Absolute configuration of pentahydroxy bile alcohols excreted by patients with cerebrotendinous xanthomatosis: a circular dichroism study

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Abstract The absolute configurations of the C_{27} pentahydroxy bile alcohols present in bile and feces of two patients with cerebrotendinous xanthomatosis (CTX) were determined by circular dichroism (CD) spectroscopy. The CD spectra of 5β-cholestane-3α,7α,12α,24α,25-pentol in the presence of Eu(fod)$_3$ [tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato) europium (III)] exhibited a negative Cotton effect and was assigned the 24*R* absolute configuration. Conversely, 5β-cholestane-3α,7α,12α,24β,25-pentol showed a strong positive Cotton effect and was assigned the 24*S* configuration. These assignments were based upon comparison with a model compound, 5-cholestene-3β,24(*R*),25-triol, whose single-crystal X-ray structure has been determined. The importance of these data is to establish a structural mechanism for the conversion of 5β-cholestane-3α,7α,12α,24*S*,25-pentol rather than 5β-cholestane-3α,7α,12α,24*R*,25-pentol into cholic acid in man as well as in animals.

Supplementary key words Cotton effects · epimeric bile alcohols

In patients with the rare sterol storage disease cerebrotendinous xanthomatosis (CTX) bile acid synthesis is impaired and, as a consequence, relatively large amounts of C_{27} bile alcohols are excreted in bile and feces (1, 2). Analysis of the bile alcohol fraction disclosed the presence of two major components, 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,23,25-pentol, and several minor components among which 5β-cholestane-3α,7α,12α,24,25-pentol predominated (2). The currently accepted pathway of cholic acid synthesis from cholesterol does not include 25-hydroxylated intermediates, and side chain cleavage is thought to proceed via 26-hydroxylation of 5β-cholestane-3α,7α,12α-triol (3). Nevertheless, the existence of an alternate pathway of cholic acid biosynthesis in man and rat involving bile alcohols with 25-hydroxyl functions was recently demonstrated in our laboratory (2). The quantitative significance of this pathway in the normal mammalian organism remains to be established.

The insertion of hydroxyl groups into the 23- or 24-position of 5β-cholestane-3α,7α,12α,25-tetrol was found to be stereospecific. For example, in the case of the two 5β-cholestane-3α,7α,12α,24,25-pentols, epimeric at C-24, only the more dextrorotatory compound [$(\alpha)_D^{25}$ + 44.8°] was found in bile and feces. This compound was tentatively assigned the 24"α" configuration on the basis of molecular rotation studies (4). The more levorotatory pentol [$(\alpha)_D^{25}$ + 28.7°], 24"β", was not detected in bile or feces since it was rapidly converted to cholic acid in vivo and in vitro (5).

MATERIALS AND METHODS

Preparation of substrates

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,24α,25-pentol (mp 212–214°C) and 5β-cholestane-3α,7α,12α,24β,-25-pentol (mp 203–205°C) were synthesized from cholic acid and purified as described by Dayal, et al. (6). Cholic acid was converted into its higher homologue, homocholic acid, by the Arndt-Eistert method which on further treatment with diazomethane gave methyl homocholate. A Grignard reaction of methyl mag-

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl; CD, circular dichroism; CTX, cerebrotendinous xanthomatosis; MS, mass spectrometry.

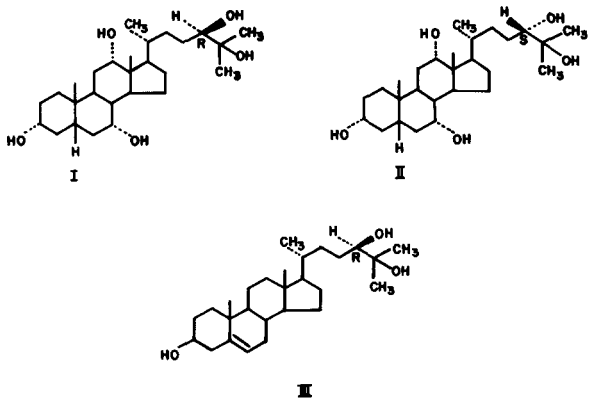

Fig. 1. Structures of isomeric bile alcohols. I, 5β-cholestane-3α,7α,12α,24α,25-pentol; II, 5β-cholestane-3α,7α,12α,24β,25-pentol; and III, cholest-5-ene-3β,24*R*,25-triol.

nesium iodide with methyl homocholate yielded 5β-cholestane-3α,7α,12α,25-tetrol (mp 189–191°C), which was dehydrated to form a mixture of 5β-cholest-24-en-3α,7α,12α-triol and the corresponding Δ^{25} compound. Oxidation of the Δ^{24} compound with OsO_4 yielded 5β-cholestane-3α,7α,12α,24,25-pentol. This mixture of two pentols epimeric at C-24 was separated by thin-layer chromatography (6).

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

5β-Cholestane-3α,7α,12α,24α,25-pentol was isolated from the bile and feces of two patients with CTX (2, 4). The compound was purified by thin-layer chromatography (Table 1), and crystallized from ethyl acetate–methanol as previously described (4, 6).

