

GEORGIA INSTITUTE OF TECHNOLOGY  
OFFICE OF RESEARCH ADMINISTRATION  
RESEARCH PROJECT INITIATION

*Remits fill  
action  
of you*

Date: July 12, 1974

Project Title: **Exzymatic Epoxidation**

Project No: **G-33-682**

*Gr ed  
mess*

Principal Investigator **Dr. Sheldon W. May**

Sponsor: **Research Corporation**

Agreement Period: From 6/1/74 Until Open

Type Agreement: **Grant**

Amount: **\$10,050**

Reports Required: **Annual Progress  
Final**

Sponsor Contact Person (s):  
**Mr. Sam C. Smith  
Vice President - Grants  
Research Corporation  
405 Lexington Avenue  
New York, N. Y. 10017**

Assigned to: Chemistry

COPIES TO:

- |  |                                 |
|--|---------------------------------|
| Principal Investigator   | Library                         |
| School Director  | Rich Electronic Computer Center |
| Dean of the College  | Photographic Laboratory         |
| Director, Research Administration                                    | Project File                    |
| Director, Financial Affairs (2)                                      |                                 |
| Security-Reports-Property Office <input checked="" type="checkbox"/> |                                 |
| Patent Coordinator   | Other _____                     |

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 12/12/86

Project No. G-33-682

School/~~OXH~~ Chem.

Includes Subproject No.(s) N/A

Project Director(s) S. W. May GTRC / ~~OXH~~

Sponsor Research Corporation

Title Enzymatic Epoxidation

Effective Completion Date: Open (Performance) \_\_\_\_\_ (Reports) \_\_\_\_\_

Grant/Contract Closeout Actions Remaining:

- None
- Final Invoice or Final Fiscal Report
- Closing Documents
- Final Report of Inventions
- Govt. Property Inventory & Related Certificate
- Classified Material Certificate
- Other \_\_\_\_\_

Continues Project No. \_\_\_\_\_ Continued by Project No. \_\_\_\_\_

COPIES TO:

- Project Director
- Research Administrative Network
- Research Property Management
- Accounting
- Procurement/GTRI Supply Services
- Research Security Services
- Reports Coordinator (OCA)
- Legal Services

- Library
- GTRC
- Research Communications (2)
- Project File
- Other I. Lashley
- A. Jones
- R. Embry

**REPORT OF RESEARCH CORPORATION GRANT**

(Please check one)

(Submit original and one legible copy)

G 33-682

 Interim Report Terminal Report

## INSTITUTION AND ADDRESS

Georgia Institute of Technology  
Atlanta, Georgia 30332

PRINCIPAL INVESTIGATOR Dr. Sheldon W. May

PHONE (404) 894-4052

ACADEMIC RANK AND DEPARTMENT Assistant Professor of Chemistry - Dept. of Chemistry

SHORT TITLE OF RESEARCH SUPPORTED BY GRANT

Enzymatic Epoxidation

STARTING DATE June 6, 1974

SUMMARY OR PRINCIPAL FINDINGS AND THEIR SIGNIFICANCE (State succinctly in language understandable to one not necessarily expert in this field. Include extent to which original goals have been realized and any changes to original plan made or contemplated.)

During the past year, funds from Research Corporation have allowed us to set up our laboratory and begin work on the olefin epoxidation system of Pseudomonas oleovorans. We have isolated and stabilized a strain of this organism with very high epoxidative activity which is suitable as a source of enzymes. Our enzyme isolation work has progressed well, and very recently we have observed a new protein which complexes with rubredoxin during the isolation procedure. It is possible that this protein, which does contain heme, is involved in the epoxidation reaction in some as yet undefined manner. A number of affinity resins for our ligand-specific chromatography work have been prepared and we will soon be testing their utility in isolation procedures. Our specificity and mechanistic work has been initiated by the successful synthesis of trans-trans-dideutero-1,7-octadiene, a development which we did not foresee at the outset of this work. This substrate will allow us to determine by NMR techniques whether the cis-trans isomerism of the double bond is preserved during epoxidation, and thus will represent the first direct test of the mechanism of active oxygen attack in an enzymatic system.

