Potential bile acid metabolites. 14. Hyocholic and muricholic acid stereoisomers

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Abstract The complete set of the eight theoretically possible stereoisomeric 3,6,7-trihydroxy-5β-cholanic acids, four of which are new, related to hyocholic and muricholic acids were prepared from chenodeoxycholic acid. The principal reactions used were *1*) *cis*-dihydroxylation of Δ^6-compounds with osmium tetroxide/*N*-methylmorpholine *N*-oxide; *2*) *trans*-dihydroxylation of 6α,7α-epoxy compounds with boron trifluoride etherate in *N,N*-dimethylformamide; *3*) inversion of equatorial 3α-hydroxylated compounds to the corresponding 3β-epimers with diethyl azodicarboxylate/triphenylphosphine/formic acid; and *4*) stereoselective reduction of 7-keto derivatives with zinc borohydride (or sodium borohydride) and by metallic potassium/*tert*-amyl alcohol.—**Iida, T., T. Momose, T. Tamura, T. Matsumoto, F. C. Chang, J. Goto, and T. Nambara.** Potential bile acid metabolites. 14. Hyocholic and muricholic acid stereoisomers. *J. Lipid Res.* 1989. **30:** 1267–1279.

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Of the eight theoretically possible stereoisomeric 3,6,7-trihydroxy-5β-cholanic acids (compounds **1–8, Fig. 1**), the four 3α-hydroxy isomers are natural products and their syntheses are known (1–5). A review of the occurrence, biological importance, and syntheses of these acids, hyocholic (**1**; 3α,6α,7α), ω-muricholic (**2**; 3α,6α,7β), α-muricholic (**3**; 3α,6β,7α), and β-muricholic (**4**; 3α,6β,7β), is in the literature (6). These compounds continue to be of biological and chemical interest, but with the exception of **1**, which is moderately accessible from pig bile but may be prepared from chenodeoxycholic (3α,7α-dihydroxy-5β-cholanic) acid (**9**) (7), the others are in very short supply.

As part of our ongoing program of synthesis of new and scarce potential bile acids, we have devised improved procedures for the preparation of the four known 3α,6ξ,7ξ-trihydroxy acids, and now describe the successful syntheses of the new 3β-hydroxy epimers (and their methyl esters) to complete the set of eight stereoisomers of the 5β-series.

The key intermediates in our work are essentially the same ones used in the previous syntheses (6) of the 3α-hydroxy acids, namely, methyl 3α,6α-dihydroxy-7-oxo-5β-cholanate (**13a**), and methyl 3α-cathyloxy-Δ^6-5β-cholenate (**15a**). Both were prepared from **9a** by slight modifications of literature methods; the ester **13a** is obtained in 48% overall yield from **9**, through the ester **9a**, and successively via the 3α-cathylate **10a** (8), the 3α-cathyloxy-7-oxo ester **11a**, the corresponding 6α-bromo derivative **12a** (9), and the acid **13** (10) (**Scheme 1**).

The other key compound **15a** requires as intermediate the 6α-bromo-3α-cathyloxy-7α-hydroxy ester **14a**, which can be prepared by sodium borohydride in methanol reduction of **12a**, and re-esterification. However, reduction of **12a** by zinc borohydride (11) offers a more practical one-step route to **14a**; the less basic zinc reagent (12) leaves the C-24 ester group intact during the reduction at C-7. Furthermore, the reduction proceeds faster and more cleanly than with sodium borohydride. Treatment of **14a** with zinc powder in boiling acetic acid (1) led to satisfactory yield of **15a**: the overall yield of **15a** from **9** was 40%.

Known 3α,6ξ,7ξ-trihydroxy acids

Hyocholic acid (**1**, $\alpha\alpha\alpha$) with an equatorial-axial *cis*-glycol structure, one of the earliest 3,6,7-trihydroxy acids

Abbreviations: IR, infrared; NMR, nuclear magnetic resonance; MS, mass spectra; TLC, thin-layer chromatography; GLC, gas–liquid chromatography. 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 various compounds in Fig. 1 and Schemes 1–7 are designated by a **bold face** number. The corresponding methyl esters at C-24 are designated "a" after the compound number.

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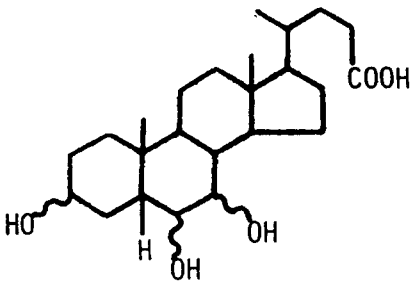


	C-3	C-6	C-7
1	α	α	α
2	α	α	β
3	α	β	α
4	α	β	β
5	β	α	α
6	β	α	β
7	β	β	α
8	β	β	β

Fig. 1.

known, has been synthesized by a number of different methods (2, 6), the most efficient of which appears to be the direct reduction of **13** by sodium borohydride (6). We have found that by use of zinc borohydride reduction of **13a**, as in the preparation of compounds **14a**, followed by alkaline hydrolysis, **1** can be obtained in 84% yield without need for column chromatographic purification (**Scheme 2**).

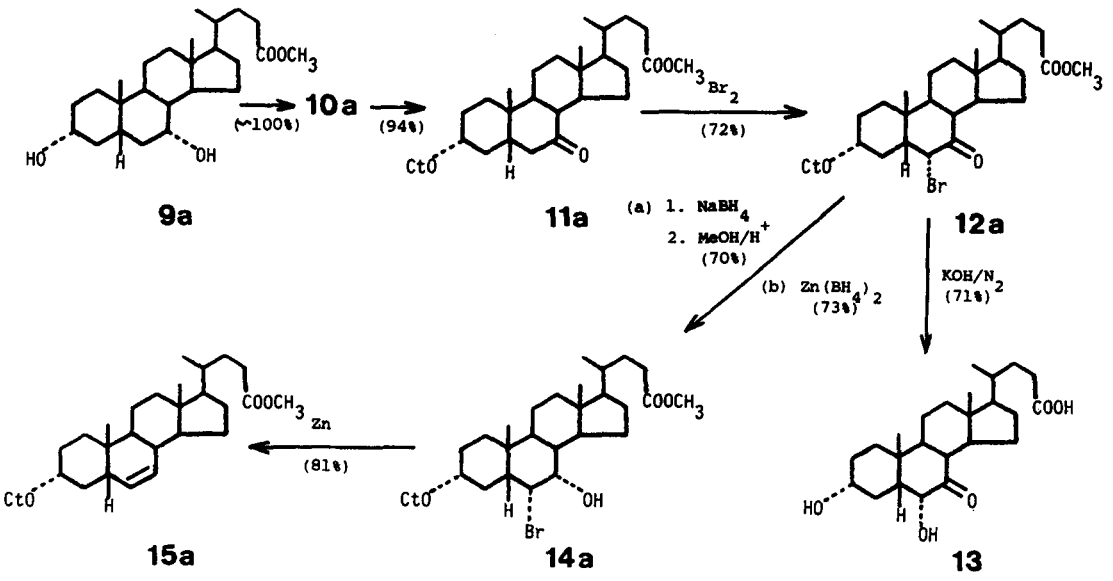
ω-Muricholic acid (**2**, $\alpha\alpha\beta$) with a diequatorial *trans*-glycol structure had been prepared from the 3α,6α-dihydroxy-7-oxo ester **13a** through a series of steps that first involved formation of the 3α- and 6α-ditetrahydropyranyl ether derivative, in order to prevent possible allomerization at the C-5 junction during the subsequent reduction with sodium in *n*-propanol (4, 6). The process required many steps and the final yield was low. We have devised an analogous procedure that is simpler and gives a higher yield, based on the use of recently introduced reagents (**Scheme 3**). The ester **13a** was disilylated at C-3 and C-6 by use of *tert*-butyldimethylsilyl chloride/imidazole in *N,N*-dimethylformamide-pyridine solution (13), and the resulting di-*tert*-butyldimethylsilyl ether **16a** was readily hydrolyzed to acid **16**. The ether linkage at position C-6 in **16** would prevent the formation of a 6-keto intermediate and hence would also prevent allomerization of the A/B-ring junction (4, 6). In fact, **16** was reduced by metallic potassium in boiling *tert*-amyl alcohol (14) to afford **2a** after cleavage of the *t*-butyldimethylsilyl ether linkage by conc. hydrochloric acid, methyl esterification, and then column chromatographic purification. Alkaline hydrolysis of **2a** afforded the corresponding acid **2** (overall yield from **13a** was 37%).

α-Muricholic acid (**3**, $\alpha\beta\alpha$) with a diaxial *trans*-glycol structure has previously been synthesized by *trans*-opening of a 6α,7α-epoxy intermediate to give the 6β,7β,7α-dihydroxy derivative (1). Our attempts to obtain the 3α,6β,7α-trihydroxy isomer directly from the Δ^6-ester **15a** by *trans*-dihydroxylation with iodine/silver benzoate reagent or with periodic acid (15) were unsuccessful; both methods gave complex mixtures of products. However, **15a** via the 6α,7α-epoxide **17a**, easily obtainable by treatment with *m*-chloroperoxybenzoic acid/4,4-thiobis-(6-*tert*-butyl-3-methylphenol) (16), by a change of reagent to boron trifluoride etherate in *N,N*-dimethylformamide (17), proceeded smoothly to yield the sterically homogeneous 3α-cathyloxy-6β-formyloxy-7α-hydroxy ester **18a** in excellent yield without need for chromatographic separation. The overall yield of acid **3** from **15a** after hydrolysis of **18a** was 72% (**Scheme 4**).

