Model Systems for Cytochrome P450 Dependent Mono-oxygenases. Part 2.[1,2] Kinetic Isotope Effects for the Oxidative Demethylation of Anisole and [*Me*-$^2H_3$]Anisole by Cytochrome P450 Dependent Mono-oxygenases and Model Systems

John R. Lindsay Smith * and Paul R. Sleath
Department of Chemistry, The University of York, York YO1 5DD

Anisole, [*Me*-$^2H_3$]anisole, and [^{18}O]anisole have been used as substrates to study the mechanisms of oxidative demethylation by model systems for the cytochrome P450 dependent mono-oxygenases. The size of the kinetic isotope effect for the demethylation can be used as a sensitive probe of the oxidation mechanism and as a method for classifying the chemical systems. By this procedure 17 mono-oxygenase models and four microsomal systems have been examined. Two systems involving iron(III) porphyrins and iodosylbenzene show large kinetic isotope effects comparable with those from the microsomal oxidations and can be considered as good models for the biological process. The remaining systems exhibit smaller isotope effects, k_H/k_D 1—3.4. Alternative mechanisms for the oxidative demethylations are discussed and the major routes are shown to be either radical *ipso*-substitution or attack on the C–H bond of the methoxy-group.

Oxidative *O*-demethylation is one of the several types of oxidation brought about by the cytochrome P450 dependent mono-oxygenases.[3] For phenolic ethers the generally accepted mechanism involves hydroxylation of the α-carbon to give a hemi-acetal (1) which subsequently breaks down to a phenol and an aldehyde or ketone [3a] [reaction (1)]. Analogous schemes have been proposed for enzymic *N*- and *S*-oxidative dealkylation.[3h,4]

Support for this mechanism comes from [^{18}O] labelling and kinetic isotope effect studies. In the oxidative *O*-demethylation of 4-methoxyacetanilide with rat liver microsomes in the presence of $^{18}O_2$ or $H_2{}^{18}O$ none of the label is incorporated in the product 4-hydroxyacetanilide.[5] These results show that cleavage of the *O*-alkyl bond must occur in the reaction and that the alkoxy-group is not displaced by the incoming hydroxy in an *ipso*-substitution.

In vitro studies with aryldeuteriomethyl ethers have shown that the oxidative demethylation proceeds with a kinetic isotope effect (k_H/k_D 2—10).[6] Although there is a wide range of values for the isotope effect the data clearly indicate that C–H bond cleavage is occurring during enzymic *O*-dealkylation.

In contrast with the biological reactions above, pulse radiolysis studies suggest that the demethylation of 1,4-dimethoxybenzene by the hydroxyl radical proceeds by *ipso*-substitution *via* the intermediate hydroxycyclohexadienyl radical (2; R = OCH_3) [reaction (2)].[7]

This difference in behaviour towards arylmethyl ethers by the hydroxyl radical and the active oxidant in the cytochrome P450 dependent mono-oxygenases (this is currently thought to act as an oxyl radical [3g,8]) led us to examine the oxidative *O*-demethylation of anisole, [*Me*-$^2H_3$]anisole, and [^{18}O]anisole by a selection of model systems. We report here the results from this study and how measurement of the kinetic isotope effect for demethylation provides a simple method for classifying the model systems.

Results

Measurement of the Kinetic Isotope effect for the Oxidative Demethylation of Anisole and [Me-$^2H_3$]*Anisole.*—(a) *Rat liver microsomes.* The major products from the oxidation of anisole with rat liver microsomes in the presence of NADPH † and dioxygen are phenol and 4-methoxyphenol as reported by

