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SYNTHESIS OF RACEMIC [2-^{14}C] INSECT JUVENILE HORMONE III

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SUMMARY

Both racemic [2-^{14}C] juvenile hormone III (JH III; methyl-10,11-epoxy-3,7,11-trimethyl-2(E),6(E) [2-^{14}C]dodecadienoate) and its 2-(Z) isomer were synthesised on a 1 millimolar scale by forming the 9,10 epoxide of geranyl acetone and reacting this with the anion of trimethylphosphono-[2-^{14}C]acetate. After purification the yield of [2-^{14}C] JH III in the radiosynthetic step was 14% (with purity >93% by GC, specific activity 0.4mCi/mmol) and 4.7% (>97%, 0.4mCi/mmol) for the 2-(Z) isomer.

KEYWORDS

Racemic [2-^{14}C] juvenile hormone III, Preparative GC, Radiosynthesis, Microscale.

INTRODUCTION

Insects rely upon the timely internal production of controlled amounts of juvenile hormones for normal development. Exogenous application of these hormones or synthetic analogues can interrupt development, is often lethal, and so provides a potential insect control method. In order to investigate the effect and safety of these agents their fate in both insects and food must be studied. Such studies are greatly assisted by the use of radioisotopes, for example carbon-14. From various examples of Coleopteran, Orthopteran and Dictopteran pests investigated only JH III has been isolated and identified

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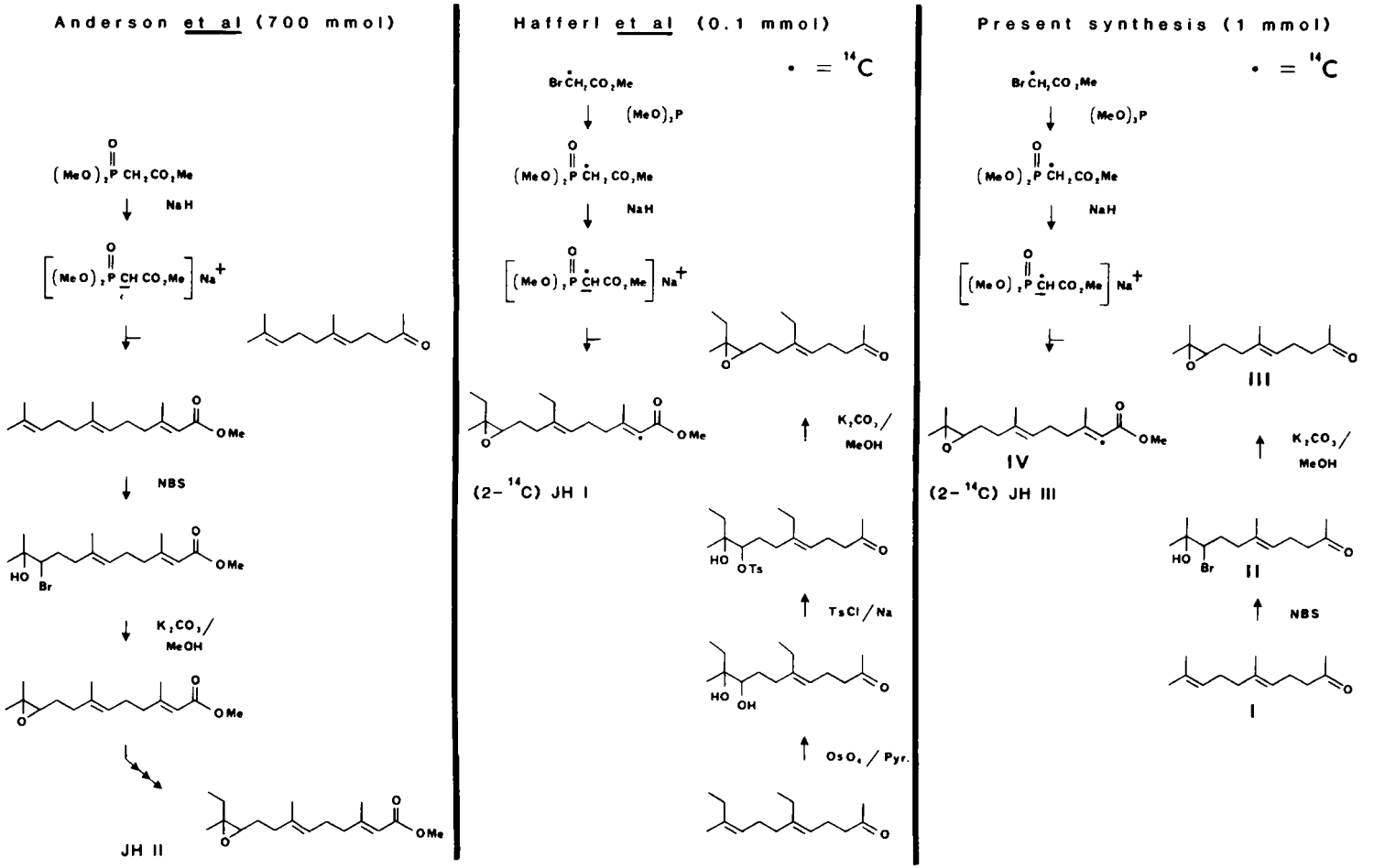
FIG. 1 BASIS OF THE PRESENT SYNTHESIS OF (2 - ^{14}C) JH III



