Structural and biosynthetic studies of a principal bile alcohol, 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, in human urine

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Abstract The stereochemistry at C-24 and C-25 of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, a principal bile alcohol in human urine, and its biosynthesis are studied. Four stereoisomers of the C_{26}-24,25-pentols were synthesized by reduction with $LiAlH_4$ of the corresponding epoxides prepared from (24*S*)- or (24*R*)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol. The stereochemistries at C-25 were deduced by comparison of the C_{26}-24,25-pentols with the oxidation products of (24*Z*)-27-nor-5β-cholest-24-ene-3α,7α,12α-triol with osmium tetraoxide. On the basis of this assignment, the principal bile alcohol excreted into human and rat urine was determined to be (24*S*,25*R*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, accompanied by a lesser amount of (24*R*,25*R*)-isomer. To elucidate the biosynthesis of the C_{26}-24,25-pentol, a putative intermediate, 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one, derived from 3α,7α,12α-trihydroxy-24-oxo-5β-cholestanoic acid by decarboxylation during the side-chain oxidation of 3α,7α,12α-trihydroxy-5β-cholestanoic acid, was incubated with rat liver homogenates. The 24-oxo-bile alcohol could be efficiently reduced to yield mainly (24*R*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol. If a 25R-hydroxylation of the latter steroid occurs, it should lead to formation of (24*S*,25*R*)-C_{26}-24,25-pentol. Now it has appeared that a major bile alcohol excreted into human urine is (24*S*,25*R*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, which might be derived from 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one via (24*R*)-27-nor-5β-cholestane-3α, 7α,12α,24-tetrol.—Une, M., S. Takenaka, T. Kuramoto, K. Fujimura, T. Hoshita, and K. Kihira. **Structural and biosynthetic studies of a principal bile alcohol, 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, in human urine. *J. Lipid Res.* 2000.** 41: **1562–1567.**

Supplementary key words bile alcohol • bile acid • absolute configuration • human urine • cholesterol • biosynthesis • hydroxylation

In lower vertebrates bile alcohols are excreted into the bile as end products of cholesterol elimination. Bile alcohols in mammals are classified as biosynthetic intermediates in the formation of bile acid from cholesterol. It has been shown that bile alcohols are excreted in large amounts into the bile and urine of patients with defects of bile acid biosynthesis, such as cerebrotendinous xanthomatosis (CTX) (1) and giant cell hepatitis (2). Studies have also demonstrated that bile alcohols are excreted in substantial amounts in the urine of healthy humans (3, 4) as well as patients with liver disease (5–7), where the major constituent is 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, accounting for 70–80% of the total. While the metabolic origin of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol has been shown to be cholesterol (8), the biosynthetic route is still speculative. At the present time the biosynthetic route of the C_{26}-24,25-pentol is postulated as shown in **Fig. 1** (3, 9). Thus, the biosynthetic route is thought to be identical with that of cholic acid (**VII**), the most common bile acid in mammals, up to the intermediate 3α,7α,12α-trihydroxy-24-oxo-5β-cholestanoyl-CoA (**VI**). In this stage the hydrolysis and decarboxylation of the latter would have occurred, and then the resulting 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one (**IX**) would be reduced to give 27-nor-5β-cholestane-3α,7α,12α,24-tetrol (**X**), and finally converted to 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (**XI**).

Although the 24*S* configuration of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (**XI**) isolated from feces and urine of a patient with CTX has been assigned by means of circular dichroism (10), its stereochemistry in the side chain still remains to be established, because four stereoisomers at C-24 and C-25 of the pentol (**XI**) are present.

Therefore, to investigate the stereochemistry of the side chain of naturally occurring 27-nor-5β-cholestane-3α,7α,

Abbreviations: CMR, ^{13}C nuclear magnetic resonance; CTX, cerebrotendinous xanthomatosis; GLC, gas-liquid chromatography; mp, melting point; MS, mass spectrometry; PMR, proton nuclear magnetic resonance; TLC, thin-layer chromatography; TMS, trimethylsilyl.

