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Metal Ion Catalyzed Oxidation of Steroids. III.[1] Reactions of Cholesterol with Ferrous Ions- and Titanous Ions-Hydrogen Peroxide Systems in Acetonitrile[2]

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The reactions of cholesterol (I) with hydrogen peroxide in the presence of transition-metal salts were carried out at moderate temperature in an acetonitrile medium. When ferrous or titanous sulphate was employed, 5,6α-epoxy-5α-cholestan-3β-ol (II) (and/or epimeric β-epoxide, XII), 5α-cholestane-3β,5,6β-triol (III), cholest-5-ene-3β,7α-diol (IV), and cholest-5-ene-3β,7β-diol (V) were formed in rather poor yields. The radical scavenger, α-tocopherol, inhibited the hydroxylation at the allylic position and thus no formation of IV and V was observed.

On the contrary, titanium trichloride-hydrogen peroxide system was found to consume entirely the substrate (I) giving considerable amounts of III and the chlorinated products such as 5,6β-dichloro-5α-cholestan-3β-ol (VI), 5-chloro-5α-cholestane-3β,6β-diol (VII), 6β-chloro-5α-cholestane-3β,5-diol (VIII), and 6α-chloro-5β-cholestane-3β,5-diol (IX). The epoxides (II and XII) were separated solely as the artifacts from the monochlorides (VII and VIII) through column chromatography on alumina. The yields of III, VI, the mixture of VII and VIII, and of IX were in an approximate ratio of 2: 1: 2: 4.

In 1950, Clemo, *et al.*[4] reported the formation of 5α-cholestane-3β,5,6β-triol (III) and 3β-acetoxy-5α-cholestane-5,6β-diol from cholesterol (cholest-5-en-3β-ol) (I) by Fenton's reagent in an acetic acid medium. They understood these results as a general tendency for the hydroxyl radicals to be added to the isolated double bond in the C-5: 6 position. In the preceding paper of this series,[1] more reliable explanation was presented that ferrous ions accelerated the reaction of acetic acid with hydrogen peroxide to form peracetic acid which then epoxidized cholesterol in an usual fashion of heterolysis and the triol (III) as well as their acetates were formed by the subsequent solvolysis of the epoxide (5,6α-epoxy-5α-cholestan-3β-ol) (II).

Titanous ions-hydrogen peroxide system has also been postulated as generating hydroxyl radicals.[5] Armstrong[6] reported that the relative reaction rates of the oxidizing species from Fe(II)/H_2O_2 (Fenton's reagent), Ti(III)/H_2O_2, and γ-radiolysis of water are identical for a variety of substartes and these three systems can produce hydroxyl radicals at pH 1.0. Electron spin resonance (ESR) spectroscopic studies on aqueous Ti(III)-H_2O_2 systems, however, suggested the presence of the species formed by the interaction of hydroxyl radicals with Ti(IV) peroxide complexes.[5c,7] The present paper deals with the reactions of cholesterol (I) with

1) Part II: M. Kimura, M. Tohma, and T. Tomita, *Chem. Pharm. Bull.* (Tokyo), **20**, 2185 (1972).
2) A part of this work was presented at the 92nd Annual Meeting of the Pharmaceutical Society of Japan (April, 1972, in Osaka).
3) Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*
4) C.R. Clemo, M. Keller, and J. Weiss, *J. Chem. Soc.*, **1950**, 3470.
5) *a*) W.T. Dixon and R.O.C. Norman, *Nature*, **196**, 891 (1962); *idem*, *J. Chem. Soc.*, **1963**, 3119; *idem*, *ibid.*, **1964**, 3625, 4850, 4857; *b*) C.R.E. Jefcoate and R.O.C. Norman, *J. Chem. Soc.* (*B*), **1968**, 48; *c*) R.O.C. Norman and P.R. West, *J. Chem. Soc.* (*B*), **1969**, 389.
6) W.A. Armstrong, *Can. J. Chem.*, **47**, 3737 (1969).
7) *a*) Y.S. Chiang, J. Graddock, D. Mickewich, and J. Turkevich, *J. Phys. Chem.*, **70**, 3509 (1966); *b*) H. Fischer, *Ber. Bunsenges. Phys. Chem.*, **71**, 685 (1967); *c*) K. Takakura and B. Ranby, *J. Phys. Chem.*, **72**, 164 (1968); *d*) R.E. Florin, F. Sicilio, and L.A. Wall, *ibid.*, **72**, 3154 (1968); *e*) R.E. James and F. Sicilio, *ibid.*, **74**, 2294 (1970); *f*) Y. Shimizu, T. Shiga, and K. Kuwata, *ibid.*, **74**, 2929 (1970).

ferrous ions- and titanous ions-hydrogen peroxide systems in acetonitrile media, which can hardly be assumed to form the peracid under the conditions attempted, and deals also with the formation of several chlorinated products in the similar reaction using titanium trichloride.

