Configuration at C-25 in 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid by X-ray crystallography

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Abstract The two diastereoisomers at carbon-25 of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid, a key intermediate in the biosynthetic pathway of cholic acid, were obtained in pure form by a combination of fractional crystallization and thin-layer chromatography. The configuration at C-25 of these two isomers was established by X-ray crystallography as 25*S* for one diastereoisomer (mp 199–201°C) and 25*R* for the other (mp 180–182°C). These findings permit us to determine, unequivocally, the configuration of this naturally occurring C_{27}-bile acid in man and other animals and to establish the stereospecificity of the microsomal and mitochondrial ω-hydroxylation pathway for the side-chain oxidation of cholesterol to bile acids.—**Batta, A. K., G. Salen, J. F. Blount, and S. Shefer.** Configuration at C-25 in 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid by X-ray crystallography. *J. Lipid Res.* 1979. **20:** 935–940.

Supplementary key words diastereoisomers · thin-layer chromatography

3α,7α,12α-Trihydroxy-5β-cholestan-26-oic acid (THCA) has been postulated as a key intermediate in the biosynthesis of cholic acid from cholesterol (1). This C_{27}-bile acid has been shown to be formed from 5β-cholestane-3α,7α,12α-triol by mouse and rat liver homogenates (2, 3) and to be metabolized to cholic acid in man and in the rat (1, 4). The hydroxylation of this triol introduces an asymmetric center at C-25 to form the 25*R* and 25*S* isomers of 5β-cholestane-3α,7α,12α,26-tetrol which upon oxidation yield the corresponding isomers of THCA (**Fig. 1,** *a* and *b*). In vitro experiments in the rat demonstrated that the catabolism of cholesterol resulted in the formation of both isomers of THCA (5, 6). Furthermore, Mendelsohn and Mendelsohn (5) isolated both isomers of this acid from the bile of *Crocodylus niloticus*, while Haslewood (7) obtained only one isomer from the bile of *Alligator mississippiensis*. This latter isomer was also isolated from human bile (8, 9). It has been suggested that the drastic procedures involved in the hydrolysis of the taurine conjugate of THCA, the principal bile acid of the Crocodylidae might cause isomerization at C-25, thus giving rise to both diastereoisomers (10).

Up to now, fractional crystallization was employed to separate the two forms of THCA which differed in melting point and optical rotation. However, no definite assignment to either the *R* or the *S* configuration could be made. Popják et al. (11) using ^{13}C-NMR spectroscopy proposed, for purposes of nomenclature, that the pro-(*R*)-methyl group at C-25 (derived from C-2 of mevalonate) be numbered 26 and the pro-(*S*)-methyl group (derived from C-3′ of mevalonate) be numbered 27. On the basis of this nomenclature, Gustafsson and Sjöstedt (6) reported that rat liver microsomes hydroxylate the C-26 methyl group of 5β-cholestane-3α,7α,12α-triol to form 5β-cholestanetetrol which is then oxidized to THCA carboxylated at C-26.

The present report describes a solvent system that can separate the two diastereoisomers of THCA by thin-layer chromatography (TLC) (**Fig. 2**). Both isomers were obtained in pure form by a combination of fractional crystallization and preparative TLC and their configurations at C-25 were established by X-ray crystallography.

EXPERIMENTAL METHODS

Methods

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

Abbreviations: THCA, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl.

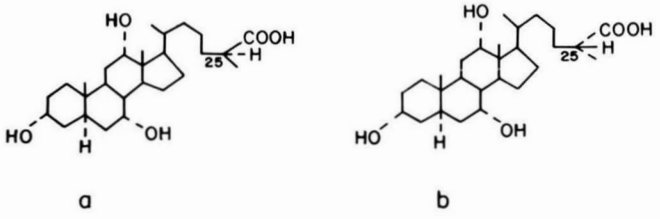

Fig. 1. Structures of isomeric 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acids. *a*, 25*R*; *b*, 25*S*.

