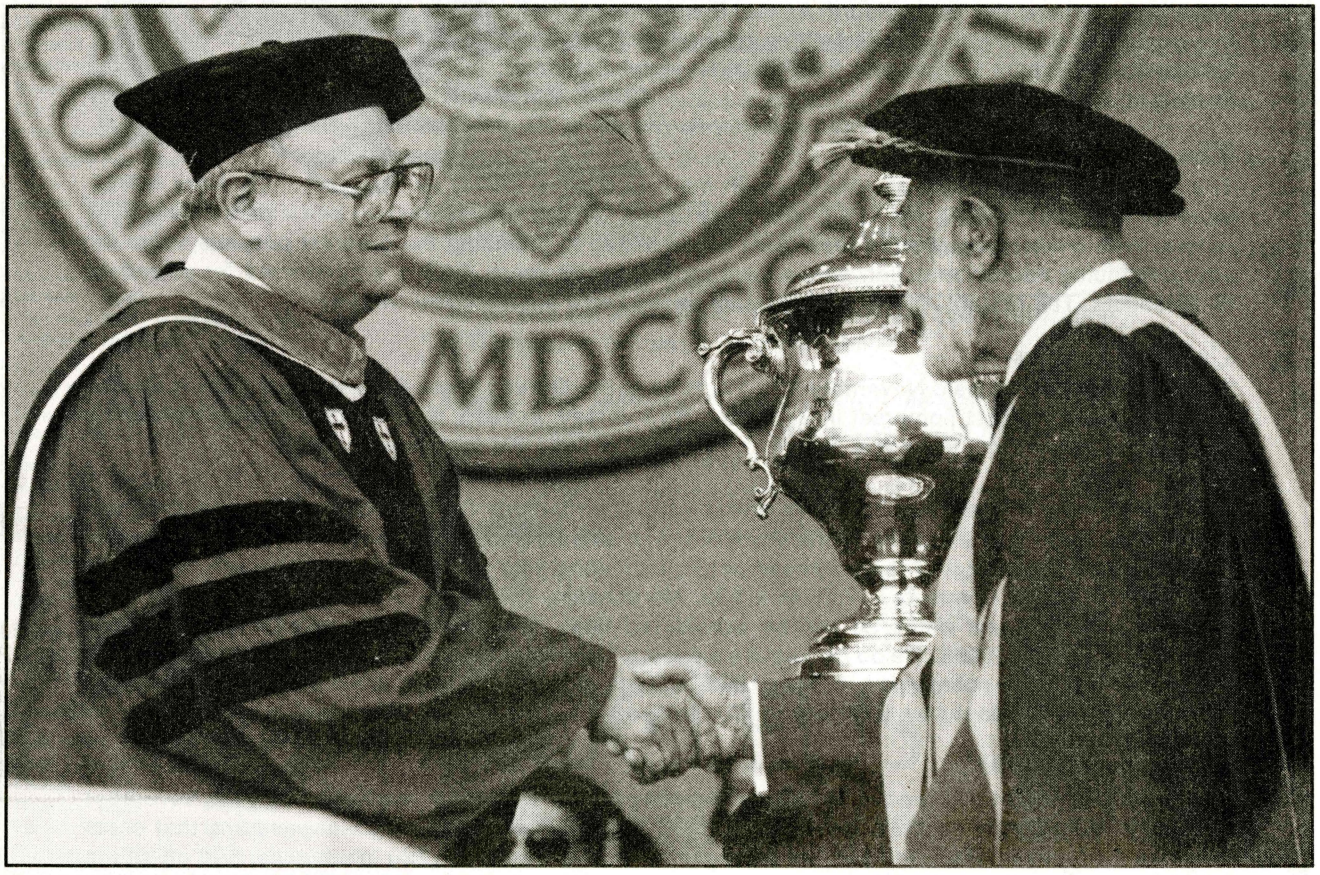
THE NUCLEUS

February 1995 *Of the Northeastern Section of the American Chemical Society* *Vol. LXXIII, No. 6*



Monthly Meeting

M.Z. Hoffman on excited Ruthenium II diimine complexes

Summer Scholar's Report

Glutathione-S-Transferase from Oysters – Purification, Characterization

Meeting Report

Prof. Jacobsen at the December meeting: Catalysis for Asymmetric Synthesis

NESACS Amendments

Proposal to increase the Board of Directors by adding six at-large directors

The Northeastern Section of the American Chemical Society, Inc.

Office: Marilou Cashman, 23 Cottage St., Natick, MA 01760. 1-800-872-2054
(Voice or FAX) or (508) 653-6329.
Any Section business may be conducted via the business office above.
Washington, D.C. ACS Hotline: 1-800-227-5558

Contents

Cover: *Prof. Morton Z. Hoffman receives the Metcalf Cup award for excellence in teaching from Dr. Metcalf, Chairman, Boston University Board of Trustees (May 1994). (Photo by Boston University Photo Services*

Deadlines: *April 1995 issue: February 22, 1995*

THE NUCLEUS

Dedicated to the Memory of James Flack Norris
Published monthly from September to May by the Northeastern Section of the American Chemical Society, Inc.

The Nucleus is distributed to the members of the Northeastern Section of the American Chemical Society, to the secretaries of the Local Sections, and to editors of all local publications. Forms close for advertising on the 1st of the month of the preceding issue. Text must be received by the editor six weeks before the date of issue.

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Nominations

1995 James Flack Norris Award for Outstanding Achievement in the Teaching of Chemistry

Nominations are being received for the 1995 James Flack Norris Award for Outstanding Achievement in the Teaching of Chemistry. The Norris Award, one of the oldest awards given by a section of the American Chemical Society, is presented annually by the Northeastern Section. It consists of a certificate and an honorarium of $3000. Nominees must have served with special distinction as teachers of chemistry at any level: secondary school; college level or graduate school. This must be attested by broad evidence of their students' subsequent careers in chemistry and/or other evidence of wide-ranging effects on the teaching of chemistry. Since 1951 past winners have included eminent as well as less widely known, but equally effective teachers at all levels. The awardee for 1994 was Dr. Samuel P. Massie, Prof. emer. of the U.S. Naval Academy, Annapolis, Md.

