Bile Acids and Steroids. XXI[1]*. Studies on the Hog Bile Acids (Part 5)*[1]*. On the Oxidation of Epimeric 3,6-Dihydroxycholanoic Acids with N-Bromosuccinimide*[*1]

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(Received September 30, 1960)

Previously, it was described that α-hyodeoxycholic acid is selectively oxidized at C-3 with *N*-bromosuccinimide (NBS) to give 3-oxo-6α-hydroxy-5β-cholanoic acid[2], whereas it has been reported earlier by Wieland[3] that it was preferentially oxidized at C-6 with chromic acid. From this reverse result, it was of interest to investigate the oxidation of epimeric 3,6-dihydroxy-5β-cholanoic acids with NBS. Oxidation was carried out in both dioxane-water (9:1) and acetone-water (7:3) in order to obtain information depending on polarity of the solvent and with 1 and 2 molar equivalents of NBS respectively in order to obtain information depending on selectivity. As seen in Table I, the order of susceptibility to oxidation obtained from the results was as follows:

$$6\beta > 3\beta \ \& \ 3\alpha > 6\alpha \quad (\mathrm{A/B: cis})$$

The 6β-hydroxyl group was most rapidly oxidized, the 6α-hydroxyl group was not attacked and the ease of oxidation of both the 3β- and the 3α-hydroxyl groups were in between. However, the difference between 3β- and 3α-hydroxyl group was not definable in such an experiment. Although there is a method in which the reagent such as NBS is volumetrically titrated, it is presumed to complicate the results on account of two factors, both a consumption by the substrates and a decomposition by the solvent. Therefore, the author examined the oxidation using the absorption band at 1720 cm^{-1} corresponding to the carbonyl group of the six-membered ketone in the infrared spectrum. This band is so strong that it is useful for determination. Since the reaction itself was assumed to be very complicated as mentioned above, it was most reliable to use the optical densities of the six-membered carbonyl band in the infrared spectrum for this purpose. In this case, as methyl 3-oxo-6α-hydroxy-5β-cholanoate was oily, it was unsuitable for preparing the calibration curve. Consequently, methyl lithocholate[7] and methyl 3β-hydroxy-5β-cholanoate[8] were used as starting materials. Ester is also suitable to avoid the overlap of the acid carbonyl band with the six-membered ketone band in the infrared spectrum. As the six-membered carbonyl band was further somewhat overlapped with that of

TABLE I. THE POSITION ATTACKED AND THE YIELD IN EACH EPIMER

Solvent	Dioxane-Water (9:1)		Acetone-Water (7:3)	
Mol. equiv. of NBS	1	2	1	2
Reaction time at room temperature (20±2°C)	1.5 hr.	1.5 hr.	Overnight	4 days
3α, 6α	3(27.2%)	3(30.1%)	3(41.7%)	3(61.8%)
3β, 6α[4]	3(21.3%)	3(45.7%)	3(31%)	3(62.5%)
3β, 6β[5]	6(77%)	6(56.6%) 3,6(14.3%)	6(77.5%)	6(55%) 3,6(19.3%)
3α, 6β[6]	6(70%)	6(60%) 3,6(7.5%)	6(70%)	6(59.4%) 3,6(16%)

[*1] The rest is abbreviated as NBS.

[1] Part XX: J. Kawanami, This Bulletin, 34, 509 (1961).

[2] K. Takeda and J. Kawanami, *J. Biochem.* (*Japan*), 40, 477 (1953).

[3] H. Wieland and E. Dane, *Z. Physiol. Chem.*, 212, 41 (1932).

[4] β-Hyodeoxycholic acid was prepared from 3-oxo-6α-hydroxy-5β-cholanoic acid according to Moffett method. (R. B. Moffett and W. Hoehn, *J. Am. Chem. Soc.*, 69, 1995 (1947)).

[5] Synthesis of 3β, 6β-dihydroxy-5β-cholanoic acid was described in the preceding part XX.

[6] 3α, 6β-Dihydroxy-5β-cholanoic acid was prepared from methyl 3α-acetoxy-6-oxo-5β-cholanoate according to Moffett method. (R. B. Moffett and W. Hoehn, *J. Am. Chem. Soc.*, 68, 1855 (1946)). This compound was also prepared in good yield by using sodium borohyride in place of Raney nickel.

[7] Methyl lithocholate was prepared from cholic acid according to the usual method. (S. Sarel and Y. Yanuka, *J. Org. Chem.*, 24, 2018 (1959)).

[8] Methyl 3β-hydroxy-5β-cholanoate was prepared from both Methyl 3-oxo-cholanoate and Methyl 3α-tosyloxy-5β-cholanoate according to Fieser's and Reindel's method respectively. (F. Reindel and K. Niederlandre, *Ber.*, 68, 1243 (1935); L. F. Fieser and J. C. Babcock, *J. Am. Chem. Soc.*, 74, 5474 (1952)).

