Substrate-dependent changes of the oxidative *O*-dealkylation mechanism of several chemical and biological oxidizing systems

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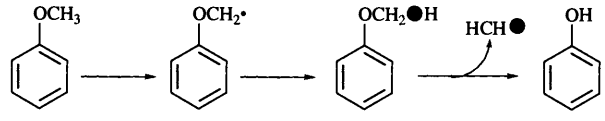
The *O*-dealkylation mechanisms of a series of alkyl aryl ethers, mediated by several chemical and biological oxidizing systems, *i.e.* Cu^{2+}–ascorbic acid–O_2, γ-radiolysis and rat liver microsomes–NADPH/O_2, were examined. In every oxidizing system, the *O*-dealkylation mechanisms changed dramatically depending on the nature of the substrates. In the Cu^{2+}–ascorbic acid–O_2 system and γ-radiolysis, electron density at the *ipso*-position and the ease of H atom abstraction from the alkyl moiety of the substrates were critical to determine the *O*-dealkylation mechanism. In the cytochrome P450-dependent monooxygenases, the determinant was whether or not the substrate has a phenolic hydroxy group at an *ortho*- or *para*-position relative to the alkoxy group. The results have led us to propose a new *O*-dealkylation mechanism involving the initial formation of a phenoxyl radical.

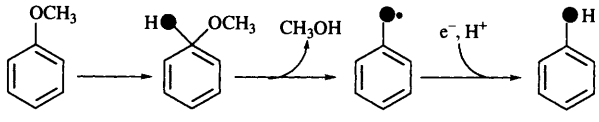
Introduction

Oxidative *O*-dealkylation of alkyl aryl ethers is one of the major metabolic reactions catalysed by cytochrome P450.[1,2] Many investigators have tried to clarify the mechanisms of this reaction using rat liver microsomes[3,4] and various model systems,[5] and there are two generally accepted mechanisms, *i.e.*, the H atom abstraction mechanism [reaction (1)] and the *ipso*-substitution mechanism [reaction (2)]. (In the reaction scheme,

Reaction 1



Reaction 2



● indicates that the oxygen atom originated from the active oxidizing intermediate.) For anisole the H atom abstraction mechanism involves hydroxylation of the α-carbon to give a hemiacetal *via* a phenoxymethyl radical, followed by breakdown to a phenol and formaldehyde [reaction (1)].[3,4] In this mechanism, the phenolic oxygen arises from the substrate, not from the active oxygen species. The *ipso*-substitution mechanism involves the initial formation of a hydroxycyclohexadienyl radical which is subsequently converted into a phenol *via* a phenoxyl radical with release of methanol [reaction (2)],[6] here, the phenolic oxygen arises from the active oxygen species. Many studies have been reported pointing out mechanistic differences among chemical model systems and biological oxidizing systems. So far, it is thought that the mechanism which actually operates depends on the oxidizing system used, namely in biological reactions such as the cytochrome P450-dependent enzymic reaction, the former mechanism operates, and in the hydroxyl radical-mediated reactions, the latter does.[5] However, little is known about the effects of different substrates and model compounds. We report herein that the mechanisms are dependent not only on the oxidizing system, but also largely on the characteristics of the substrates. That is, even in *in vitro* studies with rat liver microsomes, *O*-dealkylation of certain alkyl aryl ethers proceeds in the *ipso*-substitution manner, and even in the hydroxyl radical-mediated reaction, H atom abstraction can occur.

