Application of Chemical P450 Model Systems to Studies on Drug Metabolism. I. Phencyclidine: A Multi-functional Model Substrate[1)]

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Cytochrome P450 model and liver microsomal oxidations of drugs were compared using phencyclidine. In general, the chemical reaction systems produced many oxidation products. Besides the formation of the cyclohexane-4-hydroxyl compound (2), hydroxylation of the aromatic ring was favored in the Fenton reaction system ($Fe^{2+}+H_2O_2$). Formation of an *m*-hydroxylated product (*m*-5) was the main aromatic oxidation pathway in the Udenfriend reaction (Fe^{2+}–ascorbic acid–O_2), and 2, the piperidine-3-hydroxyl compound (3), and the piperidine-4-hydroxyl compound (4) were also formed. In the system using *meso*-tetraphenylporphinatoiron chloride (Fe(III)TPPC1) with an oxidant, the main product was the piperidine-3-oxo compound (8). In the liver microsomes system, 2, 4, 8, and *m*-5, which were all generated by the chemical oxidation reactions, were detected as metabolites of phencyclidine. They were formed by cytochrome P450-dependent reactions. Chemical oxidation systems can be used to study drug metabolism; they can reveal some tendencies of the real metabolic reactions, are easy to operate, and yield sufficient amounts of product.

Keywords cytochrome P450-dependent reaction; phencyclidine metabolism; Fenton reaction; Udenfriend reaction; *meso*-tetraphenylporphinatoiron(III) chloride

Introduction

The metabolism of drugs and foreign compounds has been extensively studied in recent years. Many model systems have been used to elucidate the molecular mechanisms of biological oxygen activation and the insertion of the oxygen atom into a substrate. However, only very simple compounds are usually used as substrates, for example, cyclohexene, adamantane, *etc.* We focused on a more complex compound, which is also used as an actual drug, to study and to compare microsomal oxidation with chemical oxidation systems. The advantages of using model systems to understand drug metabolism are as follows: 1) metabolite candidates are available in relatively large amounts and can be used to identify the real *in vivo* metabolites and to provide samples for pharmacological testing; 2) the mode of metabolism can be clarified, for example, an unstable metabolite can be isolated under selected and controlled reaction conditions; and 3) the use of experimental animals can be minimized.

For this study, phencyclidine (PCP) (**1**) was chosen as a drug because 1) it is an anesthetic agent, which is involved in widespread drug abuse; 2) despite being a small molecule, it has an aromatic ring, an aliphatic ring, and a N-containing heterocyclic ring moiety, which make it very interesting from the viewpoint of organic and metabolic chemistry; and 3) some data are available on its *in vivo* metabolism.[2,3)]

Results and Discussion

Gas chromatography/mass spectrometry (GC/MS) was used for detection, and gas chromatography/selected ion monitoring analyses (GC/SIM) which can sensitively detect specified mass fragment ions were used for quantitation.

The mass spectra of the derivatized authentic compounds were measured by GC/MS (Fig. 1) and confirmed the efficiency of the GC/MS and GC/SIM detection. Consideration of various analysis conditions showed that good separation could be obtained, as shown in Fig. 2.

Chemical Oxidation Systems (Cytochrome P450 Model Systems) As shown in Table I, every reaction model system produced many types of oxidation products, phenolic, alcoholic, and ring-opened amino acids.

Besides the formation of the cyclohexane-4-hydroxyl compound (**2**) which is a known phencyclidine metabolite, hydroxylation of the aromatic ring was favored in the Fenton reaction system ($Fe^{2+}+H_2O_2$).[4)] This system is known to produce a hydroxyl radical in an aqueous solvent and ferryl ions in a less aqueous solvent as active species.[5)]

The Udenfriend reaction (Fe^{2+}–ascorbic acid–O_2) system was the first model of the Fe–O complex and it is known to catalyze the hydroxylation of cyclohexane and the aromatic ring of tyramine.[6)] Cyclohexane-4-hydroxyl (**2**), piperidine-3-hydroxyl (**3**), and piperidine-4-hydroxyl (**4**) compounds were formed in relatively good yields. Compound **4** is also one of the known phencyclidine metabolites *in vivo*.[2,3)] The *meta* position of the aromatic ring of PCP was extensively hydroxylated in the Udenfriend reaction system. This selectivity may be caused by the steric effects of the Fe–ascorbic acid complex.

Groves's group[7)] investigated *meso*-tetraphenylporphinatoiron(III) chloride (Fe(III)TPPCl) as a cytochrome P450 model. It produces $(Fe^{IV}=O)^{+\cdot}$ (Fe-oxenoid) as the ultimate active species when iodosylarene is used as the oxidant. The system was also explored by Mansuy's group[8)] using cumene hydroperoxide as the oxidant. Interestingly, Fe(III)TPPCl leads to the production of more of the piperidine ring-3-oxo compound than any other product if either iodosylxylene or cumene hydroperoxide is used as the oxidant. This product was quantitated as the hydroxyl derivative after reduction by sodium borohydride ($NaBH_4$) because the piperidine-3-oxo compound is unstable.[9)] Ketone formation in other positions was not observed. The piperidine-3-oxo compound was also identified directly by GC/MS (Fig. 3) compared with an authentic sample (**8**) synthesized from **3** by Swern oxidation.[10)] As shown in Fig. 3, the molecular ion peak ($M^{+\cdot}$, m/z 257) and all fragment peaks of the authentic compound (**8**) were observed in the MS of the product.

