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Autoxidation of Taurocholanic Acid in Aqueous Solution of Ferrous Ions[1)]

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Taking into account the possible effect of the polar group located in the side chain, the autoxidation of taurocholanic acid in neutralized aqueous solution was carried out with the ferrous ions-molecular oxygen system at 70° for 4 hours. The taurocholanates of the reaction mixture were collected on a column packed with XAD-2 resin, then saponified to the free bile acids, and finally esterified for convenience of purification to obtain the methylated products.

Attack of the hypothetical ferrous dioxygen complex seemed to occur in the ring D as expected, since the structure of the product B was assumed to be methyl 15α-hydroxy-5β-cholan-24-oate. Thin-layer as well as gas-liquid chromatograms of the product A were in good agreement with those of authentic methyl lithocholate, indicating that the complex can also attack the ring A from α-side of the steroidal skeleton.

Keywords——autoxidation; bile acid; ferrous ion; hydroxylation; molecular oxygen; taurocholanic acid

During the course of the studies of this series, it was reported that oxygen function was introduced into C_{15} of deoxycholic,[3)] nordeoxycholic,[4)] and taurodeoxycholic[4)] acids when their aqueous solutions were submitted to the autoxidation in the presence of ferrous ions. Although the mechanism of such oxygenation has not been clarified, it might be assumed that the carboxyl or carbonyl group in the side chain and/or C_{12}-α hydroxyl group of the substrates can be cooperative with the ferrous dioxygen complex[5)] to attack the C_{15} position where is located rather closely to these polar groups. Along with this direction, an investigation has been undertaken to determine the effect of polar group present in the side chain of steroidal substrates. The present paper deals with the introduction of oxygen function by the Fe(II)/O_2 system into taurocholanic acid (Ia) which is devoid of polar group in the steroidal skelton.

Results and Discussion

Since cholanic acid is actually insoluble in neutral water, its taurine-conjugate (Ia) was employed as a soluble substrate for the autoxidation reaction. The phosphate buffer which has been usually used in the studies of this kind was excluded, as it tends to consume ferrous

1) This paper constitutes Part VII of the series entitled "Metal Ion Catalyzed Oxidation of Steroids"; Part VI: M. Kawata, M. Tohma, T. Sawaya, and M. Kimura, *Chem. Pharm. Bull.* (Tokyo), **24**, 3109 (1976). Following trivial names are used: cholic acid, 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholan-24-oic acid; nordeoxycholic acid, 24-nor-3α,12α-dihydroxy-5β-cholan-23-oic acid; cholanic acid, 5β-cholan-24-oic acid; taurocholanic acid, tauro-5β-cholan-24-oate; methyl lithocholate, methyl 3α-hydroxy-5β-cholan-24-oate; methyl apocholate, methyl 3α,12α-dihydroxy-5β-chol-8(14)-en-24-oate.

2) Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*

3) *a*) M. Kimura, M. Kawata, M. Tohma, A. Fujino, and K. Yamazaki, *Tetrahedron Letters*, **1970**, 2021. *b*) M. Kimura, M. Kawata, M. Tohma, A. Fujino, K. Yamazaki, and T. Sawaya, *Chem. Pharm. Bull.* (Tokyo), **20**, 1883 (1972).

4) M. Kimura, A. Fujino, K. Yamazaki, and T. Sawaya, *Chem. Pharm. Bull.* (Tokyo), **24**, 2301 (1976).

5) J.E. Baldwin and J. Huff, *J. Am. Chem. Soc.*, **95**, 5757 (1973); see also references cited therein.

ions in forming the precipitates which reduce the solubility of the substrate and prevent stirring of the reaction mixture. Thus, a ferrous sulfate solution was added dropwise to the aqueous solution of Ia, which was bubbled with oxygen at 70° for four hours under stirring and was neutralized with alkali every thirty minutes. Filtrate of the reaction mixture was then passed through a column packed with XAD-2 resin which was developed with methanol to collect the steroidal reactants. After saponifying them with alkali, the hydrolysates were treated with diazomethane for the convenience of purification. Thin-layer chromatography (TLC) of the methyl esters thus obtained revealed, as shown in Fig. 1, that at least three

