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Isolation of New C_{26} Bile Alcohols from Bullfrog Bile[1-3]

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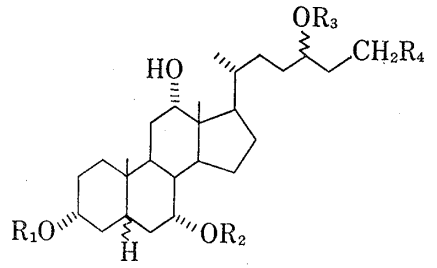
The bile alcohol constituents of bullfrog were examined to give four new C_{26} bile alcohols along with three known bile alcohols, 5α-cyprinol, 5α-ranol, and 5β-ranol. On the basis of the spectral data and the direct comparison with synthetic samples, the structures of the new bile alcohols were established to be four 27-norcholestane-3α,7α,12α,24-tetrols isomeric at C-5 and C-24, (I), (III), (VII), and (VIII).

Earlier studies on the bile acids and bile alcohols of bullfrog have led to the isolation of two important compounds. The first of these is 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid,[5] which is shown to be an intermediate in the course of cholic acid biosynthesis from cholesterol in mammals including man.[6] The second, 5β-ranol (IX) is a C_{26} bile alcohol containing a biogenetically unprecedented side chain.[7] 5β-Ranol is also formed from cholesterol in bullfrog.[8] Hence, the biosynthetic pathway of the C_{26} steroid must include presumably a hitherto unrecognized reaction involving loss of only one carbon atom from the terminal portion of the cholesterol side chain. Our interest in the biochemistry of 5β-ranol led us to re-examine the bile alcohol constituents of bullfrog. We report here isolation and characterization of four new C_{26} bile alcohols structurally related to 5β-ranol.



I (5α, 24α, $R_1=R_2=R_3=R_4=H$)
II (5α, 24α, $R_1=R_2=R_3=Ac$, $R_4=H$)
III (5α, 24β, $R_1=R_2=R_3=R_4=H$)
IV (5α, 24β, $R_1=R_2=R_3=Ac$, $R_4=H$)
V (5α, 24ξ, $R_1=R_2=R_3=H$, $R_4=OH$)
VI (5α, 24ξ, $R_1=R_2=R_3=Ac$, $R_4=OAc$)
VII (5β, 24α, $R_1=R_2=R_3=R_4=H$)
VIII (5β, 24β, $R_1=R_2=R_3=R_4=H$)
IX (5β, 24ξ, $R_1=R_2=R_3=H$, $R_4=OH$)

Chart 1. C_{26} Bile Alcohols

Hydrolysis of the bile extract of bullfrog, *Rana catesbeiana*, afforded a mixture of bile alcohols, which was fractionated by column chromatography to give seven principal bile alcohols, designed as A—G in order to increasing polarity (Chart 2).

1) Partly presented at the 93rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1973.
2) This paper is Part VI of a series entitled "Comparative biochemical studies of bile acids and bile alcohols." Part V: T. Hoshita, M. Yasuhara, K. Kihira, and T. Kuramoto, *Steroids*, **27**, 657 (1976).
3) The following trival names of bile acids and bile alcohols have been used: allocholic and cholic acids, 3α,7α,12α-trihydroxy-5α- and 5β-cholan-24-oic acids; 5α- and 5β-cyprinols, 5α- and 5β-cholestane-3α,7α,-12α,26,27-pentols; 5α- and 5β-bufols, 5α- and 5β-cholestane-3α,7α,12α,25,26-pentols; 5α- and 5β-ranols, 27-nor-5α- and 5β-cholestane-3α,7α,12α,24ξ,26-pentols.
4) Location: *Kasumi 1-2-3, Hiroshima.*
5) Y. Kurauti and T. Kazuno, *Z. Physiol. Chem.*, **262**, 53 (1939).
6) T. Komatsubara, *Seikagaku*, **27**, 519 (1955); R.J. Bridgwater and S. Lindstedt, *Acta Chem. Scand.*, **11**, 409 (1957); E. Staple and J.L. Rabinowitz, *Biochim. Biophys. Acta*, **59**, 735 (1962); J.B. Carey Jr., *J. Clin. Invest.*, **43**, 1443 (1964).
7) T. Kazuno, T. Masui, and K. Okuda, *J. Biochem.* (Tokyo), **57**, 75 (1965).
8) T. Masui, *J. Biochem.* (Tokyo), **51**, 112 (1962); S. Betsuki, *ibid.*, **60**, 411 (1966); S. Betsuki, *Hiroshima J. Med. Sci.*, **15**, 25 (1966).