Finally, the Fe(III)TPPCl–Zn–acetic acid–O_2 system

This paper is dedicated to Professor Haruaki Yajima on this occasion of his retirement from Kyoto University in March 1989.

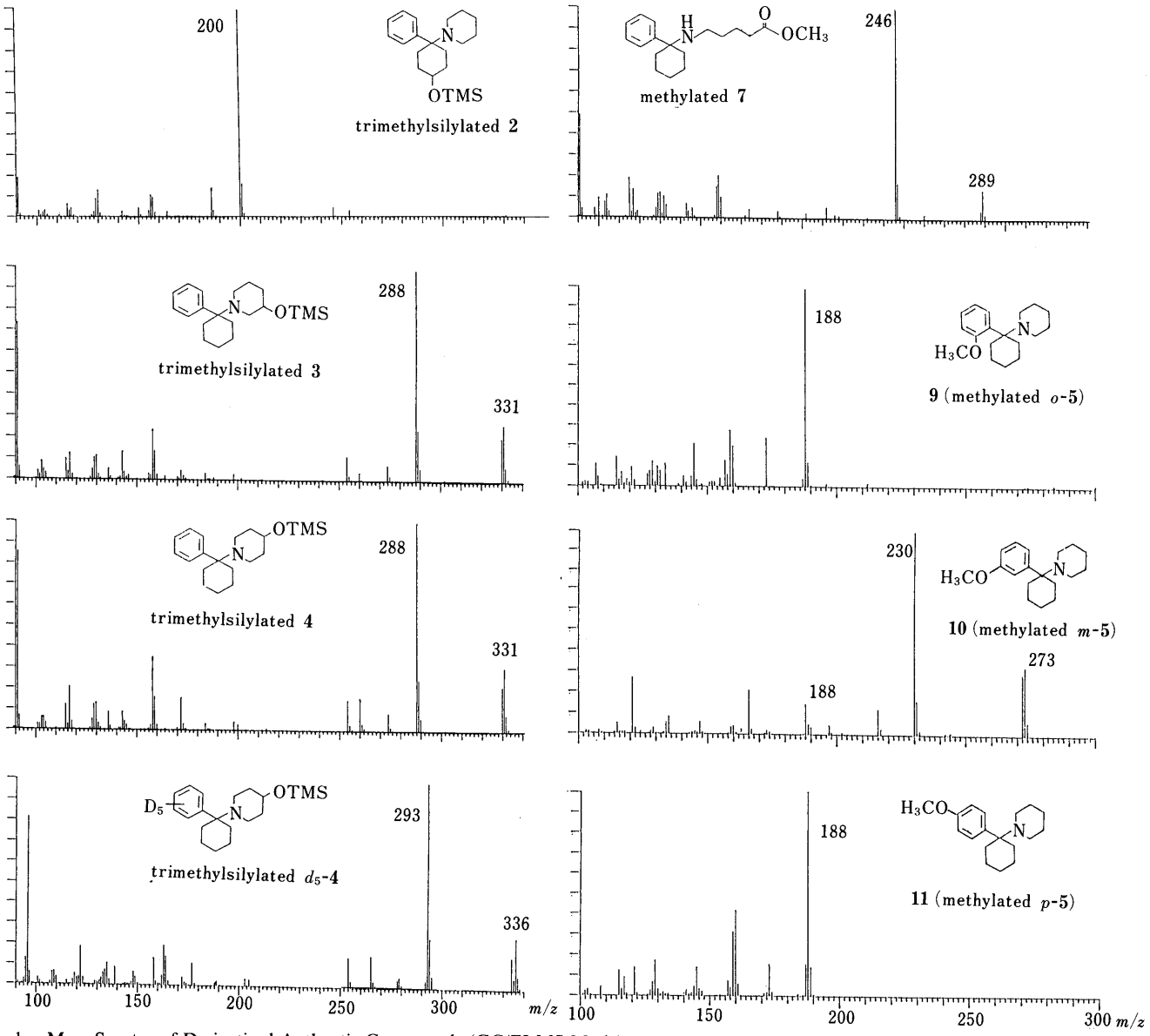


Fig. 1. Mass Spectra of Derivatized Authentic Compounds (GC/EI-MS Mode)

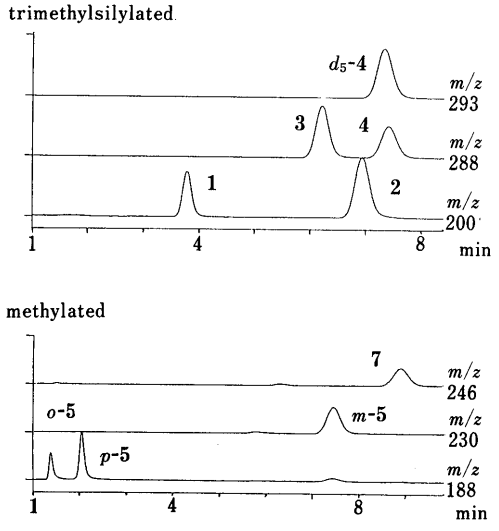


Fig. 2. GC/SIM Chromatogram of Derivatized Authentic Compounds

GC/SIM mode; column, 3% OV-17 2 m × 2 mmϕ; injection temperature, 250 °C; column temperature, 210 °C; ionization energy, 70 eV.

