[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE AMERICAN UNIVERSITY OF BEIRUT]

Unsaturated Bile Acid Derivatives. I. Some Transformation Products from Hyodesoxycholic Acid[1]

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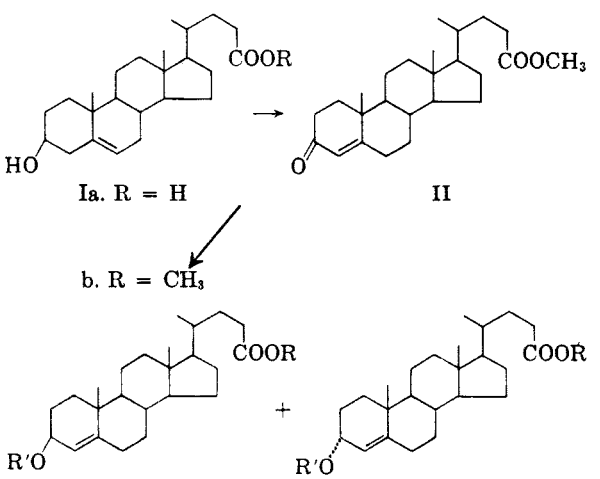
Methyl 3-keto-Δ^4-cholenate, readily obtainable from hyodesoxycholic acid, is reduced by sodium borohydride to a mixture of the 3-hydroxy epimers in which the 3β-epimer predominates. A preparation of methyl $\Delta^{3,5}$-choladienate from hyodesoxycholic acid is described.

$\Delta^{3,5}$-Cholestadiene and the two epimeric 3-hydroxy-Δ^4-cholestenes are well known compounds in the sterol series. However, relatively little work has been done on the corresponding compounds in the bile acid series. A recent method[3] for the preparation of these derivatives, by selenium dioxide oxidation of Δ^3-cholenic acid, gives a mixture of products and involves tedious separations. The present paper describes the preparation of $\Delta^{3,5}$-choladienic acid and the two epimeric 3-hydroxy-Δ^4-cholenic acids from the readily available hyodesoxycholic acid.

Our interest in hyodesoxycholic acid as a possible starting material was aroused by a recent article[4] describing an elegant method for the conversion of this compound to 3β-hydroxy-Δ^5-cholenic acid (Ia). Compound I was our key intermediate for the preparation of the two epimeric 3-hydroxy-Δ^4-cholenic acids (IIIa and IVa).

Oppenauer oxidation of Ib furnished methyl 3-keto-Δ^4-cholenate (II), which, upon reduction with sodium borohydride gave methyl 3β- and 3α-hydroxy-Δ^4-cholenate (IIIc and IVc) in the approximate ratio of 7:1. The predominance of the β-epimer in this reduction is not surprising in view of the high degree of stereospecificity in the reduction of Δ^4-3-ketones by metal hydrides.[5]



Ia. R = H
II
b. R = CH_3

IIIa. R = R′ = H
b. R = CH_3; R′ = Ac
c. R = CH_3; R′ = H

IVa. R = R′ = H
b. R = CH_3; R′ = Ac
c. R = CH_3; R′ = H

Separation of the β-epimer from the mixture *via* the digitonide left the α-epimer, which was isolated as the acetate (IVb) and purified by chromatography. The structure of IIIc as a Δ^4-3-ol was proved by its easy conversion to II by manganese dioxide at room temperature.[3,6] Further confirmation of the structures of the epimers is furnished by the molecular rotations, discussed later on.[7]

Sodium borohydride was chosen as the reducing agent in this investigation since it does not affect ester linkages. We later learned of the interesting finding of Sondheimer and Klibansky[5d] that sodium borohydride reduction of Δ^4-3-ketones may result partly in the saturation of the double bond. No such complication was experienced during the present investigation, although it is entirely possible that small amounts of the saturated product were formed but could not be isolated.

Dehydrotosylation of methyl hyodesoxycholate ditosylate offers a convenient but low-yield route to methyl $\Delta^{3,5}$-choladienate, easily recognizable by its ultraviolet spectrum and the strong levo rotation.[3] Work is being directed at present toward the preparation of this compound in better yield from other intermediates.

During this investigation we occasionally experienced difficulty in handling the free unsaturated hydroxy acids. The presence of the carboxyl group apparently enhances the tendency of the Δ^4-3-ol system to undergo dehydration or etherification in the presence of methanol. Similar difficulties were experienced previously by one of us[3] and have also

(1) Abstracted in part from the M.S. thesis of M. J. H., American University of Beirut, June 1959.

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(7) For further discussion of the molecular rotation data see ref. (3).

been reported by others.[8] For this reason, chemical transformations were carried out, wherever possible, on the methyl esters.

The shift in molecular rotation in passing from II (M_D 348 chloroform) to IIIb (M_D 43 chloroform) is found to be −305, which is in close agreement with the values −301, −307 and −300 reported[5d,9] for the corresponding difference in molecular rotation between the Δ^4-3-ketone and the Δ^4-3β-OAc for the compounds Δ^4-cholesten-3-one, 17α-methyltestosterone and progesterone respectively. On the other hand, the shift in molecular rotation in passing from II to IVb (M_D 754 chloroform) is found to be +406, in agreement with the value +418 calculated[9] for the difference in passing from Δ^4-cholesten-3-one to 3α-acetoxy-Δ^4-cholestene.

