Chem. Pharm. Bull. 24(12)3109—3113(1976)

UDC 547.922.04 : 542.943.04

A New Product of Cholesterol by Metal-free Autoxidation in Aqueous Dispersion[1]

Meiji Kawata,[2a] Masahiko Tohma, Takuji Sawaya, and Michiya Kimura[2b]

The National Institute for Environmental Studies[2a] and Faculty of Pharmaceutical Sciences, Hokkaido University[2b]

(Received April 19, 1976)

Chemical structure was studied on the compound A which was one of the autoxidation products of cholesterol in an aqueous dispersion employing sodium cholesteryl sulfate as solubilizer. 5α-Cholestane-3β,5,6β,7α-tetrol and its 7β-epimer were prepared as the authentic specimens. Contrary to the 7α-tetrol and the compound A, the epimer gave an acetonide and was susceptible to the oxidation with periodic acid. Identity of the compound A with the 7α-tetrol was concluded to be unequivocal by comparing the analytical data of these compounds and of their derivatives.

During the course of study of this series,[1] metal-free autoxidation of cholesterol (I) was examined in aqueous dispersions employing different kinds of detergent such as sodium stearate, sodium taurocholate, sodium cholesteryl hemisuccinate, and sodium cholesteryl sulfate, at 70° for 20 hr. Numbers of product were detected in thin-layer chromatography (TLC) and those identified were cholest-5-ene-3β,7α-diol, cholest-5-ene-3β,7β-diol, 5α-cholestane-3β,5,6β-triol, cholest-5-en-3β-ol-7-one, and cholesta-3,5-dien-7-one. A polar product was also formed when I was autoxidized in an aqueous dispersion employing sodium cholesteryl sulfate as solubilizer. The present paper deals with the elucidation of chemical structure of this polar product, the compound A.

Results and Discussion

Since the compound A (II), $C_{27}H_{48}O_4$, colorless needles, mp 248—250°, gave a triacetate retaining a free hydroxyl group, II seemed to be a tetrol compound. Considerable amounts of cholest-5-ene-3β,7α-diol (IIIa), cholest-5-ene-3β,7β-diol (IVa), and 5α-cholestane-3β,5,6β-triol (V) were obtained at the same time when II was produced in the autoxidation of cholesterol (I).[1] It was, therefore, assumed that chemical structure of the tetrol (II) was closely related to those of such products as IIIa, IVa, and V and had an additional tertiary hydroxyl group probably at C_5. The nuclear magnetic resonance (NMR) as well as mass (MS) spectral data, which are described in the experimental part, indicated that II was likely to be of 5α-cholestane-3β,5,6β,7ξ-tetrol and hence the authentic epimers at C_7 were prepared as described below.

A mixture of epimeric cholest-5-ene-3β,7ξ-diols (IIIa and IVa) was obtained when 3β-acetoxycholest-5-en-7-one (VI) was reduced with $NaBH_4$ in 80% dioxane at 100° for 3 hr, though their 3β-acetates (IIIb and IVb) were formed with the same reagent at 20° for 22 hr as reported.[3] When IIIa and IVa were refluxed in benzene with *tert*-butyl hydroperoxide in

1) This paper constitutes Part VI of the series entitled "Metal Ion Catalyzed Oxidation of Steroids" by M. Kimura and co-workers; Part V: M. Kimura, M. Kawata, and T. Sawaya, *Chem. Pharm. Bull.* (Tokyo), **24**, 2258 (1976).

