Identification of bile alcohols in urine from healthy humans[1]

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Abstract Bile alcohols present in urine from healthy adults were studied. Urine was extracted with octadecylsilane-bonded silica gel, and a neutral fraction and a glucuronide fraction were isolated by ion exchange chromatography on piperidinohydroxypropyl Sephadex LH-20. Following hydrolysis of the glucuronide fraction with β-glucuronidase and purification by silica gel column chromatography, the bile alcohols were analyzed by a combined gas–liquid chromatography–mass spectrometry. By direct comparison with reference standards, the structures of four major bile alcohols were elucidated as follows: 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol; 5β-cholestane-3α,7α,12α,24,25-pentol; 5β-cholestane-3α,7α,12α,25,26-pentol; and 5β-cholestane-3α,7α,12α,26,27-pentol. The daily excretion of these four bile alcohols in urine was 0.5–1.0 μmoles. After purification by silica gel column chromatography, the neutral fraction was analyzed by a combined gas–liquid chromatography–mass spectrometry. The major bile alcohol excreted in urine as the unconjugated form was 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol. The daily excretion of the unconjugated C_{26} pentol (0.02–0.05 μmoles) was far lower than that of the conjugated C_{26} pentol (0.34–0.86 μmoles).—**Kuwabara, M., T. Ushiroguchi, K. Kihira, T. Kuramoto, and T. Hoshita.** Identification of bile alcohols in urine from healthy humans. *J. Lipid Res.* 1984. **25:** 361–368.

Supplementary key words gas–liquid chromatography–mass spectrometry

Although bile alcohols are found in some primitive vertebrates such as fishes and amphibians as the major bile constituents (1), it had been supposed, until recently, that such substances did not occur in humans. At present, there are several reports on the occurrence of bile alcohols in humans. The accumulation of bile alcohols in bile and feces of patients with the rare inherited sterol storage disease, cerebrotendinous xanthomatosis (CTX), was first described in 1974 by Setoguchi et al. (2). Subsequent studies (3–7) have demonstrated that these bile alcohols are 5β-cholestanes hydroxylated at C-3α, C-7α, C-12α, C-25, and elsewhere in the side chain. Kibe et al. (8) have described a case of cholestasis in which the patient had considerable amounts of three different bile alcohols in his bile: 26,27-dinor-5β-cholestane-3α,7α,12α,24,25-pentol, 24-nor-5β-cholestane-3α,7α,12α,25-tetrol, and 3α,7α,12α-trihydroxy-26,27-dinor-5β-cholestan-24-one. Summerfield, Billing, and Shackleton (9) found in blood plasma and urine from patients with cholestasis a cholestanetriol, probably 5β-cholestane-3α,7α,26-triol, in addition to 24- and 26-hydroxycholesterols and large numbers of bile acids. Furthermore, Karlaganis et al. (10, 11) reported that urine from healthy infants and children as well as from patients with liver disease contained a number of bile alcohol glucuronides, and the major urinary bile alcohol was identified as 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol.

In order to obtain at least partial information about the biogenesis of bile alcohols in man and the mechanisms of the accumulation of the bile alcohols in certain pathological conditions, a more complete knowledge of bile alcohols in healthy subjects is required. The present report describes identification of bile alcohols excreted in urine from healthy adults.

MATERIALS AND METHODS

General

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

Infrared (IR) spectra were obtained on a Shimadzu model IR-408 spectrophotometer as KBr discs. Absorption frequencies are quoted in reciprocal centimeters.

Proton nuclear magnetic resonance (PMR) spectra, in δ ppm,

Abbreviations: CTX, cerebrotendinous xanthomatosis; IR, infrared; PMR, proton nuclear magnetic resonance; GLC, gas–liquid chromatography; TMS, trimethylsilyl; GLC–MS, gas–liquid chromatography–mass spectrometry; PHP-LH-20, piperidinohydroxypropyl Sephadex LH-20; RRT, relative retention time.

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were recorded on a Hitachi model R-40 spectrometer at 90 MHz using tetramethylsilane as an internal standard.

Gas–liquid chromatography (GLC) was carried out on a Shimadzu model GC-6A gas chromatograph using a glass column (2 m × 3 mm) packed with 2% OV-1, 3% OV-17, 3% QF-1, or 2% Poly I-110 on 80–100 mesh Gas Chrom Q. All retention times were given relative to the trimethylsilyl (TMS) ether of methyl cholate. Quantitation was accomplished by mixing a known quantity of 5α-cholestane with a measured aliquot of the sample and then comparing areas under the GLC curve. Measurements of peak areas were accomplished with a Shimadzu E1A automatic integrator.

