Dehydration of Bile Acid and their Derivatives. VIII. Synthesis of 3α-Hydroxy-Δ$^{8(14)}$-cholenic and 3α-Hydroxy-Δ^{14}-cholenic Acids. A Note on "β"-Cholenic Acid

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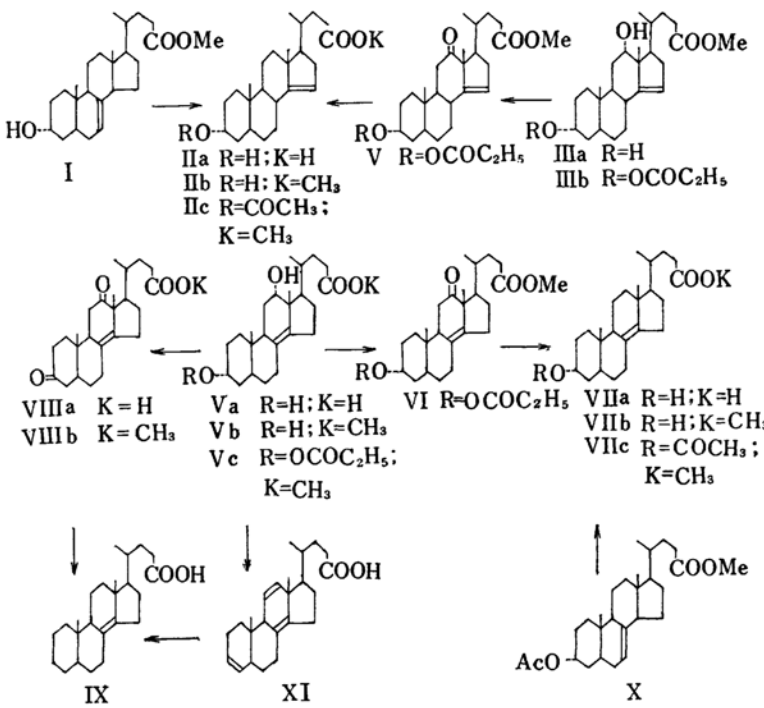
In the preceding[1] paper it has been reported that methyl 3α-hydroxy-Δ^{7}-cholenate (I) in chloroform was isomerized into 3α-hydroxy-Δ^{14}-cholenate (IIb) by means of hydrochloric acid gas. It is here shown that this new hydroxycholenic acid can also be prepared by another route: methyl dihydroxycholenate (IIIa) of Bödecker[2], 3-hydroxy group of which was selectively protected by its conversion into an ethoxycarbonyloxy grouping IIIb, was oxidized and the resulting 12-keto compound IV was then subjected to the Huang-Minlon reduction. The physical properties of the product obtained as well as of its derivatives were in fairly good agreement with those of the hydroxycholenic acid prepared by the isomerization[1].

Apocholic acid[2], 3α, 12α-dihydroxy-Δ$^{8(14)}$-cholenic acid (Va), 12-hydroxy group of which was eliminated by a procedure similar to that mentioned above, was found to be converted into another 3α-hydroxycholenic acid (VIIa) of melting point 167~168°C, $\alpha_D^{15} = +41 \pm 2°$ (chf.). It is reasonably assumed that during the Huang-Minlon reduction cource of methyl 3α-ethoxycarbonyloxy-12-keto-Δ$^{8(14)}$-cholenate(VI), one of the intermediary products of this route, no shift of its double bond did happen to occur, since the same reduction procedure of methyl 3, 12-diketocholenate (VIIIb) derived from apocholic acid (Va) gave "β"-cholenic acid (IX) without shift of its double bond (see below).

This assumption was further confirmed by the fact that the extinction quotient in the ultraviolet region of the above-mentioned hydroxycholenic acid was high enough ($\varepsilon_{max}^{EtOH} =$



1) R. Osawa and K. Yamasaki, This, Bulletin, 32, 1302 (1959).

2) Fr. Bödecker, *Ber.*, 53, 1852 (1920).

