Identification of 5β-cholestane-3α,7α,12α,24,25,26-hexol in human urine

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Abstract A new bile alcohol, 5β-cholestanehexol, was identified in the urine of healthy humans as the glucuronide. The bile alcohol glucuronide fraction was isolated by an ion exchange chromatography on piperidinohydroxypropyl Sephadex LH-20. After enzymatic hydrolysis, the bile alcohols were converted into trimethylsilyl ether derivatives and analyzed by a combination of gas-liquid chromatography and mass spectrometry. The major bile alcohol was 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol. As minor constituents the following C_{26} and C_{27} bile alcohols were identified: 27-nor-5β-cholestane-3α,7α,12α,24,25,26-hexol, 5β-cholestane-3α,7α,12α,24,25-pentol, 5β-cholestane-3α,7α,12α,24,26-pentol, 5β-cholestane-3α,7α,12α,25,26-pentol. In addition to these bile alcohols, a new bile alcohol was identified as a sixth component of the urinary bile alcohols. The structure was assigned as (24S)-5β-cholestane-3α,7α,12α,24,25,26-hexol by the direct comparison of mass spectral data and chromatographic properties with synthetic standard. The average daily excretion of the new bile alcohol was 28.6 μg and 3.0% of the total bile alcohols. The presence of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol and 27-nor-5β-cholestane-3α,7α,12α,24,25,26-hexol suggests that 26-hydroxylation of 5β-cholestane-3α,7α,12α,24,25-pentol is most likely for the biosynthesis of this new bile alcohol. — **Hiraoka, T., K. Kihira, G. Kajiyama, T. Kuramoto, and T. Hoshita.** Identification of 5β-cholestane-3α,7α,12α,24,25,26-hexol in human urine. *J. Lipid Res.* 1987. **28:** 895–899.

Supplementary key words bile alcohol

Many bile alcohols are present as the sulfated form in bile of lower vertebrates (1). Patients with cerebrotendinous xanthomatosis (CTX) excrete large amounts of 23-, 24-, and 25-hydroxylated bile alcohols in bile, feces, and urine (2). Substantial amounts of bile alcohols are also excreted mainly as glucuronides in urine of healthy subjects and the excretion is elevated in patients with liver diseases (3–7). The bile alcohols identified in urine of healthy subjects are 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, 27-nor-5β-cholestane-3α,7α,12α,24,25,26-hexol, 5β-cholestane-3α,7α,12α,24,25-pentol, 5β-cholestane-3α,7α,12α,24,26-pentol, 5β-cholestane-3α,7α,12α,25,26-pentol, and 5β-cholestane-3α,7α,12α,26,27-pentol. The major urinary bile alcohol in healthy humans is 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol. Although 27-nor-5β-cholestane-3α,7α,12α,24,25,26-hexol, the 26-hydroxylated derivative of the major bile alcohol, has been found, 5β-cholestane-3α,7α,12α,24,25,26-hexol, the corresponding derivative of 5β-cholestane-3α,7α,12α,24,25-pentol, has not been detected. Moreover, the cholestanepentols possessing the 26-hydroxyl group have been identified in urine. These findings strongly suggest the presence of 5β-cholestane-3α,7α,12α,24,25,26-hexol in human urine.

The present study deals with the identification and synthesis of 5β-cholestane-3α,7α,12α,24,25,26-hexol in human urine.

MATERIALS AND METHODS

General

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

Proton nuclear magnetic resonance (PMR) spectra, in δ ppm, were recorded as solutions of pyridine-d_5 on a Hitachi model R-40 spectrometer at 90 MHz using tetramethylsilane as an internal standard.

Thin-layer chromatography (TLC) was carried out on precoated silica gel G plates (0.25 mm thickness, Merck). The spots were visualized by spraying 10% phosphomolybdic acid in ethanol and heating at 110°C for 5 min.

Gas–liquid chromatography (GLC) was carried out on a Shimadzu model GC-8A gas chromatograph equipped with a flame ionization detector. The columns used were

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

glass columns (3mm × 2 m) coated with 2% OV-1 or 3% OV-17. The samples were separated as their trimethylsilyl (TMS) ether derivatives and the retention times are described relative to that of the TMS ether derivative of methyl cholate (1.00).

Gas-liquid chromatography-mass spectrometry (GLC-MS) was carried out on a Shimadzu model QP-1000 gas chromatograph-mass spectrometer using the following conditions: column, a capillary column coated with OV-1 (0.35 mm × 25 m, Gasukoro Kogyo Inc., Japan); column temperature, 265°C; ion source temperature, 250°C; ionizing energy, 70 eV; trap current 60 μA.

