Identification of new bile alcohols, 5β-cholestane-3α,7α,24,26-tetrol, 5β-cholestane-3α,7α,25,26-tetrol, and 5β-cholestane-3α,7α,26,27-tetrol in human gallbladder bile

M. Une,* Y. Shinonaga,* N. Matoba, S. Kuroki,** K. Kihira,* and T. Hoshita[1,]***

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,* Kasumi 1-2-3, Minami-ku, Hiroshima, 734, Japan, and The First Department of Surgery, Kyushu University Faculty of Medicine,** Maidashi 3-1-1, Higashi-ku, Fukuoka, 812, Japan

Abstract The nature of cholestanetetrols present as the glucurono-conjugates in human gallbladder bile was studied. Glucurono-conjugated bile alcohols were isolated by ion exchange chromatography and, after enzymatic hydrolysis, were fractionated by reversed phase partition chromatography to give a fraction containing tetrahydroxy bile alcohols which was analyzed by gas–liquid chromatography and mass spectrometry. Along with the three previously identified bile alcohols, 5α- and 5β-cholestane-3α,7α,12α,24-tetrols, and 5β-cholestane-3α,7α,12α,26-tetrol, three new cholestanetetrols, possessing two hydroxyl groups in the ring system and two in the side chain, were detected in the tetrahydroxy bile alcohol fraction. These new bile alcohols were identified as 5β-cholestane-3α,7α,24,26-tetrol, 5β-cholestane-3α,7α,25,26-tetrol, and 5β-cholestane-3α,7α,26,27-tetrol by direct comparison of their gas–liquid chromatographic behaviors and mass spectral data with those of authentic standards prepared from chenodeoxycholic acid by partial synthesis.—**Une, M., Y. Shinonaga, N. Matoba, S. Kuroki, K. Kihira, and T. Hoshita.** Identification of new bile alcohols, 5β-cholestane-3α,7α,24,26-tetrol, 5β-cholestane-3α,7α,25,26-tetrol, and 5β-cholestane-3α,7α,26,27-tetrol in human gallbladder bile. *J. Lipid Res.* 1986. **27:** 1318–1323.

Supplementary key words chenodeoxycholic acid • gas–liquid chromatography–mass spectrometry

In 1974, Setoguchi et al. (1) found that patients with cerebrotendinous xanthomatosis excreted considerable amounts of bile alcohols in bile and feces, which had long been thought to occur only in primitive vertebrates such as fishes and amphibians. Since then, several investigations (2–9) have revealed the occurrence of bile alcohols in biological fluids from healthy and from diseased humans. Recently, we have found (10) that a large number of bile alcohols are present, although in trace amounts, in human gallbladder bile. Most of them have a ring system related to cholic acid, i.e., carrying three hydroxyl groups in positions at 3α, 7α, and 12α, and have been characterized with certainty by direct comparison with reference standards. However, a few of them are cholestanetetrols having two hydroxyl groups in the ring system and two in the side chain, and have not been identified conclusively because reference compounds were not available.

As an extention of our previous study, the present investigation was performed in order to confirm the structure of these cholestanetetrols. This was achieved by the re-examination of human biliary bile alcohols, and by the chemical synthesis of reference bile alcohols.

MATERIALS AND METHODS

General

Melting points (mp) were determined with a Kofler hot-stage apparatus and are uncorrected. Infrared (IR) spectra were taken on a JASCO IRA-1 spectrometer as chloroform solutions. Proton magnetic resonance (PMR) spectra were measured at 90 MHz on a Hitachi R-40 spectrometer. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane internal standard.

Gas–liquid chromatography (GLC) was carried out on a Shimadzu GC-6A gas chromatograph using a glass column (2 m × 3 mm i.d.) packed with 2% OV-1 or 3% OV-17 on 80–100 mesh gas chrome Q or a FS-WCOT capillary column (25 m × 0.25 mm i.d.) chemically bonded with OV-1 (Gasukuro Kogyo Inc., Japan). All retention times are given relative to the TMS ether of methyl cholate.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry; TMS, trimethylsilyl; TBDMS, *tert*-butyldimethylsilyl; PMR, proton magnetic resonance; ODS, octadecylsilyl; RRT, relative retention time.

[1] To whom reprint requests should be addressed.

