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Biomimetic Hydroxylation using Oxo-iron Systems: Correlation between the Nature of the Active Species and Distribution of the Oxidized Products

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Biomimetic hydroxylation using several oxo-iron systems was applied to bencyclane (I) in order to examine the correlation between the nature of the active species and the distribution of the oxidized products.

Aromatic hydroxylation was found to occur predominantly in the ferrous ion- H_2O_2 system containing a ligand such as phenol or catechol, whereas regioselective aliphatic hydroxylation was observed in the ferrous ion- H_2O_2 system with detergent or cyclodextrin. The ferrous ion- H_2O_2 system with cyclodextrin was found to produce the bencyclane cycloheptane ring-hydroxylated metabolite (II γ -*cis*) with fairly good regio- and stereoselectivity.

Keywords—biomimetic hydroxylation; bencyclane; oxo-iron system; cytochrome P-450; aromatic hydroxylation; aliphatic hydroxylation; regio- and stereoselectivity

Studies on biomimetic hydroxylation have contributed to the understanding of oxygenase-catalyzed reactions by providing valuable mechanistic concepts for enzymologists, and have also contributed to synthetic organic chemistry by providing useful synthetic methods. Furthermore such studies are particularly important in relation to drug metabolism, since hydroxylation is one of the principal metabolic pathways of drugs. Thus, such studies might also be helpful to medicinal chemists in predicting of the structure of metabolites and in synthesizing them for pharmacological evaluation.

A number of oxo-iron species have been extensively studied as biomimetic model systems which mimic aliphatic or aromatic enzymatic hydroxylations observed with cytochrome P-450. Reported studies, however, have mostly been concerned with basic aspects of the hydroxylation using relatively simple molecules such as cyclohexane, aniline and toluene as substrates.¹⁾ Thus, little information has hitherto been available concerning the applicability of biomimetic hydroxylation to medical drugs with more complex structures. Thus, we have attempted to investigate the applicability of these biomimetic hydroxylations to medical drugs. For

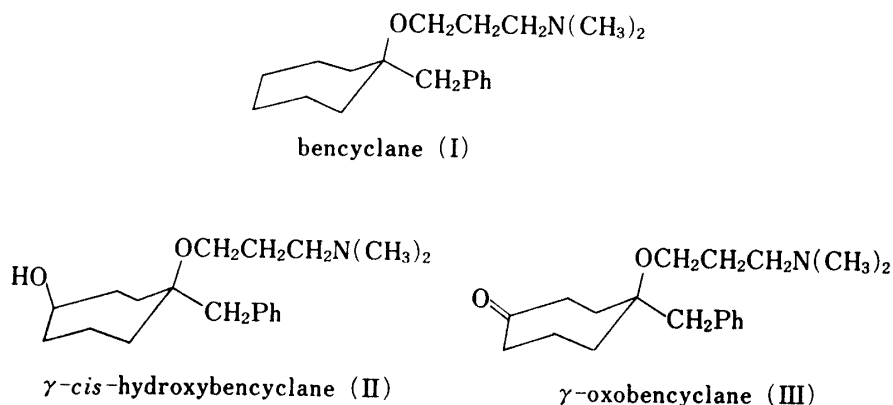


Chart 1

this study, we chose bencyclane (I), a clinically useful vasodilator,²⁾ as a model substrate since we had already found in our previous work that the main metabolites of I were cycloheptane ring-oxygenated products of I, namely, the γ -*cis*-hydroxy product (II) and the γ -oxo product (III).^{3,4)} Furthermore, the structure of bencyclane is such that it should be suitable as a model substrate to examine the selectivity between aromatic and aliphatic hydroxylation.

Here we wish to report the correlation between the nature of the active species in several oxo-iron systems and the distribution of oxidized products of I, and we discuss the regio- and stereoselectivity of these biomimetic hydroxylations.

Results and Discussion

Oxidizing Systems and Determination of Oxidized Products

Cytochrome P-450 consists of ferric ion, ligand and protein moiety, and therefore in this study three types of oxidizing systems were examined: 1) oxo-iron systems without ligands, 2) oxo-iron systems with several ligands and, 3) oxo-iron systems with detergent and cyclodextrin.

As oxidizing systems without ligands, the following four systems were chosen: System A is known as Fenton's reagent, which is reported to generate the hydroxy radical.^{1,5)} System B is Fenton's reagent in a less aqueous medium, in which the active species was reported by Groves *et al.*^{5,6)} to be the ferryl ion. System C is one of the systems having ferric ion instead of ferrous ion, and system D is considered to be a source of the hydroxy cation.^{1,7)}

