# Selective reduction of oxo bile acids: synthesis of 3β-, 7β-, and 12β-hydroxy bile acids

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**Abstract** Preparation of some biologically important keto bile acids is described. Advantage is taken of the preferential ketalization of 3-oxo group in bile acids over 7- and 12-oxo groups for the selective reduction of these keto groups. The method was found to be specially useful for preparation of 7β-, 12α-, and 12β-[$^3$H]-3-oxo bile acids. Improved methods are also described for the preparation of epimers of naturally occurring bile acids at C-3, C-7, and C-12. 3β-Hydroxy bile acids (iso-bile acids) were prepared with the use of diethylazodicarboxylate/triphenylphosphine/formic acid. Iso-bile acids were obtained in excellent yields (80–95%) except during synthesis of isoursodeoxycholic acid (yield, 50%). Isoursodeoxycholic acid was, however, prepared in very good yield via epimerization of 3α-hydroxyl group in 7-oxolithocholic acid followed by stereoselective reduction of 7-oxo group. A highly efficient method for the reduction of 7-oxo and 12-oxo groups was developed. Thus, 7-oxolithocholic acid and 7-oxoisolithocholic acid on reduction with potassium/tertiary amyl alcohol yielded ursodeoxycholic acid and isoursodeoxycholic acid in yields of 96% and 94%, respectively, while reduction of 7-oxodeoxycholic acid resulted in ursocholic acid in 93% yield. In a similar manner, reduction of 12-oxolithocholic acid and 12-oxochenodeoxycholic acid yielded 3α,12β-dihydroxy-5β-cholanoic acid (lagodeoxycholic acid; 92% yield) and 3α,7α,12β-trihydroxy-5β-cholanoic acid (lagocholic acid; 86% yield). —**Batta, A. K., S. K. Aggarwal, G. Salen, and S. Shefer.** Selective reduction of oxo bile acids: synthesis of 3β-, 7β-, and 12β-hydroxy bile acids. *J. Lipid Res.* 1991. **32:** 977–983.

**Supplementary key words** bile acids • ursodeoxycholic acid • iso-bile acids • lagodeoxycholic acid • lagocholic acid

During their intestinal transit, a portion of bile acids is exposed to the colonic bacteria where, among other modifications, bile acids are oxidized at C-3, C-7, and C-12 positions to form various oxo bile acids. These oxo compounds are further reduced to the epimeric hydroxy compounds, thus giving rise to a variety of secondary bile acids (1). The secondary bile acids are brought into enterohepatic circulation via intestinal absorption and can be further modified by hepatic enzymes. Thus, it has been shown that 7-oxolithocholic acid is effectively converted into chenodeoxycholic acid (2) and iso-bile acids are transformed back into 3α-hydroxy bile acids (3). However, the 7β-hydroxy bile acids are not epimerized in the liver. Recently, several oxo bile acids have been reported in the serum and urine of patients with cholestatic liver disease (4). In order to study the metabolism of these intestinal products of bile acids, we required the synthesis of some of these compounds to be used as substrates or as reference standards for characterization purposes. In this report, we describe convenient syntheses of several oxo bile acids and various epimers of naturally occurring bile acids.

## EXPERIMENTAL

### Materials

Cholic acid, deoxycholic acid, and lithocholic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI). Chenodeoxycholic acid was purchased from Canada Packers (Toronto, Canada); ursodeoxycholic acid was a gift from Tokyo Tanabe, Japan. All other chemicals were reagent grade and were purchased from Aldrich Chemical Co. and solvents were from Fisher Scientific Co. (Springfield, NJ).

3,7-Dioxo- and 3,12-dioxo-5β-cholanoic acids were prepared by oxidation of chenodeoxycholic acid and deoxy-

Abbreviations: lithocholic acid, 3α-hydroxy-5β-cholanoic acid; isolithocholic acid, 3β-hydroxy-5β-cholanoic acid; 7-oxolithocholic acid, 3α-hydroxy-7-oxo-5β-cholanoic acid; 12-oxolithocholic acid, 3α-hydroxy-12-oxo-5β-cholanoic acid; 7-oxoisolithocholic acid, 3β-hydroxy-7-oxo-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; ursodeoxycholic acid, 3α,7β-dihydroxy-5β-cholanoic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid; isochenodeoxycholic acid, 3β,7α-dihydroxy-5β-cholanoic acid; isoursodeoxycholic acid, 3β,7β-dihydroxy-5β-cholanoic acid; lagodeoxycholic acid, 3α,12β-dihydroxy-5β-cholanoic acid; 7-oxodeoxycholic acid, 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid; 12-oxochenodeoxycholic acid, 3α,7α-dihydroxy-12-oxo-5β-cholanoic acid; cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; lagocholic acid, 3α,7α,12β-trihydroxy-5β-cholanoic acid; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; MS, mass spectrometry.

