Potential Bile Acid Metabolites; 12[1]. Synthesis of Stereoisomeric 3,7,12-Trihydroxy-5α-cholanic Acids and Related Compounds[2]

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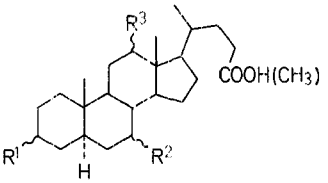
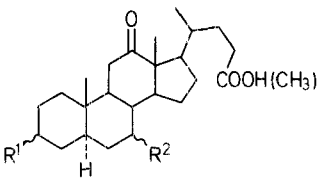
The complete set of the eight theoretically possible stereoisomeric 3,7,12-trihydroxy-5α-cholanic acids, five of which are new, were prepared by reduction of the four possible 3,7-dihydroxy-12-oxo-5α-esters. These esters, obtained by use of the inverting reagents, potassium superoxide-18-crown-6 ether and/or dimethylformamide, by stereoselective reduction with sodium borohydride-palladium dichloride or *t*-butylamine-borane complex yielded the eight desired stereoisomeric trihydroxy compounds. With this work, all the 5β- and 5α-cholanic acids substituted by one to three hydroxy groups at positions 3, 7 and 12, 26 in each series, are now known and have been synthesized.

This paper describes the synthesis of the five remaining hitherto unreported stereoisomers of the eight theoretically possible 3,7,12-trihydroxycholanic acids of the 5α ("allo") series. Since all the other 5α-cholanic acids substituted by one to three hydroxyl groups in the 3, 7 and 12 positions are known[1,3-10] and the preparation of all twenty-six of the analogous acids of the 5β ("normal") series was culminated recently[11-12], this work completes the literature record of the preparation and characterization of the entire set of the fifty-two possible stereoisomers (12 monohydroxy, 24 dihydroxy, and 16 trihydroxy) of the 5β- and 5α-series. In addition, the methyl esters of the new acids and several related compounds are reported herewith.

As suggested by previous work in the 5β-series[12], all the eight of the 5α-3,7,12-trihydroxy acids **1–8**, including the three





1	R^1 = β-OH, R^2 = R^3 = α-OH
2	R^1 = R^2 = R^3 = α-OH
3	R^1 = R^3 = α-OH, R^2 = β-OH
4	R^1 = R^3 = β-OH, R^2 = α-OH
5	R^1 = R^2 = β-OH, R^3 = α-OH
6	R^1 = R^2 = R^3 = β-OH
7	R^1 = α-OH, R^2 = R^3 = β-OH
8	R^1 = R^2 = α-OH, R^3 = β-OH
9	R^1 = β-OH, R^2 = α-OH
10	R^1 = R^2 = β-OH
11	R^1 = α-OH, R^2 = β-OH
12	R^1 = R^2 = α-OH

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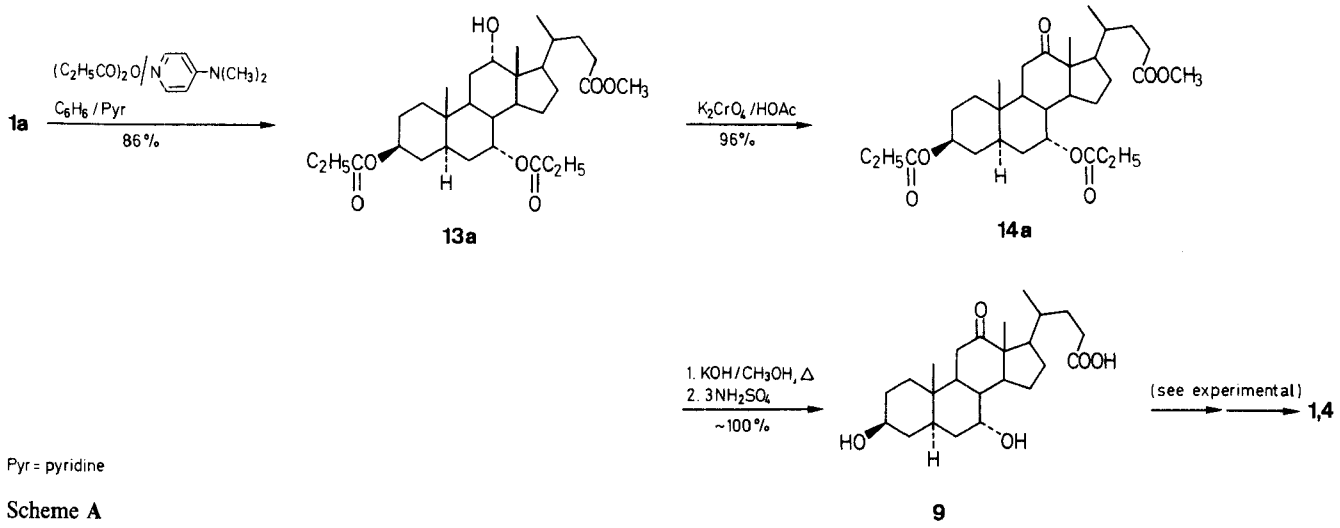
known ones **1**–**3**[3–7], should be obtainable by selective reduction of the four 12-keto derivatives **9a**–**12a**. Thus our primary effort consisted of the synthesis of these ketones, which to be effective and practical, had to originate from an available 5β-acid.

