[Chem. Pharm. Bull. 31(4)1207—1212(1983)]

Autoxidation of Cholanic Acid in the Presence of Ferrous Ions[1)]

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(Received September 27, 1982)

Autoxidation of cholanic acid in an aqueous acetone solution containing 0.2 M acetate buffer (pH 5.0) and ferrous sulfate gave three C(12)-keto products, A, B, and C. The main product A was elucidated to be 12-oxo-5β-cholan-24-oic acid. The minor product B was probably 12,23-dioxo-5β-cholan-24-oic acid. The other carbonyl function of the diketo minor product C was assumed to be situated in ring A or B at a position strongly affected by the angular C(19)-methyl group.

No autoxidation occurred when the acidic function of cholanic acid was blocked by methylation to give the ester or by reduction to the alcohol.

Keywords——cholanic acid; 12-oxo-cholanic acid; 7,12-dioxo-cholanic acid; 12,23-dioxo-cholanic acid; 24-cholanol; autoxidation; ferrous sulfate; ferrous ions-molecular oxygen system oxyfunctionalization

Previously, we reported that an oxygen function was introduced at the C(15)-position of deoxycholic,[2)] nordeoxycholic,[3)] taurodeoxycholic,[3)] and taurocholanic[4)] acids when their aqueous solutions were subjected to autoxidation in the presence of ferrous ions. We speculated that the positively charged species,[5)] $[Fe\text{-}O_2]^{2+}$, can approach the ring D with an electronegative group in the side chain and attack the substrate from the α-side, which is sterically unhidered.[4)] However, the mechanism of such regioselective oxy-functionalization remains to be proved. In this study, we report the oxygenation of cholanic acid(Ia) with the Fe^{2+}-O_2 system in an aqueous acetone solution that can dissolve steroidal substrates of sparing water solubility.

Results and Discussion

Autoxidation of cholanic acid(Ia) in aqueous acetone was carried out in the presence of ferrous sulfate for three hours at 40°C. Chromatography of the methylated reaction mixture gave three peaks, A (main, t_R 8.9), B (t_R 11.0), and C (t_R 12.5), as shown in Fig. 1. Products A, B, and C were isolated by column chromatography on alumina and purified by preparative thin-layer chromatography (TLC).

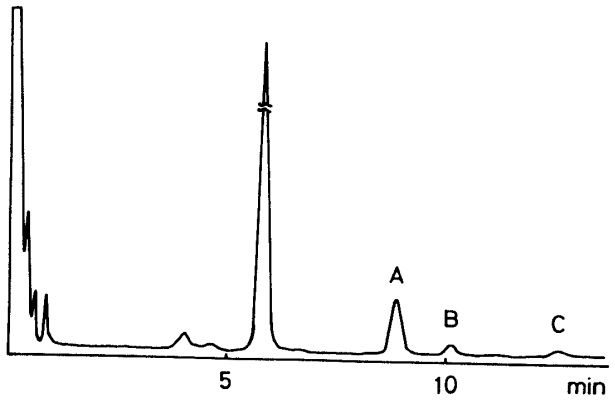


Fig. 1. Gas-Liquid Chromatogram of a Reaction Mixture

Product A, colorless needles, mp. 109.5—110°C, 180 mg, about 7% yield, gave the mass spectrum (MS) shown in Fig. 2. The parent peak at *m/e* 388 may indicate that a carbonyl function was introduced into the molecule of cholanic acid (Ia). The peak at 273 may be due to a fragment ion formed by cleavage of the side chain. An oxo group seemed, therefore, to be introduced into the steroidal skeleton of Ia. The base peak at 233 is

due to the cleavage of ring D, and is characteristic of C(12)-ketosteroids, as reported by Djerassi *et al.*[6] In the proton magnetic resonance (^{1}H NMR) spectrum of product A, the signals due to C(18)- and C(19)-methyl groups were shifted down-field by 0.38 and 0.10 ppm, respectively, as summarized in Table I. These values are similar to those shown by C(12)-ketosteroids, as reported by Bhacca *et al.*[7] From these results, a probable structure of product A was assumed to be methyl 12-oxo-5β-cholanoate (II), and thus, an authentic specimen of II was prepared, as well as specimens of the C(3)-, C(7)-, and C(15)-monoketones (V, IV, and III). The relative retention times (Rt_R) of these specimens in gas-liquid chromatography (GLC) are summarized in Table II, where product A, the C(12)-ketone, and the C(15)-ketone are shown to give the same Rt_R of 1.53. The C(15)-ketone (III) was, however, different from product A in the MS; III gave a peak at *m/e* 246 and a large peak at 218 due to cleavage of the C(15)–C(16) and C(14)–C(15) bonds, respectively.[4] The MS and ^{1}H NMR specta of the authentic C(12)-ketone (II), mp 112—113°C, were identical with those of product A as shown and summarized in Fig. 2 and Table I, respectively. Thus, the oxy-functionalization in the title reaction seemed to occur mainly at the C(12)-position of the steroidal skeleton.