Isolation of bile alcohols from urine. Urine was collected during a 24-hr period. A 200-ml portion of urine was passed through a glass filter and four Sep-Pak C_{18} cartridges (50 ml on each cartridge). After washing with 10 ml of water the adsorbed materials were eluted with 20 ml of methanol. The combined methanolic eluates were evaporated. The resulting residue was dissolved in 5 ml of 90% ethanol and the solution was passed through a column of piperidinohydroxypropyl Sephadex LH-20 (PHP-LH-20, 1 ml) (8) and followed by 5 ml of 90% ethanol to remove neutral compounds. A glucuronide fraction was then obtained by elution with 8 ml of 0.2 M formic acid in 90% ethanol. After evaporation of the solvent, the resulting residue was subjected to enzymatic hydrolysis at 37°C in 20 ml of a solution of 5,000 units of β-glucuronidase (EC 3.2.31., Sigma Chemical Co., Type H-1) in 0.1 M sodium acetate buffer, pH 5.0. After a 48-hr incubation period, the mixture was extracted with Sep-Pak C_{18} cartridges as described above for urine. The methanolic eluate was evaporated, dissolved in 5 ml of 90% ethanol, and then passed through a column of PHP-LH-20 (1 ml). The effluent and additional wash with 5 ml of 90% ethanol were collected to give a fraction of deconjugated bile alcohols.

Reference bile alcohols. 27-Nor-5β-cholestane-3α,7α,12α,24,25-pentol (7), 27-nor-5β-cholestane-3α,7α,12α,24,25,26-hexol (9), 5β-cholestane-3α,7α,12α,24-tetrol (10), 5β-cholestane-3α,7α,12α,24,25-pentol (11), 5β-cholestane-3α,7α,12α,25,26-pentol (12), and 5β-cholestane-3α,7α,12α,24,26-pentol (13) were synthesized or isolated from natural sources according to the methods reported previously.

Usual workup refers to extraction with an organic solvent, washing with water to neutrality, drying over anhydrous Na_2SO_4, filtration, and evaporation under reduced pressure to dryness.

Synthesis of 5β-cholestane-3α,7α,12α,24,25,26-hexol

5β-Cholest-25-ene-3α,7α,12α,24-tetrols (IIa and IIb). A solution (50 ml) of 3α,7α,12α-triacetoxy-5β-cholan-24-al (I, 10 g) prepared as described previously (10) in anhydrous benzene was added to a tetrahydrofuran solution (150 ml) containing propenyl-2-magnesium bromide prepared from 6 g of magnesium and 25 ml of 2-bromopropene. The reaction mixture was stirred for 4 hr at room temperature. After acidification with 1 N HCl, the usual workup (ethyl acetate, 200 ml × 2) gave a residue. The residue was hydrolyzed with 5% methanolic KOH (100 ml) for 2 hr by warming on a water bath (50°C). The reaction mixture was diluted with five volumes of water and the usual workup (ethyl acetate, 100 ml × 2) afforded an oily residue. The residue was chromatographed using ethyl acetate graded by acetone as an eluting solvent on a silica gel column (150 g). By monitoring on TLC, appropriate fractions containing (24R and 24S)-5β-cholest-25-ene-3α,7α,12α,24-tetrols (IIb and IIa) were collected and the solvent was removed under reduced pressure. The resulting residue (1.5 g) was rechromatographed on a silica gel column (Lober column LiChroprep Si 60, 310 × 25 mm, 40-63 μm, Merck) using chloroform-acetone-methanol 35:15:1 (by vol) as an eluting solvent. Fractions that eluted from 520 ml to 660 ml were collected and the solvent was evaporated. Repeated crystallization of the residue from methanol gave colorless crystals (210 mg) of (24S)-5β-cholest-25-ene-3α,7α,12α,24-tetrol (IIa): mp, 190.0-190.5°C; R_f on TLC, 0.33 (solvent system, chloroform-acetone-methanol, 7:3:2); RRT on GLC (as TMS ether), 1.33 (OV-1); PMR, 0.83 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.26 (3H, d, J=6 Hz, 21-CH_3), 1.88 (3H, s, 27-CH_3), 3.40-4.50 (4H, m, 3β-, 7β-, 12β-, and 24-H), 4.94 and 5.18 (2H, s, 26-H_2).