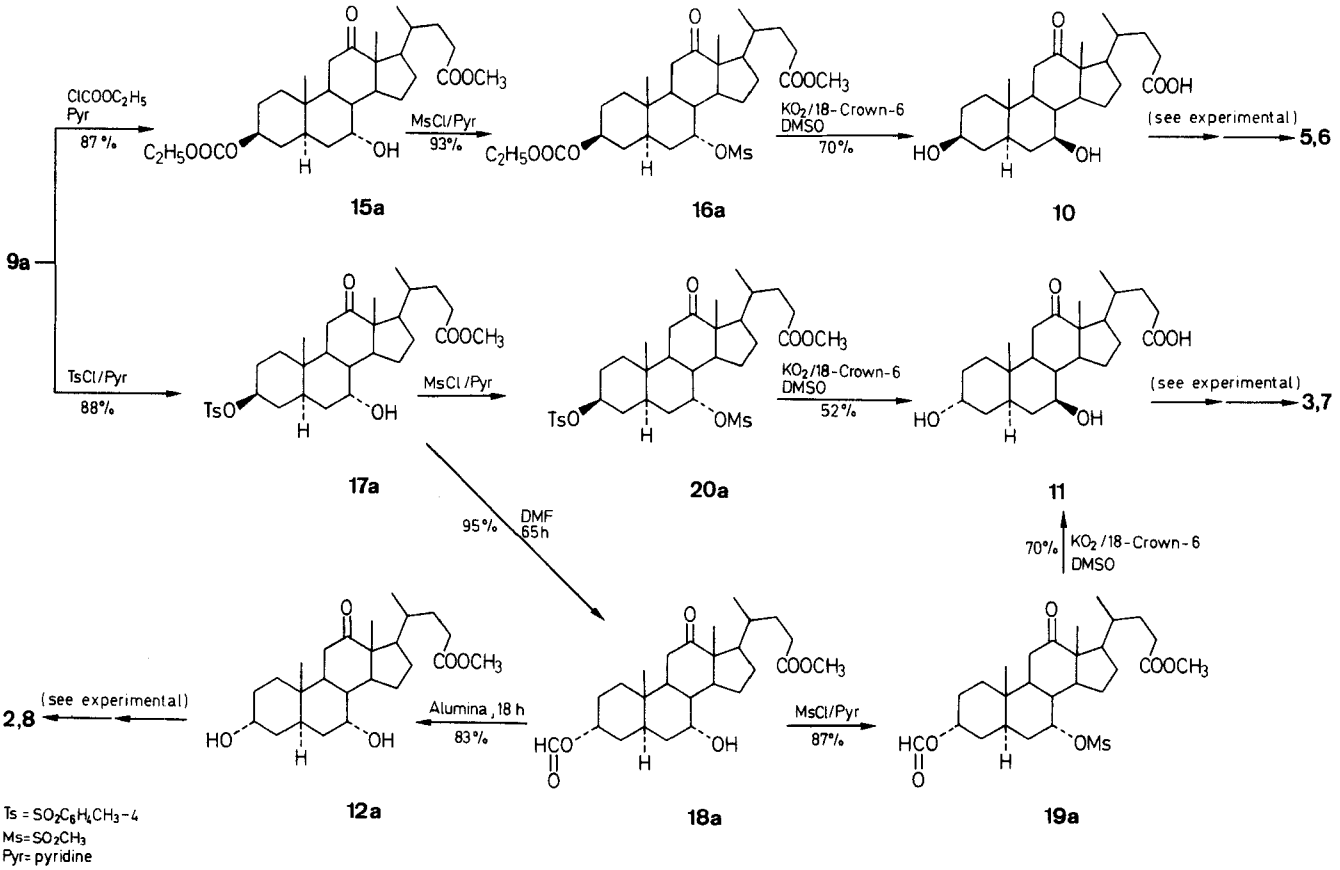
Fortuitously an improved method of allomerization to 5α-compounds[1] offered a feasible route to an adequate supply of the key intermediate, 3β,7α,12α-trihydroxy-5α-cholanic acid (**1**, yield: 24 %) starting from the abundantly available cholic acid (3α,7α,12α-trihydroxy-5β-cholanic acid). Selective acylation at C-3 and C-7 of the ester **1a** by acetic anhydride/pyridine in benzene, which proceeds readily in the 5β-analogs[13], was unsuccessful, but **1a** reacted with propionic anhydride/4-dimethylaminopyridine in benzene/pyridine to yield smoothly the 3β,7α-dipropionate (**13a**). Oxidation of **13a**, followed by alkaline hydrolysis of the resulting ketone **14a**, gave the desired 3β,7α-dihydroxy-12-keto acid (**9**) (overall yield from **1a**, 83 %) (Scheme **A**).

The other 12-ketones were obtained from **9a** following procedures devised for the preparation of the corresponding stereoisomers in the 5β-series[12], in which inversion of *equatorial* hydroxyl at C-3 was achieved by treatment with dimethylformamide[14] and of *axial* hydroxyl at C-7 with potassium superoxide/18-crown-6 ether on appropriate derivatives of **9a** (Scheme **B**).

The C-3 cathylate **15a** of **9a** was converted to the C-7 mesylate **16a** without difficulty. The ester **16a** in the



Scheme **A**



Scheme **B**

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potassium superoxide/crown ether reaction caused inversion[15] at C-7 with simultaneous hydrolyses of the C-3 acyloxy group and the C-24 methyl ester to yield the 3β,7β-dihydroxy-12-keto acid **(10)** in 70% isolated yield.

The 3α,7β-dihydroxy-12-keto acid **(11)** was made by two routes. The first involved the reaction of the 3β-monotosylate **17a** with dimethylformamide to give the inverted 3α-monoformate **18a**, which in turn was converted to the 3α-formate-7α-mesylate **19a**. Reaction of **19a** with potassium superoxide/crown ether reagent resulted in the formation of the expected ketone **11** in 70% yield. The second synthesis of **11** consisted of a one-step reaction discovered in the 5β-work[16] in which the potassium superoxide/crown ether reagent causes simultaneous inversion of the *equatorial* 3β-tosyloxy and 7α-mesyloxy groups of the ester **20a** to yield the desired acid **11** in 52% yield.

The fourth 3α,7α-dihydroxy-12-keto acid **12** was prepared as its methyl ester **12a** by selective hydrolysis at C-3 of the 3α-formate ester **18a** by allowing it to standing in a column of neutral alumina (activity II) in 83% eluted yield.

With the four stereoisomeric 12-ketones in hand, the eight possible trihydroxy acids, as planned, were obtained by use of stereoselective reducing agents found to be effective in our previous work. With sodium borohydride/palladium dichloride[10] all four ketones gave the corresponding *axial* 12α-hydroxy epimers in at least 92% yield. With *t*-butylaminoborane complex[17] the 12β/12α-ratio of the hydroxy product varied according to overall structure of the ketones, but in all cases the major product was the *equatorial* 12 β-epimer. Separate chromatography on either alumina or silica gel of the reduction products resolved the pure esters which on hydrolysis gave the eight desired acids (Table).

