# SYNTHESIS OF BIOLOGICAL PRECURSORS OF CHOLIC ACID II*

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## ABSTRACT

This paper describes the partial syntheses of 3α,7α,12α-trihydroxy-5β-cholestan-26-al, 7α,12α,26-trihydroxy-5β-cholestan-3-one and 7α,12α-dihydroxy-3-oxo-5β-cholestan-26-al via $Ag_2CO_3$/Celite oxidation of 5β-cholestane-3α,7α,12α,26-tetrol. These bile alcohols were resolved by analytical and preparative TLC, characterized by gas-liquid chromatography and mass spectrometry. These compounds will be useful to delineate further the mechanism of oxidation of 5β-cholestane-3α,7α,12α,26-tetrol on the pathway to cholic acid.

## INTRODUCTION

Recent studies from our laboratory have shown that in patients with rare sterol storage disease, cerebrotendinous xanthomatosis (CTX), bile acid production is subnormal (1), but considerable quantities of $C_{27}$ bile alcohols are excreted in bile and feces (2,3). Three bile alcohols, namely 5β-cholestane-3α,7α,12α,25-tetrol, 5β-cholestane-3α,7α,12α,23α,25-pentol and 5β-cholestane-3α,7α,12α,24α,25-pentol, have been identified conclusively and thus it appears that diminished bile acid synthesis in CTX results from defective oxidation of the cholesterol side chain (4-8). However, according to current views in bile acid biosynthesis, bile alcohols hydroxylated at carbon 25 are not considered major precursors of bile acids (9,10). Evidence has been obtained to indicate 5β-cholestane-3α,7α,12α-triol and 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid are intermediates in the biological formation of cholic acid from cholesterol. The conversion of 5β-cholestane-3α,7α,12α-triol into 3α,7α,12α-trihydroxy-5β-cholestan-26-oic

-acid has been shown to occur by means of the intermediate formation of 5β-cholestane-3α,7α,12α,26-tetrol (10). But no adequate information is available concerning the possibility of 3α,7α,12α-trihydroxy-5β-cholestan-26-al as an intermediate in the oxidation of 5β-cholestane-3α,7α,12α,26-tetrol. In order to investigate the major metabolic pathway of cholic acid biosynthesis, we required the synthesis of the hypothetical intermediates 3α,7α,12α-trihydroxy-5β-cholestan-26-al, 7α,12α,26-trihydroxy-5β-cholestan-3-one and 7α,12α-dihydroxy-3-oxo-5β-cholestan-26-al (compounds III, IV and V, fig. 1).

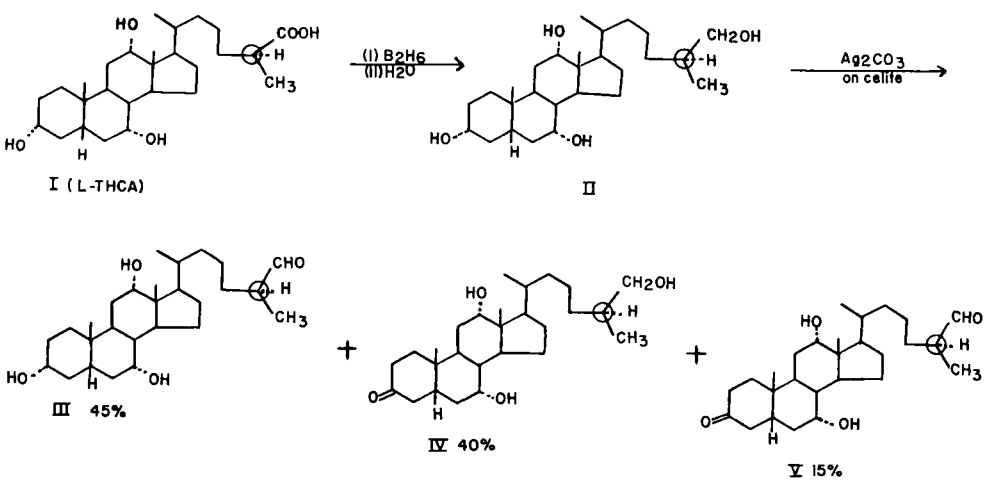


FIG. 1

## METHODS

PHYSICAL MEASUREMENTS: Melting points were determined on a thermolyne apparatus, model MP-12600, and are uncorrected.

