A facile synthesis of [^{14}C]epicholesterol

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Summary A facile procedure is described for the preparation of [^{14}C]epicholesterol from [^{14}C]cholesterol. Cholesterol is first converted to cholesteryl mesylate, which is treated with cesium acetate and 18-crown-6 in refluxing toluene to give epicholesteryl acetate. The latter is hydrolyzed, without isolation, with potassium hydroxide in tetrahydrofuran–methanol to give epicholesterol, which is obtained in pure form by preparative thin-layer chromatography. **—Yan, J., and R. Bittman.** A facile synthesis of [^{14}C]epicholesterol. *J. Lipid Res.* 1990. **31:** 160–162.

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Epicholesterol (5-cholesten-3α-ol) has been used extensively in model membranes to elucidate the role of cholesterol in regulating the permeability and lipid packing of biological membranes (1–4). One approach to the study of phospholipid-cholesterol interactions involves the use of isotopically labeled cholesterol (5–9). The only isotopically labeled derivative of epicholesterol that has been used so far in membrane research is [3β-^{2}H]cholest-5-en-3α-ol, which was prepared by sodium borodeuteride reduction of cholest-5-en-3-one (10); this compound was shown by ^{2}H NMR spectroscopy to be oriented differently than cholesterol with respect to the normal to the bilayer plane (10). We report here a new method of synthesis of [^{14}C]epicholesterol from [^{14}C]cholesterol. Biophysical studies of the movement of this compound between membranes will be reported elsewhere.

NMR relaxation measurements with [4-^{13}C]cholesterol have been used to estimate the exposure of the hydroxyl group to the aqueous phase in bilayer vesicles (5, 6) and lipoproteins (7, 8). The method we describe here for the preparation of [^{14}C]epicholesterol can be used to prepare [4-^{13}C]epicholesterol from the readily available [4-^{13}C]-cholesterol, thus making possible ^{13}C NMR studies of the segmental motions of the epicholesterol C_4 atom in bilayers.

MATERIALS AND METHODS

[4-^{14}C]Cholesterol (57.5 Ci · mol^{-1}) was purchased from DuPont-New England Nuclear (Boston, MA). Cholesterol was obtained from Sigma Chemical Co. (St. Louis, MO) and was recrystallized twice from ethanol with 5% acetone. Methanesulfonyl chloride and cesium carbonate were obtained from Aldrich Chemical Co. (Milwaukee, WI). 18-Crown-6 was from Fluka Chemical Corp. (Ronkonkoma, NY). The solvents were dried as follows. Dichloromethane was distilled from calcium hydride and stored over 4A molecular sieves. Toluene and triethylamine were distilled from and stored over calcium hydride. Methanol was dried over type 4A molecular sieves. Cesium acetate was prepared by adding 4 drops of glacial acetic acid to 70 mg (0.22 mmol) of cesium carbonate (Aldrich) in 2.5 ml of dry methanol. After the mixture was stirred for 1 h at room temperature, the solvents were removed under vacuum, leaving 82 mg (0.43 mmol) of cesium acetate on thorough drying in a vacuum desiccator.

Chromatography

Silica gel GF thin-layer chromatography (TLC) plates (250- and 1000-μm) were purchased from Analtech (Newark, DE). The TLC plates were developed in ether–petroleum ether 1:1 (v/v), and the spots were visualized by exposing the plates to iodine. In some experiments, the spots were visualized by spraying the plate with 10% H_2SO_4, followed by charring of the plate on a hot plate. High-pressure liquid chromatography (HPLC) was carried out with a Perkin-Elmer model 410 liquid chromatograph equipped with a model LC235 diode array detector and LCI100 recorder (Perkin-Elmer, Norwalk, CT). The HPLC column used was 5 μm C_{18}-Carbosphere (250 mm × 4.6 mm), which was purchased from Phenomenex (Rancho Palos Verdes, CA).

