Potential bile acid metabolites. 13. Improved routes to 3β,6β- and 3β,6α-dihydroxy-5β-cholanoic acids[1]

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Abstract New synthetic routes to three possible stereoisomers of hyodeoxycholic (3α,6α-dihydroxy-5β-cholanic) acid are described. The principal reactions involved were inversion at C-3 of 3α-hydroxy-6-oxo derivatives with diethyl azodicarboxylate–triphenylphosphine–formic acid and with N,N-dimethylformamide, without allomerization to the more stable 5α form. On the basis of physical and chromatographic data, previously reported 3β,6α-dihydroxy-5β-cholanic acid and its methyl ester are shown to be C-3 epimeric mixtures. The ^{13}C nuclear magnetic resonance spectra were of key importance in characterizing the stereoisomers and estimating their purity. — **Iida, T., T. Momose, T. Tamura, T. Matsumoto, F. C. Chang, J. Goto, and T. Nambara.** Potential bile acid metabolites. 13. Improved routes to 3β,6β- and 3β,6α-dihydroxy-5β-cholanoic acids. *J. Lipid Res.* 1988. **29:** 165–171.

Supplementary key words 3,6-dihydroxy-5β-cholanic acid stereoisomers • hyodeoxycholic acid • diethyl azodicarboxylate–triphenylphosphine–formic acid reaction • N,N-dimethylformamide reaction • capillary GLC • HPLC • ^{13}C-NMR

Hyodeoxycholic (3α,6α-dihydroxy-5β-cholanic) acid (1, **Scheme 1**), historically of key importance in the elucidation of the epimeric relationship between the 5β ("normal") and 5α ("allo") steroids (1), is a commercial product isolated from hog bile. However, its three possible 3,6-dihydroxy-5β-stereoisomers (2–4), although known for many years (2–5), are not easily available products. As part of our ongoing program of synthesis of new and scarce potential bile acid metabolites, a need for a supply of these stereoisomers prompted us to re-examine the known procedures for their preparation.

All three compounds have been prepared starting from the available 1. Compound 3 has been synthesized from 1 by Kawanami (2) via a Δ^5-cholenate intermediate, and by Moffatt (3) from coprostan-3β,6β-diol. Both of these are multi-step low yield processes.

An exploratory experiment to obtain 3 by direct inversion through the ditosylate 5a, suggested by the successful inversion of various 3α-tosyloxy-5β-cholanates at C-3 by reaction with DMF (6), failed. A similar attempt with the inverting reagent, diethyl azodicarboxylate-triphenylphosphine-formic acid (7–9), was unsuccessful. In both reactions, elimination of the C-6 substituent took place (10–13) and the major product was the 3β-formyloxy-Δ^5-compound 6a.

Both compounds 3 and 4 were finally obtained indirectly by first applying the inversion reaction to the 3α-hydroxy-6-oxo ester 7a. Compound 7a in the diethyl azodicarboxylate reaction was smoothly converted to the inverted 3β-formate 8a in excellent isolated yield (88%), while the tosylate (9a) of 7a in the DMF reaction gave 8a in 66% yield. To obtain pure 8a, silica gel was used as column chromatographic adsorbent, which does not result in allomerization at C-5 during the purification of 8a. When neutral alumina was the chromatographic adsorbent, allomerized methyl 3β-hydroxy-6-oxo-5α-cholanate (10a) was the major product (55%), indicating that allomerization at C-5 takes place with the 6-oxo compound when chromatographed on alumina (14), and does not involve the inversion process at C-3.

Reduction of 8a with $NaBH_4/PdCl_2$ (6) gave the corresponding 6-hydroxy epimers 11a and 12a in the ratio of 3.7 to 1; with *tert*-butylamine-borane complex (6) as reducing agent, the ratio was approximately 1 to 1. The resulting C-6 epimeric formate mixtures were readily separated by chromatography on silica gel. The individual formates could be partially hydrolyzed in 5% conc. HCl

Abbreviations: IR, infrared; NMR, nuclear magnetic resonance; MS; mass spectrometry; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; GLC, gas-liquid chromatography; DMF, N,N-dimethylformamide; $NaBH_4$, sodium borohydride; $PdCl_2$, palladium dichloride.