Gas–liquid chromatography–mass spectrometry (GLC–MS) was carried out on a Shimadzu model GCMS-7000S gas chromatograph–mass spectrometer. The following operating conditions were employed: column, 1% OV-1 (2 m × 3 mm); column temperature, 280°C; ion source temperature, 310°C; ionization voltage, 70 eV; ionization current, 300 μA; carrier gas, helium; carrier flow rate, 40 ml/min.

Silica gel ODS G-3 was supplied by Fujigel Hanbai Co. This octadecylsilane-bonded silica gel was used for extraction of bile alcohols from urine samples as mentioned below. Preliminary recovery experiments using [G-^{3}H]24-nor-5β-cholestane-3α,7α,12α,25-tetrol and its 3-glucuronide gave results for the unconjugated bile alcohol of 89–99% (mean, 94%) and for the bile alcohol glucuronide of 84–96% (mean, 91%).

Ion exchange chromatography on piperidinohydroxypropyl Sephadex LH-20 (PHP-LH-20) was performed according to the procedure reported by Goto et al. (12). Preliminary experiments showed that by this method unconjugated and glucurono-conjugated bile alcohols were eluted stepwise with 90% ethanol and 0.2 M formic acid in 90% ethanol, respectively. The recovery was better than 98% as determined for both unconjugated and glucurono-conjugated bile alcohols in multiple runs.

'*The usual work-up*' refers to extraction with organic solvent, washing to neutrality, drying over anhydrous Na_2SO_4, filtration, and evaporation under reduced pressure.

Analysis of bile alcohols in urine

Extraction and isolation of bile alcohols from urine. Urine was collected during a 24-hr period. Five hundred ml of urine was percolated through 4 g of silica gel ODS G-3 in a column (4 cm × 2.2 cm i.d.) at a rate of about one drop every 2 sec. The column was washed with 50 ml of water and then with 200 ml of methanol. The methanol eluate was evaporated in vacuo. The resulting residue was dissolved in 10 ml of 90% ethanol and the solution was passed through a column of PHP-LH-20 (7.5 ml). The effluent and an additional wash with 20 ml of 90% ethanol were collected to give a fraction of neutral compounds. The column was then eluted with 60 ml of 0.2 M formic acid in 90% ethanol to give a fraction of bile alcohol glucuronides.

Enzymatic hydrolysis of bile alcohol glucuronides. The glucuronide fraction eluted from the PHP-LH-20 column was evaporated in vacuo. The residue was subjected to hydrolysis at 37°C for 48 hr in 20 ml of a solution of 10,000 units of β-glucuronidase ([EC 3.2.1.31.], Sigma Chemical Co., Type IX) in 0.1 M phosphate buffer, pH 6.8. The incubation mixture was extracted with silica gel ODS G-3 (4 g) as described above for urine. The methanol eluate was taken to dryness, redissolved in 10 ml of 90% ethanol, and passed through a column of PHP-LH-20 (7.5 ml). The effluent and an additional wash with 20 ml of 90% ethanol were collected to give a fraction of deconjugated bile alcohols.

Purification by silica gel column chromatography

The deconjugated bile alcohol fraction was evaporated in vacuo, and the residue was transferred to a column of silica gel 60 (Merck) (5 g). The column was successively eluted with benzene (10 ml), ethyl acetate (20 ml), acetone (20 ml), acetone–ethanol 1:1 (by vol) (10 ml), and ethanol (20 ml). The fractions were taken to dryness in vacuo and each residue was treated with pyridine–hexamethyldisilazane–trimethylchlorosilane 5:2:1 (by vol) for 2 hr at room temperature. The resulting TMS ether derivative was analyzed by GLC and GLC–MS. The neutral fraction was also chromatographed on a column of silica gel 60 (5 g), and analyzed by GLC and GLC–MS as described above for the deconjugated bile alcohol fraction.

Reference bile alcohols

5β-Cholestane-3α,7α,12α,24,25-pentol was prepared according to the method described previously (13).

5β-Bufol (5β-cholestane-3α,7α,12α,25,26-pentol) and 5β-cyprinol (5β-cholestane-3α,7α,12α,26,27-pentol) were isolated from the solvolyzed biles of the toad, *Bufo vulgaris formosus* (14) and the frog, *Rana nigromaculata* (15), respectively.

[G-^{3}H]24-Nor-5β-cholestane-3α,7α,12α,25-tetrol and its 3-glucuronide, which were used for preliminary recovery experiments, were prepared according to the procedures described previously (16).