Nominations for 1995 will be received until April 15, 1995. Nominating material must be limited to 30 pages and focus specifically on the nominee's contribution to and effectiveness in teaching chemistry. This should include a thorough *curriculum vitae* with listings of honors, awards and such publications as relate to education. There must be a nominating letter and as many seconding letters as are necessary to convey how the nominee's teaching inspired students to spend their professional life in chemistry, with broad student testimonial. They will also attest to the influence of other activities in chemical education such as textbooks, articles or other activities at national level. Materials should be standard 8½ x 11 size for binding but should not include reprints or books.

Nominating material should be sent before April 15th to Cynthia B. McGowan, 5 Heritage Dr., Bedford, MA 017330-0480. ◇

Henry A. Hill Award

Nominations for the Henry A. Hill Award for Outstanding Service to the Northeastern Section should be sent to the Administrative Secretary, NESACS, 23 Cottage St., Natick, MA 01760 by June 1. A resume of professional activities and description of the candidate's contributions to the Northeastern Section should be included. ◇

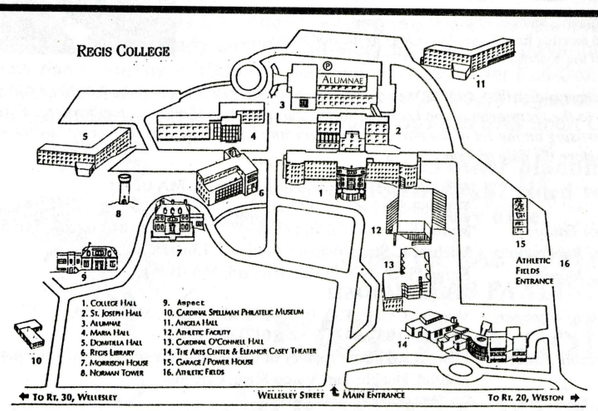
An additional nominating notice is on page 13.

Directions to Regis College

From the North: Take Rte. 128 to Exit 26. Take Rte. 20 West (right turn where Rte. 20 West and East divide). After 1¼ Mi take School St. south. It becomes Wellesley St. After 1¾ Mi, the Regis College entrance is on your right. Follow signs for parking and the Student Lounge in Alumnae Hall.

From the South: Take Rte. 128 north to Exit 24 ("I 90 and Rte. 30"). Stay straight where the access to Rte. I 90 diverges right. Take the following right branch to Rte. 30, then left turn into Rte. 30 West. (*) After 2 Mi. take Wellesley St. north, 1/2 Mi. to the Regis College entrance. Follow signs for parking and the student lounge.

From Boston, Newton: Take the Mass Turnpike Extension to the Rte. 128/ Rte. 30 Exit. Follow the Cloverleaf toward Rte. 128 North, but stay right to take the exit to Rte. 30, then Left turn to 30 West. Follow directions above for approach from the south following the (*). ◇



Monthly Meeting

The 768th Meeting of the Northeastern Section of the American Chemical Society

Thursday, February 9, 1995
Regis College, 235 Wellesley St., Weston, MA
Upper Student Lounge, Alumnae Hall

5:30 Social Hour

6:30 Dinner

8:00 Evening Meeting, Valerie Wilcox, Chairman, presiding
Prof. Morton Z. Hoffman, Boston University: *The World as Seen by the Excited States of Ruthenium(II)-Diimine Complexes*

Refreshments will be served after the program.

Dinner reservations should be made no later than noon, February 2. Please call or fax Marilou Cashman at (800) 872-2054. Reservations not cancelled at least 24 hours in advance must be paid. Members, $21.00; Non-members, $23.00; Retirees, $12.50; Students, $8.00. THE PUBLIC IS INVITED. Anyone who needs special services or transportation, please call Marilou Cashman a few days in advance so that suitable arrangements can be made.

Parking on Campus: Follow signs. See directions on p. 4.

Next meeting: March 9, 1995, U. Mass.-Lowell. Dr. Peter Setlow, Biochemistry Dept., U. of Connecticut, Health Division, Farmington, CT to speak on SASTs: Proteins that protect DNA by altering its conformation.

Biography

Morton Z. Hoffman is a native of New York City, where he attended the Bronx High School of Science. He received an A.B. degree from CUNY-Hunter College, an M.S. and in 1960 a Ph.D. in physical chemistry from the University of Michigan. Professor Richard B. Bernstein was his Ph.D. mentor. He spent a year as a postdoctoral research associate with Prof. George Porter at Sheffield University, England, where he applied flash photolysis to the study of nonradiative decay of aromatic triplet states. Since 1961 he has been on the faculty of Boston University where he is Professor of Chemistry. Currently he is Director of the Center for Teaching Excellence in the College of Liberal Arts. He is an Alternate Councilor of NESACS and Chairman of the Chemistry Education Committee, a member of the ACS SOCED Task Force on Undergraduate Programming at National ACS Meetings, and a member of the ACS International Activities Committee, and the Program Committee of the Division of Chemical Education. His research interests include the photochemistry and photophysics of transition metal coordination complexes, photochemical storage and conversion of solar energy, the photochemistry of environmental systems, and the application of fast kinetic techniques. He has held a Senior Postdoctoral Research Associateship from the U.S. National Academy of Sciences, and is a Fellow of the AAAS. He was awarded the 1994 Metcalf Cup and Prize for Excellence in Teaching from Boston University. ◇

Abstract

The metal-to-ligand charge transfer states of Ru(II)-diimine complexes, which possess neutral ligands, are cationic and polar. On the other hand, the aromatic ligands are generally hydrophobic and can possess peripheral heterocyclic nitrogen bases. As a result, the ground and excited states display a wide and varying range of interactions with electrolytes, acids, neat and mixed aqueous and non-aqueous solvents, and solutes that can engage in hydrogen-bonding, π-stacking, or intercalation. Ru(II) complexes show marked solution, medium and temperature dependencies of their NMR spectra, emission spectra, excited-state lifetimes, electron transfer quenching rate constants, and cage escape yields of redox products; they serve as effective luminescent reporters of the surrounding environment. Recent results will be presented for compounds with 2,2'-bipyridine, 2,2'-bipyrimidine, and 2,2'-bipyrazine ligands. ◇

Board of Directors

NOTE: Board meetings are held on the meeting day at 4:30 p.m. Section members are invited to attend.

