Iron(III)Picolinate-Catalyzed Oxygenation of Cholesteryl Acetate with Hydrogen Peroxide or Peracetic Acid[1)]

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The reaction of cholesteryl acetate 1 with a Fe^{III}(PA; picolinate)$_3$/H_2O_2/MeCN system (reagent system A), a simple model system for mono-oxygenases, gave mainly the 7α-hydroxylation product 2a, along with 7-ketonization product 3 and the 5,6-epoxidation product 4. On the other hand, reaction of 1 using a Fe(PA)$_3$/peracetic acid (AcOOH)/MeCN system (reagent system C) or a $Fe^{III}(ClO_4)_3 \cdot 9H_2O$–picolinic acid(PAH)–pyridine(Py)/AcOOH/MeCN system (reagent system F), provided 4 predominantly without formation of 2a. The former reaction may proceed *via* the dimeric Fe^{III}–Fe^{V} manifold complex, $(PAH)(PA)_2Fe^{III}$–O–O–Fe^{V}=O$(PA)_2$ (VII) as a hypothetically active species and a nonradical pathway, and the latter may proceed through monomeric iron complexes, $[(PAH)(PA)_2Fe^{V}=O]^+$ (IX) and $[(PAH)(PA)_2Fe^{V}(OH)(OOH)]^+$ (X).

Key words 7α-hydroxylation; 5,6-epoxidation; cholesteryl acetate; iron(III) picolinate; hydrogen peroxide; peracetic acid

Stereoselective 7α-hydroxylation of 3β-acetoxy-Δ^5-steroids is interesting in terms of synthesis of the recently discovered cytotoxic 3β,7α-dihydroxy-Δ^5-steroids from *Pseudobersama mossambicensis*[2)] and the metabolism of cholesterol by the cytochrome P-450 species 7α-hydroxylase in the livers of most mammals. Many investigations of oxygenation reactions using simple, readily available reagent systems mimicking mono-oxygenase enzymes have been carried out. Of these, a study using iron(II) or iron(III) picolinate (PA) complexes as catalysts of oxygenation reactions raised challenging problems since it was noted that oxidations with H_2O_2 catalyzed by these complexes varied depending on the solvents used.[3)]

Although many studies on iron picolinate complex/H_2O_2/solvent systems have been reported by the Sawyer[3)] and Barton groups,[4)] there has been no report on oxidation reactions using the Fe^{III}(PA picolinate)$_3$/H_2O_2/MeCN system.

Recently, we reported the modified system Fe(PA)$_3$/H_2O_2/MeCN (reagent system A), as an alternative to the Gif model system (GoAgg^{III}), which is effective for stereoselective 7α-hydroxylation of 3β-acetoxy-Δ^5 steroids.[1)] Further investigations to characterize this reagent system using alternative oxygen sources in MeCN as solvent were then carried out. We describe herein the details of oxygenation of cholesteryl acetate (**1**) utilizing reagent systems A—H and a revision of the previously proposed mechanism and active species for the formation of the oxygenation products.

Crystals of $Fe^{III}(PA)_3$ (**I**),[3a)] the iron(III)–picolinate complex used in reagent system A can be prepared conveniently by reaction of $Fe(ClO_4)_3 \cdot 9H_2O$ (1 mol) with sodium picolinate (3 mol) in water, followed by recrystallization of the resulting $Fe^{III}(PA)_3(H_2O)$ (**II**)[5)] from anhydrous MeCN or MeOH. Recrystallization of the hydrous form **II** with 1 M aqueous MeCN gave predominantly the hydrous form **II**. In the recrystallization of **II** with 2 M aqueous MeCN, a hydroxyl bridged dimer, $[(PA)_2Fe(OH)]_2$ (**III**)[3a)] was mainly obtained (Chart 1).

By exposure to moisture in air, this complex exists as a mixture of the hydrous form **II** and anhydrous form **I** in wet MeCN in an equilibrium. The iron complex **II** may possess a seven coordination structure[5,6)] similar to $Mn(PA)_3(H_2O)$[7)] confirmed by X-ray crystallographic analysis.[8)] The six coordinated anhydrous form, complex **I** transforms to the seven coordinated monohydrate complex **II** by the addition of a small amount of water in MeCN.

The iron(III)–picolinate complex in the $Fe^{III}(ClO_4)_3 \cdot 9H_2O$–picolinic acid (PAH)–pyridine(Py)/H_2O_2/MeCN system (reagent system E), was used as a solution of components in place of the complex itself in the $Fe^{III}(PA)_3(H_2O)$/H_2O_2/MeCN (reagent system B).

First, the re-examination of oxidation of **1** with reagent system A was carried out under various conditions according to the following procedure (Chart 2, Table 1). Three 0.1-ml portions of a 30% aqueous H_2O_2 solution (3—5 mmol) were added dropwise every 30 min to a stirred solution of **1** (1 mmol) and Fe(PA)$_3$ (0.1—0.5 mmol) in MeCN (70 ml) at room temperature under a nitrogen atmosphere and the reaction mixture was stirred for 0.5—3 h at room temperature.