β-Muricholic acid (**4**, $\alpha\beta\beta$) with an axial-equatorial *cis*-glycol structure had also been synthesized by several methods, especially during the studies to clarify the structure of the isomeric forms (2, 3). The method finally selected by the Hsia group was by treatment of methyl 7α-bromo-3α,6β-diacetoxy-5β-cholanate with silver acetate in refluxing acetic acid–water solution (6). Our preparation of **4** is a modification of an earlier synthesis (3) by application of a β-face *cis*-dihydroxylation procedure introduced by VanRheenen, Kelly, and Cha (18) (Scheme 4). The cathylate **15a** by treatment with *N*-methylmorpholine *N*-oxide in a *tert*-butyl alcohol–tetrahydrofuran–water mixture (19) and a catalytic amount of osmium tetroxide, gave the cathylate **19a** in isolated yield of 81%, and by the usual hydrolysis **4** was obtained (93%). The initial product **19a** had a dark brown contaminant which was not removed by Norite treatment, and direct crystallization, nor by silica gel chromatography, but was effectively removed by chromatography over neutral alumina.

New 3β,6ξ,7ξ-trihydroxy acids

In principle, the corresponding 3β-hydroxylated acids should be able to be prepared by simple treatment of each of the 3α-hydroxy epimers with diethyl azodicarboxylate/triphenylphosphine/formic acid, an inverting reagent that has been used successfully in earlier studies (20, 21) to directly invert an equatorially oriented hydroxyl group of cholanic acids at C-3; however, the presence at C-6 and C-7 of additional hydroxyl groups would interfere with selective reaction at C-3. Our syntheses of the C-3 epimers, therefore, involve alternate routes with inversions at different stages



Scheme 1.

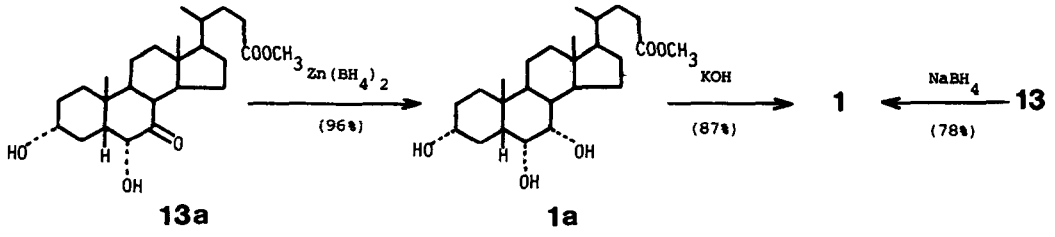
of the intermediates on protected C-6 and C-7 hydroxyls.

3β,6α,7α-Trihydroxy acid **5**, the C-3 epimer of hyocholic acid (**1**), was prepared from the ester **1a** through the 6α,7α-acetonide **20a** (22), which then could be inverted at C-3 by use of the inverting reagent. The resulting 3β-formate-6,7-acetonide by treatment with HCl in methanol, by simultaneous hydrolysis at C-3 and cleavage of the acetonide group, affords the desired ester **5a** (56%), accompanied by a small amount of methyl 6α,7α-dihydroxy-Δ^3-5β-cholenate (28%). The usual hydrolysis of **5a** gives the acid **5** in 52% overall yield from **1a** (**Scheme 5**).

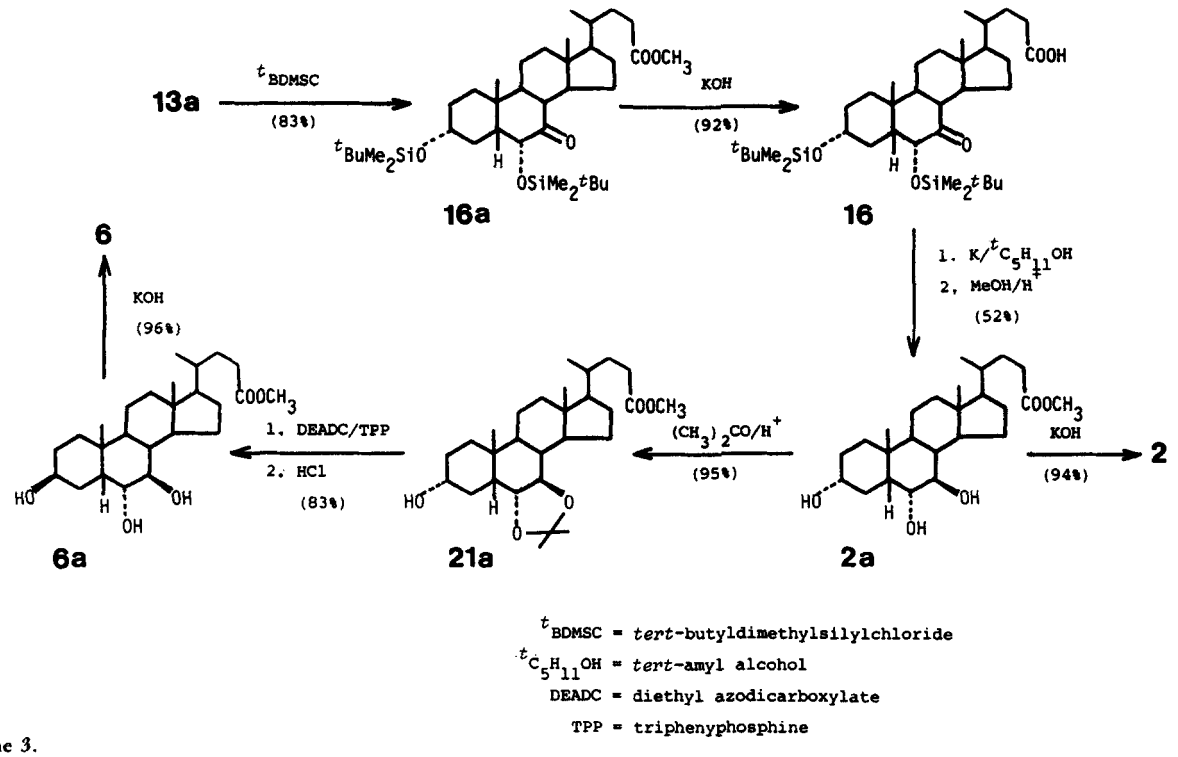
3β,6α,7β-Trihydroxy acid **6** was prepared by conversion of **2a** to the 6α,7β-acetonide **21a** as for the 6α,7α-acetonide **20a** in the preparation of **5a**. Despite the *trans* orientation of the 6α- and 7β-hydroxyl groups in **2a**, their diequatorial relationship apparently allows the acetonide **21a** to be formed under the conditions used. (This contradicts a statement in a recent publication (23) that ω-muricholic acid (**2a**) does not form an 6α,7β-acetonide by dimethoxypropane/0.1 N HCl reagent system.) The steps from the acetonide **21a** to the acid **6** follow exactly the procedure for obtaining acid **5** from **20a** (above). The overall yield of **6** from **2a** was 76% (Scheme 3).

3β,6β,7α-Trihydroxy acid **7** was prepared starting from **15a** proceeding through the Δ^6-ester **22a** and inversion at C-3 to **23a** and hydrolysis of the formate group to **24a**. Subsequent steps to the desired **7** followed those involved in the synthesis of *trans*-6β,7α-dihydroxy acid 3. Compound **24a** was converted to the 6α,7α-epoxide **25a**, subjected to cleavage to the *trans*-6β-formyloxy-7α-hydroxy ester **26a**, which on hydrolysis gave acid **7** from **24a** in overall yield of 74% (**Scheme 6**).

3β,6β,7β-Trihydroxy acid **8** was synthesized by two routes starting with the esters **22a** and **4a**. (a) The 3β-formate-Δ^6-ester **23a**, obtained smoothly from **22a** with the inverting reagent, was *cis*-dihydroxylated by osmium tetroxide/*N*-methylmorpholine *N*-oxide/*tert*-butyl alcohol–tetrahydrofuran–water reagent (19) to methyl 3β,6β,7β-trihydroxy-5β-cholanate 3-formate **27a**. (Ester **24a**, easily obtained from **23a** by contact with a column of neutral alumina, underwent similar *cis*-dihydroxylation to the ester **8a**.) Both **27a** and **8a** were hydrolyzed nearly quantitatively to the desired acid **8** in good yields (**Scheme 7**). (b) The second route to **8**, from **4a**, consists of formation of 6β,7β-acetonide



Scheme 2.

