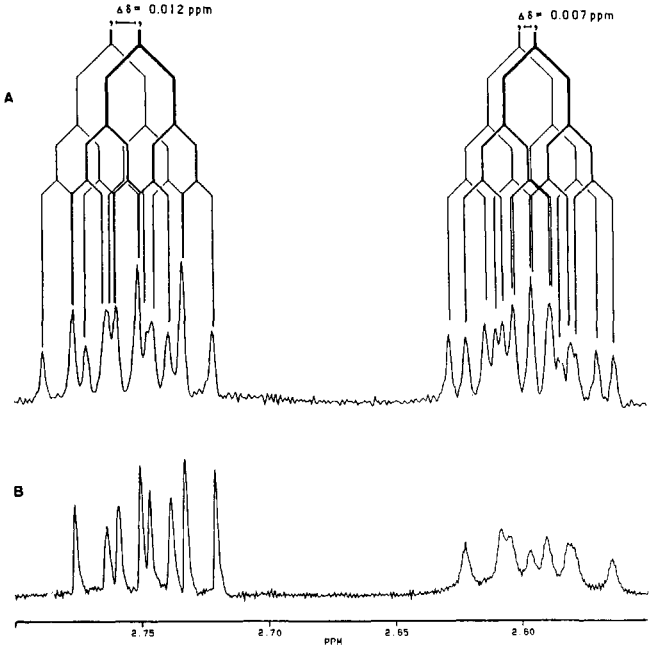

Figure 1. Effects of the addition of 3 equiv of (*S*)-(+)-α-methoxyphenylacetic acid (**4**) on ^{1}H NMR (500 MHz) resonances due to (A) the α-sulfinyl protons of racemic **2b** and (B) the α-sulfinyl protons of biologically produced **2b**.

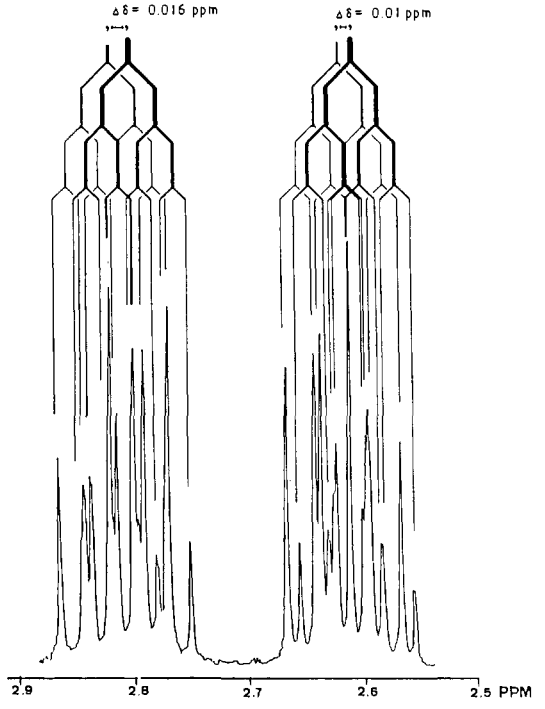

Figure 2. Effects of the addition of 3 equiv of (*S*)-(+)-α-methoxyphenylacetic acid (**4**) on ^{1}H NMR (300 MHz) resonances due to the α-sulfinyl protons of predominantly (*R*)-butyl butyl-d_9 sulfoxide (**5**).

fatty acid sulfoxide; however, in this case, the *upfield* half of each doublet was reduced in intensity.[9] (See Figure 2.) It clearly follows that the disposition of the labeled and unlabeled methylene groups surrounding the sulfinyl group of biologically produced **2b** is opposite to that of **5**. The absolute configuration of the parent sulfoxide **2a** is therefore *R*, a stereochemical result that is consistent with that obtained for a benzyl analogue (**6**).[10]

(9) The ee of **5** was estimated to be 47%, in excellent agreement with the known de (47%) of the starting menthyl sulfinate mixture.[8]

(10) Buist, P. H.; Marecak, D. M.; Partington, E. *J. Org. Chem.* **1990**, *55*, 5667. The absolute configuration of **6** has recently been confirmed by synthesis (unpublished results).