[1]. Thus JH III labelled with carbon-14 would provide a useful experimental tool and (since the compound was otherwise unavailable) its synthesis was proposed.

The synthesis of juvenile hormones is well established in the literature although none has yet been reported for carbon-14 labelled JH III. The present synthesis was based on two published syntheses; that for racemic [2-^{14}C] JH I [2] and racemic, non-labelled JH II [3] (Fig 1). In both cases the desired carbon chain length was achieved by coupling the trimethylphosphonoacetate anion to a terpenoid ketone of the appropriate geometry using a form of the Wittig reaction. This gave the required 2E esters preferentially, although both groups reported the formation of significant amounts of the 2Z isomers. Despite this, the coupling permitted use of the readily available methyl [2-^{14}C]bromoacetate as the source of radiocarbon. For the introduction of the epoxide group, Hafferl *et al* [2] had to use a three-step route in order to retain the required Z-configuration. In the present synthesis, where there was no such requirement, the simpler two-step route of Anderson *et al* [3] was used. However the epoxide was introduced before the Wittig coupling so that the concomitant loss of material (expected to be about 20%) did not involve the valuable radiolabel.

EXPERIMENTAL AND RESULTS

Unless otherwise stated all chemicals used were analytical grade and all solvents were fractionally distilled and dried.

GERANYL ACETONE BROMOHYDRIN (II): N-Bromosuccinimide (NBS, 99%, 2.58g, 16.9mmol) was added over 5 mins to a mixture of geranyl acetone (I) (Fluka AG, >99.5%, 3.0g, 15.5mmol), 1,2-dimethoxymethane (glyme,33ml) and H_2O (17ml) at 0°C. After 90 mins the reaction mixture was poured into brine (50ml) and extracted with Et_2O (3x50ml). The extracts were combined, washed (H_2O, 3x50ml) and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave an orange coloured oil which was chromatographed on fine silica (Reeve-Angel, PTLC grade, 150g) with mixtures of $CHCl_3$ and MeOH, giving the bromohydrin (II)

(2.16g, 7.44mmol, 48%) as a colourless oil: TLC R_F 0.23 (petrol bpt 60-80°: EtOAc 2:1) cf geranyl acetone (I) 0.54; >98% pure by GC, R_T 21mins (3% OV101 @ 175°C) cf geranyl acetone (I) 3.3mins; IR (cm^{-1})(film) 3440 broad (b) (OH), 2900 b (C-H), 1710 very strong (vs)(C=O), 1670 weak (w)(C=C), 1440 w, 1370 b (O-H), 1120 w (C-O), 910 vs, 730 vs (C-Br); NMR δH ($CDCl_3$) 1.35 (6H singlet(s), 2xC-10 Me), 1.65 (3H s C-6 Me), 1.7-2.2 (6H multiplet(m), C-4,7,8 CH_2), 2.15 (3H s C-1 Me), 2.4 (2H triplet(t), J 7Hz C-3 CH_2), 3.9 (1H m C-9 H), 5.1 (1H t J 7Hz C-5 H); MS m/z (70eV) 274, 272 (1%, M^+-H_2O), 193 (27, $(M^+-H_2O)-Br$), 135 (75), 93 (34), 81 (20, ^{81}Br), 79 (16, ^{79}Br), 56 (46), 43 (100, MeC=O).