TABLE I. Chemical Shifts of Angular Methyl Protons

Compound[a]	18-CH_3 (ppm)	19-CH_3 (ppm)
Methyl 3-one (V)	0.70 (0.05)	1.02 (0.09)
Methyl 7-one (IV)	0.66 (0.01)	1.18 (0.25)
Methyl 12-one (II)	1.03 (0.38)	1.03 (0.10)
Methyl 3, 7-dione (VIII)	0.71 (0.06)	1.32 (0.39)
Methyl 3, 12-dione (VII)	1.06 (0.41)	1.16 (0.23)
Methyl 7, 12-dione (VI)	1.04 (0.39)	1.30 (0.37)
Product A	1.03 (0.38)	1.03 (0.10)
Product B	0.97 (0.32)	1.03 (0.10)
Product C	1.05 (0.40)	1.29 (0.36)
Methyl cholanoate (Ib)	0.65	0.93

a) In $CDCl_3$.

TABLE II. Relative Retention Times of Products and Authentic Specimens

Authentic specimen	Rt_R	Product[a]	Rt_R
Methyl cholanoate (Ib)	1.00[b]		
Methyl 12-one (II)	1.53	A	1.53
Methyl 15-one (III)	1.53		
Methyl 7-one (IV)	1.64		
Methyl 3-one (V)	1.90	B	1.90
Methyl 7, 12-dione (VI)	2.28	C	2.16
Methyl 3, 12-dione (VII)	2.83		
Methyl 3, 7-dione (VIII)	2.84		

a) As methyl ester.
b) 5.8 min.

Since product B gave the same Rt_R of 1.90 as the authentic C(3)-ketone (V) in GLC and a peak at *m/e* 402 (M^+) in the MS as shown in Fig. 2, two carbonyl groups were assumed to be introduced into the molecule of Ia. The authentic specimens of C(3,7)-, C(3,12)-, and C(7,12)-diketones (VIII, VII, and VI) gave, however, different Rt_R values from that of product B, as summarized in Table II. In the MS of product B, a peak at *m/e* 315 due to α,β-bond cleavage of the ester indicated that one of the carbonyl groups may be located at the C(23)-position. Since the spectrum also gave the same peaks at *m/e* 273 and 233 (base) as those given by product A, the other oxo function was concluded to be at the C(12)-position. In the ^{1}H NMR of product B, the signals due to the C(18)- and C(19)-methyl groups were