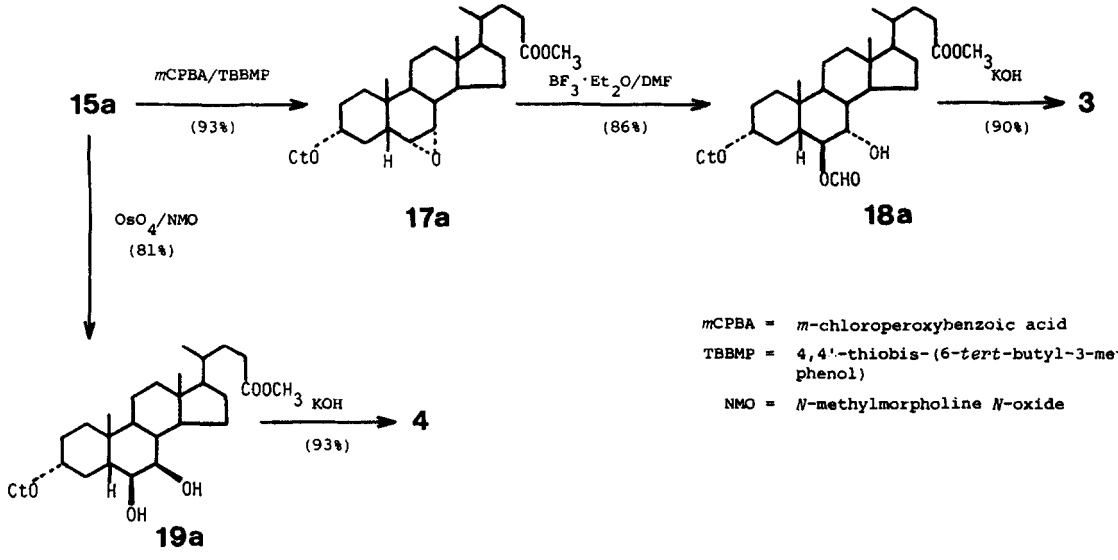


^{t}BDMSC = *tert*-butyldimethylsilylchloride
$^{t}C_5H_{11}OH$ = *tert*-amyl alcohol
DEADC = diethyl azodicarboxylate
TPP = triphenyphosphine

Scheme 3.

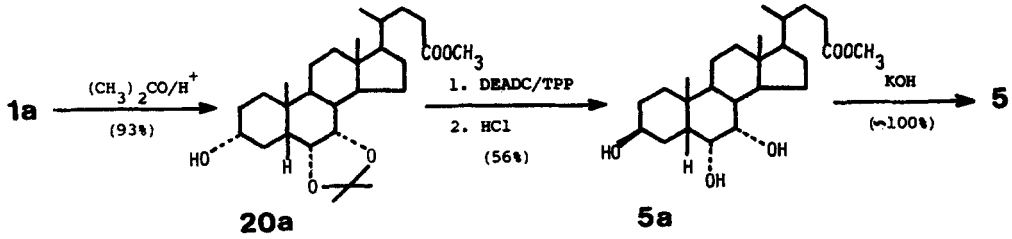
28a, followed by treatment with the inverting reagent to give the 3β-formyloxy-6β,7β-acetonide. The latter, without need for purification at this stage, on reaction with HCl in methanol, underwent simultaneous hydrolysis of the formate group and cleavage of the acetonide group to the ester **8a** in 60% isolated yield after chromatography.

Chemical evidence for the vicinal glycol structure (23), stereochemical configuration of hydroxyls, A/B-*cis* ring junction (24, 25) and purify of the eight stereoisomers (**1a–8a**) was further confirmed by high-resolution ^{1}H- and ^{13}C-NMR (**Table 1** and **Table 2**). The signal assignment of each signal was based, to a large extent, on the work of Kuroki et al. (23) who recently reported the ^{1}H- and ^{13}C-NMR signal assigments of the acids **1–4**. Slight differences



*m*CPBA = *m*-chloroperoxybenzoic acid
TBBMP = 4,4'-thiobis-(6-*tert*-butyl-3-methyl-phenol)
NMO = *N*-methylmorpholine *N*-oxide

Scheme 4.



Scheme 5.

in the chemical shifts of corresponding signals can be accounted for by the differences in the free acids measured in pyridine-d_5 (23) and the corresponding methyl esters measured in $CDCl_3$.

EXPERIMENTAL PROCEDURES AND RESULTS

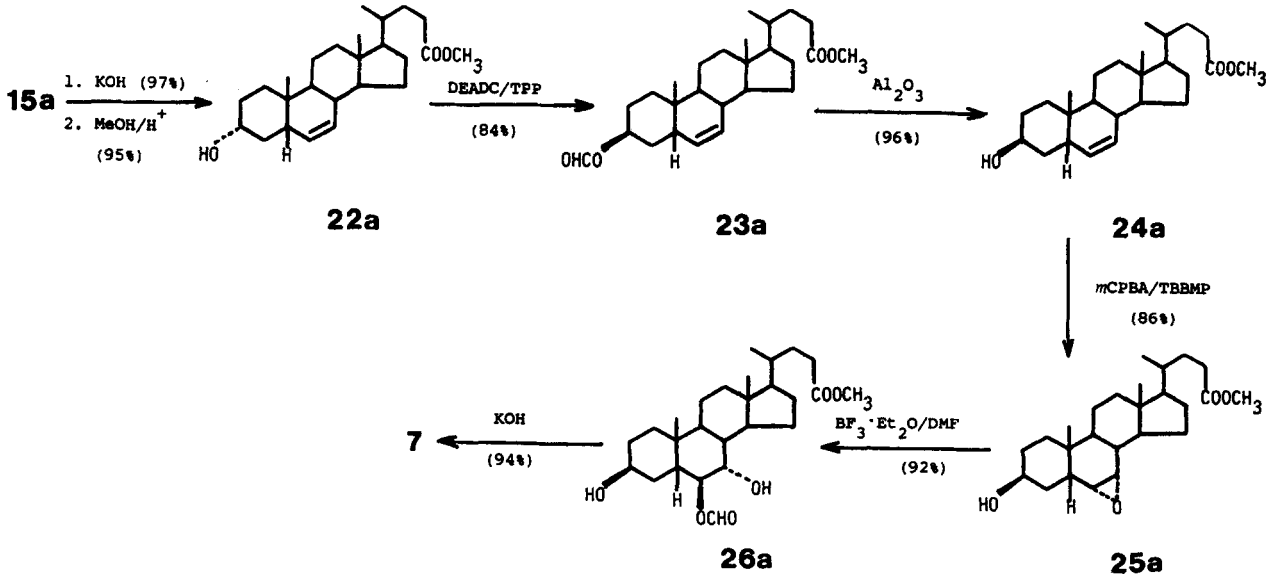
Melting points (mp) were determined on an electric micro hot stage and are uncorrected. IR spectra were obtained on a JASCO IRA-II double-beam spectrophotometer. ^1H- and ^{13}C-NMR spectra were obtained on a JEOL FX-90Q instrument at 90 and 22.53 MHz, respectively, with $CDCl_3$ containing 1% Me_4Si as the solvent except where otherwise indicated. The high resolution ^1H-NMR spectra were also recorded on a JEOL GSX-500 instrument at 500 MHz. High resolution MS and low resolution GLC–MS were recorded on a JEOL DX-303 mass spectrometer at 70 ev. Analytical TLC was performed on precoated silica gel (20 cm × 20 cm, 0.25 mm layer thickness; Merck). All compounds were dried by azeotropic distillation before use in reactions.

General procedure for the hydrolysis of methyl esters to free acids

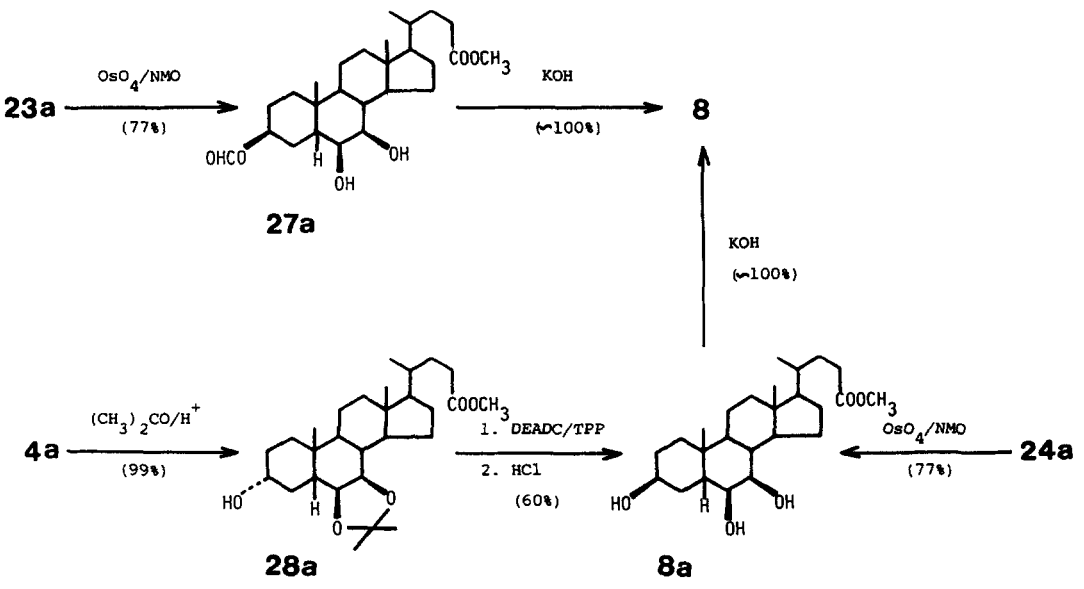
The ester (300 mg) was refluxed in 5% methanolic KOH (9 ml) for 1 h. Most of the solvent was evaporated, and the residue was dissolved in water, cooled in an ice-bath, and acidified with 10% H_2SO_4 with stirring. The precipitated solid was filtered, washed with water, and recrystallized from an appropriate solvent.

General procedure for the esterification of free acids to methyl esters

p-Toluenesulfonic acid (30 mg) was added to the free acid (300 mg) in methanol (9 ml) and the mixture was allowed to



Scheme 6.



Scheme 7.

stand overnight at room temperature. Most of methanol was evaporated, and the residue was extracted with CH_2Cl_2. The organic extract was washed successively with water, 5% $NaHCO_3$, and water, dried with Drierite, and evaporated to give the corresponding ester which was crystallized from an appropriate solvent.

