Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 2531–2539

BIOORGANIC & MEDICINAL CHEMISTRY

Rat Liver Microsomal Enzyme Catalyzed Oxidation of 1-Cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine

Zhiyang Zhao,[b] Stéphane Mabic,[a] Simon Kuttab,[a] Christelle Franot,[a] Kay Castagnoli[a] and Neal Castagnoli, Jr.[a,*]

[a]*Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0212, USA*
[b]*Drug Metabolism Research, Pharmacia and Upjohn, Inc., 333 Portage Road, Kalamazoo, MI 49001-0199, USA*

Received 21 May 1998; accepted 16 October 1998

Abstract—NADPH supplemented rat liver microsomal enzyme preparations catalyze the conversion of 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine to the *p*-hydroxyphenyl (low yield), descyclopropyl (high yield) and 2,3-dihydropyridinium and, subsequently, pyridinium (intermediary yield) metabolites. When the methine proton of the cyclopropyl group was replaced with a deuteron, a normal deuterium isotope effect (1.4) was observed on the formation of the decyclopropylated metabolite and an inverse isotope effect (0.6) on the dihydropyridinium metabolite. A larger deuterium isotope effect (3.6) was observed on the ring α-carbon oxidation pathway with the 2,2,6,6-d_4 analogue as substrate. These results and the observation that the ratios of the rates of these two α-carbon oxidation pathways are independent of initial substrate concentrations suggest that both pathways are catalyzed by the same active site of one form of P450. These transformations are discussed in terms of metabolic pathways that have been proposed for the cytochrome P450 catalyzed α-carbon oxidation of amines.

Introduction

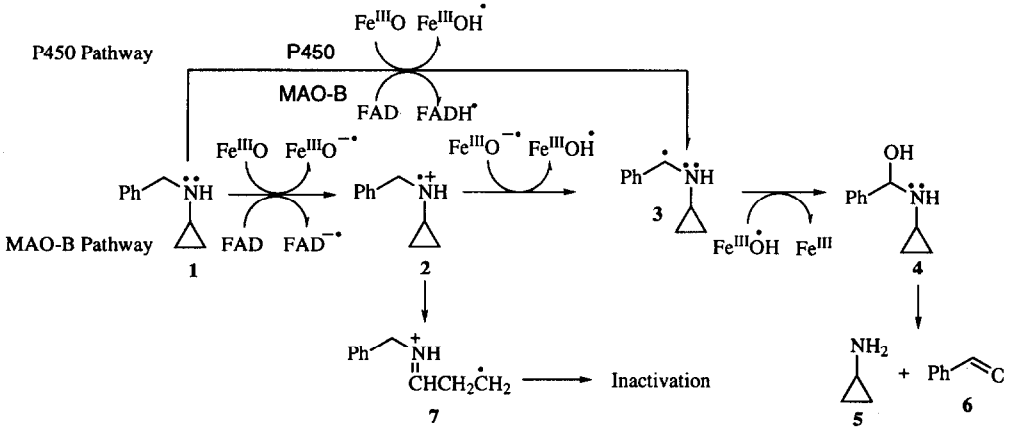
The cytochrome P450 catalyzed oxidative N-dealkylation of amines, illustrated in Scheme 1 for the oxidative N-debenzylation of N-benzylcyclopropylamine (**1**), has been proposed to proceed via an initial single electron transfer (SET) step from the nitrogen lone pair to the P450 iron oxo group followed by deprotonation of the resulting aminyl radical cation (**2**) to form an α-carbon radical (**3**). Radical recombination leads to a carbinolamine intermediate (**4**) that cleaves to yield the dealkylated amine (**5**) and an aldehyde (**6**).

The evidence supporting the SET pathway has been summarized in a recent paper by Guengerich and Macdonald.[1] Particularly persuasive are the mechanism based inactivator properties of cyclopropylamines such as **1**.[2] In this case the SET generated cyclopropylaminyl radical cation **2** ring opens to form the primary carbon centered radical **7** that is thought to mediate the inactivation by alkylating an enzyme active site functionality. This pathway is consistent with the results of kinetic studies showing that a cyclopropylaminyl radical undergoes ring opening at a rate of $7\times10^{11}\,s^{-1}$ or faster.[3] The corresponding rates for cyclopropylaminyl radical cations appear to be too fast to measure.[4]

Despite the evidence quoted above, the SET pathway remains a subject of debate with some investigators favoring a hydrogen atom transfer (HAT) pathway (**1**→**3**, Scheme 1).[1,5] Chen et al. recently reported that treatment of *p*-cyclopropyl-*N,N*-dimethylaniline with horseradish peroxidase or P450 or under model SET reaction conditions leads exclusively to N-demethylation with no evidence of ring opening.[6] A possible explanation for the absence of ring opened products was based on ab initio calculations showing that the majority of the charge and unpaired spin in the radical cation of the aniline derivative are centered on the nitrogen atom rather than the aromatic ring. On the other hand, the P450 catalyzed oxidations of phenylcyclopropane and *p*-methoxyphenylcyclopropane undergo benzylic hydroxylation rather than ring opening reactions[7] even though the photochemically generated radical cations

*Corresponding author. Tel: +1-540-231-8202; Fax: 540 231-8890; E-mail: ncastagnoli@chemserver.chem.vt.edu

0968-0896/98/$ - see front matter
PII: S0968-0896(98)00223-5



Scheme 1. Proposed pathways for the cytochrome P450 catalyzed α-carbon oxidation and mechanism based inactivation of *N*-benzylcyclopropylamine (**1**).

derived from phenylcyclopropane and *p*-methoxyphenylcyclopropane undergo ring opening reactions.[8] Unlike the case with the aniline derivative, ab initio calculations are consistent with the direct formation of the benzylic radical by a HAT pathway.[9] Results of studies from the laboratories of Jones and Dinnocenzo in which deuterium isotope effect profiles observed for chemical models of the HAT and SET pathways of a series of amine substrates were compared with the corresponding profiles observed in the P450 catalyzed oxidations stongly argue in favor of thc HAT pathway.[10] Finally, comparisons of the regio, stereochemical and deuterium isotope effect outcomes of the P450 catalyzed oxidation of (*S*)-nicotine-(*R*)-5-d_1 and (*S*)-nicotine-(*S*)-5-d_1 with the results from electrochemical and photochemical model reactions have led Carlson et al. to argue in favor of a HAT pathway.[11]