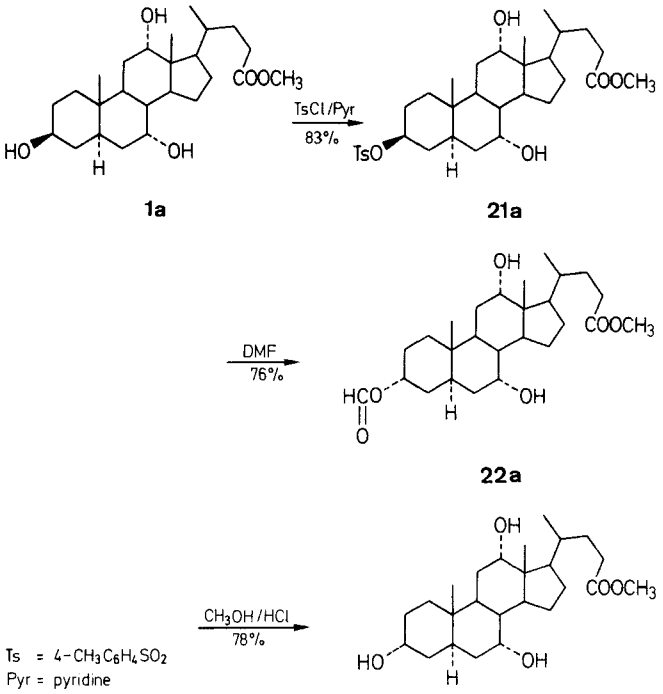
Table. Reduction of 12-Ketones **9a–12a**[a]

Ketone	$NaBH_4-PdCl_2$		*t*-Butylamine-Borane	
	12α-OH [%]	12β-OH [%]	12α-OH [%]	12β-OH [%]
9a	97	3	36	64
10a	93	7	19	81
11a	93	7	26	74
12a	92	8	23	77

[a] The value of the resulting epimers (corresponding to ketones reduced) was determined by HPLC. HPLC was carried out by using a C_{18} reversed phase column, methanol-water mixtures of 70 : 30 (v/v) and flow rate of 0.8 ml/min.

Allocholic acid (**2**; 3α,7α,12α-trihydroxy-5α-cholanic acid), one of the earliest 5α-acids known, had been synthesized by several lengthy and low-yield procedures all of which involved obtaining 3- or 7-keto intermediates which were then reduced by reagents known to favor *axial* hydroxyl products[4–7].

Our preparation described above similarly involves reduction of the 12-ketone **12a**, with sodium borohydride/palladium dichloride. We report an additional preparation of **2** which does not involve reduction of an intermediate ketone, and which we consider to be the method of choice for preparing **2** because of its simplicity and the ready availability of **1a** as starting material. The ester **1a** with an *equatorial* hydroxyl at C-3 readily forms the 3β-monotosylate **21a** which undergoes inversion in the presence of dimethylformamide to the 3α-monoformate **22a** (yield, 76%) and its subsequent hydrolysis with methanol-HCl yields **2a** (Scheme C).



Scheme C

Melting points were determined on an electric micro hot stage and are uncorrected. IR spectra were obtained on Model IRA-II JASCO double-beam spectrophotometer. ^{1}H-NMR spectra were obtained on a JEOL FX-90Q (90 MHz) instrument. HPLC was carried out on a Waters Associates system (M-45 pump; U6K sample loop injector; R401 differential refractometer) using a Nova-Pak C_{18} reversed-phase column (15 cm × 3.9 mm I.D.) with methanol-water mixtures as mobile phase.

General Procedure for the Hydrolysis of Methyl Esters to Free Acids:
The methyl ester (100 mg) is refluxed in 5% methanolic potassium hydroxide (3 ml) of 1 h. Most of the solvent is evaporated, and the residue is dissolved in water (7 ml), cooled in an ice-bath, and acidified with 3 normal sulfuric acid (3 ml) with stirring. The precipitated solid is filtered and recrystallized from an appropriate solvent (see below).

General Procedure for the Esterification of Free Acids to Methyl Esters:
To the free acid (100 mg) in methanol (3 ml) is added *p*-toluenesulfonic acid (10 mg), and the mixture is stirred overnight at room temperature. Most of the solvent is evaporated, and the residue is extracted with dichloromethane (2 × 10 ml). The organic extract is washed successively with water (10), 5% sodium hydrogen carbonate (10 ml), water (10 ml), dried with Drierite, and evaporated to give the crude ester which is crystallized from an appropriate solvent (see below).

Methyl 3β,7α,12α-Trihydroxy-5α-cholanate (1a):
3-Oxo-7α,12α-dihydroxy-4-cholenic acid (prepared from cholic acid as described previously[1]) is reduced with lithium/ammonia as described[4] and the reaction is quenched by adding methanol as a proton source. After treatment of the residual bile salts by sulfuric acid, and then purification by chromatography on alumina (eluent, benzene/ethyl acetate), the desired ester **1a** is obtained as the main product; yield: 56%; m.p. 200–202°C (acetone/hexane) (Lit., 186–187°C[4] and 198–199°C[5]).

IR (KBr): ν = 3400, 1035 cm^{-1} (OH); 1743 (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.69 (s, 3 H, 18-CH_3); 0.80 (s, 3 H, 19-CH_3); 0.98 (d, 3 H, J = 6.3 Hz, 21-CH_3); 3.61 (br m, 1 H, 3-H); 3.66 (s, 3 H, $COOCH_3$); 3.82 (m, 1 H, 7-H); 3.95 ppm (m, 1 H, 12-H).

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3β,7α,12α-Trihydroxy-5α-cholanic Acid (1):
M.p. 236–238 °C (acetone/hexane) (Lit.[4], 241–242 °C).

IR (KBr): ν = 3420, 1035 (OH); 1705 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6): δ = 0.68 (s, 3H, 18-CH_3); 0.79 (s, 3H, 19-CH_3); 0.99 (d, 3H, J = 5.4 Hz, 21-CH_3); 3.53 (br m, 1H, 3-H); 3.78 (m, 1H, 7-H); 3.91 ppm (m, 1H, 12-H).

Methyl 3β,7α-Dipropionyloxy-12α-hydroxy-5α-cholanate (13a):
To a stirred solution of the ester **1a** (1.5 g, 3.5 mmol) in benzene (30 ml) and pyridine (13 ml) is added in one portion propionic anhydride (1.9 g, 14 mmol) and 4-dimethylaminopyridine (1.2 g). The mixture is stirred for 5 min at room temperature, and then poured into water (30 ml). The organic layer is washed with 10% hydrochloric acid (30 ml) and water (60 ml), dried with Drierite, and evaporated to dryness. Crystallization from acetone/hexane affords colorless needles of the title compound; yield: 1.63 g (86%); m.p. 149.0–150.5 °C.

$C_{31}H_{50}O_7$	calc.	C 69.63	H 9.43
(534.7)	found	69.74	9.53

IR (KBr): ν = 3540, 1015, 970 (OH); 1735, 1700 (C=O); 1215, 1173 cm^{-1} (C–O).

