

On the Reaction of Cholesteryl Acetate with *tert*-Butyl Hydroperoxide in the Presence of Tris(acetylacetonato)iron(III)¹⁾

MICHIYA KIMURA and TOSHIKI MUTO

Faculty of Pharmaceutical Sciences, Hokkaido University²⁾

(Received July 20, 1978)

A model reaction was investigated for the biological oxyfunctionalization of steroid. The benzene solution of cholesteryl acetate (I), *tert*-butyl hydroperoxide, and tris(acetylacetonato)iron(III) in a molar ratio of 1.2:9.2:0.13 was refluxed under argon atmosphere and time-course of the reaction was followed for 24 hours. The products identified were 3 β -acetoxycholest-5-en-7-one(II), 3 β -acetoxycholest-5-en-7-ols (III; trace), 3 β -acetoxy-5,6-epoxycholestanes (IV), and the alkyl peroxides (Va, Vb, and Vc). These peroxides showed maximum concentration in the early period of the reaction and most of the substrate (I) was consumed by this time. The structures of Va and Vb were elucidated to be 3 β -acetoxycholest-5-ene-7 α -*tert*-butylperoxide and its 7 β -epimer, respectively. The product Vc was assumed to be 3 β -acetoxycholest-6-ene-5 ξ -*tert*-butylperoxide. The ketone(II) was a major product when the peroxides (Va and Vb) came into contact with the oxidation system mentioned above; the yields from Va and Vb were 56 and 94%, respectively. Thus it was concluded that the major final product (II) may be formed through the intermediary peroxides (V) in the titled reaction.

Keywords—metal complex; hydroperoxide; epoxidation; allylic oxidation; 3 β -acetoxycholest-5-ene-7-*tert*-butylperoxides; ketone from alkylperoxide; quantitative TLC; time-course

In a series of studies on the model reactions of the biological hydroxylation of steroids, we have reported on the stereoselective 15 α -hydroxylation of deoxycholic acid with ferrous sulfate and molecular oxygen (Udenfriend's system).³⁾ We studied also the oxidation of cholesterol with ferrous sulfate and hydrogen peroxide (Fenton's system) affording 5 α -cholestane-3 β ,5,6 β -triol.⁴⁾ The hydrogen peroxide/tris(acetylacetonato)iron(III) system was found to bring about a stereoselective β -epoxidation of cholesterol and its analogues.⁵⁾ The reactions of other olefins such as stilbene, methyl octadecenoate, and octadecenol were examined also with this system and it was concluded that the reaction proceeded *via* an intermediate, in which the C-C bond rotated around in attaining conformational equilibrium.⁶⁾ As to the biological oxygenation of steroidal substrates, it was reported that cytochrome P-450 of rat liver microsomes can catalyze the organic hydroperoxide-dependent hydroxylation in the absence of NADPH and molecular oxygen.⁷⁾ Recent progress in the studies of the lipid peroxidation prompted us to investigate the similar reactions using a model system. We show in this paper that epoxide formation as well as allylic oxidation occur in the reaction of cholesteryl acetate (I) with *tert*-butyl hydroperoxide and tris(acetylacetonato)iron(III) in benzene and that the alkyl peroxide of the substrate is likely the intermediate which leads to formation of the products oxygenated at C(7) atom.

- 1) This paper constitutes Part VIII of the series entitled "Metal Ion Catalyzed Oxidation of Steroids;" Part VII: M. Kimura and T. Sawaya, *Chem. Pharm. Bull.* (Tokyo), **25**, 1073 (1977).
- 2) Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*
- 3) M. Kimura, M. Kawata, M. Tohma, A. Fujino, and K. Yamasaki, *Tetrahedron Lett.*, **1970**, 2021.
- 4) M. Kimura, M. Tohma, and T. Tomita, *Chem. Pharm. Bull.* (Tokyo), **20**, 2185 (1972).
- 5) M. Tohma, T. Tomita, and M. Kimura, *Tetrahedron Lett.*, **1973**, 4359.
- 6) T. Yamamoto and M. Kimura, *J.C.S. Chem. Commun.*, **1977**, 948.
- 7) E.G. Hrycay, J.-A. Gustafsson, M.I.-Sundberg, and L. Ernster, *Eur. J. Biochem.*, **61**, 43 (1976).