Reader surveys have indicated that members do not wish extensive Board of Director meeting reports. We shall comply with the readers' wishes by only giving essential board items and reporting actions taken. Consequently we are changing the heading to "Notes". We invite your comments.

Notes, Meeting of November 10, 1994

Committee Reports:
Program: The Esselen Award meeting will be at Harvard on April 13, 1995

Constitution and Bylaws: Dr. Hopkins presented amendments of Article VI and VIII of the Constitution prepared at the request of members who felt that the Board of Directors should be broadened to include some Section members who are not officers, Councilors/Alternate Councilors or standing committee chairmen. (The text is elsewhere in this issue). The board VOTED approval of this amendment.

Education: Dr. Hoffman MOVED that an application for a grant-in-aid for an undergraduate student to attend the Anaheim, CA ACS meeting in April

continued on page 13

Amendments to the Constitution of the Northeastern Section

Additions underlined, deletions in brackets [ ].
Rationale: *Proponents of this amendment seek to increase participation in the governance of the Section by adding to the Board of Directors six elected members who are not officers, Councilors, Alternate Councilors or chairmen of committees.*

Article VI - Board of Directors

Section 1. The Board of Directors shall consist of the officers of the Northeastern Section, the immediate past Chairman, the members of the Board of Trustees, the Chairmen of the Standing Committees, the Editor of the Official Publication of the Northeastern Section *ex officio*, the Archivist, the Councilors and Alternate Councilors who have been elected by the Northeastern Section to represent the Section in the Council of the SOCIETY *ex officiis*, [and] any other Councilors of the SOCIETY residing in the territory of the Northeastern Section *ex officiis* and six directors-at-large elected from among the members of the Section.

Section 4. The meetings of the Board of Directors shall ordinarily be held monthly except during June July and August and may be held at the call of the Presiding Officer or of three Directors. Notice of meetings shall be sent by the Secretary to each member of the Board at least one week previous to the date of said meeting, and the principal items of business to be presented shall be stated in the notice. [Twelve] Fifteen members shall constitute a quorum.

Article VIII - Elections

Section 1. *change subsection (i) to (j) and insert a new subsection (i):*

(i) Two directors-at-large shall be elected every year for a term of three years.

Section 4. Provisions shall be made for dissemination of biographical data supplied by the nominees, and, in the case of nominees for officers, directors-at-large, Trustees, Councilors and Alternate Councilors, also for position statements provided by the nominees. ...

The above amendments have been approved by the Board of Directors at its meeting of November 10, 1994. In accordance with the provisions for amendments, Article XX, they can be voted by the members at the March meeting, or at any subsequent meeting of the Section. Amendments take effect after approval by the ACS Committee on Constitution and Bylaws. ◇

NESACS

Committee Chairmen for 1995

Archivist Myron Simon
Awards: Phyllis Brauner
Bd. of Publications: Joe Lima
Budget: *James Piper
Chemistry Education: Morton Hoffman
Constit. & Bylaws: Esther Hopkins
Continuing Education: Alfred Viola
Environmental Improvement, Public Affairs & Service: James Golen
***Esselen Award:** Arthur Obermayer
Hospitality: David Howell
Long Range Planning: Valerie Wilcox
Membership: Iclal Hartman
Natl. Chemistry Week: Albertha Paul
***Nominating Committee:** *James Kaufman
***Norris Award:** Cynthia McGowan
Professional Relations: Leon Rubin
Program: *Patricia Samuel
Project SEED: Dorothy Phillips
***Richards Committee:** Cynthia Friend
Safety: Frank Wagner
Speakers Bureau: Michael Dube

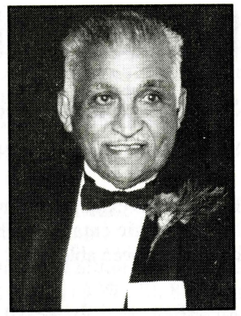
* Elected committees or *ex officio* chairmen ◇

From the November Meeting

Cynthia McGowan (1995 Chairman of the Norris Award Committee), Norris Awardee Samuel Massie, Gloria Massie, Elmer Jones (1994 Chairman of the Norris Award Committee)

Dr. Massie delivering his award address, November 10, 1994: "The Arkansas Traveler"

(Photos by A. Fingland)



B.U. Student Affiliate Chapter Cited

The A.C.S. has announced that the Student Affiliates Chapter at Boston University *(Chemia)* has been cited as "Outstanding" for its activities in 1993-94 by the Society Committee on Education. *Chemia* is one of 23 chapters at colleges and universities in the U.S. and Puerto Rico, and the only one within the Northeastern Section, to have been so designated. Professors Morton Z. Hoffman and Patricia L. Samuel served as faculty advisors, and Matthew Russell, currently a student at the University of Massachusetts Medical School, was president. This is the third year in a row that Chemia has received an "Outstanding" award; it was honored as "Commendable" for 1990-91.

Evaluations are based on the information provided by the chapters in their annual reports. There are 890 chartered student affiliates chapters, of which 325 submitted reports. The following NESACS chapters (faculty advisor) in addition to Chemia, submitted reports for 1993-4: Framingham State College (K. Whitburn), Merrimack College (M. Singh), Simmons College (I. Hartman), Stonehill College (L. Liotta), Suffolk University (D. Lewis), U. of Massachusetts-Lowell (R. Tanner), University of New Hampshire (R. Planalp), Wellesley College (M. Hearn).

