A Hypersensitive Mechanistic Probe for Distinguishing between Radical and Carbocation Intermediates

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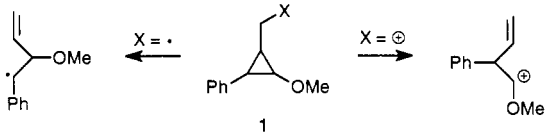
Mechanistic studies of chemical and biochemical processes often include reactions of probe substrates. The mechanistic probe is a potential precursor to a reactive intermediate that will suffer a characteristic reaction, usually a structural rearrangement or isomerization, and the detection of rearranged or isomerized products from the probe provides evidence that the intermediate was produced. Our interest in the mechanisms of oxidations of unactivated hydrocarbons by the iron-containing cytochrome P-450 and methane monooxygenase enzymes focused our attention on radical probes. In order to compete with potentially very fast pseudo-first-order processes that can capture a radical intermediate, "hypersensitive" probes that give radicals that rearrange rapidly are desired. Several very fast radical rearrangements based on cyclopropylcarbinyl radical ring openings have been calibrated,[1] and probes based on these reactions have been applied in studies of enzyme oxidations of unactivated hydrocarbons.[4] However, for most radical probes in general and for the hypersensitive probes in particular, the structural reorganizations of the radicals formed from these compounds are the same as those that would occur from the corresponding carbocations. Such probes cannot provide qualitative evidence that permits one to distinguish between radical and cationic intermediates, and a conclusion in this regard must be deduced from kinetics or from studies of more than one substrate. We report here a probe design that maintains hypersensitive reactivity and allows one to differentiate between a radical and carbocation intermediate.[5]

The probe design was based on the simple notion that, in ring openings of cyclopropylcarbinyl systems, a phenyl group will stabilize an incipient radical center more strongly than does an alkoxy group, but the converse will be true for an incipient carbocation. The architecture in **1** was appropriate for our objective, and the question revolved around the extent of the product energy differences that would be reflected in the ring-opening transition states. In practice, the discrimination factor for **1**, i.e., the ratio of products from a radical rearrangement times the ratio from a cationic rearrangement, exceeds 1×10^5.



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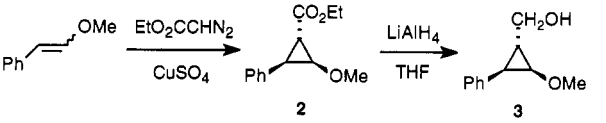
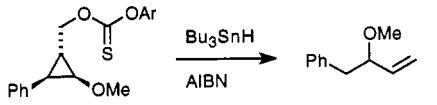
Reaction of β-methoxystyrene (mainly *cis*) with ethyl diazoacetate gave the cyclopropane ester **2**[6] (23% yield); ^{1}H NMR NOE experiments and elemental analysis confirmed the structure and identity. Reduction of **2** gave alcohol **3**[6] (100% yield); the mass spectrum of **3** does not contain a molecular ion, but the $(M - 18)^+$ peak from loss of water had an appropriate HRMS.



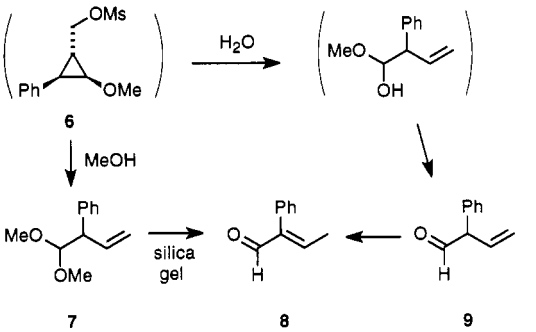
2 3

The radical chemistry of **1** was studied under Barton–McCombie deoxygenation conditions. Alcohol **3** was converted[7] to thionocarbonate **4**[6a,b] (72%), which was reduced in a tin hydride-mediated reaction at 80 °C in benzene with AIBN initiation. Barton–McCombie deoxygenations at primary centers are difficult because the intermediate formed by addition of the stannyl radical to the thione sulfur atom is trapped by tin hydride in competition with fragmentation to the primary radical, but product **5**[6,8] was isolated in an acceptable 47% yield.