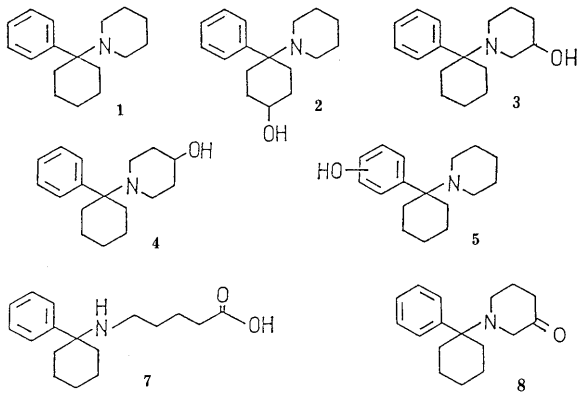
originally discovered by our group[11] and Mansuy's group[12] was found to hydroxylate preferentially the cyclohexane and piperidine rings. Only the *meta* position of the aromatic ring was hydroxylated.

Liver Microsomes–Oxidant System The cytochrome P450 catalyzed oxidation cycle requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) and O_2. The oxidation cycle also operates with some oxidants instead of NADPH and O_2. To probe the cytochrome P450 reaction mechanisms, various groups have studied the relative reactivities of the liver microsomes–oxidant, purified cytochrome P450–oxidant and cytochrome P450–NADPH/O_2 systems.[13] While similarities have been found, differences have also been found.

In this study, we examined the reaction of PCP in the liver microsomes–cumene hydroperoxide or -iodosylxylene system and compared the product profiles with those obtained from the liver microsomes–NADPH/O_2 system and from the chemical oxidation systems.

Microsomes–oxidant systems were constituted from three kinds of liver microsomes of rats (non-treated, sodi-

TABLE I. Oxidation of Phencyclidine (**1**) by Several P450 Model Systems



Reaction system	Amount of product (μg/reaction mixture)							
	o-**5**	*m*-**5**	*p*-**5**	**2**	**3**	**4**	**7**	**8**
$Fe^{2+}+H_2O_2$	0.345	76.5	24.2	87.70	3.38	N.D.	0.98	—
in $H_2O:CH_3CN=8:3$	(0.2)	(39.6)	(12.5)	(45.4)	(1.8)	(0.0)	(0.5)	—
$Fe^{2+}+H_2O_2$	1.475	107.0	28.2	99.88	12.0	0.697	1.00	—
in $H_2O:CH_3CN=1:9$	(0.6)	(42.7)	(11.3)	(39.9)	(4.8)	(0.3)	(0.4)	—
$Fe^{2+}+O_2$	0.089	0.740	Trace	50.11	21.71	33.53	N.D.	—
+ascorbic acid	(Trace)	(0.7)	(Trace)	(47.2)	(20.4)	(31.6)	(0.0)	—
Fe(III)TPPCl	9.87	0.196	0.152	1.48	2.40	0.679	0.43	250.4
+iodosylxylene	(3.7)	(Trace)	(Trace)	(0.6)	(0.9)	(0.3)	(0.2)	(94.3)
Fe(III)TPPCl	1.31	0.264	0.203	1.90	3.49	1.79	N.D.	93.3
+cumene hydroperoxide	(1.3)	(0.3)	(0.2)	(1.9)	(3.4)	(1.8)	(0.0)	(91.2)
Fe(III)TPPCl	N.D.	0.328	N.D.	40.85	15.77	25.45	N.D.	—
$+Zn+AcOH+O_2$	(0.0)	(0.4)	(0.0)	(49.6)	(19.1)	(30.9)	(0.0)	—

Fe(III)TPPCl=tetraphenylporphinatoiron(III) chloride. —, not measured. N.D., not detected. Trace, <0.1. Relative yields (%) are given in parentheses.

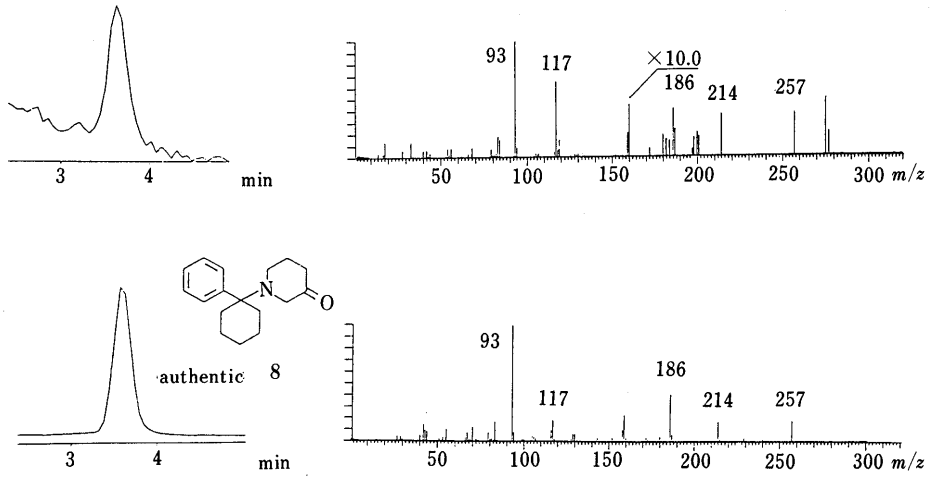


Fig. 3. Identification of **8** in Fe(III)TPPCl–Iodosylxylene System

GC/EI-MS mode; column, 1% OV-17 1 m×2 mm (i.d.); injection temperature, 220 °C; column temperature, 200 °C; ionization energy, 70 eV.

um phenobarbital (PB)-treated, and 3-methylcholanthrene (3-MC)-treated), non-treated mouse liver microsomes, and two kinds of oxidants(cumene hydroperoxide and iodosylxylene).