^{1}H-NMR ($CDCl_3$): δ = 0.68 (s, 3H, 18-CH_3); 0.83 (s, 3H, 19-CH_3); 0.98 (d, 3H, J = 5.4 Hz, 21-CH_3); 1.12, 1.16 (2 t, 3H, each; J = 7.2 Hz, 3- and 7-$OCOCH_2C\underline{H}_3$); 2.28 (q, 4H, J = 9.9 Hz, 3- and 7-$OCOC\underline{H}_2CH_3$); 3.66 (s, 3H, $COOCH_3$); 3.96 (m, 1H, 12-H); 4.73 (br m, 1H, 3-H); 4.90 ppm (m, 1H, 7-H).

Methyl 3β,7α-Dipropionyloxy-12-oxo-5α-cholanate (14a):
To a stirred solution of the ester **13a** (1.0 g, 1.9 mmol) in acetic acid (30 ml), is added potassium chromate (1.0 g, 5.1 mmol) dissolved in water (2 ml). After the mixture is stirred overnight at room temperature, water (50 ml) is added to the dark brown solution, and the oily product separated is extracted with dichloromethane (2 × 40 ml). The combined extract is washed with water to neutrality, dried with Drierite, and evaporated to an oily residue; yield: 0.96 g (96%), which crystallizes gradually from acetone/hexane as colorless prisms; m.p. 159.5–161.0 °C.

$C_{31}H_{48}O_7$	calc.	C 69.89	H 9.08
(532.7)	found	70.14	8.83

IR (KBr): ν = 1730, 1700 (C=O); 1183 cm^{-1} (C–O).

^{1}H-NMR ($CDCl_3$): δ = 0.85 (d, 3H, J = 5.4 Hz, 21-CH_3); 0.94 (s, 3H, 18-CH_3); 1.03 (s, 3H, 19-CH_3); 1.12 (t, 6H, J = 7.2 Hz, 3- and 7-$OCOCH_2C\underline{H}_3$); 2.29, 2.31 (q, 2H each, J = 9.9 Hz, 3- and 7-$OCOC\underline{H}_2CH_3$); 3.66 (s, 3H, $COOCH_3$); 4.69 (br m, 1H, 3-H); 4.99 ppm (m, 1H, 7-H).

3β,7α-Dihydroxy-12-oxo-5α-cholanic Acid (9):
A solution of the crude ester **14a** (1.5 g, 2.8 mmol) in 10% methanolic potassium hydroxide (35 ml) is refluxed for 12 h. Most of the solvent is evaporated, the residue is dissolved in water (70 ml), cooled in an ice-bath, and acidified with 3 normal sulfuric acid (50 ml). The precipitated solid; yield: 0.76 g (~100%) is recrystallized from aqueous methanol; m.p. 272–273 °C.

$C_{24}H_{38}O_5$	calc.	C 70.90	H 9.42
(406.6)	found	70.72	9.70

IR (KBr): ν = 3450, 1032 (OH); 1695 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6): δ = 0.84 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.88 (s, 3H, 18-CH_3); 1.01 (s, 3H, 19-CH_3); 3.49 (br m, 1H, 3-H); 3.81 ppm (m, 1H, 7-H).

Methyl 3β,7α-Dihydroxy-12-oxo-5α-cholanate (9a):
M.p. 189–190 °C (acetone/hexane).

$C_{25}H_{40}O_5$	calc.	C 71.39	H 9.59
(420.6)	found	71.47	9.48

IR (KBr): ν = 3560, 3475, 1040, 975 (OH); 1720, 1700 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.87 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.90 (s, 3H, 18-CH_3); 1.03 (s, 3H, 19-CH_3); 3.58 (br m, 1H, 3-H); 3.66 (s, 3H, $COOCH_3$); 3.93 ppm (m, 1H, 7-H).

Methyl 3β-Cathyloxy-7α-hydroxy-12-oxo-5α-cholanate (15a):
To a stirred solution of the ester **9a** (1.0 g, 2.4 mmol) in dioxane (16 ml) and dry pyridine (2.5 ml) cooled in an ice-bath is added dropwise ethyl chloroformate (3 ml). After standing for 1 h at room temperature, water (35 ml) and concentrated hydrochloric acid (2.5 ml) are added to the mixture with stirring. The precipitated solid is filtered, washed with water (100 ml), and recrystallized from acetone/hexane as colorless fine needles; yield: 2.54 g (87%); m.p. 135–136 °C.

$C_{28}H_{44}O_7$	calc.	C 68.26	H 9.00
(492.6)	found	68.12	9.01

IR (KBr): ν = 3475, 1033, 1010 (OH); 1730, 1685 (C=O); 1262 cm^{-1} (C–O).

^{1}H-NMR ($CDCl_3$): δ = 0.87 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.91 (s, 3H, 18-CH_3); 1.02 (s, 3H, 19-CH_3); 1.30 (t, 3H, J = 7.2 Hz, 3-$OCOOCH_2C\underline{H}_3$); 3.66 (s, 3H, $COOCH_3$); 3.92 (m, 1H, 7-H); 4.17 (q, 2H, J = 10.8 Hz, 3-$OCOOC\underline{H}_2CH_3$); 4.56 ppm (br m, 1H, 3-H).

Methyl 3β-Cathyloxy-7α-mesyloxy-12-oxo-5α-cholanate (16a):
To a stirred solution of the ester **15a** (1.65 g, 3.3 mmol) in dry pyridine (25 ml) is added dropwise methanesulfonylchloride (1.6 ml, 14 mmol). Stirring is continued for 1 h and the mixture is allowed to stand overnight at room temperature. The dark brown solution is added dropwise onto a mixture of ice chips in water (50 ml) with stirring. The precipitated solid is filtered, washed with water (120 ml), and extracted with dichloromethane (2 × 50 ml). The combined extract is washed with water (100 ml), decolorized with Norite, and evaporated to give the title compound, which is recrystallized from dichloromethane/isopropyl ether as colorless prisms; yield: 1.78 g (93%); m.p. 162–164 °C.

$C_{29}H_{46}O_9S$	calc.	C 61.03	H 8.13
(570.7)	found	61.11	8.13

IR (KBr): ν = 1735, 1695 (C=O); 1330, 1175, 890 cm^{-1} (SO_2).

^{1}H-NMR ($CDCl_3$): δ = 0.84 (d, 3H, J = 5.4 Hz, 21-CH_3); 0.95 (s, 3H, 18-CH_3); 1.04 (s, 3H, 19-CH_3); 1.30 (t, 3H, J = 7.2 Hz, 3-$OCOOCH_2C\underline{H}_3$); 3.02 (s, 3H, SO_2CH_3); 3.66 (s, 3H, $COOCH_3$); 4.17 (q, 2H, J = 10.8 Hz, 3-$OCOOC\underline{H}_2CH_3$); 4.60 (br m, 1H, 3-H); 4.95 ppm (m, 1H, 7-H).