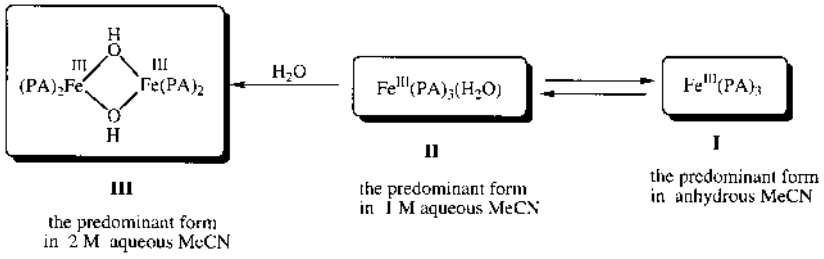

Chart 1

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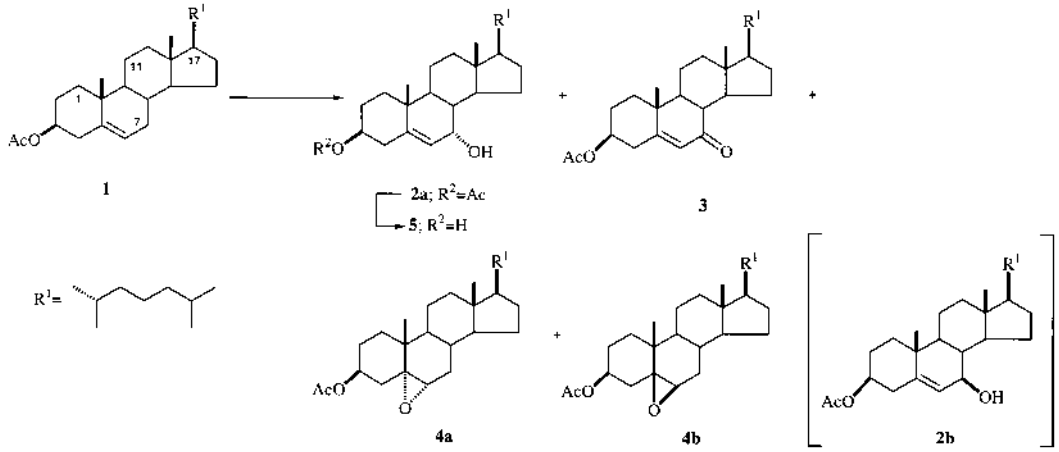

Chart 2

Table 1. Oxygenation of Cholesteryl Acetate (**1**) with Reagent System A under Various Conditions

Run	Molar ratio			Product (yield, %)[a]			Recovery (%)
	1 : $Fe(PA)_3$: H_2O_2			**2a**	**3**	**4a**+**b**	**1**
1	1	0.1	3.0	14.3	6.6	8.2	54.7
2	1	0.5	3.0	40.0	19.8	13.1	22.6
3	1	0.5	5.0	37.0	34.3	12.0	8.2
4	1	1.0	3.0	22.9	6.6	6.9	54.7

a) Isolated yields by column chromatography.

The above reaction afforded 7α-hydroxy derivative **2a** without the formation of 7β-hydroxy derivative **2b**, 7-oxo derivative **3**, 5,6-α and β epoxides, **4a** and **4b**, and recovered **1** in all cases, and the product yields varied with the reaction conditions used, as shown in Table 1. In our previous paper,[1] a trace amount of the 7β-hydroxylated compound was reported to be obtained by oxidation with reagent system A. However, re-examination indicated that this compound was not the 7β-hydroxylated compound **2b**, but it was an unidentified compound; the structure was not assigned.

In this investigation, we found that the most efficient and stereoselective 7α-hydroxylation reaction was obtained using a molar ratio of substrate **1** : $Fe(PA)_3$: 30% H_2O_2 = 1 : 0.5 : 3.0 to give **2a** (40%) together with **3** (19.8%) and **4** (13.1%). Structural identification of **2a** was performed by comparing the physical data of the corresponding compound **5**[9a—d] prepared by hydrolysis, with an authentic sample.[9a—c] The structures of **3**,[9b—e] **4a**, and **4b**[9e] were identified by comparison with the respective authentic samples.[9b,d,e]

Subsequently, reactions of **1** with reagent system A under an oxygen atmosphere and air were performed. These reactions gave the same results as under a nitrogen atmosphere, as described above. Furthermore, we investigated the reaction of **1** with the Gif system ($GoAgg^{III}$) to compare with the present reagent system A. However, stereoselective 7α-hydroxylation reaction of **1** did not proceed in this case.[10] These results showed that the present reagent system A has different reactivity in comparison with the Gif system ($GoAgg^{III}$) reported by Barton previously.[11]

We next investigated reactions of **1** using reagent system B, as an alternative to reagent system A (run 2 in Table 2). Reagent system A is considered to be similar to reagent system B, although the iron(III) picolinate complex was used as the hydrous form, in place of the anhydrous form employed in reagent system A. This similarity may be due to the predominant form of the iron(III) picolinate complex in MeCN, which was used as the solvent. When crystals of the hydrous form **II** are dissolved in anhydrous MeCN, the equilibrium prefers the anhydrous form, because **I** was easily obtained from **II** by recrystallization from anhydrous MeCN as described above.

Subsequently, reactions with reagent system E afforded a small amount of **3** and **4** together with a large amount of recovered starting material **1**. The low efficiency of the oxidation using reagent system E may be due to the formation of the inactivated iron complex **III** from the active iron complex **II** by the effect of H_2O contained in $Fe^{III}(ClO_4)_3 \cdot 9H_2O$.

In fact, the reaction of **1** using reagent system E afforded large amounts of complex **III** over time. Accordingly, the iron(III) complex in reagent system E may exist predominantly as the dimer form **III**, and include only a small amount of the hydrous form **II**.