[1]In uniformity with the nomenclature of the previous papers in 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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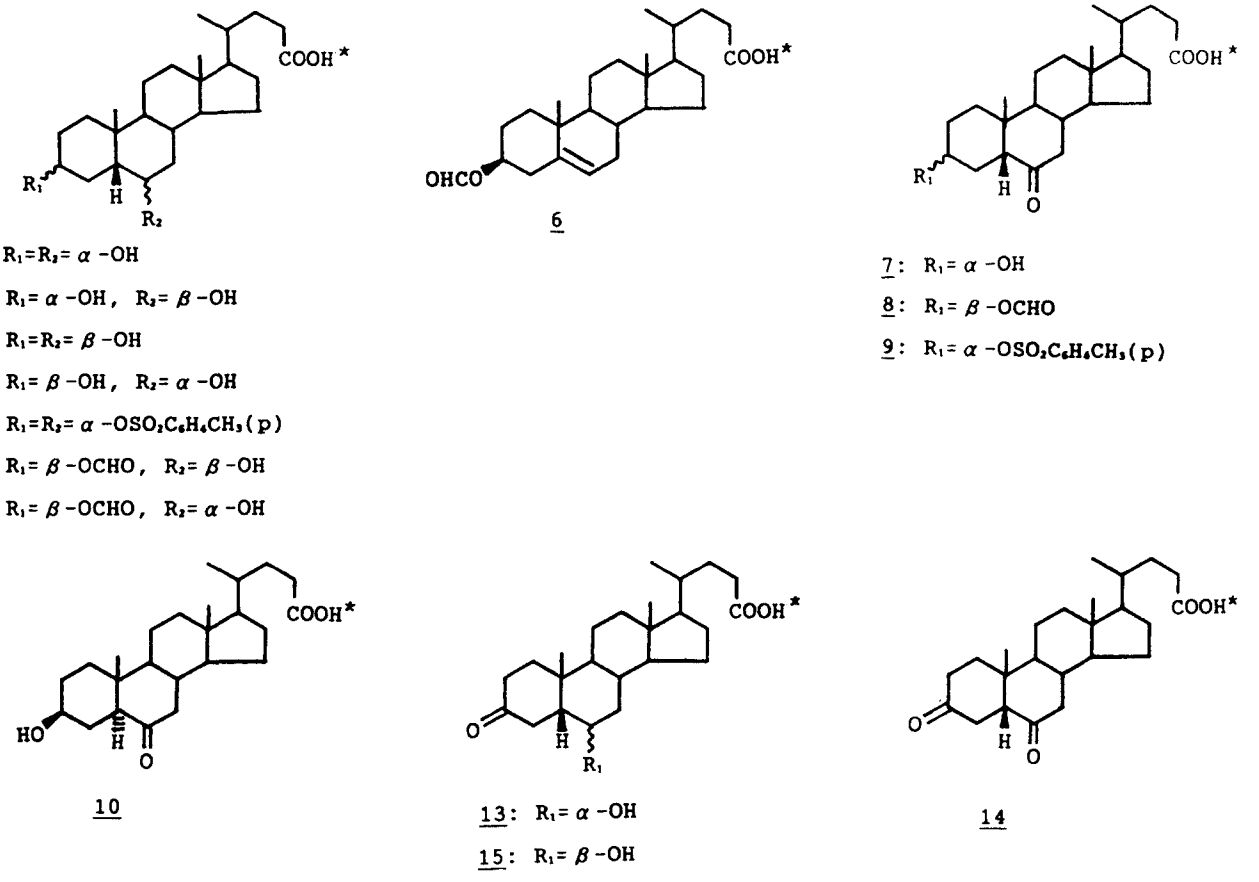

Scheme 1. (*) The corresponding methyl esters are designated "a".

in methanol to either the esters 3a or 4a, or to the acids 3 or 4 by alkaline hydrolysis followed by acidification.

Discrepancies between the physical properties of 4 and 4a as prepared by us and those reported in the literatures (4, 5) led us to suspect the purity of the previous products. Our acid 4 crystallized from aqueous methanol or acetone, and melted at 198–199°C. The product from acetone as prepared by catalytic reduction of the 3-oxo acid 13 (4) under acidic conditions or by Meerwein-Ponndorf reduction of the 3,6-dioxo analog 14 (5) melted at 190°C. The same 190°C melting point had been reported for 4 isolated from hog bile (15, 16). Additionally, our ester 4a was crystallized readily from acetone–hexane, while the product prepared by the reduction of 14 could not be crystallized (5).

That the previous products were not homogeneous, but were contaminated by traces of 1, was confirmed by repeating the procedure of Moffett and Hoehn (4) in which 13 was catalytically hydrogenated in acetic acid with platinum oxide and a trace of hydrobromic acid. The crystalline product, according to HPLC analysis, was a mixture of 4 and 1 (88%:12%), which could not be separated by fractional crystallization; it had a melting point of 188–191°C in accord with the literature value. Furthermore, the methyl ester of the mixture was an oily product which resisted attempts at separation by crystallization as well as by TLC and column chromatography on either silica gel or alumina. Additional support for the heterogeneity of the earlier preparations of 4 was from analyses by HPLC, capillary GLC, and ^{13}C-NMR, methods not available to the previous investigators.