In conclusion, we have demonstrated that biosulfoxidation of a quasisymmetrical thia fatty acid analogue is a highly enantioselective process.

Acknowledgment. We thank the NSERC for a postgraduate scholarship to D.M. and Carleton University for financial support of this work. The assistance of Dr. Barbara Blackwell, John Nikiforuk (Agriculture Canada), and Professor G. W. Buchanan (Carleton University) is greatly appreciated.

Pseudomonas oleovorans Monooxygenase Catalyzed Asymmetric Epoxidation of Allyl Alcohol Derivatives and Hydroxylation of a Hypersensitive Radical Probe with the Radical Ring Opening Rate Exceeding the Oxygen Rebound Rate

Hong Fu,[†] Martin Newcomb,*[,‡] and Chi-Huey Wong*[,†]

Department of Chemistry, Scripps Research Institute
La Jolla, California 92037
Department of Chemistry, Texas A&M University
College Station, Texas 77843

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The non-heme monooxygenases are NAD(P)H/O_2-dependent metalloenzymes which often contain iron(s) in the active site for catalysis.[1] These enzymes catalyze the incorporation of molecular oxygen into unactivated organic molecules in a selective manner.[2] It has been demonstrated, with support from model studies,[3] that the putative "iron-oxo" species[4] generated from the reduced enzyme and molecular oxygen are like that of the heme-containing monooxygenases[5] and capable of hydroxylation of alkanes, epoxidation of olefins, oxidation of heteroatoms such as S or N, and O-demethylation of methyl ethers. *Pseudomonas oleovorans*

[†] Scripps Research Institute.
[‡] Texas A&M University.

(1) For reviews of monooxygenases, see: Davies, H. G.; Greene, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in preparative organic chemistry: The use of isolated enzymes and whole cell systems in synthesis*; Academic Press: New York, 1989; pp 169-219. Hayaishi, O. *Molecular Mechanisms of Oxygen Activation*; Academic Press: New York, 1974.

(2) (a) Colbert, J. E.; Katopodis, A. G.; May, S. W. *J. Am. Chem. Soc.* **1990**, *112*, 3993. (b) Katopodis, A. G.; Wimalasena, K.; Lee, J.; May, S. W. *J. Am. Chem. Soc.* **1984**, *106*, 7928. (c) Baldwin, J. E.; Abraham, E. P. *Nat. Prod. Rep.* **1988**, *5*, 129. (d) Corey, E. J. In *Stereochemistry of organic and bioorganic transformations*; Bartmann, W., Sharpless, K. B., Eds.; VCH: Deerfield Beach, FL, 1986; pp 1-12. (e) Fox, B. G.; Borneman, J. G.; Wackeh, L. P.; Lipscomb, J. D. *Biochemistry* **1990**, *29*, 6419. (f) Ruzicka, F.; Huang, D.-S.; Donnelly, M. I.; Frey, P. A. *Biochemistry* **1990**, *29*, 1696. (g) Ericson, A.; Hedman, B.; Hodgson, K. O.; Green, J.; Dalton, H.; Bentsen, J. G.; Beer, R. H.; Lippard, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 2330.

(3) (a) Lippard, S. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 344. (b) Barton, D. H. R.; Csuhai, E.; Doller, D.; Ozbalik, N.; Balavoine, G. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3401. (c) Sheu, C.; Richert, S. A.; Cofrē, P.; Ross, B., Jr.; Sobkowiak, A.; Sawyer, D. T.; Kanofsky, J. R. *J. Am. Chem. Soc.* **1990**, *112*, 1936. (d) Sheu, C.; Sawyer, D. T. *J. Am. Chem. Soc.* **1990**, *112*, 8212. (e) Kitajima, N.; Fukui, H.; Moro-oka, Y. *J. Chem. Soc., Chem. Commun.* **1988**, 485.

