Configuration at C-25 in 5β-cholestane-3α,7α,12α,25,26-pentol excreted by patients with cerebrotendinous xanthomatosis: circular dichroism and ^{13}C-NMR studies[1]

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Abstract The configuration at C-25 in 5β-cholestane-3α,7α,12α,25,26-pentol isolated from the bile and feces of patients with cerebrotendinous xanthomtosis (CTX) was determined from the lanthanide-induced circular dichroism (CD) Cotton effects and ^{13}C-NMR measurements. Under anhydrous conditions, CD spectra of 5β-cholestane-3α,7α,12α,25,26-pentol in the presence of Eu(fod)$_3$ exhibited a large induced negative Cotton effect at 320 nm. On the basis of the empirical rule (primary-tertiary-α-diols) in which R compounds have positive Cotton effects and S compounds have negative Cotton effects at 320 nm, it was concluded that 25,26-pentol has the 1,2,glycol structure with C-25 having the S-configuration. This assignment was based upon comparison with model compounds, 25(R and S),26-dihydroxy cholesterols and 25(R and S),26-dihydroxy cholecalciferols whose single-crystal X-ray structure and ^{13}C-NMR studies have been performed. It is suggested that these data may be helpful to clarify the stereospecificity of the hydroxylation of the terminal methyl group of the cholesterol side chain in CTX.—**Dayal, B., G. Salen, V. Toome, and G. S. Tint.** Configuration at C-25 in 5β-cholestane-3α,7α,12α,25,26-pentol excreted by patients with cerebrotendinous xanthomatosis: circular dichroism and ^{13}C-NMR studies. *J. Lipid Res.* 1986. **27:** 1328–1332.

Supplementary key words pentahydroxy bile alcohol

In patients with the rare sterol storage disease cerebrotendinous xanthomatosis (CTX), bile acid synthesis is impaired; as a consequence, relatively large amounts of C_{27} bile alcohols are excreted in bile and feces (1–6). These compounds have been conclusively identified by chemical synthesis and their structures have been determined by various spectroscopic methods as 5β-cholestane-3α,7α,-12α,24(R) tetrol, 5β-cholestane-3α,7α,12α,24(S)tetrol, 5β-cholestane-3α,7α,12α,25-tetrol, 5β-cholestane-3α,7α,12α,23(R),25-pentol, and 5β-cholestane-3α,7α,-12α,24(R),25-pentol (7–9).

We recently described the isolation of as yet another unrecognized pentahydroxy bile alcohol from the feces of CTX patients and provisionally assigned its structure as 5β-cholestane-3α,7α,12α,25ξ,26-pentol (10). The present studies elucidate the stereochemical configuration at C-25 by circular dichroism and ^{13}C-NMR spectroscopy.

MATERIALS AND METHODS

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, IA) model MP-12600 and are uncorrected.

Gas–liquid chromatography (GLC). The bile alcohols, as the trimethylsilyl (TMSi) derivatives, were analyzed on a 180 cm × 4 m column packed with 3% OV-17 on 80/100 mesh Gas-Chrom Q (Applied Science Laboratories, State College, PA); column temp. 270°C, N_2 flow 40 ml/min (Hewlett-Packard model 7610 gas chromatograph, Palo Alto, CA).

Mass spectra (MS) of the bile alcohols were obtained with a Varian MAT-5 and Varian MAT-111 gas chromatograph–mass spectrometer as described previously (11).

Thin-layer chromatography (TLC). The bile alcohols were separated on silicia gel G plates (Brinkman, Westbury, NJ, 0.25 mm thickness) and the spots were visualized either with iodine or phosphomolybdic acid (3.5% in isopropanol).

Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer (Norwalk, CT) model 141 polarimeter.

^{1}H- and ^{13}C-NMR spectrometry. Nuclear magnetic resonance spectra (^{1}H- and ^{13}C-NMR) were recorded on a Varian Associates XL-200 Spectrometer equipped with Fourier transform mode. All NMR spectra were taken in ($CDCl_3$ + CD_3OD) solution with Me_4Si as the internal standard. All the CMR spectra were recorded in the proton noise-decoupled mode to obtain exact chemical shifts, and the degree of substitution at each carbon was determined by experiments in the single-frequency off-resonance (SFOR) decoupled mode (12).