TABLE 1. Physical characteristics of bile acids

| Bile Acid | TLC | GLC[a] | Melting Point |
|---|---|---|---|
| | | | °C |
| Isolithocholic acid | 0.64[b] | 1.33 | 176–177 |
| Iso-$\Delta^6$lithocholic acid | 0.64[b] | 1.27 | 162–163 |
| Lithocholic acid | 0.62[b] | 1.34 | 184–186[c] |
| 7-Oxo-isolithocholic acid | 0.35[b] | 1.66 | 247–248 |
| 12-Oxo-isolithocholic acid | 0.43[b] | 1.68 | 218–220 |
| Isoursodeoxycholic acid | 0.56[d,e] | 1.55[f] | 166–167 |
| Isochenodeoxycholic acid | 0.57[d] | 1.46 | 193[c] |
| Ursodeoxycholic acid | 0.56[d,e] | 1.55[f] | 203–204 |
| Chenodeoxycholic acid | 0.51[d] | 1.48 | 145–146[c] |
| Ursocholic acid | 0.24[d] | 1.59 | 127–129 |
| Cholic acid | 0.18[d] | 1.51 | 196–198[c] |
| Deoxycholic acid | 0.53[d] | 1.44 | 176–177[c] |
| 12$\beta$-Hydroxylithocholic acid | 0.64[d] | 1.38 | 172–174 |
| 12$\beta$-Hydroxychenodeoxycholic acid | 0.22[d] | 1.47 | 197–198 |

[a]A fused silica CP-Sil-5 CB capillary column (20 m × 0.22 mm) was used for GLC. Chromatographic conditions: injector temperature, 260°C; detector temperature, 290°C; column temperature, 100°C for 2 min, then programmed at 35°C/min to a final temperature of 265°C. Helium was used as carrier gas at a flow rate of 1.5 ml/min. Retention times are given relative to that of 5$\alpha$-cholestane. Retention time of 5$\alpha$-cholestane, 13.39 min.

[b]Solvent system: hexane–ethyl acetate–acetic acid 10:15:0.2 (v/v) (solvent system A).

[c]Literature melting point (27).

[d]Solvent system: chloroform–methanol–acetic acid 30:3:1.5 (v/v) (solvent system B).

[e]Ursodeoxycholic acid and isoursodeoxycholic acid were resolved when the plate was developed three times in solvent system A. $R_f$, ursodeoxycholic acid, 0.48, and isoursodeoxycholic acid, 0.51.

[f]Relative retention times for isoursodeoxycholic acid and ursodeoxycholic acid were 1.80 and 1.82, respectively, when injected into a fused silica CP-Sil-19 CB capillary column (25 m × 0.22 mm) under identical chromatographic conditions as above. When this column was used, the retention time of 5$\alpha$-cholestane was 11.56 min.

cholic acid, respectively, with Jones' reagent (prepared by adding 2.2 g chromium trioxide to 2.8 ml concentrated sulfuric acid and diluting to 10 ml with water) followed by crystallization. 7-Oxolithocholic acid and 12-oxolithocholic acid were prepared from chenodeoxycholic acid and deoxycholic acid, respectively, via formation of 3$\alpha$-hemisuccinates [refluxing with succinic anhydride in carbon tetrachloride/pyridine (5)]. The hemisuccinates were then oxidized with Jones' reagent followed by alkaline hydrolysis. The 7- and 12-oxo compounds obtained were pure as judged by thin-layer chromatography (TLC) and were obtained in above 90% isolated yield.