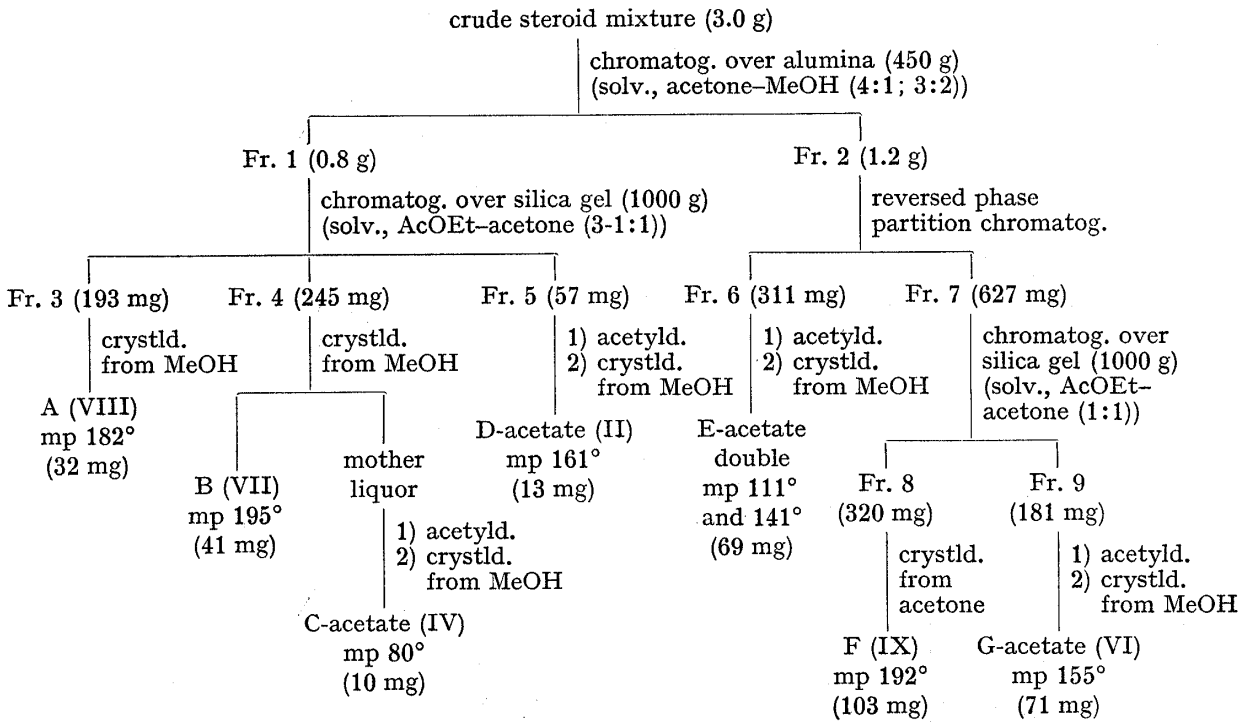


Chart 2. Fractionation of Bile Alcohols obtained from Bullfrog Bile

The mass spectrum of bile alcohol A (VIII), mp 182°, depicted a molecular ion at *m/e* 422 corresponding to an empirical formula $C_{26}H_{46}O_4$. The spectrum also showed fragment ions at *m/e* 404 ($M-H_2O$), 386 ($M-2H_2O$), 368 ($M-3H_2O$), 350 ($M-4H_2O$), 271 ($M-(2H_2O+$side chain)), and 253 ($M-(3H_2O+$side chain)), indicating that VIII contains four hydroxyl groups, three on the nuclear and one on the side chain. Infrared (IR) spectrum of VIII closely resembled that of cholic acid in the region between 850 cm^{-1} and 1300 cm^{-1}, indicating that VIII has the cholic acid nucleus.[9] Nuclear magnetic resonance (NMR) spectrum of VIII exhibited four methyl resonances: two singlets at δ 0.79 and 0.99, and a doublet at δ 1.27 ascribable to the C-18, C-19, and C-21 methyl groups, respectively; a triplet δ 1.15 must be part of the side chain since the mass spectral evidence cited above supports the C_{19} nucleus. The NMR spectrum also showed four carbinyl hydrogen signals, indicating that the hydroxyl group on the side chain as well as three nuclear such groups is secondary. Irradiation at δ 1.70 in methylene envelope results in conversion of the triplet at δ 1.15 into a singlet and a broad multiplet at δ 3.64 into a triplet-like signal. This indicates the presence of a $-CHOH-CH_2-CH_3$ moiety in the side chain. These physico-chemical data suggest that bile alcohol A might be a 27-nor-5β-cholestane-3α,7α,12α,24ξ-tetrol.

Bile alcohol B (VII), mp 195°, depicted IR, NMR, and mass spectra essentially identical to those of bile alcohol A (VIII). Gas chromatographic properties of both the bile alcohols as their trimethylsilyl (TMS) ethers were identical to each other. Since our experience suggests that 24-epimeric bile alcohols do not separate on gas–liquid chromatography (GLC), and can't distinguish in their spectra, bile alcohol B (VII) might be the 24-epimer of bile alcohol A (VIII).

The structures of bile alcohols A and B were confirmed by the direct comparison with synthetic samples of two 27-nor-5β-cholestane-3α,7α,12α,24-tetrols epimeric at C-24 which were prepared as follows (Chart 3). Cholic acid (Xb) was converted into the formyl ester using formic acid. Reaction with thionyl chloride of the formate yielded the acid chloride which was treated with diethylcadmium followed by alkaline hydrolysis to remove the protective formyl

9) G.A.D. Haslewood, "Bile Salts," Methuen and Co., London, 1967, p. 34.

