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Facile Synthesis, Aggregation Behavior, and Cholesterol Solubilization Ability of Avicholic Acid

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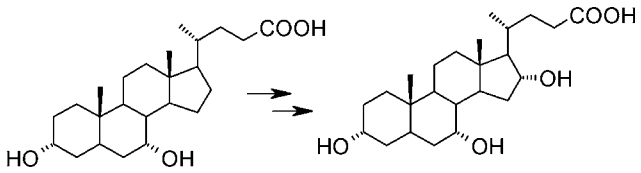
ABSTRACT



Chenodeoxycholic acid
(found in human bile)

Avicholic acid
(found in avian bile)

Avicholic acid, a major constituent of the bile of several avian species, was synthesized in eight steps from readily available chenodeoxycholic acid in 9% overall yield using Breslow's remote functionalization strategy in a key step. Micelle formation and equilibrium cholesterol solubilization properties were studied for avicholate in aqueous solution.

Bile acid science (cholanology) continues to have importance in physiology and medicine.[1] Bile acids conjugated with taurine and/or glycine form mixed micelles with fatty acids and cholesterol in the intestine.[2] Bile acids from different species chemically differ in two respects: (i) the side-chain structure and (ii) the distribution of the number, position, and stereochemistry of the hydroxyl groups in the steroid nucleus. Several decades ago, Haslewood addressed the issue of considering the bile acid structure as an aid to the understanding of the evolutionary process.[3] It has been noted that the bile acid structure shows a pattern of progressive molecular development along the line of vertebrate evolution. It has been suggested that the most evolved mammalian bile acids have a 5β configuration with hydroxyl groups at 3α, 7α, and 12α.[4] An unusual 3α,7α,16α-trihydroxy bile acid was recently isolated from storks and herons by Hagey et al.[5] It was called avicholic acid to signify that it is a class that has to date been identified only in avian species. This bile acid was a major constituent (>90%) of biliary bile acids in the Shoebill stork and several herons. It was also suggested that 16α-hydroxy is a primitive bile acid, whereas 12α-hydroxy is a more evolved bile acid. The first and so far the only chemical synthesis of avicholic acid was achieved by Iida et al. from chenodeoxycholic acid.[6] This synthesis utilized a somewhat selective 17α-hydroxylation (ca. 15%) of acetylated methyl chenodeoxycholate by dimethyldioxirane. The overall yield of avicholic acid was <1%, clearly suggesting the need for an improved synthetic route. Herein,

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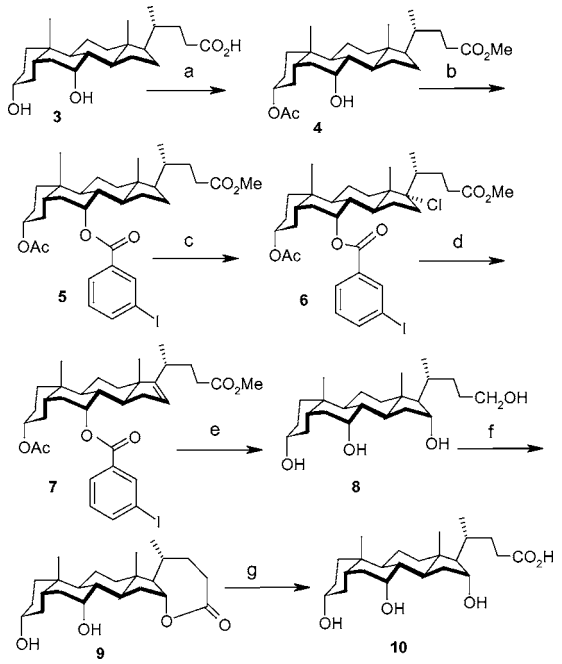
we report a facile synthesis of avicholic acid from readily available chenodeoxycholic acid in about 9% overall yield (Scheme 1). The aggregation behavior and the equilibrium cholesterol solubilizing ability of avicholate in aqueous media are also reported.