Methyl 3β-Tosyloxy-7α-hydroxy-12-oxo-5α-cholanate (17a):
To the ester **9a** (2.0 g, 4.8 mmol) dissolved in dry pyridine (12 ml) is added freshly recrystallized *p*-toluenesulfonyl chloride (2.0 g, 10.5 mmol) in dry pyridine (6 ml) in one portion. After being allowed to stand for 24 h at room temperature, ice-water (30 ml) is added, and the reaction product is extracted with dichloromethane (2 × 50 ml). The combined extract is washed with water (70 ml), 3 normal hydrochloric acid (50 ml), and water (100 ml), dried over Drierite, and evaporated to dryness. The oily residue is crystallized from benzene/hexane as colorless round tufts of needles; yield: 2.42 g (88%); m.p. 168–169 °C.

$C_{32}H_{46}O_7S$	calc.	C 66.87	H 8.07
(574.7)	found	67.15	8.02

IR (KBr): ν = 3500, 1025 (OH); 1740, 1705 (C=O); 1595 (C=C_{arom}); 1325, 1165, 925 cm^{-1} (SO_2).

^{1}H-NMR ($CDCl_3$): δ = 0.84 (d, 3H, J = 5.4 Hz, 21-CH_3); 0.87 (s, 3H, 18-CH_3); 1.00 (s, 3H, 19-CH_3); 2.45 (s, 3H, $C_6H_4-C\underline{H}_3$); 3.65 (s, 3H, $COOCH_3$); 3.90 (m, 1H, 7-H); 4.38 (br m, 1H, 3-H); 7.32, 7.78 ppm (AA'BB', 2H each, J = 9.0 Hz, H_{arom}).

Methyl 3α-Formyloxy-7α-hydroxy-12-oxo-5α-cholanate (18a):
The ester **17a** (2.3 g, 4.0 mmol) dissolved in dry dimethylformamide (110 ml) and kept at 80 ± 1 °C for 65 h. The straw-colored solution is diluted with water to near turbidity, and allowed to stand till crystallization is over. A second crop is obtained on further dilution of the mother liquor and refrigeration; yield: 1.70 g (95%); m.p. 188.0–189.5 °C (colorless thin plates from acetone/hexane).

$C_{26}H_{40}O_6$	calc.	C 69.61	H 8.99
(488.6)	found	71.03	9.15

IR (KBr): ν = 3450, 1030 (OH); 1745, 1730, 1695 (C=O); 1180, 1155 cm^{-1} (C–O).

^{1}H-NMR ($CDCl_3$): δ = 0.87 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.90 (s, 3H, 18-CH_3); 1.03 (s, 3H, 19-CH_3); 3.66 (s, 3H, $COOCH_3$); 3.95 (m, 1H, 7-H); 5.20 (m, 1H, 3-H); 8.04 ppm (s, 1H, 3-OCHO).

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Methyl 3α-Formyloxy-7α-mesyloxy-12-oxo-5α-cholanate (19a):
The ester **18a** (1.5 g, 3.3 mmol) is treated with methanesulfonyl chloride (1.5 ml, 13 mmol) as described above. After being processed analogously, the oily product is crystallized from dichloromethane/isopropyl ether as colorless needles; yield: 1.53 g (87%); m.p. 152–153°C.

$C_{27}H_{42}O_8S$ (526.6) calc. C 61.58 H 8.04 found 61.85 8.08

IR (KBr): ν = 1745, 1725, 1705 (C=O); ν = 1190, 1110 (C–O); 1320, 1168, 873 cm^{-1} (SO_2).

^{1}H-NMR ($CDCl_3$): δ = 0.86 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.93 (s, 3H, 18-CH_3), 1.04 (s, 3H, 19-CH_3), 3.00 (s, 3H, SO_2CH_3), 3.66 (s, 3H, $COOCH_3$), 4.97 (m, 1H, 7-H), 5.21 (m, 1H, 3-H), 8.05 ppm (s, 1H, 3-OCHO).

Methyl 3β-Tosyloxy-7α-mesyloxy-12-oxo-5α-cholanate (20a):
20a is prepared from the ester **19a** by mesylation as described above. Although it is shown to be homogeneous by HPLC and ^{1}H-NMR, it could not be recrystallized.

$C_{33}H_{48}O_9S_2$ (652.7) calc. C 60.72 H 7.41 found 60.53 7.33

IR ($CHCl_3$): ν = 1740, 1715 (C=O); 1600 (C=C_{arom}); 1355, 1175, 935, 895 (SO_2).

^{1}H-NMR ($CDCl_3$): δ = 0.83 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.91 (s, 3H, 18-CH_3); 1.01 (s, 3H, 19-CH_3); 2.45 (s, 3H, $C_6H_4CH_3$); 3.00 (s, 3H, SO_2CH_3); 3.65 (s, 3H, $COOCH_3$); 4.44 (br m, 1H, 3-H); 4.93 (m, 1H, 7-H); 7.31, 7.79 ppm (AA'BB', 2H each, J = 9.0 Hz, H_{arom}).

3β,7β-Dihydroxy-12-oxo-5α-cholanic Acid (10):
To a stirred suspension of potassium superoxide (1.0 g, 14 mmol) in dimethyl sulfoxide (50 ml) is added under nitrogen atmosphere 18-crown-6 ether (0.3 g, 1.2 mmol), and the mixture is stirred additionally for 1.5 h at room temperature. To this solution is added the ester **16a** (1.78 g, 3.1 mmol) dissolved in dry dimethyl sulfoxide (15 ml). After stirring the mixture for 1.5 h at room temperature under nitrogen, saturated brine (45 ml) is added gradually under ice-bath cooling (ice-bath), and the resulting solution is extracted with benzene (50 ml). The aqueous layer is cooled in an ice-bath and acidified with 10% sulfuric acid. The precipitated solid is filtered, washed with water, and recrystallized from ethyl acetate/hexane containing a few drops of methanol as colorless needles; yield: 0.89 g (70%); m.p. 249–251°C.

$C_{24}H_{38}O_5$ (406.5) calc. C 70.90 H 9.42 found 70.62 9.34

IR (KBr): ν = 3530, 3160, 1040, 980 (OH); 1690 (C=O).

^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6): δ = 0.84 (d, 3H, J = 5.4 Hz, 21-CH_3); 0.92 (s, 3H, 18-CH_3); 1.05 (s, 3H, 19-CH_3); 3.41 ppm (br m, 2H, 3- and 7-H).

