(2) N. I. Karev, N. G. Blokhina, E. K. Vozny, and M. P. Pershin, *Neoplasma*, **19**, 347–350 (1972).
(3) N. Valdivieso, G. P. Bodey, J. A. Gottlieb, and E. J. Freireich, *Cancer Res.*, **36**, 1821–1824 (1976).
(4) A. M. Cohen, *Drug Metab. Dispos.*, **3**, 303 (1975).
(5) S. Fihii, H. Okuda, A. Akazawa, Y. Yasuda, Y. Kawaguchi, Y. Fukunaga, and H. Nishikawa, *Yakugaku Zasshi*, **95** 732–740 (1975).
(6) S. Fujimoto, A. Akao, B. Itoh, I. Koshizuka, K. Kayano, Y. Kitsukawa, M. Takahashi, T. Minami, H. Ishigami, Y. Nomura, and K. Itoh, *Cancer Res.*, **36**, 33–36 (1976).
(7) A. Z. Smolyan-skaya and O. A. Tugarinov, *Neoplasma*, **19**, 341–345 (1972).
(8) J. A. Benvenuto, K. Lu, S. W. Hall, R. S. Benjamin, and T. L. Loo, *Cancer Res.*, **38**, 3867–3870 (1978).
(9) A. T. Wu, H. J. Schwandt, C. Finn, and W. Sadee, *Res. Commun. Chem. Pathol. Pharmacol.*, **14**, 89–102 (1976).
(10) J. A. Benvenuto, D. Farquhar, E. J. Freireich, and T. L. Loo, *Proc. Am. Assoc. Cancer Res.*, **18**, 145 (1977).
(11) A. T. Wu, J. L. Au, and W. Sadee, *Cancer Res.*, **38**, 210–214 (1978).
(12) T. L. Loo, R. S. Benjamin, K. Lu, J. A. Benvenuto, S. W. Hall, and E. M. McKelvey, *Drug Metab. Rev.*, **8**, 137–150 (1978).
(13) F. Sweet and R. K. Brown, *Can. J. Chem.*, **44**, 1571–1576 (1966).
(14) U. Niedballa and H. Vorbruggen, *Angew. Chem., Int. Ed. Engl.*, **9**, 461 (1970)
(15) A. F. Casy, "PMR Spectroscopy in Medicinal and Biological Chemistry", Academic Press, London and New York, 1971.
(16) T. Nishimura and B. Shimizu, *Chem. Pharm. Bull.*, **13**, 803 (1965).
(17) S. Hattori and K. Matsumoto, *Yuki Gosei Kagaku Kyokai Shi*, **19**, 461–463 (1961).
(18) S. Hattori, *Yuki Gosei Kagaku Kyokai Shi*, **19**, 453–460 (1961).
(19) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 4934 (1965).

Mechanism of the Dealkylation of Tertiary Amines by Hepatic Oxygenases. Stable Isotope Studies with 1-Benzyl-4-cyano-4-phenylpiperidine

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The microsomal oxidative dealkylation of 1-benzyl-4-cyano-4-phenylpiperidine has been studied and the source of oxygen shown to be molecular oxygen. The rate of debenzylation was decreased by substituting deuterium for hydrogen in the methylene portion of the benzyl group. The isotope effect was measured by comparison of the reaction rates of the d_0 and d_2 compounds **1a** and **1b** and also of the d_5 and d_7 compounds **1c** and **1d**. Determination of the reaction rates for various mixtures of labeled and unlabeled species allowed the rates for 0 (k_H) and 100 mol % (k_D) to be accurately obtained. A primary isotope effect of 1.46 was observed when the methylene hydrogens of benzyl were replaced by deuterium. No secondary isotope was observed when the aromatic hydrogens of benzyl were replaced by deuterium. The results of this study are consistent with a mechanism involving direct hydroxylation at the benzyl methylene position in a rate-determining step.