2) Location: *a) P.O. Yatabe, Ibaraki-ken, 300-21, Japan; b) Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*

3) C.W. Shoppe and B.C. Newman, *J. Chem. Soc.* (*C*), **1968**, 981.

the presence of vanadyl acetylacetonate, $VO(acac)_2$,[4] their epoxides (VIIa and VIIIa) were readily produced, respectively. With the same reagents the epimeric 3β-acetoxyepoxides (VIIb and VIIIb) were also obtainable from the corresponding acetates (IIIb and IVb) in an almost quantitative yield. NMR spectra of a series of stereoisomeric 4,5-epoxycholesten-3-ols showed that C_4-proton in *cis* 4,5-epoxy-3-alcohols appeared at 2.97—3.05 ppm as a doublet (J=4.0—4.5 Hz) while in the *trans* isomers the proton appeared at 2.70—2.75 ppm as a singlet.[5] Since the epoxides (VIIb and VIIIb) exhibited the signal attributable to C_6-proton at 3.21 (doublet, J=4.5 Hz) and 3.13 (singlet) ppm, respectively, they were both presumed to be *cis* 5,6-epoxy-7-alcohol. An examination of Dreiding model indicated the dihedral angle of about 80° between $C_6\alpha$–H and $C_7\alpha$–H in the β-epoxide. Consequently, VIIb was assumed as 3β-acetoxy-5α,6α-epoxycholestan-7α-ol and VIIIb as 3β-acetoxy-5β,6β-epoxycholestan-7β-ol. Compared with the usual way employing the peracid such as *m*-chloroperbenzoic acid, this epoxidation seemed to be stereo-selective, since the mixture of IIIb and IVb gave that of the epoxides (VIIb and VIIIb) without formation of any other epimers such as the 5α,6α-epoxy-7β-ol and 5β,6β-epoxy-7α-ol. It was also found to be highly advantageous for the preparation or identification of such epimers at C_7 as described above and below, that they were best separated in the chromatography on silica gel when they were in the form of epoxide.