Here we used three oxidizing systems, namely rat liver microsomes–NADPH/O_2, Cu^{2+}–ascorbic acid–O_2 and γ-radiolysis. The Cu^{2+}–ascorbic acid–O_2 system is often used as an active oxygen species generating system to degrade biomolecules such as amino acids, proteins and DNA.[7–9] In contrast to other Fenton-like systems, oxidative conversion of *ortho*-methoxyphenols to catechols and hydroxylation of phenols to catechols and hydroquinones could be conducted with high selectivity in moderate yield with this system.[10] γ-Radiolysis was employed as a purely hydroxyl radical generating system. The γ-irradiation of water generates two main active species, the hydroxyl radical and the hydrated electron. Further, when irradiation is carried out under nitrous oxide, at pH > 5, the hydrated electron is converted into a hydroxyl radical *via* the following reactions: $e_{aq}^- + N_2O \longrightarrow N_2 + O^-$, $O^- + H^+ \longrightarrow {}^{\bullet}OH$. The resulting active species is therefore only a hydroxyl radical.[11]

To investigate the mechanism of the *O*-dealkylations, widely accepted techniques, *i.e.* ^{18}O labelling techniques and evaluation of intramolecular isotope effects (k_H/k_D) were used, and ^{18}O incorporation and k_H/k_D were determined by GC/SIM from the M^+ peak area ratio.

Experimental

Materials

Methoxyphenols, dimethoxybenzenes and 1,2-methylenedioxybenzene were purchased from Tokyo Kasei Kogyo Co. Ltd., ascorbic acid from Wako Pure Chemical Industries Ltd., cupric perchlorate hexahydrate from Nacalai Tesque, Inc., nitrous oxide from Showa Denko Co. Ltd., and cofactor-I from Oriental Yeast Co. Ltd. [$^2H_3$]Iodomethane (Aldrich Chemical Co. Ltd.) was 99.5% deuteriated. [^{18}O]Dioxygen gas and [^{18}O]water (both from Isotec Inc.) were 97.9% and 97.1% enriched, respectively. Acetone was distilled over potassium permanganate. All the substrates were purified on an alumina or silica column, or by being washed with aqueous NaOH (2 mol dm^{-3}), in order not to contaminate the corresponding *O*-dealkylation products.

Three trideuteriomethoxyanisoles (**14–16**) (substrates for estimating the kinetic isotope effects on the *O*-demethylation of dimethoxybenzenes) were prepared from the corresponding

methoxyphenol and [$^2H_3$]iodomethane in the usual way. 2-(Trideuteriomethoxy)-6-methoxyphenol (**17**) was prepared following Aihara *et al.*[10d] *o*-Benzyloxyanisole (**10**) was synthesized from *o*-methoxyphenol and benzyl bromide in the usual way.

Catechol monoformate (**13**) was extracted directly from the reaction mixture of the Cu^{2+}–ascorbic acid–O_2 system with methylene chloride. The organic layer was dried (Na_2SO_4), evaporated and dried *in vacuo*, to give a crude mixture of catechol monoformate, 1,2-methylenedioxybenzene and catechol. Because catechol monoformate was easily hydrolysed in the course of purification, the ^1H NMR spectrum of catechol monoformate was assigned from that of the crude mixture: δ_H(400 MHz, $CDCl_3$, Me_4Si) 8.33 [s, 1 H, O(CO)H], 7.13–7.18 (td, 1 H, J_o = 8.1 Hz, J_m = 1.5 Hz, Ar-H, dd, 1 H, J_o = 8.1 Hz, J_m = 1.5 Hz, Ar-H), 7.01 (dd, 1 H, J_o = 8.1 Hz, J_m = 1.5 Hz, Ar-H), 6.94 (td, 1 H, J_o = 8.1 Hz, J_m = 1.5 Hz, Ar-H); [lit.,[12] δ_H(90 MHz, $CDCl_3$) 8.8 (s, 1 H), 7.0–7.2 (m, 4 H)].

Cu^{2+}–Ascorbic acid–O_2 system

The *O*-dealkylation of various alkyl aryl ethers by the Cu^{2+}–ascorbic acid–O_2 system was carried out as follows. A yellow-green solution of a substrate (50 mM), cupric perchlorate hexahydrate (50 mM), and ascorbic acid (500 mM) in acetone–water was stirred vigorously under a pure oxygen atmosphere at room temperature.[10e] The yields of *O*-dealkylation products were determined by HPLC. HPLC was performed with a reverse-phase column (Tosoh TSKgel ODS-12OT, 4.6 × 150 mm) under the following conditions: eluent; acetonitrile–0.1% H_3PO_4 aq. = 1:3 (v/v), flow rate; 1.0 ml min^{-1}, detection; UV 280 nm.