Gas–liquid chromatography–mass spectrometry (GLC–MS) was carried out on a Shimadzu GCMS-1000 gas chromatograph–mass spectrometer equipped with a data-processing system (Shimadzu GCMSPAC-90) and Van den Berg's solventless injector. The following conditions were employed: column OV-1 (12 m × 0.25 mm i.d.); injection port temperature, 270°C; column oven temperature, 240–270°C, 2°C/min; separator temperature, 270°C; ionization source temperature, 290°C; flow rate of helium carrier gas, 1.5 ml/min; ionization energy, 70 eV; trap current, 60 μA.

Column chromatography using an octadecylsilyl (ODS) column (10 cm × 22 mm i.d., Kusano Scientific Co., Japan) was carried out for the group separation of bile alcohols. Bile alcohols were dissolved in methanol (0.5 ml) and transferred onto an ODA column. The column was eluted successively with 120 ml of 80% aqueous methanol, 140 ml of 85% aqueous methanol, and 200 ml of 90% aqueous methanol to give fractions containing penta-, tetra-, and trihydroxy bile alcohols, respectively.

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.

Analysis of bile alcohols in bile

The gallbladder bile was obtained from a patient (60-year-old Japanese woman) with gallstones by needle aspiration during an abdominal operation. The clinical data indicated that the liver functions of the patient were not severely affected.

The bile sample (10 ml) was extracted with 100 ml of ethanol at room temperature. The ethanolic extract was concentrated to dryness under reduced pressure. The resulting residue (1.1 g) was dissolved in 10 ml of 90% ethanol and applied to a column (15 cm × 3 cm i.d.) of piperidinohydroxypropyl Sephadex LH-20 (PHP-LH-20). The column was washed with additional 300 ml of 90% ethanol, and then eluted with 800 ml of 0.2 M formic acid in 90% ethanol.

The second fraction, which contained glucurono-conjugated bile alcohols along with glycine-conjugated bile acids, was evaporated in vacuo. The residue was dissolved in 40 ml of 0.1 M phosphate buffer, pH 6.8, containing 10,000 units of β-glucuronidase (EC 3.2.1.31) (Shigma Chemical Co., Type IX) and two drops of chloroform, and the mixture was incubated at 37°C for 48 hr. The incubation mixture was lyophilized and extracted with 100 ml of methanol. The methanolic extract was evaporated to dryness and the residue was dissolved in 5 ml of 90% ethanol and the solution was applied on a column (10 cm × 1.6 cm i.d.) of PHP-LH-20. The column was eluted with 100 ml of 90% ethanol to give deconjugated bile alcohols. Further fractionation of the deconjugated bile alcohols was carried out using an ODS column. The tetrahydroxy bile alcohol fraction obtained was taken to dryness in vacuo and the residue was treated with pyridine–hexamethyldisilazane–trimethylchlorosilane 5:2:1 for 2 hr at room temperature. The resulting trimethylsilyl (TMS) ether derivatives were analyzed by GLC and GLC–MS.

Reference compounds

5α-Cholestane-3α,7α,12α,24-tetrol (11), 5β-cholestane-3α,7α,12α,24-tetrol (12), 5β-cholestane-3α,7α,-12α,26-tetrol (13), 5α-cholestane-3α,7α,26,27-tetrol-(12-deoxy-5α-cyprinol) (14), and 5β-cholestane -3α,7a,-12α,24,26-pentol (5β-chimaerol) (15) were prepared according to methods described previously. 5β-Cholestane-3α,7α,25,26-tetrol (16) was kindly supplied by Dr. E. H. Mosbach.

Synthesis of 5β-cholestane-3α,7α,24,26-tetrol (VI) (Fig. 1)

