papers and notes on methodology

Potential bile acid metabolites. 9. 3,12-Dihydroxy- and 12β-hydroxy-5α-cholanoic acids[1]

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Abstract Syntheses of the heretofore unreported 3α,12β-, 3β,12β-dihydroxy-, and 12β-hydroxy-5α-cholanic acids of the 5α-series, their methyl esters, and some related derivatives are described. In addition, allodeoxycholic (3α,12α-dihydroxy) acid was prepared by a new route. The principal reactions involved were the stereoselective reduction of C-12 ketones with an amino-borane reagent and of a C-3 ketone with K-Selectride®, and inversion of a 3β-tosylate derivative with N,N-dimethylformamide. — **Iida, T., T. Tamura, T. Matsumoto, and F. C. Chang.** Potential bile acid metabolites. 9. 3,12-Dihydroxy- and 12β-hydroxy-5α-cholanoic acids. *J. Lipid Res.* 1985. **26:** 874–881.

Supplementary key words allo bile acids

As a result of the work reported in earlier papers of this series (1–4), the 26 theoretically possible 5β-cholanic acids substituted with one to three hydroxyl groups at positions 3, 7, and 12 are now known and characterized. Since the "allo" cholanic (5α, A/B *trans*) acids are also potential metabolites (5),[3] we are extending our program to include the synthesis of some of the 5α analogs that have not yet been reported. Of these we have chosen initially to prepare three which can be derived from the available 5β compound, deoxycholic acid [5β-3α,12α-$(OH)_2$; **Scheme 1**, I] first producing the inverted 5α-intermediates by using known and improved methods, and finally employing several methods developed for the syntheses of the 5β analogs.

Two of the four possible stereoisomeric 3,12-dihydroxy cholanic acids of the 5α series, the 3β,12β- (II) and 3α,12β- (III) epimers and one of the four possible monohydroxy isomers, the 12β-hydroxy acid (IV), have not been reported. In this publication we describe the synthesis of these new acids, their methyl esters, and some related derivatives. Also we report a new route to allodeoxycholic acid [3α,12α-dihydroxy-5α-cholanic acid:V] (6, 7).

EXPERIMENTAL PROCEDURES AND RESULTS

Melting points were determined on an electric micro hot stage and are uncorrected. IR spectra were obtained on a Model IRA-II JASCO double-beam spectrophotometer as KBr tablets. UV spectra were determined in ethanol solution using a Model UV-200 Shimadzu double-beam spectrophotometer. ^1H- and ^{13}C-NMR spectra were obtained on Hitachi R-22 (90 MHz) and JEOL FX-90Q (22.53 MHz) instruments, respectively, with $CDCl_3$ containing 1% Me_4Si as the solvent except where otherwise indicated. Chemical shifts are expressed in δ ppm relative to Me_4Si. High resolution mass spectra were recorded on a Hitachi RMU-7M mass spectrometer using the following conditions: ion source temperature, 180°C; ionizing voltage, 70 eV. HPLC was carried out on a Waters Associates system (M-45 pump; U6K sample loop injector; R401 differential refractometer or Shimadzu Model SPD-2A UV detector) using a Nova-Pak C_{18} (15 cm × 3.9 mm I.D., 5 μm; Waters Associates) reversed phase column with methanol–water mixtures as mobile phase. Analytical TLC was performed on pre-coated silica gel (20 cm × 20 cm, 0.25 mm layer thickness; Merck).

Abbreviations: IR, infrared; UV, ultraviolet; NMR, nuclear magnetic resonance; MS, mass spectrometry; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; DMF, N,N-dimethylformamide; THF, tetrahydrofuran.

[1] In uniformity with the nomenclature of the previous papers of this series, the older name "cholanic" is used in place of the newer IUPAC-suggested "cholanoic" acids. The corresponding methyl esters at C-24 are designated "a" after the compound number.

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[3] Derivatives of cholanic acid in which the hydrogen at C-5 is α-oriented rather than β-oriented as in the large number of naturally occurring bile acids. Elliott has compiled a comprehensive chapter on allo bile acids (ref. 5).

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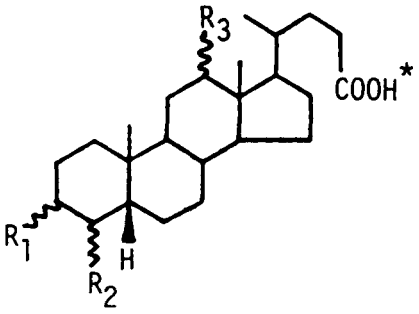

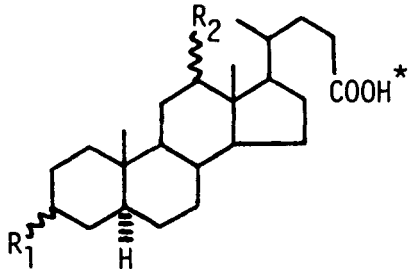

	R_1	R_2	R_3
I	α−OH	H	α−OH
VI	=O	β−Br	α−OH
VII	=O	H	α−OH
VIII	=O	Δ^4	α−OH

	R_1	R_2
II	β−OH	β−OH
III	α−OH	β−OH
IV	H	β−OH
V	α−OH	α−OH
IX	β−OH	α−OH
X	=O	α−OH
XI	=O	=O
XII	β−OAc	α−OH
XIII	β−OAc	=O
XIV	β−OAc	β−OH
XV	OMe, OMe	=O
XVI	=O	β−OH
XVII	β−OTs	α−OH
XVIII	α−OCHO	α−OH
XIX	H	α−OH
XX	H	=O

Scheme 1. ★ The corresponding methyl esters are designated "a".