(4) (a) For a detailed discussion, see: Ortiz de Montellano, P. R. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 217-271. Also see: (b) Mcurry, T. J.; Groves, J. T.; In Cytochrome P-450: *Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 3-5. (c) Groves, J. T.; McClusky, G. A. *J. Am. Chem. Soc.* **1976**, *98*, 859.

(5) (a) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315. (b) Sligar, S. G.; Kennedy, K. A.; Pearson, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1240. (c) Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B. J. *J. Am. Chem. Soc.* **1981**, *103*, 2884. (d) Groves, J. T.; Watanabe, Y. *J. Am. Chem. Soc.* **1986**, *108*, 507. (e) Ortiz de Montellano, P. R.; Stearns, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3415. (f) Castellino, A. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1988**, *110*, 7512. (g) Collman, J. P.; Brauman, J. I.; Meunier, B.; Hayashi, T.; Kodadek, T.; Raybuck, S. A. *J. Am. Chem. Soc.* **1985**, *107*, 2000. (h) Traylor, T. G.; Xu, F. *J. Am. Chem. Soc.* **1988**, *110*, 1953. (i) Valentine, J. S.; Burstyn, J. N.; Margerum, L. D. In *Oxygen Activation by Transition Metals*; Sawyer, D. T., Ed.; Plenum: New York, 1989; pp 175-187.

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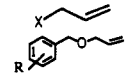
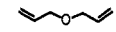
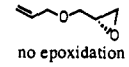
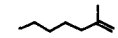
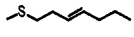
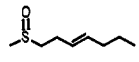
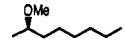
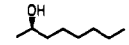
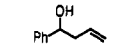
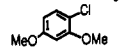
Table I. *Pseudomonas oleovorans* (ATCC 29347) Monooxygenase Catalyzed Oxidations[a]

Entry	Substrate	Product	% ee[b]
1	 X = Cl, OH	no reaction	—
2	R = H		75
3	R = *p*-MeO-		80[c]
4	R = *m*-MeO-		32
5	R = *o*-MeO-	no reaction	—
6	R = *p*-Cl-		90
7	R = *p*-F-		86
8	R = *p*-CH_3	several products	—[d]
9	R = MeO		98
10	R = F		99
11	R = $CH_3OCH_2CH_2$-		98[e]
12			81
13		no epoxidation	—[f]
14			86[g]
15			100[g]
16	Ph-CH_3	Ph-CH_2OH	—
17			23[h]
18		no reaction	