3α,7α-Diacetoxy-5β-cholan-24-ol (II). Chenodeoxycholic acid diacetate (I) was prepared from chenodeoxycholic acid in the usual manner. Triethylamine (8.3 ml) and ethyl chlorocarbonate (5.8 ml) were added to a solution of the chenodeoxycholic acid diacetate (I) (20 g) in freshly distilled tetrahydrofuran (280 ml) with stirring in an ice bath. The mixture was stirred for 1 hr, then a suspension of $NaBH_4$ (10 g) in water was added to the solution and the whole was kept at 0°C for 2 hr with stirring. The usual workup (ethyl acetate) gave a residue (19.7 g). Recrystallization of the residue from methanol gave crystals (15.3 g) of II: mp 159°C; IR (cm^{-1}), 3400, 1720; PMR (δ ppm), 0.61 (3H, s, 18–CH_3), 0.84 (3H, s, 19–CH_3), 0.91 (3H, d, J = 6 Hz, 21–CH_3), 2.00 (6H, s, 3α– and 7α–$OCOCH_3$), 3.77 (2H, t, J = 6 Hz, 24–CH_2OH), 4.71 (1H, m, 3β–H), 4.94 (1H, m, 7β–H).

3α,7α-Diacetoxy-5β-cholan-24-al (III). To a solution of II (7 g) in freshly distilled dichloromethane (100 ml), pyridium chlorochromate (5 g) and sodium acetate (0.4 g) were added with stirring at 0°C. After standing for 2 hr at 0°C, the reaction mixture was diluted with dry ether and filtered. The filtrate was percolated through a Florisil column (50 g). The column was further eluted with 500 ml of ether. The eluates were combined and concentrated to dryness. Recrystallization of the residue from ether gave crystals (6.6 g) of III; mp 153–154°C; IR (cm^{-1}), 1720; PMR (δ ppm), 0.59 (3H, s, 18–CH_3), 0.85 (3H, s, 19–CH_3), 0.87 (3H, d, J = 6 Hz, 21–CH_3), 2.01, 2.03 (6H, s, 3α– and 7α-$OCOCH_3$), 4.70 (1H, m, 3β–H), 4.93 (1H, m, 7β–H), 9.65 (1H, t, J = 4 Hz, –CHO).

Methyl 3α,7α,24-trihydroxy-5β-cholestanoate (V). A solution of III (6.2 g) in dry benzene (50 ml) was added to a

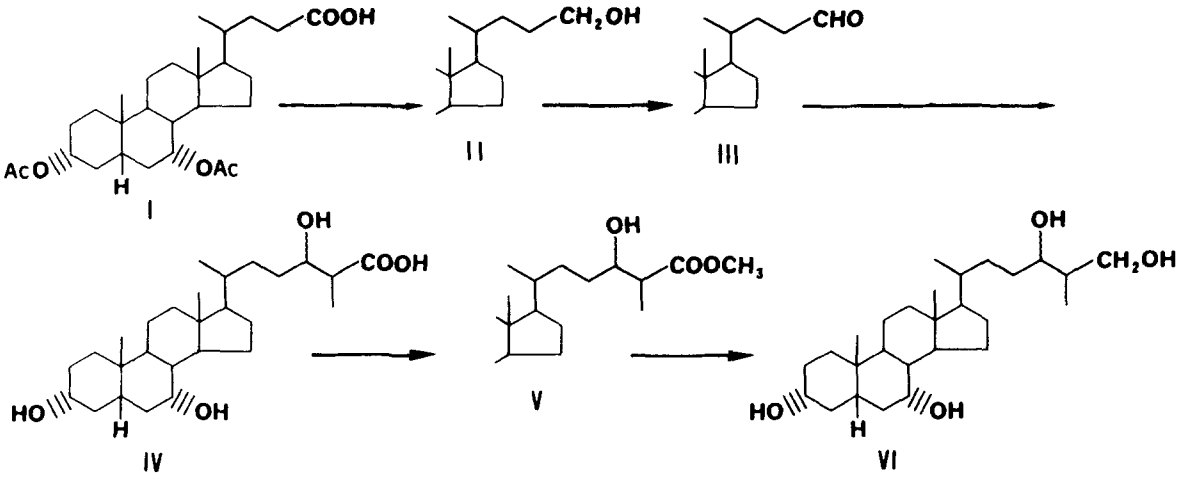

Fig. 1. Synthesis of 5β-cholestane-3α,7α,24,26-tetrol. I, 3α,7α-Diacetoxy-5β-cholanoic acid; II, 3α,7α-diacetoxy-5β-cholan-24-ol; III, 3α,7α-diacetoxy-5β-cholan-24-al; IV, 3α,7α,24-trihydroxy-5β-cholestanoic acid; V, methyl 3α,7α,24-trihydroxy-5β-cholestanoate; VI, 5β-cholestane-3α,7α,24,26-tetrol.

