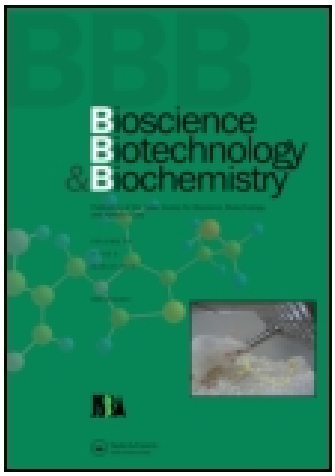

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Rate Parameter Changes by Added Albumin in the Microsomal Oxidative Demethylation of Deuteriated and Non-deuteriated 4-Methoxyanisole

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Bovine serum albumin (BSA) added to the reaction medium for the oxidative demethylation of 4-methoxyanisole and its "di-CD_3" isotopomer ([d_6]methoxyanisole), when catalyzed by liver microsomes from untreated rats, decreased the K_m values and increased the V_{max}/K_m ($= V/K$) values. The V_{max} values were not markedly altered. The values for the deuterium isotope effect on V_{max} and V/K for the reaction with this isotopomer were between 2.2 and 2.8, and that on K_m was close to unity. The magnitude of the isotope effect was not significantly changed by adding BSA. The intramolecular isotope effect with [mono-CD_3]4-methoxyanisole ([d_3]methoxyanisole) in liver microsomes from untreated rats was between 10.3 and 10.8, which was not significantly changed by BSA. Liver microsomes from rats treated with phenobarbital resulted in the intramolecular isotope effect value in the absence of BSA being between 7.2 and 9.1, which was not significantly altered by BSA. Based on these data, the calculated apparent rate constant for the enzyme-substrate complex formation was markedly increased by up to about 1.9- and 3.5-fold by 1% and 2% of BSA added, respectively.

Key words: albumin effect; isotope effect; methoxyanisole demethylation; microsomal P450 reaction; oxidative demethylation

The metabolic mechanism for pesticides and other xenobiotics involving C-H bond cleavage may be elucidated by measuring the intrinsic deuterium isotope effects.[1-3] However, the magnitude of the observed kinetic isotope effect, namely the ratio of the reaction rate parameters observed when using a pair of deuteriated and nondeuteriated substrates, is often heavily suppressed to a value lower than that of the intrinsic isotope effect. This is due to various noncatalytic steps involved in the reaction sequence when they are slow and rate-limiting (at least partially rate-determining) in the overall reaction sequence. As indicated by Northrop,[1] the most orthodox means for obtaining the magnitude of the intrinsic deuterium isotope effect is to measure the tritium isotope effect on the same reaction. However, this measurement is often laborious and difficult due to the requirement for a substrate labeled with tritium of high specific radioactivity at a specific position. On the other hand, if a noncatalytic step such as enzyme-substrate-(ES-)complex dissociation or enzyme-product-(ES-)complex dissociation could be accelerated, a larger observed isotope effect value, which would often be close enough to the intrinsic isotope effect, could be obtained. One way to achieve this is to measure the intramolecular isotope effect, and several successful attempts have been described.[4-7] However, determination of the intramolecular isotope effect is not always possible, since it requires a substrate having a symmetrical structure and equivalent reacting groups in symmetrical positions.

We have reported that adding a noncatalytic protein such as bovine serum albumin (BSA) to the incubation medium for methoxychlor, 1,1-bis-(4-methoxyphenyl)-2,2,2-trichloroethane, a hydrophobic insecticide, affected the rate of some noncatalytic steps in its metabolism, resulting in a decrease of the observed isotope effect on parameter V/K.[8,9] To find a means for enhancing the magnitude of the observed isotope effect, we examined the effect of BSA on the magnitude of the observed isotope effect in 4-methoxyanisole as a model for a simple hydrophobic substrate.

Typically 4-methoxyanisole is oxidatively demethylated to 4-methoxyphenol when catalyzed by liver microsomes. An early study by Foster *et al.*[4] has demonstrated that the intrinsic deuterium isotope effect value was very close to 10.0 by means of an intramolecular isotope effect measurement that used liver microsomes obtained from phenobarbital-(PB)-treated rats. They discussed the difference in magnitude between the intermolecular and intramolecular isotope effects.

