Aromatization of Tetralone Derivatives by $Fe^{III}PFP(Cl)/PhIO$ and Cytochrome P-450*cam*: A Model Study on Aromatase Cytochrome P-450 Reaction

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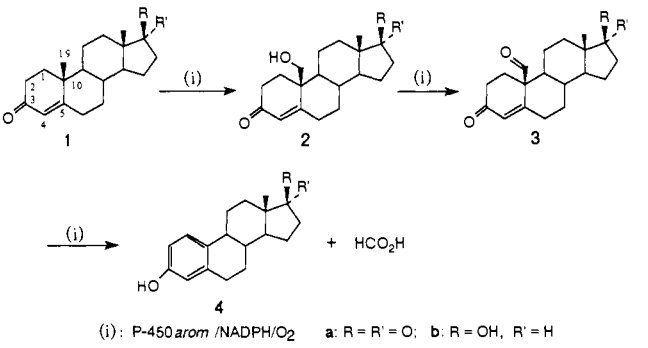
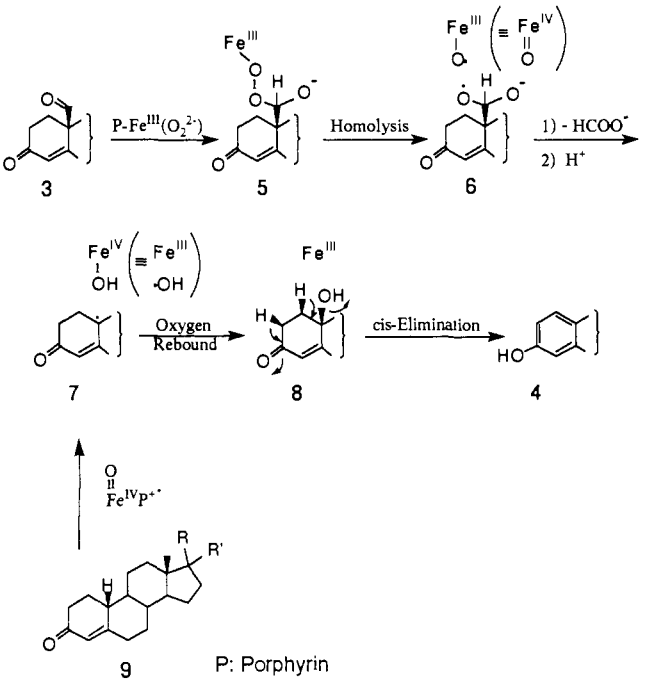
Aromatase cytochrome P-450 (P-450*arom*) is responsible for the biosynthesis of the female sex hormone, i.e., the transformation of androgens (**1**) to estrogens (**4**), at the expense of 3 mol each of NADPH and O_2 according to the stepwise reaction shown in Scheme I.[1] The reaction is initiated by C-19 hydroxylation of **1**, and subsequent oxidation gives the C-19 oxo intermediate (**3**).[2] The final step in the reaction is the oxidation of **3** yielding **4** and formic acid. For this unique reaction, several mechanisms including an alkylperoxo-iron(III) complex[3] (**5**) and 2β-hydroxide[4] intermediates have been proposed for the transformation of **3** to **4**. Meanwhile, recent studies on the aromatase reaction have demonstrated that 19-norandrogen (**9**) is aromatized by a reconstituted enzyme system with purified human placental P-450*arom*.[5] These observations have led us to postulate the formation of common intermediates (**7**, **8**) in the aromatase reactions (Scheme II).

The proposed reaction mechanism begins with addition of the peroxo-iron intermediate of cytochrome P-450 $[Fe^{III}(O_2^{2-})]$[6] to the C-19 carbonyl of **3** followed by homolytic O-O bond cleavage of **5** to **6**. The reactions of peroxo complexes of metalloporphyrins with electrophiles such as acid halides and CO_2 have been observed.[7] The homolytic O-O bond cleavage of alkyl (acyl) peroxo-metalloporphyrin complexes has also been reported.[7b,8] Similar to the decarboxylation of acyloxyl radicals,[9] release of formate yields the C-10 radical intermediate (**7**) which is then captured by the oxygen bound to the heme iron to produce the C-10 hydroxide (**8**).[8] Regiospecific and stereospecific oxidation of the C-19 hydrogens of **1** by P-450*arom*[1,2] is suggestive of the C-10 hydrogen of **9** to be also very close to the active center.[7a] Furthermore, the C-10 position seems chemically more reactive than others, since it is allylic and tertiary. Thus, when **9** is the substrate for P-450*arom*, the oxidation could afford **8**. Stereospecific enolization of the C-3 ketone with 2β-hydrogen and 1,10-cis-dehydration of **8**[10] eventually give **4**.