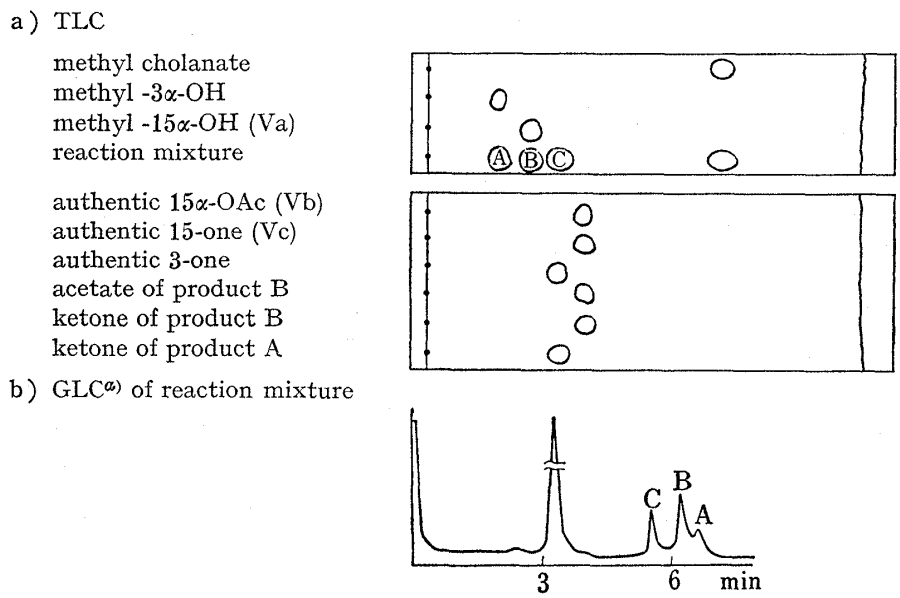

Fig. 1. Chromatograms of Products (A, B, C)[b] and Authentic Specimens

a) under the condition 1 *b*) as methyl esters,

products (A,B, and C) were formed by autoxidizing Ia in the presence of ferrous ions. Gas-liquid chromatogram (GLC) of the methylated mixture was also obtained and is shown in the same figure. The steroidal mixture was submitted to column chromatography on silica gel and the fractions were purified further by preparative TLC. *Rf* as well as t_R values of the product A and the ketone which was obtained by oxidizing A with Jones reagent were in good agreement with those of authentic methyl lithocholate and its 3-keto derivative, respectively (Fig. 1 and Table I). These results may indicate that the hydroxylation can take place at the C_3-position when the steroidal substrate has no functional group there.

Table I. Relative Retention Times of Products and Authentic Specimens

Authentic specimen	R.R.T.	t_R (min)[a]	Product[b]	R.R.T.	t_R (min)[a]
Methyl cholanate	1.00	12.20			
3α-OH	2.01	24.52	A	1.98	24.16
3-one	2.19	26.72	ketone of A	2.18	26.60
15α-OH (Va)	1.80	21.96	B	1.80	21.96
15-one (Vc)	1.72	20.98	ketone of B	1.73	21.11
			C	1.52	18.54

a) under the condition 2 *b*) as methyl ester

Introduction of hydroxyl group to the C_{15}-position of Ia was, on the other hand, reasonably anticipated, since such stereospecific hydroxylation has ever occurred in the metal catalyzed autoxidation of various bile acids.[3,4] Preparation of methyl 15α-hydroxy-5β-cholan-24-oate (Va) was, therefore, required, which would necessarily be the authentic

specimen for the second product B. The hydroxyl group at C_7 of cholic acid was eliminated by zinc chloride in acetone to give the mixture of isomeric mono-olefins (II) as shown in Chart 1. Methyl ester of the Δ^{14}-olefin thus obtained was oxidized into the diketone (III)