The oxidation of cholic acid with N-bromosuccinimide as described by Fieser and Rajagopalan (6) yielded impure 7-oxo-3$\alpha$,12$\alpha$-dihydroxy-5$\beta$-cholanoic acid in less than 40% yield and the pure compound could be obtained only after elaborate column chromatography. This compound was prepared in over 80% isolated yield by the following modified route. Cholic acid (4 g) was refluxed for 3 h with succinic anhydride (2.5 g) in carbon tetrachloride (25 ml) containing pyridine (5 ml). Solvents were evaporated under reduced pressure and the residue was taken up in ethyl acetate and washed with water. Evaporation of ethyl acetate yielded 4.5 g of 3$\alpha$-hemisuccinate-7$\alpha$,12$\alpha$-dihydroxy-5$\beta$-cholanoic acid as a colorless solid. The 3$\alpha$-hemisuccinate (4.5 g) was dissolved in 80 ml of 3% sodium bicarbonate and 4.0 g N-bromosuccinimide was slowly added with stirring, over a period of 15 min at 70°C. After 1 h at 70°C, the contents were cooled to room temperature and stirring was continued for 18 h. Temperature was then raised to 70°C for 1 h, and finally the reaction mixture was left overnight at room temperature. The reaction mixture was then acidified with 25% hydrochloric acid and the colorless solid obtained was filtered, washed with water, and hydrolyzed with 5% methanolic sodium hydroxide to yield 3.6 g of a colorless solid which showed one major spot on TLC. Crystallization from ethyl acetate yielded pure 7-oxo-3$\alpha$,12$\alpha$-dihydroxy-5$\beta$-cholanoic acid. 3$\alpha$,7$\alpha$-Dihydroxy-12-oxo-5$\beta$-cholanoic acid was prepared from cholic acid via oxidation of 3$\alpha$,7$\alpha$-diacetoxy-12$\alpha$-hydroxy-5$\beta$-cholanoic acid as described by Fieser and Rajagopalan (7). Melting points were determined on a Thermolyne model MP-12600, and are uncorrected.

### Thin-layer chromatography (TLC)

The TLC of the various bile acids was performed on silica gel O plates (Analabs, New Haven, CT) in a solvent system of hexane–ethyl acetate–acetic acid 10:15:0.2 (v/v/v) (solvent system A) for monohydroxy and monohydroxy-oxo bile acids (8). A solvent system of chloroform–methanol–acetic acid 30:3:1.5 (v/v/v) (solvent system B) was used for TLC of other bile acids used in this study (9). Spots were visualized by spraying the plate with 10% $H_2SO_4$ followed by a solution of 3.5% phosphomolybdic acid and then heating at 110°C for 2 min.

### Gas–liquid chromatography (GLC)

The various bile acids and their oxo derivatives were methylated with 3% methanolic HCl and the methyl esters were silylated with 100 $\mu$l of Sil-Prep (Alltech Associates, Inc., Deerfield, IL) for 30 min at 55°C. After evaporation of solvents under $N_2$, the silyl ethers were dissolved in 100 $\mu$l of hexane and 1–5 $\mu$l was injected into a Hewlett-Packard model 5890A gas chromatograph equipped with a split/splitless device for capillary column. A fused silica CP-Sil-5 CB capillary column (20 m; i.d., 0.20–0.22 mm) was used and helium was used as carrier gas. The GLC operating conditions were as follows: injector and detector temperatures were 265°C and 290°C, respectively. After injection, oven temperature was kept at 100°C for 2 min, then programmed at a rate of 35°C/min to a final temperature of 280°C. The retention times of some of the synthesized bile acid derivatives are given in **Table 1.**

TABLE 2. Yields of synthesized bile acids

| Bile Acid | Yield | Literature Yield[a] |
|---|---|---|
| | % | % |
| Isolithocholic acid[b] | 95[c] | 90 (12) |
| Iso-$\Delta^6$lithocholic acid[b] | 95[c] | 84 (19) |
| 7-Oxo-isolithocholic acid[b] | 85[c] | 85 (12) |
| 12-Oxo-isolithocholic acid[b] | 80[c] | 80 (12) |
| Isoursodeoxycholic acid[d] | 94[e] | 79 (18)[f] |
| Ursodeoxycholic acid[d] | 96[e] | 48 (18)[g] |
| Ursocholic acid[d] | 93[e] | 33 (18)[h] |
| 12$\beta$-Hydroxylithocholic acid[d] | 92[e] | 64 (21) |
| 12$\beta$-Hydroxychenodeoxycholic acid[d] | 86[e] | 64 (21) |

[a]Literature reference in parentheses.
[b]Synthesized via epimerization of 3$\alpha$-hydroxyl group.
[c]Isolated yield. Yield is based on the corresponding 3$\alpha$-hydroxy compound that was used for epimerization.
[d]Synthesized via reduction of 7- or 12-oxo bile acid with potassium/tert. amyl alcohol.
[e]Yield estimated by GLC analysis of the reaction product. Yield is based on the corresponding 7- or 12-oxo bile acid that was used for reduction with potassium/tert. amyl alcohol.
[f]The reported yield was based on methyl 3$\beta$-acetoxy-7$\alpha$-mesyloxy-5$\beta$-cholanoate as the starting material.
[g]The reported yield was based on methyl chenodeoxycholate as the starting material.
[h]The yield reported is approximate and was calculated from ref. 18 with methyl 3$\alpha$-cathyloxy-7$\alpha$-mesyloxy-12-oxo-5$\beta$-cholanoate as the starting material.