mixture of ethyl DL-α-bromopropionate (5 ml), dry benzene (50 ml), granulated zinc (8.6 g), a few crystals of iodine, and a small amount of powdered copper. When the mixture was refluxed on a steam bath, a vigorous reaction occurred and the solution became cloudy. Gentle refluxing was continued for 1.5 hr with rapid stirring. The reaction mixture was then cooled, poured into ice water, acidified with 10% H_2SO_4, and extracted with ether (300 ml × 3). After the usual workup (ether), the product was dissolved in 100 ml of 5% methanolic KOH and the solution was allowed to reflux for 2 hr. After acidification with dilute HCl, the usual workup (ethyl acetate) of the hydrolyzates afforded a residue (5 g) which was methylated with freshly prepared ethereal diazomethane solution. The resulting methylated material was chromatographed on a silica gel column (100 g) using a solvent system of acetone graded into ethyl acetate. Elution with 10% acetone in ethyl acetate gave V (2.3 g) as a gum; IR (cm^{-1}), 3300, 1710; PMR (δ ppm), 0.70 (3H, s, 18–CH_3), 0.95 (3H, s, 19–CH_3), 0.98 (3H, d, J = 6 Hz, 21–CH_3), 1.43 (3H, d, J = 6 Hz, 27–CH_3), 3.60 (3H, s, –$COOCH_3$), 3.50–4.10 (2H, m, 3β–H and 24–H), 3.95 (1H, m, 7β–H); GLC (as TMS ether), RRT, 2.27 (3% OV-17); GLC–MS, m/z (relative intensity), 500(M–180)(85), 485(M–180–15)(21), 410(M–270)(38), 395(M–270–15)(31), 343(M–side chain–90)(31), 323(32), 296(48), 281(46), 255(63), 253(M–side chain–180)(100), 243(38), 189(77).

5β-Cholestane-3α,7α,24,26-tetrol (VI). $LiAlH_4$ (200 mg) was added to a solution of V (310 mg) in dry ether (40 ml). The resulting suspension was refluxed for 2 hr. The mixture was cooled to 0°C, then crushed ice (100 g) and 2% H_2SO_4 (20 ml) were added to decompose the excess $LiAlH_4$. The usual workup (ethyl acetate) gave VI (220 mg) as a noncrystalline material. IR (cm^{-1}), 3350; PMR (δ ppm), 0.70 (3H, s, 18–CH_3), 0.96 (3H, s, 19–CH_3), 1.00 (3H, d, J = 6 Hz, 21–CH_3), 1.23 (3H, d, J = 6 Hz, 27–CH_3), 3.50–4.20 (5H, m, 3β–, 7β–, and 24–H and 26–CH_2OH).

Synthesis of 5β-cholestane-3α,7α,26,27-tetrol (XII) (Fig. 2)

*Methyl chenodeoxycholate 3-*tert*-butyldimethylsilyl ether (VIII).* To a solution of methyl chenodeoxycholate (VII) (10 g) in dimethylformamide (120 ml) were added imidazol (20 g) and *tert*-butyldimethylsilyl chloride (17 g), and the solution was allowed to stand at room temperature for 5 hr. After dilution of the reaction mixture with ether, the usual workup (ether) gave a residue. Recrystallization of the residue from ethyl acetate gave crystals (6.5 g) of VIII, mp 145–146°C; IR (cm^{-1}), 3300, 1725; PMR (δ ppm), 0.13 (6H, s, Si–$CH_3)_2$), 0.66 (3H, s, 18–CH_3), 0.93 (3H, s, 19–CH_3), 0.93 (3H, d, J = 6 Hz, 21–CH_3), 0.93 (9H, s, –Si–$C(CH_3)_3$), 3.55 (1H, m, 3β–H), 3.56 (3H, s, –$COOCH_3$), 3.93 (1H, m, 7β–H).

