C-3 CHOH), 3.90 (1 H, m, C-7 CHOAc), 7.32 and 7.88 (each 2 H, J = 8 Hz, para-disubstituted phenyl).

Methyl 3α,7β-dihydroxy-12-oxocholanate tosylhydrazone (26a) was prepared from 1.6 g of 3α,7β-dihydroxy-12-oxocholanate (**25a**)[20] by the general procedure. The residue crystallized from methanol as colorless thin plates: 1.44 g (64%); mp 244.0–247.0 °C; IR (KBr) 1718 (C=O), 3106, 1618, 1587, 1321, 1166 (s), 816 (tosylhydrazone) cm^{-1}; NMR ($CDCl_3$ + 10% Me_2SO-d_6) δ 0.79 (3 H, s, C-18 Me), 0.99 (3 H, s, C-19 Me), 2.41 (3 H, s, ArMe), 3.50 (2 H, br m, C-3 and C-7 CHOH), 3.64 (3 H, s, COOMe), 7.26 and 7.78 (each 2 H, d, J = 8 Hz, para-disubstituted phenyl).

Anal. Calcd for $C_{32}H_{48}N_2O_6S \cdot {}^1/_3CH_3OH$: C, 64.81; H, 8.30. Found: C, 64.81; H, 8.21.

Methyl Di-*O*-acetylchenodeoxycholate (16a).[5] To a magnetically stirred solution of acetic acid (80 mL) containing 4.04 g (0.006 mol) of **13a** was added 2.27 g (0.06 mol) of $NaBH_4$ (pellets) at a rate which did not allow the reaction temperature to exceed 60 °C (ca. 1 h). Stirring was continued at room temperature for 3 h, and then with the flask immersed in an ice bath, ice chips were gradually stirred in. The precipitated and filtered solid, after being washed with water, crystallized from aqueous methanol as fine needles: 1.58 g (53%); mp 133.0–133.5 °C (further characterization in ref 5).

Methyl Chenodeoxycholate (1a). (a) Reduction of 3α,7α-dihydroxy-12-oxocholanic acid tosylhydrazone (**14**) by the general procedure afforded a precipitate which did not crystallize, but according to TLC, **1** was predominant. The crude residue was esterified by MeOH–HCl treatment and chromatographed on a Florisil column. A fraction (72%) according to NMR, TLC and HPLC was homogeneous **1a**.

(b) Reduction of methyl 3α,7α-dihydroxy-12-oxocholanate tosylhydrazone (**14a**) by the general method when diluted with ice–H_2O precipitated as an oil. The aqueous residual mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract, neutralized with $NaHCO_3$ solution, dried (Drierite), and chromatographed on an alumina (activity II) column, gave **1a** (57%), as confirmed by TLC, NMR, and hydrolysis to acid **1**.

Methyl Ursodeoxycholate (2a). Methyl 3α,7β-dihydroxy-12-oxocholanate tosylhydrazone (**26a**, 510 mg) reduced by the general method, yielded an oily residue which did not crystallize. Chromatographed on a Florisil column, the fractions eluted (33 mg) with CH_2Cl_2/EtOAc (1:1) were olefinic (tetranitromethane test). Further elution with EtOAc yielded **2a** which crystallized from aqueous methanol [182 mg (52%); mp 159–161 °C] and was identical with authentic methyl urosodeoxycholate according to mixture melting point, TLC, NMR, and HPLC comparisons.

7α-Acetoxy-3α,24-cholanediol (21). (a) Reduction of **13a** (1.0 g), according to the Cagliotti and Grasseli conditions,[17] by $NaBH_4$ in dioxane under reflux for 6 h yielded, after processing and crystallization from aqueous acetone, 412 mg (66%) of **21** as needles: mp 86.0–88.5 °C; IR ($CHCl_3$) 1721 (C=O), 1070, 969 (COH)8 1015 (OAc) cm^{-1}; NMR δ 0.67 (3 H, s, C-18 Me, 0.92 (3 H, s, C-19 Me), 2.03 (3 H, s, OCOMe), 3.45 (1 H, br m, C-3 CHOH), 3.56 (2 H, t, CH_2OH), 4.88 (1 H, m, C-7 CHOAc).

Anal. Calcd for $C_{26}H_{44}O_4 \cdot 0.30C_3H_6O$: C, 70.67; H, 10.51. Found: C, 70.56; H, 10.45.

(b) The tosylhydrazone **15a**, after reduction by $NaBH_4$–dioxane[29] as in part a, was chromatographed on activity II alumina. The CH_2Cl_2–MeOH (99:1) eluted material crystallized from aqueous MeOH as fine needles identical with the product of part a by mixture melting point, NMR, and HPLC comparisons.

Acknowledgment. This work was supported in part by a grant from the National Large Bowel Cancer Project. Ms. Susan Brannan contributed able technical assistance. We thank Dr. A. Krekel of Pharmazell Diamalt AG for generous supplies of cheno- and ursodeoxycholic acid.

Registry No. **1a**, 3057-04-3; **2a**, 10538-55-3; **3**, 566-24-5; **3a**, 28050-38-6; **4**, 78919-26-3; **4a**, 73465-45-9; **5a**, 81857-19-4; **6a**, 81857-20-7; **7a**, 81938-69-4; **8**, 81875-58-3; **9**, 81857-21-8; **9a**, 81938-70-7; **10a**, 81875-59-4; **11a**, 28192-93-0; **12**, 2458-08-4; **12a**, 10538-64-4; **13a**, 76927-60-1; **14**, 79580-95-3; **14a**, 81875-60-7; **15a**, 81875-61-8; **16a**, 2616-71-9; **17a**, 71837-87-1; **18a**, 28535-81-1; **20**, 81857-22-9; **23a**, 81089-18-1; **24a**, 81857-23-0; **25a**, 81655-85-8; **26a**, 81857-24-1; **28a**, 19684-68-5; **30a**, 81857-25-2.

(29) Reduction of **15a** (amorphous) by the general procedure ($NaBH_4$–HOAc) afforded a complex mixture which contained methyl 7-*O*-acetylchenodeoxycholate (**29a**) as the major product, according to HPLC and NMR comparisons with authentic **29a**. However, the best fractions of **29a** obtained by column chromatography still contained impurities which inhibited crystallization. This route to chenodeoxycholic acid (**1**) was deemed inferior to the published method[5] and was not further pursued.

(30) Corrected values: F. C. Chang, *J. Org. Chem.*, **46**, 2603 (1981).

Potential Bile Acid Metabolites. 7.[1] 3,7,12-Trihydroxy-5β-cholanic Acids and Related Compounds

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With this work, the complete set of the eight possible stereoisomeric (5β)-3,7,12-trihydroxy acids are now known and characterized. The key intermediates methyl 3α,7β-dihydroxy-12-oxo- and 3β,7β-dihydroxy-12-oxocholanate have been synthesized, and their reductions by *tert*-butylamine–borane complex and by $NaBH_4$ are described.

The 3,7,12-trihydroxy stereoisomers of cholic (3α,7α,12α-trihydroxycholanic[3]) acid (**1**, Chart I) are of sustained interest in metabolic studies of the bile acids.[4] As part of a program to make available new and known, but generally unavailable, potential bile acid metabolites for such studies, three of the seven possible stereoisomers [3α,7α,12β (**2**), 3β,7α,12α (**3**), and 3β,7α,12β (**4**)] were synthesized and previously reported.[5] The present paper describes the syntheses of the four remaining members of this group and several related compounds and, in addition, presents improved preparations of **2** and **4**.

The 3α,7β,12α acid **5** was prepared by Samuelsson some years ago[6] by adaptation of a route used to obtain urso-

(1) Part 6 of this series: T. Iida and F. C. Chang, *J. Org. Chem.*, accompanying paper in this issue.

(2) On leave, Nihon University, Japan.