Results and Discussion

Cholesteryl acetate (I), *tert*-butyl hydroperoxide, and tris(acetylacetonato)iron(III) in a molar ratio of 1.2:9.2:0.13 were dissolved in benzene and the reaction mixture was refluxed for 24 hours under argon atmosphere. The products identified in thin-layer chromatography (TLC) were 3β -acetoxycholest-5-en-7-one (II), 3β -acetoxycholest-5-en-7-ols (III; trace), and 3β -acetoxy-5,6-epoxycholestanes (IV). Unidentified spots, V and VI (trace), were also detected in TLC and the former was separated into three spots (Va, Vb, and Vc) when it was developed with a different solvent system. The products Va and Vb were shown to be the epimeric C(7)-alkylperoxides as described below. The third one (Vc) failed to be purified and was assumed to be the C(5)-peroxide on the basis of infrared (IR) spectrum as well as nuclear magnetic resonance (NMR) data.

Aliquots of the reaction mixture were taken at varying times and the contents were submitted to TLC. The relative amounts of the products and the substrate (I) still remaining were then determined by using the hydrogen-flame-ionization detector. The time-course of the reaction was followed for 24 hours and is shown in Fig. 1. As can be seen in this figure, the peroxides (Va, Vb, and Vc) gave the maximum value after approximately 5 hours. The substrate (I) was highly consumed during this period and became undetectable after approximately 15 hours. The amounts of the C(7)-ketone (II), which predominated in the final products, increased rather rapidly with decrease in those of the peroxides. Formation of the epoxide (IV) was slow, in contrast to that of II.

In order to elucidate the chemical structures of the peroxides (Va, Vb, and Vc), the reaction was carried out for 5 hours and the reaction mixture was submitted to TLC in preparative scale. After worked up as described in the experimental section, two peroxides, Va, $C_{33}H_{56}O_4$, mp 112–113°, 1198 cm^{-1} (C–O–O–C) and Vb, $C_{33}H_{56}O_4$, mp 102–104°, 1200 cm^{-1} (C–O–O–C), were obtained. Lithium aluminum hydride reduction of Va and Vb gave cholest-5-ene- $3\beta,7\alpha$ -diol and its 7β -epimer, respectively. From these results and the analytical data, it may be concluded that the peroxide Va is 3β -acetoxycholest-5-ene- 7α -*tert*-butylperoxide and the other one (Vb) is its 7β -epimer.

When the peroxide (V) was refluxed with the *tert*-butyl hydroperoxide/tris(acetylacetonato)iron(III) system in benzene for 24 hours under argon atmosphere, the C(7)-ketone(II) was formed as a major product and small amounts of the C(7)-hydroxide(III) as well as the unidentified product (VI) were also obtained, without any formation of the epoxide (IV). Tris (acetylacetonato) iron(III) and *tert*-butyl hydroperoxide were cooperative in decomposing V, since their absence or independent use in the refluxing reaction mixture was invalid or far less effective. The system Cr(III) acetylacetonate and *tert*-butyl hydroperoxide was proposed as a free-radical initiator yielding *tert*-butoxy radical.⁸⁾ Although enough evidence has not been accumulated to discuss the mechanism, it may be plausible that II was produced from V through elimination of the allylic hydrogen at C(7) attacked by such radical species. Steric hindrance due to the bulky *tert*-butyl group was effective so that the formation of II was much easier from the C(7) β -peroxide (Vb) than its epimer (Va); the apparent yields were 94 and 56%, respectively. In the reaction of cholesteryl acetate (I) with the present oxygenation system, it may be concluded that the major final product (II) is formed through the intermediary peroxide (V).

In the reactions of olefins with the organic peroxides such as *tert*-butyl and cumene hydroperoxides, the epoxide formation was predominant when Ce(IV) salt or complex of the metal such as V, Mo, Cr, Co, Cu, Mn, or W was used as a catalyst.^{9–12)} Allylic oxidation became

8) N. Indictor, T. Jochsberger, and D. Kurnit, *J. Org. Chem.*, **34**, 2855 (1969).