We found that the major product and the reaction efficiency changed drastically with the $Fe^{III}(PA)_3$/AcOOH/MeCN system (reagent system C) and the $Fe^{III}(ClO_4)_3 \cdot 9H_2O$–PAH–Py/AcOOH/MeCN system (reagent system F) using peracetic acid (AcOOH) in place of H_2O_2 as the oxygen source (runs 3 and 6). In the reaction of **1** with reagent system C, the epoxide **4** (83.8%) was predominantly produced along with the 7-oxo compound **3** (6.4%) in good reaction efficiency. A similar reaction using reagent system F gave mainly **4** (72.8%), together with a small amount of **3** (8.0%). The reaction of **1** with reagent system E in the presence of acetic anhydride (Ac_2O) was performed to give mainly **4** (78.6%), along with **3** (11%) (run 7).

On the other hand, no reaction occurred with the $Fe(PA)_3$/*tert*-BuOOH/MeCN (reagent system D) using *tert*-butyl hydroperoxide (TBHP) as the oxygen source, which is an alternative to the Gif model system ($GoAgg^{IV}$) (run 4).[12] Reactions with reagent systems G[13] and H, which do not have picolinic acid and pyridine were examined, but no transformation occurred (runs 8 and 9).

In summary, these results had the following feature: in the oxygenation of **1**, 7-hydroxylation proceed predominantly

Table 2. Fe(III)–Picolinate Complex Catalyzed Oxygenation of Cholesteryl Acetate (**1**) in MeCN

Run	Reagent system	Reaction time (h)	Product (yield,%)			Recovery (%)
			7-Hydroxy **2a**	7-Oxo **3**	5,6-Epoxy **4** (**a**/**b**)	**1**
1	$Fe(PA)_3/H_2O_2$/MeCN (A)[a]	3	40.0	19.8	13.1 (4.7/8.4)	22.6
2	$Fe(PA)_3(H_2O)/H_2O_2$/MeCN (B)[b]	3	37.5	20.5	11.0 (4.8/6.2)	24.7
3	$Fe(PA)_3$/AcOOH/MeCN (C)[b]	0.5	0.0	6.4	83.8 (34.3/49.5)	0.0
4	$Fe(PA)_3$/*tert*-BuOOH/MeCN (D)[b]	24	0.0	0.0	0.0	100.0
5	$Fe(ClO_4)_3 \cdot 9H_2O$–PAH–Py/H_2O_2/MeCN (E)[b]	3	0.0	0.7	1.3 (0.5/0.8)	91.7
6	$Fe(ClO_4)_3 \cdot 9H_2O$–PAH–Py/AcOOH/MeCN (F)[b]	0.5	0.0	8.0	72.8 (29.0/43.8)	2.1
7	Reagent system (E)+Ac_2O[b]	0.5	0.0	11.0	78.6 (28.0/50.6)	3.4
8	$Fe(ClO_4)_3 \cdot 9H_2O/H_2O_2$/MeCN (G)[b]	3	0.0	0.0	0.0	100.0
9	$Fe(ClO_4)_3 \cdot 9H_2O$/AcOOH/MeCN (H)[b]	3	0.0	0.0	0.0	100.0

a) Isolated yields after silica gel column chromatography followed by HPLC. *b*) Yields were determined by HPLC analysis.

