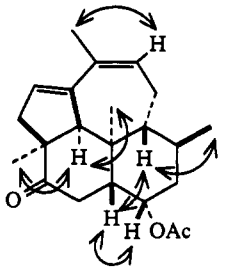

Figure 1. Critical observed NOE interactions for synthetic (±)-**2**.

oxy-1-(trimethylsilyl)methyllithium, conversion of the intermediate enol ether to the thermodynamically favored aldehyde **6**, and subsequent Wittig reaction to yield diolefin **7**. Selective hydroboration with disiamylborane[6] followed by oxidative workup gave the primary alcohol, which was converted into dibenzyl ether **8**. Hydroboration of the remaining double bond with diborane, followed by oxidation,[7] yielded **9** as a mixture of diastereomers, which was treated with pyridinium bromide perbromide in cold THF.[8] The resulting bromides were subjected to elimination to give **10** and **11** respectively. Bromo ketone **11** was converted into **10** by dehalogenation with Bu_3SnH.[9] Compound **10** contains five centers in the desired relative configuration and the requisite substituted enone required for the next annulation.

With enone **10** at hand, the construction of the five-membered ring was initiated, advantage being taken of the steric effect of the angular methyl group (Scheme II). As expected, cycloaddition of isoprene to **10** proved difficult but was eventually accomplished by using $EtAlCl_2$ as an acid catalyst[10] to give a mixture of two regioisomers favoring **12** by 2.6:1; their separation was accomplished by HPLC. No products arising from addition to the other face of **10** could be detected, thus, two more stereocenters were fixed. The double bond in **12** was dihydroxylated,[11] and the benzyl protecting groups were removed, furnishing tetrol **13** as a mixture of diastereomers. Glycol cleavage with $NaIO_4$ afforded labile **14**, which was converted upon treatment with acid into dienol ether **15**. The expected initial aldol product could not be isolated under any circumstances. However, the eight-membered-ring ether proved very useful as an intramolecular blocking group for the primary hydroxyl function.[12] Acetylation of **15** followed by hydrolysis of the enol ether and oxidation of the liberated primary alcohol gave enone aldehyde **16**. Finally, Ti^0-induced coupling[2] completed the construction of the tetracyclic skeleton to give (±)-kempene-2, which exhibited the same spectral properties as reported for the natural product.[1]

The correctness of the relative configuration at each stereocenter was further proven by a 2D-NOESY experiment (Figure 1). It is of particular interest that neither the carbonyl nor the ester moiety was affected by the last synthetic manipulation, confirming the utility of the Ti^0-induced coupling reaction in highly functionalized systems.

Acknowledgment. This research was supported by National Science Foundation Grant No. 8618303.

Supplementary Material Available: Table with comparison of NMR data for both natural and synthetic **2** and 2D-COSY and NOESY spectra and MS for (±)-**2** (7 pages). Ordering information is given on any current masthead page.

(5) Magnus, P.; Roy, G. *Organometallics* **1982**, *1*, 553.
(6) Brown, H. C.; Moerikofer, A. W. *J. Am. Chem. Soc.* **1961**, *83*, 3417.
(7) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.
(8) Djerassi, C.; Scholz, C. R. *J. Am. Chem. Soc.* **1948**, *70*, 417.
(9) Neumann, W. P. *Synthesis* **1987**, 665.
(10) Fringuelli, F.; Pizzo, F.; Taticchi, A.; Wenkert, E. *J. Org. Chem.* **1983**, *48*, 2802.
(11) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* 1976, 1973.
(12) Attempts to conduct the aldol reaction with protected hydroxyls failed.

DNA Modification: Intrinsic Selectivity of Nickel(II) Complexes

Xiaoying Chen, Steven E. Rokita,* and Cynthia J. Burrows*

Department of Chemistry
State University of New York at Stony Brook
Stony Brook, New York 11794-3400

Received December 14, 1990

Naturally occurring and laboratory-designed agents for DNA modification often rely upon transition-metal ions as promoters for nucleic acid oxidation.[1-8] Simple metal complexes may themselves show site specificity in their reactions with DNA based on (i) intercalative, groove-binding, or hydrogen-bonding interactions of the metal's ligands with the DNA[3-5] or (ii) the intrinsic reactivity of certain bases or sequences with the oxidant.[7] Alternatively, metal complexes may be tethered to known DNA-binding drugs or proteins in order to effect site specificity.[8] The identification of new metal complexes for reaction with DNA through nondiffusible species would aid in the development of new sequence-specific or conformation-specific DNA cleaving agents.

