the antitumor activity of *cis*-[Pt(ammine)$_2$X$_2$] complexes decreases markedly along the series $NH_3 > NH_2R \geq NHR_2 >> NR_3$.[18]

In closing, we wish to emphasize that the present conclusions are based on a theoretical analysis with the program AMBER. They show what is possible and do not necessarily reveal the actual structures. Further theoretical and experimental studies of platinated oligonucleotides are in progress.

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Supplementary Material Available: Table S1 summarizing conformational energies and G5,G6 phase angles for platinated oligonucleotides **1** and **2** (1 page). Ordering information is given on any current masthead page.

(18) Cleare, M. J.; Hoeschele, J. D. *Bioinorg. Chem.* **1973**, *2*, 187–210.

Cytochrome P-450 Catalyzed Oxidation of Quadricyclane. Evidence for a Radical Cation Intermediate[1]

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The question of whether oxygen transfer occurs in a single two-electron step or is preceded by electron transfer from the substrate to the activated oxygen complex is one of the more elusive aspects of the catalytic mechanism of cytochrome P-450. The large isotope effect and the epimerization associated with the cytochrome P-450 catalyzed hydroxylation of tetradeuterated norbornane,[2] and the scrambling of regiochemistry observed in the allylic hydroxylation of unsaturated hydrocarbons,[3] require an intermediate (probably a radical) in hydrocarbon hydroxylation reactions. The available data,[4,5] particularly the finding that the cytochrome P-450 catalyzed oxidation of 4-alkyl-1,4-dihydropyridines results in release of the 4-alkyl groups as free radicals,[4] furthermore suggest that electron transfer is the initial event in the oxidation of nitrogenous substrates. We now report evidence that quadricyclane (**1**) is oxidized by cytochrome P-450 to a radical cation that is captured in a distinct step by the activated oxygen species.

The strain in the quadricyclane structure and the extremely low oxidation potential (0.9 V) inherent in that strain[6] make quadricyclane a unique hydrocarbon. Quadricyclane, as a result, is isomerized to norbornadiene by metal catalysts[7] and readily undergoes oxidative addition reactions.[8] The quadricyclane radical cation, the probable intermediate in the oxidative addition reactions, is in equilibrium with the norbornadiene radical cation.[9]

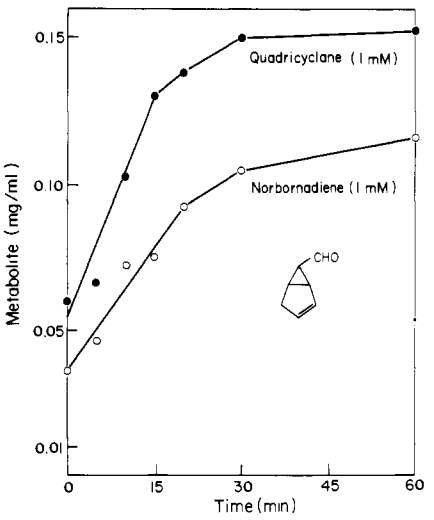

Figure 1.

Incubation of quadricyclane (1 mM) and an NADPH regenerating system with liver microsomes at 37 °C for 30 min, followed by extraction with ether and analysis by gas–liquid chromatography, provides nortricyclanol (**2**) and rearranged aldehyde **3** as the principal metabolites.[10] Quantitative studies with 5-norbornen-2-ol as an internal standard and studies with inhibitors demonstrate that the formation of nortricyclanol is independent of cytochrome P-450 whereas that of aldehyde **3** depends strictly on catalytic turnover of the enzyme (Table I). The oxidation of quadricyclane to nortricyclanol is equally well catalyzed by reduced and unreduced microsomes, aqueous $FeCl_3$, aqueous $FeCl_2$ plus H_2O_2, or 0.1 M $NaKPO_4$ (pH 7.4) buffer,[11] but not by double glass distilled water. The oxygen in nortricyclanol, as shown by studies with ^{18}O-labeled water and oxygen, derives from the medium. The aldehyde, in contrast, is only formed if microsomes, oxygen, and NADPH are present, although oxygen and NADPH can be replaced by iodosobenzene (Table I).

The oxidative microsomal metabolism of norbornadiene, the valence tautomer of quadricyclane, yields **3** but no nortricyclanol. The possibility that quadricyclane isomerizes to norbornadiene before the hydrocarbon is oxidized to aldehyde is ruled out by the finding that (a) more aldehyde is formed from 1 mM quadricyclane than from 1 mM norbornadiene (Figure 1),[12] (b) norbornadiene, as shown by NMR analysis of extracts, does not accumulate in incubations of quadricyclane (1 mM) with the microsomal system, (c) the spectroscopically determined binding constants (K_s) for quadricyclane and norbornadiene are both approximately 100 μM, and (d) the aldehyde is not formed in detectable amounts with low (μM) concentrations of norbornadiene.