There were also 47 "Commendable" and 31 "Honorable Mention" awards. ◇

Historical Notes

by Edward R. Atkinson, Amherst, Mass.

Conclusion of obituary notices of members started in the December 1994 issue and continued in the January 1995 issue.

Bernard Kopelman, 77, died on August 6, 1994. A Boston native, he attended the Boston Latin School and then received the B.S. and Ph.D. degrees from Clark University. During World War II he was employed on the Manhattan Project and was a member of the U.S. delegation to the Atomic Energy Commission conference in Paris. He became chief engineer of the atomic energy division of Sylvania Electric Products, then was director of market development for the Sylvania–Corning Nuclear Corporation. He retired in 1982 as vice president for research of the lighting division of the GTE Corporation.

Mr. Kopelman was the inventor of the Magic Cube flash device for cameras, author of "Materials for Nuclear Reactors" (1954), and the author of 15 patents. He served as a trustee of the Hammond Castle Museum in Gloucester.

Paul R. Shafer, 70, emeritus professor of chemistry at Dartmouth College, died on April 6, 1994. He was an Ohio native who earned the B.S. at Oberlin College in 1947 and the Ph.D. at the University of Wisconsin in 1951. Prior to his college work he served as a B-26 pilot with the U.S. Army Air Force and spent a few months in a German prison camp after being shot down over France. During his graduate study years he was a Captain in the Wisconsin Air National Guard. Shafer joined the Dartmouth faculty in 1952 and retired in 1988. He served as Chairman of the Division of Sciences (1961-1964), Chairman of the Chemistry Department (1967-1969), and Associate Dean of the college (1969-1973). He was a NSF Fellow at Caltech in 1959 and established a NMR laboratory at Dartmouth in the 1960's. He taught outdoor skills to generations of Dartmouth students and led a 25,000 mile 4-vehicle safari on a circumnavigation of the African continent in 1973.

John M. Smith, 64, died on January 19, 1994 at his home in Quincy, Mass. He was a native Scot who obtained the M.S. degree in chemistry from Strathclyde University. After serving in the British army (1952-1954) defusing bombs still remaining in Germany, he joined the explosives division of Imperial Chemical Industries. In 1959 he was recruited by the Ensign-Bickford Industries, Simsbury, Conn. and devel-

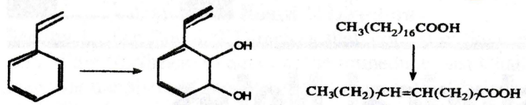
continued on page 9

Meeting Report

Synthetic and Biological Catalysts for Asymmetric Synthesis

From the address by Eric N. Jacobsen at the December 15, 1994 NESACS-Medicinal Chemistry Group Meeting

Biology has examples of awe-inspiring syntheses produced by enzymatic catalysis which synthetic organic chemists have never been able to do. E.g.:

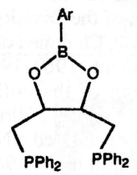


Enzymatic catalysis involves precoordination:

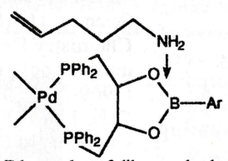
$$E + S \rightleftharpoons E \cdot S \rightleftharpoons E \cdot P \rightleftharpoons E + P$$

Characteristics of enzyme catalysis are 100% yields, 100% selectivity, under a narrow range of, usually physiological, conditions. Synthesis requires broad ranges of substrates, mild, usually tunable conditions, low molecular weight catalysts, high turnover numbers, i.e. minimal product inhibition.

Some effective biomimetic catalysts which involve substrate precoordination have been developed, notably by Sharpless (epoxidation of allylic alcohols). In a relatively newer strategy Hayashi has devised difunctional ligands for asymmetric synthesis, one function to bind the substrate, the second function to carry out the transformation. Jacobsen has also developed bis chelating ligands such as "dibop"

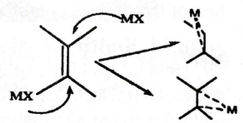


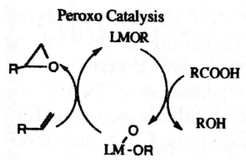
Dibop

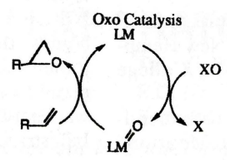


Pd complex of dibop and substrate

Bimolecular reactions without precoordination:

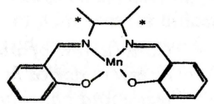


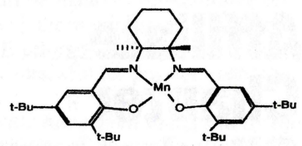




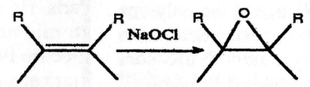
Manganese Salen Catalysts

Cytochrome P-450 is a heme protein, protoporphyrin IX, with an iron atom in the center. Its mechanism for epoxidation may be symbolized by the oxo diagram. Various chemists have synthesized synthetic porphyrins, lacking a protein molecule, notably Coleman, 1993. Jacobsen took a different approach, making catalysts in which the active site, the metal atom, is close to the stereogenic centers.



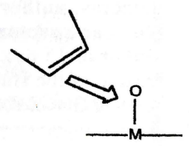


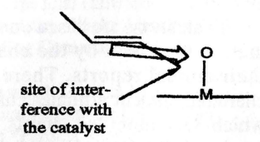
The asterisks show the stereogenic centers. The second structure has been scaled up at Sepracor for large scale manufacturing use. The manganese atom is uniquely useful as the metal atom in these catalysts. These "manganese salen" catalysts permit epoxide formation from cis-disubstituted and some trisubstituted olefins with high selectivity. E.e.'s are above 84% and such purity allows crystallization to obtain 100% e.e, often in a single step.



pH=10-11.5. organic solvent, pyridinium oxide catalyst "promoter"

This is a biomimetic catalyst that functions on simple olefins. Trans olefins are less reactive, which can be explained by the sidewise approach of the olefin to the oxygen atom.