As our initial approach to this goal, we chose to investigate a series of square-planar nickel(II) complexes, some of which have been shown previously to catalyze oxygen atom transfer chemistry (e.g., olefin epoxidation) using iodosylbenzene, NaOCl, or $KHSO_5$ (oxone) as terminal oxidant.[9] Since, in the case of olefin epoxidation, the ability of Ni^{II} complexes to catalyze oxygen atom transfer was found to be highly ligand dependent, it suggested a course of study for the design of Ni^{II} complexes as catalysts for DNA oxidation. Interestingly, square-planar Ni^{II} complexes of tetraazamacrocycles such as the Schiff base complex NiL_1^{2+} and nickel cyclam, NiL_3^{2+}, were found to be highly active agents for DNA modification under oxidative conditions compared to related copper complexes or octahedral Ni^{II} complexes. Both $KHSO_5$ (oxone) and magnesium monoperoxyphthalate (MMPP) were effective as oxidants, but peracetic acid displayed a diminished activity and H_2O_2 with ascorbate was ineffective. Furthermore,

(1) For recent reviews, see: *Metal-DNA Chemistry*; Tullius, T. D., Ed.; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989.
(2) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107-1136.
(3) Fleisher, M. B.; Mei, H.-Y.; Barton, J. K. In *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, D. M. J., Eds.; Springer-Verlag: Berlin, 1988; Vol. 2, pp 65-84.
(4) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180-186.
(5) (a) Van Atta, R. B.; Bernadou, J.; Meunier, B.; Hecht, S. M. *Biochemistry* **1990**, *29*, 4783-4789 and references therein. (b) Ward, B.; Skorobogaty, A.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 6875-6883. (c) Groves, J. T.; Farrell, T. P. *J. Am. Chem. Soc.* **1989**, *111*, 4998-5000.
(6) Tullius, T. D. In *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, D. M. J., Eds.; Springer-Verlag: Berlin, 1989; Vol. 3, pp 1-12.
(7) Frantz, B.; O'Halloran, T. V. In *Metal-DNA Chemistry*; Tullius, T. D., Ed.; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989; pp 97-105.
(8) (a) For references to FeEDTA work, see: Sluka, J. P.; Griffin, J. H.; Mack, J. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 6369-6374. (b) Mack, D. P.; Iverson, B. L.; Dervan, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 7572-7574. (c) Mack, D. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 4604-4606.
(9) (a) Kinneary, J. F.; Albert, J. S.; Burrows, C. J. *J. Am. Chem. Soc.* **1988**, *110*, 6124-6129. (b) Yoon, H.; Burrows, C. J. *J. Am. Chem. Soc.* **1988**, *110*, 4087-4089. (c) Wagler, T. R.; Fang, Y.; Burrows, C. J. *J. Org. Chem.* **1989**, *54*, 1584-1589. (d) Yoon, H.; Wagler, T. R.; O'Connor, K. J.; Burrows, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 4568-4570.
(10) Metal complexes were prepared according to the following references. $[NiL_4](ClO_4)_2$ ((2,12-dimethyl-2,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),2,11,13,15-pentaene)nickel(II) perchlorate): Karn, J. L.; Busch, D. H. *Nature (London)* **1966**, *211*, 160-162. $[CuL_1][ZnCl_4]$: Rich, R. L.; Stucky, G. L. *Inorg. Nucl. Chem. Lett.* **1965**, *1*, 61-64. $[NiL_4][ZnCl_4]$ ((2,3-dimethyl-1,4,8,11-tetraazacyclotetradeca-1,3-diene)nickel(II) tetrachlorozincate): Tait, A. M.; Busch, D. H. *Inorg. Synth.* **1978**, *18*, 27-29. $[NiL_5](I)$ ((11,13-dimethyl-1,4,7,10-tetraazacyclotrideca-10,12-dienato)nickel(II) iodide): Cummings, S. C.; Sievers, R. E. *Inorg. Chem.* **1970**, *9*, 1131-1136. Other ligands were commercially available. Cyclam complexes, NiL_3 and CuL_3, were prepared as the bis-trifluoromethanesulfonate salts. Cyclen and tren complexes, NiL_6 and NiL_7, are discussed below.[13]

