Four recent studies in cytochrome P450 modelings: A stable iron porphyrin coordinated by a thiolate ligand; a robust ruthenium porphyrin–pyridine N-oxide derivatives system; polypeptide-bound iron porphyrin; application to drug metabolism studies

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Abstract

(1) A distinctive structural feature of P450 is the unusual thiolate coordination to heme. We have succeeded in the preparation of the first synthetic thiolato–iron porphyrin (**SR** complex) which retains its structure during catalytic oxidation. Experiments using **SR** complex have revealed that the thiolate ligand greatly accelerates the rate of the O–O bond cleavage and its heterolysis even in highly hydrophobic media. (2) Heteroaromatic N-oxides were found to be excellent oxidants in the presence of ruthenium porphyrin. 2,6-disubstituted pyridine N-oxides plus a catalytic amount of Ru porphyrin oxidized olefins and sulfides to afford epoxides and sulfoxides, respectively, in high yields. The system in the presence of hydrogen halide effectively oxidized unactivated alkanes and arenes to give alcohols (or ketones) and p-quinones in high yields with high selectivity and an extremely high catalyst turnover number (up to 1.2×10^5). (3) A polypeptide-bound porphyrinatoiron complex was prepared. The polymer complex exhibited greater P450-like activity than non-bound Fe(TPP)Cl in the oxidation of olefin and aniline derivatives. (4) P450 mimics were applied to drug metabolism studies. These model systems were effective for one-step preparation of unstable metabolic intermediates, 'candidate metabolites', and for the discovery of novel modes of metabolism.

Keywords: Cytochrome P450; Porphyrin; Thiolate; Axial ligand; O–O bond; Ruthenium; Pyridine N-oxide; Epoxidation; Hydroxylation; Quinone; Polypeptide; Decarboxylation

Cytochrome P450s are ubiquitous enzymes which play important roles in diverse in vivo processes. The enzymes catalyze the synthesis of physiologically significant biomolecules including various steroids and prostaglandins, and are mainly responsible for metabolism of xenobiotics. The mechanism of their catalytic activities and structure–function relationships have been the subject of extensive investigation in the field of biomimetic chemistry (for recent reviews, see Ref. [1]). We have succeeded in the construction of several novel model systems of cytochrome P450, and have employed them in

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studies of P450 functions, as well as in synthesis and drug metabolism studies.

We report here our recent results in four areas: synthesis of a unique iron porphyrin coordinated by a thiolate anion and evaluation of the relative effect of the axial thiolate ligand by use of the complex [2]; a highly reactive oxidizing system using heteroaromatic N-oxides catalyzed by ruthenium porphyrin [3]; synthesis of polypeptide-bound iron porphyrin and examination of the modifying effect of the macromolecule on P450-like reactivities [4]; the application of appropriately designed P450 mimics to drug metabolism studies [5].

1. Pronounced effects of axial thiolate ligand on the reactivity of iron porphyrin

The distinctive structural features of P450 as a hemoprotein are the unusual thiolate coordination to heme and also the extreme hydrophobicity of its active site. Among many heme enzymes, only P450 can hydroxylate unactivated alkanes and arenes. Our interest has been focused on the relative effect of an axial thiolate ligand on the reactivity of heme in lipophilic media as a model of the P450 pocket. The iron porphyrin ligated by alkylthiolate anion (SR complex) prepared by us is a unique model which retains its axial thiolate coordination dur-

ing catalytic oxidation reactions (Fig. 1) [2]. The UV–Vis and EPR spectra, elemental analysis and FAB mass spectra all supported the structure shown in the figure. EXAFS analysis of SR complex (Fe^{3+}) indicated an Fe–S bond length of 2.20 Å, which is quite close to that in native ferric P450 enzymes [6]. SR complex could be stored at room temperature under air for several months.

1.1. The axial ligand effect on the rate of O–O bond cleavage

To clarify the axial ligand effect on the oxygen activation by P450, which is a multistep reaction, the effect at each step should be evaluated using the SR complex. First of all, we examined the catalytic activity of SR on the peroxide shunt reaction of P450 using alkyl hydroperoxides and compared it with that of Fe(TPP)Cl to investigate the relative effect of a thiolate ligand on O–O bond cleavage. 2,4,6-Tri-tert-butylphenol (TBPH) and 1,1-diphenyl-2-picrylhydrazine (DPPH) were chosen as substrates because both are known to trap reactive intermediates with high efficiency to produce almost stoichiometrically the 2,4,6-tri-tert-butylphenoxyl (TBP) or 1,1-diphenyl-2-picrylhydrazyl (DPP) radical [7]. Toluene was used as the solvent, taking into account the highly hydrophobic environment of the active site of
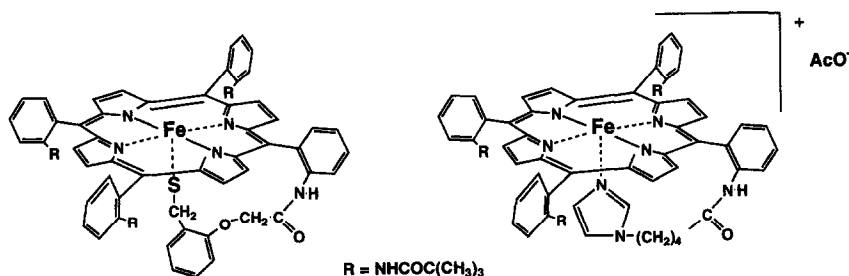

R = NHCOC(CH₃)₃

Fig. 1. SR complex (left side): UV–Visible spectra of SR(Fe^{II})–CO complex λ_{max} 383 nm (Soret), 459 nm (Soret). EPR spectra of SR(Fe^{III}) at 77 K: g_x 1.96, g_y 2.21, g_z 2.32. Rhombicity = 1.02. XAFS spectra of SR (Fe^{III}): Fe–S 2.20 Å, Fe–N 2.00 Å. Elemental analysis. Calcd C: 69.51, H: 5.66, N: 9.40, S: 2.68. Found: C: 69.23, H: 5.52, N: 9.38, S: 2.60. FAB mass spectra $(M_+H^+)^+$ 1160.
N.P. complex (right side): UV–Visible spectra of N.P.(Fe^{II})–CO complex λ_{max} 423 nm (Soret). EPR Spectra of SR(Fe^{III}) at 77 K. Elemental analysis g = 6.0, 2.0. Calcd for [Fe(Por)Im]$^+ \cdot$ AcO$^-$ C: 57.69, H: 5.45, N: 9.34. Found: C: 57.87, H: 5.00, N: 9.27. High-resolution FAB mass. observed m/e 1131.4668 (M–OAc)$^+$. Calcd for $(C_{67}H_{67}N_{10}O_4Fe)$ 1131.4696.

Table 1
Observed initial rates of TBP˙ or DPP· formation on the oxidation of TBPH or DPPH with alkyl hydroperoxides catalyzed by SR or FeTPPCl.

$$ROOH + 2ArOH \xrightarrow{\text{iron porphyrin}} ROH + 2ArO˙ + H_2O \quad (1)$$

Substrate	Oxidant	V (turnover number/min) [a]		$V_{SR}/V_{FeTPPCl}$
		SR	FeTPPCl	
TBPH	$PhC(CH_3)_2OOH$	21	0.35	58
	t-Bu–OOH	8.5	0.080	110
DPPH	$PhC(CH_3)_2OOH$	20	0.085	235
	t-Bu–OOH	7.5	0.041	182

Conditions: solvent = toluene; [TBPH] = 0.2 M; [DPPH] = 0.1 M; [Oxidant] = 5×10^{-2} M; [SR] = [FeTPPCl] = 10^{-4} M. These reactions were carried out at 20° under an argon atmosphere.
[a] Observed initial rates of the reactions were based on the catalysts (turnover number of catalysts/min).

