Steroid-bismetalloporphyrin as enzyme model of cytochrome P-450 monooxygenase

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The model compounds of androgenic steroid-bismetalloporphyrins 3a—3d and androgenic monometalloporphyrins 2a—2d have been synthesized. Catalytic study in two-phase condition on epoxidation of styrene under the catalysis of model catalysts shows that the catalytic performances of steriod-bismetalloporphyrin is superior to steroid-metalloporphyrin and the corresponding simple metalloporphyrin. The excellent catalytic property of steriod-bismetalloporphyrin can be rationalized as the hydrophobic action of steroid framework and the cooperative action of two metalloporphyrins in catalysis.

Keywords Steroid, porphyrin, androgenic steroid-bisporphyrin, enzyme model, catalytic epoxidation

Introduction

Enzymes catalyze reactions with distinguished reaction specificity, high substrate selectivity, and high efficiency under mild conditions. Similar to enzymatic catalysis, biomimetic catalysis offers high selectivity and efficiency. Thus, the biomimetic chemistry and the closely related techniques are in accord with the principles of green chemistry, since it can effectively accomplish the goals of Green Chemistry.[1]

As a prosthetic group porphyrin plays improtant role in oxygen metabolism, electron transport and photosynthesis. Recent advances in biomimetic chemistry indicate that the porphyrin dimers cantaining potential cavities can be used to construct the artificial enzymes capable of binding, recognition and catalysis. Various types of porphyrin dimers have been reported.[2-7] In some cases, however the linkage is subjected to breakage under operating conditions.[4] The rigidity of steroid is advantageous to form the dimers with stable conformation. At the same time, the hydrophobic action of steroid residue is advantageous to enhance the catalytic activities.[8] Therefore, we have designed and synthesized the novel type of steroid-bisporphyrin dimers as enzyme model with multiple site of recognition.

In the previous communication[9] we reported the synthesis and conformation of androgenic steroid-bisporphyrin. In this paper we reported mainly that the synthesis of androgenic steroid-bismetalloporphyrin complexes **3a—3d** and their catalytic property. As a comparison, steroid-monometalloporphyrin **2a—2d** and the corresponding simple metalloporphyrin **1a—1d** were also synthesized. We chose two-phase system with styrene as substrate to examine the catalytic performance of the synthesized steroid-bismetalloporphyrin as model for cytochrome P-450 monooxygenese. The structures of these models are illustrated in Scheme 1.

Experimental

General

Melting points were determined on a micro-melting apparatus and uncorrected. Ultraviolet spectra were determined on a Perkin-Elmer Lambda 4B spectrophototer in chloroform. Infrared spectra were obtained on a NICOLET FT IR 1705X spectrometer with KBr disks.

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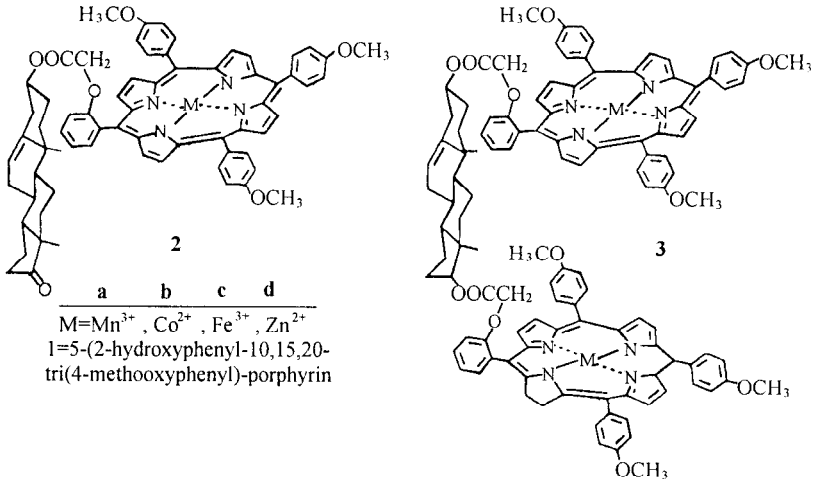
Nuclear magnetic resonance spectra were determined on a JUN-FX 90Q spectrometer. Microanalyses were carried out using a CARO ERBA 1106 for C, H and N determination. Gas chromatography was performed on an SC-6 chromatograph with CDMC-ICX data computer.