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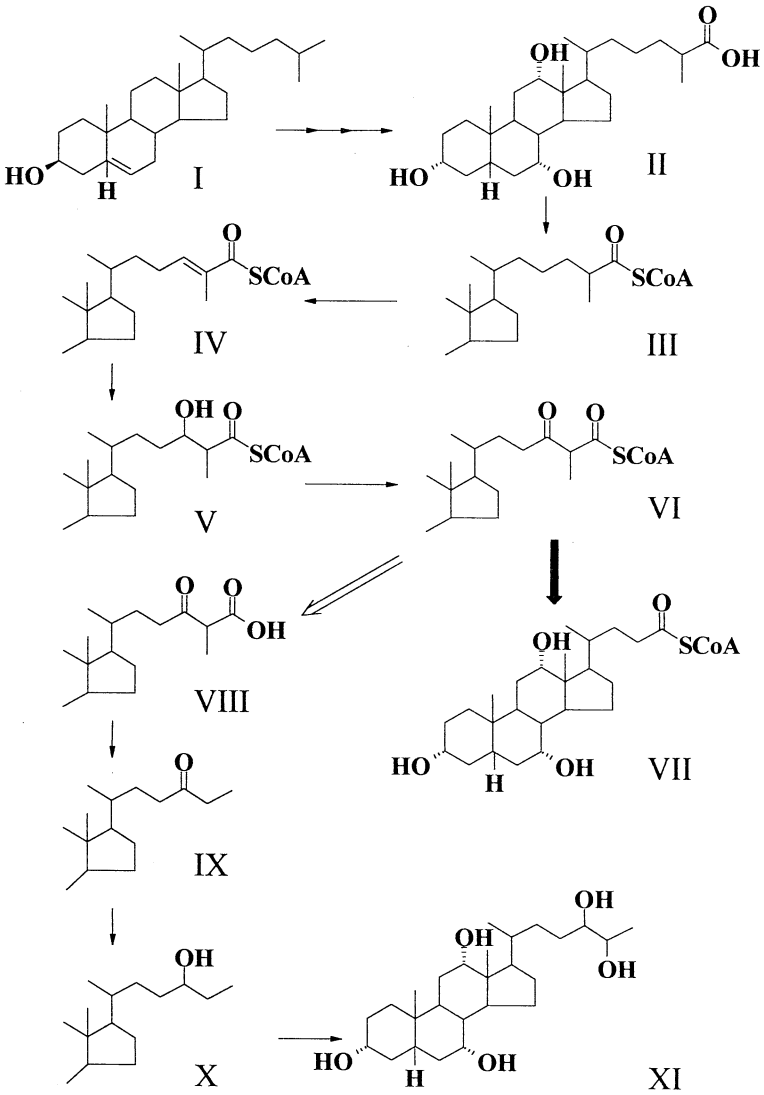

Fig. 1. Possible pathway for the formation of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol from cholesterol. **I**, Cholesterol; **II**, 3α,7α,12α-trihydroxy-5β-cholestanoic acid; **III**, 3α,7α,12α-trihydroxy-5β-cholestanoyl-CoA; **IV**, 3α,7α,12α-trihydroxy-5β-cholest-24-enoyl-CoA; **V**, 3α,7α,12α,24-tetrahydroxy-5β-cholestanoyl-CoA; **VI**, 3α, 7α,12α-trihydroxy-24-oxo-5β-cholestanoyl-CoA; **VII**, cholanoyl-CoA; **VIII**, 3α,7α,12α-trihydroxy-24-oxo-5β-cholestanoic acid; **IX**, 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one; **X**, 27-nor-5β-cholestane-3α,7α,12α,24-tetrol; **XI**, 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol.

12α,24,25-pentol, we prepared reference standards of known absolute configuration. In addition, we examined its biosynthetic sequence, using rat liver homogenates.

MATERIALS AND METHODS

General

Melting points (mp) were determined on a Kofler hot-stage apparatus, and are uncorrected.

Proton nuclear magnetic resonance (PMR) and ^{13}C nuclear magnetic resonance (CMR) spectra were measured on a JEOL GX-400 spectrometer in solutions of deuterated methanol with tetramethylsilane as an internal standard.

Thin-layer chromatography (TLC) was carried out on precoated silica gel G plates (0.25-mm thickness; Merck, Rahway, NJ). The solvent systems used were ethyl acetate–acetic acid–water 7:2:1 (solvent system A) and chloroform–ethanol 8:2 (solvent system B). The spots were detected by spraying phosphomolybdic acid (10% in ethanol) and heated at 110°C for 5 min.

