by TLC and GC: IR($CHCl_3$) 3030(HC=C), 1750 (CO, ester), 1240 1215 and 1190 (CO, methyl ester), 985 (C=C, trans) cm^{-1}; NMR ($CDCl_3$) δ0.93 (CH_3), 3.70 (CH_3OC=O, s), 5.45 (vinylic); HRMS (70eV), m/z 264.2098 (M^+, $C_{17}H_{28}O_2$, 66%). 235.1686 ($C_{15}H_{23}O_2$, 6%), 233.1920 ($C_{16}H_{25}O$, 24%), 221.1514 ($C_{14}H_{21}O_2$, 13%), 207.1386 ($C_{13}H_{19}O_2$, 4%) 177.1642 ($C_{13}H_{21}$, 13%), 149.1324 a.m.u. ($C_{11}H_{17}$, 100.0%).

(E)-6-Hexadecen-11-yn-1-yl acetate (6). The enyne ester 5 (1.51 g, 5.7 mmol) was reduced with lithium aluminum hydride (215.0 mg, 5.7 mmol) in 100 ml of dry ether (20°, 16 h). Workup as usual and vacuum distillation gave 1.19 g (88%) of the pure alcohol as a colorless liquid (bp 113°C/0.10 torr). This material was dissolved in 3 ml of dry pyridine and 3 ml of acetic anhydride was added. After stirring 16 h at 20°C, the product was isolated as usual, purified by column chromatography and vacuum distillation, bp 155-157°C/0.2 torr to give 905 mg (8.0% overall yield) of pure enyne acetate 6. The acetate 8 was homogeneous on TLC (5% $AgNO_3$-SiO_2), R_f = 0.32 (ethyl acetate:benzene 5:95) and GLC analysis was performed on both polar (Carbowax 20 M) and non-polar

(Apiezon L)columns; IR($CHCl_3$) 3015(HC=C), 1740(CO, acetate), 1235 (acetate), 980 (C=C, trans)cm^{-1}; NMR ($CDCl_3$) δ0.95 (CH_3), 2.10 (CH_3CO_2, s), 4.10 (CH_2O, t, J = 7.0 Hz), 5.50 (vinylic); MS(70eV), m/z 278.2238 (M^+,$C_{18}H_{30}O_2$, 31%), 221.1494 ($C_{14}H_{21}O_2$, 5%) 177.1586 ($C_{13}H_{21}$, 6%), 161.1328 ($C_{12}H_{17}$, 100%), 149.1326 ($C_{11}H_{17}$, 37%), 123.1146 (C_9H_{15}, 6%); C,H,O anal. (±0.2%) for $C_{18}H_{30}O_2$.

(E,Z)-6,11-Hexadecadienyl acetate. The enyne acetate 6 (303.4 mg, 1.09 mmol) was dissolved in 10 ml of anhydrous methanol and hydrogenated at room temperature and atmospheric pressure using 70 mg of 5% Pd/$BaSO_4$ deactivated by the addition of 3 drops of freshly distilled quinoline to the reaction mixture. After 1 hr the H_2 uptake became negligible and the hydrogenation was stopped after a total uptake of 23.7 ml of H_2. The solution was filtered through Celite in vacuo, concentrated, and chromatographed, and evaporatively distilled (75°/0.02 torr) to give 245 mg (80%) of pure pheromone 1; IR (thin film) 3010(HC=C), 1755(CO, acetate), 1245(acetate), 975(RHC=CHR, trans), 730(RHC=CHR, cis)cm^{-1}; NMR ($CDCl_3$) δ0.90 (CH_3), 2.05 (CH_3CO_2, s) 4.05 (CH_2O, t, J = 6.5 Hz), 5.40 (vinylic); MS(70eV) m/z 280.2390 ($C_{18}H_{32}O_2$, 100%), 220.2140 ($C_{16}H_{28}$, 15%), 163.1494 ($C_{12}H_{19}$, 18%), 149.1350 (C_9H_{13}, 37%), 107.0846 a.m.u. (C_8H_{11}, 4%). The synthetic (E,Z)-6,11-hexadecadienyl acetate 1 was proven to be identical (and of 95% purity) to authentic samples of the A. polyphemus pheromone supplied by W.L. Roelofs and by H. Bestmann using GLC analysis on one polar and two non-polar packed columns, and on two capillary columns.

(E,Z)-6,11-Hexadecadienyl diazoacetate (2). A solution of 70 mg of diene acetate 1 in 2 ml of 4:1 CH_3OH : 3 N NaOH was stirred 2 h at 20°. Then, 6 ml of H_2O was added, the mixture was extracted with three 4 ml portions of 1:1 ether-hexane, the extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo to give 50 mg of TLC-homogeneous enynol which was used without further purification.