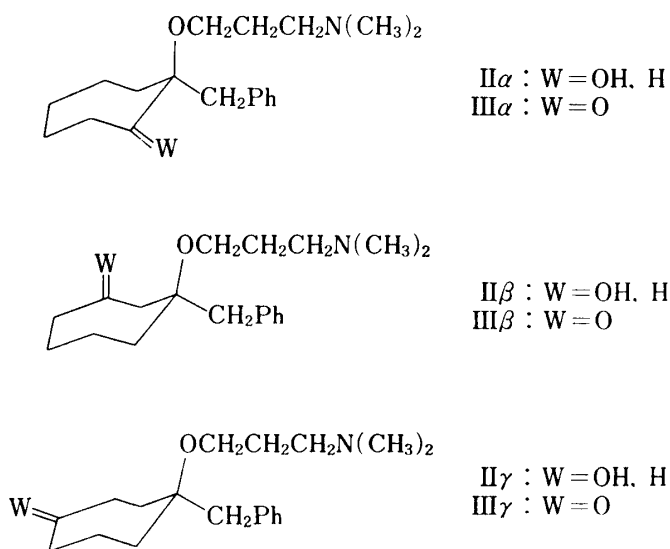


Chart 2

Oxidation of I in one of the above systems was carried out according to the reported procedures with some modifications,^{5,7)} and the whole reaction product was subjected to gas chromatographic analysis in order to determine the distribution of oxidized products. Assignment of the peaks in the gas chromatogram of oxidized products could be done by comparing the peaks with those of authentic samples as well as by GC-MS analysis. The quantification of the oxidized products was carried out by the normalization method of GC. Since the peaks of geometrical isomers (*cis* and *trans*) of α -, β - or γ -hydroxybencyclane were indistin-

guishable in GC due to their similar retention times, α -, β -, and γ -hydroxybencyclane were each determined as a *cis-trans* isomeric mixture. The *cis/trans* ratio of γ -hydroxybencyclane was determined by high performanic liquid chromatography (HPLC) analysis.

Synthesis of the authentic samples, cycloheptane ring-oxidized derivatives (II α , II β , II γ , III α , III β , III γ) was reported previously,⁴⁾ and three phenyl ring-hydroxylated derivatives were synthesized as follows. Benzyloxybenzyl chloride (V), prepared by the chlorination of benzyloxybenzyl alcohol, was converted to 1-(benzyloxy)benzylcycloheptanol (VI) by Grignard reaction with cycloheptanone, and VI was then alkylated with an excess of 3-(*N,N*-dimethylamino)propyl chloride in the presence of sodium hydride to give the dimethylamino-propyl ether (VII). Debenzylation of VII by catalytic hydrogenation gave the hydroxylated bencyclane (IV).

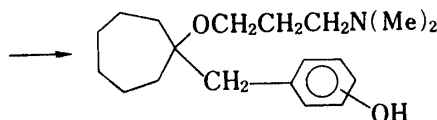
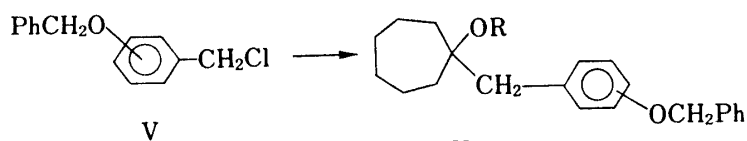


Chart 3

TABLE I. Product Distribution in Hydroxy Cation, Hydroxy Radical and Ferryl Ion Systems

Reaction conditions		Relative ratio ^{a)}								Yield ^{b)} (%) II γ +III γ
		II+III			IV			$\frac{\text{II}\gamma}{\text{III}\gamma}$	$\frac{\text{IVp}}{\text{II}\gamma+\text{III}\gamma}$	
		α	β	γ	o	m	p			
A	Fe(ClO ₄) ₂ 20% CH ₃ CN 20—30 °C	1	6	17.3	<1	<1	<1	>5	<0.1	4.0
B	Fe(ClO ₄) ₂ 90% CH ₃ CN -20—-30 °C	1	5.4	12.8	<1	<1	<1	≐ 1	<0.1	12.8
C	Fe(ClO ₄) ₃ 20% CH ₃ CN 20—30 °C	1	4.4	9.1	<1	<1	<1	≐ 0.4	<0.1	1.1
D	K ₄ Fe(CN) ₆ 20% CH ₃ CN 20—30 °C	1	2.8	9.8	<21	5.6	10	≐ 0.2	1.0	0.3

a) Values indicate amounts of oxidative product relative to that of a-oxo- and a-hydroxybicyclicane (II α +III α). b) Yield based on bicyclicane. In all cases, hydrogen peroxide was used as the oxidizing agent.

Comparative Study of Systems A, B, C and D

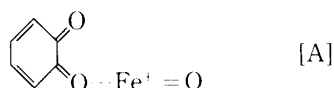
The results obtained in the four systems (A, B, C and D) are summarized in Table I.

In the case of systems A, B and C, aliphatic hydroxylation similar to metabolism in man occurred preferentially, whereas such aliphatic preference was less in the case of system D. On the other hand, large differences in the (II γ /III γ) ratio were observed among these systems, and in the case of systems C and D the formation of oxo-bicyclicane (III) occurred predominantly. Although it is rather difficult to correlate the variation of oxidized products with the nature of these oxidizing systems, the difference in these results suggests that the active species involved were different.

Since the ferryl ion is now generally accepted as the most probable active species in enzymatic hydroxylation,⁶⁾ system B, which is reported to generate the ferryl ion, is thought to be the most biomimetic one. However, systems A and B both afforded almost the same regioselectivity, although system A has been claimed to generate the hydroxy radical as its active species.