The spots were detected by spraying with 0.5% vanillin in H_2SO_4–ethanol 4:1 (v/v) and heated at 110°C. All compounds were dried by azeotropic distillation before use in reactions.

General procedure for hydrolysis to free acid

The methyl ester was refluxed in 5% methanolic KOH (100 mg of ester / 3 ml of base) for 1 hr. Solvent was evaporated, and the residue was dissolved in water, cooled in an ice-bath, and acidified with 3 N H_2SO_4 with stirring. The precipitated solid was filtered and crystallized in an appropriate solvent.

Methyl 4β-bromo-12α-hydroxy-3-oxo-5β-cholanate (VIa)

Methyl 12α-hydroxy-3-oxo-5β-cholanate VIIa (45 g; mp, 145–147°C), prepared from I (8), was dissolved in 380 ml of DMF containing 3.15 g of *p*-toluenesulfonic acid, and treated dropwise (ca. 5 hr) with a solution of 25 g of bromine in 85 ml of DMF. The mixture was then stirred overnight at room temperature. Water was added and the precipitated oil was extracted with CH_2Cl_2 (× 2). The CH_2Cl_2 extract was washed with 5% $Na_2S_2O_3$ solution and water, dried (Drierite), and evaporated to dryness. The light yellow residue, when treated with EtOAc-hexane, afforded 34.45 g of prismatic needles (64%, in two crops): mp, 131.5–132.5°C (lit. 134.0–134.5°C (9)); $\nu_{max.}$ 1735 (C=O), 3590, 1073, 1033, 983, 962 (OH), 555 (C-Br); ^{1}H-NMR, 0.74 (3H, s, C-18 Me), 1.00 (3H, d, j = 6.3 Hz, C-21 Me), 1.09 (3H, s, C-19 Me), 3.65 (3H, s, COOMe), 3.99 (1H, m, C-12 C<u>H</u>OH), 4.97 (1H, d, j = 11.7 Hz, C-4 H).

Methyl Δ^4-12α-hydroxy-3-oxo-cholenate (VIIIa)

To a solution of 4β-bromo compound VIa (20 g) in 800 ml of dioxane was added a solution prepared by dissolving 14.6 g of semicarbazide hydrochloride and 10 g of sodium acetate in 120 ml of water. The mixture was stirred for 3 hr under N_2 at room temperature. Pyruvic acid (58 ml) in 220 ml of water was then added and the mixture was further stirred overnight at room temperature. Water was added gradually to near turbidity to cause crystallization of the Δ^4-3-ketone ester (VIIIa). Recrystallization from aqueous acetone gave 15.04 g (90%) of an analytical sample: mp, 150.0–151.5°C (lit. 144–145°C (9) and 151–152 (10)); $\nu_{max.}$ 1715, 1660 (C=O), 1608, 860, 773 (Δ^4), 3530, 1048, 972, 958 (OH): $\lambda_{max.}$ 242 nm (ε 14,400); ^{1}H-NMR, 0.74 (3H, s, C-18 Me), 0.99 (3H, d, j = 5.4 Hz, C-21 Me), 1.18 (3H, s, C-19 Me), 3.63 (3H, s, COOMe), 4.01 (1H, m, C-12 C<u>H</u>OH), 5.66 (1H, s, C-4 H).

Δ^4-12α-Hydroxy-3-oxo-cholenic acid (VIII) was obtained quantitatively from the corresponding ester VIIIa by the

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general hydrolysis method described at the beginning of the Experimental section: light yellow crystals of VIII crystallized from aqueous methanol, mp, 235–238°C (lit. 247–250°C (8)); $\nu_{max.}$ 1695, 1630 (C=O), 1610 (Δ^4), 3470, 1042, 975, 957 (OH); $\lambda_{max.}$ 240 nm (ϵ 14,200); ^{1}H-NMR ($CDCl_3$ + 20% Me_2SO-d_6), 0.70 (3H, s, C-18 Me), 0.97 (3H, d, j = 6.3 Hz, C-21 Me), 1.14 (3H, s, C-19 Me), 3.90 (1H, m, C-12 CHOH), 5.62 (1H, s, C-4 H).