Spectrometry

^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on IBM-Bruker 200-MHz and GE QE 300-MHz spectrometers using tetramethylsilane as the reference. Proton-decoupled ^{13}C NMR spectra of epicholesterol and cholesterol (60 mg/ml) were recorded on a GE QE 300 MHz spectrometer at 75 MHz with a resolution of 1.2 Hz using a 90° pulse, 1000 scans, 1 s repetition rate; δ values refer to the central line of $CDCl_3$ (δ = 77.00 ppm). Infrared spectra were obtained with a Perkin-Elmer Model 598 spectrophotometer. Mass spectra were recorded on a Hewlett-Packard 5988A GC-quadrupole mass spectrometer. Optical rotations were measured in a 1-dm cell on a Jasco DIP-140 polarimeter. Radioactivity was counted using a Packard 2000CA liquid scintillation counter.

Melting point

Melting points were measured on a Fisher-Johns apparatus and are uncorrected.

Abbreviations: TLC, thin-layer chromatography; HPLC, high pressure liquid chromatography.

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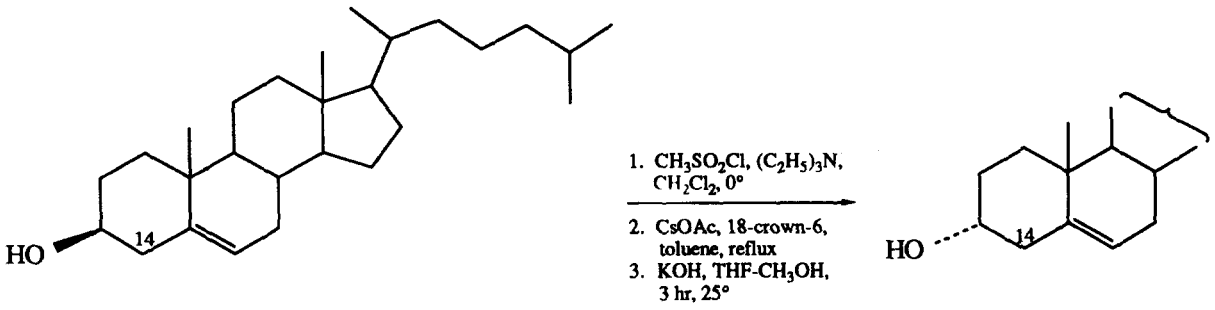

Scheme. 1. Reaction sequence for the preparation of [4-^{14}C]epicholesterol from [4-^{14}C]cholesterol.

Synthesis of [^{14}C]epicholesterol (Scheme 1)