Methyl 3α-cathyloxy-7-oxo-5β-cholanate (11a)

To a stirred solution of the ester **10a** (10 g, 20.9 mmol) (prepared nearly quantitatively from **9a** (**8**)) in acetic acid (200 ml) was added dropwise potassium chromate (7.0 g, 36.0 mmol) dissolved in water (20 ml). After the mixture was stirred overnight at room temperature, the dark brown solution was diluted with water to near turbidity, and allowed to stand until crystallization was complete. The precipitated solid was filtered, washed with water, and recrystallized from aqueous methanol as colorless thin plates; yield, 9.33 g (94%); mp, 143–144°C. IR V_{max} cm^{-1}: 1735 (C=O), 1265 (=C-O). ^{1}H-NMR δ: 0.65 (s, 3H, 18-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 1.21 (s, 3H, 19-Me), 1.29 (t, 3H, J = 7.2 Hz, 3-$OCOOCH_2\underline{CH_3}$), 3.66 (s, 3H, COOMe), 4.16 (q, 2H, J = 7.2 Hz, 3-$OCOO\underline{CH_2}CH_3$), 4.54 (brm, 1H, 3-H). Anal. calcd. for $C_{28}H_{44}O_6$: C, 70.55; H, 9.31. Found: C, 70.67; H, 9.55.

Methyl 6α-bromo-3α-cathyloxy-7-oxo-5β-cholanate (12a)

To a stirred solution of the ester **11a** (4.5 g, 9.4 mmol) dissolved in acetic acid (100 ml) containing 47% hydrobromic acid (3 ml) was added dropwise a solution of bromine (2.0 g, 12.5 mmol) in acetic acid (10 ml). After stirring overnight at room temperature, the mixture was poured gradually onto ice-water, and the precipitated solid was filtered and then washed with water. Several recrystallizations from EtOAc-hexane gave an analytically pure **12a** as

TABLE 1. 500 MHz ^{1}H-NMR spectral data for stereoisomeric methyl 3,6,7-trihydroxy-5β-cholanates (**1a–8a**)[a]

	18-Me[b]	19-Me[b]	21-Me[c]	COOMe[b]	3-H[c]	6-H[c]	7-H[c]
1a (3α,6α,7α)	0.66	0.90	0.93(d, 6.5)	3.67	3.42–3.48(brm)	3.85(m)	3.85(m)
2a (3α,6α,7β)	0.67	0.95	0.92(d, 6.0)	3.67	3.55–3.61(brm)	3.79(dd, 9.3 and 5.1)	3.40(t, 9.4)
3a (3α,6β,7α)	0.69	1.08	0.93(d, 6.0)	3.66	3.47–3.53(brm)	3.72(m)[d]	3.73(m)[d]
4a (3α,6β,7β)	0.69	1.10	0.93(d, 5.0)	3.67	3.58–3.64(brm)	3.70(dd, 3.6 and 2.5)	3.55(dd, 9.8 and 3.8)
5a (3β,6α,7α)	0.69	0.94	0.93(d, 6.5)	3.66	4.12(m)	3.95(m)	3.86(m)
6a (3β,6α,7β)	0.68	0.99	0.92(d, 6.5)	3.67	4.14(m)	3.86(dd, 9.3 and 5.3)	3.38(t, 9.1)
7a (3β,6β,7α)	0.70	1.12	0.93(d, 5.0)	3.67	4.06(m)	3.68(m)[d]	3.73(m)[d]
8a (3β,6β,7β)	0.70	1.13	0.93(d, 5.0)	3.67	4.07(m)	3.65(dd, 3.6 and 2.7)	3.50(dd, 10.1 and 3.6)

[a] In ppm downfield from Me_4Si.
[b] Singlet.
[c] Values in parentheses refer to coupling constant (J in Hz): d, doublet; t, triplet; m, multiple (or broad singlet); brm, broad multiplet.
[d] Assignments in each line may be interchanged.

TABLE 2. ^{13}C-NMR spectal data for stereoisomeric methyl 3,6,7-trihydroxy-5β-cholanates (**1a–8a**)[a]

Carbon	**1a** 3α,6α,7α	**2a** 3α,6α,7β	**3a** 3α,6β,7α	**4a** 3α,6β,7β	**5a** 3β,6α,7α	**6a** 3β,6α,7β	**7a** 3β,6β,7α	**8a** 3β,6β,7β
1	35.5	35.3	35.7	35.4	30.0	29.9	30.6	30.1
2	30.4	30.9	30.1	29.9	27.4	27.3	27.7	27.4
3	71.7	71.2	71.4	70.9	66.0	65.6	66.3	65.7
4	32.6	29.9	36.1	35.4	29.3	27.8	33.2	32.5
5	47.8	47.6	48.0	47.2	42.4	42.3	42.8	42.4
6	71.9[b]	73.0	76.5	75.4	71.8[b]	73.0	76.6	75.0
7	69.6[b]	75.4	72.7	73.5	69.5[b]	75.6	73.0	73.4
8	38.6	41.7	35.3	38.5[b]	38.4	41.6	35.2	38.5[b]
9	32.5	39.7	32.9	39.6[b]	32.0	38.9	33.0	39.2[b]
10	35.9	35.3	34.7	33.8	36.4	35.8	35.2	34.2
11	20.5	21.2	20.4	20.8	20.9	21.5	20.8	21.0
12	39.5	40.0	39.5	39.9	39.4	40.1	39.5	39.9
13	42.7	43.8	42.6	43.7	42.7	43.8	42.6	43.5
14	50.1	55.0	49.9	54.9	50.2	55.1	49.8	54.9
15	23.0	26.7	23.6	27.0	23.4	26.7	23.7	26.9
16	28.1	28.5	28.1	28.4	28.0	28.5	28.1	28.4
17	55.8	55.9	55.8	55.5	55.7	56.0	55.8	55.6
18	11.7	12.2	11.6	12.0	11.6	12.2	11.7	12.0
19	23.5	23.6	25.2	25.4	23.6	24.2	25.4	25.6
20	35.3	35.3	35.1	35.2	35.3	35.2	35.2	35.1
21	18.2	18.4	18.3	18.4	18.2	18.4	18.2	18.3
22	31.0	31.1	30.9	31.0	30.9	31.1	31.0	30.9
23	31.0	31.1	30.9	31.0	30.9	31.1	31.0	30.9
24	174.6	174.6	174.6	174.6	174.6	174.6	174.7	174.6
25	51.4	51.3	51.4	51.4	51.4	51.4	51.4	51.3

[a]In ppm downfield from Me_4Si.
[b]Assignments in each column may be interchanged.

colorless needles; yield, 3.77 g (72%); mp, 117–118°C. IR V_{max} cm^{-1}: 1748 (C=O), 1248 (=C-O). ^{1}H-NMR δ: 0.66 (s, 3H, 18-Me), 0.92 (d, 3H, J = 6.3 Hz, 21-Me), 1.28 (s, 3H, 19-Me), 1.29 (t, 3H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 3.66 (s, 3H, COOMe), 4.17 (q, 2H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 4.53 (brm, 1H, 3-H), 5.18 (d, 1H, J = 4.5 Hz, 6-H). Anal. calcd. for $C_{28}H_{43}O_6Br$: C, 60.53; H, 7.80. Found: C, 60.46; H, 8.00.

3α,6α-Dihydroxy-7-oxo-5β-cholanic acid (13)

To a stirred suspension of the ester **12a** (4.0 g, 7.2 mmol) in methanol (100 ml) was added KOH (6.0 g) dissolved in methanol (70 ml), and the mixture was stirred overnight under N_2 at room temperature. Most of the solvent was evaporated under reduced pressure, and the residue was dissolved in water and then acidified with 10% H_2SO_4. The precipitated solid was filtered, washed with water, and dried. The crude acid was recrystallized twice from methanol (or EtOAc) as colorless needles; yield, 2.08 g (71%); mp, 184–186°C (lit. mp, 187–189 (2), 186–187 (6), and 183–185°C (10)). IR V_{max} cm^{-1}: 3400, 1055, 1018 (OH), 1718 (C=O). ^{1}H-NMR δ: 0.66 (s, 3H, 18-Me), 0.94 (d, 3H, J = 6.3 Hz, 21-Me), 1.23 (s, 3H, 19-Me), 3.55 (brm, 1H, 3-H), 4.52 (d, 1H, J = 6.3 Hz, 6-H). High resolution MS: 406.2697 (M$^+$, $C_{24}H_{38}O_5$ requires 406.2720).

Methyl 3α,6α-Dihydroxy-7-oxo-5β-cholanate (13a)

This compound was prepared nearly quantitatively from the acid **13** by the general esterification method. Although **13a** was homogeneous according to TLC and ^{1}H-NMR analyses, it could not be crystallized.[2] IR V_{max} cm^{-1}: 3400, 1060, 1022 (OH), 1738, 1718 (C=O). ^{1}H-NMR δ: 0.66 (s, 3H, 18-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 1.23 (s, 3H, 19-Me), 3.52 (brm, 1H, 3-H), 3.66 (s, 3H, COOMe), 4.50 (d, 1H, J = 6.3 Hz, 6-H). High resolution MS: 420.2856 (M$^+$, $C_{25}H_{40}O_5$ requires 420.2876).

Methyl 6α-bromo-3α-cathyloxy-7α-hydroxy-5β-cholanate (14a)

(a) To a stirred solution of the crude ester **12a** (9.0 g, 16.1 mmol) dissolved in CH_2Cl_2 (25 ml) and methanol (150

[2]The purity and A/B-*cis* ring structure were further confirmed by the ^{13}C-NMR, which showed the 18- and 19-methyl signals at 11.9 and 23.1 ppm, respectively (24, 25).

ml) sodium borohydride (4.5 g, 119 mmol) was added gradually with ice-bath cooling. Stirring was continued at room temperature for 2 h, and ice chips were gradually stirred in; the resulting solution was extracted with CH_2Cl_2.

