Dehydration of Bile Acids and their Derivatives. VII. Isomerization of Methyl 3α-Hydroxy-Δ^7-cholenate to Methyl 3α-Hydroxy-Δ^{14}-cholenate

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Yamasaki[1] reported previously that the action of concentrated hydrochloric acid on chenodeoxycholic acid gave three isomeric 3α-hydroxycholenic acids, but the localization of their double bonds has not yet been settled. In order to obtain any further information about these acids, the authors have attempted to isomerize 3α-hydroxy-Δ^7-cholenic acid derived from chenodeoxycholic acid and to compare the product obtained with the isomers reported by Yamasaki[1]. Methyl 3α-ethoxycarbonyloxy-7α-hydroxycholanate (Ib) was prepared from methyl chenodeoxycholate (Ia) according to Fieser et al.[2] and dehydrated with phosphorus oxychloride in pyridine[3] to give methyl 3α-ethoxycarbonyloxy-Δ^7-cholenate[4] in a fairly good yield, m. p. 136°C, $\alpha_D^{24}=+81.20°$ (Chf.) [Herz[5]: m. p. 126°C, $\alpha_D^{21}+80\pm2°$ (Di)].

Methyl 3α-hydroxy-Δ^7-cholenate (IIc)

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[1] K. Yamasaki and K. Takahashi, *Z. physiol. Chem.*, **256**, 21 (1938); K. Yamasaki, *J. Chem. Soc. Japan, Pure Chm. Sec.* (*Nippon Kagaku Zasshi*), **72**, 680 (1951).

[2] L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romero and T. Utne, *J. Am. Chem. Soc.*, **74**, 3309 (1952).

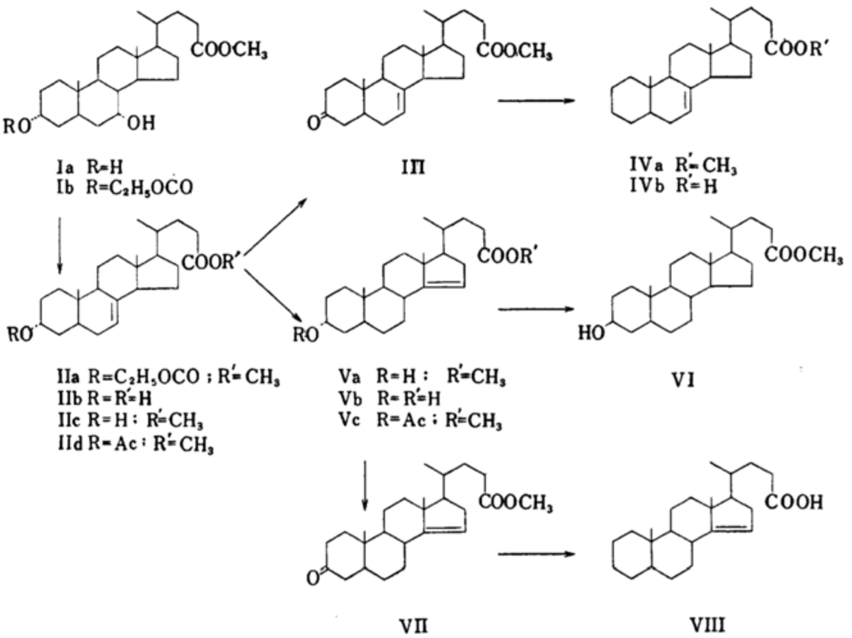
[3] E. Berner, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **30**, 1542 (1947).

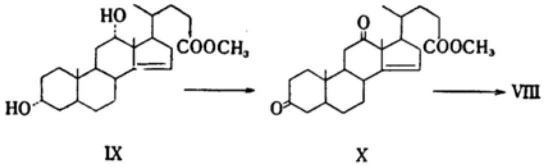
[4] Although the proposed position (Δ^7) of the double bond of this acid has not yet been studied, the position is highly probable, because the C_7 OH-group of chenodeoxycholic acid is apt to be dehydrated (*trans*-dehydration) exactly like cholic acid[3], and any possible shift of the double bond then formed can be excluded under the condition here described.

[5] J. E. Herz, A thesis submitted to the Department of Chemistry, Harvard University (1951).