(3) All cholanic acid derivatives in this work are of the 5β series; the 5β designations are omitted in their names. The older name cholanic acid is used throughout in place of the newer IUPAC-suggested "cholanoic acid".

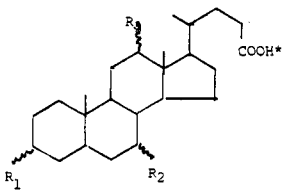
(4) There is epidemiological evidence that bile acid metabolites are implicated in colon carcinogenesis, and gastroenterological research has been involved for many years in exploring the role of bile acids in normal and abnormal metabolism.

(5) F. C. Chang, *J. Org. Chem.*, **44**, 4567 (1979).

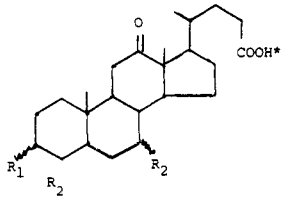
(6) B. Samuelsson, *Acta Chem. Scand.*, **14**, 7 (1960). The number of requests received from investigators for samples of **5** attests to its rarity and need.

0022-3263/82/1947-2972$01.25/0

Chart I



	R_1	R_2	R_3		R_1	R_2	R_3
1	α-OH	α-OH	α-OH	11	β-OH	α-OH	H,H
2	α-OH	α-OH	β-OH	12	β-OH	β-OH	H,H
3	β-OH	α-OH	α-OH	13	α-OH	=O	α-OH
4	β-OH	α-OH	β-OH	14	α-OCO_2Et	=O	α-OH
5	α-OH	β-OH	α-OH	16	α-OCO_2Et	α-OH	α-OH
6	α-OH	β-OH	β-OH	17	α-OCO_2Et	α-OMs	α-OH
7	β-OH	β-OH	α-OH	18	α-OCO_2Et	α-OH	α-OMs
8	β-OH	β-OH	β-OH	32	β-OCHO	=O	α-OH
9	α-OH	α-OH	H,H	33	β-OH	=O	α-OH
10	α-OH	β-OH	H,H	37	β-OCHO	α-OH	α-OH



	R_1	R_2		R_1	R_2
15	α-OCO_2Et	=O	27	α-OTs	β-OH
19	α-OH	α-OH	28	β-OH	α-OH
20	α-OCO_2Et	α-OH	29	β-OCHO	α-OH
21	α-OAc	α-OH	30	β-OCHO	α-OMs
22	α-OCO_2Et	α-OMs	31	β-OH	β-OH
23	α-OAc	α-OMs	34	=O	=O
24	α-OH	β-OH	35	α-OH	=O
25	α-OTs	α-OH	36	β-OH	=O
26	α-OTs	α-OMs			

* The corresponding C-24 methyl esters are designated "a".

deoxycholic (**10**) from chenodeoxycholic (**9**) acid, namely, by sodium–alcohol reduction of the 7-oxo analogue. Our attempts to repeat the procedure starting from cholic acid were discouraging; low yields of acid **5** were obtained only after a laborious multistep process. Both of the key steps were troublesome; selective oxidation at C-7 was less straightforward than indicated in the literature, and the reduction with sodium–alcohol, as commonly experienced with this type of reaction, was experimentally exacting and inelegant and additionally required separation of the resulting epimers and numerous byproducts by column chromatography.

The most promising of the literature preparations of the keto acid **13** appeared to be the Fieser and Rajagopalan procedure[7] in which selective oxidation of methyl 3-(cathyloxy)cholate (**16a**) was reported to give in reasonable yield the corresponding 7-oxo derivative of good purity, although the product had variable melting points. In their paper they concluded that samples from various preparations, some differing widely in melting points, were pure monooxocathylate **14a** on the basis of evidence of elemental analytical results and the excellence of the crystalline form of the preparations. In repeating the work, assisted by instrumentation (NMR, HPLC, GC) not available at the time of the Fieser and Rajagopalan efforts, we have found that efficient selective oxidation at C-7 is not feasible. Even under much milder conditions than have been reported in the literature, in which some methyl 3-(cathyloxy)cholate remains untouched, 7,12-dioxo compound **15a** is formed. The analytical evidence is misleading because the differences in the carbon and hydrogen percentages between the mono- and dioxocathylates are not sufficiently large for positive differentiation. By careful fractional crystallization followed by analysis by HPLC and NMR we have found that well-shaped crystalline products can have monooxo/dioxo ratios varying from 10:1, 7:1, and 4:1 to 1:1. The "selective" oxidation of methyl 3β-(formyloxy)-7α,12α-dihydroxycholanate[5] was similarly unsatisfactory. Mixtures of the 7-oxo and 7,12-dioxo 3-formates resulted. However, the corresponding 3β-hydroxy esters could be obtained and separated by alumina column chromatography of the formate mixture.

Thus, we were prompted to explore a route to 7β-hydroxy acids which did not involve an intermediary 7-ketone. The recently reported successful inversion of a hydroxyl group in a sensitive position of a prostaglandin encouraged us to try the superoxide–crown ether method,[8] though many previous efforts to directly invert 7α- to 7β-hydroxy steroids had been unsuccessful. As a model experiment, the conversion of chenodeoxycholic acid (**9**) to ursodeoxycholic acid (**10**) was chosen. The successful inversion summarized in Scheme Ia has been published.[9]

The success of the above inversion promised new access to other 7β-hydroxy bile acid derivatives, in particular to the desired 3α,7β,12α isomer **5**, by extension of the procedure to methyl 3-(cathyloxy)cholanate (**16a**) via the corresponding 7-mesylate (**17a**). Since **16a** had previously been selectively monoacetylated at C-7,[10] analogous monomesylation to **17a** was expected. However, in the actual mesylation of **16a**, although monomesylation did take place to give a clean crystalline product, the latter proved unexpectedly to be the methyl 3-(cathyloxy)-12-(mesyloxy)cholanate (**18a**).

The finding of a more efficient reagent for reducing 12-oxocholanes to 12β-hydroxy analogues[11] led to the successful synthesis of **5** as well as the remaining three unreported stereoisomers. Improved synthesis **2** and **4** also resulted.

Both methyl 3α-(cathyloxy)-7α-hydroxy-12-oxocholanate (**20a**) and the corresponding 3-acetate **21a** prepared from the readily available ester **19a**[5] underwent mesylation without difficulty to **22a** and **23a**, respectively. Both mesylates reacted in the KO_2–crown ether reaction, as previously reported[9] for the 12-methylene analogue, to give inversion at C-7 and simultaneous hydrolysis of the acyloxy group at C-3 and the methyl ester at C-24, forming 3α,7β-dihydroxy-12-oxocholanic acid (**24**). In contrast with

(7) L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **72**, 5530 (1950). The other methods tried [G. A. R. Halsewood, *Biochem. J.*, **27**, 109 (1943); W. H. Hoehn and J. Linsk, *J. Am. Chem. Soc.*, **67**, 312 (1945); H. Heusser and H. Wuthier, *Helv. Chim. Acta*, **30**, 2165 (1947)] all had their shortcomings: under- or overoxidations, difficulty of isolation of product, and/or low yields. However, the monoketo acid **13** has been obtained pure but generally circuitously and in poor yields. Our choice is to oxidize methyl 3-*O*-cathylcholate with 1.2 equiv of *N*-bromosuccinimide which leaves a part of the starting cathylate unoxidized. Hydrolysis of the cathylate mixture and reesterification affords a mixture of **1a** and methyl 3α,12α-dihydroxy-7-oxocholanate (**13a**), which can be separated by column chromatography.

(8) E. J. Corey, K. C. Nicolaou, M. Shibasaki, M. Machida, and C. S. Shiner, *Tetrahedron Lett.*, 3185 (1975).