γ-Radiolysis

γ-Ray irradiation experiments were performed in closed glass vessels containing 10 ml of buffered solution (0.2 M NaPi, pH 6.0) of a substrate (5 mM) and ascorbic acid (10 mM).[10e,13] Solutions were saturated with nitrous oxide before being placed in the ^{60}Co γ-source at the Research Center for Nuclear Science and Technology, University of Tokyo. The dose rate was *ca.* 20 Gy min^{-1}, and the total radiation dose was 600 Gy. All the products were extracted with methylene chloride.

Rat liver microsome–NADPH/O_2 system

Preparation of microsomes was carried out according to the following procedures. Rats (5 weeks, *ca.* 160 g) received intraperitoneal injection of 60 mg kg^{-1} sodium phenobarbital once daily for 3 days, then were starved overnight and killed by exsanguination from the abdominal aorta. The livers were removed immediately, perfused with ice-cold phosphate buffer (10 mM phosphate buffer containing 0.15 M KCl, pH 7.4) and homogenized in 3 vol of the same buffer. Microsomes were prepared according to the method of Omura and Sato.[14] The protein concentration of microsomal preparation was determined by the method of Lowry *et al.* with bovine serum albumin as a standard.[15]

The *O*-demethylation of various anisoles by the rat liver microsomes–NADPH/O_2 system was carried out as follows. The standard mixtures containing Cofactor-I (final concentration: 8.6 mM $MgCl_2$, 17.0 mM KCl, 2.6 mM G-6-P, 2.0 mM NADPH, 2.2 mM NADH, 42.1 mM Na_2HPO_4, 10.3 mM NaH_2PO_4), glucose-6-phosphate dehydrogenase (10 units), 5 mg of microsomal protein (10.8 nmol P450), phosphate buffer (0.1 M, pH 7.4) and a substrate (0.25 mM) were incubated at 37 °C for 15 min. The reaction mixture was chilled followed by addition of cold methylene chloride for extraction.

^{18}O Incorporation experiments (from $^{18}O_2$)

The reaction conditions in each oxidizing system were identical to those mentioned above, except that the reactions were carried out under an $^{18}O_2$–Ar (molar ratio *ca.* 1:4) atmosphere by employing a vacuum line. In each case, the *O*-dealkylation products were extracted with methylene chloride and trimethylsilylated with a bis(trimethylsilyl)trifluoroacetamide–trimethylsilyl chloride–pyridine = 5:1:4 (v/v/v) mixture before analysis. GC/SIM analyses were conducted by use of a Hewlett-Packard 5890 series II gas chromatograph with a 30 m × 0.25 mm × 0.25 mm RTX-5 column (Restek Co.) and a JEOL JMS-SX102A mass spectrometer. ^{18}O Incorporation values were calculated from [^{18}O]product/[^{16}O]product ratios and the values were corrected by considering the actual ^{18}O content of $^{18}O_2$ used and the isotopic natural abundance.

^{18}O Incorporation experiments (from $H_2{}^{18}O$)

The reaction and analytical conditions were identical to those mentioned above, except that the reactions were carried out under a $^{16}O_2$ atmosphere in aqueous medium containing $H_2{}^{18}O$.