Perhaps the most exciting development in our work has been our development of procedures for preparing cell-free preparations with high epoxidizing activity from cells of P. oleovorans. These preparations are easy to obtain in large quantity, and allow one to carry out the oxygenation of organic substrates on a preparative scale. Thus, for example, optically active epoxides can be prepared overnight from molecular oxygen and olefins. Reactions such as these are impossible to carry out using non-enzymatic catalysts, and thus we are confident that our results will represent a significant contribution to the techniques of organic chemistry.

STUDENT PARTICIPATION (Give names of students working on the project, their role in the research, their achievements and their career plans.)

M.S. Steltenkamp, Ph.D. candidate. (Mechanistic and Stereochemical studies on epoxidation). He has synthesized the deuterium labeled substrates and is developing enzymatic systems for use in organic synthesis.

J.Y. Kuo, (Isolation, Ligand-specific chromatography, protein chemistry). He has developed the high-activity strain, synthesized affinity resins, and is carrying out isolation procedures.

Since both of these students are only in their second year of graduate school, it is too early to evaluate their career goals.

PAPERS AND SCIENTIFIC TALKS (Give titles and references to papers or talks resulting from the work. Attach two copies of any reprints available, if not previously forwarded.)

"Enzymatic Epoxidation and Oxygen Activation." Invited paper presented at the Fifth Conference on Catalysis in Organic Reactions, Boston, April, 1975. Paper is being published by Academic Press, Inc.

OTHER SUPPORT (List amounts and sources—including institutional—of other contributions received or expected for this work.)

National Science Foundation, \$50,000 grant, beginning 1/1/75.

EXPENDITURE OF RESEARCH CORPORATION GRANT FUNDS (The terminal report should be approved by an authorized officer of the institution.)

a. Equipment, supplies (Itemize major expenditures)

UV Monitor for fraction collector, \$1,985. Assorted smaller items and supplies, \$800.

b. Stipends (Academic status, rates, periods of appointment)

Half-time support for M. Steltenkamp and J. Kuo, both graduate students, Summer, 1974, \$1,110 total.

c. Other expenditures (Itemize and give purpose)

.....  
Signature of principal investigator

6/2/75  
.....  
Date

.....  
Signature of authorized officer of institution (required for terminal report only)

.....  
Date

.....  
Name and position of authorized officer of institution

6-336082

# REPORT OF RESEARCH CORPORATION GRANT

(Please check one)

(Submit original and one legible copy)

Interim Report

Terminal Report

## INSTITUTION AND ADDRESS

Georgia Institute of Technology  
Atlanta, Georgia 30332

PRINCIPAL INVESTIGATOR Dr. Sheldon W. May

PHONE (404) 894-4052

ACADEMIC RANK AND DEPARTMENT Assistant Professor of Chemistry, School of Chemistry

SHORT TITLE OF RESEARCH SUPPORTED BY GRANT

Enzymatic Epoxidation

STARTING DATE June 6, 1974

SUMMARY OR PRINCIPAL FINDINGS AND THEIR SIGNIFICANCE (State succinctly in language understandable to one not necessarily expert in this field. Include extent to which original goals have been realized and any changes to original plan made or contemplated.)

During the past year, we have made substantial progress on several fronts on this research program. The funds remaining from the Research Corporation grant have provided flexibility to purchase materials and supplies for which funds from other sources are not readily available. Among the major results which we have obtained to date are the following:

(1) Rubredoxin has been obtained in high yield and purity by a novel technique and has been successfully immobilized on a water-insoluble support. This represents the first known example of the immobilization of an enzyme of this class and we have now established that the ability of this immobilized protein to accept electrons from reducing agents and its reduction potential are very similar to the soluble enzyme. Furthermore, we have isolated ferredoxin-NADP reductase from spinach leaves and shown that this enzyme effectively complexes with immobilized rubredoxin and transfers electrons to it. We have developed specialized techniques for accurately examining the spectrum of the immobilized rubredoxin and have carried out procedures such as spectral and anaerobic titrations of the enzyme while attached to the solid support. These findings establish the basis for the use of such enzymes in affinity chromatography of complex multienzyme systems and suggest possible applications of immobilized electron transfer proteins in biochemical fuel cell applications.

(2) We have completed a study of the epoxidation of deuterated octadiene using partially relaxed Fourier transform, NMR spectroscopy. This project represents the first direct mechanistic test of the oxygen insertion process in a reaction of this type, and our results indicate that the currently popular "oxenoid" mechanism cannot be correct for this enzymatic reaction. Our results have provided clear criteria by which mechanistic proposals for enzymatic oxygen insertion can now be evaluated.