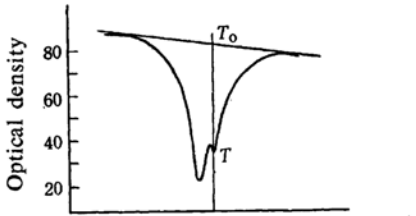

Fig. 1. Baseline method.

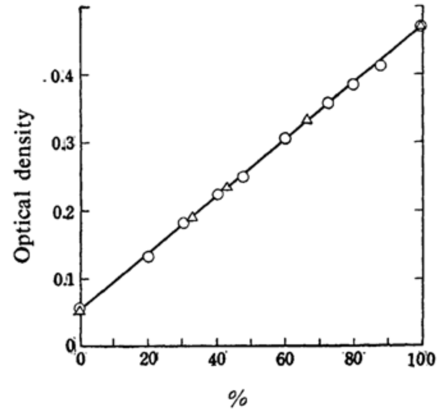

Fig. 2. Calibration curve.
—○— 3α-epimer; —△— 3β-epimer

the ester group, T_0 was estimated according to the baseline method (Fig. 1).

The calibration curve was made by using a variety of mixtures consisting of each methyl 3β- or 3α-hydroxy-5β-cholanoate and methyl 3-oxo-5β-cholanoate. As shown in Fig. 2, the calibration curve plotting the relationship between the optical density and the concentration (W/W%) of the oxidation product, 3-oxo-5β-cholanoate to each of methyl 3β- or 3α-hydroxy-5β-cholanoate, formed a straight line which indicated its usefulness for a quantitative analysis. The values thus obtained are shown in Tables II and III. Then, the

TABLE II. RELATIONSHIP BETWEEN OPTICAL DENSITY AND RATIO (W/W%) OF THE 3-OXO DERIVATIVE TO THE 3α-EPIMER

Percentage of 3-oxo deriv.	3-Oxo mg.	3α-OH mg.	CCl_4+ Sample mg.	W %	$\overline{T}_0$	$\overline{T}$
0	0	5.0	589.0	0.850	88.3	79.4
20	0.8	3.2	472.0	0.848	87.8	67.7
30	1.8	4.2	717.2	0.838	87.6	61.7
40	2.0	3.0	604.0	0.828	88.0	57.9
48	4.5	4.9	1163.1	0.807	87.7	55.2
60	3.0	2.0	594.0	0.842	87.7	48.5
72	4.3	1.7	704.5	0.852	87.3	43.5
80	4.0	1.0	588.0	0.850	87.3	41.3
87.4	6.9	1.0	942.9	0.837	87.1	39.6
100	6.3	0	792.4	0.795	87.2	36.6

TABLE III. RELATIONSHIP BETWEEN OPTICAL DENSITY AND RATIO (W/W%) OF THE 3-OXO DERIVATIVE TO THE 3β-EPIMER

Percentage of 3-oxo deriv.	3-Oxo mg.	3β-OH mg.	Sample +CCl_4 mg.	W %	$\overline{T}_0$	$\overline{T}$
0	0	7.2	897.3	0.801	87.8	80.0
33.3	2.7	5.4	924.9	0.875	87.7	60.0
43.4	3.7	4.9	948.9	0.908	87.4	53.6
66.7	6.2	3.1	1081.8	0.859	87.1	45.5
100	6.3	0	792.4	0.795	87.2	36.6

TABLE IV. RELATIONSHIP BETWEEN THE YIELD OF OXIDATION PRODUCTS AND TIME AT 25°C

No.*	t min.	Sample mg.	Sample +CCl_4 mg.	W %	D	Yield
1	30	45.5	5342.3	0.851	—	—
2	30	46.2	5490.0	0.841	—	—
3	50	41.2	4850.6	0.850	0.122	20
4	50	45.0	5305.0	0.848	0.105	15
5	70	35.0	4099.5	0.854	0.183	38
6	70	44.3	5215.3	0.851	0.144	27
7	90	44.6	5232.6	0.852	0.257	60
8	90	44.0	5171.0	0.850	0.227	51.5

* Odd numbers are from the 3β-epimer and even numbers from the 3α-epimer.

TABLE V. THE OXIDATION OF BOTH EPIMERS FOR 60 min. AT 25°C

Starting material	Sample mg.	Sample +CCl_4 mg.	W %	D	Yield of 3-oxo deriv. %
3β	41	4.919	0.831	0.262	63.8
3α	43.5	5.050	0.862	0.219	48.3

