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Autoxidation of Nor- and Tauro-deoxycholic Acids in the Presence of Ferrous Ions[1)]

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Reactions with the simple aerobic hydroxylating system at moderate temperature were studied on the aqueous solutions of two varieties of bile acid. Ferrous sulfate was added to a buffered solution (pH 6.5—6.7) of nordeoxycholic acid which was bubbled with molecular oxygen, as reported.[3b)] The structures of the methylated products were elucidated as methyl 24-nor-3α,12α,15α-trihydroxy-5β-cholan-23-oate and methyl 24-nor-3α,12α-dihydroxy-15-oxo-5β,14β-cholan-23-oate. Oxygen function was introduced also stereospecifically at C_{15} and in an α-configuration when taurodeoxycholic acid was treated with the same hydroxylating system.

As has been reported previously,[3)] a new bile acid, 3α,12α,15α-trihydroxy-5β-cholan-24-oic acid (I), was derived when an aqueous solution of deoxycholic acid (II) was treated with Udenfriend's system, Fe(II)/ethylenediaminetetraacetic acid (EDTA)/ascorbic acid/O_2,[4)] as well as with the more simple system, Fe(II)/O_2. Matkovics, *et al.*,[5)] on the other hand, demonstrated the transformation of II into cholic acid by the ascorbic acid-O_2 (or H_2O_2) system in the presence of ferrous ions and EDTA. These results offer some interesting and complicated problems on the mechanism of the reaction which is likely to transform II into the different trihydroxy derivatives with different conditions. Further investigations on these hydroxylation reactions have thus been undertaken in this laboratory. The present paper deals with the 15α-hydroxylation of nor- and tauro-deoxycholic acids, which were carried out in aqueous solution with the most simple aerobic hydroxylating system, ferrous sulfate and molecular oxygen, at moderate temperature.[3b)]

Ferrous sulfate solution was added dropwise for four hours to a phosphate buffer solution (pH 6.5—6.7) of nordeoxycholic acid (III), which was kept at 40° and bubbled with molecular oxygen, as reported.[3b)] The products were methylated and submitted to column chromatography on silica gel, giving two esters, $C_{24}H_{40}O_5$ (IV), mp 298—300°, and $C_{24}H_{38}O_5$ (V), mp 249—250°. In the mass (MS) spectrum of IV, the presence of base peak *m/e* 253 which was similarly given by the methyl ester of the hydroxylation product of II[3)] seemed to indicate that the third hydroxyl group was introduced in the steroidal nucleus of III (Chart 1). Chromate oxidation of IV in acetone gave the product (VI), $C_{24}H_{34}O_5$, mp 195—197°, which showed infrared (IR) absorption at 1739 and 1713 cm^{-1} in chloroform. MS spectrometry of VI gave the peaks at *m/e* 402 (M^+), 247, and 197 (Chart 1) which seemed to be in a similar

1) This paper constitutes Part IV of the series entitled "Metal Ion Catalyzed Oxidation of Steroids"; Part III: M. Kimura, M. Tohma, and T. Tomita, *Chem. Pharm. Bull.* (Tokyo), **21**, 2521 (1973). Following trivial names are used: nordeoxycholic acid, 24-nor-3α,12α-dihydroxy-5β-cholan-23-oic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholan-24-oic acid; cholic acid, 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; Taurodeoxycholic acid, tauro-3α,12α-dihydroxy-5β-chlan-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) S. Udenfriend, C.T. Clark, J. Axelrod, and B.B. Brodie, *J. Biol. Chem.*, **208**, 731 (1954).

5) B. Matkovics, P. Pénzes, and G, Göndös, *Steroids*, **5**, 451 (1965).