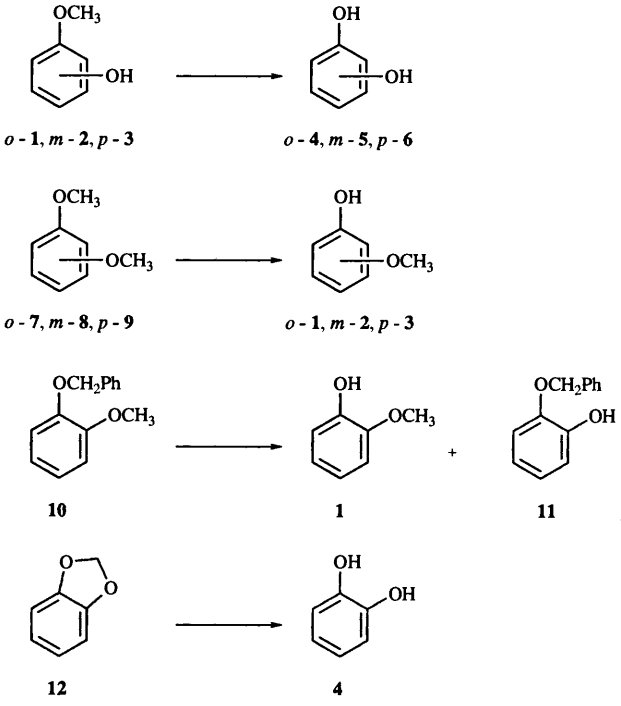
Kinetic isotope effect (k_H/k_D) experiments

The reaction and analytical conditions were identical to those used in the ^{18}O incorporation experiments, except that anisoles bearing a trideuteriomethoxy moiety were used as substrates. The k_H/k_D values were calculated from [$^2H_3$]product/[H_3]-product ratios and the values were corrected by considering the isotopic natural abundance.

Results and discussion

Cu^{2+}–Ascorbic acid–O_2 system (*O*-demethylation)

In the Cu^{2+}–ascorbic acid–O_2 system, methoxyphenols and dimethoxybenzenes were *O*-demethylated in moderate yield and with high selectivity (almost 100% yield based on consumed substrates), except for the *meta*-isomers (Table 1). When



meta-isomers (**2**, **8**) were used as substrates, some aromatic hydroxylation products were also formed, so the selectivities were low (runs 2, 5). For example, *m*-dimethoxybenzene (**8**) was converted not only to *m*-methoxyphenol (**2**) (yield = 4.7%), but also to 2,4- and 2,6-dimethoxyphenol (yield = 3.1% and 3.0%, respectively).

To investigate the *O*-demethylation mechanisms in the Cu^{2+}–ascorbic acid–O_2 system, ^{18}O incorporation into the *O*-demethylation products from $^{18}O_2$ was measured. In the case of *ortho*- and *para*-isomers (**1**, **3**, **7**, **9**), high ^{18}O incorporations

Table 1 *O*-Dealkylation of various alkyl aryl ethers by the Cu^{2+}–ascorbic acid–O_2 system[a]

Substrate	Product	Substituent position	Yield (%)	Conversion (%)	^{18}O Incorporation from $^{18}O_2$ (%)
O-Demethylation of methoxyphenols					
1	**4**	*ortho*	20.0	100	90
2	**5**	*meta*	5.7	25	33
3	**6**	*para*	20.3	93	66
O-Demethylation of dimethoxybenzenes					
7	**1**	*ortho*	18.7	94	77
8	**2**	*meta*	4.7	33	17
9	**3**	*para*	17.2	93	65
O-Dealkylation of *ortho*-disubstituted benzenes					
		Reaction type			
10	**11**	*O*-Demethylation	4.7	30	70
	1	*O*-Debenzylation	5.1	33	35
	PhCHO		3.2		
12	**4**	*O*-Demethylenation	16.0	69	11

[a] These reactions were carried out under the following conditions. For the determination of yield, a mixture of a substrate (50 mM), $Cu(ClO_4)_2$ (50 mM) and ascorbic acid (500 mM) in acetone–water was stirred under a pure O_2 atmosphere at room temperature for 24 h. Yields were determined by HPLC. For the evaluation of ^{18}O incorporation into the *O*-dealkylation products from $^{18}O_2$, reaction conditions were identical to those described above, except that stirring was done under an $^{18}O_2$–Ar mixture for 2–4 h. All the products were extracted with chloroform and trimethylsilylated before analysis. ^{18}O Incorporation was determined by GC/SIM based on M^+ peak area ratio.