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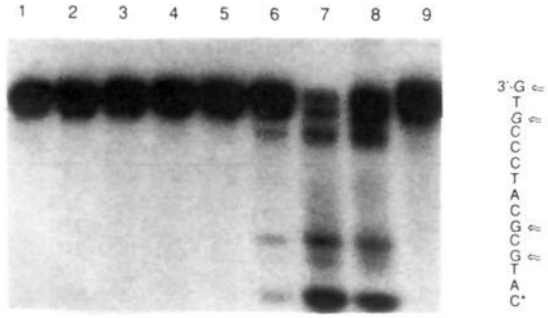
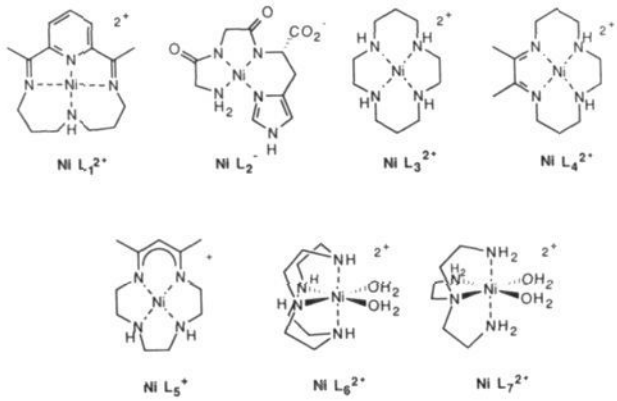


Figure 1. Autoradiogram of 20% polyacrylamide gel (denaturing 7 M urea) showing control studies and cleavage products obtained with NiL_1, oxidant, and piperidine treatment. The 15-base oligonucleotide **1** was labeled at the 5'-terminus with ^{32}P. Solutions (100 μL) buffered to pH 7.0 (10 mM potassium phosphate, 100 mM NaCl) containing 3 μM oligonucleotide (10 nCi) DNA, 3 μM metal complex, and 100 μM oxidant were maintained under ambient conditions and quenched after 30 min. with 20 mM Na_2SO_3. The samples were individually dialyzed against 1 mM EDTA at pH 8 (2 × 3 h) and water (1 × 12 h), lyophilized, treated with 0.2 M piperidine for 30 min at 90 °C, lyophilized again, and resuspended in 80% formamide for electrophoresis. Lane 1: NiL_1^{2+} *only, no oxidant. Lanes 2–4: control studies with oxidants alone,* namely, 1:1 H_2O_2/ascorbate, MMPP, and oxone, respectively. Lane 5: NiL_1 with 1:1 H_2O_2/ascorbate. Lane 6: NiL_1^{2+} with MMPP. Lane 7: NiL_1^{2+} with oxone. Lane 8: Maxam–Gilbert G lane.[11] Lane 9: As for lane 7 with omission of piperidine treatment.

oxidative DNA modification leading to strand scission after alkaline treatment occurred with high base specificity for guanine.



Oligonucleotides were chosen for study since they are small enough to permit a detailed study of reaction products yet large enough to display the base specificity for nucleotide oxidation. Reactions were carried out with a purified oligonucleotide **1** having the 15-base sequence of d(CATGCGCTACCCGTG). The 5'-terminus was labeled with [^{32}P]phosphate for analysis by gel electrophoresis and autoradiography. Samples of **1** were incubated with various metal complexes and an excess of oxidant, either oxone, MMPP, or a 1:1 mixture of H_2O_2/ascorbate. Optimized reaction conditions and control studies for DNA cleavage reactions with NiL_1^{2+} are presented in Figure 1. Lane 7, representing the reaction of **1** with 3 μM NiL_1^{2+} and 100 μM oxone, exhibits a fragmentation pattern equivalent to that of the Maxam–Gilbert *G lane (lane 8).*[11] *Cleavage products were observed only after* treatment with piperidine (compare lanes 7 and 9). Control studies verified that neither the nickel complex alone nor the oxidants alone generated base-labile products (lanes 1–4), and a comparison of oxidants (lanes 5–7) showed that oxone produced the most reaction with DNA. These studies lead to the conclusion that NiL_1^{2+} is an excellent promoter of oxidative DNA modification at G residues, giving rise to base-specific cleavage upon alkaline workup. The intermediate product of this transformation is likely formed by a net hydroxylation of guanine.[12]

(11) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499–560.