TABLE I. 3α-HYDROXYCHOLENIC ACIDS DERIVED FROM CHENODEOXYCHOLIC ACID

3α-Hydroxy-cholenic acid	m. p. °C	$[\alpha]_D$	Methyl ester, m. p., °C	Methyl ester acetate, m. p. °C
β-Apochenodeoxycholic acid[1]	195～196	+83.67° (alc.)	—	83～84
γ-Apochenodeoxycholic acid[1]	182～183	+101.73° (MeOH)	—	77～78
Chenocholenic acid[1]	211～213	+30.0° (MeOH)	—	—
3α-Hydroxy-Δ^7-cholenic acid	189～190	+62.0° (Di.)	108～109	127
3α-Hydroxy-Δ^{14}-cholenic acid	160	+62.8° (Chf.)	140～141	110





was subjected to isomerization by the action of dry hydrogen chloride gas[6], and a crystalline mixture was obtained. A fraction melting higher than the starting material was purified to afford crystals of m. p. 140～141°C, $\alpha_D^{20}=+55.8°$ (Chf.). The isomerized ester showed a distinct depression of the melting point on admixture with the starting substance and gave a negative selenium dioxide test, while the latter did a positive test, contrary to Fieser's report[7].

The corresponding free acid dissolved in ethanol showed an absorption maximum at 211 mμ with an extinction coefficient of $\varepsilon_{\max}^{\text{EtOH}}$ 2390, while $\Delta^{8(14)}$-cholenic acid as a reference substance, obtained from apocholic acid[8], showed an extinction coefficient of $\varepsilon_{\max}^{\text{EtOH}}=6170$. According to Bladon et al.[9], this finding suggests that the new 3α-hydroxycholenate possesses either a tri- or a disubstituted ethylenic grouping. As was expected, the ester absorbed one mole of hydrogen on catalytic hydrogenation to give methyl lithocholate (VI) (m.p. 130°C). These results provide a strong evidence for the Δ^{14}-structure (V) of the new acid.

In order to ascertain the structure, the 3-hydroxy-ester (Va) was transformed into a cholenic acid by the Huang-Minlon reduction[10] of the corresponding 3-keto-ester (VII). The cholenic acid obtained was identified with Δ^{14}-cholenic acid (VIII) which was derived from 3α, 12α-dihydroxy-Δ^{14}-cholenic acid[11] (IX) through its dehydro-acid (X).

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[6] K. Yamasaki, *Z. physiol. Chem.*, **233**, 10 (1935).

[7] L. F. Fieser, *J. Am. Chem. Soc.*, **75**, 4395 (1953).

[8] To be published.

[9] P. Bladon, H. B. Henbest and G. W. Wood, *J. Chem. Soc.*, **1952**, 2737.

[10] Huang-Minlon, *J. Am. Chem. Soc.*, **71**, 3301 (1949).

[11] Cf. L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene", 3rd Ed., Reinhold Publishing Corp., New York (1949), p. 118.

Thus it is concluded that the isomerized product from methyl 3α-hydroxy-Δ^7-cholenate (IIc) is methyl 3α-hydroxy-Δ^{14}-cholenate (Va), but, as shown in Table I, the 3α-hydroxy-Δ^{14}-cholenic acid (Vb) was found to be different from any of the three 3α-hydroxycholenic acids obtained by Yamasaki[1]. The expected presence of the isomeric 3α-hydroxy-$\Delta^{8(14)}$-cholenate together with the Δ^{14}-ester in the isomerization product is now under investigation.

Experimental

Preparation of 3α-Hydroxy-Δ^7-cholenic Acid and Derivatives. — *Methyl 3α - ethoxycarbonyloxy-7α-hydroxycholanate (Ib).* — Two grams of methyl chenodeoxycholate (Ia) (the free acid, m.p. 140°C, was prepared according to Fieser and Rajagopalan[12] from cholic acid and the non-crystalline methyl ester was prepared with diazomethane as ususl) dissolved in 20 ml. of dioxane containing 3.2 ml. of pyridine was treated with 4 ml. of ethyl chloroformate with cooling in ice water. After standing at room temperature for 2 hr., the mixture was diluted with water, and extracted twice with ether. The combined extract was washed with dilute hydrochloric acid and with water, dried over sodium sulfate, and evaporated to dryness in vacuo. The residue was treated with a small quantity of methanol, warmed and allowed to stand at room temperature to crystallization. Recrystallization from methanol gave the ethoxycarbonyl derivative (Ib) in colorless blades, m. p. 136°C, $\alpha^{20}_{\mathrm{D}}=+30.55^\circ$ (Chf.).

