LABELED BILE ACIDS VI: [22,23,24-$^{13}C_3$]-METHYL 3α,7α-DIACETOXY-5β-CHOLAN-24-OATE, [22,23,24-$^{13}C_3$]-METHYL 3β-ACETOXYCHOL-5-EN-24-OATE, AND [16,17,22,23-$^{3}H_4$]-METHYL 3β-ACETOXYCHOL-5-EN-24-OATE (1)

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SUMMARY

A general short procedure for the introduction of ^{13}C to the side chain of bile acids is described. Suitable (Z)-pregn-17(20)-enes are key intermediates, while the isotope is introduced by an ene reaction with [1,2,3-$^{13}C_3$]-methyl propiolate. For the labeling with tritium, the unlabeled product of the ene synthesis, a $\Delta^{5,16,22}$-triene was saturated selectively at 16,17 and 22,23 with tritium gas.

Key Words: Bile acids, [22,23,24-$^{13}C_3$]-methyl-3α,7α-diacetoxy-5β-cholan-24-oate, [16,17,22,23-$^{3}H_4$]-methyl-3β-acetoxychol-5-en-24-oate, carbon-13-labeled, tritium-labeled.

INTRODUCTION

In two previous publications (2,3) we described a general method for the labeling of the side chain of bile acids with ^{13}C at the 2 terminal positions (C-23 and 24). In the present paper we report a method which allows the labeling of the last 3 terminal positions, thereby increasing the utility of thusly labeled substrates (4), which are useful in the study of the catabolism of cholesterol in the liver. In addition we selectively tritiated a $\Delta^{5,16,22}$ intermediate with tritium to give [16,17,22,23-$^{3}H_4$]-methyl 3β-acetoxychol-5-en-24-oate (7) of very high specific activity.

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DISCUSSION

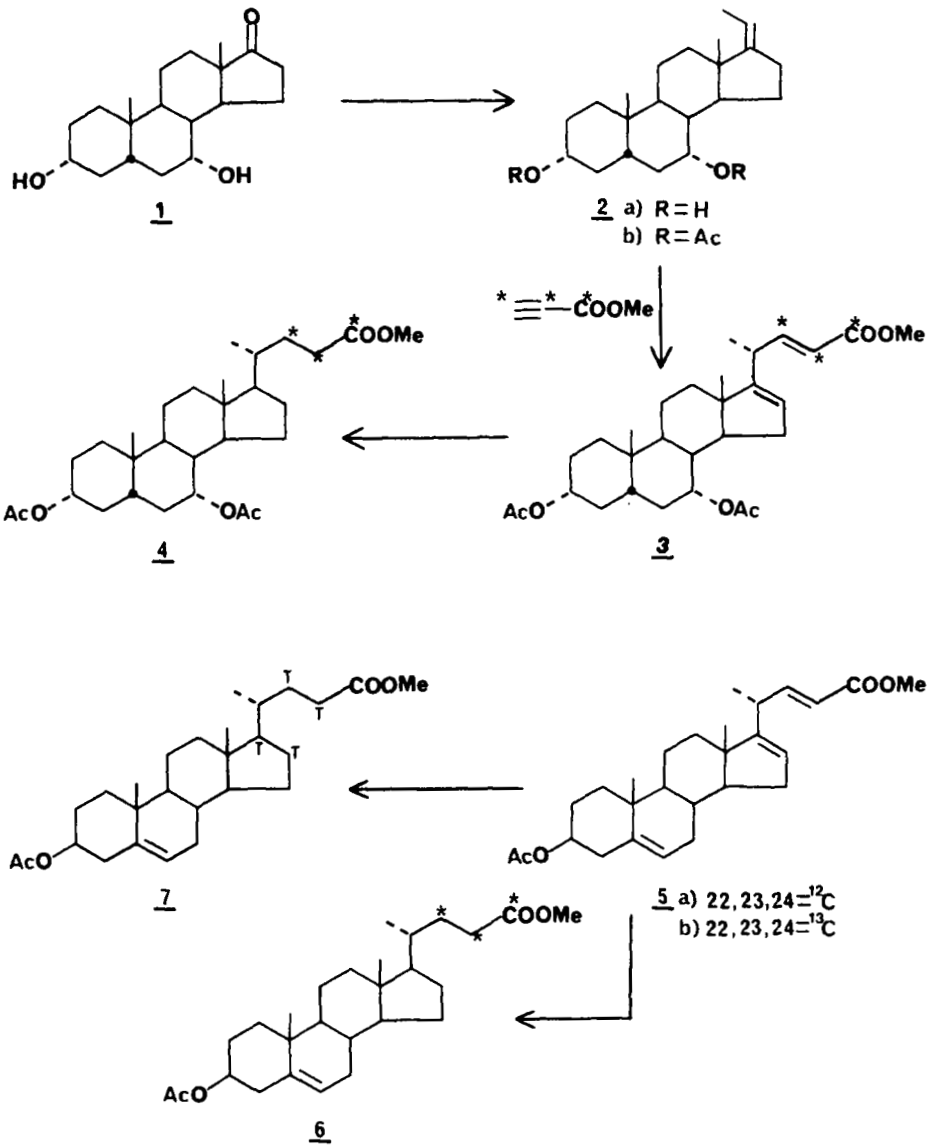
A recent publication (5) describes the preparation of the starting material, 3α,7α-dihydroxy-5β-androstan-17-one (1), which was used for the synthesis of [22,23,24-$^{13}C_3$]-chenodeoxycholic acid, as already described (6) recently for unlabeled material. The diol 1 was reacted with ethyledenetriphenylphosphorane to give the (Z)-olefin 2a which was then acetylated to yield the diacetate 2b. The ethylene diacetate was subjected to an ene reaction ([1,2,3-$^{13}C_3$]-methyl propiolate and ethylaluminum dichloride) to give [22,23,24-$^{13}C_3$]-methyl 3α,7α-diacetoxy-5β-chola-16,22-dien-24-oate (3). Catalytic hydrogenation with 5% palladium-on-charcoal in ethyl acetate gave the chenodeoxycholic acid derivative 4 which was identical (HPLC, IR and TLC) to an authentic, unlabeled material (7).