### Gas-liquid chromatography-mass spectrometry (GLC-MS)

When required, the GLC-MS of the bile acids and their derivatives was performed on a Hewlett-Packard model 5988 capillary gas-liquid chromatograph-mass spectrometer (Paramus, NJ) operating in the electron impact mode with an energy of 70 electron volts (10). The GLC operating conditions were identical to those described above.

### Selective reduction of oxo bile acids: preparation of 3-oxo bile acids

3,7-Dioxo- or 3,12-dioxo-5$\beta$-cholanoic acid (0.1 mmol) was dissolved in 0.2 M methanolic solution of europium chloride hexahydrate (0.5 ml) in a screw-cap tube and 75 mg of trimethylorthoformate was added (11). The solution was cooled (0°C to −15°C) for 15 min and then 10 mg of sodium borohydride was added. After 15 min, the solution was acidified to pH 2 with 1 N hydrochloric acid and the contents were shaken for 30 min. Water (2 ml) was then added and the white precipitate was collected (yield, 36 mg). The product showed one major spot on TLC and was of the order of 90% pure as judged by GLC.

### Preparation of [7$\beta$-$^3$H]7$\alpha$-hydroxy-3-oxo-5$\beta$-cholanoic acid

3,7-Dioxo-5$\beta$-cholanoic acid (5 mg) was dissolved in 0.2 ml of a methanolic solution of europium chloride hexahydrate (0.2 M) in a small screw-cap tube and the solution was cooled to 0°C to −15°C. Ten milligrams of trimethylorthoformate was then added and after 15 min 1 mg of sodium borotritiide (50 mCi) was added. After 15 min more, 2 mg of sodium borohydride was added and the contents were kept at 0°C for 1 h. Hydrochloric acid (1 N, 0.1 ml) was then added and the contents were shaken for 30 min at room temperature. Water (0.5 ml) was added and the precipitate obtained was filtered, washed with water, and dried; yield, 5 mg; TLC, one major spot; $R_f$, 0.38 (solvent system, A); sp act, 102.5 $\mu$Ci.

### Preparation of 3$\beta$-hydroxy bile acids

In a typical reaction, diethylazodicarboxylate (0.65 ml) was dissolved in anhydrous benzene (0.5 ml) and added dropwise to a solution of the methyl ester of 3$\alpha$-hydroxy bile acid (or 7-oxo-3$\alpha$-hydroxy- or 12-oxo-3$\alpha$-hydroxy bile acid) (0.5 g) and triphenylphosphine (1.0 g) in 5 ml benzene containing 0.2 ml 98% formic acid (12). The resulting pale yellow solution was refluxed for 48 h and then cooled to room temperature when a white solid crystallized out. The solid was filtered off and washed with a small amount of benzene and the combined mother liquors were concentrated to obtain a pale yellow solid. The solid was dissolved in anhydrous ether (3 ml) and poured onto 20 ml hexane. The white solid formed was again filtered off and washed with hexane and the mother liquors were concentrated. The residue was dissolved in 10 ml 5% methanolic potassium hydroxide and refluxed for 3 h. Water (10 ml) was added and the contents were concentrated to half the volume at 40°C. The solution was cooled on ice and acidified with dilute hydrochloric acid. The resulting white solid was collected, washed with water to neutrality, and dried. The product was crystallized from a chloroform-methanol mixture; yield 80–95% (**Table 2**). Products were usually pure as judged by thin-layer chromatography and gas-liquid chromatography, and appreciable amounts of the unreacted material were observed only during the synthesis of isoursodeoxycholic acid from ursodeoxycholic acid where up to 50% ursodeoxycholic acid remained unreacted. The 3$\beta$-hydroxy compounds obtained were characterized by a combination of their melting points, TLC, GLC (Table 1), and mass spectral fragmentation patterns (**Table 3**).