One of the important reactions mediated by the membrane-bound oxygenases of liver is the oxidative N-dealkylation of amines. The products of the conversion, the dealkylated amine and an aldehyde, are thought to arise from the spontaneous dissociation of an intermediate carbinolamine [$R_2NCHOHR \rightarrow R_2NH + RCHO$].[1,2] The formation of the carbinolamine has been considered to occur by direct hydroxylation of the α-carbon atom;[1] i.e., oxidative dealkylation is really another example of hydroxylation at an aliphatic carbon atom. Cytochrome P 450 mediated aliphatic hydroxylation, as typified by the conversion of ethylbenzene to predominantly (*R*)-methylphenylcarbinol, occurs by direct oxygen insertion (without inversion). Molecular oxygen serves as a source of the oxygen atom and a deuterium isotope effect of about 1.8 can be demonstrated.[3] In an early study, Elison, Elliott, Look, and Rapoport[4] found an isotope effect for the demethylation of *N*-(trideuteriomethyl)morphine of 1.4, consistent with a hydroxylation mechanism. The possibility that microsomal dealkylation might occur via intermediate *N*-oxides has been discussed but is considered less likely, since the dealkylation of *N*-oxides by cytochrome P 450 has been shown to involve an initial step in which *N*-oxide is reduced to tertiary amine, which in turn is dealkylated by the hydroxylation mechanism.[5] In contrast to tertiary amines, dealkylation of secondary amines may involve more than one mechanism. Prough and Ziegler[6] have recently presented data which suggest that certain secondary amines, such as benzphetamine, may undergo dealkylation via an *N*-hydroxy intermediate.

In the present study, the debenzylation of 1-benzyl-4-cyano-4-phenylpiperidine (**1a**) was investigated with respect to both source of oxygen and deuterium isotope effects. The study of deuterium isotope effects has become of increasing importance not only because of mechanistic implications but also because of the great increase in the use of deuterium labeling in biological research. It becomes important to know when primary and secondary deuterium isotope effects can be expected and when they would be unlikely.

The primary isotope effect of the debenzylation of **1a** would normally be measured by comparison of the reaction rates of **1a** and **1b**, in which deuterium has been substituted for hydrogen in the *methylene* portion of the benzyl group. In order to increase the accuracy of the measurement of the primary isotope effect, it was also determined by a second method, comparing the reaction rates of **1c** and **1d**, which correspond to **1a** and **1b** with the addition of having deuterium substituted for hydrogen in the *aromatic* portion of the benzyl moiety. The presence

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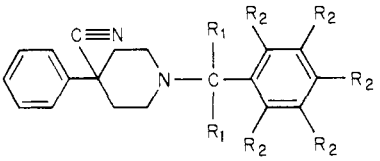


1a, $R_1 = R_2 = H$
b, $R_1 = {}^2H$, $R_2 = H$
c, $R_1 = H$, $R_2 = {}^2H$
d, $R_1 = R_2 = {}^2H$

or absence of a secondary isotope effect in the debenzylation, possibly caused by the deuteration of the aromatic part of the benzyl group, was monitored by the comparison of the reaction rates of **1a** vs. **1c** and also of **1b** vs. **1d**.

Chemistry. Benzyl-*1,1-d*$_2$ alcohol prepared by LAD (99% ^2H) reduction of methyl benzoate[7] had 97% isotopic purity. Material of higher isotopic incorporation (99% ^2H) was obtained by utilizing the Cannizzaro reaction from benzaldehyde-d_1, readily prepared by several methods.[9] Since the reaction involves an intermolecular hydride transfer,[8] the benzyl-*1,1-d*$_2$ alcohol retained all the deuterium in the system.

For the preparation of the variously deuterated analogues **1a–d**, the appropriate benzyl bromide (obtained from the correspondingly labeled benzyl alcohol by treatment with phosphorus tribromide) was condensed with 4-cyano-4-phenylpiperidine. The crystalline products **1a–d** were characterized by their IR, NMR, and EIMS spectra. Deuterium incorporation was uniformly good, ranging from 98.0 to 98.5%.