The high axial selectivity found in the reduction of 6-oxo (8a) to 6β-OH (11a) with $NaBH_4/PdCl_2$ suggested its use for an improved preparation of ester 2a. Indeed, when the reduction reaction was carried out on the 3α-hydroxy-6-oxo ester 7a, the desired 3α,6β-dihydroxy isomer 2a was obtained in a ratio of 3.6 to 1 over its epimer 1a, with an isolated yield of 72%.

Since the improved routes to all three target stereoisomers resulting from the present work depend on the starting ketone 7a, a review of its preparation was indicated. The logical route to a clean oxidation of 1 at C-6 via an initially protected 3α-hydroxy group was closed because selective acylation (acetylation, formylation, carbethoxylation, and tosylation) of 1 at C-3 would fail, presumably because both hydroxyls in the compound are

equatorial. The products of these acylation reactions are the diacylated derivatives, which conversely are not susceptible to selective hydrolysis.

However, since the ester 7a had been prepared by selective oxidation of 1a (14), by modifying the reactants and conditions of the reaction we succeeded in obtaining 7a in better yield and under less stringent conditions. With the ester 1a in acetic acid and K_2CrO_4 as oxidant, at room temperature, 7a was obtained in 70% yield. Even under these conditions the 3,6-dioxo compound 14a (11%) was formed and column chromatography was needed for separation of the products.

In contrast, the reagent, silver carbonate-Celite, which has been known to selectively oxidize a number of bile acids and other steroids (17, 18), was found to react with 1a to give a 73% yield of the 6α-hydroxy-3-oxo ester 13a, and with 2a, 70% of the 6β-hydroxy epimer 15a. From both reactions, each desired compound was obtained by direct crystallization of the crude product; chromatography was not needed.

Table 1 shows the mobilities of the four 3,6-dihydroxy stereoisomers on TLC, reversed phase HPLC, and capillary GLC. HPLC and GLC of the stereoisomers were carried out as their 4-nitrophthalimidemethyl ester derivatives (19) and methyl ester-dimethylethylsilyl ether derivatives (20), respectively, because of the excellent chromatographic properties reported previously. While each of the C-6 epimeric pairs [3α,6α- vs. 3α,6β-$(OH)_2$ and 3β,6α- vs. 3β,6β-$(OH)_2$] was well separated on TLC, each C-3 epimeric pair was not. In contrast, each C-3 pair of epimers was resolved completely on reversed phase HPLC and capillary GLC; but the 3β,6β- versus 3β,6α-$(OH)_2$ pair on HPLC, and the 3α,6α- versus 3β,6β-$(OH)_2$ pair on capillary GLC, were not resolved.

TABLE 1. Chromatographic data for 3,6-dihydroxy stereoisomers

Configuration of Hydroxyls	TLC[a] (R_f Values)		HPLC[b] (rk' Values)	GLC[c] (RRT)
	Free Acid	Me-Ester		
3α,6α(1)	0.31	0.38	1.00	1.00
3α,6β(2)	0.45	0.55	0.65	0.97
3β,6β(3)	0.44	0.53	1.27	1.00
3β,6α(4)	0.30	0.36	1.27	1.08

[a]The samples were developed in hexane–EtOAc–acetic acid 10:40:2 (v/v/v).

[b]The samples were analyzed as the C-24 4-nitrophthalimidemethyl esters (19) under the following conditions: column, Nova-Pak C_{18}; detector, UV at 254 nm; mobile phase, MeOH–water 85:15 (v/v); flow rate, 0.5 ml/min. Capacity factors (k') are expressed relative to that of the 3α,6α-dihydroxy ester.

[c]The samples were analyzed as the methyl ester–dimethylethylsilyl ethers (20) under the following conditions: column, HiCap-CBP1 (18 m × 0.2 mm i.d.); column temp., 280°C; helium linear velocity, 32cm/s; splitting ratio, 1:80. Retention times are expressed relative to that of the 3α,6α-dihydroxy ester.