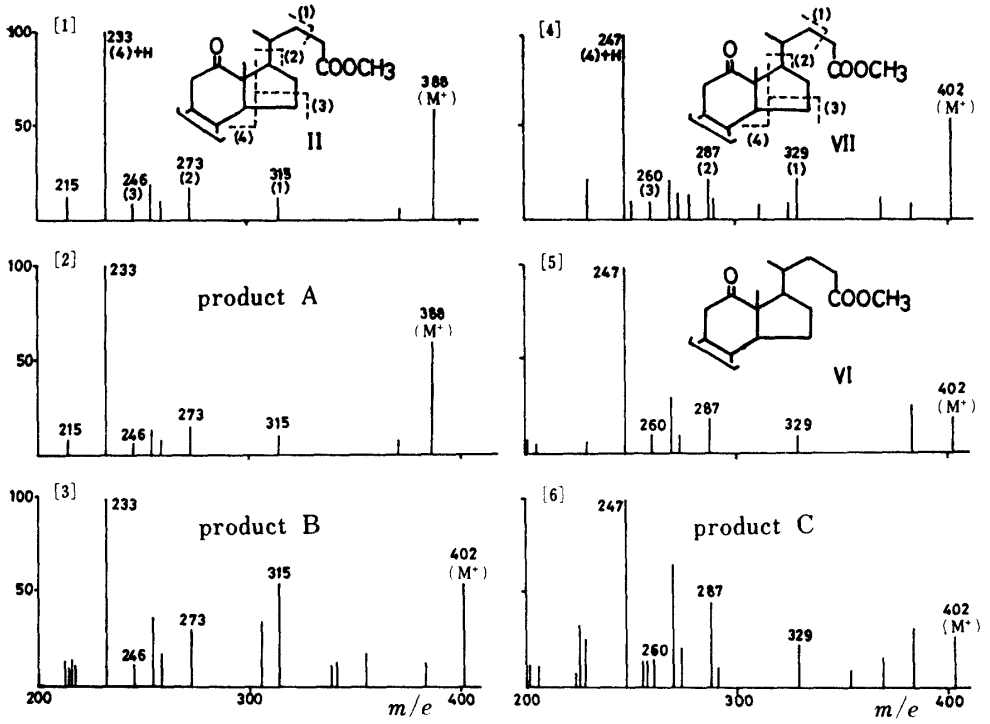


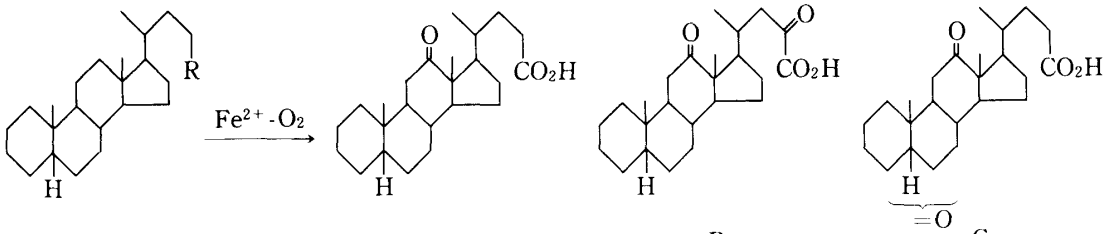
Fig. 2. Mass Spectra of Products and Authentic Specimens

shifted downfield by 0.32 and 0.10 ppm, respectively, similarly to those shown by the C(12)-monoketone (II) and as summarized in Table I. The chemical structure of product B may, therefore, be methyl 12,23-dioxo-5β-cholanoate; the oxy-functionalization in the title reaction also seemed to occur in the side chain of the steroidal skeleton.

In the MS of product C, the parent peak was at *m/e* 402, as shown in Fig. 2 and thus two carbonyl groups seemed to be introduced into the cholanic acid molecule. The Rt_R of product C in GLC was different from those of the diketones, VI, VII, and VIII, as summarized in Table II. Since the MS also gave fragment ion peaks *m/e* 329 and 287 due to cleavage of the side chain and the base peak at 247 due to the cleavage of ring D, it was assumed that the two carbonyl groups are located on rings A or B and C, and that one of them is at the C(12)-position. In fact, authentic C(3,12)- and C(7,12)-diketones (VII and VI) both gave the same base peak at 247 and fragment patterns similar to that of product C, as shown in Fig. 2. The signals due to the C(18)- and C(19)-methyl groups were shifted downfield by 0.40 and 0.36 ppm, respectively, as summarized in Table I. Since the downfield shift of the signal due to the C(19)-methyl group was much larger than that of the C(12)-monoketone (II), product C was assumed, in addition to the C(12)-carbonyl function, to have another one in ring A or B at a position, other than C(3) and C(7), which is strongly affected by the C(19)-methyl group. Complete structure elucidation of product C, however, was not achieved owing to the low yield.

In order to examine the function of the carboxylic acid moiety of the substrate (Ia) in the title reaction, the methyl ester (Ib) and alcohol derivative (Ic) were prepared and subjected to autoxidation in the presence of ferrous ions. No peak, except for those due to the substrates, was seen in GLC of the reaction mixtures and thus no oxygenation seemed to occur when the cholanic substrate lacks the free carboxyl group.

