LABELED BILE ACIDS II: METHYL 3β-HYDROXYCHOL-5-EN-24-OATE, LABELED WITH ^{13}C AND/OR ^{2}H (1)

Chang Yon Byon, Marcel Gut* and Chee Kong Lai

The Worcester Foundation for Experimental Biology

Shrewsbury, MA 01545

SUMMARY

The incorporation of ^{13}C, and/or $^{2}H_2$, into methyl 3β-hydroxychol-5-en-24-oate to give products with an abundance of the isotopic label at a specific position, useful for metabolism studies, has been accomplished.

Key Words: [22,23,24-$^{13}C_3$]-methyl-3β-hydroxychol-5-en-24-oate, [23,24-$^{13}C_2$-22,23-$^{2}H_2$]-methyl-3β-hydroxychol-5-en-24-oate.

INTRODUCTION

The synthesis of the tritiated title compound has recently been described (2). However, as already indicated in our preceding paper (3), there is an immense advantage in labeling with stable isotopes, since these molecules can be used in the clinic with control subjects in any desired amounts. In most, if not all, cases the stable isotopes are cheaper than their radioactive counterparts, work is easier, and a mass + 2 will already suffice for obtaining meaningful results, as we have already discussed previously (3).

Although the introduction of ^{18}O (3) into the title compound is easier, the application of that method is somewhat limited. For every biological application the possibility of loss of label by either elimination followed by hydration, or by oxidation and equilibration of the intermediary ketone, followed by reduction, has to be checked.

*Author to whom correspondence is to be addressed.

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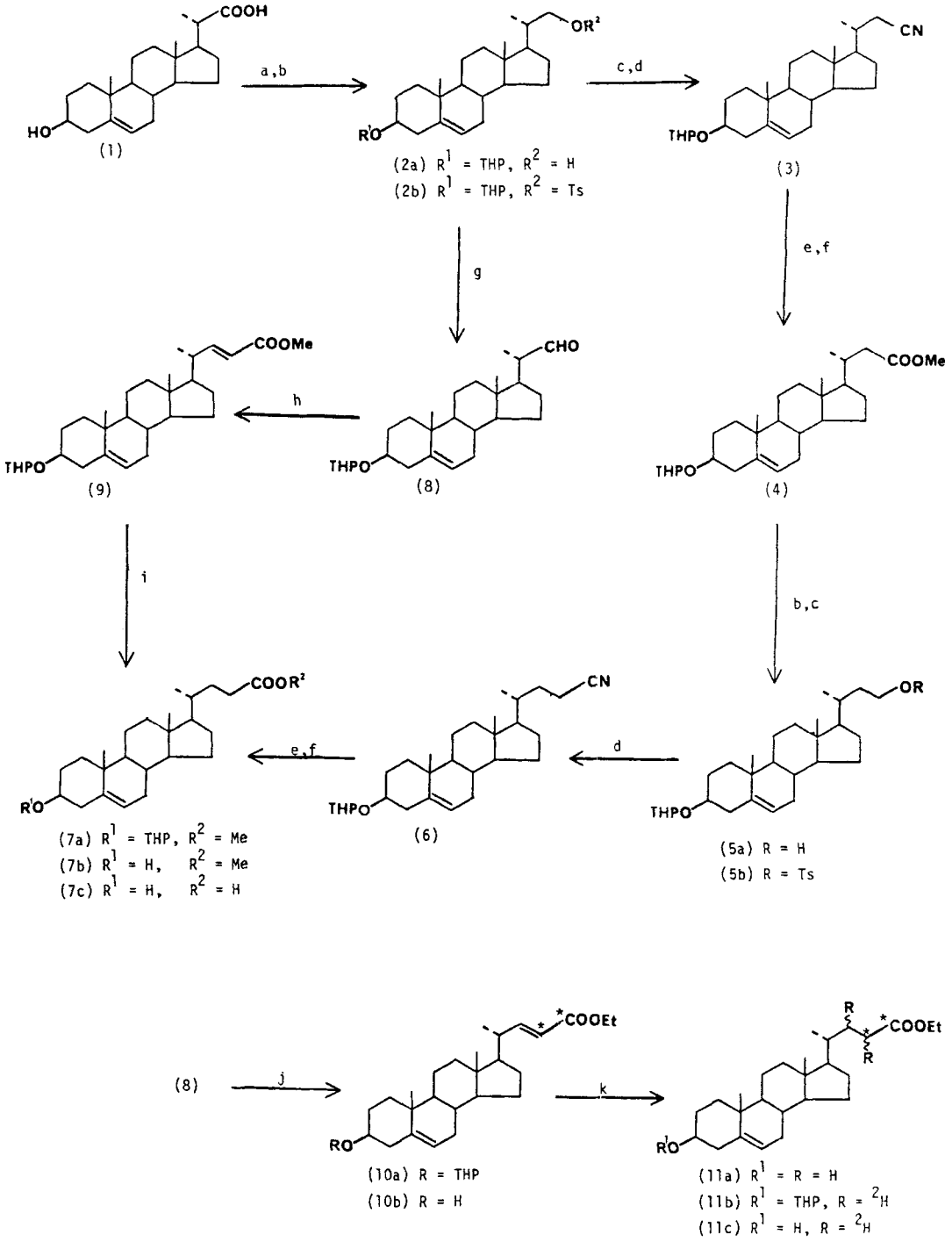