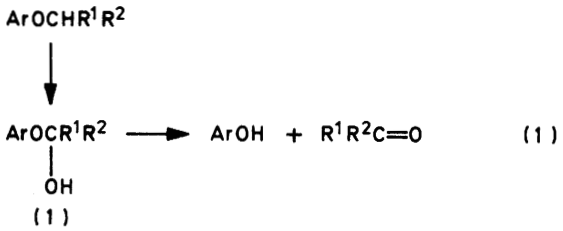
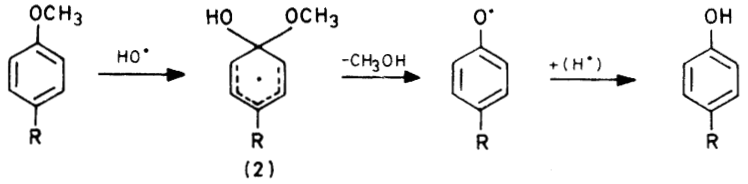


Table 1. Yields of phenol and 4-methoxyphenol from the *in vitro* metabolism of anisole and [*Me*-$^2H_3$]anisole with rat liver microsomes–NADPH–dioxygen

Substrate	Incubation time (min)	Yield of phenol (μmol)	Yield of 4-methoxyphenol (μmol)
Anisole	15	0.183	0.498
[*Me*-$^2H_3$]Anisole	15	0.025	0.714
Anisole	30	0.214	0.615
[*Me*-$^2H_3$]Anisole	30	0.028	0.900
Anisole	60	0.235	0.609
[*Me*-$^2H_3$]Anisole	60	0.034	0.890
Anisole	60	0.221	0.698
[*Me*-$^2H_3$]Anisole	60	0.027	0.906
Anisole	60	0.244	0.629
[*Me*-$^2H_3$]Anisole	60	0.036	0.865

incubations performed at 0 °C, or with heat denatured microsomes or when anisole was omitted from the reaction. The results show that after 30 min incubation the rate of oxidation becomes very slow. The 60 min incubation was repeated with three separate batches of microsomes and the results show good reproducibility. Pairs of identical reaction mixtures, one containing anisole and the other [*Me*-$^2H_3$]anisole, were used to obtain the kinetic isotope effects. The yield of phenol from the deuterioanisole is markedly less than that from anisole revealing a large kinetic isotope effect for demethylation.

† The following abbreviations are used in this paper: NADP and NADPH for nicotinamide adenine dinucleotide and the reduced coenzyme, EDTA, ethylenediaminetetra-acetic acid disodium salt, Fe^{III}TPPCl tetraphenylporphinatoiron(III) chloride, and Fe^{III}-



(2)

(2)

Furthermore the yield of 4-methoxyphenol from [*Me*-$^2H_3$]-anisole is significantly larger than that from the undeuteriated substrate.

The magnitude of the kinetic isotope effect obtained from a comparison of the absolute yields of phenol from each pair of reactions is independent of the incubation time, k_H/k_D 7.4. Alternatively, the isotope effect can be calculated from the yield of phenol relative to that of 4-methoxyphenol from a pair of reactions. The latter method assumes that deuteriation of the methoxy-group of anisole has no influence on the yield of 4-methoxyphenol and uses the yield of this product as an internal standard for the reaction. It was assumed that the latter method, which should eliminate minor differences in enzyme activity, would give a better measure of the kinetic isotope effect, k_H/k_D 10.6. However, in view of the observed increase in 4-hydroxylation with methoxy-deuteriation this assumption may be invalid.

The oxidations with rat liver microsomes were repeated with added oxidants (iodosylbenzene, t-butyl hydroperoxide, or 3-chloroperbenzoic acid) in the absence of NADPH. As described above, the experiments were carried out in pairs, one containing anisole and the other [*Me*-$^2H_3$]anisole. The initial rates of oxygenation with these systems are higher than those with NADPH and dioxygen but the oxidations are only sustained for a short time. Thus the yields are low and give rise to larger errors in the product analyses. Indeed the quantity of phenolic products from the iodosylbenzene supported system was too low to quantify the isotope effect. The results from these modified microsomal systems, calculated as described above, are the same within experimental error as those from the NADPH–dioxygen supported system (Table 2). The modified systems also show the effect of increased yield of 4-methoxyphenol when [*Me*-$^2H_3$]anisole is used in place of anisole.