To obtain information on the rate constants for the steps involved in this enzyme reaction, we measured the intermolecular isotope effects in the noncompetitive mode, using liver microsomes from untreated rats in the presence and absence of BSA. Microsomes from both untreated and PB-treated rats were used to also measure the intermolecular isotope effect values with and without BSA in a competitive mode involving the co-incubation of deuteriated and non-deuteriated substrates. To obtain the magnitude of the intrinsic isotope effect on this reaction, we also determined the intramolecular isotope effect by using [d_3]-methoxyanisole catalyzed by liver microsomes from untreated and PB-treated rats. Based upon the data obtained from these determinations, we conclude that, in the reaction

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Abbreviations: BSA, bovine serum albumin; PB, phenobarbital.

catalyzed by untreated rat liver microsomes, the apparent rate constant for the formation of an ES-complex was markedly increased by adding BSA, whereas the other rate constants were not significantly affected. The magnitude of the observed isotope effect on any rate parameter was not significantly altered, which we will explain by a simple kinetic model of an enzyme-catalyzed reaction.

Materials and Methods

Compounds. 1,4-Bis-(trideuteriomethoxy)benzene ([d_6]methoxyanisole), 4-trideuteriomethoxyphenol ([d_3]methoxyphenol) and 4-trideuteriomethoxyanisole ([d_3]methoxyanisole) were respectively prepared by the complete and partial trideuteriomethylation of hydroquinone and 4-methoxyphenol, using CD_3I (with a D content of over 99.5%, see Foster *et al.*[4]). The mass spectral data for these deuteriated compounds were as follows. [d_6] ($C_8H_4D_6O_2$): *m/z* 144 $(M)^+$, 126 $(M-CD_3)^+$, 98 $(M-CD_3-CO)^+$; [d_3]methoxyphenol ($C_7H_5D_3O_2$): *m/z* 127 $(M)^+$, 109 $(M-CD_3)^+$, 81 $(M-CD_3-CO)^+$; [d_3]methoxyanisole ($C_8H_7D_3O_2$): *m/z* 141 $(M)^+$, 126 $(M-CH_3)^+$, 123 $(M-CD_3)^+$, 98 $(M-CH_3-CO)^+$, 95 $(M-CD_3-CO)^+$. The D content of the CD_3 group(s) in these synthesized compounds was over 99.5% when judged from their mass spectral data. Commercially available chemicals of reagent grade (including 4-methoxyanisole = [d_0]methoxyanisole) were all purchased from Waken Yaku Co. and Nacalai Tesque Co. (Kyoto, Japan).

Microsomes. Liver microsomes were prepared by a conventional procedure from untreated male Wistar rats of about 200 g in body weight. The liver was briefly perfused with 0.9% NaCl, dissected out, homogenized with 1.15% KCl, centrifuged at 10,000 × *g* for 30 min, and then the supernatant was centrifuged at 105,000 × *g* for 90 min. The resulting pellet was homogenized with 0.2 M potassium phosphate (pH 7.4), and centrifuged again at 105,000 × *g* for 90 min. This pellet was suspended in the same phosphate buffer and used for the reaction.

The rats were given an intraperitoneal injection of an aqueous solution of sodium phenobarbital (16 mg/200 μl) 72, 48, and 24 h before their sacrifice (*i.e.*, an 80 mg/kg dose once a day for 3 days). The liver was dissected out, and the PB-treated liver microsomes were prepared in the same way as that described above for the untreated type.

Cytochrome P450 levels were measured spectrophotometrically as described by Omura and Sato.[10] The protein content of microsomal suspensions was determined as described by Lowry *et al.*[11] The cytochrome P450 concentration in the microsomes was 0.6 to 0.8 nmol/mg of protein in the untreated rat liver, and 1.5 to 2.0 nmol/mg of protein in the PB-treated rat liver.