The labeling of methyl 3β-acetoxychol-5-en-24-oate was carried out as already described (8,9) for the unlabeled compound. (Z)-3β-Acetoxypregna-5,17(20)-diene was reacted (10) with [1,2,3-$^{13}C_3$]-methyl propiolate to give [22,23,24-$^{13}C_3$]-(Z)-methyl 3β-acetoxychola-5,16,22-trien-24-oate (5b). Selective catalytic reduction of the triene at positions 16 and 22 (possibly due to the difference in the steric hinderance) gave the desired [22,23,24-$^{13}C_3$]-methyl 3β-acetoxychol-5-en-24-oate (6).

For the labeling with tritium, the unlabeled (Z)-methyl 3β-acetoxychola-5,16,22-trien-24-oate (5a) was selectively reduced [5% platinum-on-charcoal (8,9)] to give the desired tritiated 3β-acetoxycholenate 7 with a specific activity of 148 mCi/mg (64.8 Ci/mmol).

EXPERIMENTAL

Melting points are uncorrected. The NMR spectra were obtained in deuteriochloroform solution using tetramethylsilane as an internal reference and were recorded for ^1H on a 90 MHz Varian EM-390 spectrometer, and for ^{13}C on a Jeol FX-90 Q spectrometer. Mass spectra were recorded on a Nuclide 1290 G spectrometer. The IR spectra of crystals were determined as KBr-pellets, and of oils as a film on sodium chloride windows (Perkin Elmer Model 237). Tritiation was done by New England Nuclear/Dupont Products. Radiogram TLC was run on Model 930 Auto-Scanner of Vangard systems.



(Z)-5β-Pregn-17(20)-ene-3α,7α-diol (2a). To 3 mL of anhydrous t-butanol under a stream of nitrogen was added 250 mg (6.9 mmol) of potassium. The mixture was warmed to 60°C until all potassium was dissolved. Then the excess solvent was evaporated in vacuo, 10 mL of dry tetrahydrofuran was added, followed by the addition of 1 g (6.3 mmol) of ethyltriphenylphosphonium bromide. The characteristic bright orange solution was kept at 60°C for 30 min and then a solution of 190 mg (0.62 mmol) of the ketone 1 in 5 ml of dry tetrahydrofuran was added.