TLC. The two isomers of THCA were separated on silica gel G plates (Brinkmann, 0.25 mm thickness). The spots were detected with phosphomolybdic acid (3.5% in isopropanol) and sulfuric acid (20%).

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

Infrared spectra were determined in chloroform solution on a Perkin-Elmer model 421 grating spectrophotometer.

GLC. The two isomers of THCA, as their methyl ester TMSi-derivatives, were analyzed on a 180-cm × 4-mm column packed with 3% QF-1 on 80/100 mesh Gas Chrom Q, column temp. 230°C (Hewlett-Packard model 7610 gas chromatograph).

Mass spectra. Mass spectra were obtained with a Varian MAT-111 gas chromatograph–mass spectrometer (Varian Associates, Palo Alto, CA).

X-ray diffraction data were collected using a Hilger-Watts diffractometer (Ni filtered Cu$K\alpha$ radiation, θ-2θ scans, with pulse height discrimination). The size of the crystal used was approximately 0.75 × 0.34 × 0.16 mm. The crystals were monoclinic, of space group $P2_1$; cell dimensions: $a = 9.776(2)$Å, $b = 7.697(2)$Å, $c = 17.933(4)$Å, $\beta = 102.41(2)°$ and $d_{calc} = 1.135$ g cm^{-3} for $Z = 2$. Of the 1942 independent reflections for $\theta < 57°$, 1876 were considered to be observed [$I > 2.5\ \sigma$ (I)].

Isolation of the two diastereoisomers of THCA

i) Bile of *Alligator mississippiensis* (100 ml) was deproteinized (5) and the greenish brown residue thus obtained was dissolved in 100 ml of diethylene glycol containing 25 g of sodium hydroxide. The reaction mixture was heated to 190–200°C for 30 min. After cooling, 200 ml of water was added, the solution was acidified to pH 1 with 6 N HCl and extracted six times with 100-ml portions of ethyl acetate. The combined ethyl acetate extracts were washed to neutrality with water (4 × 20 ml), dried over anhydrous sodium sulfate, and evaporated; yield approximately 8 g. After trituration with *n*-hexane (4 × 40 ml) the residue was dissolved in 100 ml of ethanol. After decoloration (boiling for 1 min with 10 g of charcoal) the resulting filtrate was evaporated. Crystallization from ethyl acetate (5°C) yielded colorless prisms (mp 195–199°C). This crystalline material (A) was found by TLC analysis, using silica gel G plates and a solvent system of chloroform–acetone–methanol 70:50:10 (v/v/v, developed twice), to have two components with R_f values of 0.41 and 0.44 (see Fig. 2), the latter predominating. After crystallization from ethyl acetate to constant melting point [199–201°C; $[\alpha]_D^{25} = 45.6°$ (*c*, 1.75 in ethanol)] only one spot (R_f 0.44) was seen. In this fashion the faster-moving isomer of THCA was isolated in pure form. The supernatant from (A) was crystallized from ethyl acetate to yield colorless prisms (B) (mp 184–185°C,

