SYNTHESIS OF ^{14}C-LABELED 10,11-EPOXYFARNESYL DIAZOACETATE, A POTENTIAL PHOTOAFFINITY LABELING REAGENT FOR INSECT JUVENILE HORMONE BINDING PROTEINS.

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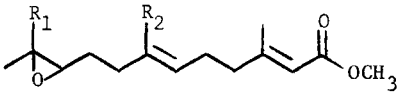
SUMMARY

The synthesis of [^{14}C]-10,11-Epoxy-(2E,6E)-farnesyl diazoacetate in one vessel, starting from [^{14}C]-glyoxylic acid, is described. This compound is useful as a potential photoaffinity labeling agent for juvenile hormone binding sites.

Key Words: Juvenile Hormone Analog, Photoaffinity Labeling, Diazoacetate

INTRODUCTION

Juvenile hormone (JH) 1 is one of the major regulators of development and differentiation in insects, and the interaction of JH with various JH binding proteins which are thought to mediate its biological activity has been the object of recent investigations. We have reported the synthesis of a number of



Juvenile Hormone

1a (R_1 = Et, R_2 = Et)
1b (R_1 = Et, R_2 = Me)
1c (R_1 = Me, R_2 = Me)

JH analogs, and we have measured their binding interactions with the hemolymph carrier protein from the tobacco hornworm, Manduca sexta.[1] Several of these analogs contain reactive functional groups and were designed as affinity labeling reagents to study the interactions of JH with this and other JH binding proteins. One analog in particular, 10,11-epoxyfarnesyl diazoacetate (2), exhibited high binding affinity for the hemolymph carrier protein (40% relative to C-18 JH 1a)[2] and was considered to be an excellent candidate as a photoaffinity label. In this paper we report an efficient, single vessel synthesis of 2

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in ^{14}C radiolabeled form.

EXPERIMENTAL

Materials

Chemicals were obtained from the following sources: Aldrich Chemical Co. - p-toluenesulfonyl hydrazine, thionyl chloride, glyoxylic acid monohydrate; Eastman Chemical Co. - triethylamine; Amersham - ^{14}C-glyoxylic acid, sodium salt (5.5 mCi/mmole); Central Solvents and Chemical Corp. - Triton X-114. (2E,6E)-Farnesol, used in the preparation of 10,11-oxido-(2E,6E)-farnesol, was synthesized from geranyl acetone (obtained from Givaudan as a mixture of geranyl and neryl acetones and separated by distillation) as described by Reich.[2]

Methods

All preparative column chromatography was performed on a medium pressure liquid chromatograph (MPLC), designed and built in our laboratory.[3] Analytical high performance liquid chromatography (HPLC) was conducted on a Varian Model 4100 liquid chromatograph, equipped with an ultraviolet detector (254 nm). Proton magnetic resonance (^1H-NMR) spectra were obtained on Varian Associates EM-390 (90 MHz) spectrometer and are expressed as parts per million downfield from tetramethylsilane as an internal standard (δ scale). Infrared (IR) spectra were obtained with a Beckman IR-12 or a Perkin-Elmer Model 137 spectrophotometer, and data are presented as cm^{-1}. Mass spectra were obtained on a Varian Associates MAT CH-5 spectrometer at 10 or 70 eV. Data are reported in the form: m/e (intensity relative to base peak = 100). High resolution electron impact mass spectroscopy for exact mass determination was performed on a Varian Associates MAT 731 mass spectrometer.

Radioactivity was measured in a Nuclear Chicago Isocap 300 liquid scintillation counter, in minivials using 5 mL of a xylene-base cocktail containing 0.55% 2,5-diphenyloxazole, 0.01% p-bis[2-(5-phenyloxazolyl)]benzene, and 25% Triton X-114. Radiochemical purity was determined by chromatography on plastic-backed neutral aluminum oxide thin-layer plates (Eastman chromogram sheet without fluorescent indicator). The labeled material was spotted on top of

unlabeled carrier. After development, the carrier spot was visualized by exposure to iodine vapor, and the chromatogram was cut into strips, which were then placed in minivials for radioactivity determination.

