IDENTIFICATION OF (23S)-5β-CHOLESTANE-3α,7α,12α,23,25-PENTOL IN CEREBROTENDINOUS XANTHOMATOSIS[1,2]

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The synthesis of (23R)- and (23S)-5β-cholestane-3α,7α,12α,23,25-pentols is described. Norcholyl aldehyde was converted into the cholestanepentols by a Reformatsky reaction with ethyl bromoacetate followed by a Grignard reaction with methylmagnesium iodide. One of the synthetic pentols, the 23S epimer was identical with a bile alcohol isolated from patients with cerebrotendinous xanthomatosis.

Patients with cerebrotendinous xanthomatosis have an impaired capacity to convert cholesterol to C_{24} bile acids and excrete considerable amounts of C_{27} bile alcohols, two of which were identified positively as 5β-cholestane-3α,7α,12α,25-tetrol and (24R)-5β-cholestane-3α,7α,12α,24,25-pentol by comparison with known reference compounds (3,4). The third compound was a new bile alcohol different from all those hitherto described, and was identified provisionally by infrared spectrometry, nuclear magnetic resonance spectrometry and mass spectrometry as 5β-cholestane-3α,7α,12α,23ξ,25-pentol (4).

In order to confirm the structure of the new bile alcohol, the chemical synthesis of (23R)- and (23S)-5β-cholestane-3α,7α,12α,23,25-pentols was undertaken.

Norcholyl aldehyde (I) was prepared from cholic acid according to the method of Yashima (5), and was converted into the tetrahydropyranyl derivative because of the poor solubility of the hydroxy-aldehyde (I) in benzene and toluene. Treatment of the tetrahydropyranyl derivative with an excess of ethyl bromoacetate and zinc in benzene and toluene followed by acid hydrolysis and alkaline hydrolysis provided a mixture of 23-epimeric 3α,7α,12α,23-tetrahydroxy-26,27-bisnor-5β-cholestan-25-oic acids (II).

Treatment of II with ethereal diazomethane followed by a Grignard reaction with methylmagnesium iodide in benzene afforded a 1:1 mixture of 23-epimeric 5β-cholestane-3α,7α,12α,23,25-pentols (IVa) and (IVb). Separation of these epimers was achieved by silica gel column chromatography.

The infrared spectrum, nuclear magnetic resonance spectrum and mass spectrum (Fig. 1) of the more polar compound (IVb) permitted positive identification of the structure. The less polar compound (IVa) resisted crystallization but its purity was ascertained by thin-layer chromatography and gas-liquid chromatography. The mass spectrum of IVa was indistinguishable from that of IVb, indicating the former was the 23-epimer of the latter.

IVb has the molecular rotation +222, and the increment of +60 for the introduction of the 23-hydroxyl group into

5β-cholestane-3α,7α,12α,25-tetrol was compatible with the molecular rotation difference (+75) found between lanosterol and 23β-hydroxylanosterol (23S) (Table I). Hence, IVb may be assigned the 23β configuration, (23S)-5β-cholestane-3α,7α, 12α,23,25-pentol.

By direct comparison with the specimen synthesized in the present work, the third bile alcohol isolated from the patients with cerebrotendinous xanthomatosis was shown to be (23S)-5β-cholestane-3α,7α,12α,23,25-pentol (IVb). The biosynthetic bile alcohol had the same melting point, optical rotation, chromatographic properties, infrared spectrum, nuclear magnetic resonance spectrum and mass spectrum (Fig. 1) as the reference compound.

EXPERIMENTAL

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-180 polarimeter at room temperature. Infrared spectra were taken with a JASCO IRA-1 spectrometer. Nuclear magnetic resonance spectra were obtained at 100 MHz on a JEOL JNM-PS-100 spectrometer using pyridine-d_5 as solvent. Chemical shifts are given in the δ(ppm) scale with tetramethylsilane as internal standard. Signal multiplicities are represented by s(singlet), d(doublet) and m(multiplet). Molecular weights were determined from the molecular ions using high-resolution mass spectra, which are recorded with a JEOL JMS-01SG mass spectrometer with an accelerating potential of 10 kV, an ionization potential of 70 eV and a source temperature of 130°. Thin-layer chromatography was performed on Kieselgel G nach Stahl (Merck) using 10% phosphomolybdic acid in ethanol (spraying followed by heating) as detection reagent. Column chromatography was carried out with silica gel (Woelm). Gas-liquid chromatography was run on a Shimadzu GC-6A gas chromatograph with a flame ionization detector using glass columns (2 m x 4 mm) packed with 3% OV-17 or 3% QF-1 on Gas-Chrom Q (80-100 mesh) from