Cyclopropylamines such as **1** also are mechanism based inactivators of the flavoprotein monoamine oxidase B (MAO-B),[12] an outer membrane mitochondrial enzyme that catalyzes the α-carbon oxidation of a variety of neurotransmitter substances.[13] A SET reaction pathway with flavin adenine dinucleotide (FAD) serving as the electron acceptor also has been proposed for these reactions.[14] Strong support for the SET mechanism comes from the recent report that treatment of MAO-B with a cyclopropylamine mechanism based inactivator produces a cysteinyl residue modified on the thiol group with the three carbon atoms derived from the cyclopropyl moiety.[15]

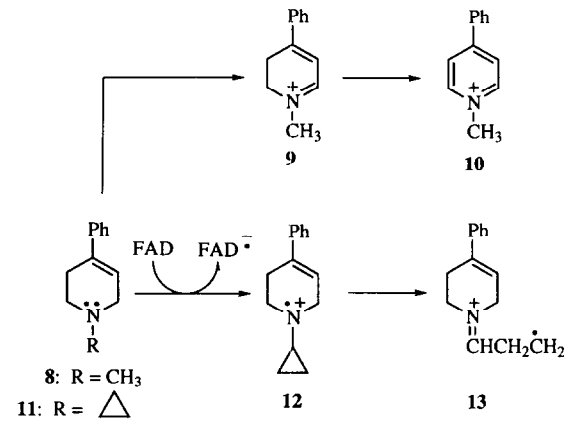
Our interests in the MAO-B catalyzed metabolic bioactivation of the parkinsonian inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (**8**) to the neurotoxic 1-methyl-4-phenylpyridinium metabolite MPP^+ (**10**) via the corresponding dihydropyridinium intermediate **9**,[16] prompted us to examine the MAO-B inactivating properties of 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine (**11**). Consistent with a SET pathway, **11** proved to be an efficient mechanism based inactivator of this enzyme.[17] The inactivation pathway presumably proceeds via the cyclopropylaminyl radical cation **12** and the subsequent bioalkylation of the enzyme active site by the ring opened carbon centered radical **13** (Scheme 2). Related studies with a variety of 1-cyclopropyl-4-substituted-1,2,3,6-tetrahydropyridinyl derivatives, however, have clouded this picture since some of these analogues display poor or no inactivator properties but instead are readily converted to the corresponding dihydropyridinium metabolites, behavior which may be more consistent with a HAT than a SET mechanism since the HAT pathway obviates the possibility of the ring opening reaction leading to enzyme inactivation.[18] Chemical model studies have confirmed that **11** and related 1-cyclopropyl-4-substituted-1,2,3,6-tetrahydropyridinyl derivatives undergo only allylic α-carbon oxidation under model HAT conditions. When examined under SET reaction conditions, however, only products generated via intermediates formed from ring opening of the cyclopropylaminyl radical cation were observed.[19]

In an attempt to gain additional information on the comparative features of the P450 versus MAO-B catalyzed oxidations of 1-cyclopropyltetrahydropyridinyl derivatives, studies were undertaken to characterize the P450 catalyzed oxidation of **11** using NADPH supplemented rat liver microsomal enzyme preparations.

Results and Discussion

The metabolites derived from **11** were characterized by comparison of their liquid chromatographic-diode array

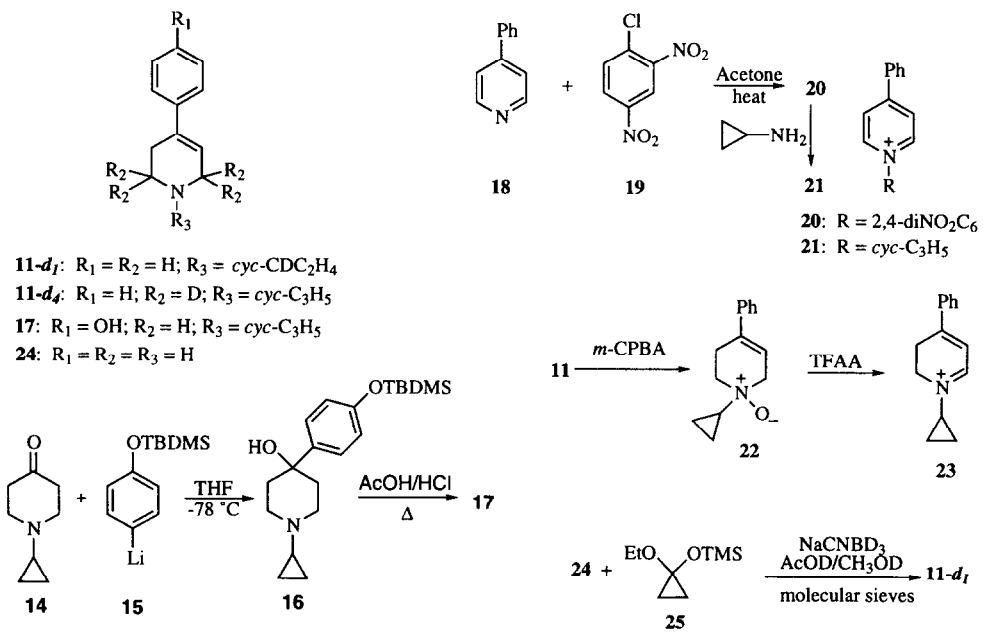
(LC-DA) and liquid chromatographic-electrospray ionization (LC-ESI) mass spectral properties with those of authentic standards which were prepared as follows (Scheme 3). **M1**, the *p*-hydroxyphenyl metabolite **17**, was obtained by reaction of 1-cyclopropyl-4-piperidone (**14**)[20,21] with the lithiated silyloxyphenyl species **15**[22,23] followed by acid catalyzed dehydration and desilylation of the resulting carbinol **16**.[24] **M2**, the decyclopropylated metabolite **24**, was commercially available. **M3**, the pyridinium metabolite **21** which is produced from the initially formed but unstable dihydropyridinium intermediate **23**, was obtained by reaction of cyclopropylamine with the Zincke salt **20**, prepared from 4-phenylpyridine (**18**) and 2,4-dinitrochlorobenzene (**19**).[25,26] Oxidation of **11** with *m*-CPBA to the N-oxide **22** followed by treatment with trifluoroacetic anhydride gave the dihydropyridinium intermediary metabolite **23**.[16,19] As observed previously with related 2,3-dihydropyridinium compounds,[27] **23** was stable only under acidic conditions. Therefore, isolation of this compound was achieved by the addition of 70% aqueous $HClO_4$ to the reaction mixture and purification of the resulting perchlorate salt.



