U. D. C. 547.92 : 542.944.

66. **Ken'ichi Takeda, Taichiro Komeno, and Kikuo Igarashi :** Bile Acids and Steroids VII.* Bromination of Methyl 3α-Acetoxy-7, 12-dioxocholanate.

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In 1945, Reichstein and Grand[1] obtained methyl 3α-acetoxy-x-bromo-7, 12-dioxocholanate by the bromination of methyl 3α-acetoxy-7, 12-dioxocholanate (I), but could not determine the position and the configuration of the bromine atom introduced.

In the present communication are described the dibromination of (I) and the dehydrobrmination of the mono- and dibromides of (I).

Methyl 3α-acetoxy-7, 12-dioxocholanate (I) was predominatingly brominated to a monobromide (II), m.p. 180°, with 1 mole of bromine in glacial acetic acid, in the same manner as reported by Reichstein and Grand. Since this compound agreed with their monobromide in respect to the melting point and the crystal form, the two compounds should be identical. The ultraviolet spectrum of (II) shows the absorption maximum at 288 mμ and the infrared spectrum exhibits peaks at 5.79 and 5.87 μ, while the ultraviolet and the infrared spectra of the parent diketone (I) show the absorption maximum at 291 mμ and peaks at 5.77, 5.83, and 5.88 μ. The shift[2] at 5.83 μ (C_7-ketone) to 5.79 μ in the infrared spectrum indicates that the bromine atom introduced into (II) is located at C_6 and that the configuration is α (equatorial). Furthermore, Baba and Kazuno[3] reported that monobromination of methyl 3α-ethoxycarbonyl-7, 12-dioxocholanate gave two kinds of monobromide and that these substances are epimers caused by the configuration of bromine atom at C_6. The structures of these epimeric pairs were confirmed from the fact that these substances were converted to α-hyodesoxycholic acid by saponification with alkali-methanol followed by Huang-Minlon reduction. It appears that monobromination of 7, 12-dioxo compound does not occur at C_{11} but at C_6. Therefore, it is assumed that the monobromide (II) obtained by Reichstein and the authors is methyl 3α-acetoxy-6α-bromo-7, 12-dioxocholanate.

The dibromination of (I) with two moles of bromine in acetic acid gave a dibromo compound (III), m.p. 206°, together with a small amount of the low melting compound, which could not be purified by fractional recrystallization. The ultraviolet spectrum of (III) shows the absorption maximum at 280 mμ and its infrared spectrum exhibits a single peak at 5.77 μ, which means the shift of 5.83 μ (C_7-ketone) and 5.87 μ (C_{12}-ketone) to 5.77 μ and indicates that the two bromine atoms introduced into (III) are located at C_6 and C_{11} and that both the configurations are α(equatorial). Hence it is assumed that (III) is methyl 3α-acetoxy-6α,11α-dibromo-7, 12-dioxocholanate.

Previously, the authors[4] attempted the reduction of methyl 3α, 12α-diacetoxy-6α-bromo-7-oxocholanate by the method described by Huang-Minlon[5] and obtained desoxycholic acid in a 50% yield. Nishikawa, Hagiwara, and Morita[6] obtained $\Delta^{9(11)}$-litho-

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[1] R. Grand, T. Reichstein : Helv. Chim. Acta, **28**, 344(1945).