Scheme 1



Materials

The chemicals are of analytical grade or reagent grade. Solvents were purified before use. The precursors of models **3** and **2** were prepared as reported.[9] 5-(2-Hydroxyphenyl)-10,15,20-tri(4-methoxyphenyl)-porphyrin (**1**) was prepared according to the literature.[10] The structures of **1**, **2** and **3** were identified by UV-Vis, IR, ^{1}H NMR and elemental analysis. Styrene oxide used as standard sample in GC was prepared by the reaction of peroxyacetic acid with styrene.

Catalytic epoxidation of styrene and product analysis

All reactions were carried out at 21 ± 0.5℃ (except the ones in which the effect of temperature on reaction was investigated) in a 25 mL Schlenk equipped with a stirrer. A solution of 1.1 mmol of styrene and 4 mL of dichloromethane was added successively to a mixture of 2.0×10^{-3} mmol of catalyst and 0.018 mmol of benzyldimethyltetradecylammonium chloride. Then 1 mL of NaOCl (0.32 mol/L) was added to the organic phase. Magnetic stirring was stopped before each aliquot (4 μL) was withdrawn from the organic phase for product analysis. Analytical condition of GC: oven temperature, 230℃; column temperature, 80℃; flowing speed of the hydrogen gas, 30 mL/min.

Syntheses of models

Steroid-bisporphyrin manganese complex **3a** $Mn(Ac)_2 \cdot 4H_2O$ (53.9 mg, 0.22 mmol) and porphyrin **3** (108.7 mg, 0.06 mmol) were refluxed in DMF (20 mL) at 150—152℃ for 30 min with stirring (The completion of the reaction was checked by UV-vis spectra). The crude reaction mixture was poured into aqueous solution of NaCl with stirring and allowed to stand, and then filtered under reduced pressure. The green solid was dissolved in chloroform-methanol and two drops of concentrated hydrochloric acid were added to the solution. The solution was concentrated to dryness under reduced pressure. It was again dissolved in chloroform and chromatographed on a silica gel column using $CHCl_3$-CH_3OH (10:1 *V/V*) as an eluting agent. The second band was collected. Its recrystallization from chloroform-acetone-petroleum ether afforded a yellow-green crystal (42.5 mg, 71.5%). mp > 300℃. λ_{max} ($CHCl_3$): 621.3, 479.8 (soret band) nm. ν_{max}: 950 (N—M)

cm^{-1}, [3312 cm^{-1}(NH) disappeared]. $\delta_H(CDCl_3)$: 8.84(16H, s, porphyrin ring); 8.17—7.25(32H, m, Ph-H); 4.7—4.95(1H, d, steroidal ring-C = C-H); 4.03(18H, s, OCH_3); 3.84(2H, s, C-CH_2 O); 0.96 (3H, s, 19-CH_3); 0.17 (3H, s, 18-CH_3); 0.14—2.35(19H, m, steroidal ring-CH_2, CH) (these features are similar to those of the ligand **6** except the absence of the internal pyrrole proton). Anal. $C_{117}H_{98}N_8O_{12}Mn_2Cl_2$. Calcd: C, 70.66; H, 4.97; N, 5.63. Found: C, 71.21; H, 5.11; N, 5.84.

Steroid-bisporphyrin cobalt(II) *complex* **3b** Co $(Ac)_2 \cdot 4H_2O$ (54.8 mg, 0.22 mmol), **3** (108.7 mg, 0.06 mmol) and DMF (20 mL) were refluxed at 150—152℃ with stirring for 15 min. The crude reaction mixture was poured into aqueous solution of NaCl with stirring and allowed to stand, and then filtered under reduced pressure. The purple-red solid was dissolved in chloroform and chromatographed on a silica gel column using $CHCl_3$ as an eluting agent. The second band was collected. Its recrystalization from petroleum ether afforded a purple-red crystal (87.8 mg, 76%). mp > 300℃. $\lambda_{max}(CHCl_3)$: 544.8, 431.4(soret band)nm. ν_{max}: 950 cm^{-1} (N—M), [3312 cm^{-1} (NH) disappeared]. $\delta_H(CDCl_3)$: the spectral features are similar to those of the ligand **3** except the absence of the internal pyrrole proton (NH). Anal. $C_{117}H_{98}N_8O_{12}Co_2$. Calcd: C, 72.97; H, 5.13; N, 5.82. Found: C, 72.58; H, 5.24; N, 5.66.

