LABELED BILE ACIDS I: SYNTHESIS OF ^{18}O-METHYL HYDROXY-5β-CHOLAN-24-OATES (1)

Warren G. Anderson (2), Chang Yon Byon, Charles R. Eck (2),
and Marcel Gut*
The Worcester Foundation for Experimental Biology
Shrewsbury, MA 01545

SUMMARY

The incorporation of ^{18}O into the 3-hydroxyl of bile acids has been accomplished by hydrolysis of the acetal corresponding to the 3-oxo bile acid with $H_2{}^{18}O$, followed by hydride reduction of the ketone to the labeled hydroxyl function.

Key Words: [3-^{18}O] bile acids, 3-oxo bile acids cyclic 3-(1,2-ethanediyl acetal)

INTRODUCTION

The availability of instrumentation sufficiently sensitive for high resolution mass measurement makes labeling with stable isotopes especially attractive for the study of metabolic products in the normal and diseased human organism. In particular, interest in the biosynthesis and metabolism of bile acids as well as their relation to intracellular transport in vivo, has prompted the development of new methodology for analysis of picogram quantities (3). For these purposes, it seemed worthwhile to find easy synthetic access routes to molecules with a minimum of a mass + 2 since the natural abundance of such a mass would be approximately 3% (4) for a C_{24} compound. If one were to consider using bile acids enriched with two ^{13}C atoms then two problems would be encountered, namely, synthesis and percent

*Author to whom correspondence is to be addressed.

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enrichment. Normally, commercially available ^{13}C specimens contain no more than 90% enrichment which would lead to a maximum of 81% for mass + 2. For these reasons we considered labeling with ^{18}O, available commercially with 99% atom enrichment.

DISCUSSION

The starting materials for the ^{18}O labeling of selected bile acids were the 3-keto methyl esters which can be conveniently prepared (5,6) by Fétizon oxidation (silver carbonate-celite) of the corresponding alcohols. These keto esters were transformed into their acetals with ethylene glycol catalyzed by p-toluenesulfonic acid. Using this strategy, the following compounds were prepared: Methyl 3-oxo-12α-hydroxy-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal), methyl 3-oxo-7α-hydroxy-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal), methyl 3-oxo-7β-hydroxy-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal), and methyl 3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal) (7).

The initial incorporation experiments were modeled after earlier experiments (8) in which the acetal was hydrolyzed with $H_2{}^{18}O$ in dioxane at 140°C for 24 hr. However, when a dioxane solution of methyl 3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal) was treated with a 10 molar excess of $H_2{}^{18}O$ and the [3-^{18}O]-3-keto-5β-cholan-24-oate isolated, the mass analysis revealed a galaxy of enrichment masses from M + 5 down to M + 2. A slight modification of this procedure led to a more uniform product of a mass + 2 (9): Thus, when the surface of the acetal solution in dioxane containing a 10 molar excess of $H_2{}^{18}O$ (20% atom enrichment) was exposed to a stream of hydrogen chloride gas for 5 seconds, stirred and let stand for 18 hr in a stoppered test tube, a specifically labeled product having a mass + 2, enrichment of 18% was obtained in good yield. Sodium borohydride reduction likewise provided a homogeneous sample of the corresponding 3-hydroxide which showed a M + 2 with 19% enrichment.

It is of interest to note that when a Corey oxidation (pyridinium chlorochromate) of methyl-3α-hydroxy-5β-cholan-24-oate was attempted (see Experimental), the corresponding 3,3-dimethyl acetal was obtained. Acetal

formation was apparently due to the fact that the crude product contained a sufficient quantity of acid that during recrystallization from methanol, acetalization was favored. The identical acetal was also obtained (not described in the Experimental) by refluxing a methanolic solution of methyl 3-oxo-5β-cholan-24-oate containing p-toluenesulfonic acid monohydrate.

This reaction enrichment sequence was of general utility for use with any of the normal bile acids. Use of this methodology for the preparation of bile acids with high atom enrichment (99%) and their biochemical application will be described in a future report.