Gas-liquid chromatography (GLC) was carried out on a Hewlett-Packard (Palo Alto, CA) 4890 gas chromatograph equipped with a flame ionization detector. The column used was a capillary column (0.25 mm × 30 m) coated with HP-1. Bile alcohol samples were analyzed as trimethylsilyl (TMS) ether derivatives under the following conditions: a column oven temperature of 240–290°C at a rate of 2°C/min. 27-Nor-5β-cholestane-3α,7α,12α,24,25-pentols (**XI**) were analyzed after conversion to the 24,25-acetonide (11) followed by TMS derivatization. The column oven temperature was 200–290°C at a rate of 0.7°C/min.

Gas-liquid chromatography/mass spectrometry (GC/MS) was carried out on a Hewlett-Packard 5890 gas chromatograph and a JEOL JMS-SX 102 mass spectrometer under the following conditions: a capillary column (0.25 mm × 30 m) coated with HP-1, a column oven temperature of 220–290°C at a rate of 2°C/min, an injection port temperature of 280°C, an ion source temperature of 250°C, a flow rate of 2.0 ml of helium carrier gas per minute, an ionizing energy of 70 eV, and an ionizing current of 300 mA.

The "usual workup" refers to dilution with a large amount of water, extraction with organic solvent, washing to neutrality with water, drying over anhydrous Na_2SO_4, filtration, and evaporation of the solvent under reduced pressure.

Reference compounds

Cholic acid was a commercial product. 3α,7α,12α-Trihydroxy-5β-cholestanoic acid (12) and 3α,7α,12α-trihydroxy-5β-cholest-24-enoic acid (13), (24*R*)- and (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrols (14), (24*R*)- and (24*S*)-27-nor-5β-cholest-25-ene-

3α,7α,12α,24-tetrols (14), and 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one (15) were synthesized as reported previously.

Labeled compounds

3α,7α,12α-Trihydroxy-27-nor-5β-[24-^{14}C]cholestan-24-one (1.6×10^7 dpm/μg) and (24*RS*)-27-nor-5β-[24-^{3}H]cholestane-3α,7α,12α,24-tetrol (7.8×10^7 dpm/μg) were kept as stocks in our laboratory.

Synthesis of four stereoisomers of (24*R*)- and (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentols

(See structures **XIa–d** in **Fig. 2**.) Two epimers at C-24 of 27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol (**XIIa** and **XIIb**) were prepared from cholic acid according to the method reported previously (14).

To a solution of (24*S*)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol (**XIIa**, mp 175°C) (50 mg) dissolved in CH_2Cl_2 (10 ml) was added *m*-chloroperbenzoic acid (50 mg), and the solution was stirred at room temperature for 7 h. The reaction mixture was poured into water (100 ml) and extracted with ethyl acetate (100 ml × 2). The ethyl acetate layer was washed with 10% Na_2SO_3 (100 ml), 5% $NaHCO_3$(100 ml), and water (100 ml × 3), and evaporated to dryness. The residue (61 mg) containing (24*S*)-25,26-epoxy-27-nor-5β-cholestane-3α,7α,12α,24-tetrol was dissolved in dry ethyl ether (100 ml). $LiAlH_4$ (500 mg) was added to the solution in small portions over a 30-min period. The resulting suspension was heated for 2 h under reflux, and after acidification with ice-cold dilute HCl the usual workup (ethyl acetate) gave a white powder (51 mg). The residue was chromatographed on a reversed-phase partition column (Lober RP-8, 2.5 cm × 31 cm; Merck), using 80% methanol solution as the moving phase to give a mixture (27 mg) of (24*S*,25*S*)- and (24*S*,25*R*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentols (**XIa** and **XIb**). Although GLC analysis of TMS derivatives of the mixture revealed two peaks at 24.84 and 25.62 min, only one spot was detected on TLC (R_f value 0.30, solvent system A). Both mass spectra as TMS derivatives showed fragment ions at m/z 253 (M-side chain-90 × 3), 343 (M-90 side chain-90 × 2), 321 (M-117-90 × 4, base peak), 411 (M-117-90 × 3), 501 (M-117-90 × 2), 591 (M-117-90), 681(M-117), 798 (M^+). Mass spectra of acetonide-TMS derivatives of two isomers also gave identical fragment ions at 253 (M-side chain-90 × 3, base peak), 343 (M-90-side chain-90 × 2), 424 (M-90 × 3), 514 (M-90 × 2), 604 (M-90), 694 (M^+).

