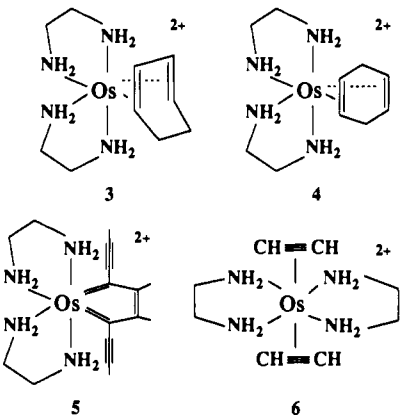
Table I. Selected Data for the Structure of **2** and of Other Alkene–Alkyne Metal Complexes[a]

	M–C(alkyne)	M–C(alkene)	Δ[b]	C≡C	C=C	∠C≡C–R, deg
trans-$[Os(en)_2(CH{\equiv}CH)(CH_2{=}CH_2)]^{2+}$, **2**	2.155(4)	2.175(4)	0.02	1.199(9)	1.352(9)	157.4(1)
cis-W(ma)(PhC≡CH)$(S_2CNEt)_2$[9a]	2.006(8)	2.233(9)	0.24	1.32(1)	1.41(1)	136.6(8)
ma = maleic anhydride	2.01(1)	2.261(8)				
$[CpMo(PPh_2C_6H_4CH{=}CH_2)(MeC{\equiv}CMe)]^{+}$ [9b]	1.996(8)	2.266(7)	0.25	1.293(10)	1.425(11)	142.0(8)
	2.037(7)	2.265(8)				136.7(7)
cis-$W(PhC{\equiv}CPh)(CH_2{=}CH_2)(Cl)_2(PMe_3)_2$[9c]	2.033(5)	2.248(5)	0.22	1.330(10)	1.399(13)	135.7(5)
	2.033(5)	2.248(5)				128.0(5)

[a] The bond distances are in Å. [b] Δ is the average of M–C(alkene) – M–C(alkyne).

to our work only three alkene–alkyne metal compounds had been structurally characterized (see Table I).[9a-c] Comparison of the structure of **2** with the structures of these compounds reveals two striking differences. In contrast to their disposition in **2**, the alkene and alkyne ligands in the Mo or W complexes are cis and lie parallel. Moreover, in **2**, the metal–C(alkene) and –C(alkyne) bond distances are almost identical, but in the other compounds, the metal–C(alkene) bond distances are more than 0.2 Å longer than the metal–C(alkyne) bond distances. It is likely that the alkyne ligand in the W and Mo complexes acts as a $4e^-$ donor, while in **2** the ethyne ligand is a $2e^-$ donor, a change which leads to bond lengthening. Coordination of ethyne to the osmium center causes severe distortion of the molecule. The two C–H bonds bend away from the original linear configuration with a C–C–H angle of 153.4°, and the ligand loses planarity, the dihedral H–C–C–H angle being 60.5°.

When the dihydrogen complex **1** is treated with 1,3-cyclohexadiene or 1,4-cyclohexadiene, trans–cis isomerization occurs and both **3** and **4**, respectively, are isolated and characterized. To find out if a similar trans–cis isomerization occurs in the ethene–ethyne complex **2**, it was heated at 79 °C in D_2O. After 20 min, ^1H NMR spectroscopy showed decomposition of the compound with no indication of the formation of alkene–alkyne coupling products. **1** reacts with a conjugated dialkyne, 2,4-hexadiyne, to give a metallacyclopentatriene complex, **5**, an alkyne–alkyne coupling product analogous to that isolated from



the reaction of **1** with 2-butyne.[4c,d] In contrast to the behavior of the internal alkynes just described, the reaction of **1** with ethyne leads to a *trans*-bis(ethyne) complex, **6**. *trans*-Bis(alkyne) complexes have not been observed as intermediates for the formation of metallacyclopentatrienes. Studies toward an understanding of the factors controlling alkene and alkyne coupling on the osmium center are under way.

Acknowledgment. Financial support from the National Institutes of Health (GM-13638-25) is gratefully acknowledged. We also thank Prof. H. Hope at the University of California—Davis for providing the X-ray facility.

