Identification of pentahydroxy bile alcohols in cerebrotendinous xanthomatosis: characterization of 5β-cholestane-3α,7α,12α,24ξ,25-pentol and 5β-cholestane-3α,7α,12α,23ξ,25-pentol

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Abstract This paper describes studies dealing with the nature of the C_{27} pentahydroxy bile alcohols present in the bile and feces of two patients with cerebrotendinous xanthomatosis (CTX). The presence of a bile alcohol having the structure 5β-cholestane-3α,7α,12α,24α,25-pentol was confirmed by separation of the two 24-hydroxy epimers of 5β-cholestane-3α,7α,12α,24,25-pentol and characterization of the epimers by gas–liquid chromatography and infrared and mass spectrometry. Tentative assignment of the 24α and 24β configuration was made on the basis of molecular rotation differences. A second major bile alcohol excreted by the CTX subjects was 5β-cholestane-3α,7α,12α,23ξ,25-pentol. Its structure was determined by infrared spectrometry, proton magnetic resonance spectrometry, and mass spectrometry because a reference compound was not available.

Supplementary key words 5β-cholestane-3α,7α,12α,24α,25-pentol · 5β-cholestane-3α,7α,12α,24β,25-pentol · 5β-cholestane-3α,7α,12α,25-tetrol · bile acid precursors · infrared spectrometry · mass spectrometry · NMR · PMR

Patients with cerebrotendinous xanthomatosis (CTX) have an impaired capacity to convert cholesterol to bile acids (1). The decreased bile acid production in these subjects is attributed to incomplete oxidation of the cholesterol side chain, because bile alcohols accumulate in bile and feces of these patients. One of the main components of the bile alcohol fraction in all three CTX subjects studied, so far, was a tetrahydroxy bile alcohol, 5β-cholestane-3α,7α,12α,25-tetrol, which was positively identified by comparison with the known compound. Other tetrahydroxy bile alcohols were not present in appreciable amounts, with the possible exception of 5β-cholestane-3α,7α,12α,23ξ-tetrol in one of the patients (1). The pentahydroxy bile alcohol fraction in all patients was more complex, consisting of two major and several minor components. The weight ratio between the tetrahydroxy and the pentahydroxy bile alcohol fractions appeared to be variable from patient to patient. Bile alcohols have not been detected in bile or feces of normal subjects.

In a previous publication (1) we described the isolation of a pentahydroxy bile alcohol from the feces of CTX patients that was provisionally assigned the structure 5β-cholestane-3α,7α,12α,24ξ,25-pentol. The possibility that the pentol was substituted in the 3α, 7α, 12α, 23ξ, and 25 positions could not be excluded because its mass spectrum exhibited a series of fragment ions that were more consistent with the structure of a 23-hydroxylated compound. The present studies indicate that the pentahydroxy bile alcohol fraction in two CTX patients consisted of 5β-cholestane-3α,7α,12α,23ξ,25-pentol and 5β-cholestane-3α,7α,12α,24α,25-pentol, with the former predominating. In the course of these studies the two 5β-cholestane-3α,7α,12α,24,25-pentols, epimeric at C-24, were prepared and characterized. Provisional assignment of the configuration of the C-24 hydroxyl groups was made on the basis of molecular rotation differences.

EXPERIMENTAL PROCEDURES

GLC. The isomeric pentols were analyzed as the TMSi derivatives on 180 cm × 4 mm columns packed with 3% QF-1 or 1% Hi-Eff 8BP on 80–100 mesh Gas-Chrom Q; column temperature, 230°C (Hewlett-Packard model 7610

Abbreviations: CTX, cerebrotendinous xanthomatosis; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl; NMR, nuclear magnetic resonance; PMR, proton magnetic resonance.

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gas chromatograph). The retention times relative to 5α-cholestane are summarized in Table 2 (Results section).

Mass spectra of the bile alcohols were obtained with a Varian MAT-111 gas chromatograph–mass spectrometer (Varian Associates, Palo Alto, Calif.) as described previously (1).

Infrared spectra (KBr discs) were obtained with a Perkin-Elmer model 421 grating spectrophotometer.