Synthesis of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (Fig. 1)

27-Nor-5β-cholest-24-ene-3α,7α,12α-triol (II). A suspension of ethyl triphenylphosphonium bromide (4 g) and potassium *tert*-butoxide (1.5 g) in benzene (80 ml) was added to a solution of 3α,7α,12α-triacetoxy-5β-cholan-24-al (I). The reaction mixture was refluxed for 2 hr.

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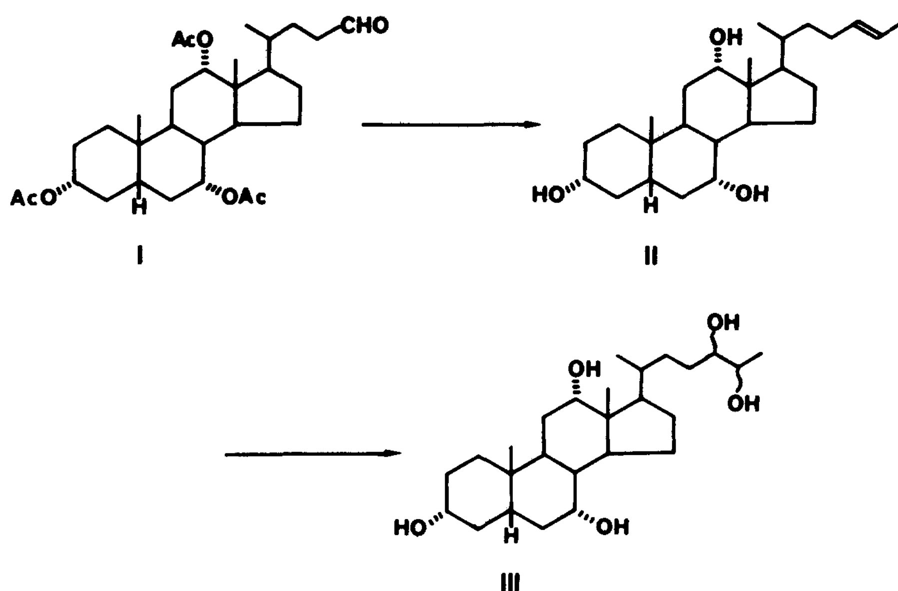

Fig. 1. Partial synthesis of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol. I, 3α,7α,12α-Triacetoxy-5β-cholan-24-al; II, 27-nor-5β-cholest-24-ene-3α,7α,12α-triol; III, 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol.

After acidification with diluted HCl, the mixture was extracted with ether. The usual work-up afforded a residue, which was dissolved in 2 N methanolic KOH, and refluxed for 2 hr. After dilution with water and the usual work-up (ethyl acetate), silica gel column chromatography (Merck) (80 g) using 20% acetone in ethyl acetate as eluting solvent followed by recrystallization from ethyl acetate gave 360 mg of colorless needles of II: mp 182°C; IR: 3400 (OH); PMR (pyridine-d_5,δ): 0.81 (3H, s, 18–CH_3), 0.99 (3H, s, 19–CH_3), 1.21 (3H, d, J = 6 Hz, 21–CH_3), 1.62 (3H, m, 26–CH_3), 3.45–4.40 (3H, m, 3β-H, 7β-H, and 12β-H), 5.46 (2H, m, –CH=CH–).

27-Nor-5β-cholestane-3α,7α,12α,24,25-pentol (III). Acetic anhydride (5 ml) was added to a solution of II (145 mg) in pyridine (3 ml) and the reaction mixture was refluxed for 12 hr. After dilution with water and acidification with diluted HCl, the usual work-up (ether) gave a residue (171 mg), which was dissolved in a mixture of 30 ml of ether and 2 ml of pyridine. Two hundred mg of osmium tetraoxide was added to the solution. The reaction mixture was allowed to stand in a closed flask at room temperature for 24 hr. After removal of the solvents in vacuo, the residue was refluxed with 20 ml of aqueous 50% ethanol and 2 g of sodium sulfite for 3 hr. The mixture was then diluted with a large amount of ethanol and filtered. The filtrate was evaporated to dryness and the residue was refluxed with 2 N methanolic KOH for 2 hr. After the usual work-up (ethyl acetate), recrystallization from ethyl acetate gave colorless needles of III (12 mg): mp 230°C; IR: 3350 (OH); PMR (pyridine-d_5,δ): 0.82 (3H, s, 18–CH_3), 1.00 (3H, s, 19–CH_3), 1.26 (3H, d, J = 6 Hz, 21–CH_3), 1.52 (3H, d, J = 6 Hz, 26–CH_3), 3.55–4.40 (5H, m, 3β-H, 7β-H, 12β-H, 24-H, and 25-H).