Methyl 3β,12α-dihydroxy-5α-cholanate (IXa) and methyl 12α-hydroxy-3-oxo-cholanate (Xa)

Δ^4-12α-Hydroxy-3-oxo acid VIII (12 g) was reduced by the method of Kallner (11), using methanol at the end of the reaction. After the treatment of the residual bile salts by acidification followed by methylation, the crude reaction product (10.23 g) was chromatographed over neutral alumina (activity grade II; 360 g). The fractions eluted by benzene–EtOAc 8:2 (v/v) gave 2.68 g (21%) of the 12α-hydroxy-3-oxo ester Xa: mp, 141.5–143.0°C (from acetone–hexane) (lit. 134–136°C (6) and 144–145°C (12)); $\nu_{max.}$ 1725, 1708 (C=O), 3470, 1030, 1017, 967, 955 (OH); ^{1}H-NMR, 0.72 (3H, s, C-18 Me), 1.01 (3H, s, C-19 Me), 3.65 (3H, s, COOMe), 3.97 (1H, m, C-12 CHOH).

Continuous elution with benzene–EtOAc 1:1 (v/v) gave a major fraction (6.89 g; 54%) of the 3β,12α-dihydroxy ester IXa, which crystallized from aqueous methanol as fine needles: mp, 140–142°C (lit. 137–138°C (6, 12)); $\nu_{max.}$ 1715 (C=O), 3500, 1075, 1035, 980, 955 (OH); ^{1}H-NMR, 0.71 (3H, s, C-18 Me), 0.82 (3H, s, C-19 Me), 0.98 (3H, d, j = 5.4 Hz, C-21 Me), 3.57 (1H, brm, C-3 CHOH), 3.65 (3H, s, COOMe), 3.97 (1H, m, C-12 CHOH); MS, m/z (relative intensity), 388 (5%, M – H_2O), 273 (100%, M – H_2O – S.C.), 255 (28%, M – $2H_2O$ – S.C.), 249 (4%, M – S.C. – ring D), 213 (5%, M – $2H_2O$ – S.C. – ring D).

The above ester IXa, hydrolyzed by the usual KOH–methanol method, gave the corresponding acid IX: mp, 228.5–229.5°C (from aqueous methanol) (lit. 228°C (6) and 231–232°C (12)); $\nu_{max.}$ 1715 (C=O), 3530, 1042, 1017, 982, 958 (OH); ^{1}H-NMR ($CDCl_3$ + 20% Me_2SO-d_6), 0.68 (3H, s, C-18 Me), 0.80 (3H, s, C-19 Me), 1.00 (3H, d, j = 5.4 Hz, C-21 Me), 3.52 (3H, brm, C-3 CHOH), 3.92 (1H, m, C-12 CHOH).

Methyl 3β,12β-dihydroxy-5α-cholanate (IIa)

To a magnetically stirred solution of 380 mg of methyl 3,12-dioxo-5α-cholanate XIa [prepared from IXa or Xa (12)] in 20 ml of CH_2Cl_2 was added 200 mg of *tert*-butylamine-borane reagent. The clear solution, after some effervescence, was allowed to stand at room temperature for 4 hr and then acidified by 6 N HCl. The CH_2Cl_2 solution was shaken with 10% $NaHCO_3$ and then with water, filtered through phase-separating paper, and evaporated to give 378 mg of an oily residue, which by HPLC consisted of a mixture of two components. Chromatography of the oil over a column of alumina (activity III, 20 g) resulted in two components. The first fractions eluted with benzene–EtOAc 7:3 (v/v) gave 72 mg (19%) of homogeneous oil which was crystallized from aqueous methanol and found to be identical by melting point, TLC, and ^{1}H-NMR comparisons with the 3β,12α-dihydroxy ester IXa.

The second fractions eluted with benzene–EtOAc 1:1 (v/v) gave 248 mg (65%) of the main component which was identified with the desired 3β,12β-dihydroxy ester IIa and crystallized as colorless fine needles from aqueous methanol. It partially melted at 139–140°C, resolidified, and remelted at 159.0–160.0°C; $\nu_{max.}$ 1730 (C=O), 3450, 1077, 1040, 1003, 975 (OH); ^{1}H-NMR, 0.72 (3H, s, C-18 Me), 0.81 (3H, s, C-19 Me), 1.01 (3H, d, j = 6.3 Hz, C-21 Me), 3.39 (2H, brm, C-3 and C-12 CHOH), 3.64 (3H, s, COOMe); MS, m/z (relative intensity), 388 (9%, M – H_2O), 373 (5%, M – H_2O – CH_3), 357 (5%, M – H_2O – methoxy), 273 (100%, M – H_2O – S.C.), 255 (25%, M – $2H_2O$ – S.C.), 249 (5%, M – S.C. – ring D).

Anal. calcd. for $C_{25}H_{42}O_4 \cdot 1/4\ H_2O$: C, 73.04; H, 10.42. Found: C, 72.80; H, 10.25

3β,12β-Dihydroxy-5α-cholanic acid (II), obtained quantitatively from the ester IIa by the general hydrolysis procedure, crystallized from aqueous methanol as colorless fine needles: mp, 223.5–225.5°C; $\nu_{max.}$ 1715 (C=O), 3250, 1075, 1040, 1027, 975 (OH); ^{1}H-NMR ($CDCl_3$ + 20% Me_2SO-d_6), 0.71 (3H, s, C-18 Me), 0.81 (3H, s, C-19 Me), 1.03 (3H, d, j = 7.2 Hz, C-21 Me), 3.38 (2H, brm, C-3 and C-12 CHOH).