(3) We have completed a detailed examination of the stereochemistry of both mono- and diepoxide production by this enzyme system. We have established that the highly stereoselective synthesis of both these types of compounds can be readily accomplished on a preparative scale using procedures which we have developed, feats which cannot be duplicated by any known chemical epoxidizing agents. The synthetic potential of this enzymatic system has thus been greatly enhanced. Furthermore, stereochemical studies have revealed that the configuration of a preformed asymmetric center at one end of a substrate molecule can completely alter the stereochemical consequences of epoxidation at the other end, and thus the two ends of a substrate molecule do not act independently in this process. This finding establishes the basis for control of this enzymatic reaction and also provides insight regarding the mode of substrate binding at the active site.

(4) As a prelude to immobilization of complex multienzyme systems using the novel liquid membrane system developed in our laboratory, we have successfully demonstrated

that coenzymes can be incorporated into, and recycled using coupled enzyme systems within liquid membrane emulsions.

**REPORT OF RESEARCH CORPORATION GRANT**

Page 2

**STUDENT PARTICIPATION** (Give names of students working on the project, their role in the research, their achievements and their career plans.)

M. S. Steitenkamp and J. Y. Kuo (both Ph.D. candidates) are still involved in this project as outlined in the last report.

Laura L. Landgraff (M.S. candidate) is carrying out experiments on the immobilization of multienzyme systems using liquid membrane emulsions.

**PAPERS AND SCIENTIFIC TALKS** (Give titles and references to papers or talks resulting from the work. Attach two copies of any reprints available, if not previously forwarded.)

See attached sheet.

**OTHER SUPPORT** (List amounts and sources—including institutional—of other contributions received or expected for this work.)

- (1) National Science Foundation - see last report
- (2) NIH Biomedical Sciences Support grants (2) - \$10,000

**EXPENDITURE OF RESEARCH CORPORATION GRANT FUNDS** (The terminal report should be approved by an authorized officer of the institution.)

a. Equipment, supplies (Itemize major expenditures)

Miscellaneous supplies: Approximately \$5,000.

b. Stipends (Academic status, rates, periods of appointment)

No additional stipends since last report.

c. Other expenditures (Itemize and give purpose)

Signature of principal investigator

Date

7/17/76

Signature of authorized officer of institution (required for terminal report only)

Date

Name and position of authorized officer of institution

PUBLICATIONS AND PAPERS SINCE LAST REPORT

1. "Structural Effects on the Reactivity of Substrates and Inhibitors in the Epoxidation System of *Pseudomonas oleovorans*," S. W. May, R. D. Schwartz, B. J. Abbott and O. R. Zaborsky, Biochimica Biophysica Acta, 403, 245 (1975).
2. "Enzymatic Oxygen Activation", S. W. May, Catalysis Org. Reactions, p. 101-111, Academic Press, N. Y. (1976).
3. "Cofactor Recycling in Liquid Membrane-Enzyme Systems", S. W. May and L. L. Landgraff, Biochem. Biophys. Res. Commun., 68, 786 (1976).
4. "Enzymatic Epoxidation of trans,trans-1,8-dideutero-1,7-octadiene: Analysis using Partially Relaxed FT NMR", S. W. May, S. L. Gordon and M. S. Steltenkamp, Submitted to J. Amer. Chem. Soc.
5. "Stereoselective Formation of Diepoxides by an Enzyme System of *Pseudomonas oleovorans*," S. W. May, M. S. Steltenkamp and R. D. Schwartz, submitted to J. Amer. Chem. Soc.
6. "Preparation and Properties of Immobilized Rubredoxin", S. W. May and J. Y. Kuo, to be submitted to J. Biol. Chem.
7. "Mechanism of Enzymatic Oxygen Activation", S. W. May, S. L. Gordon and M. S. Steltenkamp, Fed. Proceedings, 35, 924 (1976) presented at the American Society of Biological Chemists Meeting, San Francisco, June 1976.
8. "Enzyme-Liquid Membrane Systems", S. W. May and L. L. Landgraff, presented at the Symposium on Liquid Membranes, Centennial ACS meeting, New York, April, 1976 (invited symposium presentation).