RESULTS

Urine samples from six healthy subjects were examined for bile alcohols. Each sample was extracted with silica gel ODS G-3 and the extract was submitted to ion exchange chromatography on PHP-LH-20; a neutral fraction and a glucuronide fraction were obtained. The latter fraction was treated with β-glucuronidase and was again chromatographed on PHP-LH-20 to give a fraction of deconjugated bile alcohols. Since preliminary GLC–MS analysis of this fraction revealed a complex mixture of neutral compounds, further fractionation of the mixture was undertaken by means of silica gel column chromatography. GLC–MS analysis showed that the last (ethanol-eluted) fraction from the silica gel column contained at least four pentahydroxy bile alcohols which were tentatively designated 1–4 in order of increasing retention time. The pentahydroxy bile alcohol profiles in all the urine samples examined resembled one another. The approximate amounts of the individual pentahydroxy bile alcohols in the urine samples from six healthy subjects, which were measured from the peak areas on GLC using OV-1 column, are shown in **Table 1,** with their relative retention times (RRTs) on GLC using four different columns. The mass spectra of these bile alcohols as the TMS ether derivatives are shown in **Fig. 2.**

The mass spectrum of the TMS ether of bile alcohol 1 was identical in all respects to that reported for the TMS ether of 27-nor-5β-cholestane-3α,7α,12α,24,25-

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TABLE 1. Glucurono-conjugated pentahydroxy bile alcohol excretion in healthy adults

Subject	Age (yr)	Sex	Daily Urinary Bile Alcohol Excretion (μmol) 1	2	3	4	Total
S.K.	28	M	0.86	0.07	0.06	0.05	1.04
M.H.	34	M	0.49	0.05	0.03	0.02	0.59
M.K.	27	M	0.43	0.08	0.03	0.02	0.56
M.U.	29	M	0.42	0.14	0.05	0.03	0.64
I.M.	22	F	0.53	0.14	0.04	0.02	0.73
M.K.	24	F	0.34	0.06	0.03	0.02	0.45
RRT on GLC							
OV-1			2.00	2.41	2.72	3.06	
OV-17			1.30	1.57	1.73	2.05	
QF-1			0.98	1.18	1.29	1.41	
Poly-I 110			1.00	1.21	1.39	1.68	

The bile alcohols 1, 2, 3, and 4 were identified as 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, 5β-cholestane-3α,7α,12α,24,25-pentol, 5β-cholestane-3α,7α,12α,25,26-pentol, and 5β-cholestane-3α,7α,12α,26,27-pentol, respectively. RRTs are given relative to TMS ether of methyl cholate (RRT = 1.00).

pentol (10). In the mass spectrum (Fig. 2) of the TMS ether of bile alcohol 1, the molecular ion was seen at m/z 798. There were three series of fragment ions: one at m/z 708, 618, 528, 438, and 348, a second at m/z 693, 603, and 513, a third at m/z 681, 591, 501, 411, and 321. The first series results from the successive loss of trimethylsilanol (TMS-OH) molecules from the molecular ion. The seond series results from the loss of a methyl group plus one, two, and three TMS-OH molecules. The third series results from scission of the bond between C-24 and C-25 followed by the successive loss of TMS-OH molecules. The ions at m/z 371 and 281 result from scission of the bond between C-20 and C-22 plus loss of two and three nuclear TMS-OH groups, respectively. The ions at m/z 343 and 253 represent loss of the side chain plus two and three nuclear TMS-OH groups, respectively. The peaks at m/z 219 and 129 are side chain fragments resulting from scission of the bond between C-23 and C-24 and then the loss of a TMS-OH group,