Anal. calcd. for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.20; H, 9.96.

Methyl 3β-acetoxy-12α-hydroxy-5α-cholanate (XIIa)

To the 3β,12α-dihydroxy ester IXa (600 mg) dissolved in 6 ml of benzene and 2.5 ml of anhydrous pyridine was added 1.2 ml of acetic anhydride. After standing for 12 hr at room temperature, the solution was poured into ice-water, the flask was rinsed with ethyl ether, and the combined ether layer was washed with water, 3 N HCl, and then water, dried (Drierite), and evaporated to dryness. The residue was crystallized from aqueous methanol as colorless fine needles: mp, 161.5–163.0°C; 505 mg, 70%; $\nu_{max.}$ 1732, 1710 (C=O), 3570, 3460, 985, 958 (OH), 1260, 1043, 1021 (acetate); ^{1}H-NMR, 0.72 (3H, s, C-18 Me), 0.85 (3H, s, C-19 Me), 1.00 (3H, d, j = 5.4 Hz, C-21 Me), 2.03 (3H, s, OCOMe), 3.64 (3H, s, COOMe), 3.92 (1H, m, C-12 CHOH), 4.63 (1H, brm, C-3 CHOAc).

Anal. calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.26; H, 9.68.

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Methyl 3β-acetoxy-12-oxo-5α-cholanate (XIIIa)

The 3β-acetoxy-12α-hydroxy ester XIIa (600 mg) was oxidized with potassium chromate in acetic acid by the procedure of Fieser and Rajagopalan (13). The dark brown solution, after standing overnight at room temperature, was diluted with water to near turbidity, and on standing, crystals separated. A second crop was obtained on further dilution with water of the mother liquor: total of the two crops, 592 mg (99%). The ester recrystallized from aqueous methanol as colorless thin plates, mp, 163.5–165.0°C; $\nu_{max.}$ 1730, 1700 (C=O), 1250, 1240, 1040, 1030 (acetate); ^{1}H-NMR, 0.86 (3H, d, j = 7.2 Hz, C-21 Me), 0.93 (3H, s, C-18 Me), 1.05 (3H, s, C-19 Me), 2.06 (3H, s, OCOMe), 3.65 (3H, s, COOMe), 4.64 (1H, brm, C-3 CHOAc).

Anal. calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.78; H, 9.29.

Methyl 3β-acetoxy-12β-hydroxy-5α-cholanate (XIVa)

The 3β-acetoxy-12-oxo ester XIIIa (540 mg), reduced with *tert*-butylamine-borane and processed as described for the preparation of IIa, yielded 512 mg of crude oil which by HPLC consisted of XIVa and XIIa in the 12β/12α ratio of 5.0. The two epimers were separated cleanly by chromatography on alumina (activity II; 35 g). The less polar compound (82 mg, 15%) eluted with benzene–EtOAc 9:1 (v/v) was identified with XIIa, according to TLC and ^{1}H-NMR.

The more polar compound (388 mg, 72%) with benzene–EtOAc 8:2 (v/v) was characterized as the desired XIVa which crystallized from aqueous methanol as dense prisms: mp, 120.5–122.0°C; $\nu_{max.}$ 1730 (C=O), 3540 (OH), 1250, 1237, 1023 (acetate); ^{1}H-NMR, 0.75 (3H, s, C-18 Me), 0.86 (3H, s, C-19 Me), 1.02 (3H, d, j = 6.3 Hz, C-21 Me), 2.02 (3H, s, OCOMe), 3.38 (1H, brm, C-12 CHOH), 3.64 (3H, s, COOMe), 5.45 (1H, brm, C-3, CHOAc).

Anal. calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.21; H, 9.79.

The above ester XIVa, hydrolyzed in the usual manner, yielded the corresponding acid nearly quantitatively, identical, according to melting point, TLC, and ^{1}H-NMR comparisons, to II prepared as described above.

Methyl 12-oxo-3,3-dimethoxy-5α-cholanate (XVa)

To the 3,12-dioxo ester XIa (3.07 g) dissolved in 150 ml of methanol was added 1.5 g of *p*-toluenesulfonic acid and 35 g of molecular sieve (4 Å), and the mixture was stirred vigorously for 4 hr at room temperature. Molecular sieve was filtered off, and the mother liquor was diluted with water and extracted with ethyl ether–EtOAc 1:1 (v/v) (× 2). The combined extract was washed with water to neutrality, dried with Drierite, and evaporated to afford XVa. Recrystallization from methanol yielded 2.86 g (84%) of pure XVa as colorless fine needles: mp, 151.5–153.0°C; $\nu_{max.}$ 1735, 1695, (C=O), 1168, 1133, 1110, 1093, 1070, 1045 (C-O-C-O-C); ^{1}H-NMR, 0.88 (3H, d, j = 5.4 Hz, C-21 Me), 0.92 (3H, s, C-18 Me), 1.05 (3H, s, C-19 Me), 3.13 and 3.19 (each 3H, s, C-3 OMe), 3.65 (3H, s, COOMe).

Anal. calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.31; H, 9.62.