Metabolic reactions. The reaction mixture consisted of liver microsomes [containing 5 nmol cytochrome P450 (7 to 8 mg of protein for the untreated sample, and 3 to 4 mg protein for the PB-treated sample) or 2.5 nmol P450 for the noncompetitive reaction, to obtain such rate parameters as V_{max} and K_m] and the NADPH-generating system (10 μmol of glucose 6-phosphate, 1.0 unit of glucose 6-phosphate dehydrogenase, and 1 μmol of $NADP^+$) in a total of 2 ml of 0.2 M potassium phosphate (pH 7.4). BSA (Cohn fraction V, with a purity of over 95% and not defatted) was included to a final concentration of 1 or 2% when necessary. After a 1-min incubation of the mixture at 37 °C, a defined total quantity of the substrate (between 125 nmol and 2 μmol) in ethanol (10 μl) was added, and the mixture shaken for 30 min at 37 °C. In the noncompetitive runs to determine the intermolecular isotope effect values, the [d_0]- and [d_6]-methoxyanisole were separately incubated at various concentrations. In the competitive determination, a mixture of the [d_0] and [d_6] substrates at a single one-to-one ratio was used. The reaction was terminated by adding 500 μl of 1 N HCl, triplicate runs being conducted for the respective substrate concentrations. After adding an internal standard (2,6-xylenol), the metabolites, substrate and the added internal standard were extracted with ethyl acetate (3 ml) by shaking for 30 min, and then centrifuged for 20 min to separate the layers. The intramolecular isotope effect study was conducted on [d_3]methoxyanisole as the substrate at two or three concentrations (triplicate runs for each concentration), and the metabolites were extracted without adding an internal standard.

GC-MS analysis of the metabolite mixture. The ethyl acetate extract was condensed to a small volume (less than 100 μl) by gently introducing dry nitrogen gas, and the concentrate (about 5 μl) was injected under the splitless mode into a GC-MS instrument (Hewlett-Packard MSD model 5970 equipped with an HP-1 capillary column of 12 m). The oven temperature was programmed from 50 °C to 125 °C at 10 °C per minute. The mass fragments at *m/z* 124 ($C_7H_8O_2$, M^+ of [d_0]methoxyphenol), 127 ($C_7H_5D_3O_2$, M^+ of [d_3]methoxyphenol), and *m/z* 122 ($C_8H_{10}O$, M^+ of 2,6-xylenol) were monitored in the selected ion monitoring mode. The peak area was used to calculate the amount of the substance by means of a standard curve generated by plotting the area against the injected amount of the standard compound. Rate parameters V_{max}, K_m, and V/K were obtained in the noncompetitive reactions by using a Lineweaver Burk plot.

Results

The V_{max}, K_m, and V_{max}/K_m ($= V/K$) values for [d_0]- and [d_6]methoxyanisole in the absence and presence (1% and 2%) of BSA are listed in Table I. The ratios of these values for the d_0 and d_6 substrates, that is, the noncompetitively determined intermolecular isotope effects, are also shown.

V_{max} did not change significantly between the runs with 0% and 1% (and between 1% and 2%) added BSA, but slightly decreased when 2% BSA was added when compared to the runs without BSA. The K_m values for both substrates decreased, and the V/K values consequently increased upon the addition of BSA to the incubation medium. Since the decreases in K_m values (and the increase in V/K values) for the d_0 and d_6 substrates were similar, the ratio of K_m (and V/K) for the d_0 and d_6 substrates {= observed intermolecular isotope effect on K_m (and V/K) which we denote as ^DK (and $^D(V/K)$), according to Northrop[1]} was not significantly altered. The observed intermolecular isotope effect on V_{max} ($= {}^DV$) also remained essentially unaltered, irrespective of the presence or absence of BSA.

The $^D(V/K)$ values were also determined competitively, the results each being expressed as the average value (± standard deviation) and shown in Table II. With the liver microsomes from untreated rats, the average $^D(V/K)$ values were apparently altered according to the added BSA concentration, but an examination of the alteration for

Table I. Rate Parameters Determined by Using a Lineweaver–Burk Plot for the Oxidative Demethylation of [d_0]- and [d_6]Methoxyanisole and Intermolecular Deuterium Isotope Effects (Noncompetitively Determined) in the Absence and Presence of BSA[a]

	Untreated rat liver microsomes				(PB)[b]	
	BSA (%)	d_0	d_6	d_0/d_6	(d_0/d_6)	
V_{max} mol/mol/min	0	1.43 ± 0.29	0.538 ± 0.073	2.66	(2.10)	^DV[c]
	1	1.24 ± 0.16	0.555 ± 0.088	2.23		
	2	1.01 ± 0.12	0.362 ± 0.029	2.79		
K_m[d] (mM)	0	0.407 ± 0.102	0.415 ± 0.053	0.981	(1.29)	^DK[c]
	1	0.163 ± 0.017	0.193 ± 0.021	0.845		
	2	0.122 ± 0.020	0.107 ± 0.002	1.140		
V/K	0	3.51	1.30	2.70	(1.64)	$^D(V/K)$[c]
	1	7.63	2.87	2.66		
	2	8.29	3.37	2.46		