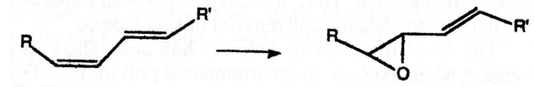




The ligand system is rigid above the ethylenediamine bridge, but the larger portions of the catalyst, further away from the stereogenic centers, are flexible and can limit approach to the small rigid center. The dissymmetry of the ligand area in the bridge is subtle, but only 2-3 kcal/mol are necessary to effect high enantioselectivity. In contrast to this catalytic system, the porphyrin catalysts are rigidly locked at the pyrrole groups, but flexible on the external groups where the asymmetric groups are.

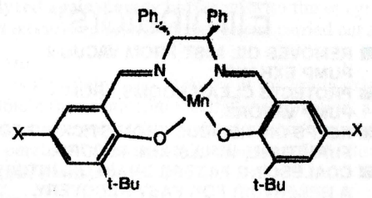
Cis olefins give both cis and trans epoxides, showing that the two bonds are formed stepwise. Jacobsen obtained evidence that the first step forms a short-lived radical (10^{-8} sec.) which closes to either epoxide. Evidence includes secondary isotope effects and the finding that styrene is a good substrate, while vinyl cyclohexane is not. This is explicable by reasoning that the first step is endothermic, leading to the radical whose structure now resembles product, a two hump energy curve with the first peak the higher.

Formation of trans epoxides has valuable consequences. A 1993 publication reports that modification of the epoxidation method has provided a highly enantioselective route to trans epoxides from cis olefins.



This method employs the standard conditions in conjunction with a hydrophobic ("greasy") phase transfer catalyst (PTC), e.g. $C_6H_5CH(OH)CH(CH_3)N^+(CH_3)C_{12}H_{25}$. Selectivity in the generation of trans epoxides from cis olefins was increased above 92% using NaOCl, chlorobenzene, the Mn salen catalyst and the PTC to epoxidize stilbene. Treatment of the epoxide with ammonia gave very valuable ethanolamines in two steps from an inexpensive starting material.

Electronic effects on the catalyst were unexpected but substantial.



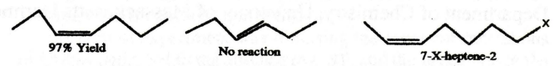
In the structure shown, when X=OMe, 96% e.e. was obtained, while $X=NO_2$ gave e.e. of only 22%. Other substituents acted similarly, and a straight-line Hammett plot could be drawn.

Biological Catalyst

Chloroperoxidase (CPO) isolated from *caldariomyses fumago* (Hager, 1966, 1989) with hydrogen peroxide and chloride ion converts $Me_2C = CMe_2$ to $Me_2C(Cl)C(OH)Me_2$ in an unselective manner. In the absence of chloride ion the system is known to epoxidize cyclohexene. The advantage to such epoxidizing reagent is that only water is a by-product; there is no need to regenerate a reductant, e.g. NADH.

Jacobsen discovered that CPO is a highly enantioselective epoxidation catalyst. Because hydrogen peroxide inactivates the CPO, it is necessary to add the hydrogen peroxide at a slow rate. By so doing, yields can be raised from 5% to 70%, and using acetone as cosolvent allows 100% conversion and 97% e.e. The enzyme survives 100,000 turnovers.

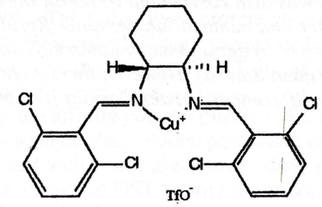
Cis olefins undergo this reaction, while trans olefins do not:



Electronic effects play a part in this reaction, for in the 2-heptene shown, when X=OMe, yield is 90%; Cl, 87%; Br, 85%; N_3, 85%; OH, 0%; NH_2, 0%; NHR, 0%. Shortening the chain between X and the double bond still shows effects: 5-bromo-pentene-2, 76%; 4-bromo-butene-2, 67%.

Alkene Aziridination

There are no enzyme models for aziridination of alkenes. Using a copper salen catalyst with chlorine or methyl groups ortho to the aldimino group gave good aziridination of both cis and trans disubstituted olefins.



Reported by M.S. Simon ◇

Historical Notes

continued from page 7

oped methods for the manufacture, fabrication, and activation of explosive devices and detonating fuses. He became Senior Vice President of Ensign-Bickford and then President of a subsidiary, the Darworth Company. Among his 8 patents were procedures for the fabrication of a safe detonating fuse that could be activated on site for reliable explosive propagation.

Louis E. Stahl, 80, died on August 29, 1994. After early life in Boston and Peabody he obtained the S.B. in Chemistry at M.I.T. in 1936. He joined his father's company, Stahl Finish, which manufactured dyes for the leather industry and helped the company expand from a kitchen stove-top enterprise to a company with an international trade. During this period Mr. Stahl also founded Polyvinyl Chemicals, a firm manufacturing various vinyl plastic goods for international trade.

Mr. Stahl sold both companies to Beatrice Foods Chemical Division. He helped the company to diversify by acquisition of companies in the U.S. and abroad. After retirement in 1980 he became President of the Stahl Investment Corp. and was active in support of Jewish charities and his *alma mater,* M.I.T. ◇

Summer Scholar's Report

Purification and Characterization of Glutathione-S-Transferase from Oyster

by Jie Ge[1] and Bal Ram Singh
Department of Chemistry, University of Massachusetts Dartmout

Abstract

A novel glutathione S-transferase (GST) was purified from oyster by ammonium sulfate precipitation and affinity chromatography. The purified enzyme had a specific activity of 3.77 μmol· min^{-1}· mg^{-1} protein with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Kinetic analysis revealed the K_m and V_{max} values of the purified GST to be 0.75 mM and 0.016 mM· min^{-1}, respectively. The inhibition pattern of oyster GST by tetrapyrroles was investigated both in crude extract and in purified forms. Inhibition of rat liver GST by tetrapyrroles was also carried out to reveal the differences between oyster and mammalian enzymes. Results indicate similar pattern of oyster and mammalian GST inhibition by tetrapyrroles quantitatively. However, there were significant quantitative differences between oyster and mammalian GST's.