9) N. Indictor and W.F. Brill, *J. Org. Chem.*, **30**, 2074 (1965).

10) G. Sosnovsky and D.J. Rawlinson, "Organic Peroxides," Vol. 2, ed. by D. Swern, John Wiley and Sons, Inc., New York, 1971, pp. 195–211.

11) R.A. Sheldon and J.A. van Doorn, *J. Catal.*, **31**, 427 (1973).

12) R.A. Sheldon and J.K. Kochi, *Adv. Catal.*, **25**, 272 (1976).

predominant in the same reactions, when the catalyst was salt of the metal ion in lower valence such as Cu(I), Co(II), Mn(II), Ni(II), or Fe(II).^{8,10,12)} Predominance of the allylic oxidation was also clarified in the reaction of cholesteryl acetate (I) with the *tert*-butyl hydroperoxide/tris(acetylacetonato)iron(III) system as described above. The epoxidation was exclusive, on the contrary, when hydrogen peroxide took the place of organic hydroperoxide in this system.⁵⁾ The type of oxyfunctionalization in I may be dependent on the hydroperoxide employed. Further studies are thus in progress by using the hydroperoxides with different types of structures.

Experimental

General Methods—Melting points were taken on a micro hot-stage apparatus and are uncorrected. IR spectral measurements were run on JASCO Model IRA-2 spectrometer. NMR spectra were measured by Hitachi Model R-24A spectrometer at 60 Mc using tetramethylsilane as an internal standard. TLC was carried out on silica gel (Wakogel B-5F) plate developed by the solvent system of benzene/hexane (4: 6, triplicate develop.; system I) or benzene/AcOEt (19: 1; system II) and by staining with 10% H₂SO₄ and

heating at about 130° for 5 min. The peak areas in TLC were determined by using a hydrogen-flame-ionization detector on Iatron Model TFG-10 Thinchrograph. The compositions (%; Fig. 1) or the apparent yields of steroidal components in the reaction mixtures were calculated from the ratio of the peak areas thus obtained, where the relative sensitivity of each steroid to the substrate was tentatively taken as unity. Abbreviations used: s=singlet, d=doublet, and m=multiplet.

Materials—The commercially available tris(acetylacetonato)iron(III), mp 183° (lit.¹³⁾ 182°) and *tert*-butyl hydroperoxide (92% purity determined by iodometry) were obtained from Dozin Yakkagaku Lab. and Nakarai Chem. Co., respectively and employed without purification. The authentic specimens of 3 β -acetoxycholest-5-en-7-one (II), mp 154–156° (lit.¹⁴⁾ 157–158°), cholest-5-ene-3 β ,7 α -diol, mp 184–187° (lit.¹⁵⁾ 188–189°), and cholest-5-ene-3 β ,7 β -diol, mp 172–175° (lit.¹⁵⁾ 172–176°/180–181°) were prepared as reported. The authentic acetate of

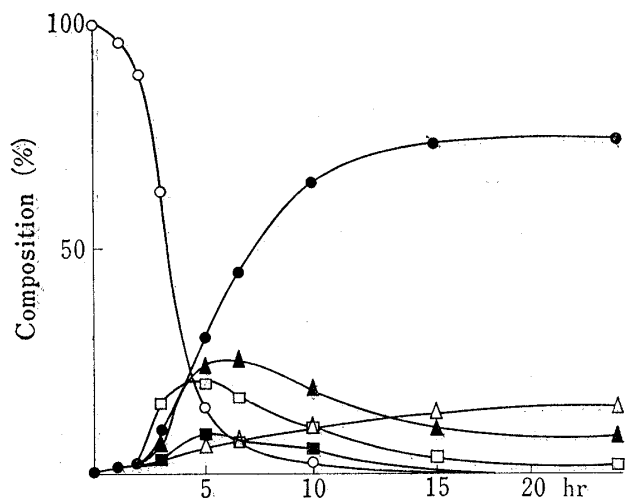


Fig. 1. Time-course of Reaction

○: I; ●: II; △: IV; ▲: Va; □: Vb; ■: Vc.

