notes on methodology

A convenient synthesis of 3-keto bile acids by selective oxidation of bile acids with silver carbonate–Celite

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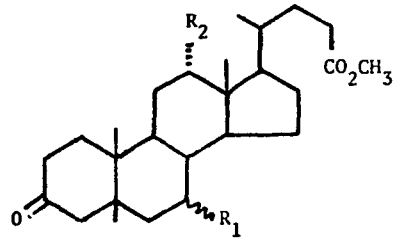
Summary A number of 3-keto bile acids were synthesized by the selective oxidation of bile acid methyl esters with silver carbonate–Celite in refluxing toluene. The pure 3-keto bile acids were isolated simply by filtering the reaction mixture and concentrating the filtrate. The relation of the bile acid structure to the oxidation rate is also discussed.

Supplementary key words bile acid methyl esters

Bile acids are known to undergo bacterial oxidation in the intestine to form keto bile acids (1); this can be demonstrated by the presence of such keto bile acids in feces (2). However, the quantitation of these keto bile acids is difficult, because the destruction during alkaline hydrolysis and the enol ester formation during derivatization produce artifacts. In our investigations of the conditions necessary for avoiding artifact formation, authentic keto bile acids were needed, and, in our program to develop an inverse isotope dilution assay for total bile acids in plasma by gas–liquid chromatography–mass spectrometry (3), 3-keto bile acids were required to prepare deuterated bile acids as internal standards by $NaBD_4$ reduction.

3-Keto bile acids have traditionally been synthesized by the Oppenauer oxidation of bile acid esters with aluminum *tert*-butoxide and acetone (4–6). Although Jones, Webb, and Smith (4) reported a moderate yield and an uncomplicated isolation procedure, the experience with this technique in this and other laboratories (5, 6) has been unsatisfactory. The Oppenauer oxidation of bile acids results in a complicated mixture of products and column chromatography is needed to isolate the desired 3-keto bile acids from the mixture (5, 6). Consequently, the yields are usually low. The alternative procedure for the synthesis of 3-keto bile acids is the oxidation of protected bile acids, such as 3α-hydroxy formyloxy (7) or 3α-hydroxy acetoxy bile acids (8), but the long synthetic sequence of this procedure renders it unattractive.

In a preliminary communication, Fetizon and Golfier (9) described the facile synthesis of methyl 3-oxo-7α,12α-dihydroxy-5β-cholan-24-oate (*Ia*, **Scheme 1**) from methyl cholate by silver carbonate–Celite oxidation. This provides an attractive route to the general synthesis of 3-keto bile acids. Silver carbonate–Celite has been known to be selective in the oxidation of alcohols (10); however, the condition used by Fetizon and Golfier (9) is not practical for synthetic application. They used 30 mmol of silver carbonate–Celite to oxidize 1 mmol of methyl cholate by refluxing in benzene for 5 hr. For the synthesis of gram quantities of keto bile acid, several hundred grams of the reagent would have to be used, which is unpractical and even impossible for larger scale syntheses. Attempts were made to modify the procedure by reducing the amount of silver carbonate–Celite used, but no reaction occurred even after refluxing for several days in benzene. However, when toluene was substituted for benzene, methyl cholate was readily oxidized in 8 hr at the temperature of refluxing toluene with as little as 2 equivalents of the reagent, while methyl chenodeoxycholate, methyl deoxycholate, and methyl ursodeoxycholate were oxidized within 3 hr. The isolation of the product was accomplished simply by filtering off the reagent, concentrating the filtrate, and then crystallizing the product from solvents. The yields were between 70 and 90%.



I, a, $R_1 = R_2 = OH$ (α)

b, $R_1 = H$, $R_2 = OH$

c, $R_1 = OH(\alpha)$, $R_2 = H$

d, $R_1 = OH(\beta)$, $R_2 = H$.

Scheme 1. Methyl esters of 3-keto bile acids.

Abbreviation: TLC, thin-layer chromatography.