Methyl 3α-ethoxycarbonyloxy-Δ^7-cholenate (IIa).— The above ester (Ib) (1.8 g.) was dissolved in 20 ml. of pyridine, 6 ml. of phosphorous oxychloride was added, and the mixture was kept at room temperature overnight. The reaction mixture was poured cautiously into 300 ml. of ice water, and extracted 3 times with ether. The combined ethereal extract was washed with water, dilute hydrochloric acid, aqueous sodium hydroxide solution and again water, successively, and dried over sodium sulfate. Removal of the solvent gave a crystalline residue, to which was added a small quantity of methanol, and the solution was warmed and allowed to stand at room temperature to give a crystalline product, m.p. 120~121°C. Yield, 1.65 g. Recrystallization from acetone gave the product in blades, m. p. 132~133°C, $\alpha^{24}_{\mathrm{D}}=+81.2^\circ$ (Chf.).

Anal. Found: C, 73.00; H, 9.63. Calcd. for $C_{28}H_{44}O_5$ (460.32): C, 73.01; H, 9.89%.

3α-Hydroxy-Δ^7-cholenic acid (IIb). — The above ester (IIa) (1.5 g.) was dissolved in 20 ml. of methanol containing 10 ml. of 10% aqueous potassium hydroxide and the solution was refluxed for 1.5 hr. The hydrolyzate was diluted with water and acidified with dilute hydrochloric acid. The precipitates were collected, washed with water and dried to give the free acid, m. p. 183 ~184°C. Yield, 1.17 g. Recrystallization from ethyl acetate and methanol gave the substance in needles, m. p. 189~190°C, $\alpha^{21}_{\mathrm{D}}=+62.0^\circ$ (Di). The selenium dioxide test was positive.

Anal. Found: C, 76.64; H, 10.05. Calcd. for $C_{24}H_{38}O_3$ (374.54): C, 76.94; H, 10.23%.

Methyl 3α-hydroxy-Δ^7-cholenate (IIc).—The above acid (IIb) (m. p. 190~191°C, 0.5 g.) was refluxed with 10 ml. of methanol containing two drops of concentrated hydrochloric acid for 1/2 hr., and the solution was concentrated by passing a stream of air to crystallization. After completion of crystallization, the crystals were collected and washed with a small volume of methanol, m. p. 104~105°C. Yield, 0.49 g.

Recrystallized twice from aqueous methanol, the substance was obtained in glistening leaflets, m. p. 108~109°C, $\alpha^{24}_{\mathrm{D}}=+61.8\pm1.4^\circ$ (Chf.).

Methyl 3α-acetoxy-Δ^7-cholenate (IId).—The above ester (IIc) (200 mg.) in 4 ml. of pyridine was treated with 2 ml. of acetic anhydride. After standing for 24 hr., water and concentrated hydrochloric acid (3.5 ml.) were added and the mixture was warmed for 20 min. on a steam bath. The solid was collected, and weighed 200 mg. Recrystallized once from methanol, the substance was obtained in glistening plates, m. p. 126~127°C, $\alpha^{23}_{\mathrm{D}}=+86.0^\circ$ (Chf.), λ_{max} 211 mμ, $\varepsilon^{\mathrm{EtOH}}_{\mathrm{max}}$ 2075.

Anal. Found: C, 74.80; H, 9.64. Calcd. for $C_{27}H_{42}O_4$ (430.61): C, 75.30; H, 9.85%.

Methyl 3-keto-Δ^7-cholenate (III). — Methyl 3α-hydroxy-Δ^7-cholenate (IIc) (800 mg.) was oxidized in acetone with the chromic acid-H_2SO_4 mixture according to Baldon et al.[13] When the reaction was completed, the solution was diluted with water. After completion of crystallization in an ice box, the crystals were collected, m. p. 123~124°C. Yield, 770 mg. Recrystallized once, the substance was obtained in a pure state, m. p. 125°C, $\alpha^{23}_{\mathrm{D}}=+53.3$ (Chf.).

Anal. Found: C, 77.22; H, 9.77. Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.67; H, 9.91%.

Methyl Δ^7-cholenate (IVa). — Methyl 3-keto-Δ^7-cholenate (III) (0.51 g.) was heated with a mixture of triethylene glycol (30 ml.), hydrazine hydrate (1.5 ml.) and potassium hydroxide (1 g.) by the modified Wolff-Kishner method (Huang-Minlon[10] reduction).

The reaction mixture was diluted with water and acidified with dilute hydrochloric acid. The solid was collected, washed with water, and dried. The crude acid in methanol was treated with an ether solution of diazomethane and concentrated. The first crop (0.37 g.) showed m. p. 95.5~97°C, and the second (0.05 g.), m. p. 85~86°C.

