Magnitude of the Kinetic Isotope Effect for the Oxidative Demethylation of Anisole and [Me-$^2H_3$]Anisole: a New and Simple Criterion for assessing Model Systems for Cytochrome P450 Dependent Mono-oxygenases

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The data from the oxidative demethylation of anisole, [Me-$^2H_3$]anisole and [^{18}O]anisole by rat liver microsomes and ten model systems shed light on the mechanisms of *O*-demethylation and can be used to classify the model systems.

The current interest in the mechanisms of oxygenation by cytochrome P450 dependent mono-oxygenases has led to the development of numerous 'chemical models'.[1] The logic for studying the model systems is the hope that a knowledge of the mechanisms of the chemical systems will lead to a clearer understanding of the analogous biological processes. The relevance of a particular model depends on how closely its reactions resemble those of the mono-oxygenase and this has been assessed by such comparative criteria as the range of oxidations brought about by both systems and their product distributions,[1,2] stereochemical studies,[1d,3] the origin of the oxygen,[1b–d] and the magnitude of the NIH shift.[1c,4] Here we report how the mechanism of demethylation of anisole and [Me-$^2H_3$]anisole, as assessed by kinetic isotope effect studies, provides a new and simple method of classifying the model systems.

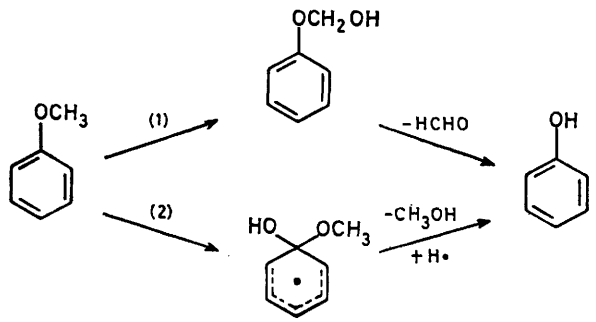
The kinetic isotope effects were obtained by comparing the ratio of the yield of phenol (demethylation) to that of 2-methoxyphenol (hydroxylation) from equivalent oxidations of the two substrates. We argue that whereas the former process might show a kinetic isotope effect the latter process should not and should serve as an internal standard for the oxidation. Thus, comparison of the yields of demethylation with those of hydroxylation minimises a major source of error, that of variable yields of oxidation, which would have put limitations on obtaining the kinetic isotope effects from the measurement of demethylation yields alone.

Table 1 records the values of k_H/k_D obtained from a selection of model systems and, for comparison, the values obtained with rat liver microsome preparations. The biological system shows a large kinetic isotope effect in agreement with the generally accepted mechanism for biological *O*-dealkylation of aromatic ethers involving an initial rate-determining C–H bond cleavage (reaction 1).[11] The magnitude of this isotope effect lies between the values reported[12,13] for

Table 1. Kinetic isotope effects from the oxidative demethylation of anisole and [Me-$^2H_3$]anisole by rat liver microsomes and a selection of model systems.

Oxidising System	k_H/k_D[a]
Rat liver microsomes/NADPH/O_2 (15 min)[b] (ref. 5)	7·3±0·7
Rat liver microsomes/NADPH/O_2 (30 min)[b]	7·6±0·7
Rat liver microsomes/NADPH/O_2 (60 min)[b]	8·3±0·7
Fe^{2+}/H_2O_2 (Fenton's reagent) (ref. 6)	1·0±0·1
Fe^{3+}/H_2O_2/catechol (Hamilton's system) (ref. 7)	1·3±0·1
Fe^{2+}/EDTA/ascorbic acid/O_2 (Udenfriend's system) (ref. 8)	1·2±0·1
Fe^{2+}/EDTA/O_2 (ref. 1a)	1·0±0·1
Reduced flavin mononucleotide/O_2 (ref. 4b)	1·0±0·1
Fe^{3+}/*N*-benzyl-1,4-dihydronicotinamide/O_2 (ref. 1a)	1·2±0·1
Fe^{2+}/2-mercaptobenzoic acid/O_2 (Ullrich's system) (ref. 2d)	2·2±0·1
Trialkylphosphite/$h\nu$/O_2 (ref. 9)	2·1±0·2
Sn^{2+}/sodium pyrophosphate/O_2 (ref. 2c)	2·1±0·1
Tetraphenylporphinatoiron(III) chloride/PhIO (Groves' system) (ref. 10)	9·0±3·0

[a] The kinetic isotope effect was calculated from the yields of phenol and 2-methoxyphenol measured by g.l.c. analysis. [b] Time of incubation in parentheses.



the *O*-demethylation of 4-[$^2H_3$]methoxyanisole, k_H/k_D = 10 and 3·3. Alkane hydroxylations mediated by cytochrome P450 enzymes also show large primary kinetic effects.[14]

It is noteworthy that the model systems fall into three groups: i, the systems that show k_H/k_D = *ca.* 1·0, ii, those with a medium sized isotope effect, 2—3, and iii, the tetraphenylporphinatoiron(III) chloride–iodosylbenzene system which shows an isotope effect comparable with that of the biological system.

The absence of an isotope effect from dealkylation by Fenton's reagent was not unexpected since pulse radiolytic studies have shown that the hydroxyl radical dealkylates 1,4-dimethoxybenzene by *ipso*-substitution (reaction 2).[15] Presumably a similar *ipso*-substitution is brought about by the hydroxyl or a related oxy-radical with all the systems where k_H/k_D = *ca.* 1·0. *ipso*-Substitution was confirmed for Fenton's and Udenfriend's systems by the absence of ^{18}O in the phenol derived from [^{18}O]anisole [3·1% ^{18}O].

The medium-sized isotope effects could arise by a combination of *ipso*- and side-chain attack or by side-chain oxidation by a process exhibiting a low kinetic isotope effect such as has been observed for C–H bond cleavage by reactive radicals.[16] For the tin(II) pyrophosphate system the latter explanation seems correct since the phenol from [^{18}O]anisole retains > 90% of the isotopic label. This conclusion is in agreement with the kinetic isotope effect, k_H/k_D = 1·9, from the hydroxylation of [$^2H_{11}$]cyclohexane by this system.[14c]

We conclude that, of the model systems we have studied, only the tetraphenylporphinatoiron(III) chloride–iodosylbenzene system can be considered a suitable model for cytochrome P450 mediated oxidations.

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