### Preparation of 7$\beta$- and 12$\beta$-hydroxy bile acids

*Ursodeoxycholic acid and ursocholic acid.* To a boiling solution of 7-oxolithocholic acid (1 g) in tert. amyl alcohol (25 ml) was added 0.9 g of potassium metal over a period of 10 min. The solution was refluxed for 30 min, cooled to room temperature, and diluted with 25 ml water. The solution was concentrated to half its volume under reduced pressure at 40°C, cooled on ice, and acidified with dilute hydrochloric acid. The white solid formed was

TABLE 3. Mass spectral fragmentation pattern of some synthesized 3β-, 7β-, and 12β-hydroxy bile acids

| Bile Acid | Characteristic Mass Ion Fragments (m/z; %)[a] | | | | |
|---|---|---|---|---|---|
| | $M^+-15$ | $M^+-90$ | $M^+-2\times90$ | $M^+-3\times90$ | Others |
| Isochenodeoxycholic acid | | 460 (0.2)[b] | 370 (100) | | 355 (24), 255 (21), 73 (13) |
| Isoursodeoxycholic acid | 545 (1) | 460 (37) | 370 (100) | | 355 (42), 255 (27), 73 (32) |
| Ursodeoxycholic acid | 535 (3) | 460 (100) | 370 (63) | | 355 (38), 255 (47), 73 (31) |
| Ursocholic acid | 623 (1) | 548 (3) | 458 (14) | 368 (17) | 433 (7), 343 (26), 253 (100), 73 (35) |
| 12β-Hydroxylithocholic acid | 535 (8) | 460 (4) | 370 (25) | | 355 (13), 345 (18), 255 (100), 73 (17) |
| 12β-Hydroxychenodeoxycholic acid | 623 (7) | 548 (1) | 458 (48) | 368 (25) | 343 (76), 253 (100), 73 (50) |

[a]Mass spectra of the various bile acids were performed on a capillary gas chromatograph–mass spectrometer (electron impact mode) with an energy of 70 electron volts. The GLC conditions were identical with those reported in Table 1.
[b]Values in parentheses represent percentages of the various fragment ions relative to the base-ion fragment.

filtered, washed with water to neutrality, and dried; 0.9 g of product was obtained. The product showed one major spot on thin-layer chromatography ($R_f$, 0.56; solvent system B) while on gas–liquid chromatography two peaks were observed in a ratio of 96:4 with relative retention times (1.55 and 1.48; Table 1) identical with those for ursodeoxycholic acid and chenodeoxycholic acid, respectively. Crystallization from ethyl acetate yielded 0.85 g of pure ursodeoxycholic acid (**Fig. 1**), melting point and mixed melting point with standard sample, 203–204°C. Similar reduction of 7-oxodeoxycholic acid (1 g) in tert. amyl alcohol with potassium yielded 0.9 g of a white product that showed one major spot on TLC ($R_f$, 0.24, solvent system B). The GLC analysis of this product showed the presence of ursocholic acid and cholic acid in a ratio of 93:7 (relative retention times, 1.59 and 1.51, respectively; Table 1). The product was crystallized twice from ethyl acetate to obtain 0.8 g of chromatographically pure ursocholic acid, melting point, 127–129°C, undepressed when admixed with standard sample of ursocholic acid.

*3β-Hydroxy-7-isoursodeoxycholic acid.* 3β-Hydroxy-7-oxo-5β-cholanoic acid (Fig. 1. IV; 0.5 g) in refluxing tert. amyl alcohol (15 ml) was reduced with potassium (0.5 g) for 30 min and the product was isolated as described above (480 mg); TLC (solvent system B), one major spot; $R_f$, 0.56. The product showed two peaks on GLC. The major component (94%) had a retention time identical with that of standard isoursodeoxycholic acid on both CP-Sil-5 CB and CP-Sil-19 CB capillary columns (Table 1) and its mass spectral fragmentation pattern (Table 3) was compatible with the structure. The minor component (6%) was confirmed as isochenodeoxycholic acid (GLC and GC–MS). Two crystallizations of the crude product from ethyl acetate yielded 0.42 g of pure isoursodeoxycholic acid (Fig. 1. VI) melting at 166°–167°C, literature melting point, 166°–168.5°C (13). The structure of the compound was confirmed from study of its mass spectral fragmentation pattern (Table 3).

*Lagodeoxycholic acid and lagocholic acid.* Reduction of 12-oxolithocholic acid (1 g) with tert. amyl alcohol/potassium as described above produced 0.9 g of a mixture of lagodeoxycholic acid (3α,12β-dihydroxy-5β-cholanoic acid) and deoxycholic acid (92:8 as shown by GLC). Pure lagodeoxycholic acid was obtained by crystallization from ethyl acetate; yield, 0.77 g [melting point, 172°–174°C, literature melting point, 172.5°–173.6°C (14)]. Reduction of 12-oxochenodeoxycholic acid under the above conditions yielded 0.88 g of a mixture of lagocholic acid (3α,7α,12β-trihydroxy-5β-cholanoic acid) and cholic acid in a ratio of 86:14 (GLC) from which 0.68 g of pure lagocholic acid was obtained after two crystallizations from ethyl acetate [melting point, 197°–198°C, literature melting point, 200°–201.5°C (15)]. The mass spectral fragmentation patterns of the two compounds are given in Table 3.