The organic layer was washed with 10% HCl and water, dried with Drierite, and evaporated to an oily residue. A solution of the oil redissolved in methanol (90 ml) containing *p*-toluenesulfonic acid (450 mg) was allowed to stand overnight at room temperature. The precipitated solid was filtered and washed with ice-cold methanol. A second crop was obtained on concentration of the mother liquor; yield, 6.33 g (70%); mp, 126–127°C (colorless prisms from methanol). IR V_{max} cm^{-1}: 3470, 1000 (OH), 1742 (C=O), 1252 (=C-O). ^{1}H-NMR δ: 0.65 (s, 3H, 18-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 0.99 (s, 3H, 19-Me), 1.30 (t, 3H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 3.66 (s, 3H, COOMe), 3.87 (m, 1H, 7-H), 4.18 (q, 2H, J = 7.2 Hz, 3-OCOOCH$_2$ CH$_3$), 4.44 (brm, 1H, 3-H), 4.70 (m, 1H, 6-H). Anal. calcd. for $C_{28}H_{45}O_6Br$: C, 60.31; H, 8.14. Found: C, 60.12; H, 8.04.

(b) To a stirred solution of zinc borohydride in Et_2O (30 ml, 4.8 mmol), freshly prepared by the procedure of Gensler, Johnson, and Sloan (11), a solution of the crude ester **12a** (1.0 g, 1.8 mmol) in benzene–Et_2O (60 ml; 1:1, v/v) was added dropwise under N_2. After further stirring for 30 min at room temperature, the mixture was poured into water, and the organic layer was washed with 10% HCl and water, dried with Drierite, and evaporated to dryness. The light yellow residue was recrystallized twice from aqueous acetone as colorless prisms; yield, 0.73 g (73%). This compound was found to be identical, according to TLC and ^{1}H-NMR comparisons, to **14a** prepared as described above (a).

Methyl 3α-cathyloxy-Δ^6-5β-cholenate (15a)

To a refluxing solution of the bromohydrine **14a** (3.0 g, 5.4 mmol) in acetic acid (60 ml), zinc powder (3.5 g, 53.5 mmol) was added in small portions. After further refluxing for 30 min, the excess zinc was removed by filtration. The mother liquor was diluted with water to near turbidity, and on standing, crystals separated. The precipitated solid was recrystallized from methanol as colorless needles; yield, 2.02 g (81%); mp, 100–101°C. IR V_{max} cm^{-1}: 1738 (C=O), 1273 (=C-O). ^{1}H-NMR δ: 0.69 (s, 3H, 18-Me), 0.86 (s, 3H, 19-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 1.29 (d, 3H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 3.66 (s, 3H, COOMe), 4.17 (q, 2H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 4.54 (brm, 1H, 3-H), 5.46 (s, 2H, 6- and 7-H). Anal. calcd. for $C_{28}H_{44}O_5$: C, 73.00; H, 9.63. Found: C, 73.07; H, 9.67.

Methyl 3α,6α,7α-trihydroxy-5β-cholanate (1a)

The 7-keto ester **13a** (1.0 g, 2.4 mmol) was subjected to the reduction with zinc borohydride and processed as described for the preparation of **14a**, to afford **1a**; yield, 0.96 g (96%). Although this compound was homogeneous by TLC and ^{1}H-NMR analyses, it could not be crystallized. IR V_{max} cm^{-1}: 3400, 1050(OH), 1737 (C=O). ^{1}H-NMR δ: 0.65 (s, 3H, 18-Me), 0.91 (s, 3H, 19-Me), 0.93 (d, 3H, J = 4.5 Hz, 21-Me), 3.46 (brm, 1H, 3-H), 3.66 (s, 3H, COOMe), 3.83 (m, 2H, 6- and 7-H). Low resolution MS, *m/z* (relative intensity): 422 (16%, M$^+$), 404 (24%, M-H_2O), 386 (23%, M-2H_2O). High resolution MS: 422.2984 (M$^+$, $C_{25}H_{42}O_5$ requires 422.3033).

3α,6α,7α-Trihydroxy-5β-cholanic acid (1)

(a) Compound **1** was prepared from **1a** by the general hydrolysis procedure; yield, 87%; mp, 185–187°C (colorless needles from aqueous methanol) (lit. mp, 187–188 (2), 183–185 (22), and 188–189°C (26)). IR V_{max} cm^{-1}: 3430, 1050 (OH), 1718 (C=O). ^{1}H-NMR (CDCl + 10% DMSO-d_6) δ: 0.65 (s, 3H, 18-Me), 0.90 (s, 3H, 19-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 3.39 (brm, 1H, 3-H), 3.82 (m, 2H, 6- and 7-H). Anal. calcd. for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.39; H, 10.07.

(b) To a stirred solution of the acid **13** (500 mg, 1.2 mmol) in methanol (15 ml) sodium borohydride (450 mg, 11.9 mmol) was added gradually. After the mixture was further stirred for 2 h at room temperature, water was added, the solution was acidified with 10% H_2SO_4, and the reaction product was extracted with EtOAc. The combined extracts were washed with water to neutrality, dried with Drierite, evaporated to an oily residue, which crystallized slowly from aqueous methanol. Two recrystallizations from aqueous methanol afforded an analytical pure **1:** yield, 387 mg (78%).

Methyl 3α,6α-dihydroxy-7-oxo-5β-cholanate 3,6-di-*tert*-butyldimethylsilyl ether (16a)

To a solution of the ester **13a** (1.6 g, 3.8 mmol) in anhydrous *N,N*-dimethylformamide (2 ml) and pyridine (1 ml) was added imidazole (2.6 g, 38.2 mmol) and *tert*-butyldimethylsilylchloride (1.2 g, 8 mmol) (13), and the mixture was stirred at 50°C for 1.5 h. The reaction mixture was poured onto ice-water and extracted with EtOAc. The organic layer was washed with water, dried with Drierite, and evaporated to give the desired ester **16a**, which was crystallized from methanol as colorless fine needles; yield, 2.04 g (83%); mp, 133–134°C. IR V_{max} cm^{-1}: 1742, 1730 (C=O), 1256, 858, 839, 779 (Si-C), 1088 (Si-O). ^{1}H-NMR δ: −0.02, 0.02, 0.08, 0.86, and 0.89 (s, 30H, 3- and 6-OSiC(CH$_3$)$_3$(CH$_3$)$_2$), 0.64 (s, 3H, 18-Me), 1.18 (s, 3H, 19-Me), 3.49 (brm, 1H, 3-H), 3.66 (s, 3H, COOMe), 4.51 (d, 1H, J = 5.4 Hz, 6-H). Anal. calcd. for $C_{37}H_{68}O_5Si_2$: C, 68.46; H, 10.56. Found: C, 68.39; H, 10.62.

3α,6α-Dihydroxy-7-oxo-5β-cholanic acid 3,6-di-*tert*-butyldimethylsilyl ether (16)

This compound was prepared from the ester **16a** (1.5 g, 2.3 mmol) by the general hydrolysis method; yield, 1.35 g (92%); mp, 201–202°C (colorless needles from aqueous methanol). IR V_{max} cm^{-1}: 1730, 1705 (C=O), 1252, 858,

838, 778 (Si-C), 1088 (Si-O). ^{1}H-NMR δ: −0.01, 0.02, 0.08, 0.86, and 0.89 (s, 30H, 3- and 6-OSiC$(CH_3)_3(CH_3)_2$), 0.64 (s, 3H, 18-Me), 1.18 (s, 3H, 19-Me), 3.46 (brm, 1H, 3-H), 4.51 (d, 1H, J = 5.4 Hz, 6-H). Anal. calcd. for $C_{36}H_{66}O_5Si_2$: C, 68.09; H, 10.48. Found: C, 67.87; H, 10.60.

Methyl 3α,6α,7β-trihydroxy-5β-cholanate (2a)

To a refluxing solution of the acid **16** (1.27 g, 2.0 mmol) dissolved in *tert*-amyl alcohol (30 ml) metallic potassium (0.8 g, 20 mmol) (14) in small pieces was added with vigorous stirring. After the potassium was consumed (ca. 10 min), the mixture was cooled, diluted with water, and then acidified with 10% H_2SO_4. The reaction product was extracted with EtOAc, and the organic layer was washed with water to neutrality, dried over Drierite, and evaporated to an oil. A solution of the oil in methanol (30 ml) containing conc. HCl (0.4 ml) was allowed to stand at room temperature overnight. Most of the solvent was evaporated and the residue was extracted with CH_2Cl_2. The organic extract was washed with 5% $NaHCO_3$ and water, dried with Drierite, and evaporated to give an oily residue (812 mg), which by TLC consisted of a mixture of two components. Chromatography of the oil over a column of silica gel (32 g) resulted in two components. The first fraction eluted with benzene–EtOAc 2:8 (v/v) gave an homogeneous oil (256 mg) which was crystallized from aqueous ethanol as colorless needles and found by TLC and ^{1}H-NMR comparisons to be identical with an authentic methyl ursodeoxycholate (methyl 3α,7β-dihydroxy-5β-cholanate).

