RADIOSYNTHESIS OF HIGH SPECIFIC ACTIVITY TRITIUM-LABELED PRECOCENE II

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SUMMARY

The insect antijuvenile hormone precocene II (6,7-dimethoxy-2,2-dimethylchromene) was radiosynthesized, incorporating tritium specifically in the gem-dimethyl group at high specific activity. 7-Hydroxycoumarin was methylated (CH_3I/KOH) and then oxidized at 6-position ($K_2S_2O_8$/NaOH). The resulting 6-hydroxy-7-methoxycoumarin was methylated (CH_3I/KOH) and reacted with excess C^3H_3MgI to give precocene II-[2-methyl-^{3}H] at a specific activity of 32 Ci/mmol. This synthetic scheme offers access to radiolabeled preparations of a wide variety of mono- and dialkoxy-substituted analogues of precocene II.

Key Words: Antijuvenile hormones; precocene II-[2-methyl-^{3}H]; 6,7-dimethoxy-2,2-dimethylchromene-[2-methyl-^{3}H].

INTRODUCTION

Precocene II (6,7-dimethoxy-2,2-dimethylchromene) is one of two related chromenes isolated from Ageratum houstonianum that possess antijuvenile hormone activity in insects (1,2). Radiolabeled precocenes are essential for metabolism and mode of action studies in insects, and several labeled preparations have been described. Initial radiosyntheses gave precocene II labeled with carbon-14 at either the gem-dimethyl position (3) or the 4-position of the chromene ring (4). These preparations were useful in establishing the metabolic fate of precocene II in in vitro systems (3-5) but were of insufficient specific activity to be of use

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in in vivo metabolism or mode of action studies. Precocene II-[4-^{3}H] at 16 Ci/mmol was synthesized by NaB^3H_4 reduction and subsequent dehydration of the corresponding chromanone (6), but this preparation proved to be unstable in biological systems even after 16-fold dilution with unlabeled carrier (7). Recently, [6-methoxy-^{3}H]-labeled (8) and [2-methyl-^{3}H]-labeled (9) preparations of precocene II have been reported. The general utility of these compounds is limited by the metabolic lability of the label in the case of the methoxy-labeled preparation (5) and by their low specific activities. Because of the limitations of previous labeled preparations, we sought a radiosynthetic route that would yield precocene II labeled with tritium at a high specific activity in a position expected to be refractory to metabolic attack. We now report the synthesis of precocene II specifically labeled with tritium at the gem-dimethyl position at a specific activity of 32 Ci/mmol.

DISCUSSION

Previous studies established the feasibility of labeling the gem-dimethyl group with excess radiolabeled Grignard reagent (3) and demonstrated that the gem-dimethyl position was not a site of oxidative attack (5). Accordingly, we prepared 6,7-dimethoxycoumarin (IV) for reaction with C^3H_3MgI (Figure 1). 7-Hydroxycoumarin (I) was first methylated and then oxidized with potassium persulfate to give 6-hydroxy-7-methoxycoumarin (III), which upon methylation gave IV. This sequence has broad radiosynthetic utility in this series, offering ready access to a variety of precocene analogs having different alkoxy substituents in which the radiolabel is introduced in the final synthetic step. For example, ethylation of 7-hydroxycoumarin with ethyl iodide, followed by the subsequent oxidation and methylation steps, would yield 7-ethoxy-6-methoxycoumarin, the precursor of ethoxy-precocene II (7-ethoxy-6-methoxy-2,2 dimethylchromene), a potent synthetic analog (10), whereas the use of II in the Grignard reaction would yield labeled precocene I (7-methoxy-2,2-dimethylchromene), the other naturally occurring antihormone (1,2).

Efficient incorporation of the tritium label in the last step gave a product

with the highest specific activity (32 Ci/mmol) of any labeled precocene reported to date. Labeling at this position is advantageous since there is little likelihood of metabolic loss of the label from the parent carbon skeleton (5). Also, the gem-dimethyl position is much less reactive than the highly activated 3-4 double bond, suggesting that the gem-dimethyl labeled preparation might be more stable than that labeled at the 4-position. Preliminary observations suggest that the gem-dimethyl-labeled precocene II exhibits increased stability, but the relative rates of degradation of these two high specific activity preparations under a variety of conditions have not been determined.

EXPERIMENTAL

7-Methoxycoumarin (II). 7-Hydroxycoumarin (I, 6g, 0.037 mole; Aldrich, Milwaukee, WI, USA) in anhydrous dimethylformamide (125 ml) was stirred with

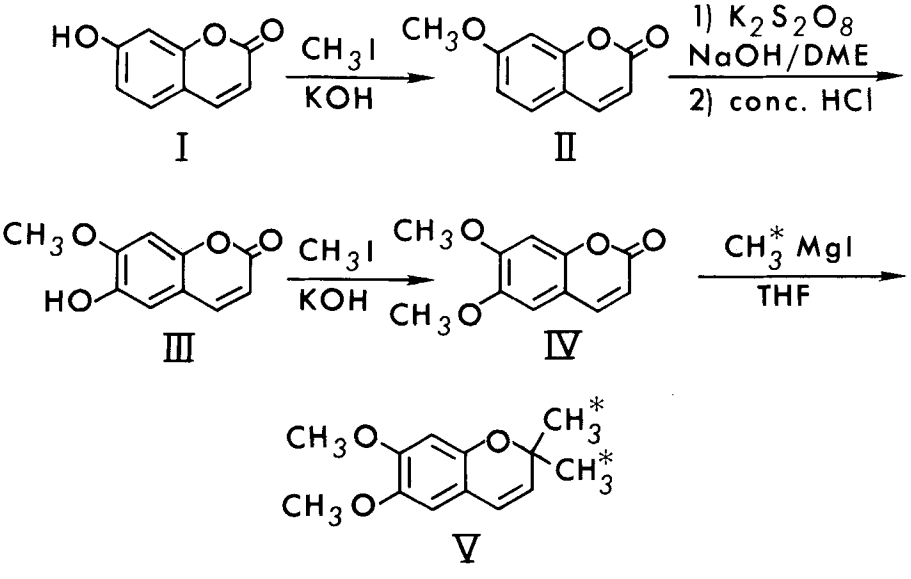

Figure 1. Synthetic route to precocene II labeled with tritium at the gem-dimethyl position. Asterisks indicate positions of tritium labeling.