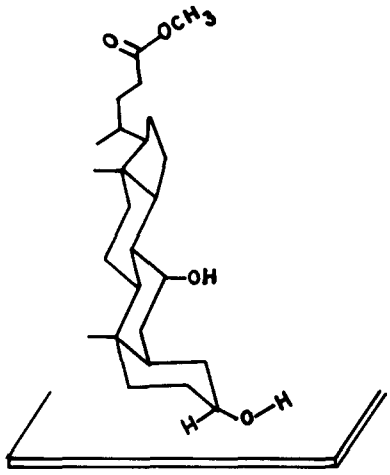


Fig. 1. The adsorption geometry of bile acid on the oxidizing surface needed to have oxidation take place.

To explain the selective oxidation and different oxidation rates of various compounds, it has been proposed (10) that the adsorption of alcohol on the solid surface of the oxidizing reagent has to have the three-dimensional arrangement shown in **Fig. 1**, in which both the oxygen and 3β-hydrogen are attached to the oxidizing surface, in order to have oxidation take place. The authors further documented that other hydroxyl groups and π-electron system-containing groups on the same molecule would affect the reaction rate by altering the adsorption geometry on the oxidizing surface. Thus, the 7- and 12-hydroxyl groups and the carboxylate group on bile acids would tend to make the molecule assume the configuration of **Fig. 2**, in which only the oxygen is attached to the oxidizing surface, while the 3β-hydrogen is projected away from the surface. This arrangement prevents oxidation from taking place.

According to the proposed mechanism, methyl cholate should be the least reactive, since occurrence of both the 7α- and 12α-hydroxyl groups on the same side of the molecule favors the configuration of Fig. 2. Methyl deoxycholate, methyl chenodeoxycholate, and methyl ursodeoxycholate would have similar reaction rates all of which would be faster than that of methyl cholate. This reaction sequence is indeed what was observed. The reaction time for the completion of oxidation is as follows: methyl lithocholate, 1 hr; methyl deoxycholate, 1.5 hr; methyl chenodeoxycholate, 2 hr; methyl ursodeoxycholate, 2.5 hr; and methyl cholate, 7 hr. Methyl lithocholate is the most reactive, as would be expected from the proposed mechanism. Methyl deoxycholate is oxidized slightly faster than either methyl chenodeoxycholate or methyl ursodeoxycholate. This difference can be explained by the steric shielding of the 12α-hydroxyl group by the side chain (11), which makes this group less available for chemiabsorption to assume the configuration of Fig. 2. The reaction rates given above can be used as an approximate guide when oxidations of other bile acids or bile alcohols are to be performed.

When performing oxidation with silver carbonate on Celite, it is generally advised (12) that freshly prepared reagent be used; however, we did not detect any change in reactivity whether the reagents were freshly prepared or stored for more than one year (in an amber colored container). It was also found that a trace amount of water in the reaction mixture does not affect the reaction, but a significant amount of water can lower the reaction temperature by forming a lower boiling azeotropic mixture with toluene and thus slow down the reaction. Thus, the reagent used for oxidation does not necessarily need to be absolutely dry, and a Dean-Stark apparatus can be attached to the reaction vessel to remove the water during reflux.

Materials and methods

Melting points were determined on a Fisher-Jones melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was carried out on precoated silica gel plates (Kontes Q_1, Kontes, Vineland, NJ) using benzene–acetone 70:30 and benzene–dioxane–acetic acid 75:20:2 (13). The plates were visualized by spraying with 10% H_2SO_4 in ethanol and heating at 120°C. Infrared spectra were determined on a Perkin-Elmer 337 infrared spectrophotometer.

Deoxycholic acid was purchased from Aldrich Chemical Co., Milwaukee, WI. Chenodeoxycholic acid (Diamalt, Germany) and ursodeoxycholic acid (Tokyo Tanabe, Japan) were gifts of Dr. A. F. Hofmann of the Mayo Clinic. These three bile acids were all used without further purification. Cholic acid (Eastman Kodak, Rochester, NY) was recrystallized from ethanol and dried at 150°C in vacuo overnight.

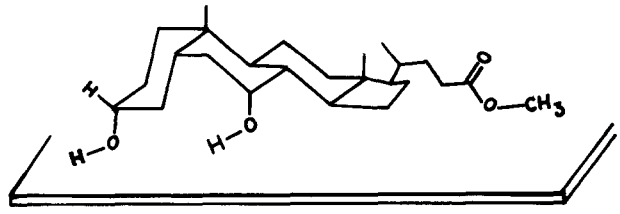


Fig. 2. The adsorption geometry of bile acid on an oxidizing surface that prevents oxidation from taking place.