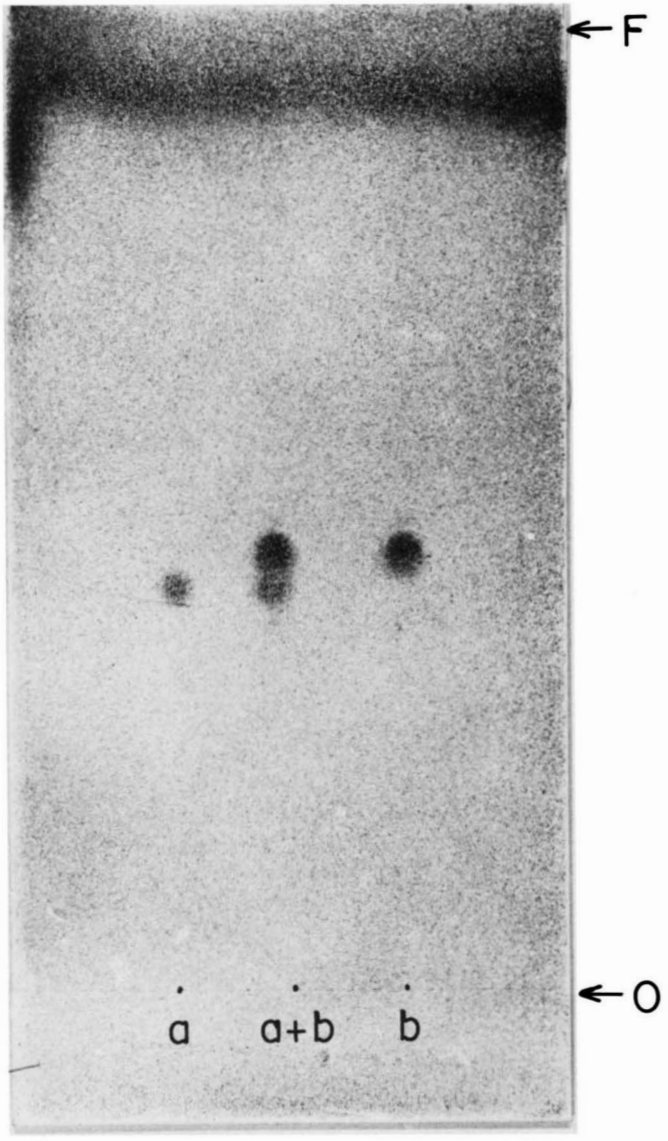

Fig. 2. Thin-layer chromatogram of the isomeric 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acids. Silica gel G plates, developed twice in chloroform–acetone–methanol 70:50:10 (v/v/v). *a*, 25*R*; *b*, 25*S*; *O*, origin; and *F*, solvent front.

unchanged upon repeated crystallization). However, TLC analysis of (B) indicated the presence of the two compounds with R_f values of 0.41 and 0.44 in approximately equal amounts. In order to obtain the slower-moving compound, (B) was subjected to preparative TLC using the above solvent system. The compound with R_f 0.41 was eluted with methanol and crystallized to a constant melting point of 180–182°C, $[\alpha]_D^{25} = +30.2°$ (c, 1.70 in ethanol). The supernatant from (B) yielded an additional crop of colorless prisms (mp 173–175°C) (C) which upon TLC also indicated the presence of the above two compounds. These two compounds (R_f 0.41 and 0.44) had identical infrared spectra and showed identical mass-spectral fragmentation patterns which agreed with that reported for THCA (12).

ii) THCA was synthesized via Arndt-Eistert rearrangement of 3α,7α,12α-triformyloxy-5β-cholane-24-carboxylic acid chloride (13) and, upon TLC in chloroform–acetone–methanol 70:50:10 (v/v/v, developed twice), showed two spots of equal intensity with R_f values of 0.41 and 0.44. The compounds corresponding to these two spots were isolated by preparative TLC, using the same solvent system, and were found to be identical with those obtained from *Alligator mississippiensis* (mp, mixed mp, TLC, $[\alpha]_D$, infrared and mass spectroscopy). Only the faster-moving isomer yielded sufficiently large crystals suitable for X-ray crystallography.

RESULTS AND DISCUSSION

The thin-layer chromatographic solvent system and the fractional crystallization procedure described above enabled us to isolate the two diastereoisomers of THCA in pure form. That the two compounds were isomeric at C-25 was proved by their synthesis

TABLE 1. Physical properties of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) isomeric at C-25

	THCA	
	25*R*	25*S*
mp	180–182°C[a]	199–201°C[b]
$[\alpha]_D^{25}$[c]	+30.2°[d]	+45.6[e]
TLC[f]	0.41	0.44
GLC[g]	2.63	2.63
Molecular ion (M^+)	450	450

[a] Lit. mp 172°C (5); 183–184°C (15).
[b] Lit. mp 195°C (5); 199–201°C (15).
[c] Determined in methanol: THCA (25*R*), 17.5 mg/ml; THCA (25*S*), 17.0 mg/ml.
[d] Lit. $[\alpha]_D^{20} = +27{\cdot}8 \pm 2°$ (5); +28° (15).
[e] Lit. $[\alpha]_D^{20} = +43 \pm 2°$ (5); +44° (15).
[f] Solvent system: chloroform–acetone–methanol 70:50:10 (v/v/v, developed twice); silica gel G, 0.25 mm thick (Brinkmann).
[g] Retention time of TMSi ethers relative to 5α-cholestane. Column: 1% HiEFF 8BP, column temperature 240°C, N_2 flow 40 cc/min, retention time of 5α-cholestane 5·80 min.