The second fraction eluted with EtOAc–methanol 95:5 (v/v) gave 438 mg (52%) of homogeneous oil which was identified as the desired ester **2a** and could not be crystallized. IR V_{max} cm^{-1}: 3430, 1050 (OH), 1735(C=O). ^{1}H-NMR δ: 0.68 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 0.96 (s, 3H, 19-Me), 3.45 (brm, 2H, 3- and 7-H), 3.66 (s, 3H, COOMe), 3.70 (brm, 1H, 6-H). Low resolution MS, *m/z* (relative intensity): 422 (7%, M$^+$), 404 (74%, M−H_2O), 386 (83%, M−2H_2O), 371 (16%, M−2H_2O-Me), 368 (23%, M−3H_2O). High resolution MS: 422.3019 (M$^+$, $C_{25}H_{42}O_5$ requires 422.3033).

3α,6α,7β-Trihydroxy-5β-cholanic acid (2)

The ester **2a**, hydrolyzed by the usual method, recrystallized from acetone–hexane as colorless needles; yield, 94%; mp, 162–164°C (lit. melted at 150–153°C, resolidified at about 160°C, and remelted at 184–188°C (4)). IR V_{max} cm^{-1}: 3400, 1045 (OH), 1690 (C=O). ^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6) δ: 0.66 (s, 3H, 18-Me), 0.93 (s, 3H, 19-Me), 3.40 (brm, 2H, 3- and 7-H), 3.68 (brm, 1H, 6-H). Anal. calcd. for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.37; H, 9.97.

Methyl 3α-cathyloxy-6α,7α-epoxy-5β-cholanate (17a)

A mixture of the Δ^6-ester **15a** (1.5 g, 3.3 mmol), 4,4'-thiobis-(6-*tert*-butyl-3-methylphenol) (30 mg, 0.1 mmol) and *m*-chloroperoxybenzoic acid (2.25 g, 13.0 mmol) in 1,2-dichloroethane (70 ml) (16) was refluxed for 1.5 h. The organic layer was washed with 5% sodium thiosulfate, 5% $NaHCO_3$, and water, dried over Drierite, and evaporated to dryness. The oily residue was crystallized from hexane containing a few drops of acetone as colorless crystals; yield, 1.45 g (93%); mp, 104–105°C. IR V_{max} cm^{-1}: 1738 (C=O), 1272 (=C-O). ^{1}H-HMR δ: 0.69 (s, 3H, 18-Me), 0.84 (s, 3H, 19-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 1.30 (t, 3H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 3.08 (s, 2H, 6- and 7-H), 3.67 (s, 3H, COOMe), 4.18 (q, 2H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 4.57 (brm, 1H, 3-H). High resolution MS: 476.3096 (M$^+$, $C_{28}H_{44}O_6$ requires 476.3138).

Methyl 3α-cathyloxy-6β-formyloxy-7α-hydroxy-5β-cholanate (18a)

To a solution of the ester **17a** (1.3 g, 2.7 mmol) in anhydrous *N,N*-dimethylformamide (50 ml) boron trifluoride ethyl ether complex (2.2 ml, 15.5 mmol) was added dropwise (17). After standing overnight at room temperature, water was added, and the reaction product was extracted with CH_2Cl_2. The combined extracts were washed with water, dried with Drierite, and evaporated to dryness. The oily residue was crystallized from aqueous methanol as colorless thin plates; yield, 1.22 g (86%); mp, 131–132°C. IR V_{max} cm^{-1}: 3530, 1000 (OH), 1725 (C=O), 1268 (=C-O), 1190 (C-O). ^{1}H-NMR δ: 0.69 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 1.02 (s, 3H, 19-Me), 1.29 (t, 3H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 3.66 (s, 4H, 7-H and COOMe), 4.17 (q, 2H, J = 7.2 Hz, 3-OCOOCH$_2$CH$_3$), 4.46 (brm, 1H, 3-H), 4.84 (m, 1H, 6-H), 8.03 (s, 1H, 6-OCHO). Anal. calcd. for $C_{29}H_{46}O_8 \cdot 1/4H_2O$: C, 66.07; H, 8.89. Found: C, 66.04; H, 8.91.

3α,6β,7α-Trihydroxy-5β-cholanic acid (3)

The ester **18a**, hydrolyzed by the usual method, recrystallized from aqueous acetic acid. Recrystallization from acetone–hexane gave the acid **3** as colorless neeedles; yield, 90%; mp, 200–202°C (lit. mp, 199–200 (1) and 196–197°C (5)). IR V_{max} cm^{-1}: 3400, 1045, 1018 (OH), 1710 (C=O). ^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6) δ: 0.69 (s, 3H, 18-Me), 0.94 (d, 3H, J = 5.4 Hz, 21-Me), 1.08 (s, 3H, 19-Me), 3.40 (brm, 1H, 3-H), 3.69 (m, 2H, 6- and 7-H). Anal. calcd. for $C_{24}H_{40}O_5 \cdot 1/2H_2O$: C, 69.03; H, 9.90. Found: C, 69.32; H, 9.90.

Methyl 3α,6β,7α-trihydroxy-5β-cholanate (3a)

This was prepared nearly quantitatively from the acid **3** by the general esterification method; mp, 97–98°C (colorless needles from acetone–hexane). IR V_{max} cm^{-1}: 3420, 1042, 1018 (OH), 1740 (C=O). ^{1}H-NMR δ: 0.69 (s, 3H, 18-Me), 0.94 (d, 3H, J = 5.4 Hz, 21-Me), 1.08 (s, 3H, 19-Me), 3.44 (brm,1H,3-H), 3.66 (s, 3H, COOMe), 3.71 (m, 2H, 6- and 7-H). Low resolution MS, *m/z* (relative intensity): 422(1%,M$^+$), 404(41%, M−H_2O), 386(60%,

M-2H_2O), 371(15%, M-2H_2O-Me). Anal. calcd. for $C_{25}H_{42}O_5$: C, 71.05; H, 10.02. Found: C, 71.09; H, 9.86.

Methyl 3α-cathyloxy-6β,7β-dihydroxy-5β-cholanate (19a)

To the ester **15a** (1.5 g, 3.3 mmol) dissolved in *tert*-butyl alcohol–tetrahydrofuran–water (15 ml; 10:3:1, v/v/v) was added *N*-methylmorpholine *N*-oxide (1.12 g, 8.3 mmol) and osmium tetroxide (30 mg, 0.1 mmol) (18, 19); and the mixture was allowed to stand overnight at room temperature. The dark brown solution was poured onto water, and extracted with CH_2Cl_2. The organic layer was washed successively with water, 10% HCl, 5% $NaHCO_3$, and water, dried with Drierite, and evaporated to an oily residue. Chromatography of the oil on a column of neutral alumina (45 g, activity II) and elution with benzene–EtOAc 3:7 (v/v) afforded compound **19a** which crystallized from benzene–hexane as colorless crystals; yield, 1.31 g (81%); mp, 147–149°C. IR V_{max} cm^{-1}: 3470, 1020 (OH), 1742 (C=O), 1258 (=C-O). ^{1}H-NMR δ: 0.69 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 1.11 (s, 3H, 19-Me), 1.30 (3H, J = 7.2 Hz, 3-OCOOCH_2CH_3), 3.49 (brm, 1H, 7-H), 3.66 (s, 4H, 6-H and COOMe), 4.18 (q, 2H, J = 7.2 Hz, 3-OCOOCH_2CH_3), 4.50 (brm, 1H, 3-H). Anal. calcd. for $C_{28}H_{46}O_7$: C, 67.98; H, 9.37. Found: C, 67.71; H, 9.50.

3α,6β,7β-Trihydroxy-5β-cholanic acid (4)

The ester **19a** hydrolyzed with 5% methanolic KOH by the usual method, crystallized from aqueous methanol as colorless thin plates; yield, 93%; mp, 225–227°C (lit. mp, 226–228 (3) and 225–226°C (5)). IR V_{max} cm^{-1}: 3425, 1052 (OH), 1695 (C=O). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.68 (s, 3H, 18-Me), 0.94 (d, 3H, J = 6.3 Hz, 21-Me), 1.08 (s, 3H, 19-Me), 3.50 (brm, 2H, 3- and 7-H), 3.61 (m, 1H, 6-H). Anal. calcd. for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.61; H, 10.12.

Methyl 3α,6β,7β-trihydroxy-5β-cholanate (4a)

This was prepared nearly quantitatively from the acid **4** by the general esterification method. Although **4a** was homogeneous by TLC and ^{1}H-NMR, it could not be crystallized. IR V_{max} cm^{-1}: 3410, 1058 (OH), 1740 (C=O). ^{1}H-NMR δ: 0.70 (s, 3H, 18-Me), 0.94 (d, 3H, J = 6.3 Hz, 21-Me), 1.10 (s, 3H, 19-Me), 3.54 (brm, 2H, 3- and 7-H), 3.67 (s, 3H, COOMe), 3.70 (m, 1H, 6-H). Low resolution MS, *m/z* (relative intensity): 422 (1%, M^+), 404 (100%, M-H_2O), 368 (62%, M-2H_2O), 371 (15%, M-2H_2O-Me). High resolution MS: 422.3026 (M^+, $C_{25}H_{42}O_5$ requires 422.3033).