[a] See the procedures for the noncompetitive runs of metabolic reactions described in Materials and Methods in the text.
[b] PB-treated rat liver microsomes; data cited from ref. 4.
[c] Observed isotope effects corresponding to d_0/d_6 ratio values; see the text for the notation.
[d] Compare these values with the K_m values for [d_0]methoxychlor: 0.53 μM (in 1% BSA) and 1.03 μM (in 2% BSA).[12]

Table II. Competitively Determined Deuterium Isotope Effects on 4-Methoxyanisole Demethylation[a]

Substrate concentration ($[d_0]\mu$M/$[d_6]\mu$M)	125/125	250/250	500/500	Average values for the data at various concentrations $^{D}(V/K)$
Untreated rat liver microsomes				
BSA 0%	2.89±0.19	3.09±0.20	3.15±0.18	3.04±0.14
BSA 1%	2.62±0.07	2.38±0.16	3.54±0.09	2.85±0.61
BSA 2%	2.13±0.08	2.43±0.17	2.30±0.05	2.29±0.15
PB-treated rat liver microsomes				
BSA 0%	1.83±0.26	2.13±0.12	2.56±0.25	2.17±0.37
BSA 1%	1.50±0.08	2.16±0.28	2.20±0.09	1.95±0.39
BSA 2%	1.70±0.12	1.94±0.07	1.91±0.31	1.85±0.13

[a] See the procedures for the competitive runs of metabolic reactions described in Materials and Methods in the text. The ratios for the d_0 and d_3 metabolite, *i.e.*, the rate ratios, are listed, which correspond to $^{D}(V/K)$ values (see ref. 1). Standard deviations of triplicate runs are also listed.

Table III. Intramolecular Isotope Effect on the Microsomal Oxidative Demethylation of $[d_3]$Methoxyanisole[a]

Substrate (μM)	250	500	1000	Average values of the data at various concentrations ($\approx k_{3H}/k_{3D}$)
Untreated rat liver microsomes				
BSA 0%	11.54±1.01	9.99±0.24	9.39±0.22	10.31±1.11
BSA 1%	12.64±0.67	10.39±0.41	9.59±0.44	10.86±1.56
BSA 2%	11.90±0.35	9.20±0.62	10.99±0.30	10.70±1.37
PB-treated rat liver microsomes				
Substrate (μM)	62.5	125		Average values of the data at various concentrations ($\approx k_{3H}/k_{3D}$)
BSA 0%	7.73±0.32	9.65±0.85		8.69±1.36
BSA 1%	7.94±0.81	10.20±1.08		9.09±1.60
BSA 2%	7.40±0.08	7.05±0.70		7.23±0.25

[a] See the procedures for the intramolecular study of metabolic reactions described in Materials and Methods in the text. The ratios of $[d_3]$ and $[d_6]$methoxyphenol are listed, which correspond to the observed intramolecular isotope effect values, and are considered to be close to intrinsic isotope effect values k_H/k_D (see the text). Standard deviations of triplicate runs are also listed.

each substrate concentration revealed that this was not a general trend. With the PB-treated microsomes, the $^{D}(V/K)$ values under various conditions were similar, although the values seem a little smaller than those with untreated liver microsomes.

To evaluate the magnitude of the intrinsic isotope effect on the demethylation of methoxyanisole, intramolecular isotope effect values were determined by using $[d_3]$methoxyanisole. The results are each the average value (± standard deviation) of triplicate determinations for the respective substrate concentrations and are shown in Table III. These ratios of the products resulting from intramolecular competition should be close to the magnitude of the intrinsic isotope effect. In fact, they were between 10.3 and 10.9 for the untreated, and between 7.2 and 8.7 for the PB-treated rat liver microsomes, respectively. These values for magnitude are close to those reported (10.0) for PB-treated rat liver microsomes.[4]

Discussion

Based on a simple model of an enzyme-catalyzed reaction,

$$E+S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} EP \xrightarrow{k_4} E+P$$

($k_3 = k_{3H}$ and k_{3D} for the d_0 and d_6 substrates, respectively)

in which E, S, P, ES, and EP are the enzyme, substrate, product(s), enzyme-substrate complex and enzyme-product complex, respectively, the following relationships can be derived, assuming a steady state ES and EP concentration:

$$V_{max(H)} = (k_{3H}k_4E_t)/(k_{3H}+k_4)$$
$$V_{max(D)} = (k_{3D}k_4E_t)/(k_{3D}+k_4)$$
$$K_{m(H)} = [k_4(k_2+k_{3H})]/[k_1(k_{3H}+k_4)]$$
$$K_{m(D)} = [k_4(k_2+k_{3D})]/[k_1(k_{3D}+k_4)]$$

in which E_t is the total concentration of enzyme-containing species, *i.e.*, [E]+[ES]+[EP].