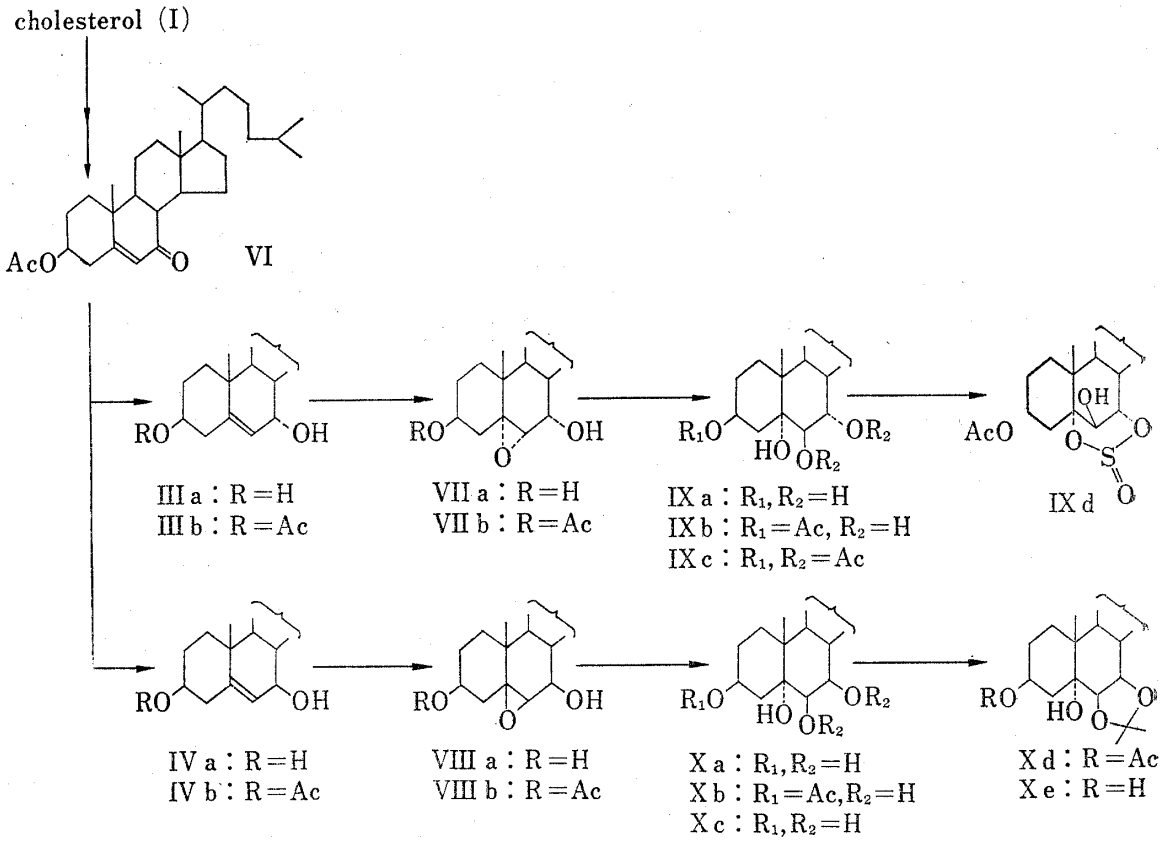


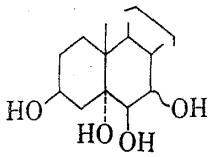
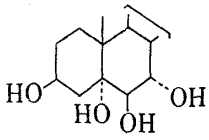
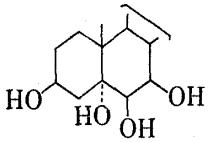
Chart 1

Hydrolysis of the epoxide VIIb with 70% $HClO_4$ in acetone gave 3β-acetoxy-5α,6β,7α-triol (IXb) which was derived to the triacetate (IXc) and the 5,7-sulfite (IXd) by acetic anhydride and thionyl chloride, respectively. When the epimeric epoxide (VIIIb) was hydrolyzed in the same conditions, an acetonide (Xd) was derived, which gave readily the

4) M.N. Sheng and J.G. Zajacek, *J. Org. Chem.*, **35**, 1839 (1970); K.B. Sharpless and R.C. Michaelson, *J. Am. Chem. Soc.*, **95**, 6136 (1973).
5) D.J. Collins and J.J. Hobbs, *Tetrahedron Letters*, **1963**, 632.

3β-acetoxy-5α,6β,7β-triol (Xb) by treating with 80% acetic acid. NMR spectrum of Xd showed sharp signals at 1.28 and 1.46 ppm, characteristic to the acetonide–methyl group.[6] MS spectrum of Xd revealed the base peak 503 (M^+-CH_3), which is also characteristic to

Table I. Some Properties of the Compound A and the Specimens Concerned

	Compound A(II)	7α-Tetrol (IXa)	7β-Tetrol (Xa)
Structure			
mp	248—250°	250—251°	219—222°
NMR C_{13}–CH_3	0.79	0.80	0.77
C_{10}–CH_3	1.59	1.63	1.58
MS M^+	436	436	436
M^+-H_2O	418	418	418
M^+-2H_2O	400	400	400
M^+-3H_2O	382	382	382
HIO_4 oxidation	—	—	+
Acetonidation	—	—	+
Triacetate NMR –OAc	1.97	2.00	1.89
	2.05	2.09	2.00
	2.09	2.11	2.08

the acetonide derivative.[6b] The tetrols (IXa and Xa) were obtainable by the hydrolysis of the epoxides (VIIa and VIIIa) with 70% $HClO_4$ in methyl ethyl ketone, respectively. When they came into contact with HIO_4, the 7α-tetrol (IXa) having the vicinal hydroxyl groups in a *trans*-diaxial orientation both at $C_{5,6}$ and $C_{6,7}$ remained intact. The epimeric tetrol (Xa) having axial hydroxyl group at C_6 and equatorial one at C_7, on the contrary, was susceptible to the oxidative cleavage.