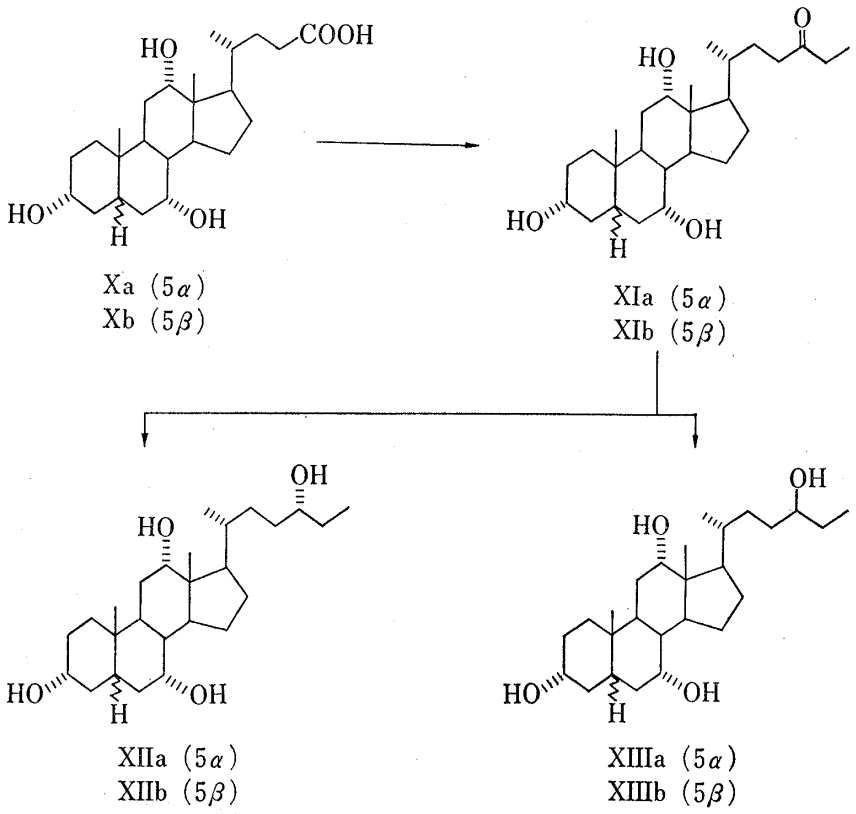


Chart 3. Synthesis of Norcholestanetetrols

groups to produce 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one (XIb). Reaction with sodium borohydride of the 24-ketone (XIb) gave a mixture of two 27-nor-5β-cholestane-3α,7α,12α,24-tetrols epimeric at C-24. The separation of these epimers was achieved by silica gel column chromatography. The less polar epimer (XIIIb) was identical with bile alcohol A in melting point, optical rotation, chromatographic properties, and spectral data. The more polar epimer (XIIb) was identical in all respects with bile alcohol B. Tentative assignment of the 24α and 24β configurations was made on the basis of optical rotation differences and mobilities on thin-layer chromatography (TLC). As shown in Table I, among pairs of bile alcohols epimeric at C-24, the 24α-epimers possessed highly positive optical

Table I. Optical Rotations and Relative Mobilities on Thin-Layer Chromatograms of 24-Hydroxylated Bile Alcohols

Bile alcohol	$[\alpha]_D$	Relative mobility[a]
5β-Cholestane-3α,7α,12α,24α-tetrol[b]	+39°	1.00[c]
5β-Cholestane-3α,7α,12α,24β-tetrol[b]	+24°	1.20[c]
5β-Cholestane-3α,7α,12α,24α,25-pentol[d]	+45°	1.00[e]
5β-Cholestane-3α,7α,12α,24β,25-pentol[d]	+29°	1.13[e]
27-Nor-5α-cholestane-3α,7α,12α,24α-tetrol (I=XIIa)		1.00[c]
27-Nor-5α-cholestane-3α,7α,12α,24β-tetrol (III=XIIIa)		1.26[c]
27-Nor-5β-cholestane-3α,7α,12α,24α-tetrol (VII=XIIb)	+36°	1.00[c]
27-Nor-5β-cholestane-3α,7α,12α,24β-tetrol (VIII=XIIIb)	+27°	1.25[c]

a) relative to italicized reference compound
b) T. Masui and E. Staple, *Steroids*, **9**, 443 (1967)
c) solvent system; EA-2
d) S. Shefer, B. Dayal, G.S. Tint, G. Salen, and E.H. Mosbach, *J. Lipid Res.*, **16**, 280 (1975)
e) solvent system: CAM