Fractions that eluted from 1300 ml to 2000 ml were combined and the solvent was evaporated to dryness. Repeated crystallization of the residue from ethyl acetate gave colorless crystals (220 mg) of (24R)-5β-cholest-25-ene-3α,7α,12α,24-tetrol (IIb): mp, 168.5-170°C; R_f on TLC, 0.29 (same solvent system for IIa); RRT on GLC (as TMS ether), 1.33 (OV-1); PMR, 0.84 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.26 (3H, d, J=6 Hz, 21-CH_3), 1.88 (3H, s, 27-CH_3), 3.40-4.50 (4H, m, 3β-, 7β-, 12β-, and 24-H), 4.98 and 5.20 (2H, s, 26-H_2).

5β-Cholestane-3α,7α,12α,24-tetrols (IIIa and IIIb). A solution of (24S)-5β-cholest-25-ene-3α,7α,12α,24-tetrol (IIa, 10 mg) dissolved in 10 ml of methanol was hydrogenated with PtO_2 (10 mg) as the catalyst at room temperature. After removal of the catalyst from the reaction mixture by filtration the solvent was evaporated to dryness. Crystallization of the residue gave crystals that were identical with authentic (24S)-5β-cholestane-3α,7α,12α,24-tetrol (IIIa) on TLC and GLC: mp, 186.0-187.0 (reported 186.0-186.5°C) (10).

(24R)-5β-Cholest-25-ene-3α,7α,12α,24-tetrol (IIb, 10 mg) was hydrogenated as described for IIa and gave crystals that were identical with authentic (24R)-5β-cholestane-3α,7α,12α,24-tetrol (IIIb) on TLC and GLC: mp, 184.5-185.0°C (reported, 185.0-185.5°C) (10).

5β-Cholestane-3α,7α,12α,24,25,26-hexol (IVa and IVb). (24S)-5β-Cholest-25-ene-3α,7α,12α,24-tetrol (IIa, 200 mg) was acetylated with acetic anhydride (20 ml) by

heating at 100°C for 18 hr. After dilution with ten volumes of water, the usual workup (ether, 200 ml × 2) gave a residue. The residue was dissolved in 5 ml of anhydrous ether and 0.5 ml of pyridine. A solution of OsO_4 (400 mg) in anhydrous ether (5 ml) was added. The reaction mixture was kept at room temperature for 60 hr and the solvent was evaporated. The resulting residue was dissolved in 20 ml of 50% ethanol containing 2 g of $NaHSO_3$ and refluxed for 2 hr. The reaction mixture was diluted with three volumes of ethanol and filtered. The filtrate was evaporated and hydrolyzed with 5% methanolic KOH (20 ml) at 60°C for 2 hr. The hydrolyzate was diluted with five volumes of water and the usual workup (ethyl acetate, 200 ml × 2) gave a residue. Repeated crystallization from ethyl acetate gave colorless crystals (122 mg) of (24S)-5β-cholestane-3α,7α,12α,24,25,26-hexol (IVa): mp, 208.0–209.0°C; R_f on TLC, 0.21 (solvent system, ethyl acetate–acetic acid–water, 17:2:1); RRT on GLC (as TMS ether), 3.71 (OV-1) and 2.29 (OV-17); PMR, 0.77 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 1.20 (3H, d, J=6 Hz, 21-CH_3), 1.45 (3H, s, 27-CH_3), 3.40–4.50 (4H, m, 3β-, 7β-, 12β-, and 24-H), 3.92 (2H, s, 26-H_2).

(24R)-5β-Cholest-25-ene-3α,7α,12α,24-tetrol (IIb, 200 mg) was treated with OsO_4 as described for the 24S isomer (IIa). Repeated crystallization of the product from ethyl acetate gave colorless crystals (110 mg) of (24R)-5β-cholestane-3α,7α,12α,24,25,26-hexol (IVb): mp, 204.0–206.0°C; R_f on TLC, 0.18 (same solvent system for IVa); RRT on GLC (as TMS ether), 3.53 (OV-1) and 2.21 (OV-17); PMR, 0.80 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.23 (3H, d, J=6 Hz, 21-CH_3), 1.53 (3H, s, 27-CH_3), 3.40–4.50 (4H, m, 3β-, 7β-, 12β-, and 24-H), 4.00 (2H, s, 26-H_2).

RESULTS AND DISCUSSION

Urine samples from eight healthy humans (six males and two females, age: 41.1 ± 7.9 years old) were fractionated by an ion exchange chromatography on PHP-LH-20 and enzymatically hydrolyzed by β-glucuronidase treatment. The isolated bile alcohols were converted into TMS ether derivatives and analyzed by GLC and GLC-MS.