P450. EPR spectra of the reaction solution (15 s after the start of the reaction at 25°C) exhibited low-spin spectra of the thiolate-ligated iron porphyrin in addition to the signal of the DPP· formed, and the high-spin signal ($g = 6$) increased very slightly. All of the results supported the conclusion that **SR** catalyzes the oxidation of the substrates by organic hydroperoxides while the axial thiolate ligand remains essentially unoxidized. Comparison of the rates of the reactions (Table 1: this table is reproduced from Ref. [2]) shows the cytochrome P450 model **SR** to have about 60–240 times higher catalytic activity than Fe(TPP)Cl. Thus, the acceleration of the catalytic reaction by thiolate ligation is undoubtedly due to the enhancement of O–O bond scission, because the concentration of the peroxides used in these reactions is so high that the O–O bond cleavage step is rate-determining. The cyclic voltammogram of **SR** in DMF showed a reversible reduction couple (Fe(III)/Fe(II)) at -0.45 V versus SCE, which is more negative than that for Fe(TPP)Cl (-0.27 V versus SCE) (Table 2).
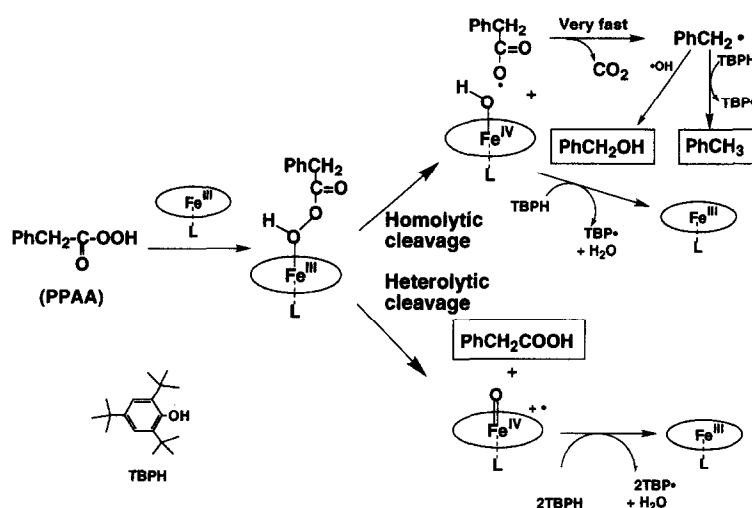

Fig. 2. PPAA as a probe for the determination of the mode of O–O bond cleavage.

The negativity of the redox potential of **SR** is probably due to electron donation from the thiolate to the iron atom.

1.2. The axial ligand effect on the mode of the O–O bond cleavage

Next, we investigated the effect of thiolate ligand on the mode of the O–O bond cleavage. A new iron porphyrin axially and intramolecularly coordinated by imidazole, termed the NP complex, was prepared in order to compare the effect of imidazole as an axial ligand with that of thiolate [8]. The structure of **NP** is a modification of the imidazole-ligated heme prepared by Collman et al. [9]. The UV–Vis and EPR spectra, elemental analysis and high-resolution FAB mass spectra all supported the structure shown in Fig. 1. We compared the catalytic activity of **NP** with that of **SR** complex in an oxidation system using peroxyphenylacetic acid (PPAA). PPAA is a useful probe for the determination of the mode of the O–O bond cleavage, as illustrated in Fig. 2 [10]. **SR** gave PAA quantitatively, while Fe(TPP)Cl mainly catalyzed the formation of toluene, benzyl alcohol, and carbon dioxide (Table 3: this table is reproduced from Ref. [8]). Complex **NP** showed moderate reactivity, intermediate between those of **SR** and Fe(TPP)Cl. Therefore we can unambiguously conclude that **SR** breaks the O–O bond of peroxyacids *heterolytically in benzene*. This result indicates that the thiolate ligand enhances heterolytic cleavage of peroxyacid–iron porphyrin complex even in highly hydrophobic media without the assistance of acid or base. In contrast, it was deduced from our data that Fe(TPP)Cl catalyzes the homolysis of peroxyacid in benzene. The conclusion concerning the O–O bond homolysis by Fe(TPP)Cl in benzene is consistent with the results described by Groves, Watanabe and co-workers [11]. It is expected that a more strongly electron-donating axial ligand would increase both the proportion and rate of heterolytic O–O bond scission.

Table 2
Redox potentials (Pt versus SCE) of the iron porphyrins

Iron porphyrin	Fe(III)P/Fe(II)P	Fe(II)P/Fe(I)P
SR (DMF)	~0.45 (V)	~1.26 (V)
SR (CH$_2$Cl$_2$)	~0.54	n.d.
Fe(TPP)Cl(DMF)	~0.27	~1.08

In 0.1 M n-Bu$_4$NClO$_4$/solvent at Pt versus NaSCE reference. n.d.: not determined.

Therefore, the order of donative character of the examined ligands can be estimated to be as follows: thiolate > imidazole ≫ chloride anion. These results are supported by a related, and independent work [12] which was published in the same year as ours (Ref. [8]). White et al. proposed a mechanism involving homolysis of the O–O bond for P450, based on the result of experiments using the enzyme itself and PPAA as a peroxide probe [10]. They observed benzyl alcohol formation in the oxidation of aliphatic substrates with PPAA catalyzed by P450s. Therefore, the mode of the O–O bond cleavage by P450 remains controversial. Our study using **SR** complex should contribute to the discussion.

Further, the reactivity of the active species formed by cleavage of the O–O bond of peroxyacid–iron porphyrin complexes was examined by using unactivated alkanes as substrates in benzene (Table 3). The **SR** complex catalyzed the hydroxylation of adamantane so efficiently that the yield of adamantanols based on the used mCPBA reached 88% (run 1). In the reaction, 80% of **SR** was confirmed to remain undecomposed by EPR and UV–Vis examinations. On the other hand, only a low or moderate yield of adamantanol was obtained by catalysis with Fe(TPP)Cl or **NP**, although mCPBA was completely consumed (runs 2, 3). Both Fe(TPP)Cl and **NP** also retained their structures after completion of the reactions. The degree of activity of iron porphyrins for heterolysis of PPAA in benzene is thought to correlate positively with the degree of hydroxylation activity toward alkanes.

Table 3
Oxidation of substrate with peroxyacid catalyzed by iron porphyrins

Run	Iron porphyrin	Substrate	Peroxyacid	Products (yield %)[a]			
1	SR	TBPH	PPAA	PAA (100)	PhCH$_3$ (0)	PhCH$_2$OH (0)	TBP· (kcat = 12.5)[b]
2	Fe(TPP)Cl	TBPH	PPAA	PAA (7)	PhCH$_3$ (61)	PhCH$_2$OH (28)	TBP· (kcat = 0.043)[b]
3	NP	TBPH	PPAA	PAA (58)	PhCH$_3$ (15)	PhCH$_2$OH (0)	TBP· (kcat = 0.33)[b]
4	SR	TBPH	PPAA + PAA[c]	PAA (98)[d]	PhCH$_3$ (0)	PhCH$_2$OH (0)	TBP· (kcat = 12.1)[b]
5	Fe(TPP)Cl	TBPH	PPAA + PAA[c]	PAA (85)[d]	PhCH$_3$ (1)	PhCH$_2$OH (14)	TBP· (kcat = 0.083)[b]
6[e]	SR	adamantane[g]	mCPBA		1-adamantanol (80)	2-adamantanol (8)	
7[e]	Fe(TPP)Cl	adamantane[g]	mCPBA		1-adamantanol (9)	2-adamantanol (2)	
8[e]	NP	adamantane[g]	mCPBA		1-adamantanol (34)	2-adamantanol (5)	
9[e]	SR	adamantane[g]	PPAA		1-adamantanol (65)	2-adamantanol (7)	
10[e]	Fe(TPP)Cl	adamantane[g]	PPAA		1-adamantanol (11)	2-adamantanol (2)	

These reactions were carried out in benzene at 25° under argon for 10 min. PPAA: PhCH$_2$CO$_3$H, PAA: PhCH$_2$CO$_2$H. [Fe(Por)] = 0.10 mM: [peracid] = 1.0 mM: [TBPH] = 10 mM. Benzaldehyde was not detected. Otherwise as noted.
[a] Yields are based on the oxidants used. Products were determined by GLC and/or GCMS.
[b] kcat: the initial rate of TBP· formation (turnover number/s).
[c] One equivalent amount of PAA to PPAA was added.
[d] Yield based on two equivalent amounts to the added PPAA.
[e] [Fe(Por)] = [peracid] = 1.0 mM.
[f] Ketone form product was not detected in any case.
[g] [adamantane] = 0.50 M.