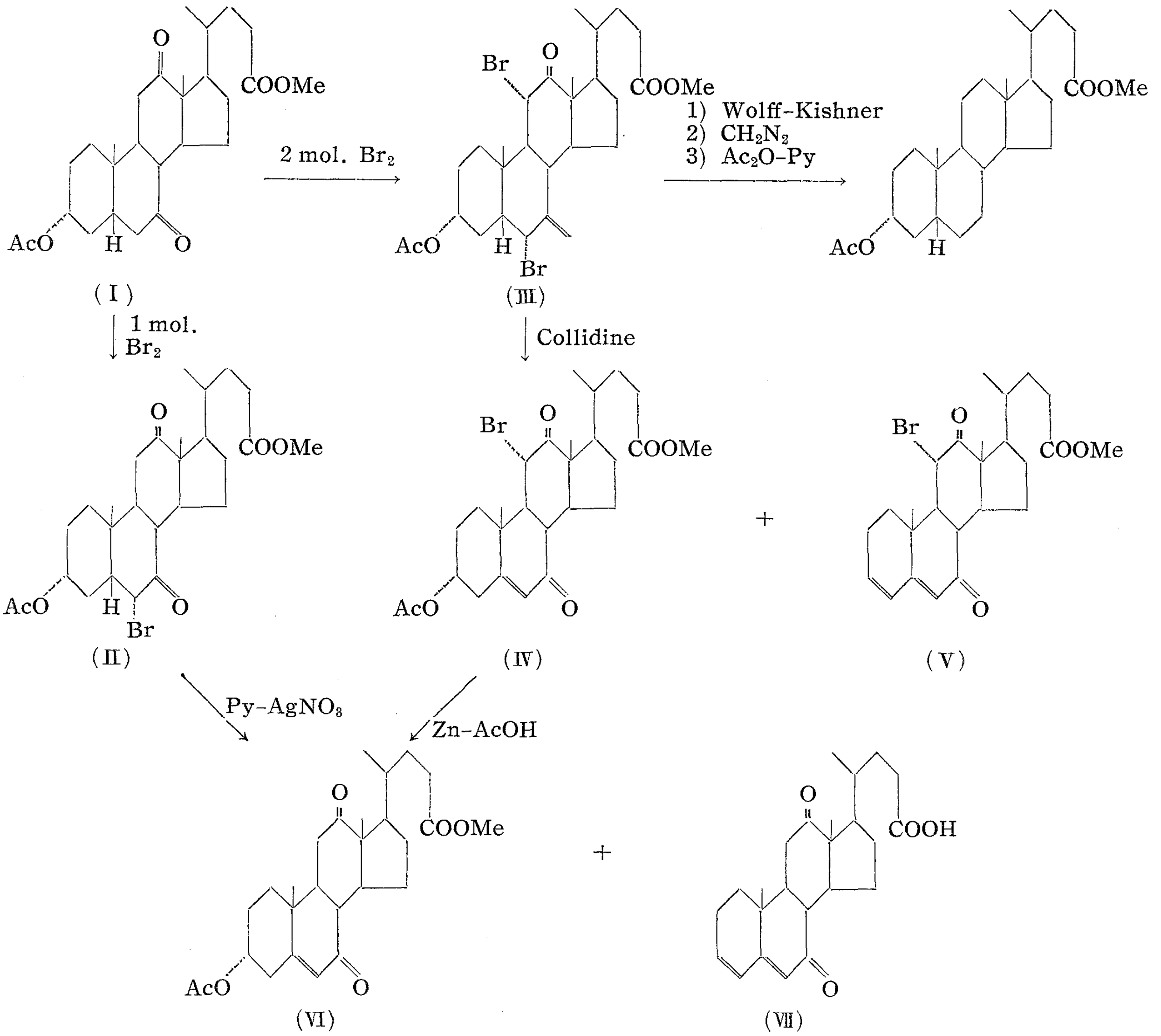
[2] R. N. Jones : J. Am. Chem. Soc., **74**, 2828(1952); E. J. Corey : *Ibid.*, **76**, 175(1954); R. C. Cookson: J. Chem. Soc., **1954**, 282; Ken'ichi Takeda, Kikuo Igarashi, Taichiro Komeno : This Bulletin, **2**, 348(1954).

[3] T. Baba, T. Kazuno : Paper read at the 27th General Meeting of the Biochemical Society of Japan, April 5, 1950.

[4] Ken'ichi Takeda, Taichiro Komeno : Unpublished.

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

[6] M. Nishikawa, H. Hagiwara, K. Morita : J. Pharm. Soc. Japan, **74**, 1081(1954).



cholenic acid by the same reduction of methyl 3α-acetoxy-11α-bromo-12-oxocholanate. Therefore, an attempt was made to reduce (III) in the same way as above and a compound, m.p. 134°, was obtained by esterification followed by acetylation. It showed a yellow color reaction with tetranitromethane, but its melting point was not depressed on admixture with methyl lithocholate acetate. Their infrared spectra were in close agreement even in finger-print regions and the presence of the $\Delta^{9(11)}$-compound could not be recognized spectroscopically.

