Preparation of 24(*R*)- and 24(*S*)-5β-cholestane-3α,7α,24-triols and 25(*R*)- and 25(*S*)-5β-cholestane-3α,7α,26-triols by a hydroboration procedure

B. Dayal, A. K. Batta, S. Shefer, G. S. Tint, G. Salen, and E. H. Mosbach

Department of Medicine, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ 07103; The Public Health Research Institute of the City of New York, Inc., New York, NY 10016; and the Veterans Administration Hospital, East Orange, NJ 07019; and Cabrini Health Care Center, New York, NY 10003.

Abstract This report describes a new and convenient method for the preparation of 5β-cholestane-3α,7α,24-triol (24*R* and 24*S*) and 5β-cholestane-3α,7α,,26-triol (25*R* and 25*S*) starting from 5β-cholestane-3α,7α,,25-triol. Dehydration of the latter with acetic anhydride and glacial acetic acid yielded a mixture of 5β-cholest-24-ene-3α,7α-diol and the corresponding Δ^{25} compound. Hydroboration and oxidation of the Δ^{24} unsaturated bile alcohol resulted in the formation of 5β-cholestane-3α,7α,24-triol. 5β-Cholestane-3α,7α,26-triol and 5β-cholestane-3α,7α-diol were obtained from the Δ^{25} bile alcohol. In each case the bile alcohols epimeric at C-24 and C-25 were resolved by analytical and preparative thin-layer chromatography and characterized by gas–liquid chromatography, infrared-, proton magnetic resonance-, and mass spectrometry. Tentative assignment of the 24*R*, 24*S* and 25*R*, 25*S* configurations was made on the basis of molecular rotation differences. These epimeric bile alcohols will be useful for biological studies of chenodeoxycholic acid biosynthesis.

Supplementary key words bile alcohols · chenodeoxycholic acid biosynthesis

The mechanism whereby cholesterol is converted into chenodeoxycholic acid in vertebrates has not been studied in adequate detail. C_{27} bile alcohols have been postulated as intermediates in the formation of this primary bile acid and the individual steps in the formation of chenodeoxycholic acid are presumably similar to those involved in cholic acid synthesis (1). The pathway for the degradation of the sterol side chain is thought to involve 26-hydroxylation as an initial step (2). Recent studies from this and other laboratories have indicated that 25-hydroxylation of the side chain may also play a role in bile acid synthesis (2,3). In order to investigate the major metabolic pathway of chenodeoxycholic acid biosynthesis and the sequence of side-chain hydroxylations, we required the hypothetical intermediates 5β-cholestane-3α,7α,26-triol (25*R* and 25*S*), and the isomeric 5β-cholestane-3α,7α,24(*R*)-triol and 5β-cholestane-3α,7α,24(*S*)-triol (**Fig. 1**).

Reported syntheses (4, 5) of 5β-cholestane-3α,7α,26-triol involve the electrolytic coupling of chenodeoxycholic acid with the half ester of methylsuccinic acid and subsequent reduction with $LiAlH_4$. The product resulting from electrolysis is a complex mixture that has to be separated by preparative thin-layer or column chromatography and yields are very low (5).

Utilizing the sequence illustrated in **Fig. 2,** we have shown that it is possible to produce 5β-cholestane-3α,7α,24-triol and 5β-cholestane-3α,7α,26-triol by a hydroboration reaction (6). Small amounts of 5β-cholestane-3α,7α-diol are also formed. The bile alcohols epimeric at C-24 or C-25 were separated to obtain chromatographically pure products.

EXPERIMENTAL

Methods

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, IA), model MP-126000, and are uncorrected.

Infrared spectra were recorded on a Perkin-Elmer (Norwalk, CT) model 421 grating spectrophotometer as KBr discs. Absorption frequencies are quoted in reciprocal centimeters.

NMR spectra, in Hertz, were obtained in deuterated chloroform ($CDCl_3$) solution using a JEOL (Medford,

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethyl silyl; NMR, nuclear-magnetic resonance; IR, infrared; THF, tetrahydrofuran.

