of (+)-pentalenene [(+)-1] is stereocontrolled, short, and effective and should be applicable to the constructions of isocomene,[20] silphinene,[21] senoxydene,[22] pentalenic acid, and pentalenolactone. The hydrolytic ring closure reaction of vinyl sulfide and ketones seems to be general and should prove to be a valuable new reaction tool in other syntheses. Utilization with more highly oxidized members of the sesquiterpene is being explored.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compound **1-3**, **10**, **11**, and **14-21** (21 pages). Ordering information is given on any current masthead page.

(20) (a) Zalkow, L. H.; Harris, R. N., III; Van Derveer, D.; Bertrand, J. A. *J. Chem. Soc., Chem. Commun.* **1977**, 456. (b) Bohlmann, F.; LeVan, N.; Pickhardt, J. *Chem. Ber.* **1977**, *110*, 3777. (c) Zalkow, L. H.; Harris, R. N., III; Burke, N. I. *J. Nat. Prod.* **1979**, *42*, 96.
(21) Bohlmann, F.; Jakupovic, J. *Phytochemistry* **1980**, *19*, 259.
(22) Bohlmann, F.; Zdero, C. *Phytochemistry* **1979**, *18*, 1747.

Hydrogen-Deuterium Exchange during Propylene Epoxidation by Cytochrome P-450

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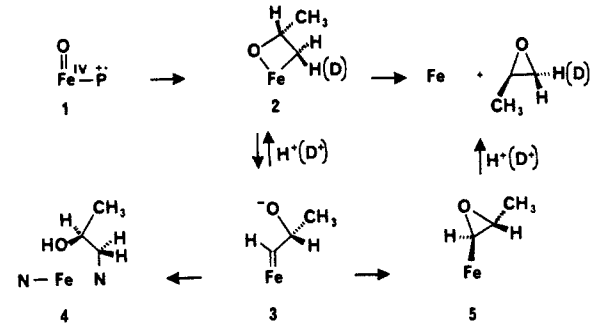
The nature of the "active oxygen species" and the mechanism of the oxygen-transfer event in the catalytic cycle of cytochrome P-450[1] have been the subject of extensive investigation and speculation. A powerful approach to the study of such elusive intermediates has been the use of substrate molecules designed to reveal the chemistry of the ultimate oxidant. We have previously demonstrated that epimerization[2] and allylic rearrangement[3] occur during the aliphatic hydroxylation by cytochrome P-450. We report here that the epoxidation of *trans*-1-deuteriopropylene by a reconstituted cytochrome P-450 system proceeds with significant loss of the deuterium label. Further, the epoxidation of propylene by this enzymic system in D_2O affords predominantly *trans*-1-deuteriopropylene oxide.

In a typical experiment cytochrome $P\text{-}450_{LM2}$ (5 nmol), cytochrome P-450 reductase (10 nmol), and dilauroyl-GCP (150-220 μL of a 1 mg/mL solution) were mixed and then diluted with 0.5 mL of phosphate buffer (pH 7.4) and 0.5 mL of water in a manner identical with that which we have previously described.[3,4] NADPH (250 μL of a 10 mg/mL solution) was added from a bent side neck and the mixture was cooled to 4 °C. After removal of air on a vacuum line, propylene (1.9-2.2 mmol) or *trans*-1-deuteriopropylene (0.9-1.0 mmol, 70% d_1) was admitted to the reaction vessel and the mixture was allowed to equilibrate for 15-30 min. After propylene was removed to 0.5 atm and replaced with oxygen, the mixture was allowed to stand at 4 °C overnight. After removal of oxygen from the frozen reaction mixture, volatile products were transferred to a cold trap. Unreacted propylene was removed from the reaction products by distillation, and propylene oxide was either condensed into a sample bulb or isolated from the product fraction by preparative gas chromatography on a 20% Carbowax column. The amount of propylene oxide was determined manometrically at this point. Simultaneous, quantitative analysis of propylene oxide, *trans*-1-deuteriopropylene oxide, *cis*-1-deuteriopropylene oxide, and unreacted propene was carried out by microwave spectroscopy as we have previously described.[5] Blank samples were determined before and after each run to assure that the vacuum line, gas chromatograph, and microwave cavity were free of contamination.