(a) TsOH/dihydropyran, (b) LAH, (c) TsCl/Py, (d) KCN/DMSO, (e) KOH/EtOH, (f) CH_2N_2, (g) PCC/CH_2Cl_2
(h) ϕ_3P = CH-COOMe, (i) PtO_2/H_2/EtOH, (j) ϕ_3P = *CH-*COOEt, (k) PtO_2/D_2/EtOD, "*" indicates ^{13}C

DISCUSSION

In order to study the metabolites of 3β-hydroxychol-5-en-24-oic acid in humans we prepared that bile acid labeled with stable isotopes.

In our first approach, in which C-23 and C-24 are introduced in sequence, we started from the known 3β-hydroxy-22,23-bisnorchol-5-en-24-oic acid (1) which was first transformed to its 3β-tetrahydropyranyl ether and then reduced to 22,23-bisnorchol-5-ene-3,24-diol 3β-tetrahydropyranyl ether 2a. This alcohol was tosylated to its ester 2b and the tosyloxy group displaced by cyanide to give the nitrile 3. Hydrolysis of the nitrile gave a carboxylic acid the ester of which was reduced with lithium aluminum hydride to its alcohol 5a. Repetition of this reaction sequence, namely a) tosylation, b) displacement by cyanide, c) hydrolysis, gave the desired product 7. The procedure is described in detail in EXPERIMENTAL PART, however not with labeled reagent ($K^{13}CN$), since we developed simultaneously more efficient methods of labeling, described below.

The aldehyde 8 (4) was condensed (5) with carbomethoxymethylenetriphenylphosphorane (6) to yield methyl 3β-tetrahydropyranyloxychola-5,22-dien-24-oate (9). The desired cholenate 7b was prepared by the selective hydrogenation of the C-22 double bond, followed by the hydrolysis of the tetrahydropyranyl ether. This sequence (8 → 9 → 7b) was repeated by using [1,2-$^{13}C_2$]-ethyl bromoacetate, obtained from 90 Atom % $^{13}CO_2$ for the preparation (5) of [1,2-$^{13}C_2$]-carboethoxymethylenetriphenylphosphorane. In order to obtain masses of higher order than mass + 2 the saturation of the double bond was carried out with deuterium. Differences of the products labeled with ^{13}C and of the unlabeled species as revealed in the IR and NMR spectra are given in the EXPERIMENTAL.

EXPERIMENTAL

Melting points are uncorrected. Nuclear magnetic resonance (NMR) spectral data are reported in parts per million (ppm) deshielded with respect to tetramethylsilane and were recorded for ^1H on a 90 MHz Varian EM-390 spectrometer, and for ^{13}C on a Joel FX-60 spectrometer. Mass spectra were recorded on a Nuclide 1290 G spectrometer.