To 40 mg (0.104 mmol) of cholesterol containing 10 μCi of [4-^{14}C]cholesterol in 3 ml of dichloromethane was added 157 mg (216 μl, 1.55 mmol) of triethylamine. After the solution was cooled to 0°C, a solution of 29 mg (0.25 mmol) of methanesulfonyl chloride in 1 ml of dichloromethane was added dropwise over a 20-min period under nitrogen. The reaction mixture was stirred for 3 h at 0°C, then diluted with ether and washed with 5% aqueous HCl (10 ml) and water (3 × 10 ml). The organic layer was dried (Na_2SO_4) and concentrated on a rotary evaporator. The residue of [4-^{14}C]cholesteryl mesylate was dissolved in 10 ml of toluene and was added to 82 mg (0.43 mmol) of cesium acetate and 30 mg (0.11 mmol) of 18-crown-6. After the mixture was refluxed for 88 h, toluene was removed under reduced pressure. The resulting epicholesteryl acetate was hydrolyzed without isolation by addition of 1 ml of THF and 2 ml of 5% KOH in methanol, followed by stirring for 3 h at room temperature. The solvents were removed and the residue was extracted with ether several times. Removal of the ether left a residue that was dissolved in a minimum volume of chloroform. Purification of the crude product by preparative TLC gave [4-^{14}C]epicholesterol (R_f 0.53) and recovered [4-^{14}C]cholesterol (R_f 0.42) in a ratio of 6:1 (w/w). The purified [^{14}C]epicholesterol thus obtained was then analyzed by TLC by scraping zones from the TLC plate and counting in a liquid scintillation counter; all of the radioactivity was found to correspond to the R_f value of unlabeled epicholesterol. The overall yield of [4-^{14}C]epicholesterol from repitition of this experiment a number of times ranged from 40 to 53%. The infrared and ^{1}H NMR spectra of unlabeled epicholesterol prepared by this procedure were identical to those obtained with the commercially available sterol; mp 141.5–142°C; MS 386; IR (KBr) 3200–3600 cm^{-1}, 1640 cm^{-1}; HPLC R_t 16.8 min (elution with acetonitrile-2-propanol, 4:1; flow, 1 ml/min); [α] −35.87° (c 1.0, C_2H_5OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.00 (1 H, m, $C_{3\beta}$-H), 5.45 (1 H, C=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.50 (C_5), 123.96 (C_6), 67.07 (C_3), 56.70 and 56.07 (C_{14}, C_{17}), 50.30 (C_9), 42.26 (C_{13}), 39.81, 39.72, 39.48, 37.30, 36.15, 35.77, 33.18, 31.95, 31.80, 28.87, 28.20, 27.99, 24.24, 23.80, 22.81, 22.54, 20.75, 18.69 and 18.63 (C_{19}, C_{21}), 11.82 (C_{18}). [For comparison, cholesterol exhibits the following differences in HPLC and spectra: R_t 22.1 min (elution with acetonitrile-2-propanol, 4:1; flow, 1 ml/min); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.52 (1 H, m, $C_{3\alpha}$-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.70 (C_5), 121.68 (C_6), 71.75 (C_3), 42.25 (C_4, C_{13}), 19.37 (C_{19}). (For assignments of the chemical shifts of cholesterol in chloroform, see refs. 11, 12.) It is noted that in epicholesterol the low-intensity resonance of the quaternary C_{13} atom is observed at δ 42.26 since the C_4 signal is shifted to δ 39.81; the C_4 and C_{13} signals overlap in cholesterol. The most marked shift, however, in the ^{13}C NMR spectra of cholesterol and epicholesterol is observed for the C_3 signal (δ 67.07 in epicholesterol, δ 71.75 in cholesterol), in agreement with a previous report (10).] Integration of the ^{1}H NMR spectrum of epicholesterol on an expanded scale in the region δ 3.2–4.2 indicated that contamination of cholesterol was <1% in the product obtained after preparative TLC.

DISCUSSION

We have reported a simple procedure for inversion of the configuration of [^{14}C]cholesterol. Acetate ion, which is solubilized in refluxing toluene in the presence of the Cs^+-18-crown-6 complex, was found to be an effective nucleophile for the displacement of the mesylate group and inversion of configuration at C-3. Lower yields were found when toluene was replaced by xylene or dimethylformamide, when dicyclohexano-18-crown-6 was used instead of 18-crown-6, and when the trifluoromethanesulfonate (triflate) group was used in place of the mesylate group.

Previous procedures for inversion of the configuration of the secondary hydroxyl group of cholestanol and C_{27}-steroid bile acids have been reported to proceed in high yield (13–17). However, inversion of the C_3-hydroxyl group is complicated by the presence of the Δ^5-bond of cholesterol; for example, the Mitsunobu reaction of cholesteryl tosylate resulted in retention of configuration, apparently via inversion of the initially formed 3,5-cyclocholestan-6α-yl derivative (18). Indeed, formation of this cyclic product is

recognized as an important side product in displacement reactions of cholesterol derivatives (18, 19). The removal of the 3β-toluenesulfonate group of cholesterol by superoxide displacement is accompanied by the formation of cholestadiene as a byproduct (20). The method of preparation of epicholesterol presented here did not give rise to byproducts involving elimination and homoallylic rearrangement. The facile method we describe for the inversion of the C_3-hydroxyl group of ^{14}C-labeled cholesterol is likely to be preferred to the process of reduction of cholest-5-en-3-one (21, 22), since the latter method is lengthier [requiring oxidation of cholesterol, isolation of the 3-keto product, and reduction to give of C_3-axial and equatorial hydroxyl groups (10)].

Studies of the movement of [^{14}C]epicholesterol between membranes are in progress. The possibility of preparing [^{13}C]epicholesterol from commercially available [^{13}C]cholesterol offers the opportunity to examine epicholesterol-phosphatidylcholine and epicholesterol-protein interactions with further definition than reported previously (e.g., refs. 23, 24).

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