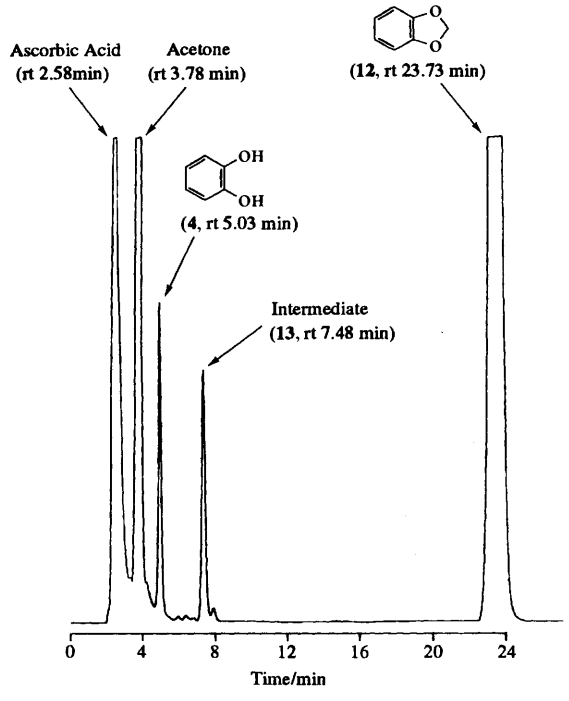


Fig. 1 HPLC Profile of *O*-demethylenation of 1,2-methylenedioxybenzene (**12**) by the Cu^{2+}–ascorbic acid–O_2 system

from $^{18}O_2$ were observed, which indicated that the reactions proceeded predominantly by the *ipso*-substitution mechanism (Table 1). On the other hand, *meta*-isomers (**2**, **8**) were *O*-demethylated with low ^{18}O incorporations from $^{18}O_2$. In the case of these *meta*-isomers (**2**, **8**), ^{18}O incorporations from $H_2{}^{18}O$ were also examined and it turned out that no oxygen atom of the resulting phenolic hydroxy group had arisen from water (data not shown). Thus, the *O*-demethylation of *m*-methoxyphenol (**2**) and *m*-dimethoxybenzene (**8**) proceeded mainly by the H atom abstraction mechanism.

These substrate-dependent changes of the *O*-demethylation mechanism were supposed to depend on the electron-density at the *ipso*-position of the substrates. As both methoxy and hydroxy groups are strong electron-donating groups, the electron-density at the *ipso*-position of *ortho*- and *para*-isomers is expected to be high. So *ipso*-addition of the oxidative intermediate is fast, and *O*-demethylation proceeds in the *ipso*-substitution manner. On the other hand, the electron-density at the *ipso*-position of *meta*-isomers is low, so H atom abstraction predominates. Instead, the electron-densities at the 2,4- and 6-positions of *meta*-isomers are high, so aromatic hydroxylated products were obtained in relatively high yields.

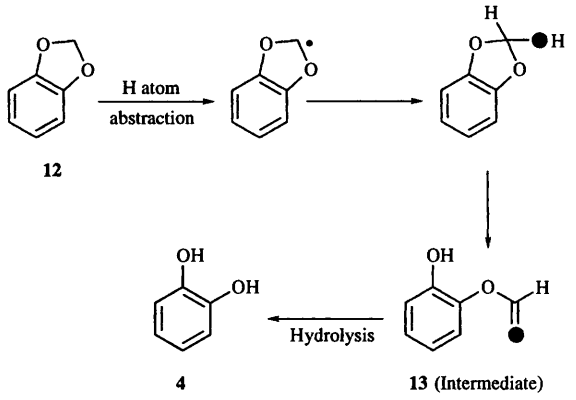
Thus, the electron-density at the *ipso*-position influences the *O*-demethylation mechanism in the Cu^{2+}–ascorbic acid–O_2 system.