Silver carbonate–Celite reagent was prepared according to Fetizon and Golfier (9).

Preparation of methyl esters of bile acids. The appropriate bile acid (20 g) was suspended in 100 ml of absolute methanol, and then 3 ml of conc. HCl was added. The solution was mixed well, and then 50 ml of DMP (2,2-dimethoxypropane, Aldrich Chemical Co., Milwaukee, WI) was added. The solution was mixed and kept at 25°C overnight. Methyl cholate was obtained as prisms from this solution. Methyl chenodeoxycholate was obtained by concentrating the solution to a syrup, and then drying in vacuo to a crystalline powder. Methyl deoxycholate was obtained as needles from the concentrated solution. Methyl ursodeoxycholate was obtained as needles by diluting the reaction mixture with water and crystallizing the precipitate from aqueous methanol. The yields of the methyl esters were between 90 and 95% and were more than 98% pure as judged by thin–layer chromatography.

In preparing methyl chenodeoxycholate, it was important that the reaction mixture not be heated. Otherwise, a significant amount of by-products was produced. When used directly in the oxidation, these products prolong the reaction time. In one instance, as long as 24 hr was required for complete oxidation instead of less than 3 hr. Presumably the by-products inhibit the oxidation by competing for the oxidizing surface (10).

Methyl 3-oxo-12α-hydroxy-5β-cholan-24-oate (Ib, Scheme 1). To a solution of methyl deoxycholate (10.15 g, 0.025 mol) in 350 ml of toluene was added silver carbonate–Celite (28.5 g, 0.050 mole). The reaction vessel was then attached to a Dean-Stark apparatus and heated with magnetic stirring in a 135°C constant temperature bath. After refluxing for 3 hr, the reaction mixture was filtered and washed with hot toluene; the combined filtrate was then evaporated to an oil. Crystallization of this oil from aqueous methanol (water–methanol 10:50, v/v) afforded 9.14 g (90%) of the compound, mp 148–149°C, lit. mp (petroleum ether) 140–142°C (4), 142–145°C (5). It was identical with the authentic sample synthesized by the Oppenauer oxidation (4).

Methyl 3-oxo-7α-hydroxy-5β-cholan-24-oate (Ic, Scheme 1). The same procedure as that used for *Ib* was employed. After evaporation of toluene, the oily residue was crystallized from petroleum ether (60–110°C boiling range) as prisms. The product weighed 8.5 g (84% yield), mp 128–129°C, lit. mp 125°C (ethyl acetate–petroleum) (5), 126°C (benzene–petroleum) (6). It showed no difference from an authentic sample (5, 6).

Methyl 3-oxo-7β-hydroxy-5β-cholan-24-oate (Id, Scheme 1). The same procedure as that used for *Ib* was employed. After evaporation of toluene the residue was crystallized from benzene–petroleum ether (60–110°C range) as prisms. The product weighed 9.13 g (90% yield), mp 103–104°C. Its free acid form was identical with the authentic sample (7).

Methyl 3-oxo-7α,12α-dihydroxy-5β-cholan-24-oate (Ia, Scheme 1). This compound was obtained the same way as *Ib*, except that 7 hr of refluxing was needed. The concentration of the toluene filtrate to a small volume produced crystalline *Ia* in 72% yield, mp 181–183°C, lit. mp 171–172°C (MeOH) (4), 172°C (5), 174°C (ethyl acetate–heptane) (6). It was identical with the authentic sample (4).

Determination of reaction time for complete oxidation. Bile acid methyl esters (0.0025 mol) were oxidized the same way as described above. Aliquots (0.2 ml each) of the reaction mixture were withdrawn with a syringe with a long needle every 0.5 hr during the first 3 hr of reaction, then every hour afterwards. Those aliquots were examined by TLC. The times required for the complete disappearance of starting materials are reported above.

There has been some concern about the feasibility of obtaining kinetic data from a heterogenous reaction (10). Factors such as stirring rate and reagent batch are said to influence the reaction rate. We used the same batch of reagent and kept the reaction conditions as uniform as possible. The reaction times obtained above were reproducible over several runs. At the temperature of refluxing toluene, the agitation of the refluxing suspension probably cancelled out the subtle differences in the stirring rate, which is the major factor contributing to the variation of kinetic data (10).

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