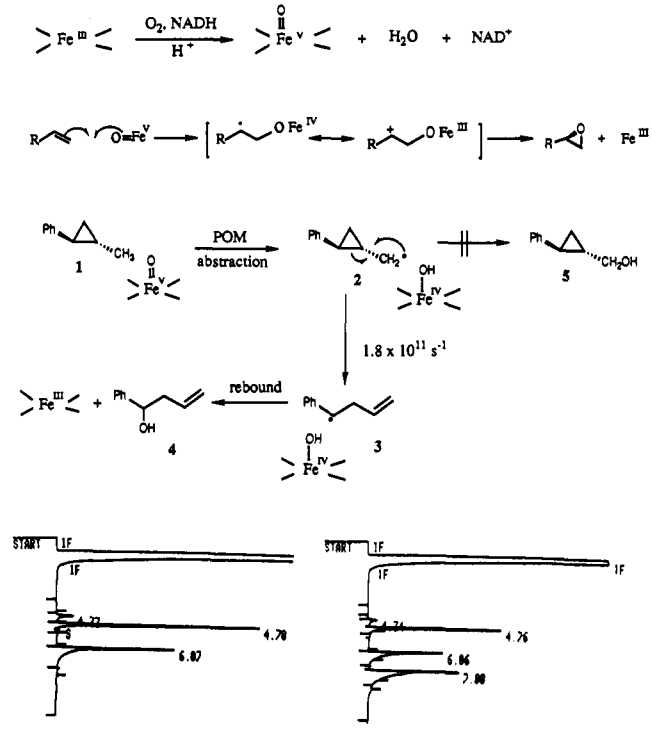
[a] All reactions were carried out with growing cells unless otherwise indicated. [b] Determined by using HPLC with a Chiralcel OC column (0.46 × 25 cm) and a UV detector (254 nm). The mobile phase was hexane/2-propanol (19:1 for entries 2 and 6; 9:1 for entries 3, 4, and 9; 49:1 for entries 7 and 10). The absolute stereochemistry for the epoxides of entries 2 and 12 was determined by comparison of the retention times with those of the authentic samples prepared previously.[9] The other epoxides were also separated by HPLC and assumed to have the same stereochemistry. All the products were further confirmed by ^{1}H NMR and HRMS, and the data were consistent with those expected. [c] A small portion (~20%) of the substrate was oxidatively O-demethylated. [d] Oxidation of the ring methyl group and epoxidation occurred. [e] Johnston, S. L.; Phillips, G. T.; Robertson, B. W.; Watts, P. D.; Bertola, M. A.; Koger, H. S.; Mark, A. F. In *Biocatalysis in organic media*; Laane, C., Tramper, J., Lilly, M. D., Eds.; Elsevier: New York, 1987; p 387. [f] Only oxidations of the terminal methyl groups occurred. [g] Katopodis, A. G.; Smith, H. A.; May, S. W. *J. Am. Chem. Soc.* **1988**, *110*, 897. [h] The reaction was carried out with resting cells and with the crude enzyme preparation.

monooxygenase (POM) is one of the most studied non-heme monooxygenases.[2a,b] All of the aforementioned reactions have been conducted with POM using resting or growing cells, as well as partially purified POM coupled with NADPH regeneration.

To further exploit the synthetic utility of POM,[6] we report our initial study on the enzyme-catalyzed epoxidation of allyl alcohol derivatives[7] and hydroxylation of the designed hypersensitive radical probe *trans*-2-phenyl-1-methylcyclopropane.[8]

As shown in Table I, although allyl alcohol (and allyl chloride) is not epoxidized,[9] its *O*-alkyl derivatives are oxidized to the corresponding (*R*)-epoxides with high enantiomeric excess (ee).[10]

Scheme I. Proposed Mechanisms for POM Reactions[a]



[a] At bottom of scheme: GC analyses of the products from POM reaction with **1** (left) and authentic compounds **1** (t_R 4.76 min), **4** (t_R 6.07 min), and **5** (t_R 7.08 min) (right).

Both the enzyme reactivity and enantioselectivity in the epoxidation of benzyl allyl ether derivatives were affected by the substituent in the aromatic group. The para-substituted benzyl derivatives gave higher ee (80% ee for the *p*-methoxybenzyl (entry 3) and 90% ee for the *p*-chlorobenzyl derivatives (entry 6)) than that of the unsubstituted (75% ee, entry 2) and the *m*-methoxybenzyl derivatives (32% ee, entry 4), and no epoxidation was observed for the *o*-methoxybenzyl derivative (entry 5). Very high enantioselectivity was observed for the epoxidation of aryl allyl ether (entries 9–11). Monoepoxidation of diallyl ether was observed with 81% enantioselectivity. Internal olefins and 2,2-disubstituted terminal olefins, however, are not oxidized, providing a useful regioselectivity in POM-catalyzed reactions. The enantioselective epoxidations illustrated here together with other reactions indicated in Table I suggest that POM is a synthetically useful enzyme. Although the free enzyme is too unstable to be used in large-scale reactions,[2a,b] the crude enzyme or cell preparations[2a,b,11] seem to be useful for a number of transformations.