The ^{13}C-NMR chemical shifts of 1a–4a, together with the data for monohydroxylated methyl 5β-cholanates, assigned on the basis of a previous paper (21), are shown in **Table 2.** The signal assignments were further confirmed by the distortionless enhancement by polarization transfer (DEPT) spectra. The shielding data of the α-carbon absorptions (3α, 71.0 ppm; 3β, 66.7 ppm; 6α, 68.4 ppm; 6β, 73.7 ppm) in the lower field region are of particular importance in characterizing the number, position, and configuration of the hydroxyl groups. Since these sharp signals are unequivocally identified and separated completely from each other, the shielding data provide a straightforward identification of each isomer as well as an estimation of purity. The ^{13}C-NMR spectra also afforded the confirmatory evidence of the stereochemistry of the A/B-ring junction of 5α- and 5β-series. Thus the 19-methyl signal resonates at lower fields in 1a–4a (23.4–26.1 ppm) than in methyl 3β,6α- and 3β,6β-dihydroxy-5α-cholanates[3] (13.5 and 15.8 ppm, respectively) prepared from 10a, in accord with the previous findings of analogous 5α (22) and 5β (21) bile acid methyl esters. A slight downfield shift (ca. 2 ppm) observed for the 19-methyl signal in 2a and 3a, compared to 1a and 4a, is ascribed to 1,3-diaxial interaction with 6β-hydroxyl group.

EXPERIMENTAL

Melting points were determined on a micro hot-stage apparatus and are uncorrected. IR spectra were obtained on a JASCO IRA-II double-beam spectrometer as KBr tablets. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL FX-90Q (90 and 22.53 MHz, respectively) with $CDCl_3$ containing 1% Me_4Si as the solvent; chemical shifts are expressed in δ (ppm) relative to Me_4Si. In addition, the DEPT spectra were also recorded on a JEOL JNM-GX 500 Fourier transform spectrometer at 125.65 MHz. Mass spectra were recorded on a Hitachi M-80B mass spectrometer under the following conditions: ion source temperature, 180°C; ionizing voltage, 70eV. HPLC was carried out on a Waters Associates system (M-45 pump; U6K sample loop injector) combined with a Shimadzu SPD-2A UV detector using a Nova-Pak C_{18} reversed-phase column (15 cm × 3.9 mm i.d., 5 μm; Waters Associates) with MeOH–water mixture as the mobile phase. A Shimadzu GC-7A gas chromatograph equipped with a flame ionization detector was used isothermally. It was fitted with a chemically bonded fused-silica capillary column [HiCap-CBP1 (equivalent to

[3]Unpublished data: α-carbon signals occur at 71.2 (3β)and 69.4 (6α) ppm in methyl 3β,6α-dihydroxy-5α-cholanate and 71.7 (3β) and 72.0 (6β) ppm in methyl 3β,6β-dihydroxy-5α-cholanate.

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

	Position and Configuration of Hydroxyls							
Carbon	3α	3β	6α[b]	6β[b]	3α,6α(1a)	3α,6β(2a)	3β,6β(3a)	3β,6α(4a)
1	35.1	29.8	37.6	37.8	35.5	35.8	30.2	30.0
2	30.1	27.8	21.0	20.8	30.2	30.1	27.5	27.5
3	71.0	66.7	26.2	26.7[c]	71.5	71.2	66.1	66.0
4	36.0	33.4	19.2	27.2[c]	29.2	36.5	33.7	26.3
5	41.8	36.3	50.0	50.2	48.4	48.6	43.6	43.0
6	26.9	26.5	68.4	73.7	67.9	73.1	73.2	67.8
7	26.2	26.1	35.0	34.7	34.8	34.7	34.5	34.6
8	35.5	35.5	34.8	30.7	34.8	30.7	30.6	34.6
9	40.1	39.6	39.8	40.7	39.8	40.7	40.1	39.1
10	34.2	34.9	36.6	35.0	35.9	34.3	34.8	36.4
11	20.5	20.9	20.7	20.5	20.7	20.6	20.9	21.0
12	39.9	40.2	40.0	40.1	39.8	40.0	40.1	40.0
13	42.2	42.6	42.8	42.7	42.8	42.7	42.8	42.9
14	56.2	56.4	56.2	56.3	56.1	56.3	56.5	56.2
15	23.9	24.0	24.1	24.1	24.1	24.2	24.1	24.1
16	27.8	28.0	28.0	28.1	28.0	28.1	28.1	28.0
17	55.6	55.8	55.9	56.0	55.9	56.0	56.1	56.0
18	11.7	11.9	12.0	12.0	11.9	12.0	12.1	12.0
19	23.1	23.7	24.2	26.4	23.4	25.5	26.1	23.9
20	35.0	35.2	35.2	35.3	35.3	35.3	35.4	35.3
21	17.9	18.1	18.2	18.2	18.2	18.3	18.3	18.2
22	30.7	30.8	30.9	31.0	30.9	31.0	31.0	30.9
23	30.7	30.8	30.9	31.0	30.9	31.0	31.0	30.9
24	174.2	174.4	174.5	174.6	174.5	174.6	174.7	174.6
25	51.0	51.2	51.3	51.3	51.3	51.4	51.4	51.4

[a] In ppm downfield from MeSi.
[b] Assignments were confirmed by the DEPT spectra at 125.65 MHz.
[c] Assignments may be interchanged.