In this study, cholanic acid (Ia) was shown to give the C(12)-ketone derivative (A) as a major product and the minor diketones (B and C), when it was autoxidized in the presence of ferrous ions. Since all of these products retain an oxygen function at the C(12)-position of the steroidal skeleton, the active oxygen species seems first to attack this position. Oxy-function-



Ia: $R=CO_2H$
Ib: $R=CO_2CH_3$
Ic: $R=CH_2OH$

Chart 1

alization at C(15), on the other hand, occurred when autoxidation of deoxycholic,[2] nordeoxycholic,[3] taurodeoxycholic,[3] or taurocholanic acids[4] was carried out in an aqueous solution without any organic co-solvent. It may, from these findings, be assumed that an oxygen molecule bound with ferrous ion to form a positively charged species, $[Fe^{2+}\text{-}O_2]$,[5] which interacted with the negative carboxyl group in the side chain of the substrate molecule, and the active oxygen species thus formed then attacked the C(12)- or C(15)-position, which is located in a region sterically favorable to make contact with the species.

Experimental

General Methods——Melting points were taken on a micro hot-stage apparatus and are uncorrected. Infrared (IR) spectra and MS were measured with JASCO IRA-2 and Hitachi RMU-6E spectrometers, respectively. ^1H NMR spectra were measured with a Hitachi H-6013 spectrometer, in deuterochloroform with tetramethylsilane as an internal standard. Preparative TLC was carried out on silica gel (Wakogel B-5F) plates with the solvent system *n*-hexane–Et_2O (2: 3) for the methyl esters. GLC was carried out by using a Shimadzu GC-4BMPF gas chromatograph equipped with a flame ionization detector and a glass tube (3 mm i.d. × 2 m) packed with 1.5% SE-30 on Shimalite W (60—80 mesh); under the following conditions: N_2 carrier gas (50 ml/min), column temperature at 260°C, detector temp. at 270°C. Abbreviations used are: s=singlet, t=triplet, br s=broad singlet, m=multiplet.

Materials——Cholanic acid (Ia), mp 167—169°C (lit.[8] 164—165°C), methyl cholanoate (Ib), mp 86—87°C (lit.[9] 86—87°C), and 24-cholanol (Ic), mp 131.5—132.5°C (lit.[10] 129.5—130.5°C) were prepared from cholic acid by the usual methods.

Oxygenation of Cholanic Acid (Ia)——Ferrous solution (3.00 g of $FeSO_4 \cdot 7H_2O$ in 50 ml of H_2O) and iron powder (1.00 g) were added to a mixture of acetone (250 ml), 0.2 M acetate buffer (pH 5.0, 150 ml), and Ia (200 mg, 0.56 mol). Oxygen was bubbled through the solution for 3 h at 40°C with vigorous stirring. In total, 2.4 g of Ia was treated in this way. Acetone was then evaporated off to give an aqueous solution, which was acidified with 2 N HCl and extracted with CH_2Cl_2. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The brown oil (2.5 g) thus obtained was methylated with diazomethane in Et_2O–MeOH to give a brown oil (2.5 g). The gas chromatogram of the final product is shown in Fig. 1.

Isolation and Identification of Products——The methylated residue (2.5 g) was subjected to column chromatography on alumina (100 g). Elution with *n*-hexane–benzene (1: 1, 600 ml) and benzene (300 ml) yielded the methyl ester (1954 mg) of Ia. Elution with benzene (300 ml), benzene–CH_2Cl_2 (9: 1, 500 ml), and then CH_2Cl_2 (500 ml) gave a yellow oily residue (346 mg). The residue was subjected to preparative TLC to yield products A (180 mg), B, and C (trace amount).

Product A: Recrystallization of crude A (180 mg) from acetone gave 87 mg of colorless needles, mp 109.5—110°C. GLC, ^1H NMR, and MS data are summarized in Tables I and II and shown in Fig. 2. *Anal.* Calcd for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.04; H, 10.34.

Products B and C: GLC, ^1H NMR, and MS data are shown in Fig. 2 and summarized in Tables I and II.

Preparation of Authentic Specimens——Methyl 3-oxo-5β-cholanoate (V), mp 119—120°C (lit.[11] 116—117°C), methyl 3,7-dioxo-5β-cholanoate (VIII), mp 160—163°C (lit.[12] 153—160°C), and methyl 3,12-dioxo-5β-cholanoate (VII), mp 134—135.5°C (lit.[13] 133—134°C), were prepared from the corresponding methyl esters of bile acids by means of Jones oxidation. Methyl 15-oxo-5β-cholanoate (III) was prepared as reported.[4] Methyl 7-oxo-5β-cholanoate (IV), methyl 12-oxo-5β-cholanoate (II), and methyl 7,12-dioxo-5β-cholanoate (VI) were prepared from methyl chenodeoxycholate, methyl deoxycholate, and methyl cholate, respectively, by selective reduction of the 3α-hydroxyl group, employing Jones oxidation and methylation with diazomethane,[14] as follows.