(b) *Chemical model systems for cytochrome* P450 *dependent mono-oxygenases.* Anisole and [*Me*-$^2H_3$]anisole were oxidised with chemical model systems and the kinetic isotope effects for demethylation were obtained. Since deuteriation of the methoxy-group of anisole should not influence the rate of 2-hydroxylation we used the yield of 2-methoxyphenol as an internal standard for each oxidation. Thus the kinetic isotope effects were obtained by comparing the ratio of the yields of phenol (demethylation) and 2-methoxyphenol (hydroxylation) from equivalent oxidations of the two substrates. This method of analysis is simple and convenient since the two oxidation products have similar retention times using g.c. analysis and it minimises errors arising from variable yields of oxidation.

We investigated 17 model systems and kinetic isotope effects from oxidations by 13 of these are given in Table 3. Each value of k_H/k_D is the average of all the analyses from at least two experiments and the quoted errors represent the spread of the results from these analyses. The large error for Groves' system and the approximate value for the modified system with Fe^{III}TFPPCl reflect the low yield of phenolic products from these oxidations. This, coupled with a large kinetic isotope effect, gives low yields of phenol from the deuteriated anisole and poor quantitation of the results.

Unlike the enzymic systems, none of the chemical models shows an increase in the yield of methoxyphenols when [*Me*-

Table 2. Kinetic isotope effects for the oxidative demethylation of anisole and [*Me*-$^2H_3$]anisole with rat liver microsomes supported by a selection of oxidants

Oxidant	Incubation time (min)	k_H/k_D from ratio of phenol yields	k_H/k_D from ratio of relative yield of phenol to 4-methoxyphenol
NADPH–O_2	15	7.3 ± 0.9	10.5 ± 0.7
NADPH–O_2	30	7.6 ± 0.9	11.2 ± 0.7
NADPH–O_2	60	7.3 ± 0.9	10.0 ± 0.7
t-Butyl hydroperoxide	10	6.0 ± 2.0	12.3 ± 2.5
3-Chloroperbenzoic acid	10	5.8 ± 1.8	8.4 ± 3.0

Table 3. Kinetic isotope effects for the demethylation of anisole and [*Me*-$^2H_3$]anisole by chemical model systems for the cytochrome P450 dependent mono-oxygenases

Oxidising system	k_H/k_D [a]
Fe^{2+}–H_2O_2 (Fenton's reagent) [10]	1.0 ± 0.1
Fe^{2+}–H_2O_2–CH_3CN (Non-aqueous Fenton's reagent) [11]	1.0 ± 0.3
Fe^{3+}–H_2O_2–catechol (Hamilton's system) [12]	1.3 ± 0.1
Fe^{2+}–EDTA–ascorbic acid–O_2 (Udenfriend's system) [13]	1.2 ± 0.1
Fe^{2+}–EDTA–O_2 [14]	1.0 ± 0.1
Fe^{3+}–*N*-benzyl-1,4-dihydronicotinamide–O_2 [14]	1.2 ± 0.1
Reduced flavin mononucleotide–O_2 [15]	1.0 ± 0.1
Fe^{2+}–2-mercaptobenzoic acid–O_2 (Ullrich's system) [16]	2.2 ± 0.1
Trialkylphosphite–*hν*–O_2 [17]	2.1 ± 0.2
Sn^{2+}–pyrophosphate–O_2 [18]	2.1 ± 0.1
Diazofluorene–*hν*–O_2 [19]	3.4 ± 0.4
Tetraphenylporphinatoiron(III) chloride–PhIO (Groves' system) [20]	9.0 ± 3.0
Tetrakis(pentafluorophenyl)porphinatoiron(III) chloride–PhIO [21]	8.0

[a] The kinetic isotope effect was calculated from yields of phenol and 2-methoxyphenol (see text).