GERANYL ACETONE EPOXIDE (III): A mixture of bromohydrin (II) (0.29g, 1mmol) and MeOH (15ml) was treated with K_2CO_3 (0.55g, 4 mmol). After 30 mins the solid was filtered off, the filtrate poured into brine (50ml) and extracted with Et_2O (3x50ml). The extracts were combined, washed and dried ($MgSO_4$). Removal of the solvent gave the epoxide (III) (0.193g, 0.92 mmol, 92%): TLC R_F 0.38 cf bromohydrin (II) 0.25; >98% pure by GC, R_T 6.3 mins (conditions as above) cf bromohydrin (II) 21 mins; >99% pure by capillary GC, R_T 1.8 mins (10m SE30 @ 150°C); IR (cm^{-1}) (film) 2900 b (C-H), 1720 vs (C=O), 1670 w (C=C), 1440 w, 1390 W, 1250 w (epoxide) 870 w; NMR δ_H($CDCl_3$) 1.2 (3H s C-10 Me), 1.25 (3H s C-11 Me), 1.5-1.9 (2H m C-8 CH_2), 1.65 (3H s C-1 Me), 1.9-2.5 (4H m C-4,7 CH_2), 2.15 (3H s C-1 Me), 2.4 (2H t J 7Hz C-9 H), 5.1 (1H t J 7Hz C-5 H); MS m/z (70eV) 210 (2%,M^+), 192 (4, M^+-H_2O), 109 (11), 95 (22), 85 (41, $M^+ - C_5H_9O$), 81 (24), 71 (26), 59 (22), 43 (100, Me-C=O).

TRIMETHYL [2-^{14}C] PHOSPHONOACETATE: Trimethylphosphite (0.316g, 2.55mmol) in Et_2O (1ml) was added to methyl [2-^{14}C]bromoacetate (0.153g, 1mmol, 0.5mCi, 0.5 mCi/mmol) in Et_2O (1ml). The mixture was stirred at 120°C for 4 hrs; after this time excess trimethylphosphite, unreacted bromoacetate and solvent were removed *in vacuo* (0.1mm Hg, 25°C) leaving the product as a colourless oil (0.151g, 0.83mmol, 83%; 0.410mCi, 82%): TLC R_F 0.64 (EtOH) cf. methyl bromoacetate 0.44 and trimethylphosphite 0.58; GC R_T 9.0 mins (10m SE30 @ 150°C) cf methyl bromoacetate 1.5 mins and trimethylphosphite 0.6 mins; IR

(cm^{-1})(film) 3500 w, 2900 b (C-H), 1740 vs (C=O), 1440 b, 1270 b, 1120 s (P=O), 1050 b, 900 s, 850 w, 800 w; NMR δH ($CDCl_3$)3.0 (2H d J 20Hz CH_2), 3.7 (3H s C(O)Me), 3.8 (6H d J 10Hz 2xP(O)Me); MS m/z (70eV) 182 (12%, M^+), 151 (99, M^+-MeO), 142 (32), 123 (53, $(MeO)_2PO$), 79 (27), 74 (21), 31 (16, OMe).