Recrystallization of the combined crops from methanol-acetone gave thin rectangular plates of m. p. 98~99°C, undepressed by the sample obtained by the phosphorous oxychloride dehydration of methyl 7-hydroxycholanate (Herz[5]); $\alpha^{23}_{\mathrm{D}}=+53\pm3^\circ$ (Chf.) [$\alpha^{21}_{\mathrm{D}}=+56\pm2^\circ$ (Di.) (Herz[5])].

Anal. Found: C, 80.25; H, 10.58. Calcd. for $C_{25}H_{40}O_2$ (372.57): C, 80.62; H, 10.82%.

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[12] L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **72**, 5530 (1950).

[13] P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch and G. W. Wood, *J. Chem. Soc.*, **1951**, 2402.

Δ^{7}-Cholenic acid (IVb).—The above ester (IVa) (100 mg.) was refluxed for 1 hr. with 7 ml. of methanol and 3 ml. of 10% aqueous potassium hydroxide, and the reaction mixture was diluted with water and acidified with dilute hydrochloric acid. The crystalline solid was collected. Recrystallized twice from methanol, Δ^{7}-cholenic acid was obtained in a pure state, m. p. 150～151°C, undepressed on admixture with the specimen (m. p. 151°C) prepared by Herz[5].

Anal. Found: C, 80.78; H, 10.47. Calcd. for $C_{24}H_{38}O_2$ (558.54): C, 80.80; H, 10.68%.

Isomerization of Methyl 3-Hydroxy-Δ^{7}-cholenate. —*Methyl 3α-hydroxy-Δ^{14}-cholenate (Va).*—A stream of dry hydrogen chloride gas was passed into a solution of methyl hydroxy-Δ^{7}-cholenate (IIc) (1.0 g.) in anhydrous chloroform (20 ml.) for 2 hr. at 0°C. The solution was diluted with chloroform with cooling in ice water, washed with water, soda solution and again with water, successively, and dried over sodium sulfate. Removal of the solvent gave a crystalline residue. This was dissolved in 1 ml. of benzene, passed throngh a Brockmann-alumina column and eluted with petroleum ether, benzene, ether and methanol, successively. The first portions of the elute contained the original substance, and a substance of higher melting point was obtained from the fractions eluted with ether. Rechromatography of the fractions of the higher melting point gave an ester of m. p. 139°C, and recrystallization from ethanol-water afforded crystals of m. p. 140～141°C, $\alpha^{20}_{\mathrm{D}}=+55.8^\circ$ (Chf.), which showed a depression in the melting point on admixture with the starting substance and was negative in the selenium dioxide test.

Anal. Found: C, 77.40; H, 10.13. Calcd. for $C_{25}H_{40}O_3$ (388.32): C, 77.69; H, 9.91%.

Methyl 3α-acetoxy-Δ^{14}-cholenate (Vc). — Methyl 3α-hydroxy-Δ^{14}-cholenate (Va) (m. p. 141°C, 100 mg.) in 2 ml. of pyridine was treated with 1 ml. of acetic anhydride. After standing for 24 hr., water and concentrated hydrochloric acid (3.5 ml.) were added to the reaction mixture, and the whole was warmed for 20 min. on a steam bath. The solid was collected. Yield, 100 mg. Recrystallized once from methanol, the product was obtained in glistening leaflets of m. p. 110°C, $\alpha^{20}_{\mathrm{D}}=+86.7^\circ$ (Chf.).

Anal. Found: C, 75.30; H, 9.85. Calcd. for $C_{27}H_{42}O_4$ (430.61): C, 75.30; H, 9.85%.

3α-Hydroxy-Δ^{14}-cholenic acid (Vb).—Methyl 3α-hydroxy-Δ^{14}-cholenate (Va) (100 mg.) was dissolved in 7 ml. of methanol containing 3 ml. of 10% aqueous potassium hydroxide and the mixture was refluxed for 1 hr. The solution was acidified with dilute hydrochloric acid. The crystalline solid was collected. Recrystallized twice from methanol containing ethyl acetate, the substance was obtained in a pure state, m. p. 161°C, $\alpha^{24}_{\mathrm{D}}=+62.8^\circ$ (Chf.), $\lambda_{\max}$ 211 mμ, $\varepsilon^{\mathrm{EtOH}}_{\max}$ 2390.

Anal. Found: C, 76.27; H, 10.37. Calcd. for $C_{24}H_{28}O_3$ (374.54): C, 76.94; H, 10.23%.