Effect of Several Ligands

In comparison with the enzymatic systems, systems A, B and C lack a ligand, such as the porphin ring and the thiolate anion, which are present in cytochrome P-450. Many hydroxylation systems using several ligands have already been reported. For example, fairly simple molecules such as phenol, catechol and phenylenediamine were used as ligands in previous studies. Thus, Hamilton *et al.*⁸⁾ have reported the effect of catechol on aromatic hydroxylation using the Fe^{3+} /catechol/ H_2O_2 system. They suggested a mechanism involving a complex (A) which is a radical-like reagent.



They also reported that phenol or resorcinol showed no catalytic activity.

On the other hand, Mimoun and de Roch⁹⁾ have shown that a modified Udenfriend system consisting of FeCl_2 , hydrazobenzene (or *o*-phenylenediamine), benzoic acid and oxygen, can oxidize hydrocarbons such as cyclohexane, giving cyclohexanol and cyclohexanone, and toluene, giving benzylalcohol and cresol. They suggested a mechanism involving Fe^{3+}OOH as the active species. In spite of extensive studies on hydroxylation model systems with ligands, little is known about the effect of the ligand on the selectivity between aromatic and alicyclic

TABLE II. Effect of Various Ligands on the Product Distribution.

		II γ +III γ	IVo	IVm	IVp	Yield (%)
A ^{a)}	$\text{Fe}(\text{ClO}_4)_2$, 20% CH_3CN 20–30 °C	1	<0.1	<0.1	<0.1	4.0 II γ +III γ
E ^{b)}	A+catechol	0	1.1	0.4	1	0.6 IVp
F ^{b)}	A+phenol	0	1.5	0.6	1	0.5 IVp
G ^{b)}	A+phenylenediamine	0	1.1	0.5	1	1.1 IVp
H ^{b,c)}	A+cysteine	0.2	/	1.2	1	0.3 IVp
I ^{b)}	A+thiophenol	0	6	2.5	1	0.2 IVp
B ^{a)}	$\text{Fe}(\text{ClO}_4)_2$, 90% CH_3CN	1	<0.1	<0.1	<0.1	12.8 II γ +III γ
J ^{b)}	B+phenol	3.5	<1.8	0.7	1	0.8 IVp
K ^{b)}	B+catechol	0	1.9	0.4	1	3.4 IVp
C ^{a)}	$\text{Fe}(\text{ClO}_4)_3$, 20% CH_3CN	1	<0.1	<0.1	<0.1	1.1 II γ +III γ
L ^{b)}	C+phenol	0	1.4	0.5	1	0.8 II γ +III γ
M ^{a,c)}	A+2, 6-di <i>tert</i> -butylphenol	1	/	<0.1	<0.1	4.4 II γ +III γ

a) Relative ratio to γ -oxo- and γ -hydroxybicyclane (II γ +III γ).

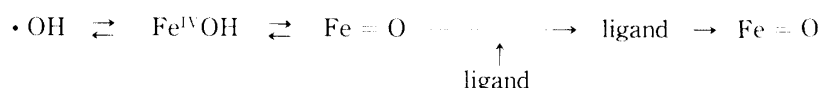
b) Relative ratio to *para*-hydroxybicyclane (IVp).

c) *ortho*-Hydroxybicyclane could not be determined exactly because of the formation of the other products. In all cases, hydrogen peroxide was used as the oxidizing agent.

hydroxylations. Thus, we have studied the effect of several ligands on the selectivity of hydroxylation, and the results are shown in Table II.

When phenol or catechol (equimolecular) was added to system A, the selectivity between aliphatic and aromatic hydroxylation changed greatly, leading to predominant aromatic hydroxylation, as shown in Table II, E or F. The same ligand effect was observed in the

case of systems G, H, and I, having phenylenediamine, cysteine and thiophenol, respectively. When phenol was added to system B, aromatic preference was also observed, but its selectivity in system B (less aqueous medium) was lower than that in system A (aqueous medium). On the other hand, the effect of catechol, a divalent ligand, was almost the same in systems A and B. This difference between phenol and catechol could be explained by an inadequate coordination of phenol in the less aqueous medium. A sterically hindered phenol, 2,6-di *tert*-butylphenol, did not accelerate the aromatic hydroxylation. From these effects of the ligand on selectivity, we assumed that the predominant aromatic hydroxylation might be due to the stabilization of the ferryl ion by ligands such as phenol or catechol. That is, the addition of ligands to systems A and B would result in the formation of a stable ligandferryl ion complex with an electrophilic property, leading to predominantly aromatic hydroxylation.



We next examined the selectivity in the system containing a porphin ring as the ligand, and the results are shown in Table III.

Hemin System

In the TPPFeCl-PhIO system (R)¹⁰⁾ or hemin iodosobenzene system (Q), hydroxylated products could not be detected. In these systems, *N*-demethylation might occur in preference to aliphatic or aromatic hydroxylation owing to the neutral nature of the reaction systems. On the other hand, hydroxylation was observed in Sakurai's hemin-cysteine system (N)¹¹⁾ (a system closer to cytochrome P-450), in the hemin-ascorbic acid system (P) and in the TPPFeCl-cysteine system (O). Although the selectivity was not satisfactory, preferential aromatic hydroxylation was observed in these hemin systems in contrast to system A or B, and this result seems to give additional support to the assumption that the stabilization of the ferryl ion by ligands should result in predominant aromatic hydroxylation.