Chemical Syntheses

10,11-Epoxy-3,7,11-trimethyl-2(E),6(E)-dodecadien-1-yl Diazoacetate (2) (10,11-Epoxyfarnesyl Diazoacetate)[2] - A solution of 119 mg (0.500 mmol) of 10,11-epoxyfarnesol (4)[2,4] and 50.5 mg (0.500 mmol) of triethylamine in 1.0 ml dry dichloromethane was added dropwise to a solution of 130 mg (0.500 mmol) of the p-toluenesulfonylhydrazide of glyoxylic acid chloride 3[5] in 2 ml dichloromethane a 0°C under N_2 in the absence of light. After stirring for 2 hr, an additional 50.5 mg (0.5 mmol) of triethylamine was added, followed by stirring for 3 hr at 0°C. Workup and purification were done in the dark. After addition of ether, the mixture was filtered and the solid washed several times with ether. The combined filtrate and wash was evaporated and the residue purified by preparative MPLC on a cyanopropyl functionalized silica column (0.5" x 15") with 4:1:1 hexane:ether:dichloromethane as the eluting solvent. The solvent was removed under high vacuum at 0°C, to give 102 mg (66%) of light yellow oil (2). ^{1}H-NMR δ 1.21, 1.24 (2s, 6H, C-11, CH_3), 1.48 (m, 2H, 2 x H-9), 1.62 (s, 3H, C-7, CH_3), 1.71 (s, 3H, C-3, CH_3), 2.06 (m, 6H, CH_2: C-8,5,4), 2.49 (t, J=6 Hz, 1H, H-10), 4.58 (d, J=7 Hz, 2H, 2 x H-1), 4.62 (s, 1H, CHN_2), 5.07 (t(broad), 1H, H-6), 5.29 (t, J=7 Hz, 1H, H-2). IR 3110 ($\underline{CH}N_2$), 2115 (N_2), 1690 (C=O), 1235 and 1185 (C-O) cm^{-1}.

High resolution mass. Calcd. for $C_{17}H_{26}N_2O_3$: 306.4084. Found: 306.4078.

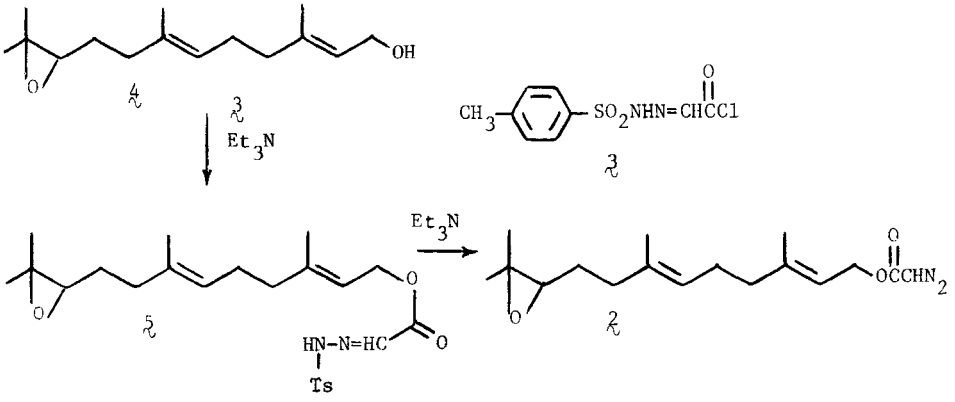
Radiochemical Preparation of 10,11-Epoxyfarnesyl ^{14}C-Diazoacetate (2) - ^{14}C-glyoxylic acid sodium salt (0.21 mg, 1.82 μmol, 10.0 μCi) in 200 μl water was treated with 1.69 mg (10 μmole) p-toluenesulfonylhydrazine in 85 μl of 3N HCl at 60°C for 30 min. Evaporation of water in vacuo left a colorless solid residue, which was suspended in 500 μl chloroform, and treated with excess (10 μl) thionyl chloride under reflux for 45 min. The solvent and excess thioyl chloride were removed in vacuo, and the residue was dissolved in

CH_2Cl_2. After cooling to 0°C, a solution of 300 µl triethylamine and 4.31 mg (13.1 µmol) 10,11-epoxyfarnesol (4)[2,4] in 1500 µl CH_2Cl_2 was added. After 2.5 hr, the reaction was treated with an additional 30 µl triethylamine and stirring was continued for 3 hr. The solvent was removed under a slow stream of N_2, and the residue redissolved in 100 µl chloroform. Purification by HPLC on a 2.1 mm x 25 cm cyanopropyl silica gel (Varian Micropack CN-10) column with a 0-75% gradient of 80:20 CH_2Cl_2:isopropanol vs hexane afforded 2 in 61.2% radiochemical yield. Radiochemical purity, as determined by TLC analysis on neutral alumina plastic-backed plates, was 92.6%. The material was stored at -20°C in nanograde hexane under argon.