The reaction mixture was stirred at 60°C under nitrogen for 3 days and then

poured into an ice cold 10% HCl solution. The product was exracted with CH_2Cl_2 (5 x 20 mL). The methylene chloride solution was concentrated and the crude product chromatographed on a preparative TLC (Rf=0.68; 50% acetone/hexane) to yield the olefin 2a (156 mg, 79%). An analytical sample was recrystallized from ethyl acetate, m.p. 158-160° (Lit. (6) 161-162°C), and was identical to an authentic sample (IR, NMR, and mixed mp).

[22,23,24-$^{13}C_3$]-Methyl 3α,7α-diacetoxy-5β-chola-16,22-dien-24-oate (3). To a stirred solution of [1,2,3-$^{13}C_3$]-methyl propiolate (50 µl) in methylene chloride (1.5 mL) under a nitrogen atmosphere was added 25% ethylaluminum dichloride in toluene (0.3 mL), immediately followed by the addition of a solution of 100 mg of (Z)-5β-pregn-17(20)-ene-3α,7α-diacetate (2b) in 1.5 mL of CH_2Cl_2. After 2 hours the reaction mixture was poured into an ice cold saturated sodium dihydrogenphosphate solution. The mixture was extracted with methylene chloride. The organic phase was washed with water and brine, then dried and evaporated to yield syrupy material. The purification on PLC (Rf=0.39; 20% acetone in hexane) gave 80 mg of a syrup 3 and 20 mg of starting material; ^1H NMR: δ 0.75 (3H, s, 18-Me), 0.95 (3H, s, 19-Me), 2.00 and 2.03 (3H, s, 3 and 7-OAc), 3.72 (3H, d, J = 3 Hz, 24-CO_2Me), 4.60 (1H, m, 3-H), 4.97 (1H, br. s, 7-H), 5.06 (1H, br. s, 16-H); ^{13}C*** NMR: δ 118.737 (dd, J = 74.470 and 70.840 Hz, ^{13}C-23), 153.519 (d, J = 69.580 Hz, ^{13}C-22), 167.281 (d, J = 74.470, ^{13}C-24); MS of 3α,7α-OTMS (11) m/e 459 [(M+3)-TMSOH], 458 [(M+2)-TMSOH], 457 [(M+1)-TMSOH]; Isotopic Purity: 61% (M+3), 24% (M+2), 15% (M+1).

[22,23,24-$^{13}C_3$]-Methyl 3α,7α-diacetoxy-5β-cholan-24-oate (4). To a solution of 3 (70 mg) in 3 mL of EtOAc was added 10 mg of 10% Pd/C. The suspension was stirred under a hydrogen atmosphere until the theoretically calculated amount of hydrogen was absorbed. The mixture was filtered through a bed of celite. the solvent was evaporated to give 62 mg of product (4). This material was identical (HPLC, IR and TLC) to an authentic (7) unlabeled sample.

[16,17,22,23-$^3H_4$]-Methyl 3β-acetoxychol-5-en-24-oate (7). To 80 mg (0.19 mmol) of the triene 5a in 2 mL of ethyl acetate was added 14 mg of 5% Pt-C cata-

*** In ppm (± 1.3 Hz) downfield from TMS.

lyst and 25 Ci (0.42 mmol) of tritium gas and the reaction mixture stirred at room temperature for 24 h (12). The total activity of the crude after removing the labile tritium amounted to 1.4 Ci, corresponding to a specific activity of 178 mCi/mg. An aliquot was chromatographed on a celite partition column using aqueous methanol as stationary and hexane as mobile phase. The clean material, isolated in ca. 17% yield, had a specific activity of 148 mCi/mg (64.8Ci/mmol), and was identical to an authentic material (Isotope dilution showed no change in specific activity from initial value after three recrystallizations and TLC; Rf=0.35, 10% ethylacetate/hexane).

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