Scheme I



Typical data for a series of propylene oxidations presented as intensity ratios for five prominent mirowave transitions are shown in Table I. Product yields and isotopomer distributions are presented in Table II. Remarkably, the epoxidation of *trans*-1-deuteriopropylene afforded 95% propylene-d_0 oxide. This apparent deuterium exchange was confirmed by the runs performed with unlabeled propylene in D_2O which gave greater than 80% *trans*-1-deuteriopropylene oxide. Thus, *the epoxidation of propylene with this reconstituted cytochrome P-450 system proceeded with a concomitant, stereospecific hydrogen/deuterium exchange from the aqueous milieu.*

Electrophoretically homogeneous cytochrome P-450 alone catalyzed the epoxidation of propylene with iodosylbenzene to give an isotopomer ratio indistinguishable from that of the starting propylene. Further, the incubation of propylene oxide with the fully reconstituted P-450 system in D_2O for 3 days caused no more than 10% exchange. The epoxidation of *trans*-1-deuteriopropylene with Fe(TPP)Cl or Mn(TPP)Cl and iodosylbenzene in wet methylene chloride gave products consistent with complete retention of configuration with the iron porphyrin and significant trans-cis rearrangement with the manganese porphyrin but with no H-D exchange. Similar results have been obtained for these synthetic porphyrin catalysts with other substrates.[6]

A number of mechanisms have been suggested for the substrate oxygenation catalyzed by cytochrome P-450.[1] No simple oxygen-transfer process would have predicted the stereospecific exchange of a carbon-bound hydrogen observed here, however. Two processes that do allow for the observed results are shown in Scheme I. We have described the preparation and characterization of an oxoiron(IV) porphyrin cation radical species (**1**) which is capable of epoxidizing olefins at low temperature.[7] Such a

†Current address: Department of Chemistry, Princeton University, Princeton, NJ 08544.
(1) (a) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315. (b) Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9-16. (c) Groves, J. T. *Adv. Inorg. Biochem.* **1979**, *1*, 119-145.
(2) (a) Groves, J. T.; McClusky, G. A.; White, R. E.; Coon, M. J. *Biochem. Biophys., Res. Commun.* **1978**, *81*, 154-160. (b) Gelb, M. H.; Heimbrook, D. C.; Mälkönen, P.; Sligar, S. G. *Biochemistry* **1982**, *21*, 370-377.
(3) (a) Groves, J. T.; Subramanian, D. V. *J. Am. Chem. Soc.* **1984**, *106*, 2177-2181. (b) Groves, J. T.; Akinbote, O. F.; Avaria, G. E. In *Microsomes, Drug Oxidations and Chemical Carcinogens*; Coon, M. J., et al., Eds.; Academic Press: New York, 1980; Vol. 1, pp 253-261.
(4) (a) Haugen, D. R.; Coon, M. J. *J. Biol. Chem.* **1976**, *25*, 7929-7939. (b) Coon, M. J.; van der Hoeven, T. A.; Dahl, S. B.; Haugen, D. A. *Methods Enzymol.* **1978**, *52*, 109-117.
(5) (a) Larrabee, A. L.; Kuczkowski, R. L. *J. Catal.* **1978**, *52*, 72-80. (b) Imachi, M.; Egashira, M.; Kuczkowski, R. L.; Cant, N. W. *J. Catal.* **1981**, *70*, 177-181. (c) Imachi, M.; Kuczkowski, R. L. *J. Mol. Struct.* **1982**, *96*, 55-60. (d) Lide, D. R., Jr.; Christensen, J. *J. Chem. Phys.* **1961**, *35*, 1374. (e) Lide, D. R., Jr.; Mann, D. E. *J. Chem. Phys.* **1957**, *27*, 868.
(6) (a) Groves, J. T.; Kruper, W. J.; Haushalter, R. C. *J. Am. Chem. Soc.* **1980**, *102*, 6375. (b) Groves, J. T.; Nemo, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 5786.
(7) Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B. J. *J. Am. Chem. Soc.* **1981**, *103*, 2884-2885.

