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# "STUDY OF THE CHEMISTRY OF NATURAL PRODUCTS"

## COLLECTED REPRINTS 1970 - 1992

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Thesis submitted for the degree of Doctor of Science in the Faculty of Science, The University of Adelaide, 1992.

Awarded 1993

CONTENTS

PREFACE

In support of my candidature, I submit a thesis comprising fifty published works in the general theme of "Study of The Chemistry of Natural Products." The papers are grouped, essentially in chronological order as described under the "Statement on Authorship and Contribution." Subject to the "Statement," the publications report original work undertaken by myself, alone or in collaboration, or work carried out under my supervision in my laboratory at Massey University, New Zealand or at Genentech Inc., San Francisco.

This collection of papers represents my interest over a period of more than twenty years in the study of natural products, either isolated from natural sources or produced by chemical synthesis and by biosynthesis. I have been fortunate to work at a time when the study of natural products has undergone tremendous changes. During this period my interests have progressed from terpenoid natural products to polypeptides. In these studies, structural analysis and synthesis have been associated in a synergistic manner and have given a common theme to the work. It would have been difficult to predict in 1970, however, advances such as the development of polypeptide synthesis on a polymeric support or novel biosynthetic techniques derived from recombinant DNA-technology.

In a similar manner, analytical techniques such as high performance liquid chromatography and mass spectrometry have been applied to biological macromolecules. Such techniques have developed to the point where subtle structural differences can be detected in a protein, despite the presence of hundreds of functional groups. One consequence of these rapid developments in technology has been a cross-fertilization between the different branches of chemistry. My work has been greatly aided by disciplines such as analytical chemistry and biochemistry, as well as molecular biology.

Such broad advances in chemistry have made valuable contributions in many fields, for example in medicine with better understanding of disease processes. My work has contributed to the production and clinical trials of protein therapeutics produced by recombinant DNA-technology. I have found that structural organic chemistry is intimately involved in the demonstration that a complex molecule produced by novel biological process has the structural and biological properties to permit clinical trials.

DECLARATION

This thesis contains no material submitted for another degree or diploma in any University except where due reference is made in the "Statement on Authorship and Contribution." I consent to the thesis being made available for photocopying and loan if accepted for the award of the degree.

Signed Date 8.28.92

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I wish to acknowledge support by way of National Heart Foundation of New Zealand, Medical Research Council of New Zealand, and University Grants Committee of New Zealand. Some of the manuscripts were prepared and some research initiated during periods of leave, 1976 -- Australian National University, 1980 -- Baylor College of Medicine and 1984 -- US. Food and Drug Administration. I wish to thank Professor D. A. Buckingham, Dr. J. Sparrow, and Dr. E. Titus for the hospitality of their laboratories. During my work at Massey University I was fortunate to have Dr. David Harding as a close associate. Also many of the early HPLC studies were carried out in collaboration with Dr. M. Hearn's group in Dunedin. I wish to express my appreciation for the guidance given to me by my Ph.D. supervisors, Dr. R. A. Massy - Westropp and Dr. L. N. Mander. In my post-doctoral studies I was fortunate to work in the laboratories of Dr. P. R.Vagelos and Dr. G .Marshall. During my career, I have enjoyed the friendship and support from several distinguished scientists, Professor R. D. Batt, Dr. B. R. Karger, Dr. C. Horvath, Dr. B. Welander, and Dr. A. Ramel.

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SUMMARY OF WORK

The organic chemistry of natural products has undergone tremendous changes over the past twenty years and the candidate has been fortunate to participate in this field over such a stimulating period. My Ph.D. studies were directed at the total synthesis of a diterpenoid, rosenonolactone (7,17). During this period, the chemical synthesis of peptides had been advanced dramatically by the introduction of the Solid Phase Method of Merrifield. For my postdoctoral studies I given the opportunity to work on the chemical synthesis of a seventy four residue polypeptide, Acyl Carrier Protein. Publications 2 and 5 described the synthesis of the protein as well as structure-function studies that were carried out on analogs prepared by the solid phase method (6).

After joining the chemistry department at Massey University, I continued studies on chemical synthesis of proteins, culminating with the total synthesis of apolipoprotein CI (14 ). Other publications described improvements in the solid phase approach to peptide synthesis(9-12), as well as a new method based on cobalt chelates (48,49). Another project involved the semisynthesis of Acyl Carrier Protein, in which an amino-terminal hexapeptide was combined with the corresponding fragment of the native protein(44).

In 1976 the use of High Performance Liquid Chromatography (HPLC) for peptide and protein analysis and purification was studied in my laboratory (18,19) and resulted in the first publication on peptide mapping by this technique (22). A new method of activation of polysaccharide matrices was developed to facilitate affinity chromatography purifications (28). This method was granted international patents and licensed to Pierce Chemical Co. under the product name of Reacti-Gel. Affinity chromatography was also used to purify and characterize antithyroglobulin antibodies (50).

A sabbatical at the Baylor College of Medicine and the subsequent support of the National Heart Foundation of New Zealand allowed studies on the structure of lipoproteins and the causes of heart disease. These studies used the combination of chemical synthetic approaches (64,65) and HPLC (45,47,57,67) to study lipoprotein structure in normal and hyperlipemic subjects. Using these techniques, the first animal model of Type I hyperlipoproteinemia was characterized in collaboration with Mr. B. Jones of Massey University (58,69).;

In 1985 the candidate joined a biotechnology company, Genentech to apply protein characterization technology, in particular HPLC (77,89,90), to the novel proteins produced by recombinant-DNA technology. These new analytical procedures were invaluable in the approval process for medical use of human growth hormones (treatment of dwarfism) and tissue plasminogen

activator (myocardial infarction). The degradation pathways for protein pharmaceuticals were studied(R12,74,80) and characterization of glycoproteins was described in the following publications (79,89). Recently new analytical methods have also been applied to biosynthetic proteins, such as Hydrophobic Interaction Chromatography (84), Mass Spectrometry (86,88), Capillary Electrophoresis (R17,81,86).

## STATEMENT ON AUTHORSHIP AND CONTRIBUTION

The candidate states that all publications referred to in this thesis were carried out either by the candidate, or under supervision in his laboratory, or in collaboration with colleagues who have been acknowledged in the previous section.

## PUBLISHED WORKS

The following publications were selected to be representative of the work carried out by the candidate and have been grouped together into 5 sections: Synthesis of a diterpenoid (A), Chemical synthesis of polypeptides (B), HPLC of polypeptides (C), New matrices for affinity chromatography (D), Structure-function studies on lipoproteins (E), Analytical Biotechnology(F).

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