Supplementary Material Available: Listings of analytical and spectroscopic data for compounds **2–6** and tables of atomic coordinates and equivalent isotropic displacement coefficients, bond lengths, bond angles, heavy atom anisotropic displacement coefficients, hydrogen atom coordinates, and isotropic displacement coefficients for **2** (5 pages); table of observed and calculated structure factors for **2** (5 pages). Ordering information is given on any current masthead page.

(8) Selected examples of metal-mediated alkyne–alkene coupling reactions: (a) Negishi, E.; Holmes, S. J.; Tour, J. M.; Miller, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 2568. (b) Negishi, E.; Cerderbaum, F. E.; Takahashi, T. *Tetrahedron Lett.* **1986**, *27*, 2829. (c) Nugent, W. A.; Calabrese, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 6422. (d) Tamao, K.; Kobayashi, K.; Ito, Y. *J. Am. Chem. Soc.* **1988**, *110*, 1286. (e) Vollhardt, K. P. C. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 523. (f) Trost, B. M.; Lautens, M. *J. Am. Chem. Soc.* **1985**, *107*, 1781. (g) Trost, B. M.; Tour, J. M. *J. Am. Chem. Soc.* **1987**, *109*, 5268. (h) Trost, B. M.; Pfrengle, W.; Urabe, H.; Dumas, J. *J. Am. Chem. Soc.* **1992**, *114*, 1923. (i) Klang, J. A.; Collum, D. B. *Organometallics* **1988**, *7*, 1532. (j) Smith, G.; Schrock, R. R.; Churchill, M. R.; Youngs, W. J. *Inorg. Chem.* **1981**, *20*, 387. (k) Lee, G. C.; Tobias, B.; Holmes, J. M.; Harcourt, D. A.; Garst, M. E. *J. Am. Chem. Soc.* **1990**, *112*, 9330. (l) Herrmann, W. A.; Fischer, R. A.; Herdtweck, E. *Organometallics* **1989**, *8*, 2821. (m) Silverberg, L. J.; Heck, R. F. *J. Organomet. Chem.* **1991**, *409*, 411. (n) Yu, J. S.; Fanwick, P. E.; Rothwell, I. P. *J. Am. Chem. Soc.* **1990**, *112*, 8171.

(9) (a) Morrow, J. R.; Tonker, T. L.; Templeton, J. L. *J. Am. Chem. Soc.* **1985**, *107*, 6956. (b) Allen, S. R.; Green, M.; Moran, G.; Orpen, A. G.; Taylor, G. E. *J. Chem. Soc., Dalton Trans.* **1984**, 441. (c) Clark, G. R.; Nielson, A. J.; Rae, A. D.; Rickard, C. E. F. *J. Chem. Soc., Chem. Commun.* **1992**, 1069. (d) Green, M.; Nagle, K. R.; Woolhouse, C. M.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1987**, 1793.

(10) Several unstable nickel(0)–phosphine–ethene–ethyne complexes were made at −78 °C, and they all decompose below 0 °C. Pörschke, K. R. *J. Am. Chem. Soc.* **1989**, *111*, 5691.

(11) Crystal data for **2** (140 K): $C_8H_{22}N_4OsCl_2{\cdot}2H_2O$, monoclinic, $C2/c$, a = 10.549(3) Å, b = 10.885(3) Å, c = 13.261(6) Å, V = 1522.1(9) Å^3, Z = 4, D_{calcd} = 2.057 g cm^{-3}, μ = 8.742 mm^{-1}. A colorless block (0.70 × 0.30 × 0.30 mm) was cut from a needle-shaped crystal and used for data collection (Siemens R3m/V, 0.0° < 2θ < 50.0°, Mo Kα). Of 2865 reflections collected, 1331 were independent (R_{int} = 3.50%) and empirically corrected for absorption (XEMP and XABS programs were used). The atoms were located by Patterson and difference Fourier methods and refined by full-matrix least squares. Hydrogen atoms on ethylenediamines were placed in calculated positions, while hydrogens on ethene and ethyne were refined freely. All hydrogens had fixed isotropic U. R = 2.09%, R_w ($=\sum||F_o| - |F_c||w^{1/2}/\sum|F_o|w^{1/2}$) = 2.33%, all data R = 2.09%, wR = 3.20%, GOF ($=[\sum(w||F_o| - |F_c||^2)/(M-N)]^{1/2}$) = 0.88. All computer programs and sources of scattering factors are contained in SHELXTL PLUS (G. M. Sheldrick, A Program for Crystal Structure Determination, Version 4.0, 1989, Siemens Analytical X-ray Instruments, Madison, WI).

