Identification of 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol) in patients with Smith-Lemli-Opitz syndrome

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Abstract Cholesta-5,8-dien-3β-ol (8-dehydrocholesterol) and cholesta-5,7-dien-3β-ol (7-dehydrocholesterol) were isolated from the fecal neutral sterol fraction from homozygotes with Smith-Lemli-Opitz syndrome. The structures of the sterols were conclusively established from their mass spectra and ^{1}H and ^{13}C nuclear magnetic resonance spectra. It is probable that 8-dehydrocholesterol arises from 7-dehydrocholesterol and is not a direct precursor of cholesterol.—**Batta, A. K., G. S. Tint, S. Shefer, D. Abuelo, and G. Salen.** Identification of 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol) in patients with Smith-Lemli-Opitz syndrome. *J. Lipid Res.* 1995. **36:** 705–713.

Supplementary key words 7-dehydrocholesterol • cholesterol • ^{1}H and ^{13}C nuclear magnetic resonance • mass spectrometry • biosynthesis

Smith-Lemli-Opitz syndrome is a devastating, often fatal autosomal recessive disorder characterized by a large number of birth defects affecting nearly every organ system (1–6). The most discernible abnormalities are a set of distinctive dysmorphic facial features: microcephaly, micrognathia, cataracts, ptosis, wide nasal bridge with anteverted nares, and low set posteriorly rotated ears. Limb abnormalities are also common with syndactyly of the second and third toe noted in at least 75% of cases and polydactyly seen in 20–25% of patients. The most severely affected patients exhibit genital disorders, especially cryptorchidism, hypospadias, and microphalus and often die prematurely. Also reported are widespread defects in many endocrine glands (complete absence of lipid in adrenal cortex), liver, kidneys and urinary system, heart, lungs, and skeleton. These children are mentally retarded and they often show severe failure to thrive and frequently require a gastrostomy feeding tube. The condition is estimated to be the third most common autosomal recessive disorder among North American Caucasians after cystic fibrosis and phenylketonuria; and its prevalence is approximately 1 out of 20,000 births (6).

We have recently described a severe abnormality in cholesterol biosynthesis in five homozygotes with this syndrome in which we found extremely low plasma cholesterol levels associated with the accumulation of the cholesterol precursor, cholesta-5,7-dien-3β-ol (7-dehydrocholesterol) as well as an unidentified isomeric dehydrosterol (7–10). In this report, we describe the unequivocal characterization of this sterol as cholesta-5,8-dien-3β-ol (8-dehydrocholesterol) (**Fig. 1**) based on its ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral studies.

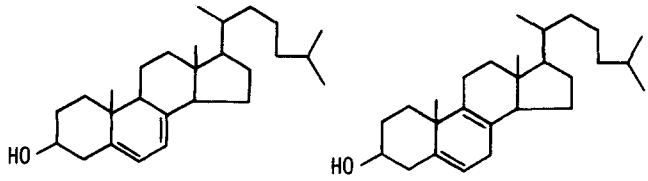
EXPERIMENTAL

Reference standards of cholesterol and 7-dehydrocholesterol were purchased from Steraloids, Inc. (Wilton, NH). Cholesta-8,14-dien-3β-ol was prepared by isomerization of 7-dehydrocholesterol with hot dilute hydrochloric acid (11) and it was purified by crystallization. The compound showed ultraviolet absorption maxima and mass spectral fragmentation pattern identical to those reported in literature for cholesta-8,14-dien-3β-ol (11). All reagents and solvents were reagent grade and were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Methods

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, IA) model 12,600 and are uncorrected.

Abbreviations: NMR, nuclear magnetic resonance; IR, infrared spectroscopy; UV, ultraviolet spectroscopy; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; GLC, gas–liquid chromatography; TMS, trimethylsilyl; rrt, relative retention time; DEPT, distortionless enhancement by polarization transfer. The following trivial nemes were used: cholesterol, cholest-5-en-3β-ol; 7-dehydrocholesterol, cholesta-5,7-dien-3β-ol; 8-dehydrocholesterol, cholesta-5,8-dien-3β-ol; lathosterol, 5α-cholest-7-en-3β-ol.



7-Dehydrocholesterol Cholesta-5,8-dien-3β-ol

Fig. 1. Structures of 7-dehydrocholesterol and cholesta-5,8-dien-3β-ol (8-dehydrocholesterol).

Optical rotations were obtained on a Perkin-Elmer (Norwalk, CT) model 141 polarimeter with ethanol as the solvent.

Infrared (IR) spectra were obtained on a Perkin-Elmer model 421 spectrophotometer as KBr discs.

Ultraviolet spectroscopy (UV)

The UV spectra of the various compounds were obtained in chloroform solution with a Hewlett-Packard model HP 8450 spectrophotometer. The plasma or fecal sterol extract from the patients was subjected to preparative TLC on plates impregnated with 10% silver nitrate and the pertinent bands were eluted with chloroform–methanol 95:5 and first subjected to gas–liquid chromatography (GLC) as the trimethylsilyl ethers. Bands with the desired GLC retention times were then repurified by argentation TLC and were finally dissolved in known volumes of chloroform. Aliquots were used for UV spectroscopy.