EXPERIMENTAL

Melting points were determined on a Kofler melting point apparatus and are uncorrected. The ^1H NMR spectra were determined in deuteriochloroform solution in a Jeol Fx 90 Q, using tetramethylsilane as an internal reference, and the positions of the proton signals are expressed in parts per million downfield from tetramethylsilane.

General Method:

a) Acetalization: To a solution of 500 mg of the appropriate 3-keto bile acid methyl ester in 50 mL of benzene was added 75 mg of p-toluenesulfonic acid monohydrate and 0.75 mL of ethylene glycol and the mixture heated under reflux overnight in a Dean-Stark apparatus. After cooling, the benzene solution was washed with a saturated solution of sodium bicarbonate, dried and evaporated. The acetals were purified either by preparative HPLC or by preparative TLC.

b) Hydrolysis: To a solution of 100 mg of the acetal in 2 mL dioxane was added 0.5 mL of H_2O. Then a stream of gaseous hydrogen chloride was passed over the surface of the liquid, the test tube stoppered and left at room temperature overnight. The mixture was extracted with methylene chloride, the combined extracts washed several times with a saturated solution of sodium

bicarbonate, dried and evaporated. The resulting ketones were purified either by preparative HPLC or by preparative TLC.

c) Reduction: To a solution of 100 mg of the ketone in 10 mL of 95% ethanol was added a solution of 5 mL ethanolic sodium borohydride (50 mg). After stirring for 2 hr at room temperature the solution was poured into water, the mixture acidified with acetic acid and extracted with methylene chloride. The organic extract was washed with a saturated solution of sodium bicarbonate, dried and the solvent evaporated. The resulting 3α-alcohol was purified by either preparative TLC or by preparative HPLC, and the stereoselectivity was in all cases above 90%.

The following acetals were produced, then hydrolyzed with $H_2{}^{18}O$ and reduced, as described above.

Methyl 3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal), mp 161-162°C (lit (7), mp 160-161°C); IR ν 1700 cm^{-1} ($COOCH_3$); NMR δ 0.67 (s, 3H, 18-CH_3), 0.97 (s, 3H, 19-CH_3), 3.68 (s, 3H, -$COOCH_3$), 3.95 (s, 4H, -OCH_2CH_2O-).

[3-^{18}O]-Methyl 3-oxo-5β-cholan-24-oate.-Methyl 3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal) was hydrolyzed, as described above, with $H_2{}^{18}O$ (20 Atom %). The crude product was purified on preparative TLC to give clean ketone in 87% yield with an enrichment (M+2) of 19%.

[3-^{18}O]-Methyl 3α-hydroxy-5β-cholan-24-oate.-The reduction of the 3-ketone according to the described procedure gave, after TLC, the desired 3α-alcohol in 90% yield [17 Atom % enrichment (M+2)].

Methyl 12α-hydroxy-3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal), mp 103-105°C; IR ν 3450 (OH) and 1690 cm^{-1} ($COOCH_3$); NMR δ 0.70 (s, 3H, 18-CH_3) 0.95 (s, 3H, 19-CH_3), 3.67 (s, 3H, -$COOCH_3$), 3.93 (s, 4H, -OCH_2CH_2O-).

Anal. Calcd for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.16; H, 9.83.

[3-^{18}O]-Methyl 12α-hydroxy-3-oxo-5β-cholan-24-oate.-Methyl 12α-hydroxy-3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal) was hydrolyzed with $H_2{}^{18}O$ (20 Atom %) as described above, to give after preparative TLC, the free ketone in a yield of 85%. The observed M+2 peak corresponded to 19% enrichment.

[3-^{18}O]-Methyl 3α,12α-dihydroxy-5β-cholan-24-oate.-The above free 3-ketone was reduced as described, and gave after purification on TLC the desired 3α-alcohol in 90% yield. The observed enrichment amounted to 17 Atom %.