TABLE I. $\Delta^{8(14)}$-CHOLENIC ACID

Designated as	M. p., °C	$[\alpha]_D$	Preparative method	Starting material	Ref.
Apocholanic acid	144.5～145.5	+37.0° (chf.)	CrO_3-oxidation +Clemmensen reduction	Apocholic acid	6
β-Cholenic acid	142～143	+38.0°(chf.)*	Pyrolysis+Hydrogenation	Apocholic acid	7
β-Cholenic acid	141～142	+40.7°(alc.)	Pyrolysis+Hydrogenation	Apochenodeoxy-cholic acid	5
β-Cholenic acid	145～146	+37.0°(chf.)	Hydrogenation	α-Cholatrienic acid (II)	9
$\Delta^{8(14)}$-Cholenic acid	140～141	+38.8±5° (chf.)	CrO_3-oxydation+Huang-Minlon reduction	Apocholic acid	

* H. Wieland and E. Dane, *Z. physiol. Chem.*, **210**, 268 (1932).

5550) to indicate that its double bond was tetra-substituted[3], (Bladon et al.) like its mother substance, apocholic acid.

According to Reichstein et al.[4] 3α, 12α-dihydroxy-Δ^7-cholenic acid is easily isomerized into apocholic acid by treating it with platinum and hydrogen gas. It was found, however, that isomerization of methyl 3α-acetoxy-Δ^7-cholenate (X) by the same treatment was unexpectedly slow in rate, and that even with a 30 hrs' shaking with the catalyst in hydrogen-atmosphere not more than 40% of the starting material was isomerized into 3α-hydroxy-$\Delta^{8(14)}$-cholenate (VIIc).

"α-Apochenodeoxycholic acid[5]" had been obtained by hydrolysis of a crystalline taurine-conjugated acid (m. p. 210°C) which separated from the chicken bile treated with a higher concentrated hydrochloric acid (about 18%). Notwithstanding some discrepancies in physical property, this acid must be identical with the above-mentioned hydroxycholenic acid (VIIa) derived from apocholic acid, because the former was reported[5] to give just the same cholenic acid, "β"-cholenic acid (IX) as apocholic acid gave[6,7].

"β"-Cholenic acid had been first derived from apocholic acid, two hydroxyl groups of which were eliminated either by the usual chromium trioxide-oxidation VIIIa followed by the Clemmensen reduction[6] or by pyrolytic dehydration XI followed by catalytic hydrogenation[6,7]. As shown in Table I, apocholic acid (Va) afforded the same cholenic acid, "β"-cholenic acid, by the methods involving even such drastic procedure as pyrolysis or heating in concentrated hydrochloric acid.

While Borsche et al.[6] had failed to obtain any of the diketo derivatives of apocholic acid in a crystalline state, the chromium trioxide-sulfuric acid oxidation of its methyl ester gave a crystalline diketo ester VIIIb which was converted into "β"-cholenic acid (IX) by the Huang-Minlon reduction.

Experimental

$\Delta^{8(14)}$-Cholenic Acid from Apocholic Acid.—*Methyl Apocholate (Vb).*—Apocholic acid (m. p. 171～173°C) was methylated with diazomethane as usual and recrystallized from methanol, m. p. 88～90°C.

Methyl 3,12-Diketo-$\Delta^{8(14)}$-cholenate (VIIIb).—Methyl apocholate (1 g.) in acetone was oxidized with the CrO_3–H_2SO_4 mixture (Bladon et al.[8]). The reaction product was extracted with ether, the extract was washed successively with dilute sodium carbonate solution and water, and dried over magnesium sulfate. To the concentrated ether extract was added a small volume of petroleum ether, and the deposited crystals were collected and washed with small portions of a petroleum ether-ether mixture (1 : 1). The crystals melted at 114～115°C. From the filtrate a second crop of m. p. 113～115°C was obtained. The total yield was 0.61 g. After recrystallization from methanol, the product melted at 119～120°C.

Found: C, 74.17; H, 9.19. Calcd. for $C_{25}H_{36}O_4$ (400.563): C, 74.96; H, 9.05%.