Introduction

Glutathione S-transferases (GSTs, EC 2.5.1.18), also called ligandins, are a family of multifunctional enzymes found in almost all living organisms examined. GST catalyzes the conjugation of glutathione (GSH, γ-glutamylcysteinylglycine) to electrophilic compounds (e.g. benzene epoxide, aflatoxin B_1-8,9-oxide, and benzopyrene-7,8-dihydrodiol-9,10-oxide)[2] so that they become more soluble and more easily excreted. Thus, this enzyme plays an important role in the detoxification and removal of xenobiotics.

The level of GSTs in animals has been shown to increase when exposed to environmental pollution[3–5]. For example, GST activity was elevated in mussels and crabs from polluted sites[3,4]. It is also reported that GST synthesis in primary cultured rat liver cells was induced upon exposure to the potent carcinogens, polychlorinated biphenyl (PCB) congeners[6], which are known to be the major environmental problem in New Bedford harbor and its surrounding area.

As a first step to use glutathione S-transferase for monitoring local marine animal health and environment[7–9], we studied the enzyme activity, kinetics, and inhibition characteristics of glutathione S-transferase from oyster, a typical marine animal from the local area.

Materials and Methods

Crude Extract Preparation

Freshly shucked oysters were purchased from Fairhaven Chowder House, Fairhaven, MA. Crude extract of oyster was prepared according to Singh and Shaw[10]. The oysters were combined with a grinding buffer (100 mM sodium phosphate, 300 mM sucrose, 1mM EDTA, pH 6.5) and phenylmethylsulfonate (PMSF), a protease inhibitor, and were blended in a cold (4°C) Waring blender at low speed for 30 seconds. The resulting mixture was centrifuged at 10,000 rpm for 20 min in an SS-34 rotor at 4°C using a Sorvall RC-5B centrifuge. The supernatant was collected and spun for 5 min in an Eppendorf centrifuge to remove any small particles. The supernatant was finally diluted 10 times with assay buffer (22 mM sodium phosphate, pH 7.0) before assaying for GST activity.

Purification

The crude extract was treated with 39.1 g ammonium sulfate per 100 mL supernatant. The protein precipitate was separated by centrifuging the mixture at 12,000 rpm for 20 min at 4°C using an SS-34 rotor. The supernatant was discarded, and the white precipitate redissolved in assay buffer. This solution was then centrifuged again at 12,000 rpm for 20 min to remove any cell debris.

A 10 mL glutathione-agarose (Sigma Chemical Co., St. Louis, MO) column[12,13] was first equilibriated with assay buffer. After applying the redissolved protein sample to the column, 40 mL of assay buffer was used to wash the column. Glutathione S-transferase was eluted with 0.05 M Tris buffer (pH 9.6) containing 5 mM glutathione. The fractions containing purified GST were then dialyzed against assay buffer prior to the enzyme activity assay. All procedures described above were carried out at 4°C.

Protein Assay

The total protein concentration of the crude extract, redissolved protein from ammonium sulfate precipitation, and purified GST were determined by the Bradford method[13], with the protein assay kit purchased from Bio-Rad (Hercules, CA). Bovine serum albumin (BSA) was used as standard.

SDS-PAGE

The molecular weight and purity of the purified GST were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 8-25% gradient gel on the Phast-System (Pharmacia). The low range molecular weight standards (Bio-Rad) used were lysozome (14.4 kDa), trypsin inhibitor (21.5 kDa), carbonic anhydrase (31.0 kDa), ovalbumin (45.0 kDa), serum albumin (66.2 kDa), and phosphorylase b (97.4kDa).

Enzyme Assay and Inhibition by Tetrapyrroles

The enzyme activity was measured by adding 50 μL of 10-fold diluted crude extract, 50 μL of 20 mM 1-chloro-3,5-dinitrobenzene (CDNB), and 50 μL of 20 mM reduced glutathione (GSH) to a 1 mL reaction mixture and by monitoring the absorbance change at 340 nm[10]. In order to determine the inhibition of GST activity by tetrapyrroles, 50 μL of 0.1 mg/mL solution of each tetrapyrrole (bilirubin, biliverdin, chlorophyllin, and hemin) was added to the reaction mixture before the addition of reduced GSH.

CDNB, GSH, and the four tetrapyrroles were purchased from Sigma Chemical Co. (St. Louis, MO). All solutions were freshly prepared immediately before the enzyme activity assay.

Enzyme Kinetics

The initial rate kinetics of oyster GST with respect to glutathione was studied by measuring the initial reaction rate of the conjugation reaction while varying the GSH concentration and keeping CDNB concentration constant.

A control experiment was run using the same concentrations of CDNB and GSH in the absence of GST, and the initial rate in the presence of GST was corrected for the control rate. A Lineweaver-Burk (double reciprocal) plot was generated by plotting 1/V vs. 1/[GSH], then k_m and V_{max} values were determined from the slope (k_m/V_{max}) and y-intercept ($1/V_{max}$) of the plot.

Results and Discussion

The crude extract (100 mL) prepared from 227 g of oysters contained 7.8 mg/mL total protein, which corresponds to a total protein of 3.44 mg/g wet weight in oysters. It exhibited a specific activity of 0.102 μmol·min^{-1}·mg^{-i} protein. Protein recovery from ammonium sulfate precipitation was 89%. The redissolved protein showed a specific activity of 0.075 μmol·min^{-1}·mg^{-1} protein, indicating at least a 26% loss in specific activity of GST during the precipitation and redissolving process.