Steroid-bisporphyrin iron (III) *complex* **3c** A solution of **3** (48.9 mg, 0.027 mmol), $Na_2S_2O_4 \cdot 2H_2O$ (23.1 mg, 0.11 mmol) and *N*, *N*-dimethylformamide (20 mL) were heated at 150—152℃ with stirring, and then $FeCl_2 \cdot H_2O$ (15.7 mg, 0.11 mmol) was quickly added. After 5 min, the mixture was poured into aqueous solution of NaCl and allowed to stand, then filtered under reduced pressure. The solid was washed to neutrality, dried and dissolved in chloroform and two drops of concentrated hydrochloric acid were added to the solution, then the mixture was stirred for a moment and the solvent was evaporated under reduced pressure to dryness. The solid was dissolved in chloroform and chromatographed on a silica gel column using $CHCl_3$-CH_3OH (10:0.5 *V/V*) as an eluting agent. The second band was collected. Its recrystalization from chloroform-petroleum ether afforded a yellow crystal of **3c** (36.2 mg, 67.4%). mp > 300℃. $\lambda_{max}(CHCl_3)$: 678.2, 452.4 (soret band) nm. ν_{max}: 950 cm^{-1} (N—M), [3312 cm^{-1}(NH) disappered]. $\delta_H(CDCl_3)$: the spectral features are similar to those of the ligand **3** except the absence of the internal pyrrole proton (NH). Anal. $C_{117}H_{98}N_8O_{12}Fe_2Cl_2$. Calcd: C, 70.59; H, 4.96; N, 5,63. Found: C, 70.23; H, 5.02; N, 5.44.

Steroid-bisporphyrin zinc (II) *complex* **3d** Prepared in the same way as used in the synthesis of **3b** and chromatographed on a silica gel column ($CHCl_3$-CH_3OH = 10: 0.3, *V/V*) to give **3d** as purple-red crystal (yield 82.2 %). mp > 300℃. $\lambda_{max}(CHCl_3)$: 550.8, 425.9(soret band)nm. ν_{max}: 950 cm^{-1}(N—M), [3312 cm^{-1}(NH) disappeared]. $\delta_H(CDCl_3)$: the spectral features are similar to those of the ligand **3** except the absence of the internal pyrrole proton (NH). Anal. $C_{117}H_{98}N_8O_{12}Zn_2$. Calcd: C, 72.48; H, 5.09; N, 5.78. Found: C, 72.25; H, 5.26; N, 5.80.

Steriod-porphyrin mangance (III) *complex* **2a** Prepared in the same way as used in the synthesis of **3a**. yield 64.6%. mp > 300℃. ν_{max} ($CHCl_3$): 620.0, 473.4 nm. ν_{max}: 985 cm^{-1}(N—M), [3311 cm^{-1}(NH) disappeared. $\delta_H(CDCl_3)$: the spectral features are similar to those of the ligand **2** except the absence of the internal pyrrole proton (NH). Anal. $C_{68}H_{62}N_4O_7MnCl$. Calcd: C, 71.79; H, 5.49; N, 4.92. Found: C, 71.23; H, 5.61; N, 5.08.

Steriod-porphyrin cobalt (II) *complex* **2b** Prepared in the same way as used in the synthesis of **3b**. yield 65.6%. mp > 300℃. ν_{max} ($CHCl_3$): 620.0, 473.4 nm. ν_{max}: 985 cm^{-1}(N—M), [3311 cm^{-1}(NH) disappeared]. δ_H ($CDCl_3$): the spectral features are similar to those of the ligand **2** except the absence of the internal pyrrole proton (NH). Anal. $C_{68}H_{62}N_4O_7Co$. Calcd: C, 73.83; H, 5.65; N, 5.06. Found: C, 73.87; H, 5.32; N, 5.42.

Steriod-porphyrin iron (III) *complex* **2c** Prepared in the same way as used in the synthesis of **3c**. yield 64.6%. mp > 300℃. λ_{max} ($CHCl_3$): 524.2, 432.5 nm. ν_{max}: 985 cm^{-1}(N—M), [3311 cm^{-1}(NH) disappeared]. δ_H ($CDCl_3$): the spectral features are similar to those of the ligand **2** except the absence of the internal pyrrole proton (NH). Anal. $C_{68}H_{62}N_4O_7FeCl$. Calcd: C, 71.74; H, 5.49; N, 4.92. Found: C, 71.96; H, 5.84; N, 4.90.