Scheme 1. Synthesis of **10** from **3**[a]



[a] (a) (i) MeOH/HCl, rt, 10 h, (ii) MeCOCl/pyridine/CH_2Cl_2, rt, 5 h (78%); (b) 3-iodobenzoyl chloride/CaH_2/$BnNEt_3^+Cl^-$/PhMe, reflux, 13 h (50%); (c) $PhICl_2$ (2.5 equiv)/^{t}BuOH (0.3 M)/CH_2Cl_2/*hν*; (d) pyridine, reflux, 14 h (65% from **3**); (e) (i) BH_3·THF, (ii) NaOH−H_2O_2, (iii) 5% KOH in MeOH, rt, 12 h (63%); (f) CH_2Cl_2−H_2O (carbonate buffer, pH 8.6) NCS-TEMPO/$BnNEt_3^+Cl^-$, rt, 4 h (63%); (g) (i) 5% KOH in MeOH, rt, 13 h, (ii) H_3O^+ (90%). Overall yield: 9%.

Breslow has pioneered (covalently linked) iodoaryl template directed chlorination of cholestanol derivatives.[7] Compound **1a** and **1b** were shown to selectively chlorinate C-9 and C-17, respectively, under radical relay conditions (Figure 1).[7] Despite the 5β-configuration, we envisioned that 3-iodobenzoate attached at C-7 (e.g., compound **2**) would selectively chlorinate C-17. Our modeling studies (AM1) indicated

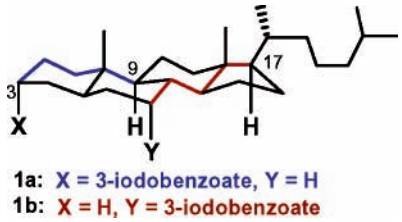


Figure 1. Template-directed radical-relay chlorination developed by Breslow.[7]

that the calculated distance from C-7 oxygen to the attached chlorine in **2** (the reactive species for the template directed chlorination) is 4.96 Å, and the C-7 oxygen to C-17 hydrogen distance is 4.91 Å (Figure 2).[8]

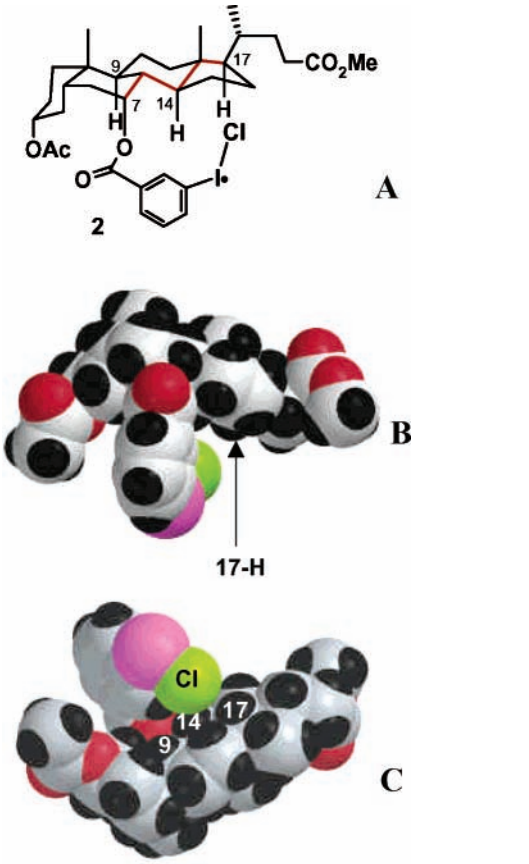


Figure 2. (A) Perspective representation of the reactive species **2**. (B) Optimized structure of **2** using semiempirical AM1 method. (C) A view from the α-face of **2**. Cl, 9-H, 14-H and 17-H are labeled.