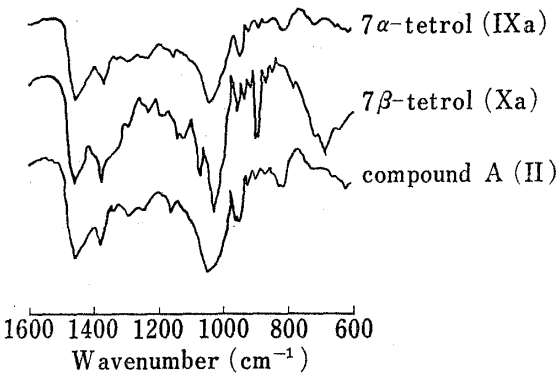


Fig. 1. IR Spectra of the Compound A and the Specimens Concerned

Experimental data of the compound A (II), the 7α- (IXa), and 7β-tetrol (Xa) are summarized comparatively in Table I and their infra red (IR) spectra are shown in Fig. 1. On the basis of these results, it seems reasonable to draw a conclusion that the chemical structure of the compound A is 5α-cholestane-3β,5,6β,7α-tetrol.

Experimental

Melting points were taken on a micro hot-stage apparatus and are uncorrected. IR and NMR spectral measurements were run on JASCO Model DS-301 Spectrometer and Hitachi Model H-6013 Spectrometer at 60 Mc using tetramethylsilane as an internal standard, respectively. MS spectra 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) unless otherwise stated and spots were stained by sprying H_2SO_4 and heating on a hot-plate. Abbreviation used s=singlet, d=doublet, m=multiplet, and br=broad.

6) *a*) H.H. Sauer, E.K. Weiss, and T. Reichstein, *Helv. Chim. Acta.*, **49**, 1632 (1966); *b*) T. Abe and A. Kambegawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 1295 (1973).

Materials and Reagents——3β-Acetoxycholest-5-en-7-one (VI), mp 160—161°, was prepared by the method of Heusler, *et al.*[7] 3β-Acetoxycholest-5-en-7α-ol (IIIb), mp 137°, and 3β-acetoxycholest-5-en-7β-ol (IVb), mp 110—111°, were obtained as reported by Shoppe, *et al.*[3] Cholest-5-ene-3β,7α-diol (IIIa), mp 165—168°, and cholest-5-ene-3β,7β-diol (IVa), mp 172—175°, were prepared by the modification of the Shoppe-Newman's method at 100° for 3 hr and purified by column chromatography.

$HClO_4$ Reagent: To methyl ethyl ketone (18 ml) was added 70% $HClO_4$ (0.1 ml). Other reagents were of commercial sources.

5β,6β-Epoxycholestane-3β,7β-diol (VIIIa)——A solution of *tert*-BuOOH (0.6 ml) in benzene (6 ml) was added dropwise for 20 min to a refluxing mixture of the diol (IVa, 1.927 g), VO $(acac)_2$ (26.5 mg), and benzene (40 ml). After the reaction mixture was refluxed for further 4 hr, the organic solvent was evaporated into dryness *in vacuo* to give a residue which was then dissolved in ether. The ether solution was washed with saturated aq. Na_2SO_3, sat. aq. NaCl, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue (2.405 g) obtained was purified by column chromatography on silica gel using the solvent system of AcOEt–benzene (15–25: 85–75) and then recrystallized from MeOH to colorless crystalls, mp 170—171°, 1.31 g. *Anal.* Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.52; H, 10.97. NMR($CDCl_3$) δ: 3.48 (m, $C_3\alpha$–H, $C_7\alpha$–H), 3.12 (s, $C_6\alpha$–H), 0.99 (s, C_{10}–CH_3), 0.64 (s, C_{13}–CH_3).

5α,6α-Epoxycholestane-3β,7α-diol (VIIa)——was similarly derived from the epimeric diol (IIIa). mp 183—185° (from MeOH). *Anal*, Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.47; H, 11.16. NMR ($CDCl_3$) δ: 3.85 (m, $C_3\alpha$–H, $C_7\beta$–H), 3.22 (d, J=4.5 Hz, $C_6\beta$–H), 1.06 (s, C_{10}–CH_3), 0.60 (s, C_{13}-CH_3).