Result

Reactions with Ferrous Sulphate- and Titanous Sulphate-Hydrogen Peroxide Systems

When cholesterol (I) was oxidized by ferrous sulphate-hydrogen peroxide system in acetonitrile, the epoxide (II and/or 5,6β-epoxy-5β-cholestan-3β-ol, XII), triol (III), 7α-hydroxycholesterol (cholest-5-ene-3β,7α-diol) (IV), and its 7β-epimer (cholest-5-ene-3β,7β-diol) (V) were detected in thin-layer chromatography (TLC) of the products as shown in Fig. 1. When α-tocopherol which is one of the popular radical scavengers was present in the reaction mixture, neither 7α-(IV) nor 7β-hydroxylated product (V) was detected by TLC (Fig. 1). Similar results were obtained when Fenton's reagent was displaced by the titanous sulphate-hydrogen peroxide system. The isotope experiment using cholesterol-4-^{14}C and ferrous system revealed the formation of epoxide (II and/or its β-isomer, XII) and triol (III) in the respective yield of about 10 and 7 per cent. In the case of titanous system, these yields were calculated to be roughly 9 and 8 per cent, respectively. The amounts of IV and V were, in both cases, as smaller as counted to be rather of trace, thugh they could be detected in TLC by staining with hot sulfuric acid.

Reactions with Titanous Ions-Hydrogen Peroxide System in the Presence of Chloride Anions

An interesting reaction occured in the presence of chloride anions. When hydrogen chloride was added to the reaction mixture of the substrate (I), titanous sulphate, and hydrogen peroxide in acetonitrile, the different results were observed in TLC and these were given more distinctively when titanium trichloride was employed in place of the sulphate in the Ti(III)/H_2O_2 system as shown in Fig. 2. The reaction mixture in a preparative scale was treated as described in the experimental part and the organic contents were then submitted to column chromatography on alumina. The products thus separated were the epoxides (II and XII), triol (III), 5,6β-dichloro-5α-cholestan-3β-ol (VI), and a monochloride (IX). Although the formation of IV, V, 5-chloro-5α-cholestane-3β,6β-diol (VII), and 6β-chloro-5α-cholestane-3β,5-diol (VIII) was recognized in TLC of the reaction mixture (Fig. 2), the preparative column chromatography gave none of them but a mixture of II and XII, which could not entirely be detected in TLC. The isotope experiment using radio TLC scanner revealed that the reaction was likely to proceed almost completely and the yields of III, VI, the mixture of VII and VIII, and of IX were in an approximate ratio of 2: 1: 2: 4, respectively.

The monochloride, $C_{27}H_{47}O_2Cl$, mp 140.5—142.5°, (IX) gave a monoacetate (X) which retained a free hydroxyl group and was dehydrated with thionyl chloride yielding an olefinic product (XI); one of the hydroxyl groups in IX was thus tertiary in nature. By heating in an alkaline solution, IX turned to an epoxide (XII). The tertiary hydroxyl group in IX was thus likely to be located at C_5 and of β-configuration. Oxidation of IX with Jone's reagent gave a hydroxyketone (XIII) which was converted into 6α-chloro-cholest-4-en-3-one (XIV) when it was heated in acetic acid. From these results, the chemical structure of the compound (IX) was elucidated to be 6α-chloro-5β-cholestane-3β,5-diol.

Discussion

Polar aprotic solvents have often been employed in such type of reaction as discussed in the present study; for example, N,N-dimethylacetamide was employed for the oxygenation

8) B.R. James and E. Ochiai, *Can. J. Chem.*, **49**, 975 (1971); B.R. James and F.T.T. Ng, *Chem. Commun.*, **1970**, 908.
9) R.C. Paul, K.S. Sooch, O.C. Vaidya, and S.P. Narula, *Anal. Chim. Acta*, **46**, 131 (1969).

reactions catalyzed by rhodium complexes[8] and formamide was the solvent used for the oxidation with lead salts.[9] Dimethyl sulfoxide can easily produce methyl radical, $\cdot CH_3$, and methyl sulfonyl radical, $\cdot CH_3\text{-}SO_2$, when it is mixed with Ti(III)/H_2O_2 system.[10] Although acetonitrile is known also to produce the radical, $\cdot CH_2CN$, through the electrochemical oxidation of anions such as perchlorate, tetrafluoroborate, and hexafluorophosphate,[11] a variety of organic reactions using inorganic reagents has commonly been carried out in nonaqueous as well as aqueous media of this solvent; for example, the reactions of peroxides and alkenes with copper salts,[12] the use of Fe(III)/Fe(II) and Cu(II)/Cu(I) couples as the analytical oxidant as well as the reagents for investigating the oxidation of organic compounds,[13] and the oxygenase-model reaction with stannous phosphate complex.[14] Also in acetonitrile medium, the reaction using benzoyl peroxide and cuprous chloride was carried out even at higher temperature of 80° for three hours.[15] From the results in the present experiment, it may thus be reasonable to assume that the peracid and radical, which might be generated from the solvent molecule, can not be the principal attacking species in such reactions studied here.