Cu^{2+}–Ascorbic acid–O_2 system (*O*-debenzylation and *O*-demethylenation)

O-Dealkylation of *o*-benzyloxyanisole (**10**) and 1,2-methylenedioxybenzene (**12**) by the Cu^{2+}–ascorbic acid–O_2 system was examined. In both cases, the major reaction was *O*-dealkylation (Table 1). It is thought that the *ipso*-addition of the oxidative intermediate to these two substrates is as fast as to *o*-dimethoxybenzene, because they are also *o*-dialkoxybenzenes and have similar electron-densities at the *ipso*-position. In contrast, they are dissimilar in terms of the ease of H atom abstraction from the alkyl moiety.[16]

In the Cu^{2+}–ascorbic acid–O_2 system, *o*-benzyloxyanisole (**10**) was *O*-demethylated to give *o*-benzyloxyphenol (**11**) with high ^{18}O incorporation from $^{18}O_2$, while it was *O*-debenzylated to give *o*-methoxyphenol (**1**) with low ^{18}O incorporation (Table 1). These data suggested that *O*-debenzylation proceeded mainly by the H atom abstraction mechanism. This was also supported by the yield of benzaldehyde, the final form of the benzyl moiety in the case of the H atom abstraction mechanism. The yield of benzaldehyde based on the *O*-debenzylation product, was 64%. This value directly gives the ratio of the H atom abstraction mechanism, and agrees well with the value calculated from the ^{18}O incorporation from $^{18}O_2$, which gives the ratio of the *ipso*-substitution mechanism.

1,2-Methylenedioxybenzene (**12**) was *O*-demethylenated with lower ^{18}O incorporation from $^{18}O_2$, which showed that the H atom abstraction mechanism prevailed. Further, it was observed that the *O*-demethylenation of **12** proceeded mainly *via* an intermediate which was detected by HPLC (Fig. 1). The intermediate was readily hydrolysed in acidic water (pH 1) to give catechol. From these results and its ^1H NMR spectrum, the intermediate was concluded to be catechol monoformate (**13**). This observation also suggested the predominance of the H atom abstraction mechanism (Scheme 1).

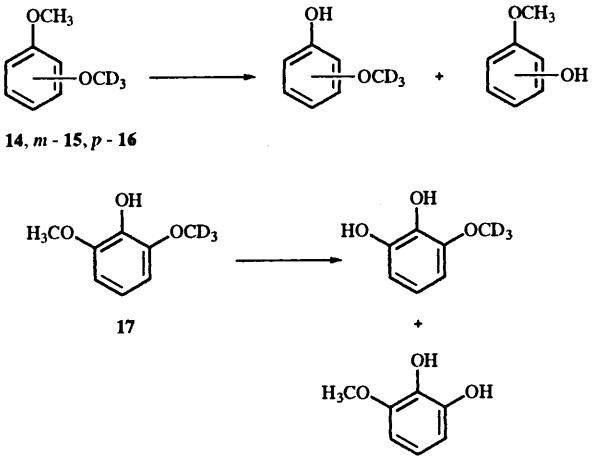


Scheme 1 H atom abstraction mechanism in the *O*-demethylenation of 1,2-methylenedioxybenzene (**12**) by the Cu^{2+}–ascorbic acid–O_2 system

As described above, the ease of H atom abstraction from the alkyl moiety, as well as the electron density at the *ipso*-position, influences the mechanism of *O*-dealkylation.

γ-Radiolysis

The mechanisms of hydroxyl radical-mediated *O*-demethylation of dimethoxybenzenes and 2,6-dimethoxyphenol were examined by using the γ-radiolysis technique and compared to that of the Cu^{2+}–ascorbic acid–O_2 system. The involvement of *O*-demethylation mechanisms was estimated from the intramolecular isotope effects (k_H/k_D). In the case of *ortho*-, *para*-trideuteriomethoxyanisoles (**14**, **16**) and 2-(trideuteriomethoxy)-6-methoxyphenol (**17**), low intramolecular isotope effect (k_H/k_D) values were obtained in both γ-radiolysis and the Cu^{2+}–ascorbic acid–O_2 system (Table 2). These data clearly showed that the reaction proceeded mainly by the *ipso*-substitution mechanism. On the other hand, in the case of *m*-trideuteriomethoxyanisole (**15**), slightly higher k_H/k_D values were obtained (Table 2). These values suggested that in



both systems, the ratio of the H atom abstraction mechanism was increased in the *O*-demethylation of *m*-dimethoxybenzene because of its low electron-density at the *ipso*-position, in accordance with the conclusion based on the ^{18}O incorporation data. In this regard, these two oxidizing systems resemble each other.