Experimental Section

Unless otherwise indicated, all reactions were performed under nitrogen and solvents were concentrated on a rotary evaporator under vacuum. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were measured with a Perkin-Elmer Model 337 spectrophotometer. NMR spectra were recorded in a Model A-60A Varian spectrometer using $CDCl_3$ as solvent and tetramethylsilane as an internal standard (Me_4Si = 0.0 ppm; abbreviations used: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet). Low-resolution EI mass spectra were run on an AEI MS12 instrument. Deuterium analysis by mass spectrometry was performed on the molecular ions. Literature melting or boiling points refer to the undeuterated compounds.

Benzyl-*1,1-d*$_2$ Alcohol. To a solution of sodium hydroxide (1 g) in water (1 mL) was added benzaldehyde-d_1 (1.5 g, 14 mmol), and the mixture was well shaken until a stable emulsion was formed. A moderate rise in temperature was observed. The flask was connected to a reflux condenser and heated at 45 °C (bath temperature) with stirring for 1.5 h. After cooling, the mixture was diluted with 20 mL of water and extracted with ether (2 × 25 mL). The extract was washed with saturated sodium bisulfite solution (2 × 15 mL) and dried ($MgSO_4$), the ether was removed on a rotatory evaporator, and the residue was distilled, bp 68–70 °C (0.5 mm), lit.[7] bp 88 °C (10 mm). The product (0.6 g, 82%) contained 99% deuterium on the α carbon by NMR integration: IR (neat) 3300 (br, OH), 2200 (C-D), 2080 (C-D) cm^{-1}; NMR δ 4.09 (s, 1 H, OH), 7.22 (s, 5 H, aromatic); EIMS *m/e* (relative intensity) M$^+$ 110 (100%), 109 (63%), 81 (64%), 80 (54%).

Benzyl-*1,1-d*$_2$ Bromide. To a solution of freshly distilled benzyl-*1,1-d*$_2$ alcohol (4 g, 36 mmol) in 80 mL of dry ether containing 1 mL of dry pyridine were added dropwise 3.3 g (12 mmol) of freshly distilled phosphorus tribromide with stirring. The reaction mixture was gently refluxed at 50 °C (bath temperature) for 2 h and cooled. The organic layer was washed with water, a saturated solution of sodium bicarbonate, and water. The combined extracts were dried ($MgSO_4$) and distilled to give the pure bromide: yield 4.75 g (76%); bp 65 °C (0.5 mm), lit.[7] 80–81 °C (14 mm); IR (neat) 2200 cm^{-1} (C-D); NMR δ 7.22 (s, aromatic).

Benzyl-*2',3',4',5',6'-d*$_5$ Bromide. This was prepared similarly from benzyl-*2',3',4',5',6'-d*$_5$ alcohol (Merck, Sharp & Dohme, 98.5% ^2H) in 76% yield: IR (neat) 2950 (C-H), 2260 (C≡C-D), 1210 (C-H) cm^{-1}; NMR δ 4.6 (s, CH_2).

Benzyl-*1,1,2',3',4',5',6'-d*$_7$ Bromide. Obtained from benzyl-*1,1,2',3',4',5',6'-d*$_7$ alcohol (Merck, Sharp & Dohme, 98.5% ^2H) in 87% yield: IR (neat) 2260 cm^{-1} (C≡C-D), 2175 (C-D), 930 (C-D) cm^{-1}.