TABLE 2. Final atomic parameters for 25*S*-THCA

Atom	X	Y	Z	B
O(1)	0.4248(4)[a]	0.2804	-0.0914(2)[a]	[b]
O(2)	0.4410(3)	0.0443(6)[a]	0.1579(2)	[b]
O(3)	0.6034(3)	0.5984(6)	0.2263(2)	[b]
O(4)	1.1565(4)	0.2640(7)	0.7248(2)	[b]
O(5)	1.2860(4)	0.5029(8)	0.7482(2)	[b]
C(1)	0.2102(5)	0.5403(8)	0.0195(2)	[b]
C(2)	0.3255(5)	0.5059(8)	-0.0222(3)	[b]
C(3)	0.3114(5)	0.3223(8)	-0.0566(3)	[b]
C(4)	0.3061(5)	0.1915(7)	0.0053(3)	[b]
C(5)	0.1938(4)	0.2250(7)	0.0497(3)	[b]
C(6)	0.1940(5)	0.0852(8)	0.1108(3)	[b]
C(7)	0.3162(5)	0.0972(8)	0.1799(3)	[b]
C(8)	0.3282(4)	0.2831(7)	0.2131(3)	[b]
C(9)	0.3312(5)	0.4235(8)	0.1524(2)	[b]
C(10)	0.1998(5)	0.4119(7)	0.0846(3)	[b]
C(11)	0.3488(5)	0.6051(8)	0.1907(3)	[b]
C(12)	0.4796(5)	0.6177(7)	0.2554(2)	[b]
C(13)	0.4752(4)	0.4819(7)	0.3179(2)	[b]
C(14)	0.4603(4)	0.3010(7)	0.2769(2)	[b]
C(15)	0.4826(5)	0.1713(8)	0.3417(3)	[b]
C(16)	0.6018(5)	0.2528(8)	0.4012(3)	[b]
C(17)	0.6163(5)	0.4460(7)	0.3772(2)	[b]
C(18)	0.3572(5)	0.5194(8)	0.3576(3)	[b]
C(19)	0.0644(5)	0.4519(9)	0.1095(3)	[b]
C(20)	0.6599(5)	0.5623(8)	0.4479(2)	[b]
C(21)	0.6544(6)	0.7578(8)	0.4305(3)	[b]
C(22)	0.8090(5)	0.5092(9)	0.4892(3)	[b]
C(23)	0.8529(5)	0.5661(9)	0.5715(3)	[b]
C(24)	0.9964(5)	0.4975(9)	0.6088(3)	[b]
C(25)	1.0395(5)	0.5324(9)	0.6952(3)	[b]
C(26)	1.1751(5)	0.4359(10)	0.7264(3)	[b]
C(27)	1.0578(7)	0.7267(12)	0.7131(4)	[b]
HO(1)	0.459	0.376	-0.111	5.0
HO(2)	0.516	0.040	0.198	5.0
HO(3)	0.593	0.658	0.182	5.0
HO(4)	1.240	0.210	0.742	6.0
H(1)A	0.118	0.539	-0.018	5.0
H(1)B	0.225	0.660	0.043	5.0
H(2)A	0.417	0.514	0.015	5.0
H(2)B	0.320	0.592	-0.064	5.0
H(3)	0.223	0.315	-0.097	4.0
H(4)A	0.292	0.072	-0.018	5.0
H(4)B	0.401	0.191	0.042	5.0
H(5)	0.103	0.216	0.013	4.0
H(6)A	0.200	-0.036	0.085	4.0
H(6)B	0.104	0.087	0.128	4.0
H(7)	0.300	0.013	0.221	4.0
H(8)	0.244	0.304	0.236	4.0
H(9)	0.418	0.400	0.131	4.0
H(11)A	0.353	0.695	0.153	5.0
H(11)B	0.265	0.625	0.214	5.0
H(12)	0.482	0.734	0.279	4.0
H(14)	0.543	0.287	0.252	4.0
H(15)A	0.511	0.054	0.324	5.0
H(15)B	0.396	0.156	0.362	5.0
H(16)A	0.693	0.188	0.404	5.0
H(16)B	0.580	0.249	0.454	5.0
H(17)	0.694	0.447	0.348	5.0
H(18)A	0.374	0.632	0.386	6.0
H(18)B	0.351	0.424	0.396	6.0
H(18)C	0.266	0.525	0.320	6.0
H(19)A	-0.017	0.442	0.067	6.0
H(19)B	0.069	0.573	0.132	6.0
H(19)C	0.053	0.368	0.152	6.0
H(20)	0.597	0.536	0.485	5.0
H(21)A	0.718	0.783	0.395	6.0
H(21)B	0.690	0.825	0.480	6.0
H(21)C	0.558	0.793	0.408	6.0
H(22)A	0.874	0.558	0.459	6.0
H(22)B	0.814	0.377	0.486	6.0
H(23)A	0.856	0.694	0.575	7.0
H(23)B	0.786	0.519	0.602	7.0
H(24)A	1.067	0.554	0.583	7.0
H(24)B	0.999	0.368	0.599	7.0
H(25)	0.963	0.485	0.722	6.0
H(27)A	1.136	0.771	0.688	7.0
H(27)B	1.083	0.749	0.768	7.0
H(27)C	0.970	0.790	0.689	7.0