Scheme 2. SET pathway proposed for the MAO-B catalyzed oxidation of 1,4-disubstituted 1,2,3,6-tetrahydropyridine derivatives.

Deuterium isotope effect studies employed the known 2,2,6,6-tetradeutero analogue **11-d_4**[28] and the 1-(1-deuterocyclopropyl) analogue **11-d_1** which was synthesized by a recently reported reductive amination reaction[29] of amine **24** with 1-ethoxy-1-(trimethylsilyloxy)cyclopropane (**25**) and $NaCNBD_3$. The deuterium content of this product was estimated to be ≥95% by GC-EIMS and ^1H NMR analysis using CH_3OD and AcOD but only 60% with non-deuterated solvents. This method constitutes a simple and efficient alternative to the multistep synthesis reported by Corey for the preparation of cyclopropylamine derivatives deuterated in the methine position.[30]

LC-DA analysis of rat liver microsomal incubation mixtures documented the time and NADPH dependent decrease in concentration of the substrate **11** (20.5 min, λ_{max} 245 nm) and the concomitant appearance of three metabolites designated **M1** (6.9 min, λ_{max} 255 nm), **M2**



Scheme 3. Synthesis of metabolites and the deuterium labeled substrate **11-d_1**.

(9.9 min, λ_{max} 245 nm) and **M3** (11.5 min, λ_{max} 299 nm). The 299 nm chromophore of **M3** led us to suspect that this metabolite was the 1-cyclopropyl-4-phenylpyridinium species **21** since the λ_{max} value for the corresponding 1-methylpyridinium compound **10** also is 299 nm. This tentative assignment was supported by the identical LC-DA spectrum observed with a synthetic sample of **21**. The biotransformation of **11** to **21** was shown to proceed via the dihydropyridinium intermediate **23** since the LC-DA spectrum of the incubation mixture at $t < 15$ min revealed a peak with the same retention time (12.7 min) and UV diode array spectrum (λ_{max} 340 nm) as those observed with synthetic **23**.

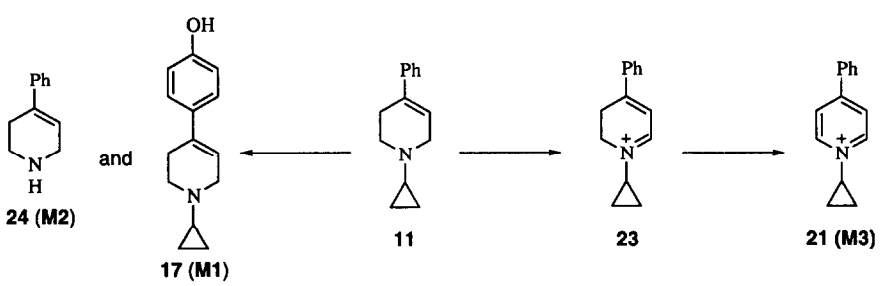
The UV spectral features of **M1** and **M2** were not distinctive and therefore LC-ESI mass spectral data were obtained. Peaks corresponding to all three metabolites were observed in the total ion current chromatogram. The same retention time and parent ion ($M^+ = 196$ Da) observed for **M3** and the synthetic standard confirmed the structure of this metabolite as the pyridinium species **21**. **M2** displayed a parent ion at m/z 160 which corresponds to MH^+ of the N-descyclopropyl species **24**. An authentic sample of **24** displayed LC-DA and LC-ESI mass spectral properties identical to those of **M2** confirming this assignment. Finally, the difference of 16 Da for MH^+ of the most polar metabolite **M1** ($m/z = 216$) and that of the substrate molecule **11** ($MH^+ = 200$ Da) indicated a monooxygenated product. Based on literature reports documenting the NADPH dependent rat liver microsomal catalyzed N-oxidation of MPTP,[31–33] the N-oxide **22** was tentatively proposed as the structure of **M1**. Comparison of the LC-DA behavior of synthetic **22** (which did not elute during the 30 min chromatographic run) with that of **M1**, however, ruled out this possibility. Subsequently the structure of **M1** was shown to be the *p*-hydroxy derivative 1-cyclopropyl-4-(4-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (**17**) by comparing its LC-DA and LC-ESI mass spectral characteristics of **M1** with those of synthetic **17**. The metabolic pathway documented for **11** in this study is summarized in Scheme 4.

Estimates of the initial rates of formation of these three metabolites (Table 1) were obtained by LC-DA analysis of aliquots of incubation mixtures taken from 0 to 5 min during which time the rates of metabolite formation were constant.[34] The initial rate of formation of the descyclopropyl metabolite **24** (4.9 ± 0.4 nmol/min-mg protein) was about 3 times faster than the corresponding rate for the pyridinium metabolite **21** (1.7 ± 0.1 nmol/min-mg protein). In an attempt to determine if these 2 α-carbon oxidations may be catalyzed by the same active site, we estimated the ratios of the rates of formation of **21** and **24** at different initial substrate concentrations (500, 250, 125 and 62.5 μM). Since the resulting ratios (3.3, 3.2, 3.3 and 3.1, respectively) were very similar, it seems likely that a single catalytic site mediates both pathways.

We also examined the deuterium isotope effects of these biotransformations with the aid of the 1-(1-deuterocyclopropyl) and 2,2,*6*,*6*-tetradeutero analogues, **11-*d*$_1$** and **11-*2*,*2*,*6*,*6*-*d*$_4$**, respectively (Table 1). The absence of an inverse isotope effect on the rate of formation of the phenolic metabolite **17** suggests that a form of P450 different from that responsible for the α-carbon oxidative transformations catalyzes the *p*-hydroxylation of **11**. Normal isotope effects (1.4 ± 0.1, for the pathway leading to loss of the cyclopropyl group, and of 3.4 ± 0.1, for the

Table 1. Initial rates and isotope effects observed in the oxidative metabolism of **11**