1-Benzyl-4-cyano-4-phenylpiperidine (1a). To a solution of 4-cyano-4-phenylpiperidine (6.6 g, 35 mmol) in 100 mL of dry benzene was added dropwise 3.1 g (18 mmol) of freshly distilled benzyl bromide with stirring. The hydrobromide salt of 4-cyano-4-phenylpiperidine separated immediately. The reaction mixture was refluxed gently for 6 h and cooled. The white precipitate was filtered off and the filtrate evaporated to dryness. The residue crystallized on addition of a few drops of ethanol, and recrystallization from methanol–hexane gave white needles: yield 4.7 g (94%); mp 74–75 °C. The hydrochloride salt (from methanol–ethyl acetate) had mp 261–262 °C, lit.[10] 260–262 °C; IR (KBr) 2230 cm^{-1} (C≡N); NMR δ 2.09 (m, 4 H, CH_2CCH_2), 2.77 (m, 4 H, CH_2NCH_2), 3.6 (s, 2 H, NCH_2Ph), 7.42 (m, 10 H aromatic); EIMS *m/e* (relative intensity) M$^+$ 276 (29%), 199 (16%), 185 (31%), 146 (12%), 91 (100%).

1-(Benzyl-*1,1-d*$_2$)-4-cyano-4-phenylpiperidine (1b). Prepared similarly from benzyl-*1,1-d*$_2$ bromide in 92% yield, mp 71–72 °C. The hydrochloride had mp 261–262 °C; IR (KBr) 2230 (C≡N), 2200 (C-D) cm^{-1}; NMR δ 2.3 (m, 4 H, CH_2CCH_2), 2.70 (m, 4 H, CH_2NCH_2), 7.34 (m, 1 OH, aromatic); EIMS *m/e* (relative intensity) M$^+$ 278 (35%), 201 (16%), 185 (35%), 148 (15%), 93 (100%). Deuterium incorporation: 98% *d*, of which 97.5% was d_2 and 2.5% d_1.

1-(Benzyl-*2',3',4',5',6'-d*$_5$)-4-cyano-4-phenylpiperidine (1c). Prepared similarly from benzyl-*2',3',4',5',6'-d*$_5$ bromide in 94% yield, mp 74–75 °C. The hydrochloride had mp 260–261 °C; IR (KBr) 2950 (CH), 2260 (C≡C-D), 2230 (C≡N), 2260 (C≡C-D), 22330 (C≡N) cm^{-1}; NMR δ 2.1 (m, 4 H, CH_2CCH_2), 2.7 (m, 4 H, CH_2NCH_2), 3.59 (s, 2 H, NCH_2Ph), 7.42 (m, 5 H, aromatic); EIMS *m/e* (relative intensity) M$^+$ 281 (43%), 280 (20%), 199 (19%), 186 (19%), 185 (23%), 97 (23%), 96 (100%). Deuterium incorporation: 98.6%, of which 94.1% was d_5, 4.9% d_4, and 1.0% d_3.

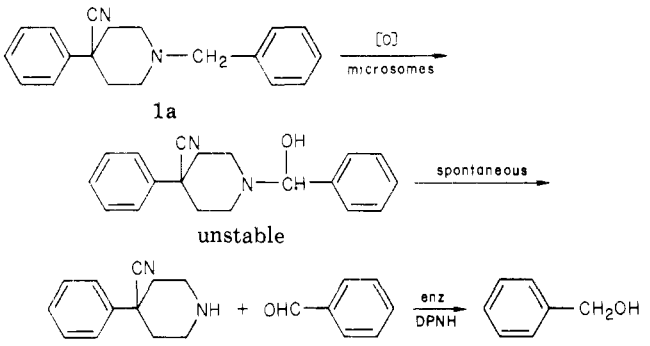
1-(Benzyl-*1,1,2',3',4',5',6'-d*$_7$)-4-cyano-4-phenylpiperidine (1d) was prepared from benzyl-*1,1,2',3',4',5',6'-d*$_7$ bromide. The hydrochloride (96%) had mp 261–262 °C; IR (KBr) 2260 (C≡C-D), 2230 (C≡N), 2175 (C-D) cm^{-1}; NMR δ 2.1 (m, 4 H, CH_2CCH_2), 2.78 (m, 4 H, CH_2NCH_2), 7.49 (m, 5 H, aromatic); EIMS *m/e* (relative intensity) M$^+$ 283 (42%), 282 (17%), 201 (20%), 186 (23%), 185 (23%), 99 (24%), 98 (100%). Deuterium incorporation: 98.1%, of which 89% was d_7, 9% d_6, and 2% d_5.