The products formed are shown in Table II. In both systems, aromatic hydroxylation occurred preferentially at the *meta* position. The piperidine-3-hydroxyl compound (**3**), which was generated in the chemical oxidation systems, was not generated in the microsomal systems, while the ketone compound (**8**) was.

As described in the section on chemical oxidation, the Fe(III)TPPCl–iodosylarene system produces Fe–oxenoid as the active species. It has been reported that the cytochrome P450–iodosylarene system produced the oxenoid intermediate in its reaction cycle.[13b] On the other hand, it was suggested that homolytic scission of the O–O bond of the (cumeneperoxy-Fe) complex occurred, and the resulting cumylalcoxyl radical and Compound I like Fe–O· served as the active species in the Fe(III)TPPCl–cumene hydroperoxide system.[14] It is very likely that the same reaction as described above occurs at the heme center of the cytochrome P450 of the microsomes with cumene hydroperoxide. In these microsomes–oxidant systems, the catalyst is cytochrome P450, which is different from syn-

TABLE II. Oxidation of Phencyclidine (**1**) by Microsomes–Oxidant Systems

Microsomes	Oxidant	Amount of product (μg/reaction mixture)			
		m-**5**	**2**	**4**	**8**
Rat non-treated	A	0.40 (4.3)	0.96 (10.4)	4.2 (45.4)	3.7 (40.0)
	B	0.38 (5.4)	3.5 (49.4)	1.7 (24.0)	1.5 (21.2)
Rat PB-treated[a]	A	0.36 (10.0)	2.4 (66.7)	0.37 (10.3)	0.47 (13.1)
	B	0.34 (3.4)	6.0 (59.2)	1.2 (11.8)	2.6 (25.6)
Rat 3-MC-treated[b]	A	0.42 (20.7)	0.89 (43.8)	0.28 (13.8)	0.44 (21.7)
	B	0.38 (2.2)	1.6 (9.1)	8.3 (47.2)	7.3 (41.5)
Mouse	A	0.87 (31.4)	1.3 (46.9)	0.24 (8.7)	0.36 (13.0)
	B	0.34 (3.9)	2.6 (30.1)	3.0 (34.7)	2.7 (31.3)

Oxidant: A) cumene hydroperoxide, B) iodosylxylene. *a*) Phenobarbital Na 60 mg/kg d for 3 d i.p. *b*) 3-Methylcholanthrene 30 mg/kg d for 3 d i.p. Relative yields (%) are given in parentheses.

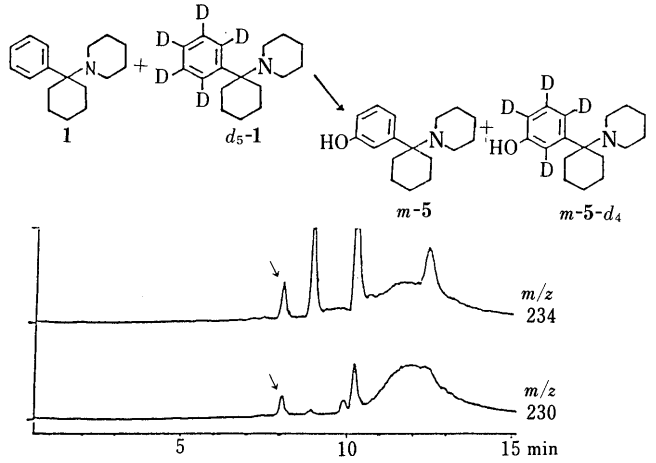


Fig. 4. GC/SIM Chromatogram of an Equimolar Mixture of PCP (**1**) and Deuterium-Labeled PCP (d_5-**1**) in Rat Liver Microsomes (PB-Treated)–NADPH/O_2 System

GC/SIM conditions were as given in the legend to Fig. 2.

thetic catalysts. Since the synthetic catalysts (for example, Fe(III)TPPCl) are not held by the apoprotein, which is buried in the lipid membrane. Thus the fact that the product profiles obtained from these systems were different from those obtained from the chemical oxidation systems might be mainly due to the existence of the surrounding protein, which restricts the mode of substrate interaction and its direction of access to the heme.

Complete depression of *ortho* and *para* hydroxylations of the aromatic ring and of the piperidine-3-hydroxylation was observed in these reactions. Together with the results of the chemical oxidation systems, these results suggest that if the same active species were generated in both Fe(III)-TPPCl- and microsomes–oxidant systems, then the regioselectivities of the aromatic hydroxylations were mainly due to the existence of the ferrous chelators (porphyrins), and those of the aliphatic hydroxylations, mainly due to the existence of the enzyme protein.