NMR. Structural studies of the pentols were carried out using proton magnetic resonance spectroscopy with a Varian XL-100 spectrometer equipped with Fourier transform capability.

Optical rotations were determined in methanol on a Carey model 60 spectropolarimeter.

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

Patient material. Studies were conducted in two patients with CTX (E.E. and E.S.) (1). The subjects ate a regular diet and did not receive medication for the duration of this study. Bile specimens were obtained from each subject after an overnight fast. A Rehfuss tube was positioned in the duodenum, and bile flow was stimulated by the intravenous injection of cholecystokinin (obtained from Professor Victor Mutt, Karolinska Institute, Stockholm, Sweden). Stools were collected as 2-day pools and were stored at −20°C.

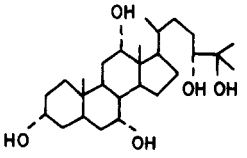
Preparation of 5β-cholestane-3α,7α,12α,24α,25-pentol and 5β-cholestane-3α,7α,12α,24β,25-pentol (Fig. 1, I and II)

5β-Cholestane-3α,7α,12α,24ξ,25-pentol (III) (mp 199–200°C, reported 191.5°C [2]) was prepared from 5β-cholestane-3α,7α,12α,25-tetrol (3) (mp 189–191°C) by the method of Hoshita (2).

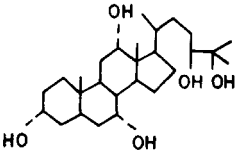
Separation of I and II (Fig. 1)

A. *By column chromatography.* For the separation of I and II in the 100–500 mg range, column chromatography on alumina was employed. 20 × 2.0 cm columns containing 50 g of neutral alumina, activity grade V (Bio-Rad, AG 7), were eluted with increasing proportions of methanol in ethyl acetate. The column fractions were monitored by TLC (see below). In a typical separation of 150 mg of III, a fraction containing 10 mg of the pure, less polar epimer II was eluted with 7.5% methanol in ethyl acetate (100 ml). Elution with increasing concentrations (7.5–12.5%) of methanol in ethyl acetate (600 ml) produced 100 mg of a mixture of the two epimers (I and II). When the concentration of methanol was increased to 15%, 20 mg of the chromatographically pure, more polar epimer (I) was obtained.

B. *By thin-layer chromatography.* For the separation of epimers I and II on a milligram scale, III was applied as a band to a 0.25-mm-thick silica gel G plate (Brinkmann).



5β-Cholestane-3α,7α,12α,24α,25-pentol (I)



5β-Cholestane-3α,7α,12α,24β,25-pentol (II)

Fig. 1. Structures of epimeric 5β-cholestane-3α,7α,12α,24,25-pentols. I, 5β-cholestane-3α,7α,12α,24α,25-pentol (24*R*); II, 5β-cholestane-3α,7α,12α,24β,25-pentol (24*S*).

The plate was developed with chloroform–acetone–methanol 35:25:7.5 (v/v/v), and the bands were made visible with iodine vapor. The more polar epimer (I) had an R_f of 0.30, and the less polar epimer (II), an R_f of 0.34. The bile alcohols were extracted from the silica gel with methanol.

C. *By crystallization.* Repeated crystallization of III from ethyl acetate–acetone 25:75 (v/v) or from ethyl acetate was monitored by TLC and resulted after a minimum of four crystallizations in epimer I, colorless prisms (mp 212–214°C), shown to be homogeneous by TLC and GLC. Work-up of the mother liquors by column or thin-layer chromatography was required to obtain epimer II, colorless prisms from ethyl acetate (mp 203–205°C).

D. *By gas–liquid chromatography.* TMSi derivatives of the epimers (I and II) were resolved incompletely by GLC on two different phases (Table 2, Results section). GLC could be used for the quantitative analysis of the individual pure epimers. Partial resolution of the epimers was achieved with Hi-Eff 8BP columns.

Infrared spectrometry

Fig. 2 shows the infrared spectra of I and II in KBr discs. Both spectra have the characteristic hydroxyl band at 3420 cm^{-1}. The spectra are very similar but show some differences in the region from 900 to 1050 cm^{-1} (930, 962, 995 cm^{-1}).