5,6-epoxy-cholestan-3 β -ol (mixture of α -epoxide and β -epimer in a molar ratio of 3: 1, determined by NMR) were prepared by oxidizing cholesteryl acetate (I) with *m*-chloroperbenzoic acid.

Oxyfunctionalization of Cholesteryl Acetate (I)—1. Benzene solution (20 ml) of cholesteryl acetate (I, 500 mg, 1.2 mmol), tris(acetylacetonato)iron(III) (45 mg, 0.13 mmol), and *tert*-butyl hydroperoxide (1 ml, 9.2 mmol) was refluxed for 24 hr under argon atmosphere. To the reaction mixture was added NaHSO₃ (10 ml) and the aqueous layer was extracted with ether (50 ml $\times$ 3). The benzene layer of the reaction mixture was combined with the ether solutions and then washed with saturated aqueous NaCl, dried on anhydrous Na₂SO₄, filtered, and finally evaporated to dryness. The extract was submitted to preparative TLC with the solvent system II, giving the fractions 1-1 (*R_f* 0.2; II), 1-2 (*R_f* 0.3; IV), and 1-3 (*R_f* 0.5; V; discarded); III and VI showed *R_f*s 0.1 and 0.4, respectively, and were trace in quantity.

2. Benzene solution (20 ml) of I (500 mg), tris(acetylacetonato)iron(III) (45 mg), and *tert*-butyl hydroperoxide (1 ml) was refluxed for 5 hr under argon atmosphere. The reaction mixture was then worked up as described above. Through 6 runs of the reaction in the same scale, 3 g of I were oxidized. The products were separated by preparative TLC developed with the solvent system I, giving the fractions 2-1 (*R_f* 0.20; Va), 2-2 (*R_f* 0.25; Vb), and 2-3 (*R_f* 0.45; Vc); I showed *R_f* 0.55 and II, III (trace), IV, and VI (trace) were almost immobile.

13) E.W. Berg, *Anal. Chim. Acta*, **32**, 245 (1965).

14) A. Nickon and W.L. Mendelson, *J. Am. Chem. Soc.*, **87**, 3921 (1965).

15) C.W. Shoppee and B.C. Newman, *J. Chem. Soc. (C)*, **1968**, 981.

3 β -Acetoxycholest-5-en-7-one (II)—The fraction 1-1 was extracted with CHCl_3 and the extract was crystallized from EtOH to give colorless needles, mp 152–154°. NMR (10% solution in CDCl_3) δ : 0.69 (s, 3H, C(13)- CH_3), 1.23 (s, 3H, C(10)- CH_3), 2.04 (s, 3H, C(3 β)-Ac), 4.70 (m, 1H, C(3 α)-H), 5.71 (s, 1H, C(6)-H). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 1725, 1665 (C=O), 1630 (C=C).

3 β -Acetoxycholest-5 ξ ,6 ξ -epoxycholestane (IV)—The extract of the fraction 1-2 was oily, 46.6 mg, a mixture of epimeric epoxides in a molar ratio of α : β =1:4 (determined by NMR). NMR (10% solution in CDCl_3) of α -epoxide δ : 0.66 (s, 3H, C(13)- CH_3), 0.95 (s, 3H, C(10)- CH_3), 1.93 (s, 3H, C(3 β)-Ac), 2.76 (d, J =2.2 Hz, 1H, C(6 β)-H), 4.69 (m, 1H, C(3 α)-H). NMR (10% solution in CDCl_3) of β -epoxide δ : 0.66 (s, 3H, C(13)- CH_3), 0.95 (s, 3H, C(10)- CH_3), 1.93 (s, 3H, C(3 β)-Ac), 2.94 (d, J =2 Hz, 1H, C(6 α)-H), 4.69 (m, 1H, C(3 α)-H).