2. Versatile, highly efficient oxidations with heteroaromatic N-oxides catalyzed by ruthenium porphyrin

Oxidative functionalization of hydrocarbons is a key area of metalloporphyrin chemistry from the viewpoints of model study of cytochrome P450, and utilization of hydrocarbon resources. Many oxidation systems using metalloporphyrins have been reported for oxidation of hydrocarbons. However, the product yields based on substrate used are too low for synthetic purposes in most cases. We have focused on the development of new metalloporphyrin-catalyzed oxidizing systems to serve this purpose. It is thought to significant which oxidant is chosen as an oxygen atom donor to a metallo-

porphyrin, because the formed highly reactive intermediate, which can hydroxylate unactivated alkanes, would oxidize not only hydrocarbon, but also the oxidant itself. It is therefore considered to be preferable for an oxygen atom donor to have the following properties.

(1) The donor (X–O) should readily react with a metalloporphyrin and form reactive species such as a high-valent oxo–metal porphyrin complex.
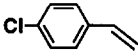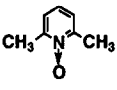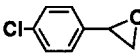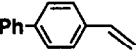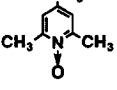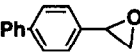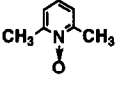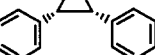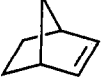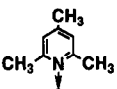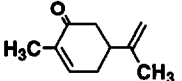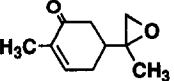
(2) X–O and its deoxygenated product (X) should have only low reactivities toward the formed active intermediate.

(3) X–O and X should be unreactive toward substrates and oxidized products.

(4) The leaving group X should not strongly coordinate to the metal of the catalyst.

Table 4
Epoxidation of various olefins with 2,6-substituted pyridine N-oxides catalyzed by Ru(TMP)(O)$_2$

Olefin	Oxidant	Epoxide	Isolated Yield[a]
Cl-⟨⟩-CH=CH₂	CH₃-⟨N⁺-O⟩-CH₃	Cl-⟨⟩-epoxide	99 %
Ph-⟨⟩-CH=CH₂	CH₃,CH₃-⟨N⁺-O⟩-CH₃	Ph-⟨⟩-epoxide	92 %
cis-stilbene	CH₃,CH₃-⟨N⁺-O⟩-CH₃	stilbene oxide	98 %
norbornene	CH₃,CH₃-⟨N⁺-O⟩-CH₃	norbornene oxide	97 %[b]
carvone	Cl-⟨N⁺-O⟩-Cl	carvone epoxide	70 %

These reaction were carried out in benzene at RT under Ar overnight. [olefin] = 170 mM; [oxidant] = 180 mM; [Ru(TMP)(O)₂] = 0.5–2.0 mM.
[a] Based on olefins.
[b] Determined by GLC.

Almost none of the previously reported X–O satisfies all four requirements. We are interested in heteroaromatic N-oxides, which are represented by pyridine N-oxide derivatives. These N-oxides are generally stable at ambient temperature. Pyridine N-oxide is unable to oxidize thiols and phosphines at room temperature [13]. Pyridine is resistant to reactive oxidizing reagents such as permanganate, and indeed is frequently used as a solvent in permanganate oxidations. Many nitrogen-containing heterocycles as leaving groups strongly coordinate to metals. However, 2,6-dimethylpyridine has almost no coordinating ability. Therefore, we adopted the N-oxides of heterocycles of which the nitrogen atoms are hindered by introducing substituents at the neighboring positions, as candidates for X–O, though heteroaromatic N-oxides had not previously been used as effective oxidants for reactions of this kind owing to their

Table 5
The catalytic activity of various metal complexes for the oxygen transfer reactions from pyridine N-oxides
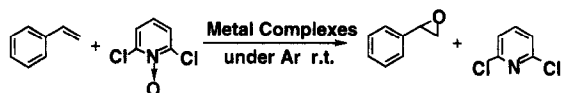
Metal complex	Yield [a]	
	epoxide	pyridine
$Mn^{III}(TPP)Cl$	0%	0%
$Fe^{III}(TPP)Cl$	0	0
$Co^{II}(TPP)$	0	0
$Mo^{V}(TMP)(O)OH$	0	0
$Ru^{VI}(TMP)(O)_2$	100	95
$Ru^{VI}(T_{2,6di}FPP)(O)_2$	94 [b]	97 [b]
$Ru^{II}(TPP)CO$	26	55
$Ru^{II}(TMP)CO$	99	93
$Ru^{II}Cl_2(PPh_3)_3$	0	0
$Ru^{II}Cl_2(PPh_3)_4$	0	0
$Rh^{III}(TMP)Cl$	0	0
$Rh^{II}(TMP)$	0	0
$Os^{VI}(TMP)(O)_2$	trace	0

[Metal complex] = 1 mM, [styrene] = 170 mM, [2,6-diCl-pyridine N-oxide] = 180 mM in benzen under Ar at RT.
[a] Based on starting materials.
[b] Carried out in CH_2Cl_2.

Table 6
Oxygen atom transfer activity of pyridine N-oxide derivatives to Ru porphyrin

Pyridine R_1	N-oxide R_2	Time	Pyridine yield[a,b]	Epoxide yield[a,c]
H	H	o.n. [d]	n.d. [e]	trace
CH_3	H	o.n.	n.d.	26%
Cl	H	o.n.	100%	94
CH_3	CH_3	6 h	n.d.	95
Cl	Cl	2 h	95	100
Br	Br	6 h	100	98
Ph	Ph	o.n.	n.d.	trace

These reactions were carried out in benzene at room temperature under Ar ([olefin] = 170 mM, [pyridine N-oxide] = 180 mM, [RuTMP(O)$_2$] = 1 mM).
[a] Detected by GLC.
[b] Based on pyridine N-oxides.
[c] Based on olefins.
[d] Overnight (16 ~ 20 h).
[e] Not determined.

low oxidation abilities compared to the above oxidants.