A solution of 38 mg (0.16 mmole) of enynol in 2 ml of dry CH_2Cl_2 containing 34 µl (0.25 mmole) of distilled triethylamine was cooled to 0°C under N_2, and then 65 mg (0.25 mmole) of the acid chloride of glyoxylic acid tosylhydrazone

(10) was added. The pale yellow mixture was stirred at 0 to 20° for 1.5 h, then 70 µl (0.50 mmole) of triethylamine was added and the yellow-orange solution stirred an additional 2 hr. The solvent was removed in vacuo, the sticky residue was taken up in 5% ethyl acetate-hexane, and this solution was chromatographed on flash grade silica to give TLC-homogeneous diazoacetate 2 (4). The spectral (IR, UV, NMR) properties were consistent with the presence of the diazoacetate reported in the literature (4,10). Diagnostic ^{1}H-NMR signals occurred at δ4.73 (s, C(O)CHN$_2$) and 4.15 (t, 7 Hz, -C(O)OCH$_2$-). ^{1}H-NMR also shows the presence of a tosyl-containing impurity (ca. 10-30%) (δ2.42, tosyl CH$_3$ and characteristic A$_2$X$_2$ aromatic pattern at δ7.3-7.6) which cannot be readily removed by chromatography.

[11,12-^{3}H$_2$]-(E,Z)-6,11-Hexadecadienyl acetate ([^{3}H]-1). A solution of 10 mg of enyne acetate 6 (0.035 mmole) in 3 ml of dry methanol containing 2.2 mg of 5% Pd/BaSO$_4$ and 1.3 mg of quinoline was freeze-degassed on the tritium line at the LBL Tritiation Facility. The line was charged with ca. 200 Ci of carrier-free T$_2$ (desorbed from U^{3}H$_3$) and was maintained at 740 torr as the sample thawed. The mixture was stirred 15 min at 0-20°C, during which time the catalyst suspension remained light beige. After 20-25 min, a slight greyish color began to appear, signaling incipient overreduction which occurred if the catalyst color went black. The reaction was thus frozen with liquid N$_2$, degassed to a T$_2$ storage tank, and concentrated in vacuo to 2 ml. The catalyst was removed by centrifugation, the solvent was removed in vacuo (20°, 0.1 torr). TLC (Machery-Nagel Polygram Sil G/UV254 4 x 8 cm plates, developed in 20% ethyl acetate-hexane and visualized with I$_2$) showed >60% conversion of slower moving enyne (R$_f$ 0.58) to the desired tritiated diene (R$_f$ 0.61). This material was first chromatographed to remove polar materials with 10% ethyl acetate-hexane on flash silica in a disposable pipet. The fractions containing 6 and 1 were concentrated with N$_2$ and rechromatographed on 20% AgNO$_3$-SiO$_2$ (flash) with 2.5% ethyl acetate-hexane to give pure [^{3}H]-1, ca. 2 Ci at 58 Ci/mmol.

[11,12-$^3H_2$]-(E,Z)-6,11-Hexadecadienyl diazoacetate (^3H-2). A sample of approximately 0.5 Ci (ca. 8 μmole) was carried on at this stage, while the remaining [^3H]-1 was saved for studies of pheromone-protein interactions (2,3, 14,15). Thus, hydrolysis in 0.5 ml of 4:1 CH_3OH : 3 N NaOH followed by workup as before, gave the TLC-homogeneous [11,12-$^3H_2$]-dienol (R_f 0.33) which was used without further purification. Thus, a solution of the dienol in 2 ml of dry CH_2Cl_2 was added to a stirred, 0°C solution of 45 mg of the acid chloride of glyoxylic acid tosylhydrazone in 2 ml of dry CH_2Cl_2 under N_2. Triethylamine (20 μl) was added last to the cold solution (Important: solution should be cold and only a faint yellow color may develop). The solution was stirred 1.5 h at 0 to 20°C, then 40 μl of triethylamine was added and the now orange-yellow solution was stirred at 20°C for 2 hr. Solvents were removed under a stream of N_2, the dark residue was triturated with 15% ethyl acetate-hexane, and these washings were passed through flash silica to remove polar impurities. The fractions containing the diazoacetate 2 were combined and rechromatographed with 4% ethyl acetate in hexane to give TLC-homogeneous diazoacetate [^3H]-2 (R_f 0.55), ca. 0.4 Ci at 58 Ci/mmole.

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