$Eu(fod)_3$

[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.

TABLE 1. Physical properties of 5β-cholestanepentols epimeric at C-24

	5β-Cholestane-3α,7α,12α,24α,25-pentol	5β-Cholestane-3α,7α,12α,24β,25-pentol
mp	212–214°C	203–205°C
TLC[a]	0.30	0.34
GLC	4.23[b] (1.47)[c]	4.35[b] (1.55)[c]
$(\alpha)_D^{25}$[d]	+44.8°	+28.7°
$(M)_D$	+203°	+130°

[a] Solvent system: chloroform–acetone–methanol 35:25:7.5 (v/v/v); silica gel G plates, 0.25 mm thick (Brinkman).

[b] Retention time of TMSi ethers relative to 5α-cholestane. Column: 3% QF-1, column temperature 230°C, retention time of 5α-cholestane 2.95 min.

[c] Column: 1% HI-EFF 8BP, column temperature 230°C, retention time of 5α-cholestane 5.42 min.

[d] Determined in methanol (5β-cholestane-3α,7α,12α,24α,25-pentol, 3.39 mg/ml; 5β-cholestane-3α,7α,12α,24β,25-pentol, 1.62 mg/ml; 5β-cholestane-3α,7α,12α,23,25-pentol, 2.54 mg/ml).

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 $[\Theta] = 3300\ \Delta\epsilon$ (7). 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 hydroxy groups (8).

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

In **Table 1** are listed the physical properties of 5β-cholestane-3α,7α,12α,24α,25-pentol and 5β-cholestane-3α,7α,12α,24β,25-pentol. The purity of each compound was established by mp, TLC, GLC, and MS (4, 6). The optical rotations correspond to molecular rotations of 203° and 138° respectively for 5β-cholestane-3α,7α,12α,24α,25-pentol and 5β-cholestane-3α,7α,12α,24β,25-pentol. The 24α and 24β configurations had previously been assigned on the basis of the molecular rotation differences (4).

The absolute configuration of the 5β-cholestane-3α,7α,12α,24,25-pentol was assigned on the basis of the circular dichroism spectra (**Figs. 2** and **3** and **Table 2**). For the 5β-cholestane-3α,7α,12α,24,25-pentols, the 24α-pentol showed $\Delta\epsilon_{308} = -13.5$ degree $\times$ cm^2 $\times$ dmol^{-1}, while the 24β compound showed $\Delta\epsilon_{308} = +9.5$ degree $\times$ cm^2 $\times$ dmol^{-1} (Figs. 2 and 3). These corresponding negative and positive Cotton effects indicate the respective chirality at C-24 as 24*R* and 24*S*.

The chirality of the model compound, 24,25-dihydroxy cholesterol (Fig. 1, III), was established by Partridge, Toome, and Uskoković (9) by CD (Table 2). The $\Delta\epsilon_{308} = -11.5$ degree $\times$ cm^2 $\times$ dmol^{-1}, and thus the chirality at C-24 was assigned *R*. This structure was confirmed independently by X-ray diffraction studies (9).

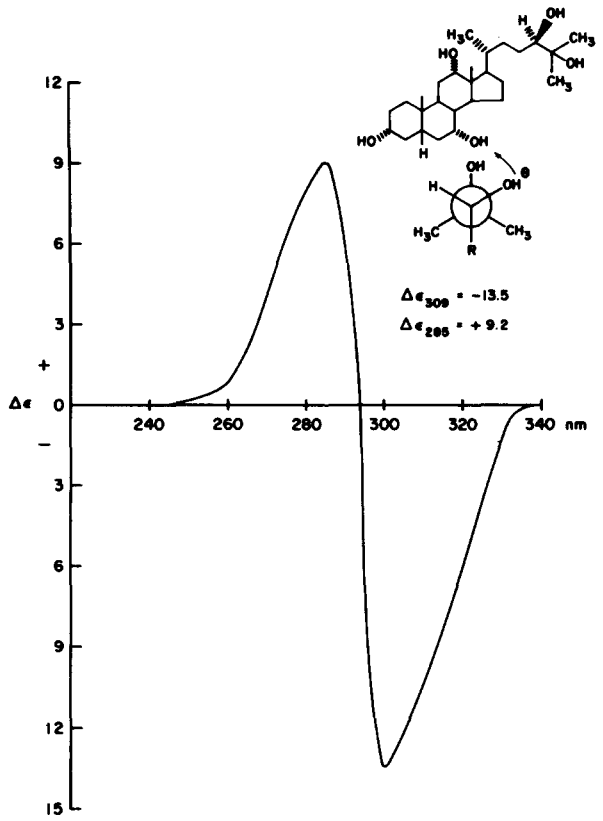

Fig. 2. Circular dichroism of 2×10^{-4} M 5β-cholestane-3α,7α, 12α,24α,25-pentol and 2×10^{-4} M $Eu(fod)_3$ in dry $CHCl_3$ at ambient temperature within 30 min after mixing.