Thin-layer chromatography (TLC)

TLC of the various sterols was carried out on silica gel O plates (Analabs, New Haven, CT) in a solvent system of chloroform–acetone 96:4 (v/v) (solvent system A) and the spots were visualized by spraying the plate with phosphomolybdic acid (3.5%, in isopropanol) and sulfuric acid (20%) and heating at 110°C for 2 min. Argentation thin-layer chromatography was carried out in the dark at 4°C on thin-layer plates impregnated with 10% silver nitrate (the plates were immersed in a 10% solution of silver nitrate in methanol–water 1:1, air-dried for 15 min in the dark, and then heated at 110°C for 1 h) and developed in a solvent system of chloroform–acetone 85:15 (v/v) (solvent system B). The spots were visualized by spraying with 20% sulfuric acid and heating at 110°C for 5–10 min. When preparative TLC (regular or argentation TLC) was carried out, the plates were developed in the appropriate solvent system, compounds were visualized by spraying the plates with distilled water, the opaque bands were marked, and plates were dried at room temperature in the dark. Compounds were isolated by scraping the bands and soaking the silica gel overnight in ethyl acetate.

Nuclear magnetic resonance spectroscopy (NMR)

The high-resolution proton NMR spectra of the sterols were obtained at 400 MHz in $CDCl_3$ on a Varian Associates XL-400 spectrometer equipped with Fourier transform mode, and tetramethylsilane was used as the internal standard. The ^{13}C-NMR spectra were performed at 50.4 MHz and the solvent signal of $CDCl_3$ was used as the internal standard. The chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane and are accurate to $\pm$ 0.05 ppm (δ $CDCl_3$ = 76.9 ppm). The spectra were recorded in a proton noise-decoupled mode in order to measure the exact chemical shifts of all ^{13}C nuclei present. In order to obtain carbon multiplicities, 90° and 135° DEPT (distortionless enhancement by polarization transfer) spectra were recorded. In this mode, only the primary, secondary, and tertiary carbons appeared, the primary and tertiary carbons above the baseline and the secondary carbons below the baseline. The positions of the quaternary carbons were determined by subtraction from the noise-decoupled spectra.

High-performance liquid chromatography (HPLC)

HPLC of the sterols was performed on a Waters Associates (Millford, MA) Model M-6000 reciprocating pump and a Model UK6 loop injector. A Waters Associates Model 401 differential refractometer was used and the detector response was recorded with a Spectra-Physics (San Jose, CA) Model SP 4290 integrator. A Waters Associates Radial-Pak μ Bondapak C_{18} reversed-phase column (100 × 8 mm I.D., 5 μm particle size) was used for the chromatography. A guard column (Waters Associates) with C_{18} reversed-phase material was placed before the separation column. Ten μg of the sterol dissolved in 5 μl chloroform was injected into the HPLC column. A solvent system consisting of ethanol–methanol–water 160:40:10 (v/v/v) was used (12) and the flow rate was maintained at 2 ml/min (operating pressure, ca 10.3 × 10^3 KPa).

Capillary gas–liquid chromatography

A Hewlett-Packard (Palo Alto, CA) model 5890A gas chromatograph equipped with a flame ionization detector and an injector with a split/splitless device for capillary columns was used for all separations. The chromatographic column consisted of a chemically bonded fused silica CP-Sil 5CB column (25 m × 0.32 mm I.D.; stationary phase, 100% dimethylsiloxane) or CP-Wax 57CB (Carbowax PEG) capillary column (25 m × 0.22 mm I.D.) (Chrompack, Inc., Raritan, NJ) and helium was used as the carrier gas. The GLC operating conditions were as follows: injector and detector temperatures were 260°C and 290°C, respectively. After injection, oven temperature was kept at 100°C for 2 min, then programmed

at a rate of 35°C/min to a final temperature of 265°C when using a CP-Sil 5CB column (13), while the chromatography was performed isothermally at 225°C in the case of the CP-Wax 57CB column (8). Flow rate of the carrier gas was maintained at 1 ml/min for both columns. The sterols (5–10 μg) were reacted with 100 μl of Sil-Prep (hexamethyldisilazane–trimethylchlorosilane–pyridine 3:1:9; Alltech Associates, Inc., Deerfield, IL) for 20 min at 55°C. Solvents were evaporated at 55°C under N_2 and the trimethylsilyl (TMS) ether derivative formed was taken up in 100 μl of hexane. One microliter was injected into the GLC column simultaneously with 5α-cholestane used as the internal standard.

Gas chromatography–mass spectrometry (GC–MS)

Mass spectra of the various sterols were carried out on a Hewlett-Packard Model 5988 gas chromatograph–mass spectrometer using a 25 m CP-Sil 5CB capillary column.