22,23-Bisnorchol-5-ene-3,24-diol 3β-tetrahydropyranyl ether (2a) from 1. To the stirred mixture of 10 g of 3β-hydroxy-22,23-bisnorchol-5-en-24-oic acid (1) and 200 mg of p-toluenesulfonic acid in 100 mL tetrahydrofuran was added 7 mL of dihydropyran. After 20 min all solids were in solution and after 2 hr the solution was poured into a saturated sodium bicarbonate solution. The mixture was extracted with ethyl acetate, washed with water, dried and evaporated. The crude residue (7.45 g) was directly reduced with excess lithium aluminum hydride in tetrahydrofuran (4 hr reflux). The crude product, after recrystallization from methanol, gave 6.85 g of 23,24-bisnorchol-5-ene-3β,22-diol 3β-tetrahydropyranyl ether (2a), m.p. 155-157°C, IR ν 3500 (-OH), 1090 and 980 cm^{-1} (THP-ether); NMR δ 0.73 (s, 3, 18-CH_3), 1.02 (s, 3, 19-CH_3), 1.05 (d, 3, J = 6 Hz, 21-CH_3), 3.47 (m, 2, CH_2OH), 5.35 (m, 1, 6-H).

Anal. Calcd for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.84; H, 10.82.

24-Tosyloxy-22,23-bisnorchol-5-en-3β-ol 3-tetrahydropyranyl ether (2b) from 2a. A solution of 6.0 g of the alcohol 2a and 7.0 g of p-toluenesulfonyl chloride in 50 mL pyridine was left standing for 18 hr at 22°C. Then water was added and the precipitate was filtered off and washed with water. Two recrystallizations from methanol gave 6.74 g tosylate 2b, m.p. 185-187°C; IR ν 1160 (tosylate), 1020 and 930 cm^{-1} (-OTHP); NMR δ 0.73 (s, 3, 18-CH_3), 0.97 (d, 3, J = 6 Hz, 21-CH_3), 0.98 (s, 3, 19-CH_3), 2.43 (s, 3, aromat. CH_3), 5.33 (m, 1, 6-H), 7.20-7.80 (m, 4, aromat. H).

Anal. Calcd for $C_{34}H_{50}O_5S$: C, 71.55; H, 8.33; S, 5.61. Found: C, 71.47; H, 8.66; S, 5.64.

3β-Hydroxy-22,23-bisnorchol-5-ene-24-carbonitrile 3-tetrahydropyranyl ether (3) from 2b. To the solution of 5.9 g tosylate 2b in 200 mL of anhydrous dimethylsulfoxide was added 1.0 g potassium cyanide (1.5 equval) and the mixture stirred at 90°C for 5 hr under nitrogen. After cooling water was added slowly while stirring and the crystalline precipitate was filtered off. The 4.0 g of crude nitrile gave, after recrystallization from acetone, 2.93 of pure 3, m.p. 170-171°C; IR ν 1040 and 970 cm^{-1} (-OTHP); NMR δ 0.69 (s, 3, 18-CH_3), 0.99 (s, 3, 19-CH_3),

1.16 (d, 3, J = 6 Hz, 21-CH_3), 2.31 (m, 2, -CH_2CN), 5.33 (m, 1, 6-H).

Anal. Calcd for $C_{28}H_{41}O_2N$: C, 79.38; H, 9.76, N, 3.31. Found: C, 79.26; H, 10.12; N, 3.21.

Methyl 3β-tetrahydropyranyloxy-23-norchol-5-en-24-oate (4) from 3. The mixture of 7.0 g of nitrile 3 in 300 mL of 20% potassium hydroxide in ethanol was heated on a steam bath for 24 hr. After cooling the solution was poured into water and the neutral material was extracted with ether. The ether extract yielded 810 mg of crude amide which was hydrolyzed further, as described above. The crude amide gave IR ν 3500, 3150, 1650, 1610 (amide), 1020 and 975 cm^{-1} (-OTHP). The crude acid was isolated from the combined acidified hydrolysis solution and was esterified with diazomethane to give, after filtration through a short alumina column and recrystallization from acetone, 5.1 g pure ester 4, m.p. 106-107°C; IR ν 1740 (-$COOCH_3$), 1020 and 980 cm^{-1} (-OTHP); NMR δ 0.71 (s, 3, 18-CH_3), 0.97 (d, 3, J = 6 Hz, 21-CH_3), 1.01 (s, 3, 19-CH_3), 3.65 (s, 3, -$COOCH_3$), 5.34 (m, 1, 6-H).

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.98; H, 10.21.