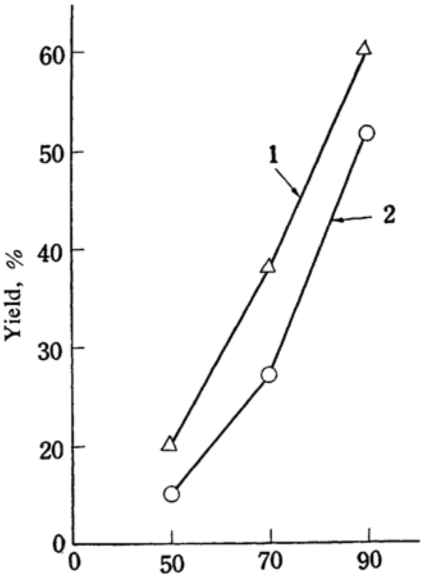

Fig. 3. Relationship between time and yield in each epimer.
(1) 3β-epimer; (2) 3α-epimer

oxidation of epimeric methyl 3-hydroxy-5β-cholanoates was carried out with 1.3 molar equivalents of NBS in dioxane-water (9 : 1) solution at 25°C. There is a marked difference in the yield between 3β- and 3α-hydroxyl derivatives (Fig. 3). The values obtained are shown in Tables IV and V. Accordingly, the order of susceptibility to oxidation obtained from the results described above was as follows:

6β(a) > 3β(a) > 3α(e) > 6α(e) (A/B: cis)

In order to obtain the further support on the influence of steric factors in this reaction, the oxidation of two 3,6-dihydroxy-5α-cholanoic acid (3β,6β-dihydroxy-5α-cholanoic acid and 3β,6α-dihydroxy-5α-cholanoic acid) was examined. The oxidation was carried out as in the previous case. While the oxidation of the former gave a 6-oxo derivative, the oxidation of the latter gave a 3-oxo derivative. From these results in A/B-trans series, the 6β-hydroxy group was also rapidly oxidized but the 6α-hydroxyl group was not affected under such a condition and the ease of oxidation of the 3β-hydroxyl group was in between. Accordingly, the order of the susceptibility to the oxidation of hydroxyl groups in A/B-trans series was also as follows:

6β(a) > 3β(e) > 6α(e) (A/B: trans)

Discussion on the Reaction Mechanism

Prior to considering the reaction mechanism of oxidation with NBS from the above result, the outline of reports on the reaction mechanism of chromic acid oxidation should be described. Westheimer and Nicolaides⁹⁾ minutely studied the reaction mechanism of chromic acid oxidation and they found that the rate-determining step is not the formation of a chromate ester but the cleavage of the carbon hydrogen bond.

(A) $R_2CHOH+HCrO_4^-+H^+$

$$\overset{\text{fast}}{\rightleftarrows} R_2CHOCrO_3H+H_2O$$

(B) $R_2\underset{\substack{|\\ H}}{C}\text{-}O\text{-}CrO_3H+H_2O$

$$\xrightarrow{\text{slow}} R_2C{=}O+[HCrO_3^-]+H_3O^+$$

Barton¹⁰⁾ found that the conformational factor determined nearly always the order of reactivity in oxidation among several hydroxyl groups and established a general rule that an axial alcohol is ordinarily more susceptible than the corresponding equatorial alcohol. There are, however, some cases in which an equatorial alcohol having a less accessible axial carbon hydrogen bond is faster oxidized than an axial alcohol (e. g. compare equatorial 11α-hydroxyl with 3-axial hydroxyl group). Schreiber and Eschenmoser¹¹⁾ measuring the reaction rates of oxidation of a number of hydroxy-5α-cholestanes, confirmed again a general rule proposed by Barton and further defined a second factor determining reaction rates. That is, the transformation of the tetrahedral carbon of an alcoholic (or chromate ester) group to a trigonal carbonyl carbon often eliminates some or all of the repulsive interactions leading to instability. The reaction rates are thus dependent upon the extent of decrease in such interactions. A example in A/B-trans series is as follows:

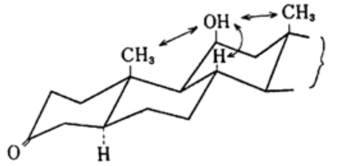

Fig. 4

As seen in Fig. 4, the 11β-hydroxyl group is under steric strain from the repulsions between the four groups, 11β-hydroxyl, C-10-methyl, C-13-methyl and 8β-hydrogen. Relief of this strain provides a driving force sufficient to promote the oxidation.

On the other hand, oxidation with reagents such as *N*-bromosuccinimide or *N*-bromoacetamide has been less clear from both mechanistic and conformational view points¹²⁾. On the basis of the studies described above, the reaction mechanism of oxidation with NBS could be considered. The order of susceptibility to oxidation of the epimeric 3,6-dihydroxycholanoic acids with NBS was as follows:

(C) 6β(a) > 3β(a) > 3α(e) > 6α(e)
(A/B: cis)

(D) 6β(a) > 3β(e) > 6α(e)
(A/B: trans)

On the other hand, the order in chromic acid oxidation of hydroxy-5α-cholestanes by Schreiber at the corresponding position was as follows:

(E) 6β(a) > 3α(a) > 6α(e) > 3β(e)
[36]* [3.0]* [2.0]* [1.0]*
(A/B: trans)

* Relative rates to 3β-hydroxy-5α-cholestane.