Preparation of Enzyme. Livers were removed from albino rats (200–230 g), weighed, cut into pieces and placed in 3 volumes of cold 0.1 M phosphate buffer (pH 7.4) containing 0.002 M $MgCl_2$. The liver was then homogenized in a glass homogenizer with a Teflon pestle in the usual manner, and the 100000*g* microsomal pellet was prepared by differential centrifugation. The microsomal pellet was then resuspended in 3 volumes of the same buffer and rehomogenized. This suspension of microsomal enzyme contained the equivalent of 250 mg of liver (wet wt)/mL. It was stored at −20 °C and was stable for at least a week without loss of activity. Samples were thawed just before use.

Isotopic Oxygen Studies. A reaction mixture containing 10.5 mL of rat liver microsomal fraction (from male rats), equivalent to 2.6 g of liver; 35 mg of isocitric acid; 1 mg of isocitric acid dehydrogenase; 21 μM of substrate, 1-benzyl-4-cyano-4-phenylpiperidine hydrochloride; 42 μL of 0.1 N sodium hydroxide; and sufficient pH 7.4 buffer to make a final volume of 21 mL was placed in a round-bottom flask, together with a magnetic stirrer, frozen in liquid nitrogen, and attached to a vacuum line. The flask was evacuated (0.01 mmHg) and then thawed under vacuum. After refreezing in liquid nitrogen, the flask was removed from the vacuum line and, while still frozen, 7 mg of NADPH and 70 mg of NADH were added. The flask, still frozen, was returned to the vacuum line and reevacuated. To the evacuated flask was then added 15 mL of oxygen-18 (91 mol %) and the flask closed off. The reaction mixture was stirred for 0.5 h at 37 °C to complete the reaction.

The reaction was terminated by the addition of 1 mL of 10% deoxycholic acid, and the benzyl alcohol formed in the reaction was recovered by extraction into dichloromethane and by

Scheme I



evaporation. The crude yield of benzyl alcohol was about 200 μg (a percent yield of about one-half of that obtained in the small-scale analytical procedure described immediately below). This suggests that activity retention during the freeze–thaw procedure was reasonably good. The extract was brought to a volume of 500 μL, and 1-μL samples were used for GC–MS analysis (LKB model 9000 instrument). A comparison of the ion distribution of experimentally formed benzyl alcohol with that of unlabeled benzyl alcohol allowed a direct calculation to be made of the percentage of benzyl alcohol containing ^{18}O.

Deuterium Isotope Effects. Each incubation mixture contained 1.5 mL of rat liver microsomes, 1 mg of NADP, 5 mg of isocitric acid, 0.1 mg of isocitric acid dehydrogenase, 10 mg of NADH, 6 μL of 0.1 N sodium hydroxide, 3 μmol of substrate (unlabeled and/or labeled), and sufficient pH 7.4 buffer to give a final volume of 3 mL. Incubations were carried out in air at 37 °C for 30 min, and the reaction was terminated by addition of 0.3 mL of 10% sodium deoxycholate. The yield of benzyl alcohol was determined by the following procedure.

Gas Chromatographic Method for Benzyl Alcohol. To each reaction mixture was added 60 μg of 2-phenethyl alcohol as an internal standard. The reaction mixtures were then extracted with 3 mL of dichloromethane and the extract reduced in volume to about 300 μL. Analyses were performed on an OV-225 3% GC column held at 100 °C. Benzyl alcohol content was calculated from the relative peak heights of benzyl alcohol and of an internal standard utilizing a previously prepared standard curve.

Results and Discussion

Source of Oxygen. ^{18}O Studies. The reaction being studied is summarized in Scheme I. The first step, hydroxylation, is catalyzed by the cytochrome P 450 enzyme bound in the endoplasmic reticulum of the liver cell. If this reaction is indeed a typical aliphatic hydroxylation, it would be expected that the oxygen atom being transferred would originate from molecular oxygen rather than from water.[3] Preliminary studies[11] using 1-benzyl-4-carbethoxy-4-phenylpiperidine as substrate indicated that this was so. However, because yields of product were low and loss of oxygen from benzaldehyde by exchange may have been substantial, the result was considered to be provisional.

