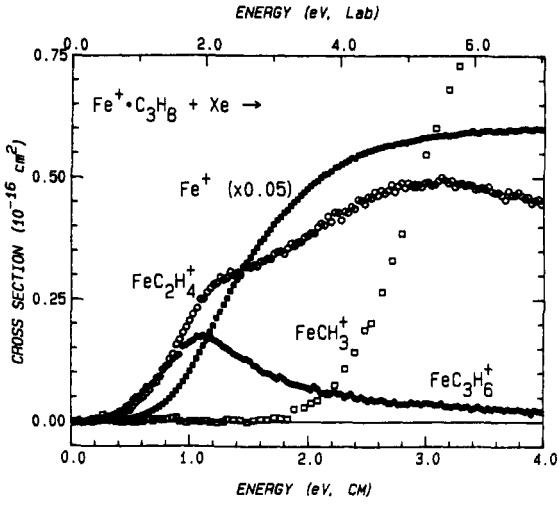

Figure 1. Cross sections for TCA of $Fe^+ \cdot C_3H_8$ as a function of relative energy (lower x axis) and laboratory energy (upper x axis).

ditional experiments also imply that the parent ion derives from the $Fe^+(^6D,3d^64s)$ ground state rather than the $Fe^+(^4F,3d^7)$ first excited state. Specifically, recent measurements find that bond energies for Co^+–alkane complexes are consistently stronger than the analogous Fe^+–alkane species,[7,9] contrary to the similar alkane bond strengths expected for $Co^+(3d^8)$ and $Fe^+(3d^7)$.

Since reactions 1a and 1b (which are exothermic by 19 and 11 ± 5 kcal/mol,[10-12] respectively) exhibit no activation barriers,[2] reactions 2a and 2b must have lower thermodynamic thresholds than process 2c, as is evident from Figure 1. Analyses of these two products find a threshold of 0.47 ± 0.12 eV (11 ± 3 kcal/mol) for both processes, even though reactions 2a and 2b have different reaction enthalpies, ~0 and 8 kcal/mol, respectively, given $D^\circ(Fe^+-C_3H_8)$ = 19 kcal/mol. Clearly, the observed threshold for reaction 2a is *not* the thermodynamic threshold, but rather the activation barrier associated with the rate-limiting step in the bimolecular process. It seems likely that this is also true for reaction 2b. In analogy with recent work on the Co^+ + propane system (based on completely different experimental techniques),[13] we propose that this barrier corresponds to the activation of a C–H bond of propane by Fe^+.

Further insight into the PES can be obtained from the kinetic energy dependence of reactions 2a and 2b. For the bimolecular system, reaction 1a is favored by a factor of 3 at all kinetic energies for both the ^6D and ^4F electronic states of Fe^+,[2] consistent with the overall thermodynamics. In contrast, the branching ratio of reactions 2a and 2b is highly energy dependent. At the lowest energies, reaction 2b is slightly favored, indicating that the product branching ratio is under kinetic control or that reaction 2b has a slightly lower threshold. Cross sections for both $FeC_2H_4^+$ and $FeC_3H_6^+$ exhibit a sudden change at ~1 eV. Since dissociation of these products cannot begin until 1.7 and 2.1 eV,[12] respectively, this abrupt change must be due to competition with direct dissociation, reaction 2c. Above this energy, cross sections for reactions 2a and 2b deviate strongly from one another, suggesting that a new pathway for reaction 2a (but not reaction 2b) becomes

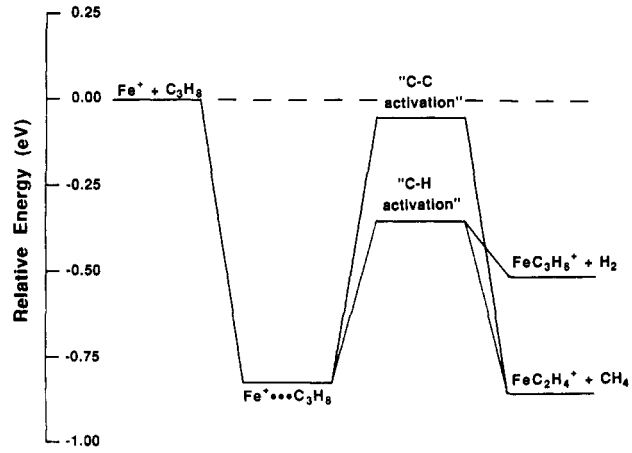

Figure 2. Thermodynamics of the Fe^+–propane potential energy surface. The "C–C activation" barrier is derived by scaling the cross section for reaction 2b to that of reaction 2a and fitting a threshold to the remainder.

