69. Stereoselective Introduction of Steroid Side Chains. Synthesis of Chenodeoxycholic Acid

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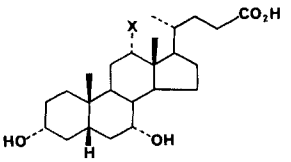
Summary

A new short route to chenodeoxycholic acid has been developed. The synthesis is based on the stereoselective introduction of the steroidal side chain *via* an ene reaction of methyl acrylate and a (17*Z*)-ethylidene steroid prepared from androstenedione.

Recent interest in chenodeoxycholic acid (**1a**) has blossomed due to its efficacy in the non-surgical dissolution of cholesterol gallstones [1]. In addition to isolation from animal sources, basically two routes to this important bile acid have been explored. The more traditional approach involves the deoxygenation of another bile acid, cholic acid (**1b**) [2], while the total synthesis carried out by *Kametani et al.* [3] features the application of *o*-quinodimethane-*Diels-Alder* methodology to build the ring systems. A key strategic point in the present work was to make use of intermediates which could be readily obtained from abundant plant steroids. Application of our previous work [4] involving an ene reaction to stereospecifically attach the cholic acid side chain has now led to a short, practical synthesis of chenodeoxycholic acid (**1a**).



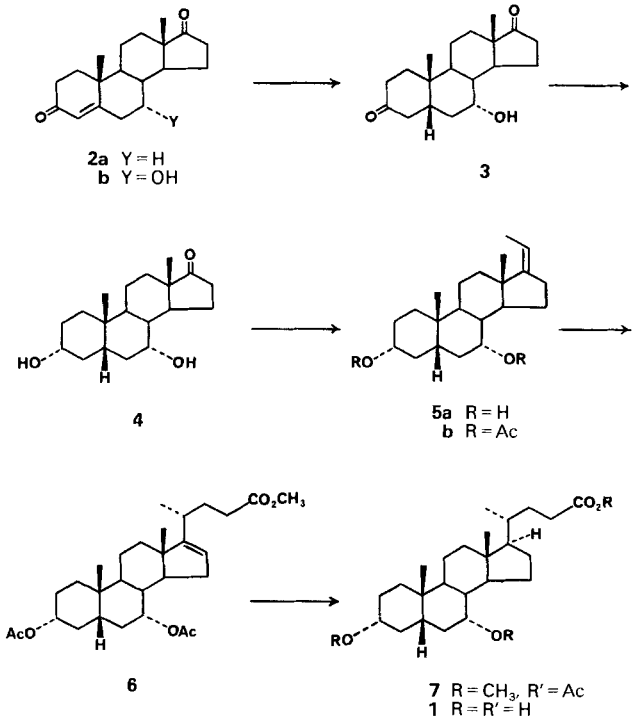
1a X=H
b X=OH

The incorporation of the ene methodology into the synthetic plan necessitated the production of an appropriately substituted (17*Z*) steroid alkene. For this purpose, we chose the 7*α*-hydroxy enone **2b** as the starting material since it is readily accessible from the commercially available androst-4-ene-3,17-dione (**2a**) by microbial hydroxylation [5]. A further requisite of this strategy was the differentiation of the C(3) and C(17) carbonyl groups in **2b**. While model studies on the (17*Z*)-ethylidene derivative of **2a** [6] indicated that indeed, the ene reaction could be successfully executed without interference from the enone moiety, the difficulty encountered in preparing the (17*Z*)-ethylidene derivative of **2b** with its C(3) carbonyl *and* 7*α*-hydroxy group prompted the

investigation of alternatives. The solution was ultimately achieved through selective reduction of the C(3) carbonyl.