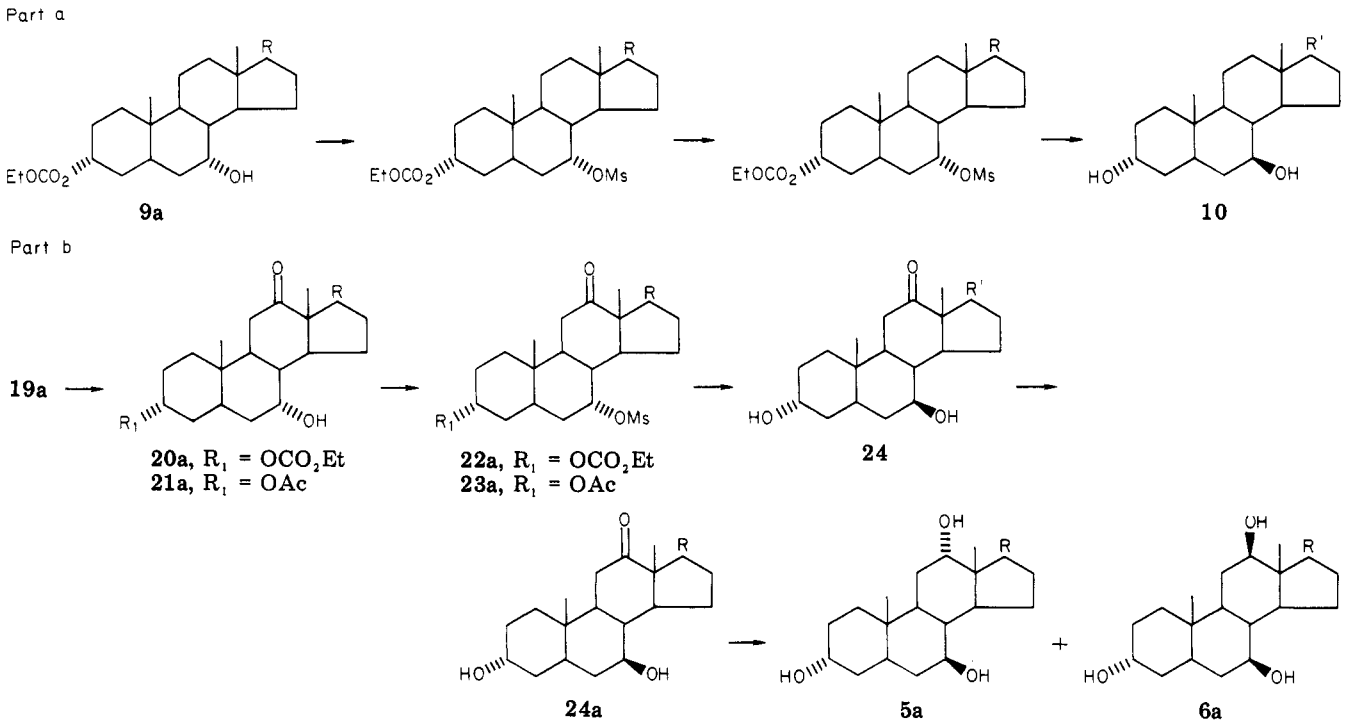
(9) T. Iida and F. C. Chang, *Lipids*, **16**, 863 (1981).

(10) L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romero, and T. Utne, *J. Am. Chem. Soc.*, **74**, 3309 (1952).

(11) F. C. Chang, *Synth. Commun.*, 11, 875 (1981).

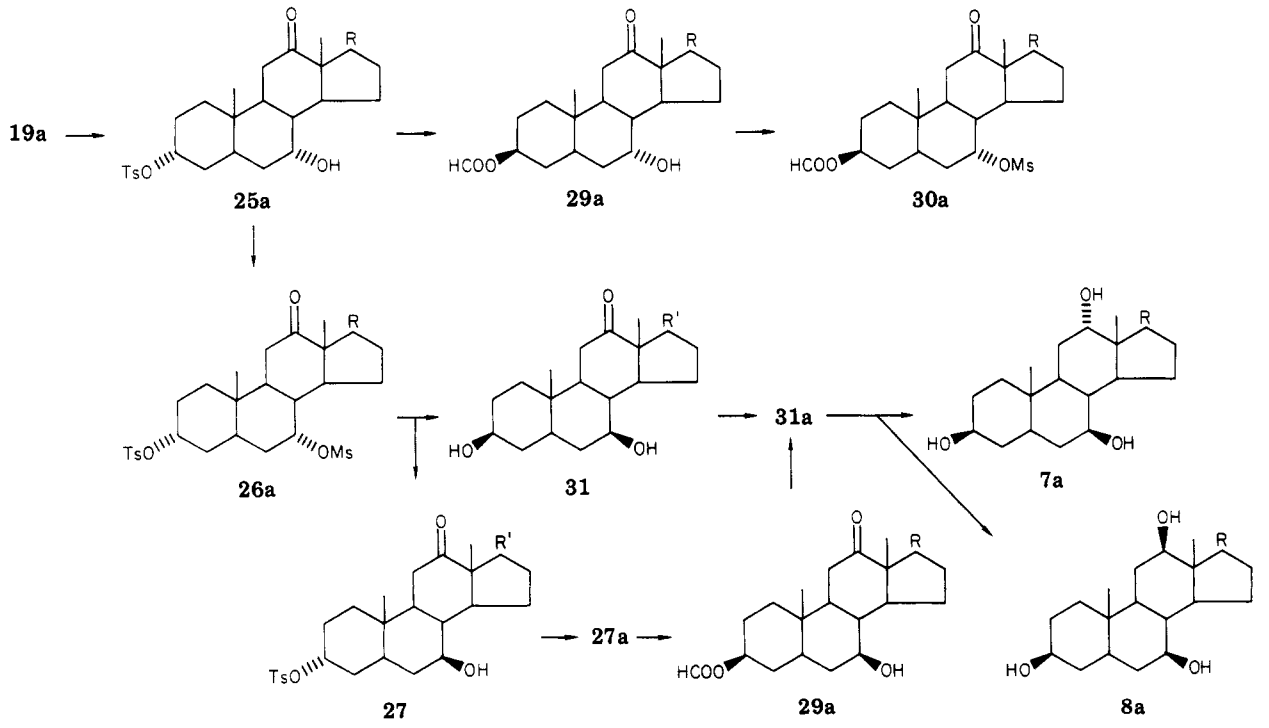
(12) F. C. Chang, *Tetrahedron Lett.*, 2085 (1979), Ref 5.

Scheme I[a]



[a] R = CH(Me)CH$_2$CH$_2$COOMe and R′ = CH(Me)CH$_2$CH$_2$COOH.

Scheme II[a]



[a] R = CH(Me)CH$_2$CH$_2$COOCH$_3$ and R′ = CH(Me)CH$_2$CH$_2$COOH.

previous work[12] in which **19a**, the C-7 epimer of **24a**, when reduced by $NaBH_4$ afforded almost exclusively the 12α-hydroxy product, cholic acid (**1**), similar reduction of either **24** or its methyl ester **24a** yielded a 12-hydroxy epimeric mixture in which the 12β/12α ratio was ca. 0.55. Column chromatography of the methyl ester mixture readily resolved the epimers **5a** or **6a** (Scheme Ib).

The *tert*-butylamine–borane reagent, which had been found to stereoselectively reduce 12-oxocholanic acids to the corresponding 12β-hydroxy compounds, with **24a** gave the epimers in a β/α ratio of ca. 3.7.

Thus the 3β,7β,12α (**7**) and 3β,7β,12β (**8**) acids became available by an analogous route via methyl 3β,7β-dihydroxy-12-oxocholanate (**31a**). The ester **31a** was made by the two procedures developed in the synthesis of the corresponding 12-methylene compound (**12a**[1] Scheme II).