Methyl lithocholate (VI).—Methyl 3α-hydroxy-Δ^{14}-cholenate (Va) (m. p. 141°C, 50 mg.) in 10 ml. of glacial acetic acid was shaken for 3 hr. with 10 mg. of PtO_2 (Adams) and hydrogen, 3.1 ml. of hydrogen being absorbed (the calculated amount: 3.07 ml.). Concentration of the solution, addition of a small amount of methanol and heating gave needles. Recrystallization from methanol afforded a sample of m. p. 130°C, $\alpha^{20}_{\mathrm{D}}=+24.2^\circ$ (Chf.). This showed no depression in the melting point on admixture with authentic methyl lithocholate (m. p. 129～130°C).

Anal. Found: C, 77.48; H, 10.17. Calcd. for $C_{25}H_{42}O_2$ (375.58): C, 76.78; H, 10.83%.

Methyl 3-keto-Δ^{14}-cholenate (VII). — Methyl 3α-hydroxy-Δ^{14}-cholenate (Va) (m. p. 141°C, 200 mg.) in 20 ml. of acetone was oxidized with the chromic acid-H_2SO_4 mixture according to Bladon et al.[13] After completion of oxidation, the excess of the oxidant was destroyed by addition of a small quantity of methanol. The solution was diluted with water and extracted with ether. The ether extract was washed with water, soda solution and again with water, successively, dried over sodium sulfate and evaporated. The residue was triturated with a small quantity of methanol, and kept at room temperature. The yield of colorless needles, m. p. 78～79°C, was 155 mg. Recrystallized twice from aqueous methanol, the substance was obtained in a pure state, m. p. 89～90°C, $\alpha^{24}_{\mathrm{D}}=+38.8^\circ$ (Chf.).

Anal. Found: C, 77.40; H, 10.13. Calcd. for $C_{25}H_{38}O_3$ (386.55); C, 77.69, H. 9.09%.

Δ^{14}-Cholenic acid (VIII).—Reduction of methyl 3-keto-Δ^{14}-cholenate (VII) (100 mg.) was conducted according to the modified Huang-Minlon procedure[10] with a mixture of triethylene glycol (10 ml.), hydrazine (1 ml.) and potassium hydroxide (1 g.). The reaction mixture was diluted with water and acidified with dilute hydrochloric acid. The solid was collected, washed with water, dried over sodium sulfate, and evaporated. Recrystallization from ethyl acetate and then from aqueous methanol afforded colorless needles of m. p. 143～144°C, $\alpha^{22}_{\mathrm{D}}=+61.8^\circ$ (Chf.), $\varepsilon^{\mathrm{EtOH}}_{\max}$ 3404.

Anal. Found: C, 80.44, H, 10.71. Calcd. for $C_{24}H_{38}O_2$ (358.54): C, 80.39; H, 10.68%.

Derivation of Δ^{14}-cholenic Acid from 3, 12-Dihydroxy-Δ^{14}-cholenic Acid.—*Methyl 3, 12-diketo-Δ^{14}-cholenate (X).*—Methyl 3, 12-dihydroxy-Δ^{14}-cholenate (IX) (200 mg.) was oxidized with the chromic acid-H_2SO_4 mixture according to Bladon et al.[13] Recrystallization from methanol gave fine needles of m. p. 134°C, $\alpha^{22}_{\mathrm{D}}=+60.3$ (Chf.). Yield, 0.175 g.

Anal. Found: C, 75.18; H, 9.15. Calcd. for $C_{25}H_{36}O_4$ (400.56): C, 74.96; H, 9.06%.

Δ^{14}-Cholenic acid (VIII).—The above diketoester (X) (200 mg.) was reduced by the Huang-Minlon procedure[10] (25 ml. of triethylene glycol, 2 ml. of hydrazine hydrate, 2 g. of potassium hydroxide). Recrystallization from acetone and then from acetic acid gave crystals of m.p. 142～143°C, $\alpha^{20}_{\mathrm{D}}=+62.1^\circ$ (Chf.). This acid showed no depression of the melting point on admixture with the specimen obtained above from 3α-hydroxy-Δ^{14}-cholenic acid.

Anal. Found: C, 79.59; H, 10.75. Calcd. for $C_{24}H_{38}O_2$ (358.54): C, 80.39; H, 10.68%.

The preparation of methyl Δ^7-cholenate from methyl 3α-hydroxy-Δ^7-cholenate was carried out, when Yamasaki was working in L. F. Fieser's Laboratory, Harvard University. The elementary analyses were carried out by Mr. Okada of the Analysis Room of Shionogi Laboratory, to whom the authors' gratitude is due.

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