Enhanced Site-Specific Cleavage of the Tetracycline Repressor by Tetracycline Complexed with Iron

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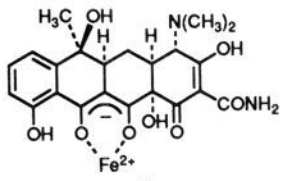
Received December 21, 1992

The last several years have seen a serious increase in the emergence of bacterial infections resistant to previously effective antimicrobial agents.[1] Rational drug design based on a molecular

0002-7863/93/1515-2546$04.00/0

understanding of the interaction of the antibiotic with the protein associated with resistance is essential to combat this development. Resistance to tetracycline antibiotics in gram-negative organisms is based primarily on the active efflux of the drug from the cells.[2] In these organisms the Tet repressor protein[3] controls transcription of the genes conferring resistance and is negatively regulated by tetracycline (Tc), which functions as an inducer in the presence of Mg^{2+}.[4] In this communication we describe the functional replacement of Mg^{2+} by the redox-active Fe^{2+} (**1**) and its use to map the binding site of the drug on the Tet repressor protein.[5]



1

An overproducing strain of Tet repressor resistance determinant class B was grown in a fermenter, and the (46.6 kDa dimeric) Tet repressor was purified to homogeneity by standard chromatographic methods.[6] Its binding activity was measured by Tc fluorescence and determined to be 95% of the total amount of protein.[4] Cleavage of the Tet repressor was initiated by incubating equimolar amounts of repressor monomer with Fe^{2+} and a 2-fold excess of Tc for 3 min in a cleavage buffer (50 mM NaCl, 20 mM Tris, pH 7.0) followed by addition of 10 equiv of Na ascorbate and H_2O_2 to a final volume of 30 μL. After 15 s at room temperature the reaction was quenched by the addition of glycerol (0.5 M) and the same volume of gel loading buffer.[7] The reaction products were monitored by SDS-PAGE[8,9] with silver staining.[10] Examination of the gel revealed that the Tc·Fe^{2+} complex selectively produced two major bands approximating molecular weight markers of about 10 and 9 kDa (Figure 1a) with minor bands of 17–14, 7, and 5 kDa.

N-Terminal sequence determination[11] and ion-spray mass spectrometry (ISMS)[12] showed that the 10- and 9-kDa fragments originated from cleavage of the peptide bonds between Arg-104 and Pro-105 (7% yield)[13] and between Pro-105 and Thr-106 (5%). The 10-kDa band is actually a mixture of two fragments of 103

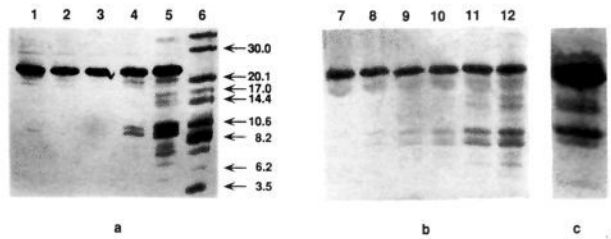


Figure 1. Silver-stained SDS-PAGE gels of Tet repressor cleavage by Tc·Fe^{2+}. (a) Lane 1: denatured Tet repressor dimer (11 μM) incubated with Fe^{2+} (22 μM), Tc (44 μM), and ascorbate/H_2O_2 (0.22 mM). Lanes 2–5: native Tet repressor with ascorbate/H_2O_2 in the absence of Tc·Fe^{2+} (lane 2); without Fe^{2+} (lane 3); without Tc (lane 4); and together with Tc·Fe^{2+} (lane 5). Lane 6: marker proteins and horse heart myoglobin fragments. (b) Competition of Fe^{2+} with Mg^{2+}. Lane 7: native Tet repressor dimer (11 μM) with Mg^{2+} (44 μM), Tc (22 μM), and ascorbate/H_2O_2. Lanes 8–12: same as in lane 7 but in the presence of Fe^{2+} (22 μM) postincubated for 30 s before addition of ascorbate/H_2O_2 (lane 8); for 1 min (lane 9); for 5 min (lane 10); for 15 min (lane 11); and for 1 h (lane 12). (c) Coomassie Blue-stained PVDF membrane.