The first involved the DMF inversion reaction[5] of the 3α-tosylate **25a** to the corresponding 3β-formate **29a**, which

in turn was converted to the 3β-formate 7α-mesylate **30a**. Reaction of **30a** with the KO_2–crown ether reagent resulted in the expected 3β,7β-dihydroxy-12-oxocholanic acid (**31**). The second synthesis of **31** consisted of a one-step inversion at both C-3 and C-7 in which the tosylate mesylate keto ester **26a** undergoes the KO_2–crown ether reaction to yield acid **31**. As found with the corresponding reaction to produce **12a** (above),[1] inversion at C-7 of the mesyloxy group is considerably faster than at C-3 of the tosyloxy group, and in an intentionally incomplete reaction (4 h), the intermediary 3α-(tosyloxy)-7β-hydroxy-12-oxocholanic acid (**27**) as well as the desired acid **31** were obtained and isolated. The methyl ester of **27** when treated with DMF[5] afforded the expected mixture of 3β-formate **29a** and olefinic product. When this mixture was allowed to stand overnight on an alumina column, hydrolysis of the formate takes place, and on elution the ester **31a** and the olefinic material are readily resolved.

Reduction of **31a** to give the βββ and ββα compounds was straightforward; with $NaBH_4$ the ratio of βββ/ββα obtained was ca. 0.64; with *tert*-butylamine the ratio was ca. 2.7. Both reactions are rapid and clean, and workup and separation of the epimers by column chromatography are easy.

The previous preparations[5] of the two 12β-hydroxy acids **2** and **4** depended on reduction of the appropriate ketones by Raney nickel catalytic hydrogenation, the disadvantages of which have been summarized.[11] In the case of **19a** the major objection was the low yield of the 12β epimer (ratio β/α, ca. 1:1). In the amino–borane complex reduction the ratio obtained was ca. 5.1. The Raney nickel reduction of **28a** to give **4** presented a more serious problem;[5] the long reaction time needed for completion caused inversion of the axial 3β-hydroxy group, resulting in the formation of four stereoisomers. With *tert*-butylamine–borane, **28a** is facilely reduced to give a product with a 12β/12α ratio of 4.5, from which **4** is readily isolated; no inversion at C-3 takes place.

Initially, we had visualized that by using reducing agents with varying degrees of stereoselectivity, the easily available 3,7,12-trioxo analogue **34** would yield product mixtures of the trihydroxy isomers, and by chromatographic techniques, one might be able to isolate and characterize the isomers. Trials with several common reducing agents soon convinced us that the approach was impractical; the problems encountered were (1) available reagents had little stereoselectivity,[13] (2) chromatographic separation even of the few isomers actually obtained was difficult, and (3) only three of the eight stereoisomers were available as reference standards.

The first problem became less formidable when it was found that the *tert*-butylamine–borane reagent which reduces 12-keto derivatives stereoselectively to 12β-hydroxy compounds[11] also reduces 3-keto and 7-keto acids to the corresponding β-hydroxy "unnatural" isomers to a significant, though small, extent. Due to this development combined with the fact that as a result of the present and previously reported work[5] the complete set of eight stereoisomers (**1–8**) was now at hand as reference standards and, moreover, since conditions for clean resolution of the eight isomers by HPLC[14] had been determined, another try at reduction of the trioxo ester **34a** seemed justified. However, in actual experiments with the amine–borane reagent, although the reduction proceeds cleanly to a mixture of trihydroxy esters, HPLC analysis showed that the product consisted largely of 3α-hydroxy isomers, with compound **2a** (ααβ) being the predominent component (52%) and the remainder being **1a** (ααα, 23%), **6a** (αββ, 14%), **4a** (βαβ, 6%), **5a** (αβα, 5%), and **3a** (βαα, 2%) (percentages were estimated by HPLC).[11] The isomers **7a** (ββα) and **8a** (βββ) were not detected. Compounds **1a** and **3a** are easily separated by column chromatography, but the other isomers have very close R_f values, with **2a** and **4a** being nearly inseparable.

Both the 3α- and 3β-hydroxy-7,12-dioxo acids **35** and **36** are known compounds, and reduction of each would limit the products to the four stereoisomers with the C-3 configuration of the starting diketone. Compound **35a** is easily prepared,[5] and **36a** can be obtained by inversion of **35a**[5] or by oxidation of methyl 3β-formoxy-7α,12α-dihydroxycholanate (**37a**).[5] Reduction of **36a** with the amine–borane reagent in fact did yield the four 3β,7ξ,12ξ-trihydroxy esters in approximately the expected proportions,[11] with **4a** (βαβ) decidedly the predominant isomer. However, isolation of the stereoisomers remained as the obstacle. By column chromatography **3a** (βαα) is easily separated, but the remaining three have very close retention times. Those of **4a** (βαβ), **8a** (βββ), and **7a** (ββα) are nearly identical, and repeated careful chromatography would be required to adequately separate them.

Similar reduction of the 3α-hydroxy-7,12-dioxo ester **35a** yielded analogous results; **1a** (ααα) separate easily, and **2a** (ααβ) and **5a** (αβα) are the recalcitrant pair.

Thus, for the present,[15] we feel that the most practical routes to the trihydroxy acids **2** and **4–8** lie in reduction of the 12-oxo analogues **19**, **24**, **28**, and **31** (prepared from either **1** or **3**) by $NaBH_4$ or *tert*-butylamine–borane, even though more steps are required in the processes and column chromatographic separation is still ncessary for the final step. However, in all instances separation of the 12α- from 12β-hydroxy analogues is easy.

Experimental Section[16]

General Reactions. Each of the following reactions is used for several preparations in this work; they are described fully only in the first such preparation: (1) mesylation of 7α-hydroxy derivatives (see compound 22a), (2) inversion of 7α-mesylates by treatment with KO_2–crown ether (see compound **24**), (3) reduction of 12-oxo esters by $NaBH_4$ (see compounds **5a** and **6a**), and (4) reduction of 12-oxo esters by *tert*-butylamine–borane (see compounds **5a** and **6a**).

(13) Catalytic hydrogenation with PtO_2 afforded mainly cholic acid (**1**) and only trace amounts of other α-hydroxy isomers; with Raney nickel the reaction was exceedingly slow and yielded a complex mixture with **1** predominating; with $NaBH_4$ as reducing agent the product mixture was largely **1**;[12] with K-Selectride no reduction at C-12 occurred; with Na–ROH reduction of the acid, although considerable formation of 7β-hydroxyl resulted, the product contained a complex mixture of numerous unidentified components.

(14) The HPLC instrument used was a Waters Associates assembly with a septumless injector and refractive index detector. Of the C-18 reverse-phase columns tried, Bondapak (Waters), Radial Compression (Waters), and Excalibar (Applied Science), the last two gave excellent resolutions, but the Radial Compression column allowed the chromatography to be performed at a lower pressure (a desirable feature). The methyl esters of the stereoisomers emerged in the following order: ααβ, βββ, βαβ, βαα, αβα, ααβ, βαα, and finally ααα. The solvent system used was methanol–water (70:30) with a flow rate of 0.5 mL/min.

(15) With the development of preparative HPLC instrumentation it is quite conceivable that preparation of these stereoisomers can be simplified; reduction of the diketo compounds **35** and **36** would appear to be the appropriate route.

(16) Melting points were determined on an electrical micro hot stage and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer infrared spectrophotometer. NMR spectra were determined with a Perkin-Elmer R-32 instrument, with deuteriochloroform containing Me_4Si as the solvent except where otherwise indicated. For TLC the development the solvent used in the trisubstituted derivatives was $CHCl_3$–EtOAc–HOAc (45:45:10). Solvents were evaporated on a Rotavap at 50 °C under reduced pressure. All bile acid derivatives were dried by azeotropic distillation [benzene, benzene–CH_2Cl_2, CH_2Cl_2, or CH_2Cl_2–MeOH (acids)] before use in reactions. Refer to Table I of the accompanying paper[1] for NMR assignments.