2.1. Olefin epoxidation

Among various metalloporphyrins, only ruthenium (Ru) porphyrins are excellent catalysts for transfer of the oxygen atom of N-oxide to an olefin [3]. We have found that the Ru porphyrin-2,6-disubstituted pyridine N-oxide couple efficiently epoxidizes various olefins (Table 4). No metal complexes examined, except for Ru(por), worked as catalysts (Table 5). An olefin and a slight excess amount of 2,6-dimethylpyridine N-oxide were mixed in benzene, then the olefin and the N-oxide were completely converted into the epoxide and 2,6-dimethylpyridine by the addition of the catalytic amount (0.5 mol%) of $Ru^{VI}(TMP)(O)_2$ to the mixture. cis-stilbene gave cis-stilbene oxide selectively in the reaction. The turnover number reached 17,000. The reaction did not proceed with nonsubstituted pyridine N-oxide, as expected. 2-

Table 7
Reactivity of various heteroaromatic N-oxides as oxidants

N-Oxide	Yield of Epoxide	N-Oxide	Yield of epoxide
(2,6-dimethylpyridine N-oxide)	95 %	(4-nitroquinoline N-oxide)	99 %
(tetramethylpyrazine N-oxide)	100	(acridine N-oxide)	59
(trimethylpyrazine N-oxide)	99	(3,6-dichloropyridazine N-oxide)	28
(2-methylquinoline N-oxide)	46		

Table 9
Selective epoxidation of natural dienol acetates

Run	Terpenes	6,7-Epoxide	Yield[a] 2,3-Epoxide	Diepoxide
1	(geranyl acetate) OAc	79%[b] (90%)[c]	1% (1%)	3% (3%)
2	(neryl acetate) OAc	75% (78%)	2% (2%)	16% (17%)
3	(linalyl acetate) OAc	97% (97%)	0%[d] (0%)	0% (0%)

These reactions were carried out in benzene at room temperature under Ar for 2 d ([terpene] = 170 mM, [lutidine N-oxide] = 250 mM, [RuTMP(O)$_2$] = 1 mM).
[a] Determined by 400 MHz NMR.
[b] Based on terpenes.
[c] Based on conversion.
[d] 1,2-epoxide.

methylpyridine N-oxide worked as an oxidant to some extent. 2,6-Disubstituted pyridine N-oxide generally had excellent activities to epoxidize olefins quantitatively (Table 6). The only exception was 2,6-diphenylpyridine N-oxide, which had almost no oxidizing activity. This inertness is probably due to the steric hindrance by the phenyl groups. The addition of pyridine (only 2 equivalent to Ru(Por)) to the usual system completely inhibited the reaction. Therefore, these results indicate the significance of inhibition by coordination of the formed pyridines. Halogen-substituted pyridine N-

oxides had higher reactivity than methyl-substituted ones in terms of yield and reaction rate. N-oxides of heteroaromatic compounds other than pyridine are also effective as oxidants, provided that the coordination abilities of their deoxygenated heteroaromatic are sufficiently low (Table 7) [14]. This N-oxide/Ru(Por) system stereospecifically epoxidized a cis-olefin to afford a cis-epoxide (Table 8). Competitive oxidation of cis- and trans-β-methylstyrene resulted in the selective conversion of cis-β-methylstyrene to the cis-epoxide. This cis-olefin selectivity has also been observed in the study of the Fe(TMP)Cl–PhIO system by Groves and Nemo [15]. Acetates of natural dienols, i.e., geraniol, linalol, and nerol, were selectively

Table 8
Competitive epoxidation of 1:1 mixture of cis- and trans-olefin

1:1 mixture of cis- and trans-olefin	Yield of epoxide		Recovery	
Ph⁀⁀Ph (stilbene)	Ph⁀Ph (cis-epoxide) 87 %	Ph⁀Ph (trans-epoxide) 1 %	Ph⁀Ph (cis) 2 %	Ph⁀Ph (trans) 99 %
Ph⁀⁀CH₃ (β-methylstyrene)	Ph⁀CH₃ (cis-epoxide) 89 %	Ph⁀CH₃ (trans-epoxide) 5 %	Ph⁀CH₃ (cis) 7 %	Ph⁀CH₃ (trans) 91 %

These reactions were carried out in benzene at room temperature (stilbene: for 4 h; β-methylstyrene: o.n.) ([cis-olefin] = [trans-olefin] = [2,6-lutidine N-oxide] = 170 mM). Yields were based on used cis(trans)-olefins.

Table 10
Oxidation of adamantane with Ru porphyrin-2,6-diCl-pyridine N-oxide system

Run	Catalyst	Additive	Time	Temp.	Products (% yield)[a,b]		
					Adam.-1-ol (a)	Adam.-1,3-diol (b)	Adam.-2-one (c)[i]
1	RuTMP(O)$_2$	HCl	24 h	r.t.	68	25	1
2	RuTMP(O)$_2$	HBr	24 h	r.t.	63	15	2
3	RuTMP(O)$_2$	—	24 h	r.t.	4	n.d.[f]	trace[g]
4	—	HCl	24 h	r.t.	1	n.d.	n.d.
6	RuTPP(CO)	HBr	6 h	r.t.	66	27	trace
8[c,d]	RuTPP(CO)	HBr	15 h	40°	66 (6600)[h]	22 (4400)	0.5 (100)
10[c,e]	RuTPP(CO)	HBr	6 h	80°	59 (59,000)	29 (58,000)	1.5 (3000)

These reactions were carried out in benzene under Ar. The reaction mixtures contained adamantane (200 mM), 2,6-dichloropyridine N-oxide (260 mM), catalyst (1.0 mM), 36% HCl aq. (200 ~ 410 mM) or 48% HBr aq. (120 ~ 240 mM), and molecular sieves 4A (100 mg/ml).
[a] Yields were based on starting adamantane (%).
[b] Determined by GLC.
[c] [adamantane] = 1.0 M, [N-oxide] = 1.3 M.
[d] [catalyst] = 100 μM.
[e] [catalyst] = 10 μM.
[f] Not detected.
[g] < 0.5%.
[h] The turnover number per catalyst (turns).
[i] Adamantan-2-ol was not detected.

mono-epoxidized (Table 9), in all cases, at the 6,7-double bonds. The steric and electronic effects of acetoxy groups may cause the highly selective epoxidation. Sulfides were also oxidized to afford the sulfoxides and the sulfones in good yields. However, the sulfide oxidation required more time or higher temperature for completion than epoxidation. This retardation is probably attributable to coordination of a sulfide or a sulfoxide to the Ru atom [16,17].

Table 11
Hydroxylation and/or ketonization of various alkanes and alcohols

| Run	Substrate	Condition	Product (Yields are based on substrates)[a]			
1	(methylcyclohexane)	cat. = RuTPP(CO) add. = HBr [N-oxide] = 2.0 equiv. r.t., 6 h	(1-hydroxy-1-methylcyclohexane) OH	94 %	(methylcyclohexanone)	(2- keto / 3- keto / 4- keto) trace[d]
2	(methylcyclohexane)	cat. = RuTMP(O)₂ add. = HBr [N-oxide] = 2.2 equiv. 40°, 9 h	(1-hydroxy-1-methylcyclohexane) OH	77 %	(methylcyclohexanone)	(2-keto / 3-keto / 4-keto) 2- : 3 % 3- : 2 % 4- : 1 %
3	(1-adamantanol) OH	cat. = RuTMP(O)₂ add. = HBr [N-oxide] = 1.1 equiv. 60°, 4 h	(1,3-adamantanediol) OH ... OH	74 %[b] (90 %[c])		
4	(decalin)	cat. = RuTPP(CO) add. = HBr [N-oxide] = 1.2 equiv. r.t., 6 h	(9-decalol) OH	72 %[b] (80 %[c])		
5	(ethylbenzene)	cat. = RuTMP(O)₂ add. = HCl [N-oxide] = 2.2 equiv. r.t., 24 h	(acetophenone) O	88 %	(1-phenylethanol) OH	n.d.[e]
6	(cyclooctane)	cat. = RuTPP(CO) add. = HBr [N-oxide] = 3.0 equiv. r.t., 40 h	(cyclooctanone) O	77 %	(cyclooctanol) OH	2 %
7	(cyclohexanol) OH	cat. = RuTMP(O)₂ add. = HCl [N-oxide] =1.1 equiv. r.t., 24 h	(cyclohexanone) O	88 %		
8	(2-adamantanol) OH	cat. = RuTMP(O)₂ add. = HBr [N-oxide] = 1.1 equiv. r.t., 24 h	(2-adamantanone) O	84 %[b]		
9	(4-methylcyclohexanol) OH	cat. = RuTMP(O)₂ add. = HCl [N-oxide] = 1.1 equiv. r.t., 24 h	(4-methylcyclohexanone) O	81 %[b]		