Methyl 7-Oxo-5β-cholan-24-oate (IV)——Methyl 3α-Tosyloxy-7α-hydroxy-5β-cholan-24-oate (IX): A pyridine solution (10 ml) of *p*-toluenesulfonyl chloride (4.0 g) was added to a stirred pyridine solution (50 ml) of methyl chenodeoxycholate (3.0 g) at 5°C and the mixture was stirred for 4 h. A few ml of water was added to the reaction mixture, which was then concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with 2 N HCl, 5% $NaHCO_3$, and water, and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a residue (3.5 g), which was crystallized from MeOH to provide colorless needles, mp 122—123°C. IR ν_{max}^{Nujol} cm^{-1}: 3550, 1730, 1600, 1375, 1170. NMR δ: 0.65 (3H, s, C(18)-H_3), 0.88 (3H, s, C(19)-H_3), 2.45 (3H, s, aromatic CH_3), 3.68 (3H, s, CO_2CH_3), 3.85 (1H, br s, C(7)β-H), 4.29 (1H, m, C(3)β-H), 7.37 (2H, d, J=8.5 Hz), and 7.85 (2H, d, J=8.5 Hz, aromatic H). MS *m/e*: 388, 370, 255. *Anal.* Calcd for $C_{32}H_{48}O_6S$: C, 68.54; H, 8.63; S, 5.71. Found: C, 68.45; H, 8.69; S, 5.52.

7α,24-Dihydroxy-5β-cholane (X): A solution of IX (3.7 g) in dry Et_2O (40 ml) was added dropwise to a stirred and refluxing suspension of $LiAlH_4$ (1.0 g) in dry Et_2O (50 ml) and the mixture was stirred for 3 h. Work-up of the reaction mixture gave the crude product (2.6 g), which was subjected to column chromatography on silica (50 g). Elution with $CHCl_3$ and evaporation of the solvent from the organic layer gave crude X, which was recrystallized from *n*-hexane as colorless crystals, mp 70—71°C (lit.[15] 88—90°C). IR ν_{max}^{Nujol} cm^{-1}: 3350. NMR δ: 0.67 (3H, s, C(18)-H_3), 0.91 (3H, s, C(19)-H_3), 3.58 (2H, t, J=5.5 Hz, C(24)-H_2), 3.81 (1H, br s, C(7)β-H). MS *m/e*: 362 (M^+), 344. *Anal.* Calcd for $C_{24}H_{42}O_2$: C, 79.50; H, 11.68. Found: C, 79.65; H, 11.72.

7-Oxo-5β-cholan-24-oic Acid (XI): Jones reagent (2 ml) was added to a stirred solution of X (500 mg) in acetone (50 ml) under ice-cooling, and the mixture was allowed to stand for 20 min. Usual work-up of the reaction mixture gave XI (546 mg). IR ν_{max}^{Nujol} cm^{-1}: 1700—1730. NMR δ: 0.67 (3H, s, C(18)-H_3), 1.19 (3H, s, C(19)-H_3).

Methylation with diazomethane of XI (500 mg) gave the ester (IV), which was crystallized from MeOH as colorless plates, mp 86—88°C (lit.[16] 92—93°C). IR ν_{max}^{Nujol} cm^{-1}: 1740, 1710. NMR δ: 0.66 (3H, s, C(18)-H_3), 1.18 (3H, s, C(19)-H_3), 3.65 (3H, s, CO_2CH_3). MS *m/e*: 388 (M^+), 273, 245 (base peak). *Anal.* Calcd for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.36; H, 10.28.

Methyl 12-Oxo-5β-cholan-24-oate (II)——Methyl 3α-Tosyloxy-12α-hydroxy-5β-cholan-24-oate (XII): Selective 3α-tosylation of methyl deoxycholate (2.1 g) with *p*-toluenesulfonyl chloride (2.7 g) as described above gave a crude product (2.5 g) of XII, which was crystallized from Et_2O as colorless needles, mp 142—145°C. IR ν_{max}^{Nujol} cm^{-1}: 3430, 1730, 1595, 1370, 1170. NMR δ: 0.66 (3H, s, C(18)-H_3), 0.88 (3H, s, C(19)-H_3), 2.44 (3H, s, aromatic CH_3), 3.65 (3H, s, CO_2CH_3), 3.95 (1H, s, C(12)β-H), 4.46 (1H, m, C(3)β-H), 7.31 (2H, d, J=8.5 Hz), and 7.78 (2H, d, J=8.5 Hz, aromatic H). MS *m/e*: 388, 255 (base peak). *Anal.* Calcd for $C_{32}H_{48}O_6S$: C, 68.54; H, 8.63; S, 5.71. Found: C, 68.41; H, 8.45; S, 5.59.