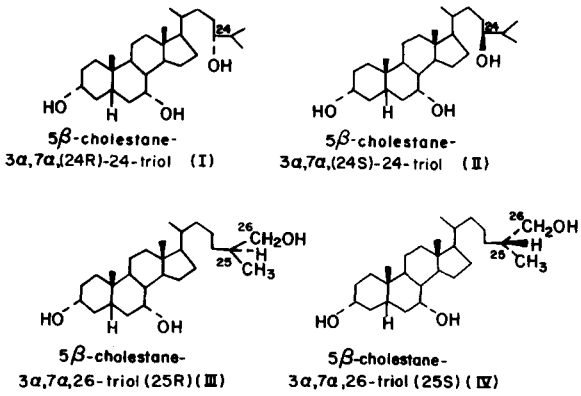


Fig. 1. Structures of epimeric 5β-cholestane-3α,7α,24-triols (24*R* and 24*S*) and 5β-cholestane-3α,7α,26-triols (25*R* and 25*S*).

MA) PS-100 spectrometer equipped with Fourier transform capability.

Optical rotations were determined in methanol on a Perkin-Elmer (Norwalk, CT) model 141 polarimeter.

GLC. The bile alcohols, as the TMSi derivatives, were analyzed on a 180 cm × 4 mm column packed with 3% QF-1 or 1% HI-EFF 8BP on 80/100 mesh Gas Chrom Q; column temp. 240°C (Hewlett-Packard model 7610 gas chromatograph) (Hewlett-Packard, Palo Alto, CA.).

Mass spectra of the bile alcohols were obtained with a Varian MAT-111 gas chromatograph–mass

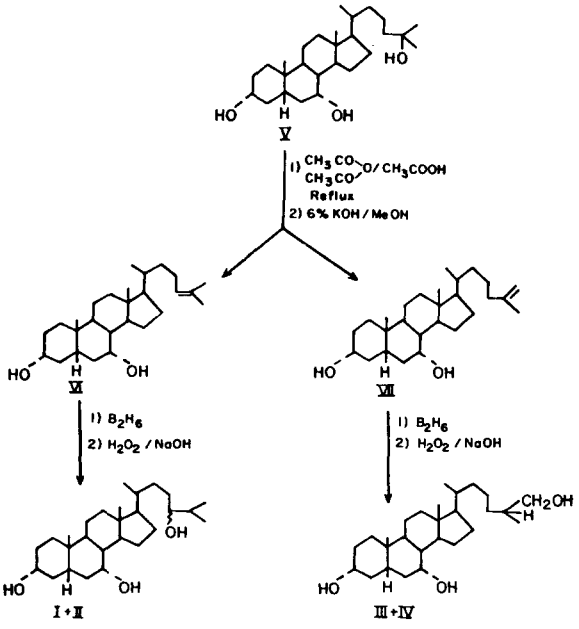


Fig. 2. Synthesis of 5β-cholestane-3α,7α,24-triol and 5β-cholestane-3α,7α,26-triol. *V*, 5β-cholestane-3α,7α,25-triol; *VI*, 5β-cholest-24-ene-3α,7α-diol; *VII*, 5β-cholest-25-ene-3α,7α-diol; *I + II*, 5β-cholestane-3α,7α,24ξ-triol; *III + IV*, 5β-cholestane-3α,7α,26-triol.

spectrometer (Varian Associates, Palo Alto, CA) at an ion source pressure of 2–3 × 10^{-6} mm and an electron energy of 70 eV, as described previously (7). High resolution mass spectra of bile alcohols were determined on a model CEC-110 mass spectrometer (Consolidated Electrodynamics, Monrovia, CA).

TLC. The bile alcohols were separated on silica gel G plates (Brinkmann Instruments, Westbury, NJ, 0.25 mm thickness), or on precoated plastic sheets (Brinkmann, 0.25 mm silica gel without gypsum) with the solvent system, chloroform–acetone–methanol 35:25:0.75 (v/v/v). The spots were made visible either with iodine or with water.

Dehydration of 5β-cholestane-3α,7α,25-triol: 5β-cholest-24-ene-3α,7α-diol (VI) and 5β-cholest-25-ene-3α,7α-diol (VII, Fig. 2)