Eu(fod)$_3$. Tris-1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-octane-4,6-dionato) europium (III) (Thompson-Packard, Fort Lee, NJ) was used as a complexing agent without further purification.

Preparation of substrates. 5β-Cholestane-3α,7α,12α,-23(R),25-pentol (mp 210–211°C) and 5β-cholestane-3α,7α,12α,24(R),25-pentol (mp 214°C) were isolated, synthesized, and characterized as described previously (3, 6–10). 5β-Cholestane-3α,7α,12α,24(S),25-pentol (mp

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl; CD, circular dichroism; CTX, cerebrotendinous xanthomatosis; MS, mass spectrometry; NMR, nuclear magnetic resonance; SFOR, single-frequency off-resonance decoupling.

[1] Dayal, B., G. S. Tint, V. Toome, and G. Salen. 1983. Structure and stereochemistry of 5β-cholestane-3α,7α,12α,25(S)26-pentol excreted by patients with cerebrotendinous xanthomatosis (CTX). A circular dichroism study presented in part at the 29th IUPAC Congress, Cologne, West Germany, September 1983.

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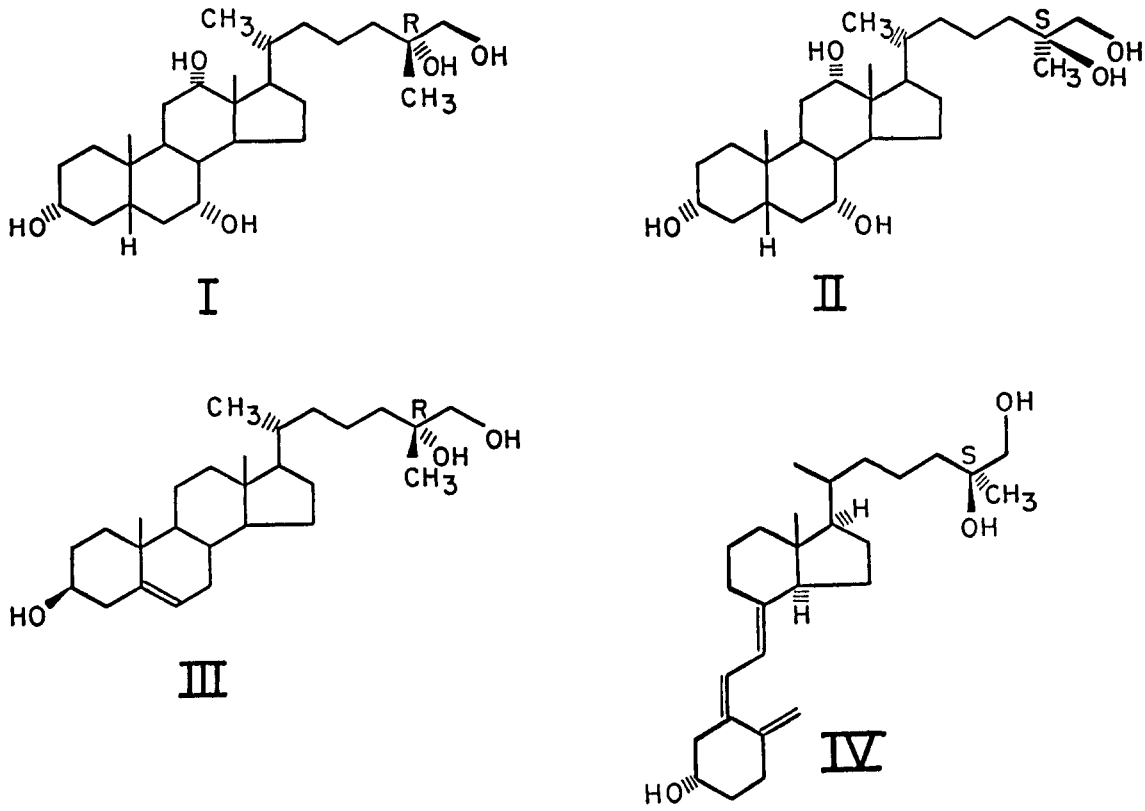

Fig. 1. Structure of isomeric bile alcohols. I, 5β-Cholestane-3α,7α,12α,25(R),26-pentol; II, 5β-cholestane-3α,7α,12α,25(S),26-pentol; III, cholest-5-ene-3β,25(R),26-triol; IV, 25(S),26-dihydroxycholecalciferol.