3β-Acetoxy-5α,6α-epoxycholestan-7α-ol (VIIb)——A solution of *tert*-BuOOH (0.64 ml) in benzene (16 ml) was added as described above to a benzene solution (34 ml) of VO $(acac)_2$ (26.6 mg) and the mixture (2.349 g) of 3β-acetoxycholest-5-en-7-ols (IIIb and IVb), which was obtained by reducing VI. The reaction mixture was also worked up as mentioned above and finally gave a residue (2.456 g). A benzene solution of the residue was then submitted to column chromatography on silica gel (170 g) with subsequent elution by AcOEt–benzene (5: 95). Fractions of 25 ml were collected and the eluates were examined by TLC. Fract. Nos. 144—212 were combined and evaporated to dryness *in vacuo* to give a residue (420 mg) which was recrystallized from MeOH to colorless needles, mp 144—145°. *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.37; H, 10.50. NMR ($CDCl_3$) δ: 4.90 (m, $C_3\alpha$–H), 3.85 (s, $C_7\beta$–H), 3.21 (d, J=4.5 Hz, $C_6\beta$–H), 2.01 (s, $C_3\beta$-OAc), 1.00 (s, C_{10}–CH_3), 0.62 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 460 (M^+).

3β-Acetoxy-5β,6β-epoxycholestan-7β-ol (VIIIb)——Fraction Nos. 263—395 in the above-mentioned chromatography were combined and evaporated to dryness *in vacuo* to give a residue (1.438 g) which was recrystallized from MeOH to colorless crystalls, mp 130—133°. *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.89; H, 10.59. NMR ($CDCl_3$) δ: 4.80 (m, $C_3\alpha$–H), 3.51 (m, $C_7\alpha$–H), 3.13 (s, $C_6\alpha$–H), 2.00 (s, $C_3\beta$–OAc), 0.92 (s, C_{10}–CH_3), 0.64 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 460 (M^+).

3β-Acetoxy-5α-cholestane-5,6β,7α-triol (IXb)——A mixture of VIIb (100 mg) and $HClO_4$ reagent (7 ml) was allowed to stand for 3 hr at room temperature. After the reaction mixture was worked up as usual, the residue obtained was purified by preparative TLC and finally recrystallized from aq. MeOH to give colorless needles, mp 225—226°. *Anal.* Calcd. for $C_{29}H_{50}O_5$: C, 72.76; H, 10.53. Found: C, 72.69; H, 10.36. NMR ($CDCl_3$) δ: 5.15 (m, $C_3\alpha$–H), 3.79, 3.63 (2H, $C_6\alpha$–, $C_7\beta$–H), 2.05 (s, $C_3\beta$–OAc), 1.17 (s, C_{10}–CH_3), 0.70 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 460 (M^+-H_2O), 442 (M^+-2H_2O), 424 (M^+-3H_2O).

Triacetate (IXc): mp 90—92° (from aq. MeOH). NMR ($CDCl_3$) δ: 5.16 (m, $C_3\alpha$–H), 4.8—4.9 (2H, $C_6\alpha$–, $C_7\beta$–H), 2.11, 2.09 ($2CH_3$, $C_6\beta$–, $C_7\alpha$–OAc), 2.00 (s, $C_3\beta$–OAc), 1.15 (s, C_{10}–CH_3), 0.69 (s, C_{13}–CH_3).

Sulfite (IXd): To a pyridine solution (1 ml) of IXb (78.3 mg) was added a mixture of $SOCl_2$ (1 ml) and pyridine (1 ml) under ice-cooling. After 5 min the reaction mixture was worked up as usual and the residue (83.6 mg) obtained was purified by preparative TLC (cyclohexane–AcOEt=2: 1), and finally recrystallized from MeOH–$CHCl_3$ to give colorless crystalls, mp 220—224°. IR ν_{max}^{Nujol} cm^{-1}: 3480 (OH), 1730 (CO), 1195 (SO). NMR ($CDCl_3$) δ: 5.1—5.5 (2H, $C_3\alpha$–, $C_7\beta$–H), 4.40 (br. s, $C_6\alpha$–H), 0.89 (s, C_{10}–CH_3), 0.70 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 442 ($M^+-H_2SO_3$), 382 (442−AcOH), 364 (382−H_2O).