[a] Number in parentheses denotes standard deviation.
[b] Anisotropic thermal parameters are given in Table 3.

TABLE 3. Final anisotropic thermal parameters[a] for 25S-THCA

Atom	$B11 \times 10^4$[b]	$B22 \times 10^4$[b]	$B33 \times 10^4$[b]	$B12 \times 10^4$[b]	$B13 \times 10^4$[b]	$B23 \times 10^4$[b]
O(1)	179(5)	127(7)	29(1)	-3(5)	17(2)	0(2)
O(2)	113(4)	102(6)	33(1)	17(4)	-10(2)	-2(2)
O(3)	109(4)	125(6)	25(1)	-21(4)	-4(2)	7(2)
O(4)	131(5)	211(10)	51(2)	17(6)	-20(2)	8(3)
O(5)	106(5)	256(10)	59(2)	-3(6)	-25(2)	16(4)
C(1)	112(6)	93(8)	26(2)	11(6)	-11(2)	7(3)
C(2)	129(6)	88(8)	25(2)	-2(6)	-9(3)	3(3)
C(3)	112(6)	112(9)	24(2)	-7(6)	-9(2)	-3(3)
C(4)	120(6)	78(8)	28(2)	-14(6)	-9(3)	-3(3)
C(5)	85(5)	94(9)	28(2)	-12(6)	-13(2)	0(3)
C(6)	102(6)	113(9)	32(2)	-32(6)	-7(3)	1(3)
C(7)	104(6)	89(8)	29(2)	-26(6)	-6(3)	5(3)
C(8)	86(5)	95(8)	26(2)	-4(5)	-4(2)	5(3)
C(9)	90(5)	79(7)	22(1)	-4(5)	-4(2)	2(3)
C(10)	84(5)	93(8)	26(2)	-1(5)	-13(2)	1(3)
C(11)	113(6)	74(8)	25(2)	0(6)	-9(3)	1(3)
C(12)	111(6)	66(7)	24(2)	-6(6)	-6(3)	1(3)
C(13)	89(5)	91(9)	23(1)	10(5)	-6(2)	3(3)
C(14)	88(5)	81(8)	22(2)	-3(5)	-7(2)	1(3)
C(15)	122(6)	107(9)	31(2)	-2(6)	-2(3)	15(3)
C(16)	149(7)	101(9)	28(2)	7(7)	-11(3)	16(3)
C(17)	106(6)	95(8)	21(2)	16(6)	-9(2)	7(3)
C(18)	124(6)	149(10)	27(2)	9(7)	-1(3)	5(4)
C(19)	98(6)	188(12)	34(2)	15(7)	-6(3)	6(4)
C(20)	116(6)	124(10)	23(2)	14(6)	-8(3)	1(3)
C(21)	159(7)	122(10)	29(2)	-1(8)	-12(3)	-7(4)
C(22)	114(6)	176(11)	30(2)	20(7)	-15(3)	-14(4)
C(23)	136(7)	235(14)	29(2)	61(8)	-12(3)	-13(4)
C(24)	115(6)	212(13)	31(2)	39(8)	-15(3)	-14(4)
C(25)	106(6)	225(13)	30(2)	22(8)	-6(3)	-6(4)
C(26)	104(7)	236(14)	25(2)	20(8)	-5(3)	11(4)
C(27)	179(9)	297(18)	50(3)	66(11)	-16(4)	-46(6)