12α,24-Dihydroxy-5β-cholane (XIII): Reduction of XII (2.5 g) with $LiAlH_4$ (1.0 g) gave the diol XIII (1.8 g), which was recrystallized from EtOAc as colorless needles, mp 114—115°C (lit.[17] 113—115°C). IR ν_{max}^{Nujol} cm^{-1}: 3325. NMR δ: 0.69 (3H, s, C(18)-H_3), 0.91 (3H, s, C(19)-H_3), 3.60 (2H, t, J=5.5 Hz, C(24)-H_2), 3.97 (1H, br s, C(12)β-H). MS *m/e*: 344, 257 (base peak).

12-Oxo-5β-cholan-24-oic Acid (XIV): Jones oxidation of the diol XIII (130 mg) gave the acid XIV (123 mg), colorless powder (from AcOEt), mp 184.5—187°C (lit.[18] 187°C). IR ν_{max}^{Nujol} cm^{-1}: 1720, 1700. NMR δ: 1.07 (3H, s, C(19)-H_3), 1.12 (3H, s, C(18)-H_3). MS *m/e*: 374 (M^+), 273, 233 (base peak). *Anal.* Calcd for $C_{24}H_{38}O_3$: C, 76.96; H, 10.02. Found: C, 76.78; H, 10.03.

Methylation of XIV (120 mg) gave the ester (II), which was crystallized from EtOH as colorless needles, mp 112—113°C (lit.[19] 109.5°C). IR ν_{max}^{Nujol} cm^{-1}: 1745, 1710. NMR δ: 1.03 (6H, s, C(18)-H_3 and C(19)-H_3), 3.70 (3H, s, CO_2CH_3). MS *m/e*: 388 (M^+), 273, 233 (base peak). *Anal.* Calcd for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.29; H, 10.28.

Methyl 7,12-Dioxo-5β-cholan-24-oate (VI)——Methyl 3α-Tosyloxy-7α,12α-dihydroxy-5β-cholan-24-oate (XV): Selective 3α-tosylation of methyl cholate (3.0 g) with *p*-toluenesulfonyl chloride (4.0 g) gave crude XV, which could not be crystallized. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1730, 1595, 1370, 1170. NMR δ: 0.67 (3H, s, C(18)-H_3), 0.85 (3H, s, C(19)-H_3), 2.43 (3H, s, aromatic CH_3), 3.65 (3H, s, CO_2CH_3), 3.80 (1H, br s, C(7)β-H), 3.90 (1H, br s, C(12)β-H), 4.27 (1H, s, C(3)β-H), 7.29 (3H, d, J=8.5 Hz), and 7.73 (2H, d, J=8.5 Hz, aromatic H).

7α,12α,24-Trihydroxy-5β-cholane (XVI): Reduction of XV (3.9 g) with $LiAlH_4$ (1.0 g) gave the triol XVI (2.6 g), which was crystallized from AcOEt as colorless needles (1.4 g), mp 191.5—193.5°C (lit.[15] 198—200°C). IR ν_{max}^{Nujol} cm^{-1}: 3250. NMR (in pyridine-d_5) δ: 0.84 (3H, s, C(18)-H_3), 1.02 (3H, s, C(19)-H_3), 3.88 (2H, br s, C(24)-H_2), 4.1- (1H, br s, C(7)β-H), 4.30 (1H, br s, C(12)β-H). MS *m/e*: 344. *Anal.* Calcd for $C_{24}H_{42}O_3$: C, 76.14; H, 11.18. Found: C, 76.09; H, 11.07.

7,12-Dioxo-5β-cholan-24-oic Acid (XVII): Jones oxidation of the triol XVI (400 mg) gave the acid XVII, colorless needles (from benzene-*n*-hexane), mp 180—182.5°C. IR ν_{max}^{Nujol} cm^{-1}: 1720—1710, 1700. NMR δ: 1.05 (3H, s, C(18)-H_3), 1.31 (3H, s, C(19)-H_3). MS *m/e*: 388 (M^+), 370, 287, 247 (base peak). *Anal.* Calcd for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 73.92; H, 9.32.