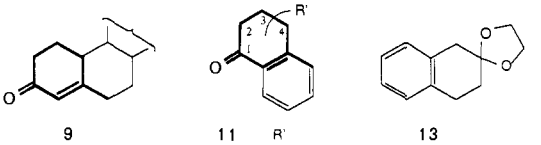
Scheme I



Scheme II



In order to examine the above postulate, i.e., the intermediacy of **8** in the aromatization reaction, we have employed a $Fe^{III}PFP(Cl)$/iodosobenzene/tetralone derivatives (**11**) system as a simple model of P-450*arom*/NADPH/19-nortestosterone. As shown below, **11** shares the critical structure for aromatization with **9**.



Hydroxylation of tetralone derivatives by the model system: In a typical run, oxidation of 4-methyl-1-tetralone (**11a**, 160 mg, 1 mmol) in 3 mL of CH_2Cl_2 took place with aliquot addition of PhIO (total 2-4 equiv) in the presence of a catalytic amount of 5,10,15,20-(tetrakis(pentafluorophenyl)porphyrinato)iron(III) chloride ($Fe^{III}PFP(Cl)$) (3 mg, 2.8 μmol) at room temperature for a few hours. The corresponding 4-hydroxy derivative (**12a**),

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(8) The homolytic O-O bond cleavage of an (acylperoxo)-$Fe^{III}TMP$ and an (acylperoxo)-$Mn^{III}TMP$ gives the corresponding alkyl alcohols after decarboxylation; ref 7b and Groves, J. T.; Watanabe, Y. *J. Am. Chem. Soc.* **1986**, *108*, 7836-7837.

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Table I. Oxidation Products of Tetralone Derivatives

substrate	oxidation system	product(s) yield,[a] %			total conversion,[b] %
			4-hydroxide	4-one	
11a	$Fe^{III}PFP(Cl)/PhIO$	**12a**	17		
	$Fe^{III}TPP(Cl)/PhIO$	**12a**	1.2		
	P-450*cam*/NADH/O_2	**12a**	(11.5)[c]		
11b	$Fe^{III}PFP(Cl)/PhIO$	**12b**	13	2.2	4.0
		12b	10	5.8	22
		12b	5.7	8.4	36
11c	$Fe^{III}PFP(Cl)/PhIO$	**12c**	11	5.9	25
		12c	8.5	9.3	36
13	$Fe^{III}PFP(Cl)/PhIO$	**14**	7.6		

[a] Yields based on PhIO used were determined by GLC. [b] Total conversion of substrate to products (4-hydroxide + 4-one). [c] Turnover number (product mol/P-450 mol/min).

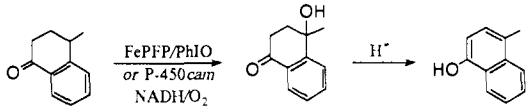
Table II. Oxidation of 1-Tetralone Trimethylsilyl Enol Ethers

substrate	oxidation system	products,[a] %	
15a	$Fe^{III}PFP(Cl)/PhIO$	**16a**, 8.9	**17a**, 27
15b	$Fe^{III}PFP(Cl)/PhIO$	**16b**, 39	**17b**, 13
15b	P-450cam/NADH/O_2	**16b**, 65[c]	**17b**, 35[c] (10)[b]

[a] Yields based on PhIO used were determined by GLC. [b] Overall turnover number (products mol/P-450/min). [c] Ratio between **16b** and **17b**.

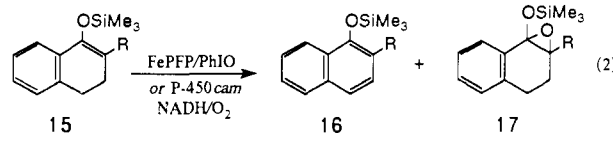
equivalent to **8**, was isolated by column chromatography ($SiO_2/CHCl_3$ and AcOEt) as the sole product. The structure of **12a** was determined by ^1H NMR and mass spectroscopy.[11] Representative results of the oxidation are summarized in Table I.

As shown, only the benzylic position (C-4) of tetralones is reactive for the hydroxylation. The oxidation of **11a** by a reconstituted system with purified cytochrome P-450*cam*[12] (P-450*cam*/*putidaredoxin*/*putidaredoxin reductase*/NADH/O_2) in phosphate buffer (pH 7.4, 0.2 M) at room temperature also afforded **12a** with a turnover number of 11.5 per min. The smaller turnover number[12] suggests that **11a** is not a good substrate for P-450*cam* and binding of **11a** in the active site of the enzyme is not as tight as that for *d*-camphor. While **12**'s are stable upon addition of weak acids such as 6 N HCl (aq) and trifluoroacetic acid, treatment of **12a** in methylene chloride with 12 N HCl gives 4-methyl-1-naphthol in ca. 30% yield with some other unidentified products. When 2-tetralone ethylene ketal (**13**) was oxidized by the model system, the 4-hydroxy derivative (**14**) was obtained (Table I). Hydrolysis of **14** with 1-3 N HCl (aq) readily affords β-naphthol. These results are consistent with the proposed mechanism for the aromatization of **9**.