Optical rotation

The molecular rotation of 5β-cholestane-3α,7α,12α,24,25-pentol can be considered to be made up of the molecular rotation of 5β-cholestane-3α,7α,12α,25-tetrol (+160.82° = A) and the molecular rotation due to the additional asymmetric center ± B (at C-24). The molecu-

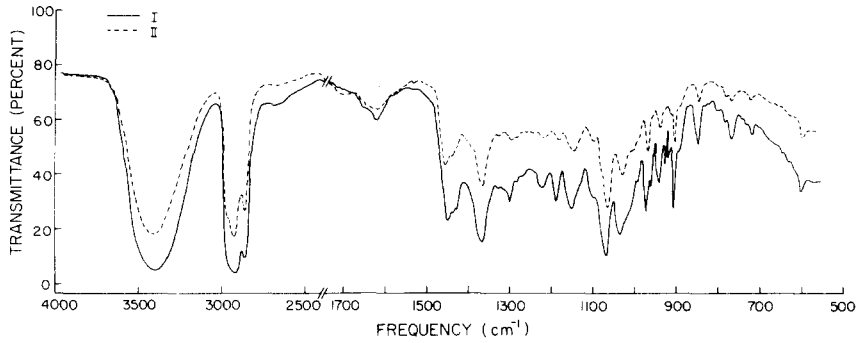


Fig. 2. Infrared spectra of 5β-cholestane-3α,7α,12α,24α,25-pentol (I, *solid line*) and 5β-cholestane-3α,7α,12α,24β,25-pentol (II, *dashed line*).

lar rotations of the two epimers differed significantly, as shown in **Table 1.** Epimer I may be assigned the 24α configuration, 5β-cholestane-3α,7α,12α,24α,25-pentol; epimer II, being the more levorotatory of the two epimers, is assigned the 24β configuration, 5β-cholestane-3α,7α,12α,24β,25-pentol (4–6).

Isolation of bile alcohols from bile and feces of CTX patients

Ethyl acetate extracts of the neutral steroid fraction (containing sterols and bile alcohols) were prepared exactly as described previously (1). The fraction was subjected to column chromatography on neutral alumina, grade V (Bio-Rad, AG 7). The nonpolar neutral sterols were eluted with ethyl acetate, the tetrahydroxy bile alcohol fraction was eluted with 5% (v/v) methanol in ethyl acetate, and the pentahydroxy bile alcohols were eluted with 7.5–15% methanol in ethyl acetate. The column effluents were monitored by TLC (see below).

TLC of biological pentols

For monitoring the bile alcohol fractions obtained by alumina column chromatography (see preceding section), TLC on silica gel G plates (Brinkmann, 0.25 mm thick) was employed. The solvent system was chloroform–acetone–methanol 35:25:7.5 (v/v/v), and the following R_f values were observed: 5β-cholestane-3α,7α,12α,25-tetrol, 0.57; 5β-cholestane-3α,7α,12α,25,26-pentol, 0.30; 5β-cholestane-3α,7α,12α,24α,25-pentol, 0.30; and 5β-cholestane-3α,7α,12α,24β,25-pentol, 0.34 (cholesterol, 0.95). All column fractions containing pentahydroxy bile alcohols were combined and purified further from traces of 5β-cholestane-3α,7α,12α,25-tetrol and from unidentified, more polar compounds by preparative TLC (silica gel G plates, 1 mm thick, Analtech; chloroform–acetone–methanol 35:25:7.5 [v/v/v]). The bands were made visible with iodine vapor, and the pentahydroxy bile alcohol bands were eluted with methanol.

The pentol fraction was separated into its components by preparative TLC on a 0.25-mm-thick layer of silica gel G (Brinkmann) with the solvent system chloroform–acetone–methanol 35:25:7.5. The bands were removed from the plate and eluted with methanol. The isomeric bile alcohols were crystallized from ethyl acetate to constant melting point.