These reactions were carried out under Ar in benzene ([substrate] = 200 mM, [catalyst] = 1 mM, HBr aq. (47%) or HCl aq. (36%) = 30 μl/ml, molecular sieves 4A = 100 mg/ml).
[a] Yields are based on substrates (detected by GLC).
[b] Isolated yield.
[c] Based on conversion.
[d] < 0.5%.
[e] Not detected.
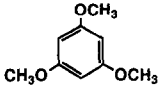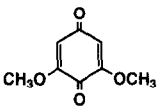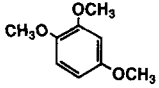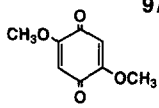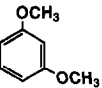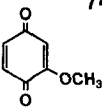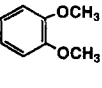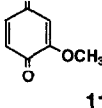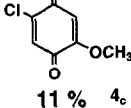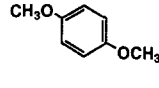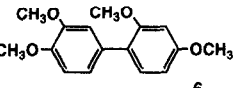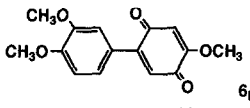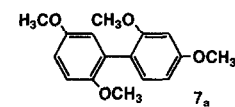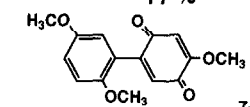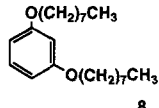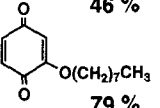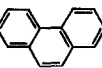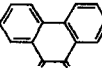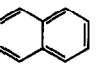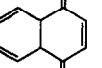
2.2. Alkane hydroxylation

We next examined the effect of additives upon the oxidation reactivity of this system, with the aim of developing a novel oxidation system for alkanes and other unreactive compounds. We found that the catalytic ability of ruthenium porphyrins was enhanced by the

Table 12
Oxidation of aromatic compounds by ruthenium porphyrin-2,6-dichloropyridine N-oxide system

presence of a small amount of HCl or HBr and that the oxidation of alkanes or aliphatic alcohols with pyridine N-oxides was also catalyzed by ruthenium porphyrins with high efficiency in the presence of these acids (Table 10) [18,19]. Under this condition, adamantane was almost wholly consumed to afford adamantan-1-ol, adamantane-1,3-diol, and adamantan-2-one in yields of 68%, 25%, and 1% based on adamantane, respectively (run 1). The role of HBr or HCl has not yet been fully clarified. However, these acids are considered to work as donors of an axial ligand (Br^- or Cl^-) of Ru porphyrin, and also as activators of an active intermediate, by protonation. Details are described in the Mechanistic studies section. Ruthenium catalysts were extremely stable under these conditions. Adamantane was reacted with 2,6-dichloropyridine N-oxide in the presence of 1.0×10^{-4} eq. of Ru(TPP)(CO) and HBr at 40°C. The oxidant was almost exhausted in 15 h, at which time the turnover number was 11,100 (run 8). At higher temperature, Ru(TPP)(CO) gave a turnover number of 120,000 during 6 h (run 9). The turnover frequency for the last case was 5.6/s. Several alkanes were oxidized with this system. The results are shown in Table 11. 1-Methylcyclohexanol was selectively obtained with high efficiency in the oxidation of methylcyclohexane (yield = 94%) (run 1). The oxida-

tion of cis-decalin proceeded efficiently and cis-decalol was isolated in 72% yield (run 4). The stereo isomer, trans-decalol, was not detected by GLC analysis. Ethylbenzene was converted into acetophenone in 88% yield (run 5). Cyclooctane, which is less reactive than a tertiary alkane or benzyl alkane, was oxidized to afford cyclooctanone in reasonable yield (yield = 77%) (run 6). Aliphatic alcohols were also oxidized to afford the corresponding ketones (runs 7, 8, 9). These results indicated that the ketones were generated via alcohols in the oxidation of secondary alkanes.

2.3. Selective quinone formation by arene oxidation [20]

This highly reactive Ru(Por)-N-oxide system was also expected effectively to oxidize rather unreactive aromatic compounds. We examined oxidation of aromatic compounds with the Ru(Por)-2,6-disubstituted pyridine N-oxide system in the presence of acid, and found selective quinone formation [20]. Oxidation of various arenes was examined with 2,6-dichloropyridine N-oxide catalyzed by Ru(Por) in the presence of hydrogen halide. Alkoxybenzene derivatives were oxidized to afford mainly p-benzoquinones in yields that greatly depended upon the structure of the substrates (Table 12). Compound 1_a gave the best result (1_b: 97%). The turnover number of Ru(Por) reached 33,000 in the case of 1_a. m-dimethoxybenzene (abbreviated as m-DMB) (3_a) was converted into 2-methoxy-p-benzoquinone (3_b) in good yield (run 3). In contrast, o-DMB (4_b) afforded 3_b and the chlorinated quinone 4_c in low yield (run 4), and p-DMB 5_a gave almost no product (run 5). Competitive oxidation of m-DMB and o-DMB resulted in an exclusive oxidation of m-DMB and recovery of o-DMB (Fig. 3). The reaction rate of m-DMB was much higher than that of o-DMB in the oxidizing system. Therefore, the chemo-selectivity of the oxidizing system was examined by using compounds 6_a and 7_a, which possess both m-DMB and o-DMB or p-DMB
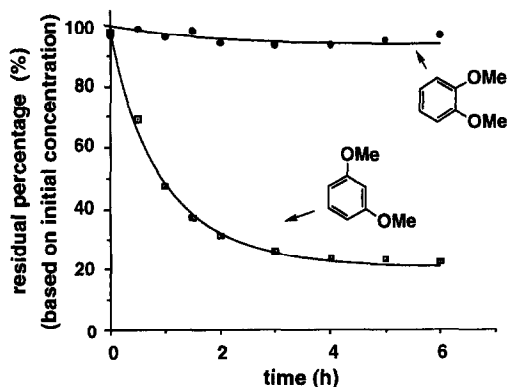
Fig. 3. Competitive oxidation of o- and m-dimethoxy-benzenes by Ru(TPP)CO-2,6-dichloropyridine N-oxide system in the presence of HBr.

structure, as substrates. In the case of 6_a, 2-methoxy-5-(3,4-dimethoxyphenyl)-p-benzo-quinone 6_b was afforded in high yield as a single product (run 6). The oxidation of 7_a gave an analogous result (7_b) (run 7). This type of selectivity is unique among oxidizing reagents commonly used in quinone synthesis (for recent reviews, see Ref. [21]). For example, cerium (IV) ammonium nitrate (CAN) preferentially oxidizes p-dimethoxybenzene structures. Our attempt to oxidize 6_a and 7_a with CAN resulted in the formation of a complex mixture of products, in each case. The only isolable product in the CAN oxidation of 7_a was 2-(2,4-dimethoxyphenyl)-p-benzoquinone (yield = 8%) as expected.

The hydrocarbon oxidations by the present oxidizing system are the first examples of pyridine N-oxide derivatives working as oxidants in a catalytic oxidation of hydrocarbons.