Methyl 3α-hydroxy-6α,7α-isopropylidenedioxy-5β-cholanate (20a)

To the ester **1a** (1.1 g, 2.6 mmol) in acetone (120 ml) was added *p*-toluenesulfonic acid (300 mg) and molecular sieve (20 g, 4 Å), and the mixture was stirred at room temperature for 6 h. The molecular sieve was filtered off, most of the acetone was evaporated, and the reaction product was extracted with CH_2Cl_2. The combined extract was washed with water, 5% $NaHCO_3$, and water, dried over Drierite, and evaporated to afford **20a**, which was homogeneous according to TLC and ^{1}H-NMR but failed to crystallize (22); yield, 1.12 g (93%). IR V_{max} cm^{-1}: 3470, 1050 (OH), 1735 (C=O). ^{1}H-NMR δ: 0.65 (s, 3H, 18-Me), 0.89 (s, 3H, 19-Me), 0.93 (d, 3H, J = 6.3 Hz, 21-Me), 1.32 and 1.51 (s, each 3H, 6,7-acetonide Me), 3.66 (s, 3H, COOMe), 3.70 (brm, 1H, 3-H), 4.19 (m, 2H, 6- and 7-H). High resolution MS: 447.3130 (M^+-CH_3, $C_{27}H_{43}O_5$ requires 447.3111).

Methyl 3β,6α,7α-trihydroxy-5β-cholanate (5a)

A solution of diethyl azodicarboxylate (590 mg, 3.4 mmol) in benzene (1 ml) was slowly added dropwise to a solution of the acetonide **20a** (460 mg, 1.0 mmol), triphenylphosphine (890 mg, 3.4 mmol) and formic acid (150 mg, 3.4 mol) in benzene (5 ml) (20,21). After refluxing for 48 h and then cooling, the precipitated solid (diethyl hydrozodicarboxylate) was removed by filtration, and the filtrate was evaporated and redissolved in Et_2O–hexane. The precipitate (triphenylphosphine oxide) was filtered off, and the filtrate was evaporated to an oily product. A solution of the oil in methanol (20 ml) containing conc. HCl (0.2 ml) was allowed to stand at room temperature for 1 h. Most of the solvent was evaporated and the residue was extracted with CH_2Cl_2. The organic layer was washed with 5% $NaHCO_3$ and water, dried with Drierite, and evaporated to give an oil, which by TLC consisted of a mixture of two components. Chromatography of the oil over a column of silica gel (18 g) resulted in two components. The less polar compound eluted with benzene–EtOAc 1:1 (v/v) was identified as methyl 6α,7α-dihydroxy-Δ^3-5β-cholenate; yield, 113 mg (28%); mp, 124–126°C (colorless needles from aqueous methanol). IR V_{max} cm^{-1}: 3380 (OH), 1738 (C=O). ^{1}H-NMR δ: 0.67 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 0.98 (s, 3H, 19-Me), 3.66 (s, 3H, COOMe), 3.74 (m, 1H, 7-H), 3.92 (m, 1H, 6-H), 5.51–5.99 (m, 2H, 3- and 4-H). Anal. calcd. for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.47; H, 10.08.

The more polar compound eluted with EtOAc–methanol 95:5 (v/v) was identified as the desired ester **5a**, which was homogeneous according to TLC and ^{1}H-NMR analyses but failed to crystallize; yield, 235 mg (56%). IR V_{max} cm^{-1}: 3425, 1040 (OH), 1740 (C=O). ^{1}H-NMR δ: 0.66 (s, 3H, 18-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 0.94 (s, 3H, 19-Me), 3.66 (s, 3H, COOMe), 3.85 (m, 1H, 7-H), 3.93 (brm, 1H, 6-H), 4.11 (m, 1H, 3-H). Low resolution MS, *m/z* (relatively intensity): 422 (7%, M^+), 404 (34%, M-H_2O), 386 (57%, M-2H_2O), 371 (11%, M- 2H_2O-Me). High resolution MS: 422.3023 (M^+, $C_{25}H_{42}O_5$ requires 422.3033).

3β,6α,7α-Trihydroxy-5β-cholanic acid (5)

The compound was prepared nearly quantitatively from the ester **5a** by the general hydrolysis method. The crude acid **5** was recrystallized from aqueous methanol as colorless needles; mp, 214–217°C. IR V_{max} cm^{-1}: 3440, 1048 (OH), 1708 (C=O). ^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6) δ: 0.66 (s, 3H, 18-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 0.94 (s, 3H, 19-Me), 3.84 (m, 1H, 7-H), 3.90 (brm, 1H, 6-H), 4.08 (m, 1H, 3-H). Anal. calcd. for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.31; H, 9.80.

Methyl 3α-hydroxy-6α,7α-isopropylidenedioxy-5β-cholanate (21a)

The ester **2a** (420 mg, 1.0 mmol) was converted to its 6,7-acetonide **21a** by the method described for the preparation of **20a**; yield, 437 mg (95%). Although this compound was homogeneous according to TLC and ^{1}H-NMR analyses, it could not be crystallized. IR V_{max} cm^{-1}: 3480(OH), 1735(C=O). ^{1}H-NMR δ: 0.68 (s, 3H, 18-Me), 0.93 (d, 3H, J = 7.2 Hz, 21-Me), 0.97 (s, 3H, 19-Me), 1.34 and 1.40 (s, each 3H, 6,7-acetonide Me), 3.31–3.81 (m, 3H, 3-, 6-, and 7-H). High resolution MS: 447.3109 (M^+–CH_3, $C_{27}H_{43}O_5$ requires 447.3111).

Methyl 3β,6α,7β-trihydroxy-5β-cholanate (6a)

The ester **21a** (230 mg, 0.5 mmol) was treated with diethyl azodicarboxylate/triphenylphosphine/formic acid, followed by conc. HCl as described for the preparation of **5a**. After being processed analogously, the oily product was purified by a column of silica gel (10 g). Elution with EtOAc–methanol 95:5 (v/v) gave compound **6a**; yield, 174 mg (83%), which was homogeneous according to TLC and ^{1}H-NMR but failed to crystallize. IR V_{max} cm^{-1}: 3440, 1022 (OH), 1735 (C=O). ^{1}H-NMR δ: 0.68 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 0.98 (s, 3H, 19-Me), 3.35 (brm, 1H, 7-H), 3.66 (s, 3H, COOMe), 3.82 (brm, 1H, 6-H), 4.11 (m, 1H, 3-H). Low resolution MS, *m/z* (relative intensity): 422 (8%, M^+), 404 (60%, M–H_2O), 386 (73%, M–2H_2O), 371 (13%, M–2H_2O-Me), 368 (16%, M–3H_2O). High resolution MS: 422.3007 (M^+, $C_{25}H_{42}O_5$ requires 422.3033).

3β,6α,7β-Trihydroxy-5β-cholanic acid (6)

The compound was prepared from the ester **6a** by the general hydrolysis procedure; yield, 96%; mp, 129–131°C (colorless crystals from acetone–hexane). IR V_{max} cm^{-1}: 3410, 1025 (OH), 1710 (C=O). ^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6) δ: 0.66 (s, 3H, 18-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 0.95 (s, 3H, 19-Me), 3.33 (brm, 1H, 7-H), 3.74 (brm, 1H, 6-H), 4.00 (m, 1H, 3-H). Anal. calcd. for $C_{24}H_{40}O_5 \cdot 1/2H_2O$: C, 69.03; H, 9.90. Found: C, 69.29; H, 9.79.

3α-Hydroxy-Δ^6-5β-cholenic acid (22)

This acid was prepared from the ester **15a** by the general hydrolysis method; yield, 97%; mp, 218–219°C (colorless thin plates from methanol). IR V_{max} cm^{-1}: 3330, 1062 (OH), 1708 (C=O). ^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6) δ: 0.69 (s, 3H, 18-Me), 0.84 (s, 3H, 19-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 3.52 (brm, 1H, 3-H), 5.46 (s, 2H, 6- and 7-H). Anal. calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 77.20; H, 10.40.

Methyl 3α-hydroxy-Δ^6-5β-cholenate (22a)

This was prepared from the acid **22** by the general esterification method: yield, 95%; mp, 104–105°C (colorless fine needles from aqueous methanol). IR V_{max} cm^{-1}: 3520, 1005 (OH), 1716 (C=O). ^{1}H-NMR δ: 0.69 (s, 3H, 18-Me), 0.85 (s, 3H, 19-Me), 0.93 (d, 3H, J = 6.3 Hz, 21-Me), 3.53 (brm, 1H, 3-H), 3.66 (s, 3H, COOMe), 5.46 (s, 2H, 6- and 7-H). Anal. calcd. for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.24; H, 10.39.

Methyl 3β-formyloxy-Δ^6-5β-cholenate (23a)

The ester **22a** (2.83 g, 16.2 mmol) was subjected to the inversion reaction with diethyl azodicarboxylate/triphenylphosphine/formic acid and processed as described for the preparation of **5a** to yield an oily residue. The oil was chromatographed on a silica gel column (100 g) and eluted with benzene–EtOAc 9:1 (v/v). Crystallization of the product from aqueous methanol gave **23a** as colorless thin plates; yield, 1.98 g (84%); mp, 86–88°C. IR V_{max} cm^{-1}: 1722 (C=O), 1208 (C-O). ^{1}H-NMR δ: 0.70 (s, 3H, 18-Me), 0.89 (s, 3H, 19-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 3.66 (s, 3H, COOMe), 5.16 (m, 1H, 3-H), 5.47 (s, 2H, 6- and 7-H), 8.07 (s, 1H, 3-OCHO). Anal. calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 74.71; H, 9.72.