TABLE 1. Molecular rotation of epimers I and II[a]

5β-Cholestane	$[\alpha]_D^{25}$	M_D Calcd	M_D Found
3α,7α,12α-triol (4)	+30.4°	+133°[b]	+128°
3α,7α,12α,25-tetrol	+36.8°		+161°
3α,7α,12α,24α,25-pentol (I)	+44.8°	+203°[c]	+203°
3α,7α,12α,24β,25-pentol (II)	+28.7°	+138°[c]	+130°
Mixture III[d]	+38.8°		+176°

[a] Determined in methanol (5β-cholestane-3α,7α,12α,25-tetrol, 9.83 mg/ml; I, 3.39 mg/ml; II, 1.62 mg/ml; III, 2.70 mg/ml).
[b] Calculated from Fieser and Fieser (5), p. 179.
[c] Calculation based on M_D of 5β-cholestane-3α,7α,12α-triol (+128°) (4), ΔM_D 24α-ol, +42° (4); ΔM_D 24β-ol, −23° (4); and ΔM_D 25-ol, +33°.
[d] 5β-Cholestane-3α,7α,12α,24ξ,25-pentols, synthesized by the method of Hoshita (2) (epimeric composition unknown, but calculated from the molecular rotations to be a mixture of 62.4% of epimer I and 37.6% of epimer II).

TABLE 2. GLC and TLC characteristics of pentahydroxy bile alcohols

	R_f[a]	Retention Time of TMSi Ethers Relative to 5α-Cholestane[b] 3% QF-1	1% Hi-Eff 8BP
Band 1	0.30	4.23	1.47
Band 2	0.32	3.94	1.32
5β-Cholestane-3α,7α,12α,24α,25-pentol	0.30	4.23	1.47
5β-Cholestane-3α,7α,12α,24β,25-pentol	0.34	4.35	1.55
5β-Cholestane-3α,7α,12α,25,26-pentol	0.30	4.63	

[a] Solvent system: chloroform–acetone–methanol 35:35:7.5 (v/v/v); silica gel G plates, 0.25 mm thick (Brinkmann).
[b] Retention time of 5α-cholestane on 3% QF-1, 2.95 min; on Hi-Eff 8BP, 5.42 min; column temp, 230°C.

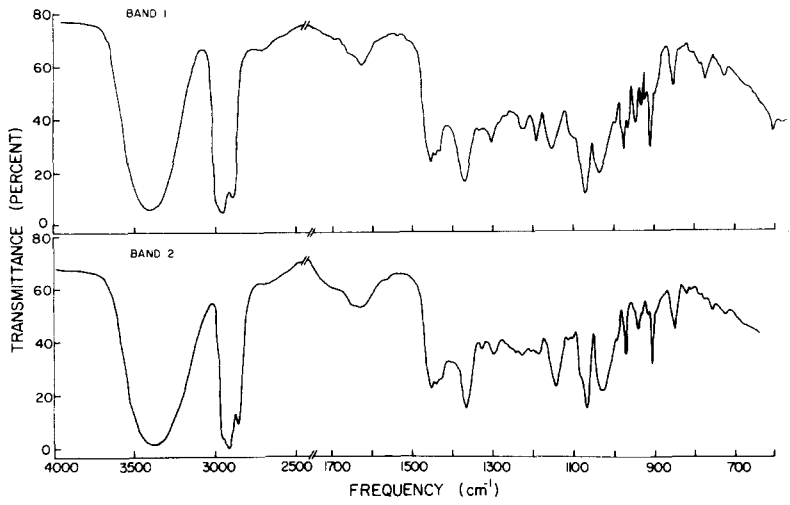


Fig. 3. Infrared spectra of 5β-cholestane-3α,7α,12α,24α,25-pentol (band 1) and 5β-cholestane-3α,7α,12α,23ξ,25-pentol (band 2).

RESULTS

Isolation and purification of pentahydroxy bile alcohols

The pentahydroxy bile alcohol fraction obtained from bile and feces of patients E.S. and E.E. was subjected to preparative TLC, yielding bands of R_f 0.30 (band 1) and R_f 0.32 (band 2) (**Table 2**).