In spite of numerous investigations on Fenton's reagent, the Haber-Weiss's mechanism[16] remains uncertain except the initial step[17]: $Fe^{2+}+H_2O_2 \rightarrow FeOH^{2+}$(ion-pair)$+\cdot OH$. Titanous ions-hydrogen peroxide system has also been postulated as generating hydroxyl radicals,[5] through which both systems bring about oxidation.[5c] It was reported that the relative reaction rates of the oxidizing species from Fenton's reagent, Ti(III)/H_2O_2, and the γ-radiolysis of water are identical for a variety of substrates and these three systems can produce hydroxyl radicals at pH 1.0.[6] Electron spin resonance (ESR) spectroscopic studies on aqueous Ti(III)-hydrogen peroxide systems suggested the presence of the species formed by an interaction of hydroxyl radicals with Ti(IV) peroxide complex,[5c,7] specifically Ti(IV)-O-O$\cdot^{3+}$(titanium peroxyl radical).[6,7d] In the oxidation reaction of alcohols or ethers using Fe(II)/EDTA/H_2O_2 system in neutral phosphate buffer, the ratios of the concentrations of the various possible radicals as observed by their ESR spectra were quite different from those observed when Ti(III)/H_2O_2 system was used.[5c] In contrast, no differences have been observed between the hydroxylating entities in the Fenton's and Ti(III)/H_2O_2 systems when some benzenoid substrates were oxidized.[5c] In the present experiment employing ferrous sulphate- or titanous sulphate-hydrogen peroxide system, the isomers (IV and V) of 7-hydroxycholesterol were commonyl produced from cholesterol (I) and their formation was inhibited by the presence of α-tocopherol, the radical scavenger (Fig. 1). Hydroxy radicals are considered to show electrophilic properties when they come into contact with the π-electron system.[18] The formation of IV and V may, threfore, be realized as the result of this kind of attack at the allylic position in I by these radicals.

Another products common to all systems employed in the present study were the epoxide (II and/or XII) and the triol (III) in considerable amounts. When cholesterol (I) was incubated with Fe(III)(acetylacetonate)$_3$/H_2O_2 system in acetonitrile medium, stereoselective

10) W. Damerau, G. Lassmann, and K. Lohs, *Z. Chem.*, **9**, 343 (1969).
11) M. Fleischmann and D. Pletcher, *Tetrahedron Letters*, **1968**, 6255.
12) J.K. Kochi and H.E. Mains, *J. Org. Chem.*, **30**, 1862 (1965).
13) B. Kratochvil and R. Long, *Anal. Chem.*, **38**, 770 (1966); *idem, ibid.*, **40**, 442, 2120 (1968); *idem, ibid.*, **42**, 43 (1970); B. Mruthyunjaya and A.R.V. Murthy, *Anal. Chem.*, **41**, 186 (1969); J.K. Senne and B. Kratochvil, *Anal. Chem.*, **44**, 585 (1972).
14) V. Ullrich and Hj. Staudinger, *Z. Naturforsch.*, **24b**, 583 (1969).
15) S. Hashimoto, W. Koike, and Y. Matsuda, *Kogyo Kagaku Zasshi* (*J. Chem. Soc. Japan, Ind. Sect.*), **72**, 2277 (1969).
16) F. Haber and J. Weiss, *Naturwiss.*, **20**, 948 (1932); N. Uri, *Chem. Rev.*, **50**, 375 (1952).
17) F. Basolo and R.G. Pearson, "Mechanism of Inorganic Reactions," 2nd ed., J. Wiley and Sons, Inc., New York, 1967, Chapter VI.
18) L.M. Dorfman, A. Taub, and R.E. Büchler, *J. Chem. Phys.*, **36**, 305 (1962); R.O.C. Norman and G.K. Radda, *J. Chem. Soc.*, **1963**, 2897; J.H. Fendler and G.L. Gasowski, *J. Org Chem.*, 33, 1865, 2755 (1968).

