SYNTHESIS OF BIOLOGICAL PRECURSORS OF CHOLIC ACID*

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ABSTRACT

This paper describes a new and convenient procedure for the synthesis of 5β-cholestane-3α,7α,12α,24-tetrol(24R and 24 S) and 5β-cholestane-3α,7α,12α,26-tetrol starting from 5β-cholestane-3α,7α,12α,25-tetrol. Dehydration of the 25-hydroxytetrol with glacial acetic acid and acetic anhydride yielded a mixture of 5β-cholest-24-ene-3α,7α,12α-triol and the corresponding Δ^{25} compound. Hydroboration and oxidation of the mixture of Δ^{24} and Δ^{25} unsaturated bile alcohols resulted in the formation of 5β-cholestane-3α,7α,12α,24ξ-tetrol and 5β-cholestane-3α,7α,12α,26-tetrol. In addition, smaller amounts of 5β-cholestane-3α,7α,12α,23ξ-tetrol and 5β-cholestane-3α,7α,12α-triol were also obtained.

The bile alcohols epimeric at C-24 were resolved by analytical and preparative TLC, characterized by gas-liquid chromatography and mass-spectrometry. Tentative assignments of the 24R and 24S configuration was made on the basis of molecular rotation differences. These compounds will be useful for biological studies of cholic acid biosynthesis.

INTRODUCTION

The mechanism whereby cholesterol is converted into bile acids in vertebrates has been studied extensively in recent years (1). C_{27} bile alcohols have been postulated as intermediates in the formation of the primary bile acids: cholic acid and chenodeoxycholic acid. The pathway for the degradation of the sterol side chain is thought to involve

C-26 hydroxylation as an initial step (2-4). Recent studies from our laboratory have indicated that 25-hydroxylation of the side chain may also play a role in bile acid synthesis (5). In order to investigate the major metabolic pathway of cholic acid biosynthesis and the sequence of the side chain hydroxylations we required the synthesis of the hypothetical intermediates 5β-cholestane-3α,7α,12α,26-tetrol and the isomeric 5β-cholestane-3α,7α,12α,24α-tetrol and 5β-cholestane-3α,7α,12α,24β-tetrol (compounds IV and V, fig. 1).