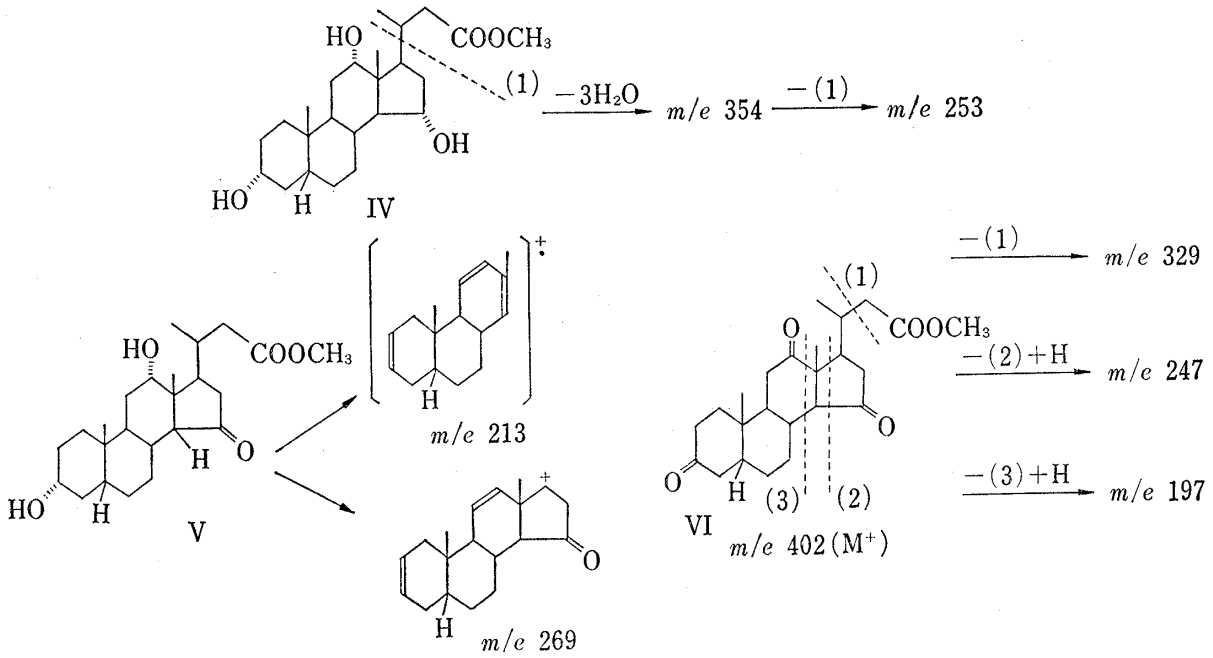

Chart 1

pattern corresponding to those, *m/e* 416, 247, and 211, observed in methyl 3,12,15-trioxo-5β-cholan-24-oate.[3b] These results and that of high-resolution MS spectrometry of VI are best interpreted to indicate that the hydroxyl group under consideration in IV is situated at C_{15}. In nuclear magnetic (NMR) studies on steroids, signal shifts of the angular methyl groups were observed in connection with the hydroxyl groups in the verious positions.[6] The chemical shifts observed in IV and the methyl esters of its homologous bile acids are summarized in Table I. It was observed previously that the remarkable downfield shift (−0.45 ppm) of the

Table I. Chemical Shifts of Angular Methyl Protons

Methyl ester	Site of hydroxyl group	C_{18}–H (δ)	C_{19}–H (δ)
Deoxycholate	3α, 12α	0.70	0.93
Nordeoxycholate	3α, 12α	0.72	0.94
Cholate	3α, 7α, 12α	0.77	0.97
Norcholate	3α, 7α, 12α	0.78	0.97
Compound I	3α, 12α, 15α	0.80	0.97
Compound IV	3α, 12α, ?	0.82	0.99

Spectra were taken in pyridine solution containing tetramethylsilane as an internal standard.

signal peak due to C_{18}–H was caused by C_{15}–β OH and the effect of C_{15}–α OH, on the contrary, was as smaller as −0.10 ppm.[3b] The third hydroxyl group in IV may thus be rather in an α-configuration. Consequently, the chemical structure of the ester (IV) may be elucidated as methyl 24-nor-3α,12α,15α-trihydroxy-5β-cholan-23-oate.

Another methyl ester (V) of the hydroxylation product from III gave MS spectrum showing the peaks *m/e* 406 (M^+), 388, 370, 269, and 213 (Chart 1) which seemed to correspond to those, *m/e* 420 (M^+), 402, 384, 269, and 213, of methyl 3α,12α-dihydroxy-15-oxo-5β,14β-cholan-24-oate (VII).[3b] High-resolution MS spectrometry of the latter two peaks indicated the presence of C_{15}=O. In NMR spectrum of V, the chemical shifts (δ 0.99) of angular methyl protons at both C_{18} and C_{19} were observed to be identical also with those of VII. The structure of V may, therefore, be elucidated as methyl 24-nor-3α,12α-dihydroxy-15-oxo-5β,14β-cholan-23-oate.