$\Delta^{8(14)}$-CholenicAcid (IX).—Methyl 3,12-diketo-$\Delta^{8(14)}$-cholenate (0.21 g.) (VIIIb) in 2.0 ml. of triethylene glycol was reduced together with 1 ml. of hydrazine and 0.8 g. of potassium hydroxide according to the Huang-Minlon method. The product melted at 140～141°C after recrystallization from acetone or acetic acid; $\alpha_D^{24}=+38.8\pm5°$ (chf.); $\varepsilon_{max}^{EtOH}=6170$.

Found: C, 79.68; H, 10.49. Calcd. for $C_{24}H_{38}O_2$ (358.568): C, 80.39; H, 10.68%.

Preparation of 3α-Hydroxy-$\Delta^{8(14)}$-cholenic Acid.—*Methyl 3α-Ethoxycarbonyloxy-12α-hydroxy-$\Delta^{8(14)}$-cholenate (Vc).*—Methyl apocholate (5 g.) (Vb) was dissolved in 25 ml. of dioxane and 4 ml. of pyridine, and then 5 ml. of ethyl chlorocarbonate was added with cooling. The reaction mixture was allowed to stand at room temperature for 30 min. and then diluted with water; 2 ml. of concentrated hydrochloric acid was added and heated on a water bath for 30 min. The product was extracted with ether and the ether extract was washed with water, dried over sodium sulfate and then evaporated in vacuo.

3) P. Bladon, H. B. Henbest, and G. W. Wood, *J. Soc., Chem.*, **1952**, 2737.
4) E. Berner, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **30**, 1542 (1947).
5) K. Takahashi, *Z. physiol. Chem.*, **255**, 277 (1938).
6) W. Borsche and A. R. Todd, ibid., **197**, 173 (1931).
7) H. Wieland and V. Deulofeu, ibid., **198**, 127 (1931).
8) P. Bladon, J. M. Favian, H. B. Henbest, H. P. Koch and G. W. Wood, *J. Chem. Soc.*, **1951**, 2402.
9) T. Shimizu, T. Oda and H. Makino, *Z. physiol. Chem.*, **213**, 136 (1932).

Yield, 5.5 g. The product was subjected to the following procedure without crystallization.

Methyl 3α-Ethoxycarbonyloxy-12-keto-$\Delta^{8(14)}$-cholenate (VI).—The above-mentioned ester (5.5 g.) was dissolved in 550 ml. of acetone and the CrO_3–H_2SO_4 mixture of Bladon et al.[8] was added carefully in 3 min. at 30~35°C, and the excess of chromic acid was reduced with methanol. The mixture was diluted with water and a crystalline matter deposited. After several recrystallizations from methanol a pure substance melting at 116~117°C was obtained. Yield, 2 g.

Found: C, 70.67; H, 9.09. Calcd. for $C_{28}H_{42}O_6$ (474.644): C, 70.85; H, 8.91%.

Methyl 3α-Acetoxy-$\Delta^{8(14)}$-cholenate (VIIc).—Methyl 3α-ethoxycarbonyloxy-12-keto-$\Delta^{8(14)}$-cholenate (1.2 g.) (VI) was subjected to the Huang-Minlon reduction (triethylene glycol, 12 ml.; potassium hydroxide, 1.2 g.; and 80% hydrazine hydrate, 1.2 ml.). The reaction mixture was acidified with dilute hydrochloric acid and a crystalline precipitate was collected. Yield, about 1 g. The product was converted into methyl ester (amorphous) with an ethereal solution of diazomethane, and the ester in 10 ml. of acetic anhydride was heated for 1 hr. on a water bath. After several recrystallizations from methanol, colorless crystals of m. p. 81~82°C were obtained. Yield, 0.5 g. $\alpha_D^{27}=+56\pm2°$ (chf.); $\varepsilon_{max}^{EtOH}=5410$ ($\lambda_{max}=215$ mμ).

Found: C, 75.09; H, 9.77. Calcd. for $C_{27}H_{42}O_4$ (430.633): C, 75.30; H, 9.83%.