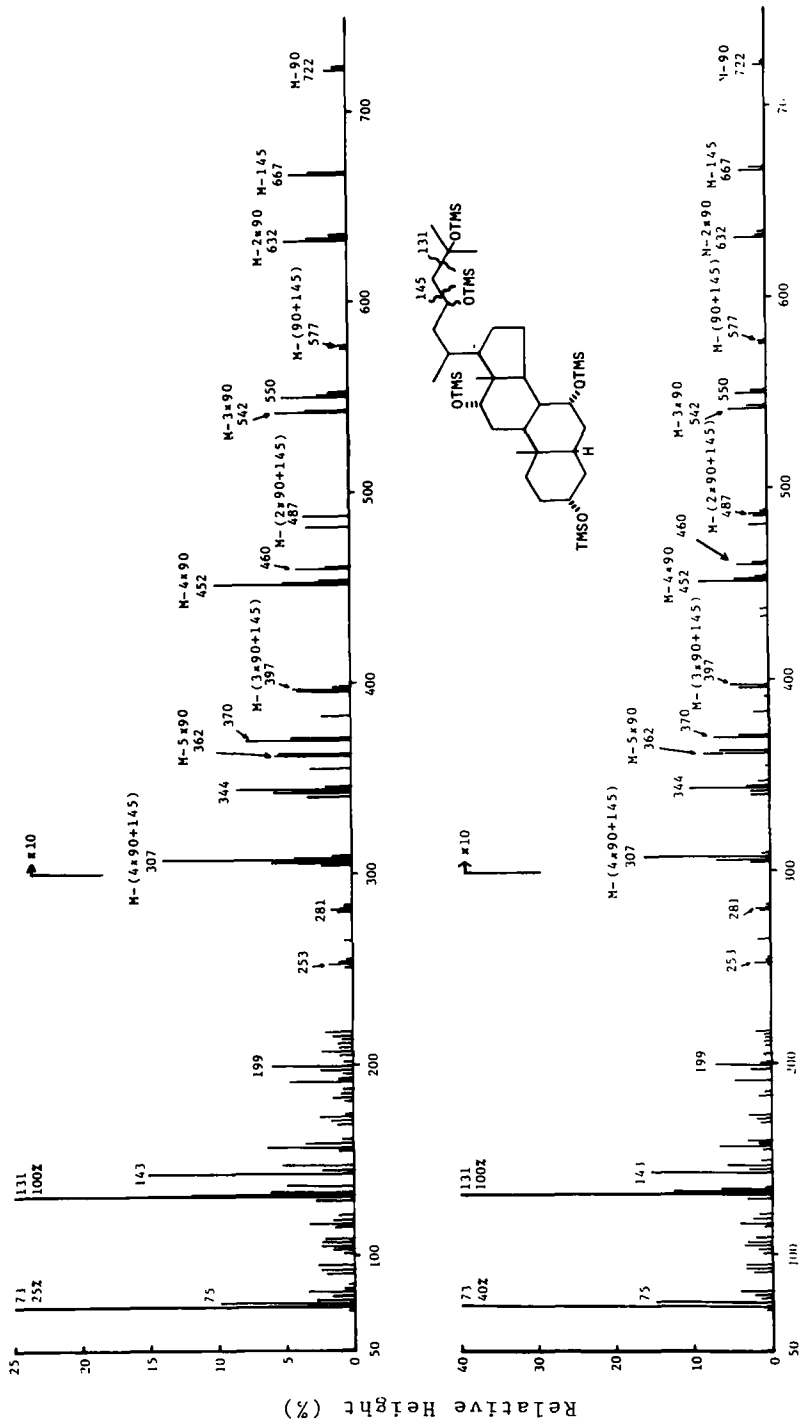
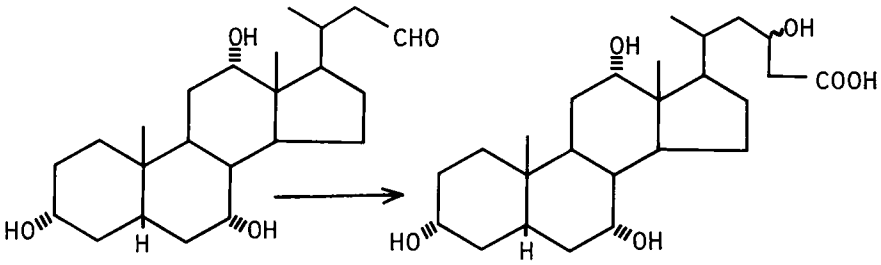
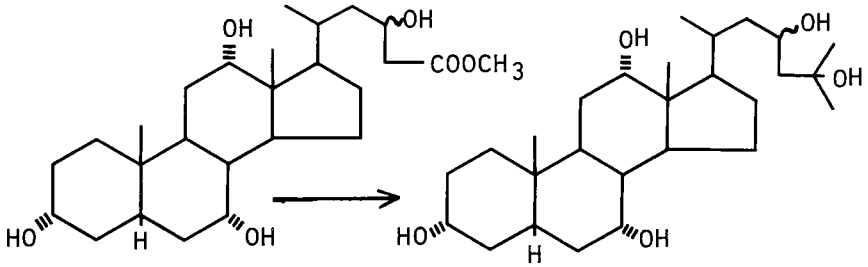