From the results on the oxidation of α-hyodeoxycholic acid with chromic acid, there are also the following positions.

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9) F. H. Westheimer and N. Nicolaides, ibid., **71**, 25 (1949).

10) D. H. R. Barton, *Experientia*, **6**, 316 (1950); *J. Chem. Soc.*, **1953**, 1027.

11) J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta*, **38**, 1929 (1955).

12) H. J. E. Loenthal, *Tetrahedron*, **6**, 269 (1959).

(F) $6\alpha(e) > 3\alpha(e)$ (A/B: cis)

Compared with the above cited four expressions C, D, E and F, it is analogous in conformational view in accord with the Barton rule. In other words, an axial alcohol is more susceptible to oxidation than the corresponding equatorial epimer ($6\beta > 6\alpha$, $3\beta > 3\alpha$: A/B cis). Accordingly, in the case of the axial hydroxyl group, it is clear that the oxidation with NBS proceeds likewise to chromic acid oxidation, because both intermediates similarly have a more accessible C–H bond (equatorial) and a repulsive intermediate ester (axial). The concept described above will be also supported by the following examples.

cholestane-3β, 5α, 6β-triol—
6-oxo derivative[13]

3β, 6β-dihydroxy-5α-cholanoic acid—
6-oxo derivative*2

5α-cholestane-3β, 7α-diol—
7-oxo derivative[14]

11β-hydroxytigogenine—11-oxo derivative[15]

11β-hydroxyprogesterone—
11-oxo derivative[16]

(11α-hydroxyprogesterone was not affected under same condition).

On the other hand, in the series of the generally less reactive equatorial hydroxyl group, e. g. in the case of α-hyodeoxycholic acid, the order with NBS was the reverse of that with chromic acid as seen in expressions C and F. This difference is supposed to depend on a driving force in the intermediate, because it is clear that the conformation of C–H bond (e. g. the 6β-H in Figs. 5 and 6) is equivalent in each case. In the chromic acid oxidation, the intermediate ester has a strong driving force which is derived from both a large space-claim ("Raumbeanspruchung") and an inductive factor (Fig. 5). On the other hand, in the oxidation with NBS, it is assumed that the intermediate ester has a little driving force which resulted from a smaller space-claim and from almost no inductive factor. (Fig. 6). The further explanation was as follows: in the chromic acid oxidation, since the 6β-hydrogen suffers the driving force sufficient to promote the reaction as mentioned above, it can be more easily eliminated than the 3β-hydrogen, whereas in the case of NBS, the driving force of the intermediate hypohalite is very insufficient. For this reason, an attack of the base on a hydrogen atom attached to the hydroxyl carbon is indispensable for the promotion of the reaction as well as the elimination of this hydrogen. Consequently, it may be deduced that the most important factor in the oxidation of the less reactive equatorial alcohol with NBS depends upon the extent of easiness of the proximity of base to the C–H bond of the alcoholic carbon being oxidized. Therefore, in the oxidation of α-hyodeoxycholic acid with NBS, the 3α-hydroxyl group is more easily oxidized than the 6α-hydroxyl group owing to the vulnerability of the 3β-hydrogen. This concept may be further supported by the fact that the less accessible C–H bonds such as in the 6α- or 11α-alcohol are not affected.

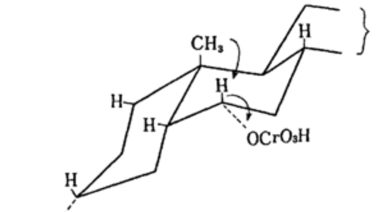

Fig. 5

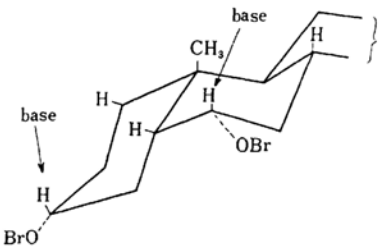

Fig. 6

Examples which show the concept described above are as follows:

3β, 6α-dihydroxy-5α-cholanoic acid—
3-oxo derivative*2

5β-androstane-3α, 11α, 17β-triol—
3, 17-dioxo derivative[17]

11α-hydroxypregnane-3, 20-dione is recovered unchanged after treatment with *t*-butylhypochlorite.

3β, 11α-dihydroxy-5α-pregnan-20-one—
3, 20-dioxo derivative[18]

That desoxycholic acid was not affected with

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[13] L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3938 (1949).

*2 See experimental part.

[14] L. F. Fieser and S. Rajagopalan, ibid., **74**, 3309 (1952).

[15] S. G. Brooks, J. S. Hunt, A. G. Long and B. Mooney, *J. Chem. Soc.*, **1957**, 1175.

[16] A. R. Hanze, G. S. Fonken, A. V. McIntosch, A. M. Searcy and R. H. Levin, *J. Am. Chem. Soc.*, **76**, 3179 (1954).