OV-1); 18 m × 0.2 mm i.d., film thickness, 0.25 μm; Shimadzu]. Analytical TLC was performed on pre-coated silica gel (20 cm × 20 cm, 0.25 mm layer thickness; E. Merck AG) using hexane–EtOAc–acetic acid mixture as the developing solvent. All compounds were dried by azeotropic distillation before use in reactions.

Methyl 3β-formyloxy-5-cholenate (6a)

(a) The ester (1a; 200 mg) was subjected to the diethyl azodicarboxylate-triphenylphosphine-formic acid inversion procedure as described for the preparation of 8a. The mixture was processed, and the resulting product was crystallized from acetone to give 6a (143 mg; 69%) as colorless needles: mp 106–107°C, IR V_{max} cm^{-1}: 1730 (C=O), 1372 (C=C), 1192 (ester). ^{1}H-NMR δ:0.68 (3H,s,18-H), 0.93 (3H,d,J=6.3 Hz,21-H), 1.03 (3H,s,19-H), 3.66 (3H,s,COOMe), 4.72 (1H,brm,3-H), 5.39 (1H,d,J=4.5 Hz,6-H), 8.04 (1H,s,CHO). Anal. calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 74.91; H, 9.65.

(b) The ditosylate (5a; 620 mg), obtained from 1a (10), was subjected to the DMF inversion reaction as described below. After 48 hr at 80°C, the mixture was processed, and the resulting product was identified as 6a (190 mg; 52%) by melting point, TLC, and ^{1}H-NMR comparisons.

Methyl 3α-hydroxy-6-oxo-5β-cholanate (7a)

A solution of K_2CrO_4 (1.2 g) in water (2.5 ml) was added to a stirred solution of 1a (2.0 g) in acetic acid (30 ml). The mixture was further stirred at room temperature for 6 hr and poured onto water. The reaction product was extracted with CH_2Cl_2, and the combined CH_2Cl_2 layer was washed with 10% $NaHCO_3$ and water, dried over Drierite, and evaporated. The oily residue was chromatographed on silica gel (80 g). Elution with benzene–EtOAc 7:3 (v/v) gave 0.21 g (11%) of a solid, which was characterized as methyl 3,6-dioxo-5β-cholanate (14a). mp 143–145°C (aqueous MeOH) (lit. mp 139°C (23)).

Further elution with benzene–EtOAc 4:6 (v/v) and crystallization of the eluate from acetone–hexane gave the main product 7a (1.41 g; 71%) as colorless needles: mp 143–144°C (lit. mp 144–146°C (14)). IR V_{max} cm^{-1}: 1725, 1695 (C=O), 3510, 1064 (OH). ^{1}H-NMR δ:0.66 (3H,s,18-H), 0.84 (3H,s,19-H), 0.93 (3H,d,J=5.4 Hz, 21-H), 3.57 (1H,brm,3-H), 3.66 (3H,s,COOMe).

Methyl 3α,6β-dihydroxy-5β-cholanate (2a)

$NaBH_4$ (1.51 g) was added to a suspension of 7a (1.5 g) and $PdCl_2$ (0.5 g) in MeOH (70 ml) over a period of 30 min at room temperature. After further stirring for 2 hr, the precipitated Pd was removed by filtration and washed with MeOH. The filtrate and washings were combined

and evaporated, and the residue was extracted with CH_2Cl_2. The combined extract was washed with water, dried over Drierite, and evaporated. The oily residue, estimated by GLC to be a 3.6:1 mixture of the epimers (2a and 1a), was chromatographed on neutral alumina (activity II, ratio 70:1). Elution with benzene–EtOAc 1:1 ~1:9 (v/v) provided two well-separated fractions. The less polar fraction was recrystallized from aqueous MeOH to give 2a (1.08 g; 72%) as colorless crystals: mp 114–115°C (lit. mp 114°C (5)). IR V_{max} cm^{-1}: 1740 (C=O), 3400, 3360, 1040, 1010 (OH). ^{1}H-NMR δ:0.68 (3H,s,18-H), 0.92 (3H,d,J=5.4 Hz,21-H), 1.11 (3H,s,19-H), 3.56 (1H,brm,3-H), 3.66 (3H,s, COOMe), 3.75 (1H,m,6-H). MS m/z (relative intensity): 388 (66%, M-H_2O), 370 (33%, M-2H_2O), 355 (20%, M-2H_2O-CH_3), 273 [17%, M-H_2O-side chain (SC)]. Anal. calcd. for $C_{25}H_{42}O_4 \cdot 1/3H_2O$: C, 72.77; H, 10.34. Found: C, 72.58; H, 10.13.