GLC: 5β-cholestane-3α,7α,12α,26-tetrol, 3α,7α,12α-trihydroxy-5β-cholestan-26-al, 7α,12α,26-trihydroxy-5β-cholestan-3-one and 7α,12α-dihydroxy-3-oxo-5β-cholestan-26-al (compounds II-V, fig. 1) as TMSi derivatives were analysed on a 180 cm x 4 mm column packed with 3% QF-1 and 1% Hi-EFF-8BP, column temp. 230°C (Hewlett-Packard model 7610 gas chromatograph).

MASS SPECTRA: were obtained with a Varian MAT-111 gas chromatograph mass spectrometer (Varian Associates, Palo Alto, Ca.). High resolution mass spectra were recorded on a model CEC-110 (Consolidated Electrodynamics Corp., Monrovia, Ca.).

TLC: Performed on silica gel "G" plates (Brinkmann, 0.25 mm thickness). The spots were detected with phosphomolybdic acid (3.5% in isopropanol), sulphuric acid (10%) and heating for one minute at 110°C. Bands on preparative TLC were made visible with iodine or water.

## EXPERIMENTAL

(A) Preparation of (25R) 5β-cholestane-3α,7α,12α,26-tetrol (Compound II fig. 1) via hydroboration of (25R)-3α,7α,12α-trihydroxy-5β-cholestane-26-oic acid THCA (compound I, fig. 1) ( 11-13).

An oven-dried 25 ml flask fitted with a side arm dropping funnel, a magnetic stirring bar and a reflux condenser connected to a mercury bubbler was cooled to room temperature under dry nitrogen. Then 20 mg of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) was dissolved in 4 ml of absolute tetrahydrofuran (THF) was placed into reaction flask. The flask was immersed into the ice-bath cooled to 0°C. To this 1.5 ml (1.5 mmol) of 1.0 M borane solution in THF was slowly added during a 15 minutes period. Hydrogen evolution started almost instantaneously, which corresponded to 1 mmol of hydrogen per mmol of the acid. The mixture was stirred well. The ice-bath was removed and replaced (0.5 hours after) by a water bath (25°C). At the end of 45 minutes, 0.2 ml of the reaction mixture was hydrolysed with water and analysed by GLC on 3%-QF-1 column, indicating the presence of 5β-cholestane-3α,7α, 12α,26-tetrol. At the end of one hour a 99% yield of the 26-tetrol was realized. After this indication from the whole reaction mixture the THF layer was evaporated and water was added. The aqueous layer was extracted with three 12 ml portions of ethyl acetate. During aqueous workup protonolysis of the organoborane intermediate took place. The combined organic phase was dried over anhydrous $Na_2SO_4$ and after careful removal of the ethyl acetate solvent, 15 mg of 5β-cholestane-3α,7α,12α,26-tetrol was obtained mp 196° - 198° (lit. mp 201-203°C) (14,15).Single spot on TLC, GLC and mass spectral characteristics matched those in the literature.

(B) $Ag_2CO_3$/Celite Oxidation of 5β-cholestane-3α,7α,12α,26-tetrol(fig. 1, compd. II). Preparation of 3α,7α,12α-trihydroxy-5β-cholestan-26-al, 7α,12α,26-trihydroxy-5β-cholestan-3-one and 7α,12α-dihydroxy-3-oxo-5β-cholestan-26-al (fig. 1, compds. III, IV and V).

Fifteen mg of 5β-cholestane-3α,7α,12α,26-tetrol was placed in a 25 ml round bottom flask and dissolved in about 5 ml of dry benzene, 310 mg (4.0 mmoles) of oxidant ($Ag_2CO_3$/Celite) was added, and flask was fitted with a Dean-Stark receiver and a condenser (16,17). The mixture was refluxed azeotropically to remove any water from the reaction mixture while stirring magnetically. Progress of the reaction was monitored by TLC, using solvent system chloroform:acetone:methanol:70:20:7 (v/v/v). After about ½ hour to 45 minutes of refluxing the mixture was worked up by filtering out the solid material and careful washing with fresh benzene. The solvent was evaporated on a rotary evaporator and the residue (10 mg) was resolved by analytical and preparative TLC silica gel "G"; $CHCl_3$;$(CH_3)_2CO$; MeOH; 70:20:7 (v/v/v). The following $R_f$ values were obtained: III, 0.32; IV, 0.50; V, 0.68. These compounds (III=4.5 mg, IV=4 mg, V=1.5 mg) as their TMSi derivatives were separated and quantitated by GLC on 3% QF-1 and 1% Hi-EFF 8 BP. A comparison of their relative GLC retention times are recorded in Table I and their mass spectral characteristics are recorded in Tables 2 and 3.