Therefore, the relative rate constants can be expressed by the following equations:

$$k_1/k_{3D} = \{(^{D}V-1)\times{}^{D}(V/K)\}/\{[1-{}^{D}(V/K)\times(1/{}^{D}k)]\times{}^{D}V\times K_{m(D)}\} \quad (mM^{-1})$$
$$k_2/k_{3D} = [{}^{D}(V/K)-1]/[1-{}^{D}(V/K)\times(1/{}^{D}k)]$$
$$k_4/k_{3D} = [{}^{D}V-1]/[1-{}^{D}V\times(1/{}^{D}k)].$$

In these equations, $^{D}k = k_{3H}/k_{3D}$, that is, the intrinsic isotope effect, and ^{D}V and $^{D}(V/K)$ denote the ratios $[V_{max(H)}/V_{max(D)}]$ and $\{[V_{max(H)}/K_{m(H)}]/[V_{max(D)}/K_{m(D)}]\}$, respectively. The equations and data in Tables I to III enabled the relative rate constants to be calculated as shown in Tables IV and V. In these Tables, we also include the values for rate of catalysis R and commitment to catalysis C, which denote the values for k_{3H}/k_4 and k_{3H}/k_2, respectively. R represents the ratio of the rate of the catalytic step to the rate of the other forward steps contributing to the maximal velocity, while C is the tendency of the enzyme-substrate complex to go forward through catalysis rather than backward to free enzyme and substrate.[1] In these calculations, we have assumed that $^{D}k = 10$ for simplicity, while values between 7.2 and 10.9 (the experimentally obtained minimum and maximum values) for ^{D}k would not yield any markedly different conclusions.

Several characteristics of the rate parameters were revealed. BSA suppressed the magnitude of the apparent

Table IV. Relative Rate Constants for the Enzyme-Catalyzed Demethylation of 4-Methoxyanisole Calculated from the Magnitude of the Non-competitively Determined Intermolecular Isotope Effect, and the Rates (and Rate Ratios) Observed in the Noncompetitive Experiments (Assuming $k_{3H}=10k_{3D}$)[a]

	Untreated rat liver microsomes				
	k_1/k_{3D} (mM^{-1})	k_2/k_{3D}	C^b ($=k_{3H}/k_2$)	k_4/k_{3D}	R^b ($=k_{3H}/k_4$)
BSA 0%	5.56	2.33	4.29	2.26	4.42
BSA 1%	10.51	2.26	4.42	1.58	6.33
BSA 2%	19.56	1.94	5.15	2.48	4.03

[a] See Discussion section in the text.
[b] C=commitment to catalysis, R=ratio of catalysis; see Discussion section in the text.

Table V. Relative Rate Constants for the Enzyme-catalyzed Demethylation of 4-Methoxyanisole Calculated from the Competitively Determined Magnitude of the Intermolecular isotope Effect (Assuming $k_{3H}=10k_{3D}$)[a]

	Untreated rat liver microsomes		PB-treated rat liver microsomes	
	k_2/k_{3D}	C^b ($=k_{3H}/k_2$)	k_2/k_{3D}	C ($=k_{3H}/k_2$)
BSA 0%	2.93	3.41	1.49	6.31
BSA 1%	2.59	3.86	1.18	8.47
BSA 2%	1.67	5.99	1.04	9.61

[a] See Discussion section in the text.
[b] See Table IV and Discussion section in the text.

K_m values, this suppression being considered to be due to the enhancement of k_1 (the rate constant of ES-complex formation) by BSA. The magnitude of constants k_2 and k_4 was not markedly altered. Thus, the magnitude of observed isotope effects ^DV and $^D(V/K)$ did not alter. Although constant k_2 based on the average $^D(V/K)$ values in the competitive intermolecular isotope effect study seems to have markedly decreased in the presence of increasing BSA concentration (Table V), its significance is doubtful because the $^D(V/K)$ values determined experimentally and listed in Table II did not necessarily show a regular trend. For example, for the substrate combination 500 μM/500 μM with untreated liver microsomes, the value was increased from 3.15 to 3.54 by adding BSA.