Band 1. The material in this band, which amounted to approximately 20% of the total pentol fraction in both bile and feces, was eluted with methanol and chromatographed repeatedly until it was pure, as monitored by TLC and GLC (Table 2). The material had the same relative retention time on two different columns and the same R_f value when subjected to analytical TLC as 5β-cholestane-3α,7α,12α,24α,25-pentol. When crystallized from ethyl acetate, the substance in band 1 gave colorless prisms, mp 212–214°C, unchanged when mixed with known 5β-cholestane-3α,7α,12α,24α,25-pentol.

Infrared spectrometry. The infrared spectrum of the crystalline material from band 1 (**Fig. 3**) was identical in all respects with that of the known 5β-cholestane-3α,7α,12α,24α,25-pentol (Fig. 2) (hydroxyl vibration at 3420 cm^{-1} and other prominent peaks in the fingerprint region at 1070, 1035, 970, 945, and 910 cm^{-1}).

Mass spectrometry. The mass spectrum of the TMSi derivative of the crystalline material from band 1 was identical with that of known 5β-cholestane-3α,7α,12α,24α,25-pentol (**Fig. 4**) and 5β-cholestane-3α,7α,12α,24β,25-pentol. A molecular ion was observed at *m/e* 812, and peaks were present at *m/e* 797 (M − 15) and 722 (M − 90). The base peak at *m/e* 131 is ascribed to the cleavage of the C-24,25 bond. A peak was also present at *m/e* 681 (M − 131). The ions at *m/e* 591 (M − 131 − 90), 501 (M − 131 − [2 × 90]), 411 (M − 131 − [3 × 90]), and 321 (M − 131 − [4 × 90]) were formed by successive losses of trimethylsilanol (TMSiOH, 90 amu), a fragmentation pattern characteristic of trimethylsilyl ether derivatives. The intensities of the major ion fragments of I and II (Fig. 1) were so similar that mass spectrometry probably cannot be used to differentiate between the two epimers.

Band 2. The material in this band amounted to approximately 80% of the pentol fraction in both bile and feces. The TLC and GLC characteristics of this material differed from those of the reference compounds available to us (Table 2). The compound crystallized from ethyl acetate as colorless needles; mp 210–211°C; $[\alpha]_D^{25}$ 45.7°.

Infrared spectrometry. The infrared spectrum of the crystalline material (Fig. 3) showed a prominent hydroxyl

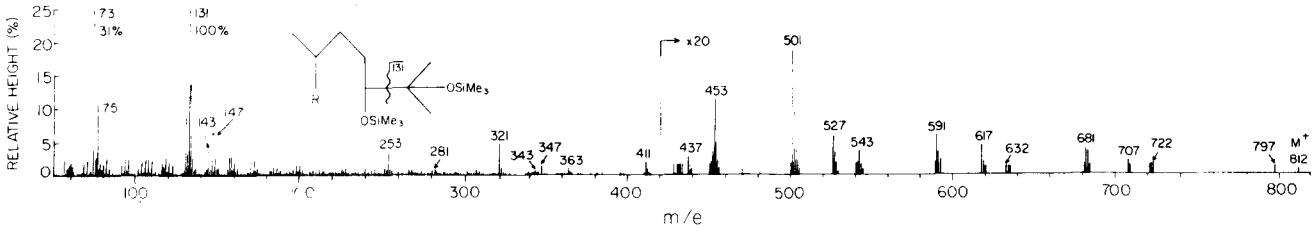


Fig. 4. Mass spectrum of 5β-cholestane-3α,7α,12α,24α,25-pentol (I) TMSi ether. R = 3α,7α,12α-trimethylsilyloxy-5β-etiocholanyl.