rotations and moved with lesser mobilities on TLC than their 24β-counterparts. Hence, the more polar epimer (bile alcohol B, VII=XIIb), being the more dextrorotatory of the two epimers may be assigned the 24α configuration, 27-nor-5β-cholestane-3α,7α,12α,24α-tetrol; the less polar epimer (bile alcohol A, VIII=XIIIb) is assigned the 24β configuration, 27-nor-5β-cholestane-3α,7α,12α,24β-tetrol.

Bile alcohols C (III) and D (I) were isolated as their acetates (IV), mp 80° and (II), mp 161°, respectively. IR spectra of II and IV were identical to each other, and closely resembled that of 5α-ranol 3,7,24,26-tetraacetate (VI), suggesting that these acetates have the same nuclear structure, *i.e.* the 3,7-diacetylated allocholic acid nucleus. Mass spectrum of II was completely identical with that of IV, and similar to that of 5α-ranol tetraacetate (VI) with respects of peak intensities and fragmentation pattern. The only difference was that a series of peaks (*m/e*: 488, 470, 428, 410, 368, and 350) in the spectra of II and IV are at *m/e* ratio consistently lower by 58 mass units than their counterparts, *m/e*: 546 (M−AcOH), 528 (M−(AcOH+H_2O)), 486 (M−2AcOH), 468 (M−(2AcOH+H_2O)), 426 (M−3AcOH), and 408 (M−(3AcOH+H_2O)), in the spectrum of 5α-ranol tetraacetate (VI). On GLC, the TMS ethers of bile alcohols C and D can't resolved and eluted together with a shorter retention time than the TMS ether of bile alcohol A or B. The ratios (Table II) of the retention times between bile alcohol C (=D) and bile alcohol A (=B) were in good agreement with the constant separating factors found in pairs of 5α-bile alcohols and their 5β-counterparts under the employed conditions. These findings indicate that bile alcohols C and D might be the 5α-isomers of bile alcohols A and B.