The work reported in the present paper utilizes compound **1a** as a substrate, since it is a substantially more active substrate than the ester analogue used earlier.[11] In addition, the loss of ^{18}O from benzaldehyde was minimized by coupled enzymatic reduction to benzyl alcohol. The microsomal fraction from rat liver has been shown to contain a NADH-dependent reductase which will reduce benzaldehyde to benzyl alcohol.[12] This enzyme differs from the soluble aromatic aldehyde reductase described earlier[13] which was NADPH dependent. It is also distinct from alcohol dehydrogenase in that it has the opposite stereospecificity with respect to hydride transfer from NADH. The reaction mixture used in the present study contained a very large excess of NADH to ensure rapid and complete reduction to benzaldehyde as soon as it was formed by spontaneous dissociation of the intermediate carbinolamine.

Table I

mol % labeled compd[b]	yield of benzyl alcohol, μg/30 min, from mixtures of:[a]			
	1a/1b-d_2	1a/1b-d_2	1a/1c-d_5	1a/1d-d_7
0	79 ± 1.4[d]	20.5 ± 0.2[c]	56 ± 1.1[d]	55 ± 0.8[d]
24.5	75 ± 0.03	17 ± 0.4	53 ± 1.8	49 ± 0.4
49.0	66 ± 0.9	16.2 ± 0.4	53 ± 1.5	45 ± 0.6
73.5	61 ± 0.8	14.5 ± 0.2	54 ± 0.6	41 ± 0.7
98.0	55 ± 0.9	14.5 ± 0.1	55 ± 0.1	38 ± 0.2
k_H/k_D[e]	1.46	1.43	1.01	1.47

[a] For details of the method used, see the Experimental Section. Three separate determinations were made. The results represent the median result ± 1 SD. [b] Mole percent was calculated based on an average purity of each labeled compound of 98 mol %. [c] Microsomes for this experiment were prepared from female rats. [d] Microsomes for these experiments were prepared from male rats. A different enzyme preparation was used for the 1b and for the 1c and 1d studies. [e] The data in each column were fitted to the best straight line and rates at 0 and 100 mol % were estimated for calculation of k_H/k_D ratios.

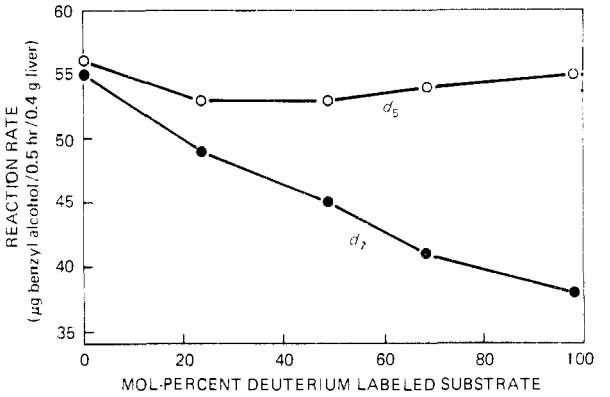


Figure 1. N-Debenzylation of mixtures of labeled and unlabeled 1-benzyl-4-cyano-4-phenylpiperidine. See Table I and Experimental Section for details.

Substrate (**1a**) was incubated with rat liver microsomes in the presence of oxygen containing 91 mol % $^{18}O_2$. Benzyl alcohol was recovered and its isotopic content determined by combined GC–MS. The benzyl alcohol was found to contain 78 mol % (corrected for isotopic purity of oxygen used) of benzyl alcohol-^{18}O. This result contrasts with 28 mol % incorporation observed in the earlier study[11] in which exchange of oxygen with water was thought to be very substantial.