Steriod-porphyrin zinc (II) *complex* **2d** Prepared in the same way as used in the synthesis of **3d**. yield 73.2%. mp > 300℃. λ_{max} ($CHCl_3$): 558.8,

428.4 (soret band) nm. ν_{max}: 985 cm^{-1} (N—M), [3311 cm^{-1}(NH) disappeared]. $\delta_H(CDCl_3)$: the spectral features are similar to those of the ligand **2** except the absence of the internal pyrrole proton (NH). *m/z*: 1111(M^+). Anal. $C_{68}H_{62}N_4O_7Zn$. Calcd: C, 73.41; H, 5.62; N, 5.03. Found: C, 72.98; H, 5.13; N, 5.38.

Syntheis of metal complexes of **1**

1a, **1b**, **1c** and **1d** were prepared according to the method described in the literature.[11-13] Their structures were all confirmed by UV-vis, IR, ^1H NMR spectra, and elemental analyses.

Results and discussion

Catalytic properties on the epoxidation of styrene

The olefin epoxidation using manganese porphyrins as catalyst and NaOCl as oxidant under phase-transfer conditions has been well-established.[14-16] We therefore chose two-phase system (H_2O-CH_2Cl_2) with styrene as substrate and benzyldimethyltetradecylammonium chloride as phase transfer catalyst to examine the performance of the synthesized steriod-porphyrin complexes as the model of cytochrome P-450 monooxygenase.

The epoxidations were carried out with 6.8×10^{-3} mmol of catalyst, 4 mL of CH_2Cl_2, 0.32 mmol of NaOCl, and 0.018 mmol of phase-transfer catalyst at 20 ± 0.5℃. The oxidation products were analyzed by means of GC. The catalytic properties of three types of metalloporphyrin complexes were compared. The results showed that the catalytic activity of bismetalloporphyrin is superior to that of steroid-monometalloporphyrin and that of the corresponding simple metalloporphyrin. The manganses complex of steroid-bisporphyrin is the best.

Effects of various factors, such as concentrations of substrate, catalyst, sodium hypochloride, temperature and axial ligand on the catalytic property *etc*. were investigated. Herein our report is focused mainly on the comparison of catalytic property of models, the effects of axial ligands, the stability of model catalyst **3a** and the investigation of the cooperative action of bisporphyrins in catalysis.

Comparison of catalytic properties

The conversion of styrene and the selectivity to epoxide formation in the presence of the metal complexes of **3**, **2** and **1** are shown in Table 1. The catalytic properties (based on conversion and selectivity) decrease generally in the following order: steroid-bismetalloporphyrin > steroid-metalloporphyrin > corresponding simple metalloporphyrin for the same metal, and the catalytic properties of different metal ions decrease in the order of $Mn^{3+} > Co^{2+} > Fe^{3+}$, Zn^{2+} for the same ligand. Among all the catalysts **3a** is the most efficient and its stability is superior.

Table 1 Styrene epoxidation catalyzed by metal complexes of **3**, **2** and **1**

	Conversion of styrene (%)			Selectivity to epoxide (%)		
Catalyst	60	90	150 (min)	60	90	150 (min)
3a	44.8	54.5	68.9	99.8	99.8	99.7
3b	2.6	3.8	4.5	96.2	96.0	95.8
3c	1.7	2.0	2.6	94.8	93.1	88.8
3d	1.5	1.9	2.7	93.4	93.3	92.0
2a	31.1	48.6	58.3	99.7	99.5	96.8
2b	6.5	9.4	12.6	53.2	54.0	49.0
2c	1.3	2.3	3.1	80.9	59.7	57.8
2d	1.5	1.8	2.3	94.6	92.9	92.1
1a	5.4	11.1	12.9	97.0	97.0	96.9
1b	0.06	0.95	2.8	0	88.8	60.4
1c	0.06	0.12	1.6	0	0	44
1d	0.04	0.18	0.20	0	0	0

Conditions: styrene, 1.1 mmol; [cat] = 6.8×10^{-3} mmol; NaOCl, 1.0 mL (0.32 mol/L); PTC, 0.018 mmol; solvent, CH_2Cl_2 (4 mL); Temp., 20 ± 0.5℃.