Mechanistic studies of POM-catalyzed epoxidation of terminal olefins suggest that iron–oxo attack at C-1 gives a cationic or radical intermediate that closes preferentially from the *Si* face of C-2 to give the (*R*)-epoxide (Scheme I).[2a,b] POM-catalyzed hydroxylation of alkanes, however, has not been investigated mechanistically, and thus, the oxidation of *trans*-2-phenyl-1-methylcyclopropane (**1**) is of particular interest. 1-Phenyl-3-buten-1-ol (**4**) (23% ee) was the only product detected to a limit of 1–2%,[12] suggesting that the hydroxylation proceeds through a nonconcerted radical process similar to that implicated for heme-containing cytochrome P-450[5e] and non-heme *Methylosinus trichosporium* (OB3b) monooxygenase (MTM).[3f] As shown in

(6) For a recent review on enzymatic organic synthesis, see: Wong, C.-H. *Science* **1989**, *244*, 1145.

(7) Prepared by reaction of allyl bromide and substituted benzyl alcohol in the presence of NaOH and the phase-transfer catalyst tetrabutylammonium hydrogen sulfate. All reactions were unoptimized with 10–20% yield. No other byproducts were obtained. For chemical epoxidation, see: Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

(8) Prepared via a carbene insertion from *trans*-methyl-β-styrene and CH_2I_2 in the presence of Zn–Cu. (Simmons, H. E.; Cairns, T. L.; Vladuchick, S. A. *Org. React. (N.Y.)* **1973**, *20*, 1.) The unreacted substrate was oxidized to the epoxide with MCPBA and separated fromt he desired product by using silica gel chromatography with hexane as eluent.

(9) Hydroxylated compounds are normally not oxidized (Schwartz, R. D.; McCoy, C. J. *Appl. Environ. Microbiol.* **1977**, *34*, 47).

(10) Both the ee and stereochemistry were determined according to the procedure described previously (Pederson, R. L.; Liu, K. K.-C.; Rutan, J. F.; Chen, L.; Wong, C.-H. *J. Org. Chem.* **1990**, *55*, 4897.

(11) Fu, H.; Shen, G.-J.; Wong, C.-H. *Recueil*, in press.

(12) Confirmed by ^{1}H NMR [($CDCl_3$) δ 7.25–7.36 (m, 5 H), 5.70–5.89 (m, 1 H), 5.11–5.19 (m, 2 H), 4.68–4.75 (m, 1 H), 2.47–2.54 (m, 2 H), 2.14 (s, 1 H)] and GC analysis with a DB-5 column (15 × 0.522 cm, 50–250 °C, 20 °C/min, t_R = 6.07 min) and compared to the authentic compound. Compounds **1** and **4** were the only products isolated, in 21% yield in a 1:1 ratio. GC analysis showed that **5** was not formed.

Scheme I, hydrogen atom abstraction from **1** by the putative iron–oxo species would give radical **2**, which can ring open to radical **3** ($k = 2 \times 10^{11}$ s^{-1} at 30 °C);[13] subsequent hydroxyl transfer from the Fe(IV) species to **3** (oxygen rebound) would give product **4**. This mechanism was further supported by the observed kinetic isotope effect of 7.8 when the monodeuteriomethyl derivative of **1** was used as substrate, and it suggested only a tentative limit of $<4 \times 10^9$ s^{-1} for the rate constant for the oxygen rebound step.[14] Nevertheless, the simple preparation of **1**, the easy recovery of **1** and its products from the reaction medium, and the apparent regiospecific oxidation of **1** suggest that aryl-containing cyclopropanes will be useful for study of various monooxygenases and their models.[16]

Supplementary Material Available: Physical constants (^{1}H NMR, HRMS) for the epoxides and detailed procedures for the preparation of **1** and **4** (6 pages). Ordering information is given on any current masthead page.