2.4. Mechanistic studies

In the catalytic reaction with cytochrome P450, iron–oxo porphyrin complexes are considered to be the active intermediates for the

oxidation of substrates (for recent reviews, see Ref. [1]). Since $Ru(TMP)(O)_2$ was synthesized and characterized by Groves and Quinn [22], this complex has been regarded as analogous to the active intermediate of cytochrome P450, and the oxidation reactivity of this dioxo complex has attracted much interest [22,23]. Considering the difference in reactivity between the $Ru(TMP)(O)_2-O_2$ system and the $Ru(TMP)(O)_2$-N-oxide system, it seemed likely that some active intermediate other than $Ru(TMP)(O)_2$ is generated during the oxidation with our system. The rate constant of the reaction between $Ru(TMP)(O)_2$ and 2-vinyl-naphthalene was determined in a mechanistic study [16]. It is clear from the kinetic experiments that the catalytic reaction proceeded at a much faster rate than would be predicted from the reactivity of $Ru(TMP)(O)_2$, which indicates that an active intermediate other than $Ru(TMP)(O)_2$ mainly epoxidizes olefins during this catalytic reaction. A study on incorporation of ^{18}O from labeled water into the epoxide also supported the above proposal [16].

In contrast, it might be possible to understand the mechanism in the presence of HCl or HBr
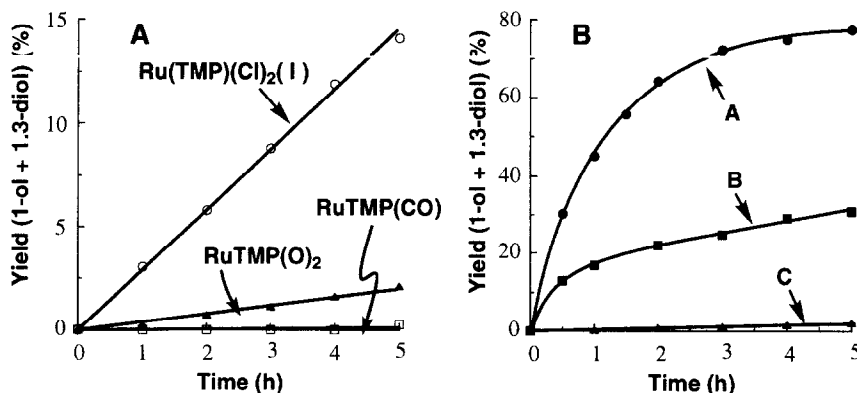
Fig. 4. (A) The hydroxylation of adamantane with 2,6-dichlorpyridine N-oxide catalyzed by RuTMP(Cl)$_2$, RuTMP(O)$_2$, and RuTMP(CO). These reactions were carried out in benzen under Ar at 40°C ([adamantane] = [2,6-dichloropyridine N-oxide] = 100 mM, [catalyst] = 0.4 mM). Neither HCl (or HBr) nor molecular sieves were added to the reaction mixtures. Yields (based on starting adamantane) were determined by GLC. (B) The hydroxylation of adamantane with 2,6-dichloropyridine N-oxide catalyzed by RuTMP(O)$_2$ in the presence or the absence of HCl. These reactions were carried out in benzen under Ar at 40°C ([adamantane] = [2,6-dichloropyridine N-oxide] = 100 mM, [RuTMP(O)$_2$] = 0.4 mM). A saturated HCl solution of benzene was added to these reaction mixtures (A: 10 ml/l, B: 5 ml/l, C: 0 ml/l). Molecular sieves were not added to the mixture, because the added HCl solution was anhydrous. Yields (based on starting adamantane) were determined by GLC.

by considering the effect of these acids, because the acids greatly affected the reactivity of the catalytic oxidation. To elucidate the effect of HCl, Ru(TMP)(O)$_2$ was allowed to react with HCl without substrate or oxidant, and the resulting ruthenium porphyrin complex (I) was isolated (y. 82%). It is probable that I is the trans-dichloro complex of ruthenium porphyrin, formulated as Ru(TMP)(Cl)$_2$, as judged from its NMR data [19].

The catalytic abilities of a series of complexes, I, Ru(TMP)(O)$_2$, and Ru(TMP)(CO), were compared in the absence of HCl or HBr at 40°C. As shown in Fig. 4, I was the most efficient catalyst among them, suggesting that Ru(TMP)(O)$_2$ and Ru(TMP)(CO) acted as efficient catalysts after conversion into I or into complexes which could be generated more easily from I than from Ru(TMP)(O)$_2$ or Ru(TMP)(CO). However, the oxidation with Ru(TMP)(O)$_2$ in the presence of HCl at 40°C proceeded far more efficiently than that with I in the absence of acid, and the amount of added HCl affected the efficiency of the oxidations

(Fig. 4). It is probable that the active intermediates are the ruthenium–oxo porphyrin complexes formulated as [O=Ru^V(por)(X)] (X = Cl or Br) (b), and the acids accelerate the formation of the Ru–oxo intermediate from the N-oxide and ruthenium porphyrin in reduced form. The present results do not allow us to identify the mechanism or the active intermediate. Nevertheless, the catalytic activity of ruthenium porphyrins appears to be influenced drastically by the nature of the axial ligands. Further investigation of the mechanistic details is needed and is in progress in our laboratory.

3. Synthesis of polypeptide-bound iron porphyrin and effect of the macromolecule on P450-like reactivities

We designed a novel polypeptide-bound porphinato iron complex to investigate the environmental effect in heme enzyme. This polymer porphyrin (PP), was synthesized from poly-γ-methyl-L-glutamate (MW: 100,000) by cou-
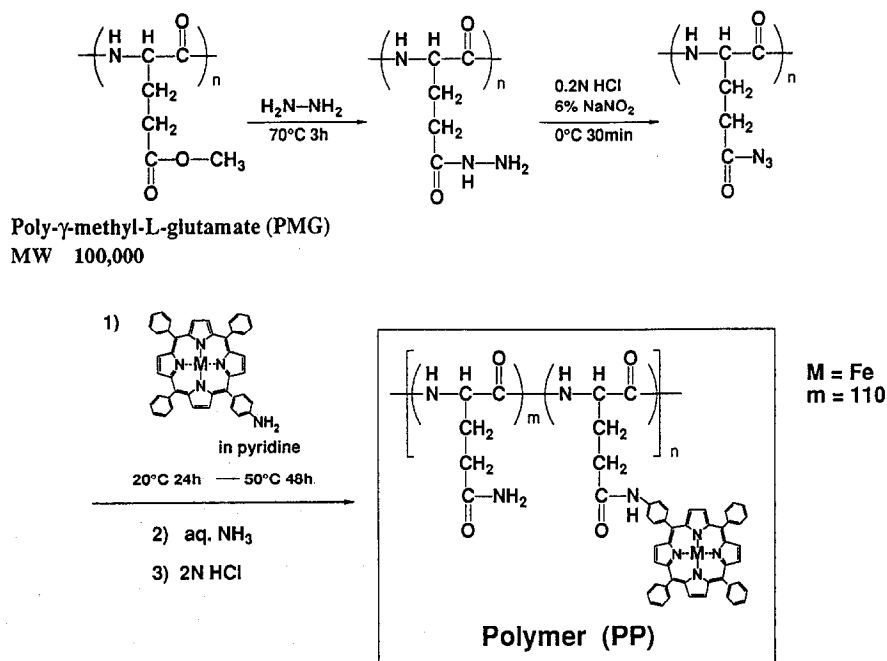
Fig. 5. Synthesis of polypeptide-bound porphinatoiron.

pling with Fe(TPP)Cl[4]. The ratio of glutamine residues to Fe(TPP)Cl was estimated to be about 110 by atomic absorption analysis (Fig. 5). We have already reported that this polymer complex exhibited more P450-like activities than the non-bound Fe(TPP)Cl in the olefin oxidation with molecular oxygen in the presence of $NaBH_4$ [4].