Autooxidation of quadricyclane to nortricyclanol with incorporation of oxygen from the medium is explained by addition of water to the radical cation to give the nortricyclyl radical that abstracts a hydrogen or is reduced and protonated (Figure 2). The rearranged aldehyde in the cytochrome P-450 catalyzed oxidation, in contrast, is most reasonably explained by oxidation of quadricylane to the radical cation followed by reaction with the concomitantly generated equivalent of a hydroxyl radical to give the nortricyclyl *cation*, which is known to rearrange to aldehyde **3**.[13] Alternatives such as concerted exo addition to give the epoxide of norbornadiene or insertion of oxygen into the carbon–carbon bond to give an oxetane have no chemical or bio-

(1) This research was supported by NIH Grants GM 25515 and GM 32488.

(2) Groves, J. T.; McCluskey, G. A.; White, R. A.; Coon, M. J. *Biochem. Biophys. Res. Commun.* **1979**, *81*, 154–160.

(3) Groves, J. T.; Subramanian, D. V. *J. Am. Chem. Soc.* **1984**, *106*, 2177–2181.

(4) Augusto, O.; Beilan, H. S.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1982**, *257*, 11288–11295.

(5) Guengerich, F. P.; Willard, R. J.; Shea, J. P.; Richards, L. E.; Macdonald, T. L. *J. Am. Chem. Soc.* **1984**, *106*, 6446–6447.

(6) Gassman, P. S.; Yamaguchi, R.; Koser, G. F. *J. Org. Chem.* **1978**, *43*, 4392–4393.

(7) Schwendiman, D. P.; Kutal, C. *J. Am. Chem. Soc.* **1977**, *99*, 5677–5682.

(8) Baggaley, A. J.; Brettle, R.; Sutton, J. R. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1055–1059.

(9) Roth, H. D.; Schilling, M. L. M.; Jones, G. *J. Am. Chem. Soc.* **1981**, *103*, 1246–1248.

(10) The chromatographic analysis was carried out at 125 °C on a glass column packed with 10% Carbowax 20M on 120/140 mesh gaschrome Q. The structures of the metabolites were confirmed by mass spectrometry.

(11) Traces of iron and other metal ions are present in phosphate buffers.

(12) Product is present at the "zero" time point because it takes approximately 1–2 min to quench the reaction.

(13) Meinwald, J.; Labana, S. S.; Chadha, M. S. *J. Am. Chem. Soc.* **1963**, *85*, 582–585.

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Table I. Cytochrome P-450 Catalyzed Oxidation of Quadricyclane

incubation conditions	yield of 3, nmol/nmol of P-450
control[a]	370
-NADPH	ND
+argon	95
+argon, -NADPH, +PhIO (1 mM)	1570
+argon, -NADPH, -microsomes, +PhIO	57
+CO	8
+1-aminobenzotriazole (1 mM)	11
+SKF 525A[b] (100 μM)	18
+benzphetamine (0.5 mM)	15

[a] Incubation contained phenobarbital-induced rat liver microsomes (1.0 mg of protein/mL), glucose-6-phosphate dehydrogenase (0.5 units/mL), glucose 6-phosphate (4.5 mM), NADP (0.5 mM), $MgCl_2 \cdot H_2O$ (2 mM), KCl (1.0 mM), diethylenetriaminepentaacetic acid (1.0 mM), and quadricyclane (1 mM) in 0.1 M $NaKPO_4$ (pH 7.4) buffer (20-mL incubation volume). Mixtures were incubated at 37 °C for 30 min (ND, not detectable). The argon experiments were carried out with microsomes from a different set of rats. [b] SKF 525A is (diethylamino)ethyl 2,2-diphenylpentanoate hydrochloride.

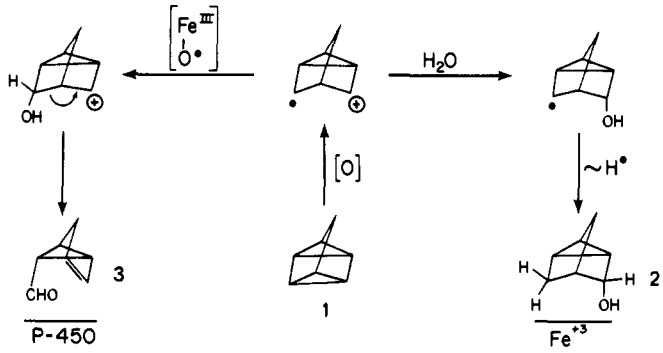

Figure 2.

chemical precedent and are therefore unlikely. The novel reactivity observed in the oxidation of quadricyclane by cytochrome P-450 indicates that the enzyme can oxidize hydrocarbons that have low oxidation potentials by sequential one-electron steps. The net outcome is oxidation of an otherwise unactivated carbon–carbon *single* bond.