Four of the model systems were not amenable to the analysis described above. (i) Trifluoroperacetic acid [22] hydroxylates anisole in high yield but does not bring about demethylation. (ii) The photoactivation of pyridine *N*-oxide gives both phenol and methoxyphenols with anisole in aqueous solution as reported by Jerina *et al.*[23] However, the low pressure u.v. source needed for the photoactivation also brings about the photodemethylation of anisole. The phenol from this reaction of anisole accounts for most, if not all, of the phenol yield from the model system. (iii) The oxidation of anisole in the vapour phase with triplet oxygen atoms, $O(^3P)$, from the mercury-photosensitised decomposition of nitrous oxide [24] gives phenol and methoxyphenols but, as with the *N*-oxide system above, the former product comes predominantly, if not entirely, from the photodecomposition of anisole. (iv) Attempts to oxidise anisole with a carbonyl oxide generated by the photosensitised formation of singlet dioxygen, $^1O_2$, in the presence of diazofluorene [25] were unsuccessful. No phenolic products were

Table 4. [^{18}O] Content of 2-methoxyphenol and phenol produced from the oxidation of [^{18}O]anisole (3.19 ± 0.2%) by model systems for the cytochrome P450 dependent mono-oxygenases

Model system	[^{18}O] content of 2-methoxyphenol (%)	[^{18}O] content of phenol (%)	Loss of [^{18}O] in oxidative demethylation (%)
Fe^{2+}–H_2O_2 (Fenton's reagent)	3.1	0.3	91
Fe^{2+}–EDTA–ascorbic acid (Udenfriend's system)	3.0	0.0	100
Fe^{3+}–H_2O_2–catechol (Hamilton's system)	3.2	0.7	78
Sn^{2+}–pyrophosphate–O_2	3.2	2.9	9

Demethylation of [^{18}O]*Anisole with Model Systems.*—The origin of the oxygen in the phenol from demethylation of anisole by four of the model systems namely, Fenton's reagent and Udenfriend's, Hamilton's, and the tin(II)–pyrophosphate–dioxygen systems was determined by the use of [^{18}O]anisole as substrate. These systems which were selected as representatives of oxidants exhibiting small or medium-sized isotope effects, only require small amounts of substrate to produce sufficient phenol for g.c.–m.s. analysis. Table 4 shows that no [^{18}O] label is lost during the formation of 2-methoxyphenol. However, for three of the systems most, if not all, the label is removed during demethylation. With the fourth model, tin(II)–pyrophosphate–dioxygen, 91% of the isotopic label is retained in the phenol.

Discussion

Although it is generally accepted that *O*-demethylation of arylmethyl ethers by cytochrome P450 dependent mono-oxygenase occurs *via* a hemi-acetal intermediate (1), the mechanism of the latter oxygenation remains unclear. The formation of phenol from anisole by model systems has been noted but the mechanism of this reaction has received very limited attention.

Six possible routes for the oxidative *O*-demethylation are given below [reactions (3)—(5) and (7)—(9)]. All these need to be considered for the model systems; however, only reactions (3), (4), and (7) involve an intermediate hemi-acetal and are possible alternatives for the biological process.

Reaction (3).—Oxygen is inserted directly into the methoxy C–H bond by a singlet oxenoid species to give the hemiacetal (1; $R^1 = R^2 = H$).

Reaction (4).—Hydrogen is abstracted from the methoxy-group (most probably as a hydrogen atom) followed by hydroxylation of the aryloxymethyl radical (3).

Reaction (5).—*ipso*-Hydroxylation by an oxy-radical results in a cyclohexadienyl intermediate (4) which is aromatised to give phenol *via* the phenoxyl radical.

Reaction (6).—Electron abstraction gives the anisole radical cation (5) which might be demethylated by one of three mechanisms [reactions (7)—(9)].

Reaction (7).—This involves loss of a proton from the methoxy-group followed by hydroxylation as in reaction (4).

Reaction (8).—*ipso*-Hydroxylation of the radical cation gives a hydroxycyclohexadienyl radical (1; $R^1 = R^2 = H$) which is aromatised by loss of methanol to give the phenoxyl radical.