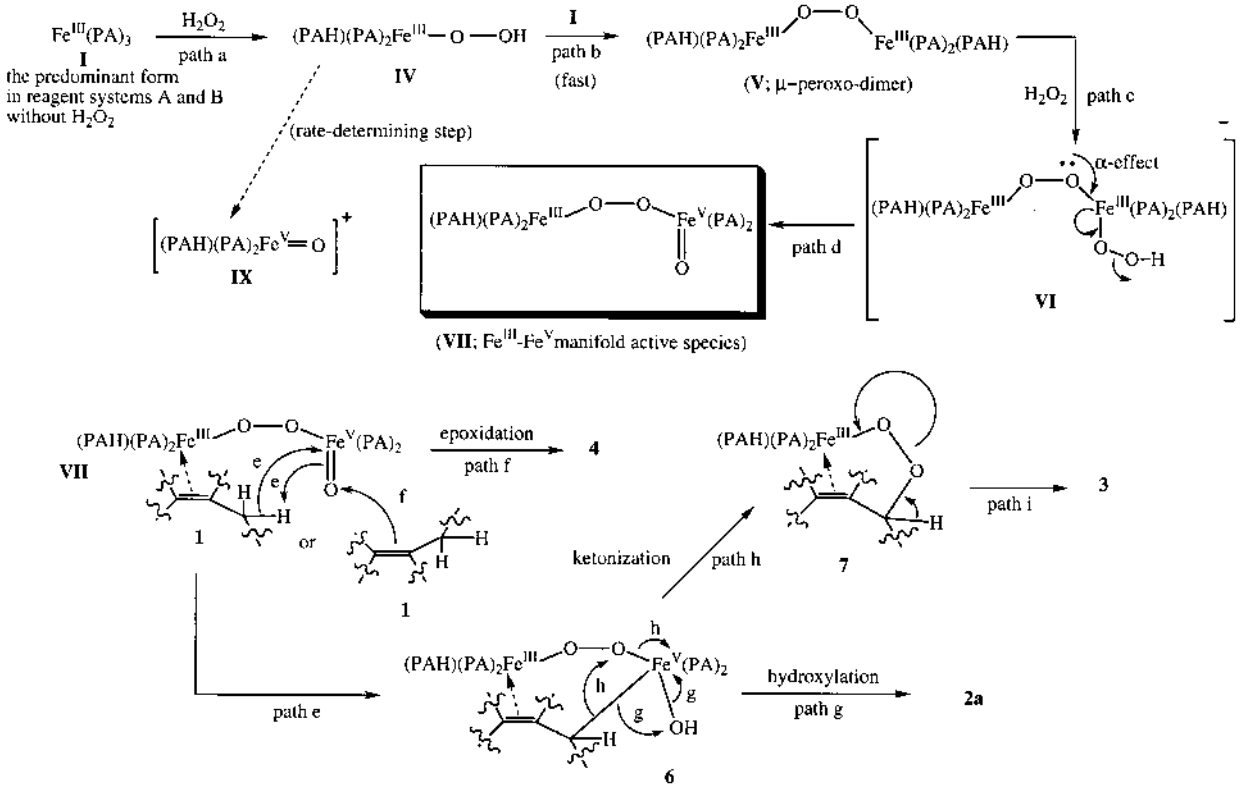

Chart 3. Proposed Mechanism for the Reaction of Cholesteryl Acetate (**1**) Using Reagent Systems A and B

with reagent systems A and B, compared to 5,6-epoxidation with the other reagent systems.

Barton *et al.*[14] reported that (i) pyridine base plays an important role in Gif systems such as $GoAgg^{III}(FeCl_3 \cdot 6H_2O$–$PAH/H_2O_2$/HOA–pyridine); it is not only used as a solvent, but also has a major effect on efficient ketonization since it is a ligand on the Fe^{III} complex;[14a] (ii) NMR studies of the iron(III) complex show two picolinic acid ligands (PAH) and one pyridine ligand (Py) must be bonded per iron atom;[14b,c] (iii) acetic acid (AcOH) is also necessary for ketone formation; (iv) the active species for ketone formation using $GoAgg^{III}$ in pyridine is the dimeric Fe^{III}–Fe^{V} manifold complex (Fe^{III}–O–O–Fe^{V}=O), generated by reaction between the Fe^{III} complex and H_2O_2, followed by heterolytic O–O bond cleavage of the resulting dimer (Fe^{III}–O–O–Fe^{III}–O–O–H) as the current hypothesis.[14b] On the other hand, it is known that oxygenation reaction modes involving O–O bond cleavage vary depending on the kind of ligands per iron atom, the solvents, and the oxidants used, as shown in various P-450 model systems, and other chemical[3c,4b,14a,15] and electrochemical systems.[16a] Additionally, oxygenation reactions using these systems are accelerated by the addition of acids (AcOH and CF_3CO_2H, *etc.*,[16a,b]), heteroaromatic *N*-bases (imidazole, *etc.*,[16c,d]), and acid anhydrides (acetic anhydride, *etc.*,[17]) as activators.

Although the present reagent system A is analogous to the Gif system ($GoAgg^{III}$ system), they differ in the following three ways; (i) the complexes present in solution (ligands per iron atom) [$Fe(PA)_3$ *vs.* $Fe(PA)_2(Py)$], (ii) solvents (MeCN *vs.* pyridine/AcOH), and (iii) the absence or presence of an acid such as AcOH. The differences in the reaction environments between reagent system A and the $GoAgg^{III}$ system, and the

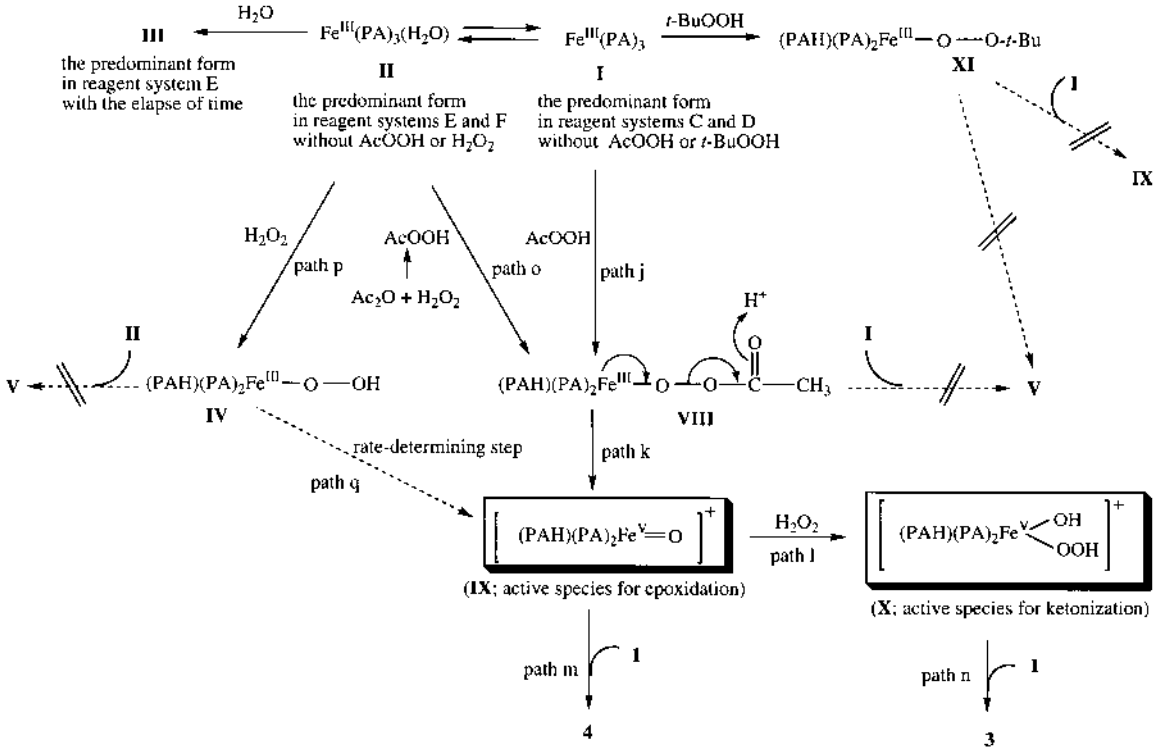

Chart 4. Proposed Mechanism for the Reaction of Cholesteryl Acetate (**1**) Using Reagent Systems C, D, E, F, and Reagent System E in the Presence of Ac_2O

above information allow some suggestions for the reaction mechanism of oxygenation of **1** with reagent system A.