## RESULTS AND DISCUSSION

The hydroxyl groups at C-3, C-7, and C-12 positions in the bile acids are of unequal reactivity and specific oxidation and acylation are possible (7). Reduction of these oxo compounds with sodium borohydride usually yields bile acids with an equatorial hydroxyl group while sodium/alcohol reduction or catalytic hydrogenation results predominantly in bile acids with an axial hydroxyl group. Thus, all possible isomers of chenodeoxycholic acid, deoxycholic acid, and cholic acid have been reported in literature (13–15). However, in most cases, mixtures of epimeric bile acids are obtained and pure compounds are obtained only after extensive column chromatography. We report herein simple routes to the synthesis of some of the biologically important oxo bile acids and epimers of naturally occurring bile acids.

3-Oxo hydroxy bile acids have been prepared in good yields by selective reduction of hydroxyl group at C-3 with silver carbonate/Celite (16, 17). However, the method is quite cumbersome for preparation of radiolabeled 3-oxo bile acids where microgram quantities of reactants are used. Gemal and Luche (11) reported the use of rare-earth chlorides for the preferential in situ ketalization of a less hindered oxo group in presence of a more hindered oxo

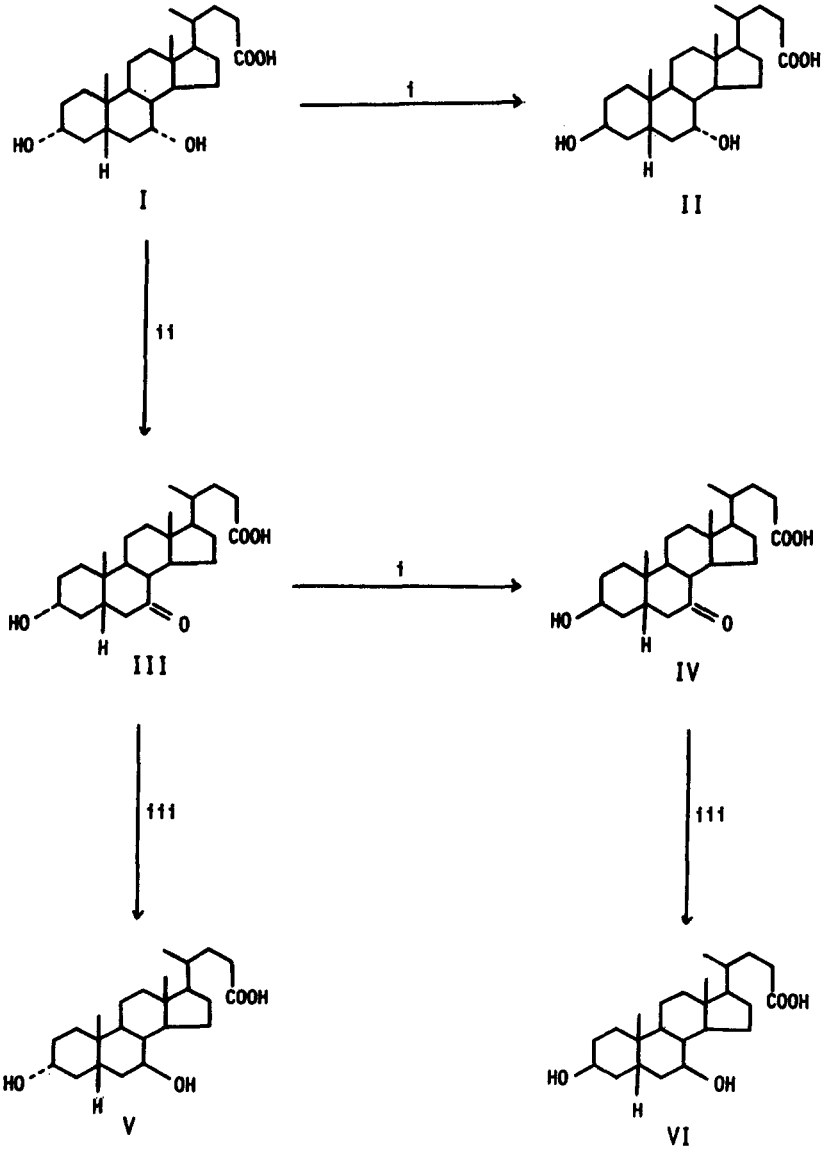


**Fig. 1.** Synthesis of some iso-bile acids. I, Chenodeoxycholic acid; II, isochenodeoxycholic acid; III, 7-oxolithocholic acid; IV, 7-oxoisolithocholic acid; V, ursodeoxycholic acid; VI, isoursodeoxycholic acid. i, Diethylazodicarboxylate/triphenylphosphine/formic acid; ii, $CrO_3$ oxidation on 3$\alpha$-hemisuccinate; iii, potassium/tert. amyl alcohol.