The more polar fraction (0.22 g; 15%) was identified as 1a by TLC, melting point, and ^{1}H-NMR comparisons.

3α,6β-Dihydroxy-5β-cholanic acid (2)

This was prepared from 2a by the usual method with 5% methanolic KOH. Recrystallization of the product from aqueous MeOH gave 2 as colorless needles: mp 214–215°C (lit. mp 208°C (5) and 209–210°C (24)). IR V_{max} cm^{-1}: 1715 (C=O), 3410, 3300, 1050, 1028 (OH). ^{1}H-NMR δ($CDCl_3$+30%DMSO-d_6): 0.68 (3H,s,18-H), 0.93 (3H,d,J=5.4 Hz, 21-H), 1.11 (3H,S,19-H), 3.57 (1H,brm,3-H), 3.74 (1H,m,6-H). Anal. calcd. for $C_{24}H_{40}O_4 \cdot 1/6H_2O$: C, 72.87; H, 10.24. Found: C, 72.84; H, 10.02.

Methyl 6α-hydroxy-3-oxo-5β-cholanate (13a)

A suspension of 1a (2.0 g) and silver carbonate-Celite (5.0 g) in toluene (70 ml) was refluxed for 7 hr. The precipitate was filtered off, and the filtrate was decolorized with Norite and evaporated. The residual oil, when treated with acetone–hexane, afforded 13a (1.45 g; 73%) as colorless needles: mp 99–100°C (lit. mp 121–122°C (23)). IR V_{max} cm^{-1}: 1738, 1720 (C=O), 3450, 1042 (OH). ^{1}H-NMR δ: 0.68 (3H,s,18-H), 0.93 (3H,d,J=5.4 Hz,21-H), 1.01 (3H,s,19-H), 3.66 (3H,s,COOMe), 4.08 (1H,brm,6-H). Anal. calcd. for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.17; H, 9.76.

Methyl 6β-hydroxy-3-oxo-5β-cholanate (15a)

This was prepared from 2a (1.0 g) by the silver carbonate-Celite method as described for the preparation of 13a. Crystallization from acetone–hexane gave 15a (0.70 g; 70%) as colorless needles: mp 124–125°C. IR V_{max} cm^{-1}: 1740, 1710 (C=O), 3400, (OH). ^{1}H-NMR δ: 0.72 (3H,s,18-H), 0.93 (3H,d,J=5.4 Hz,21-H), 1.22 (3H,s,19-H), 3.67 (3H,s,COOMe), 3.72 (1H,m,6-H). Anal. calcd. for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.12; H, 9.84.

Methyl 3α-tosyloxy-6-oxo-5β-cholanate (9a)

Tosyl chloride (4.0 g) was added to a solution of 7a (4.0 g) in dry pyridine (25 ml) and stirred for 12 hr at room temperature. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with 10% HCl and water, dried over Drierite, and evaporated. The residual oil (4.53 g; 82%), although homogeneous on TLC and ^{1}H-NMR analyses, could not be crystallized. IR V_{max} cm^{-1}: 1722, 1702 (C=O), 1160, 842, 668 (phenyl), 1362 (SO_2). ^{1}H-NMR δ:0.64 (3H,s,18-H), 0.81 (3H,s,19-H), 0.93 (3H,d,J=6.3 Hz,21-H), 2.45 (3H,s,C_6H_4Me), 3.66 (3H,s,COOMe), 4.35 (1H,brm,3-H), 7.30 and 7.77 (each 2H,d,J=9.0 Hz,pra-disubstituted phenyl). Anal. calcd. for $C_{32}H_{46}O_6S \cdot 1/3H_2O$: C, 68.06; H, 8.33. Found: C, 68.06; H, 8.01.