6) K. Tori and K. Aono, *Ann. Rept. Shionogi Res. Lab.*, **14**, 136 (1964); *idem*, *Steroids*, **4**, 713 (1964).

Hydroxylation reaction of taurodeoxycholic acid (VIII) was similarly carried out with the ferrous sulfate-molecular oxygen system. Hydrolyzates of the products with alkali were derived to their methyl esters and were then submitted to column chromatography on silica gel. The esters were thus purified into the colorless needles, $C_{25}H_{42}O_5$ (IX), mp 256—259°, and $C_{25}H_{40}O_5$ (X), mp 244—245°, and they were found to be identical with the authentic methyl 3α,12α,15α-trihydroxy-5β-cholan-24-oate[3b] and methyl 3α,12α-dihydroxy-15-oxo-5β,14β-cholan-24-oate,[3b] respectively. The C/D-*cis* configuration in V and X is interpreted in terms of the ring inversion which occurred in the C_{15}-oxo products through enolization.

As has been observed in II previously,[3b] the C_{15}-α hydroxylation was found to occur also in the reactions of III and VIII with the Fe(II)/O_2 system as described above. Although the mechanism of this stereospecific hydroxylation can not be clarified at present, it may be assumed that the carboxyl or carbonyl group in the side chain and/or C_{12}-α hydroxyl group of II, III, and VIII can be cooperative with the ferrous dioxygen complex[7] to attack C_{15} position. In fact, methyl 3α,12α-dihydroxy-5β-chol-14-en 24-oate gave considerable amounts of 3α,12α,15α,24-tetrahydroxy-5β-cholane,[8] $C_{24}H_{42}O_4$, mp 317—318°, in the hydroboration-oxidation,[9] contrary to the methyl ester of 3α,12α-dihydroxy-5β-cholan-7-en-24-oate[10] and the usual esters which are scarecely susceptible to the same reductant. It is of interest, in this respect, that reduction of ester group can occur when it is situated in the position which permit the formation of cyclic borane complex with olefin in the same molecule.[11] In a series of bile acids (II, III, and VIII) examined so far, C_{12}-α hydroxyl group has an *axial* configuration and is also located rather closely to the C_{15} position to be attacked by the hypothetical ferrous dioxygen complex. Detailed discussion is, however, not appropriate untill more evidence has been accumulated and further studies are now in progress.

Experimental[12]

Materials——Nordeoxycholic acid (III), mp 217—218° (lit.[13] 213—214°), and taurodeoxycholic acid (VIII), mp 165—170° (lit.[14] 171—175°), were prepared and purified as reported.

Hydroxylation Reaction——In the same manner as reported,[3b] the stirred buffer solution (0.1 M Na_2HPO_4, pH 6.5—6.7) of III (6.0 g) or VIII (8.95 g) was treated with the Fe(II)/O_2 system at 40° for 4 hr.

Chromatography of the Products from III——The reaction mixtures were combined, acidified to pH 3 with dil. H_2SO_4, and extracted with AcOEt. Evaporation of solvent *in vacuo* from the organic layer left a residue (5.0 g) which was then methylated with CH_2N_2. The products (5.6 g) were submitted to column chromatography on silica gel (166 g), eluting with benzene (1 liter), benzene–AcOEt (4: 1; 2 liter), benzene–AcOEt (1: 1; 4 liter), benzene–AcOEt (1: 4; 2 liter), AcOEt (1.5 liter), and finally AcOEt–MeOH (19: 1; 4 liter). Fractions 1 (0.26 g), 2 (2.30 g), 3 (1.96 g), 4 (0.54 g), and 6 (0.73 g) were thus obtained respectively.

Methyl 24-Nor-3α,12α,15α-trihydroxy-5β-cholan-23-oate (IV)——The fraction 5 was crystallized from acetone–MeOH to give colorless needles, mp 298—300°. *Anal.* Calcd. for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.30; H, 9.83. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1740 (COOMe). Mass Spectrum *m/e*: 390 (M^+-18), 372 ($M^+-2\times18$), 354 ($M^+-3\times18$), 271, 253 (Chart 1). NMR: as given in Table I.