By the same procedure (24*R*)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol (**XIIb**, mp 188°C) (50 mg) gave a mixture (15 mg) of (24*R*,25*R*)- and (24*R*,25*S*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentols (**XIc** and **XId**); R_f value on TLC: 0.30 (solvent system A). The TMS derivatives of the mixture also gave two peaks at 25.04 and 25.62 min on GLC (HP-1). Their mass spectra also showed the same fragment ions observed in those of (24*S*)-isomers.

Synthesis of (24S,25R)- and (24R,25S)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentols

(See structures **XIb** and **XId** in Fig. 2.) 3α,7α,12α-Triacetoxy-5β-cholan-24-al (**XIII**, 5.0 g) and (ethylidene)-triphenylphosphorane (mp 211–212°C) were dissolved in dry benzene and the solution was refluxed for 3 h in an atmosphere of nitrogen. The usual workup (ethyl ether) gave an oily residue. The resulting residue was then hydrolyzed in 5% methanolic KOH solution. After dilution with dilute HCl, the usual workup (ethyl acetate) gave a syrup, which was crystallized from ethyl acetate. Recrystallization from methanol-water gave crystals of (24*Z*)-27-nor-5β-cholest-24-ene-3α,7α,12α-triol (**XIV**), mp 180–180.5°C, PMR (δ ppm): 0.71 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 1.03 (3H, d, J = 6.6 Hz, 21-CH_3), 1.59 (3H, d, J = 5.9 Hz, 26-CH_3), 3.37 (1H, m, 3β-H), 3.80 (1H, m, 7β-H), 3.96 (1H, m, 12β-H), 5.34–5.42 (2H, m, 24 and 25-H).

A solution of **XIV** (50 mg) in pyridine (10 ml) and acetic anhydride (10 ml) was heated at 100°C for 24 h in a steam bath. The usual workup (ethyl ether) gave a residue (61 mg) containing the triacetate of **XIV.** To the solution of the triacetate of **XIV** in anhydrous ethyl ether (20 ml) and pyridine (1.5 ml), a solution of OsO_4 (150 mg) in anhydrous ethyl ether was added. The

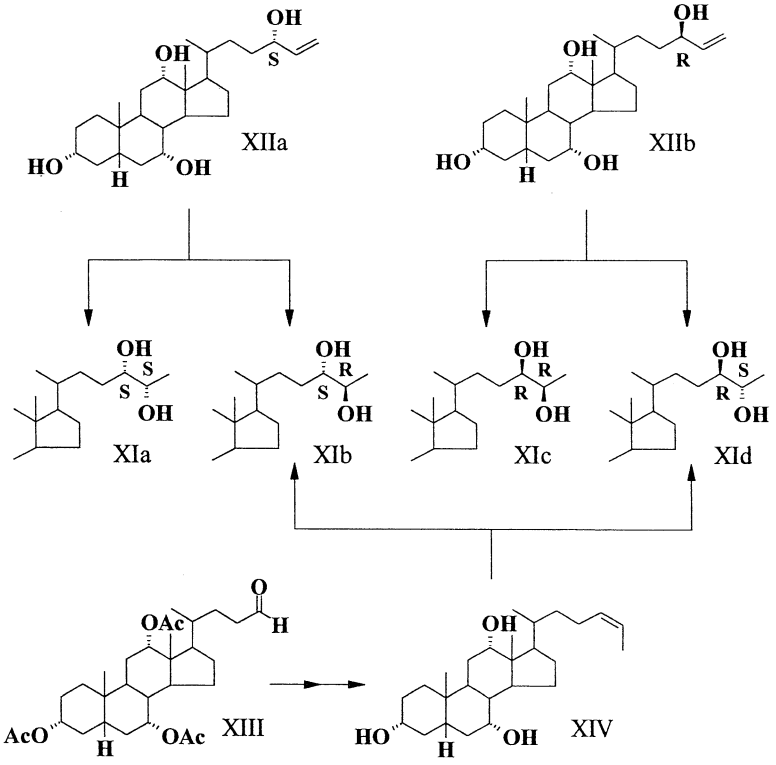

Fig. 2. Synthesis of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol. **XIa**, (24*S*,25*S*)-27-Nor-5β-cholestane-3α,7α,12α,24,25-pentol; **XIb**, (24*S*,25*R*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentol; **XIc**, (24*R*,25*R*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentol; **XId**, (24*R*,25*S*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentol; **XIIa**, (24*S*)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol; **XIIb**, (24*R*)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol; **XIII**, 3α,7α,12α-triacetoxy-5β-cholan-24-al; **XIV**, (24*Z*)-27-nor-5β-cholest-24-ene-3α,7α,12α-triol.