A probable mechanism is illustrated in Chart 3. This is similar to that proposed for reaction with reagent system B. The previously proposed intermediate species (**IX**)[1)] has been revised to the dimeric Fe^{III}–Fe^{V} manifold complex,[18)] (PAH)$(PA)_2Fe^{III}$–O–O–Fe^{V}=O$(PA)_2$ (**VII**).

It is considered that heterolytic O–O bond cleavage of **IV** leading to **IX**, previously proposed, may be the rate-determining step in reactions without activators, as described for reagent system A, although the details are not clear. This is also postulated from the results obtained from the cyclic voltammogram (CV) experiments, as described below. Therefore, complex **IV** reacts more rapidly with complex **I** in preference to transformation into **IX** to give the μ-peroxo-dimer complex **V**. Subsequently, complex **V** reacts further with H_2O_2 to form complex **VI**, followed by heterolytic O–O bond cleavage to generate a hypothetically active species **VII** (path a $\rightarrow$ path b $\rightarrow$ path c $\rightarrow$ path d). This O–O bond cleavage for generation of **VII** may be due to the effect of the α oxygen atom adjacent to two iron atoms (Fe^{III}–O–O–Fe^{III}) (the so-called "α effect"[19)]) in **VI**.

Furthermore, the reaction between **VII** and cholesteryl acetate (**1**) may proceed to produce the oxygenative products, **2a**, **3**, and **4** through various pathways, as shown in Chart 3. Compound **4** is formed *via* path f. In the rearrangement of **6**, formation of **2a** *via* path g takes place in preference to formation of **3** *via* path h.

Although active species **VII** and intermediate **6** in the present reagent system A are similar to those in the $GoAgg^{III}$ system proposed by Barton *et al.*,[4b)] the major products differ. We believe that this may be attributable to differences in the kinds of ligands on the two iron atoms (Fe^{III} and Fe^{V} atoms) and to different solvents. Therefore, path g may proceed predominantly to give **2a** in the case of reagent system A, whereas path h may be the main process in the case of the $GoAgg^{III}$ system, leading selectively to **3** after rearrangement of **6**.

From these considerations, we have now revised the previously proposed active species **IX** for the formation of **2a**, **3**, and **4**, to the complex **VII**.[1)]

Many reports on the mechanism of epoxidation of olefins by P-450 model systems, chemical methods[20a,b)] and by electrochemical methods,[17)] with various metalloporphyrins and oxidants have appeared. It was reported that epoxidation of olefins using an electrochemical system such as Fe^{III}TPPCl (*meso*-tetra-phenylporphinato iron(III) chloride) or Mn^{III} TPPCl/molecular oxygen/dimethylformamide (DMF) in the presence of Ac_2O, proceeds *via* a high-valent metal oxo porphyrin complex. For example, by Fe^{V}=O or Mn^{V}=O generated by O–O bond heterolysis of metallo-acylperoxy complexes. On the other hand, studies on heterolytic O–O bond cleavage of monomeric metallo-acylperoxy complexes have been reported.[20c)]

Based on these studies, the proposed mechanism for reaction of **1** using reagent system C is illustrated in Chart 4. This mechanism is analogous to that proposed for reaction with reagent systems E and F in the presence of Ac_2O, except for the formation process of the active species, [(PAH)$(PA)_2Fe^{V}$=O]$^+$ (**IX**) and [(PAH)$(PA)_2Fe^{V}$(OH)(OOH)]$^+$ (**X**). The active species for formation of **3** and **4** in reactions with these reagent systems is considered to be iron complexes **IX** and **X**. The active species **IX** is considered to be formed *via* specific pathways as follows; (i) *via* reaction of **I** with AcOOH, followed by heterolytic O–O bond cleavage of **VIII**, without formation of **V** by reaction between **VIII** and **I**

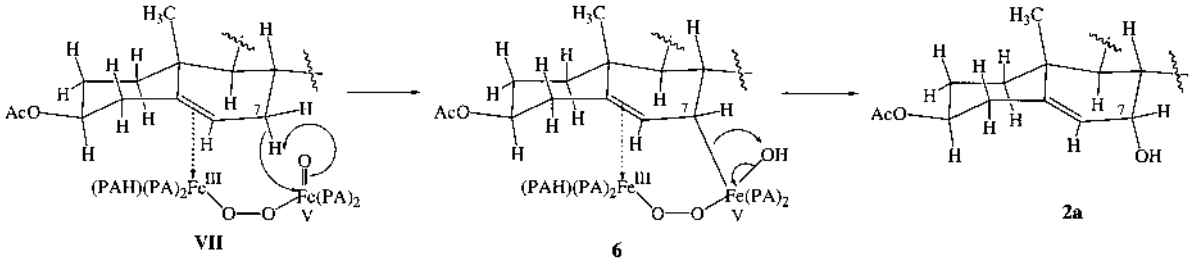

Chart 5. Proposed Mechanism for Stereoselective 7α-Hydroxylation of Cholesteryl Acetate (**1**)

in reagent system C (path j → path k), (ii) *via* path o → path k in reagent system F, and (iii) *via* reaction of **II** with AcOOH generated by reaction between Ac_2O and H_2O_2, followed by O–O bond cleavage of **VIII** in reagent system E in the presence of Ac_2O (path o → path k). On the other hand, compounds **3** and **4**, prepared by reaction with reagent system E, may be formed through **IX** and **X**, respectively (path p → path q → path l → path n, and path p → path q → path m).