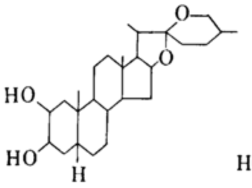
[17] H. L. Herzog, M. Jevnik. and E. B Hershberg, ibid., **75**, 269 (1953).

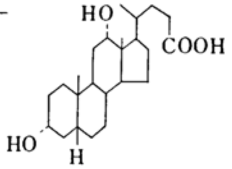
[18] O. Mancera, J. Romo, F. Sondheimer, G. Rosenkranz and C. Djerassi, *J. Org. Chem.*, **17**, 1066 (1952).

NBS in aqueous acetone or in aqueous sodium hydrogen carbonate[19] but with NBA in aqueous *t*-butanol[20], certainly appears to be governed principally by steric effects coupled with size of the reagent and solvating power of the solvent. Thereupon, all hydroxyl groups in which α-hydrogen permits the approach of the base, should be oxidized and this theory was also supported by the following examples.

Ethyl $3\beta, 5\beta$, 19-trihydroxyetianate—3-oxo derivative[21]
Methyl 3α-hydroxy-$9\alpha, 11\alpha$-epoxy-5β-cholanoate—3-oxo derivative[22]

The hydroxyl group at C_3 in samogenine[23] (I) and in desoxycholic acid[19] (II), however, was not affected with NBS The reason is not clear in details but it perhaps depends upon the intermediate permitting no release of α-hydrogen on account of vicinal effect or another interactions.


(I)


(II)

Experimental[24]

Oxidation of Epimeric 3, 6-Dihydroxy-5β-cholanoic Acids with NBS—General procedure of oxidation was performed by almost the same means as the method previous described[2].

1) **The Case of α-Hydroxycholic Acid.**—*1.1) With 1 Molar Equivalent of NBS in Dioxane-Water.*—A mixture consisting of α-hyodeoxycholic acid (761 mg.), dioxane-water (9 : 1) (15 ml.) and NBS. (380 mg. ; 1.1 mol. equiv.) was allowed to stand for 1.5 hr. at room temperature (18°C). The mixture was changed from colorless to yellow and to orange successively in coloration. To the solution, 10% of aqueous sodium bisulfite was added. The ether extract was washed well with water and evaporated to dryness to afford oil (920 mg.) which was followed by esterification with methanol (4 ml.) and 10% of methanolic hydrochloric acid (0.5 ml.) overnight at room temperature. The ether extract, after isolation in the usual way, gave a yellow oil (777 mg.) which was chromatographed on alumina to afford methyl 3-oxo-6α-hydroxy-5β-cholanoate 3-acetal, 237 mg. (27.2%), m. p. 153~154°C. Identity was established by a mixed melting point and the infrared spectrum with the authentic sample. Infrared spectrum : $\nu_{\max}$ 3564 (OH); 1724 cm^{-1} (C=O).

Found : C, 72.10 ; H, 10.30. Calcd. for $C_{27}H_{46}O_5$: C, 71.96 ; H, 10.29%.

1.2) With 2 Molar Equivalents of NBS in Dioxane-Water.—A mixture consisting of α-hyodeoxycholic acid (605 mg.), dioxane-water (9 : 1) (10 ml.) and NBS (600 mg. ; 2.2 mol. equiv.) was treated as in the previous case. The mixture gave methyl 3-oxo-6α-hydroxy-5β-cholanoate 3-acetal, m. p. 152~153°C, 209 mg. (30.1%). This compound was identified by a mixed melting point and the infrared spectrum with the authentic sample.

1.3) With 1 Molar Equivalent of NBS in Acetone-Water.—A mixture consisting of α-hyodeoxycholic acid (579 mg.), acetone-water (7 : 3) (13 ml.) and NBS (290 mg. ; 1.1 mol. equiv.) was treated as in the previous case after being allowed to stand overnight at room temperature to furnish crystals, 224 mg., m. p. 146~151°C. The residue from the filtrate was chromatographed on alumina in the usual manner to afford the acetal with m. p. 149~151°C (53 mg.). Total yield were 41.7%. This sample was also identical in a mixed melting point and the infrared spectrum.

1.4) With 2 Molar Equivalents of NBS in Acetone-Water.—The mixture consisting of α-hyodeoxycholic acid (929 mg.), pyridine (500 mg.), acetone-water (7 : 3) (20 ml.) and NBS (926 mg. ; 2.2 mol. equiv.) was treated in a way similar to that of the case in the preceding section after being kept for four days at room temperature (20°C) to give the acetal with m. p. 150~152°C (660 mg. ; 61.8%). Identity was established by a mixed melting point and the infrared spectrum. Found : C, 72.16 ; H, 10.26%.

2) **The Case of β-Hyodeoxycholic Acid.**—*2.1) With 1 Molar Equivalent of NBS in Dioxane-Water.*—After treatment as in the case of section 1.1 the above acetal with m. p. 152~153°C was obtained in 21.3% yield. This compound was identical with the authentic methyl 3-oxo-6α-hydroxy-5β-cholanoate 3-acetal in a mixed melting point and the infrared spectrum.