reaction mixture was kept for 24 h in a dark place at room temperature. After removing the organic solvent under reduced pressure, a 50% ethanol solution (20 ml) containing Na_2SO_3 (1.5 g) was added to the residue and the solution was refluxed for 3 h. The reaction mixture was diluted with a large amount of ethanol and filtered. The filtrate was evaporated to dryness and the residue was refluxed in 2 N ethanolic KOH solution (30 ml) for 2 h in a steam bath. The residue obtained after the usual workup (ethyl acetate) was subjected to reversed-phase partition column chromatography as described above. The effluents containing the pentols were evaporated together to dryness to give a mixture (15.4 mg) of (24*S*,25*R*)- and (24*R*,25*S*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentols (**XIb** and **XId**) as a white powder; R_f value on TLC: 0.30 (solvent system A). The TMS derivatives of the mixture gave one peak at 25.62 min. However, the separation could be achieved on GLC as the acetonide-TMS derivatives (83.17 and 83.59 min).

Preparation of rat liver subcellular fractions

Male Wistar rats (180–200 g) were used. Each liver was minced and put into 5 volumes of 0.25 M sucrose, 1 mM EDTA, 0.1% (v/v) ethanol, and 10 mM HEPES-NaOH buffer (pH 7.4) and homogenized by one stroke in a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 800 *g* for 12 min. The pellet was rehomogenized and the suspension was recentrifuged under the same conditions. The combined supernatants were centrifuged at 2,500 *g* for 13 min (heavy mitochondrial fraction). The 2,500 *g* supernatant was centrifuged at 20,000 *g* for 22 min. The resulting pellet (light mitochondrial fraction) was washed once and resuspended in the homogenizing medium. The light mitochondrial fraction was layered on top of a sucrose gradient (d = 1.15–1.25), centrifuged at 74,700 *g* for 3 h, and fractionated into 1.5-ml volumes, taken sequentially from the bottom. The fractions with the highest amount of peroxisomal enzyme marker were pooled and diluted 1:8 with 0.25 M sucrose-10 mM HEPES (pH 7.4). The peroxisomes were subsequently sedimented at 20,000 *g* for 30 min. The pellet was resuspended in 0.2 ml of 0.25 M sucrose-10 mM HEPES (pH 7.4) and used for the enzyme assay. In another experiment the 20,000 *g* supernatant was centrifuged at 100,000 *g* to obtain microsomal pellets.

Incubation, extraction, and analytical procedures

Metabolism of 3α,7α,12α-trihydroxy-5β-cholestanoic acid in rat liver peroxisomes. The incubation mixture contained the following in 1 ml of 0.1 M Tris-HCl buffer (pH 8.0): 7.5 μmol of ATP, 7.5 nmol of FAD, 1.5 μmol of NAD, 0.1 mmol of CoASH, 10 μmol of $MgCl_2$, and 1.0 mg of protein. After preincubation at 37°C for 10 min, the reaction was started by the addition of 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA, 100 mg), and the incubation was continued for 120 min. The reaction mixture was directly subjected to a Sep-Pak C_{18} cartridge (Waters, Milford, MA) and the reaction products were eluted by methanol after washing with water. An aliquot of the extract was converted to methyl ester-trimethylsilyl ether derivatives, and analyzed by GLC and GLC/MS.

Metabolism of 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one in rat liver subcellular fractions

The incubation mixture contained the following in 2.0 ml of potassium phosphate buffer: 1.75 μM $MgCl_2$, 3 μM NADPH, and 1.0 mg of heavy mitochondrial, microsomal, or cytosolic protein. After preincubation at 37°C for 15 min, the reaction was initiated by adding 50 μg of 3α,7α,12α-trihydroxy-27-nor-5β-[24-^{14}C]cholestan-24-one, and the incubation was continued for 2 h. The reaction products were extracted in the same manner described above. An aliquot of the extracts was analyzed by TLC using solvent system B. Samples were applied as bands, and reference compounds were spotted simultaneously. After development of the plates, the bands and spots were made visible by spraying the reagent. To locate the radioactivity on the plate, each TLC plate was cut into 1-cm sections from origin to solvent front, and each section was then placed in a scintillation vial and the radioactivity was determined with a liquid scintillation counter (LSC-3500; Aloka, Tokyo, Japan) in a xylene-based scintillator.

