A CONVENIENT METHOD TO SYNTHESIZE SPECIFICALLY LABELLED CHOLESTEROL WITH TRITIUM

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SUMMARY

A simple method is described to label cholesterol with tritium. Cholesterol was first oxidized to 5-cholesten-3-one (2) which was then purified by HPLC. Its structure was established by electron impact (EI) mass spectrometry and ^{1}H-NMR spectroscopy. The ketone (2) was reduced with NaB^3H_4 to give specifically labelled cholesterol (C-3^3H) at low specific activity.

Key words: Cholesterol, tritium, specific labelling, oxidation, reduction

INTRODUCTION

Cholesterol is a major sterol of mammalian tissues. Its role as precursor to various steroids and bile acids and its association with various human diseases has captured the attention of chemists and biochemists over a long period of time (1,2). Radiolabelled cholesterol and its derivatives have been used to determine various metabolic pathways in mammals and in plants.

Tritium labelled cholesterol is widely used in chemistry and bio-organic sciences. There are several advantages of using tritium over ^{14}C. The most important is the higher molar radioactivity of tritium labelled compounds than their ^{14}C analogs. Commercially available tritiated cholesterol is commonly labelled at C-1, 2, 6 or 7. We decided to label cholesterol at a specific position (C-3) via an intermediate, 5-cholesten-3-one (2). The ketone (2) was reduced with NaB^3H_4 to give radioactive cholesterol (3).

EXPERIMENTAL

Synthesis of 5-cholesten-3-one (2):

Cholesterol (10mg, 0.025mmol) was stirred at room temperature with excess of pyridinium chlorochromate (PCC) in dichloromethane (5ml, dry) for 2 hrs. The resulting mixture was filtered through florisil. Ketone (2) was purified by HPLC using a reversed-phase column (Waters, ODS, 10μm, 25cm x 10mm i.d.). Methanol was used as a mobile phase at a flow rate of 1.5ml/min. The retention time for (2) was 17.2 min. Its EI mass spectrum indicated the molecular ion peak at m/z 384 (M^+) with other prominent peaks occurring at m/z 342, 275, 271, 229 and 124. ^{1}H-NMR (200MHz, $CDCl_3$, δppm), 5.33 (m, 1H, C-6H), 1.19 (s, 3H, C-19H), 0.70 (s, 3H, C-18H), 0.86 (d, 3H, J=6.4Hz, C-26H) and 0.92 (d, 3H, J=6.4Hz, C-27H).

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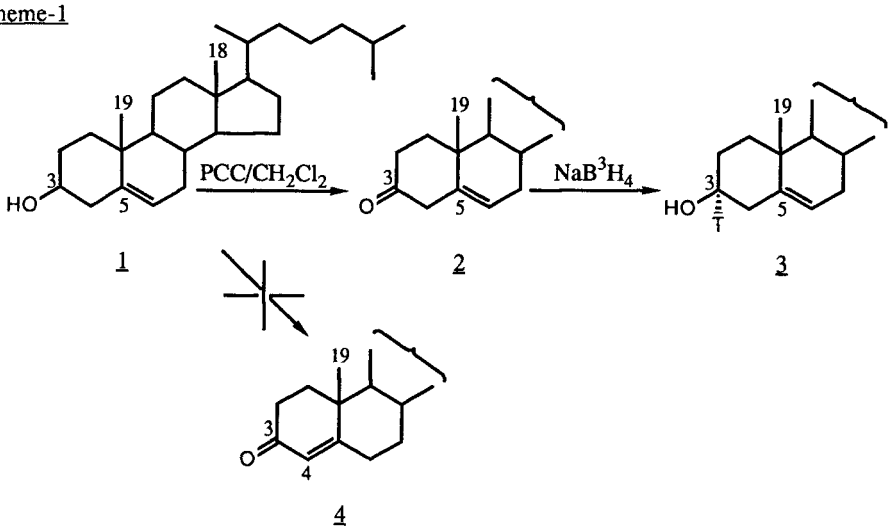
Synthesis of tritium labelled cholesterol (3) from 5-cholesten-3-one (2):

5-cholesten-3-one (2mg, 0.005mmol) was dissolved in a small amount of ethanol and NaB^3H_4 (10mCi, 600mCi/mmol) was added. Reaction mixture was stirred at r.t. for 3 hrs. $NaBH_4$ (2mg) was added to this mixture and stirring was continued for 1hr. Wet ether was added and the combined solvents were removed with stream of nitrogen. The residue was dissolved in diethyl ether and filtered. The resulting solution was subjected to a small silica gel column using 5% ethyl acetate in hexane as eluent. This resulted in 85% of (3) with a small amount of its C-3 epimer (14:86 ratio). The specific activity of purified radioactive cholesterol (3) was 1088μCi/mmol.

RESULTS AND DISCUSSION

The introduction of tritium or deuterium in place of hydrogen into an organic compound can be achieved in several ways. We used a simple strategy to label cholesterol (Scheme-1) in which cholesterol was oxidized to a stable intermediate, 5-cholesten-3-one (2), using pyridinium chlorochromate (PCC) as an oxidizing reagent (3,4). A more commonly used method to oxidize sterols to ketones is Jones' oxidation (5,6). The use of PCC in place of Jones' reagent makes the reaction simple and easy to workup. The purification of ketone (2) was achieved by reversed-phase HPLC and the structure of purified ketone was established by EI mass spectrometry and ^{1}H-NMR spectroscopy.

Scheme-1



The mass spectrum of ketone (2) indicated the molecular ion peak at m/z 384 with other prominent peaks occurring at m/z 342, 275, 271, 229 and 124. The occurrance of a peak at m/z 275 was characteristic for the presence of Δ^5 double bond in the steroid molecule (7). The structure of 2 was further substantiated by its ^{1}H-NMR spectrum which showed a multiplet centered at δ5.33 for an olefinic proton (C-6H) and a three proton singlet resonating at δ1.18 (C-19H).

A closely related isomer of 2 , with an identical molecular weight (M^+ 384), is 4-cholesten-3-one (4). The distinction between 2 and 4 was made on the basis of their mass and ^{1}H-NMR spectra. The mass spectrum of steroid (4) did not show any peak at m/z 275, suggesting the absence of Δ^5 double bond (8). In the ^{1}H-NMR spectrum of (4), the olefinic proton (C-4H) was found to resonate significantly down field, at δ5.71 in comparison to the olefinic proton of ketone (2) which resonated at δ5.33. Finally the ketone (2) was reduced with NaB^3H_4 in ethanol (9) to give labelled (C-3^{3}H) cholesterol (3). The position of the label at C-3 was proved by oxidizing labelled cholesterol (3) with PCC to a ketone (2). This ketone was reduced with $NaBH_4$ to give cholesterol (1) which had no radioactivity, confirming the label specificity.

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