(13) Newcomb, M.; Manek, M. B. *J. Am. Chem. Soc.* **1990**, *112*, 9662.
(14) A rate constant of 2×10^{10} s^{-1} has been estimated for rebound in P-450 oxidation,[5e,15] but rebound in MTM oxidation appears to be slower.[2f]
(15) Bowry, V. W.; Lusztyck, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1989**, *111*, 1927.
(16) Castellino and Bruice had reported that (*Z*)-1,2-bis(*trans*-2,*trans*-3-diphenylcyclopropyl)ethene was oxidized by a P-450 model to the corresponding (*trans*-2,*trans*-3-diphenylcyclopropyl)carbinyl radical to homoallyl radical with the radical rearrangement estimated as $\geq 2 \times 10^{10}$ s^{-1} (Castellino, A. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1988**, *110*, 7512).
(17) This research was supported by NSF grants to M.N. (CHE8816365) and C.-H.W. (CHE8996249). We thank Dr. M. B. Manek for assistance with the synthesis of **1**.

Radical Type Reactivity in a γ-Distonic Radical Cation: A Gas-Phase Experimental Study

Krista G. Stirk and Hilkka I. Kenttämaa*

Department of Chemistry, Purdue University
West Lafayette, Indiana 47907

Received February 21, 1991

In sharp contrast to the extensive work carried out on radical cations that are generated by removal of an electron from a stable, neutral molecule,[1] ions with *spatially separated radical and charge sites* (distonic ions[2]) have only recently become the focus of attention. Interest in these ions was sparked by the discovery[3] that they can be more stable than their conventional isomers. Reactions of various ions with the radical and charge sites formally on adjacent atoms (α-distonic ions) have been investigated in detail.[2] However, it is difficult to distinguish the role of the charge and radical sites in these reactions.[4] The current knowledge on distonic ions with the charge and radical sites separated by at least one heavy atom is almost exclusively limited to unimolecular chemistry of highly excited and short-lived species.[2] The results obtained thus far on bimolecular reactions of low-energy β-distonic ions suggest that they predominantly involve the charge site,[2,4,5] although some recent results[4b] could also be explained in terms of a radical type mechanism. We report here the first study on the bimolecular gas-phase reactions of a stable γ-distonic ion. Conclusive evidence is presented for a radical type reaction occurring remotely from the charge site.

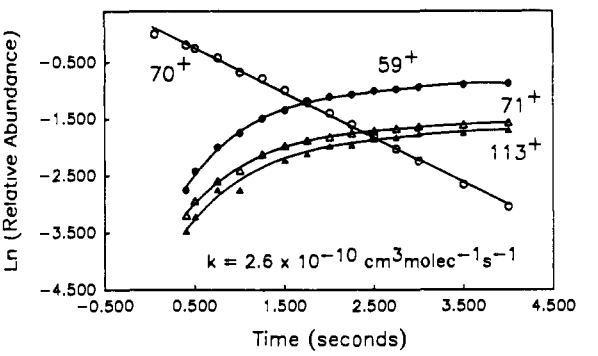
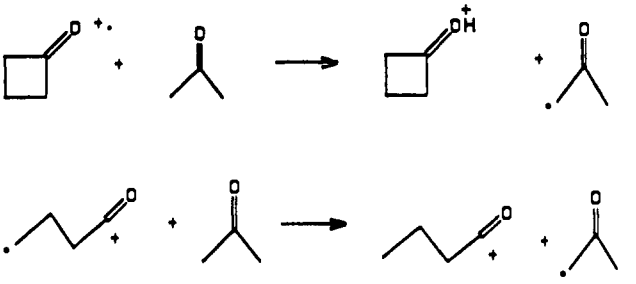


Figure 1. Reaction of ionized cyclobutanone with acetone (9×10^{-8} Torr).