Clinical

All four patients had many of the features and limb abnormalities typical of the Smith-Lemli-Opitz syndrome and all were mentally retarded. Their case histories have been reported previously (8).

Sample collection

The plasma and stool samples from the patients were obtained on dry ice and stored in the dark at −20°C until used.

Sterol analysis

Plasma sterols were isolated from 0.1–0.5 ml plasma after saponification with 1 N sodium hydroxide (3 ml) for 1 h and extraction of the free sterols with n-hexane (3 ml × 3). After evaporation of solvent under nitrogen, the residue containing the sterols was directly derivatized and used for quantification by GLC. The lyophilized feces (1 g) were exhaustively extracted with ethanol containing 0.5% ammonium hydroxide in a Soxhlet extractor and solvent was removed under reduced pressure. The residue was taken up in 10 ml 0.5 N NaOH and extracted with n-hexane (10 ml × 4). The hexane layer was washed once with water (10 ml) and evaporated to dryness under nitrogen. The residue containing the fecal neutral sterols was dissolved in chloroform (10 ml) and aliquots were used for TLC and GLC.

Isolation of 8-dehydrocholesterol and 7-dehydrocholesterol

The total neutral sterol fraction obtained from 5 g lyophilized feces from a 1-year-old patient with Smith-Lemli-Opitz syndrome was subjected to preparative TLC. The crude sterol extract was dissolved in 0.8 ml chloroform and applied as a thin layer on four silica gel TLC plates impregnated with 10% silver nitrate and the plates were developed in chloroform–acetone 85:15. A reference standard of 7-dehydrocholesterol was applied as a marker near a corner of the plates. Bands due to various sterols were visualized by spraying the plates with distilled water and the opaque bands were marked, plates were dried at room temperature in the dark, and silica gel corresponding to the appropriate bands was scraped into glass vials. Compounds were isolated by soaking the silica gel in ethyl acetate overnight and then filtering. Two compounds were obtained in this way; TLC R_f value, 0.45 and 0.60, respectively (silica gel plate, impregnated with 10% silver nitrate; solvent system B). The compound with TLC R_f 0.45 was crystallized by trituration of the residue obtained by evaporation of solvents with a drop of anhydrous methanol. The colorless microscopic crystals formed were filtered (5.5 mg), melting point, 148.5–150°C; $[\alpha]_D^{25°}$, −117° (c = 0.88%; ethanol). The melting point of the compound was undiminished when mixed with standard pure 7-dehydrocholesterol [melting point of 7-dehydrocholesterol, 150°C (14)]. It showed a single spot on TLC (silica gel O plate; solvent system A), R_f, 0.60 and the R_f value on the plain silica gel plate as well as on silver nitrate-impregnated TLC plate was identical with that of standard 7-dehydrocholesterol. The two compounds were inseparable when applied together on the TLC plate. On GLC, it showed a single peak on two capillary columns [CP-Sil 5CB column, retention time, 17.25 min (retention time of 5α-cholestane, 12.56 min); CP-Wax 57CB column, retention time, 14.57 min (retention time of 5α-cholestane, 6.59 min)]. When co-chromatographed with a reference standard of 7-dehydrocholesterol, the peak height due to the fecal 7-dehydrocholesterol was enhanced on both columns, while the peak width was unchanged. On reversed-phase HPLC, this compound eluted at 30.3 ml (elution volume for standard 7-dehydrocholesterol, 30.3 ml). The compound showed UV absorbance λ_{max} at 274, 283, and 294 nm, and its infrared spectrum was superimposable over that for standard 7-dehydrocholesterol (**Fig. 2**). The ^{1}H- and ^{13}C-NMR signals for the sterol are reported in **Table 1** and **Table 2,** respectively. The TMS ether of the compound showed the following major fragments in the mass spectrum: *m/z* 456 (18%; M^+), 366 (20%; M^+–90), 351 (100%; M^+–90–15), 325 (92%; M^+–131), 253 (15%; M^+–side chain), 211 (25%; M^+–side chain–42).

The second compound with R_f value of 0.60 on the silver nitrate-impregnated TLC plate (8-dehydrocholesterol) was crystallized from methanol as colorless plates (1.8 mg), melting point, 104–106°C. It showed a single spot on TLC (silica gel O plate; solvent system A), R_f, 0.60, GLC retention time, 16.46 min on CP-Sil 5CB column (retention time on CP-Wax 57CB column, 12.31 min) and HPLC retention volume, 28.6 ml. The compound did not show UV absorption maxima above 220 nm. The ^{1}H-