Metabolism of (24*RS*)-27-nor-5β-cholestanane-3α,7α,12α,24-tetrol in rat liver subcellular fractions

The incubation was carried out under the same conditions, except for using (24*RS*)-27-nor-5β-[24-^{3}H]cholestane-3α,7α,12α,24-tetrol as a substrate.

RESULTS AND DISCUSSION

(24*S*)- and (24*R*)-5β-Cholest-25-ene-3α,7α,12α,24-tetrols (**XIIa** and **XIIb**) were synthesized from cholic acid and purified according to the previous report (14). A mixture of two epimers at C-25 of (24*S*)-5β-cholestane-3α,7α,12α,24,25-pentols (**XIa** and **XIb**) was obtained from the (24*S*)-tetrol (**XIIa**) by epoxidation followed by reduction with $LiAlH_4$. The acetonide-TMS derivatives of the two epimers showed two peaks (peaks 1 and 2; **Fig. 3**) at 78.2 and 83.2 min on GLC. By the same procedure, the acetonide-TMS derivatives of the products prepared from (24*R*)-5β-cholest-25-ene-3α,7α,12α,24-tetrol (**XIIb**) gave two peaks (peaks 3 and 4) at 78.6 and 83.6 min on GLC. While these four epimers could be partially separated even as TMS derivatives on GLC, two epimers (compounds corresponding to peaks 2 and 4 in Fig. 3) could not be resolved completely.

When the Wittig reagent prepared from ethyl bromide was reacted with 3α,7α,12α-triacetoxy-5β-cholan-24-al (**XIII**), two isomeric 27-nor-5β-cholest-24-en-3α,7α,12α-triols (**XIV**) were obtained in a ratio of about 8:2 as judged by GLC (16.4 and 15.9 min, respectively). Recrystallization from methanol-water gave the major product in pure form. In the CMR spectrum, the carbon signal at C-26 appeared at 12.8 ppm. The spectrum of the crude filtrate showed two signals corresponding to C-26 methyl carbon at 12.8 and 18.1 ppm. From these results the geometry of the major product could be assigned as the 24*Z* form because signals from a methyl carbon neighboring a *cis* double bond appear slightly upfield compared with those in a *trans* double bond (16), as shown in the cases of 2-hexene (*trans*, 17.51 ppm; *cis*, 12.29 ppm) and 2-pentene (*trans*, 17.34 ppm; *cis*, 12.01 ppm). Oxidation of **XIV** with osmium tetraoxide gave two epimeric pentols. From the known mechanism (preparing *cis*-glycol) these could be determined as (24*S*,25*R*)- and (24*R*,25*S*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentols, of which the acetonide-TMS derivatives were identical with peaks 3 and 4 as regards chromatographic behavior on GLC, respectively. It has now been established that absolute configurations of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentols giving rise to peaks 1, 2, 3, and 4 are (24*S*,25*S*), (24*S*, 25*R*), (24*R*,25*R*), and (24*R*,25*S*), respectively.

To elucidate the stereochemistry of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol on the basis of the present results, bile alcohols were extracted from urine of healthy humans