11a **12a** (1)

Oxidation of 1-tetralone trimethylsilyl enol ethers: We have further prepared 1-tetralone trimethylsilyl enol ethers (**15**)[13] to compare the reactivity of **11** with the corresponding enolates. Oxidation of **15a** by the PhIO/$Fe^{III}PFP$ system directly affords aromatized product, **16a**, along with **17a**.[14] Introduction of methyl group at the C-2 position (**15b**) suppresses epoxide formation[15] (Table II). In these reactions, no hydroxylated products were observed. The oxidation of **15b** was also carried out with the P-450*cam* system and the reaction products were obtained as shown in Table II. Cole and Robinson have recently reported importance of an enolate in the aromatization reaction of 19-alkyl peroxo derivatives of testosterone.[16]



15 **16** **17** (2)

a: R = H; b: R = Me

Recent X-ray crystal structure of P-450*cam* has shown that the C-5 methylene of *d*-camphor is fixed right above the heme iron by hydrogen bonding interaction between Tyr 96 and carbonyl oxygen of *d*-camphor.[17] The active site structure of P-450*arom* has not been delineated yet; however, similar interaction between the C-3 carbonyl oxygen of **1** and amino acid residues such as Tyr and His in the active site would allow the regiospecific oxidation of the C-19 hydrogens. The hydrogen bonding interaction may favor keto-enol equilibrium toward the enol side of the steroid. Once the C-1 position of **8** becomes allylic, dehydration will proceed smoothly to yield **4** as observed in the model compounds.

Finally, the reaction of **3a** and a model complex of peroxo-iron(III) intermediate of P-450, $Fe^{III}PFP(O_2^{2-})$, was found to produce **9a** and an unidentified product (**10**) as major products accompanied by a small amount of **4a**.[18] The structure of **10** is not clear yet; however, that the treatment of the reaction mixture with 3 N HCl (aq) readily gave **4a** is indicative of the structure of **10** being **8a**.[19] Isolation and characterization of **10** is currently under investigation.

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(11) ^1H NMR ($CDCl_3$) for **4**-Me 1.63 ppm (3 H, s); *m/e* 176 (M^+), 161 (base), 148, 105. The other products and synthesized compounds in this paper gave satisfactory ^1H NMR and mass spectroscopic data.

(12) Cytochrome P-450*cam*: cytochrome P-450 isolated from the bacterium *Pseudomonas putida*. It catalyzes oxidation of *d*-camphor to the 5-*exo*-hydroxy derivative with a turnover number of ca. 1000/min; Tyson, C. A.; Lipscomb, J. D.; Gunsalus, I. C. *J. Biol. Chem.* **1972**, *247*, 5777-5784.

(13) **11b** and **11c** were treated with LDA in THF at -78 °C followed by the addition of trimethylsilyl chloride to afford **15a** and **15b**.

(14) Authentic samples were synthesized as follows. Oxidation of **15** by mCPBA in the presence of $NaHCO_3$ (powder) was carried out in CH_2Cl_2 at room temperature and **17** was isolated in quantitative yield. **16** was prepared by the reaction of lithium α-naphtholate and trimethylsilyl chloride in THF at -78 °C.

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(18) $Fe^{III}PFP(O_2^{2-})$ [λ_{max}, nm: 445 (Soret), 555, 586] was prepared by the reaction of $Fe^{III}PFP(Cl)$ and KO_2 in the presence of 18-crown-6 (less than 0.8 equiv to FePFP to avoid contamination of free O_2^-) in acetonitrile according to Valentine's method: McCandlish, E.; Miksztal, A. R.; Nappa, M.; Sprenger, A. Q.; Valentine, J. S.; Stong, J. D.; Spiro, T. G. *J. Am. Chem. Soc.* **1980**, *102*, 4268-4271. The ratio of **9a**/**10** is 0.5-2, depending on the reaction condition.

(19) That the retention time of **10** is shorter than that of **3a** on GLC also indicates the loss of 19-oxo group by the reaction.

β-Hydroxyalkyl σ-Metalloporphyrins: Models for Epoxide and Alkene Generation from Cytochrome P-450

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The mechanism by which cytochrome P-450 mediated alkane hydroxylation occurs is now well understood,[1] but there are still

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