203–205°C) and 5β-cholestane-3α,7α,12α,25,26-pentol (mp 180–182°C) were synthesized and characterized according to Dayal et al. (6–10).

*Isolation of 5β-cholestane-3α,7α,12α,25(S),26-pentol (**Fig. 1**, compound II).* 5β-Cholestane-3α,7α,12α,25(S),26-pentol was isolated from the feces of two patients with CTX (10). The purity of the compound was established by melting point, TLC, $[\alpha]_D$, GLC, and MS (10) (GLC chromatogram of the isolated pentol fraction shown in **Fig. 2**) in which 25,26-pentol comprised 41% of the total bile alcohol fraction. The compound was purified by thin-layer chromatography and crystallized from ethyl acetate–methanol as previously described (10). The CD spectrum of the compound in the presence of Eu(fod)$_3$ showed $\Delta\epsilon_{320} = -1.29$ degree $\times$ cm^2 $\times$ dmol^{-1} (first Cotton effect), and $\Delta\epsilon_{288} = +0.98$ degree $\times$ cm$_2$ $\times$ dmol^{-1} (second Cotton effect) (see compound 4, **Table 1** and **Fig. 3**).

Optical measurements. The CD measurements were carried out on a Jasco J-20 instrument at 24°C, under a stream of high purity, dry N_2, with a cell thickness of 0.1 cm. The coefficient of dichroic absorption, $\Delta\epsilon$, was calculated from the molar ellipticity (θ) by the following equation: molar ellipticity $[\theta] = 3300\ \Delta\epsilon$. Both the molar ellipticity $[\theta]$ and $\Delta\epsilon$ are expressed in degree $\times$ cm^2 $\times$ dmol^{-1}. The Cotton effect was measured at its maximum value, around 310 nm, and was found to correlate with the chirality of the two hydroxyl groups (8, 9, 13–18).

Experimental procedure. A 1:1 mixture of the bile alcohol and complex Eu(fod)$_3$ was made in dry chloroform (ethanol-free) so that the concentration of the solutes was 2×10^{-4} M. The CD was then measured after 30–60 min at 24°C, under a stream of high purity, dry N_2, with a cell thickness of 0.1 cm.

RESULTS AND DISCUSSION

Recently we have elucidated the absolute stereochemistry at C-23 and C-24 of 5β-cholestane-3α,7α,12α,23,25-pentol and the isomeric 5β-cholestane-3α,7α,12α,24,25-pentol from the lanthanide-induced circular dichroism Cotton effect measurements (8, 9). These experiments conclusively defined the chirality of these pentahydroxy bile alcohols having 1,2 and 1,3 glycol systems in the side chain. Using Eu(fod)$_3$ under anhydrous conditions, we obtained the desired CD spectra exhibiting very large induced split Cotton effects (compounds 1–3, Table 1). On