Hydrogenation of **2b** over 5% Pd/C in DMF produced the 5β-dione **3** *(Scheme)*. The conversion of **3** to keto-diol **4** was best carried out with lithium tri-*t*-butoxyaluminum hydride in THF although some over-reduction was still observed. The removal of small amounts of the triol by-product was found to be much simpler when the separation was deferred until after the next step. Therefore, the crude reduction product was treated with ethylidenetriphenylphosphorane to generate the desired (*Z*)-alkene **5a**[1]), which was subsequently bis-acetylated under the standard conditions to give **5b**. On exposure to an excess of methyl acrylate and ethylaluminum dichloride, ethylidene diacetate **5b** smoothly underwent the ene reaction to give the adduct **6** in 72% yield. Hydrogenation over 5% Pd/C in i-PrOH gave the chenodeoxycholic acid derivative **7** which was identical by TLC, NMR and mixed m.p. with authentic material prepared from chenodeoxycholic acid[2]). Basic hydrolysis then provided pure chenodeoxycholic acid (**1a**).

Scheme



[1]) While not observed by ^{1}H-NMR, the presence of *ca.* 3–5% of the (*E*)-alkene could not be excluded. Nevertheless, small quantities of the (*E*)-isomer was of minor concern since, as noted previously [7], (*E*)-isomers typically react substantially more slowly than the (*Z*)-isomers.

[2]) A comparison between the synthetic and authentic materials at this point was appropriate since chenodeoxycholic acid itself can retain solvent on crystallization [2] thereby obfuscating a mixed m.p. determination. This problem was not encountered with **7**.

In summary, chenodeoxycholic acid has been prepared by a new short route from androst-4-ene,3,17-dione. In a single step, the cholic acid side chain was appended and the natural C(20)-configuration formed stereoselectively by a *Lewis*-acid-catalyzed ene reaction of a (17*Z*)-ethylidene steroidal substrate and methyl acrylate. Catalytic hydrogenation generated the stereo center at C(17) and subsequent ester hydrolysis yielded chenodeoxycholic acid.

We wish to express our gratitude to the members of the Physical Chemistry Department for determination of spectral and analytical data and to Mr. *C. W. Despreaux* and Mrs. *K. R. Rittweger* of the Microbiology Department for carrying out and improving the microbial hydroxylation of **2a**. We also thank Mr. *A. Williams* for technical assistance.

Experimental Part

General. Melting points (m.p., °C) were determined on a *Thomas-Hoover* melting point apparatus and are uncorrected. Specific rotations ($[\alpha]_D$) were measured in $CHCl_3$ at 25°. IR spectra (cm^{-1}) were obtained on a *Digilab* model *FTS-15E* spectrometer. The ^{1}H-NMR were recorded on a *Varian XL-100* (100 MHz) spectrometer in $CDCl_3$ with TMS as the internal standard. The spin-spin coupling constants (J) are given in Hz.

(5β,7a)-7-Hydroxyandrostane (**3**). A mixture of enone **2b** [5] (4.25 g, 14 mmol) and 5% Pd/C (0.425 g) in 75 ml of DMF was stirred under H_2 until the uptake ceased. The mixture was filtered through *Celite* and the solvent removed *in vacuo*. The residue was dissolved in 400 ml of AcOEt and washed with H_2O (2 × 100 ml) and dried (Na_2SO_4). Filtration and removal of solvent under reduced pressure gave a solid which on crystallization from AcOEt yielded 3.7 g (86%) of **3** [a second crop yielded an additional 0.4 g (9%) of **3**]. The analytical sample was recrystallized from CH_3CN, m.p. 231–232°, $[\alpha]_D = +79.4°$ ($c = 1.07$). IR ($CHCl_3$): 1735, 1707. ^{1}H-NMR: 0.89 (*s*, 3H); 1.03 (*s*, 3H); 3.44 (*dd*, $J = 13, 15$, 1H); 4.10 (br. *s*, 1H). Anal. calc. for $C_{19}H_{28}O_3$ (304.43): C 74.96, H 9.27; found: C 74.83, H 9.10.