Liver Microsomes–NADPH/O_2 System The microsomes for the liver microsomes–NADPH/O_2 system were from the same sample of microsomes used for the microsomes–oxidant system. As we described in our preliminary report,[1] the formation of phenolic metabolite (*m*-**5**) was followed by a GC/SIM, ion cluster technique. A 1 : 1 mixture of unlabeled PCP and deuterium labeled (phenyl-d_5-labeled) PCP was used as the substrate. In the GC/SIM spectra (Fig. 4), an ion cluster with *m*/*z* 230 appeared

TABLE III. Inhibition of *m*-**5** and **8** Formation in Microsomes–NADPH/O_2 Systems Under Various Incubation Conditions

Condition	Relative activities (%)	
	m-**5**	**8**
Complete	100	100
− NADP	0	4
+ SKF525A 3 mM	24	12
+CN^- 2 mM	6	26
Boiled microsomes	6	3
No derivatization	6	0

Phenobarbital Na-treated rat liver microsomes were used.

TABLE IV. Formation of Phencyclidine Metabolites by Microsomes–NADPH/O_2 Systems

Microsomes	Formation of metabolite (nmol/mg of protein)			
	m-**5**	**2**	**4**	**8**
Rat non-treated	0.15	6.6	5.1	2.8
Rat PB-treated[a]	0.2	16.7	6.5	3.8
Rat 3-MC-treated[b]	1.9	4.3	3.0	2.1
Mouse	0.2	5.0	0.43	0.70

a) Phenobarbital Na 60 mg/kg d for 3 d i.p. *b*) 3-Methylcholanthrene 30 mg/kg d for 3 d i.p.

together with an ion cluster at *m*/*z* 234. Both peaks (*m*/*z* 230, 234) had a retention time identical to that of an authentic sample of methylated *m*-OH PCP (**10**). No other aromatic position (*ortho*, *para*) was hydroxylated. The formation of *m*-**5** was effectively inhibited when SKF525A (a commonly used inhibitor of cytochrome P450) and CN^- were added. When NADP was not included or boiled microsomes were used, *m*-**5** was not generated. Thus, this reaction is dependent on cytochrome P450 (Table III). The amount of *m*-**5** formation was maximal when 3-MC-treated rat liver microsomes were used (Table IV).

The piperidine-3-hydroxyl compound (**3**) was also not found in the microsomes–NADPH/O_2 system. But, when the reaction mixture was reduced by $NaBH_4$, the peak of **3** on GC/SIM could be clearly detected. Thus the ketone compound (**8**) was also formed in the liver microsomes–NADPH/O_2 system. The amount of **8** formed was determined as **3** after reduction. The amount of **8** was maximal when PB-treated rat microsomes were used (Table IV). SKF525A and CN^- inhibited **8** formation, and the boiled microsomes system or the system without NADP could not generate **8** (Table III). Thus, this reaction is also

cytochrome P450-dependent.

Although *m*-**5** is only a minor metabolite compared to known *in vivo* metabolites, it has been synthesized and tested for pharmacological activity by Kamenka's group.[15] Interestingly *m*-**5** possesses far more potent pharmacological activity than PCP. The other previously unidentified metabolite (**8**) is interesting from the point of view that the piperidine ring of PCP is oxidized quantitatively to the level of a ketone at the β-position, while initial hydroxylation at the γ-position proceeds no further. The metabolism of the piperidine ring by rat liver microsomes is under current investigation with a simple piperidine derivative (*N*-benzylpiperidine) as the substrate in order to probe the generality of the biotransformation of the piperidine ring.

No major differences were observed in the product profiles obtained by employing NADPH/O_2 instead of oxidants. The differences that do exist might be due to differences of the catalytic cycle and active species.

Conclusion

Chemical oxidation systems using PCP as a substrate can generate many kinds of oxidation products. Among them, two compounds could also be generated as new metabolites of PCP by rat liver microsomes in studies using standard liver microsomal systems. The chemical oxidation systems should be useful for further studies on PCP biotransformation.

The fact that *m*-**5** is far more pharmacologically potent than PCP may explain the long-term effects of PCP, despite the fact that *m*-**5** is a minor metabolite.

Although Zbaida's group very recentry investigated the role of iron chelates in the selectivities of Fenton reagent in oxidations of cimetidine,[16] the use of various chemical oxidation systems as a general methodology to study drug metabolism has not previously been attempted, to our knowledge. However, chemical oxidation systems can lead to the formation of various metabolic products, are easy to operate, and can yield products in sufficient amounts for isolation and further study. Thus, their use as an approach to drug metabolism would appear to merit further investigation. For example, this approach led to the discovery of the unstable ketone compound (**8**) and active phenolic metabolite (*m*-**5**).

Experimental

General Methods Elemental analyses were performed by the Microanalytical Laboratory at the University of Tokyo. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Mass spectra were measured on a JEOL DX-300 mass spectrometer operating in the direct injection-electron impact mode. Infrared (IR) spectra were recorded on a JASCO DS-701G spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Hitachi R-24B (60 MHz) or a JNM FX-100 (100 MHz) pulse Fourier transform NMR spectrometer. Chemical shifts are expressed in units (ppm) downfield of internal tetramethylsilane. Splitting patterns are designated as follows: s, singlet; m, multiplet. Ultraviolet (UV) and visible absorption spectra were recorded on a Hitachi 557 double beam spectrophotometer equipped with thermostatic cell compartments, at 37 °C.