powdered potassium hydroxide (2.5 g, 0.047 mole) for 30 min. To this was added iodomethane (6.3 g, 0.047 mole) in one portion and the reaction was stirred for 2 h at 80°C. The cooled mixture was diluted with water and extracted twice with ether. The combined extracts were washed with brine and dried over anhydrous magnesium sulfate. Removal of ether *in vacuo* gave 6.4 g (98%) of a solid residue that behaved as a single component upon analysis by thin layer chromatography (0.25 mm silica gel chromatoplate developed in benzene-ethyl acetate, 3:1) and gas-liquid chromatography (3% QF-1, 185°C). This product was used without further purification. NMR spectrum (δ, $CDCl_3$): 3.90 (s,3H), 6.27 (d,1H, J= 9 Hz), 6.90 (m,2H), 7.33 (s,1H), 7.58 (d,1H, J= 9 Hz).

6-Hydroxy-7-methoxycoumarin (III). 7-Methoxycoumarin (II; 2.64 g, 0.015 mole) dissolved in a solution of 10% sodium hydroxide (30 ml) and dimethoxyethane (45 ml) was stirred in an ice bath (to maintain a temperature below 15°C) during the dropwise addition of potassium persulfate (4.05 g, 0.015 mole) in water (80 ml) after which the reaction was stirred at room temperature for 16 hours. The mixture was acidified to pH 2 with conc. hydrochloric acid and extracted twice with ether. Conc. hydrochloric acid (10 ml) was added to the aqueous portion, which was stirred on the steam bath (80°C) for 1.5 h, cooled to room temperature, saturated with sodium chloride, and extracted twice with methylene chloride. The methylene chloride extracts were combined, dried over anhydrous magnesium sulfate, and concentrated *in vacuo* to give a solid residue (0.81 g, 31%). NMR spectrum (δ, acetone-d_6): 3.41 (br s,1H,OH), 4.02 (s,3H), 6.23 (d,1H, J= 9 Hz), 7.00 (s,1H), 7.10 (s,1H), 7.86 (d,1H, J= 9 Hz).

6,7-Dimethoxycoumarin (IV). 6-Hydroxy-7-methoxycoumarin (III; 0.5 g, 0.0026 mole) in dimethylformamide (10 ml) was stirred with powdered potassium hydroxide (0.17 g, 0.0030 mole) for 30 min. To this was added iodomethane (0.44 g, 0.0030 mole) in one portion. After stirring for 16 h at room temperature, the reaction was diluted with 20 ml water and extracted twice with ether. The combined ether extracts were washed with brine and dried over anhydrous magnesium sulfate. Removal of the solvent *in vacuo* gave an impure product (0.5 g), which was

purified by column chromatography over Florisil. Upon elution with hexane-methylene chloride (1:1) a single pure component homogenous by thin layer chromatography (0.41 g, 76%) was obtained. NMR spectrum (δ, $CDCl_3$): 3.95 (s,3H), 3.98 (s,3H), 6.25 (d,1H, J= 9 Hz), 6.90 (s,2H), 8.66 (d,1H, J= 9 Hz).

6,7-Dimethoxy-2,2-dimethylchromene-[2-methyl-^{3}H] (V). Methyl iodide-[^{3}H] (3 mmol, diluted to approx. 15 Ci/mmol) was manometrically transferred to a 25 ml flask containing Mg (3.2 mmol, 0.078 g) and anhydrous ether (5 ml). After formation of the Grignard reagent, the solvent was removed *in vacuo* and replaced with anhydrous tetrahydrofuran (3 ml; distilled from $LiAlH_4$). 6,7-Dimethoxycoumarin (IV; 0.053 g, 0.25 mmol) in 5 ml of anhydrous tetrahydrofuran was injected into the flask and the mixture was stirred overnight at room temperature. After quenching with 0.5 M ammonium chloride (1 ml), the reaction residue was extracted with diethyl ether (10 ml) and the combined ether extracts were transferred to a Carius tube. After removal of the ether under reduced pressure, the residue was dried by the sequential addition and removal of three portions (5 ml) of benzene. Glacial acetic acid (1 ml) was added, and the tube was evacuated, cooled (-78°C), sealed, and then heated at 100°C for 1 h. After cooling and removal of glacial acetic acid *in vacuo*, the crude product (5.2 Ci) was dissolved in benzene (100 ml) for storage. An aliquot (0.9 Ci) was purified using high pressure liquid chromatography (microparticulate silica column eluted with benzene), yielding 200 mCi of V-[^{3}H] (purity, 96%; 32 Ci/mmol). ^{1}H NMR spectrum (δ, $CDCl_3$): 1.38 (s,5.2H), 3.80 (s,6H), 5.46 (d,1H, J= 10 Hz), 6.23 (d,1H, J= 10 Hz), 6.40 (s,1H), 6.52 (s,1H); ^{3}H NMR spectrum (δ, $CDCl_3$): 1.35 (s), no other signals.

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