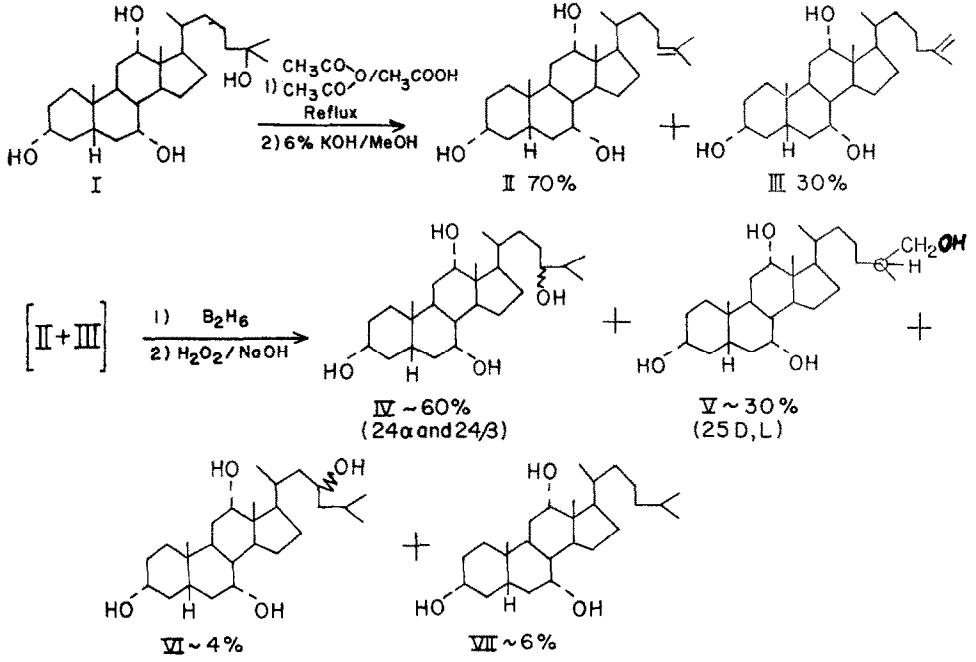

Figure 1

Previously reported syntheses (6) of 26-hydroxytetrol involve the electrolytic coupling of cholic acid with the half ester of methyl succinic acid and subsequent reduction with $LiAlH_4$. The product resulting from an electrolysis reaction is a complex mixture and its separation by preparative thin layer chromatography results in very low yields of the 26-tetrol.

Utilizing the sequence illustrated in Fig. 1, we have shown that it is possible to produce a mixture of 60% 5β-cholestane-3α,7α,12α,24ξ-tetrol, 30% 5β-cholestane-3α,7α,12α,26-tetrol, 4% 5β-cholestane-3α,7α,12α,23ξ-tetrol, and 6% 5β-cholestane-3α,7α,12α-triol by a hydroboration reaction (7).

METHODS

Physical measurements: Melting points were determined on a Thermolyne apparatus, model MP-12600, and are uncorrected.

Optical rotations were determined at 25°C in methanol on a Carey model 60 spectropolarimeter.

GLC: The bile alcohols, as the TMSi-derivatives, were analyzed on a 180cm x 4mm column packed with either 3% QF-1 230°C (Hewlett-Packard model 7610 gas chromatograph).

Mass Spectra of the bile alcohols 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: The bile alcohols were separated 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°. Bands on preparative TLC were made visible with iodine or water.

EXPERIMENTAL

(A) Dehydration of 5β-cholestane-3α,7α,12α,25-tetrol: 5β-cholest-24-ene-3α,7α,12α-triol (II, fig.1) and 5β-cholest-25-ene-3α,7α,12α-triol (III, fig.I).

A solution of 220 mg. of 5β-cholestane-3α,7α,12α, 25-tetrol (8) in 6 ml of glacial acetic acid was refluxed for 3 hrs. Four ml of acetic anhydride was added and the reaction mixture was further refluxed for 12 hrs. The solution was evaporated to dryness in vacuo. The pale yellow semisolid (220 mg) obtained was subjected to column chromatography on neutral alumina which on elution with benzene-ethyl acetate 80:20 provided 200 mg of a mixture of 5β-cholest-24-ene-3α,7α,12α-triacetate and 5β-cholest-25-ene-3α,7α, 12α-triacetate. This was hydrolysed by refluxing with 10 ml of 6% methanolic potassium hydroxide for 1.5 hrs., and the mixture poured into a beaker containing crushed ice with vigorous stirring which on filtration gave a white precipitate consisting of 5β-cholest-24-ene-3α,7α,12α-triol and 5β-cholest-25-ene-3α,7α,12α-triol (II and III, fig. 1).

(B) Hydroboration of a mixture (70:30) of 5β-cholest-24-ene-3α,7α,12α-triol and 5β-cholest-25-ene-3α,7α,12α-triol.