epoxidation occurred in an extremely high yield.[19] It has been known that cyclohexene was epoxidized by Fenton's reagent.[20] From the irradiated aqueous solution of I, the triol (III) was obtained and this result was explained by the addition of hydroxyl radicals to the C-5: 6 double bond, since no evidence could be obtained for the presence of an epoxide among the products.[21] The radical scavenger was, however, incapable of preventing the formation of II and III, though it could inhibit that of IV and V (Fig. 1). The triol (III) was also produced mainly through the epoxide (II) once formed from I by Fenton's reagent in acetic acid.[1] It may be reasonable to assume that the epoxide(s) (II and/or its β-isomer) was (were) the initial product(s) under the conditions attempted in the present study and a part of the epoxide(s) thus formed was also hydrolyzed subsequently to give the triol (III). Epoxidation by peracid occurs readily in nonpolar solvent and the most satisfactory mechanism is explained by the formation of intermediary π-complexes of olefins with peroxide.[22] It seems consequently that free hydroxyl radicals cannot be the main attacking species in the present reactions but some unknown peroxidic intermediate complex of the metal ion, which may force highly polarlized the O-O bond of such peroxidic species, might be favorable for epoxidation. Since the reaction was, contrary to those in the other two systems, likely to proceed almost completely in the titanium trichloride-hydrogen peroxide system as described above, the complex-formation of titanium chloride with acetonitrile[23] might have some advantage in forming such peroxidic complex.

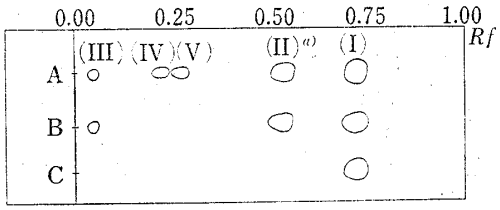


Fig. 1. Thin-Layer Chromatogram of Reaction Mixture with Ferrous System

A: reaction mixture
B: containing radical scavenger
C: lacking metal salt
a) and/or β-isomer
solvent system: AcOEt/cyclohexane (1: 1)
stain: spraying with 2N sulfuric acid and heating

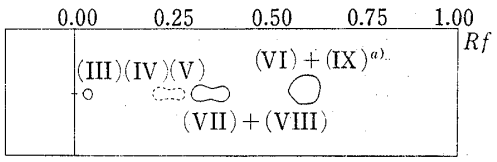


Fig. 2. Thin-Layer Chromatogram of Reaction Mixture with Titanium Trichloride System

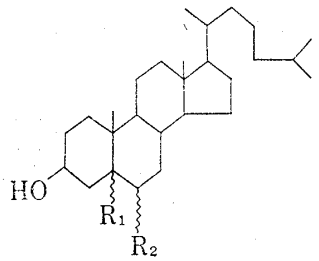
solvent system: AcOEt/cyclohexane (1: 1)
stain: spraying with 2N sulfuric acid and heating
a) *Rf* values of VI and IX in the solvent system of AcOEt/cyclohexane (1: 7) were 0.45 and 0.17, respectively.

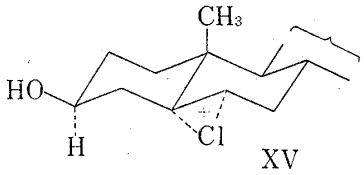
In the presence of chloride anions the reactions were rather complicated. The present experiment employing cholesterol-4-^{14}C with titanium trichloride-hydrogen peroxide system revealed that the reaction proceeded almost completely and the higher yields of III, VI, a mixture of VII and VIII, and of IX were in an approximate ratio of 2: 1: 2: 4. The formation of III, VII, and VIII may indicate that the epoxides (II and XII) are the initial products as in the other systems lacking chloride ions. It has been known that chlorohydrin can generally be derived from the corresponding epoxide and *vice versa*. In fact, VII and VIII were derived from the authentic specimens of XII and II by using hydrochloric acid, respectively and were then turned back to the corresponding epoxides by mixing with alumina under the conditions employed in the column chromatography, as described in the experimental part. It seemed consequently that the epoxides initially formed were consumed subsequently to give III, VII, as well as VIII in the acidic reaction mixture and that hydrochloric acid was eliminated from

19) M. Kimura, M. Tohma, and T. Tomita, to be published.
20) C.A. Hamilton and R.J. Workman, *J. Am. Chem. Soc.*, **86**, 3390 (1964).
21) M. Keller and J. Weiss, *J. Chem. Soc.*, **1950**, 2709.
22) E.S. Gould, "Mechanism and Structure in Organic Chemistry," H. Holt and Co., New York, 1959, Chapter XIII.
23) G.W.A. Fowles and K.F. Gadd, *J. Chem. Soc.*, (*A*), **1970**, 2232; B. Hessett and P.G. Perkins, *ibid.* (*A*), **1970**, 3329.