2.2) With 2 Molar Equivalents of NBS in Dioxane-Water.—Treatment as in the case of section 1.2 afforded the acetal with m. p. 150~152°C in 45.7% yield. Identification was carried out by a mixed melting point and the infrared spectrum.

2.3) With 1 Molar Equivalent of NBS in Acetone-Water.—Treatment similar to that in the case of section 1.3 gave the acetal with m. p. 150~152°C in 31% yield. This compound was also identified

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[19] L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3535 (1949).

[20] H. Reich and T. Reichstein, *Helv. Chim. Acta*, **26**, 562 (1943).

[21] P. Herzig and M. Ehrenstein, *J. Org. Chem.*, **17**, 713 (1952).

[22] L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **73**, 118, 5252 (1951).

[23] H. Ōsaka, private communication.

[24] All melting points are uncorrected. Infrared spectra were recorded in Nujol Mulls with a "Koken DS 301" double beam infrared spectrophotometer, unless otherwise noted, and the ultraviolet spectra were taken in 95% ethanol solution using a "Hitachi EPS 2" spectrophotometer. Optical rotations were determined in a 1-dm. tube for chloroform solutions, unless otherwise specified. Alumina used for chromatography in this experiment was Merk's reagent grade standardized according to Brockmann and the chromatography was usually performed according to the method described by T. Reichstein. (T. Reichstein and W. Schoppee, *Diss. Faraday Soc.*, **1949**, 305) Extracts were dried over anhydrous sodium sulfate before evaporation unless stated otherwise.

by a mixed melting point and the infrared spectrum.

2.4) With 2 Molar Equivalents of NBS in Acetone-Water. — Isolation similar to that in the case of section 1.4 furnished the acetal with m. p. 148∼150°C in 62.5% yield. Identity was established by a mixed melting point and the infrared spectrum.

3) The Case of 3β, 6β-Dihydroxy-5β-cholanoic Acid.—In this experiment, methyl 3β, 6β-dihydroxy-5β-cholanoate was used on account of insolubility of its acid in acetone-water (7 : 3).

3.1) With 1 Molar Equivalent of NBS in Dioxane-Water.—A mixture consisting of methyl 3β, 6β-dihydroxy-5β-cholanoate (350 mg.), dioxane-water (9 : 1) (10 ml.) and NBS (169 mg.; 1.1 mol. equiv.) was allowed to stand for 1.5 hr. at room temperature (22°C). To the solution, 10% of aqueous sodium bisulfite was added to decolorize it. The extract with ether was washed well with water and evaporated to dryness to yield orange oil (494 mg.) which was acetylated with pyridine (4 ml.) and acetic anhydride (4 ml.) under reflux for 2 hr. After cooling, the mixture was carefully diluted with water to afford crystals which were collected, washed throughly with water and recrystallized from methanol to methyl 3β-acetoxy-6-oxo-5α-cholanoate, 235 mg., scales, m. p. 156∼158°C. Ether extraction from the above aqueous and methanolic filtrate afforded a yellow oil (156 mg.) which was followed by chromatography on alumina. The eluate with petroleum ether-benzene (2 : 8 and 1 : 1) and benzene gave the above 6-oxo derivative with m. p. 157.5∼158°C (61 mg.). Total yield was 77%. This compound was identified by a mixed melting point and the infrared spectrum with the authentic methyl 3β-acetoxy-6-oxo-5α-cholanoate. $[\alpha]_D^{24} = -14 \pm 2°$ (*c* 1.038). Infrared spectrum: ν_{max} 1730, 1710 (C=O); 1252, 1239, 1169, 1039 cm^{-1} (C–O).

Found: C, 72.65; H, 9.48. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48%.

3.2) With 2 Molar Equivalents of NBS in Dioxane-Water.—A mixture consisting of methyl 3β, 6β-dihydroxy-5β-cholanoate (347 mg.) dioxane-water (9 : 1) (10 ml.) and NBS (320 mg.; 2.1 mol. equiv.) was treated as in the previous subsection to give an orange oil (413 mg.) which was followed by chromatography on alumina. The eluate with benzene-petroleum ether (1 : 1), benzene and benzene-chloroform (9 : 1) furnished methyl 3β-acetoxy-6-oxo-5α-cholanoate, 216 mg. (56.6%), scales, m. p. 158∼159°C. This was identical with the above specimen in a mixed melting point and the infrared spectrum. The benzene-chloroform (8 : 2 and 1 : 1) elution afforded methyl 3,6-dioxo-5α-cholanoate, 49 mg. (14.3%), m. p. 146∼148°C. Zimmermann test gave cherry-red coloration. Infrared spectrum: ν_{max} 1745, 1711 cm^{-1} (C=O).

Found: C, 74.19; H, 9.58. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52%.