By comparison of the structures of compounds **3**, **2** and **1**, it reveals that the catalytic property of steroid-metalloporphyrin is more effective than the corresponding metalloporphyrin, which might be due to the hydrophobic action of steroid framework.[8,17] The catalytic property of steroid-bismetalloporphyrin with two porphyrin rings is higher than that of the corresponding steroid-metalloporphyrin with one porphyrin. This phenomenon could be rationalized by the cooperation of the two metallophyrins.[2,6]

The nature of the metal has important influence on the catalytic properties of porphyrin complexes. The factors involved whereby may be very complicate. It possibly concerns with the formation of Π-feedback bonding between d-electron and ligand as well as stabilizing energy of complex.[18,19]

Study of the stability of model catalysts

In order to study the stability of model catalyst, the catalyst **3a** was recovered and reused for three times (see Table 2). The result showed that catalytic activity of **3a** had been slightly decreased. The structure of **3a** was unchanged via UV-Vis and/or ^{1}H NMR spectroscopy examination whereas the simple metalloporphyrin was demolished after being used once. The oxidative demolition is a major drawback of general simple metallo-tetraarylporphyrin[20] and the linkage of some porphyrin dimers is also subjected to breakage under operating conditions.[4] It is obvious that the stability of steroid-bisporphyrin catalyst is superior because of the existence of steroid.

Table 2 The result for model **3a** reused in epoxidation

Reused time	Conversion of styrene (%)	Selectivity to epoxide (%)
1	99.4	99.7
2	96.4	99.7
3	94.6	99.7

Condition: axial ligand (imidazole): 0.36 mmol, Time: 300 min, other conditions are as given in Table 1.

Study of the concerted action

In order to examine the concerted action of bismetalloporphyrin in catalysis, the reactions were repeated by varying the amount of **3a** and **2a**. It has been found that **3a** is much better than **2a** (Table 3). This fact demonstrates that two matelloporphyrins which are in appropriate arrangement in the model **3a** can act in cooperation in catalysis.

Table 3 Influence of the amounts of catalyst **3a** or **2a** on the catalytic behavior

No.	Catalyst	Mole number (mmol)	Conversion of styrene (%)	Selectivity to epoxide (%)
1	**3a**	6.80×10^{-3}	99.4	99.7
2	**3a**	1.96×10^{-3}	95.0	99.0
3	**2a**	6.8×10^{-3}	64.7	93.5

Conditions: axial ligand (imidazole): 0.36 mmol; reaction time: 300 min; Other conditions are as given in Table 1.

Influence of axial ligands

Several different heterocycles as axial ligand were added to the styrene epoxidation catalyzed by **3a**/NaOCl system so as to examine the effect upon the epoxidation reaction. The results (see Table 4) indicated that three axial ligands all could significantly affect the catalytic activity. The order of auxocatalysis is : Im > Py > Tri-phIm. Such a sequence is consistent with the decreasing electron-donating ability. Generally the stronger the electron donating ability of the axial ligand, the more effective the auxocatalysis.[21] Since the electron density of five-membered heterocyclic rings is higher than that of the six-membered rings, so the electron donating ability of imidazole is stronger than that of the pyridine. It is noteworthy that when sterically-hindered 2, 4, 5-triphenylimidazole is used as axial ligand, the steric interference may become predominant action.

Table 4 Influenece of axial ligands an epoxidation of styrene

No.	Axial ligand	Conversion of styrene (%)	Selectivity to epoxide (%)
1	--	72.1	99.7
2	Im	99.4	99.7
3	Py	93.3	99.7
4	Triph-Im	79.7	99.6

Condition: axial ligand: 0.136 mmol; Time: 300 min; Other conditions are as given in Table 1.

Abbreviation: Im, imidazole; Py, pyridine; Tri-phIm = 2,4,5-triphenylimidazole.

There are two parameters that enhance the catalysis, namely electronic factor that affects electron back-feed and that promotes the high valence oxomanganese

complex intermediate forming. Secondly, the steric interaction of axial ligand that prevents the two porphyrin moieties from forming μ-oxodimer.[21,22]

It is obvious that the coordination of axial bases with metal ions is related to both electronic effect and steric interference.

Conclusion

1. A novel type of steroid-bismetalloporphyrin model compound has been synthesized.

2. The catalytic properties of steroid-bismetalloporphyrin towards epoxidation of styrene have been investigated. Comparison of catalytic performance demonstrates that the catalytic property of steroid-porphyrin metal complexes decreases generally in the following order: steroid-bismetalloporphyrin > steroid-metalloporphyrin > corresponding simple metalloporphyrin. Steroid-bisporphyrin mangnese(III) complex is the most efficient among all the catalysts and its stability is superior. The excellent catalytic property of model compounds may be attributed to the hydrophobic action of steroid framework and the cooperation action of two metalloporphyrins in catalysis.

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