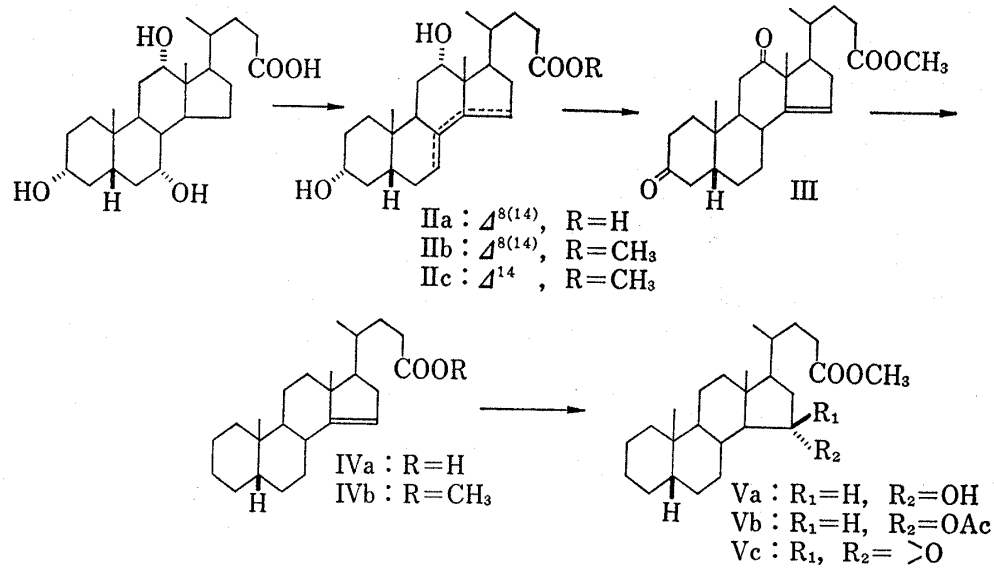

Chart 1

which was then reduced by Huang-Minlon method and methylated again to give the olefin (IVb). Hydroboration-oxidation[3b,6] of IVb gave a hydroxy derivative (Va). Mass (MS) spectrum of the ketone (Vc) which was derived by oxidizing Va revealed that the oxygen function was situated at C_{15}.[4,7] The remarkable downfield shift (−0.45 ppm) of the signal peak due to C_{18}-H, that is characteristic to C_{15}-β-OH,[3b,8] was not observed in nuclear magnetic resonance (NMR) spectrum of Va. Consequently, these results are best interpreted to indicate that the hydroxy compound thus prepared is the desired 15α-hydroxycholanate (Va).

The product B readily gave the acetate as well as the ketone by the usual methods. Their *Rf* and t_R values were in good agreement with those of the corresponding authentic specimens (Va, Vb, and Vc), respectively, as shown in Fig. 1 and Table I. The same was also in the comparison of NMR spectra of the product B and its acetate with those of Va and Vb, as summarized in Table II. Although no other comparative examinations such as those

TABLE II. Chemical Shifts of Angular Methyl Protons

Compound[a]	C_{18}–H (ppm)	C_{19}–H (ppm)	$COCH_3$ (ppm)
Methyl lithocholate (3α-OH)	0.62(+0.01)	0.91(−0.01)	
Authentic 15α-OH (Va)	0.67(+0.06)	0.93(+0.01)	
Authentic 15α-OAc (Vb)	0.60(−0.01)	0.88(−0.04)	2.01
Product B[b]	0.66(+0.05)	0.90(−0.02)	
Acetate of product B[b]	0.64(+0.03)	0.90(−0.02)	1.99
Methyl cholanate	0.61	0.92	

a) in pyridine *b*) as methyl ester

6) H.C. Brown and K.A. Keblys, *J. Am. Chem. Soc.*, **86**, 1795 (1964).
7) C. Djerassi, G. von Mutzenbecher, J. Fajkos, D.H. Williams, and H. Budjikiewicz, *J. Am. Chem. Soc.*, **87**, 817 (1965).
8) K. Tori, T. Komeno, and T. Nakagawa, *J. Org. Chem.*, **29**, 1136 (1964).

in MS and infrared (IR) spectra and on admixture of melting point were carried out because of the poor yield of B, it may be concluded from these results that the characteristic hydroxylation at C_{15}-position as well as in α-configuration occurred also in this instance.

The third product C might be anticipated to have a tertiary hydroxyl group, owing to its larger *Rf* and shorter t_R values than those of the other two products as shown in Fig. 1 and Table I. Studies on the chemical structure of this product are now in progress and further details will be reported later.

Hydroxyl group has been stereospecifically introduced into deoxycholic acid when it was treated with aqueous Fe(II)/O_2 system.[3] Taking into account the possible effect of the polar groups such as axial OH at $C_{12}\alpha$ and -CONH- as well as $-SO_3^-$ in the side chain, the autoxidation of nordeoxycholic and taurodeoxycholic acids was subsequently examined in the same system and the characteristic hydroxylation was also observed to occur at C_{15}-position and α-configuration.[4] When the substrate has no hydroxyl group in its steroidal skeleton, on the contrary, the group seems, as observed in the present study, to be rather unspecifically introduced to the ring carbon atoms. Although the predominance of the hydroxylation at C_{15} was not clarified enough in this case, it might be plausible that the positively charged species,[5] $[Fe\text{-}O_2]^{2+}$, can approach more easily to the environment of the ring D having electronegative groups in its side chain and attack the substrate (Ia) from the α-side where is rather unhindered sterically.