Methyl 3β-Hydroxy-7,12-dioxocholanate (36a) and Methyl 3β,12α-Dihydroxy-7-oxocholanate (33a). Methyl 3β-(formyloxy)-7α,12α-dihydroxycholanoate (**37a**,[5] 1.4 g) was subjected to the selective oxidation procedure of Fieser and Rajagopalan[7] in which potassium chromate in HOAc–NaOAc was used supposedly to selectively oxidize methyl 3-(cathyloxy)cholate (**16a**). Under the same conditions the product was a crystalline mixture of the 3β-formate of the 7,12-dioxo and 7-oxo-12-hydroxy esters. The ratio of the two esters, **36a/33a**, estimated by HPLC, was 0.83. The mixture, left overnight on a neutral alumina column, was hydrolyzed to the 3β-hydroxy esters; elution with CH_2Cl_2–MeOH (99:1) yielded 0.38 g of methyl 3β-hydroxy-7,12-dioxocholanate (**36a**): mp 151.0–153.0 °C (from methanol) (lit.[17] mp 152–154 °C); NMR δ 1.04 (3 H, s, C-18 Me), 1.33 (3 H, s, C-19 Me), 3.65 (3 H, s, COOMe), 4.07 (1 H, m, C-3 CHOH).

Continued elution yielded 0.46 g of methyl 3β,12α-dihydroxy-7-oxocholanate (33a): mp 165.5–167.0 °C (from $CHCl_3$–acetone); NMR δ 0.69 (3 H, s, C-18 Me), 1.22 (3 H, s, C-19 Me), 3.66 (3 H, s, COOMe), 4.02 (2 H, m, C-3 and C-12 CHOH).

Anal. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59. Found: C, 71.75; H, 9.91.

3β,12α-Dihydroxy-7-oxocholanic Acid (33). Ester **33a**, hydrolyzed by the usual method, was crystallized from aqueous ethanol as thin needles: mp 217.5–219.0 °C; NMR δ 0.69 (3 H, s, C-18 Me), 1.21 (3 H, s, C-19 Me), 4.01 (2 H, m, C-3 and C-12 CHOH).

Anal. Calcd for $C_{24}H_{38}O_5 \cdot C_2H_6O$: C, 68.99; H, 9.80. Found: C, 69.15; H, 9.65.

Methyl 3α-(cathyloxy)-7α-hydroxy-12-oxocholanate (20a) was prepared by the cathylation method[7] of Fieser and Rajagopalan, 410 g of **19a** yielding 4.45 g (95%) of **20a** which crystallized from methanol as thin plates: mp 181.5–184.0 °C; IR 1730 (ester C=O), 1701 cm^{-1} (C=O); NMR δ 1.09 (6 H, s, C-18 and C-19 Me), 1.32 (3 H, t, J = 7 Hz, $OCO_2CH_2CH_3$), 3.70 (3 H, s, COOMe), 3.98 (1 H, m, C-7 CHOH), 4.20 (2 H, q, J = 7 Hz, $OCO_2CH_2CH_3$), 4.49 (1 H, br m, C-3 CHOH).

Anal. Calcd for $C_{28}H_{44}O_7$: C, 68.26; H, 9.00. Found: C, 68.66; H, 9.21.

Methyl 3α-(Tosyloxy)-7α-hydroxy-12-oxocholanate (25a). Tosylation of methyl 3α,7α-dihydroxy-12-oxocholanate (**19a**) was by the usual tosyl chloride–pyridine method.[18] The monotosylate obtained essentially quantitatively crystallized from CH_2Cl_2–hexane as colorless needles: mp 165.6–167.0 °C; NMR δ 0.94 (3 H, s, C-18 Me), 0.99 (3 H, s, C-19 Me), 2.41 (3 H, s, ArMe), 3.63 (3 H, s, COOMe), 3.92 (1 H, m, C-7 CHOH), ca. 4.3 (1 H, br m, C-3 CHOTs), 7.31 and 7.78 (each 2 H, d, J = 8 Hz, *para*-disubstituted phenyl).

Anal. Calcd for $C_{32}H_{46}O_7S \cdot {}^1/_6C_6H_{14}$ C, 67.28; H, 8.27. Found: C, 67.57; H, 8.26.

Methyl 3β-(formyloxy)-7α-hydroxy-12-oxocholanate (29a) was prepared from **25a** (1.2 g) by the DMF inversion method[5] (80 °C, 60 h) and crystallized from methanol: mp ~200 °C dec (dependent on rate of heating); 0.71 g (75%); IR 1724 (ester C=O), 1709 (C=O), 1190, 1174 (3β-formate), 1017 cm^{-1} (OH); NMR δ 1.04 (6 H, s, C-18 and C-19 Me), 3.64 (3 H, s, COOMe), 3.96 (1 H, m, C-7 CHOH), 5.13 (1 H, m, C-3, CHOCHO), 8.05 (1 H, s, OCHO).

Anal. Calcd for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 69.94; H, 9.09.

Mesylation Reactions. Methyl 3α-(Cathyloxy)-7α-(mesyloxy)-12-oxocholanate (22a).[19] To methyl ester **20a** (4.0 g), in a nitrogen atmosphere, magnetically stirred in 40 mL of pyridine was added dropwise 20 mL of methanesulfonyl chloride. Stirring was continued for 4 h. The light brown product was very slowly dripped into a stirred ice-cold solution of dilute HCL (about 2 L) with periodic addition of HCl to maintain an acidic medium. A nearly colorless fluffy solid is precipitated if the pyridine solution is dripped very slowly. The solid was filtered through a sintered-glass funnel and dissolved in CH_2Cl_2. The CH_2Cl_2 solution was extracted successively with dilute $NaHCO_3$ solution and H_2O and filtered through phase-separating paper. The amorphous solid (4.2 g) resisted crystalization attempts but according to HPLC, TLC, and NMR was homogeneous: IR 1730 (ester C=O), 1709 (C=O), 1333, 1172 (SO_2), 948, 895, cm^{-1} (OMs); NMR δ 1.08 (6 H, s, C-18 and C-19 Me), 1.32 (3 H, t, J = 7 Hz, $OCO_2CH_2CH_3$), 3.01 (3 H, s, SO_2Me), 3.67 (3 H, s, COOMe), 4.18 (2 H, q, J = 7 Hz, $OCO_2CH_2CH_3$), 4.50 (1 H, br m, C-3 CHOH), 5.01 (1 H, m, C-7, CHOH).

Anal. Calcd for $C_{29}H_{46}O_9S$: C, 61.03; H, 8.13. Found: C, 60.94; H, 8.18.

Methyl 3α-(tosyloxy)-7α-(mesyloxy)-12-oxocholanate (26a) was obtained from **25a** by the general mesylation method in nearly quantitative yield, but although it was shown to be homogeneous by TLC, HPLC, and NMR, it resisted crystallization attempts: IR 1730 (ester C=O), 1709 (C=O), 1351, 1333, 897 cm^{-1} (mesylate); NMR δ 1.00 (6 H, s, C-18 and C-19 Me), 2.44 (3 H, s, ArMe), 2.96 (3 H, s, SO_2Me), 3.64 (3 H, s, COOMe), ~4.4 (1 H, br m, C-3 CHOTs), 4.96 (1 H, m, C-7 CHOMs), 7.33 and 7.77 (each 2 H, d, J = 8 Hz, paradisubstitued phenyl).

Anal. Calcd for $C_{33}H_{48}O_9S_2$: C, 60.72; H, 7.41. Found: C, 60.99; H, 7.46.

Methyl 3α-Acetoxy-7α-(mesyloxy)-12-oxocholanate (23a). Methyl 3α-acetoxy-7α-hydroxy-12-oxocholanate[20] (**21a**, 4.5 g) was mesylated by the general method. The product, after azeotropic distillation in benzene, was a slightly colored oil (5.1 g, 98%) which was homogeneous according to NMR and HPLC but failed to crystallize: IR 1721 (ester C=O), 1704 (C=O), 1250, 1029 (OAc), 1328, 1170 (SO_2), 973,898 cm^{-1} (mesylate); NMR δ 1.04 (6 H, s, C-18 and C-19 Me), 1.99 (3 H, s, OCOMe), 3.00 (3 H, s, SO_2Me) 3.64 (3 H, s, COOMe), 4.56 (1 H, br m, C-3 CHOAc), 4.98 (1 H, m, C-7 CHOMs).