3.3) With 1 Molar Equivalent of NBS in Acetone-Water. — Isolation as in the section 3.1 after being kept overnight at room temperature (18°C) gave methyl 3β-acetoxy-6-oxo-5α-cholanoate, m. p. 157∼158°C in 77.5% yield. Zimmermann test was negative. This compound was also identical with the above specimen by a mixed melting point and the infrared spectrum.

3.4) With 2 Molar Equivalents of NBS in Acetone-Water.—Treatment as in the previous case furnished methyl 3β-acetoxy-6-oxo-5α-cholanoate, m. p. 157∼158°C in 55% yield from the elution with benzene-petroleum ether (1 : 1) and benzene, and methyl 3,6-dioxo-5α-cholanoate, m. p. 148°C in 19.3% yield from the benzene-chloroform (9 : 1) eluate. These samples were also identified by a mixed melting point and the infrared spectrum.

4) The Case of 3α, 6β-Dihydroxy-5β-cholanoic Acid. — *4.1) With 1 Molar Equivalent of NBS in Dioxane-Water.*—To a solution of 3α, 6β-dihydroxy-5β-cholanoic acid (377 mg.) in dioxane-water (9 : 1) (10 ml.), NBS (188 mg.; 1.1 mol. equiv.) was added and kept for 1.5 hr. at room temperature (20±2°C). Isolation, esterification with ethereal diazomethane and acetylation in the usual way gave methyl 3α-acetoxy-6-oxo-5β-cholanoate as prisms, m. p. 150∼153°C, 260 mg. $[\alpha]_D^{17} = -21 \pm 2°$ (*c* 1.052, dioxane). Infrared spectrum: ν_{max} 1703 (C=O); 1241, 1230, 1164, 1036, 1026 cm^{-1} (C–O).

Found: C, 72.62; H, 9.59. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48%.

By chromatography on alumina, the filtrate from the above crystals gave further 6-oxo derivative, 40 mg., m. p. 155∼165°C (from methanol), a mixture of 5α- and 5β-series.

Found: C, 72.71; H, 9.53%. Total yield was 70%.

4.2) With 2 Molar Equivalents of NBS in Dioxane-Water. — Isolation in the usual way gave a mixture of 6-oxo derivative in 5α- and 5β-series with m. p. 143∼147°C in 60% yield from the eluate with benzene-petroleum ether (1 : 1), benzene and benzene-chloroform (9 : 1). Infrared spectrum: ν_{max} 1741, 1712 (C=O); 1249, 1175, 1025 cm^{-1} (C–O).

Found: C, 72.75; H, 9.43. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48%.

The benzene-chloroform (8 : 2 and 1 : 1) eluate gave methyl 3,6-dioxo-5α-cholanoate, m. p. 146∼148°C, in 7.5% yield. Infrared spectrum: ν_{max} 1740, 1710 (C=O); 1171 cm^{-1} (C–O).

Found: C, 74.19; H, 9.64. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52%.

4.3) With 1 Molar Equivalent of NBS in Acetone-Water. — The methyl ester was used in this case because the acid was not dissolved in acetone-water (7 : 3). Treatment as in section 3.3 gave 6-oxo derivative, m. p. 150∼155°C, in 70% yield. The identification was carried out by a mixed melting point and the infrared spectrum.

Found: C, 72.55; H, 9.64%.

4.4) With 2 Molar Equivalents of NBS in Acetone-Water. — Reaction and isolation as in section 3.4 afforded 6-oxo derivatives in 5α- and 5β-series with m. p. 155∼160°C and m. p. 169∼170°C, respectively in 59.4% yield from the eluate with benzene and benzene-chloroform (9 : 1). Zimmermann test was negative. Identity was established by a mixed melting point and the infrared spectrum.

Found: C, 72.92; H, 9.57%.

Benzene-chloroform (9 : 1) elution gave methyl 3,6-dioxo-5β-cholanoate, m. p. 128∼129°C, in 16% yield. Infrared spectrum: ν_{max} 1739, 1712 (C=O), 1171 cm^{-1} (C–O).

Found: C, 74.07; H, 9.44%.

Oxidation of 3β, 6α-Dihydroxy-5α-cholanoic Acid[*3]. — To a solution of 3β, 6α-dihydroxy-5α-cholanoic acid (140 mg.) and pyridine (37.3 mg.) in acetone (1.96 ml.) and water (0.84 ml.) was added NBS (82.5 mg.; 1.3 mol. equiv.). The resulting mixture was refluxed for 4.5 hr., and then the solvent was removed under reduced pressure to the point of distinct turbidity. After a large volume of water was added, the precipitate was collected and dried. This crude keto acid (46 mg.) was recrystallized from acetone to afford 3-oxo-6α-hydroxy-5α-cholanoic acid, fine needles, m. p. 195∼197°C (20 mg.). Zimmermann test was positive. $[\alpha]_D^{32}=+4\pm2°$ (c 1.059, pyridine).

Found: C, 73.21; H, 9.85. Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81%.