Experimental[9]

Materials——Taurocholanic acid (Ia), mp 243—244.5° (lit. 241—243°), was prepared according to the method of Norman.[10] The authentic specimens of methyl lithocholate, mp 128.5—129.5° (lit.[11] 127—128°), and its 3-ketone, mp 119—120° (lit.[12] 116—117°), were prepared by the usual methods. Methyl 3α,12α-dihydroxy-5β-chol-14-en-24-oate (IIc), mp 76—78°, was prepared from cholic acid through apocholate (IIb) as reported.[3,13]

Oxygenation of Taurocholanic Acid (Ia)——To a stirred aqueous solution (500 ml) of Ia (50 mg, 0.11 mmole) was added dropwise an aqueous solution (50 ml) of $FeSO_4 \cdot 7H_2O$ (2.0 g, 7.2 mmole) during 4 hr at 70° under bubbling oxygen and the solution was neutralized with alkali at every 30 min. In the same procedure, 720 mg of Ia in total amounts was treated. The reaction mixtures were combined and filtrated. The aqueous solution was poured onto a column packed with XAD-2 resin and was then eluted with MeOH. Evaporation of the methanolic eluates *in vacuo* left the residue which was saponified with 2N KOH–EtOH at 120° for 6 hr and then neutralized with 2N HCl. After evaporation of EtOH *in vacuo*, the neutral solution was acidified with 2N HCl to pH 1.0 and extracted with ether. The organic layer was worked up as usual and evaporated to give the brown residue which was then treated with diazomethane in ether–MeOH. Evaporation of the reaction mixture gave 521 mg of the methylated products.

The mixture of methyl esters (521 mg) thus obtained was submitted to chromatography on silica gel (28 g) and eluted in turn with benzene (1954 ml), benzene–$CHCl_3$ (98: 2, 300 ml), and benzene–$CHCl_3$ (90: 10, 150 ml) to give 7 fractions. The first fraction (104 ml, 266 mg of methyl cholanate) was discarded and the other fractions were combined. Evaporation of the solvent from the combined solution *in vacuo* left a residue (86 mg) which was then submitted to preparative TLC and separated into the fractions A, B, and C.

9) Melting points were taken on a micro hot-stage apparatus and are uncorrected. IR spectral measurements were run on JASCO Model IR-S spectrometer. NMR and MS spectra were measured by Hitachi Models H-6013 and RMU-6E spectrometers, respectively. TLC was carried on silica gel (Wakogel B-5F) plate by the solvent system *n*-heptane–ether (40: 60) for the methyl esters; *Rf* values were given by staining with 10% phosphomolybdic acid–MeOH and heating at 110°. Gas–liquid chromatography (GLC) was carried out by using a Shimadzu Model GC-4APF gas chromatograph equipped with a flame ionization detector and U-shaped stainless steel tube (3 mm i.d.) packed with 1.5% SE-30 on Shimalite W (60—80 mesh). N_2 was used as a carrier gas. Following column-length, -temperature, and flow rate of N_2 were employed: 2 m, 240°, 50 ml/min (condition 1) and 3 m, 220°, 30 ml/min (condition 2).

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11) A. Nickon, *J. Am. Chem. Soc.*, **79**, 243 (1957).

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

13) K. Yamazaki, *Z. Physiol Chem.*, **233**, 10 (1935); R.K. Callow, *J. Chem. Soc.*, **1936**, 462; A.W. Devor and H.W. Marlow, *J. Am. Chem. Soc.*, **68**, 2101 (1946); D.H.R. Barton, *J. Chem. Soc.*, **1946**, 1116; E. Berner, A. Lardon, and T. Reichstein, *Helv. Chim. Acta*, **30**, 1542 (1947).

Product A——The fraction A obtained as described above gave the chromatographic data coincident with those of the authentic methyl lithocholate as follows: Rf=0.16 and t_R=6.6 min (condition 1) and 24.16 min (condition 2). Ketone of Product A: The fraction A was oxidized with Jones reagent in acetone. Preparative TLC of the crude product thus obtained gave colorless needles. Rf=0.29. t_R=26.60 min (condition 2).