Table II. Ratios of the Gas Chromatographic retention times between 5α-Bile Alcohols and 5β-Bile Alcohols

Bile alcohol[a)]	Relative retention time					
	5α		5β		5α/5β	
	OV-1	OV-17	OV-1	OV-17	OV-1	OV-17
Cholestane-3α,7α,12α-triol	0.77	0.56	0.82	0.62	0.94	0.90
Cholestane-3α,7α,12α,26-tetrol	1.64	1.10	1.73	1.25	0.95	0.88
Ranol	2.16	1.42	2.28	1.57	0.95	0.90
Bufol	2.54	1.55	2.68	1.71	0.95	0.91
Cyprinol	2.83	1.82	2.99	2.01	0.95	0.90
Bile alcohol A (=B)			1.22	0.88	0.94	0.89
Bile alcohol C (=D)	1.15	0.78				

a) The bile alcohols were chromatographed as their TMS derivatives.

The structures of bile alcohols C (III) and D (I) were established by the direct comparison with synthetic samples of two 27-nor-5α-cholestane-3α,7α,12α,24-tetrols epimeric at C-24, which were prepared from allocholic acid (Xa) according to the procedure described above for the 5β-isomers, (XIIb) and (XIIIb) (Chart 3). The less polar epimer, 27-nor-5α-cholestane-3α,7α,12α,24β-tetrol (XIIIa) depicted GLC retention times and TLC mobilities identical to those of bile alcohol C (III). The IR and mass spectra, TLC mobilities, melting point, and optical rotation of the 3,7,24-triacetylated derivative of the synthetic tetrol (XIIIa) compared well with those of C-acetate (IV). The more polar epimer, 27-nor-5α-cholestane-3α,7α,12α,24α-tetrol (XIIa) and its 3,7,24-triacetate were completely identical in all respects with bile alcohol D (I) and its acetate (II), respectively.

Bile alcohols E, F, and G were identified as 5α-cyprinol, 5β-ranol (IX), and 5α-ranol (V), respectively, by the direct comparison with known reference compounds.

The stereochemistry at C-24 in 5α- and 5β-ranols is not known. It can, however, be assumed that the 24-hydroxyl group of either 24α- or 24β-epimer of 27-nor-5α(or 5β)-cholestane-

3α,7α,12α,24-tetrols has the same configuration to that of 5α(or 5β)-ranol. The structural relationship between the tetrols and the pentols, and their co-existence suggest that the formers are the direct biosynthetic precursors of the latters. Biosynthesis and metabolism of these C_{26} bile alcohols in bullfrog are now under investigation.