these chlorides during course of the chromatography on alumina to give the corresponding epoxides (II and XII) as the artifacts. Chloride ions in such an oxidizing system, on the other hand, might produce chlorine molecules which might directly add to the olefinic substrate (I) forming the dichloride (VI). It is interesting in these respects that chlorine dissolved in an aqueous *tert*-butanol converted cholesterol (I) to various chlorinated derivatives such as VI (3% in yield), VII (36%), VIII (trace), and IX (15%) which was isolated merely as the 3β-acetate.[24]

Contrary to the cases of VI, VII, and VIII, the formation of 6α-chloro-5β-cholestane-3β,5-diol (IX) seems to be in a particular fashion of stereochemistry and it is of great interest that an equatorial attack of azide anion occurred on the 3β-acetate of the β-epoxide (XII) yielding the 5β-hydroxy-6α-azide[25a] and that the *trans*-diequatorial addition products were also formed in the bromination as well as chlorination of unsaturated steroids.[25b] On the other hand, the aluminum chloride-hydrogen peroxide system[25c] was assumed to involve a hydroxyl cation species, highly polarlized $AlCl_3$–H_2O_2 complex, and the chlorination was presumed to occur *via* hypochlorous acid which was formed by attack of chloride anoins on the complex and acted as the precursor for the chloronium cation. The chlorine atoms or Cl_2^- radicals can be formed by the reaction of hydroxyl radical with chloride anions.[25d] As far as hypochlorous acid concerned, the addition to isobutene, $(CH_3)_2C{=}CH_2$, has been known to yield as the predominant product the α-chlorohydrin, $(CH_3)_2C(OH)$-CH_2Cl.[26] It should be reasonable that hypochlorous acid was formed also in the aq. butanol solution of chlorine, in which traces of 6β-chloride (VIII) and considerable amounts of 6α-epimer (IX) were thus produced from I.[24] When the 3β-acetate of I was the substrate, on the contrary, the yield of the 6α-chloride was highly reduced to 6% contrary to an increase in that of the 6β-epimer up to 8% in the same chlorine system.[24] It might, from these results, be possible to assume that the intermediary cyclic chloronium ions (XV) of α-configuration, which might be formed with chloronium cation from hypochlorous acid, were attacked by water molecules from the β-side of the molecule resulting the unusual formation of such diequatorial chlorohydrin (IX). Further discussion is not appropriate untill more evidence has been accumulated.





XV

VI : R_1=α-Cl R_2=β-Cl
VII : R_1=α-Cl R_2=β-OH
VIII: R_1=α-OH R_2=β-Cl
IX : R_1=β-OH R_2=α-Cl
II : R_1, R_2=α-epoxy
XII: R_1, R_2=β-epoxy

Experimental

Materials

All reagents employed were of reagent-grade. Cholesterol (I) and acetonitrile were obtained from commercially available sources and purified by the ordinary methods. The authentic specimens of 5,6α-epoxy-

24) B.O. Lindgren, *Acta, Chem. Scand.*, **21**, 1397 (1967); *idem, Svensk Papperstid.*, **70**, 532 (1967) [*C.A.*, **68**, 3096a (1968)].
25) *a*) J.G.L. Jones and B.A.M. Marples, *J. Chem. Soc.* (*C*), **1968**, 2698; *b*) G.H. Alt and D.H.R. Barton, *J. Chem. Soc.*, **1954**, 4284; *c*) M.E. Kurz and G.J. Johnson, *J. Org. Chem.*, **36**, 3184 (1971); *d*) T.J. Sworski, *Radiat. Res.*, **2**, 26 (1955); M. Anbar and J.K. Thomas, *J. Phys. Chem.*, **68**, 3829 (1964).
26) A. Michael and V.L. Leighton, *Chem. Ber.*, **39**, 2157 (1906).

5α-cholestan-3β-ol (II),[27] 5,6β-epoxy-5β-cholestan-3β-ol (XII),[28] 5α-cholestane-3β,5,6β-triol (III),[29] Cholest-5-ene-3β,7α-diol (IV)[30] cholest-5-ene-3β,7β-diol (V),[31] 5,6β-dichloro-5α-cholestan-3β-ol (VI),[32] 5-chloro-5α-cholestane-3β,6β-diol (VII),[33] and 6β-chloro-5α-cholestane-3β,5-diol (VIII)[33] were prepared and purified as reported. The toluene scintillator was made by dissolving 2,5-diphenyloxazole (2500 mg) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (150 mg) in toluene (500 ml).