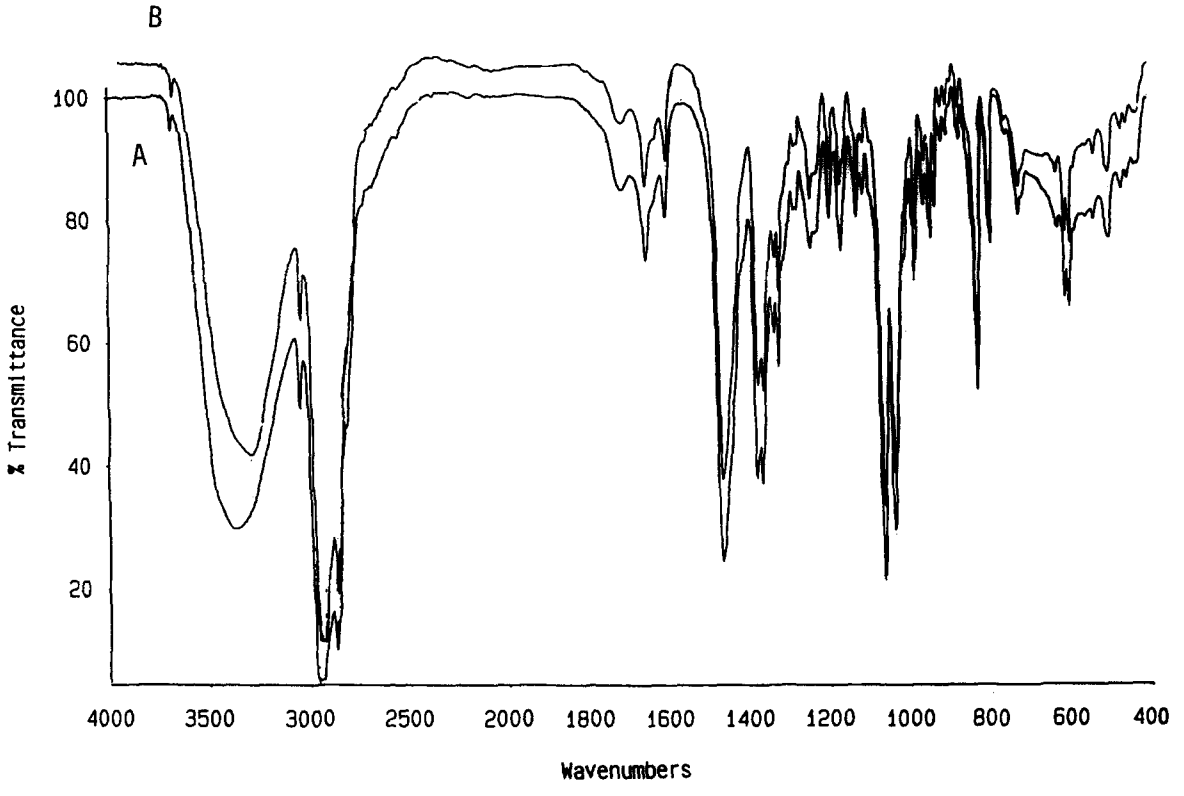


Fig. 2. Infrared spectra of 7-dehydrocholesterol isolated from a homozygote with Smith-Lemli-Opitz syndrome (A) and standard 7-dehydrocholesterol (B).

methyl signals and the ^{13}C-NMR signals of the compound are given in Tables 1 and 2, respectively, and the complete ^{1}H-NMR spectrum is shown in **Fig. 3.** The TMS ether showed the following major fragments in the mass spectrum: *m/z* 456 (16%; M^+), 366 (18%; M^+-90), 351 (90%; M^+-90-15), 325 (100%; M^+-131), 253 (15%; M^+-side chain), 211 (25%; M^+-side chain-42).

RESULTS

The mass spectra of the TMS ethers of 7-dehydrocholesterol and the isomeric sterol were almost identical and, therefore, mass spectral fragmentation pattern could not be used to unequivocally characterize these isomeric cholestadienols (8). To better characterize these compounds, pure samples were obtained from fecal extracts from a homozygote with Smith-Lemli-Opitz syndrome by argentation TLC and their purity was ascertained by TLC, HPLC, and GLC. The biological 7-dehydrocholesterol was identified by melting point, thin-layer chromatography, GLC, HPLC, UV absorption maxima characteristic of the 5,7-diene system, mass spectral fragmentation pattern, and the ^{1}H- and ^{13}C-NMR spectral data which were all completely compatible with those ob-

TABLE 1. Methyl signals in the high resolution ^{1}H-NMR spectra of cholesta-5,8-dien-3β-ol, cholesta-5,7-dien-3β-ol, and cholesta-8,14-dien-3β-ol[a]

	Signal (δ, ppm)[b]			
Compound	C-18	C-19	C-21	C-26,27
Cholesta-5,8-dien-3β-ol	0.652 (s, 3H)	1.115 (s, 3H)	0.942, 0.926 (d, J = 6.4 Hz, 3H)	0.877, 0.860 (d, J = 6.8 Hz, 6H)
Cholesta-5,7-dien-3β-ol	0.619 (s, 3H)	0.945 (s, 3H)	0.945, 0.930 (d, J = 6.0 Hz, 3H)	0.876, 0.860 (d, J = 6.4 Hz, 6H)
Cholesta-8,14-dien-3β-ol	0.816 (s, 3H)	0.990 (s, 3H)	0.945, 0.929 (d, J = 6.4 Hz, 3H)	0.877, 0.860 (d, J = 6.8 Hz, 6H)

[a] Four hundred MHz spectra.
[b] Chemical shifts (δ) in ppm relative to tetramethylsilane in $CDCl_3$ solution.