The procedure used was similar to that of Dayal, et al. (7, 8). A solution of 300 mg of 5β-cholestane-3α,7α,25-triol (9) in 6 ml of glacial acetic acid was refluxed for 3 hr. Four ml of acetic anhydride was added and the reaction mixture was further refluxed for 12 hr. The reaction mixture was evaporated to dryness in vacuo. The pale yellow semisolid (300 mg) obtained was subjected to column chromatography on 10% $AgNO_3$–silicic acid (50 g). Elution with benzene yielded pure 5β-cholest-24-ene-3α,7α-diacetate (208 mg), mp 67–69°C, ν_{max}^{KBr} 826 cm^{-1} (>C=CH–). The compound was hydrolyzed by refluxing with 10 ml of 6% methanolic potassium hydroxide for 1.5 hr, and the reaction mixture was poured into a beaker with crushed ice. The 5β-cholest-25-ene-3α,7α-diol (VI, Fig. 2) obtained weighed 180 mg. Further elution of the column with benzene–chloroform 75:25 provided pure 5β-cholest-25-ene-3α,7α-diacetate (70 mg), mp 69–72°C, ν_{max}^{KBr} 890 cm^{-1} (>C=CH_2). Hydrolysis of this compound with 5 ml of 6% methanolic potassium hydroxide yielded 60 mg of 5β-cholest-25-ene-3α,7α-diol (VII, Fig. 2).

Preparation of 5β-cholestane-3α,7α,24(*R*)-triol and 5β-cholestane-3α,7α,24(*S*)-triol, via hydroboration of 5β-cholest-24-ene-3α,7α-diol[1]

5β-Cholest-24-ene-3α,7α-diol (100 mg) obtained by the hydrolysis of the diacetate was used without further purification. The compound was dissolved in 15 ml of absolute tetrahydrofuran (THF), the solution was cooled to 0°C, and a 1 M borane solution in dry

[1] The preparation and handling of organoboranes require techniques and precautions similar to those used for the Grignard reaction. Although the necessity for a nitrogen atmosphere has not been established, hydroboration reactions are normally carried out under nitrogen. It is convenient to transfer solutions of diborane and dialkylboranes by means of a hypodermic syringe.

TABLE 1. GLC and TLC characteristics of trihydroxy bile alcohols

	TLC	GLC	
		Retention Time of TMSi Ethers Relative to 5α-Cholestane[a]	
	R_f	3% QF-1	1% HI-EFF 8BP
5β-Cholestane-3α,7α,24-triol (24*R*)	0.56[b]	2.86 ± 0.01	1.70
5β-Cholestane-3α,7α,24-triol (24*S*)	0.61[b]	2.90 ± 0.01	1.68
5β-Cholestane-3α,7α,26-triol (25*R*)	0.57[c]	3.33	2.27
5β-Cholestane-3α,7α,26-triol (25*S*)	0.59[c]	3.35	2.28

[a] Retention time of 5α-cholestane on 3% QF-1, 2.65 min; on 1% HI-EFF 8BP, 4.1 min; column temp., 240°C; N_2 flow 40 ml/min.
[b] Solvent system: chloroform–acetone–methanol 35:25:0.75 (v/v/v). Silica gel G plates, 0.25 mm thick (Brinkmann).
[c] Solvent system: chloroform–acetone–methanol 35:25:0.75 (v/v/v). Precoated silica sheets (Brinkmann, 0.25 mm silica gel without gypsum).

THF (2.25 ml; 2.25 mmol)[2] (Aldrich Chemical Co., Inc., Milwaukee, WI) was added (10). The mixture was stirred at 0°C for 1 hr followed by stirring at 25°C for 15 min. 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 THF in vacuo, extraction with ethyl acetate, two washings with saturated NaCl solution, and evaporation to dryness yielded 80 mg of an amorphous powder. Purification by column chromatography on neutral alumina (grade IV) with ethyl acetate gave 40 mg of 5β-cholestane-3α,7α,24(*S*)-triol, mp 78–79°C. High resolution mass spectrum: 402.3487; $M^+ - H_2O$ (calculated for $C_{27}H_{48}O - H_2O$: 402.3498). A single spot on TLC; $[\alpha]_D$, GLC, NMR, and mass spectrum are given in **Tables 1–4.** Elution of the column with 1% methanol in ethyl acetate yielded 32 mg of 5β-cholestane-3α,7α,24(*R*)-triol, mp 125–127°C. Single spot on TLC. TLC, GLC, $[\alpha]_D$, NMR, mass spectral characteristics are given in Tables 1–4.

[2] In the hydroboration procedure the presence of one or more free hydroxyl groups required a corresponding excess of the hydroborating agent. The boric esters formed did not interfere with the hydroboration and were hydrolyzed subsequently to free hydroxyls during the product isolation work-up.