*5β-Cholane-3α,7α,24-triol 3-*tert*-butyldimethylsilyl ether (IX).* The silyl ether (VIII, 5 g) was dissolved in dry ether (50 ml). $LiAlH_4$ (2.5 g) was added to the solution in small portions over a 30-min period with cooling (ice water) during each addition. The resulting suspension was heated for 2 hr under reflux. After dilution with ice-cold 10% Rochelle salt solution, the usual workup (ether) gave IX (4 g) as a colorless gum; IR (cm^{-1}), 3350; PMR (δ ppm), 0.15 (6H, s, Si–$(CH_3)_2$), 0.69 (3H, s, 18–CH_3), 0.93 (3H, s, 19–CH_3), 0.93 (9H, s, –Si–$C(CH_3)_3$), 1.00 (3H, d, J = 6 Hz, 21–CH_3), 3.55 (1H, m, 3β–H), 3.78 (2H, t, J = 6 Hz, 24–CH_2OH), 3.95 (1H, m, 7β–H).

3α,7α-Dihydroxy-5β-cholestane-26,27-dioic acid diethyl ester (XI). To an ice-cold solution of IX (4 g) in dry pyridine (20 ml), a solution of *p*-toluenesulfonyl chloride (2 g) was

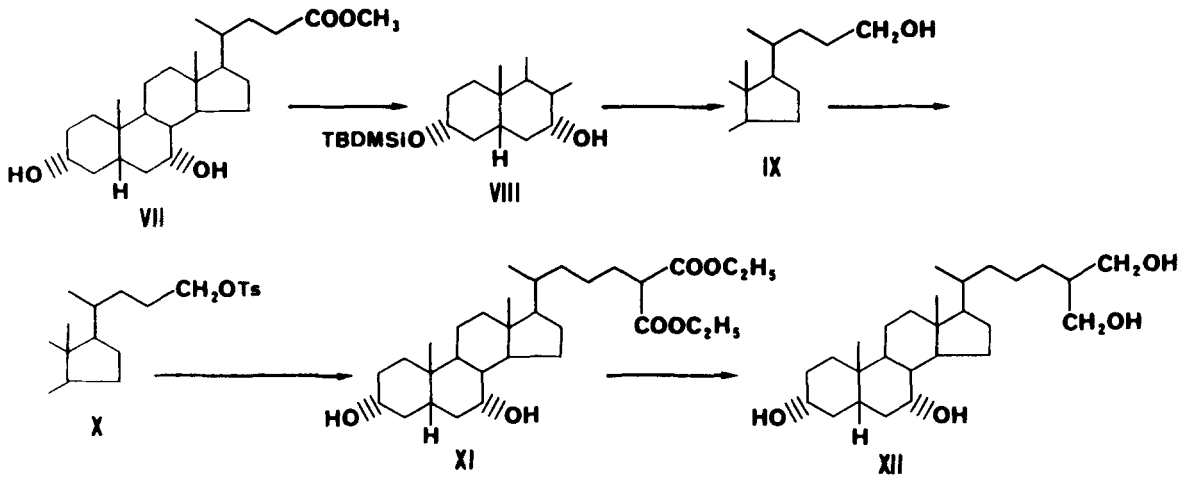

Fig. 2. Synthesis of 5β-cholestane-3α,7α,26,27-tetrol. TBDMSi = $(CH_3)_3C(CH_3)_2Si$; Ts = $CH_3C_6H_4SO_2$-; VII, methyl chenodeoxycholate; VIII, methyl chenodeoxycholate 3-*tert*-butyldimethylsilyl ether; IX, 5β-cholane-3α,7α,24-triol 3-*tert*-butyldimethylsilyl ether; X, 5β-cholane-3α,7α,24-triol 3-*tert*-butyldimethylsilyl ether 24-monotosylate; XI, 3α,7α-dihydroxy-5β-cholestane-26,27-dioic acid diethyl ester; XII, 5β-cholestane, 3α,7α,26,27-tetrol.

added dropwise with stirring. Stirring was continued at room temperature for 16 hr. After dilution with ice-cold dilute HCl, the usual workup (ether) gave the crude 24-monotosylate of IX (3.5 g) in semi-solid form, which was used for the next step without further purification or characterization.