TABLE 2. ^{13}C signals in the 50.4 MHz ^{13}C-NMR spectra of cholesta-5,8-dien-3β-ol, cholesta-5,7-dien-3β-ol, and cholesta-8,14-dien-3β-ol[a,b]

Carbon	Sterol		
	Cholesta-5,8-dien-3β-ol	Cholesta-5,7-dien-3β-ol δ (ppm)	Cholesta-8,14-dien-3β-ol
1	35.60	38.37	35.31
2	31.96	31.98	31.67
3	71.37	70.41	70.95
4	42.21	40.78	38.27
5	138.92	141.47	40.91
6	119.43	119.47	25.28
7	29.00*	116.14	26.58
8	126.49	139.77	123.15
9	132.12	46.21	140.89
10	37.41	37.06	36.60
11	22.28	21.12	21.84
12	36.81	39.18	36.92
13	42.02	42.98	45.10
14	51.79	54.45	151.14
15	22.98	23.01	117.30
16	28.81*	28.08	35.89
17	54.73	55.86	57.20
18	11.32	11.82	15.68
19	18.72	16.27	18.37
20	36.22	36.12	34.04
21	18.72	18.85	18.87
22	36.11	36.09	36.07
23	23.90	23.87	23.74
24	39.47	39.47	39.48
25	28.00	28.01	27.99
26	22.53	22.55	22.54
27	22.83	22.81	22.80

[a] Spectra: 50.4 MHz. Chemical shifts (δ) in ppm referenced to $CDCl_3$ at 76.9 ppm in $CDCl_3$ solution.
[b] Assignments marked with an asterisk may be interchanged.

tained for standard 7-dehydrocholesterol. Further, the infrared spectrum of the biological 7-dehydrocholesterol was completely superimposable over that obtained for authentic 7-dehydrocholesterol (Fig. 2). Therefore, the structure of this sterol in patients with Smith-Lemli-Opitz syndrome is unequivocally confirmed as 7-dehydrocholesterol.

Both the ^1H-NMR spectrum of the second sterol and the mass spectrum of its TMS ether suggested that it was

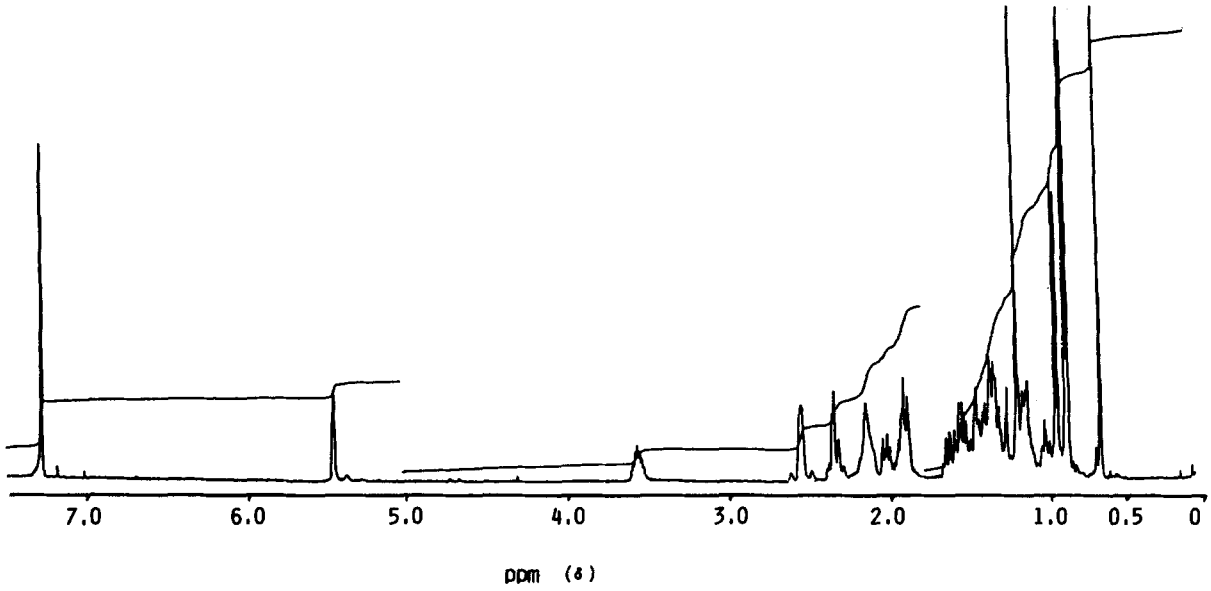


Fig. 3. High resolution proton NMR spectrum of 8-dehydrocholesterol isolated from a patient with Smith-Lemli-Opitz syndrome.