[a] The anisotropic temperature factor has the form $\exp(-(h^2B11 + k^2B22 + l^2B33 + 2hkB12 + 2hlB13 + 2klB23))$.

[b] Number in parentheses denotes standard deviation.

from 3α,7α,12α-trihydroxy-5β-cholanoic acid (13) that involved a nonstereospecific introduction of asymmetry at C-25. The physical properties of these two diastereoisomers are illustrated in **Table 1.** To determine the configuration at C-25 in the two diastereoisomers of THCA, a single crystal of the less polar diastereoisomer (R_f, 0.44; mp 199–201°C) was examined by X-ray crystallography. The structure of this isomer was solved by a multiple solution procedure (14) and was refined by full-matrix least squares. The positions of the hydrogen atoms were calculated, based on the molecular geometry, after anisotropic refinement of the nonhydrogen atoms. In the final refinement, anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were

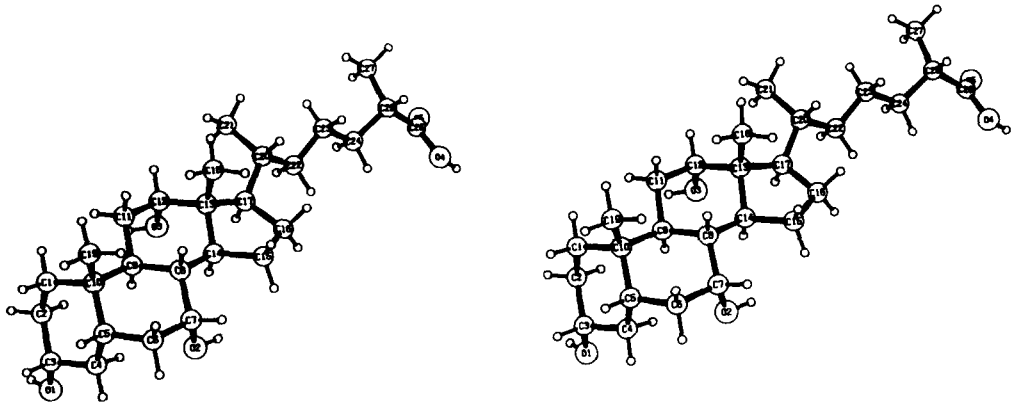

Fig. 3. A stereoscopic view of the 25S diastereoisomer of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid, as found in the crystal structure.