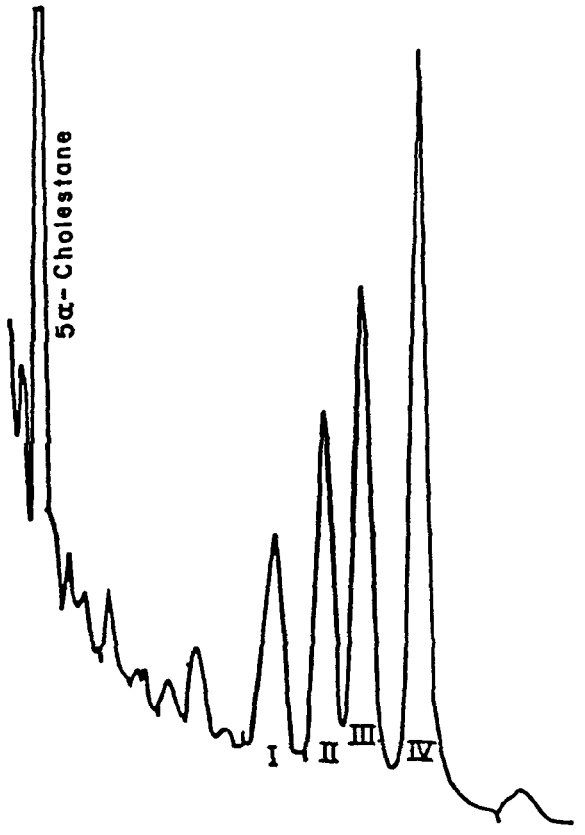

Fig. 2. GLC chromatogram of the TMSi ether derivatives of pentahydroxy bile alcohols isolated from the feces of a patient with cerebrotendinous xanthomatosis. Column: 3% OV-17 at 270°C and N_2 flow 40 ml/min. 0, 5α-Cholestane; I, 5β-cholestane-3α,7α,12α,23ξ,25-pentol; II, 5β-cholestane-3α,7α,12α,23α,25-pentol; III, 5β-cholestane-3α,7α,12α,24α,25-pentol; IV, 5β-cholestane-3α,7α,12α,25S,26-pentol.

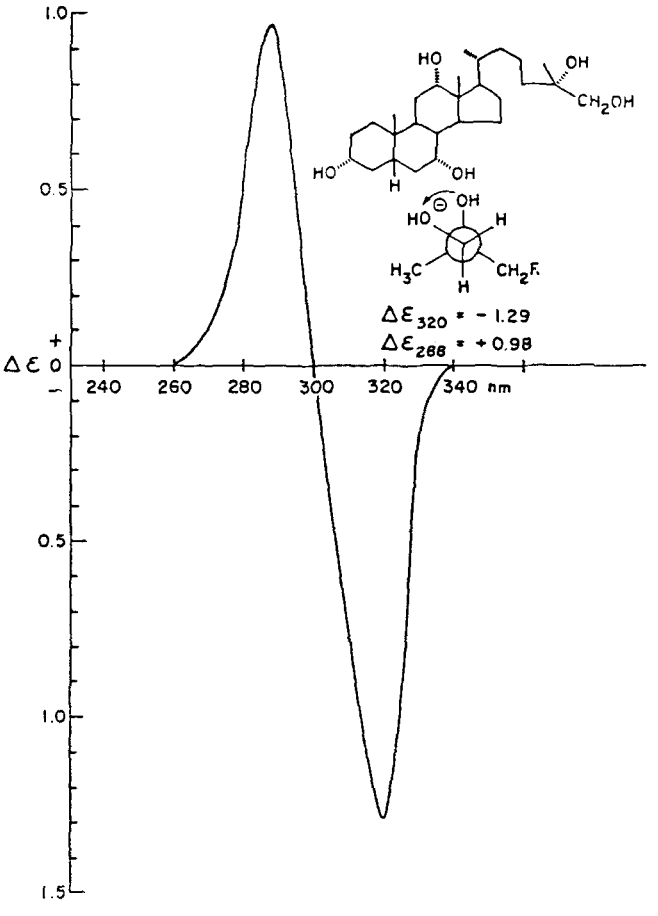

Fig. 3. Circular dichroism of (2×10^{-4} M) 5β-cholestane-3α,7α,12α,25(S),26-pentol and (2×10^{-4} M) $Eu(fod)_3$ in dry $CHCl_3$ at ambient temperature within 30 min after mixing.

the basis of the empirical rule of Nakanishi et al. (13) and Nakanishi and Dillon (14, 15),[3] the bile alcohols 24α,25-pentol and 24β,25-pentol were assigned the 24R and 24S configurations, respectively, and 5β-cholestane-3α,7α,12α,23α,25-pentol was shown to possess the 23R configuration (compounds 1–3, Table 1). These assignments were fully confirmed by comparison with "24R,25-dihydroxycholesterol", a model compound whose single-crystal X-ray structure had been determined (16).