In order to obtain information to support the proposed mechanisms described above, cyclic voltammograms (CV) of reagent systems A, C and D, without cholesteryl acetate (**1**), were measured. Monitoring of CV changes in reagent systems A and C indicated that the reversible wave of complex $Fe^{III}(PA)_3$ (**I**) ($E_{1/2}=-0.04$ V *vs.* SCE) changed to an irreversible one upon addition of 30% H_2O_2 or AcOOH, and returned back to the original wave upon consumption of the oxidant. In the case of reagent system D, using TBHP in place of H_2O_2, the reversible wave of complex **I** changed to an irreversible one but did not return to the original wave. These CV changes showed that reactions between $Fe(PA)_3$ and the oxidants, namely H_2O_2, AcOOH, and TBHP, proceed as the first step to give the corresponding peroxy complexes, **IV**, **VIII**, and **XI** in all cases. However, transformation of **XI** into **V** or **IX** can not occur in the case of reagent system D. This may be due to differences in the functional group in the above peroxy complexes. That is, complex **VIII** having electron-withdrawing substituents such as an acetyl group, undergoes predominantly O–O bond cleavage due to the electron-withdrawing effect, however, complex **XI** possessing an electronic donor group, such as a *tert*-butyl group in reagent system D, does not undergo O–O bond cleavage or reaction with complex **I**. Based on this postulate, complex **IV** without the above functional groups, may react mainly *via* the complex **I** in preference to O–O bond cleavage. The results from CV experiments provide information which supports the proposed mechanisms.

The mechanism of stereoselective 7α-hydroxylation leading to formation of **2a** can be postulated as follows (Chart 5). The σ bond formation between the C-7α-position of **1** and the Fe^{V} atom in **VII** *via* a nonradical pathway,[4] with chelation between the π bond in **1** and the Fe^{III} atom in **VII** may take place stereoselectively to yield **6** under stereoelectronic control[21] and *via* steric hindrance. Further, cleavage of the σ bond between the Fe atom and the C7-position in **6** including rearrangement of the hydroxyl group, may proceed to afford **2a** with retention of configuration.

Finally, as a control, reactions of **1** with known allylic acetoxylation reagents, CuBr/*tert*-BuOOCOPh/HOAc,[2,22] $Pd(OAc)_2/Fe(NO_3)_3\cdot 9H_2O/O_2$/HOAc,[23] and $Pb(OAc)_4$/HOAc[24] reported previously were performed, however, 7α-hydroxylated compound was not obtained stereoselectively.

In conclusion, the present results suggest that the different reactivity in these reagent systems may be due to structural differences between $Fe^{III}(PA)_3$ (**I**) and $Fe^{III}(PA)_3(H_2O)$ (**II**) complexes in MeCN as solvent, as well as between the peroxy complexes, **IV**, **VIII**, and **IX**, generated by reaction between **I** or **II** and oxidants. The formation of **3** and **4** by reaction with reagent systems A and B may be different from those with reagent systems C, E, F, and reagent system E in the presence of Ac_2O. Furthermore, these results provide a new example of oxidative 7α-hydroxylation of Δ^5-steroids, one of the major metabolic reactions catalyzed by cytochrome P-450.

Further studies on the reaction mechanisms in these reagent systems are in progress.

Experimental

All melting points are uncorrected. Infrared (IR) spectra were recorded with a JASCO IR-700 spectrometer, and ^{1}H- and ^{13}C-NMR spectra with JEOL JNM-EX90, JNM-GX270 and JNM-GSX500 spectrometers, with tetramethylsilane as an internal standard ($CDCl_3$ solution). Mass spectra were recorded on a JEOL JMS-D300 spectrometer. Elemental analyses were done using a Yanaco CHN-MT-3 apparatus. Wako silica gel C-200 (200 mesh) and Merck Kieselgel 60 F_{254} were used for column chromatography and thin-layer chromatography (TLC), respectively. Each organic extract was dried over Na_2SO_4. Preparative HPLC (high-performance liquid chromatography) was carried out with a JASCO HPLC system (pump, JASCO 880; RI-detector, JASCO 830) using a silica-3301-N (Senshu Pac, 8ϕ×300 mm i.d.) column.

Preparation of Iron(III) Picolinate Complexes, $Fe(PA)_3$, $Fe(PA)_3(H_2O)$, and $[(PA)_2Fe(OH)]_2$ A solution of $Fe(ClO_4)_3\cdot 9H_2O$ (25.8 g, 50 mmol) in H_2O (100 ml) was added in one portion with stirring to a mixture of picolinic acid (18.5 g, 150 mmol) and NaOH (6 g, 150 mmol) in H_2O (100 ml), and the whole was stirred for 5 min. The resulting pale yellow precipitate was collected by filtration, and washed with a small amount of water and acetone to give the crude product $Fe(PA)_3(H_2O)$. The crude product was recrystallized from anhydrous MeCN to yield 19.0 g (90%) of the iron complex $Fe(PA)_3$ (**I**) as pale yellow-green crystals, mp 285—287 °C.