GEORGIA INSTITUTE OF TECHNOLOGY  
ATLANTA, GEORGIA 30332

CHEMISTRY

March 1, 1982

Mr. John B. Grutzner  
Program Officer for  
Chemical Dynamics Program  
National Science Foundation  
Washington, DC 20550

Dear John:

Thank you for your letter of February 1. I am a little late with respect to your three week deadline, however, it seems responsibilities came in bunches. I have just returned from a trip and your request was first on my list of things to do.

The area of electron transfer in organic reactions has blossomed according to our hopes and I believe we are in a most unusual, timely and productive area of research. Many thanks to you and NSF for making this work possible.

Attached are the four statements you requested. I hope you find everything in order.

Sincerely,

E.C. Ashby, Regents' Professor of Chemistry

ECA:ctm

Enclosures:



(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING)

**SUMMARY PROPOSAL BUDGET**

FOR NSF USE ONLY

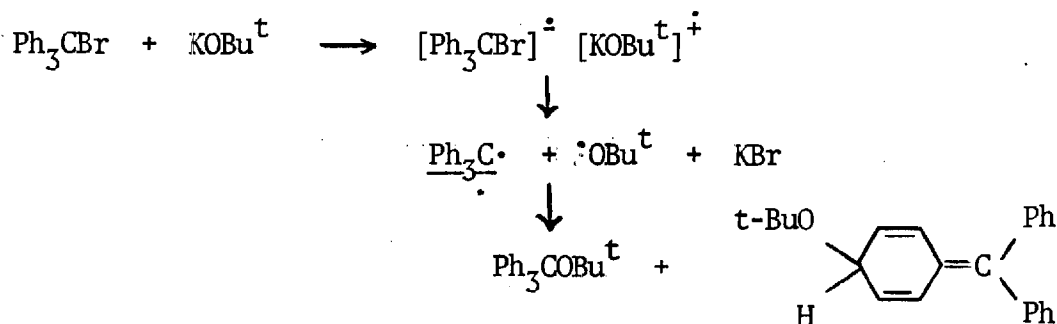
ORGANIZATION <b>Georgia Tech Research Institute</b>		PROPOSAL NO.		DURATION (MONTHS)	
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR <b>E.C. Ashby</b>		AWARD NO.		Proposed	Granted
A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title; A.6. show number in brackets)		NSF FUNDED PERSON-MOS.		FUNDS REQUESTED BY PROPOSER	
		CAL.	ACADSUMR	FUNDS GRANTED BY NSF (IF DIFFERENT)	
1. <b>E.C. Ashby</b>			1.5	\$ 5,589	\$
2.					
3.					
4.					
5. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)					
6. ( ) TOTAL SENIOR PERSONNEL (1-5)					
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. ( <b>2</b> ) POST DOCTORAL ASSOCIATES		12		28,000	
2. ( ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)					
3. ( <b>2</b> ) GRADUATE STUDENTS				14,518	
4. ( ) UNDERGRADUATE STUDENTS					
5. ( ) SECRETARIAL-CLERICAL					
6. ( ) OTHER					
TOTAL SALARIES AND WAGES (A+B)				48,107	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) <b>11.59% of A1</b>				648	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				48,755	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$1,000; ITEMS OVER \$10,000 REQUIRE CERTIFICATION)					
TOTAL PERMANENT EQUIPMENT					
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS)				600	
2. FOREIGN					
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS \$ _____					
2. TRAVEL _____					
3. SUBSISTENCE _____					
4. OTHER _____					
TOTAL PARTICIPANT COSTS					
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				5,000	
2. PUBLICATION COSTS/PAGE CHARGES				1,000	
3. CONSULTANT SERVICES					
4. COMPUTER (ADPE) SERVICES					
5. SUBCONTRACTS					
6. OTHER					
TOTAL OTHER DIRECT COSTS				55,355	
H. TOTAL DIRECT COSTS (A THROUGH G)					
I. INDIRECT COSTS (SPECIFY) <b>On Campus: 55% of Modified Total Direct Costs</b>					
TOTAL INDIRECT COSTS				30,445	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				85,800	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS GPM 252 AND 253)				-0-	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				\$ 85,800	\$

PI/PD TYPED NAME & SIGNATURE*		DATE	FOR NSF USE ONLY		
<b>E.C. Ashby</b>		<b>3/4/82</b>	INDIRECT COST RATE VERIFICATION		
INST. REP. TYPED NAME & SIGNATURE*		DATE	Date Checked	Date of Rate Sheet	Initials - DGC
<b>D. Hutchison</b>		<b>3/5/82</b>			