Methyl 3β-formyloxy-6-oxo-5β-cholanate (8a)

(a) A solution of diethyl azodicarboxylate (6.43 g) in benzene (5 ml) was slowly added dropwise to a solution of 7a (5.0 g), triphenylphosphine (9.68 g), and formic acid (1.7 g) in benzene (50 ml). After refluxing for 48 hr and then cooling, the precipitated solid (diethyl hydrazodicarboxylate) was filtered, and the filtrate was evaporated and crystallized from Et_2O–hexane. The precipitate (triphenylphosphine oxide) was filtered off, and the filtrate was chromatographed on silica gel column (250 g) and eluted with benzene–EtOAc 8:2 (v/v). Recrystallization of the product from acetone–hexane gave 8a (4.69 g; 88%): mp 122–123°C. IR V_{max} cm^{-1}: 1722, 1702 (C=O), 1198 (ester). ^{1}H-NMR δ: 0.66 (3H,s,18-H), 0.89 (3H,s,19-H), 0.93 (3H,d,J=6.3 Hz,21-H), 3.66 (3H,s,COOMe), 5.25 (1H,m,3-H), 8.03 (1H,s,CHO). Anal. calcd. for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 72.17; H, 9.26

(b) A solution of 9a (1.0 g) in DMF (45 ml) was kept at 80°C ± 1°C for 75 hr. The reaction mixture was diluted with water and the product was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with water, dried over Drierite, and evaporated. The residual oil, purified by column chromatography on silica gel as described in part a, gave 8a (511 mg, 66%), identical with that prepared above, according to melting point, TLC, and ^{1}H-NMR comparisons.

Methyl 3β-hydroxy-6-oxo-5α-cholanate (10a)

The ester 9a (500 mg) was subjected to the DMF reaction as described for the preparation of 8a (part b). The resulting oily product was chromatographed on neutral alumina (activity II, 25 g) and eluted with benzene–EtOAc 1:1 (v/v). Recrystallization of the product from acetone–hexane gave 10a (195 mg; 54%) as colorless needles: mp 152–153°C. IR V_{max} cm^{-1}: 1710 (C=O), 3400, 1068 (OH). ^{1}H-NMR δ: 0.66 (3H,s,18-H), 0.75 (3H,s,19-H), 0.92 (3H,d,J=5.4 Hz,21-H), 3.57 (1H,brm, 3-H), 3.66 (3H,s,COOMe). Anal. calcd. for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.13; H, 9.89.

Methyl 3β-formyloxy-6β-hydroxy-5β-cholanate (11a) and methyl 3β-formyloxy-6α-hydroxy-5β-cholanate (12a)

(a) *tert*-Butylamine–borane complex (1.42 g) was added to a solution of 8a (3.0 g) in CH_2Cl_2 (120 ml). The mixture was stirred at room temperature for 3 hr and then acidified with 3 N HCl. The CH_2Cl_2 layer was washed with 10% $NaHCO_3$ and water, and then dried over Drierite and evaporated. The oily residue, estimated by GLC to be a 1:1 mixture of the epimers (12a and 11a), was chromatographed on silica gel (150 g). Elution with benzene–EtOAc 8:2 (v/v) provided two well-separated fractions. The less polar fraction was recrystallized from acetone–hexane to give 11a (1.38 g; 46%) as colorless prisms: mp 153–154°C. IR V_{max} cm^{-1}: 1730 (C=O), 3430, 1016 (OH), 1182 (ester). ^{1}H-NMR δ: 0.69 (3H,s,18-H), 0.92 (3H,d,J=5.4 Hz,21-H), 1.16 (3H,s,19-H), 3.66 (3H,s,COOMe), 3.70 (1H,m,6-H), 5.19 (1H,m,3-H), 8.06 (1H,s,CHO). Anal. calcd. for $C_{26}H_{42}O_5$: C, 71.85; H, 9.74. Found: C, 71.75; H, 9.67.

The more polar fraction was recrystallized from acetone–hexane to give 12a (1.36 g; 45%) as colorless prisms: mp 129–130°C. IR V_{max} cm^{-1}: 1710 (C=O), 3410, 1024 (OH), 1170 (ester). ^{1}H-NMR δ:0.65 (3H,s,18-H), 0.93 (3H,d,J=6.3 Hz,21-H), 0.96 (3H,s,19-H), 3.66 (3H,s,COOMe), 4.12 (1H,brm,6-H), 5.30 (1H,m,3-H), 8.06 (1H,s,CHO). Anal. calcd. for $C_{26}H_{42}O_5$: C, 71.85; H, 9.74. Found: C, 71.75; H, 9.68.

(b) When 11a and 12a were prepared from 8a by the $NaBH_4/PdCl_2$ method as described for the preparation of 2a, the crude product was estimated by GLC to be a 3.7:1 mixture of the epimers (11a and12a).