Recrystallization of above crude product with 1 M aqueous MeCN or 2 M aqueous MeCN gave iron complex, $Fe(PA)_3(H_2O)$ (**II**) as a pale yellow-green powder, mp 282—283 °C (decomp.) and the hydroxyl bridged dimer, $[(PA)_2Fe(OH)]_2$ (**III**), as a pale yellow powder, respectively. The melting point of **III** was greater than 300 °C. **I**[3a,5]: IR (KBr) cm^{-1}: 1672, 1602, 1563. *Anal*. Calcd for $C_{18}H_{12}O_6N_3Fe$: C, 51.21; H, 2.87; N, 9.95. Found: C, 50.97; H, 3.09; N, 9.72. **II**[5]: IR (KBr) cm^{-1}: 3526, 3458, 1675, 1603. **III**[3a]: IR (KBr) cm^{-1}: 3450 (br), 3250 (br), 1673, 1641, 1602.

Oxygenation of Cholesteryl Acetate (1) with Various Reagent Systems and Hydrolysis of the Oxygenation Products (2a) with the $Fe(PA)_3$/H_2O_2/MeCN System (Reagent System A) Three 0.1-ml portions of 30% aqueous H_2O_2 solution (0.3 ml, 3 mmol) were added dropwise every 30 min to a vigorous stirred solution of **1** (428 mg, 1 mmol) and $Fe(PA)_3$ (211 mg, 0.5 mmol) in MeCN (70 ml) at room temperature and the reaction mixture was then stirred for 3 h. The mixture was then poured into ice water and extracted with ether. The organic layer was washed with 10% HCl and brine. The ethereal solution was dried and concentrated and the residue purified by column chromatography on silica gel with hexane–AcOEt (10 : 1, v/v). The first eluate gave 97 mg (22.7%) of recovered starting material (**1**). The sec-

ond eluate gave 58.2 mg (13.1%) of a mixture of **4a** and **4b**. The third eluate gave 87.5 mg (19.8%) of 3β-acetoxy-5-cholesten-7-one (**3**), colorless crystals (ethanol), mp 156—158 °C. The final eluate gave 177.6 mg (40%) of 3β-acetoxy-5-cholesten-7α-ol (**2a**), colorless crystals (ether–hexane), mp 140—141 °C. The mixture of **4a** and **4b** was further subjected to preparative HPLC with hexane–AcOEt (10 : 1, v/v). The first eluate afforded 37.3 mg (8.4%) of 3β-acetoxy-5,6β-epoxycholestane (**4b**), colorless crystals (ethanol), mp 111—112 °C. The second eluate afforded 20.9 mg (4.7%) of 3β-acetoxy-5,6α-epoxycholestane (**4a**), colorless crystals (ethanol), mp 84—85 °C.

A solution of **2a** (177.6 mg, 0.4 mmol) in 5% alcoholic NaOH (10 ml) was heated at 60 °C for 10 min. The reaction mixture was then poured into ice-water, acidified with aqueous 10% HCl and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried and concentrated. The residue was recrystallized from ethanol to yield 160.8 mg (95%) of 5-cholesten-3β,7α-diol (**5**), colorless crystals, mp 194—195 °C. Analysis by HPLC with hexane–AcOEt (10 : 1, v/v, 3 ml/min) confirmed the formation of **1** (retention time (rt), 4.57 min), **4b** (rt, 5.94 min), **4a** (rt, 6.58 min), **3** (rt, 9.73 min), and **2a** (rt, 23.98 min). **4a**: IR (KBr) cm^{-1}: 1726. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.61 (3H, s, C18-H), 0.86 (3H, d, J=6.7 Hz, C26-H), 0.86 (3H, d, J=6.4 Hz, C27-H), 0.89 (3H, d, J=6.7 Hz, C21-H), 0.92—1.98 (28H, m, aliphatic-H), 1.07 (3H, s, C19-H), 2.01 (3H, s, C3-Ac), 2.89 (1H , d, J=4.6 Hz, C6β-H), 4.91—4.98 (1H, m, C3α-H). HR-MS Calcd for $C_{29}H_{48}O_3$: 444.3604. Found: 444.3638. MS m/z: 444 (M^+). **4b**: IR (KBr) cm^{-1}: 1726. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.63 (3H, s, C18-H), 0.85 (3H, d, J=6.6 Hz, C26-H), 0.86 (3H, d, J=6.7 Hz, C27-H), 0.89 (3H, d, J=7.0 Hz, C21-H), 1.00 (3H, s, C19-H), 1.12—2.40 (28H, m, aliphatic-H), 2.03 (3H, s, C3-Ac), 3.08 (1H , d, J=2.1 Hz, C6α-H), 4.71—4.83 (1H, m, C3α-H). HR-MS Calcd for $C_{29}H_{48}O_3$: 444.3604. Found : 444.3600. MS m/z: 444 (M^+). **3**: IR (KBr) cm^{-1}: 1733, 1668. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.68 (3H, s, H-18), 0.86 (3H, d, J=6.7 Hz, C26-H), 0.87 (3H, d, J=6.7 Hz, C27-H), 0.92 (3H, d, J=6.4 Hz, C21-H), 0.99—2.61 (26H, m, aliphatic-H), 1.21 (3H, s, C19-H), 2.05 (3H, s, C3-Ac), 4.70 (1H, m, C3-H), 5.70 (1H , d, J=1.5 Hz, C6-H). HR-MS Calcd for $C_{29}H_{46}O_3$: 442.3447, Found: 442.3413. MS m/z: 442 (M^+). **2a**: IR (KBr) cm^{-1}: 3406, 1726, 1643. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.68 (3H, s, C18-H), 0.86 (3H, d, J=6.7 Hz, C26-H), 0.87 (3H, d, J=6.7 Hz, C27-H), 0.92 (3H, d, J=6.4 Hz, C21-H), 1.20—2.44 (27H, m, aliphatic-H), 1.59 (3H, s, C19-H), 2.04 (3H, s, C3-Ac), 3.85 (1H, br s, C7-H), 4.65 (1H, m, C3-H), 5.63 (1H , d, J=5.2 Hz, C6-H). HR-MS Calcd for $C_{29}H_{48}O_3$: 444.3604. Found: 444.3648. MS m/z: 444 (M^+). **5**: IR (KBr) cm^{-1}: 3404. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.69 (3H, s, C18-H), 0.86 (3H, d, J=6.7 Hz, C26-H), 0.87 (3H, d, J=6.7 Hz, C27-H), 0.93 (3H, d, J=6.7 Hz, C21-H), 0.98—2.43 (28H, m, aliphatic-H), 1.00 (3H, s, C19-H), 3.59 (1H, m, C3-H), 3.85 (1H, br s, C7-H), 5.60 (1H , d , J=5.2 Hz, C6-H). HR-MS Calcd for $C_{27}H_{46}O_2$: 402.3498, Found: 402.3539. MS m/z: 402 (M^+).