(3a,5β,7a)-3,7-Dihydroxyandrostan-17-one (**4**). A solution of dione **3** (1.217 g, 4 mmol) in 60 ml of dry THF was added under Ar to a solution of lithium tri-*t*-butoxyaluminum hydride (1.588 g, 5.6 mmol) in 20 ml of dry THF at 0°. The mixture was stirred at 0° for 3½ h and then quenched by the addition of 25 ml of 1N HCl. Then 100 ml of AcOEt was added, the mixture washed with brine (3 × 50 ml) and then dried (Na_2SO_4). Filtration and evaporation of solvent *in vacuo* gave 1.282 g of crude **4**. A calibrated liquid chromatography indicated a purity of 81.6% which corresponds to an overall yield of 85.4%. This material was suitable for use in the next step since the major impurity was the product of over-reduction. An analytical sample was obtained by chromatography on silica gel eluting with AcOEt. The diol could be precipitated from hexane as an amorphous solid. $[\alpha]_D = +67.7°$ ($c = 0.97$). IR ($CHCl_3$): 1733. ^{1}H-NMR: 0.84 (*s*, 3H); 0.92 (*s*, 3H); 3.44 (*m*, 1H); 3.96 (br. *s*, 1H). Anal. calc. for $C_{19}H_{30}O_3$ (306.43): C 74.47, H 9.87; found: C 74.39, H 9.92.

The corresponding diacetate of **4** crystallized readily from hexane. M.p. 146° $[\alpha]_D = +68.2°$ ($c = 0.52$). IR (KBr): 1738. ^{1}H-NMR: 0.85 (*s*, 3H); 0.96 (*s*, 3H); 2.00 (*s*, 3H); 2.04 (*s*, 3H); 4.58 (*m*, 1H); 5.04 (br. *s*, 1H). Anal. calc. for $C_{23}H_{34}O_5$ (390.52): C 70.74, H 8.78; found: C 71.01, H 8.83.

(Z)-(3a,5β,7a)-Pregn-17(20)-ene-3,7-diol (**5a**). A solution of the crude diol **4** (1.282 g, *ca.* 3.4 mmol) in 5 ml of dry THF was added to a mixture of *t*-BuOK (1.875 g, 16.7 mmol) and ethyltriphenylphosphonium bromide (6.215 g, 16.7 mmol) in 113 ml of dry THF. The mixture was stirred at r.t. under Ar for 72 h and then poured into 750 ml of H_2O containing 250 g of ice. After 30 min, the crude product (3.0 g) was collected by suction filtration, dried *in vacuo* and then chromatographed on silica gel eluting with hexane/AcOEt (1:2) to yield 0.888 g (70% based on two steps) of **5a**. Trace amounts of impurities visible by TLC were removed by precipitating the material from hexane containing a small amount of CH_2Cl_2. The analytical sample was recrystallized from acetonitrile. M.p. 160–161°, $[\alpha]_D = +14.7°$ ($c = 0.99$). ^{1}H-NMR: 0.86 (*s*, 3H); 0.90 (*s*, 3H); 1.65 (*dm*, $J = 7$, 3H); 3.42 (*m*, 1H); 3.87 (br. *s*, 1H); 5.12 (*qm*, $J = 7$, 1H). Anal. calc. for $C_{21}H_{34}O_2$ (318.5): C 79.19, H 10.76; found: C 79.00, H 10.77.

(Z)-(3a,5β,7a)-Pregn-17(20)-ene-3,7-diol diacetate (**5b**). Under standard conditions (acetic anhydride, pyridine, dimethylaminopyridine) diol **5a** was converted to the diacetate **5b** in 75% recrystallized (MeOH) yield. M.p. 92–93°, $[\alpha]_D = +38.4°$. IR ($CHCl_3$): 1735. ^{1}H-NMR: 0.86 (*s*, 3H); 0.94 (*s*, 3H); 1.63 (*dm*, $J = 7$, 3H); 2.00

(*s*, 6H); 4.55 (*m*, 1H); 4.90 (br. *s*, 1H); 5.13 (*qm*, $J = 7$, 1H). Anal. calc. for $C_{25}H_{38}O_4$ (402.58): C 74.59, H 9.51; found: C 74.68, H 9.60.