a cholestane derivative with two double bonds in the ring system. A sterol with double bonds at either 7,9(11) or 7,14 or 8,14 was ruled out by the intense ion-fragment at *m/z* 325 which strongly suggested that both double bonds were in ring B. The absence of conjugated double bonds was further indicated from lack of UV absorption above 220 nm which also ruled out a $\Delta^{6,8}$-diene system. The structure of dehydrosterol A was finally confirmed as 8-dehydrocholesterol from a study of its ^{1}H-NMR spectrum (Fig. 3) and comparison with the ^{1}H-NMR spectra for 7-dehydrocholesterol and cholesta-8,14-dien-3β-ol (Table 1). Thus, the sterol showed only one unsaturated proton (at C-6) at δ 5.437 ppm which suggested that the other double bond must be tetrasubstituted. The C-18 methyl signal, usually the most shielded in sterols, appeared at δ 0.619 ppm in the spectrum of 7-dehydrocholesterol and at δ 0.652 ppm in 8-dehydrocholesterol. This ruled out a $\Delta^{8(14)}$ double bond or a double bond in ring D since such a double bond has strong deshielding effect on the C-18 methyl signal, e.g., δ 0.816 ppm in cholesta-8,14-dien-3β-ol (Table 1 and ref. 15). A highly deshielded C-19 methyl signal in 8-dehydrocholesterol appearing at δ 1.155 strongly suggests the tetrasubstituted double bond to be at C-8(9) (15) which leaves only C-5(6) as the site for the isolated trisubstituted double bond. As expected, the side chain methyl groups at C-21 and C-26,27 appeared at approximately the same positions in the spectra of all three compounds. Thus, the ^{1}H-NMR spectrum of this sterol is in complete agreement with the structure cholesta-5,8-dien-3β-ol (16).

The position of the double bonds in the sterol was further confirmed by the 50.4 MHz ^{13}C-NMR spectrum and comparison with the values obtained for 7-dehydrocholesterol and cholesta-8,14-dien-3β-ol (Table 2) and literature values (16–18). Thus, the noise-decoupled ^{13}C-NMR spectrum of 8-dehydrocholesterol showed three low field = C < carbons and only one = CH–carbon, thereby supporting a tri- and a tetrasubstituted double bond system in the compound. The signal at δ 119.59 ppm was due to C-6 (= CH–) carbon and it appeared at almost the same position as that observed in the spectrum of 7-dehydrocholesterol (δ 119.60 ppm). The = C < carbons appearing at δ 138.93, 126.50, and 132.12 ppm, respectively, were for C-5, 8, and 9, respectively, from comparison with the signals due to C-5 at δ 139.78 in the spectrum of 7-dehydrocholesterol and δ 123.15 ppm for C-8 in cholesta-8,14-dien-3β-ol. The C-9 signal in cholesta-8,14 appeared downfield (δ 140.89 ppm) due to the proximity of double bond at C-14(15) position. The effect of the double bonds was clear on the methyl signals also. Thus, the C-21,26,27 signals appeared at δ 18.72, 22.52, and 22.84 ppm, respectively, in the spectrum of 8-dehydrocholesterol and their positions were virtually unchanged in the spectra of 7-dehydrocholesterol and cholesta-8,14-dien-3β-ol. The C-18 signal appeared at δ 11.32 and 11.81 in 8-dehydrocholesterol and 7-dehydrocholesterol, respectively, and downfield at δ 15.69 in cholesta-8,14-dien-3β-ol. The C-19 signal at δ 23.02 ppm was downfield in 8-dehydrocholesterol due to the more profound τ_a interaction due to the $\Delta^{8(9)}$-bond as compared with $\Delta^{7(8)}$-bond (18). The remaining signals were all comparable to those obtained for 5α-cholest-8-en-3β-ol (16).

Both 7-dehydrocholesterol and 8-dehydrocholesterol were present in plasma and stool from all patients studied and the substantially larger amounts of 8-dehydrocholesterol in the plasma suggest a hepatic origin for the compound (**Table 3**). 8-Dehydrocholesterol was previously isolated by Fumagalli et al. (19) from the livers of litters of pregnant rats given AY 9944 [*trans*-1,4-bis(2-dichlorobenzylaminoethyl)cyclohexane dichloride], a drug that interferes with the last steps of cholesterol biosynthesis, where they found as much as 13% (0.21 mg/g tissue) of the total sterols as 8-dehydrocholesterol and up to 58% (0.93 mg/g tissue) as 7-dehydrocholesterol. Both the ^{1}H-NMR and ^{13}C-NMR spectra of our compound are in agreement with those reported by them for the acetate of 8-dehydrocholesterol. A structure of 8-dehydrocholesterol was tentatively proposed by Axelson (12) to a sterol, detected in less than 50 ng/ml in the plasma of healthy humans, from similarity of the mass spectrum of its TMS ether derivative and 7-dehydrocholesterol coupled with lack of UV absorption above 220 nm. In the 6-month-old patient (patient 1), bacterial metabolites of the sterols were not observed, whereas the older patients had substantial amounts of coprostanol and coprostanone and the metabolites of plant sterols (Table 3). In patient 2 we could also confirm the presence of small amounts of Δ^7-coprostanol based on the mass spectral fragmentation pattern of its TMS ether. This compound is likely to be a bacterial metabolite of 7-dehydrocholesterol (20). Although the proportions of 7- and 8-dehydrocholesterols varied in patients, the ratio of the dehydrocholesterols versus cholesterol plus its bacterial metabolites in both plasma and stool was higher in the more seriously affected patients than in the less severely affected patients (Table 3). 7-Dehydrocholesterol, 8-dehydrocholesterol, or Δ^7-coprostanol could not be detected in lyophilized stool from four control subjects when analyzed in an identical manner.