The k_H/k_D values obtained in the Cu^{2+}–ascorbic acid–O_2 system were always slightly larger than those in γ-radiolysis. It is considered that the oxidizing intermediate of the Cu^{2+}–ascorbic acid–O_2 system has more powerful H atom abstracting ability or less powerful *ipso*-addition ability. This implies that the oxidizing intermediate of the Cu^{2+}–ascorbic acid–O_2 system is not a hydroxyl radical but a hydroxyl radical equivalent intermediate, such as a crypto-type hydroxyl radical.

Table 2 Intramolecular isotope effects[a] on the *O*-demethylation reactions in the Cu^{2+}–ascorbic acid (AA)–O_2 system, γ-radiolysis and the rat liver microsome (Ms)–NADPH/O_2 system

	k_H/k_D, Oxidizing system		
Substrate	Cu^{2+}–AA–O_2	γ-Radiolysis	Ms–NADPH/O_2[b]
14	1.0	1.0	6.2 ± 0.1
15	2.2	1.3	12.6 ± 0.4
16	1.3	1.1	11.9 ± 0.1
17	1.4	1.1	1.0 ± 0.0

[a] Kinetic isotope effect (k_H/k_D) was determined by GC/SIM based on M^+ peak area ratio. [b] The data represent the mean ± s.e. of three experiments.

Table 3 ^{18}O Incorporation into *O*-demethylation products by the rat liver microsomes–NADPH/O_2 system[a]

			^{18}O Incorporation (%)	
Substrate	Product	Substituent position	from $^{18}O_2$	from $H_2{}^{18}O$
Methoxyphenols				
1	**4**	*ortho*	>99	n.d.[b]
2	**5**	*meta*	3	3
3	**6**	*para*	84	n.d.
Dimethoxybenzenes				
7	**1**	*ortho*	4	<1
8	**2**	*meta*	<1	<1
9	**3**	*para*	1	<1

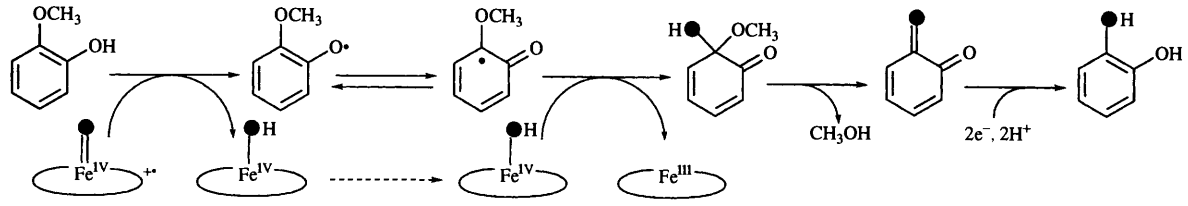
[a] These reactions were carried out under an $^{18}O_2$–Ar mixture at 37 °C for 10 min. Analytical conditions were identical to those described in Table 1. [b] Not determined.