A mixture of unsaturated triols (compounds II and III, fig. 1) (180 mg, 0.43 mmol) was dissolved in 20 ml absolute tetrahydrofuran. The solution was cooled to 0°C, and 1 M borane solution in dry tetrahydrofuran (2.25 ml; 2.25 mmol) was added. The mixture was kept at 0°C for 1 hr. and at 25°C for 15 minutes. Aqueous 3 N NaOH, 0.6 ml, at 0°C was mixed with a precooled solution of 30% H_2O_2 (0.7 ml). The cold basic peroxide was gradually added (30 min.) to the organoborane solution at 0°C and stirring was continued overnight at room temperature. Dilution with water, removal of tetrahydrofuran in vacuo, extraction with ethyl acetate, two washings with saturated NaCl solution and evaporation to dryness yielded 150 mg of an amorphous powder. This residue was purified by column chromatography on neutral alumina grade IV followed by preparative TLC [$CHCl_3$-$(CH_3)_2CO$-MeOH, 70:50:15 (V/V/V)]. The compound from the zone with R_f 0.70 was crystallized from methanol to yield 12 mg of 5β-cholestane-3α,7α,12α-triol, m.p. 185-186°C (lit. m.p. 186-188°C) (9), $[\alpha]_D^{25}$ = + 30.4°. The compound from the zone with R_f 0.40 (40 mg) was crystallized from acetone to yield 33 mg of 5β-cholestane-3α,7α,12α,24β-tetrol (24S), m.p. 181-183°C (lit. m.p. 186-187°C) (10), $[\alpha]_D^{25}$ = + 5.2°, and the material from the zone with R_f 0.34 yielded after two crystallizations from acetone 16.0 mg of 5β-cholestane-3α,7α,12α,24α-tetrol (24R), m.p. 180-182°C (lit. m.p. 184-186°C (10), $[\alpha]_D^{25}$ = + 34.21°. The mother liquor after re-

peated crystallizations from acetone gave 17.0 mg of 5β-cholestane-3α,7α,12α,26-tetrol, m.p. 200-202°C (lit. m.p. 204° C) (11). The fraction having R_f = 0.27 m/e TMSi 724 could not be crystallized.

DISCUSSION

Tentative assignment of the 24α and 24β configuration was made by reference to known bile steroids (7,10,12,13). A comparison of the relative retention times of these compounds and different types of bile alcohols required for this study is given in Table 1.

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

Compound	3% QF-1[a]	1% Hi-EFF 8BP[b]
5β-Cholestane-3α,7α, 12α-triol	1.64	0.70
5β-Cholest-24-ene-3α, 7α,12α-triol	1.80	0.91
5β-Cholest-25-ene-3α, 7α,12α-triol	1.81	0.89
5β-Cholestan-3α,7α,12α, 22ξ-tetrol	2.37	0.97
5β-Cholestan-3α,7α,12α, 24ξ-tetrol	2.65	1.11
5β-Cholestan-3α,7α,12α, 25-tetrol	1.98	1.25
5β-Cholestan-3α,7α,12α, 26-tetrol	3.24	1.56
5β-Cholestan-3α,7α,12α, 23ξ,25-pentol	3.90	1.58
5β-Cholestan-3α,7α,12α, 24α,25-pentol	4.22[c]	1.65
5β-Cholestan-3α,7α,12α, 24β,25-pentol	4.35[c]	1.76

a Column 235 C; N 40ml/min: Retention time of 5β-cholestane-2.75 min.
b Column 235 C; N 40ml/min: Retention time of 5β-cholestane-7.08 min.
c The RRT for these two epimers are different with $p<0.01$ on both columns (13).

The identification of these tetrols via mass spectrometry was facilitated by earlier studies of Cronholm and Johansson (4), who observed major fragment ions as m/e 145 and 159 in the mass spectra of the TMSi ethers of 5β-cholestane-3α,7α,12α,24ξ- and 3α,7α,12α,23ξ-tetrol, respectively. The base peak for the TMSi ether of 5β-cholestane-3α,7α,12α, 26-tetrol was m/e 253. The fragment ions, 145, 159, and 253 appeared as base peaks in spectra of TMSi ethers of 5β-cholestane-3α,7α,12α,24α-, 3α,7α,12α,24β-, 3α,7α,12α,23ξ-, and 3α,7α,12α,26-tetrols respectively (Table 2).

TABLE 2
% RELATIVE INTENSITY FOR MAJOR FRAGMENTS OF THE TMSi ETHERS OF THE 5β-CHOLESTANE TETROLS (14).