During the affinity column purification, GST activity appeared as a single sharp elution peak when the bound protein was eluted with 5 mM glutathione. However, there was a significant amount of GST activity recovered during application of the crude extract to the affinity column. Interestingly, when this fraction was reapplied to the affinity column, almost no GST bound to the affinity column. We are currently investigating this observation further to explore the presence of isoenzyme of GST. Kinetic and inhibition studies presented in this article will mostly be dealing with the one isoenzyme preparation that binds preferably to the affinity column, and shows a single homogeneous band on SDS-PAGE gel.

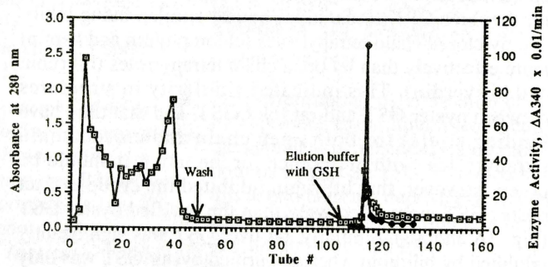


Figure 1. A typical elution profile of glutathione affinity chromatography in terms of the GST activity and protein content as indicated by absorbance at 280 nm. Arrows indicate application of the sample wash buffer and elution buffer. Each fraction collected contained 25 drops, ~1 mL.

The purified enzyme, which was 0.047 mg/mL in concentration, showed a specific activity of 3.77 μmol·min^{-1}·mg^{-1} protein towards CDNB. This activity was

about 50-fold higher compared to the activity of the protein fraction before affinity column purification. This specific activity is relatively low compared with GSTs from two other marine animals: 140 μmol•min^{-1}•mg^{-1} protein for GST isoenzyme I, 35.3 μmol•min^{-1}•mg^{-1} protein for GST isoenzyme II from shrimp eye[14], and 820 μmol•min^{-1}•mg^{-1} protein for GST from squid[15]. Also, specific activity of rat liver GST was determined to be 17.1 μmol•min^{-1}•mg^{-1} protein.

Analysis of the purified oyster GST on SDS-PAGE suggested a molecular weight of 26 kDa, which is consistent with MWs for other glutathione S-transferases (22-30 kDa)[6,9,14,15].

Kinetic study (Fig. 2) indicated a K_m value of 0.75 mM and V_{max} value of 0.016 mM•min^{-1} for the purified oyster GST. From the literature, GST isoenzyme I from shrimp eye"[14] has a K_m of 0.19 mM, and GST isoenzyme II has a K_m of 0.17 mM, both of which are lower than the K_m value for oyster GST.

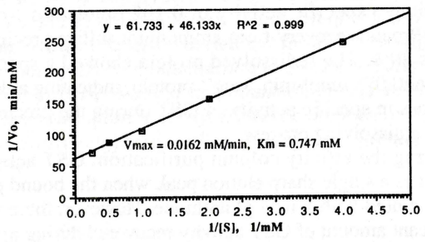


Figure 2. Lineweaver-Burk Plot of 1/v vs. 1/[GSH] for Purified Oyster GST. Initial reaction rates (Vo) were determined from the slope of ΔA_{340} vs. time plots at different concentrations of GSH. All the experiments were carried out at room temperature (25°C).

Inhibition of enzyme activity of oyster crude extract, purified GST, and rat liver GST by four tetrapyrroles was performed. Inhibition patterns were compared in order to understand the structural and functional properties of this ubiquitous family of enzymes. In general, inhibition patterns for the three GST preparations appear similar, being inhibited by closed chain tetrapyrroles (chlorophyllin and hemin) more effectively than by open chain tetrapyrroles (bilirubin and biliverdin). This indicated similarity in structures between oyster GST and rat liver GST, and that they have binding site(s) for both open chain and closed chain tetrapyrroles, with preference for the latter. It should be noted, however, that bilirubin inhibited the crude extract oyster GST more effectively than the purified oyster GST (Fig. 3A and 3B). Similarly, rat liver GST was significantly inhibited by bilirubin whereas purified oyster GST was only marginally inhibited (Fig. 3B and 3C). The reason for this observation could be either a different inhibitor to enzyme concentration ratio for oyster GST (ratio=2.1) and rat liver GST (ratio=21), or some real structural difference between shellfish and mammalian GSTs. The exact reason is still to be investigated.

In summary, it has been found that glutathione S-transferase exists in oysters and has similar structural characteristics as mammalian GST. This provides a basis for future studies including detailed structural investigations, feasibility of some environmental pollutants as substrate of this enzyme, and correlations between GST level and health conditions of marine organisms.

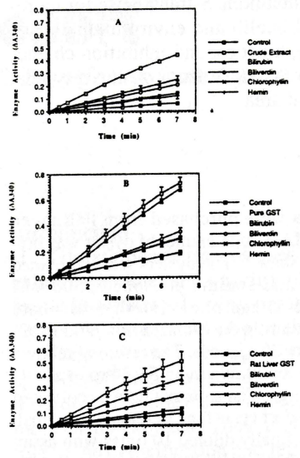


Figure 3. Inhibition of enzyme activity of (A) Oyster Crude Extract, (B) Purified Oyster GST, and (C) Rat Liver GST by tetrapyrroles.

Acknowledgements

This research project was supported by the 1994 James Flack Norris Summer Scholarship from the Northeastern Section of the American Chemical Society. We would also like to thank the Center for Marine Science and Technology, UMass Dartmouth for partial support of the project.