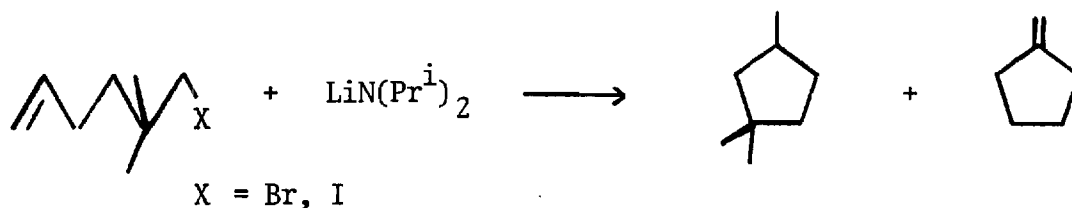
## ELECTRON TRANSFER - A MAJOR REACTION PATHWAY

This past year has been the most successful of any year in the history of our research efforts. This period has not only been very productive as evidenced by the appearance of twelve papers as a result of our work, but during this same time period we have made another very unusual discovery in addition to the one reported a year ago which produced such interesting results.

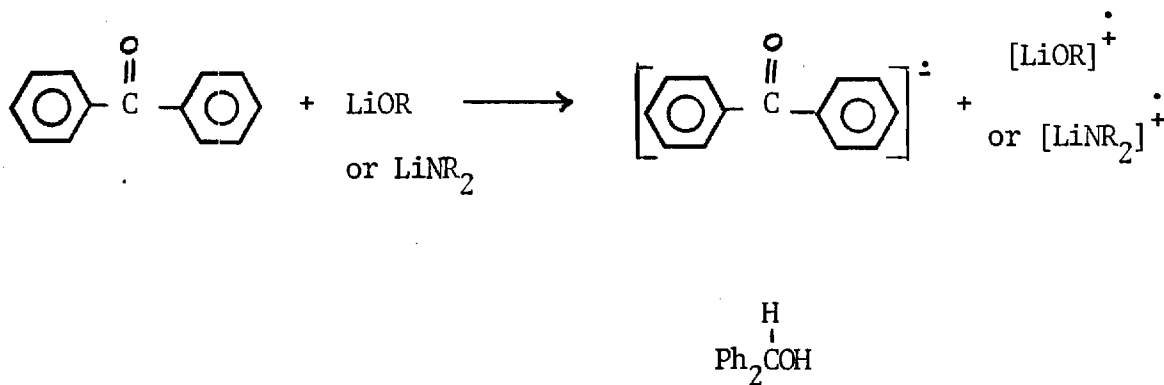
First, during the past year we have reported that not only nucleophiles such as carbanions ( $R^-$ ) and hydrides ( $H^-$ ) behave as electron transfer agents, but also more classic nucleophiles such as alkoxides ( $OR^-$ ) and dialkylamides ( $NR_2^-$ ). The following examples demonstrate the principles involved.



The appearance and disappearance of  $\text{Ph}_3\text{C}\cdot$  was observed by ESR and hence the integrity of this mechanism established. In addition to direct observation of radical intermediates, the use of probes was also used to establish the intermediary in reactions involving typical nucleophiles. For example:



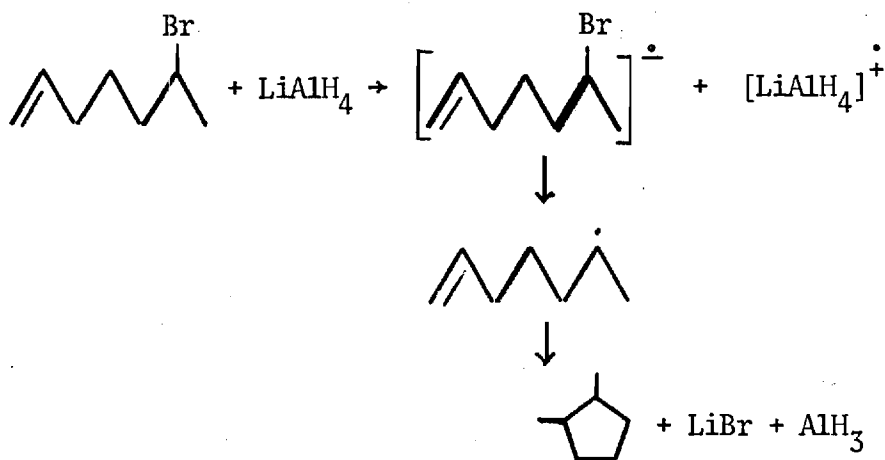
When ketones are used as a substrate, the ketone itself becomes the probe which is directly observable by ESR.