Methods

Melting points were taken on a micro hot-stage apparatus and uncorrected. Infrared (IR) spectral measurement was run on JASCO Model IR-S spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained by Hitachi Model H-6013 spectrometer at 60 Mc. Mass spectra (MS) were measured by Hitachi Model RMU-6E spectrometer. TLC was carried out on silica gel (Wakogel B-5) plate by the solvent system of AcOEt/cyclohexane (1: 1) and by staining with 2N H_2SO_4 and heating at 130° for 5 min. The radioactive spots were detected by Aloka Model GTC-223 radio thin-layer chromatography (TLC) scanner. The radioactivity was measured by Aloka Model LSC-501 liquid scintillation counter employing the toluene scintillator.

Standard Procedures of the Reactions

Procedure 1——For 45 min at 40° under stirring in nitrogen atmosphere, an aqueous solution (10 ml) of the metal salt (5—7×10^{-4} mole) and 30% H_2O_2 (2 ml, 1.94×10^{-2} mole) were added individually in capillary streams to the acetonitrile solution (40 ml) of cholesterol (I) (20 mg, 5.17×10^{-5} mole). After the reaction mixture was stirred for another 75 min, aq. 10% Na_2SO_3 (20 ml) was added and the organic layer formed was separated from the aqueous one which was then extracted with ether. The organic layers thus obtained were combined, washed with 5% $NaHCO_3$, then water, and dried over anhydrous Na_2SO_4. Evaporation of solvent from the solution gave a residue which was submitted to TLC for the examination of the products.

Procedure 2——Acetonitrile solution (10 ml) of I (5.0 mg, 1.30×10^{-5} mole) containing cholesterol-4-^{14}C (2.18×10^{4} cpm) was treated with an aqueous solution of the metal salt as well as with 30% H_2O_2 (0.5 ml, 4.88×10^{-3} mole) and the reaction mixture was worked-up, in the same manner as described above. The residue thus obtained was dissolved in 10 ml of EtOH and 0.5 ml of this solution was employed for measuring the recovery (mean of two determinations) of radioactivity. The remains of the residue were then submitted to TLC for estimating the formation-ratio of the products. After scanning the TLC plate, each of the spots detected was extracted with EtOH. The extracts was dissolved again in 10 ml of EtOH and 0.5 ml of this solution was employed for measuring the radioactivity (mean of two determinations) retained in each products.

Reactions with Ferrous System (Fig. 1)

In the standard procedure 1, 200 mg (7.19×10^{-4} mole) of $FeSO_4\cdot7H_2O$ was employed. No product was observed under the conditions lacking the metal salt. When the scavenger reaction was examined (B in Fig. 1), about 100 mg of α-tocopherol was mixed with this reaction mixture.

For the determination of the products, aqueous solution (2.5 ml) of $FeSO_4\cdot7H_2O$ (50 mg, 1.80×10^{-4} mole) was employed in the standard procedure 2. The recovered radioactivity was 17831 cpm (82%) and the activities retained in I, II+β-isomer, and III were 12035, 1450, and 1015 cpm, respectively.

Reaction with Titanous System

In the standard procedure 1, 79.6 mg (5.21×10^{-4} mole) of $Ti_2(SO_4)_3$ was employed. The reaction in the presence of chloride anions was carried out with this reaction mixture by adding 1N HCl (1.25 ml).

Aqueous solution (1.25 ml) of $Ti_2(SO_4)_3$ (19.90 mg, 5.18×10^{-5} mole) was employed in the standard procedure 2 for the determination of the products. The recovered radioactivity was 18789 cpm (86%) and the activities retained in I, II+β-isomer, and III were 11877, 1260, and 1200 cpm, respectively.

When 20% aqueous solution (0.04 ml) of $TiCl_3$ (5.18×10^{-5} mole) was employed in the procedure 2, about 94% (20474 cpm) of the radioactivity was recovered. No spot of the substrate (I) was observed in TLC. The scanner was entirely insensitive to the spots of IV and V, though they were detectable by staining with hot 2N H_2SO_4 (Fig. 2). The radioactivities retained in III, VI+IX, and VII+VIII were 3440, 6576, and 3256 cpm, respectively. Since VI and IX gave the identical *Rf* value by the solvent system of EtOAc/cyclohexane (1: 1), the extract from their piled spots (6576 cpm) were acetylated with Ac_2O–pyridine and submitted again to TLC with the solvent system of AcOEt/cyclohexane (1: 7). The activities retained in the acetates of VI and IX were 599 and 2465 cpm, respectively; the formation of VI and IX being thus in an approximate ratio of 1: 4.