TABLE 4. Bond lengths (Å) in 25*S*-THCA[a]

O(1)- C(3)	1.422	C(10)-C(19)	1.517
O(2)- C(7)	1.420	C(11)-C(12)	1.533
O(3)-C(12)	1.424	C(12)-C(13)	1.541
O(4)-C(26)	1.335	C(13)-C(14)	1.567
O(5)-C(26)	1.189	C(13)-C(17)	1.575
C(1)- C(2)	1.504	C(13)-C(18)	1.507
C(1)-C(10)	1.549	C(14)-C(15)	1.513
C(2)- C(3)	1.537	C(15)-C(16)	1.535
C(3)- C(4)	1.507	C(16)-C(17)	1.563
C(4)- C(5)	1.511	C(17)-C(20)	1.536
C(5)- C(6)	1.535	C(20)-C(21)	1.535
C(5)-C(10)	1.564	C(20)-C(22)	1.541
C(6)- C(7)	1.527	C(22)-C(23)	1.511
C(7)- C(8)	1.544	C(23)-C(24)	1.514
C(8)- C(9)	1.538	C(24)-C(25)	1.539
C(8)-C(14)	1.537	C(25)-C(26)	1.517
C(9)-C(10)	1.571	C(25)-C(27)	1.532
C(9)-C(11)	1.551		

[a] Estimated standard deviation for a typical C–C bond length is 0.009 Å.

not refined. The final discrepancy indices were $R = 0.058$ and $\omega R = 0.069$ for the 1876 observed reflections. The final atomic coordinates, thermal parameters, bond lengths, bond angles, and torsion angles are given in **Tables 2–6.**[1] The crystal structure analysis proved the compound to be the 25*S* diastereoisomer of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid, a stereoscopic view of which, showing its conformation in the crystalline state, is shown in **Fig. 3.**[2] Consequently the more polar diastereoisomer (R_f 0.41; mp 180–182°C) must be the 25R isomer.

Mendelsohn and Mendelsohn (5) reported the isolation of two isomers of THCA from gall bladder bile of *Crocodylus niloticus*. They assigned the 25-L configuration to the compound with the higher melting point (195°C) and the 25-D configuration to the compound with the lower melting point (172°C) (see note to Table 1). Briggs (15) synthesized both isomers of THCA by electrolytic coupling of cholic acid and the optically active forms of the half ester of methyl succinic acid and obtained compounds that melted at 199–201°C (25-L) and 183–184°C (25-D) (see note to Table 1). It is possible that the above mentioned 25-L compound corresponds to the 25*S* isomer in our study, while Briggs' 25-D compound could correspond to the 25*R* isomer.

It is noteworthy that in the course of our studies concerning the biosynthesis of chenodeoxycholic acid in man and other mammals, 5β-cholestane-3α,7α-diol was converted predominantly to one diastereoisomer of 3α,7α,26-triol by hepatic mitochondria, while the microsomal fraction yielded both diastereoisomers (16, 17). These two diastereoisomers were tentatively characterized by their optical rotation differences (18). It may now be possible to establish the absolute configuration of these two isomeric 5β-cholestane-3α,7α,26-triols by their synthesis from THCA of known absolute configuration.

Finally, the establishment of the configuration at C-25 in the two diastereoisomers of THCA and their resolution by TLC permit us to study their stereo-

TABLE 5. Bond angles (°) in 25*S*-THCA[a]