Methylation of the acid XVII (200 mg) with diazomethane gave the ester VI, colorless needles (from MeOH), mp 136.5—137.5°C (lit.[20] 137—138°C). IR ν_{max}^{Nujol} cm^{-1}: 1730, 1710, 1690. NMR δ: 1.04 (3H, s, C(18)-H_3), 1.30 (3H, s, C(19)-H_3), 3.68 (3H, s, CO_2CH_3). MS *m/e*: 402 (M^+), 287, 247 (base peak). *Anal.*

Calcd for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.54; H, 9.55.

Oxygenation of Methyl Cholanoate (Ib) and 24-Cholanol (Ic)——Iron powder (1.0 g) and ferrous solution (1.0 g of $FeSO_4 \cdot 7H_2O$ in 10 ml of H_2O) were added to a mixture of acetone solution (70 ml) of Ib or Ic (40 mg, 0.11 mmol) and 0.2 M acetate buffer (pH 5.0, 40 ml). The mixture thus obtained was examined for oxygenation under the same conditions as those for Ia described above. After work-up as usual, the organic extract from the reaction mixture gave no peak on a gas liquid chromatogram other than that due to the substrate.

Acknowledgement We wish to thank the staff of the Center for Instrumental Analysis, Hokkaido University, for the elemental analysis and the measurements of NMR and mass spectra. A part of this work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, for which we are grateful.

References and Notes

1) This paper constitutes Part XIX of the series entitled "Metal ion-catalyzed Oxidation of Steroids." Part XIIX: T. Muto, C. Urano, T. Hayashi, T. Miura, and M. Kimura, *Chem. Pharm. Bull.*, **31**, 1166 (1983).
2) M. Kimura, M. Kawata, M. Tohma, A. Fujino, and K. Yamazaki, *Tetrahedron Lett.*, **1970**, 2021; M. Kimura, M. Kawata, M. Tohma, A. Fujino, K. Yamazaki, and T. Sawaya, *Chem. Pharm. Bull.*, **20**, 1883 (1972).
3) M. Kimura, A. Fujino, K. Yamazaki, and T. Sawaya, *Chem. Pharm. Bull.*, **24**, 2301 (1976).
4) M. Kimura and T. Sawaya, *Chem. Pharm. Bull.*, **25**, 1073 (1977).
5) J.E. Baldwin and J. Huff, *J. Am. Chem. Soc.*, **95**, 5757 (1973) and references cited therein.
6) C. Djerassi and L. Tökes, *J. Am. Chem. Soc.*, **88**, 536 (1966).
7) N.S. Bhacca and D.H. Williams, in "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., 1964, p. 22.
8) A. Norman, *Arkiv för Kemi*, **8**, 331 (1955).
9) M. Schenck, *Hoppe-Seyler's Z. Physiol. Chem.*, **286**, 270 (1951).
10) Y. Shalon, Y. Yamuka, and S. Sarel, *Tetrahedron Lett.*, 957 (1969).
11) L.F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **72**, 5530 (1950).
12) E. Berner, A. Lardon, and T. Reichstein, *Helv. Chim. Acta*, **30**, 1542 (1947).
13) H.P. Sigg and T. Reichstein, *Helv. Chim. Acta*, **39**, 1507 (1956).
14) R.T. Blickenstaff and F.C. Chang, *J. Am. Chem. Soc.*, **80**, 2726 (1958).
15) S. Ahmed, M. Alauddin, B. Caddy, M. Martin-Smith, and W.T.L. Sidwell, *Aust. J. Chem.*, **24**, 251 (1971).
16) S. Hara, *Yakugaku Zasshi*, **78**, 1030 (1958).
17) G.V. Rao and C.C. Price, *J. Org. Chem.*, **27**, 205 (1962).
18) H. Wieland and O. Schlichting, *Hoppe-Seyler's Z. Physiol. Chem.*, **150**, 267 (1925).
19) S. Kuwada, S. Yoshiki, and M. Kuwashima, *Proc. Imp. Acad.* (Tokyo), **20**, 381 (1944).
20) S. Kuwada and S. Morimoto, *Bull. Chem. Soc. Jpn.*, **17**, 147 (1942).