Experimental

Melting points were determined with a Yanaco micromelting point apparatus and are uncorrected. Optical rotations were taken in MeOH solution with a JASCO DIP-180 polarimeter at room temperature. IR spectra were taken with a JASCO IRA-1 spectrometer. NMR spectra were obtained at 100 MHz on a JEOL JNM-PS-100 spectrometer using pyridine-d_5 as solvent, and chemical shifts are given in δ (ppm) scale with tetramethylsilane as internal standard. Signal multiplicities are represented by s(singlet), d(doublet), t(triplet), and m(multiplet). Molecular weights were determined from the molecular ion using high-resolution mass spectra, which were recorded on a JEOL JMS-01SG-2 mass spectrometer with an accelerating potential of 9.45 kV, an ionization potential of 75 eV, and a source temperature of 120—140°. GLC was run on a Shimadzu GC-6A gas chromatograph using glass columns (2 m×4 mm) packed with 3% OV-1, OV-17, or QF-1 on Gas-Chrom Q (80—100 mesh) from Applied Science Laboratories. TMS ethers were prepared with N-trimethylsilylimidazol at 90° for 1 hr. Retention times are reported relative to methyl cholate or its TMS derivative. TLC was carried out on Kieselgel G nach Stahl (Merck) using 10% solution of phosphomolybdic acid in EtOH (spraying followed by heating) as detection reagent. The following solvent systems were used: EA-2, AcOEt–acetone (7: 3); CAM, $CHCl_3$–acetone–MeOH (70: 50: 15); BE, benzene–AcOEt (3: 1). Adsorption column chromatography was carried out using alumina (300 mesh, Wako) or silica gel (70—325 mesh, Merck). Reversed phase partition column chromatography was performed according to the procedure previously reported[10] with the following solvent system: MeOH–H_2O (1: 1) as the moving phase; $CHCl_3$–isooctanol (1: 1) as the stationary phase. Hostalene (100 g, polyethylene powder, Farbwerke Hoechst, A.G.) was employed as the supporting material for the stationary phase (60 ml). The ratios of solvents in mixture are given in v/v.

Isolation of Bile Alcohols from Bullfrog Bile——The EtOH extractive (4.6 g) obtained from gall-bladders of about 200 bullfrogs was treated with a 40% CCl_3COOH in dioxan according to the procedure described in a previous paper of this series[11] to yield a neutral steroid mixture (3.1 g). The crude steroid mixture was fractionated, under monitoring by GLC and TLC, to give seven kinds of homogeneous compounds as shown in Chart 2.

Bile Alcohol A (VIII)——Evaporation of the solvent from Fr. 3 and recrystallization from MeOH gave crystals of bile alcohol A (VIII), mp 182°, $[\alpha]_D$ +27° (c=0.26). TLC: *Rf* 0.30 (EA-2). GLC: rrt 1.06 (QF-1), 1.22 (as the TMS, OV-1), 0.88 (as the TMS, OV-17). *Anal.* high-resolution mass spectrum, Calcd. for $C_{26}H_{46}O_4$ (M^+, *m/e*): 422.33961. Found: 422.33102. Mass Spectrum *m/e* (rel. int.): 404 (1), 386(35), 368(8), 350(5), 271(100), 253(99). IR ν_{max}^{KBr} cm^{-1}: 3350(OH). NMR: 0.79 (3H, s, 18-C$\underline{H}_3$), 0.99 (3H, s, 19-C$\underline{H}_3$), 1.15 (3H, t, 26-C$\underline{H}_3$), 1.27 (3H, d, 21-C$\underline{H}_3$), 3.5—4.4 (4H, m, C$\underline{H}$–OH). Bile alcohol A was identified with an authentic sample of synthetic 27-nor-5β-cholestane-3α,7α,12α,24β-tetrol (XIIIb) by comparison of TLC, GLC, IR, NMR, and mass spectra, and by mixed melting point determination.

Bile Alcohol B (VII)——Fr. 4 was crystallized from MeOH to give crystals of bile alcohol B (VII), mp 195°, $[\alpha]_D$ +35° (c=0.26). TLC: *Rf* 0.24 (EA-2). GLC: rrt 1.06 (QF-1), 1.22 (as the TMS, OV-1), 0.88 (as the TMS, OV-17). *Anal.* high-resolution mass spectrum, Calcd. for $C_{26}H_{46}O_4$ (M^+, *m/e*): 422.33961. Found: 422.33567. Mass Spectrum *m/e* (rel. int.): 404 (1), 386(67), 368 (21), 350 (4), 271 (100), 253 (75). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH). NMR: 0.84 (3H, s, 18-C$\underline{H}_3$), 1.01 (3H, s, 19-C$\underline{H}_3$), 1.17 (3H, t, 26-C$\underline{H}_3$), 1.28 (3H, d, 21-C$\underline{H}_3$), 3.5—4.4 (4H, m, C$\underline{H}$–OH). Identified with an authentic sample of synthetic 27-nor-5β-cholestane-3α,-7α,12α,24α-tetrol (XIIb) by the comparison of their chromatographic properties and the spectral data and by mixed melting point determination.