group. We used europium chloride hexahydrate for selective ketalization of an oxo group at C-3 in presence of oxo groups at C-7 and C-12 in bile acids. In this way, we could successfully prepare 3-oxo,7$\alpha$-hydroxy-5$\beta$-cholan-24-oic acid and 3-oxo,12($\alpha$ and $\beta$)-hydroxy-5$\beta$-cholan-24-oic acid by ketalization of 3-oxo group in 3,7-dioxo-5$\beta$-cholan-24-oic acid (or 3,12-dioxo-5$\beta$-cholan-24-oic acid), reduction of the unprotected oxo group with sodium borohydride, followed by deketalization with mineral acid. Reduction of the 12-oxo group with sodium borohydride resulted in a mixture of the 12$\alpha$- and 12$\beta$-hydroxy epimers, which on chromatographic separation yielded 3-oxo,12$\alpha$-hydroxy-5$\beta$-cholan-24-oic acid and 3-oxo,12$\beta$-hydroxy-5$\beta$-cholan-24-oic acid in a ratio of 2:1. The reactions could be performed in the same flask without the need to isolate intermediates and with the use of tritium-labeled sodium borohydride, 3-oxo bile acids specifically labeled with tritium at C-7 or C-12 could be prepared. Ketalization was usually complete at 0°C to −15°C, while incomplete ketalization resulted at ambient temperatures. Overall yields were usually of the order of 80% and only occasionally small amounts of the ketal accompanied the final product, probably due to the formation of heterogeneous medium during deketalization. However, since the ketal was quite easily resolved from the sodium borohydride reduction product by TLC, the method was

found to be specially useful for preparation of specifically tritium-labeled 3-oxo bile acids. On the other hand, reduction of the 3-oxo group with sodium borotritiide would yield 3α-tritiated iso bile acids.

For preparation of the epimeric bile acids on a preparation scale, literature methods were modified. Thus, it was found that 7-oxolithocholic acid and 12-oxolithocholic acid could be prepared in over 90% isolated yields via protection of the hydroxyl group at C-3 as hemisuccinate and then oxidation of the hydroxyl group at C-7 or C-12 with Jones' reagent. Oxidation of cholic acid or its 3α-cathylate with N-bromosuccinimide (6, 18) yielded mixtures from which pure 7-oxodeoxycholic acid could be obtained by chromatography in less than 40% isolated yield. However, protection of hydroxyl group at C-3 as hemisuccinate followed by careful N-bromosuccinimide oxidation consistently produced 7-oxodeoxycholic acid in almost 80% isolated yield and the pure compound could be obtained without resorting to chromatography.

Isolithocholic acid, isochenodeoxycholic acid, and isodeoxycholic acid could be prepared in good to excellent yields according to the method of Bose et al. (12). Less than 20% of the 3α-hydroxy epimer remained unreacted and both isolithocholic acid and iso-$\Delta^6$-lithocholic acid were prepared in near quantitative yields. However, in an attempt to prepare isoursodeoxycholic acid by inversion of 3α-hydroxyl group in ursodeoxycholic acid, as much as 50% of ursodeoxycholic acid remained unreacted. The pure iso compound could not be obtained by column chromatography and was always contaminated with ursodeoxycholic acid. Iida, Taneja, and Chang (18) prepared this compound in 79% yield by potassium superoxide-18 Crown ether inversion reaction on methyl 3β-acetoxy-7α-mesyloxy-5β-cholanoate (prepared from 3β-acetate of isochenodeoxycholic acid). However, both isochenodeoxycholic acid and its 3β-acetate had to be synthesized from chenodeoxycholic acid and were obtained only in moderate yields. We found that although the inversion of 3α-hydroxyl group in ursodeoxycholic acid was largely incomplete, 7-oxolithocholic acid could be effectively epimerized into 7-oxo-isolithocholic acid (Fig. 1. IV). The latter compound on reduction with potassium in tert. amyl alcohol (19) yielded isoursodeoxycholic acid (VI) and isochenodeoxycholic acid in a ratio of 94:6 from which isoursodeoxycholic acid was easily obtained by crystallization. The overall yield of isoursodeoxycholic acid starting from chenodeoxycholic acid was found to be above 70% in three consecutive preparations.