Substrate (250 μM)	Metabolite	Rate (nmol/mg protein/min)	Isotope Effect
	17	0.9 ± 0.1	–
11-*d*$_0$	**24**	4.9 ± 0.2	–
	21	1.7 ± 0.1	–
	17	0.9 ± 0.2	1.0 ± 0.3
11-*d*$_1$	**24**	3.6 ± 0.3	1.4 ± 0.1
	21	2.7 ± 0.1	0.6 ± 0.1
	17	0.9 ± 0.1	1.0 ± 0.1
11-*d*$_4$	**24**	5.4 ± 0.3	0.9 ± 0.1
	21	0.6 ± 0.1	3.4 ± 0.1



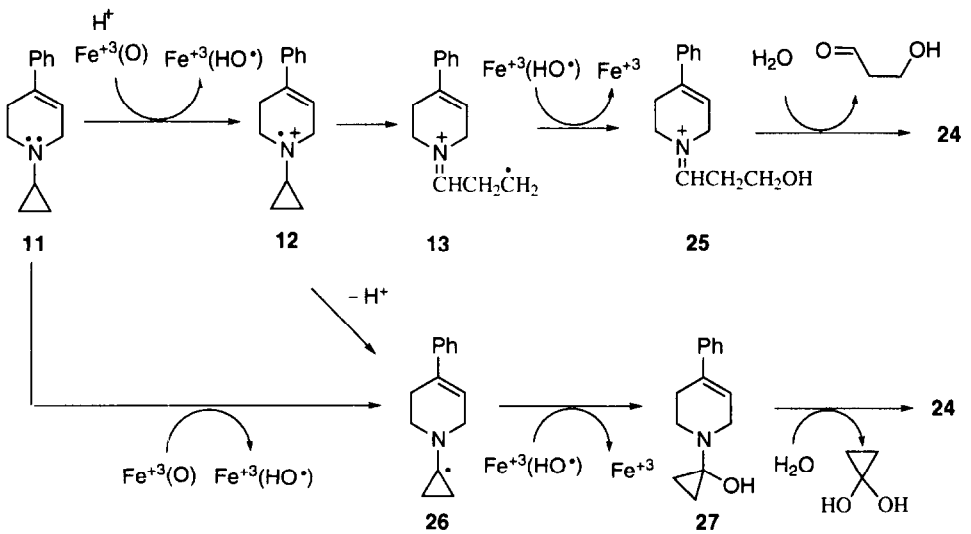
Scheme 4. Metabolic pathway for the rat liver microsomal catalyzed oxidation of 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine (**11**).

ring α-carbon oxidation pathway) were observed for the transformations involving the C–D versus C–H bond cleavages. An inverse isotope effect (0.6±0.1) was observed for the ring α-carbon oxidation of **11-d_1**. This 'isotope dependent metabolic shifting' also is consistent with the proposal that these two biotransformations are catalyzed by the same enzyme active site since one would expect a relatively large inverse isotope effect when the metabolic shifting involves a normal isotope effect on the major pathway, in this case the pathway leading to N-dealkylation. A much smaller inverse isotope effect (0.9) was observed on the decyclopropylation pathway with the d_4 substrate as would be expected for this minor pathway.

The formation of the N-dealkylated metabolite **24** could proceed through the SET pathway by radical recombination of the distonic radical cation **13** to form the carbinol **25** followed by hydrolysis of the iminium functionality (Scheme 5). The theoretical maximum value for the secondary isotope that would be expected for the formation of **13** is 1.36.[35,36] The experimentally determined isotope effect of 1.4±0.1 therefore does not rule out this pathway although secondary isotope effects for processes involving conversion of an sp^3 center to an sp^2 center are reported to be about 1.2.[36,37] On the other hand, a normal (and, perhaps masked) deuterium isotope effect would be expected if the intermediate radical cation **12** were to undergo deprotonation to form the carbon radical **26** which would eventually yield **24** via the carbinolamine **27**. A normal isotope effect also would be expected with the HAT pathway in which the P450 iron-oxo species abstracts the methine proton from the cyclopropyl group to form the carbon radical **26**. Both pathways also would account for the metabolic shift to the dihydropyridinium metabolite **23** observed with **11-d_1** as substrate.

The present data do not provide a clear distinction between the SET and HAT pathways. If the cyclopropylaminyl radical cation **12** is an obligatory intermediate in these cytochrome P450 catalyzed oxidations, then the α-carbon deprotonation steps must proceed faster than the rate of ring opening estimated to be at least $7\times10^{11}\,s^{-1}$. Since opening of the cyclopropyl ring requires appropriate alignment of the half-filled p-orbital of the radical cation with the p-type orbitals of the cyclopropyl group (the bisected conformer $\mathbf{11}_{bisec}$ shown in Figure 1), ring opening could be retarded if the cyclopropyl radical cation were constrained in the active site to the higher energy perpendicular conformer ($\mathbf{11}_{perpen}$).

Calculations performed at the semiempirical level (AM1) on the aminium radical species **11** estimated a difference in energy of 1.75 kcal/mol in favor of the bisected conformation. An electron spin density map—contribution of the unpaired electron on each atom—also was generated. It clearly shows a transfer of spin density from the C–C cyclopropyl bonds in the bisected conformation (Fig. 2(A)) to the methine H in the perpendicular conformation (Fig. 2(B)). Values of spin densities for hydrogen atoms at the C6 position of the tetrahydropyridinyl ring and the C1 position of the cyclopropyl ring are reported in Table 2. While the contribution of the two C6 hydrogen atoms remains similar for both conformations, the contribution of the methine H is multiplied by a factor of 16 for the bisected



Scheme 5. Possible pathways for the cytochrome P450 catalyzed oxidative N-decyclopropylation of the tetrahydropyridine derivative **11**.

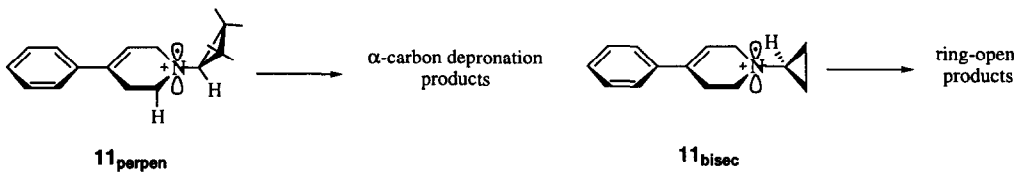


Figure 1. Conformations of the N-cyclopropyl moiety.

conformation relative to that of the perpendicular conformation making the spin density on this hydrogen atom similar to that present on the C6 hydrogen atoms. These results argue not only for a much decreased rate of ring opening in the perpendicular conformation but also for a potentially higher reactivity for deprotonation of the methine H in the perpendicular conformation. These results also are compatible with the observed competitive pathways involving loss of the C6 proton, to form the dihydropyridinium metabolite **23**, and the cyclopropyl methine proton to form, eventually, the dealkylated metabolite **24**.