and 104 amino acids (11 705 and 11 834 Da by ISMS; theoretical: 11 758 and 11 854) with the N-terminal sequence of the Tet repressor.[3] The 9-kDa band accounts for the original C-terminal portion of the protein and consists of two fragments corresponding to 102 and 101 amino acids with new N-termini of Pro-Thr-Glu-Leu-Gln- and Thr-Glu-Leu-Gln-Tyr, which are uniquely located at residues 105–109 and 106–110 of the sequence. Interestingly, the amino acids at the newly formed N-terminal cleavage sites are not modified, consistent with recent results reported by the Meares's group using redox-active Fe^{2+}·EDTA chelates.[5a,b,14]

The N-terminal sequence of the minor bands above and below the main cleavage bands were consistent with fragments originating from the N-terminal portion of the repressor. ISMS measurements indicated masses of 16 667, 16 545, 15 358, and 6140 kDa (theoretical: 16 632, 16 516, 15 360, and 6144) attributable to cuts between Glu-147 and Asp-148, Asp-148 and Glu-149, Ala-136 and Val-137, Ala-56 and Ile-57 for the smallest fragment.

In a control experiment, Tet repressor was incubated with free Fe^{2+} in the absence of drug, showing only two faint bands (1–2% yield)[15] matching those of the major cleavage fragments observed above. No cleavage was observed when both Fe^{2+} and Tc were omitted from the reaction mixture. Control experiments with denatured repressor (Figure 1a, lane 1; 1% SDS, 4 mM DTT, boiling water for 3 min) and Tc·Fe^{2+} showed very faint cleavage, demonstrating the specificity of the observed chain scission and its dependence on the tertiary structure of the protein. In addition, no cleavage of the Tet repressor was observed by the non-redox-active Tc·Mg^{2+} complex (Figure 1b).[16] However, incubation of this mixture for at least 1 h in the presence of an equivalent amount of Fe^{2+} gave a cleavage yield equivalent to that of the reaction without Mg^{2+}.[17] These results strongly suggest an identical binding site for both cationic complexes. The fact that site-specific cleavage occurs to a small extent without Tc (Figure

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(4) Takahashi, M.; Altschmied, L.; Hillen, W. *J. Mol. Biol.* **1986**, *187*, 341–348.

(5) For recent examples of this technique, see: (a) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1991**, *113*, 1859–1861. (b) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1990**, *112*, 2457–2458. (c) Schepartz, A.; Cuenoud, B. *J. Am. Chem. Soc.* **1990**, *112*, 3247–3249. (d) Hoyer, D.; Cho, H.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 3249–3250.

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(7) Gel loading buffer: 2.5% SDS, 32 mM DTT, 80 μM EDTA, 0.01% Bromophenol Blue, and 0.4 M Tris, pH 8.0. The samples were denatured in boiling water for 5 min, and 2-iodoacetamide was added at a final concentration of 2%.

(8) The SDS-PAGE (Pharmacia PhastGel high density: 20% acrylamide, 2% bis(acrylamide), and 30% ethylene glycol) was run and stained on a Pharmacia PhastSystem.

(9) Schägger, H. von Jagow, G. *Anal. Biochem.* **1987**, *166*, 368–379.

(10) Heukeshoven, J.; Dernick, R. *Electrophoresis* **1985**, *6*, 103–112.

(11) Cleaved fragments were blotted onto a ProBlott PVDF membrane by Pharmacia PhastTransfer and Coomassie Blue-stained. N-Terminal analyses were carried out with a Pulsed liquid Applied Biosystems 477A sequencer.

(12) This work was done on a Sciex API-III triple quadrupole mass spectrometer at the University of Tübingen, Institute of Organic Chemistry, FRG. The details of this work will be published separately.