Methyl (3a,5β,7a)-3,7-Diacetoxychol-16-en-24-oate (**6**). To a solution of methyl acrylate (0.108 ml, 1.31 mmol), ethylaluminum dichloride (25% solution in hexane, 1.092 ml, 1.61 mmol) and 3 ml of CH_2Cl_2 (stored over 4 Å prior to use) at 0° and under Ar was added a solution of diacetate **5b** (0.22 g, 0.55 mmol) in 1 ml of CH_2Cl_2. The cooling bath was removed and the mixture stirred at r.t. for 72 h. The reaction was quenched by addition of 5 ml of 30% potassium sodium tartrate, transferred to a separatory funnel containing 50 ml of 15% potassium sodium tartrate solution and extracted with CH_2Cl_2 (4 × 25 ml). The combined CH_2Cl_2 extracts were washed with H_2O, brine, and then dried (Na_2SO_4). Filtration and removal of solvent *in vacuo* gave 0.277 g of crude product, which, on silica gel chromatography eluting with hexanes/AcOEt (3:1), yielded 0.162 g (72%) of ene adduct **6**. The analytical sample was recrystallized from hexane/AcOEt, m.p. 109–110°, $[\alpha]_D = +1.36°$ ($c = 1.03$). IR (KBr): 1735. ^{1}H-NMR: 0.71 (*s*, 3H); 0.95 (*s*, 3H); 1.01 (*d*, $J = 7$, 3H); 2.00 (*s*, 3H); 2.02 (*s*, 3H); 3.64 (*s*, 3H); 4.60 (*m*, 1H); 4.95 (br. *s*, 1H); 5.28 (br. *s*, 1H). Anal. calc. for $C_{29}H_{44}O_6$ (488.67): C 71.28, H 9.08; found: C 71.31, H 9.01.

Methyl 3a,7a-diacetoxy-5β-cholan-24-oate (**7**). A mixture of ene adduct **6** (0.15 g, 0.31 mmol), 0.05 g of 5% Pd/C and 10 ml of i-PrOH were stirred under H_2 until uptake ceased. The mixture was filtered through *Celite*, evaporated under reduced pressure and the residue chromatographed on silica gel eluting with hexane/CH_2Cl_2/AcOEt (9:9:2) to yield 0.112 g (72%) of **7**. Recrystallization from MeOH gave m.p. 129.5–130° which did not depress on mixture with authentic material prepared from chenodeoxycholic acid. $[\alpha]_D = +15.6°$ ($c = 0.75$), authentic material $[\alpha]_D = +16.4°$ ($c = 1.02$). IR (KBr): 1735. ^{1}H-NMR: 0.65 (*s*, 3H); 0.92 (*d*, $J = 7$, 3H); 0.94 (*s*, 3H); 2.02 (*s*, 3H); 2.04 (*s*, 3H); 3.65 (*s*, 3H); 4.57 (*m*, 1H); 4.87 (*m*, 1H). Anal. calc. for $C_{29}H_{46}O_6$ (490.66): C 70.99, H 9.45; found: C 71.15, H 9.35.

Chenodeoxycholic acid (**1a**). A solution of diacetate **7** (0.294 g, 0.6 mmol), 3 ml of MeOH and 5 ml of i-PrOH was refluxed under Ar for 4 h, then left overnight at r.t. The mixture was acidified with 1N HCl and extracted with AcOEt (3 × 25 ml). The combined extracts were washed with H_2O, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was recrystallized from AcOEt [2] to yield 0.163 g (69%) of chenodeoxycholic acid (**1a**), identical with authentic material by TLC, NMR and mixed m.p. of authentic material also recrystallized from AcOEt.

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