It is well known that cytochrome P450 catalyzes the demethylation of alkylamines [24]. However, when the described polypeptide-bound porphyrin was used, unexpected products, such as N–N coupling and C–N coupling products, were obtained besides the normal demethylation product, aniline (Table 13) [4]. Table 13 shows the result of this reaction under air in the presence of $NaBH_4$. The coupling compounds were produced in relatively high yield compared with the case of the free Fe(TPP)Cl. Based on this result, we presumed that these products may be unknown metabolites in the P450 system. So we attempted to detect these compounds in a rat liver microsomal reaction mixture using the GC-SIM method [25]. Two rat liver microsomal systems, the $NADPH/O_2$ and the cumene hy-

droperoxide-supported shunt path, were employed. As expected, these coupling compounds were also found as metabolites of N-methylaniline in the microsomal system. A significant amount of the coupling product was formed, though the yield was lower than that of the demethylation product.

In addition, P450-specific inhibitors, SKF-525A and metyrapone, suppressed the formation of these coupling products (data not shown). These results indicate that the coupling reaction is P450-dependent. Thus, P450 chemical model systems are available for investigating new metabolic pathways.

4. Application of P450 mimics to drug metabolism studies and evaluation of their utility

Metabolism of most xenobiotics involves cytochrome P450 in vivo. It is widely recognized that heme in the enzyme is mainly responsible for its chemistry. Chemical models can be useful in studies on drug metabolism, because it is

Table 13
Coupling products formation catalyzed by cyt. P450 model systems

Catalyst	N,N	p-C,N	o-C,N (μmol/μmol cat.)	Aniline
Polymer-FeTPPCl	1.84	0.12	1.61	8.92
Free FeTPPCl	0.06	trace	0.12	17.0

The reaction mixture contained catalyst (1 μmol), N-methylaniline (0.2 mmol), tetramethylammonium hydroxide (0.2 ml of 10% methanol solution), $NaBH_4$ (0.5 mmol), and methanol (1.3 ml). The reaction mixture was stirred vigorously under air for 3 h at RT.

Table 14
Oxidation of PCP by iron porphyrin–mCPBA system
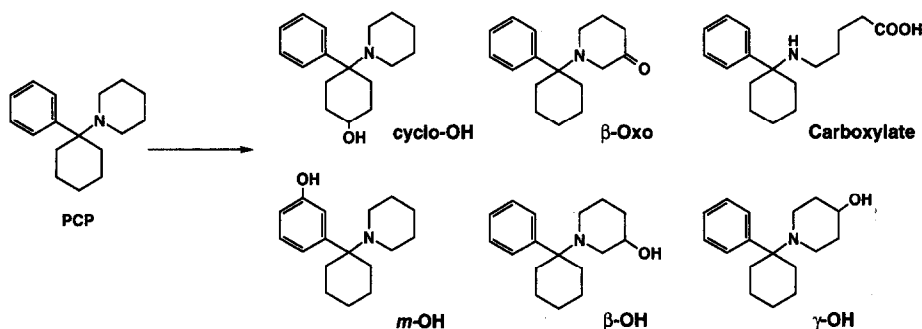
Catalyst	Yield (based on oxidant)					
	m-OH	β-OH	γ-OH	cyclo-OH	β-oxo	carboxylate
SR	n.d.	1.85%	41%	0.44%	18.5%	n.d.
NP	n.d.	2.7	41	0.59	0.41	6.5%
Fe(TPP)Cl	n.d.	0.18	22	0.75	1.2	trace

Reaction conditions; [cat.] = 1 mM, [mCPBA] = 1 mM, [PCP] = 500 mM at RT under argon.

usually laborious and expensive to obtain sufficient amounts of drug metabolites by using animals, during the development of new medicines. Some oxidation products identical with metabolites are expected to be afforded by the catalytic oxidation of drugs with metalloporphyrin systems, since their reactivities are thought to resemble, at least to some extent, that of cytochrome P450. The advantages of using model systems to understand drug metabolism are as follows: (1) metabolite candidates can be obtained in relatively large amounts and used to identify the real in vivo metabolites and provide samples for pharmacological testing; (2) the mode of metabolism can be clarified, for example, unstable metabolites can be isolated under selected and controlled reaction conditions; and (3) the usage of experimental animals can be reduced.

As the first example for study, we chose the anesthetic agent phencyclidine (PCP), because,

despite being a small molecule, it has an aromatic ring, an aliphatic ring and an N-containing heterocyclic ring moiety, and is expected to exhibit a diversity of reactions in various model systems. As shown in Table 14, many types of oxidation products were produced by using various chemical model systems; phenolic, alcoholic, and ring-opened amino acids [5].

The SR and NP complexes gave similar products in combination with mCPBA as shown in Table 14. It is noteworthy that the piperidine 3-oxo compound was predominantly obtained in the SR catalyst system.

We then examined the reaction of PCP in the liver microsomal systems (data not shown). Microsomes–oxidant systems were constituted from three kinds of rat liver microsomes, i.e., non-treated, phenobarbital (PB)-treated and 3-methylcholanthrene (3MC)-treated, as well as non-treated mouse liver microsomes, and two kind of oxidants, cumene hydroperoxide (A)
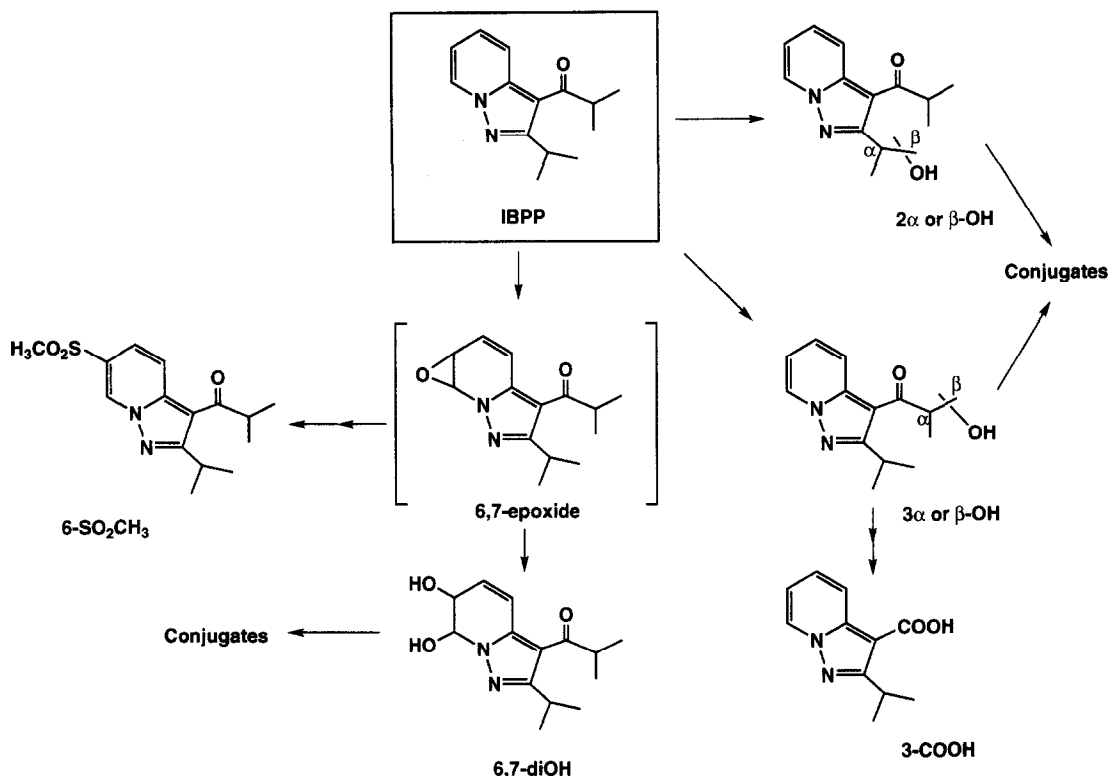
Fig. 6. Metabolic pathways of 3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine (IBPP) in humans and animals.

and iodosylxylene (B). The identity of all products (Table 14) was confirmed by comparison with authentic samples obtained from the chemical model systems. The piperidine-β-oxo compound was generated, as in the chemical model systems.