TABLE 2. Molecular rotation of epimeric bile alcohols[a]

5β-Cholestane-	$[\alpha]_D^{25}$	M_D	
		Calculated	Found
3α,7α-diol[b]	+14.2°	+40°[c]	+57.5°
3α,7α,24-triol (24*R*) (I)	+25.8°	+99.5°[d]	+108.5°
3α,7α,24-triol (24*S*) (II)	+4.0°	+34.5°[d]	+16.8°
3α,7α,26-triol (25*R*) (III)	+15.9°	+86°[e]	+66.9°
3α,7α,26-triol (25*S*) (IV)	+8.8°	+38.5°[f]	+37.0°

[a] Determined in methanol (5β-cholestane-3α,7α-diol, 11.3 mg/ml; I, 4.8 mg/ml; II, 5.5 mg/ml; III, 10.7 mg/ml; IV, 0.8 mg/ml).
[b] See reference 13.
[c] Calculated from Fieser and Fieser (13), p. 179.
[d] Calculation based on M_D of 5β-cholestane-3α,7α-diol (+57.5°), ΔM_D 24(*R*)-ol, +42° (14) and ΔM_D 24(*S*)-ol, −23° (14).
[e] Calculations based on M_D of 5β,25(*R*)-cholestane-3α,7α,12α26-tetrol = +157° (15), M_D of 5β-cholestane-3α,7α,12α-triol = +128° (16) and ΔM_D of 26-ol = 157° − 128° = +29°.
[f] Calculations based on M_D of 5β-cholestane-3α,7α,12α,26-tetrol = +109° (15), M_D of 5β-cholestane-3α,7α,12α-triol = +128° (16) and ΔM_D of 26-ol = 109° − 128° = −19°.

TABLE 3. NMR spectra of C_{27} bile alcohols[a]

Compound	C-18	C-19	C-21	C-26	C-27
	Hz	*Hz*	*Hz*	*Hz*	*Hz*
5β-Cholestane-3α,7α,24-triol (24*R*)	66.0	91.0	91.0	91.5	91.5
5β-Cholestane-3α,7α,24-triol (24*S*)	65.0	90.0	88.5	92.5	92.5
5β-Cholestane-3α,7α,26-triol[b]	66.0	90.0	91.0		92.5

[a] Determined as described in Experimental section.
[b] A mixture of the 25*R* and 25*S* diastereomers was used.

Preparation of 5β-cholestane-3α,7α,26-triol (25*R* and 25*S*) via hydroboration of 5β-cholest-25-ene-3α,7α-diol

5β-Cholest-25-ene-3α,7α-diol (50 mg) was dissolved in 6 ml of absolute tetrahydrofuran. The solution was cooled to 0°C and a 1 M borane solution in THF (0.65 ml) was added. The mixture was stirred at 0°C for 1 hr and at 25°C for 15 min. One ml of NaOH (3 N), cooled to 0°C, was mixed with 0.2 ml of H_2O_2

TABLE 4. Percent relative intensity for major fragments of the TMSi ethers of 5β-cholestane triols

Fragment Ions	*m/e*	% Relative Intensity	
		3α,7α,24-triol[a]	3α,7α,26-triol[b]
M^+	636		
M − 15	621		
M − 43	593		
M − 90	546	0.4	1.2
M − (90 + 15)	531	0.2	0.6
M − (90 + 43)	503	6.6	
M − (2 × 90)	456	3.7	23.7
M − (2 × 90 + 15)	441	1.0	10.2
M − (2 × 90 + 43)	413	28.8	
M − (3 × 90)	366	3.4	4.1
M − (3 × 90 + 15)	351	3.3	6.9
M − (3 × 90 + 43)	323	11.3	
M − (2 × 90 + 201)	255	15.0	34.9
Charged side chain fragment	145	100	
$Si(CH_3)_3$	73	51.2	100

[a] The mass spectra of the 24*R* and 24*S* epimers were identical.
[b] The mass spectra of 25*R* and 25*S* were identical.