TABLE 1:

RETENTION TIMES OF THE TMSi ETHER DERIVATIVES OF SOME BILE ALCOHOLS RELATIVE TO 5α-CHOLESTANE ON 3% QF-1 & 1% Hi-EFF 8 BP

| Compound | 3% QF-1[a] | 1% Hi-EFF 8 BP[b] |
|---|---|---|
| 5β-cholestane-3α,7α,12α-triol | 1.64 | 0.70 |
| 5β-cholestane-3α,7α,12α,23-tetrol | 2.37 | 0.97 |
| 5β-cholestane-3α,7α,12α,24-tetrol | 2.65 | 1.11 |
| 5β-cholestane-3α,7α,12α,25-tetrol | 1.98 | 1.25 |
| 5β-cholestane-3α,7α,12α,26-tetrol | 3.24 | 1.56(1.75)[c] |
| 3α,7α,12α-trihydroxy-5β-cholestan-26-al | 3.88[d] | 2.55[c] |
| 7α,12α,26-trihydroxy-5β-cholestan-3-one | ---- | 5.69[c] |
| 7α,12α-dihydroxy-3-oxo-5β-cholestan-26-al | ---- | 10.39[c] |

a. Column temp. 235°C; $N_2$=40 ml/min:
Retention time: 5α-cholestane = 2.75 min.

b. Column temp. 235°C; $N_2$=40 ml/min.
Retention time: 5α-cholestane = 7,08 min.

c. Column temp. 240°C; $N_2$=40 ml/min.
Retention time: 5α-cholestane = 5.84 min.

d. Column temp. 240°C; $N_2$= 40 ml/min:
Retention time: 5α-cholestane = 2.95 min.

TABLE 2

Relative intensities of ion fragments from 3α,7α,12α-trihydroxy-5β-cholestan-26-al. TMSi ether derivative; GLC-MS Underivatized; direct injection, 170°C.

TMSi ether derivative

| ION | M/e | % |
|---|---|---|
| M+ | 650 | --- |
| M-15 | 635 | --- |
| M-90 | 560 | --- |
| M-(90+15) | 545 | 2 |
| M-2x90 | 470 | 14 |
| M-(2x90+15) | 455 | 7 |
| M-3x90 | 380 | 35 |
| M-(3x90+15) | 365 | 7 |
| M-(2x90+127 side chain) | 343 | 22 |
| M-(3x90+127 side chain) | 253 | 100 |
| M-(3x90+C-16,17) | 226 | 15 |
| M-(3x90+C-15,16,17) | 211 | 10 |

UNDERIVATIZED

| ION | M/e | % |
|---|---|---|
| M+ | 434 | 1 |
| M-15 | 419 | --- |
| M-18 | 416 | 4 |
| M-(17+15) | 402 | 2 |
| M-2x18 | 398 | 20 |
| M-(17+18+15) | 384 | 13 |
| M-3x18 | 380 | 12 |
| M-(3x18+15) | 365 | 7 |
| M-(18+127 side chain) | 289 | 12 |
| M-(2x18+127 side chain) | 271 | 98 |
| M-(3x18+127 side chain) | 253 | 100 |
| M-(3x18+C-16,17) | 227 | 32 |
| M-(3x18+C-15,16,17) | 211 | 23 |

TABLE 3

Relative intensities of ion fragments from the TMSi ether derivative of 7α,12α,26-trihydroxy-5β-cholestan-3-one (GLC-MS) and underivatized 7α,12α-dihydroxy-3-oxo-5β-cholestan-26-al (direct injection, 160°C).

TMSi 7α,12α,26-trihydroxy-5β-cholestan-3-one

| ION | M/e | % |
|---|---|---|
| M+ | 650 | 0.6 |
| M-15 | 635 | 2 |
| M-90 | 560 | 3 |
| M-(90+15) | 545 | 0.6 |
| M-2x90 | 470 | 16 |
| M-(2x90+15) | 455 | 2 |
| M-(2x90+C-1 to C-6+C-10) | 347 | 5 |
| M-(2x90+201 side chain) | 269 | 100 |
| M-(2x90+18+201 side chain) | 251 | 8 |

7α,12α-dihydroxy-3-oxo-5β-cholestan-26-al

| ION | M/e | % |
|---|---|---|
| M+ | 432 | 5 |
| M-15 | 417 | -- |
| M-18 | 414 | 10 |
| M-(18+15) | 399 | 1 |
| M-2x18 | 396 | 7 |
| M-(2x18+15) | 381 | 4 |
| M-(18+127 side chain) | 287 | 15 |
| M-(2x18+C-1 to C-6+C-10) | 273 | 9 |
| M-(2x18+127 side chain) | 269 | 100 |
| M-(3x18+127 side chain) | 251 | 10 |

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