energetically accessible. An obvious choice is C–C bond activation. This then implies that the 11 kcal/mol activation barrier corresponds to an initial primary C–H bond activation step which can lead to both reactions 2a and 2b. Secondary C–H bond activation is also presumably occurring but apparently exhibits no independent feature in the TCA spectrum. The insensitivity of the branching ratio of reactions 1a and 1b to kinetic energy can be explained, since in reaction 1 (but *not* reaction 2), the reactants enter with enough energy to surmount either of the two activation barriers. These details are again similar to those suggested for reaction of Co^+ with C_3H_8.[13]

The combination of all these results leads to the PES shown in Figure 2. This diagram clearly does not indicate all of the complications of the system, in particular the surfaces of differing spins that arise from the two lowest energy states of Fe^+.[2] Further work to probe the PES of this interesting reaction system is underway.[11] Such studies will include examination of the reverse of reactions 2a and 2b and, if possible, TCA of other purported intermediates on the PES.

Acknowledgment. This work was supported by the National Science Foundation, Grant No. CHE-8917980.

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Primary and Secondary Oxygen-18 Isotope Effects in the Alkaline and Enzyme-Catalyzed Hydrolysis of Phosphotriesters

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Phosphotriester hydrolysis occurs by an associative mechanism with nucleophilic attack of hydroxide.[1] This associative process can occur via two limiting mechanistic alternatives: the formation

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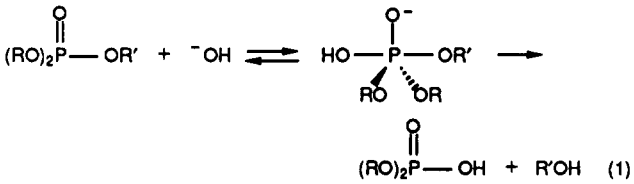
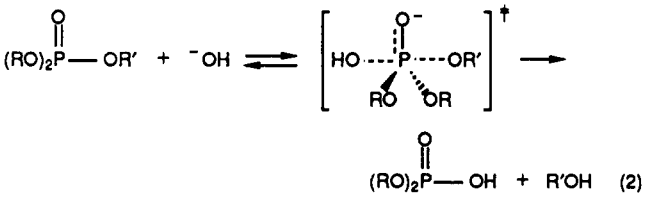
of a pentavalent phosphorane intermediate (eq 1) or a direct S_N2-like reaction (eq 2). With cyclic phosphotriesters where



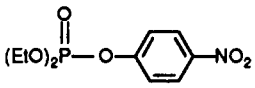
(1)



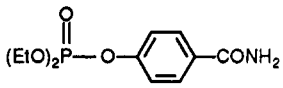
(2)

pseudorotation is necessary for the leaving group to leave axially, a phosphorane intermediate is predicted.[1,2] However, for the chemical hydrolysis of acyclic phosphotriesters, neither mechanistic pathway has been eliminated. The utilization of primary and secondary ^{18}O isotope effects has now provided direct evidence for a transition state where there are significant bond-order changes to both the phosphoryl oxygen and the phenolic leaving group.

A phosphotrieasterase has been recently isolated and purified[3] which exhibits a very broad specificity for substrate hydrolysis.[4] The limitation in rate for the enzymatic hydrolysis of the insecticide paraoxon (I) is a combination of diffusional and chemical steps.[5] On the contrary, the rate of hydrolysis of the nonsticky substrate *O,O*-diethyl *O*-4-carbamoylphenyl phosphate (II) is limited by bond breaking.[5] In an effort to further define the



I



II

enzymatic mechanism, as well as to provide evidence for the mechanism of alkaline hydrolysis of phosphotriesters, ^{18}O isotope effects for the phenolic and phosphoryl oxygen atoms were measured. The magnitude of the ^{18}O isotope effects at these two positions should provide direct information on the changes that have occurred in these phosphorus–oxygen bonds in the transition state.