(30%) precooled to 0°C. The cold basic peroxide was gradually added to the organoborane solution at 0°C, and stirring was continued overnight at room temperature. Dilution with water, removal of THF in vacuo, extraction with ethyl acetate (two NaCl washings), drying, and concentration in vacuo gave a semisolid. This was passed through a column of neutral Al_2O_3 (15 g) and 26 mg of a material was obtained that showed two major spots on TLC [($CHCl_3$–CH_3COCH_3–MeOH 70:50:1.5) developed twice]. The compound with R_f 0.57 [5β-cholestane-3α,7α,26-triol (25*R*)] crystallized from methanol and had a mp of 155–157°C; that with R_f 0.59 [5β-cholestane-3α,7α,26-triol (25*S*)] melted at 138–140°C (from methanol–acetone). The TLC, GLC, $[\alpha]_D$, NMR, and mass spectral characteristics are given in Tables 1–4. The two compounds were separated by preparative TLC using precoated plastic sheets (Brinkmann, 0.25 mm silica gel without gypsum). The plates were developed twice in $CHCl_3$–CH_3COCH_3–MEOH 70:50:1.5. The two compounds were eluted from the sheets, checked by analytical TLC and were found to be homogeneous in the above solvents. Their TMSi derivatives had identical retention times on QF-1 columns and had identical mass spectra (Tables 1 and 4). High resolution mass spectra: 5β-cholestane-3α,7α,26-triol (25*R*), 420.3540; M^+ (calculated for $C_{27}H_{48}O_3$: 420.3603); 5β-cholestane-3α,7α,26-triol (25*S*), 420.3634; M^+ (calculated for $C_{27}H_{48}O_3$: 420.3603).

Hydroboration of a mixture (75:25) of 5β-cholest-24-ene-3α,7α-diol and 5β-cholest-25-ene-3α,7α-diol

A mixture of unsaturated diols (compounds VI and VII, Fig. 2) (180 mg; 0.43 mmol) was dissolved in 20 ml of absolute THF. The solution was cooled to 0°C and hydroborated with 1 M borane solution in dry THF (2.25 ml; 2.25 mmol) to yield 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 70:50:4 (v/v/v)]. The compound from the zone with the R_f 0.74 was crystallized from methanol to yield 12 mg of 5β-cholestane-3α,7α-diol, mp 82–84°C (lit. mp 84–86°C) (11).

The compound from the zone with R_f 0.61 (40 mg) was crystallized from acetone to yield 33 mg of II(24*S*), mp 78–79°C, and the material from the zone with R_f 0.56 yielded, after two crystallizations from acetone, 16.0 mg of I(24*R*), mp 125–127°C. The mother liquor contained 35 mg of 5β-cholestane-3α,7α,26-triol (III + IV) and, after repeated crystallizations from acetone, gave 17.0 mg of a product, mp 154–155°C (lit. mp 149–151°C) (12), which was a mixture of the 25*R* and 25*S* isomers.

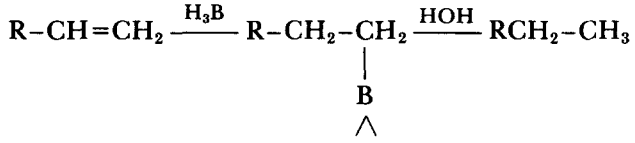
RESULTS AND DISCUSSION

This paper describes a new synthetic procedure for the preparation of the following C_{27}-steroid alcohols: 5β-cholestane-3α,7α,24-triol (24*R* and 24*S*) and 5β-cholestane-3α,7α,26-triol (25*R* and 25*S*) (Fig. 1). These bile alcohols will be useful both as substrates and as reference compounds in studies dealing with chenodeoxycholic acid biosynthesis.

The previous methods for the synthesis of 5β-cholestane-3α,7α,26-triol (25*R* and 25*S*) have employed electrolytic coupling (Kolbe reaction). For example, in the preparation of 3α,7α-dihydroxy-5β-cholestan-26-oic acid, the half ester of methyl succinic acid is coupled with chenodeoxycholic acid resulting in a mixture of 25*R* and 25*S* epimers (12). Bridgwater (4) attempted to prepare the isomeric coprostanoic acids (25*R* and 25*S*) by coupling the bile acid with optically active methyl succinic acid esters. The compounds obtained were not considered pure with respect to configuration at C-25, suggesting that racemization might have occurred at some stage or that some coupling of $HOOC–CH(CH_3)–CH_2–COOCH_3$ may, in fact, have taken place, in either case yielding a mixture (4). Thus the use of the optical enantiomorphs of methyl succinic acid half esters cannot be regarded as reliable. In 1970, Briggs (5) improved the Bridgwater procedure by preparing optically active methyl succinic acid from methyl allyl acetic acid, and synthesized 25*R*- or 25*S*-trihydroxycoprostanoic acid (THCA) and dihydroxycoprostanoic acid (DHCA) methyl esters. These methyl esters could be reduced with $LiAlH_4$ to obtain the corresponding 26-hydroxy bile alcohols. The method adopted by Briggs of optical resolution of the starting material is cumbersome and time consuming and the yields of the final products in the electrolytic coupling process are usually quite low (3–5%). For this reason we worked out a synthesis of the 26-hydroxylated bile alcohol from the Δ^{25} unsaturated intermediate by a hydroboration reaction. The bile alcohol obtained was a mixture of the 25*R* and 25*S* forms of 5β-cholestane-3α,7α,26-triol. Since these compounds are diastereoisomers, they could be separated by thin-layer chromatography (7). Similarly, the 24-hydroxy epimers of 5β-cholestane-3α,7α,24-triol could be prepared by hydroboration of the Δ^{24} compound and the two epimeric constituents were likewise separable by TLC.