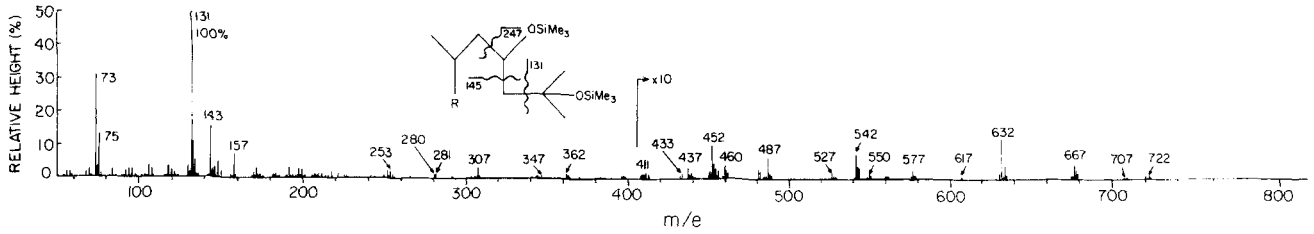


Fig. 5. Mass spectrum of 5β-cholestane-3α,7α,12α,23ξ,25-pentol (band 2) TMSi ether. R = 3α,7α,12α-trimethylsilyloxy-5β-etiocholanyl.

vibration at 3380 cm^{-1} and differed from the spectra of 5β-cholestane-3α,7α,12α,24α,25-pentol (band 1) and 5β-cholestane-3α,7α,12α,24β,25-pentol at 1080, 1020, 930, and 920 cm^{-1}.

Mass spectrometry. The mass spectrum of the crystalline material from band 2 (TMSi derivative) (**Fig. 5**) exhibited fragment ions at *m/e* 667, 577, 487, 397, and 307, which are consistent with a pentol hydroxylated at C-23. The fragment ions at *m/e* 550, 460, 370, 280, and 281 may arise as follows. With a TMSi ether at C-23, the side chain apparently is induced to provide sizable fragments by cleavage at C-20–C-22, yielding the side chain moiety as mass 261, $-CH_2CH(OSiMe_3)CH_2C(CH_3)_2(OSiMe_3)$. The charge at C-20 is probably stabilized by loss of the 17α-hydrogen to the above fragment, with generation of the ion *m/e* 550. Subsequent loss of trimethylsilanol in the ring system results in *m/e* 460 (M − [90 + 262]), 370 (M − [2 × 90 + 262]), and 280 (M − [3 × 90 + 262]). The ion at *m/e* 281 (253 + 28) conceivably represents the desaturated nucleus with C-20 and C-21 and is found in both spectra (Figs. 4 and 5). The underivatized material gave a molecular ion at *m/e* 452 consistent with a pentahydroxy C-27 bile alcohol (1).

NMR

The chemical shifts for the C-27, C-26, C-21, C-19, and C-18 methyl groups of the crystalline material from band 2, 5β-cholestane-3α,7α,12α,25-tetrol, and 5β-cholestane-3α,7α,12α,24ξ,25-pentol are given in **Table 3** and **Fig. 6**. The proton resonances of the angular methyl groups at C-19 and C-18 are almost identical in all three compounds. The C-21 methyl group appears as a doublet (at 102.6 Hz in 5β-cholestane-3α,7α,12α,25-tetrol, at 103.6 Hz in the 5β-cholestane-3α,7α,12α,24ξ,25-pentol, and at 104.8 Hz in the material from band 2) with a coupling constant of 6 Hz.

The methyl groups at C-27 and C-26 appear as a singlet at 118.4 Hz in 5β-cholestane-3α,7α,12α,25-tetrol, whereas the chemical shifts for these methyl groups appear at 116.8 and 114.4 Hz in 5β-cholestane-3α,7α,12α,24ξ,25-pentol and at 126.8 and 124.0 Hz in the material isolated from band 2.

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

Compound	C-18	C-19	C-21	C-26	C-27
	Hz	*Hz*	*Hz*	*Hz*	*Hz*
Band 2[b]	72.6	92.0	d 104.8	124.0	126.8
5β-Cholestane-3α,7α, 12α,25-tetrol[c]	72.0	92.8	d 102.6	118.4	118.4
5β-Cholestane-3α,7α, 12α,24ξ,25-pentol[d]	72.4	92.2	d 103.6	114.4	116.8

[a] Solvent CD_3OD.
[b] Crystalline material isolated from band 2, biological 5β-cholestane-3α,7α,12α,23ξ,25-pentol.
[c] Synthetic compound (3).
[d] Synthetic compound (2).