Fig. 1 Mass spectra of the TMS ethers of (23S)-5ß-cholestane-3α,7α,12α,23,25-pentol (lower) and of the biosynthetic bile alcohol (upper). The two spectra are identical in all important respects.

Table I. Molecular Rotation Differences between 5β-Cholestane-3α,7α,12α,25-tetrol, Lanosterol and Their 23-Hydroxylated Derivatives

Steroid	$[\alpha]_D$	M_D	ΔM_D
5β-Cholestane-3α,7α,12α,25-tetrol (4)	+37°	+162	——
IVb	+49°	+222	+60
Lanosterol (6)	+58°	+247	——
23α-Hydroxylanosterol (23R)(6)	+53°	+234	-13
23β-Hydroxylanosterol (23S)(6)	+73°	+322	+75





Applied Science Laboratories. The samples were injected into the gas chromatograph as their trimethylsilyl ethers which were prepared with N-trimethylsilylimidazol at 90° for 1 hr. All retention times are reported relative to trimethylsilyl ether of methyl cholate (relative rt=1.00). Combined gas chromatography-mass spectrometry was performed on a Shimadzu-LKB 9000 instrument. The following operating conditions were employed: column, 1.5% OV-17 (1 m x 3 mm); column temperature, 220°; molecular separator temperature, 260°; helium gas flow, 20 ml/min; ion source temperature, 290°; ion source current, 60 μA; electron energy, 70 eV.

3α,7α,12α,23-Tetrahydroxy-26,27-bisnor-5β-cholestan-25-oic Acid (II)

Norcholyl aldehyde (I) (2.3 g) was dissolved in 10 ml of dihydropyrane containing two drops of conc HCl. The solution was allowed to stand for 12 hrs, and then diluted with ether. The ethereal solution was washed with water until free from HCl and the solvent was evaporated to dryness. The resulting oily residue was added to a mixture of ethyl bromoacetate (12 ml), granulated Zn (20 g), benzene (40 ml), toluene (20 ml), a few crystals of I_2 and a small amount of Cu powder. The reaction mixture was refluxed for 2 hrs. After cooling to 0° in ice, 20 ml of dil H_2SO_4 and 500 g of crushed ice were added to decompose the Reformatsky product. The solution was extracted with ethyl acetate. The extract was washed with water until the washings were neutral, dried over Na_2SO_4 and then evaporated to dryness. The residue was dissolved in 50 ml of 70% acetic acid and refluxed for 1 hr. The solution was diluted with water and extracted with ethyl acetate. The extract was washed with 5% Na_2CO_3 solution to remove acetic acid and the solvent was evaporated to dryness. The resulting residue was refluxed with 50 ml of 15% methanolic potassium hydroxide for 2 hrs. After the methanol was evaporated from the hydrolysate, water was added, and the alkaline solution was filtered in order to remove unreacted norcholyl aldehyde. The filtrate was acidified with dil HCl and the resulting precipitate was extracted with ethyl acetate. Evaporation of the solvent from the washed and dried extract left 1.8 g of II as semi-crystalline material which gave two spots with Rf values 0.20 and 0.29 on tlc (solvent system, benzene-isopropanol-acetic acid, 30:10:1).