The above crude acid was esterified with ethereal diazomethane to yield its methyl ester, melting at 90∼97°C undefinitely. Zimmermann test was positive. $[\alpha]_D^{27}=+55\pm2°$ (c 1.038). Infrared spectrum: $\nu_{max}^{CHCl_3}$ 3524 (OH); 1731, 1711 cm^{-1} (C=O).

Found: C, 72.86; H, 10.04. Calcd. for $C_{25}H_{40}O_4 \cdot 1/2H_2O$: C, 72.61; H, 9.91%.

Acetylation of this methyl ester gave methyl 3-oxo-6α-acetoxy-5α-cholanoate, plates, m. p. 115∼116°C (from ether-petroleum ether). Zimmermann test was positive. $[\alpha]_D^{33}=+48\pm4°$ (c 0.608)[*4]. Infrared spectrum: $\nu_{max}^{CHCl_3}$ 1729, 1712 (C=O); 1249, 1175, 1024 cm^{-1} (C-O).

Found: C, 72.92; H, 9.81. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48%.

Oxidation of 3β, 6β-Dihydroxy-5α-cholanoic Acid[*5]. — To a solution of 3β, 6β-dihydroxy-5α-cholanoic acid (73 mg.) in acetone (6 ml.), water (1 ml.) and methanol (1.5 ml.), was added NBS (75 mg.; 2.3 mol. equiv.). After the resulting mixture was kept for 3 hr. at room temperature, aqueous sodium bisulfite was added, and the extract with ether was washed with water and evaporated to furnish needles with m. p. 235∼237°C. This crude acid was recrystallized from acetone to methyl 3β-hydroxy-6-oxo-5α-cholanoic acid, m. p. 244∼245°C, 23 mg. Zimmermann test was negative. Ultraviolet spectrum: λ_{max}^{EtOH} 287 mμ (ε=45). Infrared spectrum: ν_{max} 1727, 1689 cm^{-1} (C=O).

Found: C, 73.61; H, 9.68. Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81%.

Its methyl ester was obtained as scales from methanol, melting at 93∼95°C. Acetylation of this methyl ester furnished methyl 3β-acetoxy-6-oxo-5α-cholanoate, m. p. 155∼157°C, scales. A mixed melting point with methyl 3α-acetoxy-6-oxo-5β-cholanoate with m. p. 156∼157°C was melted at 130∼135°C. $[\alpha]_D^{32}=-9\pm2°$ (c 0.91, methanol).

Found: C, 72.62; H, 9.45. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48%.

Spectroscopic Determination of the Order of Reactivity in the Oxidation of Methyl 3β- and 3α-Hydroxy-5β-cholanoate with NBS. — *Measurement.*—For the measurement of the optical densities, a "Koken DS 301" (double beam) was used. Infrared spectra were measured by used of a 0.25 mm. cell and at the concentration of about 37% transmission of carbonyl stretching absorption band of the ester group which was controlled by dilution. The speed in the measurements was adjusted to 50 cm^{-1} per 36 sec. at about 1700 cm^{-1} for the purpose of a quantitative analysis. The optical densities of carbonyl band at 1720 cm^{-1} in carbon tetrachloride solution was calculated by the base line method (Fig. 1). T_0 and T were a mean value of three measurements.

Procedure. — A mixture consisting of 3-hydroxy compounds (210 mg.), NBS (126 mg.; 1.3 mol. equiv.) and dioxane-water (9 : 1) (22 ml.) in a 50 ml. reaction flask with stopper was allowed to stand at 25°C with use of thermostat. Each 5 ml. of the reaction mixture was pipetted at various times and the reaction in each aliquot was stopped with aqueous sodium bisulfite, extracted with ether and successively washed with water, soda and water (ten times). The ethereal layers were evaporated and finally dried in vacuo for 12 hr. at 60°C. The obtained residue (about 45 mg.) was dissolved in so much carbon tetrachloride as to make an adequate concentration giving optical density of the carbonyl band in the ester group at about 37% transmission. The yield of the oxidation product was estimated from the calibration curve prepared above (Fig. 2). These values are shown in Tables IV and V (Fig. 3).

Summary

(1) The order of susceptibility of the hydroxyl groups in epimeric 3, 6-dihydroxycholanoic acids to the oxidation with NBS was determined and the results were as follows:

6β(a) > 3β(a) > 3α(e) > 6α(e) (A/B: cis)

6β(a) > 3β(e) > 6α(e) (A/B: trans)

(2) Reaction mechanism of the oxidation with NBS was discussed.

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[*3] Synthesis of this compound was described in preceding part XX.

[*4] Optical rotation of methyl 3-oxo-6α-acetoxy-5β-cholanoate was as follows: $[\alpha]_D^{31}=-29\pm2°$ (c 1.057).

[*5] As optical rotation of this compound had not been reported, it was measured by the author and its value was found to be as follows: $[\alpha]_D^{33}=-0.1\pm2°$ (c 1.057, pyridine).