23-Norchol-5-ene-3β,24-diol 3-tetrahydropyranyl ether (5a) from 4. The solution of 5.0 g ester 4 in 30 mL tetrahydrofuran was added dropwise to a mixture of 1.0 g of lithium aluminum hydride in 350 mL of anhydrous ether and the solution heated under reflux for 1 1/2 hr. The usual work-up gave 4.9 g of crude alcohol. A small sample was recrystallized from methanol to give an analytical sample of the alcohol 5a with m.p. 127-131°C; IR ν 3500 (-OH), 1020 and 980 cm^{-1} (-OTHP). NMR δ 0.69 (s, 3, 18-CH_3), 0.95 (d, 3, J = 6 Hz, 21-CH_3), 0.98 (s, 3, 19-CH_3), 5.30 (m, 1, 6-H).

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.79. Found: C, 78.30; H, 10.79.

24-Tosyloxy-23-norchol-5-en-3β-ol 3-tetrahydropyranyl ether (5b) from 5a. The solution of 5.0 g of the crude alcohol 5a in 50 mL pyridine containing 6.0 g p-toluenesulfonyl chloride was left standing for 18 hr. Slow addition of water while stirring gave a crystalline precipitate which was filtered off and washed

with water and air-dried. Recrystallization from hexane gave 5.6 g pure tosylate 5b, m.p. 137-138°C; IR ν 1160 (-OTs), 1020 and 950 cm^{-1} (-OTHP); NMR δ 0.64 (s, 3, 18-CH_3), 0.84 (d, 3, J = 6 Hz, 21-CH_3), 1.00 (s, 3, 19-CH_3), 2.44 (s, 3, aromat. CH_3), 5.34 (m, 1, 6-H), 7.20-7.83 (m, 4, aromat. H).

Anal. Calcd for $C_{35}H_{52}O_5S$: C, 71.88; H, 8.96; S, 5.48. Found: C, 72.15; H, 9.25; S, 5.88.

3β-Hydroxy-23-norchol-5-ene-24-carbonitrile 3-tetrahydropyranyl ether (6) from 5b. Following exactly the procedure detailed for the synthesis of the nitrile 3, 5 g of tosylate 5b gave, after recrystallization from acetone, 2.4 g pure nitrile 6, m.p. 151-152°C; IR ν 2250 (-CN), 1030 and 975 cm^{-1} (-OTHP); NMR δ 0.69 (s, 3, 18-CH_3), 0.87 (d, 3, J = 6 Hz, 21-CH_3), 1.00 (s, 3, 19-CH_3), 2.34 (m, 2, -CH_2CN), 5.34 (m, 1, 6-H).

Anal. Calcd for $C_{29}H_{43}O_2N$: C, 79.58; H, 9.90; N, 3.20. Found: C, 79.45; H, 10.17; N, 3.29.

Methyl 3β-tetrahydropyranyloxychol-5-en-24-oate (7a) from 6. The nitrile 6 was hydrolyzed as described above for its nor derivative 3 and the resulting crude acid was converted to its methyl ester. This 2.0 g of nitrile 6 gave, after recrystallization from methanol, 1.40 g ester 7a, m.p. 112-113°C; IR ν 1740 (-$COOCH_3$), 1030 and 975 cm^{-1} (-OTHP); NMR δ 0.67 (s, 3, 18-CH_3, 0.93 (d, 3, J = 6 Hz, 21-CH_3), 1.00 (s, 3, 19-CH_3), 3.65 (s, 3, -$COOCH_3$), 5.34 (m, 1, 6-H).

Anal. Calcd for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.29; H, 10.49.

Methyl 3β-hydroxychol-5-en-24-oate (7b) from 7a. To the solution of 1.0 g of the ether 7a in 10 mL tetrahydrofuran was added 2 drops of conc. hydrochloric acid and the solution was kept at 50°C for 2 hr. Then it was poured on ice, the solid filtered off, washed with water and air-dried. Recrystallization from methanol gave 840 mg of pure ester 7b, m.p. 144-146°C, identical in all respects with an authentic sample.

3β-Hydroxychol-5-en-24-oic acid (7c) from 7b. Hydrolysis of the ester 7b with 2N methanolic potassium hydroxide at 23°C for 20 hr gave the acid 7c, which

was recrystallized from methanol-water, m.p. 240-242°C and was identical with its standard in all respects.