TABLE III. Product Distribution in Hemin Systems

		II γ +III $\gamma^{a)}$	IV $^{a)}$			$\frac{\text{II}\gamma}{\text{III}\gamma}$	Yield (%) II γ +III γ
			o	m	p		
N	Hemin-Cys, 80% acetone 50—60 °C	1	0.9	0.4	1.0	<<1	0.07
O	TPPFeCl-Cys, 80% acetone 50—60 °C	1	0.6	0.1	0.3	<<1	0.11
P	Hemin-ascorbic acid 50—60 °C, acetone	1	<1.0	0.3	0.6	<1	0.18
Q $^{b)}$	Hemin-PhIO, CH ₂ Cl ₂ 20—30 °C	—	—	—	—	—	—
R $^{b)}$	TPPFeCl-PhOI, CH ₂ Cl ₂ 20—30 °C	—	—	—	—	—	—

a) Relative ratio to γ -oxo- and γ -hydroxybicyclane (II γ +III γ).

b) Hydroxylated products could not be determined. Hemin: protoporphin IX chloride, Cys, cysteine; PhIO, iodosobenzene; TPPFeCl, chloro iron (III) tetraphenylporphin. In systems N, O and P, molecular oxygen was used as the oxidizing agent.

Detergent (Micellar) and Cyclodextrin Systems

The oxidizing systems of the ferryl ion with ligands are expected to be the most similar to that of P-450, but the results obtained in E, F, J, K or hemin systems were not in accord with those obtained in the metabolism of bicyclane in man. Thus, we assumed that some factors other than the nature of the active species might also influence the selectivity of hydroxylation.

Next, we examined the effect of the addition of cyclodextrin or surface-active agents, the favorable effect of which had been reported in several biomimetic reaction systems.¹²⁾

TABLE IV. The Effect of Detergent or Cyclodextrin on the Product Distribution

		$\frac{\text{II}+\text{III}}{\beta \quad \gamma}$		$\frac{\text{IV}}{o \quad m \quad p}$			$\frac{\text{II}\gamma}{\text{III}\gamma}$	Yield (%)
A ^{a)}	Fe(ClO ₄) ₂ 20% CH ₃ CN 20–30 °C	0.35	1	<0.1	<0.1	<0.1	>5	4.0 II γ +III γ
S ^{a)}	A+CTMABr	0.13	1	<0.1	<0.1	<0.1	<<1	1.2 II γ +III γ
F ^{b)}	A+Phenol	0	0	1.5	0.6	1.0	—	0.5 IV _p
T ^{a)}	F+CTMABr	^{c)}	0.4	<0.1	0.4	1.0	$\approx$ 1	0.5 IV _p
U ^{c)}	A+LAS	—	—	—	—	—	—	—
V ^{a)}	A + β -CD	0.2	1	<0.1	0.1	0.2	$\approx$ 1	13.7 II γ +III γ

a) Relative ratio to γ -oxo- and γ -hydroxybicyclic (II γ +III γ).

b) Relative ratio to *para*-hydroxybicyclic (IV_p).¹⁾ Hydroxylated product was not detected.

c) β -Hydroxybicyclic could not be determined exactly because of interference by CTMABr.
In all cases, hydrogen peroxide was used as the oxidizing agent.

It is of interest to note that predominant aliphatic hydroxylation with fairly good regioselectivity (γ -orientation) was observed in the micellar system. That is, in the presence of CTMABr (cetyl trimethyl ammonium bromide), γ -oxo-bicyclic was the main product, with fairly good regioselectivity (Table IV, S). In the case of system T, some aliphatic hydroxylation was induced by addition of CTMABr to system F, in which aliphatic hydroxylation did not occur. In the system containing LAS (sodium lauryl sulfate) however, the reactivity was greatly suppressed.

The increase of aliphatic hydroxylation in the micellar systems (S) and (T) might be explained by the assumption of incorporation of the phenyl ring of bicyclic into the micelles. Should this be the case, this system would be a good model for the binding of substrate in a biological system.

The β -cyclodextrin system (V) might also be considered as a model system which mimics the binding of substrate at the protein site of cytochrome P-450. When β -cyclodextrin was added to system A, fairly regioselective aliphatic hydroxylation in good yield (13.7%) was observed, a result which could be explained by incorporation of the phenyl ring of bicyclic

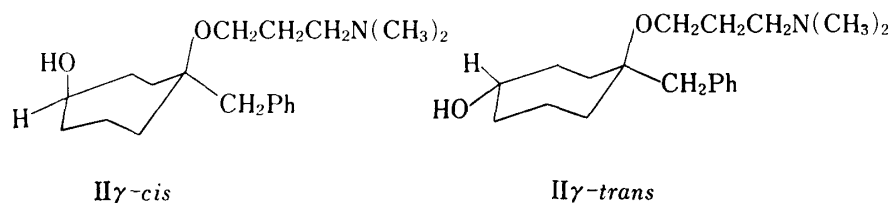


Chart 4

TABLE V. Stereo-selectivity in Cyclodextrin Systems

			II γ <i>cis</i>	II γ <i>trans</i>	Yield (%) II γ +III γ	
A	Fe (ClO ₄) ₂	20% CH ₃ CN (25 ml)	1	:	1	4.0
V	A+ β -CD	20% CH ₃ CN (25 ml)	1.5	:	1	13.7
W	A+ β -CD	H ₂ O (15 ml)	6—7	:	1	17.6