Bile Alcohol C (III)——The mother liquor free from the crystals of bile alcohol B, after being evaporation, gave a residue, which was treated with a mixture (1: 1) of Ac_2O and pyridine at room temperature overnight. Isolation in the usual manner and crystallization from MeOH afforded crystals of C-acetate (IV), mp 80°, $[\alpha]_D$ +16° (c=0.24). TLC: *Rf* 0.50 (BE). GLC: 2.06 (QF-1). Mass Spectrum *m/e* (rel. int.): 488 (7), 470 (96), 428 (32), 410 (46), 368 (9), 350 (25), 313 (100), 253 (75). IR ν_{max}^{KBr} cm^{-1}: 3560 (OH), 1720, 1255 (AcO). Treatment with 5% methanolic KOH of the acetate (IV) by the usual method gave bile alcohol C as a solid forming only gelatinous amorphous precipitates from organic Solvents. TLC: *Rf* 0.24 (EA-2). GLC: rrt 1.13 (QF-1), 1.15 (as the TMS, OV-1), 0.78 (as the TMS, OV-17). III and IV were identified (TLC, GLC, IR, mass spectrum, and mixed mp) with samples of synthetic 27-nor-5α-cholestane-3α,7α,12α,24β-tetrol (XIIIa) and its 3,7,24-triacetate, respectively.

10) S. Bergström and J. Sjövall, *Acta Chem. Scand.*, **5**, 1267 (1951).
11) T. Kuramoto, H. Kikuchi, H. Sanemori, and T. Hoshita, *Chem. Pharm. Bull.* (Tokyo), **21**, 952 (1973).

Bile Alcohol D (I)——Fr. 5 was treated with Ac_2O in pyridine and worked up in the usual manner. Crystallization from MeOH gave crystals of D-acetate (II), mp 161°, $[\alpha]_D$ −1° (c=0.23). TLC: *Rf* 0.50 (BE). GLC: rrt 2.01 (QF-1). Mass spectrum *m/e* (rel. int.): 488 (10), 470 (100), 428 (37), 410 (44), 368 (11), 350 (23), 313 (100), 253 (71). IR ν_{max}^{KBr} cm^{-1}: 3560 (OH), 1720, 1255 (AcO). Alkaline hydrolysis of the acetate (II) gave bile alcohol D as solid forming only gelatinous amorphous precipitates from organic solvents. TLC: *Rf* 0.19 (EA-2). GLC: rrt 1.13 (QF-1), 1.15 (as the TMS, OV-1), 0.78 (as the TMS, OV-17). Respectively identified (TLC, GLC, IR, mass spectrum, and mixed mp) with samples of synthetic 27-nor-5α-cholestane-3α,7α,12α,24α-tetrol and its 3,7,24-triacetate.

Bile Alcohol E——Fr. 6 was treated with Ac_2O in pyridine and worked up in the usual manner to give crystals of E-acetate, double mp 111° and 141° (MeOH), which was identified (TLC, GLC, IR, mass spectrum, and mixed mp) with an authentic sample of 5α-cyprinol 3,7,26.27-tetraacetate.

Bile Alcohol F (IX)——Fr. 8 was crystallized from acetone to give crystals of bile alcohol F (IX), mp 192°, $[\alpha]_D$ +26° (c=0.33). Identified (TLC, GLC, IR, NMR, mass spectrum, and mixed mp) with an authentic sample of 5β-ranol.

Bile Alcohol G (V)——Fr. 9 was treated with Ac_2O in pyridine and the product was crystallized from MeOH to give crystals of G-acetate (VI), mp 155°, which was identified (TLC, GLC, IR, mass spectrum, and mixed mp) with an authentic sample of 5α-ranol 3,7,24,26-tetraacetate.