[2-^{14}C] JUVENILE HORMONE III (IV): Sodium hydride (50% dispersion in oil, 50mg, 1mmol) was rinsed twice with Et_2O (under N_2) and the rinsings discarded. More Et_2O (5ml) was added and the mixture stirred. Trimethylphosphono[2-^{14}C]-acetate (0.151g, made up to 0.182g, 1 mmol with non-labelled material) in Et_2O (3ml) was added slowly (via a septum using a syringe) and the mixture refluxed for 4 hrs. After cooling geranyl acetone epoxide (III) (0.21g, 1mmol) was added (via the septum) and the mixture refluxed for 20 hrs. The mixture was diluted with MeOH (1ml), extracted with Et_2O (2x50ml), the combined extracts washed with brine (2x20ml) then H_2O (2x20ml) and dried ($MgSO_4$). Removal of the solvent in vacuo gave a colourless oil (0.179g) shown by GC to contain 2 unknowns, epoxide (III) and a minor impurity. The unknowns were isolated from the crude material (0.179g) in hexane (2ml) by preparative GC (2m x 6mm, Carbowax 20M @ 200°C) with thermal conductivity detection. Four cooled (liq N_2) glass U-tubes were placed in turn at the detector effluent exit port and used in rotation to collect the 2 unknowns, the unreacted epoxide (III) and waste. The contents of each tube were recovered by rinsing (Et_2O, 5ml) after every 6 injections (of 30 μl). Each solution was assessed by GC (10m,SE30 @ 150°C) prior to pooling. The 4 fractions consisted of unknown 1 (R_T 14.4 mins, 37.2mg; 59.4 μCi, 14%), unknown 2 (R_T 11.8 mins, 12.6mg; 19.4 μCi, 4.7%), epoxide (III) (R_T 4.8 mins, 48.2mg) and waste (52mg, 51.4 μCi); recovery of 84% of the applied mass, 81% of the applied radioactivity. Subsequent analysis confirmed that unknowns 1 and 2 were [2-^{14}C] JH III and its [2-Z] isomer respectively. [2-^{14}C] JH III (unknown 1) : TLC R_F 0.51 (petrol bpt 60-80° : : EtOAc 2:1), >94% pure by GC (R_T 4.0 mins, 10m, SE30 @ 170°C); NMR δ_H($CDCl_3$) 1.2 (3H s C-11 Me), 1.25 (3H s C-12 Me), 1.5-1.9 (2H m C-9 CH_2), 1.65 (3H s C-7 Me), 1.9-2.5 (6H m C-4,5,6, CH_2), 2.2 (3H s C-3 Me), 2.7 (1H t J 7Hz

C-10 H) 3.7 (3H s CO_2Me), 5.15 (1H m C-6 H), 5.7 (1H s C-12 H); NMR $\delta_C(CDCl_3)$ 16 (C-7 Me), 18.8 (C-3, 11 Me), 24.9 (C-12), 25.9 (C-9), 27.5 (C-5), 36.3 (C-8), 40.8 (C-4), 50.8 (C-1, OMe), 58.2 (C-11), 64.1 (C-10), 115.3 (C-2), 123.4 (C-6), 135.3 (C-7), 159-9 (C-3), 167.2 (C-1); MS m/z(70eV) 266 (14%, M^+), 163 (12), 153 (12), 135 (65), 121 (39), 114 (52), 107 (40), 95 (27), 93 (50), 91 (20), 81 (100), 71 (53), 69 (70), 55 (40), 43 (71, Me-C=O). Similar analytical data were obtained with racemic, non-labelled JH III (commercially obtained from Calbiochem Inc.).

[2-Z] isomer of [2-^{14}C] JH III (unknown 2): TLC R_F 0.51, >98% pure by GC (R_T 2.8 mins, 10m SE30 @ 170°C); NMR $\delta_H(CDCl_3)$ as for [2-^{14}C] JH III except 1.8-2.4 (4H m C-5,8 CH_2), 1.9 (3H s C-3 Me), 2.5-2.85 (2H m C-4 CH_2); NMR δC ($CDCl_3$) as for [2-^{14}C] JH III except 25.3(C-3 Me), 26.7 (C-9), 33.4 (C-4), 115.9 (C-2), 124.1 (C-6), 134.9 (C-7), 160.3 (C-3), 176.1 (C-1); MS m/z (70eV) 266 (3%, M^+), 248 (20), 173 (13), 163 (18), 153 (14), 149 (24), 135 (96), 121 (67), 114 (60), 107 (60), 95 (35), 93 (60), 91 (39), 81 (100), 71 (43), 69 (69), 55 (49), 43 (75, Me-C=O).