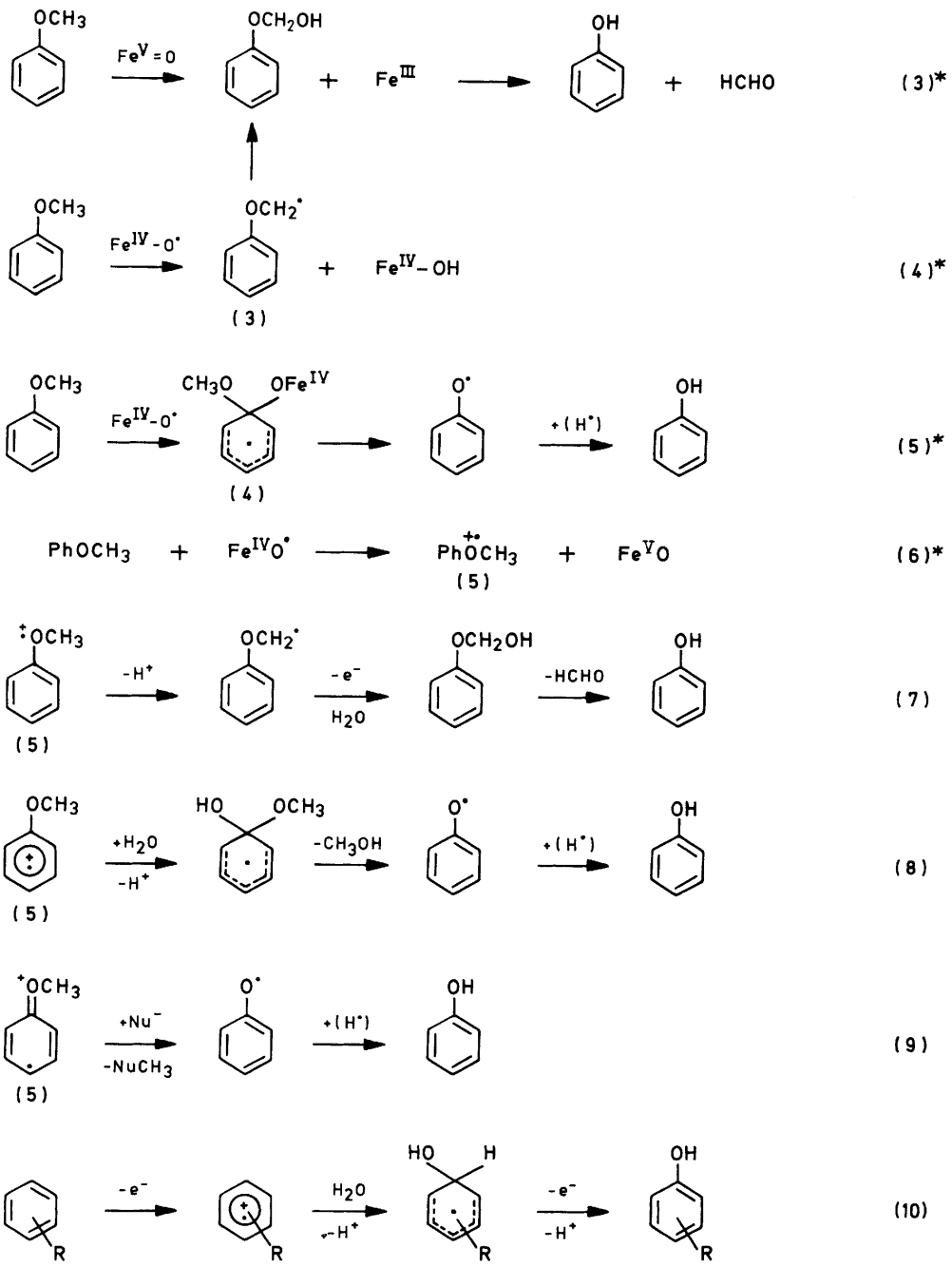
Reaction (9).—Nucleophilic substitution on the methyl group of the radical cation gives the phenoxyl radical directly. This last process is analogous to the non-oxidative dealkylation of arylmethyl ethers by nucleophiles with acid catalysis.