The two-step synthesis of 5β-cholestane-3α,7α,24(*R*)-triol and 5β-cholestane-3α,7α,24(*S*)-triol involves the hydroboration of the Δ^{24} compound with B_2H_6 in THF (6). The diborane intermediate was oxidized with alkaline hydrogen peroxide to give the mixture of two isomeric triols (I and II, Fig. 1).

The formation of small amounts of 5β-cholestane-3α,7α-diol (see Experimental) is attributed to the protonolysis (6) of the organoborane intermediate as follows:



Similarly, the synthesis of 5β-cholestane-3α,7α,26-triol (25*R* and 25*S*) was achieved by hydroboration of the Δ^{25} compound, followed by oxidation with alkaline hydrogen peroxide of the intermediate organoborane. The isomeric 5β-cholestane-3α,7α,26-triols (25*R* and 25*S*) were separated by preparative TLC using silica gel G plates or precoated plastic sheets, and crystalline products were obtained. These purified bile alcohols were employed to determine certain physical properties.

The nuclear magnetic resonance spectra of 5β-cholestane-3α,7α,24(*R*)- and 5β-cholestane-3α,7α,24(*S*)-triol (Table 3) were essentially identical except for some differences in the chemical shift of the C-21 methyl group, which appeared as a doublet at 0.91 ppm for 24(*R*)-triol and 0.88 ppm for 24(*S*)-triol. In the NMR spectrum of 5β-cholestane-3α,7α,26-triol, the C-26 methyl signal was absent and, instead, a multiplet at δ 3.45 was observed for two protons of the CH_2–OH group.

The TMSi derivatives of the bile alcohols were quantitated by GLC on 3% QF-1 or 1% HI-EFF 8BP. The retention times of the TMSi ethers of the epimeric 5β-cholestane-3α,7α,24-triols were very similar, as were those of the two 5β-cholestane-3α,7α,26-triols (Table 1).

The identification of the triols was done by mass spectrometry of their TMSi derivatives. The fragment ions 145 and 255 appeared as base peaks in the spectra of the TMSi ethers of 5β-cholestane-3α,7α,24-triol and 5β-cholestane-3α,7α,26-triol, respectively (Table 4). The mass spectra of the TMSi ethers of 5β-cholestane-3α,7α,24-triol and 5β-cholestane-3α,7α,24(*S*)-triols were identical. Likewise the two 5β-cholestane-3α,7α,26-triols (25*R* and 25*S*) had identical mass spectra (Table 4).

Assignment of configuration at C-24 and at C-25 of 5β-cholestane-3α,7α,24-triol (24*R* and 24*S*) and 5β-cholestane-3α,7α,26-triol (25*R* and 25*S*) was carried out by the method of molecular rotation differences. Introduction of a new center of asymmetry changes the optical rotation of bile alcohols in a manner that causes systematic differences in molecular rotation (M_D). These M_D increments (ΔM_D) should be the same for a series of bile alcohols having the same asymmetry at a given carbon atom. This assumes no vicinal effects, which is probably the case with the bile alcohols under study in which the side-chain hydroxyl is well separated from the rest of the molecule. In the case of 5β-cholestane-3α,7α,24-triol the parent compound was 5β-cholestane-3α,7α-diol, M_D = 57.5°. The compound with the more positive rotation (M_D=108.5°) was assigned the 24*R* configuration as previously suggested by Masui and Staple (15, 16) for 5β-cholestane-3α,7α,12α,24α-tetrol. In the case of 5β-cholestane-3α,7α,26-triol the ΔM_D attributable to the 26-hydroxyl group was based upon the known molecular rotations of 5β-cholestane-3α,7α,12α,26-tetrol (25*R*=+157° and 25*S*=+109°). We have tentatively assigned the 25*R* configuration to the more dextrorotatory of the two epimeric 5β-cholestane-3α,7α,26-triols (Table 2).