The GLC analysis confirmed the presence of five bile alcohols that have been known to be present in urine. These bile alcohols were identified as 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, 27-nor-5β-cholestane-3α,7α,12α,24,25,26-hexol, 5β-cholestane-3α,7α,12α,24,25-pentol, 5β-cholestane-3α,7α,12α,24,26-pentol, and 5β-cholestane-3α,7α,12α,25,26-pentol by direct comparison with authentic samples. In addition to these bile alcohols, GLC analysis also showed the presence of a new bile alcohol with RRT of 3.71 and 2.29 (OV-1 and OV-17, respectively).

Fig. 1 shows the mass spectrum of the new bile alcohol. A series of fragments at m/z 797, 617, 527, 437, and 347 is thought to arise by the loss of CH_2OTMS moiety (103 mass units) followed by subsequent losses of trimethylsilanoxyl (TMSOH) groups from the molecular ion (900). The analogous series was observed at m/z 709, 619, 529, 439, and 349 in the mass spectrum of 5β-cholestane-3α,7α,12α,25,26-pentol (M^+ = 812 as TMS ether) and arise by the scission of the bond between C-25 and C-26 losing the CH_2OTMS moiety (103 mass units) and subsequent losses of TMSOH groups (14). The spectrum showed the fragments characteristic for 3α,7α,12α-trihydroxy-5β-steroid nucleus at m/z 343 and 253 that arose by losses of the entire side chain and nuclear TMSOH groups (15). This fact indicates that the new bile alcohol has the cholic acid type nucleus as well as the other urinary bile alcohols. The base peak was observed at m/z 219. This ion was also present in the mass spectrum of 5β-cholestane-3α,7α,12α,25,26-pentol and assigned as the side chain fragment consisting of C-25 to C-27. The fragment at m/z 129 was derived by the loss of a TMSOH group from the base ion. Therefore, the new bile alcohol seems to be a derivative of 5β-cholestane-3α,7α,12α,25,26-pentol with an additional hydroxyl group in the side chain. Another series of fragments at m/z 591, 501, 411, and 321 was also observed in the mass spectrum of the new bile alcohol. This series is characteristic for bile alcohols possessing three hydroxyl groups in the nucleus and two in the side chain at C-24 and C-25 and arose by the scission of the bond between the C-24 and C-25 followed by subsequent losses of TMSOH groups. The new bile alcohol was considered to have 24,25-dihydroxyl moiety in the side chain. As mentioned above the new bile alcohol exhibited the base ion at m/z 219. This fact also

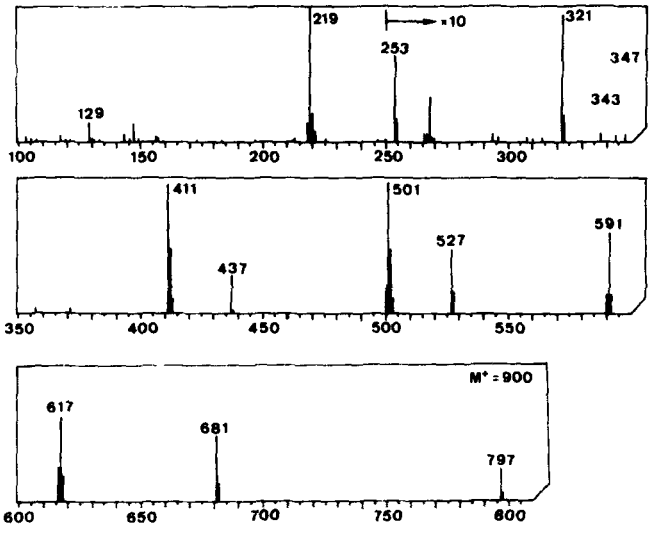

Fig. 1. Mass spectrum of the new bile alcohol, (24S)-5β-cholestane-3α,7α,12α,24,25,26-hexol as TMS ether derivative.