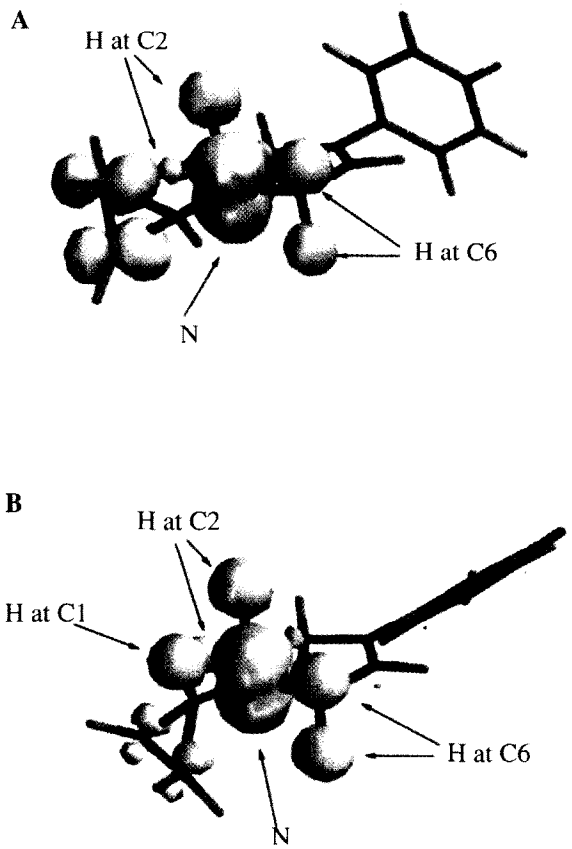


Figure 2. Spin density maps of bisected (A) and perpendicular (B) conformers of the cyclopropylaminyl radical cation **12**.

Experimental

General

The HPLC systems consisted of a Perkin–Elmer Series 410 quaternary gradient HPLC pump, ISS-200 autosampler, LC-235C diode array (LC-DA) detector. A Zorbax RX C_8 HPLC column (4.6 mm i.d. × 25 cm) employing an isocratic mobile phase (0.1 M ammonium acetate adjusted to pH 5.0 with 0.1 M acetic acid:-acetonitrile (80/20))and a flow rate of 1.0 mL/min. The detection wavelengths were 250, 300 and/or 350 nm. LC-Electrospray Ionization Mass Spectrometry (LC-ESIMS) was performed on a Finnigan TSQ 7000 mass spectrometer directly coupled to the LC system which consisted of a Hewlett Packard 1050 Series pump, an autosampler and a Thar (Thar Designs Inc., Pittsburgh, PA) two position actuator. Chromatographic separation conditions were the same as described above except that the flow rate was 0.5 mL/min. The mass spectrometer was controlled using Instrument Control Language (ICL, version 8.0) and the data were processed using Interactive Chemical Information System (ICIS, version 8.1.1) software. Data were collected on a DEC 3000 model 300X computer running OSF/1 version 2.0 as the operating system.

Microsomal preparations

Microsomal fractions were prepared from saline rinsed whole livers, freshly obtained and pooled from 30 untreated male CRL:CD BR Sprague–Dawley rats (250–300 g) following standard procedures.[38] A suspension of the final microsomal pellet in 20% glycerol solution (20 mM potassium phosphate buffer, pH 7.4 in 20% w/v glycerol containing 0.1 mM EDTA) was stored at −65 °C until use. Microsomal protein concentrations

Table 2. Spin densities on selected hydrogen atoms in the bisected conformation (0°) and perpendicular conformation (90°) of **11**

	0°	90°
H6a	0.0481	0.0496
H6b	0.0434	0.0576
HcPr	0.0028	0.0470

were determined by the bicinchoninic acid method[39] and standardized relative to bovine serum albumin. Total cytochrome P450 content was measured by absorption at 450 nm of the dithionite-reduced CO difference spectrum using $\varepsilon_{450-490} = 91\ mM^{-1}\ cm^{-1}$.[40]

Microsomal incubations

Typical microsomal metabolism studies were conducted in an incubation system consisting of 1 μM total cytochrome P450 in a final volume of 1 mL 50 mM *Tris*-HCl buffer containing 0.1 mM EDTA, pH 7.4 at 37 °C. Substrates (25 mM) were added in 10 μL CH_3OH for a final concentration 250 μM. After pre-incubation at 37 °C for 2 min, the reaction was initiated by addition of 100 μL of an NADPH generating system [5 units isocitric dehydrogenase (ICDH), 10 mM $NADP^+$] to maintain a steady state concentration of 1 mM NADPH in the incubation mixture. Aliquots (150 μL) taken at 0, 2, 5, 10, 20, and 40 min were quenched by the addition of an equal volume of CH_3CN and the resulting mixtures were centrifuged at 4 °C for 5 min (Eppendorf centrifuge 5402, Rotor F-45-18-11, 15,800 *g*). The supernatants were analyzed directly by HPLC or LC-ESIMS (100 μL injections). Isotope effect measurements, employing **11-*d*$_0$**, **11-*d*$_1$** and **11-*d*$_4$**, and measurements of the effects of substrate concentration on the rates of formation of metabolites **18** and **21** (see below) were carried out in the same manner. Control incubations were conducted in the absence of either drug or the NADPH generating system under which conditions no evidence of metabolite formation was observed.

Chemistry

The general section is the same as reported previously.[20] The N-cyclopropyltetrahydropyridinyl substrate **11** as its oxalate salt was prepared in methanol by $NaBH_4$ reduction of the corresponding pyridinium species **21**.[41] The melting point (185–186 °C) and spectral properties of this compound were identical to those reported previously for this compound prepared by an independent route.[17] The HCl salts 4-phenylpyridine (**18**) and 4-phenyl-1,2,3,6-tetrahydropyridine (**24**) were purchased from Aldrich; 1-cyclopropyl-4-piperidone (**14**)[21] and **11-*2,2,6,6-d*$_4$**[28] were prepared as previously reported. Calculations were performed using AM1 embedded in the software MacSpartan (Wavefunction, Inc.). The unrestricted Hartree–Fock method was used (multiplicity: 2, charge 1) to determine the energy and spin density of **11**.