Scheme I



Ionized cyclobutanone was recently suggested[6] to have the γ-distonic, open-chain structure $^{\bullet}CH_2CH_2CH_2C{=}O^+$. We observed that this ion abstracts a hydrogen atom from acetone in a dual-cell Fourier transform ion cyclotron resonance instrument (a prototype Extrel FTMS-2001).[7] The product ion (*m*/*z* 71) corresponds to 30% of the total product ion distribution; other major ionic products include protonated acetone (*m*/*z* 59, 50%) and an ion (*m*/*z* 113, 20%) formed by loss of a methyl radical from the complex of ionized cyclobutanone and acetone (Figure 1). For d_6-acetone, abstraction of a deuterium atom by ionized cyclobutanone to yield a positive ion of *m*/*z* 72 was observed, in addition to protonated d_6-acetone (*m*/*z* 65) and an ion of *m*/*z* 116. Great care was taken to avoid protonation of neutral cyclobutanone since protonated cyclobutanone has the same mass value as the reaction product of interest (*m*/*z* 71). Cyclobutanone was introduced into one of the differentially pumped reaction regions (cell 1) for the ionization event only (pulsed sample introduction[8]). After electron ionization, ionized cyclobutanone was transferred into the other reaction region (cell 2), where it was allowed to react with acetone. No neutral cyclobutanone was in the instrument at this time. A double-resonance experiment that involved ejection of ionized cyclobutanone from the cell confirmed that this ion was the origin of all the product ions mentioned above.

Assuming that ionized cyclobutanone has the proposed[6] acyclic structure (calculated[6b] to be 18 kcal/mol lower in energy than the cyclic structure), the observed hydrogen atom abstraction from acetone represents the first radical type reaction observed for a γ-distonic radical cation in the gas phase. However, no conclusive experimental evidence existed at the time of this work for the proposed acyclic structure.[9] Hydrogen atom abstraction from acetone would be near thermoneutral for the original cyclic structure[10,11] as well as for the acyclic structure.[11,12] Our results

(1) See, for example: Hammerich, O.; Parker, V. D. *Phys. Org. Chem.* **1984**, *20*, 55.
(2) A review: Hammerum, S. *Mass Spectrom. Rev.* **1988**, *7*, 123.
(3) (a) Bouma, W. J.; Nobes, R. H.; Radom, L. *J. Am. Chem. Soc.* **1982**, *104*, 2929. (b) Bouma, W. J.; MacLeod, J. K.; Radom, L. *J. Am. Chem. Soc.* **1982**, *104*, 2930. (c) Holmes, J. L.; Lossing, F. P.; Terlouw, J. K.; Burgers, P. C. *J. Am. Chem. Soc.* **1982**, *104*, 2931.
(4) (a) Rusli, R. D.; Schwarz, H. *Chem. Ber.* **1990**, *123*, 535. (b) Wittneben, D.; Grutzmacher, H.-F. *Int. J. Mass Spectrom. Ion Processes* **1990**, *100*, 545. (c) De Koster, C. G.; Van Houte, J. J.; Van Thuijl, J. *Int. J. Mass Spectrom. Ion Processes* **1990**, *98*, 235.
(5) Zeller, L.; Farrell, J.; Vainiotalo, P.; Kenttämaa, H. I., submitted for publication.
(6) (a) Heinrich, N.; Koch, W.; Morrow, J. C.; Schwarz, H. *J. Am. Chem. Soc.* **1988**, *110*, 6332. (b) Dass, C.; Gross, M. L. *Org. Mass Spectrom.* **1990**, *25*, 24.
(7) Farrell, J. T., Jr.; Lin, P.; Kenttämaa, H. I. *Anal. Chim. Acta* **1991**, *246*, 227.
(8) Carlin, T. J.; Freiser, B. F. *Anal. Chem.* **1983**, *55*, 571.
(9) Rearrangement of $^{\bullet}CH_2CH_2CH_2C{=}O^+$ to $CH_3CH_2CH{=}C{=}O^{\bullet+}$ can be ruled out: H$^{\bullet}$ abstraction from acetone and protonation of acetone would be endothermic for this structure (≥3 kcal/mol[11,12]) (for $CH_3CH{=}C{=}O^{\bullet+}$, $\Delta H_{rxn} \geq 0$ kcal/mol for both reactions).

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