DISCUSSION

In the present synthesis geranyl acetone (9,10) epoxide is formed from geranyl acetone (I) *via* the bromohydrin. van Tamelen and Curphey [4] noted that NBS generates HOBr in aqueous polar solvents eg, BuOH, THF and glyme, and that this reagent shows some selectivity towards reaction with compounds with multiple double bonds. NBS in aqueous glyme has been used to convert farnesyl acetate to its (10,11) epoxide *via* the bromohydrin [5] and in a similar procedure to give JH I [6][7]. In their synthesis of JH II (Fig 1) Anderson *et al* used THF as solvent for bromohydrin formation. In the present synthesis optimum yields were obtained using glyme; although conversion to the bromohydrin (II) was incomplete, the product was easily isolated using liquid chromatography. Instability of bromohydrins in the presence of a weak base (eg K_2CO_3) was used to advantage in the formation of the epoxide (III) *via* a Williamson (cyclic) ether synthesis. Use of high purity bromohydrin (II)

(>98%) obviated the necessity to purify the epoxide (III) prior to use. The optimum reaction conditions for the synthesis of trimethylphosphonoacetate (Arbusov synthesis) [8] were investigated using both non-labelled methyl bromoacetate and that labelled with a small amount of ^{14}C-activity (50 μCi). Gastight reaction vessels were essential to retain the minute quantity of methyl bromoacetate used at the elevated temperature necessary for successful reaction. The relative involatility of the phosphonoacetate (bp 118°C @ 0.85 mmHg) cf trimethyl phosphite (bp 116°C @ 760 mmHg) enabled the unreacted phosphite to be easily removed (in vacuo) from the reaction mixture.

[2-^{14}C] JH III was synthesised by forming the stabilised carbanion of trimethylphosphonoacetate and reacting this with the carbonyl moiety of geranyl acetone epoxide (III) (Wittig-Horner reaction). Stabilisation and reaction conditions were selected to give, predominantly, the 2-(E) isomer although formation of a significant amount of the 2(Z) isomer was expected (and desirable since there would be an opportunity to isolate and biologically assess both isomers). For successful formation of the carbanion with NaH it was found essential to use thoroughly dry and gas-tight apparatus (to prevent inactivation of NaH by moisture and retain Et_2O during the lengthy reflux period). Initially, non-labelled JH III was synthesised to establish synthetic and separatory techniques, then [2-^{14}C] JH III incorporating a small amount of activity (30 μCi) was made to assess radioyield and specific activity. Finally syntheses using larger amounts of activity (2x400 μCi) were performed to provide sufficient material for biological testing. As expected both [2-^{14}C] JH III and its 2-(Z) isomer were obtained (in the approximate ratio, 2:1) with 64% conversion of the epoxide (III).

The use of preparative GC to isolate juvenile hormones has been attempted by other workers with varied success. Hafferl et al used "microscale" prep. GC (ie 50 μg per injection) to isolate [2-^{14}C] JH I but attempts to accommodate 1mg injections failed; only polar rearrangement products being isolated. Mori [9] isolated geometric isomers of a JH I precursor (2(E),6(E),10(E) and

2(Z),6(E),10(E) 7-ethyl-3,11-dimethyltridecatrienoates) (1g) with no apparent degradation. The main disadvantages in using prep. GC are the relatively high injector and column temperatures which increase the risk of thermal rearrangement/degradation and the limited amount of material which can be applied in a single injection. Nevertheless in the present study practical conditions were found by which injections containing 2.8mg of reaction product (in 30 µl of solvent) could be made and the components isolated without evidence of rearrangement/degradation. Although the process was time-consuming (64 injections @ 3 per hr) the components were obtained isomerically pure with the expected specific activities.

REFERENCES

1. Schooley D.A. - In Analytical Biochemistry of Insects (Ed. R.B. Turner) Elsevier (USA) : 241 (1977)
2. Hafferl W., Zurflüh R. and Dunham L. - J. Labelled Comp. VII (3) : 331 (1971)
3. Anderson R.J., Henrick C.A., Siddall J.B. and Zurflüh R. - J. Amer. Chem. Soc. 94 : 5379 (1972)
4. van Tamelen E.E. and Curphey T.J. - Tetrahedron Lett. 121 : 1 (1962)
5. van Tamelen E.E., Sterni A., Hessler E.J. and Schwarz M.J. - J. Amer. Chem. Soc. 85 : 3295 (1963)
6. van Tamelen E.E. and McCormick J.P. - J. Amer. Chem. Soc. 92 : 737 (1970)
7. Corey E.J., Katzenellenbogen J.A., Gilman N.W., Roman S.A. and Erickson B.W. - J. Amer. Chem. Soc. 90 : 5618 (1968)
8. Arbusov E.A. - Zhur. Russ. Fiz. Khim. Obchestra 38 : 687 (1906).
9. Mori K. - Tetrahedron 28 : 3747 (1972)