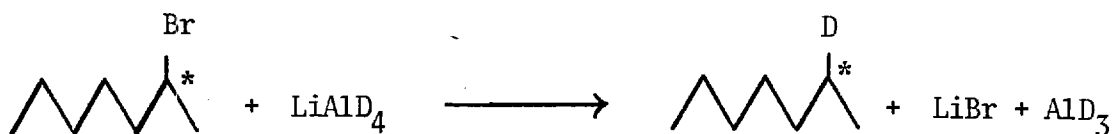
In these reactions benzophenone ketyl is observed in high concentration and the yields of alcohol are nearly quantitative. Similar observations were made using aluminum alkoxides (Meerwein-Ponndorff-Verley Reductions).

In the past three months we have also demonstrated the presence of electron transfer in Aldol Condensations, the Wittig Reaction and the Cannizzao Reaction. Details of these studies will be presented at a later time, however, it is sufficient to say the ESR observations establish the existence of electron transfer at least in the model systems studied.

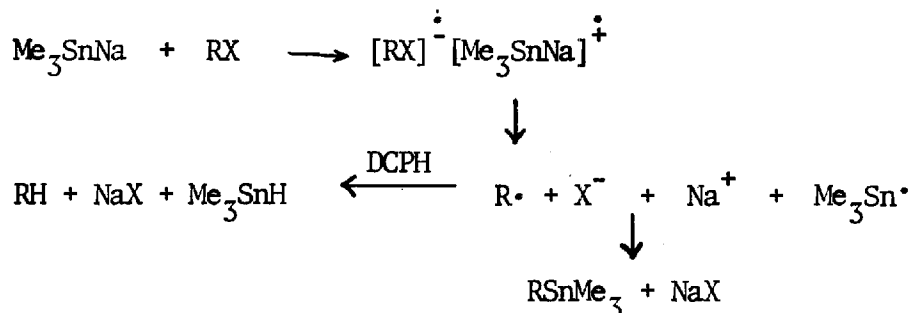
Possibly the most important finding has been the proof gathered that electron transfer and reactions proceeding by a radical intermediate can proceed with substantial inversion of configuration. For example, we have shown that the reduction of 2° halides with  $\text{LiAlH}_4$  proceeds to a large extent by an electron transfer pathway.



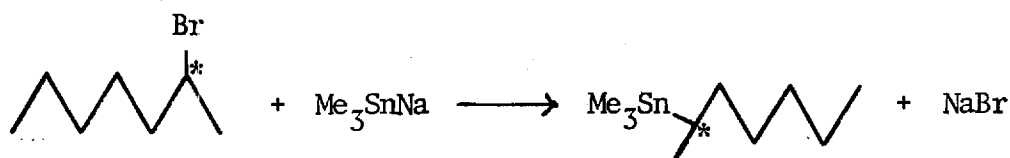
Yet when we carry out a very similar reaction we are able to show that the reaction proceeds with predominant inversion of configuration.



In addition to this system, we have been able to show that the reaction reported by Kuivila to proceed by SET and reported by San Filippo actually does proceed by SET.



We have shown that the reaction does proceed by a SET pathway yet proceeds by predominant inversion of configuration..



77% Inversion



70% Cyclized Product

(3) It appears that we will have no uncommitted funds remaining at the end of the current period.

(4) Current support for our entire research effort is two fold (1) NSF and (2) PRF.

"Organometallic Reaction Mechanism. The Importance of Single Electron Transfer Pathways" NSF Grant (CHE 78-00757), \$85,000/yr, 6/81-6/84 80% Effort

"New Reagents and New Reactions in Organometallic Chemistry" PRF Grant (12545-AC1), \$15,000/yr. 9/80-9/81 20% Effort

There is no relationship between the NSF and PRF proposals or effort.

Pending Proposal: PRF Renewal Proposal

"Single Electron Transfer in Organic Reactions"

The reactions proposed here are mainly organometallic in nature with emphasis on preparation of probes needed to detect SET. There is no overlap between this proposal and the present work sponsored by NSF.