Methyl 12β-hydroxy-3-oxo-5α-cholanate (XVIa)

The 12-oxo-3,3-dimethoxy ester XVa (2.6 g) was similarly reduced with the amine–borane reagent as described above. The crude reaction product (2.51 g), which consisted of three major components as judged by TLC, was chromatographed on an alumina column (activity III; 150 g).

Elution with benzene–EtOAc 8:2 (v/v) afforded 860 mg (37%) of the desired 12β-hydroxy-3-oxo ester XVIa which was crystallized from aqueous methanol as colorless fine needles: mp, 129.5–131.0°C; $\nu_{max.}$ 1725, 1690 (C=O), 3440, 1053, 1023, 973, 952 (OH); ^{1}H-NMR, 0.76 (3H, s, C-18 Me), 1.01 (3H, d, j = 7.2 Hz, C-21 Me), 1.02 (3H, s, C-19 Me), 3.40 (1H, brm, C-12 CHOH), 3.64 (3H, s, COOMe).

Anal. calcd. for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.11; H, 9.94.

The more polar component (431 mg, 18%) eluted with the same solvent system was identified with Xa, according to TLC and ^{1}H-NMR.

Further elution with benzene–EtOAc 1:1 (v/v) yielded 882 mg (37%) of the IIa, identical with that prepared above, according to TLC and ^{1}H-NMR comparisons.

Methyl 3α,12β-dihydroxy-5α-cholanate (IIIa)

To a solution of the 12β-hydroxy-3-oxo ester XVIa (570 mg) in 5 ml of dry THF, at −40°C under N_2, was added slowly 2.3 ml of a 1 M solution of K-Selectride® in THF. The mixture was further stirred under N_2, at −40°C for 4 hr. The crude reaction product (544 mg), after the treatment with NaOH solution followed by H_2O_2 as previously reported (14), was chromatographed on an alumina column (activity III, 30 g). The fractions eluted with benzene–EtOAc 8:2 (v/v) gave 73 mg (13%) of the starting ketone XVIa.

The fractions eluted with benzene–EtOAc 6:4 (v/v) were collected and evaporation of the solvent gave 423 mg (74%) of the desired 3α,12β-dihydroxy ester IIIa as the main product. This crystallized from acetone–hexane as colorless fine needles: mp, 143–144°C; $\nu_{max.}$ 1740 (C=O), 3370, 995, 965 (OH); ^{1}H-NMR, 0.73 (3H, s, C-18 Me), 0.79 (3H, s, C-19 Me), 1.01 (3H, d, j = 7.2 Hz, C-21 Me), 3.37 (1H, brm, C-12 CHOH), 3.65 (3H, s, COOMe), 4.01 (1H, m, C-3 CHOH); MS, m/z (relative intensity), 388 (9%, M − H_2O), 357 (5%, M − H_2O − methoxy),

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273 (100%, M – H_2O – S.C.), 255 (57%, M – $2H_2O$ – S.C.), 249 (7%, M – S.C. – ring D), 213 (6%, M – $2H_2O$ – S.C. – ring D).

Anal. calcd. for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found: C, 73.60; H, 10.11.

3α,12β-Dihydroxy-5α-cholanic acid (III), obtained quantitatively from the ester IIIa by the general hydrolysis procedure, crystallized from aqueous methanol as colorless needles: mp, 206.5–207.5°C; $\nu_{max.}$ 1680 (C=O), 3440, 1050, 1028, 1013, 1000, 970 (OH); ^{1}H-NMR ($CDCl_3$ + 20% Me_2SO-d_6), 0.72 (3H, s, C-18 Me), 0.79 (3H, s, C-19 Me), 1.03 (3H, d, j = 7.2 Hz, C-21 Me), 3.39 (1H, brm, C-12 CHOH), 3.99 (1H, m, C-3 CHOH).

Anal. calcd. for $C_{24}H_{40}O_4 \cdot H_2O$: C, 70.20; H, 10.31. Found: C, 70.41; H, 10.22.

Methyl 12α-hydroxy-3β-tosyloxy-5α-cholanate (XVIIa)

To the 3β,12α-dihydroxy ester IXa (700 mg), dissolved in 5 ml of anhydrous pyridine, was added 1.0 g of freshly recrystallized tosyl chloride in 10 ml of pyridine in one portion. After being allowed to stand for 12 hr at room temperature, ice-water was added, and the reaction product was extracted with CH_2Cl_2 (× 2). The CH_2Cl_2 extract was washed with water, 3 N HCl, and then water, dried (Drierite), and evaporated to dryness. The residue was crystallized from benzene–hexane as colorless fine needles: mp, 156–157°C, 792 mg (82%); $\nu_{max.}$ 1740 (C=O), 3600 (OH), 1173, 912 (tosylate); ^{1}H-NMR, 0.66 (3H, s, C-18 Me), 0.77 (3H, s, C-19 Me), 0.96 (3H, d, j = 5.4 Hz, C-21 Me), 2.44 (3H, s, $C_6H_4CH_3$), 3.65 (3H, s, COOMe), 3.96 (1H, m, C-12 CHOH), 4.36 (1H, brm, C-3 CHOTs), 7.31 and 7.79 (each 2H, d, j = 9.0 Hz, *para*-disubstituted phenyl).