Using identical conditions when the CTX bile alcohol 5β-cholestane-3α,7α,12α,25,26-pentol was examined by CD spectroscopy in the presence of $Eu(fod)_3$, it exhibited an induced split Cotton effect at ca. 320 nm $\Delta\epsilon_{320} = -1.20$ degree $\times$ cm^2 $\times$ dmol^{-1} and $\Delta\epsilon_{288} = +0.98$ degree $\times$ cm^2 $\times$ dmol^{-1} (compound 4, Table 1 and Fig. 3). These observed Cotton effects could not predict the absolute configuration at C-25 based on the empirical model (secondary-tertiary α-diols) of Nakanishi et al. (13). However, recent studies by Partridge et al. (17) have unequivocally shown that, for examples of primary-tertiary α-diols (compounds 5a–5d, Table 1 and compounds III and IV, Fig. 1), the R compounds have positive Cotton effects and the S compounds have negative Cotton effects at 318 nm. (This observation seems to be a general phenomenon for primary-tertiary α-diols.)[4] From these observations, the stereochemical configuration at C-25 of 5β-cholestane-3α,7α,12α,25,26-pentol (compound 4, Table 1 and compound II, Fig. 1) was assigned S based on the 1,2-glycol system in the side chain.

The chirality of the model compounds 25(R/S),26-dihydroxycholesterols and (25(R/S),26-dihydroxycholecalciferols (Fig. 1, compounds III and IV; compounds 5a–5d, Table 1) was established by Partridge et al. (17) by

[3] In these cases, secondary-tertiary α-diols were studied.

[4] The stereochemistry of 26 (or 27)-nor-5β-cholestane-3α,7α,12α,24S,25ξ-pentol was recently established by us from the lanthanide-induced circular dichroism Cotton effect measurements. In this case of secondary-secondary acyclic glycol system, the sign for predicting chirality was also opposite to the glycol chirality of the conformer having the large groups to the rear (18).

TABLE 1. Circular dichroism of 5β-cholestane pentols

Entry	Compound	Origin of Sample	Molar Ratio Substrate Eu(fod)$_3$	Solvent	CD[a] Δε[b]	CD[a] λ, nm[c]	Chirality
1	5β-Cholestane-3α,7α,12α,24R,25-pentol	a) Isolated from CTX patient b) Synthesized in our laboratory[d]	1:1	$CHCl_3$	−13.5 +9.2[e]	309 285	24R
2	5β-Cholestane-3α,7α,12α,24S,25-pentol	Synthesized in our laboratory	1:1	$CHCl_3$	+9.5 −5.9[e]	308	24S
3	5β-Cholestane-3α,7α,12α,23R25-pentol	Isolated from CTX patient	1:1	$CHCl_3$	−2.7 +2.7[e]	320 290	23R
4	5β-Cholestane-3α,7α,12α,25(S),26-pentol	a) Isolated from CTX patients b) Synthesized in our laboratory[d]	1:1	$CHCl_3$	−1.29 +0.98[e]	320 288	25S
5	a) Cholest-5-ene-3β,25,26-triol (25R)		1:1	$CHCl_3$	+2.6	318	25R
	b) Cholest-5-ene-3β,25,26-triol (25S)				−2.6	318	25S
	c) 25(R),26-dihydroxy-cholecalciferol	(Ref. 16)	1:1	$CHCl_3$	+2.6	318	25R
	d) 25(S),26-dihydroxy-cholecalciferol		1:1	$CHCl_3$	−2.6	318	25S

[a] The sign of the longer wavelength Cotton effect (first Cotton effect) (compounds 1–3) is in agreement with the chirality of the secondary/tertiary acyclic compounds, whereas for acyclic primary/tertiary 1,2 glycols (compound 4 and compounds 5a–5d) the signs are opposite to the glycol chirality (refs. 16, 18).

[b] The Δε is the coefficient of dichroic absorption and is expressed by D/Cl, where D is the observed difference in the values of absorbance between left and right circular, polarized light, C is the molar concentration, and l is the path length of the cell in cm.

[c] The conformer with the bulkier groups to the rear is used to define the chirality of acyclic glycols.

[d] Compounds synthesized in our laboratory resulted in equivalent parameters.

[e] A second Cotton effect of opposite sign is observed around 290 nm.

CD. The $\Delta\epsilon_{318} = +2.6$ degree × cm^2 × dmol^{-1}, $\Delta\epsilon_{318} = -2.6$ degree × cm^2 × dmol^{-1}, and thus the chirality at C-25 were assigned as R and S, respectively. The structure of the 25(R),26-dihydroxycholesterol (Fig. 1, compound III) with the +Ve CD was then confirmed independently by X-ray diffraction studies.