Electron Paramagnetic Relaxation as a Probe for Structural Characterization of Paramagnetic Oxyanions

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The purpose of this note is to show that in some structural studies using electron paramagnetic resonance (EPR) spectroscopy, where conventional identification procedures (based on g values and hyperfine splitting) are insufficient for distinguishing between two species, the temperature-dependent line-width behavior may provide an important criterion. This situation may arise for a complex in which the ligand atoms exhibit little or no hyperfine structure, for example, paramagnetic oxyanions (CrO_3^-, CrO_4^{3-}, WO_4^{3-}, etc.). The direct determination via EPR of the number of oxygen ligands is difficult since the dominant oxygen isotope (^{16}O) has nuclear spin $I = 0$ and hence exhibits no hyperfine structure, and the natural abundance of its magnetic isotope ^{17}O ($I = 5/2$) is too low (~0.04%) to be normally detected by EPR. We experienced such difficulty during the initial stages of studies aimed at characterizing the Cr(V) oxyanions CrO_3^- and CrO_4^{3-}.

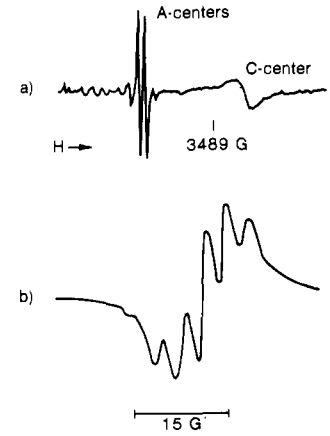

Figure 1. EPR spectrum of (a) γ-irradiated $K_2Cr_2O_7$ oriented with $H//a^*$-axis and (b) CrO_4^{3-} in KH_2PO_4. Note the (1:4:6:4:1) quintet structure from four H's hydrogen bonded to the four oxygens of the CrO_4^{3-}, thus confirming the identity of this center. The temperature dependence of the overall line width of this signal provided an internal standard for identifying the C center. The spectra were recorded at room temperature under nonsaturating (~1 mW) microwave power. The modulation amplitude was about 0.5 G at 100 kHz.

Such Cr(V) oxyanions have been postulated as active sites in chromia–alumina and chromia–silica catalysts,[2,3] but their identity has not been confirmed. Since both CrO_3^- and CrO_4^{3-} are paramagnetic (d^1), attempts have been made to produce and characterize (using EPR) these species in well-defined crystalline chromium–oxygen lattices such as K_2CrO_4,[4,5] $K_2Cr_2O_7$,[6-8] and $Na_2Cr_2O_7$.[9] In all of these studies, the Cr(V) species were identified via electron Zeeman tensor (g) and in some cases the ^{53}Cr hyperfine tensor. However, the assignment of CrO_3^- in all of the above studies is in disagreement with recent additive ligand-field theoretical calculations of g values for a tricoordinate Cr(V) oxyanion.[10] The theoretical results also suggest that the spectral features assigned to CrO_3^- should in fact be reassigned to CrO_4^{3-}. To help solve this controversy preparation of an ^{17}O-labeled host crystal (such as $K_2Cr^{17}O_4$) was considered, but it was felt that the results might not be conclusive if the labeling were not 100% complete, a fairly difficult task. Alternatively it was decided to utilize the well-known fact that the electron spin–lattice relaxation time (T_1) for a species is dependent upon its coordination and point symmetry.[11] Under identical conditions, a tetrahedral species such as CrO_4^{3-} will exhibit a shorter T_1 than a tricoordinate species such as CrO_3^-. In EPR measurements a shorter T_1 would be observable as a larger signal line width. Since T_1 is a sensitive function of temperature (in general, sharply decreasing with increasing temperature), variation of the EPR line width with temperature should be characteristic of the coordination and symmetry.[12] Thus a comparison of the temperature dependence of the line width of an unknown with that of a selected set of known species should serve as a criterion for structural identification. The work summarized below, and described in greater detail elsewhere,[13] demonstrates the feasibility of such a procedure.

(1) Present address: Department of Chemistry, Indiana University, Bloomington, Indiana 47405.

(2) Cossee, P.; van Reijen, L. L. *Actes Congr. Int. Catal. 2nd, 1960*, **1961**, *2*, 1967.
(3) Boreskov; Bukanaeva; Dzisko; Kazansky; Pecherskaya *Kinet. Catal. (Engl. Transl.)* **1964**, *5*, 379.
(4) Debuyst, R.; Apers, D. J.; Capron, P. C. *J. Inorg. Nucl. Chem.* **1972**, *34*, 1541.
(5) Debuyst, R.; Ladriere, J.; Apers, D. J. *J. Inorg. Nucl. Chem.* **1972**, *34*, 3607.
(6) Krishnamurthy, M. V. *J. Phys. Chem. Solids* **1974**, *35*, 606.
(7) Alybakov, A. A.; Gubajdullin, Z. H.; Gubanova, V. A. *Krist. Tech.* **1974**, *9*, 51.
(8) Alybakov, A. A.; Gubanova, V. A.; Tojchiev, N. *Krist. Tech.* **1978**, *13*, 1399.
(9) Alybakov, A. A.; Gubanova, V. A.; Tojchiev, N. *Krist. Tech.* **1980**, *15*, 317.
(10) Vanquickenborne, L. G.; Gorller-Walrand, C.; Debuyst, R. *J. Magn. Reson.* **1978**, *29*, 275.

0002-7863/85/1507-4082$01.50/0