Lead tetraacetate oxidation

The material in band 1 was cleaved by lead tetraacetate (forming cholyl aldehyde). The compound in band 2 did not respond to the test under identical conditions, suggesting that the hydroxyl groups in the side chain were not adjacent.

DISCUSSION

In the bile and feces of the two CTX patients studied, the pentahydroxy bile alcohol fraction consisted of two major components. The predominant bile alcohol of the pentol fraction was 5β-cholestane-3α,7α,12α,23ξ,25-pentol, amounting to approximately 80% by weight, while 5β-cholestane-3α,7α,12α,24α,25-pentol accounted for approximately 20% of this fraction. The evidence leading to the identification of these bile alcohols was as follows. The less abundant pentol was shown to be identical with 5β-cholestane-3α,7α,12α,24α,25-pentol, which had been prepared from 5β-cholestane-3α,7α,12α,25-tetrol. This biosynthetic pentol had the same melting point, TLC and GLC properties, and infrared and mass spectra as the reference compound.

The identification of the predominating pentol as 5β-cholestane-3α,7α,12α,23ξ,25-pentol was more difficult because a known reference compound was not available. Previous mass spectrometric studies (1) had shown that the underivatized bile alcohol had a molecular ion at *m/e* 452 and a prominent peak at *m/e* 253, suggesting a 5β-cholestanepentol with three hydroxyl substituents in the ring sys-

tem (7). The mass spectrum of the TMSi derivative indicated that there were five hydroxyl groups, three in the ring system and two in the side chain. The presence of a hydroxyl group at C-25 seemed highly probable because of the fragment ion at *m/e* 131. The presence of a hydroxyl group at C-23 was suggested by the series of fragment ions at *m/e* 667, 577, 487, 397, and 307. Additional confirmation of the structure of the pentol in band 2 was obtained by NMR. The chemical shift of the C-21 methyl group in the bile alcohol series appears as a doublet (at 102.6 Hz in 5β-cholestane-3α,7α,12α,25-tetrol and at 103.6 and 104.8 Hz in the 5β-cholestane-3α,7α,12α,24ξ,25-pentol and the crystalline material from band 2, respectively) with a coupling constant of 6 Hz. The presence of a hydroxyl group at C-22 (α to the C-21 methyl group) in the material from band 2 was excluded for the following reasons.

(*1*) The C-21 doublet was not shifted appreciably (approx. 1 Hz) compared with its position with 5β-cholestane-3α,7α,12α,24ξ,25-pentol. One would have expected a shift of 7–10 Hz for the C-21 doublet if the hydroxyl group had been situated at C-22 (deshielding effect) (8).

(*2*) The data in Table 3 and Fig. 6 show that in the presence of a hydroxyl group at the C-25 position only (as in 5β-cholestane-3α,7α,12α,25-tetrol) the C-26 and C-27 methyls appear as a sharp single peak because of their equal environment and free rotation around the C-24 bond. The introduction of a hydroxyl group at the C-24 position (as in 5β-cholestane-3α,7α,12α,24ξ,25-pentol) produces a difference in the chemical shift of C-26 and C-27 methyls of 2–3 Hz. This suggests that there is a restricted rotation along the C-24,25 bond so that the two methyl groups do not have equal environment. The same situation would have been exhibited by a 23-hydroxylated pentol. The inspection of a molecular model of 5β-cholestane-3α,7α,12α,23ξ,25-pentol shows that the introduction of a hydroxyl group at C-23 causes the methyl groups at C-26 and C-27 to be deshielded to a greater extent than by the introduction of a hydroxyl group at C-24. If the pentahydroxy bile alcohol in band 2 were substituted at C-22, as in ecdysone, then the C-26 and C-27 methyls would again be equivalent because their environment is not affected by the C-22 hydroxyl group, which is four carbon atoms distant. One would then expect free rotation around the C-24 bond, as in 5β-cholestane-3α,7α,12α,25-tetrol, and a sharp singlet for the C-26 and C-27 methyls.