DISCUSSION

In addition to identical mass spectral fragmentation and GLC characteristics, identification of 7-dehydrocholesterol in patients with Smith-Lemli-Opitz syndrome has been based on melting point, $[\alpha]_D$, characteristic UV absorption, superimposable IR spectrum, co-thin-layer chromatography, and co-gas–liquid chromatography of a

TABLE 3. Fecal neutral sterols in patients with Smith-Lemli-Opitz syndrome

Sterol	Patient[a] 1	2	3	4
	mg/g dry feces			
Cholesterol	1.7	1.3	6.3	2.3
7-DHC[b]	7.4	1.4	0.8	1.1
8-DHC[c]	2.4	0.8	0.03	0.3
Coprostanol + coprostanone	n.d.[d]	0.5	0.8	10.1
Δ^7-Coprostanol	n.d.	0.05	n.d.	n.d.
Plant sterols[e]	7.1	5.7	0.8	4.4
Total	18.6	9.75	8.73	18.2
7-DHC/8-DHC	3.1	1.8	26.7	3.7
7-DHC + 8-DHC/cholesterol and metabolites[f]	5.8	1.2	0.12	0.11
7-DHC/8-DHC in plasma[g]	1.3	1.4	1.5	2.1
7-DHC + 8-DHC/cholesterol in plasma[g,h]	6.9	1.6	0.38	0.34

[a]Patients 1–4 correspond to patients 1–4, respectively, as described in ref. 8.
[b]7-DHC, 7-dehydrocholesterol.
[c]8-DHC, 8-dehydrocholesterol.
[d]n.d., not detected.
[e]Plant sterols include sitosterol and campesterol and their 5β-saturated derivatives.
[f]Ratio of the sum of 7-DHC and 8-DHC and the sum of cholesterol and its bacterial metabolites (coprostanol, coprostanone).
[g]Values calculated from Table 1, ref. 8.
[h]Bacterial metabolites of cholesterol were not found in plasma.

sample isolated from the feces of a patient compared with those for an authentic reference standard of 7-dehydrocholesterol. The isomeric sterol was also obtained in pure form and its structure has now been established as 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol) from analysis of its ^{13}C- and ^{1}H-NMR spectra.

Finding large quantities of 7-dehydrocholesterol and 8-dehydrocholesterol in plasma and stool not only signifies abnormal cholesterol biosynthesis but can be used to establish the biochemical diagnosis of the condition. The severity of the biochemical defect can be assessed from the proportions of these sterols in the plasma and feces where a higher ratio of 7-dehydrocholesterol plus 8-dehydrocholesterol to cholesterol is obvious in more severely affected patients (8). Substantially larger proportions of 8-dehydrocholesterol relative to 7-dehydrocholesterol are found in the plasma of all patients than in the stool. This may result from reduced biliary secretion of 8-dehydrocholesterol as compared to cholesterol and 7-dehydrocholesterol (21) coupled with varied degrees of metabolism of 8-dehydrocholesterol by intestinal bacteria. A direct isomerization of the Δ^8-double bond to Δ^7-double bond probably does not take place in the intestine, as large amounts of 8-dehydrocholesterol are excreted in the stool of the 6-month-old patient 1, in whom other bacterial metabolites are minimal (Table 3).