Anal. calcd. for $C_{32}H_{48}O_6S \cdot 1/6\ C_6H_{14}$: C, 68.92; H, 8.82. Found: C, 68.96; H, 8.50.

Methyl 3α-formyloxy-12α-hydroxy-5α-cholanate (XVIIIa)

The 3β-tosylate ester XVIIa (700 mg) in 32 ml of DMF was kept at temperature of 80°C for 72 hr. The product was extracted with CH_2Cl_2 (× 2), and the extract was washed with water, decolorized with Norite, dried (Drierite), and evaporated to afford the desired XVIIIa. Crystallization from acetone–hexane yielded 456 mg (84%) of pure XVIIIa as colorless prisms: mp, 126–128°C; $\nu_{max.}$ 1718 (C=O), 3560, 1028, 1010, 985, 975 (OH), 1195, 1160 (formate); ^{1}H-NMR, 0.69 (3H, s, C-18 Me), 0.80 (3H, s, C-19 Me), 0.97 (3H, d, j = 5.4 Hz, C-21 Me), 3.64 (3H, s, COOMe), 3.96 (1H, m, C-12 CHOH), 5.13 (1H, m, C-3 CHOCHO), 8.02 (1H, s, C-3 OCHO).

Anal. calcd. for $C_{26}H_{42}O_5 \cdot 1/6\ C_6H_{14}$: C, 72.23; H, 9.95. Found: C, 72.43; H, 9.69.

3α,12α-Dihydroxy-5α-cholanic acid (V), obtained from the 3α-formyloxy-12α-hydroxy ester XVIIIa by the general hydrolysis procedure, was crystallized from aqueous methanol: mp, 220–221°C (lit. 214–215°C (6) and 215–216°C (12)); $\nu_{max.}$ 1690 (C=O), 3350, 1060, 1013, 1003, 975, 955 (OH); ^{1}H-NMR ($CDCl_3$ + 20% Me_2SO-d_6), 0.68 (3H, s, C-18 Me), 0.77 (3H, s, C-19 Me), 1.00 (3H, d, j = 5.4 Hz, C-21 Me), 3.95 (2H, m, C-3 and C-12 CHOH).

Esterification of V with methanol and concentrated HCl afforded the corresponding ester Va: mp, 179.0–180.5°C (from aqueous methanol) (lit. 174–176°C (6) and 179–180°C (7)); $\nu_{max.}$ 1735 (C=O), 3470, 1025, 1008, 970, 957, (OH); ^{1}H-NMR, 0.71 (3H, s, C-18 Me), 0.79 (3H, s, C-19 Me), 0.98 (3H, d, j = 5.4 Hz, C-21 Me), 3.63 (3H, s, COOMe), 3.96 (2H, m, C-3 and C-12 CHOH); MS, m/z (relative intensity), 388 (6%, M – H_2O), 373 (2%, M – H_2O – CH_3), 357 (4%, M – H_2O – methoxy), 273 (100%, M – H_2O – S.C.), 255 (40%, M – $2H_2O$ – S.C.), 249 (2%, M – S.C. – ring D), 213 (6%, M – $2H_2O$ – S.C. – ring D).

Methyl 12-oxo-5α-cholanate (XXa)

The 12α-hydroxy ester XIXa (1.25 g) [prepared from Xa (12)] was oxidized by the potassium chromate–acetic acid procedure as described for the preparation of XIIIa to give 1.21 g (98%) of the desired XXa as colorless thin plates: mp, 112.5–114.0°C (from aqueous methanol); $\nu_{max.}$ 1703, 1743 (C=O); ^{1}H-NMR, 0.86 (3H, d, j = 6.3 Hz, C-21 Me), 0.89 (3H, s, C-18 Me), 1.02 (3H, s, C-19 Me), 3.65 (3H, s, COOMe).

Anal. calcd. for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.28; H, 10.40.

Methyl 12β-hydroxy-5α-cholanate (IVa)

The 12-oxo ester XXa (600 mg) was reduced with *tert*-butylamine-borane, as described for the preparation of IIa, to yield a crude oil which was judged by HPLC to be a mixture of IVa and XIXa in the 12β/12α ratio of 6.5 to 1. The oil, on standing in a small volume of aqueous methanol at –10°C, gradually crystallized. Several recrystallizations from aqueous methanol afforded 249 mg (41%) of the 12β-hydroxy ester IVa as colorless thin plates which partially melted at 84–86°C, and then 101–102°C: $\nu_{max.}$ 1733 (C=O), 3480, 1023, 1002, 984 (OH); ^{1}H-NMR, 0.71 (3H, s, C-18 Me), 0.79 (3H, s, C-19 Me), 1.00 (3H, d, j = 7.2 Hz, C-21 Me), 3.37 (1H, brm, C-12 CHOH), 3.63 (3H, s, COOMe); MS, m/z (relative intensity), 372 (5%, M – H_2O), 357 (5%, M – H_2O – CH_3) 257 (100%, M – H_2O – S.C.), 233 (8%, M – S.C. – ring D), 215 (6%, M – H_2O – S.C. – ring D).