3β-Tetrahydropyranyloxy-22,23-bisnorchol-5-en-24-al (8) from 2a. To 950 mg (2.28 mmol) alcohol 2a in 200 mL anhydrous methylene chloride at 0°C was added finely grinded pyridinium chlorochromate (1.2 equivalent).

The reaction was allowed to proceed at 25°C the progress being followed by TLC. When all material was converted, the mixture was filtered through a column of florisil and evaporation of solvent at room temperature gave 750 mg of aldehyde 8 (80%), m.p. 136-138°C (Lit. (4) 137-139°C).

(E)-Methyl 3β-tetrahydropyranyloxychola-5,22-dien-24-oate (9) from 8. To the solution of 3.4 g of aldehyde 8 in 150 mL of benzene was added 8.4 g (1.5 mole) of methyl (triphenylphosphoranylidene) acetate (6) and the solution left standing at 23°C for 10 days. The benzene was removed in vacuo and the residue was partitioned between hexane and 75% methanol/water. The aqueous methanol layer was extracted with hexane and the combined hexane solutions dried and evaporated. Silica gel chromatography yielded, after recrystallization from methanol, 3.0 g of 9, m.p. 129-130°C; IR ν 1710 (ester), 1050 and 980 (ether) and 910 cm^{-1} (olefin); NMR δ 0.71 (s, 3, 18-CH_3), 1.00 (s, 3, 19-CH_3), 1.05 (d, 3, J = 6 Hz, 21-CH_3), 3.71 (s, 3, -$COOCH_3$), 5.35 (m, 1, 6-H), 5.73 (d, 1, 23-H), 6.86 (dd, 1, 22-H), 5.69 (d, 1, J = 16 Hz, 23-H), 6.79 (dd, 1, J = 9 Hz, J = 16 Hz, 22-H).

Anal. Calcd for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85. Found: C, 76.64; H, 9.86.

[1,2-$^{13}C_2$]-Carbethoxymethylene triphenylphosphorane. To a solution of 5 g of triphenylphosphine in 40 mL dry benzene was added slowly 3 g of [1,2-$^{13}C_2$]-ethylbromo acetate, 91 Atom %, and the solution heated on a steam bath under reflux for 16 h. After cooling the mixture was filtered and the residue washed with hexane. The salt was air-dried to give 7.0 g of (carbethoxymethyl)triphenylphosphonium bromide, m.p. 156-158°C, decomp. Without further recrystallization these 7.0 g were dissolved in 150 mL water and to the stirred solution was

added 2N sodium hydroxide to pH 9 (phenolphthalein). The oily precipitate was extracted with methylene chloride and the organic extract dried over sodium sulfate and the solvent evaporated. Recrystallization from ethyl acetate-hexane gave 4.9 g of the phosphorane, m.p. 122-125°C. IR ν 2900 (C-H), 1550 (^{13}C=O), 1280, 1080 (^{13}C-O), 860 (phenyl) cm^{-1}. ^{1}H NMR δ 1.03 (t, 3, J = 6 Hz, -^{13}COOCH$_2$CH$_3$), 3.96 (dq, 2, J = 6 Hz, J_{*CH} = 3 Hz, -^{13}COOCH$_2$CH$_3$), 6.53 (m, 15 H, P-φH); ^{13}C NMR δ 171.3 (dd, J_{C^*CP} = 12.2 Hz, J_{C^*C} = 86.7 Hz, -^{13}CH-^{13}COOEt), 133.2, 132.7, 131.8, 129.0, 128.5 (s, C-phenyl rings), 57.0 (s, OCH$_2$CH$_3$), 30.0 (dd, J_{*CP} = 125.7 Hz, J_{C^*C} = 86.7 Hz, -p$^{\delta+}$ - ^{13}C$^{\delta-}$-H ^{13}COOEt), 15.0 (s, OCH$_2$CH$_3$). % Isotopic labeling: M+2 (87%), M+1 (12%).