C(2)- C(1)-C(10)	116.1
C(1)- C(2)- C(3)	110.4
O(1)- C(3)- C(2)	111.9
O(1)- C(3)- C(4)	109.0
C(2)- C(3)- C(4)	109.4
C(3)- C(4)- C(5)	114.7
C(4)- C(5)- C(6)	111.5
C(4)- C(5)-C(10)	113.5
C(6)- C(5)-C(10)	111.5
C(5)- C(6)- C(7)	114.6
O(2)- C(7)- C(6)	109.1
O(2)- C(7)- C(8)	111.9
C(6)- C(7)- C(8)	110.5
C(7)- C(8)- C(9)	112.9
C(7)- C(8)-C(14)	110.7
C(9)- C(8)-C(14)	108.8
C(8)- C(9)-C(10)	111.8
C(8)- C(9)-C(11)	109.7
C(10)- C(9)-C(11)	112.9
C(1)-C(10)- C(5)	106.8
C(1)-C(10)- C(9)	111.9
C(1)-C(10)-C(19)	107.1
C(5)-C(10)- C(9)	108.4
C(5)-C(10)-C(19)	110.1
C(9)-C(10)-C(19)	112.4
C(9)-C(11)-C(12)	112.8
O(3)-C(12)-C(11)	110.6
O(3)-C(12)-C(13)	110.7
C(11)-C(12)-C(13)	111.0
C(12)-C(13)-C(14)	106.0
C(12)-C(13)-C(17)	116.9
C(12)-C(13)-C(18)	110.9
C(14)-C(13)-C(17)	98.1
C(14)-C(13)-C(18)	112.9
C(17)-C(13)-C(18)	111.3
C(8)-C(14)-C(13)	114.1
C(8)-C(14)-C(15)	118.2
C(13)-C(14)-C(15)	104.0
C(14)-C(15)-C(16)	103.1
C(15)-C(16)-C(17)	107.3
C(13)-C(17)-C(16)	103.6
C(13)-C(17)-C(20)	120.2
C(16)-C(17)-C(20)	110.6
C(17)-C(20)-C(21)	114.3
C(17)-C(20)-C(22)	107.9
C(21)-C(20)-C(22)	110.2
C(20)-C(22)-C(23)	115.7
C(22)-C(23)-C(24)	111.9
C(23)-C(24)-C(25)	114.3
C(24)-C(25)-C(26)	108.3
C(24)-C(25)-C(27)	112.1
C(26)-C(25)-C(27)	110.3
O(4)-C(26)- O(5)	123.2
O(4)-C(26)-C(25)	111.9
O(5)-C(26)-C(25)	124.9

[a] Estimated standard deviation for a typical C–C–C bond angle is 0.7°.

[1] A list of final *Fo* and *Fc* values is available at the Department of Medicine, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ 07103.

[2] The configuration at C-25 of the diastereoisomer illustrated in Fig. 3 is specified *S*, since, if we visualize this structure in such a way that the hydrogen attached to the asymmetric carbon at C-25 is directed away from us, in the view down the C(25)–H bond there is a counterclockwise arrangement.

TABLE 6. Torsion angles (°) in 25*S*-THCA[a]

C(10)- C(1)- C(2)- C(3)	57.4
C(1)- C(2)- C(3)- C(4)	-54.5
C(2)- C(3)- C(4)- C(5)	54.7
C(3)- C(4)- C(5)-C(10)	-53.9
C(4)- C(5)-C(10)- C(1)	49.0
C(5)-C(10)- C(1)- C(2)	-52.8
C(10)- C(5)- C(6)- C(7)	-54.8
C(5)- C(6)- C(7)- C(8)	51.3
C(6)- C(7)- C(8)- C(9)	-51.5
C(7)- C(8)- C(9)-C(10)	56.0
C(8)- C(9)-C(10)- C(5)	-56.5
C(9)-C(10)- C(5)- C(6)	55.2
C(14)- C(8)- C(9)-C(10)	179.3
C(11)-C(12)-C(13)-C(14)	57.0
C(12)-C(13)-C(14)- C(8)	-59.7
C(13)-C(14)- C(8)- C(9)	59.6
C(17)-C(13)-C(14)-C(15)	49.1
C(13)-C(14)-C(15)-C(16)	-39.9
C(14)-C(15)-C(16)-C(17)	14.3
C(15)-C(16)-C(17)-C(13)	16.1
C(16)-C(17)-C(13)-C(14)	-38.5
C(17)-C(20)-C(22)-C(23)	-161.3
C(20)-C(22)-C(23)-C(24)	176.0
C(22)-C(23)-C(24)-C(25)	-173.0
C(23)-C(24)-C(25)-C(26)	172.3
C(24)-C(25)-C(26)- O(5)	109.6

[a] Estimated standard deviation for a typical C–C–C–C torsion angle is 0.8°.

specific formation from cholesterol and their transformation into cholic acid.

This work was supported by United States Public Health Service grants AM-26756, AM-18707, and HL-17818.

Manuscript received 11 December 1978; accepted 2 April 1979.

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