(23R)- and (23S)-5β-Cholestane-3α,7α,12α,23,25-pentols (IVa) and (IVb)

A solution of II (1.1 g) in methanol was treated with ethereal diazomethane solution at 0°; was allowed to stand at room temperature for 12 hrs. Evaporation of the solvents and excess diazomethane gave the methyl ester (III) as an oily residue.

The ester (III) was dissolved in 40 ml of dry benzene and the solution was added dropwise to a solution of methylmagnesium iodide (prepared from 7.5 ml of methyl iodide and 2.5 g of Mg) dissolved in 100 ml of ether. The reaction mixture was refluxed for 1 hr, the ether was distilled off, and the mixture was further refluxed for 30 min. After cooling to 0° in ice, 20 ml of dil H_2SO_4 and 500 g of crushed ice were added to decompose the Grignard product. The solution was extracted with a mixture (1:1) of n-butanol and ethyl acetate. The extract was washed with water, dried over Na_2SO_4 and then evaporated to dryness. When examined by tlc (solvent system, chloroform-acetone-methanol, 70:70:15), the resulting residue (1.3 g) consisted chiefly of almost equal amounts of two products (IVa, Rf value 0.50; IVb, Rf value 0.33). These were separated in the following manner.

The residue was mixed with a small amount of silica gel with the aid of methanol and dried, placed on a column of silica gel (Activity IV, 50 g), and chromatographed eluting with ethyl acetate-acetone mixtures. The column fractions were monitored by tlc. Successive elution with a 90:10 mixture gave the chromatographically pure less polar product (IVa) (351 mg) and with a 85:15 mixture produced a mixture (96 mg) of both the products (IVa) and (IVb), and finally with a 80:20 mixture yielded the more polar product (402 mg), which was recrystallized from ethyl acetate to give crystals (46.9 mg) of (23S)-5β-cholestane-3α,7α,12α,23,25-pentol (IVb) with the following properties: mp 209-210°; $[\alpha]_D^{25}$ +49° (methanol, c=1.7); single spot on tlc with Rf value 0.33 (solvent system, chloroform-acetone-methanol, 70:70:15) or 0.35 (solvent system, ethyl acetate-acetone, 7:3); single peak on glc of the TMS derivative with relative rt 1.13 (3% QF-1 column) or 1.41 (3% OV-17 column); Anal. high-resolution mass spectrum, Calcd. for $C_{27}H_{48}O_5$ (M^+, m/e): 452.348. Found: 452.350; nuclear magnetic resonance spectrum (δ): 0.82 (s, 3H, 18-C$\underline{H_3}$), 0.96 (s, 3H, 19-C$\underline{H_3}$), 1.38 (d, 3H, 21-C$\underline{H_3}$), 1.44 and 1.50 (s, 3H x 2, 26-C$\underline{H_3}$ and 27-C$\underline{H_3}$), 3.40-4.60 (m, 4H, C$\underline{H}$-OH); infrared spectrum $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3340 (hydroxyl), 1075, 1020, 990, 920, 894 [cholic acid-type nucleus (7)].

The non-crystalline less polar product (IVa) gave a single spot on tlc with Rf value 0.50 (solvent system, chloroform-acetone-methanol, 70:70:15) or 0.51 (solvent system, ethyl acetate-acetone, 7:3), and a single peak on glc of the TMS derivative with relative rt 1.11 (3% QF-1 column) or 1.38 (3% OV-17 column). Its mass spectrum was identical to that of IVb.

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REFERENCES

1. The following IUPAC names apply to the steroids discussed in this manuscript:
   cholesterol = cholest-5-en-3β-ol;
   cholic acid = 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid;
   norcholyl aldehyde = 3α,7α,12α-trihydroxy-24-nor-5β-cholan-23-al;
   lanosterol = 5α-lanosta-8,24-dien-3β-ol;
   23-hydroxylanosterol = 5α-lanosta-8,24-dien-3β,23-diol.

2. This paper is Part V of a series entitled 'Comparative biochemical studies of bile acids and bile alcohols'. Part IV, Kihira, K., Kuramoto, T. and Hoshita, T. STEROIDS, in press

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