Methyl 3β,7β-Dihydroxy-12-oxo-5α-cholanate (10a):
M.p. 198.5–200.5°C (acetone/hexane).

$C_{25}H_{40}O_5$ (420.6) calc. C 71.39 H 9.59 found 71.14 9.45

IR (KBr): ν = 3470, 1028, 1000, 975 (OH); 1605 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.84 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.93 (s, 3H, 18-CH_3); 1.05 (s, 3H, 19-CH_3); 3.40 (br m, 2H, 3- and 7-H); 3.66 (s, 3H, $COOCH_3$).

3α,7β-Dihydroxy-12-oxo-5α-cholanic Acid (11):
From **19a**: The ester **19a** (1.4 g, 2.7 mmol) is converted to the title compound **11** by the potassium superoxide/18-crown-6 ether inversion reaction described for the preparation of the acid **10**; yield: 0.76 g (70%); m.p. 264–266°C (aqueous methanol) (Lit.[6], 247–248°C).

IR (KBr): 3400, 1028, 1003 (OH); 1732, 1695 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6): δ = 0.84 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.88 (s, 3H, 18-CH_3); 1.05 (s, 3H, 19-CH_3); 3.25 (br m, 1H, 7-H); 3.96 ppm (m, 1H, 3-H).

From **20a**: The ester **20a** (300 mg, 0.5 mmol) subjected to the same potassium superoxide/crown ether inversion method, required 8 h for complete reaction. After the mixture is processed as described above, the reaction product is found to be identical with the acid **11** prepared from **19a** according to m.p., HPLC and ^{1}H-NMR analyses; yield: 130 mg (52%).

Methyl 3α,7β-Dihydroxy-12-oxo-5α-cholanate (11a):
This is prepared from the acid **11** by the general esterification method. Although it is found to be homogeneous according to HPLC and ^{1}H-NMR analyses it could not be crystallized.

$C_{25}H_{40}O_5$ (420.6) calc. C 71.39 H 9.59 found 71.13 9.55

IR ($CHCl_3$): ν = 3500, 1025, 1005 (OH); 1690, 1675 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.85 (d, 3H, J = 6.3 Hz, 21-CH_3); 0.89 (s, 3H, 18-CH_3); 1.06 (s, 3H, 19-CH_3); 3.37 (br m, 1H, 7-H); 3.66 (s, 3H, $COOCH_3$); 4.07 ppm (m, 1H, 3-H).

Methyl 3α,7α-Dihydroxy-12-oxo-5α-cholanate (12a):
A solution of the ester **18a** (500 mg, 1.0 mmol) dissolved in benzene (5 ml) is poured onto a column of neutral alumina (25 g, activity II) and allowed to stand for 18 h. Elution with ethyl acetate gives the title compound; yield: 390 mg (83%), which is homogeneous according to HPLC and ^{1}H-NMR but failed to crystallize.

$C_{25}H_{40}O_5 \cdot \frac{2}{3} H_2O$ (420.6+) calc. C 69.41 H 9.63 found 69.47 9.67

IR ($CHCl_3$): ν = 3360, 1008 (OH); 1726, 1690 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.84 (d, 3H, J = 5.4 Hz, 21-CH_3); 0.87 (s, 3H, 18-CH_3); 1.02 (s, 3H, 19-CH_3), 3.66 (s, 3H, $COOCH_3$); 3.92 (m, 1H, 7-H); 4.07 ppm (m, 1H, 3-H).

3α,7α-Dihydroxy-12-oxo-5α-cholanic Acid (12):
M.p. 248–250°C (ethyl acetate).

$C_{24}H_{38}O_5 \cdot \frac{3}{4} H_2O$ (406.5) calc. C 68.62 H 9.48 found 68.41 9.49

IR (KBr): ν = 3400, 998 (OH); 1700, 1685 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6): δ = 0.86 (s, 3H, 18-CH_3); 1.02 (s, 3H, 19-CH_3); 3.83 (m, 1H, 7-H); 4.07 ppm (m, 1H, 3-H).

Methyl 3β-Tosyloxy-7α,12α-dihydroxy-5α-cholanate (21a):
The ester **1a** (500 mg, 1.2 mmol) is converted to its tosylate **21a** by the method as described for the preparation of the ester **17a**; yield: 560 mg (83%); m.p. 145–148°C (colorless prisms from aqueous methanol).

$C_{32}H_{48}O_7S$ (576.7) calc. C 66.64 H 8.39 found 66.47 8.46

IR (KBr): ν = 3540, 1025 (OH); 1735 (C=O); 1594 (C=C_{arom}); 1325, 1172, 902, 858 cm^{-1} (SO_2).

^{1}H-NMR ($CDCl_3$): δ = 0.67 (s, 3H, 18-CH_3), 0.77 (s, 3H, 19-CH_3), 0.96 (d, 3H, J = 5.4 Hz, 21-CH_3), 2.44 (s, 3H, $C_6H_4CH_3$), 3.65 (s, 3H, $COOCH_3$), 3.79 (m, 1H, 7-H); 3.93 (m, 1H, 12-H); 4.37 (br m, 1H, 3-H); 7.31, 7.78 ppm (AA'BB', 2H each, J = 9.0 Hz, H_{arom}).

Methyl 3α-Formyloxy-7α,12α-dihydroxy-5α-cholanate (22a):
The ester **21a** (450 mg, 0.8 mmol) subjected to the inversion reaction with dimethylformamide and processed as described for the preparation of the ester **18a**, affords the title compound (in two crops); yield: 270 mg (76%); m.p. 157.5–159.0°C (aqueous methanol).

$C_{26}H_{42}O_6$ (450.6) calc. C 69.30 H 9.40 found 69.01 9.15

IR (KBr): ν = 3380, 1028 (OH); 1728, 1710 (C=O); 1195 and 1155 cm^{-1} (C–O).

^{1}H-NMR ($CDCl_3$): δ = 0.69 (s, 3H, 18-CH_3), 0.80 (s, 3H, 19-CH_3), 0.98 (d, 3H, J = 5.4 Hz, 21-CH_3), 3.65 (s, 3H, $COOCH_3$), 3.82 (m, 1H, 7-H), 3.96 (m, 1H, 12-H), 5.18 (m, 1H, 3-H); 8.04 ppm (s, 1H, 3-OCHO).