3α-Hydroxy-$\Delta^{8(14)}$-cholenic Acid (VIIa).—The acetyl methyl ester mentioned above was dissolved in 2% alcoholic solution of potassium hydroxide and let stand at room temperature for 24 hr. The free acid so obtained was recrystallized from methanol. Colorless plates of m. p. 167~168°C; $\alpha_D^{15}=+41\pm2°$ (chf.); $\varepsilon_{max}^{EtOH}=5550$ ($\lambda_{max}=214$ mμ).

Found: C, 77.01; H, 10.24. Calcd. for $C_{24}H_{38}O_3$ (374.568): C, 76.95; H, 10.22%.

Taurine-conjugation of 3α-Hydroxy-$\Delta^{8(14)}$-cholenic Acid.—The above-mentioned acid (100.25 mg.) was treated with a mixture of tri-*n*-butylamine (0.06 ml.) in 0.5 ml. of dioxane and ethyl chlorocarbonate (0.024 ml.). The mixture was allowed to stand at room temperature for 15 min., and cooled down to 10°C; a solution of taurine (33.6 mg.) in 0.25 ml. of 1 N sodium hydroxide was added and left for 1 hr. One milliliter of water was added and then the solvent was removed by reduced pressure. The residue was dissolved in water and acidified with dilute hydrochloric acid. Crystalline substance so obtained was collected and recrystallized from dilute alcohol. M. p. 207°C. Yield, 30 mg.

Found: N, 2.87. Calcd. for $C_{26}H_{43}O_5NS$ (481.701): N, 2.90%.

"Tauroapochenodeoxycholic acid" of Takahashi[5] was reported to melt at 210°C.

Isomerization of Methyl 3α-Acetoxy-Δ^7-cholenate into its $\Delta^{8(14)}$-Isomer.—*Methyl 3α-Acetoxy-$\Delta^{8(14)}$-cholenate (VIIc).*—Methyl 3α-acetoxy-Δ^7-cholenate (m. p. 123~124°C) (300 mg.) (X) in 10 ml. of glacial acetic acid was shaken with platinum dioxide (300 mg.) for 30 hr. in hydrogen atmosphere. The catalyst was filtered off. The filtrate was diluted with water and crystals separated.

The first crop (230 mg.) melted at 90~95°C and repeated recrystallization gave 80 mg. of the pure starting material (m. p. 123~124°C).

From the combined filtrate fractions of recrystallization a crude crystalline matter (m. p. of 90~100°C) weighing 120 mg. was obtained, and a carefull repetition of recrystallization afforded silky crystals of m. p. 84~85°C, $\alpha_D^{23}=+61\pm2°$ (chf.). They showed no melting point depression on admixture with methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate mentioned above.

Found: C, 75.19; H, 9.69. Calcd. for $C_{27}H_{42}O_4$ (430.633): C, 75.30; H, 9.83%.

3α-Hydroxy-$\Delta^{8(14)}$-cholenic Acid (VIIa).—The above-mentioned ester (220 mg.) was dissolved in 10 ml. of methanol with 3 ml. of 10% solution of potassium hydroxide, and refluxed on a water bath for 30 min. After cooled, the product was deposited with dilute hydrochloric acid and collected. Yield, 130 mg. The free acid was recrystallized from methanol and melted at 168~169°C, exactly like the acid prepared from apocholic acid. $\alpha_D^{25}=+33\pm5°$ (di.).

Preparation of 3α-Hydroxy-Δ^{14}-cholenic Acid.—*Methyl 3α,12α-Dihydroxy-Δ^{14}-cholenate (IIIa).*—3α,12α-Dihydroxy-Δ^{14}-cholenic acid (700 mg.) was methylated with ethereal solution of diazomethane as usual. Colorless prisms of m. p. 91~92°C. Yield, 500 mg.