Methyl 7α-hydroxy-3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal), mp 109-110°C; IR ν 3500 (OH) and 1700 cm^{-1} ($COOCH_3$), NMR δ 0.68 (s, 3H, 18-CH_3), 0.95 (s, 3H, 19-CH_3), 3.67 (s, 3H, -$COOCH_3$), 3.92 (s, 4H, -OCH_2CH_2O-).

Anal. Calcd for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: 72.19, H, 10.13.

[3-^{18}O]-Methyl 7α-hydroxy-3-oxo-5β-cholan-24-oate.-Methyl 7α-hydroxy-3-oxocholan-24-oate cyclic 3-(1,2-ethanediyl acetal) was hydrolyzed, as described above, with $H_2{}^{18}O$ (20 Atom %). The purified material gave the following mass spectrum: M/e 404 (amplitude 2080), M/e + 2, 406 (amplitude 456) 18% enrichment.

[3-^{18}O]-Methyl 3α,7α-dihydroxy-5β-cholan-24-oate.-The reduction of [3-^{18}O] methyl 7α-hydroxy-3-oxocholan-24-oate, as described under "General Method" gave [3-^{18}O]-methyl 3α,7α-dihydroxycholan-24-oate which was observed to have an M+2 peak corresponding to 15% enrichment. M/e 406 (amplitude, 1918), M/e + 2, 408 (amplitude 340).

Methyl 7β-hydroxy-3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal), amorphous; IR ν 3500 (OH) and 1700 cm^{-1} ($COOCH_3$), NMR δ 0.70 (s, 3H, 18-CH_3), 0.98 (s, 3H, 19-CH_3), 3.68 (s, 3H, -$COOCH_3$), 3.92 (s, 4H, -OCH_2CH_2O-).

Anal. Calcd for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.14, H, 9.60.

[3-^{18}O]-Methyl 7β-hydroxy-3-oxo-5β-cholan-24-oate.-Methyl 7β-hydroxy-3-oxo-5β-cholan-24-oate cyclic 3-(1,2-ethanediyl acetal) was hydrolyzed, as described above, with $H_2{}^{18}O$ (20 Atom %). The crude product was purified on TLC to yield (89%) clear ketone with an enrichment (M+2) of 18%.

[3-^{18}O]-Methyl 3α,7β-dihydroxy-5β-cholan-24-oate.-The reduction of the 3-oxo group with $NaBH_4$ according to the above described procedure gave, after TLC, the desired 3α-alcohol in a yield of 85% [16 Atom % enrichment (M+2)].

Methyl 3,3-dimethoxy-5β-cholan-24-oate.-A solution of 390 mg of methyl 3α-hydroxycholan-24-oate in 2 mL of methylene chloride was added to a solution of

320 mg of pyridinium chlorochromate in 2 mL of methylene chloride and the resulting mixture was stirred for 2 hr. Dilution with 20 mL of ether and filtration through a column of florisil gave, after evaporation 379 mg of a crude solid which was recrystallized from methanol to give 238 mg of the 3α,3β-dimethoxy acetal, mp 97°C. IR ν 1700 cm^{-1} ($COOCH_3$); NMR δ 0.67 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 3.15 (s, 3H, 3β-OCH_3), 3.22 (s, 3H, 3α-OCH_3), 3.67 (s, 3H, -$COOCH_3$).

Anal. Calcd for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.40; H, 10.42

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4. Considering the case for C_{24} bile acids and ascribing higher masses exclusively to the 1.11% natural abundance of ^{13}C, we calculated the probability for mass + n = $(\frac{24!}{n!(24-n)!})(\frac{1.11}{100})^n(\frac{100-1.99}{100})^{24-n}$ Hence for mass + 1 = 0.20; mass + 2 = 0.026; mass + 3 = 0.0021. Natural abundance of deuterium is negligible and the probability of contribution to the mass from ^{18}O is very small, i.e. for M + 3 = 3.4×10^{-8}.
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9. For a stable isotope labeled analog, it is assumed that the substance is identical in all respects, except mass, to the parent compound, i.e., physically and chromatographically. Consequently, the peak intensities in the mass spectrum will represent the percentages of the two compounds in the mixture