Methyl 3β-hydroxy-Δ^6-5β-cholenate (24a)

The ester **22a** (1.0 g, 2.4 mmol) was subjected to the inversion reaction with diethyl azodicarboxylate/triphenylphosphine/formic acid and processed as described for the preparation of **5a**. A solution of the crude product dissolved in benzene (5 ml) was poured onto a column of neutral alumina (40 g, activity II) and allowed to stand overnight. Elution with benzene–EtOAc 7:3 (v/v) gave compound **24a**; yield, 0.81 g (81%); mp, 119–120°C (colorless needles from aqueous methanol). IR V_{max} cm^{-1}: 3280, 1042 (OH), 1740 (C=O). ^{1}H-NMR δ: 0.70 (s, 3H, 18-Me), 0.89 (s, 3H, 19-Me), 0.93 (d, 3H, J = 6.3 Hz, 21-Me), 3.66 (s, 3H, COOMe), 4.06 (m, 1H, 3-H), 5.47 (s, 2H, 6- and 7-H). Anal. calcd. for $C_{25}H_{40}O_3$: C,77.27; H, 10.38. Found: C, 77.36; H, 10.29.

Methyl 3β-hydroxy-6α,7α-epoxy-5β-cholanate (25a)

The ester **24a** (1.4 g, 3.6 mmol) was converted to compound **25a** by epoxidation with *m*-chloroperoxybenzoic acid

as described for the preparation of **17a**; yield, 1.26 g (86%); mp, 141–143°C (colorless needles form aqueous methanol). IR V_{max} cm^{-1}: 3380, 1042, 1002 (OH), 1738 (C=O). ^{1}H-NMR δ: 0.70 (s, 3H, 18-Me), 0.86 (s, 3H, 19-Me), 0.92 (d, 3H, J = 6.3 Hz, 21-Me), 3.09 (s, 2H, 6- and 7-H), 3.66 (s, 3H, COOMe), 4.13 (m, 1H, 3-H). Anal. calcd. for $C_{25}H_{40}O_4 \cdot 3/4H_2O$: C, 71.82; H, 10.00. Found: C, 71.84; H, 9.93.

Methyl 3β,7α-dihydroxy-6β-formyloxy-5β-cholanate (26a)

The ester **25a** (1.0 g, 2.5 mmol), subjected to the hydroxylation reaction with boron trifluoride etherate and processed as described for the preparation of **18a**, afforded compound **26a**; yield, 1.02 g (92%). Although this compound was homogeneous by TLC and ^{1}H-NMR, it could not be crystallized. IR V_{max} cm^{-1}: 3500, 1042 (OH), 1720 (C=O), 1180 (C-O). ^{1}H-NMR δ: 0.69 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 1.04 (s, 3H, 19-Me), 3.66 (s, 3H, COOMe), 4.07 (m, 1H, 3-H), 4.82 (m, 1H, 6-H), 8.04 (s, 1H, 6-OCHO). High resolution MS: 450.2992 (M^+, $C_{26}H_{42}O_6$ requires 450.2982).

3β,6β,7α-Trihydroxy-5β-cholanic acid (7)

The ester **26a**, hydrolyzed by the usual method, recrystallized from EtOAc–hexane as colorless crystals; yield, 94%; mp, 214–216°C. IR V_{max} cm^{-1}: 3420, 1038, 1020 (OH), 1720 (C=O). ^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6) δ: 0.69 (s, 3H, 18-Me), 0.94 (d, 3H, J = 5.4 Hz, 21-Me), 1.11 (s, 3-H, 19-Me), 3.66 (m, 2H, 6- and 7-H), 4.02 (m, 1H, 3-H). Anal. calcd. for $C_{24}H_{40}O_5 \cdot 1/4H_2O$: C, 69.78; H, 9.88. Found: C, 69.64; H, 9.97.

Methyl 3β,6β,7α-trihydroxy-5β-cholanate (7a)

This was prepared nearly quantitatively from the acid **7** by the general esterification method. Although this ester was homogeneous according to TLC and ^{1}H-NMR analyses, it could not be crystallized. IR V_{max} cm^{-1}: 3410, 1040 (OH), 1720 (C=O). ^{1}H-NMR δ: 0.69 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 1.10 (s, 3H, 19-Me), 3.66 (s, 5H, 6- and 7-H and COOMe), 4.01 (m, 1H, 3-H). Low resolution MS, *m/z* (relative intensity): 422 (3%, M^+), 404 (84%, M-H_2O), 386 (24%, M-2H_2O), 371 (7%, M-2H_2O-Me). High resolution MS: 422.3022 (M^+, $C_{25}H_{42}O_5$ requires 422,3033).

Methyl 3β-formyloxy-6β,7β-dihydroxy-5β-cholanate (27a)

The 3β-formyloxy-Δ^6-ester **23a** (390 mg, 1 mmol), subjected to the *cis*-dihydroxylation reaction with osmium tetroxide and *N*-methylmorpholine *N*-oxide and processed as described for the preparation of **19a**, afforded compound **27a**; yield, 323 mg (77%); mp, 168–169°C (colorless crystals from aqueous methanol). IR V_{max} cm^{-1}: 3475, 1058 (OH), 1720 (C=O), 1190 (C-O). ^{1}H-NMR δ: 0.71 (s, 3H, 18-Me), 0.94 (d, 3H, J = 6.3 Hz, 21-Me), 1.14 (s, 3H, 19-Me), 3.49 (brm, 1H, 7-H), 3.62 (m, 1H, 6-H), 3.66 (s, 3H, COOMe), 5.19 (m, 1H, 3-H), 8.07 (s, 1H, 3-OCHO). Anal. calcd. for $C_{26}H_{42}O_6$: C, 69.30; H, 9.40. Found: C, 69.07; H, 9.43.

Methyl 3α-hydroxy-6β,7β-isopropylidenedioxy-5β-cholanate (28a)

The ester **4a** (550 mg, 1.3 mmol) was converted to its 6,7-acetonide **28a** by the method as described for the preparation of **20a**; yield, 594 mg (99%). Although this compound was homogeneous according to TLC and ^{1}H-NMR analyses, it could not be crystallized. IR V_{max} cm^{-1}: 3420, 1050 (OH), 1742 (C=O). ^{1}H-NMR δ: 0.66 (s, 3H, 18-Me), 0.92 (d, 3H, J = 5.4 Hz, 21-Me), 1.10 (s, 3H, 19-Me), 1.30 and 1.47 (s, each 3H, 6,7-acetonide Me), 3.50 (brm, 1H, 3-H), 3.66 (s, 3H, COOMe), 3.63–3.95 (m, 2H, 6- and 7-H). High resolution MS: 447.3102 (M^+-CH_3, $C_{27}H_{43}O_5$ requires 447.3111).

Methyl 3β,6β,7β-trihydroxy-5β-cholanate (8a)

(a) This compound was prepared from the Δ^6-ester **24a** (450 mg, 1.1 mmol) by *cis*-dihydroxylation with osmium tetroxide and *N*-methylmorpholine *N*-oxide as described for the preparation of **19a**. Purification by alumina (13 g) chromatography eluting with EtOAc–methanol 95:5 (v/v) gave the desired ester **8a** (378 mg, 77%) which was homogeneous according to TLC and ^{1}H-NMR analyses, but failed to crystallize. IR V_{max} cm^{-1}: 3420, 1038 (OH), 1740 (C=O). ^{1}H-NMR δ: 0.70 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 1.13 (s, 3H, 19-Me), 3.48 (brm, 1H, 7-H), 3.63 (m, 1H, 6-H), 3.66 (s, 3H, COOMe), 4.07 (m, 1H, 3-H). Low resolution MS, *m/z* (relative intensity): 422 (6%, M^+), 404 (86%, M-H_2O), 386 (40%, M-2H_2O), 371 (9%, M-2H_2O-Me). High resolution MS: 422,2989 (M^+, $C_{25}H_{42}O_5$ requires 422.3033).

(b) The acetonide **28a** (460 mg, 1.0 mmol) was treated with diethyl azodicarboxylate/triphenylphosphine/formic acid, followed by conc. HCl as described for the preparation of **5a**. After being processed analogously, chromatography of the oily product over a column of silica gel (18 g) resulted in two components. The less polar compound eluted with benzene–EtOAc 1:1 (v/v) and was characterized as methyl 6β,7β-dihydroxy-Δ^3-5β-cholenate, which could not be crystallized; yield, 99 mg (25%). IR V_{max} cm^{-1}: 3480 (OH), 1735 (C=O). ^{1}H-NMR δ: 0.71 (s, 3H, 18-Me), 0.93 (d, 3H, J = 5.4 Hz, 21-Me), 1.12 (s, 3H, 19-Me), 3.26–3.40 (m, 1H, 7-H), 3.66 (s, 3H, COOMe), 3.81 (m, 1H, 6-H), 5.62–5.75 (m, 2H, 3- and 4-H). High resolution MS: 404,2892 (M^+, $C_{25}H_{40}O_4$ requires 404.2927).

The more polar compound eluted with EtOAc–methanol 95.5 (v/v) and was identical to the desired ester **8a** as prepared above (a); yield, 253 mg (60%).

,3β,6β,7β-Trihydroxy-5β-cholanic acid (8)

The compound was prepared nearly quantitatively from the ester **8a** or **27a** by the general hydrolysis method. The crude acid **8** was recrystallized from aqueous methanol as colorless needles; it melted at 165–167°C, resolidified at about 170°C, and then remelted at 189–190°C. IR V_{max} cm^{-1}: 3425, 1038 (OH), 1713 (C=O). ^{1}H-NMR ($CDCl_3$ + 10% DMSO-d_6) δ: 0.69 (s, 3H, 18-Me), 0.94 (d, 3H, J = 6.3 Hz, 21-Me), 1.12 (s, 3H, 19-Me), 3.43 (brm, 1H, 7-H), 3.61 (m, 1H, 6-H), 4.03 (m, 1H, 3-H). Anal. calcd. for $C_{24}H_{40}O_5 \cdot 1/4H_2O$: C, 69.78; H, 9.98. Found: C, 69.82; H, 9.72.

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