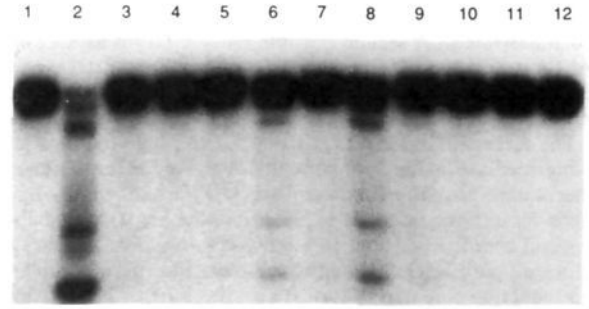


Figure 2. Autoradiogram of denaturing polyacrylamide gel comparing efficacy of various metal complexes (3 μM) and $KHSO_5$ (100 μM) as oxidant for cleavage of oligonucleotide **1** (3 μM). Reaction conditions and electrophoretic analysis were identical with those described in Figure 1 and included piperidine treatment as described. Lane 1: $Ni(OAc)_2$. Lane 2: NiL_1^{2+}. Lane 3: CuL_1^{2+}. Lane 4: Ni–GGH (NiL_2). Lane 5: Cu–GGH (CuL_2). Lane 6: $[Ni(cyclam)]^{2+}$ (NiL_3^{2+}). Lane 7: $[Cu(cyclam)]^{2+}$ (CuL_3^{2+}). Lane 8: NiL_4^{2+}. Lane 9: NiL_5^{+}. Lane 10: $Ni(cyclen)(NO_3)_2$ (NiL_6X_2). Lane 11: $Ni(tren)(OAc)_2$ (NiL_7X_2). Lane 12: cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$).

The activities of various metal complexes under the conditions described for oxone in Figure 1 are shown in Figure 2. A qualitative comparison of Ni^{II} complexes showed that the pyridine-containing Schiff base complex, NiL_1^{2+}, was the most active, followed by NiL_4^{2+}, another Schiff base complex (compare lanes 2 and 8), while nickel cyclam, NiL_3^{2+}, was less active (lane 6). The Ni^{II} and Cu^{II} complexes of the tripeptide GGH, NiL_2 and CuL_2, have been demonstrated by Dervan and co-workers[8b,c] to give highly site specific DNA cleavage with MMPP and H_2O_2, respectively, when the tripeptide is appended to a DNA-binding protein fragment. In the present case, however, neither NiL_2^- nor CuL_2^- led to significant amounts of DNA modification as judged by piperidine treatment (lanes 4 and 5). Apparently these anionic square-planar metal complexes do not interact sufficiently with DNA and the oxidant in the absence of a DNA-binding agent to yield substantial DNA reactivity. Accordingly, the three reactive Ni^{II} complexes are those that carry a +2 charge and are square-planar complexes of neutral tetradentate ligands. Surprisingly, the Cu^{II} analogues of these ligands, CuL_1^{2+} and CuL_3^{2+}, *were inactive for DNA oxidation under these conditions* (lanes 3 and 7). For comparison, the monocationic complex NiL_5^+ was tested, and faint evidence of reaction was observed in comparison to background (lane 9). The octahedral complexes $[NiL_6(H_2O)_2]^{2+}$ and $[NiL_7(H_2O)_2]^{2+}$[13] (lanes 10 and 11) were not detectably active under the same conditions. Finally, comparison was made with the DNA-binding drug cisplatin, which has been shown to bind to N-7 of guanines. No evidence of G-specific oxidative reactivity was obtained (lane 12).

Two possible roles for Ni^{II} can be envisioned in the oxidation chemistry of DNA. One is a redox role in which formation of a high oxidation state of nickel is part of the catalytic cycle. For example, Ni^{III} is readily accessible for the nickel complexes of L_1–L_5 ($E_{1/2}^{III/II}$ = 1.03,[14] 0.95,[15] 0.67,[14] 0.86,[14] and 0.27[14] V vs Ag/Ag^+ in CH_3CN), and square-pyramidal or octahedral Ni^{III} complexes should result from oxidation by oxone. Their subsequent reaction with G residues would be well-explained by the observation that guanine is the most easily oxidized of the four heterocyclic bases as well as the fact that N-7 of guanine is the best binding site for Ni^{II} or Ni^{III} (see structure A).[16] In this type of reaction, $Ni^{III}L_1$ would be the strongest oxidant. Whether or not a discrete metal–oxygen intermediate is formed remains to

(12) (a) Kasprzak, K. S.; Hernandez, L. *Cancer Res.* **1989**, *49*, 5964–5968. (b) Kouchakdjian, M.; Bodepudi, V.; Shibutani, S.; Eisenberg, M.; Johnson, F.; Grollman, R. P.; Patel, D. G. *Biochemistry* **1991**, *30*, 1403–1412.