Oxidative *N*- and *S*-demethylations by electron-transfer pathways analogous to reactions (6) and (7) are well documented for model systems [26] and have been proposed for the biological processes also.[4,6c,27] However, these oxidations should occur more easily for tertiary amines and sulphides which have lower oxidation potentials than the corresponding ethers. The hydroxylation of an aromatic compound by reaction of its radical cation with water [reaction (10), analogous to the first step of reaction (8)] has been observed for the reactions of benzene and toluene with strong oxidants.[28] Very recently Torii *et al.*[29] proposed *ipso*-hydroxylation of the 4-methoxytoluene radical cation leading to the 4-methylphenoxyl radical [*cf.* reaction (8)] as a pathway in the oxidation of 4-methoxytoluene by cerium(IV) ammonium nitrate in aqueous methanol or acetic acid. However, Eberhardt reports that the persulphate radical anion does not hydroxylate anisole [28a] and he suggests that the radical cation from anisole is not susceptible to nucleophilic attack by water. O'Neill *et al.*[30] who reach a similar conclusion about the hydration of the anisole radical cation from pulse radiolysis studies, also suggest that the removal of a proton from the radical cation to give the phenoxymethyl radical [reaction (7)], in an analogous manner to the radical cations of methylbenzenes, is an unfavourable process. We conclude that it is unlikely that anisole is oxidised by an electron-transfer process either in the biological system, in agreement with Oae and his co-workers,[6c] or in the model systems.

We argued that the remaining three mechanisms [reactions (3)—(5)] might be distinguishable by a combination of [^{18}O] labelling and kinetic isotope effect studies. Thus, for the direct insertion and hydrogen abstraction processes the phenolic oxygen is that in the starting anisole but the latter process might show a larger kinetic isotope effect, the value depending on the extent of C–H bond breakage in the transition state.[31] By analogy with C–H insertion with singlet carbenes the kinetic isotope effect for an oxene insertion would be small, k_H/k_D *ca.* 1—2.5.[32] The *ipso*-substitution should not show a kinetic isotope effect and the oxygen of the anisole would be lost in forming phenol.

Microsomal Oxidative O-*Demethylation.*—The microsomal demethylations, whether supported by NADPH–dioxygen, t-butyl hydroperoxide, or 3-chloroperbenzoic acid, show large kinetic isotope effects. The effect is independent of incubation time and within experimental error, which is large for the peroxide and peroxyacid supported systems, it is also independent of the oxygen source.

The value obtained from a direct comparison of the yield of phenol from separate experiments with anisole and [*Me*-$^2H_3$]-anisole (k_H/k_D 7.4) is an intermolecular isotope effect. This value is larger than the intermolecular isotope effects for *O*-demethylation reported for a selection of arylmethyl ethers (k_H/k_D *ca.* 2).[6a,b] However, recently Watanabe *et al.*[6c] obtained a value of 5.1 for the monodemethylation of 1,4-dimethoxybenzene and [*Me*-$^2H_6$]-1,4-dimethoxybenzene with rabbit liver microsomes. The absence of an observable kinetic isotope effect or its suppression in intermolecular competition



* Reactions (3)—(6) are illustrated with the iron-oxy-species considered to be the active oxidant in the cytochrome P450 mono-oxygenase.[8] This is abbreviated to $Fe^{V}=O$ and $Fe^{IV}-O\cdot$

experiments with mono-oxygenases can be attributed to the oxidation being part of a multistep process in which the rate-determining step may not be the C–H bond cleavage of the substrate.[33]