3β-Acetoxy-5α-cholestane-5,6β,7β-triol (Xb)——A mixture of VIIIb (157 mg) and $HClO_4$ reagent (10 ml) was allowed to stand for 30 min at room temperature. The reaction mixture was worked up similarly to the case of IXb and gave finally colorless crystalls, mp 179—180° (from MeOH). *Anal.* Calcd. for $C_{29}H_{50}O_5$: C, 72.76; H, 10.53. Found: C, 72.52; H, 10.45. NMR ($CDCl_3$) δ: 5.10 (m, $C_3\alpha$–H), 3.78, 3.46 ($C_6\alpha$–, $C_7\alpha$–H), 2.02 (s, $C_3\beta$–OAc), 1.16 (s, C_{10}–CH_3), 0.70 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 460 (M^+-H_2O), 442 (M^+-2H_2O), 424 (M^+-3H_2O).

Triacetate (Xc): mp 100—103° (from MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1735 (CO). NMR ($CDCl_3$) δ: 5.25—4.98 (3H, $C_3\alpha$–, $C_6\alpha$–, $C_7\alpha$–H), 2.08, 2.00, 1.89 (s, $C_3\beta$–, $C_6\beta$–, $C_7\beta$–OAc).

Acetonide (Xd): A mixture of VIIIb (228 mg), 70% $HClO_4$ (0.1 ml), and acetone (18 ml) was allowed to stand at room temperature for 24 hr. After the reaction mixture was worked up as usual, the residue (265.3 mg) obtained was purified by column chromatography (cyclohexane–AcOEt=1: 1) on silica gel and finally recrystallized from MeOH–$CHCl_3$ to give colorless crystalls, mp 81—82°. *Anal.* Calcd. for $C_{32}H_{54}O_5$:

7) K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **35**, 284 (1952).

C, 74.09; H, 10.49. Found: C, 73.95; H, 10.37. NMR ($CDCl_3$) δ: 5.12 (m, $C_3\alpha$–H), 3.94, 3.74 ($C_6\alpha$–, $C_7\alpha$–H), 2.00 (s, $C_3\beta$–OAc), 1.46 (s, acetonide-CH_3), 1.28 (s, acetonide–CH_3), 1.16 (s, C_{10}–CH_3), 0.66 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 503 (M^+-CH_3), 443 (503−AcOH), 425 (443−H_2O). Hydrolysis of the acetonide by refluxing with 80% AcOH for 1.5 hr gave also Xb.

5α-Cholestane-3β,5,6β,7α-tetrol (IXa)——A mixture of VIIa (219 mg) and $HClO_4$ reagent (18 ml) was allowed to stand at room temperature for 3 hr. After ether was added, the reaction mixture was washed with 5% $NaHCO_3$, sat. NaCl, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The oily residue was purified by preparative TLC (acetone–cyclohexane=1: 1) and then crystallized from MeOH to colorless crystalls, mp 250—251°. *Anal.* Calcd. for $C_{27}H_{48}O_4$: C, 74.26; H, 11.08. Found: C, 74.16; H, 11.03. IR ν_{max}^{KBr} cm^{-1}: 3360 (OH). NMR (pyridine-d_5) δ: 1.63 (s, C_{10}–CH_3), 0.80 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 436 (M^+), 418 (M^+-H_2O), 400 (M^+-2H_2O), 382 (M^+-3H_2O).