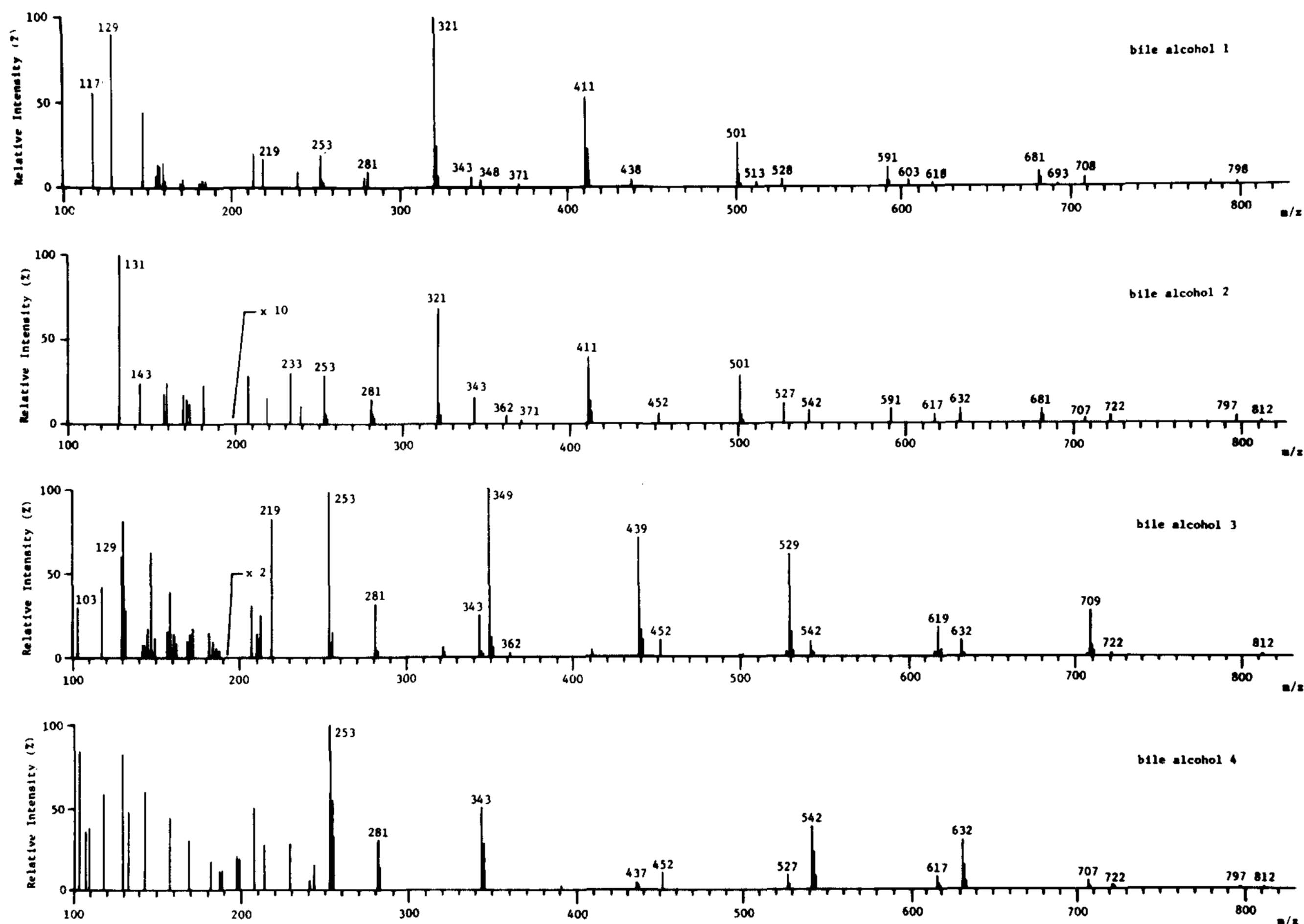

Fig. 2. Mass spectra of the TMS ether of urinary bile alcohols 1, 2, 3, and 4, which were identified as 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, 5β-cholestane-3α,7α,12α,24,25-pentol, 5β-cholestane-3α,7α,12α,25,26-pentol, and 5β-cyprinol (5β-cholestane-3α,7α,12α,26,27-pentol), respectively.

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respectively. The peak at m/z 117 is the side chain fragment resulting from scission of the bond between C-24 and C-25.

Confirmation of this structural assignment for bile alcohol 1 was attempted by synthesis (Fig. 1). 3α,7α,12α-Triacetoxy-5β-cholan-24-al (I) was prepared from cholic acid as described previously (17). Wittig reaction with ethyl triphenylphosphonium bromide of the triacetoxylcholanal (I) followed by alkaline hydrolysis afforded 27-nor-5β-cholest-24-ene-3α,7α,12α-triol (II). Osmium tetraoxide oxidation of the norcholestenetriol (II) gave the desired 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (III). The RRTs on GLC using four different columns and the mass spectrum of the synthetic bile alcohol were completely identical with those of the natural bile alcohol 1.

Bile alcohol 2 was identified as 5β-cholestane-3α,7α,12α,24,25-pentol. The TMS ether of bile alcohol 2 had the same RRT and mass spectrum of the authentic compound. The mass spectrum (Fig. 2) of the TMS ether of bile alcohol 2 was similar to that of the corresponding derivative of bile alcohol 1 with respect to peak intensities and fragmentation patterns. The only difference was that some peaks in the spectrum of bile alcohol 2 TMS ether were shifted 14 mass units upfield because of the presence of the additional C-27 methyl group.