The ^{18}O isotope effects were measured by the remote label method,[6] where the $^{15}N,^{18}O$ compound and the ^{14}N (^{15}N depleted) compound were combined to give a resulting mixture very near the natural abundance ratio for nitrogen. The ^{18}O isotope effect was determined by separating the phenol from the residual substrate after about 50% hydrolysis (hydrolysis performed with 0.1 N KOH or the phosphotriesterase), and then each was subsequently converted separately to diatomic nitrogen by Kjeldahl digestion, alkaline steam distillation, and oxidation. In the case of paraoxon, the nitro group was first reduced in the presence of sodium thiosulfate.[7] The mass ratio of the isolated nitrogen was measured by using an isotope ratio mass spectrometer. The oxygen isotope effects are presented in Table I.

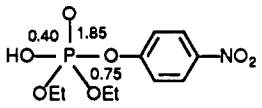
The ^{18}O isotope effects of 1.006 and 1.0063 for the alkaline hydrolysis of paraoxon provide strong evidence that significant bond-order changes have occurred to both the phosphoryl and

Table I. Primary and Secondary Isotope Effects for the Alkaline and Enzyme-Catalyzed Hydrolysis of Paraoxon (I) and *O,O*-Diethyl *O*-4-Carbamoylphenyl Phosphate (II)[a]

compd	isotope effect	W^b	^{18}k
I	1° alkaline	1.0058	1.0060[c]
I	1° enzymatic	1.0017	1.0020[c]
I	2° alkaline	1.0056	1.0063 ± 0.0001
I	2° enzymatic	1.0016	1.0021 ± 0.0004
II	1° alkaline	1.023	1.027 ± 0.002
II	1° enzymatic	1.031	1.036 ± 0.002
II	2° alkaline	1.020	1.025 ± 0.002
II	2° enzymatic	1.0144	1.0181 ± 0.0007

[a] The following equation was utilized to calculate the ^{18}O isotope effect (^{18}k) (ref 14): $^{18}k = WY/[A - (W - A)[(1 - B)Z/(BX)] - W(1 - Y)]$, where W = the observed isotope effect $[^{15,18}(V/K)]$, A = the ^{15}N isotope effect at the remote labeled position determined with natural abundance phosphotriester (1.0007 ± 0.0001 for the chemical hydrolysis of I,[9] 1.000 for the enzymatic hydrolysis of I and II, and 1.000 for the chemical hydrolysis of II), B = the fraction of $^{15}N,^{18}O$-containing phosphotriester in the final mixture or pseudo natural abundance (0.003 851 for I 1°, 0.003 602 for I 2°, 0.003 630 for II 1°, and 0.003 629 for II 2°), Y = the fraction of ^{18}O in the $^{15}N,^{18}O$ phosphotriester (0.88 for I 1°, 0.80 for I 2°, 0.89 for II 1°, and 0.82 for II 2°), X = the fraction of ^{15}N in the $^{15}N,^{18}O$ phosphotriester (0.99), and Z = the fraction of ^{15}N in the $^{14}N,^{16}O$ phosphotriester (0.0001). [b] The observed isotope effects were determined from comparative isotopic analysis between either the residual phosphotriester, the phenol product, or both after approximately 50% reaction, and the initial phosphotriester. [c] Only one determination of this number was made.