To this crude product (3.5 g) dissolved in ethanol (20 ml) was added 15 ml of an ethanol solution that contained 0.7 g of sodium as the sodium ethoxide and 6.7 g of diethyl malonate. The reaction mixture was refluxed for 6 hr. The residue obtained after the usual workup (ether) was dissolved in tetrahydrofuran (50 ml) containing tetra-*n*-butylammonium fluoride (3 g) in order to remove the protecting *tert*-butyldimethylsilyl group. The solution was allowed to stand at room temperature for 10 hr. The usual workup (ether) gave a residue, which was purified by silica gel (100 g) column chromatography with increasing amount of ethyl acetate in benzene. The fractions eluted with 30% ethyl acetate in benzene gave XI (1.6 g) as a gum; IR (cm^{-1}), 3400, 1720; PMR (δ ppm), 0.69 (3H, s, 18–CH_3), 0.95 (3H, d, J = 6 Hz, 21–CH_3), 0.97 (3H, s, 19–CH_3), 1.17 (6H, t, J = 6 Hz, 2 × OCH_2CH_3), 3.67 (1H, m, 3β–H), 3.95 (1H, m, 7β–H), 4.17 (4H, q, J = 6 Hz, 2 × OCH_2CH_3).

5β-Cholestane-3α,7α,26,27-tetrol (XII). $LiAlH_4$ (500 mg) was added to a solution of the diester (XI, 800 mg) in dry ether (30 ml). The resulting suspension was heated for 2 hr under reflux. The usual workup (ethyl acetate) gave a residue. Recrystallization of the residue from ethyl acetate gave crystals (420 mg) of XII, mp 136.5–138°C; IR (cm^{-1}), 3300; PMR (δ ppm), 0.70 (3H, s, 18–CH_3), 0.93 (3H, d, J = 6 Hz, 21–CH_3), 0.95 (3H, s, 19–CH_3), 3.75 (1H, m, 3β–H), 3.97 (1H, m, 7β–H), 4.02 (4H, d, J = 6 Hz, 26 and 27–CH_2OH).

RESULTS AND DISCUSSION

A gallbladder bile sample obtained from a patient with cholelithiasis was examined for cholestanetetrols which were partially identified in our previous study (10). Since these bile alcohols have been known to exist as the major components of the glucurono-conjugated bile alcohol fraction (10), the crude bile salts were first subjected to ion-exchange chromatography to isolate bile alcohol glucuronides, which were then treated with β-glucuronidase. The resulting deconjugated bile alcohols were further fractionated by a reversed phase partition chromatography to give a fraction containing tetrahydroxy bile alcohols, which were analyzed as the TMS ether derivatives by GLC and GLC–MS.

As shown in **Fig. 3,** GLC analysis revealed the presence in the tetrahydroxy bile alcohol fraction of at least six tetrahydroxy bile alcohols corresponding to GLC peaks 1–6. The retention times and mass spectra of peaks 1, 2, and 3 were identical with those of the TMS ethers of authentic 5α-cholestane-3α,7α,12α,24-tetrol, 5β-cholestane-3α,7α,12α,24-tetrol, and 5β-cholestane-3α,7α,12α,26-tetrol, respectively. The occurrence and identification of these cholestanetetrols possessing three nuclear hydroxyl groups in human bile have been reported in our previous paper (10). The retention times and mass spectra of peaks 4, 5, and 6 were the same as those of three of the partially identified bile alcohols, tentatively named as bile alcohols 22, 23, and 27, respectively, in our previous study (10). The mass spectra of peaks 4–6 showed no molecular ion (**Fig. 4**). However, there was a series of fragment ions at m/z 544, 454, and 364, except for peak 5 in which only m/z 454 was observed, that arose from the consecutive loss of two to four TMS-OH