Anal. Calcd for $C_{28}H_{44}O_8S \cdot {}^1/_6C_6H_6$: C, 62.90; H, 8.19. Found: C, 62.89; H, 8. 9.

Methyl 3β-(formyloxy)-7α-(mesyloxy)-12-oxocholanate (30a) was prepared from **29a** (0.65 g) by the general mesylation procedure. After being processed, the product was a light yellow oil which was homogeneous according to TLC, HPLC, and NMR analyses but failed to crystallize (isopropyl ether, final solvent): NMR δ 1.04 (3 H, s, C-18 Me), 1.07 (3 H, s, C-19 Me), 3.00 (3 H, s, SO_2Me), 3.65 (3 H, s, COOMe), 4.98 (1 H, m, C-7 CHOMs), 5.18 (1 H, m, C-3, CHOCHO), 8.05 (1 H, s, OCHO).

Anal. Calcd for $C_{27}H_{42}O_8S \cdot 0.5C_6H_{14}O$: C, 62.90; H, 8.62. Found: C, 63.19; H, 8.27.

Methyl 3-(Cathyloxy)-12-(mesyloxy)cholanate (18a). Methyl 3-(cathyloxy)cholanate (**16a**,[10] 1.28 g), subjected to the general procedure for C-7 mesylation, yielded an amorphous solid which crystallized from methanol [mp 82–110 °C dec; 1.04 g (70%)] and was characterized as a 12-mesylate: NMR δ 0.74 (3 H, s, C-18 Me), 0.88 (3 H, s, C-19 Me), 1.35 (3 H, t, J = 7 Hz, $OCO_2CH_2CH_3$), 3.02 (3 H, s, SO_2Me), 3.62 (3 H, s, COOMe), 3.85 (1 H, m, C-7 CHOH), 4.12 (2 H, q, J = 7 Hz, $OCO_2CH_2CH_3$), 4.45 (1 H, br m, $CHOCO_2Et$), 5.11 (1 H, m, C-12 CHOMs).[1]

Anal. Calcd for $C_{29}H_{48}O_9S \cdot 1.5CH_3OH$: C, 59.00; H, 8.70. Found: C, 58.75; H, 8.39.

KO_2–Crown Ether Inversion Reactions. 3α,7β-Dihydroxy-12-oxocholanic Acid (24). (1) Methyl 3α-(cathyloxy)-7α-(mesyloxy)-12-oxocholanate (**22a**, 3.1 g) dissolved in 20 mL of Me_2SO was gradually added to a solution previously prepared as follows.[19] To powered KO_2[21] (2.0 g) in 250 mL of Me_2SO stirred under N_2 for 10 min was added 1.2 g of 18-crown-6. Stirring under N_2 was continued for 5 min and throughout the subsequent reaction as the solution of **22a** was added. After 3 h, the reaction flask was immersed in an ice bath, and 120 mL of saturated NaCl solution was added to the stirred solution. The aqueous layer, after extraction with benzene (3 × 100 mL) was cooled in an ice bath, acidified with 3 N HCl, and extracted with EtOAc (3 × 100 mL). The EtOAc extract was washed with water, dried (Drierite), and evaporated to an oil which crystallized from EtOAc: 1.52 g (65%); mp 128–131 °C. Recrystallization from aqueous ethanol afforded colorless needles: mp 138.0–139.5 °C; IR 1740, 1670 cm^{-1} (C=O); NMR ($CHCl_3$ + 10% Me_2SO-d_6) δ 1.08 (6 H, s, C-18 and C-19 Me), 3.58 (2 H, br m, C-3 and C-7 CHOH).

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(19) The procedure is slightly modified from the conditions used in ref 9 for the 12-methylene analogue.
(20) J. F. Baker and R. T. Blickenstaff, *J. Org. Chem.*, **40**, 1579 (1975).
(21) Alfa Products, No. 54107.

Anal. Calcd for $C_{24}H_{38}O_5$: C, 70.90; H, 9.42. Found: C, 70.76; H, 9.48.

(2) By the same general inversion method, methyl 3α-acetoxy-7α-(mesyloxy)-12-oxocholanate (**23a**, 3.75 g) was converted to acid **24**, crude yield 2.1 g. Examination by TLC indicated that the product is essentially acid **24** but contains[5] several faster running minor components, probably Δ^3 or Δ^7 olefinic derivatives. Preparation of acid **24** and **23a** thus appears to be less satisfactory than that from **22a**.

Methyl 3α,7β-dihydroxy-12-oxocholanate (24a) was prepared from **24** by the usual MeOH–HCl esterification method. The product, although prepared from crystalline acid, subjected to column chromatography and found to be homogeneous according to HPLC and NMR analyses, resisted crystallization attempts (final solvent, EtOAc): NMR δ 1.04 (6 H, s, C-18 and C-19 Me), 3.56 (2 H, br m, C-3 and C-7 CHOH), 3.64 (3 H, s, COOMe).

Anal. Calcd for $C_{24}H_{40}O_5 \cdot 0.4C_4H_8O_2$: C, 70.08; H, 9.55. Found: C, 70.24; H, 9.67.

3β,7β-Dihydroxy-12-oxocholanic Acid (31). Method 1. By the general inversion reaction, the 3-formate 7-mesylate **30a** (1.0 g) was converted to acid **31**: fine needles from aqueous ethanol; mp 258.0–259.0 °C; 0.36 g (46%); IR (KBr) 1730 (ester C=O), 1672 (COOH), 3636, 3300, 1032, 986, 969, 959 cm^{-1} (OH); NMR ($CDCl_3$ + Me_2SO-d_6 + D_2O) δ 1.05 (6 H, s, C-18 and C-19 Me), 3.49 (1 H, br m, C-7 CHOH), 4.00 (1 H, m, C-3 CHOH).

Anal. Calcd for $C_{24}H_{38}O_5$: C, 70.90; H, 9.42. Found: C, 70.91; H, 9.58.

Method 2. Methyl-3α-(Tosyloxy)-hydroxy-12-oxocholanate (27a) and Methyl 3β,7β-Dihydroxy-12-oxocholanate (31a). 3-Tosylate 7-mesylate **26a** (14 g) was subjected to the inversion reaction and monitored by TLC. After 48 h the product consisted of two major components (TLC). After the usual processing, the mixture (5.48 g) was esterified by methanol–HCl treatment. The methyl ester mixture (5.32 g) was chromatographed on a Florisil column (ratio 50:1). The fractions eluted by benzene–EtOAc (4:1) consisted of an oil which crystallized from acetone as fine prisms [1.33 g (11%); mp 161.0–162.0 °C] and which was characterized as **27a**: IR 1724 (ester C=O), 1701 (C=O), 1351, 1186, 1175, 948, 935, 885 (SO_2), 1018 cm^{-1} (OH); NMR δ 1.02 (6 H, s, C-18 and C-19 Me), 2.44 (3 H, s, ArMe), ~3.5 (1 H, br m, C-7 CHOH), ~4.4 (1 H, br m, C-3 CHOTs), 7.34 and 7.79 (each 2 H, d, J = 8 Hz, para-disubstituted phenyl).

Anal. Calcd for $C_{32}H_{46}O_7S$: C, 66.87; H, 8.07. Found: C, 66.66; H, 8.13.

Contiued elution with benzene–EtOAc (1:1) gave a residual oil which crystallized from acetone as dense crystals in two crops [2.02 g (22%); mp 177.0–178.0 °C] and was characterized as methyl 3β,7β-dihydroxy-12-oxocholanate (**31a**): IR 1733 (ester C=O), 1704 (C=O), 1033, 990, 963 cm^{-1} (OH); NMR δ 1.04 (3 H, s, C-18 Me), 1.07 (3 H, s, C-19 Me), 3.58 (1 H, br m, C-7 CHOH), 3.64 (3 H, s, COOMe), 4.04 (1 H, m, C-3 CHOH).