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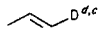
Table I. Microwave Data for Propylene Oxide Isotopomeric Mixtures

run	transition, peak intensity (trans-d_1/d_0) $9_{2,7}$–$9_{1,8}$	$10_{2,8}$–$10_{1,9}$	$11_{2,9}$–$11_{1,10}$	$12_{2,10}$–$12_{1,11}$	$13_{2,11}$–$13_{1,12}$	$n_{t\text{-}d_1}/n_{d_0}$[a]
2	38/9	41/11	54/12	66/16	81/18	4.8 (0.3)
3	17/3	21/4.5	26.5/5	32/7	40/8	5.7 (0.5)
4	0.9/15	0.9/17.5	1.1/20	1.6/28	1.8/30	0.06 (0.01)
5	0.95/21	1.11/25	1.3/31	1.6/41	2.2/48	0.05 (0.01)
6	15.5/7	19/8	21.5/10	30/12	35/14	2.5 (0.2)
7	3.0/24	3.0/29	3.5/31	4/42	5/46	0.12 (0.01)
8	24/6.8	30/7.9	37/9.6	47/11.9	61/14	4.4 (0.2)
9[b]	17/13	24/14	30/18	39/23	48/29	1.6 (0.2)

[a] Average isotopomer ratio and standard deviation calculated following ref 5a; for each transition $n_a/n_b = R^{-1}(I_a/I_b)$ where I_a is the peak intensity for species a etc. and R converts intensity ratios into the mole ratio n_a/n_b. The values of R for the five transitions are respectively 0.86, 0.87, 0.88, 0.88, and 0.89 for $n_{t\text{-}d_1}/n_{d_0}$ and 1.0, 0.99, 0.97, 0.96, and 0.95 for $n_{t\text{-}d_1}/n_{c\text{-}d_1}$. [b] Peak intensities for trans-d_1/cis-d_1; d_0 not determined.

Table II. Epoxidation of Propylene by Cytochrome P-450$_{LM_2}$

run	substrate	conditions	epoxide yield, μmol	% d_0	% *trans*-d_1
1		P-450/reductase/H_2O, NADPH/O_2	1.6	100	
2		P-450/Reductase/D_2O,	2.2	17	83[b]
3		NADPH/O_2	2.4[a]	15	85
4		P-450/Reductase/H_2O,	2.3	94	6[b]
5		NADPH/O_2	1.8	95	5[b]
6		P-450/ϕIO/H_2O	2.0	27	73[b]
7		P-450/Reductase/D_2O, NADPH 3 days		89	11[b]
8		Fe(TPP)Cl/Ph-IO	60	18	82[b]
9		Mn(TPP)Cl/Ph-IO	50	38[f]	62

[a] Recovered propylene showed 4.7% *trans*-1-deuteriopropylene. [b] Less than 1% *cis*-1-deuteriopropylene oxide was detected. Loss of deuterium was confirmed by GC–mass spectrometry (*m/e* 58, 43). [c] Starting material was 72% *trans*-1-deuteriopropylene, 28% propylene. [d] Deuterium content of recovered propylene was unchanged. [e] Starting material was 80% *trans*-1-deuteriopropylene, 20% propylene. [f] *cis*-1-Deuteriopropylene oxide; propylene oxide not determined.

species is an attractive candidate for the "active oxygen species" in the cytochrome P-450 cycle. Although the detailed mechanism of olefin epoxidation by simple oxometal species is not yet clear, the interesting suggestion that chromyl reagents[8] and oxomanganese porphyrins[9] react with carbon–carbon double bonds to form an oxametallacycle may offer an opportunity for proton exchange. Thus, the cycloaddition of propylene with **1** could reasonably form **2**. There is ample precedent that metal alkyls can be deprotonated to form metal carbenes.[10] Loss of a proton from **2** would provide access to the iron carbene **3**. Metallocarbene species have been suggested to result from the reductive metabolism of halocarbons by cytochrome P-450.[11] Further, an iron carbene such as **3** would provide a path to the *N*-alkylporphyrins **4** which are known to derive from terminal alkenes.[12]

