Synthesis of C-22, C-23-^{3}H-labeled 3α,7α,12α-trihydroxy-5β-cholestane

R. Somanathan and S. Krisans[1]

Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, CA 92182

Summary This report describes an efficient synthesis of C-22, C-23-^{3}H-labeled 3α,7α,12α-trihydroxy-5β-cholestane. — **Somanathan, R., and S. Krisans.** Synthesis of C-22, C-23-^{3}H-labeled 3α,7α,12α-trihydroxy-5β-cholestane. *J. Lipid Res.* 1985. **26:** 774–775.

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3α,7α,12α-Trihydroxy-5β-cholestane is an important intermediate in the biosynthesis of cholic acid from cholesterol (1). Two methods of synthesis have been reported for tritium-labeled trihydroxy cholestane. One method is with ^{3}H at carbons 2 and 4 obtained from cholic acid by oxidizing the 3-hydroxy group to a ketone and exchanging the acidic protons on C-2 and C-4 with tritiated water in the presence of a catalyst (2, 3).

The second method involves the preferential oxidation of a 7α-OH group to a ketone using N-bromosuccimide and then reducing it with NaB^3H_4, thereby introducing a tritium atom at C-7(4). Both methods have several major drawbacks. In the first method the exchange is never complete and at equilibrium only partial exchange of tritium at C-2 and C-4 is obtained. The major problem in both methods, however, is that reduction of the ketones at C-3 and C-7 leads to α- and β-hydroxyls and chromatographic separation is required in order to obtain the desired α-hydroxy product. Here we describe an efficient synthesis of 3α,7α,12α-trihydroxy-5β-cholestane specifically labeled at C-22 and C-23 with tritium of high specific activity. This new method of synthesis is not subject to the above disadvantages.

METHODS

Melting points were determined with a Fischer hot-stage apparatus and are uncorrected.

Thin-layer chromatography was done on Silica Gel-60F-254 plates (EM Reagents, 0.2 mm thickness). The spots were detected with sulfuric acid (10%). Preparative thin-layer chromatography was performed on a Chromatotron (Model 7924, Harrison Research, Palo Alto, CA). Infrared spectra were recorded on a Perkin-Elmer 621 spectrometer. ^{1}H-NMR-spectra were measured on a varian EM 390 (90 MHz) spectrometer. Mass spectra were determined on a Hitachi RMU 6E spectrometer. Tritiation was carried out at the New England Nuclear Laboratories, Boston, MA.

RESULTS

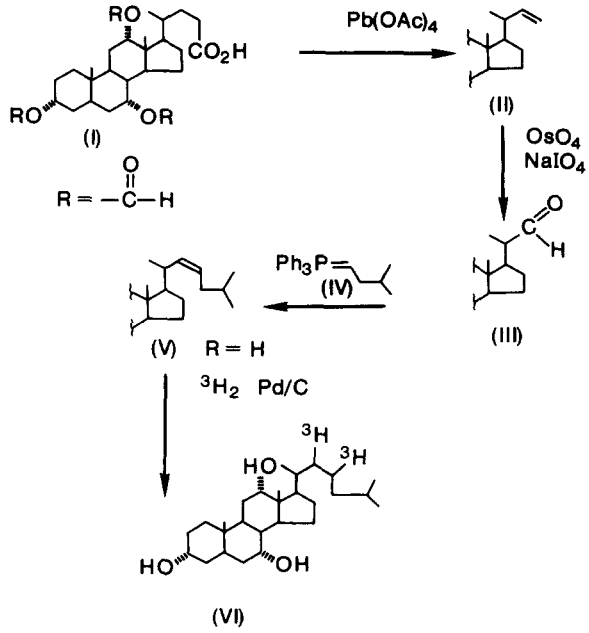
Preparation of 24-nor-5β-chol-22-ene-3α,7α,12α-triformate (II)

Compound (II) (**Scheme 1**) was synthesized in 50% yield from (I) by the method described by Carlson and coworkers (5), mp 180–184°C (lit. 5 186–188°C). NMR, δppm ($CDCl_3$) 8.15 (s, 1H,-OCOH), 8.05 (s, 1H,-OCOH), 8.00 (s, 1H,-OCOH), 5.60 (m, 1H,=CH), 5.20 (br.s, 1H, C-12 βH), 5.05 (br.s, 1H, C-7 βH), 4.85 (m, 3H, $=CH_2$, 3βH), 1.00 (s, 3H, C-19), 0.85 (s, 3H, C-18).

M/e 446 (M^+), 400 (-HCOOH), 354 (-HCOOH), 308 (-HCOOH).

Preparation of bisnorcholyl aldehyde (III)

To a stirred solution of the olefin (II) (400 mg) in dioxane (5 ml) and water (15 ml) was added a catalytic amount of osmium tetroxide (10 mg). When the solution turned deep brown, finely powdered sodium periodate (2.00 g) was added portion-wise over 30 min, maintaining the temperature at 24–26°C. After 2 hr of stirring the



Scheme 1.