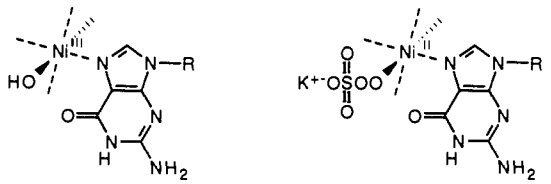
(13) Complexes $[NiL_6](NO_3)_2$ and $[NiL_7](OAc)_2$ were used in the reactions but most likely exist as bis-aquo complexes under the reaction conditions.

(14) Lovecchio, F. V.; Gore, E. S.; Busch, D. H. *J. Am. Chem. Soc.* **1974**, *96*, 3109–3116.

(15) $E°$ vs NHE at pH 9.6, from the following: Bossu, F. P.; Margerum, D. G. *Inorg. Chem.* **1977**, *16*, 1210–1214.

(16) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32–38.

be demonstrated. A second possible role for Ni^{II} is as a Lewis acid for activation of a peracid toward oxidative attack at a guanine. In this case then, Ni^{II} might serve as a template for coordination of both substrate and reactant as shown in structure B. The successful ligands, L_1, L_3, and L_4, are those that provide an intermediate ligand field strength, allowing for formation of either square-planar or octahedral species. Thus, the important criteria for intrinsic reactivity of Ni^{II} complexes are (i) availability of vacant coordination sites through a square-planar geometry, (ii) overall positive charge on the complex, and (iii) a relatively high reduction potential of the Ni^{III} state. Further verification of these hypotheses through a systematic study of ligand effects is in progress.



A B

In support of a nickel-guanine complex, oxidation is specific for only freely accessible residues. When **1** was hybridized to its complement and then subjected to oxidation, only a single G reacted, the 3'-terminal guanine (data not shown). This reagent should therefore prove to be quite useful as a probe for unusual DNA structures.[17]

Acknowledgment. Support of this work through a Seed Grant from the Stony Brook Center for Biotechnology sponsored by the New York State Science and Technology Foundation (to S.E.R. and C.J.B.) and by grants (to C.J.B.) from the National Science Foundation (CHE-9006684) and National Institutes of Health (GM-34841) is gratefully acknowledged.

(17) Wells, R. D., Harvey, S. C., Eds. *Unusual DNA Structures*; Springer-Verlag: New York, 1988.

Olefin Formation in the Oxidative Deformylation of Aldehydes by Cytochrome P-450. Mechanistic Implications for Catalysis by Oxygen-Derived Peroxide

Alfin D. N. Vaz,* Elizabeth S. Roberts, and Minor J. Coon

Department of Biological Chemistry
The University of Michigan
Ann Arbor, Michigan 48109-0606
Received January 22, 1991

We describe the cytochrome P-450 mediated oxidative deformylation of a xenobiotic aldehyde with introduction of unsaturation into the residual carbon framework. The reaction with cyclohexanecarboxaldehyde is a useful model for the demethylation reactions catalyzed by the steroidogenic P-450s, aromatase and lanosterol demethylase, where formic acid and an olefinic product are also formed.[1]

The active oxidant in P-450 catalyzed reactions is generally thought to be a pentavalent oxoiron species, or "iron oxene".[2] This concept fails, however, to account for the oxidative carbon-carbon bond cleavage step in steroid demethylation reactions. Alternatively, various investigators have suggested a role for an O_2-derived peroxide in the P-450 catalyzed cleavage of the oxysteroid intermediate.[3] However, no direct evidence for the role of peroxide in these reactions has been provided. H_2O_2 and organic peroxy compounds can be substituted for O_2 and NADPH in many P-450 catalyzed reactions,[2,4] but in the deformylation herein reported

Table I. Component Requirements and Effects of Catalase and Superoxide Dismutase on the Formation of Cyclohexene from Cyclohexanecarboxaldehyde

system[a]	act., (nmol/min)/(nmol of P-450)
Experiment 1	
complete	0.30 ± 0.04
NADPH omitted	0.02 ± 0.00
O_2 concn reduced (4.0 μM)	0.05 ± 0.00
reductase omitted	0.02 ± 0.00
P-450 LM_2 omitted	0.00 ± 0.00
DLPC omitted	0.07 ± 0.00
Experiment 2	
complete	0.26 ± 0.02
catalase added (240 units)	0.29 ± 0.02
catalase added (960 units)	0.27 ± 0.01
superoxide dismutase added (60 units)	0.28 ± 0.00
superoxide dismutase added (360 units)	0.29 ± 0.00