The kinetic isotope effect obtained by comparing the ratio of the yields of phenol and 4-methoxyphenol from each substrate is less easily defined. It is an intermolecular effect if demethylation and hydroxylation are brought about by two different enzymes and intramolecular if the same enzyme(s) study favour the latter explanation with anisole as substrate. Thus once the substrate is bound to the enzyme it is committed to oxidation, so that when demethylation is made less favourable by deuterium substitution ring hydroxylation is enhanced. This explanation accounts for the higher yield of 4-methoxyphenol from [*Me*-$^2H_3$]anisole than from anisole and for the observation that the sum of the yields of phenol and 4-methoxyphenol from equivalent experiments is independent of deuterium substitution. Mitoma *et al.*[34] reported a similar

the yields of hydroxylated products from microsomal oxidation of 4-nitrophenyl propyl ether and concluded the same enzyme(s) was(were) responsible for the formation of all the products. Relevant to this study is the recent work of Gelb *et al.*[35] who show that cytochrome $P450_{CAM}$ mono-oxygenase can remove the 4-*exo*- or 4-*endo*-hydrogen of camphor to give 5-*exo*-hydroxycamphor. They discuss at length the influence of the geometrical selection of the mono-oxygenase for the 5-*endo*- and 5-*exo*-positions on the observed isotope effect. However, studies with competitive inhibitors suggest that aromatic hydroxylation and dimethylation may be mediated by different cytochrome P450 haemoproteins.[36]

Despite the uncertainties described above, it is clear from the large isotope effects that C–H bond cleavage is occurring in the demethylation of anisole by microsomal enzymes. This conclusion is fully in agreement with the generally accepted mechanism for these oxidative *O*-demethylations.

The kinetic isotope effects from this study are comparable with the intramolecular effects for monodemethylation of 1,4-dimethoxybenzene [6b] and for alkane hydroxylation.[37] The size of these effects is in agreement with these processes occurring by hydrogen-atom abstraction by the active oxidant.[37a]

The similarity of the isotope effects exhibited by the NADPH–dioxygen system and those using t-butyl hydroperoxide or 3-chloroperbenzoic acid suggests that the same or similar active oxidants are present in all three systems. Ullrich [37b] also reached this conclusion from the intramolecular kinetic isotope effects in the hydroxylation of [$^2H_{11}$]cyclohexane. He obtained values of k_H/k_D of 7—8.6 for liver microsomal hydroxylation supported by NADPH–dioxygen, hydrogen peroxide, iodosylbenzene, 3-chloroperbenzoic acid, or cumene hydroperoxide. Likewise Groves *et al.* obtained k_H/k_D 4—6 for the hydroxylation of deuteriocyclohexenes by a reconstituted mono-oxygenase with a similar range of oxygen donors.[8b] Currently the nature of the active oxidant in the microsomal and modified microsomal systems is an active area of research and discussion.[38]

Model Systems.—With the requirements of the model system clearly delineated from the results with the microsomal systems, we investigated a wide range of model systems to examine (i) which could bring about both oxidative *O*-demethylation and aromatic hydroxylation of anisole, and (ii) which would show a large kinetic isotope effect for the demethylation.

Trifluoroperacetic acid, a typical peroxycarboxylic acid, did not demethylate anisole. So that although peroxycarboxylic acids epoxidise alkenes stereospecifically and bring about aromatic hydroxylation with large values of the NIH shift [22a] it seems unlikely that they are good models for cytochrome P450 dependent mono-oxygenases.

Two photochemical systems requiring u.v. light (aqueous pyridine *N*-oxide–*hν* and Hg–N_2O–*hν*) could not be tested because the anisole was photochemically demethylated in the absence of the oxidant. It is likely that the excited anisole loses a hydrogen atom from the methoxy-group to give a phenoxymethyl radical which reacts further to give phenol.[39]

In our hands a fourth system, that is reported to generate a carbonyl oxide from singlet dioxygen and a diazoalkane,[25b] did not oxidise anisole. This result was unexpected since the diphenyldiazomethane–*hν*–singlet dioxygen system is reported to hydroxylate naphthalene.[40]