In all cases, hydrogen peroxide was used as the oxidizing agent.

into the cavity of β -cyclodextrin.¹³⁾ In this system, however, stereoselectivity was found to be unsatisfactory ($\text{II}\gamma\text{-cis}:\text{II}\gamma\text{-trans}=3:2$). Thus, in order to complete the incorporation of bencyclane into the cavity of β -cyclodextrin, the solvent was changed from 20% aqueous acetonitrile (25 ml) to water (150 ml). In this dilute system, regio- and stereoselective aliphatic hydroxylation at the γ -position (α - and β -hydroxylated products could not be detected) in good yield (17.6%) was observed, leading to the target bencyclane metabolite ($\text{II}\gamma\text{-cis}:\text{II}\gamma\text{-trans}=6\text{--}7:1$). The process thus developed was found to be an effective method for obtaining a bencyclane metabolite.

N-Demethylation of Bencyclane

In the reaction of bencyclane (I) with oxo-iron species, *N*-demethylation was expected to occur as well as the hydroxylation described above, since various trialkylamines have recently been shown to be *N*-demethylated by some biomimetic oxidizing systems (TPPFeCl-iodosoxylene, TPPFeCl-iodosobenzene).¹⁴⁾ However, no detectable amount of the demethylated bencyclane (VIII) was formed in the oxidizing systems studied here, except in the cases of systems Q and R (yields of VIII: Q, 0.3%; R, 1.4%). It can be assumed that the acidic nature of the systems in our study (except for systems Q and R) disfavored the *N*-demethylation of I.

Conclusion

In the present study, we have found that the ferryl ion with ligand, which is accepted as the most probable active species in enzymatic hydroxylation, has an electrophilic nature, leading to aromatic hydroxylation, and that regio- and stereoselective aliphatic hydroxylation similar to that occurring in man is induced by addition of β -cyclodextrin.

These results suggest that the regio- and stereoselectivity of metabolic hydroxylation could be controlled by the binding of the substrate at the protein site rather than by the nature of the oxo-iron species.

Experimental

Melting points were obtained on a Melt-temp apparatus and are uncorrected. Proton nuclear magnetic resonance (NMR) data were recorded in solution as noted on a Varian T-60 spectrometer with Me_4Si (δ 0.0) as an internal reference, and mass spectra (MS) were determined by using a Shimadzu LKB-9000 GC/MS system at 70 eV. Gas chromatography (GC) analysis was carried out with a Hitachi 163 gas chromatograph equipped with a hydrogen flame ionization detector. HPLC analysis was carried out with a Waters model 6000A machine. Bencyclane was extracted from bencyclane fumarate (Halidor) and chloro iron(III) tetraphenylporphyrin was synthesized according to a previously described procedure.¹⁵⁾ All other materials were reagent grade.

Oxidation Procedure—1) The general procedure for the hydroxylation in systems A, B, C and D is as follows. A mixture of bencyclane (I; 0.3 g, 1.0 mmol), the iron compound (3.5 mmol) as indicated in Table I, and 70% perchloric acid (0.543 g, 3.8 mmol) in 20 ml of 87.5% or 25% aqueous CH_3CN was placed in a reaction vessel and purged with nitrogen for 10 min. Next, 30% H_2O_2 (0.39 g, 3.4 mmol) was dissolved in 5 ml of water (in case of A, C or D) or in 5 ml of CH_3CN (in case of B), and the hydrogen peroxide solution was added to the metal-bencyclane solution in small amounts over 10 min with stirring and nitrogen purging. The mixture was stirred for 1 h, then 10% aqueous NaHSO_3 (10 ml) was added to the reaction mixture, immediately followed by 28% aqueous NH_4CH (5 ml). The resulting mixture was poured into saturated aqueous NaCl (200 ml) and extracted with EtOAc (200 ml). The EtOAc layer was washed with saturated aqueous NaCl, dried (Mg_2SO_4) and concentrated to yield an oil.

2) The procedure in systems E, F, G, H, I, J, K, L or M was similar to that used in systems A, B, C or D. A solution of bencyclane (I; 0.3 g, 1.0 mmol), the iron compound (3.5 mmol) and an equivalent amount (3.5 mmol) of ligand as indicated in Table II in aqueous CH_3CN (20 ml) was purged with nitrogen, and a solution of 30% H_2O_2 was then added over 10 min. The resulting mixture was stirred for 1 h, then worked up exactly according to the procedure described above.

3) Reaction in system N or O was conducted by a modification of a previously described procedure.¹¹⁾ A solution of hemin chloride (protoporphyrin IX chloride) (30 mg) [or chloro iron(III) tetraphenylporphyrin (30 mg)], L-cysteine (0.32 g, 2.0 mmol) and bencyclane (0.6 g, 2.1 mmol) in 80% aqueous CH_3COCH_3 (20 ml) was stirred for 2 h at 50–55°C. After cooling, 10% aqueous NaHSO_3 (10 ml) was added, followed by 28% aqueous NH_4OH (5 ml) with stirring at 20–30°C. The resulting mixture was poured into saturated aqueous NaCl and extracted with EtOAc (200 ml). The EtOAc layer was washed with saturated aqueous NaCl,

dried (MgSO_4) and concentrated to yield an oil.