[a] The complete system contained 0.25 nmol each of the reductase and P-450 LM_2, 30 μg of DLPC, 50 μmol of potassium phosphate buffer, pH 7.4, 1.0 μmol of cyclohexanecarboxaldehyde, and 2.0 μmol of NADPH as the final addition in a 1.0-mL reaction volume. The vessel was sealed with a rubber septum and incubated at 37 °C for 10 min. The reactions were quenched by the addition of 100 μL of 30% perchloric acid, and the cyclohexene was quantitated by gas chromatography. Each experiment was carried out in triplicate and corrected for a blank in which the enzymes had been heat-denatured prior to addition.

Table II. Effectiveness of Other Oxidants in the Cytochrome P-450 Catalyzed Formation of Cyclohexene from Cyclohexanecarboxaldehyde

oxidant added[a]	concn, mM	cyclohexene formed, nmol
hydrogen peroxide	0.10	0.19 ± 0.06
hydrogen peroxide	0.50	0.91 ± 0.05
iodosobenzene	0.01	nd[b]
iodosobenzene	0.05	nd
m-chloroperbenzoic acid	0.01	nd
m-chloroperbenzoic acid	0.05	nd
cumyl hydroperoxide	0.10	nd
cumyl hydroperoxide	0.50	nd

[a] The reactions were as described in Table I except that the reductase, NADPH, and phospholipid were omitted. Reactions were initiated by the addition of a 10 mM aqueous solution of the oxidant or, in the case of iodosobenzene, a methanolic solution. The volume of methanol used was known not to affect the formation of cyclohexene in the complete system as described in Table I. After incubation for 3 min, reactions were quenched by the addition of 100 μL of saturated aqueous sodium thiosulfate. With H_2O_2 the reaction is linear with time for 3 min. In other experiments the inactivity of the three organic oxidants was shown not to be due to P-450 destruction. [b] Not detected (limit of detection, 50 pmol).

(1) (a) Fishman, J. *Cancer Res.* **1982**, *42*, 3277s-3280s. (b) Alexander, M.; Akhtar, M.; Boar, R. B.; McGhie, J. F.; Barton, D. H. R. *J. Chem. Soc., Chem. Commun.* **1972**, 383-385.

(2) (a) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315-356. (b) Groves, J. T.; Nemo, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 5786-5791. (c) McMurry, T. J.; Groves, J. T. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; pp 1-28. (d) Ortiz de Montellano, P. R. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; pp 217-271.

(3) (a) Akhtar, M.; Calder, M. R.; Corina, D. L.; Wright, J. N. *Biochem. J.* **1982**, *201*, 569-580. (b) Stevenson, D. E.; Wright, J. N.; Akhtar, M. *J. Chem. Soc., Perkin Trans. 1* **1988**, *1*, 2043-2052. (c) Cole, P. A.; Bean, J. M.; Robinson, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2999-3003. (d) Yoshida, Y.; Aoyama, Y.; Sonoda, Y.; Sato, Y. In *Proceedings of the VIIIth International Symposium on Microsomes and Drug Oxidations*; Ingelman-Sundberg, M., Gustafsson, J.-Å., Orrenius, S., Eds.; 1990; p 118. (e) Trzaskos, J. M.; Fischer, R. T.; Magolda, R. L.; Ko, S. S.; Brosz, C. S.; Larson, B. In *Proceedings of the VIIIth International Symposium on Microsomes and Drug Oxidations*; Ingelman-Sundberg, M., Gustafsson, J.-Å., Orrenius, S., Eds.; 1990; p 118. (f) Watanabe, Y.; Ishimura, Y. J. *J. Am. Chem. Soc.* **1989**, *111*, 410-411.

(4) (a) Kadlubar, F. F.; Morton, K. C.; Ziegler, D. M. *Biochem. Biophys. Res. Commun.* **1973**, *54*, 1255-1261. (b) Nordblom, G. D.; White, R. E.; Coon, M. J. *Arch. Biochem. Biophys.* **1976**, *175*, 524-533. (c) Blake, R. C.; Coon, M. J. *J. Biol. Chem.* **1980**, *255*, 4100-4111; (d) **1981**, *256*, 5755-5763; (e) **1981**, *256*, 12127-12133; (f) **1989**, *264*, 3694-3701.

0002-7863/91/1513-5886$02.50/0