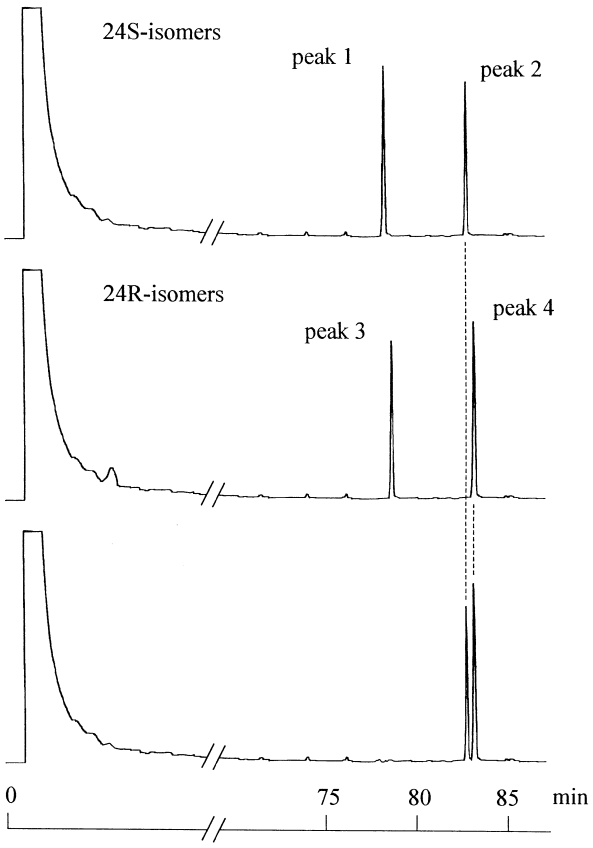

Fig. 3. Gas-liquid chromatographic separation of four stereoisomers of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentols as acetonide-TMS derivatives. Peaks 1, 2, 3, and 4 correspond to (24*S*,25*S*)-, (24*S*,25*R*)-, (24*R*,25*R*)-, and (24*R*,25*S*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, respectively.

and the C_{26}-24,25-pentol fraction was isolated on a Lober column (RP-8) after treatments with sulfatase and glucuronidase (17). GLC analysis after acetonide-TMS derivatization revealed the presence of (24*S*,25*R*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (90%) along with (24*R*,25*R*)-isomer (10%) as a minor constituent. By the same procedure it appeared that urinary bile alcohols in rats also consisted mainly of (24*S*,25*R*)-27-nor-5β-cholestane-3α,7α,12α,24,25-pentol. The stereochemistry of the naturally occurring C_{26}-24,25-pentol had previously been elucidated by Dayal et al. (10), who identified the pentol isolated from feces and urine of a patient with cerebrotendinous xanthomatosis as the 24*S* form by means of circular dichroism, while the configuration at C-25 was not assigned. The present result is consistent with their assignment for the chirality at C-24. It has also been reported that two isomers of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol existed in urine from patients with primary biliary cirrhosis and healthy volunteers (7). These isomers were described as second and third isomers on the basis of their retention times, and were present in a ratio of 1:10. These findings were also identical with our results. Further, 5β-cholestane-3α,7α,12α,24,25,26-hexol, probably a 26-hydroxylation product of 5β-cholestane-3α,7α,12α,24,25-pentol, was found in human urine, and its chirality at C-24 was also shown to be 24*S* (18), strongly suggesting the predominant formation of the (24*S*)-isomer in humans. However, it was opposite to the absolute configuration at C-24 of 5β-ranol (27-nor-5β-cholestane-3α,7α,12α,24,26-pentol), another naturally occurring C_{26}-bile alcohol, which is a principal bile alcohol of bullfrogs and has been demonstrated to be 24*R* (14).

Although 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, unlike most naturally occurring bile alcohols, possesses an unusual side chain, it has been confirmed to be formed from a common precursor, cholesterol (8). This C_{26} bile alcohol is considered to be derived from an intermediate in the biosynthetic pathway from cholesterol to cholic acid by the loss of one terminal carbon atom. We previously proposed the route involving decarboxylation of the C_{27}-oxo-bile acid, 3α,7α,12α-trihydroxy-24-oxo-5β-cholestanoic acid (**VIII**), to the C_{26}-oxo-bile alcohol (**IX**) for the biosynthetic pathway of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (**XI**) as shown in Fig. 1. To verify this assumed pathway, the metabolism of putative intermediates was estimated by using rat liver homogenates. First, 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA, **II**) was incubated with rat liver peroxisomes in the presence of ATP and CoA. Incubation products were extracted through a Sep-Pak C_{18} cartridge, and analyzed by GLC after methyl ester-TMS derivatization.