1-(2,4-Dinitrophenyl)-4-phenylpyridinium chloride (20)[25]. A mixture of 4-phenylpyridine (**18**, 7.1 g, 46 mmol) and 2,4-dinitrochlorobenzene (**19**, 14 g, 69 mmol) in 200 mL anhydrous acetone was heated under reflux for 48 h. The precipitated product was collected and washed with acetone. The filtrate was concentrated to 100 mL and additional **19** (1 g, 5 mmol) was added. This reaction mixture was heated under reflux for an additional 24 h and the second crop of precipitated product was collected and combined with the first crop to afford 15.8 g (45 mmol, 98%) of product: mp 178–178.5 °C. The ^1H NMR spectrum [(CD_3OD) δ 9.28 (1H, d, J = 2.5 Hz), 9.23 (2H, m, d like, J = 7.0 Hz), 8.91 (1H, dd, J = 2.5 Hz, J = 8.5 Hz), 8.69 (2H, m, d like, J = 7.0 Hz), 8.32 (1H, d, J = 8.5 Hz), 8.17 (2H, m), 7.73 (3H, m)] corresponded to that reported previously;[27] ^{13}C NMR (CD_3OD) δ 161.1, 151.5, 147.4, 145.2, 140.5, 135.2, 135.0, 133.4, 131.7, 130.3, 126.3, 123.7; UV (nm, MeOH) 210, 315.

1-Cyclopropyl-4-(4-*tert*-butyldimethylsilyloxyphenyl) piperidin-4-ol (16). A solution of *p-tert*-butyldimethyl silyloxybromobenzene[22] (1.58 g, 5.5 mmol) in anhydrous THF (50 mL) was converted to its lithio derivative **15** by the dropwise addition of a solution of *s*-BuLi (5.5 mmol, 1.3 M, 4.2 mL) in THF. The reaction mixture was stirred at −78 °C for 40 min and the piperidone **14** (695 mg, 5 mmol) was then added. After stirring an additional 12 h at 25 °C, a saturated solution of aqueous NH_4Cl (20 mL) was added and the resulting mixture was extracted with Et_2O (3×100 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (Hex/AcOEt 6/4) to give **16** (1.33 g, 4 mmol, 73% yield) as a white solid: mp 75–77 °C; ^1H NMR ($CDCl_3$) δ 7.32 (2H, m), 6.77 (2H, m), 2.89 (2H, bd, J = 11.3 Hz), 2.64 (2H, td, J = 12.0 Hz, J = 2.6 Hz), 2.02 (2H, td, J = 13.3 Hz, J = 4.6 Hz), 1.72 (2H, m), 1.65 (1H, m), 0.96 (9H, s), 0.43 (4H, m), 0.17 (6H, s); ^{13}C NMR ($CDCl_3$) δ 154.5, 141.3, 125.7, 119.7, 71.1, 49.7, 38.7, 38.4, 25.7, 18.2, 5.9, −4.5; GC (t_R = 11.76 min)–EIMS (*m/z*, rel int) 348 (11), 318 (20), 205 (17), 112 (47), 99 (42), 98 (40), 97 (42), 82 (89), 70 (58), 41 (100). UV (nm, MeOH) 209, 221, 272. Anal. calcd for $C_{20}H_{33}NO_2Si$: C, 69.11; H, 9.57; N, 4.03. Found: C, 68.88; H, 9.62; N, 4.02.

1-Cyclopropyl-4-(4-hydroxyphenyl)-1,2,3,6-tetrahydropyridinium oxalate [17·(CO_2H)$_2$]. The piperidinol **16** (347 mg, 1 mmol) was heated under reflux in 10 mL of a mixture of AcOH/HCl (3/1) for 3 h. After cooling, the pH was adjusted to 9 by the slow addition of 40% NaOH at 0 °C. After extraction with AcOEt (3×25 mL), the combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The residue in CH_3CN was treated with an etheral solution of oxalic acid (1.04 mg, 1.15 mmol) under anhydrous conditions. The resulting salt was recrystallized from CH_3OH to yield **17·(CO_2H)$_2$** as a white solid (235 mg, 0.77 mmol, 77% yield): mp 175–185 °C (dec.); ^1H NMR (DMSO-d_6) δ 7.25 (2H, d,

J=8.6 Hz), 6.73 (2H, d, J=8.6 Hz), 5.97 (1H, bs), 3.54 (2H, bs), 3.11 (2H, t, J=5.8 Hz), 2.53 (2H, m), 2.26 (1H, m), 0.61 (4H, m); ^{13}C NMR (DMSO-d_6) δ 165.9, 157.7, 135.9, 131.4, 127.4, 116.3, 114.1, 52.6, 51.2, 39.4, 25.2, 4.5; GC (t_R=8.73 min)–EIMS (*m/z*, rel int) 215 (42), 200 (100), 158 (30), 145 (28), 131 (46), 107 (34), 77 (33). UV (nm, MeOH) 209, 259. Anal. calcd for $C_{16}H_{19}NO_5$: C, 60.21; H, 6.14; N, 5.02. Found: C, 60.34; H, 6.19; N, 4.97.

1-Cyclopropyl-4-phenylpyridinium chloride (21·Cl). A solution of the dinitrophenylpyridinium salt **20** (7.2 g, 20 mmol) and cyclopropylamine (2.05 mL, 60 mmol) in anhydrous butanol (200 mL) was heated under reflux for 3 h and stirred for an additional 12 h at 25 °C. The dark red color that formed initially slowly disappeared during the course of the reaction. An aqueous solution of the residue obtained after removing the solvent was washed with CH_2Cl_2 (7×100 mL) and the water was removed under reduced pressure to afford the hygroscopic 1-cyclopropyl-4-phenylpyridinium chloride (**21·Cl**, 4.5 g, 19.4 mmol, 97%) which was recrystallized from water to give the colorless analytical sample: mp 101–103 °C; ^{1}H NMR (CD_3OD) δ 9.15 (2H, m, d like, J=6.8 Hz), 8.35 (2H, m, d like, J=6.8 Hz), 8.00 (2H, m), 7.60 (3H, m), 4.36 (1H, tt, J=4.1 Hz, J=7.5 Hz), 1.40 (4H, m); ^{13}C NMR (CD_3OD) δ 158.8, 147.1, 135.7, 133.9, 131.4, 129.7, 126.2, 43.9, 8.7. UV (nm, MeOH) 209, 299. Anal. calcd for $C_{14}H_{14}NCl·1.6\ H_2O$: C, 64.60; H, 6.61; N, 5.38. Found: C, 64.57; H, 6.59; N, 5.49.