EXPERIMENTAL[10]

Methyl hyodesoxycholate. Hyodesoxycholic acid (5 g., m.p. 194–197°) was refluxed with charcoal (0.5 g.) in methanol (20 ml.) for 5 min. The solution was filtered and the filtrate refluxed with another portion of charcoal, filtered, diluted with hot water to incipient cloudiness and allowed to crystallize. Yield 4.5 g., m.p. 198–199°. An ethereal suspension of the purified acid was esterified in the usual manner with diazomethane. The methyl ester, recrystallized from benzene *n*-hexane (1:1), melted at 120° with previous softening (lit.: m.p. 114°,[11] 86°,[12] 110–112°[13]).

3β-Hydroxy-Δ^5-cholenic acid (Ia) was obtained from methyl hyodesoxycholate by the method of Bharucha and coworkers.[4] Esterification with diazomethane gave Ib, needles from ether–petroleum ether (2:1), melting at 143–144° (lit.[14] m.p. 144°).

Methyl 3-keto-Δ^4-cholenate (II). To a solution of Ib (15 g.) in dry acetone (200 ml.) and dry benzene (200 ml.) at 75–80°, was added aluminum *t*-butoxide[15] (20 g.) in hot benzene (100 ml.). The mixture was kept at 80° for 11 hr., cooled, poured into 10% sulfuric acid (200 ml.) and extracted with benzene. The benzene extracts were dried and evaporated to dryness. Two recrystallizations from aqueous methanol gave prisms of II (11.5 g., 77%), melting at 125–126°; $[\alpha]_D$ +90° (*c* 1.22); $\lambda_{max}^{CH_3OH}$ 241 mμ (log ϵ 4.22) [Lit.: m.p. 126–127°,[3] 124–125°;[13,14,16] $[\alpha]_D$ +87° (*c* 1.3),[3] +66.13° (*c* 1 CH_3OH);[17] log ϵ_{241} 4.22[3,13]].

Reduction of II with sodium borohydride and isolation of products. To a solution of II (4.1 g.) in methanol (300 ml.) was added sodium borohydride (1.2 g.) and the mixture kept at room temperature (15–20°) for 24 hr., poured into water (300 ml.) and extracted with ether. The ether extracts were dried and evaporated to dryness. The residue was dissolved in 90% hot ethanol and poured into a hot solution of digitonin (14 g.) in 90% ethanol (1100 ml.). The mixture was kept at room temperature for 24 hr. and worked-up as previously described.[3]

Methyl 3-β-hydroxy-Δ^4-cholenate (IIIc) was obtained from the digitonide. Yield 3.2 g. (80%), m.p. 129–131°. The analytical sample, recrystallized from *n*-hexane, melted at 131–133°, $[\alpha]_D$ +48° (*c* 1.88).

Anal. Calcd. for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 76.99; H, 10.34.

On oxidation with manganese dioxide in chloroform (28°, 2 hr.), IIIc gave II, m.p. 125–126°.

Treatment of IIIc with acetic anhydride in pyridine gave the acetate (IIIb), long needles from methanol, m.p. 147–148°, $[\alpha]_D$ +10° (*c* 1.45).

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.04; H, 9.76.

Hydrolysis of IIIc (2.5 *N* methanolic potassium hydroxide at reflux for 1.5 hr.) gave 3β-hydroxy-Δ^4-cholenic acid. The product was recrystallized from ethyl acetate, m.p. 180–182° (lit.[3] 180–182°).

Anal. Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.79; H, 10.24.

The mother liquor and ether washings from the digitonin precipitation were evaporated to dryness under reduced pressure and the residue extracted with ether. Evaporation of the ether extract gave a crude product (0.5 g.) which was acetylated (pyridine–acetic anhydride), and chromatographed on alumina. Elution with petroleum ether–benzene (7:3, 6:4) followed by several recrystallizations from methanol gave IVb, m.p. 145–147°; $[\alpha]_D$ +175° (*c* 0.99). (lit.[3] m.p. 147–149°); $[\alpha]_D$ +178° (*c* 1.1).

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 74.96; H, 9.77.

Methyl $\Delta^{3,5}$-choladienate. Methyl hyodesoxycholate ditosylate[4] (6 g.) was refluxed with 2,6-lutidine (30 ml.) for 1 hr. and the mixture was concentrated on a water bath under reduced pressure. The residue was treated with ice and extracted with ether. The combined ether extracts were dried and evaporated to dryness, and the residue chromatographed on alumina. Elution with petroleum ether and petroleum ether–benzene (9:1, 4:1, 7:3) gave an oil which solidified when rubbed with methanol. Repeated recrystallizations from methanol gave methyl $\Delta^{3,5}$-choladienate[3] (0.82 g., 26%), m.p. 96–98°, $[\alpha]_D$ −128° (*c* 1.09), $\lambda_{max}^{CH_3OH}$ 227.5, 235, 243 mμ, (log ϵ 4.28, 4.32, 4.12).

Anal. Calcd. for $C_{25}H_{38}O_2$: C, 81.03; H, 10.34. Found: C, 81.08; H, 10.38.

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BEIRUT, LEBANON

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(10) Melting points are uncorrected. Rotations (rounded off to the nearest integer) were measured in chloroform solution, unless specified otherwise. Alumina used for chromatography was neutral, grade I "Woelm" to which 3% water was added. Ultraviolet spectra were determined in a Beckman model DU quartz spectrophotometer. Hyodesocycholic acid was obtained from Canada Packers Ltd., Toronto, Canada. Microanalyses by Pascher Mickroanalytisches Laboratorium, Bonn, Germany.

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