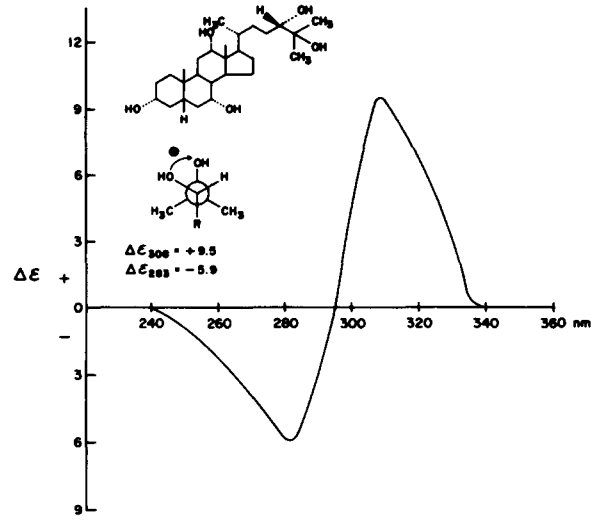

Fig. 3. Circular dichroism of 2×10^{-4} M 5β-cholestane-3α,7α, 12α,24β,25-pentol and 2×10^{-4} M $Eu(fod)_3$ in dry $CHCl_3$ at ambient temperature within 45 min after mixing.

These experiments conclusively defined the chirality of these pentahydroxy bile alcohols having a 1,2-glycol system in the side chain. Using $Eu(fod)_3$ (8) under anhydrous conditions, we obtained the desired CD spectra exhibiting very large induced split Cotton effects.

Recently, Nakanishi and Dillon (10, 11) developed a spectroscopic method for absolute configurational studies of vicinal glycols. The method consists of measuring the CD of a given compound in the presence of $Eu(fod)_3$[tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-octane-4,6-dionato) europium (III)] dissolved in a dry nonpolar solvent. The complexing of Eu with the bile alcohols prepared in this study shows an induced Cotton effect around 310 nm, presumably due to the formation of a bidentate adduct between the glycol and europium complex. The sign of this Cotton effect can be related to the chirality of the two hydroxyl groups.

The results of the CD measurements clearly indicate that the chirality at C-24 of 5β-cholestane-3α,7α,12α, 24α,25-pentol is *R*. The compound showed a prominent negative Cotton effect which was similar to the negative Cotton effect of the model compound, 24(*R*), 25-dihydroxycholesterol (Table 2). Since the chirality of the 24-hydroxyl group of the model compound was conclusively established by Partridge et al. (9) as 24*R*,

TABLE 2. Circular dichroism of 5β-cholestanepentols epimeric at C-24

Compound	Origin of Sample	Molar Ratio Substrate $Eu(fod)_3$	Solvent	CD[a] Δε[b]	CD[a] λ, nm[c]	Chirality
5β-Cholestane-3α,7α, 12α,24α,25-pentol	a) Isolated from CTX patient b) Synthesized in our laboratory	1:1	$CHCl_3$	−13.5 +9.2[d]	309 285	24*R*
5β-Cholestane-3α,7α, 12α,24β,25-pentol	Synthesized in our laboratory	1:1	$CHCl_3$	+9.5 −5.9[d]	308 283	24*S*
Cholest-5-ene-3β, 24*R*,25-triol	Ref. 10	1:1	$CHCl_3$	−11.5	308	24*R*

[a] The sign of the longer wavelength Cotton effect (first Cotton effect) is in agreement with the chirality of the acyclic α-glycol.
[b] The Δε is the coefficient of dichroic absorption and is expressed by D/C l, 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] A second Cotton effect of opposite sign is observed around 290 nm.

the 5β-cholestane-3α,7α,12α,24α,25-pentol must also be assigned the same configuration. In contrast, the 5β-cholestane-3α,7α,12α,24β,25-pentol showed a prominent positive Cotton effect ($\Delta\epsilon_{309} = +9.2$). This indicates that the chirality of this compound at C-24 is *S*.

The importance of these observations relates to the possible role of these bile alcohols as precursors of cholic acid in man and other animals. Patients with the rare lipid storage disease (CTX) excrete substantial amounts of both 5β-cholestane-3α,7α,12α,24α,25-pentol and 5β-cholestane-3α,7α,12α,23α,25-pentol (2). We have presented evidence showing that only 5β-cholestane-3α,7α,12α,24β,25-pentol and not 5β-cholestane-3α,7α,12α,24α,25-pentol or 5β-cholestane-3α,7α,12α,23ξ,25-pentol is converted to cholic acid in man and rats (5). Thus, only the 5β-cholestane-3α,7α,12α,24β,25-pentol(24*S*) is capable of being acted upon by the hepatic enzymes that catalyze side chain cleavage. The 5β-cholestane-3α,7α,12α,24α,25-pentol(24*R*) and the 5β-cholestane-3α,7α,12α,23ξ,25-pentol(23*R*) apparently do not have the structure to interact with the appropriate dehydrogenase or hydroxylase in this pathway.

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