Preparation of 27-Nor-5α-cholestane-3α,7α,12α,24α-tetrol (XIIa) and 27-Nor-5α-cholestane-3α,7α,12α,-24β-tetrol (XIIIa)——Allocholic acid (Xa) (500 mg) was dissolved in 10 ml of HCOOH and the solution was heated at 55° for 5 hr. The reaction mixture was diluted with water and extracted with ether. The ethereal extract was washed with water until free from HCOOH and the solvent was evaporated to dryness under reduced pressure. To the resulting residue was added 5 ml of $SOCl_2$, the reaction was allowed to proceed at room temperature for 2 hr. The $SOCl_2$ was removed at room temperature *in vacuo* and the resulting acid chloride was used, without purification, for the following reaction. To a flask containing diethylcadmium prepared from $CdCl_2$ (2 g) and ethylmagnesium bromide (3 g) in 20 ml of ether, a solution of the acid chloride in 20 ml of benzene was added. The reaction mixture was refluxed for 4 hr. After cooling to 0° in ice, 5% H_2SO_4 and crushed ice were added in order to decompose the reaction product. The solution was extracted with ether. The ethereal extract was washed with water, dried over Na_2SO_4 and then evaporated to dryness. The residue was dissolved in 5% methanolic KOH and was refluxed for 2 hr. The solution was diluted with water and extracted with AcOEt. Evaporation of the solvent from the washed and dried extract left a crystalline solid of 3α,7α,12α-trihydroxy-27-nor-5α-cholestan-24-one (XIa) (251 mg).

To a solution of the 24-ketone (XIa) dissolved in 50 ml of MeOH was added 0.5 g of $NaBH_4$. The reaction mixture was allowed to stand at room temperature for 5 hr and then was diluted with water. Extraction with AcOEt, and evaporation of the solvent gave a gelatinous material (187 mg), which was chromatographed on a column of silica gel (187 g) made up in AcOEt. Elution with 40% acetone in AcOEt gave 55 mg of 27-nor-5α-cholestane-3α,7α,12α,24β-tetrol (XIIIa) as a solid. Treatment with a mixture of Ac_2O and pyridine of the tetrol (XIIIa) and the usual work-up provided crystals (10.1 mg) of 3,7,24-triacetate of XIIIa, mp 81° (MeOH), $[\alpha]_D$ +15° (c=0.20). *Anal.* Calcd. for $C_{32}H_{52}O_7$: C, 70.04; H, 9.55. Found: C, 70.11; H, 9.29. Elution with 50% acetone in AcOEt gave 60 mg of XIIa, which was acetylated as described above to afford crystals (12.0 mg) of 3,7,24-triacetate of 27-nor-5α-cholestane-3α,7α,12α,24α-tetrol XIIa, mp 162° (MeOH), $[\alpha]_D$ +2° (c=0.21). *Anal.* Calcd. for $C_{32}H_{52}O_7$: C, 70.04; H, 9.55. Found: C, 70.30; H, 9.46.

Preparation of 27-Nor-5β-cholestane-3α,7α,12α,24α-tetrol (XIIb) and 27-Nor-5β-cholestane-3α,7α,12α,-24β-tetrol (XIIIb)——Synthesis from 1.3 g of cholic acid (Xb) and reduction with $NaBH_4$ (1 g) of 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one (XIb) were carried out according to the procedure described above for the 5α-isomer (XIa). The resulting mixture (550 mg) of the epimers XIIb and XIIIb was separated on a column of silica gel (550 g). Elution with 35% acetone in AcOEt gave a solid (153 mg). Recrystallization from MeOH afforded crystals (28 mg) of 27-nor-5β-cholestane-3α,7α,12α,24β-tetrol (XIIIb), mp 184°, $[\alpha]_D$ +27° (c=0.40). *Anal.* Calcd. for $C_{26}H_{46}O_4$: C, 73.88; H, 10.97. Found: C, 73.78; H, 10.87. Elution with 40% acetone in AcOEt gave a solid (162 mg) which was recrystallized from MeOH to afford crystals (32 mg) of 27-nor-5β-cholestane-3α,7α,12α,24α-tetrol (XIIb), mp 196°, $[\alpha]_D$ +36° (c=0.33). *Anal.* Calcd. for $C_{26}H_{46}O_4$: C, 73.88; H, 10.97. Found: C, 73.72; H, 10.90.