phenolic oxygen in the transition state. The isotope effect for the phenolic oxygen is very similar to the value of 1.007 ± 0.004 obtained for the hydrolysis of 2,4-dinitrophenyl dibenzyl phosphate measured by a direct spectrophotometric method.[8] A primary ^{18}O isotope effect of this size would not be expected for the formation of a discrete phosphorane intermediate since the rate-determining step is most likely the addition of hydroxide to the paraoxon.[9] The secondary ^{15}N isotope effect for the nitro group of 1.007 ± 0.0001 with alkaline hydrolysis of paraoxon reported by Hengge and Cleland[10] also reflects the phosphorus–phenolic oxygen bond order in the transition state. On the basis of the recently measured maximum equilibrium ^{15}N isotope effect of 1.0028 for the deprotonation of *p*-nitrophenol,[10] this secondary ^{15}N isotope effect represents about 25% bond cleavage in the transition state for paraoxon hydrolysis. The ^{18}O isotope effect for the phosphoryl oxygen is 1.0063 and indicates a change in bond order between the transition state and the ground state. Calculations predict a theoretical maximum secondary ^{18}O isotope effect of 4% normal[11,12] using the BEBOVIB-IV program developed by Sims.[13] On the basis of this value, the observed secondary ^{18}O isotope effect would suggest a bond order of 1.85 in the transition state. If we assume a total bond order of 5 to the phosphorus with a total distributed negative charge of 1, then the transition-state structure can be depicted with 0.40 bond order for the attacking hydroxide as illustrated. (We assume a bond order of 1 for each of the ethyl ester substituents.)



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The ^{18}O isotope effect at the phenolic oxygen for the alkaline hydrolysis of compound II, which possesses a leaving group with a higher pK_a (8.56), is 2.75% normal. Again, this is consistent with a transition state where there is significant cleavage of the bond to the leaving group and is considerably larger than that observed for paraoxon hydrolysis. This difference is rationalized on the basis of the resonance capabilities of *p*-nitrophenol where *p*-nitrophenol exists in a quinoid resonance form under the conditions of the experiment. As such, there is considerable double-bond character to the phenolic oxygen, thus compensating, qualitatively, for the bond breaking between the phosphorus and the phenolic oxygen. A similar comparison of the calculated and observed equilibrium ^{18}O isotope effect for the deprotonation of *p*-nitrophenol has been reported.[14] The secondary ^{18}O isotope effect for this compound is 1.025 and indicates a change in bond order to the phosphoryl oxygen leading to the transition state. The transition state is later relative to the effect observed with paraoxon and corresponds to a bond order of about 1.38. This is anticipated since 4-hydroxybenzamide is a somewhat poorer leaving group compared to *p*-nitrophenol.

The primary and secondary ^{18}O isotope effects measured for the enzymatic hydrolysis of paraoxon (I) are 1.0020 and 1.0021, respectively. These isotope effects are much smaller than those observed for nonenzymatic hydrolysis and are consistent with previous data which indicate that the chemical step is not totally rate limiting.[5] However, with the nonsticky substrate II, the primary and secondary ^{18}O isotope effects are 1.036 and 1.018, respectively. These values are similar to those for alkaline hydrolysis and therefore support the chemical step as totally rate limiting in the enzymatic hydrolysis of this substrate. At present, a complete interpretation of the measured isotope effects for the enzymatic mechanism is limited since the potential participation of active-site residues and a required zinc atom is unknown. Experiments are underway that will better define the role of the zinc atom in catalysis.

Acknowledgment. This work was supported by the Army Research Office (DAALO3-90-G-0045), Robert A. Welch Foundation (A-840), and the National Institutes of Health (GM18938).

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*Book Reviews**

The Chemistry of Functional Groups. Supplement A: The Chemistry of Double-Bonded Functional Groups. Parts 1 and 2. Edited by Saul Patai (The Hebrew University, Jerusalem). John Wiley & Sons: New York and Chichester. Part 1: xiv + 797 pp. $360.00. ISBN 0-471-91719-2. Part 2: xiv + 893 pp. $430.00 ISBN 0-471-92493-8.