Oxidation with Fe(PA)$_3$(H$_2$O)/H$_2$O$_2$/MeCN (Reagent System B) This reaction was carried out at room temperature according to the procedure used for oxidation with reagent system A. Analysis by HPLC confirmed the formation of **4a**, **4b**, and **3**. Yields are listed in Table 2.

Oxidation with Fe(PA)$_3$/AcOOH/MeCN (Reagent System C) A 40% AcOOH solution (0.9 ml, 3 mmol) was added dropwise to a solution of $Fe(PA)_3$ (211 mg, 0.5 mmol), and **1** (428 mg, 1 mmol) in MeCN (70 ml) at room temperature with vigorous stirring and the whole was stirred for 30 min. The reaction mixture was worked up according to the procedure used for oxygenation with reagent system A. Analysis by HPLC confirmed the formation of **4a**, **4b**, and **3**. Yields are listed in Table 2.

Oxidation with Fe(PA)$_3$/*tert*-BuOOH/MeCN (Reagent System D) *tert*-Butylhydroperoxide (0.4 ml, 3 mmol) was added dropwise to a solution of $Fe(PA)_3$ (211 mg, 0.5 mmol), and **1** (428 mg, 1 mmol) in MeCN (70 ml) at room temperature, with vigorous stirring and the whole was stirred for 24 h. The reaction mixture was worked up according to the procedure used for oxygenation with reagent system A. Analysis by HPLC confirmed only the recovered material **1**.

Oxidation with Fe(ClO$_4$)$_3$·9H$_2$O–PAH–Py/H$_2$O$_2$/MeCN (Reagent System E) Three 0.1-ml portions of a 30% aqueous H_2O_2 solution (0.3 ml, 3 mmol) were added dropwise to a solution of $Fe(ClO_4)_3\cdot 9H_2O$ (258 mg, 0.5 mmol), picolinic acid (185 mg, 1.5 mmol), pyridine (197.5 mg, 2.5 mmol), and **1** (428 mg, 1 mmol) in MeCN (70 ml) at room temperature with vigorous stirring, and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was worked up according to the procedure described for reagent system A. Analysis by HPLC confirmed the formation of **4a**, **4b**, and **3**. Yields are listed in Table 2.

Oxidation with Fe(ClO$_4$)$_3$·9H$_2$O–PAH–Py/AcOOH/MeCN (Reagent System F) A 40% AcOOH solution (0.9 ml, 3 mmol) was added dropwise to a solution of $Fe(ClO_4)_3\cdot 9H_2O$ (258 mg, 0.5 mmol), picolinic acid (185 mg, 1.5 mmol), pyridine (197.5 mg, 2.5 mmol), and **1** (428 mg, 1 mmol) in MeCN (70 ml) at room temperature with vigorous stirring and the whole was stirred for 30 min. The reaction mixture was worked up according to the procedure used for oxygenation with reagent system A. Analysis by HPLC confirmed the formation of **4a**, **4b**, and **3**. Yields are listed in Table 2.

Oxidation with Reagent System E in the presence of Ac$_2$O Three 0.1-ml portions of a 30% aqueous H_2O_2 solution (0.3 ml, 3 mmol) were added dropwise to a solution of $Fe(ClO_4)_3\cdot 9H_2O$ (258 mg, 0.5 mmol), picolinic acid (185 mg, 1.5 mmol), pyridine (197.5 mg, 2.5 mmol), and **1** (428 mg, 1 mmol) in MeCN (70 ml) and Ac_2O (1 ml) at room temperature with vigorous stirring, and the whole was stirred for 30 min. The reaction mixture was worked up according to the procedure described for reagent system A in the presence of Ac_2O. Analysis by HPLC confirmed the formation of **4a**, **4b**, and **3**. Yields are listed in Table 2.

Oxidation with Fe(ClO$_4$)$_3$·9H$_2$O/H$_2$O$_2$/MeCN (Reagent System G) This reaction was carried out at room temperature according to the procedure used for oxidation with reagent system E. Analysis by HPLC confirmed only the recovered material **1**.

Oxidation with Fe(ClO$_4$)$_3$·9H$_2$O/AcOOH/MeCN (Reagent System H) This reaction was carried out at room temperature according to the procedure used for oxidation with reagent system F. Analysis by HPLC confirmed only the recovered material **1**.

References and Notes

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