5α-Cholestane-3β,5,6β,7β-tetrol (Xa)——A mixture of Xb (124 mg), K_2CO_3 (100 mg) dissolved in a few drops of water, and MeOH (10 ml) was allowed to stand at 50—60° over night. After $CHCl_3$ was added, the reaction mixture was washed with sat. NaCl, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The residue (122 mg) obtained was recrystallized from MeOH to give colorless crystalls, mp 219—222°. *Anal.* Calcd. for $C_{27}H_{48}O_4$: C, 74.26; H, 11.08. Found: C, 74.07; H, 11.09. IR ν_{max}^{KBr} cm^{-1}: 3360 (OH). NMR (pyridine-d_5) δ: 1.58 (s, C_{10}–CH_3), 0.77 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 436 (M^+), 418 (M^+-H_2O), 400 (M^+-2H_2O), 382 (M^+-3H_2O). The tetrol (181.4 mg) was also obtained by hydrolyzing the epoxide (VIIIa, 334 mg) with $HClO_4$ reagent (18 ml).

Acetonide (Xe): A mixture of VIIIa (525 mg), 70% $HClO_4$ (0.1 mg), and acetone (15 ml) was allowed to stand at room temperature for 24 hr. After ether was added, the reaction mixture was washed with 5% $NaHCO_3$, sat. NaCl, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. An oily residue (600 mg) was submitted to column chromatography on silica gel (30 g) which was eluted successively with *n*-hexane, benzene–AcOEt (95: 5), and benzene–AcOEt (70: 30). Fractions of 25 ml were collected and each eluate was examined by TLC. Fract. Nos. 15—22 were combined and evaporated to dryness *in vacuo*. The residue (177 mg) was recrystallized from aq. MeOH to give colorless crystalls, mp 180—182° (99 mg). *Anal.* Calcd. for $C_{30}H_{52}O_4$: C, 75.58; H, 11.00. Found: C, 75.70; H, 11.00. NMR ($CDCl_3$) δ: 4.1—3.7 (3H, $C_3\alpha$–, $C_6\alpha$–, $C_7\alpha$–H), 1.46 (s, acetonide), 1.30 (s, acetonide), 1.16 (s, C_{10}–CH_3), 0.67 (s, C_{13}–CH_3).

Oxidation with HIO_4: A mixture of Xa, $HIO_4 \cdot 2H_2O$ (12 mg), dioxane (0.5 ml), and water (0.05 ml) was allowed to stand at room temperature for 24 hr. The reaction mixture was worked up as usual and gave finally a residue. UV λ_{max}^{EtOH}: 223 nm. IR ν_{max}^{KBr} cm^{-1}: 1720 (CO). TLC of the residue, contrary to the highly polar substrate (Xa, *Rf*=0.17), gave only one spot which was less polar (*Rf*=0.53) than that given by cholesterol (*Rf*=0.51).

Compound A (II)——The isolation from the autoxidation mixture of cholesterol was reported previously.[1] mp 248—250° (from aq. EtOH). Yield: 3%. *Anal.* Calcd. for $C_{27}H_{48}O_4$: C, 74.26; H, 11.08. Found: C, 74.11; H, 10.99. IR ν_{max}^{KBr} cm^{-1}: 3360 (OH). NMR (pyridine-d_5) δ: 1.59 (s, C_{10}–CH_3), 0.79 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 418 (M^+-H_2O), 400 (M^+-2H_2O), 382 (M^+-3H_2O).

Triacetate: amorphous powder. NMR ($CDCl_3$) δ: 5.13, 4.82, 4.74 (3H), 2.09, 2.05, 1.97 (s, 3OAc), 1.12 (s, C_{10}–CH_3), 0.67 (s, C_{13}–CH_3). Mass Spectrum *m/e*: 502 (M^+−AcOH), 484 (M^+−AcOH−H_2O), 442 (M^+−2AcOH), 424 (M^+−2AcOH−H_2O), 382 (M^+−3AcOH).

Acknowledgement The authors are indebted to H. Harada, T. Kawano, H. Tamura, T. Karihara, and M. Saito for technical assistance and to the staffs of the central analytical laboratory of this Institute for the measurements of NMR and MS spectra. A part of this work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, to which the authros' thanks are due.