4, Ar = *p*-C_6H_4F 5

The cation chemistry of **1** was studied by converting alcohol **3** to its mesylate (**6**) and allowing crude **6** to react in water and in methanol. Attempts to isolate mesylate **6** were unsuccessful. Reaction of **6** in methanol gave acetal **7**[6a,c] (90% crude yield), which could be purified by column chromatography (with partial reaction to give aldehyde **8**[6]). Silica gel chromatography of **7** without careful drying of the sample gave only aldehyde **8**. The reaction of **6** in water gave the unconjugated aldehyde **9** admixed with 5–10% of **8** as the only products. Aldehyde **9** isomerized to **8** and decomposed on standing, but the identity of **9** was established by comparison of the ^{1}H NMR spectrum to that reported for the known compound.[9]



In order to quantitate the tin hydride reduction of **4** and the hydrolysis reaction of **6**, authentic samples of unobserved products **10**, **11** and **12** (both diastereomers) were prepared.[10] The tin hydride reduction and the mesylate solvolysis reactions were repeated, and the crude product mixtures were analyzed by GC–

(1) These include cyclopropylcarbinyl radicals substituted with phenyl groups,[2a,b] with multiple methyl groups,[2c] and with carboalkoxy groups[3] and the cyclopropylcarbinyl radical incorporated into a highly strained bicyclic system, the bicyclo[2.1.0]pent-2-yl radical.[2c,d]

(2) (a) Newcomb, M.; Manek, M. B. *J. Am. Chem. Soc.* **1990**, *112*, 9662–9663. (b) Newcomb, M.; Johnson, C. C.; Manek, M. B.; Varick, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10915–10921. (c) Bowry, V. W.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1991**, *113*, 5687–5698. (d) Newcomb, M.; Manek, M. B.; Glenn, A. G. *J. Am. Chem. Soc.* **1991**, *113*, 949–958.

(3) Beckwith, A. L. J.; Bowry, V. W. *J. Org. Chem.* **1989**, *54*, 2681–2688. (b) Newcomb, M.; Choi, S.-Y. *Tetrahedron Lett.* **1993**, *34*, 6363–6364. (c) Choi, S.-Y.; Newcomb, M., submitted for publication.

(4) (a) White, R. E.; Groves, J. T.; McClusky, G. A. *Acta Biol. Med. Ger.* **1979**, *38*, 475–482. (b) Ortiz de Montellano, P. R.; Stearns, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3415–3420. (c) Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1991**, *113*, 5699–5707. (d) Fu, H.; Newcomb, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 5878–5880. (e) Liu, K. E.; Johnson, C. C.; Newcomb, M.; Lippard, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 939–947. (f) Atkinson, J. K.; Ingold, K. U. *Biochemistry* **1993**, *32*, 9209–9214. (g) Atkinson, J. K.; Hollenberg, P. F.; Ingold, K. U.; Johnson, C. C.; Le Tadic, M.-H.; Newcomb, M.; Putt, D. A., submitted for publication.

(5) A conceptional precedent for a cyclopropane probe that can distinguish between radical and cation intermediates exists, see: Groves, J. T.; Kruper, W. J., Jr.; Haushalter, R. C. *J. Am. Chem. Soc.* **1980**, *102*, 6377–6380.

(6) Characterized by (a) ^{1}H NMR spectroscopy, (b) ^{13}C NMR spectroscopy, (c) GC–mass spectral analysis.

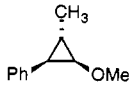
(7) Barton, D. H. R.; Jaszberenyi, J. Cs. *Tetrahedron Lett.* **1989**, *30*, 2619–2622. Method: Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059–4065.

(8) An authentic sample of **5** was prepared by addition of PhMgBr to vinyloxirane and methylation (NaH, CH_3I) of the resulting alcohol.

(9) Barbot, F.; Miginiac, P. *Synthesis* **1983**, 651–654.

0002-7863/94/1516-9753$04.50/0

mass spectrometry. The tin hydride-mediated deoxygenation of **4** produced **5**, a trace of **11**, and no detectable amount (<0.1%) of **10**; the **5**:**11** ratio was 170:1. The hydrolysis of **6** produced **9** and no detectable amount of **12**. A minimum detection limit for **12** determined with authentic mixtures of **9** and **12** was <0.1%; therefore, the ratio of **9**:**12** produced in the solvolysis of **6** exceeded 1000:1.