Bile alcohol 3 was identified as 5β-cholestane-3α,7α,12α,25,26-pentol by direct comparison of the RRT and mass spectrum with the authentic 5β-bufol. The mass spectrum (Fig. 2) of the TMS ether of bile alcohol 3 shows a weak molecular ion at m/z 812, and two series of fragment ions: one at m/z 722, 632, 542, 452, and 362, and a second at m/z 709, 619, 529, 439, and 349. The former series is formed by the successive loss of TMS-OH molecules from the molecular ion. The second series results from scission of the bond between C-25 and C-26 followed by the successive loss of TMS-OH molecules. The peaks at m/z 219 and 129 are side chain fragments resulting from scission of the bond between C-24 and C-25 and then the loss of a TMS-OH molecule, respectively. The peak at m/z 103 is the side chain fragment resulting from scission of the bond between C-25 and C-26.

Bile alcohol 4 was identified as 5β-cyprinol. The RRT and mass spectrum of the TMS ether of bile alcohol 4 were identical with those of the corresponding derivative of the authentic compound. The mass spectrum (Fig. 2) shows a weak molecular ion at m/z 812 and two series of fragment ions. One series of fragment ions at m/z 722, 632, 542, and 452 results from the successive loss of TMS-OH molecules from the molecular ion. Another series of fragment ions at m/z 797, 707, 617, 527, and 437 is attributed to the loss of a methyl group plus one, two, three, and four TMS-OH molecules.

The neutral fraction obtained from the first PHP-LH-20 column chromatography was further fractionated on a column of silica gel and the resulting subfractions were analyzed as the TMS ether derivatives by GLC and GLC–MS. GLC analysis of the last (ethanol-eluted) fraction from the silica gel column revealed several peaks having retention times corresponding to pentahydroxy bile alcohols. The major peak was found to be due to the TMS ether of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol by direct comparison of its GLC and mass spectral properties with those of the authentic compound. The unconjugated 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol was found in all urine samples examined, and constituted an average of 82% (range 71–90). However, the daily excretion of the unconjugated C_{26} pentol (average, 0.03 μmol, range 0.02–0.05) was far lower than that of the conjugated C_{26} pentol (average 0.51 μmol, range 0.34–0.86). Although the GLC retention times of some among the other peaks were identical to those of TMS ethers of known bile alcohols, they could not be characterized conclusively, since amounts of these compounds were too little to confirm their chemical structures.

DISCUSSION

The present studies confirm and extend the results of previous investigations (10, 11) which showed that healthy infants and children excrete bile alcohols in urine as glucuronides, and the major urinary bile alcohol was identified as 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol on the basis of a combination of microchemical reactions and GLC–MS analysis. The present studies have shown the occurrence of at least four different pentahydroxy bile alcohols in urine from healthy adults, which were present as conjugates, probably glucuronides. The most abundant bile alcohol excreted in urine from healthy adults was found to be the same as that excreted in urine from infants and children. The structure of this C_{26} bile alcohol was confirmed as 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol by direct comparison of its GLC and mass spectral data to those of the authentic compound which was synthesized from cholic acid in the present studies. However, the configuration of the hydroxyl groups at C-24 and C-25 of the natural C_{26} pentol have remained obscure, because the chemically synthesized 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol is one of the four 24,25-isomers produced in the course of the synthesis, and the absolute configurations at C-24 and C-25 have not been elucidated. It is also possible that the synthetic C_{26} pentol is a mixture of two or more of the four stereoisomers. The other three bile alcohols were identified as 5β-cholestane-3α,7α,12α,24,25-pentol, 5β-cholestane-3α,7α,12α,25,26-pentol, and 5β-cyprinol with certainty by direct compar-

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ison of their GLC properties and mass spectra to those of authentic compounds. Since GLC and mass spectral analysis could not differentiate stereoisomers due to configurations of substitutes in the steroid side chain, stereochemistries at C-24 of the 24,25-pentol and at C-25 of the 25,26-pentol remained to be established. Since intact conjugates have not been isolated in the present studies, it is not known whether the site of the conjugation is the nucleus or the side chain. Apparently, further studies are required to elucidate the stereochemistries at C-24 and C-25 of the bile alcohols excreted in human urine, and to isolate and characterize intact conjugates.