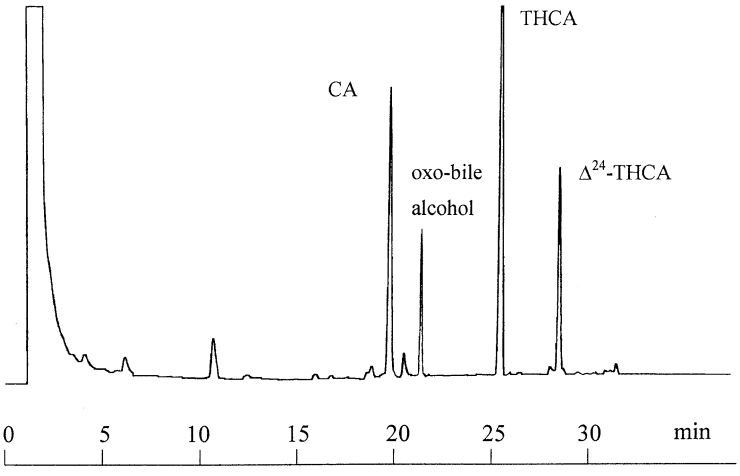

Fig. 4. Gas-liquid chromatogram of the incubation products of 3α,7α,12α-trihydroxy-5β-cholestanoic acid with rat liver peroxisomes. CA, Cholic acid; Oxo-bile alcohol, 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one; THCA, 3α,7α,12α-trihydroxy-5β-cholestanoic acid; Δ^{24}-THCA, 3α,7α,12α-trihydroxy-5β-cholest-24-enoic acid.

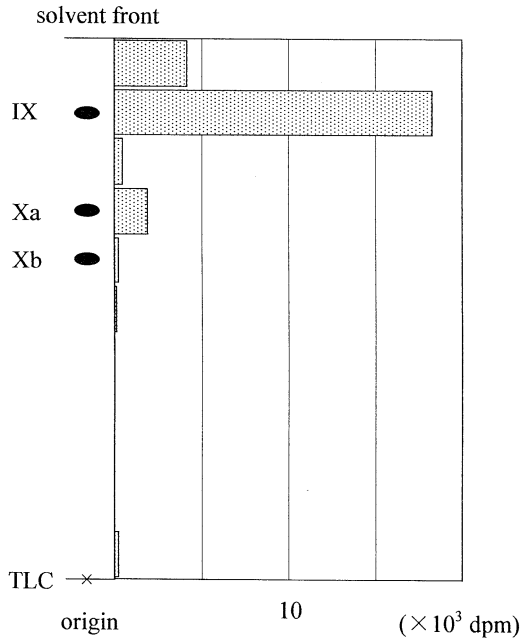

Fig. 5. Radio-TLC of labeled compounds after incubation of 3α,7α,12α-trihydroxy-27-nor-5β-[24-^{14}C]cholestan-24-one in rat liver 100,000 *g* supernatants. **IX**, 3α,7α,12α-Trihydroxy-27-nor-5α-cholestan-24-one; **Xa**, (24*R*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol; **Xb**, (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol. TLC was carried out with solvent system B.

In addition to 3α,7α,12α-trihydroxy-5β-cholest-24-enoic acid and cholic acid, the formation of 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one (**IX**) could be detected (**Fig. 4**). Although this compound was also identified previously in the incubation of THCA with rat liver homogenates, it was considered to be an artifact from 3α,7α,12α-trihydroxy-24-oxo-5β-cholestanoyl-CoA (**VI**) formed in the incubation mixture during alkaline hydrolysis and acidification for extraction with organic solvent (19). However, in this study we extracted the incubation products immediately, using a Sep-Pak C_{18} cartridge without any treatment, indicating that the decarboxylation occurred exclusively during the incubation. Second, we elucidated the metabolism of 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one (**IX**) in rat liver homogenates. When 24-^{14}C-labeled **IX** was incubated with 100,000 *g* supernatant fraction in the presence of NADPH, radio-TLC analysis of the incubation products revealed that a substantial amount of radioactivity existed in the band associated with (24*R*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (**X**), accompanied by a negligible amount of (24*S*)-epimer (**Fig. 5**). In other subcellular fractions no reduction activity could be detected under the condition. Finally, the metabolism of ^{3}H-labeled (24*RS*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (**X**) was estimated in rat liver homogenates. Although radio-TLC of the incubation products with the mitochondrial fraction, but not other subcellular fractions, revealed that only a small amount of radioactivity was found in more polar metabolites, further identification could not be achieved (data not shown). If a hydroxylation at C-25 of (24*R*)-27-nor-5β-cholestane-3α,7α,12α, 24-tetrol, however, were to occur, the absolute configuration at C-24 of the pentol (**XI**) would be called as *S* form. With the exception of a 25-hydroxylation of (24*R*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (**X**), we have demonstrated all the individual steps in the suggested mechanism for formation of the pentol (**XI**).

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