Model Systems with k_H/k_D *ca.* 1.0.—Seven of the oxidising systems give kinetic isotope effects near unity and consequently cannot be considered to be good models for the cytochrome P450 dependent mono-oxygenases. It is unlikely that oxidative *O*-demethylation by any of them involves a rate-determining cleavage of the methoxy C–H bond. This result was not unexpected for Fenton's reagent in which the active oxidant is the hydroxyl radical since, as described above, the hydroxyl radical has been shown to demethylate 1,4-dimethoxybenzene by *ipso*-substitution and not by hydrogen-atom abstraction from the methoxy-group.[7] This mechanism was confirmed by the complete loss of [^{18}O] label in the conversion of [^{18}O]-anisole to phenol. It is probable that for the oxidising systems with k_H/k_D *ca.* 1.0 all, or almost all, the demethylation is by an oxyl radical *ipso*-substitution. This was confirmed for Udenfriend's system with [^{18}O]anisole. However, with Hamilton's system [^{18}O] labelling studies suggest that although *ipso*-substitution is the major pathway (*ca.* 80%), possibly involving the hydroxyl radical, a small proportion of the reaction may take place with retention of the oxygen label. If 20% of the oxidation occurs by hydrogen-atom abstraction this could account for the kinetic isotope effect being greater than unity. A kinetic isotope effect of 8.0 for the minor reaction would give an overall isotope effect of 1.2.

Model Systems with k_H/k_D 2—3.4.—The four systems that show medium-sized isotope effects may bring about demethylation by a combination of *ipso*-substitution and side-chain attack or alternatively solely by side-chain attack by a mechanism exhibiting a medium-sized isotope effect. From [^{18}O] labelling experiments it is clear that for the tin(II)–pyrophosphate–dioxygen system the retention of the oxygen from anisole in the phenol is only compatible with the latter explanation.

The kinetic isotope effects in this study for the tin(II)–pyrophosphate–dioxygen and diazoalkane–*hν*–dioxygen systems agree well with values reported for the hydroxylation of cyclohexane and deuteriated cyclohexane. Ullrich obtained an isotope effect for the former system of 1.9 [37b] and Hamilton and Giacin a value of 4.6 for the latter [19] as compared with 2.1 and 3.4, respectively from this study.

The side-chain reaction could involve hydrogen-atom abstraction by a radical or oxene insertion into the methoxy C-H bond. In this respect it is noteworthy that three of the systems, namely Ullrich's [16,41] and those using trialkyl phosphite–*hν*–dioxygen [17] and diazoalkane–*hν*–dioxygen [19] are thought to oxidise organic compounds by radical mechanisms. However, Ullrich and Staudinger have proposed an oxenoid mechanism for the tin(II)–pyrophosphate–dioxygen system.[18]

The size of the kinetic isotope effect to be expected in an oxene insertion into a C–H bond is uncertain [42] but by analogy with equivalent carbene insertions it is unlikely to be >2.5. For hydrogen-atom abstraction it will depend on the position of the transition state on the reaction profile which in turn defines the extent of C–H bond cleavage in the transition state.[31] For early or late transition states k_H/k_D should be small. The kinetic isotope effect should be maximal for a thermoneutral process with a symmetrical transition state in which the C–H bond would be approximately half-broken.[31,43] Thus radical chlorination of toluene by the reactive chlorine atom has an early transition state with little C–H bond cleavage and exhibits a small kinetic isotope effect (k_H/k_D 1.3 at 77 °C).[44] However, the equivalent bromination has a larger value (k_H/k_D 4.9 at 77 °C) as would be predicted for this nearly thermoneutral process.[44] The majority of kinetic isotope effects for C–H bond cleavage by hydrogen-atom abstraction in solution have k_H/k_D values between 2 and 8.[43,45]

If these oxidative *O*-demethylations are initiated by hydrogen-atom abstraction from the methoxy-group it is likely that the transition state is early on the reaction profile. However, although the authors are in favour of radical mechanisms for these oxidations, the present data cannot distinguish con-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.