4) The procedure in system P was similar to that described above (system N), except that L-cysteine was replaced by ascorbic acid (0.36 g, 2.0 mmol) and 70% perchloric acid (0.543 g, 3.8 mmol).

5) Reaction in system Q or R was conducted by a modification of a previously described procedure.¹⁰⁾ Iodosylbenzene (100 mg, 0.5 mmol) was added in portions to a solution of hemin chloride (30 mg) [or chloroiron(III) tetraphenylporphyrin (30 mg)] and benzocyclane (0.6 g, 2.1 mmol) in CH_2Cl_2 (2 g) with stirring under a nitrogen atmosphere at 20–30°C. After being stirred for 20 min at 20–30°C, the resulting mixture was worked up exactly according to the procedure described for system N or O.

6) The procedure in system S or T was similar to that described in system A or F, respectively, except for the addition of CTMABr (0.5 g, 1.4 mmol).

7) The procedure in system U was similar to that described in system A, except for the addition of LAS (1 g, 3.5 mmol).

8) The procedure in system V was similar to that described in system A, except for the addition of β -cyclodextrin (1.1 g, 1.0 mmol) instead of 70% perchloric acid.

9) The procedure in system W was similar to that described in system V, except for the replacement of 20% aqueous CH_3CN (25 ml) by H_2O (150 ml).

Analysis of Oxidation Products—The composition of the reaction mixture obtained in each reaction system was determined by GC analysis. When the hydroxyl compound (II) and oxo compound (III) were both formed, GC analysis was carried out after conventional NaBH_4 reduction of the crude mixture. The identification of oxidation products was conducted by GC or TLC comparison with authentic samples or by GC/MS analysis. GC analysis was carried out on three types of columns as follows: 1) 3% SE-30-glass column (300 $\times$ 0.3 cm) at 220°C; 2) 2% PEG-20M-glass column (50 $\times$ 0.3 cm) at 200°C; 3) 2% polyester F.F-glass column (200 $\times$ 0.3 cm) at 220°C. Retention times on 3% SE-30 column: II α (7.25 min), II β (7.95), II γ (8.81), III α (6.25), III γ (8.81), IVo (7.95), IVm (10.35), IVp (11.15). Retention times on 2% PEG-20M column: II α (3.48), II β (6.74, 8.27), II γ (9.15), III α (2.84), II β (6.74, 8.27), II γ (9.15), III α (2.84), III β (5.66), III γ (7.33), IVo (5.45), IVm (25.72), IVp (29.86). Retention times on 2% polyester F.F column: II α (15.72), II β (31.11, 38.31), II γ (46.97), III α (12.59), III β (26.93), III γ (34.12).

GC/MS analysis was carried out in the cases of systems B, D, E, S and V. III β : mass spectrum, m/z (rel intensity) 304 (1), 212 (18), 102 (100), 91 (14), 86 (58), 58 (68). III γ : 304 (1.5), 303 (0.4), 212 (36), 102 (100), 91 (16), 86 (62), 58 (84). II γ : 306 (0.6), 214 (38), 102 (100), 91 (17), 86 (72), 58 (59). IVo: 305 (5), 202 (15), 198 (4), 107 (48), 102 (74), 95 (26), 86 (60), 58 (100). IVm: 202 (12), 198 (4), 107 (34), 102 (100), 95 (29), 86 (60), 58 (100). IVp: 202 (9), 198 (11), 107 (46), 102 (50), 95 (11), 86 (100), 58 (74).

HPLC analysis was carried out on a Lichrosorb SI-60 (10) column using a 1:30:80:100 mixture of 28% aqueous NH_3 , MeOH, EtOAc and *n*-hexane as the eluant; retention time at 1 atm. II γ -*trans*, 12.37 min, II γ -*cis*, 15.84 min.

Synthesis of Phenyl Ring-hydroxylated Benzocyclane 4-Benzoyloxybenzyl Chloride (Vp)—A mixture of 4-benzoyloxybenzyl alcohol (10 g, 47 mmol) and thionyl chloride (10 ml, 137 mmol) in benzene (30 ml) was refluxed for 2.5 h. The solution was evaporated to dryness and the residue was crystallized from petroleum ether (20 ml) to yield 7.6 g (33 mmol, 70%) of Vp as slightly yellow crystals: mp 80.1–81.1°C, lit.¹⁶⁾ mp 79–80°C.

3-Benzoyloxybenzyl Chloride (Vm)—This compound was prepared from 3-benzoyloxybenzyl alcohol¹⁷⁾ by a procedure similar to that used to prepare Vp. The product was purified by silica gel column chromatography using ether as an eluant to give an 80% yield of viscous oil: $^1\text{H-NMR}$ (CCl_4) δ : 4.43 (s, 2H, CH_2Cl), 4.97 (s, 2H, CH_2O), 6.73–7.0 (m, 3H, aromatic), 7.07–7.47 (m, 6H, aromatic). *Anal.* Calcd for $\text{C}_{14}\text{H}_{13}\text{ClO}$: C, 72.25; H, 5.64; Cl, 15.24. Found: C, 72.61; H, 5.68; Cl, 14.99.