[23,24-^{13}C$_2$]-(E)-Ethyl 3β-hydroxychola-5,22-dien-24-oate (10b). This product was synthesized exactly as described above, except that [1,2-^{13}C$_2$]-carbethoxymethylene triphenylphosphorane, prepared as described (4) was used. The product was obtained, after hydrolysis of the THP-ether, in comparable yield: m.p. 61-63°C; UV 223 nm, ε 7593; IR ν 3500 (OH), 1700 (-^{12}COOEt, weak), 1670 (^{13}COOEt, strong), and 910 cm^{-1} (olefin). ^{1}H NMR δ 0.69 (s, 3, 18-CH$_3$), 0.87 (d, 3, J = 6 Hz, 21-CH$_3$), 1.00 (s, 3, 19-CH$_3$), 1.27 (t, 3, J = 6 Hz, 24-^{13}COOCH$_2$CH$_3$), 3.47 (br m, 1, w/2 = 27 Hz, 3α-H), 4.10 (dq, 2, J_{CH} = 6 Hz, J_{*CH} = 3 Hz, ^{13}COOCH$_2$CH$_3$), 5.33 (m, 1, 6-H), 5.69 (complex multiplet, 1,23-^{13}CH), 6.79 (complex multiplet, 1,22-CH); ^{13}C NMR δ 167 (d, J_{C^*C} = 70.8 Hz, ^{13}C-24), 118 (d, J_{C^*C} = 70.8 Hz, ^{13}C-23); MS m/e 384 [(M+2)-H$_2$O]$^+$, 383 [(M+1)-H$_2$O]$^+$, 255 [M-(H$_2$O + side chain)]$^+$. % Isotopic labeling: M+2 (85%), M+1 (15%).

Methyl 3β-tetrahydropyranyloxychol-5-en-24-oate (7a) from 9. The solution of 3.0 g of the olefin 9 in 100 mL of 95% ethanol was hydrogenated at one atmosphere with 300 mg of prereduced platinum oxide catalyst. After 120% of the calculated hydrogen uptake had been consumed the catalyst was filtered and the solvent evaporated *in vacuo*. Two recrystallizations from methanol gave 2.8 g of 7a, identical with the material obtained previously (from 6).

[23,24-$^{13}C_2$]-Ethyl 3β-hydroxychol-5-en-24-oate (11a). This compound was prepared by the hydrogenation of the labeled olefin, followed by hydrolysis, as described above, for the transformation of 9 → 7a → 7b: IR ν 3400 (-OH), 1720 (-$^{12}COOCH_3$, weak), 1660 (-$^{13}COOCH_3$, strong) cm^{-1}; NMR δ 0.69 (s, 3, 18-CH_3), 0.87 (d, 3, J = 6 Hz, 21-CH_3), 1.00 (s, 3, 19-CH_3), 1.27 (t, 3, J = 6 Hz, -$^{13}COOCH_2\underline{CH_3}$), 3.53 (m, 1, w/2 = 27 Hz, 3α-H), 4.10 (dq, 2, J = 6 Hz, $J_{\star_{CH}}$ = 3 Hz, -$^{13}COO\underline{CH_2}CH_3$), 5.37 (m, 1, 6-H). % Isotopic labeling: M+2 (36%), M+1 (14%).

[23,24-$^{13}C_2$-22,23,$^2H_2$]-Ethyl 3β-hydroxychol-5-en-24-oate (11c). The reduction of the ^{13}C labeled olefin was carried out with deuterium, followed by the hydrolysis of the THP-ether. Recrystallization from ethanol gave m.p. 93-94°C. IR ν 3450 (-OH), (-^{12}COOEt, weak, 1665 -^{13}COOEt, strong) cm^{-1}; NMR δ 0.67 (s, 3, 18-$\underline{CH_3}$), 0.92 (d, 3, J = 6 Hz, 21-$\underline{CH_3}$), 1.00 (s, 3, 19-$\underline{CH_3}$), 1.23 (t, e, J = 6 Hz, -$^{13}COOCH_2\underline{CH_3}$), 3.47 (br m, 1, w/2 = 27 Hz, 3α-H), 4.11 (dq, 2, J = 6 Hz, $J_{\star_{CH_{1r}}}$ = 3 Hz, -$^{13}COO\underline{CH_2}CH_3$), 5.33 (m, 1, 6-H). MS m/e 406 $(M+4)^+$, 405 $(M+3)^+$, 404 $(M+2)^+$, 388 $[(M+4)-H_2O]^+$, 387 $[(M+3)-H_2O]^+$, 386 $[(M+2)-H_2O]^+$, 255 $[M-H_2O$ + side chain)$]^+$. % isotopic labeling: M+4 (81%), M+3 (16%), M+2 (3%).

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