The presence of a hydroxyl group not vicinal to the C-25 hydroxyl is further suggested by the failure of lead tetraacetate to cleave the side chain. In contrast, lead tetraacetate oxidation of 5β-cholestane-3α,7α,12α,24α,25-pentol (band 1) produced a cleavage of the side chain with the formation of cholyl aldehyde.

We have previously suggested that the occurrence of bile alcohols hydroxylated at position 25 in CTX patients indicates the presence of an alternative pathway of bile acid

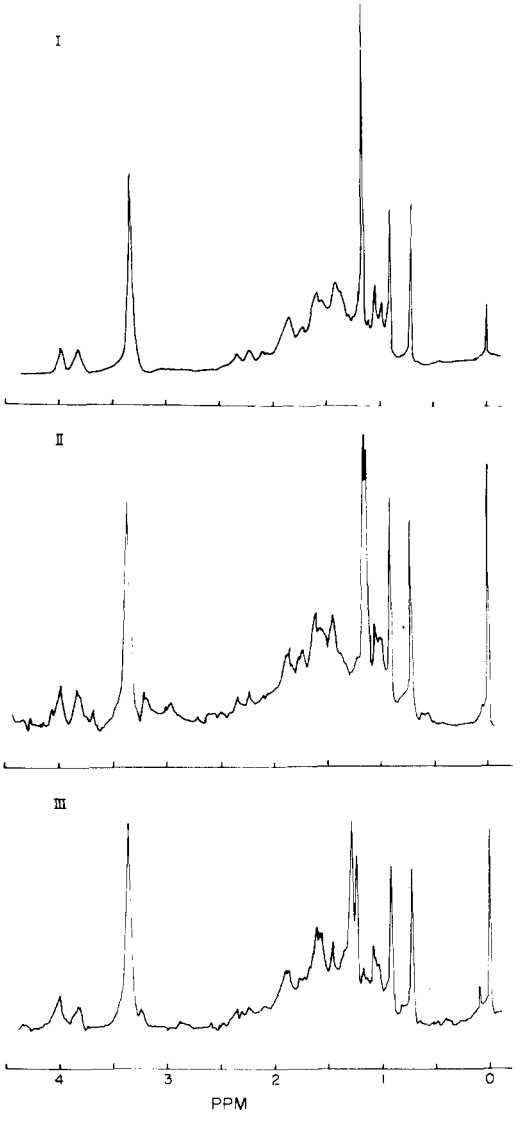


Fig. 6. 100-MHz PMR spectra (in CD_3OD) of 5β-cholestane-3α,7α,12α,25-tetrol (I), 5β-cholestane-3α,7α,12α,24ξ,25-pentol (II), and 5β-cholestane-3α,7α,12α,23ξ,25-pentol (III).

synthesis via 25-hydroxylated intermediates (1). The identification of 5β-cholestane-3α,7α,12α,24α,25-pentol indicates that cholic acid arises from the cleavage of a 24,25-glycol. It is not certain from the data currently available whether 5β-cholestane-3α,7α,12α,24α,25-pentol is the biologically active intermediate or whether both the 24α and 24β epimers are formed from 5β-cholestane-3α,7α,12α,25-tetrol. The presence of the 24α epimer might then indicate that the 24β epimer was preferentially metabolized to cholic acid and was therefore not detected. On the other hand, it seems unlikely that 5β-cholestane-3α,7α,12α,23ξ,25-pentol is a precursor of the normally occurring bile acids. If further metabolism of this bile alcohol takes place, it could conceivably lead to the formation of α-hydroxy or $\Delta^{22,23}$ bile acids. These compounds are not

known to be present in normal bile or in the bile of CTX patients. We ascribe the formation of 5β-cholestane-3α,7α,12α,23ξ,25-pentol to the impaired bile acid production that is known to exist in CTX patients. It seems probable that 5β-cholestane-3α,7α,12α,25-tetrol remains in the liver cell for a relatively prolonged time, thus exposing it to the action of a 23-hydroxylase that is normally too inactive to compete with the 24-hydroxylase for substrate.

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