Chenodeoxycholic acid (**3**) was converted to ester **5** in two steps (Scheme 1). Photolysis was performed on **5** (10 mM) in 0.3 M ^{t}BuOH[7d]/dichloromethane (deoxygenated) in the presence of dichloroiodobenzene to yield 17-Cl-steroid (**6**). Regioselective introduction of the Δ^{16} double bond was achieved by refluxing **6** in pyridine.[7e] Differences in the chemical shift value of angular methyl groups were in accordance with the literature data for other Δ^{16}-steroids (Table 1).[9]

Hydroboration-oxidation of **7** (BH_3·THF, followed by H_2O_2/NaOH) yielded side chain reduced product **8**

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Table 1. Differences in ^{1}H NMR Chemical Shift Value for Steroid **5** and Δ^{16}-Steroid **7** (in $CDCl_3$)

	18-CH_3	19-CH_3	21-CH_3
5	0.68	0.99	0.92
7	0.76	1.02	1.01
$\Delta\delta$	−0.08	−0.03	−0.09

(3α,7α,16α, 24-tetrol), as observed earlier by Iida et al.[6] Spectral data of **8** matched with the data published in ref 6, confirming the site-specific introduction of the hydroxyl group at the 16α-position. Our next aim was to oxidize the primary alcohol (24-OH) in the presence of three secondary alcohols. In the earlier work by Iida et al., the tetrol was oxidized to the triketo carboxylic acid,[6] but the selective reduction of the oxo groups of the triketo methyl ester did not lead to satisfactory selectivity in favor of α-OH groups (yield 16%). Therefore, we felt the need for an efficient method of oxidation of **8** to the final product. Initially, the 24-OH was selectively protected (trityl), followed by the protection of the secondary alcohols (acetate) and subsequent deprotection/oxidation of the CH_2OTr under acidic condition (Jones reagent).[10] The final product (**10**) was then obtained by cleaving the acetate groups (not shown).

Unsatisfactory overall yield and long reaction times (2 days for 24-O-tritylation) compelled us to go for an alternative oxidation methodology. Einhorn et al.[11] had successfully performed the oxidation of a primary diol to the corresponding lactone using TEMPO-mediated *N*-chlorosuccinimide oxidation in a biphasic mixture using a phase transfer catalyst such as $BnNEt_3^+Cl^-$. By employing the same methodology (with slight variation in the condition; see Scheme 1 and Supporting Information) a one-step oxidation of **8** to avicholic lactone (**9**) has been accomplished. Spectral data for **9** matched with the data reported for 3α,7α-dihydroxy-5β-cholane *O*-24,16α-lactone.[6] Cleavage of the lactone (5% KOH/MeOH), acidification (cold 1 M HCl), and quick extraction yielded **10**. Longer exposer of **10** to an acidic aqueous medium always led to partial lactonization. This facile lactonization of 16α-hydroxy bile acid was also reported for pythocholic (3α,12α,16α-trihydroxy) acid.[12] It was suggested that the 16-hydroxyl group is in a geometrically favorable position to form an ϵ-lactone. After the avicholic acid was obtained, the triacetoxy methyl ester derivative was prepared to compare with the data published for the same compound from avicholic acid Hagey et al. isolated from the avian bile (Table 2).

Since there was no report on the aggregation behavior of avicholate in aqueous medium, we decided to study the micellization of avicholate in aqueous solution. Pyrene was used as a fluorescent probe to measure the CMC (critical micellar concentration) of avicholate. The ratio of the two