27) L.F. Fieser and M. Fieser, "Reagents for Organic Synthesis," John Wiley and Sons, Inc., New York, 1967, p. 136.
28) A.T. Rowland and H.R. Nace, *J. Am. Chem. Soc.*, **82**, 2833 (1960).
29) L.F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3938 (1949).
30) C.W. Shoppe and B.C. Newman, *J. Chem. Soc.*, **1968**, 981.
31) L.F. Fieser, M. Fieser, and R.N. Chakravarti, *J. Am. Chem. Soc.*, **71**, 2226 (1949).
32) D.H.R. Barton and E. Miller, *J. Am. Chem. Soc.*, **72**, 370 (1950).
33) Y. Ueno, *J. Pharm. Soc. Japan*, **72**, 1620 (1952); *idem*, [*C. A.*, **47**, 8764 (1953)].

Formation and Isolation of Products from $TiCl_3$-H_2O_2 System

Cholesterol (I) (200 mg, 5.17×10^{-4} mole) was dissolved in the mixture of acetonitrile (400 ml) and 0.8N HCl (50 ml). To the stirred solution thus obtained, were added individually 20% aqueous solution of $TiCl_3$ (1.6 ml, 2.07×10^{-3} mole) and 30% H_2O_2 (20 ml, 1.94×10^{-1} mole) in capillary streams during the range of 45 min at 40° under N_2. After the reaction mixture was stirred for another 90 min, 10% $NaHSO_3$ (200 ml) was added and extracted with ether (300 ml). The organic layer was worked-up as usual and finally the solvent was evaporated to give a residue. Numbers of the same reaction were carried out and 8 g of the substrate (I) in total amount gave 12.3 g of the residue which was then submitted to column chromatography on neutral alumina (480 g). The eluting solutions employed were in successive order of hexane–benzene (1: 1 and 3: 7), benzene, $CHCl_3$, and MeOH, giving seven fractions (1—7).

5,6β-Dichloro-5α-cholestan-3β-ol (VI)——Evapotraion of solvent *in vacuo* from the fraction 1 and 2 (hexane: benzene=1: 1, 7400 ml) left a residue (1423 mg) which was recrystallized from $CHCl_3$–MeOH to give colorless needles, mp 144—145° (lit.[32] 143—144°), no depression on admixture with authentic specimen. Beilstein reaction: positive. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH). NMR ($CDCl_3$) τ: 9.34 (C_{13}–CH_3), 8.61 (C_{10}–CH_3), 6.51 ($C_3\alpha$-H, m), 5.62 ($C_6\alpha$-H, t, J=2 Hz). *Anal.* Calcd. for $C_{27}H_{46}OCl_2$: C, 70.87; H, 10.13; Cl, 15.50. Found: C, 71.05; H, 10.21; Cl, 15.32. Acetate: colorless needles (from MeOH), mp 102—104° (lit.[32] 89—90°). IR ν_{max}^{Nujol} cm^{-1}: 1748 (C=O). NMR ($CDCl_3$) τ: 9.30 (C_{13}–CH_3), 8.63 (C_{10}–CH_3), 7.97 ($COCH_3$), 5.62 ($C_6\alpha$-H, t, J=2 Hz), 4.61 ($C_3\alpha$-H, m).

6α-Chloro-5β-cholestane-3β,5-diol (IX)——Evaporation of solvent *in vacuo* from the fraction 4 (hexane: benzene=3: 7 and benzene, 5000 ml in total volume) left a residue (1408 mg) which was recrystallized from aq. MeOH to give colorless needles, mp 140—142.5°. Beilstein reaction: positive. IR ν_{max}^{Nujol} cm^{-1}: 3585, 3440 (OH). NMR ($CDCl_3$) τ: 9.36 (C_{13}–CH_3), 9.03 (C_{10}–CH_3), 5.85 ($C_3\alpha$-H, m). Mass Spectrum *m/e*: 438 (M^+). *Anal.* Calcd. for $C_{27}H_{47}O_2Cl$: C, 73.85; H, 10.79; Cl, 8.07. Found: C, 74.04; H, 10.69; Cl, 8.21. Acetate (X): colorless needles (from MeOH), mp 128.5—130.5° (lit.[24] 126—128.5°). IR ν_{max}^{Nujol} cm^{-1}: 3534 (OH), 1723 (C=O), 1270 (COC). NMR ($CDCl_3$) τ: 9.34 (C_{13}–CH_3), 9.01 (C_{10}–CH_3), 7.93 ($COCH_3$), 4.73 ($C_3\alpha$-H, m). Mass Spectrum *m/e*: 480 (M^+). *Anal.* Calcd. for $C_{29}H_{49}O_3Cl$: C, 72.39; H, 10.27. Found: C, 72.77; H, 10.42.