2-Benzoyloxybenzyl Chloride (Vo)—This compound was prepared from 2-benzoyloxybenzyl¹⁸⁾ alcohol by a procedure similar to that used to prepare Vp. The product was purified by silica gel column chromatography using ether as an eluant to give a 75% yield of viscous oil: $^1\text{H-NMR}$ (CCl_4) δ : 4.6 (s, 2H, CH_2Cl), 4.98 (s, 2H, CH_2O), 6.7–6.9 (t, 2H, aromatic), 7.03–7.47 (m, 7H, aromatic). *Anal.* Calcd for $\text{C}_{14}\text{H}_{13}\text{ClO}$: C, 72.25; H, 5.64; Cl, 15.24. Found: C, 72.67; H, 5.64; Cl, 14.64.

1-(4-Benzoyloxybenzyl)cycloheptanol (VIp)—A solution of Vp (4.6 g, 20 mmol) in THF (12 ml) was added to a stirred mixture of Mg (1 g, 41 mmol), ethyl iodide (0.03 ml) and a trace amount of iodine in THF (10 ml) at 0–10°C. The mixture was stirred for 45 min at 0–10°C, then a solution of cycloheptanone (2.2 g, 20 mmol) in THF (10 ml) was added over a period of 20 min at 10–15°C. After being refluxed for 30 min, the mixture was poured into saturated aqueous NH_4Cl and extracted with ether. The ether layer was washed with water, dried (MgSO_4) and concentrated, and the residual oil was chromatographed on silica gel using ether as an eluant to yield 2.8 g (9 mmol, 45%) of VIp as white crystals: mp 45–51°C; $^1\text{H-NMR}$ (CDCl_3) δ : 1.2–1.8 (br, 13H, cycloheptane ring, OH), 2.63 (s, 2H, CH_2Ph), 4.97 (s, 2H, OCH_2Ph), 6.7–7.5 (m, 9H, aromatic). *Anal.* Calcd for $\text{C}_{21}\text{H}_{26}\text{O}_2$: C, 81.23; H, 8.46. Found: C, 80.95; H, 8.34.

1-(3-Benzoyloxybenzyl)cycloheptanol (VIm)—This compound was prepared from Vm by a procedure similar to that used to prepare VIp. The product was purified by silica gel column chromatography using ether as an eluant to give a 16% yield of viscous oil: $^1\text{H-NMR}$ (CCl_4) δ : 1.2–1.8 (br, 13H, cycloheptane ring, OH), 2.58 (s, 2H, CH_2Ph), 4.90 (s, 2H, OCH_2Ph), 6.5–7.4 (m, 9H, aromatic). *Anal.* Calcd for $\text{C}_{21}\text{H}_{26}\text{O}_2$:

C, 81.23; H, 8.46. Found: C, 81.09; H, 8.55.

1-(2-Benzoyloxybenzyl)cycloheptanol (VIo)—This compound was prepared from Vo by a procedure similar to that used to prepare VIp. The product was purified by silica gel column chromatography using ether as an eluant to give an 18% yield of viscous oil: $^1\text{H-NMR}$ (CCl_4) δ : 1.2—1.8 (br, 13H, cycloheptane ring, OH), 2.78 (s, 2H, CH_2Ph), 4.97 (s, 2H, OCH_2Ph), 6.7—7.4 (m, 9H, aromatic). *Anal.* Calcd for $\text{C}_{21}\text{H}_{26}\text{O}_2$: C, 81.23; H, 8.46. Found: C, 81.20; H, 8.40.

1-(4-Benzoyloxybenzyl)-1-[3-(*N,N*-dimethylamino)propoxy]cycloheptane (VIIp)—Sodium hydride 6(0%, 0.7 g, 17.5 mmol) was added in portions to a solution of VIp (2.8 g, 9 mmol) and 3-(*N,N*-dimethylamino)propyl chloride (2.2 g, 18 mmol) in dry toluene (30 ml) with stirring at 20—30°C. After being refluxed for 6 h, the mixture was poured into water and extracted with EtOAc. The EtOAc layer was washed with water, dried (MgSO_4) and concentrated, and the residual oil was chromatographed on silica gel using a 1:10:25:25 mixture of 28% aqueous NH_3 , MeOH, *n*-hexane and EtOAc as an eluant to yield 2.1 g (5.3 mmol, 59%) of VIIp: $^1\text{H-NMR}$ (CDCl_3) δ : 2.23 (s, 6H, Me), 2.7 (s, 2H, CH_2Ph), 3.49 (t, 2H, $J=7$ Hz, OCH_2), 5.03 (s, 2H, OCH_2Ph), 6.8—7.5 (m, 9H, aromatic). *Anal.* Calcd for $\text{C}_{26}\text{H}_{37}\text{NO}_2$: C, 78.92; H, 9.44; N, 3.54. Found: C, 77.70; H, 9.70; N, 3.70.