RESULTS

The synthesis of unlabeled 10,11-epoxyfarnesyl diazoacetate 2 is shown in Scheme 1. Esterification of the p-toluene sulfonylhydrazone of glyoxylic acid 3, via its acid chloride, with 10,11-epoxyfarnesol 4, afforded the ester 5, which was immediately subjected to Et_3N induced elimination of p-toluenesulfinic acid to afford the diazoacetate 2 in 43% yield from 3.

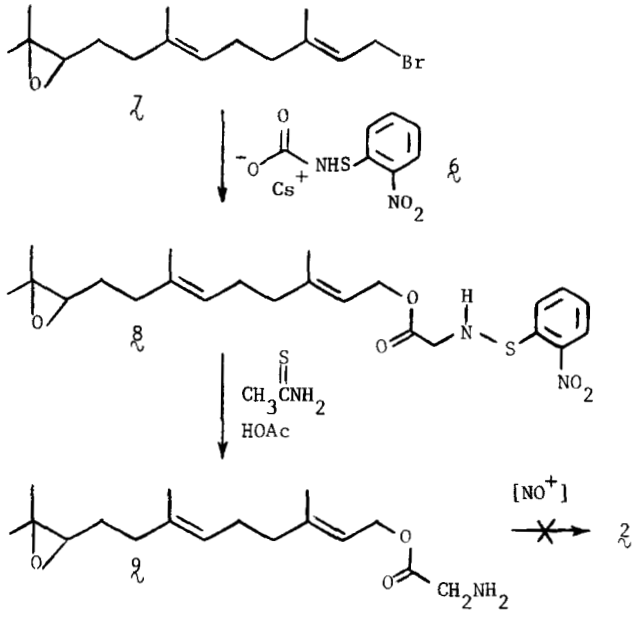
Scheme 1



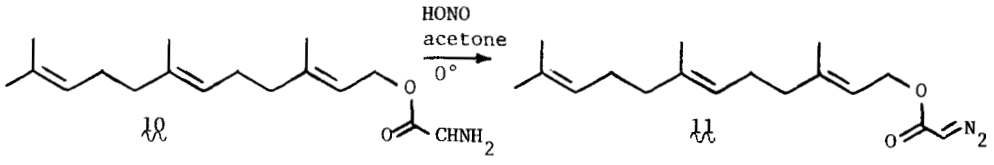
A synthesis of 2, utilizing glycine as a potential radiolabeled starting material was attempted, as shown in Scheme 2. The cesium salt of the protected glycine derivative 6 was alkylated with 10,11-epoxyfarnesyl bromide (7) in DMF to afford the ester 8 in 93%. Thiophilic removal of the o-nitrophenylsulfenyl

group with thioacetamide in acetic acid afforded 10,11-epoxyfarnesyl glycinate 9 in 36%.

Scheme 2



Scheme 3



Diazotization of farnesylglycinate 10 with $NaNO_2$ in 1:1 acetone:$4NH_2SO_4$ at 0°C afforded in 56% yield farnesyl diazoacetate (11). However, when the epoxide 9 was subjected to these conditions, epoxide hydrolysis occurred at a rate comparable with the desired diazotization. Attempts to buffer the reaction, use of organic nitrites (e.g. amyl nitrite, 3-butoxy-1-propylnitrite) or inorganic nitrosating agents (e.g. N_2O_5/CCl_4, NOCl, $NOPF_6$) failed to preserve the epoxide function while effecting diazotization of the amine.

The successful radiochemical synthesis of ^{14}C-labeled 10,11-epoxyfarnesyldiazoacetate was achieved utilizing ^{14}C-labeled glyoxylic acid as the starting material. The aforementioned procedure was modified so that all of the four

transformations be conducted in a single reaction vessel. Condensation of [^{14}C]-glyoxylic acid with p-toleuensulfonyl hydrazine at elevated temperature (60°C) with an acid catalyst (HCl) afforded the [^{14}C]-hydrazone 2, which, after removal of water in vacuo, was heated to reflux with thionyl chloride in benzene. Removal of excess thionyl chloride and solvent in vacuo, esterification with 10,11-epoxyfarnesol and elimination of the sulfinic acid with triethylamine furnished the [^{14}C]-diazoacetate (2). Purification by gradient elution from a CN-silica gel HPLC column afforded the ^{14}C labeled compound 2 in 62% radiochemical yield with a radiochemical purity of 93%, as determined by thin-layer chromatographic analysis. The specific activity of this material was diluted to 1 mCi/mmole with unlabeled 2.

This simple synthesis provides access in high yield and potentially high specific activity to a valuable photoaffinity reagent that may prove to be capable of specifically labeling JH binding proteins and probing the mode of JH activity.

ACKNOWLEDGMENT

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