Conversion to the Epoxide (XII)——After a mixture of IX (72 mg), MeOH (7 ml), and 30% KOH (0.2 ml) was refluxed for 30 min, it was diluted with H_2O and extracted with ether. The organic layer was worked-up as usual and solvent was evaporated *in vacuo*. The residue (59 mg) was recrystallized from aq. EtOH to give colorless needles (27 mg), mp 133—133.5° (lit.[28] 130.5—133°). IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH). NMR ($CDCl_3$) τ: 9.37 (C_{13}–CH_3), 9.01 (C_{10}–CH_3), 6.95 ($C_6\alpha$-H, d, J=2.5 Hz), 6.35 ($C_3\alpha$-H, m). Mass Spectrum *m/e*: 402 (M^+). *Anal.* Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.46; H, 11.38.

3β-Acetoxy-6α-chlorocholest-4-ene (XI)——To a solution of X (75 mg) in pyridine (2 ml) was added $SOCl_2$ (0.2 ml) at −5——10° and the reaction mixture was kept at room temperature for 1 hr. The mixture was diluted with H_2O and was extracted with ether. The organic layer was acidified with 2N HCl and washed with 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and finally the solvent was evaporated *in vacuo* to give the crude product. NMR ($CDCl_3$) τ: 9.33 (C_{13}–CH_3), 8.92 (C_{10}–CH_3), 7.94 ($COCH_3$), 4.12 (C_4-H, s).

5-Hydroxy-6α-chloro-5β-cholest-3-one (XIII)——To a solution of IX (100 mg) in acetone (22 ml) was added dropwise Jone's reagent (0.17 ml) at 2° and the reaction mixture was stirred for 4 min at the same temperature. After the mixture was extracted with ether, the organic layer was worked up as usual. Evaporation *in vacuo* of solvent from the layer left a residue which was recrystallized from MeOH to give colorless needles (71 mg), mp 180.5—182.5°. IR ν_{max}^{Nujol} cm^{-1}: 3530 (OH), 1730 (C=O). NMR ($CDCl_3$) τ: 9.32 (C_{13}–CH_3), 8.97 (C_{10}–CH_3), 5.65 ($C_6\beta$-H, t, J=2 Hz). ORD: a=+13.8 (c=0.587, dioxane). Mass Spectrum *m/e*: 436 (M^+), 438 (M+2). *Anal.* Calcd. for $C_{27}H_{45}O_2Cl$: C, 74.19; H, 10.38; Cl, 8.11. Found: C, 74.24; H, 10.28; Cl, 8.22.

6α-Chlorocholest-4-en-3-one (XIV)——A solution of X (98 mg) in AcOH (9 ml) was heated at 97—100° for 2 hr. The mixture was then diluted with H_2O and extracted with ether. After the organic layer was worked up as usual, evaporation of solvent *in vacuo* left a residue which was recrystallized from AcOEt–MeOH to give colorless needles (46 mg), mp 124—125° (lit.[32] 125—126°). IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1682 (C=O), 1610 (C=C).

Mixture of 5,6α-Epoxy-5α- (II) and 5,6β-Epoxy-5β-cholestan-3β-ol (XII)——Evaporation of solvent *in vacuo* from the fraction 6 (benzene and $CHCl_3$, 6400 ml in total volume) left a residue (508 mg) which was recognized from the NMR data to be a mixture of II and XII in an approximate ratio of 1: 1.

5α-Cholestane-3β,5,6β-triol (III)——The contents (4614 mg) of the fraction 7 (MeOH, 2500 ml) were submitted again to column chromatography on neutral alumina and the crude substance thus obtained was recrystallized from MeOH to give colorless needles, mp 236—242°, no depression on admixture with the authentic specimen.[29] IR ν_{max}^{Nujol} cm^{-1}: 3358 (OH). NMR (pyridine) τ: 9.27 (C_{13}–CH_3), 8.78 (C_{10}–CH_3).

Mutual Conversion between Epoxides (II and XII) and Chlorohydrins (VII and VIII)——A solution of the epoxide (II or XII, several milligrams) in $CHCl_3$ (1 ml) saturated with HCl was allowed to stand for 2 hr at room temperature. After worked-up as usual, solvent was evaporated *in vacuo* from the solution. TLC of the residue revealed the formation of the corresponding chlorohydrin (VIII, Rf=0.36 or VII, Rf=0.32). Under stirring for 24 hr at room temperature, alumina (200 mg) was suspended in $CHCl_3$ solution (2 ml) of the chlorohydrin thus obtained. Evaporation *in vacuo* of solvent from the filtrate of the mixture left a re-

sidue which was then submitted again to TLC. The spot ($Rf=0.54$) was observed showing the formation of the epoxide (II or XII).

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