Abbreviation: TLC, thin-layer chromatography.

[1]To whom correspondence should be addressed.

reaction mixture was extracted with diethylether (3 × 100 ml) and the combined organic layers were washed several times with a saturated solution of sodium thiosulfate (800 ml) followed by potassium bicarbonate (2 × 50 ml) and water. The ether layer was dried over $MgSO_4$; removal of solvent gave (III) as a crystalline solid (272 mg), 68% mp 162–165°C, IR (KBr) 1715, 1460, 1390, 1180, 950, 900 cm^{-1}. NMR δppm ($CDCl_3$) 9.35 (d, 1H, J3Hz, -CHO), 8.05 (s, 1H, -OCOH), 7.95 (s, 1H, -OCOH), 7.90 (s, 1H, -OCOH), 5.12 (br.s, 1H, C-12βH), 5.00 (br.s, 1H, C-7βH), 4.60 (m, 1H, C-3βH), 1.05 (d, 3H, J6Hz, C-21), 0.95 (s, 3H, C-19), 0.75 (s, 3H, C-18).
M/e 448 (M^+), 402(-HCOOH), 356(-HCOOH), 310(-HCOOH).

Preparation of 3α,7α,12α-trihydroxy-5β-cholest-22-ene (V)

To a suspension of isoamyltriphenylphosphonium bromide (700 mg) in dry tetrahydrofuran (50 ml) at −78°C was added excess n-butyllithium (1.5 ml, 1.5 M solution in hexane, Aldrich). The solution turned bright orange due to ylide (IV) formation, dissolving into the solution. The aldehyde (III) (430 mg) in dry tetrahydrofuran (5 ml) was added dropwise to the stirred solution and mixed for an additional 3 hr. During this period the reaction was followed by TLC, using benzene–acetone 1:1. At the end of the reaction the mixture was carefully diluted with methanol and the solvent was removed under reduced pressure. The residue was extracted with dichloromethane and separated on the Chromatotron using carbon tetrachloride–acetone (3:1 as eluant to give (V) as a crystalline solid in 48% yield, mp 155–158°C. IR (KBr) 3400, 1450, 1375, 1080, 1045, 980, 950, 915, 860, 610 cm^{-1}. NMR δppm ($CDCl_3$) 5.15 (m, 1H, =CH), 3.85 (m, 1H, =CH) 1.00 (d, 3H, J6Hz, C-21) 0.85 (s, 3H, C-18).
M/e 418 (M^+), 400 ($-H_2O$), 382 ($-H_2O$), 364 ($-H_2O$), 272 ().

Preparation of 22,23-^{3}H-3α,7α,12α-trihydroxy-5β-cholestane (VI)

Titration was carried out in the laboratories of New England Nuclear by stirring the olefin (V) (42 mg) with Pd/C (50 mg) in dry ethyl acetate under an atmosphere of tritium gas for 2.5 hr. The labile tritium was removed by washing with chloroform and methanol to give (VI) with a specific activity of 19.4 Ci/mmol.

In order to verify the location of the tritium atoms, ^{3}H-NMR analysis of the compound was performed in the laboratories of New England Nuclear. The results showed the saturated compound (3α,7α,12α-trihydroxy-5β-cholestane) was predominantly 22,23-tritiated material, formed mainly as the 22-mono-^{3}H and 23-mono-^{3}H, with small amounts of 22,23-di-^{3}H present. In addition, trace amounts of 20-^{3}H and 24-^{3}H material were also detected, most likely formed by allylic exchange at either of these sites before the reduction of the double bond. About 15% of the preparation after tritiation remained in the unsaturated form (3α,7α,12α-trihydroxy-5β-cholest-22-ene). ^{3}H-NMR analysis of this compound showed ^{3}H labels on 22-mono-^{3}H and 23-mono-^{3}H with only a very small amount of 22,23-ditritiated-22-ene present.

DISCUSSION

Using the method described by Carlson and co-workers (5), cholic acid triformate (I) was converted to the olefin (II) using lead tetraacetate. The olefin was then oxidized to the aldehyde (III) using a catalytic amount of osmium tetroxide and sodium periodate (6). This method gave excellent yields of the aldehyde and eliminated an extra step compared to the method described by Kihira, Kuramoto, and Hoshita (7). The aldehyde (III) was then condensed with the ylide (IV) (8), generated in situ with n-butyllithium, to give the olefin (V) in 48% yield. The olefin (V) from the above reaction was tritiated (New England Nuclear) to give the desired trihydroxy cholestane (VI) with ^{3}H labels at C-22 and C-23 and a specific activity of 19.4 Ci/mmol.

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