The observed preference for trans protonation can be accommodated in Scheme I either by a stereoselective protonation of **3** to regenerate **2** or by ring closure to form the metallooxirane **5**. Epoxide production in the former process would result from reductive elimination from **2** while protolysis of the carbon metal bond would produce the epoxide from **5**. No definitive choice between these possibilities can be made with the data at hand, but mechanisms for propylene epoxidation which do not allow for proton exchange must be eliminated on the basis of these results. Efforts to detect intermediates such as **2**, **3**, and **5** in enzymic and model porphyrin-mediated epoxidations are currently under way.[13]

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Registry No. P-450, 9035-51-2; Fe(TPP)Cl, 16456-81-8; Mn(TPP)Cl, 32195-55-4; CH_2=$CHCH_3$, 115-07-1; (*F*)-CHD=$CHCH_3$, 1560-60-7; iodosulbenzene, 536-80-1; monooxygenase, 9038-14-6; propylene oxide, 75-56-9; *trans*-1-deuteriopropylene oxide, 34074-743.

(8) Sharpless, K. B.; Teranishi, A. Y.; Bäckvall, J. E. *J. Am. Chem. Soc.* **1977**, *99*, 3120.
(9) Collman, J. P.; Brauman, J. I.; Meunier, B.; Raybuck, S. R.; Kodedek, T. *Proc. Natl. Acad. Sci. U.S.A.* 3245–3248.
(10) (a) Schrock, R. *Acc. Chem. Res.* **1979**, *12*, 98. (b) We thank R. Grubbs for an insightful discussion of this process.
(11) (a) Mansuy, D. *Pure Appl. Chem.* **1980**, *82*, and references therein. (b) Mansuy, D.; Lange, M.; Chottard, J.-C. *J. Am. Chem. Soc.* **1978**, *100*, 3213. (c) Mansuy, D.; Battioni, J.-P.; Chottard, J.-C.; Ullrich, V. *J. Am. Chem. Soc.* **1971**, *101*, 3971. (d) Mansuy, D.; Lange, M.; Chottard, J.-C. *J. Am. Chem. Soc.* **1979**, *101*, 6437.
(12) (a) Ortiz de Montellano, P. R.; Correia, M. A. *Annu. Rev. Pharmacol. Toxicol.* **1983**, *23*, 481 and references cited therein. (b) Ortiz de Montellano, P. R.; Mangold, B. L. K.; Wheller, C.; Kunze, K. L.; Reich, N. D. *J. Biol. Chem.* **1983**, *258*, 4208. (c) Kunze, K. L.; Mangold, B. L. K.; Wheller, C.; Beilan, H. S.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1983**, *258*, 4202. (d) Consistent with these reports the epoxidation of 1-hexene in D_2O by reconstituted cytochrome P-450 resulted in negligible deuterium incorporation. The oxidation of 1-butene with the reconstituted P-450 system in D_2O has been shown by microwave spectroscopy and mass spectrometery to give 1-butene oxide with *partial* deuterium incorporation.
(13) Groves, J. T.; Watanabe, Y. *J. Am. Chem. Soc.* **1986**, *108*, 507–508.

Scanning Electrochemical and Tunneling Ultramicroelectrode Microscope for High-Resolution Examination of Electrode Surfaces in Solution

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We describe here a new apparatus for examination of conductor (or semiconductor) surfaces immersed in solution by measurement of either the electrochemical or tunneling current that flows when an ultramicroelectrode tip is scanned above the surface. Results with a Pt-coated test structure yield a spacial resolution of about 30 nm. Most methods for examination of solid surfaces, e.g., scanning electron microscopy (SEM) or the various electron spectroscopies, require the sample to be in an ultrahigh vacuum (UHV) environment and are not suitable for in situ electrochemical studies. Optical techniques for in situ electrode surface studies exist (e.g., infrared and surface-enhanced Raman spectroscopy) but they are capable of only limited spacial resolution, as governed by diffraction limitations and source beam size. An alternative approach was suggested by recent investigations with scanning tunneling microscopy (STM) that demonstrate the possibility of direct determination of surface structure with atomic resolution.[1-5] In this method a very sharp metal tip is brought

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