Rat liver microsome–NADPH/O_2 system

We first examined the *O*-demethylation mechanism in the rat liver microsome–NADPH/O_2 system using intramolecular isotope effects (k_H/k_D). Trideuteriomethoxyanisoles (**14**–**16**) were all *O*-demethylated with high k_H/k_D values (Table 2), suggesting that the reactions proceeded by the H atom abstraction mechanism. On the other hand, in the case of 2-(trideuteriomethoxy)-6-methoxyphenol (**17**), a low k_H/k_D value was obtained (Table 2). This result shows that *ipso*-substitution can occur even in *in vitro* studies with rat liver microsomes, a system in which only the H atom abstraction mechanism has previously been believed to operate. To clarify precisely the substrate dependencies of the *O*-demethylation mechanism, ^{18}O incorporation experiments were conducted using dimethoxybenzenes and methoxyphenols as substrates. Dimethoxybenzenes (**7**–**9**) were all *O*-demethylated with low ^{18}O incorporation from $^{18}O_2$ and with no ^{18}O incorporation from $H_2{}^{18}O$ (Table 3), which indicated that the reactions proceeded by the H atom abstraction mechanism. These results were compatible with those obtained using kinetic isotope effects. In the case of methoxyphenols, only *ortho*- and *para*-isomers (**1**, **3**) were *O*-demethylated with high ^{18}O incorporation from $^{18}O_2$ (Table 3), that indicated the reactions proceeded in the *ipso*-substitution manner. On the other hand, *m*-methoxyphenol (**2**) was *O*-demethylated with low ^{18}O incorporation either from $^{18}O_2$ or from $H_2{}^{18}O$ (Table 3), and it has become apparent that the reaction proceeded by the H atom abstraction mechanism. In summary, the *O*-dealkylation mechanisms in the rat liver microsome–NADPH/O_2 system depend on (*a*) whether the substrate has a phenolic hydroxy group or not, and (*b*) the position of the phenolic hydroxy group.

The above-mentioned substrate-dependent changes of the *O*-dealkylation mechanisms cannot be explained in terms of

Reaction 3



reactions (1) and (2). Here we propose a new *O*-dealkylation mechanism involving the initial formation of a phenoxyl radical [reaction (3)] in the cytochrome P450-dependent monooxygenase reaction for alkyl aryl ethers bearing a phenolic hydroxy group at an *ortho*- or *para*-position relative to the alkoxy group. The resulting phenoxyl radicals delocalize at the *ipso*-position, and the compound II equivalent rebinds to it. Elimination of alcohol gives benzoquinone derivatives and they are reduced NADPH-dependently to *O*-dealkylation products. As an active oxygen atom is incorporated into the *O*-dealkylation product, this mechanism is classified as an *ipso*-substitution mechanism, but it is apparently different from the known *ipso*-substitution mechanism involving initial addition to the *ipso*-position [reaction (2)]. Very recently, a similar mechanism was proposed by Koymans *et al.* for the cytochrome P450-mediated oxidation of 2,6-di-*tert*-butyl-4-methylphenol (BHT).[17] However, the present paper is the first to point out that the third mechanism, reaction (3), operates in the *O*-dealkylation of certain alkyl aryl ethers, on the basis of precise examination of substrate-dependent changes of the *O*-dealkylation mechanisms.

Conclusions

We have precisely examined the *O*-dealkylation mechanisms of various alkyl aryl ethers using an ^{18}O labelling technique and kinetic isotope effects. In every chemical and biological oxidizing system examined, the *O*-dealkylation mechanisms changed dramatically depending on the nature of the substrates. In the hydroxyl radical (or its equivalent)-generating systems, electron density at the *ipso*-position and the ease of H atom abstraction from the alkyl moiety of the substrates were critical to determine the *O*-dealkylation mechanism. In the cytochrome P450-dependent monooxygenase system, the determinant is whether or not the substrate has a phenolic hydroxy group at an *ortho*- or *para*-position relative to the alkoxy group. These results have led us to propose a new *O*-dealkylation mechanism involving the initial formation of a phenoxyl radical [reaction (3)].

These substrate-dependent changes of the *O*-dealkylation mechanism can be utilized as a basis for the precise classification of the chemical models of cytochrome P450 and biological oxidizing systems. From this viewpoint, the classification of various iron porphyrins-oxidant systems, which are among the most commonly used model systems for cytochrome P450, is in progress.

Acknowledgements

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