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Calendar

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February 21
Prof. W. Dean Harman (Univ. of Virginia)
Osmium-Based De-aromatization Agents in Organic Synthesis"
Boston University
590 Commonwealth Ave., SCI 107
at 4:00 pm

February 22
Dr. Richard Laursen (Boston University)
"Coping with the Cold: Fish Antifreeze Polypeptides:
UMass Dartmouth
Rm. 305 Sci. & Eng. Bldg. (Gr. II)
at 4:00 pm

February 23
Prof. Richard J. Saykally (UCal, Berkeley)
"VRT Spectroscopy of Water Clusters: Toward a Genuine Molecular Model of the Liquid"
Harvard University
12 Oxford St., Mb-23 Seminar Room
at 5:00 pm

February 27
Prof. Michael Snyder (Yale University)
"Large Scale Functional Analysis of the Yeast Genome"
Boston University
590 Commonwealth Ave., SCI 107
at 4:00 pm

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Memorabilia of activities of the Northeastern Section of the American Chemical Society may be submitted to the archivist for possible addition to the NESACS archives.

Jie Ge

by Bal Ram Singh

Jenny, as she is known in the research group, is a senior at UMass Dartmouth. A year ago, she received a summer assistantship at Rockefeller University where she learned basic techniques in protein chemistry.

She is interested in working on challenging biochemical projects relevant to human health. Since Glutathione-S-transferases are detoxifying enzymes ubiquitously present in animals and plants, but have not been detected or characterized in the oyster, this project may provide quite novel results. The Norris Summer Fellowship has facilitated her plan to work on this project. Her work on this project is continuing during the current year and she is currently preparing a manuscript for publication in a peer-reviewed journal.

Her level of scientific interaction and grasp of experimental plan and data analysis are at least at the level of a good second year graduate student. She is hoping to join one of the prestigious graduate schools. ◇

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In order to continue bringing high quality symposia speakers and symposia to NESACS, support from this region is essential. Any companies or individuals interested in supporting the 1995 program of the group, please contact Aloke K. Dutta (617-932-4142). ◇

Nominations

Philip L. Levins Memorial Prize

Nominations for the Philip L. Levins Memorial Prize for outstanding performance by a graduate student on the way to a career in chemical science should be sent to the Executive Secretary, NESACS, 23 Cottage St., Natick, MA 01760 by March 1, 1995. The graduate student's research should be in the area of organic analytical chemistry and may include other areas of organic analytical chemistry such as environmental analysis, biochemical analysis, or polymer analysis.

Nominations may be made by a faculty member, or the student may submit an application. A biographical sketch, transcripts of graduate and undergraduate grades, a description of present research activity and three references must be included. The nomination should be specific concerning the contribution the student has made to the research and publications (if any) with multiple authors.

The award will be presented at the May 11, 1995 Section Meeting. ◇

Board of Directors

continued from page 5

for presenting a poster-session paper be approved, even though technically the applicant is outside the geographic bounds of this Section, provided that this action does not deprive a deserving applicant within the Section of a grant. The motion was VOTED.

New business: The board ELECTED Dr. D. Rickter to fill the vacancy in the list of Alternate Councilors resulting from the resignation of A. Heyn, who had been elected as Councilor for the term 1995-1997. The board ELECTED M. Chen to the Nominating Committee. ◇

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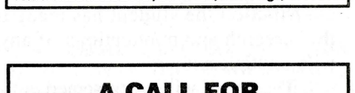

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"Modern Analytical Chemistry – A Judicious Mixture of Reaction Chemistry, Instrumentation and Chemometrics"
UMass Dartmouth
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at 4:00 pm

February 2

Dr. Stanley Stein (Center for Advanced Biotechnology and Medicine)
"Oligopeptide-Oligonucleotide Conjugates for Antisense Applications"
Northeastern University
129 Hurtig Building at 4:00 pm

February 6

Dr. Harold V. Meyers (Sphinx Pharmaceuticals)
"Multiple Simultaneous Syntheses of Small Molecule Libraries"
Boston University
590 Commonwealth Ave., SCI 107
at 4:00 pm

February 8

Dr. M. A. Altabet (Woods Hole Oceanographic Institution)
"Use of Stable Isotope Ratios to Study Nitrogen Cycling in the Oceans"
UMass Dartmouth
Rm. 305 Sci. & Eng. Bldg. (Gr. II)
at 4:00 pm

Prof. Judith Herzfeld (Brandeis Univ.)
"Solid State NMR Studies of Spectral Tuning and Proton Transport in Bacteriorhodopsin"
Harvard University
12 Oxford St., Mb-23 Seminar Room
at 4:00 pm

February 9

Prof. Thomas Pochapsky (Brandeis Univ.)
"Structure and Dynamics of Metal-Containing Proteins by NMR"
Northeastern University
129 Hurtig Building at 4:00 pm

Prof. Geoffrey Bodenhausen (Florida State University)
"Macromolecular Structures by Solution-State NMR; Suppression of Deleterious Effects of Spin Diffusion"
MIT
Room 2-105 at 5:00 pm

February 13

Prof. Edwin Vedejs (Univ. of Wisconsin at Madison)
TBA
Harvard University
12 Oxford St., Mb-23 at 4:15 pm

Prof. Kevin Jarrell (BU Medical School)
"The Mechanism of Group II Intron Self-Splicing"
Boston University
590 Commonwealth Ave., SCI 107
at 4:00 pm

February 15

Dr. Harry Hays (Tambrands, Inc.)
"Options in Industry with a Technical Degree – A Personal Viewpoint"
UMass Dartmouth
Rm. 305 Sci. & Eng. Bldg. (Gr. II)
at 4:00 pm

Dr. Jay Troutman (AT&T Bell Laboratories)
"Near-field Time-resolved Sprectroscopy of Single Molecules"
Harvard University
12 Oxford St., Mb-23 Seminar Room
at 4:00 pm

February 16

Prof. J. S. Moore (Univ. of Illinois-Urbana)
"Nonlinear Repetitive Synthesis with Phenylacetylene Monomers – Organic Nanoarchitecture for Function and Assembly"
Northeastern University
Room 129 Hurtig Building at 4:00 pm

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