In the present study that used methoxyanisole as a substrate, BSA enhanced the rate constant of ES-formation (k_1), whereas in a similar oxidative demethylation of methoxychlor, k_1 was decreased by the addition of BSA.[9] This latter observation was interpreted as the reduction of the free substrate concentration due to hydrophobic binding of methoxychlor with BSA.[9]

This reversed effect of BSA on the k_1 magnitude of these two substrates must have been due to the large difference between the magnitude of the concentrations used in the different incubations because of the large difference in their hydrophobicity: log P (octanol/water) was 4.83 for methoxychlor,[12] whereas the value for methoxyanisole was between 2.07 and 2.09 (calculated from log P of benzene and the π-value of the methoxyl group[13]). This hydrophobicity difference is reflected in the K_m values for methoxyanisole and methoxychlor (122 to 407 μM for methoxyanisole versus 0.53 to 1.03 μM for methoxychlor). In the incubation mixture, the substrates could partition into either the P450 catalytic site, the phospholipids of the microsomal membrane, or the hydrophobic portion (lipid core or some hydrophobic domain of the protein) of BSA, which was not defatted and may contain some lipids. The concentration range used in the present incubation (and K_m values) for the methoxyanisole substrate was comparable to BSA concentrations of 1% and 2% (corresponding to approximately 150 and 300 μM). These concentrations were much higher than those of P450 (5 nmol/2 ml = 2.5 μM) and methoxychlor in the incubation medium. Therefore, the present methoxyanisole substrate should have had much greater probability than methoxychlor of encountering a P450 catalytic site in the presence of BSA. When methoxychlor has once been dissociated from microsomal lipids or BSA, it would have a greater chance to be trapped again by lipids or another BSA molecule than by a P450 catalytic site. (In fact, methoxychlor not bound to microsomes can be assumed to be mostly trapped to BSA molecules in its presence.)[12] Another interpretation is that BSA caused a rearrangement of the lipid molecules surrounding P450 on the microsomal membrane, and that this may have altered the affinity of some hydrophobic substrates to P450. If so, the degree of such an affinity alternation of a substrate to P450 may considerably differ among substrates with largely different levels of hydrophobicity. These notions could account for the enhanced k_1 value for methoxyanisole and decreased k_1 value for methoxychlor by BSA, and could be proven in a future study.

Under our conditions, the values for rate constants k_2 (ES dissociation) and k_4 (EP dissociation) were always larger than that for k_{3D}, but smaller than that for k_{3H}. These relatively slow noncatalytic steps not involving covalent bond cleavage or formation (in other words, relatively large C and R) are the basis for the observed isotope effect values that were much smaller than those of the intrinsic isotope effect. Although we could not alter the rate of these steps, incubation under certain conditions may afford some means for enhancing these rates (see Northrop[1] for successful trials).

In this investigation, by using appropriate deuteriated and nondeuteriated substrate isotopomers, and by assuming a simple model of an enzyme-catalyzed reaction, we calculated the relative values for the rate constants of each unit step in the microsome-catalyzed reaction. We have revealed that increasing the concentration of BSA in the incubation medium increased the rate constant of the enzyme-substrate complex formation in methoxyanisole demethylation. The other rate constants did not change, and thus the observed isotope effect values remained unaffected.

There are various molecular species of cytochrome P450 in rat liver, and several P450 species may catalyze the present reaction. Moreover, they may exhibit different substrate affinities and different rate parameters for methoxyanisole. The differences in some values in Tables I to III between untreated and PB-treated liver microsomes suggest such a feature. Besides, we have also observed a case in a metabolism study with methoxychlor as the substrate that indicated the presence of two categories of P450 species,

one with low affinity and the other with high affinity to the substrate as well as differing stereoselectivity in metabolite formation.[12,14] Thus, while kinetic studies on the present substrate conducted with a reconstituted system and using purified P450[14] would give further invaluable information on the reaction, a simple approach using microsomes such as that used in the present study was useful for studying the kinetics of various enzyme-catalyzed biotransformations of xenobiotics.

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