The present studies also demonstrated the urinary excretion of unconjugated bile alcohols. The major constituent of the unconjugated bile alcohol fraction was 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol. However, the excretion rate of the C_{26} pentol was far lower as the unconjugate (average, 0.03 μmol) than as the glucuronide (average, 0.51 μmol). Of particular interest is the fact that bile alcohols are excreted mainly as glucurono-conjugated forms in human urine. The occurrence of bile alcohol glucuronides has been found in the bile of patients with CTX (6), who have impaired capacity to convert cholesterol to bile acids at the stage of the side chain oxidation (2). When 24-nor-5β-cholestane-3α,7α,12α,25-tetrol and 3α,7α,12α-trihydroxy-26,27-dinor-5β-cholestan-24-one were administered to bile fistula rats, these C_{26} and C_{25} bile alcohols were not converted to bile acids but were excreted as glucurono-conjugates in the bile (18). Thus, glucuronidation of bile alcohols, which forms highly polar metabolites with increased solubility in water, produces a more excretable form of bile alcohols in urine as well as in bile.

Although 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (III), the most abundant bile alcohol in urine both from adults and from children, has not previously been found in any other natural source, structurally related C_{26} bile alcohols, 5β-ranol (27-nor-5β-cholestane-3α,7α,12α,24,26-pentol) (XV), 26-deoxy-5β-ranol (27-nor-5β-cholestane-3α,7α,12α,24-tetrol) (XIV), and 24-dehydro-26-deoxy-5β-ranol (3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one) (XIII), have been found in the bile of bullfrog, *Rana catesbeiana* (19, 20). These ranols are thought to be formed in the bullfrog by the route shown in **Fig. 3** involving decarboxylation of 3α,7α,12α-trihydroxy-24-oxo-5β-cholestan-26-oic acid (VIII) (20), which has been proposed as an intermediate in the biosynthetic sequence between cholesterol (IV) and cholic

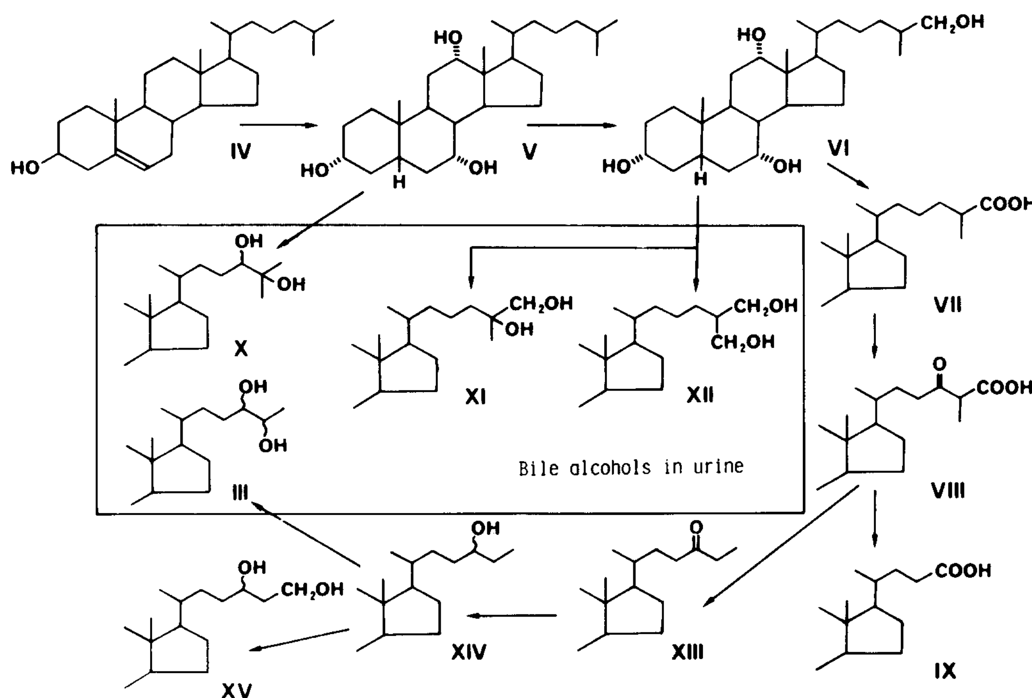

Fig. 3. Possible pathway for the formation of urinary bile alcohols in healthy humans. III, 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol; IV, cholesterol; V, 5β-cholestane-3α,7α,12α-triol; VI, 5β-cholestane-3α,7α,12α,26-tetrol; VII, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; VIII, 3α,7α,12α-trihydroxy-24-oxo-5β-cholestan-26-oic acid; IX, cholic acid; X, 5β-cholestane-3α,7α,12α,24,25-pentol; XI, 5β-cholestane-3α,7α,12α,25,26-pentol (5β-bufol); XII, 5β-cholestane-3α,7α,12α,26,27-pentol (5β-cyprinol); XIII, 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one (24-dehydro-26-deoxy-5β-ranol); XIV, 27-nor-5β-cholestane-3α,7α,12α,24-tetrol (26-deoxy-5β-ranol); XV, 27-nor-5β-cholestane-3α,7α,12α,24,26-pentol (5β-ranol).