TABLE 1. Urinary bile alcohol of healthy humans

Bile Alcohol[a]	μg/Day[b]	RRT[c] OV-1	RRT[c] OV-17
27-Nor-5β-cholestane-3α,7α,12α,24,25-pentol	466.5 ± 111.5	1.94	1.30
27-Nor-5β-cholestane-3α,7α,12α,24,25,26-hexol	90.3 ± 36.4	2.88	1.82
5β-Cholestane-3α,7α,12α,24,25-pentol	152.7 ± 62.1	2.32	1.56
5β-Cholestane-3α,7α,12α,24,26-pentol	115.2 ± 41.1	2.26	1.59
5β-Cholestane-3α,7α,12α,25,26-pentol	105.4 ± 39.6	2.83	1.72
5β-Cholestane-3α,7α,12α,24,25,26-hexol	28.6 ± 12.1	3.71	2.29
Total	957.7 ± 219.8		

[a] Bile alcohols excreted as glucuronides.
[b] Mean ± SD (n = 8).
[c] Bile alcohols were analyzed as TMS ethers and RRTs are given relative to the TMS ether of methyl cholate.

indicated that the presence of an additional hydroxyl group at C-24 in the new bile alcohol facilitated the cleavage of the bond between C-24 and C-25 compared to 5β-cholestane-3α,7α,12α,25,26-pentol whose base ion was observed at m/z 253 but not at m/z 219. From all evidence obtained by the mass spectral analysis, the new bile alcohol was shown to have three hydroxyl groups at 3α, 7α, and 12α positions in the ring system and three in the side chain at C-24, C-25, and C-26.

To confirm the postulated structure of the new bile alcohol, the synthesis of 5β-cholestane-3α,7α,12α,24,25,26-hexol was performed. The configurations at C-24 of two isomers of 5β-cholest-25-ene-3α,7α,12α,24-tetrols (IIa and IIb) synthesized from 3α,7α,12α-triacetoxy-5β-cholan-24-al (I) were assigned by direct comparison with authentic (24R and 24S)-5β-cholestane-3α,7α,12α,24-tetrols, whose configurations at C-24 were determined previously (10), after hydrogenation. The less polar unsaturated tetrol (IIa) was assigned as the 24S-isomer and the more polar isomer as the 24R-isomer. The two unsaturated tetrols (IIa and IIb) of known absolute configuration at C-24 were individually hydroxylated at C-25 and C-26 by treatment with OsO_4. The less polar tetrol (IIa, 24S) gave the less polar hexol, (24S)-5β-cholestane-3α,7α,12α,24,25,26-hexol (IVa), and the more polar tetrol (IIb, 24R) gave the more polar hexol, (24R)-5β-cholestane-3α,7α,12α,24,25,26-hexol (IVb).

Both hexols (as TMS ethers) had mass spectra that were identical with that of the biological sample. On GLC the biological sample showed the same RRT with (24S)-isomer on both OV-1 and OV-17 columns. Consequently, the new bile alcohol was identified as (24S)-5β-cholestane-3α,7α,12α,24,25,26-hexol. The configuration at C-25 could not be assigned in the present study.

Wolthers et al. (16) reported the presence of a bile alcohol postulated as 5β-cholestane-3α,7α,12α,24,25,26-hexol in the urine of CTX patients by means of GLC–MS. The mass spectrum reported by them exhibited the base ion at m/z 131. However, the ion should be due to $(CH_3)_2COTMS$ moiety of the terminal side chain. As described above, the mass spectra of the synthetic and biological 5β-cholestane-3α,7α,12α,24,25,26-hexols showed the base ion at m/z 219, and the ion at m/z 131 was not seen in considerable intensity in the mass spectra (Fig. 1). The mass spectrum reported by Wolthers et al. (16) also showed a series of ions at m/z 720, 630, 540, and 450 due to the losses of TMSOH groups from molecular ion of TMS ether of cholestanehexol (M^+ = 900), but the series was not observed in the mass spectra of the synthetic and biological hexols. Therefore, the bile alcohol found by Wolthers et al. (16) in the urine of CTX patients could not be identified as 5β-cholestane-3α,7α,12α,24,25,26-hexol with their data.

Table 1 shows the daily excretion of the bile alcohols as glucuronides in urine estimated by GLC. The new bile alcohol, (24S)-5β-cholestane-3α,7α,12α,24,25,26-hexol, was found as the sixth urinary bile alcohol in all subjects examined here. The average excretion of this cholestanehexol was 28.6 μg daily and 3.0% of the total urinary bile alcohols.

The biosynthesis of 5β-cholestane-3α,7α,12α,24,25,26-hexol is postulated as a single hydroxylation at C-24, C-25, or C-26 of the cholestanepentols found in urine, 5β-cholestane-3α,7α,12α,25,26-pentol, 5β-cholestane-3α,7α,12α,24,26-pentol, and 5β-cholestane-3α,7α,12α,24,25-pentol, respectively.