Refluxing of (III) in collidine for 40 minutes formed about 1 mole of collidine hydrobromide and gave two compound, (IV) m.p. 185°, and (V), m.p. 205°, in a 12% and 22% yield, respectively, which were isolated by chromatography. Both (IV) and (V) still possess bromine atom. The ultraviolet spectrum of (IV) shows the absorption maximum at 235 mμ (monoenone type) and that of (V) at 278 mμ (linear dienone type). The infrared spectrum of (V) does not show a peak at about 8 μ (acetate). From these optical data, it seems probable that the elimination of the bromine atom occurred at C_6 in (III). The structure of (IV), therefore, is assumed to be methyl 3α-acetoxy-11α-bromo-Δ^5-7, 12-dioxocholenate and that of (V) is assumed to be methyl 11α-bromo-$\Delta^{3,5}$-7, 12-dioxocholadienate.

In order to confirm these structures, the authors attempted the reduction of the bromine atom in (IV) with zinc and glacial acetic acid, and obtained a compound (VI), m.p. 150°, in a 39% yield. The saponification of the mother liquor gave a free acid (VII), m.p. 190°, in a 12% yield. Both compounds did not contain bromine. While the ultraviolet spectrum of (VI) shows the absorption maximum at 234 mμ, that of (VII) shows the absorption maximum at 278 mμ.

These structures were identified by the following way. The refluxing of (II) and silver nitrate in pyridine for 12 hours gave methyl 3α-acetoxy-Δ^5-7, 12-dioxocholanate, m.p. 150°, in a 24% yield. The saponification of the mother liquor gave 7, 12-dioxo-$\Delta^{3,5}$-choladienic acid, m.p. 205°, in a 28% yield. The infrared spectra of both compounds were in close agreement respectively with (VI) and (VII). The results of the mixed melting point determination of these products also indicated the indentity of the corresponding compounds.

Reichstein, Seebeck, and Alther7) reported that the 11β-bromine atom introduced into 12-oxocholanic acid or 12-oxolithocholic acid could be eliminated by pyridine. However, this method failed to eliminate the 11α-bromine atom by pyridine and was effected by collidine. They8) also described that (II) was decomposed partly by refluxing with collidine for 3 hours but that most of (II) was recovered. These facts were duly confirmed.

From the reported experiment by Reichstein *et al.*, it seems probable that the initial dehydrobromination of (III) may occur between 11α-bromine atom (e) and 9α-hydrogen atom (a). Nevertheless, the result of the foregoing experiments revealed that the initial dehydrobromination of (III) occurred between 6α-bromine atom (e) and 5β-hydrogen atom (e) and did not occur between 11α-bromine atom (e) and 9α-hydrogen atom (a).

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Experimental9)

Monobromination of Methyl 3α-Acetoxy-7, 12-dioxocholanate (I)—To a solution of 8 g. of (I) dissolved in 40 cc. of glacial AcOH was added 3 g. of Br_2 dissolved in 20 cc. of AcOH containing several drops of boron fluoride, the mixture was warmed at 50~55° on a steam bath until Br_2 color disappeared, and then poured into water. The solid that appeared was collected by filtration and dissolved in benzene. The benzene solution was washed with aq. Na_2CO_3 solution and water, dried, and evaporated *in vacuo*. The residue was crystallized from ether to give 5 g. of leaflets, m.p. 168~173°, and 2.1 g. of needles, m.p. 140~150°. Recrystallization of the leaflets from acetone-MeOH gave (II), m.p. 178~180°; λ_{max}^{EtOH} 288 mμ (log ε 1.81); λ_{max}^{Nujol} 5.79, 5.87 μ. *Anal.* Calcd. for $C_{27}H_{39}O_6Br$: C, 60.11; H, 7.29; Br, 14.81. Found: C, 60.14; H, 7.36; Br, 14.63.