Methyl 3β,6β-dihydroxy-5β-cholanate (3a)

A mixture of 11a (200 mg) in MeOH (2 ml) and conc. HCl (0.23 ml) was allowed to stand at room temperature for 1 hr. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over Drierite, and evaporated. The residue was recrystallized from aqueous MeOH to give 3a (170 mg) as colorless needles: mp 124–125°C (lit. mp 127°C (2)). IR V_{max} cm^{-1}: 1720 (C=O), 3380, 1038, 1022 (OH). ^{1}H-NMR δ: 0.68 (3H,s,18-H), 0.92 (3H,d,J=5.4 Hz,21-H), 1.14 (3H,s,19-H), 3.66 (3H,s,COOMe), 3.71 (1H,m,6-H), 4.09 (1H,m,3-H). MS m/z (relative intensity): 406 (2%, M^+), 388 (94%, M-H_2O), 373 (20%, M-H_2O-CH_3), 370 (15%, M-2H_2O), 355 (11%, M-2H_2O-CH_3), 273 (14%, M-H_2O-SC). Anal. calcd. for $C_{25}H_{42}O_4 \cdot 1/4H_2O$: C, 73.04; H, 10.42. Found: C, 72.96; H, 10.30.

Methyl 3β,6α-dihydroxy-5β-cholanate (4a)

This was prepared from 12a (200 mg) by the hydrolysis procedure described above. Recrystallization of the product from acetone–hexane gave 4a (162 mg) as colorless needles: mp 74–75°C. IR V_{max} cm^{-1}: 1740 (C=O), 3375, 1038 (OH). ^{1}H-NMR δ: 0.65 (3H,s,18-H), 0.91 (3H,d,J=5.4 Hz,21-H), 0.94 (3H,s,19-H), 4.10 (1H,brm,6-H), 4.16 (1H,m,3-H). MS m/z (relative intensity): 406 (3%, M^+), 388 (78%, M-H_2O), 373 (24%, M-H_2O-CH_3), 370 (23%, M-2H_2O), 355 (11%, M-2H_2O-CH_3), 273 (13%, M-H_2O-SC). Anal. calcd. for $C_{25}H_{42}O_4 \cdot 1/4H_2O$: C, 73.04; H, 10.42. Found: C, 73.15; H, 10.22.

3β,6β-Dihydroxy-5β-cholanic acid (3)

This was prepared from 11a (200 mg) by the usual method with 5% methanolic KOH. Recrystallization of the product from aqueous MeOH gave 3 (175 mg) as colorless thin plates: mp 260–261°C (lit. mp 258°C (2,5) and 250°C (3)). IR V_{max} cm^{-1}: 1710 (C=O), 3440, 3400, 1448, 1038, 1018 (OH). ^{1}H-NMR δ ($CDCl_3$+30% DMSO-d_6): 0.68 (3H,s,18-H), 0.92 (3H,d,J=5.4 Hz,21-H), 1.13 (3H,s,19-H), 3.64 (1H,m,6-H), 4.02 (1H,m,3-H). Anal. calcd. for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.17; H, 10.19.

3β,6α-Dihydroxy-5β-cholanic acid (4)

This was prepared from 12a (200 mg) by the usual method with 5% methanolic KOH. Recrystallization of the product from aqueous MeOH gave 4 (170 mg) as colorless needles: mp 198–199°C (lit. mp 191–192°C (4) and 190°C (5)). Crystals obtained from acetone (4) melted at 120°C, resolidified at 134°C, and then melted at 198–199°C. IR V_{max} cm^{-1}: 1702 (C=O), 3398, 1040 (OH). ^{1}H-NMR δ ($CDCl_3$+30% DMSO-d_6): 0.65 (3H,s,18-H), 0.92 (3H,d,J=5.4 Hz,21-H), 0.93 (3H,s,19-H), 3.99 (1H,brm,6-H), 4.09 (1H,m,3-H). Anal. calcd. for $C_{24}H_{40}O_4 \cdot 1/4H_2O$: C, 72.59; H, 10.28. Found: C, 72.47; H, 10.04.

Reduction of 6α-hydroxy-3-oxo-5β-cholanic acid (13) (4)

The 3-oxo acid (13; 200 mg) in acetic acid (4 ml) was catalytically hydrogenated with PtO_2 (20 mg) and a few drops of 47% hydrobromic acid at a slight positive pressure for 48 hr. The catalyst was filtered off, and the mother liquor was diluted with water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with water to neutrality, dried over Drierite, and evaporated to an oily product. After the treatment of the oil by hydrolysis with 5% methanoic KOH followed by acidification with 10% H_2SO_4, the crude precipitate was recrystallized from acetone to give crystals (136 mg). The product was estimated by HPLC to be a mixture of 4 and 1 (88%:12%); mp 188–191°C.

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