3 β -Acetoxycholest-5-ene-7 α -tert-butylperoxide (Va)—In order to avoid contamination by the more polar products, only the most reliable zone of the fraction 2-1 was carefully scratched up and extracted with CHCl_3 . The extract was crystallized from acetone to give colorless needles, 575.2 mg, mp 112–113°. Anal. Calcd. for $\text{C}_{33}\text{H}_{56}\text{O}_4$: C, 76.69; H, 10.92. Found: C, 76.54; H, 11.12. NMR (10% solution in CDCl_3) δ : 0.65 (s, 3H, C(13)- CH_3), 0.98 (s, 3H, C(10)- CH_3), 1.21 (s, 9H, C(7 α)-C(CH_3) $_3$), 2.02 (s, 3H, C(3 β)-Ac). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 1725 (C=O), 1198 (C-O-O-C), 1650 (C=C).

Tetrahydrofuran (THF) solution (5 ml) of these needles (49.6 mg) and LiAlH_4 (56.4 mg) was refluxed for 12 hr and the reaction mixture was worked up as usual. The product was identified with cholest-5-ene-3 β ,7 α -diol by comparing its IR and NMR spectra with those of the authentic specimen.

3 β -Acetoxycholest-5-ene-7 β -tert-butylperoxide (Vb)—The fraction 2-2 was extracted with CHCl_3 and the extract was crystallized from EtOH to give colorless needles, 937.3 mg, mp 101.5–102.5°. Anal. Calcd. for $\text{C}_{33}\text{H}_{56}\text{O}_4$: C, 76.69; H, 10.92. Found: C, 76.56; H, 10.82. NMR (10% solution in CDCl_3) δ : 0.67 (s, 3H, C(13)- CH_3), 1.04 (s, 3H, C(10)- CH_3), 1.20 (s, 9H, C(7 β)-C(CH_3) $_3$), 2.01 (s, 3H, C(3 β)-Ac), 4.08 (d, J =7 Hz, 1H, C(7 α)-H), 4.57 (m, 1H, C(3 α)-H), 5.73 (s, 1H, C(6)-H). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 1735 (C=O), 1200 (C-O-O-C), 1670 (C=C).

THF solution (5 ml) of these needles (46.5 mg) and LiAlH_4 (53.3 mg) was refluxed for 23 hr and the reaction mixture was then worked up as usual. The product was identified with cholest-5-ene-3 β ,7 β -diol by comparing its IR and NMR spectra with those of the authentic specimen.

3 β -Acetoxycholest-6-ene-5 ξ -tert-butylperoxide (Vc)—Although the extract from the fraction 2-3 was failed to be crystallized, the structure of the product was assumed to be 3 β -acetoxycholest-6-ene-5 ξ -tert-butylperoxide from the results of IR and NMR analyses. NMR (10% solution in CDCl_3) δ : 0.68 (s, 3H, C(13)- CH_3), 0.93 (s, 3H, C(10)- CH_3), 1.17 (s, 9H, C(5 ξ)-C(CH_3) $_3$), 2.00 (s, 3H, C(3 β)-Ac), 5.11 (m, 1H, C(3 α)-H), 5.58 (s, 2H, C(6), (7)-H). IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1735 (C=O), 1200 (C-O-O-C), 1643 (C=C).

Degradation of Peroxides (V) to Ketone (II)—Benzene solution (3 ml) of 3 β -acetoxycholest-5-ene-7 α -tert-butylperoxide (Va, 50 mg, 9.7×10^{-2} mmol), tris(acetylacetonato)iron(III) (4.5 mg, 1.3×10^{-2} mmol), tert-butyl hydroperoxide (0.1 ml, 0.92 mmol) was refluxed for 24 hr under argon atmosphere. The reaction mixture was worked up as usual and the crude products were submitted to TLC with the solvent system II. The degradation products identified were 3 β -acetoxycholest-5-en-7-one (II, 56% apparent yield) and 3 β -acetoxycholest-5-en-7 ξ -ol (3%); an unidentified substance (VI, 11%) was also detected.

Benzene, 3 β -acetoxycholest-5-ene-7 β -tert-butylperoxide (Vb), tris(acetylacetonato)iron(III), and tert-butyl hydroperoxide were mixed and treated as described above. The degradation products identified were the ketone (II, 94%) and the 7 ξ -ols (trace); an apparent yield of the unidentified substance (VI) was 4%.

Acknowledgement The authors are indebted to the staffs of the central analytical laboratory of this Institute for elemental analysis and spectral measurements. 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 author's thanks are also due.