(13) These yields are based on the amount of transferred cleavage products available for N-terminal sequence analysis. The comparatively low yield of cleavage (12–15%) of Tet repressor is possibly due to oxidative degradation of the drug. As a control, Tc was incubated with Fe^{2+} and ascorbate/H_2O_2 in the cleavage buffer without any protein. After 1 min the absorbance at 365 nm decreased to about half when monitored by HPLC diode array detection.

(14) Rana, T. M.; Meares, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10578–10582. Rush, J. D.; Koppenol, W. H. *J. Am. Chem. Soc.* **1988**, *110*, 4957–4963. Cleavage in the presence of radical scavengers such as glycerol *tert*-butyl alcohol, mannitol, or thiourea had no observable effect on cleavage. This is consistent with a peroxide-activated Tc·Fe^{2+} complex which produces a highly nucleophilic oxygen species in the binding pocket of Tet repressor, probably similar to that proposed by Meares and co-workers for other iron chelate-mediated proteolysis studies.

(15) These numbers are based on N-terminal sequencing of a Coomassie Blue-stained blot. In addition, a reverse-phase HPLC analysis of both cleavage reactions (chelated Fe^{2+} and free metal-ion) clearly showed an approximate 10-fold increase of the cleavage yield when the reaction was run in the presence of Tc as determined by integration of the separated peaks (monitored at 214 nm).

(16) The association constant of Fe^{2+} with Tc ($2 \cdot 10^5$ M^{-1}) (see: Martin, R. B. *Metal Ions in Biological Systems*; Marcel Dekker, Inc.: New York and Basel, 1985; Vol. 19, Chapter 2) is about 80 times greater than that for Tc·Mg^{2+} (2.7×10^3 M^{-1}).[4]

1a, lane 4 compared to lane 5) suggests that Tc recognizes a metal binding site and that the binding of the metal to the protein is considerably weaker than that of the chelated complex. Alternatively, the difference may relate to an induced and noninduced conformation of the repressor protein regulated by the allosteric effector Tc. Thus in the drug-bound conformation, the redox active Fe^{2+} may be more localized and in closer proximity to the peptide backbone of the binding pocket.

In conclusion, these experiments show for the first time that the redox-active Tc·Fe^{2+} complex binds to a specific site on the Tet repressor protein and can be used to define contact points between Tc and the repressor by site-specific proteolysis. It is noteworthy that the most prominent cleavage site between Arg-104 and Pro-105 is very near His-100, consistent with a pH-dependent binding study which suggested that a proton on an imidazole and Mg^{2+} compete for the same binding site on Tc.[4] The cleavage site between Glu-147 and Asp-148 points to possible binding between the carboxylate of either one of these amino acids and the Tc metal chelate. Most likely these contacts represent coordination sites to the chelated metal in which Tc serves as a template. Together with residues Ala-56 and Ile-57, they probably form the protein surface in contact with the Fe^{2+}. X-ray studies now in progress will obviously provide more insight into the binding of Tc with its repressor.

(17) The Mg^{2+}/Fe^{2+} competition experiments are consistent with a k_{diss} of 1.2×10^{-2} min^{-1} or a $t_{1/2}$ of 1 h for the dissociation of Tet repressor Tc·Mg^{2+} complex measured by Tc fluorescence.[4]

Acknowledgment. We thank Jeff Hulmes and Sjaak Reumkens for N-terminal sequencing and useful comments, Jörg W. Metzger for performing the ISMS analyses, and Winfried Hinrichs, Michael Niederweis, and Wei-Dong Ding for helpful discussions. This work was supported by a grant from the Wilhelm Sander Stiftung.

Additions and Corrections

Amide Cuprate Reagents as a New Class of Nitrogen Nucleophiles. Application to Asymmetric Synthesis of β-Lactams [*J. Am. Chem. Soc.* **1992**, *114*, 5427–5429]. YOSHINORI YAMAMOTO,* NAOKI ASAO, and TADAO UYEHARA

Page 5428: The *R* configuration is produced in the R^3R^4NM reaction whereas the *S* configuration is obtained in the high pressure induced reaction of R^3R^4NH:[9] This sentence should read as follows: It is noteworthy that the sense of chiral induction in the R^3R^4NM addition to the 8-phenylmenthyl derivatives (**1f**) is opposite to that in the high pressure induced reaction of R^3R^4NH.[9] We thank Professor J. d'Angelo for calling this error to our attention.