Anal. calcd. for $C_{25}H_{42}O_3$: C, 76.87; H, 10.84. Found: C, 77.16; H, 10.59.

Hydrolysis of IVa, by the usual manner, afforded quantitatively the corresponding acid IV which was recrystallized

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from aqueous methanol as colorless fine needles: mp, 128.0–129.5°C; $\nu_{max.}$ 1700 (C=O), 3420, 1000 (OH); ^{1}H-NMR, 0.71 (3H, s, C-18 Me), 0.77 (3H, s, C-19 Me), 1.00 (3H, d, j = 6.3 Hz, C-21 Me), 3.38 (1H, brm, C-12 CHOH).

Anal. calcd. for $C_{24}H_{40}O_3$: C, 76.55; H, 10.71. Found: C, 76.26; H, 10.52.

DISCUSSION

Two basically different procedures for allomerization of 5β-bile acids are known. The first consists of treatment of 3-hydroxy or 3-keto 5β-compounds with Raney nickel in boiling *p*-cymene (10, 12, 15) and the second involves the reduction of a Δ^4-3-keto analogue by Li in ammonia (11). On considering the two methods as applied to the preparation of the 3,12-dihydroxy 5α-compounds, we chose the latter as being more promising and more amenable for improvement. The Raney nickel reduction, although superfically straightforward, is erratic, and results generally in a complex mixture which requires extensive laborious chromatographic separation, whereas the other method is stereoselective, and affords reasonable yields.

It seemed to us that the successful application of this method would depend on improving the preparation of the Δ^4-3-keto acid VIII. We have been able to do this by adapting known procedures for two steps. By carrying out the bromination of 12α-hydroxy-3-oxo ester VIIa in DMF (16) instead of the commonly used acetic acid (9) to give 4β-bromo derivative VIa, and subsequent dehydrohalogenation of the semicarbazide derivative of VIa in pyruvic acid (17, 18), a 58% overall yield of VIIIa was obtained. Hydrolysis of VIIIa gave quantitatively the corresponding acid VIII.

Metal-ammonia reduction of the acid VIII according to the Kallner procedure (11) proceeded satisfactorily, affording the required 5α-intermediates, 3β,12α-dihydroxy (IXa) and 12α-hydroxy-3-keto (X) esters, for synthesis of the new compounds, II, III, and IV.

3β,12β-Dihydroxy-5α-cholanic acid (II)

This stereoisomer was prepared by two methods: from 3β-acetoxy-12-keto ester XIIIa, obtained from the 3β-acetoxy-12α-hydroxy ester XIIa, by reduction with *tert*-butylamino-borane complex (19); and by direct reduction of the 3,12-dioxo ester XIa by the same reagent. [Reduction of the 12-ketones in 5α-series yielded principally 12β-hydroxy products, as expected from the work in the 5β-series (20)]. In the second method esters IIa and IXa are formed in the ratio of 3.5 to 1. Separation of the two by column chromatography was clean, and the amounts of the other two possible stereoisomers were negligible. Of the two methods for the preparation of II, we felt that the second route was the one of choice because of its simplicity and the ready availability of the dioxo ester XIa.

3α,12β-Dihydroxy-5α-cholanic acid (III)

This unreported acid was synthesized by K-Selectride® (potassium *tri-sec*-butylborohydride) reduction of the 12β-hydroxy-3-oxo ester XVIa in analogy with the reduction of some 5α- and 5β-steroidal ketones recently reported by Contreras and Mendoza (14) and Elliott et al. (21, 22). Analogously, the C-3 oxo group in XVIa was converted stereoselectively into the *axial* hydroxyl group to give the desired 3α,12β-dihydroxy ester IIIa in reasonable yield.

3α,12α-Dihydroxy-5α-cholanic acid (V)

Previous syntheses of this acid were by catalytic hydrogenation in the presence of HCl (6, 12) or by reduction with trimethylphosphite and iridium (IV) chloride in an aqueous solution of isopropanol (11). The procedure described herein follows the previously reported method which involves the inversion of an *equatorial* 3-hydroxyl group via its tosylate by treatment with DMF (2, 23). The new synthesis is analogous to the inversion of the 5β-3α,7α,12α-trihydroxy ester (methyl cholate) to its C-3 epimer (2). The 3β,12α-dihydroxy ester IXa, which is similar to methyl cholate in having an *equatorial* hydroxyl group at C-3 and an *axial* group at C-12, also forms the monotosylate XVIIa readily, which by DMF treatment undergoes inversion to give the 3α-formyloxy-12α-hydroxy ester XVIIIa in good yield. Hydrolysis of XVIIIa afforded quantitatively the 3α,12α-dihydroxy acid V.

12β-Hydroxy-5α-cholanic acid (IV)

Synthesis of this acid was accomplished by stereoselective reduction of the 12-oxo ester XXa, prepared from the 12α-hydroxy ester XIXa, by the *tert*-butylamine-borane complex, as described above. The absence of interfering functional groups in XIXa enables both the oxidation at 12-hydroxyl and reduction of the resulting ketone to proceed smoothly. By fractional crystallization IVa is easily separated from the decidely minor 12α-hydroxy component XIXa. Alkaline hydrolysis of IVa afforded the corresponding acid.