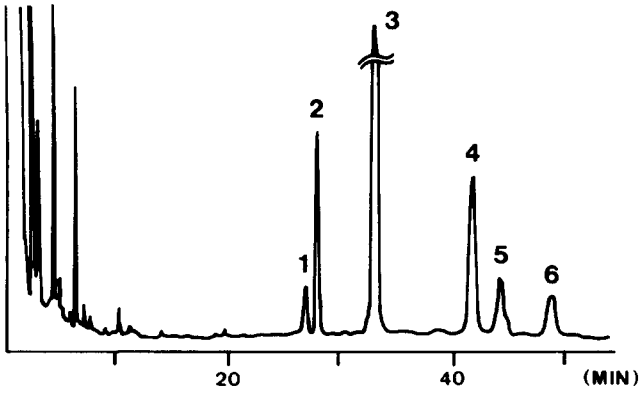

Fig. 3. Gas chromatogram of TMS ether derivatives of tetrahydroxy bile alcohols isolated from human bile. Column, capillary OV-1 (25 m × 0.25 mm i.d.); column temperature, 240–270°C, 2°C/min; 1, 5α-cholestane-3α,7α,12α,24-tetrol; 2, 5β-cholestane-3α,7α,12α,24-tetrol, 3, 5β-cholestane-3α,7α,12α,26-tetrol; 4, 5β-cholestane-3α,7α,24,26-tetrol; 5, 5β-cholestane-3α,7α,25,26-tetrol; 6, 5β-cholestane-3α,7α,-26,27-tetrol.

groups from the molecule, a fragmentation pattern characteristic of cholestanetetrol TMS ethers. These spectra also exhibited an ion at m/z 255 which represents loss of the entire side chain plus two nuclear TMS-OH groups. These data clearly indicate that the compounds giving rise to peaks 4–6 are cholestanes carrying two hydroxyl substituents in the ring system and two in the side chain.

In the mass spectrum (Fig. 4b) of peak 4, a prominent fragment ion was observed at m/z 233. The ion at m/z 233 is also seen in the spectrum (Fig. 4a) of the TMS ether of 5β-chimaerol (5β-cholestane-3α,7α,12α,24,26-pentol), and is known to be a side chain fragment formed by scission of the bond between C-23 and C-24 (17). Thus, the side chain structure of the compound eluted at peak 4 seems to be the same as that of 5β-chimaerol. This proposal is strongly supported by the occurrence of a series of fragment ions at m/z 503,413, and 323 in the spectrum (Fig. 4b) of peak 4. This could conceivably arise from scission of the bond between C-24 and C-25 followed by the successive loss of one to three TMS-OH molecules (17). Based on these mass spectral data and biogenetic considerations, the compound giving rise to peak 4 appears to be a deoxy-derivative of 5β-chimaerol, probably 5β-cholestane-3α,7α,24,26-tetrol.

To confirm this structural assignment, 5β-cholestane-3α,7α,24,26-tetrol (VI) was synthesized from chenodeoxycholic acid. Though the synthetic tetrol might be a mixture of four stereoisomers at C-24 and C-25, these could not be separated on GLC or TLC under the conditions employed. The synthetic cholestanetetrol (VI) was identical with the natural bile alcohol eluted at peak 4 as judged by GLC and mass spectrometry.

The compound giving rise to peak 5 was identified as 5β-cholestane-3α,7α,25,26-tetrol. The mass spectrum