Page 5428, ref 9: (*S*)-β-amino ester should read (*R*)-β-amino ester.

Carbon Dioxide Complexes via Aerobic Oxidation of Transition Metal Carbonyls [*J. Am. Chem. Soc.* **1992**, *114*, 6579–6580]. PENG-FEI FU, MASOOD A. KHAN, and KENNETH M. NICHOLAS*

Due to a simple calculational error the 2:1 stoichiometry reported for the reaction of $Cp'_2Nb(CO)CH_2Ph$ with O_2 (eq 1 and Figure 2, p 6580) is incorrect. The corrected concentration of **1a** is 0.18 mmol. From the corrected concentration of **1a** and several independent repetitions of the experiment a consistent stoichiometry of 1.0:1.0 ± 0.1 is obtained. We are currently seeking to identify the fate of the extra O atom.

Rapid Photopolymerization of Immunoprotective Gels in Contact with Cells and Tissue [*J. Am. Chem. Soc.* **1992**, *114*, 8311]. CHANDRASHEKHAR P. PATHAK, AMARPREET S. SAWHNEY, and JEFFREY A. HUBBELL*

In this recently published paper on the photopolymerization of water-soluble macromers for the encapsulation of tissues, we neglected to provide references on the eosin/triethanolamine photoinitiation system we used.[1-3] We regret this oversight.

(1) Chesneau, E.; Fouassier, J. P. *Angew. Makromol. Chem.* **1985**, *135*, 41.
(2) Neckers, D. C.; Raghuveer, K. S.; Valdes-Aguilera, O. *Polym. Mater. Sci. Eng.* **1989**, *60*, 15.
(3) Valdes-Aguliera, O.; Pathak, C. P.; Shi, J.; Watson, D.; Neckers, D. C. *Macromolecules* **1992**, *25*, 541.

Book Reviews*

Chemistry of Atmospheres. Second Edition. By Richard P. Wayne (University of Oxford). Clarendon Press: Oxford, England. 1991. xiii + 477 pp. $89.00 hardcover. $35.95 paperback. ISBN 0-19-655571-7.

This book provides an excellent introduction to atmospheric chemistry. The author presents a comprehensive overview of the major issues and provides the background in chemistry, physics, and biology needed to understand these issues. This second edition includes some of the important new developments of the last decade, such as the appearance of the Antarctic ozone hole and the subsequent realization that heterogeneous processes play a critical role in the chemical balance of the atmosphere. Flyby missions to the outer planets in the solar system and preliminary results are included in this book.

The first chapter discusses the composition of the atmospheres of Earth and the other planets, highlighting the special nature of Earth's atmosphere and its ability to support life. Chapters 2 and 3 provide an introduction to the topics in atmospheric physics, meteorology, and chemistry that are needed to understand atmospheric processes. Chapters 4–7 deal with different aspects of the Earth's atmosphere, starting with the ozone layer and the stratosphere in Chapter 4. Chapter 5 discusses tropospheric chemistry and air pollution, ion chemistry in the region above 60 km is covered in Chapter 6, and Chapter 7 discusses airglow due to electronically and vibrationally excited species in the atmosphere. The final two chapters of the book deal with the atmosphere of other planets and the evolution of atmospheres.

This book is highly recommended as either an introductory textbook or a general reference for the interested scientist. Anyone with an undergraduate level background in physical chemistry should find this book highly readable and a valuable resource.

Leah R. Williams, *SRI International*

Xenobiotics and Food-Producing Animals. Metabolism and Residues. Edited by D. H. Hutson (Shell Research Limited), D. R. Hawkins (Huntingdon Research Centre), G. D. Paulson (U.S. Department of Agriculture), and C. B. Struble (Hazleton Laboratories). American Chemical Society: Washington, DC. 1992. xii + 256 pp. $58.95. ISBN 0-8412-2472-2.

This book was developed from a symposium sponsored by the Division of Agrochemicals of the ACS and the International Society for the Study

*Unsigned book reviews are by the Book Review Editor.