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acid (IX) in man (1). It is not unreasonable to suppose that the pathway for synthesis of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (III) in man is closely similar to that for the synthesis of 5β-ranol (XV) in the bullfrog. The only difference is the last step: 25-hydroxylation of 26-deoxy-5β-ranol (XIV) leads to the former (III), 26-hydroxylation leads to the latter (XV).

5β-Cholestane-3α,7α,12α,24,25-pentol (X) has been found among the bile alcohols isolated after hydrolysis with β-glucuronidase of the bile of patients with CTX (3, 6). In the currently accepted pathway of cholic acid biosynthesis from cholesterol in man (1), side chain cleavage is thought to initiate by 26-hydroxylation of 5β-cholestane-3α,7α,12α-triol (V) to form 5β-cholestane-3α,7α,12α,26-tetrol (VI). However, in the CTX patients, a basic genetic defect is the relative lack of the 26-hydroxylase (21), and the substrate of the enzyme, the cholestanetriol (V), would tend to accumulate in the liver cell, thus exposing it to the action of 24- and 25-hydroxylases to form 5β-cholestane-3α,7α,12α,24,25-pentol (X). Hence, the formation of the cholestanepentol (X) in the CTX patients might represent an alternate pathway of cholesterol catabolism when the major route to form cholic acid has been blocked. The present finding that 5β-cholestane-3α,7α,12α,24,25-pentol (X) occurs in urine from healthy subjects suggests that the alternate pathway is operative even under normal conditions.

5β-Bufol (XI) and 5β-cyprinol (XII) have been found in the bile of amphibians as the major constituent (14, 15, 22, 23). The former (XI) is also present in the bile of rabbits though in much less quantity (24), but the latter (XII) has not previously been found in mammals. 5β-Bufol (XI) or 5β-cyprinol (XII) found in the primitive vertebrates is thought to be formed from 5β-cholestane-3α,7α,12α,26-tetrol (VI) by 25- or 27-hydroxylation (1). The present finding that these bile alcohols (XI and XII) are excreted in human urine suggests that the human has the capacity to synthesize 5β-bufol (or its 25-isomer) (XI) and 5β-cyprinol (XII), presumably by the same pathway as that in the primitive vertebrates. It is, therefore, conceivable that in the healthy human most of 5β-cholestane-3α,7α,12α,26-tetrol (VI) is oxidized to 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (VII) followed by a β-oxidation to give cholic acid (IX) via 3α,7α,12α-trihydroxy-24-oxo-5β-cholestan-26-oic acid (VIII); but small amounts of the intermediate (VI) can escape the normal pathway to lead cholic acid production and are exposed to the action of the 25- or 27-hydroxylase to form 5β-bufol (XI) or 5β-cyprinol (XII). In this connection, it is of interest that 3α,7α,12α,25-tetrahydroxy-5β-cholestan-26-oic acid and probably 3α,7α,12α,26-tetrahydroxy-5β-cholestan-27-oic acid have recently been found in the gastric contents from neonates with high intestinal obstruction (25). The occurrence of the tetrahydroxy C_{27} bile acids in the neonates supports the proposal that the human liver is capable of producing the pentahydroxy bile alcohols (XI and XII) because the structures of these bile acids are closely related to those of 5β-bufol (XI) and 5β-cyprinol (XII); the latter alcohols require only oxidation of a hydroxyl group at the end of the side chain to form the former acids.

In summary, all the bile alcohols excreted in human urine are thought to be side products arising by deviations from the normal pathway for the biosynthesis of cholic acid (IX) from cholesterol (I).

Addendum. After completion of this description. Ludwig-Köhn et al. (26) reported the identification of urinary bile alcohols by gas–liquid chromatography–mass spectrometry in patients with liver disease and in healthy individuals. The main bile alcohols in the urine were 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol and 5β-cholestane-3α,7α,12α,25,26-pentol. The excretion rate of the two bile alcohols in the urine of healthy individuals was approximately 0.6 μmol/day. These data are consistent with data in the present report.

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