Now, since the chirality of the 25-hydroxyl group of the model compounds 25(S),26-dihydroxycholesterol and 25(S),26-dihydroxycholecalciferol was conclusively established as 25S (having −Ve CD), the CTX pentol, 5β-cholestane-3α,7α,12α,25,26 pentol must also be assigned the same configuration. Further support of this structure was drawn from the conclusion that a 25S configuration for the chemically synthesized 5β-cholestane-3α,7α,12α,-25(S),26-pentol is compatible with stereochemical considerations of the mode of hydroxylation of the precursor 5β-cholest-25-en-3α,7α,12α-triol. The reagent, osmium-tetroxide, would most likely attack the Δ^{25}-triol from the less hindered side. Inspection of the molecular model indicates that this is the side opposite the D-ring, thus giving rise to the S configuration at carbon 25.

Additional confirmation of the structure of the biosynthetic pentol was obtained by ^{13}C-NMR. As can be seen from **Table 2,** the chemical shifts of the C-atoms C(1) to C(21) in 25,26-pentol were identical, within ±=0.04 ppm with 5β-cholestane-3α,7α,12α,25-tetrol (19). The rest of the side-chain C-atoms were assigned by consideration of

TABLE 2. ^{13}C-NMR data for bile alcohols

Cholesterol Carbon	Cholesterol ^{13}C-NMR	5β-Cholestane-3α,7α,12α,25-tetrol ^{13}C-NMR	25(S),26-Dihydroxy cholecalciferol ^{13}C-NMR	5β-Cholestane-3α,7α,12α, 25(S),26-pentol ^{13}C-NMR
C–1	38.5	35.37	33.5	35.4
C–2	32.3	30.09	36.5	30.0
C–3	72.3	71.72	70.3	71.6
C–4	43.0	39.46	46.9	39.3
C–5	143.0	41.56	137.0	41.7
C–6	122.0	34.58	122.4	34.6
C–7	33.2	68.46	118.8	68.4
C–8	33.0	39.46	142.1	39.5
C–9	51.7	26.33	29.8	26.5
C–10	37.6	34.82	146.6	34.8
C–11	22.2	28.04	23.2	28.1
C–12	41.1	73.18	41.6	73.2
C–13	43.5	46.37	46.7	46.4
C–14	58.1	41.56	57.8	41.5
C–15	25.3	23.29	24.3	23.3
C=16	29.3	27.72	28.5	27.7
C–17	57.5	47.24	57.3	47.4
C–18	12.4	12.47	12.4	12.5
C–19	19.9	22.48	112.5	22.5
C–20	37.1	35.80	37.1	35.8
C–21	19.3	17.20	19.4	17.6
C–22	37.4	36.44	37.7	36.5
C–23	24.9	20.79	21.0	20.3
C–24	40.6	44.37	39.8	39.1
C–25	29.1	71.05	73.5	73.0
C–26	22.9	29.01	70.3	69.6
C–27	23.2	20.01	23.6	22.9

multiplicity in the SFOR spectrum and by comparison of the ^{13}C-NMR chemical shifts with the 25(S),26-dihydroxycholecalciferol whose absolute configuration at C-25 has been established by X-ray crystallography (Table 2) (12, 19). Accordingly, the hydroxylated C-atoms C(25) and C(26) in 25,26-pentol were readily assigned based on their downfield chemical shifts at δ73.0) (singlet) and δ69.6 (triplet) where as C(27) exhibited highfield quartet at δ22.9, respectively. The remaining signals, triplets at δ36.5, δ20.3, and δ39.1 were assigned (by comparing with (25S),26-dihydroxycholecalciferol) to C(22), C(23), and C(24), respectively, based on the hydroxyl substituent effects on the chemical shifts of C(22), C(23), and C(24) in 25,26-pentol.[5]

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[5] The substituent effects of hydroxyl groups mentioned in this text and in reference 12 for cholesterol, 5β-cholestane-3α,7α,12α,25-tetrol, 25(S),26-pentol, and 25(S),26-dihydroxycholecalciferol will be useful in structural elucidation of new metabolites of steroids with cholesteryl side chains.