The finding that the source of oxygen in this oxidative dealkylation derives from molecular oxygen strongly supports the proposition that the reaction proceeds by direct hydroxylation; i.e., it is a typical microsomal hydroxylation in which the primary metabolite is a carbinolamine, which in turn dissociates nonenzymatically to the products, the dealkylated amine and an aldehyde.

Deuterium Isotope Effects. The rate of debenzylation of unlabeled **1a** and of its labeled analogues **1b–d** was determined by measuring the extent of benzyl alcohol production during a 30-min incubation with rat liver microsomes. Since preliminary studies indicated that the reaction was linear over this time period, the reaction rates found were considered to be initial rates. In addition to measuring the rates of debenzylation of **1a–d**, the rates of debenzylation of mixtures of **1a** with each labeled species were also determined. The data obtained are shown in

Table I. Data for two compounds (**1c** and **1d**) have also been plotted and are shown in Figure 1. As expected, the yield of benzyl alcohol declines in linear fashion as the mole percent of **1d** (the d_7 analogue) increases. The data from the experiment with **1d** were fitted to the best straight line by least-squares approximation, and rates at 0 (k_H) and at 100 mol % (k_D) were estimated. The resulting apparent isotope effect (k_H/k_D) found was 1.47. Similar calculations were made for the other data sets in Table I, and the isotope effects found are shown in that table.

The primary isotope effects found in the present study for the two methylene-labeled analogues (1.46 with **1b** and 1.47 with **1d**) are very close to the figure of 1.4 reported in 1963 for the oxidative demethylation of deuteriomorphine. This study[4] was the first to show an isotope effect in the microsomal oxygenase system. Since that time, many other reports have appeared. For example, an isotope effect of 1.8 was found for the hydroxylation of ethylbenzene,[3] 2.0 for the O-demethylation of *o*-nitroanisole,[14] 1.23 for the demethylation of ethylmorphine,[15] 1.61 and 1.86 for the O-dealkylation of phenacetin,[16,17] 1.49 for the N-deethylation of lidocaine,[18] 1.45 for the demethylation of dimethylaminophenylpropane,[19] and 1.43 for the 3-hydroxylation of biphenyl.[20]

An additional observation in the present work was that, while microsomes from female rats are much lower in activity, the isotope effect (1.43) is the same as that with microsomes derived from male rats (Table I). The relatively low values of the primary isotope effect found in these systems may be the result of a nonlinear or unsymmetrical transition state. Melander[21] and Westheimer[22] first offered an explanation of small isotope effects based on the fact that an unsymmetrical (e.g., 3-center) transition state for hydrogen transfer will have an additional isotopically sensitive zero-point energy which will offset that of the initial state and thus reduce the isotope effect. Similar arguments[23] could be applied to a possible 4-center transition state for microsomal dealkylation, such as can be postulated if molecular oxygen is activated in the oxygenase complex[24] to "oxene"[25] (isoelectronic with carbenes), which inserts directly into the C–H bond with concomitant removal of the hydrogen atom.

The results of the present study are consistent with a mechanism for N-dealkylation involving direct hydroxylation of the methylene α to nitrogen in a reaction in which a carbon–hydrogen bond is broken in the rate-determining step. Some years ago the photolytic dealkylation of *o*-nitro-*N*-alkylanilines was proposed as a chemical model for microsomal dealkylation.[26] Interestingly enough, the isotope effect in that reaction was found to be 1.59,[27] very similar to that seen in the enzymatic reaction.

The lack of a significant secondary isotope effect in the dealkylation of **1c** (in which only the aromatic ring hydrogens of the benzyl were replaced with deuterium) was not unexpected. Earlier reports indicate no secondary isotope effect for the O-deethylation of phenacetin[16,17] or for the in vivo metabolism of either (+)- or (−)-propoxyphene-d_7.[28] However, since a substantial secondary isotope effect has been reported for the N-deethylation of lidocaine,[18] further work in this area is indicated.