Methyl 3α-Ethoxycarbonyloxy-12α-hydroxy-Δ^{14}-cholenate (IIIb).—Methyl 3α,12α-dihydroxy-Δ^{14}-cholenate was dissolved in 0.8 ml. of pyridine, 1.0 ml. of ethyl chlorocarbonate was added with cooling in ice-water, and allowed to stand at room temperature for 2 hr. On addition of water, the reaction mixture was extracted with ether, and the extract was washed with water, dried over sodium sulfate and then evaporated to dryness. Crystallization from methanol gave 400 mg. of a pure ethoxycarbonyloxy compound (m. p. 150°C).

Found: C, 70.49; H, 9.34. Calcd. for $C_{28}H_{44}O_6$ (476.66): C, 70.55; H, 9.30%.

It must be pointed out here that under the same condition as methyl apocholate was treated (see above), the conversion of the isomer IIIa into the ethoxycarbonyloxy compound did not occur, almost all the starting material being recovered unchanged. It seemed to be harmful to add dioxane to the reaction mixture.

Methyl 3α-Ethoxycarbonyloxy-12-keto-Δ^{14}-cholenate (IV).—Methyl 3α-ethoxycarbonyloxy-12α-hydroxy-Δ^{14}-cholenate (350 mg.) (IIIb) in 50 ml. of acetone was oxidized exactly as the corresponding ester of apocholic acid was done. The oxidation product was recrystallized from methanol. M. p. 144~145°C; yield, 300 mg.

Found: C, 70.95; H, 8.91. Calcd. for $C_{28}H_{42}O_6$ (474.644): C, 70.85; H, 8.91%.

3α-Hydroxy-Δ^{14}-cholenic Acid (IIa).—The above-mentioned ketocholenate (250 mg.) was reduced according to the Huang-Minlon method and the crude product was purified as acetyl methyl ester (see below), and hydrolyzed in 2% of alcoholic potassium hydroxide. The free acid was recrystallized from acetone-water. M. p. 171~172°C. $\alpha_D^{14}=+65\pm2°$ (chf.); $\varepsilon_{max}^{EtOH}=2270$ ($\lambda_{max}=210$ mμ).

Found: C, 76.39; H, 10.17. Calcd. for $C_{24}H_{38}O_4$ (374.568): C, 76.96; H, 10.22%.

The physical constants of the corresponding product isomerized from methyl 3α-hydroxy-Δ^7-cholenate (Osawa et al.[1]) are as follows: m. p. 161°C, $\alpha_D^{24}=+62.8°$ (chf.).

Methyl 3α-Hydroxy-Δ^{14}-cholenate (IIb). — 3α-Hydroxycholenic acid above mentioned (20 mg.) was methylated with diazomethane as usual. The ester was recrystallized from methanol and melted at 141~142°C, undepressed on admixture with the sample obtained by Osawa et al.[1]

Found: C, 76.86; H, 10.28. Calcd. for $C_{25}H_{40}O_3$ (388.595): C, 77.27; H, 10.37%.

Methyl 3α-Acetoxy-Δ^{14}-cholenate (IIc).—Methyl 3α-hydroxy-Δ^{14}-cholenate (200 mg.) was acetylated with a mixture of acetic anhydride (3 ml.) and pyridine (0.2 ml.) as usual.

Recrystallizations from methanol gave a pure sample melting at 106~107°C, $\alpha_D^{14}=+87\pm2°$ (chf.). $\varepsilon_{max}^{EtOH}=2230$ ($\lambda_{max}=209$ mμ).

Found: C, 75.45; H, 9.90. Calcd. for $C_{27}H_{42}O_4$ (430.633): C, 75.30; H, 9.83%.

The physical properties of the product are in fairly good agreement with those of the sample obtained by Osawa et al.[1] (M. p. 110°C; $\alpha_D^{29}=+86.7°$ (chf.)).

Of the experiments mentioned above, the conversion of methyl 3, 12-diketo-$\Delta^{8(14)}$-cholenate as well as the isomerization of methyl 3α-acetoxy-Δ^7-cholenate were carried out by one of us (K. Y.) in Fieser's Laboratory during his stay at the Harvard University, U. S. A.

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