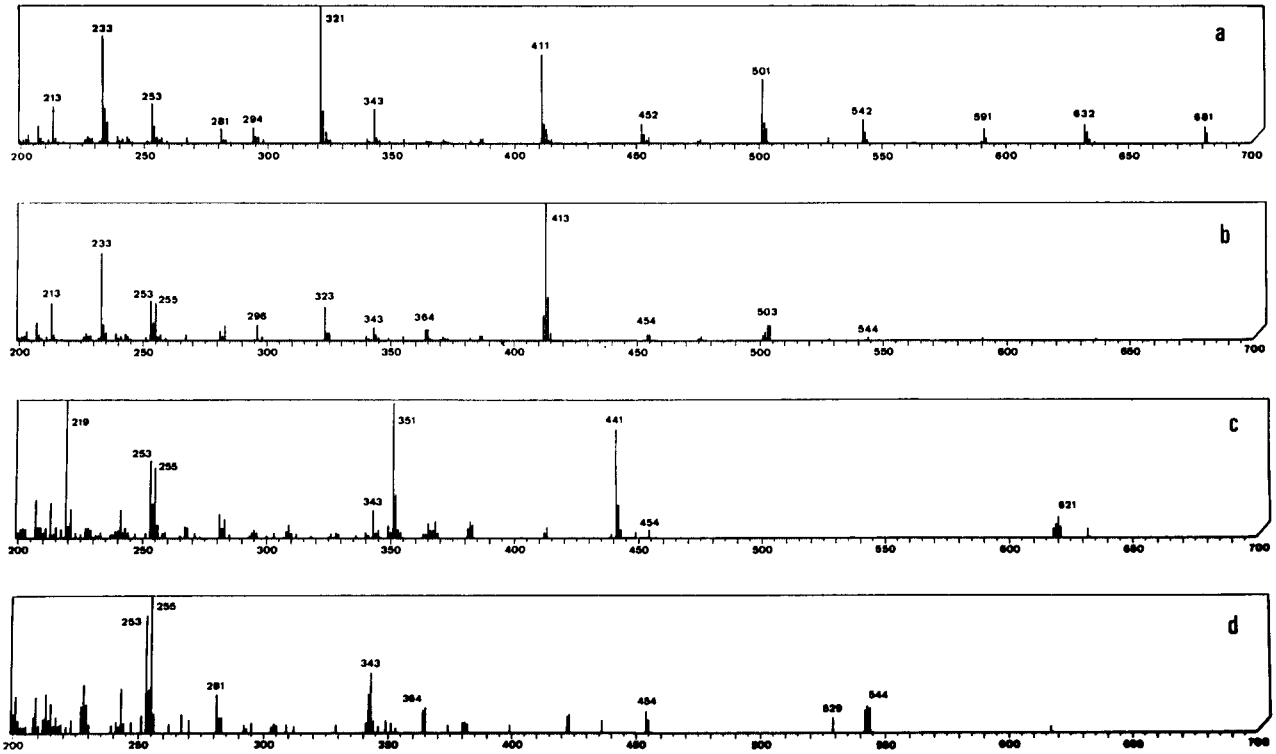

Fig. 4. Mass spectra of TMS ether derivatives of 5β-cholestane-3α,7α,12α,24,26-pentol (a) and three new bile alcohols found in human bile. Mass spectra b (peak 4), c (peak 5), and d (peak 6) were identical with those of TMS ether derivatives of authentic 5β-cholestane-3α,7α-24,26-tetrol, 5β-cholestane-3α,7α,25,26-tetrol, and 5β-cholestane-3α,7α,26,27-tetrol, respectively.

(Fig. 4c) of peak 5 exhibited the base peak at m/z 219 and a series of fragment ions at m/z 621,441, and 351. The former ion seems to be a side chain fragment formed from scission of the bond between C-24 and C-25 (17). The latter series is due to fragmentation between C-25 and C-26. This structural assignment was confirmed by direct comparison with authentic 5β-cholestane-3α,7α,25,26-tetrol. The RRTs on GLC and mass spectrum of peak 5 were identical with those of the TMS ether of the authentic sample.

The mass spectrum (Fig. 4d) of peak 6 closely resembled that of the TMS ether of 12-deoxy-5α-cyprinol (5α-cholestane-3α,7α,26,27-tetrol). The only difference between the two spectra was seen in the relative intensities of some peaks. The ratio of the GLC RRTs on OV-17 column between peak 6 (RRT = 2.51) and the deoxycyprinol (RRT = 2.34) was almost the same as that of the RRTs between the TMS ethers of methyl chenodeoxycholate (RRT = 1.12) and methyl allochenodeoxycholate (RRT = 1.01). These data suggest that the bile alcohol eluted at peak 6 is the 5β-isomer of 12-deoxy-5α-cyprinol.

To confirm this structural assignment, 5β-cholestane-3α,7α,26,27-tetrol (XII) was synthesized from methyl chenodeoxycholate. The synthetic bile alcohol (XII) was identical with the natural bile alcohol 6 as judged by GLC and mass spectrometry.

Most of the naturally occurring bile alcohols, which include those found in biological fluids from healthy or diseased humans, carry the cholic acid type of nucleus. Until the present time, only three bile alcohols having the same ring system as chenodeoxycholic acid have been found in the gallbladder bile of humans, and identified as 5β-cholestane-3α,7α,24-triol, 5β-cholestane-3α,7α,25-triol, and 5β-cholestane-3α,7α,26-triol (10). These cholestanetriols might be formed by 24-, 25-, and 26-hydroxylation of 5β-cholestane-3α,7α-diol, which is an intermediate in the biosynthetic course of chenodeoxycholic acid from cholesterol (10). The three cholestanetetrols identified in the present study might be formed from the cholestanetriols by a second hydroxylation in the side chain. Thus, these bile alcohols carrying the chenodeoxycholic acid type of nucleus appear to represent secondary metabolites of the intermediate in the formation of chenodeoxycholic acid in humans.

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