1-(3-Benzoyloxybenzyl)-1-[3-(*N,N*-dimethylamino)propoxy]cycloheptane (VIIm)—This compound was prepared from VIIm by a procedure similar to that used to prepare VIIp. The product was purified by silica gel column chromatography using a 1:10:25:25 mixture of 28% aqueous NH_3 , MeOH, *n*-hexane and EtOAc as an eluant to give a 54% yield of viscous oil: $^1\text{H-NMR}$ (CCl_4) δ : 2.17 (s, 6H, Me), 2.67 (s, 2H, CH_2Ph), 3.4 (t, 2H, $J=6.5$ Hz, OCH_2), 4.97 (s, 2H, OCH_2Ph), 6.6—6.8 (m, 3H, aromatic), 6.9—7.5 (m, 6H, aromatic). *Anal.* Calcd for $\text{C}_{26}\text{H}_{37}\text{NO}_2$: C, 78.92; H, 9.44; N, 3.54. Found: C, 77.70; H, 9.40; N, 3.50.

1-(2-Benzoyloxybenzyl)-1-[3-(*N,N*-dimethylamino)propoxy]cycloheptane (VIIo)—This compound was prepared from VIo by a procedure similar to that used to prepare VIIp. The compound was purified by silica gel column chromatography using a 1:10:25:25 mixture of 28% aqueous NH_3 , MeOH, *n*-hexane and EtOAc as an eluant to give a 22% yield of viscous oil: $^1\text{H-NMR}$ (CDCl_3) δ : 2.27 (s, 6H, Me), 2.9 (s, 2H, CH_2Ph), 3.47 (t, 2H, $J=6.7$ Hz, OCH_2), 5.05 (s, 2H, OCH_2Ph), 6.75—7.5 (m, 9H, aromatic). *Anal.* Calcd for $\text{C}_{26}\text{H}_{37}\text{NO}_2$: C, 78.92; H, 9.44; N, 3.54. Found: C, 77.60; H, 9.20; N, 4.10.

1-(4-Hydroxybenzyl)-1-[3-(*N,N*-dimethylamino)propoxy]cycloheptane (IVp)—A mixture of VIIp (2 g, 5 mmol) and 5% palladium on charcoal (0.2 g) in EtOH (50 ml) was vigorously stirred in a hydrogen atmosphere at room temperature until the consumption of hydrogen ceased. The catalyst was filtered off and the filtrate was concentrated. The residual oil was chromatographed on silica gel using a 1:10:25:25 mixture of 28% aqueous NH_3 , MeOH, *n*-hexane and EtOAc as an eluant to yield 0.7 g (2.3 mmol), 46% of IVp: mp 119.1—120.6°C; $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 2.15 (s, 6H, Me), 3.43 (t, 2H, $J=6$ Hz, OCH_2), 6.63 (d, 2H, $J=8$ Hz, aromatic), 6.98 (d, 2H, $J=8$ Hz, aromatic). *Anal.* Calcd for $\text{C}_{19}\text{H}_{31}\text{NO}_2$: C, 74.69; H, 10.25; N, 4.59. Found: C, 74.40; H, 10.60; N, 4.60.

1-(3-Hydroxybenzyl)-1-[3-(*N,N*-dimethylamino)propoxy]cycloheptane (IVm)—This compound was prepared from VIIm by a procedure similar to that used to prepare IVp. The compound was purified by silica gel column chromatography using a 1:10:25:25 mixture of 28% aqueous NH_3 , MeOH, *n*-hexane and EtOAc as an eluant to give a 68% yield of slightly yellow crystals: mp 142—144°C; $^1\text{H-NMR}$ (CDCl_3) δ : 2.63 (s, 2H, CH_2Ph), 2.77 (br, 6H, Me), 2.93—3.27 (br, 2H, CH_2N), 3.3—3.6 (br, 2H, OCH_2), 6.47—7.23 (m, 4H, aromatic). *Anal.* Calcd for $\text{C}_{19}\text{H}_{31}\text{NO}_2$: C, 74.69; H, 10.25; N, 4.59. Found: C, 74.31; H, 10.00; N, 4.61.

1-(2-Hydroxybenzyl)-1-[3-(*N,N*-dimethylamino)propoxy]cycloheptane (IVo)—This compound was prepared from VIIo by a procedure similar to that used to prepare IVp. The compound was purified by silica gel column chromatography using a 1:10:25:25 mixture of 28% aqueous NH_3 , MeOH, *n*-hexane and EtOAc as an eluant to give an 80% yield of viscous oil: $^1\text{H-NMR}$ (CDCl_3) δ : 2.22 (s, 6H, Me), 2.85 (s, 2H, CH_2Ph), 3.58 (2H, t, $J=6$ Hz, OCH_2), 6.6—7.35 (m, 4H, aromatic). *Anal.* Calcd for $\text{C}_{19}\text{H}_{31}\text{NO}_2$: C, 74.69; H, 10.25; N, 4.59. Found: C, 74.39; H, 10.24; N, 4.57.

1-Benzyl-1-[3-(*N*-methylamino)propoxy]cycloheptane (VIII)—This compound was prepared by a procedure similar to a previously described procedure.¹⁹⁾ $^1\text{H-NMR}$ (CDCl_3) δ : 1.2—1.9 (15H, m), 2.33 (3H, s, NCH_3), 2.6—2.8 (4H, m, PhCH_2 -, $-\text{CH}_2\text{N}$ -), 3.45 (2H, t, $J=6$ Hz, $-\text{OCH}_2$ -), 7.1 (5H, s, aromatic). *Anal.* Calcd for $\text{C}_{18}\text{H}_{29}\text{NO}$: C, 78.47; H, 10.63; N, 5.09. Found: C, 77.9; H, 10.3; N, 4.5.

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