Gas chromatography/mass spectral analysis was done using a JEOL DX-300 mass spectrometer with a JMA-2000 mass data analysis system. The columns were packed with 3% OV-17 on Gaschrom Q mesh 100—120 (2 m × 2 mm i.d.) and 1% OV-17 on Gaschrom Q mesh 100—120 (1 m × 2 mm i.d.) in glass columns, with 30 ml/min of helium as the carrier gas.

The structures of all known compounds synthesized in this paper were consistent with their spectral data.

Materials All solvents and reagents were commercial products of reagent-grade quality, and were used without further purification, except when otherwise noted. Ethanol was dried over Na, distilled, and stored over 4A molecular sieves. Ether was dried over Na, distilled, and stored over sodium wire. Triethylamine was dried over KOH, distilled from CaH_2 and $LiAlH_4$, and stored over 4A molecular sieves. CH_2Cl_2 was dried over $CaCl_2$, distilled from CaH_2, and stored over 4A molecular sieves. Dimethylsulfoxide was dried over $CaCl_2$ and CaH_2, distilled, and stored over 4A molecular sieves. Thin-layer chromatography (TLC) was done using precoated Silica gel 60-F_{254} (Merck No. 5554) and aluminium oxide 60-F_{254} (Merck No. 5550) sheets with fluorescence indicator. Silica gel for column chromatography was Micro Bead Silica Gel Grade 4B (100—200 mesh, neutral) purchased from Fuji-Davison Chemical Ltd.

NADP potassium salt (98%) and glucose-6-phosphate (G-6-P) disodium salt were purchased from Boehringer Mannheim GmbH, and were stored at 4 °C. Glucose-6-phosphate dehydrogenase (G-6-P DHase) (EC 1.1.1.49) from baker's yeast was purchased from Sigma Chemical Co., and was stored at −20 °C. Cumene hydroperoxide was purchased from Nakarai Chemicals Ltd. H_2O_2 (30%) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Wako Pure Chemical Industries Ltd. β-Diethylaminoethyl diphenylpropylacetate hydrochloride (SKF525A) was a gift from Smith Kline & Fujisawa Co., Ltd. CH_2N_2 was synthesized from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide.[17]

1-(1-Phenylcyclohexyl)piperidine (1) This compound was synthesized according to the method of Maddox's group.[18] The hydrochloride salt was obtained by bubbling HCl gas into an ether solution of **1**.

4-Phenyl-4-piperidino-cyclohexanol (2) and 4-Hydroxy-1-(1-phenylcyclohexyl)piperidine (4) These compounds were synthesized according to the method of Lin's group.[19]

4-Hydroxy-1-(1-d_5-phenylcyclohexyl)piperidine(d_5-4) This compound was synthesized from 1-(4-hydroxypiperidino)cyclohexane carbonitrile in a similar manner to that described for **4**, except that d_5-bromobenzene was utilized instead of bromobenzene. The product was recrystallized from *n*-hexane–ethanol, giving pale yellow needles: mp 118—120 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.7—2.2 (br m, 19H), 3.4 (br s, 1H). IR (KBr tablet) ν: 2280 (C–D) cm^{-1}. *Anal.* Calcd for $C_{17}H_{20}D_5NO$: C, 77.29; N, 5.30. Found: C, 76.99; N, 5.47.

3-Hydroxy-1-(1-phenylcyclohexyl)piperidine (3) This compound was synthesized in a manner similar to that described for **4**, except that 3-hydroxypiperidine hydrochloride was utilized instead of 4-hydroxypiperidine hydrochloride.

5-(*N*-(1-Phenylcyclohexyl)amino)pentanol (6) and 5-(*N*-(1-Phenylcyclohexyl)amino)pentanoic Acid (7) These compounds were synthesized according to the method of Baker's group.[20]

1-(1-(2-Methoxyphenyl)cyclohexyl)piperidine (9), 1-(1-(3-Methoxyphenyl)cyclohexyl)piperidine (10), and 1-(1-(4-Methoxyphenyl)cyclohexyl)piperidine (11)[21] These compounds were synthesized in a manner similar to that described for **1**, except that *o*-bromoanisole, *m*-bromoanisole, and *p*-bromoanisole were used, respectively, instead of bromobenzene.

1-(1-d_5-Phenylcyclohexyl)piperidine(d_5-1)[22] This compound was synthesized from 1.55 g of 1-piperidinocyclohexane carbonitrile in a way similar to that described for **1**, except that d_5-bromobenzene was used instead of bromobenzene. The product was recrystallized from ethanol–H_2O, giving colorless crystals (1.46 g, total 57%): mp 34—36 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.7—2.5 (br m, 20H). IR (KBr tablet) ν: 2275 (C–D) cm^{-1}. MS (EI) *m/z*: 248 ($M^{+\cdot}$), 205 (base peak). HCl gas was bubbled into an ether solution of the product to give its hydrochloride salt, which was recrystallized from ethanol–ether: mp 224—227 °C.