Three recrystallizations of the needles from acetone-MeOH gave needles, m.p. 153~155°. λ_{max}^{EtOH} 306 mμ (log ε 2.18). *Anal.* Found: C, 58.02; H, 7.27; Br, 18.23.

In addition to (II), the monobromination of (I) gave a small amount of an impure bromo compound, m.p. 155°, which showed the absorption maximum at 306 mμ in the ultraviolet spectrum. From the elemental analyses it was assumed to be a mixture of monobromo and dibromo compoumds.

Dibromination of Methyl 3α-Acetoxy-7, 12-dioxocholanate (I)—To a solution of 5 g. of (I) dissolved in 40 cc. of glacial AcOH was added 3.7 g. of Br_2 dissolved in 20 cc. of AcOH containing several drops of boron trifluoride, the mixture was warmed at 50~55° on a steam bath until Br_2 color disappeared, allowed to stand over night at room temp., and poured into water. The solid that appeared was collected by filtration and dissolved in benzene. The benzene solution was washed with aq. Na_2CO_3 solution and water. The acidic substance obtained by acidification of the aq. solution was extracted with ether, esterified with CH_2N_2 in ether, and combined with the benzene solution. The solution was dried and evaporated *in vacuo*. Crystallization of the residue from ether gave 5 g. of crystals, which was twice recrystallized from acetone-MeOH to 4 g. (III) as cubes, m.p. 204~206°; λ_{max}^{EtOH}

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7) H. B. Alther, T. Reichstein: Helv. Chim. Acta, **26**, 492 (1943); E. Seebeck, T. Reichstein: *Ibid.*, **26**, 536 (1943).

8) R. Grand, T. Reichstein: Helv. Chim. Acta, **28**, 344 (1945).

9) All melting points are uncorrected. Infrared spectra were measured with a Perkin-Elmer Single-beam, Model 12 C spectrophotometer in Nujol.

280 mμ (log ε 1.88); λ_{max}^{Nujol} 5.77 μ. *Anal.* Calcd. for $C_{27}H_{38}O_6Br_2$: C, 52.44; H, 6.19; Br, 25.85. Found: C, 52.79; H, 6.39; Br, 25.26.

Huang-Minlon Reduction of Methyl 3α-Acetoxy-6α, 11α-dibromo-7, 12-dioxocholanate (III)—A mixture of 3 g. of (III) and 10 cc. of 80% hydrazine hydrate in 100 cc. of triethyleneglycol was refluxed for 1 hr., and 12 g. of KOH was added. After additional refluxing for 1 hr., the solution was distilled until the temperature was raised to 190°, kept at 190~200° for 3 hrs., cooled, diluted with water, and acidified with dil. HCl. As the precipitated acid could not be crystallized with any solvents, it was esterified with CH_2N_2 in ether, acetylated with Ac_2O and pyridine, and chromatographed over Al_2O_3 in a benzene-petr. ether mixture (1:1). The eluate gave 0.4 g. of prismatic needles, m.p. 134°, which were recrystallized from MeOH. Although this substance colored with tetranitromethane, its melting point showed no depression on admixture with methyl 3α-acetoxycholanate and infrared spectra of both were in close agreement.

Dehydrobromination of Methyl 3α-Acetoxy-6α, 11α-dibromo-7, 12-dioxocholanate (III)—A mixture of 3 g. of (III) in 10 cc. of collidine was refluxed for 40 mins., ether was added to the reaction mixture, and 1 g. of the precipitated collidine hydrobromide corresponding to about 1 mole of the theoretical amount was collected by filtration. The ethereal solution was washed consecutively with dil. HCl, water, Na_2CO_3 solution, and water, dried, condensed to a small volume, and allowed to stand. About 1.5 g. of crystals that separated were recrystallized 4 times from acetone-MeOH to 0.3 g. of methyl 3α-acetoxy-11α-bromo-Δ^5-7, 12-dioxocholenate (IV) as needles, m.p. 183~185°(decomp.); λ_{max}^{EtOH} 235 mμ (log ε 4.04); λ_{max}^{Nujol} 5.79, 5.98, 6.15, 7.98, 8.03 μ. *Anal.* Calcd. for $C_{27}H_{37}O_6Br$: C, 60.33; H, 6.94; Br, 14.87. Found : C, 60.22; H, 6.95; Br, 14.49.