Methyl 3α,7α,12α-Trihydroxy-5α-cholanate (2a):
To the ester **22a** (220 mg, 0.5 mmol) dissolved in methanol (11 ml) is added concentrated hydrochloric acid (0.3 ml), and the mixture is allowed to stand at 25°C for 40 h (monitored by TLC, eluent: hexane/ethyl acetate/acetic acid, 10:40:2, V/V/V). Most of the solvent is evaporated under reduced pressure and the residue is extracted with dichloromethane (2 × 20 ml). The organic extract is washed with water to neutrality, dried over Drierite, and evaporated

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to give the desired ester **2a**, which is recrystallized from acetone/hexane as colorless needles; yield: 160 mg (78%); m.p. 227.0–228.5 °C (Lit.[4,5], 225–226 °C).

IR (KBr): ν = 3350, 1008 (OH); 1735 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.69 (s, 3 H, 18-CH_3); 0.77 (s, 3 H, 19-CH_3); 0.98 (d, 3 H, J = 5.4 Hz, 21-CH_3); 3.66 (s, 3 H, $COOCH_3$); 3.83 (m, 1 H, 7-H); 3.96 (m, 1 H, 12-H); 4.05 ppm (m, 1 H, 3-H).

3α,7α,12α-Trihydroxy-5α-cholanic Acid (2):
M.p. 241–243 °C (aqueous acetone) (Lit., 250–251 °C[5], 239–241 °C[6], and 238–240 °C[7]).

IR (KBr): ν = 3400, 1008 (OH); 1700 (C=O).

^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6): δ = 0.67 (s, 3 H, 18-CH_3), 0.75 (s, 3 H, 19-CH_3), 0.99 (d, 3 H, J = 5.4 Hz, 21-CH_3), 3.74 (m, 1 H, 7-H), 3.89 (m, 1 H, 12-H); 3.96 ppm (m, 1 H, 3-H).

General Procedure for Reduction of 3,7-Dihydroxy-12-oxo Esters 9a–12a:

Method A. *With Sodium Borohydride – Palladium Dichloride:* To a stirred suspension of the appropriate 12-ketone (210 mg, 0.5 mmol) and palladium dichloride (180 mg) in methanol (10 ml) is added gradually sodium borohydride (200 mg) over a period of 10 min at room temperature. After further stirring for 2 h, the precipitated palladium is removed by filtration and washed with methanol (10 ml). The filtrate and washings are combined, evaporated under reduced pressure and the residue is extracted with ethyl acetate/ethyl ether (1:1, V/V). The combined extract is washed with water (20 ml), dried with Drierite, and evaporated. The crude reduction products are estimated to be 12α:12β-mixtures (92:8 – 97:3) of the corresponding 12-hydroxy epimers as judged by HPLC (Table).

Method B. *With t-Butylamine-borane Complex:* To a stirred solution of the appropriate 12-ketone (420 mg, 1 mmol) in dichloromethane (20 ml) is added *t*-butylamine-borane complex (200 mg). The mixture is stirred at room temperature for 2 h and then acidified with 3 normal hydrochloric acid. The dichloromethane layer is washed with water (30 ml), dried with Drierite, and evaporated. The crude reduction products are estimated to 12β:12α-mixture (64:36–81:19) of the corresponding 12-hydroxy epimers as judged by HPLC.

The 12-epimeric mixtures obtained by the methods A and B as above are purified by chromatography either on a column of neutral alumina (activity III, ratio 1:70) eluted with benzene/ethyl acetate mixtures (method 1)[12] or on a column of silica gel (ratio 1:70) eluted with dichloromethane/methanol mixtures (method 2)[18]. In all cases, the less polar fraction is identified as 12β-hydroxy compounds and more polar fraction as the corresponding 12α-epimers (Table).

Methyl 3β,7α,12β-Trihydroxy-5α-cholanate (4a):
Prepared from the ester **9a** by the reduction method B and purification method 1; yield: 52%; m.p. 110–111 °C (from acetone/hexane as colorless needles).

$C_{25}H_{42}O_5 \cdot \frac{1}{3} H_2O$	calc.	C 70.06	H 10.03
(422.6+)	found	69.83	9.93

IR (KBr): ν = 3350, 1025, 1010 (OH); 1735 (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.73 (s, 3 H, 18-CH_3); 0.82 (s, 3 H, 19-CH_3); 1.01 (d, 3 H, J = 6.3 Hz, 21-CH_3); 3.46 (br m, 2 H, 3- and 12-H); 3.66 (s, 3 H, $COOCH_3$); 3.86 ppm (m, 1 H, 7-H).

3β,7α,12β-Trihydroxy-5α-cholanic Acid (4):
M.p. 242–243 °C (colorless needles from aqueous methanol).

$C_{24}H_{40}O_5 \cdot 2 H_2O$	calc.	C 64.83	H 9.98
(444.6)	found	64.78	9.74

IR (KBr): ν = 3200, 1010 (OH); 1675 (C=O).

^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6): δ = 0.68 (s, 3 H, 18-CH_3); 0.78 (s, 3 H, 19-CH_3); 1.01 (d, 3 H, J = 6.3 Hz, 21-CH_3); 3.36 (br m, 2 H, 3- and 12-H); 3.73 ppm (m, 1 H, 7-H).

Methyl 3α,7α,12α-Trihydroxy-5α-cholanate (2a):
The ester **12a**, reduced by method A and then purified by silica gel chromatography (method 2), gives the ester **2a**; yield: 76%, identical with that prepared as above, according to m.p. HPLC and ^{1}H-NMR data.

Methyl 3α,7α,12β-Trihydroxy-5α-cholanate (8a):
Prepared from the ester **12a** by the reduction method B and purification method 2; yield: 64%; m.p. 126.0–127.5 °C (colorless thin plates from acetone/hexane).

$C_{25}H_{42}O_5 \cdot \frac{1}{4}H_2O$	calc.	C 70.30	H 10.03
(422.6)	found	70.39	9.94

IR (KBr): ν = 3360, 1005 (OH); 1720 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.72 (3 H, s, 18-CH_3); 0.79 (s, 3 H, 19-CH_3); 1.01 (d, 3 H, J = 6.3 Hz, 21-CH_3); 3.41 (br m, 1 H, 12-H); 3.66 (s, 3 H, $COOCH_3$); 3.85 (m, 1 H, 7-H); 4.06 ppm (m, 1 H, 3-H).

3α,7α,12β-Trihydroxy-5α-cholanic Acid (8):
M.p. 210.5–212.0 °C (colorless needles from ethyl acetate/hexane).

$C_{24}H_{40}O_5 \cdot \frac{1}{4}H_2O$	calc.	C 69.78	H 9.88
(408.6+)	found	69.77	9.77

IR (KBr): ν = 3450, 1010, 995 (OH); 1700 cm^{-1} (C=O).