1-Cyclopropyl-4-phenyl-2,3-dihydropyridinium perchlorate (14·HClO$_4$). A solution of 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine [**10**, obtained from 1.5 g (5.2 mmol) of the corresponding oxalate salt] and 50–60% *m*-CPBA (1.25 g, 7.25 mmol) in CH_2Cl_2 (150 mL) was stirred at room temperature for 15 min. The solvent was removed under reduced pressure to give the *m*-chlorobenzoate salt of the N-oxide **16**. The free N-oxide (1.12 g, 5.2 mmol, 100%) was obtained following passage of the salt through a column of basic alumina with CH_2Cl_2.[27] To a solution of the crude N-oxide **22** (250 mg, 1.16 mmol) in CH_2Cl_2 (100 mL) was added at 0 °C trifluoroacetic anhydride (0.68 mL, 5.80 mmol). The reaction mixture was stirred for 5 min and then treated with 70% aqueous $HClO_4$ (10 mL). The solvent was removed 30 min later under reduced pressure and upon addition of CH_3OH/Et_2O (10/1) a yellow solid was obtained (157 mg, 0.527 mmol, 45%): mp 138–139 °C; ^{1}H NMR (CD_3OD) δ 8.52 (dq, 1H, J=5.0 Hz, J=1.2 Hz), 7.82–7.86 (m, 2H), 7.52–7.56 (m, 3H), 6.95 (dt-like, 1H, J=5.0 Hz, J=1.1 Hz), 4.12 (t-like, 2H, J=8.9 Hz), 4.07–4.17 (m, 1H), 3.28–3.35 (m, 2H), 1.18–1.26 (m, 2H), 1.13–1.18 (m, 2H); UV (nm, CH_3OH) 352 (ε 9000 M^{-1}). Anal. calcd for $C_{14}H_{16}ClNO_4$: C: 56.28, H: 5.42, N: 4.70. Found: C: 55.90, H: 5.33, N: 4.61.

1-(1-Deuterocyclopropyl)-4-phenyl-1,2,3,6-tetrahydropyridinium oxalate [11-d_1·(CO$_2$H)$_2$]. A solution of the freshly prepared free amine **24** (1.5 mmol, from 295 mg **24**·HCl) in CH_3OD (15 mL) was treated with AcOD (915 mg, 15 mmol), 4 Å molecular sieves (1 g), and 1-ethoxy-1-trimethylsilyloxycyclopropane (**25**, 800 mg, 4.5 mmol). $NaBCND_3$ (300 mg, 4.5 mmol) was added and the heterogeneous reaction mixture was heated under reflux with stirring for 36 h, filtered and the filtrate concentrated. A solution of the resulting residue in EtOAc (25 mL) was washed with a saturated solution of K_2CO_3 (10 mL), brine (10 mL) and dried over $MgSO_4$. After evaporation of the solvent the crude product in Et_2O:MeOH (2:1) was treated with an etheral solution of oxalic acid (149 mg, 1.65 mmol) and the resulting precipitate was recrystallized from MeOH to give 308 mg (1.06 mmol, 71%) of **11-d_1·(CO$_2$H)$_2$** as white crystals: mp 184–185 °C (mp[17] of **11-d_0** 185 °C); ^{1}H NMR (DMSO-d_6) δ 7.45 (2H, m), 7.35 (2H, tt, J=1.4 Hz, J=7.2 Hz), 7.28 (1H, tt, J=1.3 Hz, J=7.2 Hz), 6.16 (1H, m), 3.64 (2H, bd, J=3.1 Hz), 3.23 (2H, t, J=5.9 Hz), 2.64 (2H, m), 0.74 (2H, m), 0.67 (2H, m); GC (8.99 min) MS (*m/z*, rel int) 200 (41), 185 (100), 128 (39), 115 (43), 91 (24), 55 (23).

Acknowledgements

This work was supported by the National Institute of Neurological and Communicative Disorders and Stroke (NS 28792) and by the Harvey W. Peters Center for the Study of Parkinson's Disease.

References and Notes

1. Guengerich, F. P.; Yun, C.-H.; Macdonald, T. L. *J. Biol. Chem.* **1996**, *271*, 27321–27329.

2. Hanzlik, R. P.; Tullman, R. H. *J. Amer. Chem. Soc.* **1982**, *104*, 2048–2050; Macdonald, T. L.; Zirvi, K.; Burka, L. T.; Peyman, P.; Guengerich, F. P. *J. Amer. Chem. Soc.* **1982**, *104*, 2050–2052; Guengerich, F. P.; Willard, R. J.; Shea, J. P.; Richards, L. E.; Macdonald, T. L. *J. Amer. Chem. Soc.* **1984**, *106*, 6446–6447; Bondon, A.; Macdonald, T. L.; Harris, T. M.; Guengerich, F. P. *J. Biol. Chem.* **1989**, *264*, 1988–1997.

3. Musa, O. M.; Horner, J. H.; Shahin, H.; Newcomb, M. *J. Amer. Chem. Soc.* **1996**, *118*, 3862 3868.

4. Maeda, Y.; Ingold, K. U. *J. Amer. Chem. Soc.* **1980**, *102*, 328–331.

5. Karki, S. B; Dinnocenzo, J. P. *Xenobiotica* **1995**, *7*, 711–724.

6. Chen, H.; de Groot, M. J.; Vermeulen, N. P. E.; Hanzlik, R. P. *J. Org. Chem.* **1997**, *62*, 8227–8230.

7. Riley, P.; Hanzlik, R. P. *Xenobiotica* **1994**, *24*, 1–16.

8. Dinnocenzo, J. P.; Simpson, T. R.; Zuilhof, H.; Todd, W. P.; Heinrich, T. *J. Amer. Chem. Soc.* **1997**, *119*, 987–993;

Dinnocenzo, J. P.; Zuilhof, H.; Leiberman, D. R.; Simpson, T., McKechney, M. W. *J. Amer. Chem. Soc.* **1997**, *119*, 994–1004.