We have recently demonstrated that the enzyme catalyzing the reduction of 7-dehydrocholesterol to cholesterol, 7-dehydrocholesterol Δ^7-reductase, is abnormal in liver specimens from patients with Smith-Lemli-Opitz syndrome (22) and we believe this is the inherited defect underlying this recessive condition. Our contention is supported by the observation that administration of the hypocholesteremic drugs (AY 9944 and BM 15.766) that block the activity of 3β-hydroxysterol Δ^7-reductase reproduces the biochemical defect of low cholesterol coupled with elevated 7-dehydrocholesterol concentrations in plasma and cell tissues and leads to severe tissue and organ abnormalities (23–32). It is generally accepted that in the biosynthesis of cholesterol, the putative precursor cholest-8-en-3β-ol is first isomerized to lathosterol (cholest-7-en-3β-ol), then 5(6)-dehydrogenated to 7-dehydrocholesterol, followed by saturation of the Δ^7-bond to form cholesterol (33). However, using partially purified $\Delta^8 \rightarrow \Delta^7$-isomerase, Paik et al. (33) demonstrated the reversible nature of this enzyme. The accumulation of 8-dehydrocholesterol under conditions in which 7-dehydrocholesterol is not effectively metabolized to cholesterol, viz., Smith-Lemli-Opitz syndrome (8) and rats that are fed BM 15.766 (32), leads one to believe that under these circumstances reverse isomerization of 7-dehydrocholesterol to 8-dehydrocholesterol takes place at the $\Delta^{5,7}$-level (**Fig. 4**). Such a mechanism has been suggested for the accumulation of ergosta-5,8,22-trien-3β-ol (lichesterol) together with ergosterol in the common foliose lichen, *Xanthoria parientina* (16). The inhibited 7-dehydrocholesterol Δ^7-reductase results in accumulation of both 7-dehydrocholesterol and 8-dehydrocholesterol in patients with Smith-Lemli-Opitz syndrome. The 8-dehydrocholesterol probably accumulates because 7-dehydrocholesterol is poorly converted into cholesterol and also provides substrate for the conversion to 8-dehydrocholesterol.

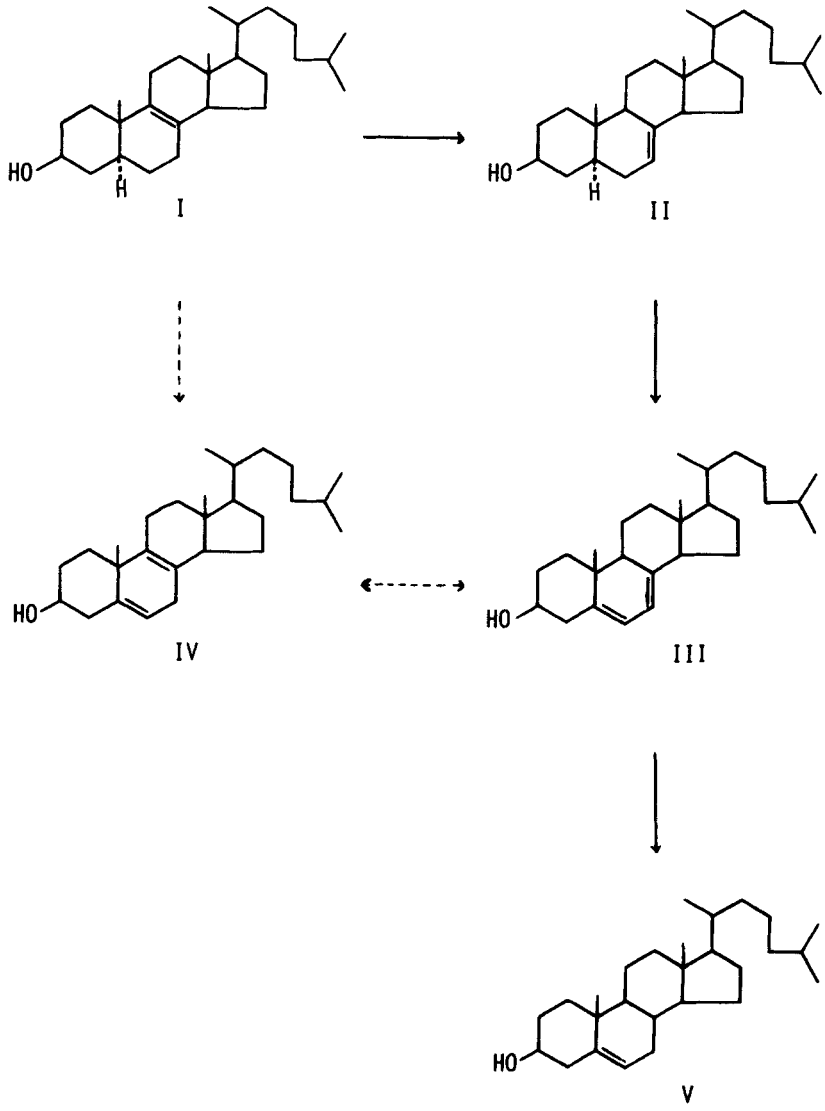


Fig. 4. Proposed block in cholesterol biosynthetic pathway in Smith-Lemli-Opitz syndrome. I, 5α-Cholest-8-en-3β-ol; II, 5α-cholest-7-en-3β-ol; III, 7-dehydrocholesterol; IV, 8-dehydrocholesterol; V, cholesterol.

In summary, we have shown that Smith-Lemli-Opitz syndrome is biochemically characterized by extremely low plasma cholesterol levels accompanied by unusually large amounts of 7-dehydrocholesterol and 8-dehydrocholesterol. Both 7-dehydrocholesterol and 8-dehydrocholesterol have been conclusively characterized. These sterols are also excreted in the feces of these patients. 8-Dehydrocholesterol is formed when 7-dehydrocholesterol is poorly transformed into cholesterol.

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