Anal. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59. Found: C, 71.38; H, 9.73.

The ester **31a**, hydrolyzed in the usual manner by methanolic KOH, acidified, and crystallized from aqueous ethanol, gave fine needles, identical according to melting point, TLC, HPLC, and NMR comparisons with the acid **31** prepared in part 1 above.

Method 3. The 3α-tosylate 7β-hyroxy ester **27a** (1.6 g), under the DMF inversion conditions,[5] yielded the expected product consisting of 3β-formate and a minor amount of olefinic material, as seen by NMR. The crude mixture was left on an alumina column as for **33a** and **36a** for hydrolysis of the formoxy group, and when eluted with benzene–EtOAc (4:1) it gave 0.26 g of an oil which according to NMR had characteristics of methyl 7β-hydroxy-12-oxo-$\Delta^3(\Delta^2)$-cholenate: NMR δ 1.04 (6 H, s, C-18 and C-19 Me), 3.27 (1 H, br m, C-7 CHOH), 5.38–5.75 [1 H, br m, $\Delta^3(\Delta^2)$ H]. Continued elution with EtOAc gave 0.53 g (45%) of a product shown to be identical with the 3β,7β-dihydroxy-12-oxo ester **31a** prepared in part 2 according to HPLC and NMR. Hydrolysis of the product by the usual method yielded the acid **31** which was crystallized from aqueous methanol (mp 258.0–259.0 °C) and found to be identical with the acid prepared in parts 1 and 2: NMR ($CDCl_3$ + 10% Me_2SO-d_6 + D_2O) δ 1.05 (6 H, s, C-18 and C-19 Me), 3.49 (1 H, br, m, C-7 CHOH), 3.99 (1 H, m, C-3 CHOH).

Reductions with $NaBH_4$ and *tert*-Butylamine–Borane.[22] **(1) 3α,7β,12α- (5) and 3α,7β,12β-Trihydroxycholanic Acid (6). (a) With $NaBH_4$.** When 1.2 g of $NaBH_4$ was added to 1.1 g of methyl 3α,7β-dihydroxy-12-oxocholanate (**24a**) in 90 mL of methanol, slight effervescence took place, and the solution soon became clear. After the mixture was allowed to stand overnight and ice–H_2O and a few drops of dilute HCl were added to acidify the solution, the solvent was removed. The residue was dissolved in CH_2Cl_2 and extracted with 10% $NaHCO_3$ solution. The CH_2Cl_2 layer was filtered through phase-separating paper and evaporated to 0.92 g of an oil, which, according to HPLC analysis, consisted essentially of the two stereoisomers **6a** and **5a** in the estimated 12β/12α ratio of 0.55. The oil (0.84 g), chromatographed over neutral alumina (activity II, ratio 50:1), yielded from the benzene–EtOAc (1:1) eluted fractions 0.26 g of an amorphous solid shown by TLC, HPLC, and NMR to be homogeneous and characterized as methyl 3α,7β,12β-trihydroxycholanate (**6a**): IR 1727 (C=O), 3448, 1046, 1010, 952, 937 cm^{-1} (OH); NMR δ 0.76 (3 H, s, C-18 Me), 0.96 (3 H, s, C-19 Me), 3.52 (3 H, br m, C-3, C-7, and C-12 CHOH), 3.64 (3 H, s, COOMe).

Anal. Calcd for $C_{25}H_{42}O_5 \cdot 0.5CH_3OH$: C, 69.82; H, 10.11. Found: C, 69.70; H, 10.08.

Continued elution with EtOAc–MeOH (95:5) gave 0.47 g of an amorphous solid, which was homogeneous by TLC, HPLC, and NMR but which resisted crystallization attempts and was characterized as methyl 3α,7β,12α-trihydroxycholanate (**5a**): NMR δ 0.71 (3 H, s, C-18 Me), 0.93 (3 H, s, C-19 Me), 3.59 (2 H, br m, C-3 and C-7 CHOH), 3.64 (3 H, s, COOMe), 3.98 (1 H, m, C-12 CHOH).

Anal. Calcd for $C_{25}H_{42}O_5 \cdot 0.5EtOAc$: C, 68.99; H, 9.80. Found: C, 69.19; H, 10.10.

(b) **With *tert*-Butylamine–Borane.** To ester **24a** (1.0 g), magnetically stirred in 40 mL of CH_2Cl_2, was added in a nitrogen atmosphere 0.4 g of the borane complex. Stirring was discontinued when the solution became clear. After being allowed to stand 4 h, the CH_2Cl_2 solution was acidified with 3 N HCl and then extracted with 10% $NaHCO_3$ solution. The CH_2Cl_2 layer was processed as in part a to yield 0.97 g of oil, which by HPLC consisted of the same two stereoisomers, **6a** and **5a**, as obtained in part a but in an estimated 12β/12α ratio of 3.7.

By column chromatography as in part a the mixture was resolved into **6a** (0.64 g) and **5a** (0.27 g). (A small intermediate fraction consisting of a mixture of **6a** and **5a** was not included in the weights.) The two stereoisomeric esters were identical with the respective compounds prepared in part a according to TLC, HPLC, and NMR.

3α,7β,12β-Trihydroxycholanic acid (6), obtained by hydrolysis of **6a**, crystallized from aqueous ethanol as colorless needles: mp 191.5–193.0 °C; IR 1701 (C=O), 3636, 3448, 1049, 1010, 954 cm^{-1} (OH); NMR ($CDCl_3$ + 10% Me_2SO + D_2O) δ 0.71 (3 H, s, C-18 Me), 0.92 (3 H, s, C-19 Me), 3.44 (3 H, br m, C-3, C-7, and C-12 CHOH).

Anal. Calcd for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.52; H, 10.06.

3α,7β,12α-Trihydroxycholanic acid (5), similarly obtained from **5a**, crystallized from EtOAc–hexane as colorless needles, mp 127.0–129.0 °C [lit.[6] mp 157–158° dec (from EtOAc-heptane)]. When crystallized according to Samuelsson[6] (MeOH, NaOH, HCl), the poorly crystallized product melted at 128.0–131.0 °C (lit.[6] mp 127–129 °C): IR (KBr) 1704 (C=O), 3448, 1042, 1010, 957 cm^{-1} (OH); NMR ($CDCl_3$ + 10% Me_2SO-d_6 + D_2O) δ 0.69 (3 H, s, C-18 Me), 0.91 (3 H, s, C-19 Me), 3.51 (2 H, br m, C-3 and C-7 CHOH), 3.91 (1 H, m, C-12 CHOH).

Anal. Calcd for $C_{24}H_{40}O_5 \cdot 0.5EtOac$: C, 67.71; H, 9.74. Found: C, 68.05; H, 9.91.

(2) 3β,7β,12α- (7) and 3β,7β,12β-Trihydroxycholanic Acid (8). (a) With $NaBH_4$. Methyl 3β,7β-dihydroxy-12-oxo-cholanate (**31a**, 0.35 g), reduced with $NaBH_4$ in methanol solution and processed as in part 1a, afforded 0.32 g of oil which by HPLC consisted of **8a** and **7a** in the 12β/12α ratio (estimated by HPLC) of 0.70. The oil, chromatographed as in part 1a, gave in the less polar fractions 0.11 g of product and in the more polar fractions 0.17 g of product. Each product was shown to be homogeneous

(22) Aldrich Chemical Co., product No. 18.021-1.

according to HPLC and NMR analyses, but crystallization was successful with neither. The less polar compound was characterized as **methyl 3β,7β,12β-trihydroxycholanate (8a)** [IR 1718 (C=O), 1024, 1000, 955 cm^{-1} (OH); NMR δ 0.76 (3 H, s, C-18 Me), 0.98 (3 H, s, C-19 Me), 3.42 (2 H, br m, C-7 and C-12 CHOH), 3.63 (3 H, s, COOMe), 4.07 (1 H, m, C-3 CHOH).