Product B——The brown oily fraction B (5 mg) of the preparative TLC described above was purified by column chromatography on alumina to the colorless product B. NMR (in $CDCl_3$) δ: 0.90 (3H, C_{19}–CH_3), 0.66 (3H, C_{18}–CH_3). Rf=0.23. t_R=6.2 min (condition 1) and 21.96 min (condition 2). Acetate: obtained from the product B (3 mg) with Ac_2O (2 ml) and pyridine (3 ml). NMR (in $CDCl_3$) δ: 1.99 (3H, –$OCOCH_3$), 0.90 (3H, C_{19}–CH_3), 0.65 (3H, C_{18}–CH_3). Rf=0.35. Ketone: obtained by oxidizing the fraction B with Jones reagent. Rf=0.35. t_R=21.11 min (condition 2).

Methyl 3,12-Dioxo-5β-chol-14-en-24-oate (III)——To a stirred acetone solution (20 ml) of methyl 3α,12α-dihydroxy-5β-chol-14-en-24-oate (IIc, 1.3 g), was added dropwise Jones reagent (4 ml) at 0°. After 10 min, small amount of water was added to give the precipitates which were collected and recrystallized from acetone to colorless needles (1.2 g), mp 131°. IR ν_{max}^{Nujol} cm^{-1}: 1735, 1700.

Methyl 5β-Chol-14-en-24-oate (IVb)——To a triethylene glycol solution (100 ml) of III (1.2 g), was added 90% hydrazine hydrate (12 ml) and KOH (12 g). After water was taken off at 120°, the solution was heated at 180° for 6 hr and then acidified by adding 2N HCl. The prcipitates formed were collected and reacrystallized from acetone–MeOH to colorless needles (IVa, 400 mg), mp 142—143°. NMR (in $CDCl_3$) δ: 0.91 (6H, C_{19}–CH_3 and C_{18}–CH_3), 5.15 (C_{15}–H, vinyl proton). The free acid (314 mg) thus obtained was methylated with diazomethane to give methyl ester (IVb). Mass Spectrum m/e: 373 (M^+), 257 (base peak).

Methyl 15α-Hydroxy-5β-cholan-24-oate (Va)——To a stirred mixture of $NaBH_4$ (200 mg), diglyme (4 ml), tetrahydrofuran (10 ml) and cyclohexene (21 ml), was added dropwise tetrahydrofuran solution (10 ml) of BF_3-etherate (1.6 g) during 30 min at 0° under N_2 stream. After the mixture was allowed to stand for 3 hr and then to come to room temperature, B_2H_6 formed was blowed off with violent bubbling of N_2. Tetrahydrofuran solution of IVb (314 mg) was added dropwise to the reaction mixture at 45—50°, which was then allowed to stand for 1 hr. Dicyclohexyl borane remained was decomposed by adding water (10 ml) and then 3N NaOH (4 ml) and water (3 ml) were added to the mixture. After heating at 40° for 3 hr, the reaction mixture was acidified with 2N HCl and extracted with ether. The extracts were submitted to chromatography on silica gel (10 g) and the fractions eluted with acetone–benzene were collected. Evaporation of the solvent *in vacuo* left the residue (62 mg) which was then saponified with methanolic KOH at 80° for 20 min to give the free acid (52 mg) as colorless needles, mp 193—195° (acetone). *Anal.* Calcd. for $C_{24}H_{40}O_3$: C, 76.55; H, 10.71. Found: C, 76.54; H, 10.73. NMR (in pyridine-d_5) δ: 0.95 (3H, C_{19}–CH_3), 0.71 (3H, C_{18}–CH_3). Mass Spectrum m/e: 376 (M^+), 358 (M^+-H_2O), 257 (base peak). The free acid was methylated with diazomethane to give the crystalline methyl ester (Va). NMR (in pyridine-d_5) δ: 0.93 (3H, C_{19}–CH_3), 0.67 (3H, C_{18}–CH_3), 3.55 (3H, $COOCH_3$). t_R=21.96 min (condition 2).

Methyl 15α-Acetoxy-5β-cholan-24-oate (Vb)——Acetylation of Va (5 mg) was carried out with Ac_2O (2 ml) in pyridine (5 ml) at room temperature. NMR (in pyridine-d_5) δ: 3.56 (3H, $COOCH_3$), 2.01 (3H, OAc), 0.88 (3H, C_{19}–CH_3), 0.60 (3H, C_{18}–CH_3).

Methyl 15-Oxo-5β-cholan-24-oate (Vc)——Oxidation of Va (5 mg) with Jones reagent gave the ketone (3 mg). Mass Spectrum m/e: 388 (M^+), 273 (base peak), 246, 218. t_R=20.98 min (condition 2).

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