^{1}H-NMR($CDCl_3$/DMSO-d_6): δ = 0.66 (s, 3 H, 18-CH_3), 0.74 (s, 3 H, 19-CH_3), 1.00 (d, 3 H, J = 6.3 Hz, 21-CH_3), 3.32 (br m, 1 H, 12-H), 3.69 (m, 1 H, 7-H), 3.90 (m, 1 H, 3-H).

Methyl 3β,7β,12α-Trihydroxy-5α-cholanate (5a):
Obtained by the reduction (method A) of the ester **10a**, followed by alumina chromatography (method 1); yield: 75%; m.p. 149–151 °C (colorless thin plates from acetone/hexane).

$C_{25}H_{42}O_5 \cdot \frac{1}{4}H_2O$	calc.	C 70.30	H 10.03
(422.6)	found	70.33	9.85

IR (KBr): ν = 3400, 1027 (OH); 1715 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.71 (s, 3 H, 18-CH_3); 0.83 (s, 3 H, 19-CH_3); 0.98 (d, 3 H, J = 5.4 Hz, 21-CH_3); 3.40 (br m, 2 H, 3- and 7-H); 3.66 (s, 3 H, $COOCH_3$); 3.97 ppm (m, 1 H, 12-H).

3β,7β,12α-Trihydroxy-5α-cholanic Acid (5):
M.p. 252–254 °C (colorless needles from aqueous methanol).

$C_{24}H_{40}O_5 \cdot \frac{1}{6} H_2O$	calc.	C 70.04	H 9.88
(408.6+)	found	70.04	9.79

IR (KBr): ν = 3440, 1027 (OH); 1680 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6): δ = 0.67 (s, 3 H, 18-CH_3); 0.79 (s, 3 H, 19-CH_3); 0.98 (d, 3 H, J = 5.4 Hz, 21-CH_3); 3.34 (br m, 2 H, 3- and 7-H); 3.87 ppm (m, 1 H, 12-H).

Methyl 3β,7β,12β-Trihydroxy-5α-cholanate (6a):
Prepared from **10a** by the reduction method B and purification method 1; yield: 66%; m.p. 165–166 °C (colorless thin plates from acetone/hexane).

$C_{25}H_{42}O_5 \cdot \frac{1}{7} H_2O$	calc.	C 70.62	H 10.03
(422.6+)	found	70.64	10.11

IR (KBr): ν = 3390, 1028 (OH); 1715 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6): δ = 0.76 (s, 3 H, 18-CH_3); 0.84 (s, 3 H, 19-CH_3); 0.99 (d, 3 H, J = 7.2 Hz, 21-CH_3); 3.40 (br m, 3 H, 3-, 7- and 12-H); 3.66 ppm (s, 3 H, $COOCH_3$).

3β,7β,12β-Trihydroxy-5α-cholanic Acid (6):
M.p. 227–228 °C (colorless needles from aqueous methanol).

$C_{24}H_{40}O_5 \cdot \frac{1}{4} H_2O$	calc.	C 69.78	H 9.88
(408.6+)	found	69.71	9.73

IR (KBr): ν = 3450, 1035, 1003 (OH); 1673 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$ + 50% DMSO-d_6): δ = 0.65 (s, 3 H, 18-CH_3); 0.77 (s, 3 H, 19-CH_3); 0.96 (d, 3 H, J = 6.3 Hz, 21-CH_3); 3.31 ppm (br m, 3 H, 3-, 7- and 12-H).

Methyl 3α,7β,12α-Trihydroxy-5α-cholanate (3a):
Obtained by the reduction (method A) of the ester **11a**, followed by chromatography on alumina (method 1); yield: 75%; m.p. 166–167 °C (colorless needles from acetone-hexane).

$C_{25}H_{42}O_5$	calc.	C 71.05	H 10.02
(422.6)	found	70.87	10.02

IR (KBr): ν = 3400, 1025, 1010 (OH); 1718 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$): δ = 0.71 (s, 3 H, 18-CH_3); 0.79 (s, 3 H, 19-CH_3); 0.98 (d, 3 H, J = 5.4 Hz, 21-CH_3); 3.35 (br m, 1 H, 7-H); 3.66 (s, 3 H, $COOCH_3$); 4.00 ppm (m, 2 H, 3- and 12-H).

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3α,7β,12α-Trihydroxy-5α-cholanic Acid (3):
M.p. 269–271 °C (aqueous methanol) (Lit., 252–253 °C[6] and 253–255 °C[7]);

IR (KBr): ν = 3500, 1025, 1000 (OH); 1675 (C=O).

^{1}H-NMR ($CDCl_3$ + 50% DMSO-d_6): δ = 0.66 (s, 3H, 18-CH_3), 0.74 (s, 3H, 19-CH_3), 0.97 (d, 3H, J = 5.4 Hz, 21-CH_3), 3.24 (br m, 1H, 7-H); 3.88 ppm (m, 2H, 3- and 12-H).

Methyl 3α,7β,12β-Trihydroxy-5α-cholanate (7a):
Prepared from the ester **11a** by the reduction method B and purification method 1; yield: 60%; m.p. 178–179 °C (colorless prisms from ethyl acetate).

$C_{25}H_{42}O_5$	calc.	C 71.05	H 10.02
(422.6)	found	71.07	9.88

IR (KBr): ν = 3280, 1028 and 1005 (OH); 1735, 1710 (C=O).

^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6): δ = 0.74 (s, 3H, 18-CH_3); 0.79 (s, 3H, 19-CH_3); 0.99 (d, 3H, J = 6.3 Hz, 21-CH_3); 3.33 (br m, 2H, 7- and 12-H); 3.65 (s, 3H, $COOCH_3$); 3.97 ppm (m, 1H, 3-H).

3α,7β,12β-Trihydroxy-5α-cholanic Acid (7):
M.p. 243–244 °C (colorless thin plates from aqueous methanol).

$C_{24}H_{40}O_5$	calc.	C 70.55	H 9.87
(408.6)		70.29	9.72

IR (KBr): ν = 3280, 1028, 1005 (OH); 1735, 1710 cm^{-1} (C=O).

^{1}H-NMR ($CDCl_3$ + 50% DMSO-d_6): δ = 0.67 (s, 3H, 18-CH_3); 0.75 (s, 3H, 19-CH_3); 0.97 (d, 3H, J = 6.3 Hz, 21-CH_3); 3.27 (br m, 2H, 7- and 12-H); 3.86 ppm (m, 1H, 3-H).

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