The residue obtained from the mother liquor of (IV) was chromatographed over Al_2O_3 as a solution in benzene-petr. ether mixture (1:2) and 0.5 g. of crystals, m.p. 194~195°(decomp.), were obtained from the eluate with benzene-petr. ether (1:2 and 1:1). The crystals were recrystallized from $CHCl_3$-MeOH to methyl 11α-bromo-$\Delta^{3,5}$-7, 12-dioxocholadienate (V) as plates, m.p. 203~205°(decomp.); λ_{max}^{EtOH} 278 mμ (log ε 4.34); λ_{max}^{Nujol} 5.80, 5.98, 6.15, 6.28 μ. *Anal.* Calcd. for $C_{25}H_{33}O_4Br$: C, 62.89; H, 6.97; Br, 16.74. Found : C, 62.48; H, 6.83; Br, 16.79.

Dehalogenation of Methyl 3α-Acetoxy-11α-bromo-Δ^5-7, 12-dioxocholenate (IV)—A mixture of 0.6 g. of (IV) and 5 g. of zinc dust in 30 cc. of glacial AcOH was refluxed for 2 hrs., filtered, and evaporated *in vacuo*. The residue was dissolved in benzene and passed through a short column of Al_2O_3. The eluate was crystallized from MeOH and 0.2 g. of crystals were recrystallized from MeOH to prisms (VI), m.p. 148~150°. The identity of this product was established by admixture with methyl 3α-acetoxy-Δ^5-7, 12-dioxocholenate and infrared spectra of both were in full agreement. λ_{max}^{EtOH} 234 mμ (log ε 4.10), λ_{max}^{Nujol} 5.76, 5.86, 5.975, 6.14, 8.00 μ. *Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found : C, 70.89; H, 8.55.

The mother liquor was saponified by refluxing with 5% KOH-MeOH for 3 hrs. The acid was crystallized from ether and recrystallized from acetone to 50 mg. of prisms, m.p. 188~190°, which was also identified by admixture with $\Delta^{3,5}$-7, 12-dioxocholadienic acid and by comparison of their infrared spectra.

Dehydrobromination of Methyl 3α-Acetoxy-6α-bromo-7, 12-dioxocholanate (II)—A mixture of 1.5 g. of (II) and 4.5 g. of $AgNO_3$ in 45 cc. of pyridine was refluxed for 12 hrs., cooled, poured into dil. HNO_3, and extracted with benzene. The extract was washed consecutively with dil. HNO_3, water, Na_2CO_3 solution, and water, condensed to a small volume, and allowed to stand. The crystals that separated were recrystallized twice from MeOH to 0.3 g. of prisms, m.p. 148~150°. λ_{max}^{EtOH} 234 mμ (log ε 4.12). *Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found : C, 71.04; H, 8.66.

The residue from the mother liquor was dissolved in benzene and passed through a short column of Al_2O_3. The ultraviolet spectrum of the eluate showed the absorption maxima at 235 and 280 mμ and so the eluate was saponified by refluxing with 5% Na_2CO_3-MeOH for 5 hrs. The acid was recrystallized from acetone to 0.3 g. of prisms, m.p. 188~190°. λ_{max}^{EtOH} 278 mμ (log ε 4.38); λ_{max}^{Nujol} 5.785, 5.96, 6.04, 6.18 6.27 μ. *Anal.* Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found : C, 75.04; H, 8.68.

Summary

1) The position of bromine atom of Reichstein's "methyl 3α-acetoxy-x-bromo-7, 12-dioxocholanate" was confirmed.

2) Dibromination of methyl 3α-acetoxy-7, 12-dioxocholanate gave 6α,11α-dibromo compound.

3) Dehydrohalogenation of this dibromo compound with collidine gave a product in which the bromine atom at C_6 had been eliminated.

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