Characterization of compounds by HPLC and ^{13}C-NMR

Recent developments in the use of HPLC and ^{13}C-NMR provide further confirmation of the stereochemical nature of a hydroxyl group present in the allo bile acid isomers as well as of their purities.

Table 1 shows the retention data of ester derivatives on HPLC, together with the R_f values on TLC. While three of the four stereoisomeric 5α-3,12-diols have very close mobility on TLC, they were well separated by HPLC,

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TABLE 1. TLC and HPLC data for mono- and dihydroxy stereoisomers of allo bile acid esters[a]

Position and Configuration of Hydroxyls	TLC[b] (R_f Values)		HPLC[c] (Retention Times, min)
	5α	5β	5α
3α	0.26	0.19	6.3
3β	0.19	0.27	5.4
12α	0.54	0.61	9.5
12β	0.55	0.56	13.2
3α,12α	0.36	0.31	29.3
3β,12α	0.34	0.38	11.5
3α,12β	0.46	0.39	15.3
3β,12β	0.35	0.46	16.2

[a]The designation 5α refers to allo cholanates, and 5β to 5β-cholanates.
[b]In TLC on silica gel; the samples were analyzed as the C-24 methyl esters; monohydroxy compounds were developed in hexane-EtOAc (80:20, v/v) and dihydroxy compounds in hexane-EtOAc-acetic acid (50:50:1, v/v).
[c]Conditions are as follows: column, Nova-Pak C_{18} (5 μm); detector, UV at 254 nm; mobile phases used were methanol-water, 90:10 (v/v; flow rate, 1.0 ml/min) for the separation of the monohydroxy compounds, and 80:20 (v/v; flow rate, 0.7 ml/min) for the dihydroxy compounds. The samples were analyzed as the 4-nitrophthalimidemethyl ester derivatives as described previously (24).

emerging from a C_{18} reversed-phase column in the following order: 3β,12α-, 3α,12β-, 3β,12β-, and 3α,12α-.

The ^{13}C-NMR chemical shifts of carbon signals in hydroxylated methyl 5α-cholanates, assigned on the basis of previous papers (25–27), are shown in **Table 2**. The shielding data of the α-carbon absorption occurring in the low-field region of 66–80 ppm and of the C-18 and C-19 signals occurring in the up-field region of 7–13 ppm in each spectrum are of particular importance. Since these very sharp signals are well separated from the main group of other carbon bands, and are sensitive to the position and configuration of the hydroxyl groups, the shielding data provide a straightforward identification of each isomer as well as an indication of purity.

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TABLE 2. ^{13}C-NMR spectral data for mono- and dihydroxy stereoisomers of methyl 5α-cholanates[a]

Carbon	Position and Configuration of Hydroxyls							
	3α	3β	12α	12β	3α,12α	3β,12α	3α,12β	3β,12β
1	32.1	36.9	38.3	38.5	31.9	36.7	32.1	36.8
2	28.9	31.3	21.9	22.0	28.7	31.5	28.8	31.4
3	66.3	71.0	26.6	26.6	66.1	70.8	66.2	70.7
4	35.8	38.0	28.4	28.8	35.7	37.9	35.7	37.7
5	39.0	44.7	46.8	46.9	38.7	44.6	38.9	44.6
6	28.5	28.6	28.4	28.8	28.3	28.4[b]	28.4	28.4
7	31.9	31.9	31.6	32.1	31.4	31.2	31.4	31.4
8	35.4	35.4	35.5	34.1	35.4	35.5	34.1	34.0
9	54.2	54.2	47.5	53.4	46.9	47.2[c]	53.0	52.9
10	35.9	35.4	35.6	36.0	35.4	34.9	35.8	35.2
11	20.7	21.1	28.4	29.4	28.3	28.7[b]	29.4	29.3
12	39.9	39.9	72.9	79.4	72.8	72.8	79.3	79.1
13	42.5	42.5	46.2	47.7	46.2	46.2	47.7	47.6
14	56.4	56.3	48.1	54.4	48.1	47.9	54.4	54.2
15	24.0	24.0	23.4	23.4	23.4	23.4	23.4	23.4
16	28.0	27.9	27.2	23.8	27.3	27.4	23.8	23.7
17	55.8	55.8	47.0	57.2	46.9	47.0[c]	57.2	57.1
18	12.0	11.9	12.5	7.6	12.5	12.5	7.8	7.7
19	11.1	12.1	11.8	12.0	10.8	12.0	11.0	12.0
20	35.2	35.2	34.9	32.6	35.2	34.9	32.6	32.4
21	18.2	18.1	17.1	20.8	17.0	17.1	20.8	20.7
22	30.9	30.9	30.9	31.5	31.0	30.9	32.1	32.0
23	30.9	30.9	30.7	31.2	30.7	30.7	31.1	31.1
24	174.4	174.5	174.5	174.4	174.4	174.4	174.5	174.4
25	51.3	51.2	51.1	51.2	51.1	51.2	51.3	51.2

[a]In ppm downfield from Me_4Si.
[b,c]Assignments along a vertical column may be interchanged.

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