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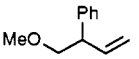

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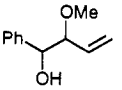

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The absence of detectable amounts of **10** in the radical reaction was not unexpected. The rate constant[12] for reaction of tin hydride with a primary alkyl radical at 80 °C is 6×10^6 M^{-1} s^{-1}, and the rate constant for ring opening of the normethoxy analog of radical **1**·, the (*trans*-2-phenylcyclopropyl)methyl radical, at this temperature is 7×10^{11} s^{-1}.[2b] It is reasonable to assume that the methoxy group at C(3) of **1**· has only a minor effect on the kinetics of the C(1)–C(2) bond cleavage, i.e., that the C(1)–C(2) bond cleavage reaction of **1**· occurs with about the same velocity as that of its normethoxy analog. Therefore, the internal competition for rearrangement of **1**· resulting in a 170:1 product mixture suggests that methoxy stabilization of the incipient product radical from C(1)–C(3) ring opening of **1**· results in a rate constant for the less favored ring opening at 80 °C of about 4×10^9 s^{-1}. At 80 °C, cleavage of one bond in the cyclopropylcarbinyl radical occurs with a rate constant[13] of 3×10^8 s^{-1}, and the kinetic acceleration of the methoxy group on a cyclopropylcarbinyl radical ring opening is somewhat greater than an order of magnitude. For comparison, the kinetic acceleration on the cyclopropylcarbinyl radical ring opening by a phenyl group is nearly 4 orders of magnitude,[2b] and the acceleration afforded by an ester group is greater than 3 orders of magnitude.[3,14]

For studies of enzyme-catalyzed oxidations, we believe that the inability of the hypersensitive probes to permit discrimination between a radical and a cationic intermediate is not a trivial shortcoming. The mechanism of cytochrome P-450 hydroxylation of unactivated hydrocarbons was thought to involve a direct insertion process until qualitative probe results indicated that an intermediate was required in many reactions and excluded a *requisite* cationic intermediate.[15] Presently, the consensus view of the P-450 mechanism is that hydrogen atom abstraction by a high-valent iron–oxo intermediate gives an alkyl radical that is subsequently captured in a homolytic substitution reaction, the oxygen rebound step.[16] However, this mechanistic reasoning contains the tacit assumption that the experimentally required intermediate is in fact an intermediate in the oxidation process and not one formed after the oxidation. Enzyme-catalyzed hydroxylations of hydrocarbons by the sequence of (1) a *concerted insertion* of oxygen followed by (2) Lewis acid-catalyzed *ionic rearrangement* of the alcohol product in the enzyme active site in competition with product escape is a mechanistic possibility not previously considered to our knowledge. Such a sequence, which is compatible with most P-450 probe results,[15] can be tested with probes that contain either the basic elements in **1** or an opposing ester and alkoxy substitution pattern;[14] the high discrimination factor should permit unambiguous conclusions in regard to the nature of the rearranging intermediate. However, there is a *caveat*: compound **10** contains several positions that might be oxidized by enzymes (i.e., the aromatic ring, the benzylic position, and the etheral α-C–H positions).[17]

Acknowledgment. We thank the National Institutes of Health (GM-48722) for support and an anonymous referee for an important commentary and reference.

(10) Compound **10**[6] was prepared by mesylation of **3** in THF at −5 °C followed by treatment of the reaction mixture with $LiBHEt_3$, treatment of the crude products with mcpba (to epoxide ring-opened olefin products), and chromatography. Compound **11**[6] was prepared by reaction of vinylmagnesium bromide with styrene oxide and methylation (NaH, CH_3I) of the resulting alcohol. A mixture of diastereomers of **12**[6] was prepared by the BF_3-mediated addition of 3-(tributylstannyl)-1-methoxypropene to benzaldehyde.[11]

(11) Koreeda, M.; Tanaka, Y. *Tetrahedron Lett.* **1987**, *28*, 143–146.

(12) Chatgilialoglu, C.; Ingold, K. U.; Scaiano, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 7739–7742.

(13) See the discussion and references in the following: Newcomb, M. *Tetrahedron* **1993**, *49*, 1151–1176.

(14) From kinetic considerations, one predicts that a cyclopropylcarbinyl system constructed with an ester group at C(2) and an alkoxy group at C(3) will also serve as a probe that permits discrimination between radical and cationic intermediates.

(15) For discussions of the mechanism of cytochrome P-450 catalyzed oxidations of unactivated hydrocarbons, see the following: McMurry, T. J.; Groves, J. T. In *Cytochrome P-450 Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; Chapter 1. Ortiz de Montellano, P. R. in *Cytochrome P-450 Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; Chapter 7. Woggon, W.-D.; Fretz, H. In *Advances in Detailed Reaction Mechanisms*; Coxon, J. M., Ed.; JAI: Greenwich, CT, 1992; Vol. 2, pp 111–147.

(16) Groves, J. T.; McClusky, G. A.; White, R. E.; Coon, M. J. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 154–160.

(17) The normethoxy analog of **10**, *trans*-2-phenylmethylcyclopropane, has been used in both cytochrome P-450 and methane monooxygenase studies.[4d-g]