1-(1-Phenylcyclohexyl)-3-piperidone (8) Oxalyl chloride (0.3 g, 2.0 mmol) in 3 ml of CH_2Cl_2 was stirred at −78 °C under an Ar atmosphere. To this solution, precooled absolute dimethyl sulfoxide (DMSO, 0.3 g, 4.0 mmol) was added. The resulting mixture was stirred for 15 min, then precooled 3-hydroxy-1-(1-phenylcyclohexyl)piperidine (**3**) (260 mg, 1.0 mmol) in 1.5 ml of CH_2Cl_2 was added. The mixture was stirred for 30 min at −78 °C under Ar. After addition of 1.0 g of triethylamine, the mixture was left standing at room temperature. Next, 50 ml of CH_2Cl_2 and 20 ml of brine (saturated aqueous NaCl) were added. The organic layer was separated and washed with brine (20 ml). After drying over anhydrous Na_2SO_4, concentration *in vacuo* gave a pale yellow oil. The product was purified by silica gel column chromatography (eluent, ether : *n*-hexane = 1 : 1), giving pale yellow crystals (217 mg, 88%): mp 42—44 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.0—3.0 (br m, 18H), 7.4 (s, 5H). IR (KBr tablet) ν: 1705 (C=O) cm^{-1}. MS (EI) *m/z*: 257 ($M^{+\cdot}$), 214, 186. This compound was stored under Ar at −20 °C.

The *O*-benzyloxime as a carbonyl derivative of **8** was prepared. A mixture of *O*-benzylhydroxylamine (61 mg), AcOH (1 drop), **8** (105 mg),

and absolute benzene (20 ml) was stirred overnight with molecular sieves 4A at room temperature and then for 4 h at 60 °C. The resulting mixture was filtered and evaporated. The obtained oil was purified by silica gel column chromatography (eluent, AcOEt : CH_2Cl_2 = 3 : 2), followed by bulb-to-bulb distillation (250 °C/4—5 mmHg), giving a colorless oil (45 mg, 31%). ^{1}H-NMR ($CDCl_3$) δ: 1.2—2.5 (br m, 16H), 2.82 and 3.20 (s × 2, total 2H), 5.0 (s, 2H), 7.05—7.40 (m, 10H). IR (Cap.) ν: 2930, 1640, 1600, 1495 cm^{-1}. *Anal.* Calcd for $C_{24}H_{30}N_2O$: C, 79.52; H, 8.34; N, 7.73. Found: C, 79.27; H, 8.34; N, 7.80.

***meso*-Tetraphenylporphinatoiron(III) Chloride (Fe(III)TPPCl)** This compound was synthesized according to the method of Adler's group.[23]

2,6-Dimethyliodosylbenzene (Iodosylxylene) This compound was synthesized according to the method of J. G. Sharefkin and H. Saltzman.[24]

Fenton Reaction ($Fe^{2+}+H_2O_2$) PCP–HCl (30 mg, 0.1 mmol), Fe-$(ClO_4)_2$ (196 mg, 0.75 mmol) and 70% $HClO_4$ (81 mg, 0.56 mmol) were dissolved in 1.25 ml of H_2O and 0.75 ml of CH_3CN (or 0.4 ml of H_2O and 2.7 ml of CH_3CN). Ar gas was bubbled into the mixture for 10 min at 37 °C. Next, a solution of 30% H_2O_2 (57 mg, 0.5 mmol) in 0.75 ml of H_2O (or CH_3CN) was added portionwise over 10 min. The mixture was incubated for 1 h under Ar at 37 °C. The reaction was terminated by adding 10% aqueous $NaHSO_3$ (1.5 ml) and 28% aqueous NH_3 (0.75 ml). The reaction mixture was worked up according to procedure A for **5** and **7** and procedure B for **2**, **3**, and **4**.

Udenfriend Reaction (Fe^{2+}–Ascorbic Acid–O_2) Ascorbic acid (25 mg, 142 μmol), $FeSO_4$ (10 mg, 15 μmol), and Na_2EDTA (30 mg, 80 μmol) were dissolved in 2.8 ml of sodium phosphate buffer (0.1 M, pH 6.0) at 37 °C. PCP–HCl (15 mg, 50 μmol) in 0.2 ml of sodium phosphate buffer (0.1 M, pH 6.0) was added. The mixture was incubated for 1 h at 37 °C, then worked up according to procedure A for **5** and **7** and procedure B (after addition of 2 ml of 1 M aqueous Na_2CO_3) for **2**, **3**, and **4**.

Fe(III)TPPCl–Oxidant System A mixture of Fe(III)TPPCl (7 mg, 10 μmol), PCP–HCl (30 mg, 0.1 mmol), 2,6-dimethyliodosylbenzene (iodosylxylene, 23 mg, 0.1 mmol) (or cumene hydroperoxide (15.2 mg, 0.1 mmol)), and 5 ml of benzene was stirred for 1 h at room temperature. The reaction mixture was worked up according to procedure C for **5** and **7**, procedure D for **2**, **3**, and **4**, and procedure E for **8**.

Fe(III)TPPCl–Zn–Acetic Acid–O_2 System A mixture of Fe(III)TPPCl (7 mg, 10 μmol), PCP–HCl (30 mg, 0.1 mmol), CH_2Cl_2 (0.5 ml), acetic acid (AcOH, 0.5 ml), and zinc (200 mg), which had been washed with 1 N HCl, was stirred vigorously for 1 h at room temperature. After addition of CH_2Cl_2 (10 ml), the mixture was washed with water (5 ml) to remove the AcOH. The CH_2Cl_2 layer was worked up according to procedure C for **5** and **7** and procedure D for **2**, **3**, and **4**.