9. de Groot, M. J.; Donné-Op den Kelder, G. M.; Commandeur, J. N. M.; van Lenthe, J. H.; Vermeulen, N. P. E. *Chem. Res. Toxicol.* **1995**, *8*, 437–443.

10. Karki, S. B.; Dinnocenzo, J. P.; Jones, J. P.; Korzekwa, K. R. *J. Amer. Chem. Soc.* **1995**, *117*, 3657–3664; Manchester, J. I.; Dinnocenzo, J. P.; Higgins, L.; Jones, J. P. *J. Amer. Chem. Soc.* **1997**, *119*, 5069–5070.

11. Carlson, T. G.; Jones, J. P.; Peterson, L.; Castagnoli, N., Jr.; Iyer, K. R.; Trager, W. F. *Drug Metab. Disp.* **1995**, *33*, 749–756.

12. Silverman, R. B. *Mechanism based enzyme inactivators*; Silverman, R. B., Ed.; Academic Press, 1995; Vol. 249, pp 240–263; Silverman, R. B. *Account Chem. Res.* **1995**, *28*, 335–342.

13. Dostert, P.; Strolin Benedetti M.; Tipton, K. F. *Med. Res. Rev.* **1989**, *9*, 45–89.

14. Silverman, R. B.; Hoffman, S. J. *J. Amer. Chem. Soc.* **1980**, *102*, 884–886; Silverman, R. B.; Zieske, P. A. *Biochemistry* **1985**, *24*, 2128–2138.

15. Zhong, B.; Silverman, R. B. *J. Amer. Chem. Soc.* **1997**, *119*, 6690–6691.

16. Singer, T. P.; Salach, J. I.; Castagnoli Jr., N.; Trevor, A. *Biochem. J.* **1986**, *235*, 785–789; Trevor, A.; Castagnoli, Jr. N.; Singer, T. P. *Toxicology* **1988**, *49*, 513–519; Rimoldi, J. M.; Wang, Y.-X.; Nimkar, S. K.; Kuttab, S. H.; Anderson, A. H.; Burch, H.; Castagnoli N., Jr. *Chem. Res. Toxicol.* **1995**, *8*, 703.

17. Hall, L.; Murray, S.; Castagnoli, K.; Castagnoli, N., Jr. *Chem. Res. Toxicol.* **1992**, *5*, 625–633.

18. Anderson, A.; Kuttab, S.; Castagnoli N., Jr. *Biochemistry* **1996**, *35*, 3335–3340; Nimkar, S. K.; Anderson, A.; Rimoldi, J. M.; Stanton, M.; Castagnoli, K. P.; Mabic, S.; Wang, X.-Y.; Castagnoli Jr., N. *Chem. Res. Toxicol.* **1996**, *9*, 1013–1022.

19. Franot, C.; Mabic, S.; Castagnoli Jr., N. *Bioorg. Med. Chem.* **1998**, *6*, 283–291.

20. Mabic, S.; Castagnoli Jr., N. *J. Med. Chem.* **1996**, *39*, 3694–3700.

21. Kuttab, S.; Kalgutkar, A.; Castagnoli Jr., N. *Chem. Res. Toxicol.* **1994**, *7*, 740–744.

22. Galan, A. A.; Lee, T. V.; Chapleo, C. B. *Tetrahedron Lett.* **1986**, *27*, 4995–4998.

23. Wright, J. L.; Downing, D. M.; Feng, M. R.; Hayes, R. N.; Heffner, T. G.; MacKenzie, R. G.; Metzer, L. T.; Pugsley, T. A.; Wise, L. D. *J. Med. Chem.* **1995**, *38*, 5007–5014.

24. Green, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; 2nd ed.; John Wiley & Sons: New York, Winchester, Brisbane, Toronto, Singapore, 1991.

25. Genisson, Y.; Mehmandoust, M.; Marazano, C.; Das, B. C. *Heterocycles* **1994**, *39*, 811–818.

26. Zincke, T. *Chem. Ber.* **1904**, 361–375.

27. Wang, Y.-X.; Castagnoli Jr., N. *Tetrahedron Lett.* **1995**, *36*, 3981. Subramanyam, B.; Woolf, T.; Castagnoli, N. J. *Chem. Res. Toxicol.* **1991**, *4*, 123–128.

28. Mabic, S.; Rimoldi, J. M.; Castagnoli Jr., N. *J. Labelled Compd. Radiopharm.* **1997**, *39*, 409–423.

29. Gillaspy, M. L.; Lefker, B. A.; Hada, W. A.; Hoover, D. J. *Tetrahedron Lett.* **1995**, *36*, 7399–7402.

30. Corey, E. J.; Atkinson, R. F. *J. Org. Chem.* **1964**, *29*, 3703–3704.

31. Parkinson, A. *Toxico. Pathol.* **1996**, *24*, 45.

32. Cashman, J. R.; Ziegler, D. M. *Mol. Pharmacol.* **1986**, *29*, 163.

33. Wu, E.; Shinka, T.; Caldera-Munoz, P.; Yoshizumi, H.; Trevor, A.; Castagnoli Jr., N. *Chem. Res. Toxicol.* **1988**, *1*, 186–194.

34. The rates of formation of all three metabolites decreased during the course of the reaction suggesting the possibility of time dependent loss of enzyme activity that could be mediated by a mechanism based inactivation process similar to that reported for cyclopropylamines. Such a process, however, would be relatively inefficient since enzyme activity is still detectable following consumption of half of the substrate at an initial concentration of 250 μM.

35. Avram, M.; Matescu, G. D. In *Infrared Spectroscopy*; Wiley Interscience; New York 1966, p 102.

36. Collins, C. F.; Bowman, N. S. In *ACS Monograph 167: Isotope Effects in Chemical Reactions*; Van Nostrand Reinhold, New York 1970, pp 162–167.

37. Melowder, L; Saunders, Jr., W. K. In *Reaction Rates of Isotopic Molecules*, John Wiley & Sons, New York 1980; pp 172–174.

38. Lu, A. Y.; Levin, W. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 1334–1339.

39. Redinbaugh, M. G.; Turley, R. B. *Anal. Biochem.* **1986**, *153*, 267–271.

40. Omura, T.; Sato, R. *J. Biol. Chem.* **1964**, *239*, 2370–2378.

41. Lyle, R. E. *The Reduction of Nitrogen Heterocycles with Complex Metal Hydrides.* In *Advances in Organic Chemistry*, 1996; Vol. 6, pp 45–93.