These two volumes are paginated continuously, and the index, covering both, is only in Part 2, although the volumes can apparently be bought separately. Their content consists of one group of chapters devoted to subjects not treated before in this series and another group of "integrative" chapters, in which a "unified and comparative treatment of several double-bonded functional groups together" is presented. The nine chapters in the latter category make up most of Part 1. They cover views on homopolar bond structure, mass spectrometry, NMR, photoelectron spectroscopy, directing and activating effects, biochemical perspectives on double bondes, intramolecular 1,3-dipolar cycloadditions, the eve reaction, and radiation chemistry. The former category includes asymmetric induction in additions to C,O and C,N double bonds, electrophilic additions to C,C double bonds, mechanisms of base-catalyzed 1,2-eliminations, carbonylation of main-group organometalic compounds, rearrangements involving allenes, 1,1-diarylalkenes, fulvenes, the thiocarbonyl group, and cycloadditions of enones.

The literature coverage is "up to about the end of 1987 and in some cases even to the middle of 1988", and the bibliographies are extensive. As is characteristic of the series, there are good tables of data and an abundance of carefully drawn structure formulas.

The writing is essentially critical, insofar as the subject matter allows, and the contributors have successfully avoided presenting a mere recitation of facts. The valuable content is made accessible by a 22-page index of professional quality, augmented by a true author index of impressive magnitude.

This is an important work of basic reference, which should be acquired by all libraries serving organic chemists.

Biotechnology. A Textbook of Industrial Microbiology. Second Edition. By Wulf Crueger and Anneliese Crueger (Bayer AG). Sinauer: Sunderland. 1990. 357 pp. $44.95. ISBN 0-87893-131-7.

This book is an English translation of a German textbook by W. Crueger and A. Crueger. Professor T. D. Brock again did a commendable job in the translation. When one goes over the English version, there is no hint or evidence whatsoever that this is a translated version. When this reviewer took Industrial Microbiology under Professor A. L. Demain at MIT, he saved most of the lecture notes and articles given by Dr. Demain. This book literally replaces all these old notes and more. The book is divided into two parts. Chapters 1–6 discuss the traditional practices by the industrial microbiologists and bioengineers. The materials are condensed but they are usually a good start for students and scientists with minimal exposure to this area. The amount of information on fermentation (Chapter 5) and product recovery (Chapter 6) may be useful as background materials for many biologists and chemists. Bioengineers on the other hand, may find them too little and too sterile of any practical use. Again, it is a good start for non-engineering scientists and students unfamiliar with these subject matters. There are sufficient references at the back of each chapter for them to explore further any interesting topics.

Chapters 7–20 are divided based on product classifications. It is striking that there is no separate chapter on recent cell culture derived or recombinant DNA technology derived bioproducts such as TPA, HGH, r-insulin, etc. Only traditional fermentation derived products are emphasized with minimal discussion on the impact of recombinant technology on those products. Waste treatment is treated in Chapter 17 with little discussion on toxic waste remediation.

Overall, the book is well-written and well-organized. The new edition did include some information of recombinant DNA techniques on strain selection and new product development. But, these additions did not seem to distinguish the new edition from the first one. It is well-suited as a reference book for undergraduate level exposure to the field of industrial microbiology. Given the broad areas that the authors try to cover, some sacrifice on substances is inevitable. It is indeed a good book for many lay researchers to start and want to join in with the biotechnology revolution.

Henry Y. Wang, *The University of Michigan*

Spectroscopic Analysis of Coal Liquids: Coal Science and Technology. Volume 12. Edited by John R. Kershaw (CSIRO Division of Materials Science and Technology). Elsevier: Amsterdam and New York. 1989. xiv + 395 pp. $152.75. ISBN 0-444-87307-4.

This volume of the *Coal Science and Technology* series provides a strong overview of many of the different spectroscopic techniques that have been applied to the analysis of coal liquids. The broad range of techniques discussed in this volume include gas chromatography (K. D. Bartle), pyrolysis and hydropyrolysis (W. Steedman), mass spectrometry (B. D. Batts and J. E. Batts), infrared spectroscopy (P. M. Fredricks), ultraviolet and luminescence spectroscopy (J. R. Kershaw), nuclear magnetic resonance (M. I. Attalla, A. M. Vassallo, and M. A. Wilson), and continuous wave and pulsed electron spin resonance spectroscopy (S. Schlick and L. Kevan). Each of the contributors provides an overview of the technique as well as an in-depth discussion of its use in the analysis

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