Next, we examined the reaction profiles of the antiasthma drug, IBPP, in various P450 chemical model systems and compared them with those of the rat or human liver microsomal

system. The company involved in this project had difficulty in developing this drug because of the diversity of its metabolic pathways including an unstable metabolite. The metabolic pathways of IBPP in vivo are shown in Fig. 6 [26]. The main metabolite is the 6,7-diol, but the 6,7-epoxide, presumed to be its precursor, could not be isolated. However, the epoxide could be obtained in good yield by using the Mn(TPP)Cl/NaOCl system [26] as shown in

Fig. 7. Synthesis of 6,7-epoxy-IBPP by a Cyt. P450 model.

Fig. 7 [27]. Usual epoxidizing reagents such as *m*CPBA did not give this epoxide. This successful isolation of the unstable epoxide was possible because the reaction medium does not contain epoxide-labile nucleophiles such as proteins and amino acids.

The reaction product profile of IBPP was compared among various cytochrome P450 model systems, rat liver microsomes, and human liver microsomes [28]. The pattern analysis of IBPP reaction products in some chemical model systems and microsomal systems is shown in Fig. 8. The relative yield of the corre-

sponding product is indicated along each axis. These diagrams, formed by linking each plot, show a characteristic pattern of each reaction system. This pattern analysis by a combination of two-dimensional TLC should make it possible to select or design a model system most suitable for the metabolism of various drugs. In this method, it is not necessary to confirm each metabolite in advance. After selection of the most suitable model system by comparison with the native P450 system, the metabolites can be chemically determined by using the model system on a large scale.

Fig. 8. Metabolic patterns of IBPP by P450 chemical models and liver microsomes.

Fig. 9. Oxidative decarboxylation of carboxylic acids.

Table 15
Oxidation products of carboxylic acids by $Fe^{III}(TPFPP)Cl/PhIO$ system

R_1	R_2	R_3	(II)	(III)	Other
I$_a$ ⟨phenyl⟩	CH$_3$	H	45.6	3.4	R$_1$,R$_2$-C-Ĉ-O-C-R$_1$,R$_2$, R$_3$ R$_3$ 2.4
I$_b$ ⟨benzoyl-phenyl⟩	CH$_3$	H	48.2	25.7	R$_1$,R$_2$-C-Cl 12.5 R$_3$
I$_c$ (CH$_3$)$_2$CHCH$_2$-⟨phenyl⟩	CH$_3$	H	51.3	20.3	R$_1$,R$_2$-C-Ĉ-O-C-R$_1$,R$_2$, R$_3$ R$_3$ 4.8
I$_d$ CH$_2$=CHCH$_2$O-⟨Cl-phenyl⟩	H	H	51.9	24.3	R$_1$COOH 5.0
I$_e$ ⟨indole deriv. CH$_3$O, CH$_3$, CO, Cl⟩	H	H	8.5	7.6	R$_1$,R$_2$-C-Ĉ-O-C-R$_1$,R$_2$, R$_3$ R$_3$ 14.2
I$_f$ ⟨indene deriv. F, CH$_3$, CH, X=SOCH$_3$⟩	H	H	30.4 (X=SO$_2$CH$_3$), 22.3 (X=SOCH$_3$)	6.3, 5.2	R$_1$COOH 1.2
I$_g$ ⟨phenyl⟩-CH=CH-	H	H	31.8		
I$_h$ ⟨phenyl⟩-CH$_2$-	CH$_3$	CH$_3$	9.2		R$_1$,R$_2$-C-Cl 18.1 R$_3$
I$_i$ Cl-⟨phenyl⟩-O-	CH$_3$	CH$_3$			Cl-⟨phenyl⟩-OH 29.5

Conditions: [substrate] = 100 mM, [PhIO] = 200 mM, [Fe^{III}(TPFPP)Cl] = 0.5 mM in CH$_2$Cl$_2$. The mixture was stirred at RT for 20 h under Ar.

Next, we have found that carboxylic acids with an aryl group or three substituents at the α carbon are readily decarboxylated oxidatively to afford the one-carbon-reduced alcohol and carbonyl products in the iron porphyrin–iodosylbenzene system (Fig. 9 and Table 15) [29]. This oxidative decarboxylation was novel among metalloporphyrin-catalyzed oxidations. FT-IR spectra of the reaction mixture in the case of ketoprofen exhibited a strong absorption due to carbon dioxide. An EPR study provided strong evidence for the formation of a carbon-centered radical in the decarboxylation. The reaction mechanism is considered to involve one-electron oxidation of a carboxyl group and a succeeding, rapid decomposition of the unstable carboxyl radical to afford CO_2 and the carbon radical.

Therefore, the findings in the model system led us to investigate the existence of this new metabolic pathway of carboxylic acids via oxidative decarboxylation in vivo, since many drugs could be affected [30]. The products derived from drugs with a carboxyl moiety could be used as authentic samples, so-called 'candidate metabolites', to identify novel metabolites in urine and bile of dosed rats or in microsomal reaction mixtures. Two metabolites of ketoprofen isolated from rat bile and urine were consistent with the authentic II$_b$ and III$_b$, in terms of the retention times and mass fragmentation in GC/MS experiments. Experiments in vitro using rat microsomes have revealed that metabolites are formed via oxidative decarboxylation in the incubation mixtures of microsomes plus indomethacin or clofibric acid or 2,2-dimethyl-3-phenylpropionic acid (Table 15). Therefore, it is highly probable that oxidative decarboxylation of carboxylic acids generally occurs as a pathway of hepatic metabolism in vivo. The results obtained provide evidence for the involvement of cytochrome P450 in the new metabolism.

Formation of novel metabolites via oxidative decarboxylation would influence the pharmacological activities and the toxicities of drugs bearing a carboxylic group, especially if the metabolites themselves are biologically active. Therefore, it is necessary to assay the activities of the metabolites, in order to clarify their role in the pharmacological effect of the parent drugs.

Fig. 10. Hydroxylactonization of γ,δ-unsaturated carboxylic acids.

The inhibitory activities of compound II_b and II_e on arachidonic acid-induced platelet aggregation in rabbits, though less than those of the mother compounds, were still significant.

Further, we have found that γ,δ- and β,γ-unsaturated carboxylic acids afford δ-hydroxy-γ-lactone and β-hydroxy-γ-lactone, respectively, in one step with the iron porphyrin-iodosylbenzene (PhIO) system (Fig. 10). This type of lactonization has not previously been reported as far as we know. We have also revealed an apparently general metabolic reaction affording γ-lactone compounds from the corresponding γ,δ- and β,γ-unsaturated carboxylic acids, catalyzed by cytochrome P450. Details of this study will be presented in another paper [31].

In summary, we have developed the **SR** complex having axial thiolate as a structural model of the cytochrome P450 active site, a robust and potent Ru porphyrin–heteroaromatic *N*-oxide system as a functional P450 model, and a polypeptide-bound iron porphyrin exhibiting effects due to the macromolecule, and we have applied P450 mimics to drug metabolism studies. We shall be well pleased if these studies contribute to the development of metalloporphyrin chemistry and also the understanding of P450 chemistry.

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