Procedure A To the mixture, 10 ml of ethanol was added. The aqueous layer was saturated with K_2CO_3. The ethanol (upper) layer was dried over anhydrous K_2CO_3 after separation. To the resulting mixture, a CH_2N_2 ether solution (*ca.* 1 g/100 ml) was added dropwise (*ca.* 3 ml) to stabilize the phenolic and amino acidic products. The mixture was left standing for 30 min at room temperature, then excess CH_2N_2 was quenched with AcOH. Tribenzylamine was added as an internal standard and the mixture was concentrated (*ca.* 0.5 ml).

Procedure B To the mixture, 10 ml of CH_2Cl_2 and a small amount of d_5-**4** (an internal standard) were added. After shaking (10 min, 5 Hz, 5-cm strokes), the CH_2Cl_2 layer was dried over anhydrous K_2CO_3 and concentrated (*ca.* 0.5 ml). The CH_2Cl_2 solution was refluxed for 1 h with BSTFA (0.25 ml) to derivatize the alcoholic products to the corresponding trimethylsilylethers.

Procedure C This was the same as procedure A without the extraction and drying operations.

Procedure D The reaction mixture was extracted with 1 N HCl (10 ml) after addition of d_5-**4** as an internal standard. The aqueous layer was neutralized with 28% aqueous NH_3 and was shaken (10 min, 5 Hz, 5-cm strokes) with CH_2Cl_2 (10 ml). The CH_2Cl_2 layer was dried over anhydrous K_2CO_3 and concentrated (*ca.* 0.5 ml). The solution was treated with BSTFA as in procedure B.

Procedure E Methanol (2 ml) and $NaBH_4$ (10 mg) were added to the extracted and concentrated CH_2Cl_2 layer obtained in procedure D, and the mixture was stirred for 30 min at room temperature. Excess $NaBH_4$ was quenched with water and the resulting mixture was extracted with CH_2Cl_2 (10 ml). The CH_2Cl_2 layer was dried over anhydrous K_2CO_3 and concentrated (*ca.* 0.5 ml). The solution was treated with BSTFA as in procedure B.

Liver Microsomes–Oxidant System Rat liver microsomes were prepared from non-treated male Wistar rats (5 weeks old, *ca.* 150 g) and from the rats that had been received intraperitoneal injection of sodium phenobarbital (PB, 60 mg/kg in saline) or 3-methylcholanthrene (3-MC, 30 mg/kg in olive oil) once daily for three consecutive days, followed by 24 h of starvation prior to killing by decapitation on the fourth day. Liver microsomes were also prepared from non-treated male ddY mice (6 weeks old). Their livers, perfused *via* the portal vein with cold 1.19% aqueous KCl were weighed, minced, and subsequently homogenized in 4 : 1 (v/w) cold sodium phosphate buffer (pH 7.4) in a Potter-Elvehjem type homogenizer. The homogenates were centrifuged at 9000 × g for 20 min at 4 °C. The supernatant fractions were centrifuged at 105000 × g for 1 h at 4 °C, then the microsomal pellet obtained was resuspended in 1 : 1 (v/w) cold sodium phosphate buffer (pH 7.4) and stored at −78 °C until use. The microsomal protein contents were assayed by Lowry's method using bovine serum albumin as the standard.[25]

An incubation mixture (3.0 ml, pH 7.4, 0.1 M sodium phosphate buffer) containing PCP–HCl (2 mM), microsomes (4 mg of protein), and iodosylxylene (or cumene hydroperoxide) (1 mM) was incubated for 20 min at 37 °C. The reaction was terminated and the mixture was worked up by one of the procedures outlined above. Procedure A was used for **5** and **7**. For **2**, **3**, **4**, and **8**, 10 ml of CH_2Cl_2 and 2 ml of saturated aqueous K_2CO_3 were added, and after shaking (10 min, 5 Hz, 5-cm strokes), the mixture was centrifuged (3000 rpm, 10 min). The CH_2Cl_2 layer was dried over anhydrous K_2CO_3 and concentrated. The solution was treated as in procedure B for **2**, **3**, and **4**, and procedure E for **8**.

Liver Microsomes–NADPH/O_2 System Liver microsomes used in this system were obtained as described above. An incubation mixture (2.5 ml, pH 7.4, 0.1 M sodium phosphate buffer) containing PCP–HCl (2 mM), microsomes (4 mg of protein), glucose-6-phosphate (G-6-P, 4 mM), glucose-6-phosphate dehydrogenase (G-6-P DHase, 10 unit), and NADP (0.4 mM) was incubated for 20 min at 37 °C. The reaction was terminated and the mixture was worked up as described above.

Analytical Procedure A 2 μl aliquot of the final solution was injected into the GC/MS system for quantitation. Calibration curves were prepared and the samples were quantitated using the JEOL MP-8300 software for automatic quantitation of selected ion monitoring data by area normalization on the peak for the internal standard; tribenzylamine with m/z 287 for the quantitation of **5** and **7**, or d_5-**4** with m/z 293 for the quantitation of **2**, **3**, and **4**.

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