We are indebted to Dr. W. Benz, Physical Chemistry Department, Hoffman-LaRoche, Nutley, NJ, for determining the high resolution mass spectra. This work was supported in part by U.S. Public Health Service grants HL-10894, HL-17818, AM-05222, AM-19696; and N.S.F. grant GB-31919X.

Manuscript received 25 January 77 and in revised form 1 August 77; accepted 14 October 1977.

REFERENCES

1. Mosbach, E. H. 1972. Hepatic synthesis of bile acids. Biochemical steps and mechanisms of rate control. *Arch. Intern. Med.* **130:** 478–487.
2. Cronholm, T., and G. Johansson. 1970. Oxidation of 5β-cholestane-3α,7α,12α-triol by rat liver microsomes. *Eur. J. Biochem.* **16:** 373–381.
3. Shefer, S., F. W. Cheng, B. Dayal, S. Hauser, G. S. Tint, G. Salen, and E. H. Mosbach. 1976. A 25-hydroxylation pathway of cholic acid biosynthesis in man and rat. *J. Clin. Invest.* **57:** 897–903.
4. Bridgwater, R. J. 1956. Partial synthesis of the two 3α,7α,12α-trihydroxy coprostanic acids and of similar bile acids with extended side chains. *J. Biochem.* **64:** 593–599.
5. Briggs, T. 1970. Partial synthesis of 25D- and 25L-cholestanoic acids from some common bile acids. *J. Org. Chem.* **35:** 1431–1434.
6. Brown, H. C. 1962. Hydroboration, W. A. Benjamin, Inc., New York, N. Y. 93–101.
7. Dayal, B., S. Shefer, G. S. Tint, G. Salen, and E. H. Mosbach. 1976. Synthesis of 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24ξ,25-pentol. *J. Lipid Res.* **17:** 74–77.
8. Dayal, B., S. Shefer, G. S. Tint, G. Salen and E. H. Mosbach. 1976. C_{26}-analogs of naturally occurring C_{27} bile alcohols. *J. Lipid Res.* **17:** 478–484.
9. Cohen, B. I., G. S. Tint, T. Kuramoto, and E. H. Mosbach. 1975. New bile alcohols—Synthesis of 5β-cholestane-3α,7α,25-triol and 5β-cholestane-3α,7α,25-24-(^{14}C)-triol. *Steroids.* **25:** 365–378.

10. Barcza, S., and C. W. Hoffman. 1975. Total synthesis of the 6-silasteroid ring system. *Tetrahedron.* **31:** 2363–2367.
11. Björkhem, I., and J. Gustafsson. 1973. ω-Hydroxylation of steroid side-chain in biosynthesis of bile acids. *Eur. J. Biochem.* **36:** 201–212.
12. Nicolau, G., B. I. Cohen, G. Salen, and E. H. Mosbach. 1976. Studies on the 12α- and 26-hydroxylation of bile alcohols by rabbit liver microsomes. *Lipids.* **11:** 148–152.
13. Fieser, L. F., and M. Fieser. 1959. Steroids. Reinhold, New York, 341.
14. Shefer, S., B. Dayal, G. S. Tint, G. Salen, and E. H. Mosbach. 1975. Identification of pentahydroxy bile alcohols in cerebrotendinous xanthomatosis (CTX). Characterization of 5β-cholestane-3α,7α,24ξ,25-pentol and 5β-cholestane-3α,7α,12α,33ξ,25-pentol. *J. Lipid Res.* **16:** 280–286.
15. Anderson, I. G., and G. A. D. Haslewood. 1970. Comparative studies of bile salts: 5α-chimaerol, a new bile alcohol from the white sucker *Catostomus commersoni Lacépède. Biochem. J.* **116:** 581–587.
16. Masui, T., and E. Staple. 1967. The separation of the stereoisomers of bile steroids, 5β-cholestane-3α,7α,12α,24α-tetrol and 5β-cholestane-3α,7α,12α,24β-tetrol, by thin layer chromatography. *Steroids.* **9:** 443–450.