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REFERENCES

1. Haslewood, G. A. D. 1978. *In* The Biological Importance of Bile Salts. Elsevier-North Holland Biochemical Press, Amsterdam. 79–96.
2. Shimazu, K., M. Kuwabara, M. Yoshii, K. Kihira, H. Takeuchi, I. Nakano, S. Ozawa, M. Onuki, Y. Hatta, and T. Hoshita. 1986. Bile alcohol profiles in bile, urine, and feces of a patient with cerebrotendinous xanthomatosis. *J. Biochem. (Tokyo).* **99:** 477–483.
3. Karlaganis, G., B. Almé, V. Karlaganis, and J. Sjövall. 1981. Bile alcohol glucuronides in urine. Identification of 27-nor-5β-cholestane-3α,7α,12α,24ξ,25ξ-pentol in man. *J. Steroid Biochem.* **14:** 341–345.

4. Ludwig-Köhn, H., H. V. Henning, A. Sziedat, D. Mattaei, G. Spiteller, J. Reiner, and H. J. Egger. 1983. The identification of urinary bile alcohols by gas chromatography–mass spectrometry in patients with liver disease and in healthy individuals. *Eur. J. Clin. Invest.* **13:** 91–98.
5. Karlaganis, G., V. Karlaganis, and J. Sjövall. 1984. Identification of 27-nor-5β-cholestane-3α,7α,12α,24ξ,25ξ,26-hexol and partial characterization of the bile alcohol profile in urine. *J. Lipid Res.* **25:** 693–702.
6. Ichimiya, H., J. Yanagisawa, and F. Nakayama. 1984. Significance of bile alcohol in urine of a patient with cholestasis: identification of 5β-cholestane-3α,7α,12α,26,27-pentol (5β-cyprinol) and 5β-cholestane-3α,7α,12α,26-tetrol (27-deoxy-5β-cyprinol). *Chem. Pharm. Bull.* **32:** 2874–2877.
7. Kuwabara, M., T. Ushiroguchi, K. Kihira, T. Kuramoto, and T. Hoshita. 1984. Identification of bile alcohols in urine from healthy humans. *J. Lipid Res.* **25:** 361–368.
8. Goto, J., M. Hasegawa, H. Kato, and T. Nambara. 1978. A new method for simultaneous determination of bile acids in human bile without hydrolysis. *Clin. Chim. Acta.* **87:** 141–147.
9. Kuroki, S., K. Shimazu, M. Kuwabara, M. Une, K. Kihira, T. Kuramoto, and T. Hoshita. 1985. Identification of bile alcohols in human bile. *J. Lipid Res.* **26:** 230–240.
10. Une, M., F. Nagai, K. Kihira, T. Kuramoto, and T. Hoshita. 1983. Synthesis of four diastereoisomers at carbons 24 and 25 of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid, intermediates of bile acid biosynthesis. *J. Lipid Res.* **24:** 924–929.
11. Hoshita, T. 1962. Synthesis of 3α,7α,12α,25ξ,26- and 3α,7α,12α,24ξ,25-pentahydroxycoprostanes. *J. Biochem. (Tokyo).* **52:** 176–179.
12. Okuda, K., T. Hoshita, and T. Kazuno. 1962. Isolation of a new bile sterol, 3α,7α,12α,25,26-pentahydroxycoprostane from toad bile. *J. Biochem. (Tokyo).* **51:** 48–55.
13. Okuda, K., S. Enomoto, K. Morimoto, and T. Kazuno. 1962. The isolation of a new bile sterol, 3α,7α,12α, trihydroxy-24,27-epoxycoprostane, from sting-ray bile. *J. Biochem. (Tokyo).* **51:** 441–442.
14. Kihira, K., M. Yasuhara, T. Kuramoto, and T. Hoshita. 1977. New bile alcohols, 5α- and 5β-dermophols from amphibians. *Tetrahedron Lett.* 687–690.
15. Elliott, W. H. 1971. *In* Biochemical Applications of Mass Spectrometry. G. R. Waller, editor. John Wiley & Sons, New York. 291–312.
16. Wolthers, B. G., M. Volmer, J. Van der Molen, B. J. Koopman, A. E. J. de Jager, and R. J. Waterreus. 1983. Diagnosis of cerebrotendinous xanthomatosis (CTX) and effect of chenodeoxycholic acid therapy by analysis of urine using capillary gas chromatography. *Clin. Chim. Acta.* **131:** 53–65.