Ursodeoxycholic acid is increasingly being used for dissolution of cholesterol gallstones (20). This bile acid is prepared from its 7α-hydroxy epimer, chenodeoxycholic acid, via either direct inversion of the 7α-hydroxyl group (9, 18) or reduction of 7-oxolithocholic acid with sodium/alcohol. However, ursodeoxycholic acid prepared in this way is always accompanied by chenodeoxycholic acid and it can be obtained in pure form only after repeated crystallization. Potassium/tert. amyl alcohol reduction was found to be an excellent method for the reduction of 7-oxolithocholic acid to ursodeoxycholic acid. Ursodeoxycholic acid and chenodeoxycholic acid were obtained in a ratio of 96:4 and pure ursodeoxycholic acid was obtained easily by crystallization. Similar success was obtained in the synthesis of ursocholic acid from 7-oxodeoxycholic acid (7β:α hydroxy epimers, 93.7). The synthesis of this bile acid was reported by Iida and Chang (21) via the inversion of methyl 3-cathyloxy-7-hydroxy-12-oxo-5β-cholanoate with potassium superoxide-Crown ether followed by sodium borohydride reduction of the 12-oxo group. The 12α- and 12β-hydroxy compounds were then chromatographically separated. The authors have reported that the method was cumbersome and the yields were low.

The preferred route for the preparation of 12β-hydroxy bile acids is by reduction of the methyl esters of the corresponding 12-oxo derivatives with borane–tert. butylamine complex (21, 22). Following this method, Iida and Chang (21, 22) have reported the 12β:12α ratios ranging from 3:2 to 5:1 in the reduction products of several 12-oxo bile acids. We found that the 12β-hydroxy bile acids could be obtained in excellent yield by potassium/tert. amyl alcohol reduction of the corresponding 12-oxo derivatives. Lagodeoxycholic acid was obtained in 92% yield from 12-oxolithocholic acid with only 8% deoxycholic acid while reduction of 12-oxochenodeoxycholic acid produced lagocholic acid and cholic acid in a ratio of 86:14 (Table 2). The structures of lagodeoxycholic acid and lagocholic acid were confirmed from a study of their mass spectral fragmentation pattern.

Thus, a simple method is reported for the preparation of tritium-labeled 3-oxo (and 3β-hydroxy) bile acids. Although unlabeled 3-oxo bile acids can be prepared in satisfactory yields using the method of Fetizon and Golfier (16), the current one-pot synthesis has distinct advantage for preparation of radiolabeled 3-oxo and 3β-hydroxy bile acids since microgram quantities of compounds can be used and the products can be easily isolated (precipitation with water). Improved yield has been obtained for the preparation of 7-oxodeoxycholic acid from cholic acid (6) by N-bromosuccinimide oxidation of 3α-hemisuccinate of cholic acid under controlled conditions. In addition, we have investigated the use of potassium/tert. amyl alcohol for reduction of 7-oxo and 12-oxo bile acids. This has resulted in improved yields for preparation of several biologically important oxo bile acids and epimers of naturally occurring bile acids. Ursodeoxycholic acid, a proven gallstone solubilizing agent, is generally prepared by reduction of 7-oxolithocholic acid with sodium/isopropanol. However, in this way, as much as 33% of the corresponding 7α-hydroxy epimer, chenodeoxycholic acid, is also formed. We have found that reduction with potassium/tert. amyl alcohol results in ursodeoxycholic acid in

96% yield. Ursocholic acid, recently shown to be a potential gallstone solubilizer (23), was obtained in 93% yield by this method. An effective method for the preparation of isoursodeoxycholic acid was developed in which the 3α-hydroxyl group in methyl 7-oxolithocholate was first epimerized according to the method of Bose et al. (12) and the resulting methyl 7-oxo-isolithocholate was then reduced with potassium/tert. amyl alcohol. Overall yield of 80% was obtained from 7-oxolithocholic acid and pure isourosdeoxycholic acid could be isolated by crystallization. There is a recent interest in both lagocholic acid and lagodeoxycholic acid since both compounds are more hydrophilic than the corresponding 12α-hydroxy epimers (24, 25). Both these compounds were prepared by potassium/tert. amyl alcohol reduction of the corresponding 12-oxo derivatives in better yields than by previously reported methods (21, 22, 26). Easy accessibility of these epimers of natural bile acids will facilitate detailed studies of their physiological properties.

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