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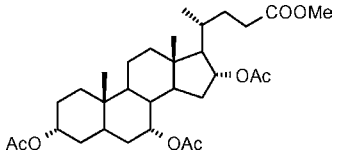
Table 2. ^{1}H NMR Chemical Shift Data for Methyl-3α,7α,16α-triacetoxy-5β-cholane-24-oate in $CDCl_3$



	derived from synthetic avicholic acid[a]	derived from naturally occurring avicholic acid[b]
18-CH_3	0.702	0.702
19-CH_3	0.928	0.928
21-CH_3	0.960 (d, 6.6 Hz)	0.960 (d, 6.5 Hz)
OAc's	1.992, 2.037, 2.043	1.997, 2.035, 2.049
CO_2Me	3.650	3.649
3β-H	4.591	4.591
7β-H	4.817	4.813
16β-H	4.938 (br t, 6.6 Hz)	4.939 (br t, 6.5 Hz)

[a] This work. [b] Reference 5.

vibronic bands (I_3/I_1) in the fluorescence spectrum of pyrene is indicative of the polarity experienced by the probe solubilized in the micellar aggregates.[13] Using this technique, we have measured the CMC values of dihydroxy (chenodeoxycholate, and 7-deoxycholate) and trihydroxy (avicholate and cholate) bile salts. The CMCs of avicholate and cholate at pH 9 were found to be ca. 15 mM (Figure 3), whereas

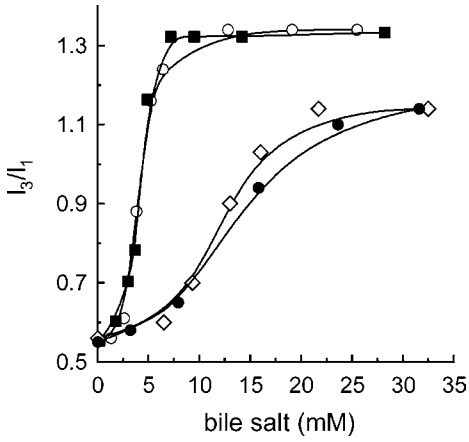


Figure 3. The ratio of vibronic bands (III/I) of pyrene fluorescence as a function of bile salt concentration at pH 9 (TRIS buffer) at 25 °C: (○) chenodeoxycholate, (■) 7-deoxycholate, (◇) cholate, (●) avicholate.

chenodeoxycholate (3α,7α-dihydroxy) and 7-deoxycholate (3α,12α-dihydroxy) had CMCs below 5 mM.[14] The introduction of the third hydroxyl group increased the aqueous

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solubility of the bile salt. Hence, CMC was higher for avicholate, as observed for cholate.[14]

Because the cholesterol solubilization ability is one of the significant properties of bile salts,[15] cholesterol solubilization ability of avicholate was also evaluated. For this study, a mixture of bile salt and solid anhydrous cholesterol was stirred at 37 °C for 1 day. After filtration, the solubilized cholesterol was assayed using a commercially available enzymatic assay (Supporting Information). Maximum aqueous solubility of cholesterol was found to be much lower with avicholate (0.8 mM, bile salt:cholesterol ~63:1) compared to that in chenodeoxycholate (2.5 mM, bile salt: cholesterol ~20:1) in 50 mM bile salt concentration at pH 10. This is not surprising because avicholate, with three hydroxyl groups, is less hydrophobic compared to chenodeoxycholate (which was also clear from the CMC data). Our data are consistent with the hypothesis that the more hydrophilic bile salts are poorer solubilizers of cholesterol.[15]

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In conclusion, we have developed a good synthetic route for chemical synthesis of avicholic acid. To the best of our knowledge this is the first demonstration of "Breslow remote funtionalization" on a bile acid backbone. This strategy may hold the promise to prepare other natural/unnatural bile acids using different combinations of substrates and templates. For the first time, we have studied the properties of avicholate in aqueous solutions. Our data show the similarity among avicholate and cholate in aqueous media. It would be useful to take up further studies on this unusual and rare bile acid.

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Supporting Information Available: Synthetic procedures, characterization data, details of the calculation, and materials and methods for the determination of CMC and cholesterol solubilization. This material is available free of charge via the Internet at http://pubs.acs.org.

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