Methyl 24-Nor-3α,12α-dihydroxy-15-oxo-5β,14β-cholan-23-oate (V)——Fraction 4 was first washed with acetone to remove the brown pigment and crystallized from acetone–MeOH to colorless needles, mp 249—250°. *Anal.* Calcd. for $C_{24}H_{38}O_5$: C, 70.90; H, 9.42. Found: C, 71.17; H, 9.35. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1740 (COOMe). NMR ($CDCl_3$) δ: 0.99 (6H, singlet, C_{18}- and C_{19}-H). Mass Spectrum *m/e*: 406 (M^+), 388 (M^+-18), 370 ($M^+-2\times18$), 269 (Calcd. for $C_{19}H_{25}O$: 269.1905. Found: 269.1874), 213 (Calcd. for $C_{16}H_{21}$: 213.1643. Found: 213.1640).

7) P. George, *J. Chem. Soc.*, **1954**, 4349.
8) M. Kimura, A. Fujino, and K. Yamazaki, unpublished.
9) G. Zweitov, N.R. Ayyanger, and H.C. Brown, *J. Am. Chem. Soc.*, **85**, 2072 (1963).
10) B. Matkovics, Z.S. Tegyey, and G.Y. Göndöus, *Steroids*, **5**, 117 (1965).
11) H.C. Brown and K.A. Keblys, *J. Am. Chem. Soc.*, **86**, 1795 (1964).
12) 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 Model H-6013 and Hitachi Model RMU-6E spectrometers, respectively.
13) B. Riegel, R.B Moffett, and A.V. Mc Intosh, "Organic Synthesis," Coll. Vol. III, ed. by E.C. Horning, John Wiley and Sons, Inc., New York, 1955, p. 234.
14) A. Norman, *Arkiv för Kemi*, **8**, 331 (1955).

Methyl 24-Nor-3,12,15-trioxo-5β-cholan-23-oate (VI)——To a solution of IV (10 mg) in acetone (10 ml), the Kiliani's chromate mixture[15] (1 ml) was added at 0° under a stream of N_2 with vigorous stirring for 10 min. After diluting with water, the reaction mixture was extracted with ether and the organic layer was washed with 5% Na_2SO_3, then with water and dried finally over anhydrous Na_2SO_4. Evaporation of solvent *in vacuo* from the ether solution left a residue (7.2 mg) which was crystallized from aqueous acetone to colorless needles, mp 195—197°. *Anal.* Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.59; H, 8.50. IR ν_{max}^{Nujol} cm^{-1}: 1740 (C-OOMe), 1710 (C=O in six-membered ring). Mass Spectrum *m/e*: 402 (M^+; Calcd. for $C_{24}H_{34}O_5$: 402.2406. Found: 402.2455), 247 (base peak; Calcd. for $C_{16}H_{23}O_2$: 247.1698. Found: 247.1749), 197 (Calcd. for $C_{11}H_{17}O_3$: 197.1178. Found: 197.1141).

Purification and Identification of the Products from VIII——After taurodeoxycholic acid (VIII, 8.95 g) was treated with Fe(II)/O_2 system, the reaction mixture was acidified to pH 1 and extracted with *n*-BuOH. Evaporation of solvent *in vacuo* from the organic layer left a residue which was then refluxed in 10% aq. NaOH for 10 hr. The alkaline solution was acidified and extracted with AcOEt. The extract (5.4 g) obtained was methylated in MeOH with dry HCl gas to give the crude esters which were then submitted to column chromatography on silica gel (290 g), eluted by acetone–benzene (7: 13; 3.43 liter, divided into 3 parts of 0.45, 0.58, and 2.4 liter), Me_2CO (0.59 liter), and finally MeOH (2 liter). Fractions 1 (1.49 g; oil), 2 (2.61 g), 3 (0.39 g), 4 (0.28 g), and 5 (0.98 g) were thus obtained respectively.

Methyl 3α,12α,15α-Trihydroxy-5β-cholan-24-oate (IX): Fraction 4 was crystallized from AcOEt–MeOH to give colorless needles, mp 256—259°, which were identified by comparing the IR, NMR, MS spectra with those of the authentic specimen.[3b]

Methyl 3α,12α-Dihydroxy-15-oxo-5β,14β-cholan-24-oate (X): Fraction 3 was crystallized from acetone–MeOH to give colorless needles, mp 244—245°, which were identified with the authentic specimen (mp 247—248°)[3b] on the basis of the spectral data.

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15) G.S. Boyd, *Biochem. J.*, **81**, 11 (1965).