References and Notes

(1) B. B. Brodie, J. R. Gillette, and B. N. LaDu, *Annu. Rev. Biochem.*, **27**, 427 (1958).
(2) R. E. McMahon, *J. Pharm. Sci.*, **55**, 457 (1966).
(3) R. E. McMahon, H. R. Sullivan, J. C. Craig, and W. E. Pereira, Jr., *Arch. Biochem. Biophys.*, **132**, 575 (1969).
(4) C. Elison, H. W. Elliot, M. Look, and H. Rapoport, *J. Med. Chem.*, **6**, 237 (1963).
(5) R. E. McMahon and H. R. Sullivan, *Xenobiotica*, **7**, 377 (1977).
(6) R. A. Prough and D. M. Ziegler, *Arch. Biochem. Biophys.*, **180**, 363 (1977).
(7) R. L. Letsinger and D. F. Pollart, *J. Am. Chem. Soc.*, **78**, 6079 (1956).
(8) T. A. Geissman, *Org. React.*, **2**, 94 (1944).
(9) (a) J. C. Craig and L. R. Kray, *J. Org. Chem.*, **33**, 871 (1968); (b) D. Seebach, B. W. Erickson, and G. Singh, *J. Org. Chem.*, **31**, 4304 (1966); (c) S. B. Matin, J. C. Craig, and R. P. K. Chan, *J. Org. Chem.*, **39**, 2285 (1974).
(10) F. Bergel, N. C. Hindley, A. L. Morrison, and H. Rinderknecht, U.S. Patent 2 398 575; *Chem. Abstr.*, **40**, 3496 (1946).
(11) R. E. McMahon, H. W. Culp, and J. C. Occolowitz, *J. Am. Chem. Soc.*, **91**, 3389 (1969).
(12) H. W. Culp, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32**, 683, abstract (1973).
(13) H. W. Culp and R. E. McMahon, *J. Biol. Chem.*, **243**, 848 (1968).
(14) C. Mitoma, D. M. Yasuda, J. Tagg, and M. Tanabe, *Biochim. Biophys. Acta*, **136**, 566 (1967).
(15) J. A. Thompson and J. L. Holtzman, *Drug Metab. Disp.*, **2**, 577 (1974).
(16) W. A. Garland, S. D. Nelson, and H. A. Sasame, *Biochem. Biophys. Res. Commun.*, **72**, 539 (1976).
(17) S. D. Nelson, W. A. Garland, J. R. Mitchell, Y. Vaishnav, C. N. Statham, and A. R. Buckpitt, *Drug Metab. Disp.*, **6**, 363 (1978).
(18) S. D. Nelson, L. R. Pohl, and W. F. Trager, *J. Med. Chem.*, **18**, 1062 (1975).
(19) M. M. Abdel-Monem, *J. Med. Chem.*, **18**, 427 (1975).
(20) R. E. Billings and R. E. McMahon, *Mol. Pharmacol.*, **14**, 145 (1978).
(21) L. Melander, "Isotope Effects on Reaction Rates", Ronald Press, New York, 1960, pp 24–32.
(22) F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).
(23) A. J. Kresge in "Isotope Effects on Enzyme-Catalyzed Reactions", W. W. Cleland, M. H. O'Leary, and D. B. Northrop, Eds., University Park Press, Baltimore, Md., 1977, pp 43–56.
(24) D. E. Hathaway, "Foreign Compound Metabolism in Mammals", Vol. 2, Specialist Periodical Report, The Chemical Society, Burlington House, London, 1972, pp 346–356.
(25) D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Underfriend, *Biochemistry*, **9**, 147 (1970).
(26) R. E. McMahon, *Tetrahedron Lett.*, 2307 (1966).
(27) R. E. McMahon, W. M. Miller, and F. J. Marshall, in "Biological Oxidation of Nitrogen Compounds", J. W. Gorrod, Ed., Elsevier, Amsterdam, 1978.
(28) R. E. McMahon and H. R. Sullivan, *Res. Commun. Chem. Pathol. Pharmacol.*, **14**, 631 (1976).