Compound	m/e	% Relative Intensity		
		3α,7α,12α, 23ξ-tetrol	3α,7α,12α 24ξ-tetrol	3α,7α,12α, 26-tetrol
M^+	724	-	0.4	0.4
M-90	634	1.1	2.2	3.2
M-(2x90)	544	6.3	13.2	52.0
M-(3x90)	454	8.2	19.5	56.1
M-(3x90+43)	411	-	17.5	-
M-(3x90+57)	397	3.0	-	-
M-(4x90)	364	11.6	17.6	7.8
M-(2x90+201 Side Chain	343	11.0	24.3	37.2
M-(4x90+43)	321	-	46.6	-
M-(4x90+57)	307	9.0	-	-
M-(3x90+201) Side Chain	253	33.0	55.4	89.1
Charged Side Chain	159	100.0	-	-
Charged Side Chain	145	-	64.1	-
Charged Side Chain	103	-	-	14.1
$Si(CH_3)_3$	73	51.6	100	100

The retention times and mass spectra of the TMSi ethers of 5β-cholestane-3α,7α,12α,24α-tetrol and 5β-cholestane-3α, 7α,12α,24β-tetrol on 3% QF-1 and 1% Hi-EFF 8 BP were identical. These epimers did not separate on gas chromatography and were found to be present in about equal amounts.

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REFERENCES

1. Mosbach, E.H., Arch. Intern. Med., 130, 478 (1972).
2. Danielsson, H., Acta Chem. Scan., 14, 378 (1960).
3. Suld, H.M., Staple, E., and Gurin, S., J. Biol. Chem., 237, 338 (1962).
4. Chronholm, T., and Johansson, G., Eur. J. Biochem., 16, 373 (1970).
5. Salen, G., Shefer,S., Setoguchi, T., and Mosbach, E.H., J. Clin. Invest., 56, 226 (1975).
6. Bridgwater, R.J., J. Biochem., 64, 593 (1956).
7. Dayal, B., Batta, A.K., Tint, G.S., Shefer, S., Salen, G., and Mosbach, E.H., J. Lipid Res., 19, 191 (1978).
8. Dayal, B., Shefer, S., Tint, G.S., Salen, G., and Mosbach, E.H., J. Lipid Res., 17, 74 (1976).
9. Bjorkhem, I., and Gustafsson, J., Eur. J. Biochem., 36, 201 (1960).
10. Masui, T., and Staple, E., Steroids., 9, 443 (1967).
11. Danielsson, H., Acta. Chem. Scan., 14, 348 (1960).
12. Dayal, B., Salen, G., Tint, G.S., shefer, S., and Mosbach, E.H., J. Lipid Res., 19, 187 (1978).

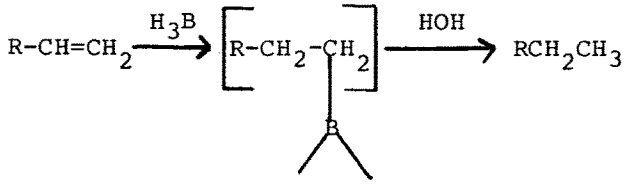
13. Shefer, S., Dayal, B., Tint, G.S., Salen, G., and Mosbach, E.H., J. Lipid Res., 16, 280 (1975).

14. Tint, G.S., Dayal, B., Batta, A.K., Shefer, S., Cheng, F.W., Salen, G., and Mosbach, E.H., J. Lipid Res., Nov. (1978) (In Press).

* Dayal, B., Shefer, S., Tint, G.S., Salen, G., and Mosbach, E.H.; Presented in part at the 172nd A.C.S. National Meeting, San Francisco, California, August, 1976.

APPENDIX

The formation of small amounts of 5β-cholestane-3α,7α, 12α-triol (See Experimental) is attributed to the protonolysis (7) of the organoborane intermediate as follows:



And the isomerization of the organoborane intermediate constituted the formation of 5β-cholestane-3α,7α,12α,23ξ-tetrol in 4% yields.

Taniguchi, H., Brener, L., and Brown, H.C., J. Amer. Chem. Soc., 98, 7107 (1976).