Anal. Calcd for $C_{25}H_{42}O_5 \cdot 0.5CH_3OH$: C, 69.82; H, 10.11. Found: C, 69.72; H, 10.09], and the more polar was characterized as **methyl 3β,7β,12α-trihydroxycholanate (7a)**: IR 1724 (C=O), 1024, 952 cm^{-1} (OH); NMR δ 0.71 (3 H, s, C-18 Me), 0.96 (3 H, s, C-19 Me), 3.58 (1 H, br m, C-7, CHOH), 3.64 (3 H, s, COOMe), 3.99 (1 H, m, C-12 CHOH), 4.06 (1 H, m, C-3 CHOH).

Anal. Calcd for $C_{25}H_{42}O_5 \cdot 0.5CH_3OH$: C, 69.82; H, 10.11. Found: C, 69.90; H, 10.18.

(b) With *tert*-Butylamine–Borane. Ester **31a** (0.50 g), reduced with the amine–borane complex and processed as in part 1b, yielded 0.46 g of oil which showed by HPLC the same two compounds, **8a** and **7a**, as in part **2a** in the 12β/12α ratio (estimated) of 1.9. Similar column chromatographic separation afforded 0.31 g of **8a** and 0.12 g of **7a**; each was identical with the corresponding product prepared in part 2a according to HPLC and NMR comparisons.

3β,7β,12β-Trihydroxycholanic Acid (8). The trihydroxy ester **8a**, hydrolyzed by the usual method, crystallized from EtOAc containing a small proportion of MeOH as small prisms: mp 164.5–167.0 °C; IR (KBr) 1695 C=O), 3448, 3333, 1020, 1004, 957 cm^{-1} (OH); NMR ($CDCl_3$ + 10% Me_2SO-d_6 + D_2O) δ 0.70 (3 H, s, C-18 Me), 0.94 (3 H, s, C-19 Me), 3.40 (1 H, br m, C-7 and C-12 CHOH), 3.93 (1 H, m, C-3 CHOH).

Anal. Calcd for $C_{24}H_{40}O_5 \cdot 0.5EtOAc$: C, 68.99; H, 9.80. Found: C, 68.88; H, 9.88.

3β,7β,12α-Trihydroxycholanic Acid (7).[23] The trihydroxy ester **7a** was hydrolyzed as above and crystallized from EtOAc: mp 164.5–165.5 °C [lit.[23] mp 154–157 °C (from EtOAc–heptane)]; IR (KBr) 1730 C=O); 3636, 3509, 1031, 1020, 990, 954 cm^{-1} (OH); NMR ($CDCl_3$ + 10% Me_2SO-d_6 + D_2O) δ 0.70 (3 H, s, C-18 Me), 0.94 (3 H, s, C-19 Me), 3.56 (1 H, br m, C-7 CHOH), 3.96 (2 H, m, C-3 and C-12 CHOH).

Anal. Calcd for $C_{24}H_{40}O_5 \cdot 0.5EtOAc$: C, 68.99; H, 9.80. Found: C, 68.99; H, 9.99.

(3) Methyl 3β,7α,12β- (4a)[5] and 3β,7α,12α-Trihydroxycholanate (3a)[5] with *tert*-Butylamine–Borane. The keto ester **36a** (1.0 g), reduced as in parts 1b and 2b, yielded an oil (1.0 g) which HPLC indicated to be a mixture of **4a** and **3a** in the 12β/12α ratio (estimated) of 5.0. The oil, on standing in a small volume of EtOAc, gradually gave minute prisms of **3a** (0.12 g) found to be identical by melting point, HPLC, and NMR comparisons with authentic 3β,7α,12α-trihydroxy ester **3a**.[5] The mother liquor contained the major product which by column chromatography with alumina as in parts 1b and 2b or with Florisil[5] yielded in the CH_2Cl_2–MeOH (98:2) cleanly eluted fractions 0.75 g of homogeneous (HPLC, NMR) but amorphous material identical with the previously prepared and characterized ester **4a**,[5] according to TLC, HPLC, and NMR. Further elution with CH_2Cl_2–MeOH (95:5) gave a negligible amount of **3a**. As contrasted with the finding in the Raney nickel reduction[5] of ester **36a**, no 3α-hydroxy analogues were detected (HPLC).

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Registry No. **1a**, 1448-36-8; **2a**, 71883-63-1; **3a**, 28050-54-6; **4a**, 71883-65-3; **5**, 2955-27-3; **5a**, 28050-56-8; **6**, 81938-67-2; **6a**, 81702-92-3; **7**, 10322-18-6; **7a**, 81702-94-5; **8**, 81873-90-7; **8a**, 81702-93-4; **16a**, 21059-36-9; **18a**, 81847-01-0; **19a**, 10538-64-4; **20a**, 81847-02-1; **21a**, 54852-57-2; **22a**, 81847-03-2; **23a**, 81847-04-3; **24**, 81873-91-8; **24a**, 81655-85-8; **25a**, 81847-05-4; **26a**, 81847-06-5; **27**, 81847-07-6; **27a**, 81847-08-7; **28a**, 71837-86-0; **29a**, 81847-09-8; **30a**, 81847-10-1; **31**, 81873-92-9; **31a**, 81644-37-3; **33**, 79732-73-3; **33a**, 81644-40-8; **33a** 3-formate, 81847-11-2; **34a**, 7727-82-4; **36a**, 81847-12-3; **36a** formate, 81847-13-4; **37a**, 42921-40-4; methyl 7β-hydroxy-12-oxo-Δ^3-cholenate, 81847-14-5; methyl 7β-hydroxy-12-oxo-Δ^2-cholenate, 81847-15-6.

(23) The 3β,7β,12α compound (7) obtained by Na–propanol reduction of methyl 3,7-dioxo-12α-hydroxycholanate in low yield was characterized by melting point and α_D (P. Eneroth, B. Gordon, and J. Sjovall, *J. Lipid Res.* 7, 524 (1966).

Solid-State Photooxidation of 21-Cortisol *tert*-Butylacetate to 21-Cortisone *tert*-Butylacetate

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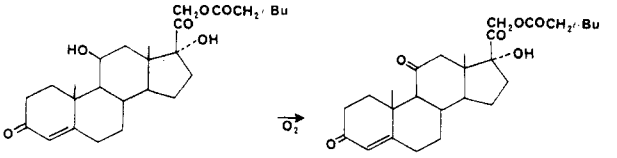
Five polymorphs of 21-cortisol *tert*-butylacetate have been prepared. Two of these forms are reactive. The hexagonal form (form I) that crystallizes as a nonstoichiometric solvate from ethanol oxidizes to 21-cortisone *tert*-butylacetate when exposed to ultraviolet light in air. A second form that crystallizes from pyridine is also reactive. Three other polymorphs are unreactive upon exposure to UV light and air. The crystal structure of the hexagonal form has been determined. The crystals belong to space group $P6_1$ with $a = b = 17.485$ Å, $c = 15.376$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$. The crystal packing of this form has steroid molecules held together by hydrogen bonding arranged in a helix along the 6_1 axis. A channel runs through the center of this helix along the 6_1 axis. The oxidation of crystals of form I is hypothesized to occur because of easy penetration of oxygen into the crystal along this channel. In addition, the crystals of the hexagonal form containing ethanol lose solvent without extensive disordering of the crystal or transformation to a new crystal form.

Introduction

The factors that render crystalline drugs reactive or unreactive are of great importance both from a fundamental and a practical standpoint.[1-4] Knowledge of these factors could lead to an improvement in our understanding of how chemical reactions occur[1-3] and to the development

Scheme I



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